

Skin

Breast

Cartilage

Bone

Joints

2018 Spring Elective

1. NEURAL

2. HEART

/Blood vessels

3. LUNGS

4. LIVER

5. PANCREAS

6. SALIVARY GLAND

7. STOMACH

8. SMALL INTESTINE

9. COLON

10. SPLEEN

11. TONSIL

12. THYMUS

13. LYMPH NODES

14. BONE MARROW

15. KIDNEY

16. BLADDER

17. TESTIS

18. PROSTATE

19. UTERUS

20. OVARY

21. BREAST

22. PLACENTA

24. SKIN

25. SKELETAL MUSCLE

**26. SMOOTH MUSCLE,
NERVES, ADIPOSE**

27. CARTILAGE

28. BONE

**29. THYROID/
Parathyroid**

30. ADRENAL

31. PITUITARY

---Eyes

---Sinuses

TUMORS

**Assess for
metastasis**

1. Plan to examine blood and serum for abnormalities before planning histopathology studies
2. Looking at multiple tissues, from multiple animals is important to help explain abnormalities in one organ, in one animal. The organs do communicate with each other in vivo !!!
3. Plan to examine animals after they have been back-crossed into at least ~8 generations, to avoid artefacts
4. Plan to examine tissues from an initial set of 24 animals:
 - 6 male and 6 female littermate;
 - 6 male and 6 female gene altered

Epidermis

Stratum corneum
Stratum lucidum
Stratum granulosum
Stratum spinosum
Stratum basale

Dermis

Papillary layer
Reticular layer

Eccrine sweat gland

Apocrine sweat gland

Root hair plexus

Hypodermis
(superficial fascia)

Meissner's corpuscle

Hair shaft

Sebaceous (oil) gland

Arrector pili muscle

Eccrine sweat gland

Apocrine sweat gland

Hair follicle

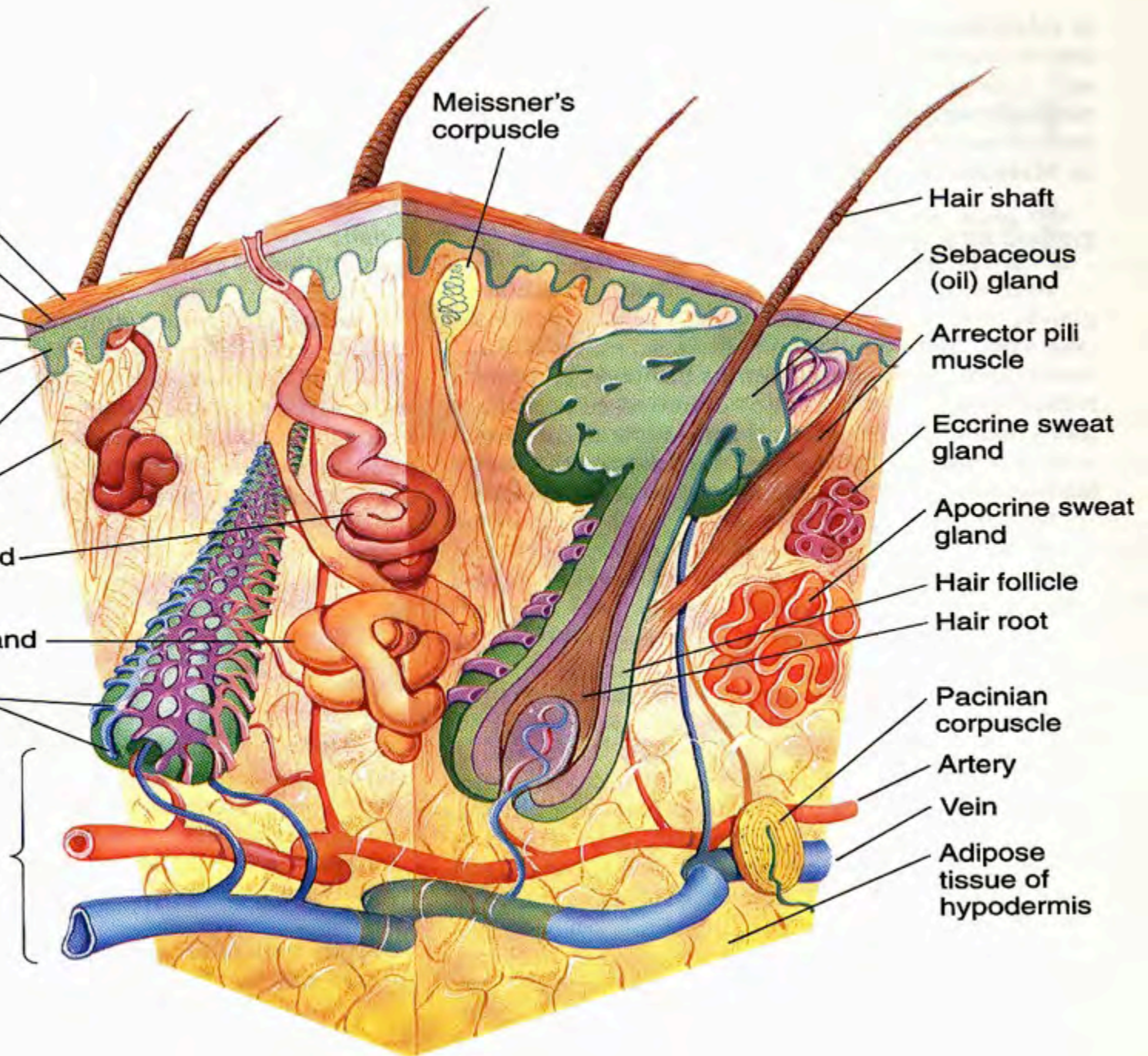
Hair root

Pacinian corpuscle

Artery

Vein

Adipose tissue of hypodermis



For skin and other biopsies:

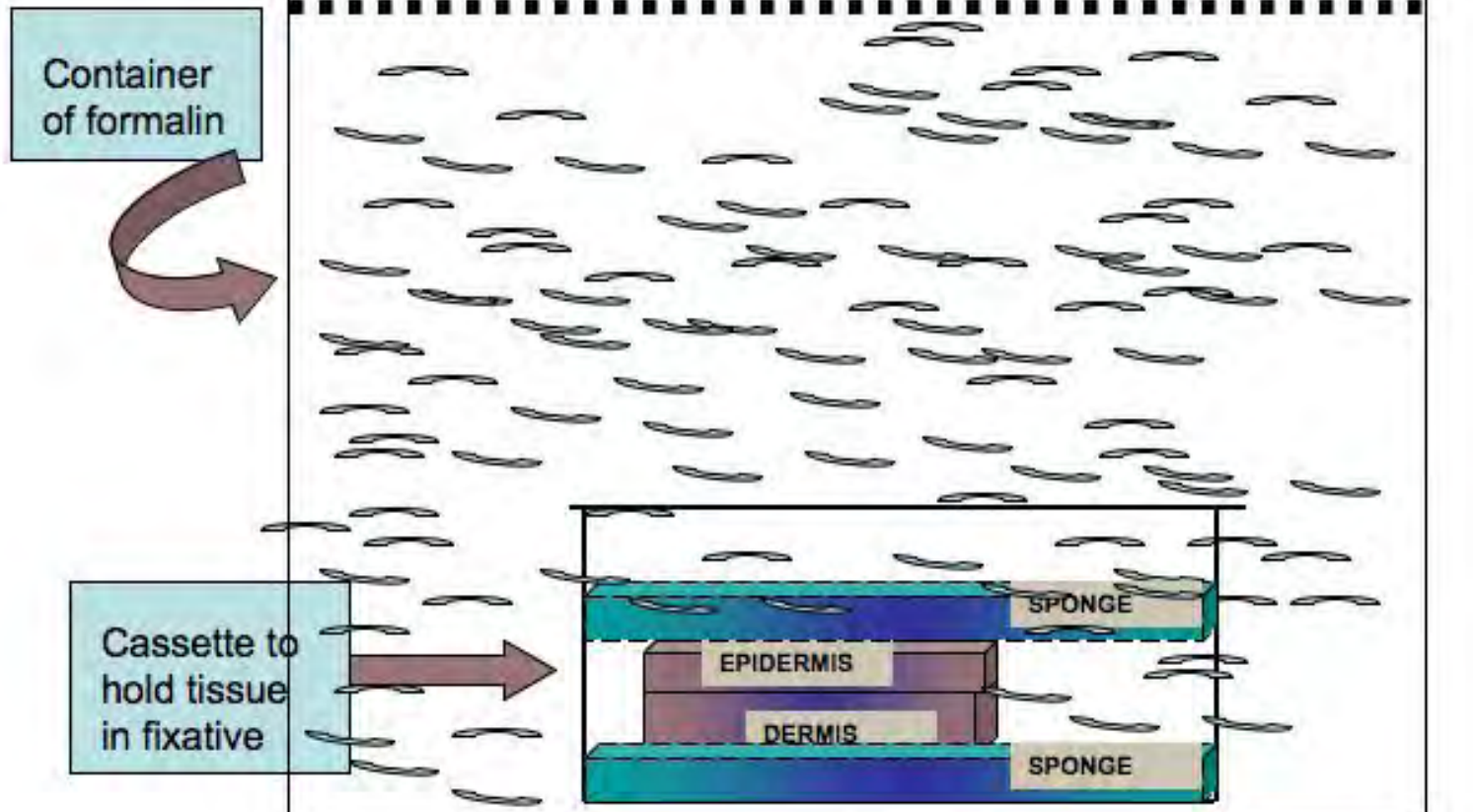
Fix flat between sponges for 24 hours

in 10 volume of

----10% buffered formalin

----Or freshly made 4% paraformaldehyde

Transfer to 70% alcohol before submitting for processing.



Squamous epithelium--
keratin positive

Cuboidal epithelium- around
sweat glands --keratin
positive

Collagen (trichrome stain
will help see this better)

Sebaceous glands

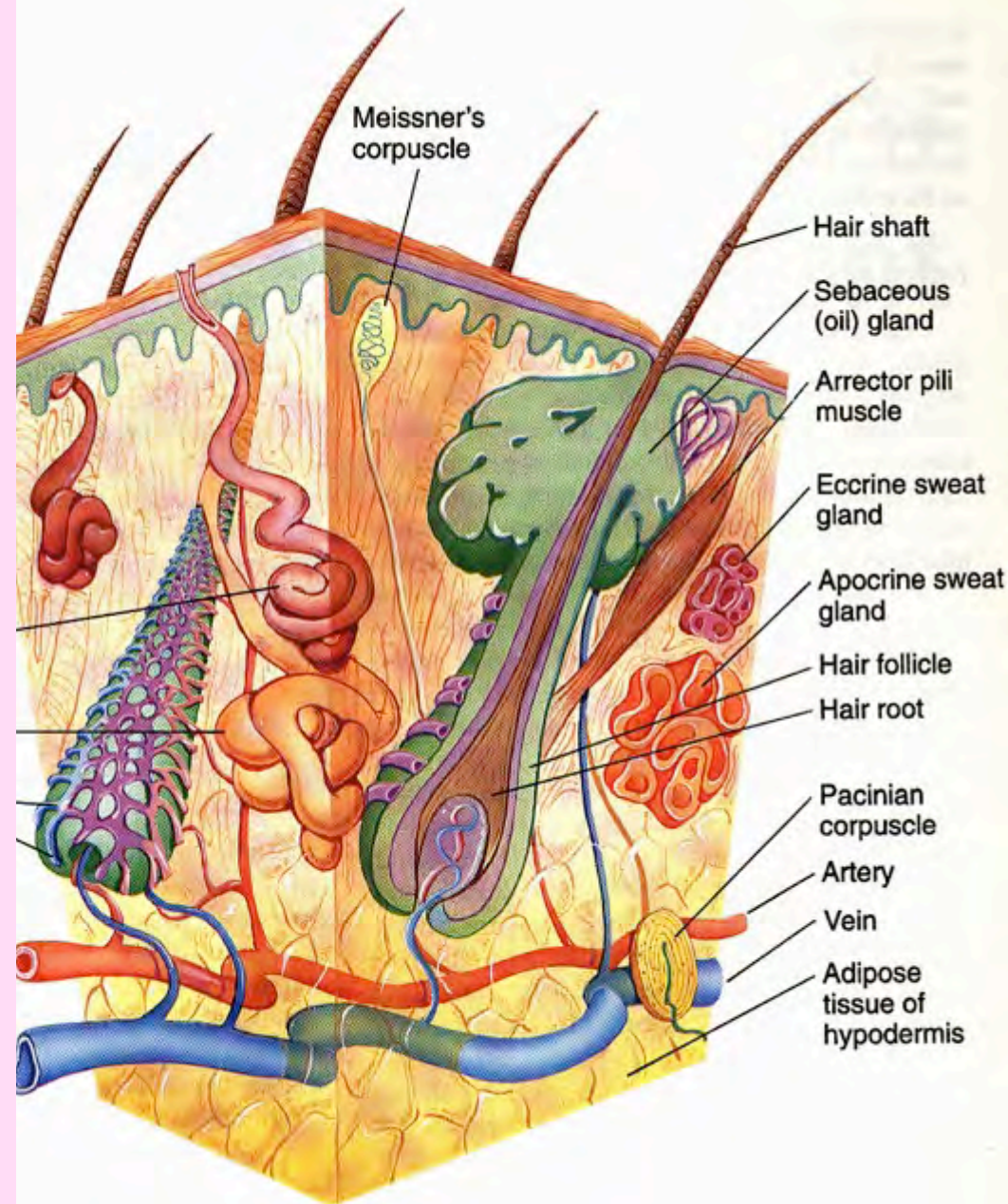
Fibroblasts--vimentin

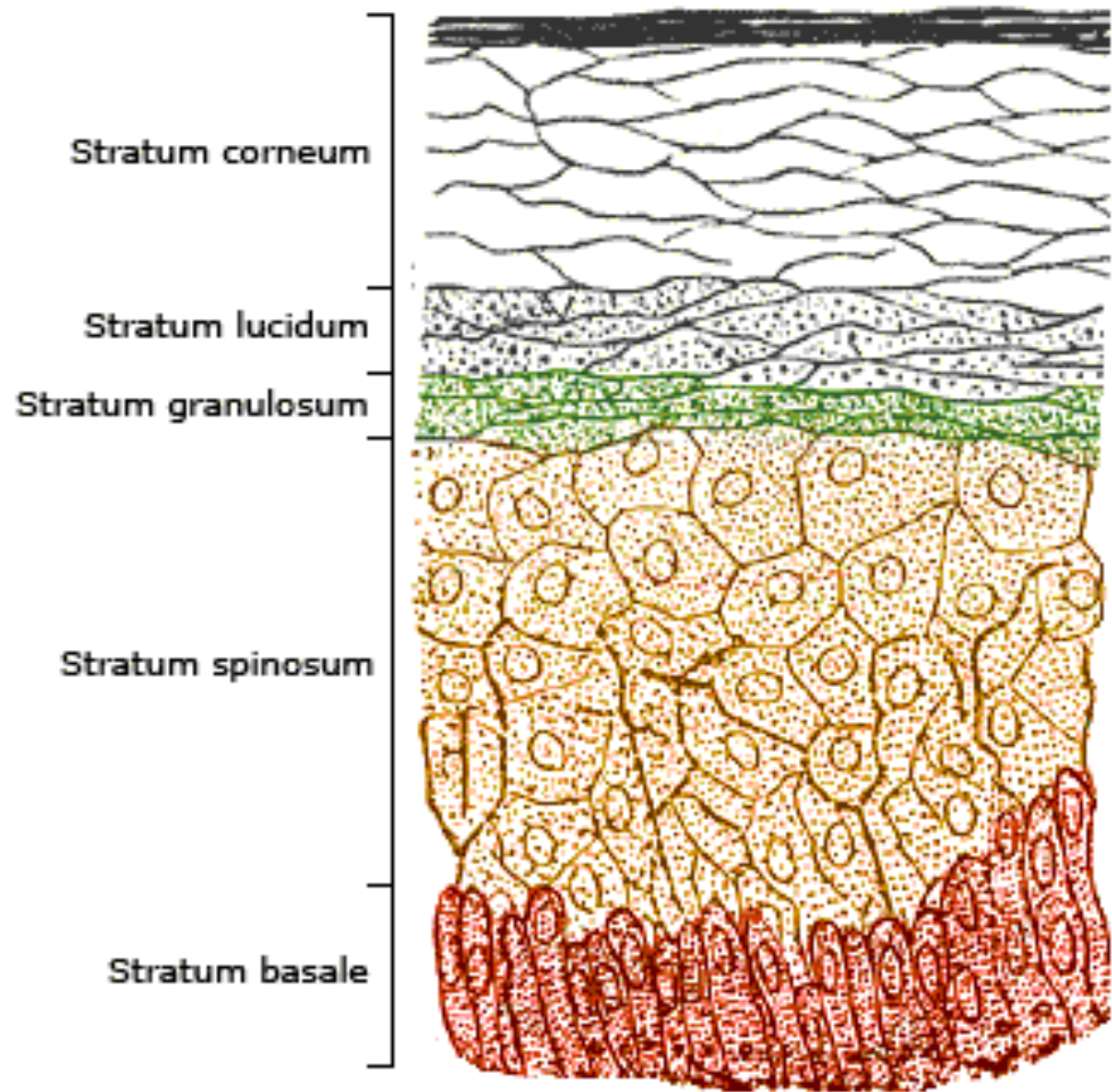
Blood vessels--CD31

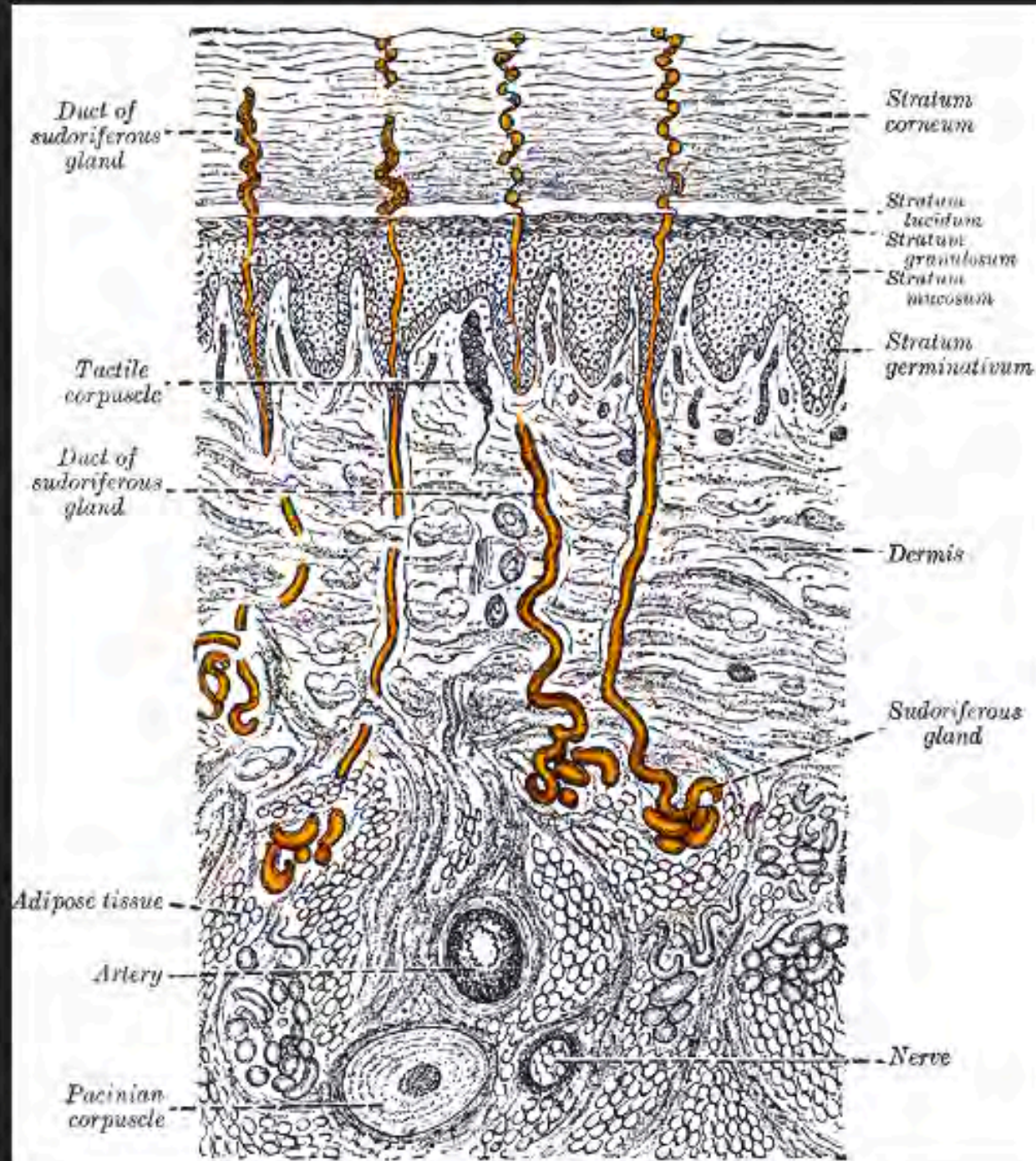
Nerve bundles

Adipose tissue

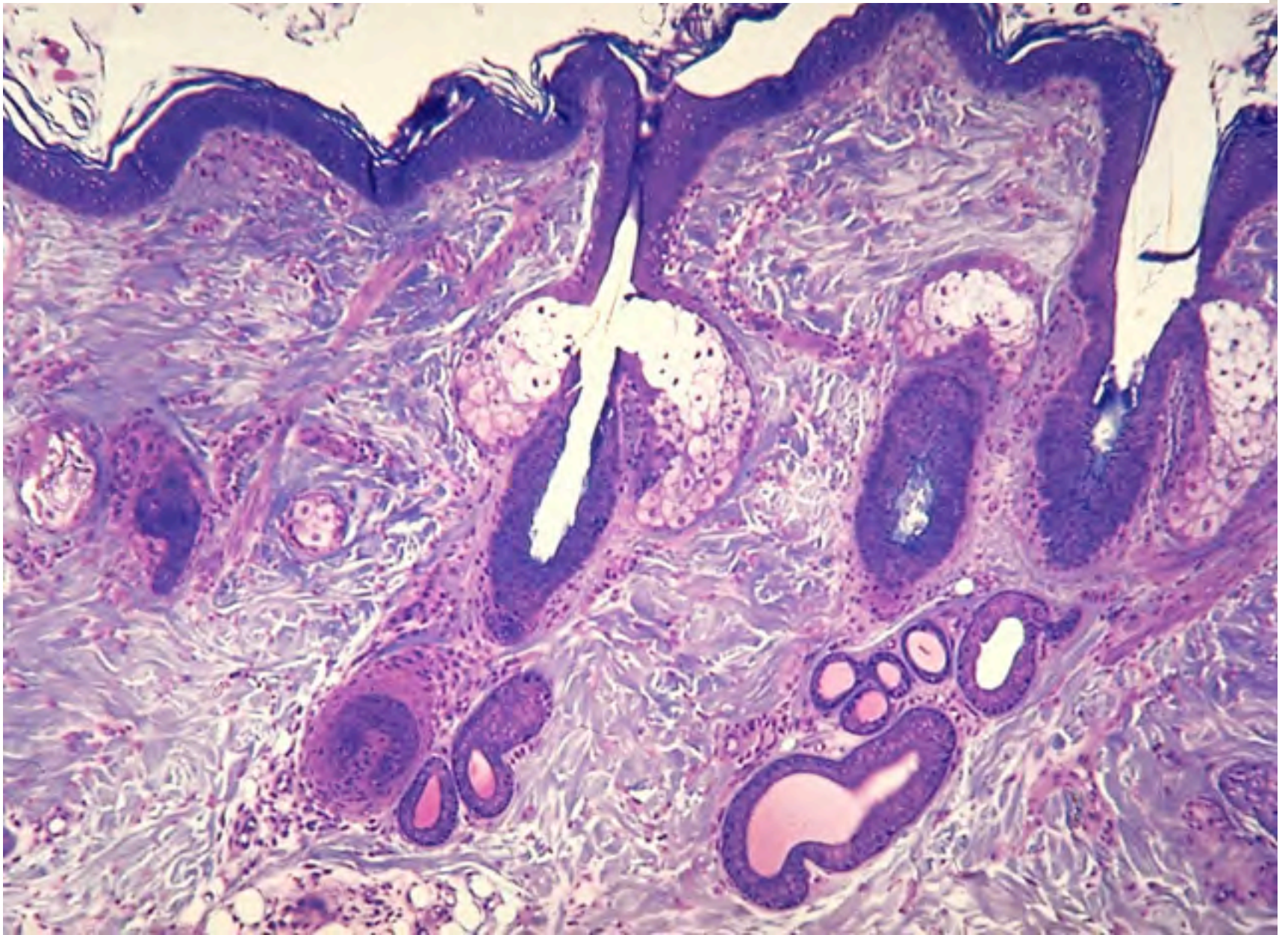
Innate immune cells--CD45
etc







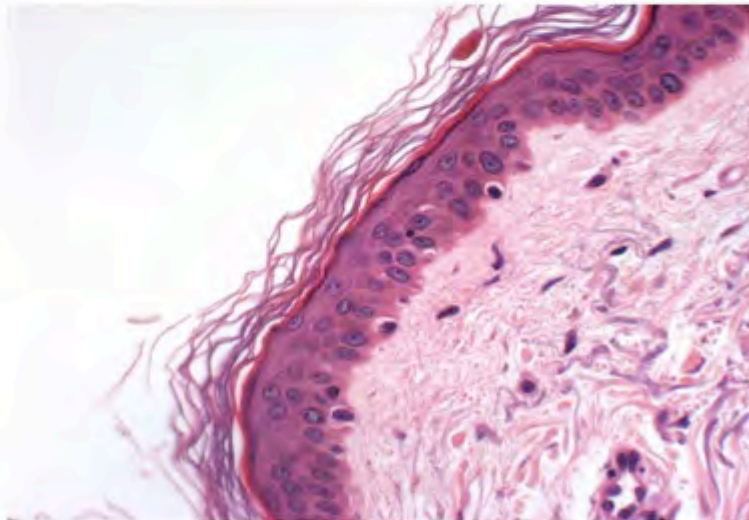
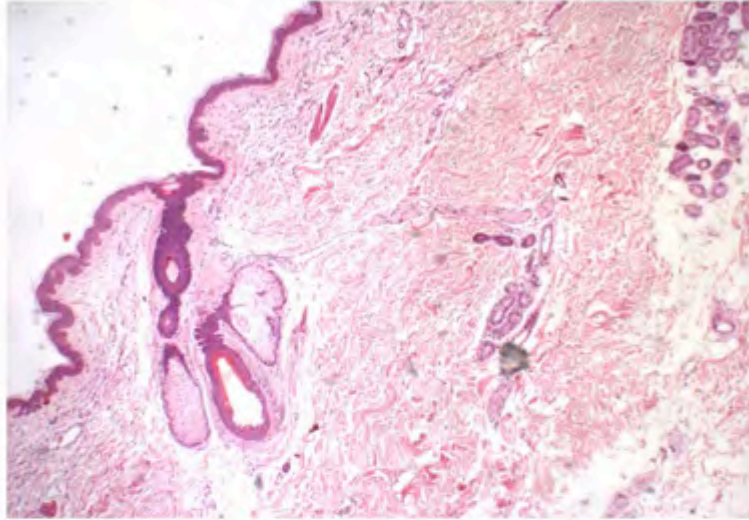
Keratinizing squamous epithelium of epidermis of skin



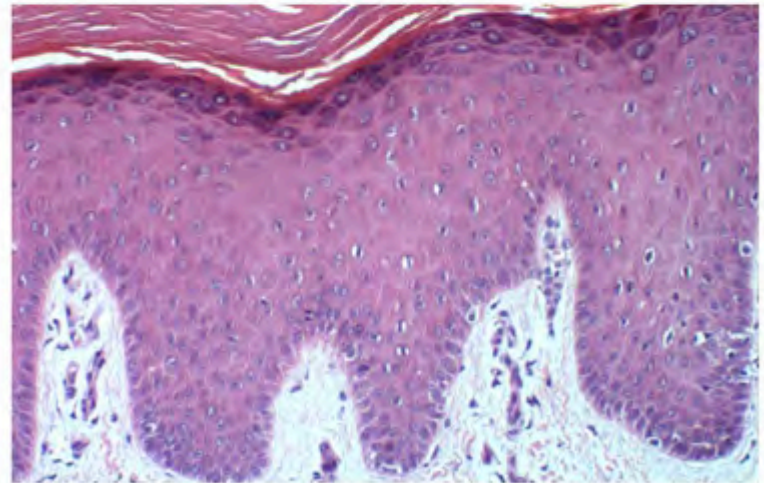
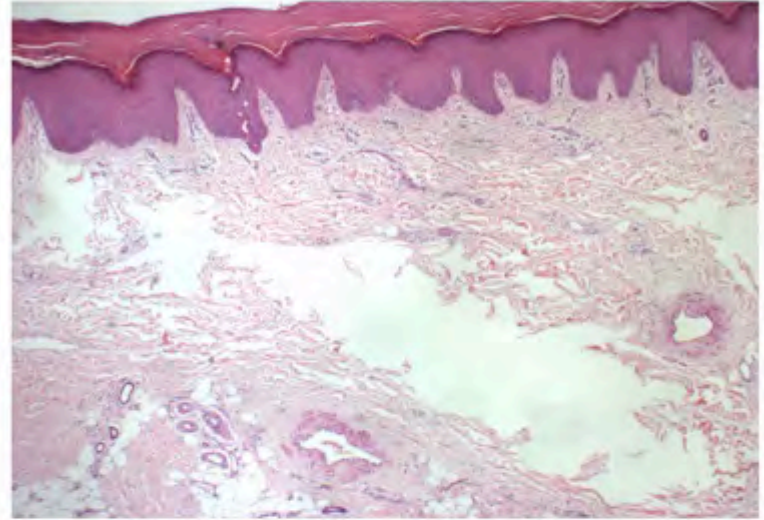
Dermis with sweat glands and sebaceous glands and hair follicles

Human Skin H&Es

Thin Skin



Thick skin



Human dermis: sweat and sebaceous glands

Sweat glands:

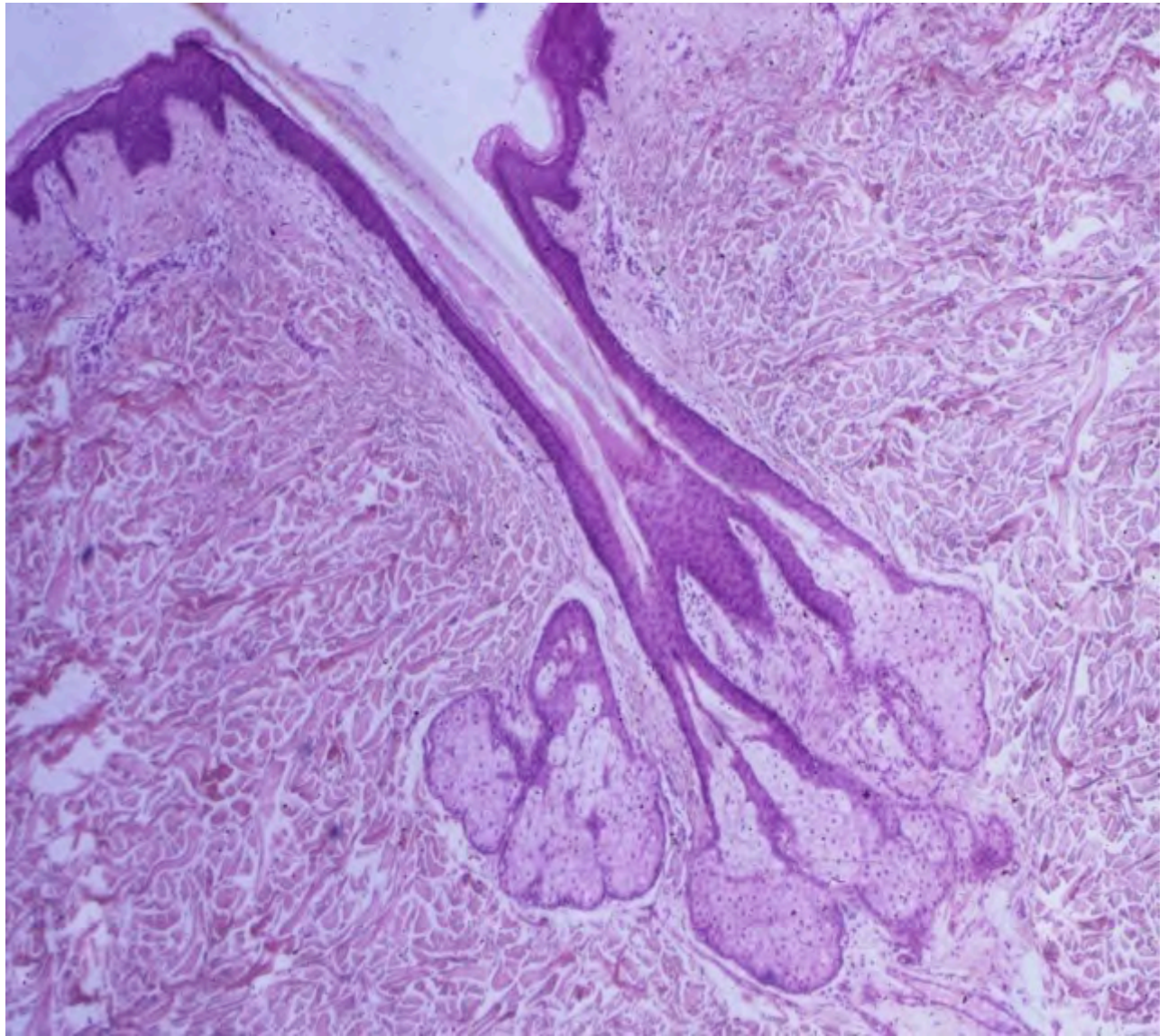
- **Eccrine** glands are found in all skin, with highest density in palms and soles.
 - Involved in Thermoregulation
- **Apocrine** glands

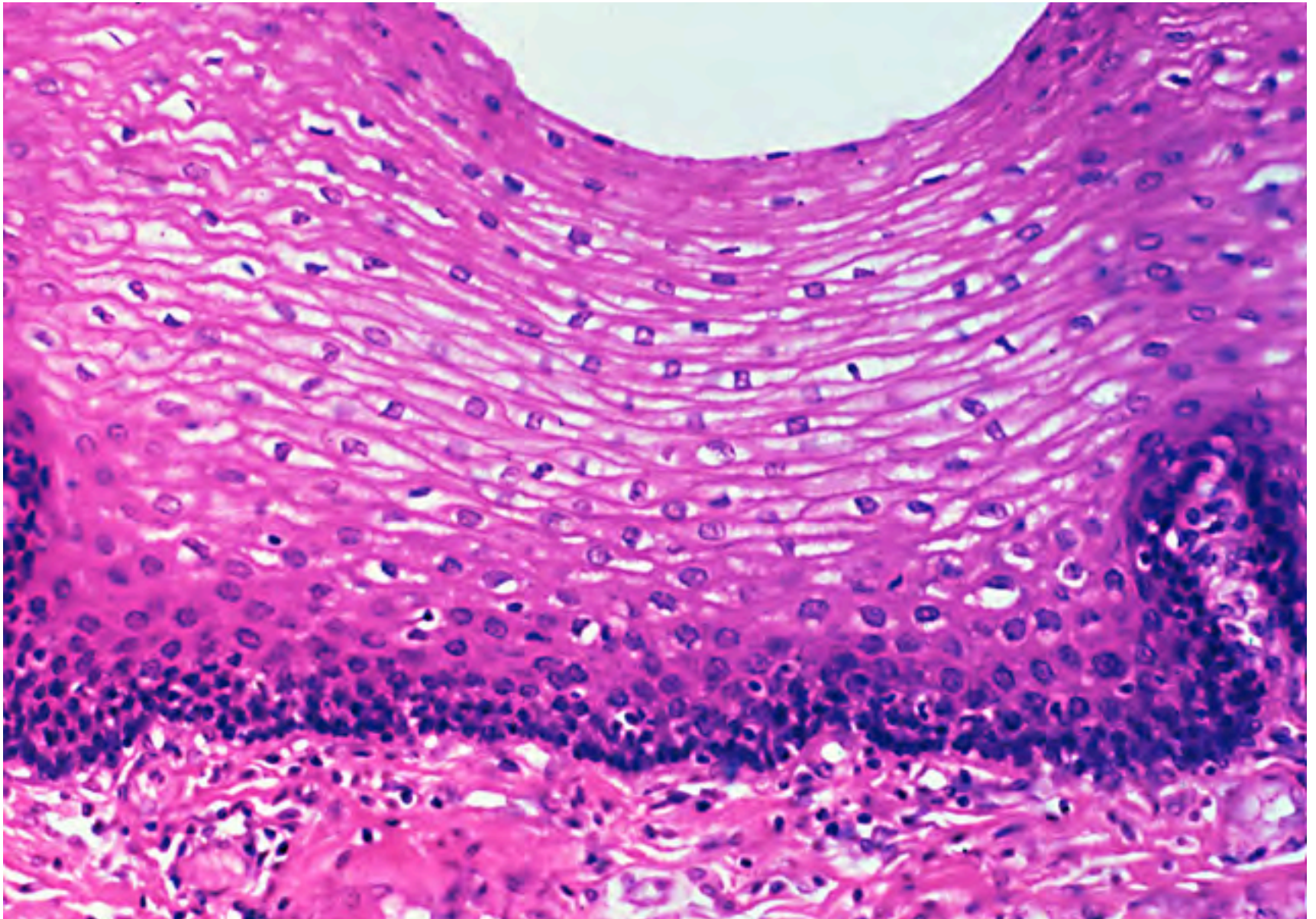
Sebaceous glands:

- Produce oily sebum
- Found on all parts of the skin except palms of hands and soles of feet in humans

Mice have sweat glands only on paws

Sebaceous
gland
surrounding
the base of
a hair
follicle



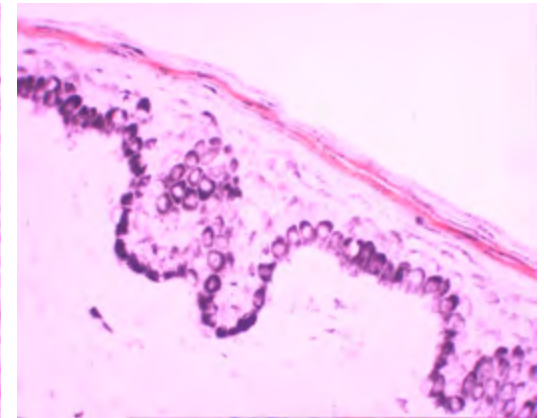
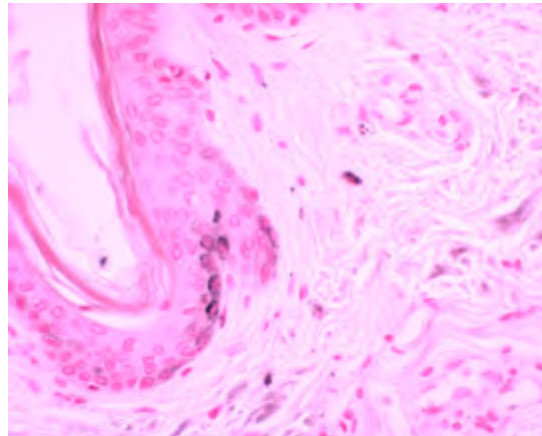
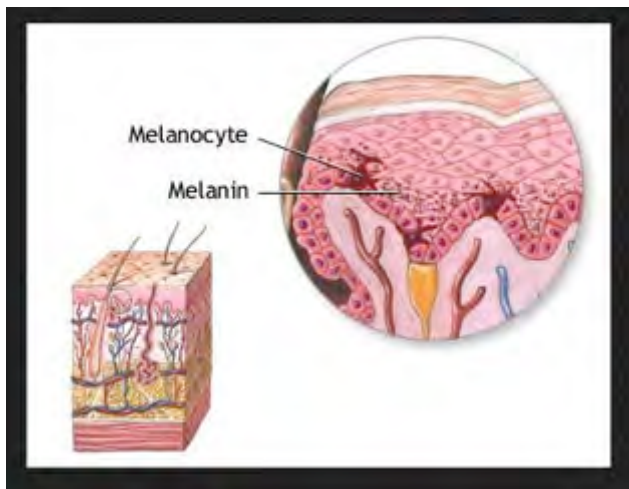


Esophagus and cervix has no keratin layer, no sweat glands, no hair follicles, no sebaceous glands etc.

Melanocytes comprise from 5% to 10% of the cells in the basal layer of epidermis. Although their size can vary, melanocytes are typically 7 μm in length.

The difference in [skin color](#) between lightly and darkly pigmented individuals is due not to the number (quantity) of melanocytes in their skin, but to the melanocytes' level of activity (quantity and relative amounts of [eumelanin](#) and [pheomelanin](#)). This process is under hormonal control, including the [MSH](#) and ACTH peptides that are produced from the precursor proopiomelanocortin.

[Albinos](#) lack an enzyme called [tyrosinase](#). Tyrosinase is required for melanocytes to produce melanin from the [amino acid tyrosine](#).^[8]



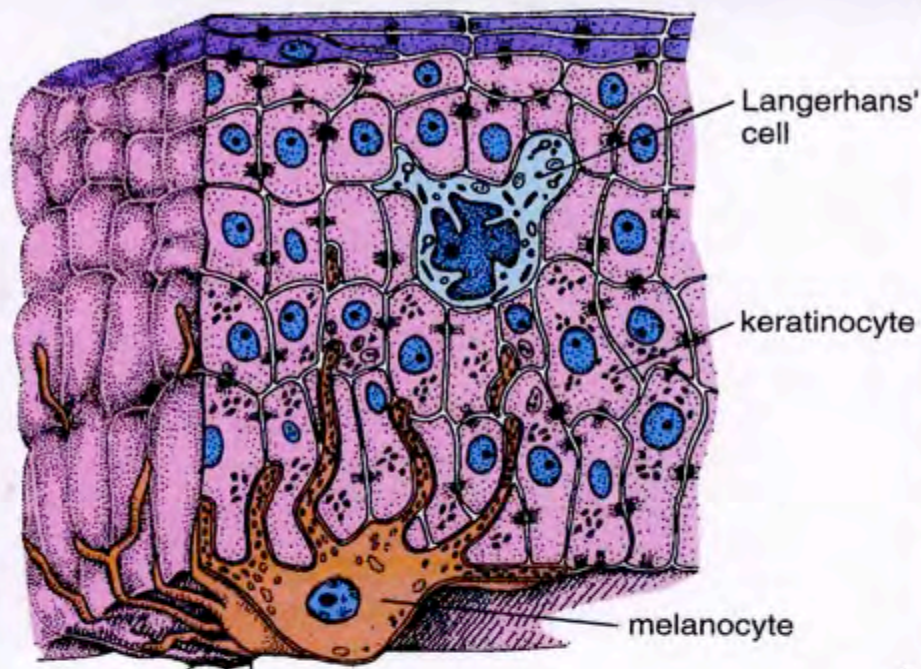


FIGURE 14.7

Diagram of the epidermis. This diagram shows a melanocyte interacting with several cells of the stratum basale and the stratum spinosum. The melanocyte has long dendritic processes that contain accumulated melanosomes and extend between the cells of the epidermis. The Langerhans' cell is a dendritic cell often confused with a melanocyte. It is actually part of the mononuclear phagocytotic system and functions as an antigen-presenting cell of the immune system in the initiation of cutaneous hypersensitivity reactions (contact allergic dermatitis). (Modified from Weiss L, ed. *Cell and Tissue Biology: A Textbook of Histology*. 6th ed. Baltimore: Urban & Schwarzenberg, 1988.)

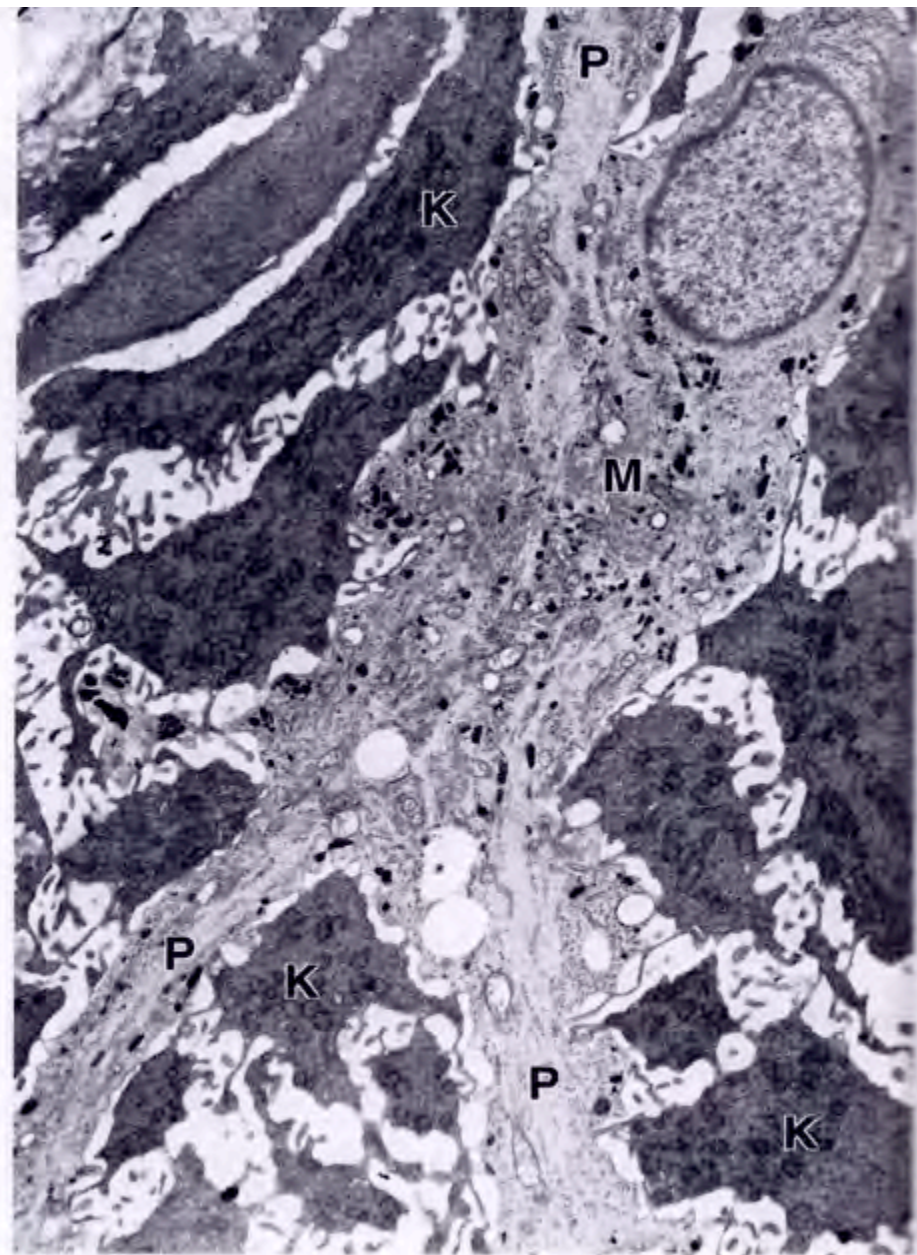
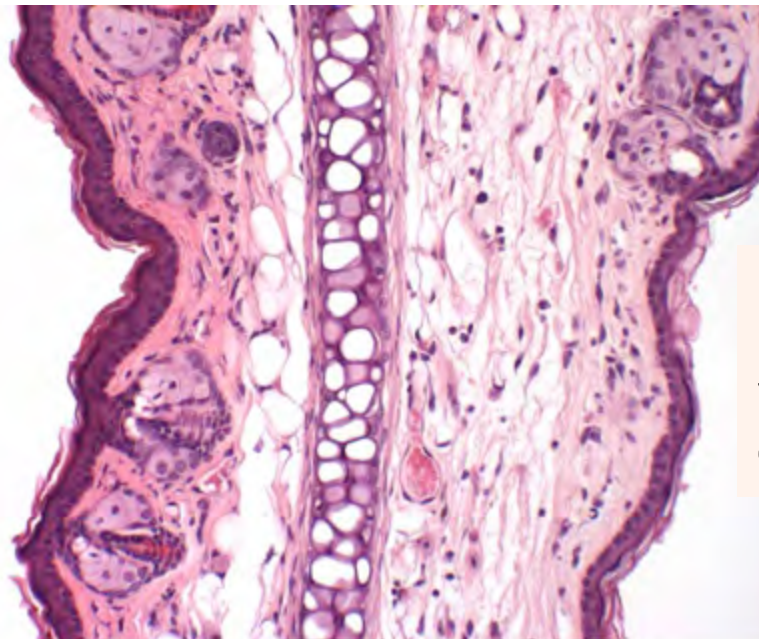
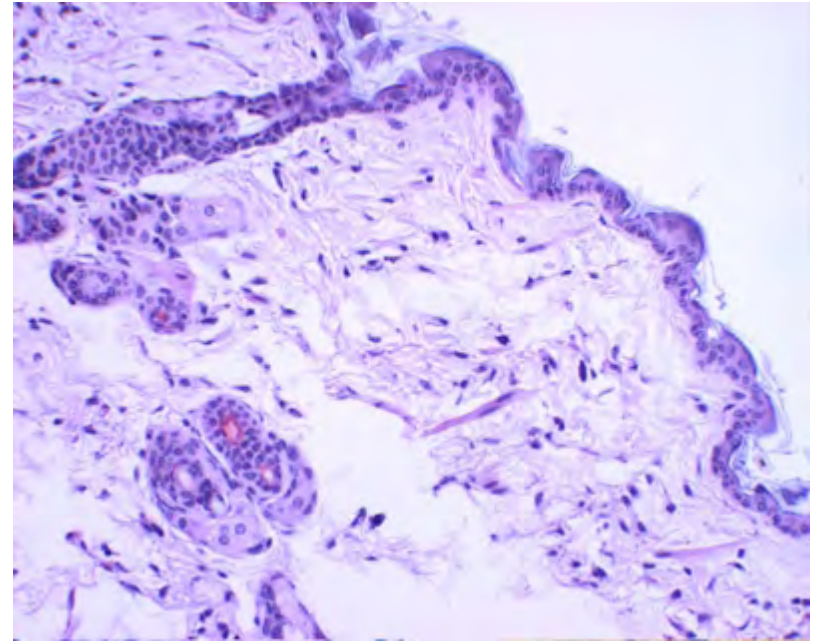
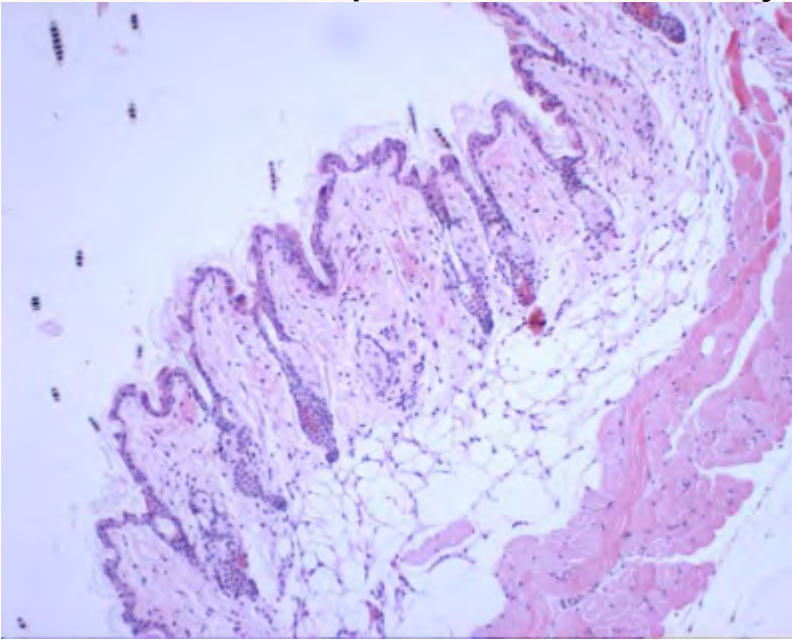


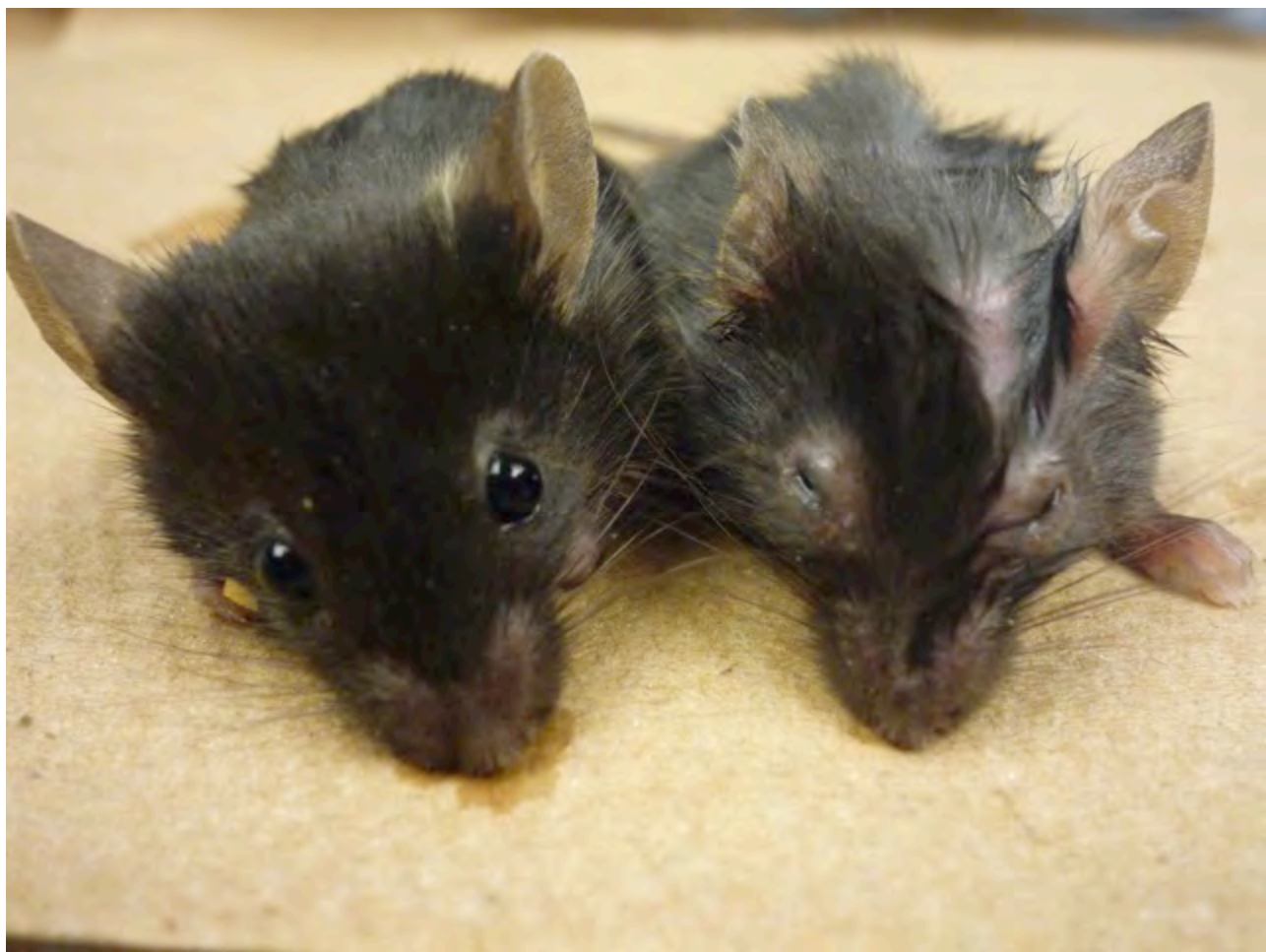
FIGURE 14.8

Electron micrograph of a melanocyte. The melanocyte (*M*) reveals several processes (*P*) extending between neighboring keratinocytes (*K*). The small dark bodies are melanosomes. $\times 8,500$. (Courtesy of Dr. [Name obscured])

Mouse skin epidermis is usually very thin



Mouse ear
skin and
cartilage

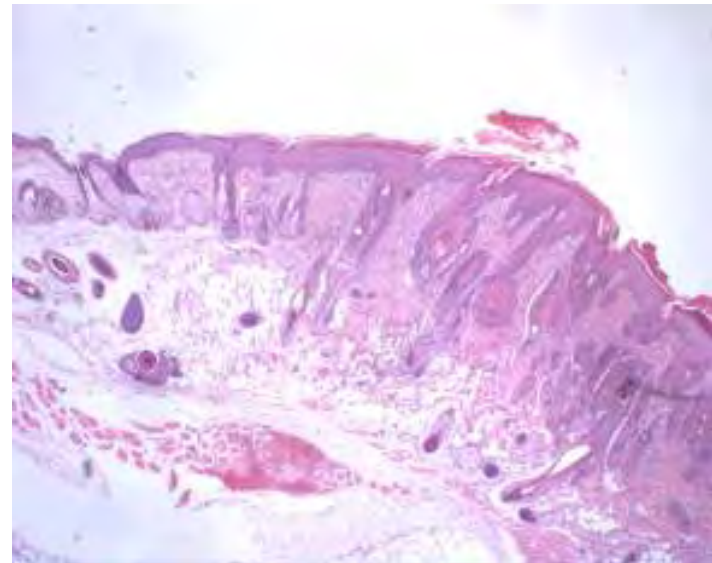
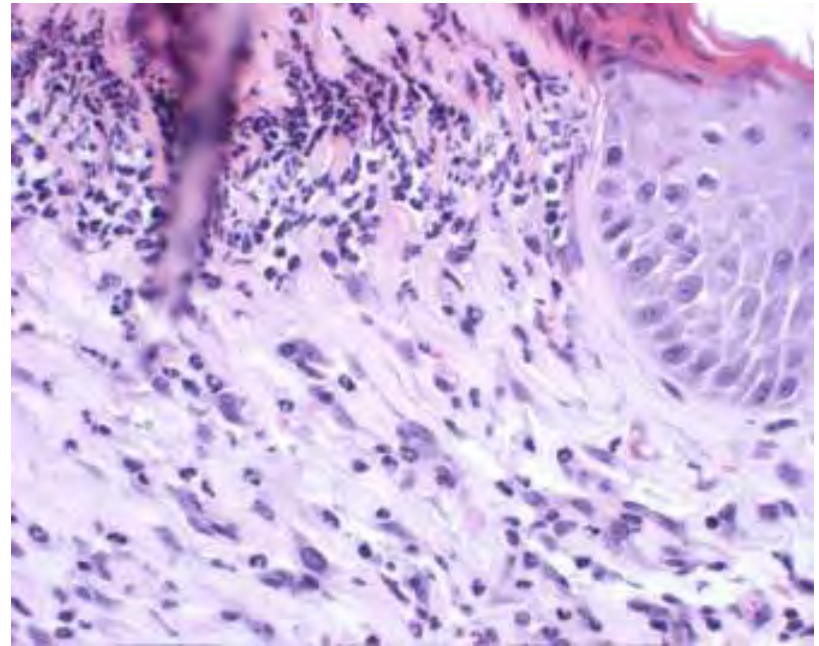


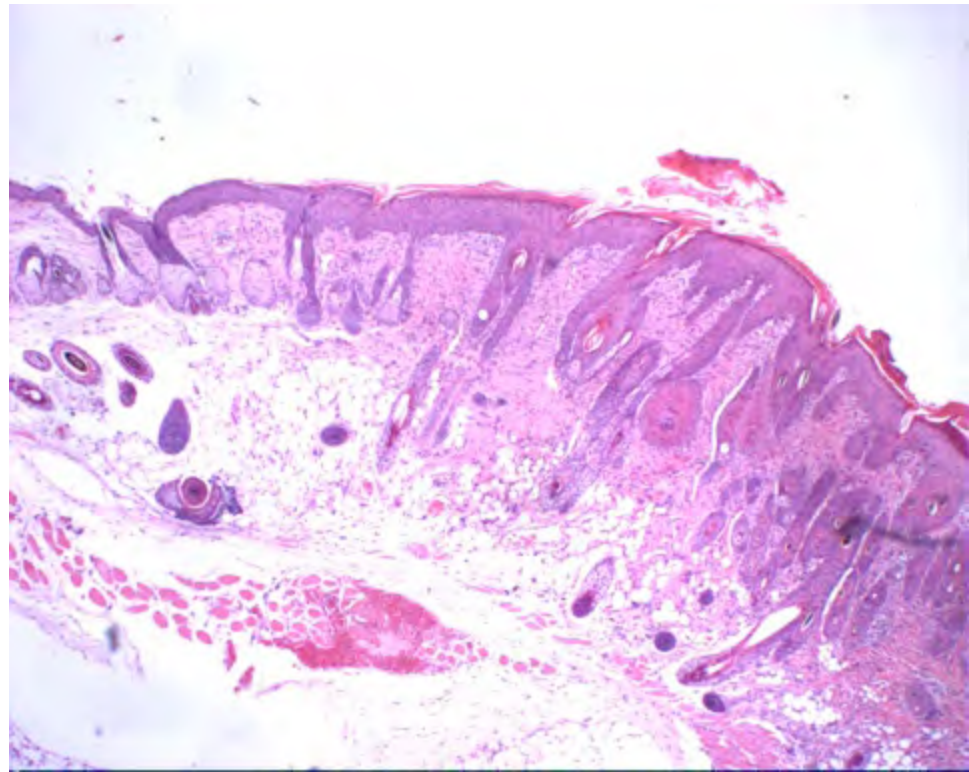
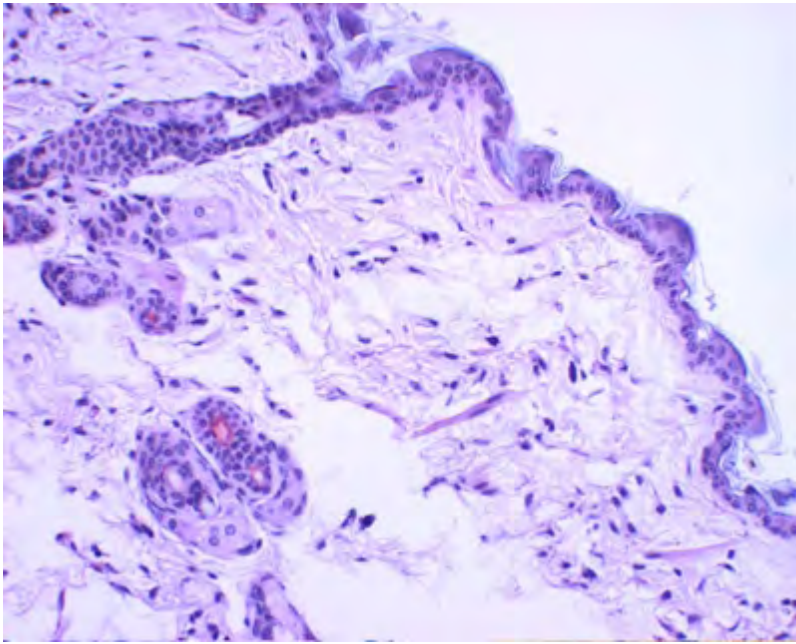
INFLAMMATION AND REPAIR

Is a protective response, where the goal is to rid the body of the initial cause of injury and the consequences

ACUTE: relatively short duration. There is an alteration of blood vessels such that there is an exudation of fluid and plasma proteins, with an emigration of leukocytes, predominantly **neutrophils**, into the focus of injury.

