

CONTINUING EDUCATION IN TOXICOLOGIC PATHOLOGY RESPIRATORY AND CARDIOVASCULAR SYSTEM

Fourth
Conference

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Pathology of the Nasal Passages in Rodents and Non-rodents

**Presented at Fourth Conference of Society of
Toxicologic Pathology - India**

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Summary of Presentation

1. Importance of nasal cavities
2. General procedures in necropsy and histology: importance of anatomy and histology basic knowledge
3. Approach to description of lesions
4. Classification of nasal cavity lesions (based on INHAND)
5. Selection of important and most frequently seen lesions (spontaneous and drug-related)
6. Induction of nasal lesions due to reflux of gavage material in rats.

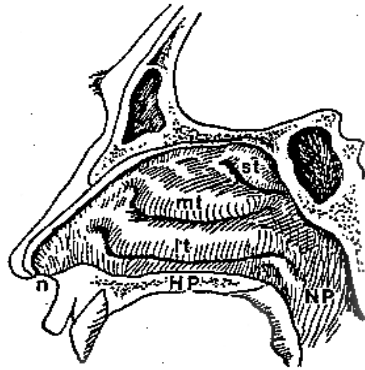
Evaluation of Nasal Cavities in Toxicology Studies

- In Inhalation studies but also in Oral Gavage studies due to reported gavage reflux lesions in nasal passages
- Species used: rats
dogs
monkeys
mice
rabbits/minipigs (recent references published)
- Types of studies: from range-finding studies to carcinogenicity studies in rats and mice

Importance of Evaluating Nasal Cavities

- First organ targeted by Test Article when given by inhalation
- Specific sites more susceptible to irritation
- Olfactory epithelium: highly metabolic site (comparable to liver based on activity per unit tissue weight)

HUMAN



MONKEY



DOG



RABBIT



RAT



Diagrammatic representation of the exposed mucosal surface of the nasal lateral wall of various species

from Harkema, 1991

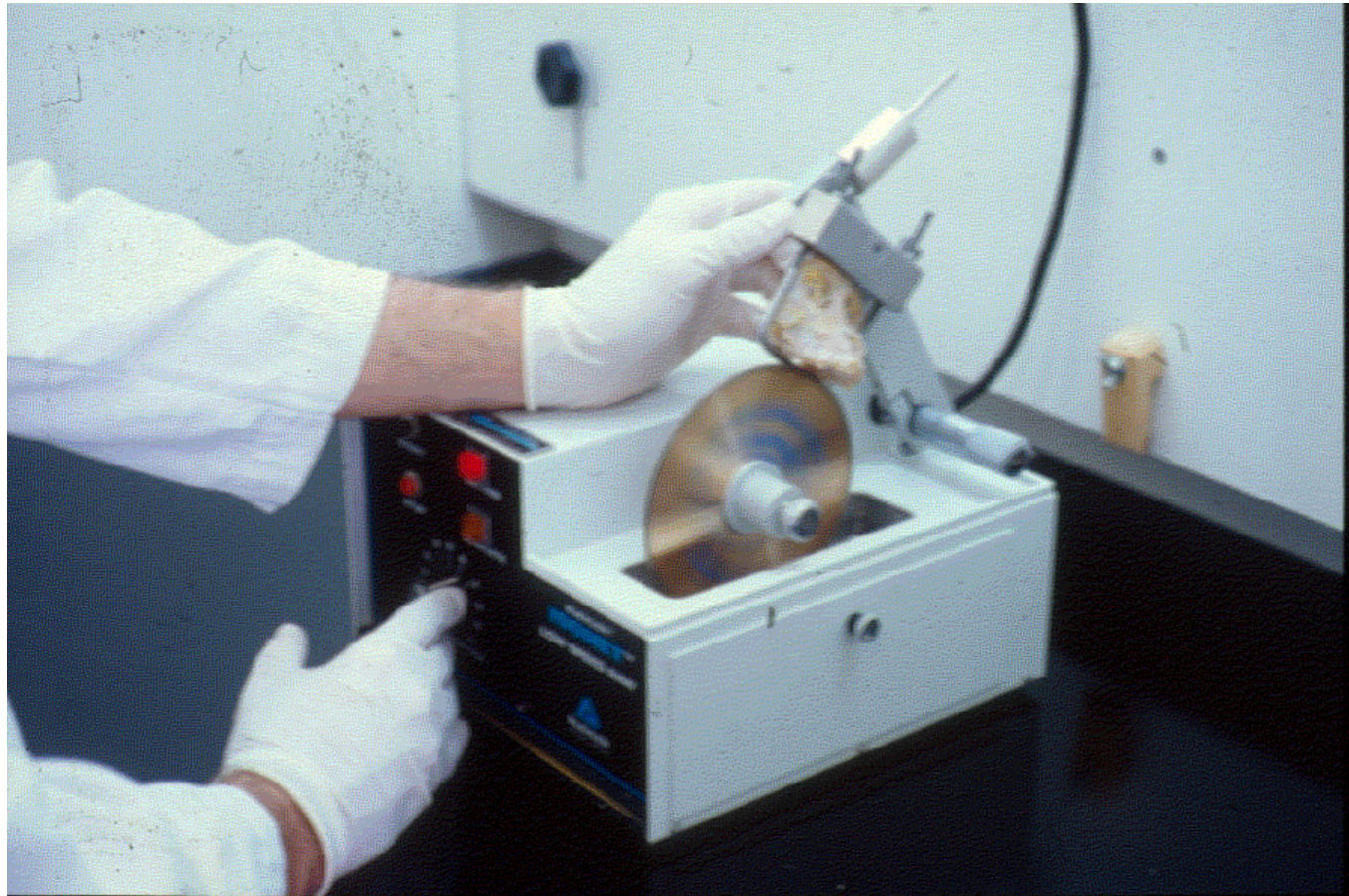
General Procedures

Fixation: Nasal cavities are perfused retrograde through the nasopharynx with formaldehyde; then head is immersed in fixative

N.B. Nasal cavity is not opened at necropsy

Decalcification: Standard sections cut with saw prior to decalcification (important in dogs and monkeys)

Standardization of nasal sections for evaluation is critical



Sectioning of nasal cavity of monkey prior to decalcification

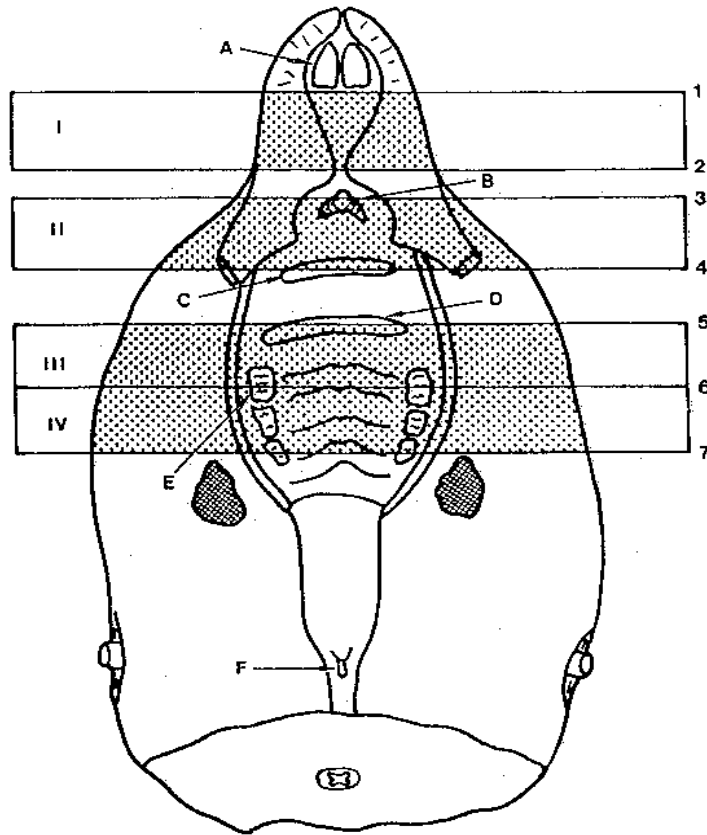
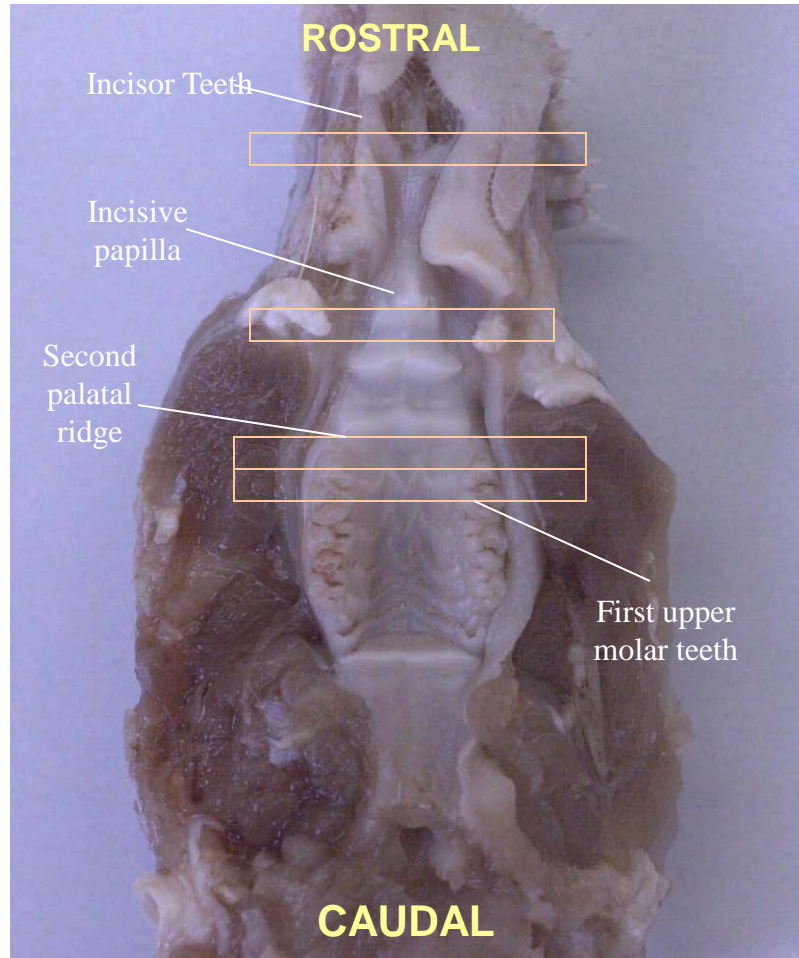


FIG. 1. Ventral view of the rat hard palate region, with the lower jaw removed, indicating the four tissue slices, I—IV (stippled areas), which will be embedded anterior face down. The numbers on the right-hand side indicate the levels of the seven cuts necessary to produce the four slices. A, upper incisor teeth; B, incisive papilla; C, first palatal ridge; D, second palatal ridge; E, first upper molar tooth; F, posterior opening of the pharyngeal duct (nasopharynx).

Anatomic
landmarks:
ventral view of the
rat hard palate
region

from Young, 1981

Nasal Cavities and sinuses (4 levels) (based on Young's technique)



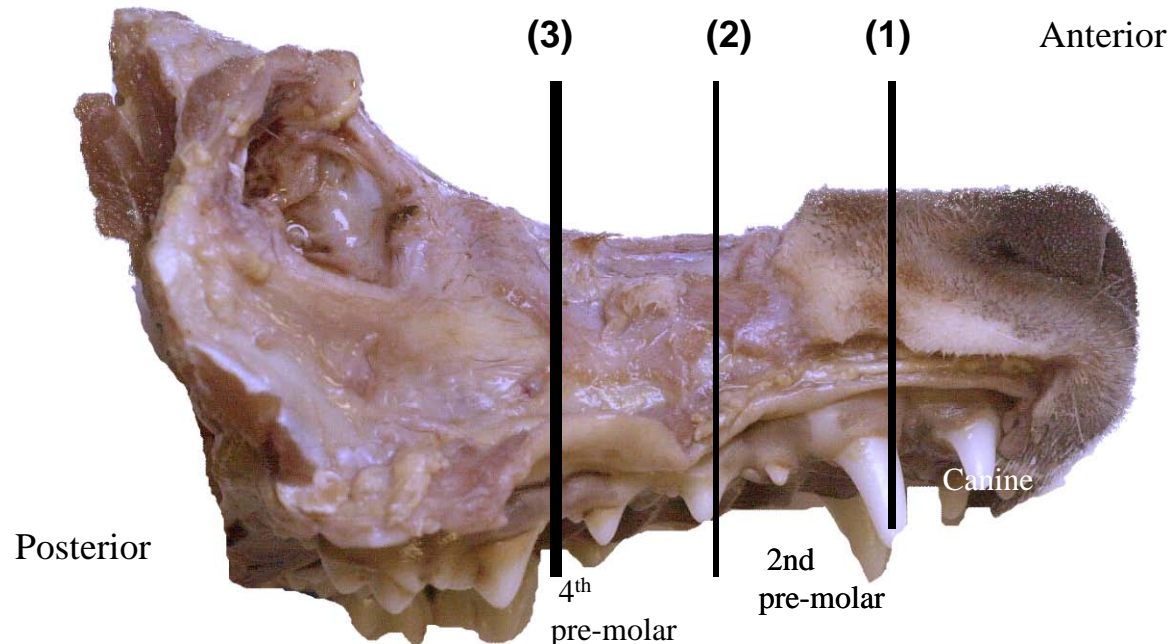
Level 1: transverse section (3-5 mm thick) immediately posterior to incisor teeth.

Level 2: transverse section (3-5 mm thick) through the incisive papilla.

Level 3: transverse section (3-5 mm thick) through the palatal ridge.

Level 4: transverse section (3-5 mm thick) through the first upper molar teeth.

Nasal cavities and Sinuses (3 levels) in DOGS

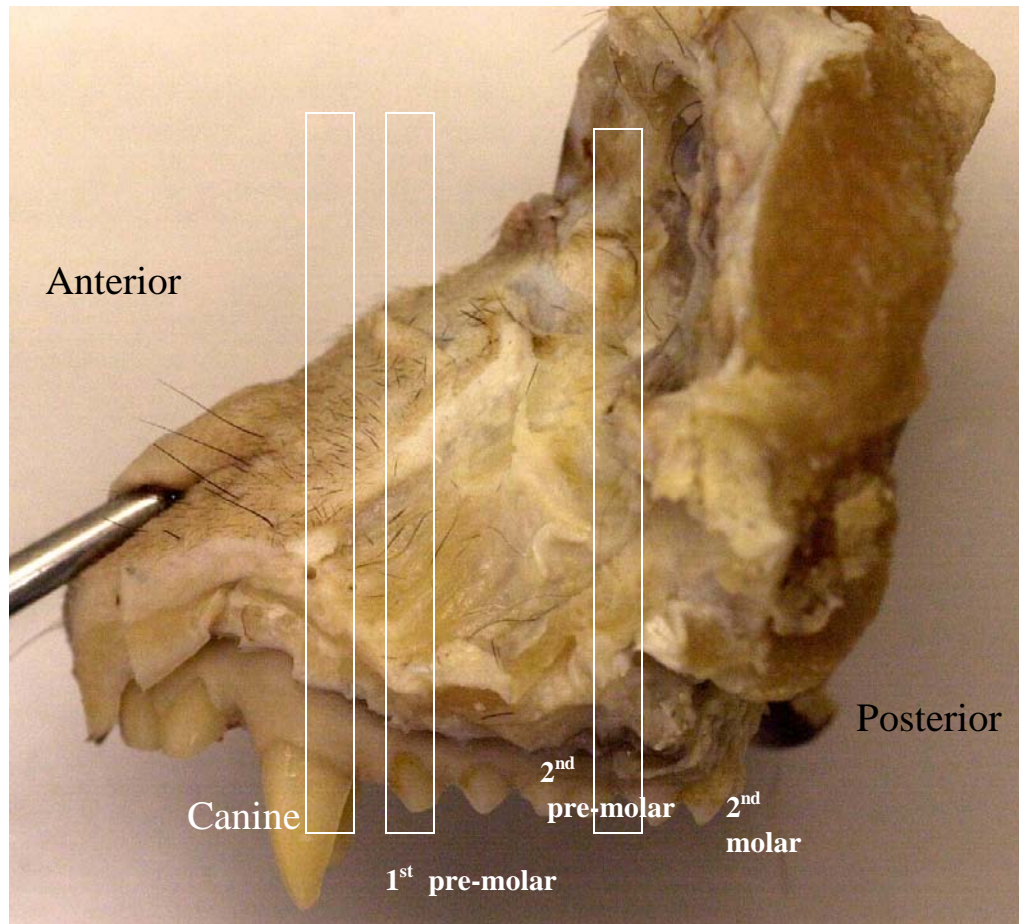


Level 1: Transverse section (3-5 mm thick) at the canine level.

Level 2: Transverse section (3-5 mm thick) at the beginning of the 2nd pre-molar teeth. Make another cut off center to include the septum with the right side.

Level 3: Transverse section (3-5 mm thick) at the beginning of the 4th pre-molar teeth. Make another cut off center to include the septum with the right side. **All sections are placed anterior face down in the cassette**

Nasal Cavities and Sinuses (3 levels) in Monkeys



Level 1: Transverse section anterior to the first premolar teeth.

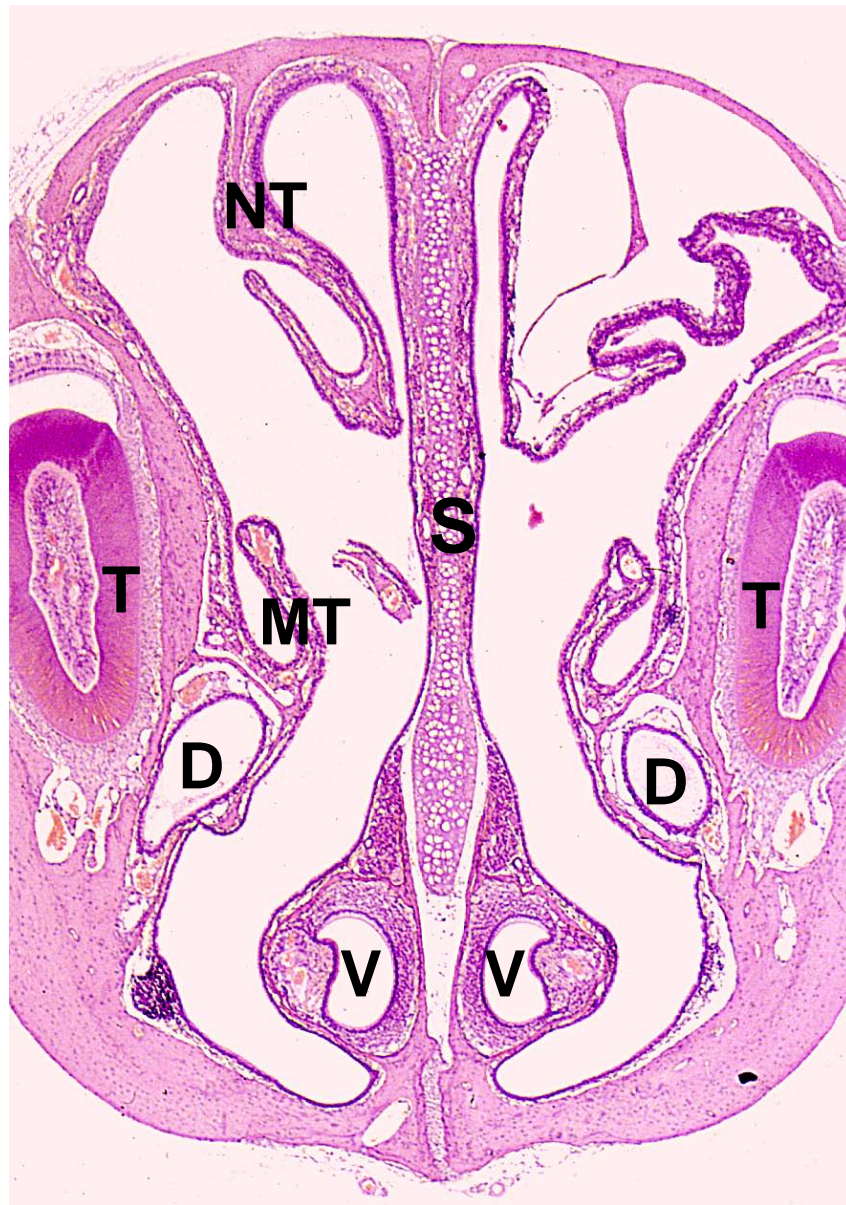
Level 2: Transverse section anterior to the second premolar teeth.

Level 3: Transverse section in the middle of the second molar teeth. Make another cut off center to include the septum with the right side.

All sections are placed anterior face down in the cassette.



