CONTINUING EDUCATION IN TOXICOLOGIC PATHOLOGY
RESPIRATORY AND CARDIOVASCULAR SYSTEM

Fourth Conference

ORGANIZED BY

SOCIETY OF TOXICOLOGIC PATHOLOGY - INDIA (STP-I)

NOVEMBER 1-3, 2012

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Spontaneous and Induced Lesions in the Larynx from Laboratory Animals

Klaus Weber, PhD, DVM, MSBiol

AnaPath GmbH, Switzerland

Literature?

Overviews:

Lewis DJ. Toxicol Pathol. 19: 352-357. 1991
Renne RA and Gideon KM. Toxicol Pathol.34: 281-285. 2006
Renne RA et al., Toxicol Pathol.35:163-169. 2007

Regurgitation Laryngitis:
Ling ZG et al. Ann Otol Rhnol. Laryngol. 116: 471-478. 2007

Important:
Germann PG et al., Toxicol Pathol. 26: 283-289. 1998

Others:
Weber K et.al., J. Toxicol Pathol, 22:229-246. 2009

Studies evaluated

- Inhalation studies in Wistar RccHan™:WIST, CD-1, Beagle, Hamster, Rabbit
- Vehicles: Lactose, Air, Saline, Mg-Stearate
- All lesions noted, most re-examined
- From negative studies, all lesions considered as spontanoues lesions
- From positive studies, only control animals used

Materials and Methods: Example

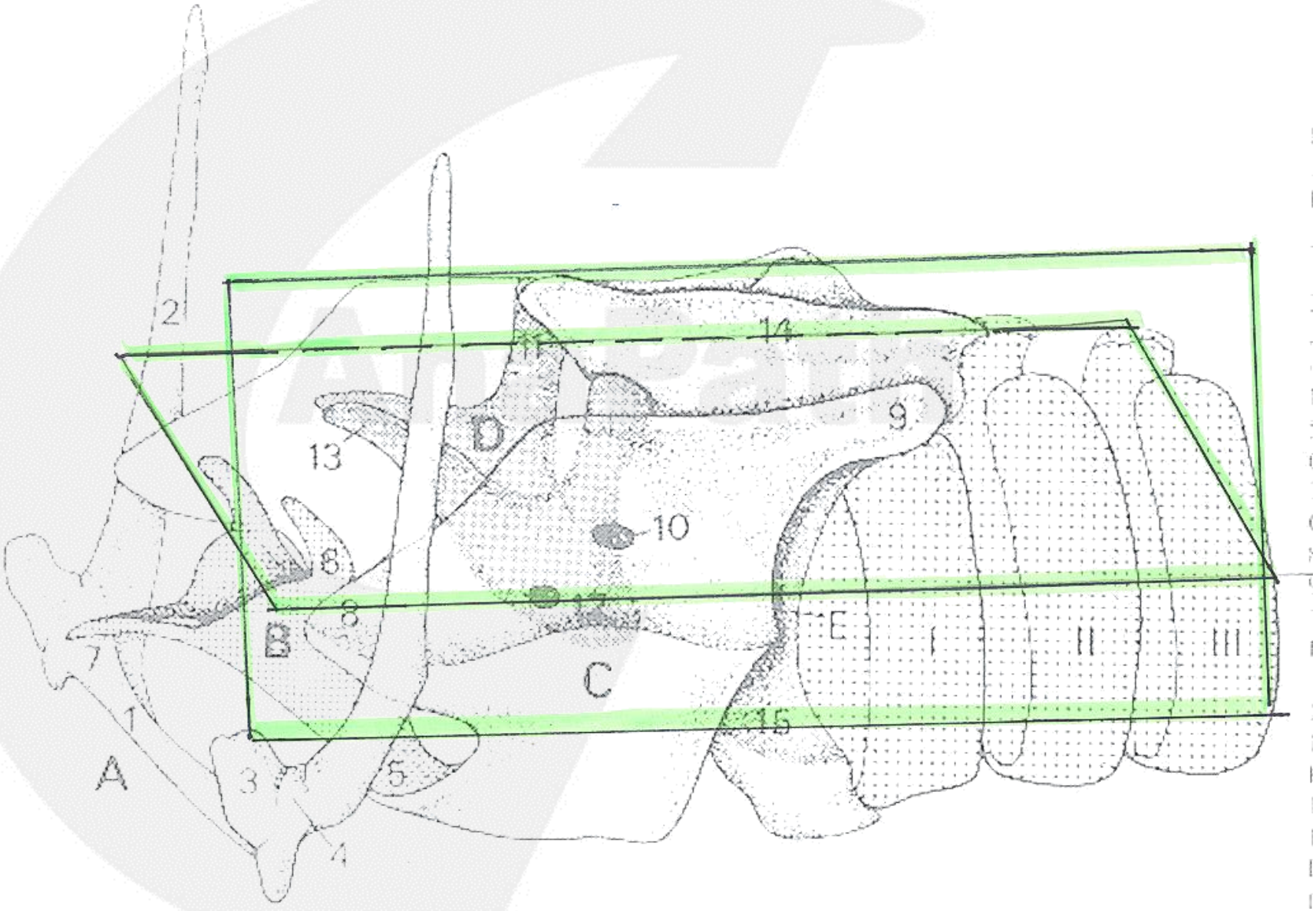
14-Day: Inhalation
*L4: Squamous metaplasia, inflammation at low to high dose; T2: squamous metaplasia at lc to high dose
** No lesion noted in study

Study ID	Vehicle	Number of Main Test Groups examined	Number of Recovery Groups examined	Main Test Animals per Sex	Recover Animals p Sex
L1	Lactose	5	-	10	-
L2**	Lactose	4	-	10	-
L3	Air	4	-	10	-
L4*	Lactose	4	-	5	-
L5**	Lactose	4	-	5	-
L6**	?	5	-	5	-
T1	Placebo Aerosol	5	-	10	-
T2*	Lactose	3	3	10	10
T3	?	4	2	10	5

Materials and Methods: Example

4-Week Inhalation Studies	Male (n=159)				Female (n=159)			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Longitudinal : Squamous metaplasia	0.2	0.5	0	1.4	0	0	0	0
Level 2: Foreign Bodies	0.5	0.9	0	2.5	0	0	0	0
Level 2: Haemorrhage	0.2	0.4	0	1.3	0	0	0	0
Level 2: Glandular dilation	8.8	23.2	0	70.0	6.9	18.2	0	55.0
Level 2: Desiccation	0.9	1.7	0	5.0	0.3	0.8	0	2.5
Level 2: Mineralization	1.6	3.3	0	10.0	0.6	1.7	0	5.0
Level 2: Mononuclear cell foci	3.1	8.3	0	25.0	2.4	4.9	0	15.0
Level 2: Glandular inflammation	0.3	0.8	0	2.5	0	0	0	0
Level 2: Ventral pouch inflammation	0.3	0.9	0	2.7	0.2	0.4	0	1.3
Level 3: Foreign Bodies	0.8	1.6	0	5.0	0	0.0	0	0
Level 3: Glandular .dilation	1.2	3.3	0	10.0	1.2	3.3	0	10.0
Level 3: Desiccation	1.6	3.3	0	10.0	1.2	2.5	0	7.5
Level 3: Mononuclear cell foci	10.1	21.0	0	65.0	12.6	27.6	0	85
Level 3: Glandular inflammation	0.9	1.7	0	5.0	0.2	0.4	0	1.2
Level 3: Granuloma	0.6	1.1	0	2.5	0	0.0	0	0
Level 3: Squamous metaplasia	2.5	6.6	0	20.0	3.1	8.3	0	25.0
Level 4: Foreign Bodies	0.8	1.2	0	2.5	0	0.0	0	0
Level 4: Haemorrhage	0.8	1.2	0	2.5	0	0.0	0	0
Level 4: Desiccation	0.8	1.2	0	2.5	0.8	1.2	0	2.5

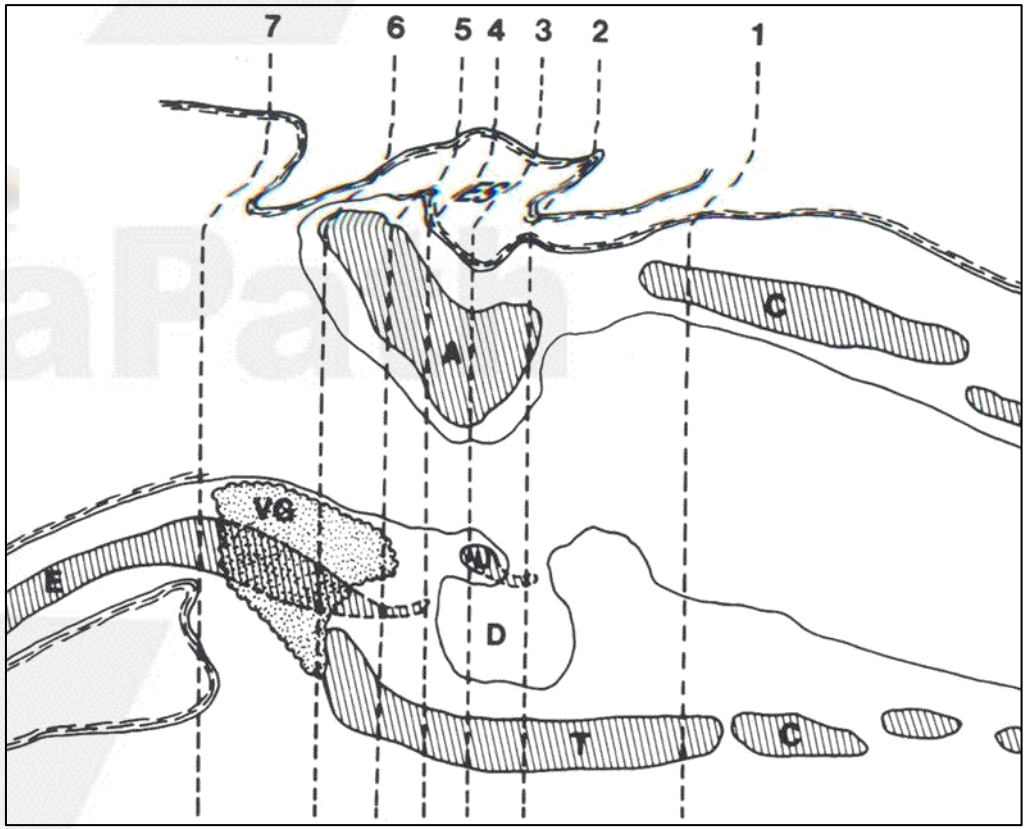
Section planes in rodents



Section planes in rodents

Section Planes: Rat

- E – Epiglottis
- VG – Ventral gland
- A – Arythenoid
- D – ventral pouch

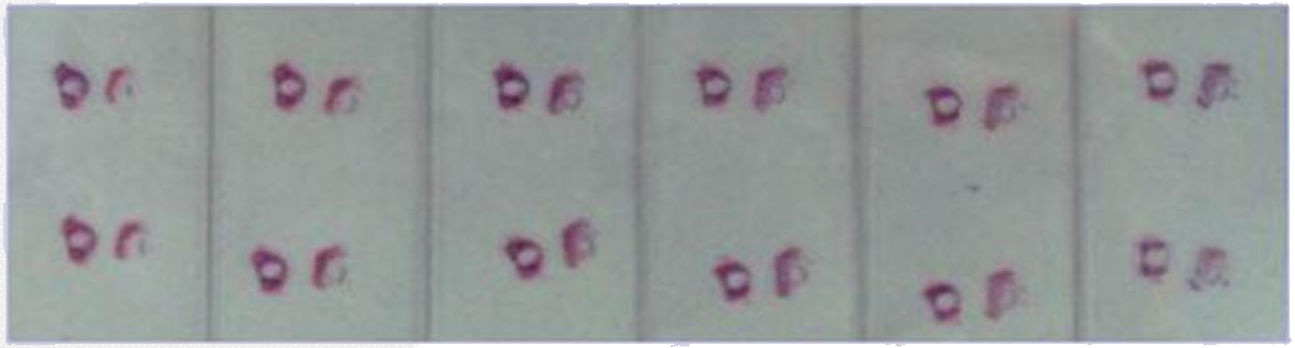


Sagartz, J.W. et al.: Histological Sectioning of the Rodent Larynx for Inhalation Toxicity Testing, Toxicol Pathol, 20: 118-121 (1992)

Larynx on Slide: Possibility

Trimming

On Slide



Material and Method: Rats, Inhalation

- **In rodents horizontolongitudinal or transversal according to Sagartz et al. (1992) sections**

- **Acute: 9 longitudinal (all negative), 4 transversal (2 positive)**
- **14-Day: 6 longitudinal (1 positive), 3 transversal (1 positive)**
- **4-Week: 9 longitudinal (1 positive), 8 transversal (3 positive)**
- **13-Week: 2 longitudinal (all negative), 7 transversal (3 positive)**
- **26-Week: 6 transversal (4 positive)**
- **104-Week: transversal (positive)**

Influence of Trimming

% of studies evaluated by horizontolongitudinal sections:

- **88.9% of all acute inhalation studies**
- **50% of all 14-Day studies**
- **75.0% of all 4-Week studies**

Not any lesion recorded!

- **vs. 100% positive outcome in all studies at these durations evaluated by transversal sections.**

Material and Method: Rats, Non-Inhalation

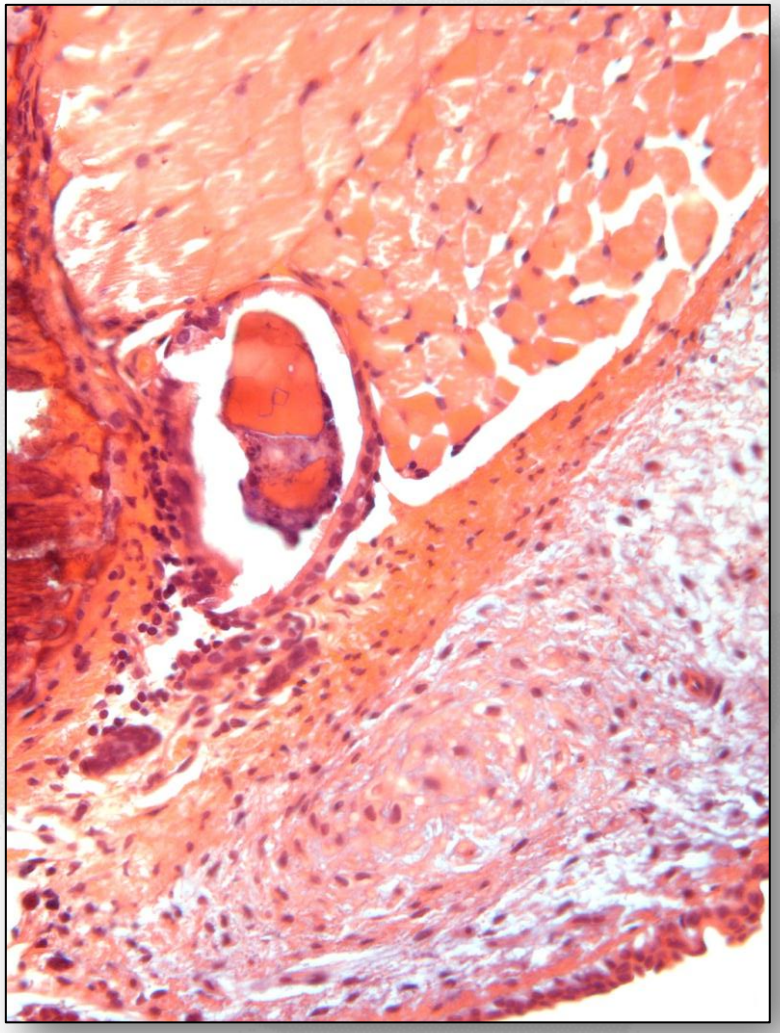
All evaluated by horizontolongitudinal sections

- **14-Day: 8 Gavage, 2 i.v., 1 intraocular**
  (1 Gavage study was positive: regurgitation laryngitis)
- **4-Week: 20 Gavage, 1 i.v., 3 dermal**
  (1 Gavage study was positive: regurgitation laryngitis)
- **13-Week: 9 Gavage, 6 feeding**
- **26-Week: 2 Gavage, 1 s.c., 1 dermal**
  (1 Gavage study positive: food impaction in high dose animals)
- **52-Week: 2 feeding**
- **104-Week: 4 feeding**

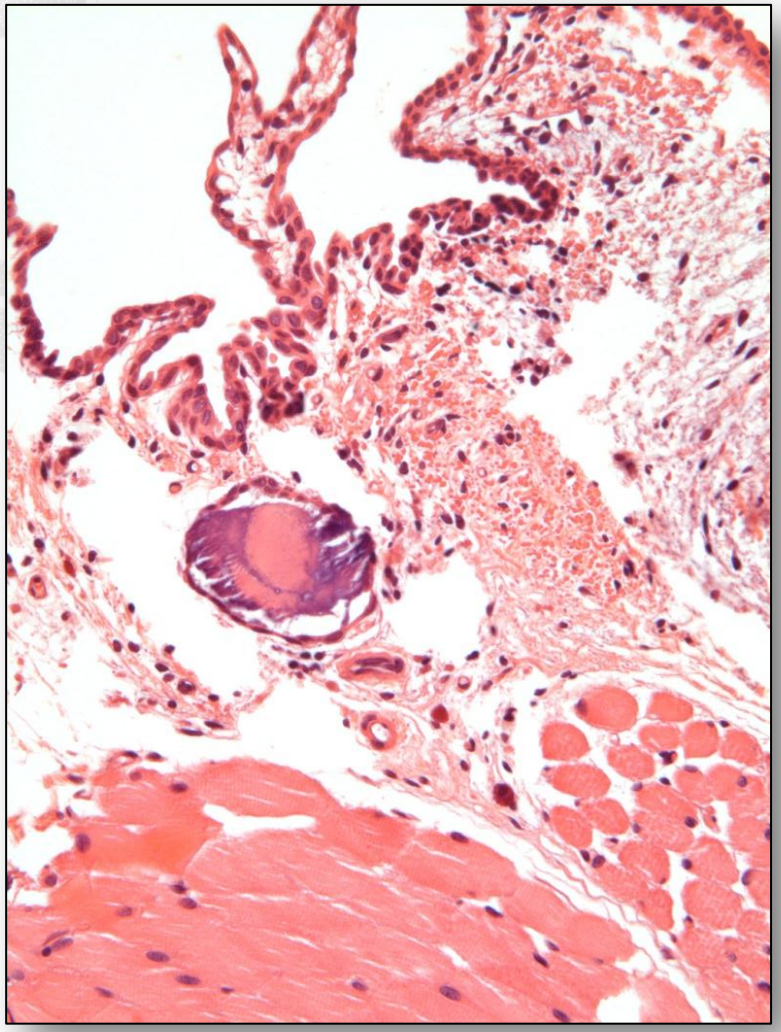
Rat: Inhalation and Non-Inhalation Studies Spontaneous Lesions

Dessicated secretion and mineralization

Level 2

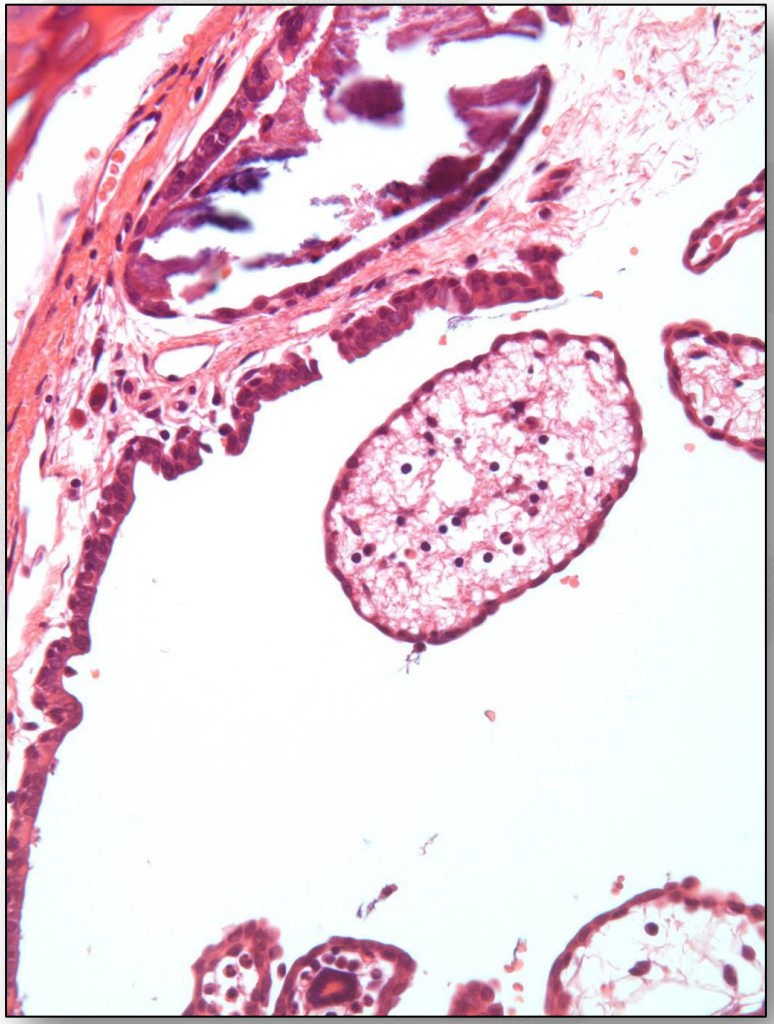


Level 3

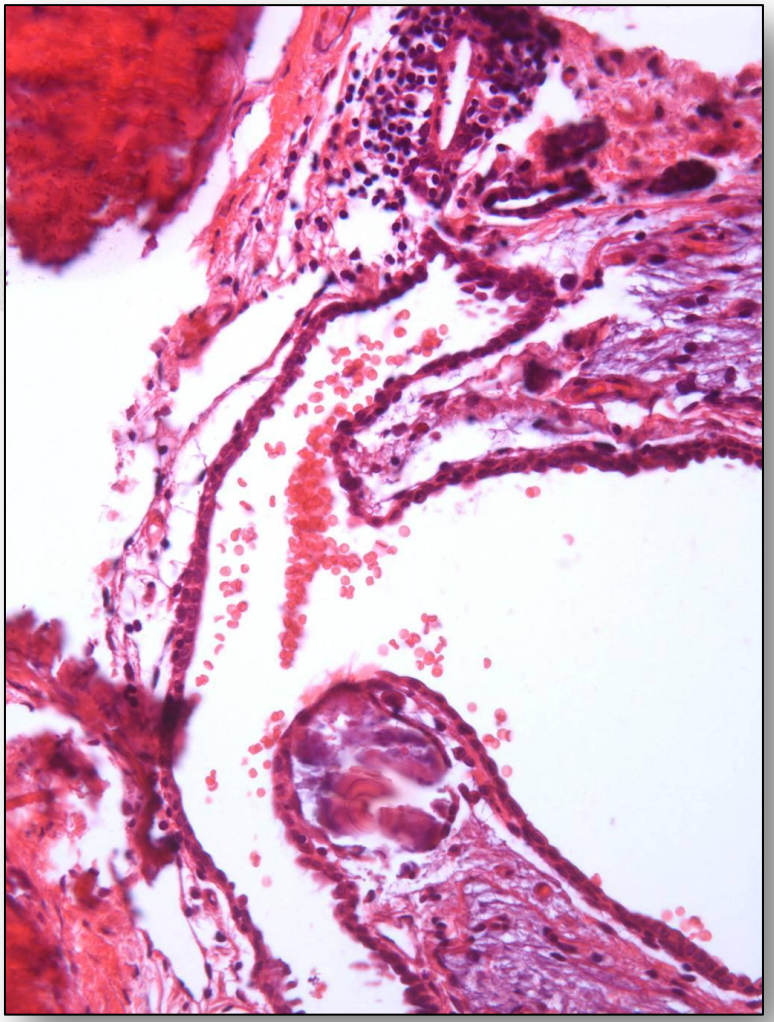


Dessicated secretion and mineralization

Mineralization of glandular duct and edema of mucosa at Level 2

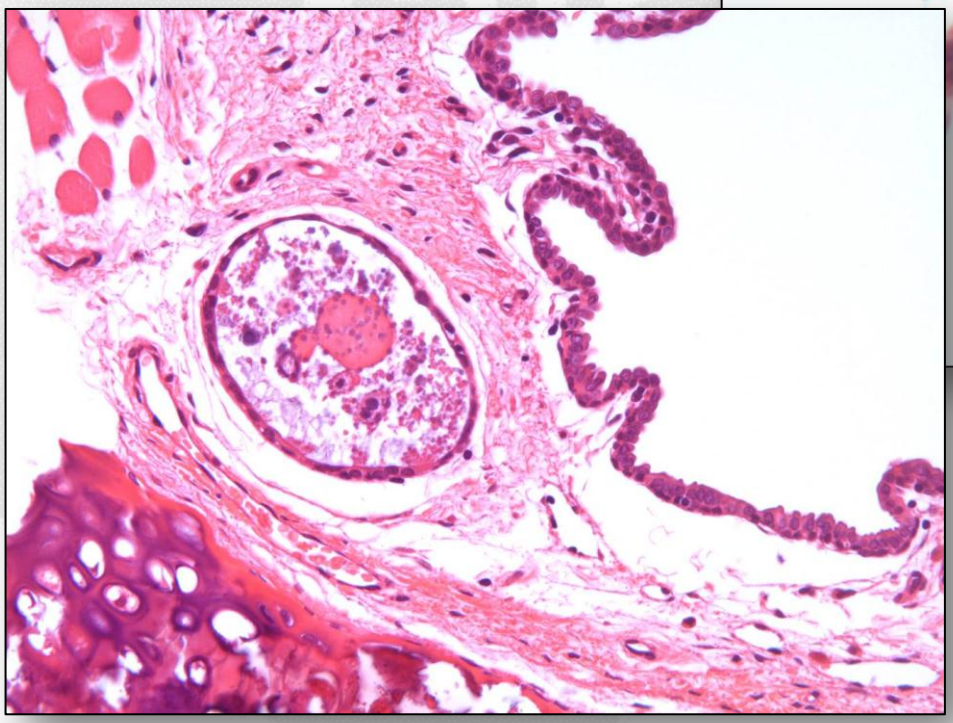


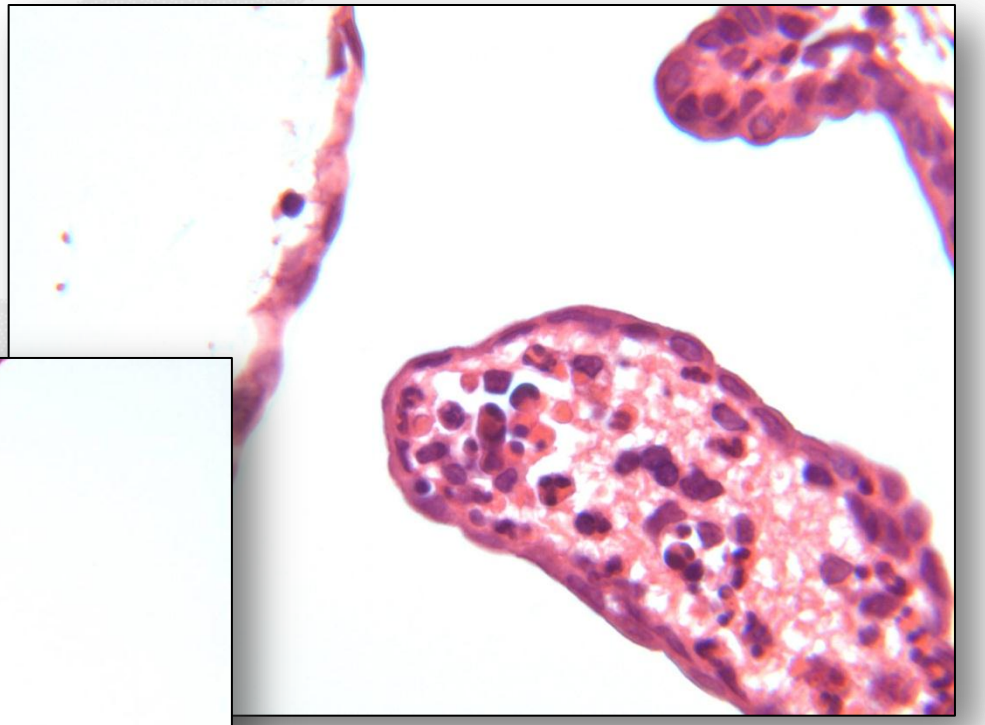
Mineralization and glandular inflammation at Level 3



Dessicated secretion and mineralization

Dessicated secretion and glandular inflammation at Level 2

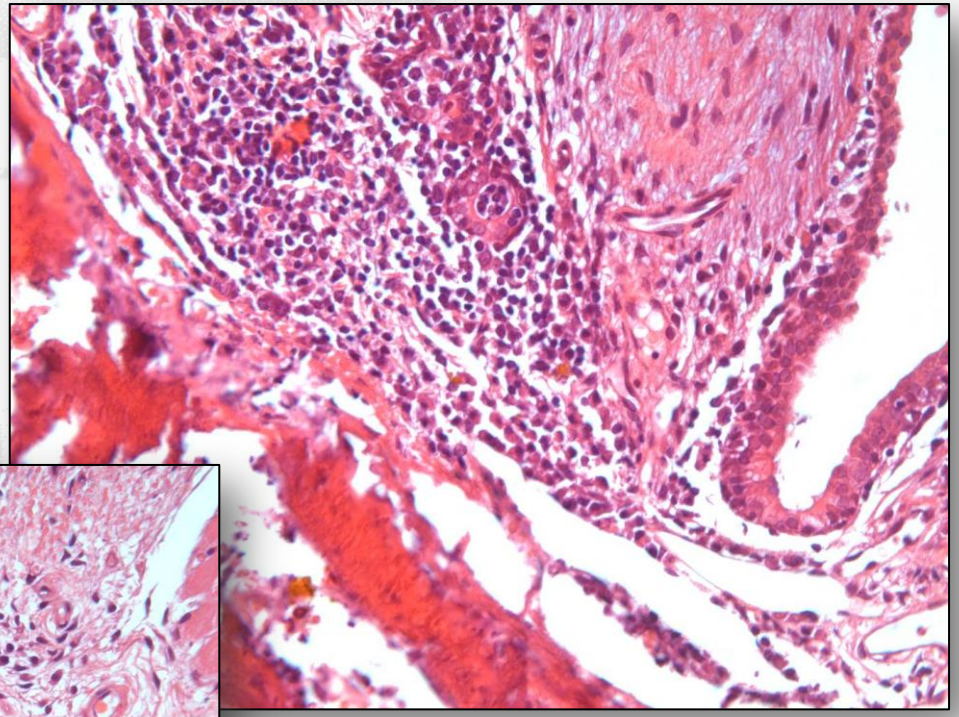


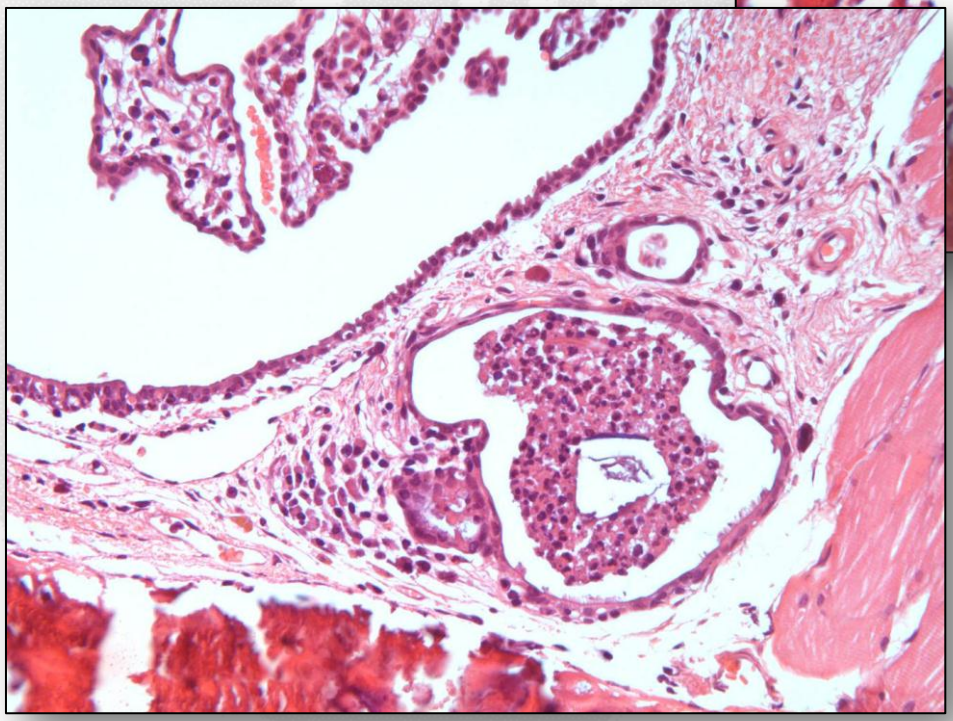


Mucosal inflammation at Level 2

Dessicated secretion and mineralization

Glandular inflammation along with focal mineralization at Level 2





Mucosal inflammation at Level 2

Dessicated secretion, mineralization, inflammation

Pathogenesis:

