Diana Weedman Molavi

The Practice of Surgical Pathology

A Beginner's Guide to the Diagnostic Process

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Dedicated to
Rameen, Claire,
and Annelise.

Preface

On This Book

Welcome to pathology. If you are reading this book, it is Iikely that you are either in pathology training or considering pathology as a specialty. This book is an attempt to bridge a gap between the way pathology is taught to medical students and the way you must learn to practice it as a resident. In medical school, with tacit acknowledgment that most students are not going to become pathologists, we teach pathology as it intersects with pathophysiology and pharmacology. *Robbins and Cotran's Pathologic Basis of Disease* is the most prominent example of this approach and is an excellent and comprehensive text for this purpose. However, the *Robbins* does not teach the more practical aspects of pathology practice, such as differential diagnoses, special stains, biopsy interpretation, the assessment of margins, and tumor grading. These are the nuts and bolts of pathology practice, the countless subtleties, shades of grey, and conventions of semantics that go into creating a patient's diagnosis. For this, the resident must turn to the huge volume of literature for practicing pathologists, from the general surgical pathology texts such as *Sternberg's Diagnostic Surgical Pathology* and *Rosai and Ackerman's Surgical Pathology* to the highly detailed organ-system texts. For the beginner, not yet fluent in the foreign dialect that is pathology, these professional-level texts are simply too much, too soon. This book, inspired by my own rocky and somewhat prolonged learning curve, is an attempt to create an intermediate step.

This book is intended to be a quick crash course in the basic facts that you are expected to know when you begin your surgical pathology rotations. In this book, you will find organ-based chapters that describe the approach to specimens, descriptions of common diagnoses, pitfalls, practical pearls, differential diagnoses, and key requirements of written diagnoses. The goal is for you to be able to read a chapter in 20 minutes and come away knowing enough about a specimen to hold an intelligent conversation with the attending at the microscope. Early in training, you do not have to get the diagnosis right to get credit—you just need to demonstrate a sound thought process and some background knowledge. If you already know the language, you can focus on asking the really practical questions, such as "How do you know it is X and not Y?" and "How do you handle this if you cannot show definite invasion?" These are the conversations that will enable you to function independently when you are finally out in the real world.

This book will also be useful to medical students rotating through pathology. Many students are given the opportunity to preview cases like a resident but will quickly find their second-year pathology course does not really help in formulating a diagnosis. This book is written at a level that should be accessible to students, enabling them to get more out of their pathology rotation by understanding the more interesting diagnostic challenges involved in even routine specimens.

On What This Book Is Not

Complete or comprehensive: This book is a very oversimplified view of pathology and, in the interests of brevity and clarity, is deliberately scant on details in many areas. Some advanced topics have been omitted entirely.

An atlas: Photographs have been chosen to complement some of the specimen descriptions, but you will get more out of this book if you have a good illustrated text or atlas to supplement your learning.

A grossing manual: For many organs, this chapter deals with either the biopsy or the organ resection, but not both, depending on which specimen type is more common or more illustrative. Therefore, while some grossing tips are included, this book complements, rather than replaces, your grossing manual.

A board review book: While you do need to know just about everything in this book to pass the boards, this text is in no way sufficient for that. However, many senior residents who have seen the material have commented that it was a good way to begin their study, to caulk up any small gaps that existed in their big-picture views.

On Learning Pathology

In pathology resident education, there are two main categories of knowledge. One is factual knowledge, and the second is experiential knowledge. To understand the difference, think about how a child learns her colors. The rote question and answer of, "What color is the sky?" "Blue!" can be taught to a child as soon as she learns to talk. She may know the standard colors of apples, leaves, bananas, and so forth, purely by repetition and games. However, when you pick up a blue block and ask her to identify the color, she may not actually know the answer. You can tell her, "This is blue," but she does not yet understand what particular quality you are pointing out. Is it the shape of the block or the texture? Is it the wood it is made from or the letter on the side? It takes many, many repetitions of pointing out different blue things (a towel, a crayon, a book) before she finally understands the quality of blue, the thing that is similar across all those different-looking items. In the same way, an intern may know that "hyperchromatic" or "atypical" are indicators of malignant cells. However, he or she will need to see countless examples of what the experts call atypical to really understand what qualities of the cell they are identifying. To that end, the more glass you see during your training, the better your eye will be. No book can give you that kind of experiential knowledge.

On the other hand, you can have the best eye in the world and misinterpret what you are seeing for lack of factual knowledge. Part of the goal of this book is to give you a head start on the factual knowledge. There are many examples in this book of very basic principles that are more or less assumed to be common knowledge and so are rarely, if ever, explicitly taught. I had multiple head-smacking moments in my own residency, when I thought in exasperation, "Why didn't anyone tell me that in the beginning?" My hope is that getting these company secrets up front will smooth the learning curve for future residents.

On Teaching Pathology

This book began over the course of a 2-year experiment at the Johns Hopkins Hospital. In my fourth year of residency, I started a weekly microscope-based slide session for interns. Each session was accompanied by a handout and approximately 20 glass slides representing the most common diagnoses in that organ. The conferences were designed purely for the interns, with the intent of creating a protected didactic environment in which no question was too basic, no prior knowledge was expected, and "zebras" (unusual or exotic diagnoses) were ignored. Sitting around a large multihead scope, we began with normal histology and the mental approach to the biopsy or resection and then covered the array of nonneoplastic entities

or changes that could simulate cancer. Finally, we looked at common tumor types and their variants, comparing and contrasting normal with tumor, low grade with high grade. This book is a compilation of those handouts, with the addition of illustrations.

The conference has now passed to a group of fourth-year residents committed to teaching and will hopefully become a sustainable tradition at Johns Hopkins. With the curriculum written, however, and the focus on common entities seen at hospitals of all sizes, this conference could easily be duplicated at other programs, either by faculty or by senior residents.

This book is an experiment in teaching, and feedback is welcome.

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Contents

1 Using the Microscope

Upon arriving in the pathology department, you will most likely be given a microscope of your own. Learning to operate the microscope effectively is the prerequisite to everything else in this book. We will begin with the basics: how not to hurt yourself.

Ergonomics

Many pathology residents have acquired new and painful musculoskeletal complaints after a few months at the microscope. Here are the general principles to avoid injury.

- A neutral neck: When looking through the eyepieces, your neck should be in a neutral position, meaning no active muscle tension is required to maintain the position. Your eyes should be pointed directly forward or slightly downward. Bad positions are those that involve flexing your neck (dropping your chin to your chest), jutting your chin forward, or turning your neck left or right. Tilt-head microscopes are optimal for this positioning, but three-ring binders under the microscope can also adjust the tilt. Your eyepieces should make no more than a 30° angle with the desk surface.
- A straight back: Your mom was right about your posture, a straight back is better than a slouch, but you will need some help in the form of a chair with a supportive back. Your chair should hold you upright so that your head and neck can sit comfortably on top of your spine, without having to crane your neck forward. This can be accomplished by either adjusting your chair back to a more vertical position or adding a support pillow. Always sit directly in front of your microscope; having it off to one side to make more room on your desk will quickly cause back and neck pain.
- Supported elbows: You will be using two hands all the time, one to drive the slide and one to focus. Either job can be done with either hand, but both elbows need to be supported on the desk. Leaving your elbows floating in space while doing fine movements with your hand will lead to a nasty parascapular back spasm. Therefore, your chair should be high enough that you can place your forearms flat on the desk in front of you, with your upper arms perpendicular to the floor and flat against your torso. This may create a new problem for your neck (see the first point) if your microscope is not tall enough to meet your eyes. A good thick book or two under the microscope should fix this problem. Shorter people may also require a footstool to maintain this chair height.
- A padded surface: Your driving hand will probably rest on its elbow, while your focusing hand will lay flat on the desk. For both arms, the point of contact with the table should be padded to avoid a compression neuropathy (often the ulnar nerve). Possible solutions involve pieces of rug or bathmat, sponges, mouse pads, or commercial gel pads designed for desk users.

- Pay attention: When something starts to hurt, take a moment to critically analyze your posture and position. Focus on which muscle group is hurting you and what action relieves it, and jury-rig a way to achieve the more comfortable position. You cannot "push through" the pain; you will only end up with a chronic repetitive motion injury that will be with you for months or years. Once the cycle of pain and muscle spasm has begun, it can be very difficult to reverse it, short of taking a few months away from the microscope.

The Parts of a Microscope

Figure 1.1 shows an Olympus BX40 microscope. The exact positions of the various knobs and rings may vary by microscope, but all of these elements should be present.

1. Light source (Light from the bulb at the back of the microscope is directed upward by a mirror, hidden within the microscope base.)
2. Field diaphragm (The width of this diaphragm is controlled by the knurled ring. Closing this diaphragm reduces the visible circle of light illuminating the image. A neutral density filter, optional and removable, sits atop this diaphragm.)
3. Screws to center condenser (one on each side)
4. Focus knobs (coarse and fine)
5. Knob to raise and lower condenser, focusing the light to achieve Köhler illumination
6. Flip knob to move the condenser out of the light path for viewing at lowest power
7. Eyepieces with diopter adjustment ring
8. Objectives
9. Stage for the slide (The slide holder has been removed from this stage, allowing free movement of the slide, which is preferred by many pathologists.)
10. Aperture diaphragm of the substage condenser (The knurled ring controls the size of the cone of light reaching the specimen, and adjusting it causes changes in image contrast and

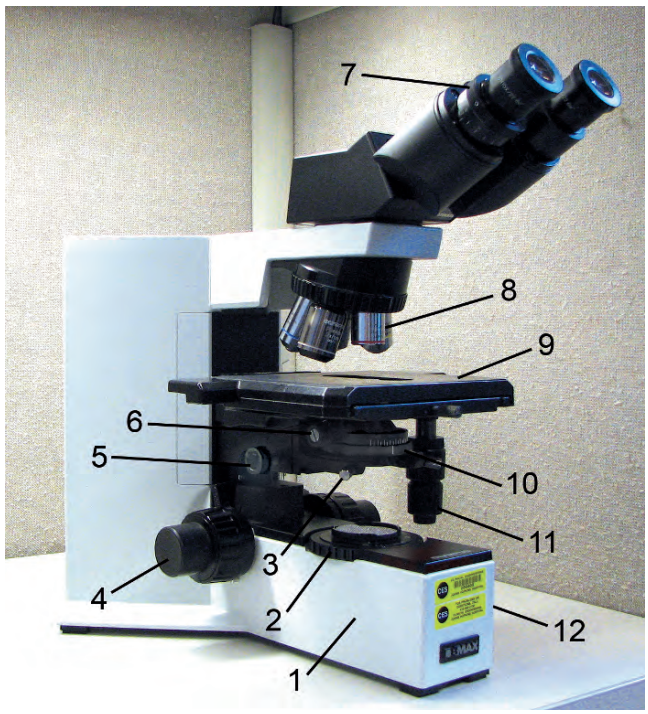

Figure 1.1. Diagram of the parts of a microscope.

quality. The substage condenser itself is the conical lens housing that sits on top of the diaphragm, hidden by the stage in this view.)
11. Knobs to move the stage (which allow for controlled X- and Y-axis movement when the slide holder is in place)
12. Light intensity adjustment (not seen; the voltage, or brightness, of the light is controlled by a knob or sliding bar)

A review of optics

There are excellent Web sites and books out there for a thorough technical review of Köhler illumination in microscopes. This is not one of them. However, the essence is that light is passed up through the microscope and focused down to a point image or spread into a wide cone through the use of lenses and diaphragms. The light originates at the light bulb at the back of the microscope, is redirected upward by a mirror, and is first shaped by the field diaphragm at the base of the microscope. Like a spotlight, this diaphragm directs a column of light up toward the slide. This column of light is concentrated into a tighter beam of light by the condenser, which results in illumination of the specimen with an even, bright, flat light.

When an image or beam of light is sent through a lens, there is a point on the other side of the lens at which the light rays converge to a point and the image is in sharp focus. In the eye, ideally, this point is at the retina, but if the eye is too long or too short relative to the lens, corrective lenses are required. In the modern microscope, there are many lenses and diaphragms in series, but there are essentially two light paths, and each one is in focus (converging to a point) at multiple different levels of the microscope.

One path is the image of the tissue. There are four points within the microscope where, if you placed a tiny projector screen, you would see a focused image of your tissue; these are called the *conjugate planes*. The conjugate planes of the image path are (1) the field diaphragm, (2) the slide or specimen, (3) the fixed diaphragm within the eyepiece (at the bottom of the removable eyepiece), and (4) a point above the microscope where you put your retina or the film of your camera.

The second path is the image of the light bulb filament. At certain points along this path, a tiny projector screen would show an image of the light source; this path is designed to have different conjugate planes than your tissue image, because, at the level of your tissue, you want a wide *unfocused* source of light. The conjugate planes of the light source are (1) the light bulb itself, (2) the condenser's aperture diaphragm, (3) the back focal plane of the objective, and (4) the "eye point" immediately above the microscope that corresponds to about where your cornea should be.

To achieve Köhler illumination is to align all of these lenses and diaphragms such that the conjugate planes are exactly where they should be, creating the best image your microscope is capable of. Fortunately, it is possible to learn this technique without fully understanding the physics behind it. You can certainly use the microscope without knowing how to do this, but the image quality will not be great, and neither will your diagnoses.

Achieving Köhler Illumination

- Place a slide on the stage. Adjust the eyepieces so that they are the correct distance apart for your eyes.
- Focus on a slide using your 10× objective. For microscopes with only one adjustable eyepiece, close the adjustable eye, and focus using the regular focusing knob. For microscopes with two adjustable eyepieces, either eye can be used first.
- Once the fixed eyepiece is in focus, shut that eye and focus the other eye with the eyepiece ring. The scale on the eyepiece ring shows the diopter adjustment; the positive direction is analogous to reading glasses, so it is easier on the eye.

- Make sure your aperture diaphragm on the substage condenser is completely open (this may be clockwise or counterclockwise, depending on the microscope).
- Close down the field diaphragm until you see a small circle or octagon of light. It should be in the center of your field of view and have a crisply focused edge. If not, you can center it using the small screws on the condenser and focus it by raising or lowering the condenser.
- Open the field diaphragm back up so that light completely fills your field of view.
- For most work, this is sufficient to give optimal viewing conditions. However, for viewing translucent (unstained) structures, or for photography, you also need to optimize the aperture diaphragm. Notice that closing it down dims the light and creates a three-dimensional quality to the image, whereas opening it up creates a flatter, brighter image. The optimal diaphragm size closes down the light path to match the diameter of the objective so that the light rays coming up from below make a straight, parallel column of light into the objective, minimizing scatter. This size is different for each objective. To find it, you must remove an eyepiece and look down into the eye tube. You will see a circle of light; close the aperture diaphragm (the ring on the condenser) until the outer one fourth of the field is black. Replace the eyepiece.

Becoming Parfocal

Parfocality means that if an image is focused at 40×, you should be able to switch to 4× and still be in focus. It is not the same as Köhler illumination. You can achieve parfocality only on a microscope with two adjustable eyepieces; it is most important on multiheaded microscopes, when the observers at the additional heads need to be in sync with the person controlling the focus. The beginning of a session with multiple users on a multihead microscope should always start with this focusing ritual.

- Start by adjusting the eyepieces on the main microscope head to the neutral position, or zero diopters. The person driving the microscope should first adjust for Köhler illumination, as above, and then go to 40× and focus on the slide. (If using a camera that projects to a TV or screen, focus the microscope such that the TV is in focus.) While the driver adjusts his or her own eyepieces, all observers should also adjust their own eyepieces to optimal focus.
- Now go to 4× without moving the slide or touching the main focus knob. While at 4×, the driver and all observers should readjust their eyepieces to be in focus. Now the screen and each individual should be in focus at each objective, or parfocal.
- If one objective is slightly "out," make sure it is tightly screwed in to the objective carriage. Sometimes one objective just can not be made perfectly parfocal, but if the above procedure is followed, at least the observers will be in sync with the driver, who can make corrections using the main focus knob.

Cool Microscope Tricks

Some things on slides do not pick up stain and therefore appear transparent or translucent on the slide. Good examples are calcium oxalate and suture material. They can be essentially invisible during normal viewing but will glow under polarized light. However, most residents' microscopes do not have polarizers. A quick and easy substitute is to flip the condenser out of the light path, just like you do when viewing at 2×. This will cause refractile material to "pop out" and be easily visible.

The knowledge of different paths of light being focused at different planes can be useful. For example, if you are looking at a slide and see debris or dust in sharp focus, that debris must be located in one of the same planes in which the image path is focused: on the surface of the field diaphragm, on the slide itself, or at the fixed eyepiece diaphragm. This diaphragm is located at the bottom of the eyepiece, in the tube, and is not usually exposed to dirt. The eyepiece diaphragm is the position where an ocular micrometer sits to superimpose a tiny ruler

on your image. On the other hand, if the debris is out of focus when the image is focused, it is more likely to be on the condenser or the top of the eyepiece.

Sometimes, at a multihead microscope session, you would like to give everyone a very low-power view of a slide, even lower than the 2× objective. The slide itself can be placed directly on the field diaphragm at the base of the microscope. This focal plane is in sync with that of a slide on the stage, so you will actually get a reasonably focused image of the entire slide. This trick also works with Kodachrome slides.

If your slide stubbornly refuses to come into sharp focus at high power, it is probably one of two problems: either the slide is upside down (coverslip on the bottom), or the objective is dirty (either a fingerprint or oil from the 100×). A dirty 40× is hard to clean, so many residents avoid ever using oil immersion on their own microscopes. If you do have a rotation that requires use of the 100×, consider arranging the objectives so that the 100× and the 40× are not next to each other, reducing the chances that you will drag the 40× through a puddle of oil. The lower power objectives are usually far enough from the slide that they pass above the oil slick.

Eyeglasses

For your average moderately nearsighted scholar, it is better to use the microscope without corrective lenses (glasses or contacts) in place. The microscope eyepieces can correct for mild to moderate vision problems, and it is easier on your eyes without an additional lens in the way. However, for more severe vision problems, or for those with astigmatism, it may be necessary to work with corrective lenses on. If you must wear glasses, there are special "high eye point" eyepieces that can be purchased. These account for the fact that because of the glasses on your face, your retina is farther from the eyepiece than if you were not wearing glasses. They may be a good investment for residents who must spend long hours looking through the microscope.

Motion Sickness

There are some unfortunate individuals out there who are very sensitive to vestibular–ocular mismatches. If you are not one of them, you may disregard this section. For some reason, having a moving image that fills most of your field of view while your body is motionless can trigger, essentially, car sickness. This phenomenon is usually only a problem when someone else is "driving," or moving the slide around, but as a resident you do quite a bit of observing while the attending drives. Some drivers are better than others; the habit of constantly moving the slide, as opposed to quick movements with long pauses, is particularly nauseating for the susceptible. Here are some suggestions to get through this unpleasant experience:

- Be reassured that you will quickly get your sea legs. Most people have to battle with this for only a few weeks before their vestibular systems adjust.
- If the experience is really bad, consider medication. There are over-the-counter medicines for this. Meclizine, sold as Bonine, does not cause as much drowsiness as Dramamine.
- If you have an unexpected episode and you are stuck at the microscope for an indefinite period of time, you need to reduce the amount of moving images hitting your eyes. If you are in a conference with the microscope hooked to a TV monitor, watch the monitor instead. Another option is to let your head sink down just enough that the images hit your eyelids, not your eyes; this is subtle, and you can straighten back up when the attending asks, "What do you think of this?" You can also close your eyes while the slide is moving, but this is a little more obvious. Studying your paperwork, looking up the patient history on the computer, answering a page, or going to get the old biopsy material can all give you momentary breaks. In desperate times, you just do what you can.

2 Descriptive Terms in Anatomic Pathology

Central to effective learning in pathology is the ability to speak the language. This chapter covers the approach to defining and describing an unknown tumor or lesion and defines histologic terms commonly used in pathology.

Interface With the Surrounding Normal Tissue

Term and definition	Appearance	Example
Circumscribed: well delineated lesion	Well-defined border between normal tissue and the lesion	Fibroadenoma
Encapsulated: surrounded by a fibrous capsule	Thick pink border surrounding the lesion	Follicular adenoma, thyroid
Infiltrative: invading into and among the surrounding normal cells	Poorly defined border with normal tissue	Prostate carcinoma
Lobular: in architecture, refers to a generally circumscribed or anatomic distribution	Circumscribed, rounded nodules of cells; simulates a normal anatomic unit	Lobular capillary hemangioma
Pushing border: expanding into and compressing the surrounding tissue	Can create the appearance of a capsule	Medullary carcinoma, breast

Cellularity (Low to High) and Mitotic Rate

Note the cellularity (by *cellularity* we often mean how blue it is, or how densely packed the nuclei are). Cellularity may be described as *hypercellular* or just *cellular* or as *hypocellular/paucicellular*. Also look for mitoses on high power. High mitotic rate may be an indicator of malignancy. Atypical mitoses (tripolar) are convincing indicators of malignancy. Estimate how many mitoses are seen per high power field (40×).

Architectural Pattern

Term and definition	Appearance	Example
Alveolar: resembling alveoli or little cells, sacs, or nests	Nested—there is structure to the lesion but no glands or ducts	Paraganglioma (Figure 2.1)
Basaloid: resembling basal cell carcinoma	A blue nested tumor (often poorly differentiated squamous) with tightly packed nuclei and palisading around the edge of the nest	Basal cell carcinoma (Figure 2.2)
Biphasic: having components of two cell lineages	Spindled cells with islands of epithelioid cells or glands	Synovial sarcoma
Cribriform: perforated, like a colander	Well-formed holes within a glandular lumen	Adenoid cystic carcinoma (Figure 2.3)
Discohesive: falling apart into single cells	No common borders among cells	Lobular carcinoma in situ
Epithelioid: composed of round to oval cells with abundant cytoplasm	Cells look plump, the opposite of sarcomatoid	Ductal carcinoma, breast (Figure 2.4)
Fascicular: composed of fascicles	Bundles of elongated, spindly cells streaming in parallel arrays	Leiomyoma (Figure 2.5)
Glandular: forming gland structures with lumens	True glands should have polarized cells radiating around a lumen	Adenocarcinoma
Glomeruloid: resembling the glomerulus	A coiled tangle of vessels, capillaries, or glands	Glioblastoma multiforme vascular proliferations
Herringbone: resembling a pattern of tweed fabric	A variant of fascicular that shows bundles alternating in a zigzag array	Fibrosarcoma (Figure 2.6)
Hobnailed: resembling a large-headed nail used in shoes	Epithelial or endothelial cells round up and protrude into the lumen as little humps	Angiosarcoma (Figure 2.7)
Indian file: cells infiltrating through the tissue in single-file lines	"Lines" may be only three to four cells long and run parallel to stromal planes	Lobular breast carcinoma
Microcystic: scattered small cystic spaces that are not ducts or tubules	Microcysts may look like glands but do not have polarized epithelial linings and are haphazard	Acinic cell carcinoma, microcystic pattern (Figure 2.8)
Micropapillary: papillary-shaped epithelial projections without true fibrovascular cores	Can have a medusa-head appearance (serous carcinoma) or lollipop projections in a duct (micropapillary ductal carcinoma in situ [DCIS])	Micropapillary serous carcinoma, ovary (Figure 2.9)
Nested: see *Alveolar*		
Pagetoid spread: single malignant cells scattered throughout a benign epidermis	Cells standing out at low power as not belonging in the epithelium	Paget's disease
Palisading: resembling a fence made of sharp stakes	Parallel arrays of nuclei catching your eye at low power as a dark border	Basal cell carcinoma (see Figure 2.2)
Papillary: an exophytic growth pattern with fibrovascular cores supporting proliferative epithelium	Cauliflower- or coral-shaped structures with branching fibrovascular cores	Papillary carcinoma, breast (Figure 2.10)
Polarized: epithelial cells that have a uniform nuclear position, either apical (lumen side) or basal (basement membrane side)	Polarized cells surrounding a true lumen should show a distinct halo of cytoplasm surrounding the lumen, if the nuclei are basal	Cribriform DCIS
Pseudopapillary: a papillary pattern caused by cell die-off in between fibrovascular septa	In some areas, the tumor will look solid or nested, but the cells at the nest centers degenerate, creating an outline of the septa	Solid pseudopapillary neoplasm, pancreas
Reticular: resembling a network or net-like array	Microcystic or honeycomb appearance	Yolk sac tumor, testes (Figure 2.11)
Rosettes: a group of nonepithelial cells that are clustered and crowned around a common center	Pseudorosettes are rosettes around a vessel; true rosettes surround a lumen or a fibrillary core	Ependymoma (Figure 2.12) and other neuroglial and neuroendocrine lesions
Spindled: composed of elongated cells with fusiform nuclei	Sheets or fascicles of fusiform cells; suggests a lesion is either a soft tissue neoplasm or a sarcomatoid variant of something else	Leiomyoma

(*continued*)

(Continued)

Term and definition	Appearance	Example
Staghorn vessels: gaping, branching vessels with thin walls, scattered throughout a lesion	Vessels should strike you as prominent at low power; the shape is unusual and the walls are disproportionately thin for the caliber	Hemangiopericytoma (Figure 2.13)
Storiform: having a cartwheel pattern—spindle cells with elongated nuclei radiating from a center point	A cellular spindled lesion with whorls of cells as opposed to parallel fascicles or right-angle bundles	Dermatofibrosarcoma protuberans (Figure 2.14)
Syncytial: having cytoplasmic continuity between the constituent cells	Looks like a collection of nuclei without recognizable cell borders	Meningioma
Tissue culture pattern: a loose aggregate of stellate (star-shaped) cells	Cells have delicate tentacles of cytoplasm	Nodular fasciitis (Figure 2.15)
Trabecular: in cord-like arrays separated by fibrous septa	Long nests and cords of cell groups	Oncocytoma (Figure 2.16)

Presence or Absence of Necrosis

Coagulative necrosis	Cells appear mummified; architecture is preserved, but there is no basophilia or cell detail	Ischemia (Figure 2.17)
Caseating necrosis	Total loss of cellular structure and architecture; basically degenerates into pink soup	Tuberculosis (Figure 2.18)
Fibrinoid necrosis	Vessels with replacement of wall by pink amorphous material	Vascular necrosis (Figure 2.19)
Fat necrosis	Grossly hard and chalky white; microscopically the fat cells are disrupted and collapsed, with foamy macrophages and giant cells	Biopsy site changes in breast (Figure 2.20)
Geographic necrosis	Describes large confluent "continent-shaped" patches of necrosis	Kikuchi's disease
Necrobiosis or gangrenous necrosis	Has a somewhat granular and blue look, with lots of fibrin deposition; loss of cellular and architectural detail	Gangrene (Figure 2.21)

Cell Shape and Size, Cytoplasm

Amphophilic: having an affinity for both acid and basic dyes	Has a unique color character, almost an iridescent purple	Pheochromocytoma (Figure 2.22)
Foamy macrophages: macrophages (histiocytes) stuffed with lipid	Macrophages have a small dark eccentric nucleus; the lipid vacuoles give a glittery granular appearance	Papillary renal cell carcinoma (Figure 2.23)
Granular: containing granules or tiny vacuoles	Color may vary, but granular texture is visible especially with lowered condenser	Granular cell tumor
Hof: a perinuclear clear zone corresponding to the Golgi apparatus	Looks like a pale spot hugging the nucleus	Plasma cells
Keratinized: keratin-producing	Keratin has a very pink and dense appearance on hematoxylin and eosin (H&E) stain	Squamous cell carcinoma (Figure 2.24)
Mucous (adj.): mucinous or producing mucin; also called *colloid*	Mucin appears clear after processing but can be stained with mucicarmine or periodic acid-Schiff–alcian blue (PAS-AB)	Adenocarcinoma
Oncocytic: large cells with cytoplasm that is granular and eosinophilic due to the presence of abundant mitochondria	Oncocytes are usually cytologically bland (uniform small dense nuclei) and look pink on H&E, mahogany on gross examination	Oncocytoma (see Figure 2.16)
Plasmacytoid: like plasma cells	Round cells with abundant cytoplasm and an eccentric round nucleus	Plasmacytoma (Figure 2.25)
Signet ring: having the shape of a ring, with a flattened nucleus compressed by a cytoplasm stuffed with mucin	Can be very hard to see but should appear on low power as a nonspecific pink "stuff" in the lamina propria	Signet-ring cell carcinoma (Figure 2.26)

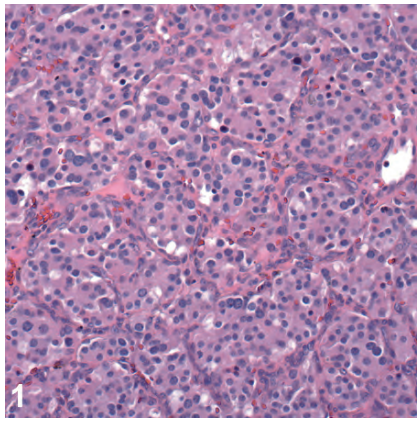

FIGURE 2.1. Alveolar pattern, paraganglioma.

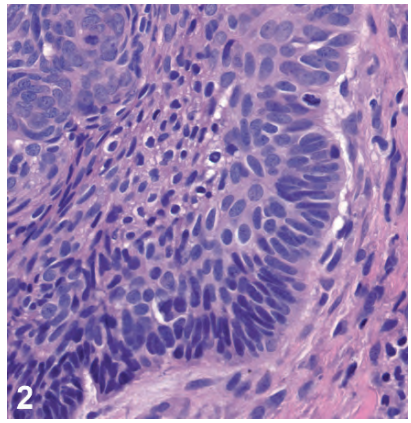

FIGURE 2.2. Basaloid pattern and palisading, basal cell carcinoma.

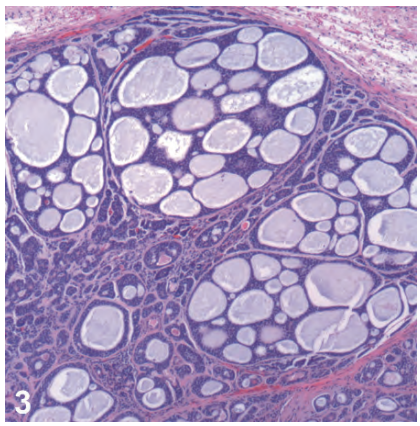

FIGURE 2.3. Cribriform pattern, adenoid cystic carcinoma.

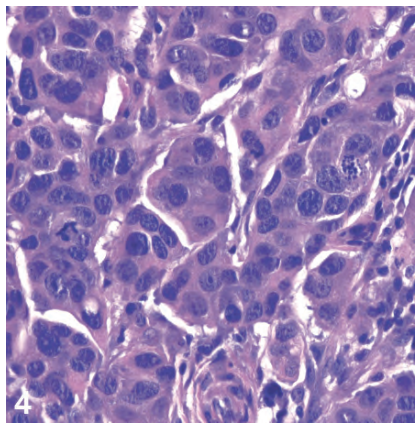

FIGURE 2.4. Epithelioid cells, breast carcinoma.

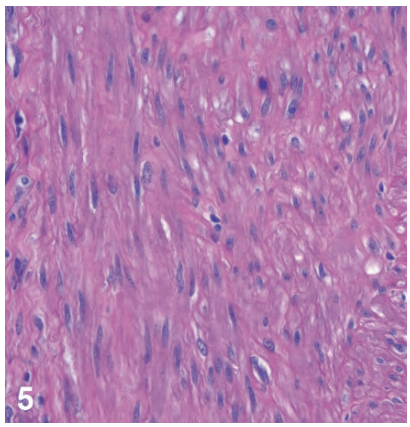

FIGURE 2.5. Fascicular pattern, leiomyoma.

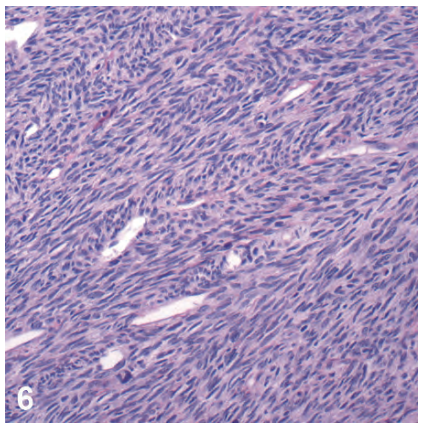

FIGURE 2.6. Herringbone pattern, fibrosarcoma.

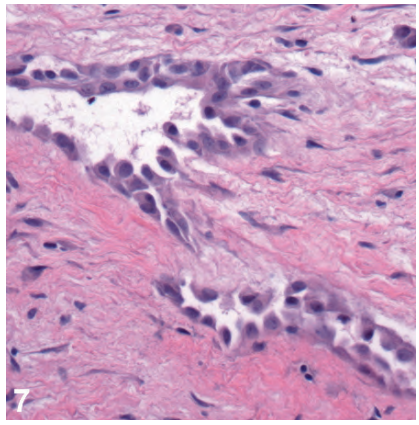

Figure 2.7. Hobnailed cells, angiosarcoma.

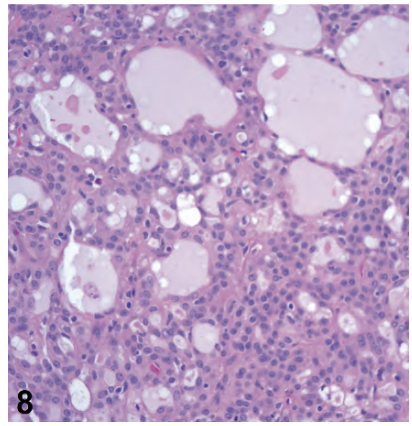

Figure 2.8. Microcystic pattern, acinic cell carcinoma.

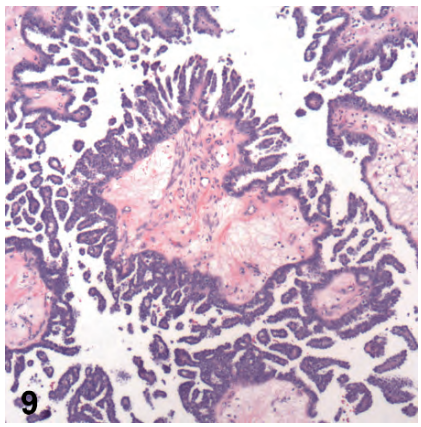

Figure 2.9. Micropapillary architecture, micropapillary serous carcinoma of the ovary.

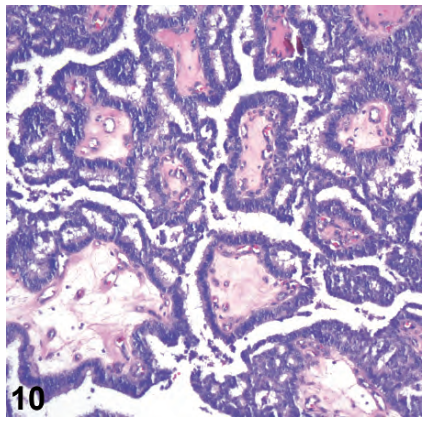

Figure 2.10. Papillary architecture, papillary carcinoma of breast.

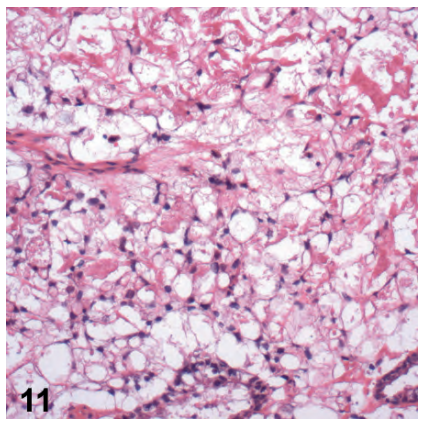

Figure 2.11. Reticular pattern, yolk sac tumor of testis.

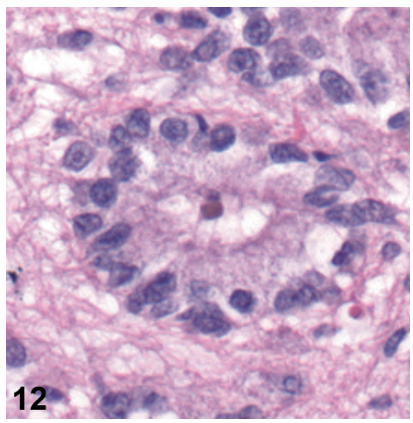

Figure 2.12. Rosette, ependymoma.

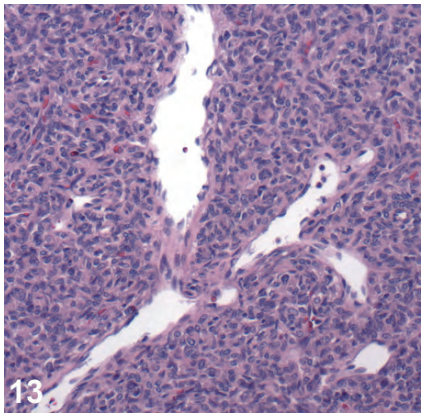

FIGURE 2.13. Staghorn vessels, hemangiopericytoma.

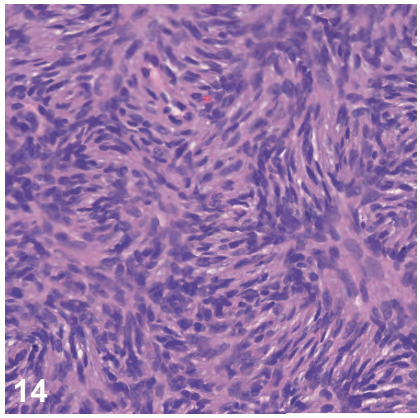

FIGURE 2.14. Storiform pattern, dermatofibrosarcoma protuberans.

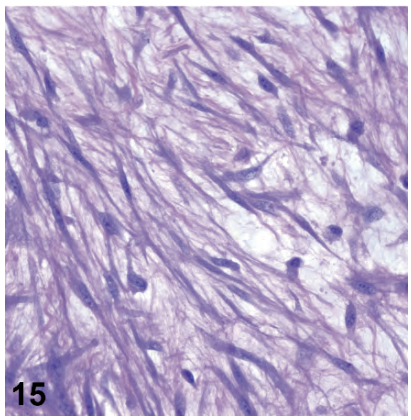

FIGURE 2.15. Tissue culture cells, nodular fasciitis.

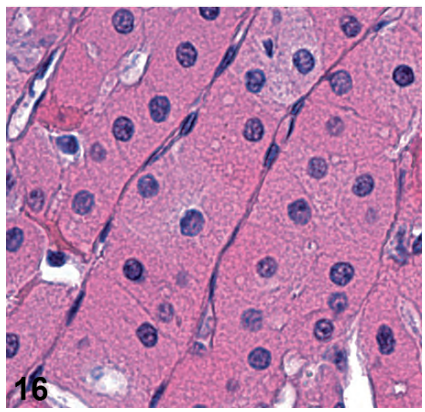

FIGURE 2.16. Trabecular pattern and oncocytes, oncocytoma.

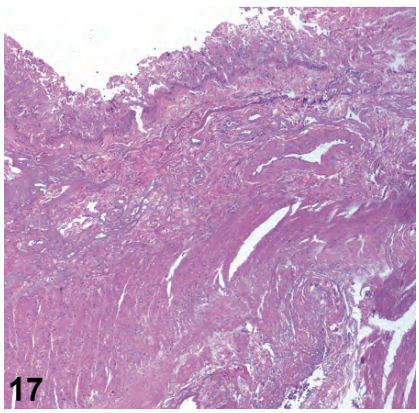

FIGURE 2.17. Coagulative necrosis, ischemic bowel.

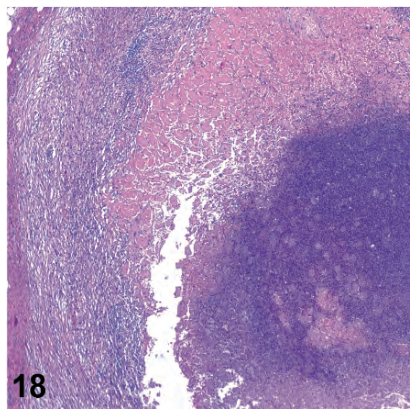

FIGURE 2.18. Caseating necrosis in a granuloma, tuberculosis.

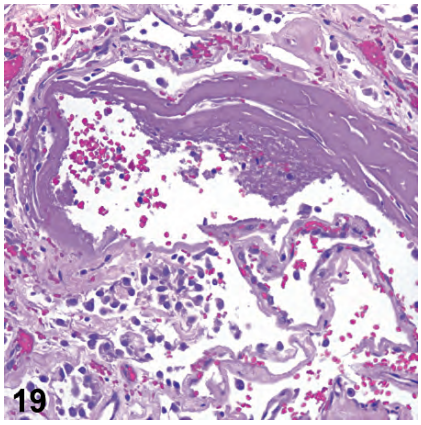

Figure 2.19. Fibrinoid necrosis, pulmonary vessel.

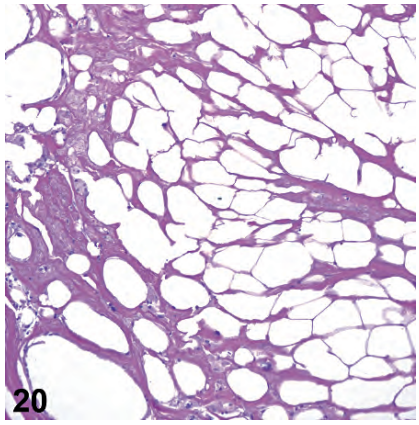

Figure 2.20. Fat necrosis, breast.

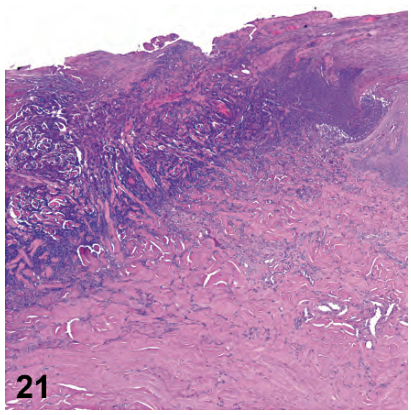

Figure 2.21. Gangrenous necrosis, toe wound.

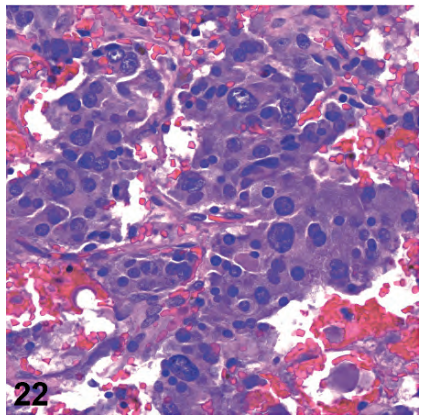

Figure 2.22. Amphophilic cytoplasm, pheochromocytoma.

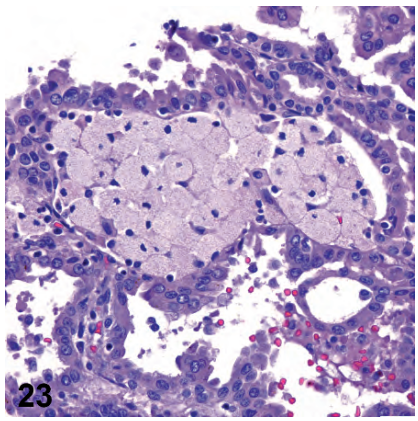

Figure 2.23. Foamy macrophages, papillary renal cell carcinoma.

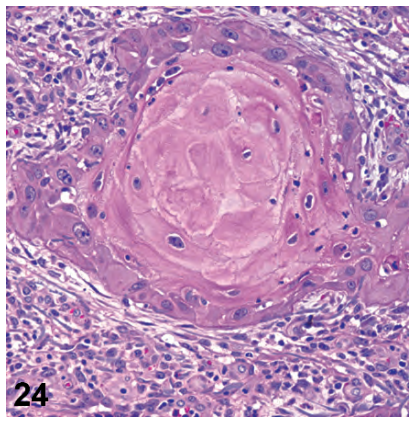

Figure 2.24. Keratin, squamous cell carcinoma.

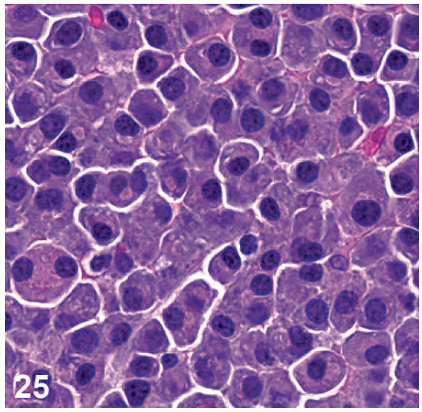

FIGURE 2.25. Plasmacytoid, plasmacytoma.

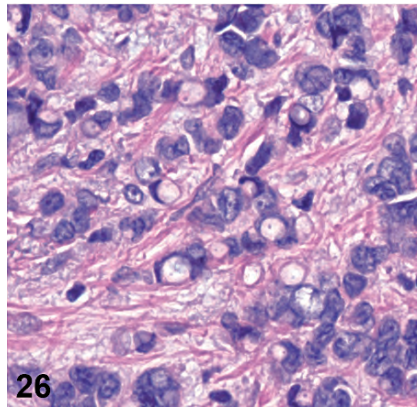

FIGURE 2.26. Signet-ring cells, breast carcinoma.

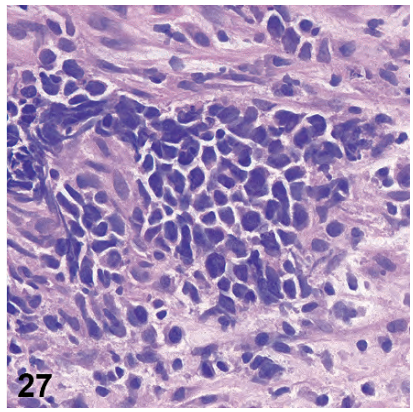

FIGURE 2.27. Nuclear molding, small cell carcinoma.

Nucleus

Clock face: evenly distributed clumped chromatin	Looks like a soccer ball	Plasma cells
Molding: nuclei that press together and indent each other due to the near absence of cytoplasm	Has a mosaic appearance and usually seen in conjunction with small dense blue nuclei	Small cell carcinoma (Figure 2.27)
Neuroendocrine: having finely speckled or salt and pepper chromatin	Nuclei should be round and bland, without nucleoli, but with occasional chromatin "chunks" or speckles	Carcinoid (Figure 2.28)
Pleomorphic: multiple sizes and shapes	Usually refers to nuclei and implies a very irregular mix of sizes and shapes	Embryonal carcinoma, testis
Vesicular: full of vesicles (bubbles)	A nucleus that is swollen and distorted by apparent bubbles in the chromatin	Various malignant neoplasms

Nucleolus

Cherry red: implies a malignant-looking nucleolus	An enlarged, solid nucleolus with a refractile reddish tinge	Prostate cancer Melanoma (Figure 2.29)

Cell Membrane

Ciliated: having cilia	If the cilia are not visible, sometimes the terminal bar is enough	Respiratory mucosa
Intercellular bridges: desmosomes	The prickles or spines between squamous cells	Normal skin

Stroma of Lesion, If Present

Myxoid: resembling mucus, but usually associated with a soft tissue lesion and hyaluronic acid	Appears as a faint pink to bluish-grey background, with a stringy mucous look, very paucicellular.	Myxoid myxofibrosarcoma (Figure 2.30)
Desmoplastic: causing edema and fibrosis in the stroma next to a neoplasm.	Appears as a pale halo around an infiltrating gland on low power; on high power fibrosis is visible	Adenocarcinoma, pancreas or colon (Figure 2.31)
Ectatic: dilated (as in a duct)	Often the duct is also filled with macrophages and debris	
Edematous: waterlogged	Water is clear on H&E so appears as lots of cleared-out space	
Fibrotic/sclerotic: replaced by collagen (fibrosis)	Collagen is pink and opaque on H&E and usually streams in parallel fibers	Sclerosed intraductal papilloma
Hyaline: clear, transparent, homogeneous	Glassy pink appearance	Amyloid, collagen, many others (Figure 2.32)

Other Noncellular Entities

Amyloid: protein deposited in a β-pleated sheet molecular structure	Appears glassy pink, stains salmon-pink with Congo red and fluoresces apple green	Medullary carcinoma, thyroid
Anthracotic pigment	Very black, very dense fine granules	Pulmonary lymph nodes
Calcium and psammoma bodies	Purple and granular, with hard edges; psammoma bodies are concentrically laminated	Papillary thyroid carcinoma
Colloid: refers to a mucin-producing neoplasm *or* the pink substance in thyroid follicles	Thyroidal colloid is a thin homogeneous pink.	
Hemosiderin	Has a glittery golden-brown refractile appearance with the poor-man's polarizer (which is waving your finger above the light source)	Old blood in any lesion (Figure 2.33)
Lipofuscin	Appears yellowish brown and globular	Seminal vesicle
Melanin	Is *not* refractile; may be brown to grey	Melanoma
Tattoo pigment	Similar to anthracotic pigment, may be multicolored	Skin with tattoos

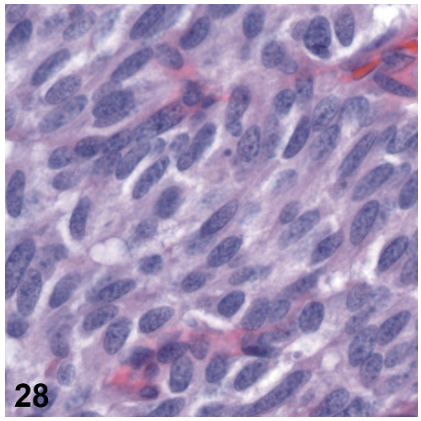

FIGURE 2.28. Neuroendocrine nuclei, carcinoid tumor.

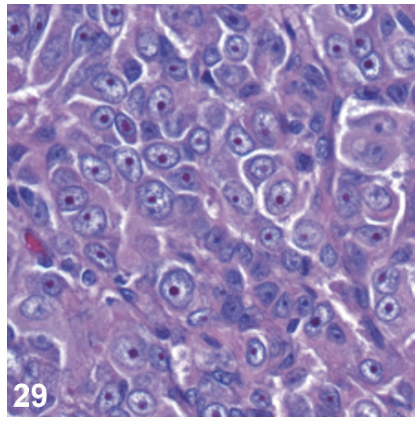

FIGURE 2.29. Cherry-red nucleolus, melanoma.

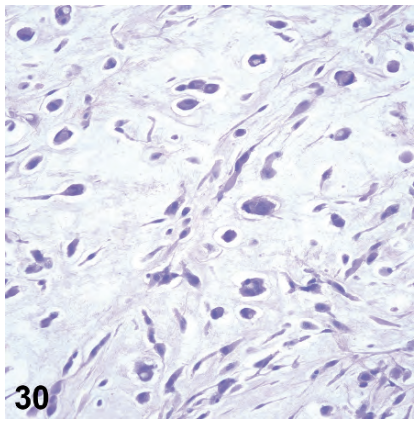

FIGURE 2.30. Myxoid stroma, myxoid myxofibrosarcoma.

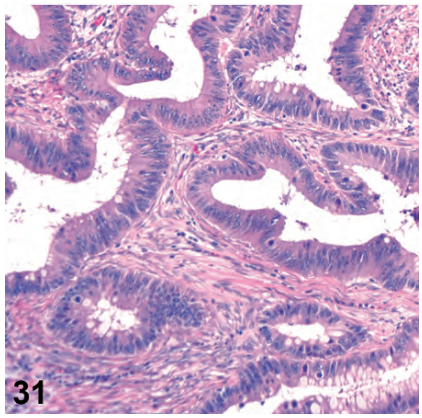

FIGURE 2.31. Desmoplastic stroma, colon cancer.

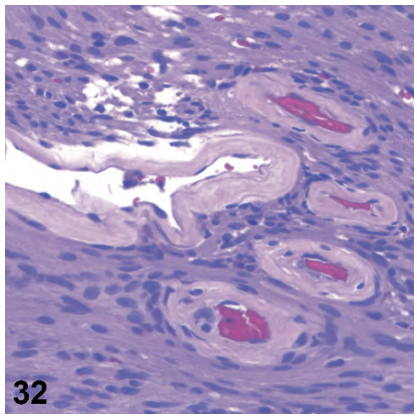

FIGURE 2.32. Hyaline deposits, vessels in schwannoma.

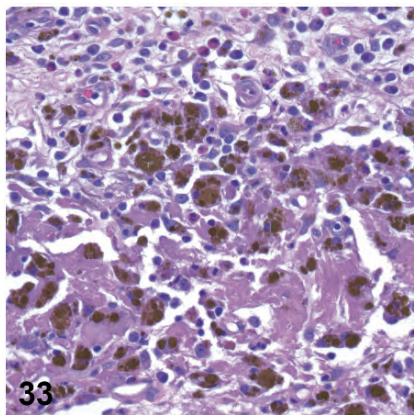

FIGURE 2.33. Hemosiderin, nasal polyp.

3 Infection and Inflammation

Let us review the types of inflammatory responses you may see. It seems very basic, but learning to differentiate inflammatory changes from dysplastic ones is a fundamental goal in pathology training.

Acute

Acute changes are the result of recent tissue damage, either from trauma, ischemia, toxins, or infection. Features include the following:

- Vascular congestion
- Edema
- Fibrinous exudate
- Tissue damage and/or necrosis
- Neutrophils ("purulence", polymorphonuclear leukocytes, polys)

Acute inflammation can be followed by resolution (healing), fibrosis or scar, abscess formation (Figure 3.1), or a chronic inflammatory stage. Evidence of recent damage and reparative changes includes granulation tissue, hemosiderin, lipid-laden macrophages, and fibroblast proliferation.

Granulation tissue has a characteristic look of a watery or myxoid background with sparse fibroblasts floating in it and a proliferation of inflammatory cells (all types) and capillaries (Figure 3.2). The endothelial cells of the capillaries can become quite prominent.

Biopsy site changes, a term often used to indicate evidence of a recent procedure, include fibroblast proliferation (early scar), foreign body–type giant cells, suture material, foamy macrophages, fat necrosis, and inflammation. They have a more solid look to them than granulation tissue (Figure 3.3).

Scar tissue implies that a dense thick collagen has replaced the normal structures. In the skin, a dermal scar is evidenced by a homogeneous pink layer of collagen and absence of adnexal structures (Figure 3.4).

Chronic

Chronic changes are the result of repetitive or sustained tissue damage due to trauma, ischemia, toxins, infection, or autoimmune processes. Features include the following:

- Increased vascularity and/or fibrosis (attempts to heal)
- Tissue destruction
- Lymphocytes, macrophages, plasma cells, eosinophils

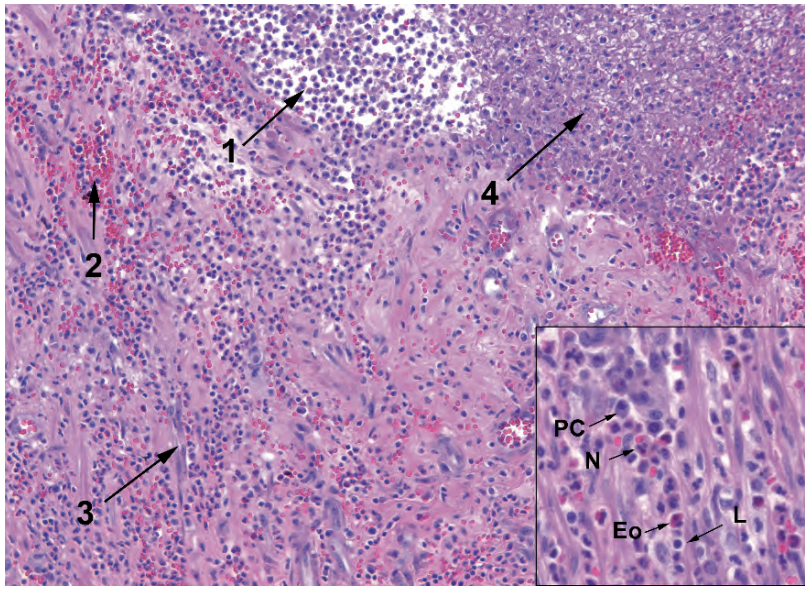

FIGURE 3.1. Acute inflammation and abscess formation. This example of the acute inflammatory response shows collections of neutrophils (abscess formation, 1), extravasated blood (2), prominent capillaries (3), and fibrin accumulation (4). **Inset**: the mixed inflammatory infiltrate includes plasma cells (PC), neutrophils (N), eosinophils (Eo), and lymphocytes (L).

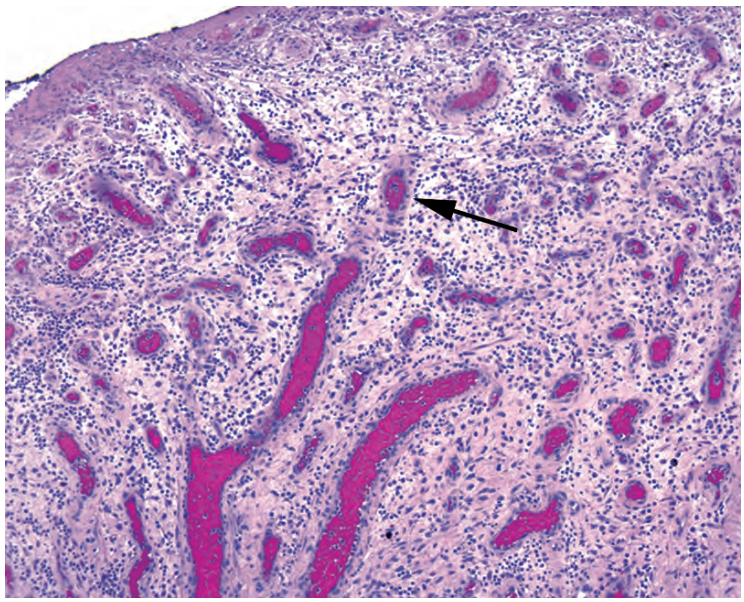

FIGURE 3.2. Granulation tissue is characterized by a loose myxoid background with fibroblasts and inflammatory cells and by prominent capillaries with plump endothelial cells and thick walls. The stroma appears condensed and thickened around the capillaries, giving them a pink halo (arrow).

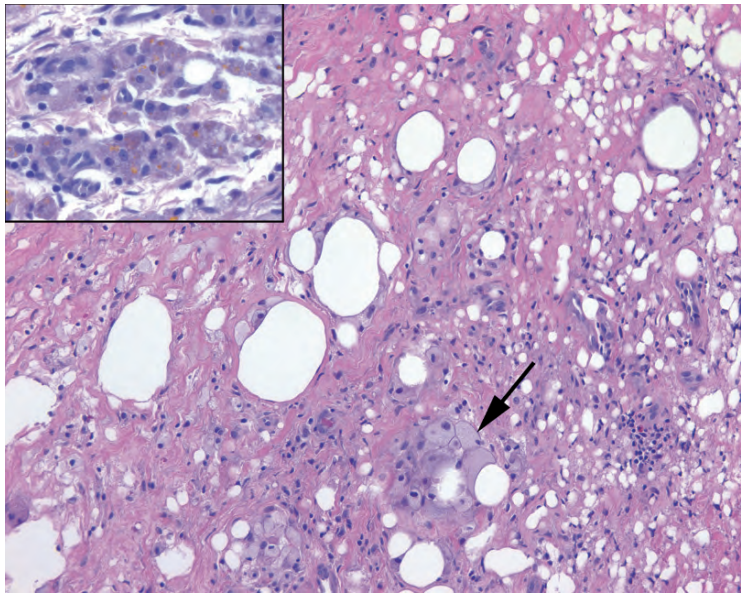

Figure 3.3. Biopsy site changes. In this subcutaneous specimen, collagen has replaced most of the fat cells, and foamy histiocytes can be seen ingesting some residual fat (arrow). **Inset**: Hemosiderin in macrophages (golden yellow to brown granules) can be seen in sites of prior trauma or bleeding.

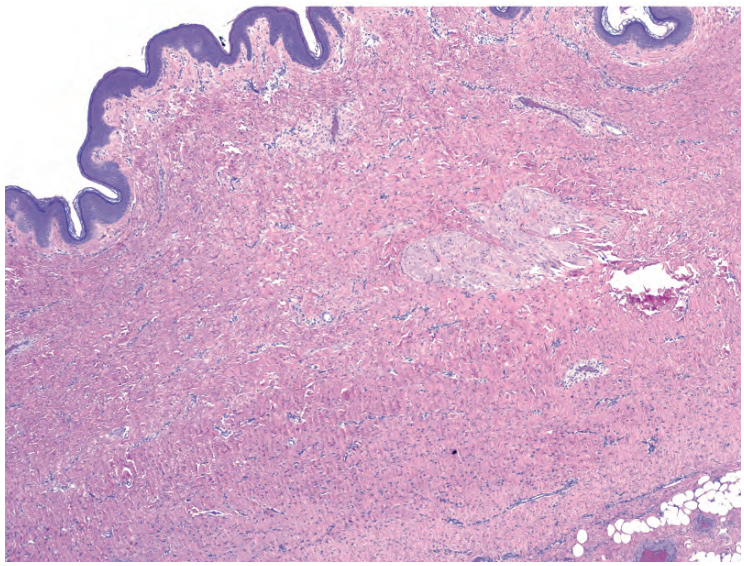

Figure 3.4. Dermal scar. Dense pink collagen has replaced the adnexal structures and displaced the subcutaneous fat in this biopsy site.

What is a *macrophage*? The precursor is a circulating monocyte, part of the myeloid lineage of blood cells (*myeloid* generally refers to cells in the granulocyte and monocyte groups, although it can also mean all cells that mature in the bone marrow, i.e., the opposite of lymphoid). The monocyte leaves the circulation and becomes a tissue macrophage. It can differentiate into organ-specific resident macrophages, such as microglia, Kupffer cells, and alveolar macrophages. It can also go to an area of inflammation and become activated, participating in the immune response. Activated macrophages are also called *histiocytes* and may be "epithelioid," as in a granuloma, or "foamy," as

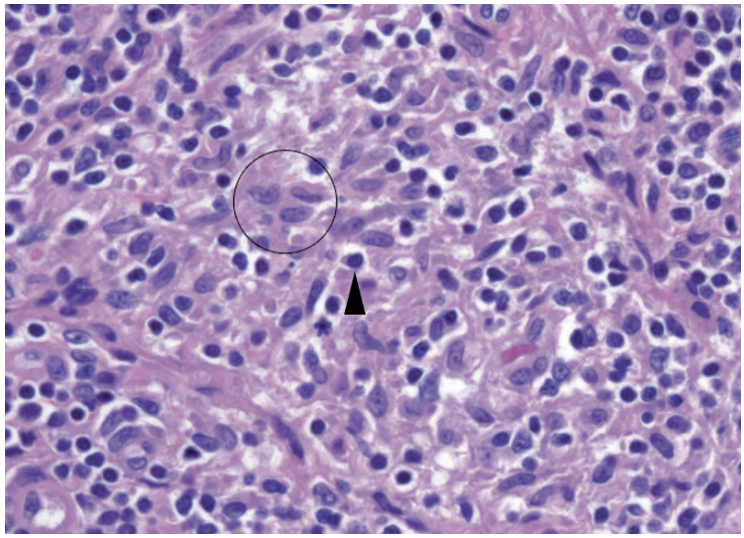

Figure 3.5. Histiocytes appear as pale folded nuclei within an area of inflammation; the cell borders are indistinct, but the nuclei are surrounded by light pink cytoplasm (circle). Compare the pale chromatin to that of the neighboring lymphocyte (arrowhead).

in lipid-laden or xanthomatous. Finally, macrophages can acquire multiple nuclei to become a Langerhans giant cell (ring of nuclei) or a foreign body–type giant cell (scattered nuclei).

Histologically, histiocytes have a bland and fade-into-the-background look to match their name (literally, "tissue cell"). They have pale-pink granular cytoplasm, sometimes with chunky phagocytosed bits of material, and indistinct cell borders (Figure 3.5). The nuclei are light with crisp outlines, oval in shape, and often grooved. In tissue, a collection of histiocytes appears as an ill-defined pink area that is easy to miss. The nuclei often stream in a circular pattern like fish swimming in a barrel. Foamy macrophages are stuffed with lipid debris or organisms, and can have an almost signet-ring appearance.

What are *eosinophils*? Eosinophils have a bilobed nucleus and big red granules. They are usually an indication of an immune/IgE response, such as to drug allergy or parasites.

We usually refer to the presence of lymphocytes as *chronic inflammation*. Lymphocytes plus neutrophils equals acute and chronic inflammation. In the gastrointestinal tract, instead of *acute* we use *active*, such as active chronic gastritis or active chronic inflammatory bowel disease. *Inactive* in the gastrointestinal tract means increased lymphocytes but no polys.

Granulomatous

A granulomatous appearance indicates a specific type of chronic inflammation with a small differential; it can be a result of mycobacteria (plus a few other bacteria), fungal infection, autoimmune disease, some toxins or irritants, and sarcoid. Granulomas are divided into caseating (usually infectious) and noncaseating.

The histologic appearance of a granuloma is a microscopic aggregate of histiocytes, with surrounding lymphocytes and plasma cells. The appearance ranges from tiny collections of histiocytes (as in Crohn's disease; Figure 3.6), to large well-circumscribed whorls of cells (sarcoid; Figure 3.7), to a layer of histiocytes surrounding a pool of caseous necrosis (tuberculosis, fungus; Figure 3.8). Giant cells are helpful but not essential. Old granulomas can become hyalinized and acellular (Figure 3.9).

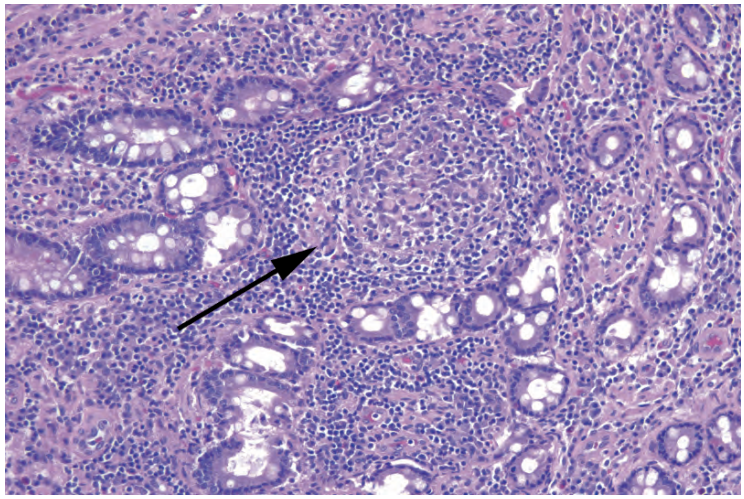

Figure 3.6. Granulomas in Crohn's disease. These granulomas of the colon are subtle (arrow), and the pale histiocytes may be seen only on high power. A surrounding collar of lymphocytes is common.

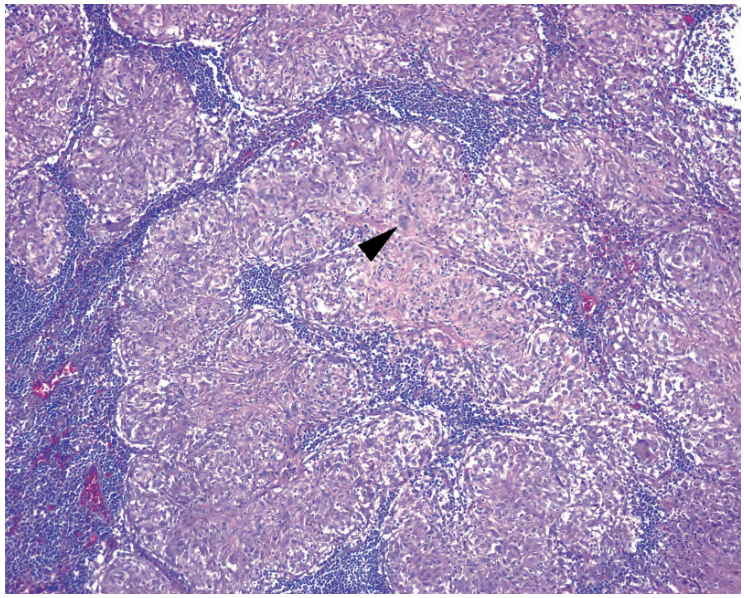

Figure 3.7. Granulomas in sarcoid. These granulomas are often more substantial and more easily recognized than those in Crohn's disease. They appear as well-defined masses of pink histiocytes. Occasional multinucleated giant cells (arrowhead) are present.

Specific Organisms and Their Stains

Fungi

Fungal organisms stain bright pink on periodic-acid Schiff (PAS) stain and black on Gomori's methenamine silver (GMS) stain. For most of these organisms, it is important to identify not just the presence and morphology of the organism but whether it is invading viable tissue or colonizing necrotic debris. Size can be helpful in identifying the various yeasts (Figure 3.10).

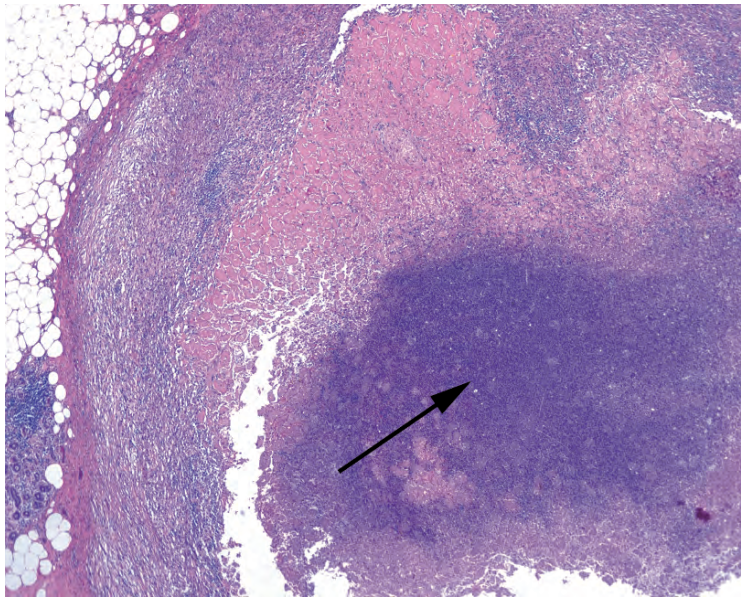

Figure 3.8. Caseating granulomas in tuberculosis. The histiocytes in these granulomas are visible only at the periphery, as the center is a mass of necrosis and cellular debris (arrow).

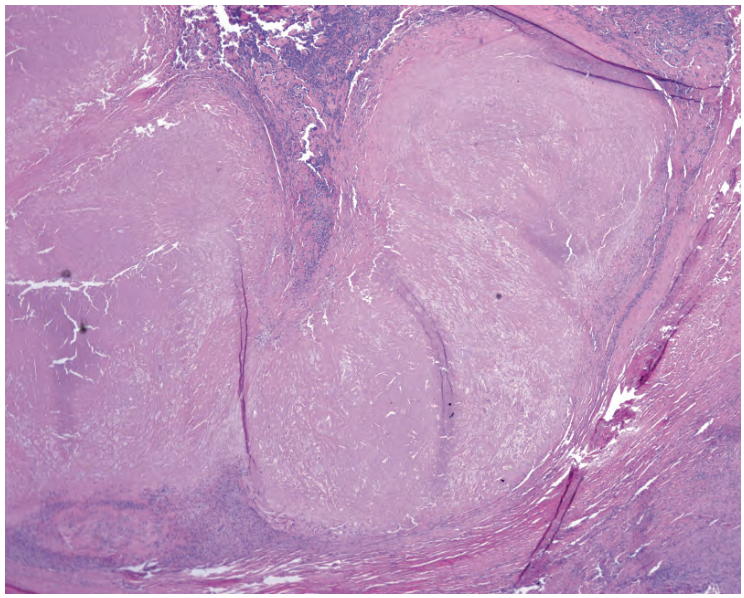

Figure 3.9. Hyalinized granuloma. The amorphous area of hyalinized collagen likely represents old, burned-out necrosis.

Candida are visible on H&E as round-to-oval yeast forms and pseudohyphae (segmented and nonbranching). They are often found in the debris at the epithelial surface (Figure 3.11).

Aspergillus are visible on H&E as long, thin hyphae with 45° branching and septations. They may appear as a solid fungal ball or as single hyphae in the tissue (Figure 3.12). Treated *Aspergillus* may have different morphology.

Mucor and zygomycetes are irregular and wide nonseptate hyphae and have the appearance of gnarled tree branch outlines with wide branch points (Figure 3.13). On H&E, they can be almost invisible, as they are essentially wide hollow spaces in the tissue. These are the bread

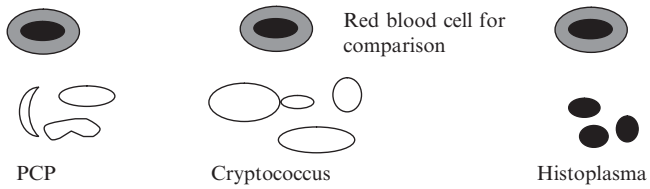

Figure 3.10. Relative size of yeasts.

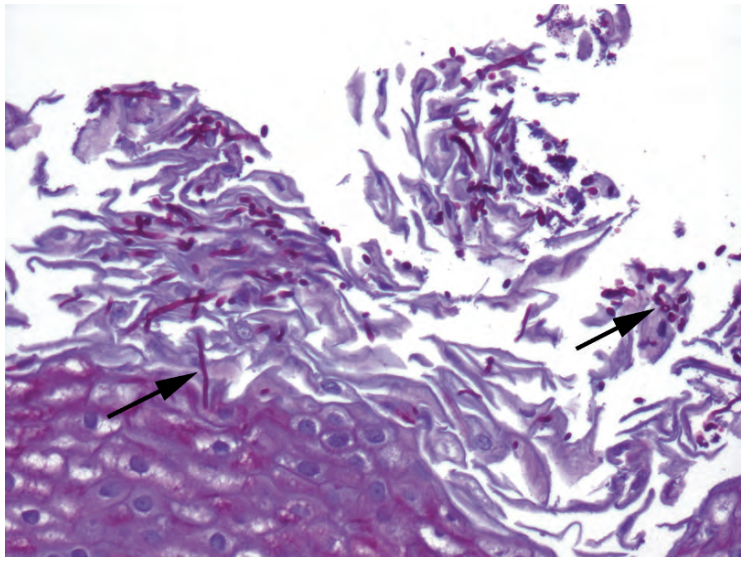

Figure 3.11. *Candida*. This example from the esophagus shows magenta pseudohyphae and yeasts (arrows, periodic-acid Schiff stain).

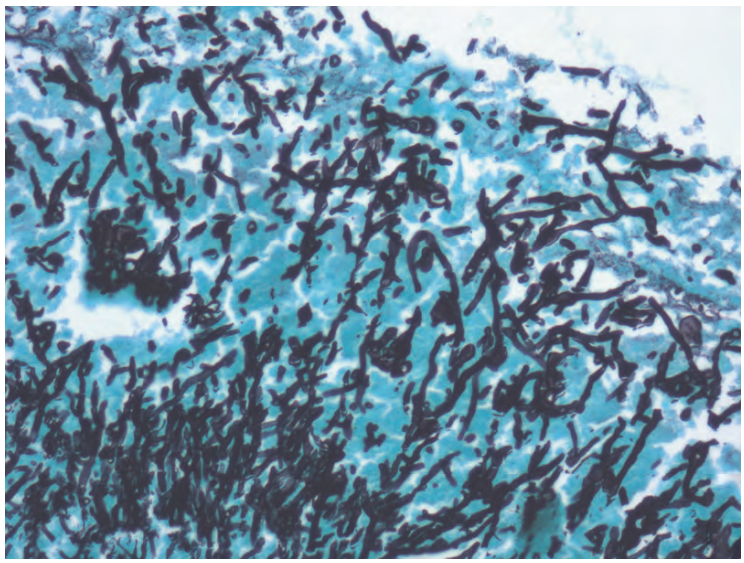

Figure 3.12. *Aspergillus*. A forest of branching hyphae are visible by Gomori's methenamine silver stain.

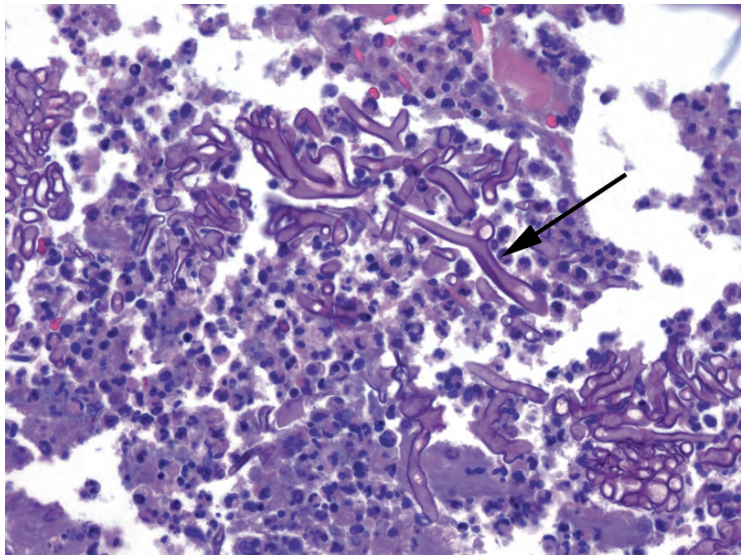

Figure 3.13. *Mucor*. A periodic-acid Schiff stain shows the thick, "hollow," irregular outlines of *Mucor* (arrow).

molds and are typically seen only in very neutropenic patients or in sinusitis in a patient with ketoacidosis.

Histoplasma are tiny intracellular yeast forms with narrow-based budding, often seen in macrophages. On H&E and Giemsa stain, these are delicate 2-μm forms in macrophages. In the context of a hyalinized granuloma, however, a silver stain shows distinct yeasts that are nearly the size of red cells (about 5 μm; Figure 3.14).

Cryptococcus are usually encapsulated yeast forms with narrow-based budding; some may be in macrophages but are often free in the tissue; on GMS the sizes are variable, and some may collapse into squashed balls (Figure 3.15). This variability in size is actually a key indicator of *Cryptococcus*. Stains for the capsule of *Cryptococcus* can differentiate it from other yeasts, including mucicarmine and Fontana-Masson. However, be aware that *Cryptococcus* can occasionally lose the capsule.

Pneumocystis may or may not be a fungus but are definitely black on GMS. They are flattened contact-lens–shaped organisms found in the alveoli (Figure 3.16). They are not visible on H&E but are usually accompanied by a foamy pink exudate.

Bacteria

Most bacteria are not found by, or identified with, stains. This is because there is little more we could say than "Gram-positive cocci in clusters," for example, which is pretty unhelpful without a culture. There are a few that are hard to culture and are best identified by stains.

Histologic evidence of *Mycobacterium* (causing tuberculosis and other diseases) is caseating granulomas. The organisms are not seen on H&E and may be very sparse in an immunocompetent patient. The conventional stain is the acid-fast (AFB) stain, which leaves the tissue unstained, with occasional pink blush in some cell types, but stains mycobacteria a bright wine red (Figure 3.17). These are tiny scattered bacilli; you need to be at 40×, at least, to spot them. Scanning the entire slide at 40× for red lint is painful but necessary to rule out infection. If clinical suspicion is high but an AFB is negative, an auramine-rhodamine is a more sensitive fluorescent stain for tuberculosis.

Mycobacterium avium-intracellulare causes infection in an immunocompromised patient. In these patients, the mycobacteria are eaten by macrophages and then multiply like crazy within the cells, giving the appearance of foamy macrophages. In the duodenum this can look just like Whipple's disease, but a PAS stain will differentiate the two (histiocytes stuffed with

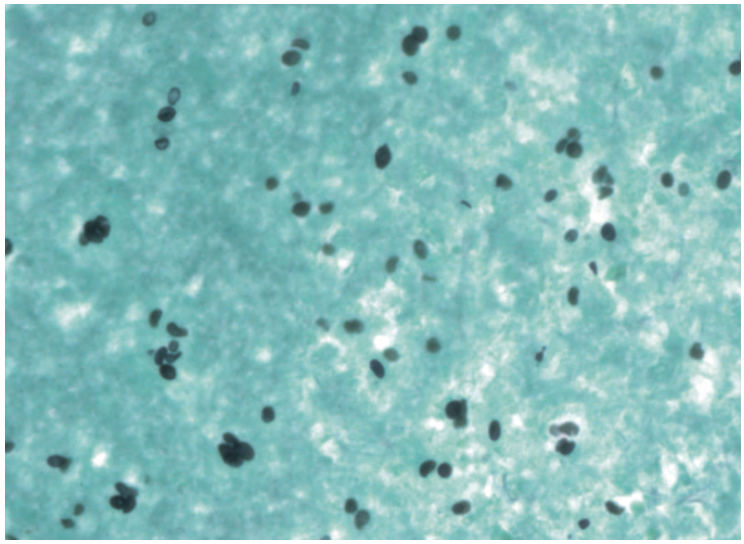

Figure 3.14. Histoplasmosis. Tiny yeasts are visible on Gomori's methenamine silver stain (40× objective).

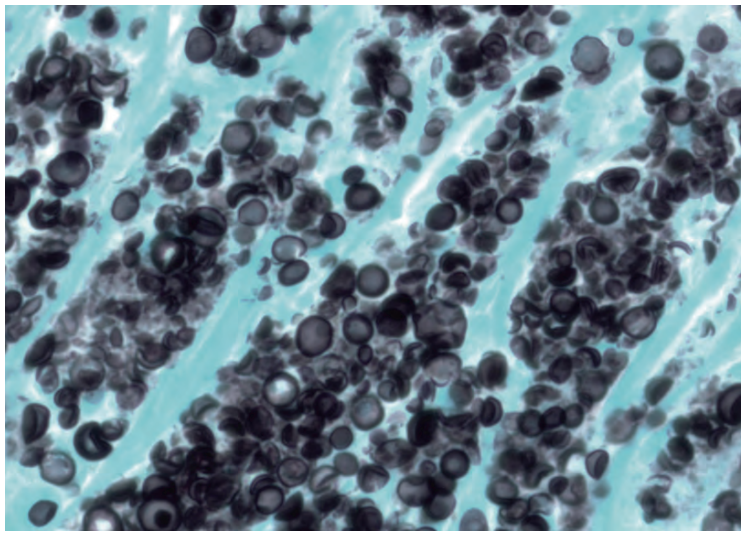

Figure 3.15. *Cryptococcus*. This photograph is taken at the same magnification as Figure 3.14. The organisms are significantly larger and show a range of sizes and shapes on Gomori's methenamine silver stain.

cranberries in Whipple's disease but with fine bacilli in *M. avium-intracellulare* infection). An AFB stain will also be positive.

Helicobacter pylori is the most common cause of gastric ulcers. Histologically you should see a chronic inflammatory infiltrate in the stomach, with a little activity here and there (polys). Infection is more common in the antrum. On Diff-Quik or Giemsa stain, look in the areas of activity. If present, *H. pylori* will be in the pit lumens or at the surface in clusters of tiny (barely visible at 20×) seagull-shaped bacilli (Figure 3.18). Sometimes you will hear them called *helicopters*. This is a phonetic joke, not a visual one. They do not look like helicopters.

Actinomyces, causing a puffball bacterial colony, is completely unremarkable in the tonsil but significant in endometrium, especially in the setting of an intrauterine device. The H&E appearance is of a granular grey-purple cloud, sometimes filamentous, with no identifiable cells or structures (Figure 3.19).

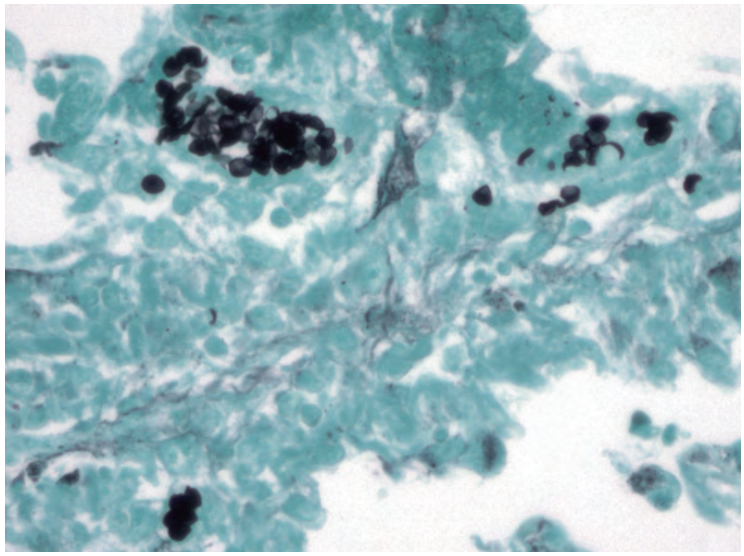

Figure 3.16. *Pneumocystis*. This photograph in the lung is taken at the same power as Figures 3.14 and 3.15. The organisms are stained with Gomori's methenamine silver stain.

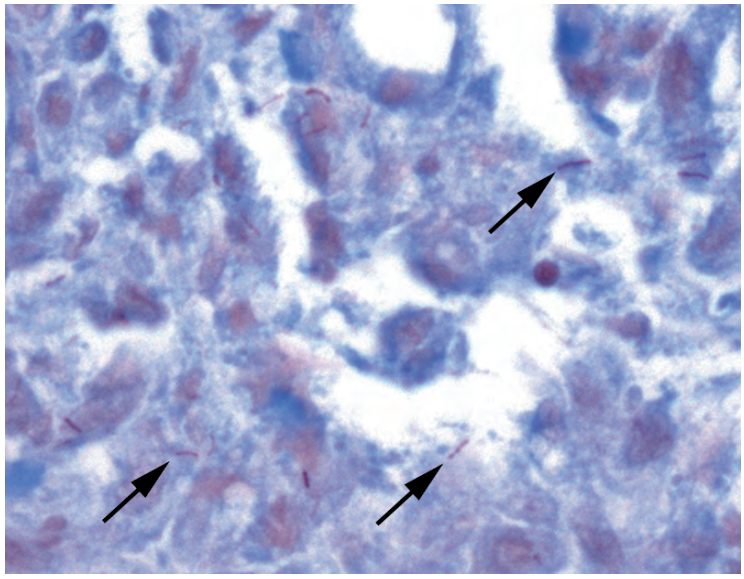

Figure 3.17. Mycobacteria on acid-fast bacteria stain. In this example, tiny wine-red rods are visible within the tissue (arrows).

Viruses

Herpes simplex virus tends to cause extensive tissue damage and ulcers. It infects the epithelium, so look in the cells immediately adjacent to the ulcer. The cells become multinucleated, with the transformed nuclei molding into each other. The chromatin is entirely displaced by glassy nuclear inclusions (viral proteins), outlined by a dark rim of residual chromatin, as though the nucleus is being digested from the inside (Figure 3.20).

Cytomegalovirus can also cause ulcers but may infect tissue without obvious localizing damage. It infects epithelial, endothelial, and mesenchymal cells. In the case of an ulcer, look

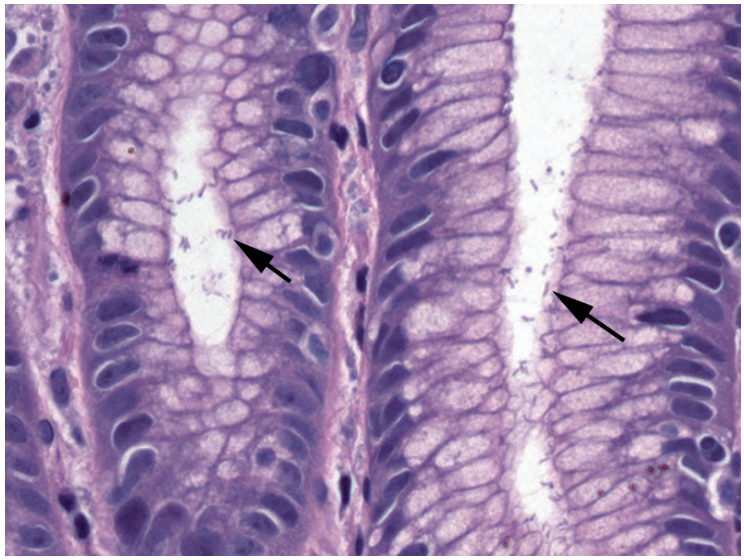

Figure 3.18. *Helicobacter pylori*. The bacilli are sometimes visible on hematoxylin and eosin stain, as seen here (arrows), in the pits of the gastric mucosa.

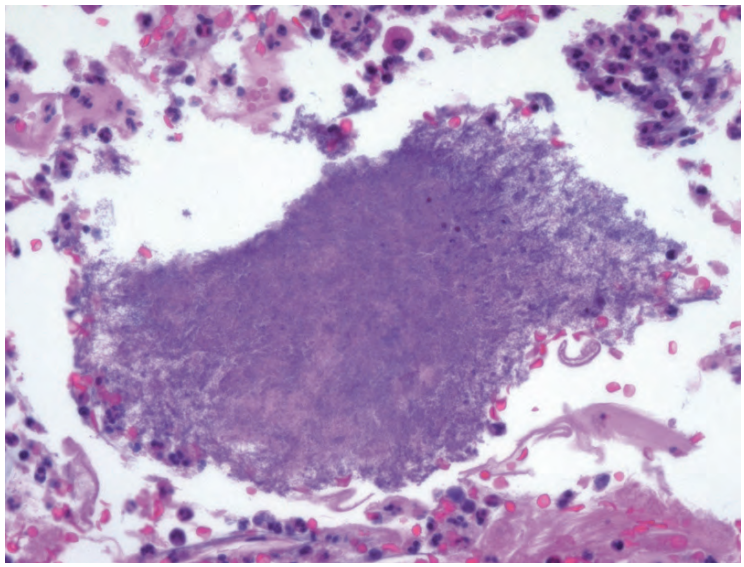

Figure 3.19. *Actinomyces*. This filamentous ball of organisms is easily overlooked, as it resembles fibrin.

in the ulcer bed, not the periphery. The virus causes enlarged cells with large nuclei. The nuclei have a very characteristic inclusion; a dark dense round/oval inclusion surrounded by a pale halo, all within the nuclear membrane (Figure 3.21). The pale halo is not always entirely visible, so finding large dark round nuclei in a group of normal cells (nonneoplastic) should prompt you to consider cytomegalovirus. Immunostains help.

Parasites

Giardia is a duodenal parasite that looks a little like a flounder with a long tail: it is kite shaped when viewed from above but a flat crescent from the side. It is found at the luminal surface of the villi and may not cause much inflammation. The parasites look very much like debris, but in a fortuitous cut

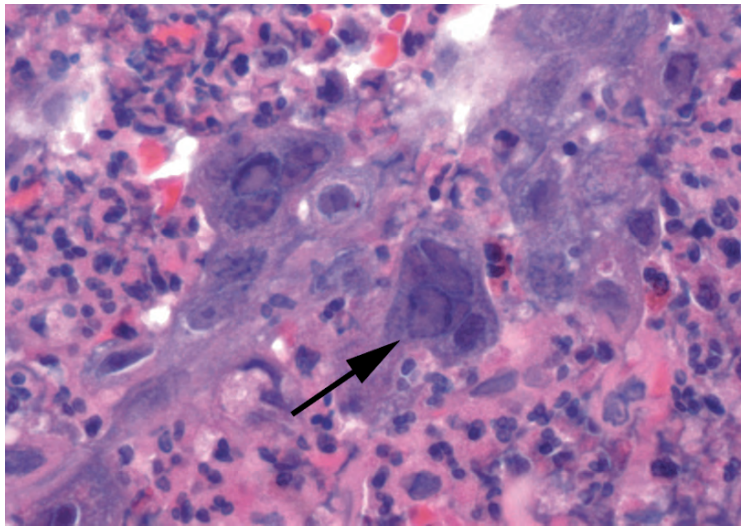

Figure 3.20. Herpesvirus. The classic nuclear changes include multiple molded nuclei with a peripheral rim of chromatin and a glassy inclusion nearly replacing the chromatin (arrow).

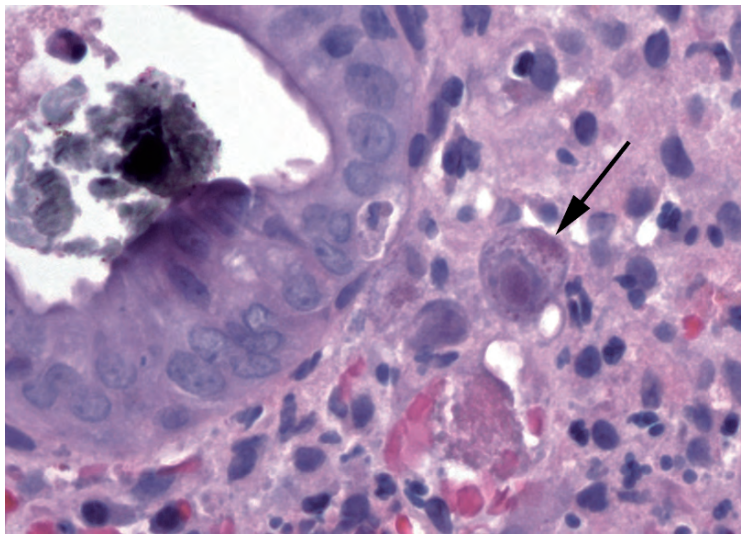

Figure 3.21. Cytomegalovirus. This infected endothelial cell in the gastrointestinal tract (arrow) shows the typical nuclear changes of cytomegalovirus, with a central reddish dense nuclear inclusion, surrounded by a clear halo and a rim of purple chromatin.

you may see the "eyes," which identify it. You will not see *Giardia* unless you look for it. It is related to *Trichomonas*, which you will see on pap smears.

Cryptosporidium is another duodenal parasite that mainly infects the immunocompromised. The tiny round parasites line up along the brush border like clinging bubbles.

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."

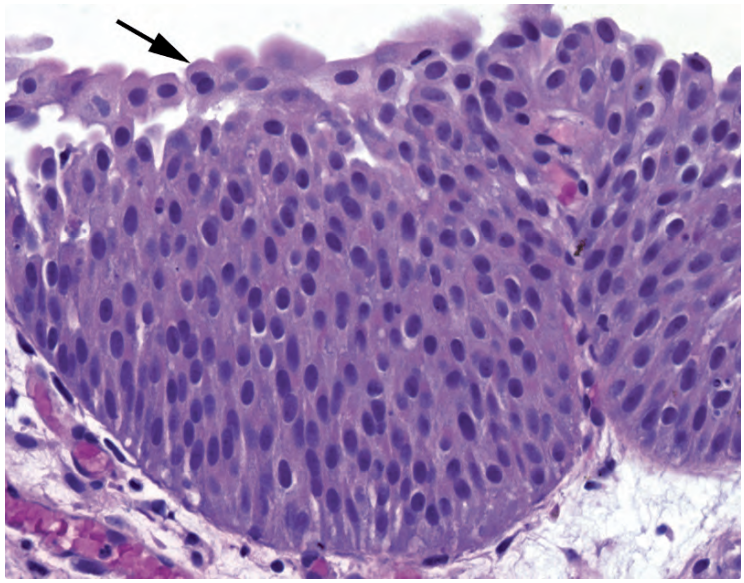

Figure 4.1. Polarity in an epithelium. In this section of urothelium, although it is thickened relative to normal, all of the nuclei can be seen to be roughly perpendicular to the surface; they "know which way is up." Plump umbrella cells are visible at the surface (arrow).

- 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.

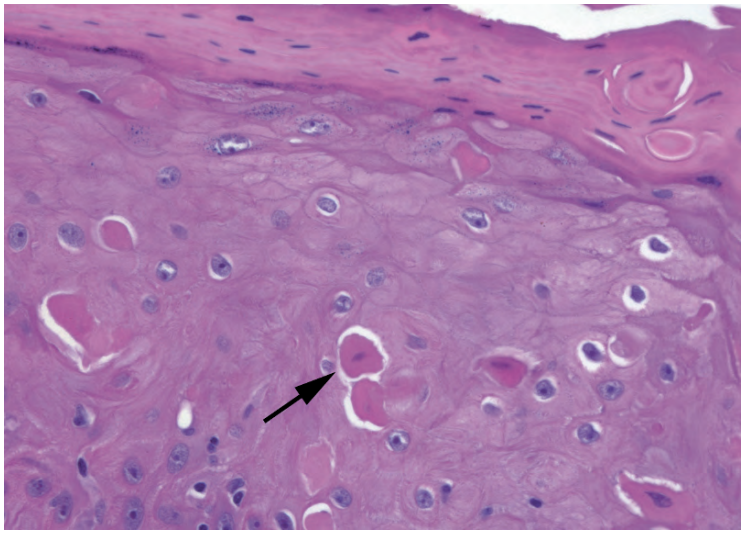

FIGURE 4.2. Dyskeratotic cells in the epidermis. These cells are essentially mummified; their nuclei are dying, and they have lost their connections to other cells. Their dense pink keratin stands out relative to the neighboring cells (arrow).

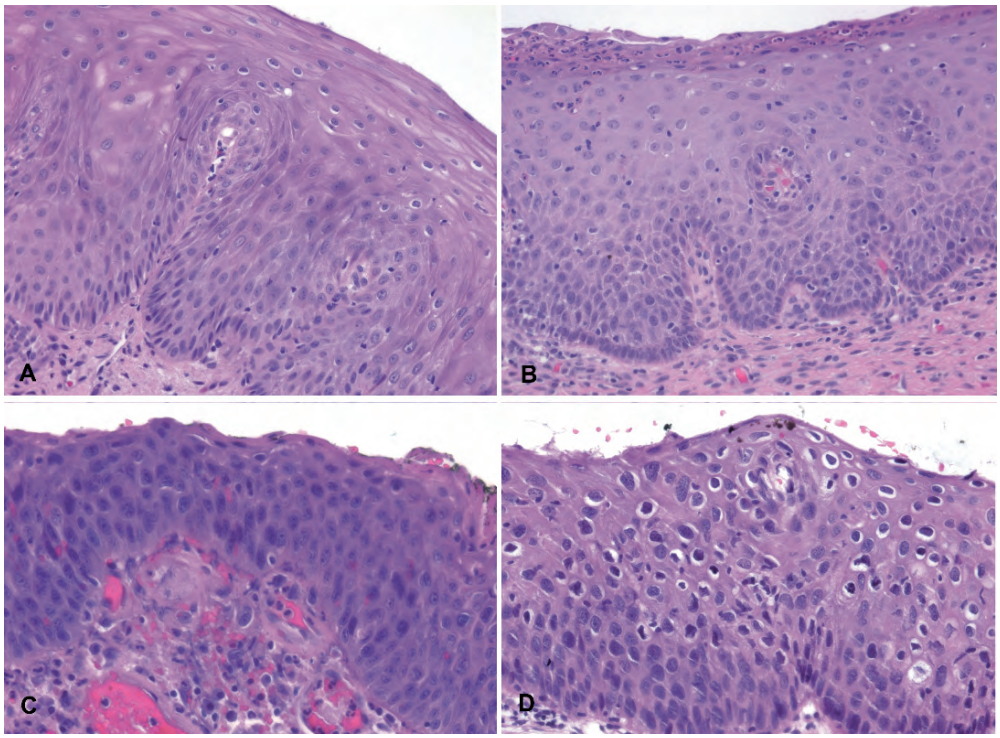

FIGURE 4.3. Examples of reactive, benign nuclei **(A, B)** and dysplastic nuclei **(C, D)**. In reactive conditions, the nuclei may be enlarged and have visible nucleoli, but the N/C ratios are still low (abundant cytoplasm), there is nuclear polarity relative to the surface, the chromatin is not too 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.

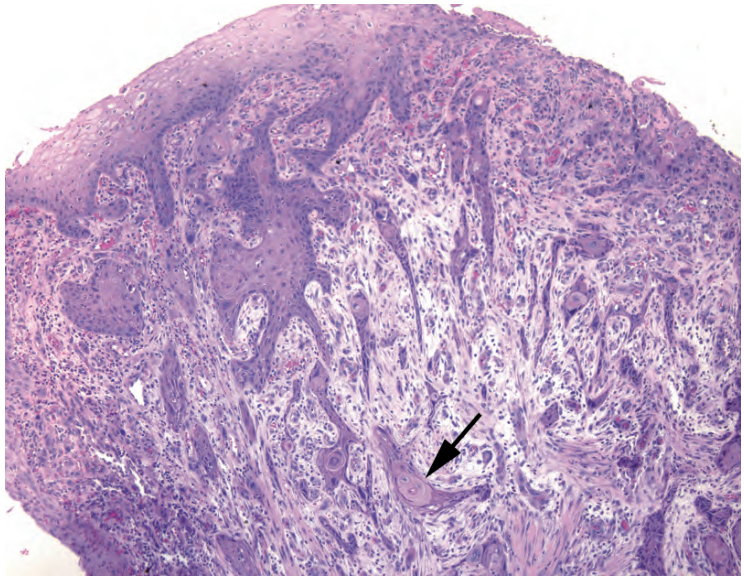

Figure 4.4. Invasive squamous cell carcinoma. Irregular nests and spicules of cells invade down into the stroma from the surface (top). Although single infiltrating cells are not visible at this magnification, the deep aberrant keratinization (arrow), in which a deep nest of cells takes on the color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

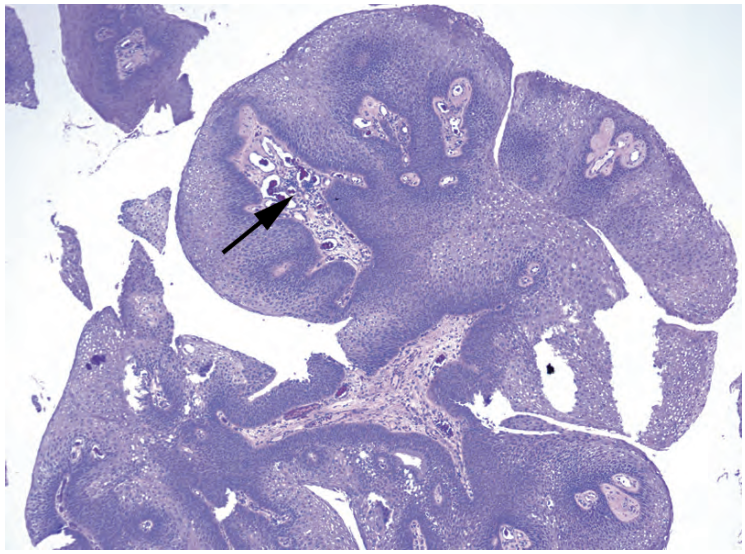

Figure 4.5. Papilloma. The squamous papilloma is defined by a squamous epithelium overlying branching fibrovascular cores (arrow).

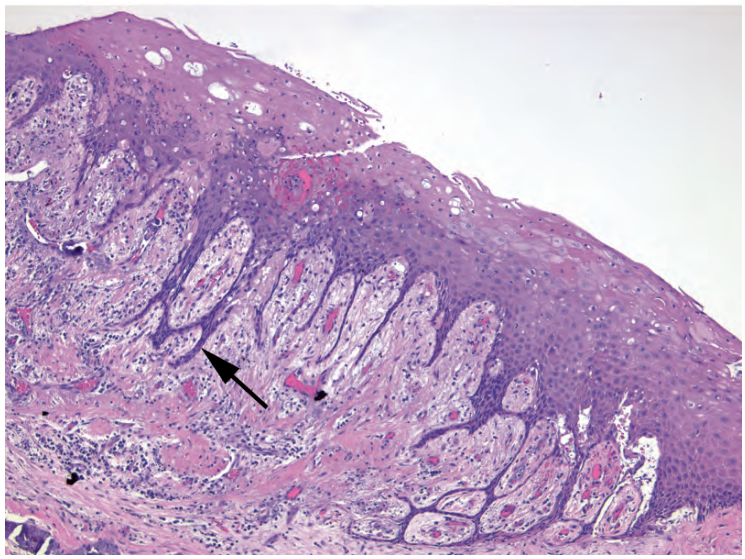

Figure 4.6. Pseudoepitheliomatous hyperplasia. In this reactive condition, thin strands of epithelium (arrow) are pulled down into the underlying dermis or lamina propria. However, the strands should not expand out into nests or show deep keratinization (compare to Figure 4.4).

(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.

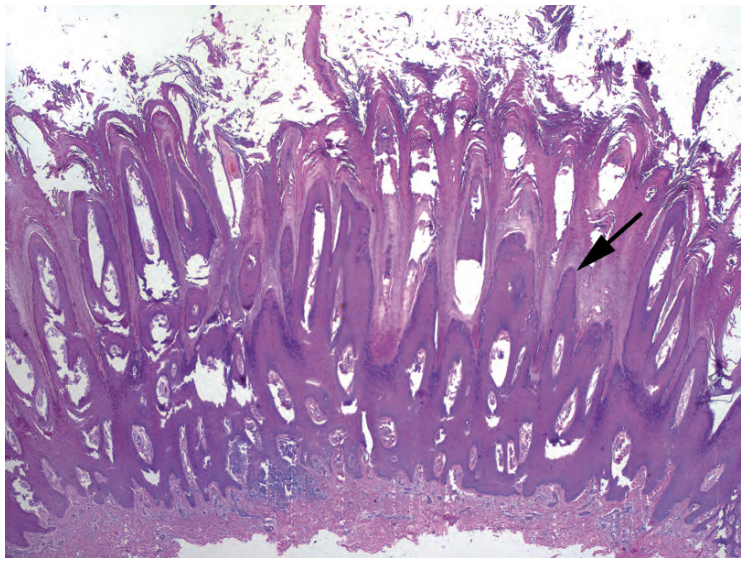

FIGURE 4.7. Verrucous pattern in a wart. Verruca vulgaris is characterized by prominent exophytic spires of the epidermis (arrow), with overlying hyperkeratosis and parakeratosis.

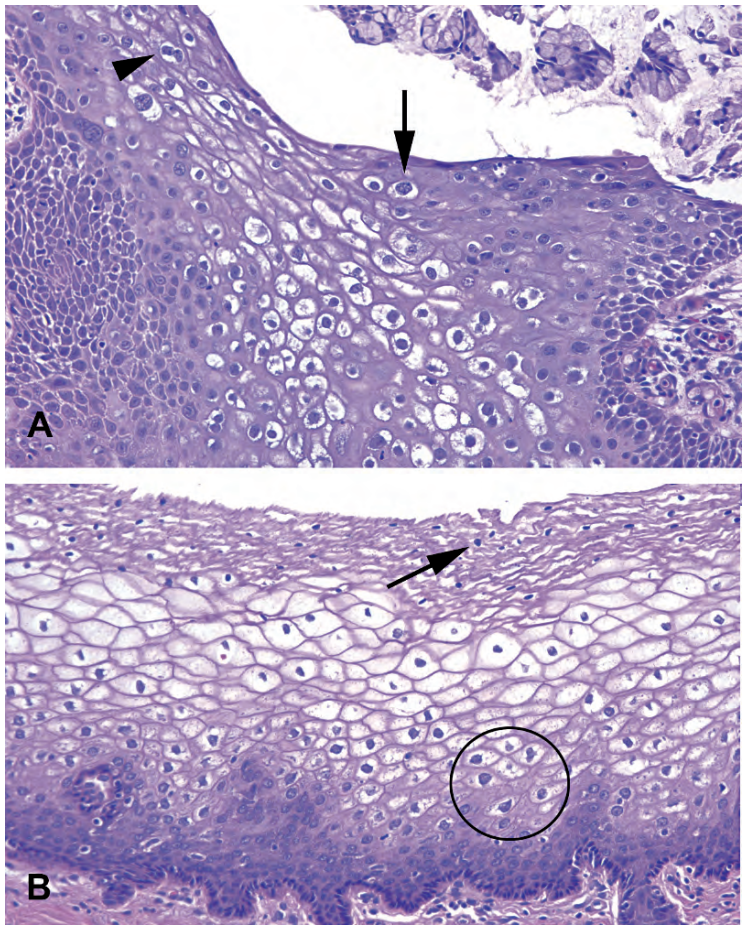

FIGURE 4.8. Viral or koilocytic atypia versus glycogen. **(A)** In this cervical lesion (low-grade squamous intraepithelial lesion [LSIL]), koilocytes are visible as large cells with prominent, crinkled, dark nuclei and perinuclear halos (arrow). Nuclei that get larger as you approach the surface are 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.

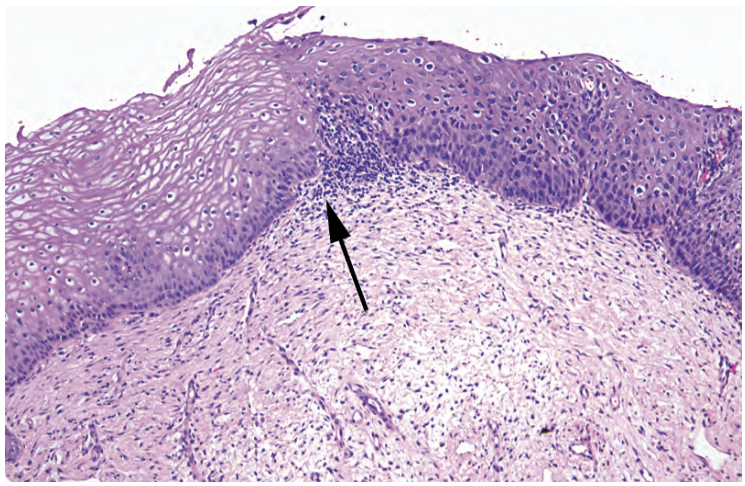

Figure 4.9. High-grade squamous intraepithelial lesion. An abrupt transition (arrow) is visible between normal (left) and dysplastic (right) epithelium. The epithelium at right shows a persistence of immature cells (large nuclei and high N/C ratios) up to the surface. Compare this to the clear distinction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

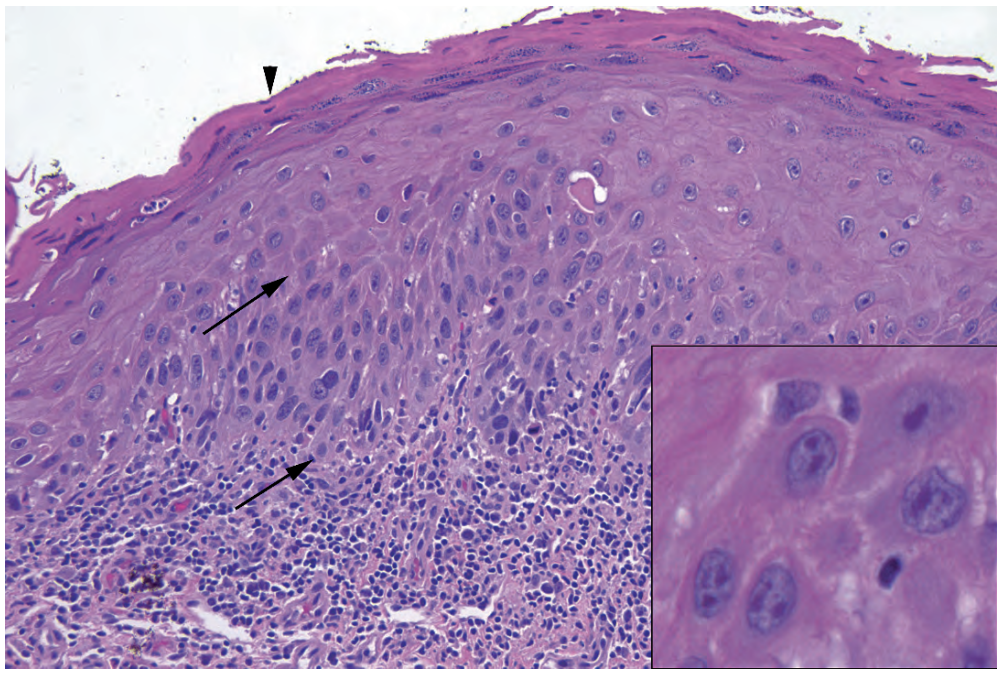

Figure 4.10. Squamous dysplasia in the mouth. In the area between the arrows, dysplastic cells with high N/C ratios and hyperchromatic, irregular nuclei can be seen occupying the lower half of the epithelium. The surface shows parakeratosis (arrowhead), which clinically will appear as a white plaque. **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.

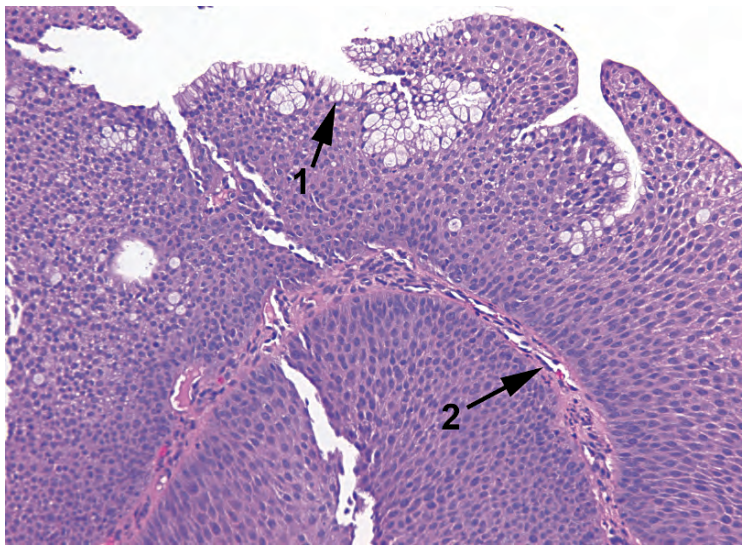

Figure 4.11. Schneiderian papilloma. The typical features are a squamous or respiratory epithelium with goblet cells (1) and neutrophils (not seen at this power). As in any papilloma, there are fibrovascular cores (2).

5 Ditzels

Ditzel (slang): A word used to describe any part of the body that is not ordinarily appropriate for everyday conversation. *"Susan is always walking down the hall with her ditzels hanging out."* (http://www.urbandictionary.com/)

Ditzels are small specimens with limited educational potential. For the purposes of this chapter, these are all specimens with no suspicion or history of malignancy. They often have about three possible diagnoses and a reduced billing charge because of their limited complexity. Until you get experience with them, they slow you down inordinately at the grossing bench and at the microscope as you struggle to get the "right" wording and obsess over whether what you see is pathologic or normal. After all, it is really embarrassing to get a ditzel *wrong*. What follows is a list of typical features, things not to miss, and a suggested wording for unremarkable specimens. However, diagnosis style may vary across institutions, so take your cues from your own attendings.

Cholesteatoma (Middle Ear)

Grossing: A small, whole specimen is usually submitted.
Histology: A cyst is formed by keratinizing epithelium and filled with flaky keratin. Other features can include inflammation, cholesterol clefts, and foreign body giant cells (Figure 5.1).
Rule out: Differential diagnosis of a middle ear mass includes inflammatory polyp, paraganglioma, middle ear adenoma, meningioma, and schwannoma.
Sample sign out: Left middle ear (excision): *Cholesteatoma* or *Fragments of keratinaceous debris (clinical cholesteatoma)*.

Sinus Contents

Grossing: Aspirate sent in a nylon bag. Submit one to two blocks, depending on volume. Use biopsy bags in cassettes.
Histology: Normal components include fragments of bone, respiratory and squamous mucosa, and mucous glands.

- Chronic sinusitis: edema, acute and chronic inflammation
- Allergic fungal sinusitis: sheets of allergic mucin (Figure 5.2) and Charcot-Leyden crystals

Rule out: If allergic mucin is present, get a periodic-acid Schiff (PAS) or Gomori's methenamine silver (GMS) stain to rule out fungus; other sinus lesions include polyps, papillomas, and unusual tumors.

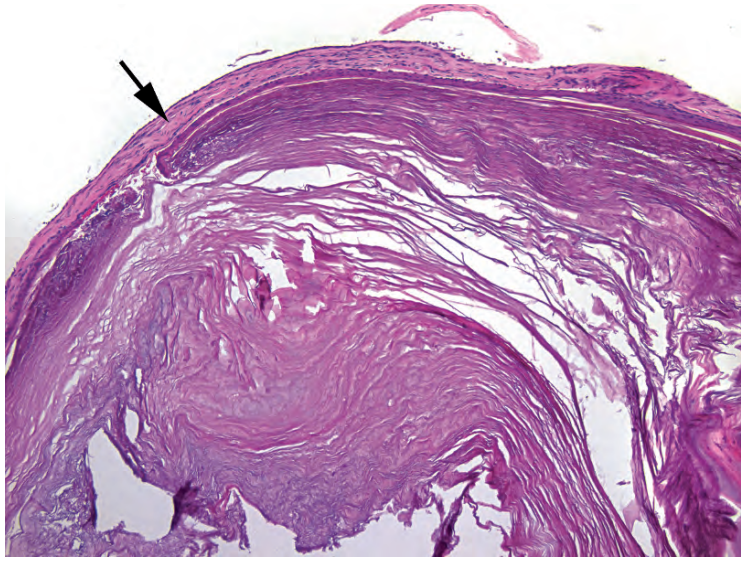

Figure 5.1. Cholesteatoma. The specimen is dominated by layers of pink keratin; the thin epithelium can be seen surrounding the keratin nodule (arrow).

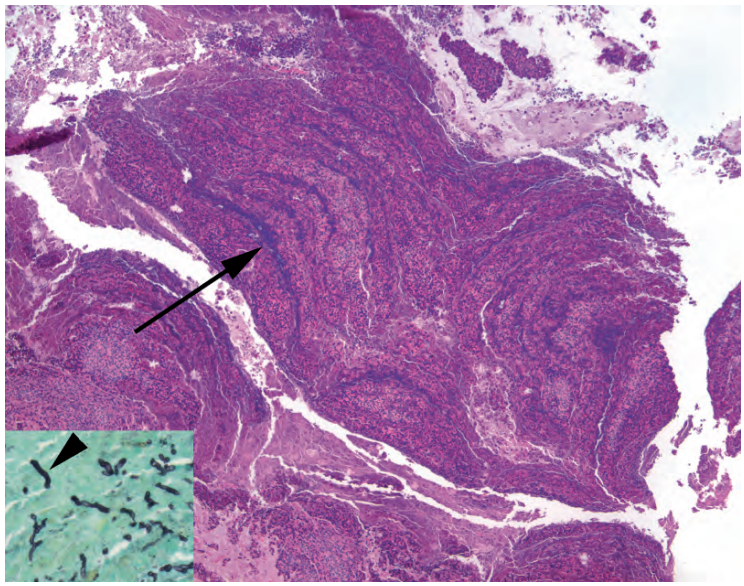

Figure 5.2. Allergic mucin in sinusitis. The allergic mucin takes on a characteristic tiger-striped appearance (arrow) as layers of eosinophils, mucin, and cell debris accumulate. **Inset**: A Gomori's methenamine silver stain shows black fungal hyphae (arrowhead).

Sample sign out: Right and left sinus contents (aspiration): *Chronic sinusitis* or *Fragments of respiratory mucosa with chronic inflammation* or *Allergic fungal sinusitis* (a PAS stain highlights fungal organisms within the mucin).

Carotid and Femoral Plaques

Grossing: Specimen is essentially a cast of the artery lumen. Take one block of representative cross sections; this usually requires light decalcification.

Histology: The inner layer of the elastic arterial wall has a variable amount of atherosclerotic debris, calcification, and/or thrombus (Figure 5.3).
Sample sign out: Carotid artery, right (endarterectomy): C*alcified atherosclerotic plaque*.

Intervertebral Disc

Grossing: Submit one block of representative or total material.
Histology: Fibrocartilage and pulpy myxoid gel (the nucleus pulposus), possibly with fragments of bone (Figure 5.4), are present.
Sample sign out: Cervical disc (excision): *Fragments of disc material*.

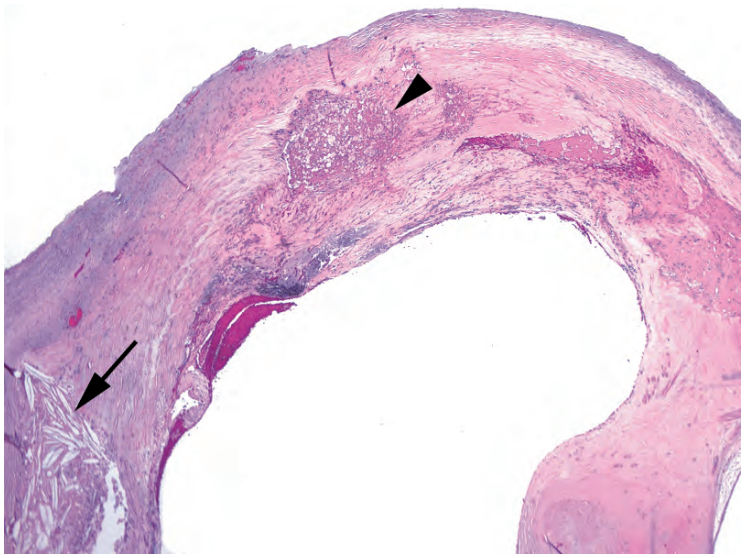

Figure 5.3. Carotid plaque. This represents the intimal surface of the artery, in which there may be calcification, foamy macrophages (arrowhead), cholesterol clefts (arrow), or inflammatory debris.

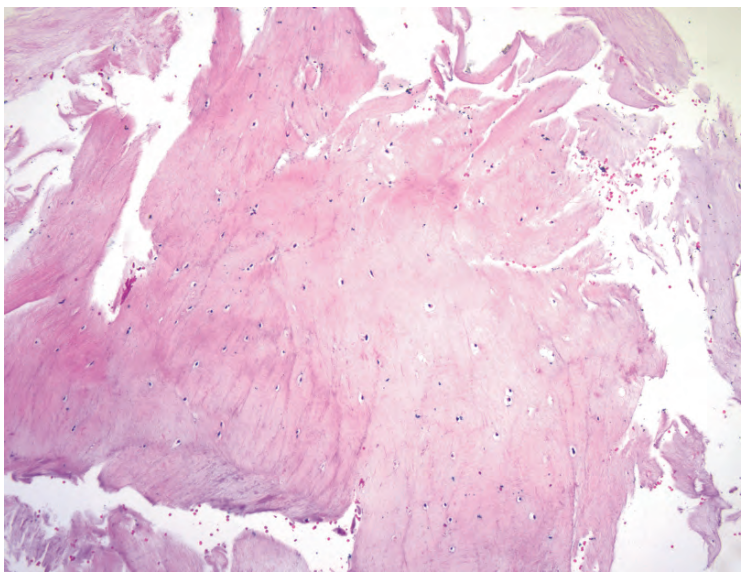

Figure 5.4. Intervertebral disc. The disc substance is paucicellular, with a homogeneous translucent stroma (ranging from myxoid to collagenous).

Thymus

Grossing: Tissue may be from incidental thymectomy (heart surgeries), biopsy, or something else (such as parathyroid). *Weigh it*. Weight is a criterion for true thymic hyperplasia. Submit in total for small specimens or representative for thymectomy.
Histology: Architecture is lobular with dark outer cortex and pale medulla (Figure 5.5). Hassall's corpuscles look like squamous nests. Germinal centers are not normal. Fatty replacement with age is normal.
Rule out: Distinguish from sheets of cells or obliterated architecture (thymoma).
Sample sign out: Thymus (thymectomy) or "left inferior parathyroid" (excision): *Histologically unremarkable thymus*.

Parathyroid

Grossing: *Weigh it*. Submit it in its entirety.
Histology: Features include monotonous round neuroendocrine cells with clear cytoplasm (chief cells) or abundant pink cytoplasm (oxyphil cells). Normal weight is around 50 mg; adenomas are usually >300 mg. *Adenoma* is a clinical diagnosis requiring evidence of normalized parathyroid hormone level after surgery. Hyperplasia and adenoma may look the same on the slide (Figure 5.6).
Rule out: Carcinoma is very rare, but dense fibrotic bands and nuclear atypia are suggestive. The diagnosis of carcinoma requires capsular or vascular invasion.
Sample sign out: Left superior parathyroid (excision):*Cellular parathyroid tissue (250 mg)*.

Heart Valves

Grossing: There is much information to be gained in grossing. Review your grossing manual for details. Note the presence of vegetations, commissural fusion, calcification, and redundancy.
Histology: Look for myxoid degeneration, calcification, and adherent vegetations (Figure 5.7).

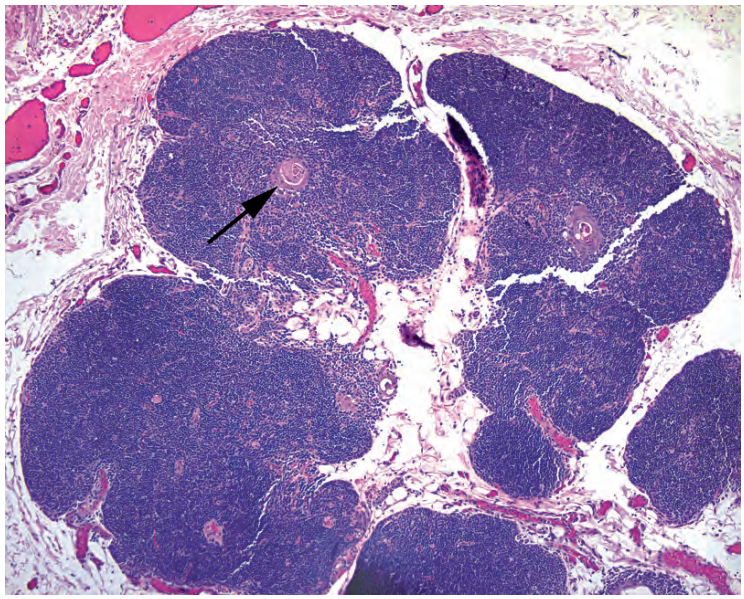

Figure 5.5. Thymus. The lobulated thymus resembles lymph node at low power but should not have germinal centers. Hassall's corpuscles (arrow) are visible as pink whorls.

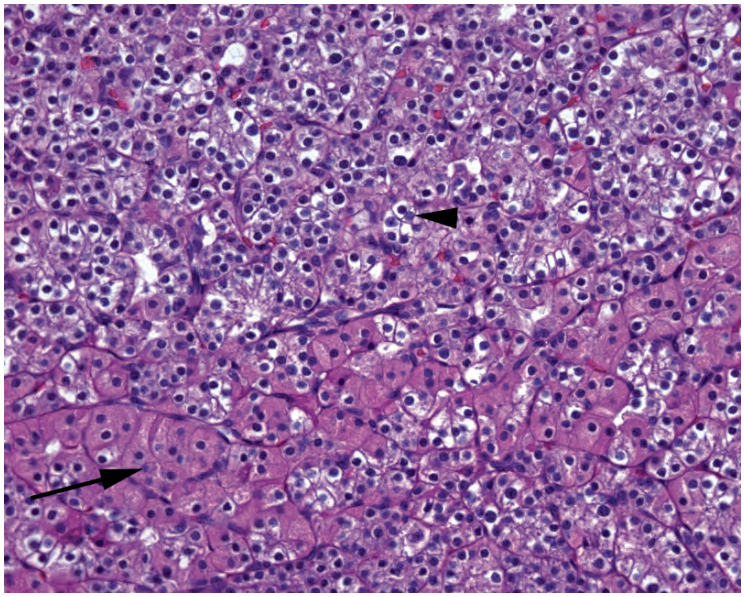

Figure 5.6. Parathyroid tissue. Normal parathyroid has two cell populations, the chief cells (arrowhead) and oxyphil cells (arrow).

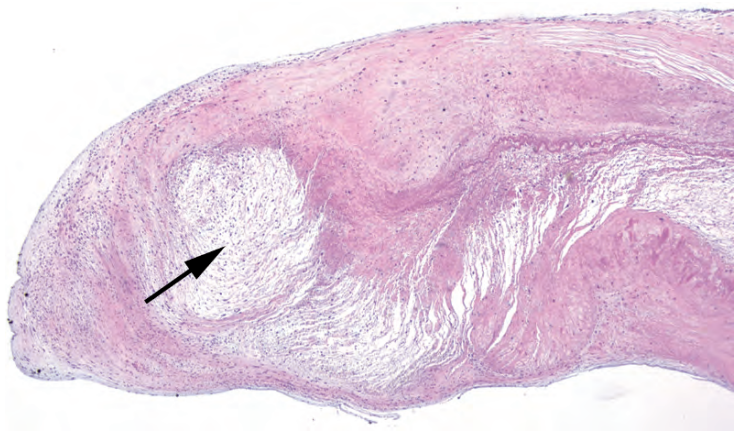

Figure 5.7. Myxoid degeneration, heart valve. In the free end of this heart valve, there is an attenuated pale area of myxoid degeneration (arrow). Calcifications and vegetations may also be seen.

Rule out: Use Gram Weigert and GMS stains on vegetations to rule out bacteria or fungus.
Sample sign out: Aortic valve (excision): *Valve with myxoid degeneration and calcification* or *Valve with adherent fibrinopurulent debris*. Numerous Gram-positive cocci are seen on Gram Weigert stain.