Monkeys: Three sections routinely examined microscopically



LEVEL I in the rat (Normal)

S = Septum

D = Nasolacrimal duct

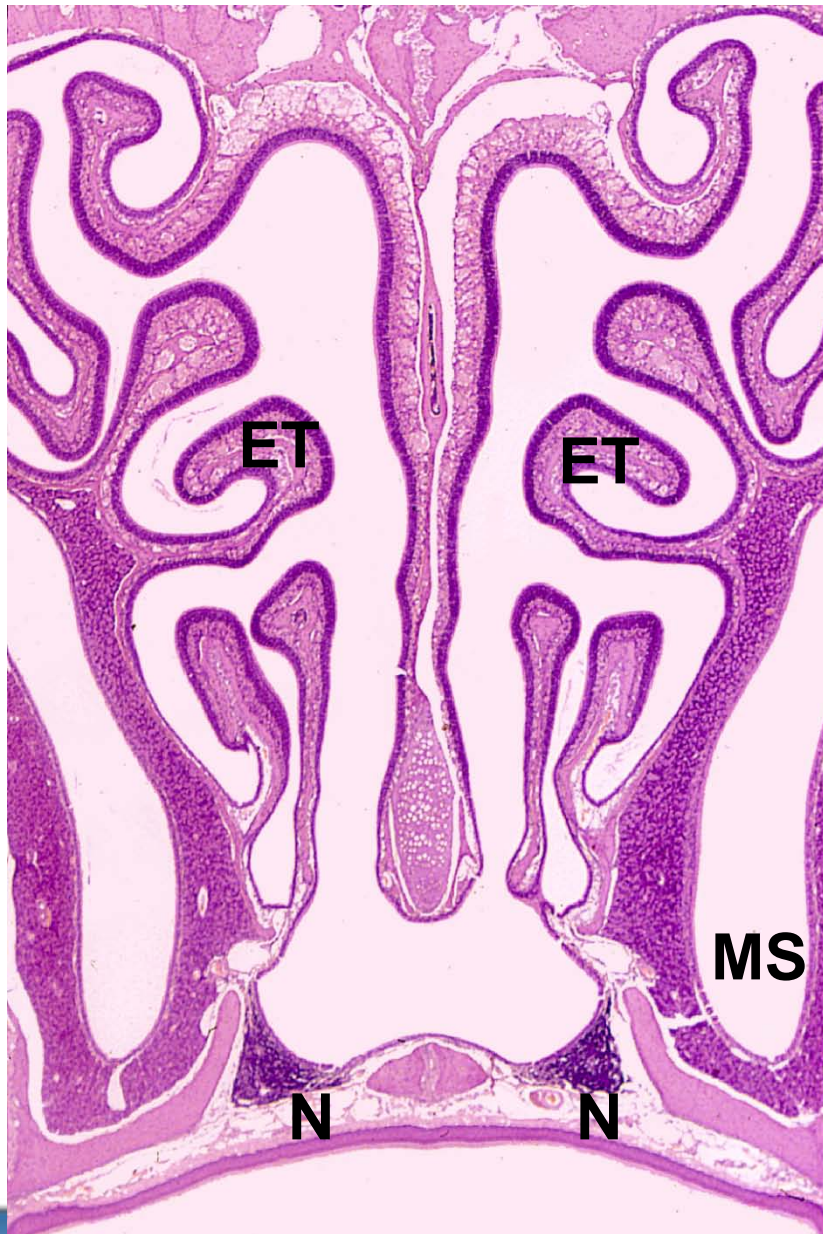
V = Vomeronasal organ

NT = Nasoturbinete

MT = Maxilloturbinete

T = Incisor teeth

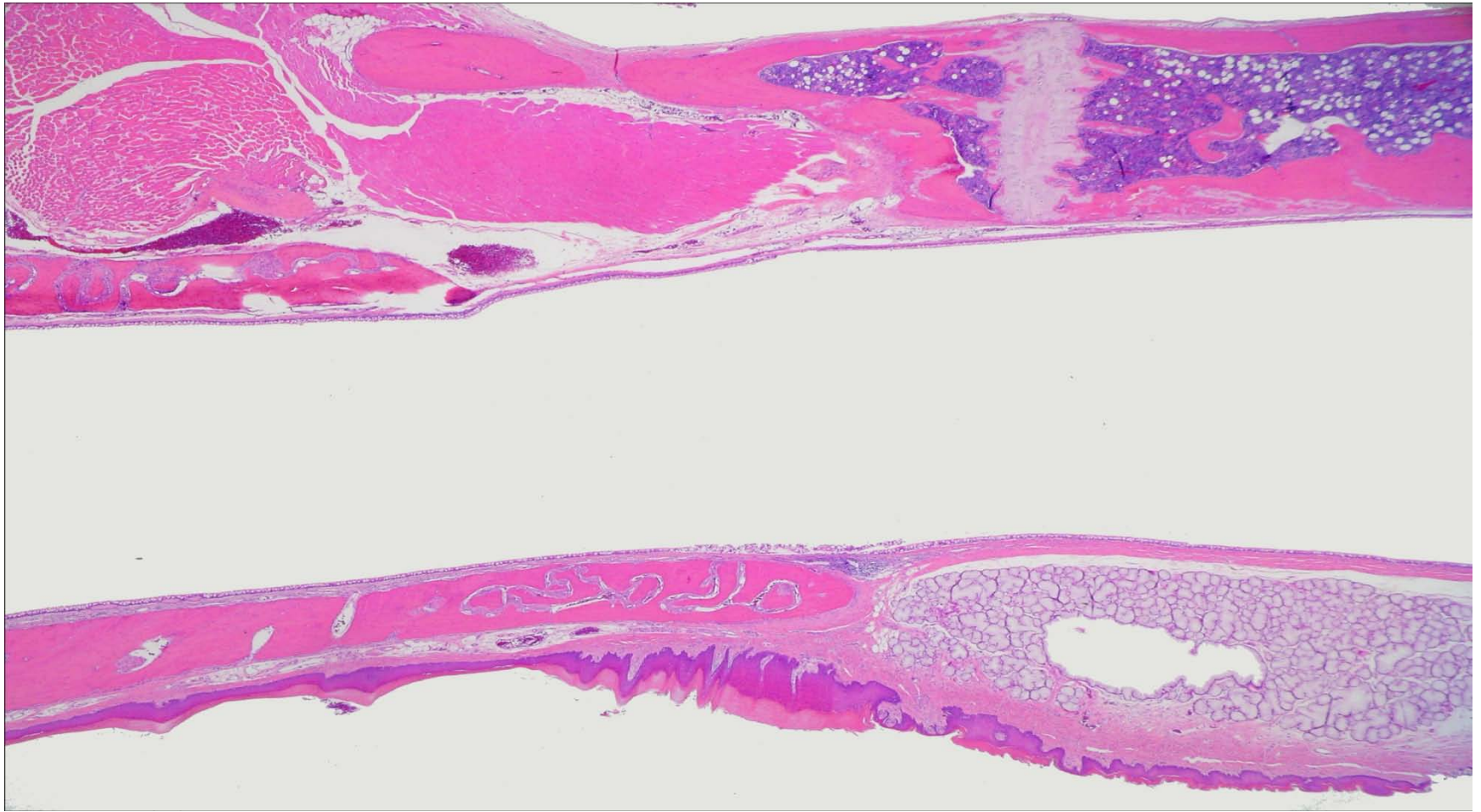
Level III In the rat (Normal)



MS = Maxillary sinus

ET = Ethmoid turbinates

N = NALT (Nasal
associated lymphoid
tissue)



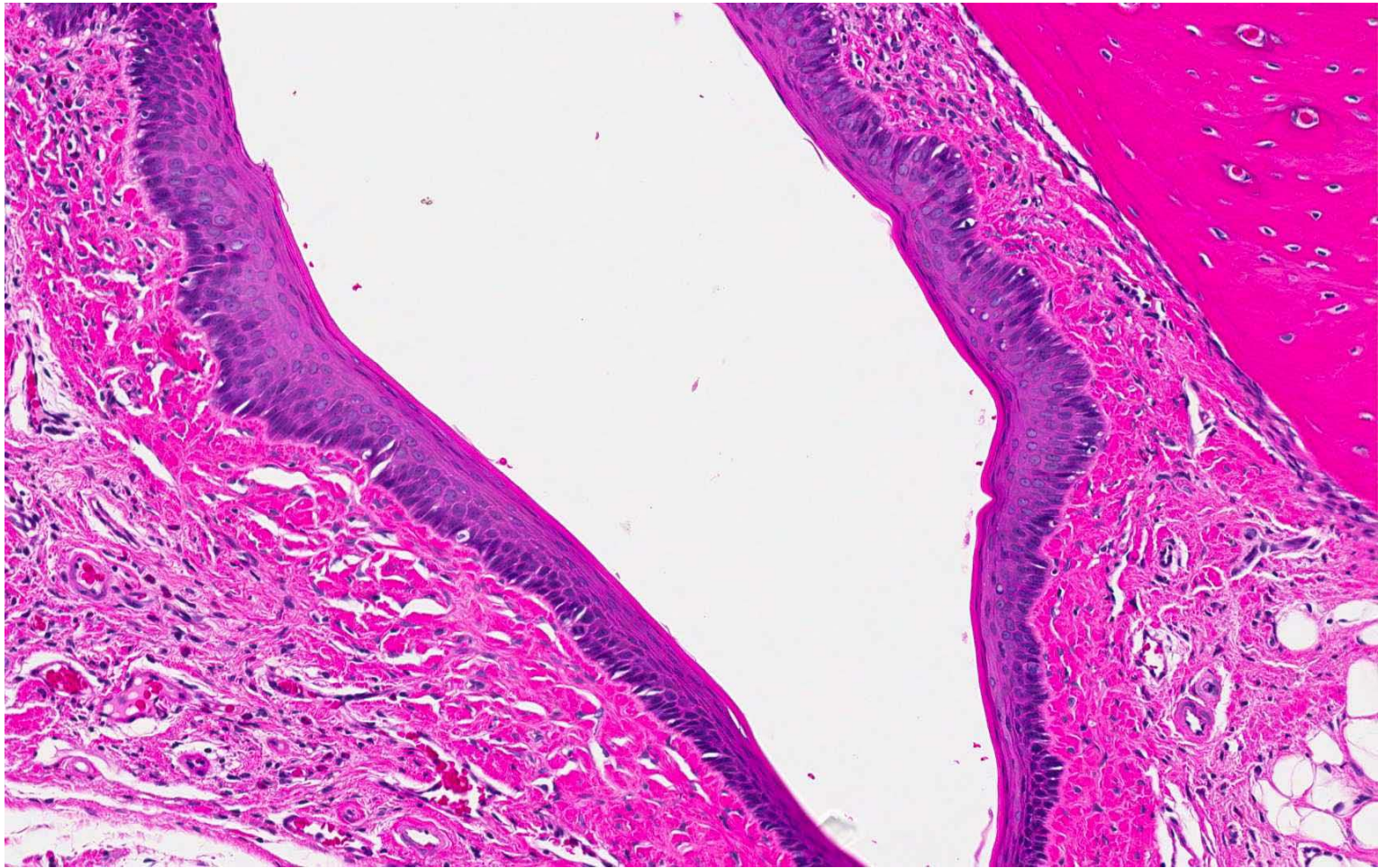
Rat: Normal Nasopharynx

Topographical areas in the nasal passages can be divided into 5 general sites:

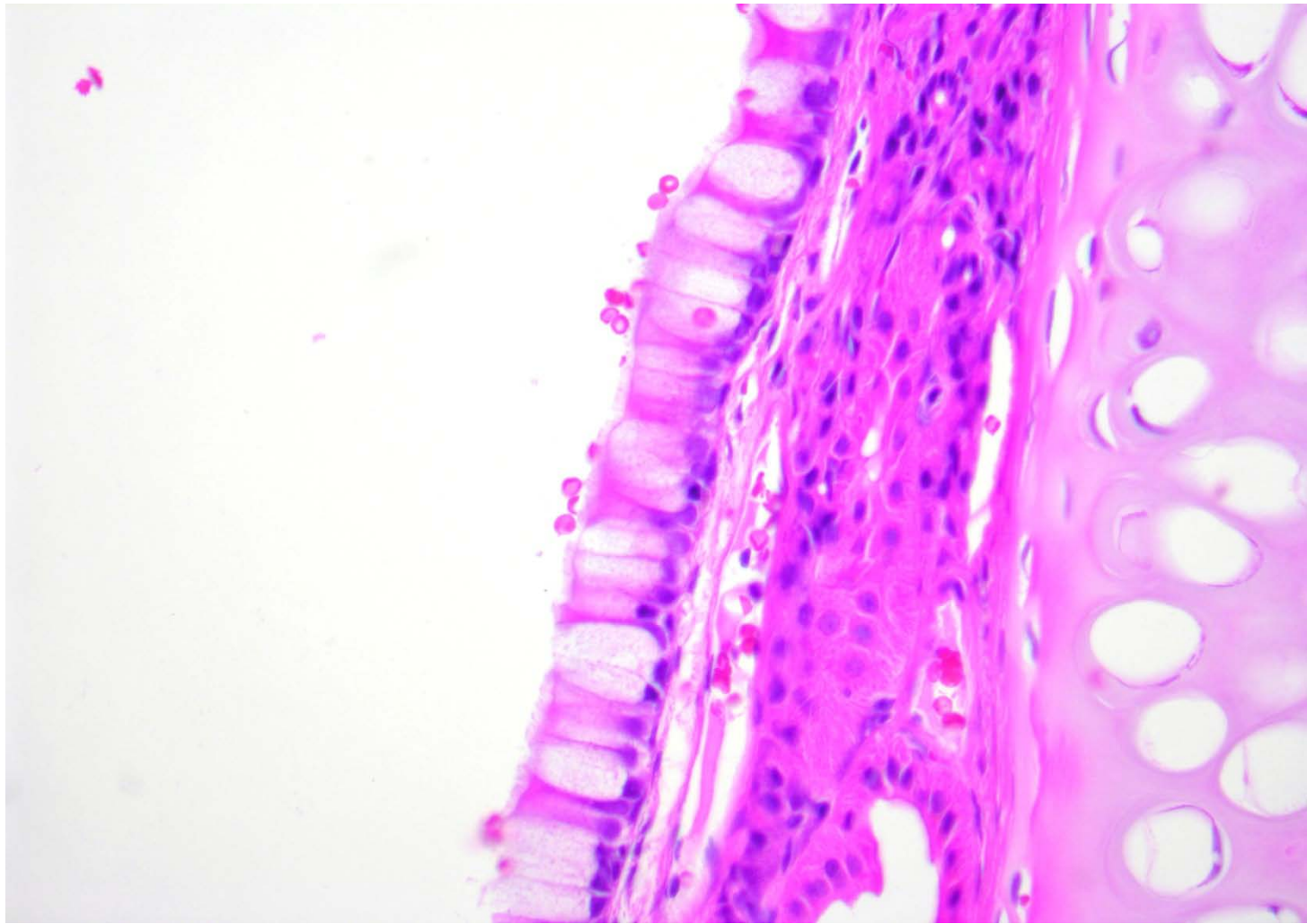
- The region of squamous epithelium (nares, vestibules, anterior ventral meatus and incisive duct);
- The region of transitional epithelium (anterior lateral wall and the anterior tips and lateral surface of the maxillary and nasal turbinates);
- The respiratory epithelial region (nasal septum and area between the transitional epithelium and the olfactory epithelium);
- The olfactory region (mid-dorsal meatus and ethmoid turbinates);
- The nasopharynx meatus

Important structures to evaluate histologically in the nasal cavities

- Mucosa: squamous epithelium
respiratory epithelium
transitional epithelium
olfactory epithelium
- Vomeronasal organ
- Nasolacrimal ducts
- Bowman's gland (and other submucosal glands)
- Teeth
- NALT (Nasal associated lymphoid tissue)



Rat: Normal squamous epithelium



Rat: Normal respiratory epithelium

Respiratory Epithelium Cell types

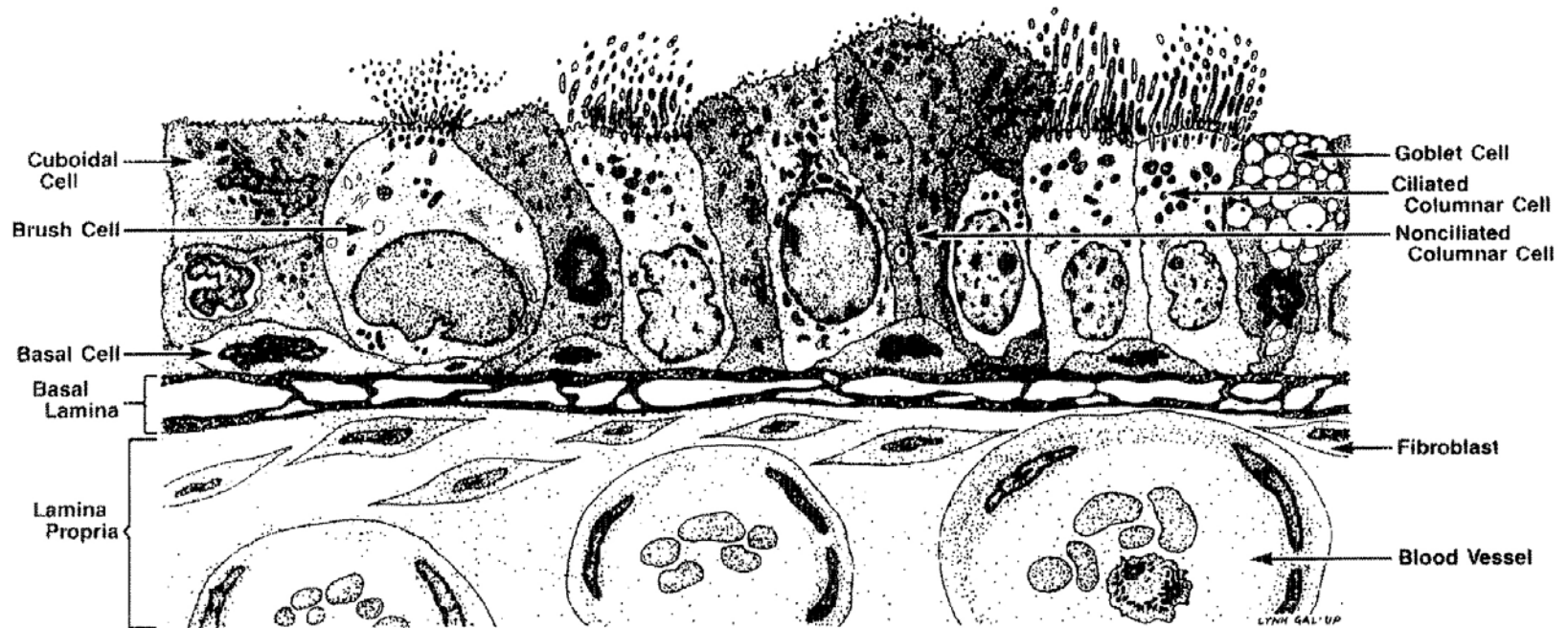
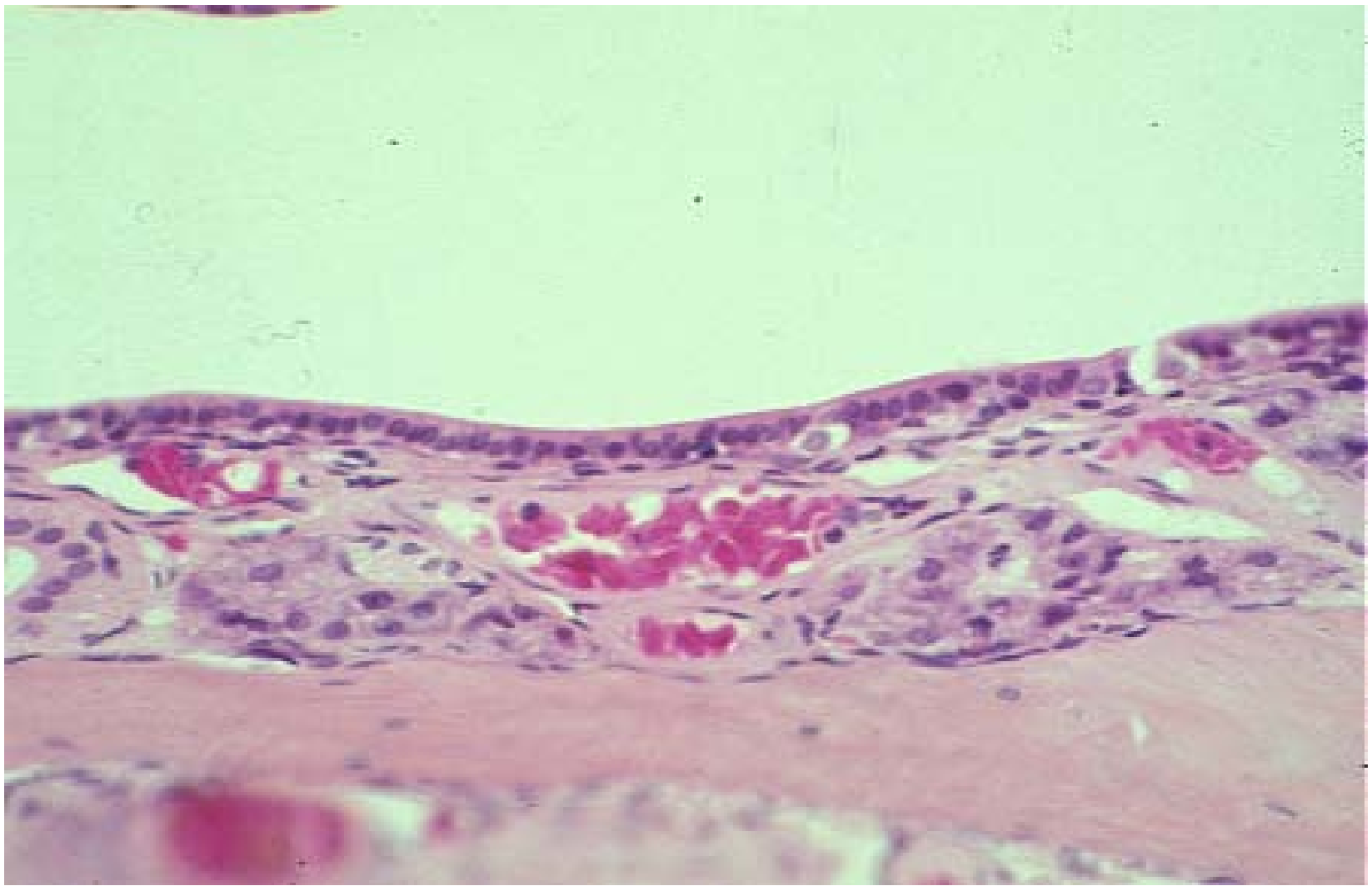
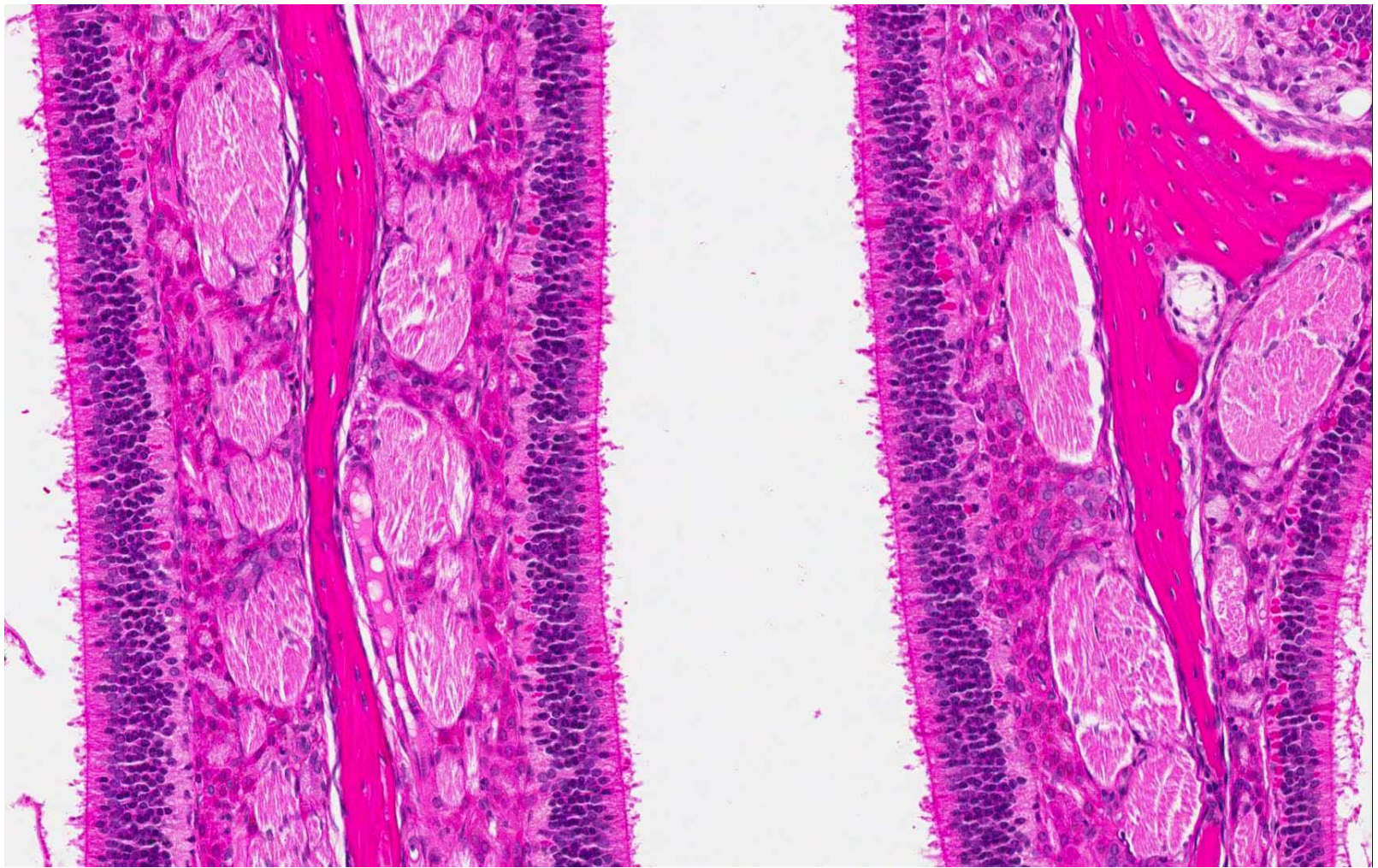