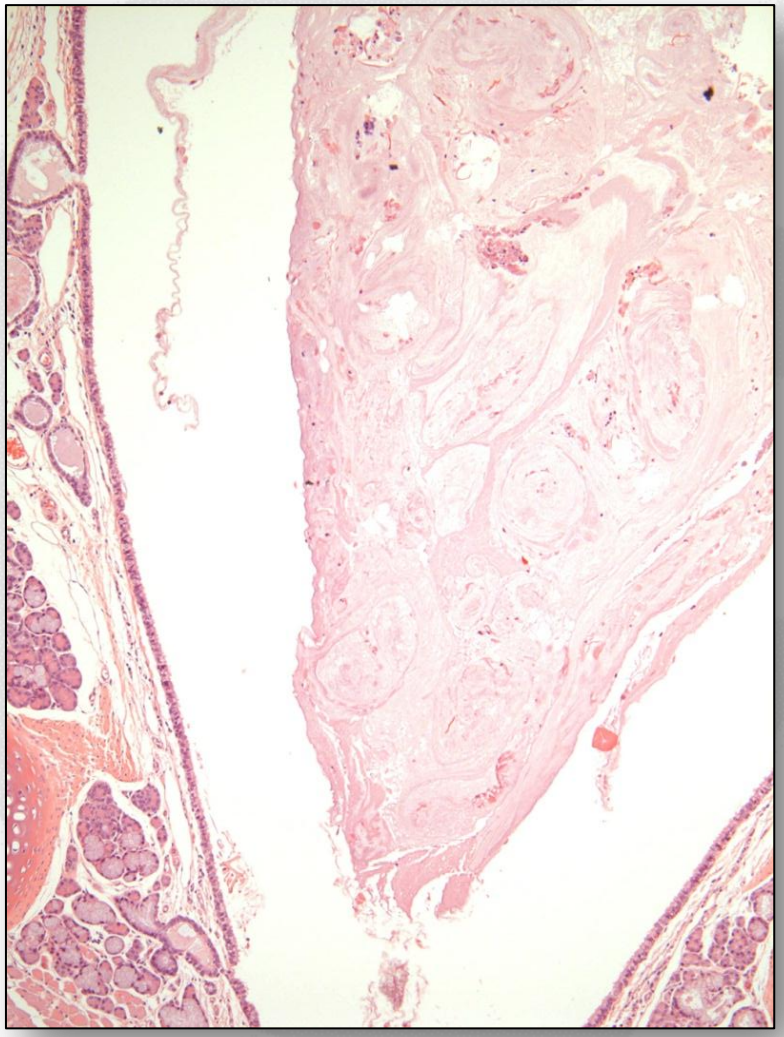
- **Dessication -> Mineralization -> Inflammation**
- **Mainly in Levels 2 and 3**

Consequences:

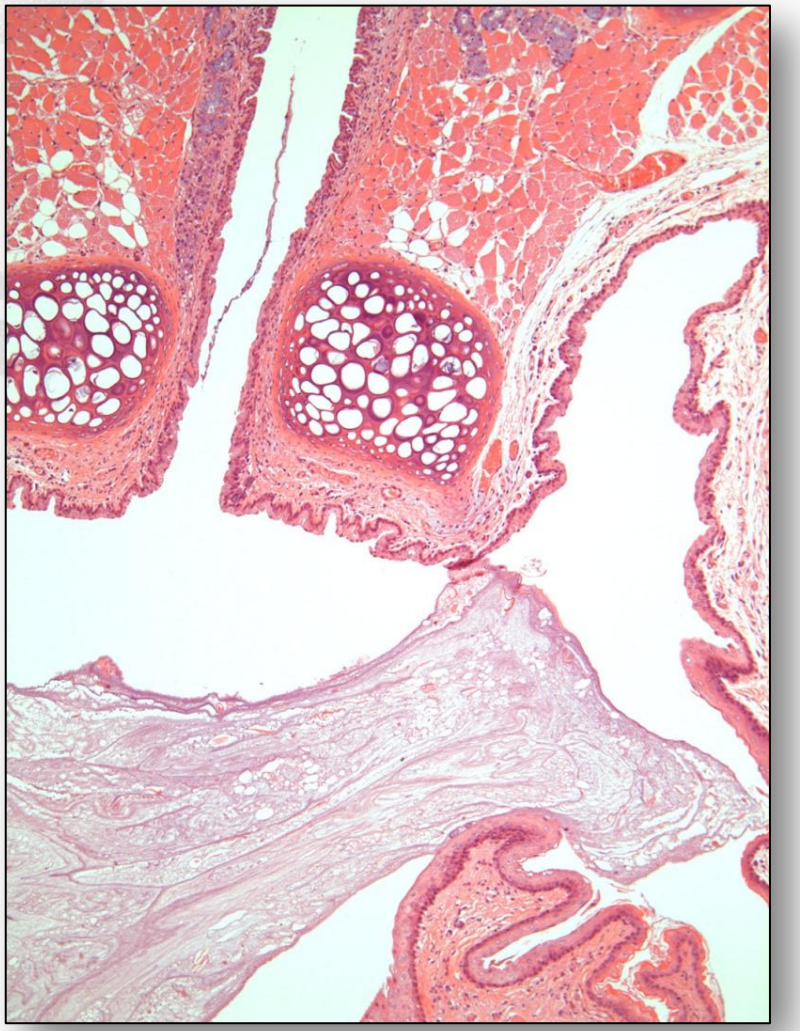
- **Dessication and inflammation at higher incidences in males (acute to 13-Week)**
- **Mineralization accompanying desiccated glandular secretion at higher incidences in females (4- and 13-Week)**
- **All differences became vague in longer lasting studies**

Aspiration, secretion

Impacted food (longitudinal section)

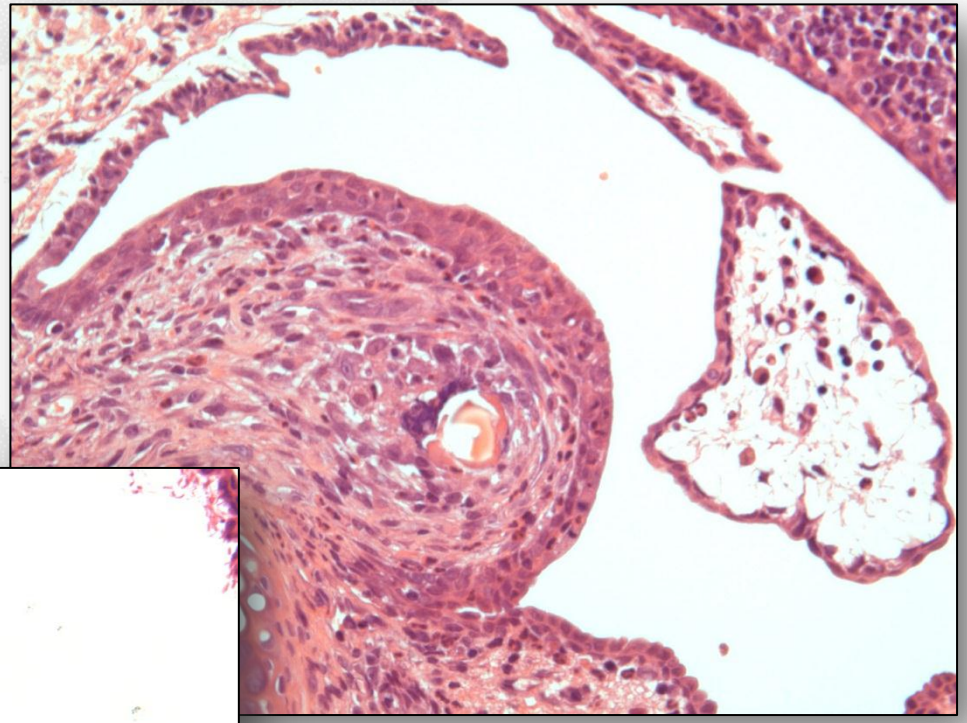


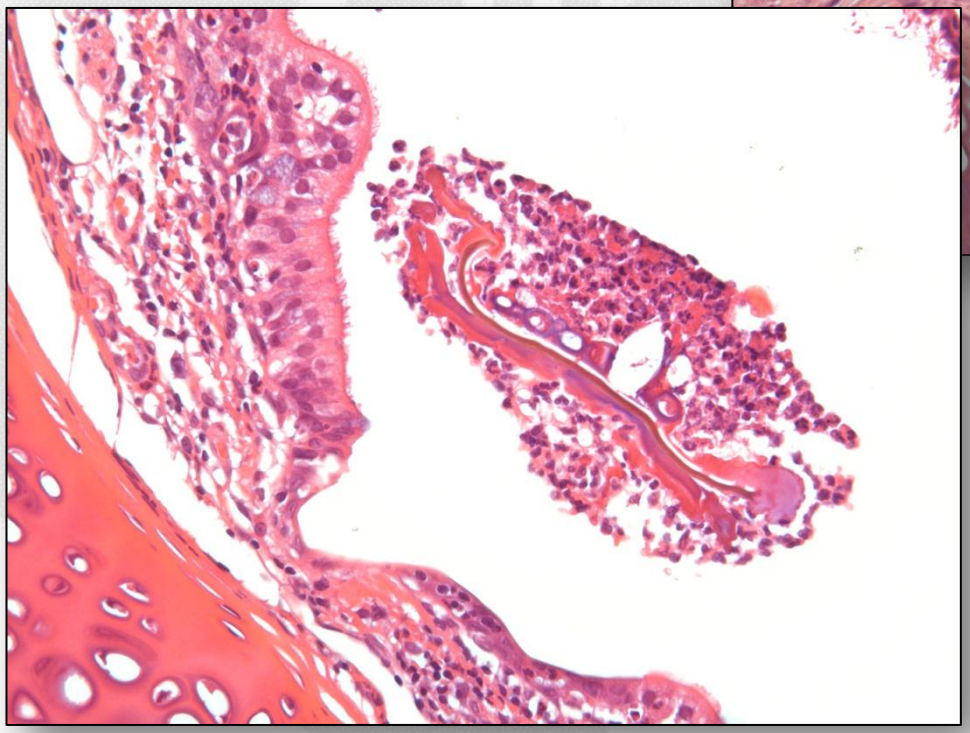
Mucous in lumen (longitudinal section)



Inflammatory lesions

Foreign body with inflammation and reactive respiratory hyperplasia at Level 2

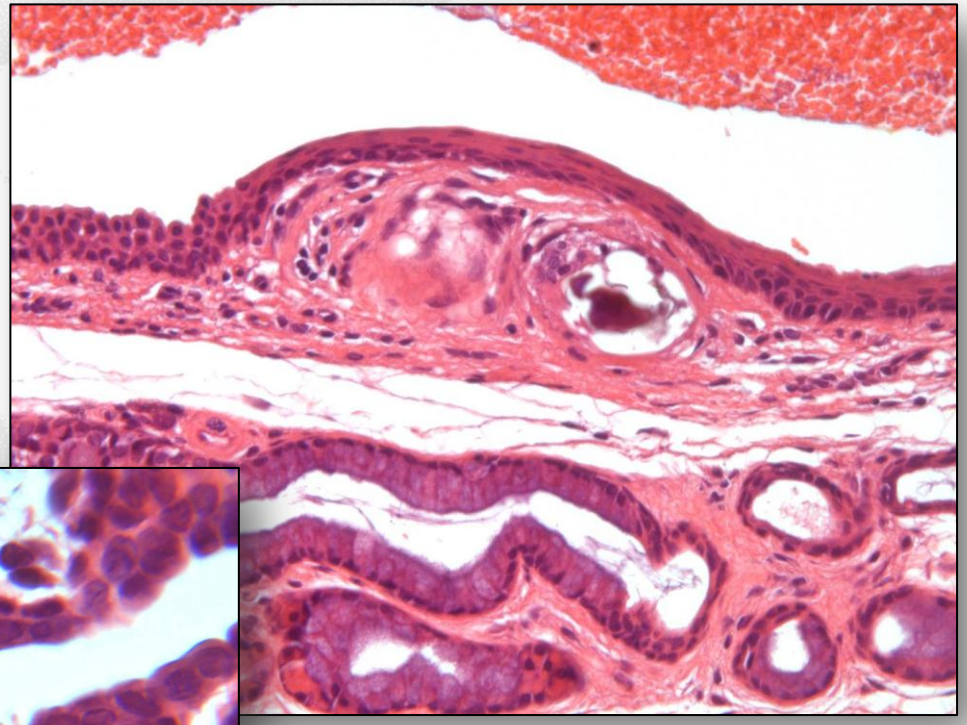


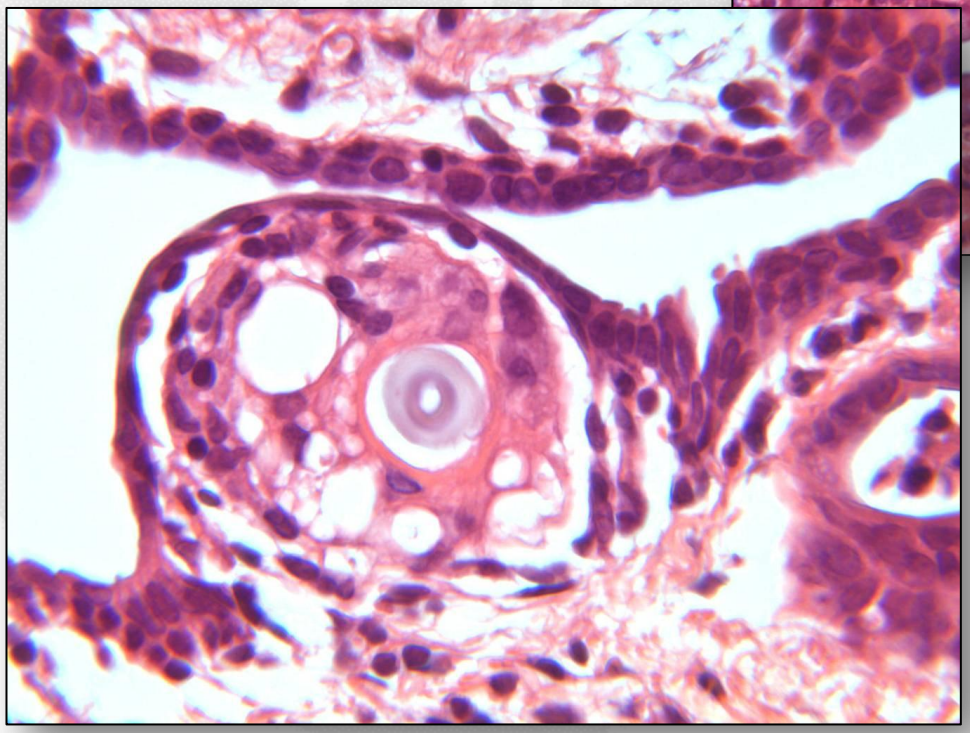


Granuloma at Level 4

Inflammatory lesions

Foreign body (probably hair) and granuloma at Level 2)

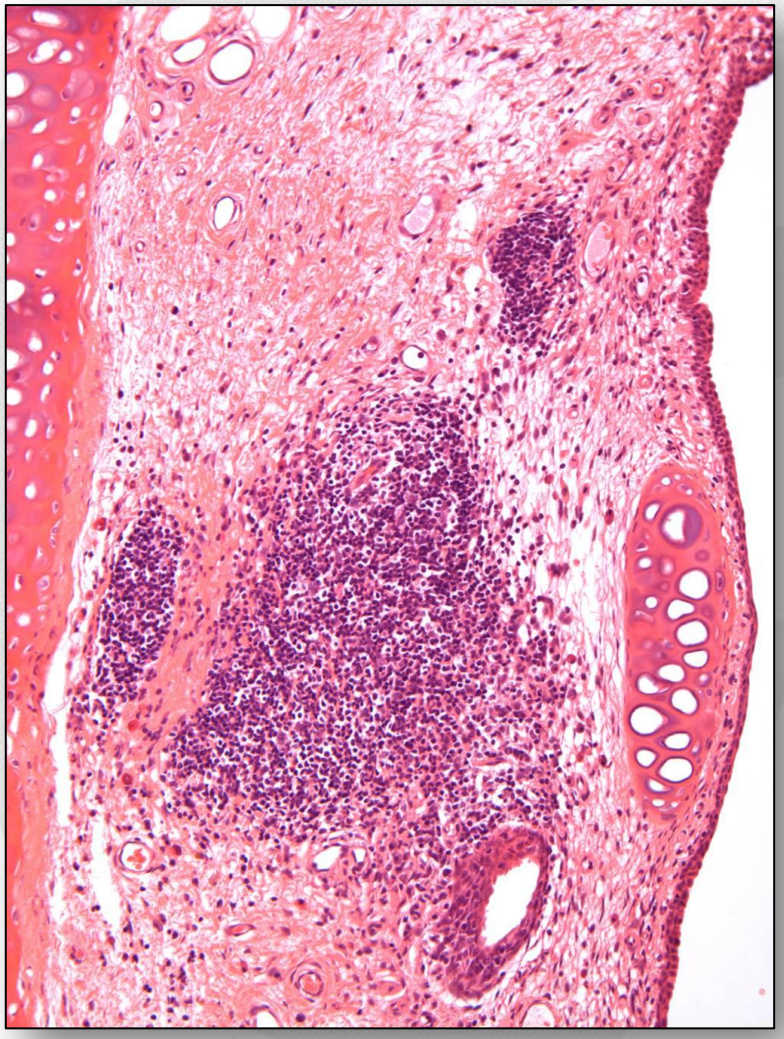




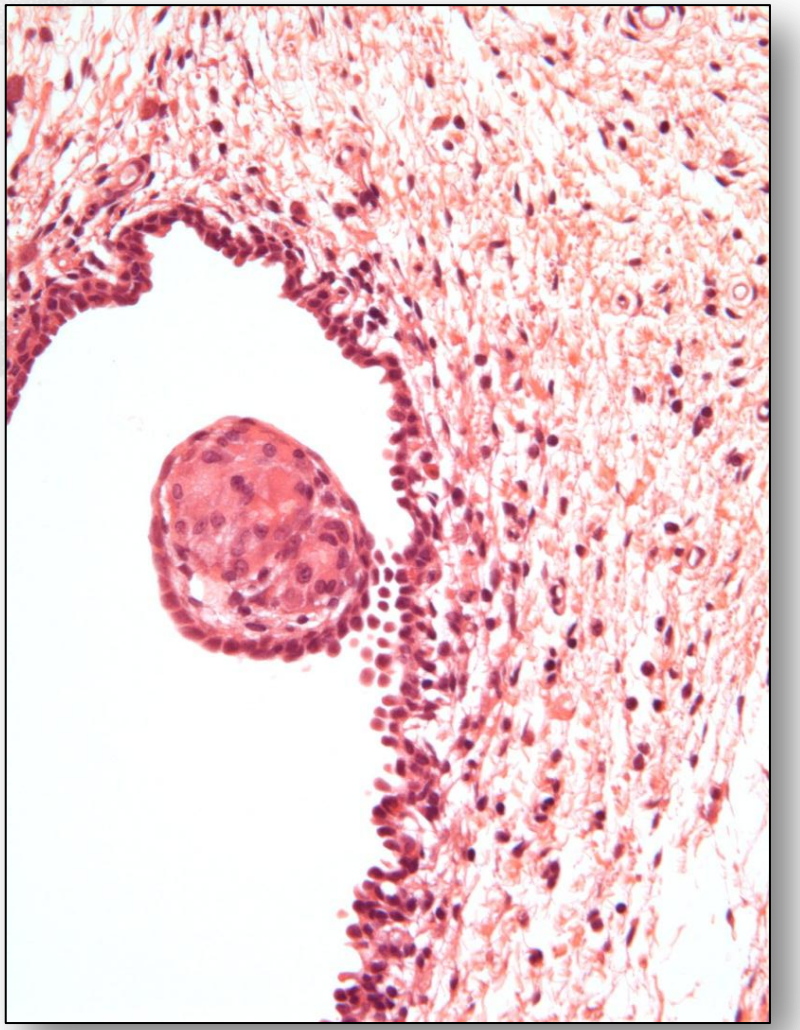
Granuloma at Level 6 with overlaying squamous metaplasia

Inflammatory lesions

Mononuclear foci at Level 5

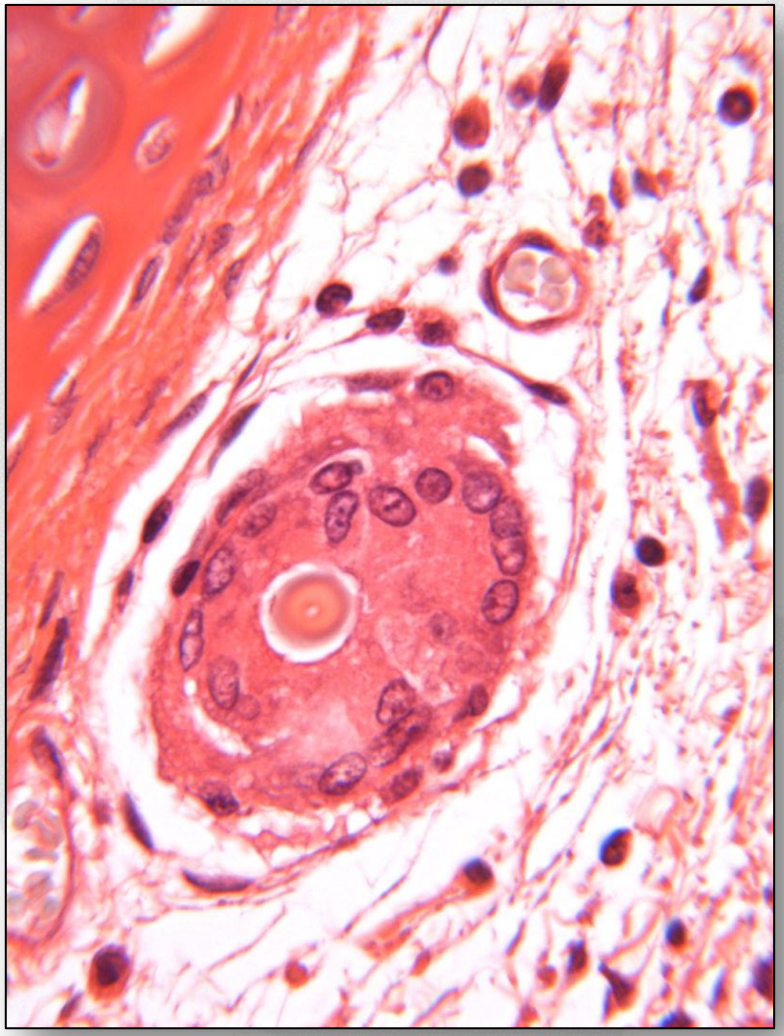


Granuloma at Level 2



Inflammatory lesions

Granuloma at Level 2



Granuloma at Level 6

Squamous metaplasia

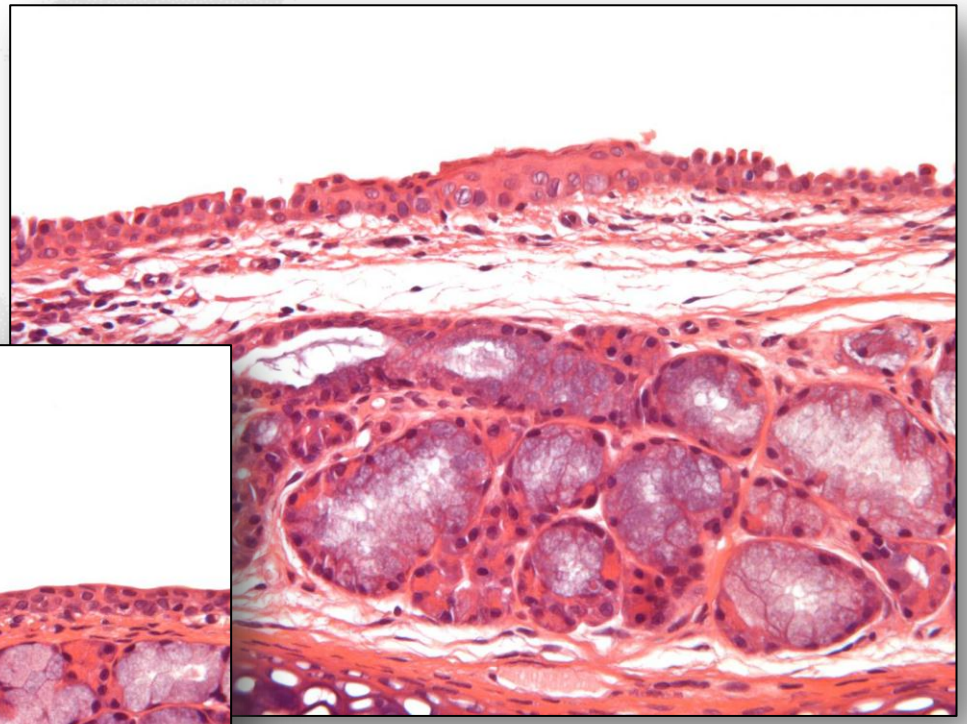
- Squamous metaplasia of the mucosa overlaying the ventral aspect of the larynx at level 6 is also a spontaneous lesion!

- 14-Day to 13-Week studies at higher incidences in control females
- 13-Week at higher incidences in control males

- Up to 20% in control males of inhalation oncogenicity study in RccHanTM: WIST

Normal aspect may become confusing

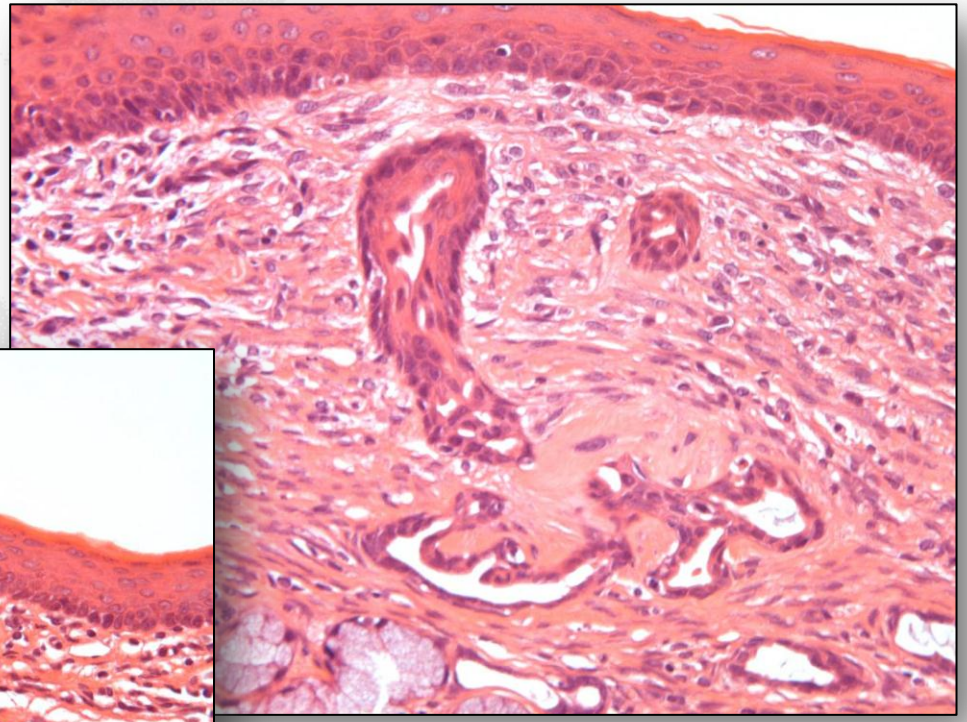
Squamoid epithelium at Level 6

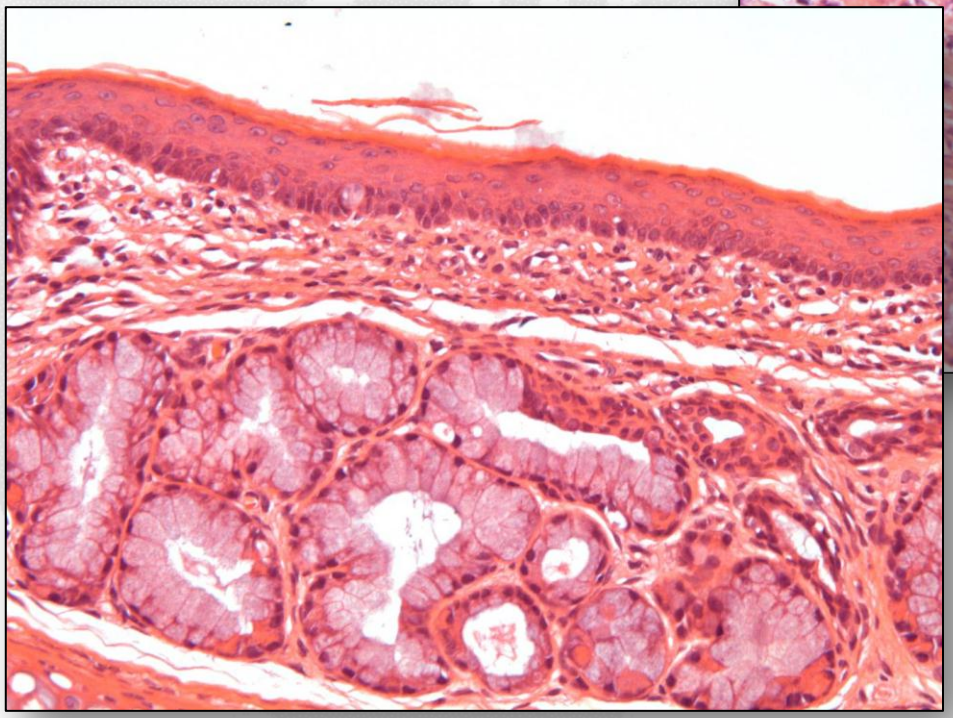


Squamous cell ‚tongue' at Level 6

Squamous metaplasia

Spontaneous squamous metaplasia at Level 6



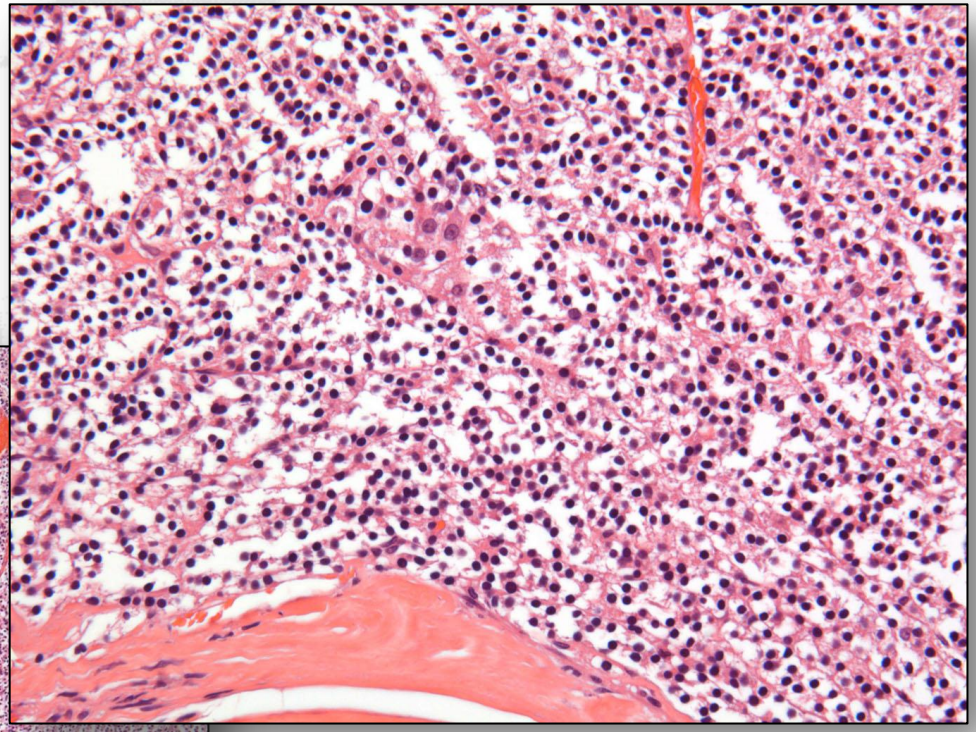


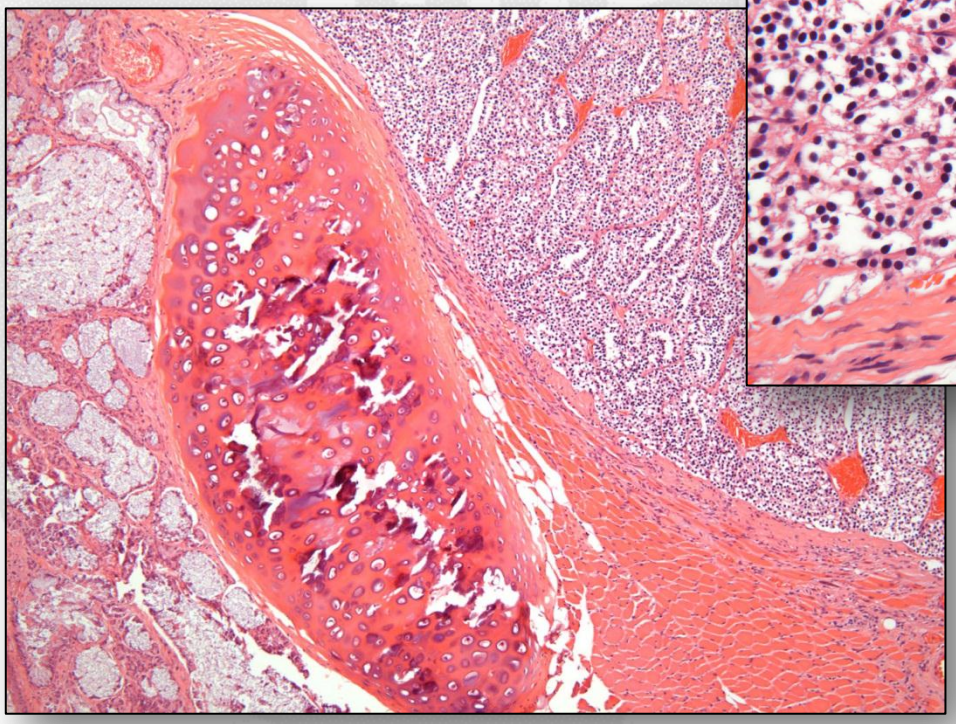
Spontaneous squamous metaplasia and submucosal fibrosis at Level 6

Neoplastic lesions

Almost no spontaneous neoplastic lesions known.