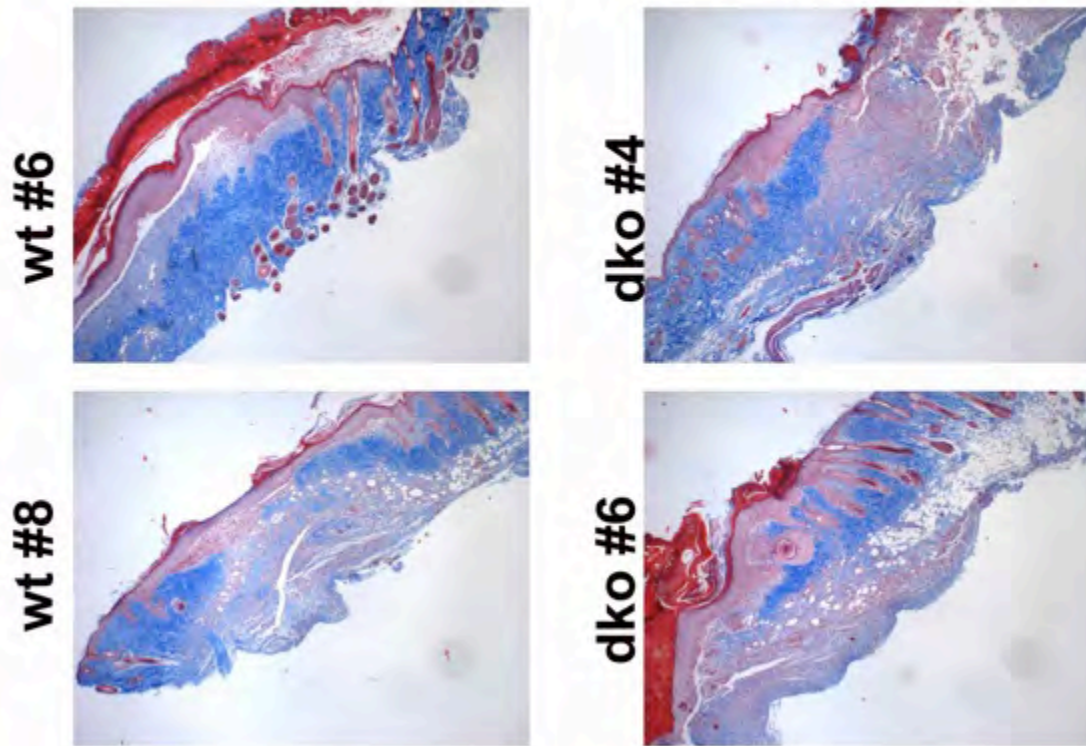
CHRONIC: is of longer duration and is associated with the accumulation of **lymphocytes and macrophages** and allowing the repair process to occur, using angiogenesis and/ or fibrosis.





An example of
abnormally thickened
mouse skin epidermis
as a result of
inflammation

grp3 d14 Trichr x40



An example of Trichrome stains being used to quantify scarring in dermal tissue

Sections of skin and gut are usually good controls for TUNEL assays for apoptosis

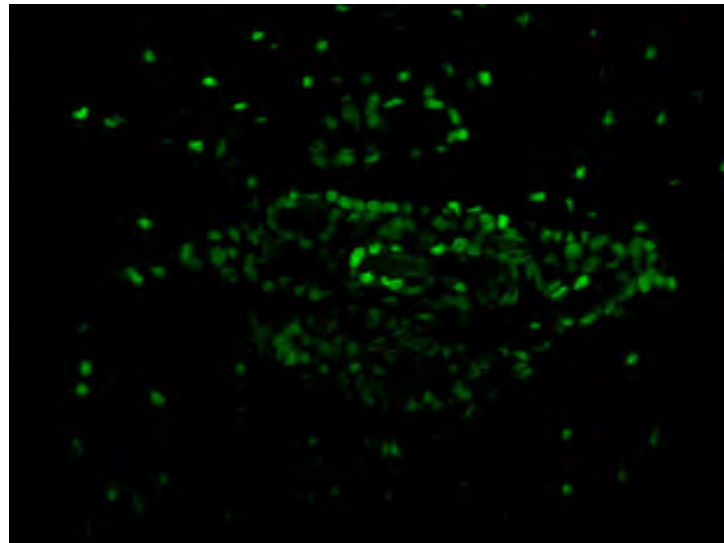
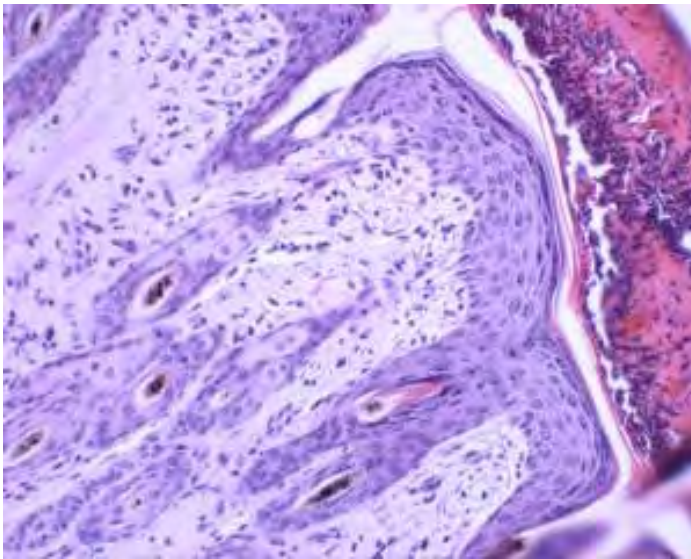
CELL DEATH:

necrosis (occurs from the progressive degradative action of enzymes on the lethally injured cells)

**apoptosis: -programmed destruction of cells
during embryogenesis**

-hormone dependent involution in the adult

**- cell deletion in proliferating cell populations,
immune cells, tumors, etc.**

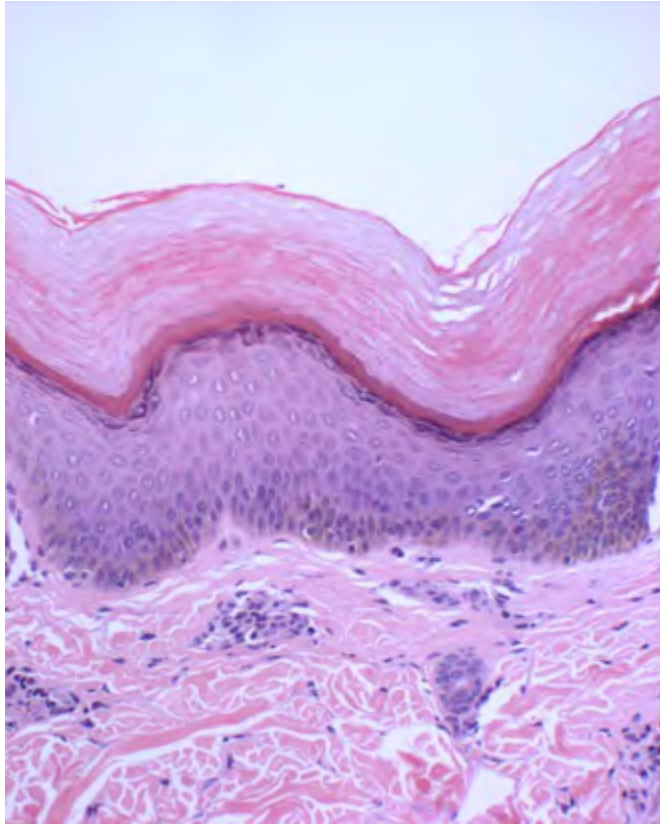


Benign tumors of skin epidermis may arise from any of the component cells:

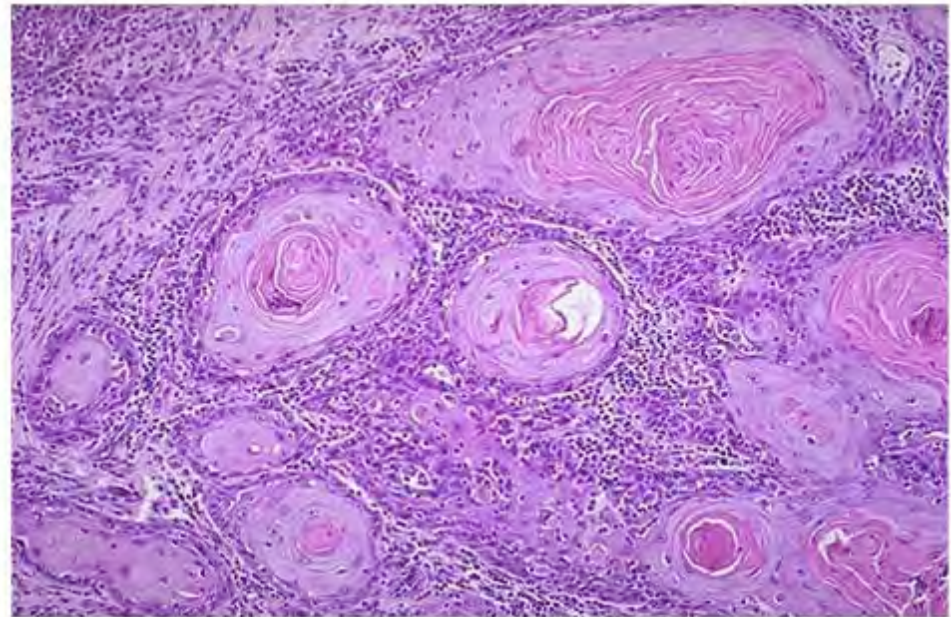
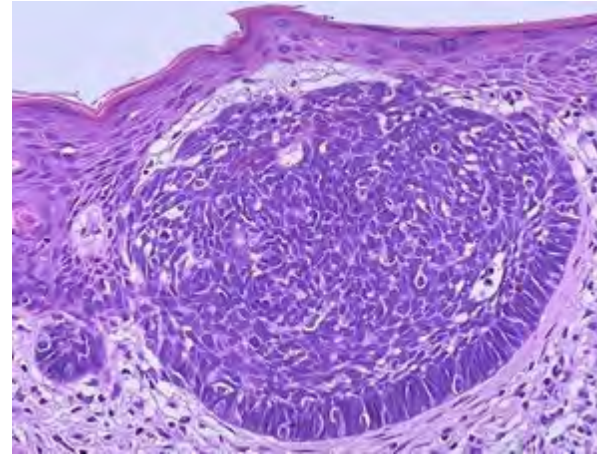
- hair follicle tumors--trichoepitheliomas
- sweat gland tumors
- vascular tumors
- tumors arising from the supporting fibroblasts

Malignancies of the skin include:

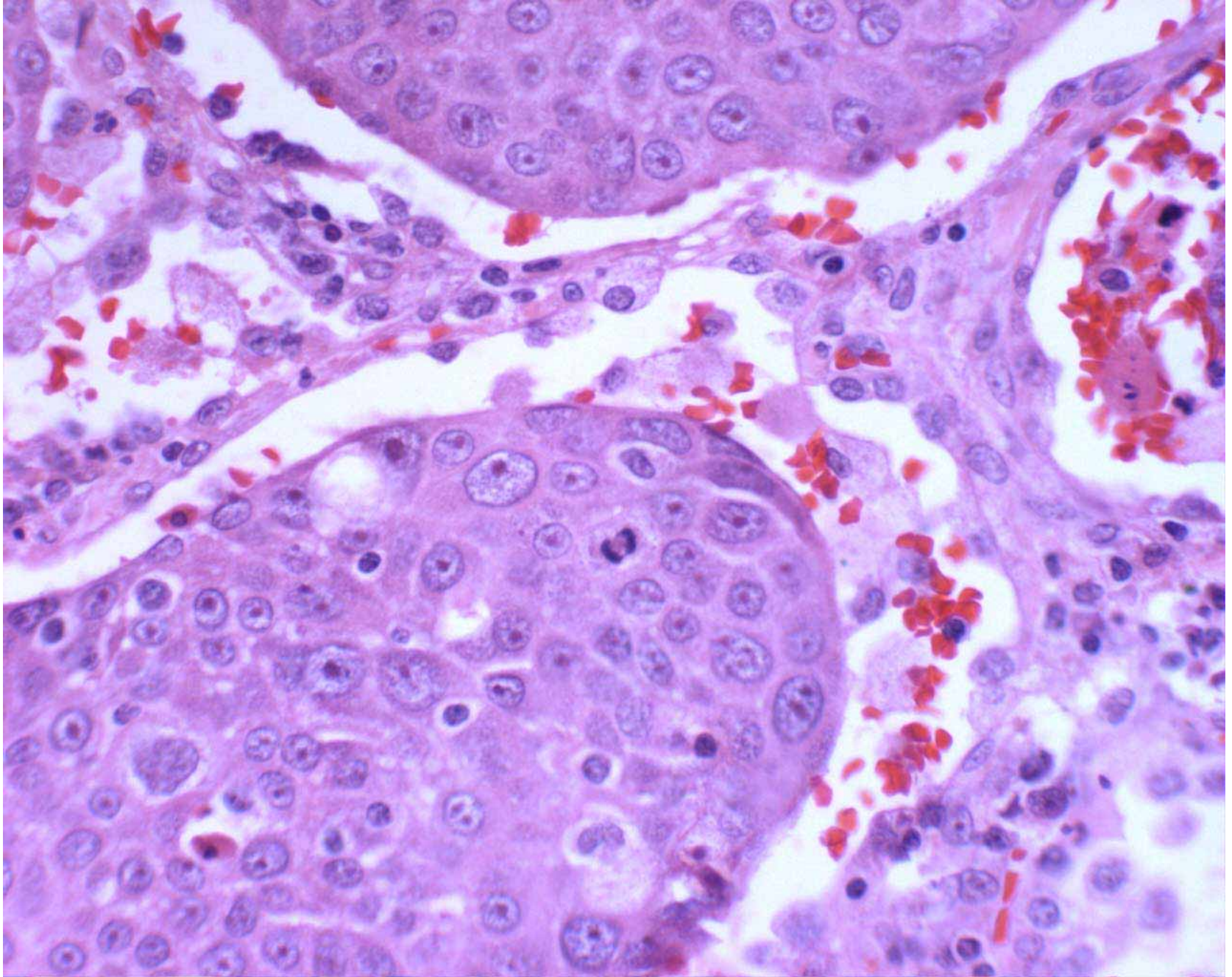
- basal cell carcinomas--only locally invasive
- squamous carcinomas
- melanomas
- metastatic malignancies



Normal skin



Squamous carcinoma



Carcinoma with mitoses

Features of Melanoma--"ABCD"



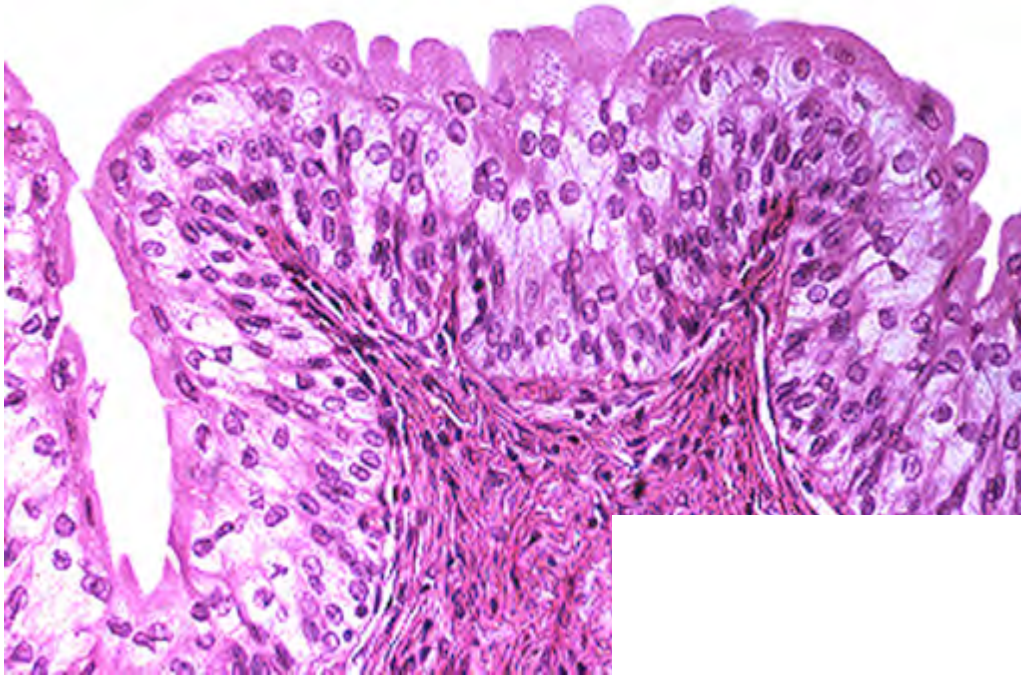
Asymmetry: If you could fold the lesion in two, the two halves would not match.

Border . Melanomas often have uneven or blurred borders.

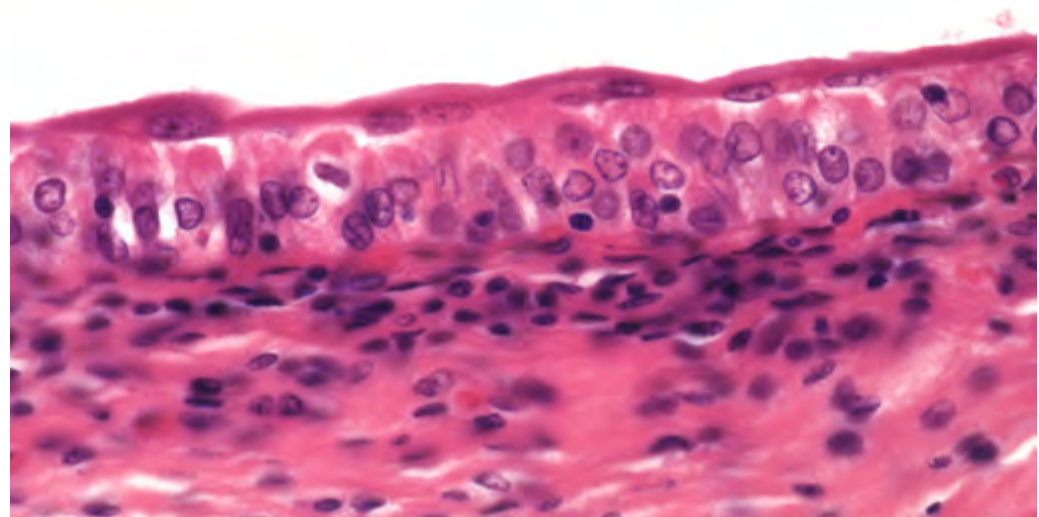
Color . Melanoma typically is not one solid color; rather it contains mixed shades of tan, brown, and black. It can also show traces of red, blue or white.

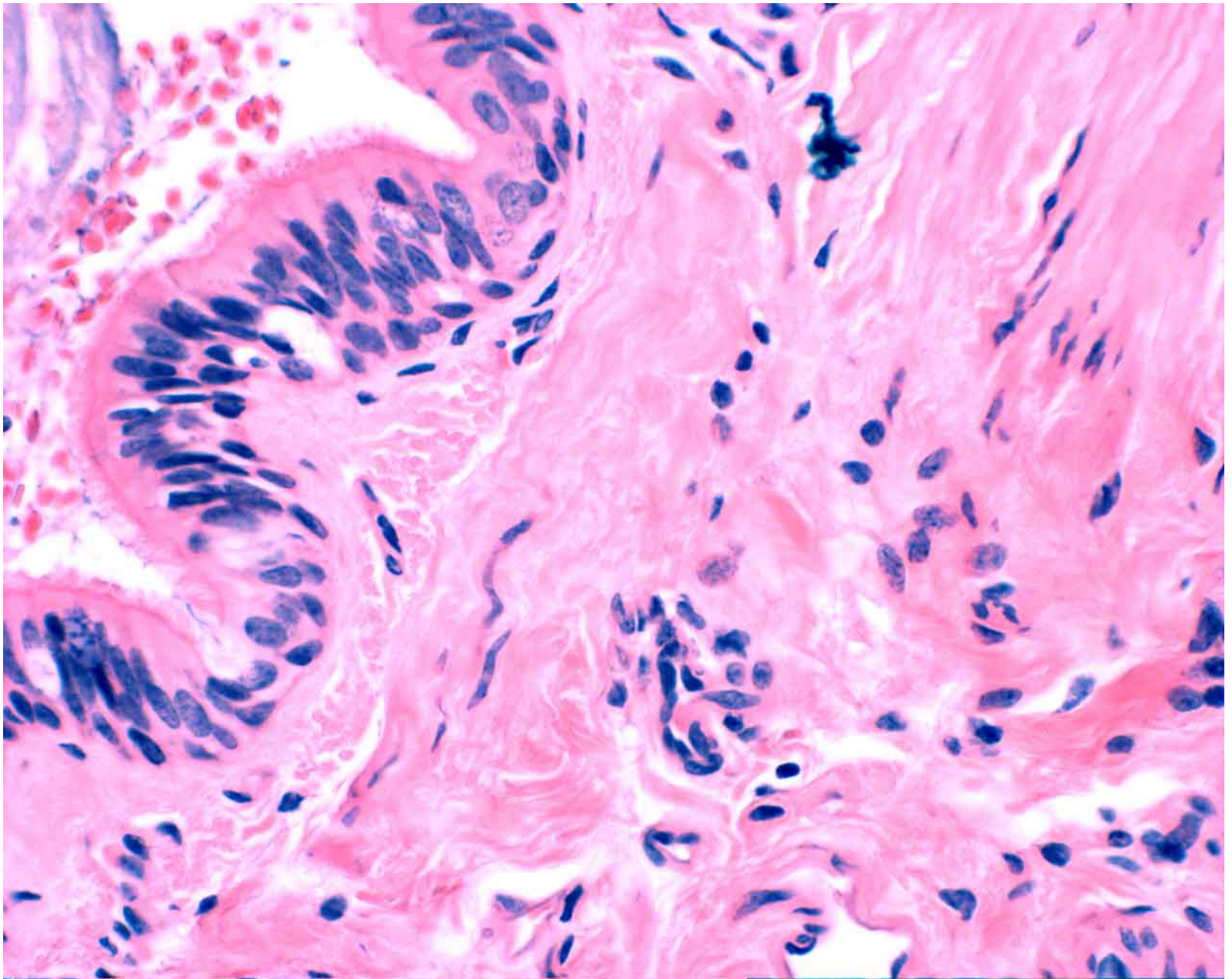
Diameter . While melanomas are usually greater than 6 millimeters (about the size of a pencil eraser) when diagnosed, they can be smaller.

If you notice a mole different from others, or which changes, itches, or bleeds even if it is smaller than 6 millimeters, you should see a dermatologist.



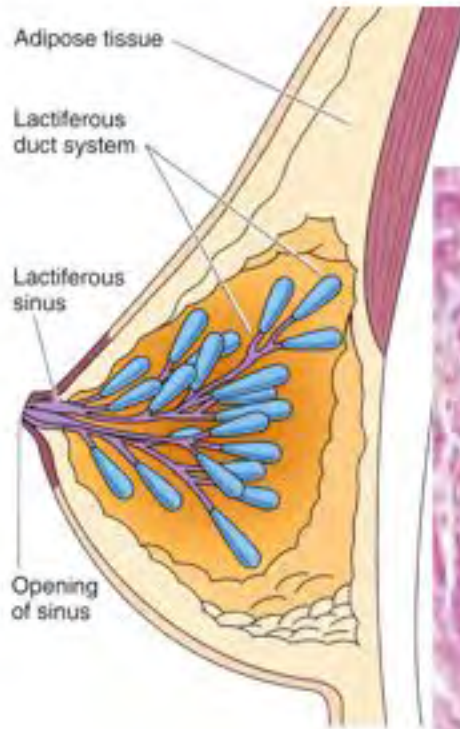
Transitional
epithelium of the
bladder



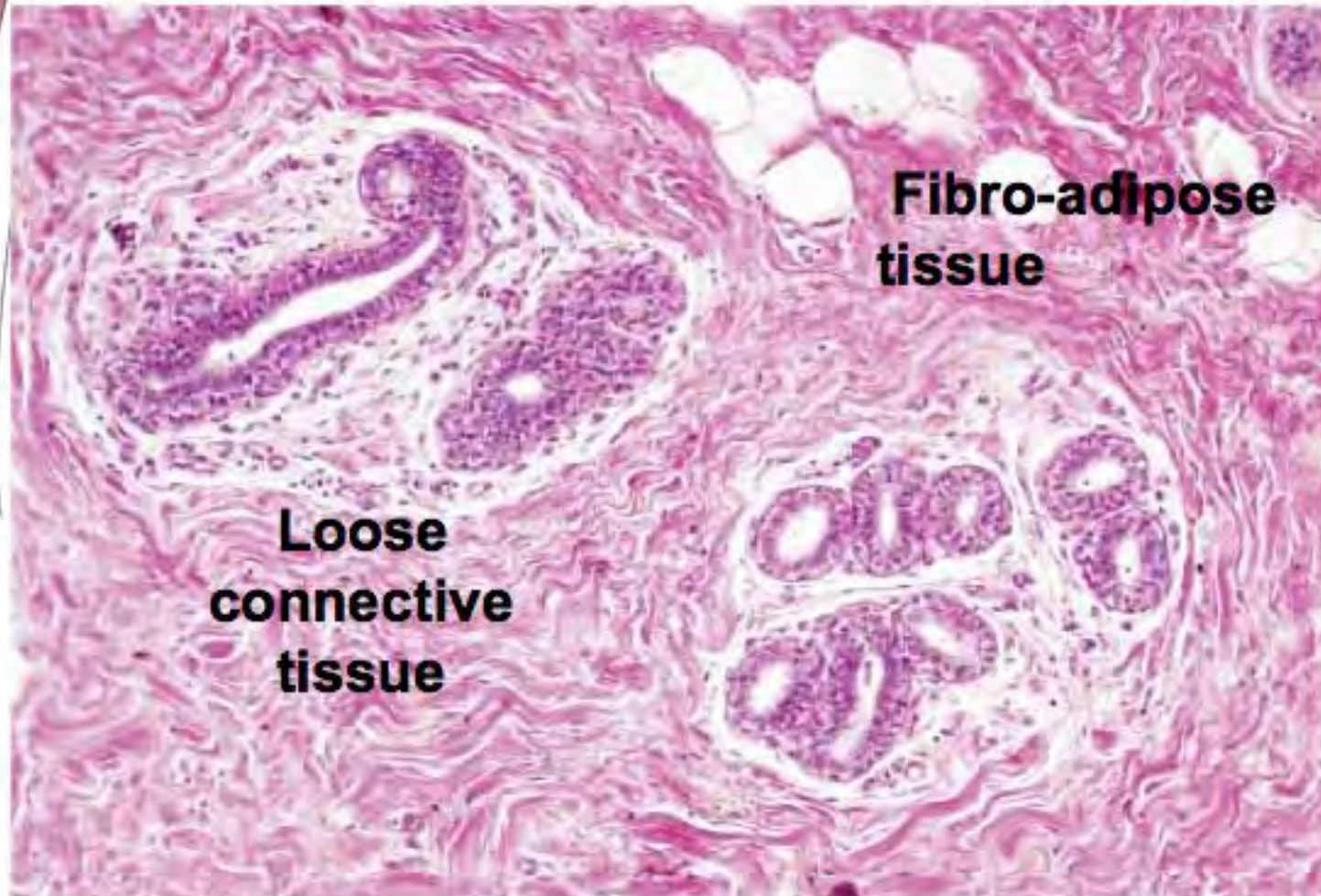


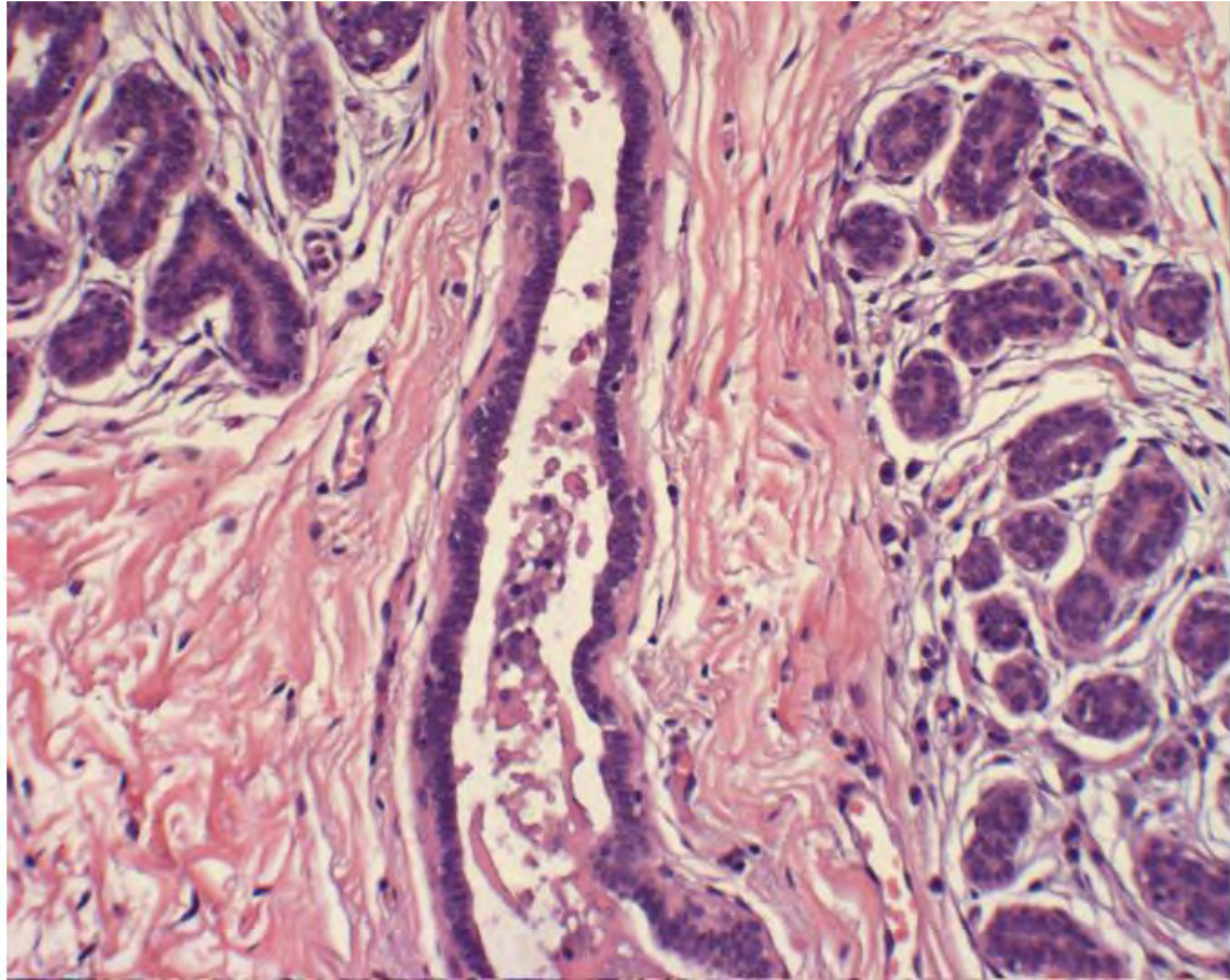
Bronchus epithelium: PseudoStratified Columnar NOT Stratified Squamous