Diagram showing the six different cell types of the respiratory epithelium and relationship to lamina propria

from Boorman, 1990



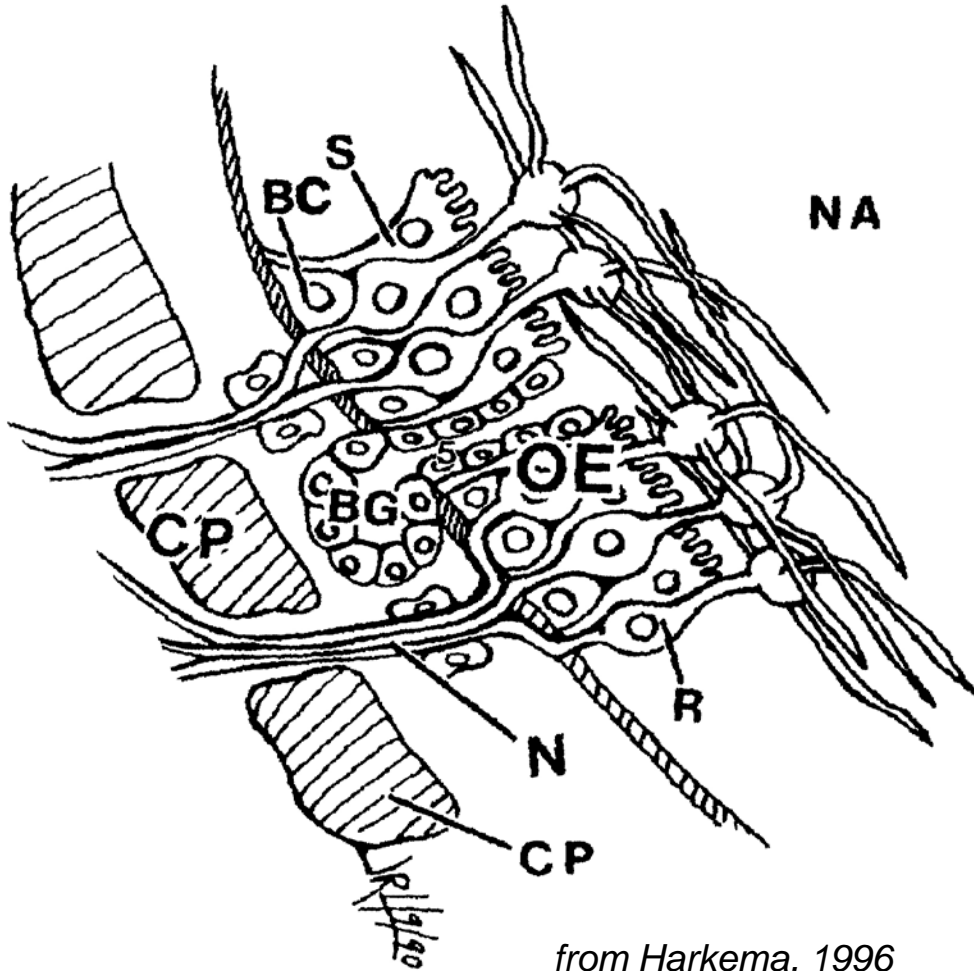
Rat - Normal transitional epithelium



Rat: Normal olfactory epithelium

Olfactory Epithelium (OE) in the nasal cavity of a F344 Rat

Diagram illustration of OE and underlying lamina propria

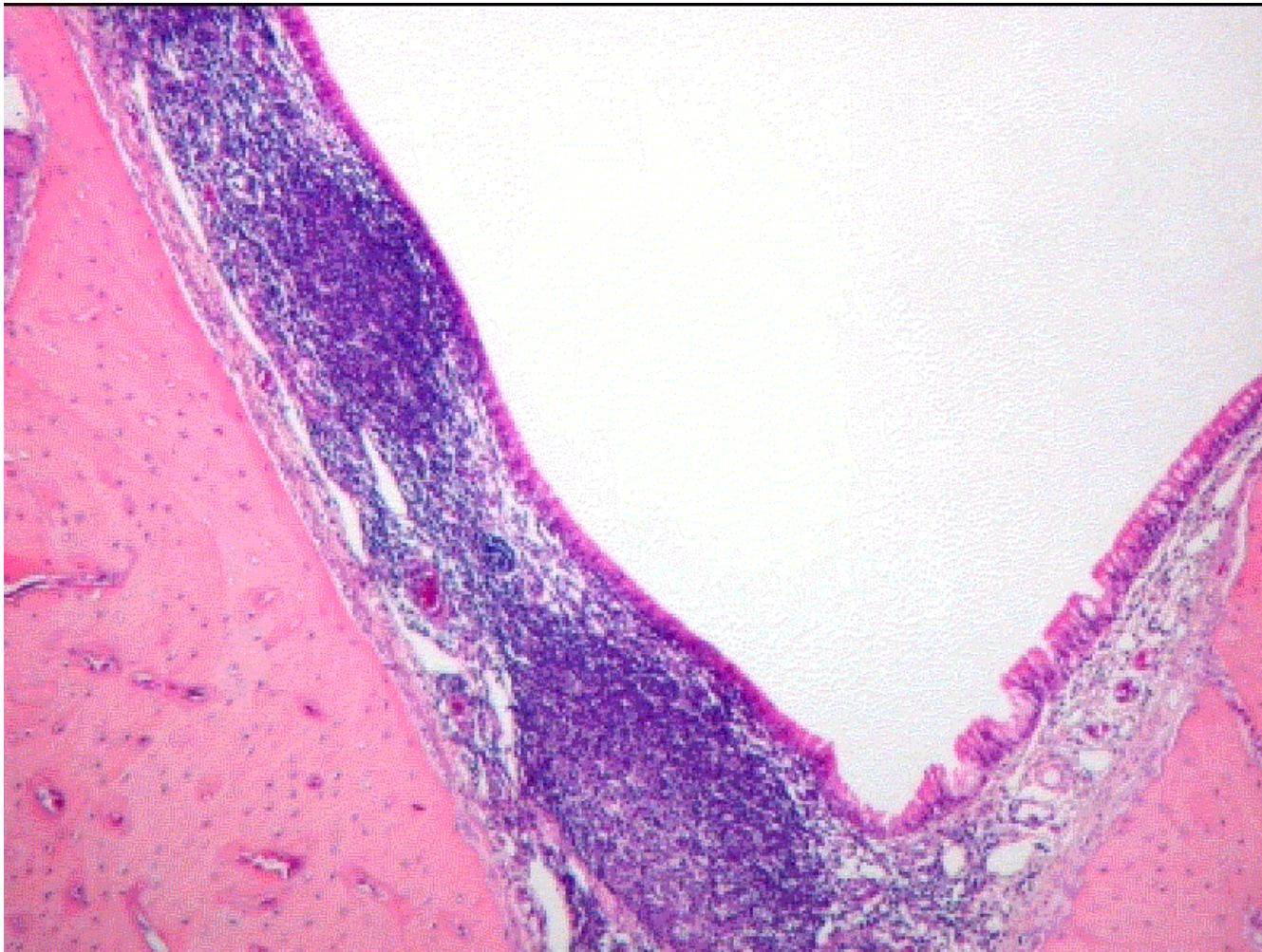


from Harkema, 1996

- OE- Olfactory Epithelium
- S - Sustentacular (support) cells
- R - Olfactory sensory receptor cells
- BC - Basal cells
- N - Olfactory nerve in lamina propria
- BG - Bowman's gland
- CP - Cribriform plate between nasal and cranial cavities
- BV - Blood vessel
- NA - Nasal airway, Bar=25 μ m



Rat: Level 4: Nasopharynx with NALT



NALT – Normal – Rat

NB: important to evaluate when compound tested is immunosuppressive

How to characterize a lesion in the nasal cavity

1. Proper morphologic diagnosis
e.g., necrosis
inflammation
tumor
2. Record severity grade (usually defined by Pathology Data Capture System)
3. Record level affected (optional, varies between species)
4. Record which structure is affected:
e.g., respiratory epithelium
Bowman's gland
Vomeronasal organ
5. Topographical modifier can be added, such as:
dorsal meatus
lateral meatus
nasal septum

International Harmonization of Nomenclature and Diagnostic Criteria (INHAND)

Proliferative and Non-Proliferative Lesions of the Respiratory Tract of the Rat and Mouse

**Toxicologic Pathology, Volume 37,
Number 7, Supplement 2009**

INHAND General Classification of Nasal Cavity Lesions

- A. Congenital Lesions
- B. Epithelial Changes
- C. Inflammation
- D. Vascular Changes
- E. Nonneoplastic Proliferative Lesions
- F. Neoplastic Proliferative Lesions

Selected Important Nasal Lesions Seen in Toxicologic Pathology

1. Eosinophilic globules
2. Corpora amylacea
3. Amyloidosis (eosinophilic substance)
4. Necrosis
5. Regeneration
6. Inflammation
7. Squamous, Metaplasia
8. Hyperplasia/Metaplasia, Mucous Cell

Eosinophilic Globules

- Cell of origin: respiratory, glandular and olfactory epithelium
- Accumulation of brightly eosinophilic cytoplasmic inclusions in sustentacular cells of olfactory epithelium, respiratory epithelial cells and epithelial cells of the nasal seromucous glands
- Usually a non-specific reaction to irritation (quite common in rats); can also be seen as an age-related change; relatively frequent change
- At EM: membrane-bound ellipsoid bodies containing homogenous electron dense matrix (presumably proteinaceous)

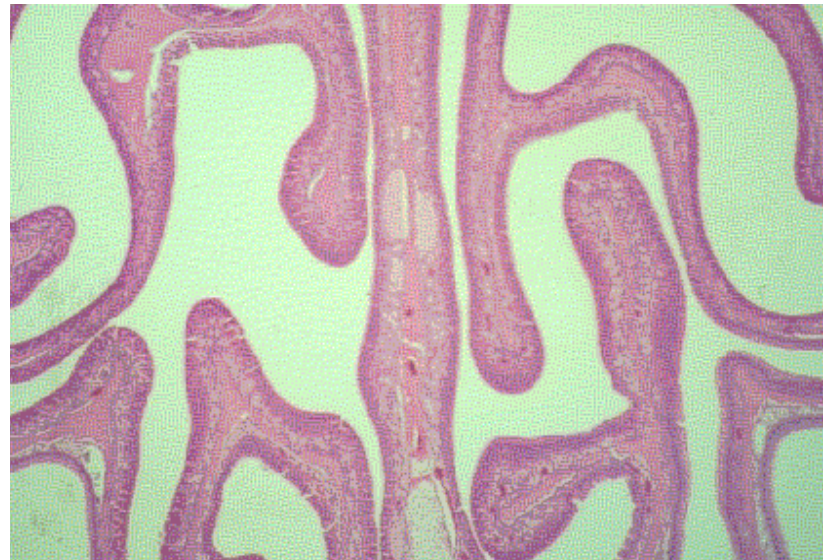
Normal olfactory
epithelium (ethmoid
turbinates)

- Rat



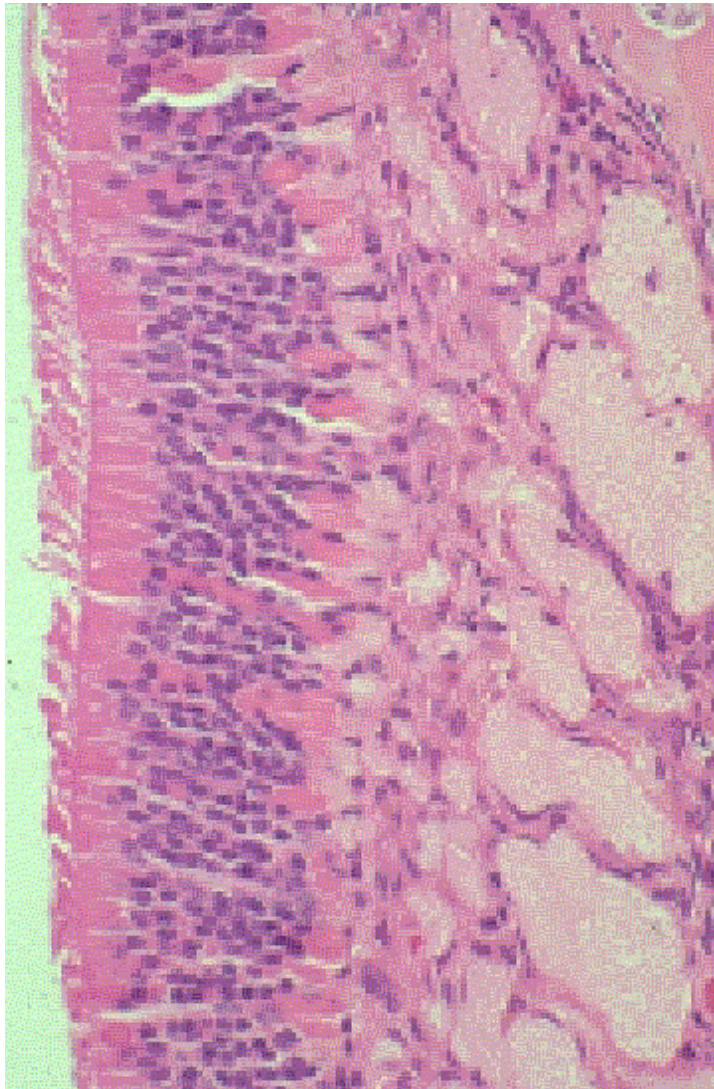
Eosinophilic globules in
the olfactory epithelium
(ethmoid turbinates)