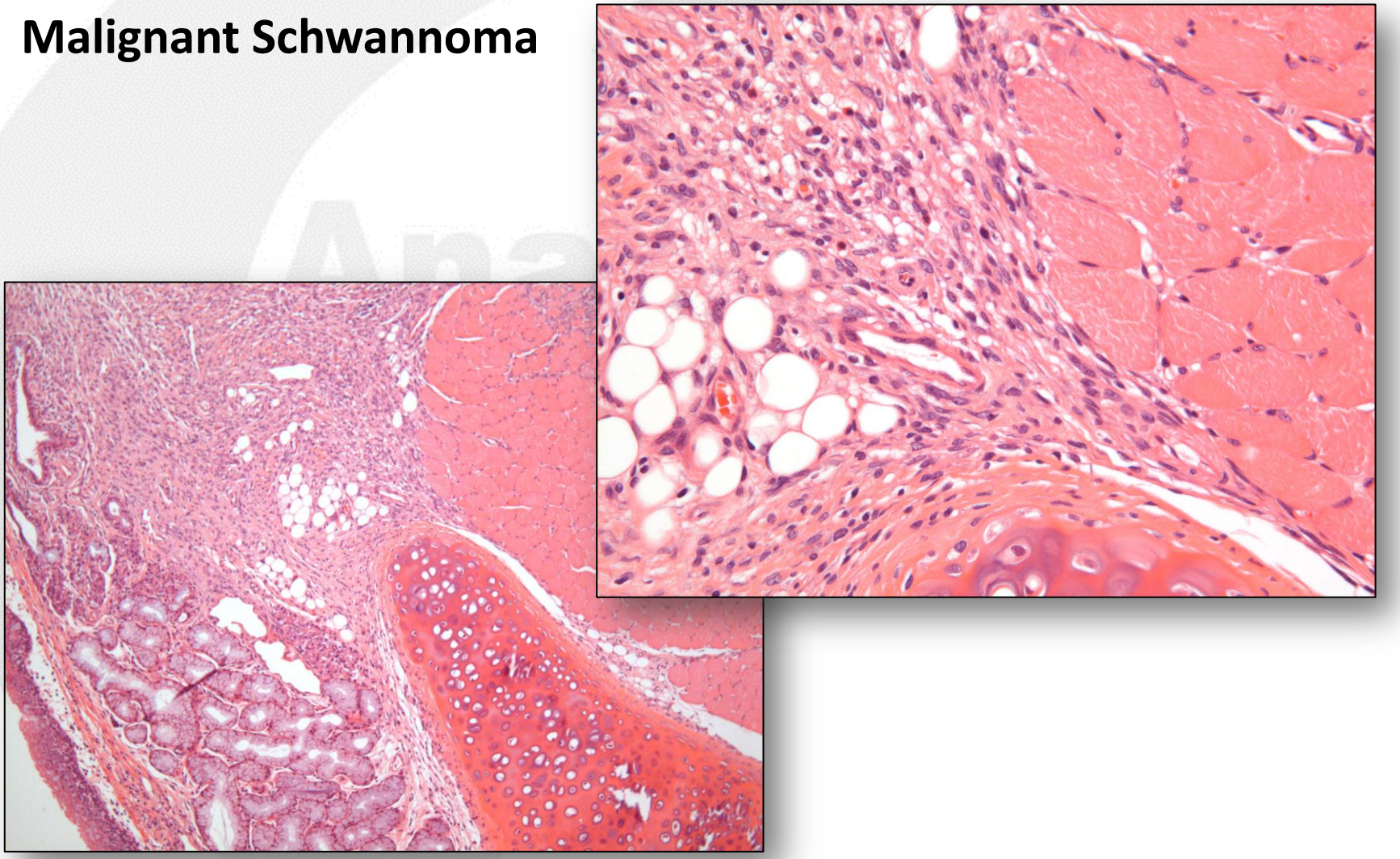
Metastatic thyroid follicular carcinoma





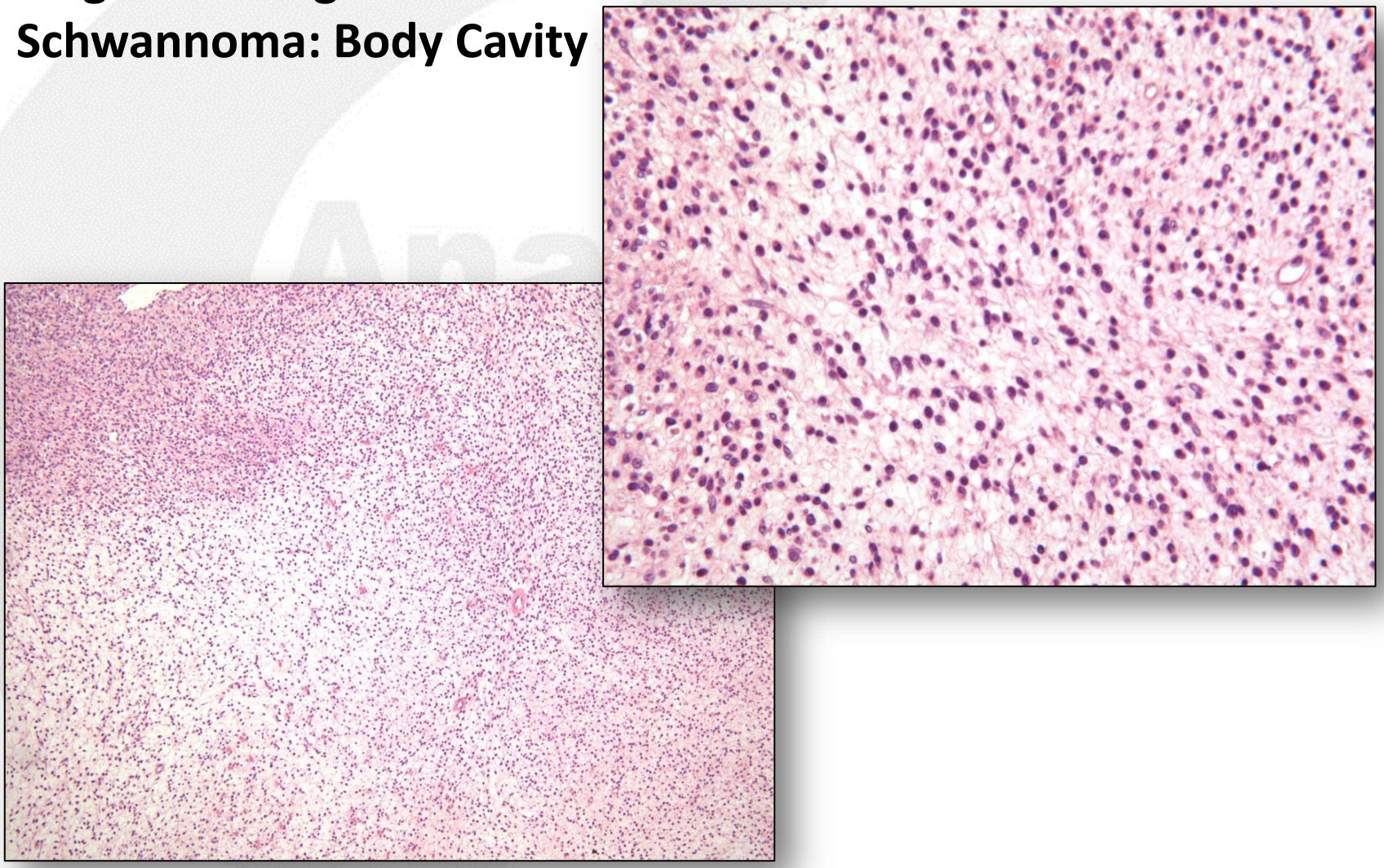
Neoplastic lesions

Metastatic Sarcoma:
Malignant Schwannoma



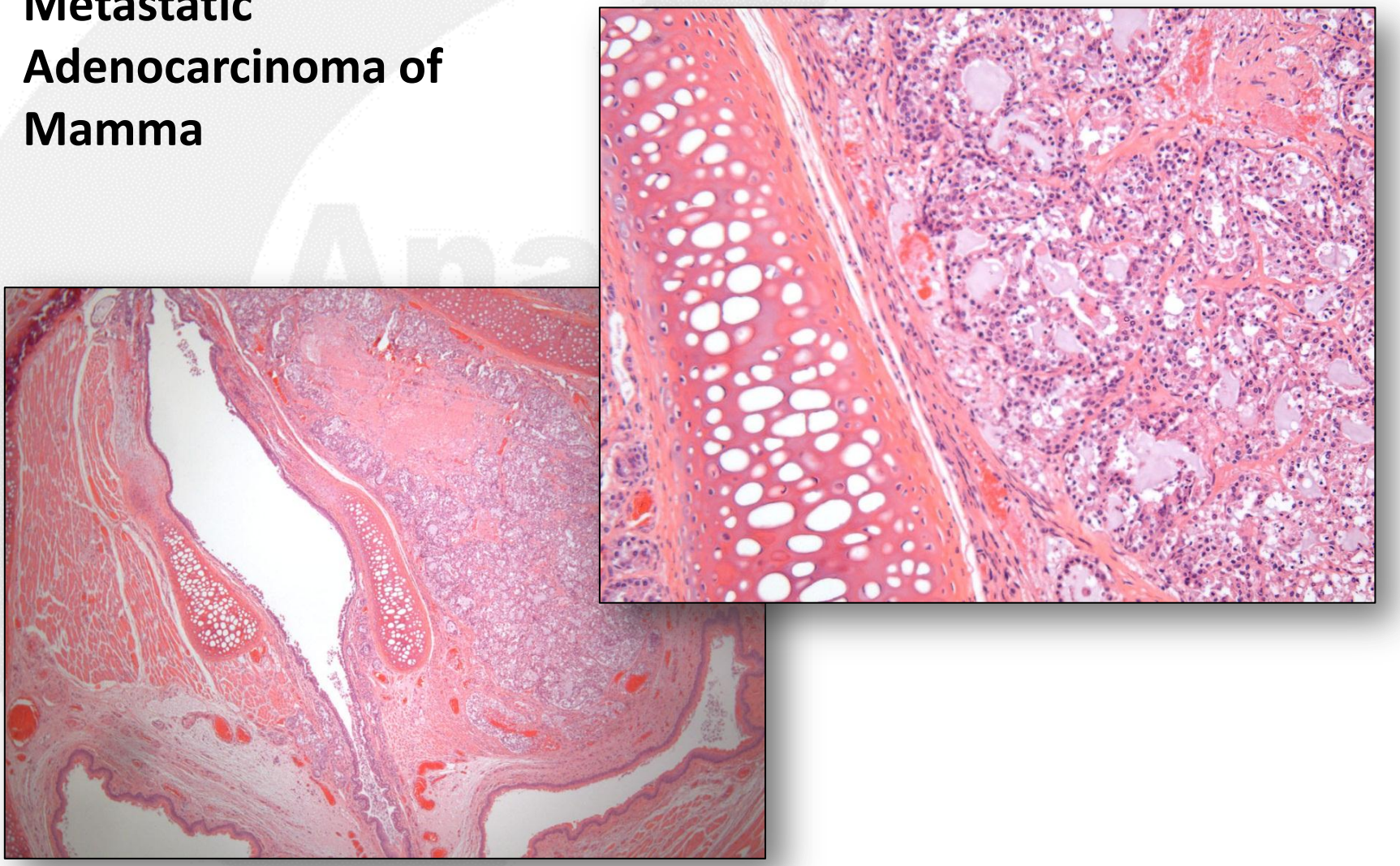
Neoplastic lesions

Origin of Malignant Schwannoma: Body Cavity



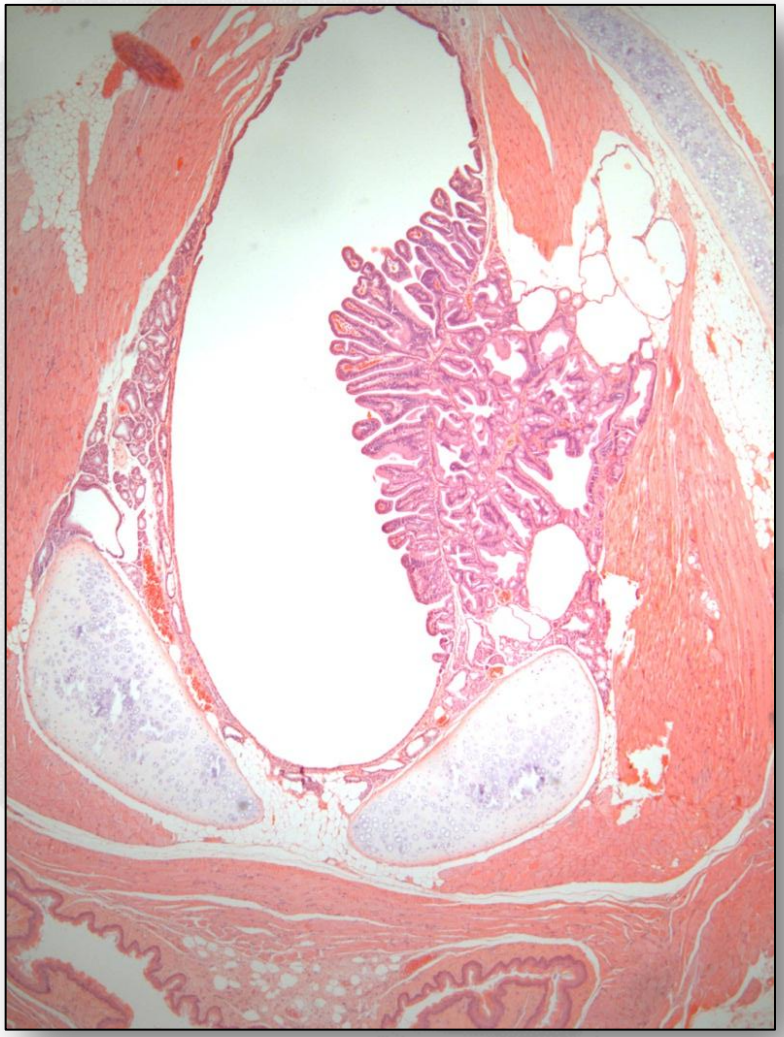
Neoplastic lesions

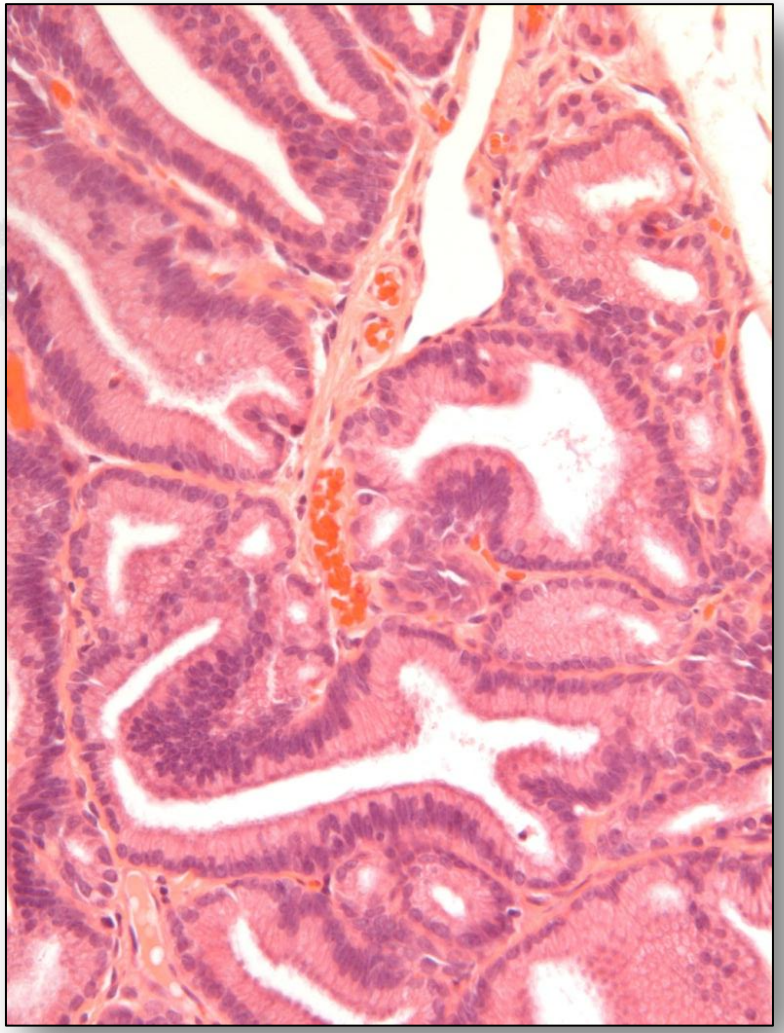
Metastatic Adenocarcinoma of Mamma



Published Spontaneous Neoplasms

F344, female, 46 weeks at age, vehicle control, gavage study (Courtesy of Paul-Georg Germann, Nycomed, 10.Schnittseminar, GTP, Gent, 1996)

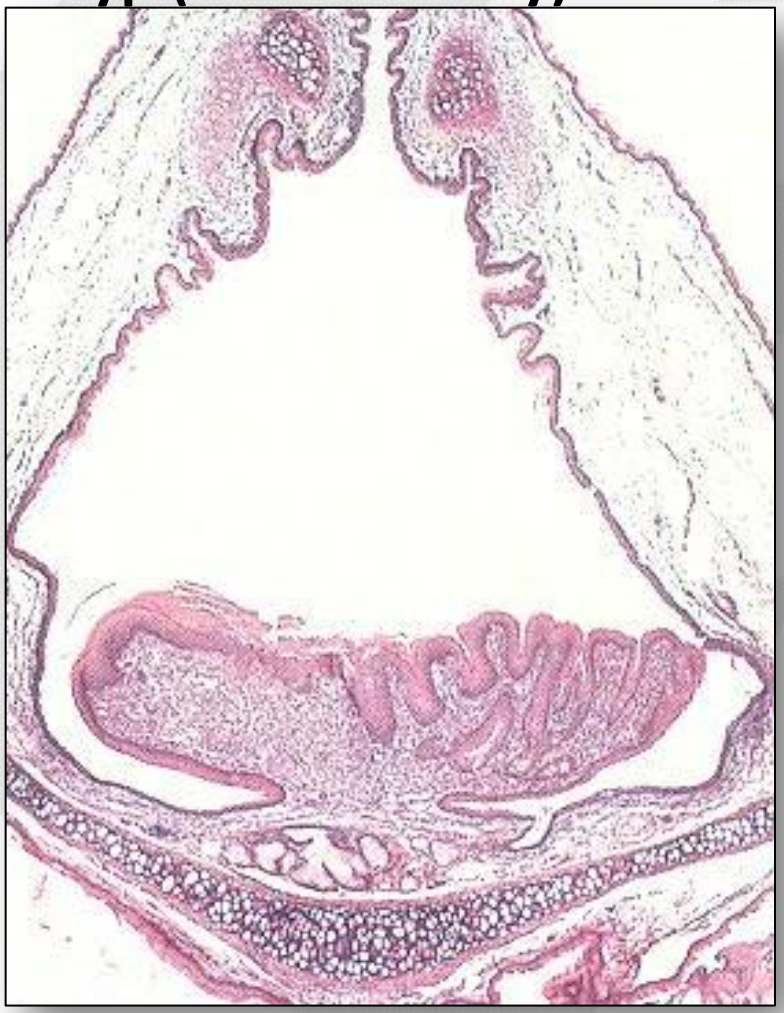




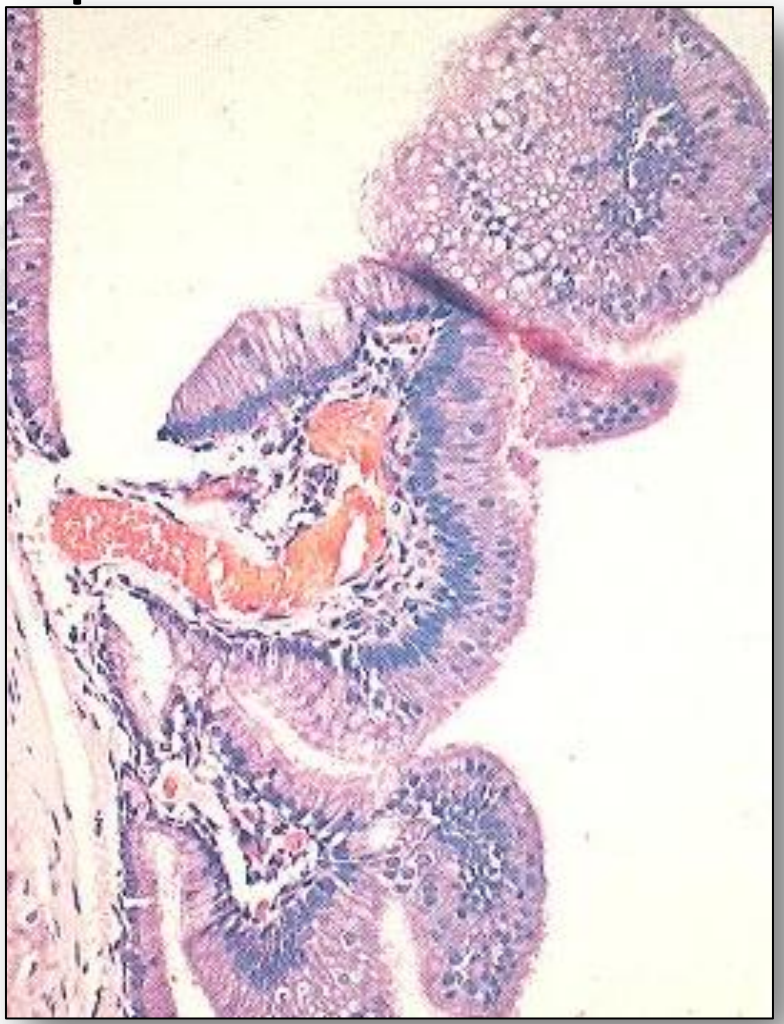
Published Spontaneous Neoplasms

Schwartz et al., IN: Guides for Toxicologic Pathology, STP/ARP/AFI
1994

Polyp (inflammatory)

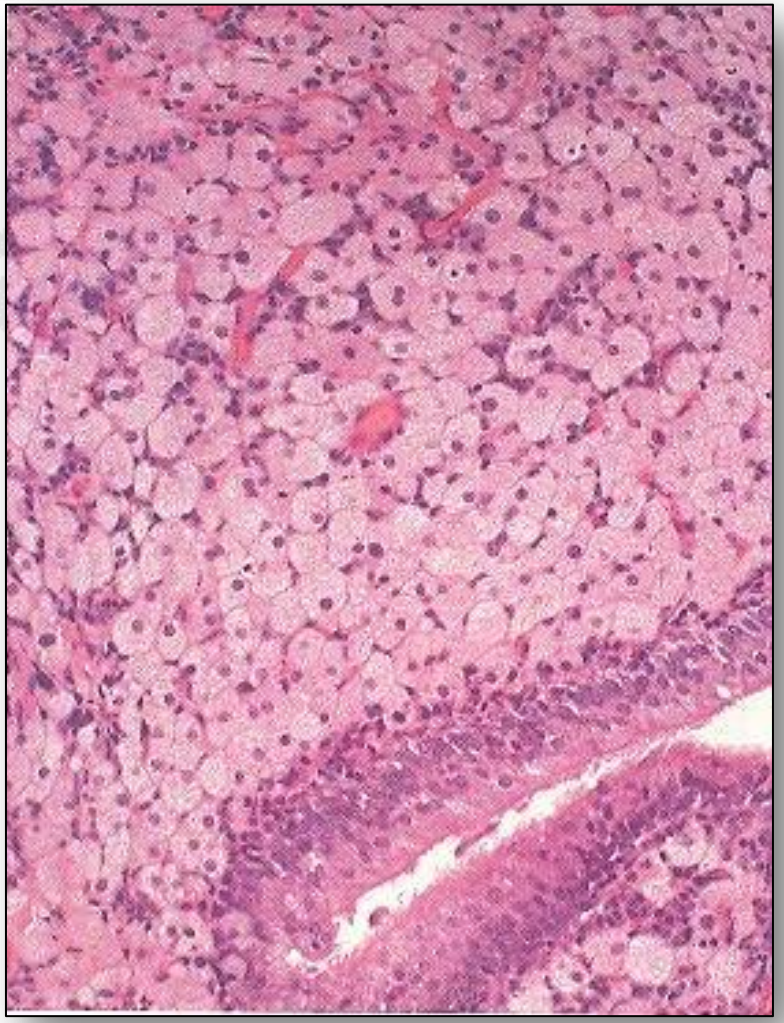


Papilloma



Published Spontaneous Neoplasms

Schwartz et al., IN: Guides for Toxicologic Pathology, STP/ARP/AFI
1994: Granular cell tumor



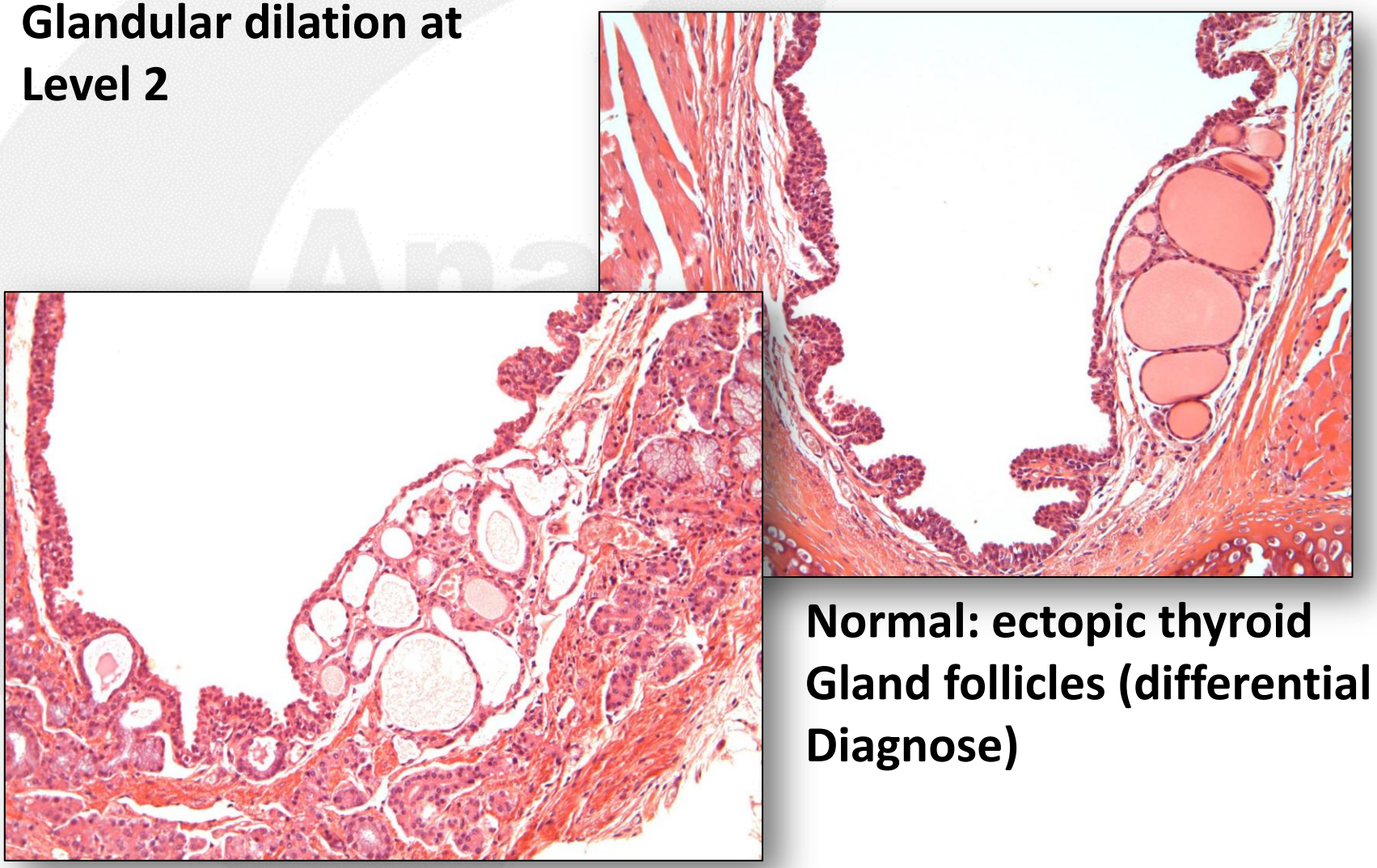
Rat: Inhalation Studies Induced Lesions

Rats: Inhalation Studies

- Squamous metaplasia recorded in all 16 positive studies, whereas in 9 studies (56.2%) it was the only lesion
- When only squamous metaplasia was recorded, there was recovery after 2-4 weeks
- Otherwise, a wide range of degenerative and inflammatory lesions

Rats: Reactive lesions

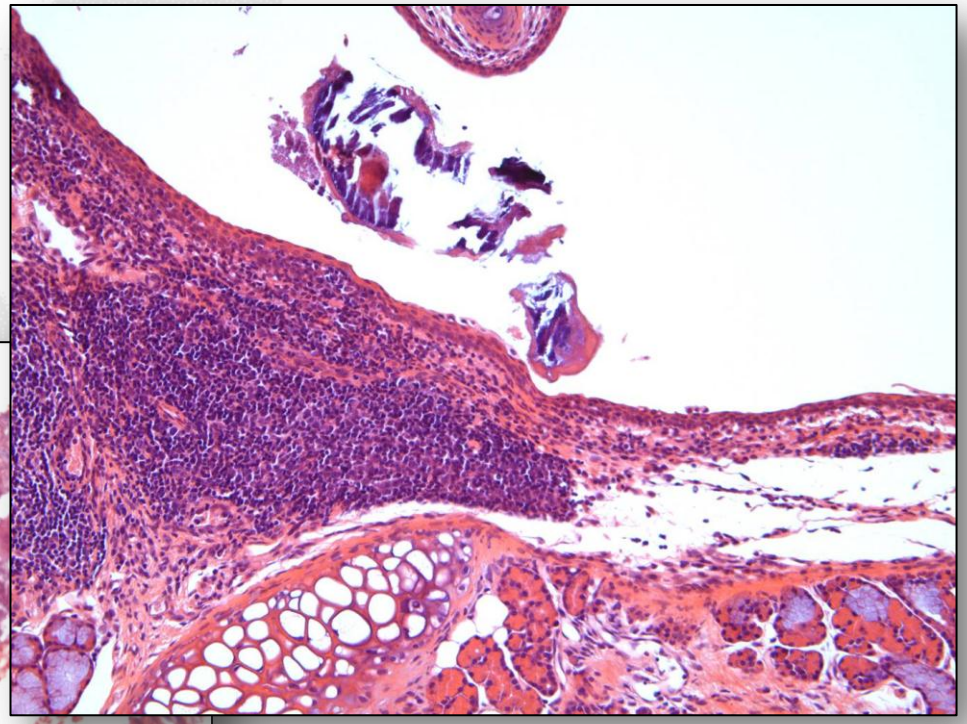
Glandular dilation at Level 2

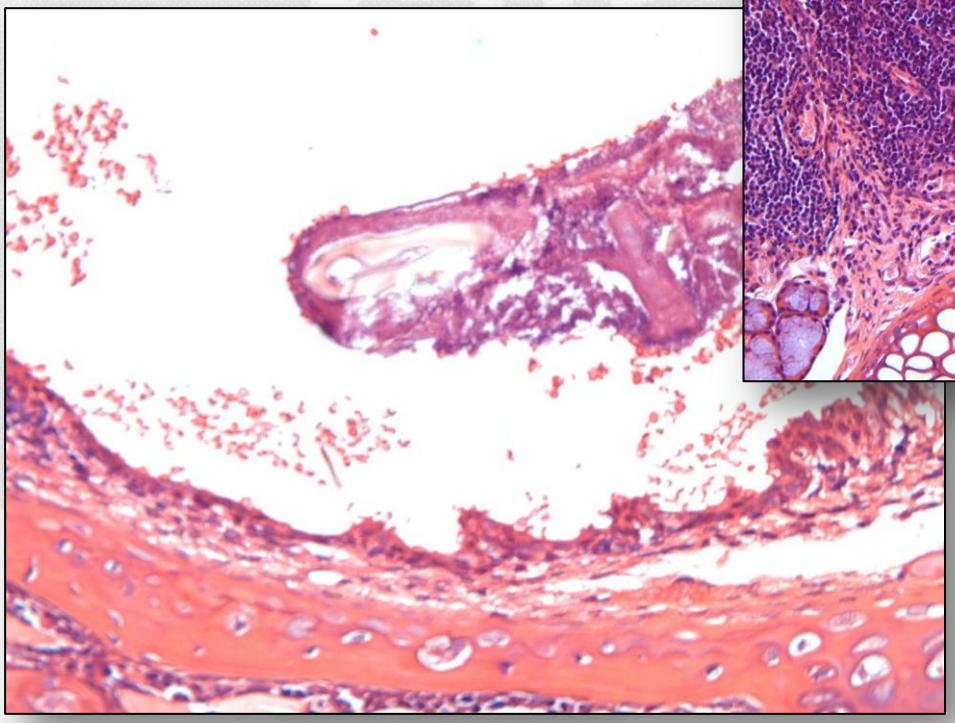


Normal: ectopic thyroid Gland follicles (differential Diagnose)

