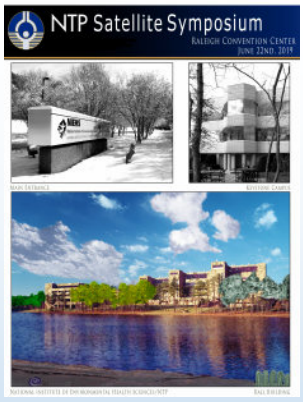

NTP Satellite Symposium Pathology Potpourri

Raleigh, NC
June 22, 2019

Purpose of Symposium

- To provide opportunity to share and learn about interesting or challenging lesions or current nomenclature or diagnostic issues
- To have audience participation
- To have fun!

- Note: Proceedings published in Toxicologic Pathology Journal issue 8, 2019

NTP National Toxicology Program

Aging Mouse Fun!

2019 NTP Satellite Symposium
Raleigh Convention Center
Raleigh, NC
June 22, 2019

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EPL, Inc.
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ILS, Inc.
Research Triangle Park, North Carolina

Background

- NTP Aging Mouse Study
  - 2 year study
  - 10 different mouse strains
    - A/J
    - C57BL/6J
    - 129S1/SvImJ
    - C3H/HeJ
    - B6C3F1/J
    - PWK/PhJ
    - NZO/HILtJ
    - WSB/EiJ
    - NOD.B10Sn-*H2*^{b}/J
    - CAST/EiJ

Background

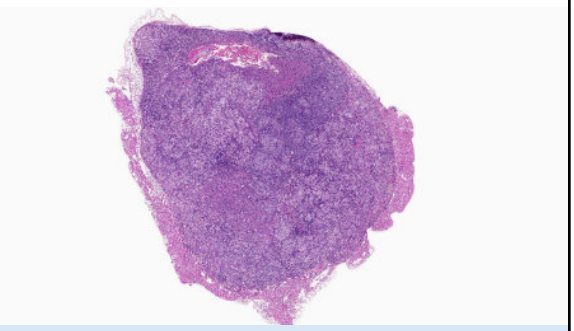
- NTP Aging Mouse Study
  - ***Preliminary Findings***
  - ***No treatment***
  - ***Zero threshold***
    - Determine strain differences in incidence of background findings
    - Identify any disease predilections or lesions that could be considered "strain-specific"

Case 1: Signalment

- Male C3H/HeJ mouse

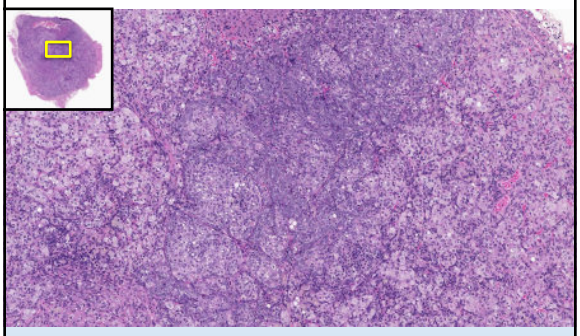
https://www.jax.org/strain/000659

Case 1: What's Your Diagnosis?



Slide 1 of 5

Case 1: What's Your Diagnosis?



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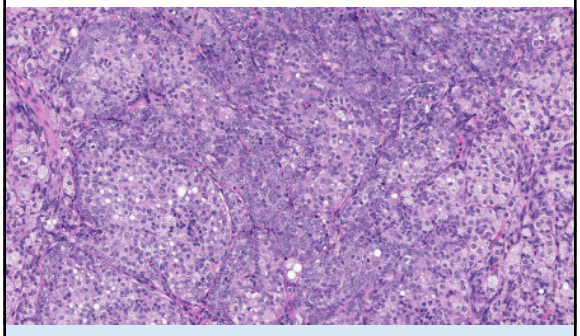
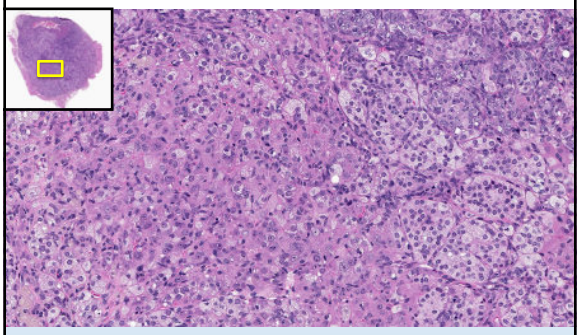
Case 1: What's Your Diagnosis?



Slide 3 of 5

Case 1: What's Your Diagnosis?



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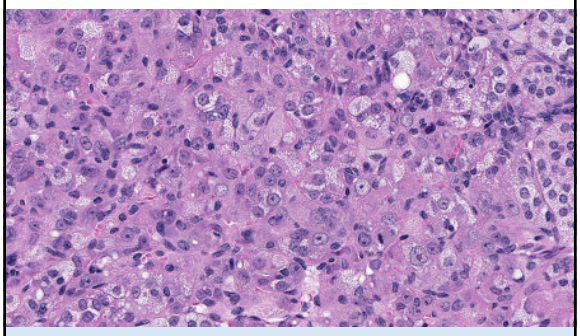
Case 1: What's Your Diagnosis?



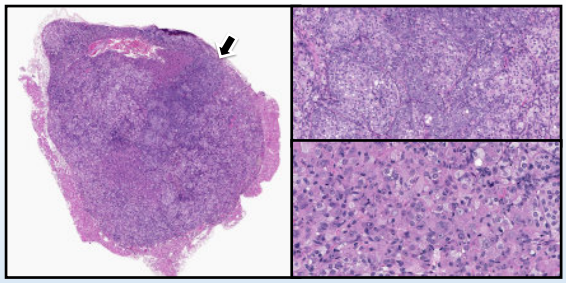
Slide 5 of 5

Case 1: What's Your Diagnosis?

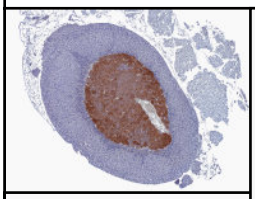
Adrenal Gland:

1. Cortex, Hyperplasia
2. Cortex, Adenoma
3. Cortex, Subcapsular Adenoma
4. Cortex, Cortical Carcinoma
5. Cortex, Subcapsular Carcinoma
6. Medulla, Hyperplasia
7. Medulla, Pheochromocytoma, Benign
8. Medulla, Pheochromocytoma, Malignant
9. Other

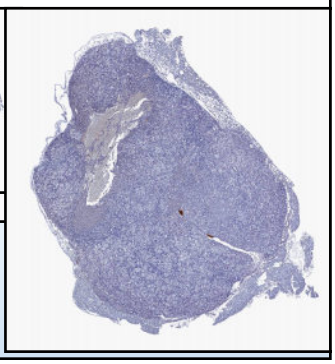
Adrenal Gland: Subcapsular Carcinoma



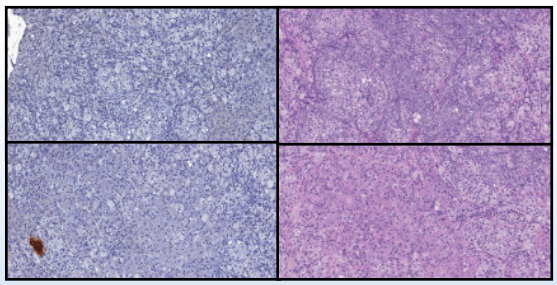
Tyrosine Hydroxylase (TH) IHC



TH Positive Control



Tyrosine Hydroxylase IHC



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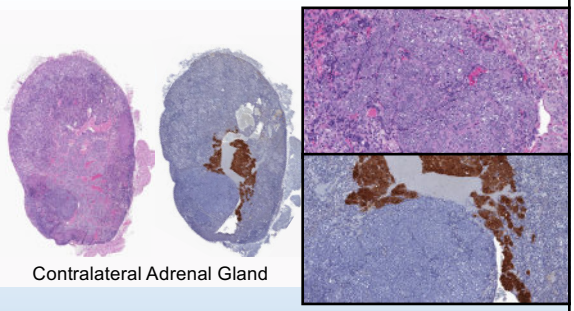


Contralateral Adrenal Gland

Cortical vs. Subcapsular Carcinoma

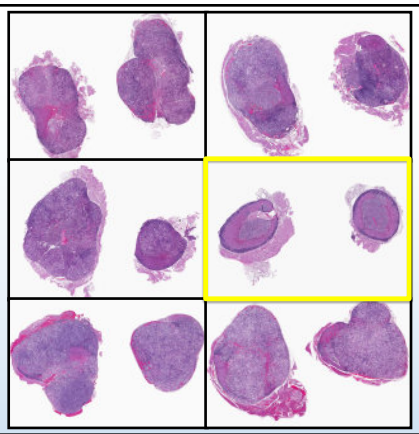
Cortical

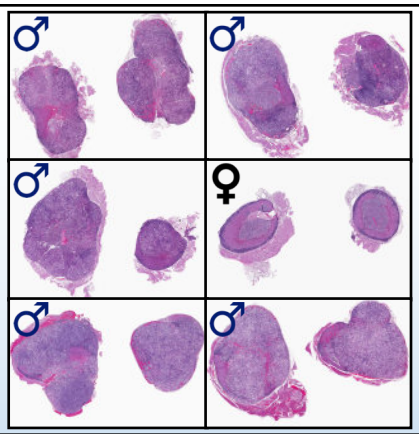
- Invasive growth into surrounding adrenal tissue
- Neoplastic cells are organized in thickened trabeculae, sheets or solid clusters with disruption of normal architecture.
- Cytoplasm is usually eosinophilic or amphophilic.
- Cellular atypia and pleomorphism are usually present.
- Mitotic figures may be numerous.
- Vacuolation, cystic degeneration, necrosis, angiectasis or hemorrhage may be present.

Subcapsular

- Presence of distinct invasion into surrounding tissues or vessels
- Tumor cell atypia, pleomorphism and mitotic figures are present.
- Cells are organized in nests, ribbons or cords.
- The modifiers are used according to the predominant A (fusiform) or B (polygonal) cell type (>70%).
- Mixed type: No predominant cell type is present.

https://www.goreni.org/





C3H/HeJ Mice: Adrenal Gland Lesions

MALE

Adrenal Cortex No. Ex.	**115**
Hyperplasia, Marked	68 (59%)
Carcinoma	20 (17%)
Adenoma	26 (23%)

FEMALE

Adrenal Cortex No. Ex.	**115**
Hyperplasia	107 (93%)
Minimal	25 (22%)
Mild	81 (70%)
Moderate	1 (0.9%)

C3H/HeJ Mice: Penile Prolapse

Adrenal Cortex No. Ex.	**115**
Hyperplasia, Marked	68 (59%)
Carcinoma	20 (17%)
Adenoma	26 (23%)
Penis No. Ex.	**64**
Prolapse	64 (56%)

Case 1: Discussion Points

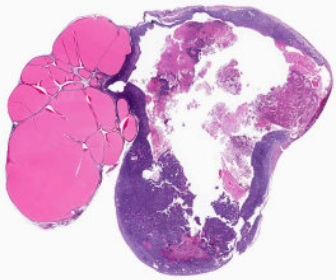
- Do you think there may be a correlation between the adrenal gland lesions and incidence of penile prolapse?

Case 2: Signalment

- Male NOD.B10Sn-*H2*^{b}/J mouse

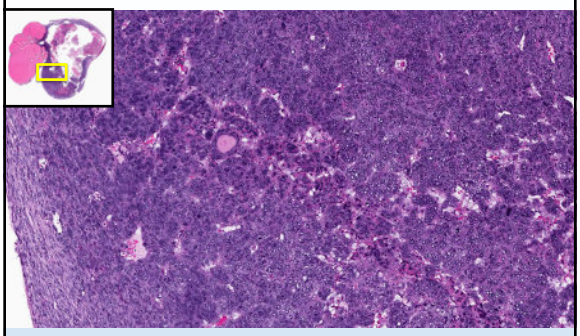
https://www.iex.org/strain/001976

Case 2: What's Your Diagnosis?



Slide 1 of 7

Case 2: What's Your Diagnosis?



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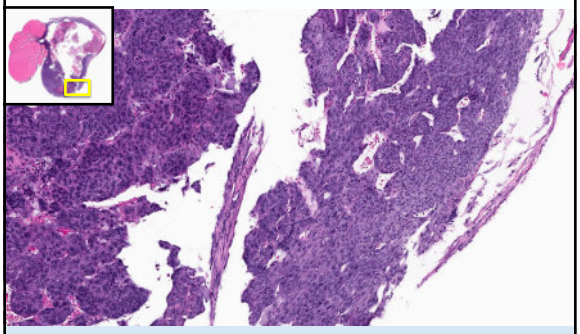
Case 2: What's Your Diagnosis?



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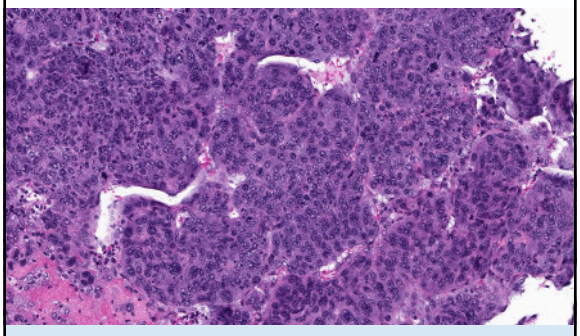
Case 2: What's Your Diagnosis?



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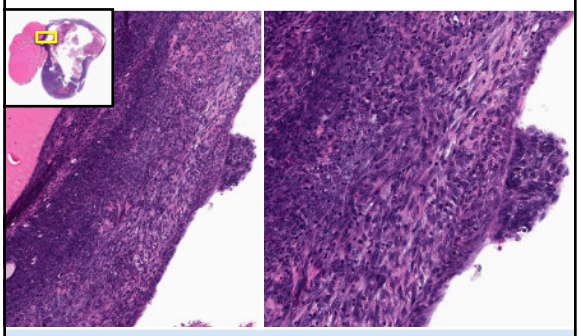
Case 2: What's Your Diagnosis?



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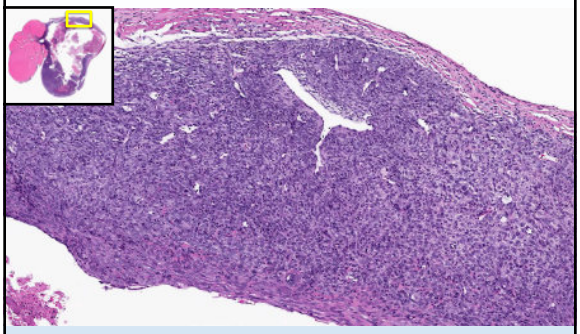
Case 2: What's Your Diagnosis?



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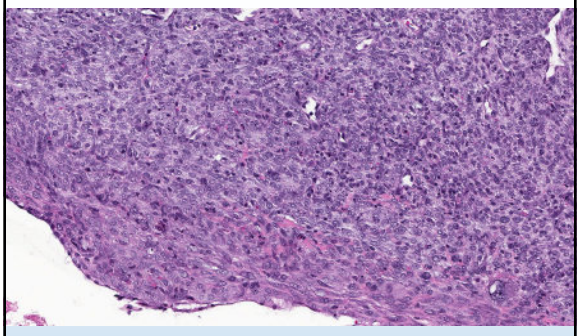
Case 2: What's Your Diagnosis?



Slide 7 of 7

Case 2: What's Your Diagnosis?

Seminal Vesicle:

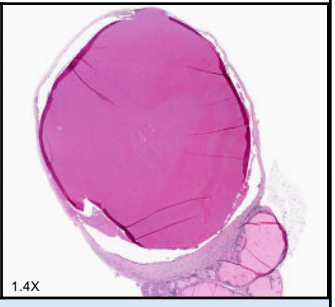
1. Inflammation
2. Fibrosis
3. Sarcoma
4. Carcinoma
5. Carcinosarcoma
6. Other

NOD.B10Sn-*H2*^{b}/J Seminal Vesicle Lesions

Seminal Vesicles No. Ex.	**115**
Dilation	95 (83%)
Fibrosis	96 (84%)
Inflammation, chronic active	66 (57%)
Inflammation, suppurative	16 (14%)
Sarcoma	**17 (15%)**
Carcinosarcoma	**1 (0.9%)**

NOD.B10Sn-*H2*^{b}/J Seminal Vesicle Lesions


1.6X


1.4X

And now…
MORE Aging Mouse Fun!

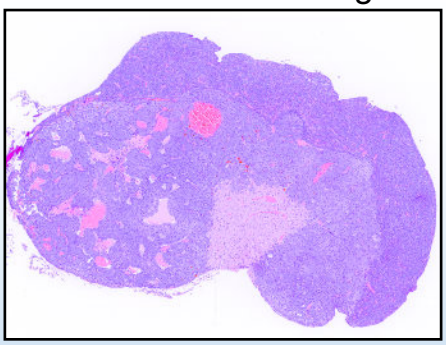
Brought to you by,
Dr. Debabrata Mahapatra
ILS

Case 3: Signalment

- > 2 yr old female mouse
- 129S1/Svlmj

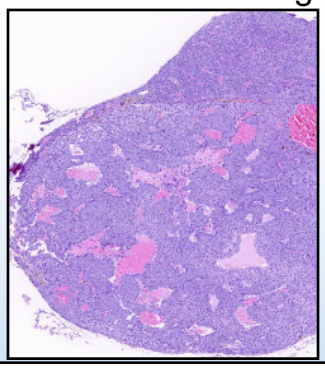
Source: www.jax.org/strain/002448

Case 3: What's Your Diagnosis?



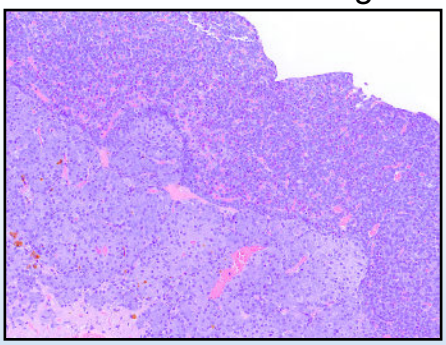
Slide 1 of 5

Case 3: What's Your Diagnosis?



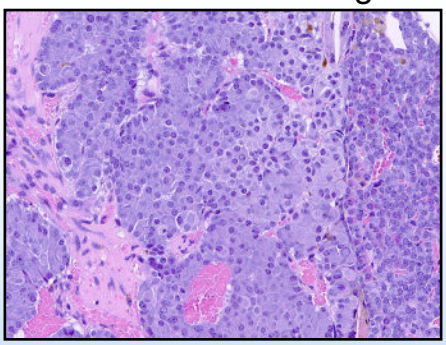
Slide 2 of 5

Case 3: What's Your Diagnosis?



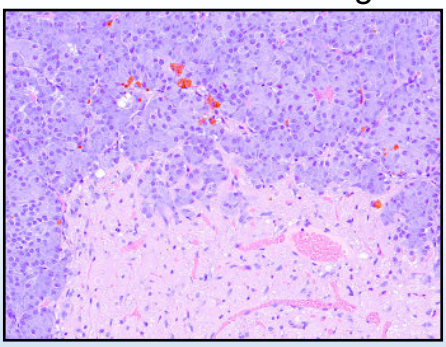
Slide 3 of 5

Case 3: What's Your Diagnosis?



Slide 4 of 5

Case 3: What's Your Diagnosis?



Slide 5 of 5

Case 3: What's Your Diagnosis?

Pituitary Gland:

1. Pars Distalis: Adenoma
2. Pars Intermedia: Hypertrophy, diffuse
3. Pars Intermedia: Hyperplasia, diffuse
4. Pars Intermedia: Adenoma
5. Pars Intermedia: Hypertrophy and Hyperplasia diffuse
6. Pars intermedia: Carcinoma
7. Other

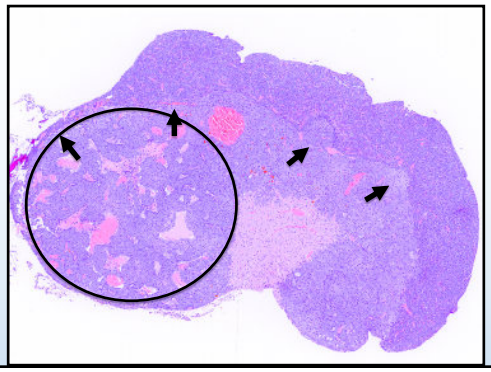
Discussion

- Uncommon in rats and mice
- Cells often extend into pars nervosa
- Cells are pleomorphic
- Compression of adjacent pars distalis is present

Diagnostic features

Pars Intermedia	Hyperplasia	Adenoma	Carcinoma
Pleomorphism	----	Yes	Yes
Compression (> 1 quadrant)	----	Yes	Yes
Extension into pars nervosa	May be present	Yes	Yes
Invasion of adjacent brain/meninges/ sphenoid bone	----	----	Yes

Discussion



Discussion

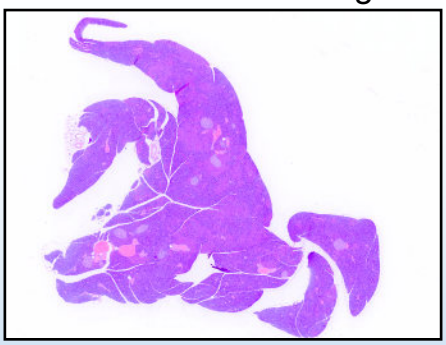
Incidence of Hyperplasia/tumors in the Pituitary Gland, Pars Intermedia (129S1/SvImj)		
	Male	Female
Adenoma	2.6%	2.6%
Hyperplasia	22.6%	10.4%

Case 4: Signalment

- ~ 2 yr old female mouse
- 129S1/SvImj

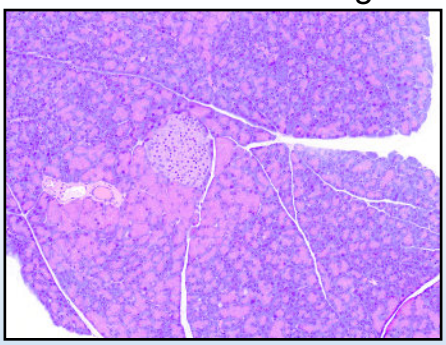
Source: www.jax.org/strain/002448

Case 4: What's Your Diagnosis?



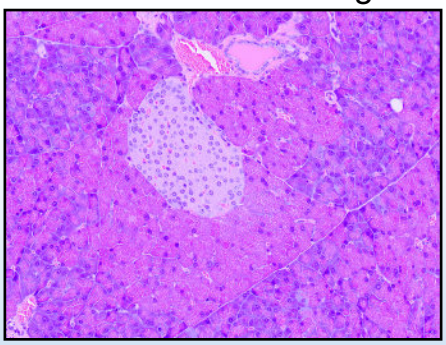
Slide 1 of 3

Case 4: What's Your Diagnosis?



Slide 2 of 3

Case 4: What's Your Diagnosis?



Slide 3 of 3

Case 4: What's Your Diagnosis?

Pancreas:

1. Islets: Hyperplasia
2. Acinus: Hypertrophy, diffuse
3. Acinus: Hyperplasia, diffuse
4. Acinus: Hypertrophy, peri-insular
5. Hepatocytes, peri-insular
6. Acinus: Hyperplasia, peri-insular
7. Acinus: Adenoma
8. Other

Discussion

- Relatively common finding
- Hypertrophy of acinar cells surrounding islets of Langerhans
- Hypertrophy often extends to distal (tele-insular) regions
- Abundant intracytoplasmic zymogen granules
- Halos result from trophic factors/hormones and (i.e., ghrelin, insulin) secreted by beta cells of the islets
- Not routinely recorded in toxicity studies

Discussion

Incidence of Acinus Hypertrophy in Pancreas (129S1/SvImj)		
	Males	Females
Pancreas acinus: Hypertrophy, peri-insular	49.5%	72%

And now…
EVEN MORE Aging Mouse Fun!

Dr. Torrie A. Crabbs

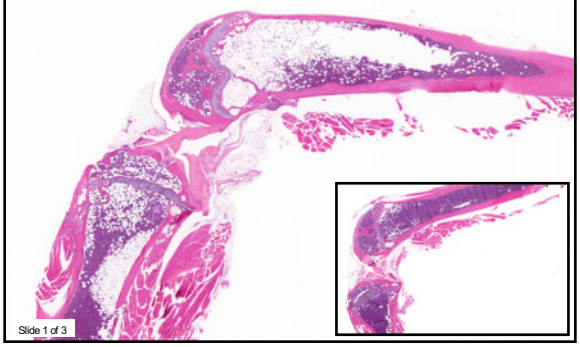
EPL

Case 5: Signalment

- Male B6C3F1/J mouse

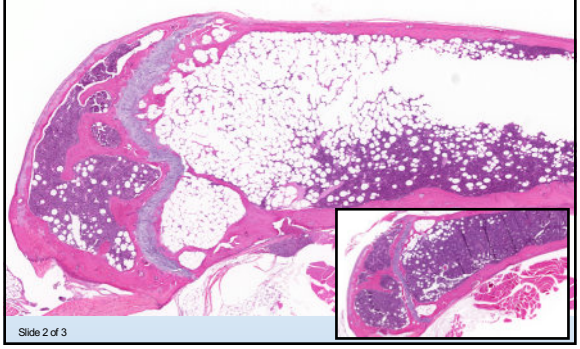
https://www.jax.org/strain/100010

Case 5: What's Your Diagnosis?



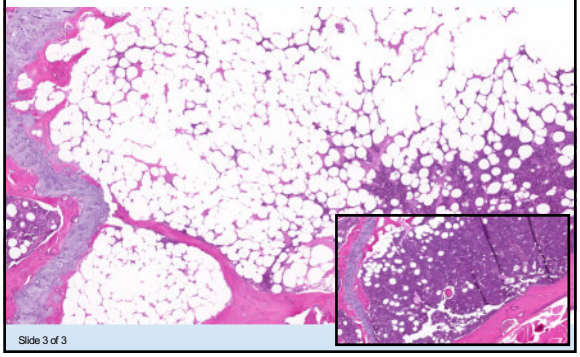
Slide 1 of 3

Case 5: What's Your Diagnosis?



Slide 2 of 3

Case 5: What's Your Diagnosis?



Slide 3 of 3

Case 5: What's Your Diagnosis?

Bone Marrow:

1. Within normal limits
2. Increased adipocytes
3. Decreased hematopoietic cells / Hypocellularity
4. Lipomatosis
5. Lipoma
6. Liposarcoma
7. Other

Incidence

- Only present in females
- Relatively localized (metaphysis)
  - Mid-diaphysis was normal to hypercellular
- Not noted in the other strains
  - Most common diagnosis = hypercellularity

	Males	Females
No. Examined	115	115
Bone Marrow – Increased Adipocytes	**0**	**49 (43%)**
Minimal	0	35 (30%)
Mild	0	10 (9%)
Moderate	0	4 (3%)

Discussion

- Relative fat content of bone marrow varies:
  - Species
  - Strain
  - Sex
  - Age
  - Anatomic site
  - Activity of hematopoietic tissue

Discussion

- Rodents generally have decreased fat and increased hematopoietic elements compared to other mammals
  - Mice > rats of the same age
- As animals age → bone marrow cellularity ↓ with a relative ↑ in adipocytes

Chicken or the Egg

- Increased adipocytes
  - Increased number or cell density of adipocytes within the medullary cavity
  - Focal, multifocal or diffuse

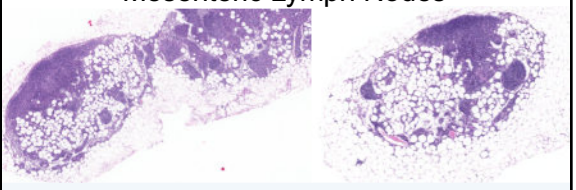
Chicken or the Egg

- Decreased hematopoietic cells / Hypocellularity
  - Reduced hematopoietic cellularity or reduced area occupied by hematopoietic cells
    - Single or multiple cell lineages may be affected
  - Real or an apparent relative increase of adipose tissue, fluid or dilated bone marrow sinuses relative to hematopoietic cells
  - Distribution may be focal, multifocal or diffuse
  - An entire cell lineage may be absent
  - Decreased cell count of affected cell type(s) may be present in peripheral blood

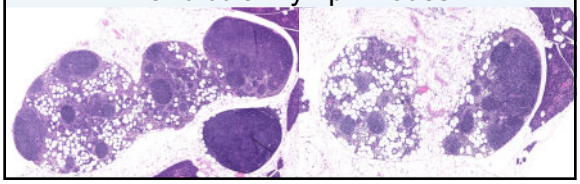
Discussion

- Per the INHAND document:
  - "It is generally more physiologically relevant to express changes in the relative proportions of adipocytes and hematopoietic cells in terms of hyperplasia or atrophy of the hematopoietic cells."
- So… why did we choose increased adipocytes over decreased hematopoietic cells…

Mesenteric Lymph Nodes



Mandibular Lymph Nodes



Incidence

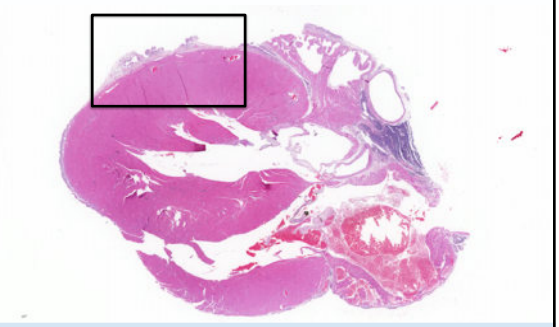
	Males	Females
No. Examined	115	115
Bone Marrow – Increased Adipocytes	**0**	**49 (43%)**
Minimal	0	35 (30%)
Mild	0	10 (9%)
Moderate	0	4 (3%)
Mesenteric Lymph Node – Infiltration, Adipocytes	**0**	**17 (15%)**
Minimal	0	17
Mandibular Lymph Node – Infiltration, Adipocytes	**0**	**25 (22%)**
Minimal	0	13
Mild	0	3
Moderate	0	1

Case 6: Signalment

- Female NZO/HlLtJ mouse

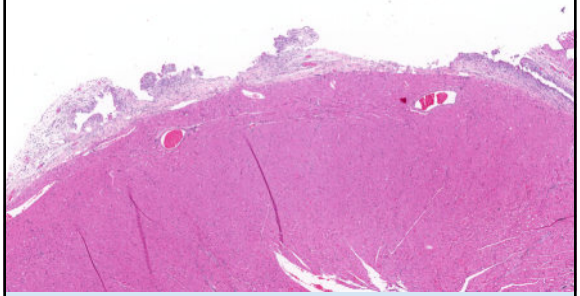
https://www.jax.org/strain/002105

Case 6: What's Your Diagnosis?