- Rat

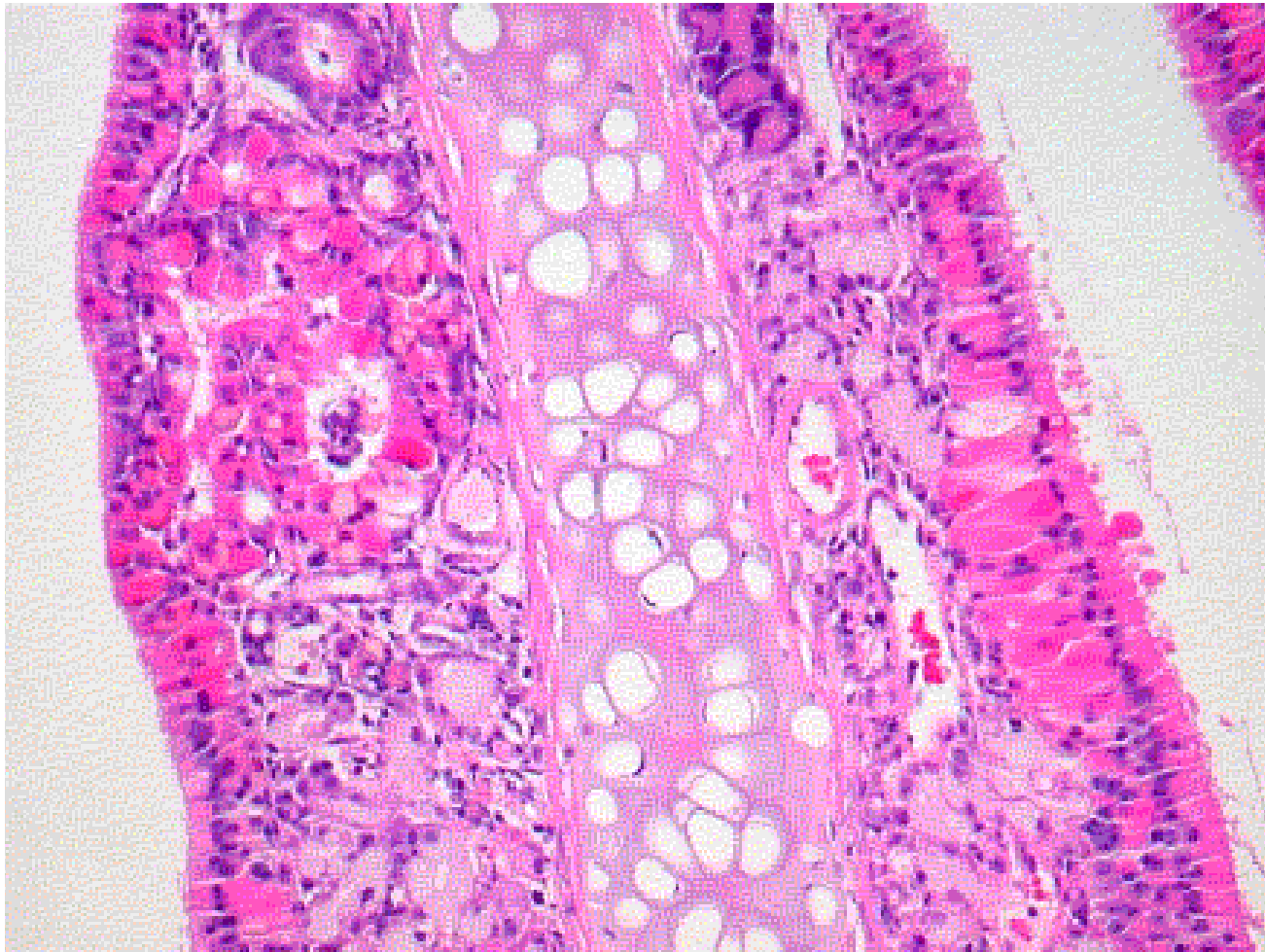




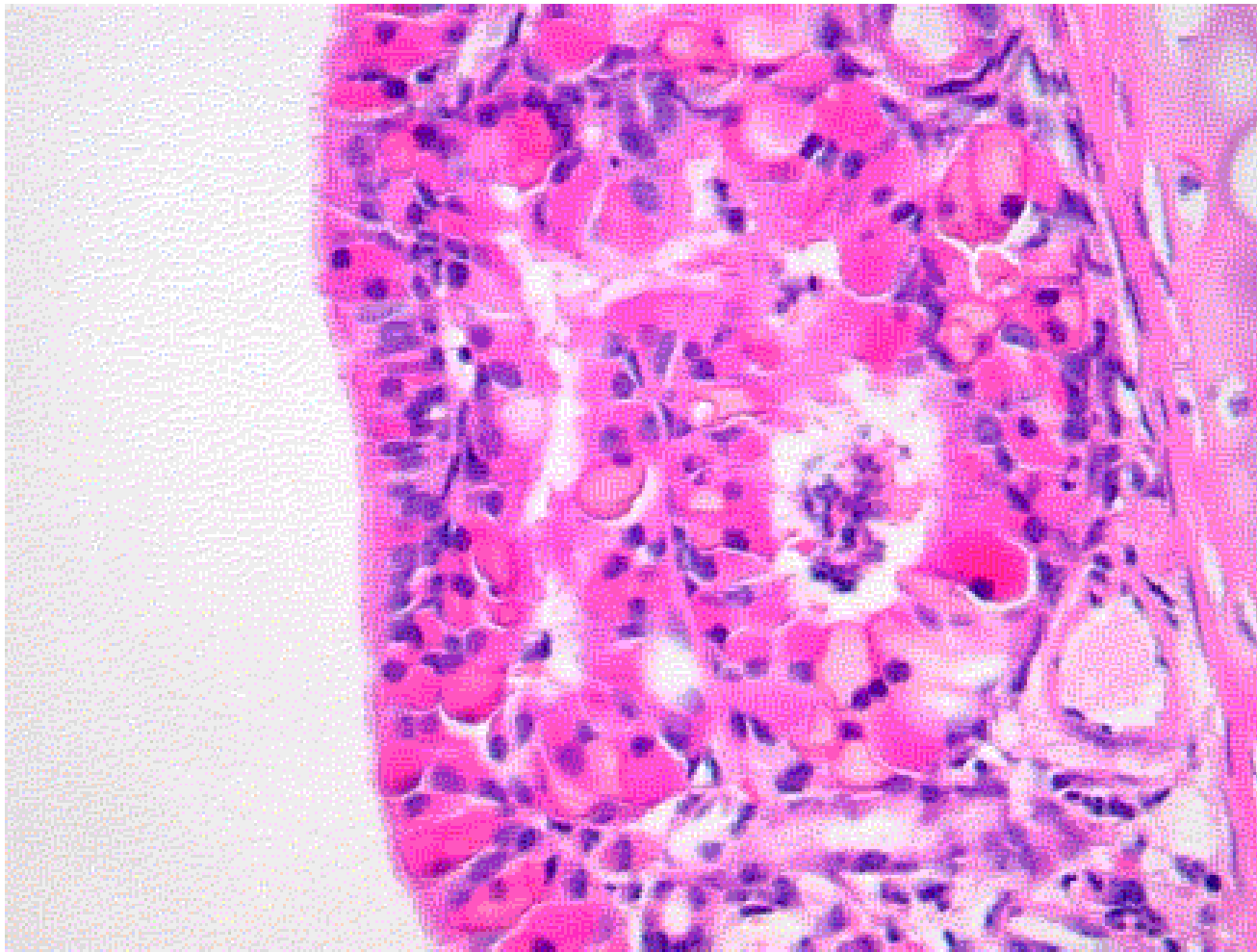
Eosinophilic
globules in the
olfactory epithelium
- Rat



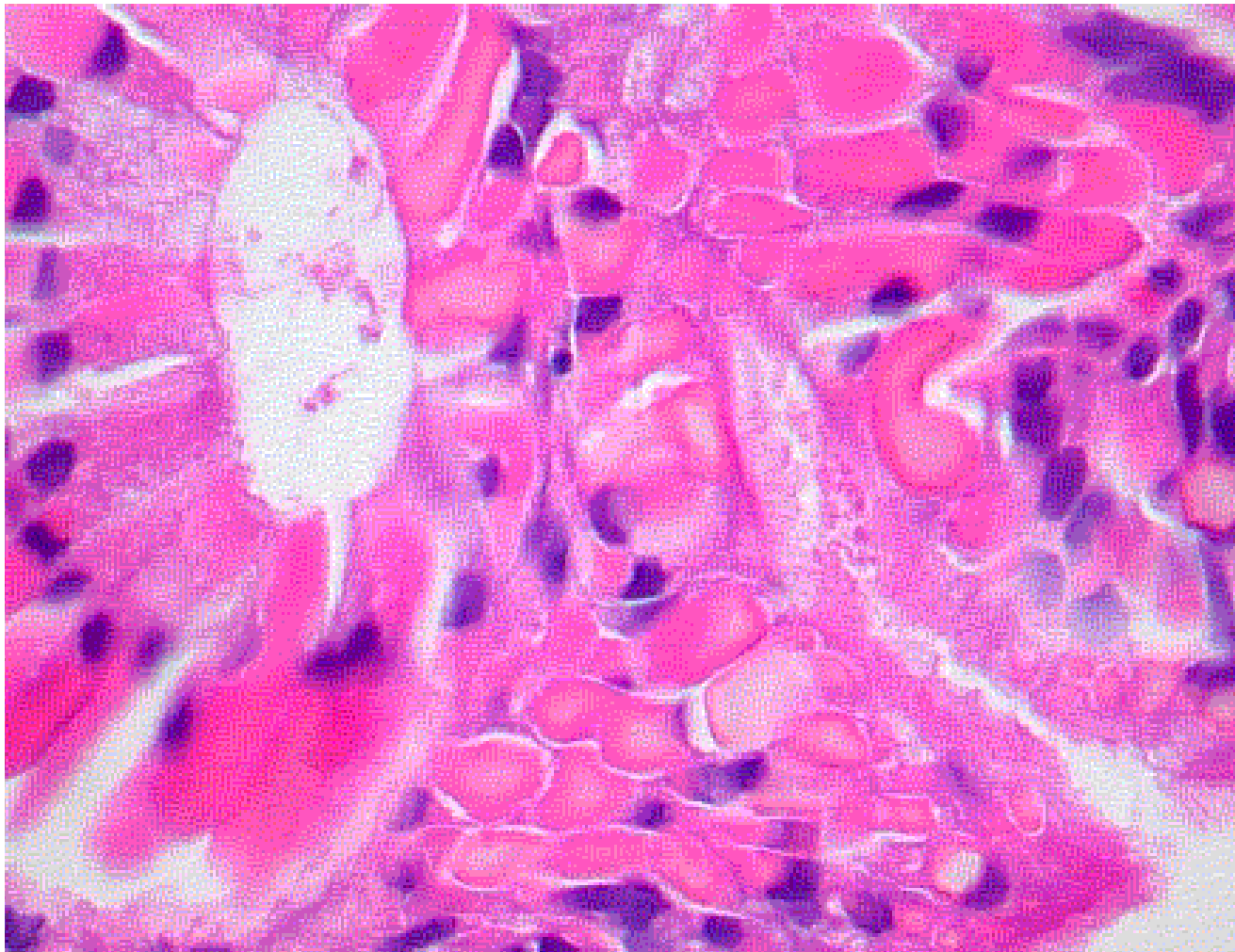
Eosinophilic
globules in the
olfactory epithelium
- Rat



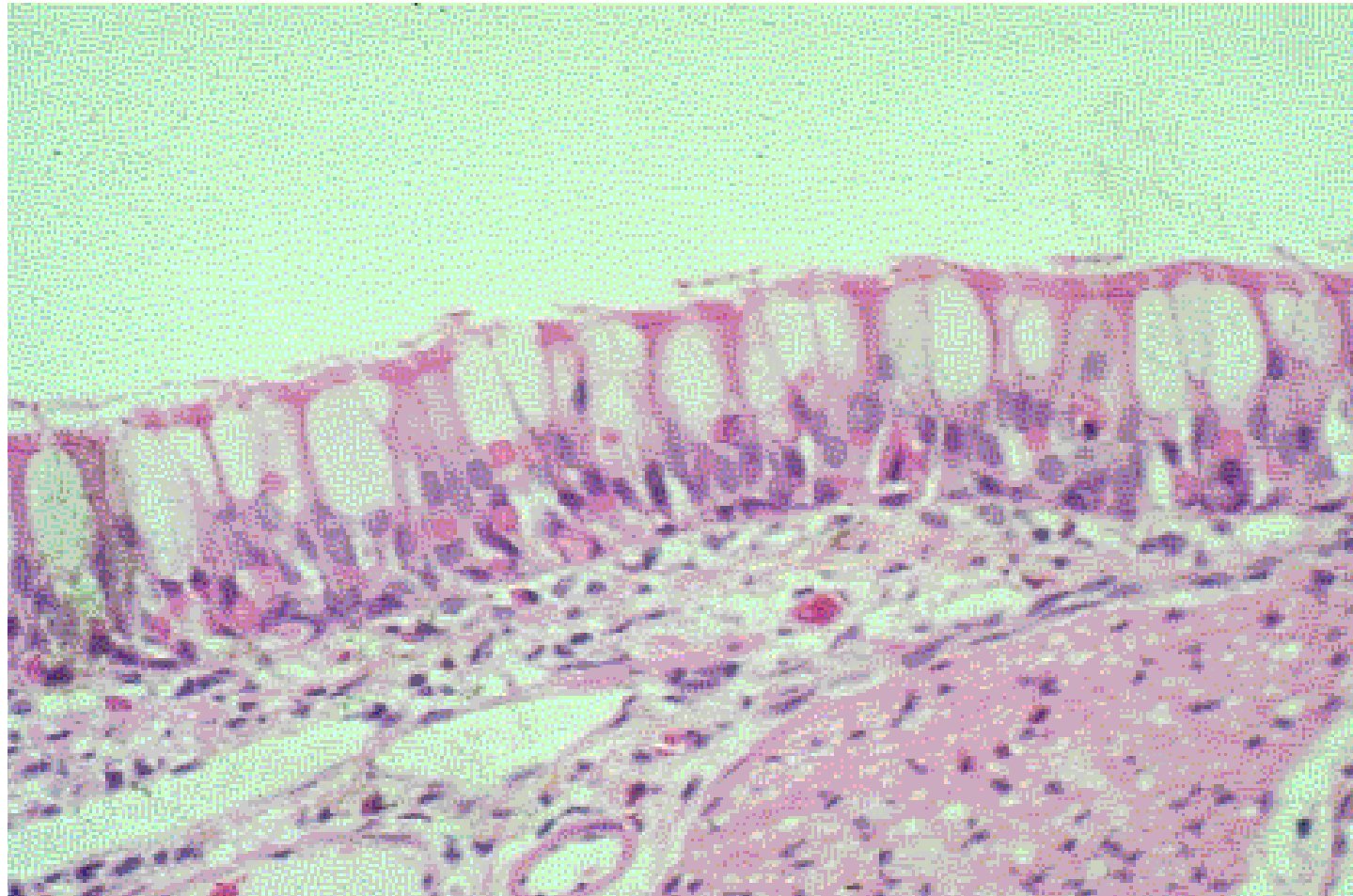
Eosinophilic globules in seromucous glands and respiratory epithelium - Mouse



Eosinophilic globules in seromucous glands and respiratory epithelium - Mouse



Eosinophilic globules in seromucous glands - Rat



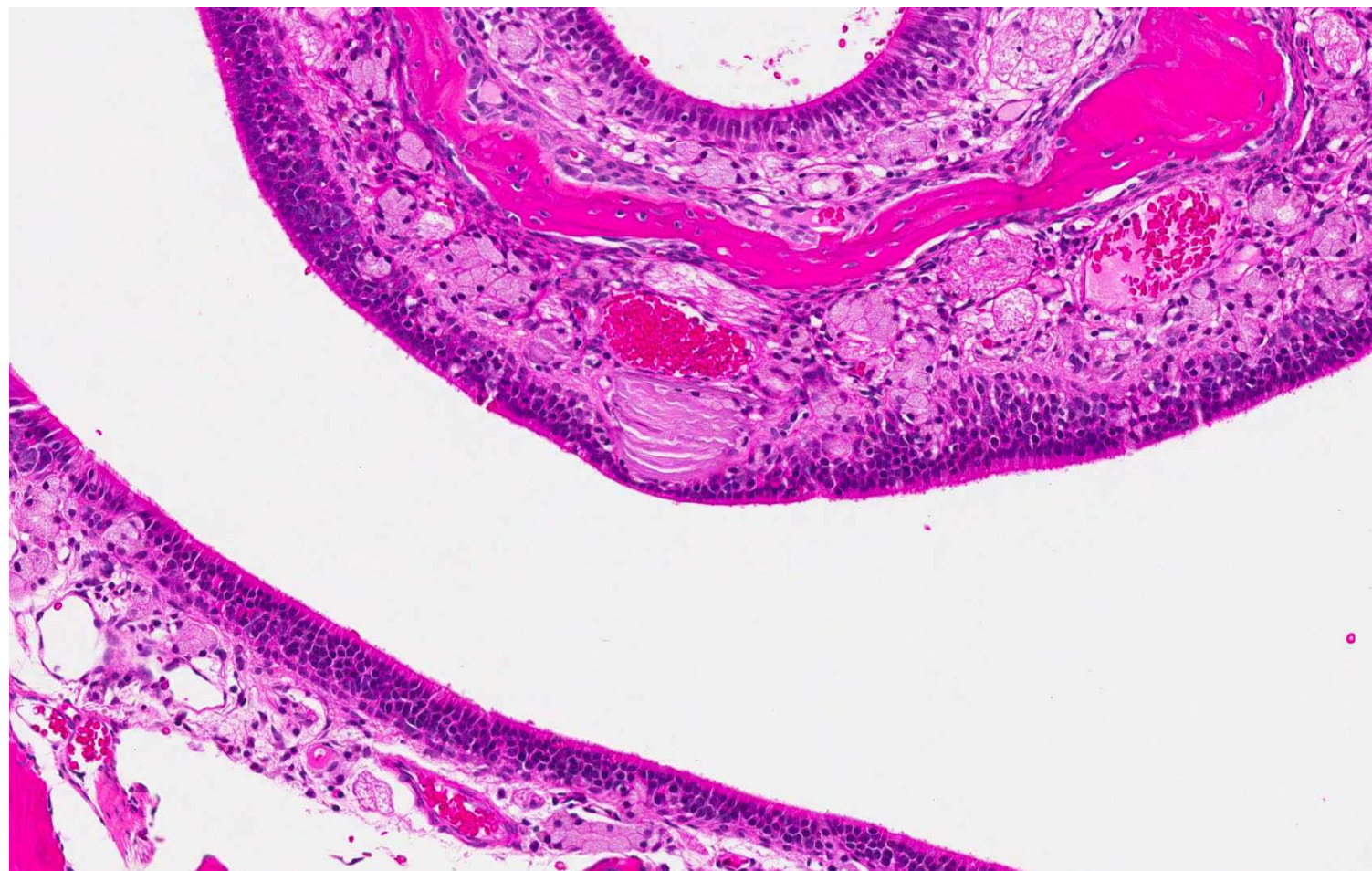
Eosinophilic globules in the respiratory epithelium-
Rat

Corpora Amylacea

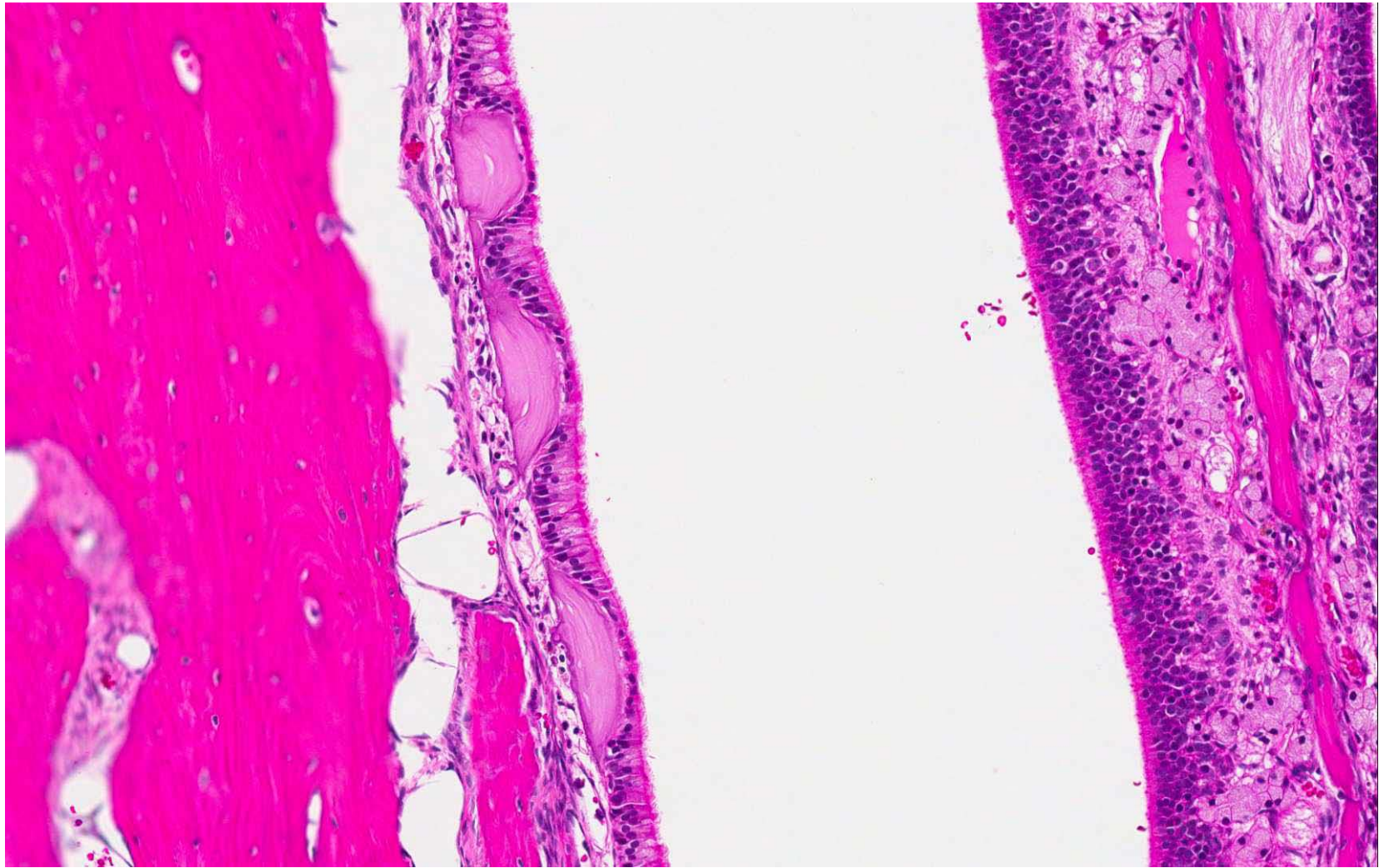
- Cell of origin: Olfactory or respiratory epithelium and adjacent lamina propria
- Diagnostic features
 - Small basophilic or amphophilic concretions
 - Often laminar with mineralized areas
- Often occurs spontaneously in rats and mice



Rat: Corpora amylacea



Rat: Corpora amylacea (olfactory epithelium)

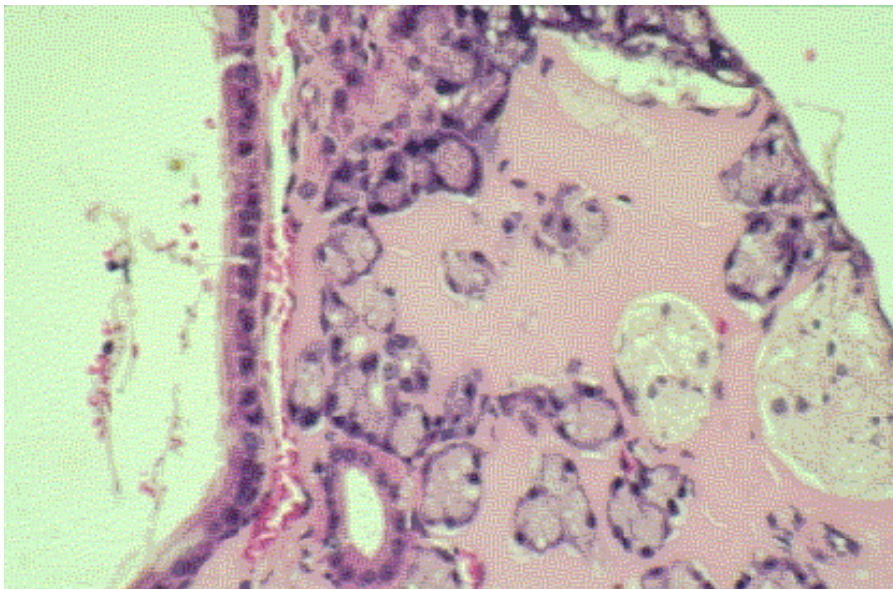
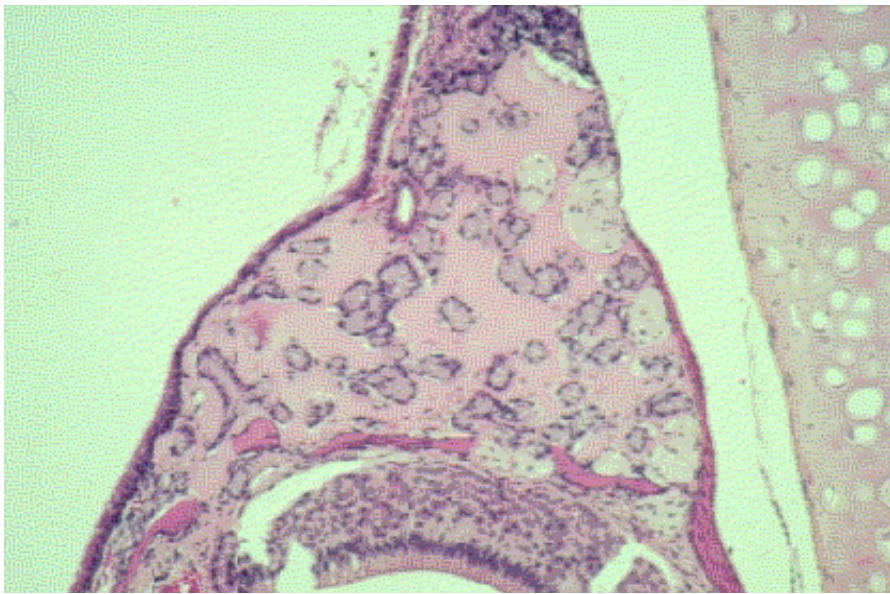


Rat: Corpora amylacea (respiratory epithelium)

Amyloidosis (Eosinophilic Substance)

- Present in ventral nasal septum of aging mice (only species affected)
- Has been recorded in textbooks as amyloid
- Recently, has been described as “eosinophilic substance” that reacts negatively to Congo Red stain with no nonbranching fibrils at EM. Results suggest that the eosinophilic substance deposition may be a physiologic phenomenon linked to the vomeronasal organ located in the vicinity (T.Do, 2010)

Diagnostic features: lightly eosinophilic amorphous extra cellular material in submucosa; may stain positively with Congo Red.