Rats: Depositions and related lesions

Food impaction and related inflammation at Level 3

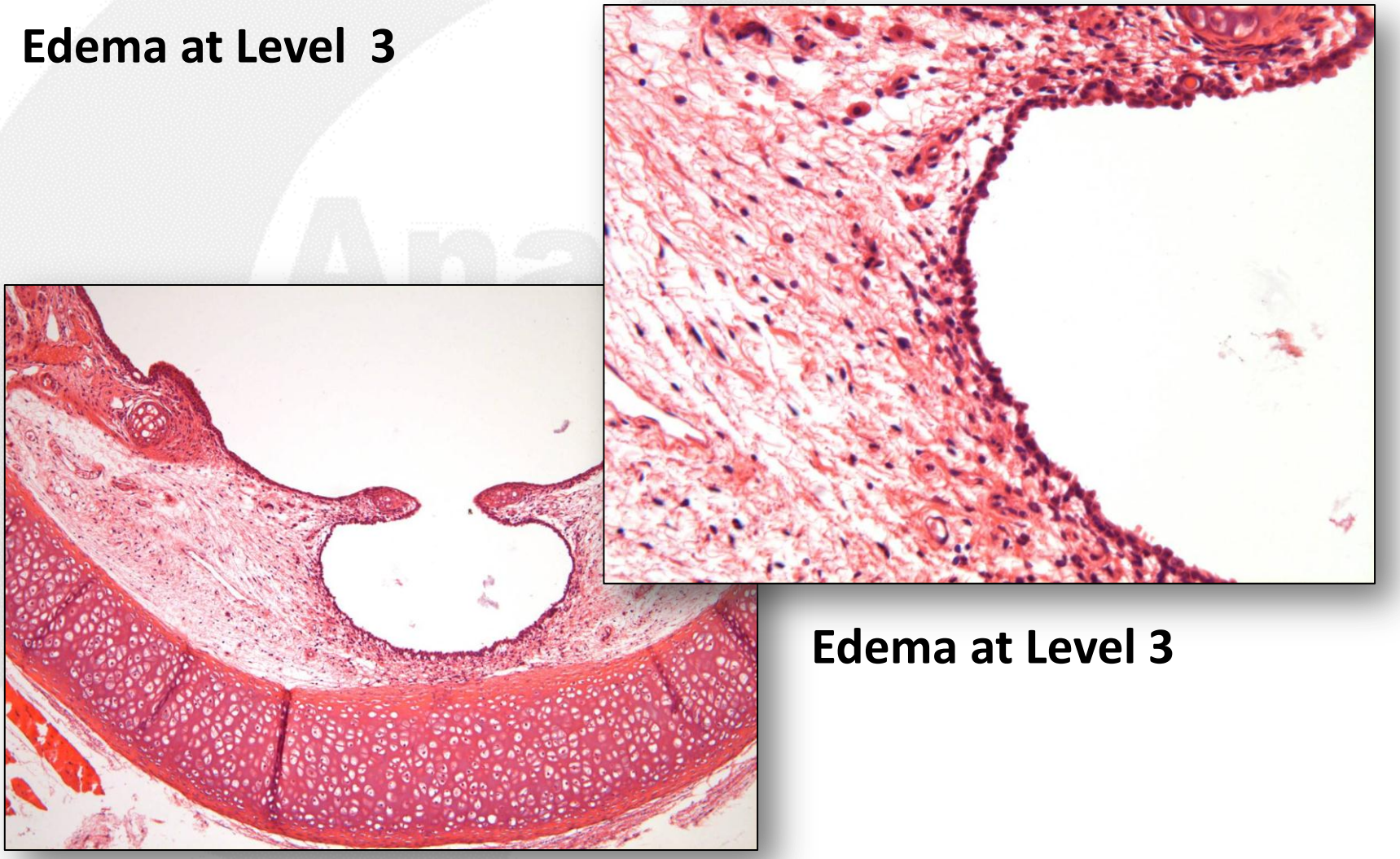




Food impaction and related inflammation At Level 6

Rats: Inflammatory lesions

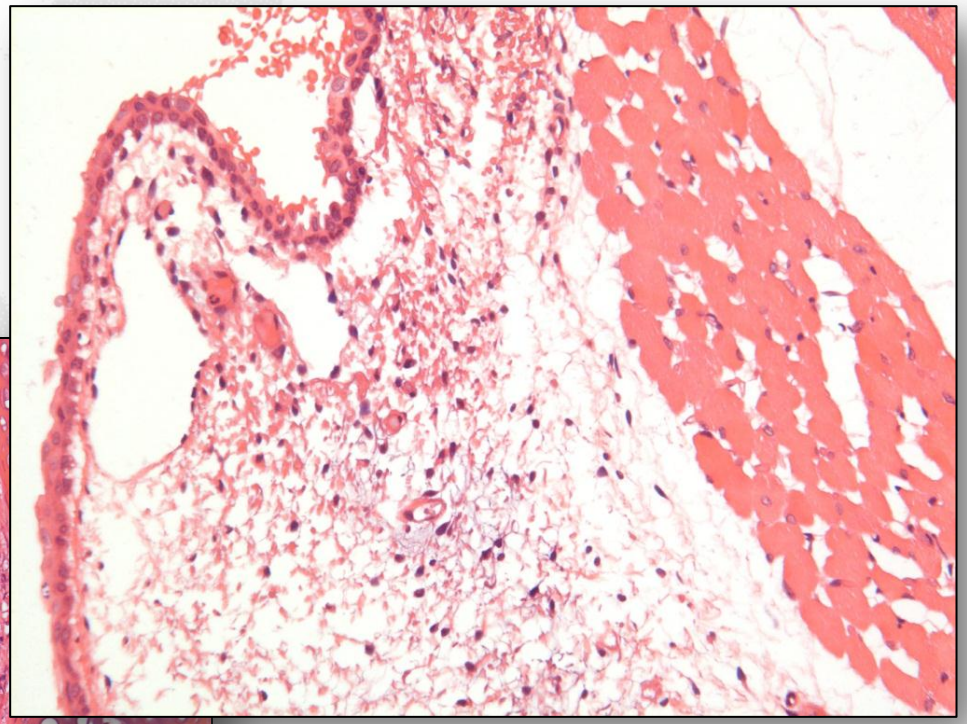
Edema at Level 3

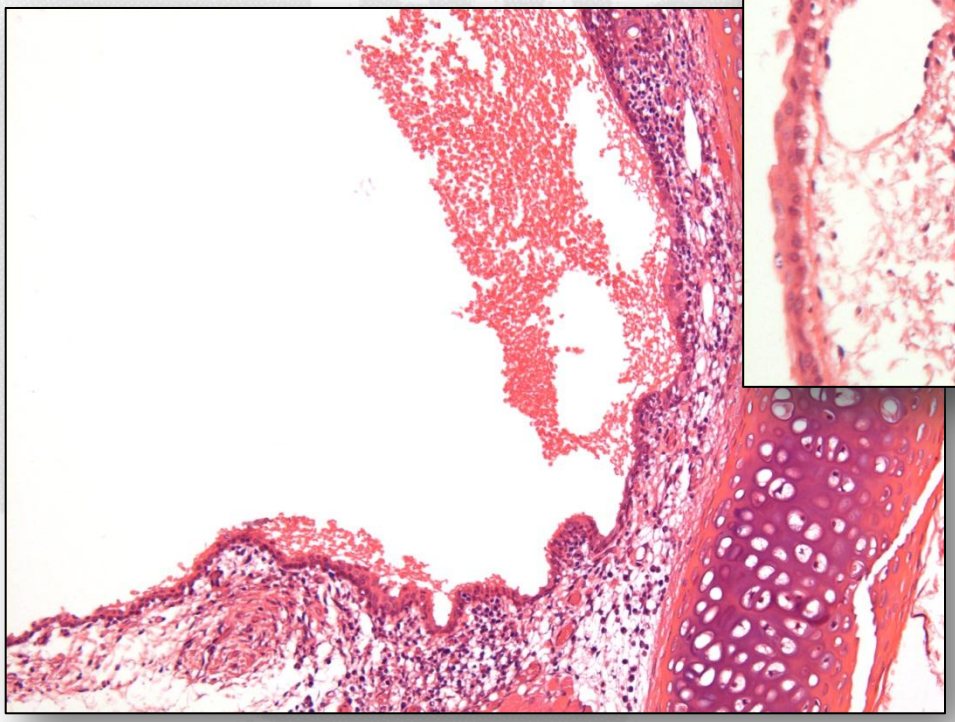


Edema at Level 3

Rats: Inflammatory lesions

Edema and inflammation at Level 2

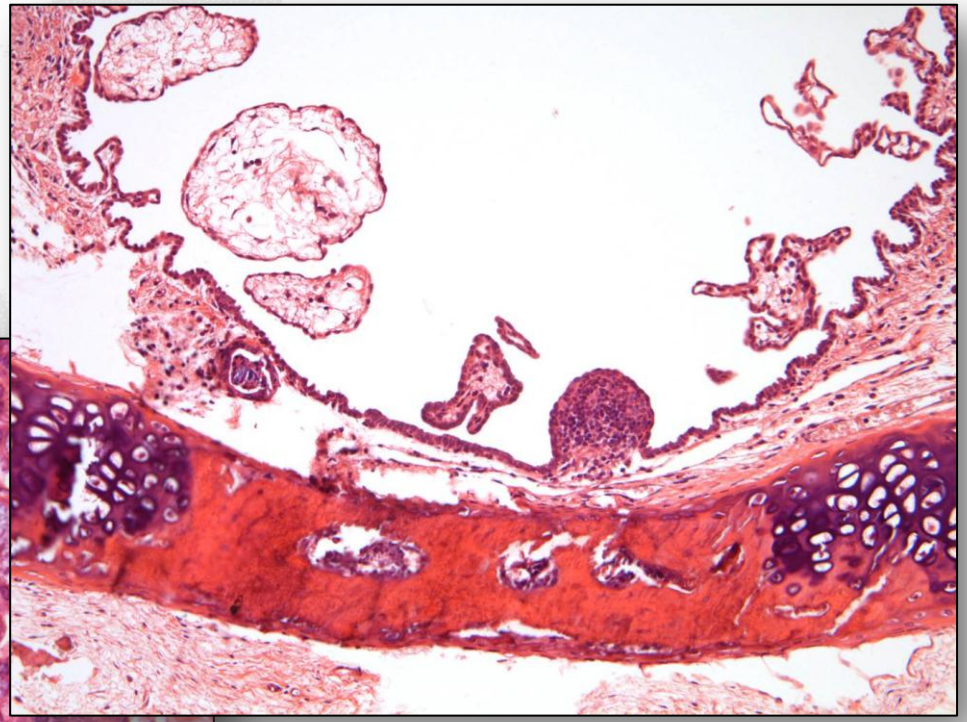


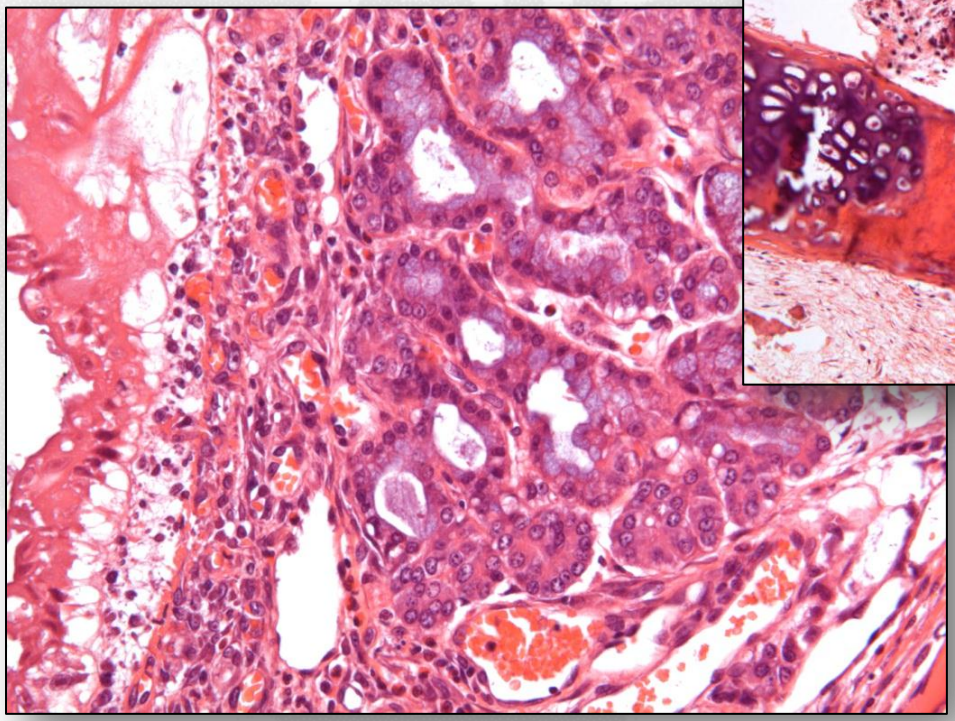


Edema and inflammatory infiltrate at Level 2

Rats: Inflammatory lesions

Mucosal necrosis, inflammation at Level 2

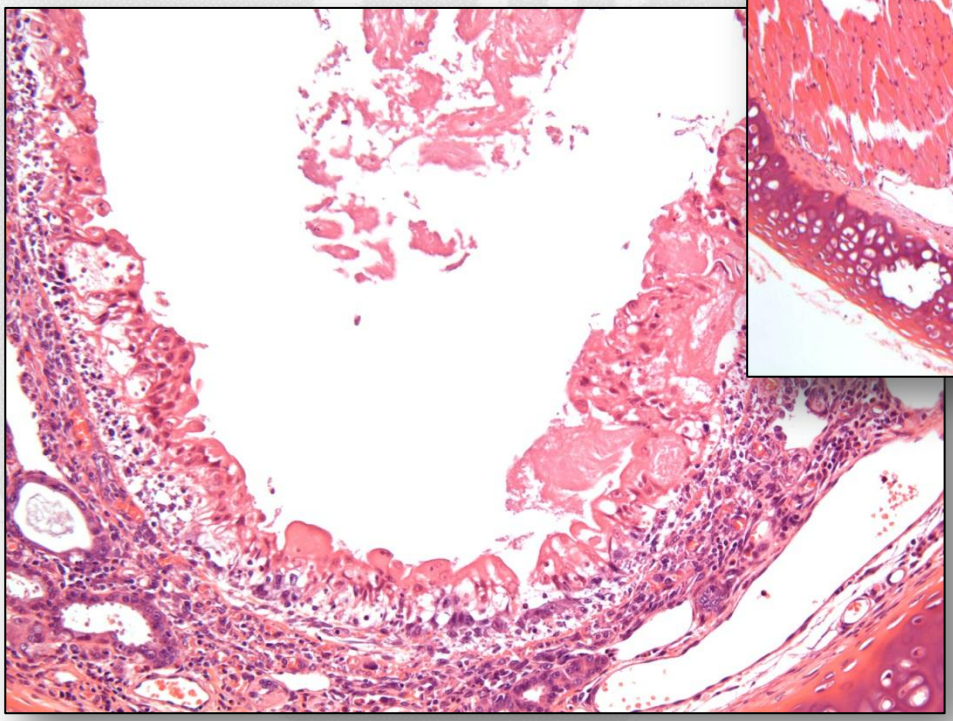


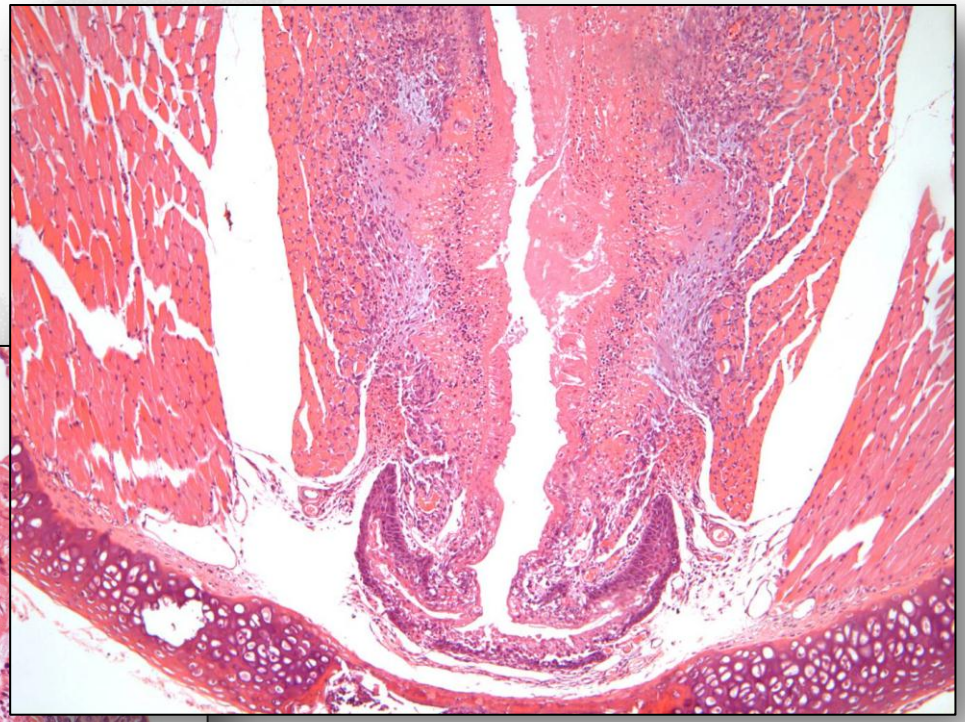


Submucosal inflammation chronic active at Level 3

Rats: Inflammatory lesions

Mucosal necrosis, Inflammation at Level 2

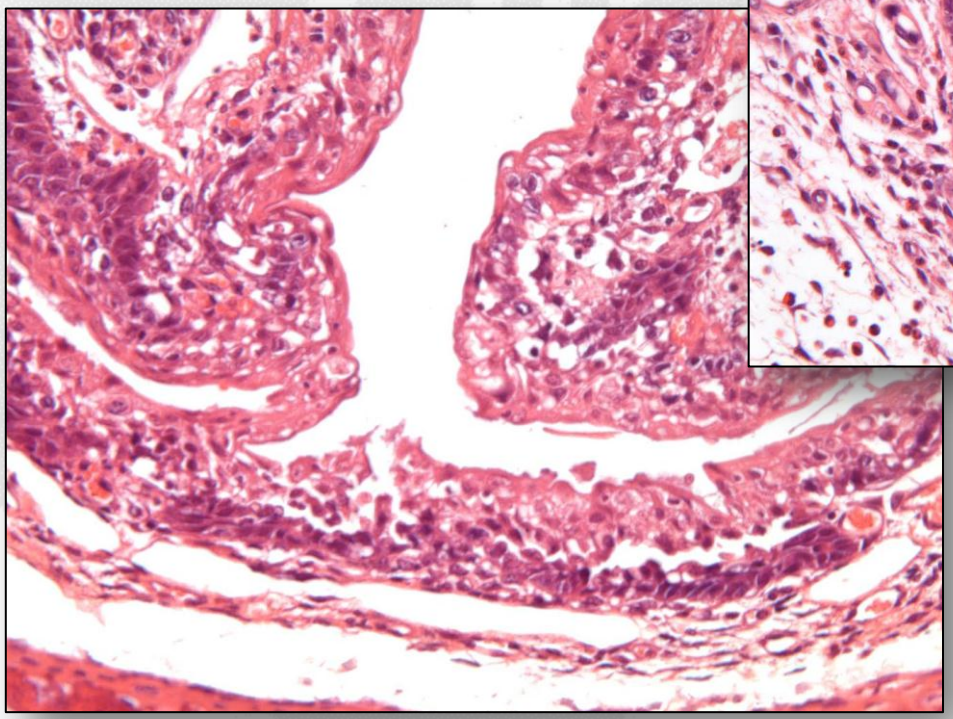


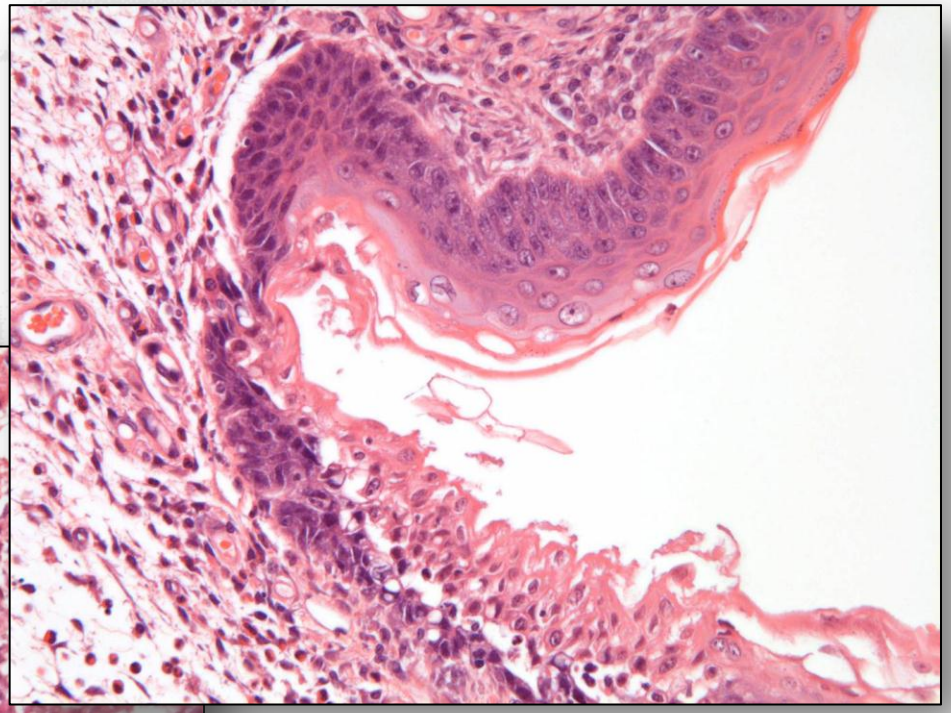


Mucosal necrosis at Level 2

Rats: Inflammatory lesions

Mucosal necrosis, Inflammation at Level 3

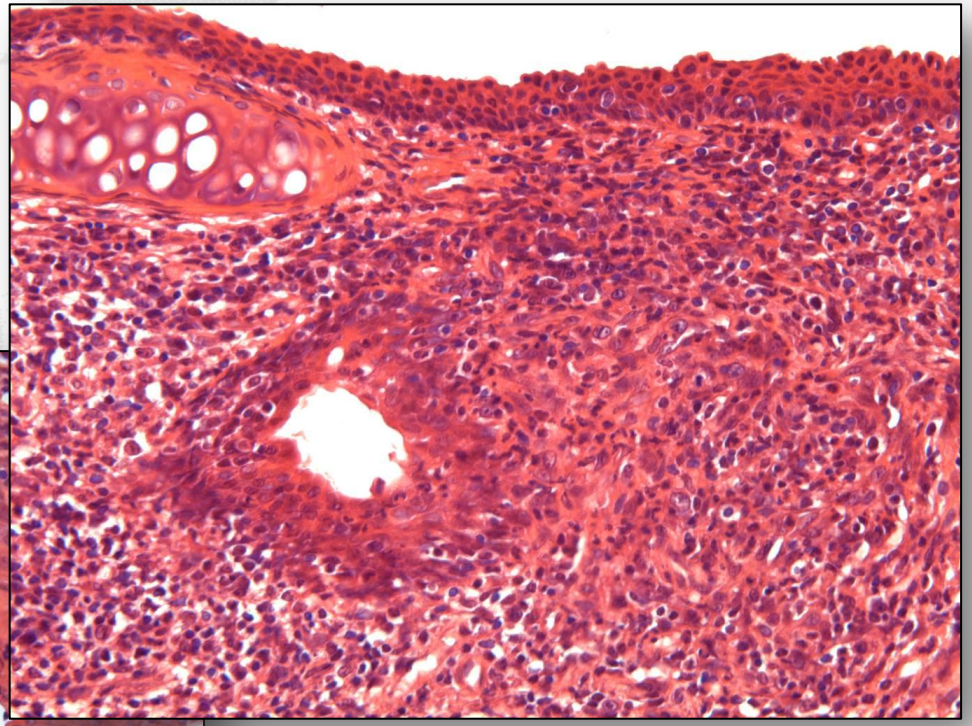


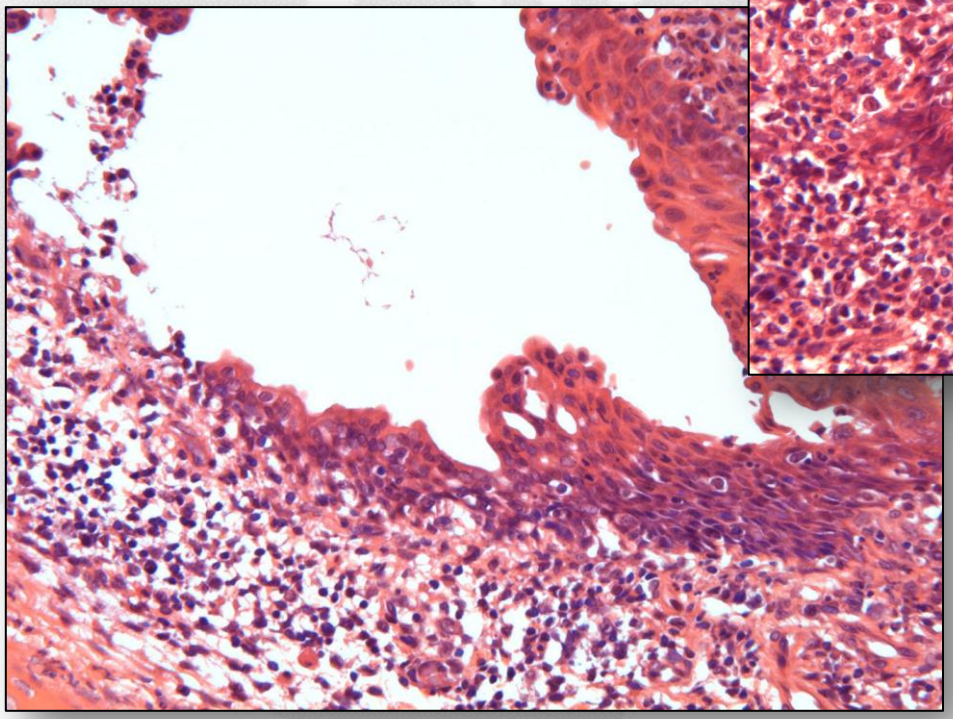


Mucosal necrosis and squamous metaplasia at Level 3

Rats: Inflammatory lesions

Mucosal necrosis, Inflammation, squamous metaplasia at Level 4

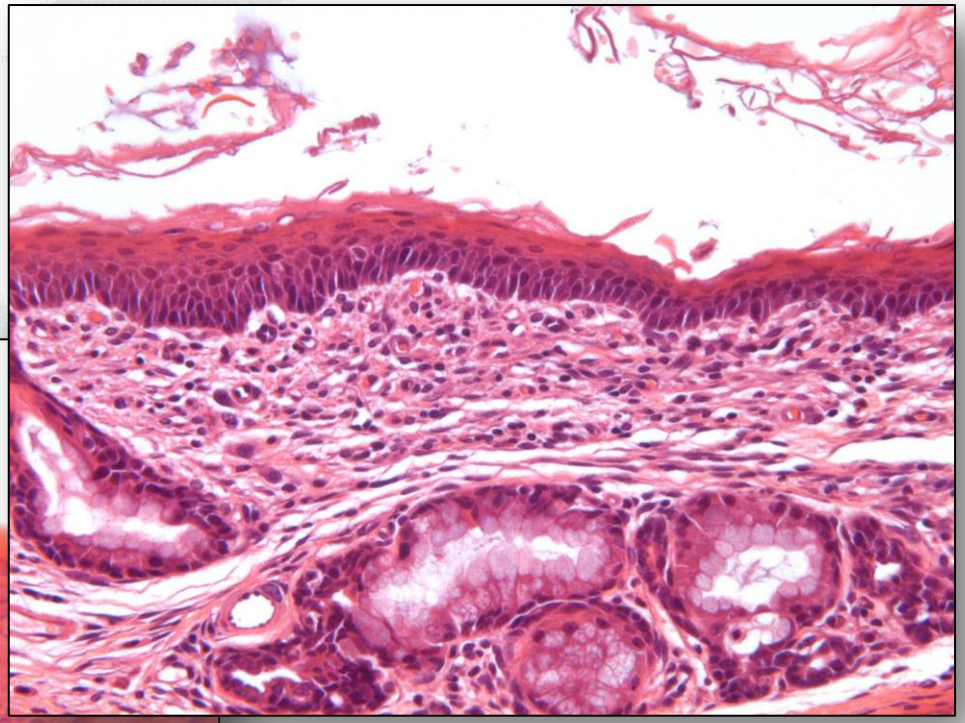


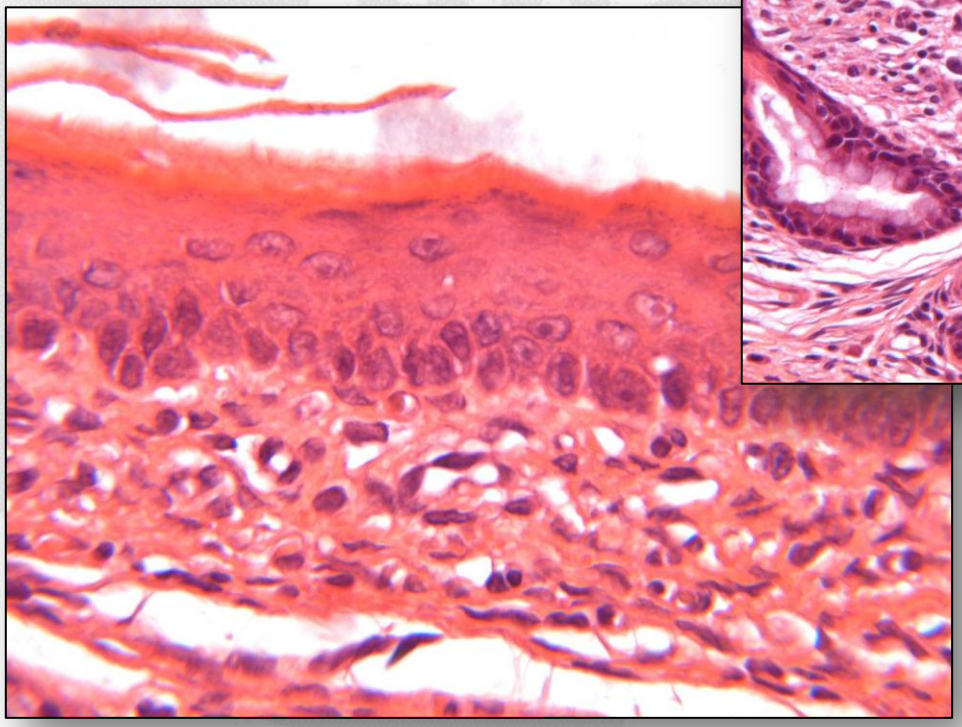


Inflammation and squamous metaplasia at Level 5

Rats: Reactive lesions

Squamous metaplasia with keratosis at Level 6

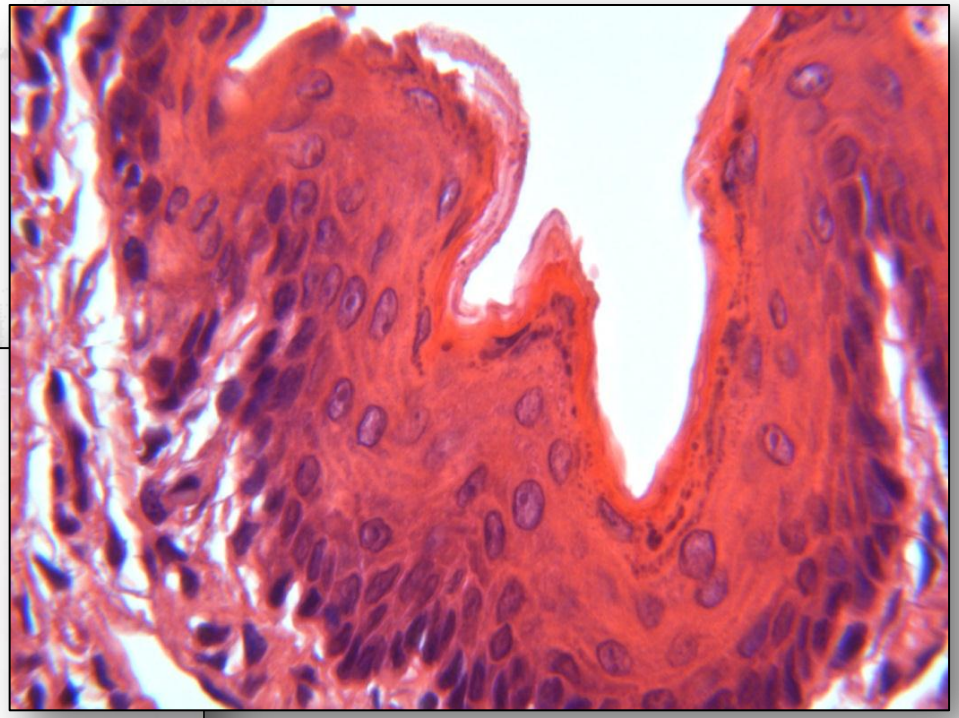


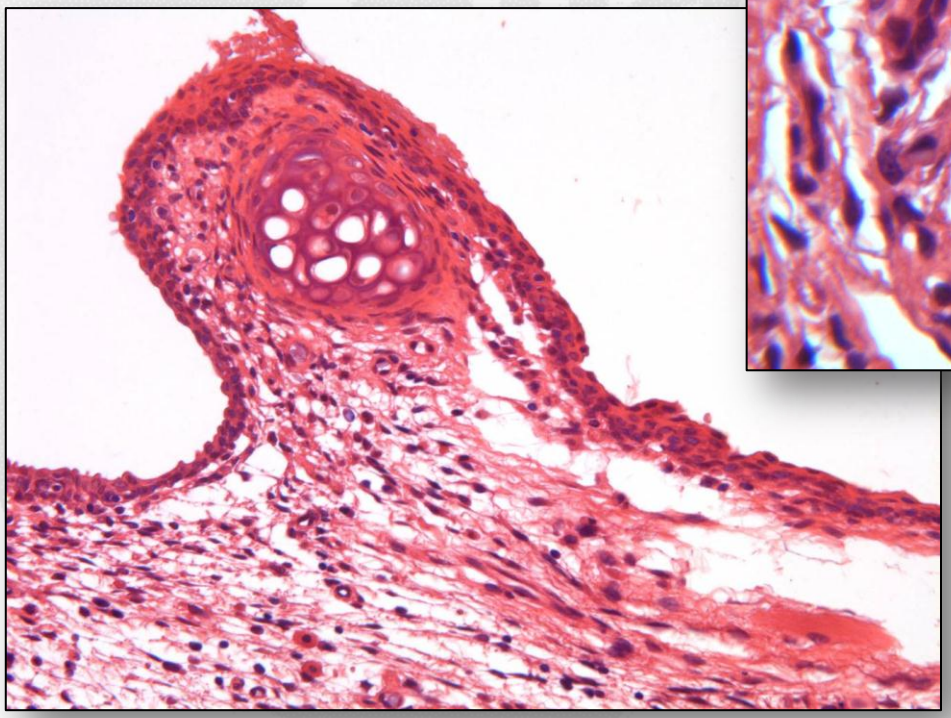


Submucosal Inflammation, squamous metaplasia and epithelial disorganization at Level 6