Mammary Glands, Inactive



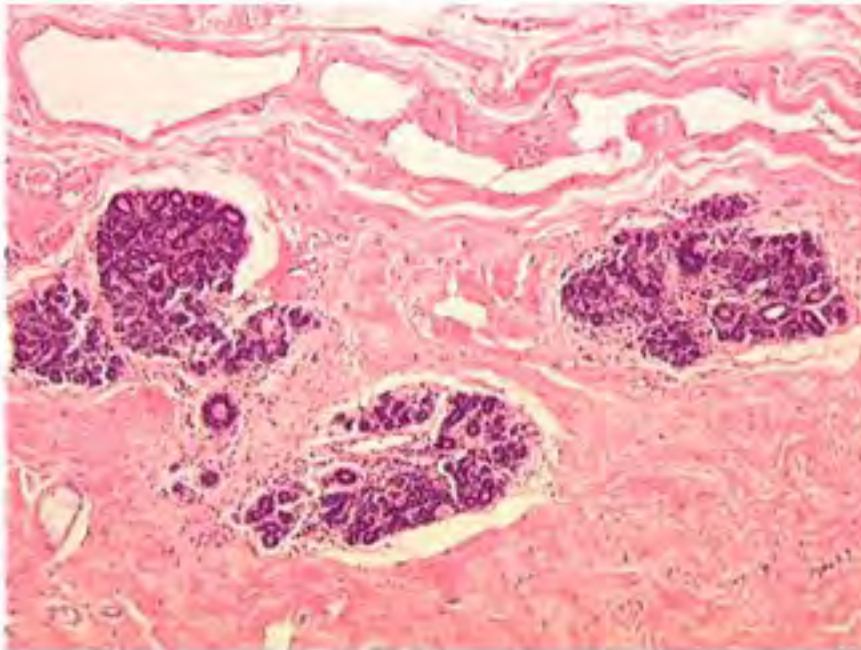
Lactiferous ducts



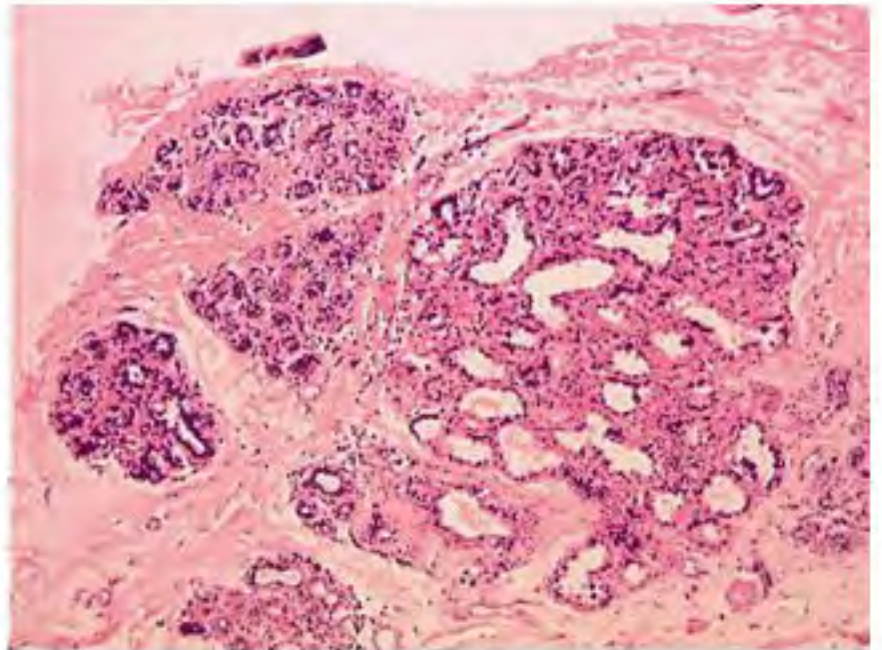


Mammary Glands

INACTIVE



ACTIVE



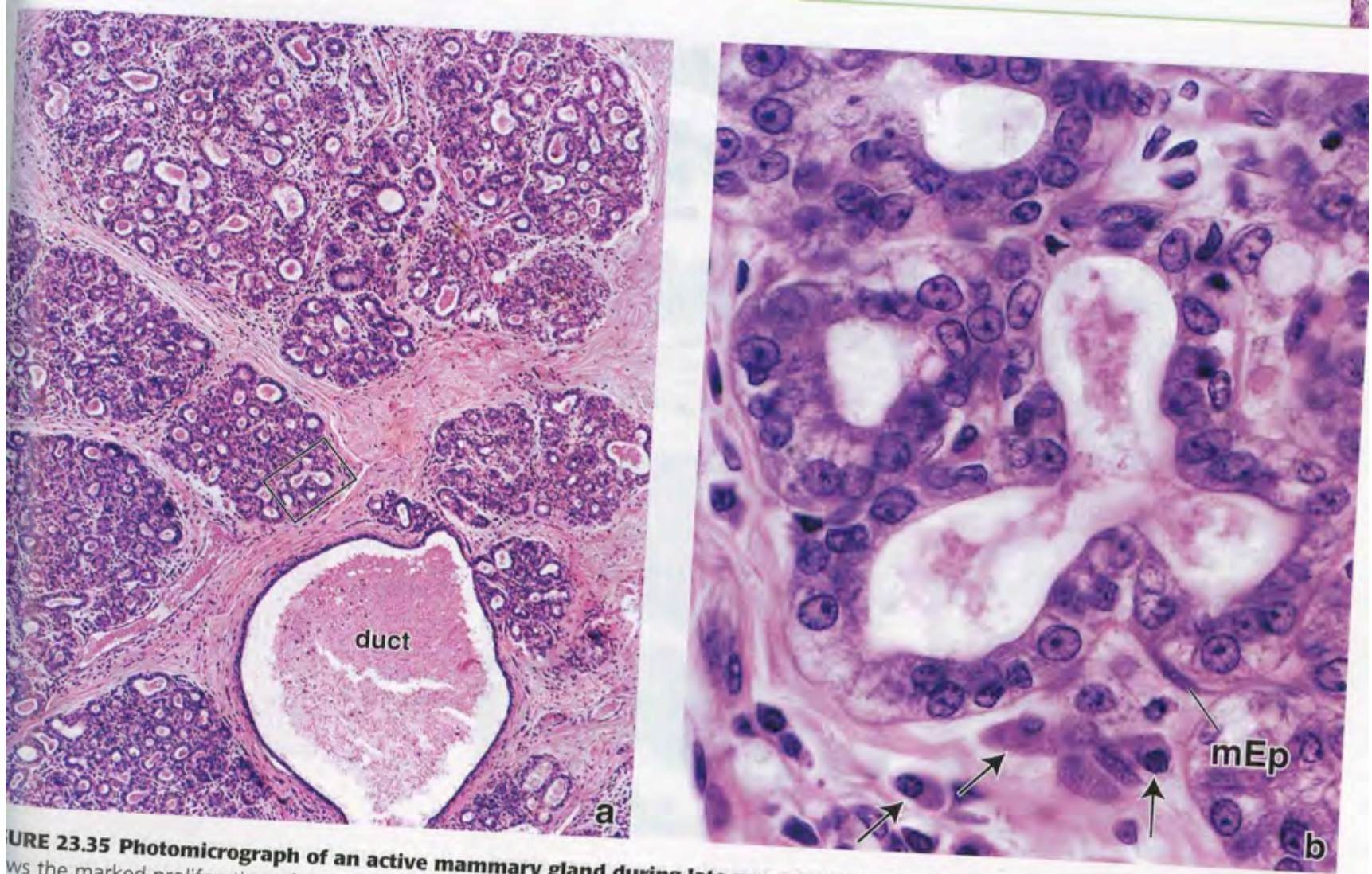
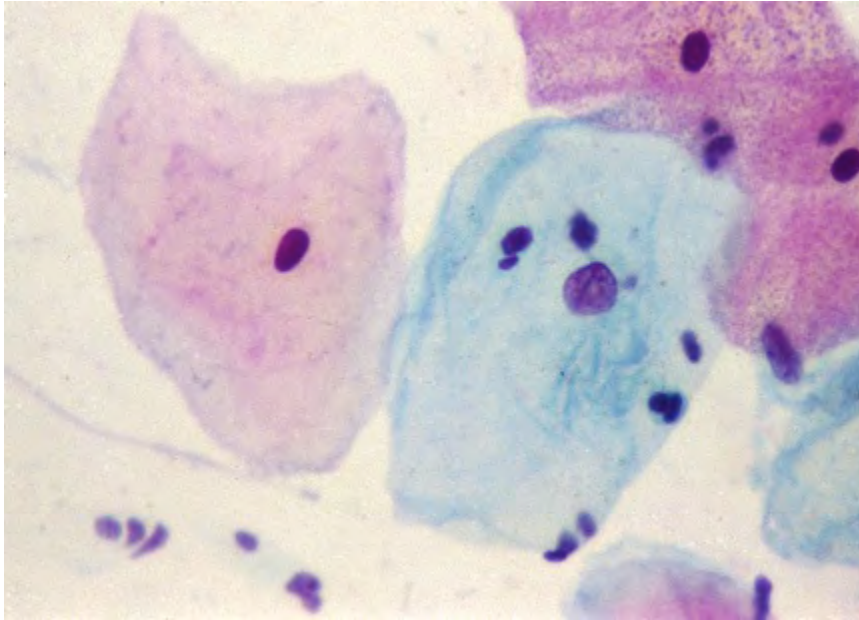
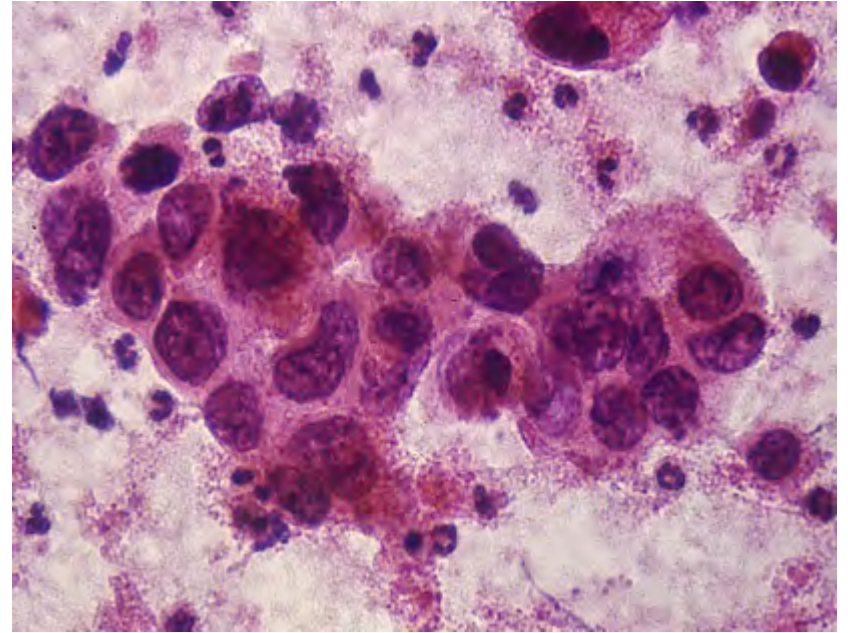


FIGURE 23.35 Photomicrograph of an active mammary gland during late pregnancy. a. This low-magnification view shows the marked proliferation of the duct system.

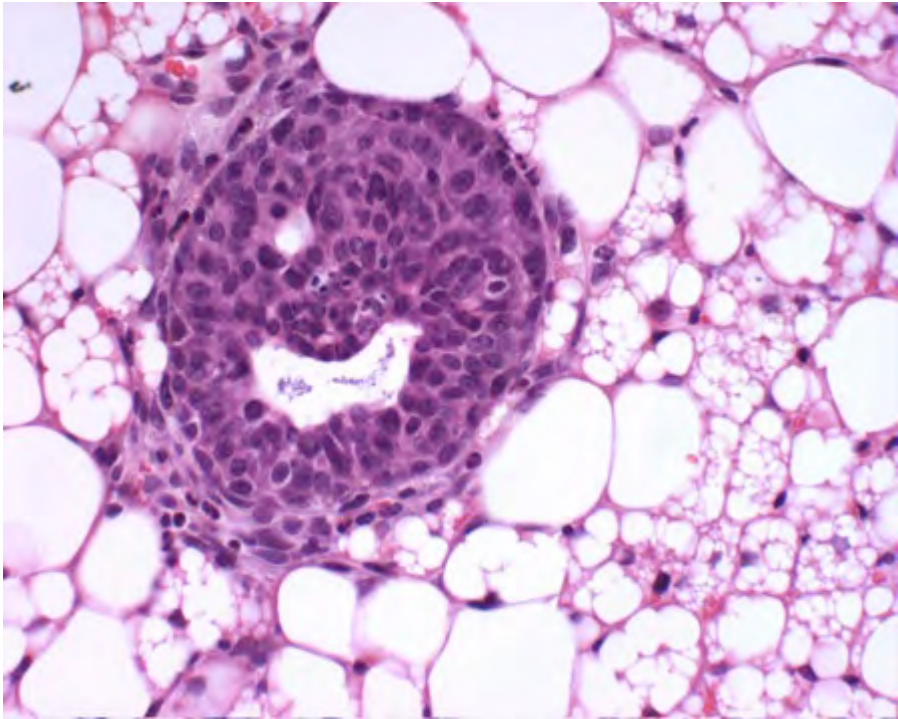
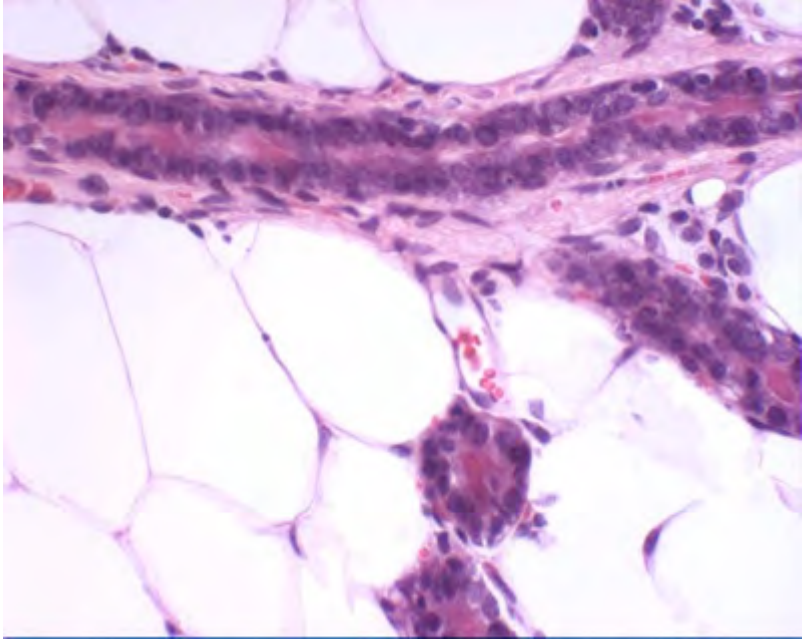
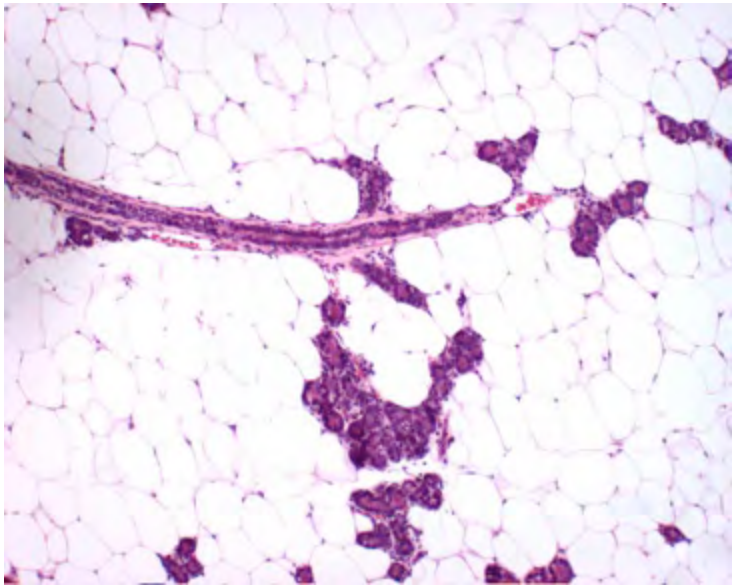
During pregnancy, there is rapid growth of the terminal ducts of the gland with hyperplasia of the epithelium. In the second half of pregnancy there is an increase in size of the parenchymal cells and the alveoli are distended with secretions.



**Normal cells
from smear of cervix**

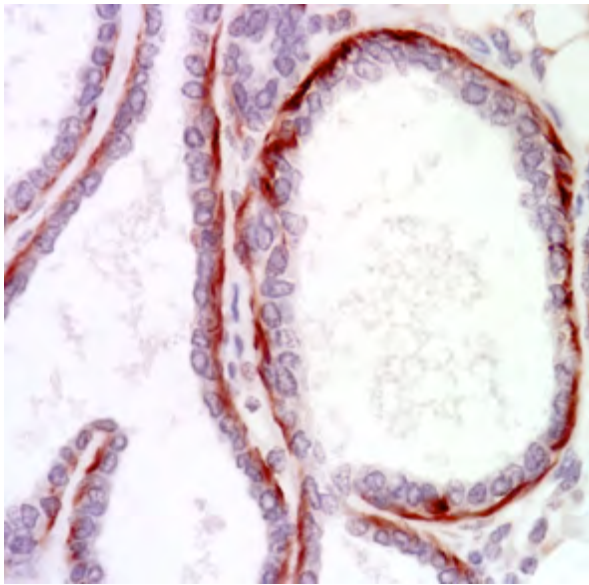


**Carcinoma cells with
altered nuclear: cytoplasmic ratio**

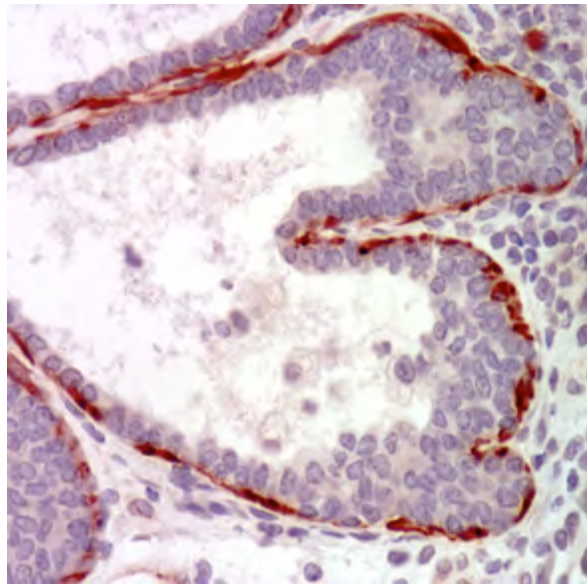


Markers of Differentiation

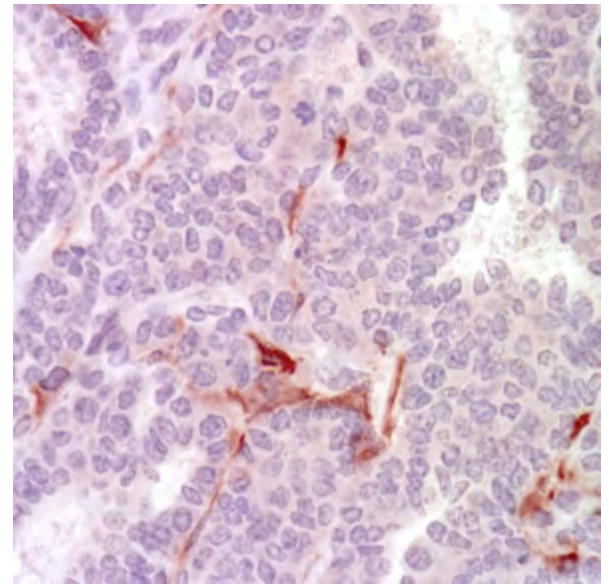
Smooth Muscle Actin (SMA)



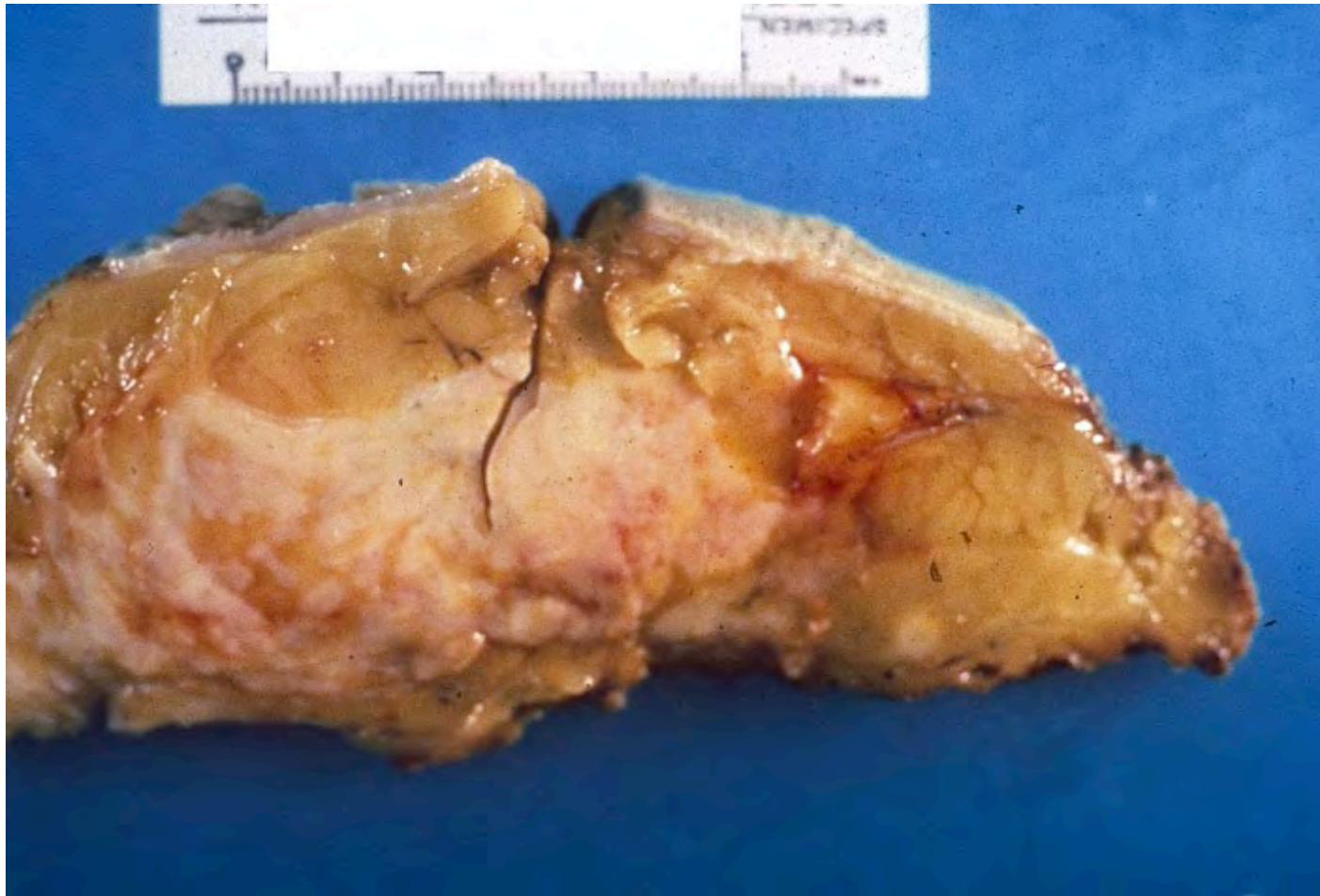
Normal



Hyperplasia



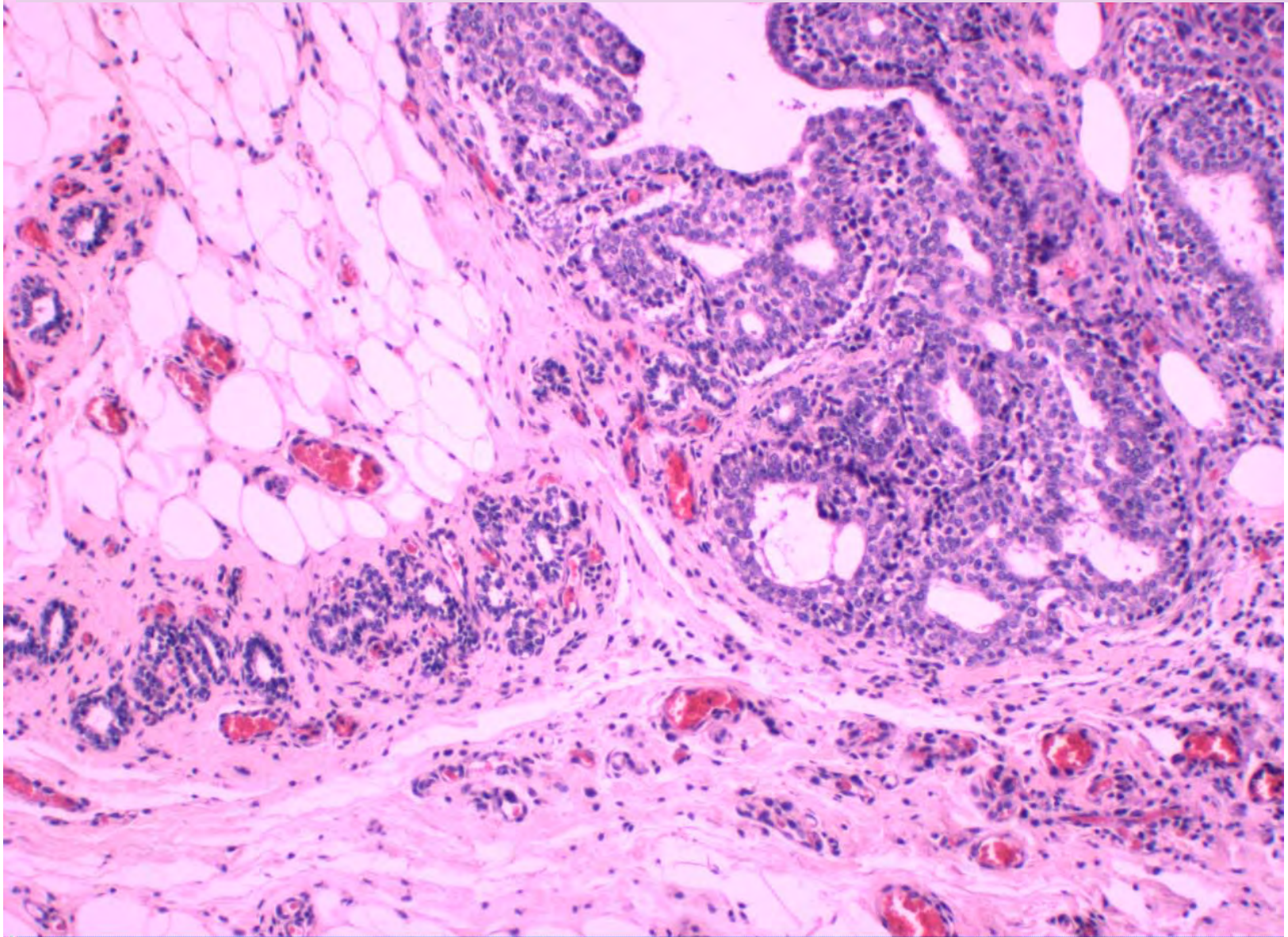
Tumor



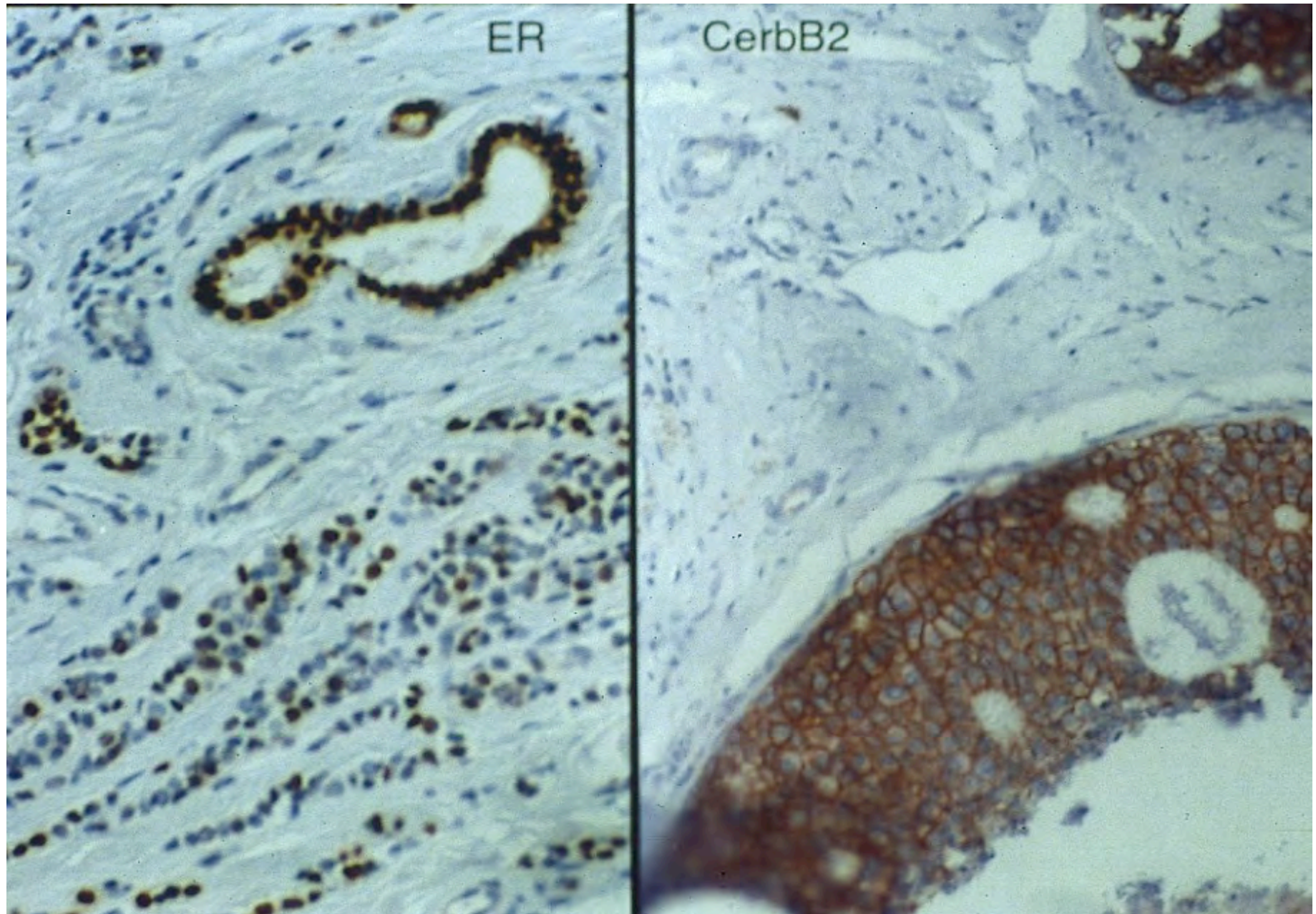
Identify organ/ lesion

Classify lesion: well-defined margins?