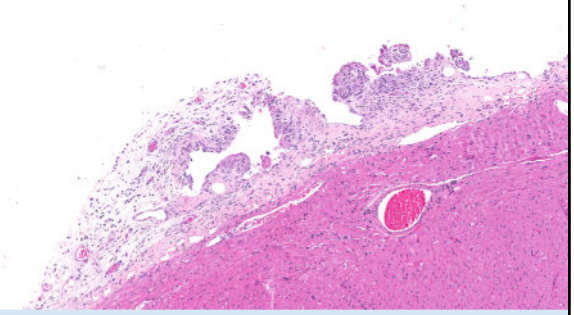
Slide 1 of 4

Case 6: What's Your Diagnosis?



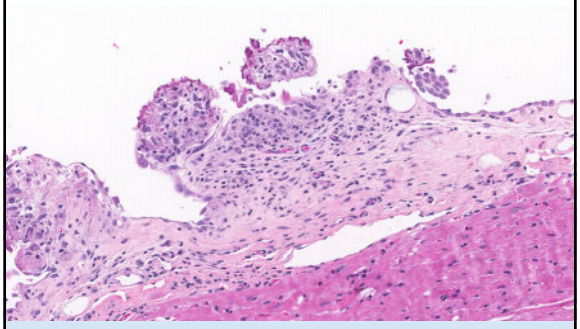
Slide 2 of 4

Case 6: What's Your Diagnosis?



Slide 3 of 4

Case 6: What's Your Diagnosis?



Slide 4 of 4

Case 6: What's Your Diagnosis?

Heart:

1. Within normal limits
2. Inflammation, chronic active
3. Hyperplasia, mesothelial
4. Both 2 and 3
5. Mesothelioma
6. Other

Case 6: What's Your Diagnosis?

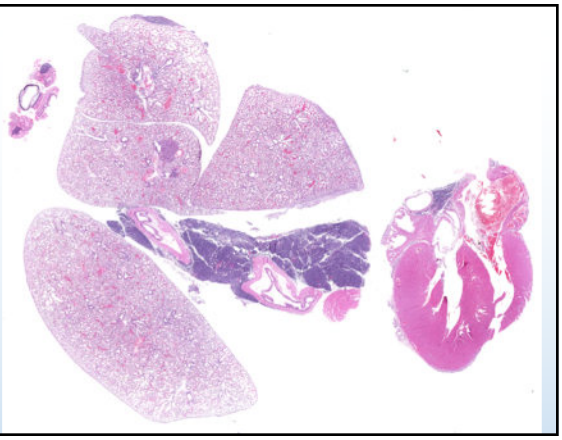
Heart:

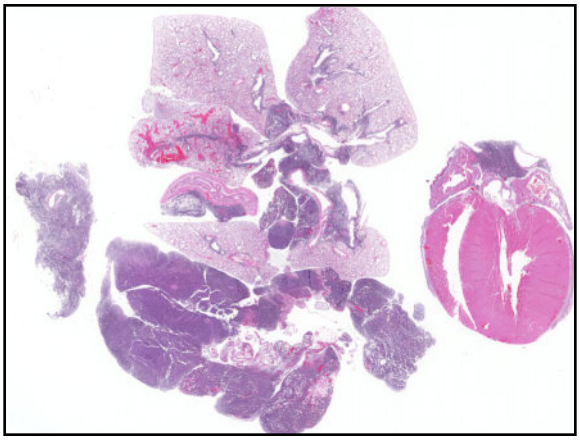
1. Inflammation, chronic active (no subsite, describe in narrative)
2. Epicardium – Inflammation, chronic active
3. Pericardium – Inflammation, chronic active
4. Myocardium – Inflammation, chronic active
5. Other

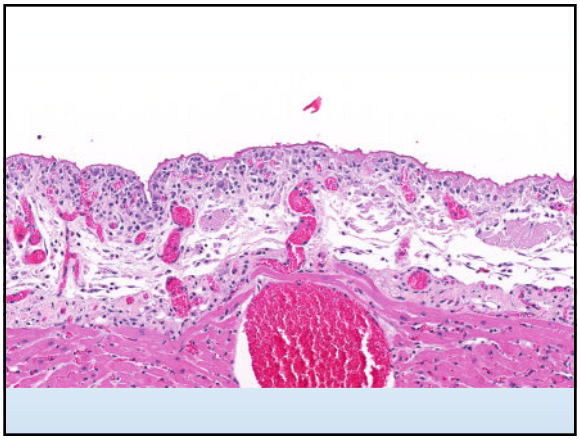
Incidence

- Present only in females
- Always accompanied by lymphoma (mediastinal)

	Males	Females
No. Examined	115	115
Epicardium – Inflammation, chronic active	**0**	**14 (12%)**
Minimal	0	4 (3%)
Mild	0	7 (6%)
Moderate	0	1 (1%)
Marked	0	2 (2%)





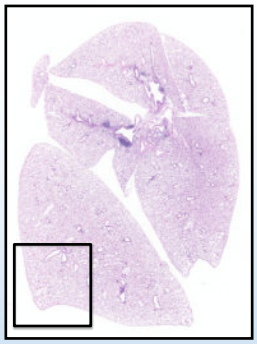


Case 7: Signalment

- Male Cast/EiJ mouse

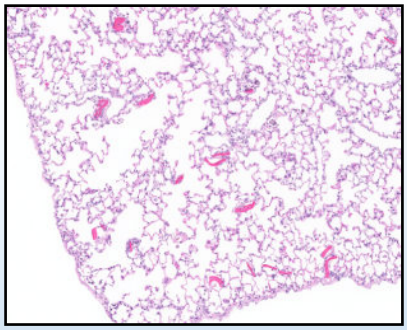
https://www.jax.org/strain/000928

Case 7: What's Your Diagnosis?



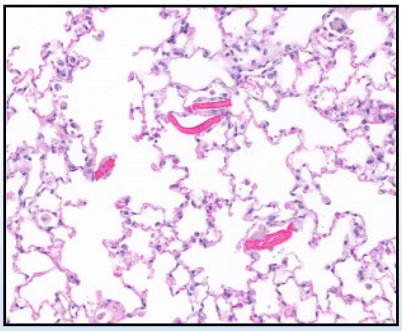
Slide 1 of 3

Case 7: What's Your Diagnosis?



Slide 2 of 3

Case 7: What's Your Diagnosis?



Slide 3 of 3

Case 7: What's Your Diagnosis?

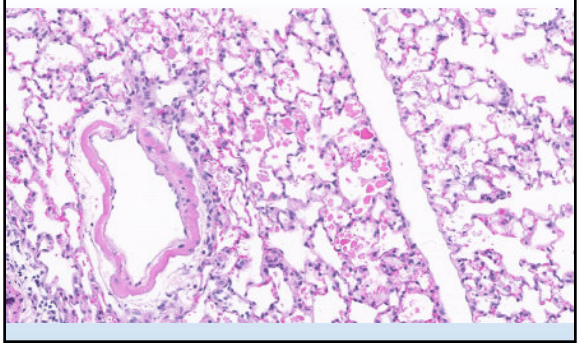
Lung:

1. Edema
2. Proteinosis
3. Eosinophilic crystals
4. Foreign Body
5. Within normal limits
6. Other

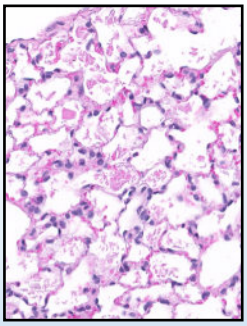
Incidence

	Males	Females
No. Examined	115	115
Crystals, eosinophilic	**103 (90%)**	**98 (85%)**
Minimal	92 (80%)	88 (77%)
Mild	11 (10%)	10 (9%)
Proteinosis	**14 (12%)**	**16 (14%)**
Minimal	14 (12%)	15 (13%)
Mild	0	1 (1%)

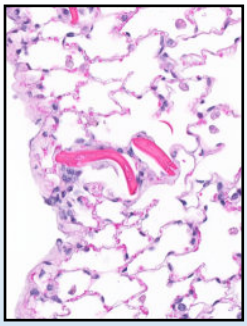
Proteinosis



Proteinosis



Eosinophilic crystals



Acknowledgements

- Kyathanahalli S. Janardhan (ILS - NTP Pathologist)
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- Maureen Paucini (EPL)
- Emily Singletary (EPL)
- Kristen Hobbie (Pathologist, ILS)
- Georgette Hill (Pathology Manager, ILS)

Discussion

Case	Tissue	Diagnosis	Mouse Strain
1	Adrenal Cortex	Subcapsular Carcinoma	C3H/HeJ
2	Seminal Vesicle	Carcinosarcoma	NOD.B10Sn-*H2*^{b}/J
3	Pituitary gland, pars intermedia	Adenoma	129S1/SvImj
4	Pancreas, acinus	Hyperplasia, peri-insular	129S1/SvImj
5	Bone marrow	Increased adipocytes	B6C3F1/J
6	Heart, epicardium	Inflammation, chronic active	NZO/HlLtJ
7	Lung	Eosinophilic crystals	Cast/EiJ

References

- Barthold, Griffey and Percey. Pathology of Laboratory Rodents and Rabbits. 2016. 4th Ed
- Brandli-baiocco et. al (2018). Non-proliferative and Proliferative lesions of Rat and Mouse endocrine system. *J Toxicol Pathol*. 31:1S-95S.
- Majka JA et. al.(1990). Proliferative lesions of the pituitary in Rats. E2. In: Guides for Toxicologic Pathology. STP/ARP/AFIP, Washington, DC.
- Nolte et. al (2016). Non-proliferative and Proliferative lesions of Gastrointestinal tract, pancreas and salivary glands of Rat and Mouse. *J Toxicol Pathol*. 29:1S-124S.
- Suttie, AW. 2018. Boorman's Pathology of the Rat, Reference and Atlas. Pituitary Gland. Chapter 31 p631- 648 Elsevier 2nd Ed.
- Yasoi Y et al. (2008). Spontaneous pituitary carcinoma of the Pars Intermedia in a B6C3F1 mouse. *J Toxicol Pathol* 21:105-108.

NTP
National Toxicology Program

It's a Phthalate Phthing

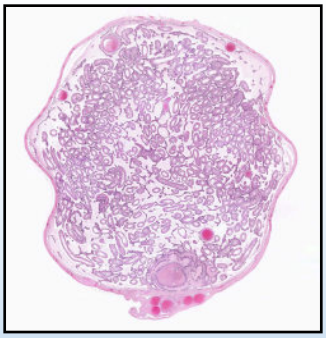
2019 NTP Satellite Symposium
Raleigh Convention Center
Raleigh, NC
June 22, 2019

Mark Cesta, DVM, PhD, DACVP
NIEHS/NTP
Research Triangle Park, NC, USA
cesta@niehs.nih.gov

Case 1: Signalment

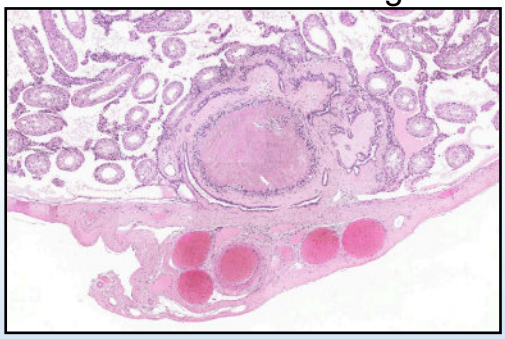
- Male Harlan Sprague-Dawley rat
- Two-year NTP carcinogenesis bioassay
- Gavage study
- A mystery phthalate

Case 1: What's Your Diagnosis?



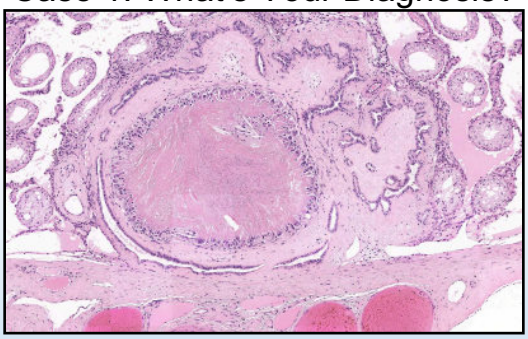
Slide 1 of 4

Case 1: What's Your Diagnosis?



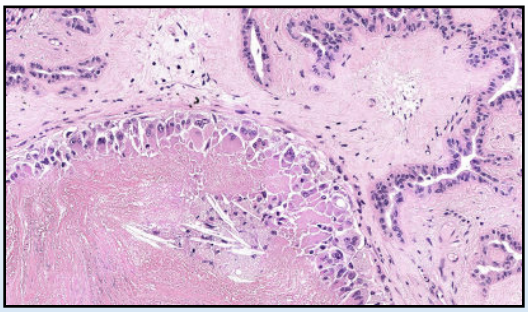
Slide 2 of 4

Case 1: What's Your Diagnosis?



Slide 3 of 4

Case 1: What's Your Diagnosis?



Slide 4 of 4

Case 1: What's Your Diagnosis for this Rete Testis Lesion?

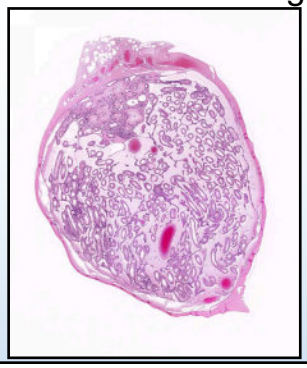
1. Sperm Granuloma
2. Fibrosis
3. Fibrosis and Sperm Granuloma
4. Spermatocoele
5. Inflammation, Chronic
6. Inflammation, Chronic and Fibrosis
7. Hyperplasia
8. I don't know
9. Is it lunch time yet?

Case 2: Signalment

- Male Harlan Sprague-Dawley rat
- Two-year NTP carcinogenesis bioassay
- Gavage study
- A mystery phthalate

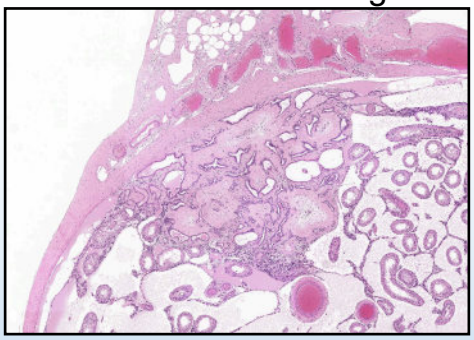
- Same study as case 1, but different rat

Case 2: What's Your Diagnosis?



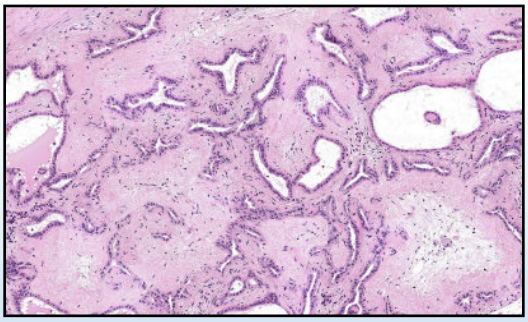
Slide 1 of 4

Case 2: What's Your Diagnosis?



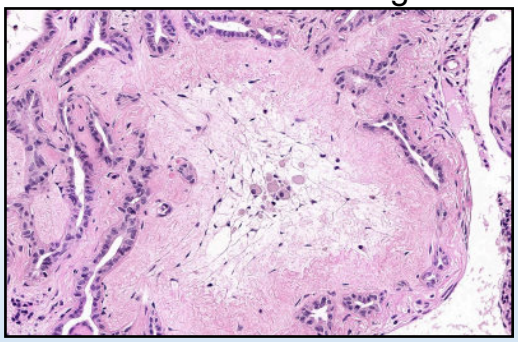
Slide 2 of 4

Case 2: What's Your Diagnosis?



Slide 3 of 4

Case 2: What's Your Diagnosis?



Slide 4 of 4

Case 2: What's Your Diagnosis for this Rete Testis Lesion?

1. Sperm Granuloma
2. Fibrosis
3. Fibrosis and Sperm Granuloma
4. Spermatocoele
5. Inflammation, Chronic
6. Inflammation, Chronic and Fibrosis
7. Hyperplasia
8. I don't know
9. I don't care; I'm leaving

The One Paper

Toxicologic Pathology, 32:79–90, 2004

ISSN: 0192-6233 print / 1533-1601 online
DOI: 10.1080/01926230490265894

Male Reproductive Tract Lesions at 6, 12, and 18 Months of Age Following in Utero Exposure to Di(*n*-butyl) Phthalate

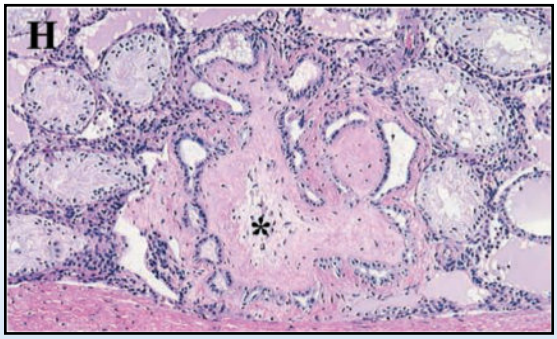
NORMAN J. BARLOW,[1] BARRY S. MCINTYRE,[2] AND PAUL M. D. FOSTER[3]

CIIT Centers for Health Research, Research Triangle Park, North Carolina, USA, and
[1]Aventis Inc., Bridgewater, New Jersey, USA
[2]Schering-Plough Research Institute, Lafayette, New Jersey, USA
[3]National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA

The One Paper

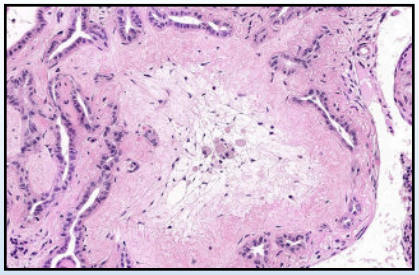
- "The rete testis was often expanded by the presence of sperm and surrounded by granulomatous inflammation. The inflammatory lesion of the rete was most prevalent on PND* 180 and 370. In the PND 370 group, inflammation often appeared to be resolving with increased amounts of fibrous connective tissue surrounding the rete. Rete fibrosis with minimal to mild granulomatous inflammation was the predominant lesion on PND 540 (Figure 1H)."

*PND = Postnatal Day



Lesion Progression

Sperm Granuloma ➡ Fibrosis



Lesion Progression

Sperm Granuloma ➡ Fibrosis

From the one paper (Barlow, et al.):

- PND 180 and 370 – Inflammation predominated
- PND 370 – Inflammation often resolving to fibrosis
- PND 540 – Fibrosis predominated

Lesion Progression

Incidences of Sperm Granuloma and Fibrosis in the Rete Testis in the NTP Study

	Control	Low Dose	Low-Mid Dose	High-Mid Dose	High Dose
# examined	49	50	50	50	50
Sperm Granuloma	0	0	0	0	2
Fibrosis	0	0	0	0	11

Association with Dysgenesis

Incidences of Sperm Granuloma or Fibrosis and Dysgenesis in the Rete Testis in the NTP Study

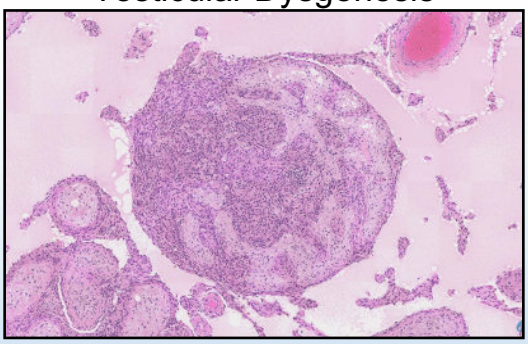
	Control	Low Dose	Low-Mid Dose	High-Mid Dose	High Dose
# examined	49	50	50	50	50
Sperm Granuloma or Fibrosis	0	0	0	0	11
Dysgenesis	0	0	0	1	9

Association with Dysgenesis

– Number of animals with rete lesion: 11
– Number of animals with dysgenesis: 9
– Number of animals with rete lesion and dysgenesis in the same testis: 5

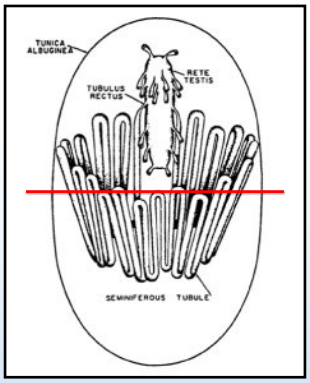
– These lesions often occur together, but not always
  • Small dysgenesis lesion may not be in section examined

Testicular Dysgenesis



Sectioning the Testis

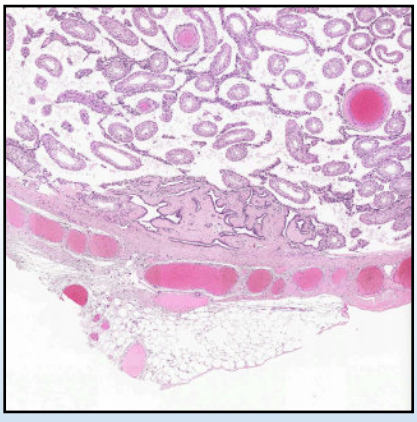
• One section in middle of testis
  – could miss the rete testis
  – could give an incomplete picture of the lesion

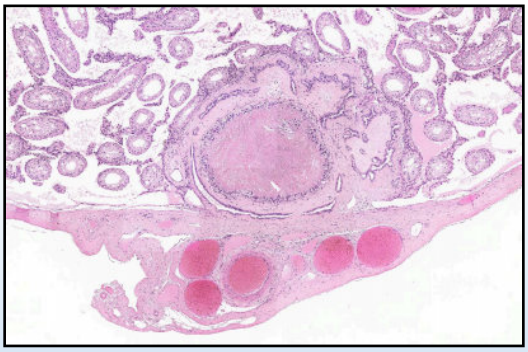


Shamelessly lifted from Foley GL, (2001). *Tox Path*, vol. 29, p. 50.

Advantages of Multiple Sections

- Less likely to miss the rete testis
- May get multiple sections of the rete testis, giving a better picture of the lesion





Pathogenesis

- Doesn't seem likely that it is caused by dysgenesis because dysgenesis is upstream of the rete testis – however, the 2 lesions do often occur in the same testis
- Could be a blockage in the efferent ducts, but these were not examined (not collected in NTP studies)
- No obstructive lesion in the epididymis

Pathogenesis

- Disruption of the wall of the rete testis?
  - DBP exposure to rats during the MPW results in disruption of seminiferous tubules with release of Sertoli and germ cells into the interstitium and development of dysgenesis

Lara NLM, van den Driesche S, Macpherson S, Franca LR, Sharpe RM. (2017). Dibutyl phthalate induced testicular dysgenesis originates after seminiferous cord formation in rats. Sci Rep 7:2521. doi: 10.1038/s41598-017-02684-2.

DBP = Dibutyl phthalate
MPW = Masculinization programming window (e15.5 – e18.5)

Pathogenesis

- Disruption of rete testis?
  - DBP exposure to rats during the MPW results in disruption of seminiferous tubules with release of Sertoli and germ cells into the interstitium and development of dysgenesis

Why Do We Care About Phthalates?

- Because they have the potential to be bad
  - Antiandrogens in rats exposed in utero
  - High level of exposure to humans
    - Soft plastics
    - Personal care products (e.g., shampoo, deodorant, hair spray)

Why Do We Care About Phthalates?

- Some lesions in rats exposed in utero
  - Testis
    - Dysgenesis
    - Leydig cell aggregation
    - Rete testis sperm granuloma/fibrosis
    - Degeneration of the germinal epithelium
  - Malformations of the epididymis, seminal vesicles, prostate and penis

Take Home Points

- Associated with phthalate administration
- Often occurs concurrently with testicular dysgenesis
- Sperm granuloma progresses to fibrosis
- Pathogenesis is unknown
- Phthalates are bad

References

- Barlow NJ, McIntyre BS, Foster PMD. (2004). Male reproductive tract lesions at 6, 12, and 18 months of age following in utero esposure to di(n-butyl) phthalate. *Toxicol Pathol* 32:79-90.
- Elmore SA, Carreira V, Labriola CS, Mahapatra D, McKea SR, Rinke M, Shackelford C, Singh B, Talley A, Wallace SM, Wancket L, Willson CJ. (2018). Proceedings of the 2018 National Toxicology Program Satellite Symposium. Toxicol Pathol 46:865-897.
- Foley GL. (2001). Overview of male reproductive pathology. *Toxicol Pathol* 29:49-63.
- Lara NLM, van den Driesche S, Macpherson S, Franca LR, Sharpe RM. (2017). Dibutyl phthalate induced testicular dysgenesis originates after seminiferous cord formation in rats. Sci Rep 7:2521. doi: 10.1038/s41598-017-02684-2.

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- Gabrielle Willson (EPL, QA/PWG Pathologist)
- Mike Meyers (as Linda Richman, host of Coffee Talk)
- Daffy Duck

NTP
National Toxicology Program

Ovarian Lesion in a Young Sprague-Dawley Rat

2019 NTP Satellite Symposium
Raleigh Convention Center
Raleigh, NC
June 22, 2019

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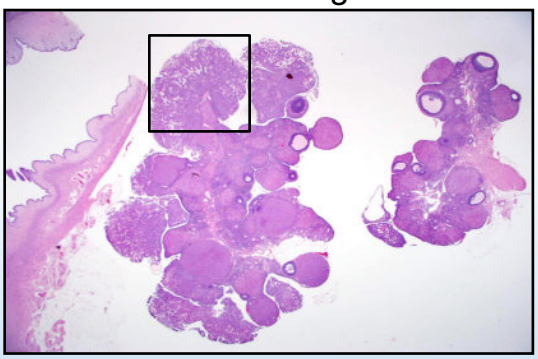
WCFP
WestChina-Frontier PharmaTech

Signalment

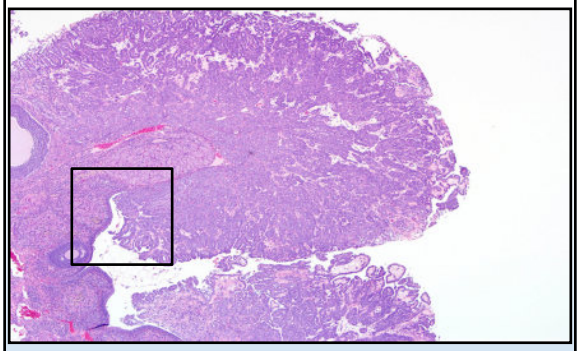
- Female Sprague-Dawley (SD) rat
  - Terminal sacrifice
  - Control group
- 13-week repeated-dose toxicity study administrated by oral gavage
  - 6~7 weeks of age at the start of the study
  - 19~20 weeks of age at scheduled sacrifice

What's Your Diagnosis?



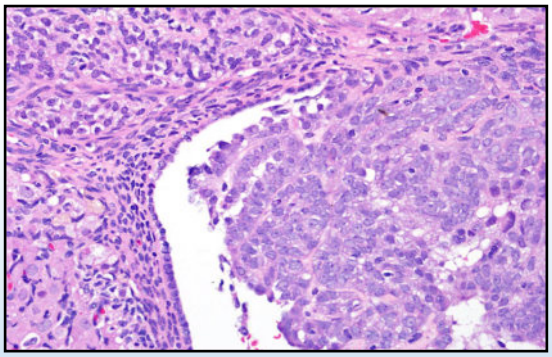
Slide 1 of 5

What's Your Diagnosis?



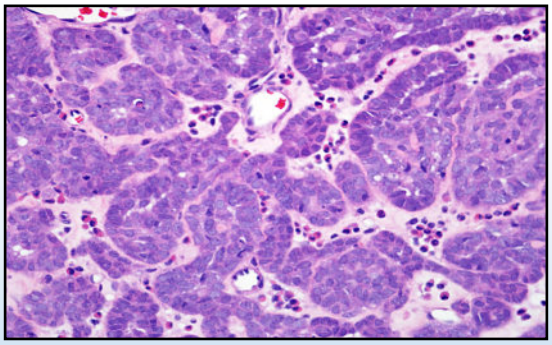
Slide 2 of 5

What's Your Diagnosis?



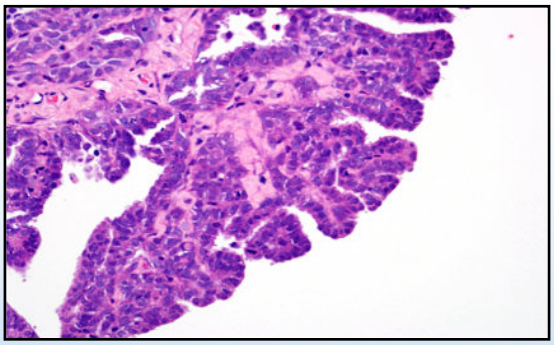
Slide 3 of 5

What's Your Diagnosis?



Slide 4 of 5

What's Your Diagnosis?



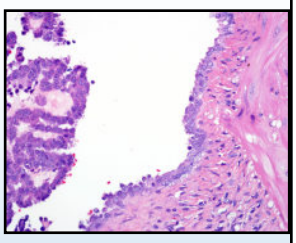
Slide 5 of 5

What's Your Diagnosis?