Rats: Reactive lesions

Squamous metaplasia at Level 3



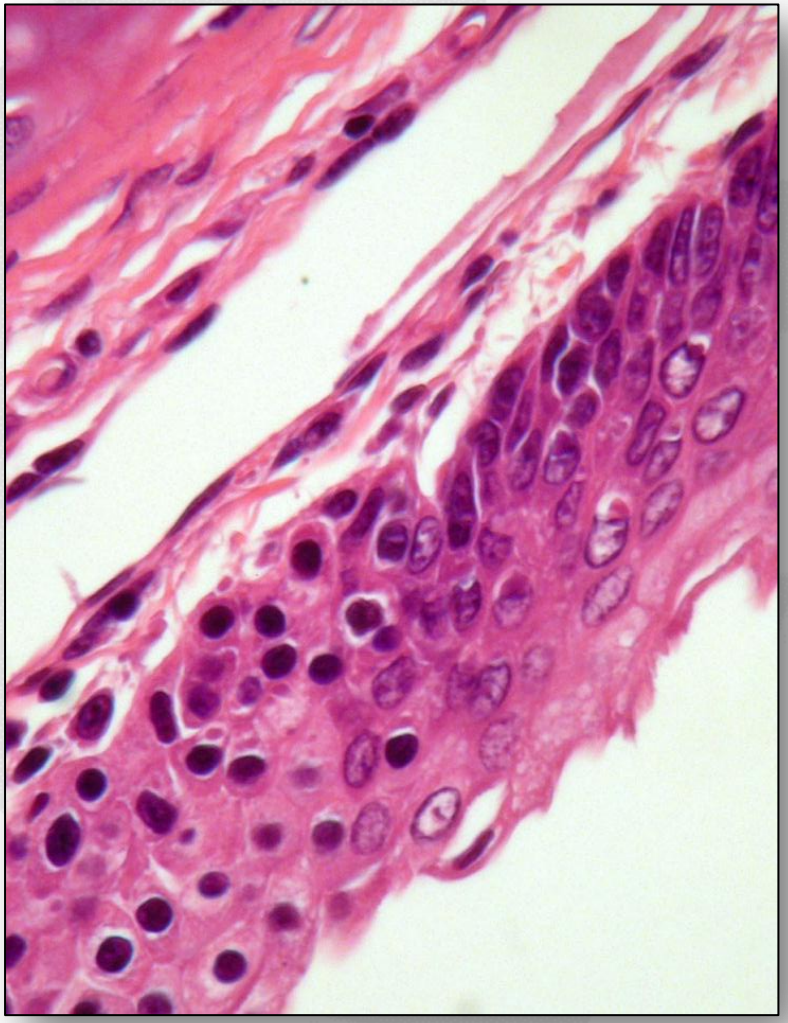


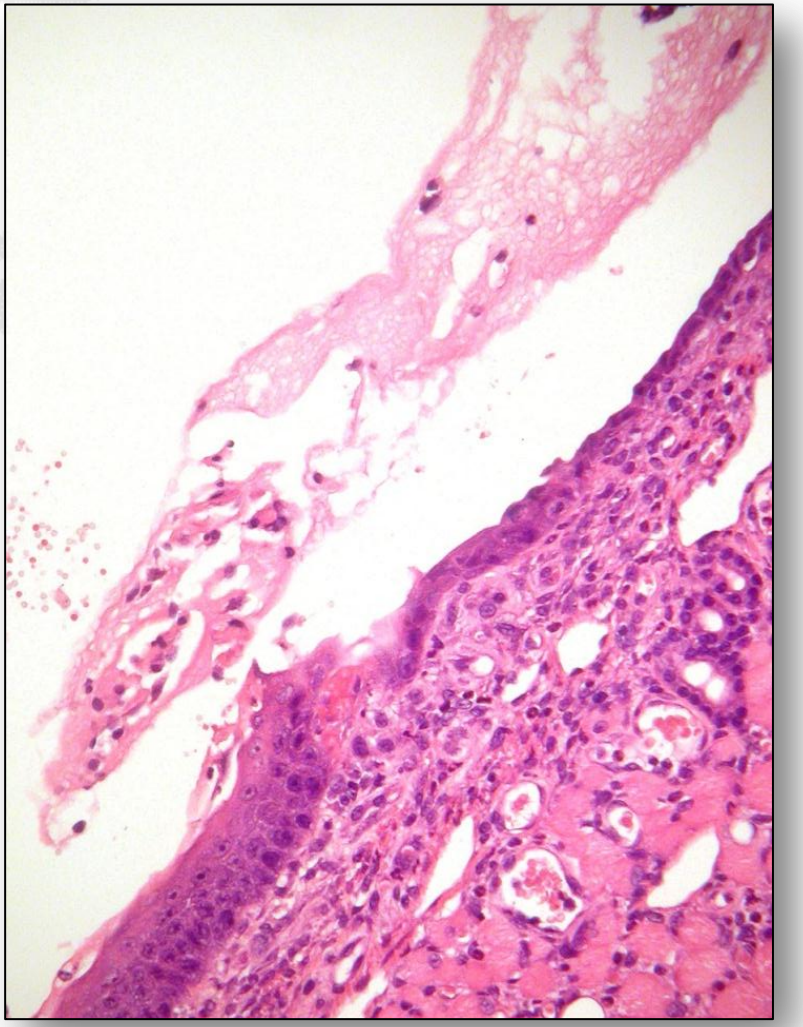
Squamous metaplasia and hyperplasia at Level 3

Rat: Inhalation Studies Longitudinal Sections: Induced Lesions

Rats: Degenerative lesions

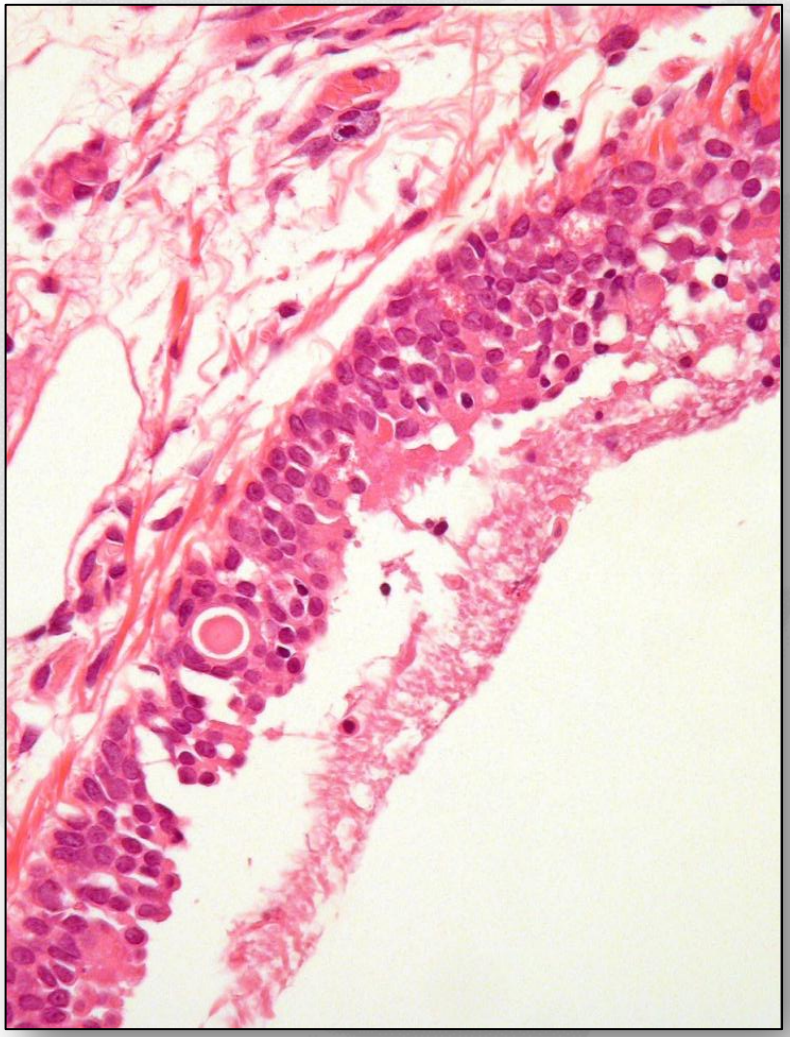
Squamous metaplasia, degeneration/disorganization of respiratory epithelium



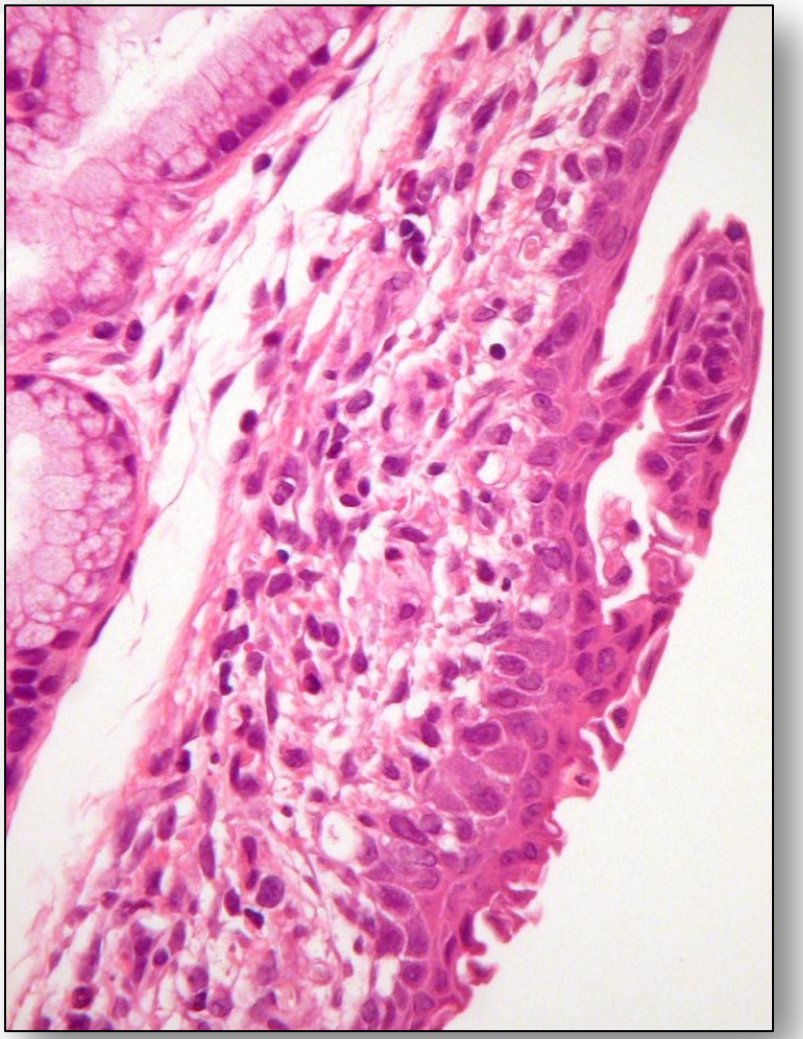


Rats: Inflammatory and reactive lesions

Necrosis



Squamous metaplasia and hyperplasia



Rat: Non-Inhalation Studies
Published Case in F344

Courtesy of
Dr. Paul-Georg German
Nycomed

Histopathology of daily gavaged F344-rats

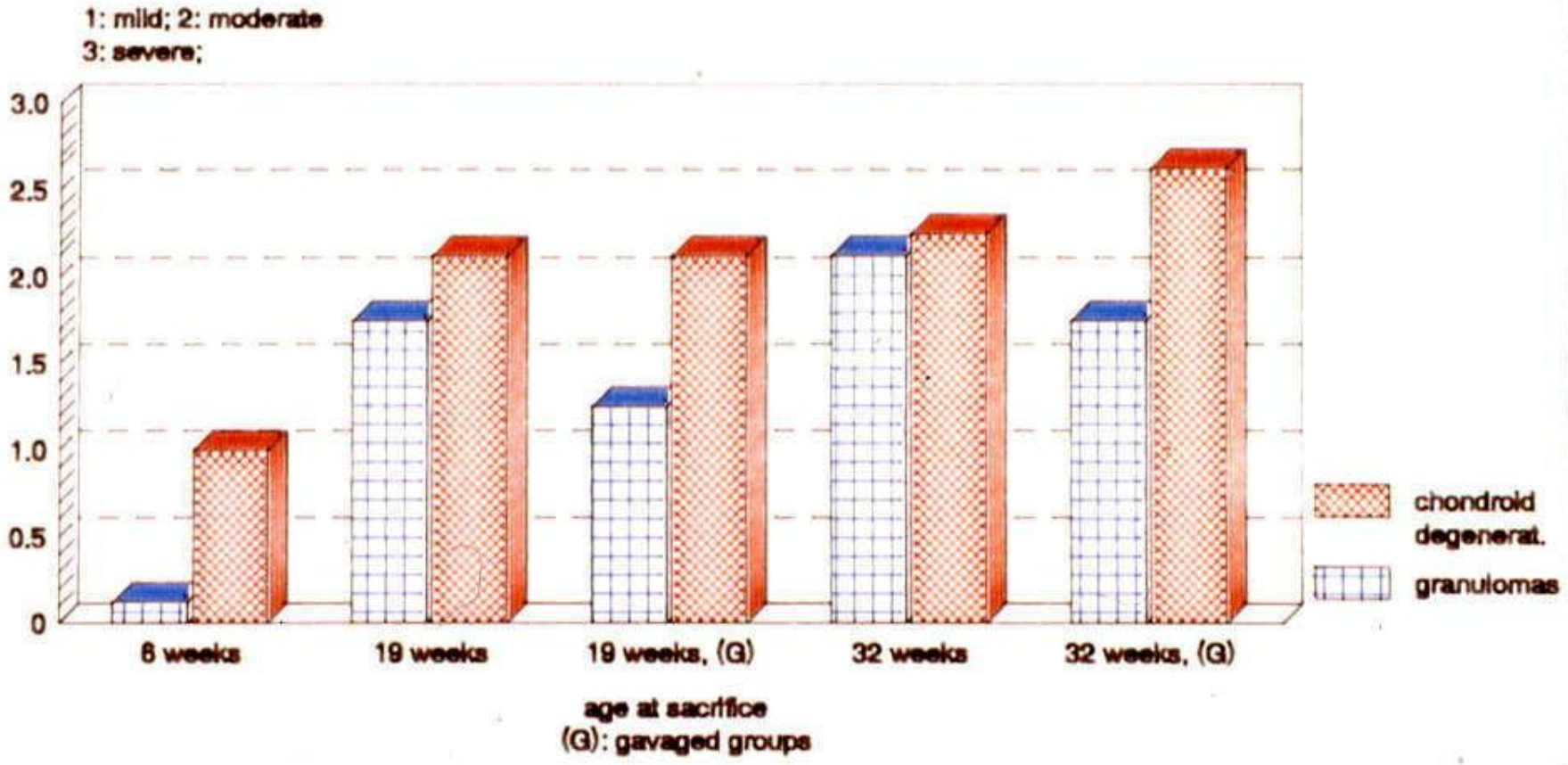
F344, n:10, age:m:51,F:45 wks, 5f/5m,	%
Foreign body granuloma in the oropharynx	78
Deformation of the tracheal cartilage	80
Papillary projections	70
Food or bedding in the oropharynx or trachea	0

Material: Comparison of strains

strain of rats	n	%	mean age (wk)
Sprague Dawley	42	7,6	42
Fischer 344	350	63,1	77
Wistar	99	17,9	20
Brown-Norway	33	6,0	9
Dark Agouti	17	3,0	98
Lewis	16	2,8	92
total	554	100	

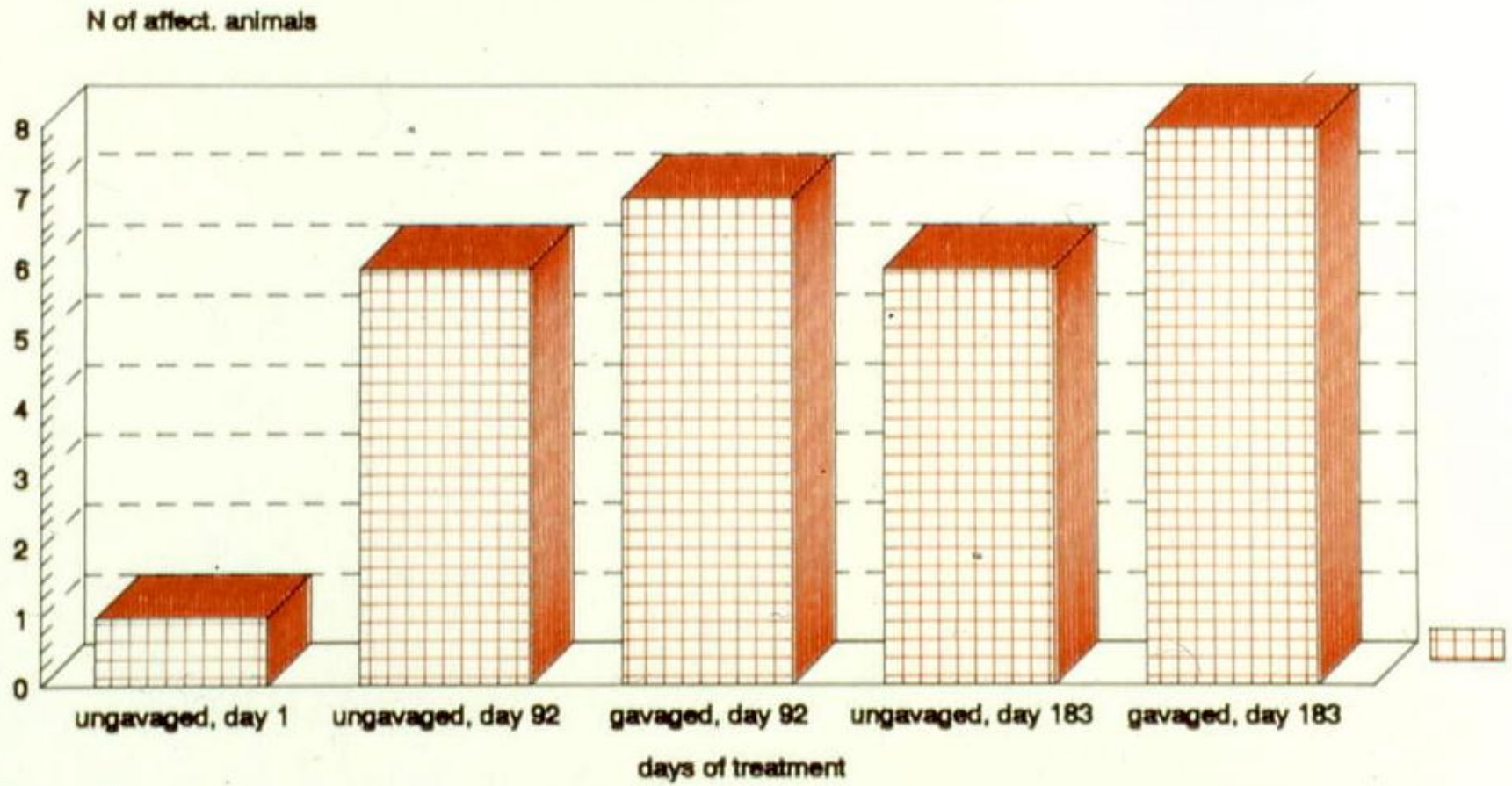
Grade of oropharyngeal granuloma & tracheal cartilage degeneration

mean: semiquantitat.grading /n per group



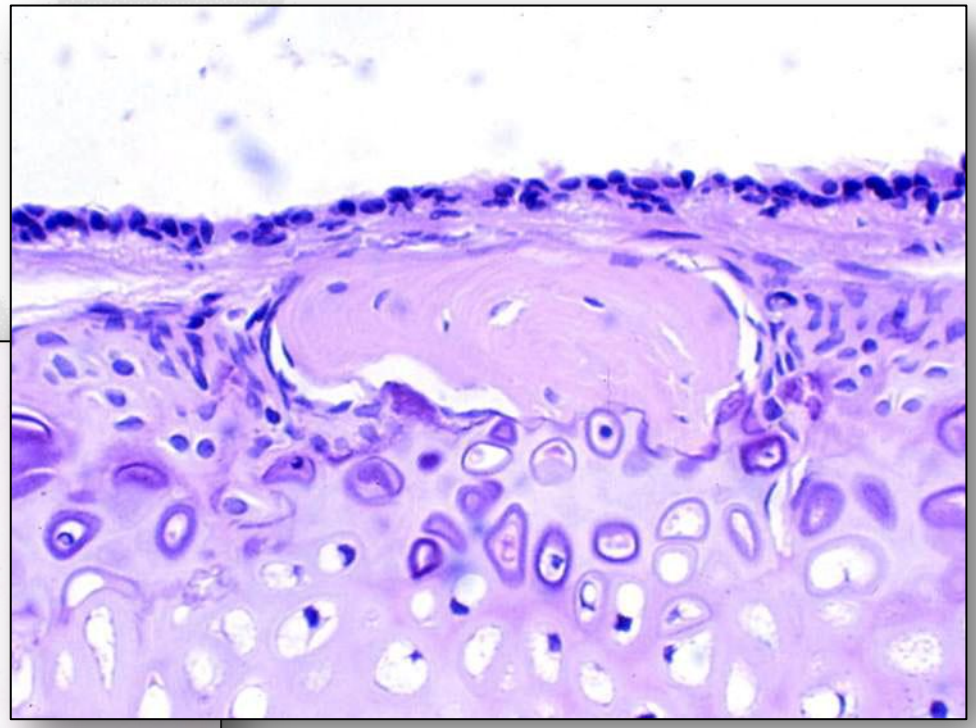
Comparison of gavaged and ungavaged F344 rats

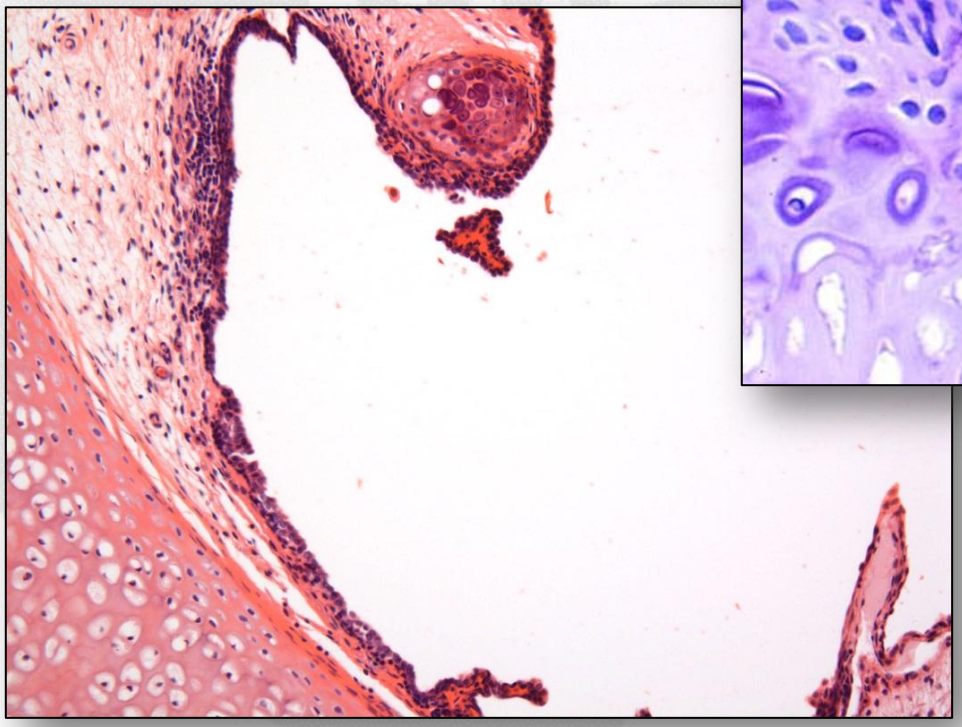
Granulomas in the orolaryngeal region



Rats: Spontaneous degenerative lesions

Degeneration of arythe-Noid cartilage (cystic necrosis, sequestration, deformation)