Amyloidosis (or
eosinophilic
substance)
of the nasal
septum
- Mouse

Necrosis

Cell of origin: all cell types

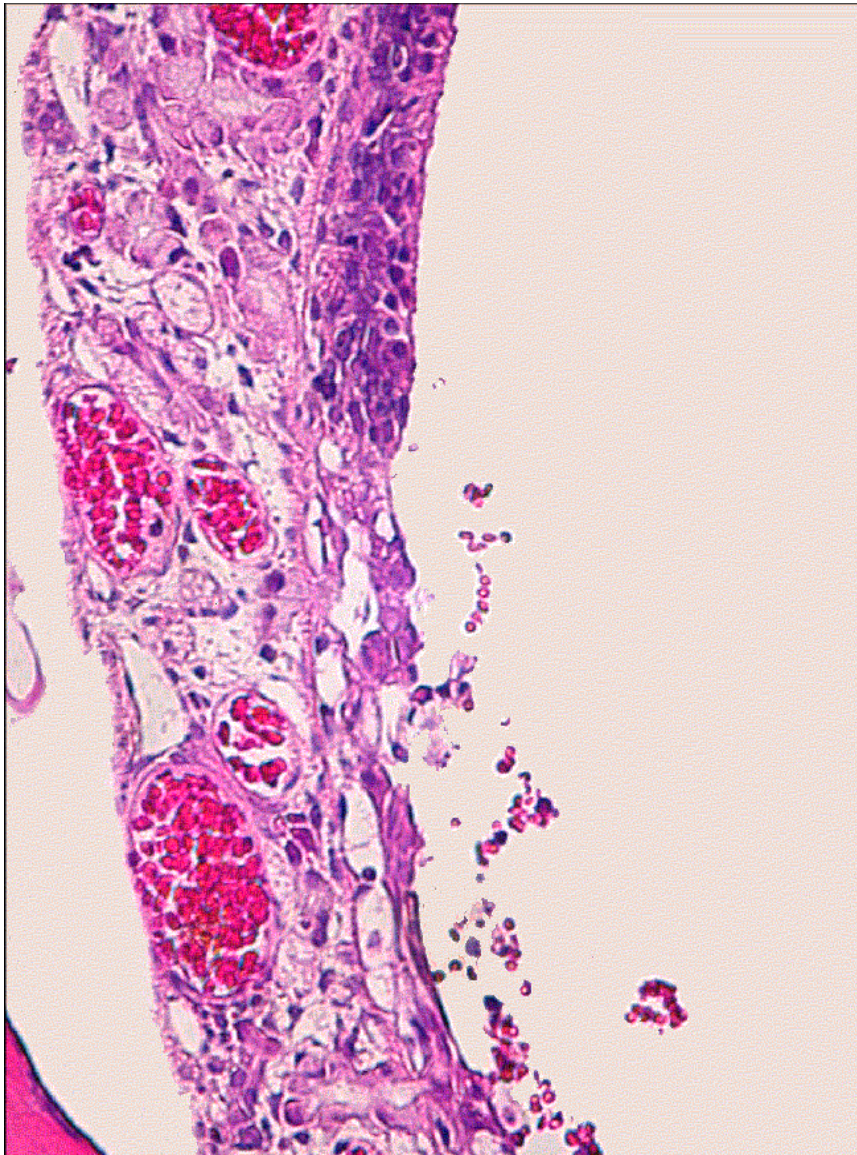
Diagnostic features

- Pyknosis or karyorrhexis of nuclei
- Cytoplasmic eosinophilia
- Cellular swelling or shrinkage
- Exfoliation of cells
- May result in erosion or ulceration
- May be associated with inflammation
- In olfactory epithelium, a frequent sequela is atrophy of nerve bundles within lamina propria

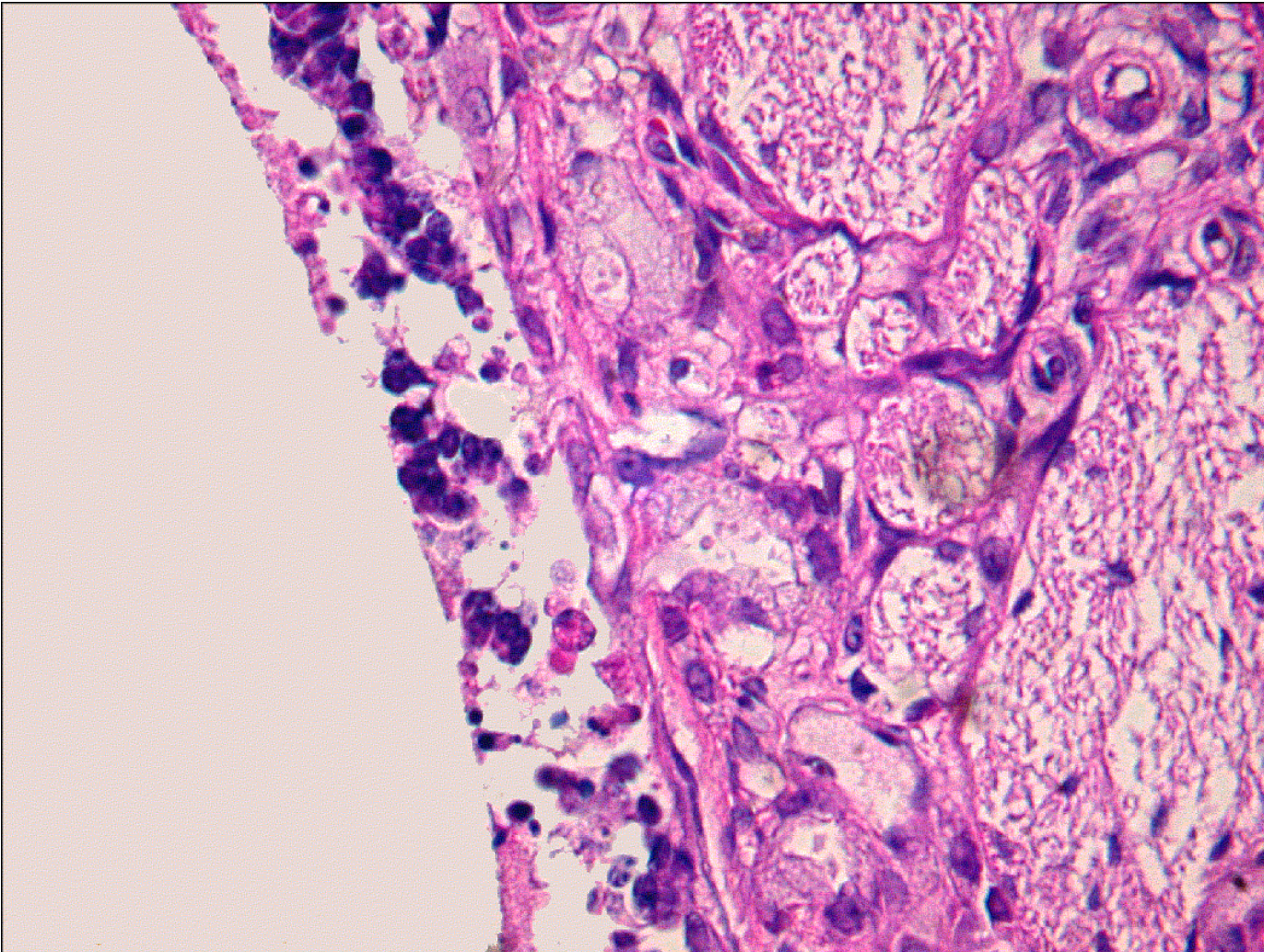
Usually drug-related



Necrosis
of the
olfactory
epithelium
- Rat



Necrosis
of the
olfactory
epithelium
- Rat



Necrosis of the olfactory epithelium - Rat

Regeneration

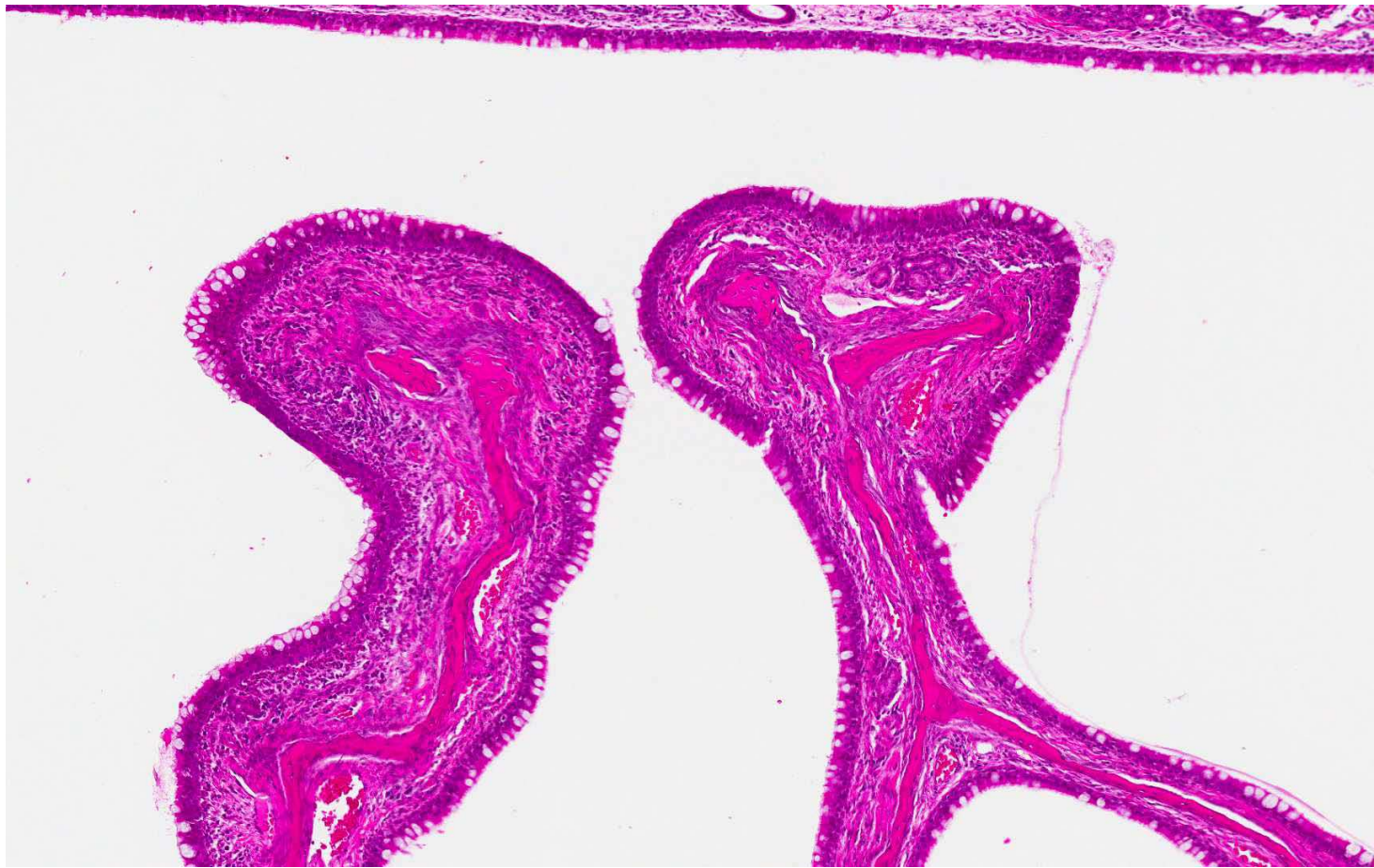
Cell of origin: all cell types

Diagnostic features

- Normal appearing epithelial cells with basophilic cytoplasm
- Increased nuclear: cytoplasmic ratio
- Epithelial architecture may remain irregular
- Adjacent to or within areas of degenerating, necrotic, hyperplastic or metaplastic epithelium

Definitions: Regeneration is a term indicating the growth of cells and tissues to replace lost or damaged structures, as opposed to hyperplasia, a term denoting an increase in the number of cells beyond normal in a tissue.

Usually drug-related



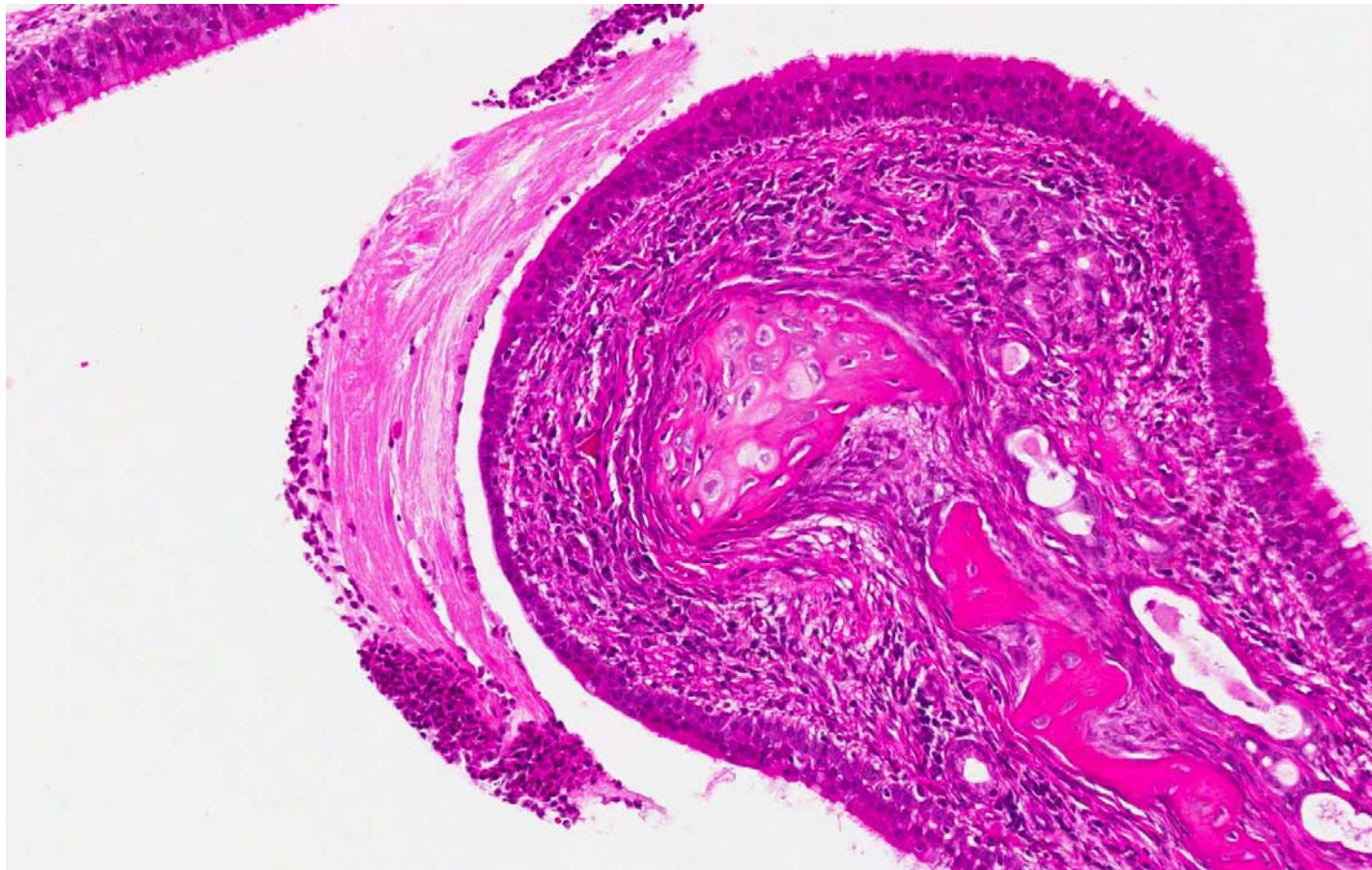
Dog: Degeneration/regeneration, respiratory epithelium, nasal turbinates



Dog: Degeneration/regeneration; respiratory epithelium, nasal turbinates



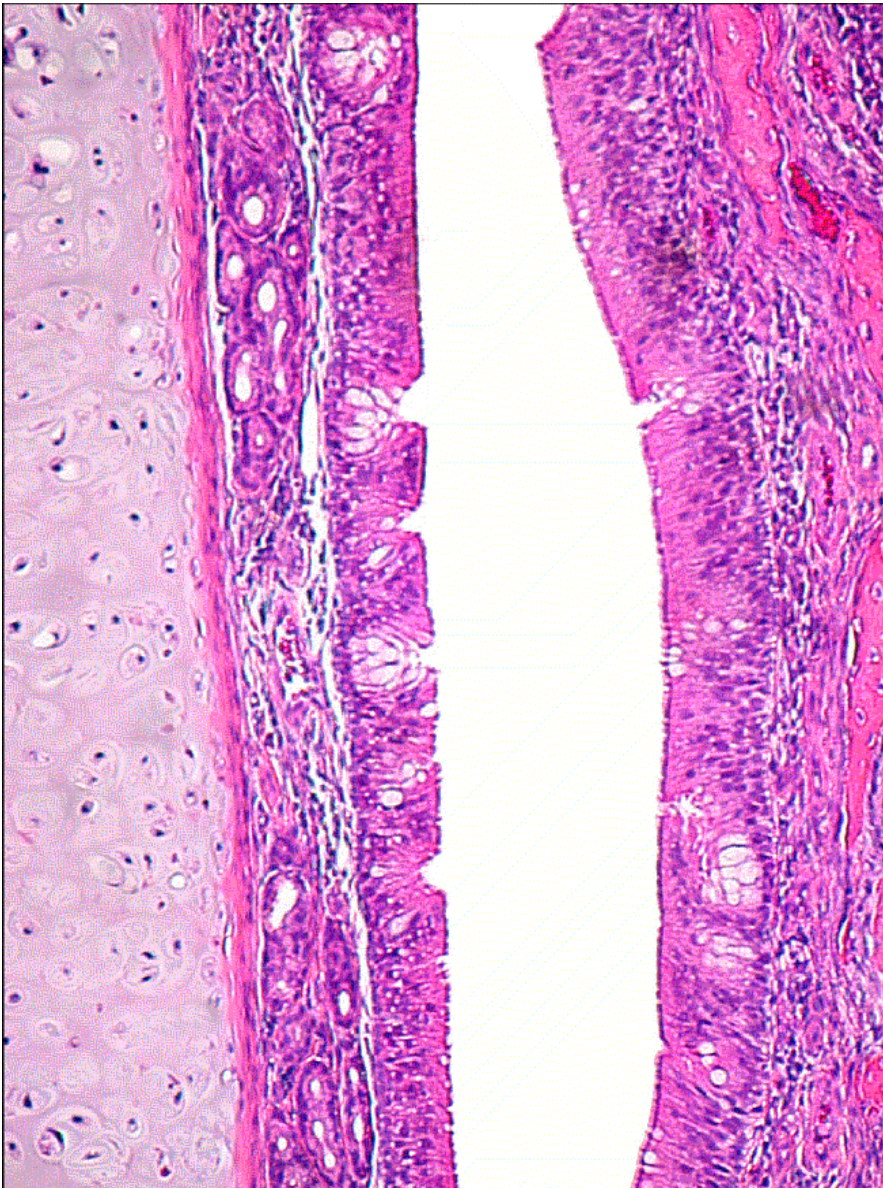
Dog: Degeneration/regeneration, respiratory epithelium, nasal turbinates