1. Hyperplasia, cystic/papillary
2. Carcinoma, tubulostromal
3. Cystadenoma
4. Cystadenocarcinoma
5. Mesothelioma, malignant
6. Other

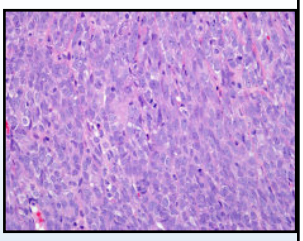
Histologic Features

- Lesion confined to the surface of the ovary
  - No infiltration into the adjacent normal ovarian tissues
- Solid, cystic and papillary structures with some ciliated epithelial cells



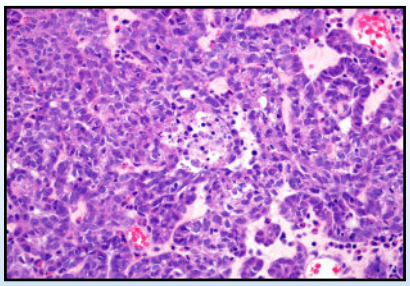
Histologic Features

- Cuboidal to low columnar cells
- Nuclear pleomorphism
- Increased nuclear/cytoplasmic ratio
- Mild mitotic figures



Histologic Features

- Necrosis, cellular debris, neutrophilic infiltration, and neovascularization



Additional Information

- No gross lesions were noted in either ovary
- Ovarian organ weight and ratios were increased

Organ	Present case	Remaining Female Controls Mean±SD (n=9)*
Terminal body weight (g)	303.1	322.9±22.7
Ovaries (g)	0.188	0.117±0.025
% organ to terminal body weight ratio (g%)	0.063	0.037±0.007
% organ to brain weight ratio (g%)	9.27	5.89±1.28

*present case compared with the mean values of the remaining female control rats in this study.
Note: g=gram.

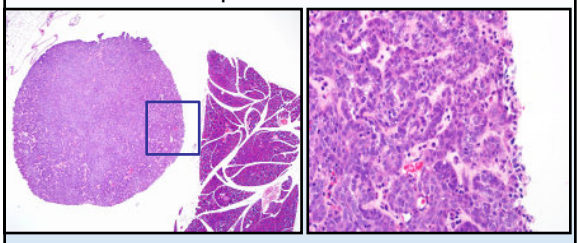
Additional Information

- Gross lesions – Only noted in pancreas
  - Several smooth, gray-white, round nodules noted on the surface of the pancreas

Numbers on ruler indicate centimeter (cm)

Additional Information

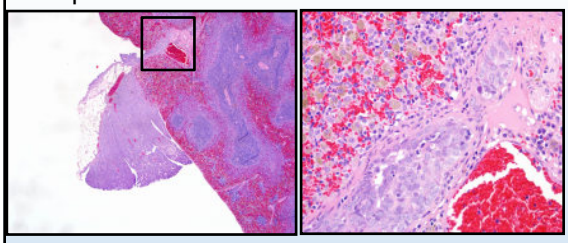
- Histological metastases were noted along the surface of pancreas



Located in the surrounding adipose tissues

Additional Information

- Histological metastases were noted at the splenic hilus



On the surface of the hilus

INHAND/goRENI Diagnostic Criteria for Cystadenocarcinoma

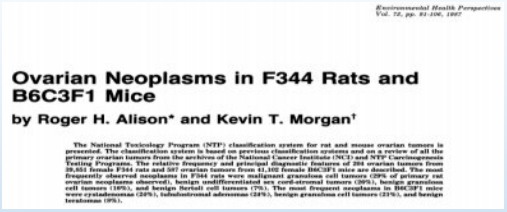
- Solid or cystic mass lined by cuboidal or low columnar pleomorphic epithelium that may be ciliated
- Mitotic figures are frequent
- Folds or papillary projections may be present

INHAND/goRENI Diagnostic Criteria for Cystadenocarcinoma

- Stromal compartment is not a prominent part of the tumor
- Infiltration of adjacent tissue is present

Is this a Common Lesion in Rodents?

- Common lesion in some mouse strains
- Uncommon in rats
  - In F344 rat – Incidence of cystadenocarcinoma in the ovary was 3% of the 204 ovarian tumors reported from 39,851 females



Environmental Health Perspectives
Vol. 72, pp. 81-100, 1987

Ovarian Neoplasms in F344 Rats and B6C3F1 Mice

by Roger H. Alison* and Kevin T. Morgan†

Is this a Common Lesion in Rodents?

- In SD rats - Cystadenocarcinoma was only reported in 1/7748 females (with 210 ovarian tumors)
  - 101 to 110 weeks of age

	Age ranges at death (weeks)								
	0-52	53-70	71-80	81-90	91-100	101-110	111-120	121-130	Total
Tumors of epithelial (ovarian coelomic mesothelium) origin									
Tubular adenoma	-	-	-	3	-	2	2	-	7
Anaplastic adenocarcinoma	-	-	1[a]	-	-	-	-	-	1
Papillary cystadenoma	-	-	-	-	-	2	-	-	2
Papillary cystadenocarcinoma	-	-	-	-	-	1[a]	-	-	1
Benign mesothelioma	-	-	-	1	-	1	-	-	2
Malignant mesothelioma	-	-	-	1	2	1	-	1	5[a]

[a] Tumors considered as factors contributory to death of the animal

Lewis, D.J. (1987) Ovarian neoplasia in the Sprague-Dawley rat. Environ Health Perspect **73**, 77-90

Is this a Common Lesion in Rodents?

- In young SD rats (10 to 32 weeks): No spontaneous ovarian cystadenocarcinomas were recoded

Occurrence of Spontaneous Tumors in Control Sprague-Dawley Rats at 10 to 32 Weeks of Age

Organ/system	Tumor type	Age (weeks)	10		19		32	
		Sex	Male	Female	Male	Female	Male	Female
		No. of animals	782	770	857	878	842	874
Pituitary	Anterior adenoma		-	-	-	1(0.1)	1(0.1)	3(0.3)
Thyroid	Follicular adenocarcinoma		-	-	1(0.1)	-	-	-
	C cell adenoma		-	-	-	1(0.1)	-	-
Tongue	Hemangiosarcoma		-	-	-	-	1(0.1)	-
Submandibular gland	Adenocarcinoma		-	-	-	-	1(0.1)	-
Spleen	Histiocytic sarcoma		-	-	-	-	1(0.1)	-
Kidney	Nephroblastoma		-	-	-	1(0.1)	-	-
Brain	Oligodendroglioma		-	-	-	-	1(0.1)	2(0.2)
Skin	Basal cell tumor		-	-	1(0.1)	-	-	-
Mammary gland	Adenocarcinoma		-	-	-	-	1(0.1)	6(0.7)*
	Fibroadenoma		-	-	-	-	-	2(0.2)*
Hemolymphoreticular system	Malignant lymphoma		-	-	1(0.1)	-	-	-
Number of tumor-bearing rats			0	0	3	3	6	13
Incidence of rats bearing tumors (%)			0	0	0.4	0.3	0.7	1.5

Numbers in parentheses indicate incidence (%). * The combined tumor incidence was 0.9%.

Ikezaki, S., Takagi, M., Tamura, K. (2011) Natural occurrence of neoplastic lesions in young sprague-dawley rats. J Toxicol Pathol **24**, 37-40

Summary

- Ovarian cystadenocarcinoma is one of the tumors of epithelial origin in the ovary
  - Ovarian coelomic mesothelium
- In our present case, an ovarian cystadenocarcinoma was found in a control Sprague-Dawley female rat nearly 20 weeks of age
- To our knowledge, this is the first report of an ovarian cystadenocarcinoma in a young rat

References

- Alison, R.H., Morgan, K.T. (1987) Ovarian neoplasms in F344 rats and B6C3F1 mice. Environ Health Perspect **73**, 91-106.
- Lewis, D.J. (1987) Ovarian neoplasia in the Sprague-Dawley rat. Environ Health Perspect **73**, 77-90.
- Gregson, R.L., Lewis, D.J., Abbott, D.P. (1984) Spontaneous ovarian neoplasms of the laboratory rat. Vet Pathol **21**,292-299.
- Ikezaki, S., Takagi, M., Tamura, K. (2011) Natural occurrence of neoplastic lesions in young sprague-dawley rats. J Toxicol Pathol **24**, 37-40.
- Dixon, D., Alison, R., Bach, U. et al. (2014) Nonproliferative and proliferative lesions of the rat and mouse female reproductive system. J Toxicol Pathol **27**(3-4 Suppl), 1S-107S.

Acknowledgements

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- Torrie A. Crabbs (EPL, Senior Pathologist)
- Tom Steinbach (EPL, Senior Pathologist)

NTP National Toxicology Program

A Periocular Predicament

2019 NTP Satellite Symposium
Raleigh Convention Center
Raleigh, NC
June 22, 2019

Gregory Krane, DVM, DACVP
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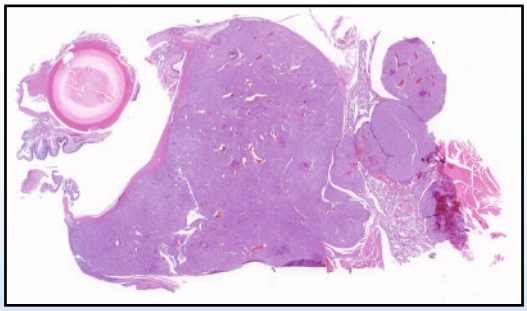
NC STATE UNIVERSITY

Signalment

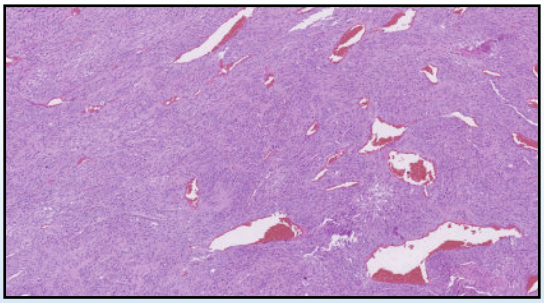
- Male F344/N treated rat (high dose)
- Two year carcinogenesis bioassay
  - 1-2 – Epoxybutane
  - Stabilizer in chlorinated hydrocarbon solvents
- Inhalation study
- Only animal with this tumor in study

What's Your Diagnosis?



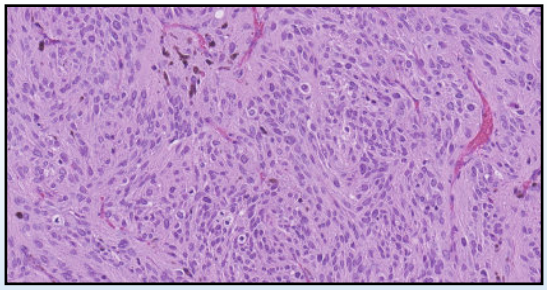
Slide 1 of 4

What's Your Diagnosis?



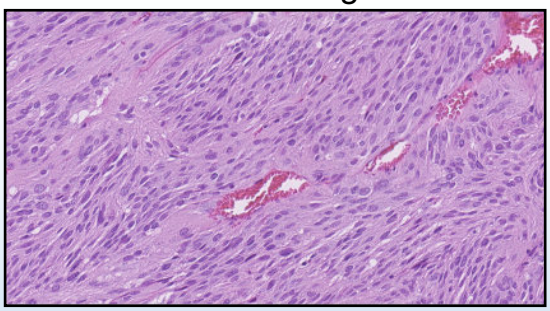
Slide 2 of 4

What's Your Diagnosis?



Slide 3 of 4

What's Your Diagnosis?

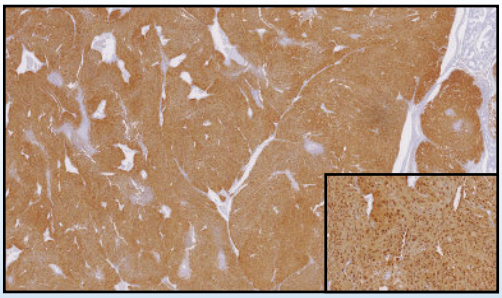


Slide 4 of 4

What's Your Diagnosis?

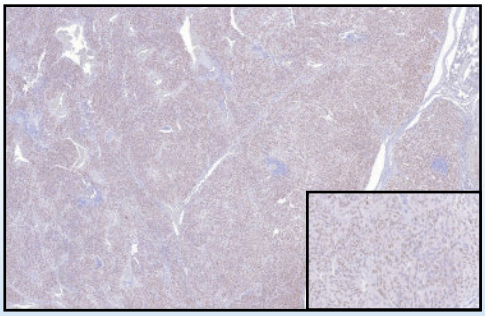
1. Leiomyoma
2. Melanoma
3. Meningioma
4. Neurofibroma
5. Perineurioma
6. Peripheral Nerve Sheath Tumor
7. Sarcoma
8. Schwannoma
9. Other / Need More Information

What's Your Diagnosis?
S-100



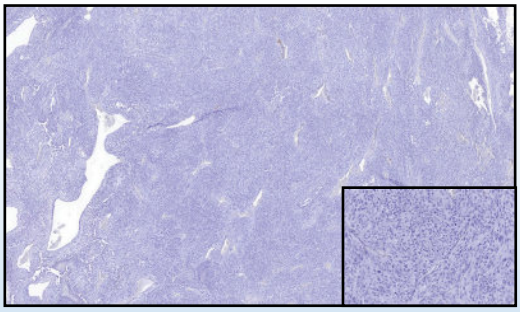
Slide 1 of 3

What's Your Diagnosis?
SOX-10



Slide 2 of 3

What's Your Diagnosis?
EMA



Slide 3 of 3

What's Your Diagnosis?

1. Leiomyoma
2. Melanoma
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4. Neurofibroma
5. Perineurioma
6. Peripheral Nerve Sheath Tumor
7. Sarcoma
8. Schwannoma
9. Other / Need More Information

Tumor Features

- Retro-orbital
- Bundles and streams of spindle cells
- Fibrovascular stroma
- Low pleomorphism and mitotic index

IHC Marker	Reactivity
S-100	+++
Sox-10	+
EMA	-

Background: Schwannoma

- Peripheral nerve sheath tumors
  - Benign and malignant variants
- Previously used nomenclature:
  - Neurinoma, neurilemmoma
- Common in domestic species (K9, Bovine)
- Associated with neurofibromatosis in man
  - *NF1* and *NF2* mutations (chromosome 22)
- Rarely metastasize
  - Can be locally invasive and recur

Rodent Schwannoma

- Rat: heart, pinna, head/neck
- Can be induced
  - N-nitrosoethylurea (trigeminal nerve)
    - *neu/erdB-2* mutation (nucleotide 2012)
    - Acid hydrolase levels
    - BDIX vs BDIV strains
  - Methyl-methane sulfonate
  - 7,12-dimethylbenz[α]anthracene
  - N-nitrosomethylurea
    - body cavities, pancreas, prostate, thymus, heart
- Mouse models of neurofibromatosis
  - *NF1* and/or *NF2* mutations

Rat Head-Neck Schwannoma

- Brain (Meninges, Pituitary Gland)
- Trigeminal Nerve & Ganglion
- Eye (Including Retrobulbar Region)
- Harderian Gland
- Nose (Including Ethmoid Nerve Bundles)
- Salivary Gland
- Mandibular Lymph Nodes
- Thyroid Gland
- Skin / Subcutis

Courtesy Dr. Maggie Gruebbel (EPL)

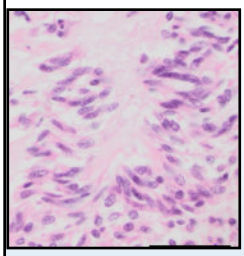
Gross Pathology: Schwannoma

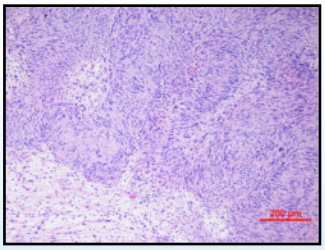
- Nodular masses or thickenings of nerves
  - Spinal, paraspinal, or cranial nerves
- Variable firmness
- White to gray, shiny and smooth
- Generally unilateral
  - Bilateral CN VIII in neurofibromatosis (man)
- Intra or extradural
- Subcutaneous or intradermal
- Intracardiac (rat, cow)

Histopathology: Schwannoma

- Densely packed fusiform cells
- Scant cytoplasm, poorly defined borders
- Interwoven bundles, streams, or whorls
- Wallerian degeneration
- +/- Antoni A and B configurations
- +/- Verocay bodies
- +/- Osseous or cartilagenous differentiation

Histopathology: Schwannoma

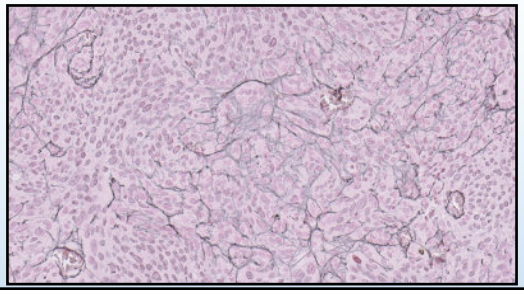




Paraschou G et al. *J Comp Pathol* 2016. Tan QT et al. *J Surg Case Rep* 2014.

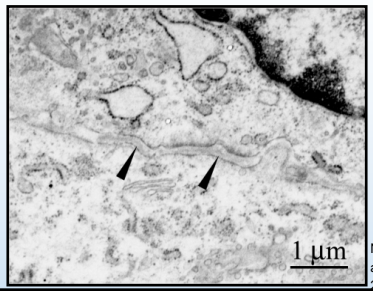
Special Stains

- Continuous basal lamina (reticulin)



Electron Microscopy

- Continuous basal lamina



Nagatani M et al. *Tox Path* 2009

Immunohistochemistry

	Schwannoma	Meningioma	Neurofibroma	Perineurioma
S-100	+++	+/-	+/-	-
Sox-10	+	-	nr	nr
EMA	-	+	-	+

nr = not reported

Immunohistochemistry

- S-100
  - Originally isolated in CNS
  - Wide distribution of tissues
  - Calcium flux regulator
  - Useful in Schwann cell, melanocytic, or chondrocytic lineage

Immunohistochemistry

- Sox-10
  - Neural crest transcription factor
  - Required for Schwann cell and melanocytic differentiation and survival
- EMA
  - Antiepithelial membrane antigen
  - Thought to be involved in cell secretion
  - Positive in perineurioma, meningioma
    - Synovial sarcoma, chordoma, myoepithelioma, plasmacytoma

Schwannoma Variants

- Cellular
  - Primarily Antoni A without Verocay bodies
- Granular
  - Similar to granular cell tumor
- Melanotic
  - Pigmented melanosomes
- Plexiform
  - Multinodular pattern in various nerve branches

Differential Diagnoses

- Leiomyoma
- Malignant Schwannoma
- Melanoma
- Meningioma
- Neurofibroma
- Perineurioma

Heart Findings (Rats)

- Cardiomyopathy
  - Significantly increased frequency rt. ventricle
    - GSM: Male and Female
    - CDMA: Male
- Schwann Cell Hyperplasia
  - Non-significant increase in males
- Schwannoma
  - Significant increase in males
- Clear evidence of carcinogenicity

Take Home Points

- Schwannomas may be present in a variety of tissues
- Schwannomas have multiple differentials
- Differentials reflect different behavior
- Determining specific tumor type may be warranted in particular circumstances
- Definitive diagnosis may require IHC

References

- Ariza A, Bilbao JM, Rosai J. *Am J Surg Pathol* 12: 1988.
- Boulagnon-Rombi C et al. *J Neuropathol Exp Neurol* 76, 2017.
- Dabbs DJ. Diagnostic Immunohistochemistry. 3rd ed, 2010.
- Elmore SA et al. *Tox Path* 26: 2013.
- Elmore SA et al. *Tox Path* 45: 2016.
- Gering KM et al. *Cancer Res* 66: 2006.
- Hirose T et al. *Mod Path* 16: 2003.
- Hornick JL, Fletcher CDM. *Am J Surg Pathol* 29: 2005.
- Kaufmann W et al. *Tox Path* 40: 2012.
- Koestner A et al. *Am J Pathol* 63: 1971.
- Kumar VK, Abbas AK, Aster JC. Pathologic Basis of Disease. 9th ed, 2015.

References

- Macarenco RS, Ellinger FE, Olivieira AM. *Arch Pathol Lab Med* 131: 2007.
- Maxie MG. Pathology of Domestic Animals. 6th ed, 2016.
- Meuten DJ. Tumors in Domestic Animals. 5th ed, 2017.
- Nagatani M et al. *Tox Path* 37: 2009.
- Nikitin AY et al. *Proc Natl Acad Sci USA* 88: 1991.
- NTP Tehnical Report 329, 1988.
- NTP Technical Report 595, 2018.
- Rodriguez FJ et al. *Acta Neuropathol* 123: 2012.
- Suttie AW. Pathology of the Rat. 2nd ed, 2015.
- Swenberg JA et al. *J Natl Cancer Inst* 55: 1975.
- Yoshida M. *J Comp Pathol* 106: 1992.
- Yoshizawa et al. *Exp Toxicol Pathol* 68: 2016.

	Schwannoma	Meningioma	Neurofibroma	Perineurioma	Leiomyoma
CNPase	+	nr	nr	nr	nr
S-100	+++	+/-	+/-	-	nr
Laminin	+	-	nr	+	nr
Sox-10	+	-	nr	nr	nr
GFAP	+/-	-	nr	+/-	-
PLP	+	nr	nr	nr	nr
PMP22	+	nr	nr	nr	nr
EMA	-	+	-	+	nr
Periaxin	+	nr	nr	nr	nr
CD57 (Leu-7)	+/-	nr	nr	nr	nr
Schwann / 2E	+	-	-	-	-
Desmin	-	nr	nr	nr	+
SSTR2A	-	+	nr	nr	nr
Vimentin	+	nr	nr	+	nr
Collagen IV	+	nr	nr	+	nr
Claudin-1	-	nr	+	+	nr
GLUT-1	-	nr	nr	+	nr

nr = not reported

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- Dr. David Malarkey, NIEHS/NTP
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- Dr. Maggie Gruebbel, EPL
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- NIEHS / NCSU Colleagues & Mentors
- Dr. Gordon Flake, NIEHS/NTP

NTP
National Toxicology Program

Unusual Lesions in the Pancreas and Liver of HSD Rats

2019 NTP Satellite Symposium
Raleigh Convention Center
Raleigh, NC
June 22, 2019

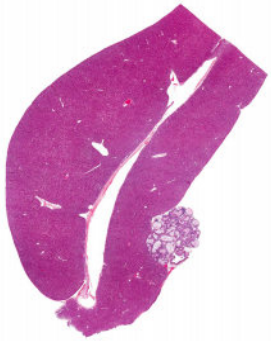
Torrie A. Crabbs, DVM, DACVP
EPL, Inc
Research Triangle Park, NC, USA

EPL

Case 1: Signalment

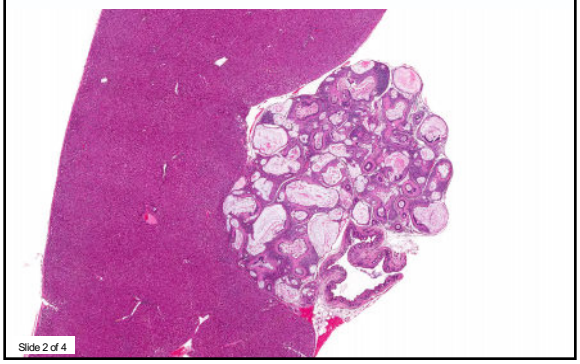
- Female Harlan Sprague Dawley (HSD) rat
  - Terminal sacrifice animal
  - High-dose group
    - Non test article-related finding
- Two year chronic toxicity study

Case 1: What's Your Diagnosis?



Slide 1 of 4

Case 1: What's Your Diagnosis?



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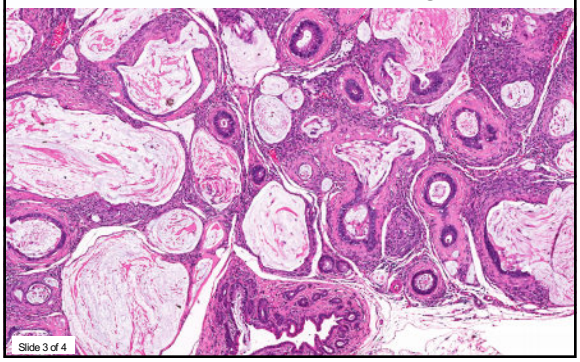
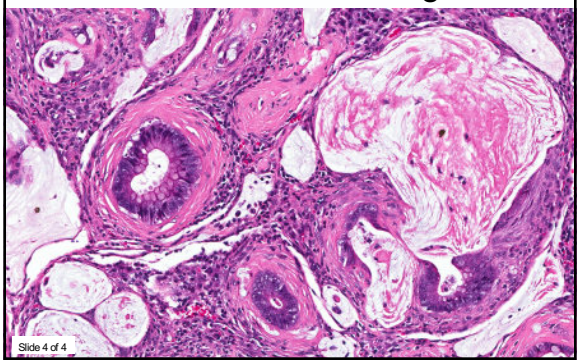


Case 1: What's Your Diagnosis?



Case 1: What's Your Diagnosis?

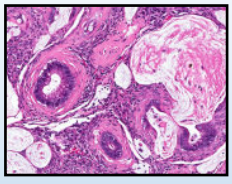
1. Chronic inflammation
2. Cholangiofibrosis
3. Periductal cholangiofibrosis
4. Cholangiocarcinoma
5. Adenocarcinoma
6. Fibrosis
7. Other

Cholangiofibrosis

- Controversial lesion with inflammatory, proliferative, and metaplastic components
- Can resemble cholangiocarcinoma (CCA)
  - Often misdiagnosed
  - CCA diagnosis based on:
    - Extensiveness of tissue involvement
    - Features of malignancy
    - Invasion
  - Reports of progression to CCA
    - Unequivocal metastasis have not been confirmed in most cases
- Not observed in humans

Pathogenesis

- Remains somewhat elusive
- Exposure to hepatotoxic xenobiotics
- Pronounced hepatocellular injury
- Oval cell proliferation



Incidence

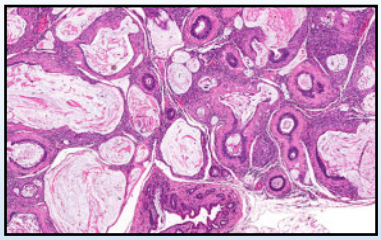
- Historically not considered a spontaneous lesion
  - No reports in untreated control F344 rats from the NTP Archives Database
- Primarily occurs in rats treated with xenobiotics
  - Dioxins, furan, related chemicals
- Long Evans Cinnamon (LEC) rat
  - "Spontaneous" incidences reported

Long Evans Cinnamon Rat

- Rat model of Wilson's disease
  - Characterized by:
    - Ceruloplasmin deficiency
    - Hepatic copper accumulation
    - Hepatocellular injury
- In other words - not a "true" spontaneous occurrence
  - Underlying hepatocellular injury

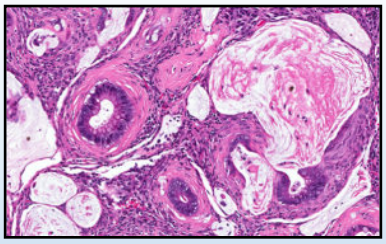
Histologic Features

- Dilated to cystic bile ducts filled with mucous and cellular debris



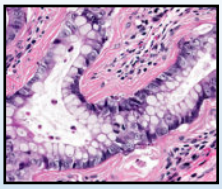
Histologic Features

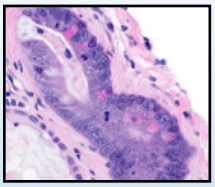
- Ducts are surrounded by inflammatory cell infiltrates and dense connective tissue



Histologic Features

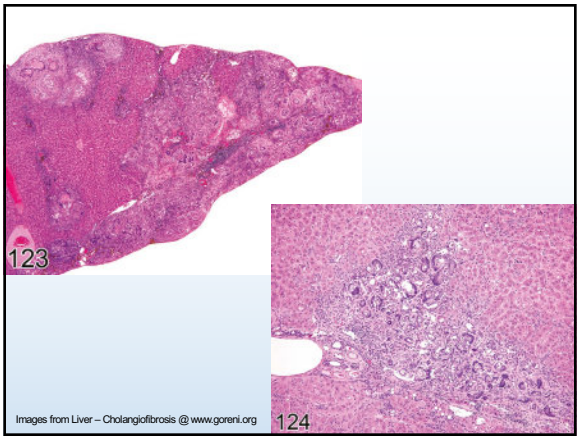
- Ducts lined by a single layer of flattened to tall columnar basophilic cells
- Intestinal metaplasia
  - Goblet cells ± Paneth cells





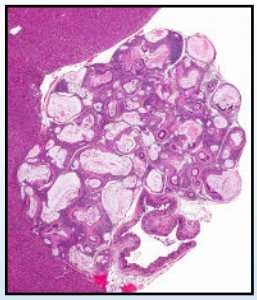
Histologic Features NOT Present in this Case

- Can occupy large interconnecting areas of a lobe without markedly disturbing the lobe outline
- Growth typically involves contraction with retraction of surrounding parenchyma
- Older lesions may be shrunken from the liver surface and appear as scars
- Regenerative hepatocellular hyperplasia may be present when there is extensive parenchymal involvement



Unique Features in this Case

- Focal / Nodular
- Extrahepatic
  - Outside the capsule
- Periductal
  - Adjacent to the common bile duct
- Non-treatment-related
- No evidence of underlying hepatocellular injury



Liver, Periductal – Cholangiofibrosis

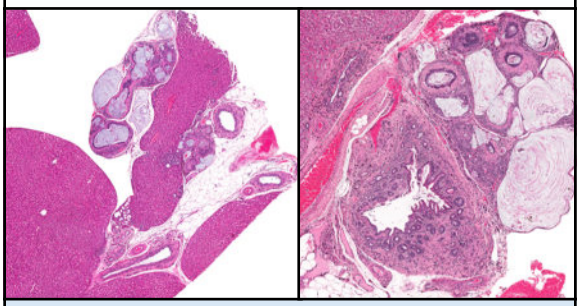
		Unexposed Controls	Low-Dose Exposed	Mid-Dose Exposed	High-Dose Exposed
No. Examined		90	90	90	90
MALES	Arm 1	2 (2.2%)	1 (1.1%)	1 (1.1%)	1 (1.1%)
	Arm 2		2 (2.2%)	2 (2.2%)	0
Total		2	3	3	1

		Unexposed Controls	Low-Dose Exposed	Mid-Dose Exposed	High-Dose Exposed
No. Examined		90	90	90	90
FEMALES	Arm 1	1 (1.1%)	0	0	1 (1.1%)
	Arm 2		1 (1.1%)	1 (1.1%)	1 (1.1%)
Total		1	1	1	2

Liver, Periductal – Cholangiofibrosis

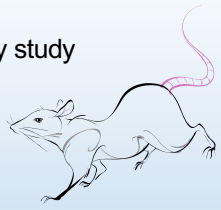
	Unexposed Controls (%)	Combined Exposed (%)
No. Examined	90	540
Males	2.2	1.3
Females	1.1	0.7

Liver, Periductal – Cholangiofibrosis

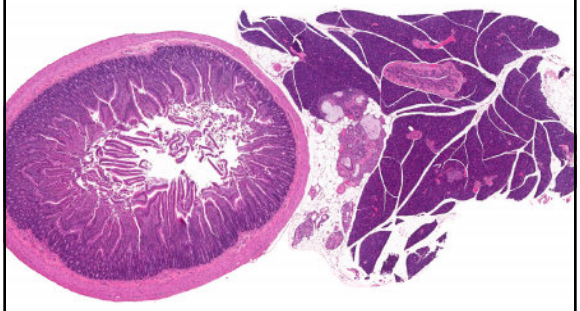


Case 2: Signalment

- Female Harlan Sprague Dawley (HSD) rat
  - Terminal sacrifice animal
  - Low-dose group
- Two year chronic toxicity study
  - Same as Case #1

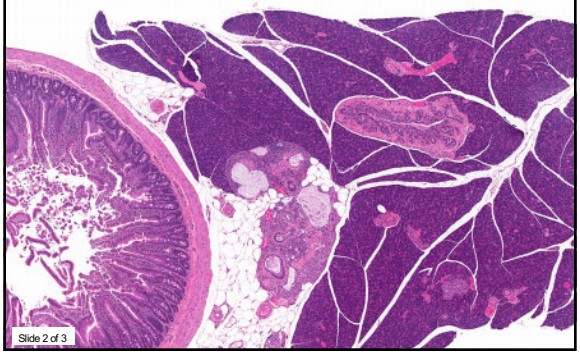


Case 2: What's Your Diagnosis?