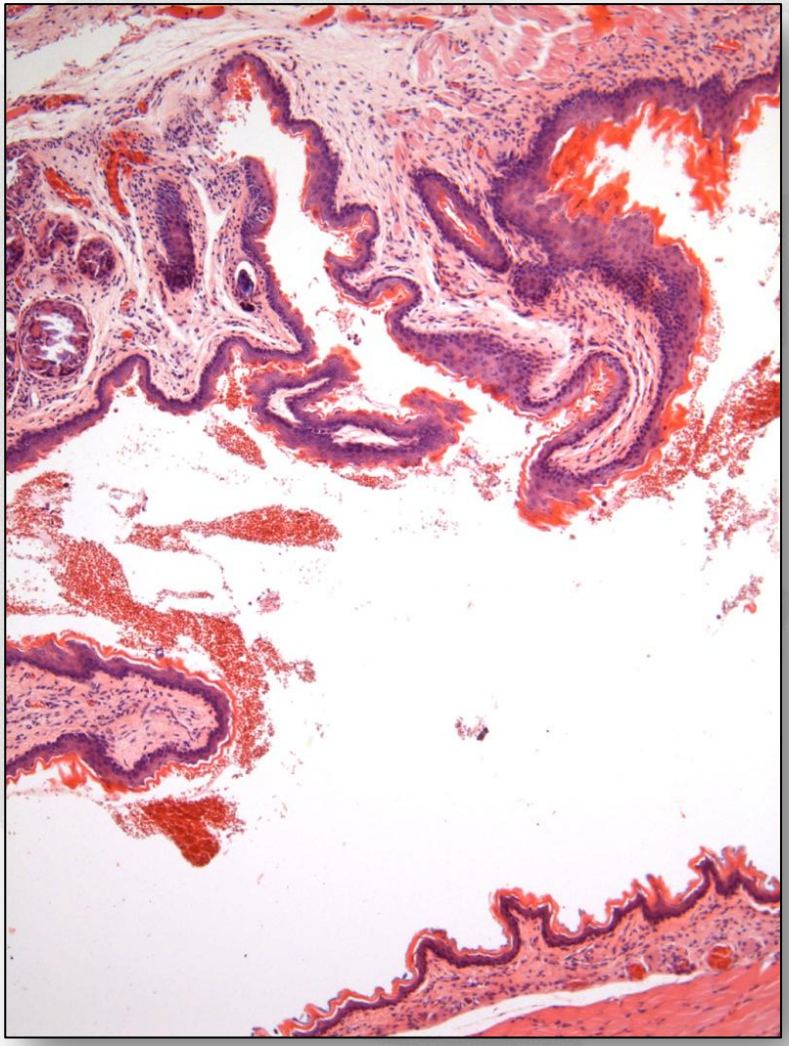


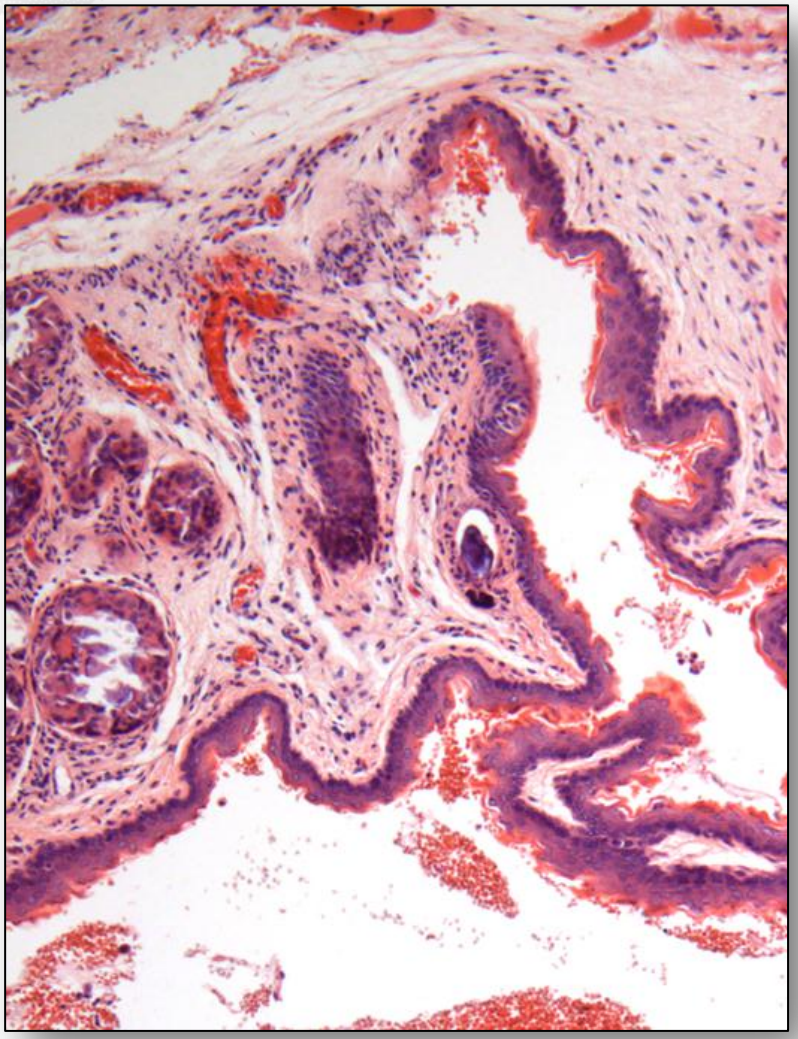


Osseous metaplasia in tracheal cartilage

Rats: Spontaneous degenerative lesions

Mineralization and cranuloma formation in submucosal seromucinous glands

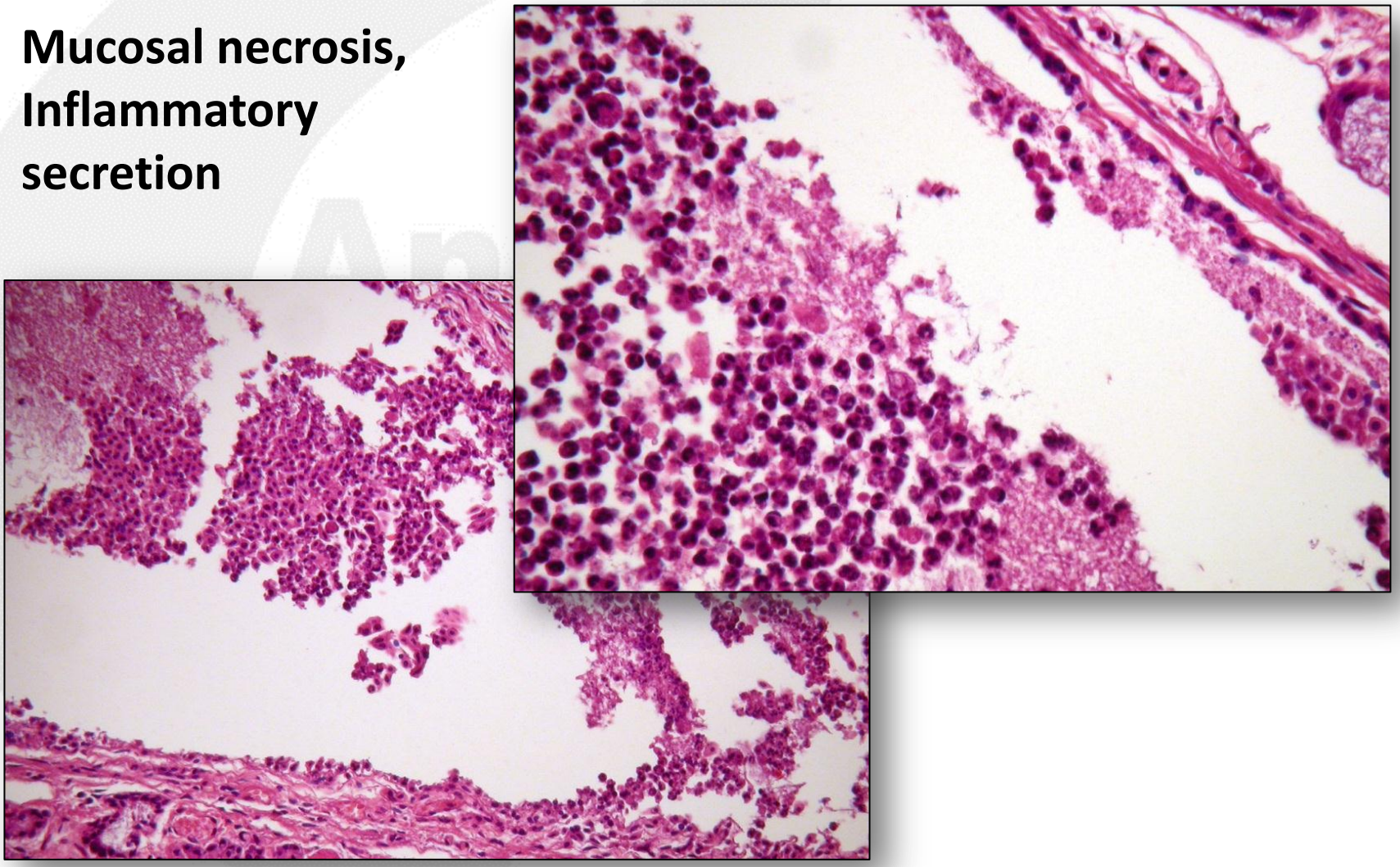




Rat: Further Lesions in Non-Inhalation Studies: Regurgitation Laryngitis

Rats: Regurgitation laryngitis

Mucosal necrosis, Inflammatory secretion



Rats: Summary

- Background lesions are not too rare
- Strain-dependent
- Trimming technique-dependent
- Some lesions increase in incidence with age
- At different ages is gender-preference
- No influence by vehicle (except by regurgitation)
- Induced lesions differ from background alterations by range of degenerative/inflammatory lesions

Rats: Summary

- **Squamous metaplasia is a consistent induced lesion (reversible)**
- **is not a preneoplastic lesion but indicator for specific sensitivity of rodent larynx**

- **Spontaneous neoplastic lesions extremely rare including mestastases**

Hamster: Inhalation Study

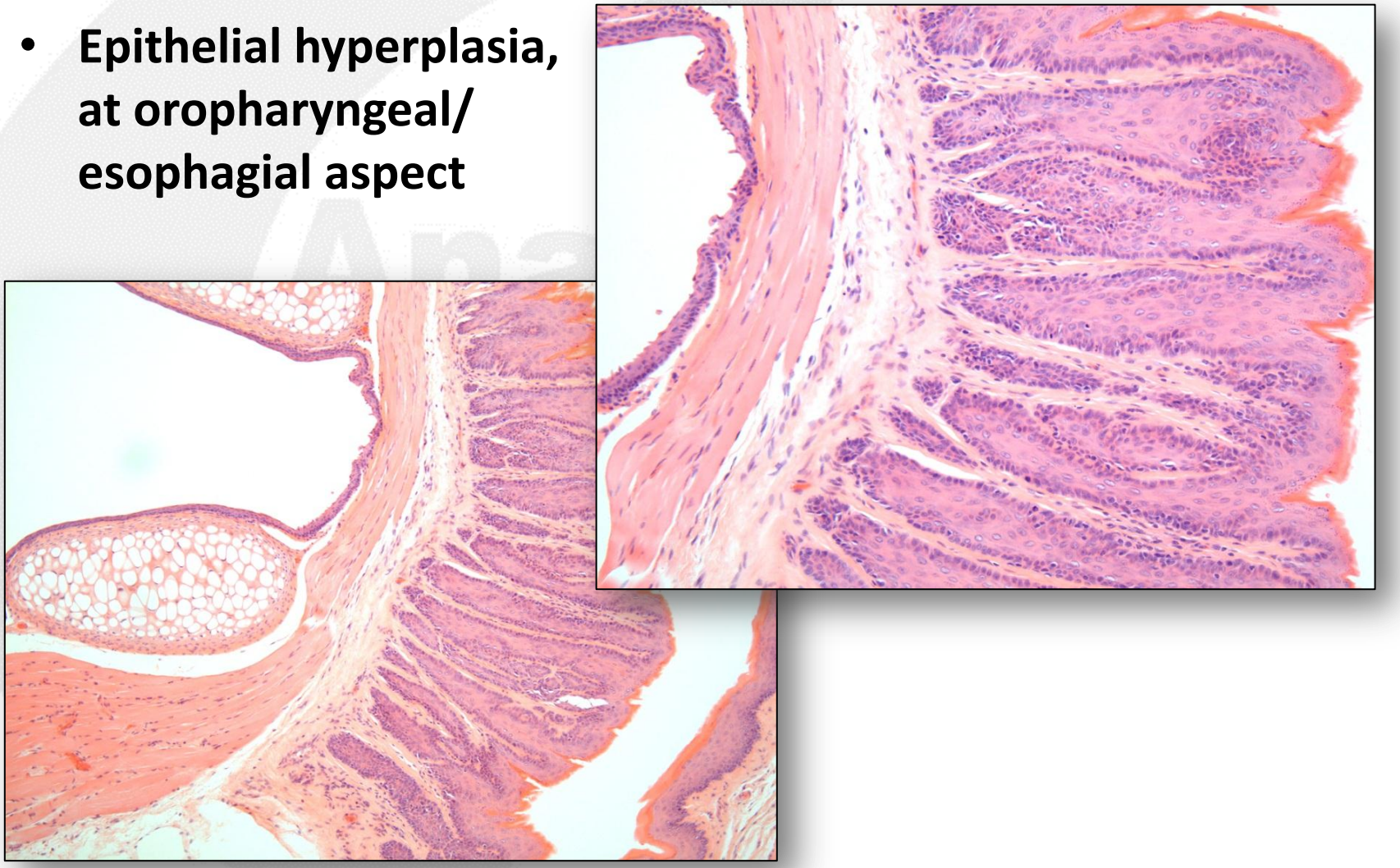
Hamster: Incidence in feeding study

- LakLVG(SYR)BR Outbred VAF/Plus
- 104-Week, Feeding

Lesion	Male	Female
Inflammation	6.0 %	12.0 %
Squamous metaplasia	1.0 %	0.3 %
Squamous carcinoma	0.3 %	

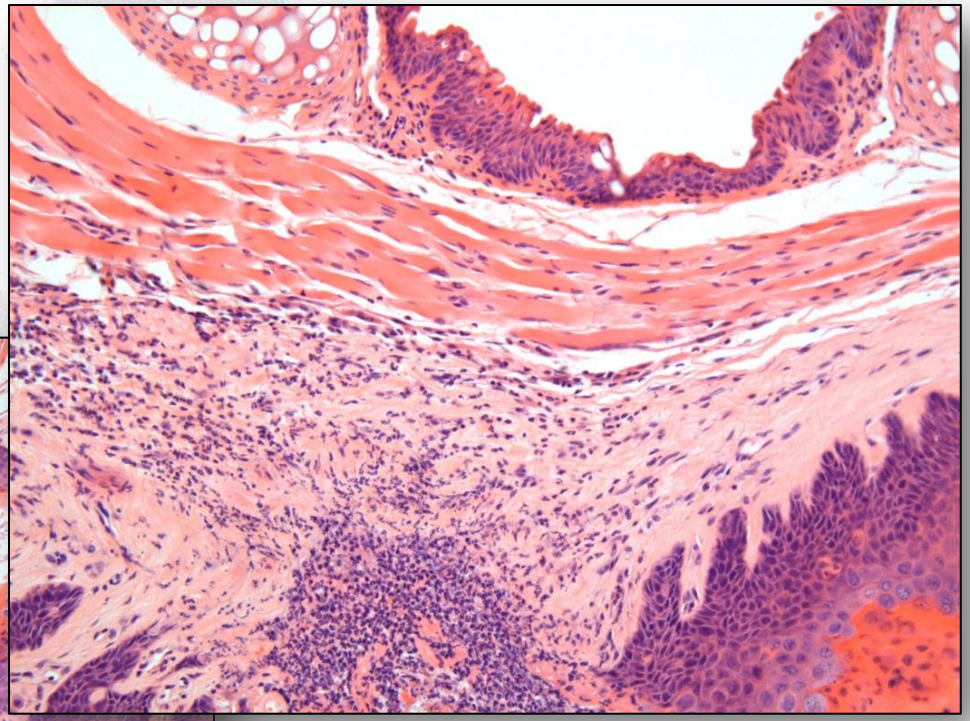
Hamster: Hyperplastic lesion

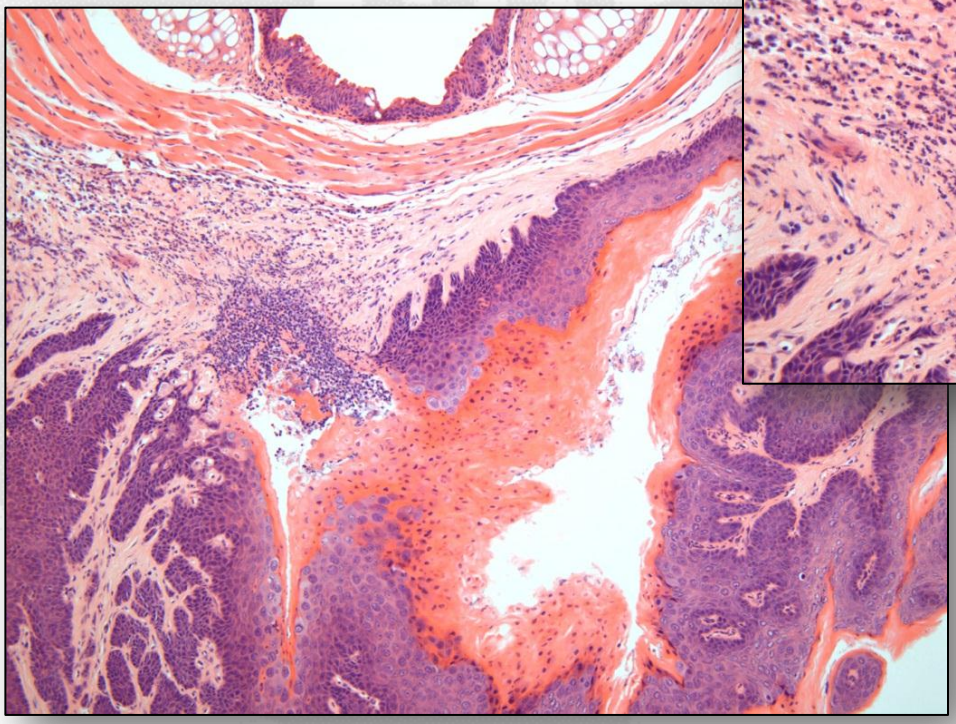
- **Epithelial hyperplasia, at oropharyngeal/ esophagial aspect**



Hamster: Neoplastic lesion

- **Squamous carcinoma at oropharyngeal/ esophagial aspect**





Mice: Inhalation Studies

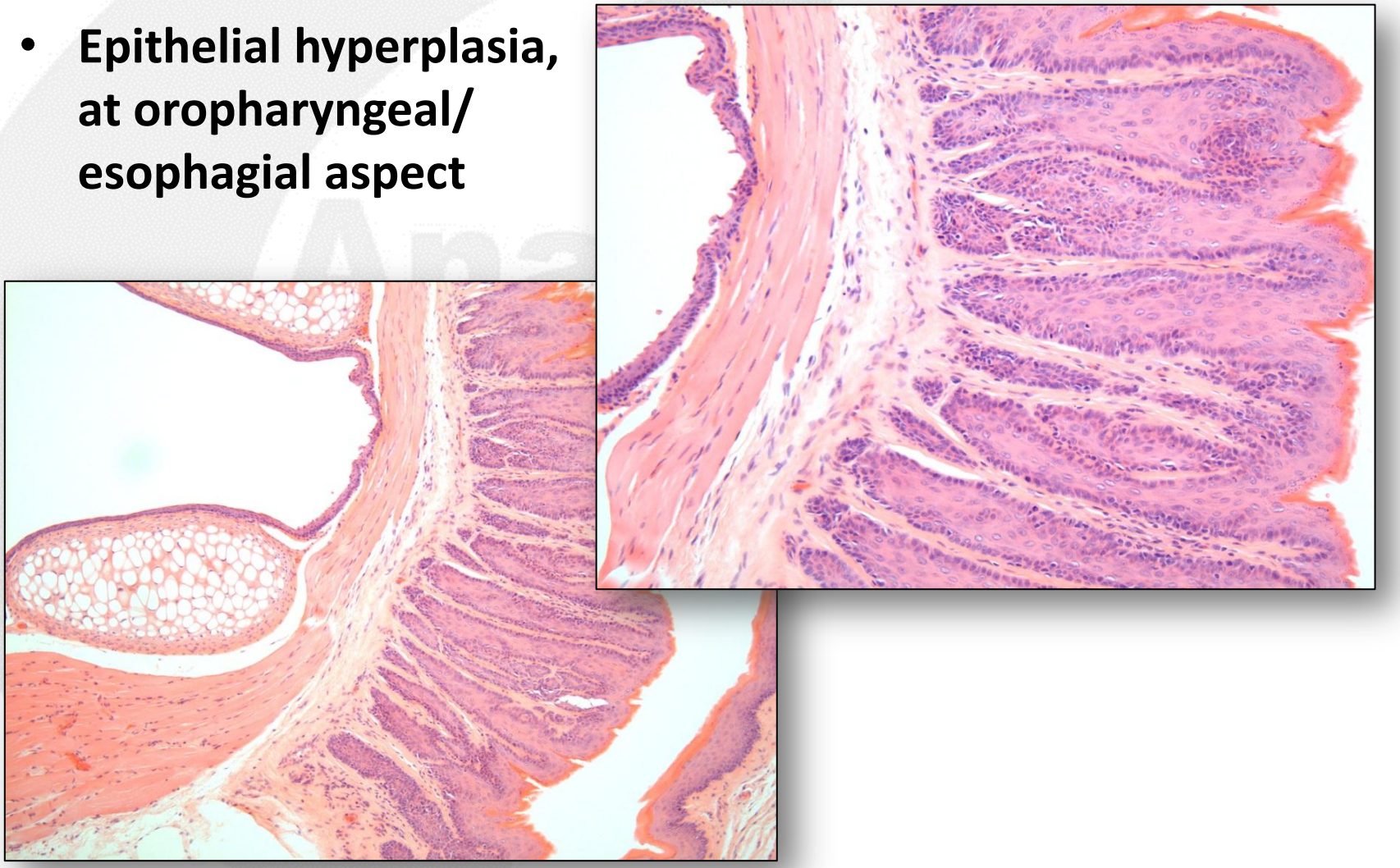
Materials and Methods

- **Sectiond horizontolongitudinal or transversal (as in rat according to Sagartz et al., 1992)**

- **Acute: 1 longitudal (negative)**
- **4-Week: 1 longitudinal (negative)**
- **13-Week: 2 longitudinal (all negative)**
- **104-Week: 1 transversal (negative)**

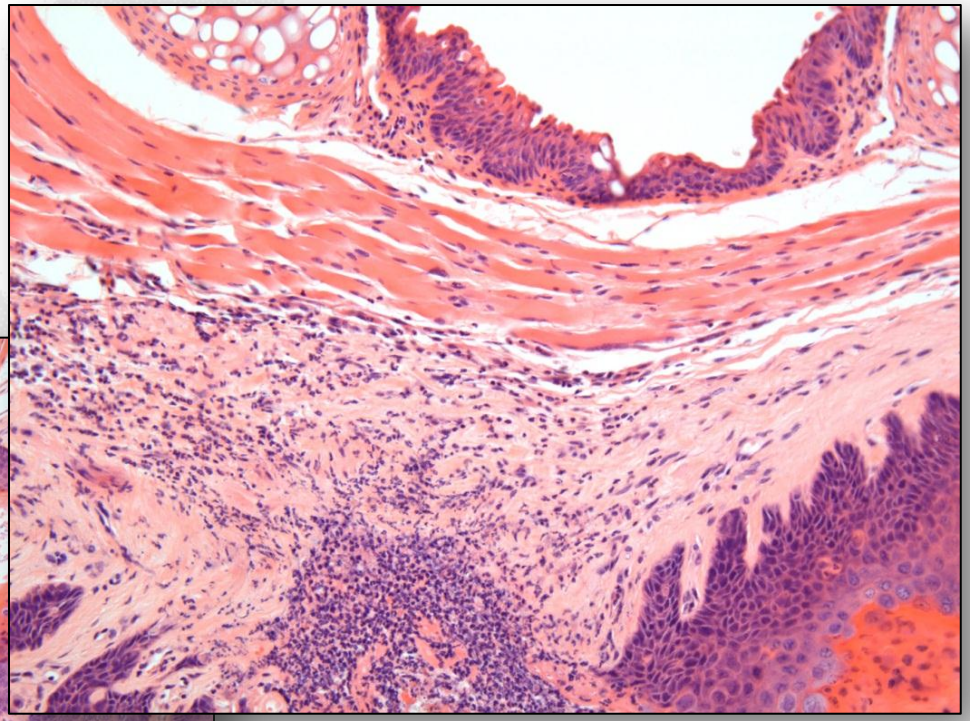
Hamster: Hyperplastic lesion

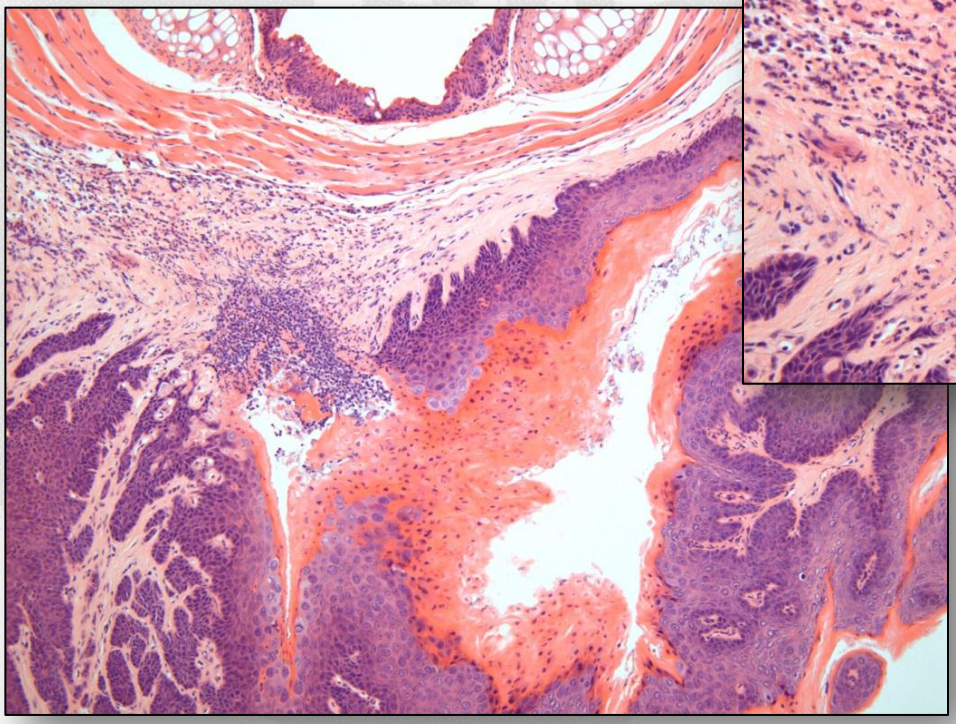
- **Epithelial hyperplasia, at oropharyngeal/ esophagial aspect**



Hamster: Neoplastic lesion

- **Squamous carcinoma at oropharyngeal/ esophagial aspect**





Mice: Inhalation Studies

Materials and Methods

- **Sectiond horizontolongitudinal or transversal (as in rat according to Sagartz et al., 1992)**

- **Acute: 1 longitudal (negative)**
- **4-Week: 1 longitudinal (negative)**
- **13-Week: 2 longitudinal (all negative)**
- **104-Week: 1 transversal (negative)**

Mice: Non-Inhalation Studies

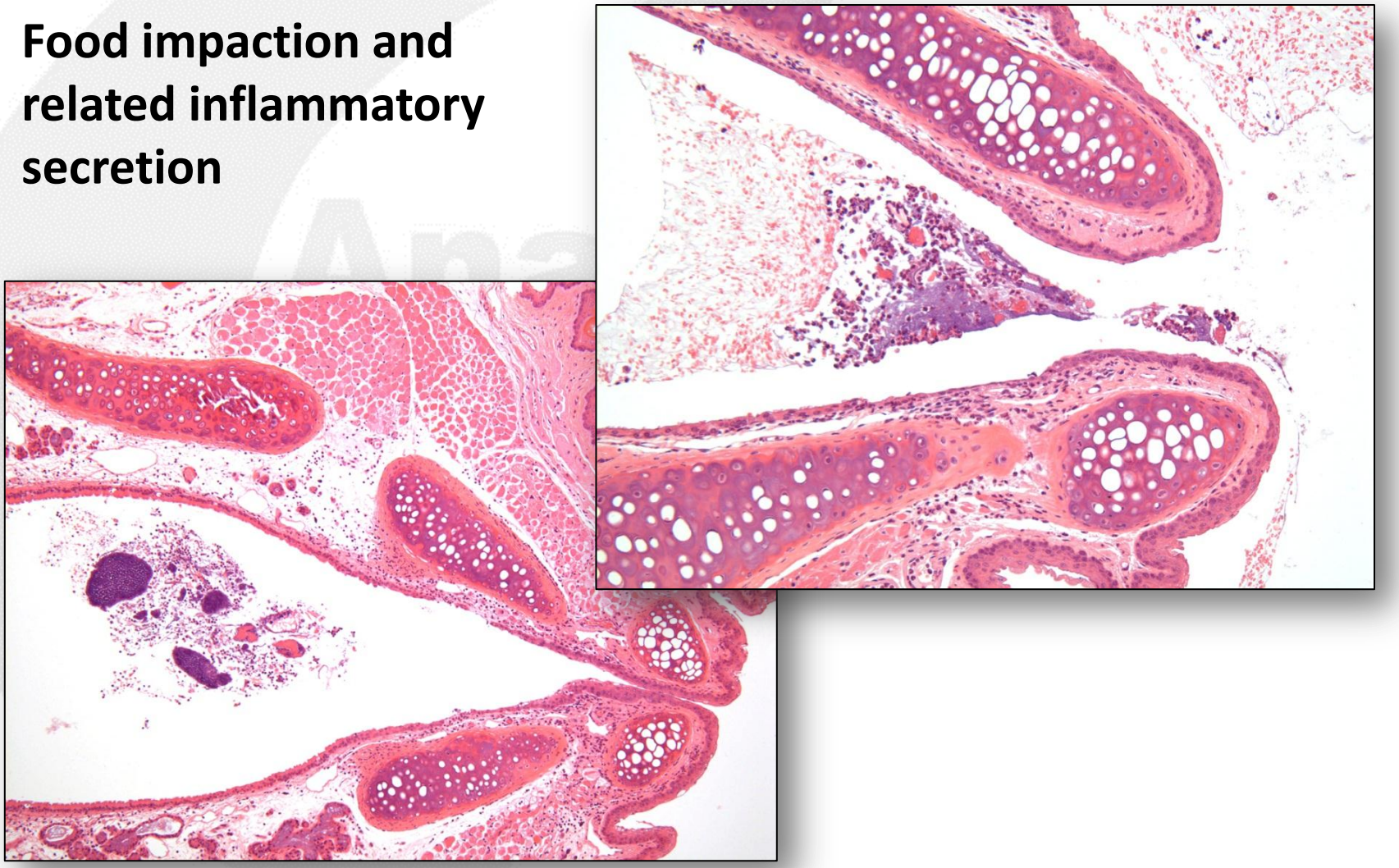
Materials and Methods

- All horizontolongitudinal sections

- 14-Day: 3 (Feeding)
- 4-Week: 1 (Gavage)
- 13-Week: 1 Gavage, 1 Dermal, 5 Feeding
- 26-Week: 1 Gavage, 1 s.c. , 52-Week: 2 feeding
- 78-Week: 6 Feeding
- 104-Week: 3 Feeding