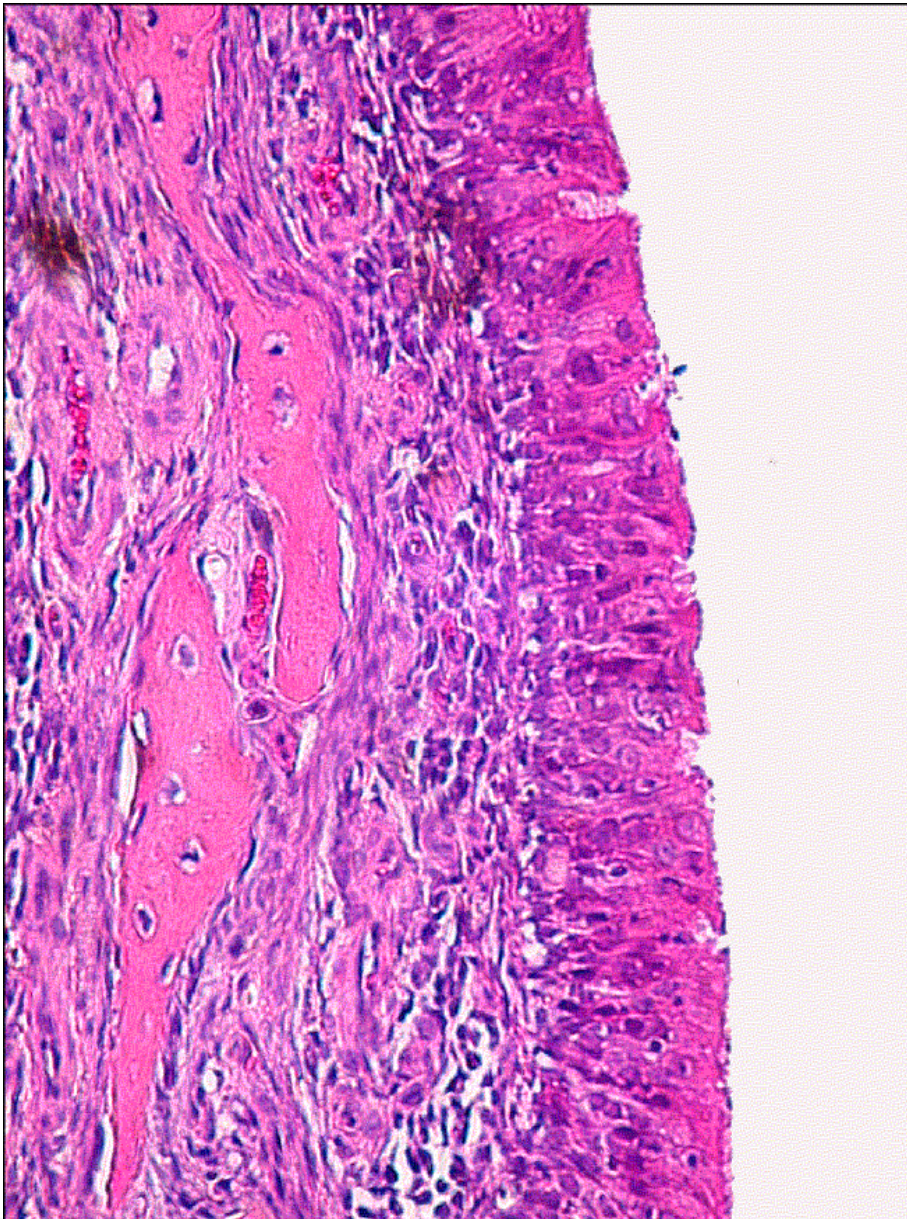
Dog: Degeneration/regeneration luminal exudate,
respiratory epithelium, nasal turbinates

Inflammation

- Various causes have been identified:
 - Infections
 - Chemicals
 - Foreign body
- In large animals (dogs, monkeys), a minimal degree of inflammation is often seen, specially in rostral sections of the nasal cavities. Needs to be differentiated from chemical – induced lesions.

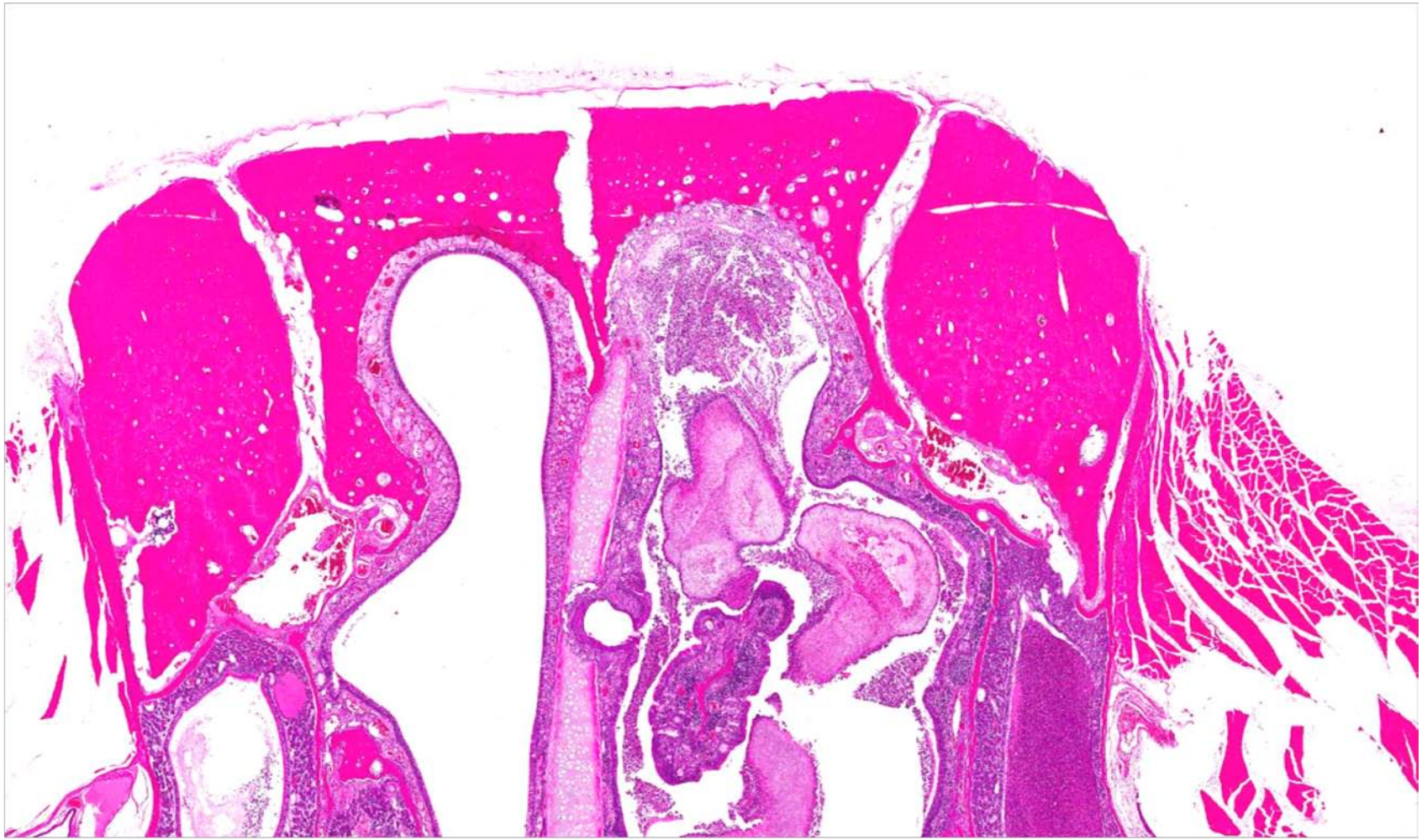


Minimal subacute
inflammation of
spontaneous
origin in the dog

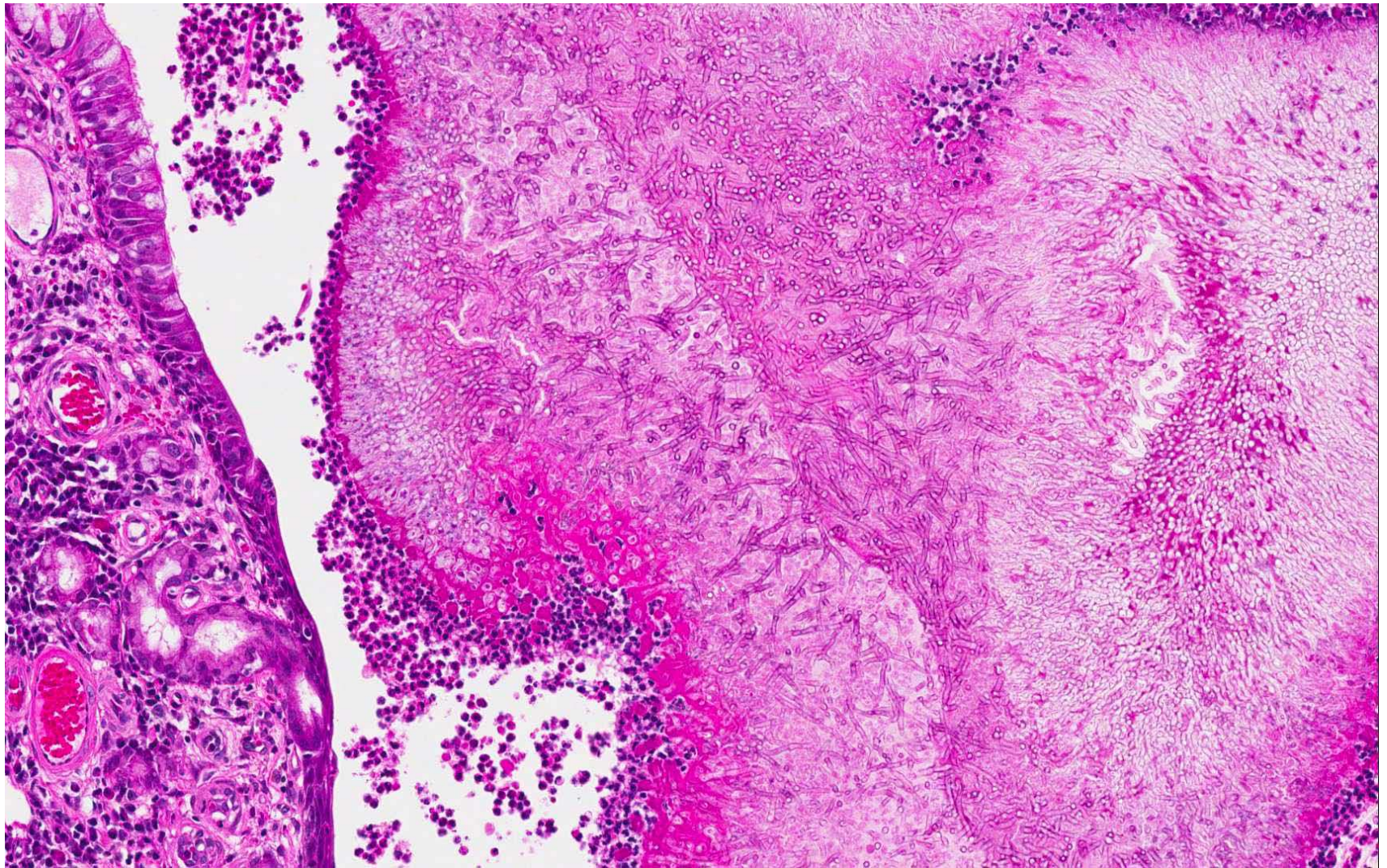


Inflammation

- Dog



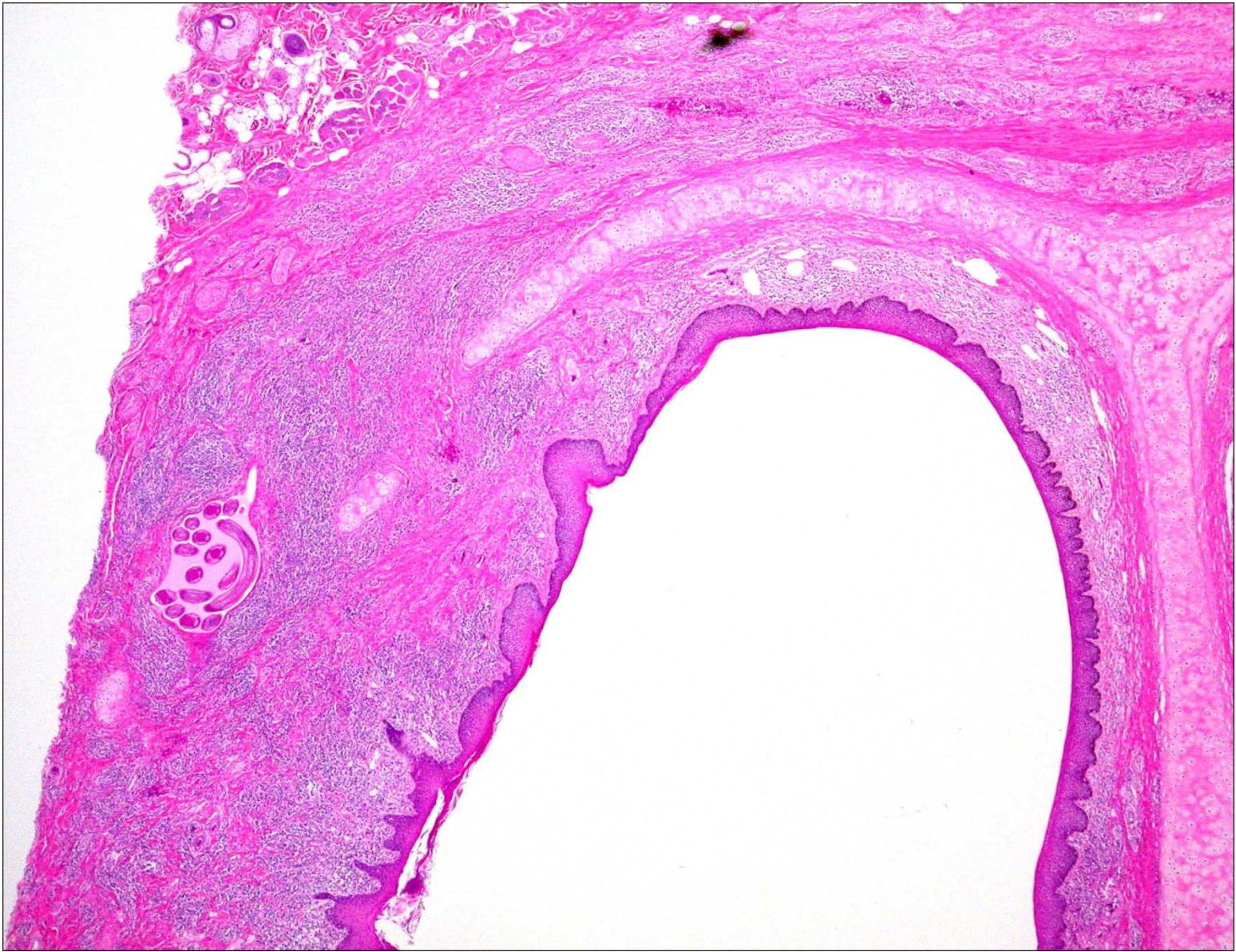
Rat: Inflammation with fungus

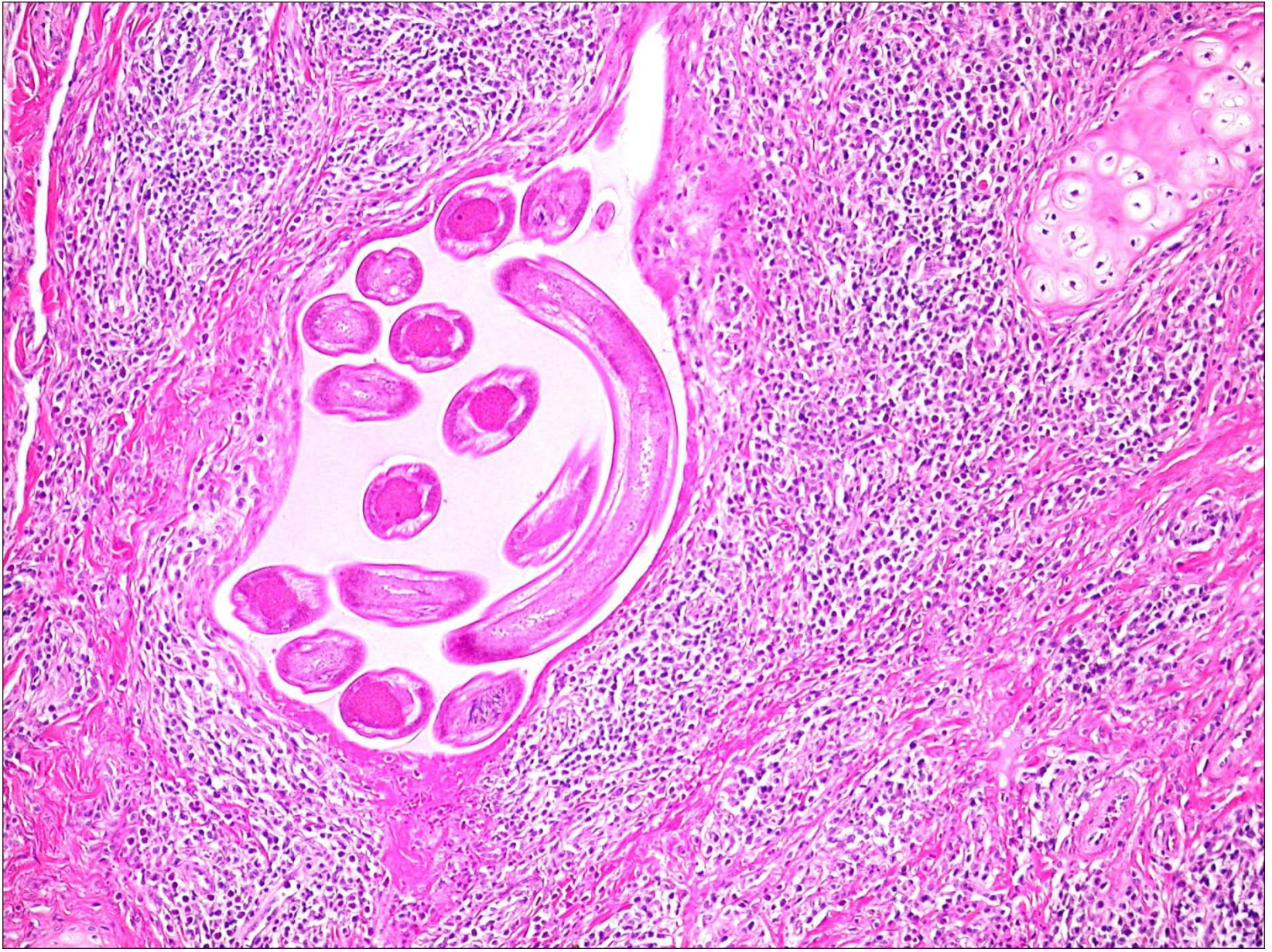


Rat: Inflammation with fungus



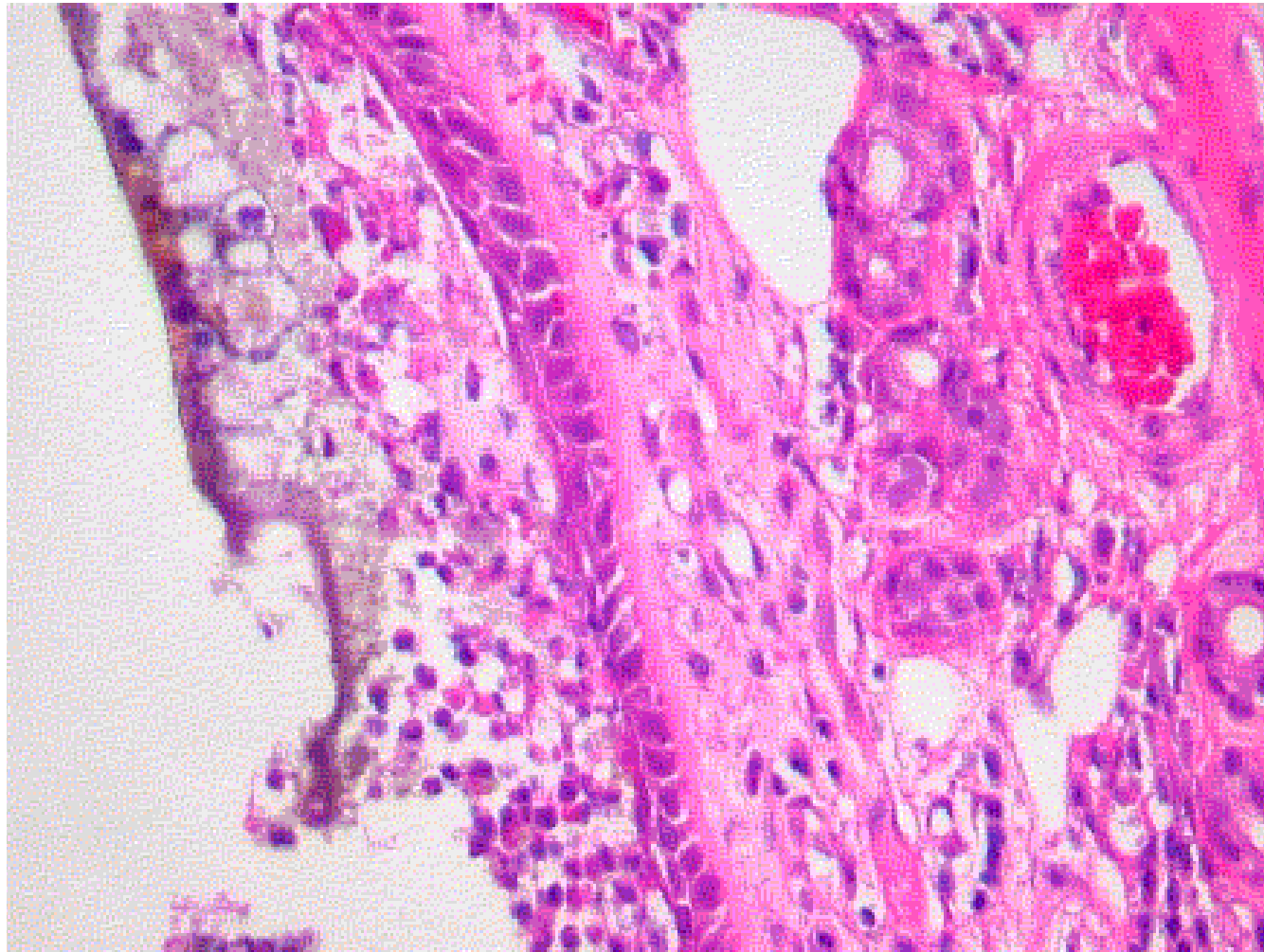
Monkey: Severe inflammation with parasite