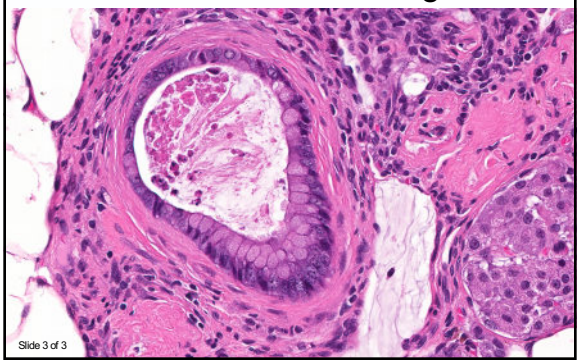
Slide 1 of 3

Case 2: What's Your Diagnosis?



Slide 2 of 3

Case 2: What's Your Diagnosis?



Slide 3 of 3

Case 2: What's Your Diagnosis?

1. Chronic inflammation
2. Cholangiofibrosis
3. Periductal cholangiofibrosis
4. Cholangiocarcinoma
5. Adenocarcinoma
6. Fibrosis
7. Other

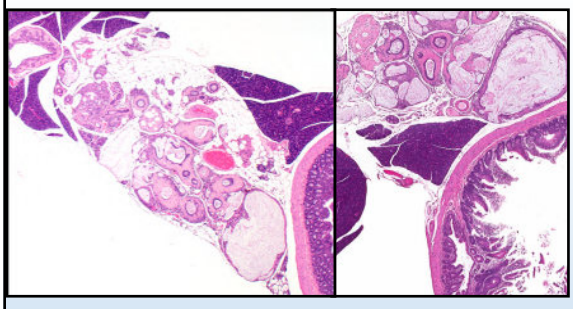
Pancreas, Periductal - Cholangiofibrosis

		Unexposed Controls	Low-Dose Exposed	Mid-Dose Exposed	High-Dose Exposed
No. Examined		90	90	90	90
FEMALES	Arm 1	0	0	7 (7.8%)	4 (4.4%)
	Arm 2		3 (3.3%)	2 (2.2%)	1 (1.1%)
Total		0	3	9	5

	Unexposed Controls (%)	Combined Exposed (%)
No. Examined	90	540
Males	0	0
Females	0	3.0*

*Non-dose-related response; Reviewed by PWG and considered non-exposure related

Pancreas, Periductal - Cholangiofibrosis



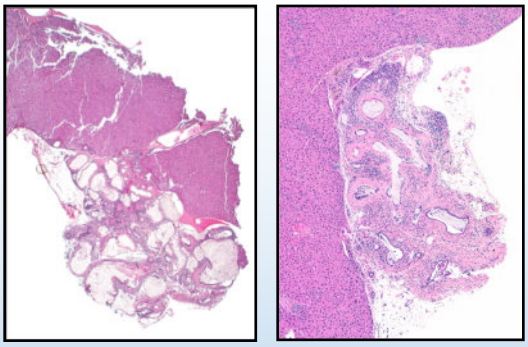
Summary – Current Study

- 90 control animals/sex and 540 treated animals/sex
- Focal periductal cholangiofibrosis was noted in the liver or pancreas
  - 9 males
    - 9/9 (100%) in the liver
  - 21 females
    - 16/21 (76%) in the pancreas
- 3 animals with liver lesions (2 males; 1 female) were unexposed controls
- Incidences in exposed groups were low, sporadic, and unrelated to dose
  - Regarded as incidental and unrelated to exposure

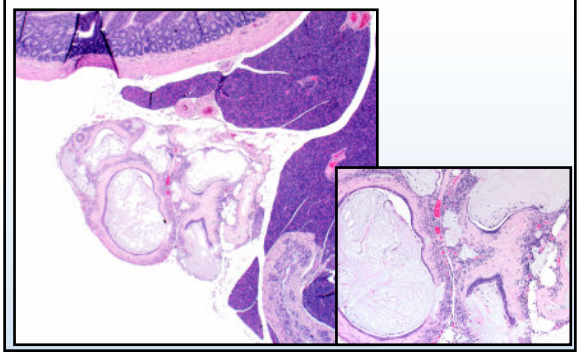
Retrospective Review

- Findings prompted a retrospective review of recent NTP two-year carcinogenicity/chronic toxicity studies in HSD rats
  - 9 studies
    - 260 males
    - 705 females
  - Liver and Pancreas were examined in all untreated / vehicle control animals
    - Included the pancreas section typically sectioned with the duodenum
- Four additional periductal cholangiofibrosis cases were noted:
  - 3 Liver = 2 females; 1 male
  - 1 Pancreas = 1 male
- Given all are control animals → spontaneous/incidental

Control Females



Control Male



Spontaneous Cholangiofibrosis in a Wistar Rat

Toxicologic Pathology
2019, Vol. 47(4) 556-560

DOI: 10.1177/0192623319842549
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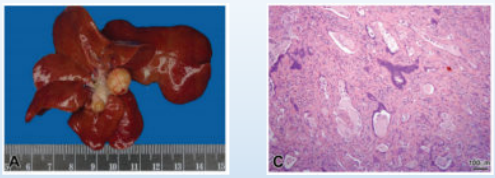
SAGE

Tao Chen[1], Ke Chen[1], Shaung Qiu[1], and Peter C. Mann[2]

Abstract

In a 2-year carcinogenicity study, we identified a spontaneous cholangiofibrosis in a control male Wistar rat. This lesion has long been considered as a compound-related change, with no spontaneous cases reported in the Wistar rat. In addition to routine hematoxylin and eosin stains evaluation, we applied Masson's trichrome staining, Alcian blue-periodic acid–Schiff staining, and OV-6 immunohistochemistry staining. The special staining demonstrated the fibrous component in the interstitium and intestinal metaplasia of the epithelium (presence of goblet cells), while the positive anti-OV-6 reaction indicated the bile duct origin of the epithelium. These results help to confirm the diagnosis of cholangiofibrosis in this case. We report this rare case to alert pathologists that spontaneous cholangiofibrosis does occur in Wistar rats.



Discussion Points

- Are these focal / nodular lesions different than the more diffuse lesions associated with hepatotoxicity?
- Thoughts on "periductal" as a designation for these findings
  - In the liver?
  - In the pancreas?

Take Home Points

- Spontaneous / Non-treatment-related incidences of cholangiofibrosis can occur
- Histologic features are similar to induced cholangiofibrosis
- Unique features include that they are often:
  - Focal / Nodular
  - Extracapsular / Extrahepatic
  - Periductal
  - Lack of underlying hepatocellular injury
- Possible sex predilection for pancreatic lesion??

References

- Chen, T., Chen, K., Qiu, S., and Mann, P.C. (2019). Spontaneous cholangiofibrosis in a Wistar Rat. *Toxicol Pathol* 47(4), 556-560.
- Elmore, L.W. and Sirica, A. E. (1991). Phenotypic characterization of metaplastic intestinal glands and ductular hepatocytes in cholangiofibrotic lesions rapidly induced in the caudate liver lobe of rats treated with furan. *Cancer Res* **51**, 5752 – 59.
- Hailey, J.R., Walker, N.J., Sells, D.M., Brix, A.E., Jokinen, M.P., and Nyska, A. (2005). Classification of proliferative hepatocellular lesions in Harlan Sprague-Dawley rats chronically exposed to dioxin-like compounds. *Toxicol Pathol* **33**, 165–74.
- Hickling, K.C., Hitchcock, J.M., Chipman, J.K., Hammond, T.G., and Evans, J.G. (2010) Induction and progression of cholangiofibrosis in rat liver injured by oral administration of furan. *Toxicol Pathol* **38**, 213-29.

References

- Schilsky, M.L., Quintana, N., Volenberg, I., Kabishcher, V., and Sternlieb, I. (1998). Spontaneous cholangiofibrosis in Long-Evans Cinnamon rats: A rodent model for Wilson's disease. *Lab Anim Sci* **48**, 156-61.
- Tatematsu, M., Kaku, T., Medline, A., and Farber, E. (1985). Intestinal metaplasia as a common option of oval cells in relatin to chloangiofibrosis in the liver of rats exposed to 2-acetylsminogluorene. *Lab Investig* **52**, 354 – 62.
- Thoolen, B., Maronpot, R.R., Harada, T., Nyska, A., Rousseaux, C., Nolte, T., Malarkey, D., Kaufmann, W., Küttler, K. Deschl, U., Nakae, D., Gregson, R., Vinlove, M. P., Brix, A.E., Singh, B., Belpoggi, F., and Ward, J.M. (2010). Proliferative and nonproliferative lesions of the rat and mouse hepatobiliary system. *Toxicol Pathol* **38**, 5S-81S.

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- Emily Singletary (EPL)

NTP National Toxicology Program

Are too Many Eggs a Risk for the Dog?

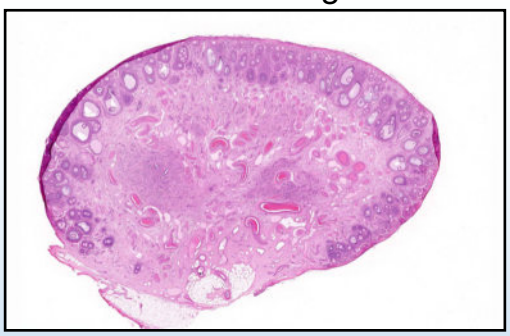
2019 NTP Satellite Symposium
Raleigh Convention Center
Raleigh, NC
June 22, 2019

Matthias Rinke, DVM, FIATP
Retired Head of Pathology and Clinical Pathology
from Bayer AG
Wuppertal and Berlin, Germany
Matthias.Rinke@t-online.de

Signalment

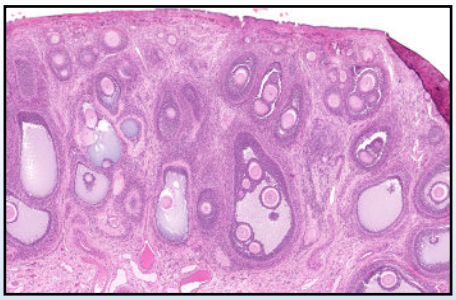
- Young female Beagle dogs
- Age at study start: 3 to 5 months
- 13-week feeding study with an agrochemical

What's Your Diagnosis?



Slide 1 of 4

What's Your Diagnosis?



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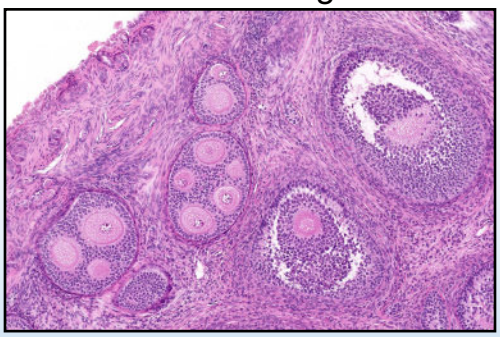
What's Your Diagnosis?



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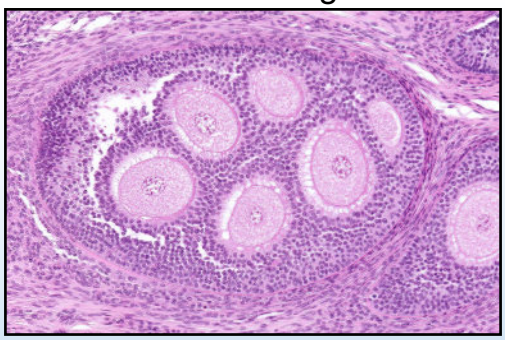
What's Your Diagnosis?



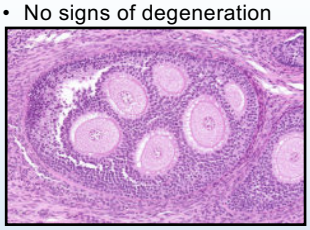
Slide 4 of 4

What's Your Diagnosis?

1. Malformation
2. Normal, no corpora lutea developed
3. Increased number of follicular oocytes
4. Multioocyte follicle (MOF) (also "pluriovular")
5. Follicle, polyoocytic
6. Follicle, polyovular (POF; INHAND Term)

Cellular Features

- Multiple oocytes surrounded by granulosa cells within a common follicle
- No signs of degeneration



Fate of the follicles: Frequently undergo degeneration, but at pre- and peripubertal ages polyovular follicles are found which persist into maturity (McDougall K et al., 1997).

What's Your Assessment?

1. Adverse finding (to be recorded and reported)
2. Non-adverse finding (to be recorded)
3. Potentially adverse finding (to be recorded)
4. Normal variance in (young) dogs (not to be recorded)
5. Normal variance in (young) dogs (to be recorded)
6. Don't know

Discussion

- Majority of follicles in all mammalian species contain one single oocyte
- Attention on bi- or multiovular follicles is present since the description of the oocyte by van Baer in 1827

100 years later:

1926: Opossum (Hartman)
1928: Ferret (Mainland)

POLYNUCLEAR OVA AND POLYOVULAR FOLLICLES IN THE OPOSSUM AND OTHER MAMMALS, WITH SPECIAL REFERENCE TO THE PROBLEM OF FECUNDITY

CARL G. HARTMAN

Department of Zoölogy, The University of Texas, and the Carnegie Laboratory of Embryology, Baltimore, Maryland

THIRTY-SIX FIGURES

PREFACE

The literature upon polyovular follicles and polynuclear ova is somewhat extensive, as a glance at the appended literature indicates. ...

„... For this species the occurrence of polynuclear ova is the rule rather than the exception, and in some ovaries they occur by hundreds. Polyovular follicles are, moreover, often found in astounding numbers and of almost every variety yet reported for other mammals."

Frequency of POFs in Mammals*

	% of Growing Follicles Containing x Oocytes		
Species	**2**	**3**	**4+**
Mouse	< 0.1 %	< 0.1 %	< 0.1 %
Rat	< 0.1 %	< 0.1 %	< 0.1 %
Sheep	< 0.1 %	< 0.1 %	< 0.1 %
Marmoset	< 0.1 %	< 0.1 %	< 0.1 %
Rabbit	0.91 %	< 0.1 %	< 0.1 %
Rhesus	1.49 %	0.3 %	< 0.1 %
Human	2.72 %	0.2 %	< 0.1 %
Cat	3.61 %	0.45 %	< 0.1 %
Dog	8.89 %	2.97 %	2.08 %
Otter	?	?	?

*Adapted from Telfer and Gosden (1987)

Discussion

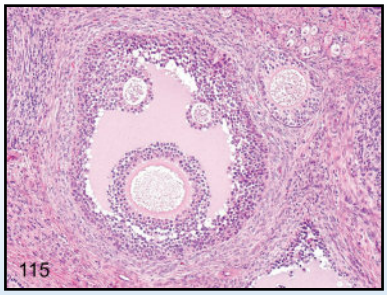


Fig. 115. Ovary: Polyovular follicle. Multiple ova occur occasionally in one ovarian follicle. Each oocyte has its own pellucid zone and radiate corona.
(from Sato J et. al. 2012)

Discussion

- Prepubertal and young dogs < 1 year have more polyovular follicles than mature ones:

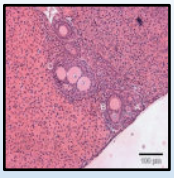
Age Group*	Animals with POFs
Prepubertal	68.4 %
Under 1 year	62.2 %
7 to 8 years	30.4 %
> 10 years	14.3 %

- Mongrels have more POFs than pure-bred dogs (52.3% vs. 25.5 %)
- This also seems to be true for pigs in which the number of polyovular follicles was higher in gilts than in sows

*Data from Payan-Carreira R and Pires MA(2008)

Comparison with Other Species

- Folliculogenesis in rodents begins soon after birth, accompanied by programmed oocyte death and germ cell loss
- In mice, neonatal exposure to a synthetic estrogen, diethylstilbestrol (DES), induces polyovular follicles, which contain two or more oocytes per ovarian follicle; it is reported that the estrogen receptor beta (ESR2) mediates DES signaling in polyovular follicle induction
- However, mouse lines selected for high fecundity show also a higher occurrence of polyovular follicles without being exposed to estrogens



Multi-oocyte follicles of the mouse. A: Primary follicle with 3 oocytes. B: Secondary follicle with 2 oocytes. C: Early antral follicle with 3 oocytes. Scale bar=100 µm (from Alm et al. 2010)

Discussion

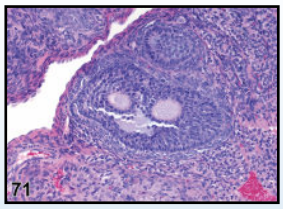


Fig. 71:
Polyovular Follicle, Ovary, rat, 20x
INHAND: Polyovular follicles occur with a low frequency in mice and rats though there may be some slight differences among the various strains.

The administration of compounds with estrogenic activity to neonatal mice (before Day 5 post-partum) results in an increase in the incidence of polyovular follicles. This has been attributed to estrogenic dysregulation of genes involved in break-down of germ cell cysts during the formation of primordial follicles.

Results of 13-wk Study (1)

Dose Group	Control	Low Dose	Mid Dose	High Dose
Increased Number of Polyovular Follicles	0 of 4	0 of 4	1 of 4	2 of 4

Do we have a treatment-related effect?

Results of 13-wk Study (2)

- One H&E section from each ovary
- Only large primary, secondary and tertiary follicles counted
- At least one oocyte in POFs with a nucleus

Control Animal	Ovary 1	Ovary 2
Monovular Follicles	52	45
Polyovular Follicles	16	12
Polyovular Follicles (2 oocytes)	7	5
Polyovular Follicles (3 oocytes)	4	2
Polyovular Follicles (4 oocytes)	5	1
Polyovular Follicles (5+ oocytes)	0	4
HD-Animal	**Ovary 1**	**Ovary 2**
Monovular Follicles	39	44
Polyovular Follicles	41	38
Polyovular Follicles (2 oocytes)	15	15
Polyovular Follicles (3 oocytes)	15	10
Polyovular Follicles (4 oocytes)	7	9
Polyovular Follicles (5+ oocytes)	4	4

Take Home Points

- Folliculogenesis covers the sequential steps in the development of a follicle, from primordial to preovulatory
- Most of the time, one follicle contains a single oocyte, but some follicles are polyovular in that they contain several
- The origin of the alteration is still unknown; failure of germ cell breakdown during early stages of folliculogenesis is proposed
- Developmental rate might be faster than differentiation of surrounding somatic cells resulting in inclusion of several germ cells in one follicle
- Polyovular follicles are a normal feature in young dogs; they are less frequently seen in older animals but still occur
- In comparison to rodents, the toxicological significance of this finding in dogs is (most likely) negligible

Discussion Points

- What is your experience with this finding?
- If recorded, have you even been asked by regulators about the finding in case of skewed incidences?
- How to deal with the term for non-rodent INHAND (Primates, Dogs, Minipig)?
- Other questions/comments?

References

- Alm H, Kuhlmann S, Langhammer M, Tuchscherer A, Torner H and Reinsch N (2010) Occurrence of Polyovular Follicles in Mouse Lines Selected for High Fecundity. J. Reprod. Dev. **56**: 449–453
- Dixon D, Alison R, Bach U, Colman K, Foley GL, Harleman JH, Haworth R, Herbert R, Heuser A, Long G, Mirsky M, Regan K, van Esch E, Westwood FR, Vidal J, Yoshida M (2014) Nonproliferative and proliferative lesions of the rat and mouse female reproductive system. *J Toxicol Pathol* **27:** 1S–107S
- Hartman CG (1926) Polynuclear ova and polyovular follicles in the opossum and other mammals with special reference to the problem of fecundity. Am J Anat **37:**1–52
- Kim H, Nakajima T, Hayashi S, Chambon P, Watanabe H, Iguchi T, Sato T (2009) Effects of diethylstilbestrol on programmed oocyte death and induction of polyovular follicles in neonatal mouse ovaries. Biol Reprod **81:** 1002 – 1009
- Mainland D (1928) The Pluriovular Follicle, with reference to its occurrence in the Ferret. J Anat **62:**139–58
- McDougall K, Hay MA, Goodrowe KL, Gartley CJ, King WA (1997) Changes in the number of follicles and of oocytes in ovaries of prepubertal, peripubertal and mature bitches. J Reprod Fertil Suppl. **51:** 25-31

References

- Payan-Carreira R and Pires MA (2008) Multioocyte follicles in domestic dogs: A survey of frequency of occurrence. Theriogenology **69**: 977-982
- Reynaud K, Fontbonne A, Saint-Dizier M, Thoumire S, Marnier C, Tahir MZ, Meylheuc T and Chastant-Maillard S (2012) Folliculogenesis, ovulation and endocrine control of oocytes and embryos in the dog. Reprod Dom Anim **47** (suppl 6): 66 – 69
- Sato J, Doi T, Wako Y, Hamamura M, Kanno T, Tsuchitani M, Narama I (2012) Histopathology of incidental findings in Beagles used in toxicity studies. J Toxicol Pathol **25:**103–134
- Stankiewicz T, Błaszczyk B, Udała J (2009) A study on the occurrence of polyovular follicles in porcine ovaries with particular reference to intrafollicular hormone concentrations, quality of oocytes and their in vitro fertilization. Anat Histol Embryol **38:** 233–239
- Telfer E and Gosden RG (1987) A quantitative cytological study of polyovular follicles in mammalian ovaries with particular reference to the domestic bitch (Canis familiaris). J Reprod Fert **81:** 137-147

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- Ansgar Buettner (Histovia) for scanning the slides
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- Susan Elmore for giving me the opportunity to talk

AND

- You for your attention

NTP National Toxicology Program

Discommodious Distalis

2019 NTP Satellite Symposium
Raleigh Convention Center
Raleigh, NC
June 22, 2019

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Integrated Laboratory Systems, Inc.
Research Triangle Park, NC, USA
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ILS

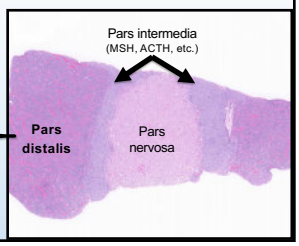
Discommodious Distalis

"Discommodious" definition:

– **inconvenient, troublesome, annoying** (Merriam-Webster)

Hormones and endocrine cells of the pars distalis

- GH, somatotrophs
- PRL, lactotrophs
- ACTH, corticotrophs
- FSH/LH, gonadotrophs
- TSH, thyrotrophs

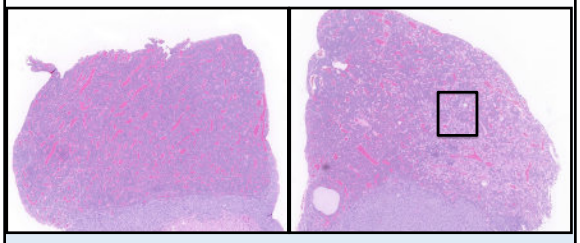


Case 1: Signalment

- 2-year-old male Hsd:Sprague Dawley (SD) rat
- Two-year NTP carcinogenicity bioassay with perinatal exposure (chemical still on study)
  – *In utero* (beginning on gestation day 6), lactation, dosed-feed (throughout life)

Case 1: What's Your Diagnosis?

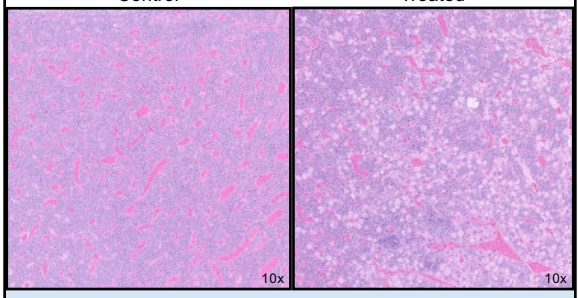
Control | Treated



Slide 1 of 4

Case 1: What's Your Diagnosis?

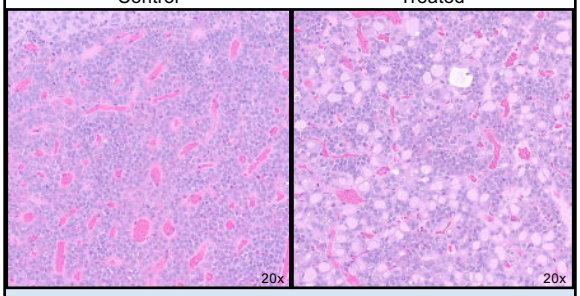
Control | Treated



Slide 2 of 4

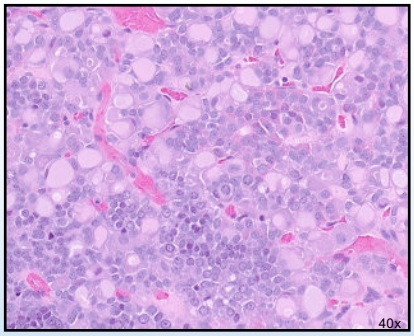
Case 1: What's Your Diagnosis?

Control | Treated



Slide 3 of 4

Case 1: What's Your Diagnosis?



Slide 4 of 4

Case 1: What's Your Diagnosis for this Pars Distalis Lesion?

1. Hyperplasia
2. Hypertrophy
3. Vacuolation
4. Cytoplasmic alteration
5. Cytoplasmic alteration and hyperplasia
6. Cytoplasmic alteration and vacuolation
7. Hyperplasia and vacuolation
8. Hypertrophy and vacuolation
9. Other

Case 1: What's Your Diagnosis for this Pars Distalis Lesion?

1. Hyperplasia
2. Hypertrophy (NTP preferred term)
3. Vacuolation
4. Cytoplasmic alteration (Original diagnosis)
5. Cytoplasmic alteration and hyperplasia
6. Cytoplasmic alteration and vacuolation
7. Hyperplasia and vacuolation
8. Hypertrophy and vacuolation (INHAND)
9. Other

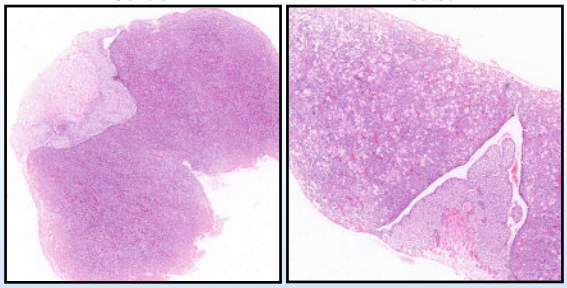
Case 2: Signalment

- Adult male Fisher 344/N rat
- 13-week feed study of ethylene thiourea (ETU)
  - Found in environment primarily as a degradation product of widely-used ethylene bis-dithiocarbamate (EBDC) fungicides

Aerial application of fungicide mancozeb® on banana plantations in Costa Rica
(Marcus Winterbauer,® Längengrad Filmproduktion GmbH)

Case 2: What's Your Diagnosis?