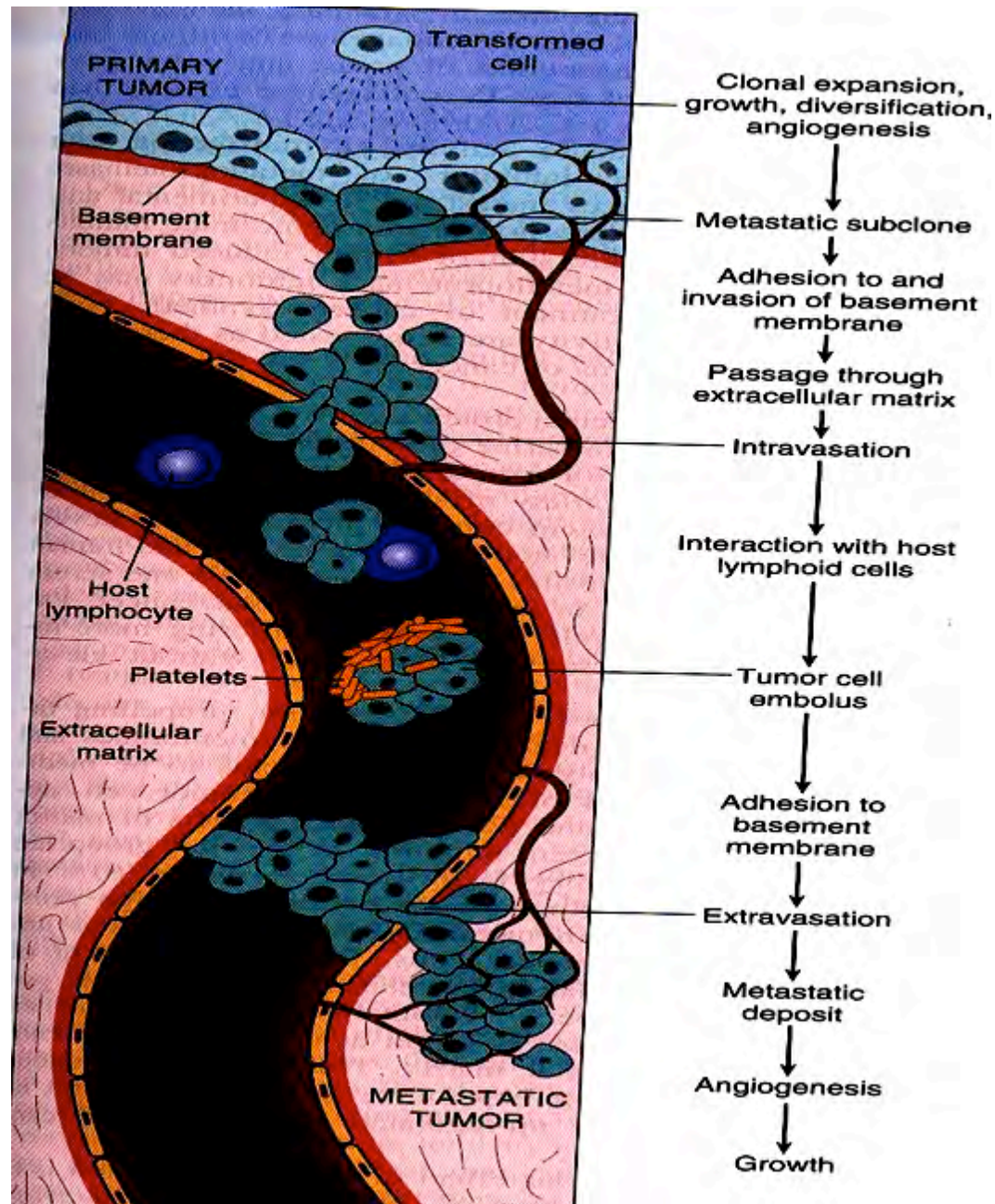
H&E of the carcinoma in a human breast



Prognostic Markers in Human Breast Cancer

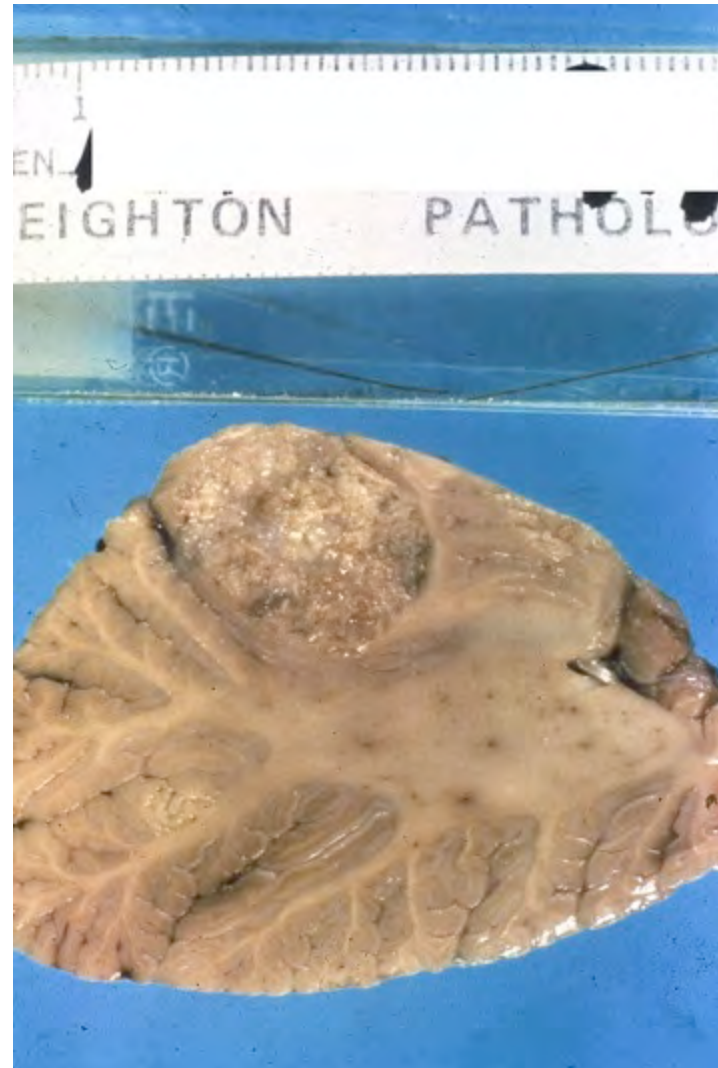


**Robbins and
Kumar
textbook of
Pathology
description
of the
process of
malignant
progression
and
metastasis**

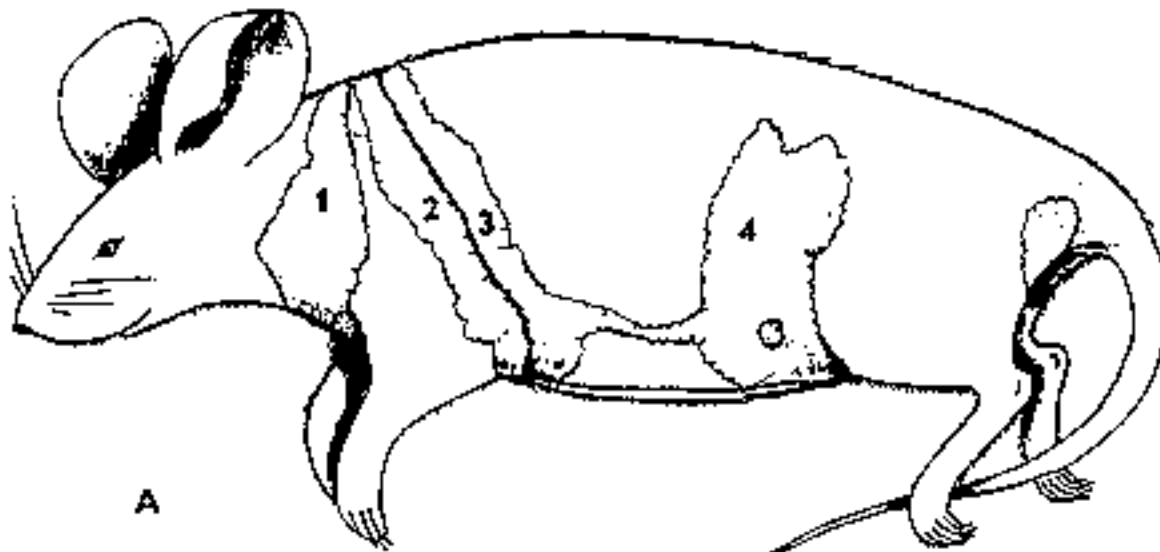




Identify organ/ lesion
Classify lesion: well-defined margins?

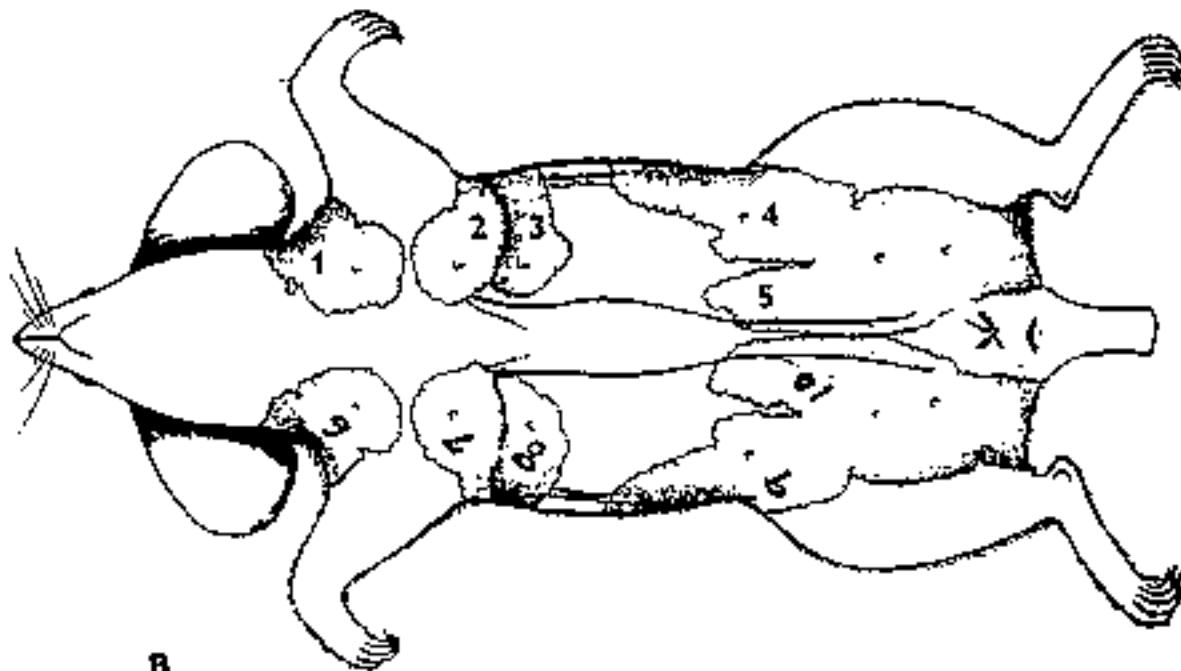


Metastasis to Brain



A

Location of the mammary glands in the mouse

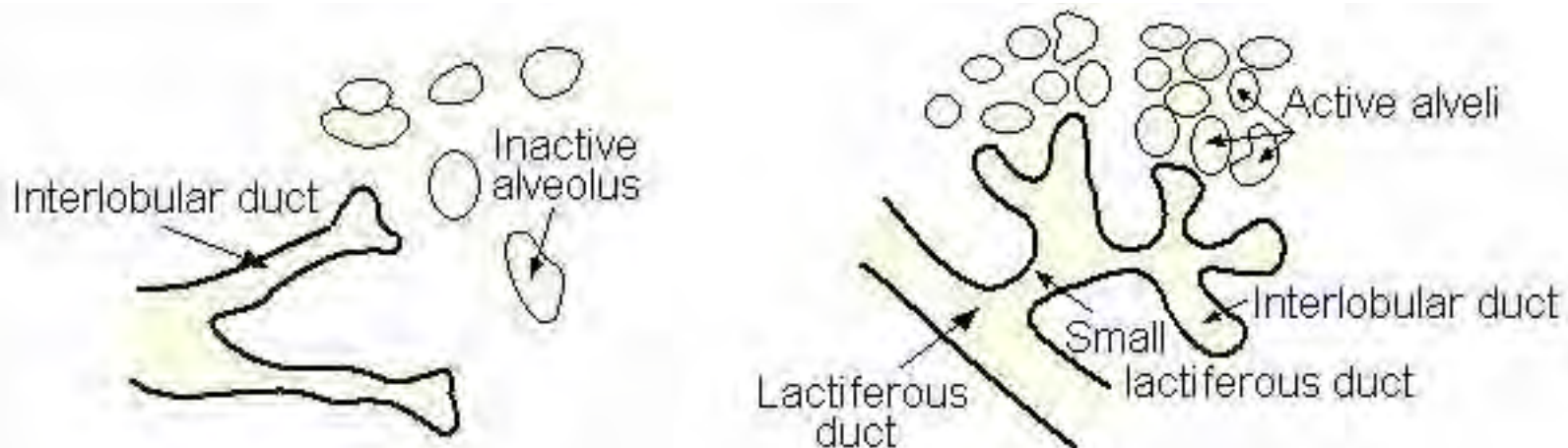


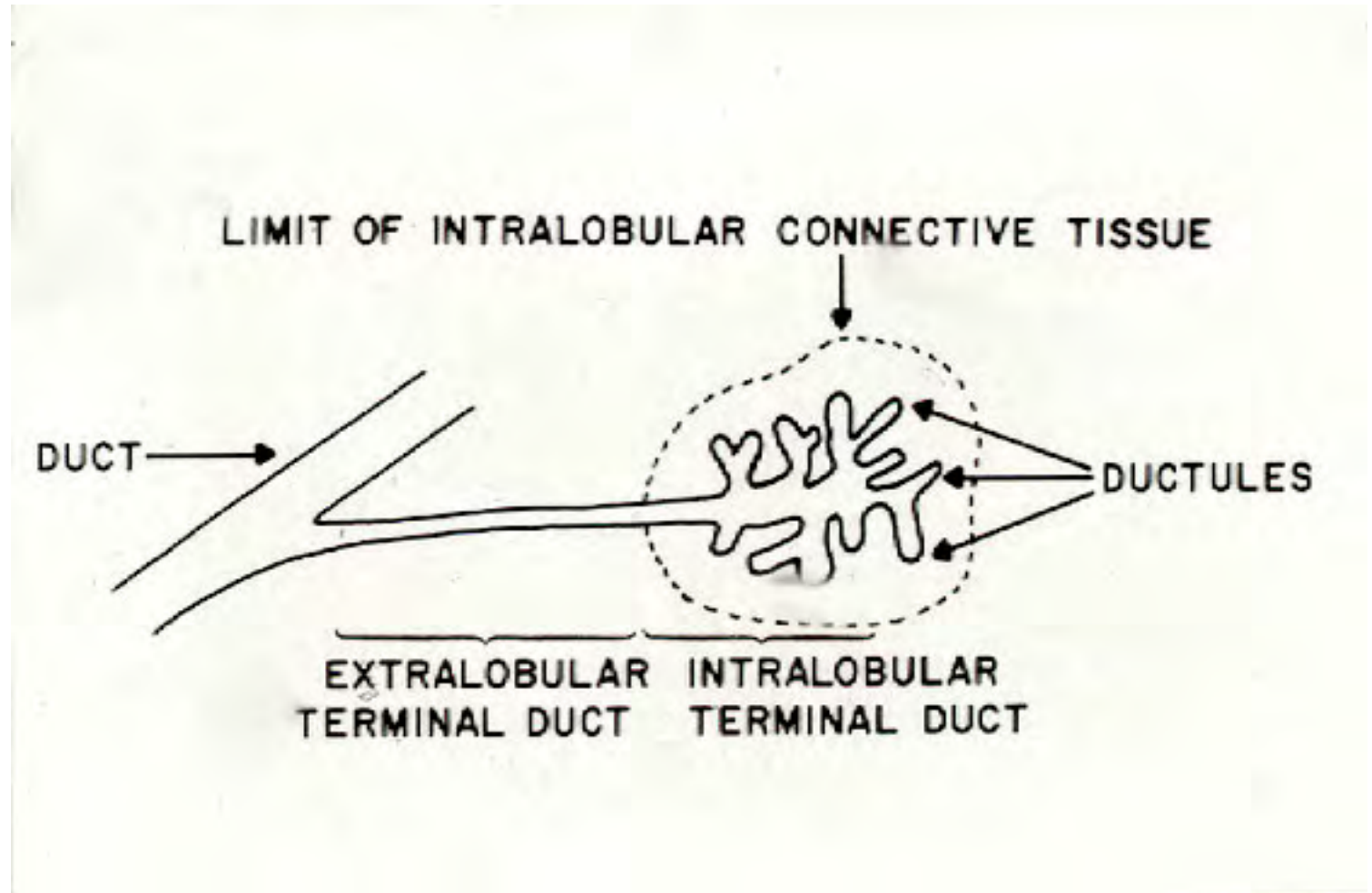
B

The mammary glands are greatly modified and enlarged sweat glands. The ducts and acini (i.e. the gland parenchyma) are formed mainly from cuboidal epithelial cells, with myoepithelial cells present to aid secretion. The connective tissue contains elastic fibres and adipose tissue. Connective tissue lamellae divide the parenchyma into lobules.

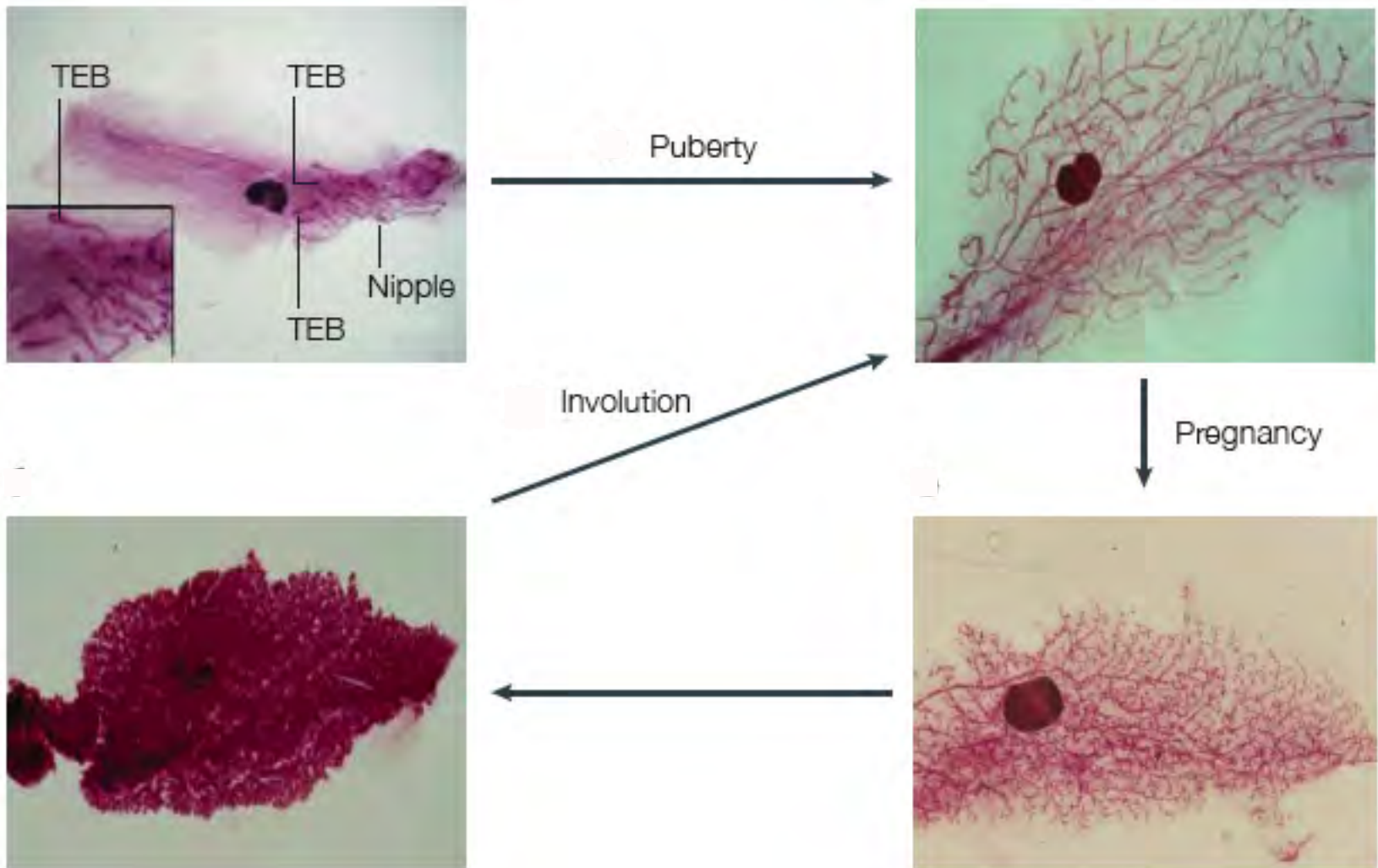
In the inactive mammary gland, the interstitial spaces between lobes and ducts are filled mainly with fat. There are a few alveoli present compared to those in the lactating gland, and the ducts and lobes of the gland are smaller than when the gland is in its active phase.

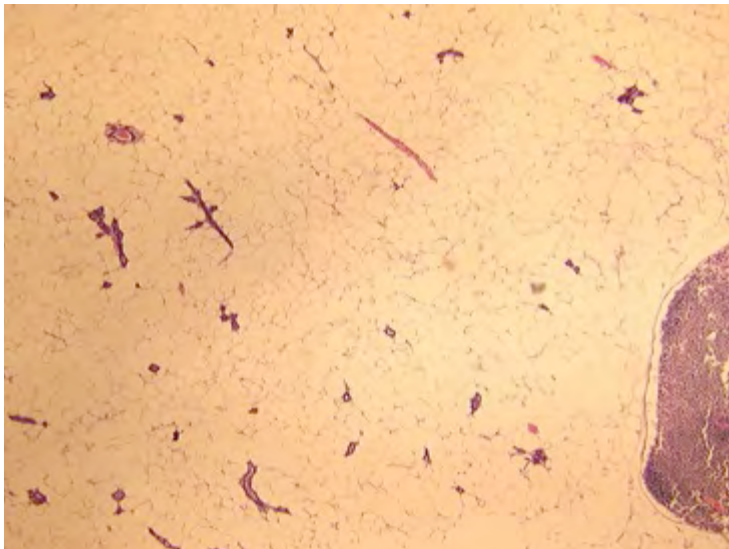
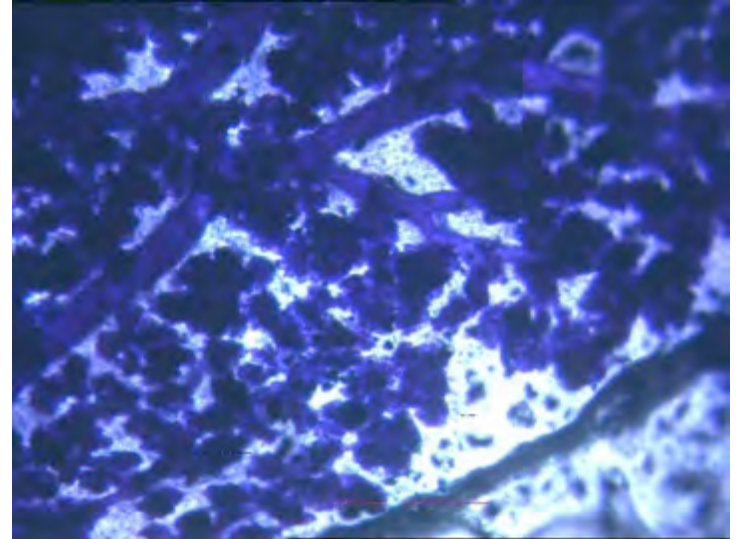
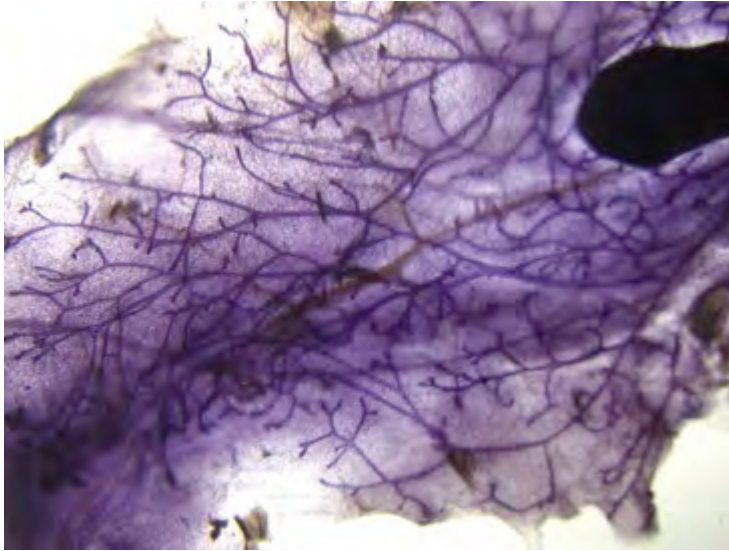
During early pregnancy, the epithelial cells of the glandular tissue proliferate rapidly to form the buds which eventually enlarge to form the alveoli. As pregnancy develops, the fat and connective tissue between lobes and ducts is replaced by secretory tissue.