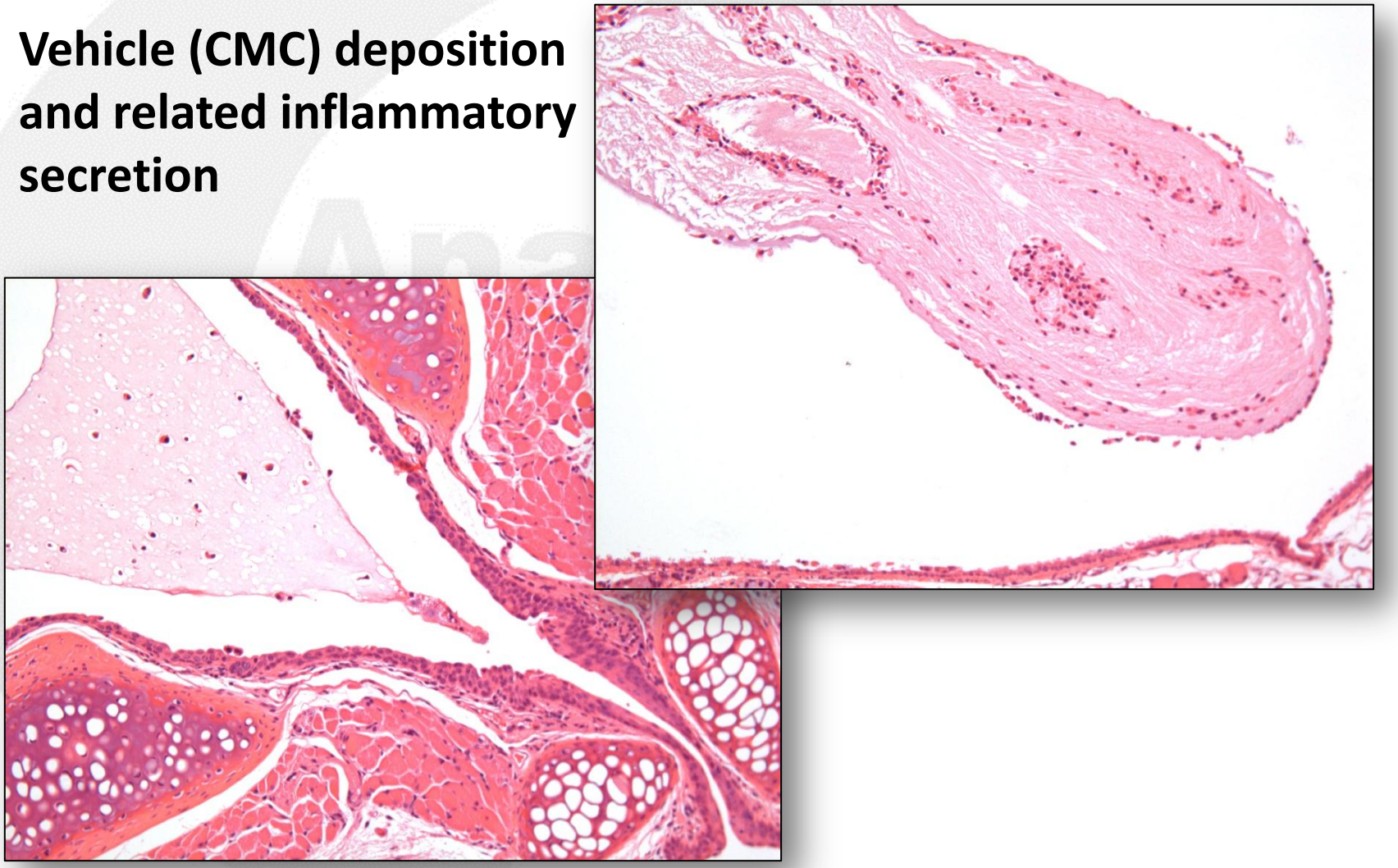
Aspiration, secretion

Food impaction and related inflammatory secretion



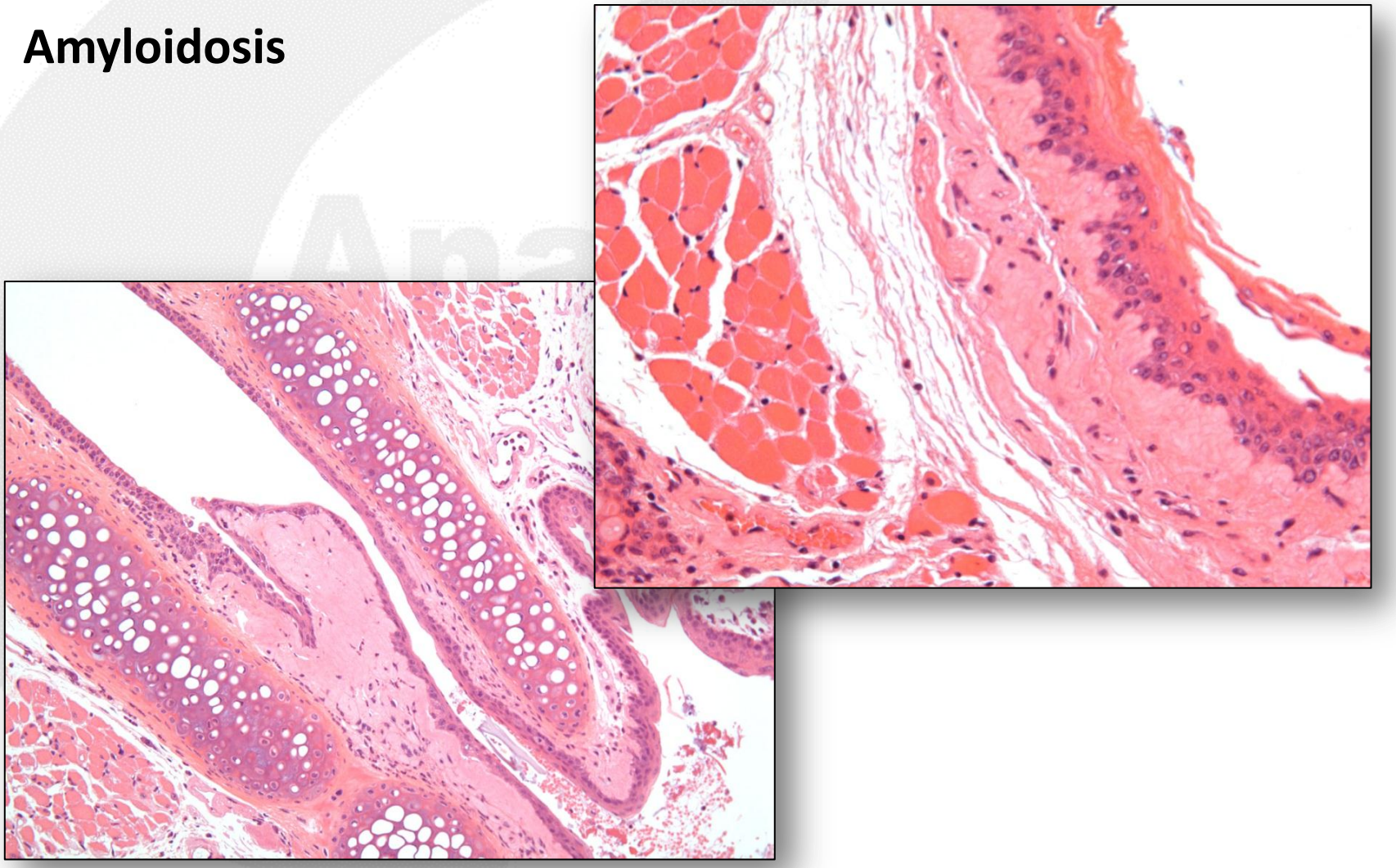
Vehicle

Vehicle (CMC) deposition and related inflammatory secretion



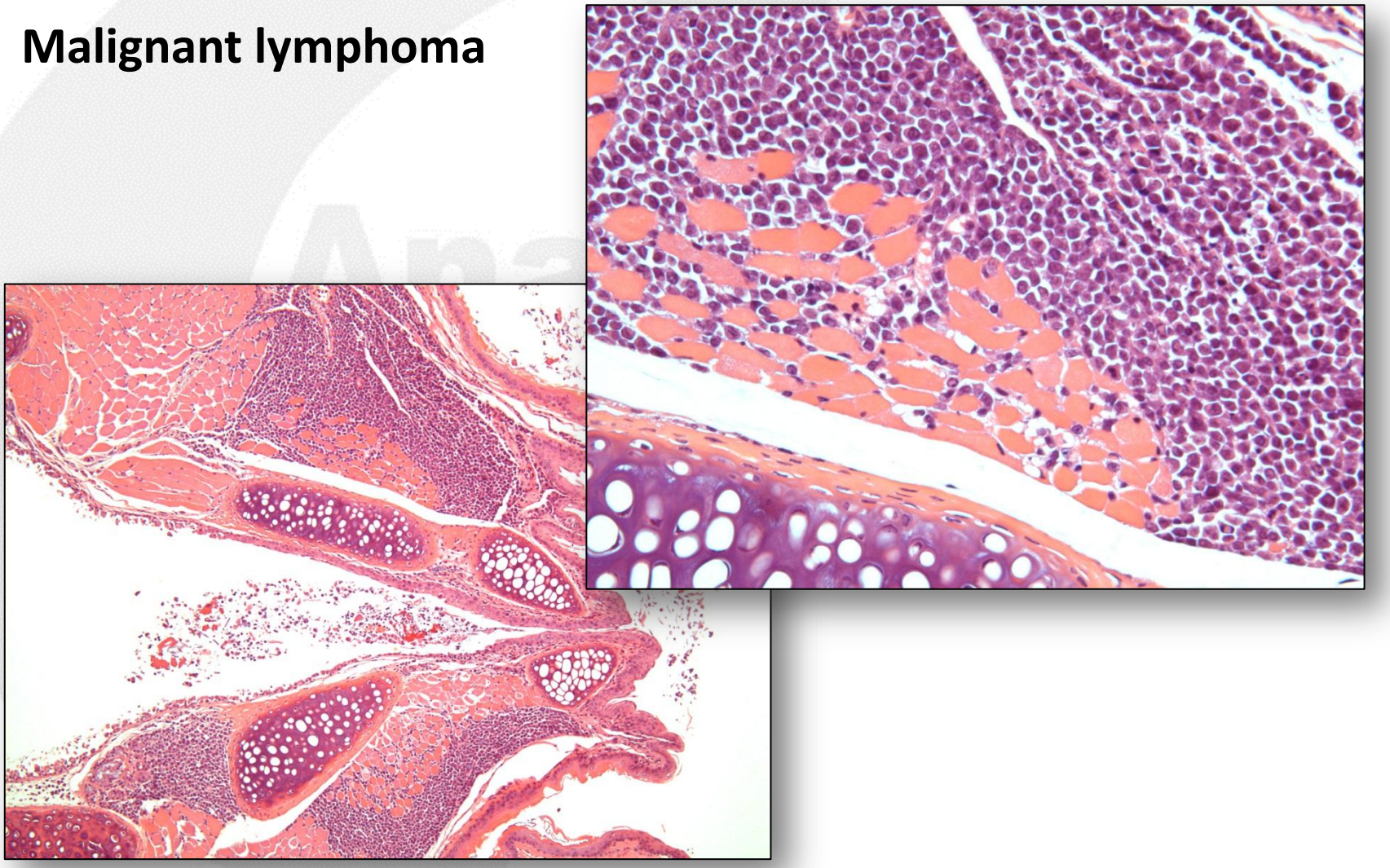
Spontaneous diseases

Amyloidosis



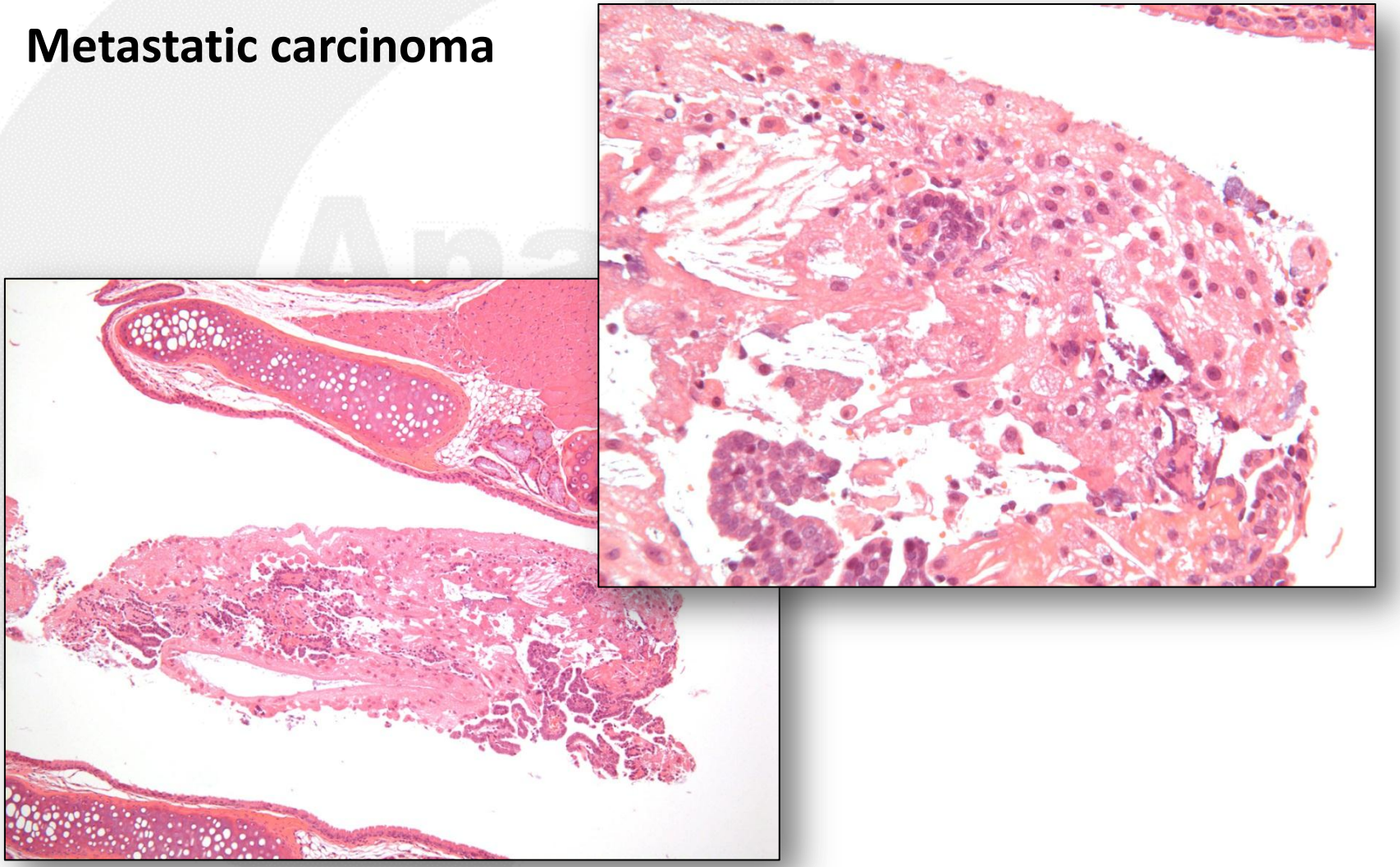
Neoplastic lesions

Malignant lymphoma



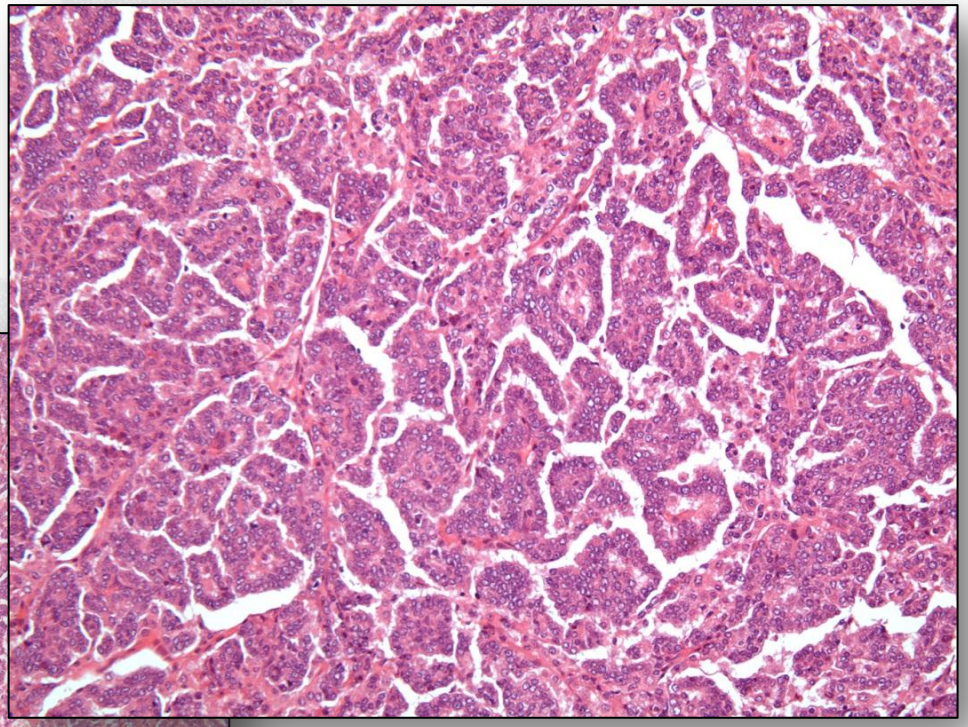
Neoplastic lesions

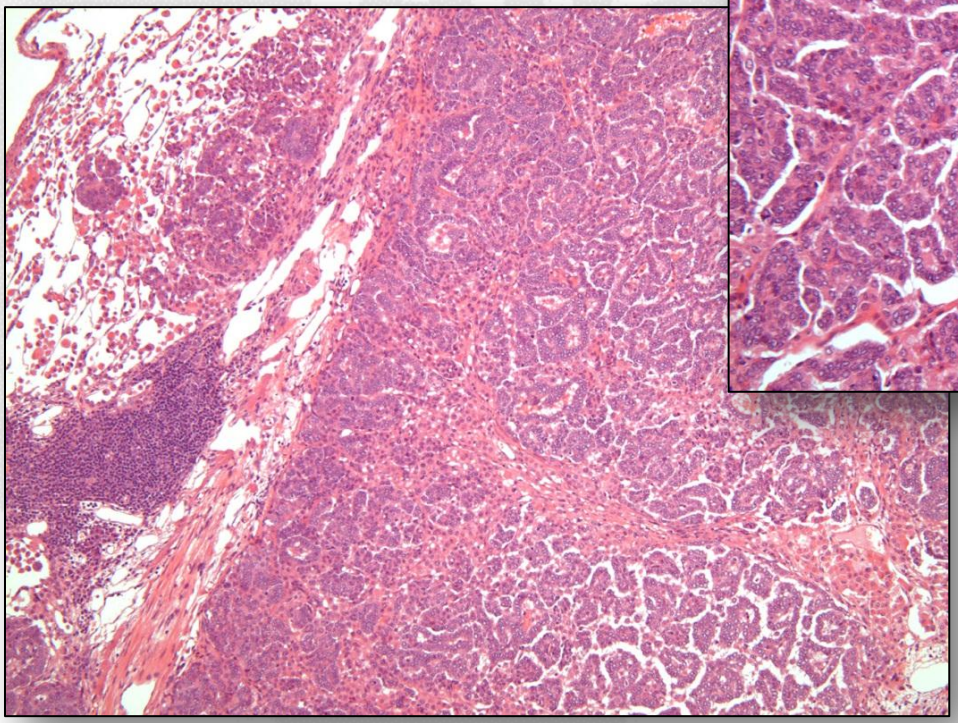
Metastatic carcinoma



Neoplastic lesions

Origin of metastasis:
Adenocarcinoma of lung





Mice: Summary

- Lesions are very rare
- No influence of vehicle (except by regurgitation)
- Lesions in inhalation and non-inhalation studies are similar
- Induced lesions were not recorded
- No age-related lesion in toxicology studies recorded, except general age-related disease and systemic neoplasia

Mice: Summary

Acute to 26-Week:

- **mainly hemorrhage, food impaction, glandular dilation**

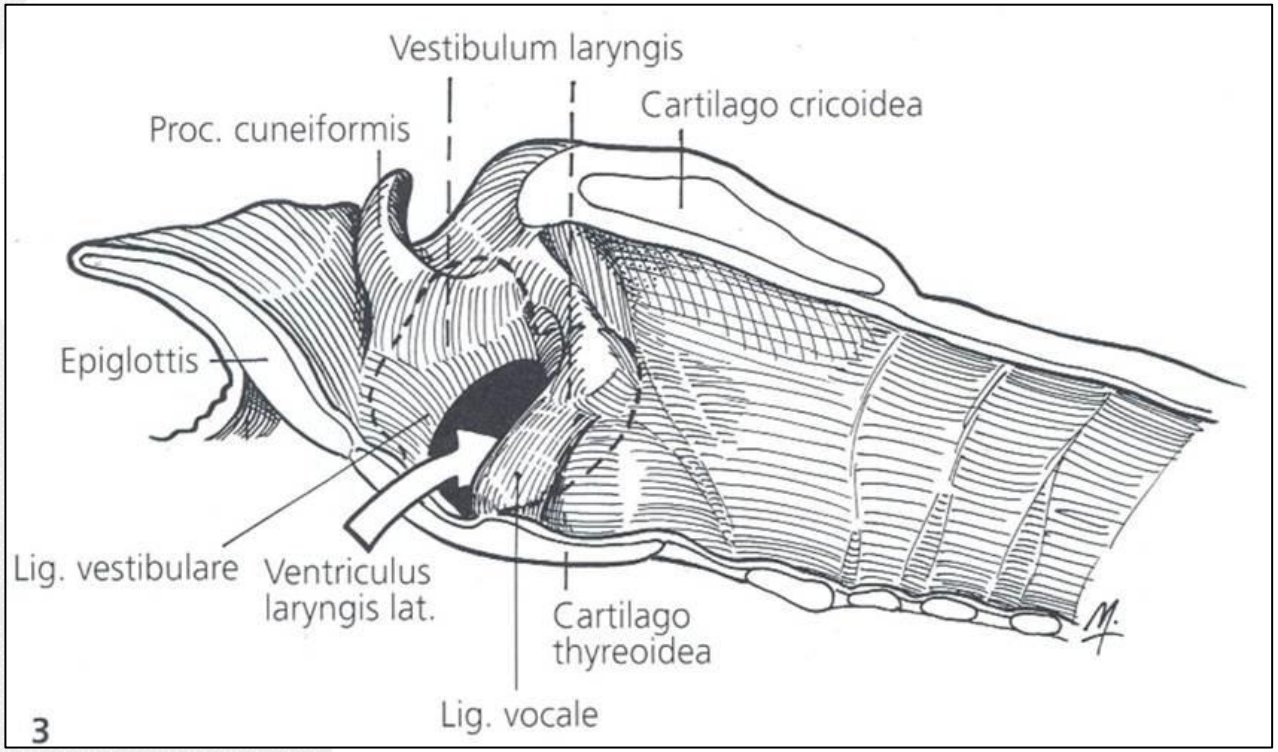
78- to 104-Week:

- **amyloidosis up to 3.0 % (strain-dependent)**
- **single cases of inflammatory secretion, thrombosis, inflammation, mononuclear foci, peri-/arteritis**
- **malignant lymphoma up to 5.2%**
- **only single cases of myeloid leukemia and histiocytic sarcoma**
- **single case of metastatic carcinoma**

Dog

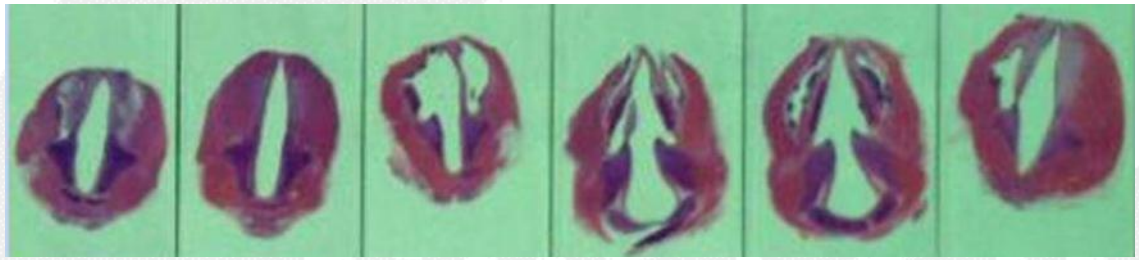
Section planes in dogs

- **Epiglottis: longitundinal**
- **Larynx:**
- **transversal through ventriculus**

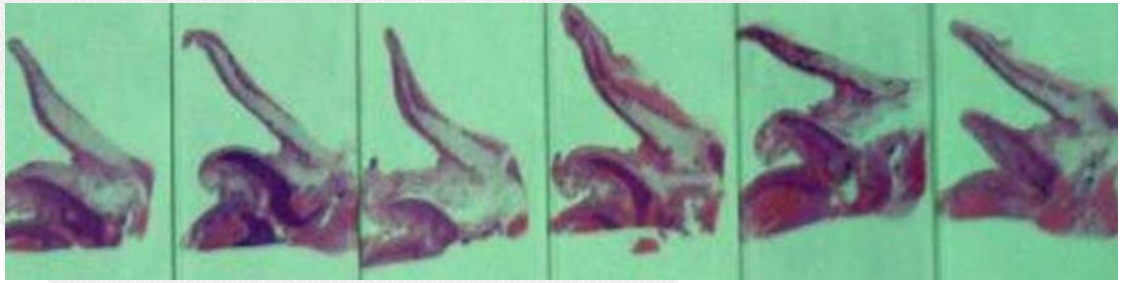


Larynx on Slide: Possibility

transversal



epiglottis



Material and Method: Inhalation studies

Saggitolongitudinal section of epiglottis and transversal section of laryngeal body

- **MTD: 6 (1 positive)**
- **14-Day: 4 (1 positive)**
- **4-Week: 11 (2 positive)**
- **13-Week: 7 (all negative)**
- **26-Week: 3 (all negative)**
- **52-Week: 2 (all negative)**

Material and Method: Non-Inhalation studies

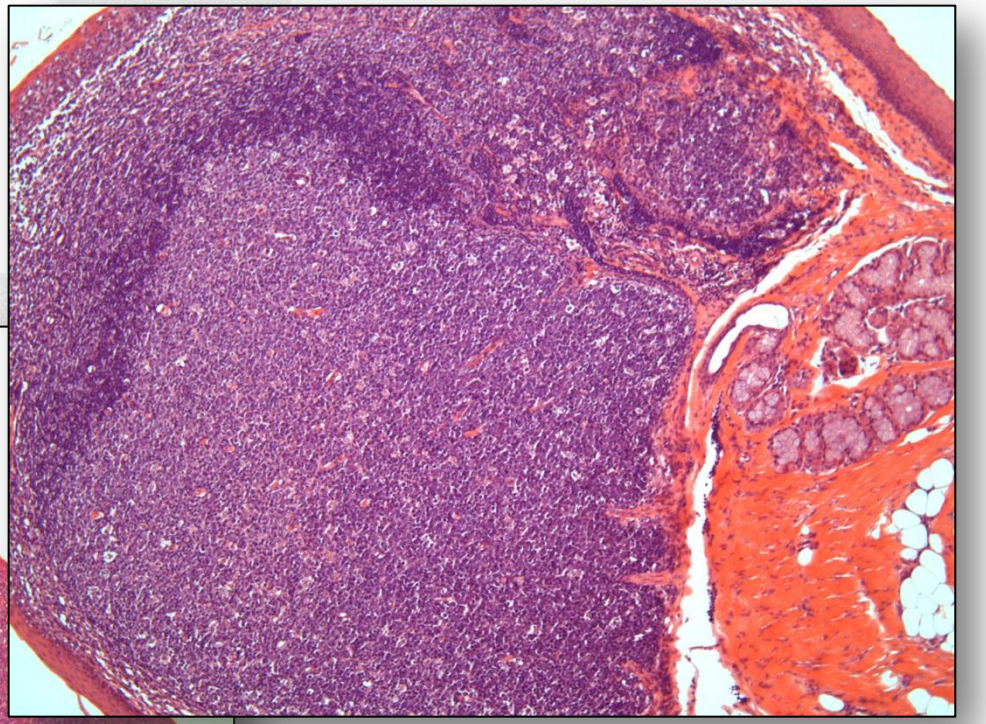
Sections as in inhalation studies

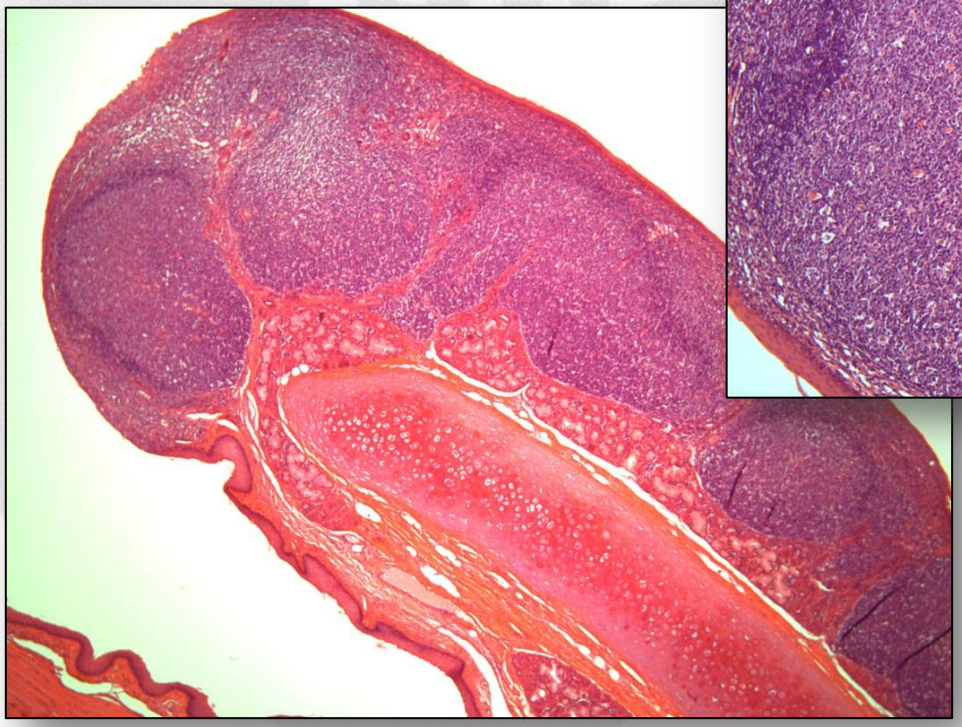
- **MTD: 8**
- **14-Day: 6**
- **4-Week: 15**
- **13-Week: 7**
- **26-Week: 1**
- **52-Week: 1**

Dog:
Spontaneous Lesions

Dog: Variations

Transversal section:
Lymphoid follicles

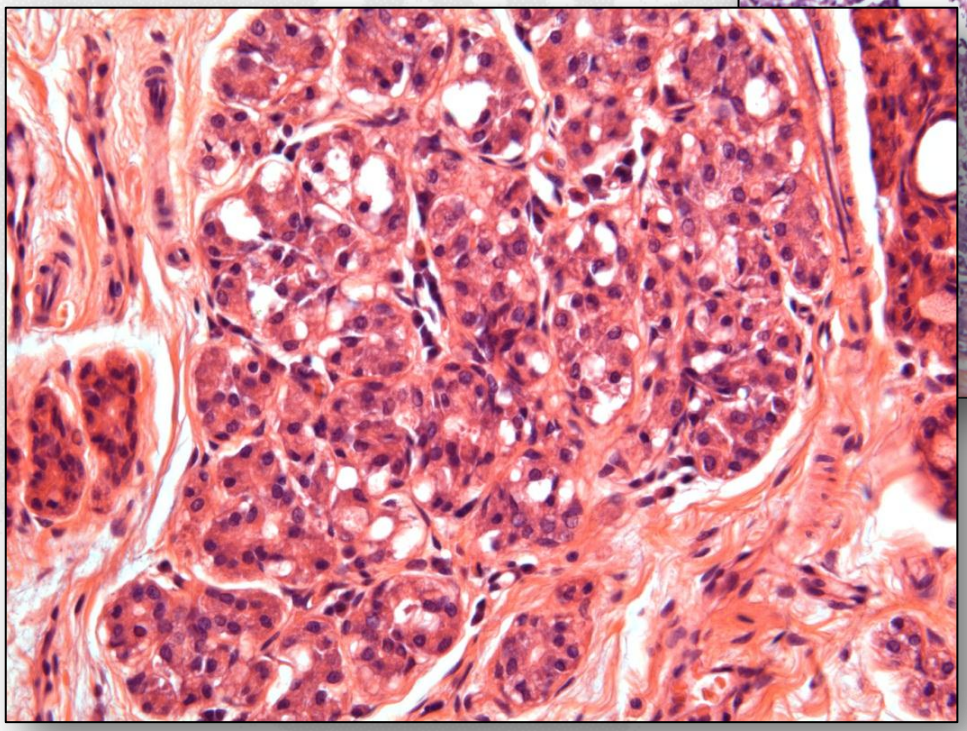


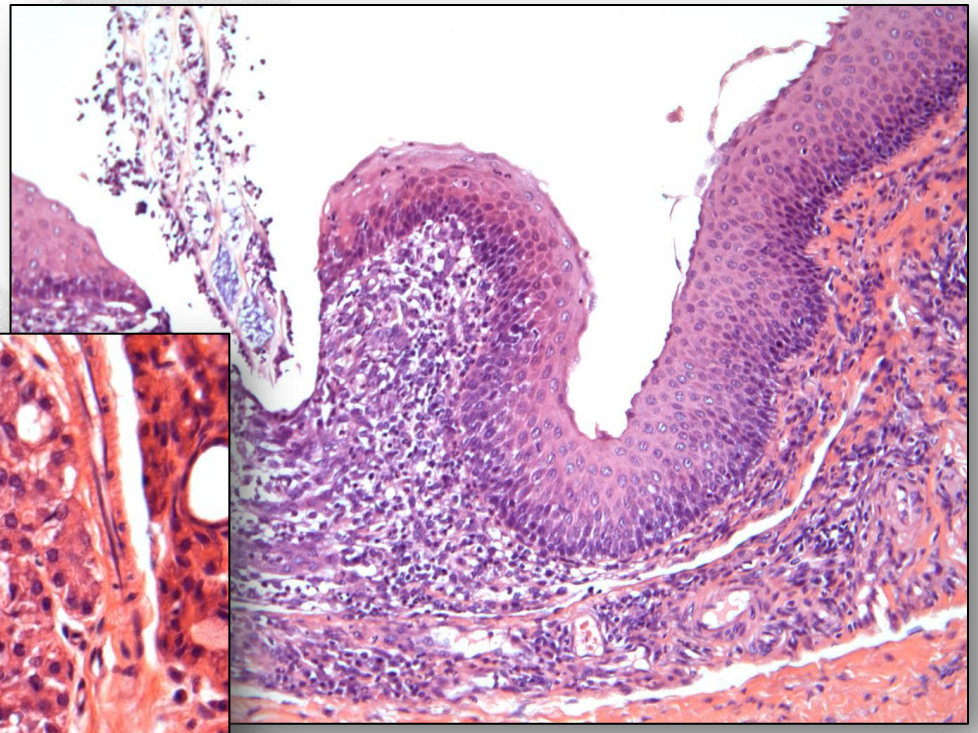


Transversal section:
Lymphoid hyperplasia

Dog: Degenerative lesions

Transversal section:
Glandular atrophy

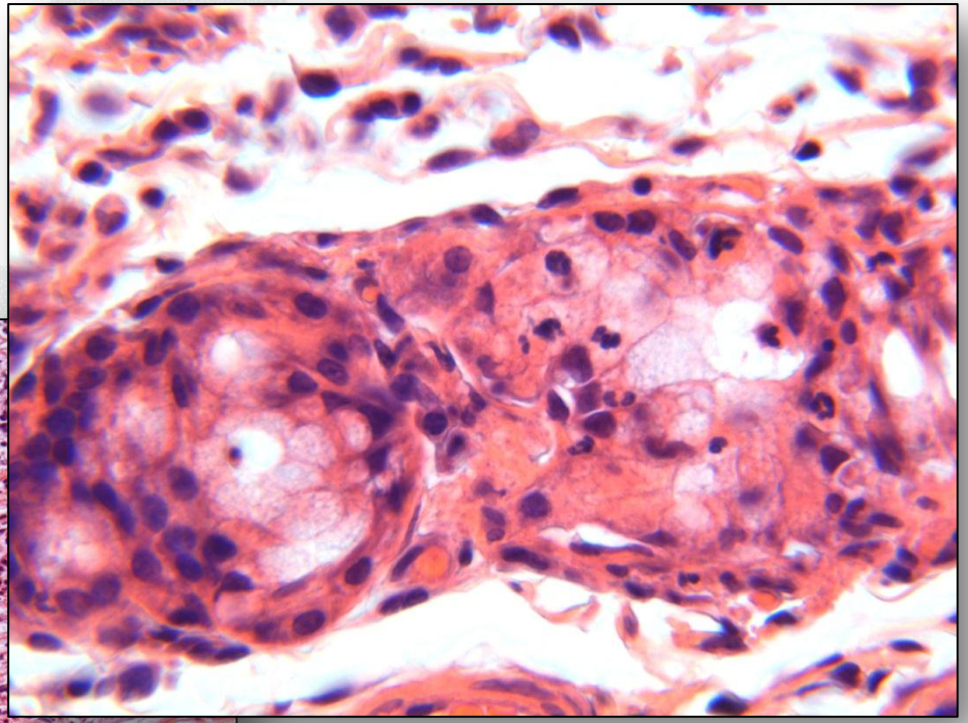


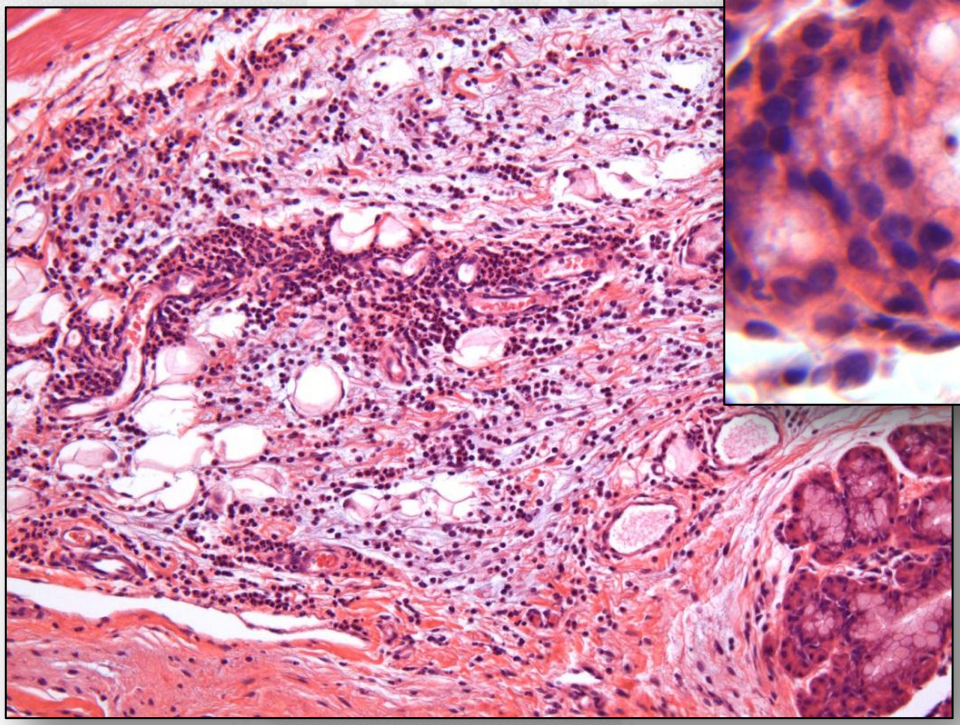


Transversal section:
Foreign body induced inflammation

Dog: Inflammatory lesions

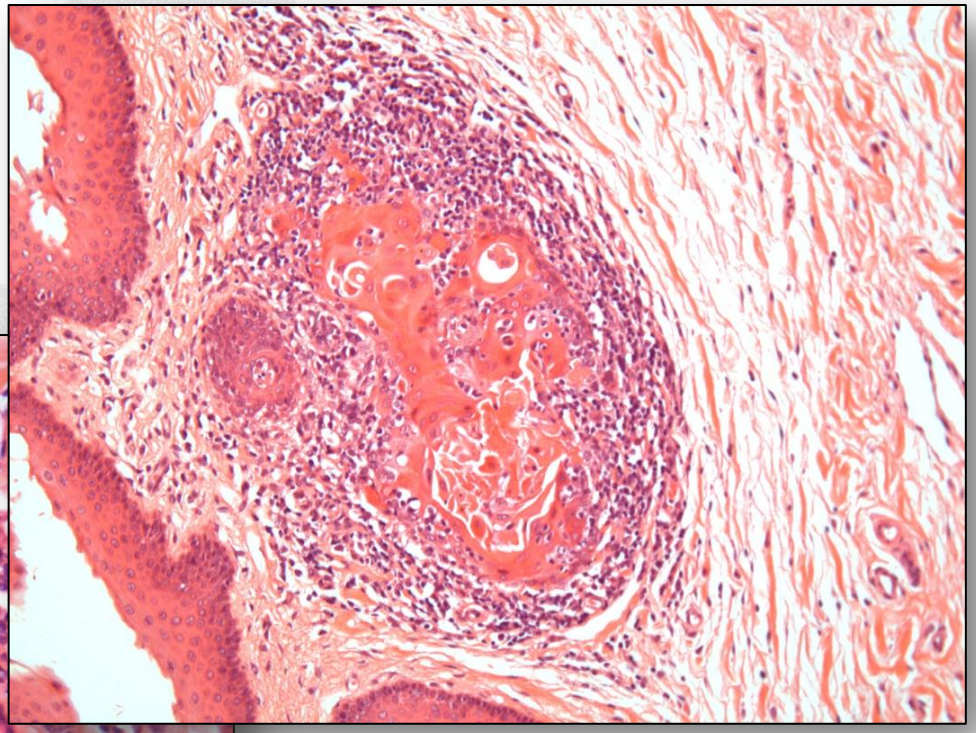
Transversal section:
Glandular inflammation