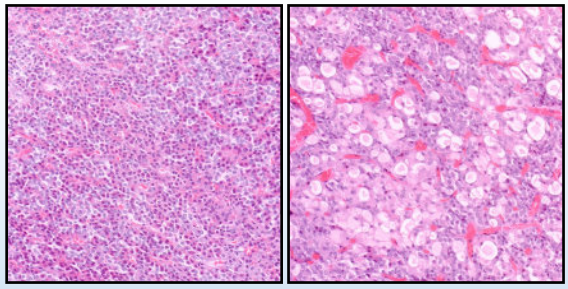
Control | Treated



Slide 1 of 3

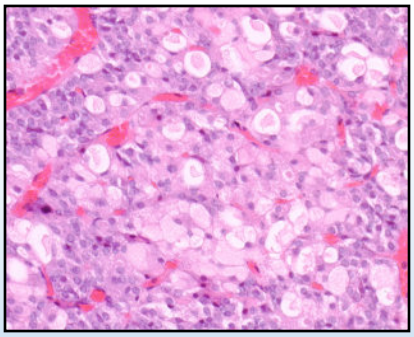
Case 2: What's Your Diagnosis?

Control | Treated



Slide 2 of 3

Case 2: What's Your Diagnosis?



Slide 3 of 3

Case 2: What's Your Diagnosis for this Pars Distalis Lesion?

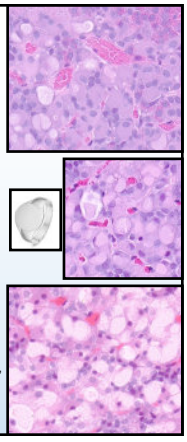
1. Hyperplasia
2. Hypertrophy
3. Cellular vacuolation
4. Cytoplasmic alteration
5. Cytoplasmic alteration and hyperplasia
6. Cytoplasmic alteration and vacuolation
7. Hyperplasia and vacuolation
8. Hypertrophy and vacuolation
9. Other

Case 2: What's Your Diagnosis for this Pars Distalis Lesion?

1. Hyperplasia
2. Hypertrophy (current NTP preferred term)
3. Cellular vacuolation (NTP, ~30 yrs ago)
4. Cytoplasmic alteration
5. Cytoplasmic alteration and hyperplasia
6. Cytoplasmic alteration and vacuolation
7. Hyperplasia and vacuolation
8. Hypertrophy and vacuolation
9. Other

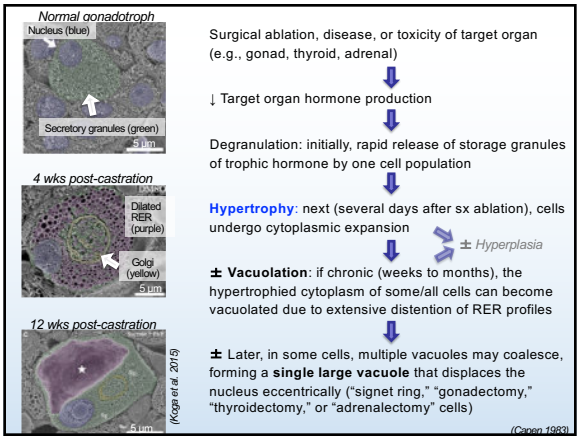
Cellular Features

- Enlargement of individual endocrine cells in the pars distalis
  - One of the more common lesions of the pars distalis in toxicity studies
  - Usually a single cell type
  - Cytoplasm (basophils and acidophils) may have decreased staining intensity
- May be accompanied by vacuolation
  - Multiple, or one large that displaces nucleus peripherally (signet ring)
- May progress to hyperplasia, but can be difficult to diagnose
  - Scattered distribution and wide range of normal
  - Variation due to staining, level of sectioning, age, sex, parity, stage of estrous cycle



Terminology

- Other terms that have been used:
  - Cytoplasmic alteration, cellular vacuolation
- NTP preferred term:
  - *Pituitary Gland, Pars Distalis – Hypertrophy*
  - Single diagnosis to maintain consistency among NTP studies
  - A continuum of the same process: all are enlarged, but not all develop vacuoles
  - Vacuolation to be described in the narrative
- INHAND (2018):
  - *Pituitary Gland, Pars Distalis – Hypertrophy*
    - Enlargement of individual endocrine cells. May be assoc. w/ cytoplasmic vacuolation.
  - *Pituitary Gland, Pars Distalis – Vacuolation*
    - Endocrine cells containing a large central or several cytoplasmic vacuoles that displace the nucleus peripherally. Vacuolated cells are usually hypertrophic.
  - *Pituitary Gland, Pars Distalis – Hypertrophy and Vacuolation*
    - Preferred terminology for vacuolated endocrine cells in the pars distalis of neutered animals, sometimes called "castration cells"



Surgical ablation, disease, or toxicity of target organ (e.g., gonad, thyroid, adrenal)

↓

↓ Target organ hormone production

↓

Degranulation: initially, rapid release of storage granules of trophic hormone by one cell population

↓

Hypertrophy: next (several days after sx ablation), cells undergo cytoplasmic expansion ± *Hyperplasia*

↓

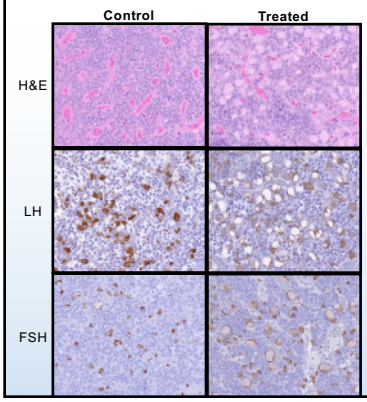
± **Vacuolation**: if chronic (weeks to months), the hypertrophied cytoplasm of some/all cells can become vacuolated due to extensive distention of RER profiles

↓

± Later, in some cells, multiple vacuoles may coalesce, forming a **single large vacuole** that displaces the nucleus eccentrically ("signet ring," "gonadectomy," "thyroidectomy," or "adrenalectomy" cells)

(Capen 1983)

Case 1: Immunohistochemistry



Basophils or acidophils may appear chromophobic on H&E if they are degranulated or actively synthesizing hormones

To ID cell type affected:

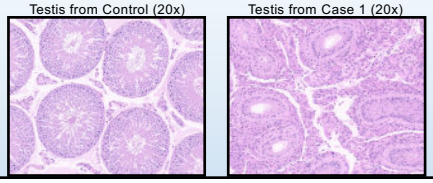
• IHC for secretory hormones

• Can infer based on peripheral tissue(s) affected

Case 1: Phthalate (in review)

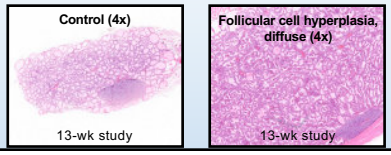
- Phthalates ↓ testosterone production by Leydig cells
  - ↓ negative feedback → ↑ GnRH → ↑ LH/FSH
- Highest dose, gross and histopathology:
  - Small: testes, epididymides, prostate glands, seminal vesicles
  - Pituitary gland, pars distalis – hypertrophy
  - Testicular atrophy, Leydig cell hyperplasia
  - Epididymal hypospermia, prostate and SV ↓ secretory fluid

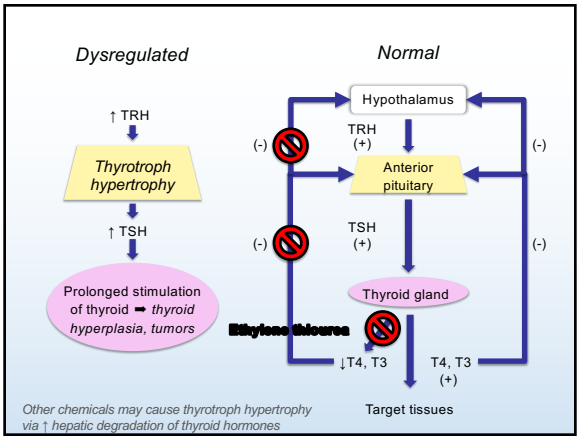
Testis from Control (20x) | Testis from Case 1 (20x)



Case 2: Ethylene Thiourea

- Ethylene thiourea accumulates in the thyroid gland
  - Interferes with thyroid peroxidase activity
  - Nongenotoxic
- NTP 2-year bioassay: *Clear evidence* of *carcinogenic activity* in M & F rats and mice
  - ↑ Incidences of **thyroid follicular cell neoplasms**
  - Nonneoplastic lesions: **follicular cell hyperplasia**
  - ↓ **T4** and **T3** (rats), ↑ **TSH** (rats and mice)
- Thyroid hypofunction → pituitary thyrotroph hypertrophy





Human Relevance: ETU

- A small number of rubber manufacturing workers exposed to ETU had ↓T4 (one w/ ↑TSH) (Smith 1984)
- ↑ TSH found in pesticide applicators in Mexico using EBDC* fungicides (metabolize to ETU) (Steenland *et al.* 1997)
- In the Agricultural Health Study (NC & IA), wives of the farmers who apply the EBDC fungicides maneb/mancozeb had an ↑ risk of either hypothyroidism or hyperthyroidism (Goldner *et al.* 2010, Shrestha *et al.* 2018)

*Ethylene bis-dithiocarbamate

Take Home Points

- Usually not a direct effect on the pituitary
  - Secondary to altered feedback pathways caused by toxicity in a peripheral target organ (e.g., testis, thyroid)
- Rather than "discommodious," this lesion can be helpful for the holistic assessment of physiology and pathology of an animal
  - Useful for interpretation of other treatment-related lesions or clinical pathology changes

Acknowledgements

- Mark Cesta (co-NTP Pathologist)
- Kyathanahalli Janardhan (ILS)
- Cynthia Shackelford and Gabrielle Willson (EPL)
- Anthony J. Skowronek (Battelle)
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References

- Brändli-Baiocco, A, *et al*. 2018. J Toxicol Pathol. 31 (3 Suppl): 1S–95S
- Capen, CC. 1983. Endocrine System. *In* Monographs on Pathology of Laboratory Animals. Jones, Mohr, Hunt (eds.) Springer-Verlag
- Goldner, WS, *et al*. 2010. Am J Epidemiol. 171:455-64
- Koga, D, *et al*. 2015. J Histochem Cytochem. 63(12) 968–79
- NTP. 1992. *Toxicology and Carcinogenesis Studies of Ethylene Thiourea (CAS No. 96-45-7) in F344 Rats and B6C3F1 Mice (Feed Studies)*. NTP Technical Report Series no. 388
- Shrestha, S, *et al*. 2018. Environment Intl. 118: 282-92
- Smith, DM. 1984. Br J Ind Med. Aug;41(3):362-6
- Steenland, K, *et al*. 1997. Environ Health Perspect. 105(10):1126-30

NTP
National Toxicology Program

A Blood Smear Dilemma

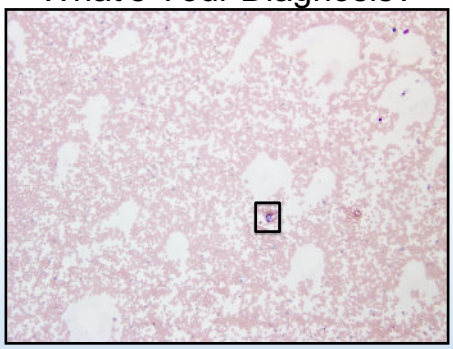
2019 NTP Satellite Symposium
Raleigh Convention Center
Raleigh, NC
June 22, 2019

Greg Travlos, DVM, DACVP
National Institute of Environmental Health Sciences
Research Triangle Park, NC, USA
travlos@niehs.nih.gov

Signalment

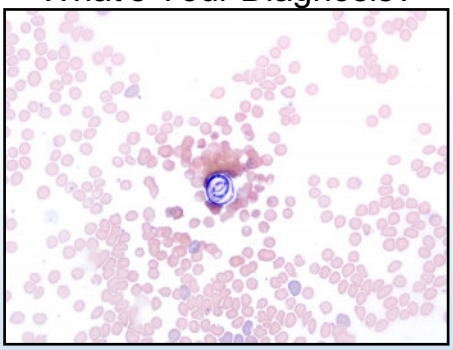
- C57BL/6 mouse
  - Male, 4-months old
- Genetically modified
  - Double KO of a fibroblast-specific gene
- Animal presented as “sick”
  - Blood submitted for a CBC
    - Romanowsky-stained blood smear

What's Your Diagnosis?



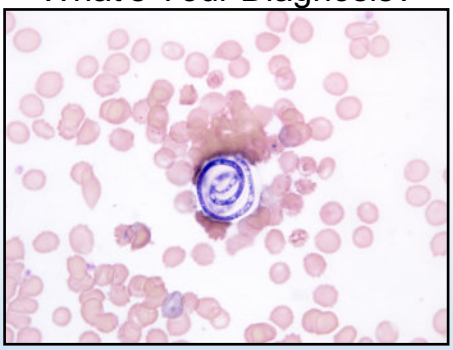
Slide 1 of 3

What's Your Diagnosis?



Slide 2 of 3

What's Your Diagnosis?



Slide 3 of 3

What's Your Diagnosis?

1. Hemoparasite in blood
2. Microfilarial microorganism
3. *Borrelia* sp. microorganism
4. *Candida* sp. microorganism
5. Trypanosomal microorganism
6. *Enterobacter* sp. microorganism
7. *Plasmodium* sp. male microgamete
8. Other – Contaminant: presumptive fungal spore
   (i.e., conidia of a helicosporous hyphomycete)

Discussion

- Helicosporous hyphomycetes
  - Several genera (e.g., *Helicomyces*, *Helicosporium*, *Helicoma*, *Helicoön*, *Spirosphaera)*
    - Saprobic fungi: decompose decaying wood, leaves, litter, etc.
    - Like moist places or around water
  - Produce various forms of coiled two- or three-dimensional conidia

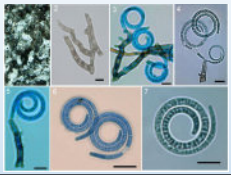
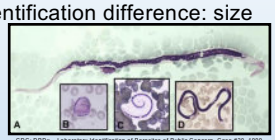


Figure: *Helicosporium xylophilous* (AMH 9744, holotype).
1. Stereoscopic view of natural colonies on dead wood.
2. Conidiophores with denticulate conidiogenous cell.
3. Branched conidiophore and coiled conidia.
4. A part of conidiophore with attached conidia.
5. A conidiophore with terminally attached conidium.
6 – 7. Magnified view of conidia.
Bars 2-7 = 10 µm.

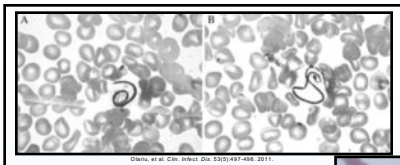
Singh and Singh, *Curr. Res. Environ. Appl. Mycol.* 6 (4): 248–255, 2016

Discussion

- Conidia are an incidental finding on blood smears
  - Contaminate blood smear samples or staining solutions
  - Contamination through air or water
- Have been erroneously identified as microfilaria
  - Report described a new species of nematode
    - *Sergentella* spiroides (Jirovec, 1956; Galliard et al., 1961)
- Primary identification difference: size

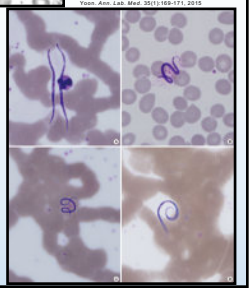


CDC: DPDx – Laboratory Identification of Parasites of Public Concern, Case #20, 1999



Otanu, et al. *Clin. Infect. Dis.* 53(5):497-498, 2011.

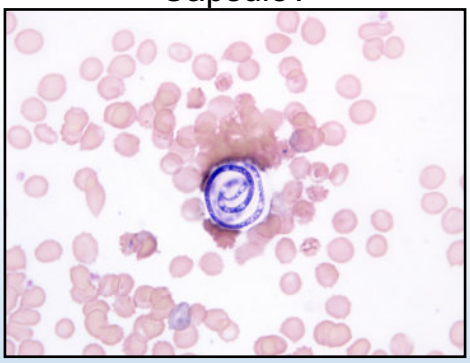
Features



Yoon. *Ann. Lab. Med.* 35(1):169-171, 2015

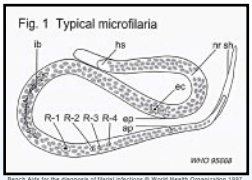
- Structure(s) observed on the blood smear
  - Characteristically coiled
  - Irregular helical
  - Thread-like
- Conidia vary from 1-2 µm in thickness x <100 µm in length
  - Microfilaria larger in size: 3-10 µm × 160-390 µm
  - RBC diameter: mouse, 4-7 µm; human, 6-8 µm
- Not observed on subsequent smears
- No internal structure/organization
- No sheath
  - May be surrounded by a capsule of irregular shape

Capsule?

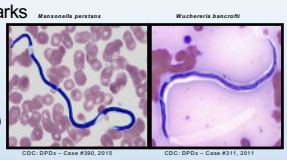


Microfilaria

- Serpentine shape
  - Body filled with nuclei
    - Density of nuclei varies
    - Head space (hs), area varies
    - Shape of tail varies
  - Some have a sheath (sh)
    - Length varies
    - Color (stain and species)
  - Other anatomical landmarks
    - Nerve ring (nr)
    - Anal pore (ap)
    - Excretory pore (ep)
    - Excretory cell (ec)
    - Amorphous inner body (ib)
    - Rectal cells (R1 – R4)



Bench Aids for the diagnosis of filarial infections © World Health Organization 1997



CDC: DPDx – Case #360, 2015 CDC: DPDx – Case #311, 2011

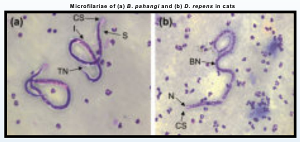
Microfilaria

- Zoonotic filariasis
  - Humans and a variety of domestic and wildlife species act as reservoirs

Microfilariae of *D. immitis* in a dog

Ruedisueli, F.L. Parasite Image Collection, Nottingham Trent University

Microfilariae of (a) *B. pahangi* and (b) *D. repens* in cats



Al-Abd, et al., *Asian Pac J Trop Med.* 8(9): 705–709, 2015

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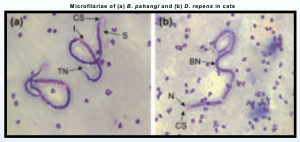
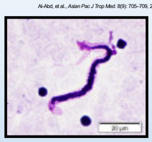


Al-Abd, et al., *Asian Pac J Trop Med.* 8(9): 705–709, 2015

- Rodent model
  - *Litomosoides sigmodontis*



Original photograph by Coralie Martin, MNHD; http://mcam.mnhn.fr/fr/annuaire/nathaly-lhermitte-vallarino-436

Besides Fungal Spores…

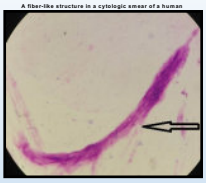
- There are a variety of potential sources of blood smear contamination
  - Hairs
  - Fibers
  - Skin cells
  - Endothelial cells
  - Stain Precipitate
  - Bacteria
  - Yeast
  - Others…

Hairs or Fibers

- May occur during smear processing
  - Can mimic microfilariae
- Appears as random, extracellular, thread-like structure of variable length, width and staining
  - May have rough and/or non-uniform borders
  - No internal structure/organization


A strand of hair in a blood smear of a dog

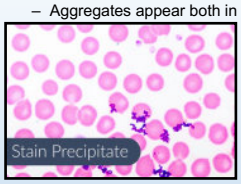
Eclinpath': http://eclinpath.com/cyg_tag/artifactcriggallery/page/4


A fiber-like structure in a cytologic smear of a human

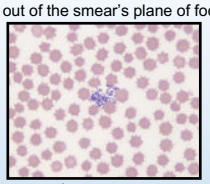
Bhatia, et al., J. Clin. Diag. Res. 12(7):DR01-DR04. 2018

Stain Precipitate

- May occur during storage or from insufficient washing of a slide after incubation
  - Can mimic bacteria but is more irregular in size and shape
- Appears as random aggregates of spherical to irregular extracellular granules
  - Uniformly dispersed throughout the smear
  - Aggregates appear both in and out of the smear's plane of focus



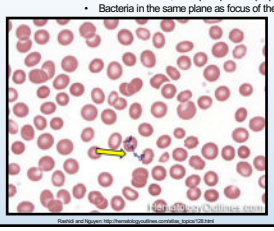
Hokamp, J.: https://www.tamucvet.org/product/evaluating-blood-smears-and-analyzing-erythrocyte-morphology/



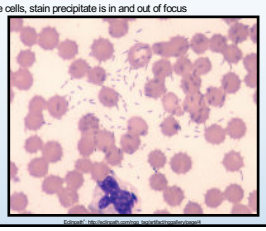
Eclinpath': http://eclinpath.com/cyg_tag/stain-precipitate/

Bacteria

- Bacterial colonies can contaminate peripheral blood smear during handling/processing
  - Commonly appears as random aggregates of extracellular cocci (presumptive Staphlococcus spp.)
- Stain precipitate mimics bacteria (e.g., cocci and *Mycoplasma* spp.)
  - Bacteria are more uniform (shape, size, staining)
    - Bacteria are blue, stain precipitate is purple
    - Bacteria in the same plane as focus of the cells, stain precipitate is in and out of focus



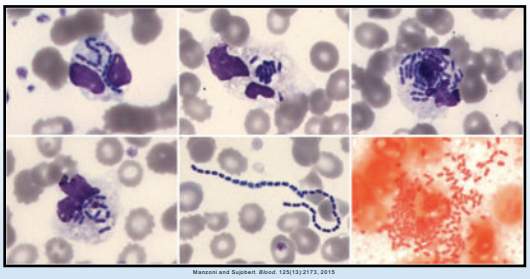
Rashid and Nguyen http://hematologyoutlines.com/atlas_topics/126.html



Eclinpath': http://eclinpath.com/cyg_tag/artifact/precipitate/page/4

Enterobacter Sp. Bacteremia

- Extracellular bacteria formed in chains
  - Contamination was considered: gram-negative bacteria in chains is uncommon



Manzoni and Sujobert. *Blood*. 125(13):2173, 2015

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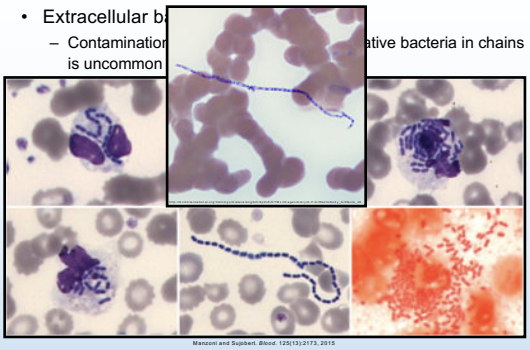


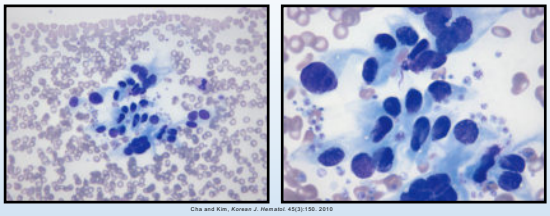
Manzoni and Sujobert. *Blood*. 125(13):2173, 2015

Endothelial Cells

- Occur related to traumatic venipuncture
  - Can mimic neoplastic process
- Increased circulating endothelial cells (CEC) occur for many conditions characterized by vascular injury or angiogenesis
  - Neoplasia
    - In humans, increased CEC with lymphoma, melanoma, glioma, breast, colonic, gastric, esophageal, renal cell, ovarian, cervical, prostate, testicular, etc. cancers
    - Chemotherapeutics (vascular-disrupting agents)
  - Vascular injury
    - Infection (viral, bacterial, rickettsial)
    - Immune-mediated (SLE)
  - Heart Disease
    - Acute myocardial infarction
    - Coronary angioplasty
  - Sickle cell anemia
  - Allogeneic stem cell transplantation

Endothelial Cells

- Seen singly or clusters
- Large, no distinct shape (often elongated)
  - Indistinct membrane with the cell edges being irregular
  - Sky blue cytoplasm, finely granular
- Eccentric round or oval nucleus (grooved)
  - Uniform moderately coarse chromatin, distinct (1-3) or indistinct nucleoli

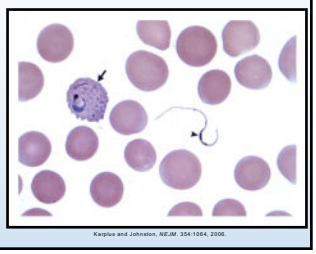


Cha and Kim, Korean J. Hematol 45(3):150. 2010

Sporozoan Microgamete

- Sporozoans have no flagella for locomotion
  - Except male gametes in the sexual phase

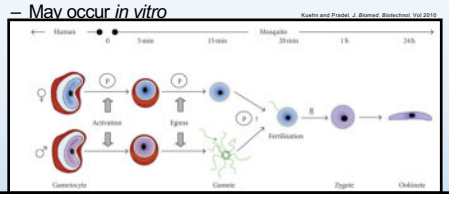
- RBC infected with *P. vivax* (arrow)
  - Ring (trophozoite)
  - Schuffner's stippling
- Microgamete (arrowhead)



Kenjiua and Johnston, NEJM. 354:1064. 2006.

Sporozoan Microgamete

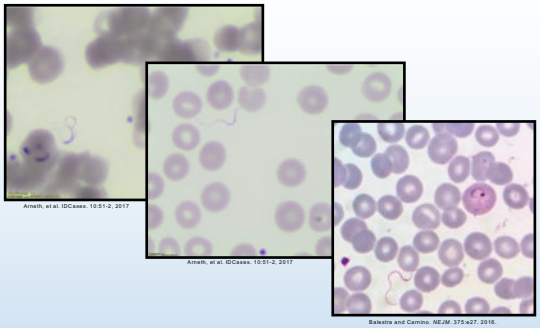
- Produced by exflagellation
  - Extrusion of nuclear material into peripheral processes
  - In mosquito
    - Decreased temp
    - Increased pH
- Rarely observed
  - May occur *in vitro*



Kuehn and Pradel. J. Biomed. Biotechnol. Vol 2010

Sporozoan Microgamete

- May resemble a flagella or spirochete



Arneth, et al. IDCases. 10:51-2, 2017

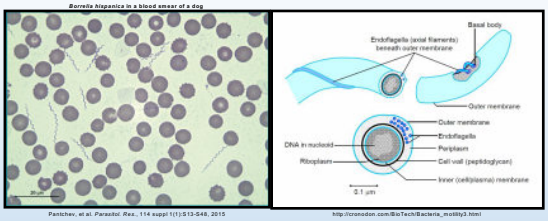
Arneth, et al. IDCases. 10:51-2, 2017

Balestra and Camino. *ME/M*. 375:e27. 2016.

Spirochetes

- Most spirochetes are long (up to 250 µm), thin (0.1 – 3 µm) and have helical (corkscrew) or flat-wave shapes
  - A multilayered outer membrane that surrounds the protoplasmic cylinder
  - Periplasmic flagella (aka. axial filaments) attached to each end of the cylinder

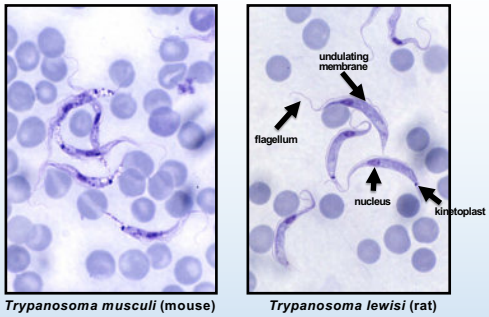
Borrelia hispanica in a blood smear of a dog



Pantchev, et al. *Parasitol. Res.*, 114 suppl 1(1):S13-S48, 2015

http://cronodon.com/BioTech/Bacteria_motility3.html

Rodent Trypanosomes

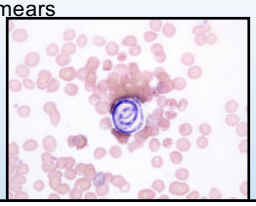


Trypanosoma musculi **(mouse)** ***Trypanosoma lewisi*** **(rat)**

Original photomicrographs by Steve J. Upton, Kansas State University: https://www.kstate.edu/parasitology/625tutorials/Protozoa07.html

Take Home Point

- Don't be fooled
  - Spores of helicosporous fungi are air-borne or water-based contaminants in laboratories and may be mistaken for microfilariae in stained blood smears



Acknowledgements

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- Debra King (CMPB, NIEHS)
- David Kurtz (CMB, NIEHS)

References

1. Al-Abd, N.M., et al. Prevalence of filarial parasites in domestic and stray cats in Selangor State, Malaysia. *Asian Pac. J. Trop. Med.* 8(9):705-709, 2015.
2. Ameth, B., et al. Malaria Exflagellation in a Human Peripheral Blood Smear. IDCases. 10:51-2, 2017.
3. Bain, O. and Babayan, S. Behaviour of filariae: morphological and anatomical signatures of their life style within the arthropod and vertebrate hosts. *Filaria. J.* 2:16, 2003.
4. Balestra, B. and Camino, L. In Vitro Exflagellation of *Plasmodium vivax*. *N. Engl. J. Med.* 375:e27, 2016.
5. Bhatia, M., et al. Microscopic Artefacts in Clinical Practice- A Diagnostic Dilemma. *J. Clin. Diag. Res.* 12(7):DR01-DR04. 2018
6. Centers for Disease Control and Prevention (CDC): Monthly Case Studies – 1999. DPDx – Laboratory Identification of Parasites of Public Concern, Case #20, 1999.
7. Centers for Disease Control and Prevention (CDC): Case #311 – November 2011. DPDx – Laboratory Identification of Parasites of Public Concern, Case #311, 2011.
8. Centers for Disease Control and Prevention (CDC): Case #390 – February 2015. DPDx – Laboratory Identification of Parasites of Public Concern, Case #390, 2015.
9. Cha, C-H and Kim, J.U. Endothelial cells in peripheral blood smear: an artifact? *Korean J. Hematol.* 45(3):150. 2010.
10. Eclinpath[©], Cornell University College of Veterinary Medicine. Bacteria. http://eclinpath.com/ngg_tag/artifact/nggallery/page/4.
11. Eclinpath[©], Cornell University College of Veterinary Medicine. Stain precipitate & echinocytes. http://eclinpath.com/ngg_tag/stain-precipitate/.
12. Eclinpath[©], Cornell University College of Veterinary Medicine. Worm mimic. http://eclinpath.com/ngg_tag/artifact/nggallery/page/4.
13. Galliard, H., et al. *Sorgentella spiroides* and conidia of helicosporoa in blood smears. *Bull. Soc. Pathol. Exot. Filiales.* 54:203–207, 1961.
14. Goon, P.K.Y., et al. Circulating Endothelial Cells, Endothelial Progenitor Cells, and Endothelial Microparticles in Cancer. *Neoplasia.* 8(2):179-88, 2006.
15. Hokamp, J. Learning Module: Evaluating blood smears and analyzing erythrocyte morphology, Texas A&M. https://www.tamucet.org/product/evaluating-blood-smears-and-analyzing-erythrocyte-morphology/.