Gallbladder

Grossing: The first section should be a cross section of the cystic duct margin. Open the gallbladder, look for stones, and take two sections of the wall. Note the wall thickness and describe the mucosa. All three fragments can go in one cassette.
Histology: The gallbladder is lined by a single layer of columnar epithelium in folds, overlying a fibromuscular layer that sometimes contains Rokitansky-Aschoff sinuses (infolded mucosa) or

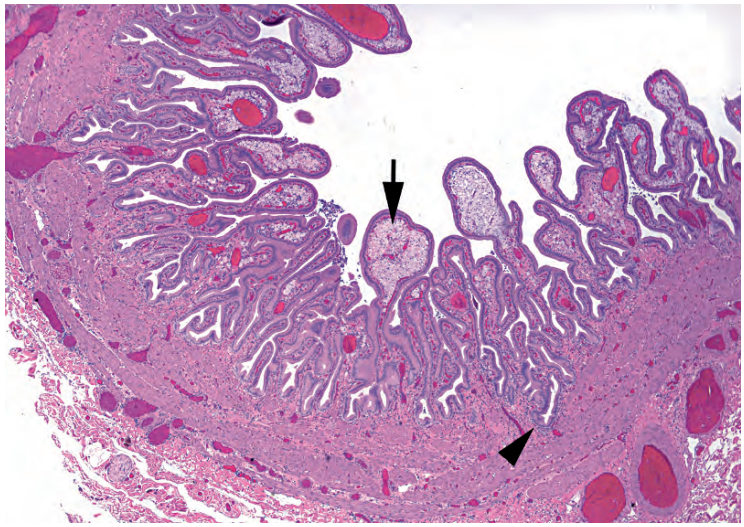

Figure 5.8. Gallbladder with cholesterolosis. The mucosal folds are distended with foamy macrophages (arrow), called cholesterolosis. Inflammation is minimal in this example. Rokitansky-Aschoff sinuses can penetrate deeply into the gallbladder wall (arrowhead).

ducts of Luschka. Cholecystitis can range from mild lymphoplasmacytic inflammation to transmural acute inflammation. Cholesterolosis is the accumulation of foamy macrophages (Figure 5.8).

Rule out: Dysplasia or carcinoma is rare in an isolated cholecystectomy.

Sample sign out: Gallbladder (cholecystectomy): *Chronic cholecystitis, cholelithiasis, and cholesterolosis* or *Acute and chronic cholecystitis*.

Appendix

Grossing: The first section should be a cross section of the proximal margin, inked or otherwise marked as margin. Then cut off the tip and take a longitudinal section (U shaped). Breadloaf the remainder, and take one to two cross sections. Look for nodules, fecaliths, hemorrhage, and pus.

Histology: Normal histology is a colonic mucosa with abundant lymphoid aggregates. Chronic inflammation is not significant, but neutrophils are, whether in the mucosa, wall (transmural inflammation; Figure 5.9), or serosa (serositis). Serositis without transmural inflammation suggests another abdominal source.

Rule out: Carcinoid in the tip and pools of mucin in the wall (as in a mucinous cystadenoma or carcinoma) should be ruled out.

Sample sign out: Appendix (appendectomy): *Acute transmural appendicitis with serositis* or *Histologically unremarkable appendix.*

Hernia Sac

Grossing: Submit a representative section.

Histology: A pouch of fibroadipose tissue is lined with mesothelium, which can be reactive or proliferative (Figure 5.10).

Rule out: A piece of the vas deferens (warrants an immediate call to the surgeon), incarcerated bowel, and metastatic tumor (especially incisional hernias) should be ruled out.

Sample sign out: Soft tissue, right inguinal (herniorrhaphy): *Hernia sac* or *Fibrovascular tissue (clinical hernia sac).*

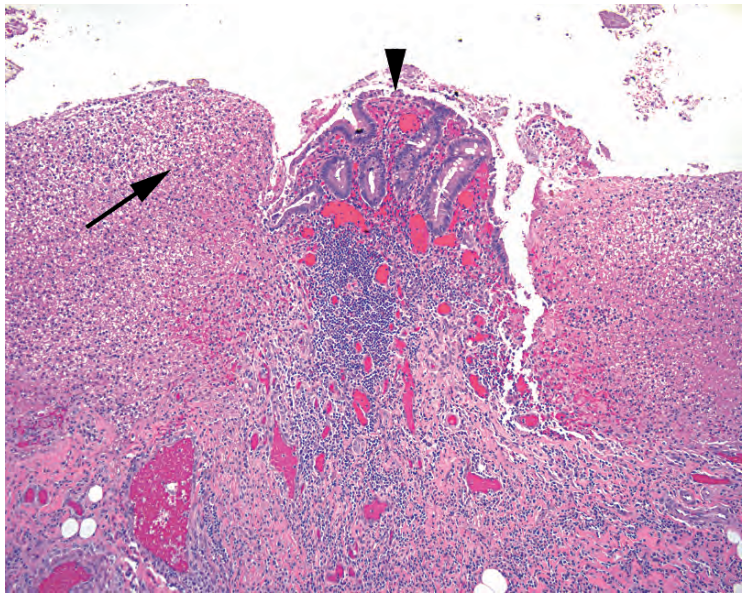

FIGURE 5.9. Appendicitis. In this close-up view, a small amount of residual colonic-type mucosa is visible (arrowhead), surrounded by mounds of fibrinopurulent debris (arrow).

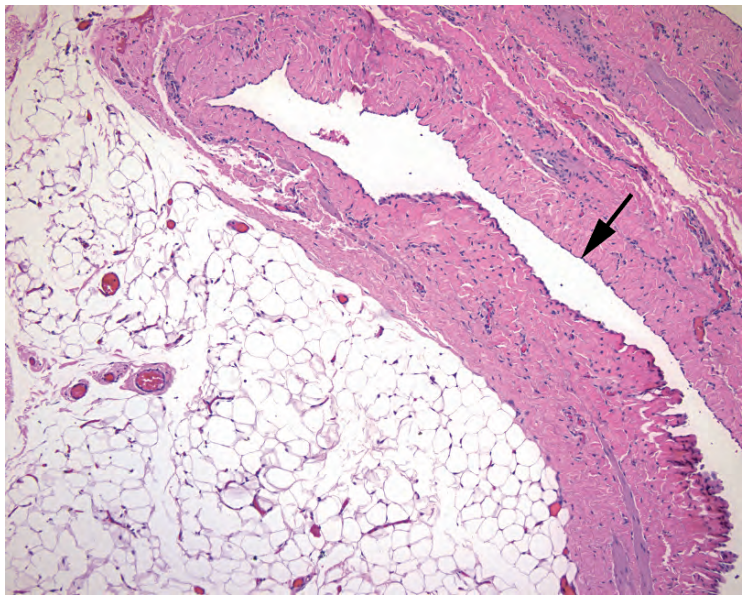

FIGURE 5.10. Hernia sac. Thick fibrous tissue and fat characterize the typical hernia sac. In this section, the delicate mesothelial lining (arrow) is visible.

Scar Revision

Grossing: Breadloaf and take representative sections through the scar. Note that this does not apply to reexcision of a skin cancer or melanoma.

Histology: Dermal scar has dense fine pink collagen, no appendages, and thin epithelium (Figure 5.11). Recent injury or surgery will show hemorrhage, foamy macrophages, inflammation and granulation tissue, suture material, and foreign body–type giant cells.

Rule out: Exclude tumor, if there is a history of tumor, and abscess.

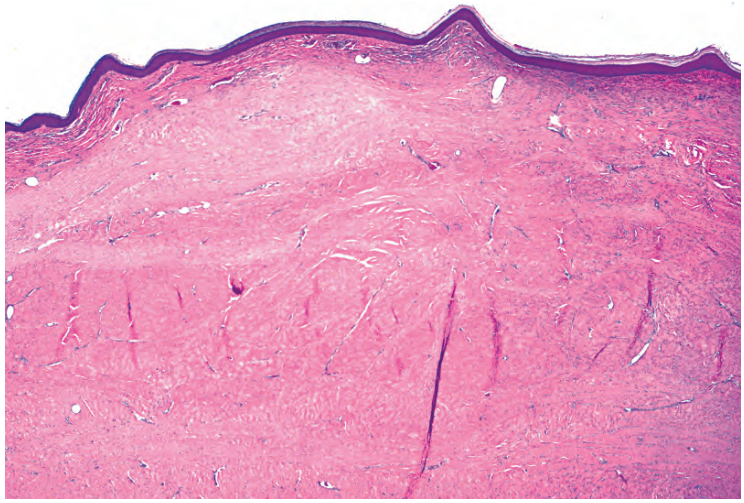

Figure 5.11. Dermal scar. Pale and homogeneous collagen underneath the epidermis, with obliteration of adnexal structures, is typical of scar formation.

Sample sign out: Left abdominal wall (scar revision): *Skin with dermal scar, negative for tumor* or *Skin with biopsy site changes and suture material*.

Femoral or Humeral Head, Knee Bones

Grossing: Use a bone saw to cross section the bone and get a 2-mm slice. Describe eburnation (absence of cartilage), osteophytes, femoral neck (fracture vs. surgical), infarcts, and subchondral cysts. Sample the articular surface, plus the margin or fracture site in fracture cases. Submit for routine decalcification.

Histology: Healthy bone has a thick cartilage layer with a smooth surface and marrow between the trabeculae. Look for the following:

- Osteoarthritis: uneven and ragged or absent cartilage, clonal nests of chondrocytes, dual tide-line, thickened and sclerotic subchondral bone, subchondral cysts, fibrosis, and granulation tissue within marrow (Figure 5.12)
- Osteonecrosis: loss of basophilia and nuclei in the marrow, fat cells, and osteocytes; fat necrosis; hemorrhage (Figure 5.13)
- Osteopenia: markedly thinned trabeculae
- Metastatic tumor: out-of-place cells in the area of fracture

Sample sign out: Right femoral head (arthroplasty): *Femoral head with osteonecrosis and fracture* or *Bone and cartilage with degenerative changes* or *Osteoarthritis*

Amputated Limbs

Grossing: It is gross, all right. Document the extent of gangrene, ulcers, venous stasis, trauma, and so forth, as well as level of amputation and the viability of the margin. In vascular or infectious disease, section the vascular margin (i.e., popliteal). Take representative sections from the worst area (soft tissue) and margin. Tissue from the bony margin is usually not necessary.

Histology: Look for gangrenous necrosis (Figure 5.14), ulceration, scar, granulation tissue, and inflammation. Evaluate vessel for atherosclerotic disease.

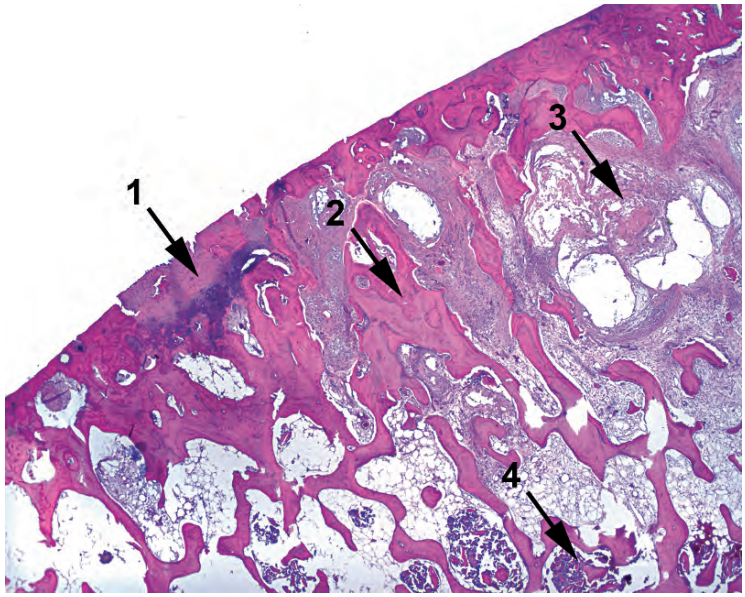

Figure 5.12. Osteoarthritis. Features include (1) eroded cartilage, in this case nearly absent, and irregular mineralization of the cartilage, seen here as a dark purple stain; (2) thickening of the subchondral bony trabeculae; (3) myxoid degeneration of the subchondral bone, forming cyst-like spaces; and (4) some residual hematopoietic marrow.

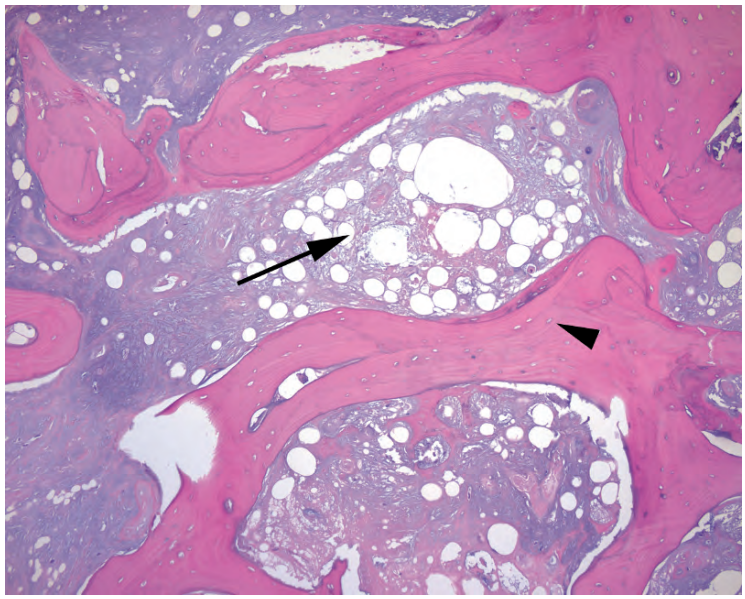

Figure 5.13. Osteonecrosis. The necrotic marrow is the most eye-catching feature (arrow), showing fat necrosis and an absence of marrow elements. On close examination, the osteocytes within lacunae are also dead or missing (arrowhead).

Rule out: Invasive fungal disease in a neutropenic patient (requires more extensive sampling of the margin) should be ruled out.

Sample sign out: Left foot (amputation): *Foot with gangrenous necrosis. Atherosclerotic vessels are identified. Surgical margin appears viable*.

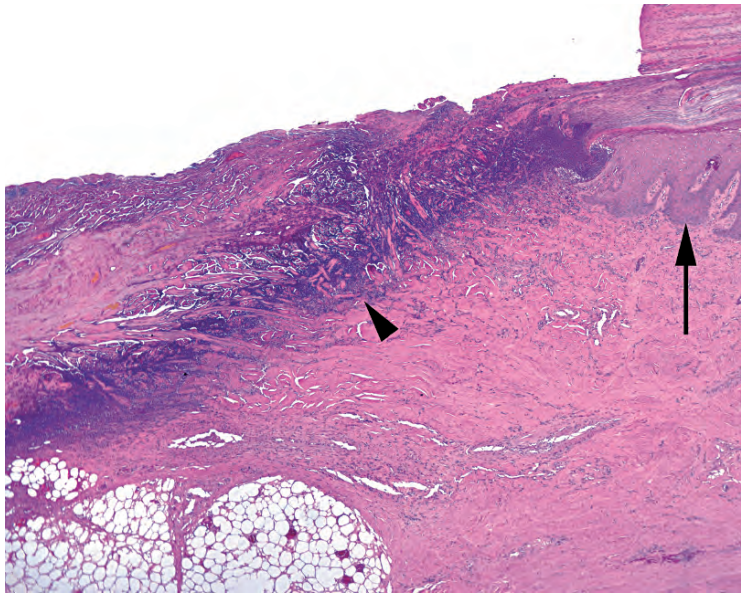

Figure 5.14. Gangrene. In this gangrenous ulcer of the toe, the epidermis is visible to the right (arrow), while the ulcer bed to the left shows an obliteration of epidermis and dermis, with a dense blue line of debris representing dying bacteria and cells (arrowhead).

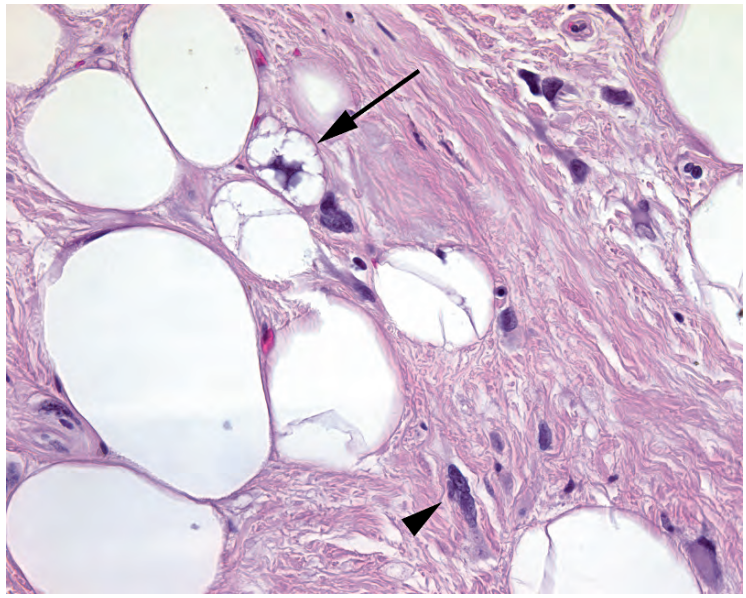

Figure 5.15. Lipoblast in an atypical lipoma. What you do not want to see in your lipoma—lipoblasts (arrow), with small fat vacuoles indenting the nucleus and atypical hyperchromatic cells within the fibrous stroma (arrowhead).

Lipoma

Grossing: Measure it. It never hurts to ink it. Submit thin slices (one per centimeter), and give them a nice long fixation time. Sample areas that are fibrous, fleshy, hemorrhagic, or otherwise nonfatty.

Histology: The definition of a lipoma is a neoplasm of mature fat cells. Fibrous tissue is okay. In fact, there are at least eight benign varieties of lipoma (fibrolipoma, myxolipoma, chondroid lipoma, myolipoma, myelolipoma, spindle cell lipoma, pleomorphic lipoma, and angiolipoma), all fat with something extra.

Rule out: Exclude liposarcoma. Clinical features that are suspicious include a large deep-seated circumscribed mass in the thigh, shoulder, retroperitoneum, or mesentery of an adult. Histologic features include chicken-wire vessels (a distinct lacy honeycomb network), atypical cells (large hyperchromatic nuclei), and lipoblasts (Figure 5.15). More details on liposarcomas are given in Chapter 28.

Sample sign out: Soft tissue, left flank (excision): *Lipoma (12 cm)* or *Fibrovascular tissue and mature adipose tissue (clinical lipoma)*

6 Esophagus

The esophagus is composed of a nonkeratinized squamous epithelium overlying a lamina propria and thin muscularis mucosa. The submucosa contains lymphatics and mucous glands with cuboidal-lined ducts running up to the luminal surface. Under the submucosa is muscularis propria (skeletal muscle proximally, smooth muscle distally), surrounded by the adventitia, which is continuous with mediastinum.

Most esophageal biopsies are performed on patients with symptoms of reflux or dysphagia and often the goal is to rule out Barrett's esophagus, a glandular metaplasia that puts the patient at increased risk for adenocarcinoma. Other common findings include reflux changes in the squamous epithelium, ulcers, or infection. Squamous dysplasia is actually uncommon.

Approach to the Slide

On low power, survey the epithelium. A normal biopsy specimen (Figure 6.1) will have a bland pink squamous epithelium and scant submucosa; muscularis propria should not be present. Occasional lymphocytes in the epithelium are typical (so-called squiggle cells because of their stretched-out appearance). The epithelium should not be interrupted or undermined by gastric-type glands, although salivary-like mucous glands are okay.

Within the squamous epithelium, look for the following:

- Basal cell hyperplasia (an increase over the normal three-cell layer) (Basal cells are the deepest layer of squamous cells and are the regenerative cell layer. They are defined by their closely packed nuclei: if you cannot squeeze a new nucleus between two existing nuclei, they are basal cells.)
- Elongated vascular papillae (over two thirds of the thickness of the epithelium)
- Balloon cell change of epithelium (an accumulation of glycogen in the cytoplasm)
- Intraepithelial neutrophils or eosinophils
- Erosions, fibrinopurulent exudate, granulation tissue
- Columnar cell mucosa or glands

Reflux Changes

A very common finding on biopsy is reflux esophagitis, which consists of the first four features in the preceding list (Figure 6.2). Not all features need be present in every case and typically are not. Severe cases may progress to erosions and ulcerations. The inflammation in reflux is mainly lymphocytic, but eosinophils may be seen; a very high number of eosinophils (or clustering of three or more) may indicate eosinophilic esophagitis. Stylistically, we often use the phrase "reactive epithelial changes" to describe changes that have some, but not all, of the features of full-blown reflux esophagitis.

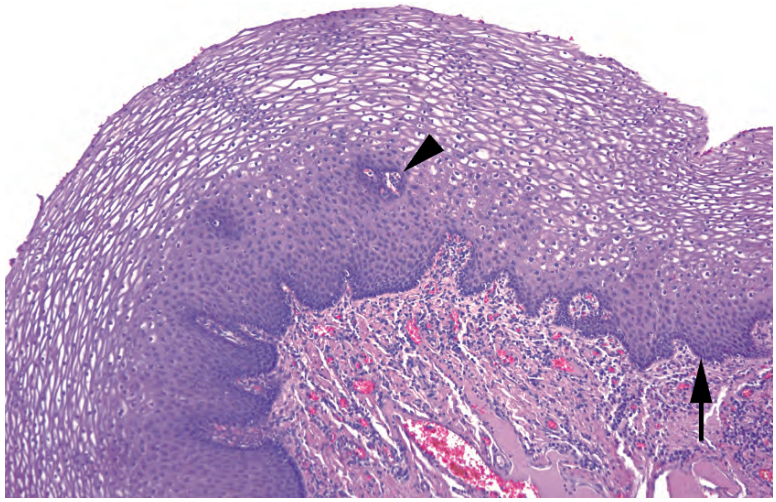

Figure 6.1. Normal esophageal mucosa. The basal layer (arrow) is seen as a crowded and blue layer at the base. The cells mature into flat nonkeratinizing squamous cells with small nuclei; the clear cytoplasm seen here is glycogen. Vascular pegs penetrate into the epithelium (arrowhead). The vascular lamina propria is visible below the basal layer.

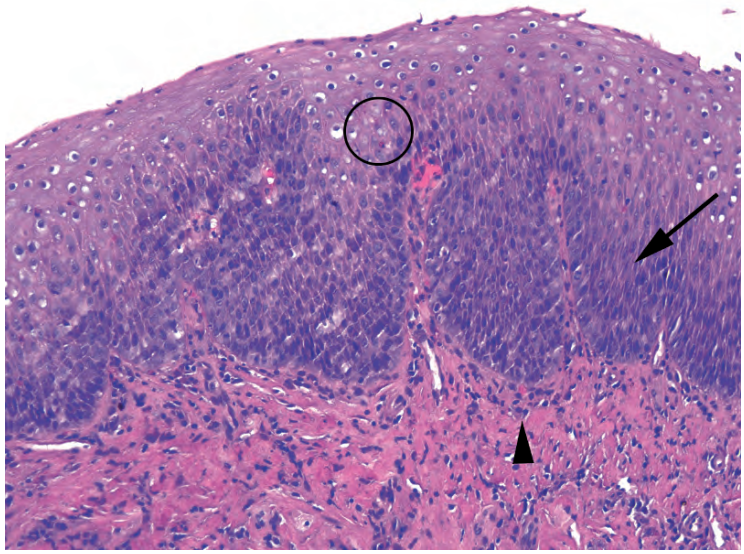

Figure 6.2. Reflux esophagitis. Compare this inflamed epithelium to the normal mucosa in Figure 6.1. The basal layer is increased in thickness (arrow), creating a more dense and blue look to the epithelium. There are inflammatory cells scattered throughout, including eosinophils (circle) and lymphocytes (arrowhead).

Neutrophils

A prominent neutrophilic infiltrate points more to an infection or acute injury rather than reflux. Look at the periodic-acid Schiff (PAS) stain to find *Candida* organisms (pseudohyphae and yeast forms in the epithelium or exudate; Figure 6.3). They may be very numerous or extremely scanty. Luminal squamous debris is another hint to look closely for *Candida*. Candidal infection is typically associated with a superficial neutrophilic infiltrate and parakeratosis (surface squames that are too red and have retained nuclei); however, some cases have almost *no* inflammation and little epithelial changes.

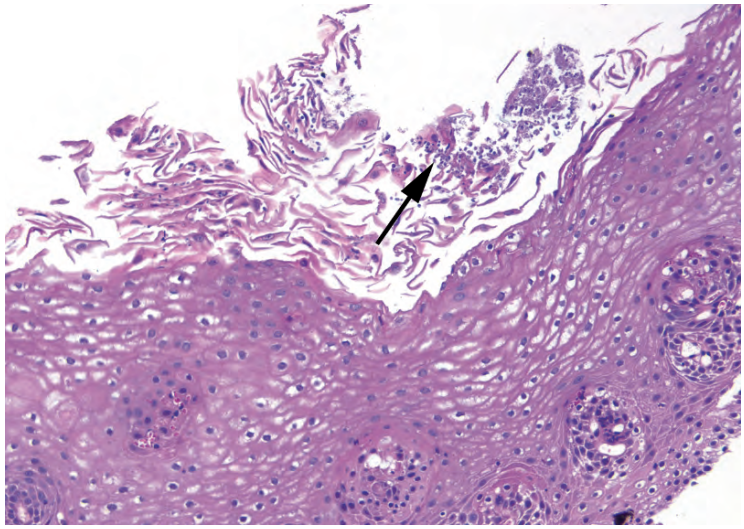

Figure 6.3. *Candida*. Tiny purple yeasts and pseudohyphae (arrow) are seen among the squamous debris at the surface of the epithelium. This is a hematoxylin and eosin stain; the yeasts are magenta on periodic-acid Schiff stain. Note that sometimes there is not a significant neutrophilic response.

Ulcers

Ulcers can be caused by severe reflux or chemical injury (especially pill esophagitis; polarize to look for pill fragments), radiation (should be accompanied by necrosis and bizarre atypia), or infection, or they can be idiopathic (particularly in acquired immunodeficiency syndrome). Viral esophagitis is rare but more common in the immunosuppressed. Herpes simplex virus and cytomegalovirus cause inflamed, punched-out ulcers.

- Herpes simplex virus infects epithelial cells. This is best seen on intact squamous mucosa adjacent to the ulcer, typically causing multinucleation with nuclear molding and glassy chromatin.
- Cytomegalovirus infects mesenchymal cells (fibroblasts, endothelial, etc.) at the ulcer base. Cytomegalovirus infection usually manifests with intranuclear and cytoplasmic red/purple inclusions that render the cells gigantic (cyto-*megalo*-virus), and thus 10× is a good objective to scan with.

The hematoxylin and eosin (H&E) slide is the best place to find the inclusions, but immunostains may help if clinical suspicion is high and H&E is not definitive (see Chapter 3 for images of viral inclusions).

Columnar Epithelium

Collections of submucosal mucous glands resembling salivary glands or Brunner's glands of duodenum are occasionally seen in mucosal biopsy material. It is more common to see the ducts from these glands. Gastric-type epithelium (foveolar surface epithelium and underlying specialized gastric glands, usually oxyntic) in an "esophagus" biopsy specimen may represent tissue inadvertently taken from proximal stomach or hiatal hernia. The presence of cardiac mucosa should be noted but does *not* equal a diagnosis of Barrett's esophagus (see later discussion). Collections of pink-purple acinar cells beneath the epithelium, resembling normal pancreas, may in fact be normal pancreas (called *pancreatic metaplasia* or *heterotopia*).

Goblet Cells

In the tubular esophagus, the presence of goblet cells (bulbous epithelial cells that are indigo-blue on PAS/alcian blue (AB) stain, clear-to-pale-blue on H&E; Figure 6.4) in glandular mucosa, otherwise known as *intestinal metaplasia*, indicates *Barrett mucosa of the distinctive type* ("Barrett's esophagus"). There are two caveats to be considered. First, intestinal metaplasia can also occur in the true cardia of the stomach as a response to inflammation. Therefore, if the location where the biopsy tissue came from is not entirely clear, some pathologists will sign out apparent Barrett's as "consistent with Barrett's mucosa if the biopsy was taken from tubular esophagus." Second, not all that stains blue with the PAS/AB is a goblet cell. Some gastric-type foveolar cells, especially at the squamocolumnar junction, will stain blue (so-called tall blues), so, to be a genuine goblet it has to stain blue *and* have goblet cell morphology (bulbous outline of goblet cells vs. the elongated foveolar cells).

Dysplasia Within Barrett's Esophagus

Like any gastrointestinal glandular mucosa, Barrett's mucosa can progress through dysplasia, intramucosal carcinoma, and invasive adenocarcinoma. As an already abnormal cell type responding to chronic injury, it is at high risk for dysplasia and is regularly screened by biopsy.

Dysplasia in Barrett's mucosa initially begins to look like a tubular adenoma of the colon (it gets blue). The cells have the following characteristics:

- Increased nuclear hyperchromatism and pleomorphism
- High nuclear-to-cytoplasmic ratio
- Loss of mucin vacuoles
- Crowding and pseudostratification
- Loss of polarity

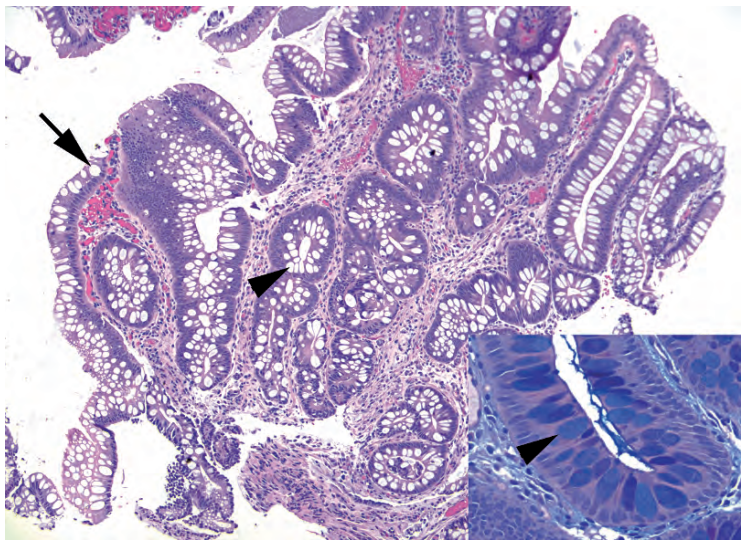

FIGURE 6.4. Goblet cells in Barrett's esophagus. The presence of columnar epithelium with goblet cells indicates Barrett's esophagus. Goblet cells are round cells that appear clear on hematoxylin and eosin stain and are typically flanked by the purplish absorptive-type cells. Back-to-back mucinous cells resembling a row of teeth are more likely to be gastric foveolar epithelium. Goblet cells may be present at the surface (arrow) or in deep glands (arrowhead). **Inset**: A periodic-acid Schiff/alcian blue stain confirms the goblet cells, which stain indigo blue (arrowhead).

True dysplasia should extend *all the way to the surface epithelium*, as in a tubular adenoma (Figure 6.5). Features that should make you back off from dysplasia include surface maturation (base looks bad, but surface looks fine), big grey-purple nuclei with prominent nucleoli (more likely to be reactive), and pronounced inflammation (also points to reactive).

Progression to high-grade dysplasia (Figure 6.6) includes increasing atypia (loss of polarity, nuclei that begin to look like boulders: large with irregular outlines), mitotic activity (although mitoses alone are not worrisome), and architectural dysplasia (glands that are crowded and complex: budding, branching, cribriform, papillary, or villiform). High-grade dysplasia tends to be diagnosed in situations in which you are worried about invasive carcinoma but cannot prove it. Think of high-grade dysplasia as synonymous with carcinoma in situ.

The next step along the malignancy progression is invasive carcinoma (Figure 6.7). As in other organs, clues to invasion include a ragged basement membrane border, single cells infiltrating, and a desmoplastic stromal response. Note that in the esophagus, unlike in the colon, intramucosal carcinoma (invasive adenocarcinoma confined to the lamina propria) *is* thought to have metastatic potential and thus is considered a "T1" lesion (not "Tis") in the TNM (tumor, node, metastasis) staging classification. This is due to the presence of lymphatics in the lamina propria of the esophagus.

Squamous Dysplasia

Within the squamous epithelium, dysplasia, carcinoma in situ, and invasive squamous cell carcinoma are diagnosed by criteria similar to those for the cervix. Dysplastic changes include enlarged, pleomorphic nuclei, increased nuclear/cytoplasmic ratio (a general blueness at low power), mitoses above the basal layer, and loss of order and polarity. Prominent nucleoli are more consistent with reactive/reparative changes. Dysplasia begins at the base and progresses to the surface. If the changes persist all the way to the surface, it is carcinoma in situ. Invasion can be hard to identify; a deep pushing front is not necessarily invasion. Look for deep aberrant keratinization (pinking up) and single cells trailing off as a clue to invasive carcinoma.

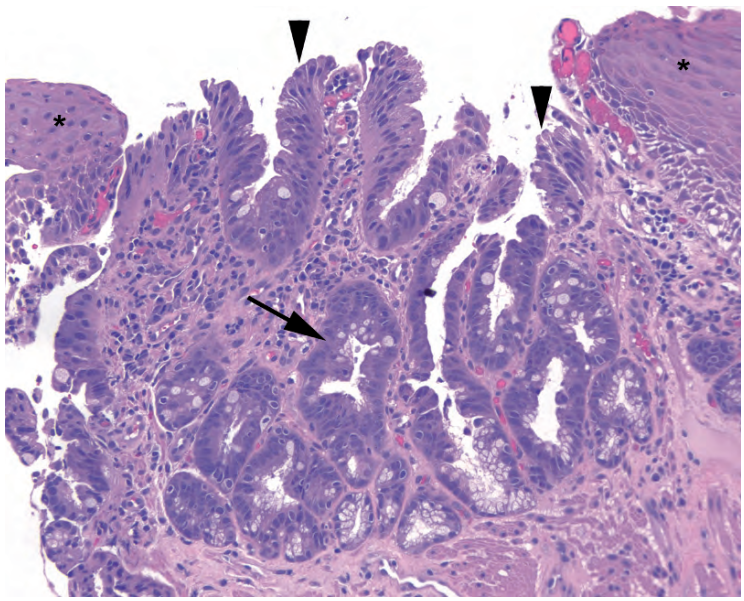

Figure 6.5. Low-grade dysplasia in Barrett's esophagus. The cells begin to lose polarity and organization, with nuclei becoming more pleomorphic and lifting up off the basement membrane (arrow). The changes must extend to the surface (arrowheads) to count. Compare with the nondysplastic epithelium shown in Figure 6.4. Adjacent squamous epithelium can be seen on either side (asterisks).

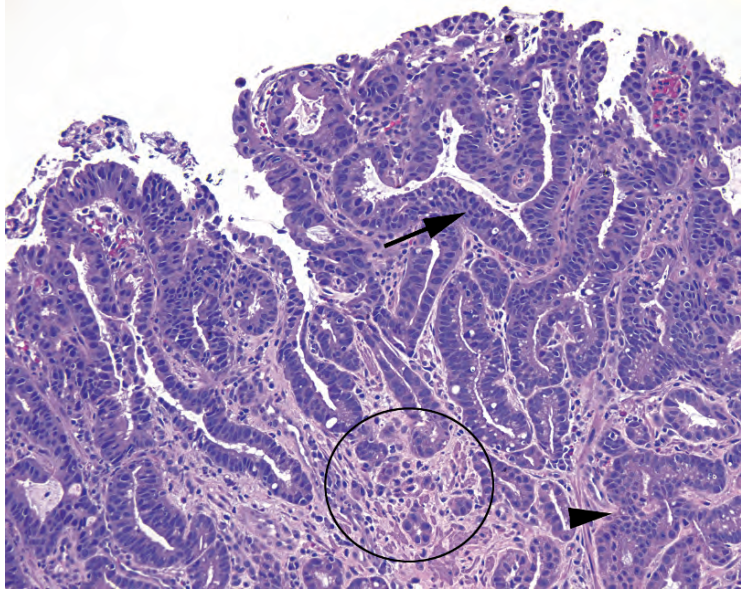

FIGURE 6.6. High-grade dysplasia. Nuclei here show marked hyperchromasia, pleomorphism, and disorganization (arrow), and the glands are beginning to show cribriform growth (arrowhead). There is a focus suspicious for invasion highlighted in the circle.

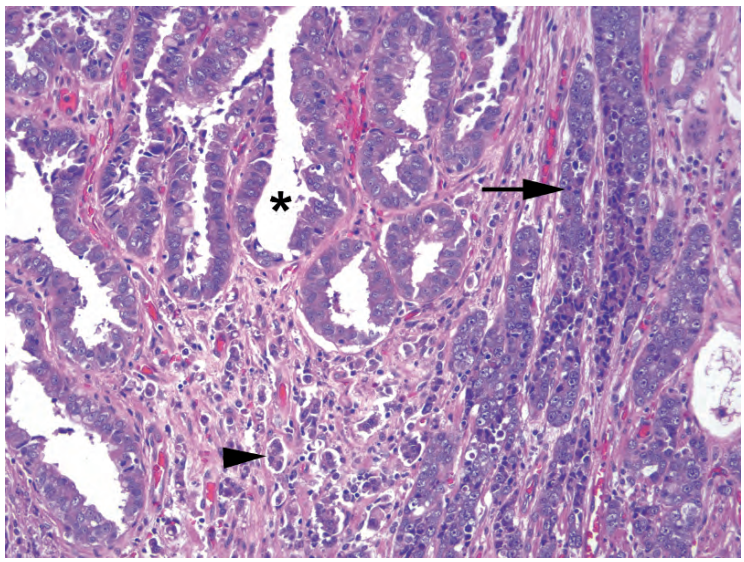

FIGURE 6.7. Invasive adenocarcinoma. The carcinoma can be seen invading the stroma as glands (asterisk), cords (arrow), and single cells (arrowhead). Notice the prominent nucleoli, which are not typically a feature of dysplasia.

Polyps

A reasonable differential for a polypoid structure in the esophagus includes the following:

- Benign
  - Inflammatory fibroid polyp: vascular, inflamed, fibrous stroma covered by benign squamous epithelium, may be ulcerated; looks like granulation tissue
  - Fibrovascular polyp: fibrovascular core covered by benign squamous epithelium, plus or minus fat

- Squamous papilloma: fibrovascular core covered by hyperplastic, but benign, squamous epithelium
- Submucosal nodules such as leiomyoma and granular cell tumor

- Malignant
  - Verrucous squamous cell carcinoma
  - Other carcinomas

Neoplasms in the Esophagus

One way to create a differential of neoplasms within an organ is to list all of the normal cell types and then think about what tumors can arise from each one. In the esophagus, the whopping majority of cancers arise from the epithelium and therefore are squamous or adenocarcinoma, but soft tissue tumors, although unusual, can occur. These include leiomyoma, granular cell tumor, hemangioma, angiosarcoma, and others.

7 Stomach and Duodenum

Stomach

The stomach is composed of several anatomic zones. Moving proximal to distal, like a piece of food, you pass (1) the gastroesophageal junction, (2) the cardia, (3) the fundus or body, (4) the antrum, and (5) the pylorus (Table 7.1 and Figure 7.1). For the pathologist, there are essentially two types of mucosa in the stomach (Figure 7.2), antral and oxyntic, as cardiac mucosa looks very similar to antrum. The entire stomach epithelium is composed of pits (invaginations from the surface) and glands (deep to the pits). The surface and pits are lined by columnar mucinous epithelium (sometimes called *foveolar type*) which stains bright pink with PAS/AB. The regions of the stomach are divided by the type of underlying glands:

- *Antral* mucosa (found in the antrum): The glands are loosely packed, mucinous, and occupy about half of the epithelial thickness. Cardiac mucosa looks very similar.
- *Oxyntic* mucosa (found in the fundus or body): The glands are tightly packed, contain granular parietal (pink, acid-secreting) and chief (purple, enzyme-secreting) cells, and occupy three fourths of the mucosal thickness.
- *Transitional* mucosa: Features of both antral and oxyntic are present. Transitional mucosa represents the overlap zone.

It is important to note what kind of epithelium is present in the biopsy tissue so that the endoscopists can correlate with what they saw. Also, there are certain processes that differentially affect mucosal types; clarifying the type of epithelium involved may change the differential.

Endocrine cells occur singly in the glands. In the body, they are mainly enterochromaffin-like cells, while in the antrum they are mixed gastrin, enterochromaffin, and somatostatin. A chromogranin stain highlights all endocrine cells. A gastrin stain should be positive only in the antrum.

The Approach to the Biopsy

Survey the glandular epithelium at low power:

- The first thing to notice is what kind of mucosa you have and whether it correlates with what the endoscopist told you. Then decide on the color of the biopsy specimen. A healthy stomach is a pretty pale pink, overall. If your general impression is blue, this probably indicates inflammation in the stroma, such as in gastritis (Figure 7.3). If your impression is that of a pink stroma with unusually dark and distinct glands, you may be looking at chemical gastritis.
- Goblet cells are usually visible from low power, especially on the PAS/alcian blue (AB) stain (as indigo-blue, bulbous cells), and indicate intestinal metaplasia, a marker of chronic

Table 7.1. Anatomic zones of the stomach.

	Esophagus	Cardia	Fundus	Antrum	Pylorus
Histology	Squamous	Cardiac (antral)	Oxyntic	Antral	Antral to duodenal
Endocrine cells	None		Enterochromaffin-like	Gastrin, somatostatin, enterochromaffin	
Common pathology	Reflux esophagitis	Reflux carditis *Helicobacter pylori*	Autoimmune gastritis	*H. pylori* gastritis Chemical gastritis	Chemical gastritis

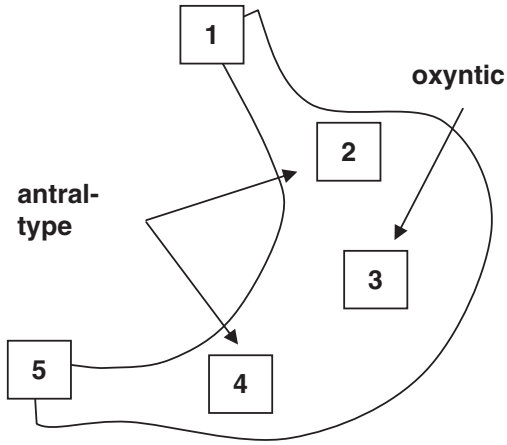

Figure 7.1. Localization of anatomic regions within the stomach: (1) the gastroesophageal junction, (2) the cardia, (3) the fundus, (4) the antrum, and (5) the pylorus. Antral-type (mucinous) mucosa is seen in the cardia and the antrum.

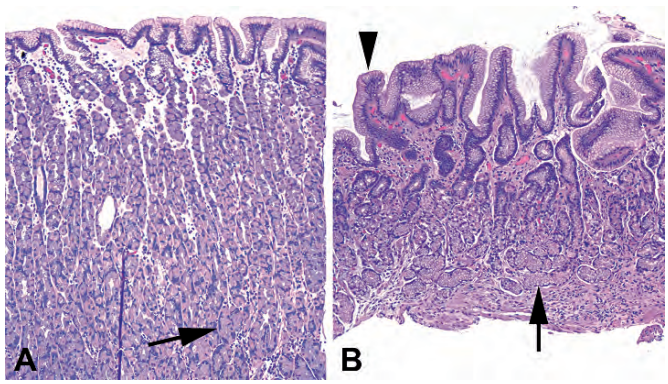

Figure 7.2. Antral and oxyntic mucosa. **(A)** Oxyntic mucosa is relatively thick, with most of the mucosa occupied by secretory cells (arrow), the parietal and chief cells. The surface is composed of mucinous foveolar epithelium. **(B)** Antral mucosa is thinner, and the glands are mucinous instead of secretory (arrow). However, the surface is still composed of foveolar epithelium (arrowhead).

irritation in the stomach. Remember that true goblet cells are usually interspersed among nonmucinous pink cells (absorptive). A row of back-to-back tall mucinous cells, even if blue on PAS/AB, is unlikely to be actual intestinal metaplasia.

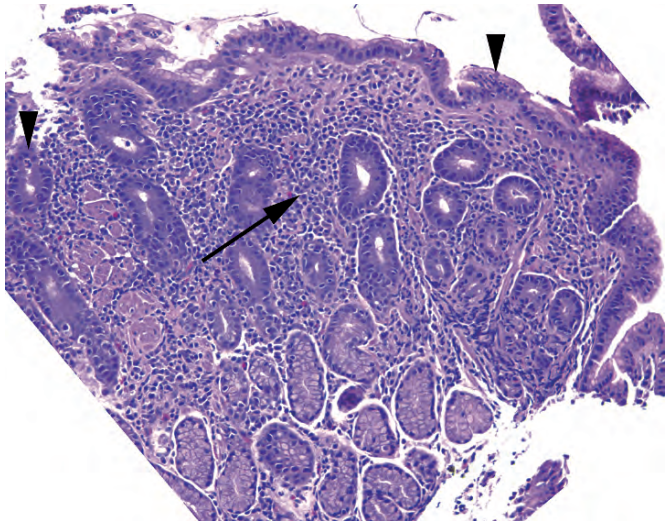

Figure 7.3. *Helicobacter pylori* gastritis, antrum. In this disease, the low-power impression is that of a "blue" biopsy due to the dense inflammatory infiltrate in the lamina propria (arrow). There are lymphocytes, plasma cells, and neutrophils. Neutrophils in the glandular or surface epithelium (arrowheads) indicate an active component to this gastritis. *Helicobacter pylori* organisms are pictured in Chapter 3.

- Areas of exudate, neutrophils, debris, and ragged-looking glands indicate an erosion or ulcer. Ulcers are discussed in more detail later.

On higher power, assess the inflammation:

- A few *lymphocytes, plasma cells*, and *eosinophils* are okay in the stomach, especially in the antrum, where there is more space between glands. However, back-to-back lymphocytes and plasma cells pushing aside or crowding the glands indicate a chronic gastritis.
- The presence of neutrophils in the stomach indicates *activity* and is not called *acute* as in other organs. If you have only mononuclear cells, you have an inactive chronic gastritis, but if there are any neutrophils embedded in the surface or glandular epithelium, you have an active chronic gastritis.
- Neutrophils, chronic gastritis, and lymphoid follicles are all associated with *Helicobacter pylori* infection. The tiny rods are visible on hematoxylin and eosin stain (see Chapter 3) but are better seen on Diff-Quik or Giemsa. They should be visible at 40× as tiny discrete seagull-shaped rods in the pit lumens or on the surface, mainly in the antrum, unless there is intestinal metaplasia, a hostile mucosa for these bugs. If you have no significant inflammation, do not work too hard looking for *H. pylori*.
- How many lymphocytes does it take to diagnose *lymphoma*? Lymphoma in the stomach often arises from mucosa-associated lymphoid tissue (MALT), is of marginal zone phenotype, and is a result of chronic *H. pylori* infection. You should see sheets of monocytoid B cells (fried egg–like) and lymphoepithelial lesions (lymphocytes embedded in epithelium) before considering this diagnosis.

Foveolar Hyperplasia

Especially in the antrum, the stomach is vulnerable to bile reflux. Bile and other sources of chemical irritation, such as nonsteroidal anti-inflammatory drugs, cause a process called *foveolar hyperplasia*. The surface mucin cells proliferate, giving the surface a papillary appearance and the pits a corkscrew profile. The mucinous cells lose mucin, and the cytoplasm becomes more dark or opaque; the nuclei also may become hyperchromatic, adding to the dark look (Figure 7.4). Smooth muscle fibers proliferate and can be seen stranding up between

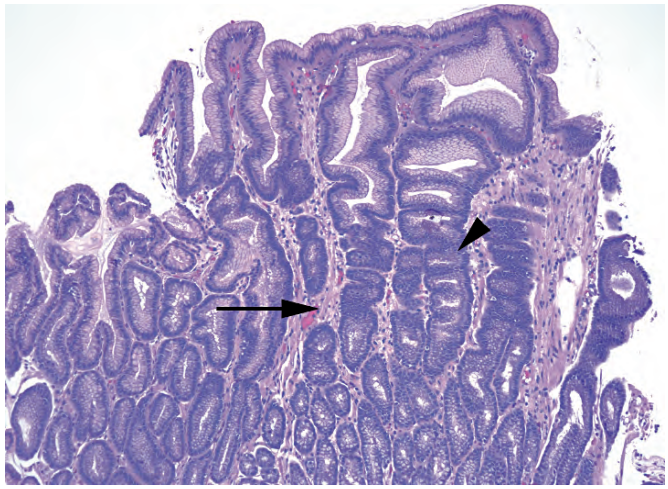

Figure 7.4. Chemical gastritis, antrum. In chemical gastritis, the lamina propria shows very little inflammation, unlike in *Helicobacter pylori* gastritis. The lamina propria is pale and sometimes edematous such that the dark reactive nuclei of the glands stand out sharply in contrast. The corkscrew profile of the hyperplastic glands is a second classic feature (arrowhead), as is the presence of thin strands of smooth muscle between the glands (arrow).

the pits. Inflammation in the stroma is not a prominent feature, so the lamina propria is often fairly pale, even edematous. This appearance is called *chemical gastritis*, and it is very common.

Atrophy

The loss of glands in the stomach, in any region, is called *atrophy*. Atrophy can be difficult to assess on any given biopsy specimen, as badly oriented sections, a healing ulcer, or dense inflammation can all lead to the appearance of loss of glands. Regardless of the cause, true atrophy, as an end-stage response to severe chronic damage, should be accompanied by intestinal metaplasia and inflammation (Figure 7.5). The two principal types of atrophy are the following:

- *Helicobacter pylori* gastritis (formerly known as *environmental metaplastic atrophic gastritis*) is secondary to *H. pylori* infection, primarily affects the antrum, and involves loss of glands in the setting of active chronic gastritis and intestinal metaplasia. You may also see lymphoid follicles and pit abscesses (pits full of neutrophils).
- Autoimmune gastritis (formerly known as *autoimmune metaplastic atrophic gastritis*), a result of the autoimmune destruction of the parietal cells in the fundus, shows loss of fundic glands in the setting of chronic inflammation and intestinal metaplasia. It is associated with a compensatory antral G-cell hyperplasia and hypergastrinemia. The hyperplasia may even progress to microcarcinoids or tumorlets. In autoimmune gastritis, you should not see activity or lymphoid follicles.

In severe autoimmune gastritis, the body of the stomach comes to resemble the antrum due to the atrophy of the secretory glands. It can therefore be difficult to decide if it is antrum or truly atrophic oxyntic mucosa. A gastrin stain, which will stain only true antrum, will help.

Lymphoma

Although diffuse large B-cell lymphomas are the most common lymphoma in the stomach, and should be considered if you see sheets of very ugly cells, differentiating them from normal inflammation is not usually a problem. Low-grade lymphomas, however, are tricky, especially given that most arise in the setting of chronic *H. pylori* gastritis. As mentioned earlier, you may see a background of lymphoepithelial lesions, which are collections of

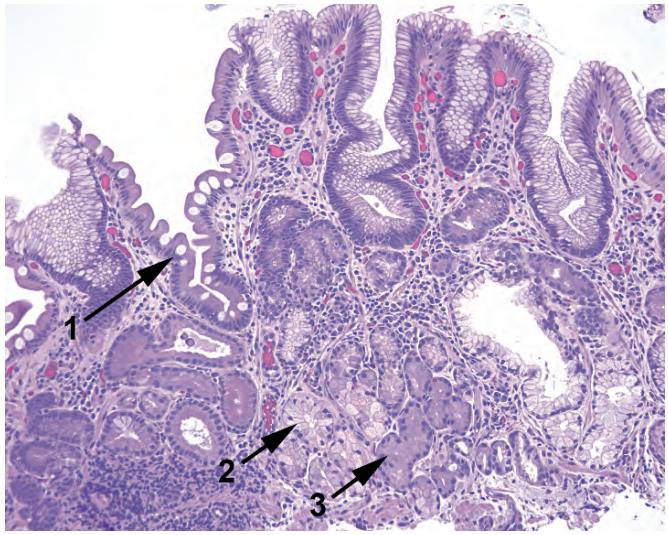

Figure 7.5. Autoimmune gastritis. This fundic biopsy specimen shows several features of atrophy. The surface shows goblet cells, which are indicative of intestinal metaplasia (1); deep to this there is inflammation and replacement of the secretory glands by mucinous, antral-type glands (2). Some residual oxyntic cells are also visible (3).

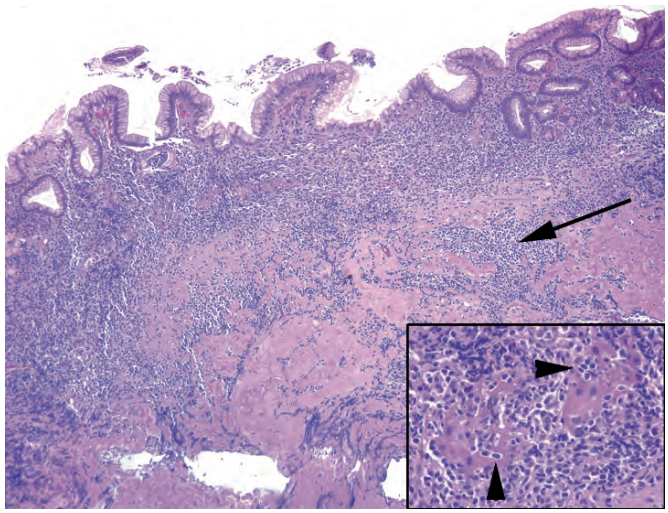

Figure 7.6. Mucosa-associated lymphoid tissue lymphoma. There are sheets of lymphocytes under the epithelium and dissecting into the muscularis mucosa (arrow). **Inset**: Lymphoepithelial lesions are typical, in which residual glands (seen here as little more than islands of pink cytoplasm) are infiltrated and destroyed by lymphocytes (arrowheads).

lymphocytes that appear to be eating glands (Figure 7.6), lymphoid follicles, and neoplastic plasma cells. Mucosa-associated lymphoid tissue lymphoma is usually of the marginal zone type, which is monocytoid in appearance (small round nuclei surrounded by a halo of clear cytoplasm).

Immunostains are often used to establish the diagnosis. In a MALT lymphoma, the majority of the cells should be B cells ($CD20^+$) that also stain for CD43. Normal T cells may also stain for CD43, so you must mentally subtract out the background T cells (shown by CD3 stain). Helpfully, in chronic gastritis, most of the lymphocytes are T cells.

Ulcers

An ulcer is a full-thickness defect of the epithelium down to muscularis mucosa (an erosion is more superficial). It is accompanied by fibrinopurulent exudate and/or granulation tissue, plus reparative glands. Search the periphery for the reason for the ulcer, including *H. pylori*, chemical gastritis, and adjacent cancer. Reparative glands appear as small, angulated glands with little mucin, and the lamina propria around them may be fibrotic. This can be difficult to distinguish from invasive carcinoma. However, reparative glands should have small or reactive nuclei and should have an overall streaming parallel arrangement, as they all want to orient to the surface (Figure 7.7).

Polyps

A reasonable differential for a polypoid structure in the stomach includes the following:

- Fundic gland polyps: Fundic gland polyps look like oxyntic mucosa but with cystically dilated glands (Figure 7.8). They are common in older people. Multiple polyps occur in familial adenomatous polyposis.
- Hyperplastic polyps: Polyps are hyperplastic, elongated, or cystic foveolar pits with mild inflammation (Figure 7.9). They are usually associated with background gastritis.
- Adenomas: These are neoplastic and dysplastic nodules that can be either gastric type or intestinal type. The gastric type is not associated with malignancy, but the intestinal type can be, such as a tubular adenoma.

Dysplasia Versus Carcinoma

Dysplasia in the stomach is assessed similarly to dysplasia in Barrett's esophagus (see Chapter 6). As in Barrett's esophagus, intestinal metaplasia is an abnormal finding but by itself is not dysplasia. Dysplasia in gastric mucosa initially begins to look like a tubular adenoma of the colon (it gets blue). The nuclei show increased hyperchromatism and pleomorphism, high nuclear/cytoplasmic ratio, loss of mucin vacuoles, crowding and pseudostratification, and loss of polarity. *High-grade dysplasia* tends to be called when invasive carcinoma is suspected but

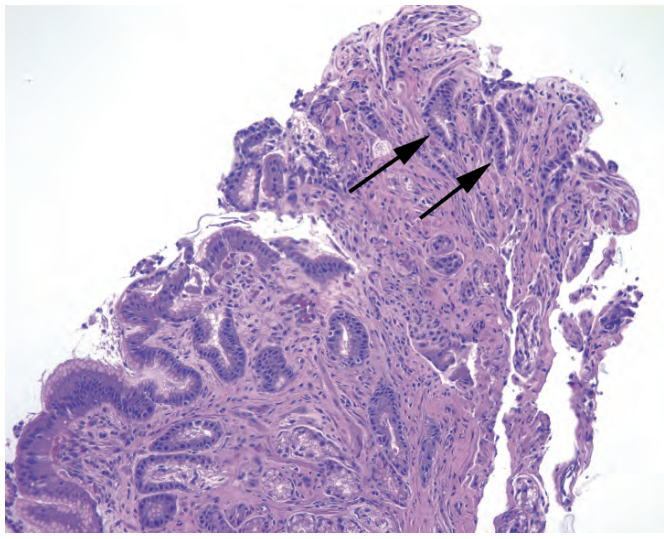

Figure 7.7. Reparative changes next to an ulcer. The tip of this fragment has a collection of poorly formed glands with an infiltrative look and minimal cytoplasm, giving the appearance of a high nuclear/cytoplasmic ratio (arrows). However, the nuclei are of about the same size and shape as the rest of the gastric glands, and these small glands stream in parallel toward the surface, consistent with regenerative or reparative glands.

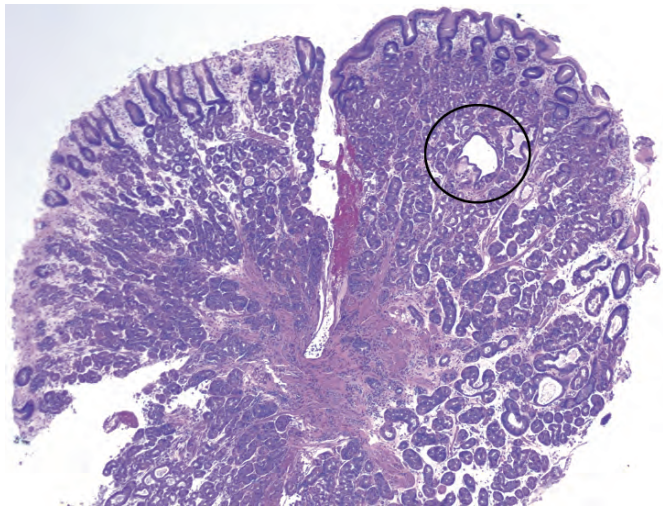

Figure 7.8. Fundic gland polyp. This polypoid fragment shows oxyntic- or fundic-type glands, with occasional dilated glands (circle).

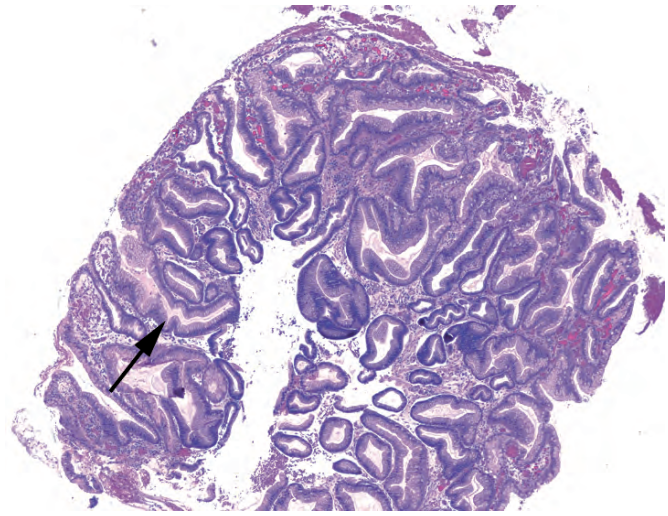

Figure 7.9. Hyperplastic polyp. This polyp is reminiscent of chemical gastritis, with corkscrew glands (arrow) and hyperplastic foveolar epithelium. Inflammation and intestinal metaplasia may be present.

cannot be proven. *Carcinoma in situ* is not used in this situation; think of high-grade dysplasia as synonymous.

Invasive adenocarcinoma comes in two types in the stomach: intestinal type (which looks like colon cancer, hence the name) and diffuse. The intestinal type is fairly easy to spot; it is usually associated with atrophy and intestinal metaplasia. The diffuse type is the poorly differentiated, often signet-ring, infiltrative cancer that can creep through the entire stomach and cause linitus plastica. Signet-ring cell carcinoma gets its name from the single vacuolated cells with displaced and indented nuclei (Figure 7.10). They can look like foamy macrophages, and they can blend almost imperceptibly into the stroma. Every stomach biopsy specimen should get a once-over at high power, such as 20×, to scan the lamina propria for signet rings. When they are there, often you will see the first one and then realize there are hundreds of them.

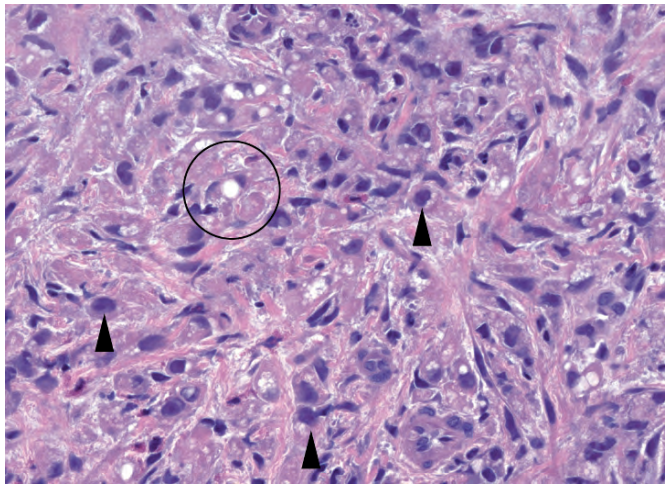

Figure 7.10. Signet-ring cell carcinoma. At low power, this sneaky tumor may be visible as little more than a slightly busy or cellular lamina propria. At high power, you can see individual signet-ring cells with single large mucin vacuoles (circle), plus other single infiltrating cells with large hyperchromatic nuclei (arrowheads). The signet-ring cells differ from fat cells by having large dark nuclei that protrude up from the surface of the central vacuole.

The Submucosa

The submucosa is not always included in a biopsy specimen. It lies below the thin muscularis mucosa. However, there are some things that are more often found in the submucosa, including the following:

- Heterotopic pancreas is a nodule of well-developed pancreas.
- Gastrointestinal stromal tumors, which arise from the interstitial cells of Cajal, are spindle-cell neoplasms that should stain for c-kit (CD117).
- Leiomyomas arise from smooth muscle cells. Leiomyoma is the second entity in the differential for are spindle-cell neoplasm. It stains for smooth muscle markers but not c-kit.
- Carcinoids may be mucosal or submucosal; they have similar morphology to carcinoids elsewhere. Carcinoids may be sporadic, they may arise in multinodular form in response to autoimmune gastritis, or they may be associated with multiple endocrine neoplasia (MEN) syndromes.

Duodenum

Duodenum is included here as duodenal tissue often accompanies stomach tissue in biopsy specimens, and the pathology in some cases is continuous. A duodenal biopsy may be performed because of combined gastritis and duodenitis, with or without peptic ulcer disease; to investigate suspected malabsorption syndromes, such as celiac disease; or to diagnose a mass lesion.

Normal duodenal mucosa is characterized by narrow villi that project above the mucosal surface. The epithelium is intestinal type, which means goblet cells are interspersed among the absorptive cells. Lymphocytes, plasma cells, and eosinophils are normal inhabitants of the lamina propria. A few intraepithelial lymphocytes may be seen, although they should not be found at the tips of the villi. Under the muscularis mucosa are collections of mucous glands called Brunner's glands, which stain bright pink on PAS/AB.

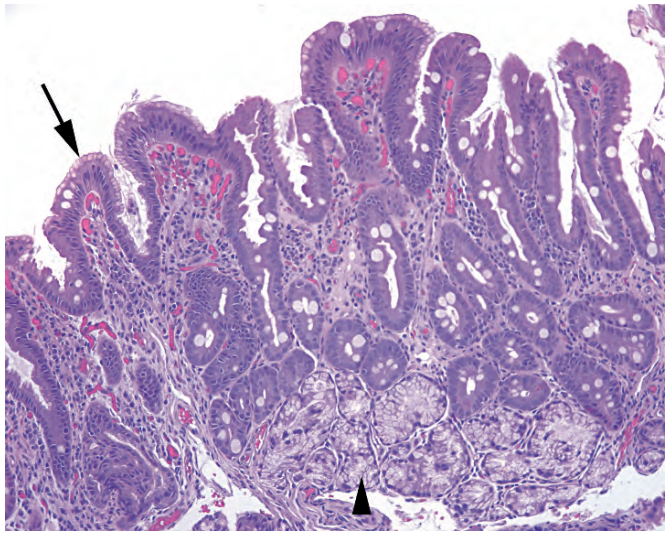

Figure 7.11. Chronic peptic duodenitis. At the surface, there is a subtle metaplastic change (arrow), where the normal absorptive and goblet cells are replaced by mucinous, foveolar-type cells, similar to those seen in antrum. There is increased chronic inflammation in the lamina propria and Brunner's gland hyperplasia (arrowhead).

Chronic Peptic Duodenitis

In severe gastritis, the inflammation and increased acid secretion may spill into the duodenum. In response to the lowered pH, the duodenum may "turn itself into stomach" or acquire gastric-type metaplasia. This shows up as metaplastic mucinous cells lining the villi (Figure 7.11) and is very obvious on PAS/AB stain because of the pink color of the gastric-type mucin. Other changes include Brunner's gland hyperplasia, which is a mucosal (as opposed to a submucosal) proliferation of glands, and increased inflammation in the lamina propria. Advanced cases may ulcerate.

Celiac Disease

Although celiac disease, or sprue, is not very common, requests to rule it out are fairly frequent. The classic picture of advanced celiac disease is that of a completely flattened mucosa, with total loss of villi, such that the duodenum mimics colon (Figure 7.12). The absorptive epithelium loses its brush border and flattens into a low cuboidal layer, hence the resulting malabsorption. However, more subtle findings include villous blunting, or loss of villous height, and prominent intraepithelial lymphocytes at the tips of the remaining villi. Evaluating villous blunting can be difficult in a poorly oriented or mangled mucosal fragment; also keep in mind that the differential for villous atrophy is long and is only diagnostic of celiac disease if the serology and clinical picture agrees.

Infections of the duodenum include those caused by *H. pylori*, which can rarely occur in the setting of gastric mucin cell metaplasia, and giardiasis. *Giardia* is very sneaky, as the organisms hide in the luminal debris and do not cause inflammatory changes. In the immunocompromised, collections of foamy histiocytes stuffing the lamina propria may represent *Mycobacterium avium-intracellulare* infection. However, the differential for stuffed macrophages also includes Whipple's disease, in which the macrophages are digesting Gram-positive rods and PAS-positive granules.

Neoplasms of the duodenum are unusual, but the most common of these are tubular adenomas (Figure 7.13), carcinoid tumors, and lymphoma (usually MALT type). Remember the intimate association of the duodenum to the pancreas and common bile duct; an adenocarcinoma found in duodenum may be originating in any of these organs.

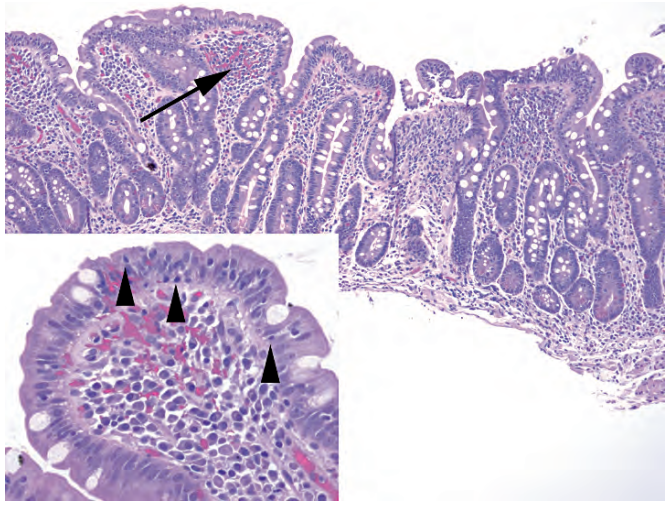

Figure 7.12. Celiac disease. The normal villi are blunted almost out of existence, with the duodenal mucosa resembling colon. There is chronic inflammation within the lamina propria (arrow). **Inset**: Increased numbers of intraepithelial lymphocytes are present (arrowheads).

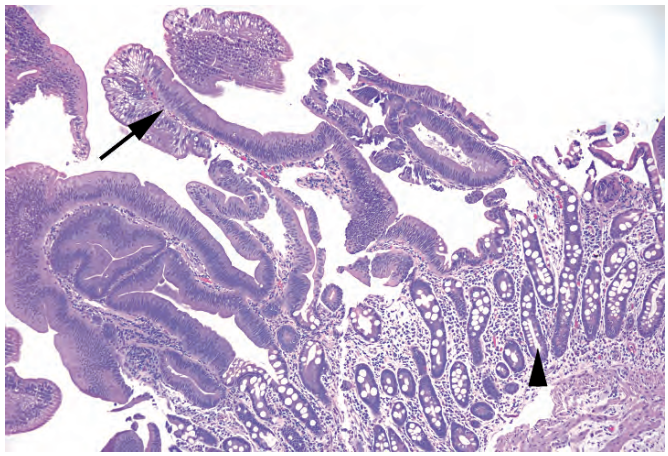

Figure 7.13. Duodenal adenoma. As in the colon, the tubular adenoma is characterized by low-grade dysplasia, showing crowded and elongated nuclei and loss of mucinous differentiation (arrow). Residual duodenal mucosa is seen underneath the adenoma (arrowhead).

8 Colon

Colon biopsies are most often performed for one of three reasons:

1. To evaluate a polyp or mass
2. To study a patient with inflammatory bowel disease and monitor dysplasia
3. To look for an explanation for diarrhea

The history is very important; you should not be diagnosing a tubular adenoma when the endoscopist did not see a polyp. You may also be missing a more ominous diagnosis (discussed later). Assuming that you have at least a succinct history or description from the endoscopist, therefore, your approach to the biopsy depends on what you are looking for.

Normal Histology

Normal colonic mucosa should have a flat surface and nicely parallel crypts, like "test tubes in a rack." The crypts are lined with goblet cells, endocrine cells, and precursor cells. Paneth cells, which are red granular cells with basal nuclei, are normal in the ascending and transverse colon but abnormal in the left colon. Immediately under the epithelium is the lamina propria, which is separated from the underlying submucosa by the muscularis mucosa (Figure 8.1). Normal constituents of the lamina propria include lymphocytes, plasma cells, and eosinophils. How many lymphocytes are too many? In general, they are assumed to be physiologic unless there is clinical or histologic evidence of chronic damage to the mucosa (see later discussion). Lymphoid aggregates are common and unremarkable.

Deep to the submucosa is the thick muscularis propria. Beyond this layer lies the serosal fat. In biopsy or polypectomy tissue, seeing adipose tissue below the muscularis propria is *not* normal and means the endoscopist may have taken a full-thickness specimen. In other words, perforated the colon. This should prompt an immediate courtesy call.

Looking for Polyps

Adenomas

An adenoma (at least in the tubular-to-villous family) is defined as a polyp with low-grade dysplasia. Low-grade dysplasia in the colon indicates a cytologic change and stands out from normal colon as looking blue on the slide. The cells lining the crypts and the surface become tall and dark (because of depleted mucin) and have cigar-shaped and/or pseudostratified hyperchromatic nuclei (Figure 8.2). Mitoses may be present but are generally not apical.

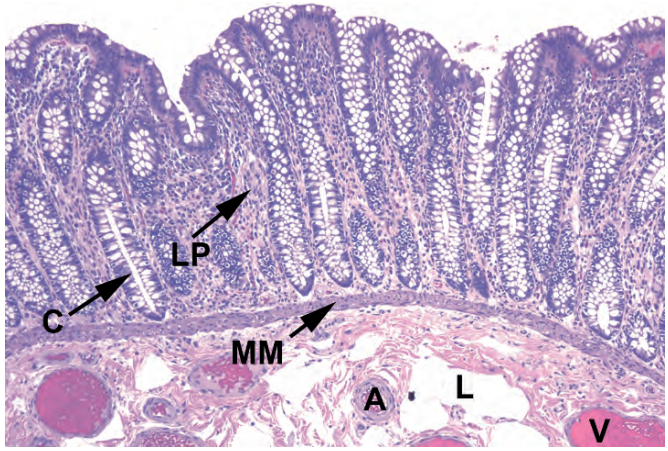

Figure 8.1. Normal colon. Section through colonic mucosa showing parallel crypts (C), lamina propria (LP), muscularis mucosa (MM), and submucosal arteries (A), veins (V), and lymphatics (L).

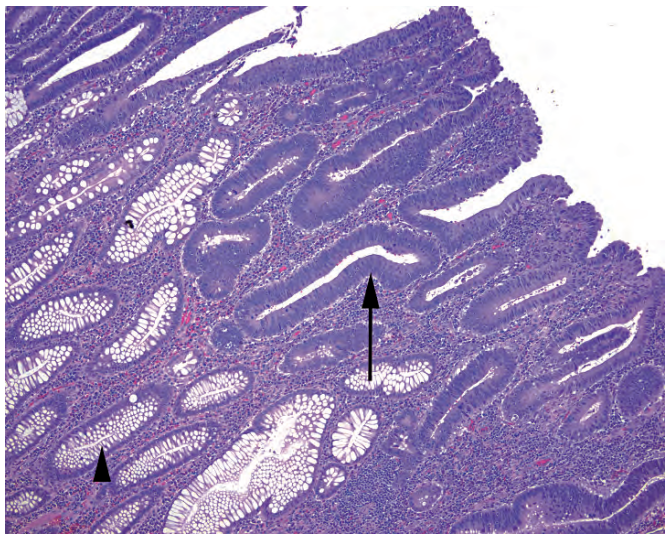

Figure 8.2. Tubular adenoma. This section shows an early tubular adenoma in which low-grade dysplasia is seen in the surface glands (arrow), while the deeper glands are uninvolved (arrowhead). The adenomatous epithelium is dark due to crowded and hyperchromatic nuclei and loss of mucinous goblet cells. Mitotic activity and neutrophils are common in adenomas but may also be seen in benign epithelium.

The dysplasia must extend all the way to the surface epithelium to qualify as an adenoma; if the epithelium shows signs of maturing as it ascends, it is more likely reactive changes.

Adenomas are subdivided by their architecture. The most common type is the *tubular adenoma*, which has a smooth surface and parallel crypts, similar to normal epithelium. A *villous adenoma* is covered in finger-like projections, whereas a tubulovillous adenoma has features of both.

Important considerations for sign out include the following:

- Margins: When an entire polyp is plucked off the colon, ideally it is cross sectioned so that you can see the stalk. Ink on the stalk is helpful, but cautery also identifies your margin. If there are identifiable margins, mention whether or not the dysplasia (adenomatous epithelium) extends to the margin.
- Dysplasia: By definition, low-grade dysplasia is present. However, *high-grade dysplasia* is equivalent to carcinoma in situ and must be noted. The diagnosis of high-grade dysplasia

is made on the basis of architecture rather than cytology. The glands should become cribriform, fused, or back to back (Figure 8.3). Usually the term *high-grade dysplasia* is reserved for areas that look so complex you are worried about carcinoma but cannot prove invasion. High-grade dysplasia is also usually accompanied by ugly cytology: total loss of nuclear polarity, significant pleomorphism, atypical mitoses, and large nucleoli.

- Carcinoma: All adenomas are considered at least premalignant lesions; sometimes you will find carcinoma arising in a polyp on biopsy. To diagnose carcinoma (as opposed to high-grade dysplasia), you must demonstrate cancer crossing the basal lamina, that is, into the lamina propria. Clues to invasion include a jagged interface with the lamina propria, individual infiltrating cells, desmoplastic response, and a pinking up of the invasive cells (Figure 8.4).
- Invasion: Invasion into the lamina propria alone is called *intramucosal carcinoma*. This may happen in a large polyp, and excision is still curative. Within the lamina propria, cancer has no metastatic potential. However, once malignant cells cross the muscularis mucosa into the

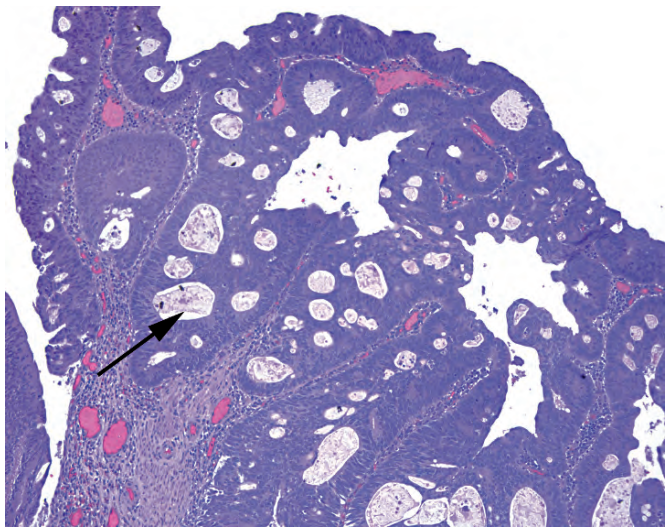

Figure 8.3. High-grade dysplasia in an adenoma. The diagnosis is largely based on architectural features, such as cribriform growth (arrow).

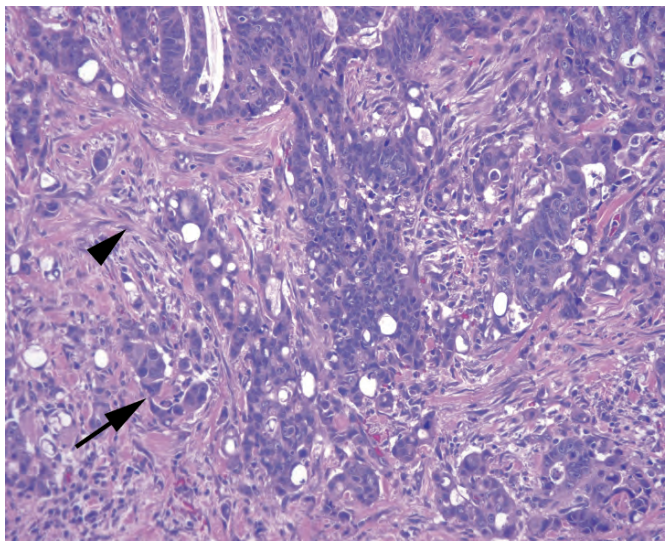

Figure 8.4. Invasive adenocarcinoma. Poorly formed glands and single cells (arrow) infiltrate through a desmoplastic stroma (arrowhead). Cells show marked pleomorphism and prominent nucleoli.

submucosa, there is at least theoretical risk of metastasis. The extent of invasion must be noted in the diagnosis.

Hyperplastic Polyps

Hyperplastic polyps are those in which the epithelial cells, although benign, begin to outgrow their available space. The glands have an increased number of goblet cells and therefore look pale or cleared out next to normal epithelium. Furthermore, because the surface area is outgrowing the lamina propria, hyperplastic polyps take on a distinctive frilly (as in a skirt) or lacy appearance (Figure 8.5). Crypts cut in cross section have a distinctive star-shaped lumen.

A polyp with adenomatous-looking cells at the base of the crypts, and frilly hyperplastic cells at the surface, is still a hyperplastic polyp. Remember that surface maturation is not consistent with a tubular adenoma. However, a true adenoma with a serrated surface profile may be a *serrated adenoma* (keep reading).

Serrated Polyps

Serrated is an adjective used to describe the frilly and undulating architecture seen in a classic hyperplastic polyp. Historically, a "serrated adenoma" was a polyp with low-grade dysplasia extending to the surface, as seen in tubular adenoma, but with serrated architecture.

Recently, large (>1 cm) hyperplastic polyps occurring in the right colon were recognized as a distinct subtype of polyp with malignant potential, associated with the microsatellite instability (as in hereditary nonpolyposis colorectal cancer [HNPCC]) cancer pathway. They are called either *sessile serrated adenomas* or *sessile serrated polyps*. The crypts have characteristic dilation and branching at the base ("duck feet"), and the epithelial cells may be more eosinophilic (less mucin) and pseudostratified than the usual hyperplastic polyp (Figure 8.6). However, mature goblet cells and the frilly surface are still evident.

The difference is in the depth of proliferation: hyperplastic polyps show mostly surface hyperplasia and expansion, whereas the sessile serrated group (remember that *sessile* means *flat*) is hyperplastic right down to the base. These are important to recognize, because they should be treated clinically like an adenoma, not just a hyperplastic polyp.

Inflammatory Pseudopolyps

Inflammatory pseudopolyps are polypoid structures that consist either of granulation tissue (when adjacent to an ulcer) or of inflamed lamina propria with distorted crypts. Given the inflammation, there can be severe reactive changes in the crypts (resembling dysplasia).

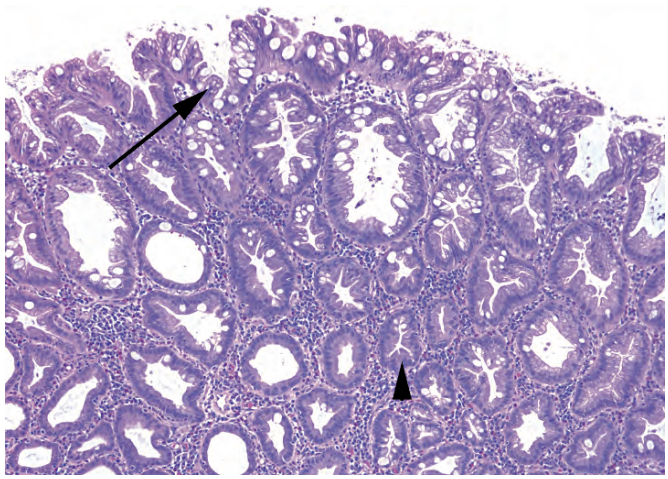

Figure 8.5. Hyperplastic polyp. The surface of the polyp shows a characteristic "frilly" appearance (arrow), with hyperplastic mucinous epithelium and prominent goblet cells. Deeper crypts (arrowhead) show star-shaped lumens.

However, surface maturation should be visible. These are common in inflammatory bowel disease. A polyp that looks like an inflammatory polyp but occurs without background inflammatory disease may be a *juvenile polyp*, a diagnosis that can be made in a patient of any age.

Prolapse-Type Lesions

Mucosal prolapse is like a tiny focus of intussusception; a protruding bulge of mucosa gets pulled, twisted, generally battered in the breeze, suffers ischemia and trauma, and begins to look fairly weird as it tries to repair itself. Features include extension of the muscularis mucosa into the lamina propria, as disorganized fibers; crypt distortion and dilation with diamond-shaped crypts; hemosiderin; and edema and inflammation with reactive atypia (Figure 8.7). This lesion may be called a *polypoid prolapsing mucosal fold* in the colon, *solitary rectal ulcer syndrome* in the rectum, or *inflammatory cloacogenic polyp* at the anorectal junction.

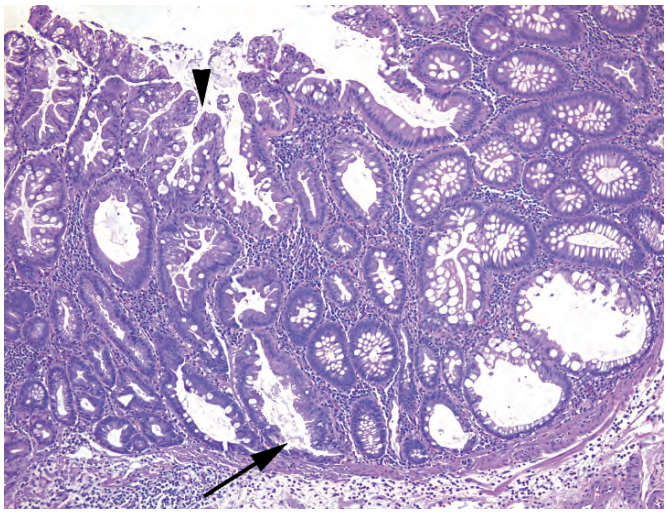

Figure 8.6. Sessile serrated adenoma. The surface looks similar to a hyperplastic polyp (arrowhead), but the base shows sideways branching of crypts (arrow) caused by proliferation at the base of the lesion.

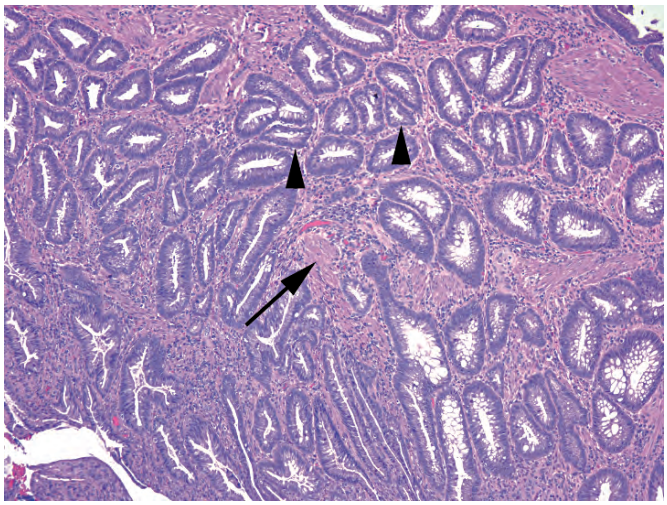

Figure 8.7. Prolapse lesion. The center of this prolapse-related polyp shows typical features, including angulated or diamond-shaped crypts (arrowheads) and smooth muscle growing between crypts (arrow).

Adenocarcinoma

Adenocarcinoma of the colon seems to follow principally two lines of tumorigenesis, highlighted by two familial cancer syndromes. Familial adenomatous polyposis (very rare) is a mutation in the *APC* gene, a tumor suppressor gene, such that the second allele is vulnerable to somatic mutations. Knockout of both genes leads to adenoma formation. These patients have thousands of tubular adenomas and inevitably progress to adenocarcinoma. *p53* is involved in this same pathway, but as a late event in the progression from adenoma to carcinoma. The resulting adenocarcinoma is of the garden variety, indistinguishable from sporadic adenocarcinoma. Approximately 85% of all colon cancers arise from this pathway (most of them sporadic, not familial, hits to these genes).

The second major pathway is seen in HNPCC patients (less rare), who have defective DNA mismatch repair genes (such as *hMLH1* and *hMSH2*). As above, these are tumor suppressor genes that are vulnerable to the "second hit" somatic mutation. The double hit leads to microsatellite instability. However, instead of tubular adenomas, these tumors tend to arise in the sessile serrated adenomas and mature into medullary, mucinous, or signet-ring cancers, usually right sided. Approximately 15% of all colon cancers fall along this pathway, most of them sporadic.

Medullary carcinoma of the colon is a distinct and rare variant with a dense associated lymphoid population (like medullary carcinoma of the breast) but a bland, almost neuroendocrine cytology (like medullary carcinoma of the thyroid), although there is probably no connection. It is worthy of mention in the colon, as medullary carcinoma in the right colon of a young person suggests HNPCC.

Carcinoid (Neuroendocrine) Tumors

The most common locations for gastrointestinal carcinoids are the appendix and small bowel, with rectum and colon further down the list. Often submucosal, carcinoid tumors may present as a mass on endoscopy or cause obstruction. The carcinoid syndrome (flushing, etc.) does not occur until and unless the tumor metastasizes to the liver. Carcinoid tumors are characterized by uniform neuroendocrine-type cytology and trabecular, spindly, or rosette-like architecture (Figure 8.8). As in other sites, the histologic features are not predictive of behavior, and a very bland-looking tumor may still metastasize. Neuroendocrine tumors are covered in more depth in Chapter 24.

Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is an idiopathic relapsing inflammatory disease affecting the colon and consists primarily of ulcerative colitis and Crohn's disease. A definitive diagnosis of IBD must include changes of chronicity. A simple acute colitis without chronic changes may be due to infection, ischemia, drugs, or bowel preparation, as well as IBD. There is a long list of descriptive diagnoses used for the colon, depending on whether there are acute or chronic changes, whether the changes are focal or diffuse, and whether the patient has a history of IBD. The changes should be pretty compelling to make a first-time diagnosis of IBD, as the patient will carry the label for life.

Features of activity (Figure 8.9) include the following:

- Polys, polys, spot the polys: neutrophils in the crypt epithelium = cryptitis.
- Neutrophils in the crypt lumen = crypt abscesses.
- Erosions and ulcers and pus are also consistent with active lesions.

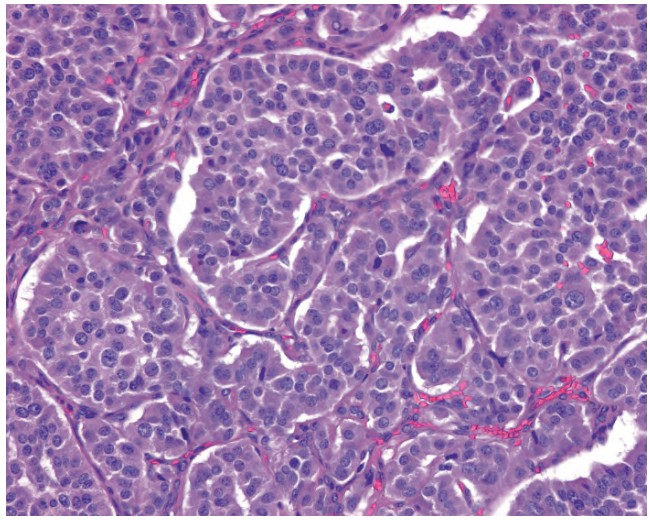

Figure 8.8. Carcinoid tumor. Nests and ribbons of cells separated by delicate fibrovascular septa are classic, as are the round and regular nuclei with finely speckled chromatin.

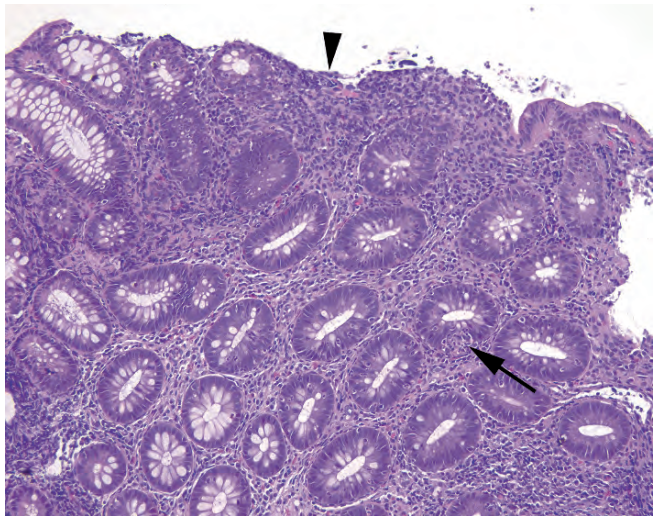

Figure 8.9. Active colitis. Neutrophils are seen in the epithelium of the crypts (arrow), and the surface is ulcerated (arrowhead).

Features of chronicity (Figure 8.10) include the following:

- Crypt distortion (branching, tortuous, or sideways crypts; test tubes warped)
- Crypt loss (test tubes missing)
- Crypt atrophy (test tubes too short)
- Basal plasmacytosis (test tubes pushed up by a dense layer of chronic inflammation)
- Paneth cell metaplasia (Paneth cells in the left colon)

Chronic inflammatory disease is usually qualified as either active (having neutrophils) or inactive. The differentiation between Crohn's disease and ulcerative colitis is difficult on biopsy; a more definitive diagnosis is made on colectomy (should it come to that). However, there are features that suggest one or the other. Remember that you must first see chronic changes to consider making the diagnosis of IBD (at least at the time of initial diagnosis).

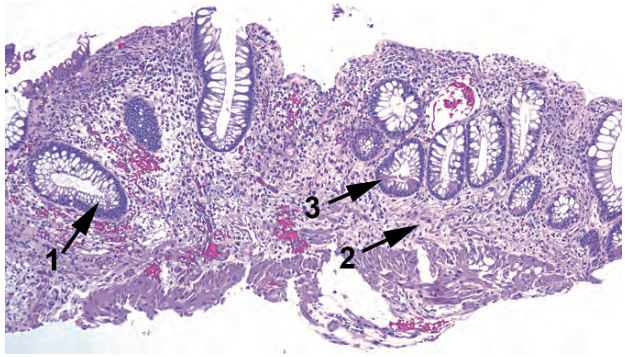

Figure 8.10. Chronic inflammatory disease. This biopsy specimen shows a loss of crypt density (atrophy); crypt distortion (1); elevation of crypts off of the muscularis mucosa (2), often accompanied by a dense basal lymphocytic infiltrate (not seen here); and Paneth cell metaplasia (3).

Features of Crohn's Disease

- Patchy mucosal involvement with skip areas
- Granulomas and/or histiocytes (Figure 8.11)
- On colectomy, transmural inflammation, cobblestoning, fissures, fistulas, and creeping fat

Features of Ulcerative Colitis

- Predominantly distal involvement or pancolonic (no skip areas)
- Diffuse mucosal inflammation, many polys

Note, however, that once a patient has begun treatment ulcerative colitis may appear patchy in activity.

Dysplasia in Inflammatory Bowel Disease

Once diagnosed, IBD must be followed both to monitor activity (response to therapy) and to look for dysplasia. The constant inflammation puts the patient at high risk for developing cancer. Therefore, the presence or absence of dysplasia should be noted in every IBD surveillance biopsy specimen.

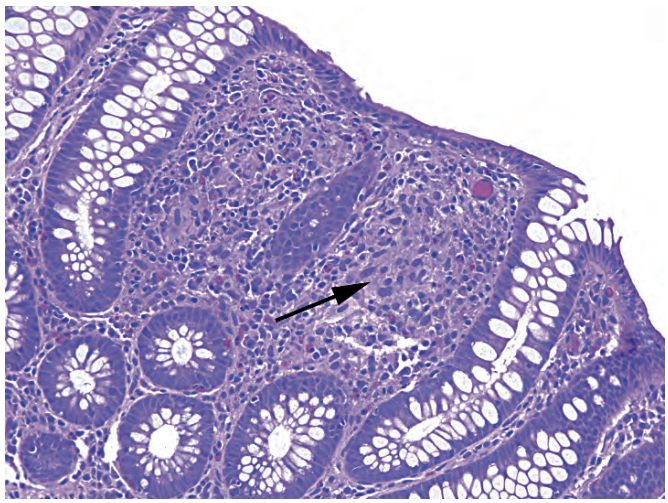

Figure 8.11. Granuloma in Crohn's disease. The granulomas in Crohn's are small and subtle. This image, taken at 40×, shows a small collection of histiocytes (arrow) between crypts.

The tricky part is that often areas of dysplasia may arise in a polypoid irregularity or fold, and it is difficult to tell a *dysplasia-associated lesion or mass* (DALM) from a sporadic and unrelated tubular adenoma. Both show the typical adenomatous epithelium. Does it matter? In fact, the DALM may be at higher risk of transforming to cancer and is usually p53 positive on immunostain (unlike an adenoma). It is managed more aggressively than a sporadic adenoma.

Flat dysplasia (not associated with a mass) can be a subtle and subjective call. Intense inflammation causes reactive changes that can look much like dysplasia. However, remember that true dysplasia does not mature at the surface. Also, be very wary of calling dysplasia in a sea of neutrophils.

Microscopic Colidities

Microscopic colidities are defined by having no abnormal endoscopic findings; they are only diagnosable under the microscope. The symptoms include chronic watery diarrhea, abdominal pain, fatigue, and weight loss. The two types are *lymphocytic colitis* and *collagenous colitis*. Both are characterized by the following:

- A lack of chronic changes (no crypt distortion, no basal plasmacytosis)
- A predominantly top-heavy lymphocytic infiltrate
- Intraepithelial lymphocytes
- Evidence of damage to the epithelium (loss of cells)

Collagenous colitis affects predominantly women and has a distinct thickened collagen band along the basement membrane. This band must be irregular and blurred into the lamina propria, not just thick. A trichrome stain confirms the diagnosis (Figure 8.12).

In the setting of a dense lymphoplasmacytic infiltrate, pay attention to whether the blueness is top heavy (more prominent under the surface) or bottom-heavy (more prominent at the base of

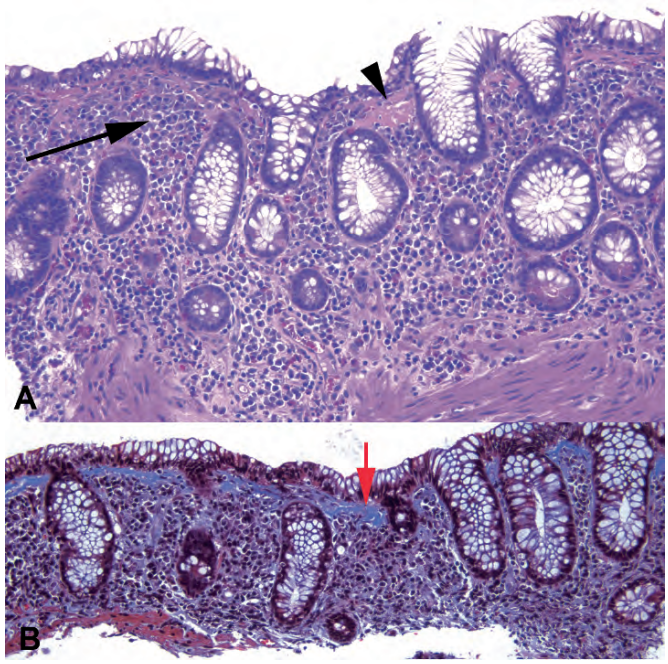

Figure 8.12. Collagenous colitis. **(A)** The hematoxylin and eosin stain shows a top-heavy lymphoplasmacytic infiltrate (arrow) accompanied by a dense pink material just under the surface epithelium (arrowhead). **(B)** A trichrome stain confirms the thickened collagen table (blue on this stain, red arrow), which has an irregular border and entraps nuclei within the lamina propria.

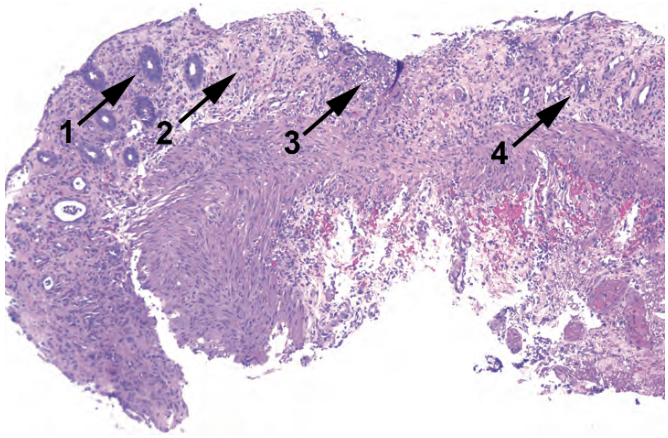

Figure 8.13. Ischemic colitis. Features include small dark regenerative crypts (1), hyalinization and fibrosis of the lamina propria (2), ulceration (3), and crypt dropout (4).

the crypts). Inflammatory bowel disease tends to be bottom-heavy and the microscopic colidities more top-heavy. This is a soft feature rather than a rule.

Other Colidities

Ischemic colitis may have many appearances, from focal active colitis to diffuse pseudomembranous colitis. Acute or transient ischemia appears as damage to the superficial surface of the mucosa, with hemorrhage and coagulative necrosis. Prolonged ischemia causes fibrosis of the lamina propria and a top-down atrophy of the crypts: they appear collapsed at the surface and regenerative at the base (Figure 8.13).

Infectious colitis is not often biopsied because of its usually self-limited course. It may range from no pathologic findings to a severe active colitis.

Diversion colitis is a special entity associated with a Hartmann pouch, which is a blind-ended rectal cavity disconnected from the fecal stream. The loss of normal colonizing flora causes a nonspecific colitis, which may be mistaken for inflammatory bowel disease.

Both drugs and the bowel preparation process may cause transient acute colitis. Acute inflammation in the absence of chronic changes is non-speficic, and should not be overinterpreted.

9 Liver

Liver biopsies are usually needle core biopsies. The most common reasons for a biopsy include monitoring disease progression in hepatitis, evaluating a transplanted patient for rejection or graft-versus-host disease (GVHD), and occasionally ruling out an additional disease process. Liver biopsies may also be done to diagnose a radiographic mass. At many institutions, all liver biopsy specimens are stained with trichrome (to evaluate fibrosis) or with an iron stain (to reveal abnormal iron in the tissue).

Anatomy

Blood comes in via the portal vein (from the gut) and the hepatic artery (from the aorta). These vessels ramify into, eventually, the arteries and veins within the portal tracts. Blood gets from the terminal portal vessels to the outgoing central veins via the sinusoids—the long channels lined by hepatocytes. Once in the central vein, blood exits the liver via the left and right hepatic veins, which join the inferior vena cava (joining blood from the lower extremities).

Bile is created by the hepatocytes and exits into the bile canaliculi, which eventually coalesce into ductules and ducts in the portal tracts. These exit the liver via the hepatic ducts, which join the cystic duct (from the gallbladder) to enter the duodenum as the distal common bile duct.

Normal Histology

The liver is composed of three main components – the *hepatocytes*, the *biliary system*, and the *vessels*. Hepatocytes are large pink polygonal cells with dense round nuclei. Nucleoli, and occasional binucleate cells, are okay. The hepatocytes are organized into plates that are one hepatocyte thick and lined by reticulin. Between these plates are the sinusoids for blood. Running perpendicular to the sinusoids, and essentially invisible to light microscopy, are the *bile canaliculi*: tiny intercellular channels between the hepatocytes.

Bile from the canaliculi makes its way to the bile ducts. The bile ducts are tubular structures with a low cuboidal epithelium (Figure 9.1). They are found in the *portal tracts*, which also contain branches of the hepatic artery and portal vein. These three components are also called the *portal triad*. Blood in both vessels is flowing *into* the liver; bile is flowing *out*. The portal tract also contains a small amount of connective tissue, which makes it stand out on a trichrome stain. The hepatocytes immediately surrounding the portal tract are called the *limiting plate*. The portal tract is usually the hotspot for inflammatory processes in the liver and so is important to identify on biopsy.

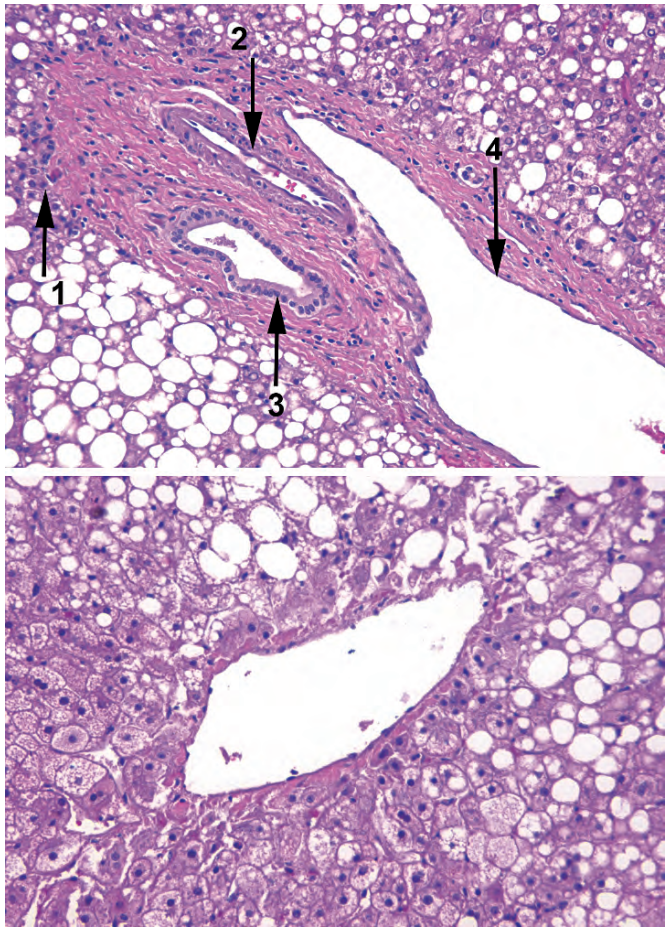

Figure 9.1. Portal tract and central vein. The upper panel shows a typical portal tract surrounded by the limiting plate of hepatocytes (1) and containing a branch of the hepatic artery (2), bile ductule (3), and portal vein (4). The lower panel shows a central vein from the same liver. Both panels show extensive steatosis.

The third vessel in the liver unit is the *central vein* or *terminal venule*. This is a thin-walled vessel surrounded by hepatocytes and nothing else (see Figure 9.1). It contains blood on its way out of the liver.

The lobule is an architectural unit with the central vein as its center and portal tracts at the periphery. *Centrilobular* refers to a process involving the central vein. This is the most easily visualized anatomic unit (Figure 9.2).

The acinus is an architectural unit with the portal tract at the base (as the source of blood flow) and the central vein at the tip. In this model, the area closest to the source of blood and oxygen is zone 1, and the most peripheral cells are zone 3. Ischemia and toxic insults affect the zones differentially. This is more of a physiologic unit and the one used when describing liver findings.

Nonneoplastic or Inflammatory Disease Categories

It is helpful to think of the different liver compartments separately, because histologic findings can often be grouped as well:

- Diseases of hepatocytes: the viral hepatitides, autoimmune hepatitis, steatohepatitis and alcoholic disease, and drug toxicity

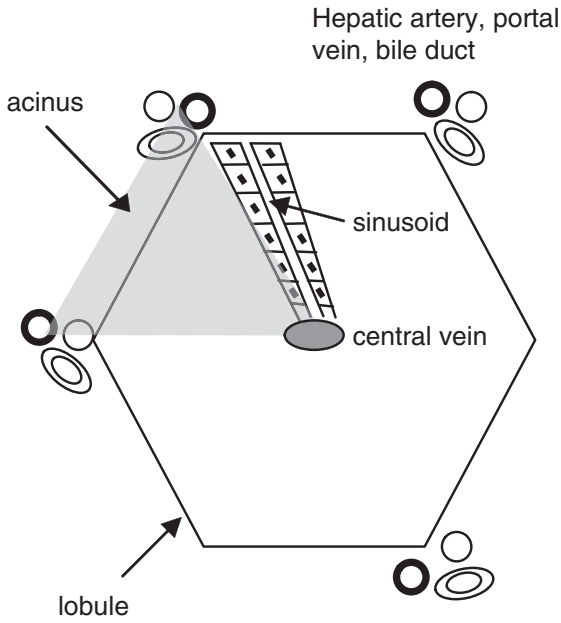

FIGURE 9.2. Liver organization. The acinus is a triangular, physiologic unit, while the lobule is a hexagonal anatomically based unit.

- Diseases of the biliary system: autoimmune biliary diseases (primary sclerosing cholangitis and primary biliary cirrhosis), obstruction, atresia, transplant rejection, GVHD, and drugs
- Diseases of the vasculature: transplant rejection, GVHD, and systemic vasculitides

The portal tract represents a collision of all three compartments. Therefore, inflammation of the portal tract is found in all of these diseases.

Pathologic Findings

The liver has only so many ways to respond to an insult or injury. An acute injury in the liver looks similar to that in any other organ: widespread edema, acute and chronic inflammation, and/or necrosis. Subacute or chronic injury generally has mainly mononuclear inflammatory cells as well as individual cell necrosis or degeneration. The final result of chronic injury from many causes is cirrhosis, or end-stage liver disease. Therefore, many diseases in the liver have histologic overlap, and, in the case of cirrhosis, often you cannot tell what the original disease process was. For this reason, the most important skill in interpreting the liver biopsy is recognizing injury to the different compartments. Attaching a diagnosis to this collection of findings requires clinical information. The most common patterns of injury are the following:

- Hepatocellular compartment
  - Portal inflammation: Inflammatory cells are present within the portal tract. In chronic hepatitis and autoimmune disorders, the infiltrate is predominantly mononuclear.
  - Interface activity (periportal hepatitis, piecemeal necrosis): Inflammation, usually lymphocytic, occurs in the limiting plate. This looks like portal inflammation spilling out into the hepatocytes (Figure 9.3). Note that the word *activity* when describing something in the liver does not mean *neutrophils*.
  - Lobular inflammation: Inflammation, usually chronic, and/or necrosis of the hepatocytes are at a distance from the portal tracts. Also called *spotty necrosis*, this appears as little clusters of lymphocytes destroying individual hepatocytes out in the lobules. Do not count lymphocytes in the sinuses, which are physiologic.

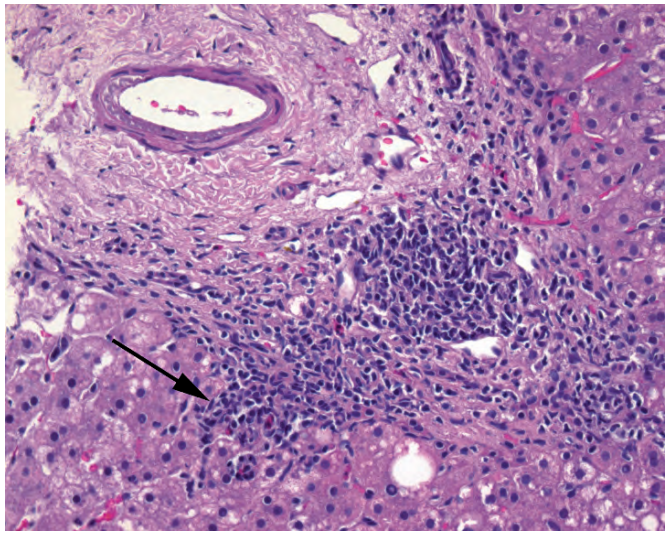

Figure 9.3. Portal inflammation. This is an example of chronic viral hepatitis. Lymphocytes in the portal tract spill out into the limiting plate of surrounding hepatocytes (arrow).

- Vacuolar degeneration (balloon cell change): This is one way in which hepatocytes can die. The cytoplasm swells and becomes feathery and pale.
- Acidophilic bodies: This is another way in which hepatocytes die. These cells are similar to dyskeratotic cells in the skin; they are bright pink, rounded up, with pyknotic nuclei.
- Fibrosis: *Fibrosis* is a general term indicating too much collagen. Fibrosis begins as an increase in collagen around the portal tract (portal fibrosis), and eventually spreads to connect adjacent portal tracts or central veins by thin webs of collagen (bridging fibrosis). The end stage of the process is cirrhosis, which is the division of the liver into individual nodules separated by thick bands of fibrosis (Figure 9.4).
- Steatosis: *Steatosis* literally means fat in the hepatocytes. Steatosis can be physiologic in small amounts (<5%), but 30%–60% involvement is considered moderate steatosis. Over 60% involvement is marked or severe disease. Macrovesicular steatosis means large single vacuoles in each hepatocyte and is typical of fatty liver, alcoholic disease, and non-alcoholic steatohepatitis. Pure microvesicular steatosis looks like foamy cytoplasm and is characteristic of mitochondrial injury such as in Reye's syndrome.
- Steatohepatitis: Steatohepatitis is steatosis plus inflammation or injury. Neutrophils are not necessary for the diagnosis, but some evidence of hepatocyte injury *is* (Figure 9.5). This includes lobular inflammation, hepatocyte necrosis, pericellular fibrosis, balloon cells, and Mallory's hyaline (see below).
- Mallory's hyaline (Mallory bodies): Mallory bodies are irregular worm-like pink blobs of condensed cytoskeleton in the cytoplasm, especially within balloon cells (Figure 9.6). They are associated with steatohepatitis, especially alcoholic disease.
- Megamitochondria: Megamitochondria are markedly enlarged mitochondria, which look like red blood cells entrapped in the hepatocyte cytoplasm.
- Iron accumulation: Abnormal levels of iron are detected with either hematoxylin and eosin or iron stain. If severe, iron accumulation may indicate hemochromatosis or be secondary to other hepatocellular processes.

- Biliary compartment
  - Cholestasis: Cholestasis is the backup of bile in the liver. This may be caused by extrahepatic obstruction to flow, intrahepatic biliary disease, or impaired excretion by the hepatocytes themselves.

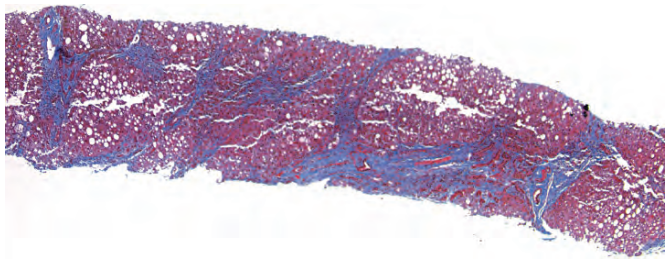

Figure 9.4. Cirrhosis in a biopsy specimen. In this trichrome stain, collagen is blue, while hepatic parenchyma is red. Collagen can be seen outlining the lobules of the liver, bridging the portal tracts and creating a nodular pattern.

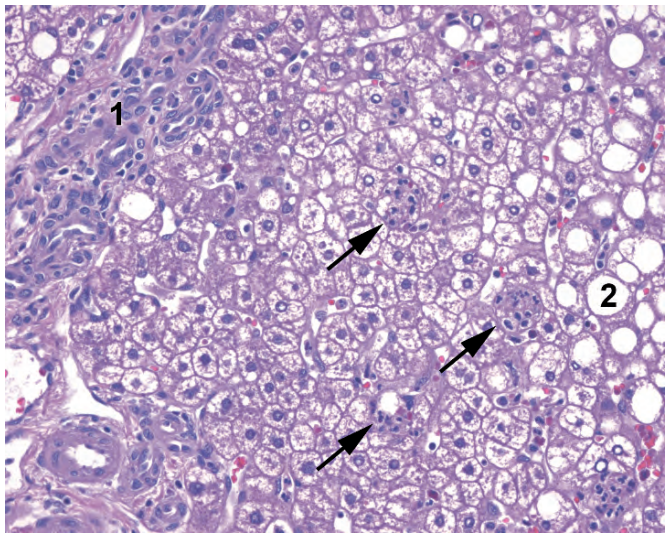

Figure 9.5. Steatohepatitis. An adjacent portal tract (1) shows minimal inflammation. In the lobule, there is macrovesicular steatosis (2) and collections of neutrophils attacking individual hepatocytes (arrows).

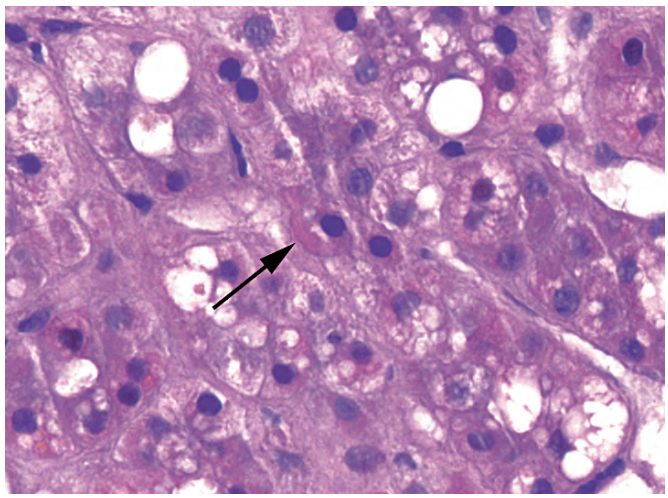

Figure 9.6. Mallory's hyaline. In the background of steatosis and inflammation, a pink refractile worm-like structure in the hepatocyte (arrow) is evidence of cytoskeletal collapse.

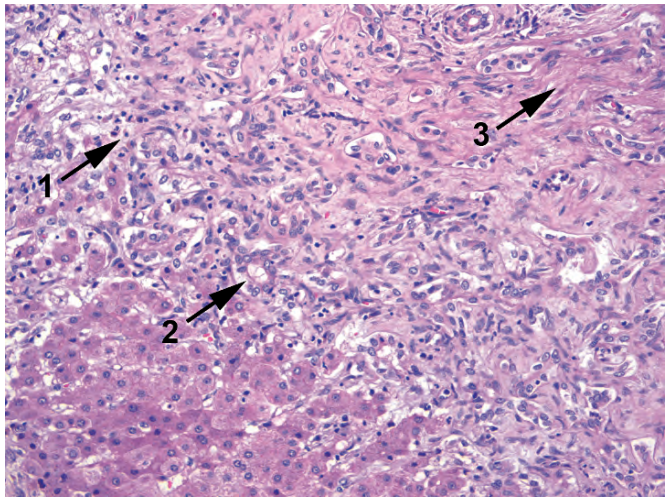

Figure 9.7. Bile stasis. In this example of congenital biliary atresia, the downstream obstruction to flow creates the triad of acute inflammation (1), a proliferation of poorly formed bile ductules (2), and the accumulation of golden globs of bile (not seen here). This will progress to fibrosis (3) and eventually loss of ductules.

- Bile duct proliferation: An increase in the number of bile duct profiles occurs in each portal tract; on average, there should be one to two per tract. Many of the new ductules are small, peripheral, and poorly formed. Bile duct proliferation occurs as a response to obstruction to flow. Other findings in obstruction include visible bile in hepatocytes or canaliculi, edema and inflammation (especially acute) in the portal tracts, eventually ductular atrophy, and finally widespread fibrosis (Figure 9.7).
- Bile duct injury: Bile duct injury is identified by lymphocytes in the bile duct epithelium and vacuolar degeneration or dropout of the epithelial cells. The end stage is ductopenia. Injury to the bile ducts can indicate a biliary disease, such as autoimmune (primary biliary cirrhosis) or rejection. Bile duct injury is usually patchy, so multiple portal tracts must be examined.
- Ductopenia: Ductopenia is loss of bile ducts, an indicator of chronic damage to the biliary system. Recognizing ductopenia, a diagnosis of absence, requires a conscious effort to look for bile ducts. Finding a bile duct in less than 80% of the portal tracts is abnormal.

- Vascular compartment
  - Venulitis (endothelitis): Venulitis is damage to the endothelium of the portal or central veins by inflammatory cells. It is usually an indication of rejection or GVHD.
  - Extramedullary hematopoiesis: Hematopoietic precursors (megakaryocytes are the most distinctive) are present in the liver sinuses. It is generally an indication of bone marrow disease (but is physiologic in fetuses and infants).

Chronic Hepatitis

Biopsies in chronic hepatitis, generally hepatitis C, are done to track disease progression, with the ultimate endpoint being cirrhosis. Sign out of a hepatitis biopsy specimen should include three key prognostic factors: *etiology* (if known), *grade* (degree of inflammation and necrosis), and *stage* (degree of fibrosis), plus any other disease process present (such as steatohepatitis).

There are many different scoring systems used to quantify grade and stage, as all clinicians love a number. However, most numeric scoring systems can be translated to or from adjectives, which convey the same information (for example, scores 0 to 4 corresponding to none,

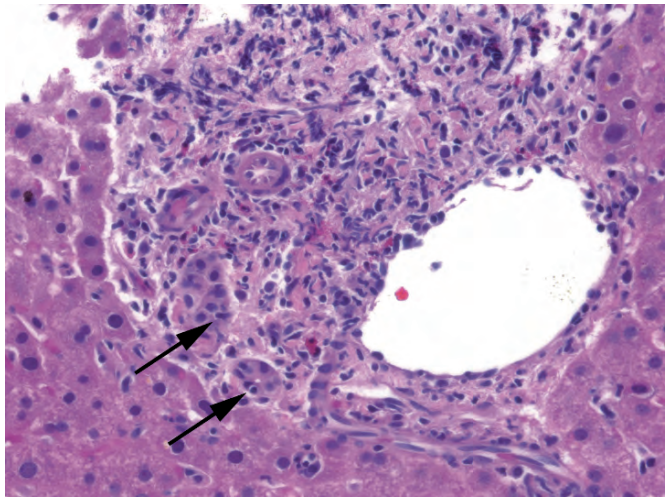

Figure 9.8. Acute rejection. Acute rejection refers to the attack on the bile ducts and venules by lymphocytes, which are seen invading the duct epithelium (arrows).

minimal, mild, moderate, or severe inflammation; and none, portal, periportal, bridging, or cirrhotic fibrosis). Calibrating these levels takes some experience, and thresholds may vary by institution.

The changes in viral hepatitis are nonspecific. The differential includes most other hepatocellular processes.

Transplant Biopsy for Rejection or Graft-Versus-Host Disease

The changes seen in cellular rejection and GVHD are histologically similar; one occurs in the setting of a liver transplant and the other in a bone marrow transplant. Both are divided into acute and chronic. (Hyperacute rejection implies an antibody response and is rare and immediate, not usually diagnosed by biopsy.)

- Acute cellular rejection: Acute rejection usually occurs 5–30 days after transplant, but can be longer. Changes include the following:
  - Mixed portal tract inflammation, including lymphocytes, neutrophils, and eosinophils
  - Venulitis
  - Bile duct inflammation and damage (Figure 9.8)
- Chronic rejection: Chronic rejection usually occurs after more than 1 year. Changes are primarily those of ductopenia and fibrosis.

Note that the changes of rejection must be differentiated from recurrent hepatitis C, an inevitable occurrence in patients who lost their first liver to hepatitis C (occurs from 3 to 9 months after transplantation).

Primary Biliary Cirrhosis and Primary Sclerosing Cholangitis

Primary biliary cirrhosis and primary sclerosing cholangitis are hard to keep straight. The bullet version is as follows:

- Primary biliary cirrhosis (occurs much more often in women than in men):
  - Primary biliary cirrhosis is a chronic destructive *intrahepatic* cholangitis (inflammation of the intrahepatic bile ducts).
  - Cirrhosis is an end-stage feature.
  - It is associated with antimitochondrial antibody.

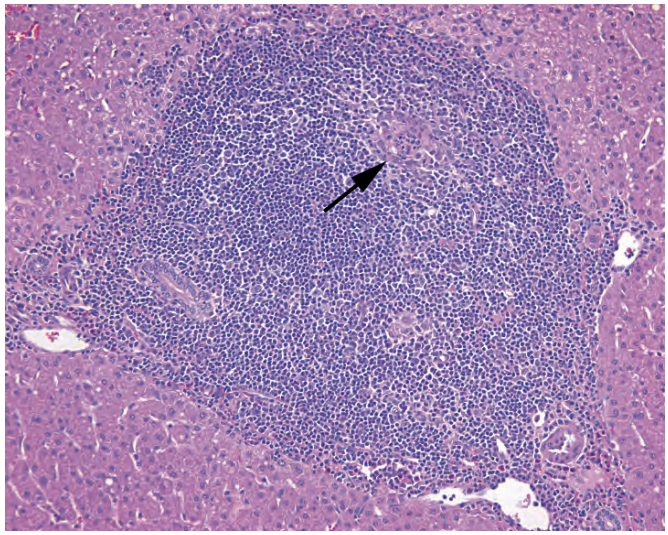

Figure 9.9. Primary biliary cirrhosis. There is a granulomatous inflammation of the portal tract, with destruction of a bile ductule (arrow).

- Findings are nonspecific and patchy but include inflammation and injury to the bile ducts, especially granulomatous, followed by proliferation and cholestasis, then eventually ductopenia and cirrhosis (Figure 9.9).
- The etiology is direct damage to bile duct epithelium.

- Primary sclerosing cholangitis (occurs more often in men than in women):
  - Primary sclerosing cholangitis is an inflammatory disease of the *extrahepatic* (and large intrahepatic) ducts.
  - It leads to patchy stricturing lesions, visible on cholangiogram.
  - It is associated with inflammatory bowel disease and p-ANCA.
  - The histologic picture is also nonspecific but dominated by ductular proliferation and cholestasis.
  - The etiology is unknown but may be a fibrotic process of the connective tissue surrounding the bile ducts, causing secondary stricture and damage.

Mass Lesions (Neoplasms)

The most common cause of mass lesions in the liver is metastatic disease. However, there are primary lesions of all three components of the liver: hepatocytes, biliary epithelium, and vessels. Within each category, it can be difficult to differentiate neoplastic from nonneoplastic, and benign from malignant, on resection, let alone on biopsy. However, here is a brief list of features that favor one over the other.

- Hepatocellular
  - Focal nodular hyperplasia: Focal nodular hyperplasia is essentially an island of cirrhosis occurring in the background of a noncirrhotic liver. This is not a clonal process, so there is more than one cell type; in addition to hepatocytes are bile ducts and fibrous septae. There is no capsule but sometimes a central scar. The lesion is composed of nodules divided by bands of fibrosis and thick vessels.
  - Adenoma: Adenomas are benign clonal neoplasms. They occur mainly in noncirrhotic livers of adult women taking oral contraceptive pills. Adenomas are circumscribed, partially encapsulated masses of uniform, bland-looking hepatocytes with no central veins or

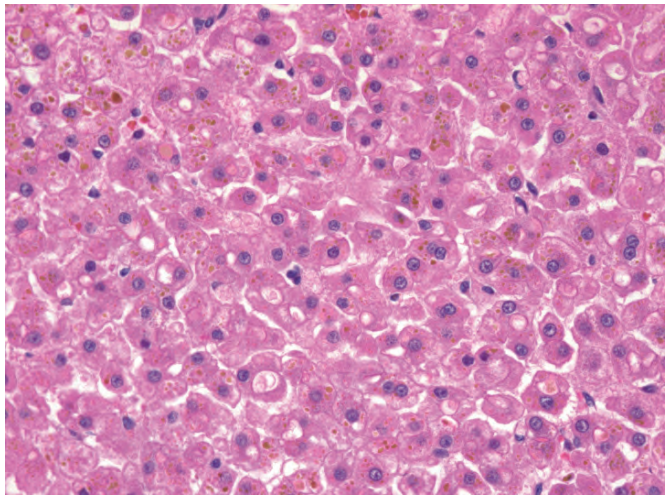

Figure 9.10. Well-differentiated hepatocellular carcinoma. Golden bile can be seen in the tumor cells, which are hard to differentiate from normal liver. Portal tracts are absent.

bile ducts (although there are diffuse prominent arterioles). The cells may be pale due to steatosis or glycogen or discolored with bile (which has no place to go). When visualized with reticulin stain, the hepatocyte plates are still only one to two cells thick (every cell touches reticulin).

- Well-differentiated hepatocellular carcinoma: Well-differentiated hepatocellular carcinoma (HCC) can be very difficult to distinguish from an adenoma histologically. However, HCC generally occurs in the setting of cirrhosis, unlike the adenoma. As with an adenoma, there are no bile ducts or central veins, and you may see intracellular bile (Figure 9.10). Nuclei may be large, hyperchromatic, and irregular. A reticulin stain shows a breakdown in architecture, and plates may be three or more cells in thickness.
- Poorly differentiated hepatocellular carcinoma: Poorly differentiated HCC can be very pleomorphic and hard to identify as hepatic. The presence of bile, if any, is still a give away.
- Fibrolamellar hepatocellular carcinoma: Fibrolamellar HCC is a variant of well-differentiated HCC occurring in children and young adults. It is typified by oncocytic cells with prominent nucleoli in a dense fibrotic stroma.

- Biliary
  - Bile duct adenoma: A bile duct adenoma is usually <1 cm and subcapsular (often sampled on frozen section), with a tangle of small simple tubules, with or without inflammation and fibrosis. It may produce mucin but not bile. Think of this as a benign biliary epithelial neoplasm (Figure 9.11).
  - Bile duct hamartoma: Also called von Meyenburg complex, a bile duct hamartoma is also usually < 1 cm and subcapsular (often sampled on frozen section). However, it generally shows more dilated and angular tubules in a loose connective tissue stroma and often produces bile. Think of this as a disordered reduplication of the portal tract. The consequences of confusing the hamartoma with the adenoma are minimal.
  - Cholangiocarcinoma: Cholangiocarcinoma is a primary malignancy of the bile ducts that appears as a nondescript adenocarcinoma infiltrating the liver. There is no definitive way to distinguish it from a metastatic lesion except by history. Although bile is *not* present in a cholangiocarcinoma, mucin is common, as is an intense desmoplastic response (Figure 9.12).

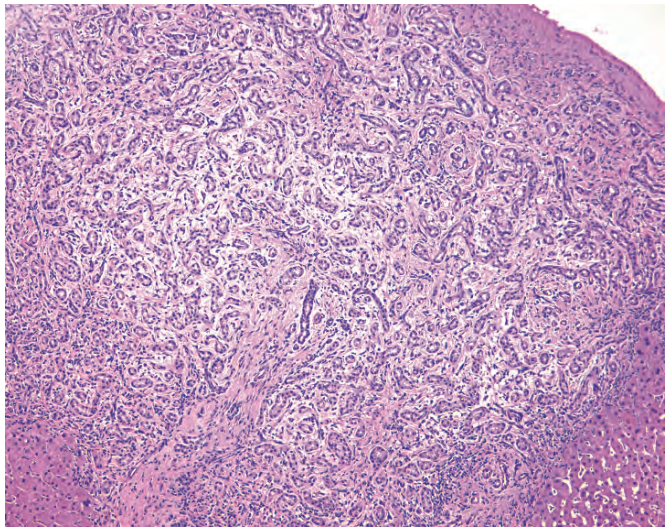

Figure 9.11. Bile duct adenoma. This is a benign tangle of proliferating bile ducts, surrounded by edema, which may mimic desmoplasia. Bile is absent, as is any cytologic atypia.