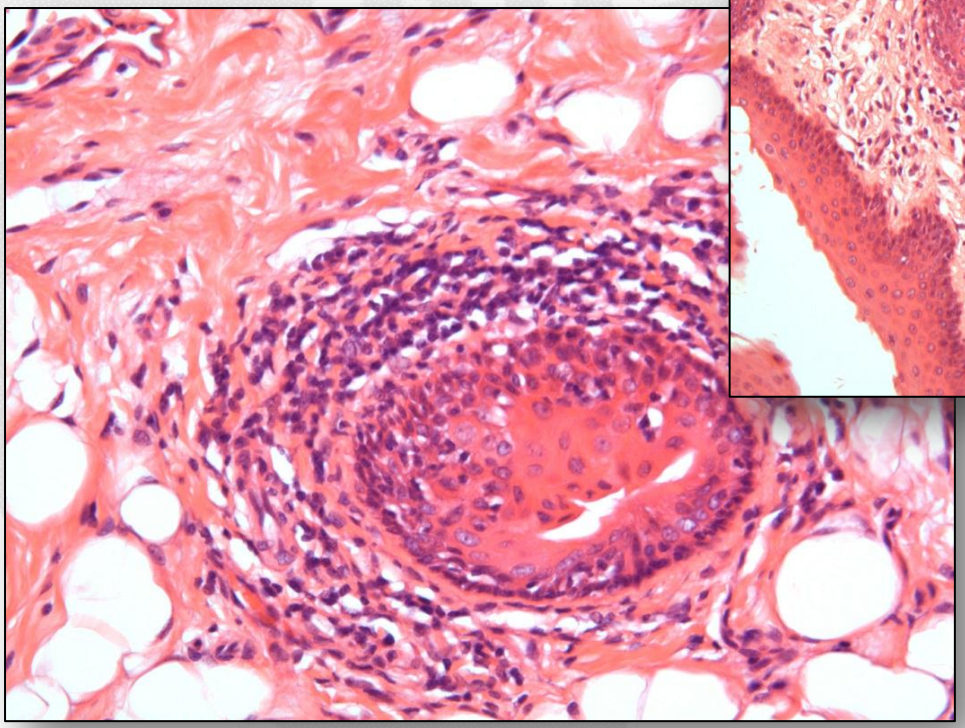




Dog: Inflammatory lesions

Epiglottis:
Glandular inflammation

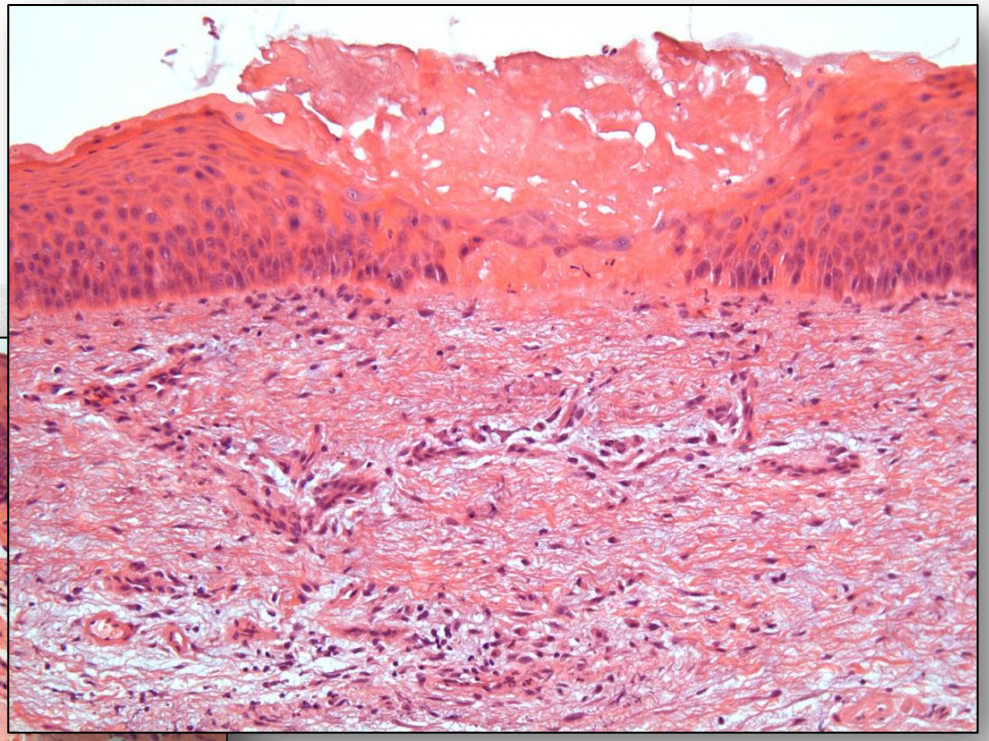


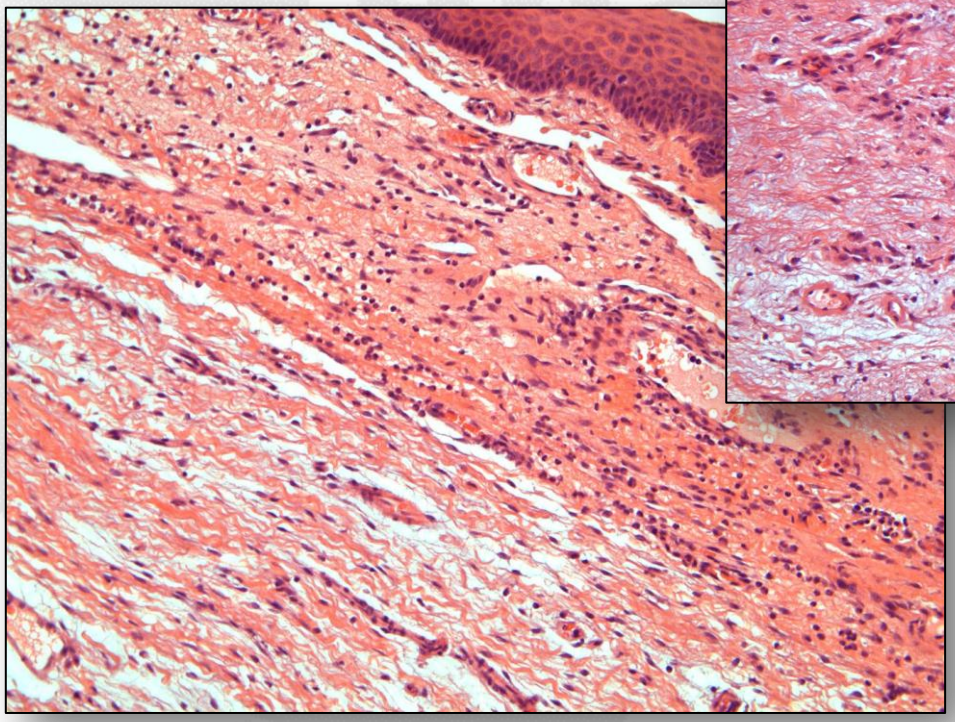


Epiglottis: Granuloma

Dog: Inflammatory lesions

Transversal section: Inflammation

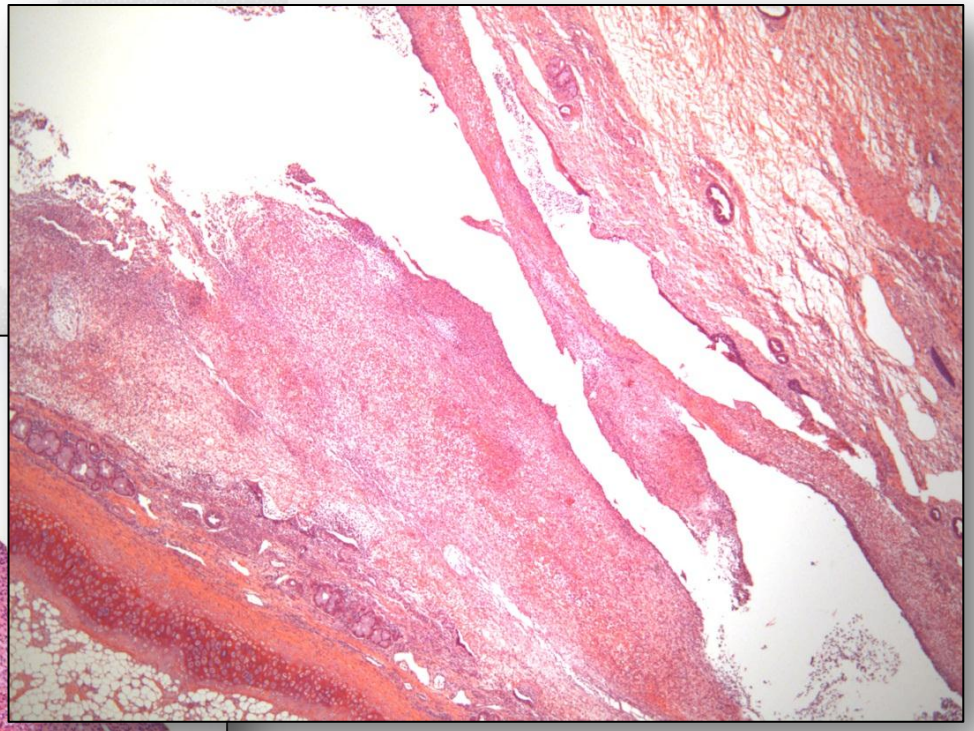


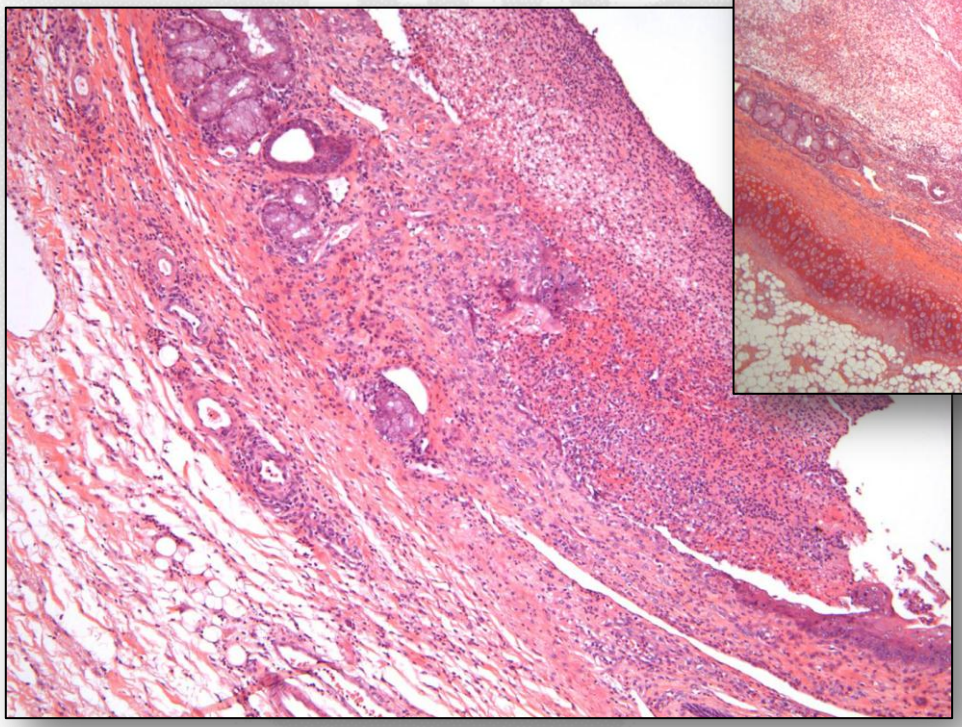


Transversal section: Erosion

Dog: Inflammatory lesions

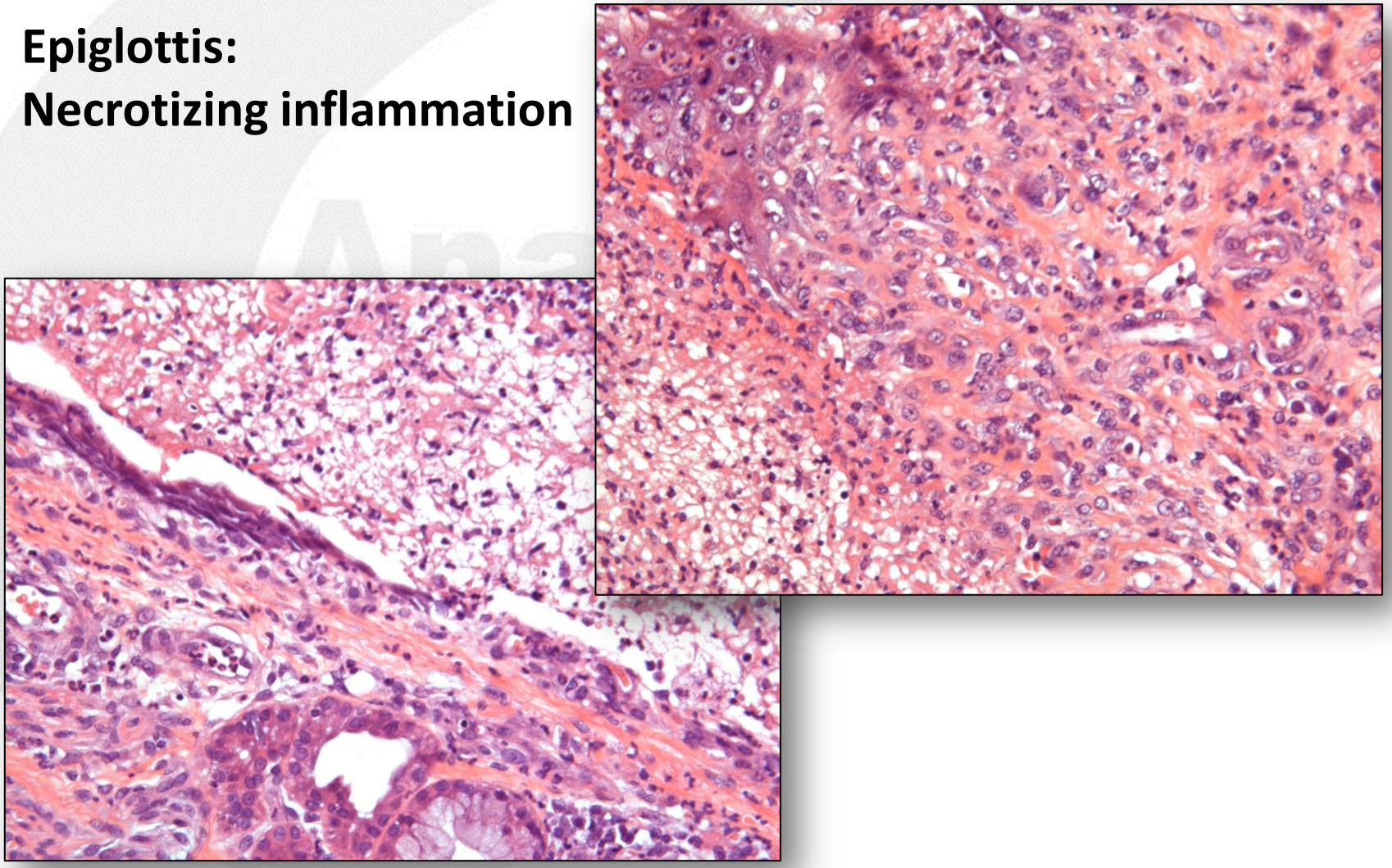
Epiglottis:
Necrotizing inflammation





Dog: Inflammatory lesions

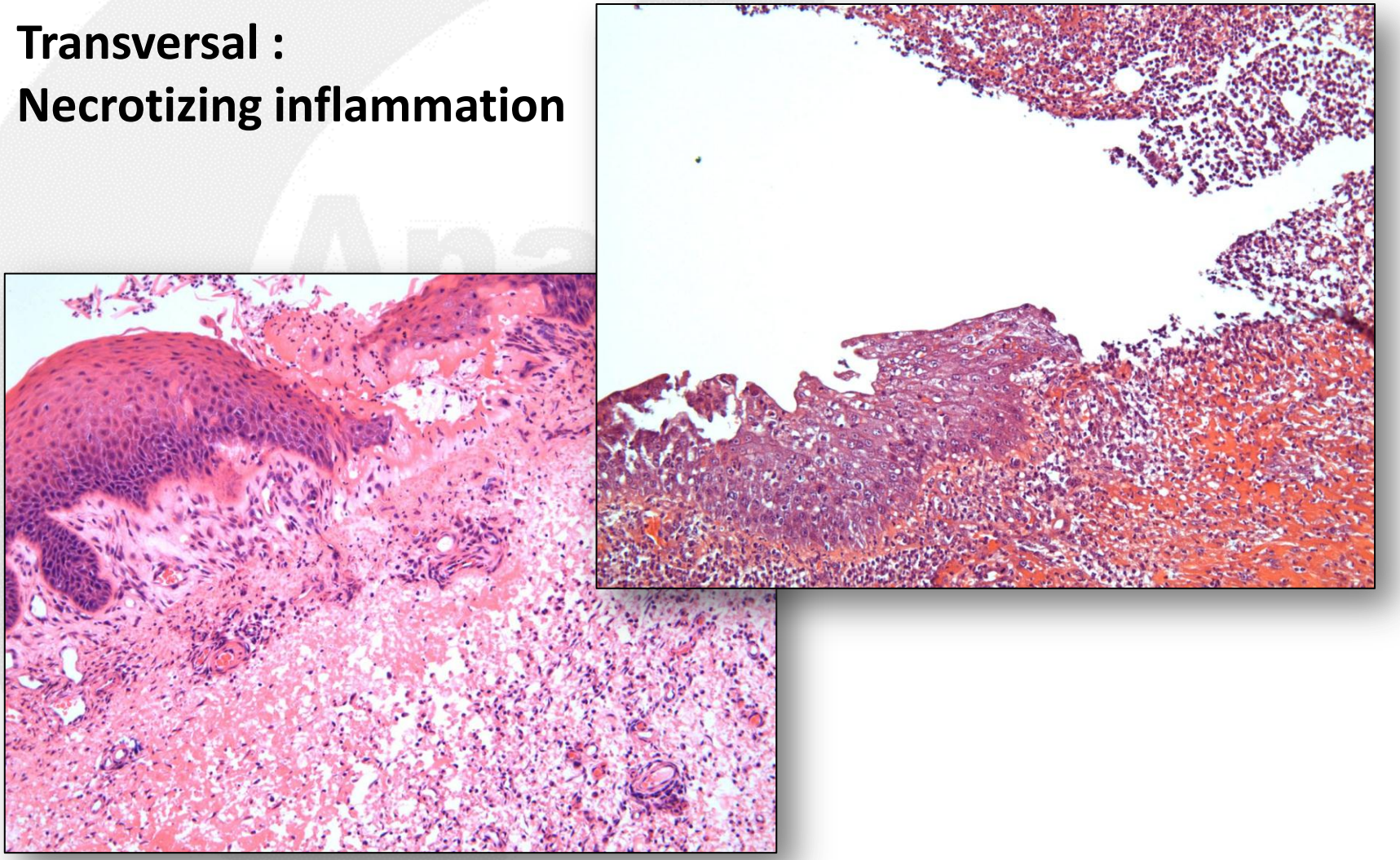
Epiglottis:
Necrotizing inflammation



Dog: Inhalation Studies. Induced Lesions

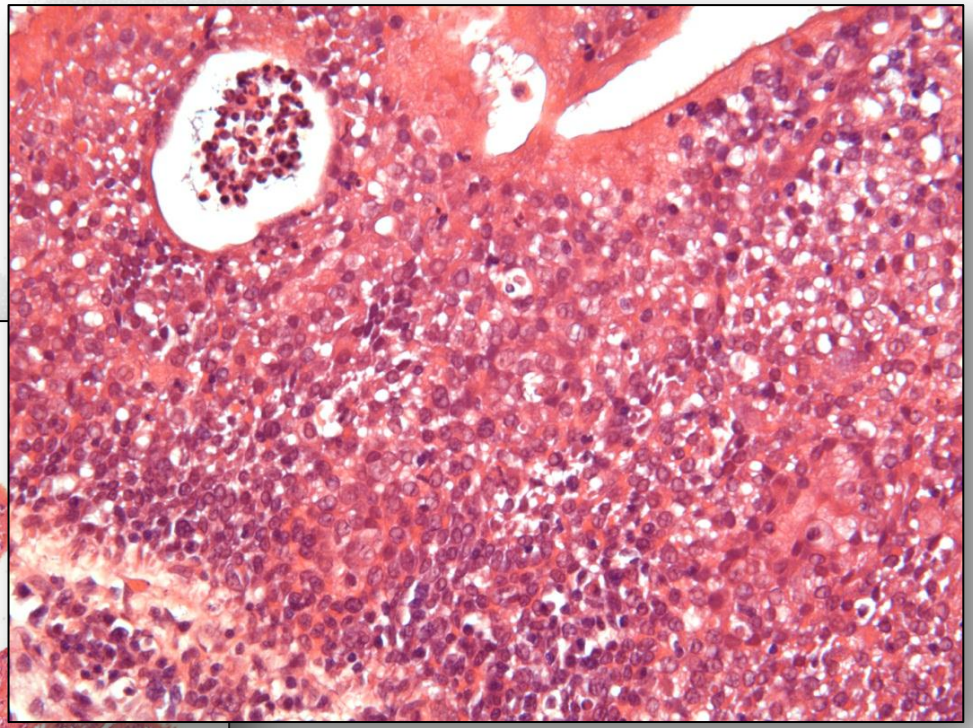
Dog: Inflammatory lesions

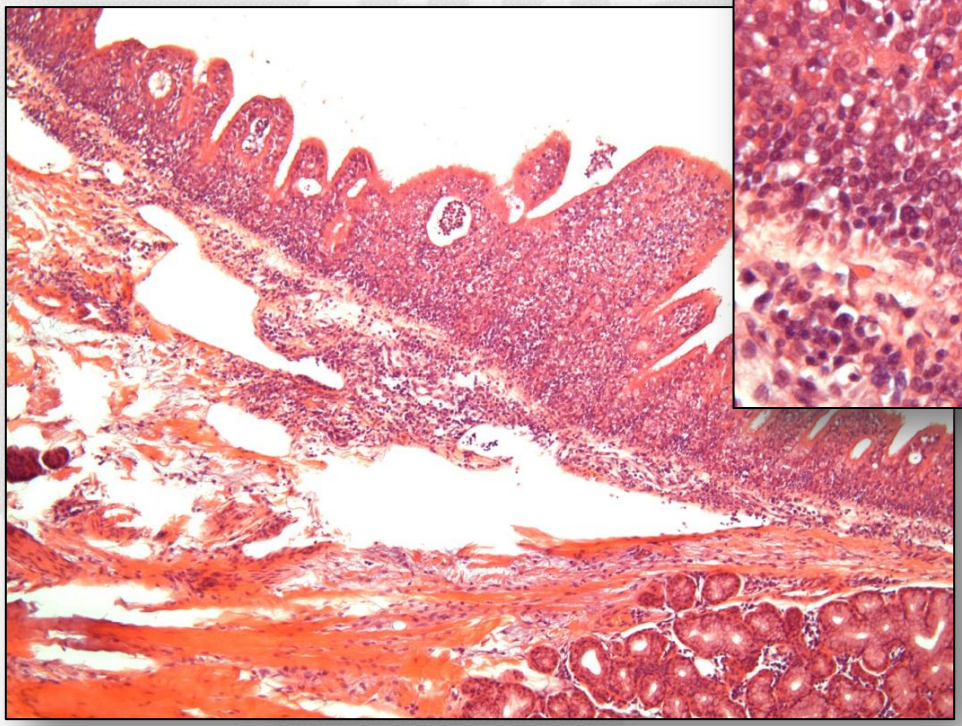
Transversal :
Necrotizing inflammation



Dog: Inflammatory lesions

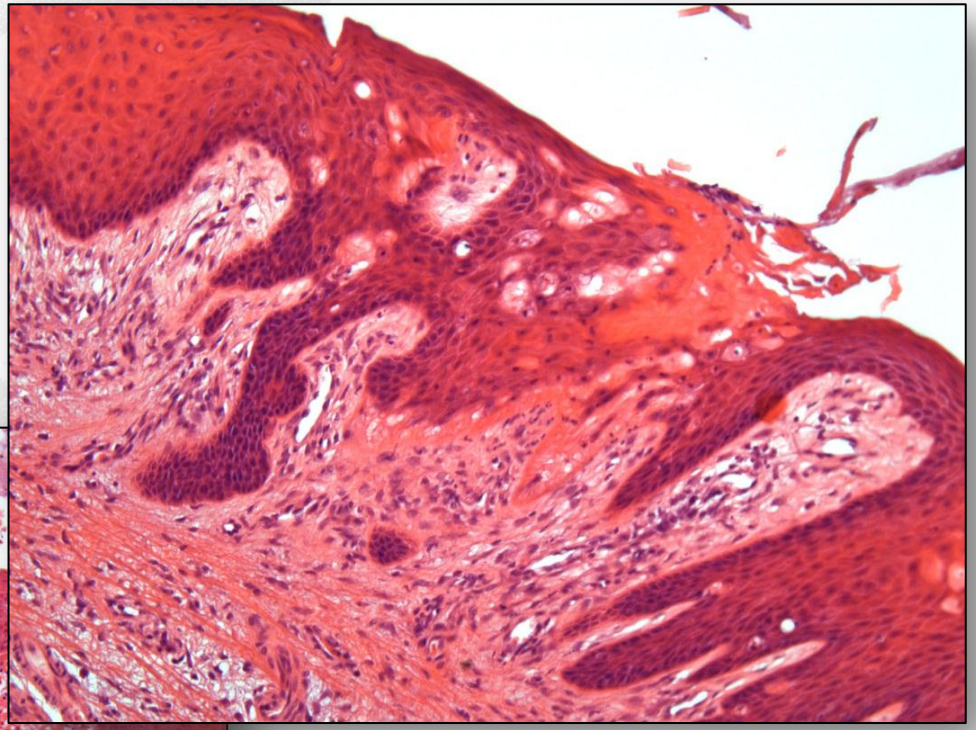
Transversal :
Necrotizing inflammation

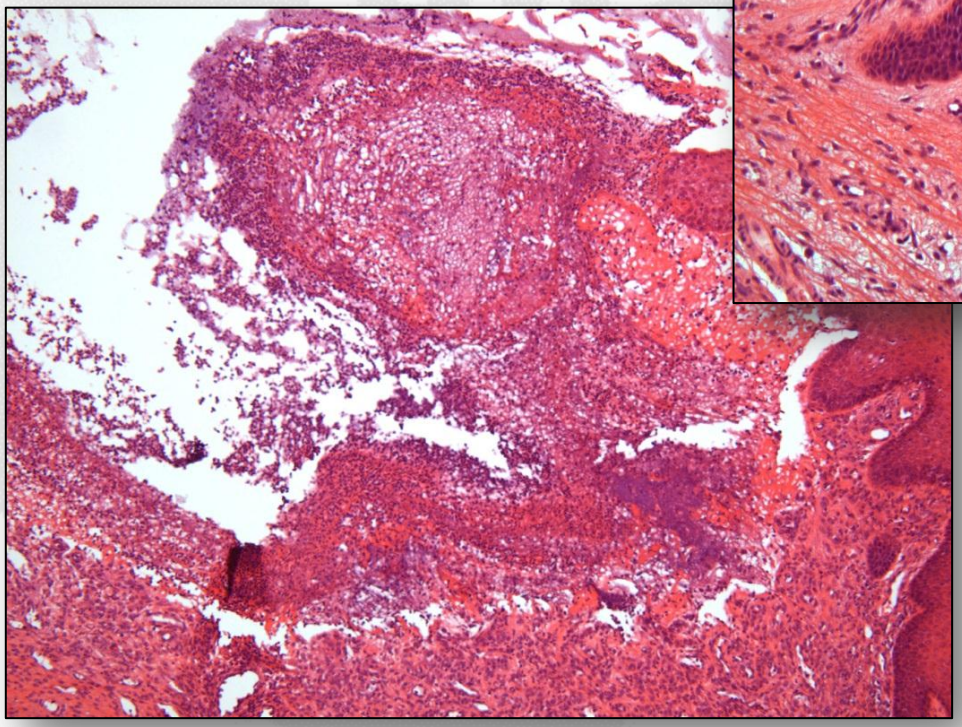




Dog: Inflammatory lesions

Transversal :
Ulceration





Transversal :
Erosion and reactive squamous hyperplasia

Dog: Summary

- Lesions are very rare
- No influence of vehicle
- Lesions in inhalation and non-inhalation studies are similar
- No age-related lesion in toxicology studies
- Technical procedures can cause lesions

The only possibility to establish test item-related lesions is the incidence!