Rodent Mammary Gland



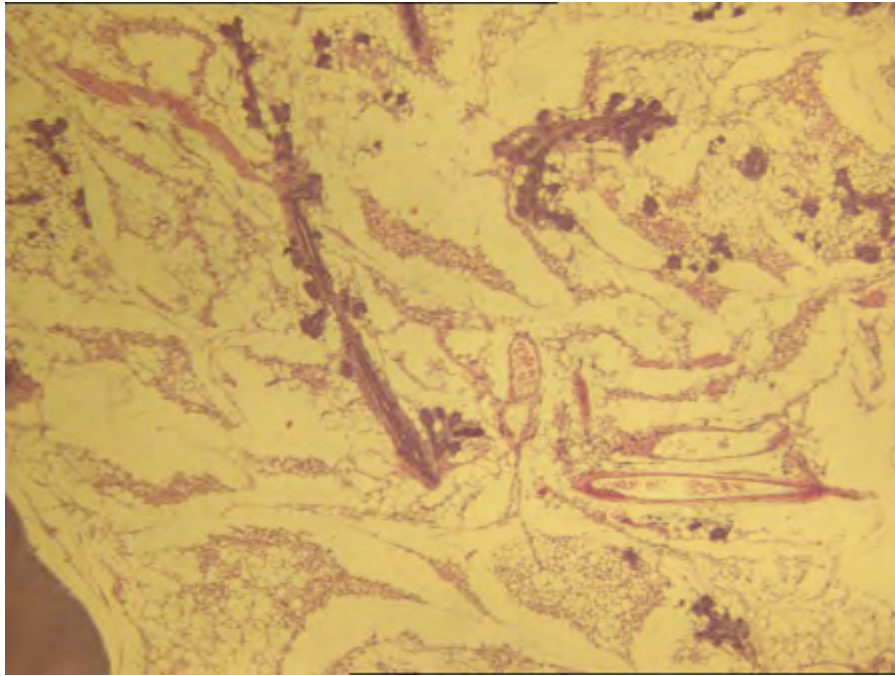


**Mouse mammary
gland stages seen
as a whole mount
preparation**

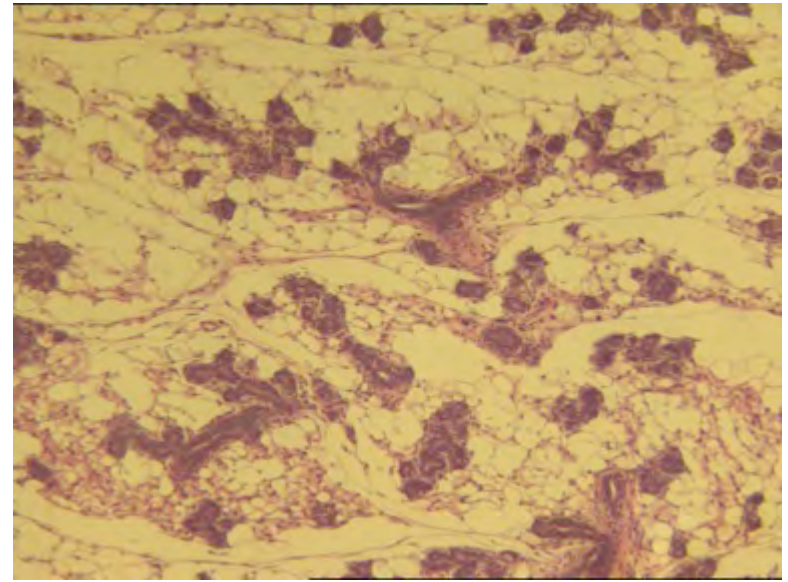
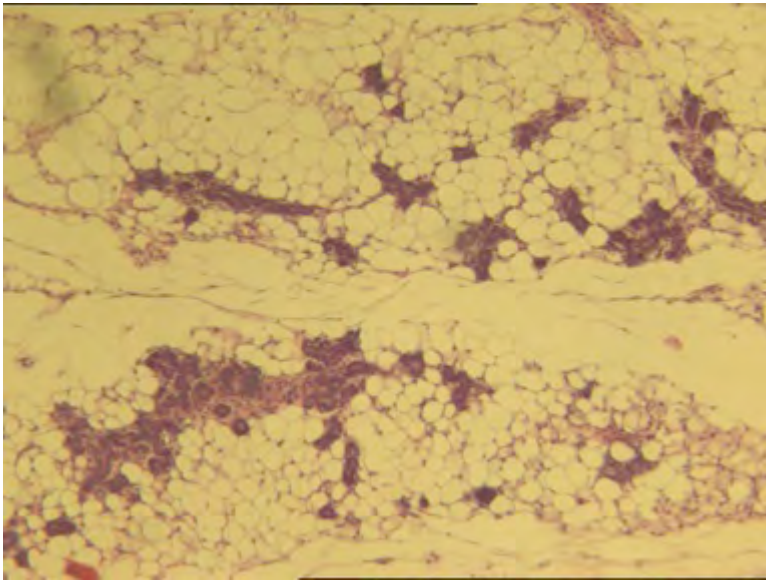
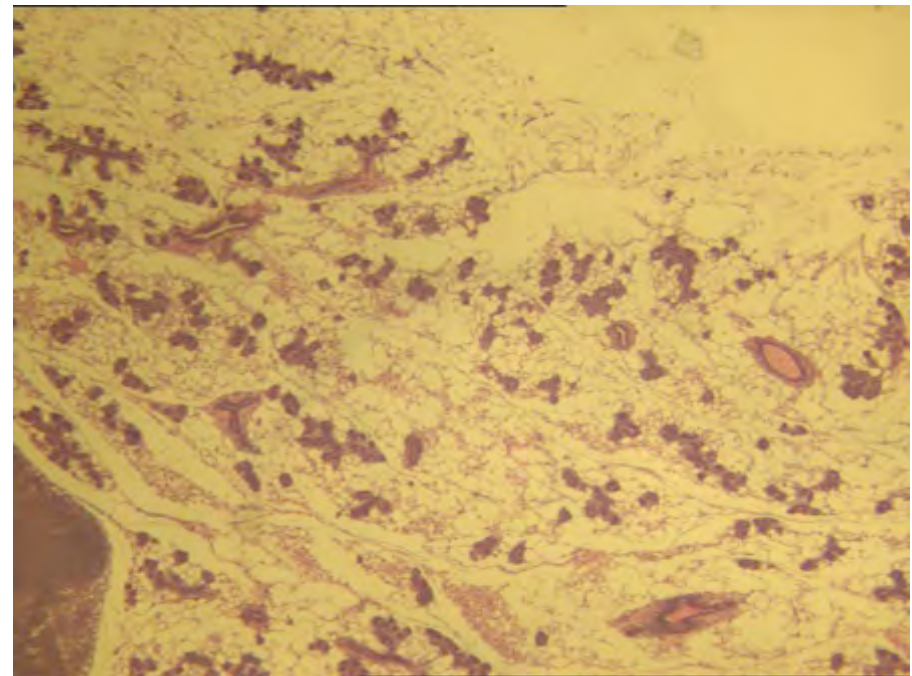
**With an example of
a paraffin section
H&E**

Defective branching of alveolar mammary glands

KO



Wt



What are the cell / tissue types you will see in breast tissue?

- Cuboidal cells--keratin positive
- Adipocytes
- Blood vessels
- Fibroblasts
- Nerve fibers
- Innate immune cells

And in mouse mammary glands:

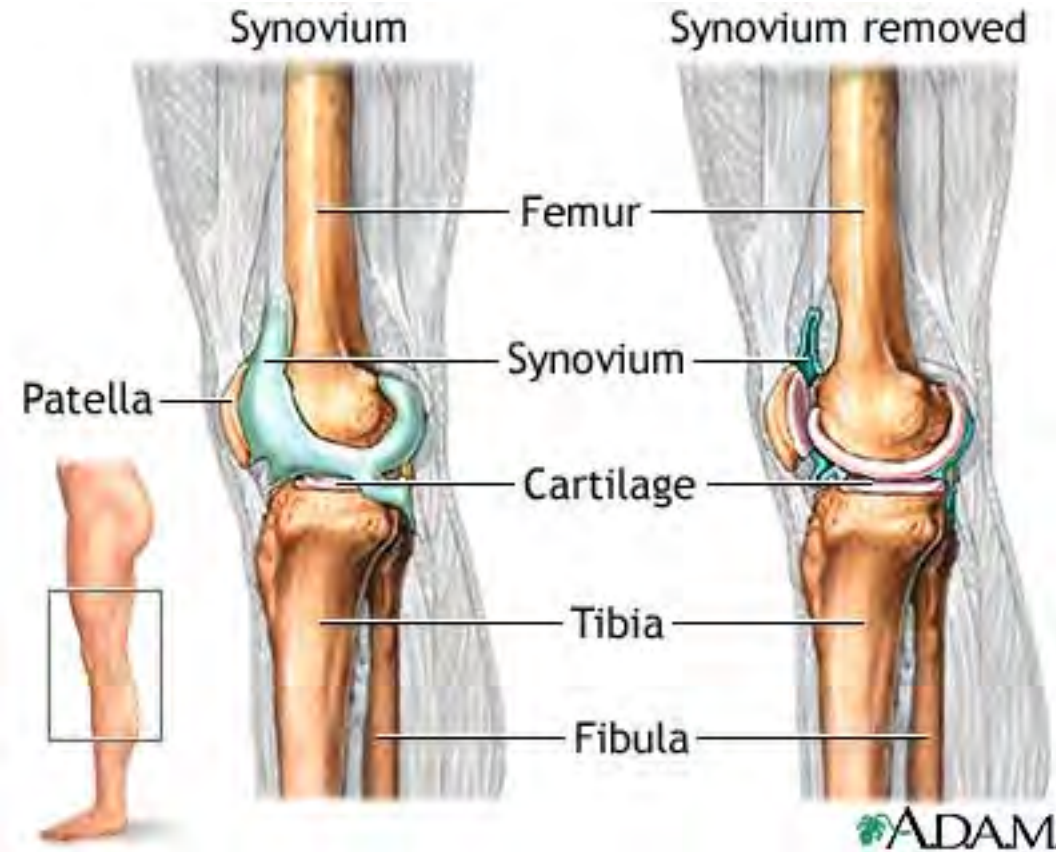
#4 has a distinctive lymph node surrounded by breast ducts and lobules

Non-epithelial supporting tissues:

Connective tissue:

- Endothelial cells and blood vessels
- Collagen, fibroblasts important stromal elements
- Elastic tissue
- Reticular tissue
- Adipose tissue
- Bone
- Cartilage
- Muscle:
 - Skeletal—voluntary, striated, parallel fibers, eccentric nuclei
 - Cardiac—involuntary, striated, central nuclei
 - Smooth muscle—involuntary, central nuclei

Bone and Joints



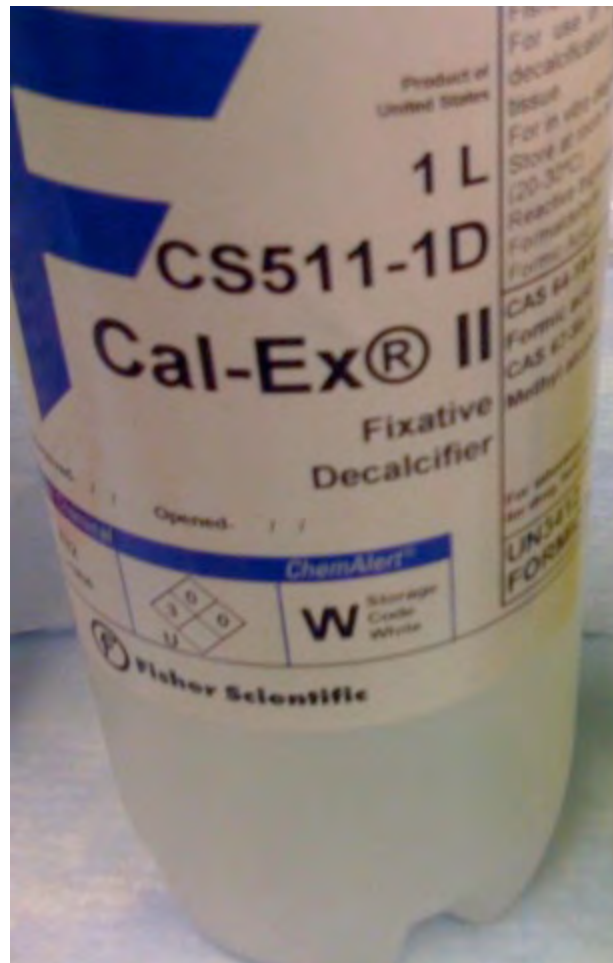
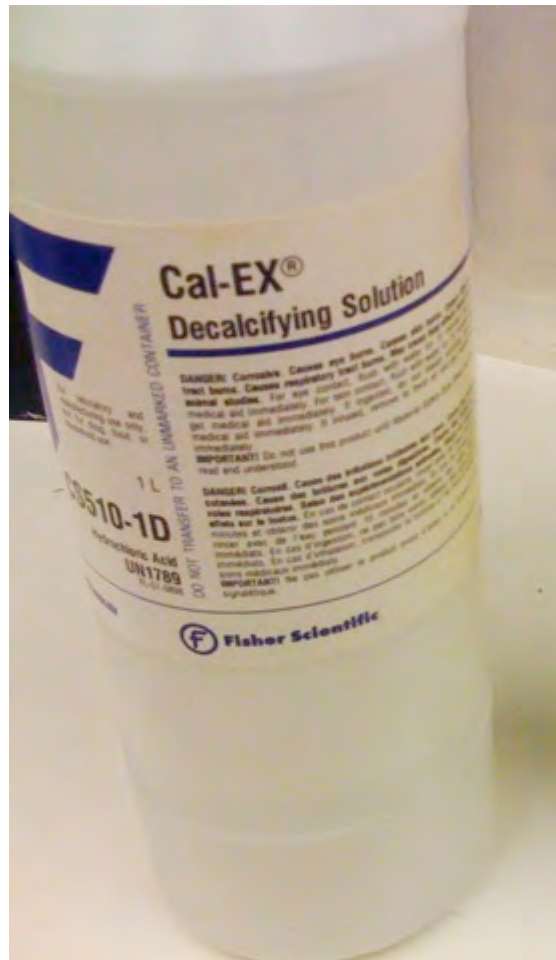
Bone has to be decalcified before it can be examined using the usual histochemical methods

REMOVE AS MUCH MUSCLE as possible

Fix in Cal-Ex II –Fisher Cat. No. CS511-1D (buffered formalin and

but do not let them be exposed to this for more than 3 days

Fix and then decalcify in EDTA if immunostains are to be done



Decalcification solutions

HCl; Formalin+ HCl; EDTA only- for slow decalcification for IHC

BONE and CARTILAGE

BONE has to be decalcified before histologic examination

Although there are orthopedic labs that use specialized microtomes to section through undecalcified bone to obtain information

CARTILAGE:

--fibro cartilage, resembles dense connective tissue, is found in intervertebral discs, the pubic symphysis, in the attachment of some tendons, and between the dense connective tissue of ligament and joint capsules

--hyaline cartilage, consists of cartilage matrix and chondrocytes embedded in cartilage cavities called the lacunae

--elastic cartilage-- in locations where support with flexibility is required as in the epiglottis, auricle of the ear etc.

Cartilage is a type of dense **connective tissue** . It is composed of cells called **chondrocytes** which are dispersed in a firm gel-like ground substance, called the **matrix**.

Cartilage is **avascular** (contains no blood vessels) and **nutrients** are **diffused through the matrix**.

The main purpose of cartilage is to provide a framework upon which bone deposition could begin.

Another important purpose of cartilage is to provide smooth surfaces for the movement of articulating bones.

Hyaline Cartilage is the most abundant type of cartilage.

Elastic cartilage

Fibrocartilage

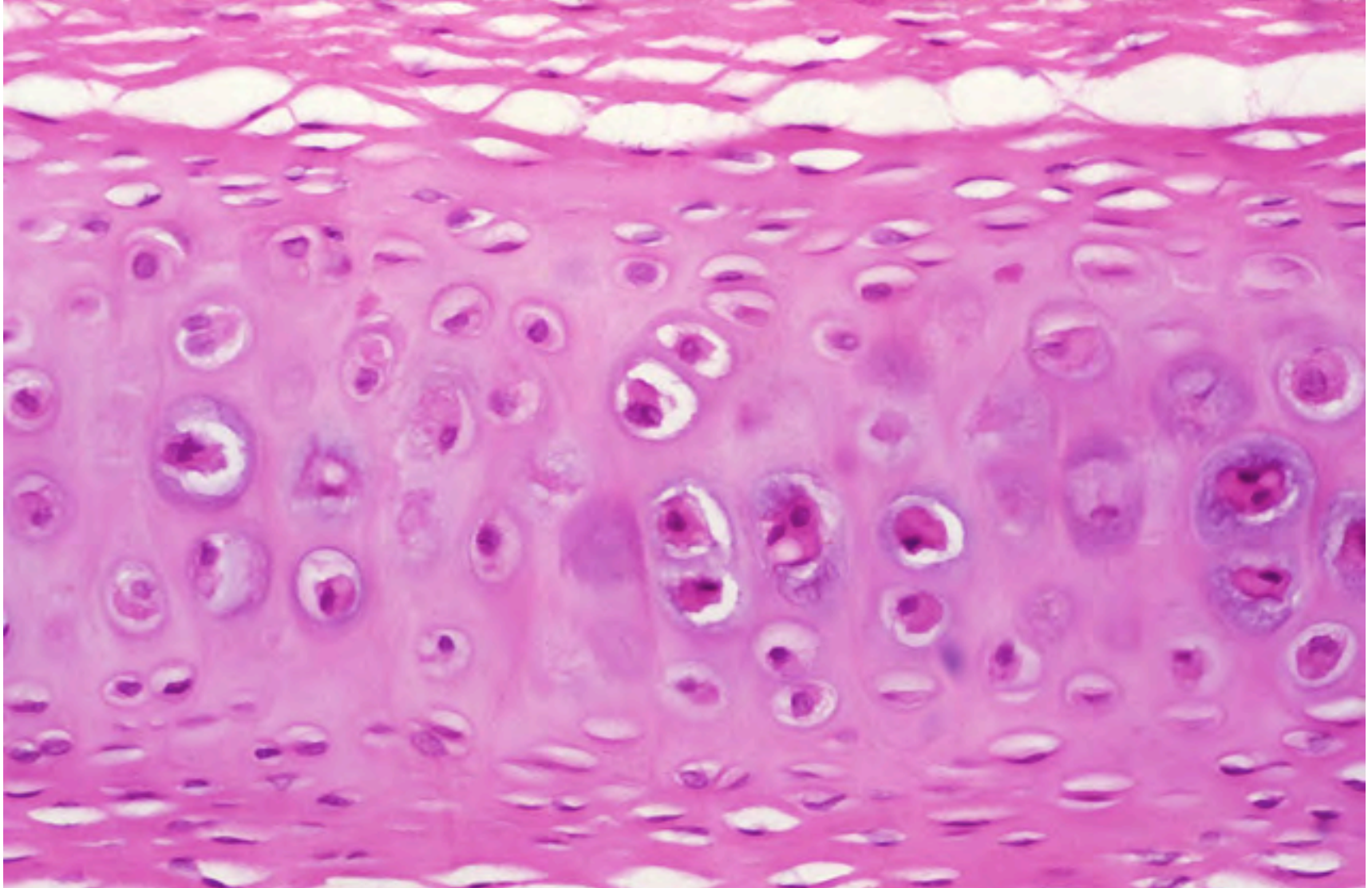
Hyaline Cartilage is the most abundant type of cartilage.

The name hyaline is derived from the Greek word hyalos, meaning glass.

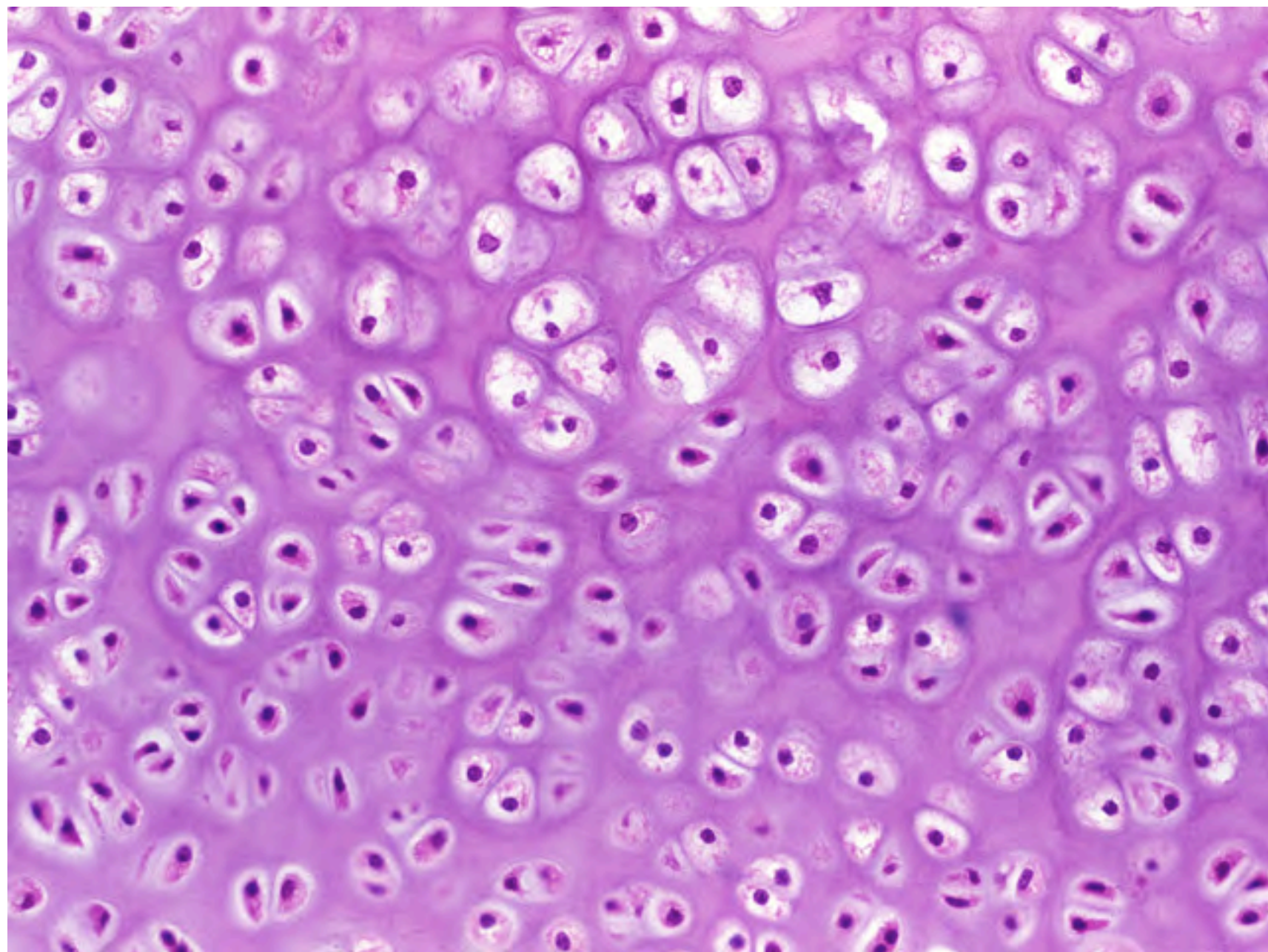
This refers to the translucent matrix or ground substance, that is made predominantly of type II collagen.

Hyaline cartilage is found lining bones in joints (articular cartilage) .

It is also present inside bones, serving as a center of ossification or bone growth. In addition, hyaline cartilage forms the embryonic skeleton.



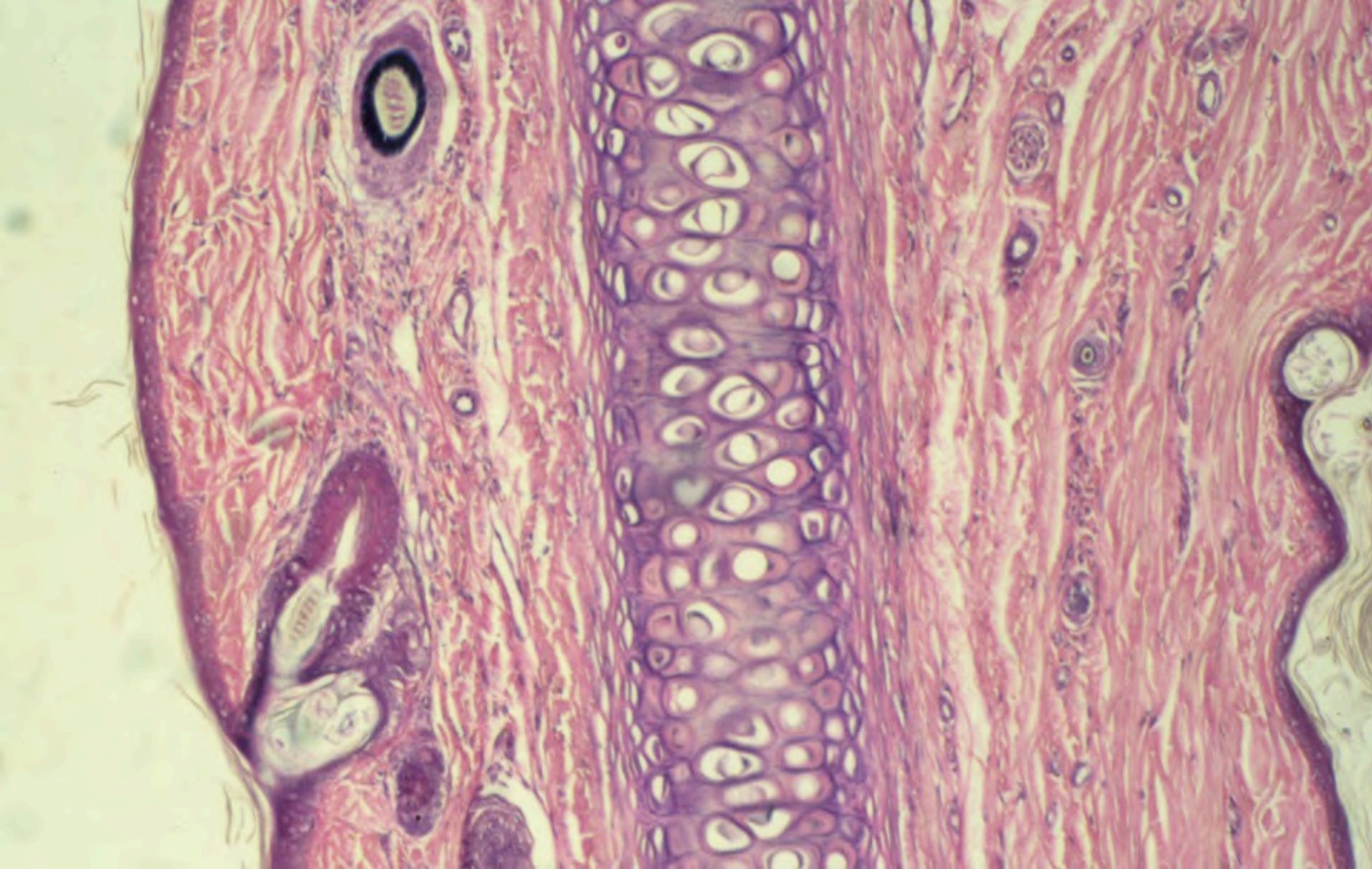
Chondrocytes develop in the perichondrium). As they mature, they are moved deeper into the cartilage. There they actually secrete the matrix that traps them. Isogenous groups (small nests of chondrocytes) result from repeated cell division.

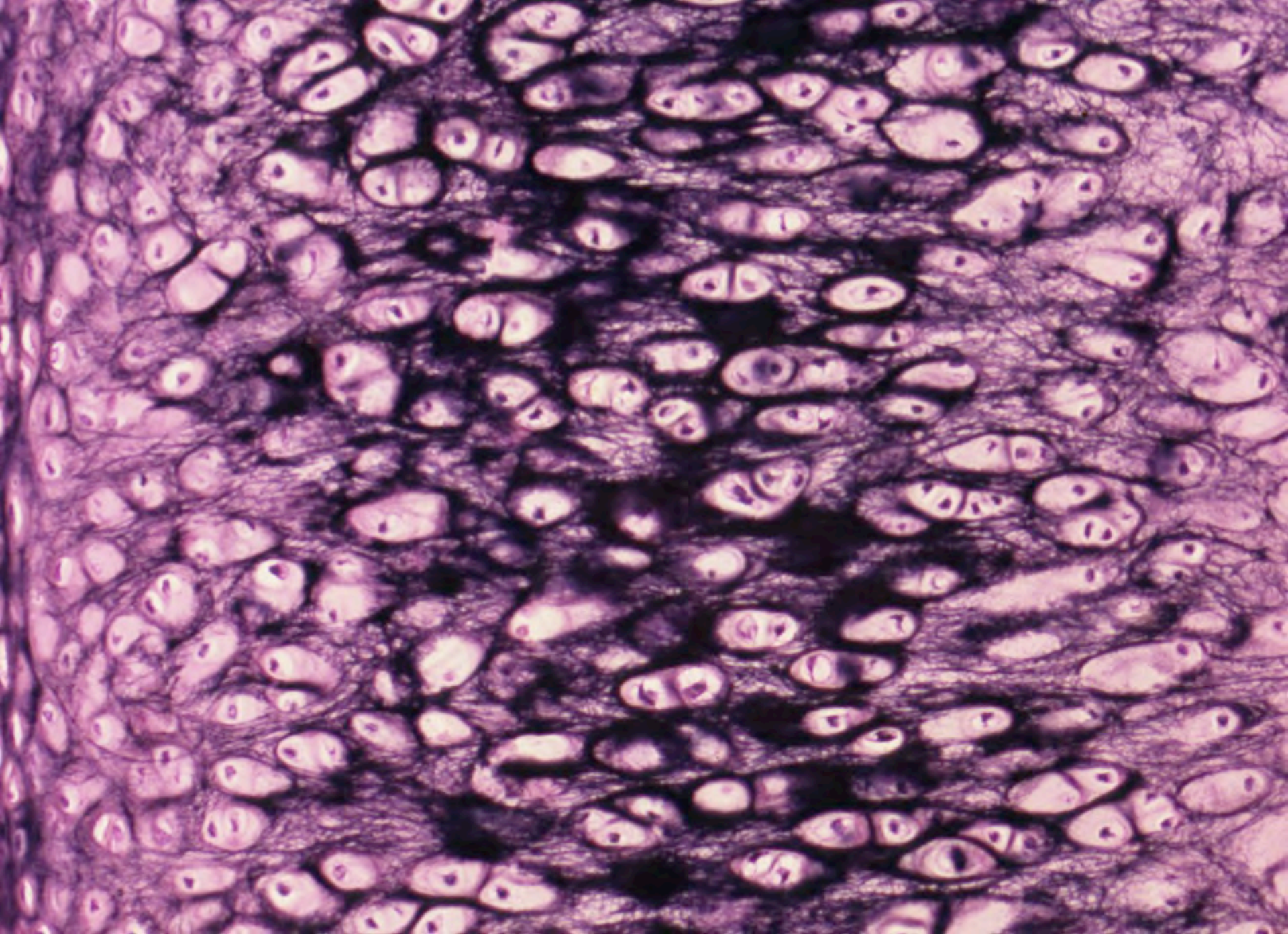


Elastic cartilage is found in the pinna of the ear and several tubes, such as the walls of the auditory and eustachian canals and larynx .