References

16. Jirovec O. *Sergentella spiroides*, a new parasite of humans. *Rev. Bras. Malariol. Doencas. Trop.* 8:203–205, 1956.
17. Karplus, R. and Johnston, S.P. *Plasmodium vivax* Microgametes. *N. Engl. J. Med.* 354:1064, 2006.
18. Kuehn, A. and Pradel, G. The Coming-Out of Malaria Gametocytes. *Journal of Biomedicine and Biotechnology*, vol. 2010, Article ID 976827, 11 pages, 2010.
19. Lam, S. and Hsia, C.C. Peripheral blood candidiasis. *Blood*. 119(21):4822, 2012.
20. Lhermitte-Vallarino, N. National Museum of Natural History (France): Original photograph: Coralie Martin, MNHD: http://mcam.mnhn.fr/fr/annuaire/nathaly-lhermitte-vallarino-436.
21. Michel, K. Animal and Human Parasite Images, Index of supplemental visual material. Original photographs: Steve J. Upton, Kansas State University. https://www.k-state.edu/parasitology/625tutorials/index.html. Last updated: 13Jan.2015.
22. Manzoni, D. and Sujobert, P. Diagnosis of bacteremia on a blood smear. *Blood*. 125(13):2173, 2015.
23. Olariu, T.R., et al. Helical Microorganisms in Blood Smears. *Clin. Infect. Dis*. 53(5):497-498. 2011.
24. Orihel, T.C., Bench aids for the diagnosis of filarial infections. Geneva, Switzerland : Division of Control of Tropical Diseases, World Health Organization, 1997.
25. Pantchev, N., et al. Tick-borne Diseases (Borrelicsis, Anaplasmosis, Babesiosis) in German and Austrian Dogs: Status quo and Review of Distribution, Transmission, Clinical Findings, Diagnostics and Prophylaxis. *Parasitol. Res.* 114 Suppl 1(1):S13–S48, 2015.
26. Rashidi, H.H. and Nguyen, J.C. HematologyOutlines.com. Bacterial contamination in peripheral blood smear. http://hematologyoutlines.com/atlas_topics/128.html.
27. Ruedisueli, F.L. Parasite Image Collection, Nottingham Trent University. http://btb.animaldisease.org/PIC/PIC/Parasite-Image-Collection/index.htm.
28. Singh, P.N. and Singh, S.K. Additions to helicoid fungi from India. *Curr. Res. Environ. Appl. Mycol.* 6 (4): 248–255, 2016.
29. Strijbos, M.H., et al. Circulating endothelial cells in oncology: pitfalls and promises. *Br. J. Cancer*. 98:1731-1735, 2008.
30. Yoon, K-H. Peripheral Blood Smear Contamination with *Helicosporium* Fungi Resembling Microfilaria. *Ann. Lab. Med.* 35(1):169-171, 2015.

NTP
National Toxicology Program

Beyond the Spectrum of Chronic Progressive Nephropathy

2019 NTP Satellite Symposium
Raleigh Convention Center
Raleigh, NC
June 22, 2019

Erin M. Quist, DVM, MS, PhD, DACVP
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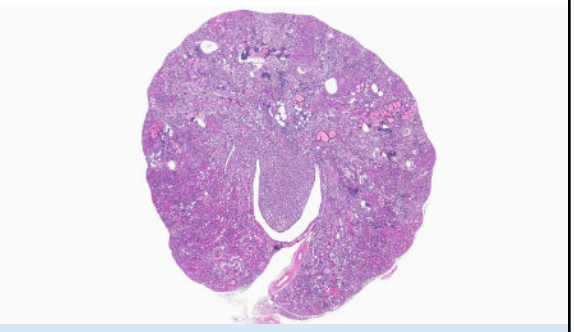
EPL

Case 1-3 Signalment

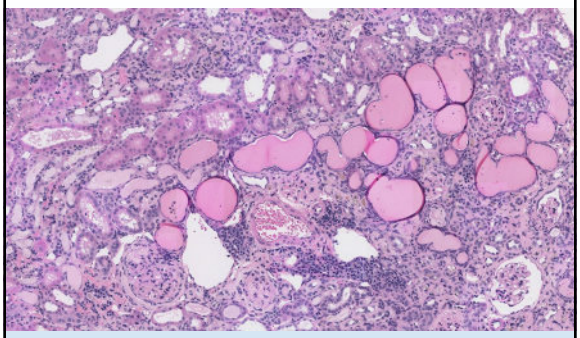
- Male, B6C3F1 mice
- 2-year toxicity/carcinogenesis bioassay
- Dosed-water study

Case 1: What's Your Diagnosis?



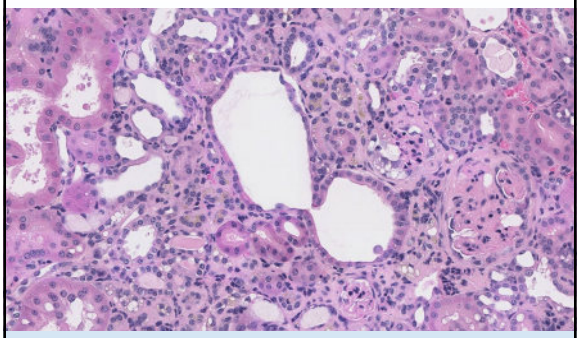
Slide 1 of 4

Case 1: What's Your Diagnosis?



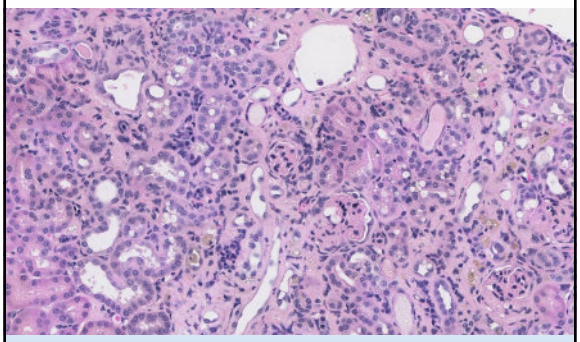
Slide 2 of 4

Case 1: What's Your Diagnosis?



Slide 3 of 4

Case 1: What's Your Diagnosis?



Slide 4 of 4

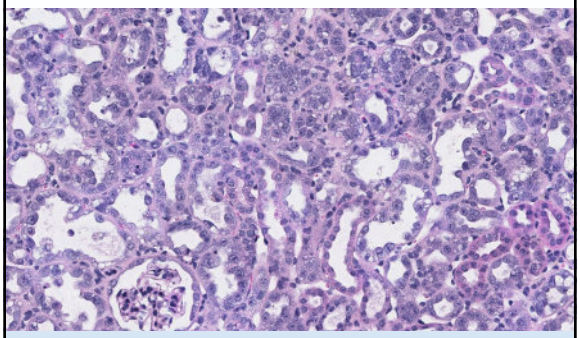
Case 1: What's Your Diagnosis?

1. Renal Tubule, Cytoplasmic Alteration
2. Renal Tubule, Degeneration
3. Renal Tubule, Regeneration
4. Renal Tubule, Basophilia
5. Renal Tubule, Hyperplasia
6. Renal Tubule, Atypia Cellular
7. Renal Tubule, Hyperplasia – Atypical
8. Chronic Progressive Nephropathy (CPN)
9. Other

Case 2: What's Your Diagnosis?

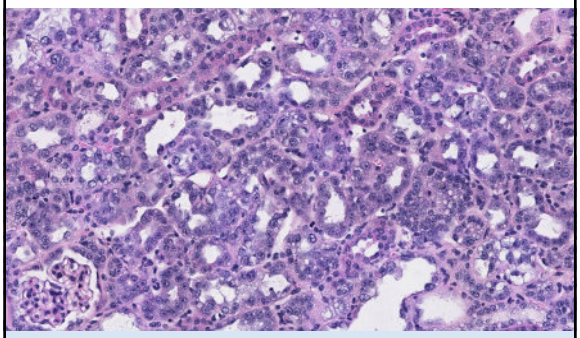
Slide 1 of 3

Case 2: What's Your Diagnosis?



Slide 2 of 3

Case 2: What's Your Diagnosis?



Slide 3 of 3

Case 2: What's Your Diagnosis?

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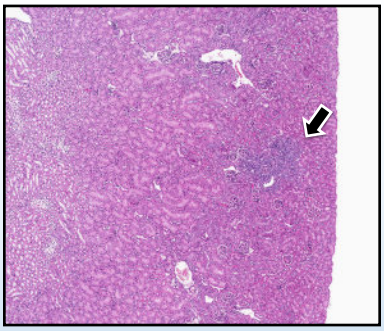
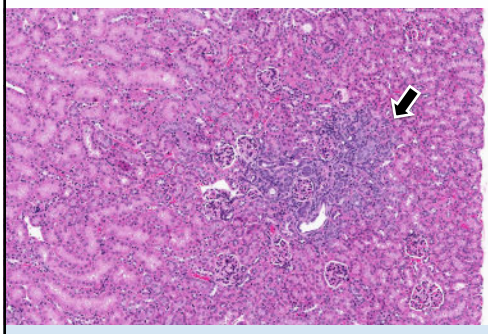
Case 3: What's Your Diagnosis?



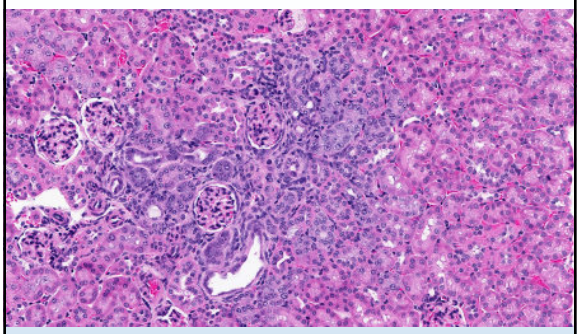
Slide 1 of 3

Case 3: What's Your Diagnosis?



Slide 2 of 3

Case 3: What's Your Diagnosis?



Slide 3 of 3

Case 3: What's Your Diagnosis?

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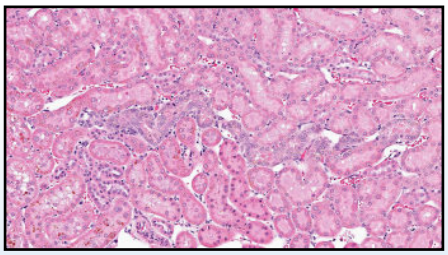
2. Renal Tubule, Degeneration
3. Renal Tubule, Regeneration
4. Renal Tubule, Basophilia
5. Renal Tubule, Hyperplasia
6. Renal Tubule, Atypia Cellular
7. Renal Tubule, Hyperplasia – Atypical
8. Chronic Progressive Nephropathy (CPN)
9. Other

Overview: Chronic Progressive Nephropathy (CPN)

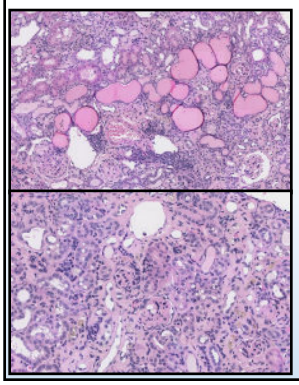
- Common spontaneous lesion of aged rodents, especially rats
- Physiological factors:
  - Age (severity increases with age)
  - Sex (male)
  - High protein diet
  - Caloric intake
  - Strain (B6C3F1 mice, Fischer and SD rats)
  - Other factors (endocrine, immunological)
- Not as well characterized in the mouse

CPN Features – Early



- Basophilic tubules
- Nuclear crowding
- Thickened basement membranes

CPN Features – Late Stage

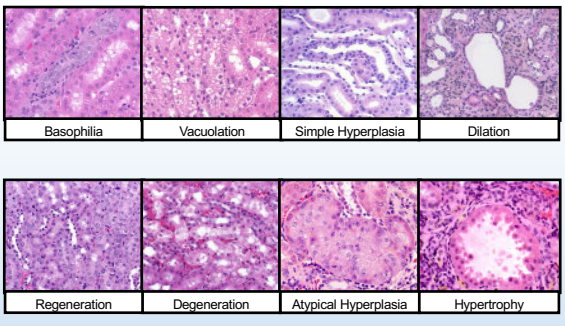


- Basophilic tubules
- Nuclear crowding
- Simple tubular hyperplasia
- Thickened basement membranes
- Glomerulosclerosis
- Tubular dilation/atrophy
- Interstitial fibrosis
- Inflammatory infiltrates
- Hyaline casts
- Pigment

CPN Differential Diagnoses

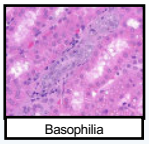
- Atypical Hyperplasia
- Simple Tubular Hyperplasia
- Tubular Regeneration
- Tubular Basophilia
- Pyelonephritis
- Obstructive nephropathy

Beyond the Spectrum of CPN



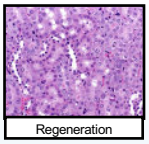
Photos: GoReni (https://www.goreni.org/) and NNLA (https://ntp.niehs.nih.gov/nnl/urinary/kidney/index.htm)

Renal Tubule Lesions



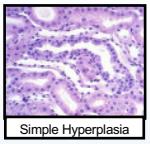
Basophilia

- Basophilic cytoplasm
- No thickening of basement membranes
- Cells may appear swollen or plump
- Increased nuclear:cytoplasmic ratio
- Increased mitoses



Regeneration

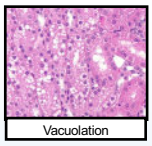
- Basophilic cytoplasm
- No thickening of basement membranes
- Flattened epithelium to low cuboidal cells
- Karyomegaly
- High mitotic index



Simple Hyperplasia

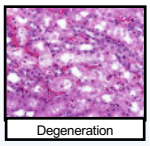
- Basophilic cytoplasm
- No thickening of basement membranes
- Nuclear crowding
- Increased cell number
- Cells do not extend into the lumen beyond a single layer

Renal Tubule Lesions



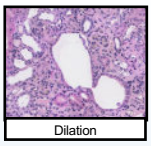
Vacuolation

- Discrete, clear cytoplasmic vacuoles (macro or micro)
- No thickening of basement membranes
- Pale, granular or swollen cytoplasm



Degeneration

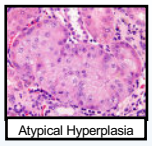
- Vacuolation
- No thickening of basement membranes
- Tinctorial change (e.g. basophilia) may be present
- Cell blebbing or sloughing



Dilation

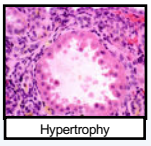
- Luminal expansion
- No thickening of basement membranes
- Normal or flattened epithelium
- May contain casts, cellular debris or inflammatory cells

Renal Tubule Lesions



Atypical Hyperplasia

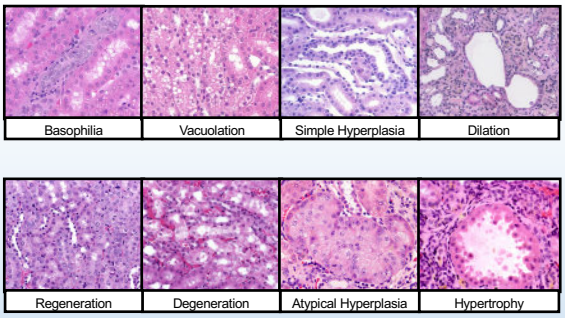
- Solid tubular profiles encircled by connective tissue
- No thickening of basement membranes
- Occurrence is solitary
- No compression of adjacent parenchyma



Hypertrophy

- Increase in cell size (without increase in cell number)
- No thickening of basement membranes
- Brightly eosinophilic cytoplasm
- Apical nuclei

Beyond the Spectrum of CPN



Photos: GoReni (https://www.goreni.org/) and NNLA (https://ntp.niehs.nih.gov/nnl/urinary/kidney/index.htm)

Terminology Challenges

- What do we call this lesion?
  - Originally diagnosed as *"Renal Tubule – Atypia Cellular"*
  - Is it a treatment-related change that should be distinguished from CPN?
  - What term will best capture the lesion?

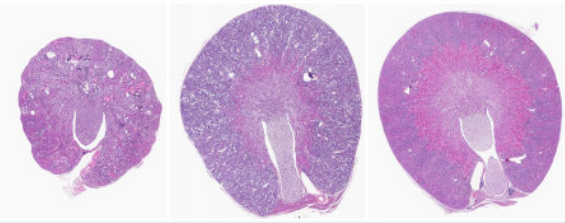
Treatment Related?

Incidence Table: 2-year Mouse Study

MALE	Control	Low	Mid	High
Kidney No. Ex.	**50**	**50**	**50**	**50**
Nephropathy, Chronic Progressive	43 [1.37]	48 [1.15]	49 [1.29]	45 [1.8]
Renal Tubule – Adenoma	0	0	1	0
Renal Tubule – Carcinoma	0	0	0	2
Renal Tubule – Atypia Cellular (*Regeneration*)	**2** [1.0]	**21** [1.38]	**30** [1.4]	**38** [1.6]
FEMALE	**Control**	**Low**	**Mid**	**High**
Kidney No. Ex.	**50**	**50**	**50**	**50**
Nephropathy, Chronic Progressive	33 [1.0]	26 [1.17]	26 [1.0]	27 [1.0]
Renal Tubule – Atypia Cellular (*Regeneration*)	**0** [-]	**1** [3.0]	**7** [1.29]	**7** [1.29]

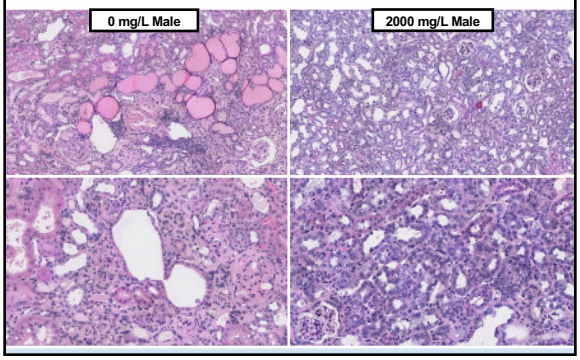
[ ] = average severity grade on a scale of 1-4

CPN or Beyond CPN?

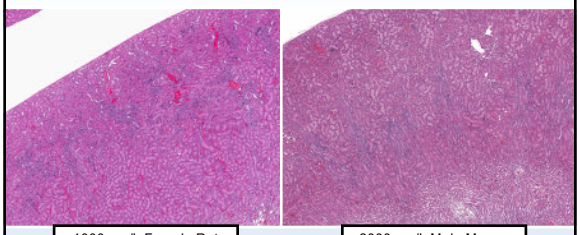


0 mg/L Male | 2000 mg/L Male | 2000 mg/L Female

CPN or Beyond CPN?



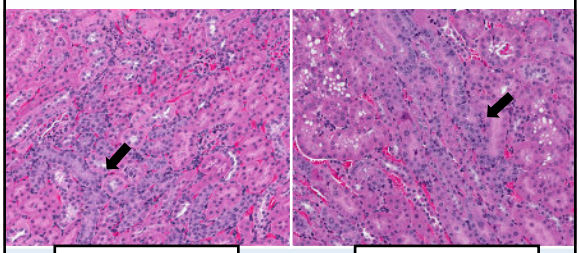
Terminology?



1000 mg/L Female Rat | 2000 mg/L Male Mouse

13-week Studies

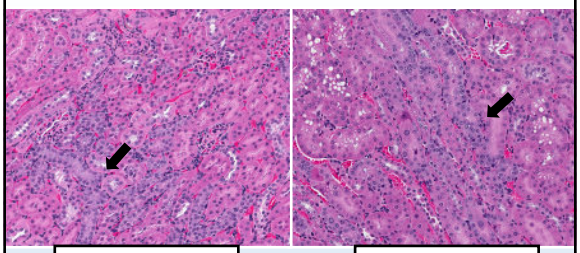
Terminology?



1000 mg/L Female Rat | 2000 mg/L Male Mouse

13-week Studies

Terminology?



1000 mg/L Female Rat | 2000 mg/L Male Mouse

Renal Tubule, Regeneration

Conclusions of the PWG

- *Renal Tubule – Regeneration*
  - Preferred terminology
    - Characteristics include:
      - Increased cytoplasmic basophilia
      - Karyomegaly
      - Hypertrophy
      - Hyperplasia
      - Degeneration
      - Increased mitoses
    - Maintains consistency across studies
    - Distinguishes this lesion from spontaneous CPN

Take Home Points

- Renal Tubule lesions can be difficult to recognize/diagnose
  - Constellation of findings
  - Overlapping morphologies between spontaneous (e.g. CPN) and toxicant-induced lesions
- Important to distinguish treatment-related lesions from spontaneous change
- It's often difficult to find terminology that captures both the morphology and suspected disease process

Discussion Points

- Do you agree/disagree with the conclusion to distinguish this lesion from CPN?
- Have you seen a similar lesion and called it something else?
- Other questions/comments?

References

- Barthold SW, Griffey SM, Percy DH (2016). Mouse – Chronic Nephropathy. In: Barthold SW, Griffey SM, Percy DH (Eds), *Pathology of Laboratory Rodents and Rabbits, Fourth Edition.* Wiley Blackwell, Ames: p. 102.
- Barthold SW, Griffey SM, Percy DH (2016). Rat – Chronic Progressive Nephropathy. In: Barthold SW, Griffey SM, Percy DH (Eds), *Pathology of Laboratory Rodents and Rabbits, Fourth Edition.* Wiley Blackwell, Ames: pp. 157-158.
- Frazier KS, Seely JC, Hard GC, Betton G, Burnett R, Nakatsuji S, Nishikawa A, Durchfeld-Meyer B, Bube A (2012). Proliferative and Nonproliferative Lesions of Rat and Mouse Urinary System. *Toxicologic Pathology 40: 14S.*
- Seely JC, Brix A (2014). Kidney – Nephropathy, Chronic Progressive. In: Cesta MF, Malarkey DE, Herbert RA, Brix A, Sills RC (Eds), *National Toxicology Program Nonneoplastic Lesion Atlas.* http://ntp.niehs.nih.gov/nnl/urinary/kidney/necp/index.htm [Accessed 22 April 2019].

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- Gabrielle Willson (EPL)

NTP National Toxicology Program

Granularity Matters for the Diagnosis of Neoplasms

2019 NTP Satellite Symposium
Raleigh Convention Center
Raleigh, NC
June 22, 2019

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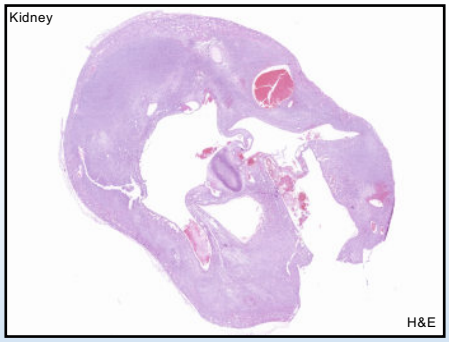
ILS

Case 1: Signalment

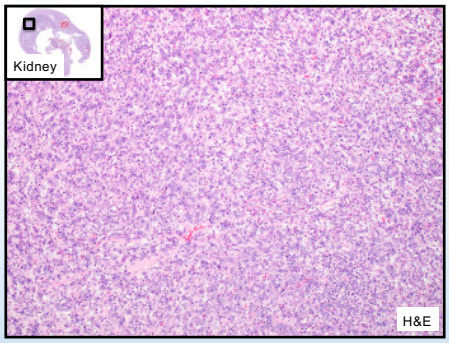
- F344/N female rat
- Two year carcinogenesis bioassay
- Dosed-water study
- 729 days on test
- Terminally euthanized
- From a treated group
- Gross lesions
  - Right kidney: 15x15x15 mm mass, white
  - Mammary gland, abdominal: 15x10x10 mm mass, firm, white

Case 1: What's Your Diagnosis?



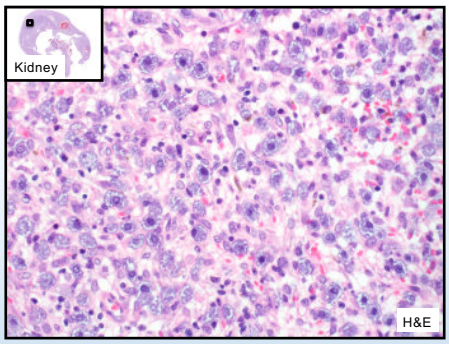
Slide 1 of 3

Case 1: What's Your Diagnosis?



Slide 2 of 3

Case 1: What's Your Diagnosis?



Slide 3 of 3

Case 1: What's Your Diagnosis?

1. Granular cell tumor
2. Histiocytic sarcoma
3. Lymphoma
4. Natural killer (NK) cell tumor
5. Mast cell tumor
6. Other

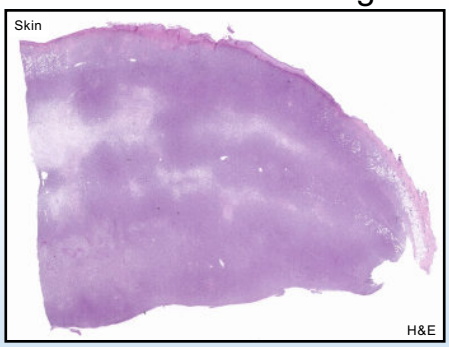
Case 2: Signalment

- Wistar Han female rat
- Two year carcinogenesis bioassay
- Inhalation study - whole body exposure
- 586 days on test
- Moribund sacrifice
- From a treated group

Case 2: Signalment

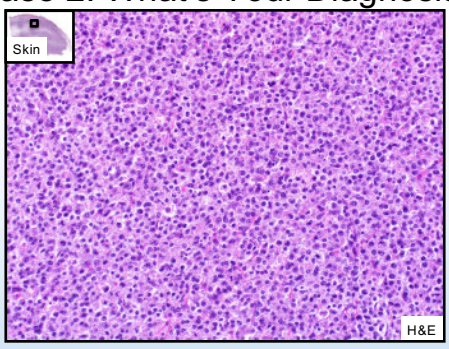
- Gross lesions
  - Pale bone marrow
  - Lung, masses, all lobes, pale, 2-3 mm
  - Lymph node, mesenteric and mediastinal, enlarged
  - Skin, subcutaneous mass, 6.3 cm x 3.3 cm
  - Pancreas, irregular 8 cm x 2 cm
  - Mesentery, pale, irregular, thick
  - Kidney, left, mass 1 cm x 1.5 cm

Case 2: What's Your Diagnosis?



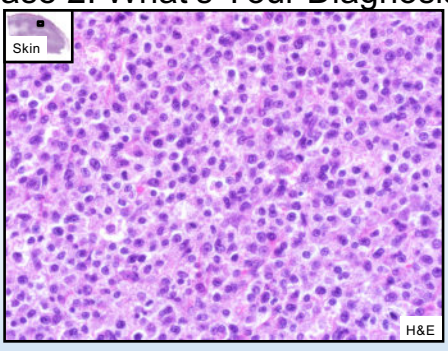
Slide 1 of 4

Case 2: What's Your Diagnosis?



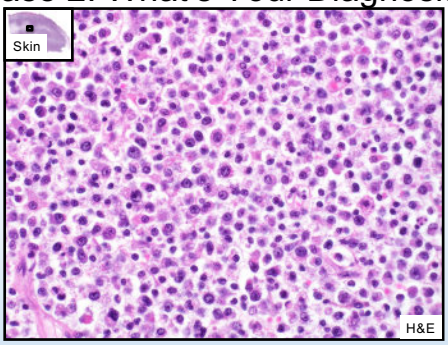
Slide 2 of 4

Case 2: What's Your Diagnosis?



Slide 3 of 4

Case 2: What's Your Diagnosis?



Slide 4 of 4

Case 2: What's Your Diagnosis?

1. Granular cell tumor
2. Histiocytic sarcoma
3. Lymphoma
4. Natural killer (NK) cell tumor
5. Mast cell tumor
6. Other

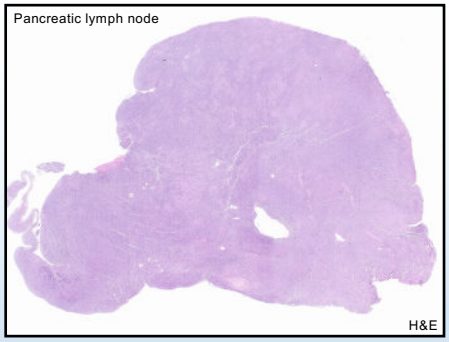
Case 3: Signalment

- F344/N male rat
- Two year carcinogenesis bioassay
- Gavage study
- 714 days on test
- Moribund sacrifice
- From a treated group

Case 3: Signalment

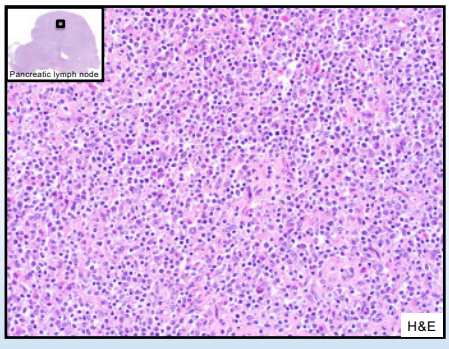
- Gross lesions
  - Lymph node, pancreatic, enlarged (10x), tan
  - Lymph node, mesenteric, enlarged (10x), tan
  - Spleen, mass, 7x6x5 mm, tan

Case 3: What's Your Diagnosis?