Nasal parasites in monkeys

- Etiology: *Anatrichosoma* ssp
- Parasite can be seen in mucosa/submucosa
- Can induce subacute to chronic inflammation which may mask changes related to compound administration
- Difficult to diagnose clinically. Usually noted only at histopathology



Monkey: Inflammation with foreign material
(test article) in nasal lumen

Metaplasia: Squamous Cell

Diagnostic features

- Characterized by replacement of transitional, respiratory or olfactory or ductal epithelium by squamous epithelium
- Keratinization might be seen in more severe cases

Differential diagnosis

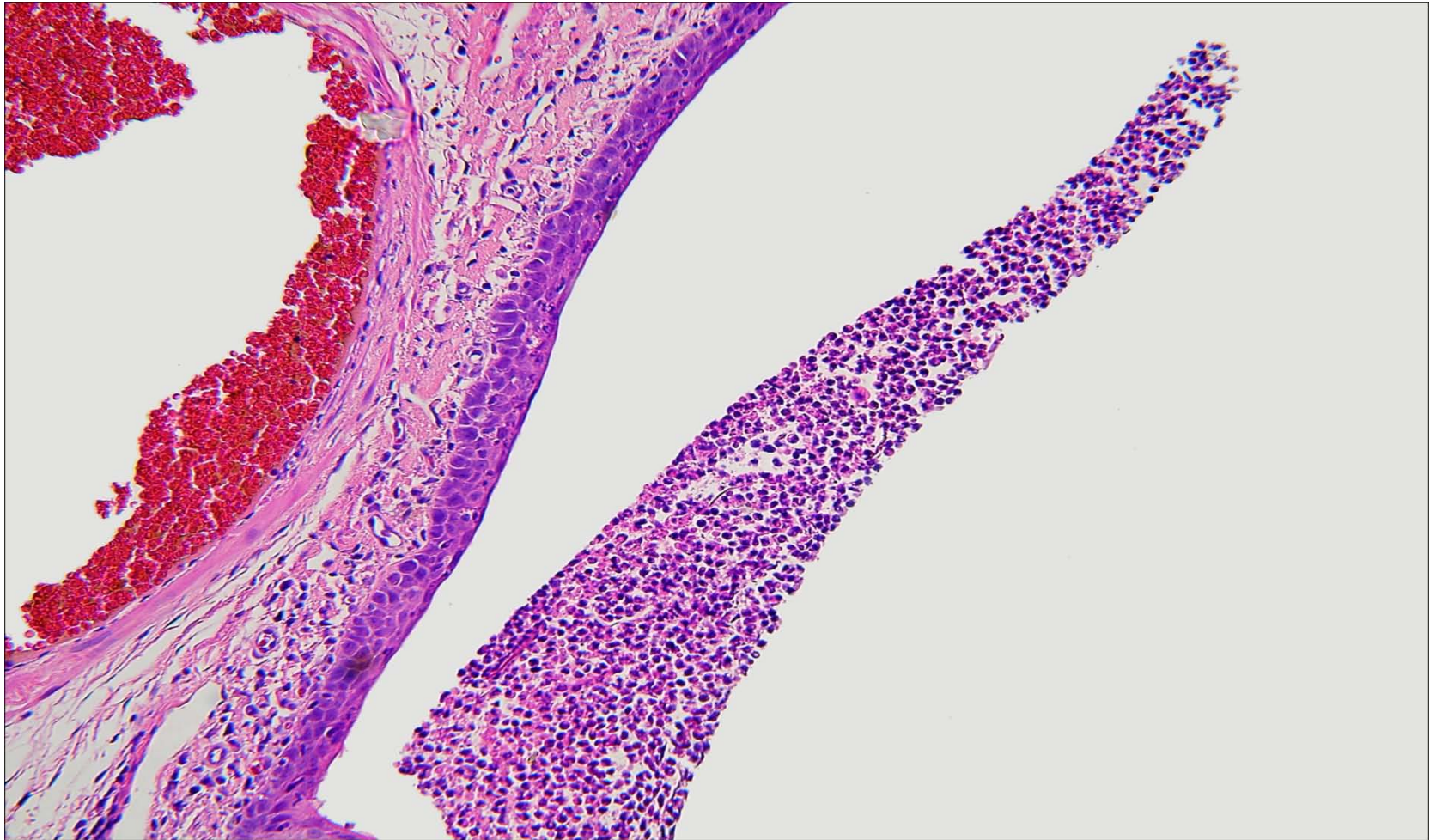
- Normal squamous epithelium. Location of change is critical
- Regeneration: usually follows acute injury (degeneration and/or necrosis); cells are one or possible two layers thick with increased basophilia with no horizontal layering of flattened cells as in squamous metaplasia

Comment:

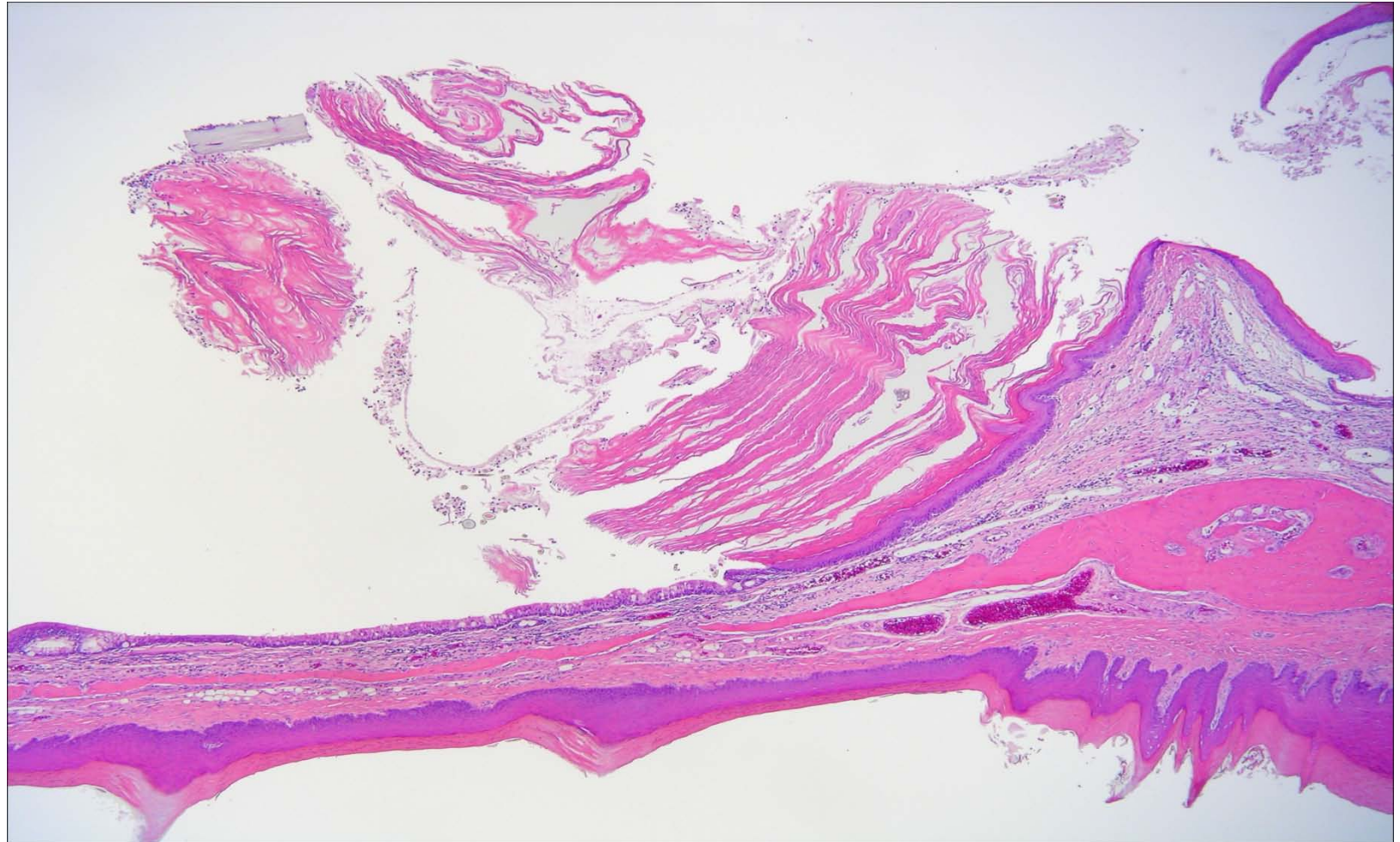
- Often occurs with inflammation
- Usually reversible



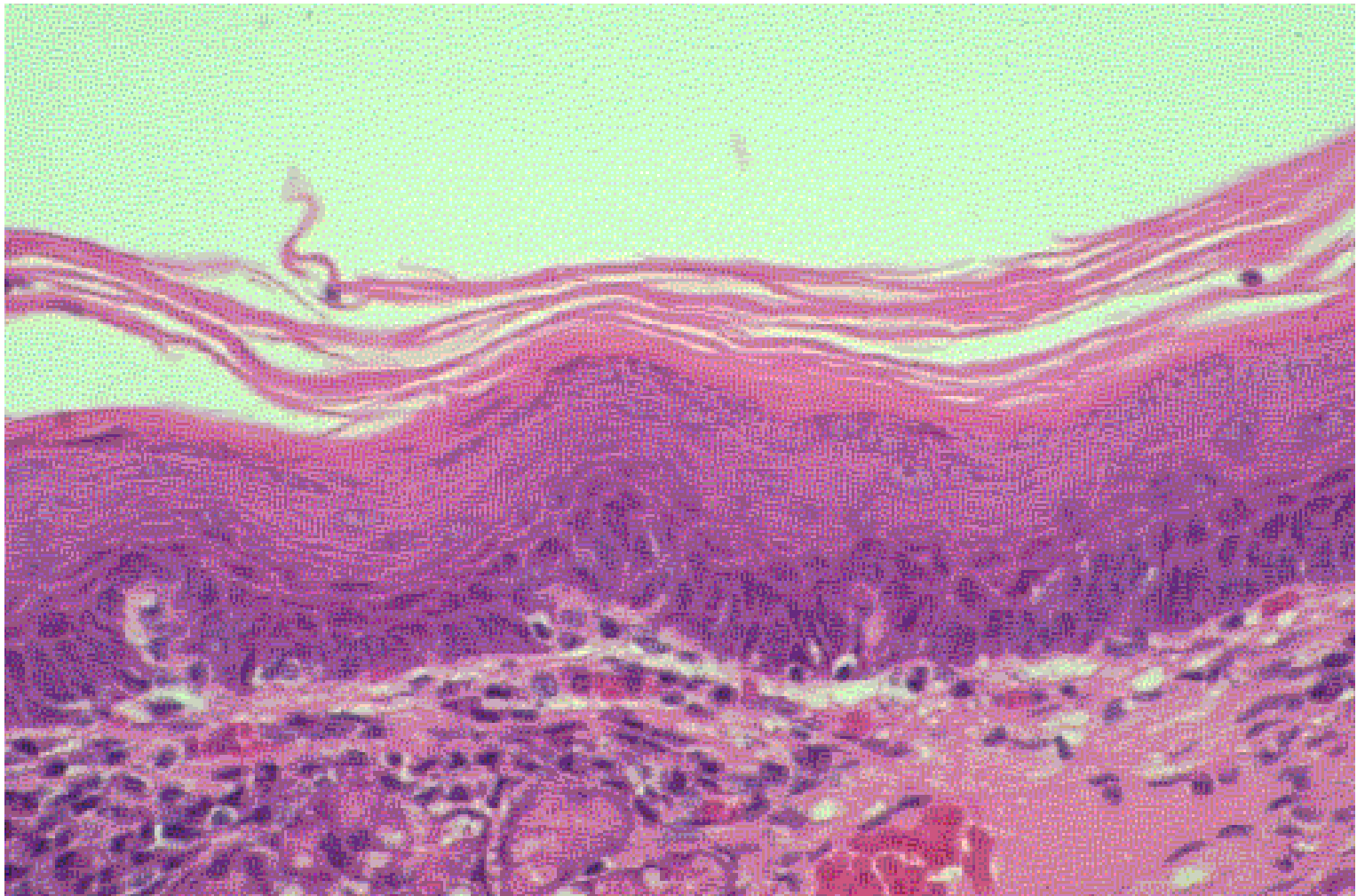
➡ Rat: Metaplasia, squamous cell, respiratory epithelium



Rat: Metaplasia, squamous cell with exudate in lumen (inflammation)



**Rat: Metaplasia, squamous cell with
keratinization, nasopharynx**



Hyperplasia/Metaplasia, Mucous Cell

Synonyms: Hyperplasia, goblet cell; metaplasia, mucous cell;
metaplasia, goblet cell

Diagnostic features

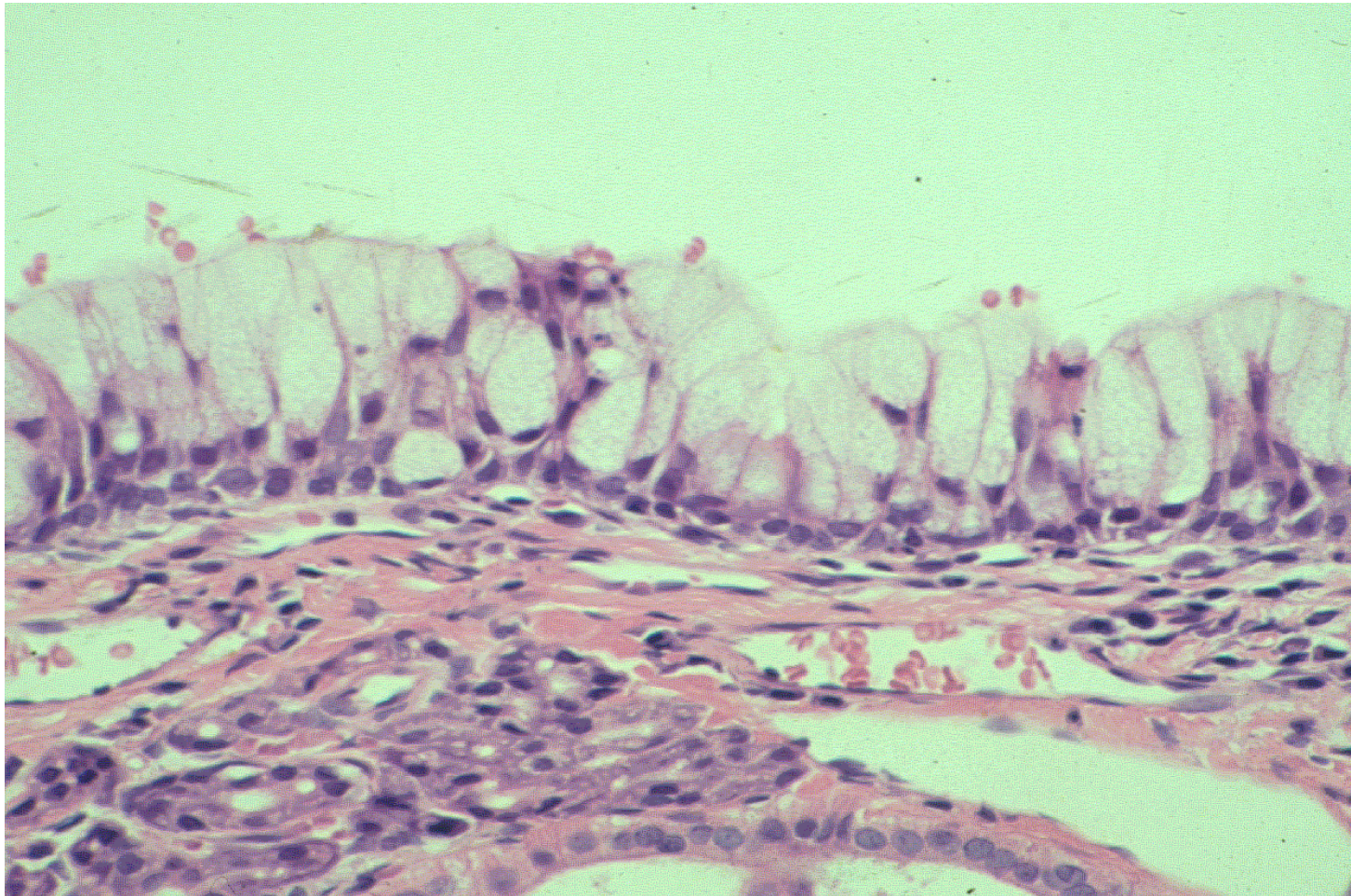
- Increased numbers of mucous cells in transitional and/or respiratory epithelium
- Metaplasia and hyperplasia are distinguished by anatomic location; significant numbers of mucous cells are not normally present in transitional epithelial zone of rodent nasal mucosa.
- Hyperplastic mucous cells in the surface epithelium may form intra epithelial glands.

Comments: Mucous cell metaplasia and hyperplasia is a very frequent change in the rostral nasal cavity in response to repeated inhalation of irritants.