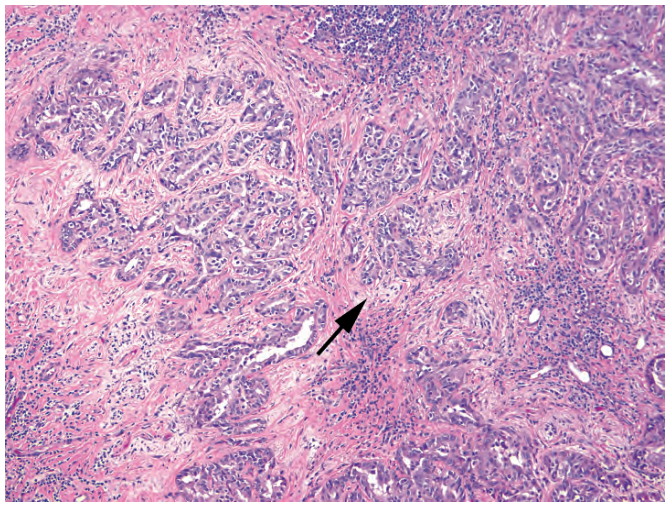

Figure 9.12. Cholangiocarcinoma. A nondescript adenocarcinoma, cholangiocarcinoma produces an intense desmoplastic response in the stroma (arrow; the pale swirling fibrosis surrounding the malignant glands).

- Vascular lesions
  - Cavernous hemangiomas are benign vascular lesions.
  - Epithelioid hemangioendotheliomas have a low malignant potential.
  - Angiosarcomas are malignant.

10 Pancreas

The Whipple Procedure

The Whipple procedure is, at minimum, a pancreaticoduodenectomy, which may or may not also include pylorus of the stomach and the gallbladder. In the pylorus-preserving Whipple procedure, the simplest version, you receive the segment of duodenum from just past the pylorus to about 20 cm beyond the ampulla of Vater. The head of the pancreas is nestled in the curve of the duodenum near the ampulla; the pancreas is shaped like a J, and the head is the base of the J, with the uncinate process as the hook. The distal common bile duct runs through the pancreas and enters the ampulla, where it is joined by the main pancreatic duct (Figure 10.1). Usually it is only the head of pancreas that comes out; if the tail is also involved, you may get the total pancreas and spleen.

There are five principal margins that are usually sampled on frozen section (see Figure 10.1). The first is the pancreatic margin, or the *pancreatic neck* (where the J is transected). This is usually taken as a shave margin, sampling the entire cross section of pancreas, and cancer anywhere on the slide is a positive margin. There is no neck margin on a total pancreatectomy.

The second margin is the *common bile duct margin*, which is a shave of the bile duct stump. This ensures that cancer is not tracking up the bile duct toward the liver.

The third is the *uncinate margin*. This is the tip of the short end of the J, and it represents the place where the pancreas sits against the major vessels. For the uncinate, you should take one representative perpendicular margin, and the edge of the tissue is inked. Cancer on the slide is okay, as long as it does not touch ink. As this tissue abuts major vessels, the surgeon often cannot resect additional tissue anyway.

The fourth and fifth margins are the *proximal* and *distal duodenal margins*. It is rare for these sections to contain tumor.

Most Whipple procedures are performed for a pancreatic mass seen radiologically. Although it is possible to get a cancer diagnosis by fine-needle aspiration, this is not always performed, and false-negative results are not uncommon. Therefore, often our first look at the tumor is during the Whipple procedure.

The Normal Pancreas

The normal pancreas is a large mixed exocrine and endocrine gland, with acinar cells arranged around ducts in lobular units. The acinar cells secrete digestive enzymes in precursor forms, which travel to the duodenum via the ducts. Normal ducts are low cuboidal epithelium, and the acinar cells are wedge-shaped granular pink and purple cells (Figure 10.2). Scattered among

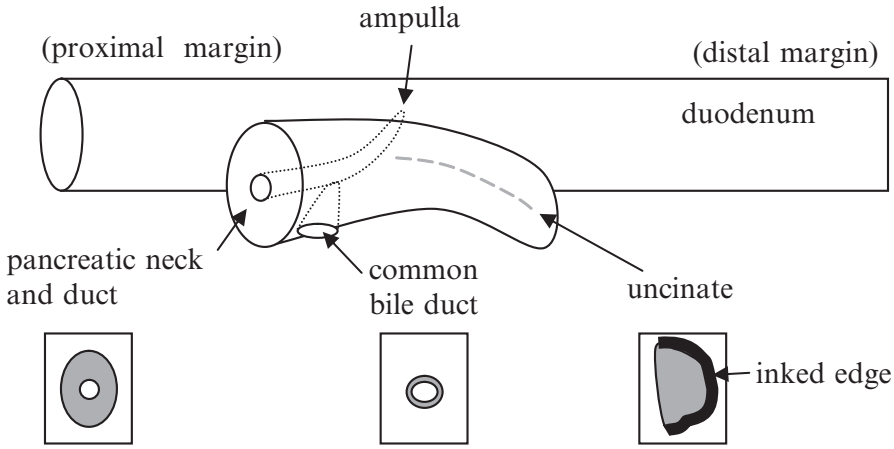

Figure 10.1. Diagram of specimen obtain during a Whipple procedure. The head of the pancreas comes out attached to a segment of duodenum. The main pancreatic duct is visible at the pancreatic neck margin (a surgical margin). The common bile duct enters the pancreas to join the pancreatic duct (also a surgical margin). The uncinate process is the tip of the pancreas, and its edge abuts major vessels (a soft tissue margin).

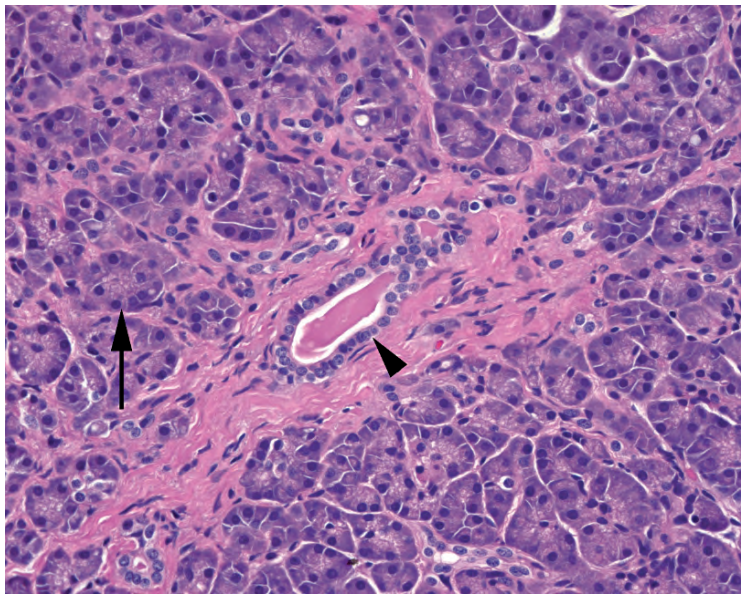

Figure 10.2. Normal pancreatic acinus. The duct is seen at the center (arrowhead), with surrounding acini of secretory cells (arrow).

them are the neuroendocrine islets of Langerhans, which show typical neuroendocrine cytology and are arranged in little nests.

Chronic Pancreatitis Versus Ductal Adenocarcinoma

Chronic pancreatitis is not an uncommon finding in a resected pancreas. The damage done to the pancreas by chronic obstruction, as with a mass, causes diffuse fibrosis, atrophy of the acinar units, reactive changes, and disruption of the normal architecture, all of which can mimic carcinoma. One of the hardest tasks (especially on frozen section) is differentiating reactive

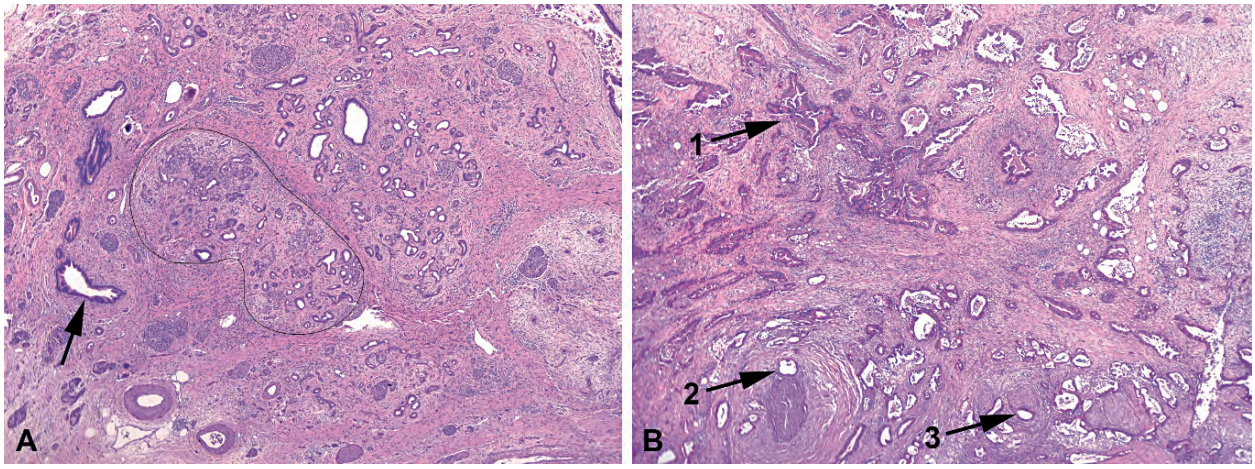

Figure 10.3. Chronic pancreatitis versus cancer, low power. **(A)** In chronic pancreatitis, the large ducts may show marked reactive changes, appearing blue and prominent, but they should still be located between lobules of acini (arrow). The acini show marked atrophy and fibrosis such that only the small ducts remain and appear infiltrative; however, the lobules retain a circumscribed outline (black line). **(B)** In adenocarcinoma, large, prominent, irregularly shaped ducts are scattered throughout, without respect to normal architecture (1). Large ducts next to vessels (2) or nerves (3) are diagnostic of cancer.

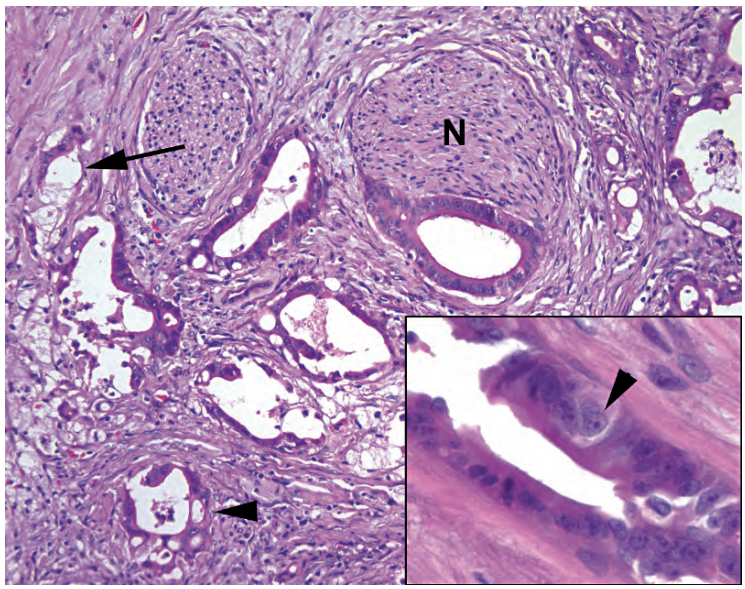

Figure 10.4. Adenocarcinoma. On high power, the infiltrative glands show incomplete lumens (arrow), cribriform growth pattern (arrowhead), and perineural invasion (N). **Inset**: Marked variation in nuclear size is diagnostic of cancer. Note the large nucleus with prominent nucleolus (arrowhead) across the gland from nuclei less than one fourth of its size.

pancreatic ducts from well-differentiated infiltrating adenocarcinoma, the most common pancreatic malignancy. Some tips include the following:

- Helpful but subjective
  - On low power, chronic pancreatitis has a lobular architecture, with large central ducts surrounded by smaller peripheral ones. Cancer is haphazard, with random and irregular distribution of glands (Figure 10.3).
  - Incomplete lumina, in which the luminal spaces are not symmetrically surrounded by nuclei, and luminal necrosis both point to a diagnosis of pancreatic cancer (Figure 10.4).

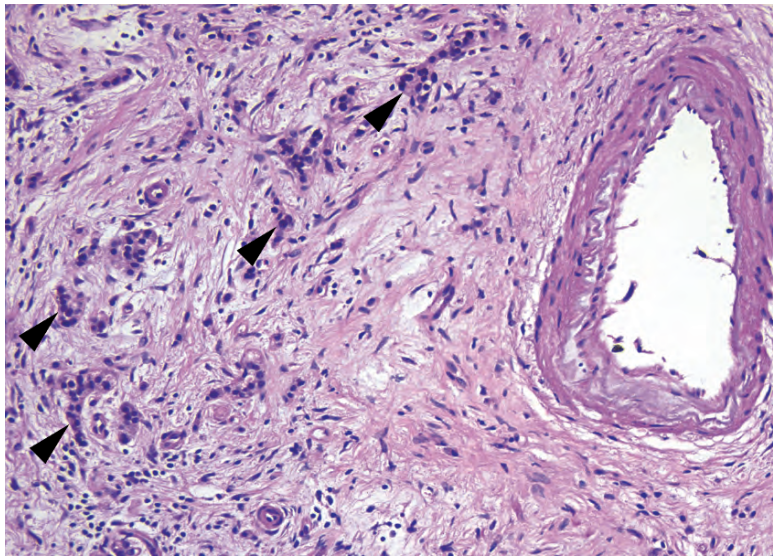

Figure 10.5. Residual islets of Langerhans. Neuroendocrine cells are among the last to go in chronic pancreatitis and appear to infiltrate through the fibrotic stroma (arrowheads). However, their small, round, dense, regular nuclei do not resemble pancreatic adenocarcinoma.

- Note cellular pleomorphism. In general, cancer tends to have hyperchromatic and irregularly shaped nuclei, mitoses, and necrosis (see Figure 10.4). You will hear of the "4:1 rule," which states that if, in one gland, one nucleus is four times the size (area) of another, it is cancer. However, chronic pancreatitis can lead to some more subtle atypia, and it does take some experience to tell the difference between a 4:1 ratio and a 3:1 ratio. Also, you will sometimes see well-differentiated pancreatic carcinoma with uniform nuclei.

- Not helpful
  - The fibrosis of chronic pancreatitis can look much like a desmoplastic stromal response. However, the pale edematous fibrosis can accentuate the lobular architecture of chronic pancreatitis, which is helpful.
  - Every intern dots all the benign islets of Langerhans on a pancreatic neck, usually missing the sneaky invasive stuff. Islets, in chronic pancreatitis, are essentially all that remains of the withered parenchyma, and therefore they look crowded, infiltrative, and haphazard (Figure 10.5). As is true for any endocrine cell, these cells can have some pleomorphism, and in some cases they can involve perineural spaces. Fortunately, the chromatin still looks neuroendocrine, so try to ignore them even though they really do look a little like lobular breast carcinoma.

- Freebies (even the beginner can interpret them)
  - Glands in a nerve, or perineural invasion, always indicate cancer.
  - Large ducts running next to a large muscular vessel almost always indicate cancer (Figure 10.6).
  - Ducts leaving the pancreas to infiltrate the duodenum always indicate cancer.

Dysplasia in the Pancreas

The pancreas is not an organ that can be evaluated with serial biopsies, and thus the natural history and malignant potential of dysplasia are not well understood. However, there are recognized grades of dysplasia within the duct system, called pancreatic intraepithelial neoplasia (PanIN). This ranges from PanIN 1, which may overlap with hyperplastic or reactive changes, to PanIN 3, which is carcinoma in situ. A lesion should always be graded by the highest level of dysplasia seen.

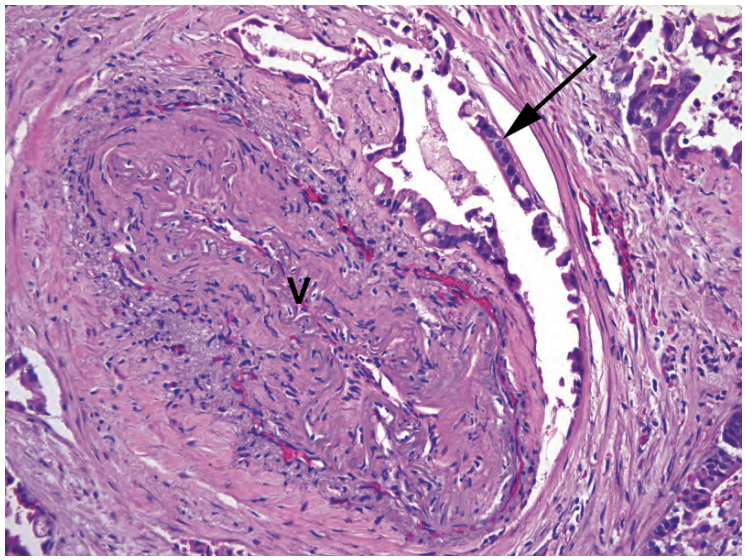

Figure 10.6. Adenocarcinoma next to a vessel. Large duct-like structures (arrow) next to a large-caliber vessel (V) are almost certainly cancer, even if deceptively well differentiated.

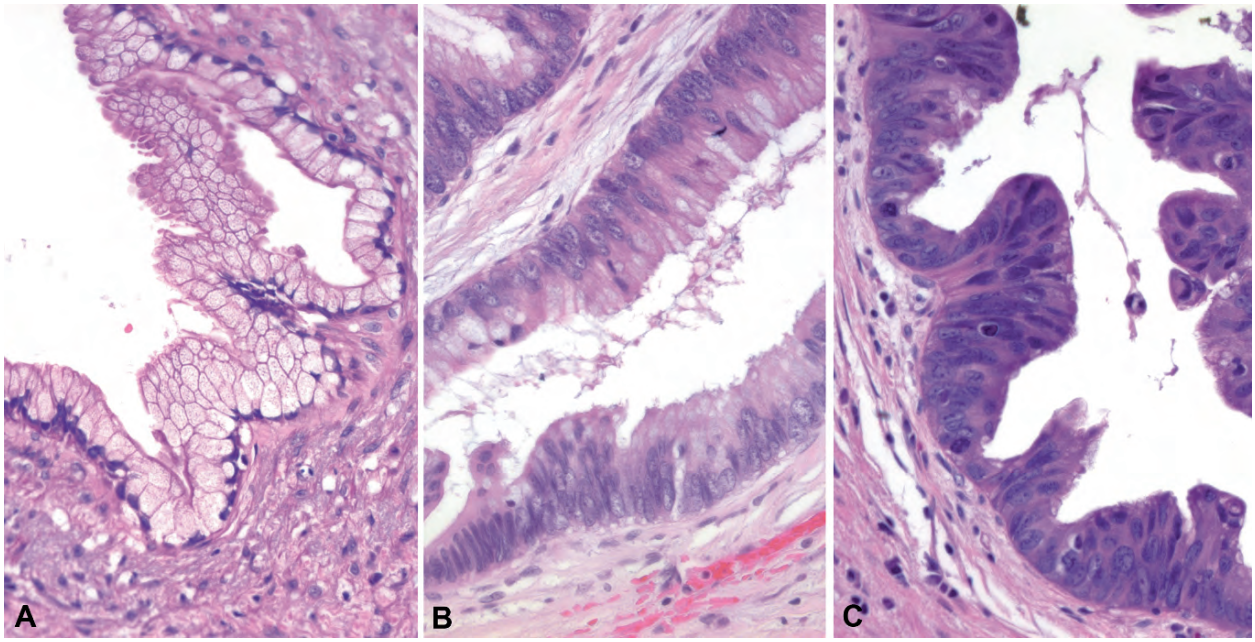

Figure 10.7. The grades of pancreatic intraepithelial neoplasia (PanIN). **(A)** PanIN 1 shows tall mucinous cells resembling endocervix. **(B)** PanIN 2 shows increasing nuclear crowding, enlargement, and atypia, suggestive of a tubular adenoma of colon. **(C)** PanIN 3 shows high-grade nuclei with loss of polarity, frequent mitoses, and loss of mucinous differentiation.

PanIN 1A has a flat layer of tall columnar cells with basal nuclei and apical mucin and no atypia. The cells are similar to normal endocervical glands (Figure 10.7). *PanIN 1B* is the same as PanIN 1A but with a papillary or undulating appearance.

PanIN 2 is flat or papillary but with nuclear abnormalities, including nuclear crowding and enlargement, stratification, hyperchromasia, and sometimes basal mitoses. This epithelium should remind you of a tubular adenoma or what would be called low-grade dysplasia in the gastrointestinal tract (see Figure 10.7).

PanIN 3 is carcinoma in situ. You may see a cribiforming, papillary, or micropapillary architecture or necrosis. Cytologic features include large ugly nuclei with prominent nucleoli, total loss of polarity, atypical mitoses, maloriented goblet cells (upside down)—essentially the same criteria you would use for high-grade dysplasia in other gastrointestinal epithelia (see Figure 10.7).

Invasive carcinoma arising out of PanIN 3 is well documented. However, remember that PanIN is a common incidental finding in a pancreas. It is not visible radiologically, it does not make a mass, and it does not cause obstruction. If you have a clinical mass, you should be thinking instead of an invasive carcinoma or intraductal papillary mucinous neoplasm (IPMN; see next section). Also, do not worry too much about the PanINs. With the exception of PanIN 3, they are of no proven clinical significance; margins with PanIN 1 or PanIN 2 lesions can safely be called negative.

Intraductal Papillary Mucinous Neoplasm

An IPMN is defined as a mucin-producing neoplasm arising in either the main pancreatic duct or a secondary (side-branch) duct. The ducts are usually dilated because they are full of a papillary proliferation and abundant mucin. The main lesion to consider in the differential diagnosis is the mucinous cystic neoplasm (discussed later). If you have a mucin-producing cystic neoplasm in the pancreas, always probe the main duct to see if the cysts are connected to it (an IPMN) or not (a mucinous cystic neoplasm). Essentially it is a gross diagnosis and may even be an endoscopic one; if mucin was seen coming out of the ampulla, the cysts must be connected to the pancreatic ducts and the lesion is more likely to be an IPMN. However, once you have identified an IPMN grossly, you must look microscopically to evaluate the level of atypia and rule out an invasive carcinoma. Intraductal papillary mucinous neoplasms are divided into three categories:

- With low-grade dysplasia: These neoplasms are cytologically bland and have the same criteria as PanIN 1.
- With moderate dysplasia (formerly known as *borderline*): These neoplasms cytologically show increasing nuclear abnormalities and have the same criteria as PanIN 2.
- With high-grade dysplasia: These neoplasms are cytologically malignant (see PanIN 3 criteria, discussed earlier). Any IPMN with high-grade dysplasia must be carefully scrutinized for invasive carcinoma slipping out of the duct.

A common question is, how can I tell PanIN in a largish duct from IPMN in a smallish side-branch duct? Features that favor an IPMN include the following:

- Long papillae, or finger-like projections with fibrovascular cores (Figure 10.8)
- Blue mucin in the lumen of the duct
- Continuity with one of the main pancreatic ducts
- Grossly or radiologically visible

When it comes right down to it, identifying the grade of dysplasia correctly right is much more important than distinguishing between an IPMN and a PanIN.

Invasive Adenocarcinoma (Ductal)

The most common form of infiltrating adenocarcinoma in the pancreas is ductal. It usually arises in the head and often invades adjacent structures before coming to clinical attention. The histologic features of ductal adenocarcinoma have been described earlier. Once you have established carcinoma, look carefully at all sections of duodenum and extrapancreatic bile duct to see if the carcinoma invades those structures (in increase in stage). The bile duct and ampullary region have numerous benign glands branching off of them, but remember that the benign glands will have a lobular and symmetric look at low power. Variants of ductal adeno-

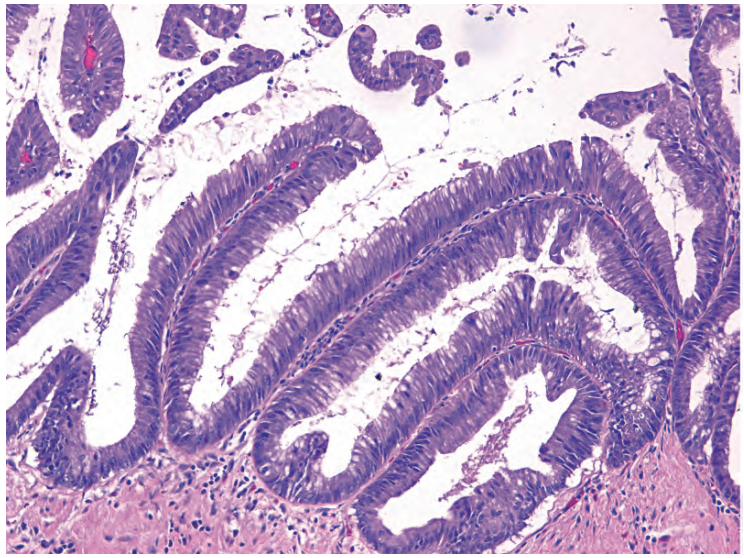

Figure 10.8. Papillary projections, intraductal papillary mucinous neoplasm with moderate dysplasia. These tall papillary fronds are covered with mucinous cells showing moderate dysplasia, similar to PanIN 2.

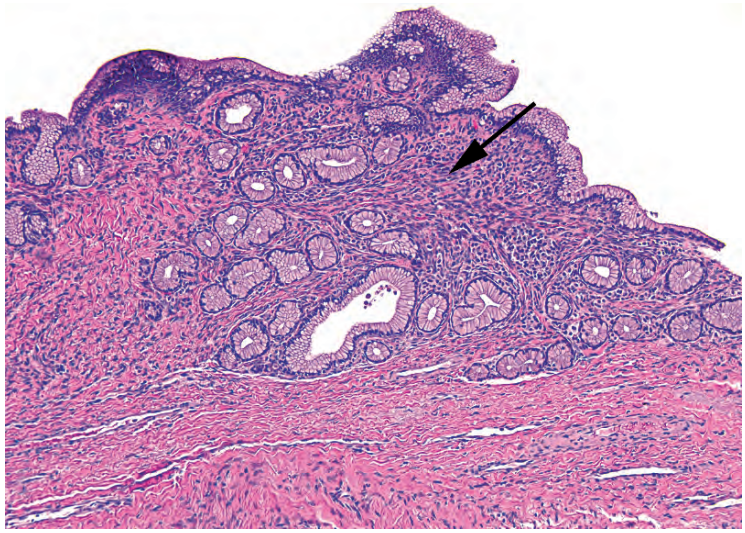

Figure 10.9. Mucinous cystic neoplasm. The cyst lining is composed of mucinous cells, benign in this example, and underlying blue spindly ovarian-type stroma (arrow).

carcinoma include adenosquamous, colloid (mucinous), hepatoid, medullary, signet ring cell, undifferentiated (anaplastic), and undifferentiated carcinoma with osteoclast-like giant cells.

Other Cystic Lesions of the Pancreas

Mucinous Cystic Neoplasm

The mucinous cystic neoplasm occurs almost always in middle-aged women, usually in the tail of the pancreas. This mucinous neoplasm produces multilocular cysts that do not communicate with the main duct system. They have, by definition, a rim of ovarian stroma (Figure 10.9), so think of them as mucinous ovarian tumors heterotopic into the pancreas. As in the ovary, they have three grades, and these grades conveniently parallel the three grades of the IPMN:

- With low-grade dysplasia: no atypia, like PanIN 1
- With moderate dysplasia: increasing nuclear atypia and/or architectural complexity, like PanIN 2
- With high-grade dysplasia: carcinoma in situ, like PanIN 3

Approximately one third of mucinous cystic neoplasms have an associated invasive carcinoma, which would be called *infiltrating moderately differentiated adenocarcinoma arising in association with a mucinous neoplasm with high-grade dysplasia*.

Serous Cystadenoma

Serous cystadenomas of the pancreas, unlike the serous cystadenomas of the ovary, are almost always microcystic. Grossly, they have a central scar and radiating small clear-fluid-filled cysts, like the cross section of a lime. Microscopically, the cysts are lined by cuboidal cells with clear cytoplasm (glycogen) and small, uniform, round nuclei (Figure 10.10). Areas of more solid or trabecular growth may look much like metastatic renal cell carcinoma, which is in fact in the differential. Serous cystadenocarcinomas exist but are extremely rare.

Solid-Pseudopapillary Neoplasm

Solid-pseudopapillary neoplasms are unusual and distinctive tumors in the differential diagnosis of cystic lesions in young women. They are malignant but extremely indolent. The cell of origin is not known, and so the neoplasm is named based on its appearance. These neoplasms start out solid but undergo cystic degeneration and therefore may present as a cyst (despite the name). The cells are characteristically noncohesive, and so the remaining solid areas show a pseudopapillary growth pattern (meaning there is solid growth along fibrovascular septa, with a dropout of the loosely cohesive cells in between septa and a resulting papillary look). The nuclei are small, oval, bland, and grooved (Figure 10.11).

The differential diagnosis for this neoplasm includes well-differentiated pancreatic endocrine neoplasm and acinar cell carcinoma, both of which are discussed later. Immunohistochemical labeling is very helpful, as solid-pseudopapillary neoplasms are CD10 positive and show nuclear labeling for β-catenin.

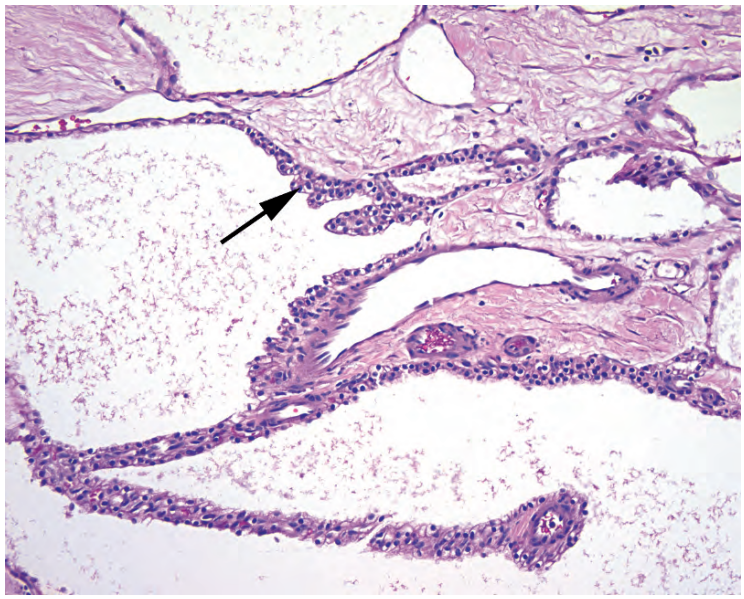

Figure 10.10. Serous cystadenoma, high power. The cells lining the multilocular cyst are small, with dense round nuclei and clear cytoplasm (arrow).

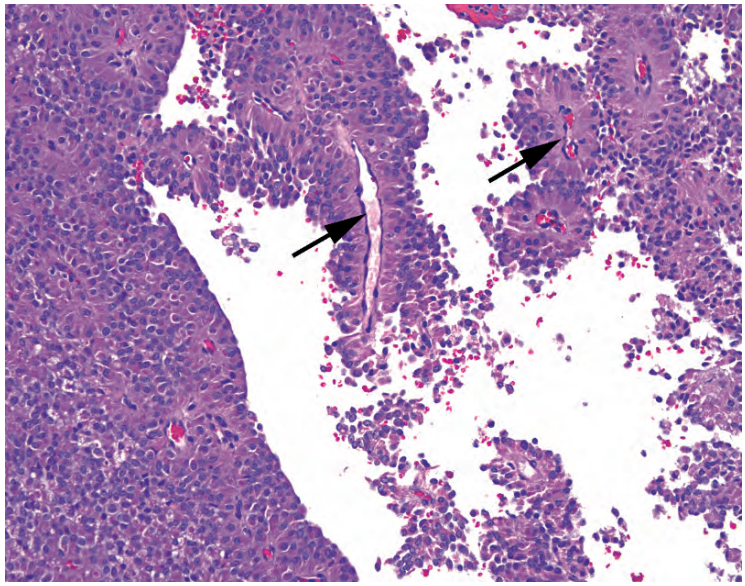

Figure 10.11. Solid pseudopapillary tumor. The small plasmacytoid cells with neuroendocrine-type chromatin could be mistaken for islet cell tumor or acinar cell carcinoma. However, this growth pattern, with rosette-like growth around fibrovascular cores (arrows) and dropout of the intervening cells, is typical of the solid pseudopapillary tumor.

Pseudocyst

The definition of a pseudocyst is "lacking an epithelial lining." This is a walled-off area of fat necrosis and granulation tissue containing high levels of pancreatic enzymes that is not usually mistaken for a malignancy, clinically or microscopically. Remember that most pseudocysts are actually extrapancreatic.

Other Solid Tumors in the Pancreas

There are only two pancreatic cell types not yet discussed (not counting soft tissue elements such as vessels and nerves): the acinar cells (exocrine secretory) and the islet cells (endocrine). Neoplasms composed of these cells are important to remember because they can release enzymes or hormones, causing dramatic clinical presentations. These tumors can also show considerable histologic overlap and may require special stains to distinguish.

Acinar Cell Carcinoma

Acinar cell carcinomas are rare tumors of older adults, usually male. The usual appearance is that of nodules and sheets of densely packed amphophilic (purple) cells with uniform round nuclei. Growth may be trabecular, nested, or acinar (arranged around tiny lumens). Prominent nucleoli are often seen and are a clue to the diagnosis. Like the benign acinar cells, these tumors are usually positive for trypsin.

Well-Differentiated Pancreatic Endocrine Neoplasm

Well-differentiated pancreatic endocrine neoplasms are simply the neuroendocrine tumors of the pancreas, also known as *islet cell tumors*. These tumors are usually well circumscribed and cellular, and the neoplastic cells tend to form nests or trabeculae. The cytology is that of a carcinoid (Figure 10.12). Some are functional and produce clinically significant levels of insulin, glucagon, somatostatin, or other peptides. Cytology is not an indication of behavior,

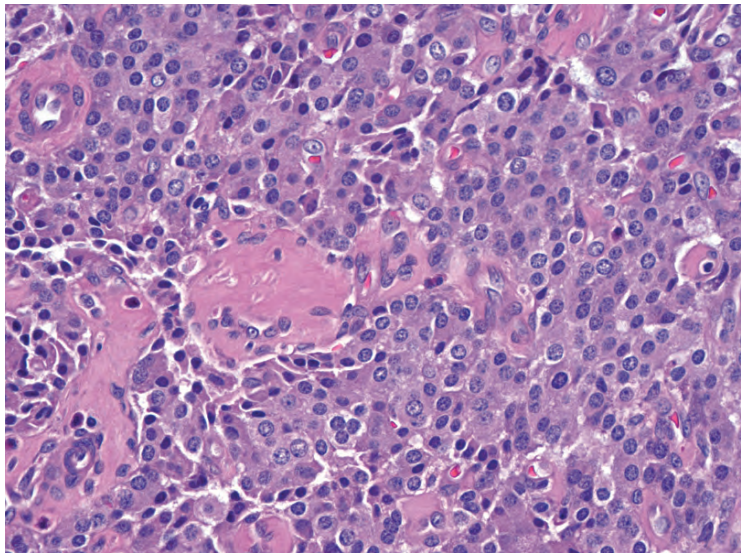

Figure 10.12. Islet cell tumor. This resembles carcinoids in other body sites, with round, well-spaced nuclei and speckled neuroendocrine-type chromatin.

usually, and a bland tumor may look just as bland when you later find it in the liver. High-grade neuroendocrine tumors are rare in the pancreas and when present are usually of the small cell variety. Well-differentiated pancreatic endocrine neoplasms express neuroendocrine markers (SYN, CHR, CD56) as well as any peptides they may be producing.

11 Prostate

Most biopsies are performed for an elevated prostate-specific antigen (PSA) level, a palpable nodule, or a history of an abnormal biopsy. In the prostate, you are generally looking only for adenocarcinoma; there are very few nonneoplastic conditions to look for.

A typical sextant biopsy is six cores from left apex, mid, and base and right apex, mid, and base. Increasingly, urology centers are sampling additional areas, 12 or more. Laboratories differ in how many cores are placed on a single slide; some laboratories may have only two slides, left and right, with a handful of cores on each slide. It is important to preserve as much detail as the urologist or laboratory gives you and to localize the cancer as much as possible.

Approach to the Core Biopsy

On 4× to 10×, scan the length of the core looking for glands that stand out and look different.

- Low-power features of prostate cancer (Figure 11.1)
  - Small individual glands infiltrating among larger benign glands (for intermediate-grade cancer, or Gleason pattern 3; see more on Gleason grades, discussed later)
  - Crowded glands (for intermediate-grade cancer)
  - An unusually cellular infiltrate (individual cells of high-grade cancer, or Gleason pattern 5)
  - Cribriform areas (high-grade cancer)
  - Sheets of cells (high-grade cancer)
  - A different color or texture to the glands (cancer may appear denser or more bluish)
  - Blue mucin, crystalloids, or dense pink secretions in the lumen
  - Absence of desmoplastic response
- Low-power features of benign glands (Figure 11.2)
  - Irregularly shaped glands with papillary infoldings (a "frilly" look)
  - Glands with a modest amount of intervening stroma
  - Corpora amylacea

At high power (20×–40×), examine the cytology of the suspicious areas. Look for features seen in carcinoma:

- Large, often cherry-red nucleoli (Figure 11.3)
- Straight, crisp luminal borders to the glands
- Enlarged and/or hyperchromatic nuclei (however, pleomorphism is minimal)
- Lack of basal cell layer (can be confirmed by immunostains)
- Mitoses (uncommon)

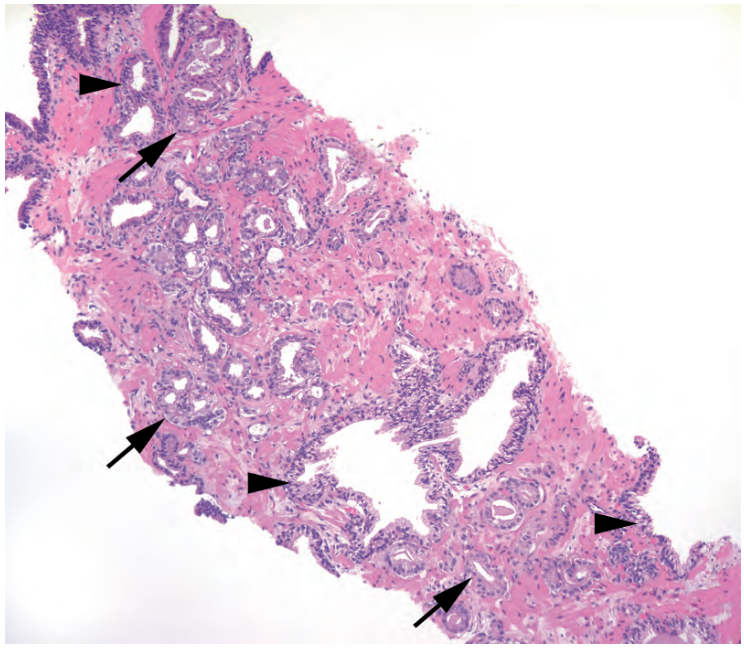

Figure 11.1. Low-power features of carcinoma. Adenocarcinoma (arrows) is seen infiltrating throughout benign glands (arrowheads) in this core biopsy specimen. The malignant glands are often back to back and have relatively denser cytoplasm, no basal layer, and straight luminal borders.

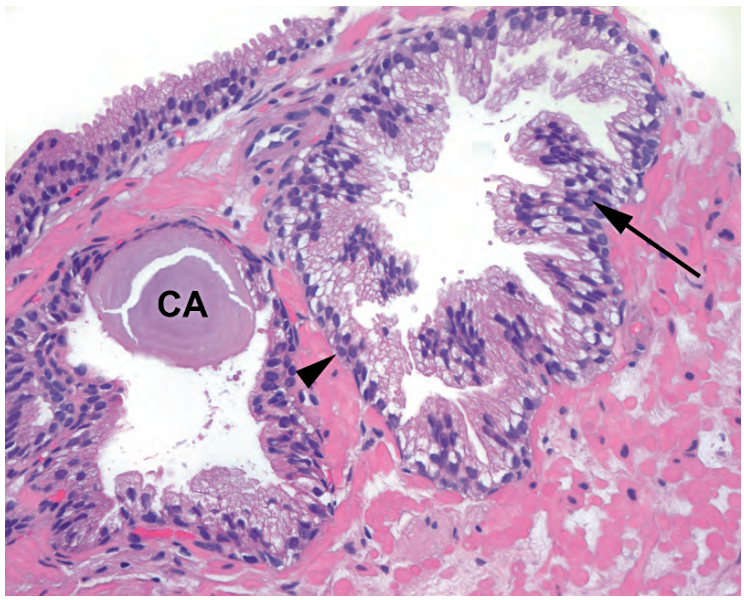

Figure 11.2. Benign prostate glands. These glands have a distinct basal cell layer underlying the epithelial cells (arrowhead) and papillary fronds in the lumen (arrow). Corpora amylacea (CA) are concentrically laminated concretions associated with benign glands.

Although none of these findings is completely sensitive and specific for cancer, having more malignant than benign features is a pretty good indication. There are three features that, although uncommon, are only seen in cancer:

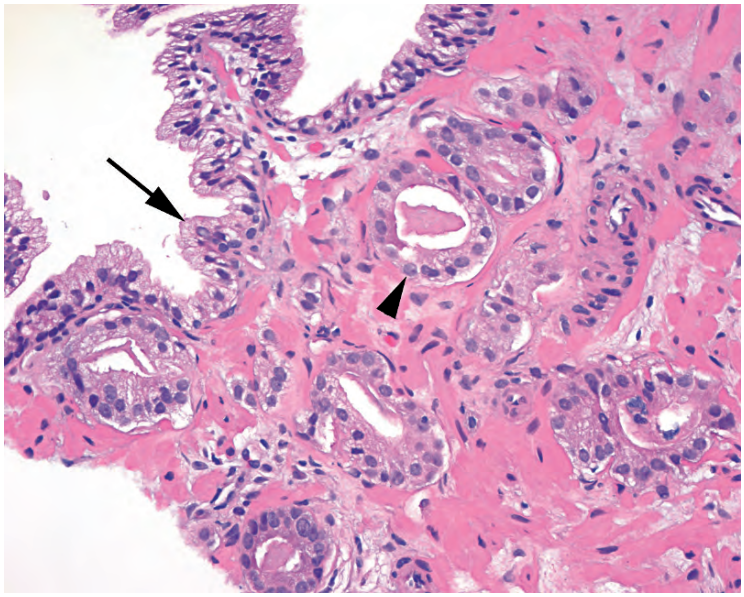

Figure 11.3. High-power features of carcinoma. Malignant glands show distinct nucleoli (arrowhead), sharp luminal borders, and an absence of basal cells. Benign glands are seen adjacent to the cancer (arrow).

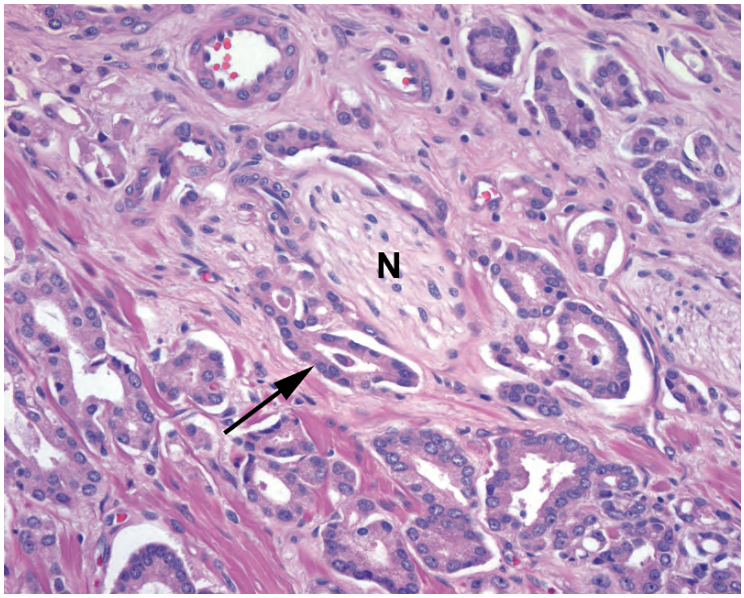

Figure 11.4. Perineural invasion. A nerve (N) is identified by the undulating axons and nerve sheath nuclei. Malignant glands are seen nearly surrounding the nerve (arrow).

1. Perineural invasion: The nerve appears as a discrete oval profile with wavy parallel stripes, almost like a fingerprint, and the malignant gland must be within the nerve sheath to count as perineural invasion (Figure 11.4). Often the gland will fill up the nerve sheath circumferentially, so the nerve appears to be floating in a gland.
2. Mucinous fibroplasia: Hyalinized whorls of organized dense secretions are present in the lumen; sometimes the surrounding gland epithelium may be compressed and indistinct.
3. Glomeruloid forms: Proliferative tangles of cells project into the larger gland lumen, resembling a glomerulus.

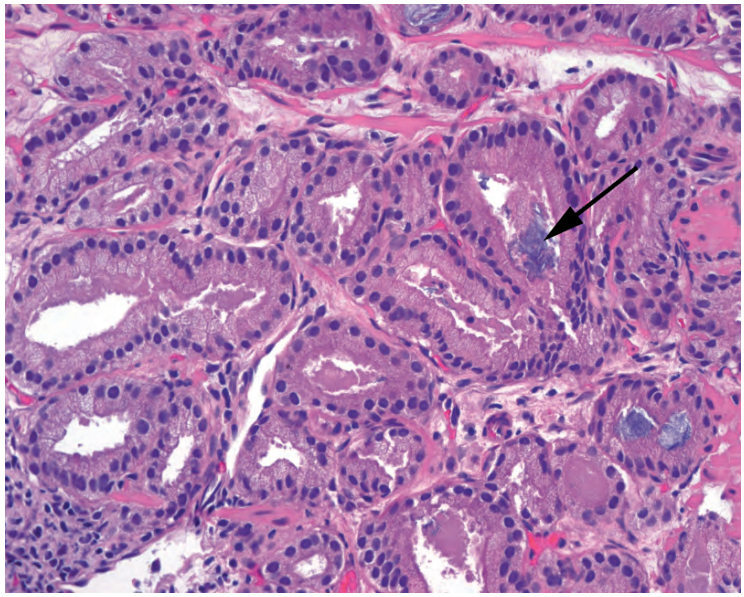

Figure 11.5. Gleason pattern 3. Individual, well-formed malignant glands make up pattern 3 cancer. Blue mucin, often associated with carcinoma, is present (arrow).

Gleason Grading

Once you have identified adenocarcinoma, you must give it a histologic grade. Prostatic adenocarcinoma is graded by the Gleason system, which is based on architectural pattern. Cytology does not affect grade. The patterns (and there may be many within a single tumor) are assigned a number from 1 to 5, with 5 being the least differentiated. After all tissue is examined, the first and second most common patterns are added together to give the Gleason score (a possible 2–10). A pure tumor of pattern 3 would be a 3 + 3 = 6; a mixture of 3 and 4 could be signed out as 4 + 3 = 7 or 3 + 4 = 7, depending on the amount of each.
You may want to take the Johns Hopkins online tutorial for prostate grading, available at http://pathology2.jhu.edu/gleason/. Other good grading websites can be found at www.isuporg.org. In summary, the pattern grades are as follows:

1. rarely used; a circumscribed nodule of uniform crowded glands
2. a circumscribed nodule of well-defined glands with minimal infiltration at the periphery; less uniform than pattern 1.
3. highly infiltrative (creeping between benign glands), with discrete and individual gland profiles such that you can mentally draw a circle around each gland (Figure 11.5).
4. fused and ill-defined glands, sheets of cribriform glands, poorly formed lumens (Figure 11.6).
5. a complete absence of glandular differentiation, solid sheets and cords of cells, single cells (Figure 11.7)

Gleason *scores* of 2–4 (i.e., a combination of patterns 1 and 2) are not diagnosed on needle biopsy, as they can only be identified in the context of surrounding tissue.

Features That Should Be Mentioned in Your Diagnosis

The following features should be mentioned in your diagnosis:

- Number of involved cores: A biopsy diagnosis should include a mention of how many cores are involved (e.g., *1 of 1 core*, or *2 of 4 cores*).

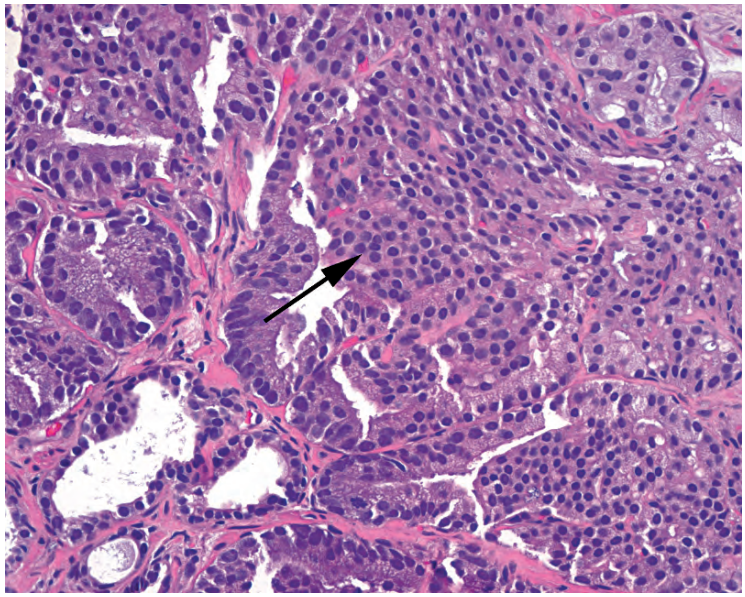

FIGURE 11.6. Gleason pattern 4. The area of cribriform growth (arrow) and adjacent fused glands is typical of pattern 4.

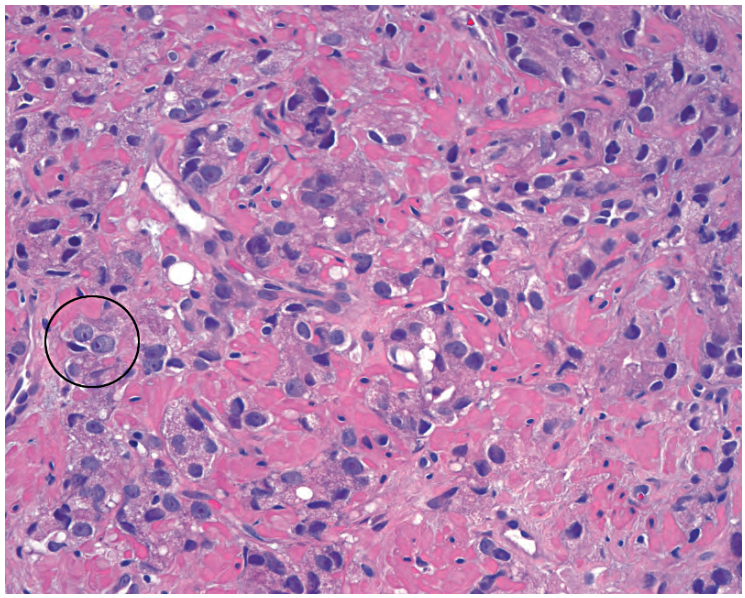

FIGURE 11.7. Gleason pattern 5. Individual malignant cells, without evidence of gland formation, are typical of pattern 5. The individual cells still cytologically resemble well-differentiated carcinoma, with round nuclei and prominent nucleoli (circle).

- Percent involvement: Note the approximate percentage involvement on each core (e.g., *involving 2 of 4 cores [30%, 60%]*). Small foci of cancer (<5% of a core) can be described as *small foci*.
- Perineural invasion: Once cancer is identified, look closely for foci of perineural invasion. The presence of perineural invasion in a biopsy specimen has adverse prognostic significance.
- Extraprostatic extension: Rarely, a core biopsy will go through the capsule of the prostate and into the fat beyond. An extremely lucky shot may show malignant glands trickling into the fat, which is diagnostic of extraprostatic extension. This also has adverse prognostic significance.

Prostatic Intraepithelial Neoplasia

Prostatic intraepithelial neoplasia (PIN) occupies a slightly uncertain place in pathology. It is considered to be a precursor to cancer and to demonstrate a generally increased risk of cancer, but, unlike precursor lesions such as high-grade squamous intraepithelial lesions (cervix) or ductal carcinoma in situ (breast), it does not warrant an immediate rebiopsy or excision. You can think of it as dysplasia in the prostate gland, but the natural history of the lesion is unclear. In any case, the lack of reproducibility and questionable significance of low-grade PIN are such that we do not mention it on biopsy. High-grade PIN, however, should be noted. Features of high-grade *PIN* include the following:

- Glands are large with prominent papillary or micropapillary luminal surfaces, similar to benign architecture. Cribriform PIN can be seen, but back-to-back glands are not PIN.
- Glands appear darker and more blue than surrounding glands (Figure 11.8).
- Nuclei are enlarged, elongated, and hyperchromatic, and by definition nucleoli are visible at 20×.
- The basal cell layer is usually still present, yet often patchy; immunostains show this nicely.

Mimickers of Prostate Cancer

There are some benign entities that may catch your eye and stand out in a biopsy specimen but that are definitely not carcinoma.

Adenosis

Adenosis literally means a proliferation of glands. Adenosis is a hyperplastic lesion, not a neoplastic one. It consists of a *lobular group* of crowded glands, which may include small suspicious-looking glands among them. The morphology of the small glands, however, should overlap with the intermixed larger, benign-looking glands; there should be a spectrum so that you cannot point to definite malignant versus benign glands. Adenosis may have visible small

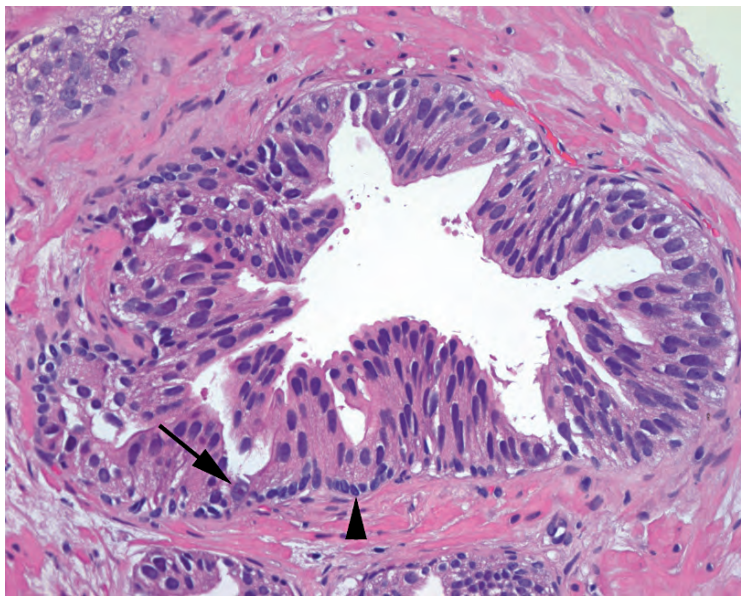

Figure 11.8. High-grade prostatic intraepithelial neoplasia. Although the papillary infoldings resemble benign prostate, the nuclei are larger and darker and show occasional prominent nucleoli (arrow). Basal cells are still present (arrowhead).

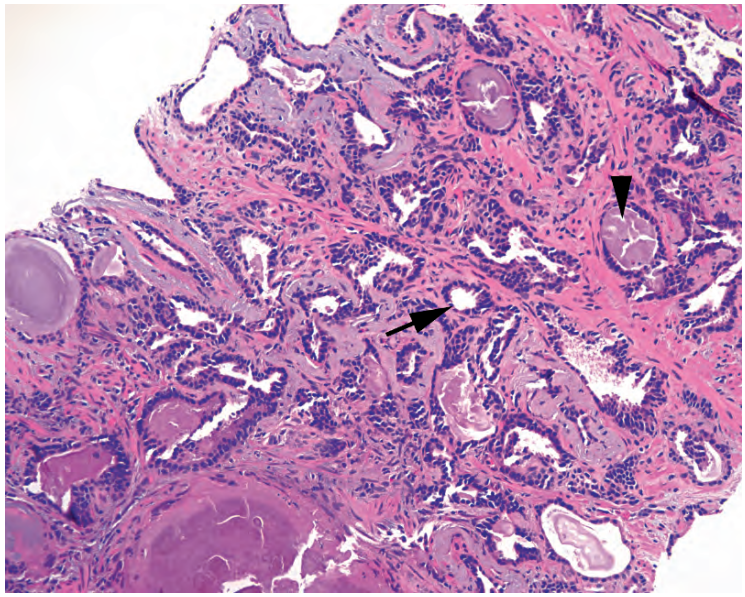

Figure 11.9. Atrophy. These glands appear hyperchromatic and infiltrative. However, the low cuboidal cells with attenuated cytoplasm (arrow) and angular gland profiles are typical of benign atrophy. Corpora amylacea are present (arrowhead).

nucleoli (how unfortunate) but by definition has a basal layer (visible by immunostains if not by H&E).

Atrophy

Atrophy is the shrinkage of the cells forming the glands. The cytoplasm shrivels down, leaving essentially rows of nuclei outlining the lumens (Figure 11.9). At low power these atrophic glands can look small and irregular, which may be suspicious. However, the lumens have an angular, almost staghorn look to them. Small- to medium-sized nucleoli may be seen, but the lack of cytoplasm should be a red flag against diagnosing cancer. Immunostains highlight a basal cell layer.

Basal Cell Hyperplasia

The basal cells that underlie the glandular cells are not usually well visualized. When they are noticeable, you can see them as sort of denim-blue, oval, regular nuclei surrounding the more purple glandular nuclei (Figure 11.10). The tricky part is that they may have nucleoli. In basal cell hyperplasia, the basal layers may proliferate and create several layers of worrisome-looking cells in the glands. The key is in recognizing the dual population; sometimes you can still see the glandular cells floating on top of the basal hyperplasia. Stains help.

Cowper's Glands

Cowper's glands are histologically normal glands (distal to the prostate, secreting directly into the urethra, and normally not sampled on needle biopsy) that consist of mucous-filled secretory glands surrounding a coil of ducts. They are lobular in architecture and have small bland nuclei. Their abundant mucin will stain with periodic-acid Schiff, and they are usually negative for the prostate markers prostate-specific antigen (PSA) prostate-specific acid phosphatase (PSAP).

Radiation Changes

Radiation atypia has a characteristic look that is difficult to describe in words. The nuclei are *too* pleomorphic to be cancer, especially when compared with the relatively uniform nuclei

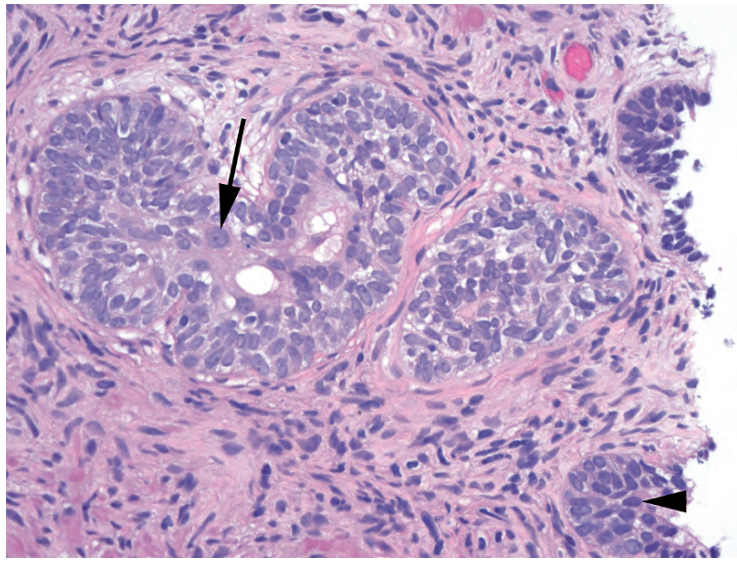

Figure 11.10. Basal cell hyperplasia. This proliferation of cells, some with prominent nucleoli (arrow), is actually an expanded basal cell layer. Comparison with benign epithelium (arrowhead) shows the relatively pale and greyish nuclei of the basal cells.

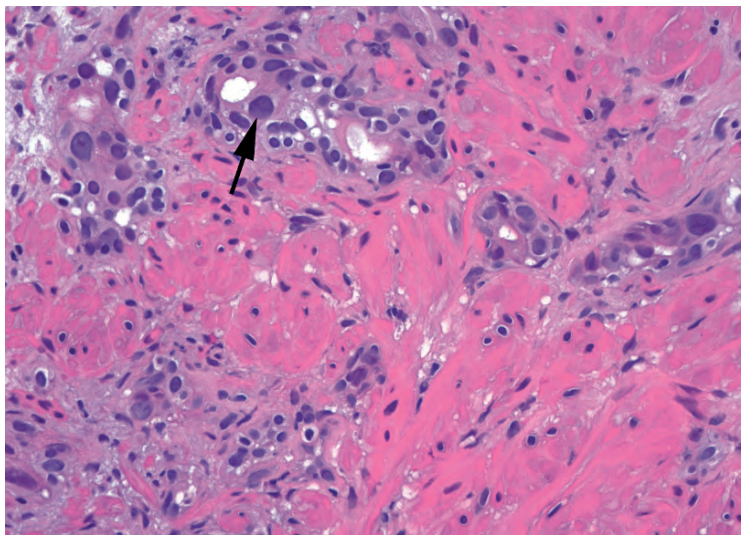

Figure 11.11. Radiation atypia in benign prostate. There is scattered and random nuclear pleomorphism (arrow). Enlarged nuclei classically have dense, uniform, smudgy chromatin.

of prostate cancer. Radiated benign glands show atrophic cytoplasm and wildly pleomorphic nuclei mixed in with normal nuclei (Figure 11.11). The nuclei may be very large, with angular shapes, and tend to have a dense smudgy chromatin without nucleoli. Identifying residual cancer in the radiated prostate is a diagnosis made largely on architecture (many individual cells with ample vacuolated cytoplasm and nuclei that ironically are often not as pleomorphic as the benign radiated nuclei).

Seminal Vesicle

The nuclei of the seminal vesicles have very pleomorphic nuclei, not unlike radiation atypia. They will definitely stand out in a needle biopsy specimen and can be very concerning based on cytology. However, remember that prostate cancer is usually not pleomorphic, and look for the telltale golden globs of lipofuscin to identify it as seminal vesicle (Figure 11.12).

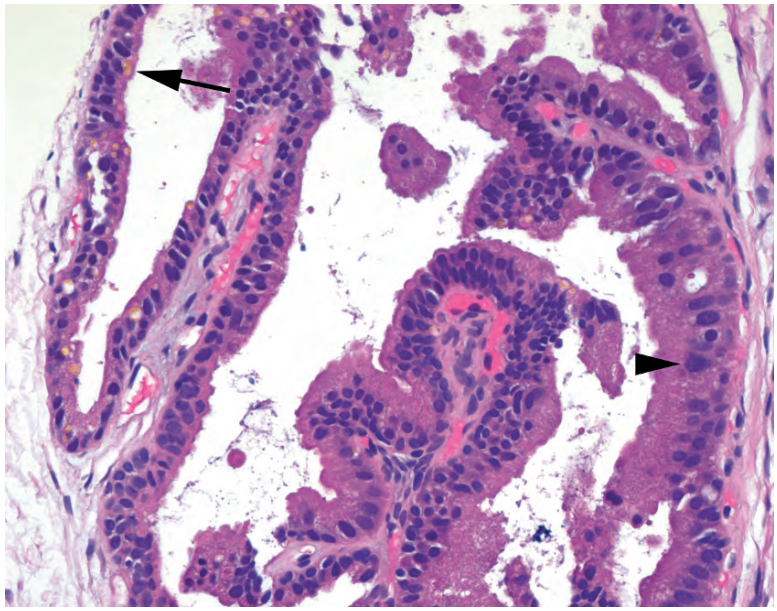

Figure 11.12. Seminal vesicle in a biopsy specimen. There are scattered large, hyperchromatic, and crowded nuclei in this gland (arrowhead). However, golden pigment is visible in the cytoplasm (arrow), identifying this as seminal vesicle.

Sclerosing Adenosis

Sclerosing adenosis is seen best in transurethral resection specimens. It is a hyperplastic and proliferative lesion that is complicated by a hypercellular stroma. The appearance is that of crowded glands and individual cells (which may have prominent nucleoli) in a background of cellular stroma. Remember that prostate cancer *does not* induce a stromal reaction.

Atypical Glands and Stains

It is not uncommon, in a needle core biopsy specimen, to stumble across one or two isolated glands that make you very nervous. However, unless several features of carcinoma are evident, most pathologists will be reluctant to make the diagnosis of cancer in that setting. One option is to sign it out as a focus of atypical glands. In the absence of definitive cancer, it will usually generate a repeat biopsy.

Immunostains may help in the diagnosis of these tiny lesions or with larger groups of glands that have some but not all of the features of cancer. Stains for the basal layer (CK903 or high-molecular-weight cytokeratin and p63) should highlight the basal cells in all benign glands and show loss of staining in malignant glands. Racemase is a newer marker that preferentially stains the cytoplasm of cancer. However, there are false-positive and false-negative results with all three of these antibodies, and so each case is interpreted in the context of the H&E appearance (which is the best approach for all immunostains).

Approach to the Radical Prostatectomy Specimen

For the radical prostatectomy specimen, the prostate is inked and bread loafed from apex (nearest the penile urethra) to base (nearest the bladder neck). Each slice is cut into four quadrants to fit them into cassettes. The margins are taken first:

1. Right and left vas deferens: These represent the true surgical margins of the vas deferens. Positive vas deferens margins are very rare, and some pathologists do not submit them.
2. Apical, or distal, margin: This is where the prostate meets the penile urethra. It is cut off as a thick tangential shave and then turned 90°, sliced, and submitted as a series of perpendicular sections that are parallel to the urethra. The presence of malignant glands is acceptable, as long as they do not touch the inked apical surface.
3. Bladder neck margin: This is where the prostate meets the bladder; it is a soft tissue margin, not a urethral margin. The urethra itself retracts back into the prostate at surgery and may not be seen on the slide. This is usually a shave margin, in which the presence of any malignant glands is considered a positive margin. It can also be treated like the apical margin, however, and sectioned perpendicularly.

After evaluating the margins, systematically examine each section of the prostate. Each full slice of prostate is halved into left and right sides. If the entire hemisection cannot fit into one block (usually it cannot), it is subdivided into anterior and posterior quadrants. Orienting the isolated quadrant can be tricky. For posterior sections, the true posterior surface should be flatter than the lateral surface. The neurovascular bundles, which sometimes come out with the prostate, are located at the posterolateral corners. For anterior sections, the anterior tip should have many smooth muscle bundles and a very poorly defined capsule. The verumontanum of the urethra (the bump on the posterior urethra) points anteriorly (Figure 11.13).

Examination of the edge of the prostate is prognostically important. There is no true organ capsule but rather the outer limit or edge of the prostate, which is best recognized posteriorly and posterolaterally as packed muscle bundles of the prostate. Extension of the cancer beyond the edge indicates extraprostatic extension (EPE) and increases the stage of the tumor. Capsular incision, where the surgeon has cut across the organ and left some prostate in the patient, becomes very important if there is cancer in the area. Malignant glands near big vessels or among skeletal muscle are not necessarily EPE, but cancer in fat is "out" by definition (Figure 11.14), as there is no intraprostatic fat. However, if you wait to diagnose EPE until seeing tumor in fat, you will miss some EPE. As cancer extends beyond the border of the prostate it is often associated with a fibrotic response to the tumor, wiping out the fat. This type of EPE is better appreciated at low power by following the contour of the edge of the prostate. Anteriorly, the muscle bundles are loose and disorganized, so it is difficult to recognize EPE except by seeing tumor in or beyond the plane of adjacent fat.

Although calling it "out" changes the tumor stage, it does not necessarily mean a positive margin. To call a positive margin, you must have glands not just really, really close to ink but actually transected by ink. The threshold is very high. In a positive margin, you must also

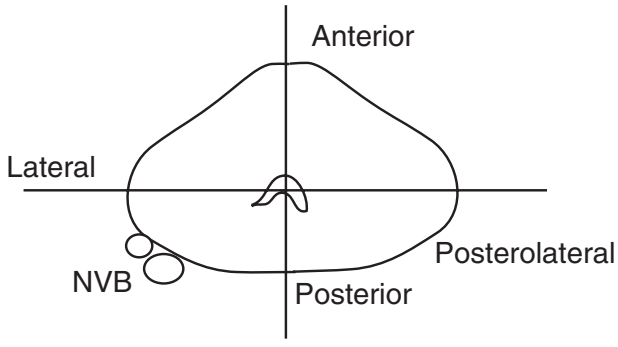

Figure 11.13. Low-power view of radical prostatectomy sections. Each cross section of prostate is cut into quadrants to fit into cassettes. The neurovascular bundles (NVB) are found at the posterolateral border of the prostate. The verumontanum points anteriorly.

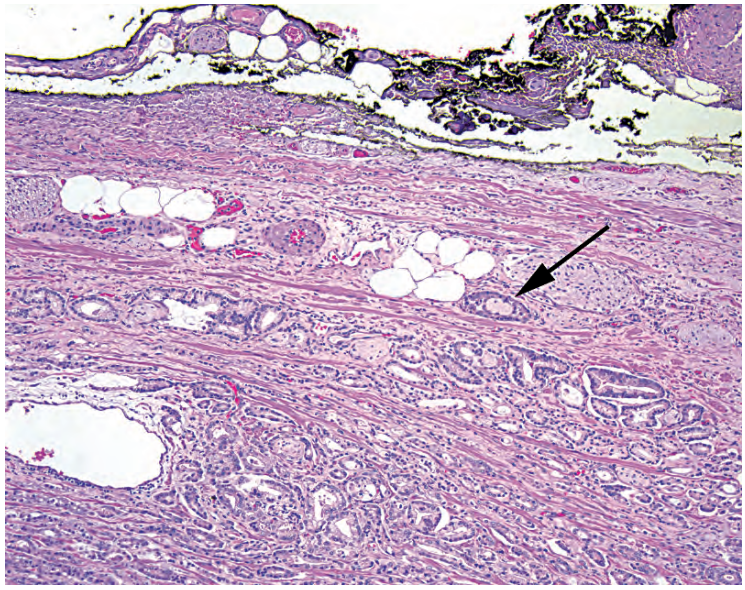

Figure 11.14. Extraprostatic extension. Malignant glands are seen wrapping around a nerve (arrow) adjacent to extraprostatic fat, diagnostic of focal extraprostatic extension. The margin, seen as the ink at the top of the photograph, is negative.

decide "why" it is positive—by noting if it occurs in an area of capsular incision or in an area of EPE. A positive margin is almost always going to be considered EPE in the anterior prostate, because the capsule is so poorly defined.

Perineural invasion is a big deal in a biopsy specimen but is taken for granted in a radical. It is not worth mentioning in the diagnosis. Grading in a radical is the same as grading in a needle core. You only have to grade the one or two biggest nodules (not the little multifocal ones), and each big nodule is given its own grade.

The seminal vesicles are examined by sampling the seminal vesicle at the point where it meets the prostate. Seminal vesicle invasion, if present, is seen on this section. Microscopically, you need to see tumor in the parenchyma of the seminal vesicle, not just next to it.

Other Prostate Neoplasms

Ductal adenocarcinoma is a variant type of adenocarcinoma that is characterized by tall, stratified columnar cells making papillary or cribriform structures (Figure 11.15). They may grow into the urethra as exophytic masses, or they may arise from more peripheral ducts in the prostate. It may be found in conjunction with conventional adenocarcinoma. It is not assigned a Gleason grade but behaves like a pattern 4 lesion.

Other types of carcinoma include mucinous carcinoma, squamous cell carcinoma, urothelial (transitional cell) carcinoma, sarcomatoid carcinoma, basal cell carcinoma, and small cell carcinoma. The more difficult-to-recognize variants of usual prostate cancer are pseudohyperplastic carcinoma (a sneaky variant that mimics the papillary architecture of benign hyperplasia), atrophic cancer (mimicking benign atrophy), and foamy gland cancer (with abundant xanthomatous appearing cytoplasm).

Spindle cell lesions may arise in the prostate. Stromal lesions arising from the unique stroma of the prostate range from the benign stromal nodules, to stromal tumors of uncertain malignant potential, to stromal sarcomas. The most common prostatic sarcoma in adults is leiomyosarcoma. Rhabdomyosarcomas occur mainly in children.

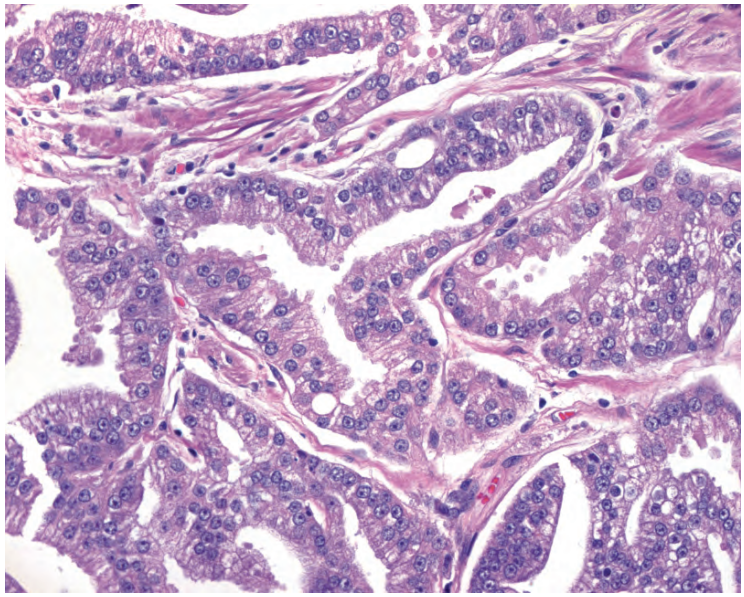

Figure 11.15. Ductal adenocarcinoma. In this variant, the tumor cells have a tall columnar morphology. The nuclei still resemble conventional prostate adenocarcinoma.

12 Bladder

Bladder biopsy specimens are usually submitted to rule out a urothelial neoplasm. The procedure may be indicated because of hematuria, an abnormal urine cytology, a history of urothelial neoplasm, or a lesion seen on cystoscopy. The cystoscopic impression is important, and you usually do not diagnose a papillary lesion if none was seen by the urologist. The bladder biopsy specimen is typically a tiny tissue fragment, so you should look at each level carefully.

The normal urothelium consists of a stratified nonsquamous epithelium, also called *transitional cell epithelium*. It consists of a five- to seven-cell thick layer of uniform cells that do not significantly mature as they reach the surface (unlike squamous epithelium) and that tend to have oblong nuclei oriented perpendicular to the surface (Figure 12.1). The nuclei are about two to three times the size of lymphocytes. Mitoses are usually seen only at the basal layer, but in the presence of inflammation and reactive changes they may be seen throughout. At the surface is a specialized cell layer called the *umbrella cells*, large pillowy cells that appear wider than the underlying urothelial cells. Umbrella cells may have atypical nuclei and should be ignored when assessing the urothelium for neoplasia.

Underneath the urothelium lies the lamina propria, a connective tissue layer that has vessels, lymphatics, occasional smooth muscle fibers, and even occasional fat. Deep to this is the thick muscularis propria, also known as the *detrusor muscle*. Beyond the muscular wall is either adventitia or, where the bladder lies against the peritoneum, peritonealized serosa.

Normal Variants

Some changes in the bladder are so common that they are essentially normal. One of these changes is the formation of *von Brunn's nests*, which are downward invaginations of the urothelium into the lamina propria (Figure 12.2). These can look alarmingly like urothelial nests that are invading the bladder, but they should have bland urothelium that looks just like normal urothelium (see below for a description of neoplastic urothelium) and have a smooth rounded border. As these nests progress, they may acquire a dilated central lumen (*cystitis cystica*), columnar cell metaplasia (*cystitis glandularis;* see Figure 12.2), and even intestinal metaplasia with mucin production. They are still benign. However, just as high-grade squamous intraepithelial lesions can involve endocervical glands, in situ urothelial carcinoma can grow down into von Brunn's nests, mimicking invasion. Another normal variant is the formation of squamous metaplasia, especially in the trigone area of the female bladder.

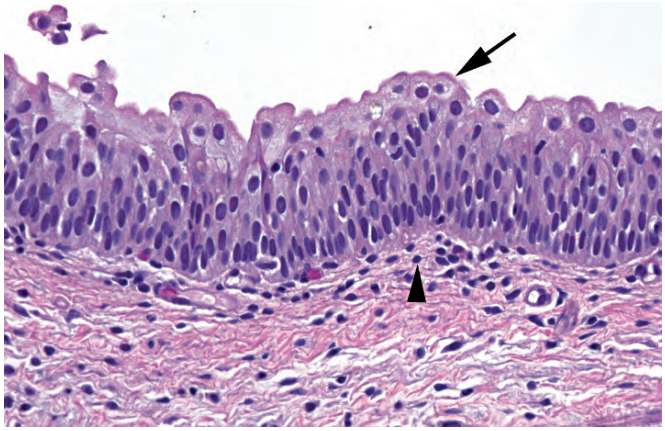

Figure 12.1. Normal urothelium. The urothelial cells form a layer five to seven cells thick, with large umbrella cells sitting on top (arrow). The urothelial nuclei are generally polarized and oriented perpendicular to the surface, with the exception of the umbrella cells. The nuclei are two to three times the size of a lymphocyte (arrowhead).

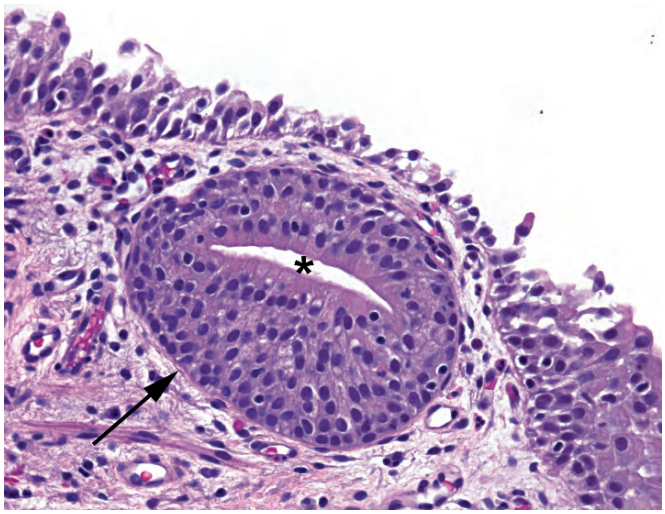

Figure 12.2. Von Brunn's nest and cystitis glandularis. The normal urothelium has invaginated down into the lamina propria, forming a rounded von Brunn's nest (arrow). The center of the nest has acquired a lumen and columnar cell metaplasia (asterisk), which is known as *cystitis glandularis*.

Inflammation (Cystitis)

There are several types of inflammatory disease that you may see. One is *granulomatous cystitis*, which once was largely caused by tuberculosis but is now more likely to be secondary to Bacillus Calmette-Guerin (BCG) therapy—a topical chemotherapy for urothelial carcinoma. The intravesical injection of BCG causes an intense inflammatory response that may wipe out the carcinoma.

Parasitic infection, most commonly by *Schistosoma* species, is still common in undeveloped countries but rare in the United States. The inflammatory response is actually not caused by the organisms but by their eggs, which are extruded into the bladder wall and cause intense foreign-body reaction. *Polypoid cystitis* is similar to an inflammatory polyp of the bladder and is associated

with any process that injures the bladder (e.g., indwelling catheters, calculi, fistula from the colon). *Interstitial cystitis* is a poorly understood disease that is mainly a cystoscopic diagnosis and more a diagnosis of exclusion for the pathologist.

Malakoplakia is one of those mysterious rare entities that most residents do not see, think about, or understand until they are studying for boards. It is a descriptive name for the yellow plaques seen on cystoscopy, which are formed by sheets of epithelioid histiocytes sporting characteristic round inclusions called Michaelis-Guttman bodies (they look like archery targets). It is caused by a defective macrophage response to infection.

Urothelial Neoplasms

Urothelial neoplasms are categorized into two cancer pathways: *flat* and *papillary*. Both can lead to invasive carcinoma, but the terminology is different. About 90% of bladder carcinomas are urothelial, so this will be the focus of this chapter.

Flat neoplasia does not form an exophytic lesion but may still be visible on cystoscopy as a red area. It progresses through dysplasia (rarely diagnosed) to carcinoma in situ. Flat urothelial carcinoma in situ is just known as *carcinoma in situ* (CIS) and can go on to high-stage invasive carcinoma without ever making an exophytic lesion, so always scrutinize the urothelium at high power, especially in denuded areas. Features of CIS include the following:

- Urothelial cells have increased nuclear size. A helpful hint is that the worst nuclei of CIS should be four to five times the size of lymphocyte nuclei (Figure 12.3).
- There are Hyperchromatic nuclei with irregular ("boulder-like") outlines.
- The urothelium has a tendency to fall apart, appearing denuded, with a few clinging cells.
- Full-thickness involvement is *not* a requirement to diagnosis CIS (even scattered malignant cells with the above features justify a diagnosis of CIS).

If a lesion has atypia that you think is neoplastic (i.e., not reactive) yet the cells are not bad enough to call CIS, then the diagnosis of dysplasia is appropriate. However, the options are limited: you should not call mild dysplasia, as urologists do not treat it, nor should you call severe dysplasia, which is the same as CIS. True dysplasia (meaning moderate dysplasia) is an uncommon diagnosis, with most bladder biopsies signed out as normal, CIS, or reactive.

Papillary neoplasia has a much wider spectrum of disease, from benign papilloma to high-grade carcinoma. The papillary lesions are unusual in nomenclature, however, in that despite being *in situ lesions*, they are conventionally just called *noninvasive papillary urothelial carcinoma*, either low or high grade. If you use the word *in situ* to refer to papillary lesions,

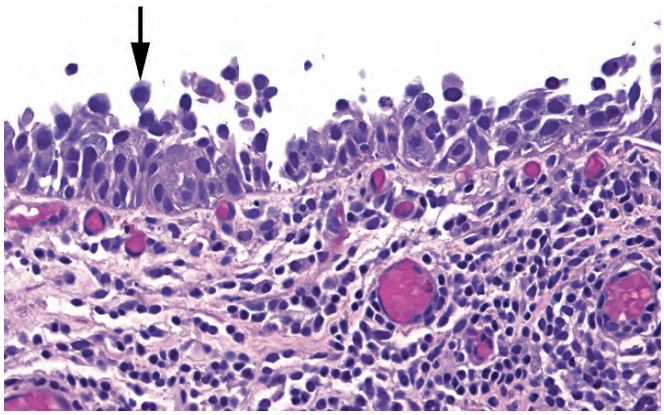

Figure 12.3. Flat carcinoma in situ. The urothelium is partially denuded (stripped of cells), but the cells that remain show enlarged, round, hyperchromatic nuclei appearing to pop off the surface (arrow). Compare the nuclear size to the underlying lymphocytes.

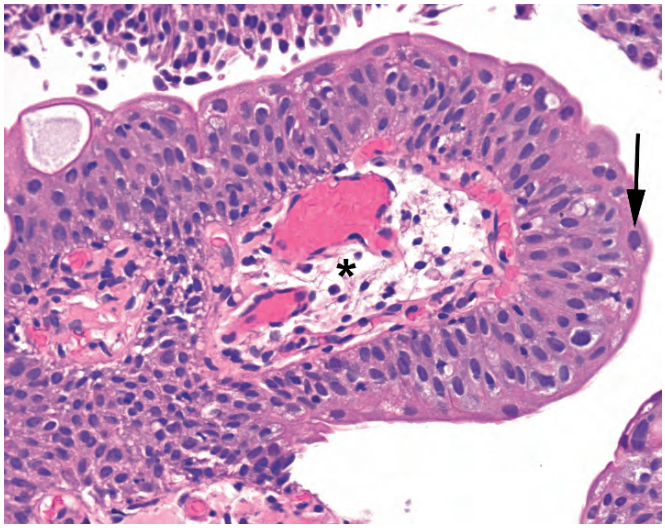

Figure 12.4. Papilloma. There is a prominent fibrovascular core (asterisk), and the urothelium resembles normal urothelium both in thickness and in bland cytology. Some large umbrella cells are visible (arrow).

urologists think you mean CIS. Once these cancers invade, they are called *invasive papillary urothelial carcinoma* and are typically high grade.

Papillary lesions all have in common a branching architecture with delicate fibrovascular cores, and they can get quite large, even filling the bladder. The classification is determined by the cytology of the urothelial lining.

Papilloma

Papillomas are defined by having a normal urothelial lining (normal thickness, well-organized and polarized, and small nuclei, often with nuclear grooves, without mitoses; Figure 12.4). They tend to be small lesions. There is no risk of malignant transformation.

Do not be fooled by papillary hyperplasia, which is an undulating wave-like urothelium without true fibrovascular cores.

Papillary Urothelial Neoplasm of Low Malignant Potential

"Papillary urothelial neoplasm of low malignant potential" (PUNLMP) was recently added to the World Health Organization classification with the intent of creating a category for those proliferative neoplasms that are larger and fuller than papillomas but do not look malignant. The urothelial lining is increased in thickness but still appears well organized, with all nuclei streaming in parallel, and has near-normal nuclear/cytoplasmic ratios (Figure 12.5). Mitoses should be exceedingly rare and confined to the basal layer, as in normal urothelium.

Low-Grade Papillary Urothelial Carcinoma

Low-grade papillary urothelial carcinomas (Figure 12.6) have the following features:

- The urothelial lining is increased in thickness and is still in general fairly organized (the cells are still mostly polarized with respect to the surface).
- There is scattered subtle nuclear atypia consisting of random slightly enlarged darker nuclei, in contrast to PUNLMP, in which every nucleus looks the same.
- Mitoses are uncommon, but typically you will see some of them, in contrast to PUNLMP.

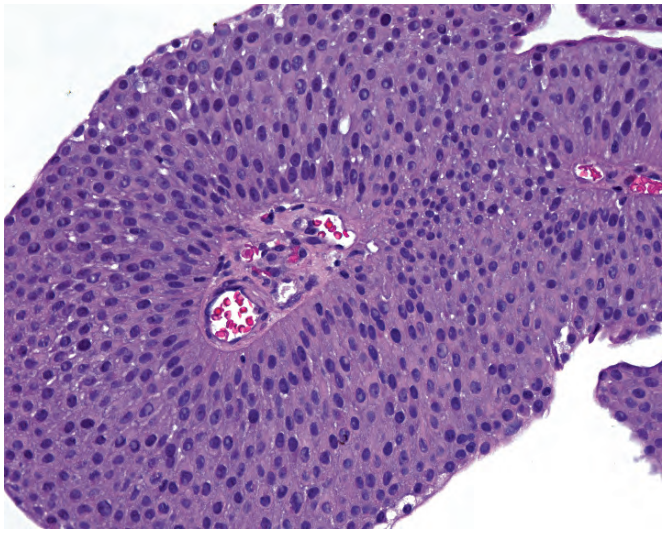

Figure 12.5. Papillary urothelial neoplasm of low malignant potential. This papillary lesion shows an increased thickness relative to normal urothelium, but the cells remain uniform and organized.

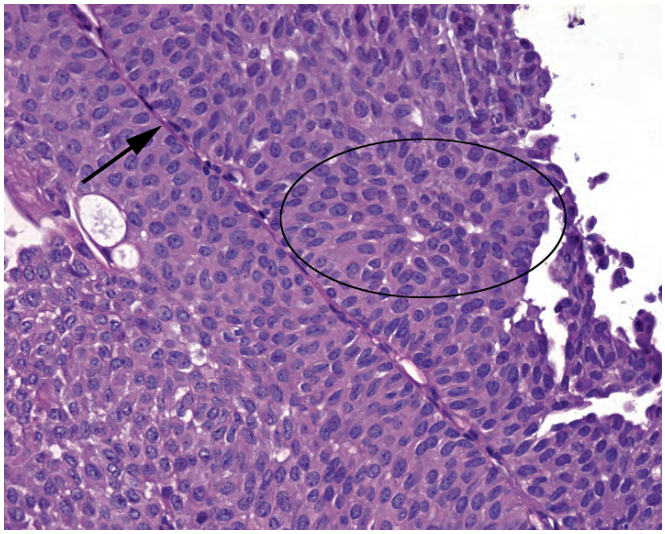

Figure 12.6. Low-grade papillary urothelial cancer. The fibrovascular cores (arrow) are lined by urothelium that is thicker than normal, increasingly disorganized (circle), and with enlarged nuclei.

High-Grade Papillary Urothelial Carcinoma

High-grade papillary urothelial carcinoma lesions are lined by cells that look like CIS. They can be noninvasive, but you have to look carefully for associated invasion, which is often present.

- The urothelium is very disorderly, with little nuclear orientation to the surface.
- Nuclei are enlarged, hyperchromatic, and pleomorphic and may have nucleoli (Figure 12.7).
- Mitoses are seen at all levels of the epithelium (in a well-oriented fragment).
- Focal nonurothelial differentiation (squamous or glandular) is possible.
- A small amount of high-grade characteristics (>5%) generally defines the entire lesion as high grade.

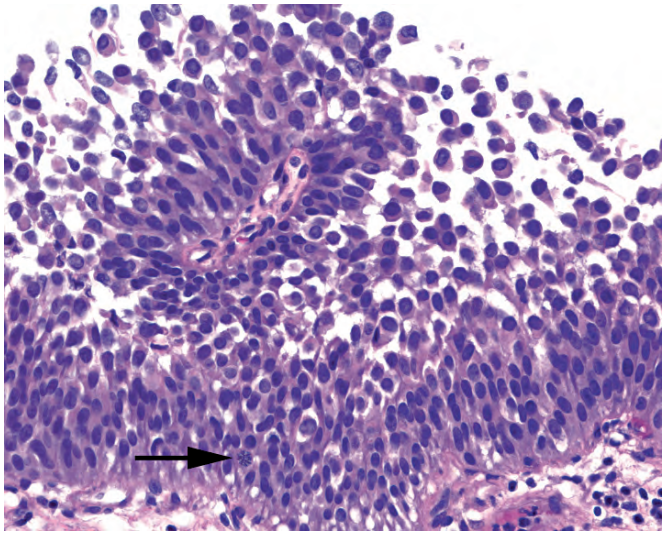

Figure 12.7. High-grade papillary urothelial cancer. This papillary lesion shows large, dark, pleomorphic nuclei popping off the surface, similar to carcinoma in situ (see Figure 12.3). A large mitotic figure is visible (arrow).

Invasive Urothelial Carcinoma (Formerly "Transitional Cell Carcinoma")

Most invasive carcinomas arise in the setting of either high-grade papillary urothelial carcinoma or CIS. Identifying invasion into the lamina propria relies on similar cues as found in other organs:

- Irregular tongues of cells or single cells pushing into the lamina propria
- "Paradoxical differentiation": the deep invasive cells acquire increased pink cytoplasm, mimicking maturing surface cells (Figure 12.8)
- Retraction artifact: the apparent cracking of the stroma away from tumor nests
- Desmoplastic response of stroma (however, often not present)

Identifying the muscularis propria (detrusor muscle), and whether the tumor invades that deeply, is critically important. Superficial carcinomas that do not invade the muscularis propria may be treated conservatively by transurethral resection (TURBT) or topical chemotherapy. Invasion of the detrusor buys the patient a cystectomy. Therefore, any diagnosis of invasive carcinoma should state whether the detrusor is (1) present on the biopsy and (2) involved. Remember that wisps of smooth muscle (the discontinuous muscularis mucosae) may be found in the lamina propria, so do not overcall detrusor invasion on that basis. The detrusor is a big slab of muscle, relatively speaking (Figure 12.9).

Also, as mentioned earlier, ugly urothelium that invades the lamina propria in broad round nests may actually be growth of CIS into von Brunn's nests or an inverted growth pattern of a noninvasive papillary urothelial carcinoma (see the following list). The difference is that in these mimickers of invasion the nests are round and even without ragged borders, and they appear basophilic, often with crowding or palisading of the outermost layer of cells.

- Conditions that look like cancer but are not
  - Inverted papilloma: As in the nose (Schneiderian papilloma), a papilloma can occasionally grow down and in, instead of up and out, creating an inside-out or inverted papilloma that is buried in stroma but does not cross the basement membrane. Like von Brunn's nests, the urothelium should look benign, but the nests may be very closely packed into a small area or coalesced into anastomosing cords.

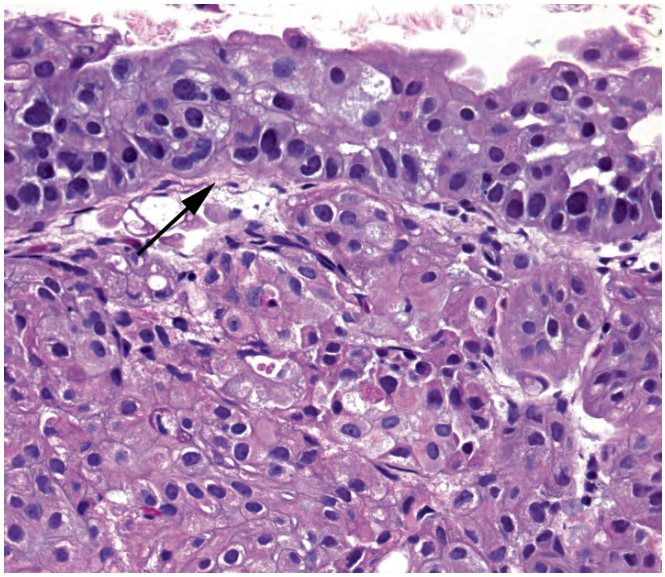

Figure 12.8. Invasive urothelial carcinoma. In this case, the carcinoma is arising out of flat carcinoma in situ, seen above the basement membrane (arrow). The nests of tumor in the lamina propria appear more pink than the surface carcinoma in situ, corresponding to paradoxical differentiation.

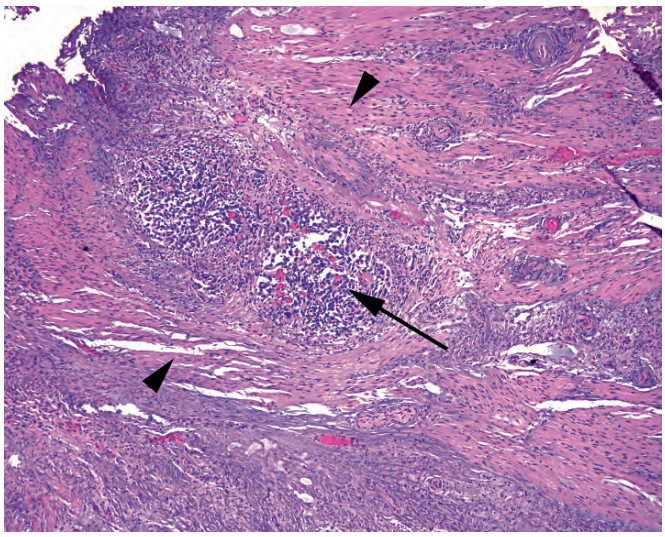

Figure 12.9. Carcinoma in detrusor muscle. Thick bands of muscle (arrowheads) are seen on either side of a nest of tumor cells (arrow).

- Reactive changes in urothelium: As in other organs, reactive changes tend to create enlarged but euchromatic nuclei; the chromatin should be evenly blue grey and the nuclear contour smooth and oval, yet nucleoli may be very large (Figure 12.10). You should raise your threshold for CIS in the presence of extensive inflammation.
- Nephrogenic adenoma: Nephrogenic adenoma is a benign proliferative neoplasm that can take on many appearances, including cuboidal cells lining papillae, hobnail cells lining vessel-like structures (Figure 12.11), small infiltrative-looking tubules, sometimes with

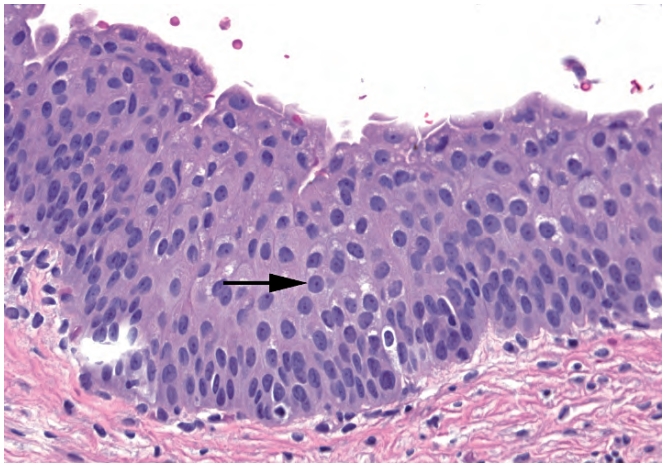

Figure 12.10. Reactive nuclei. These urothelial nuclei are somewhat enlarged and have prominent nucleoli (arrow) but retain a smooth nuclear outline and pale, even chromatin. They are benign.

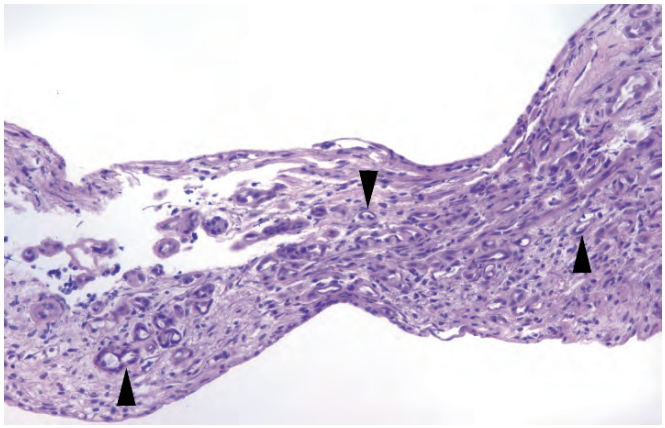

Figure 12.11. Nephrogenic adenoma. In this bladder biopsy specimen, there are multiple tiny tubules in the lamina propria (arrowheads) with prominent dark nuclei. The urothelium is not seen here.

thyroid-like accumulations of colloid, and small tubules mimicking signet-ring cell carcinoma. In all cases, these differ from urothelial lesions by being a single-layered cuboidal epithelium. There may be focal large dark nuclei, but they should have uniform dense chromatin and no mitoses.

- Conditions that look benign but are not
  - Nested transitional cell carcinoma: This is an invasive urothelial carcinoma made of small, bland nests in the lamina propria that, despite looking like von Brunn's nests, is actually an aggressive carcinoma. Great, right? Features suggestive of this lesion include an infiltrative pattern at the base of the lesion, as well as an architecturally complex pattern of closely packed small nests.
  - Lymphoepithelial-like carcinoma: While this certainly does not look like normal bladder, lymphoepithelial-like carcinoma can be very sneaky to the pathologist in training. The overall impression is that of raging inflammation and tissue destruction, with sheets

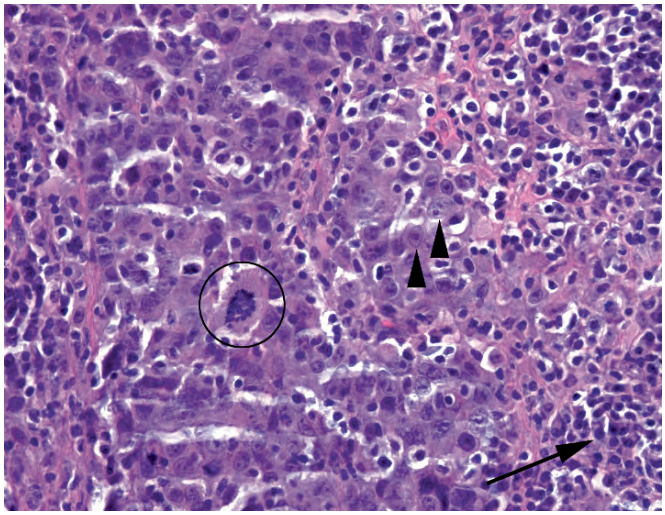

Figure 12.12. Lymphoepithelial-like carcinoma. The malignant cells (arrowheads) are almost obscured by the background of lymphocytes (arrow). Atypical mitoses are present (circle).

of lymphocytes, but the actual carcinomatous cells tend to fade into the background on H&E stain. The nuclei tend to be large and bubbly, but not particularly hyperchromatic or carcinoma-like, and the cytoplasmic borders are very indistinct, almost syncytial (Figure 12.12). A cytokeratin stain is helpful.

13 Kidney

Neoplasms

The kidney is primarily composed of glomeruli, tubules, stroma, and vasculature. However, unlike in some other organs, the neoplasms of the kidney do not faithfully reflect or recapitulate their cells of origin. Therefore, recognizing a lesion is not so much a systematic process as a pattern recognition. However, there are certain features to notice in evaluating any kidney mass, as they will help narrow the differential diagnosis in tough cases:

- Circumscription and/or encapsulation
- Presence of stroma within the tumor
- Vascular or capillary pattern
- Architectural pattern (solid, acinar, trabecular, tubular, papillary, pseudopapillary, cystic)
- Cellular pleomorphism (monotonous to bizarre)
- Mitotic activity
- Cytoplasm (clear to granular pink to densely eosinophilic; perinuclear halos)
- Nuclear size and contour (note the shape and whether the membrane is smooth or wrinkled)
- Nucleoli

When studying the kidney grossly, many details crucial to staging are identified (or lost) at the bench. Key prognostic factors include the following:

- Tumor extending through the kidney capsule and into the perirenal fat
- Tumor invading adrenal gland (always note whether the adrenal is even present)
- Gross tumor in the renal vein, both at the margin and in the renal pelvis (always open the renal vein)
- Tumor growing through Gerota's fascia (the very delicate membrane surrounding the perirenal fat; this is actually fairly uncommon but indicates stage IV disease)

Other helpful gross features include the following:

- Circumscription and presence of multiple lesions
- If cystic, multilocular versus unilocular, the presence of mural nodules, relationship to pelvis
- If solid, the homogeneity and the color(s)—yellow gold, mahogany brown, areas of hemorrhage, necrosis, fibrosis (gristle grey), or possible sarcomatoid foci (dense white)
- Site of origin (cortex vs. medulla or pelvis), if you can tell

Now that you have the key identifying features of your tumor, let us look at the differential diagnosis for tumors *in the adult*.

Cystic Lesions

Simple Cyst

Simple cysts are a very common finding, even at autopsy. The simple cyst is essentially a dilated tubule and will have a low cuboidal or flattened pink epithelial lining (Figure 13.1). It is usually unilocular. If multilocular, the septa dividing the cysts should be unremarkable stroma with no epithelial islands or nodules. *There should be no clear cells*.

Cystic Nephroma

Cystic nephroma is an uncommon lesion, but file it away as "one of those ectopic-ovarian-type-lesions in women." This is a multilocular cyst with a background of ovarian-type stroma

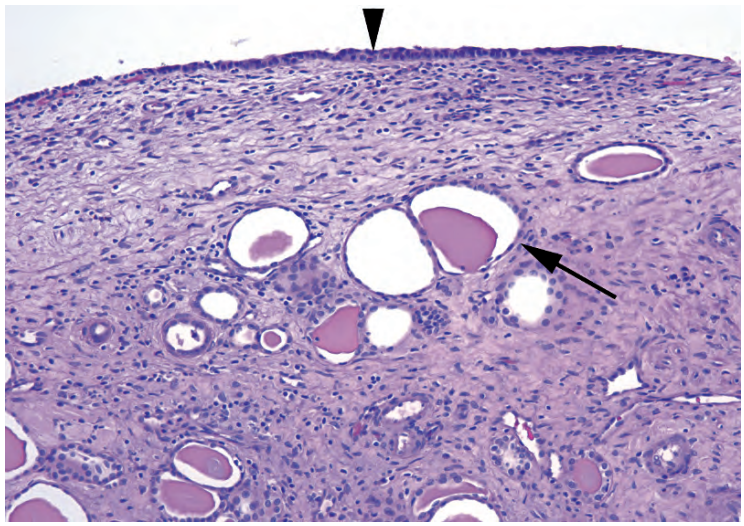

FIGURE 13.1. Simple cyst. The cyst lining (arrowhead) consists of a thin layer of cuboidal cells. Below the cyst, dilated tubules filled with proteinaceous fluid are visible (arrow).

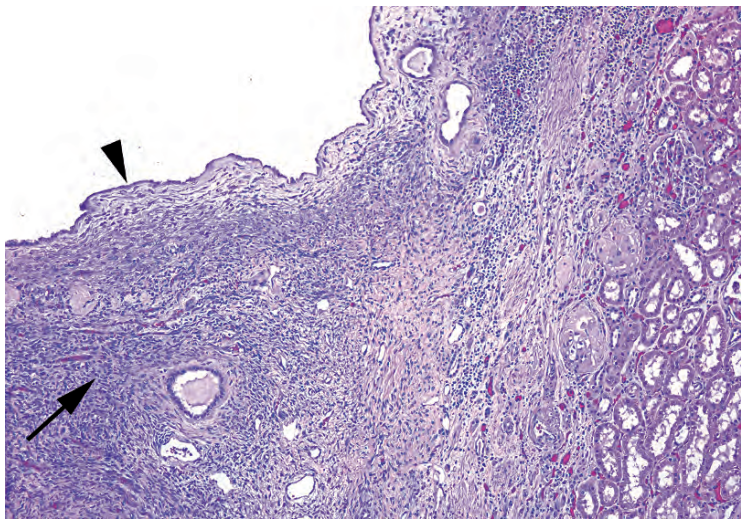

FIGURE 13.2. Cystic nephroma. Like the simple cyst, this cyst is lined with bland epithelial cells (arrowhead). However, there is adjacent spindly stroma, similar to ovarian stroma (arrow). Kidney parenchyma is seen at right.

(fairly blue, cellular, spindly, and estrogen and progesterone receptor positive; Figure 13.2). The cyst lining is cuboidal to hobnailed. *There should be no clear cells*.

Mixed Epithelial-Stromal Tumor

The mixed epithelial-stromal tumors may be cystic, but are discussed with solid lesions, below.

Renal Cell Carcinoma

Conventional (clear cell) renal cell carcinoma can present as a cyst in several ways. First, it can arise in the wall of a preexisting simple cyst. Second, it can undergo cystic degeneration of a solid tumor. Third, and most sneaky, it can occur purely as a cyst lining, usually in a multilocular cyst: this is called *multilocular cystic renal cell carcinoma*. The main indicator is *the presence of clear cells in the cyst wall* (Figure 13.3). The cyst walls may be denuded of epithelium, though, so careful sampling and hunting are essential.

Lesions With Multiple Cell Populations

Angiomyolipoma

The angiomyolipoma is, at first, a difficult lesion to recognize, because it looks like just a mishmash of normal soft tissue components. From the name, you know that it must have vessels, smooth muscle, and fat, but then so do most organs of the body. Also working against you is the fact that these lesions can have one or two components predominating, so all you see is a mass of plump spindly cells with a vessel here and there, and maybe a couple of fat cells. The key to recognizing an angiomyolipoma is knowing that you have a mass lesion and appreciating the unusual vessels that are the hallmark of this tumor.

This tumor is benign. The usual histologic features include the following:

- Large, tangled, tortuous, thick-walled, hyalinized vessels
- Smooth muscle cells (pink to clear and spindly) that seem to spin off of, or be continuous with, the vessel walls (Figure 13.4)

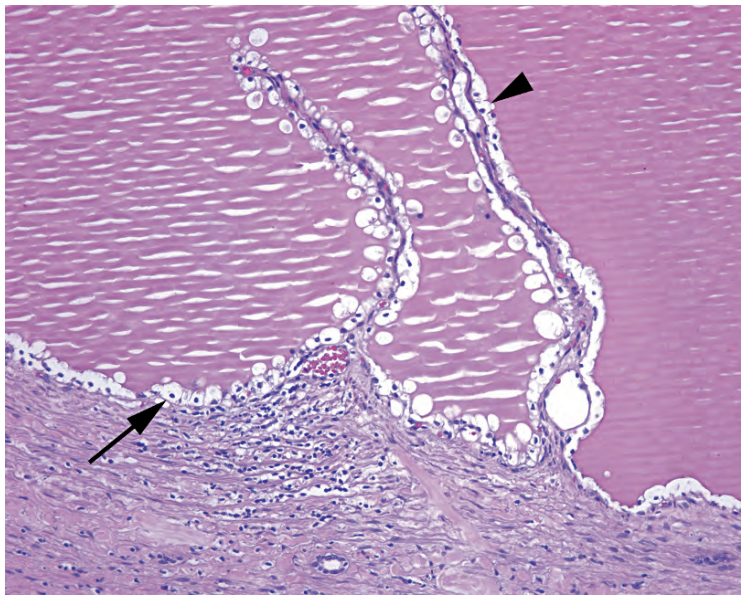

Figure 13.3. Multilocular cystic renal cell carcinoma. The cyst and fibrovascular septa (arrowhead) are lined by single clear cells with small dark nuclei (arrow); compare these cells to conventional renal cell carcinoma (see Figure 13.6).

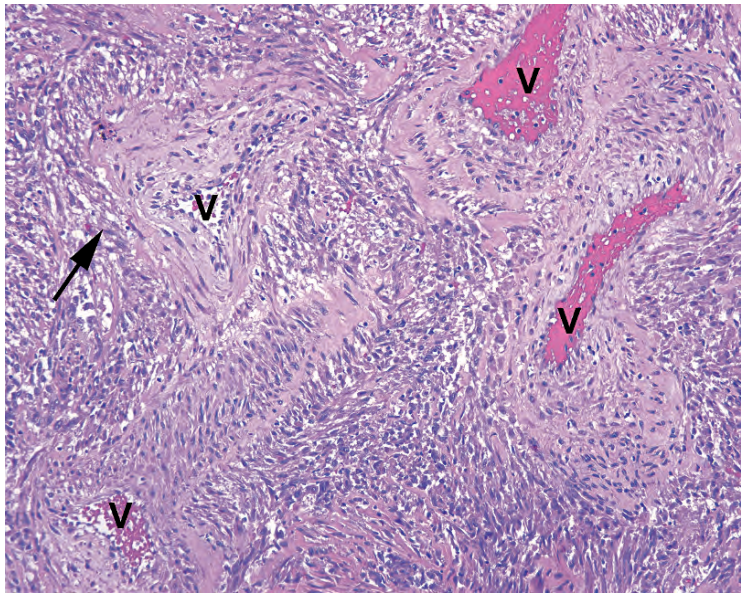

Figure 13.4. Angiomyolipoma. This example does not show the fatty component, but the prominent vessels (V) and smooth muscle components here are classic. In angiomyolipoma, the spindle cells seem to merge with, or spin off from, the thick-walled vessels (arrow).

- Mature fat cells without atypia or lipoblasts
- Pushing borders but not encapsulated
- HMB-45 and Melan-A positive (this tumor is in the perivascular epithelioid cell tumor family, all of which stain for melanoma markers)

Mixed Epithelial-Stromal Tumor

The mixed epithelial-stromal tumor, although rare, is simple in concept: it is the renal version of an adenofibroma, or a fibroadenoma, or any other benign mixture of stromal and epithelial elements. Because it can be cystic, it is also included in the differential diagnosis of cystic lesions, discussed earlier. The histologic findings include a population of cytologically benign tubules of varying shapes and sizes set in a background of bland spindled stroma, which may consist of smooth muscle, fibroblasts, or myofibroblasts. This may also be in a spectrum with cystic nephroma (discussed earlier), because it also has estrogen receptor– and progesterone receptor–positive stroma.

Solid Neoplasms

Clear Cells

The presence of clear cells in a renal tumor immediately puts renal cell carcinoma at the top of the differential. For all practical purposes, there are no benign clear cell lesions. A 3-mm clear cell focus is still a clear cell carcinoma, albeit a fairly nonthreatening one. Renal cell carcinoma is now understood to have multiple variants, but the clear cell variety is often subtitled "conventional." *Note*: Avoid the big, embarrassing, novice mistake number 1—mistaking the normal adrenal cortex for a clear cell tumor. The adrenal clear cell should have visible vacuoles that indent the nucleus, giving it a stellate outline (Figure 13.5).

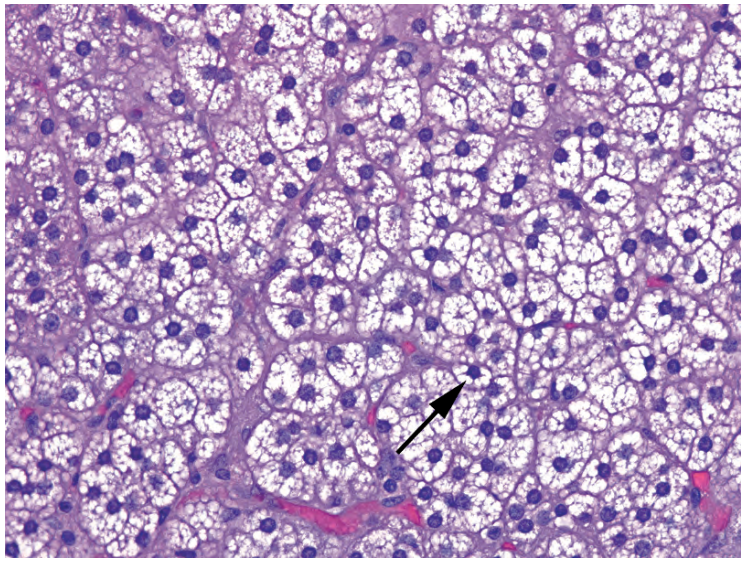

Figure 13.5. Normal adrenal cortex. Unlike clear cell carcinoma, the cells of the adrenal cortex have discrete cytoplasmic vacuoles that indent the nuclei, creating a stellate outline around the nucleus (arrow).

Renal Cell Carcinoma, Conventional or Clear Cell Type

Renal cell carcinoma is a common tumor that usually appears grossly as a granular, golden-yellowish-orange, well-circumscribed tumor, looking very much like normal adrenal tissue. It may get quite large and have areas of necrosis, hemorrhage, cystic degeneration, and fibrosis. All different-looking areas should be sampled, especially the firm solid white-to-grey areas, which could indicate sarcomatoid transformation.

Histologically, the tumor may be solid with an acinar pattern, pseudopapillary (which is an acinar pattern with centroacinar dropout), or cystic. Areas of sheeting, spindly, sarcomatoid growth will bump up the tumor to grade IV. Identifying features include the following:

- A net-like array of delicate capillaries, dividing cells into packets ("acinar" pattern)
- Clear cytoplasm, at least focally if not diffusely (Figure 13.6)
- Delicate, distinct cell membranes
- Lack of desmoplasia (although sclerosis of burned-out tumor is common)

Conventional renal cell carcinoma is graded cytologically according to *Fuhrman grade*. Low-grade tumors have clear cytoplasm, polygonal cells, and round nuclei. Higher grade tumors get pink and pleomorphic. Grade criteria, with a 10× objective (Figure 13.7), are as follows:

- Grade I: nuclei resemble lymphocytes, no nucleoli *(rarely used)*
- Grade II: nuclei still small and without nucleoli, but with open chromatin
- Grade III: easily recognizable nucleoli, larger nuclei
- Grade IV: pleomorphic and hyperchromatic nuclei with big nucleoli

Renal Cell Carcinoma, Chromophobe Variant

The chromophobe is a carcinoma that has some features of conventional renal cell carcinoma and some features of the oncocytoma. It is, overall, very pale pink under the microscope. It is not encapsulated, and it grows as a solid to papillary mass. Features include the following:

- Distinct cell membranes that give the tumor a three-dimensional texture, like alligator skin (Figure 13.8)
- Cells of varying sizes and shapes
- Pink, granular, wispy cytoplasm, often with a perinuclear clearing

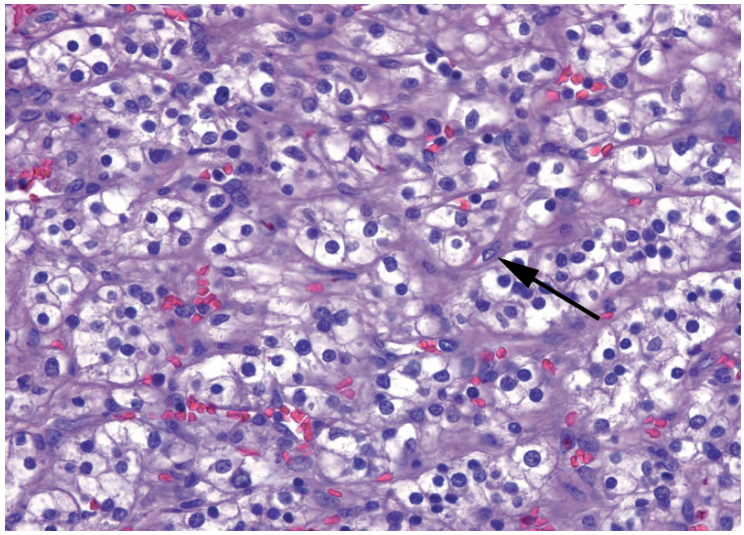

FIGURE 13.6. Clear cell renal cell carcinoma. The tumor is composed of packets of clear cells, divided by delicate fibrovascular septa (arrow). These septa are characteristic of renal cell carcinoma and are seen even in high-grade or metastatic tumors. The nuclei in this example are enlarged, but nucleoli are visible only at high power, consistent with Fuhrman grade II.

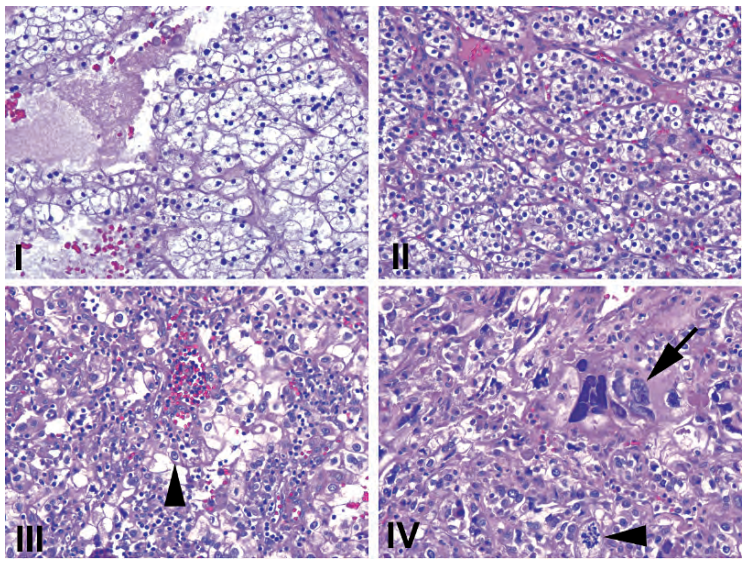

FIGURE 13.7. Fuhrman grades shown at 10×. **(I)** Nuclei are small and dense, resembling lymphocyte nuclei. **(II)** Nuclei are larger, but no nucleoli are visible at this power. **(III)** Nuclei are even larger, now with some visible nucleoli (arrowhead). **(IV)** Nuclei are frankly anaplastic (arrow) with large atypical mitoses (arrowhead). All images are taken at the same magnification.

- Nuclei that vary in size and shape and are crinkly, giving a koilocytic look (see Figure 13.8)
- Cytoplasm positive for Hale's colloidal iron
- Can transform to sarcomatoid morphology

The *eosinophilic variant of chromophobe* can look at low power like an oncocytoma, but the nuclei should still have a koilocytic flavor, unlike the very round and regular nuclei of the oncocytoma.

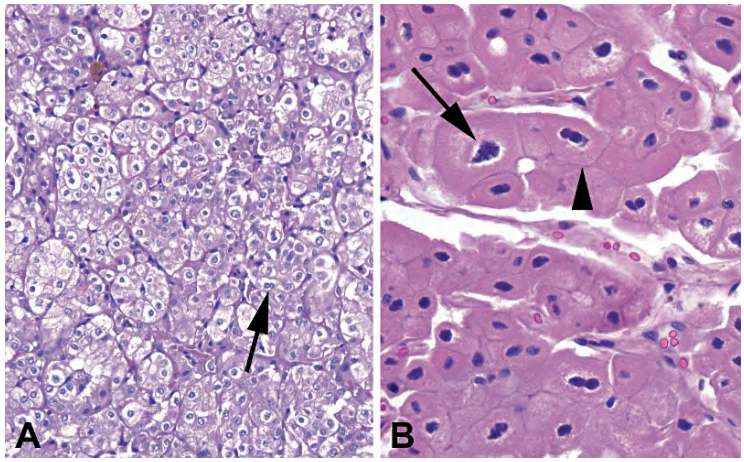

Figure 13.8. Chromophobe carcinoma. **(A)** Low-power view of a chromophobe, showing packets of cells with clear-to-pink cytoplasm, perinuclear halos, and occasional binucleate cells (arrow). The cell membranes are distinct, giving the tumor a cobblestone or alligator-skin texture. **(B)** High-power view of a chromophobe carcinoma, eosinophilic variant. Although the granular pink cytoplasm resembles an oncocytoma (see Figure 13.9), the nuclei are distinctly koilocytic, with crinkly outlines and perinuclear halos (arrow). In addition, the crisp cellular membranes are preserved (arrowhead).

Pink Cells

If the cells are not clear, your differential diagnosis includes the following:

- Chromophobe (discussed above).
- High-grade conventional renal cell carcinoma (discussed above).
- Oncocytoma: Oncocytoma is a benign tumor resembling oncocytes (or Hurthle cells) in other organs. Grossly, it is mahogany brown and well circumscribed but not encapsulated. There may be a stellate scar (a nonspecific sign of slow growth). The oncocytes are arranged in nests or cords of cells in a hypocellular stroma. The cells are round with dense pink cytoplasm and very regular, round nuclei (Figure 13.9). This regularity should strike you at low power, very different from a chromophobe. The oncocytoma is not graded. Features incompatible with this diagnosis include mitoses, papillary architecture, clear cells, and grossly identified vascular invasion.
- Papillary renal cell carcinoma: Papillary renal cell carcinoma is a cellular tumor of pink-to-blue cells (low-nuclear-grade tumors tend to be blue at low power, and high-nuclear-grade tumors tend to be pink; this seems backward) that may be arranged in papillary formations (helpful), solid sheets, or trabecular cords. The classic image is that of a fibrovascular core packed with foamy macrophages and lined by cuboidal cells with round nuclei (Figure 13.10). This image is so pathognomonic that if you find it, you are basically done. You may also see psammoma bodies, hemosiderin-laden cells, and focal clear cells.
  - Papillary adenoma: By definition, a papillary adenoma is a papillary and *non–clear cell* neoplasm of low nuclear grade and less than 5 mm in diameter.
  - Xp11: there are several translocation-defined renal cell carcinomas involving the *TFE3* gene on Xp11. They occur in young adults. Histologically, they can be summed up as clear cell tumors with papillary architecture.
- Collecting duct carcinoma: A collecting duct carcinoma is a high-grade tumor that arises in the medulla. It looks and acts much like an adenocarcinoma. The cytology is clearly malignant, there is a desmoplastic response, and it may stain for mucin and carcinoembryonic antigen. However, it is rare. Rarer still is the variant of collecting duct carcinoma found in sickle cell trait patients, the *medullary carcinoma*.

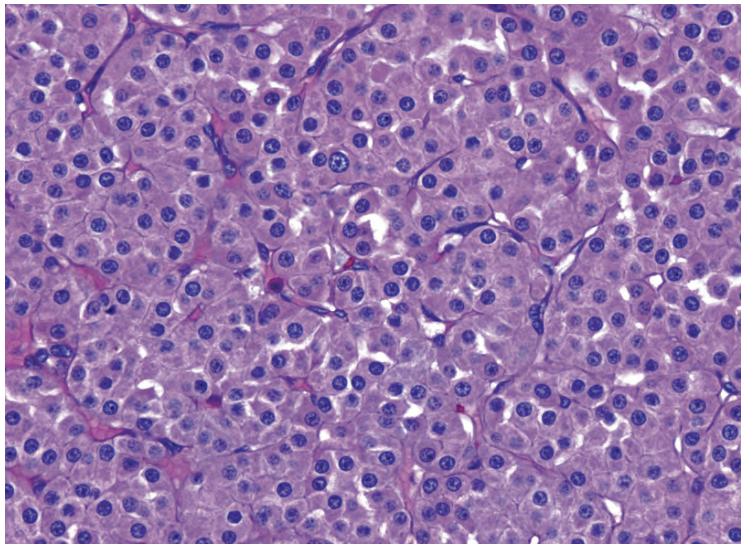

Figure 13.9. Oncocytoma. The nuclei are typically very round, uniform in size, and evenly spaced. Nucleoli may be seen, but there are no perinuclear halos. The cytoplasm is pink and granular, similar to oncocytic neoplasms elsewhere in the body.

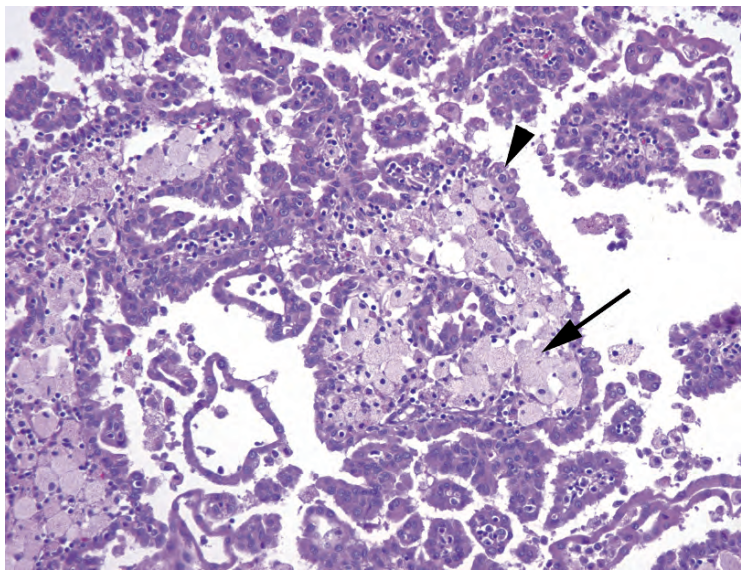

Figure 13.10. Papillary renal cell carcinoma. The tumor cells are eosinophilic, not clear, and range from cuboidal to columnar (arrowhead). This tumor may grow as solid sheets and tubules, but finding papillary structures with central cores packed with foamy histiocytes (arrow) is diagnostic. Although the tumor in this example is low grade cytologically, the cells have a relatively high nuclear/cytoplasmic ratio, and therefore this would be somewhat blue on low power.

Blue Cells

When the tumor looks blue, the differential diagnosis includes the following:

- Metanephric adenoma (blue, indigo blue, lymph node blue): Metanephric adenoma is usually a 1× diagnosis. It is a circumscribed but nonencapsulated tumor of monotonous, small, tightly packed, dense blue cells (Figure 13.11). It has little or no cytoplasm. The patterns range from tiny tubules to serpiginous gland-like structures. If this looks like a Wilms' tumor to

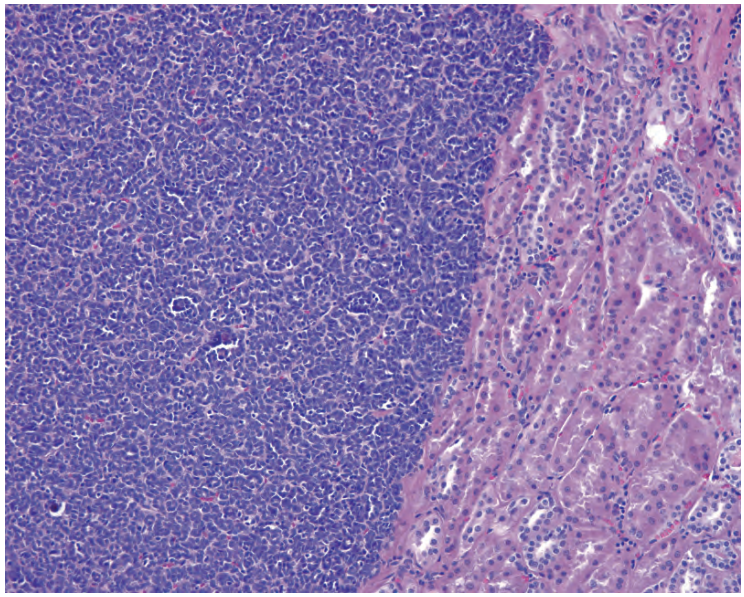

Figure 13.11. Metanephric adenoma. This benign tumor is the bluest of them all because of the very high nuclear/cytoplasmic ratio of the cells. Here you can see tiny primitive blue tubules on the left, adjacent to normal kidney on the right.

you, you are an astute observer. The metanephric adenoma may be essentially a differentiated (mature) form of a pure epithelial Wilms' tumor.

- Wilms' tumor: Wilms' tumors are unusual in adults. See the following discussion of the pediatric population.

A Brief Introduction to the Pediatric Kidney

The most common pediatric tumor is Wilms' tumor, or nephroblastoma, one of the small round blue cell tumors of childhood.

Definition of Terms

Nephrogenic rests: abnormally persistent foci of embryonal cells (small, round, and blue) that may develop into Wilms' tumor, although most do not

Blastema: sheets of undifferentiated embryonal cells in a Wilms' tumor, resembling small cell carcinoma

Anaplasia: unfavorable histology in a Wilms' tumor, defined by large, hyperchromatic nuclei and abnormal mitotic figures (tripolar)

Wilms Tumor

Wilms' tumor is defined by triphasic histology, which means you should see three components (Figure 13.12): blastema (undifferentiated, very blue), stroma (generally less cellular, more pink), and epithelium (blue like blastema but organized into tubules). One component may predominate. Histology is defined as favorable or nonfavorable, based on the presence of anaplasia. Finding foci of anaplasia requires extensive sampling and eye-grinding hunting. A Wilms' tumor may arrive at your bench post-chemotherapy. Chemotherapy changes include massive necrosis, fibrosis, histiocytic replacement, and maturation of the immature elements. One common finding is maturation to skeletal muscle cells.