Elastic cartilage is similar to hyaline cartilage but contains elastic bundles (elastin) scattered throughout the matrix.

This provides a tissue which is stiff yet elastic.





Fibrocartilage

is a specialized type of cartilage found in areas requiring tough support or great tensile strength, such as between intervertebral disks, the pubic and other symphyses , and at sites connecting tendons or ligaments to bones .

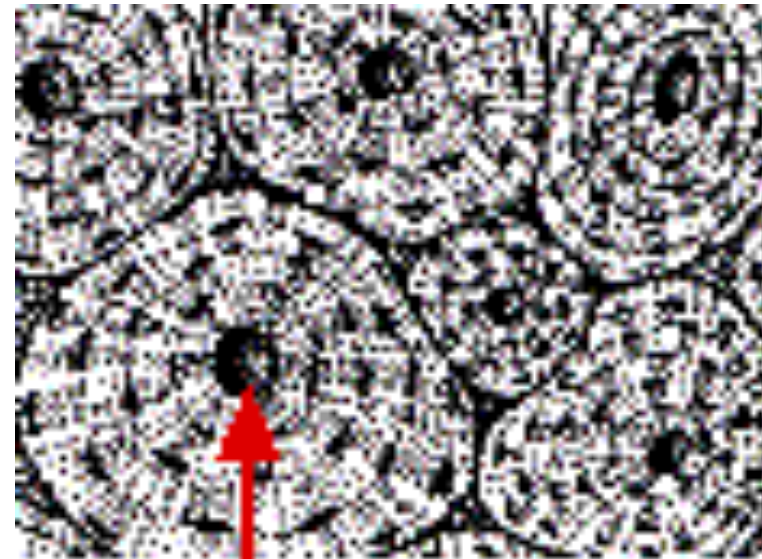
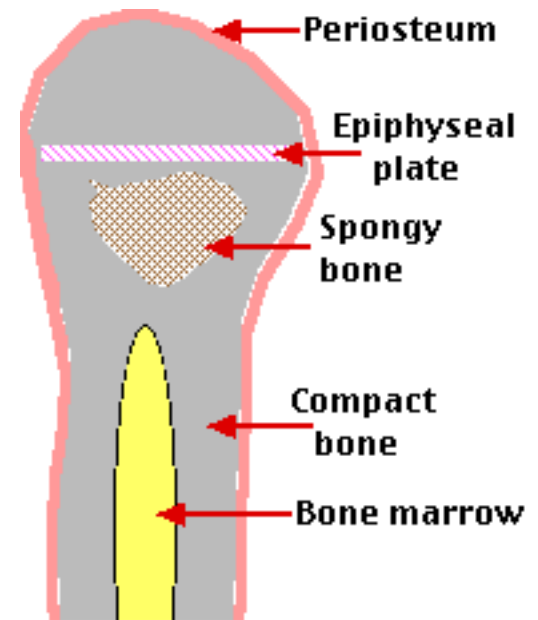
Periosteum. A tissue covering the bone that brings blood and lymph vessels, as well as nerves, to it

***Compact bone** (also known as cortical bone). Dense deposits of minerals - chiefly calcium phosphate - and collagen. These are arranged in concentric circles around a central Haversian canal through which blood and lymph vessels as well as nerves pass.

***Spongy bone** (also known as trabecular or cancellous bone). The mineral deposits are arranged as a system of struts. Bone marrow fill the spaces between.

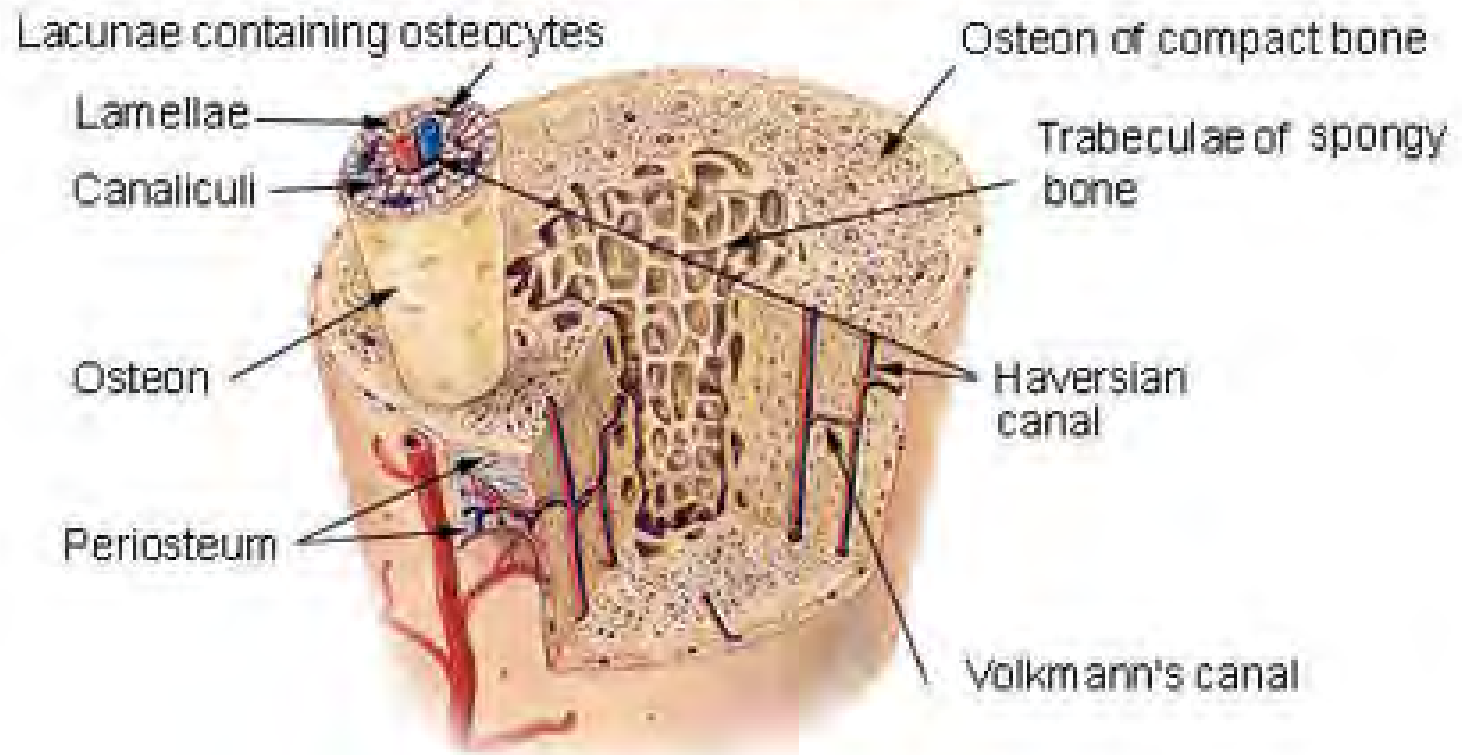
***Bone marrow.** Some bones, such as the femur, also contain a central cavity filled with bone marrow. Bone marrow contains the stem cells that gives rise to all the types of blood cells.

***Epiphyseal plate.** Prior to puberty, this disk of cartilage produces more cartilage which then is converted into more bone. In this way, the bone grows lengthwise.

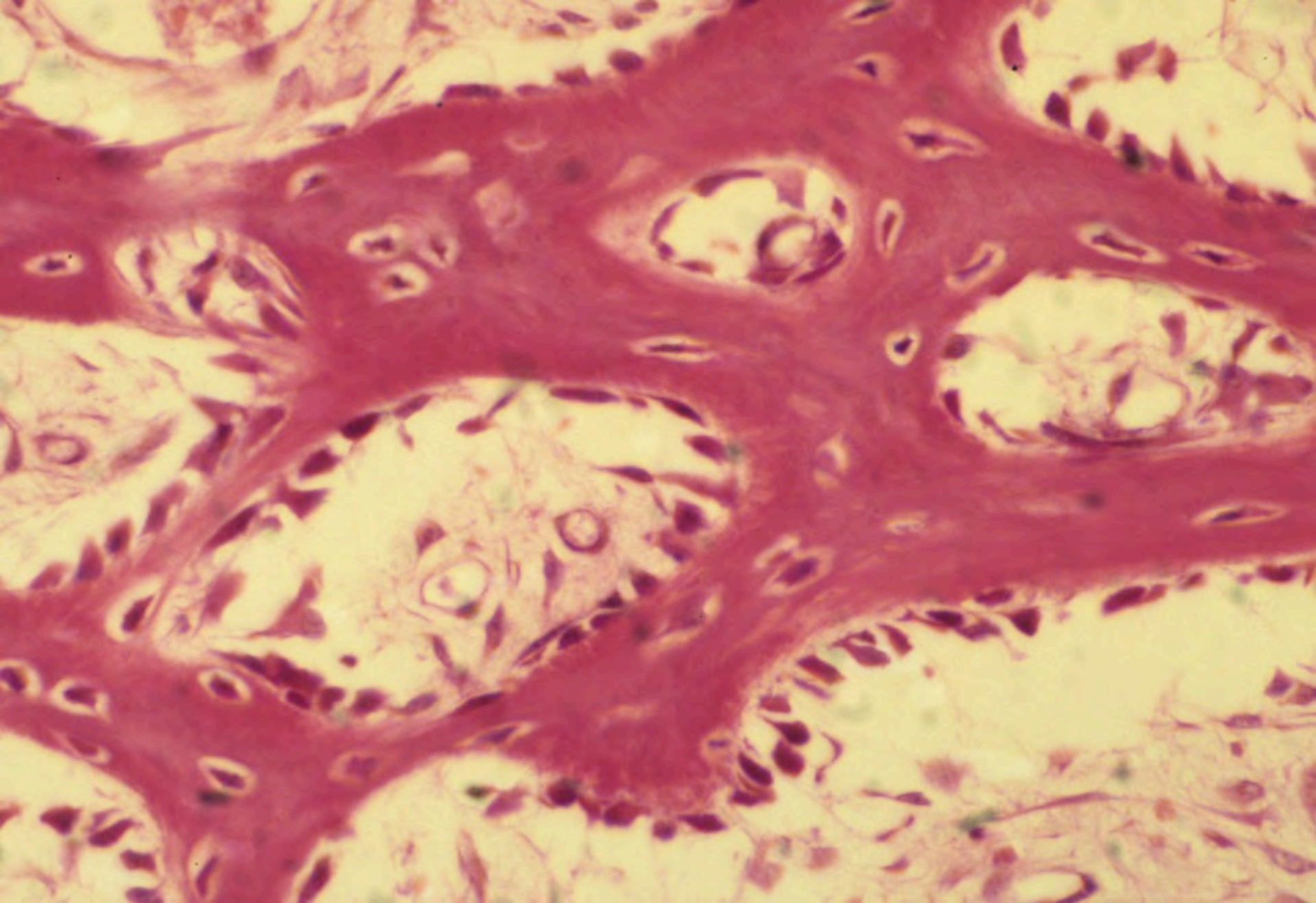


Haversian Canal

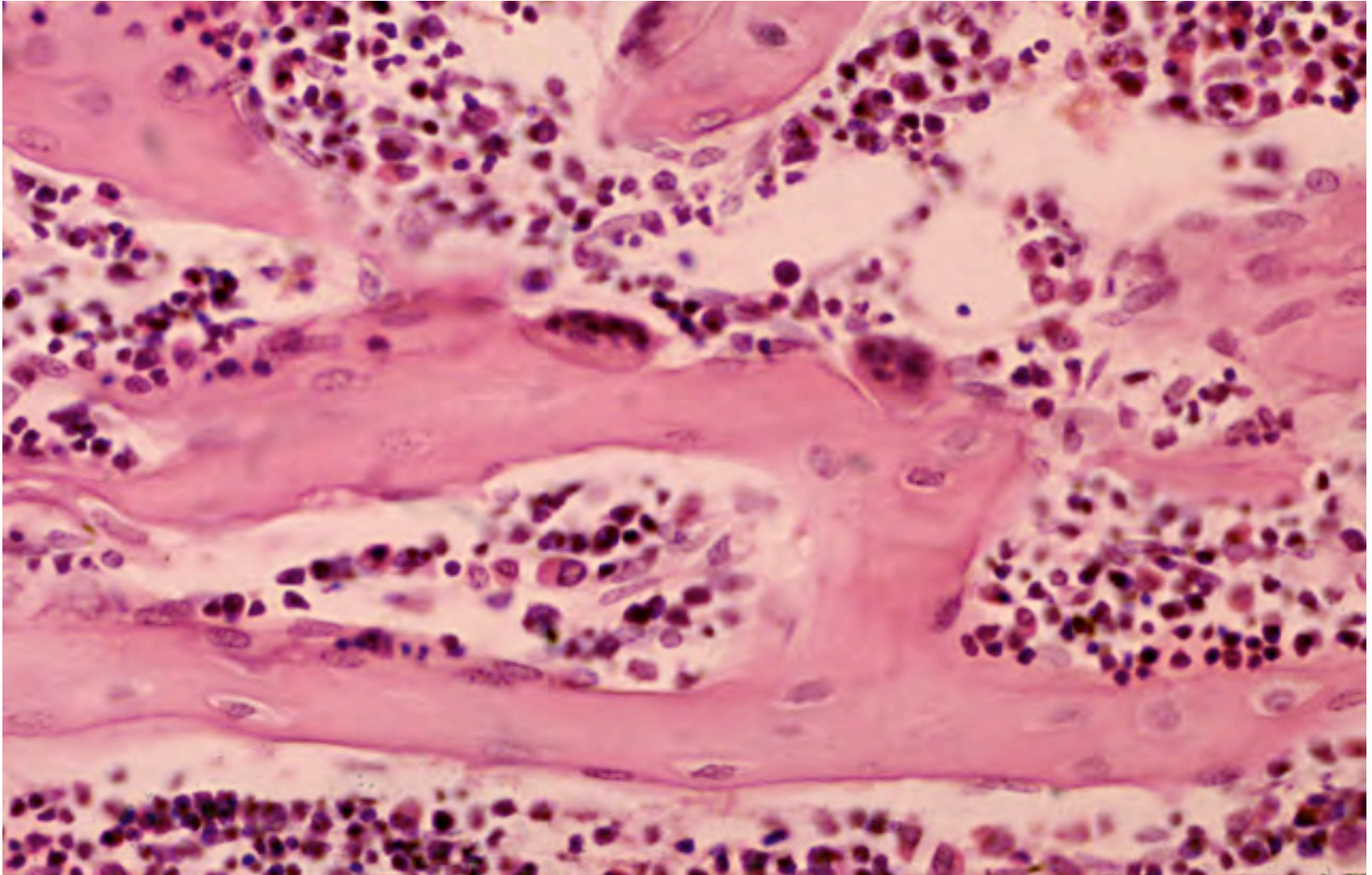
Compact Bone & Spongy (Cancellous Bone)







Osteoclasts (under the influence hormones, destroy bone) , are multinucleated giant cells, found within bone marrow, or adjacent to bone.



Osteoid: is the pink matrix composed of type 1 collagen fibers and ground substance (chondroitin sulfate and osteocalcin) and made by osteoblasts

When osteoid gets mineralized (calcium) = calcified bone

Osteoblasts secrete alkaline phosphatase

Osteoclasts secrete: Tartrate resistant acid phosphatase (TRAP)

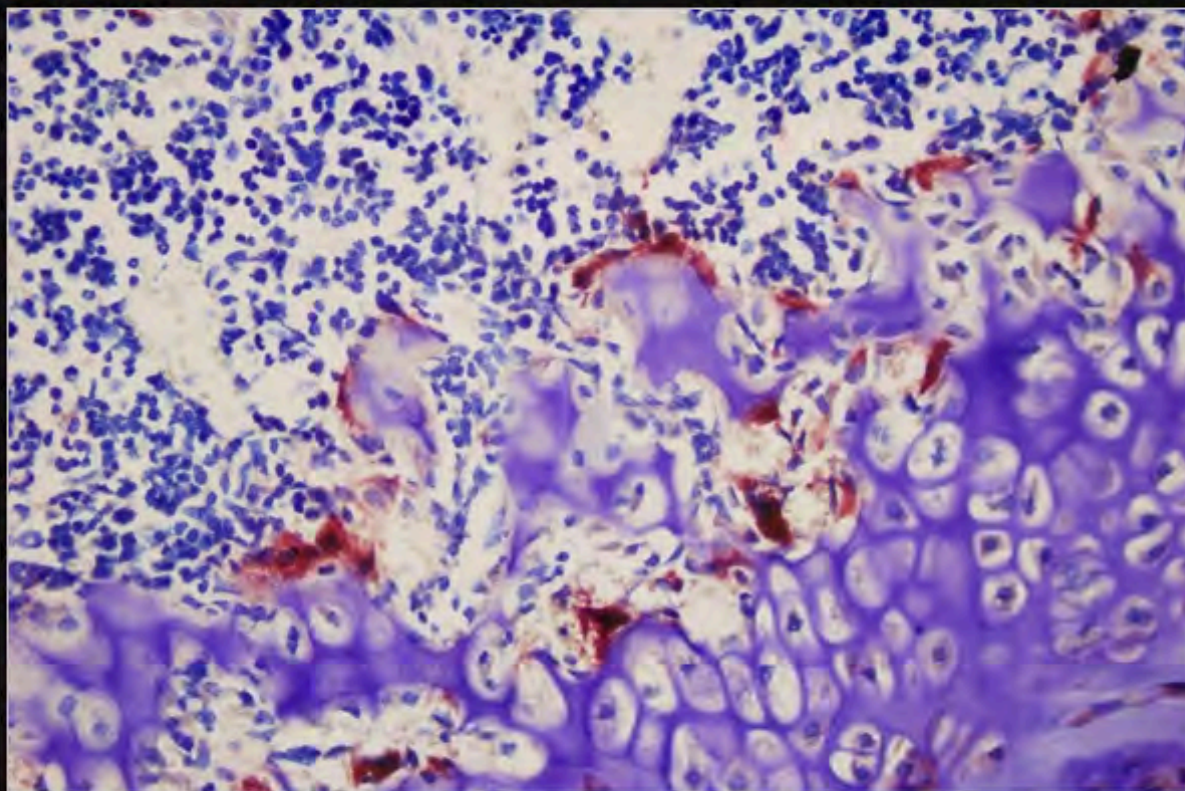
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425X magnification showing areas of tartrate resistant acid phosphatase activity

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Tartrate Resistant Acid Phosphatase 425X magnification showing areas of tartrate resistant acid phosphatase activity in proximal tibia of the mouse. Osteoclasts are the cells responsible for the resorption of bone.

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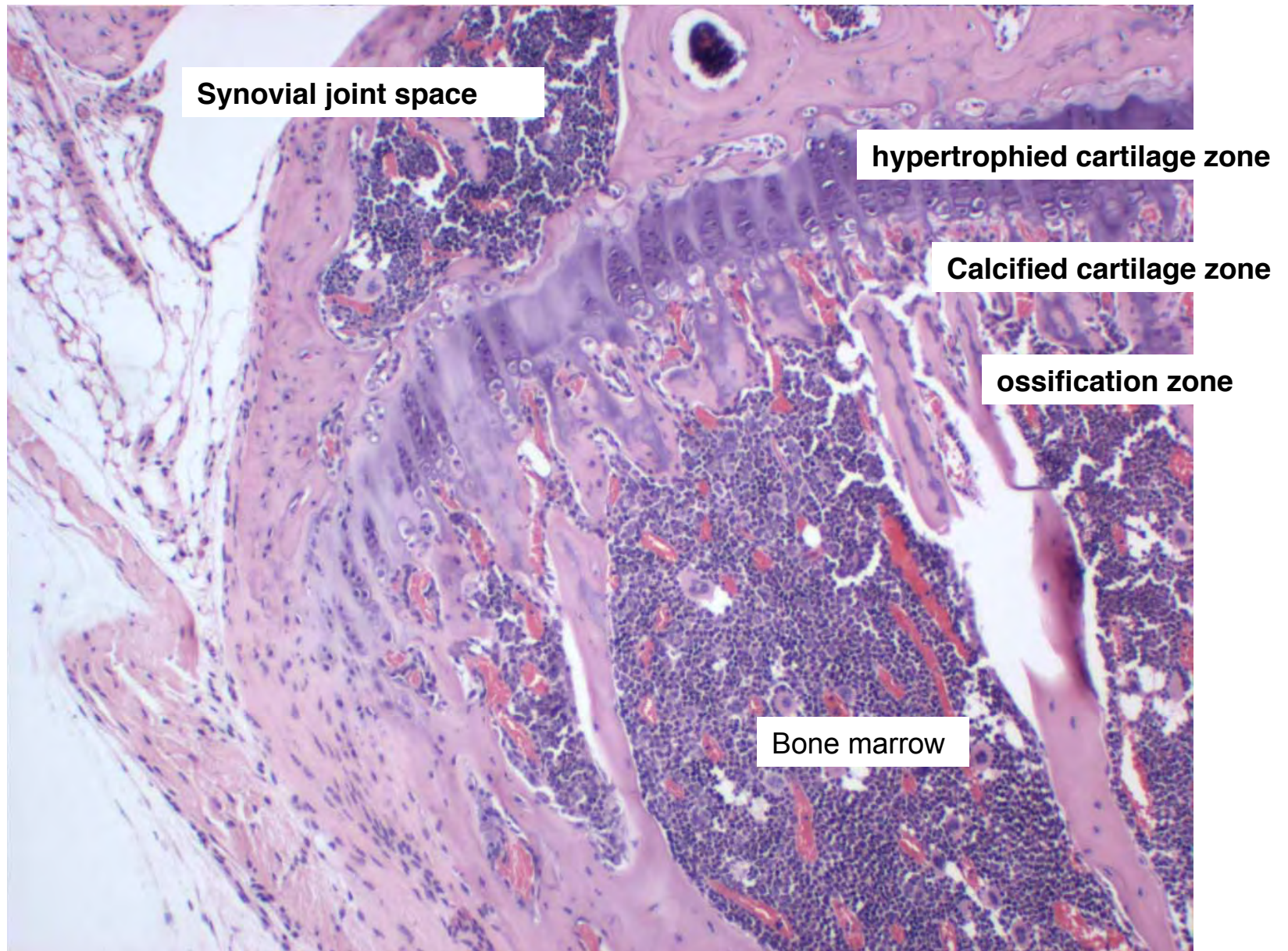
This histological image shows a section of cartilage with distinct zones of chondrocyte development. The top-left area contains resting chondrocytes, which are small and densely packed. The center features a cluster of proliferating chondrocytes, which are larger and arranged in a more organized, rounded pattern. The bottom-right area shows maturing chondrocytes, which are further differentiated and surrounded by a zone of calcification, characterized by a more granular and less cellular appearance.

Resting chondrocytes

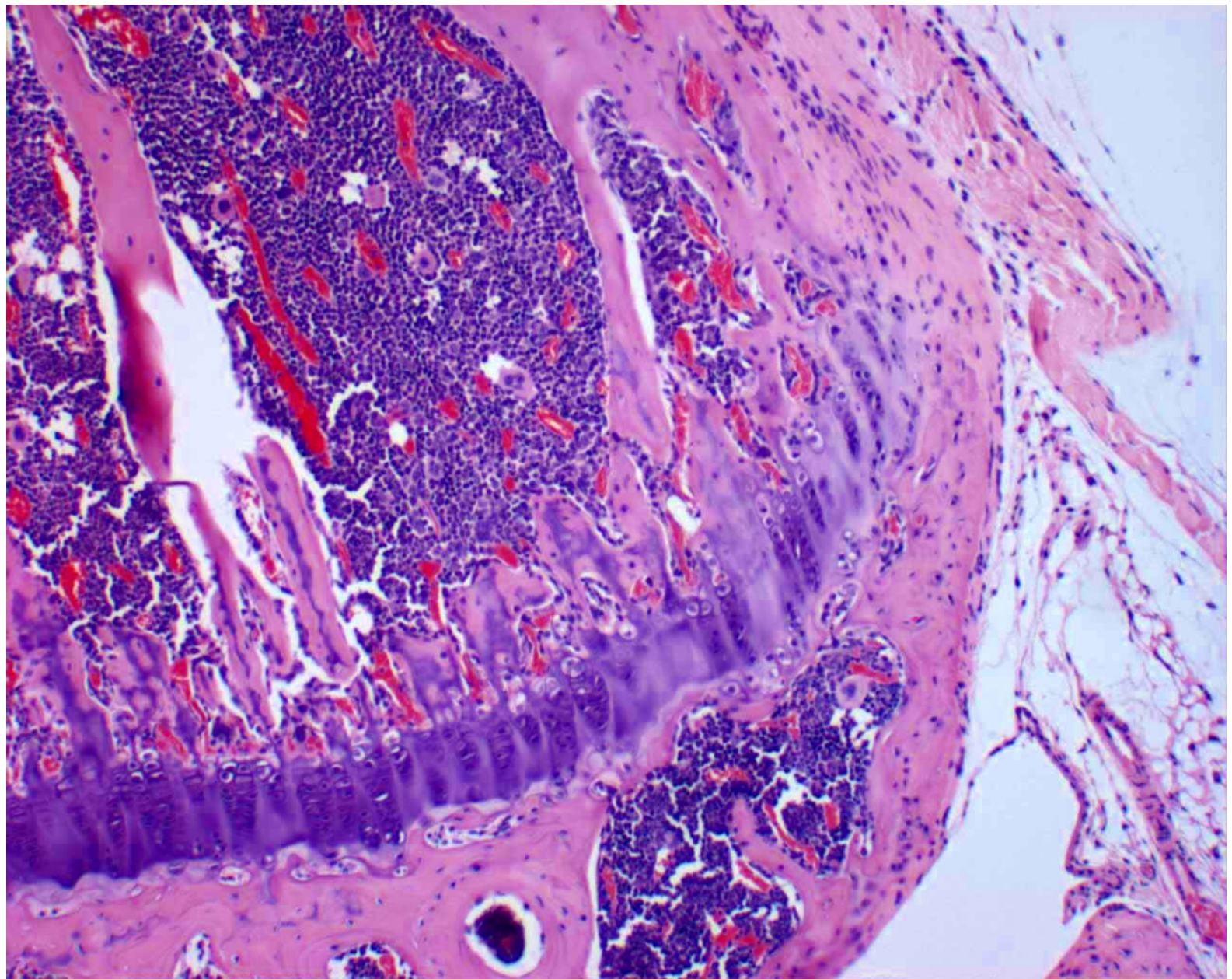
**Proliferating
chondrocytes**

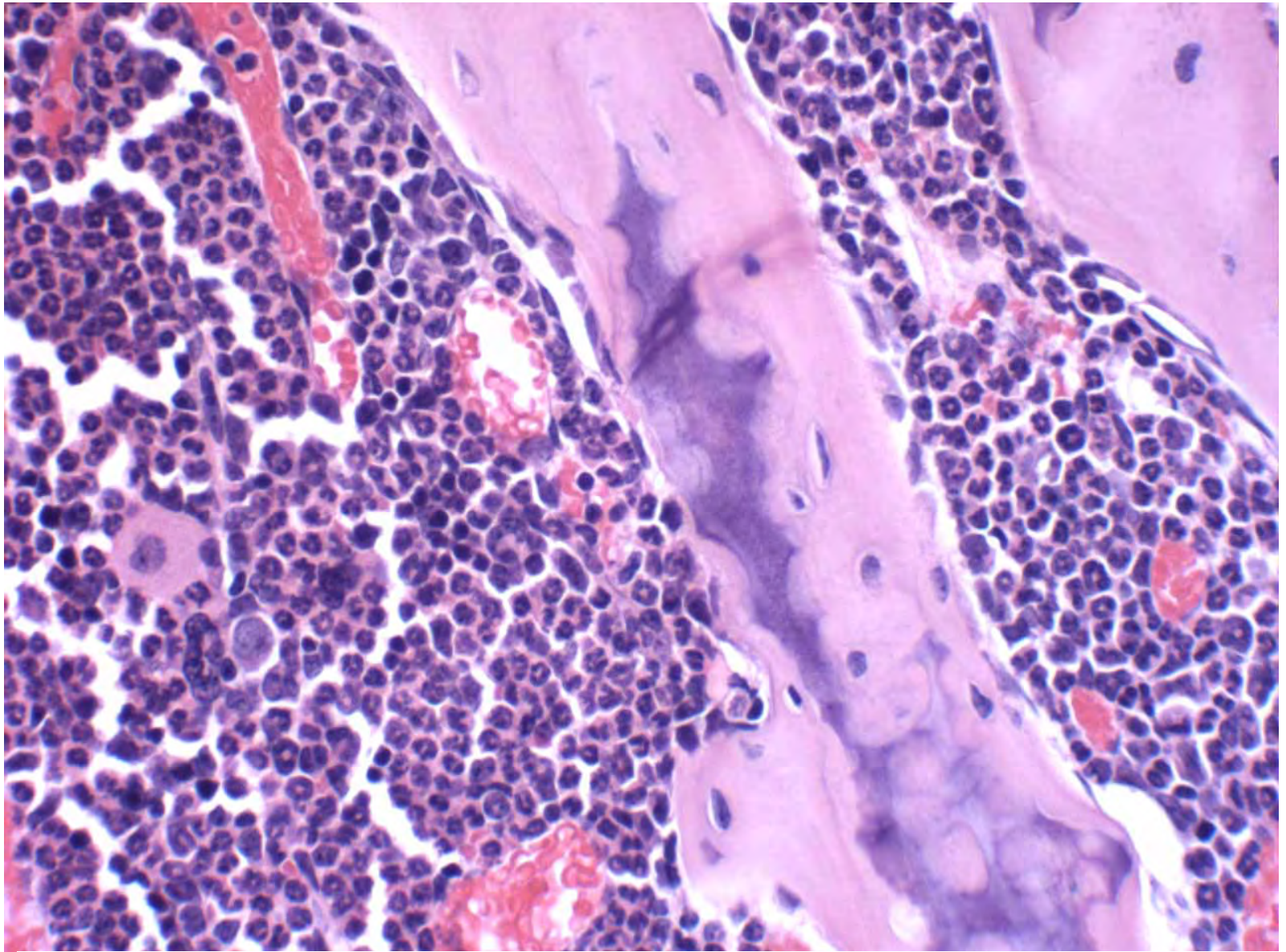
Maturing chondrocytes

Zone of calcification



Bone and marrow with precursor bone forming cells at different stages of development