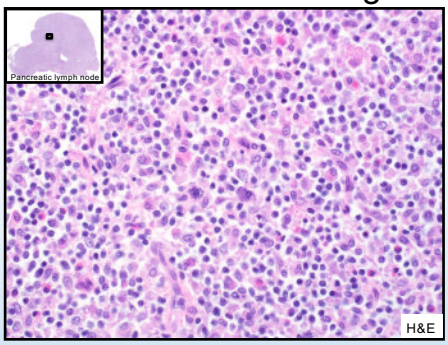
Slide 1 of 3

Case 3: What's Your Diagnosis?



Slide 2 of 3

Case 3: What's Your Diagnosis?

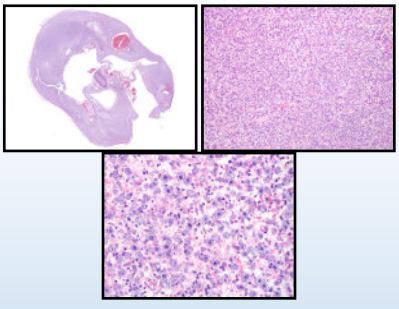


Slide 3 of 3

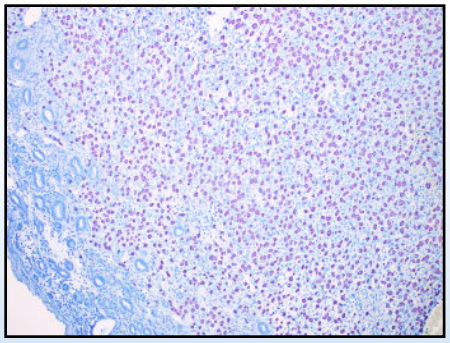
Case 3: What's Your Diagnosis?

1. Granular cell tumor
2. Histiocytic sarcoma
3. Lymphoma
4. Natural killer (NK) cell tumor
5. Mast cell tumor
6. Other

Case 1: Kidney - Mast Cell Tumor

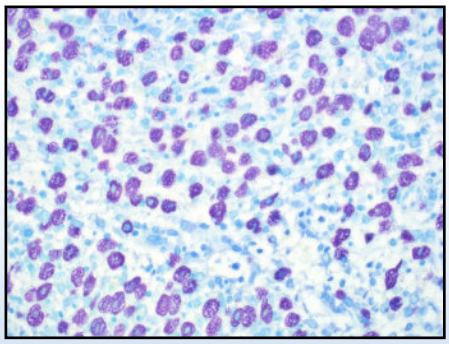


Cellular Features



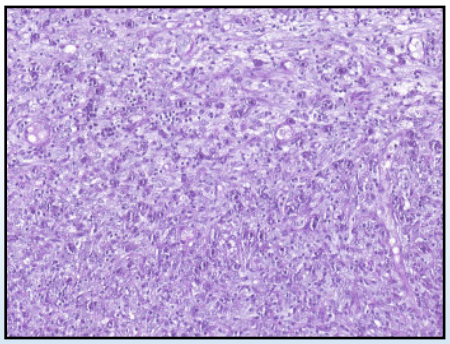
Toluidine blue

Cellular Features



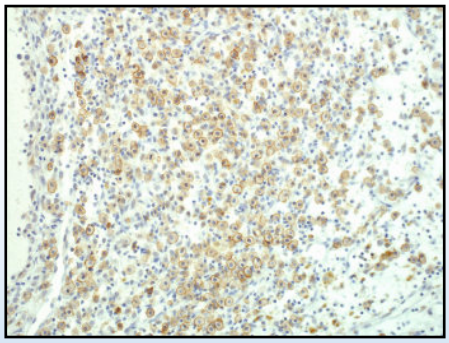
Toluidine blue

Cellular Features



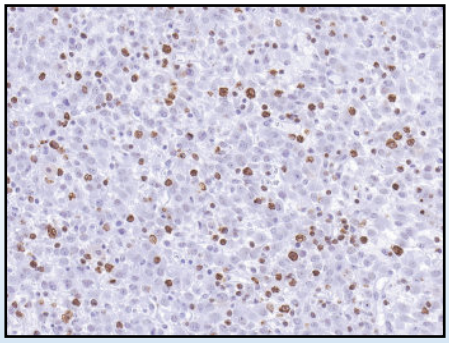
PAS

Cellular Features



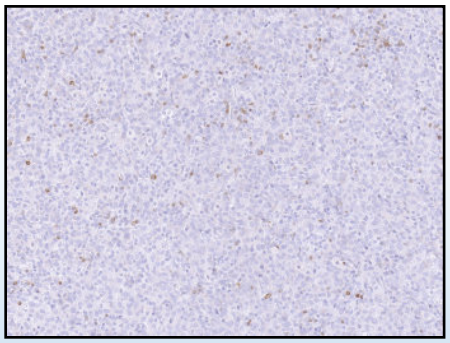
CKIT IHC; DAB chromogen

Cellular Features



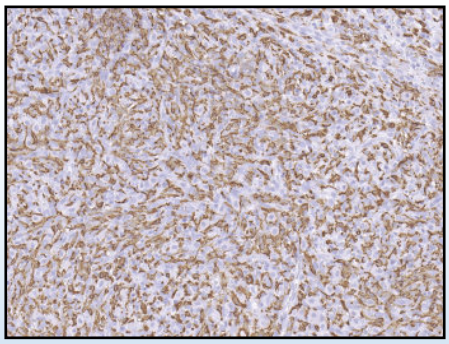
Perforin IHC; DAB chromogen

Cellular Features



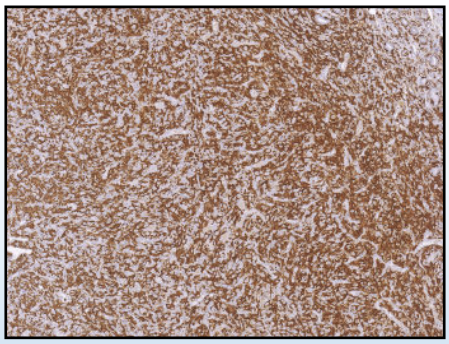
CD3 IHC; DAB chromogen

Cellular Features



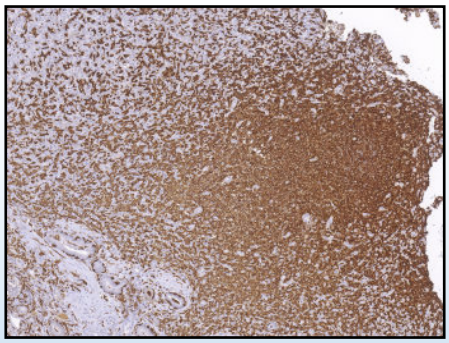
IBA1 IHC; DAB chromogen

Cellular Features



IBA1 IHC; DAB chromogen

Cellular Features

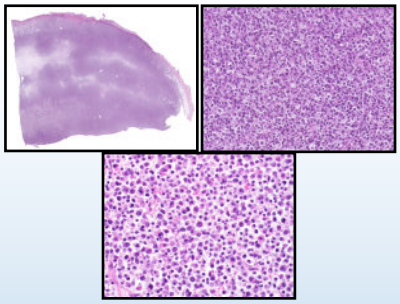


IBA1 IHC; DAB chromogen

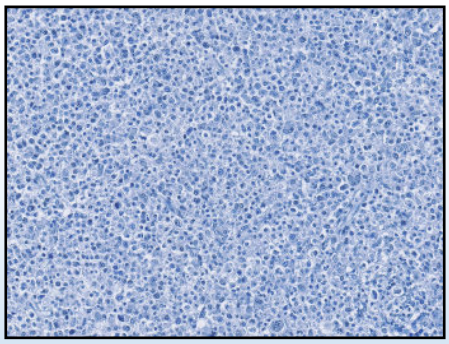
Summary

Features of neoplastic cells	Case 1
Metachromatic staining with toluidine blue	+
PAS + granules	-
CKIT	+
Perforin	-
CD3	-
IBA1	-

Case 2: Skin - NK Cell Tumor

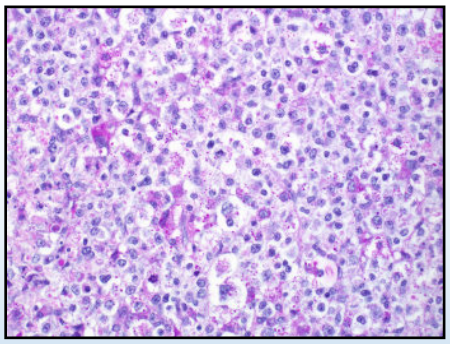


Cellular Features



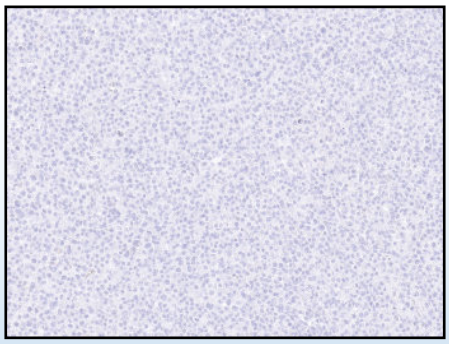
Toluidine blue

Cellular Features



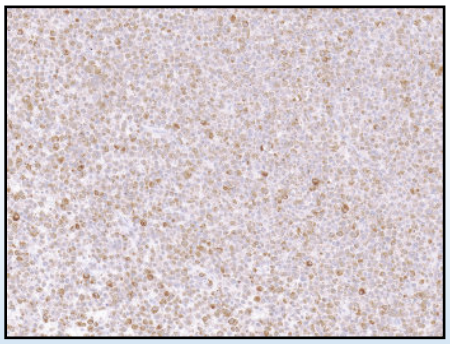
PAS

Cellular Features



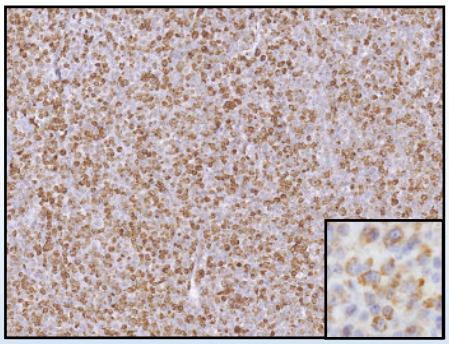
CKIT IHC; DAB chromogen

Cellular Features



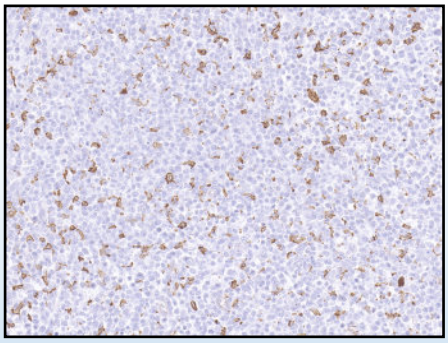
Perforin IHC; DAB chromogen

Cellular Features



CD3 IHC; DAB chromogen

Cellular Features

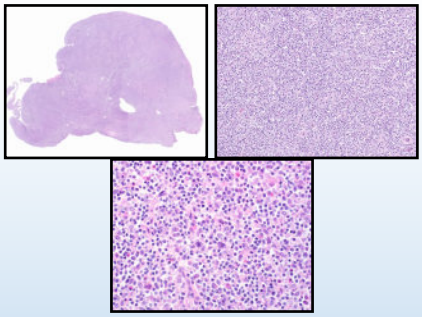


IBA1 IHC; DAB chromogen

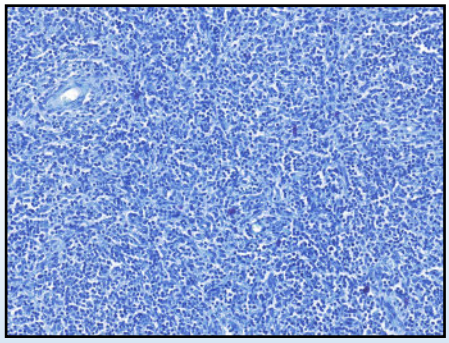
Summary

Features of neoplastic cells	Case 1	Case 2
Metachromatic staining with toluidine blue	+	-
PAS + granules	-	+
CKIT	+	-
Perforin	-	+
CD3	-	+ cytoplasmic
IBA1	-	-

Case 3: Lymph node - Histiocytic Sarcoma

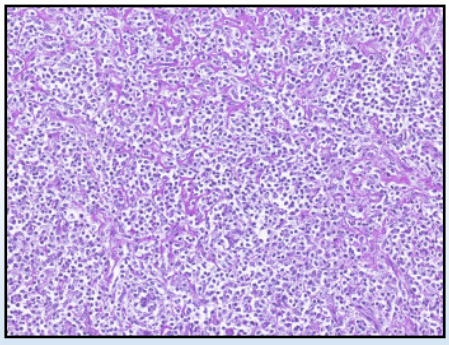


Cellular Features



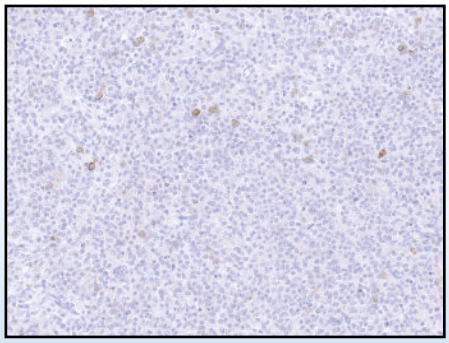
Toluidine blue

Cellular Features



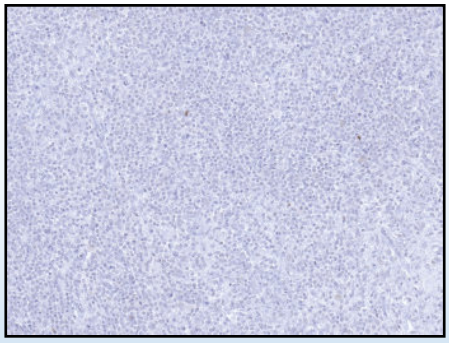
PAS

Cellular Features



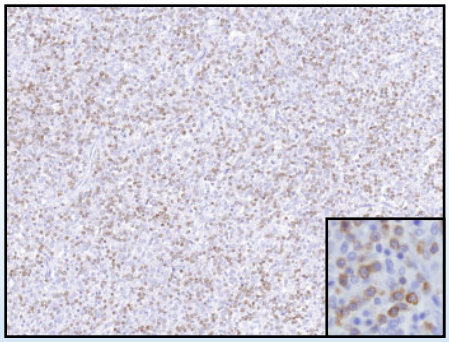
CKIT IHC; DAB chromogen

Cellular Features



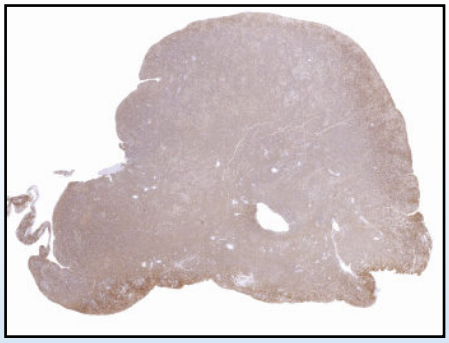
Perforin IHC; DAB chromogen

Cellular Features



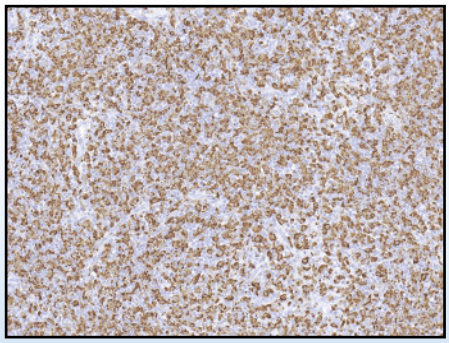
CD3 IHC; DAB chromogen

Cellular Features



IBA1 IHC; DAB chromogen

Cellular Features



IBA1 IHC; DAB chromogen

Summary

Features	Case 1: Mast cell tumor	Case 2: NK cell tumor	Case 3: Histiocytic sarcoma
Metachromatic staining with toluidine blue	+	-	-
PAS + granules	-	+	-
CKIT	+	-	-
Perforin	-	+	-
CD3	-	+ (cytoplasmic)	-
IBA1	-	-	+

Discussion

Natural killer cells

- From common innate lymphoid progenitor cells
- Group 1 innate lymphoid cells (ILCs) – IFN-γ
- In humans, NK cells are $CD56^{+}$ $CD3^{-}$
  - $CD56^{bright}$ – Fully mature, 90% of total NK cells, cytotoxicity
  - $CD56^{dim}$ – Immature, 10% of total NK cells, cytokine production, decidual tissue – angiogenesis
- Blood, spleen, liver, lung and bone marrow
- Targets cells with downregulated MHC-1
- Cytotoxic granules – granzyme and perforin

Discussion

Natural killer cells

- Most of the NK cell tumors in humans are extranodal
- Incidence of NK cell neoplasm: ~5-20%
  - More prevalent in Asians and South Americans
  - NK/T cell lymphoma, nasal (can occur in other sites)
  - Aggressive NK cell leukemia
- No CD56 expression in rodents
  - CD161 and Ly49s3 – rats
  - CD27 – mice
- Isolated cases have been reported in rats

Discussion

Natural killer T cells

- Express both NK cell and T cell markers
- Invariant (Type 1) and Variant (Type II)
- Recognize lipid antigens presented by CD1d molecule
- Have a role in tumor immunity

Mast cells

- From hematopoietic stem cells ($CD34^{+}$/$CD117^{+}$/$CD13^{+}$)
- Cytoplasmic granules, metachromatia, CKIT expression
- Neoplasms – extremely rare

Discussion

CD8 lymphocytes (cytotoxic T lymphocytes)

- CD3+ CD8+ and contain granzyme and perforin
- T cell large granular lymphocytic leukemia
- Subcutaneous panniculitis-like T cell lymphoma
- Primary cutaneous CD8-positive aggressive epidermotrophic cytotoxic T cell lymphoma
- Anaplastic large cell lymphoma (ALK^+ and ALK^-)
- Extremely rare in rats?

Discussion

Large granular lymphocytic leukemia of rats

- Cell of origin is not completely understood
- Very high incidence in F344 rats and rare in others
- Almost all cases involve spleen
- Neoplastic cells in vascular lumens of various tissues

Discussion

Granular cell tumor

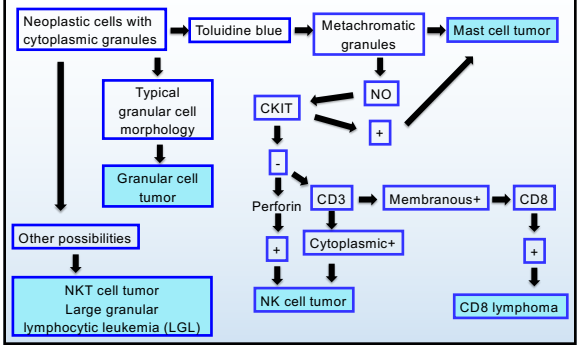
- Cell of origin not established
- Large oval to round cells
- Cytoplasmic granules
- PAS-positivity
- Diagnosis - based on the location (cervix/uterus, meninges) and typical morphology

Discussion

Histiocytic sarcoma

- Macrophages
- Liver, lung, spleen, subcutis and lymph nodes
- Incidence is very low in rats
- Ionized calcium-binding adapter molecule 1 (IBA1)- marker

Summary



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  - ASRC Federal (Data Management)
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    - Jamie Moose
    - Cari Martini
    - Ellie Sheridan
    - Isabel Lea

References

Campbell, Kerry S., and Jun Hasegawa. 2013. 'Natural killer cell biology: An update and future directions', *Journal of Allergy and Clinical Immunology*, 132: 536-44.

Dixon, Darlene, Roger Alison, Ute Bach, Karyn Colman, George L. Foley, Johannes H. Harleman, Richard Haworth, Ronald Herbert, Anke Heuser, Gerald Long, Michael Mirsky, Karen Regan, Eric Van Esch, F. Russell Westwood, Justin Vidal, and Midori Yoshida. 2014. 'Nonproliferative and Proliferative Lesions of the Rat and Mouse Female Reproductive System', *Journal of Toxicologic Pathology*, 27: 1S-107S.

Gill, Harinder, Raymond H. S. Liang, and Eric Tse. 2010. 'Extranodal Natural-Killer/T-Cell Lymphoma, Nasal Type', *Advances in Hematology*, 2010.

Hasserjian, Robert P., and Nancy Lee Harris. 2007. 'NK-Cell Lymphomas and LeukemiasA Spectrum of Tumors With Variable Manifestations and Immunophenotype', *American Journal of Clinical Pathology*, 127: 860-68.

Innginerdingen, M., L. Kveberg, C. Naper, and J. T. Vaage. 2011. 'Natural killer cell subsets in man and rodents', 78: 81-88.

Jerez, Andres, Michael J. Clemente, Hideki Makishima, Hanna Koskela, Francis LeBlanc, Kwok Peng Ng, Thomas Olson, Bartlomiej Przychodzen, Manuel Afable, Ines Gomez-Segui, Kathryn Guinta, Lisa Durkin, Eric D. Hsi, Kathy McGraw, Dan Zhang, Marcin W. Wlodarski, Kimmo Porkka, Mikkael A. Sekeres, Alan List, Satu Mustjoki, Thomas P. Loughran, and Jaroslaw P. Maciejewski. 2012. 'STAT3 mutations unify the pathogenesis of chronic lymphoproliferative disorders of NK cells and T-cell large granular lymphocyte leukemia', 120: 3048-57.

Kwong, Y. L. 2005. 'Natural killer-cell malignancies: diagnosis and treatment', *Leukemia*, 19: 2186-94.

Moon, T. C., C. D. St Laurent, K. E. Morris, C. Marcet, T. Yoshimura, Y. Sekar, and A. D. Befus. 2009. 'Advances in mast cell biology: new understanding of heterogeneity and function', *Mucosal Immunol*, 3: 111.

Nagatani, M., A. Nakamura, Y. Yamaguchi, T. Aikawa, and K. Tamura. 2001. 'Spontaneous Eosinophilic Granulated Round Cell Tumors in Rats', *Veterinary Pathology*, 38: 317-24.

Picut, Catherine A., Cynthia L. Swanson, Regina F. Parker, Kathryn L. Scully, and George A. Parker. 2009. 'The Metrial Gland in the Rat and Its Similarities to Granular Cell Tumors', *Toxicologic Pathology*, 37: 474-80.

References

Project, International T-Cell Lymphoma. 2008. 'International Peripheral T-Cell and Natural Killer/T-Cell Lymphoma Study: Pathology Findings and Clinical Outcomes', 26: 4124-30.

Rebelatto, Marlon C. 2018. 'Chapter 24 - Spleen, Lymph Nodes, and Thymus A2 - Suttie, Andrew W.' in, *Boorman's Pathology of the Rat (Second Edition)* (Academic Press: Boston).

Shey, Michael R., and Zuhair K. Ballas. 2008. 'Assessment of Natural Killer (NK) and NKT Cells in Murine Spleens and Livers.' in Laura E. Nagy (ed.), *Alcohol: Methods and Protocols* (Humana Press: Totowa, NJ).

Squire, Robert A. 1990. 'Histiocytic Sarcoma, Rat.' in Thomas Carlyle Jones, Jerrold Michael Ward, Ulrich Mohr and Ronald Duncan Hunt (eds.), *Hemopoietic System* (Springer Berlin Heidelberg: Berlin, Heidelberg).

Stewart, I. J. 1998. 'Granulated metrial gland cells in `minor' species', *Journal of Reproductive Immunology*, 40: 129-46.

Swerdlow, S. H., E. S. Jaffe, P. Brousset, J. K. Chan, L. de Leval, P. Gaulard, N. L. Harris, S. Pileri, and L. M. Weiss. 2014. 'Cytotoxic T-cell and NK-cell lymphomas: current questions and controversies', *Am J Surg Pathol*, 38: e60-71.

Swerdlow, Steven H., Elias Campo, Stefano A. Pileri, Nancy Lee Harris, Harald Stein, Reiner Siebert, Ranjana Advani, Michele Ghielmini, Gilles A. Salles, Andrew D. Zelenetz, and Elaine S. Jaffe. 2016. 'The 2016 revision of the World Health Organization classification of lymphoid neoplasms', 127: 2375-90.

Van Acker, Heleen H., Anna Capsomidis, Evelien L. Smits, and Viggo F. Van Tendeloo. 2017. 'CD56 in the Immune System: More Than a Marker for Cytotoxicity?', *Frontiers in immunology*, 8: 892-92.

Yoshida, Midori, Kenji Sasahara, Jin Ando-Lu, Masakazu Takahashi, and Akihiko Maekawa. 1997. 'Appearance of Eosinophilic Granule Cells as Pathological Changes in the Uteri of Two Aged Virgin Donryu Rats', *Toxicologic Pathology*, 25: 321-25.

NTP
National Toxicology Program

I See a Strange Confession in Thine Eye

2019 NTP Satellite Symposium
Raleigh Convention Center
Raleigh, NC
June 22, 2019

George Schaaf, DVM
Wake Forest University
School of Medicine
Winston-Salem, NC, USA
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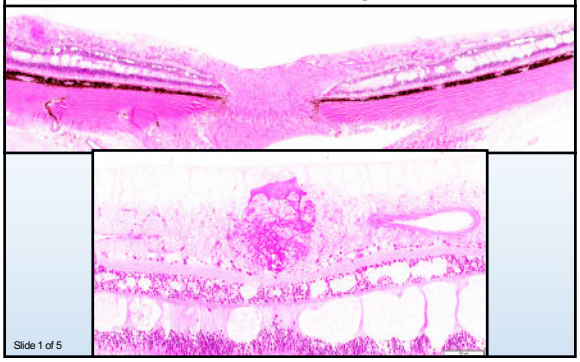
Wake Forest School of Medicine

Signalment

- 10y 5m male rhesus macaque (*Macaca mulatta)*

What's Your Diagnosis?



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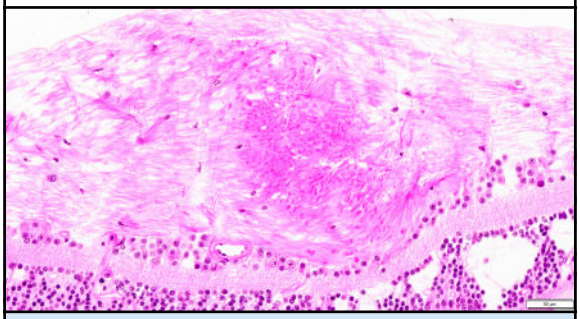
What's Your Diagnosis?



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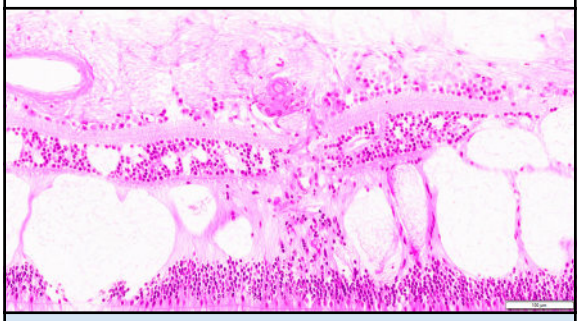

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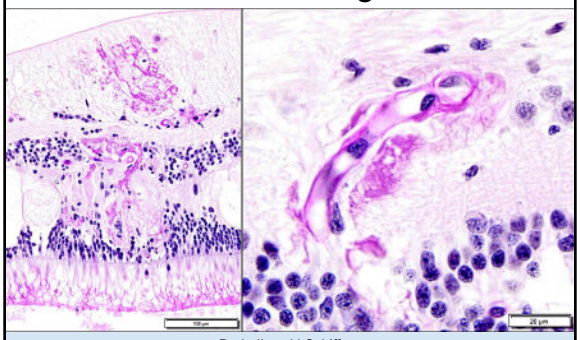
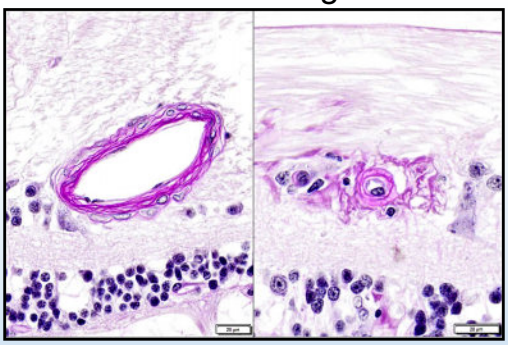
What's Your Diagnosis?



Slide 4 of 5

Periodic acid Schiff

What's Your Diagnosis?



Slide 5 of 5

Periodic acid Schiff

What's Your Diagnosis for this Diffuse Retinal Lesion?

1. Degeneration (outer nuclear layer)
2. Degeneration (inner and outer nuclear and plexiform layers)
3. Dysplasia (outer nuclear layer)
4. Dysplasia (inner and outer nuclear and plexiform layers)
5. Atrophy (outer nuclear layer)
6. Atrophy (inner and outer nuclear and plexiform layers)
7. Other

What's Your Etiologic Diagnosis?