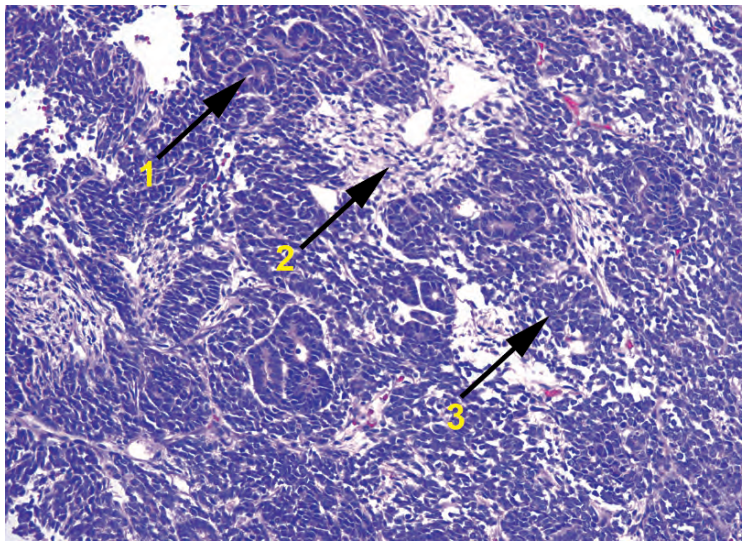

Figure 13.12. Wilms' tumor. This small round blue cell tumor classically has three components: (1) epithelium, in which the cells form primitive tubules; (2) stroma, the mesenchymal component; and (3) blastema, the most primitive and undifferentiated component. Ratios may vary by tumor.

Wilms' tumor, like renal cell carcinoma, can grossly resemble a multilocular cyst. This is called a *cystic partially differentiated nephroblastoma*. The three components are the same.

Other Pediatric Tumors

Congenital mesoblastic nephroma is a low-grade sarcoma that can resemble fibromatosis (classic type) or fibrosarcoma (cellular type). *Metanephric stromal tumor* is a spindle cell tumor that infiltrates and entraps native elements such as tubules and blood vessels. Other more aggressive tumors include *clear cell sarcoma* and *rhabdoid tumor*. Clear cell nomenclature, which is actually somewhat less than clear, is summarized in the following section.

A Note on Clear Cell Features, in General (Adults and Children)

You know about *clear cell renal cell carcinoma* (classically positive for cytokeratins and epithelial membrane antigen [EMA]). Now you also know about the *translocation tumors of the kidney*, which are clear cells on a papillary core and which arise due to translocations of the *TFE3* gene. This same gene can be translocated in the soft tissues, in which case you get *alveolar soft part sarcoma*, which may or may not be clear-cell but does resemble renal cell carcinoma because of its delicate capillary network and alveolar architecture (hence the name). This soft tissue tumor may be confused with the *clear cell sarcoma of soft tissue*, otherwise known as *melanoma of soft parts*. Like alveolar soft part sarcoma, it has an alveolar pattern and clear cells; however, it stains for the melanoma markers (S100, Melan-A, and HMB45). This tumor should not be confused with the *clear cell sarcoma of kidney*, which is totally unrelated to the clear cell sarcoma of soft tissue and is negative for most markers, including S100, cytokeratin, and EMA. However, if you are in the kidney and you have a lesion that is staining for HMB45 and Melan-A, you are most likely looking at an *angiomyolipoma*, which is a benign tumor having nothing to do with melanocytes but which does stain for the melanoma markers. Is this clear?

Medical Kidney (Non-neoplastic)

The four main compartments of the nonneoplastic kidney are the glomeruli, the tubules, the interstitium, and the vessels. The compartments are differentially affected by systemic diseases, toxins, and so forth. When evaluating the biopsy, you are looking for the following:

- In the glomeruli: the percentage of globally sclerosed glomeruli, hypercellularity (mesangial vs. endocapillary), inflammatory cells, the thickness of mesangial matrix, segmental sclerosis, hyalinosis, crescents, thrombi, and changes in the basement membrane of the capillary loops (especially by PAS and silver stains)
- In the tubules: acute and chronic inflammation in the epithelium or lumen, injury (epithelial vacuolization, necrosis, or sloughing), cellular or hyaline casts, Tamm-Horsfall protein accumulation, atrophy (dropout)
- In the interstitium: inflammation, fibrosis (especially by trichrome stain), edema
- In the arteries and arterioles: intimal thickening, hyaline deposits, emboli, thrombotic microangiopathy (fibrin thrombi, red blood cell fragments in capillary walls, fibrinoid necrosis)

This chapter does not go into great detail on these nonneoplastic entities, except to put them into the very big picture.

Acute and Chronic Damage Patterns

Nephritic Presentations

Acute injury to the glomerulus (usually immune mediated) leads to a picture of *acute glomerulonephritis* (hematuria, proteinuria, oliguria, azotemia, edema, hypertension). Histologically, the glomerulus responds with increased cellularity, which includes mesangial cells, endothelial cells, and inflammatory cells. This is a *proliferative glomerulonephritis*, and in this setting you will also see an interstitial response (edema and inflammation) and red cell casts in the tubules. Causes of this acute injury include postinfectious glomerulonephritis, IgA nephropathy, and lupus.

S*evere acute injury* causes an even more proliferative response in the form of *cellular crescents*. These are collections of epithelial and inflammatory cells in Bowman's space, hugging the glomerulus, and they are an indication of severe glomerular injury. You may also see necrosis of the glomeruli, fibrin deposition, and disruption of the basement membrane. Clinically, this appears as a *rapidly progressive glomerulonephritis* (which is the symptoms of glomerulonephritis plus acute renal failure) and causes include anti–glomerular basement membrane nephropathy (Goodpasture's syndrome), vasculitis, and anything that can cause a proliferative glomerulonephritis (see above).

Most of the above diseases are *immune-complex mediated*, so classification of the location and type of immune complex is key to subclassifying the disease. Immune complexes can be seen by electron microscopy as electron-dense areas, and their location with respect to the basement membrane is important. Immunofluorescence utilizes individual stains for IgG, IgM, IgA, and complement (C1q, C3), and their distribution also helps narrow the diagnosis. Most immune complex diseases have granular immunofluorescence staining, with the exception of anti–glomerular basement membrane disease, which has linear staining of the basement membrane.

Nephrotic Presentations

Injury that is limited to the *glomerular basement membrane* or the *podocytes* can produce a much more subtle picture. Destruction of the foot processes of the podocytes, which line the basement membrane, or disruption of the basement membrane itself, can lead to a leaky glomerulus that just shows up as proteinuria. Severe proteinuria, and the subsequent edema, hypertension, and so forth, are called the *nephrotic syndrome*. Diseases in this category include minimal change disease, focal segmental glomerulosclerosis, membranous glomerulonephritis, and membranoproliferative glomerulonephritis. Many other nonprimary renal diseases can also produce this picture, including diabetes, amyloid, lupus, drugs, and infections.

Of the four primary renal diseases listed earlier, two (minimal change and focal segmental glomerulosclerosis) are not immune complex mediated. They have little or no increase in cellularity and no immunofluorescence findings. You should see evidence of foot process damage by electron microscopy but no deposits. However, membranous and membranoproliferative are immune mediated. Both show thickened and disrupted basement membranes, granular immunofluorescence staining, and ultrastructural deposits. Membranoproliferative glomerulonephritis also has an inflammatory cellular component, so it has an added hypercellular (*hyperproliferative*) picture as well as clinical evidence of inflammation (a nephritic picture in addition to the proteinuria).

Chronic injury to the kidney produces more of a sclerotic and scarring response, as in other organs. Chronically injured glomeruli become globally sclerotic and look like whorled amorphous pink blobs in the cortex. Chronically injured tubules become flattened, sparse, and dilated, surrounded by interstitial fibrosis and chronic inflammation. When these changes are extensive, you have end-stage kidney and chronic renal failure, and it can be impossible to figure out what the original injury was.

Most of the diagnoses listed earlier are *patterns of injury*. While they can be primary renal processes, they can also represent the kidney's response to systemic diseases. Infection, drugs, and lupus are all examples of systemic diseases that can cause more than one type of kidney damage.

Diabetes and *hypertension* are common, and both are hard on the kidney. Diabetic nephropathy includes thickened basement membranes and increased mesangial matrix; it is not immune complex mediated. The hemodynamic alterations of diabetes also predispose the kidney to glomerulosclerosis, which may be nodular (the Kimmelstiel-Wilson bodies) or eventually global (end-stage kidney disease). Hypertension causes vascular changes in the kidney, including intimal fibrosis of arteries and hyaline deposits in arterioles.

Tubular diseases include acute interstitial nephritis and acute tubular necrosis. Acute interstitial nephritis is reversible damage secondary to drugs and is often associated with eosinophils. Acute tubular necrosis is acute and severe damage to the tubules causing acute renal failure. It may be caused by ischemia or a toxin.

Transplant rejection occurs in at least three forms: acute humoral, acute cellular, and chronic. Each form has specific criteria and an associated grading system. The features to look for include the following:

- In humoral rejection: glomerulitis, tubular injury, margination of neutrophils, and C4d staining in the peritubular capillaries
- In acute cellular rejection: glomerulitis, interstitial inflammation, tubulitis, and intimal arteritis
- In chronic rejection: glomerulopathy (double contours in basement membrane), mesangial matrix increase, tubular atrophy, interstitial fibrosis, intimal thickening of arteries, and hyaline thickening of arterioles

These types of rejection need to be separated from recurrence of the original disease process, preexisting donor disease (often vascular), and cyclosporine toxicity (tubular injury).

14 Testis

The testis is not a common specimen. Resections in children or young adults may be due to a nonneoplastic condition such as torsion, which produces the relatively nonspecific picture of hemorrhage and/or ischemic necrosis. Uncommonly in young children, undescended (cryptorchid) testes are removed because of an increased risk of developing germ cell tumors. There are also a few tumors that typically only occur in children. Most testicular tumors occur in men in their twenties to forties, although they also occur in the elderly.

Normal Histology

The testis is composed of a tightly packed collection of tubules. In the prepubertal testis, the tubules are lined with spindly, radially arranged Sertoli cells and rare spermatogonia. After puberty, spermatogenesis begins, and the tubules are dominated by the developing spermatocytes. Maturation is completed near the lumen of the tubules, where you can see tiny sesame seed–like spermatids (which grow tails to become spermatozoa). Polygonal pink Leydig cells in the interstitium produce testosterone (Figure 14.1).

Sperm leave the testis via the rete testis (Figure 14.2), a collection of epithelial-lined slit-like channels at the hilum of the testis, which lead into the epididymis, which eventually feeds into the vas deferens. The epididymis is lined by a pseudostratified and ciliated epithelium (Figure 14.3).

Orchiectomy in Infants and Children

In the setting of an undescended testis, the testis may be removed to prevent the development of a germ cell neoplasm. The typical *cryptorchid testis* shows small atrophic seminiferous tubules, fibrosis, and widened interstitial spaces (Figure 14.4). A related finding is the "vanishing testis syndrome" in which, upon surgical retrieval of the undescended testis, there is nothing but a nub of fibrosis and dystrophic calcification attached to an epididymal remnant. These conditions are generally signed out descriptively.

Infertility

A testis biopsy may be indicated in the workup of a persistent low sperm count (male infertility). From the pathologists' perspective, the options are the following:

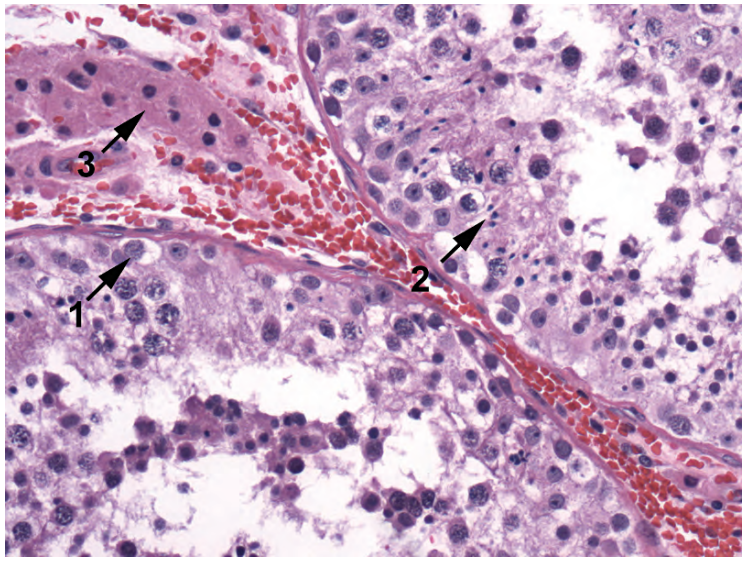

Figure 14.1. Normal seminiferous tubules. Large spermatogonia with clear cytoplasm are present at the tubule periphery (1). The developing spermatocytes have a wide range of morphologies, ending with the tiny spermatids (2), a marker of successful spermatogenesis. Plump pink Leydig cells are seen in the interstitium (3).

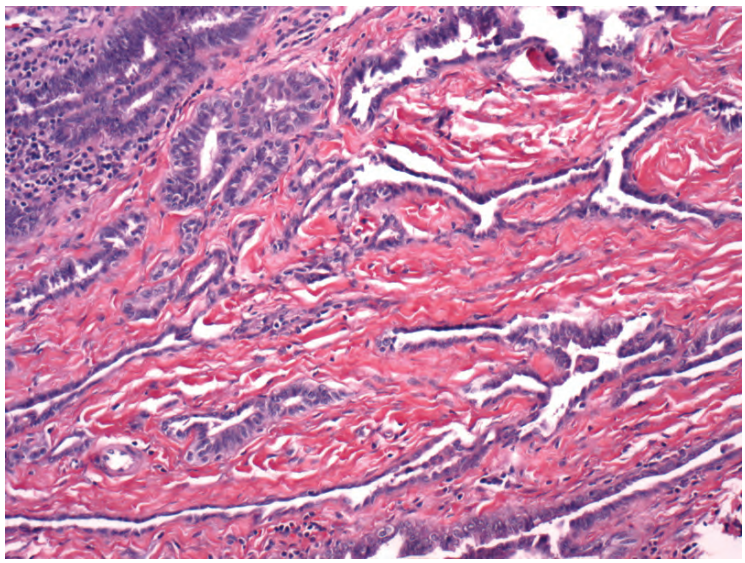

Figure 14.2. Normal rete testis. Slit-like spaces with cuboidal epithelium.

- Aplasia (or Sertoli-only syndrome, a total lack of germ cells; Figure 14.5)
- Hypospermatogenesis (decreased spermatogenesis in most tubules)
- Maturation arrest (when there is partial maturation but no spermatids produced)
- "End-stage testis" (global sclerosis and atrophy, no functioning tubules)
- Normal spermatogenesis (implying a distal obstruction)

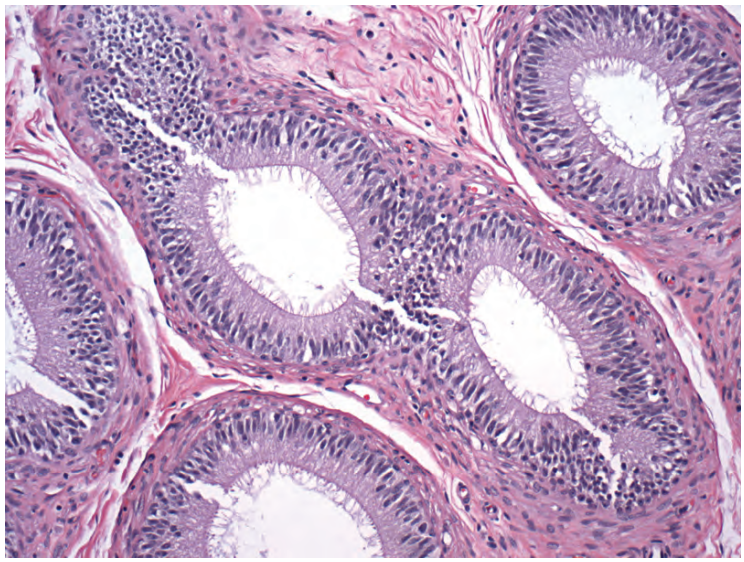

Figure 14.3. Normal epididymis. Columnar epithelium with cilia.

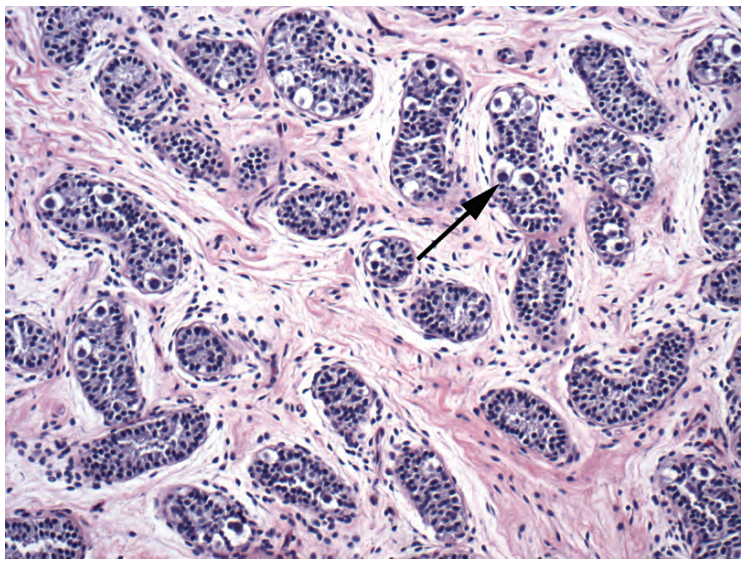

Figure 14.4. Cryptorchidism. In the infant testis, large dark spermatogonia are visible (arrow).

Tumors

Infants and children	Young adults and adults	Older adults
Yolk sac tumor	Seminoma	Spermatocytic seminoma
Teratoma	Embryonal carcinoma	Lymphoma
	Choriocarcinoma	Sex cord stromal tumors
	Teratoma	

Germ Cell Tumors

Germ cell tumors, which include seminoma, teratoma, yolk sac tumor, choriocarcinoma, and embryonal carcinoma, can all occur as pure tumors in and of themselves, but they do have a tendency to collide in adults, with the resulting mixture called a *mixed germ cell tumor*

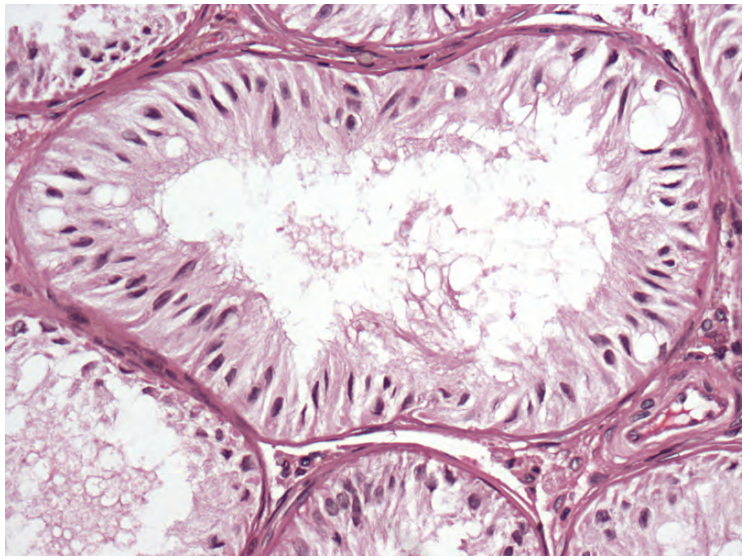

Figure 14.5. Sertoli-only syndrome in an adult. The tubules are lined with spindly Sertoli cells, and no germ cells are visible.

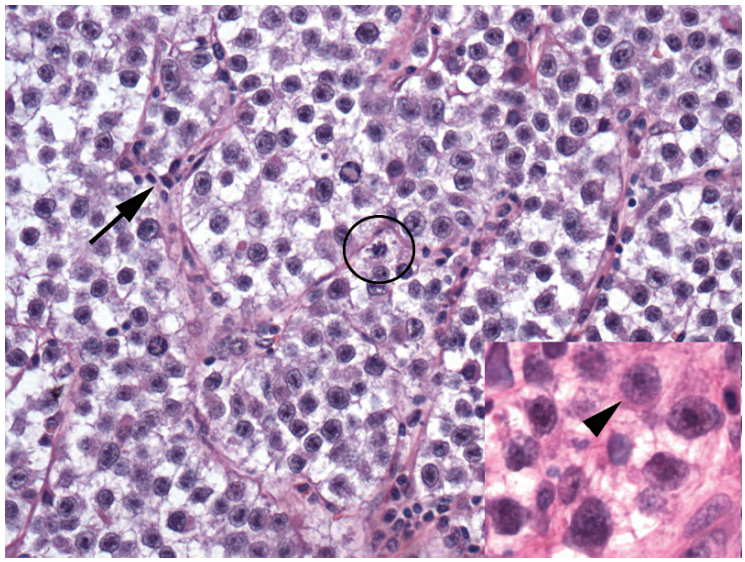

Figure 14.6. Seminoma, classic type. Delicate fibrovascular septae divide the cells into packets (arrow); collections of lymphocytes can be seen along the septae. The nuclei are widely spaced, with clear cytoplasm. Mitoses are common (circle). Nuclei have distinct nuclear membranes and prominent nucleoli (arrowhead).

(MGCT). Here, they will be described individually, but remember that for any adult neoplasm you are trying to identify every component present.

These neoplasms are notorious for their rare ability to nearly completely regress in the testis, leaving behind mainly a fibrotic scar. This does not, however, mean that they have not already metastasized.

Seminoma (Classic Type)

The seminoma is the most common germ cell neoplasm in adults. In typical form it is a large nodular mass in the testis. Microscopically, it is poorly circumscribed and infiltrates in between tubules at the periphery. The histologic features (Figure 14.6) include the following:

- An array of large, round, coarse nuclei, nonoverlapping and nonmolding, suspended in a network of delicate cell membranes
- One to two prominent central nucleoli
- Associated inflammation, especially lymphocytes, granulomas, and fibrosis
- Delicate branching fibrovascular septa
- Surrounding intratubular germ cell neoplasia (see below)

The classic type of seminoma has fairly monomorphic cells; at low power this uniformity can be deceptively bland. It may occur in pure form, but all tumors should be carefully sampled for other germ cell components (in which case it becomes an MGCT).

Intratubular Germ Cell Neoplasia

Think of intratubular germ cell neoplasia (IGCN) as the carcinoma in situ of the testis. It is actually very hard to spot, as normal spermatogenesis creates some strange looking cells. The easiest way to find IGCN is to slowly scan the tubules at 4×, looking for areas that stand out as having scattered dark, or large, or fried egg–type cells. Another approach is to study the seminomatous cells in the main tumor and look for similar cells in the adjacent tubules. An IGCN may be as subtle as a few big cells in the tubule, spreading in pagetoid fashion, or as obvious as a lumen packed with malignant cells (Figure 14.7). An IGCN is often seen next to seminomas (classic type), embryonal carcinomas, or choriocarcinomas. It is helpful because it confirms that you have a germ cell neoplasm (as opposed to a carcinoma or lymphoma). The following features help distinguish spermatogonia from IGCN:

Spermatogonia	Intratubular germ cell neoplasia
Clear cytoplasm	More abundant clear cytoplasm
Condensed chromosomes	Coarse chunky chromatin
Smooth nuclear membrane, if any	Irregular nuclear membrane
No nucleolus	Prominent nucleolus
Mature into spermatids	Little to no maturation
Placental alkaline phosphatase (PLAP) negative	c-kit, OCT3/4, and (often) PLAP positive

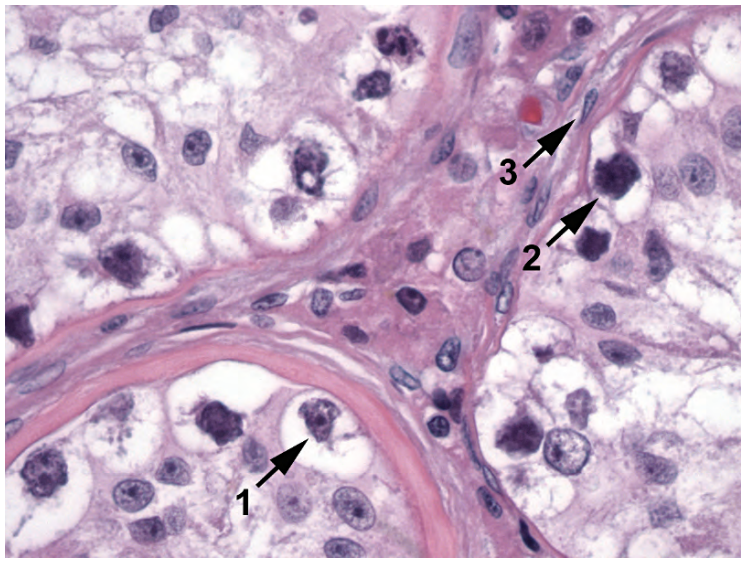

Figure 14.7. Intratubular germ cell neoplasia. Large cells with clear halos of cytoplasm and prominent nucleoli are seen at the tubule perimeter (1). Other malignant nuclei appear hyperchromatic and solid (2). Compare the malignant cells to the euchromatin of nearby endothelial cells (3).

Spermatocytic Seminoma

Many organ systems have low-grade, indolent, better differentiated versions of their neoplasms. Spermatocytic seminoma is the indolent seminoma in that it does not metastasize. It occurs in older men and has seminoma-like cells, except in three cell sizes: small, medium, and large. This tumor also lacks inflammation and PLAP positivity. It is the one tumor type not found in mixed germ cell tumors, nor is it associated with IGCN.

Embryonal Tumor

Rare as a pure tumor, embryonal tumors are a common component of MGCT. Remember embryonal as the ugly one. The cells are very pleomorphic, with hyperchromatic, angular, overlapping, or molding nuclei and large nucleoli (Figure 14.8). It looks epithelioid, like a carcinoma, and is in fact keratin positive. The architecture is solid, glandular, or papillary.

Yolk Sac Tumor (Endodermal Sinus Tumor)

Yolk sac tumor is the most common testicular neoplasm in children (in pure form), but it is also a common component of MGCT. It is famous for its many forms, especially microcystic and reticular (net-like). The pathognomonic finding is the Schiller-Duval body, a little glomeruloid form, but these are not always seen. The nuclei tend to be somewhat smaller and a little more regular than embryonal carcinoma, yet more atypical than seminoma (Figure 14.9). When found next to embryonal carcinoma, these areas look hypocellular and myxoid in comparison to the large epithelioid embryonal cells.

Choriocarcinoma

Choriocarcinoma is a rare tumor, especially in pure form. Like the placental tumor, it is characterized by two cell types (cytotrophoblast and syncytiotrophoblast), lots of blood, and human chorionic gonadotropin production. Also like the placenta, this tumor is very good at invading blood vessels, and widespread metastases are common. Syncytiotrophoblasts, the multinucleated giant cells that stain for human chorionic gonadotropin, can show up in other germ cell tumors; this does not make them choriocarcinomas. The cytotrophoblasts resemble

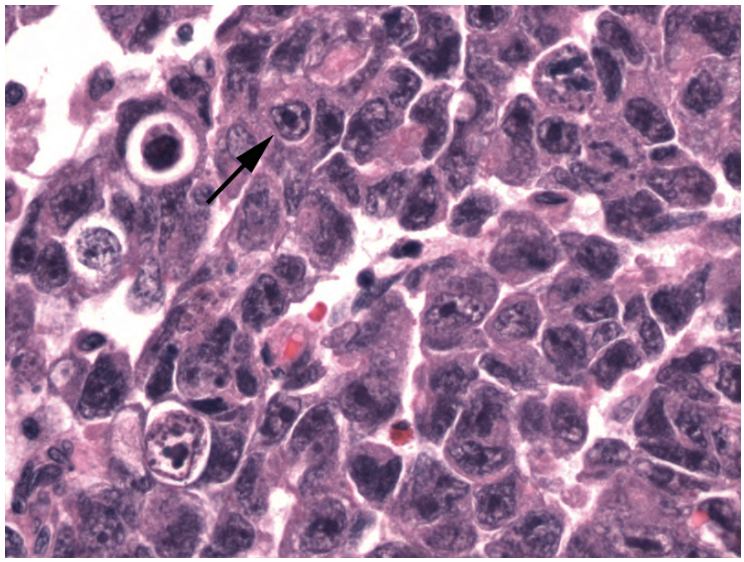

Figure 14.8. Embryonal carcinoma. Large epithelioid cells with pleomorphic nuclei grow in sheets. Unlike in seminoma, the cytoplasm is dense, and the nuclei have irregular shapes and sizes, some showing nuclear molding. Many have coarse chromatin with dark nuclear membranes and prominent nucleoli (arrow).

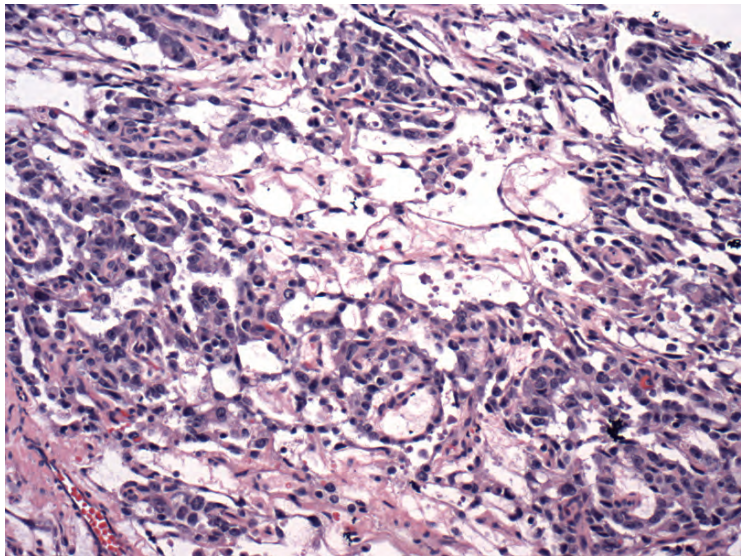

Figure 14.9. Yolk sac tumor. The cells of yolk sac tumor often appear more low grade than other germ cell tumor types. The cells are cuboidal, with pink cytoplasm, and have a tendency to pull apart into a microcystic pattern (shown here).

embryonal carcinoma, but the nuclei are smaller and not quite so pleomorphic, and the cytoplasm is pale.

Teratoma

A teratoma is a neoplasm composed of elements of the primitive germ layers: ectoderm (skin, central nervous system), mesoderm (cartilage, bone), and endoderm (gut, viscera), but not all layers have to be present in a tumor to call it a *teratoma*. Pure teratomas are found in prepubertal boys and are always considered benign in this population. In contrast, in postpubertal males, teratomas are malignant and are usually seen in the context of mixed germ cell tumors. A pure teratoma in an adult male is still malignant whether or not it contains immature elements (i.e., immature neural tissue), so there is no need to comment if a testicular teratoma is immature or mature (this is in contrast to teratomas in females). Rarely, teratomas can develop non–germ cell tumors, such as carcinomas, sarcomas, or "small blue cell tumors."

Sex Cord Stromal Tumors

The sex cord stromal tumors include the Sertoli cell and Leydig cell tumors. They are not germ cell neoplasms and are usually benign. They resemble their normal counterparts, so a tumor of oncocytic pink cells with very round nuclei is likely to be a Leydig cell tumor (Figure 14.10), and a collection of primitive tubules lined with spindly cells and oval nuclei is likely to be a Sertoli cell tumor (Figure 14.11). In both tumors, approximately 10% behave badly, but there are no hard criteria by which to predict malignancy. The usual rules apply (atypia, mitotic rate, necrosis, vascular invasion, and invasion beyond the testis all suggest poor prognosis).

Lymphoma

While uncommon in the testis, lymphoma is always in the differential diagnosis when sheets of discohesive malignant cells are present. Lymphoma can look like seminoma, yet the cells are not as homogeneous. The usual type is diffuse large B cell, which means CD20-positive cells with large nuclei, often with vesicular chromatin and large nucleoli (Figure 14.12). Lymphoma occurs in an older age group than seminoma and should not have any IGCN.

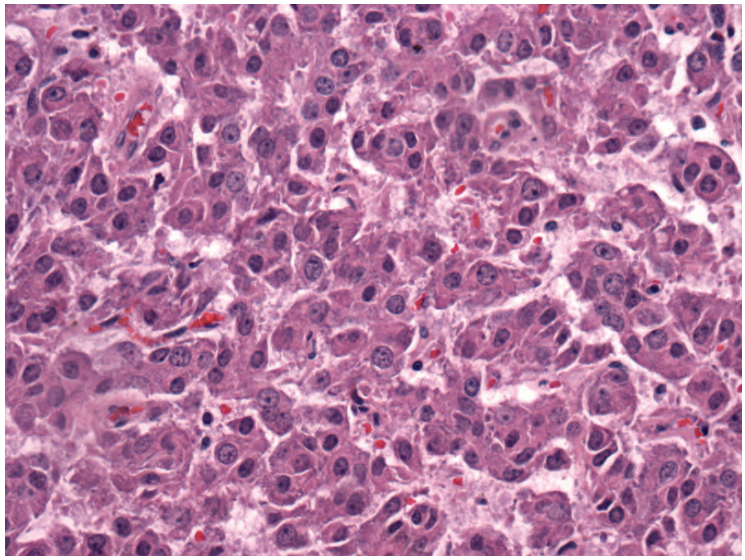

FIGURE 14.10. Leydig cell tumor. These neoplasms are reminiscent of oncocytomas in other sites. Most are benign.

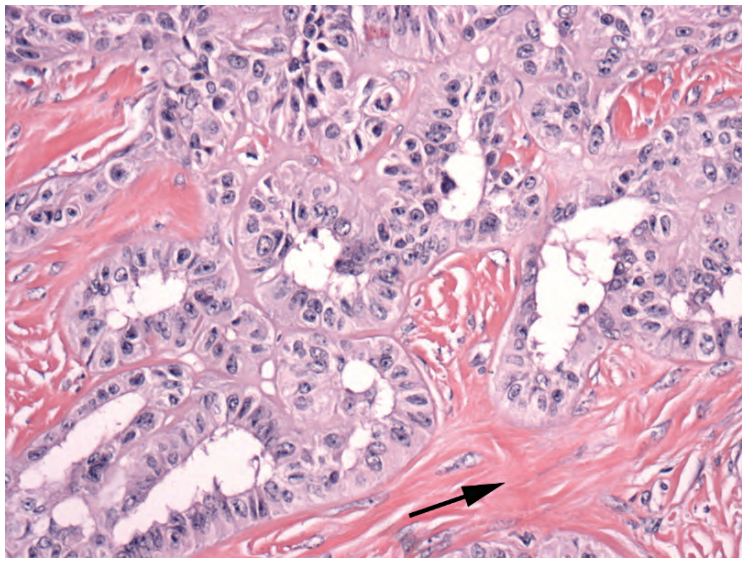

FIGURE 14.11. Sertoli cell tumor. This tumor attempts to recapitulate the seminiferous tubules. The stroma may become hyalinized (arrow).

Outside the Testis

All of the germ cell tumors can also occur in other body locations, mainly in the midline (e.g., sacrum, mediastinum, sella or pineal in brain). A germ cell tumor in the brain is called a *germinoma*. These neoplasms can also occur in the female ovary. A seminoma occurring in the ovary is called a *dysgerminoma*. Yolk sac tumors, embryonal carcinomas, and teratomas can occur in ovary. Choriocarcinoma is more commonly associated with placental tissue in women but can also arise in the ovary. Sex cord stromal tumors also arise in women, usually

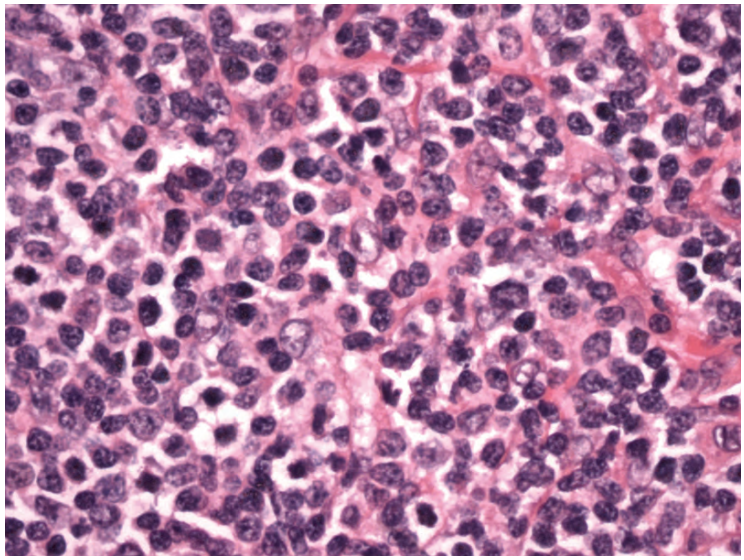

Figure 14.12. Diffuse large B-cell lymphoma. The main histologic feature is sheets of discohesive tumor cells. Nuclear chromatin is chunky.

of the thecoma and granulosa cell groups, but Sertoli and Leydig cell tumors can also develop. Strangely, you can also rarely see granulosa cell tumors in the testis. In fact, when presented with a tumor in the testis or ovary that looks like nothing you recognize, the sex cord stromal tumors are a good place to start.

15 Ovary

The ovary is a fairly straightforward organ as far as the pathologist is concerned. Find tumor, remove tumor, identify tumor. No messing around with nonneoplastic pathology, reactive lesions, and so forth. If it looks malignant, it probably is.

Normal Histology and Definitions

Surface epithelium: Surface epithelium is essentially a mesothelial lining. It is easily rubbed off the surface, so you do not always see it. Most epithelial tumors (the most common neoplasms) are thought to arise from this epithelium or from invaginations of it. Think of it as a pluripotent stem cell layer.

Stroma: The ovarian stroma is blue and spindly, with a streamy, fascicular look. Most of the cells in the stroma are fibroblasts (Figure 15.1).

Sex cord cells: Sex cord cells are the hormone-secreting supporting cells of the ovary, the thecal cells and granulosa cells. The thecal cells, under luteinizing hormone stimulation, secrete androgens, and the granulosa cells, under follicle-stimulating hormone control, convert androgens to estrogen. Together they nurture an oocyte to ovulation.

Follicles: The follicles are characterized by a halo of thecal cells outside a ring of granulosa cells (see Figure 15.1), all surrounding the giant oocyte (germ cell). In developing follicles, the granulosa cells form Call-Exner bodies, rosettes of granulosa cells surrounding pink globules.

Luteinized: Similar to decidualized, luteinized indicates cells that have become plump with abundant pink cytoplasm.

Corpus luteum: The corpus luteum is a newly ovulated follicle (Figure 15.2). The capsule of luteinized granulosa cells collapses in on itself, becoming undulating, and there is associated hemorrhage. The corpus luteum produces progesterone until (and if) the placenta takes over. If there is no pregnancy, it involutes.

Corpus albicans: The former corpus luteum ultimately hyalinizes to form cloud-shaped pink islands in the ovary, scars of old follicles (see Figure 15.2).

Walthard's rests: Walthard's rests are nests of transitional (urothelial) type epithelium in the ovary and fallopian tube.

Rete ovarii: Analogous to the rete testis in men, rete ovarii are rudimentary gland spaces located in the hilum of the ovary. They are angulated, slit-like spaces with a low cuboidal epithelium (Figure 15.3). Do not mistake them for cancer.

Follicle cyst: A follicle cyst is lined with the normal components of the follicle, the granulosa cells, and the thecal layer (Figure 15.4). A similar lesion is the hemorrhagic corpus luteum cyst, which is a blood-filled corpus luteum.

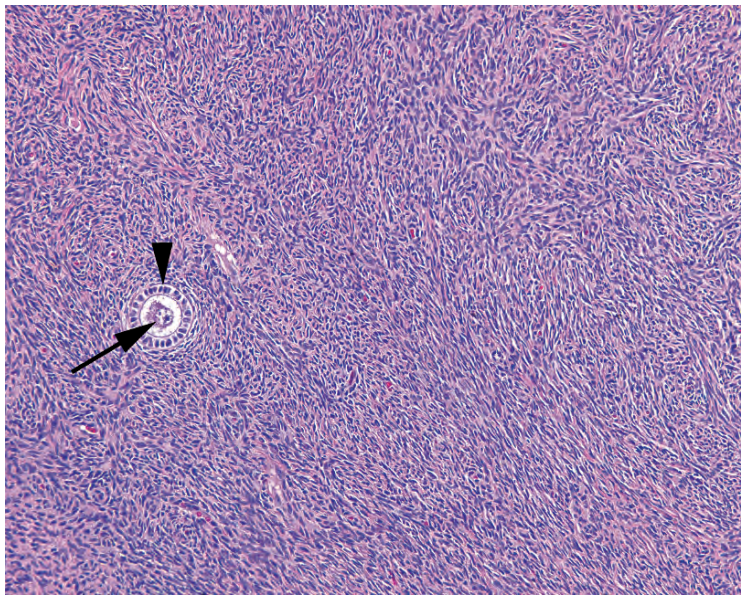

Figure 15.1. Ovarian stroma with follicle. Typical ovarian stroma is blue and cellular, with a vaguely fascicular or storiform pattern. A small primary follicle is seen with the central oocyte (arrow) and a ring of granulosa cells (arrowhead).

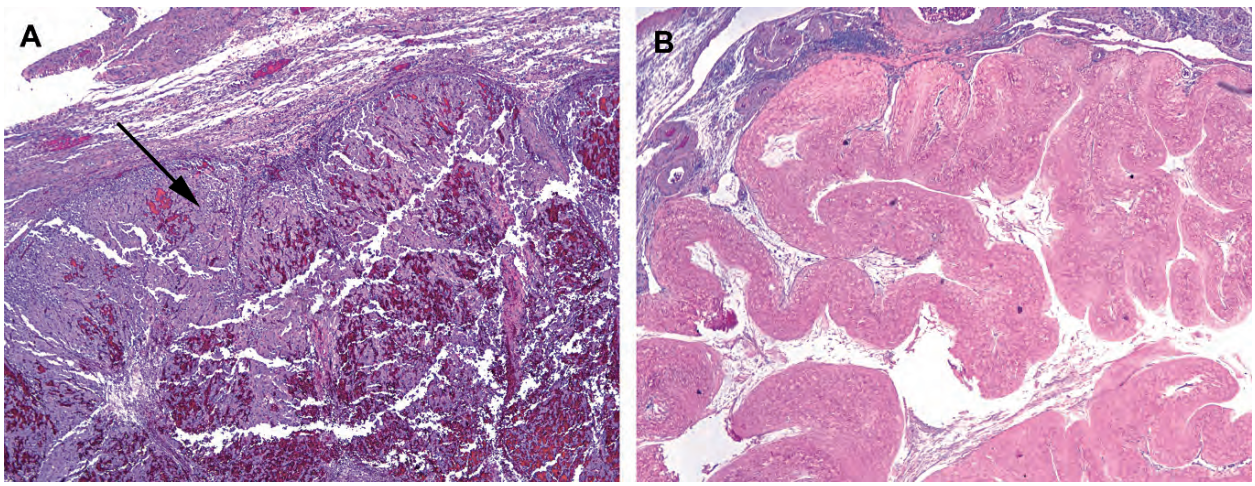

Figure 15.2. (A) Hemorrhagic corpus luteum, with undulating layers of luteinized granulosa cells (arrow) and associated blood. **(B)** A corpus albicans, the remnant of a prior corpus luteum.

Inclusion cyst: An inclusion cyst is a simple cyst lined with a cuboidal, columnar, or ciliated epithelium, often budding inward from the ovarian surface (see Figure 15.4). When small, these can be called surface inclusion cysts. However, if they are large, they are best referred to as serous cystadenomas (see later).

Neoplasms

For each cell type defined above (and then some), there are families of neoplasms that can arise. Table 15.1 lists the types of neoplasms that can occur. Those in parentheses are rare enough that we will not talk about them here.

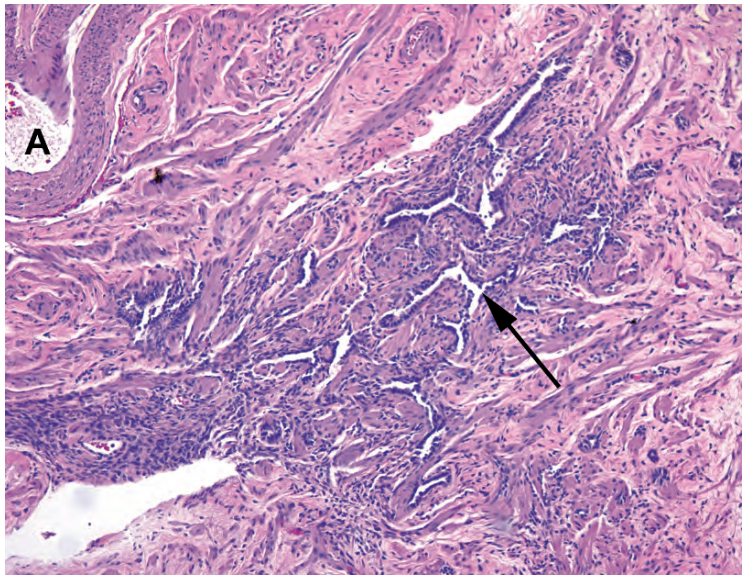

Figure 15.3. Rete ovarii. This vestigial structure is found at the hilum of the ovary, adjacent to large arteries (A) and veins. The rete consist of slit-like channels with a cuboidal cell lining (arrow).

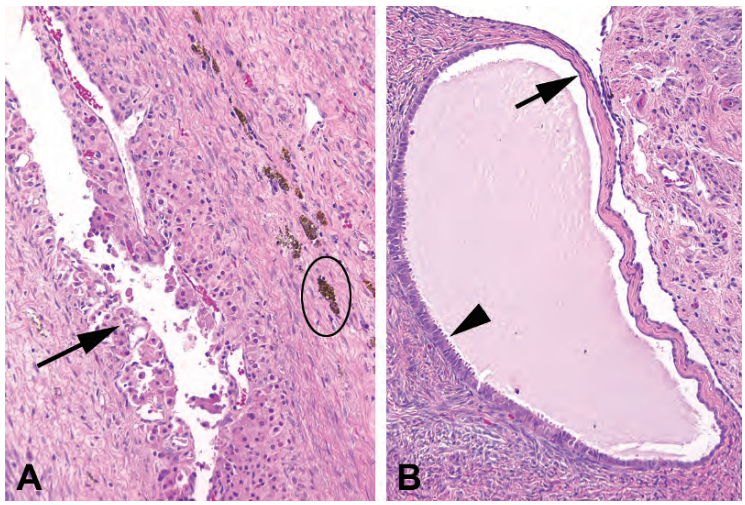

Figure 15.4. Follicular cyst versus inclusion cyst. **(A)** A follicle cyst is lined by luteinized cells, similar to those seen in the corpus luteum (arrow). There is adjacent hemosiderin (oval). **(B)** An inclusion cyst may be lined by an attenuated epithelium, similar to the surface epithelium (arrow), or may show tubal metaplasia (arrowhead).

Table 15.1. Neoplasms of the ovary.

Surface epithelial tumors	Germ cell tumors	Sex cord stromal	Metastases
Serous	Teratoma	Fibroma	Gastrointestinal
Mucinous	(Dysgerminoma)	Thecoma	Urothelial
Endometrioid	(Yolk sac)	Granulosa cell tumor	Others
Clear cell	(Choriocarcinoma)	(Sertoli cell tumor)	
Brenner or transitional	(Embryonal)	(Leydig cell tumor)	
		(Sertoli-Leydig cell tumor)	

Note: Entries in parentheses are rare tumors that are not discussed in this chapter.

Epithelial Neoplasms

Epithelial neoplasms are one of three types: benign, borderline, and malignant. Benign tumors do not metastasize, malignant ones do, and borderline tumors may recur or rarely metastasize. The nomenclature is as follows:

Adenoma: An adenoma is a benign epithelial proliferation. When cystic, as they often are, it is a *cystadenoma*. If biphasic with a secondary fibrous stromal component, it is an *adenofibroma*. If all three, it is a *cystadenofibroma*. Histologically, the cystadenomas are simple or multilocular cysts with a flat epithelial lining.

Borderline tumor (atypical proliferative tumor, low malignant potential): Borderline lesions have an increasing epithelial complexity over the adenomas. Their epithelium begins to ruffle up in papillary fronds and may "ruffle down" into the stroma in a way that looks similar to invasion. However, they do not cross the basement membrane, do not invade as single cells, and do not induce a desmoplastic reaction. Borderline tumors can shed cells into the peritoneum, which may stick onto other organs and begin to grow like weeds. However, they often do not technically invade and are called noninvasive implants, not metastases. Invasive implants can also occur and act like true metastases.

Carcinoma: Carcinomas commonly present as combination cystic/solid tumors and are called *cystadenocarcinomas*. However, there is not a significant clinical difference between calling something a *carcinoma* and a *cystadenocarcinoma*. These can be divided into low- and high-grade carcinomas, but all types can metastasize.

Carcinosarcoma: A carcinosarcoma (malignant mixed mullerian tumors in the gynecologic tract) is a carcinoma in a sarcomatous stroma. An adenosarcoma would be a benign epithelial neoplasm in a sarcomatous stroma, which is rare.

Within the surface epithelial group, there are five types of epithelial neoplasms. Each type can be subdivided into benign, borderline, or malignant, as shown in Table 15.2.

Serous (Most Common, Formerly Papillary Serous)

Cystadenoma: Cystadenomas are simple cysts lined by a tubal-like epithelium with columnar and/or ciliated cells. They can become huge, but the lining remains simple. The contents are watery.

Borderline tumor (atypical proliferative tumor; tumor of low malignant potential): Borderline tumors have increasingly complex papillary fronds, looking grossly granular. The papillary pattern is characterized by tree-like branching of smaller and smaller papillae (Figure 15.5). However, the epithelium lining the papillae is usually a single layer without significant atypia. When the papillae acquire secondary epithelial proliferations called *micropapillae* (the medusa-head look; Figure 15.6), they are on their way to *micropapillary serous carcinoma* (MPSC). A few millimeters of confluent micropapillary pattern upgrades this to an actual MPSC, in some textbooks.

Micropapillary serous carcinoma (micropapillary borderline tumor): Micropapillary serous carcinoma is a low-grade carcinoma characterized by the medusa-head pattern (see Figure 15.6). The nuclei should not be too pleomorphic. This carcinoma can occur within a confined cyst (noninvasive MPSC) or break out of the cyst and into the stroma (invasive). This perplexing concept of a noninvasive carcinoma is somewhat like the papillary urothelial carcinomas of the bladder or the papillary carcinomas of breast. Psammoma bodies are common. When

Table 15.2. Five types of epithelial neoplasms.

	Serous	Mucinous	Endometrioid	Clear cell	Transitional
Cystadenoma	60%	80%	Rare	Rare	>90%
Borderline tumor	15%	15%	Rare	Rare	Rare
Carcinoma	25%	>5%	>95%	>95%	6%

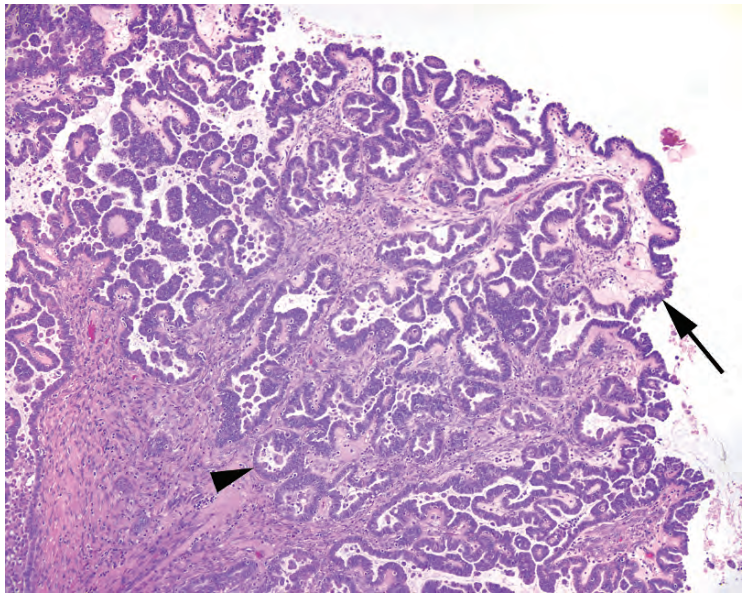

Figure 15.5. Borderline serous tumor. The epithelial lining is composed of serous, or nonmucinous, cells (arrow). The overall architecture is quite complex, with papillary branching and invaginated folds that should not be mistaken for invasion (arrowhead). However, the epithelial component is mostly a monolayer.

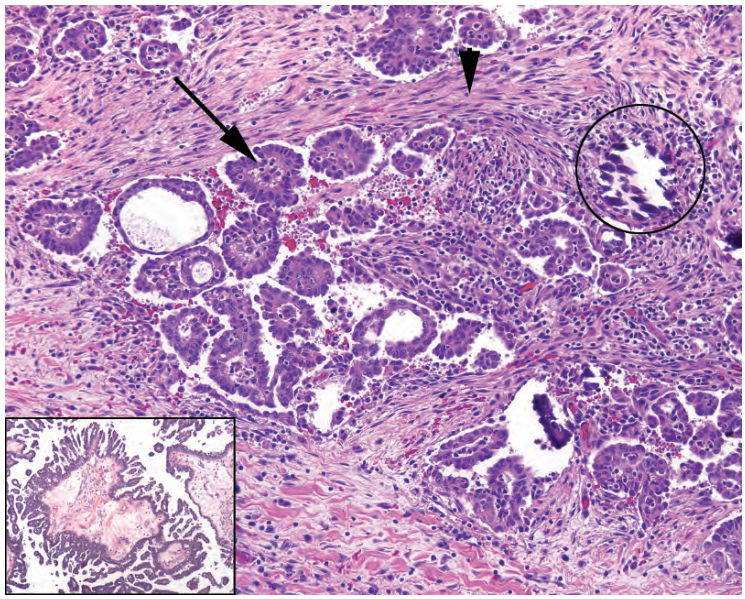

Figure 15.6. Micropapillary serous carcinoma. When invasive, micropapillary serous carcinoma looks like tiny florets of cells (arrow) in a desmoplastic stroma (arrowhead). Psammoma bodies are common (circle). **Inset**: The medusa-head, or micropapillary, pattern is indicative of micropapillary serous carcinoma. Compare the epithelial micropapillae to the simple epithelium of the borderline tumor (see Figure 15.5).

invasive, the tumor nests have a flower-like shape, with nuclei pointing outwards, and often sit in small cleft-like spaces (Figure 15.6)

High-grade serous carcinoma: High-grade serous carcinoma has very high-grade, mitotically active, apoptotic, pleomorphic blue nuclei (Figure 15.7). The architecture can be papillary, micropapillary, solid, or in nests with slit-like spaces.

Mucinous

Cystadenoma: Cysts (often multilocular) are lined with fairly flat mucinous epithelium.

Borderline tumor (atypical proliferative tumor; tumor of low malignant potential): The vast majority of these are of the intestinal type, which means they imitate intestinal epithelium, with goblet cells and glandular architecture. However, 15% are of the endocervical type, which presents as papillary architecture (like serous) with low-grade, squared-off, endocervical-like mucinous cells (Figure 15.8).

Cystadenocarcinoma: Mucinous cystadenocarcinoma is very uncommon. Mucinous carcinoma in the ovary is usually a metastasis from the gastrointestinal tract.

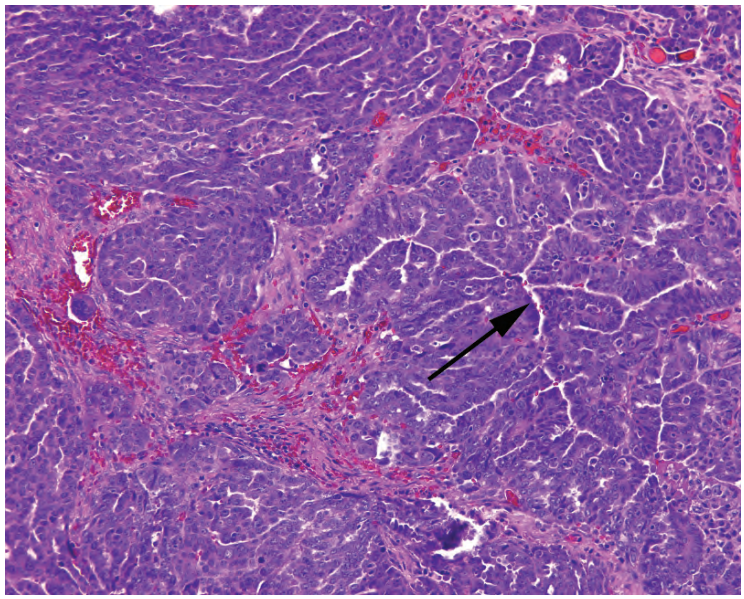

Figure 15.7. High-grade serous carcinoma. This tumor is shown invading the stroma. The cells are pleomorphic and dark, with prominent nucleoli, and grow in solid nests with slit-like spaces (arrow).

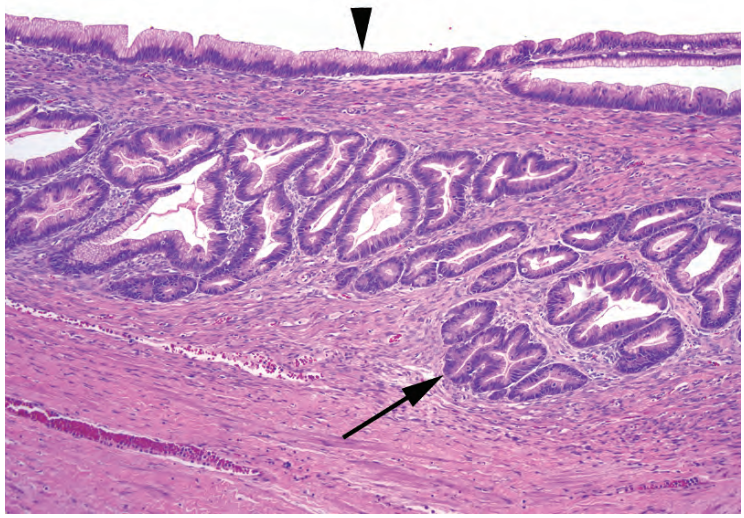

Figure 15.8. Borderline mucinous tumor. The cyst lining (arrowhead) is mucinous and resembles endocervical cells in this example. As with the borderline serous tumor, invaginations into the stroma should not be mistaken for invasion (arrow).

Endometrioid

Adenoma: How is endometrioid adenoma different from endometriosis? It has no endometrial stroma. Also, these adenomas are rare. Endometriosis is common.

Carcinoma: Endometrioid carcinomas imitate endometrial carcinoma and so have similar architecture to the patterns found in the uterus, including tubular to cribriform glands and villous structures (Figure 15.9). They may arise within endometriosis, they are often found along with endometriosis, and a concurrent endometrial carcinoma is not uncommon. They may be low or high grade.

Clear Cell

Carcinoma: Clear cell carcinomas are clear cells occurring in papillary, glandular, nested, or trabecular patterns. Cells tend to fall out of the center of the nests, leaving a hobnailed layer of cells outlining the nest (Figure 15.10). These are high grade and, like endometrioid carcinoma, are also associated with endometriosis.

Transitional

Adenoma/adenofibroma: Transitional adenomas/adenofibromas are the Brenner tumor, characterized by nests of transitional epithelium in a fibrous stroma (Figure 15.11). There may be a mucinous layer surrounding a central lumen in each nest.

Malignant Brenner tumors: Malignant Brenner tumors are characterized by very atypical cells. *Transitional cell carcinoma* (resembling that seen in the bladder) is a term used when there is no coexisting Brenner element.

How can you tell which pattern you have?

If you see…	Think…
Clear cells	Clear cell
Hobnail cells lining spaces	Clear cell
Mucinous cells with papillary fronds	Mucinous
Mucin-secreting cells	Mucinous
Papillary fronds	Serous, or any other type
Sheets of high-grade nuclei	Serous or undifferentiated
Solid growth with slit-like spaces	Serous
Squamous-like nests of round cells	Transitional
Tall villi	Endometrioid or mucinous
Tubular glands	Endometrioid

Cancer Pathways

There are thought to be two cancer pathways for serous neoplasms:

Cystadenoma → Atypical proliferative serous tumors → Micropapillary serous carcinoma → Invasive micropapillary serous carcinoma

Terribly bad luck → High-grade serous carcinoma (de novo), often p53 positive

Nonepithelial Neoplasms

For the purposes of this chapter, we will gloss over the germ cell and sex cord stromal neoplasms, except for the most common entities.

Germ Cell Neoplasms

Anything that can occur in the testis can occur in the ovary. The most common germ cell neoplasm in the ovary is the teratoma, but you can also see dysgerminoma (seminoma), yolk sac tumor, choriocarcinoma (arising unrelated to gestational trophoblastic disease), and embryonal carcinoma. Each looks similar in testis and ovary.

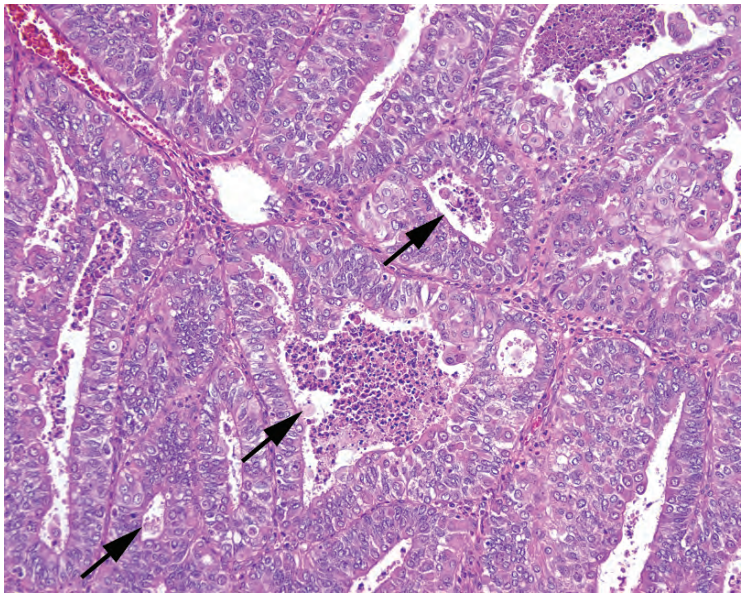

Figure 15.9. Endometrioid carcinoma. The nuclei are cleared out and pleomorphic, like endometrioid carcinoma of the endometrium. Distinct glandular spaces are visible (arrows), some with central necrosis.

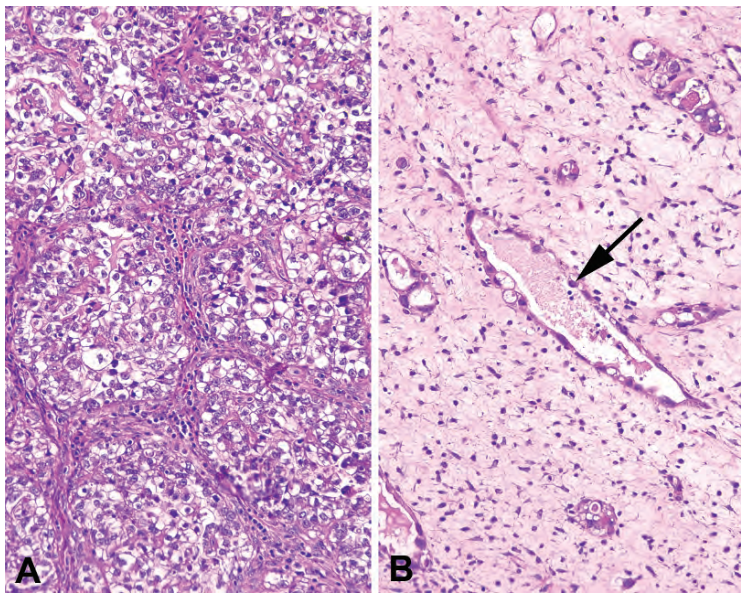

Figure 15.10. Clear cell carcinoma. **(A)** In this field, nests of clear cells are seen separated by fibrovascular septa. **(B)** A less cellular area of the same tumor shows vessel-like spaces lined by atypical cells that protrude into the lumen in hobnail fashion (arrow).

Teratomas are usually composed of at least two of three embryonic derivatives: ectoderm, endoderm, and mesodermal cells. They are often cystic (dermoid cyst) and may grow to a large size. Common elements include squamous epithelium, skin adnexal structures, hair, fat, cartilage, thyroid, brain and nerve tissue, gut epithelium, and respiratory epithelium. Primary nonovarian neoplasms can arise in teratomas, creating an endless list of case reports. Teratomas restricted to mature elements are benign in the ovary.

All teratomas must be carefully evaluated for immature (embryonal-looking) elements. The most common immature tissue type is brain. After a few products-of-conception (POC) specimens

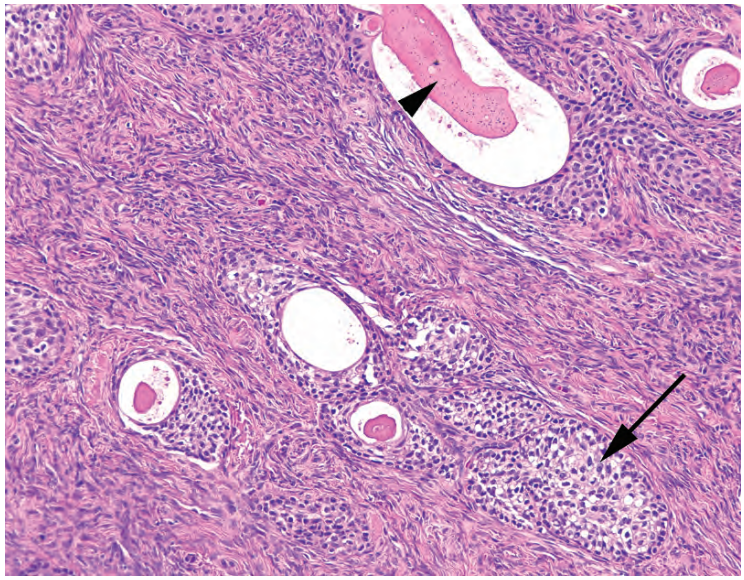

Figure 15.11. Brenner tumor. Nests of transitional-type epithelium (resembling urothelium; arrow) in a fibrotic stroma are typical. Some form gland-like spaces with pink secretions (arrowhead).

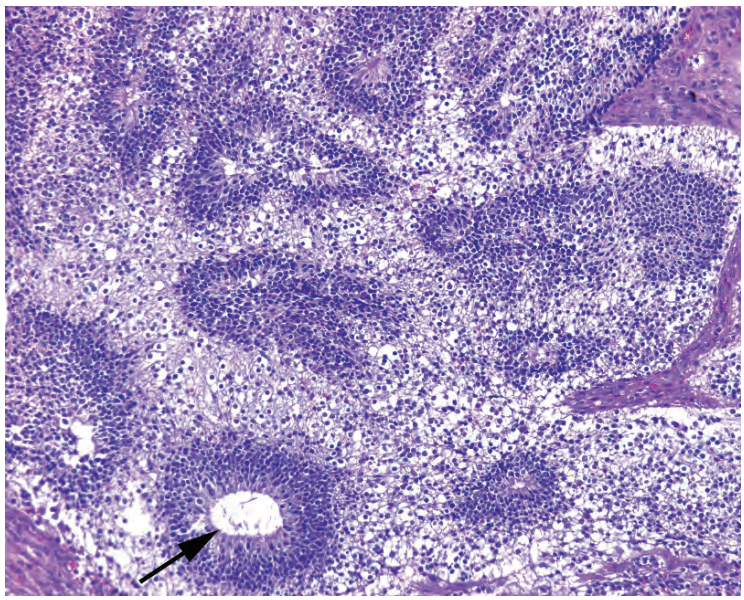

Figure 15.12. Immature neural tissue, teratoma. The combination of hypocellular areas and dense small round blue cell areas are suggestive of fetal brain. Rosettes (arrow) may also be seen. Finding this histology in a teratoma indicates an immature component.

or fetal autopsies, you should recognize fetal brain—dark blue cells in a myxoid, clear background (Figure 15.12).

Sex Cord Stromal Neoplasms

Sex cord stromal neoplasms include all of the fibrous and sex hormone cell types. The most common tumors are the fibroma/thecoma group. Granulosa cell tumors are also not uncommon. However, many of the weird and paradoxical ovarian lesions (Sertoli-Leydig?) fall into this group. Cell types in these tumors may be luteinized, just like their normal counterparts.

The fibroma/thecoma group is a spectrum of lesions from the pure fibroma, to the common mixed fibrothecoma, to the pure thecoma. Grossly they look like leiomyomas, which are very rare in the ovary. On cross section, the thecoma areas are butter-colored, and stand out from the grey-white fibroma. Histologically the tumors are also similar to leiomyomas but have more of a sheet-like pattern with bland, spindled cells (Figure 15.13). However, the tiny lipid

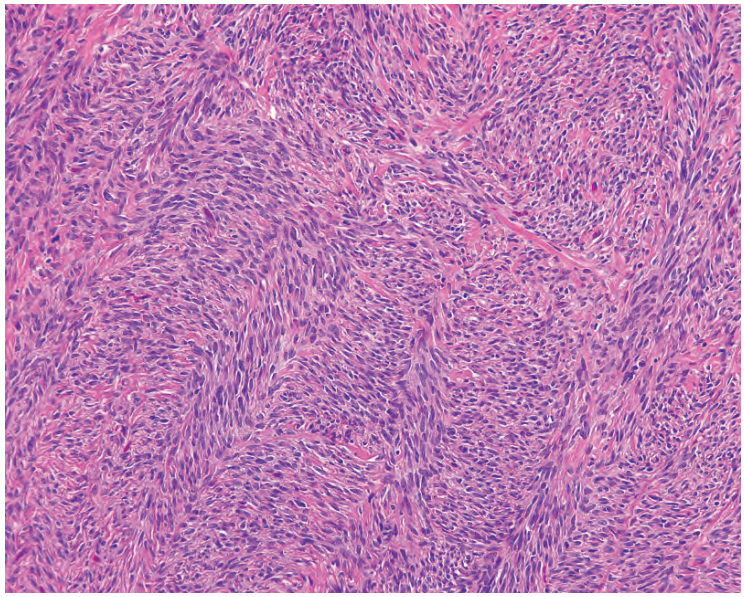

Figure 15.13. Fibrothecoma. This specimen shows mainly the fibroma component, with fascicles of bland spindled cells.

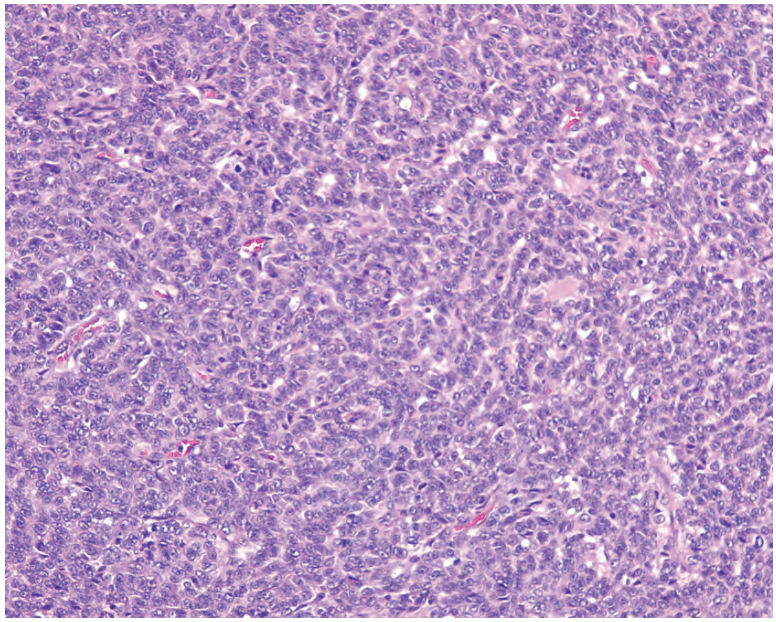

Figure 15.14. Granulosa cell tumor, low power. This section shows the characteristic cords and rows of granulosa cells, creating a pattern like watered silk, or (for those not frequenting fabric stores), a topographic map.

vacuoles that identify the thecoma component (steroid cells, remember?) are very hard to see on H&E stain, so the gold standard is an oil red O done on frozen section. Bright red lipid globules indicate a thecoma component. These are benign tumors.

Granulosa cell tumor cells appear similar to the normal granulosa cells in the ovary but have more distinctive oval folded or angulated nuclei with a longitudinal groove (the "coffee bean" nuclei). In a tumor, these cells may become more closely packed, almost giving the impression of nuclear molding, but they are not as blue, hyperchromatic, or crowded as small cell carcinoma. At low power the cells are arranged in sheets, with a zigzag "watered silk" pattern (think of a topographic map; Figure 15.14). The cells appear very uniform throughout. Rarely, you may see the pathognomonic Call-Exner bodies as seen in the developing follicle. These are technically of low malignant potential but may recur after many years.

16 Cervix and Vagina

Cervical biopsies are common and nearly always performed for the purpose of evaluating squamous or glandular dysplasia. Usually the patient will have a history of an abnormal Pap test or a prior abnormal biopsy finding. Correlation with cytology is nice, but lesions can be focal and/or transient, so perfect agreement is not required.

There are several types of specimens. The smallest is usually the endocervical curettage. This is meant to be a sampling of the endocervix, and so should contain endocervical (columnar) mucosa. These tissue scrapings may have tiny and maloriented fragments, and the tissue can be spread out over a wide area.

If a lesion is seen by the clinician on colposcopy, there may be a cervical biopsy performed, which is a crescent-shaped chunk taken out of the cervix. This tiny specimen is uninked and unoriented. A high-grade lesion requires a cone biopsy, where the transition zone is taken out in a conical fragment with the goal of completely excising the lesion. The cone biopsy may be done with cautery (loop electrosurgical excision procedure) or blade (cold-knife cone). The endocervical margin should be identified and inked to make sure the lesion is not extending up into the canal where it cannot be seen or sampled. The ectocervical margin is also inked, but a positive ectocervical margin is unusual.

Normal Histology

The cervix is covered by a nonkeratinized squamous epithelium that merges into the surrounding vaginal wall. The squamous cells may be full of glycogen and have a plump cleared-out cytoplasm (see Figure 4.8B). In postmenopausal women, the epithelium may become thin and atrophic, with an immature look.

The transition zone represents an abrupt transition to mucous-secreting columnar and glandular epithelium and may be located at the os or within the endocervical canal. Irritation or inflammation at the transition zone may lead to acute and/or chronic inflammation and squamous metaplasia overlying the glandular mucosa. This state is so universal that "chronic cervicitis and squamous metaplasia" is synonymous with normal. Squamous metaplasia, by definition, can only occur at or above the transition zone (Figure 16.1), and so mentioning it confirms that the transition zone was sampled. If you see no endocervical component at all, the specimen is simply "benign squamous mucosa."

Endocervical glands are branching and complex glands that are pale with a dark outline due to the large cytoplasmic mucin vacuole pushing aside a small crescent-shaped nucleus (Figure 16.2). Fragments of glands on endocervical curettage may have a papillary or inside-out look, which is not significant. Squamous metaplasia may fill up and replace glands and must be distinguished from invasive cancer. Crowding of otherwise benign-looking glands is not a feature of concern, as it is in the endometrium.

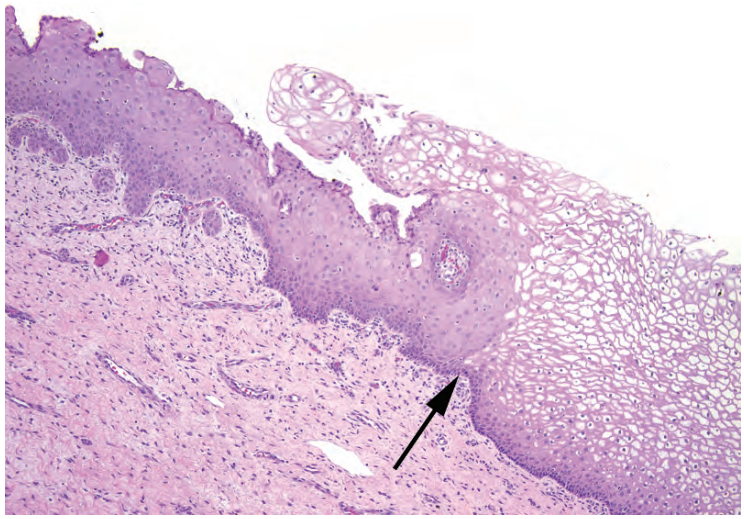

Figure 16.1. Squamous metaplasia at the transition zone. Mature squamous epithelium is seen to the right of the arrow, and squamous metaplasia is seen to the left. In squamous metaplasia, the nuclei may be larger and more immature appearing and the cytoplasm more dense.

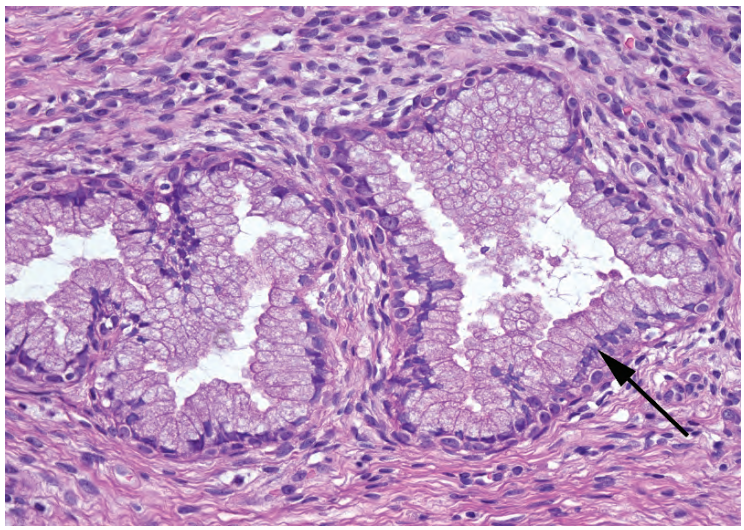

Figure 16.2. Endocervical glands. Normal endocervical glands are composed of tall columnar cells with apical mucin and small basal nuclei (arrow).

The cervical stroma is very fibrotic (as you will find when cutting through one), so it looks pink and spindly. There are a variety of normal cysts and glandular proliferations that may occur within the stroma.

The Approach to the Endocervical Curettage

First look at the slide on the tray, and circle a single level of the tissue—it may be spread out over a wide area, but may be duplicated several times on the same slide. At low power, look for fragments of squamous epithelium and endocervical glands. The presence or absence of each is noted in the diagnosis. There may be a fair amount of background mucus (stringy pale

pink amorphous substance) and inflammation. Concentrate on the actual epithelial fragments, if there are any. A smear of scattered endocervical cells (columnar cells with apical mucin) may be all you get, but ideally there are fragments of squamous epithelium to evaluate. These are evaluated by the same criteria as the biopsy, described next.

The Approach to the Biopsy

On low power, first survey the squamous epithelium and the endocervical glands to look for areas that stand out as being more disorganized, darker, denser, or more inflamed than other areas. This applies to the squamous epithelium as well as to individual endocervical glands. Any suspicious area should be studied at higher magnification to look for the following:

- Low-grade squamous intraepithelial lesion (LSIL; cervical intraepithelial neoplasia grade 1 [CIN1])
  - Low-grade squamous intraepithelial lesion is a viral cytopathic effect that affects primarily the upper cell layers of the epithelium.
  - The cells have pleomorphic, wrinkled, hyperchromatic nuclei with a perinuclear cleared halo; these are called *koilocytes*. Binucleation is common (Figure 16.3).
  - It can look haphazard at low power, but the basal layer should be maintained, and mitoses should not be higher than the lower one third.
- High-grade squamous intraepithelial lesion (HSIL; CIN2–3)
  - High-grade squamous intraepithelial lesion is a persistence of immaturity along with dysplastic changes. Essentially, the basal cells are becoming "immortalized," like a cancerous cell, and are not maturing and differentiating as they should.
  - The overall impression is of a denser and darker epithelium due to the high nuclear/cytoplasmic (N/C) ratios.

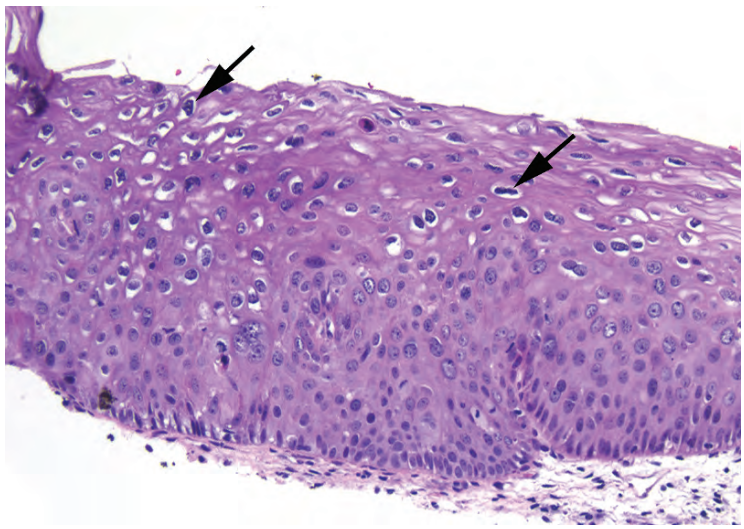

Figure 16.3. Low-grade squamous intraepithelial lesion. The hallmark of low-grade squamous intraepithelial lesion is the koilocyte, which is a squamous cell with HPV viral cytopathic effect. The nuclei are hyperchromatic (dark) and raisinoid (crinkly; see arrows), with a surrounding clear halo in the cytoplasm. Other good features include superficial nuclei that are larger than the nuclei below them and binucleated cells.

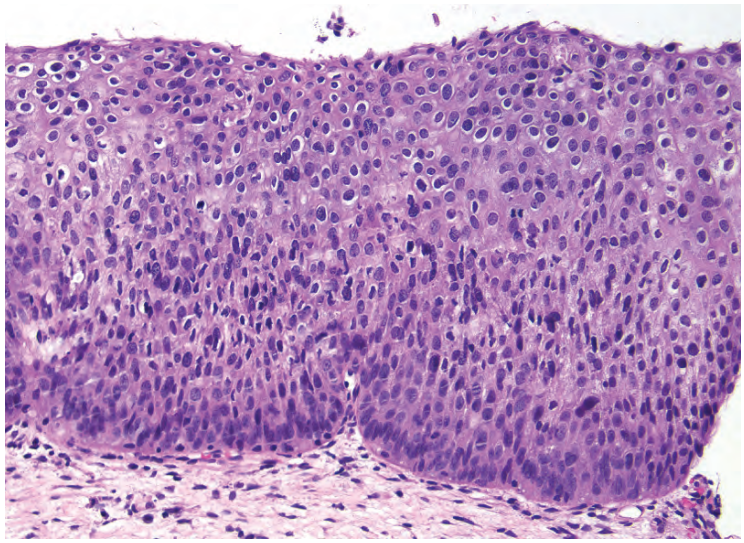

Figure 16.4. High-grade squamous intraepithelial lesion. In a high-grade lesion, paradoxically, the nuclei may not look as abnormal as in low-grade squamous intraepithelial lesion. The hallmark of high-grade squamous intraepithelial lesion is a persistence of immature-appearing cells throughout the epithelium. The nuclei are hyperchromatic and may have slightly irregular nuclear outlines, but the most striking feature at low power is the high nuclear/cytoplasmic ratios present from top to bottom.

- Atypia is seen in all cell layers, from the bottom up. The nuclei may not be as large or as bizarre as LSIL but are uniformly crowded, enlarged, and hyperchromatic with clumped chromatin and irregular membranes (boulder nuclei), and mitoses are present in the upper two thirds (Figure 16.4).
- CIN2 may show maturation at the surface or overlying LSIL.
- CIN3 shows full-thickness immaturity and atypia.
- HSIL can grow into endocervical glands, which should be mentioned in the diagnosis.

Do not confuse HSIL with *immature squamous metaplasia*, which has the following characteristics:

- Well-defined cell borders and low N/C ratios
- The "boiling mud" look (Figure 16.5)
- Usually pinker than HSIL
- Birds-egg nuclei (smooth, round, with even chromatin)
- Surface mucin or columnar layer

In very tough cases, the two can be differentiated by Ki67 and p16. Ki67 should be positive only in the basal layer in metaplasia, and p16, a marker for human papillomavirus infection, should be negative (or at most, focal).

Reactive Changes

Be wary of calling an SIL in the context of extensive acute inflammation (neutrophils). Reactive changes include the following:

- Regularly spaced nuclei with prominent nucleoli, homogeneous size, and smooth contours (Figure 16.6)
- Maturing upper layers without atypia
- Spongiotic edema

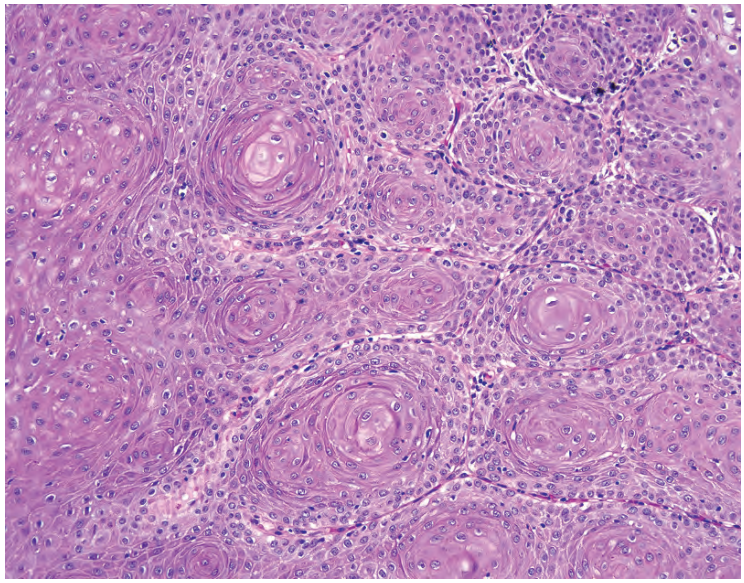

Figure 16.5. Immature squamous metaplasia. A tangential cut of squamous metaplasia can look like a lesion. However, this pattern of concentric whorls of cells with central pools of pink cytoplasm (resembling the boiling mud puddles of Yosemite) is typical of benign squamous metaplasia.

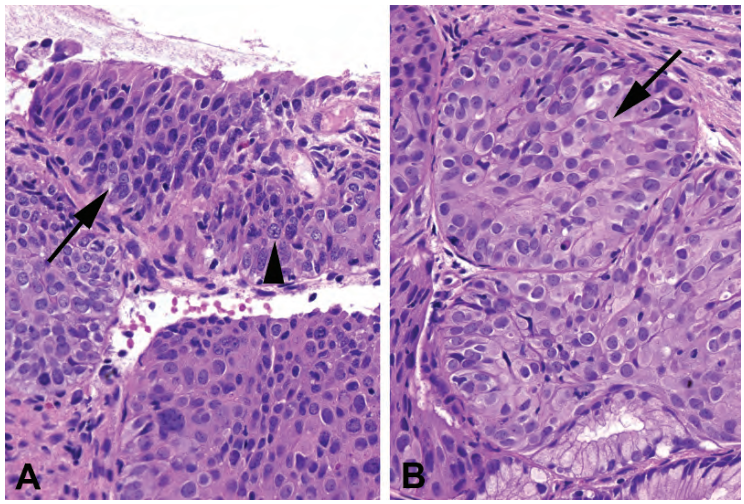

Figure 16.6. Dysplasia versus reactive changes. **(A)** In this example of high-grade squamous intraepithelial lesion, the dysplastic nuclei are irregularly shaped and appear to interlock together like stones in a wall (arrow). The quality of the chromatin is characteristic as well; it is dark and granular. Occasional nucleoli are visible (arrowhead), but they are surrounded by clumpy chromatin within the nucleus. **(B)** In reactive changes, the nuclei may be enlarged, but each nucleus remains smooth and oval in shape. The chromatin has a fine, even texture and is pale in color compared to the dysplastic cells in A. Small dense nucleoli are visible in many of the cells (arrow).

Invasive Squamous Cell Carcinoma

In the case of extensive HSIL (which is functionally carcinoma in situ), you should carefully search for evidence of invasion. As with squamous carcinoma in the skin or oropharynx, identifying invasion can be difficult and depends on multiple features. Features of invasion are similar to those in other sites:

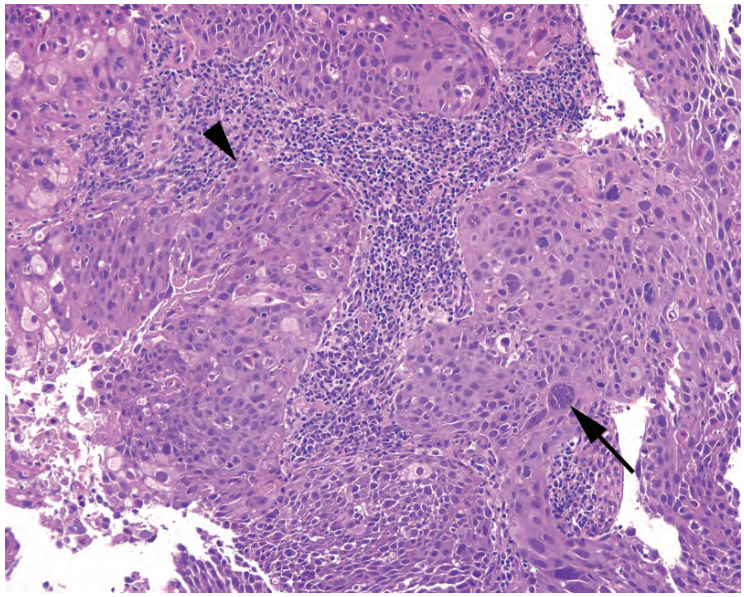

FIGURE 16.7. Invasive squamous cell carcinoma. Broad fronts of cells push into the stroma of the cervix, and at the leading edge there is a ragged border with individual infiltrating cells (arrowhead). Occasional huge and pleomorphic cells are visible (arrow).

- Deep keratinization
- Large nucleoli
- Blurred or sawtooth interface between epithelium and stroma (Figure 16.7)
- Loss of palisading basal layer
- Desmoplastic response within stroma

Invasion to a depth of less than 3 mm is considered microinvasion and has a better prognosis.

The differential diagnosis for invasion includes pseudoepitheliomatous hyperplasia, glandular involvement by HSIL, and placental site nodules. HSIL has a tendency to crawl down into endocervical glands, much like squamous metaplasia does. Although this is important and should be mentioned, it must be differentiated from invasion. Clues include remnants of columnar epithelium, a smooth rounded contour to the gland, and the lack of individual cells in the stroma.

A *placental site nodule* is a remote remnant of pregnancy—aggregates of intermediate trophoblastic cells that have large single nuclei that can look atypical, found in hyaline nodules. Their pink cytoplasm and atypical cells may remind you of deep invasive keratinizing cells (Figure 16.8). However, the cell borders should be less well defined than squamous nests, and the nuclei show bizarre degenerative atypia—large, dark, and smudgy (meaning, little chromatin detail) nuclei without nucleoli.

Miscellaneous Benign Entities

Endocervical polyps have fibrotic (spindly) stroma with a polypoid shape and normal endocervical glands or epithelium. The may have cysts, inflammation, or tubal metaplasia (luminal cilia).

Nabothian cysts are large dilated mucous-filled glands, lined with columnar epithelium. *Tunnel clusters* are lobular groups of complex branching glands (cystic or tubular), with benign columnar epithelium.

Microglandular hyperplasia is associated with oral contraceptive pills. It looks like a proliferation of small back-to-back glands lined with cuboidal or columnar cells with mucin

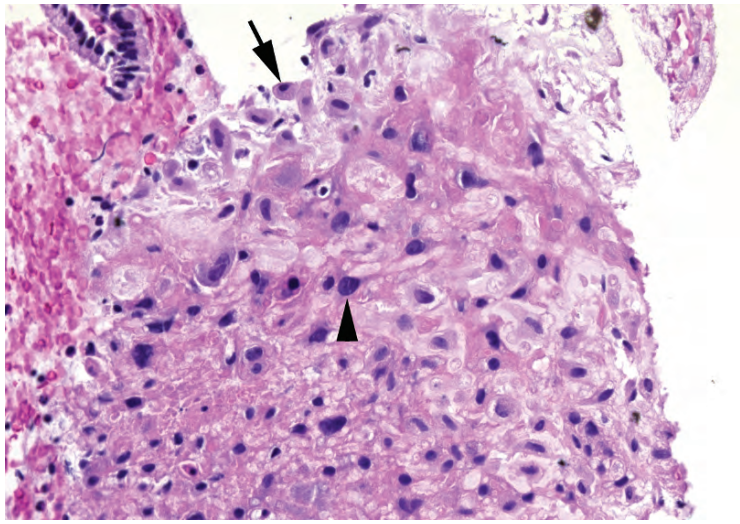

Figure 16.8. Placental site nodule, cervix. Although the dark nuclei and pink cytoplasm are concerning for squamous cell carcinoma, the nuclei are predominantly small and oval, with a few large nuclei visible (arrowhead). These large cells have dark but smudgy (blurred or indistinct) chromatin, without the chunky texture seen in HSIL (see Figure 16.6) and do not have the nuclear membrane irregularity of invasive squamous cell carcinoma (see Figure 16.7). The final clue is what appears to be a decidualized cell at the periphery (arrow).

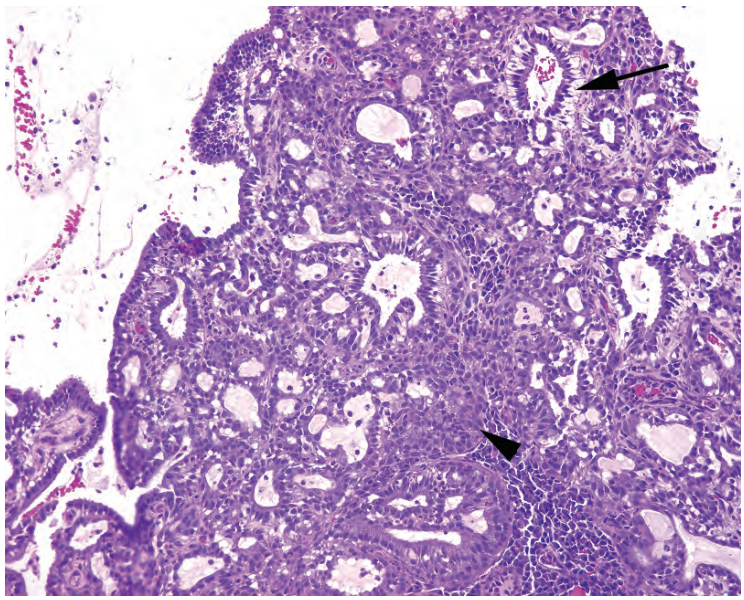

Figure 16.9. Microglandular hyperplasia. These endocervical glands show a very cellular proliferation composed of mucinous cells (arrow) and squamous metaplasia (arrowhead) and a cribriform pattern of lumens. This is benign.

vacuoles (Figure 16.9). The low-power impression is that of a cribriform architecture, but the glands should still appear overall pale and pink, in contrast to the dark blue adenocarcinoma in situ (AIS) (discussed later).

Endometriosis appears as dense blue palisaded columnar glands without mucus, surrounded by edematous endometrial-type stroma. The dark glands can be very eye catching

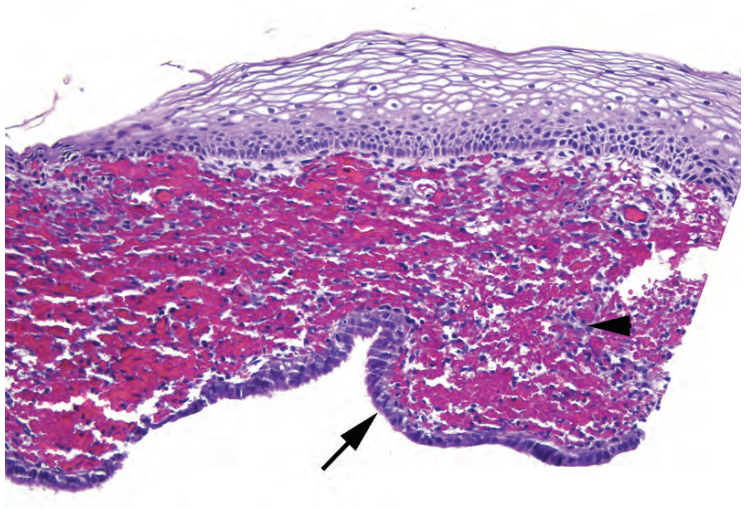

Figure 16.10. Endometriosis. This cervical biopsy specimen shows a squamous epithelium overlying stroma with hemorrhage. At the bottom of the fragment there is a dark cuboidal lining (arrow) resembling endometrial epithelium. The telltale endometrial stroma (arrowhead) is mostly obscured by blood.

and can even show mitoses, so recognizing the stroma is the key to making the diagnosis (Figure 16.10). The presence of extravasated red blood cells or hemosiderin is very helpful.

Glandular Lesions

Most pathologists do not use endocervical dysplasia as a diagnosis and only use the two ends of the spectrum, reactive atypia and AIS, for atypical noninvasive glandular lesions. Glands with AIS should stand out as being distinctly different from their benign neighbors (they look much darker). Recognizing these glands requires a conscious effort to look for them, however, because the eye tends to focus on the squamous epithelium, and AIS can be subtle at scanning magnification.

Features of AIS include the following:

- Close clusters of dark glands may resemble intestinal crypts or a tubular adenoma (Figure 16.11).
- Nuclei are tall and pseudostratified, enlarged, and hyperchromatic.
- Nucleoli may be present.
- Luminal mitoses and apoptosis are common.
- Papillary or cribriform architecture may be present and, if confluent, should raise the possibility of stromal invasion.
- Mucin may be present as scattered vacuoles or as discrete goblet cells (intestinal type).
- Ki67 staining is markedly elevated, and p16 staining should be diffusely positive (AIS is an HPV-associated lesion).

Invasive Adenocarcinoma

The most common variant of invasive adenocarcinoma is the endocervical type ("usual type"), which is an invasive form with the morphology seen in AIS. Features of invasion include the following:

- Cell clusters diving off into the stroma, as with squamous cell carcinoma
- Desmoplastic response

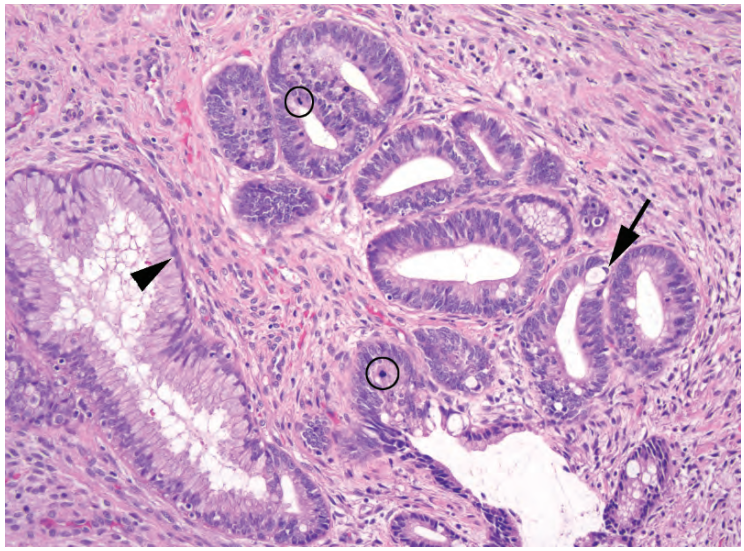

Figure 16.11. Adenocarcinoma in situ. This field shows some residual normal endocervical glands (arrowhead) adjacent to a very abnormal population with dark, elongated, crowded, and stratified nuclei representing adenocarcinoma in situ. Occasional intestinal-type goblet cells (arrow) and mitoses (circles) are present.

- Glands that are significantly deeper into the stroma than the benign glands (on perpendicular section)
- Glands with AIS features that are too crowded, or back to back

Endocervical adenocarcinoma may be hard to distinguish from endometrial adenocarcinoma. However, the endocervical variety should be diffusely p16 positive, whereas the endometrial type is usually estrogen and progesterone positive. See images and test yourself on cervical lesions at http://screening.iarc.fr/atlashisto.php.

Vaginal and Vulvar Epithelium

Human papillomavirus can cause similar lesions in the vagina and vulva. As in the cervix, the lesions are grouped into low-grade and high-grade, corresponding to vaginal intraepithelial neoplasia grade 1 (VAIN1)/vulvar intraepithelial neoplasia grade 1 (VIN1) and VAIN2–3/VIN2–3. The clinical term *bowenoid papulosis* refers to VIN3, synonymous with carcinoma in situ (Figure 16.12). As in the cervix, these lesions can progress to invasive squamous cell carcinoma.

Papillary Lesions

An exophytic viral lesion with LSIL-type changes is called a *condyloma acuminatum*. The LSIL features are somewhat subtler in a condyloma, and the nuclei may not be as obviously koilocytic. However, the presence of a verrucous (papillary, hyperkeratotic, and parakeratotic) lesion is virtually diagnostic of a condyloma (Figure 16.13). Non-HPV-related squamous papillomas also occur, usually in the vestibule, but without evidence of viral changes or hyperkeratosis. Finally, if the lesion is composed of more stroma than epithelium, it is most likely a fibroepithelial polyp (skin tag).

Inflammatory Skin Conditions

Lichen sclerosus appears as a flat, white, shiny patch clinically and in developed form looks like a bland pale swath of collagen (homogenous hyalinization) just beneath a thinned and

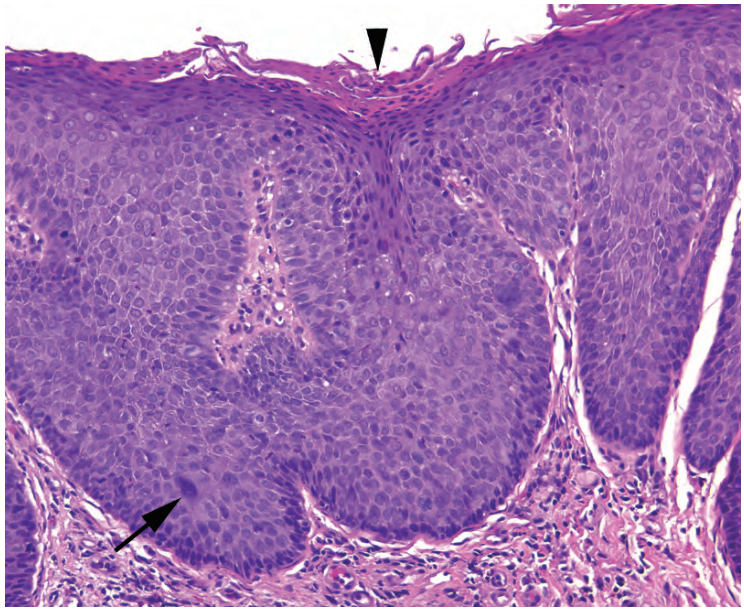

Figure 16.12. Vulvar intraepithelial neoplasia (VIN3). This biopsy specimen shows hyperkeratosis and parakeratosis (arrowhead) overlying a very blue squamous epithelium. Although the nuclear changes are not as obvious as in high-grade cervical lesions, there is loss of polarity and high nuclear/cytoplasmic ratios in the superficial epithelium. Occasional large atypical cells (arrow) are visible.

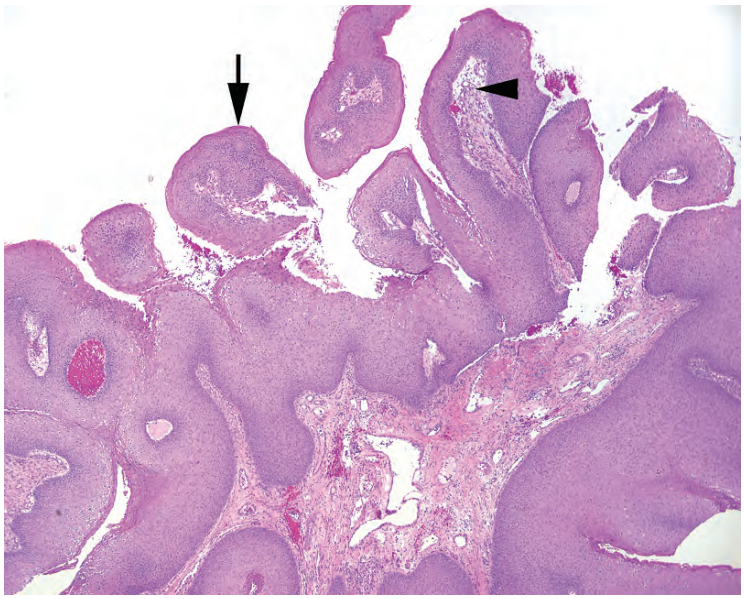

Figure 16.13. Condyloma. This exophytic lesion has prominent fibrovascular cores (arrowhead) underlying a thickened and hyperkeratotic squamous epithelium (arrow). Koilocytic or LSIL-type changes are not always obvious in condylomas.

flattened epidermis (Figure 16.14). *Lichen simplex chronicus*, on the other hand, is related to chronic spongiotic dermatitis and is characterized by epidermal thickening and hyperkeratosis over chronic inflammation in the dermis. It is a diagnosis of exclusion; you should first rule out fungal infection and squamous dysplasia.

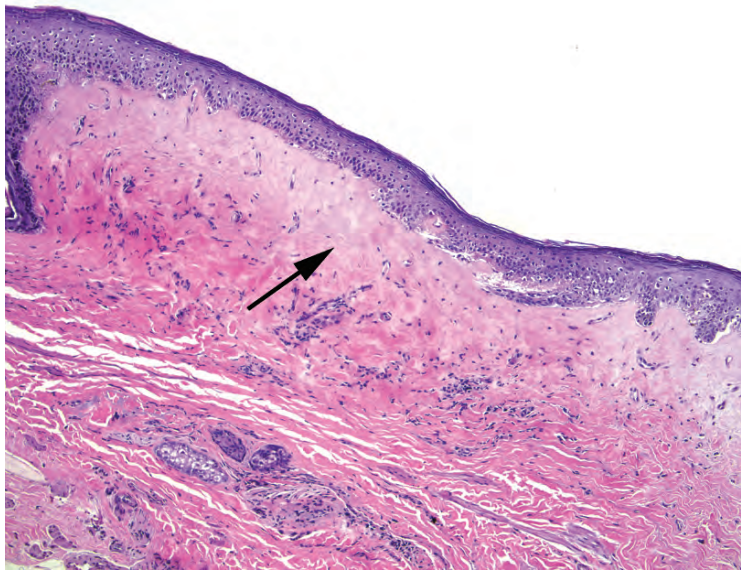

Figure 16.14. Lichen sclerosus. The epithelium is thin and atrophic, and the collagen underneath is pale, dense, and homogenized in texture (arrow). The dermal–epidermal junction is flattened, with an absence of rete.

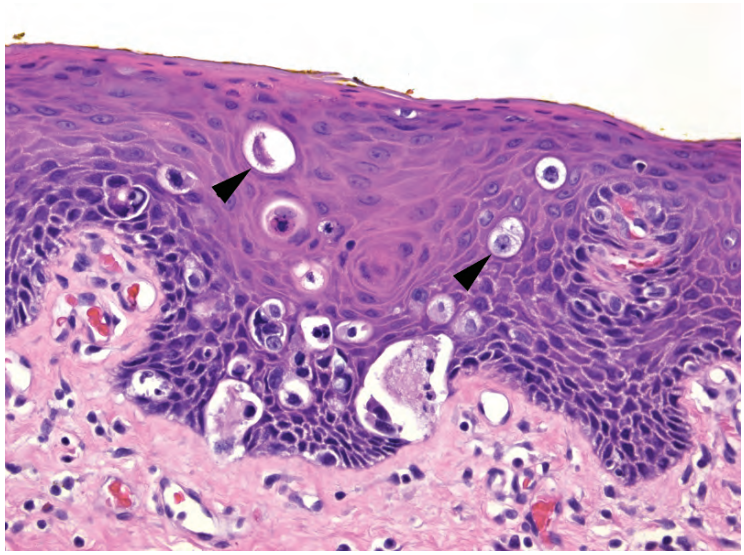

Figure 16.15. Paget's disease. Several nonsquamous cells (arrowheads) are visible within the squamous epithelium.

Paget's disease, extramammary type, is not an inflammatory skin disorder but may be mistaken for one clinically. Unlike in the breast, it does not always indicate an underlying adenocarcinoma. However, in other respects it is analogous to mammary Paget's, with large atypical carcinomatous cells percolating through a benign epidermis (Figure 16.15).

Melanoma, vulvar type, must always be in your differential diagnosis when you see pagetoid-type cells. A simple immunopanel will differentiate the two.

17 Uterus

The endometrium is a cycling glandular and stromal layer overlying the myometrium of the uterus. The appearance varies widely across different phases of the menstrual cycle, pregnancy, and menopause. Common reasons for performing an endometrial biopsy include the following:

- Abnormal vaginal bleeding
- A "thickened endometrial stripe" found on ultrasound, suggesting hyperplasia or carcinoma
- Part of an infertility workup
- Follow-up study for women with a history of hyperplasia who have been conservatively treated with hormones

The reason for the biopsy will influence your approach to the slide. Regardless of history, start by differentiating between atrophic, inactive, proliferative, secretory, and hormone-treated endometrium. On low power, survey the epithelium to get a feel for the glands and stroma:

- *Atrophic* endometrium has a low gland-to-stroma ratio, and the glands are thin, with an almost cuboidal epithelium, and no mitoses. In biopsy specimens, they tend to come off in thin strips that look like hair pins (Figure 17.1).
- *Inactive to proliferative* endometrium has a fuller, blue look to the stroma and a gland-to-stroma ratio of about 1:1 in proliferative endometrium (less in inactive). The glands are simple tubular structures that stand out as dark blue "donuts" with pseudostratified nuclei (slight variation in nuclear location, but predominantly basal) and columnar epithelium (Figure 17.2). If mitoses are readily visible in the glands, the endometrium is proliferative. Absence of mitoses indicates an inactive endometrium.
- *Secretory* endometrium has prominent spiral arterioles and variably edematous stroma so that the stromal cells look almost like naked nuclei floating in water. The glands are notable for cytoplasmic secretory vacuoles and secretions in the lumen (Figure 17.3). Later secretory stroma begins to get decidualized, or acquires pink cytoplasm, and the glands lose their vacuoles and acquire low cuboidal pink cells, ragged luminal edges, and a tortuous spiral shape. You should not see mitoses in secretory glands.
- *Progestin-treated* endometrium, like gestational endometrium, has a very decidualized stroma (plump pink cells with visible cytoplasm), but is paired with attenuated, flattened gland epithelium (Figure 17.4). These changes are due to the unopposed progesterone exposure. Unopposed estrogen, on the other hand, has a proliferative effect, and increases the chance of hyperplasia or carcinoma. (Tamoxifen acts as an estrogen agonist in the endometrium.)

Why are the endometrial characteristics important? Secretory endometrium, almost by definition, is not hyperplastic. Once you have established secretory change, you (usually) do not need to agonize over crowded glands. Because progesterone pushes the endometrium toward secretory change, it is used as treatment for hyperplasia; if you can prod the endometrium to complete the cycle and shed, the hyperplasia may go away.

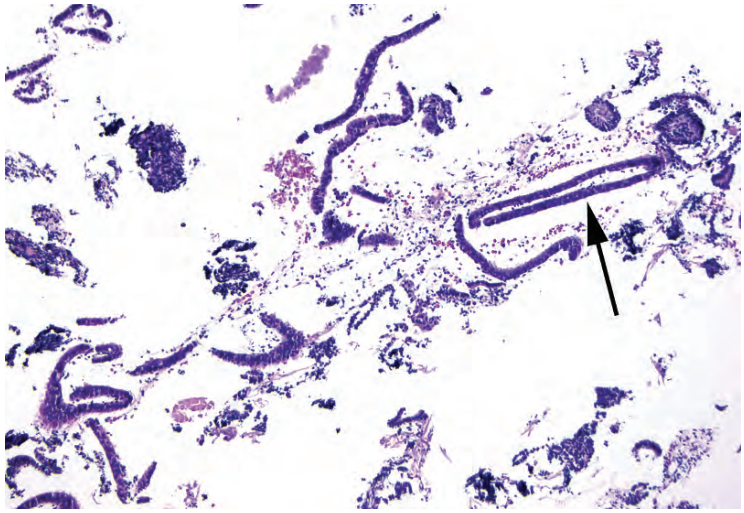

Figure 17.1. Atrophic endometrium. When curetted, the epithelium typically comes off in thin strips resembling hairpins (arrow). The specimen is also scant.

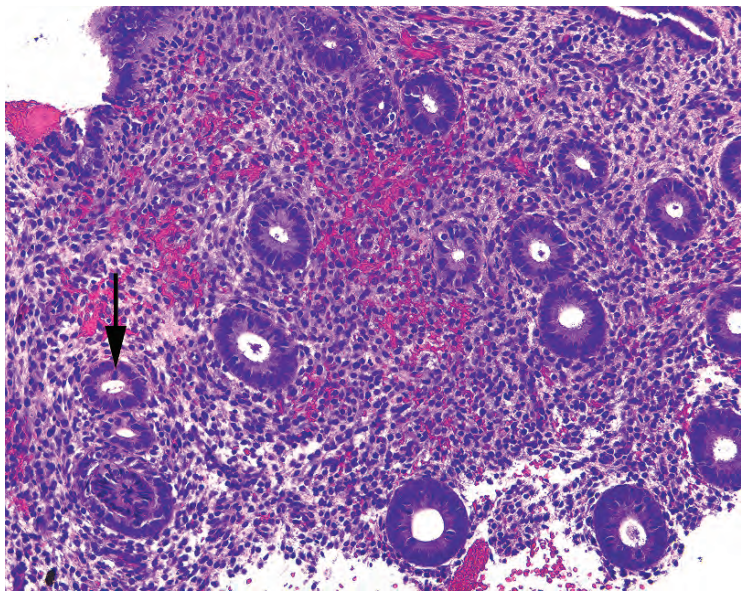

Figure 17.2. Proliferative endometrium. Multiple donut-shaped glands are visible, with dark oblong nuclei and frequent mitoses (arrow).

Next, within the biopsy fragments, look for possible causes of bleeding:

- *Benign endometrial polyp*: Benign endometrial polyps are composed of fibrotic (pink and spindly) stroma, thick-walled vessels, and usually nonfunctional (atrophic) and/or cystically dilated glands (Figure 17.5).
- *Endometrial stromal breakdown*: The stroma takes on a blurry blue look as it condenses into small dense aggregates ("blue balls"). The associated surface epithelium shows eosinophilic metaplasia, becoming almost oncocytic in appearance. Fibrin thrombi in vessels and neutrophils are also common features (Figure 17.6). The background endometrium may be end secretory (in normal menstrual bleeding) or proliferative (in dysfunctional bleeding).

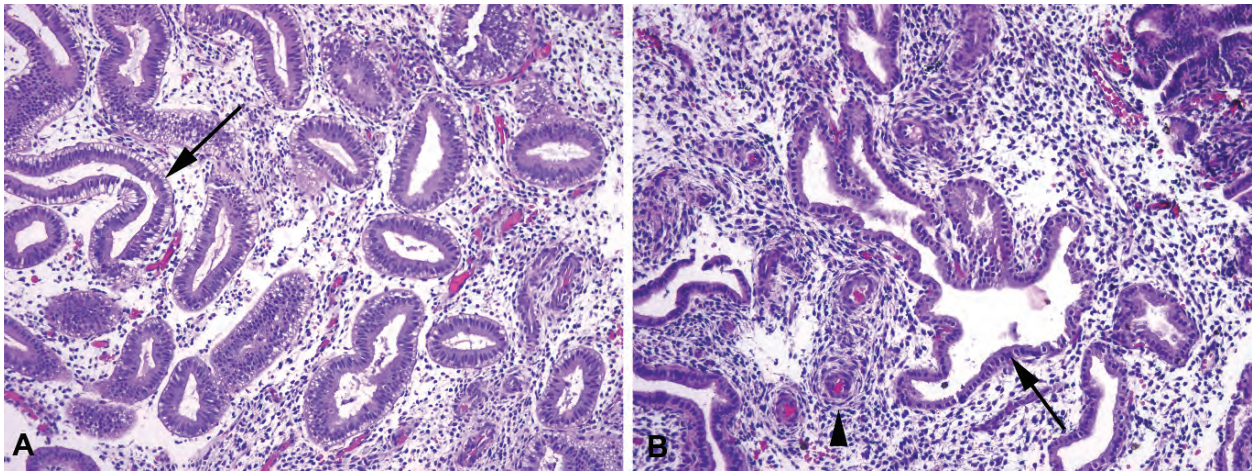

Figure 17.3. Secretory endometrium, various phases. **(A)** In early secretory endometrium, the glands have become tortuous in shape, and prominent cytoplasmic vacuoles are present (subnuclear, in this example; arrow). **(B)** Later in the secretory phase, the cytoplasmic vacuoles are gone, and the epithelium is more cuboidal in shape, with small round nuclei (arrow). The stroma is edematous, and early decidualization (accumulation of pink cytoplasm) is beginning around the spiral arteries (arrowhead).

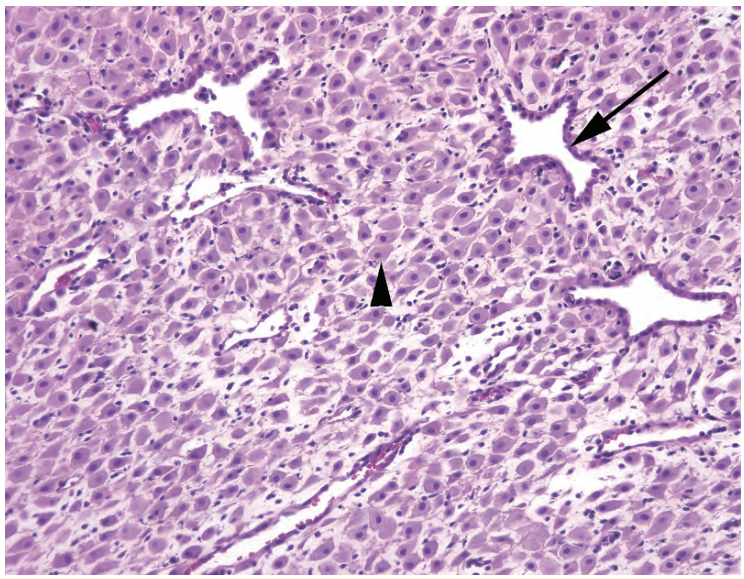

Figure 17.4. Progestin-treated endometrium. The glands are still tortuous in shape, like secretory endometrium, but the epithelium is markedly thinned (arrow). The stromal cells are decidualized (arrowhead), which means they have plump pink cytoplasm and distinct cell borders.

- Endometritis: The diagnosis of *acute endometritis* requires microabscesses and epithelial destruction; the presence of neutrophils alone may just indicate menstrual breakdown. *Chronic endometritis* is diagnosed by the presence of plasma cells. In general, the stroma takes on a blue spindly look, and there are increased numbers of lymphocytes; these features should prompt you to crawl around at 20× looking for plasma cells (Figure 17.7).
- *Disordered proliferative endometrium*: Disordered proliferative endometrium is notable for a mixture of cystically dilated, budding, and tubular glands in a proliferative setting, with only focal glandular crowding. It occurs during anovulatory cycles.
- *Atrophy*: Atrophy, described earlier, is responsible for about half of all cases of abnormal postmenopausal bleeding.

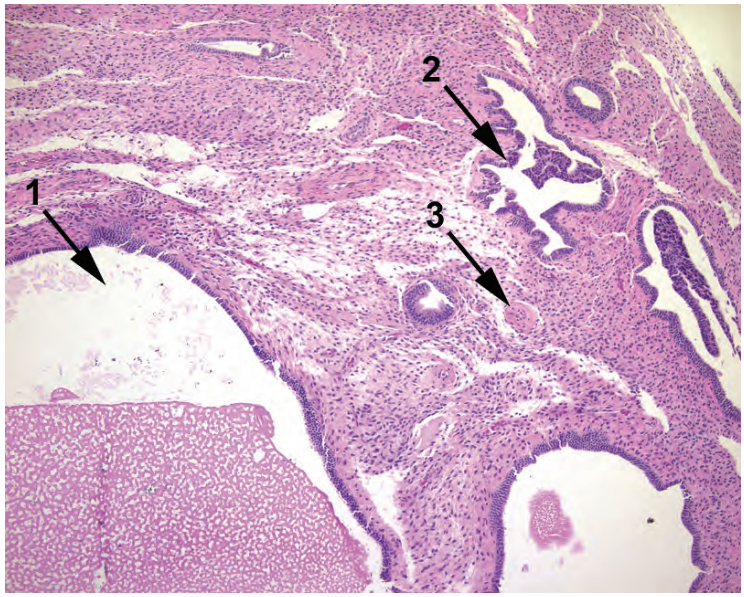

Figure 17.5. Benign endometrial polyp. This polyp shows cystic dilation of glands (1), secretory-type epithelium (2), and thickened arteries (3). The stroma is also pink, indicating a high collagen content.

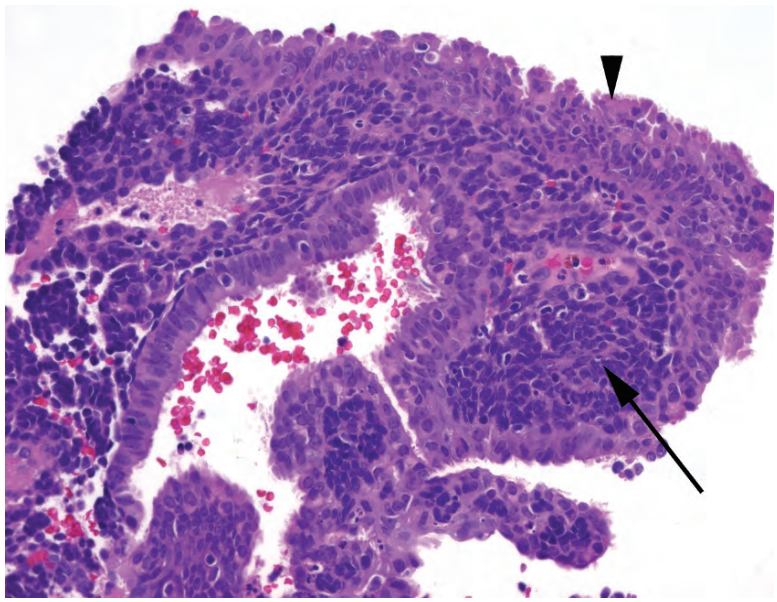

Figure 17.6. Endometrial stromal breakdown. The stroma is condensed into an extremely blue mass of tightly packed cells (arrow). The overlying epithelium is expanded into papillary tufts of pink cells, some with cilia, which is a metaplastic change (arrowhead).

Hyperplasia

Hyperplasia is defined as an increase in the gland-to-stroma ratio, and you will notice it as "crowded glands" in a proliferative setting. Endometrial hyperplasia is categorized with two criteria: architecture (simple vs. complex) and cytology (with or without atypia). The term *dysplasia* is not applied to endometrium. There are varying degrees of hyperplasia:

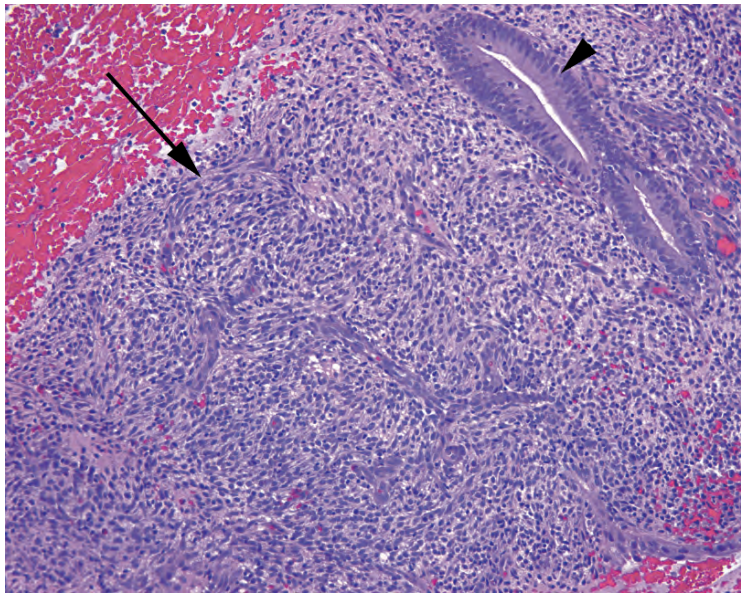

FIGURE 17.7. Chronic endometritis. At low power, the diagnostic plasma cells are not visible, but the spindly, swirling blue stroma (arrow) should be a clue to look more closely. The epithelium here is proliferative (arrowhead).

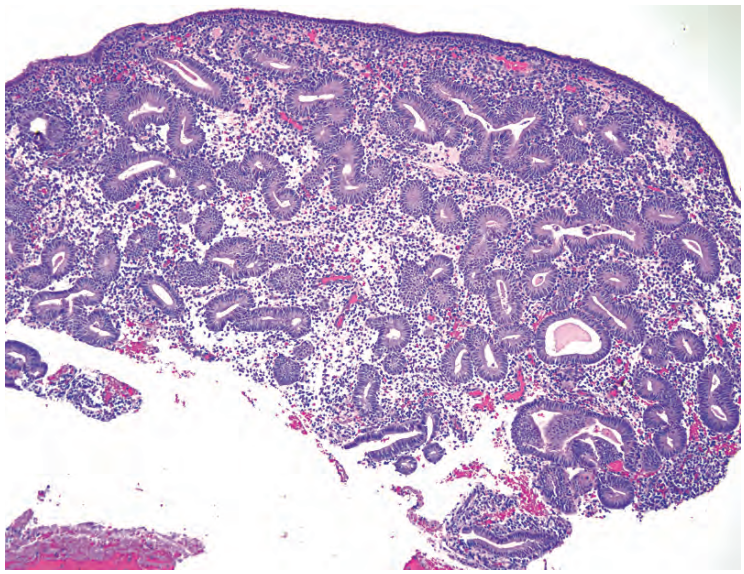

FIGURE 17.8. Simple hyperplasia. In this biopsy specimen, the glands appear proliferative and are too crowded (the gland-to-stroma ratio is greater than 1). The cells resemble normal endometrium and are not atypical.

- Simple hyperplasia: Simple hyperplasia almost always occurs without atypia. It appears as crowded, tubular, or minimally branched glands (Figure 17.8).
- Complex hyperplasia: Complex hyperplasia can occur with or without atypia. It appears as back-to-back glands with little stroma (even more crowded than in simple hyperplasia), and the glands have increasingly complex, branched outlines.
- Atypical hyperplasia: The definition of atypia varies among organs. In the endometrium, the *normal* proliferating gland has hyperchromatic, pseudostratified, elongated nuclei and

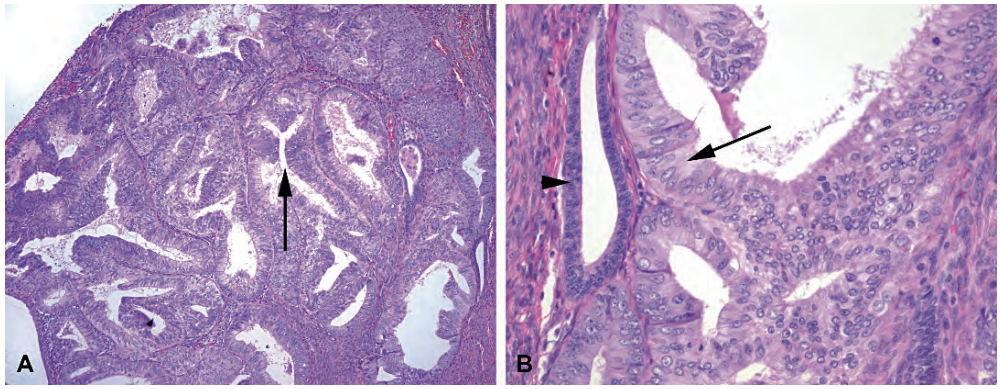

Figure 17.9. Complex atypical hyperplasia. **(A)** At low power, the glands are very crowded, even back to back, and the gland lumens have become branching and irregular (arrow). **(B)** At high power, comparing the hyperplastic epithelium (arrow) with normal residual glands (arrowhead), the hyperplastic cells have round nuclei, and pale, vesicular chromatin with prominent nucleoli, diagnostic of atypia.

frequent mitoses. This appearance in other organs, such as the colon, may make you think of low-grade dysplasia (such as a tubular adenoma). In endometrial *atypia*, the nuclei become round and pale or vesicular because of the chromatin clumping up and migrating to the nuclear membrane (Figure 17.9). Nucleoli may be prominent. The nuclei lose polarity and are seen at all levels of the epithelium (stratified). Nuclei are larger and show increased variability in size and shape. The cytoplasm becomes more eosinophilic than in nonatypical glands. Atypia is present or absent but is not graded.