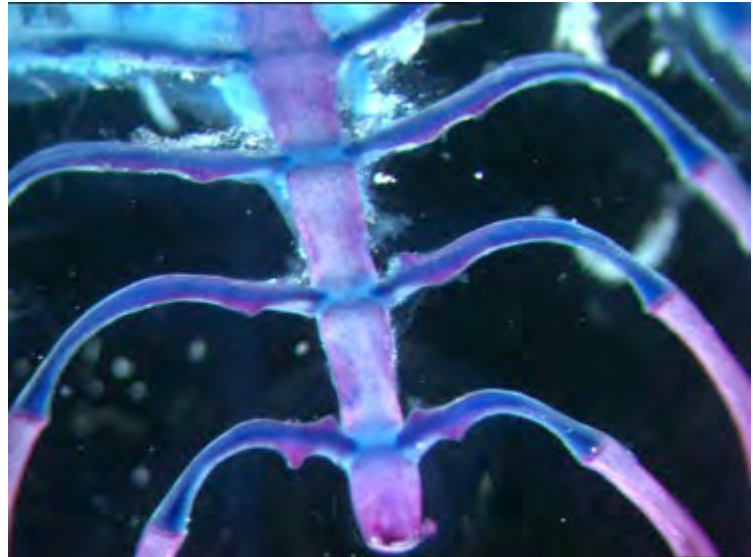
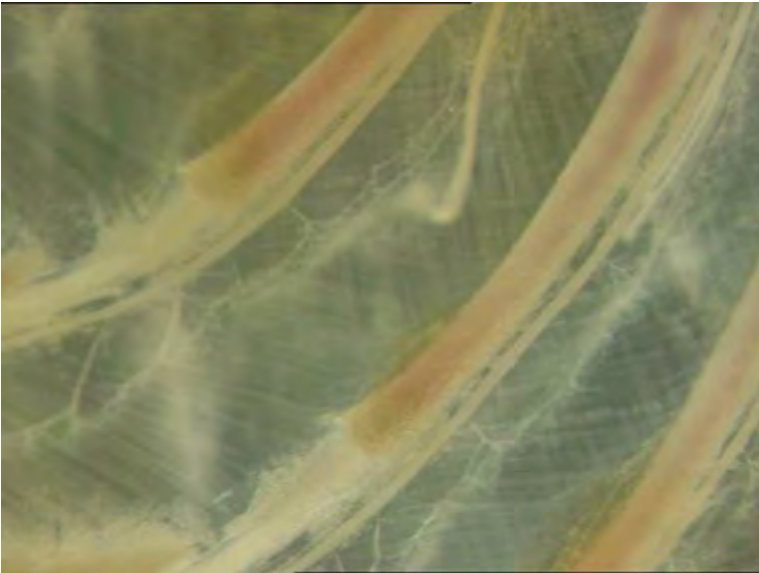


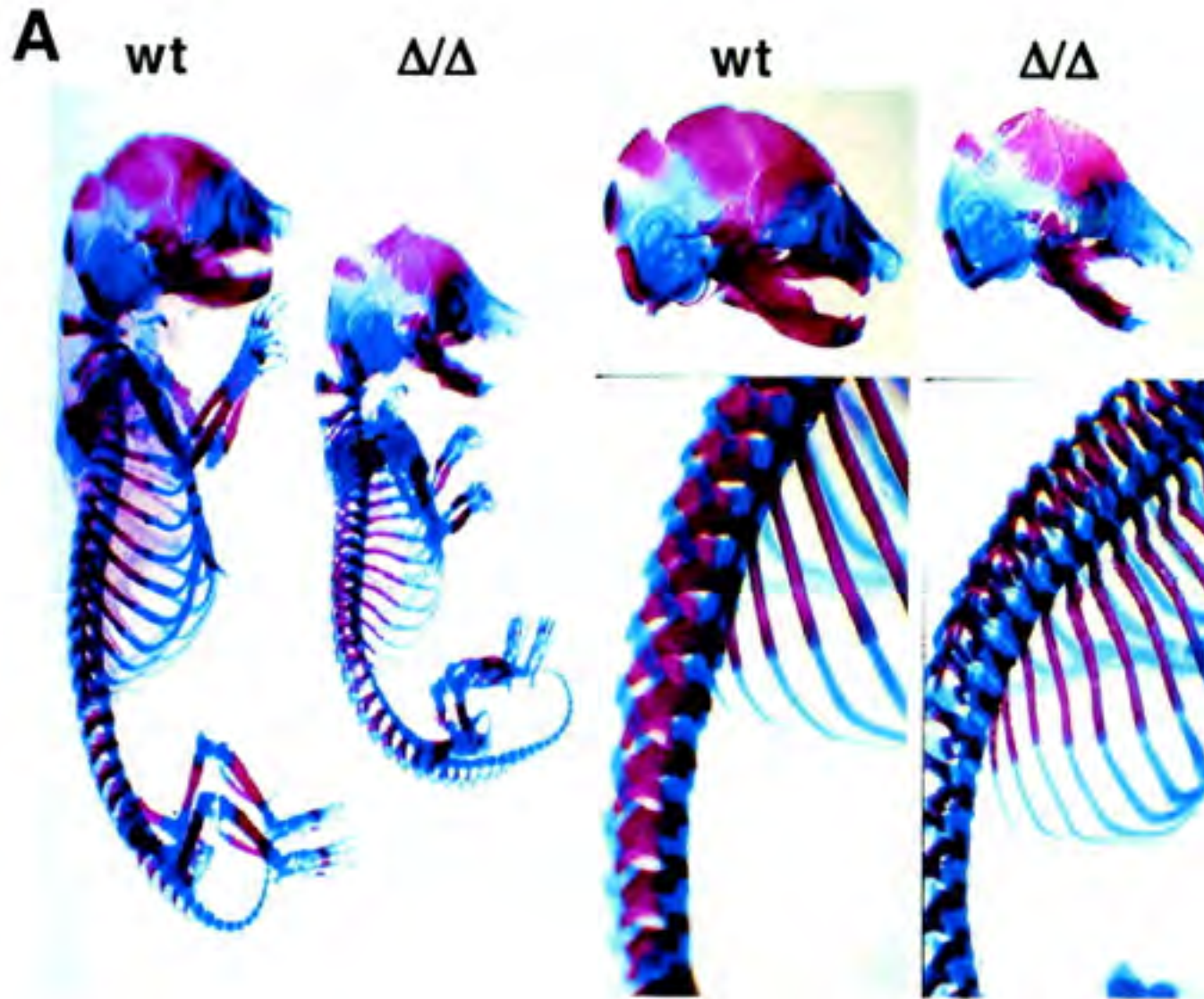
Osteoblasts (build bone) are found lining bony spicules

Histo-chemistry methods

Examples: Alizarin Red and Alcian blue on cleared embryos to examine bone and cartilage carefully

Safranin-O may also be used





Clear tissues and stain with AlizarinRed and Alcian Blue for bone and cartilage

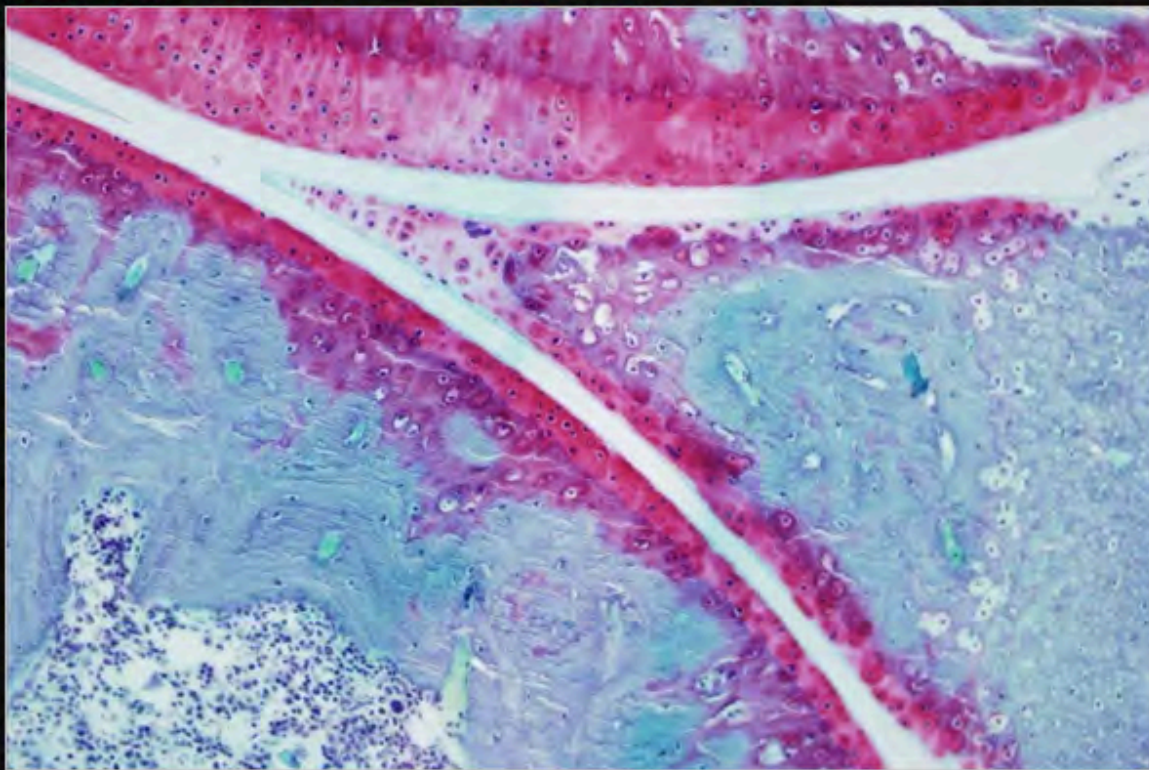
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250X magnification of mouse knee joint stained

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Safranin O 250X magnification of mouse knee joint stained with Safranin O (red) demonstrating the distribution of proteoglycans in articular cartilage. Undemineralized mouse knee was embedded in methymethacrylate and sectioned to a thickness of 5 μ prior to staining.

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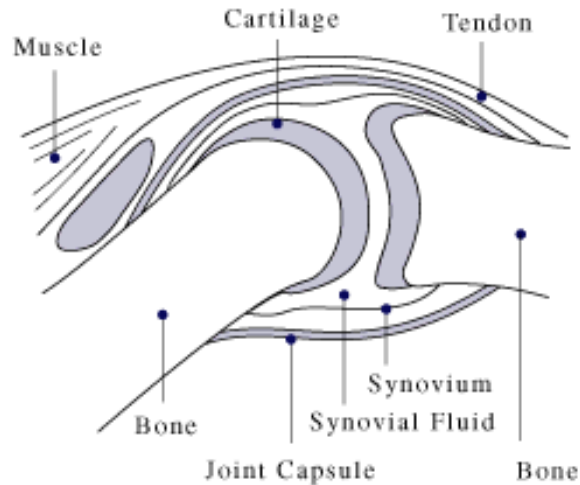
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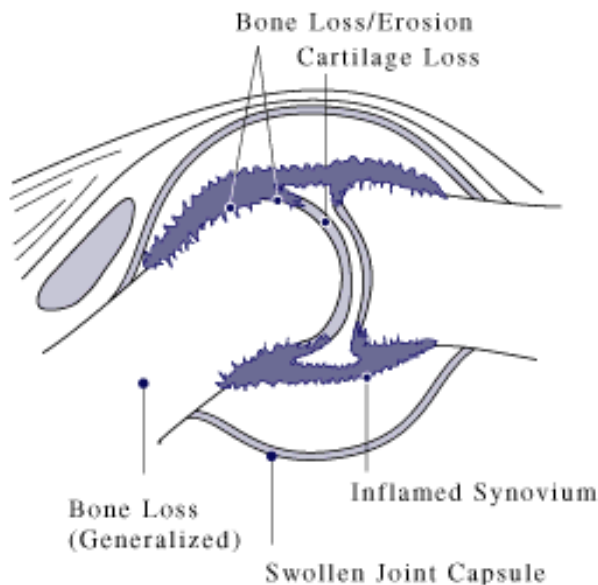
Histochemical stains review

- Alcian Blue: mucin and for cartilage—blue
- Alizarin Red: Bone—red
- Elastic stain: elastic fibers—black
- Giemsa on blood smears
- Oil Red O: for stored lipids—red--only on FROZEN sections
- PAS: basement membranes—colon control magenta mucins
- PTAH for striations—black, skeletal and cardiac
- Reticulin--black
- Safranin-O: Cartilage--red
- Sirius Red: for collagen--red
- Trichrome: for collagen—colon control (blue)
- Von Kossa: calcifications, black

Normal Joint

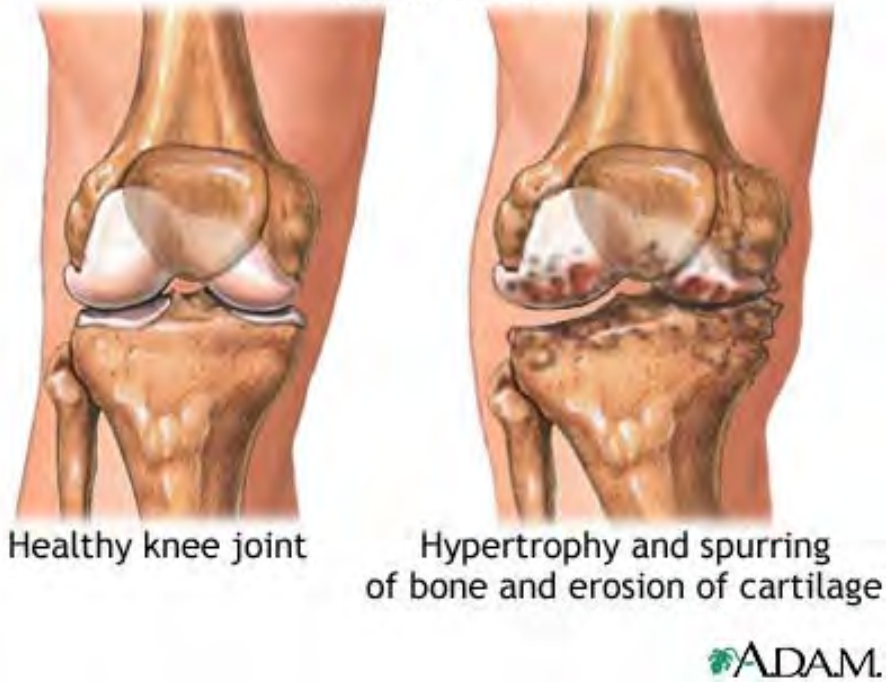


Joint Affected by Rheumatoid Arthritis

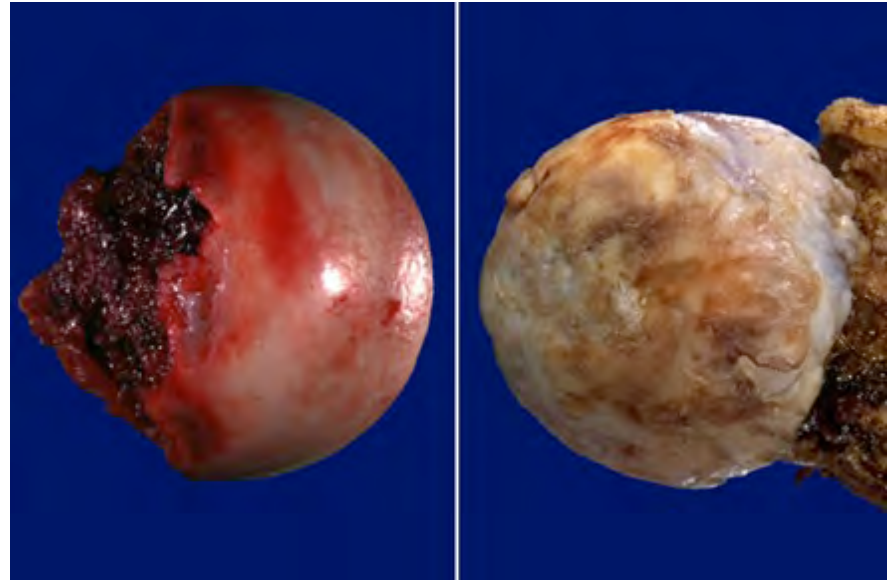


A joint (the place where two bones meet) is surrounded by a capsule that protects and supports it. The joint capsule is lined with a type of tissue called synovium, which produces synovial fluid that lubricates and nourishes joint tissues. In rheumatoid arthritis, the synovium becomes inflamed, causing warmth, redness, swelling, and pain. As the disease progresses, the inflamed synovium invades and damages the cartilage and bone of the joint. Surrounding muscles, ligaments, and tendons become weakened. Rheumatoid arthritis also can cause more generalized bone loss that may lead to osteoporosis (fragile bones that are prone to fracture).

Osteoarthritis



Degenerative Joint disease

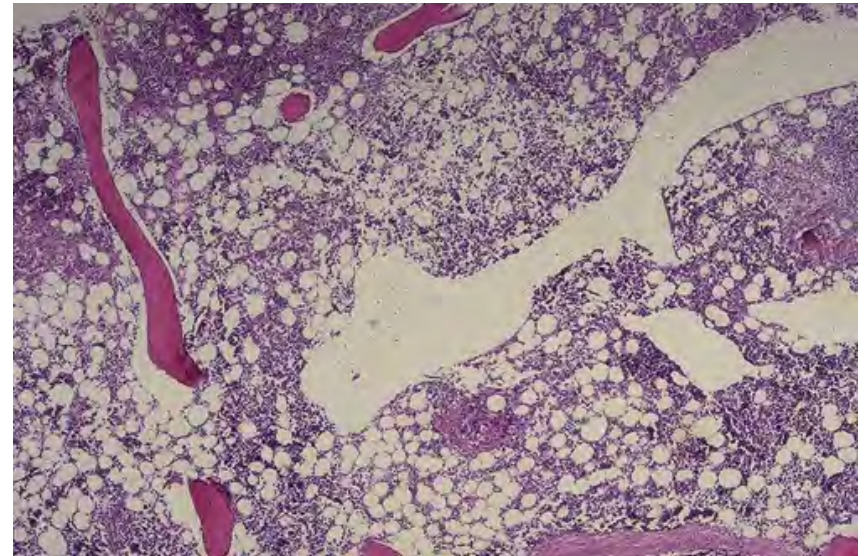
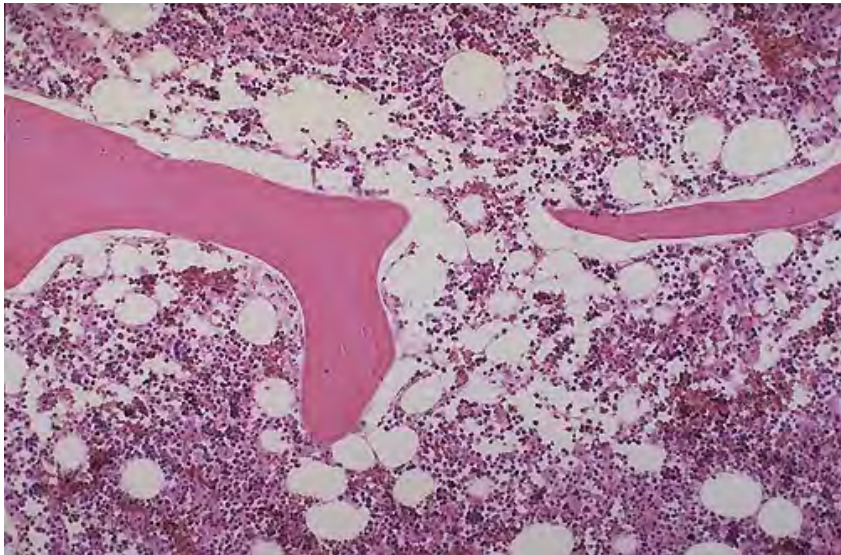


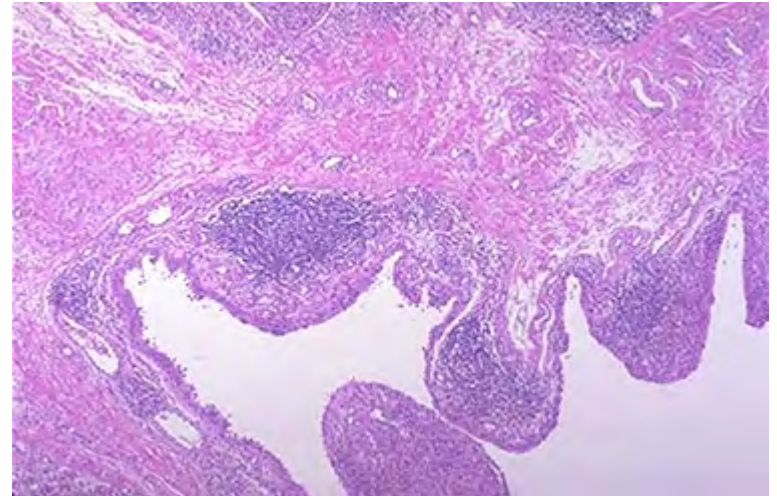
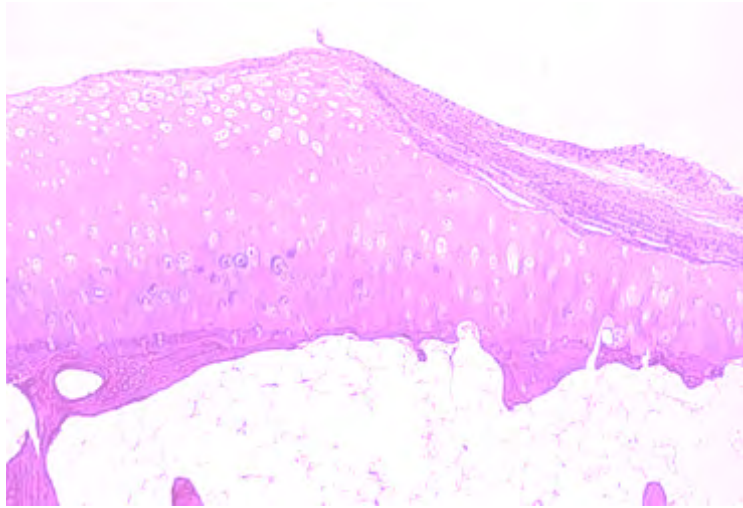
http://www.umm.edu/patiented/articles/what_osteoarthritis_000035_1.htm

Normal vertebral bone



Osteoporotic vertebral bone





This is the synovium in rheumatoid arthritis.

There is chronic inflammation with lymphocytes and plasma cells that produce the blue areas beneath the nodular proliferations.

This "pannus" is destructive and produces erosion of the articular cartilage, eventually destroying the joint.



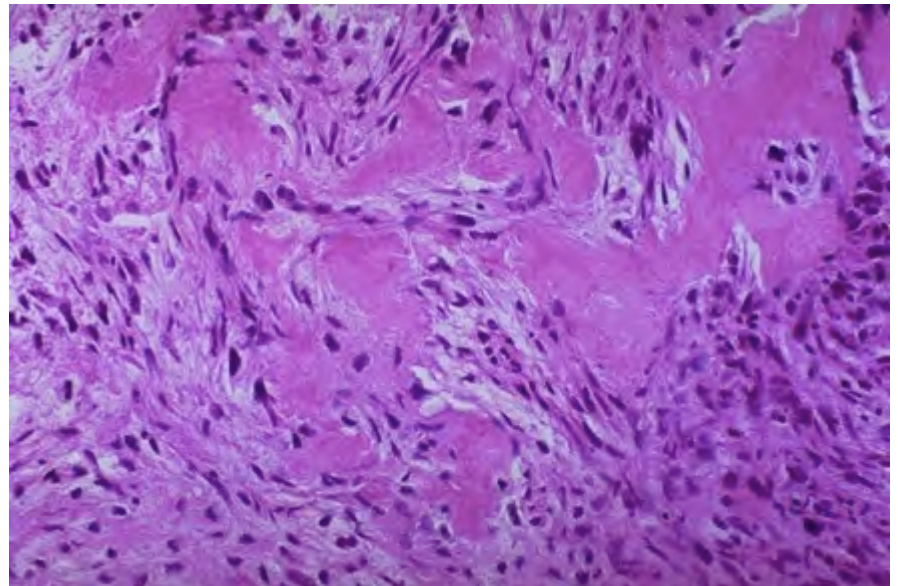
Gouty arthritis results from deposition of sodium urate crystals in joints. The joint most often affected is the first MP joint (big toe) as seen here.

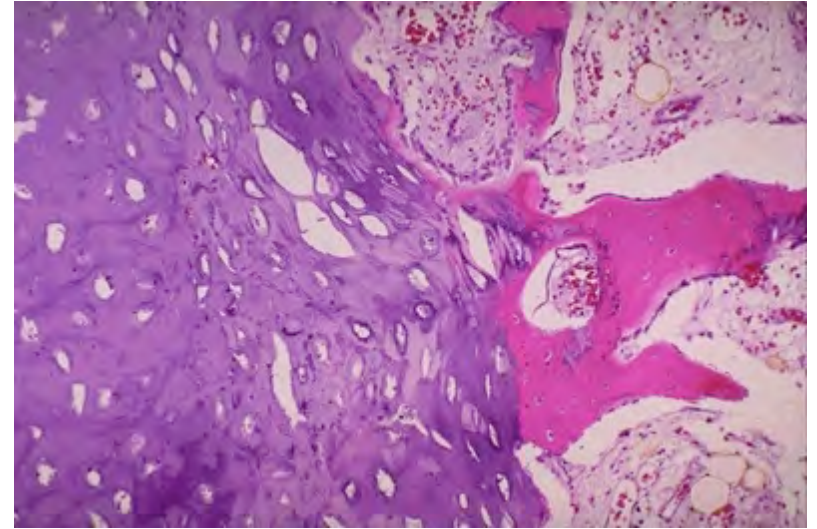
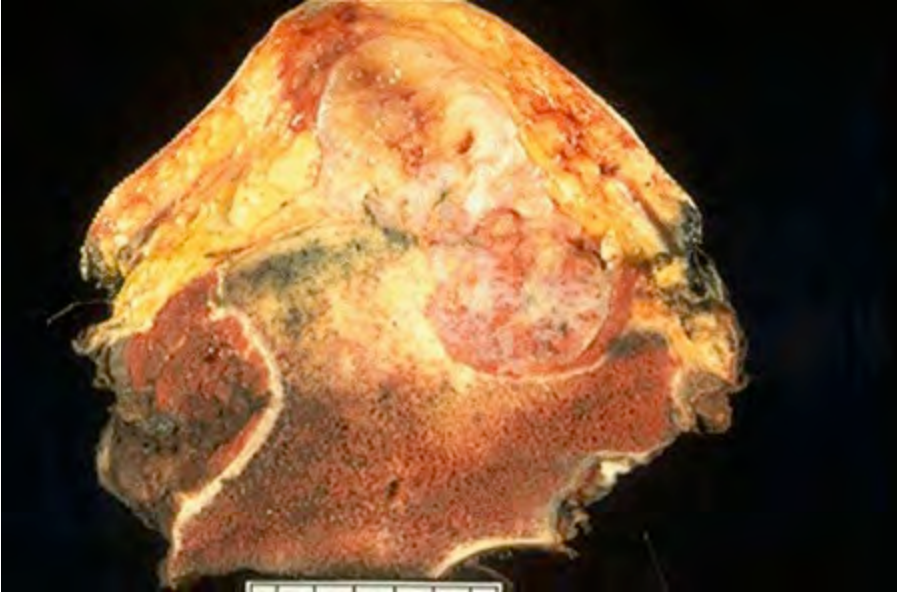


Chronic gout leads to deposition of urates into a chalky mass known as a "tophus". Such tophi can destroy the joint and adjacent bone as seen here radiographically in sequential radiographs of the same foot). In most, but not all, cases there is hyperuricemia.



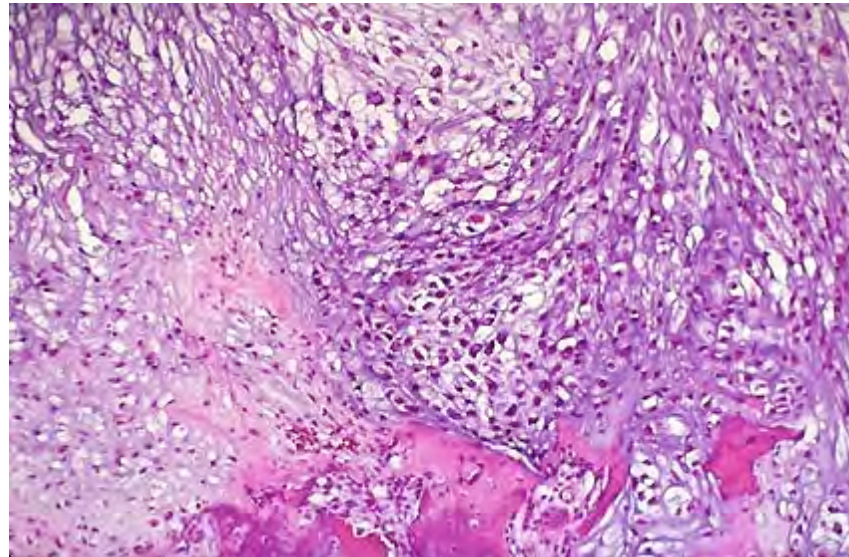
Osteosarcoma





Chondrosarcoma.

The tissue is recognizable as cartilage, and there are chondrocytes in clear spaces, but there is no orderly pattern. At the bottom, this neoplasm can be seen invading and destroying bone.



ECTODERM: Skin, mammary glands, anterior pituitary, internal ear, corneal epithelium, lens
Neuroectoderm: neural crest, melanocytes, neural tube, nerves, adrenal medulla, Schwann cells, retina, pineal body, posterior pituitary

ENDODERM: Epithelium of trachea, bronchi, lungs, GI Tract, liver, pancreas, urachus, pharynx, thyroid gland, tympanic cavity, tonsils, parathyroids

MESODERM: Muscles, connective tissue, Bone, serous membranes, blood and lymph cells, spleen, cardiovascular and lymphatic systems, urogenital system, including gonads, ducts and accessory glands.