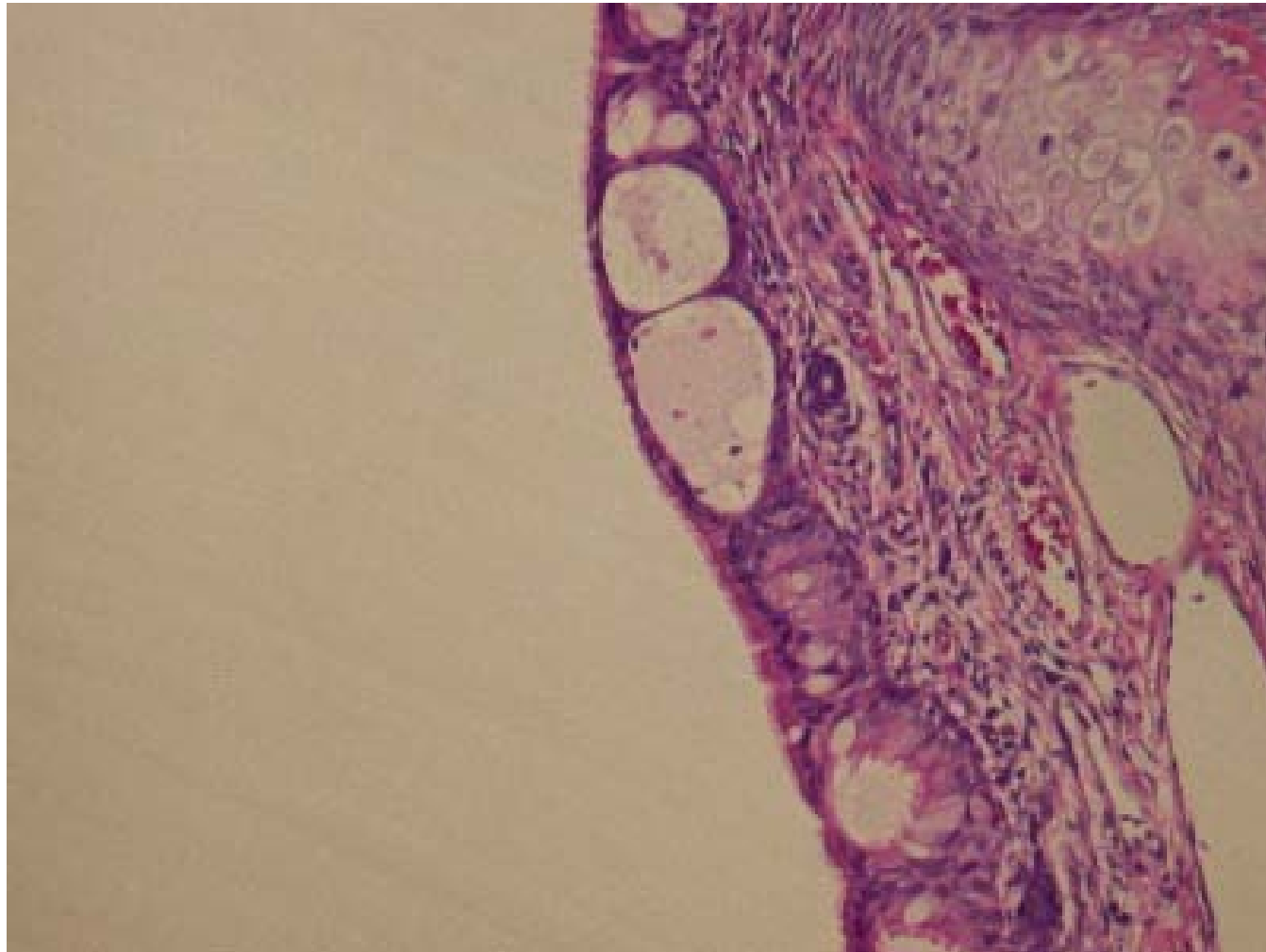


→ Nasal
septum

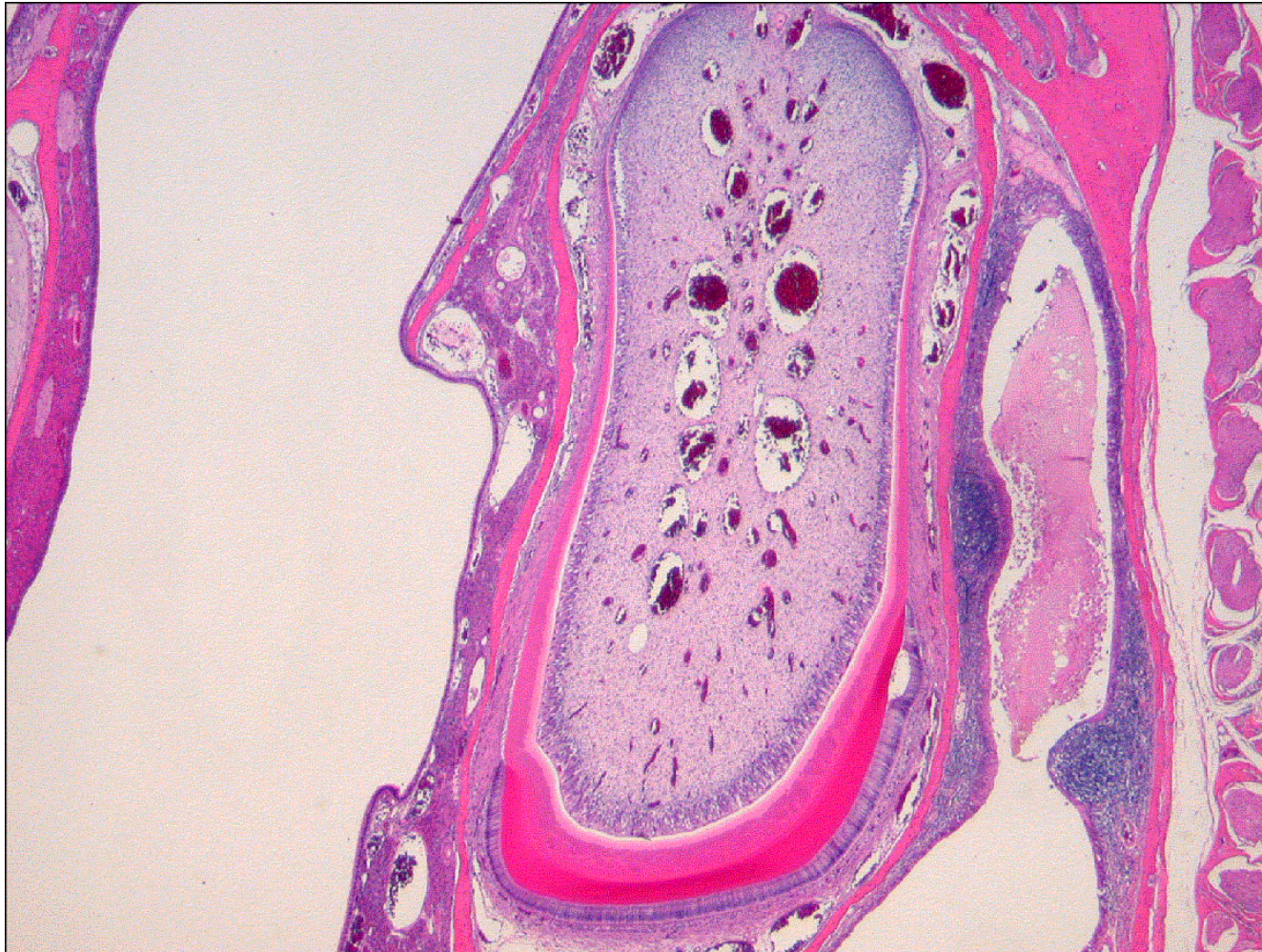
Rat: Hyperplasia, mucosa cell, respiratory
epithelium, unilateral



Rat: Hyperplasia, mucous cell, respiratory epithelium



Dog: Hyperplasia, mucous cell, respiratory epithelium with submucosal inflammation



Normal incisor tooth - Rat



Incisor teeth

- Degeneration /atrophy of the ameloblasts
- Degeneration /atrophy of the odontoblasts
- Abnormal dentin formation
- Rat



Normal incisor tooth – Rat



Incisor teeth

- ★ Enamel
- Atrophy of ameloblasts
- Abnormal dentin formation
- Degeneration of odontoblasts

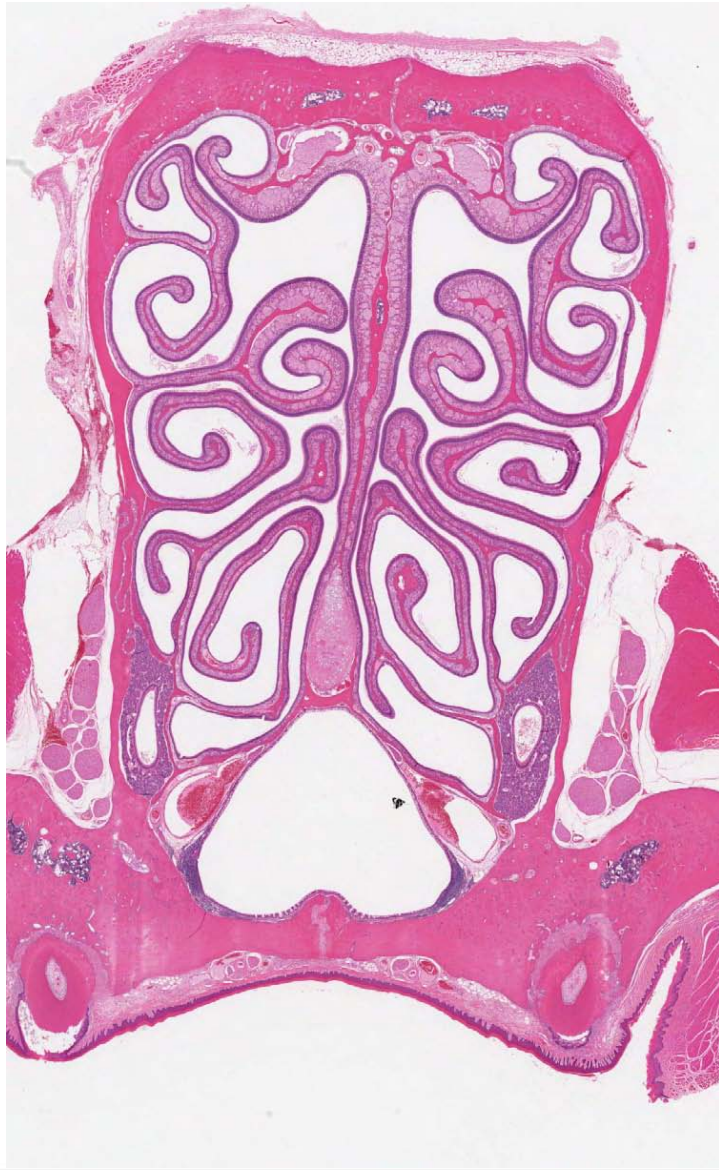
- Rat

Induction of nasal lesions due to reflux of gavage material in rats

Test article causes delayed gastric emptying thus preventing compound from entering the stomach.

Lesions are most severe in nasopharyngeal ducts and caudal portions of the nasal passages. Both respiratory and olfactory epithelia can be affected. Typically, lesions are inflammatory and degenerative.

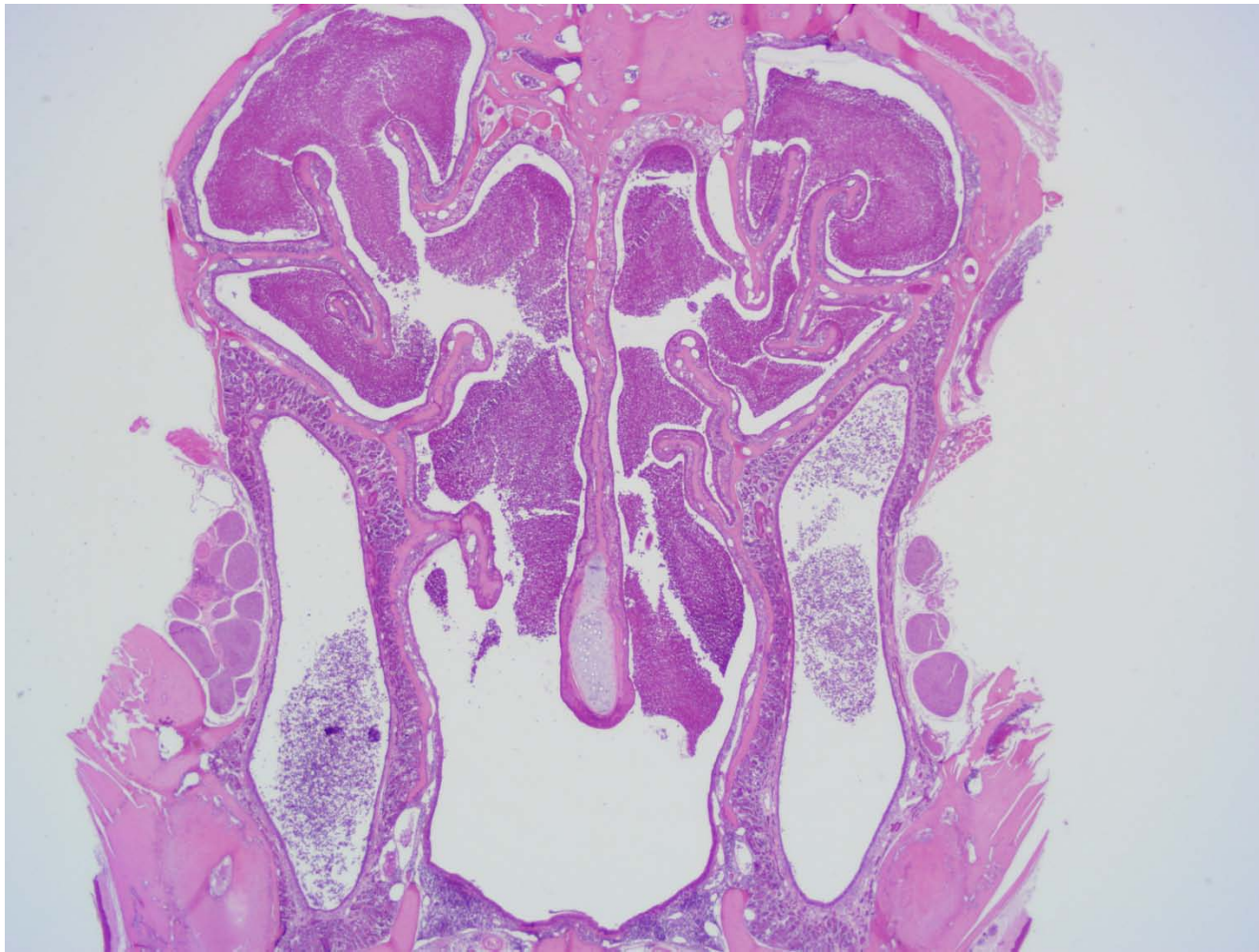
Reflux should be considered as a possible cause of nasal lesions in oral gavage studies.



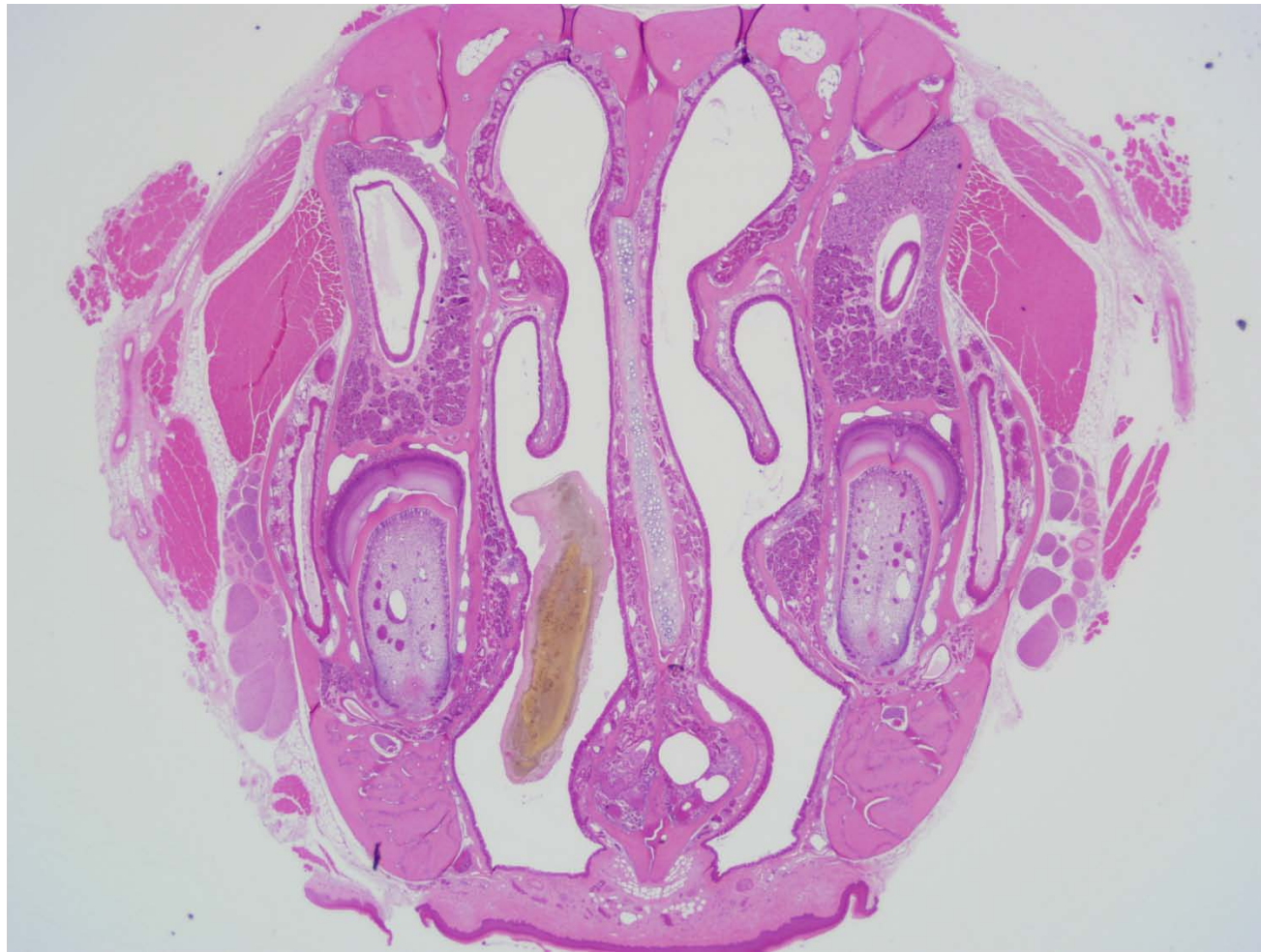
Rat: Normal (Level 3)



Rat: Acute
inflammatory
lesions (Level 3)
due to reflux



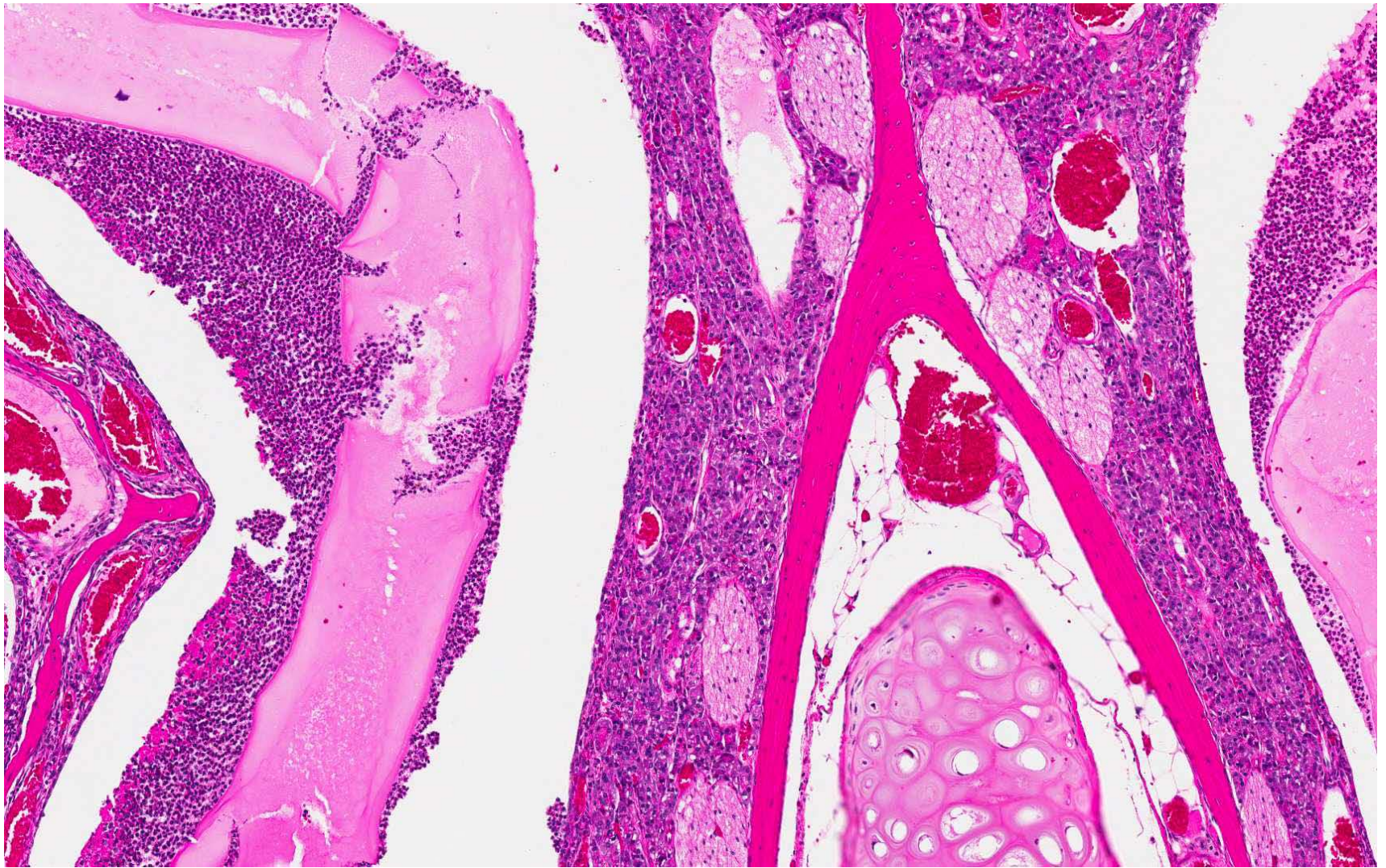
Rat: Inflammatory lesions, severe
N.B.: With approval of NTP



Rat: Inflammation with foreign material
N.B.: With approval of NTP



Rat: Inflammation with foreign material



REFERENCES

1. Doi T. et al. New Findings Concerning Eosinophilic Substance Deposition in Mouse Nasal Septum: Sex Difference and no Increase in Seniles, *Tox. Pathol.* (2010), 38, 631-636.
2. Harkema, J.R. Comparative Aspects of Nasal Airway Anatomy: Relevance to Inhalation Toxicology. *Tox. Pathol.* (1991), 19, 321-336.
3. Renne, R. (Chair), et al. Proliferative and Nonproliferative Lesions of the Rat and Mouse Respiratory Tract. *Tox. Pathol.* (2009), 37, 5S-37S
4. Young, J.T. Histopathologic Examination of the Rat Nasal Cavity. *Fundam. Appl. Toxicol.* (1981), 1, 309-312.

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- Jerry Hardisty, EPL
- Klaus Weber
- And the pathology staff at Charles River Laboratories