- Complex atypical hyperplasia: Complex atypical hyperplasia (CAH) is a precursor to carcinoma. Florid cases of CAH may in fact be hard to distinguish from well-differentiated endometrial carcinoma—so much so that experts may disagree. The concept of "carcinoma in situ" is not used in the endometrium, but CAH is fairly equivalent to it.

Do not be fooled by artifactual crowding in a biopsy. When glands are scraped out of the uterine cavity, they may clump together and look crowded. You need to find an intact piece of endometrium to evaluate the gland to stroma ratio. Also, beware of calling hyperplasia in the setting of an endometrial polyp (they are often crowded) or secretory endometrium.

The Infertility Workup

When evaluating an endometrial biopsy specimen for infertility, first rule out the conditions listed earlier that can cause bleeding. Some infertility centers request that you date the endometrium, estimating the cycle day by histology. They are looking for a luteal phase defect, which is defined as a disparity of over 3 days between the calendar date and the histologic date in two consecutive biopsy specimens.

Proliferative endometrium cannot be dated. The first secretory change occurs, on average, on day 16 or so of a 28-day cycle. This change is the appearance of clear secretory vacuoles at the base of the epithelial cells, below the nuclei. When you see just a few of these in a generally proliferative endometrium, it is called *interval endometrium*. Beyond that day, specific histologic criteria are as follows:

Days 16 to 20: glands are the most helpful feature
- Day 16: subnuclear vacuoles, pseudostratified nuclei
- Day 17: subnuclear vacuoles, but with an orderly row of nuclei (the "piano key" look)
- Day 18: vacuoles above and below nuclei
- Day 19: few vacuoles, found only above nuclei; orderly row of nuclei, no mitoses
- Day 20: peak secretions in lumen and ragged luminal border, vacuoles rare

From days 21 to 28, the glands change little. They are exhausted and appear low columnar with orderly nuclei, no mitoses, and ragged luminal edges. They may also have degenerative apical vacuoles—tricky to discern from days 19 to 20. After day 21, the stroma is the key:

Day 21: beginning of stromal edema; secretion continues
Day 22: peak stromal edema with naked nuclei
Day 23: spiral arteries become prominent
Day 24: periarteriolar cuffing with predecidua (stromal cells around the arteries begin to get plump pink cytoplasm, creating a pink halo around the vessels)
Day 25: predecidual change under the surface epithelium
Day 26: decidual islands coalesce, lymphocytes begin to infiltrate stroma
Day 27: many neutrophils in a solid sheet of decidua, with focal necrosis and hemorrhage
Day 28: prominent necrosis, hemorrhage, clumping, and break up

Changes of Pregnancy

Gestational endometrium is a solid sheet of decidua. Decidual cells are plump polygonal cells with pink to lavender cytoplasm and small oval nuclei. The glandular epithelium becomes almost papillary in nature with a hypersecretory appearance.

Well-formed glands with ballooning, cleared-out cytoplasm and wildly pleomorphic nuclei are characteristic of the Arias-Stella reaction, a normal reaction to pregnancy (Figure 17.10). The changes can be focal. The lack of mitoses or infiltration differentiates this from clear cell carcinoma, as does the age of the patient (clear cell is usually postmenopausal) and the surrounding gestational changes.

In a patient with a history of pregnancy, you may see a placental site nodule: aggregates of intermediate trophoblastic cells, which have scattered large nuclei that look atypical, within hyaline nodules. Placental site nodules should be well-circumscribed. They are the benign remnants of old implantation sites (see Figure 16.8 in Chapter 16).

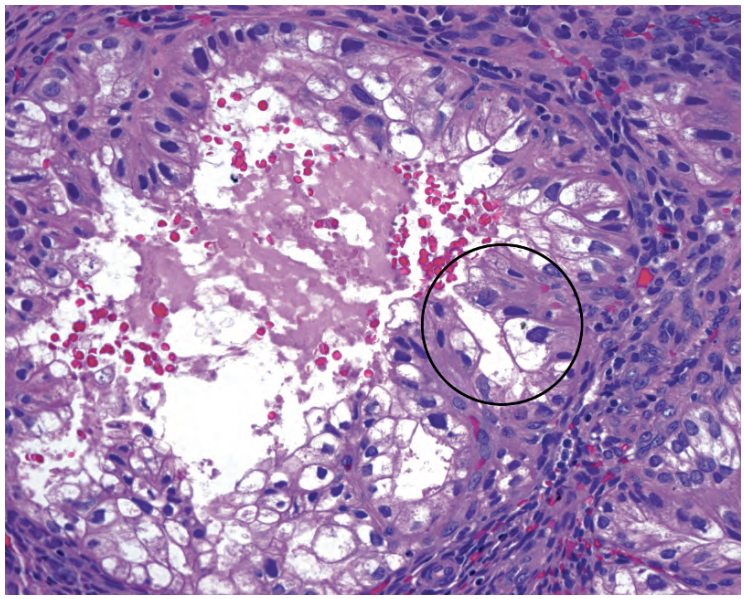

Figure 17.10. Arias-Stella reaction. Glands in the gestational endometrium can show bizarre cytology, including cleared-out cytoplasm and large hyperchromatic irregular nuclei (circle).

Types of Metaplasia

Metaplasia by itself is a benign process; however, metaplasia is often accompanied by hyperplasia. Still, it is important to recognize these cell varieties and not call them cancer. The less ominous sounding word *change* may be used instead of *metaplasia*. Cell types include the following:

- Tubal metaplasia: luminal cilia in an epithelium that looks slightly plumped up and cleared out (If you overlook the cilia, the nuclei may appear atypical.)
- Squamous metaplasia: swirling islands of immature squamous cells and, rarely, keratinization
- Mucinous metaplasia: mucinous, endocervical-type cells
- Eosinophilic metaplasia: increased eosinophilic cytoplasm; cells can proliferate in glands to the point of looking papillary (If the cells merge to form syncytial papillary tufts, it is papillary syncytial metaplasia.)
- Clear cell change: clear cells

Endometrial Malignancies

The endometrium has two cell types that can transform: glandular and stromal. Glandular cells give rise to several types of carcinoma, including endometrioid, serous, and clear cell. The stromal cells give rise to stromal sarcomas; these are entirely different from the leiomyosarcomas of the myometrium.

Endometrioid carcinoma is the most common type of endometrial cancer. It usually occurs in postmenopausal women (80% of cases), like its precursor lesion, complex atypical hyperplasia. Be cautious about diagnosing either type in a young woman, although it can happen.

Endometrioid carcinoma, in its well-differentiated form, closely resembles atypical endometrial glands. Architecturally, they are fused and complex and cover large areas without intervening stroma (Figure 17.11). The overall pattern may appear cribriform or villoglandular (like a villous adenoma of colon). The tumor may be limited to endometrium or may

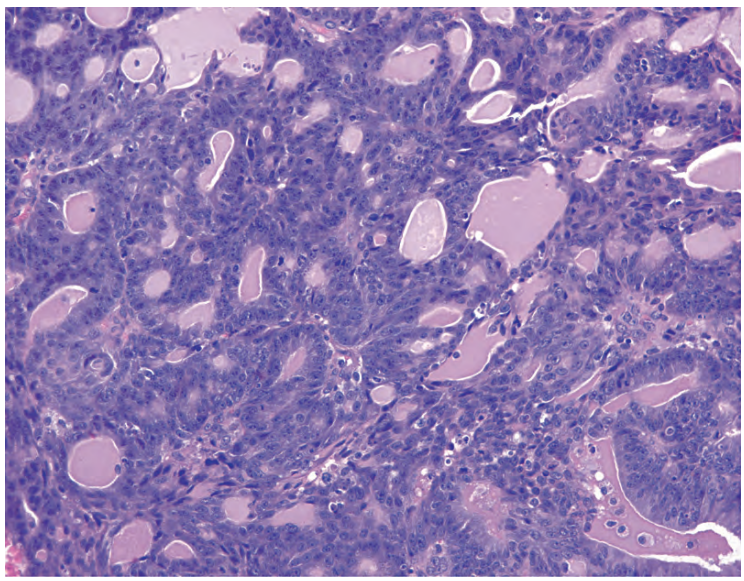

Figure 17.11. Endometrioid carcinoma. Foci of well-differentiated endometrioid carcinoma can be difficult to distinguish from complex atypical hyperplasia. However, the complicated proliferation of fused and cribriform glands in this biopsy specimen is diagnostic of carcinoma. The nuclei in this example resemble those of complex atypical hyperplasia.

invade myometrium or adjacent organs; the extent determines *stage*. The *grade* is determined by cytology and architecture. High grade tumors are equivalent to "poorly differentiated."

FIGO (International Federation of Gynecology and Obstetrics) grade 1: The tumor is <5% solid, where *solid* means sheets of cells that have lost their glandular differentiation. Areas of squamous metaplasia (common) are not counted as solid areas.
FIGO grade 2: The tumor is 6%–50% solid.
FIGO grade 3: The tumor is >50% solid.

Significant nuclear atypia (one of those features that require experience to judge) can raise the grade by one level unless the tumor is already grade 3. Variants of endometrioid carcinoma include those with squamous differentiation, a villoglandular variant, a secretory variant, and a ciliated cell variant. These variants are identified only when the majority of the tumor takes on that morphology.

Serous carcinoma is a separate tumor pathway, leading to a distinct type of carcinoma. Serous carcinoma is not associated with hormonal exposure or endometrial hyperplasia. It is considerably more aggressive than endometrioid carcinoma and tends to be diagnosed in older women. It is, by definition, high grade and therefore is not graded.

Histologically, it resembles serous carcinoma of the ovaries (formerly known as *papillary serous*). Therefore, its hallmark is a papillary architecture, although this is not required for diagnosis. The papillae have broad or fine fibrovascular cores with complex branching (Figure 17.12). The cells are notable for extreme atypia, including cherry-red nucleoli, bizarre mitoses, and multinucleated cells. As in the ovary, psammoma bodies are common.

The precursor lesion is believed to be *endometrial intraepithelial carcinoma* (EIC), a transformation of the surface epithelium, especially in polyps in older women. EIC is not quite analogous to carcinoma in situ, because EIC itself has metastatic potential. EIC, like serous carcinoma, is often associated with a *p53* mutation leading to overexpression. An immunostain for *p53* is sometimes used to confirm the diagnosis. Histologically, EIC appears as an abrupt transition on the surface from benign atrophic epithelium to pleomorphic, enlarged, atypical, mitotically active cells (Figure 17.13).

Clear cell carcinoma, like serous carcinoma, occurs primarily in older women, has no relation to hormone exposure or hyperplasia, and has a poor prognosis. Histologically, it will

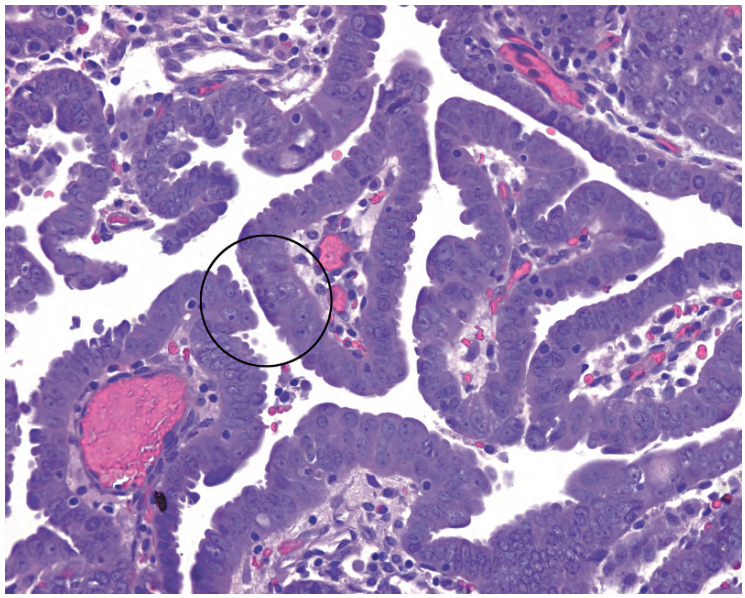

Figure 17.12. Serous carcinoma. Papillary structures are lined by atypical nuclei with prominent nucleoli (circle).

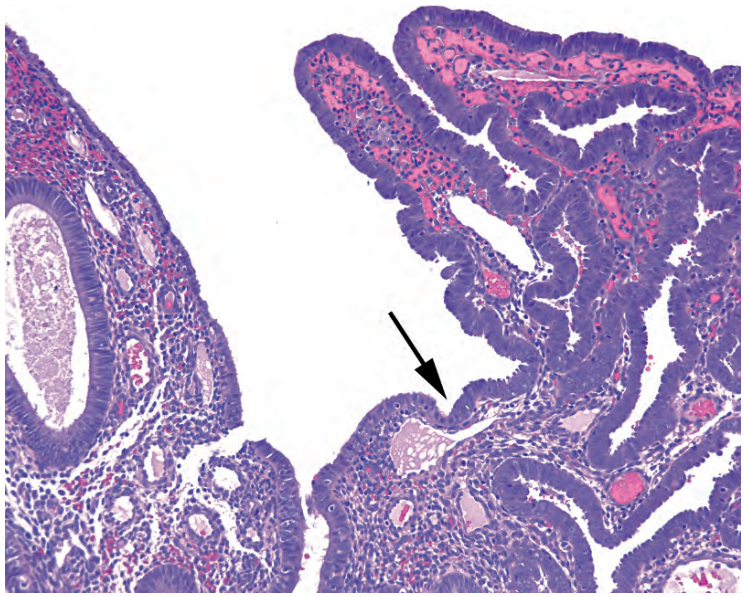

Figure 17.13. Endometrial intraepithelial carcinoma. There is an abrupt transition (arrow) from normal surface epithelium (left) to malignant cells (right). The cells of endometrial intraepithelial carcinoma resemble those of serous carcinoma.

remind you of clear cell neoplasms in other organs (such as renal cell). The cytoplasm is glycogen-rich and clear, the cell borders are distinct, and the architecture can be tubular, papillary, or solid. It may be mistaken for secretory endometrioid carcinoma but has much more nuclear pleomorphism. Like serous carcinoma, this tumor is high-grade by definition. Other rare types of carcinoma include squamous, mucinous, transitional cell, undifferentiated, and small cell carcinoma.

Endometrial stromal sarcoma is a rare malignancy of the endometrial stromal cells. The difference between a benign stromal nodule and a low-grade endometrial stromal sarcoma is in the interface with the surrounding tissue—sarcomas are infiltrative. These sarcomas have minimal atypia and few mitoses, as well as a prominent plexiform vascular proliferation (like the normal spiral arteries gone wild). The high-grade endometrial stromal sarcoma, however, has marked atypia and bizarre mitoses, like most high-grade sarcomas.

Malignant mullerian mixed tumor (carcinosarcoma) is a mixed tumor consisting of malignant glands in a sarcomatous stroma. It appears as a recognizable carcinoma, such as endometrioid type, with adjacent sarcomatous cells (large angular pleomorphic nuclei) in the stroma (Figure 17.14). Other soft tissue elements, like skeletal muscle or cartilage, may also show up. It is similar in concept to a carcinosarcoma of other organs.

In contrast, an *adenosarcoma* is a neoplasm with benign glands and a malignant stroma. An *adenofibroma* is benign glands with benign stroma. These tumors are similar to the phyllodes tumor of the breast, which can range from malignant to benign stroma.

Myometrium

The most common neoplasm of the uterus is actually the leiomyoma, a benign smooth muscle tumor of the myometrium. These tumors can be huge, multiple, myxoid, even necrotic, and still benign. Although benign, many are removed for symptomatic relief. When sampling these at the grossing bench, what you are looking for are areas that are different in texture from the typical rubbery dense consistency; areas of necrosis, hemorrhage, or dense white foci should be sampled.

The classic *leiomyoma* is a spindle cell lesion with intersecting fascicles of elongated cells, typically intersecting at right angles (Figure 17.15). The nuclei are long and thin with fine pale

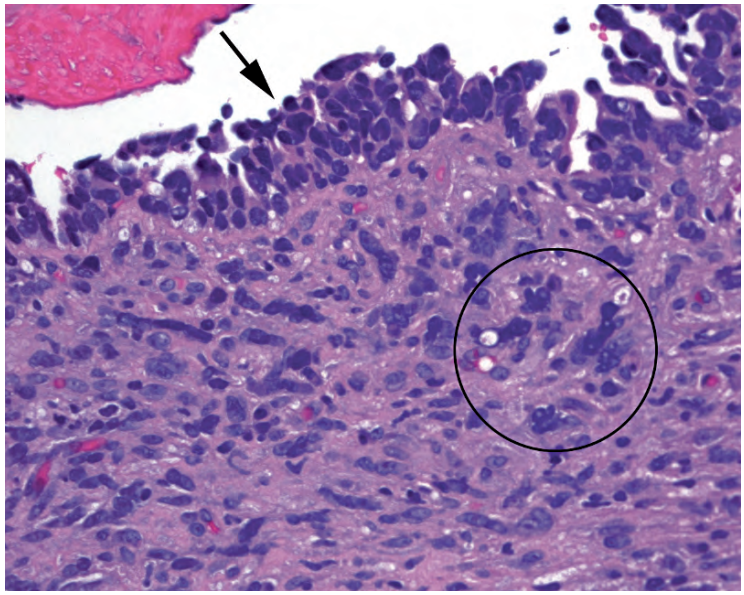

Figure 17.14. Malignant mullerian mixed tumor. This tumor is defined by the presence of carcinomatous cells in the epithelium (arrow) and sarcomatous cells in the stroma (circle). The carcinomatous cells are hyperchromatic and crowded; elsewhere in this biopsy specimen there were malignant glands. The sarcomatous cells are hyperchromatic, large, and irregular in shape, similar to malignant fibrous histiocytoma–type cells found in other sarcomas (see Chapter 28).

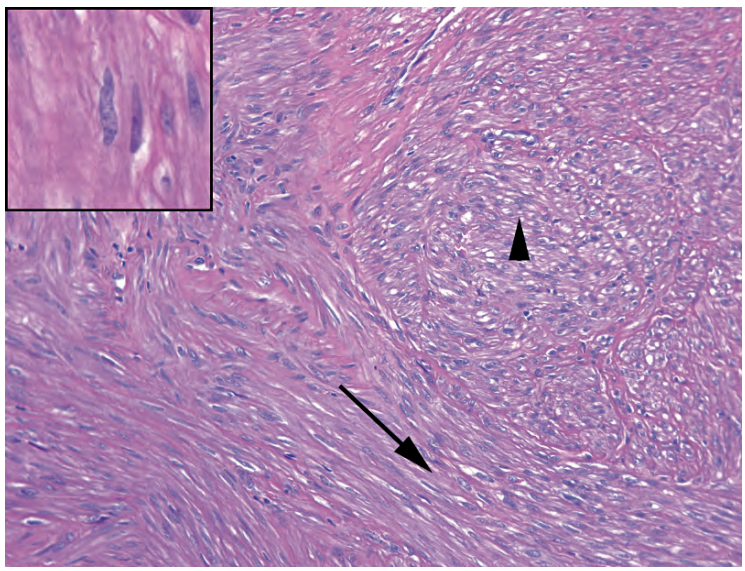

Figure 17.15. Leiomyoma. The low-power impression is that of fascicles or bundles of cells, some parallel to the slide (arrow) and some coming out at right angles (arrowhead). **Inset**: The nuclei are tapered and pale, with occasional paranuclear vacuoles, and sometimes show "corkscrew" morphology, as though the nucleus was twisted longitudinally. (Dog owners may liken this lumpy shape to something else.)

chromatin and small nucleoli. You may also see "corkscrew" nuclei, which are characteristic of smooth muscle. The stroma may be fibrotic, edematous, myxoid, or even hemorrhagic; these are all permissible degenerative changes in the absence of nuclear atypia or a high mitotic rate.

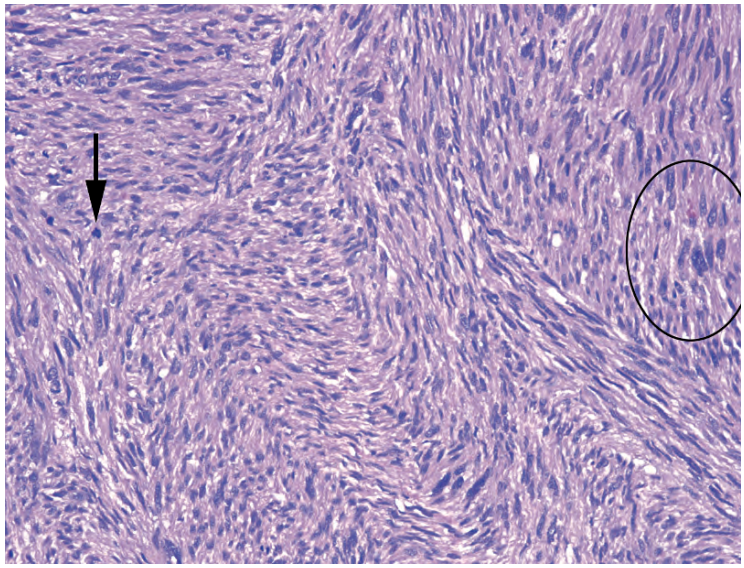

Figure 17.16. Leiomyosarcoma. The threshold for diagnosing leiomyosarcoma in the uterus is high. This lesion should be a much more cellular version of the leiomyoma, with mitoses (arrow), atypical and pleomorphic cells (circle), and necrosis (not seen here).

Leiomyosarcoma tends to present as a large, solitary mass and is not thought to arise from preexisting leiomyomas. It may resemble the fascicular leiomyoma, but mitotic activity must be high, over 10 per 10 high-power fields, and cytologic atypia should be prominent (Figure 17.16). (In sarcomas, atypia takes the form of large dark nuclei with crisp, irregularly shaped nuclear borders.) The third feature is coagulative necrosis. The threshold for diagnosing leiomyosarcoma is quite high in the uterus, unlike a leiomyomatous lesion found in the soft tissue or retroperitoneum, for example. In the uterus, atypia without mitotic activity, or mitoses without atypia, should discourage you from calling a sarcoma.

Adenomatoid tumor is a benign proliferation of mesothelial origin. It often occurs on the serosal surface of the uterus, resembling a leiomyoma both grossly and microscopically. The mesothelial tumor cells induce a smooth muscle proliferation that is probably often mistaken for leiomyoma. However, on close inspection, you will see small clefted spaces between the muscle bundles, lined by cuboidal cells forming gland-like or angiomatoid lumens. The cells can appear epithelial by histology and in fact would stain for cytokeratins. Accidentally missing it and calling it a leiomyoma does no harm to the patient; mistaking it for metastatic adenocarcinoma would be disastrous, however. Unlike adenocarcinoma, it should stain for calretinin.

18 Placenta

The gestational sac begins as a spherical structure, with the fetus surrounded by an *amnion*, a *chorion*, and placental *villi*. One surface of the gestational sac implants into the endometrium and becomes the placenta; the villi on the opposite surface degenerate. When you look at placental slides, you can see the layers of the amnion and chorion both in the membrane section (Figure 18.1) and on the fetal surface. In both locations, amnion is on the fetal side, chorion on the maternal side. The two membranes can be peeled apart grossly, because there is no tissue connection between the two.

The villi are fetal structures; they grow downward from the fetal surface in a branching architecture, like the roots of a tree. Vessels and cells inside the villi are fetal. There should not be any maternal vessels in the placenta itself. The spiral arteries of the decidua (endometrium), invaded by trophoblastic cells, spray maternal blood into the space between the villi.

Immature villi have an open and pale appearance (Figure 18.2); they are large compared with the terminal villi of the full-term placenta (when surface area is most required). They are lined by two cell layers, an outer syncytiotrophoblast and an inner cytotrophoblast layer. Very early villi may have a large intermediate trophoblastic proliferation on the surface, but it should be polar (only on one surface, like Don King's hair). Circumferential proliferation is suspicious for hydatidiform mole.

Mature villi acquire syncytial knots and perivillous fibrin (like hyaline membranes lining the villi). They become tiny—just large enough to hold a few capillaries (see Figure 18.2).

Twin placentas are divided into categories based on how many cell layers they share. Two separate eggs fertilized by two sperm will always form two separate placentas, although they may mash into each other. With two placentas you will see two chorionic plates and two complete sets of membranes (Figure 18.3); this is called diamnionic-dichorionic (di-di). An ovum that splits very early can also produce two entirely separate placentas, so a di-di placenta may be either monozygotic or dizygotic twins.

An ovum that splits a little later, after it has already formed a chorion, will produce two separate amnions and two fetuses; this is a diamnionic-monochorionic placenta (di-mo). An even later split produces two fetuses in the same amnionic sac, or monoamnionic-monochorionic (mo-mo). If the split occurs any later than this, conjoined twins will develop.

Approach to the Slides

In the *umbilical cord*, look at the vessels on low power (Figure 18.4). There should be two arteries (usually with constricted lumens) and a vein (open lumen, or the mouth on the surreal Mr. Bill faces that are found on the walls of most histology laboratories). The number of vessels is always noted on sign out, because a two-vessel cord often indicates a fetal abnormality.

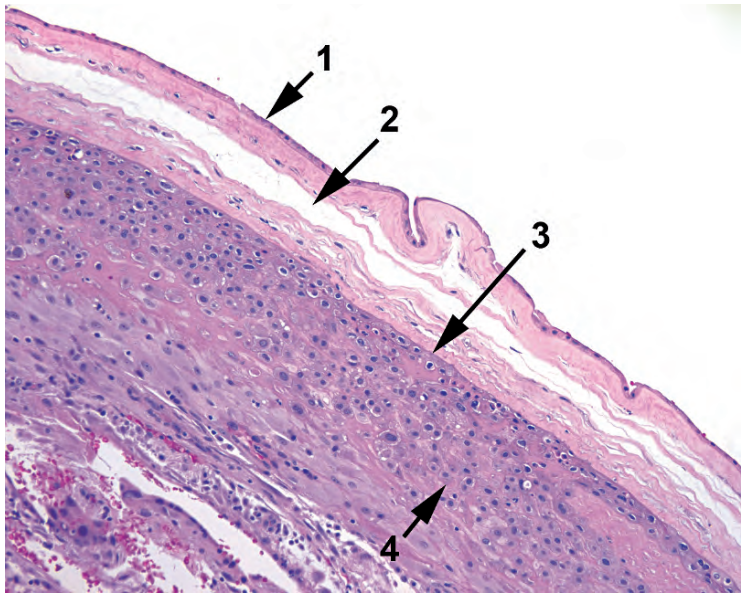

Figure 18.1. Placental membranes. In the membrane section, you can see amnion (1), an artifactual space (2) between amnion and chorion (3), and underlying decidua (4).

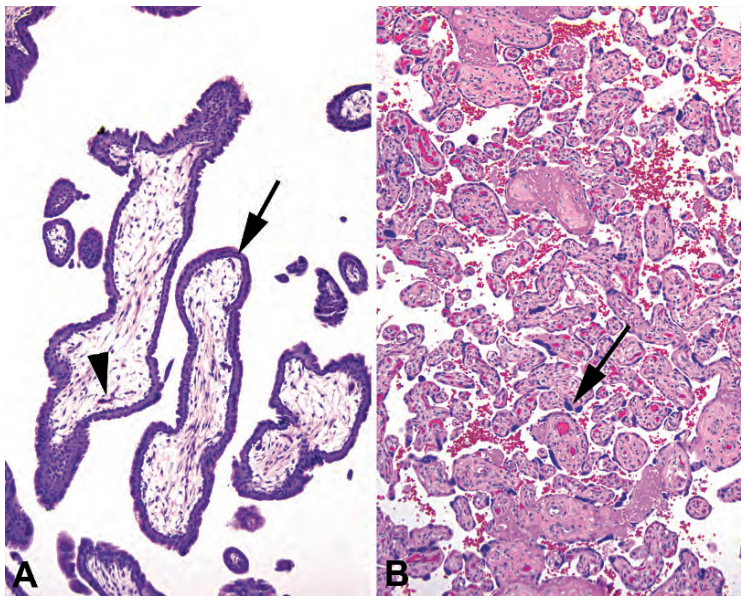

Figure 18.2. Immature villi versus terminal villi. **(A)** Villi at around 8–10 weeks are large in diameter and have a double layer of cells lining the surface (arrow). Tiny fetal capillaries have nucleated red blood cells inside (arrowhead). **(B)** Taken at the same magnification as A, this shows mature villi at approximately 38 weeks. The villi are much smaller, the fetal capillaries are more prominent, and the cytotrophoblasts have pulled away from the gas-exchange surface into syncytial knots (arrow). Maternal blood and fibrin are visible between villi.

Study the muscular wall of each vessel to look for neutrophils. Umbilical phlebitis, or neutrophils migrating into the vein wall, is an indicator of early funisitis (a fetal inflammatory response). More advanced funisitis involves the arteries (arteritis), and the most severe cases show neutrophils in the Wharton's jelly (Figure 18.5).

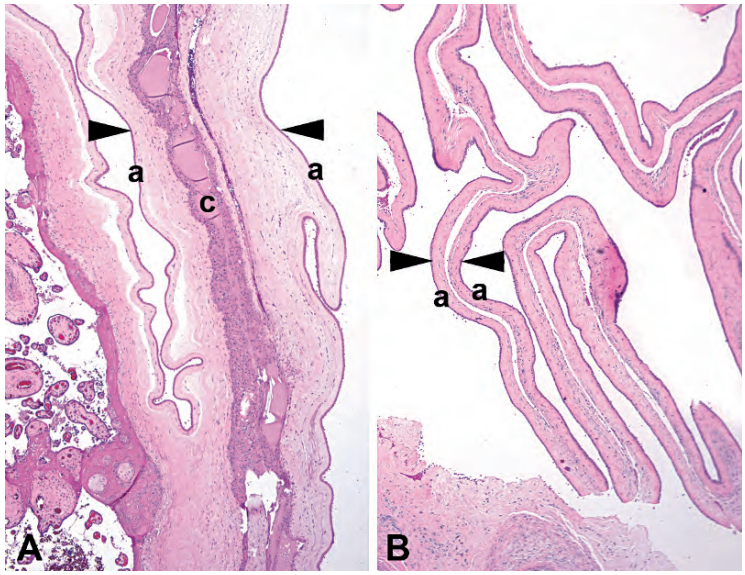

FIGURE 18.3. Twin placentas. **(A)** In a diamnionic, dichorionic placenta, the dividing membrane is captured here between the arrowheads. Amnion is seen on both surfaces (a), and a double layer of chorion is sandwiched in the middle (c). **(B)** In a diamnionic, monochorionic placenta, no chorion is present between the layers (arrowheads) of amnion (a).

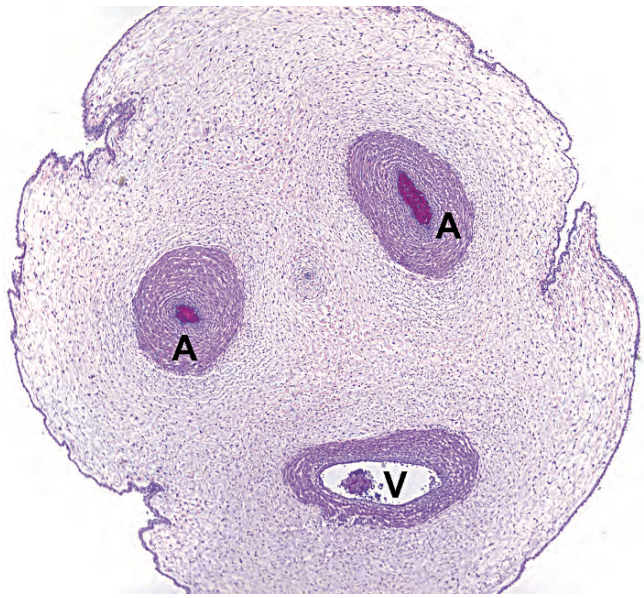

FIGURE 18.4. An umbilical cord in cross section, showing two arteries (A) and one vein (V).

The *membrane roll* is evaluated for the following:

- Chorionitis and chorioamnionitis: Look for neutrophils invading the chorion and/or amnion. Neutrophils in the decidua (below the chorion) are okay (Figure 18.6). Table 18.1 summarizes staging and grading of chorioamnionitis. Inflammation may result in a very reactive (tall, papillary) amnion. Unlike funisitis, this is a maternal response.
- Meconium staining: If, on low power, the amnion has a flat and autolyzed look, with edema separating amnion from chorion, look closely for meconiophages (Figure 18.7). These are histiocytes eating meconium (baby bile) pigment, and they appear granular and brown-gold.

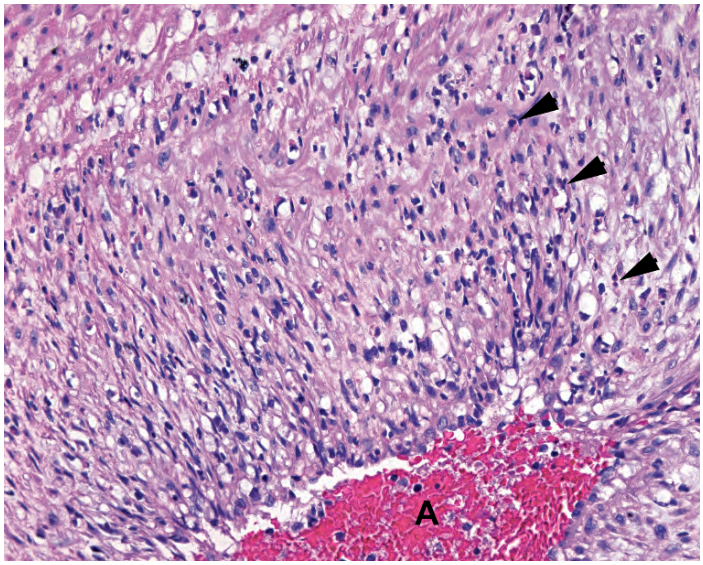

Figure 18.5. Funisitis. Neutrophils (arrowheads) can be seen squeezing through the muscular layer of an umbilical artery (A). This migration is a fetal response to infection.

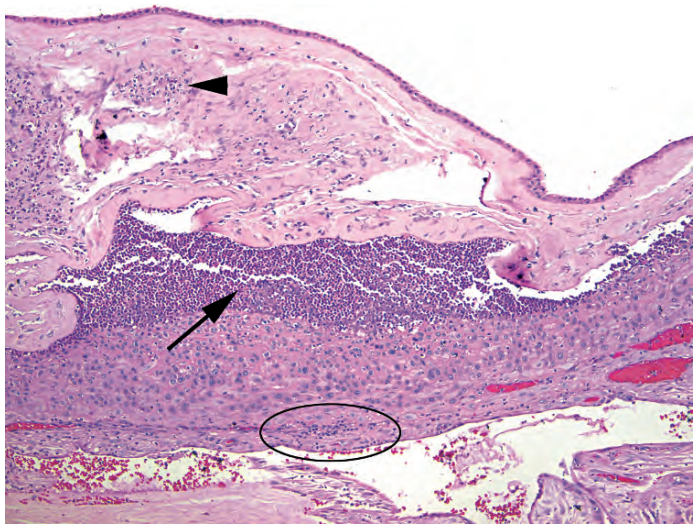

Figure 18.6. Chorioamnionitis. A collection of neutrophils (pus) has formed between the amnion and chorion (arrow). Neutrophils can also be seen beneath the amnion (arrowhead). Inflammation in the decidua (oval) may be physiologic and is not sufficient to diagnose chorioamnionitis. This is a maternal response to infection.

Table 18.1. Criteria for staging of acute chorioamnionitis and funisitis.

Stage	Maternal response	Fetal response (funisitis)
1	Subchorionitis and chorionitis: *maternal neutrophils* line up beneath the chorionic surface of either the chorionic plate or membranes	Chorionic plate vasculitis and umbilical phlebitis: *fetal neutrophils* marginate through the vessel wall
2	Chorioamnionitis: neutrophils cross the basement membrane into the connective tissue between chorion and amnion	Umbilical arteritis: neutrophils in the arterial wall
3	Necrotizing chorioamnionitis: sheets of neutrophils below the amnion, reactive or necrotic amnion, thickened amnionic basement membrane	Umbilical perivasculitis: neutrophils spread out from the vessels in a wave

Grade 1 = focal disease; grade 2 = extensive disease.

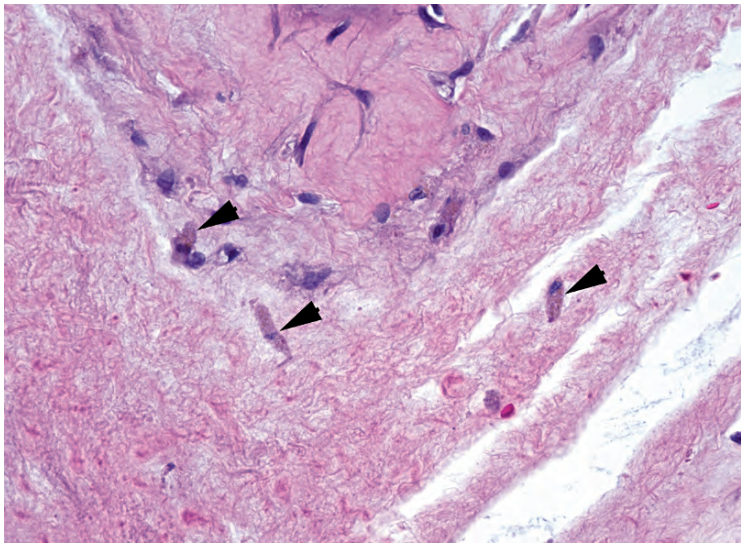

FIGURE 18.7. Meconiophages between the amnion and chorion, with deposits of brown pigment (arrowheads).

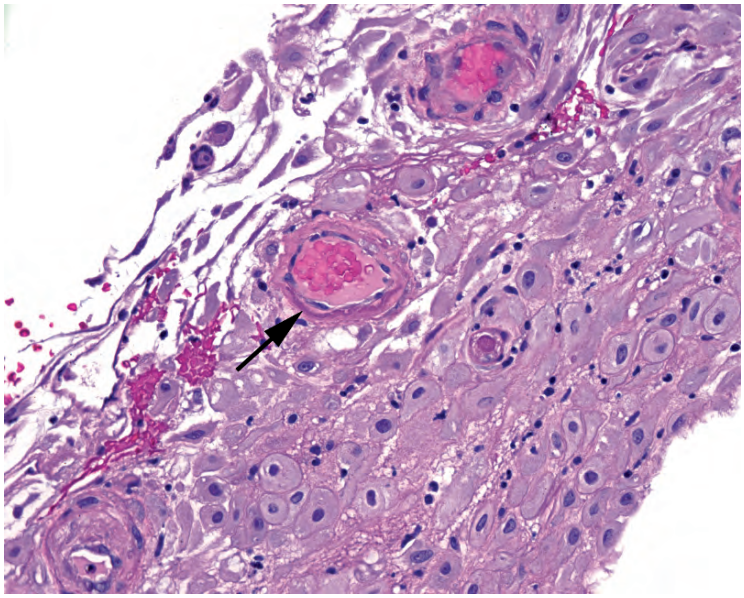

FIGURE 18.8. Fibrinoid necrosis. The dark pink condensation of the wall of this small artery (arrow) is an early sign of fibrinoid necrosis, which may be seen in preeclampsia.

The attenuated look of the amnion is due to the caustic nature of the meconium, just as gallbladders look when they have been sitting around for a day or so.

- Decidual vasculopathy: The membrane roll usually shows a nice lining of decidua, which is where you will find maternal vessels. Evaluate these for fibrinoid necrosis, a common finding in preeclampsia (Figure 18.8).

On the *fetal surface*, look for the following:

- Subchorionitis: Subchorionitis is the earliest manifestation of chorioamnionitis (maternal response). Neutrophils line up in the fibrin layer below the chorionic plate (see Table 18.1 for staging and grading chorionitis).

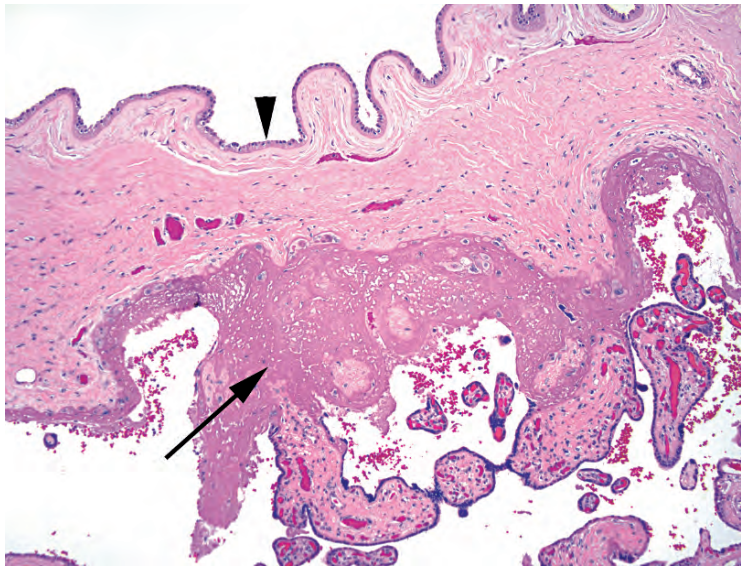

Figure 18.9. Fibrin, subchorionic. Subchorionic deposits of fibrin (arrow) are normal in a term placenta and should not be mistaken for infarct. The amnion lies atop the fetal surface (arrowhead).

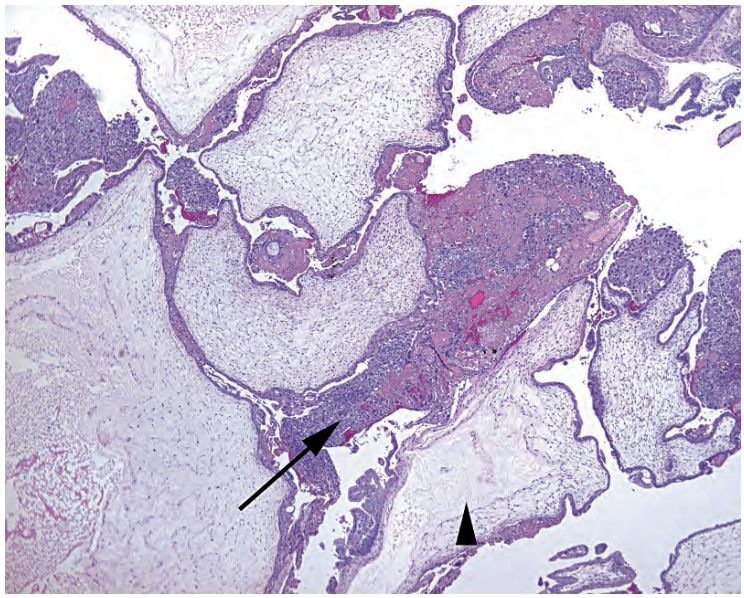

Figure 18.10. Molar villi. The villi are markedly enlarged, some with central cavities or cisterns (arrowhead). Dense trophoblastic proliferation is visible (arrow); on higher power, the cells may be very pleomorphic. This is a complete mole, so there are no fetal capillaries within the villi.

- Fetal vasculitis: The vessels that coalesce to become the umbilical vessels arborize on the fetal surface, sandwiched between the amnion and chorion. This is another place to look for a fetal phlebitis or arteritis.
- Subchorionic fibrin: Subchorionic fibrin is normal, and you may see large deposits in full-term placentas (Figure 18.9). Do not call it an infarct.

Below the fetal surface and above the maternal surface you will find the villi, the massive gas- and nutrient-exchange surface area of the placenta. In very early villi, such as in spontaneous or elective abortion, look for changes suggestive of a *hydatidiform mole* (Figure 18.10).

These changes include large swollen villi with no internal fetal vessels, circumferential and atypical trophoblast proliferation, and lack of fetal parts (in a complete mole). A complete mole is diploid with two paternal genomes and by definition has no fetus. A partial mole is triploid, one maternal and two paternal copies, and has a fetus, as well as two distinct populations of villi: normal and hydropic (edematous). An incidentally discovered early partial mole can be very subtle.

In a more mature placenta, such as an obstetric specimen, evaluate the villi for the following:

- Villous maturity: A full-term placenta should have a dense network of tiny terminal villi, each full of capillaries and lined with syncytial knots. A preterm placenta (<32 weeks or so) should have more immature villi, with larger contours, few syncytial knots, and myxoid stroma. A mismatch in gestational age and maturity is called hyper- or hypomaturity. Hypermaturity may indicate ischemia.
- Fibrin: Perivillous fibrin, which looks like hyaline membranes outlining the villi, increases with maturity, especially around the larger stem villi. Massive deposition may look like an infarct.
- Villitis: An increase in chronic inflammation within the villi may indicate a cytomegalovirus or syphilis infection. Often no organism can be found, in which case it is *villitis of uncertain etiology*.
- Infarct: Usually visible grossly as dense white patches, a true infarct has the look of coagulative necrosis (loss of nuclei and cell structure) with the mummified villi touching each other (Figure 18.11). Do not confuse this with perivillous fibrin deposition, in which fibrin encases a wide area of villi (encased villi should still show nuclear detail and be separated by abundant surrounding fibrin).
- Hematoma: A large acellular mass of fibrin, complete with lines of Zahn, is evidence of a prior hemorrhage. The hematoma may be subchorionic, intraplacental, or retroplacental (clinical abruption).
- Fetal capillaries: After prolonged death in utero, these capillaries collapse and the villi become fibrotic. Also look for nucleated red blood cells, which are abnormal in third trimester placentas.

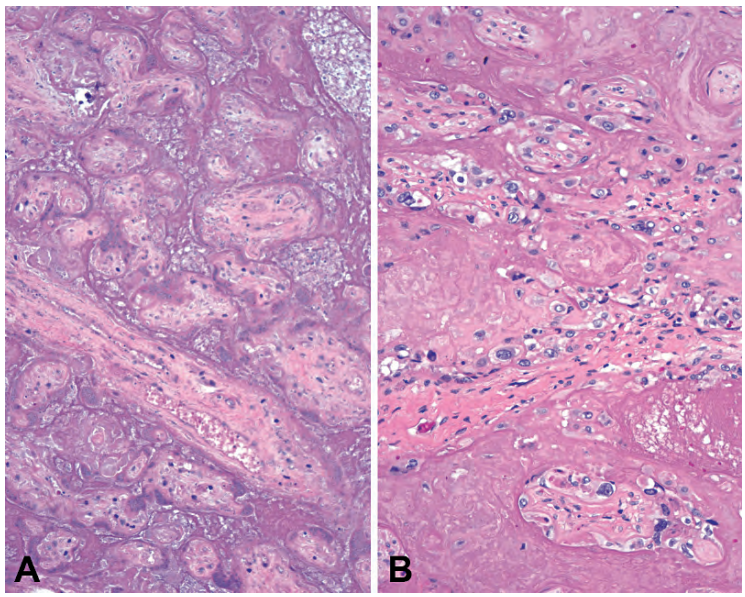

Figure 18.11. Infarct versus perivillous fibrin. **(A)** In an infarct, there is loss of basophilia and cellular detail with residual apoptotic bodies, as in coagulative necrosis elsewhere. **(B)** In a mass of perivillous fibrin, while the low power impression is a sheet of consolidated pink, on high power you can see the villi remain viable, with good nuclear detail.

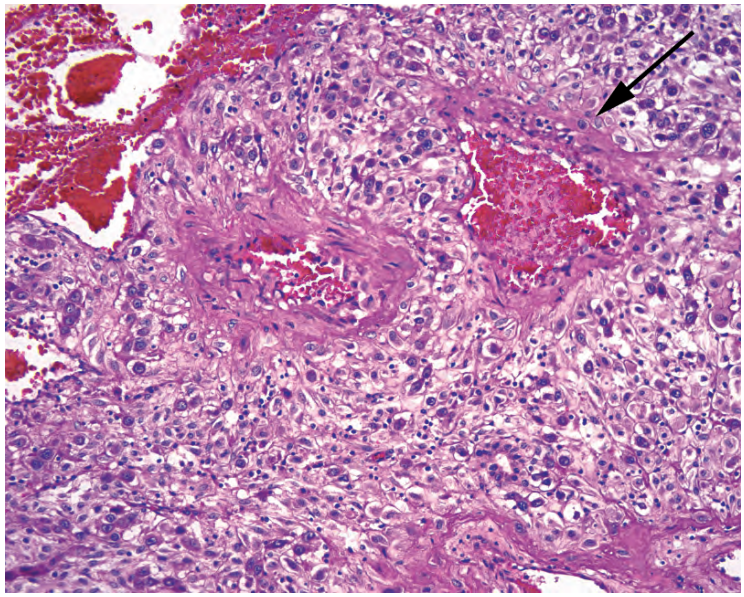

Figure 18.12. Trophoblasts in vessels. Intermediate trophoblasts (arrow) invading the wall of the maternal arteries. This is a normal process, opening fire hoses of blood to supply the placenta.

- Intervillous inflammation: Neutrophils or abscesses in the intervillous space are unusual but indicate maternal sepsis, such as from *Listeria*.

The *maternal surface* shows a layer of decidua, with implantation site changes. Trophoblasts invade the maternal muscular arteries, dissolving their muscular wall to create wide-open pipes (Figure 18.12). This invasive but normal process leaves behind a fibrinous layer—do not mistake this for fibrinoid necrosis or vasculopathy. True fibrinoid necrosis is best seen on the small maternal vessels in the membrane roll and also has an inflammatory component. However, in the maternal floor, the persistence of muscular arteries *is* a form of maternal vasculopathy, because it means the trophoblasts did not do their job, and the placenta may be ischemic. This is another component of preeclampsia.

A *maternal floor infarct* is not really a true infarct but a dense rind of fibrin encasing all of the villi along the maternal surface. *Placenta accreta* is the implantation of trophoblastic cells directly into myometrium. Histologically you may see placental villi very close to smooth muscle, with no intervening decidua. Accreta is a cause of postpartum hemorrhage.

The features of preeclampsia are the following:

- Decidual vasculopathy, also called acute atherosis: fibrinoid necrosis of decidual vessels, with accumulation of fibrin and foamy macrophages in the lumen and destruction of the arterial wall
- Hypertrophic vasculopathy (retained muscular wall) of the maternal floor vessels
- Increased perivillous fibrin, syncytial knots, and villous maturity

19 Breast

Breast biopsy specimens come in several sizes. There is the initial core biopsy, which is a large-bore needle biopsy, and the excisional biopsy, which is like a small lumpectomy. Some institutions perform cytologic studies (fine-needle aspirations), but their usefulness is limited, as many breast diagnoses are more architectural than cytologic. Biopsies are performed, with few exceptions, to rule out malignancy; there are almost no other disease processes that require tissue monitoring. A biopsy specimen with carcinoma will trigger either a lumpectomy, in which a portion of the breast is removed (a partial mastectomy, breast-conserving therapy), or a mastectomy. The mastectomy itself may include sentinel lymph nodes or, if the sentinel node is positive, an entire axillary dissection. Biopsy and most lumpectomy specimens are entirely submitted, and anything that is oriented is inked with four to six colors so we can identify all of the margins later. Mastectomies have only two margins, deep and superficial, and are representatively sampled by quadrant.

Normal Histology

The breast is sort of a giant specialized sweat gland, and so it has secretory glands (acini or lobules), arranged like grapes, and ducts, like the grape stems. A single bunch of grapes is a terminal duct lobular unit (TDLU; Figure 19.1). The ducts from these TDLUs all converge on the nipple, which has multiple large ducts and smooth muscle for ejecting the milk. The breast of a child or man will have ducts but no lobules. During lactation, the lobules fill up with fatty vacuoles of milk, giving them a very characteristic look usually called *secretory* or *lactational change*.

Each lobule and duct is composed of two cell types, the outer myoepithelial layer and the inner epithelial cells (see Figure 19.1). This is an important feature that can separate an in situ lesion (two cell types) from an invasive one (one cell type). The whole structure is bounded by a basement membrane, which is the boundary between in situ and invasive cancers. While there are unusual myoepithelial tumors, in this chapter we will only cover epithelial lesions.

Approach to the Biopsy Specimen

When signing out a core biopsy, there are certain things that should be included in the diagnosis. For malignant lesions, in situ or invasive, first, it is helpful to give an indication of *how differentiated the tumor is*. Some institutions do not Elston grade (see later) a core, but you should at least note the nuclear grade (for ductal carcinoma in situ [DCIS]) or whether it is

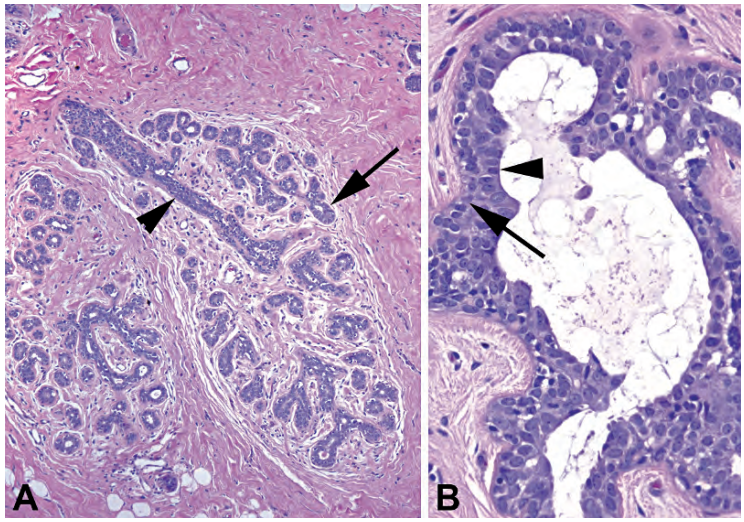

Figure 19.1. Normal breast. **(A)** The terminal duct lobular unit (TDLU) is arranged like a cluster of grapes, with the duct (arrowhead) as the stem and secretory lobules (arrow) as the grapes. The rounded and circumscribed border of the TDLU is a key feature of noninvasive lesions. **(B)** The benign breast always has two cell layers, the outer myoepithelial cells (arrow) and the inner epithelial cells (arrowhead). In situ lesions also have two cell layers.

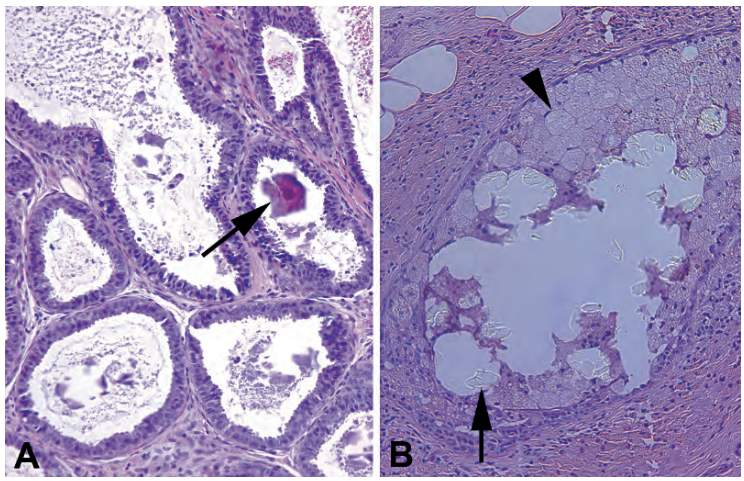

Figure 19.2. Calcifications. **(A)** Microcalcifications in this columnar cell lesion appear as tiny purple rocks (arrow), which may shatter and drag through the tissue, creating telltale scratches in the H&E stain. **(B)** Calcium oxalate does not pick up hematoxylin and therefore is only visible with a polarizer or when the condenser is flipped down, as in this photograph. The oxalate crystals (arrow) are seen in a duct space, surrounded by foamy macrophages (arrowhead).

well/moderate/poorly differentiated (for invasive cancer). These three tiers of differentiation correspond roughly to the three Elston grades.

Second, if microcalcifications were seen on mammography, you must note whether they are present in the specimen and in what context (such as, "in association with usual duct hyperplasia"). Failure to find the microcalcifications leads to x-raying the block, calling the radiologist, and so forth. Microcalcifications usually are gritty and dark purple, like calcification in other tissues, but occasionally take the sneaky form of calcium oxalate, clear refractile crystals best seen with polarized light (or flipping the condenser down; Figure 19.2).

Finally, your goal should be to identify the mass or radiographic abnormality the clinicians have detected. If there is no malignancy, you should be looking for some *explanation for their findings*. Aside from microcalcifications, which do explain a mammographic lesion, you should be looking for anything that could cause a palpable mass, such as fibrosis, cysts or cyst wall, fat necrosis, and benign tumors such as fibroadenomas.

Fibrocystic Changes

Fibrocystic changes are very common in young women, and many palpable lumps turn out to be nothing more than fibrocystic change. These are usually signed out as "Benign breast tissue with fibrocystic changes, including…" and then a list of the features. These features include the following:

- Fibrosis: Fibrosis consists of dense pink collagen among the lobules.
- Cysts: Cysts are often visible grossly, thin walled, and full of clear fluid (Figure 19.3).
- Usual duct hyperplasia: Usual duct hyperplasia is described in detail in a later section.
- Adenosis (too many glands or lobules) or sclerosing adenosis: Adenosis is a big pitfall because the lobules can look very crowded and worrisome. This is especially true of sclerosing adenosis, in which the proliferative lobules are squeezed together by fibrosis, making them look small and infiltrative (Figure 19.4). The reassuring myoepithelial cell layer can be hard to see. However, sclerosing adenosis should have an overall lobular (circumscribed and rounded) architecture, and myoepithelial cells should be visible in some glands.
- Apocrine metaplasia: Breasts are just big sweat glands, remember? Apocrine metaplasia means the epithelial cells lining the ducts look like apocrine glands (Figure 19.5); they acquire a lot of bright pink cytoplasm, can get a hobnail profile protruding into the lumen, and have enlarged nuclei with prominent nucleoli (not unlike Hurthle cell change in the thyroid). It is important to recognize this entity as a metaplastic, not a dysplastic, change.
- Fibroadenomas: A fibroadenoma is a biphasic (two cell types) proliferative lesion. The ducts are proliferating (-adenoma), as is the stroma (fibro-). (A similar lesion in the ovary is called an *adenofibroma*.) This benign tumor has thin, branching ducts set in a sparsely cellular fluffy pink stroma (Figure 19.6). The ducts often have a myxoid pale halo around them, and

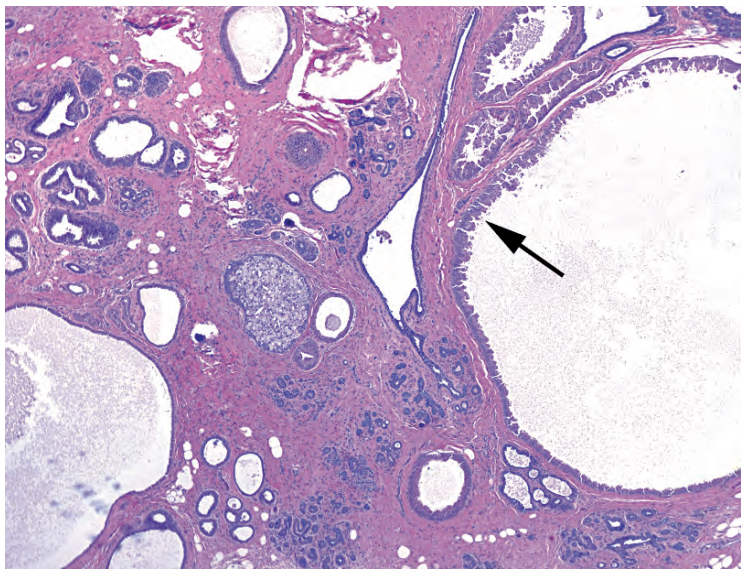

Figure 19.3. Fibrocystic disease. In this example, large dilated duct spaces are visible, some with a lining of apocrine metaplasia (arrow). The stroma is dense and fibrotic (pink).

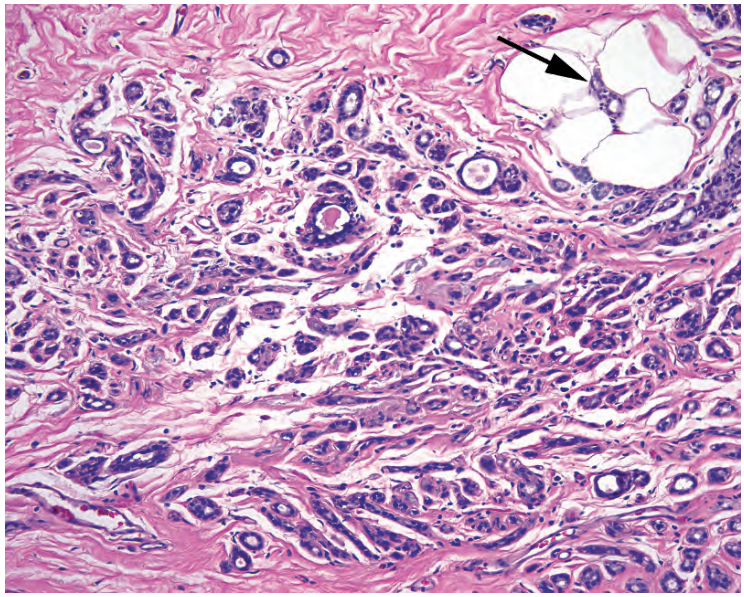

Figure 19.4. Sclerosing adenosis. On high power, this benign lesion looks infiltrative. Tiny tubules are entrapped in a fibrotic stroma, and some tubules are even seen among fat (arrow). Because of the compression, myoepithelial cells are not visible. Clues to the diagnosis include a circumscribed lesion at low power, the lack of desmoplastic (edema and fibrosis) reaction, and an intact myoepithelial cell layer seen on immunostains.

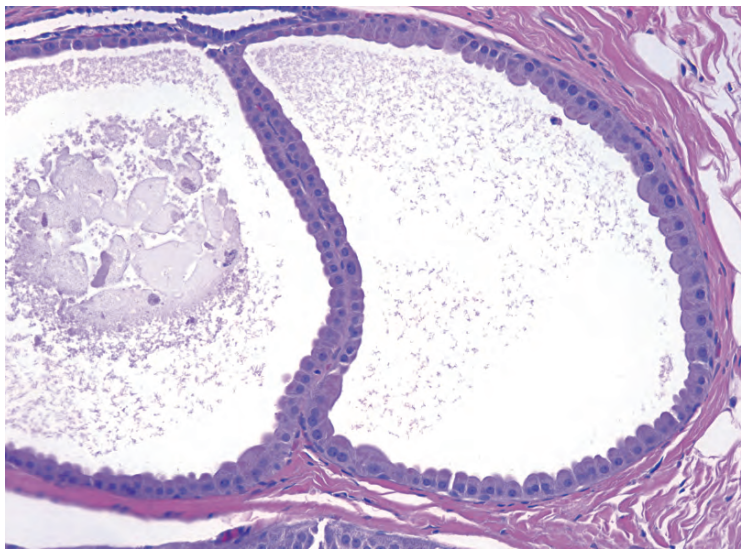

Figure 19.5. Apocrine metaplasia in fibrocystic disease. The epithelial cells lining the dilated duct are large and plump, with abundant dark pink cytoplasm, and round nuclei with prominent nucleoli. Secretions (the granular schmutz in the lumen) are common.

the proliferative stroma compresses the ducts into slits. Old fibroadenomas may become hyalinized and calcified. Fibroadenomas can occur alone or in association with fibrocystic changes.

The *phyllodes tumor* is another biphasic lesion, which has a similar appearance but a much more cellular stroma that a fibroadenoma. The phyllodes (*leaf-like*) tumor is graded based on how aggressive the stromal growth pattern is and ranges from benign to malignant. This

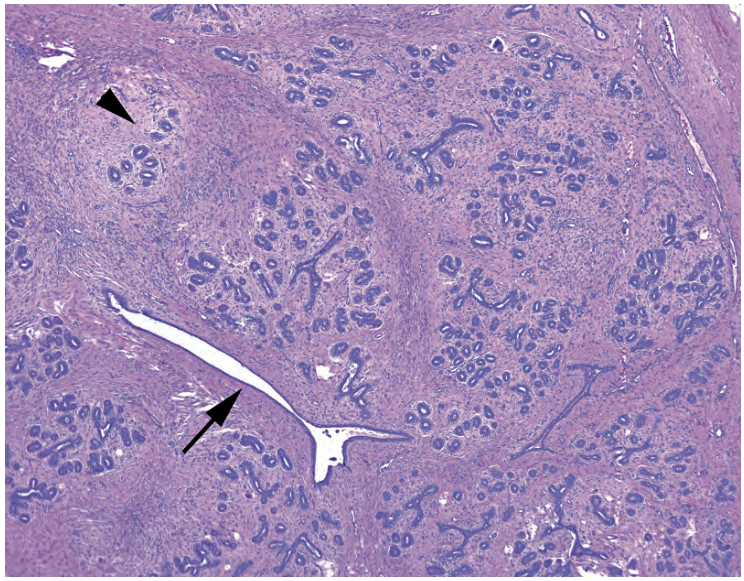

Figure 19.6. Fibroadenoma. At low power, the fibroadenoma is a well-circumscribed nodule. Within the lesion, the secretory lobules stand out in slightly edematous (pale) stroma (arrowhead), and the ducts are compressed into slit-like spaces (arrow) by the proliferative stroma.

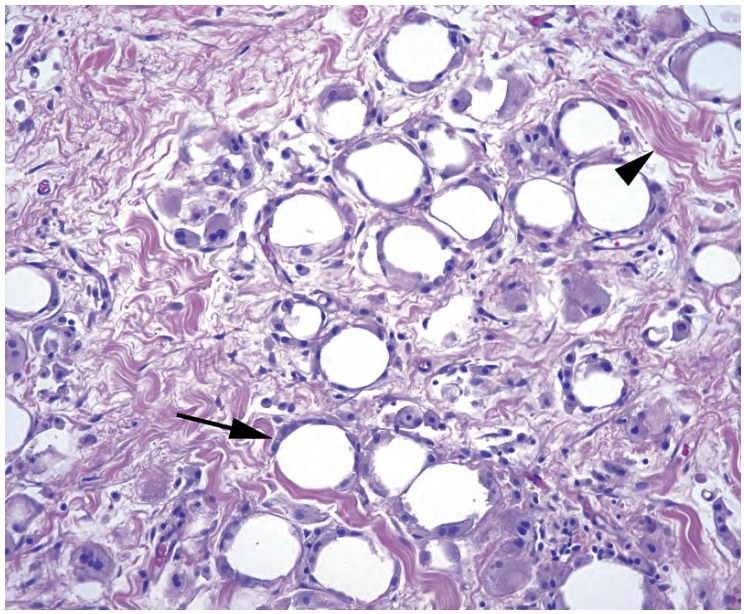

Figure 19.7. Fat necrosis. In an area of fat necrosis, secondary to trauma or surgery, the fat cells die but the globs of lipid remain. Foamy macrophages ring each dead fat cell (arrow), digesting the lipid; the spaces between the fat cells are filled in by fibrosis (arrowhead).

leaf-like pattern is often indicative of biphasic tumors with a very proliferative stroma and is seen in biphasic tumors of other organs.

Fat necrosis is evidence of a prior biopsy or other trauma. It can be hard, painful, calcified, or discolored. By clinical examination it may be very suspicious for malignancy. It is also very distracting in interpreting reexcision biopsies, where the prior surgery has left extensive fat necrosis. The key features (Figure 19.7) are as follows:

- Disrupted and irregular fat cells
- Foamy macrophages and giant cells
- Edema and hemosiderin
- Acute inflammation
- Fibrosis and calcification (in older lesions)

Intraductal Papilloma

The papilloma is composed of proliferative but benign secretory and myoepithelial cells lining a branching arbor of fibrovascular cores (Figure 19.8). The lesion is usually found in the large distal ducts and can become fibrotic (sclerosing papilloma) or calcified with age. Rarely, carcinoma can arise in a papilloma.

Ductal and Lobular Proliferative Lesions

This is the take-home point of the day: *Deciding whether a lesion is ductal or lobular has nothing to do with whether you find it in a duct or a lobule*. Lobular carcinoma in situ (LCIS) can fill a duct, and DCIS can invade a lobule, so there is no need to struggle to identify which structure you are looking at. Instead, *ductal* and *lobular* refer to *distinct morphologic patterns of in situ or invasive carcinoma*. They probably represent cancer pathways arising from a common cell type by two different mechanisms, analogous to the two cancer pathways in colon, but there are plenty of examples of "tweener" lesions (features of both) that are signed out as "mixed mammary carcinoma."

Benign hyperplasia of ductal-type epithelium (usual ductal hyperplasia) is common, whereas benign hyperplasia of lobular-type epithelium is not. However, both cell types can

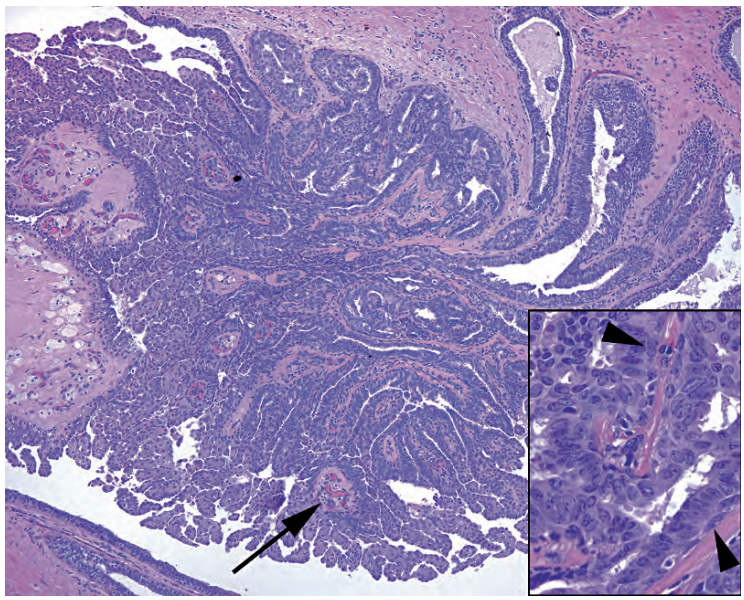

Figure 19.8. Intraductal papilloma. The branching structure fills a subareolar duct; smaller, more distal examples may be called *micropapillomas*. Although there is florid usual ductal hyperplasia, resulting in fusion of multiple branches of the papilloma, distinct fibrovascular cores are still visible (arrow). **Inset**: Along each fibrovascular core, you should still see myoepithelial cells (arrowheads), which differentiates this from a papillary carcinoma.

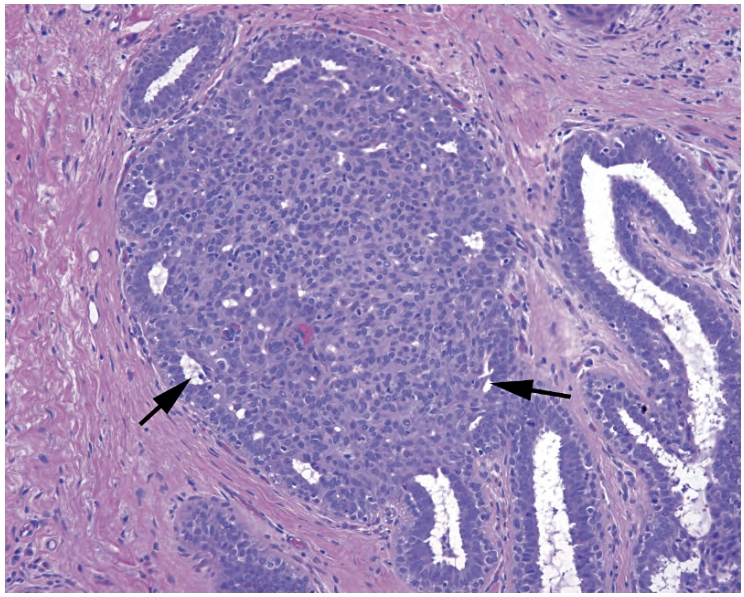

Figure 19.9. Florid usual ductal hyperplasia. The cellular proliferation entirely fills this duct, but the cell population is swirly and heterogeneous, with randomly overlapping nuclei. The peripheral ring of slit-like spaces (arrows), as though this clot of cells floated into the duct and stuck there, is very typical of usual ductal hyperplasia.

occur in an atypical proliferative phase (ADH and ALH), carcinoma in situ (DCIS and LCIS), and invasive carcinoma (IDC and ILC).

Usual ductal hyperplasia refers to a proliferation of cells within the ducts. The usual monolayer of cuboidal cells heaps up into mounds or even fills the ducts. Features of usual ductal hyperplasia include the following:

- The cells have an overall pale look; they are normochromic.
- Cells appear jumbled, overlapping, or streaming and almost syncytial (Figure 19.9).
- Heterogeneity (not to be confused with pleomorphism) is present. The nuclei, all bland with even chromatin and smooth nuclear membranes, range slightly in size and shape as though they were drawn by a sloppy artist.
- Ducts may be filled with cells and may even have a cribriform look at low power, but on higher power the nuclei should be streaming, flowing parallel to the lumens, as opposed to polarizing perpendicularly (radially) around the lumen. Luminal spaces should be slit-like or irregular, not round, and may be "fuzzy" (due to apocrine secretions).

Ductal carcinoma in situ may be low grade, which is a homogeneous population of cells, or high grade, which is a pleomorphic population of cells. In low-grade DCIS, you should get the impression that there is a monotonous, clonal population of cells, with evenly spaced dark nuclei and distinct cell borders. High-grade DCIS, although it loses its monotonous look, should still have discrete nonoverlapping cells; it also may get very pink. Irregular nuclear borders, enlarged nuclei, and nucleoli are common. Patterns of DCIS include the following:

- Cribriform: sharply punched-out round holes in the mass of cells, with cells lined up around the lumens like rosettes (Figure 19.10)
- Solid: a solid sheet of monotonous cells
- Comedo: a rim of malignant, usually high grade, cells with central necrosis (see Figure 19.10)
- Micropapillary: top-heavy lollipop protrusions into the lumen, without true fibrovascular cores; must also have cellular monotony as above.

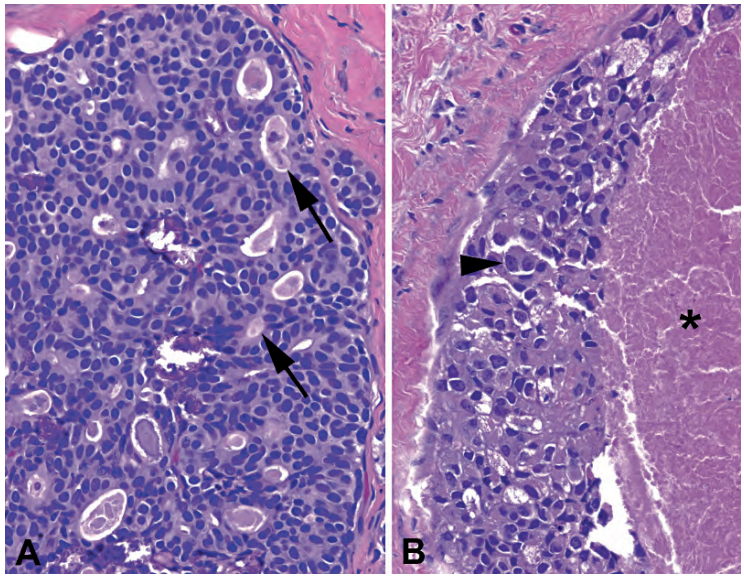

Figure 19.10. Ducal carcinoma in situ (DCIS). **(A)** In low-grade DCIS, the cells are monotonous, uniform, and largely nonoverlapping, and they form cribriform duct spaces with the cells polarized around the tiny lumens (arrows). **(B)** In high-grade DCIS, the cells have lost their monotony and are instead pleomorphic, some with prominent nucleoli (arrowhead). At the center of the dilated duct there is necrosis (asterisk), indicating comedo-type DCIS.

Remember that DCIS by definition has not crossed the basement membrane, and the outer myoepithelial layer remains intact. Ductal carcinoma in situ is treated as a precursor to malignancy, and the treatment goal is total excision. Therefore, on anything but a core biopsy, you must document its distance from each margin (adequate clearance is in the eye of the beholder, but most accept 2 mm).

Do not expect to get comfortable with the diagnosis of *atypical ductal hyperplasia* until you have mastered usual ductal hyperplasia and DCIS. Atypical ductal hyperplasia falls somewhere in between and has no definitive criteria other than "has some but not all of the features of DCIS." This diagnosis is also used in the setting of a single focus of apparent low-grade DCIS (nuclear grade 1 of 3) measuring less than 3 mm. In a core biopsy, atypical ductal hyperplasia is code for "get me more tissue." Features that can push you to DCIS in a tiny focus include high-grade nuclei and/or necrosis.

Invasive ductal carcinoma is invasive carcinoma arising from a DCIS lesion, and therefore the cells of invasive ductal look similar to those you see in DCIS. In its most common form, invasive ductal carcinoma is the cancer formerly known as *scirrhous*, so called because of the dense desmoplastic reaction generated. It is eye-catching even to the untrained eye, as a large cellular lesion with ugly cells, radiating outward in a stellate and decidedly un-TDLU-like shape (Figure 19.11). The cells are large, with large pleomorphic nuclei and substantial pink cytoplasm. Necrosis and mitoses are common. Nests of tumor cells can imitate ducts or tubules in the stroma, or acquire large necrotic centers like comedo-DCIS. For this reason it is sometimes hard to tell invasive carcinoma from DCIS or even benign tubules. Stains for myoepithelial borders are helpful here: invasive cancer does not have any.

Variants of ductal carcinoma include the following (the first five variants have a generally better prognosis than ductal carcinoma NOS):

- Tubular: a very well-differentiated cancer composed entirely of cytologically bland small angular tubules (Figure 19.12)
- Cribriform: similar to tubular, but with cribriform structures instead of tubules

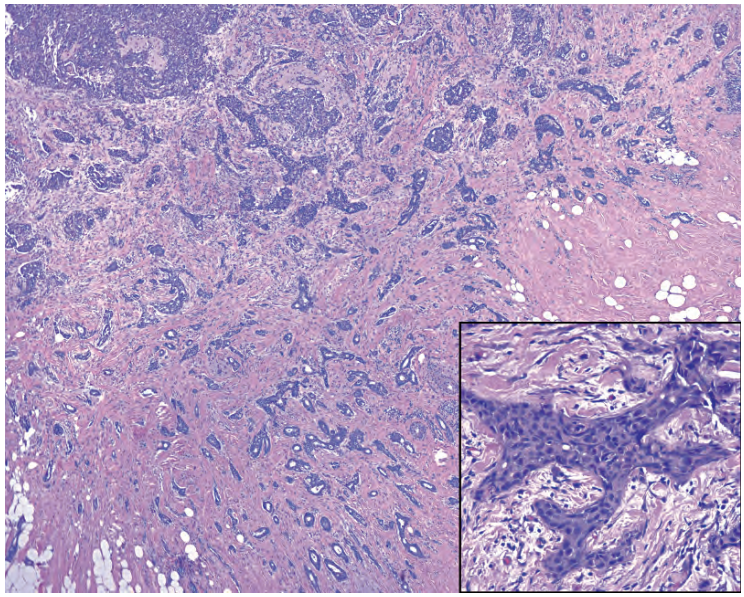

FIGURE 19.11. Infiltrating ductal carcinoma. At low power, the irregular border of the lesion is evident, with small angular tubules radiating outward into the fat. Grossly, this lesion would have a stellate appearance, and the dense stromal reaction would make the lesion very hard. **Inset**: The irregularly shaped nests of tumor cells create a desmoplastic stromal reaction, which is a combination of edema (white space) and fibrosis (pink collagen).

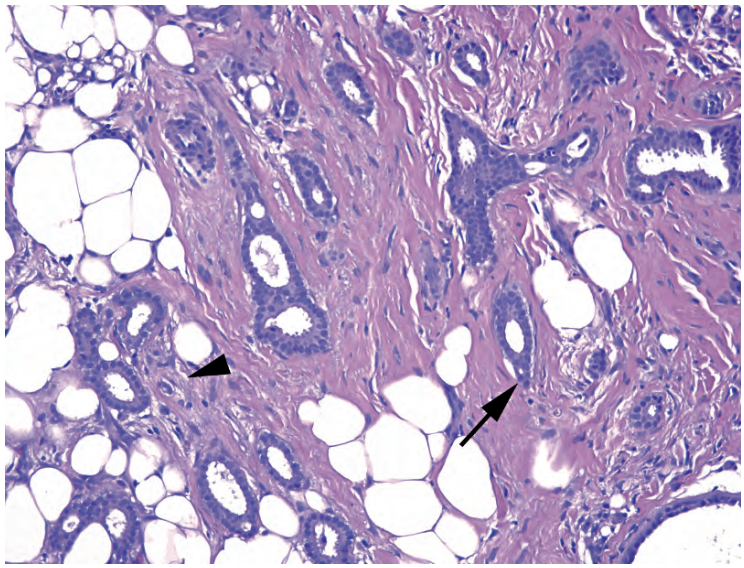

FIGURE 19.12. Tubular carcinoma. Well-formed tubules with pointed ends (arrow) and round, monotonous cells infiltrate through the stroma and fat. The myoepithelial layer is absent, both on H&E stain and by immunostain, and there is a subtle desmoplastic reaction around some of the tubules (arrowhead).

- Mucinous or colloid: characterized by pools of mucin and floating fragments of neoplastic epithelium (Figure 19.13)
- Medullary: a well-circumscribed but paradoxically ugly group of cells, with a dense lymphocytic infiltrate

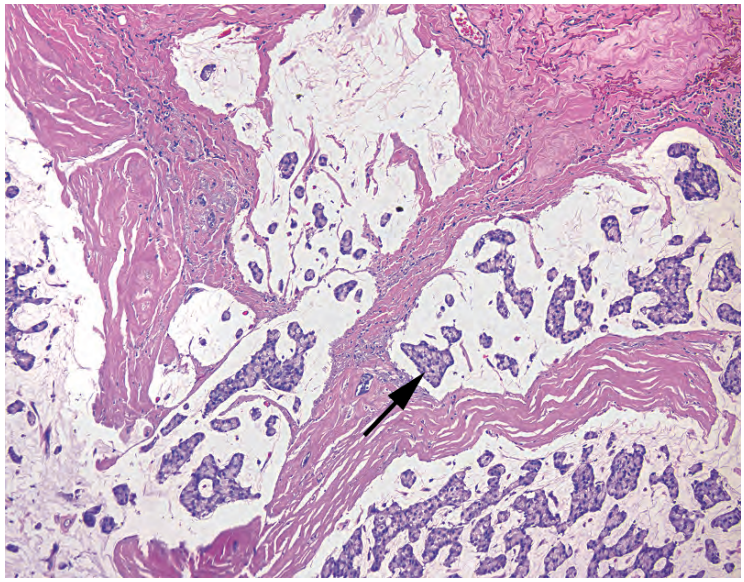

Figure 19.13. Mucinous carcinoma. Pools of extruded mucin dissect into the stroma. Although this can occur in benign mucocele-like lesions, the presence of floating clumps of cells (arrow) is diagnostic of mucinous, or colloid, carcinoma.

- Adenoid cystic carcinoma: a biphasic tumor of epithelial and myoepithelial cells, identical to the salivary gland tumor of the same name
- Metaplastic: a tumor in which there is a mesenchymal or spindle-cell component, such as cartilage, bone, or frank sarcoma, with prognosis depending on grade

In *lobular carcinoma in situ*, lobular cells, when they begin to proliferate, take on a characteristic appearance. They are homogeneous, like DCIS cells, but they have a round fried-egg shape, with a pale cytoplasm, discrete borders, and a central round nucleus (Figure 19.14). Intracytoplasmic vacuoles, even signet-ring cells, are also common. In LCIS, these cells should fill and expand the lobules, appearing at low power like a very circumscribed stippled space (such as the texture of newspaper photos under a magnifying glass). Lobular carcinoma in situ retains its bland cytology right through to invasive carcinoma.

Lobular carcinoma in situ is often multifocal and bilateral, and its progression to cancer is not considered inevitable or predictable. As a result, excision is not the goal of treatment, and so its presence at a margin is not usually noted.

Lobular carcinoma in situ is an incidental finding. It does not form masses or calcify (usually). *Atypical lobular hyperplasia* is generally code for "I'm really worried about LCIS but cannot quite get there." Like atypical ductal hyperplasia, atypical lobular hyperplasia does not have consistently agreed-upon criteria.

E-cadherin is a cell surface molecule that helps cells stick together. Lobular lesions lose expression of E-cadherin and therefore begin to appear very discohesive. You can imagine that this nonsticky surface enables the invasive lobular cells to slip through the stroma as single cells, and that is exactly what they do. Stains for E-cadherin can help to sort out LCIS (negative) from DCIS (positive) in a core biopsy specimen, as low-grade DCIS can resemble LCIS.

The cells of *invasive lobular carcinoma* look similar to those of LCIS. They are small uniform cells with bland round nuclei, pale cytoplasm, and a sometimes plasmacytoid shape with an eccentric mucin vacuole. Because of their normochromic nuclei and lack of malignant cytology, they are identified by the way they slip through the stroma. They line up as single file lines or as concentric rings around ducts and do not cause an appreciable desmoplastic

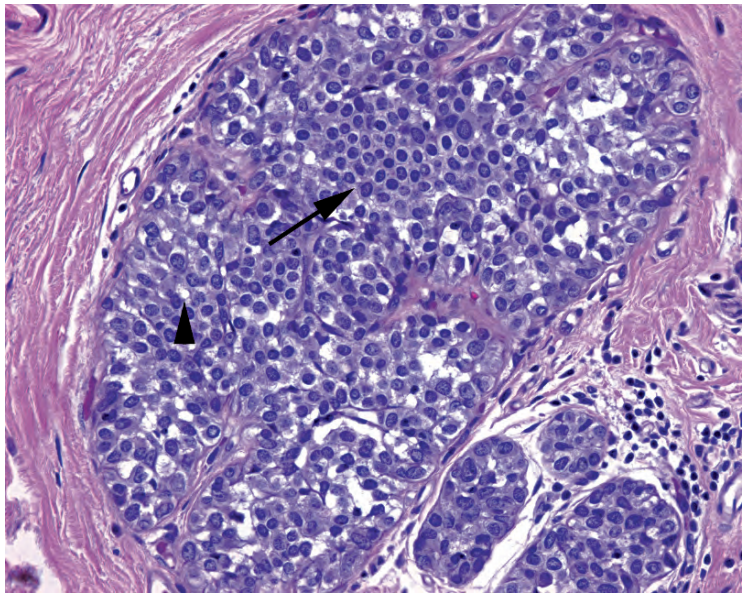

FIGURE 19.14. Lobular carcinoma in situ. The lobule is distended by a population of monotonous cells with distinct cellular borders and small round nuclei (arrow). As the lesion expands, the noncohesive cells will begin to fall apart. Cytoplasmic vacuoles (arrowhead) are typical of lobular carcinoma cells both in situ and invasive.

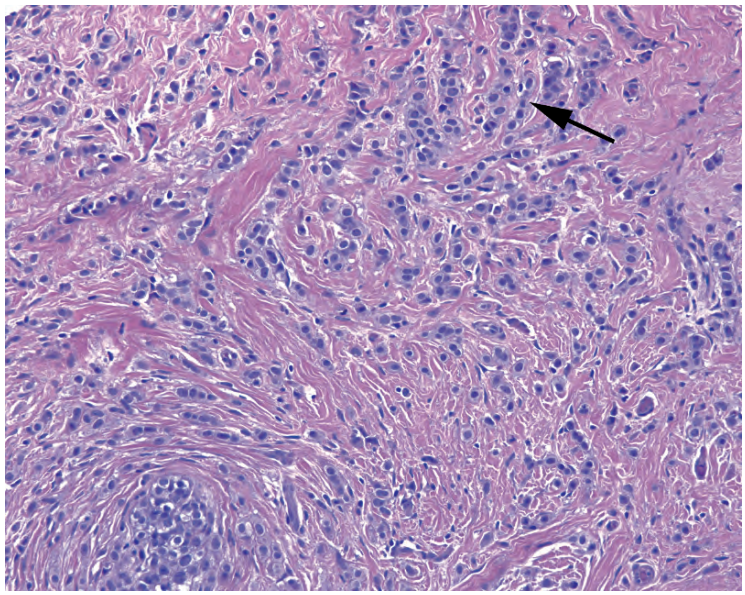

FIGURE 19.15. Invasive lobular carcinoma. The same cells as in Figure 19.14 are seen here invading through the stroma. They often form single file lines (arrow) but may also be seen as single cells or concentric circles around a duct. In some cases there is little to no desmoplastic stromal reaction, making the lesion difficult to palpate or detect.

response (Figure 19.15). They are sneaky and scary, and you have not ruled out lobular until you have looked closely at 10× or 20×. A cytokeratin stain can highlight the individual cells, as everything else in the stroma should be negative.

Invasive epithelial carcinomas must be given an *Elston grade* (A.K.A Nottingham grade, or Elston-Ellis modification of Scarff-Bloom-Richardson) when diagnosed in a lumpectomy or

mastectomy. The Elston grade is the pathologic assessment of the tumor's aggressiveness; the stage is diagnosed separately by features such as size and local extent. The Elston grade takes into account three prognostic factors:

- Tubule formation (the more tubule formation, the lower the score)
- Mitotic rate (the more mitoses, the higher the score)
- Pleomorphism (the more pleomorphic the nuclei, the higher the score)

Each characteristic is scored from 1 to 3, and then all are added, to give you a range of 3 to 9. For details on scoring, see your favorite surgical pathology text; in the beginning, just learn to look for these three features. Pleomorphism, especially, is a fairly subjective criterion that takes some experience to judge.

Papillary Nomenclature

Papillary lesions in the breast represent a confusing area. Here is the nutshell.

A *papilloma* is a benign lesion with papillary architecture. The fibrovascular cores, and the surrounding duct, are lined by myoepithelial cells. Within a papilloma, you can get usual or atypical ductal hyperplasia or DCIS, all of which are diagnosed as "arising in a papilloma." You should still have myoepithelial cells around the perimeter.

Within the DCIS family, there are several architectural types: *micropapillary* (epithelial projections without fibrovascular cores), *papillary* (epithelial projections with fibrovascular cores), and *solid papillary* (a solid ball of cells with residual entombed fibrovascular cores). None of these necessarily has anything to do with a papilloma. All usually have intact myoepithelial cells around the outside. All may be multifocal processes in the breast.

Papillary carcinoma is a specific type of carcinoma with a papillary architecture, homogeneous columnar cells, and a circumscribed profile, as though it once grew in a duct. It should be a single discrete lesion. The fibrovascular cores have no myoepithelial cells. The myoepithelial stains may also be negative around the perimeter, but it is still not really considered a true invasive carcinoma. It may be called *intracystic* or *encysted* papillary carcinoma to get this point across.

The Many Faces of Metaplastic Carcinoma

Numerous morphologies get lumped under the term *metaplastic carcinoma* and hence the struggle to learn to recognize it. You may see this diagnosis applied to the following entities:

- Squamous carcinoma: a ductal carcinoma with prominent squamous differentiation (and technically a form of metaplastic carcinoma)
- Low-grade spindle cell carcinoma: can masquerade as a hypercellular stroma, but the spindle cells should stain for cytokeratins (especially high-molecular-weight cytokeratins such as 34bE12 [CK903])
- High-grade carcinoma with spindle cell features: should also be cytokeratin positive
- Any carcinoma with coexisting sarcoma, such as chondrosarcoma or osteosarcoma (The carcinoma component will be cytokeratin positive, the sarcoma usually will not. In another organ this would be called a carcinosarcoma.)

The differential diagnosis for entities that are spindly and malignant also includes malignant phyllodes tumor and primary or metastatic sarcoma.

20 Bone Marrow

Normal Histology

The marrow biopsy specimen is usually taken from the iliac bone. It consists of bony trabeculae surrounded by a mixture of fat and hematopoietic cells. The percent cellularity (non-fat) should be roughly [100 – the patient's age]. The hematopoietic cells consist of megakaryocytes, erythroid precursors, and myeloid precursors. There may also be assorted plasma cells, lymphocytes, and histiocytes. The bone marrow is considered a reflection of what is in the peripheral blood, so the *disorders of marrow affect blood counts*. Lymphomas can involve the marrow, but generally the primary malignancies of the marrow are the leukemias.

Technically, all three of the basic "trilineage hematopoiesis" lines (megakaryocytes, erythroids, and granulocytic cells) are of the *myeloid lineage*, which differentiates them from the lymphoid lineage (B and T cells). The *myelo*proliferative diseases and *myeloid* leukemias refer to this broad classification. However, the word *myeloid*, as used at the microscope, generally refers to those cells in the granulocyte/monocyte pathway only.

Megakaryocytes are most easily identified, with their lakes of pink cytoplasm and multilobated nuclei (Figure 20.1). Erythroid precursors have a distinct rim of clear cytoplasm and centrally located, perfectly round nuclei; as they mature, the nuclei become small and dense such that erythroid islands in the marrow look like handfuls of buckshot (see Figure 20.1). Myeloid cells make up almost everything else. Myeloid precursors have more open chromatin than the red cell precursors, more cytoplasm, and more convoluted nuclei as they mature. Mature neutrophils and eosinophils should be present in normal marrow. Blasts, the most primitive hematopoietic cells, can be difficult to identify on H&E stain. Lymphoid cells, especially immature, should generally not be found in the marrow, with the exception of hematogones (nonneoplastic B-cell precursors), which can be markedly increased in children.

Usually an aspirate smear will be submitted with the biopsy specimen. The aspirate is stained with the Wright-Giemsa stain, which highlights nuclear detail. Blasts, and the successive stages of maturation, are best seen on an aspirate. The blasts are large cells with a thin rim of cytoplasm and a characteristic nucleus (Figure 20.2). The blast nucleus is large and round with a very finely textured chromatin pattern and a nonstaining nucleolus that shows up as a "hole" in the chromatin. The more differentiated precursors, such as promyelocytes and myelocytes, may have a similarly immature nucleus but acquire cytoplasmic features such as granules and a "hof" (the cleared-out Golgi zone in the cytoplasm, as in a plasma cell; see Figure 20.2).

On the aspirate, a myeloblast (as seen in acute myeloid leukemia) cannot always be distinguished from a lymphoblast (as seen in acute lymphoblastic leukemia). However, the presence of granules or Auer rods identifies a blast as myeloid. Erythroblasts have royal blue cytoplasm and very round nuclei. Monocyte precursors tend to have greyer cytoplasm and a folded or creased nucleus.

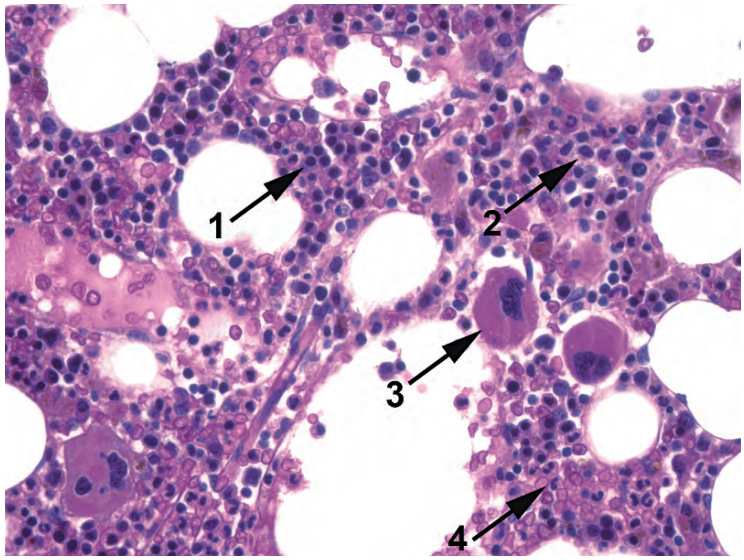

Figure 20.1. Normal megakaryocytes, erythroids, and myelocytic precursors. In this H&E stained core biopsy specimen, there are erythroid precursors (1), myeloid precursors (2), megakaryocytes (3), and maturing neutrophils (4).

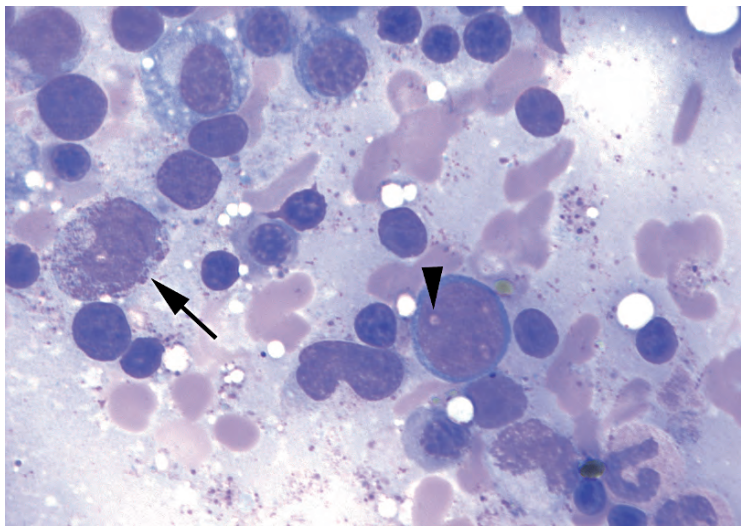

Figure 20.2. Blast on aspirate, Wright-Giemsa stain. The key to identifying a blast is the high nuclear to cytoplasm ratio and immature chromatin pattern, which consists of very finely grained, uniform chromatin with several nucleoli that show up as negative images on this stain (arrowhead). The immature cell nearby is a promyelocyte, which has the same nuclear qualities as a blast but has abundant cytoplasm with granules (arrow).

Approach to the Biopsy Specimen

A full evaluation of the specimen requires an H&E stained core biopsy, a Wright-Giemsa stained aspirate, and a peripheral smear. Beginning with the biopsy:

- On low power (4×)
  - Assess the cellularity of the marrow (Figure 20.3). A hypo- or hypercellular marrow will guide your differential diagnosis.

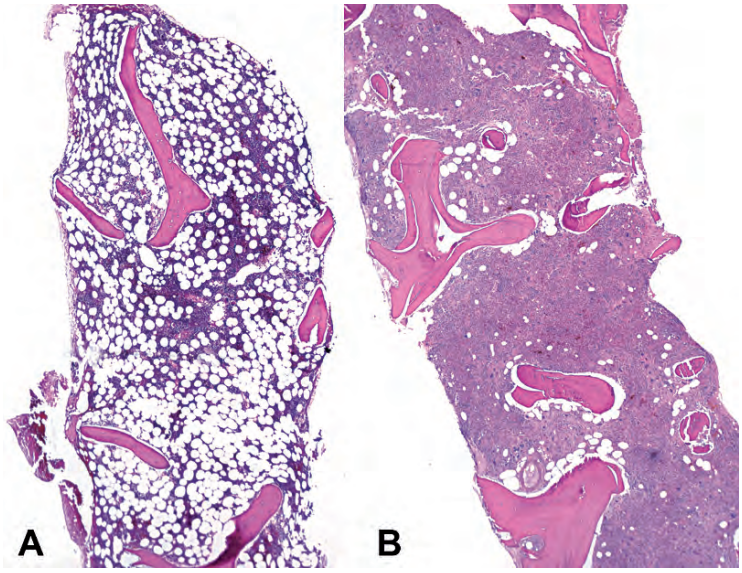

FIGURE 20.3. Marrow cellularity. **(A)** Normocellular marrow for a middle-aged adult; this cellularity is approximately 30%. **(B)** Hypercellular marrow for an adult; this cellularity is about 95% and is taken from a case of acute myeloid leukemia.

- Try to estimate the cellularity as a percentage range (i.e., 30%–40%), as clinicians sometimes follow the cellularity to monitor response to therapy.

- On medium power (10×)
  - Survey the marrow for trilineage hematopoiesis. You should see megakaryocytes, erythroid islands, and myeloid cells. Look to see if each line matures to completion: you should see mature neutrophils and red cells. Estimate the ratio of myeloid to erythroid cells (M/E ratio), which is normally about 2–4:1.
  - Look for things that do not belong in the marrow in large populations or aggregates, such as blue areas (lymphocytes), pink areas (histiocytes, plasma cells), or islands of non-heme cells (metastases). Look for fibrosis, which gives the marrow a streaming texture (Figure 20.4).

- On high power (20× and 40×)
  - Look at the individual cells, especially megakaryocytes. Small megakaryocytes with single nuclei are a feature of myelodysplasia and are also seen in chronic myeloid leukemia (Figure 20.5). Giant clustered megakaryocytes are a feature of myeloproliferative disorders. A few atypical megakaryocytes are not unusual, but a large population is significant.
  - Look for neutrophils. A packed marrow with numerous neutrophils may indicate chronic myeloid leukemia (see Figure 20.5), whereas numerous myeloid precursors with few neutrophils indicates a left-shift in maturation. Sheets of immature myeloid cells could represent anything from acute myeloid leukemia to infection; the aspirate needs to be evaluated for blasts (see next section).

Next, look at the aspirate. Hold it up to the light; an adequate aspirate will have little chunks in it (spicules) that are foci of stromal elements. Scan the slide for an optimal area of the smear. Cells should be spread out in a monolayer, with intact cytoplasm and distinct nuclei. "Naked" nuclei are not evaluable.

You have already evaluated the megakaryocytes, so, with the aspirate, focus on erythroid and myeloid cells. On high power (20× to 100×, with oil if necessary):

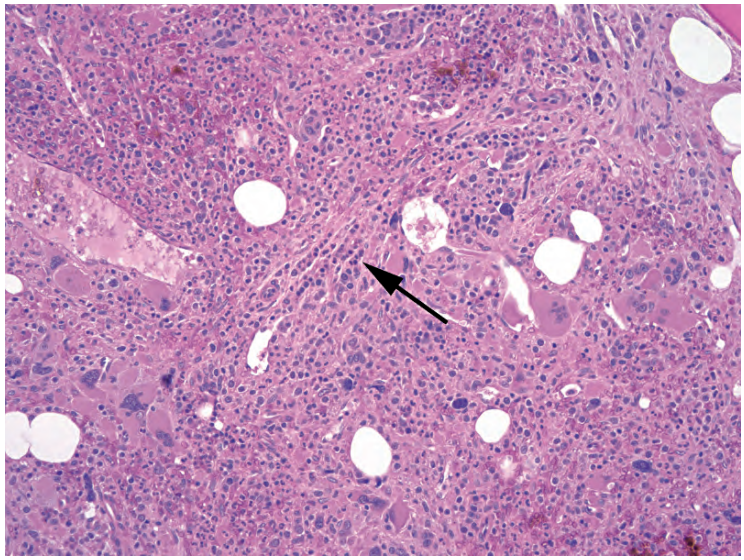

Figure 20.4. Marrow fibrosis. On hematoxylin and eosin stain, the marrow has a streaming texture (arrow), indicative of strands of collagen separating the hematopoietic cells into nests and channels.

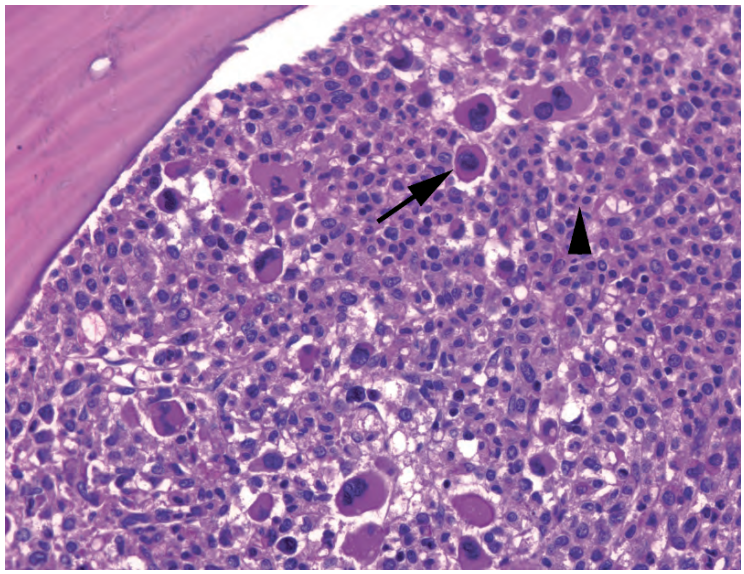

Figure 20.5. Chronic myeloid leukemia. This hypercellular marrow is full of small, hypolobated megakaryocytes (arrow) and maturing and mature neutrophils (arrowhead).

- Assess the heterogeneity of the marrow. A healthy aspirate should be a random mosaic of all different cell types at all levels of maturity. Sheets of uniform cells is a bad, bad sign (Figure 20.6).
- Look for blasts. You should be able to find scattered blasts but not clusters of them. Clumps of immature-looking cells are more commonly promyelocytes and myelocytes, as identified by their granules and cytoplasmic hofs. If you do find lots of blasts, note the shape of the nuclei, the color of the cytoplasm, and the presence of Auer rods. Presence of greater than 5% blasts is abnormal; they should be quantified by a systematic cell count of 200–500 cells. Flow cytometry will also provide a quantified percentage.

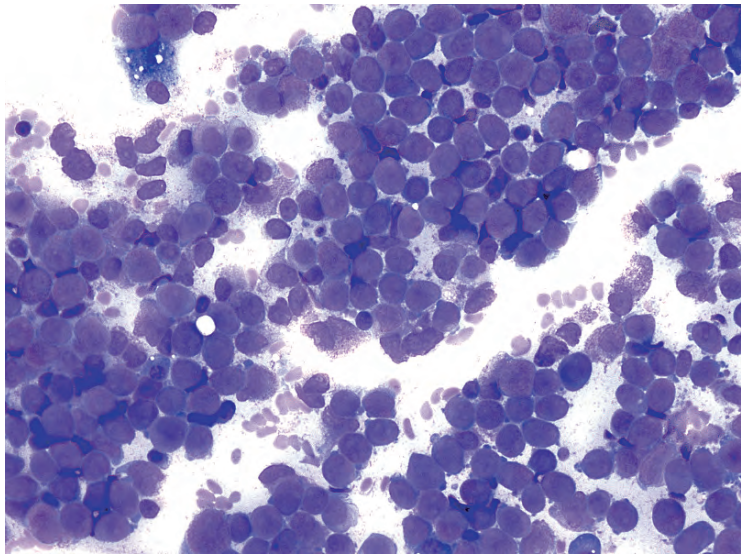

FIGURE 20.6. Monomorphic aspirate in acute leukemia. This aspirate is composed of sheets of blasts, identified by high nuclear to cytoplasmic ratios and immature chromatin.

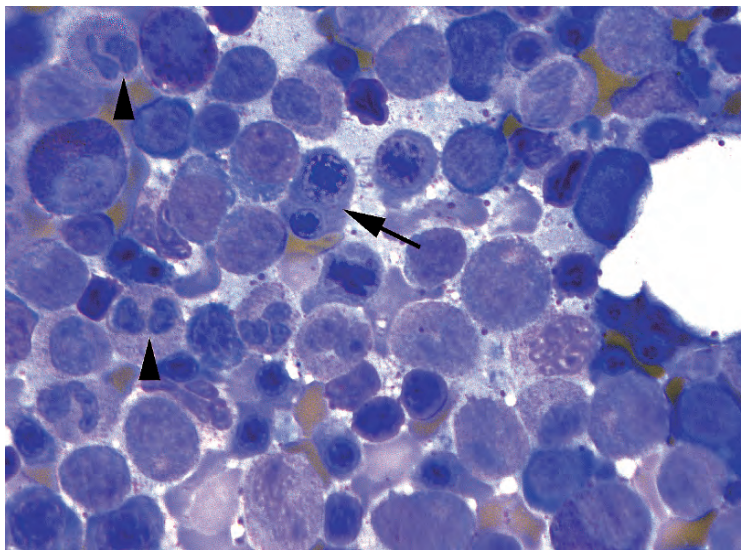

FIGURE 20.7. Dyserythropoiesis and dysgranulopoiesis. Binucleated erythroid precursors (arrow) and bilobed neutrophils without granules (arrowheads) are indicative of dysplasia.

- Evaluate the red cell precursors for dysplasia. Dyserythropoiesis shows up as binucleated red cells and red cell precursors with irregular nuclear membranes (Figure 20.7). Megaloblastoid change is a softer sign and looks like large "sliced salami" nuclei within mature (pale grey) cytoplasm.
- Dysgranulopoiesis (in neutrophils) appears as abnormalities in nuclear lobation ("Pelgeroid," which is bilobed like spectacles) and granules (absence of granules or occasionally coarse basophilic granules). Hypersegmented neutrophils can be seen in megaloblastic anemia.
- Plasma cells are easy to spot on the aspirate, with their bright blue cytoplasm and eccentric nucleus. Too many plasma cells (more than 5%–10%) may indicate a plasma cell dyscrasia.

Iron stains are performed on marrow smears to evaluate the iron stores. Hemosiderin (stainable storage iron) is found in reticuloendothelial cells and also in granules in normoblasts (developing red cell precursors). Normoblasts with iron granules in the cytoplasm are called *sideroblasts*. Sideroblasts are decreased in iron-deficiency anemia but increased in sideroblastic anemia, especially in the form of ringed sideroblasts, which have iron granules surrounding the nucleus in a ring.

After the aspirates, look at the peripheral blood film. Assess the white count, get a very rough differential, look for circulating blasts, and estimate the platelet count. (This chapter will not go into detail on peripheral smear interpretation.) Hypersegmented, hyposegmented, or hypogranular neutrophils are considered evidence of dysgranulopoiesis on the smear.

Differential Diagnosis

Once you have looked at everything, gather your facts to generate a differential diagnosis:
Hypercellular or hypocellular? (See marrow cellularity)
Dysplasia of any cell lines? (See myelodysplastic syndrome)
A prominent excess of any one cell line? (See myeloproliferative disorders)
Too many blasts? (See acute leukemia)
Lots of plasma cells? (See plasma cell dyscrasias)
Lymphocytes? (See lymphoma)

Marrow Cellularity

With hypercellular marrow, the differential diagnosis includes the following:

- Physiologic response to anemia, especially hemolytic anemia, or infection (no dysplasia, no increase in blasts)
- Ineffective hematopoiesis (such as megaloblastic anemia, human immunodeficiency virus)
- Myelodysplasia (dysplastic hematopoiesis in any cell line, <20% blasts)
- Myeloproliferative disorders (chronic myeloid leukemia, polycythemia vera (PV), essential thrombocythemia, (ET), myelofibrosis
- Acute leukemia (>20% blasts, with or without dysplasia)
- Other neoplasms (lymphoma, metastatic disease)

With hypocellular marrow, the differential diagnosis includes the following:

- Aplastic anemia
- Chemotherapy
- Infection
- Hypocellular forms of myelodysplastic syndrome (MDS)/acute myeloid leukemia (blasts are still increased)

Myelodysplastic Syndrome

Myelodysplastic syndrome includes the refractory anemia group of disorders and encompasses those diseases with *dysplasia of at least one cell line AND with blasts <20% in the marrow*. Myelodysplastic syndrome often progresses to acute myeloid leukemia, which by definition is >20% blasts. *Each dysplastic cell line has a corresponding peripheral cytopenia*, as it represents a dysfunctional hematopoiesis. Myelodysplastic syndrome tends to have an erythroid predominance in the marrow (decreased M/E ratio), as the body struggles to compensate for the anemia. The most minor disease is simple refractory anemia, which presents with anemia and shows erythroid dysplasia (see Figure 20.7). Blasts are not increased (<5%). The finding of >15% ringed sideroblasts on an iron stain indicates refractory anemia with ringed sideroblasts.

Dysplasia and cytopenias in two, or more usually three, cell lines bumps you to refractory cytopenia with multilineage dysplasia (with or without ringed sideroblasts). The dysplasia must be seen in >10% of a given cell line to be significant. Blasts are still not increased.

Once the blast percentage begins to increase, you get to refractory anemia with excess blasts (RAEB)-1 or -2 for marrow blasts 5%–9% and 10%–19%, respectively. Refractory anemia with excess blasts is otherwise known as high-grade MDS and usually progresses to acute myeloid leukemia. The presence of an Auer rod in the setting of MDS, regardless of the percentage of blasts, indicates RAEB-2. Similarly, the presence of >5% circulating or peripheral blasts indicates RAEB-2.

Myeloproliferative Disorders

The myeloproliferative disorders (MPDs), in contrast to myelodysplasia, show a superhematopoiesis. *There is little or no dysplasia, and the peripheral counts of the involved cell line(s) are high*. In chronic myeloid leukemia, the most common MPD, the polys are pushed out of the marrow so fast that they are not fully mature, so there are immature as well as mature granulocytes in circulation. Unlike MDS, there is an increased M/E ratio in the marrow. Like MDS, though, the marrow is hypercellular and the blast percentage is low (at least until it reaches a blast crisis). The gradual replacement of the bone marrow usually leads to cytopenias of the uninvolved cell lines (although chronic myeloid leukemia usually comes with a thrombocytosis), as well as extramedullary hematopoiesis and organomegaly.

Myeloproliferative disorders can affect any of the myeloid (nonlymphoid) cell lines: granulocytic (chronic myeloid leukemia), megakaryocytic (essential thrombocythemia), and erythroid (polycythemia vera). Chronic idiopathic myelofibrosis involves both the megakaryocytic and granulocytic lines. The MPDs can have overlapping features and be difficult to separate, with the exception of chronic myeloid leukemia, which is defined by unique cytogenetics (t 9;22, *bcr-abl*, the Philadelphia chromosome).

Myelodysplastic/Myeloproliferative Disorders

Of course there would be an overlap among the myelodysplastic and myeloproliferative disorders. The MDS/MPD category has features of both. The most common disorder is chronic myelomonocytic leukemia. It has the dysplasia, anemia, and thrombocytopenia of MDS, a proliferation of monocytes (monocytosis), <20% blasts, and no Philadelphia chromosome.

Acute Leukemia

Acute leukemia is defined as >20% blasts in the blood or marrow, but it is not uncommon to see >90% blasts in an initial presentation (Figure 20.8). *Acute lymphoblastic leukemia* (*ALL*) is the leukemic counterpart to acute lymphoblastic lymphoma and is primarily a disease of children in which immature lymphocyte precursors fill the marrow. Most commonly the cells are B-cell precursors (pre-B ALL), although T-cell ALL can also involve the marrow. Acute lymphoblastic leukemia can be very difficult to distinguish from acute myeloid leukemia on routine stains, as the lymphoblasts look very similar to myeloblasts. However, the diagnosis is easily confirmed by immunostains or flow cytometry.

Acute myeloid leukemia (*AML*) spans a wide category of diseases, which are roughly grouped by the type of blast that has gone bad. The classification of AML was by the FAB system for many years, which subgrouped the acute myeloid leukemias into the M0 to M7 categories (Table 20.1). The classification system used since 2001 is the World Health Organization (WHO) classification, which relies more on cytogenetic, molecular, and immunophenotypic features. In this system, some acute myeloid leukemias are defined by their cytogenetics, such as t(8;21) (ETO), t(15;17) (acute promyelocytic leukemia), and inv(16) (acute myelomonocytic leukemia with abnormal eosinophils). These are more likely to arise de novo and have a better prognosis. AML arising from MDS, however, has a poor prognosis and often deletions of chromosomes 5 and 7. Similar cytogenetics may be found in chemotherapy-related acute myeloid leukemia. The remaining AML subtypes include the leukemias previously defined in the FAB system, such as AML and acute megakaryoblastic leukemia.

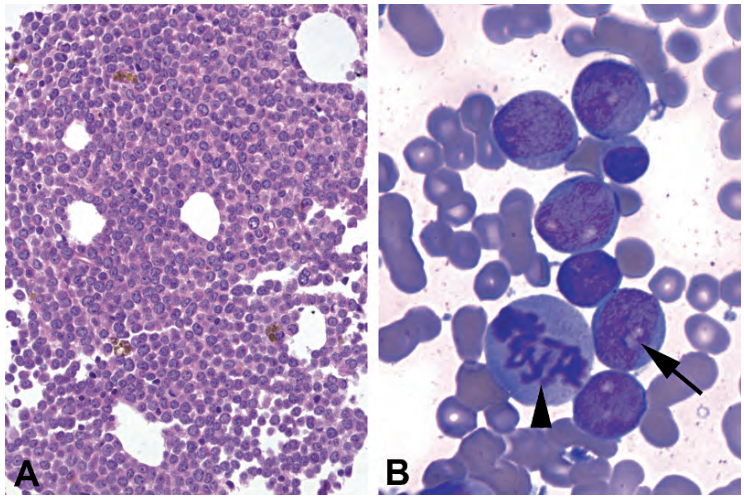

Figure 20.8. Acute myeloid leukemia. **(A)** The marrow biopsy material shows sheets of immature cells, with little or no background hematopoietic elements. Nucleoli appear dark on hematoxylin and eosin stain. **(B)** The aspirate shows clusters of myeloblasts with clear nucleoli (arrow). A large mitosis is visible (arrowhead); this in itself is not an unusual finding in the marrow.

Table 20.1. Acute myeloid leukemia subtypes according to the old FAB and the newer WHO classifications.

FAB classification	WHO classification	Blast or equivalent	Histologic hallmark
M0	AML minimally differentiated	Blast	
M1	AML without maturation	Blast	
M2	AML with maturation	Myeloblast	Granules, Auer rods, or myeloid markers
M2-ETO	AML with t(8;21)	Myeloblast with neutrophilic differentiation	Blasts with blue cytoplasm and granules, plus the immature "sunset" cells
M3	Acute promyelocytic leukemia, t(15;17)	Promyelocyte	Promyelocytes with blue granules and Auer rods
M4	AMML	Blast with myelo and mono features	Features of monocytic differentiation (grey cytoplasm, folded nuclei)
M4-Eo	AMML with inv(16)	Blast with myelo and mono features	Features of monocytic differentiation plus lots of eosinophils
M5	Acute monocytic leukemia	Monoblast	
M6	Acute erythroid leukemia	Erythroblast	
M7	Acute megakaryoblastic leukemia	Megakaryoblast	

Note: AML, acute myeloid leukemia; AMML, acute myelomonocytic leukemia; eo, eosinophils.

Histologically, acute myeloid leukemia usually presents as a hypercellular marrow mostly replaced by blasts. The differentiation of the blasts (and therefore the subclassification of the AML) is identified by morphology on the aspirate, flow cytometry, and cytogenetics. Some unique findings of the different subtypes include the following:

- t(8;21), acute myeloid leukemia–ETO: This subtype consists of a population of blasts with blue cytoplasm and granules, plus "sunset" cells, which are immature myeloid cells with salmon-pink granules, a pink cytoplasm with hof, and a peripheral blue rim. These look like a little sunset to some observers (Figure 20.9).
- inv(16): This subtype is acute myeloid leukemia with both granulocytic and monocytic differentiation (monocytic differentiation is bluish grey cytoplasm and folded or convoluted

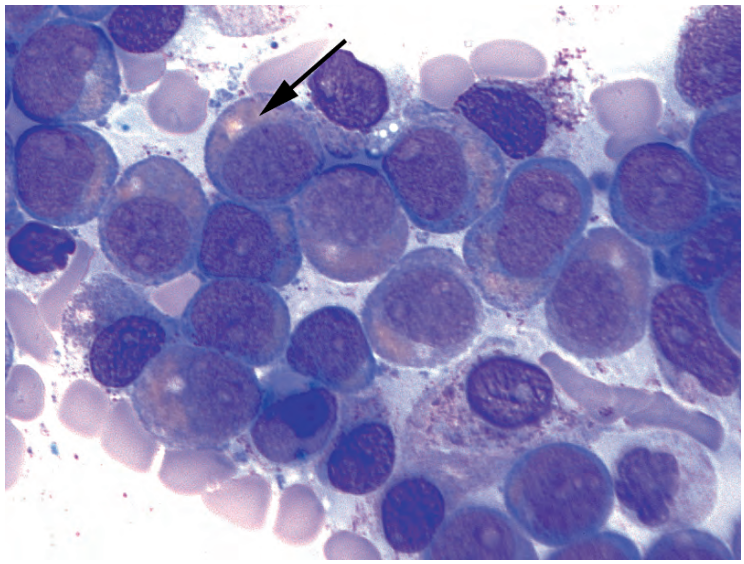

FIGURE 20.9. Acute myeloid leukemia–ETO. A population of the blasts have pink cytoplasm with pale hof, a peripheral blue rim, and pink granules (arrow).

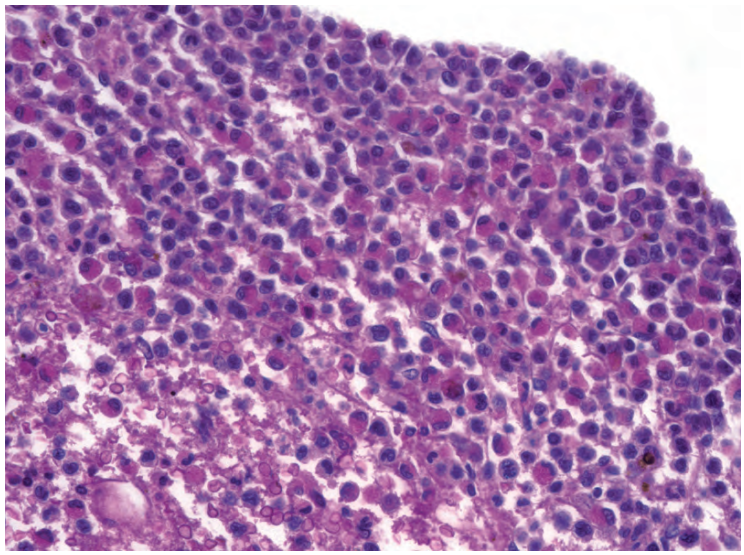

FIGURE 20.10. Acute myeloid leukemia inv (16). The marrow shows sheets of immature myeloid cells (the aspirate showed blasts) and numerous eosinophils.

nuclei) plus a high number of eosinophils in the marrow (Figure 20.10). Some of the eosinophils may have large abnormal blue granules (like basophils).

- t(15;17), acute promyelocytic leukemia: Technically there is not an increase in blasts but in promyelocytes. The nuclei are large with blast-like chromatin but folded (kidney-shaped), and the cytoplasm has large and numerous blue granules or Auer rods.

Plasma Cell Dyscrasias

A normal marrow should have 1%–2% plasma cells, scattered in a perivascular distribution. Aggregates of plasma cells and atypical forms (large nucleoli, binucleate forms) are abnormal. The diagnosis of a plasma cell myeloma requires a marrow plasmacytosis with a monoclonal gammopathy. The marrow plasmacytosis is typically >30% but can be as little

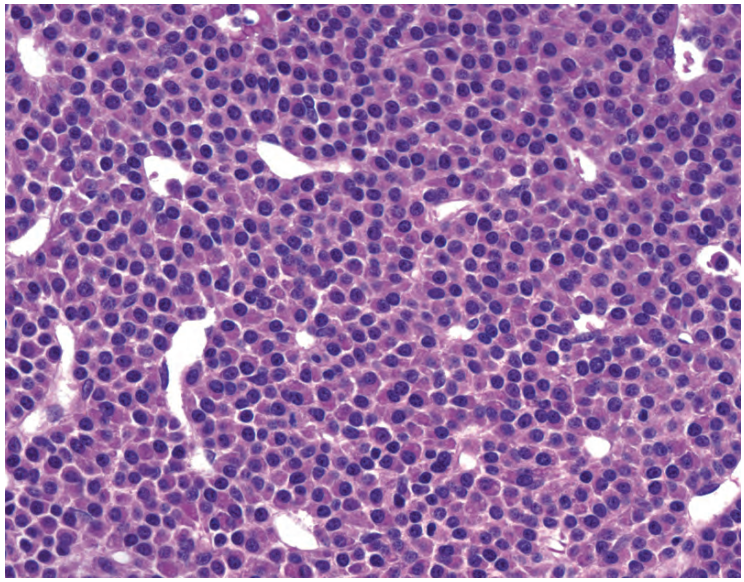

Figure 20.11. Myeloma. The marrow is replaced by sheets of plasma cells. Although the section is thick and the chromatin appears very dark, some nuclei show the distinct soccer-ball chromatin of plasma cells. The eccentric nucleus and abundant pink cytoplasm are also characteristic.

as 10% if there are also lytic bone lesions or certain other criteria (see the WHO hematopathology book for the exact diagnostic criteria and variants). A softer call is the plasma cell dyscrasia, which implies that there is a plasma cell problem but does not meet the diagnostic criteria for myeloma. A localized plasma cell lesion without systemic gammopathy is a plasmacytoma.

Sheets of plasma cells are recognizable on hematoxylin and eosin stain (Figure 20.11), but it is difficult to pick out a subtle interstitial plasmacytosis. CD138 can be used to help estimate the percentage. Immunostains for light chains kappa and lambda can identify an abnormally restricted (all kappa or all lambda) population, implying a monoclonal process. Flow cytometry tends to underestimate the number of plasma cells.

Lymphoma

Lymphocytes in the bone marrow can be a normal or reactive finding. They can also be an indication of lymphoma involvement. History is important, as the marrow is an unlikely place for a presentation of occult lymphoma (i.e., without lymphadenopathy). However, non-Hodgkin lymphoma has a 30%–50% chance of involving the marrow *at the time of diagnosis*. The subtypes most likely to go to marrow are follicular, small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL), mantle cell, lymphoblastic, Burkitt's, and peripheral T-cell lymphomas. Diffuse large B cell is less likely.

Lymphoid infiltrates can come in four basic patterns:

- Paratrabecular: This is a collection of lymphocytes that hugs the bony trabeculae. This pattern favors follicular lymphoma (Figure 20.12).
- Nonparatrabecular: This is a lymphoid aggregate that is not closely associated with a trabeculum. Benign lymphoid aggregates (discussed further below) are common in elderly patients, but small lymphocytic and chronic lymphocytic lymphomas can also have this pattern (Figure 20.13).
- Interstitial: This implies a scattered collection of lymphocytes in and among the marrow elements. It can be hard to pick out on H&E stain, because single lymphocytes will blend into the hematopoietic soup. Mantle cell and SLL/CLL tend to have this pattern, often in conjunction with nonparatrabecular aggregates.

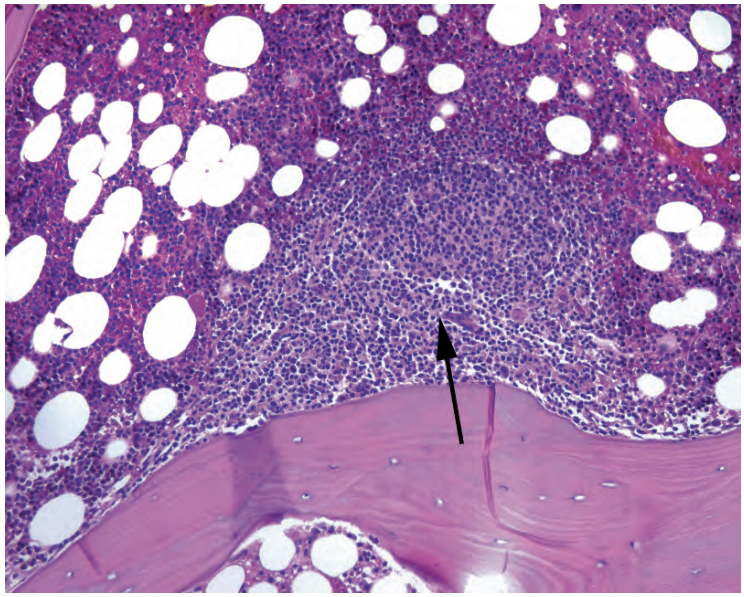

FIGURE 20.12. Paratrabecular aggregate. The arrow points to the center of this lymphoid aggregate, which appears more blue, relatively, than the surrounding marrow. One surface of the aggregate is plastered down to the bony trabeculum.

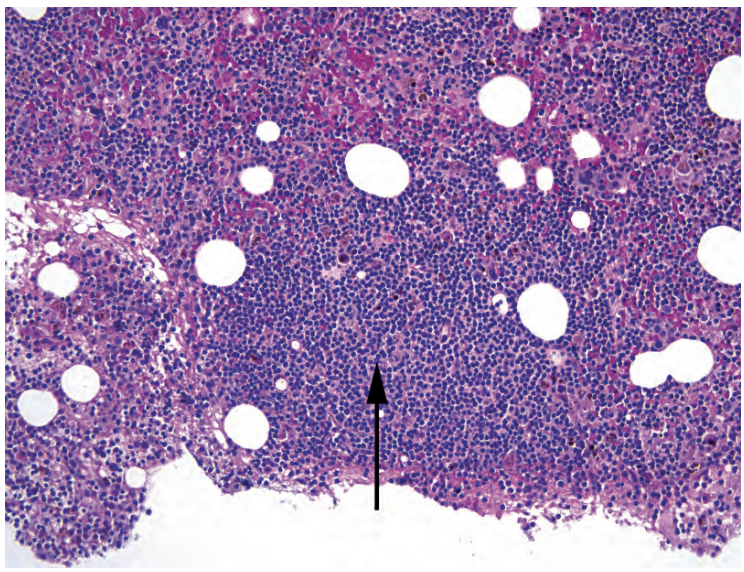

FIGURE 20.13. Nonparatrabecular aggregate in chronic lymphocytic leukemia. The arrow here points to the center of a free-floating, rounded lymphoid aggregate.

- Diffuse: This means sheets of lymphocytes replacing the marrow (Figure 20.14) and is more typical of aggressive lymphomas such as Burkitt's lymphoma, diffuse large B-cell lymphoma, or advanced SLL/CLL.

Features of a *benign lymphoid aggregate* include a nonparatrabecular site; heterogeneous mixtures of lymphocytes, plasma cells, and histiocytes; well-demarcated borders; germinal centers; and older patient age. Immunostains can also help. A benign aggregate should be a mixture of B and T cells (CD20 and CD3). Beware the CD20 stain in a patient who is taking

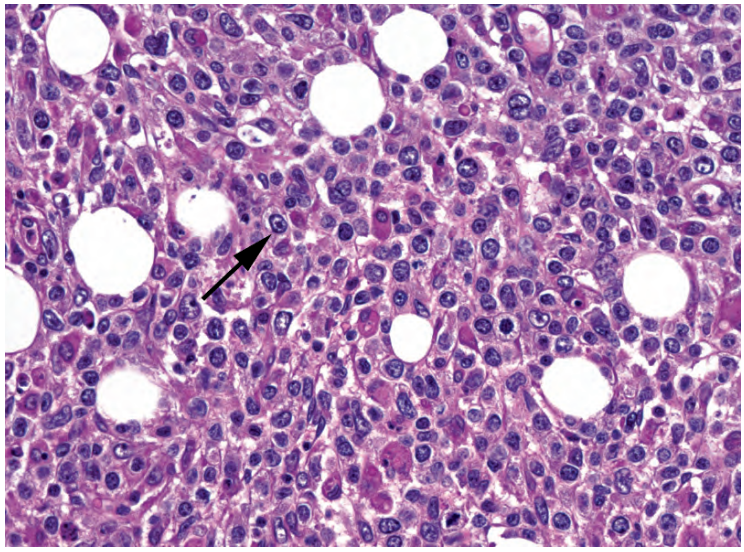

Figure 20.14. Diffuse large B-cell lymphoma in the marrow. The marrow is infiltrated by large atypical cells with prominent nucleoli, thick nuclear rims, abundant cytoplasm, and irregular nuclear membranes (arrow). This is suggestive of involvement by diffuse large B-cell lymphoma or carcinoma.

Rituximab, though, as this drug targets and eradicates CD20 expression. Use another B-cell marker such as CD22, CD79a, or PAX5.

Hodgkin's lymphoma in the marrow can be extremely subtle, as the defining trait of Hodgkin's is a mixed infiltrate with scattered neoplastic (Reed-Sternberg) cells. Focal fibrosis or granulomatous inflammation may be all that is seen initially; immunostains can often pick out the rare neoplastic cells.

21 Lymph Node

Normal Histology

The normal or benign lymph node is composed of a collection of follicles and interfollicular areas surrounded by sinuses (spaces mostly filled with histiocytes), vessels, and sometimes fat. Lymph flows from the subcapsular sinuses, through the medullary sinuses in the lymph node, and out the hilum. The follicles represent areas of maturing B cells ($CD20^+$), whereas the interfollicular areas are mostly mature T cells ($CD3^+$).

Follicles begin as primary follicles, or aggregates of antigen-naïve B cells. As they mature into secondary follicles they acquire germinal centers, which are visible as targetoid nodules within the follicles (Figure 21.1). The dark outer rim of the follicle is the displaced remains of the primary follicle and is called the *mantle zone*, still composed of antigen-naïve B cells. Once exposed to antigen, the B cells move to the germinal center and become centroblasts, large cells with primitive-looking nuclei. From there they either mature into centrocytes or die through apoptosis. Finally, B cells leave the germinal center genetically altered to circulate as memory B cells, which may ultimately differentiate into plasma cells if they meet their antigen.

Other normal germinal center components include the supporting follicular dendritic cells and tingible body macrophages, which clean up the apoptotic debris. These appear as relatively clear cells within the germinal center, with visible "dust specks" in the cytoplasm (Figure 21.2). Germinal centers may be found in any hotbed of lymphocyte activity outside the lymph nodes, but the morphology and staining pattern are preserved.

The paracortex, or interfollicular area, may occupy most of the lymph node in some cases. This absence of obvious follicles is not necessarily a reason for concern. The benign paracortex should have a mottled appearance due to the scattered pale histiocytes among the T cells.

Lymphoma, Conceptually

The word *lymphoma* means a malignancy of the lymphoid system and usually implies a solid tumor mass. However, remember that many of the lymphomas can have an associated leukemia, which is when the neoplastic cells invade the bone marrow and become circulating (Table 21.1). Among the myeloid leukemias, discussed in Chapter 20, solid tumor disease is uncommon but exists (such as the chloroma, or granulocytic sarcoma).

This chapter covers several major categories of lymphomas:

- Lymphoblastic (neoplasms of precursor cells, or lymphoblasts): These cells resemble myeloblasts and are the solid tumor counterpart to the acute lymphoblastic leukemias.

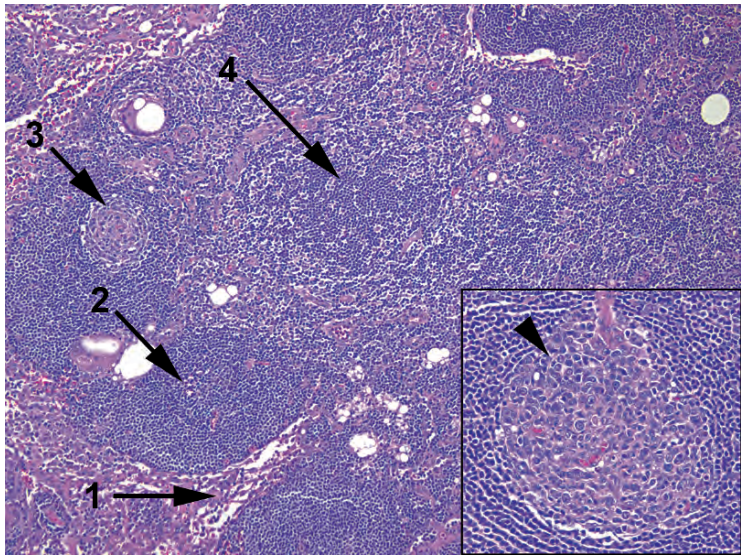

Figure 21.1. Normal lymph node. The sinuses are visible mainly as loose collections of histiocytes (1). Primary follicles (2) are collections of B cells lacking germinal centers. Secondary follicles contain germinal centers (3). The space between the follicles, or paracortex (4), is composed of T cells and shows a characteristic spotted or mottled appearance. **Inset**: A normal germinal center should be polarized, with large centroblasts clustered at one side of the follicle (arrowhead), creating a lopsided appearance.

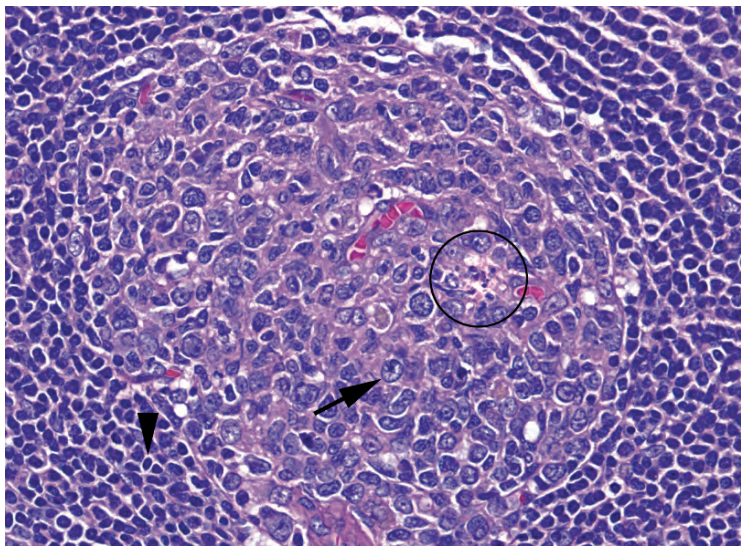

Figure 21.2. Germinal center. Other components of a benign germinal center include tingible body macrophages (circle) and large centroblasts with prominent nucleoli (arrow). The germinal center is surrounded by the mature B cells of the mantle zone (arrowhead).

- Low-grade B cell lymphomas (neoplasms of mature B cells): Like the chronic leukemias, these are indolent and simmering and are diseases of adults. They have a low mitotic rate and are therefore not susceptible to most chemotherapy.
- High-grade (aggressive) lymphomas (neoplasms of activated B cells and T cells [activated means exposed to the target antigen] or that resemble activated cells): These have a high mitotic rate but are therefore potentially curable through chemotherapy.

Table 21.1. Lymphomas and associated leukemias.

	Myeloid line	Lymphoid line	
Cell of origin	Leukemias	Lymphomas	Leukemias
Blasts	Acute myeloid leukemia, most types	Pre-B lymphoblastic lymphoma[1,2] T lymphoblastic lymphoma[1]	Pre-B lymphoblastic leukemia[1,2] T lymphoblastic leukemia[1]
Immature precursors	Acute promyelocytic leukemia	Burkitt's lymphoma[2]	
Mature cells	Chronic myeloid leukemia and myeloproliferative disorders	Low-grade lymphomas: Follicular[2] Small lymphocytic[3] Mantle cell[3] Marginal zone Lymphoplasmacytic Mycosis fungoides	Chronic lymphocytic leukemia[3] Myeloma T-cell large granular lymphocytic leukemia Sezary syndrome
Activated cells		Diffuse large B cell[4] Anaplastic large cell[4] Hodgkin's lymphoma[4]	Prolymphocytic leukemia

Markers go in batches: [1]TdT, CD34+; [2]CD10+; [3]CD5+; [4]CD30+.

- Hodgkin's lymphomas: As a group, these are neoplasms in which the neoplastic cells are a minority population, with a variable mixed inflammatory background. The prototypical tumor cell is the Reed-Sternberg cell, of which there are many variants.
- Plasmacytoma: Plasmacytomas are neoplasms of plasma cells
- Other: Other lymphomas include T-cell neoplasms and non-B, non-T cell types.

Many of the lymphomas can be placed into categories by nuclear morphology, and learning to recognize the "look" of each group is important. The lymphoblastic lymphomas have immature chromatin, which means the texture is very fine grained, with small nucleoli and an indistinct nuclear membrane, much like the myeloblasts in acute myeloid leukemia. On H&E stain, they may be mistaken for small cell carcinoma. The low-grade neoplasms resemble normal lymphocytes, with small condensed nuclei. The high-grade lymphomas show very carcinoma-like nuclei: they are large (compared with lymphocytes) and pleomorphic, with prominent nucleoli and coarse nuclear membranes. Hodgkin's lymphomas are the hardest to identify, usually, as the diagnostic cells (Reed-Sternberg cells and variants) may be few and far between. However, they do resemble the high-grade lymphoma nuclei in terms of the chromatin pattern.

Recognizing a lymphoma in an extranodal site, especially a tumor of unknown origin, takes practice. Clues to lymphoma include a relatively homogeneous, sheet-like growth of malignant cells; a lack of cell-to-cell cohesiveness or architecture; nuclei that are highly irregular in shape or contour; and an accentuation of cell density around vessels (especially in the central nervous system; Figure 21.3). Most should stain for CD45, the common leukocyte antigen, or for specific B or T markers. Positive staining for melanoma markers or cytokeratins rules out lymphoma. Sarcoma markers should be used with caution, though, as many of the familiar stains (CD117, CD34, etc.) also stain hematopoietic elements.

Diffuse large B-cell lymphoma

As the most common lymphoma, you will see diffuse large B-cell lymphoma (DLBCL) frequently. DLBCL is essentially a final common pathway in lymphoma; although it can arise spontaneously, it can also arise from the setting of any other low-grade B-cell lymphoma or from Hodgkin's lymphoma. The "diffuse" is used here as an opposite of follicular or nodular, and it implies sheet-like growth. The "large" should be interpreted with caution—what is large in hematopathology may still be fairly small next to a squamous cell.

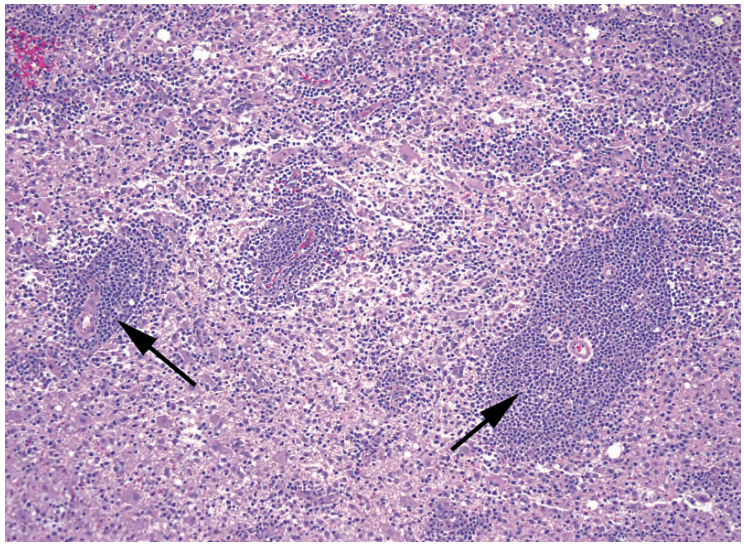

Figure 21.3. Diffuse large B-cell lymphoma in the central nervous system. The tendency of the malignant cells to cluster around blood vessels (arrows) is typical of lymphoma within the brain.

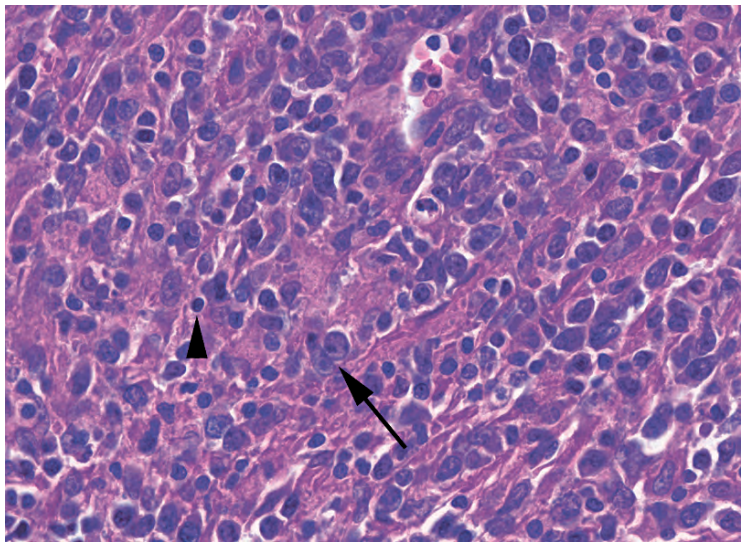

Figure 21.4. Diffuse large B-cell lymphoma. The usual appearance is that of sheets of discohesive cells that do not form any recognizable architectural patterns (such as glands or trabeculae). The cells typically have large nuclei, irregular and prominent nuclear membranes, and nucleoli (arrow). Compare the cell size to a background lymphocyte (arrowhead).

Diffuse large B-cell lymphoma is not usually mistaken for a benign entity; the nuclei are too abnormal. However, it may be mistaken for other types of malignancy, especially given its tendency to crop up in extranodal sites. As described earlier, the nuclei are very irregular in contour, with cleared-out or vesicular chromatin leaving a prominent nucleolus and thick nuclear rim (Figure 21.4). Folded, or cleaved, nuclei are common. The cells may have more cytoplasm than lymphocytes, and therefore a lower nuclear/cytoplasmic ratio.

Anaplastic large cell lymphoma is the T-cell equivalent of DLBCL. It is known for having even more elaborately folded nuclei, described as *cerebriform*, but still must be differentiated by T-specific markers.

Although at the time of this writing, DLBCL is largely a single category in the World Health Organization classification, the splitters are gaining on it. One emerging division is between those DLBCLs that are of germinal center cell origin, such as follicular lymphoma gone bad, and those of activated B-cell origin. The latter have the worse prognosis.

Follicular Lymphoma

Follicular lymphoma is the second-most common non-Hodgkin's lymphoma. Together DLBCL and follicular lymphoma account for over half of the non-Hodgkin's lymphomas. Follicular lymphoma is defined by a translocation in which bcl-2 (an anti-apoptotic factor) is abnormally upregulated. bcl-2 usually turns *off* in germinal centers, making the centroblasts and centrocytes susceptible to apoptosis. Abnormal retention of bcl-2 leads to cells that do not die, more or less, hence the malignancy. Follicular lymphoma appears as a nodular proliferation of back-to-back neoplastic follicles that fill the lymph node (Figures 21.5 and 21.6). Within these follicles are a mixture of neoplastic centrocytes (smaller) and centroblasts (larger); the relative proportion determines the grade of the lymphoma. Follicular lymphoma can also have areas of diffuse growth (the opposite of nodular), can spread to the marrow, and can transform to DLBCL. When circulating as a leukemia, the folded (cleaved) centrocyte nucleus has been compared to a "baby's butt."

The diagnostic dilemma is that benign, reactive lymphoid hyperplasia can also present as a nodular collection of follicles in an enlarged node. How to distinguish the two? The following are features of *benign follicular hyperplasia*, not seen in follicular lymphoma (see Figures 21.1 and 21.2):

- Germinal centers of variable sizes and cuffed by mantle zones (as opposed to back to back)
- Polarity of germinal centers in which the centroblasts and centrocytes tend to take up opposite positions in the follicle, creating an asymmetry
- Tingible body macrophages
- "Open" sinuses (which are not seen as open, really, but full of histiocytes)
- Abundant mitoses and apoptoses

These features should weed out the most straightforward cases. However, in very tough cases, stains help. The neoplastic follicles of follicular lymphoma can be stained with bcl-2; benign follicles should be negative.

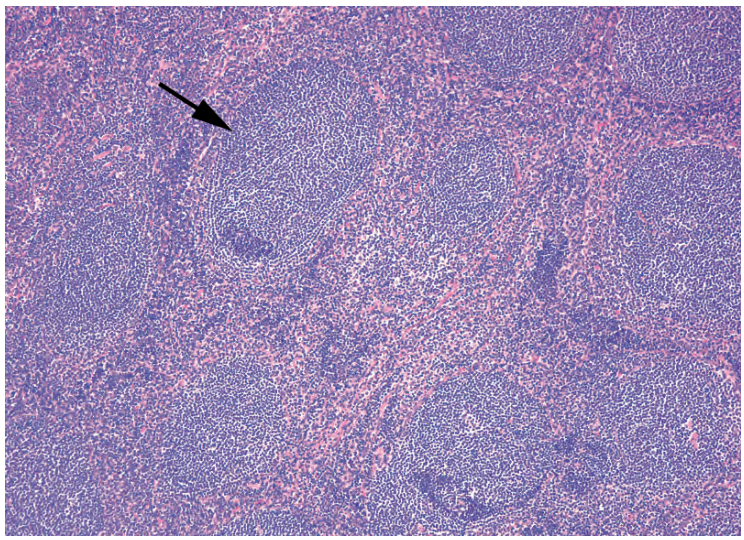

Figure 21.5. Follicular lymphoma. The lymph node is replaced by malignant follicles (arrows), which lack the mantle zones, polarization, and cell heterogeneity of germinal centers.

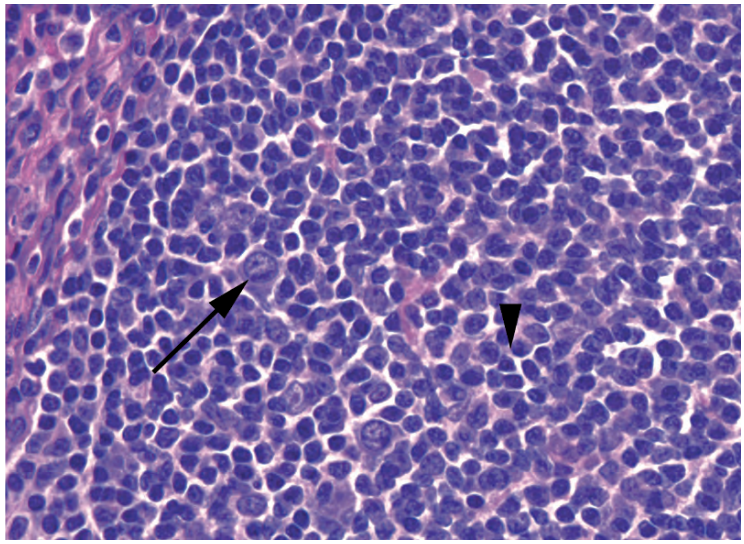

Figure 21.6. Follicular lymphoma, high power. The malignant follicles contain a mixture of small cleaved centrocytes (arrowhead) and large centroblasts (arrow).

Other Low-Grade B-Cell Neoplasms

The other three most common low-grade lymphomas, small lymphocytic lymphoma (SLL), mantle cell lymphoma (MCL), and marginal zone lymphoma (MZL), each make up less than 10% of non-Hodgkin's lymphomas. For these, and actually for most non-Hodgkin's lymphomas, flow cytometry is critical in the diagnosis. Flow cytometry can establish two things:

1. That there is a monoclonal population present. All B cells express either kappa or lambda light chain, so a significant predominance of one or the other implies a large genetically identical population (a neoplasm). A similar test can be done for T-cell neoplasms (T-cell receptor rearrangement studies) but not by flow cytometry.
2. That there are cells with an abnormal phenotype. The power of flow cytometry is that individual cells can be simultaneously tested for multiple markers, for instance, abnormal coexpression of CD20 and CD5. Doing this with immunohistochemistry is much less precise; you can estimate that the $CD5^+$ cells outnumber the normal T cells (identified by CD3) and that they are clustered in areas of B cells (identified by CD20), but you cannot see two markers on a single cell.

The interpretation of flow cytometry is beyond the scope of this chapter, but the major learning point is that saving tissue for flow cytometry will make your life much easier, so set some aside in any lymphadenopathy workup or possible extranodal lymphoma. Formalinized (fixed) tissue cannot be sent for flow cytometric studies.

In the low-grade B cell lymphomas, the low-power feature that rings alarm bells is the *effacement of the lymph node*. This means that the normal architecture, the follicles and sinuses and interfollicular areas, have been blurred out or replaced by a rather uniform population of cells. This takes some experience to judge; fortunately, every carcinoma resection comes with some bonus lymph nodes, so take the time to notice what normal looks like.

Small Lymphocytic Lymphoma

Small lymphocytic lymphoma is the solid-phase manifestation of chronic lymphocytic leukemia, and the two are often seen in concert. In lymphoma form, SLL appears at 1× as a

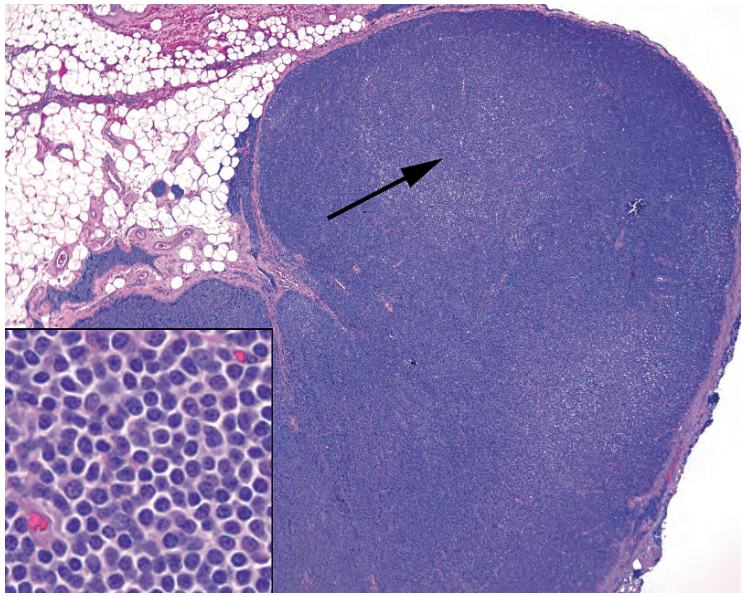

Figure 21.7. Small lymphocytic lymphoma. The lymph node, at low power, is an unnatural flat blue, without the variegation of normal sinuses and follicles. Subtle pale pseudofollicles (arrow) may be seen. **Inset**: The cells are small and nuclei are round and dense, like normal lymphocytes, except the chromatin has a chunky soccer-ball pattern, similar to a plasma cell.

very homogeneous, very blue lymph node. At low power, the follicles, paracortex, and sinuses are replaced by a sheet of what look like normal lymphocytes. There may be a vague suggestion of nodularity, called *pseudofollicles*, containing proliferating cells (Figure 21.7). On high power, the SLL cells usually have chromatin that may remind you of a plasma cell; they look like soccer balls. The nuclei are small, round, regular, and without nucleoli. Small lymphocytic lymphoma cells express CD23 and CD5.

Mantle Cell Lymphoma

Mantle cell lymphoma, although it is in the histologic differential diagnosis for the low-grade lymphomas, actually behaves more aggressively than the others in this group. On low power it is reminiscent of SLL, with sheets of small lymphocytes effacing the node. In a not-entirely-replaced node you may be able to tell that the mantle zones are expanding to engulf the germinal centers. Hyalinized vessels are typical. On high power, the cells of MCL have a chunky dark chromatin similar to the cells of SLL, but the nuclear membranes are more crinkled or angular, with more size variation (Figure 21.8). Cyclin D1 is the marker for MCL, which correlates with the translocation that defines the tumor.

Marginal Zone Lymphoma

The marginal zone of the lymph nodes is named for the more prominent and identifiable marginal zone in the spleen. In the lymph nodes, it is barely visible as a slightly attenuated zone surrounding the mantle. The cells in this zone have a prominent rim of clear cytoplasm, giving them almost a fried-egg appearance, and a pale look at low power. This morphology is called *monocytoid*. This cell type can give rise to at least three distinct lymphomas: splenic MZL (not discussed here), nodal MZL, and extranodal mucosa-associated lymphoid tissue (MALT) lymphoma of gut, salivary glands, and so forth.

MALT lymphomas are discussed in Chapter 7. Like MALT lymphomas, the cells of MZLs are monocytoid in appearance and grow in sheets or clusters, mainly in the interfollicular

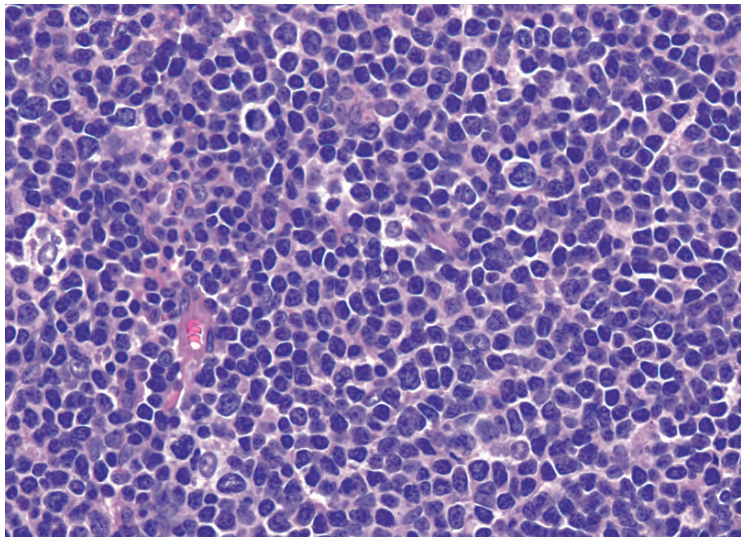

Figure 21.8. Mantle cell lymphoma. The neoplastic cells in mantle cell tend to be more irregular in shape than those of small lymphocytic lymphoma, with slightly angular nuclei. The chromatin pattern, with the soccer-ball splotches, is similar to small lymphocytic lymphoma.

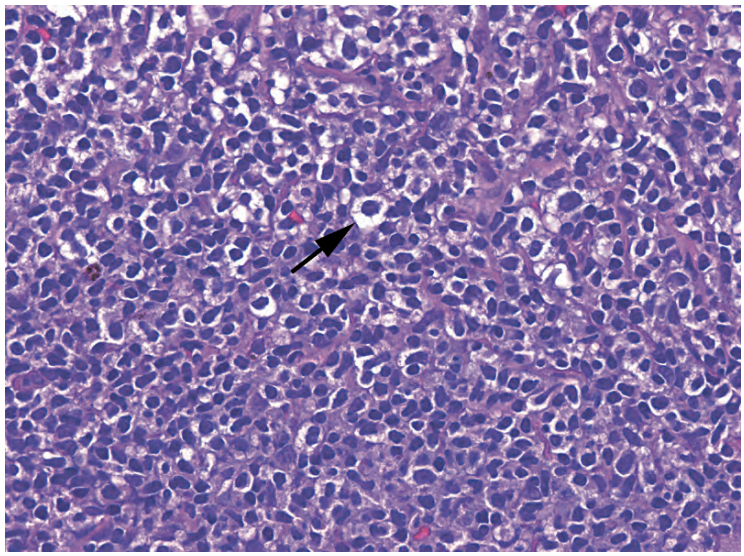

Figure 21.9. Marginal zone lymphoma. The marginal zone cells classically have a monocytoid appearance, meaning there is a distinct thin halo of clear cytoplasm (arrow).

areas (Figure 21.9). They are negative for most of the markers that identify other lymphomas but may sometimes abnormally express CD43.

Markers

Although the low-grade B-cell lymphomas do have histologic features that distinguish them, few pathologists would sign them out without confirming flow cytometry or immunostain studies. Although this book does not otherwise focus on immunostains, it is impossible to discuss the lymphomas without them. The standard panel includes CDs 3 and 20, 5 and 10, and 43, as well as some specific markers discussed earlier. CD3 identifies T cells, and

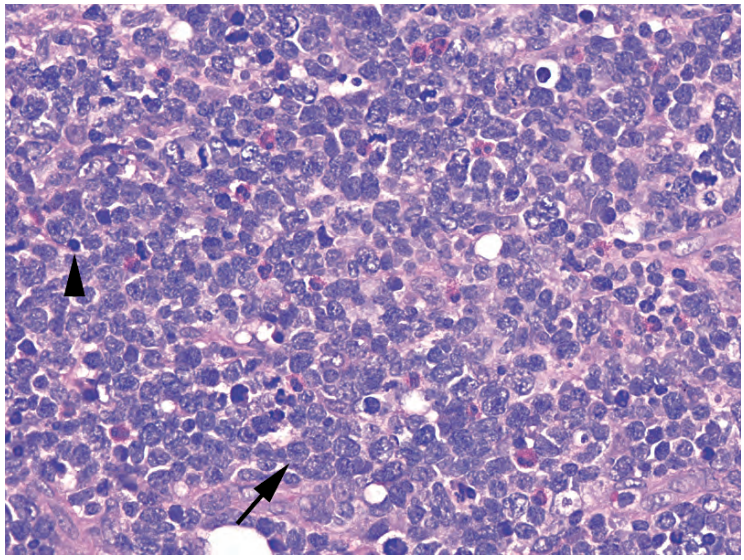

Figure 21.10. Acute lymphoblastic lymphoma. The nuclei (arrow) are larger than a normal lymphocyte (arrowhead), and the chromatin is very immature (meaning widely dispersed throughout the nucleus). Unlike large B-cell lymphoma, there are no prominent nucleoli or thick nuclear membranes.

CD20 identifies B cells. The expression of either CD5 or CD43, (T-cell markers), or CD10, (an immature B-cell/germinal center marker), in mature B cells is abnormal, and guides your differential.

Lymphoblastic Lymphoma

The lymphoblastic lymphomas are not usually diagnosed in lymph nodes. The precursor-B type more commonly presents as a leukemia (acute lymphoblastic leukemia), whereas the precursor-T type is most often seen as a mediastinal mass (remember the immature T cells are found in the thymus; Figure 21.10). Burkitt's lymphoma is also in this category, and the cells are similar in appearance; it may present anywhere, especially in the gut. Most acute lymphoblastic leukemias are now defined by cytogenetics, but they also stain for the blast markers TdT and CD34 (Burkitt's lymphoma not included). As CD45, the usual screening immunostain, is not reliably expressed in these tumors, be aware that a negative CD45 in a pediatric small round blue cell tumor may still be lymphoma. Finally, what looks like a low-grade B-cell lymphoma in a child is much more likely to be a lymphoblastic lymphoma.

Hodgkin's Lymphoma

Hodgkin's lymphoma is common, comprising 30%–40% of all lymphomas. It is now divided into two large groups, classic (most types) versus nodular lymphocyte predominant (NLPHL). Both groups share the histologic features of a dense and effacing mixed inflammatory infiltrate with scattered Reed-Sternberg (or Reed-Sternberg–like) cells. Because of the high benign background population, flow cytometry is not effective in detecting Hodgkin's lymphoma. Making the diagnosis requires either seeing the diagnostic tumor cells or demonstrating them by immunostain. In classic Hodgkin's lymphoma, the Reed-Sternberg cells are CD30 and CD15 positive while negative for CD45 and CD20. In NLPHL, the tumor cells stain exactly the opposite ($45/20^{+}$, $30/15^{-}$). In this sense, NLPHL is really analogous to a DLBCL with an associated inflammatory response.

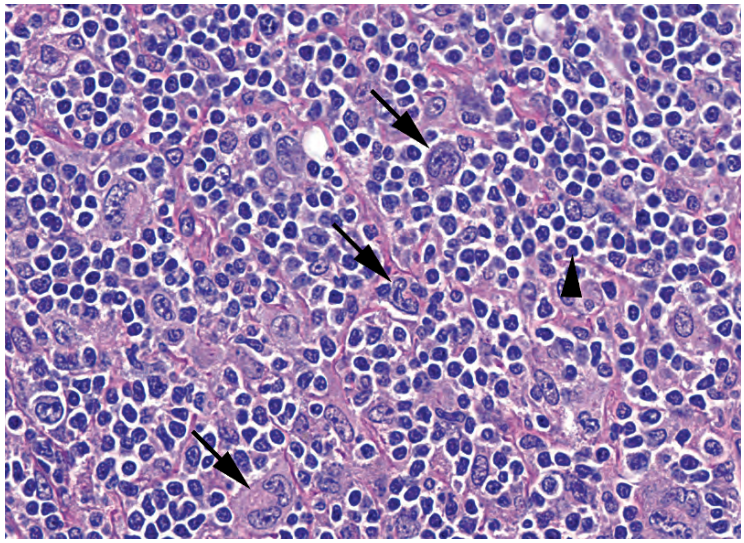

Figure 21.11. Hodgkin's lymphoma. The malignant Reed-Sternberg cells (arrows) are spread out among a background of nonneoplastic inflammatory cells, especially lymphocytes (arrowhead).

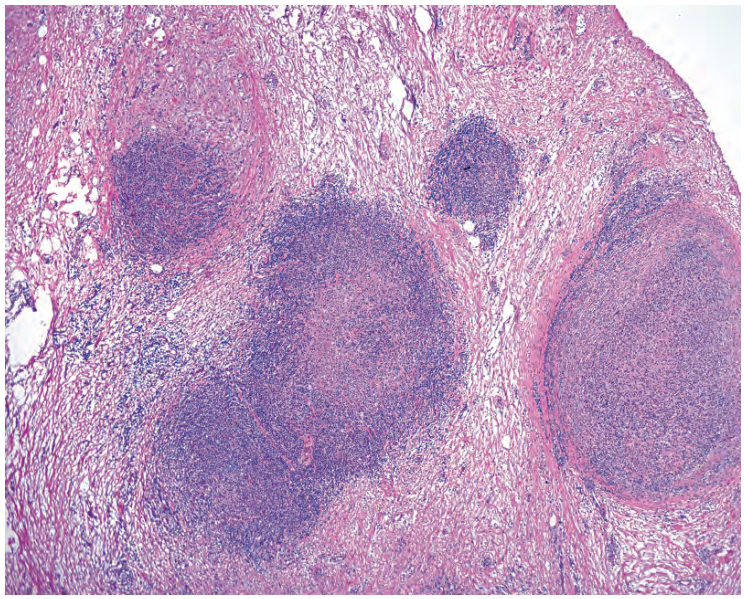

Figure 21.12. Nodular sclerosing Hodgkin's lymphoma. The aggregates of Reed-Sternberg cells and inflammation are separated by broad bands of fibrosis.

The subtypes of classic Hodgkin's lymphoma include nodular sclerosing, mixed cellularity, and the less common lymphocyte depleted and lymphocyte rich. All should have some variety of the Reed-Sternberg cells, which in classic form have at least two nuclear lobes, each with a prominent, cherry-red nucleolus and thick nuclear membrane (Figure 21.11). However, variants with single or multilobed nuclei may be seen.

In *nodular sclerosing Hodgkin's lymphoma*, at low power, the node is "cirrhotic," with nodules of mixed inflammation divided by broad fibrous bands (Figure 21.12). The node is usually also encapsulated. The Reed-Sternberg cells take the form of lacunar cells, which means the diagnostic nuclei are suspended in a retracted space or halo.

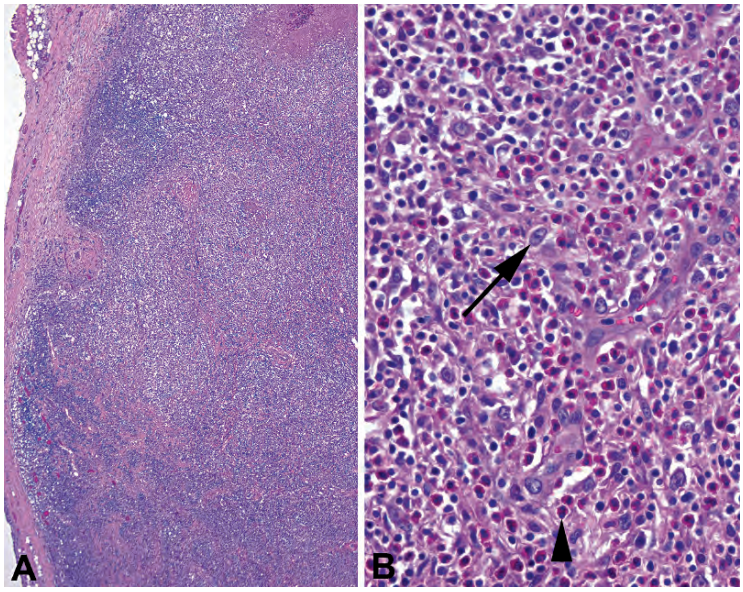

FIGURE 21.13. Mixed cellularity Hodgkin's lymphoma. **(A)** At low power, the lymph node appears to be effaced by a heterogeneous population, giving a slightly pink color to the node (compare to small lymphocytic lymphoma in Figure 21.7). **(B)** The Reed-Sternberg variants are few and far between (arrow), with a dominant population of eosinophils (arrowhead).

At low power, *mixed cellularity Hodgkin's lymphoma* appears "pink" because of the abundant histiocytes and eosinophils found in the background infiltrate (Figure 21.13). Plasma cells and lymphocytes are also common. Very subtle cases may present as granulomatous inflammation.

T-Cell Lymphomas

The incidence of T-cell neoplasms is much lower than that of B cells. Precursor T-lymphoblastic lymphoma has been discussed, as has anaplastic large cell lymphoma. The cutaneous T-cell neoplasms include mycosis fungoides/Sezary syndrome (the solid and circulating phases, respectively) and primary cutaneous anaplastic large cell lymphoma. Of those that may present in a lymph node, peripheral T-cell lymphoma, unspecified, is the most common.

Nonneoplastic Entities

Inflammation in a lymph node? It is only abnormal if it is granulomatous, acute, or necrotizing. Granulomatous inflammation may be nonnecrotizing in sarcoid or caseating in tuberculosis or fungal infection. Both of the latter should have positive findings on bug stains. Other infectious entities include infectious mononucleosis and cytomegalovirus, which can both cause dramatic follicular hyperplasia; cat scratch disease, causing an acute lymphadenitis with neutrophils; and *Toxoplasma*, which causes a follicular hyperplasia with ill-defined granulomatous inflammation. An unusual disease, called *Kikuchi's lymphadenitis*, resembles a granulomatous response, with large swaths of geographic necrosis, but on high power the necrotic areas are paradoxically devoid of neutrophils, showing only apoptotic nuclear debris.

In summary, if you see…	Think of…
Diffuse sheet of small lymphocytes	Small lymphocytic, mantle cell, or marginal zone lymphoma
Prominent nodular pattern	Follicular lymphoma
Diffuse sheet of large atypical cells	Diffuse large B-cell lymphoma
Mitotically active cells resembling small cell carcinoma	Lymphoblastic lymphoma
A pink and/or granulomatous mixed infiltrate	Mixed cellularity Hodgkin's lymphoma
Fibrous bands dividing the node	Nodular sclerosing Hodgkin's lymphoma

Knowledge of the pathway of B cells through the germinal centers, and the various markers that switch on and off, is very helpful in understanding the various B-cell neoplasms that arise from the different stages of maturation. The introductory chapter on mature B-cell neoplasms in the World Health Organization's textbook *Tumours of Haematopoietic and Lymphoid Tissues* is a very good place to start.

22 Lungs

Normal Histology

The lungs consist of principally four compartments: the large airways (bronchi), small airways and airspaces (bronchioles and alveoli), interstitium, and vessels. As in most organs, inflammatory processes tend to preferentially involve one or two compartments, so identifying the most affected area is key to the differential diagnosis. Normal histologic features include the following:

- *Bronchi*: The bronchi are lined with ciliated or columnar epithelium with scattered goblet cells. Goblet cell metaplasia is an indication of irritation, such as in bronchitis or asthma. Squamous cell metaplasia is common in smokers. Under the epithelium you should find seromucinous (salivary-type) glands, cartilage, smooth muscle, and branches of the bronchial arteries (Figure 22.1).
- *Bronchioles*: Bronchioles should have a cuboidal epithelium without goblet cells (Figure 22.2). The Clara cells are probably secretory and reserve cells, but they are difficult to see. There is no cartilage.
- *Alveoli*: The alveoli are the terminal air sacs and therefore have extremely thin walls (see Figure 22.2); in atelectasis, a common biopsy artifact, it is difficult to pick out the collapsed airspaces. Normally they are lined by nearly invisible flat type I epithelium. The presence of a cuboidal epithelium indicates type II hyperplasia (surfactant and reserve cells, which are normally sparse), seen in chronic inflammation or repair. Alveolar macrophages are often scattered throughout but macrophages packing the alveoli is pathologic (see later discussion of desquamative interstitial pneumonia).
- *Vessels*: Pulmonary arterioles run with bronchioles and have two elastic layers on Movats stain (train track appearance). Veins run in interlobular septa and have one irregular elastic lamina. Lymphatics run with arteries, veins, and in pleura.

Movats stain is a standard supplemental stain for nonneoplastic lung. On this pentachrome stain, you will see elastic laminae highlighted as black fibers (useful for identifying pleural involvement by tumors as well), hyaluronic acid or mucin in aqua blue, mature collagen in yellow, smooth muscle in dull red, and fibrinoid necrosis (as in vessels) as bright red (Figure 22.3). This stain is very useful for identifying fibroblast foci in bronchiolitis obliterans–organizing pneumonia (discussed later) because they stand out as turquoise swirls on low power. Established interstitial fibrosis will be yellow.

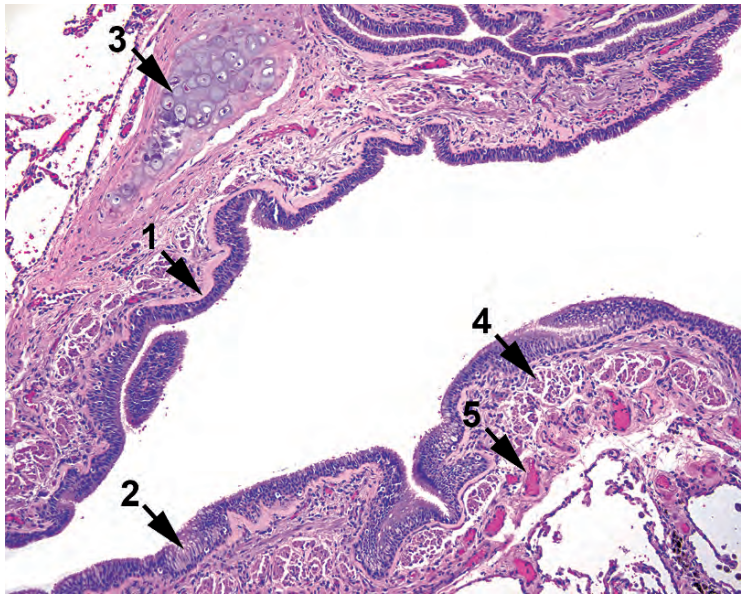

Figure 22.1. Normal bronchus. The bronchus is lined by ciliated columnar epithelium (1), foci of goblet cells (2), cartilage (3), and smooth muscle (4). The small arteries seen here (5) are branches of the bronchial artery, which carries oxygenated blood from the left ventricle.

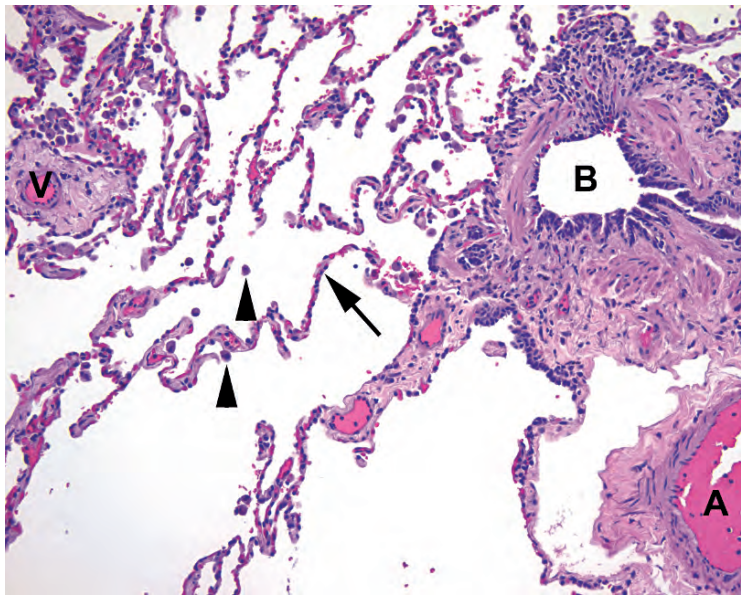

Figure 22.2. Bronchioles and alveoli. The small bronchiole (B) seen here is lined by a cuboidal epithelium and smooth muscle. The large adjacent arteriole (A) is a branch of the pulmonary artery. The veins or venules (V) run in septa. The alveolar walls (arrow) are normally lined with flat type I epithelium, of which only the nuclei are visible. Alveolar macrophages (arrowheads) are common.

A Brief Introduction to Nonneoplastic Lung

In nonneoplastic lung, within each of the four compartments you are usually looking for something that does not belong. Examples of things that do not belong include heavy mononuclear cell infiltrates (lymphocytes and macrophages), neutrophils (other than in capillaries), eosinophils, granulomas, fibrosis and fibroblast foci, and substances such as amyloid, edema fluid, and asbestos. Table 22.1 lists differential diagnoses organized by what you see and in which compartment.

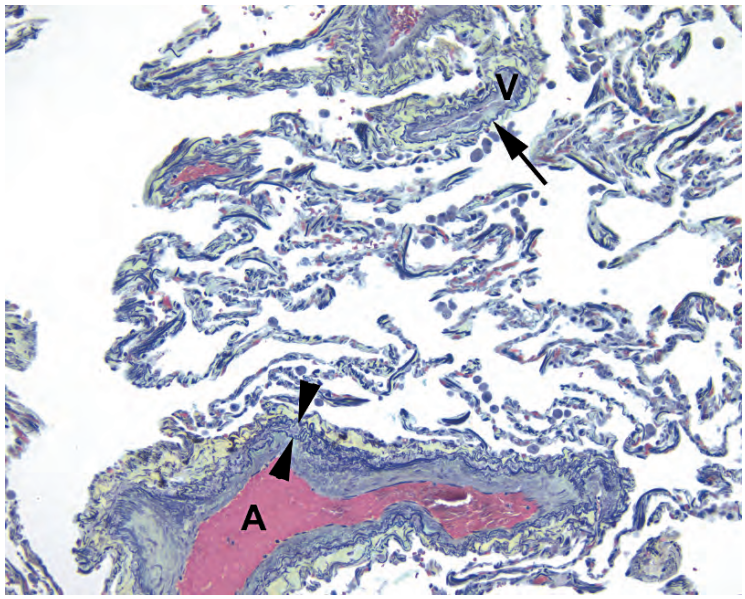

Figure 22.3. Movats stain. The pulmonary arteries (A) have two elastic layers (arrowheads), while the veins (V) have one (arrow). The collagen lining the vessels is pale yellow-green in this stain.

Table 22.1. Differential diagnoses.

	Large and small airways	Alveoli	Interstitium/septa	Vessels
Lymphocytes and mononuclear cells	Atypical/viral pneumonia EAA RB-ILD (macrophages)	Atypical/viral pneumonia DIP (macrophages) EAA	DAD (late or organizing)/AIP EAA NSIP, CIP, and LIP *Pneumocystis carinii* pneumonia UIP LCH (histiocytes)	
Neutrophils	Bronchopneumonia BCG	Bronchopneumonia	ACIP	Wegener's granulomatosis
Eosinophils	Asthma and ABPFD CEP BCG	Loeffler's syndrome CEP Churg-Strauss syndrome EAA	CEP	Churg-Strauss syndrome
Granulomas	BCG TB and fungus	TB and fungus	EAA Sarcoid Rheumatoid nodules	Churg-Strauss syndrome Invasive aspergillosis Sarcoid Wegener's granulomatosis
Fibrosis and fibroblast foci	BOOP OB	BOOP	DAD (late or organizing)/AIP DIP Pneumoconioses Sarcoid Systemic disease (RA, SLE) UIP	Pulmonary hypertension
Other substances (mucus, exudates, etc)	Asthma Chronic bronchitis	Early DAD (HM) Goodpasture's syndrome (heme) PAP (exudate) *P. carinii* pneumonia (foam)	Pneumoconioses (refractile material) Lymphangioleiomyomatosis (smooth muscle)	Amyloidosis DAD (fibrin thrombi)

ABPFD, allergic bronchopulmonary fungal disease; ACIP, active chronic interstitial pneumonitis; AIP, acute interstitial pneumonitis; BCG, bronchocentric granulomatosis; BOOP, bronchiolitis obliterans–organizing pneumonia; CEP, chronic eosinophilic pneumonia; CIP, chronic interstitial pneumonia; DAD, diffuse alveolar damage; DIP, desquamative interstitial pneumonia; EAA, extrinsic allergic alveolitis (hypersensitivity pneumonitis); HM, hyaline membranes; LCH, Langerhans cell histiocytosis; LIP, lymphocytic interstitial pneumonia; NSIP, nonspecific interstitial pneumonia; OB, obliterative bronchiolitis; PAP, pulmonary alveolar proteinosis; RA, rheumatoid arthritis; RB-ILD – respiratory bronchiolitis–interstitial lung disease; SLE, systemic lupus erythematosus; TB, tuberculosis; UIP – usual interstitial pneumonia.

Response to Injury in the Lung

It is useful to think of the three phases of injury response in the lung: acute, subacute, and chronic. *Acute injury*, which may be from infection, trauma, toxins, drugs, or a transfusion reaction, manifests as *diffuse alveolar damage*. Clinically this pattern correlates with acute respiratory distress syndrome. Idiopathic diffuse alveolar damage, when no known precipitating factor can be identified, is called *acute interstitial pneumonitis*. The histologic picture is a nonspecific indication of injury and includes interstitial edema and hemorrhage, hyaline membrane formation, type II hyperplasia, and fibrin thrombi (Figure 22.4). There should be a uniform and diffuse appearance throughout the field of view (although it may be patchy grossly).

When the initial injury begins to resolve, you see the organizing phase, which consists of new fibroblast foci forming in alveoli and bronchioles. These are the swirling nodules of stellate fibroblasts that appear myxoid on H&E stain and aqua on Movats stain (Figure 22.5). They are also the hallmark of *bronchiolitis obliterans–organizing pneumonia* (BOOP), the pattern of *subacute injury* response. It can be impossible to distinguish a primary BOOP from a resolving acute injury without the clinical context. It is also seen as a component of many other disease processes, but as a primary disease it is simply "idiopathic BOOP." Obliterative bronchiolitis is a related lesion that is really only seen in transplant patients, and is a form of either rejection or graft-versus-host disease.

Chronic and repetitive injury to the lung is like a scab on the skin that gets repeatedly picked off; there are multiple cycles of damage and repair, and the end result is chronic inflammation and fibrosis. The final common pathway of many diseases, or end-stage lung, is called honeycomb lung. A specific pattern of chronic injury that may lead to honeycomb lung is *usual interstitial pneumonia*. Usual interstitial pneumonia is a nonspecific pattern; *idiopathic pulmonary fibrosis* is the name given to idiopathic usual interstitial pneumonia.

Usual interstitial pneumonia should be *temporally heterogenous*, which means you should see evidence of all stages of injury (acute, subacute, and chronic). There is prominent interstitial fibrosis, which outlines large and angular distorted airspaces (Figure 22.6), but there should also be fibroblast foci. The airspaces are lined by plump, reactive, and scary looking type II pneumocytes. There is diffuse chronic inflammation, as well as pockets of acute inflammation.

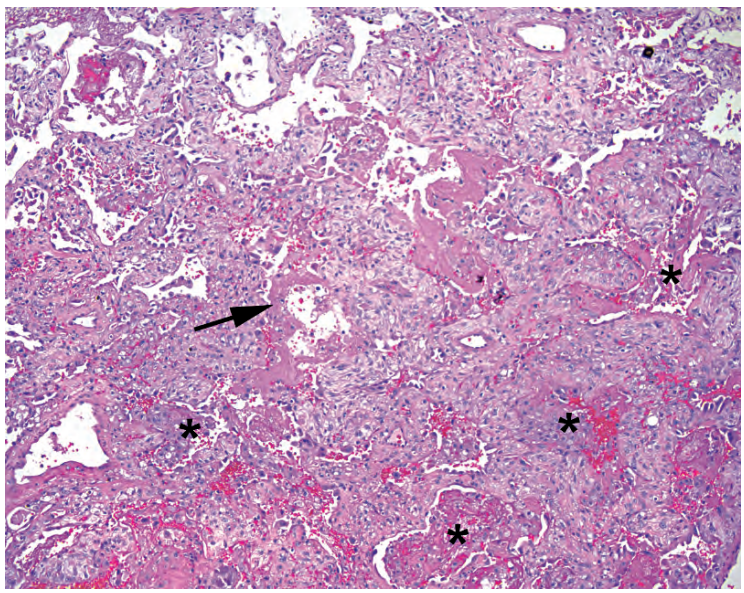

Figure 22.4. Diffuse alveolar damage. The alveolar spaces are full of fluid and blood (asterisk), which in some areas is beginning to coalesce into thick pink hyaline membranes (arrow). The interstitial spaces are thickened due to edema.

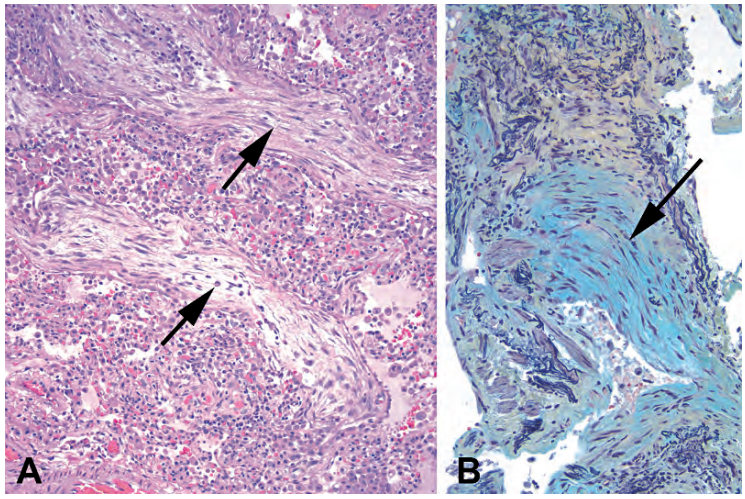

Figure 22.5. Fibroblast foci. **(A)** By H&E stain, these myxoid swirls of new fibroblasts are pale and streamy (arrows). **(B)** On Movats stain, they are turquoise (arrow).

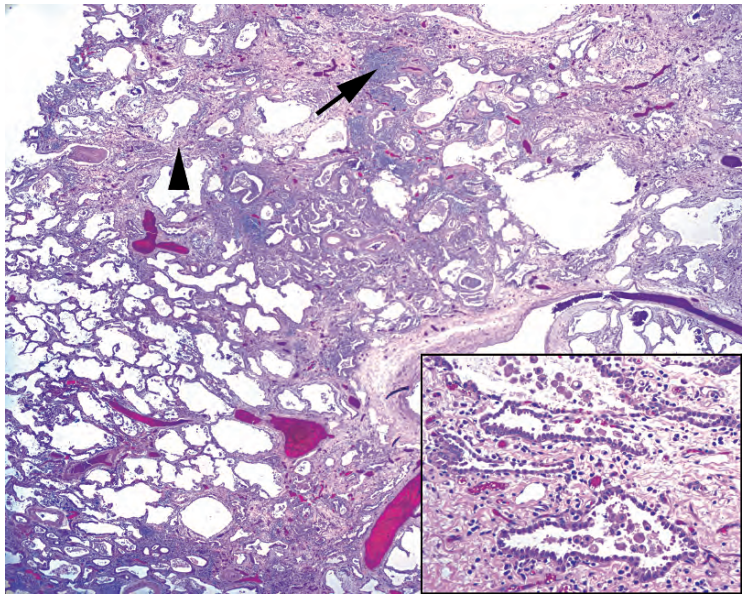

Figure 22.6. Usual interstitial pneumonia. The interstitial spaces are thickened and fibrotic (arrowhead), and there is abundant chronic inflammation (arrow). **Inset**: The scarred down, irregularly shaped, residual alveolar spaces are lined with type II pneumocytes, which protrude into the lumen and may have atypical nuclei.

Allergic Disease

There are two forms of allergic response in the lung: IgE-mediated disease and cell-mediated hypersensitivity reactions. Diseases in the first category include asthma, allergic bronchopulmonary fungal disease, bronchocentric granulomatosis (allergy to *Aspergillus*), and the eosinophilic pneumonias.

The prototypical cell-mediated hypersensitivity disease is *extrinsic allergic alveolitis*. It can have many causes and many appearances. This includes all the "(undesirable-job-here)'s lung" and "(exotic-pet-name) fancier's lung" diseases (e.g., "formalin lung" and "lizard-lover's lung"). Eosinophils do *not* feature prominently in extrinsic allergic alveolitis. The classic histologic

triad includes (1) patchy chronic interstitial pneumonia, especially peribronchiolar; (2) poorly formed small nonnecrotizing granulomas; and (3) foci of BOOP.

Diseases of Smokers

Smokers get a spectrum of interstitial lung diseases, including desquamative interstitial pneumonitis (DIP), respiratory bronchiolitis, Langerhans cell histiocytosis, and probably usual interstitial pneumonia. They also get obstructive lung disease, which includes chronic bronchitis and emphysema. DIP is a disease process in which alveolar macrophages pack the alveoli; it is usually associated with smoking, but a DIP pattern may be seen in other processes as well.

Note that Langerhans cell histiocytosis, also called *eosinophilic granuloma*, does not have traditional granulomas and may not always have eosinophils. What it does have is collections of histiocytes, identified by their pale nuclei with folds and creases (or by immunostains). This disease may occur systemically in the pediatric population, but in adults (which are 50% of cases) it is an isolated pulmonary disease of smokers.

Neoplastic Lung

Dysplasia and Carcinoma In Situ

The terms *dysplasia* and *carcinoma in situ* are not often used in pulmonary pathology. There are at least two types of epithelium that can be evaluated, the respiratory (columnar) and the squamous metaplastic. For the respiratory epithelium, the presence of cilia is a reassuring sign that all is well (Figure 22.7). However, chronically injured or irritated airspaces can get type II cell hyperplasia. On the slide, this appears as plump cuboidal to columnar eosinophilic cells, with enlarged nuclei, lining the airspaces. If this occurs as a prominent change within a small focus (less than 10 mm), it is analogous to dysplasia and is called *atypical adenomatoid hyperplasia* (Figure 22.8). Presumably these foci can go on to become bronchioloalveolar carcinoma, which is essentially adenocarcinoma in situ. Like dysplasia in other organs, these processes can be multifocal.

For squamous epithelia, although squamous dysplasia exists and is analogous to other organs, in practice it is not often caught on biopsy. Similarly, squamous carcinoma in situ

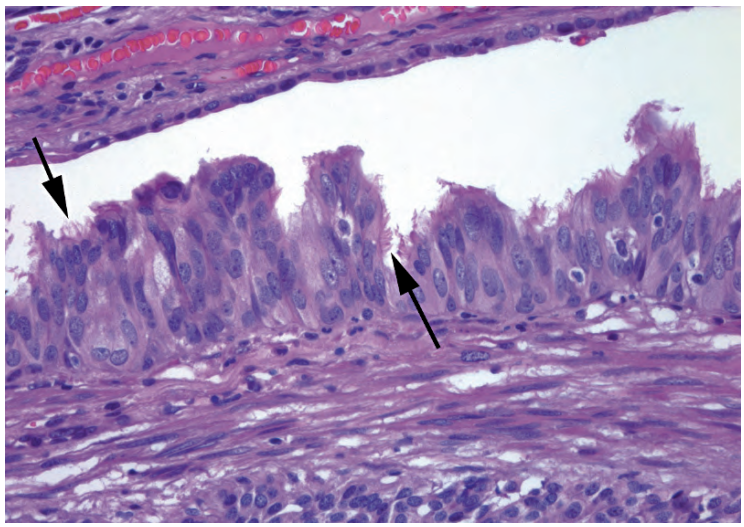

Figure 22.7. Reactive bronchial epithelium overlying a carcinoid tumor. Although the epithelium is very proliferative and has enlarged and crowded nuclei, the presence of cilia (arrows) indicates that these cells are benign.

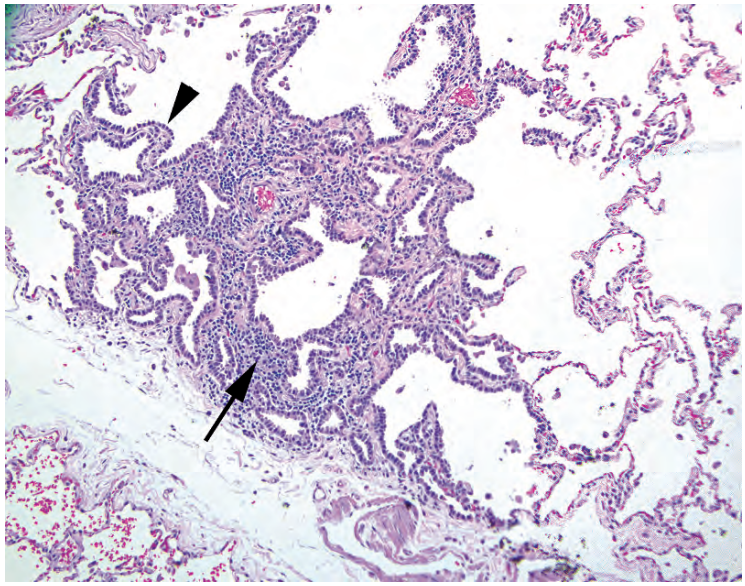

Figure 22.8. Atypical adenomatoid hyperplasia. In this tiny, limited focus, there is interstitial inflammation (arrow) and prominent type II hyperplasia (arrowhead). The adjacent alveolar walls are unremarkable.

exists in the bronchi just as in the larynx or oropharynx but is usually seen at the periphery of squamous cancers instead of as the sole finding in a biopsy specimen.

Carcinoma

Most lung biopsies in the neoplastic category are performed because a mass lesion was detected on radiology. Dysplasia and carcinoma in situ generally are not mass forming, so once you have ruled out a granulomatous process (those can form nodules), you are trying to identify the neoplasm. The most common lesions are discussed below. However, keep in mind than in lung, most tumors are a mix of tumor types or variants (pluripotent stem cells?), so you must sample well, name the tumors for their major components, and ignore small foci of different morphologies. *Non–small cell* is sort of a wastebasket term used to mean *adenocarcinoma* or *squamous cell carcinoma*, which can be grouped like that because their clinical behavior is similar.

Squamous carcinoma arises from squamous metaplasia, often in the major bronchi, and therefore is often central or hilar. The most recognizable form is the well to moderately differentiated keratinizing variety, with its pink, dense cytoplasm, keratin whorls, and distinct cell borders (Figure 22.9). It is graded on the typical well, moderately, or poorly differentiated scale. However, there are trickier variants, including the following:

- Nonkeratinizing
- Basaloid: blue and palisading, with a dense syncytial look (see Figure 22.9)
- Small cell: similar to small cell neuroendocrine, but with uglier nuclei and no neuroendocrine staining
- Spindle cell or sarcomatoid: densely cellular spindly pattern, resembling a sarcoma (Figure 22.10)
- Clear cell (adenocarcinoma can also have clear cells)
- Intrabronchial papillary: architecture like a papilloma, but malignant

Adenocarcinoma arises from multiple cell types and therefore can vary in morphology. Patterns include acinar, tubular, papillary, and solid, and they may be mucinous or nonmucinous. If you see gland formation or mucin production, it is almost certainly adenocarcinoma (Figure 22.11), and then you must decide if it is primary or metastatic.

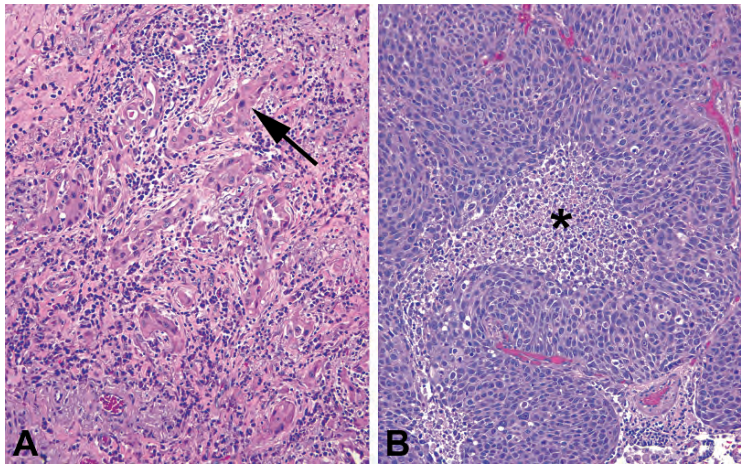

FIGURE 22.9. Squamous cell carcinoma. **(A)** Moderately differentiated squamous cell carcinoma, with irregular nests of cells with highly pleomorphic nuclei and bright pink, dense cytoplasm (arrow). Keratin pearls may also be seen in more well-differentiated tumors. **(B)** Basaloid squamous cell carcinoma, with rounded nests of very blue tumor cells with high nuclear to cytoplasmic ratio and a high mitotic rate. Central necrosis (asterisk) is common.

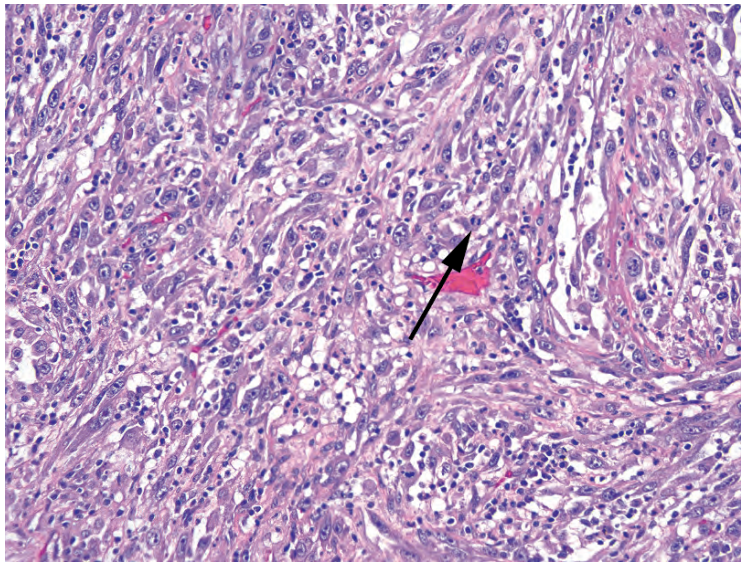

FIGURE 22.10. Sarcomatoid carcinoma. Sheets of spindled cells with large nuclei and prominent nucleoli are visible. Mitoses (arrow) are common. These cells should be positive for cytokeratin stains, confirming their epithelial origin.

As described earlier, *bronchioloalveolar carcinoma* (BAC) is the in situ form of adenocarcinoma. BAC in pure form appears to have a better prognosis than other non–small cell cancers, but, surprisingly, it still behaves, and is managed like, a full-fledged carcinoma. BAC may be mucinous (probably arising from goblet cell metaplasia) or nonmucinous. Of the two, the nonmucinous type has a better prognosis and is more often solitary.

BAC takes the form of columnar and usually eosinophilic cells growing along the bronchial and alveolar walls, outlining the structure of the airspaces (Figure 22.12). By definition, there must not be evidence of stromal invasion (irregularly shaped back-to-back glands, single cells, desmoplasia). BAC is often found at the periphery of invasive tumors, so this diagnosis should

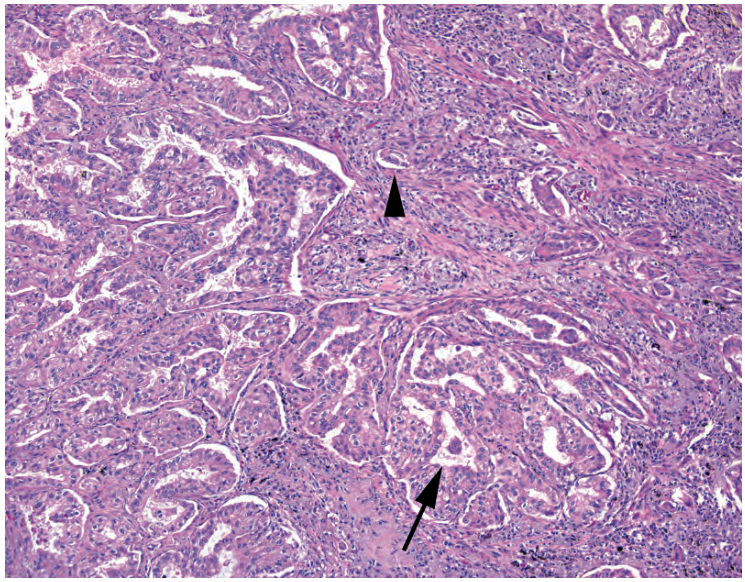

Figure 22.11. Adenocarcinoma. In some areas this tumor is forming cribriform glandular spaces (arrow), and in others small malignant glands or single cells are seen embedded in a desmoplastic stroma (arrowhead), confirming invasion.

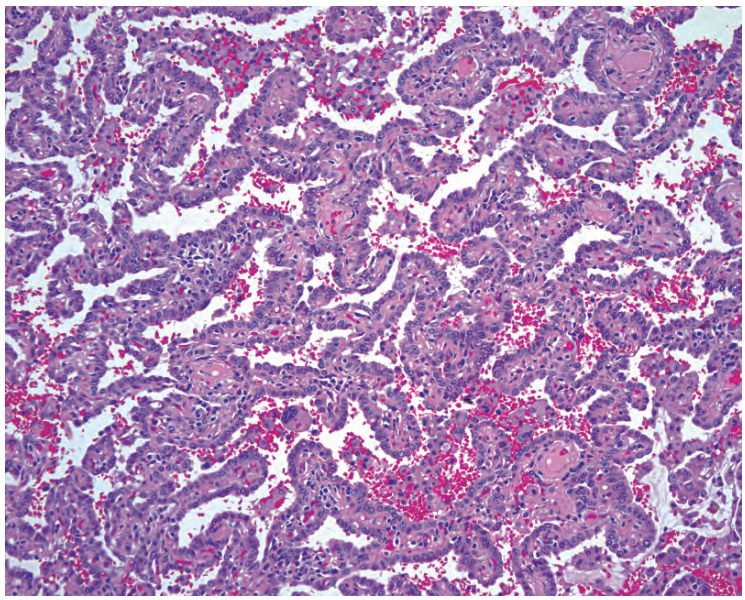

Figure 22.12. Bronchoalveolar carcinoma. The malignant cells line the alveolar walls but do not invade the stroma.

not be made on a biopsy specimen or frozen tissue or until the entire tumor has been sampled. This rule applies to most "improved-prognosis variant" tumors in pathology: you had better not label something as a good-prognosis tumor unless the entire lesion is of that type. A "BAC pattern" refers to a growth pattern of an invasive tumor that mimics BAC.

Large cell undifferentiated carcinoma arises when adenocarcinoma dedifferentiates into a very ugly tumor with no recognizable glandular features. It can also acquire pleomorphic or giant cell features. Another variety is the lymphoepithelioma-like carcinoma (scattered large

malignant cells in a sea of lymphocytes). Neuroendocrine carcinoma can have large-cell morphology; this is discussed below.

Neuroendocrine Tumors

The neuroendocrine spectrum is broad and confusing in the lung. Rosai's *Surgical Pathology* has a nice categorization of the tumor types, which includes the following:

- *Carcinoid*: A carcinoid is a well-differentiated (but not benign) neoplasm with classic neuroendocrine features, including epithelial-to-spindled architecture, regular round nuclei with fine chromatin, and no nucleoli (Figure 22.13). Despite the appearance, carcinoids can metastasize to lymph nodes.
- *Atypical carcinoid*: Atypical carcinoids are carcinoids with (1) increased mitoses, 2–10 per 10 high-power fields; (2) hyperchromatic nuclei; or (3) necrosis.
- *Small cell carcinoma*: Small cell carcinoma is a high-grade neuroendocrine neoplasm with small cell morphology, including solid-to-trabecular-to-tubular patterns, hyperchromatic finely granular (denim-blue) nuclei, no nucleoli, syncytial appearance with nuclear molding, mitoses/apoptosis/necrosis, and streaming crush artifact (Figure 22.14). Small cell carcinoma may be found in combination with other carcinomas.
- *Large cell neuroendocrine*: A large cell neuroendocrine tumor is a high-grade neuroendocrine neoplasm with some neuroendocrine features, either architectural or nuclear, and positive neuroendocrine immunostains. Note that the "large cell" refers to the presence of cytoplasm, not larger nuclei per se.
- *Non-small cell carcinoma with neuroendocrine features*: Per Rosai, this lesion looks like non–small cell by any criteria, but you happen to accidentally demonstrate that it is chromogranin positive.

Other Lesions (Incomplete Listing)

- Hamartoma: A hamartoma is a tumor-like mass composed of a disorganized mixture of the normal elements found in that organ. It is not clonal and therefore not really a neoplasm. In the lung, these are often masses of cartilage, fat, smooth muscle, and epithelium.

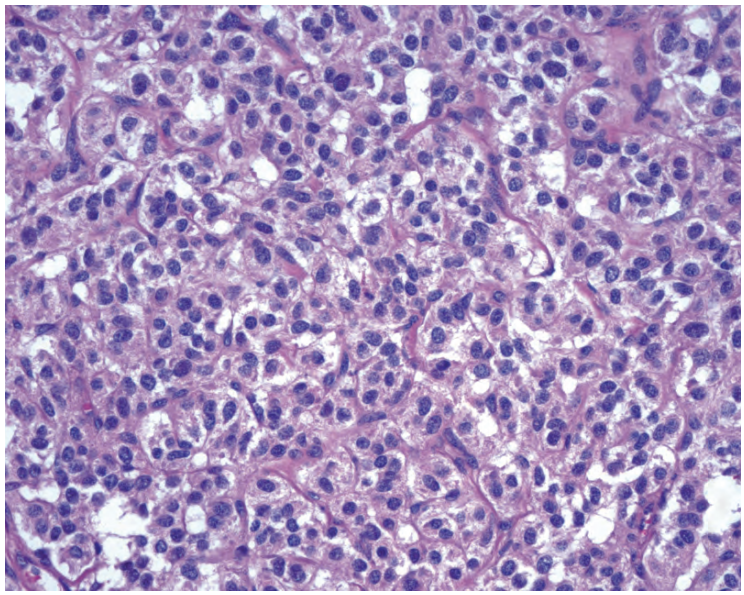

Figure 22.13. Carcinoid. This high-power view of an intrabronchial carcinoid shows a nested and trabecular pattern of cells with oval nuclei and typical "neuroendocrine" chromatin, meaning finely textured and speckled, without nucleoli or prominent nuclear membranes.

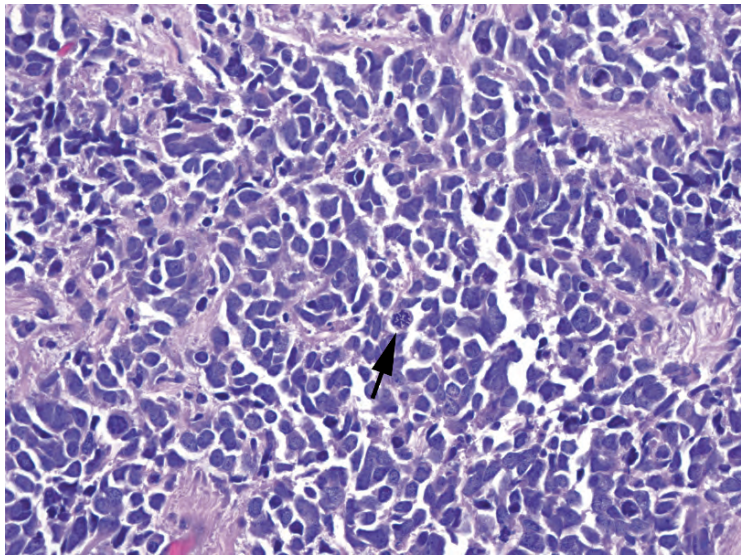

Figure 22.14. Small cell carcinoma. Sheets of nuclei appear molded together with interlocking shapes due to the near absence of cytoplasm. The chromatin, like low-grade neuroendocrine neoplasms, is uniform and lacks nucleoli. Necrosis and mitoses (arrow) are common.

- Salivary neoplasms: The seromucinous glands around the bronchi can give rise to any of the traditional salivary gland neoplasms.
- Carcinosarcoma: Carcinosarcoma is a truly biphasic malignant lesion, with a recognizable epithelial component (carcinoma) and a separate recognized form of sarcoma, such as osteosarcoma or chondrosarcoma. This is different from the sarcomatoid carcinoma, which is a pure carcinoma that has acquired spindle cell morphology.
- Pulmonary blastoma: Pulmonary blastoma is a form of carcinosarcoma in adults in which the epithelial component resembles fetal lung and the stromal component may be composed of adult-type sarcomas or immature mesenchymal tissue.
- Pleuropulmonary blastoma: Pleuropulmonary blastoma is an embryonal-type sarcoma of infancy, intrathoracic but often extrapulmonary, which may have cartilage and rhabdomyoblastic elements but not a carcinoma component.

23 Thyroid

The thyroid has two basic cell types: the follicular epithelium (TTF-1 and thyroglobulin positive) and the C cells (TTF-1, neuroendocrine-marker, and calcitonin positive; thyroglobulin negative). Normal follicular epithelium is low cuboidal. The stroma or interstitium is scant but highly vascular.

Inflammatory diseases of the thyroid are rarely seen in surgical pathology, with a few exceptions detailed in this chapter. Conceptually, they can be classified by type of response:

- Acute inflammation and necrosis: acute thyroiditis
- Foreign body giant cells and lymphocytes, diffuse: subacute thyroiditis (de Quervain's syndrome)
- Histiocytes, lymphocytes, and rare giant cells, focal: palpation thyroiditis (a reaction to physical trauma, not a primary inflammatory disease)
- Lymphocytic infiltrate with germinal centers: lymphocytic thyroiditis or Hashimoto's thyroiditis
- Dense fibrosis and chronic inflammation: sclerosing Hashimoto's versus fibrosing thyroiditis (Riedel's, a very rare entity)

Lymphocytic thyroiditis is a descriptive term implying a generalized lymphocytic infiltrate. The term *Hashimoto's thyroiditis* refers to an autoimmune process attacking the thyroid, and it is characterized by the following:

- Prominent lymphoplasmacytic infiltrate with germinal center formation (Figure 23.1)
- Small, atrophic follicles with Hurthle cell change (oncocytic change)

Scattered nuclear atypia may be seen in this setting, including large hyperchromatic Hurthle cell nuclei, as well as areas of nuclear clearing and pleomorphism that can simulate papillary carcinoma. Therefore, be cautious about diagnosing papillary carcinoma in the setting of lymphocytic thyroiditis. *However*, these patients can also get papillary carcinoma!

Graves' disease (diffuse toxic hyperplasia) is a hyperplastic, hyperthyroid condition in which autoantibodies stimulate the thyroid-stimulating hormone receptor to produce excess thyroid hormone. In treated form, more commonly seen in pathology, the follicles are large and distended, with prominent papillary infoldings (Figure 23.2). The papillary architecture can become florid, but the nuclear features are not those of papillary carcinoma (discussed later). Scalloping of the colloid is prominent. In untreated Graves' disease, on the other hand, the thyroid is highly cellular with minimal colloid.

Goiter is a nonspecific term for enlargement (hyperplasia) of the thyroid but is often used to refer to the nodular enlargement of the thyroid due to iodine deficiency (endemic goiter) or enzyme defects (sporadic goiter). *Multinodular hyperplasia* may be sampled by fine-needle aspiration (FNA) if a single nodule becomes dominant and suspicious, or the whole gland may be removed for cosmetic or physiologic reasons. The nodules usually fall on the colloid nodule-to-follicular adenoma spectrum (see later).

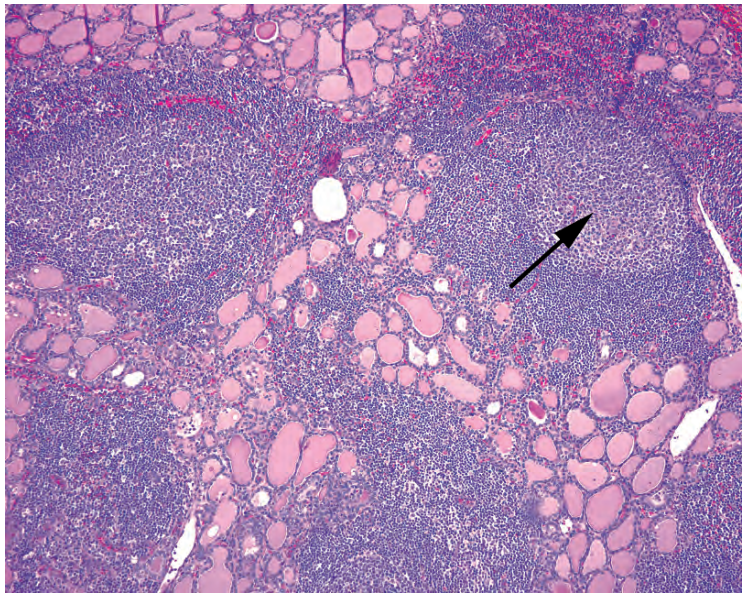

Figure 23.1. Hashimoto's thyroiditis. The thyroid follicles are displaced by germinal centers (arrow).

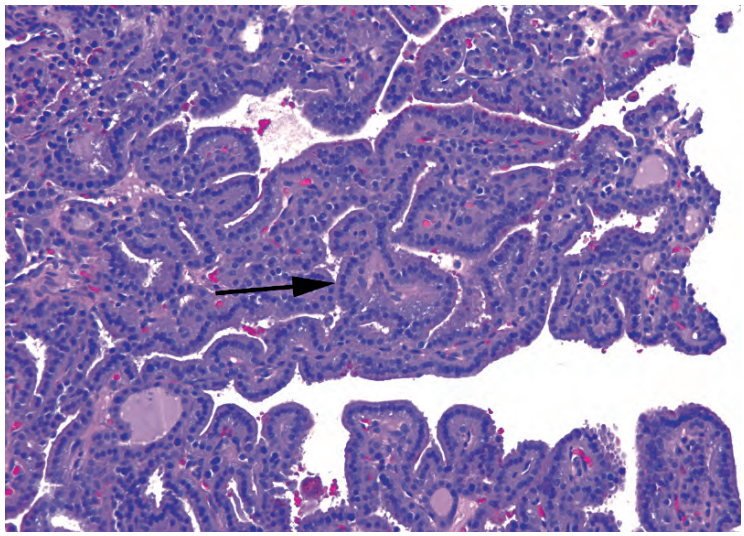

Figure 23.2. Graves' disease with papillary hyperplasia. These papillary formations are due to hyperplasia of the follicular epithelium. The follicular cells are round, fairly evenly spaced, and have dark uniform chromatin (arrow), similar to normal follicles.

The world of thyroid neoplasms can be broken down into several large categories. The first two categories arise from follicular epithelium, and they are divided in this chapter into two groups based on cytologic and nuclear features. The first category is made up of follicular-type cells that resemble normal thyroid follicular epithelium. This category includes Hurthle cells, which can be found in nonneoplastic thyroid. The second major category is the papillary carcinoma group, of which there are many variants; they have in common a set of diagnostic nuclear features. The third category of neoplasms arises from the *neuroendocrine* or C cell component of the thyroid; medullary carcinoma is the main entity in this group. Table 23.1 summarizes the architectural and cytologic features of thyroid neoplasms.

Follicular-Type Lesions

Follicular-type cells are notable for their uniformity. The nuclei tend to be round and monotonous, although they may be enlarged compared with normal thyroid. The overall impression is that of a regular array of cells, without crowded, overlapping, or irregular nuclei (Figure 23.3). The cells should respect each others' personal space, so to speak. The chromatin should be even and smooth, not cleared out, coarse, or chunky.

Colloid nodule, adenomatoid nodule, and *follicular adenoma* all describe a spectrum of hyperplastic to neoplastic lesions composed of a nodular cluster of follicular epithelium. This area is somewhat confusing as the same lesion may get different names depending on whether it is seen by FNA or on resection. A *colloid nodule* is a hyperplastic nodule of large distended follicles in which the ratio of colloid to cells is high (a key finding on FNA). A *follicular adenoma* is defined as a solitary encapsulated nodule with compression of the surrounding thyroid and is usually composed of small microfollicles with scant colloid (a low colloid to cell ratio; Figure 23.4). This lesion, seen on FNA, is called a *follicular neoplasm*, as follicular

Table 23.1. Summary of architectural and cytologic features of thyroid neoplasms.

	Macro- or normofollicular nodule	Microfollicular nodule	Papillary pattern	Solid or nested growth
"Follicular" nuclei	Hyperplastic nodule or Follicular adenoma	Follicular adenoma/ carcinoma	Graves' disease	Insular carcinoma
Hurthle cells	Hurthle cell adenoma	Hurthle cell adenoma/ carcinoma	Oncocytic variant of papillary carcinoma	
"Papillary" nuclei	Follicular variant of papillary carcinoma	Follicular variant of papillary carcinoma	Papillary carcinoma	
Pleomorphic cells				Anaplastic carcinoma
Neuroendocrine nuclei				Medullary carcinoma

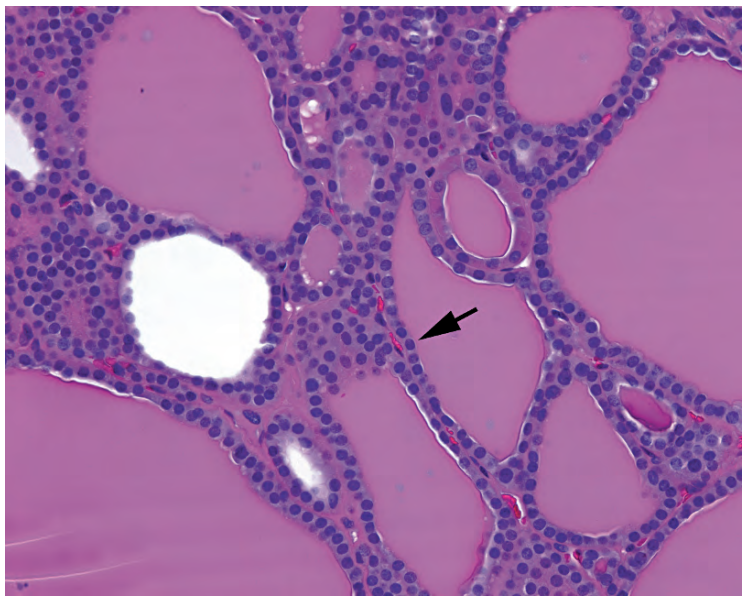

Figure 23.3. Follicular cells. Normal follicular epithelium has round uniform nuclei that tend not to overlap or crowd each other (arrow). This field is a combination of large and small follicles full of colloid and could represent normal thyroid, nodular hyperplasia, or a follicular neoplasm.

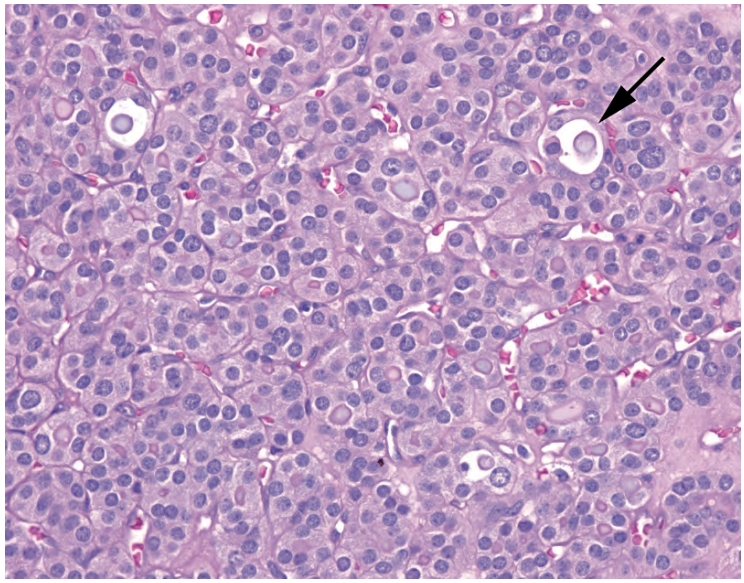

FIGURE 23.4. Follicular adenoma. This field shows a microfollicular pattern in a follicular adenoma. The capsule is not seen here. The neoplasm is composed of tightly packed small follicles (arrow) with round nuclei that, like normal follicular epithelium (see Figure 23.3), tend not to overlap or crowd. There are scattered enlarged nuclei, some with pale chromatin that should not be mistaken for true nuclear clearing.

adenoma and carcinoma can not be distinguished by FNA alone. Finally, there is the *adenomatoid nodule*, a hyperplastic lesion that has some features of the adenoma.

Before calling a lesion a follicular adenoma, however, you must submit and examine the entire capsule. Follicular carcinoma may appear histologically similar to adenoma but for the diagnostic capsular or vascular invasion. This is why you cannot make the distinction by FNA alone. You should also exclude the follicular variant of papillary carcinoma (discussed later).

Hurthle cell adenoma is very similar to a follicular adenoma in concept except the cells are large pink oncocytes with round nuclei (Hurthle cell change; Figure 23.5). Nucleoli may be prominent, and the nuclei may appear very enlarged or irregular in shape, unlike in follicular adenoma. As with follicular neoplasms, evaluating the capsule is key to calling it benign or malignant.

The defining feature of a *follicular carcinoma* (or Hurthle cell carcinoma) is the presence of capsular or vascular invasion, so examination of the capsule is critical. Atypia and necrosis, while seen in follicular carcinoma, are not sufficient to make the diagnosis.* Capsular invasion is a controversial area, and experts disagree on the exact criteria that define it; however, a mushrooming growth of tumor through the capsule is accepted by most. Vascular invasion must be found within the capsule itself or outside the capsule. The tumor deposit should be visibly attached to the vessel wall (Figure 23.6).

Follicular carcinoma comes in two strengths: minimally invasive (where you have to struggle to find the diagnostic vascular invasion) and widely invasive (where you have to dissect it off the adherent neck structures). It is not associated with radiation or thyroiditis, unlike papillary carcinoma. It spreads via the blood to lung and bone.

Insular carcinoma is rarely diagnosed and can be thought of as a poorly differentiated carcinoma. The cells grow in sheets and cords (insular pattern; Figure 23.7) and on high power resemble the round and uniform cells of follicular carcinoma. Pleomorphism is not a typical feature here, but mitoses, necrosis, vascular invasion, and infiltrative growth *are* common.

**Random pearl*: In this, the thyroid is like most other neuroendocrine organs, including parathyroid, adrenal, and pituitary. The diagnosis of malignancy is not based on atypia, which can be seen in hyperplastic conditions, but on capsular/vascular invasion or metastases.

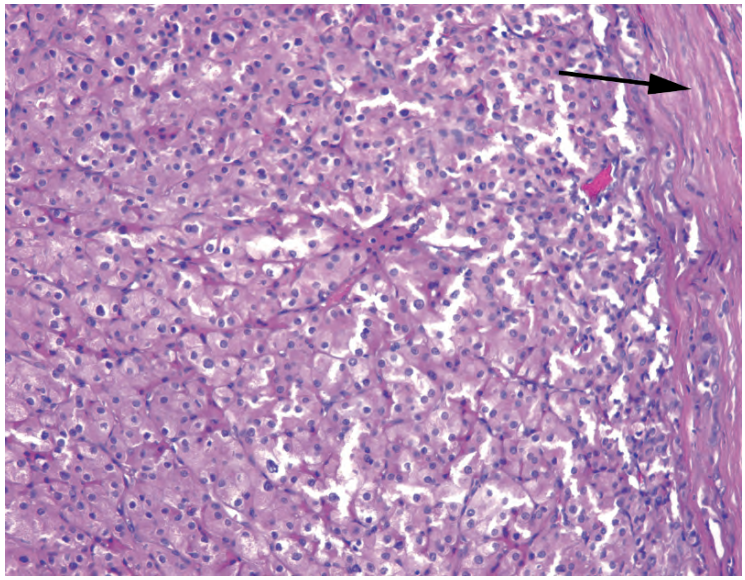

Figure 23.5. Hurthle cell adenoma. Like follicular adenomas, there is a thick fibrous capsule surrounding the neoplasm (arrow). In a Hurthle cell adenoma, the cells have abundant pink cytoplasm, and, although the nuclei are still overall round and nonoverlapping, there is increased nuclear atypia in the form of some prominent nucleoli and irregular nuclear shapes.

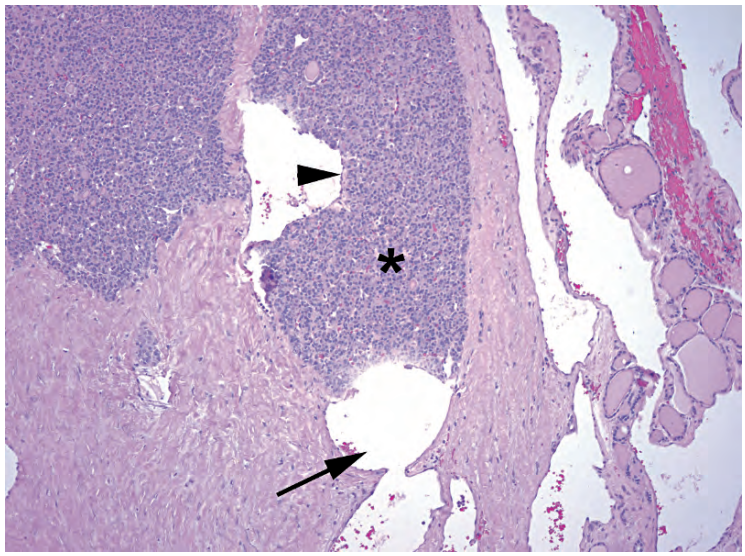

Figure 23.6. Follicular carcinoma. The neoplasm here resembles a follicular adenoma at low power, with a dense microfollicular pattern and a thick capsule. However, there is vascular invasion in the capsule, diagnostic of follicular carcinoma. A tumor plug (asterisk) is seen in the lumen of a large vessel (arrow). The surface of the tumor plug becomes endothelialized (arrowhead).

Papillary Carcinoma

Papillary carcinoma (there is no papillary adenoma), despite the name, may come with or without the papillae. The diagnosis actually rests on the nuclear features, which are consistent across variant types. The nuclear features are as follows:

- Chromatin is cleared out (resembling orphan Annie eyes). This imparts a characteristic low-power look to the lesion; the cells stand out as crisp and pale, almost glittery or glassy (Figure 23.8). It is an artifact of formalin.

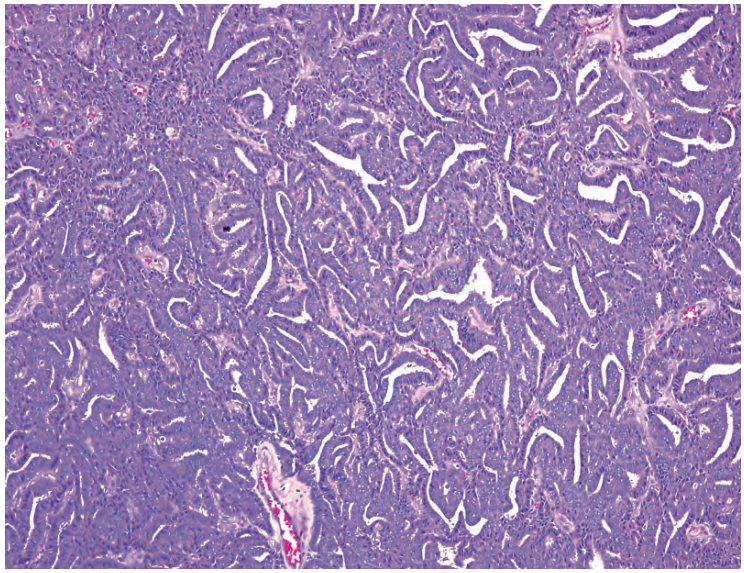

FIGURE 23.7. Insular carcinoma. Instead of microfollicles, the tumor has acquired a pattern of ribbons, cords, and slit-like spaces.

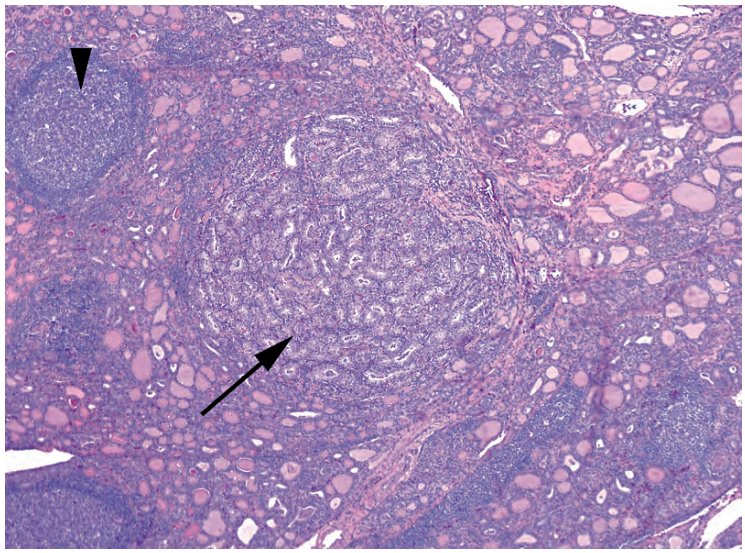

FIGURE 23.8. Papillary carcinoma, low power. The nuclear features of papillary carcinoma are eye-catching even at low power, as the clear nuclei give a translucent or glassy appearance to the tumor nodule (arrow). This is an example of an incidental microcarcinoma, arising in Hashimoto's thyroiditis (note germinal centers, arrowhead).

- Nuclei are overlapping, crowded, and pleomorphic. They often appear boxy and angular at low power, and you get the impression that too many nuclei have been stuffed into a single row (Figure 23.9); some are squeezed up and out of the row.
- Nuclear grooves (having a coffee bean appearance) are present.
- Nuclear pseudoinclusions (basically indentations of cytoplasm) are present.

Note that prominent nucleoli are not a feature of papillary carcinoma. Psammoma bodies are fairly specific for papillary carcinoma but are generally seen only in the context of papillary architecture. True psammoma bodies are dark purple, ringed like a tree, and usually found in the interstitium, not in follicles (Figure 23.10). There are several variants of papillary carcinoma.

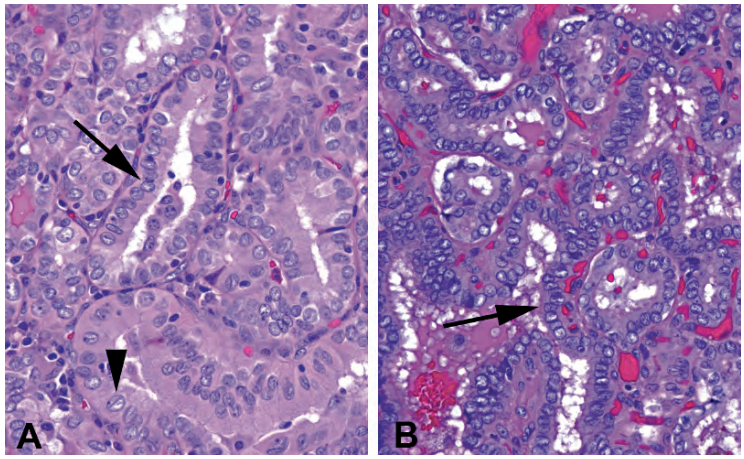

Figure 23.9. Papillary nuclei. **(A)** In this example, although the nuclear clearing is not striking, the presence of oval nuclei crowded into a row (arrow) suggests papillary carcinoma, as does the presence of nuclear grooves (arrowhead). Compare these nuclei to those of follicular epithelium see (Figure 23.3). **(B)** In this lesion the nuclear clearing is much more evident. However, the nuclei are still oval in shape and crammed together such that they mold to each other, popping up and out of their crowded rows (arrow).

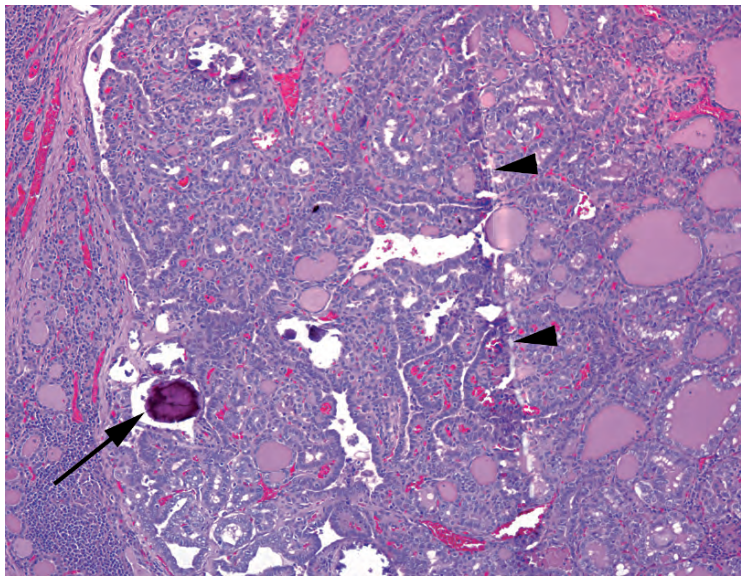

Figure 23.10. Psammoma body. This dense purple laminated calcification (arrow) is virtually diagnostic of papillary thyroid carcinoma in the thyroid or in a neck lymph node. Telltale scratches in the tissue section (arrowheads) often show where a psammoma body was dragged across the block during sectioning.

- Papillary microcarcinoma: Although histologically identical to papillary carcinoma, papillary microcarcinomas are less than 1 cm (by definition), usually incidentally discovered, and, if solitary, are considered clinically benign.
- Follicular: The follicular variant is a lesion with follicular architecture (no papillae) and papillary nuclei (Figure 23.11). It behaves like a papillary carcinoma and is now signed out as one. Differentiating between a follicular adenoma and a follicular variant of papillary carcinoma is no trivial task, as the nuclear changes can be patchy. Beware fixation artifact (which can produce nuclear clearing but not the other features) and lymphocytic thyroiditis (which produces reactive changes that can simulate papillary nuclei).

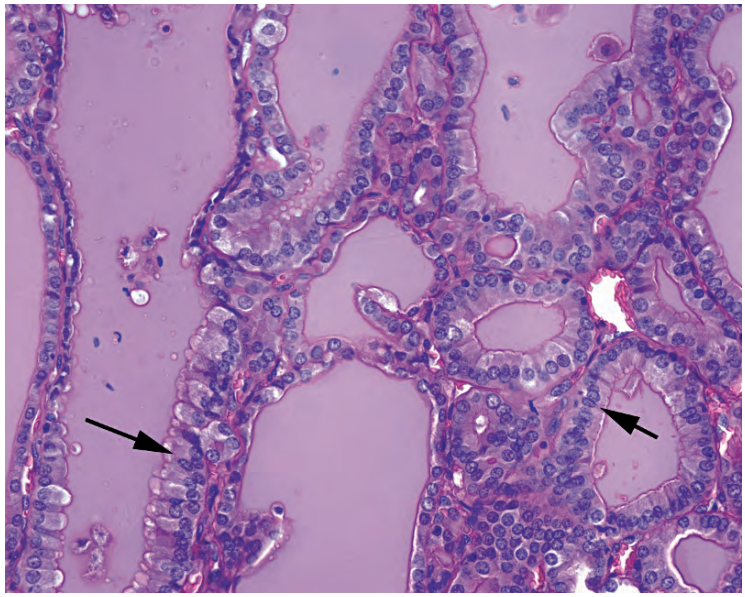

Figure 23.11. Follicular variant of papillary carcinoma. The architecture is that of a follicular adenoma, but the nuclei, oval in shape and crowded together (arrows), are those of papillary carcinoma.

- Diffuse sclerosing variant: Although rare, the diffuse sclerosing variant is important to recognize because of its worse prognosis. You can think of this variant as being widely infiltrative in its behavior, as opposed to discrete and mass-forming, and therefore more aggressive. The features include a desmoplastic or sclerotic stroma, squamous metaplasia, psammoma bodies, a dense lymphocytic infiltrate, and vascular invasion.
- Others: Other variants include tall cell, columnar cell, trabecular, cribriform, and cystic variants.

Anaplastic carcinoma is often a papillary carcinoma that has dedifferentiated (Figure 23.12). The tumor cells may appear as sheets of pleomorphic cells (truly undifferentiated), as nonkeratinizing squamous cell carcinoma (squamoid differentiation), or sarcomatoid. A background of papillary carcinoma is not uncommon, but anaplastic carcinoma may arise from other types of carcinoma as well.

The most important lessons of the papillary variants are these: not all papillary lesions are papillary carcinoma (Graves' disease, for example), and not all papillary carcinomas have papillary architecture (follicular variant, for example). Also, not all cleared out nuclei are papillary carcinoma. Beware fixation artifact (as discussed earlier), and have a very high threshold of suspicion for papillary carcinoma in the setting of lymphocytic (Hashimoto's) thyroiditis. A true carcinoma arising in Hashimoto's thyroiditis should stand out sharply from its neighbors, as in an uninflamed thyroid (see Figure 23.8). Varying degrees of nuclear clearing that come and go across the section are likely to be insignificant.

Papillary carcinomas are associated with radiation and (possibly) thyroiditis as risk factors; unlike follicular carcinoma, they spread to lymph nodes. The prognosis is usually excellent. Age is the most important prognostic factor (younger is better).

Neuroendocrine Lesions

Medullary carcinoma has features common to other neuroendocrine tumors; the growth may be nested or trabecular, and the cells range from epithelioid to spindled, with uniform finely speckled nuclei (Figure 23.13). At low power, or with poor histology, the sheet-like growth may simulate an anaplastic carcinoma. However, nuclear features or immunohistochemistry should easily tell the difference (calcitonin positivity and thyroglobulin negativity should do it). Medullary carcinomas produce prominent amyloid, which is Congo-red positive.

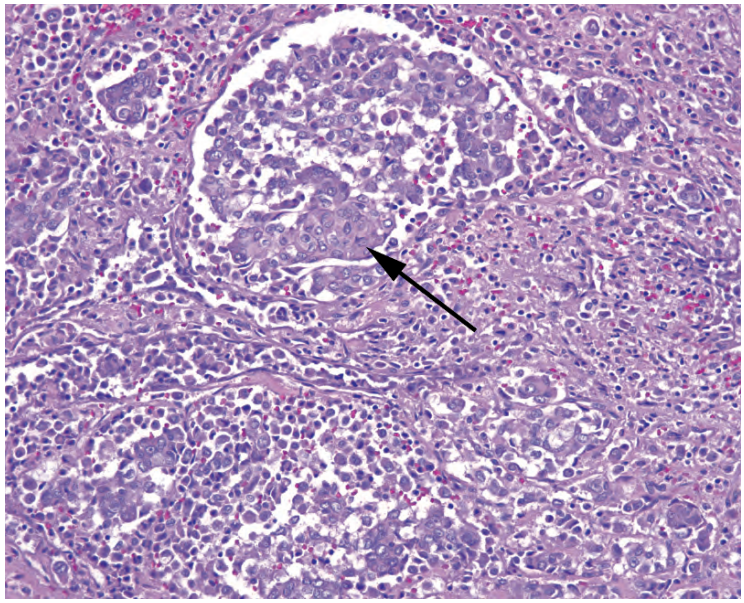

Figure 23.12. Anaplastic carcinoma. Nests and sheets of poorly differentiated carcinoma, some areas with a squamoid appearance (arrow).

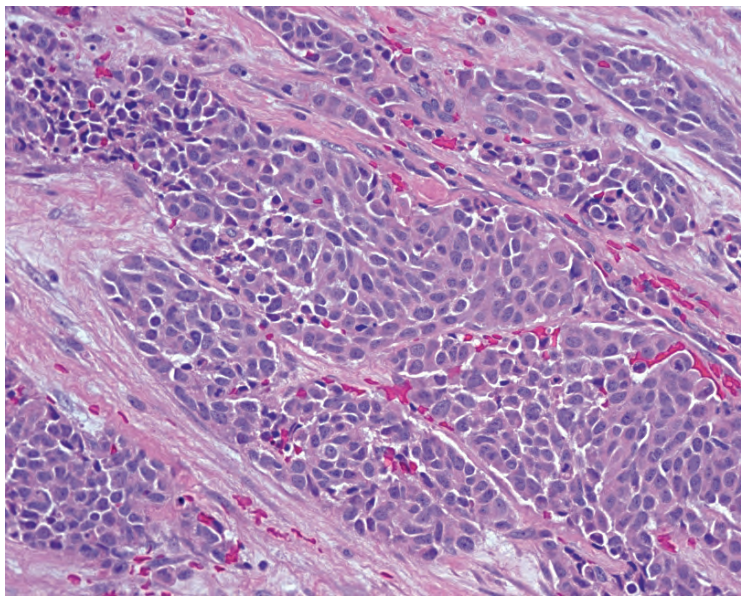

Figure 23.13. Medullary carcinoma. Although the pattern of infiltrative nests of cells may resemble anaplastic carcinoma, the nuclei are much more bland, with pale, finely speckled, neuroendocrine-type chromatin.

Cystic Nonneoplastic Lesions of the Neck

While not thyroid lesions, cystic nonneoplastic lesions of the neck are included here as they are commonly seen in surgical pathology and are sometimes mistaken clinically for a thyroid nodule. Such lesions include the following:

Thyroglossal duct cyst: a midline structure (as are the thyroid and the tongue) consisting of a cyst lined by ciliated epithelium and thyroid follicles

Branchial cleft cyst: an anterolateral structure (as are the branchial clefts) that looks somewhat tonsillar: squamous, columnar, or ciliated epithelium with a dense underlying lymphocytic infiltrate (*not* bronchial [i.e., lung] or brachial [i.e., arm])

24 Neuroendocrine Neoplasms

Natasha Rekhtman, MD, PhD*

General Definitions

The subject of neuroendocrine neoplasms, starting with the definition of what *neuroendocrine* means, is thoroughly confusing to the beginner. This chapter reviews the basic concepts and definitions pertaining to this subject.

Let us start with a definition of *neuroendocrine*. As the term implies, there are two components: "neuro" and "endocrine." The "endocrine" quality refers to the secretory nature of neuroendocrine cells: they produce and secrete peptides and amines. The "neuro" quality refers to their ultrastructural similarity to neurons: neuroendocrine cells store their secretory products in granules (i.e., dense-core granules), which bear resemblance to synaptic vesicles. Neuroendocrine cells are different from neurons structurally (no processes) and by the fact that the secretory mode is paracrine rather than synaptic. Also note that not all that secretes is neuroendocrine: for example, thyroid and adrenal cortex are not neuroendocrine because their cells do not possess neurosecretory granules (they are simply endocrine). Thus, at the most basic level, neuroendocrine cells are defined as the presence of neurosecretory granules in nonneurons. Tumors derived from these cells have a characteristic "neuroendocrine morphology" and share expression of "neuroendocrine markers."

Neuroendocrine Markers

In the past, neurosecretory granules were identified by electron microscopy and special stains. Currently these methods have been completely supplanted by immunohistochemical markers. These are called *neuroendocrine markers* and they include synaptophysin (SYN), chromogranin (CHR), neural-specific enolase (NSE), and CD56 (SYN and CHR specifically recognize dense-core granules). Note that these markers also recognize true neurons and neuroblastic cells (primitive neurons).

Neuroendocrine Morphology

Morphologically, unlike adenocarcinoma or squamous cell carcinoma, there is no single feature that defines neuroendocrine neoplasms as a group. Instead, *neuroendocrine morphology* is defined by a constellation of several cytologic and architectural features:

- Neuroendocrine cytology
  - Overall nuclear uniformity/monotony with smooth nuclear contours (unlike typical adenocarcinomas or squamous carcinomas)
  - Evenly dispersed, finely speckled "salt and pepper" nuclear chromatin without prominent nucleoli (Figure 24.1)

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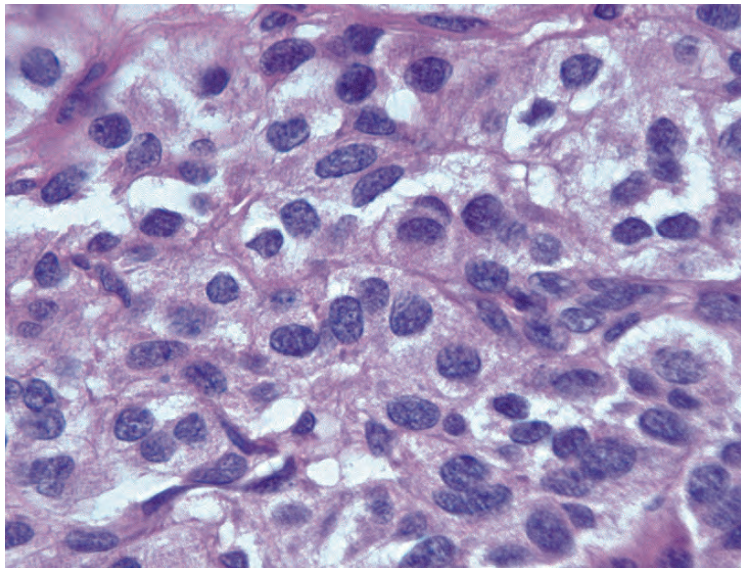

Figure 24.1. Classic neuroendocrine nuclei, with smooth oval nuclear borders, chromatin that is finely speckled throughout ("salt and pepper"), and no nucleoli.

  - Cytoplasmic granularity (corresponding to "neurosecretory granules"; variably evident)
- Neuroendocrine architecture
  - Formation of nests, rosettes, and ribbons/trabeculae
  - Prominent vascularity (in keeping with their secretory nature)

The appearance may be thought of vaguely recapitulating the normal neuroendocrine structures. Recall that nesting is a feature of normal adrenal medulla, and subtle trabecular/ribbon-like structures are present in the islets of Langerhans. The presence of rosettes is not easily explained by resemblance to normal neuroendocrine structures but may relate to their neural lineage, as many neuroglial tumors tend to form rosettes.

The morphology features listed apply to both low-grade (carcinoid, pancreatic neuroendocrine neoplasm, pheochromocytoma) and high-grade (small cell neuroendocrine carcinoma, Merkel cell carcinoma) neuroendocrine neoplasms. Whereas the defining neuroendocrine features are usually very obvious in low-grade neoplasms, they may be quite subtle to barely detectable in high-grade neuroendocrine neoplasms. Nevertheless, even when high grade, the overall uniformity and dispersed finely granular chromatin should be preserved, and at least a hint at neuroendocrine architecture (in the form of nesting, rosettes, or ribbons) is usually present.

What makes recognition of neuroendocrine neoplasms as a group so challenging is the fact that neuroendocrine morphology may be extremely subtle. For example, some neuroendocrine neoplasms rarely display typical neuroendocrine architecture (e.g., Merkel cell carcinoma typically has no nests, rosettes, or trabeculae). In addition, some neuroendocrine neoplasms frankly violate the basic rules of neuroendocrine morphology (e.g., higher grade neuroendocrine neoplasms, most notoriously large cell neuroendocrine carcinoma of the lung, do have prominent nucleoli). Therefore, diagnosis frequently hinges on recognition of subtle morphologic clues and confirmation with immunostains.

Neuroendocrine Cells and Neoplasms

What are the tissues that qualify as neuroendocrine? In addition to neuroendocrine organs (adrenal medulla and paraganglia), the neuroendocrine system includes the so-called diffuse neuroendocrine system. The term *diffuse neuroendocrine system* refers to neuroendocrine

Table 24.1. Major neuroendocrine cell types and corresponding neoplasms.

Location	Neuroendocrine cell type (secreted product)	Corresponding neoplasm	Cytokeratin expression
Intestine and appendix	EC cell (serotonin); D, L cells; other	Carcinoid	Positive
Gastric fundus	ECL cell (histamine)	Carcinoid	Positive
Gastric antrum, duodenum	G cell (gastrin)	Carcinoid	Positive
Lung	Kulchitsky (K) cell	Carcinoid	Positive
Pancreatic Islets of Langerhans	α cell (insulin)	Pancreatic endocrine neoplasm (islet cell tumor)	Positive
	β cell (glucagon)		
	δ cell (somatostatin)		
Thyroid	C cell (calcitonin)	Medullary carcinoma	Positive
Skin	Merkel cell	Merkel cell carcinoma	Positive
Anterior pituitary	Acidophil (PRL, GH)	Pituitary neoplasms	Positive
	Basophil (ACTH, TSH, FSH/LH)		
Parathyroid	(PTH)	Parathyroid neoplasms	Positive
Adrenal medulla and paraganglia	(Epinephrine, norepinephrine)	Pheochromocytoma	Negative
		Paraganglioma (i.e., extraadrenal pheochromocytoma)	
Adrenal medulla and other sites	Neuroblast (catecholamines, variable)	Neuroblastoma, PNET	Negative

ACTH, adrenocorticotropic hormone; EC, enterochromaffin; ECL, enterochromaffin-like; FSH, follicle-stimulating hormone; GH, growth hormone; LH, luteinizing hormone; PNET, primitive neuroectodermal tumors; PRL, prolactin; PTH, parathyroid hormone.

cells dispersed singly or in clusters throughout the body, including pancreas (islets of Langerhans), thyroid (C cells), lungs (Kulchitsky cells), skin (Merkel cells), gastrointestinal tract (many types), and so forth. In fact, neuroendocrine neoplasms may arise in any organ (e.g., prostate, breast, other). The broad definition of neuroendocrine cells also includes parathyroid and anterior pituitary glands. The only endocrine (peptide-secreting) organs excluded as clearly nonneuroendocrine are thyroid gland, adrenal cortex, and the steroid-producing cells of testes and ovaries. Table 24.1 summarizes the major neuroendocrine cell types and their corresponding neoplasms.

A Potentially Confusing Issue: Neuroectodermal Tumors

What about primitive neuroectodermal neoplasms (primitive neuroectodermal tumors and neuroblastoma)? Do these belong to the family of neuroendocrine neoplasms? These tumors do have neurosecretory-type granules (which are SYN/CHR$^+$), and the neuroblastoma secretes catecholamines, so technically they should qualify as neuroendocrine. However, these neoplasms also appear to possess the ultrastructural features of true primitive neurons (such as neurites). Therefore, together with medulloblastoma, they are classified under a separate heading of *primitive neural (neuroblastic/neuroectodermal) tumors*. One could think of these as neuroendocrine neoplasms with primitive neuronal phenotype.

Another Potentially Confusing Issue: Cytokeratin Expression in Neuroendocrine Neoplasms

Another potentially confusing issue with neuroendocrine neoplasms is their status as neural versus epithelial tissues. In the recent past, all neuroendocrine cells were erroneously thought to be neural (neural crest) derived; this is probably what you learned in medical school. However, it appears that neuroendocrine neoplasms actually fall into two groups: the "truly neural" group (pheochromocytoma/paraganglioma) and the "endoderm-derived/epithelial" group

(carcinoid, pancreatic endocrine neoplasm, small cell carcinoma, other). This distinction has practical implications: "neural" neuroendocrine neoplasms are cytokeratin negative, whereas "epithelial" neuroendocrine neoplasms are cytokeratin positive.

Some More (Potentially Confusing) Terminology

To complicate matters further, there are a number of terms that have been applied to neuroendocrine cells over the years. These terms are rarely in routine use today, but they may be encountered in the literature (or on the boards):

- *Amine precursor uptake and decarboxylase* (APUD) cells and *diffuse neuroendocrine system* are the terms for neuroendocrine cells scattered throughout the body (like enterochromaffin-like cells in the stomach). An *APUD-oma* is another term for carcinoid tumor.
- *Chromaffin* is a term applied to adrenal medulla because of its property to stain brown with chromic salts.
- *Enterochromaffin* refers to neuroendocrine cells of the intestine with similar properties, hence "entero" (gut).
- *Enterochromaffin-like* refers to histamine-secreting neuroendocrine cells of the gastric fundus.
- *Argentaffin* and *argyrophil* refer to the property of neuroendocrine cells to take up silver stains without or with a pretreatment step, respectively. Pretreatment gets more cells to stain. Fontana-Masson is a type of argentaffin stain (it also stains melanin), and Grimelius is a type of argyrophil stain. These stains are of historic interest only, because they have been supplanted by immunostains in practice.

Select Neuroendocrine Neoplasms

Neuroendocrine neoplasms encompass such a heterogeneous group of lesions that it may be difficult to see the common thread among them. This section attempts to highlight the common neuroendocrine qualities as well as organ-specific features of neuroendocrine neoplasms.

Note that neuroendocrine neoplasms (particularly low grade) frequently display random nuclear atypia, that is, *neuroendocrine-type atypia* or pleomorphism. Nuclei are smudgy and have bizarre shapes. Neuroendocrine atypia is degenerative in nature, probably owing to the slow growth rate of these neoplasms, and has no correlation with malignant potential. Neuroendocrine atypia is particularly prominent in pheochromocytoma and paraganglioma; do not mistake this for a feature of high-grade malignancy.

Expression of neuroendocrine markers is another defining feature of neuroendocrine neoplasms; expression is usually strong in low-grade lesions and may be weak/focal in high-grade lesions. Note that in the case of small cell carcinoma, diagnosis is based predominantly on morphology: if morphology is classic, expression of neuroendocrine markers is not required for diagnosis. The following discussion of specific neuroendocrine neoplasms emphasizes what constitutes their neuroendocrine qualities.

Well-Differentiated Neuroendocrine Neoplasm (Carcinoid)

This prototypical neuroendocrine tumor has all of the features listed earlier as the hallmarks of neuroendocrine differentiation, including finely speckled chromatin with *no* prominent nucleoli, uniform (monotonous) round nuclei with a smooth nuclear membrane, and frequently a plasmacytoid appearance (eccentrically placed nucleus). The architecture may be nests, rosettes, ribbons, or trabeculae. Delicate fibrovascular septae are characteristic. Neuroendocrine markers are usually strongly expressed.

Poorly Differentiated Neuroendocrine Carcinoma (Small Cell Carcinoma)

Despite the name, diagnosis is not based purely on size, but nuclear size is generally less than three lymphocytes in diameter. The chromatin is finely speckled but is also very dark/hyperchromatic, which may obscure the "salt and pepper" quality in a surgical specimen.

As in other neuroendocrine neoplasms, there are no prominent nucleoli. The unique features include nuclear molding, high nuclear to cytoplasmic ratio with very scant cytoplasm, numerous mitoses and apoptotic bodies, and frequent crush artifact with DNA streaming (known as the *Azzopardi phenomenon*). Nests, trabeculae, and rosettes are uncommon. The most reliable immunostain is CD56, but all may be positive. If morphology is classic, expression of neuroendocrine markers is not required for diagnosis.

Merkel Cell Carcinoma of Skin

Merkel cell carcinoma is a small round blue cell tumor. Cytology and architecture overlap with small cell carcinoma. As in small cell carcinoma, nuclear molding, crush artifact, and necrosis are usually present. Cytology is extremely high grade with numerous mitoses and apoptotic bodies (although Merkel cell carcinoma shows less molding than small cell carcinoma). Stains are usually required to distinguish the two.

Neuroendocrine morphology may be subtle, although neuroendocrine markers are reliably expressed. Hints at the neuroendocrine nature are overall nuclear monotony, despite the high nuclear grade. Although the "salt and pepper" quality of chromatin may be subtle at best, it does show dispersed granularity (so-called dusty look). Also, trabeculae and rosettes may be present (hence the former designation as trabecular carcinoma), although more commonly the pattern is diffuse.

In addition to neuroendocrine markers, Merkel cell carcinoma stains for neurofilament, which is normally a neuronal marker, and CK20 (in classic punctate perinuclear dots). In contrast, small cell carcinoma is negative for neurofilament and CK20 but is positive for TTF-1.

Medullary Carcinoma of Thyroid

Neuroendocrine cytology is as described for carcinoid; speckled chromatin may not be evident in surgical slides due to hyperchromasia but should be more apparent in cytology preparations. Plasmacytoid cytology is common. Neuroendocrine architectural features may be present. The unique features are the presence of amyloid and tendency to form large cellular islands.

Large Cell Neuroendocrine Carcinoma

Large cell neuroendocrine carcinoma is one of the neuroendocrine neoplasms that you would not guess had a neuroendocrine nature based on cytology (nuclei are vesicular, not salt and pepper, and have a single prominent nucleolus). Neuroendocrine classification is based on a subtle hint of neuroendocrine architecture (rosettes and nuclear palisading), expression of neuroendocrine markers, and ultrastructural demonstration of dense-core granules.

Pheochromocytoma (Adrenal)/Paraganglioma (Extraadrenal)

Classic neuroendocrine cytology may be barely discernible in a pheochromocytoma. Some nuclei are carcinoid-like in that they are uniform, round, and finely speckled, but many nuclei are more neuron-like by virtue of large size and single prominent nucleolus. The cytoplasm is abundant, granular, and "amphophilic" (lavender). Nuclear pseudoinclusions (cytoplasmic invaginations) are present in 30% of cases. Hyaline globules are common and, if present, distinguish pheochromocytomas from other adrenal neoplasms. Random nuclear atypia is common.

Although paragangliomas are equated with extraadrenal pheochromocytomas, the morphology is not identical. In paraganglioma, the nuclei are much more carcinoid-like. The architecture is nested and occasionally trabecular, but there are no rosettes. The nest pattern is referred to as *zellballen*, which in German literally means *cell balls*. The zellballen pattern is highlighted by S100 stain, which reacts with sustentacular/supportive cells outlining the nests (sustentacular cells are not visible on H&E stain).

Marker expression is identical in both tumors. Neuroendocrine markers are positive in the nests (chromaffin cells), and S100 highlights sustentacular cells. Unlike all other neuroendocrine neoplasms (e.g., carcinoid), these tumors are cytokeratin negative.

Neuroendocrine Differentiation in Other Types of Carcinoma

As discussed earlier, neuroendocrine neoplasms are diagnosed based on (1) morphology and (2) expression of neuroendocrine markers (electron microscopy and special stains are basically obsolete). If morphology and markers are concordant (which is usually the case), one can comfortably diagnose a neuroendocrine neoplasm. However, there are cases in which morphology and neuroendocrine marker expression are discordant. These distressing situations come in two varieties.

First, expression of neuroendocrine markers may be detected incidentally in an otherwise entirely nonneuroendocrine neoplasm (morphologically), such as a classic adenocarcinoma. In most organs, this is thought to represent a type of occult differentiation with no clear clinical significance. These lesions may be signed out as *Carcinoma with neuroendocrine differentiation by immunohistochemistry*.

Conversely, some high-grade neuroendocrine neoplasms, particularly small cell carcinoma, may express neuroendocrine markers only focally or not at all. Nevertheless, classic morphology trumps the lack of marker expression, once other small cell neoplasms have been excluded.

Note that the first scenario is different from finding small cell carcinoma as a component of another type of carcinoma, such as adenocarcinoma or squamous cell carcinoma. The latter situation is considered to be a form of dedifferentiation to a more primitive phenotype, which does carry a worse prognosis and a need for specific therapy. Such cases are signed out as *Mixed adenocarcinoma/small cell carcinoma* or *Adenocarcinoma with small cell component*.

Sign-Out Terminology for Neuroendocrine Neoplasms in Various Organs

A practical issue of note is that clinical behavior of low-grade neuroendocrine neoplasms (carcinoid, pancreatic endocrine neoplasm, pheochromocytoma) is notoriously difficult to predict based on histologic parameters. Generally, the only definitive sign of malignancy is the presence of metastases. This is why neuroendocrine neoplasms are not staged. However, there are a number of site-specific histologic features that are loosely predictive of a more aggressive phenotype. Given the uncertainty of clinical behavior, there is a range of sign-out terminology applied to neuroendocrine neoplasms (which can vary among institutions):

Primary lung neuroendocrine neoplasms may be signed out as the following:

Carcinoid tumor (low-grade neuroendocrine carcinoma): Typical neuroendocrine morphology is apparent, with <2 mitoses/10 high-power fields [hpf] and no necrosis.

Atypical carcinoid tumor (intermediate-grade neuroendocrine carcinoma): From 2 to 10 mitoses/10 hpf and/or focal necrosis are present.

Small cell (high-grade neuroendocrine) carcinoma: More than 10 mitoses/10 hpf, extensive necrosis, and specific small cell features are present.

Large cell (high-grade neuroendocrine) carcinoma: More than 10 mitoses/10 hpf, extensive necrosis, and specific features are present.

Primary gastrointestinal neuroendocrine neoplasms may be signed out as the following:

Carcinoid tumor: This indicates typical neuroendocrine morphology with no features of concern (e.g., size >1–2 cm, vascular invasion, necrosis, etc.).

Carcinoid tumor, *Malignant carcinoid tumor*, or *Well-differentiated neuroendocrine carcinoma*: Morphology is of typical carcinoid, but the tumor is behaving badly, such as already metastatic. The sign-out terminology is a contentious issue here (a matter of style). Some sign out a lesion as *carcinoid* irrespective of how malignant it behaves. Others advocate that if metastases are present, the lesion should be signed out as *malignant carcinoid* or *well-differentiated neuroendocrine carcinoma*. The third opinion is that the latter two categories should be used if *any* features of concern are present.

Poorly differentiated neuroendocrine carcinoma: The carcinoma morphologically is frankly high grade. Small cell and large cell neuroendocrine carcinomas are included here.

Primary pancreatic neuroendocrine neoplasms may be signed out as the following:

Well-differentiated pancreatic endocrine neoplasm (islet cell tumor): Typical neuroendocrine morphology is apparent, with no features of concern.

Well-differentiated malignant pancreatic endocrine neoplasm (malignant islet cell tumor): This designation is used if any one of the three definitively malignant features are present: (1) metastases, (2) large vessel vascular invasion, and/or (3) invasion of adjacent organs. Lymphatic invasion, large size, microscopically invasive border, and so forth are suspicions but not diagnostic of malignancy.

Poorly differentiated malignant pancreatic endocrine neoplasm (malignant islet cell tumor): morphologically a frankly high-grade carcinoma is present. Small cell and large cell neuroendocrine carcinoma are included here.

Note that pancreas and intestine do not have an "atypical" category corresponding to the "atypical carcinoid" category in the lung. Nevertheless, some pathologists do flag intestinal and pancreatic lesions as "atypical" based on the lung criteria (discussed earlier).

The issue of *metastatic neuroendocrine neoplasm of unknown origin* usually arises for hepatic neuroendocrine neoplasms. The differential diagnosis includes intestinal versus pancreatic metastases (primary hepatic neuroendocrine neoplasms are vanishingly rare). Intestinal carcinoids and pancreatic neuroendocrine neoplasms are histologically identical, and hormone expression is generally not reliable. Therefore clinical correlation is required. These lesions are signed out as "metastatic (well or poorly) differentiated neuroendocrine neoplasm (carcinoid vs. pancreatic endocrine carcinoma)".

One contentious issue is the use of the term *neoplasm* versus *carcinoma* as it pertains to neuroendocrine tumors that are displaying overtly malignant behavior. In principle, these tumors should be called *carcinomas* (i.e., malignant epithelial neoplasms). This terminology is indeed advocated by some authorities. Some authorities even advocate use of the term *low-grade neuroendocrine carcinoma* in place of *carcinoid* tumors to reflect the potential of any of these tumors to metastasize. In contrast, other authorities stress that the term *neoplasm* is preferable (regardless of clinical behavior or morphology) in order to draw a clear distinction between these tumors and typical carcinomas, such as pancreatic adenocarcinoma, which behave in a much more aggressive fashion than any neuroendocrine tumor.

25 Salivary Gland

Background

With the exception of the pleomorphic adenoma, salivary gland neoplasms are rare, so you will not see many during residency training. To make matters worse, there is a great deal of morphologic overlap in some of the tumors, and immunostains are not usually helpful in distinguishing them. Your goal, early in your training, should be to recognize the more classic forms of the major tumors and also to be able to create a short differential diagnosis for any given tumor. In this organ, with all the mimics and variants, it is extremely important to approach a specimen with the question, "What *else* could this be?"

Biopsies of the salivary gland are occasionally performed in search of Sjögren's syndrome; this is a complex diagnosis with specific criteria that must be met (see your favorite pathology textbook for that). Inflammatory lesions can also create a mass, such as chronic sialadenitis or a lymphoepithelial cyst.

Anatomy

There are three major and innumerable minor pairs of salivary glands. The largest, on the cheek, is the parotid, where most neoplasms arise. The smaller major glands are the sublingual and submandibular, under the tongue and jaw. In general, the smaller the gland, the higher the proportion of its neoplasms that are malignant. Salivary neoplasms can arise in virtually any part of the sinonasopharyngeal system.

Normal Histology

The first major cell type is the *secretory cell*. The salivary glands are composed of serous and mucinous secretory units and ducts (Figure 25.1). *Serous* cells are wedge shaped (like pie slices) and arranged in acini around ducts. They are full of blue to purple granules. *Mucinous* cells have basal nuclei and apical mucin, like goblet cells; these are also arranged in acinar formations. The parotid is primarily serous, the submandibular is mixed, and the sublingual is primarily mucinous.

The second major cell type is the *duct cell*. The duct system has three types of ducts: the terminal, or intercalated ducts; the intermediate-sized striated ducts; and the interlobular large ducts. Each has a different epithelium and is theoretically associated with different tumor types. The *intercalated ducts* are small profiles with low cuboidal epithelium, similar to a bile ductule (Figure 25.2). *Striated ducts* are more proximal and are larger, with pink columnar

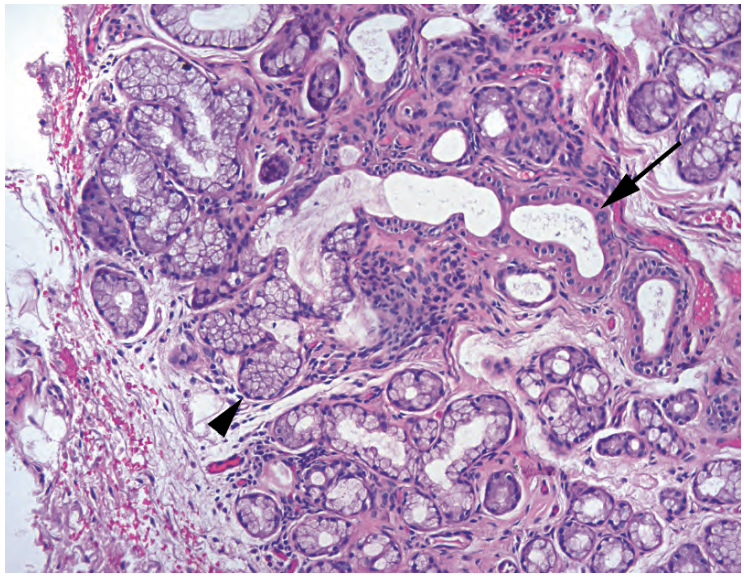

FIGURE 25.1. Normal salivary gland. In this example of mucinous salivary gland, the columnar secretory cells (arrowhead) form acini arranged around salivary ducts (arrow). Myoepithelial cells are not particularly visible on H&E stain.

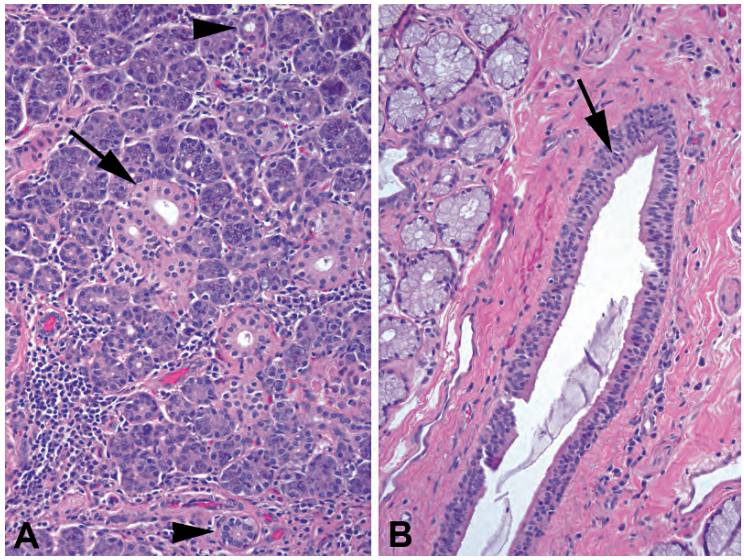

FIGURE 25.2. Types of ducts. **(A)** In the parotid, which has mainly serous glands, the terminal or intercalated ducts are visible as small tubules lined by cuboidal epithelium (arrowheads). The medium-sized striated ducts are more oncocytic in appearance, with abundant pink cytoplasm (arrow). **(B)** The large interlobular ducts have pseudostratified columnar epithelium (arrow), with occasional goblet cells, and become squamous at their junction with the gingival mucosa.

cells full of mitochondria and striated basal borders (hard to see). *Interlobular* or *excretory ducts* have pseudostratified columnar epithelium with or without goblet and squamous metaplasia. Different tumors have some morphologic similarity to these different ducts, which may help you keep all of the neoplasms straight.

The third cell major cell type is the *myoepithelial cell*. These cells, as in breast, surround acini and ducts. They are normally pale stellate cells with small nuclei and are very hard to identify in normal salivary gland. However, many neoplasms arise from the epithelial -myoepithelial

Table 25.1. Basic categories of the nine most common neoplasms.

	Probable cells of origin
Benign adenomas	
Pleomorphic adenoma (mixed tumor) and its end-of-the-spectrum variant, myoepithelioma	Epithelial–myoepithelial
Basal cell adenoma	Epithelial–myoepithelial
Warthin's tumor and oncocytoma	Striated duct cells
Low-grade malignant	
Mucoepidermoid carcinoma (low grade)	Interlobular duct cells
Polymorphous low-grade adenocarcinoma	Epithelial–myoepithelial
Acinic cell carcinoma	Serous acinar cells
Intermediate- to high-grade malignant	
Mucoepidermoid carcinoma (intermediate to high grade)	Interlobular duct cells
Adenoid cystic carcinoma	Epithelial–myoepithelial
Adenocarcinoma not otherwise specified (wastebasket of those adenocarcinomas that do not show specific differentiation)	Ducts

cell line or, more specifically, from cells that can differentiate into either line. This creates a diagnostic nightmare, because the myoepithelial cells alone can take four different forms: *spindled, plasmacytoid, epithelioid*, or *clear*. Therefore, you must recognize any of these variants as myoepithelial (their immunologic profiles change with their form, unfortunately) and lump some very different-looking tumors into the same basket. Table 25.1 lists the most common neoplasms.

Note that, in general, benign lesions are encapsulated, whereas malignant tumors are infiltrative, either as pushing fronts or as tendrils of cells (although mucoepidermoid carcinoma and acinic cell carcinoma can be deceptively well circumscribed). The first thing you should do when evaluating a salivary neoplasm is to study the periphery or capsule.

Neoplasms

This section describes the common neoplasms in order of how likely you are to see them, beginning with the most common.

Pleomorphic Adenoma (Benign Mixed Tumor)

Pleomorphic adenoma is a biphasic tumor with epithelial and myoepithelial (mesenchymal-like) components. It can occur anywhere but is very common in the parotid. The two key features to recognizing this lesion are a circumscribed, usually encapsulated tumor, and a mesenchymal-like component in the background (Figure 25.3). The mesenchymal-like stroma is often myxoid but may be chondroid or even osseous. The epithelial component can range from obviously epithelial to myoepithelial, so you may see well-defined ductular structures or pink to clear myoepithelial cells (in any of their four morphologies)—hence the designation *pleomorphic*. The individual cells, however, are notably *not* pleomorphic and in fact should be very bland (small, oval, pale nuclei).

The lesion not to miss is the polymorphous low-grade adenocarcinoma, which can look very similar but has an infiltrative periphery (see later). You should also examine the tumor for cytologically malignant cells, which may represent a *carcinoma ex-pleomorphic adenoma* (a pleomorphic adenoma gone bad).

The *myoepithelioma* is one end of the pleomorphic adenoma spectrum, with very little mesenchymal component and no ductular differentiation. As with a pleomorphic adenoma, it should be encapsulated and circumscribed. Immunostains can help you here, as myoepitheliomas should be positive for S100, cytokeratin, glial fibrillary acidic protein, and actin. *Basal cell adenoma* is also analogous to a pleomorphic adenoma, with no mesenchymal component, but with a population of basaloid cells.

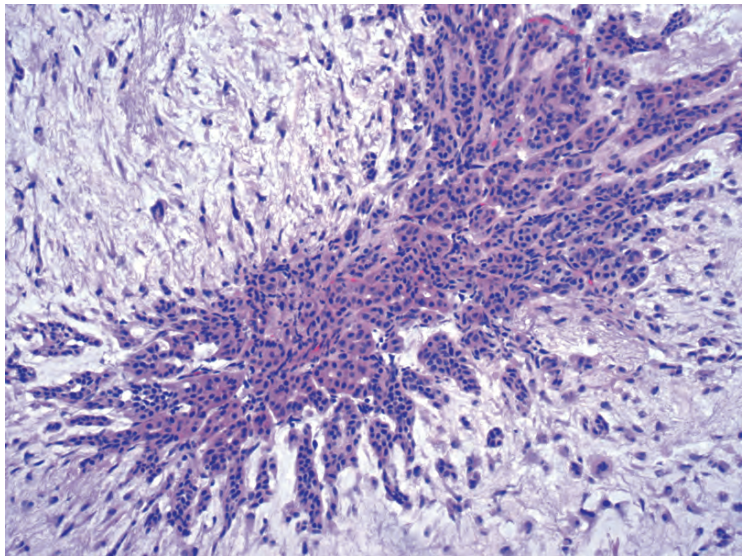

Figure 25.3. Pleomorphic adenoma. A cluster of cells is visible within the bluish myxoid stroma of a pleomorphic adenoma. The epithelial cells are small and cytologically benign, and they appear as small cords and tubules set within the stroma. The proportion of epithelial cells to stroma can vary widely.

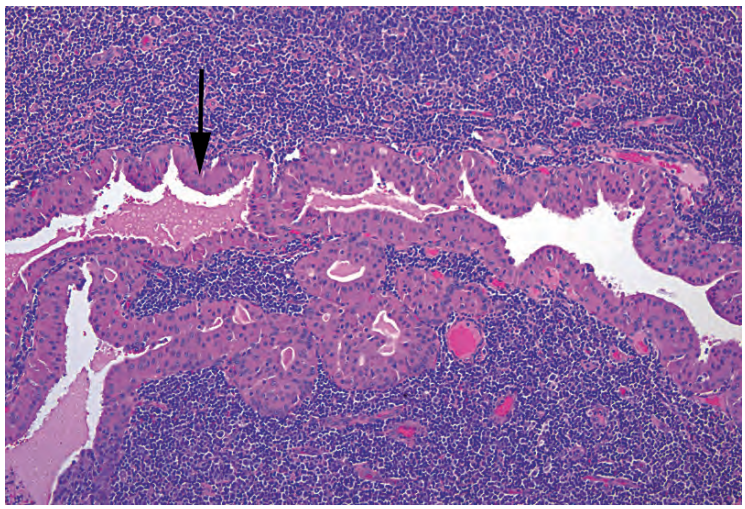

Figure 25.4. Warthin's tumor. This cyst is lined by a double layer of oncocytic cells (arrow) overlying a dense lymphoid infiltrate.

Rule of thumb: If it is an encapsulated solid lesion in the parotid, it probably fits into this category somehow.

Warthin's Tumor

Warthin's tumor is a papillary and cystic lesion lined by a double layer of oncocytic cells on top of a prominent lymphoid infiltrate with germinal centers (Figure 25.4). This usually occurs only in the parotid, but it can be bilateral. It is a low-power, 5-second diagnosis! This neoplasm (or reactive process?) may arise from striated ducts passing through intraparotid lymph nodes. The striated ducts are mitochondria rich, which explains the oncocytic nature of the lesion. This is to be differentiated from the *lymphoepithelial cyst*, a common lesion in

patients with the HIV that has a thin ragged epithelial lining instead of an oncocytic one. A related oncocytic lesion is the *oncocytoma*—a lesion composed of oncocytes that looks similar to a Hurthle cell nodule in thyroid or an oncocytoma in the kidney.

Mucoepidermoid Carcinoma

Mucoepidermoid carcinoma is the most common malignant tumor. Mucoepidermoid carcinoma has a wide range of cellularity, from cystic to solid. It can be low grade to high grade, depending on five factors: percentage of cystic component, tumor necrosis, mitoses, anaplasia, and neural invasion. The neoplasm is a mixture of squamous cells, epithelioid cells, and clear mucinous cells. However, when one cell type dominates, it can be difficult to tell this tumor from (for example) squamous cell carcinoma or a clear cell carcinoma. In these cases, recognizing intracellular mucin is the key to the diagnosis, so a periodic acid-Schiff or mucicarmine stain may be used.

The periphery should be infiltrative, not encapsulated, although the low-grade tumors may be fairly well circumscribed. This tumor may arise from the interlobular ducts (the big excretory ducts), and you will notice that an inflamed or metaplastic duct does not look that different from a little focus of low-grade mucoepidermoid carcinoma (Figure 25.5).

A low-grade cystic mucoepidermoid carcinoma must be distinguished from a mucin-containing salivary duct cyst, usually by carefully examining the cyst wall. As mentioned earlier, an intermediate-to-high grade mucoepidermoid carcinoma may be confused with metastatic or primary squamous cell carcinoma or clear cell carcinoma.

Adenoid Cystic Carcinoma

Adenoid cystic carcinoma is the prototypical cribriform tumor. It is blue, very cellular, with high nuclear to cytoplasmic ratios and fairly dense nuclei. Visually, it is reminiscent of a basal cell carcinoma of skin. It can be solid or tubular, but cribriform is the classic presentation, which makes it often instantly identifiable (Figure 25.6). Another classic feature is the balls of hyaline material found in the cribriform lumens, which are basement membrane material. It is highly infiltrative and loves nerves. Although intermediate grade at baseline, if solid growth predominates, this is a high-grade tumor.

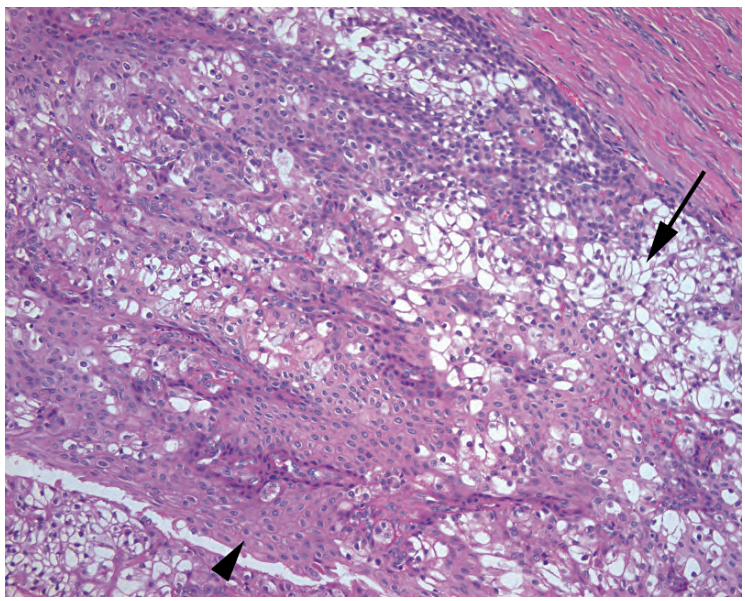

Figure 25.5. Mucoepidermoid carcinoma, low grade. This tumor resembles the metaplastic epithelium within an interlobular duct and is composed predominantly of clear goblet-like mucinous cells (arrow) and squamous cells (arrowhead).

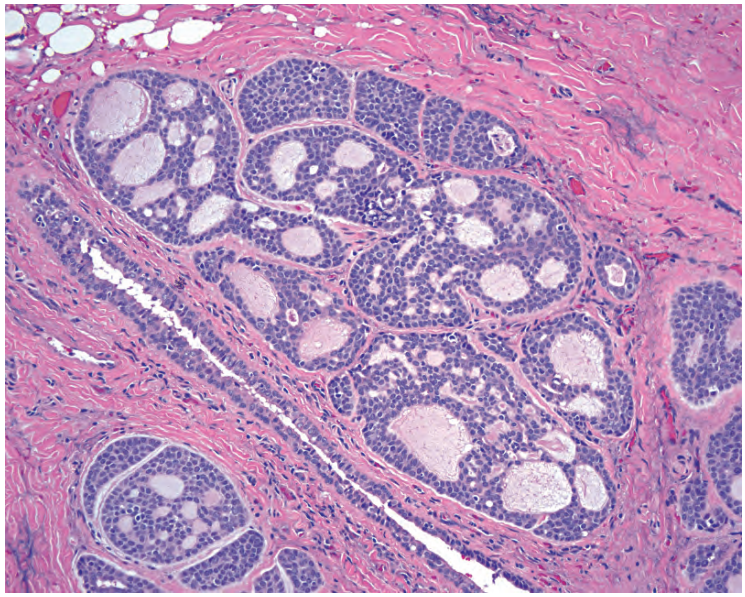

Figure 25.6. Adenoid cystic carcinoma. Although the nuclei are small, the nuclear to cytoplasmic ratio is high, making the tumor appear blue at low power. The architecture is classically cribriform, with sharply punched-out spaces full of pink secretions.

The presence of squamous areas favors a *basaloid squamous cell carcinoma*, which is in the differential diagnosis. The adenoid cystic carcinoma is very similar to the cylindroma of the skin, which can be in the differential around the ear (skin vs. tail of the parotid). Finally, if occurring in the lip or palate, rule out polymorphous low-grade adenocarcinoma, which can sometimes mimic an adenoid cystic. If you have a tumor that is cribriform and reminds you of an adenoid cystic, but is encapsulated or well-circumscribed, think instead of one of the adenomas, such as a basal cell adenoma.

Acinic Cell Carcinoma

Acinic cell carcinoma is a tumor of the serous acinar cells, and in its most differentiated form it looks quite similar to normal parotid, except without the ducts (Figure 25.7). Acinic cell is invasive, but as pushing borders rather than single cells. There are four common architectural patterns: solid, microcystic, papillary cystic, and follicular (such as thyroid). The microcystic pattern is full of little holes, but they are irregular holes that look as though the tissue has been pushed apart by expanding bubbles, very different from the rigid punched-out holes of adenoid cystic (Figure 25.8). There can be other nonserous cell types present, too, including clear cells, vacuolated cells, and ductal cells. However, finding a focus of serous differentiation (blue granular cytoplasm) pretty much seals the diagnosis.

The nonserous tumors can be quite pink and nonspecific in appearance. However, look for the microcystic pattern, the absence of cytoplasmic mucin (rules out mucoepidermoid carcinoma), and uniform well-spaced nuclei (either small and dense or medium-sized and very round with small nucleoli), similar to the follicular thyroid lesions. Note the pronunciation: it is ass-inic, not ax-inic.

Adenocarcinoma Not Otherwise Specified (NOS)

Adenocarcinoma NOS is the category for any ductal-derived carcinoma without other distinguishing features. It is the most common form of malignancy in a *carcinoma ex-pleomorphic adenoma*, which is a pleomorphic adenoma with a component of carcinoma.

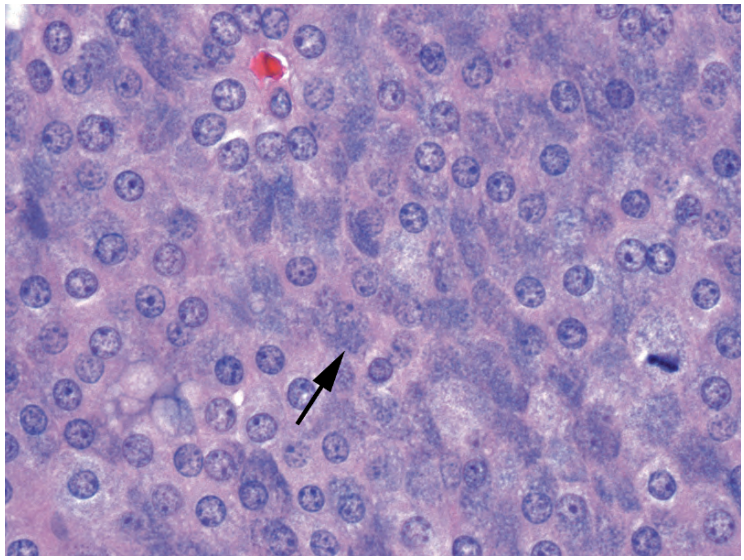

Figure 25.7. Acinic cell, solid pattern. The cells in acinic cell carcinoma replicate those of serous acini, with blue granular cytoplasm (arrow).

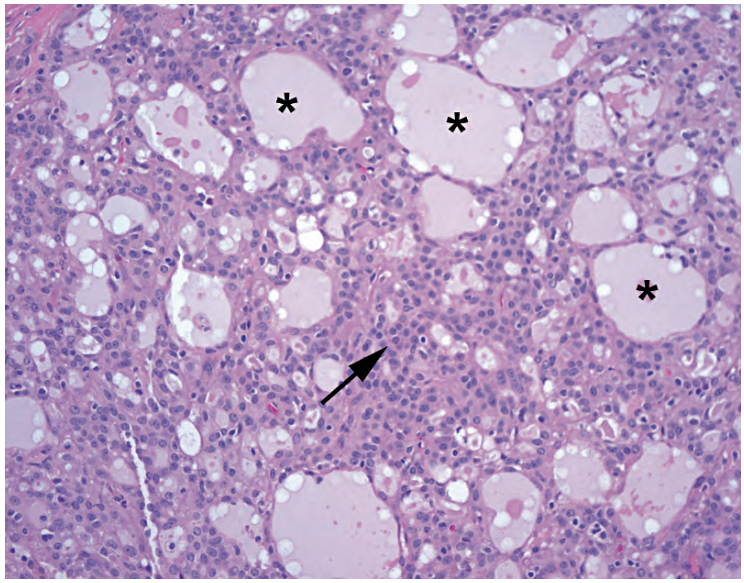

Figure 25.8. Acinic cell, microcystic pattern. In this example, the cells are nonspecifically pink (arrow) and do not have the telltale blue granules. However, the microcystic pattern, in which cells appear to be pushed apart by expanding pockets of fluid (asterisks), is typical of acinic cell. Compare these microcysts to the sharp cribriform spaces in adenoid cystic carcinoma (see Figure 25.6).

Polymorphous Low-Grade Adenocarcinoma

Importantly, polymorphous low-grade adenocarcinoma (PLGA) occurs almost exclusively in the intraoral minor salivary glands (lip and palate), so do not agonize over a funny-looking pleomorphic adenoma in the parotid! The PLGA looks very similar to a pleomorphic adenoma in terms of the epithelial component, and like a pleomorphic adenoma the cells are very bland. The key is in the margin of the tumor, which in a PLGA is infiltrative (Figure 25.9). The cells tend to spiral out of the central mass like a hurricane and may remind you of lobular carcinoma

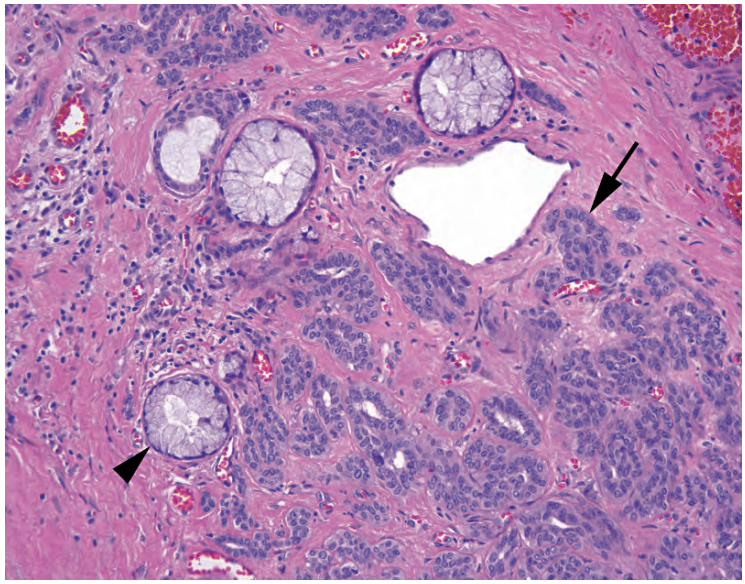

FIGURE 25.9. Polymorphous low-grade adenocarcinoma. Small tubules of bland cells (arrow) creep between benign mucinous glands (arrowhead). On high power, these infiltrative cells resemble those of the pleomorphic adenoma, but, unlike that benign tumor, the PLGA infiltrates surrounding tissues. PLGA cells may also invade as single-file lines, like lobular breast carcinoma.

(its former name, incidentally). Polymorphous low-grade adenocarcinoma can also sometimes be cribriform in appearance, mimicking an adenoid cystic.

Miscellaneous Malignant Neoplasms

Many of the benign adenomas described here have malignant counterparts, although they are rare. They include myoepithelial carcinoma, epithelial–myoepithelial carcinoma, basal cell adenocarcinoma, and oncocytic carcinoma. In general, features that favor malignancy include an infiltrative periphery, cellular pleomorphism, mitoses, or necrosis.

26 Brain

The pathology of the central nervous system is an intimidating area for pathologists. In part this is because we have virtually no role in the gross examination, just processing shreds of white pulp on gauze, and in part because of the feeling that "it could be anything at all," including a long list of exotic zebras that look just like the common things except behave completely differently. The fact that we are often asked to make our diagnoses on frozen section does not help matters. However, even in the brain, the list of likely diagnoses is still reasonably short if you have three pieces of key information: the age of the patient and the location and radiographic appearance of the tumor. Table 26.1 lists differential diagnoses that should at least put you in the right ballpark.

A strategy often proposed is to start by asking if your "lesion" could be normal tissue (i.e., the surgeon has missed). To answer this question you have to know a little normal histology, which is reviewed below. Second, you should ask if your lesion is neoplastic or nonneoplastic. The nonneoplastic lesion that many pathologists worry most about is the demyelinating lesion, which can look like a tumor by radiology. Abundant foamy macrophages, and an absence of obvious tumor cells, should make you think of a possible demyelinating lesion. Gliosis, a reactive proliferation of astrocytes, can also simulate a glioma histologically (see next section). However, once you have decided you have a neoplasm, the real work begins.

General Principles I

First, as in any organ, there are a finite number of cell types in the brain, and each cell type can give rise to a spectrum of neoplasms. In the brain, the cells and their common neoplasms are the following:

Native Cells	Tumors
Astrocytes	Astrocytoma and variants
Oligodendroglia	Oligodendroglioma
Ependyma	Ependymoma and variants
Neurons and precursors	Neurocytoma and gangliocytoma
Meninges (arachnoid cells)	Meningioma and variants, hemangiopericytoma
Choroid plexus	Choroid plexus papilloma/carcinoma
Pituitary	Pituitary adenoma
Schwann cells (in nerves)	Schwannoma
Stromal or vascular cells	Hemangioblastoma
Embryonal (immature) cells	Medulloblastoma/peripheral neuroectodermal tumors, neuroblastoma, others
Pharynx remnants*	Craniopharyngioma, Rathke's cleft cyst
Germ cell remnants*	Germinoma, teratoma, etc.
Notochord remnant*	Chordoma

Table 26.1. Differential diagnoses.

	Infants and young children	Adolescents and young adults	Adults to elderly
Cerebellum (infratentorial)	Pilocytic astrocytoma/ Medulloblastoma	Pilocytic astrocytoma/ Ependymoma/ Medulloblastoma	Diffuse astrocytoma/ Hemangioblastoma/ Metastases
Cerebellopontine angle (cranial nerves)			Schwannoma/ Meningioma
Cerebrum (supratentorial)	Neuroblastoma (rare in this location, more often an abdominal tumor)	Pilocytic astrocytoma/ Diffuse astrocytoma/ Ependymoma/ Pleomorphic xanthoastrocytoma	Diffuse astrocytoma, especially glioblastoma multiforme/ Meningioma/ Oligodendroglioma/ Mets/ Lymphoma
Sella	Craniopharyngioma	Pituitary adenoma/ Craniopharyngioma/ Germ cell tumors	Pituitary adenoma/ Craniopharyngioma (papillary type)
Pineal	Pineoblastoma	Germ cell tumors/ Pineoblastoma/ Pineal tumors/cysts	Pineal tumors/cysts
Ventricles (in or adjacent to)	Ependymoblastoma/ Choroid plexus papilloma/ carcinoma	Choroid plexus papilloma/ Ependymoma/ Pilocytic astrocytoma/ Neurocytoma/ Subependymal giant cell astrocytoma	Subependymoma
Dural based			Meningioma/ Hemangiopericytoma and solitary fibrous tumor

**Remnants* are those cell lines that do not anatomically belong in the brain but sometimes get left behind in some developmental fluke. They create midline tumors.

General Principles II

There is a broad grading system used for most central nervous system (CNS) neoplasms, the World Health Organization (WHO) tumor grade, which ranges from I (most indolent) to IV (most aggressive). In this system, grade I is equivalent to "benign," but in the CNS something cytologically benign may be clinically devastating depending on where it is growing. For this reason, CNS tumors are not described as benign versus malignant but are graded according to the WHO scale. The grade I and II tumors are sometimes referred to as "low grade," whereas grade III and IV lesions are considered "high grade." There is no TNM staging for primary brain tumors; margin status and tumor size are also not usually determined by the pathologist.

Many neoplasms are assigned to a grade by definition, but some types have a spectrum of grades based on certain histologic features. For most tumors, the following features are used to assign a higher grade to the lesion:

- Cytologic atypia (a subjective observation requiring some experience)
- Increasing cellularity relative to lowest grade tumor (again, subjective)
- Increasing numbers of mitoses (usually quantitative)
- Microvascular proliferation (objective: either present or absent)
- Necrosis (objective)

These features need to be searched for in every tumor (Table 26.2).

Table 26.2. Morphologic features in increasing order of concern.

Morphologic features in increasing order of concern							
EGBs or RFs	No atypia	No mitoses	Atypia	High cellularity	High mitoses	MVP	Necrosis
Pilocytic astrocytoma, grade I						occ. in pilocytic astrocytoma, I	
		Astrocytoma, grade II		Anaplastic astrocytoma, grade III		GBM, grade IV	
		Oligodendroglioma, grade II		Oligodendroglioma, grade III*		Oligo, IV**	
		Ependymoma, grade II		Ependymoma, grade III			
	Subependymoma, grade I						

EGBs, eosinophilic granular bodies; GBM, glioblastoma multiforme; MVP, microvascular proliferation; Occ, occasionally or rarely; RFs, rosenthal fibers.
*Microvascular proliferation is required to diagnose grade III oligodendroglioma
**Grade IV oligodendroglioma is very unusual and requires extreme malignant features.

General Principles III

Central nervous system tumors are usually initially sent for frozen section, to guide intraoperative management, and should also be evaluated with a smear or touch preparation, along with the frozen. The smear highlights some features that also narrow your differential diagnosis; on a smear it is easy to detect the fibrillary processes that identify most glial tumors, and nuclear detail is preserved. You can also get a feel for how cohesive (cell-to-cell adhesion) the tumor cells are, from very cohesive tumors (usually metastasis, schwannoma, or meningioma) to very noncohesive tumors (lymphoma, pituitary adenoma, and oligodendroglioma).

The general aim of the frozen section diagnosis is to verify that lesional tissue is present, classify the tumor into a major category (such as glioma vs. meningioma vs. carcinoma), and approximate a grade (low or high). It is often difficult and/or unwise to get more specific than this at the time frozen.

Overall, the tumors discussed in this chapter are organized based on location and age. However, astrocytomas, oligodendrogliomas, and meningiomas are discussed first, as they are so common and can arise almost anywhere along the neural axis. These are mainly tumors of adults. Note that metastases are actually the most common brain tumors in adults but are often diagnosed clinically (no biopsy, no pathologist).

Astrocytoma

Astrocytes, in their nonneoplastic form, are stellate cells with long processes that support the neurons. There are several variants of astrocytoma; the first split in categorization is between diffuse (infiltrating) types and circumscribed variants. Diffuse astrocytomas creep out into the surrounding brain so subtly that it is hard to tell where tumor ends and reactive brain begins. They come in three grades: *well differentiated* (II), *anaplastic* (III), and *glioblastoma multiforme* (IV). The distinction is by the criteria listed earlier. Hypercellularity and mitoses bump the tumor to grade III, while the presence of microvascular proliferation or necrosis is diagnostic of a glioblastoma multiforme.

Histologically, the cells of a diffuse astrocytoma are scattered in and among the normal cells of brain, with no visible architecture or cell borders (Figure 26.1). The key is in recognizing (1) that there are too many nuclei, distributed too unevenly, and (2) that many of the nuclei are hyperchromatic, coarse in texture, irregularly shaped, and large. In this sense, the astrocytomas follow the rules of other organs—you just have to learn to recognize the features of a "bad cell."

The higher the grade of tumor, the more obvious it becomes. Finding significant numbers of mitoses will generally push you to grade III. The glioblastoma multiforme, while classically shown with pseudopalisading necrosis (tumor cells lining up around a necrotic focus), can be diagnosed just by identifying a combination of malignant astrocytes, focal necrosis not associated with prior therapy, and/or microvascular proliferation, which is a characteristic expanding capillary population (Figure 26.2).

A circumscribed (nondiffuse) and very indolent form of astrocytoma is the *pilocytic astrocytoma* (grade I). It occurs in children and young adults and is usually associated with the

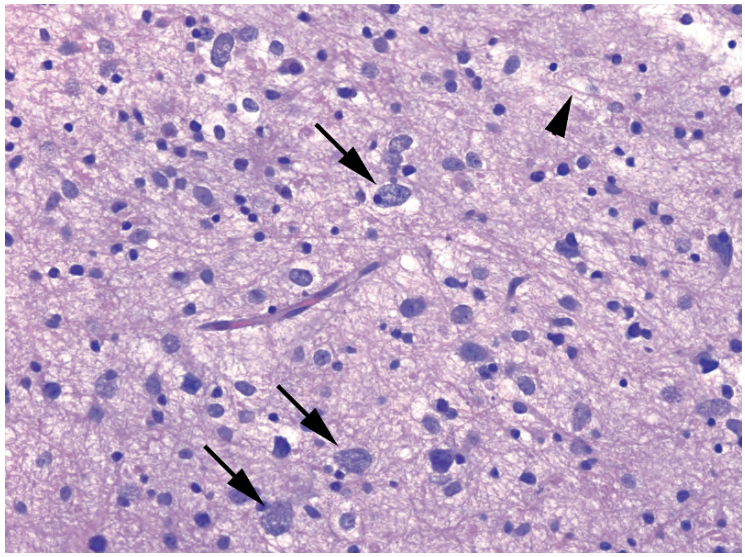

FIGURE 26.1. Astrocytoma, grade II. This field of tissue is hypercellular relative to normal brain. There are scattered large nuclei with irregular shapes and coarse chromatin (arrows); these are malignant astrocytes. The background is fibrillary (arrowhead), meaning there is a diffuse network of native neuropil and the processes of the malignant astrocytes.

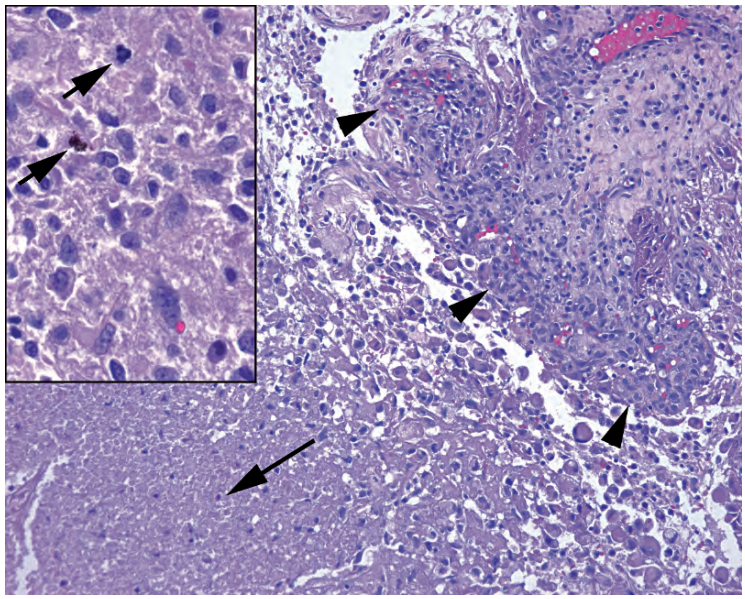

FIGURE 26.2. Glioblastoma multiforme. In this example, there is both necrosis (arrow) and microvascular proliferation (outlined by arrowheads). The microvascular proliferation is an expanding tangle of capillaries, some of which make glomeruloid forms. Many of the malignant astrocytes in this field have a gemistocytic morphology (eccentric nuclei and abundant pink cytoplasm). **Inset**: A high-power view of malignant astrocytes with enlarged and irregular nuclei and multiple mitoses (arrows).

cerebellum, optic nerve, hypothalamic region, or ventricles. The "pilo" means hair, because the fine processes create a matted-hair–like background (Figure 26.3). These tumors also show the hallmarks of a slow-growing glial process: Rosenthal fibers and eosinophilic granular bodies, both types of pink concretions seen among the tumor cells. Other, more rare, circumscribed astrocytic lesions include the *pleomorphic xanthoastrocytoma*, a seizure-causing tumor of young adults often found in the cerebral cortex, and the *subependymal giant cell astrocytoma* of tuberous sclerosis.

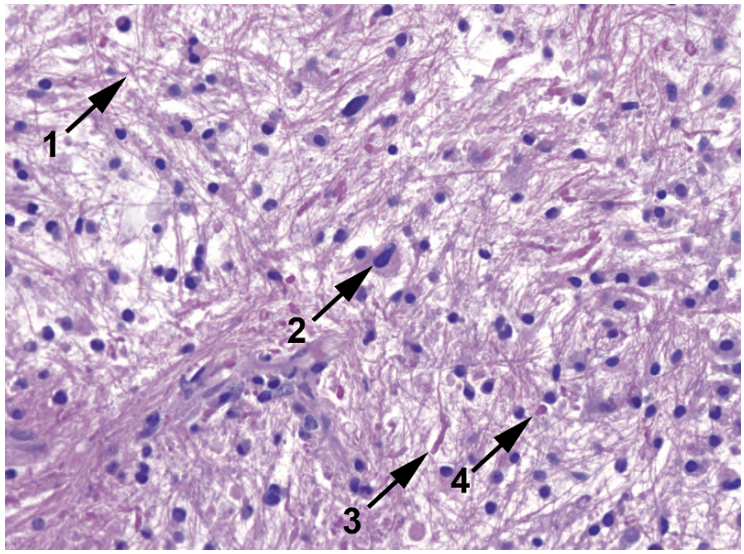

Figure 26.3. Pilocytic astrocytoma. The classic features shown here are a fibrillary or hair-like background (1), scattered large dark nuclei (2), Rosenthal fibers (3), and eosinophilic granular bodies (4).

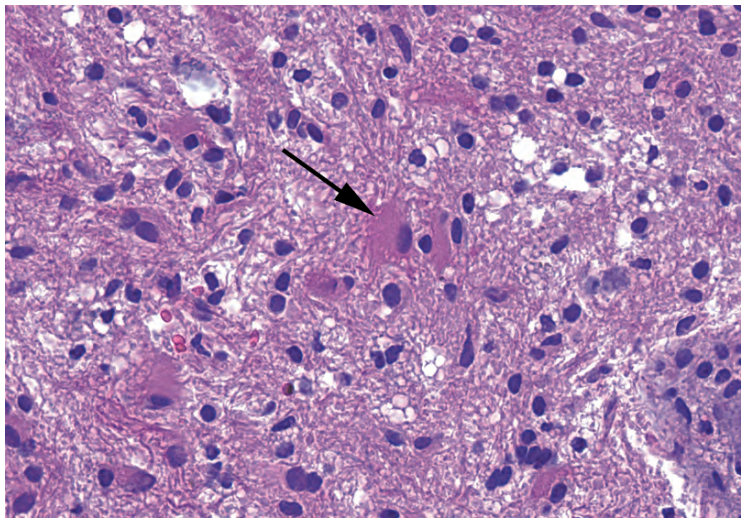

Figure 26.4. Reactive astrocytes. Normal resting astrocytes generally do not have visible cytoplasm. When responding to inflammation or injury, they become compact in shape, with dense pink cytoplasm and stubby processes (arrow).

Astrocytes can become reactive, in which their processes shorten and become more clearly visible, emphasizing their stellate shape (Figure 26.4). This nonspecific reaction to injury is called *gliosis*, and it can make nonneoplastic brain appear hypercellular. The key differential is with a well-differentiated astrocytoma, which can also resemble a subtle hypercellularity, especially around the edges. However, although some glioma cells can develop a prominent "gemistocytic" or abundant pink cytoplasm, they usually lack the multiple well-defined processes of reactive astrocytes. *Other features that favor glioma* over gliosis include the following:

- Microcystic pattern
- Calcifications
- Mitoses
- Clustering of glial cells around neurons or vessels or below the pia (satellitosis)
- Irregular distribution and crowding of glial cells

Oligodendroglioma

The oligodendroglioma is typically in the frontal/temporal lobes of adults. Usually it is a grade II tumor, although vascular proliferation and mitotic activity can push it to grade III. Histologically, it is characterized by a population of tumor cells that look like normal oligodendroglia: small round nuclei surrounded by clear halos (a retraction artifact seen only in formalin-fixed tissues). The chromatin is a little clumpy, like a plasma cell, but overall it is uniform (Figure 26.5). Architecturally, they tend to cluster around existing neurons (satellitosis). Other helpful features include a net-like capillary array, a microcystic pattern (as though torn apart by expanding bubbles), and calcifications. On a smear, these cells are not fibrillary like the astrocytoma but instead sheet out as discohesive round cells.

All useful tips, but in reality the oligodendroglioma is evolving from a morphologic diagnosis to a molecular one. "Pure" oligodendrogliomas are almost always characterized by a chromosomal deletion of 1p and/or 19q, and this same population tends to respond well to therapy. As a result, cases with anything other than classic features are often sent for cytogenetics. The idea of a mixed glioma (an oligoastrocytoma) is controversial but is included in the WHO classification.

Meningioma

The meningioma arises from arachnoid-type cells associated with the dura and is therefore almost always dural based, which may include tumors on the cerebral convexities, tumors of the falx, or tumors around the brain stem or spinal cord. It is common in adults, rare in children. The usual meningioma is a grade I tumor, but certain features or subtypes can raise the grade to II (atypical) or III (malignant).

Histologically, meningioma is one of the most protean tumors in the CNS. It has 16 subtypes and counting, very few of which have clinical significance but that must be recognized for their benign selves and not called carcinoma, sarcoma, and so forth. For this reason, before you start trying to learn subtypes, become very comfortable with the basic cytologic features of *meningothelial cells*—these do not vary much across types. The classic meningothelial cell

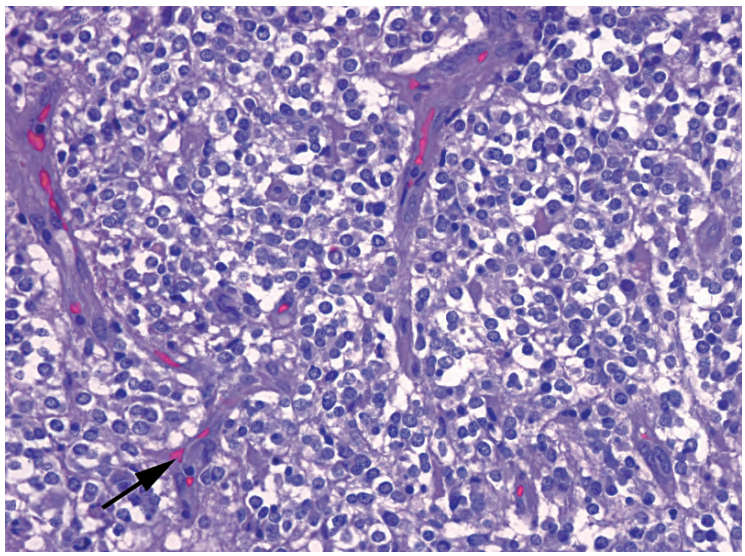

Figure 26.5. Oligodendroglioma. This is a very cellular example of an oligodendroglioma. The closely packed oligodendroglia have very round nuclei which are surrounded by clear halos. The tumor cells are suspended in a network of fine capillaries (arrow).

occurs in a syncytium with its neighbors, so cell borders are invisible. The nuclei are small, oval, and regular, with a very fine powdery chromatin, and they tend to stream in parallel in their syncytial groups (Figure 26.6). Nuclear inclusions may be seen. Meningiomas of all types often have whorls, which are spiral-shaped streams of nuclei, similar to the whorl of a fingerprint. Finally, psammoma bodies are frequently present.

The "classic" meningioma is called the *syncytial type*, but it can differentiate along more mesenchymal lines (fibrous, angiomatous) or more epithelial lines (secretory, clear cell). Important subtypes are the following:

- Clear cell (grade II): glycogen-filled cells, which therefore lose their syncytial appearance, resembling instead a clear cell carcinoma (Figure 26.7)
- Chordoid (grade II): resembles a chordoma, with a myxoid background and cords of cells

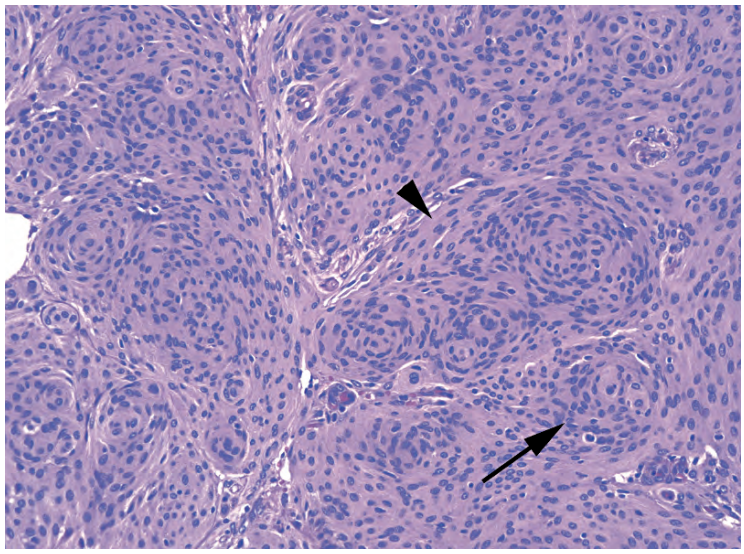

Figure 26.6. Syncytial meningioma. The meningioma nuclei are small and oval and tend to cluster in syncytial groups (arrowhead) without visible cell borders, or make whorls (arrow).

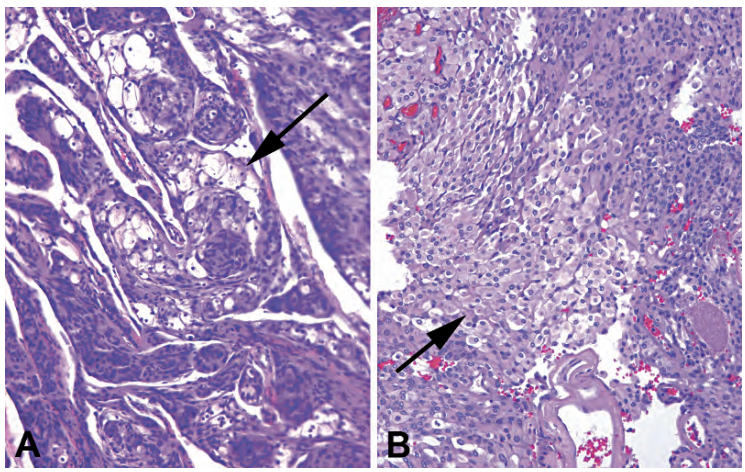

Figure 26.7. Aggressive variants of meningioma. **(A)** Clusters of distended clear cells (arrow) are visible in this meningioma, indicative of clear cell meningioma. **(B)** Rhabdoid meningioma has plump eosinophilic cells (arrow) with rhabdoid (resembling immature skeletal muscle) morphology.

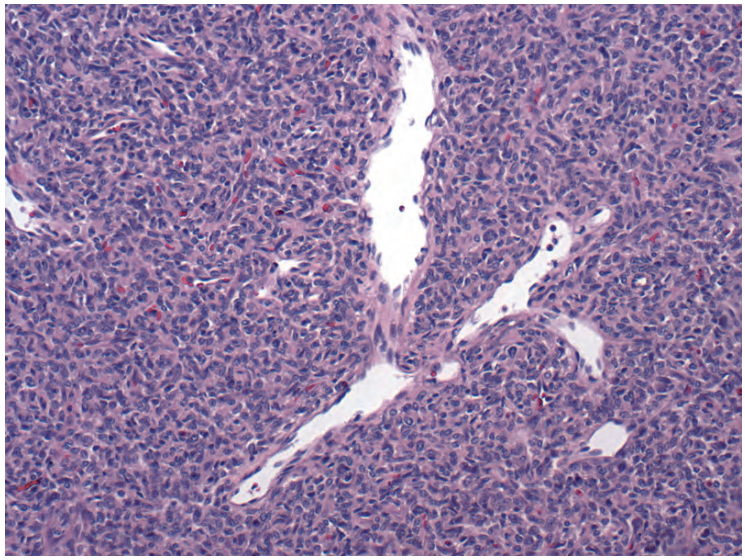

Figure 26.8. Hemangiopericytoma. A typical staghorn vessel in a background of small blue cells that are somewhere between epithelioid and spindled.

- Rhabdoid (grade III): plump pink cells with discrete cell borders, similar to rhabdomyoblasts (see Figure 26.7)
- Papillary (grade III): syncytial, meningothelial cells on arborizing fibrovascular cores

A meningioma can also be upgraded based on cytologic criteria, including cellularity, pleomorphism, mitotic rate (over 4 per 10 high-power fields [hpf]), and necrosis. Brain invasion (true infiltration into brain parenchyma) is a poor prognostic sign that previously resulted in the diagnosis of a malignant meningioma. The current WHO classification assigns these tumors a grade II "atypical" designation. Skull invasion, on the other hand, does *not* affect the tumor grade, although invasion of the skull base can be surgically problematic.

Related but much less common lesions are the *hemangiopericytoma (HPC)* and *solitary fibrous tumor*. The cell of origin is probably the same, and these are also dural-based, enhancing, well-circumscribed lesions. However, the hemangiopericytoma is the more aggressive tumor of the two. It is a blue and cellular tumor with prominent and stereotypical gaping vessels called *staghorn vessels* (Figure 26.8). The nuclei are oval but have nucleoli and more coarse chromatin than the meningioma.

Lesions of the Posterior Fossa (Infratentorial)

The main structure of the posterior fossa is the cerebellum. Never forget where you are; the granule cells of the cerebellum look tumor-like on smear if you are not expecting them. Within the cerebellum of adults, your differential usually includes gliomas, metastases, and the *hemangioblastoma*. Infratentorial tumors are much more commonly seen in children, and in this age group the big players are low-grade gliomas (*pilocytic astrocytoma* and *ependymoma*) and *medulloblastoma*: fortunately difficult to mix up on frozen section.

A special infratentorial location is along the eighth nerve, the most common site of *schwannoma* in adults. Named the *acoustic neuroma* because of its position on the auditory nerve, the schwannoma is a tumor of peripheral nerve, made up of the myelinating nonneural Schwann cells.

Hemangioblastoma is an uncommon tumor often associated with the von Hippel-Lindau syndrome (VHL). It looks a little like a renal cell carcinoma, with packets of lipidized clear tumor cells surrounded by a delicate capillary network (Figure 26.9). This is unfortunate,

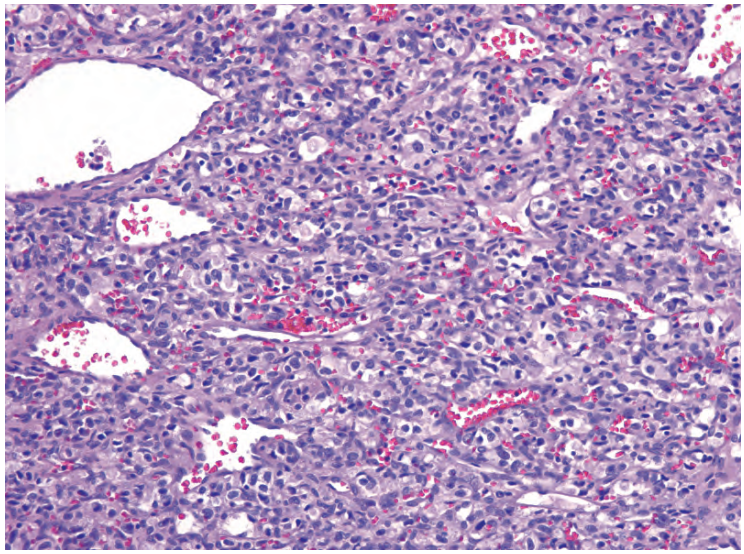

Figure 26.9. Hemangioblastoma. Clear (lipidized) cells with bland nuclei in a background of interlacing and dilated capillaries.

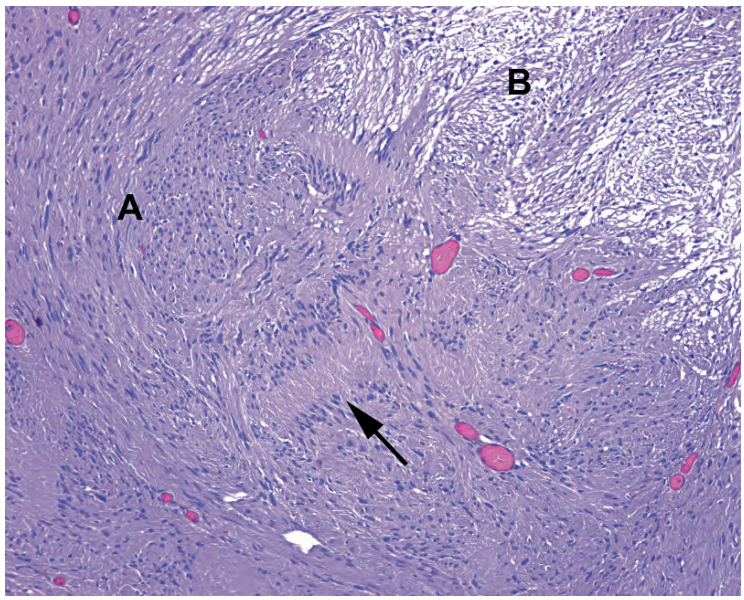

Figure 26.10. Schwannoma. This lesion shows alternating areas of high and low cellularity, called Antoni A **(A)** and B **(B)** areas. The elongated Schwann cells tend to stream in parallel groups and form opposing parallel arrays, called Verocay bodies (arrow).

because VHL patients also get renal cell carcinoma metastases. Oil Red O (a stain for fat performed only on frozen sections) and some immunostains can sort out the ambiguous cases.

Pilocytic astrocytomas (arising from the fourth ventricle) are described above in the section on astrocytomas. *Ependymoma*s (also from the ventricle) are described below, with other ventricular tumors.

Schwannoma is a benign fibrillary tumor consisting of a streaming mass of elongated nuclei (Figure 26.10). It often has alternating areas of high and low cellularity (Antoni A and B areas) and tends to make little palisaded arrays called Verocay bodies. Hyalinized (thick, pink)

vessels are common. *Medulloblastoma* is one of the aggressive small round blue cell tumors of childhood (discussed later).

Lesions of the Midline

Tumors arising from embryologic remnants tend to occur in the midline. *Germ cell tumors* include the *germinoma*, which is essentially a primary CNS seminoma, and the *teratoma*, more often found in a sacrococcygeal location than in the head. Other germ cell tumors, such as yolk sac tumor and choriocarcinoma, are rare but do occur in the CNS.

Craniopharyngioma and Rathke's cleft cyst both derive from pharyngeal tissues occurring in the sellar region. The craniopharyngioma, most common in young people, classically has an "adamantinomatous" appearance, meaning it looks like a developing tooth. The nests of cells are bounded by dark palisaded cells, with central areas of stellate cells in a myxoid stroma (Figure 26.11). There is also keratin and debris. Adults, when they get craniopharyngiomas, more often get the papillary type, resembling a nonkeratinizing squamous papilloma.

Chordomas are tumors of the notochord remnant, hence the name; note that they have nothing to do with "chondroid" or cartilage. They are most often found at the top and bottom of the spine—the clivus and the sacrum. Like intervertebral discs, another notochord remnant, they have a bluish, mucinous background. The "physaliphorous" tumor cells are typically full of clear bubbles and grow in cords.

Other sources of midline tumors include the pituitary and pineal. *Pituitary adenoma* is a common tumor of the sella. Remember that the pituitary is a very heterogenous mass of cell types, arranged in lobular nests. Therefore, cellular monotony and loss of the normal pattern of small nests of cells is the key to differentiating between an adenoma and normal pituitary (Figure 26.12). The pituitary adenoma looks similar to a neuroendocrine tumor in other sites, both cytologically and architecturally. The *pineocytoma* is the pineal adenoma. It can be very difficult to tell a pineal tumor from normal pineal, mainly because so few pathologists have actually seen a normal pineal. Tumors that are midline because they are associated with the third or fourth ventricle are discussed in the next section.

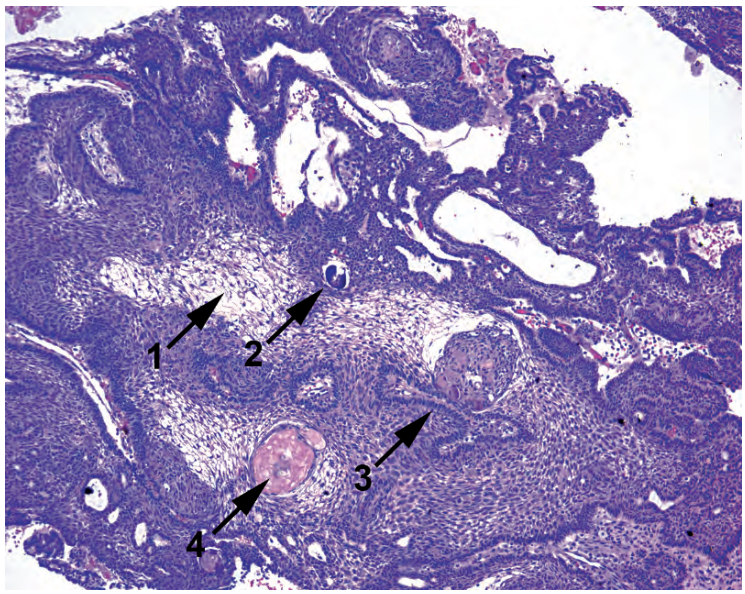

Figure 26.11. Craniopharyngioma, adamantinomatous type. There are areas of stellate reticulum (1), calcification (2), peripheral palisading (3), and accumulated "wet" keratin (4).

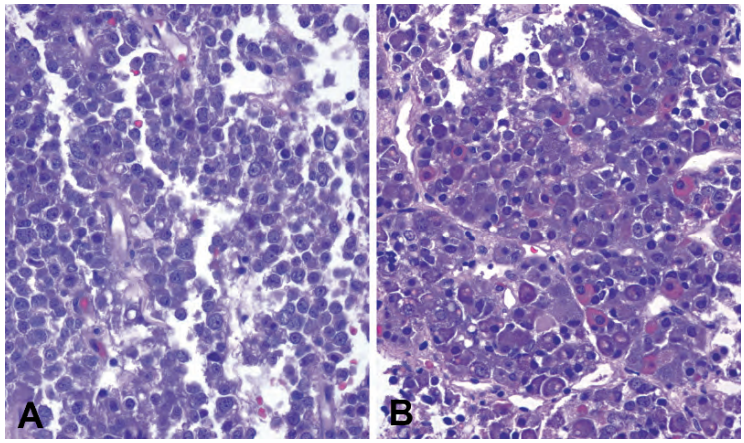

Figure 26.12. Pituitary adenoma versus normal. **(A)** In a pituitary adenoma, there is a monomorphic population of neuroendocrine-type cells. A collagen or reticulin stain would show sheets of cells no longer encircled by reticulin. **(B)** Normal pituitary is a mix of many different cell types, both eosinophilic and basophilic, and a reticulin stain would show the tissue divided into small discrete nests.

Lesions of the Ventricular/Periventricular Areas

Ependymoma

The ependymoma is a usually low-grade (II) lesion of children and young adults. Grade III anaplastic ependymomas also exist, but in most studies this pathologic distinction is not clinically meaningful. It arises from a population of cells that line the ventricles, called *ependymal cells*. When they become neoplastic, they retain this affinity for making boundaries or lumens and tend to encircle vessels.

Histologically, this is a circumscribed lesion composed of cells with pale oval nuclei that align themselves around blood vessels, sending processes down to the vessel like spokes of a wheel (Figure 26.13). The resulting structure is called a *pseudorosette*. When the cells make an array around a tiny open lumen, it is called a *true rosette*. These rosettes are the signature feature of the ependymoma.

A special type of ependymoma is the *myxopapillary ependymoma*, occurring almost exclusively at the filum terminale. It has a myxoid background and ependymal cells radiating off of papillary structures, so it was well named (Figure 26.14). A *subependymoma* is an even lower grade lesion (I) which typically occurs as a nodule on the ventricular wall.

Other Tumors

Pilocytic astrocytomas are commonly associated with ventricles, especially in children. The subependymal giant cell astrocytoma of tuberous sclerosis is a tumor of the lateral ventricles consisting of very large cells with a somewhat ganglionic appearance.

Central neurocytoma is a low-grade lesion of neural origin, typically occurring in the lateral/third ventricle area. Like other neuronal tumors, it is uncommon and mainly occurs in children and young adults. It may be mistaken for an ependymoma.

Choroid plexus papilloma is an intraventricular tumor of young children and can even be congenital. It resembles normal choroid plexus but grows in large arborizing fronds to make a mass. The bland columnar cells form a single-to-pseudostratified layer on the fibrovascular cores. When these tumors become more solid, mitotically active, and invasive, they are known as *choroid plexus carcinomas*.

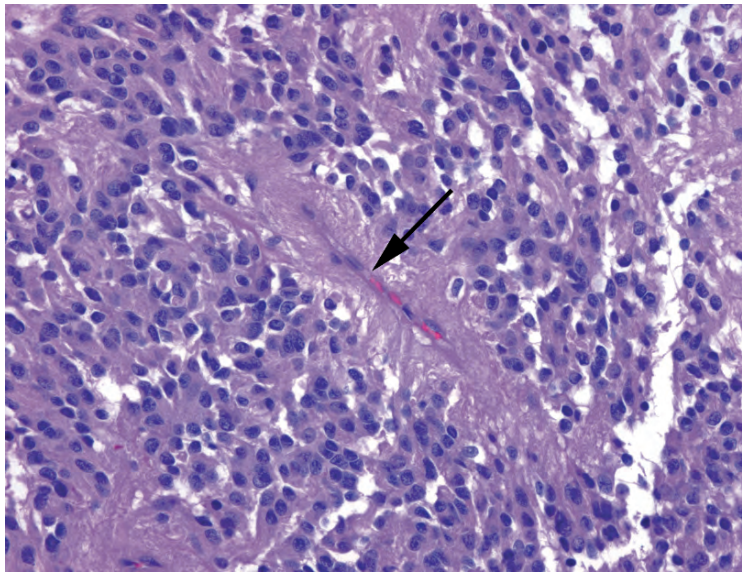

Figure 26.13. Ependymoma. There is a fibrillary background and a tendency for the cells to line up around vessels (arrow), with the ependymal cell processes extending to the vessel and the nuclei around the perimeter. This is actually an example of a pseudorosette; true rosettes have a lumen, not a vessel, at the center.

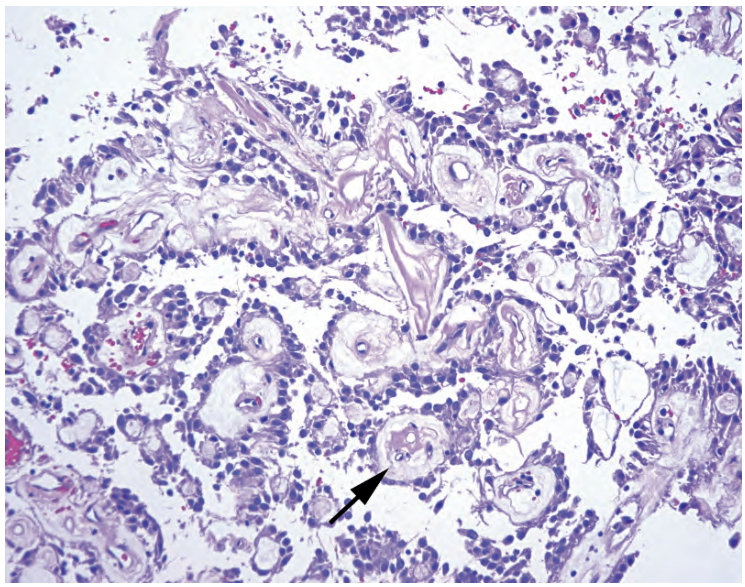

Figure 26.14. Myxopapillary ependymoma. The tumor is a papillary neoplasm with vascular cores surrounded by a myxoid stroma (arrow) and lined by ependymal cells.

Embryonal Lesions (Blastomas)

Blastomas are the small blue cell tumors of infancy and childhood, high grade and aggressive. They arise from immature stem cells. A feature they have in common is the tendency to form rosettes, similar to fetal neural tissue. This category does not include hemangio*blastoma*, or glio*blastoma* multiforme, or olfactory neuro*blastoma* (a sinonasal tumor of adults, arising from the olfactory membrane, unrelated to other things called neuroblastoma).

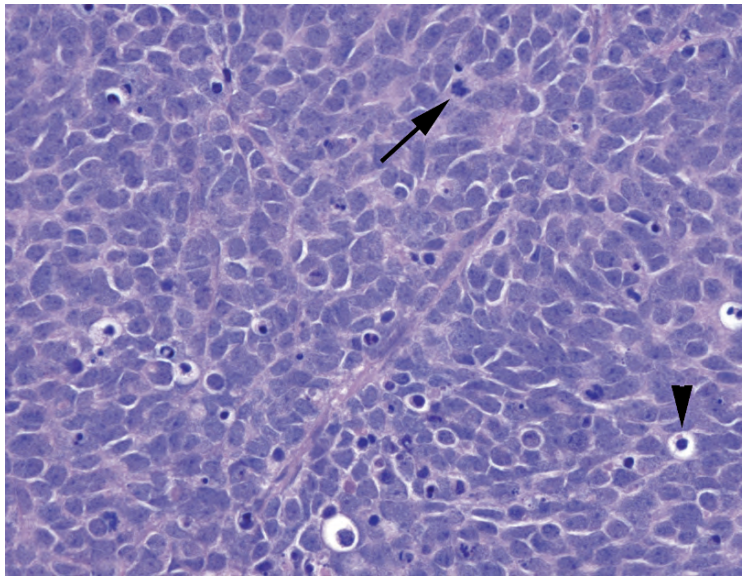

Figure 26.15. Medulloblastoma. A small round blue cell tumor composed of cells that are not actually round but more wedge-shaped or even spindly. The chromatin is very fine in texture, and there are frequent apoptoses (arrowhead) and mitoses (arrow).

Medulloblastoma is the most common of these tumors. When an identical tumor arises in the CNS outside of the cerebellum, it is called a *peripheral neuroectodermal tumor* (PNET; note that the PNET/Ewing's sarcoma tumors described outside the CNS are cytogenetically distinct from the CNS PNET see Chapter 28). These tumors are sheets of small blue cells of high nuclear to cytoplasmic ratio, high mitotic rate, and necrosis (Figure 26.15). The chromatin of the cells is fine and granular like small cell carcinoma, but the nuclei tend to be somewhat wedge or carrot shaped, especially when molded into rosettes. Unlike small cell carcinoma, there is often a fibrillary background due to the neural lineage of this tumor. Like neural stem cells, these tumors retain the ability to differentiate into neurons and glia, so these more differentiated cellular elements may be seen in the tumor.

Other tumors in this category, all rare, all grade IV, include ependymoblastoma, medullomyoblastoma, pineoblastoma, retinoblastoma, and neuroblastoma. Neuroblastoma is typically an abdominal neoplasm arising in the adrenal, but it can occur in the CNS as well, whereas retinoblastoma arises in the eye.

27 Skin

The discussion of the skin will be divided into three subsections: melanocytic lesions, non-melanocytic lesions, and inflammatory (systemic) disorders. Skin biopsies are usually performed because the clinician sees a lesion, such as a mass, a rash, or a macule. However, skin biopsies are also sometimes used to diagnose systemic conditions. Usually the history is enough to direct you to one of the major three categories. Inflammatory skin conditions are not usually diagnosed by the general surgical pathologist, but a working knowledge of their classification can be very helpful. Melanocytic lesions are also more and more the exclusive domain of dermatopathologists, but any surgical pathologist should at least be able to tackle the most benign and most malignant ends of the spectrum.

The grossing of skin biopsy specimens varies a bit by the shape, size, and purpose of the excision, but for diagnostic specimens of tumors, the margins must be entirely examined in perpendicular cuts. See your grossing manual, and consult with your attending, for the best way to cut in a specimen.

Melanocytic Lesions

Melanocytes are specialized cells in the epidermis and elsewhere that are derived from neural crest cells. They have a neuralish, dendritic morphology and stain with S100, like peripheral nerve cells. They also produce melanin pigment, which is exported from the cell and taken up by surrounding epidermal cells. Normal melanocytes do not have much visible pigment; in fact, the cytoplasm is clear, as the pigment leaves the cell (Figure 27.1). Densely pigmented cells along the basal layer of the epidermis are usually basal keratinocytes, not melanocytes. It is this pigment distribution that creates shades of skin color.

Abnormal melanocytes can accumulate pigment, and this can be a useful clue in identifying dysplastic melanocytes (discussed below) or identifying an unknown metastasis as melanoma. However, there are plenty of melanomas with no melanin to be found, so do not rely on that. Also beware the melanophage, spindly macrophages in the dermis full of chunky globs of melanin—they are eating it, not making it (Figure 27.2).

Become familiar with the melanocyte; spend a few seconds looking for them when you encounter normal skin. Melanoma is a treacherous area precisely because there are no strict diagnostic rules about when something is malignant and when it is not, and much of the diagnosis (in subtle cases) relies on recognizing atypical melanocytes that are up to no good. The only way to learn this skill is to see lots of normal melanocytes.

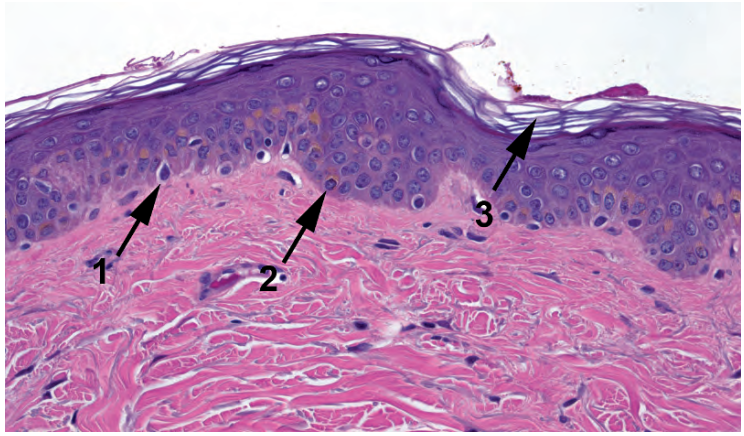

Figure 27.1. Normal melanocyte and skin. A normal melanocyte (1) stands out within a clear halo of cytoplasm. The pigmented component of the skin is actually the basal keratinocytes (2), which absorb the melanin. Typical basket weave or orthokeratin is present (3).

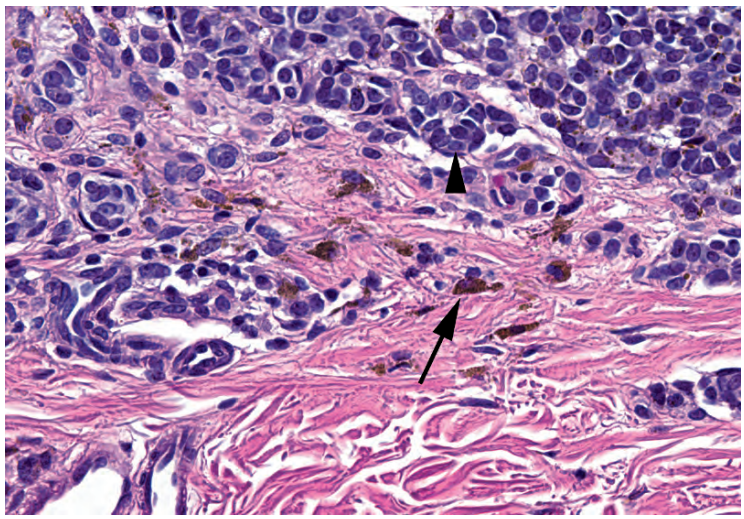

Figure 27.2. Melanophages in an intradermal nevus. The nevus cells (arrowhead) have focal small specks of pigment, but the macrophages digesting the excess melanin (arrow) stand out.

Terminology

As a pathologist, you now know that a "mole" is a type of gestational trophoblastic disease, and you are sophisticated enough to refer to a bump on the skin as a nevus. However, the word *nevus* really does mean just a *bump on the skin*, and there are things called *nevus* that have nothing to do with melanocytes. In this chapter, we will just be discussing the melanocytic nevi.

A melanocytic nevus is a proliferation of benign melanocytes. It begins along the basal layer of the epidermis, where melanocytes live, and the very earliest manifestation of this is an increased number of melanocytes along the dermoepidermal junction (DEJ) in a single layer. This produces a dark patch on the skin, and the lesion is called a *lentigo simplex* (Figure 27.3). The word *lentigo* or *lentiginous* refers to "along the DEJ" and is used in several different contexts.

The next step in the life cycle of a nevus is the proliferation of melanocytes into little nests, or *theques*, along the DEJ. These are technically intraepidermal, although it is sometimes hard to appreciate that. This lesion is called a *junctional nevus*, and it appears as little clusters of bland melanocytes hanging from the DEJ (Figure 27.4).

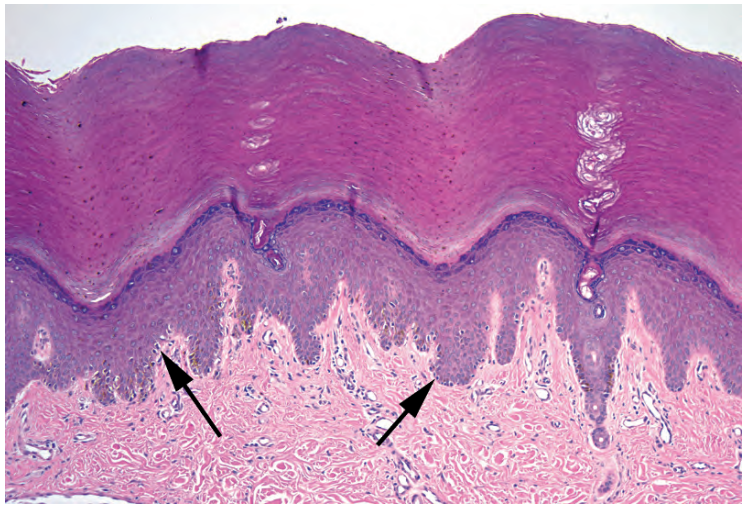

FIGURE 27.3. Lentigo simplex in acral skin. The dense keratin seen here is typical of acral skin (hands and feet). There is a linear proliferation of single benign melanocytes along the dermoepidermal junction (arrows).

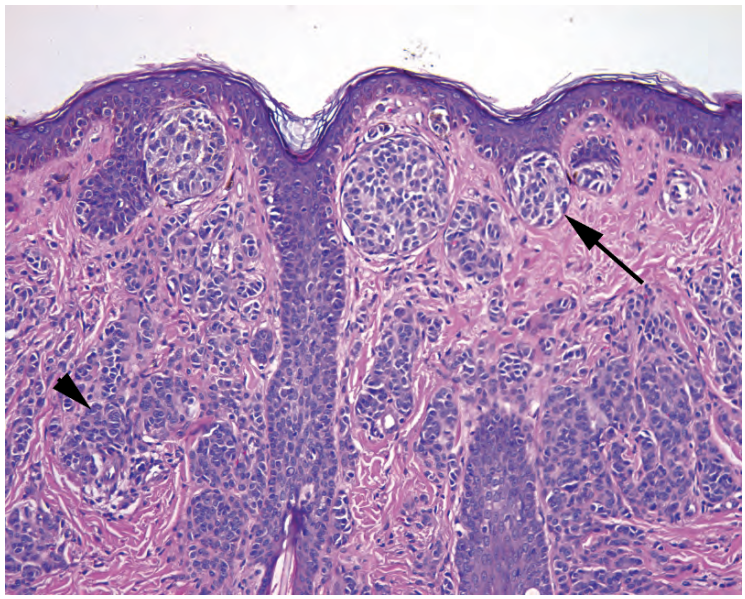

FIGURE 27.4. Compound nevus. This nevus shows nests of nevocellular (melanocytic) cells attached to the dermoepidermal junction (arrow). A nevus with only dermoepidermal junction nests would be a junctional nevus. In this example, as there are also nevus cells dropping down into the dermis (arrowhead); this is a compound nevus. In a compound nevus, the cells at the deepest point should appear slightly smaller and more bland than those at the dermoepidermal junction ("maturation").

From there, the melanocytes may begin to proliferate down into the dermis. They do so as small nests, sheets, or single cells, and they grow in a lobular pattern. Cytologically they are bland, round, clear cells, and they tend to "mature" (become smaller and more bland) the deeper into the dermis they progress. They become so numerous that they make a little nodule in the skin, forming the classic "mole" a la Cindy Crawford. Most adults have 10–20 of them. A nevus with a dermal component plus a junctional component is called a *compound nevus*. Eventually, with age, the junctional component regresses and you are left with just an *intradermal nevus* (Figure 27.5). These can be pedunculated, hyperkeratotic, hair bearing, and so forth. Note that melanoma arising in a benign intradermal nevus is vanishingly rare.

All of these phases of the nevus share some histologic features of benignity:

- Symmetry
- Size < 3 mm in diameter
- Lateral borders consisting of nests, not individual trailing melanocytes
- Lack of atypia in the melanocytes (nuclei are no larger than a keratinocyte nucleus and have small dense nucleoli, if any; multiple nuclei are okay)
- Maturation into the dermis
- Chunky brown-black pigment

Other Benign Nevi

The common *blue nevus* consists of a diffuse scattering of pigmented, dendritic (stellate), single melanocytes in the dermis (Figure 27.6). They are mixed in with melanophages.

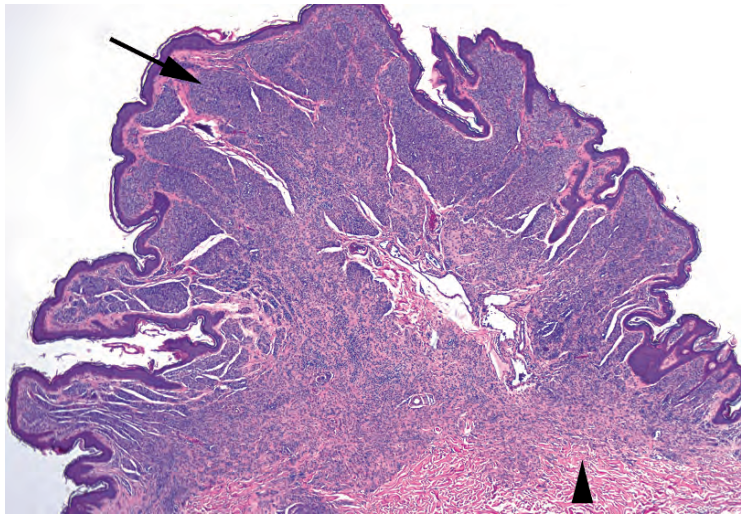

Figure 27.5. Intradermal nevus. This exophytic nevus has only dermal nests of nevus cells (arrow). The lesion is roughly symmetric, and the cells are smaller and more mature at the base (arrowhead).

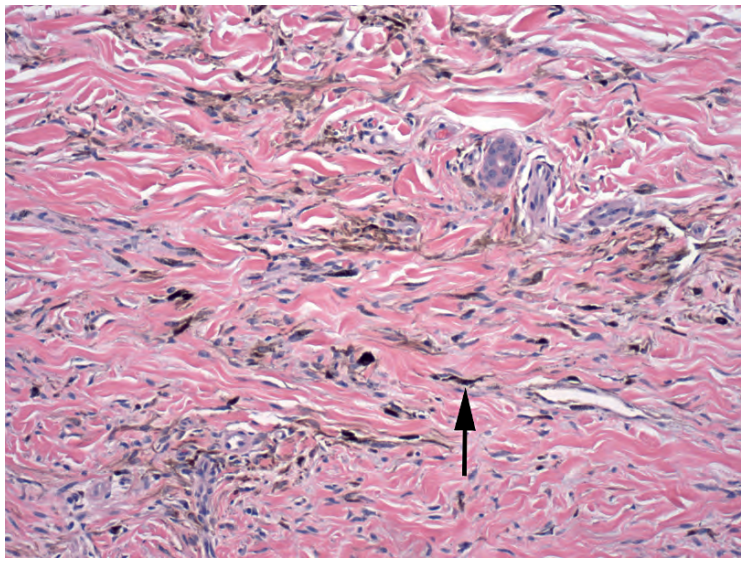

Figure 27.6. Blue nevus. Small, indistinct, pigmented cells are scattered throughout the dermal collagen (arrow). The cells are elongated and fusiform or stellate and do not make rounded nests like typical nevus cells. Some of the larger cells with chunky pigment are likely melanophages.

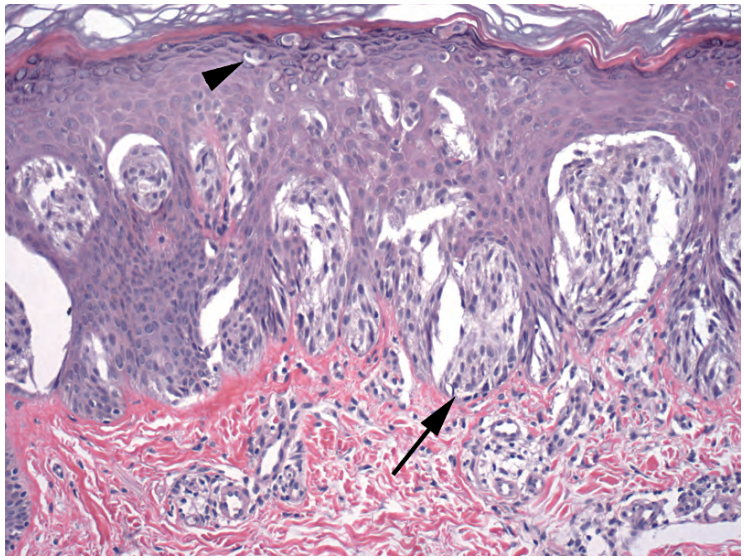

Figure 27.7. Spitz nevus. This nevus in a child shows nests of large, spindly melanocytes at the dermoepidermal junction (arrow) and rare melanocytes spreading up through the epidermis (arrowhead). In an adult, this pattern would be very worrisome.

The *Spitz nevus* is usually found on the head and neck of children and adolescents. At low power it is circumscribed and symmetric, and large nests of melanocytes are found between skinny elongated rete (Figure 27.7). Eosinophilic Kamino bodies may be seen at the DEJ. The reason this lesion is so troublesome is that the melanocytes may be large, spindled, pleomorphic, or atypical, and they may even show rare mitoses, which suggests melanoma.

Acral and genital nevi—nevi of the hands and feet, genital regions, and breasts—are allowed some atypical features. These nevi have prominent lentiginous growth, with occasional ascending cells mimicking pagetoid spread. However, they should not have cytologic atypia.

Most nevi are acquired during childhood to early adulthood, but some are congenital. To have *congenital features* means that the dermal melanocytes tend to track down the adnexal structures.

Dysplastic nevi

There are some nevi that begin to show some features more commonly associated with melanoma. These nevi are clinically distinct looking, and although they are not considered actual precursors to melanoma, patients with multiple dysplastic nevi are at significantly higher risk of developing melanoma. However, "dysplastic nevus" is a clinical diagnosis, and as pathologists we merely describe the features we see. There are two components to dysplasia in this context: architectural disorder and atypia. These lesions are signed out as, for example, *Compound nevus with architectural disorder and severe cytologic atypia*. (However, in some texts you will find this entity listed as "lentiginous melanocytic nevus.")

There are four features of *architectural disorder*: Architectural disorder is not graded but simply present or absent.

- Lentiginous spread of atypical melanocytes (along the DEJ in a creeping line)
- Shouldering (the lentiginous component is wider than the dermal component)
- Bridging of rete (nests attached to adjacent rete ridges fuse; Figure 27.8)
- Fibroplasia (a feathering of the dermal collagen that looks like pink cotton candy)

The features of *cytologic atypia* include the following:

- Hyperchromatic nuclei, increased nuclear to cytoplasmic ratio
- Large red nucleoli
- Accumulation of dusty grey-brown melanin (see Figure 27.8)
- Atypical mitoses

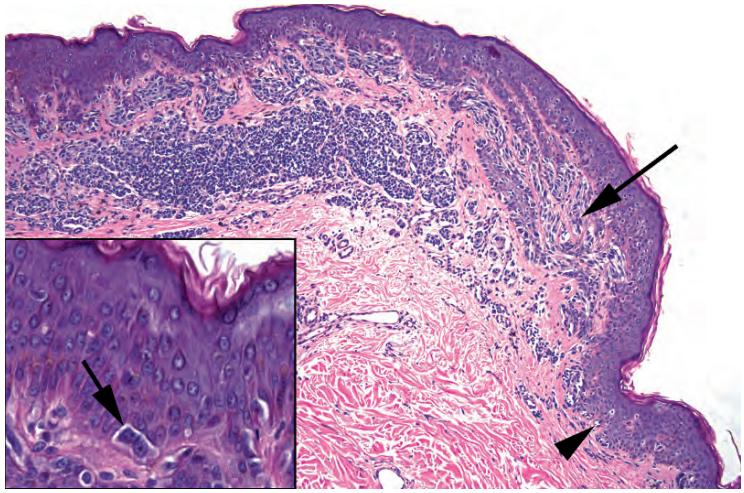

Figure 27.8. Dysplastic nevus. At low power, elongated nests of spindly melanocytes are seen bridging across adjacent rete (arrow), and single melanocytes trail off to the lateral edge of the lesion (arrowhead). These are features of architectural disorder. **Inset**: Atypical melanocytes with large nuclei and nucleoli are seen at the dermoepidermal junction.

Atypia is graded as mild, focally severe, or severe.

In general, these nevi tend to be suspicious enough that you must take a few moments to prove to yourself that they are *not* melanoma (see next section).

Melanoma

The best way to think about melanoma is as the presence of malignant melanocytes. Because melanocytes can proliferate in many ways and still be benign, it takes considerable experience to decide if a melanocyte is malignant or not. However, setting that aside for a moment, the types of melanoma include the following:

- *Lentigo maligna*: malignant melanocytes proliferating only along the DEJ.
- *Melanoma in situ*: malignant melanocytes along the DEJ, *and* percolating up through the epidermis in a pagetoid fashion (something benign melanocytes just do not do)
- *Malignant melanoma*: malignant melanocytes along the DEJ, pageting through the epidermis *and* invading the dermis
  - *Superficial spreading melanoma*: melanoma in a "horizontal growth phase," meaning it is spreading laterally along the DEJ but also involves the dermis (clinically, this is a macular lesion [flat])
  - *Nodular melanoma*: melanoma with a "vertical growth phase," meaning that it is primarily growing down into the dermis (almost like an intradermal nevus, but with malignant cells) and that it produces a raised lesion

Most melanomas have both a horizontal and a vertical component, which is the classic irregularly shaped dark macule with a central raised or ulcerated papule.

Features of Malignancy

Unfortunately there is no single feature than can rule melanoma in or out. As with many types of neoplasia, there are certain features that suggest malignancy, and the presence of enough of them can convince you of the diagnosis. Many of these criteria are subjective and require experience, which is why dermatopathology is such a booming subspecialty these days.

On low power, look for the following:

- Asymmetry
- Poorly circumscribed, pleomorphic, discohesive nests of melanocytes

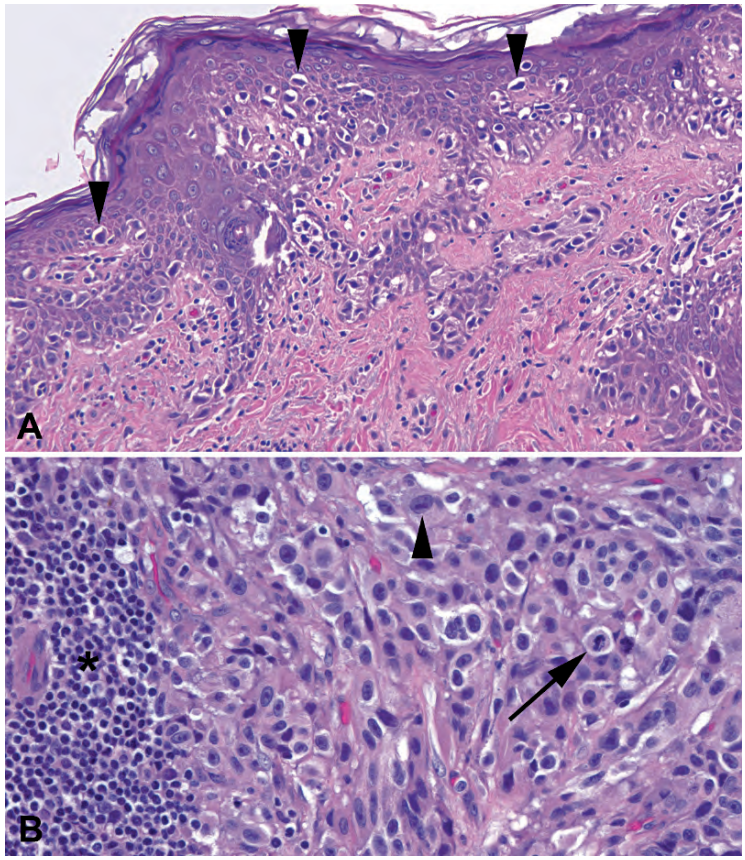

FIGURE 27.9. Melanoma. **(A)** Large, dark, irregular melanocytes can be seen infiltrating upward through the epidermis (arrowheads) in pagetoid fashion. If there were no dermal component, this would qualify as melanoma in situ. **(B)** Malignant melanocytes deep in the dermis. Large atypical nuclei with large nucleoli (arrowhead) plus the presence of mitoses (arrow) is diagnostic of invasive melanoma. Adjacent lymphocytes (asterisk left side of the field) are common.

- Shouldering (lateral spread) of atypical melanocytes
- Pagetoid spread through the epidermis (Figure 27.9)
- Associated lymphocytes, especially band-like

On high power, look for the following:

- Atypia, as described earlier
- Lack of deep maturation (for dermal component)
- Mitoses or atypia in the dermis (see Figure 27.9)
- Melanocytic necrosis

Sign Out Criteria

All diagnoses of invasive (into the dermis) melanoma must include certain prognostic features. The first is the depth of invasion. This is called Breslow's thickness and is the depth (to the hundredth of a millimeter) of invasion from the top of the epidermal granular layer to the deepest malignant cell. It is functionally equivalent to stage; the deeper the invasion, the poorer the prognosis. Clark's level is a related concept but is based on the histologic layers or levels of the dermis, not the absolute depth.

The second important prognostic feature is the presence or absence of ulceration. The third is the margin status, both deep and lateral.

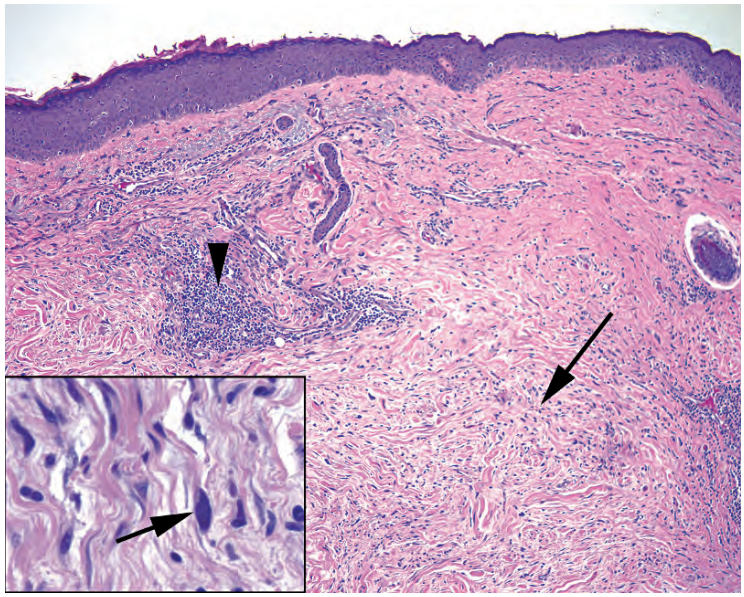

Figure 27.10. Desmoplastic melanoma. At low power, there appears to be a hypocellular scar in the dermis (arrow). The clue to melanoma lies in the collection of lymphocytes (arrowhead). **Inset**: On higher power, there are enlarged and hyperchromatic cells (arrow) in the "scar." These would be positive for S100, unlike fibroblasts.

Special Types of Melanoma

In *desmoplastic* and spindle cell melanoma, melanocytes can become very spindly and sarcomatoid. With an unidentified spindle cell lesion in the dermis, you must always rule out melanoma. The scariest and most subtle form is the desmoplastic melanoma, which is not only spindly but often sparsely cellular in a background of dense collagen; in other words, it looks just like a scar. A useful tip-off to a lurking desmoplastic melanoma is, aside from a slightly "busy" dermis, the presence of bands or clumps of lymphocytes (Figure 27.10).

Another type of melanoma is *acral lentiginous*. *Acral* refers to the distal extremities. Like the benign acral nevus, this lesion is characterized by prominent lentiginous growth. It can be very difficult to distinguish from an acral nevus.

Metastases can look like anything at all; they can be spindly, epithelioid, rhabdoid, small cell, and so forth. However, common features of metastases include alveolar (nested) architecture, large pink-to-violet cells with big nuclei and red nucleoli, and occasional melanin pigment. As a rule of thumb, if you don't know what it is, consider melanoma.

Re-excision of Melanomas

When a melanoma is diagnosed on excisional biopsy, it is nearly always given a wide re-excision. You will see these huge ellipses on surgical pathology. Pathologists differ in how much of the re-excision to submit, but at the least the entire biopsy scar, to the lateral margins, should be submitted. Carefully scan not just the epidermis but also the dermis deep to the biopsy site.

Special Stains

S100 is the workhorse stain for melanoma, as it stains all types. HMB-45 and Melan-A are also melanoma markers, but notably do not stain spindled or desmoplastic melanomas. Also remember that there is a whole family of tumors that are HMB-45 positive but not melanoma (angiomyolipoma and the perivascular epithelioid cell tumors).

Sentinel nodes for melanoma, if negative by H&E, are stained using HMB-45 and Melan-A. There are inherent S100-positive cells in lymph nodes, so it is not a good screening stain for this purpose.

There are, unfortunately, no stains that can differentiate a benign nevus from a malignant melanoma. However, in general, HMB-45 should only stain the most superficial cells in a nevus, as the deeper maturing component loses the antigen. Similarly, Ki-67, a proliferation marker, should only be positive at the surface of a nevus, not deep.

Nonmelanocytic Lesions

This section will address the lesions, both neoplastic and hyperplastic, that are not made of melanocytes. These include squamous lesions, cysts, adnexal tumors, and miscellaneous common soft tissue tumors of the dermis.

Sun Damage

The first major category of tumors is the spectrum of disease seen in sun-exposed skin, typically the face, neck, and arms of adults. A general marker of sun exposure is *solar elastosis*, which is an accumulation of grey wispy damaged elastin in the dermis (Figure 27.11). Ironically, it represents a loss of elasticity (wrinkles). One of the benign changes seen in the context of sun exposure is the *solar lentigo* (lentigo senilis, age spot), which appears as a finger-like proliferation of hyperpigmented rete growing down from the epidermis (Figure 27.12). Keratinocytes, not melanocytes, are the pigmented cells.

The solar lentigo may then develop into a dysplastic lesion called an *actinic keratosis*. Actinic keratoses have a wide variety of appearances, from very thin with subtle atypia to very hypertrophic with full-thickness atypia. However, the defining features of an actinic keratosis should include the following:

- Squamous atypia of varying thickness, often noticeable only in comparison to the surrounding uninvolved epidermis (Figure 27.13)
- Alteration of the keratinization to become pink and parakeratotic
- Sparing of the keratin above the hair follicles, classically resulting in alternating columns of parakeratosis and orthokeratosis
- Underlying solar elastosis

Actinic keratosis is regarded, conceptually, as a form of carcinoma in situ, but its natural history is unpredictable. Actinic keratoses can (rarely) invade before they reach full-thickness

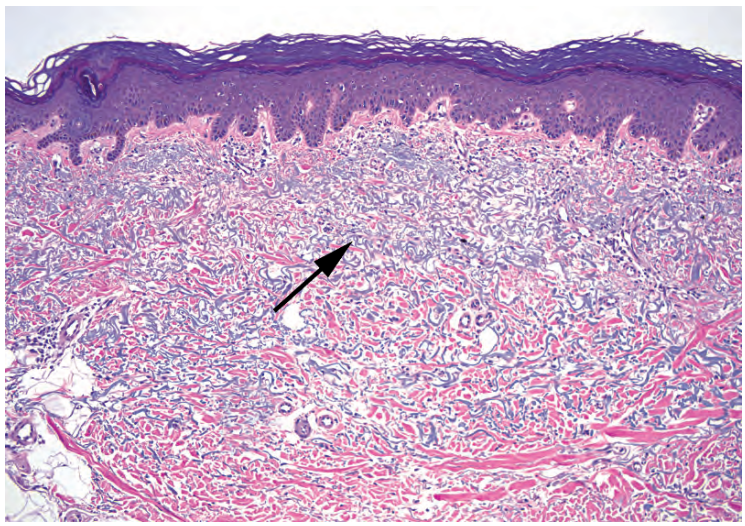

Figure 27.11. Solar elastosis. This is the typical microscopic appearance of sun-damaged skin. The collagen is replaced by wispy gray-blue strands of elastin (arrow).

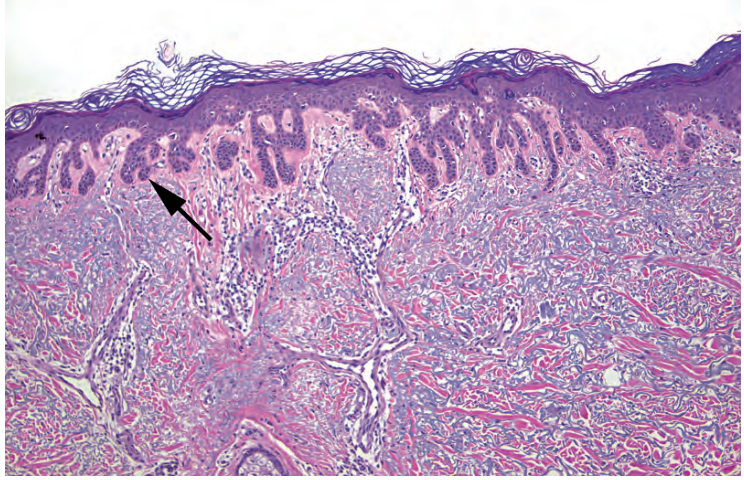

Figure 27.12. Solar lentigo. Prominent rete are growing down from the epidermis (arrow), with increased basal pigmentation (not clearly visible at this power). Notice the underlying solar elastosis. Compare this lesion to the lentigo simplex (see Figure 27.3), which, in contrast, shows a proliferation of melanocytes.

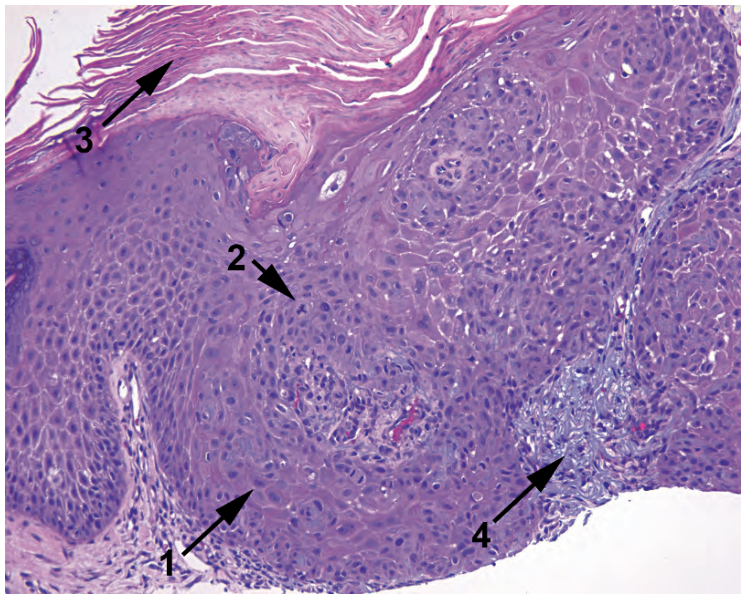

Figure 27.13. Actinic keratosis. This example shows an area of disorganized and enlarged nuclei (1) with prominent and atypical mitoses (2), consistent with dysplasia. There is overlying hyperkeratosis and parakeratosis (3) and underlying solar elastosis (4). This is a slightly tangential cut through the skin, making the lesion appear very thick.

atypia, unlike squamous lesions of the cervix. However, when the atypia does reach full thickness (assuming no invasion), these lesions may be called *bowenoid actinic keratosis* or just *squamous cell carcinoma in situ* to emphasize the severity of the lesion.

Carcinoma in situ is a simple concept in other organs, but in the skin it is a source of great debate. Most dermatopathology chapters are littered with the phrase "*we* use the term…"—meaning each camp has their own philosophy and style. Part of the problem is that the path from dysplasia to invasive carcinoma looks more like a metro subway map than a straight line. However, the basic idea is that carcinoma in situ has not yet crossed the basement membrane

and that there is some degree of epidermal dysplasia. Entities that fall under the category of carcinoma in situ include the following:

- Actinic keratosis (as discussed earlier) and bowenoid actinic keratosis
- Bowen's disease—often used as a synonym for carcinoma in situ but actually describes a particular clinical presentation that occurs on non–sun-damaged skin and does not spare the hair follicles, unlike the actinic keratosis family
- Bowenoid papulosis—a human papillomavirus–related lesion of genital sites

Invasive squamous cell carcinoma is most likely to arise from the sun-damaged, actinic keratosis–type pathway and hence is usually seen in the background of solar elastosis and actinic keratosis–like changes. Features that suggest invasion include penetration of nests deep into the dermis, accompanied by an aberrant deep keratinization (pinkness). Finding single cells invading the dermis is fairly conclusive (Figure 27.14). The appearance of squamous cell carcinoma is similar to that found in other sites (see Chapters 16 and 22).

Basal cell carcinoma is another of the common sun-related tumors. It is the most common cutaneous malignancy and, despite its reputation as a sort of ho-hum and uninteresting tumor, has a very wide range of appearances. There is also some overlap between basal cell carcinoma and benign adnexal tumors; the latter are probably often missed. Features of basal cell carcinoma include the following:

- Lobules of small, blue, basal-type keratinocytes with peripheral palisading (picket-fence) arrays of oblong nuclei (Figure 27.15)
- Formation of clefts (cracks) between the tumor nests and the stroma
- Sometimes (not always) desmoplasia, focal keratinization, or mucin production

At low power the basal cell carcinoma nests can look similar to adnexal structures, making margins challenging. However, basal cell carcinoma tumor cells should have darker chromatin, more apoptosis and mitoses, and paler cytoplasm than the hair follicles.

Special types of basal cell carcinoma include *nodular* (the usual type), *superficial multicentric*, and *sclerosing*. The superficial multicentric form tends to hang off the epidermis like stalactites, without forming a mass, and can have skip areas (harder to excise). The sclerosing form shows a prominent desmoplastic response. There are up to 20 more subtypes; a large dermatopathology atlas will show the many faces of basal cell carcinoma.

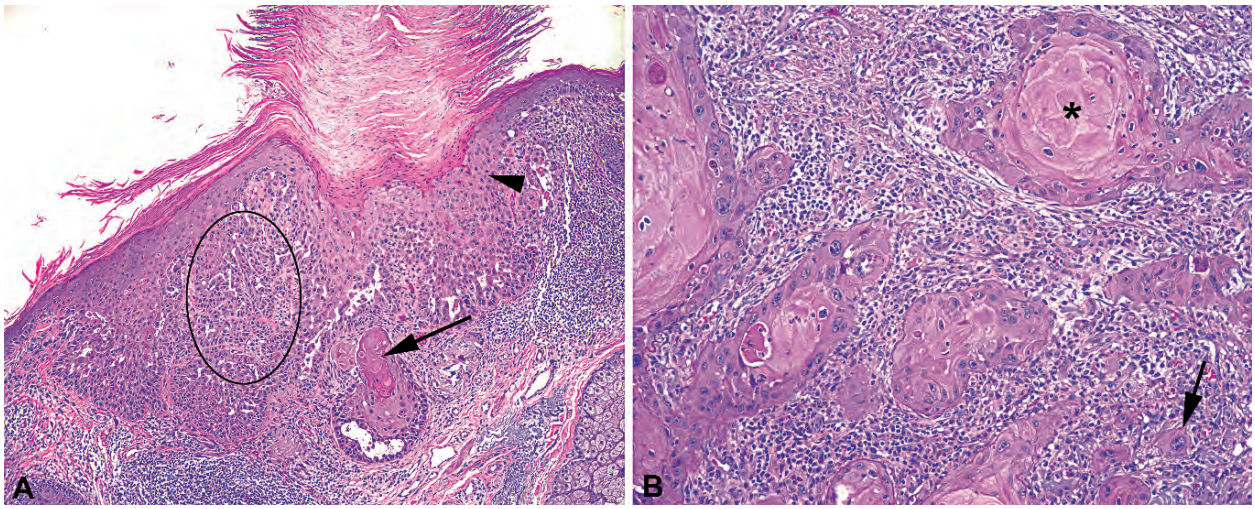

FIGURE 27.14. Squamous cell carcinoma. **(A)** Superficially invasive squamous cell carcinoma, showing the paradoxic deep keratinization (arrow), indicating an invasive nest. Actinic keratosis–type changes are seen in the overlying epidermis (arrowhead), including hyperkeratosis. In one area, the pattern of thin cords of cells infiltrating the stroma (oval) is too complex to be explained by a funny plane of section and is another pattern of invasion. **(B)** Higher power view of invasive squamous cell carcinoma, showing keratin pearls (asterisk) and infiltrating single cells (arrow).

Other Hyperkeratotic but Nonneoplastic Lesions

Seborrheic keratoses are very common, benign lesions that have many, many forms, but the usual picture is a hyperkeratotic, orthokeratotic lesion with a markedly thickened epidermis. It often forms a raised plaque on the skin; on the slide, the epidermis looks as though it was accidentally cut *en face*, with convoluted, confluent cords of epidermis. Horn cysts, which are entrapped whorls of orthokeratin, are common (Figure 27.16). (These are quite different from the squamous pearls of carcinoma, which are pink and parakeratotic.) Pigment and inflammation may be seen; atypia is not. These are not necessarily associated with sun damage.

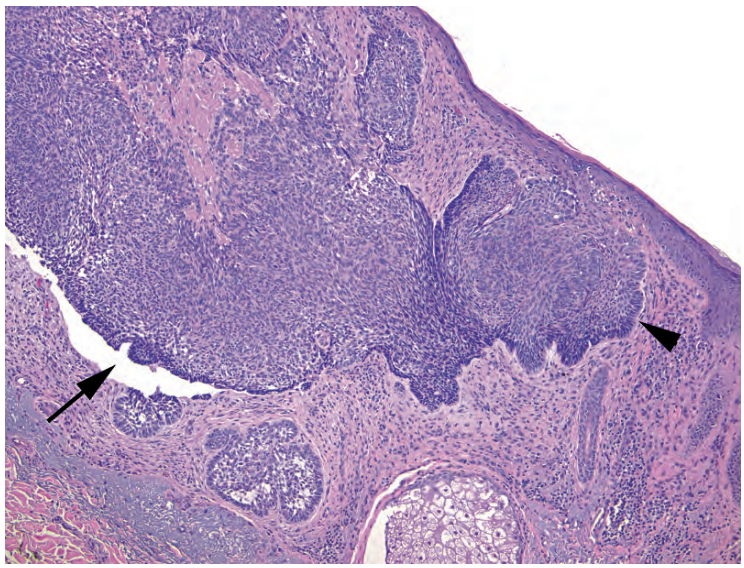

Figure 27.15. Basal cell carcinoma. Blue nests of cells appear to drop down from the epidermis. There is prominent palisading of the basal cells at the periphery of the nests (arrowhead) and clefting of the tumor cells away from the stroma (arrow).

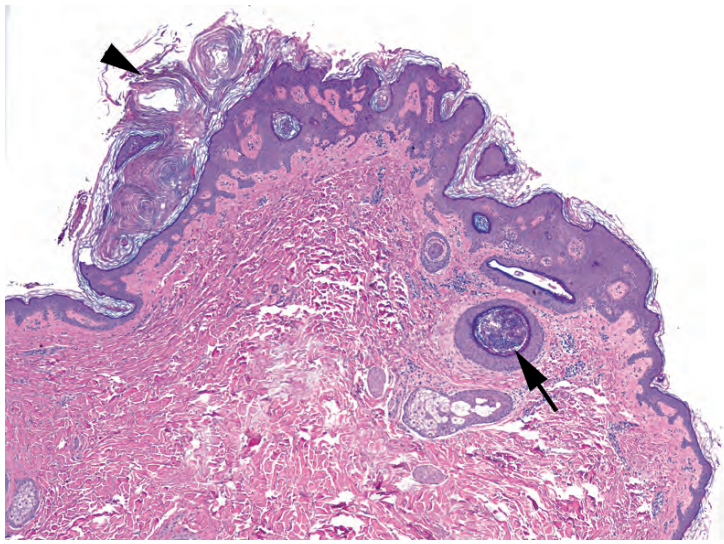

Figure 27.16. Seborrheic keratosis. This exophytic lesion shows hyperkeratosis (arrowhead) but not parakeratosis (no visible nuclei in the keratin). The epidermis takes on a complicated pattern of intertwining rete, and in some areas foci of keratin are trapped within the lesion, forming horn cysts (arrow). Compare these blue, acellular, lamellated balls of keratin with the pink keratin pearls of squamous cell carcinoma (see Figure 27.14).

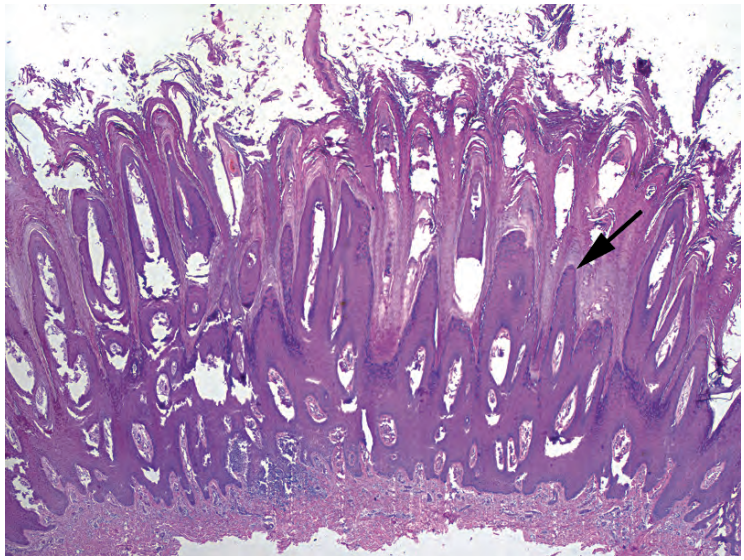

Figure 27.17. Verruca vulgaris. The epidermis in this wart is thrown up into sharply pointed spires, which are topped by hyperkeratosis and parakeratosis.

Verruca vulgaris (the common wart) is a virally induced circumscribed lesion, usually on the fingers, which shows a striking epidermal proliferation ("church spires") with overlying hyperkeratosis (Figure 27.17). The tips of the spires are often topped by parakeratosis, which can lead to a striped effect. Koilocytes (review the description of this viral change in Chapter 16) may be hard to identify. Related lesions are the planar (flat) and plantar (endophytic) warts, as well as the condylomata (genital).

If you cannot quite tell if a lesion is a seborrheic keratosis or a wart, compromise and call it a verrucous keratosis. Verrucous carcinoma, a deceptively innocuous cancer, is not usually in the differential diagnosis for skin: it is mainly seen on mucosal sites.

Adnexal Tumors

Adnexal tumors are a large, mystifying, shape-shifting group of lesions encompassing follicular, eccrine, apocrine, and sebaceous lesions. Some of the more readily identifiable tumors are listed here. Most of these are benign, although carcinomas do exist. Of the carcinomas, many are similar to those found in breast or salivary gland (similar embryologic origin), such as adenoid cystic carcinoma, mucoepidermoid carcinoma, and ductal adenocarcinomas.

The *eccrine poroma/acrospiroma/hidradenoma* group are tumors of the sweat ducts and get different names depending on where in the dermis or epidermis they arise. They are composed of cells that look similar to keratinocytes but that try to form ducts (usually tiny lumens in a sheet of cells). The cells are streamy, pale, and disorganized, not unlike usual ductal hyperplasia in the breast (Figure 27.18).

Eccrine spiroadenomas are "blue cannonballs in the dermis." Tumor balls consist of two basaloid cell lineages (often hard to separate) and have noticeable cords and droplets of hyaline basement membrane substance running through them (Figure 27.19). A related lesion is the cylindroma.

The *cylindroma* ("jigsaw puzzle") also has basaloid (blue) nests in the dermis, also with two cell populations and basement membrane matrix. However, the tumor nests are mosaic in shape.

The *syringoma* is a collection of round, dilated tubules in the dermis with characteristic comma-like or tadpole tails (Figure 27.20). *Trichoepithelioma* is a benign tumor of hair follicle differentiation that looks a lot like a basal cell carcinoma except with horn cysts, hair formation, little abortive follicles, fibrotic stroma, and a lack of clefting. *Microcystic adnexal carcinoma* (sclerosing sweat duct carcinoma), although rare, is the malignant one you do not

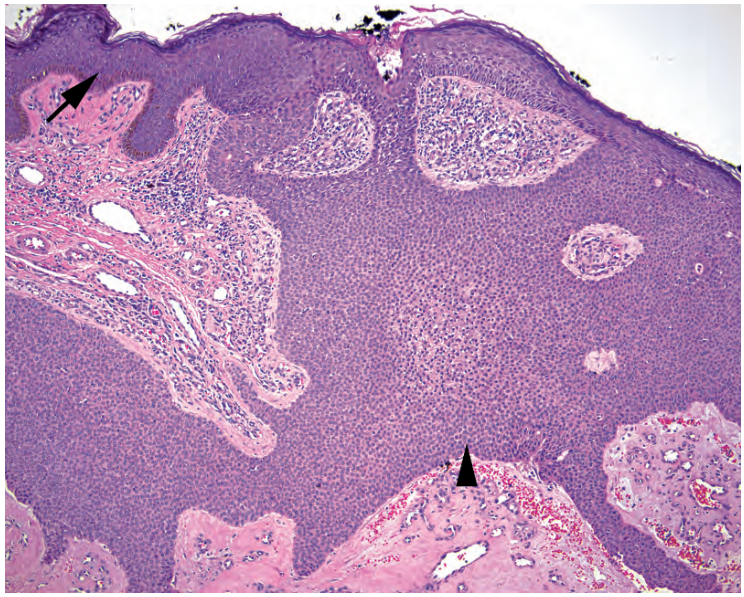

Figure 27.18. Poroma. This eccrine tumor is continuous with the epidermis, which can be seen at left (arrow). The tumor cells (arrowhead) are uniform, small, round, and pale and in some areas may form rudimentary duct spaces.

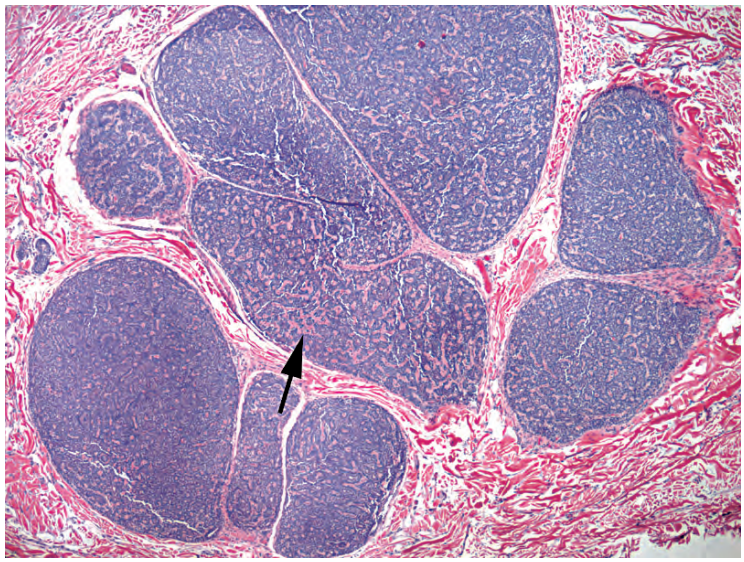

Figure 27.19. Eccrine spiroadenoma. "Cannonballs in the dermis" is the catch phrase for this tumor. Like the poroma, the cells are small and bland. Cords of hyaline pink basement membrane material are seen throughout the tumor (arrow).

want to miss. It looks similar to syringoma, with tubules and cords of bland cells, and also has horn cysts (Figure 27.21). What differentiates it from syringoma is the deep infiltration of the dermis, so dermatopathologists like to see the base of an adnexal lesion.

Cysts

The most common cysts you will see are the epidermoid cyst (sometimes called epidermal inclusion cyst) and the trichilemmal or pilar cyst (Figure 27.22). The *epidermoid cyst* is lined

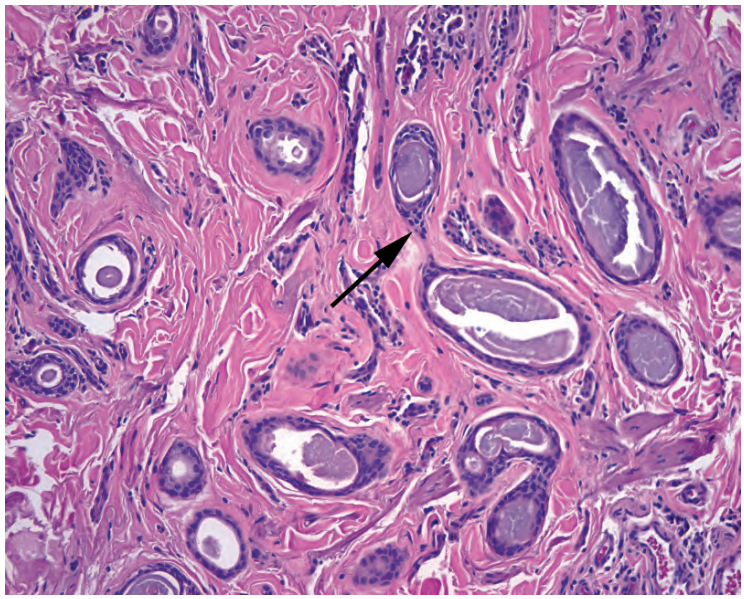

FIGURE 27.20. Syringoma. Small tubules with comma-like pointed tails within the dermis (arrow).

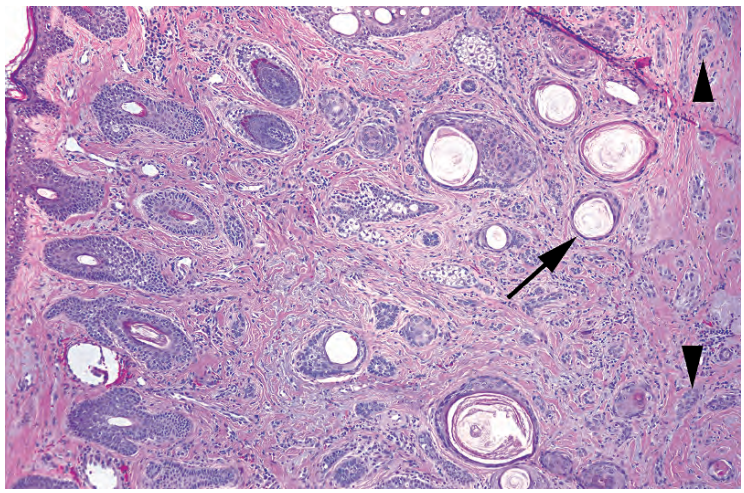

FIGURE 27.21. Microcystic adnexal carcinoma. A collection of small pale nests of cells can be seen in the dermis, some of them forming horn cysts (arrow). The feature that separates this from a benign lesion is the small nests that are infiltrating deeply into the base of the lesion at right (arrowheads). This small carcinoma infiltrated into the subcutaneous fat (not seen here).

by mature squamous epithelium with a granular layer and filled with layers of flaky keratin. The *pilar cyst* is lined by plump pillowy keratinocytes with *no granular layer* and is filled with dense compact keratin.

Dermal Tumors

Probably the three most common benign soft tissue tumors of the dermis are the dermatofibroma, the neurofibroma, and the hemangioma. More information about soft tissue tumors can be found in Chapter 28.

Dermatofibromas appear as an ill-defined blue haze in the dermis. On higher power, the blue haze is made up of tiny swarming nondescript cells that infiltrate the collagen and tend to packet it into thick bundles (Figure 27.23). The overlying epidermis may be hyperpigmented and hypertrophic (hence its presentation as a brown nodule). Dermatofibrosarcoma protuberans is the malignant counterpart of this lesion and is more deeply infiltrative, wrapping around the subcutaneous fat in a characteristic pattern (Figure 27.24).

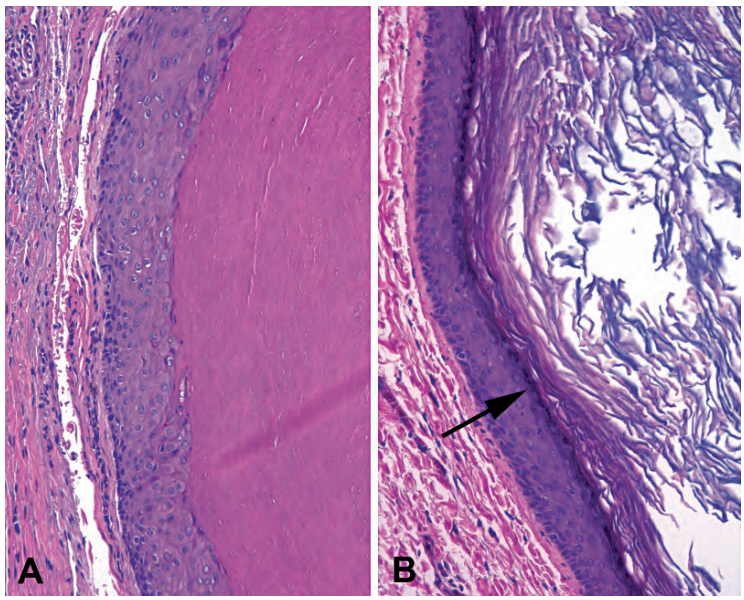

Figure 27.22. Cysts. **(A)** The trichilemmal cyst has no granular layer, with large pink puffy cells showing an abrupt transition to dense "wet" appearing keratin. **(B)** The epidermoid cyst more closely resembles epidermis, with a granular layer (arrow) and layers of "dry" flaky keratin.

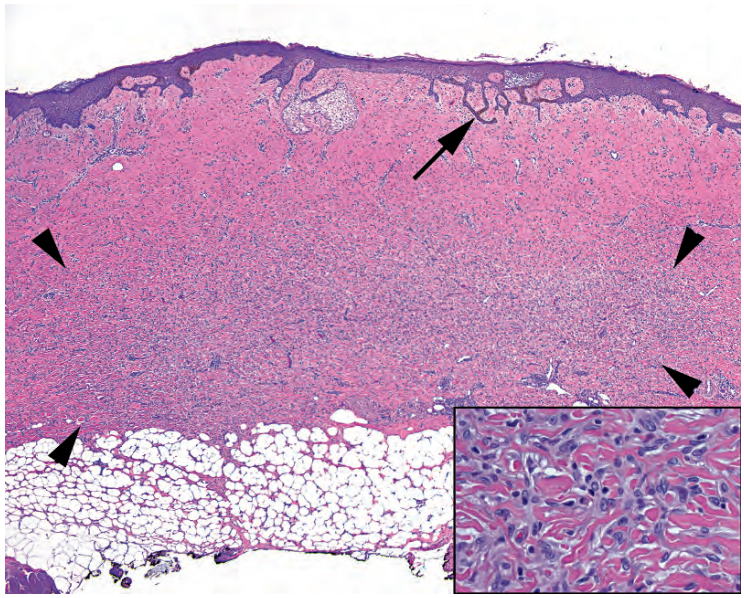

Figure 27.23. Dermatofibroma. This poorly circumscribed tumor creates a blue haze in the dermis (outlined by arrowheads), and the epidermal rete above it may become pigmented and prominent (arrow). The lesion mainly stops at the subcutaneous fat. **Inset**: The cells infiltrate between collagen bundles but have small pale round-to-oval nuclei.

Neurofibromas more often appear as a pale or grey nodule in the dermis, more defined than the dermatofibroma. It displaces the dermis rather than infiltrating it. The individual cells have wavy nuclei and wavy collagen, like overstretched elastic (Figure 27.25).

Hemangiomas are a proliferation of well-formed, dilated capillaries in the dermis (Figure 27.26). There are many variants. The malignant counterpart, angiosarcoma, is more cellular and has anastomosing channels lined by plump cells. Early Kaposi's sarcoma is so subtle it is basically invisible to the inexperienced and is not likely to simulate a hemangioma (Figure 27.27). Pyogenic granuloma, or lobular capillary hemangioma, is a common benign lesion that may be very cellular and inflamed but is identified as benign due to its rounded and circumscribed periphery.

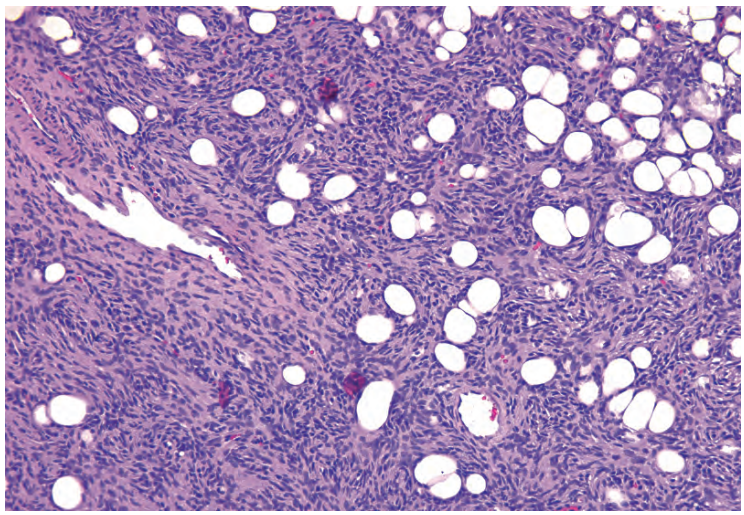

Figure 27.24. Dermatofibrosarcoma protuberans (DFSP). The DFSP is more cellular than the dermatofibroma and shows a prominent storiform pattern of spindled cells infiltrating between fat cells.

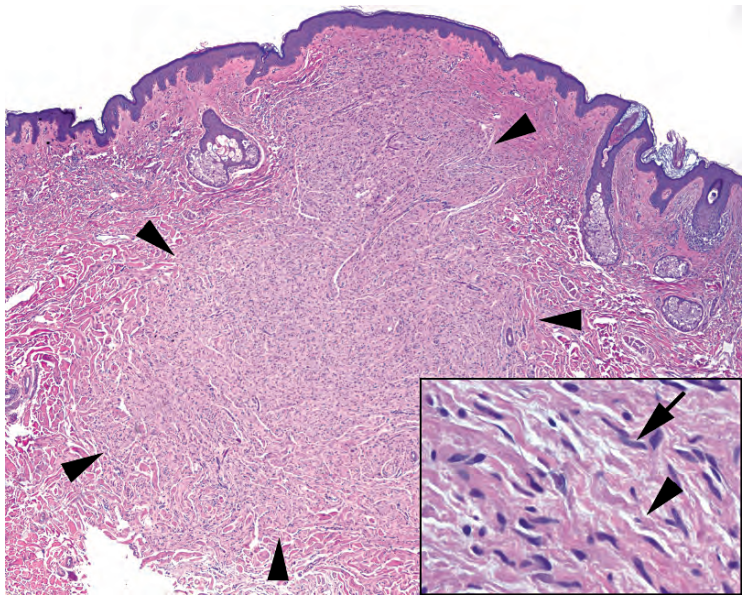

Figure 27.25. Neurofibroma. Like the dermatofibroma, this diffuse neurofibroma is a poorly defined dermal tumor (arrowheads), but, unlike the dermatofibroma, it tends to appear more pale than the surrounding dermis. **Inset**: On higher power, the tapering, undulating nuclei (arrow) are visible, as is the background of wavy collagen fibers (arrowhead).

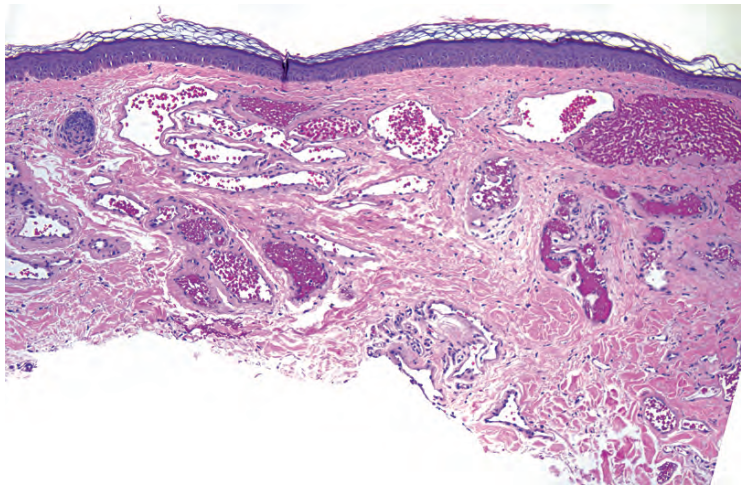

Figure 27.26. Capillary hemangioma. There is a collection of discrete, well-formed, dilated capillaries under the epidermis.

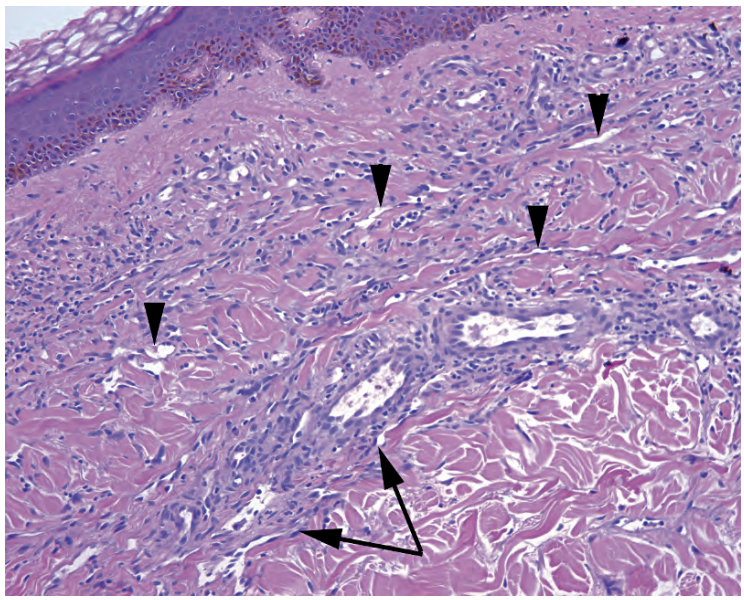

Figure 27.27. Kaposi's sarcoma. The subtle cellularity to the dermis is actually composed of slit-like vascular spaces with bland endothelium (arrowheads). The slit-like spaces are accentuated around existing capillaries (arrows), the "promontory sign."

A Brief Introduction to Nonneoplastic Skin: Patterns of Inflammation and Injury

This section is simply a primer on the terminology and classification of the inflammatory skin diseases; the details are beyond the scope of this chapter. These diagnoses are heavily influenced by the clinical presentation, so the goal here is to understand the histologic families of disease. Arriving at a specific diagnosis requires clinical information and, usually, fellowship training.

Injury to the Epidermis

1. The epidermis can become acutely damaged or inflamed. The result is edema, or *spongiosis*. This is seen as an accentuation of the spaces between keratinocytes. Severe edema can cause

intraepidermal vesicles to form (i.e., poison ivy), which have to be distinguished from true bullae (see later).

If this process continues for a while, the epidermis becomes less edematous and more hyperplastic as it responds to the chronic insult. The hyperplasia is in the form of thickening of the epidermis and elongation of the rete and is called *acanthosis*, usually accompanied by *hyperkeratosis* (a protective thickening of the keratin layer).

These changes make up the spectrum of *acute to chronic spongiotic dermatitis*, clinically eczema, and there is a large differential. Usually the pathologist signs the biopsy out descriptively, and the dermatologist combines that with the clinical features to diagnose it. *Psoriasis* fits in here, as it has histologic overlap with chronic spongiotic dermatitis, especially when partially treated.

2. Some inflammatory processes attack the basal layer of keratinocytes. This pattern is called *interface dermatitis*. Interface dermatitis has two predominant patterns that may overlap. One is an intense lymphocytic infiltrate at the DEJ, which is called *lichenoid* inflammation or dermatitis. The second is a vacuolar degeneration of the basal cells, or *vacuolar dermatitis*. Both of these patterns result in a ragged DEJ, dyskeratotic or necrotic basal cells trapped in the epidermis (colloid bodies), and a cleavage plane along the DEJ if the damage is severe enough. This can be mistaken for a bullous disease, which is a different process.

The prototypical lichenoid dermatitis is *lichen planus*. Vacuolar dermatitis has a wider differential, including *acute graft-versus-host disease, lupus*, and *erythema multiforme*.

3. A third pattern is the dissolution of the intercellular bridges that link the keratinocytes. The cells break apart and round up into individual cells, a process called *acantholysis*. This process is often antibody mediated, so immunofluorescence is important. The acantholysis can coalesce into large spaces within the epidermis, or *bullae*. Different diseases cleave the skin within different planes of the epidermis.

This group includes the inflammatory bullous diseases, such as *pemphigus, bullous pemphigoid*, and *dermatitis herpetiformis*. A noninflammatory bullous disease is *porphyria cutanea tarda*. There is also familial acantholytic disease (*Hailey-Hailey* and *Darier diseases*), transient acantholytic disease (*Grover's disease*), and focal acantholytic lesions (*warty dyskeratoma*).

Inflammation of the Dermis

The patterns of injury discussed in this section are limited to the dermis, usually with a fairly unremarkable epidermis. Many diseases begin with a nonspecific pattern of *perivascular lymphocytic inflammation* in the dermis. It is the first sign that the skin is upset. Some diseases never declare themselves beyond this stage, such as *polymorphous light eruption* and *urticaria*. If the inflammation progresses to involve neutrophils and actual damage to the vessels, the disease is called a *vasculitis*. *Leukocytoclastic vasculitis*, in which the vessels show fibrinoid necrosis and nuclear debris (karyorrhexis), has a wide clinical differential diagnosis.

Inflammatory infiltrates of the dermis are classified based on the type of infiltrate. A dense neutrophilic infiltrate is a *neutrophilic dermatosis*, such as *Sweet's syndrome*. Granulomatous inflammation may indicate infection, foreign body response, sarcoidosis, or *granuloma annulare*. *Mycosis fungoides*, or cutaneous T-cell lymphoma, can have numerous appearances, but a dense lymphocytic infiltrate should trigger a workup for mycosis fungoides.

Some diseases involve alteration of the collagen of the dermis. These include scar, keloid, *scleroderma* or *morphea*, and *lichen sclerosus. Chronic graft-versus-host disease* can look like scleroderma as well.

Inflammation of the Deep Subcutaneous Tissue (Panniculitis)

Panniculitis is divided into septal, where the inflammation is mostly in the fibrous septae between fat lobules, and lobular, where the fat itself is involved. The classic septal panniculitis is *erythema nodosum. Lupus profundus*, or deep lupus, is a lobular panniculitis.

28 Soft Tissue and Bone

Tumors of soft tissue are among the most challenging in surgical pathology. There are several reasons for this: they are rare, so you see few in training; they are overlapping in morphology; they do not always obey the principles that help you to identify malignant potential in carcinomas; and each entity has at least three names, four variants, and seven mimickers. However, we will cover some of the names you will hear most commonly. The tumors are broken down into lines of differentiation, with the caveats that there are some tumors that do not differentiate along any known lineage (grouped separately) and that many soft tissue tumors dedifferentiate into the same final common malignant pathway, the entity formerly known as *malignant fibrous histiocytoma* (MFH). In many instances, MFH is simply a generic dedifferentiated sarcoma, the high-grade form of any one of a number of precursor lesions. The good news is, once it is that high grade, the origin becomes sort of academic.

When diagnosing a soft tissue lesion, especially in its initial presentation, you must always walk yourself through the mental game of, *"what else could this be?"* It is a good habit for any organ system but especially in the field of sarcomas and spindle cell lesions. For lesions that do not look clearly malignant (by which we mean they lack nuclear atypia and necrosis), you must always consider that it might be a reactive lesion. For lesions with bizarre and huge nuclei, despite the malignant look, you must consider benign entities with degenerative atypia (such as an ancient schwannoma). For lesions in or near an organ, such as in visceral sites, you must always ask yourself if it could be a carcinoma masquerading as a sarcoma. For spindle cell lesions anywhere, you must ask yourself if it could be melanoma. Some of these questions require immunostains to answer, some just a skeptical eye.

The second question to ask, once you have ordered the cytokeratins and melanoma markers, is *"what family of soft tissue does it belong in?"* Table 28.1 lists some stereotypical features of different tumor families, seen best in low-grade (well-differentiated) lesions.

High-Grade Sarcomas

As mentioned, once sarcomas turn high grade, they may take on any number of appearances, regardless of differentiation. Some classic visual patterns are shown in Figure 28.1 and described in Table 28.2.

In high-grade sarcomas, the subtype may be clarified through immunostains, history, or the low-grade remnants of the tumor found at the periphery. Identifying the line of differentiation can be helpful in determining prognosis. Solving this puzzle is one of the more interesting games pathologists can play and breaks up the monotony of routine biopsies. However, from a clinical perspective, the oncologist is more concerned about the grade than the type, and, fortunately for all of us, high-grade sarcomas are hard to miss.

Table 28.1. Characteristics of tumor families.

Lipomatous ("lipo")	Fat cells intermixed with other elements; fat cells are identified by their crescent-shaped nuclei hugging large clear vacuoles
Fibrous ("fibro")	Fibroblasts and myofibroblasts are typically fusiform or stellate cells with pale nuclei in a collagenous (pink) matrix
Smooth muscle ("leiomyo")	Smooth muscle cells are elongated cells that run in parallel bundles, intersecting at right angles. The nuclei may be cigar or corkscrew shaped and often have paranuclear vacuoles
Skeletal muscle ("rhabdomyo")	Skeletal muscle may show either rhabdoid cells, which are plump round cells with eccentric nuclei and pink cytoplasm, or strap cells, like individual elongated myocytes with cytoplasmic cross-striations
Nerve sheath (neurofibroma, schwannoma)	Nerve sheath tumors may show delicate spindle cells with wavy nuclei in a myxoid background with thin curly tendrils of collagen, as in a neurofibroma. They may also show the dense nuclear palisading and fibrillar background of a schwannoma
Vascular ("hemangio," "angio")	Vascular tumors are characterized by a network of irregular vascular spaces, often with admixed blood. Malignant endothelial cells tend to protrude into the lumens with a hobnail appearance

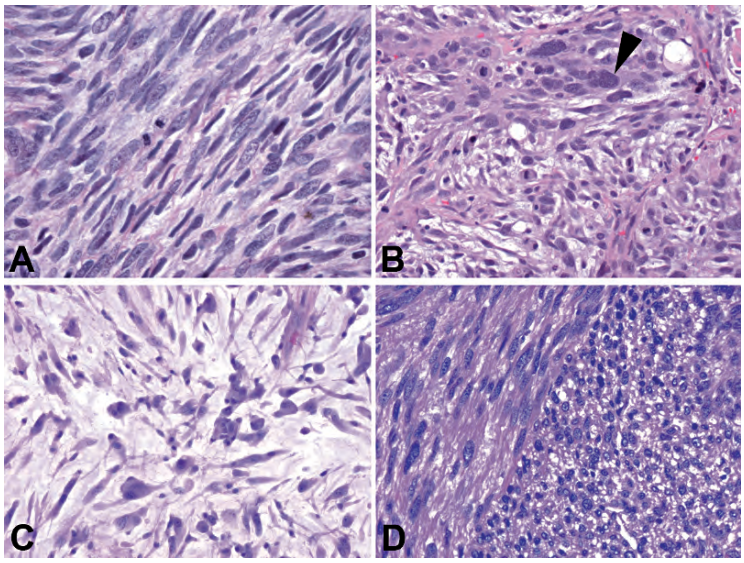

Figure 28.1. High grade sarcomas. **(A)** Fibrosarcoma, with densely packed hyperchromatic spindle cells. **(B)** Pleomorphic MFH, with very large and bizarre cells (arrowhead). **(C)** Myxoid MFH or myxofibrosarcoma, showing pleomorphic cells in a myxoid background. **(D)** Leiomyosarcoma, with perpendicular fascicles.

Table 28.2. Features of high-grade sarcoma patterns.

Fibrosarcomatous appearance	A hypercellular, fascicular tumor with a "herringbone" pattern. Atypia may not be significant. May be seen in fibrosarcoma, malignant peripheral nerve sheath tumor, synovial sarcoma, and others
Malignant fibrous histiocytoma (MFH), pleomorphic	A cellular tumor with bizarre nuclear atypia, including giant cells and highly pleomorphic and hyperchromatic nuclei. Very mitotically active, often with necrosis. Now generally called *pleomorphic undifferentiated sarcoma*
MFH, myxoid	A tumor with a myxoid or edematous background containing highly pleomorphic cells, frequent mitoses, and characteristic arcing vessels. The entity of myxoid MFH is synonymous with myxofibrosarcoma, but other sarcoma types (lipo-, leiomyo-, etc.) may take on this pattern
Leiomyosarcoma	A fascicular tumor with bundles of cells intersecting at right angles, a high mitotic rate, and significant cytologic atypia, although not as pleomorphic as the MFH. Non–smooth-muscle tumors may occasionally show this pattern
Rhabdomyosarcoma	A tumor with large eosinophilic cells with eccentric nuclei, showing significant nuclear pleomorphism (with the exception of the alveolar type). May occur as a component of other high-grade sarcomas

A reliable clue to a high-grade sarcoma is the presence of malignant nuclei. A sarcoma nucleus has some reproducible features across many tumor types. The nucleus has an irregularly shaped border and has dark, dense, granular chromatin that is fairly evenly distributed throughout the nucleus (Figure 28.2). Unlike carcinoma nuclei, prominent nucleoli and nuclear membranes are *not* a usual feature. Learning to recognize this sort of atypia is critical in identifying some of the subtle sarcomas.

Tumors of Fat

Throughout this chapter, you will find tables listing some of the more common entities, grouped by clinical behavior. Table 28.3 lists some of the common tumors of fat.

The most common soft tissue tumor is the *lipoma*. A lipoma is defined as a neoplasm of mature fat. It is histologically indistinguishable from ordinary fat; to tell the difference you must know it appeared clinically as a discrete lobular mass. There are many histologic variants of lipoma, classified based on what additional soft tissue component is present, such as the angiolipoma, fibrolipoma, angiomyolipoma, and so forth. The hibernoma is a lipoma of brown fat, in which the fat cells are full of innumerable tiny vacuoles. The lipoblastoma, despite the alarming name, is a benign pediatric tumor of mature fat and benign lipoblasts.

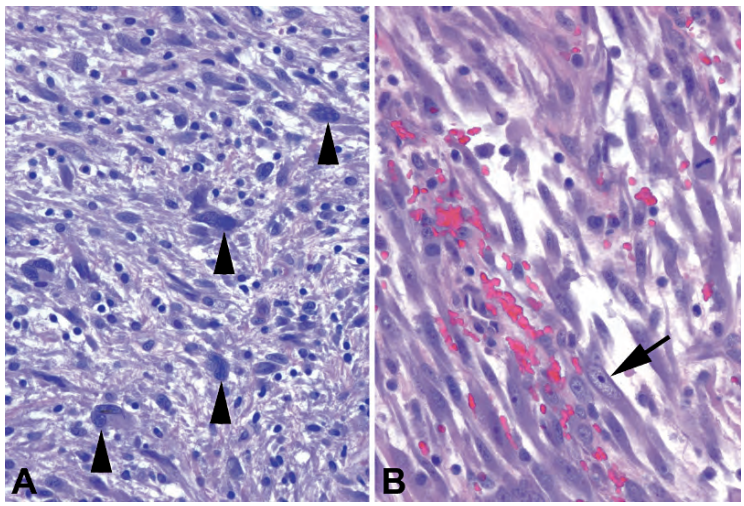

Figure 28.2. The sarcoma cell vs. the reactive cell. **(A)** Malignant cells in a MFH or other high-grade sarcoma show large nuclei with irregular shapes and very dark chromatin with a coarse texture (arrowheads). It is as though (in fact, it is likely) the nucleus has way too many chromosomes, and the nucleus is swollen and dark with the extra chromatin (truly hyperchromatic). Nucleoli are not usually prominent. **(B)** Reactive fibroblasts in nodular fasciitis have large nuclei and prominent nucleoli that stand out against a pale nucleus (arrow). The nuclear membrane is smooth and oval.

Table 28.3. Common neoplasms of fat.

Benign	Malignant but indolent	Malignant and aggressive
Lipoma, including	Atypical lipoma or	Dedifferentiated liposarcoma
Angiolipoma	Well-differentiated liposarcoma	
Angiomyolipoma	Myxoid liposarcoma	Round cell liposarcoma
Hibernoma		
Lipoblastoma (children)		Pleomorphic liposarcoma (no relation to pleomorphic lipoma)
Myelolipoma		
Spindle cell lipoma		
Pleomorphic lipoma		

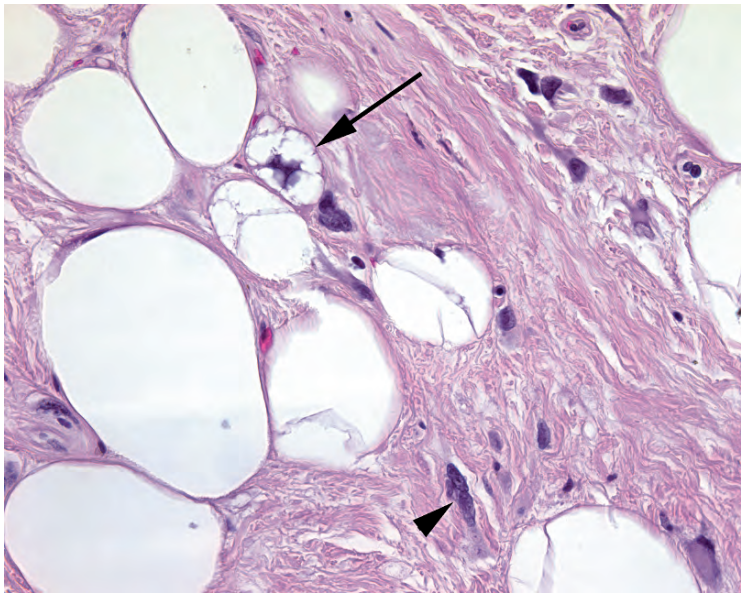

Figure 28.3. Lipoblast. Small fat vacuoles indent the nucleus of this lipoblast (arrow), seen in a well-differentiated liposarcoma. Other cells within the fibrous septa (arrowhead) have the look of sarcoma cells, with irregular, large, dark nuclei.

There is a lot of fuss about *lipoblasts*. They are immature fat cells in which the nucleus is star shaped, due to being indented on multiple sides by small bubbles of fat (Figure 28.3). They are often associated with liposarcomas, but they can also appear in the benign lipoblastoma, and they are not necessary for a diagnosis of liposarcoma (more on this later). Note that normal adipocytes are not mitotically active cells, so prominent mitoses are generally seen only in high-grade liposarcomas.

Two types of lipoma are notable for their unusual cytologic features. They are both usually found on the back or neck of elderly men and are noticeably fibrous and nonfatty on low power. These are the *pleomorphic lipoma* and *spindle cell lipoma*. The spindle cell lipoma has areas of nondescript spindle cells and collagen and may remind you of a nerve sheath tumor if there is not much fat in the lesion. The pleomorphic lipoma is similar, with the addition of large giant cells and floret cells (wreath-shaped nuclei). These giant cells (Figure 28.4) are an example of a benign lesion simulating malignant atypia; clinical information is helpful in not mistaking these for liposarcomas.

The *well-differentiated liposarcoma* (WDLS) is a tumor of adults. It looks similar to a lipoma on low power except for an increase in fibrous "interstitium" between fat cells and/or fibrous bands (Figure 28.5). A close examination of the fibrous areas reveals hyperchromatic, irregularly shaped nuclei; these are usually large and dark enough to be visible at 4×. Finding a lipoblast is a bonus. A softer feature is an assortment of differently sized fat cells, unlike the monomorphic benign lipoma. The WDLS is so named when it occurs in a nonresectable location, such as the retroperitoneum. By definition, when it occurs on an extremity, it is called an *atypical lipoma*, as the prognosis in these sites is excellent.

When the WDLS has been around for a while, especially in a recurrent or occult retroperitoneal lesion, there is a risk of the tumor transforming into a high-grade pleomorphic sarcoma. When this happens, you will see a tumor with well-differentiated lipomatous areas and an abrupt transition to a high-grade tumor (storiform/spindled, MFH-like, or even rhabdo- or leiomyosarcomatous). Regardless of morphology, this is called a *dedifferentiated liposarcoma*, and the key to diagnosis is recognizing the adjacent WDLS. Because dedifferentiated liposarcoma is the most likely diagnosis in a retroperitoneal pleomorphic sarcoma, if you are grossing such a tumor, be sure to sample anything near the tumor that looks like fat: it may be the well-differentiated component.

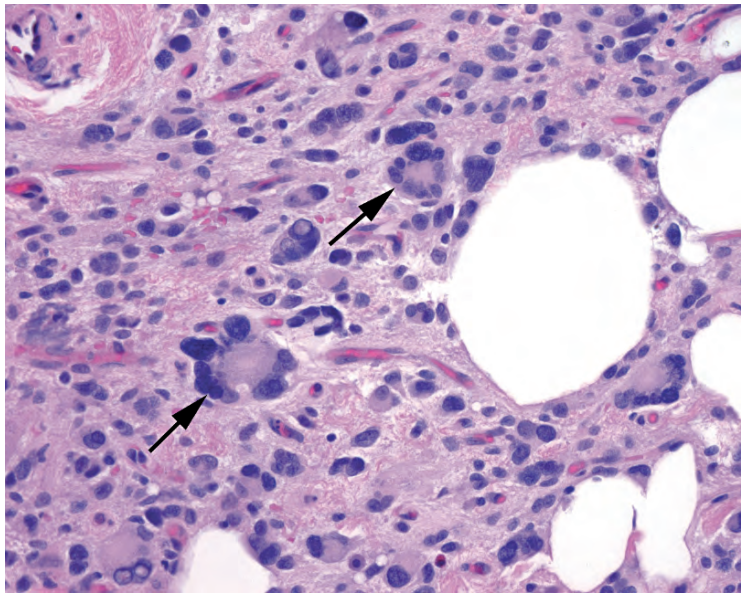

Figure 28.4. Pleomorphic lipoma. This type of benign lipoma is known for having very bizarre stromal cells that mimic sarcoma. The classic cell is the floret cell, with a circular wreath of nuclear lobes (arrows). Their presence suggests the diagnosis of pleomorphic lipoma.

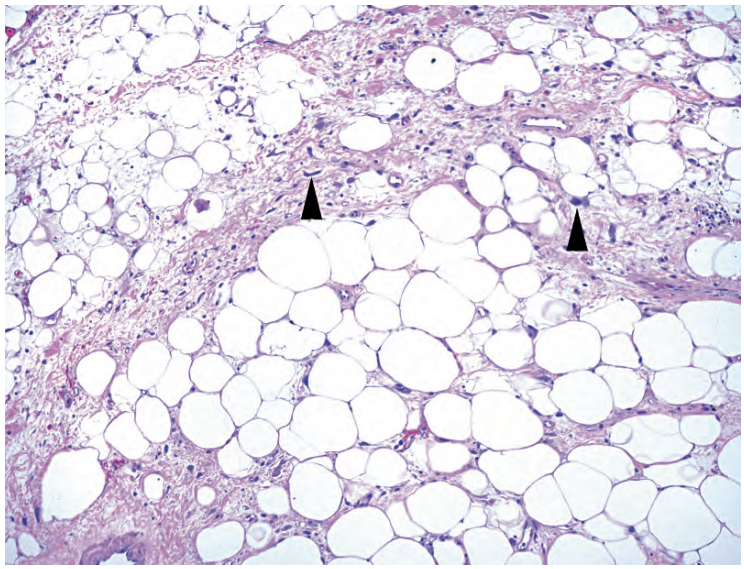

Figure 28.5. Well-differentiated liposarcoma. There is an increased amount of fibrous interstitium between fat cells, and atypical cells stand out at low power (arrowheads).

Myxoid liposarcoma is a different type of low-grade liposarcoma. It is not as clearly fatty as the WDLS, and the low-power impression is that of a gelatinous tumor with scattered fat cells and a stereotypical capillary network that has been compared to chicken-wire (Figure 28.6). These vessels are very delicate, and, unlike normal capillaries, they have little substance to their walls; they appear as a naked sleeve of endothelium stretched through the tumor. The tumor cells themselves are small spindled or rounded cells and lipoblasts, without the large atypical cells of the WDLS.

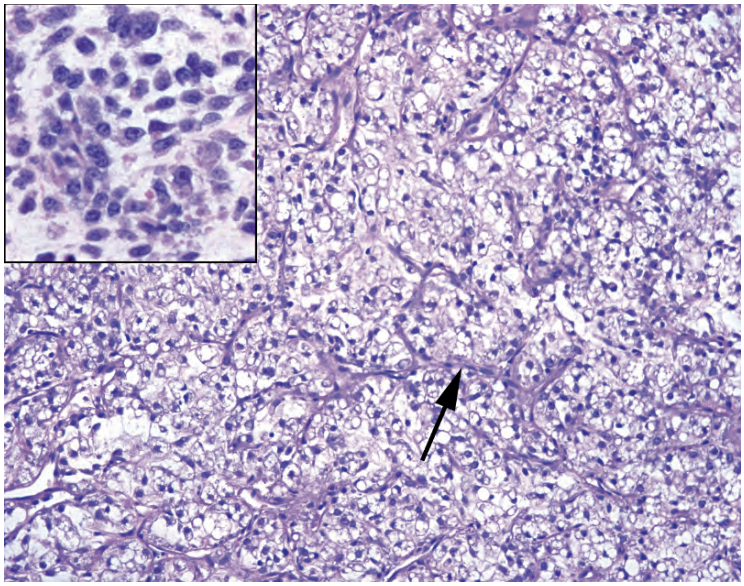

Figure 28.6. Myxoid liposarcoma. The fatty component may be very subtle in myxoid liposarcoma; the vessels are more often the tip off. The vasculature is composed of a delicate network of very thin capillaries with three- and four-way branch points, similar to chicken-wire (arrow). The cell population is composed of small cells, which may have fat vacuoles in them, and a myxoid background. Large atypical cells should not be present. **Inset**: Areas of closely packed small cells are indicative of round cell differentiation.

The myxoid liposarcoma can also transform into a higher grade lesion. When the small uniform cells become very densely packed and obscure the vascular pattern, it is indicative of round cell differentiation. The presence of more than 5% round cell differentiation is worth noting; a tumor with more than 75% is called a *round cell liposarcoma*.

The rare pleomorphic liposarcoma describes a high-grade tumor with extremely bizarre pleomorphic lipoblasts. It differs from the dedifferentiated liposarcoma in that it is still recognizable as a lipomatous tumor. It does not arise from, or have any relation to, the pleomorphic lipoma.

Fibrous Tumors and Myxoid Tumors

The fibroblast and the myofibroblast are ubiquitous cells, in charge of the reparative changes that take place in every part of the body. In resting state, they are fusiform to stellate cells with oblong pale nuclei, and they lay down a collagen matrix. Their job is to proliferate, and therefore mitotic activity is not unusual in reactive lesions. Although myofibroblasts may stain for actin (and are occasionally mistaken for smooth muscle), in general immunostains are not helpful in this tumor family.

Before we discuss the true neoplasms, we will review the collection of reactive (inflammatory or posttraumatic) lesions that present as tumors (lumps). *Keloid* is a common fibrous lesion, occurring at a site of trauma. It is similar to the normal fibroblastic proliferation of a dermal scar, except for its large size and characteristic thick cords of collagen called *keloidal* collagen. It is clinically recognizable and not usually a diagnostic dilemma.

Nodular fasciitis, on the other hand, may simulate a neoplasm clinically and is therefore more challenging for the pathologist. It is classically a rapidly growing lesion, sometimes associated with known trauma, sometimes not. On low power, it is a fairly circumscribed lesion with a hypercellular periphery, and it has a heterogeneous, as opposed to monomorphic, look. A microcystic appearance is classic. On high power the fibroblasts show a "tissue culture" appearance (fusiform to stellate with distinct cytoplasmic processes), and they float in a myxoid

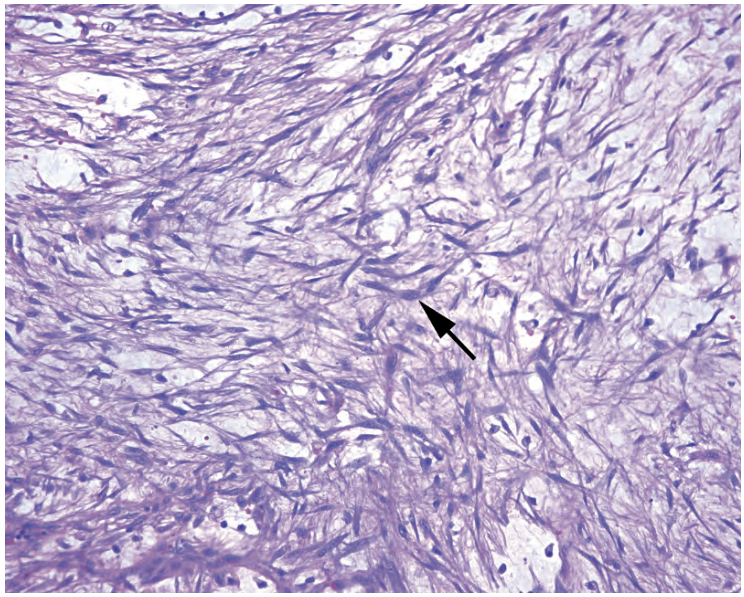

Figure 28.7. Nodular fasciitis. In this field the inflammatory component is not prominent, but the "tissue culture" pattern is seen clearly, with fusiform and stellate fibroblasts stretching delicate processes through the myxoid background (arrow).

background with surrounding red blood cells and lymphocytes (Figure 28.7). Older lesions may become more dense, collagenized, and pink and may resemble a fibromatosis (see later) with chronic inflammation. There should be no nuclear atypia, but you will see mitotic activity.

The biggest pitfall in nodular fasciitis is misinterpreting the patchy high cellularity and mitotic activity for a sarcoma. The clinical history is helpful, as is recognizing the reactive versus malignant nuclear features (something that takes practice).

Proliferative fasciitis is similar to nodular fasciitis except with the addition of large pink ganglion-like cells. *Proliferative myositis* is essentially the same lesion but in an intramuscular location. *Myositis ossificans* is a variant of proliferative myositis that shows reactive bone formation.

Inflammatory myofibroblastic tumor has gone by many names (inflammatory pseudotumor, inflammatory fibrosarcoma, plasma cell granuloma, others), but in this chapter it will be shortened to IMT. While long considered a reactive lesion, occasional cases have spread aggressively and even metastasized. Therefore, it is beginning to be regarded as a neoplasm, and will be included below, despite its histologic similarity to nodular fasciitis.

Neoplasms

The prototypical benign fibroblastic lesion is the *fibromatosis* (Table 28.4). This is a bland and indistinct tumor composed of normal-looking fibroblasts: fascicles of pink cells with pale tapering nuclei in a collagenous background (Figure 28.8). The very pale nuclei make the capillaries stand out and appear dark in comparison. It is very infiltrative around the edges, much like a normal scar. Superficial fibromatoses can occur on the palm (palmar fibromatosis, Dupuytren's contracture), sole (plantar, Ledderhose disease), or penis (Peyronie's disease), where they are benign but can recur. Axial or deep fibromatoses, such as on the chest wall or mesentery, are typically more aggressive in their expansion and are called *desmoid tumors*. The desmoid tumors are characterized by a specific immunohistochemical trait, the accumulation of β-catenin in nuclei.

Low-grade fibromyxoid sarcoma is one of those most troublesome entities; it simulates a benign lesion (fibromatosis) yet has metastatic potential. It may appear hypocellular, myxoid, or vaguely storiform, much like a fibromatosis.

Table 28.4. Fibrous and myxoid neoplasms.

Benign	Malignant but indolent	Malignant and aggressive
Fibromatosis	Low-grade fibromyxoid sarcoma	Fibrosarcoma
Palmar/plantar (superficial)	("Evans tumor")	
Desmoid tumor (deep)	Low-grade fibrosarcoma	
Dermatofibroma/benign fibrous	Dermatofibrosarcoma	
histiocytoma	Atypical fibroxanthoma	
Solitary fibrous tumor	Malignant solitary fibrous tumor	
Intramuscular myxoma	Low-grade myxofibrosarcoma	Myxofibrosarcoma
		(a.k.a. myxoid MFH)
Inflammatory myofibroblastic tumor		

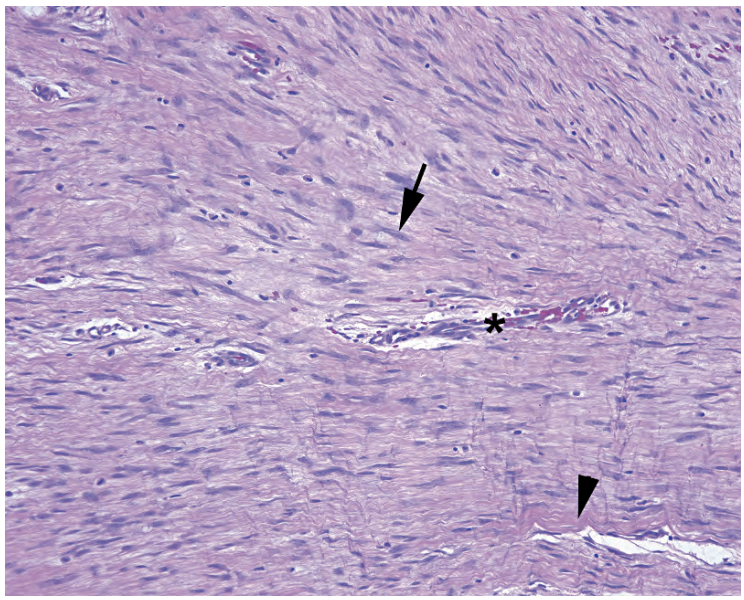

Figure 28.8. Fibromatosis. The cells in this lesion are pale and indistinct, with the small wavy nuclei (arrow) noticeably hypochromatic relative to the endothelial cells of the nearby capillary (a good internal control; asterisk). There is abundant collagen in the stroma (arrowhead).

Fibrosarcoma is the high-grade endpoint of this spectrum of lesions. It is the classic pure "herringbone" lesion, with fascicles alternating in a zigzag pattern. There is no significant collagen or inflammation to speak of. It has a high mitotic rate, but the cells are not particularly atypical: the nuclei tend to be monomorphic, oval, and euchromatic (Figure 28.9). It is mainly the cellular density and mitotic activity that set this lesion apart as malignant.

However, *what looks like fibrosarcoma is not usually fibrosarcoma*. True fibrosarcoma is quite rare, while its imitators, especially malignant peripheral nerve sheath tumor and synovial sarcoma, are more common. Therefore, fibrosarcoma is a diagnosis of exclusion.

The *solitary fibrous tumor* is included here because of its resemblance to fibroblastic tumors, but in truth the type of differentiation is not known. The solitary fibrous tumor has a unique staining pattern (CD34, CD99, and bcl-2) and typically arises from serosal surfaces. Because of its association with the pleura, it was once called the "benign mesothelioma." On low power, the tumor is described as having a patternless pattern, which evidently means nonstoriform-nonherringbone-nonfascicular. The swirling mass of uniform cells is reminiscent of ovarian stroma, but appears more pink due to abundant collagen (Figure 28.10). Collagen is laid down in parallel bundles, and the cellularity varies from one field to the next. The vessels are of the "staghorn" type, meaning they are gaping,

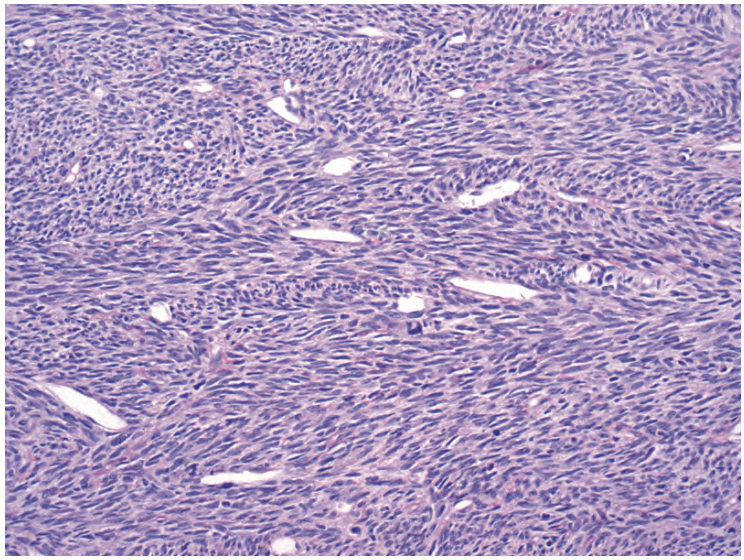

Figure 28.9. Fibrosarcoma. This field shows the typical herringbone pattern of fibrosarcoma, with zigzagging bands of spindle cells. Many other tumors can have this pattern.

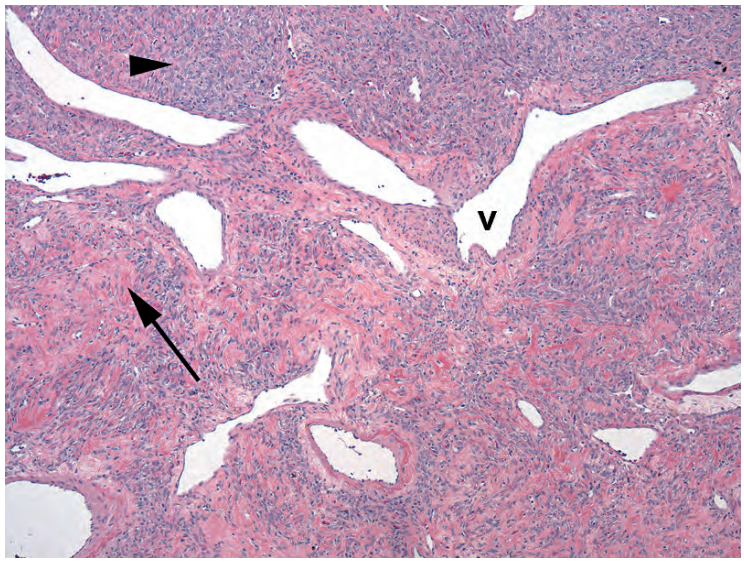

Figure 28.10. Solitary fibrous tumor. The most noticeable features at low power are the staghorn vessels (v), which this tumor shares with the related hemangiopericytoma. The tumor is composed of areas of small nondescript spindle cells (arrowhead) and collagenous stroma (arrow). The pattern of the spindle cells is described as "patternless," meaning somewhat chaotic.

branching vessels without an appreciable wall thickness: the tumor appears to extend right up to the endothelium. A mitotic rate of more than 4 per 10 high-power fields (hpf) suggests a malignant solitary fibrous tumor.

Fibrous Tumors of the Skin

The benign fibrous/fibrohistiocytic tumor of the dermis is the *dermatofibroma* or *benign fibrous histiocytoma*. It appears clinically as a little nodule, and at low power you see a diffuse, hazy, indistinct "blueness" occupying and expanding the dermis. On higher power, the dermatofibroma shows a textbook storiform pattern, with spindly cells arranged in little radial

sunbursts, like spokes on a wheel (see Figure 27.23 in Chapter 27). There is also usually accompanying inflammation, including lymphocytes, plasma cells, and foamy macrophages.

The malignant, albeit indolent, counterpart is the dermatofibrosarcoma protuberans (DFSP). This lesion is also distinctly storiform, and the tumor cells, as in the dermatofibroma, are monomorphic, fusiform, and just slightly hyperchromatic. However, the DFSP penetrates more deeply and classically infiltrates the subcutaneous fat, wrapping around fat cells in a distinctive pattern (see Chapter 27). In contrast to the dermatofibroma, the DFSP is ironically more uniform in cytology and lacks the associated inflammatory cells.

The skin also has its own MFH variant, called an *atypical fibroxanthoma*. Despite being histologically equivalent to a pleomorphic MFH, this superficial tumor is easily resected and therefore has a good prognosis.

Myxoid Tumors

The myxoid lesions included here are those that are not myxoid variants of other tumor types (such as myxoid liposarcoma). Many different lesions may converge on the myxoid phenotype, however. What we call *myxoid* is really the accumulation of hyaluronic acid, a gel-like substance that is essentially a form of solid water in the body (as seen in tissue edema). It may appear clear to a very pale blue on routine stains. A myxoid differential diagnosis would include myxoma, angiomyxoma, neurofibroma, and nodular fasciitis (all benign) and myxoid MFH (myxofibrosarcoma), myxoid liposarcoma, myxoid chondrosarcoma, myxoid leiomyosarcoma, and the low-grade fibromyxo- and myxofibro- entities (all malignant). You would also need to exclude tumors that may appear myxoid but are not, including chordoma, cartilaginous tumors, and epithelial mucinous tumors.

The *intramuscular myxoma* is a benign and nearly acellular lesion that presents as a gelatinous mass within a muscle, usually in women. There are rare small stellate cells without atypia. What separates the benign myxoma from other more worrisome lesions is its virtual lack of capillaries.

Myxofibrosarcoma is a high-grade tumor also known as *myxoid* MFH, and its low-grade counterpart is the low-grade myxofibrosarcoma (not to be confused with the fibromatosis-like low-grade *fibromyxoid* sarcoma!). The myxofibrosarcomas are tumors that are prominently myxoid but that have an increasing cellularity, nuclear pleomorphism, and mitotic rate compared to myxoma. Because of prominent vessels and bubbly cells (pseudolipoblasts), they may be mistaken for myxoid liposarcoma. However, the vessels are different. Myxofibrosarcoma vessels are "curvilinear," which means they make short thick arcs in the tumor, and the tumor cells appear to drip off of them like wax from a candle (Figure 28.11). Compare these to the delicate branching capillaries of the myxoid liposarcoma (see Figure 28.6). The nuclei of myxofibrosarcoma also set them apart; they are hyperchromatic and pleomorphic, unlike the uniform nuclei of the myxoid liposarcoma.

Inflammatory myofibroblastic tumor (IMT) is a neoplasm of mainly young people, often arising in the abdominal cavity. It is a proliferation of plump fibroblasts with abundant associated inflammation, especially plasma cells. It is very similar in appearance to a nodular fasciitis in that there are tissue culture–like fibroblasts in a myxoid, granulation tissue–like background (Figure 28.12). It differs by its visceral location and prominence of plasma cells (not seen in nodular fasciitis). The hypercellularity may be very worrisome for a high-grade sarcoma. However, while the nuclei may be enlarged, with prominent nuclear membranes or large nucleoli, you should not see the irregularly shaped hyperchromatic nuclei of MFH. Many cases show immunoreactivity for ALK.

Smooth Muscle

There are no reactive smooth muscle lesions, so we will go straight to neoplasms (Table 28.5). Smooth muscle neoplasms may be positively identified by immunoreactivity to actin and desmin but may sometimes show spurious cytokeratin or EMA staining.

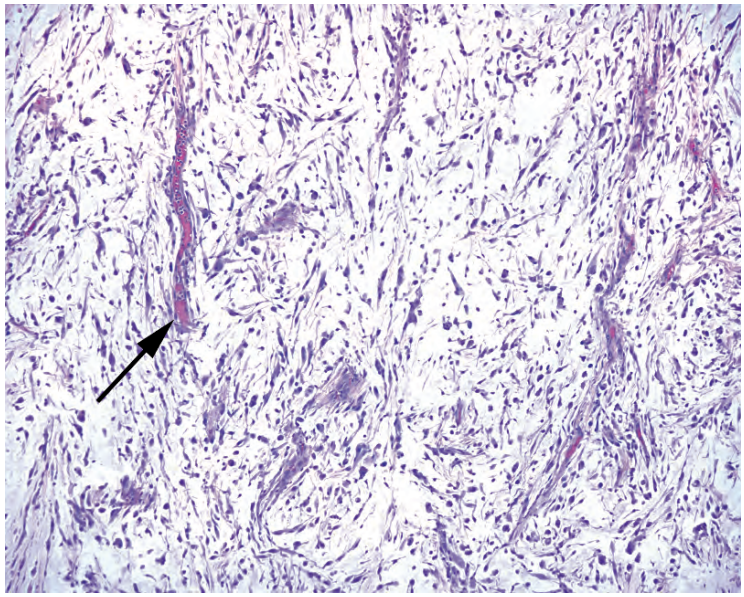

Figure 28.11. Myxofibrosarcoma. Although the cells here resemble those of pleomorphic malignant fibrous histiocytoma, the stroma is myxoid, and the vessels are unique (arrow). They appear as short arcs or segments, unlike the complex branching vessels of myxoid liposarcoma, and the tumor cells are intimately associated with the vessels, like wax dripping down the side of a candle.

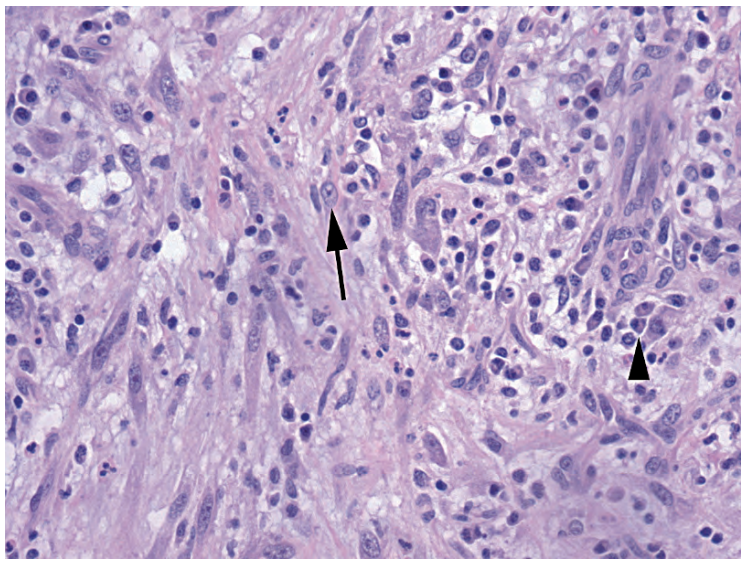

Figure 28.12. Inflammatory myofibroblastic tumor. The tumor is composed of a network of reactive-looking fibroblasts (arrow), capillaries, and inflammation, especially plasma cells (arrowhead).

Table 28.5. Smooth muscle neoplasms.

Benign	Malignant but indolent	Malignant and aggressive
Leiomyoma	Cutaneous leiomyosarcoma	Leiomyosarcoma, retroperitoneal or soft tissue

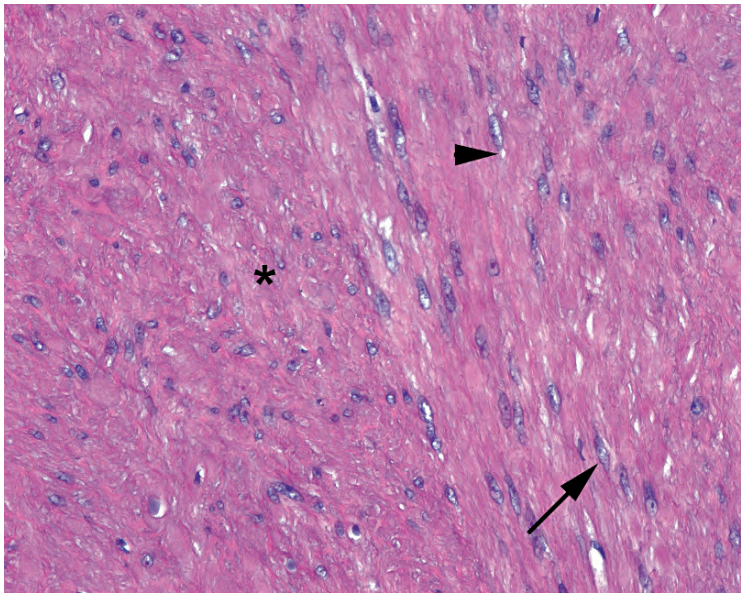

Figure 28.13. Leiomyoma of colon. As in the leiomyoma of the uterus, there are smooth muscle cells in bundles running parallel to and perpendicular to (asterisk) the slide. The features of benign smooth muscle include elongated pale nuclei with paranuclear vacuoles (arrowhead) and occasional corkscrew nuclei in which the nuclei appear twisted (arrow). Wavy pink muscle fibers are usually visible between the nuclei.

The *leiomyoma* should be familiar, as it is identical to the uterine tumor. It can occur as a primary neoplasm in cutaneous, gastrointestinal, and other sites. However, unlike in the uterus, in these body sites there is a very low threshold for bumping the lesion up to leiomyosarcoma. In general, greater than 1 mitosis per 10 hpf is worrisome.

The leiomyoma is characterized by long parallel bundles of smooth muscle cells that intersect at right angles, such that some are seen longitudinally and some cut in cross section. The nuclei are often described as cigar or box-car shaped, with blunt ends. You may also see corkscrew nuclei, which appear twisted about themselves and are associated with the contracted state. Paranuclear vacuoles are common (Figure 28.13).

Leiomyosarcomas range in appearance from something very similar to leiomyoma to a densely cellular and hyperchromatic tumor with scattered highly atypical nuclei (Figure 28.14). They can occur in the skin, where they are relatively indolent, or in the retroperitoneum, soft tissues, or any organ with smooth muscle, where they are more aggressive.

In a smooth muscle–like lesion arising anywhere near the gastrointestinal tract, you should consider the *gastrointestinal stromal tumor* (GIST) in the differential diagnosis. Many of what were once called *gastric leiomyomas* are now identified as GISTS. The cells differentiate along the line of the interstitial cell of Cajal, the pacemaker cell of the stomach, and like this cell, the GIST stains for c-kit and CD34. The GIST may take a spindle cell morphology, overlapping with leiomyoma or schwannoma, or may be epithelioid with a wide range of morphology. Clinical behaviors range from benign to malignant, depending on site and histologic factors.

Skeletal Muscle

Tumors of skeletal muscle are uncommon, and, as a terminal cell type with no stem cells or regenerative activity (like neurons), they are mainly seen in children or young adults (Table 28.6). They all get the rhabdo- prefix and should all stain with actin and desmin, plus special skeletal muscle markers myogenin and MyoD1. Other unrelated tumors in other sites sometimes are described as "rhabdoid;" remember that the –oid suffix means "looks like, but is not." Therefore, the rhabdoid meningioma or rhabdoid tumor is not of muscle origin but simply displays similar cell shape and appearance.

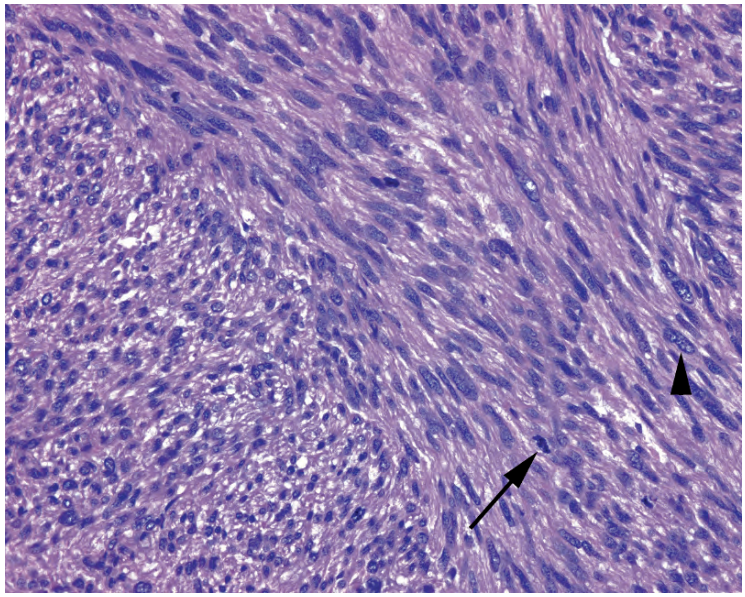

Figure 28.14. Leiomyosarcoma. A malignant version of the leiomyoma, this tumor has the architectural pattern and nuclear morphology of its benign cousin but with much higher cellularity, hyperchromatic nuclei, frequent mitoses (arrow), and large atypical cells (arrowhead).

The rhabdomyoma is a rare neoplasm of primitive skeletal muscle cells. The *fetal rhabdomyoma* generally occurs in children, typically in the head and neck, and resembles the embryonal rhabdomyosarcoma but without the atypia and mitoses. In adult men, the *adult rhabdomyoma* usually arises in the head and neck. In this variant, the cells are rhabdoid in shape, with small peripheral nuclei, and pink clumps of myofilaments in the cytoplasm, not unlike mature muscle cut in cross-section. The third variant, *genital*, occurs in adult women. This variant is predominantly composed of strap cells (elongated pink cells with cytoplasmic cross-striations), again without atypia or mitoses.

Rhabdomyosarcoma is the most common sarcoma of children and is rare in adults. Remember that any high-grade sarcoma can acquire some rhabdo- differentiation, however. Pure rhabdomyosarcomas can be grouped into three subtypes: embryonal, alveolar, and pleomorphic. The pleomorphic type is found in adults.

Embryonal rhabdomyosarcoma comprises about 80% of cases and has a significantly better outcome than the alveolar type. It is composed of sheets of rhabdomyoblasts (plump and eosinophilic with large eccentric nuclei), nonspecific spindled cells, or strap cells (Figure 28.15). The botryoid subtype of embryonal rhabdomyosarcoma refers to tumors occurring in a mucosal site, such as the genital tract.

Alveolar rhabdomyosarcoma is a very aggressive variant and equals "unfavorable histology." In this type, fibrous septa divide the tumor into packets, much like renal cell carcinoma, but the discohesive cells tend to fall apart in the middle of the packets (Figure 28.16). The solid variant may be indistinguishable from the small round blue cell tumors. As it turns out, the cytogenetic findings are different in embryonal and alveolar types. As the prognosis is so different, many centers are beginning to routinely do molecular tests on these tumors.

Table 28.6. Skeletal muscle neoplasms.

Benign	Malignant but (relatively) indolent	Malignant and aggressive
Rhabdomyoma Fetal Adult (head and neck) Genital	Embryonal rhabdomyosarcoma and Botryoid subtype	Alveolar rhabdomyosarcoma Pleomorphic rhabdomyosarcoma

Peripheral Nerve/Neuroectodermal

Nerves, as the axonal processes of terminally differentiated neurons around the spinal cord, do not actually form tumors. However, the cells associated with the nerve sheath do commonly produce neoplasms, including schwannoma and neurofibroma. Other tumors of neuroectodermal origin are included here as well.

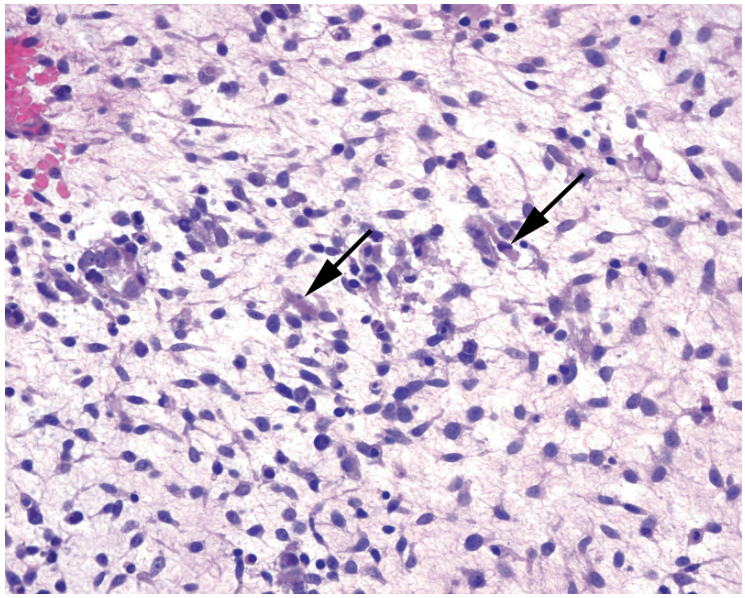

Figure 28.15. Embryonal rhabdomyosarcoma, botryoid type. The background is gelatinous, and the small spindle cells are nonspecific in appearance. Occasional strap cells are visible, with cytoplasmic muscle fibers (arrows).

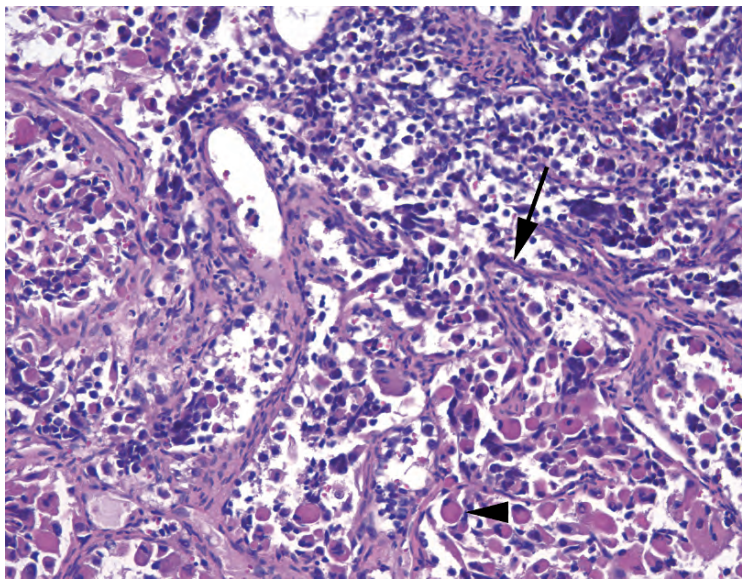

Figure 28.16. Alveolar rhabdomyosarcoma. The alveolar pattern is outlined by fibrovascular septa (arrow), and the tumor cells tend to fall out of the centers of their nests. This example shows prominent rhabdomyoblast differentiation (arrowhead), with large cells full of dense pink cytoplasm and eccentric nuclei. Other specimens may show only small round blue cell phenotype.

The only lesion that could be called reactive, in this group, is the traumatic neuroma. This is a disorganized tangle of nerve endings, including Schwann cells, perineurium, and axons, that may be found at the location of prior surgery or trauma. On the slide, it looks like a large, frayed nerve.

The benign peripheral nerve sheath tumors include the schwannoma and the neurofibroma (Table 28.7). Both of these lesions are S100 positive. Both can undergo malignant transformation into the malignant peripheral nerve sheath tumor, although this is much less common in the schwannoma. However, the nerve sheath lesions, just like the pleomorphic lipoma, may occasionally acquire bizarre cytology that does not indicate malignancy. This degenerative atypia is called *ancient change*.

The *schwannoma* is an encapsulated lesion that arises from a peripheral nerve and can therefore be found anywhere in the body. It usually shows alternating hypercellular (Antoni A) and myxoid (Antoni B) areas, as well as characteristic parallel arrays of palisading cells called *Verocay bodies* (Figure 28.17). The cells themselves have euchromatic, fusiform nuclei that stream in parallel within a pink fibrillary background. Thick-walled, hyalinized vessels are typical, which look as though they have a layer of amyloid replacing the vessel wall.

The *cellular variant* of schwannoma is still benign but can get quite hypercellular, and mitotically active (up to 10 mitoses per 10 hpf). The capsule and hyaline vessels should help to point you toward schwannoma. Foamy macrophages are common within this tumor.

The *neurofibroma*, in contrast, is an unencapsulated lesion that may appear as a nodule, a poorly circumscribed tumor, or a plexiform ("bag of worms") tangle. It is pale to pink at low power, with a myxoid background and thin curly tendrils of collagen between the cells (Figure 28.18). The nuclei are pale, thin, and slightly undulating, as in a normal nerve, and there should be no mitoses. Unlike in the schwannoma, special stains may reveal axons trapped within the lesion.

Table 28.7. Nerve-related neoplams.

Benign	Malignant
Schwannoma, neurofibroma	Malignant peripheral nerve sheath tumor
Granular cell tumor	Malignant granular cell tumor (rare)
Paraganglioma	

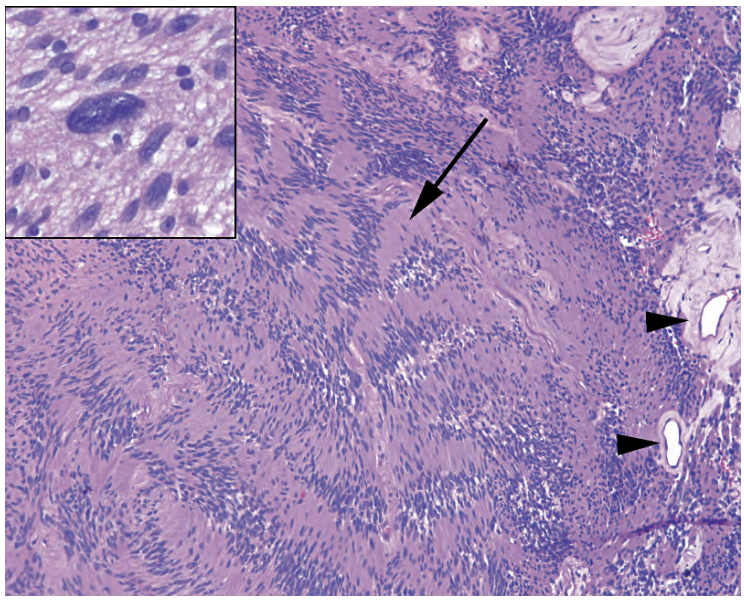

Figure 28.17. Schwannoma. In this spindle cell neoplasm, the long tapered nuclei tend to clump together and form arrays called Verocay bodies (arrow). Hyalinized vessels (arrowheads) are common. **Inset**: Occasional large atypical cells indicate ancient change, not malignancy.

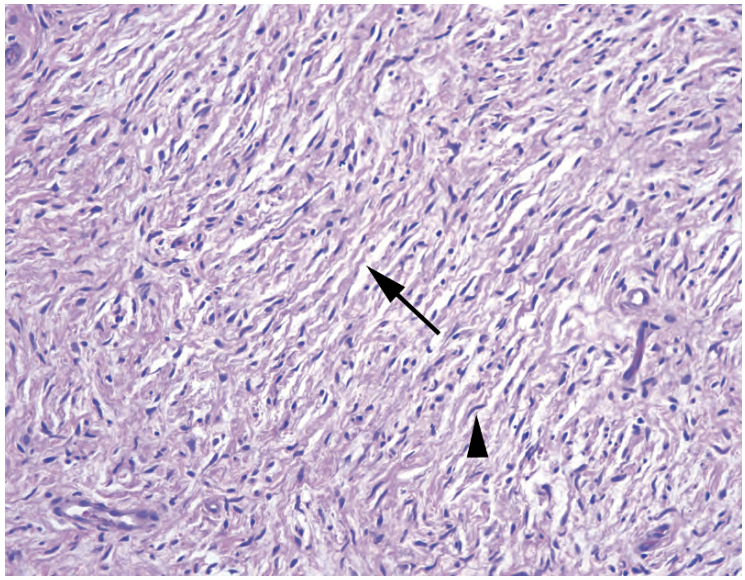

Figure 28.18. Neurofibroma. The nuclei tend to be thin and wavy (arrowhead), much like in a normal nerve. The tumor is usually paucicellular, with a myxoid background and delicate curly strands of wispy collagen (arrow).

The *malignant peripheral nerve sheath tumor* is usually a high-grade sarcoma and often takes the morphology of the fibrosarcoma (Figure 28.19). It may retain some nerve sheath features, such as the wavy nuclei, nuclear palisading, or hyalinized vessels, but tends to lose most of its S100 reactivity. Mitoses should be present, unlike in a neurofibroma.

The *granular cell tumor* is a benign tumor that shows neural differentiation but that resembles a collection of foamy macrophages. It is often associated with striking pseudoepitheliomatous hyperplasia in mucosal sites. These epithelial changes may be mistaken for squamous cell carcinoma if the subtle underlying diagnostic granular cells are overlooked.

The *paraganglioma* is actually a neuroendocrine tumor but is included here as it is sometimes presents as a soft tissue mass. It is a (usually) benign tumor with neuroendocrine-type nuclei, arranged in an alveolar pattern (Figure 28.20).

Vascular Tumors

Reactive lesions of capillaries are very common, as an inherent part of the healing process is the formation of new vasculature. Granulation tissue, which fills in a defect in the body tissues, has very prominent capillaries with large endothelial cells (see Figure 3.2 in Chapter 3). The capillaries of granulation tissue are plump and round, with at least two cell layers (endothelium and pericytes), and may be crowded but do not appear interconnected. Neoplastic vessels, on the other hand, are often lacking the pericyte component and typically form anastomotic channels and slit-like spaces with sharp angular profiles. Extravasated blood cells are common in vascular neoplasms. The immunohistochemical markers for the vascular tumors are CD31 and CD34.

Papillary endothelial hyperplasia is a pattern of organizing thrombus that may occur within a vessel or hematoma. It may be seen incidentally in a surgical specimen or represent a symptomatic small mass by itself, in which case it is called a *Masson's tumor*. It is composed of tiny fibrin papillae covered by thin endothelium (Figure 28.21).

A *hemangioma* is a benign neoplasm of vascular elements, and there are many subtypes, including the common capillary hemangioma, the cavernous hemangioma, and the juvenile hemangioma (Table 28.8). The hemangiomas generally have round, nonbranching vessels,

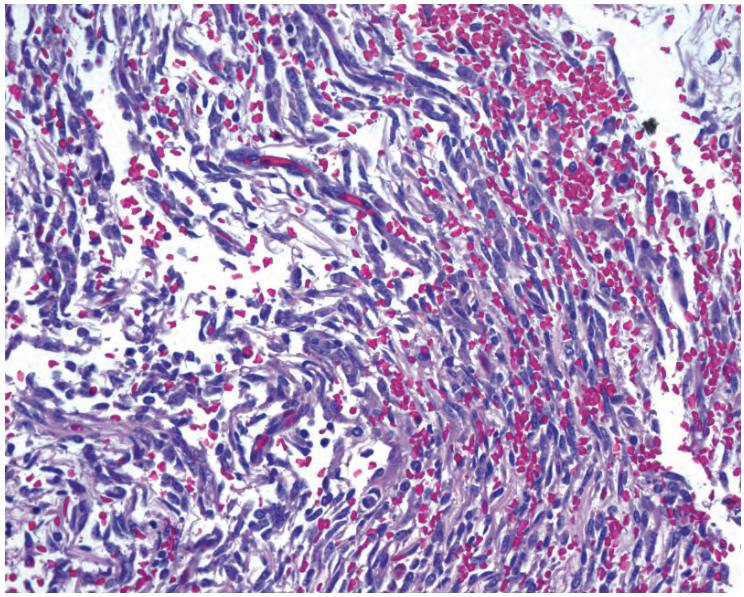

Figure 28.19. Malignant peripheral nerve sheath tumor. Although the malignant peripheral nerve sheath tumor sometimes resembles a fibrosarcoma, in this example it is more reminiscent of a neurofibroma, which was probably the origin in this case. There is a myxoid background and wavy collagen, but the cells are much more hyperchromatic and atypical than in a neurofibroma.

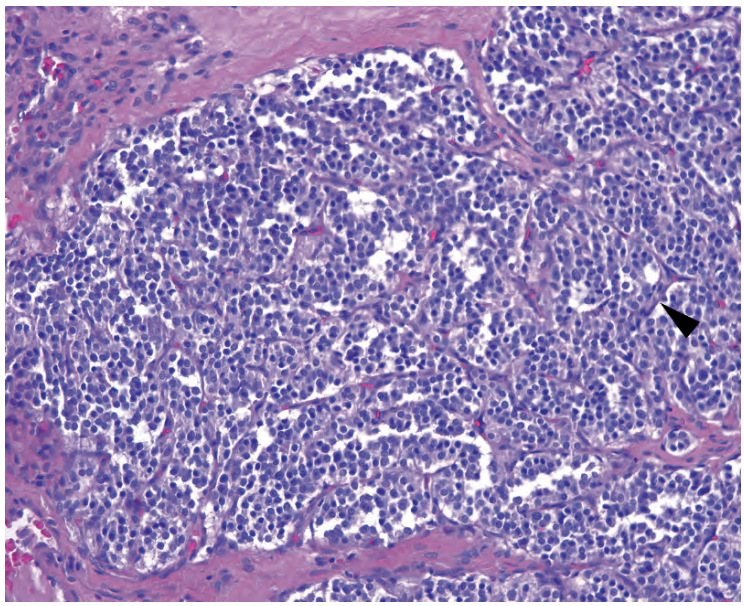

Figure 28.20. Paraganglioma. Fibrovascular septa (arrowhead) divide the neoplasm into small balls of cells (the "zellballen" pattern). The cells have small, perfectly round nuclei with neuroendocrine chromatin. Despite the paraganglioma's classification as an extraadrenal pheochromocytoma, it resembles the carcinoid tumor more closely than the pheochromocytoma.

although they may be very crowded or dilated, and the capillaries are surrounded by a pericyte layer. The *pyogenic granuloma*, once thought to be a reactive lesion, may in fact be a true neoplasm and is now called *lobular capillary hemangioma*. It is a circumscribed mass of capillaries with associated inflammation and ulceration. This lobular (circumscribed with rounded contours) appearance is characteristic of benign vascular lesions in general.

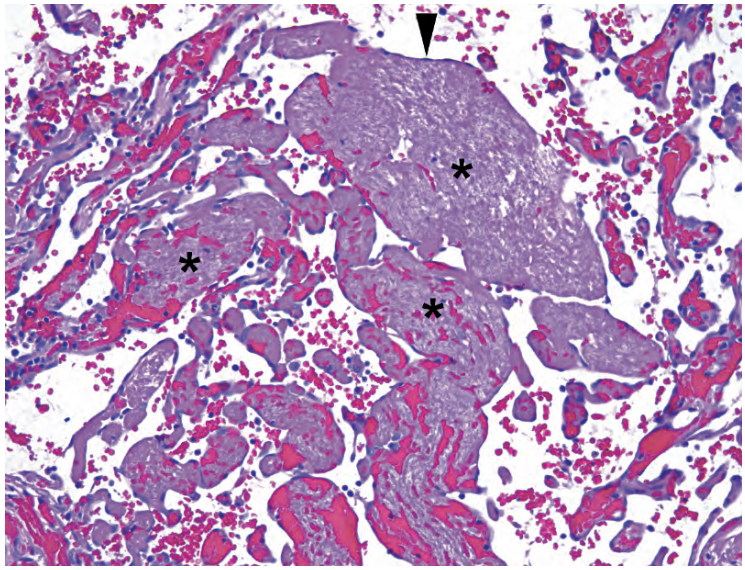

Figure 28.21. Papillary endothelial hyperplasia. Fingers of fibrin and red blood cells (asterisks), not true fibrovascular cores, are lined by bland endothelial cells (arrowhead).

Table 28.8. Vascular neoplams.

Benign	Malignant but indolent	Malignant and aggressive
Hemangioma	Hemangioendothelioma	Angiosarcoma
	Kaposi's sarcoma	
Perivascular tumors	Malignant examples of perivascular tumors are rare	
Glomus tumor		
Perivascular epithelioid cell tumor		

For every category of endothelial lesions there is an epithelioid variant, in which the endothelial cells acquire a lot of cytoplasm, becoming plump and epithelial-looking, often with cytoplasmic lumina that are their attempts at vessels. These variants are challenging because they may not look particularly vascular. Negative epithelial markers (cytokeratins, EMA) are helpful, but unfortunately some epithelioid vascular neoplasms may express some keratins.

The indolent malignant lesions of endothelium are called *hemangioendothelioma*. The epithelioid hemangioendothelioma is a sclerosing lesion with cords of vacuolated cells, some of which may contain red blood cells within the vacuoles, a diagnostic feature (Figure 28.22). It can be very difficult to distinguish from carcinoma without stains.

Kaposi's sarcoma, a virally induced (human herpesvirus type 8) low-grade sarcoma seen primarily in patients with HIV, has several stages and appearances, ranging from the most subtle of slit-like spaces in the dermis (see Chapter 27) to a dense spindle cell lesion. Because of the many variants, and a considerable array of "Kaposiform" mimickers, the differential diagnosis is beyond the scope of this chapter.

Angiosarcoma is the high-grade endothelial tumor, and it too has many variants. It can occur in organs, such as the liver or breast, especially after exposure to toxins or radiation. However, it can also arise in soft tissues de novo. Lymphedema is a recognized risk factor. Angiosarcoma classically shows branching, anastomotic irregular spaces with bulbous atypical cells lining the spaces (the hobnail pattern; Figure 28.23). Pericytes are typically absent, and at the periphery the tumor infiltrates into the surrounding tissue. This infiltrative border is very helpful in identifying malignant lesions.

Naturally, there is an epithelioid variant of angiosarcoma. The epithelioid angiosarcoma may look like a generic "very bad tumor" composed of sheets of plump cells with large nuclei

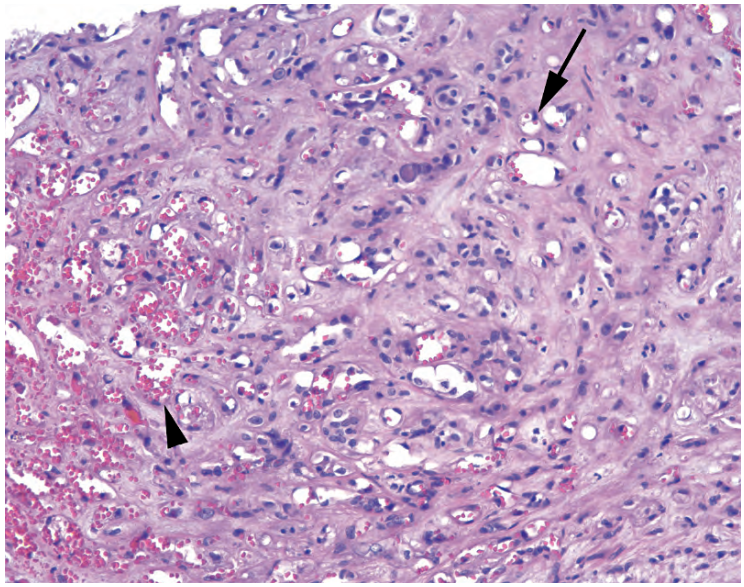

Figure 28.22. Epithelioid hemangioendothelioma. A rare but distinctive tumor, the epithelioid hemangioendothelioma is characterized by a dense fibrotic or sclerotic background, with small capillary spaces (arrowhead) and single cells with intracytoplasmic lumens complete with red blood cells (arrow).

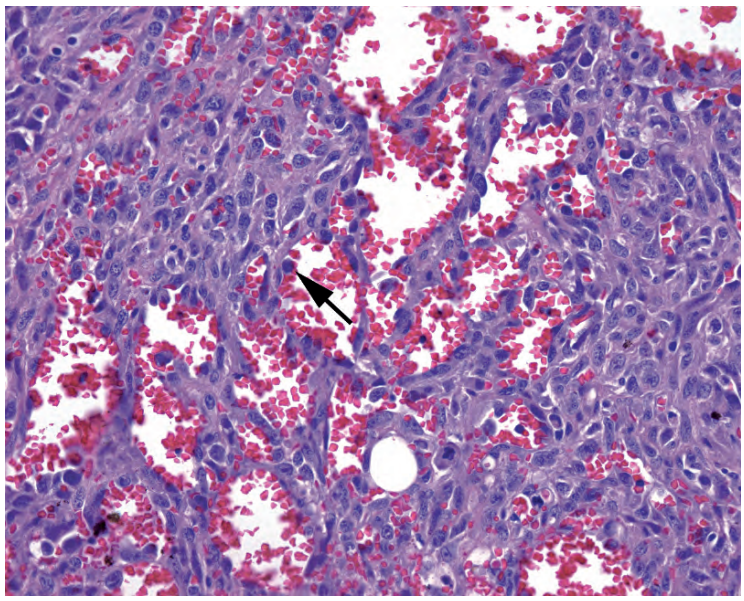

Figure 28.23. Angiosarcoma. In some areas the tumor cells have begun to grow as a solid sheet, but there are still vascular spaces visible, and full of blood. Large and hyperchromatic malignant cells protrude into the lumina (arrow) in a hobnail pattern. The tumor cells also show prominent nucleoli.

and prominent nucleoli, having almost no vascular differentiation. This sort of tumor may be identified only after a large battery of stains.

There are several tumors with *pericyte* differentiation, those cells that surround and support the endothelial cells. They are not exactly smooth muscle cells and have their own phenotype and immunostaining profile. The *glomus tumor* is one such lesion, and the hemangiopericytoma was once thought to be of pericyte origin. The perivascular epithelioid cell tumor family

of lesions are a unique group of neoplasms that share immunoreactivity for the melanoma markers HMB45 and Melan-A. This group includes the angiomyolipoma, covered in more detail in Chapter 13.

Malignant Tumors of Unknown Differentiation

The following malignant tumors of unknown differentiation are all high grade by definition and mostly occur in younger people, adolescents to people in their thirties. Most are defined by translocations, with the exception of the epithelioid sarcoma (at least to date), which may explain why they do not particularly look or stain like other mesenchymal elements we are familiar with. An interesting general rule is that translocation tumors, despite being high grade, tend to have monomorphic populations of cells. The cells may be ugly, but they are uniformly so. This is in contrast to the pleomorphic MFH like cells of other high-grade sarcomas, which show complex karyotypes.

Synovial sarcoma, despite the name, is neither synovial in origin nor found in joint spaces. It does share characteristic cleft-like spaces with some benign synovial tumors and usually arises somewhere in the vicinity of a joint. The most recognizable form is the biphasic synovial sarcoma, in which packets of cytokeratin-positive, gland-forming, epithelial cells are scattered in a spindle cell background (Figure 28.24). Not much else looks like that. However, the synovial sarcoma more commonly presents in monophasic form, which is just the spindle cell component. It is a blue and hypercellular tumor, with a monomorphic population of nondescript spindle cells, and it should be in the differential diagnosis for fibrosarcomatous or storiform tumors.

Epithelioid sarcoma is notorious for being misdiagnosed, as it does not look much like a sarcoma. It presents as ulcerated nodules on the extremities of young men and at low power resembles a large granulomatous reaction with central geographic (continent-shaped) necrosis. On higher power the tumor cells range from monomorphic spindle cells to large epithelioid cells with pink cytoplasm. Epithelioid sarcoma is unusual in that it shows reactivity to both vimentin, a sarcoma marker, and cytokeratin, a carcinoma marker.

Alveolar soft part sarcoma is a translocation tumor involving the *TFE3* gene. It is divided into small packets of cells by a capillary network, similar to a renal cell carcinoma, and in fact

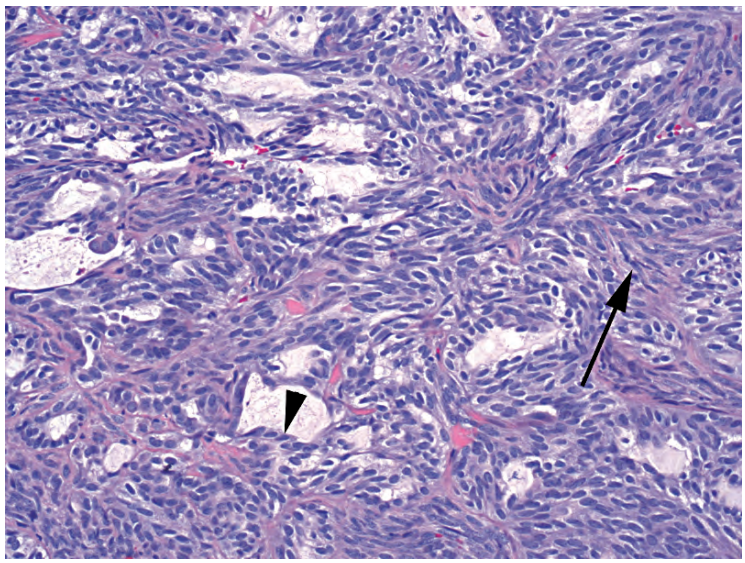

Figure 28.24. Biphasic synovial sarcoma. There are gland-like spaces surrounded by epithelial cells (arrowhead), set in a background of spindle cells (arrow). Monophasic synovial sarcoma lacks the epithelial component and can resemble fibrosarcoma.

looks somewhat carcinoma-like. The cells are large and eosinophilic with round nuclei and prominent nucleoli.

Clear cell sarcoma of soft tissue is one of several translocation tumors linked to the *EWS* gene. It is called *melanoma of soft parts*, as it stains with melanoma markers and may even produce melanin. Another *EWS* tumor is the *desmoplastic small round cell tumor*, which like it sounds is a small round blue cell tumor in a sclerotic background. The third tumor in this group is Ewing's sarcoma, which is cytogenetically identical to the peripheral neuroectodermal tumor. It is discussed with bone tumors.

Tumors of Bone

For tumors of bone, involving bone, or simulating bone, the radiograph is the gross examination. As in vascular lesions, a well-differentiated neoplasm may be classified as benign or low-grade malignant largely by the degree to which it infiltrates or invades surrounding tissue or bone. In bone, this infiltration of the periphery is best assessed by a radiologist. General features are the following:

- Benign lesions tend to be clearly defined, well circumscribed, and walled off by a layer of reactive bone (a thin rim on x-ray). Benign entities also tend to evoke a thick and smooth periosteal reaction (thickening).
- Aggressive lesions, which include infectious or malignant lesions, tend to be poorly circumscribed, reflecting their infiltration of surrounding bone. Aggressive lesions tend to produce an onion-skin, spiculated, or discontinuous periosteal reaction.

The second major principle is that primary bone tumors are rare and mainly occur in young adults and children. For any patient over 50 years, the first three items in the differential diagnosis for a bony lesion are metastasis, metastasis, and metastasis. Number four is a hematopoietic malignancy such as myeloma.

Bone-Forming Tumors

First, how does bone form? In the fetus, the main pathway is by endochondral ossification, in which new bone is laid down in the cartilage scaffolding. However, in the membranous bones of the fetal skull, and in the adult at sites of reparative bone, the first step is the synthesis of osteoid (a salmon-pink acellular matrix) by osteoblasts and its subsequent mineralization with calcium hydroxyapatite. This immature bone has a disorganized collagen framework and is called *woven bone*. Continuing development and remodeling produce bone with organized sheets of collagen visible as parallel seams within the trabeculae or cortex; this mature configuration is called *lamellar bone*. Neoplastic or reactive bone is always woven type; fragments of lamellar bone within a lesion must be entrapped native bone.

Second, how do we look at bone? Most histologic sections of bone are decalcified, so the pink fragments of "bone" you see are the osteoid left behind. Calcium phosphate itself is dark purple on H&E. In lesions with osteoid formation, which may include anything from reactive metaplastic bone to fibrous dysplasia to osteosarcoma, the osteoid (pink) can be differentiated from collagen or amyloid (also pink) by the process of mineralization, seen as a purple tinge within the seams of osteoid. Dystrophic calcification in soft tissue, such as tumoral calcinosis, is not the same as bone formation. Reactive bone formation, such as in myositis ossificans, is true bone but is not neoplastic.

The most common benign bone-forming neoplasm is the *osteoid osteoma* (Table 28.9). This lesion is composed of a small (<1.5 cm) nidus of lace-like woven bone surrounded by a dense sclerotic zone (Figure 28.25). The nidus is the source of intense bone pain. It is rarely seen on a slide these days, as radiofrequency ablation is effective treatment. The *osteoblastoma* is essentially the same lesion histologically but is larger (>1.5 cm).

At the other end of the spectrum lies *osteosarcoma*. The conventional type is a high-grade sarcoma. The usual appearance is that of a high-grade spindle cell neoplasm in which the

Table 28.9. Bone-forming tumors.

Benign	Malignant but indolent	Malignant and aggressive
Osteoma	Parosteal osteosarcoma	Osteosarcoma, conventional type
Osteoid osteoma		Periosteal osteosarcoma
Osteoblastoma		Telangiectatic osteosarcoma

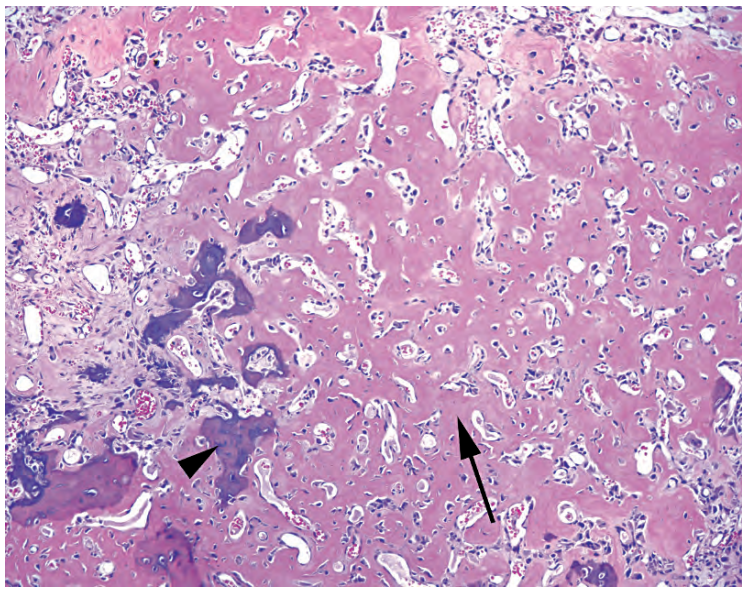

Figure 28.25. Osteoid osteoma or osteoblastoma (depending on size). At the nidus of the lesion, osteoid is laid down in a lace-like pattern (arrow) by benign osteoblasts. The hyaline pink substance can be identified as osteoid by the dark purple seams of mineralization (arrowhead).

tumor cells are associated with osteoid deposition (Figure 28.26). The osteoid is laid down in a lace-like pattern, very much like the inside of an osteoma. In fact, a well-differentiated osteosarcoma may be difficult to differentiate from an osteoblastoma. The difference is in the associated population of spindled or atypical cells and in the infiltrative periphery (best appreciated on x-ray). Osteosarcomas may have a wide range of morphologies, including chondroblastic, fibroblastic, and small cell. The unifying and defining feature is the production of osteoid by tumor cells, but osteoid may be sparse and focal. Resections of osteosarcoma are usually done postchemotherapy, at which time the goal is quantifying the amount of viable tumor that remains.

Some variants of osteosarcoma are more indolent. The *parosteal osteosarcoma* occurs on the surface of the bone, usually behind the knee of a young adult. This tumor is a low-grade sarcoma and is therefore not very cellular or atypical. It may resemble an osteochondroma, with well-formed cartilage and bone, but it is not in continuity with the marrow cavity. The similarly named but quite different *periosteal osteosarcoma* is also a surface lesion but is primarily chondroblastic and consists of a low-grade spindle cell population with cartilage formation.

Cartilage-Forming Tumors

Cartilage-forming tumors produce a characteristic fluffy or concentric-ring pattern of calcification on x-rays (Table 28.10). The *osteochondroma* is almost diagnosable by x-ray alone, as it stands out from the bone surface like a mushroom. Histologically, it is a bony stalk in continuity with the main marrow space, capped by mature cartilage, looking very much like a duplicated joint surface. Osteochondromas carry a small risk of transformation to chondrosarcoma.

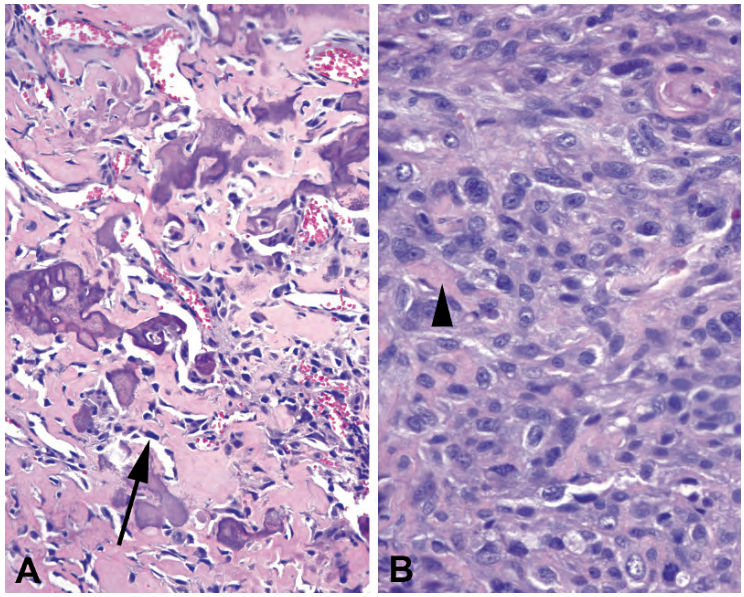

Figure 28.26. Osteosarcoma. **(A)** The most-well differentiated tumors can be very difficult to distinguish from osteoblastoma by histology alone. The osteoid deposition is similar, except the osteoblasts may appear more hyperchromatic and atypical (arrow). **(B)** A less differentiated tumor can be difficult to identify as osteosarcoma because of the focal and subtle production of osteoid (arrowhead).

Table 28.10. Cartilage-forming tumors.

Benign	Malignant
Osteochondroma	Chondrosarcoma
Enchondroma	Dedifferentiated chondrosarcoma
Chondroblastoma	
Chondromyxoid fibroma	

The *enchondroma* is merely an island of benign, hypocellular, mature cartilage occurring within the marrow space of the bone. It is usually asymptomatic in long bones but is more often found in the small bones of the hands and feet, where it leads to a visible swelling. The tumor consists of mature cartilage, which is a pale blue matrix with varying amounts of calcification (purple) and single chondrocytes sitting in bubble-like lacunae (Figure 28.27).

The *chondroblastoma* is also benign and is notable for a peculiar pattern of calcification that rings the lacunae, creating a chicken-wire or honeycomb effect. The chondromyxoid fibroma is rare but is in the differential diagnosis for a well-differentiated cartilage lesion in bone. It has both a fibrous component and a cartilaginous component.

Chondrosarcoma is typically a mass of atypical but recognizable cartilage, ranging from the low-grade chondrosarcoma (histologically resembling enchondroma) to the high-grade chondrosarcoma (Figure 28.28) based on cellularity, pleomorphism, and mitotic activity. It may be located on the surface of the bone or in the medullary cavity. Features that separate the well-differentiated chondrosarcoma from benign enchondromas include erosion of the inner cortex of bone, entrapment of trabeculae, myxoid change, and a tendency to involve the axial skeleton. Chondrosarcomas are tumors of adults (those in their thirties to fifties).

Chondrosarcomas, even high-grade ones, do not typically have a spindle cell component. A well-differentiated chondrosarcoma with an abrupt transition to high-grade sarcoma (of any pattern) is most likely a *dedifferentiated chondrosarcoma*. As in the liposarcoma family, this diagnosis relies on seeing the previous or adjacent chondrosarcoma. However, a tumor that

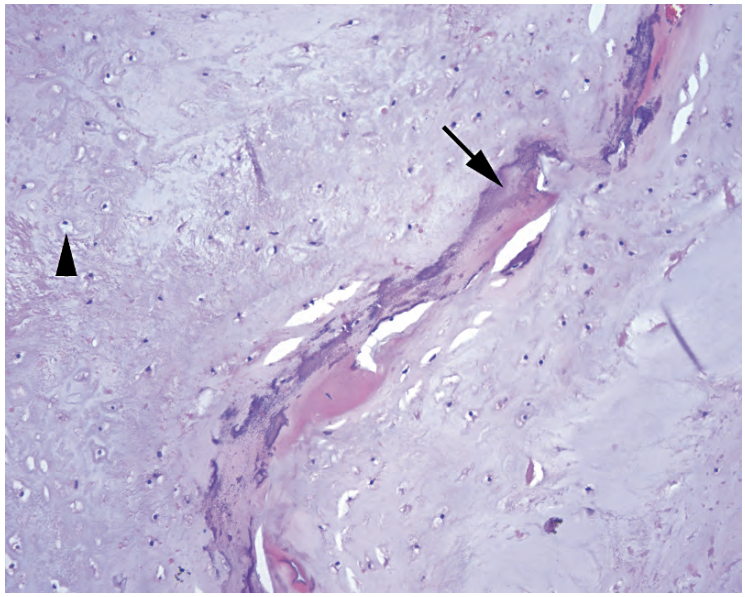

FIGURE 28.27. Enchondroma. This example shows a sheet of well-differentiated cartilage, with the characteristic blue, somewhat glassy matrix and small chondrocytes embedded in lacunae (arrowhead). The seam of mineralization (arrow) should not be mistaken for osteoid formation. A well-differentiated chondrosarcoma could look very similar histologically.

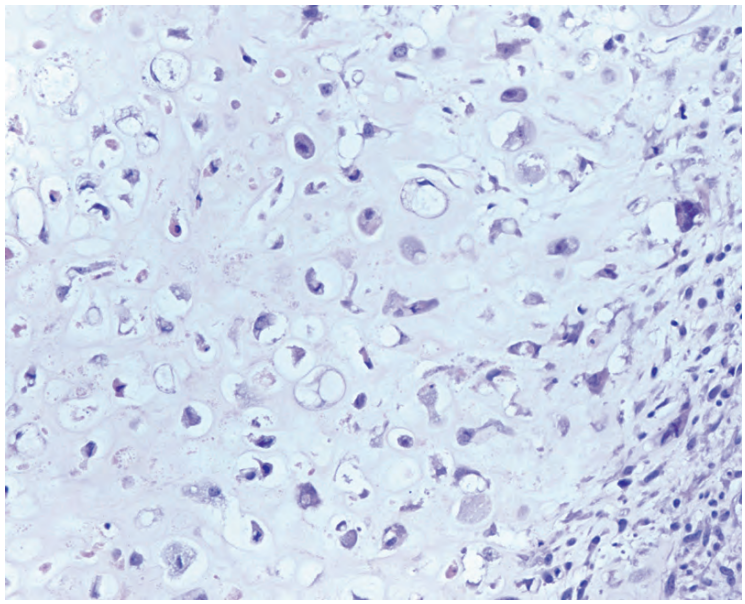

FIGURE 28.28. Chondrosarcoma. The cartilage matrix resembles normal cartilage, but the chondrocytes are pleomorphic in size and shape.

shows a gradual transition from cartilaginous areas to high-grade spindle cell areas is more likely a chondroblastic osteosarcoma. Confused? Keep reading.

Osteosarcomas often form cartilage ("chondroblastic"), and chondrosarcomas may mineralize into bone. How do we identify the primary nature of the neoplasm? Chondrosarcoma is a neoplasm of recognizable cartilage without a spindle cell sarcoma component, and the bone formation is through the direct mineralization or ossification of the cartilage, not by osteoid

deposition. A cartilage-forming osteosarcoma, on the other hand, should have areas of spindle cells that are producing osteoid. A spindle cell neoplasm intermixed with cartilage, even if the osteoid is not obvious, is more likely to be in the osteosarcoma family.

Fibrous and Miscellaneous Tumors in Bone

Fibrous dysplasia is a lytic and fibrotic lesion (a developmental abnormality, not really a neoplasm) seen mainly in long bones and craniofacial bones (Table 28.11). Microscopically, the lesion consists of a low-grade spindle cell population in which thin trabeculae of woven bone are laid down in a distinct pattern resembling (to English speakers) Chinese letters. Unlike typical reactive bone in an inflammatory lesion, osteoblasts are not visible surrounding the trabeculae. *Ossifying fibroma* is a very similar lesion that occurs in the shins (tibia, fibula) of very young children, only ossifying fibroma *does* show prominent osteoblastic rimming. Finally, we have the *nonossifying fibroma*, which is the low-grade fibroblastic population seen in the above lesions, except without the woven bone formation. It is essentially equivalent to a benign fibrous histiocytoma in other sites. The malignant correlates of malignant fibrous histiocytoma (not uncommon) and fibrosarcoma (rare) can occur in bone as well.

The *giant cell tumor of bone* is a lytic, destructive lesion seen at the ends of long bones in adults. It is composed of a mixture of osteoclast-like giant cells, often with over 50 nuclei, mixed with a background population of mononuclear cells (the true neoplastic component). Mitoses may be seen, but atypia is not. Giant cells, however, are not a unique feature, as they can be seen in almost any bony lesion. The principal differential is with the giant cell reparative granuloma.

Adamantinoma is a rare lesion of the tibia that may be composed of squamous, fibrous, or adamantinomatous (see discussion of craniopharyngioma in Chapter 26) cells. The main reason to know about it is to avoid calling it metastatic carcinoma.

Ewing's sarcoma is a tumor of adolescents and young adults and appears as a small round blue cell tumor involving the bone. Like most embryonal-type tumors, the cells have hyperchromatic, round, blue nuclei without prominent nucleoli, high nuclear to cytoplasmic ratios with scant cytoplasm, and prominent necrosis and apoptosis (Figure 28.29). It is classically positive for CD99 and overlaps with the peripheral neuroectodermal tumor, which has identical cytogenetics. The differential diagnosis includes true metastases from other small round blue cell tumors, lymphoma/leukemia, and the small cell variant of osteosarcoma.

Joint Lesions

Synovial chondromatosis is one of the few true tumors of the joint space (Table 28.12). It is characterized by the accumulation of nodules of benign cartilage in the synovium.

The *giant cell tumor of tendon sheath* actually describes two entities. The diffuse form is also known as pigmented villonodular tenosynovitis, and it is the second of the few lesions to actually involve the joint space. It is composed of a villous or papillary mass of small bland cells, multinucleated giant cells, and foamy macrophages. There are prominent clefted spaces at low power and sometimes pigment (hemosiderin).

Table 28.11. Fibrous and miscellaneous tumors in bone.

Benign	Malignant
Fibrous tumors	
Fibrous dysplasia	Malignant fibrous histiocytoma
Osteofibrous dysplasia (ossifying fibroma)	Fibrosarcoma
Cortical fibrous defect (nonossifying fibroma)	
Other tumors	
Giant cell tumor of bone	Metastatic carcinoma
Adamantinoma	Lymphoma
	Ewing's sarcoma/peripheral neuroendocrine tumor

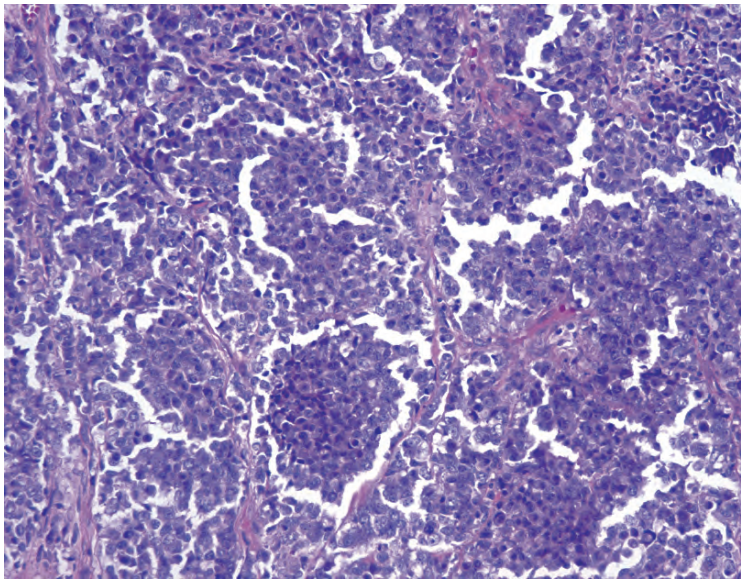

FIGURE 28.29. Ewing's sarcoma. The medullary cavity of bone is replaced by a small round blue cell tumor.

TABLE 28.12. Joint lesions.

Benign	Malignant
Giant cell tumor of tendon sheath Synovial chondromatosis	Malignant giant cell tumor (rare)

The nodular or localized giant cell tumor is also called *nodular tenosynovitis*, and, aside from presenting as a nodule on a tendon, its appearance is very similar to the diffuse giant cell tumor. The lesion is composed of bland mononuclear cells, giant cells, foamy histiocytes, cleft-like spaces, and hemosiderin, all set in a collagenous stroma. Both of the giant cell tumors of tendon sheath are distinguished from the giant cell tumor of bone by their immunoreactivity to CD68, a histiocyte marker, as well as by their clinical presentation. However, they do resemble each other on H&E.

29 A Primer on Immunostains

The use of immunostains is a highly complex field in and of itself, and most residents will need to invest in a specialized text at some point. This chapter is meant as an introduction to the most commonly used stains so that you can at least follow the thread of conversation when the acronyms begin to fly. Stains are organized by the organ system in which they are most often used.

Blood Vessels (Endothelium)

Antibody	Normal tissues stained	When it is used
CD31	Endothelial cells and megakaryocytes (cytoplasmic and membranous), also macrophages	To identify endothelial differentiation or angiosarcoma; most specific endothelial marker
CD34	Endothelial cells, fibroblasts, and hematopoietic blasts (cytoplasmic and membranous)	To identify vascular sarcomas, Kaposi's sarcoma, GIST, solitary fibrous tumor, DFSP, epithelioid sarcoma, plus some other soft tissue tumors. Synovial sarcoma is negative
FVIII	Endothelial cells, megakaryocytes, platelets (cytoplasmic)	To identify endothelial differentiation, specific but not very sensitive

DFSP, dermatofibrosarcoma protuberans; GIST, gastrointestinal stromal tumor.

Brain and Meninges

Antibody	Normal tissues stained	When it is used
EMA	Epithelial, perineural, meningothelial cells (cytoplasmic or membranous)	To identify meningioma, perineuroma, chordoma, mesothelioma, sebaceous carcinoma, plus some sarcomas (synovial sarcoma, epithelioid sarcoma) and plasma cell neoplasms. Germ cell tumors (excluding some teratomas) are negative Entities that are EMA positive, keratin negative: meningioma, perineuroma, plasma cell myeloma

(*continued*)

(Continued)

Antibody	Normal tissues stained	When it is used
GFAP	Glial cells (cytoplasmic)	To identify astrocytoma, GBM, and ependymoma; also myoepithelial tumors of salivary gland. Oligodendroglioma and neuroblastoma are negative
NSE (neuronal-specific enolase)	Neuroectodermal and neuroendocrine cells (cytoplasmic)	To identify neural differentiation but not very specific (*not* the same as nonspecific esterase, an enzyme assay for heme path). Sensitive for neuroblastoma
S100	Glial cells, Schwann cells, dendritic and Langerhans cells, melanocytes, other mesenchymal cells (nuclear and cytoplasmic)	To identify cellular schwannoma, astrocytomas/GBM, granular cell tumor, chordoma, ependymoma, MPNST, and melanocytic lesions (all types). Breast cancer may also be positive
Synaptophysin	Neuroendocrine cells (cytoplasmic)	To identify carcinoids, paraganglioma, pheochromocytomas, small cell, medullary carcinoma of thyroid, neuroblastoma, islet cell tumors, others. Differentiates neural differentiation (positive from glial (negative)

GBM, glioblastoma multiforme; MPNST, malignant peripheral nerve sheath tumor.

Breast

Antibody	Normal tissues stained	When it is used
E-cadherin	Normal ductal and lobular cells (membranous)	Loss of staining identifies lobular carcinoma (in situ and invasive); ductal lesions are positive
ER and PR	Estrogen receptor (nuclear) and progesterone receptor (nuclear)	For breast cancer prognosis (predicts response to tamoxifen) and to identify metastatic breast cancer, some gynecologic tumors, and others
GCDFP	Apocrine metaplasia of breast and apocrine sweat glands (cytoplasmic)	To identify breast carcinoma, also sweat and salivary gland carcinoma
Her2Neu	Growth factor receptor that is only weakly expressed in normal epithelial cells (membranous and cytoplasmic)	To evaluate breast carcinomas (overexpression is a poor prognostic sign but can be treated with Herceptin)
Ki67	Any proliferating cell (nuclear)	To gauge mitotic activity for prognosis
Stains that identify myoepithelial cells		
α-Actin	Smooth muscle: myoepithelial cells, blood vessels, myofibroblasts (cytoplasmic)	To delineate myoepithelial layer and rule out invasive cancer
p63	Tumor suppressor gene (nuclear)	Stains myoepithelial cells but not endothelium and fibroblasts—cleaner stain than actin/SMMHC. Also stains metaplastic carcinoma
SMMHC (smooth muscle myosin-heavy chain)	Myoepithelial cells, blood vessels, myofibroblasts (cytoplasmic)	To delineate myoepithelial layer and rule out invasive cancer
CK903	Myoepithelial cells (cytoplasmic and membranous) and usual duct hyperplasia	To differentiate usual ductal hyperplasia (positive) from ductal carcinoma in situ (negative). Also stains metaplastic carcinoma.

Cytokeratins

Antibody	Normal tissues stained	When it is used
AE1-AE3	Wide panel of keratins stains most epithelial cells (cytoplasmic), except cytokeratins 8 and 18	To identify carcinomas in general; used in conjunction with Cam 5.2 to screen for carcinoma
Cam 5.2	Low- and intermediate-molecular weight keratins 8, 18, and 19 (cytoplasmic)	Used in conjunction with AE1/AE3 to screen for carcinoma. Also to identify hepatocellular carcinoma, some adrenal cortical tumors, and some carcinomas that are negative for other keratins (undifferentiated carcinoma)
CK5/6	Two specific high-molecular-weight keratins (cytoplasmic)	To differentiate squamous cell carcinoma (positive) or mesothelioma (positive) from adenocarcinoma (negative)
CK7	A specific low-molecular-weight cytokeratin (cytoplasmic)	CK7 and CK20 are used in combination to narrow the differential of carcinoma of unknown origin. CK7 is generally positive in above-the-diaphragm carcinomas (see next table on CK7 and CK20)
CK20	A specific low-molecular-weight cytokeratin (cytoplasmic)	Generally positive in below-the-diaphragm carcinomas and in Merkel cell carcinoma (see next table on CK7 and CK20)
CK903 (34ßE12)	High-molecular-weight keratin (cytoplasmic and membranous)	To identify prostatic basal cells (loss of staining indicates carcinoma), and transitional cell (urothelial) carcinoma (positive); also metaplastic breast carcinoma

	$CK20^+$	$CK20^-$
$CK7^+$	Urothelial carcinoma Pancreatic carcinoma Ovarian mucinous carcinoma	Breast carcinoma Lung carcinoma, non–small cell Ovarian serous carcinoma Endometrial carcinoma Epithelial mesothelioma Thymoma
$CK7^-$	Colorectal carcinoma Merkel cell carcinoma	Hepatocellular carcinoma Renal cell carcinoma, clear cell type Prostate carcinoma Neuroendocrine small cell carcinoma Squamous cell carcinoma

Germ Cell and Testis

Antibody	Normal tissues stained	When it is used
AFP	Fetal tissues (cytoplasmic)	To identify yolk sac tumor and HCC. May also stain other carcinomas
c-kit	Germ cells, mast cells, interstitial cells of Cajal (cytoplasmic or membranous)	To identify seminoma (membranous) and mature teratoma, plus GIST in stomach
EMA	Epithelial, perineural, meningothelial cells (membranous)	Should be negative in seminoma, yolk sac tumor, and embryonal carcinoma
β-hCG	Human chorionic gonadotropin β-chain (cytoplasmic) in syncytiotrophoblasts	To identify choriocarcinoma and germ cell tumors, some adenocarcinoma
HPL	Trophoblasts (cytoplasmic)	To identify germ cell tumors, moles, and choriocarcinoma, also some carcinomas
Ki-1 (CD30)	Activated lymphocytes	To identify embryonal carcinoma, Hodgkin's lymphoma, and ALCL
PLAP	Placenta (cytoplasmic)	To identify germ cell tumors, intratubular germ cell neoplasia, others; does not stain spermatocytic seminoma

ALCL, anaplastic large cell lymphoma; GIST, gastrointestinal stromal tumor; HCC, hepatocellular carcinoma.

Gynecologic

Antibody	Normal tissues stained	When it is used
CA-125		To identify nonmucinous ovarian carcinoma
β-hCG	Human chorionic gonadotropin β-chain (cytoplasmic) in syncytiotrophoblasts	To identify choriocarcinoma and germ cell tumors
HPL	Trophoblasts (cytoplasmic)	To identify germ cell tumors, moles, choriocarcinoma, and some carcinomas
Inhibin	Granulosa cells, Sertoli cells, others (cytoplasmic)	To identify sex cord stromal tumors (granulosa cell, Sertoli and Leydig) and moles, choriocarcinomas, fibrothecomas, and adrenal cortical tumors
Melcam (CD146)	Intermediate trophoblasts	To identify PSTT, choriocarcinoma
Mesothelin	Mesothelial cells (membranous)	To identify serous ovarian carcinoma, mesothelioma, and pancreatic carcinoma (also a target for immunotherapy)
p16	Cells infected by HPV (nuclear)	To identify HSIL and HPV lesions of cervix and to differentiate between endocervical (positive) and endometrial (negative) adenocarcinoma
p53	Tumor suppressor gene variant that should be absent in normal cells (nuclear)	To identify EIC and serous carcinoma of endometrium

EIC, endometrial intraepithelial carcinoma; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; PSTT, placental site trophoblastic tumor.

Heme Path

Antibody	Normal tissues stained	When it is used
ALK	Fusion protein expressed by only lymphomatous cells	Stains a subset of ALCL and DLBCL. Hodgkin's lymphoma is negative
bcl-2	Inhibits apoptosis, and normally turns *off* in a germinal center (membranous and cytoplasmic stain)	To differentiate follicular lymphoma (positive) from reactive follicles (negative). Also stains mantle cell lymphoma. Burkitt's lymphoma should be negative
bcl-6	Germinal center cells (cytoplasmic)	To identify lymphomas of follicular origin (FCC, Burkitt's lymphoma)
CD1a	Thymocytes (immature T cells) and Langerhans cells (membranous)	To identify Langerhans cell proliferations, T-LBL
CDs 3, 4, 5, 7, 8	T cells	To identify T-cell lymphomas and leukemias; CD4 is also dimly positive in monocytic/histiocytic lesions
CD10	Precursor B and T cells, granulocytes (membranous)	To identify FCC, ALL, LBL, Burkitt's lymphoma, and CML; MALTomas are negative
CD20	B cells (cytoplasmic and membranous)	Used as a pan-B-cell marker; stains B-cell lymphomas, but plasmacytomas are negative
CD15 (LeuM1)	Granulocytes and macrophages (membranous and dot-like perinuclear)	To identify RS cells (classic HD), some large T-cell lymphomas and mycosis fungoides
CD23	B cells, IgE receptor (membranous)	To identify SLL/CLL; mantle cell lymphomas are negative
CD30	Activated B and T cells, immunoblasts, other nonheme cell types (cytoplasmic, membranous)	To identify RS cells, ALCL, large B- and T-cell lymphomas
CD34	Hematolymphoid blasts, fibroblasts, and endothelial cells (cytoplasmic and membranous)	To identify blasts in the marrow in acute leukemias; also many soft tissue tumors
CD45 (CLA/LCA)	Lymphocytes, granulocytes, and histiocytes, but not plasma cells (cytoplasmic, membranous)	To identify poorly differentiated neoplasms as of hematopoietic origin
CD56	Natural killer cells (membranous)	To identify natural killer/T-cell lymphomas

(*continued*)

(Continued)

Antibody	Normal tissues stained	When it is used
CD68	Histiocytes/macrophages/monocytes, granulocytes, others (cytoplasmic, membranous)	To identify histiocytic origin; also stains soft tissue tumors
CD79a	B cells and plasma cells (membranous)	To identify B-cell neoplasms negative for other B-cell markers; stains B-ALL and B lymphomas, myelomas
CD138	Plasma cells (membranous), epithelial cells	To identify plasma cell neoplasms
Cyclin D1	Nuclear stain in mantle cell lymphoma	To identify mantle cell lymphoma
EBV EBER	EBV RNA in infected B cells (nuclear)	To identify EBV-related tumors, including nasopharyngeal carcinoma, posttransplantation/AIDS lymphomas; also mononucleosis
FVIII (vWF)	Stains megakaryocytes, platelets, and endothelial cells, (cytoplasmic)	To identify megakaryocytic leukemias
Hemoglobin	Hemoglobin in erythroid cells (cytoplasmic)	To identify erythroid leukemias (rare)
Kappa and lambda	Light chain of immunoglobulins in plasma cells and B cells (cytoplasmic)	Restricted kappa or lambda staining indicates a monoclonal population of B or plasma cells
Ki67	Any proliferating cell (nuclear)	To gauge mitotic activity and identify Burkitt's lymphoma (100% positivity)
MPO	Enzyme granules in myeloid-lineage cells (cytoplasmic)	To identify AML and myeloid sarcoma (chloroma)
TdT	Immature lymphocytes (nuclear)	To identify LBL and ALL

ALCL, anaplastic large cell lymphoma; ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; DLBCL, diffuse large B-cell lymphoma; FCC, follicular center cell lymphoma; HD, Hodgkin's disease; LBL, lymphoblastic lymphoma; MALT, mucosa-associated lymphoid tissue; RS, Reed-Sternberg cells; SLL/CLL, small lymphocytic lymphoma/chronic lymphocytic leukemia.

Kidney and Bladder

Antibody	Normal tissues stained	When it is used
RCC (gp200/RTA)	Proximal renal tubules (cytoplasmic)	To identify renal cell carcinoma
TFE3	Transcription factor (nuclear)	To identify Xp11-translocation RCC and alveolar soft part sarcoma
TFE3B	Transcription factor (nuclear)	To identify t(6:11) renal cell carcinoma
Thrombomodulin	Both endothelial (cytoplasmic) and mesothelial (membranous) cells	To identify TCC, mesothelioma, some vascular tumors
WT-1	Tumor suppressor gene in developing nephrons; nephrogenic rests and adult glomerular podocytes (nuclear)	To identify Wilms' tumor; also mesothelioma, desmoplastic small round cell tumor

RCC, renal cell carcinoma; TCC, transitional cell (urothelial) carcinoma.

Liver, Pancreas, and Other Gastrointestinal

Antibody	Normal tissues stained	When it is used
α_1-antitrypsin	Histiocytes and reticulum cells (cytoplasmic)	To highlight globules of α_1-antitrypsin disease; not specific to a tumor
AFP	Fetal tissues (granular cytoplasmic)	To identify HCC and yolk sac tumors. May also stain other carcinomas

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Antibody	Normal tissues stained	When it is used
β-Catenin	APC-binding protein present in most cells (only nuclear staining is significant; indicates a mutation in APC or β-catenin)	To identify colon cancer, abdominal fibromatosis, and solid pseudopapillary tumor of pancreas (positive)
CD10	Liver canaliculi, brush border of small bowel, other tissues	A canalicular pattern in HCC
pCEA	Fetal tissues and adenocarcinomas (cytoplasmic)	A canalicular pattern in HCC (not seen with mCEA); also stains lung, colon, pancreatic carcinoma
DPC-4 clone B8	Most normal tissues (cytoplasmic)	To identify pancreatic carcinoma (55% of in situ or invasive cancers exhibit loss of staining)
EGFR	Hepatocytes, perineurium in peripheral nerves, squamous epithelium	Prediction of response to Erbitux (a monoclonal antibody) in advanced colon cancer
HepPar1 (OCH1E5)	Mitochondria in normal hepatocytes (granular cytoplasmic stain)	To identify HCC

Note: There are no immunostains that can differentiate well-differentiated HCC from normal liver tissue; the HCC stains are used to demonstrate hepatic differentiation in ambiguous or metastatic tumors. APC, activated protein C; HCC, hepatocellular carcinoma; mCEA, monocional carcinoembryonic antigen.

Lungs

Antibody	Normal tissues stained	When it is used
BerEP4	Epithelial cells (membranous)	To differentiate mesothelioma (negative) from carcinoma (positive)
mCEA and pCEA	Fetal tissues and mucin-secreting glandular tissues (cytoplasmic)	To differentiate mesothelioma (negative) from adenocarcinoma (positive)
Calretinin	Various neural and epithelial cells (cytoplasmic and nuclear)	To differentiate epithelial mesothelioma (positive) from carcinoma (negative)
LeuM1 (CD15)	Hematopoietic cells and some carcinomas (membranous and cytoplasmic)	To differentiate mesothelioma (negative) from adenocarcinoma (positive)
TTF-1	Transcription factor in lung and thyroid (nuclear)	To differentiate nonsquamous carcinoma of lung origin (including adenocarcinoma, small cell, and neuroendocrine/all positive) from nonpulmonary (negative)
WT-1	Mesothelium (nuclear)	To differentiate epithelial mesothelioma (positive) from carcinomas (negative)

Melanoma

Antibody	Normal tissues stained	When it is used
HMB45	Immature melanocytes (cytoplasmic)	To identify epithelioid melanoma, metastatic melanoma, angiomyolipoma, clear cell sarcoma, perivascular epithelioid cell tumors, and others
MART-1/ Melan-A (N2-7C10 clone)	Melanocytes (cytoplasmic)	To identify melanoma (mainly epithelioid), more sensitive than HMB45. Recognizes the same protein as Melan-A antibody
Melan-A (A103 clone)	Melanocytes (cytoplasmic)	To identify melanoma (mainly epithelioid), more sensitive than HMB45; also angiomyolipoma. Unlike MART-1, labels steroid cell tumors (adrenocortical tumors, Sertoli and Leydig cell tumors)
MitF	Melanocytes (nuclear)	To identify melanoma and melanocytic tumors, also angiomyolipoma
S100 protein	Melanocytes, glial cells, dendritic and Langerhans cells, other mesenchymal cells (nuclear and cytoplasmic)	To identify nevi and melanoma (all types, most sensitive), cellular schwannoma, granular cell tumor, glial neoplasms. Not used to screen lymph nodes, as normal dendritic cells are positive

Neuroendocrine and Endocrine

Antibody	Normal tissues stained	When it is used
Chromogranin	Neurosecretory granules (cytoplasmic, granular) in endocrine tissues and neurons	To differentiate pheochromocytoma (positive) from adrenal cortical carcinoma (negative) or to identify carcinoids, small cell, Merkel cell, and islet cell tumors
Inhibin	Adrenal cortical cells (cytoplasmic)	To identify adrenal cortical tumors, stromal sex cord tumors (granulosa cell, Sertoli and Leydig), fibrothecomas
Synaptophysin	Neuroendocrine cells (cytoplasmic), neuromuscular junction, Merkel cells	To identify carcinoids, paragangliomas, pheochromocytomas, small cell carcinoma, medullary carcinoma of thyroid, neuroblastoma, islet cell tumors, others
Various hormones	Cells that produce insulin, somatostatin, gastrin, glucagon, parathyroid hormone, etc. (cytoplasmic)	To identify products of neuroendocrine tumors, such as islet cell tumors and others

Prostate

Antibody	Normal tissues stained	When it is used
CK903 (34ßE12)	High-molecular-weight keratin (cytoplasmic and membranous) in basal cells	To identify prostatic basal cells (loss of staining indicates carcinoma) and TCC (positive)
p63	Prostatic gland basal cells (nuclear)	To identify basal cells (loss of staining indicates carcinoma)
PSA	Prostatic epithelium (cytoplasmic), but also salivary gland	To identify metastatic or ambiguous prostate cancer. Seminal vesicle is negative
PSAP (PAP)	Prostatic epithelium (cytoplasmic)	To identify metastatic or ambiguous prostate cancer and TCC; also stains rectal carcinoids
Racemase (p504s)	Prostatic carcinoma	To confirm prostate carcinoma; also stains nephrogenic adenoma of bladder

TCC, transitional cell (urothelial) carcinoma.

Soft Tissue

Antibody	Normal tissues stained	When it is used
α-Actin	Smooth muscle actin (cytoplasmic)	To identify smooth muscle differentiation, leiomyoma and leiomyosarcoma; rhabdomyosarcoma is usually negative
Actin (HHF-35)	Smooth, skeletal, and cardiac muscle, myoepithelial cells (cytoplasmic)	To identify muscle differentiation
c-kit	Mast cells, interstitial cells of Cajal (cytoplasmic and membranous)	To identify GIST, also seminoma, mature teratoma, and AML
CD34	Fibroblasts, endothelial cells, and hematopoietic blasts (cytoplasmic and membranous)	To identify GIST, SFT, DFSP, MPNST, and vascular sarcomas plus other soft tissue tumors
CD99 (O13)	A variety of mesenchymal cells (membranous)	To identify PNET/Ewing's sarcoma, lymphocytes in thymoma, plus other sarcomas and hematologic tumors. Neuroblastoma is negative
Desmin	Intermediate filaments in smooth, striated, and cardiac muscle (cytoplasmic)	To identify muscle differentiation, including rhabdomyosarcoma, some leiomyosarcomas, and others
EMA	Epithelial, perineural, meningeal cells (cytoplasmic or membranous)	To identify some sarcomas (synovial sarcoma, epithelioid sarcoma) plus chordoma, meningioma, mesothelioma, perineural tumors, and plasma cell tumors

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Antibody	Normal tissues stained	When it is used
FXIIIa and CD68	Fibrohistiocytic cells (cytoplasmic)	To identify fibrohistiocytic tumors, such as MFH and dermatofibroma (FXIIIa), giant cell tumor of tendon sheath
HMB45	Immature melanocytes (cytoplasmic)	To identify angiomyolipoma, clear cell sarcomas, PEComas, and others; also epithelioid and metastatic melanomas
Myogenin	Regenerating, but not normal, skeletal muscle (cytoplasmic)	To identify rhabdomyosarcoma
S100	Glial cells, melanocytes, dendritic and Langerhans cells, other mesenchymal cells (nuclear and cytoplasmic)	To identify cellular schwannoma, granular cell tumors, MPNST, chondrosarcoma, melanomas (all types), astrocytoma
Vimentin	Most mesenchymal cells (cytoplasmic), including fibroblasts, endothelium, smooth muscle	As an internal control for immunoreactivity and antigen preservation (the "pan-keratin" of soft tissue)
WT-1	Tumor suppressor gene in developing nephrons; nephrogenic rests and adult glomerular podocytes (nuclear)	To identify desmoplastic small round cell tumor, also Wilms' tumor

DFSP, dermatofibrosarcoma protuberans; GIST, gastrointestinal stromal tumor; MFH, malignant fibrous histiocytoma; MPNST, malignant peripheral nerve sheath tumor; PEComa, perivascular epithelioid cell tumor; PNET, primitive neuroectodermal tumor; SFT, solitary fibrous tumor.

Thyroid

Antibody	Normal tissues stained	When it is used
Calcitonin	C cells of the thyroid (cytoplasm and extracellular material)	To identify medullary carcinoma of thyroid
Thyroglobulin	Thyroid follicles (cytoplasmic)	To identify metastatic thyroid carcinoma
TTF-1	Transcription factor in lung and thyroid (nuclear)	To identify thyroid carcinoma, including follicular, papillary, and medullary; also nonsquamous carcinoma of lung (adenocarcinoma and small cell)

Differential Diagnoses

Spindle cell tumor: Differential diagnosis includes neural, muscle, fibrous, vascular, or other sarcoma, plus carcinoma and melanoma. Panel: S100, AE1/AE3, actins, desmin, CD34, c-kit.

Big pink cell tumor: Differential diagnosis includes melanoma, adrenal cortical carcinoma, renal cell carcinoma, hepatocellular carcinoma, thyroid Hurthle cell carcinoma, parathyroid, Leydig cell tumor.

Small round blue cell tumor: Differential diagnosis includes small cell carcinoma, Merkel cell, lymphoma, primitive neuroectodermal tumor/Ewing's sarcoma, alveolar rhabdomyosarcoma, desmoplastic small cell tumor, neuroblastoma, Wilms' tumor. Panel: CD45, CD99, desmin, NSE, CK20, chromogranin, myogenin, CD79a, TdT, WT-1, cytokeratin.

Alveolar pattern tumor: Differential diagnosis includes renal cell carcinoma, alveolar rhabdomyosarcoma, alveolar soft parts sarcoma, pheochromocytoma, granular cell tumor, melanoma.

Clear cell tumor: Differential diagnosis includes renal cell carcinoma, clear cell follicular thyroid carcinoma, clear cell lung carcinoma, clear cell hepatocellular carcinoma, adrenocortical carcinoma, malignant melanoma.

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