1. Diabetic retinopathy
2. Radiation retinopathy
3. Hypertensive retinopathy
4. Age-related macular degeneration
5. 2 & 3
6. 1, 2, & 3
7. All the above
8. Other

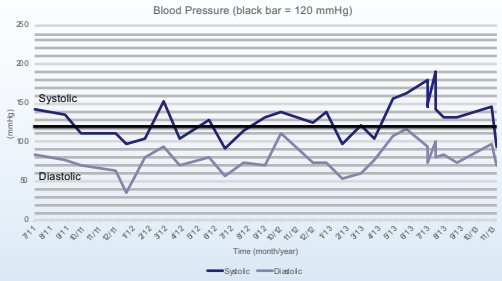
Our Morphologic Diagnosis

- Diffuse retinal degeneration (inner and outer nuclear and plexiform layers) with edema, neuronal swelling (cytoid bodies), arteriolar necrosis, degeneration and neovascularization

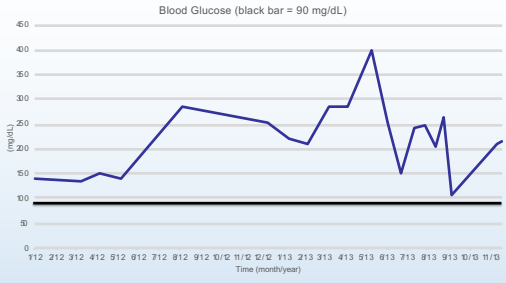
Relevant Clinical History

- Received 8.05 Gy total body irradiation six years prior to necropsy
- Diagnosed with type II diabetes mellitus one year prior to necropsy
- Two year history of hypertension

Hypertension



Hyperglycemia

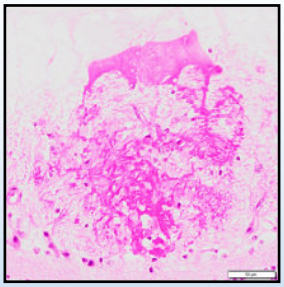


Relevant Histologic Diagnoses

- Arterial intimal & medial fibrosis: lungs, kidneys, coronary arteries, testes
- Right atrial myocardial degeneration with neovascularization and arteriolar hyperplasia
- Diffuse pancreatic islet amyloidosis

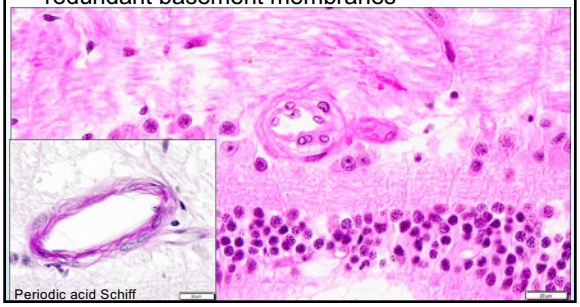
Histologic & Cellular Features

- Presumed remains of vessels with fibrinous debris in the nerve fiber layer

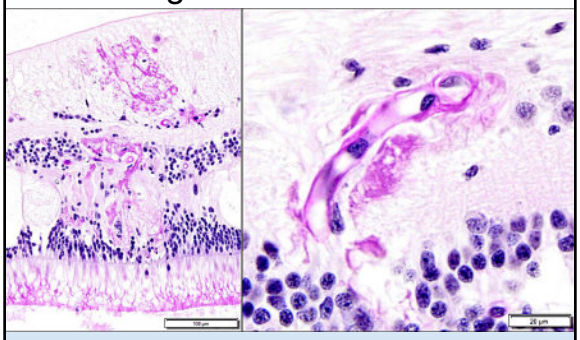


Histologic & Cellular Features

- Plump endothelial cells with vacuolation and redundant basement membranes

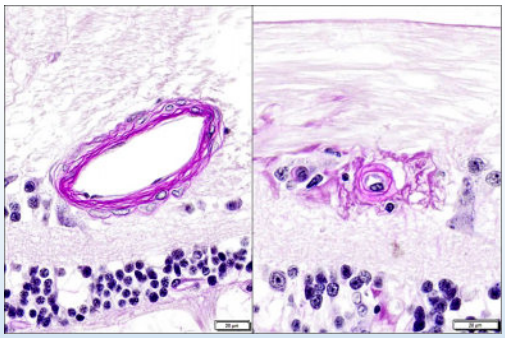


Histologic & Cellular Features



Periodic acid Schiff

Histologic & Cellular Features

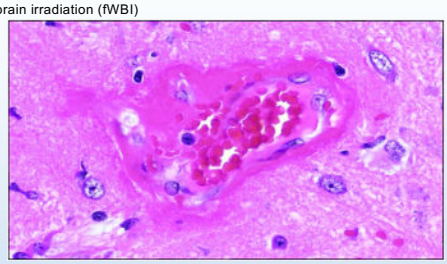


Slide 5 of 5

Periodic acid Schiff

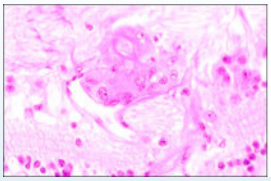
Similar Cerebrovascular Lesions

- From rhesus macaques that received 40 Gy fractionated whole-brain irradiation (fWBI)
- Perivascular accumulations of fibrin and serum

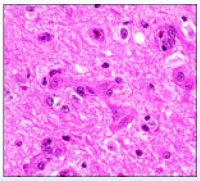


Andrews et al, 2017

Similar Cerebrovascular Lesions



A tortuous vessel from the presented case

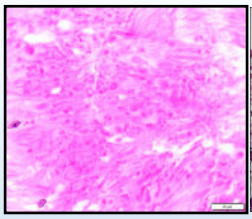


A fWBI animal showing similar lesions of the cerebrovasculature (*Andrews et al, 2017)*

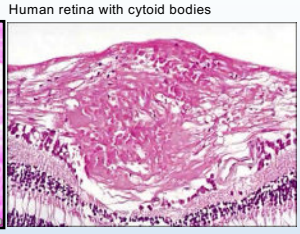
Histologic & Cellular Features

- Cytoid bodies in the nerve fiber layer

Presented Case



Human retina with cytoid bodies



Clinical Ophthalmology

"Cotton Wool Spots"

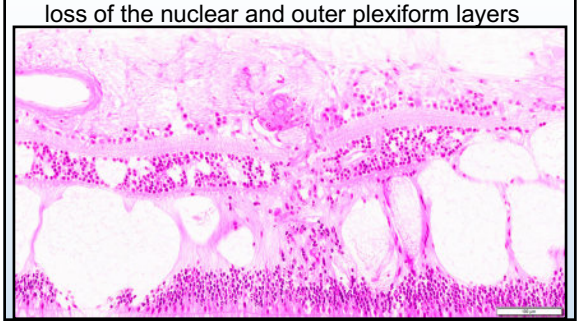
- A gross ophthalmology description
- Fuzzy white to yellow spots seen on fundoscopic exam
- Likely a result of retinal ischemia[1]
- Associated with diabetes, hypertension and radiation [1,2,3]

Human retina, American Academy of Ophthalmologists

Human retina, www.octmd.org

Histologic & Cellular Features

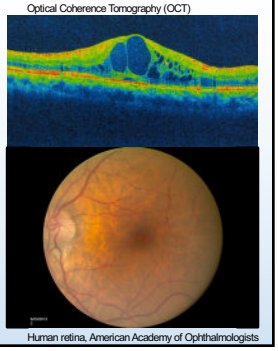
- Cystoid degeneration with edema and cellular loss of the nuclear and outer plexiform layers



"Cystoid edema"

- A common finding in diabetic and radiation retinopathies (1,3)
- Believed to be a result of ischemia (4)
- Generally occurs in the outer plexiform layer (5)
- Not a true cyst (lacks epithelial lining)

Optical Coherence Tomography (OCT)



Human retina, American Academy of Ophthalmologists

Discussion

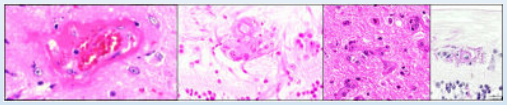
- Retinal vascular endothelial cells (RVECs) uniquely sensitive to hypertension, radiation and diabetes
  - **Hypertension:** Exaggerated autoregulation (6)
  - **Radiation:** Increased mitotic rate of RVECs (1)
  - **Diabetes:** Pericyte sensitivity to hyperglycemia (1,7)

Conclusion

- Multifactorial retinopathy
  - Radiation
  - Hypertension
  - Diabetes
- Underlying vascular pathogenesis

Take Home Points

- The response of the retina to **vascular** injury appears to be stereotypic, regardless of cause
- Radiation-induced retinal vascular injury resembles that seen in the brain



Take Home Points

- Fundoscopic screening for retinal lesions may predict or indicate concurrent cerebrovascular injury [8]

American Academy of Ophthalmologists

Discussion Points

- Thoughts on pathogenesis and the extent of contribution by comorbid conditions?
- Discrimination of underlying cause by morphologic appearance
- Other thoughts, comments, or questions?

References

1. Schmidt D: The mystery of cotton-wool spots - a review of recent and historical descriptions. Eur J Med Res **13:** 231-266, 2008
2. Archer DB, Gardiner TA: Ionizing radiation and the retina. Curr Opin Ophthalmol **5:** 59-65, 1994
3. Kim SY, Johnson MA, McLeod DS, Alexander T, Otsuji T, Steidl SM, Hansen BC, Lutty GA: Retinopathy in monkeys with spontaneous type 2 diabetes. Invest Ophthalmol Vis Sci **45:** 4543-4553, 2004
4. Stitt AW, Gardiner TA, Archer DB: Histological and ultrastructural investigation of retinal microaneurysm development in diabetic patients. Br J Ophthalmol **79:** 362-367, 1995
5. Scholl S, Kirchhof J, Augustin AJ: Pathophysiology of macular edema. Ophthalmologica **224 Suppl 1:** 8-15, 2010
6. Crispin SM, Mould JR: Systemic hypertensive disease and the feline fundus. Vet Ophthalmol **4:** 131-140, 2001
7. Jo DH, Cho CS, Kim JH, Jun HO, Kim JH: Animal models of diabetic retinopathy: doors to investigate pathogenesis and potential therapeutics. J Biomed Sci **20:** 38, 2013

References

8. Patton N, Aslam T, Macgillivray T, Pattie A, Deary IJ, Dhillon B: Retinal vascular image analysis as a potential screening tool for cerebrovascular disease: a rationale based on homology between cerebral and retinal microvasculatures. J Anat **206:** 319-348, 2005
9. Andrews RN, Metheny-Barlow LJ, Peiffer AM, Hanbury DB, Tooze JA, Bourland JD, Hampson RE, Deadwyler SA, Cline JM: Cerebrovascular Remodeling and Neuroinflammation is a Late Effect of Radiation-Induced Brain Injury in Non-Human Primates. Radiat Res **187:** 599-611, 2017
10. Andrews RN, Dugan GO, Peiffer AM, Hawkins GA, Hanbury DB, Bourland JD, Hampson RE, Deadwyler SA, Clinea JM: White Matter is the Predilection Site of Late-Delayed Radiation-Induced Brain Injury in Non-Human Primates. Radiat Res **191:** 217-231, 2019
11. Hanbury DB, Robbins ME, Bourland JD, Wheeler KT, Peiffer AM, Mitchell EL, Daunais JB, Deadwyler SA, Cline JM: Pathology of fractionated whole-brain irradiation in rhesus monkeys (Macaca mulatta). Radiat Res **183:** 367-374, 2015
12. Duane TD: Clinical ophthalmology, Rev. ed. p. v. <1 >. Medical Dept., Harper & Row, Hagerstown, Md., 1978

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- David Caudell (WFU, Associate Professor of Pathology)
- Jean Gardin (WFU, Laboratory Manager)
- Cathy Mathis (WFU, Histology Technician)
- Lisa O'Donnell (WFU, Histology Technician)

NTP National Toxicology Program

Catch of the Day from the (F)INHAND Non-Rodent Fish Working Group

2019 NTP Satellite Symposium
Raleigh Convention Center
Raleigh, NC
June 22, 2019

Jeffrey C. Wolf, DVM, DACVP
Experimental Pathology Laboratories, (EPL®) Inc.
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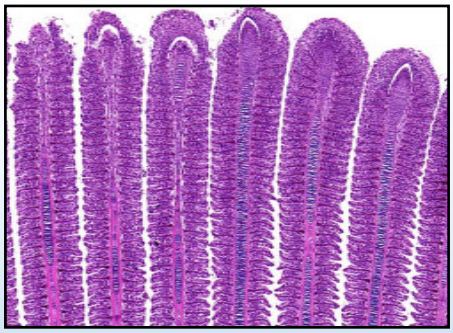
EPL

Case 1: Signalment

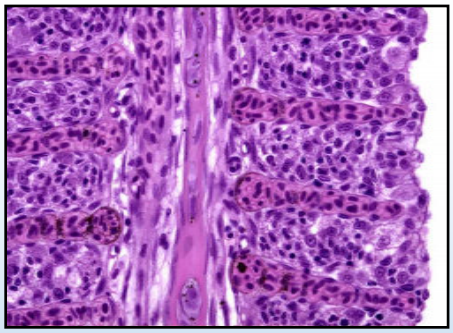
- Male and female tilapia, *Oreochromis* sp. ~107 days old
- 20 day bioassay
- Antibiotic administered in feed

Case 1: What's Your Diagnosis?



Slide 1 of 2

Case 1: What's Your Diagnosis?



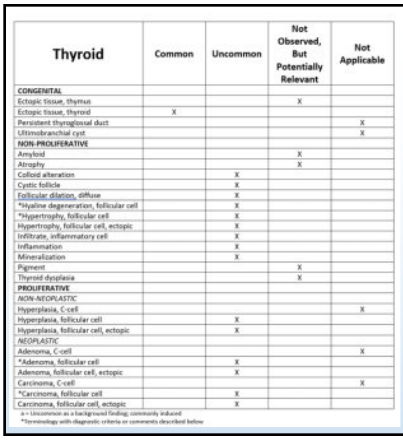
Slide 2 of 2

Case 1: What's Your Diagnosis?

1. Hyperplasia, lamellar epithelium, diffuse
2. Pseudobranch, lamellar fusion
3. Branchitis, proliferative, with mucous cell hyperplasia
4. Lamellar fusion, diffuse
5. Filament clubbing, segmental
6. Cellular infiltrate, mononuclear cell
7. 1, 4, and 6
8. 2, 3, 4, and 5
9. All of the above

INHAND Nomenclature and Diagnostic Criteria

- Joint initiative of the ESTP, BSTP, JSTP, and STP to develop an internationally accepted system of preferred diagnostic criteria and nomenclature for non-proliferative and proliferative microscopic lesions in laboratory animals
- General reliance on descriptive, rather than diagnostic, terminology
- As much as possible, attempt made to harmonize fish terms with rodent INHAND

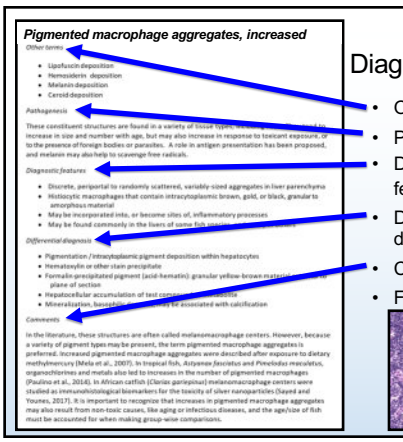


Thyroid	Common	Uncommon	Not Observed, But Potentially Relevant	Not Applicable
CONGENITAL				
Ectopic tissue, thymus			X	
Ectopic tissue, thyroid	X			
Persistent thyroglossal duct				X
Ultimobranchial cyst				X
NON-PROLIFERATIVE				
Amyloid			X	
Atrophy			X	
Colloid alteration		X		
Cystic follicle		X		
Follicular dilation, diffuse		X		
*Hyaline degeneration, follicular cell		X		
*Hypertrophy, follicular cell		X		
Hypertrophy, follicular cell, ectopic		X		
Infiltrate, inflammatory cell		X		
Inflammation		X		
Mineralization		X		
Pigment			X	
Thyroid dysplasia			X	
PROLIFERATIVE				
NON-NEOPLASTIC				
Hyperplasia, C-cell				X
Hyperplasia, follicular cell		X		
Hyperplasia, follicular cell, ectopic		X		
NEOPLASTIC				
Adenoma, C-cell				X
*Adenoma, follicular cell		X		
Adenoma, follicular cell, ectopic		X		
Carcinoma, C-cell				X
*Carcinoma, follicular cell		X		
Carcinoma, follicular cell, ectopic		X		

a – Uncommon as a background finding, commonly induced
*Terminology with diagnostic criteria or comments described below

INHAND Nomenclature

- Common
  - Seen regularly in tox studies
- Uncommon
  - Seen, but not regularly
- NO, BPR
  - Generally not seen, but could possibly occur
- Not applicable
  - e.g., tissue not found in fish



Pigmented macrophage aggregates, increased

Other terms

- Lipofuscin deposition
- Hemosiderin deposition
- Melanin deposition
- Ceroid deposition

Pathogenesis

These constituent structures are found in a variety of tissue types, [illegible] increase in size and number with age, but may also increase in response to toxicant exposure, or to the presence of foreign bodies or parasites. A role in antigen presentation has been proposed, and melanin may also help to scavenge free radicals.

Diagnostic features

- Discrete, periportal to randomly scattered, variably-sized aggregates in liver parenchyma
- Histiocytic macrophages that contain intracytoplasmic brown, gold, or black, granular to amorphous material
- May be incorporated into, or become sites of, inflammatory processes
- May be found commonly in the livers of some fish [illegible]

Differential diagnosis

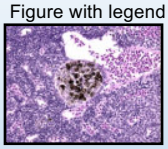
- Pigmentation / intracytoplasmic pigment deposition within hepatocytes
- Hematoxylin or other stain precipitate
- Formalin-precipitated pigment (acid-hematin): granular yellow-brown material [illegible] plane of section
- Hepatocellular accumulation of [illegible]
- Mineralization, basophilic [illegible] associated with calcification

Comments

In the literature, these structures are often called melanomacrophage centers. However, because a variety of pigment types may be present, the term pigmented macrophage aggregates is preferred. Increased pigmented macrophage aggregates were described after exposure to dietary methylmercury (Mela et al., 2007). In tropical fish, *Astyanax fasciatus* and *Pimelodus maculatus*, organochlorines and metals also led to increases in the number of pigmented macrophages (Paulino et al., 2014). In African catfish (*Clarias gariepinus*) melanomacrophage centers were studied as immunohistological biomarkers for the toxicity of silver nanoparticles (Sayed and Younes, 2017). It is important to recognize that increases in pigmented macrophage aggregates may also result from non-toxic causes, like aging or infectious diseases, and the age/size of fish must be accounted for when making group-wise comparisons.

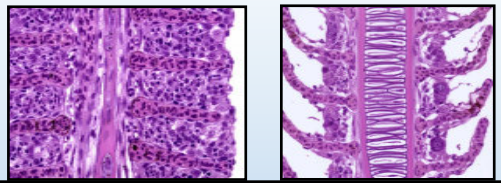
INHAND Diagnostic Criteria

- Other terms
- Pathogenesis
- Diagnostic features
- Differential diagnoses
- Comments
- Figure with legend



Discussion: Gill Findings

1. Hyperplasia, lamellar epithelium, diffuse
2. Pseudobranch, lamellar fusion
3. Branchitis, proliferative, with mucous cell hyperplasia
4. Lamellar fusion, diffuse
5. Filament clubbing, segmental
6. Cellular infiltrate, mononuclear cell



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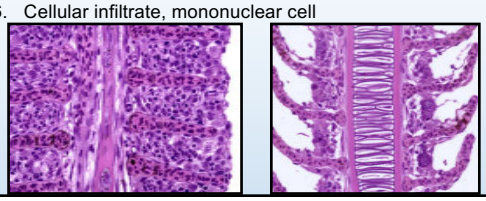

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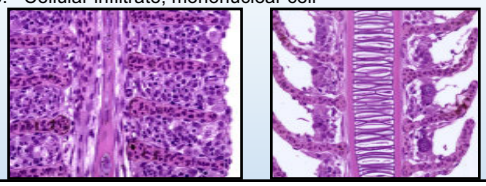
Not pseudobranch

Diagnostic vs. descriptive, and no mucous cells

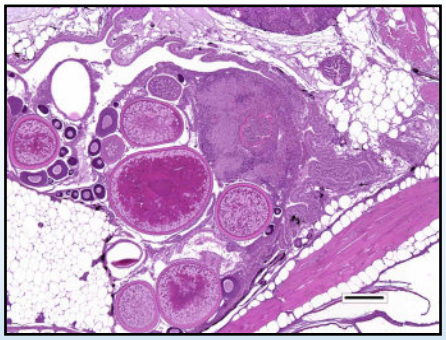
Not segmental



Case 2: Signalment

- Adult female fathead minnow *Pimephales promelas*
- 21 day bioassay
- Negative control fish

Case 2: What's Your Diagnosis?



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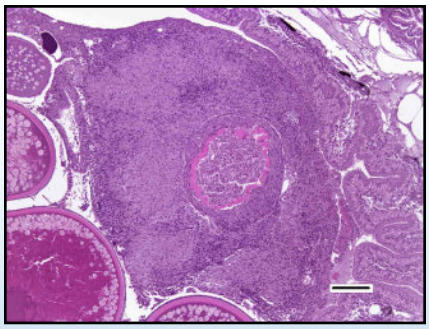
Case 2: What's Your Diagnosis?



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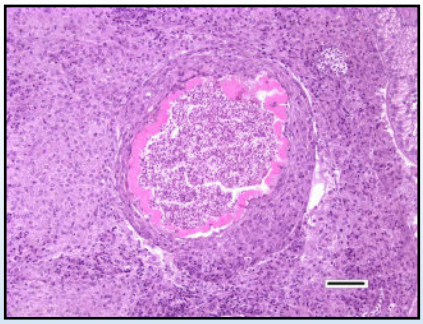
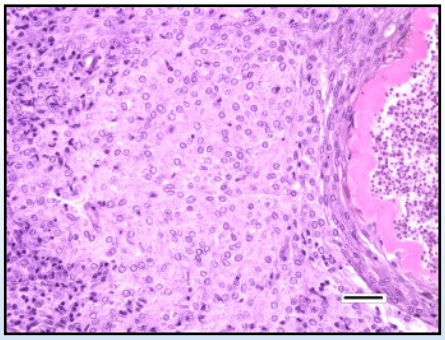
Case 2: What's Your Diagnosis?



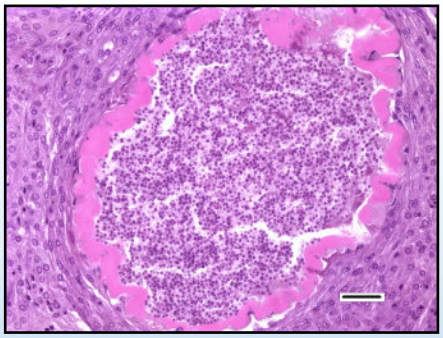
Slide 3 of 5

Case 2: What's Your Diagnosis?



Slide 4 of 5

Case 2: What's Your Diagnosis?

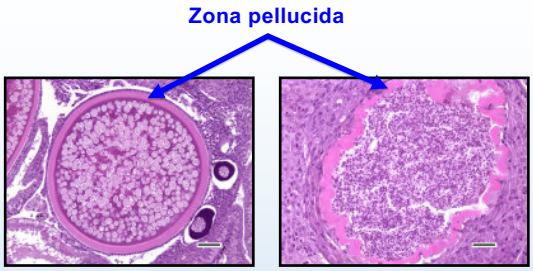


Slide 5 of 5

Case 2: What's Your Diagnosis for this Ovarian Lesion?

1. Teratoma
2. Rodlet cell tumor
3. Squamous cell carcinoma
4. Yolk degeneration and granulomatous inflammation with acid fast bacilli
5. Oocyte atresia; inflammation, granulomatous; and microsporidian oophoritis

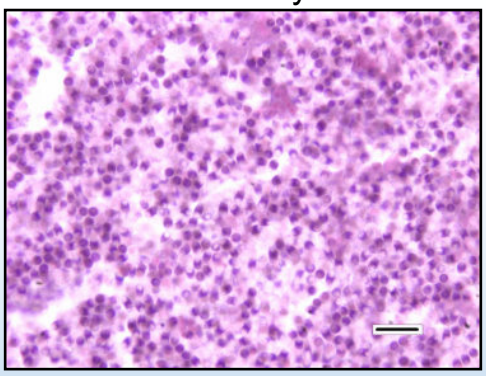
Discussion: Oocyte Atresia



Normal Oocyte

Atretic Oocyte with Microsporidia

Discussion: Oocyte Atresia



Discussion: Background Infections

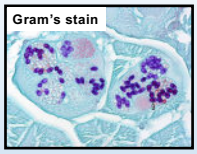
- Background infectious diseases can potentially confound the results of toxicologic studies
- Example diagnoses from the INHAND rodent nomenclature:
  - Liver, *Helicobacter* sp. hepatitis
  - Liver, murine norovirus hepatitis
  - Liver, mouse hepatitis virus hepatitis
  - Liver, Tyzzer's disease (*Clostridium piliforme* infection)

Discussion: Microsporidia

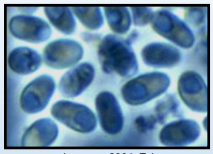
- Microsporidian infections occur commonly in a wide variety of fish species
- May be incidental, or may cause profound disease
- May target specific tissues, or less often cause systemic infections
- Examples likely to be seen in fish studies:
  - Fathead minnow ovary: *Pleistophora* spp.
  - Zebrafish spinal cord: *Pseudoloma neurophilia*
  - Zebrafish skeletal muscle: *Pleistophora hyphessobryconis*

Discussion: Microsporidia

- Gram-positive, obligate intracellular
- Lately classified with fungi vs. Protista
- Direct transmission
- Typical “bedroom slipper” appearance in wet mounts and histologic sections

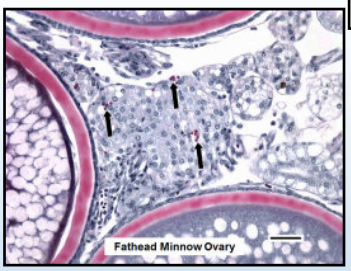


Donald K. Nichols, US Army


Larsson, 2004, ToL

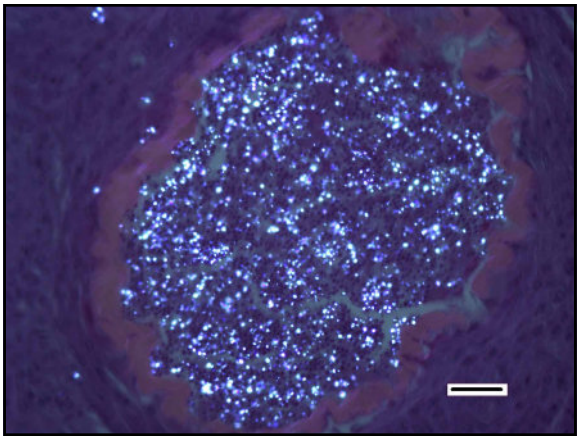
Diagnosis: Microsporidia

- Best method: Luna stain




Luna stain, an improved selective stain for detection of microsporidian spores in histologic sections

Lee G. Luna,
Chief, Histopathology
Laboratories, AFIP
1931-1992



Challenges and Considerations for Fish INHAND

- > 34,000 fish species
  - Focus on species that are most likely to be investigated in toxicology studies
  - Tox studies may involve wild species for which biological, physiological, and/or anatomical information is limited or unreliable
- Background changes and artifacts
  - Wild-caught fish and some laboratory colonies may have pathological manifestations of infectious or husbandry-related diseases
  - The fish literature contains a variety of artifacts that have been mis-reported as pathological changes

Challenges and Considerations for Fish INHAND

- Rodent tissues not found in fish:
  - ✓ Bone marrow
  - ✓ Cochlea
  - ✓ Diaphragm
  - ✓ Hair follicles
  - ✓ Lacrimal glands
  - ✓ Lungs
  - ✓ Lymph nodes
  - ✓ Mammary glands
  - ✓ Sebaceous glands
  - ✓ Thyroid gland c-cells
  - ✓ True urinary bladder
  - ✓ Uterus

Challenges and Considerations for Fish INHAND

- Rodent tissues not found in fish
- Fish tissues not found in rodents:
  - ✓ Corpuscle of Stannius
  - ✓ Dermal scales, mucus, and alarm cells
  - ✓ Gills and pseudobranch
  - ✓ Lateral line
  - ✓ Stato-acoustic organ
  - ✓ Swim bladder
  - ✓ Ultimobranchial body

Challenges and Considerations for Fish INHAND

- Rodent tissues not found in fish
- Fish tissues not found in rodents
- Fish tissues not found in all fishes:
  - ✓ Gizzard, pyloric ceca
  - ✓ Glandular stomach
  - ✓ Oral teeth
  - ✓ Pneumatic duct or gas gland
  - ✓ Renal glomeruli

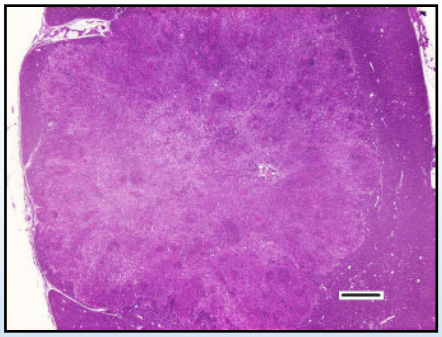
Challenges and Considerations for Fish INHAND

- Rodent tissues not found in fish
- Fish tissues not found in rodents
- Fish tissues not found in all fishes
- Fish processes not found in rodents
  - ✓ Aerobic erythrocyte metabolism
  - ✓ Cardiac muscle regeneration
  - ✓ Renal hematopoiesis
  - ✓ Sequential hermaphroditism

Case 3: Signalment and History

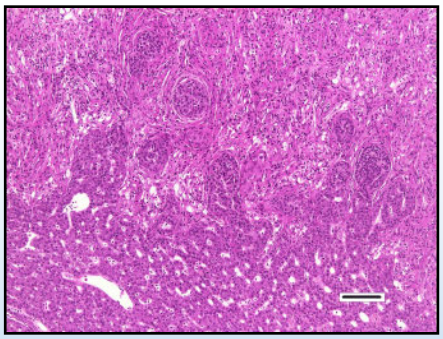
- Two female white sucker fish (*Catostomus commersonii*), ~10 years old
- Part of a collection of 100 suckers by Environment and Climate Change Canada staff from three sites within the St. Mary's River Area of Concern
- Contaminants of particular concern that are known to occur at high concentrations in St. Marys River sediment include polyaromatic hydrocarbons (PAHs) such as benzo[*a*]pyrene
- Historically, the overall prevalence of primary liver tumors is relatively high (5-10%) in white suckers obtained from this stretch of river

Case 3: What's Your Diagnosis?



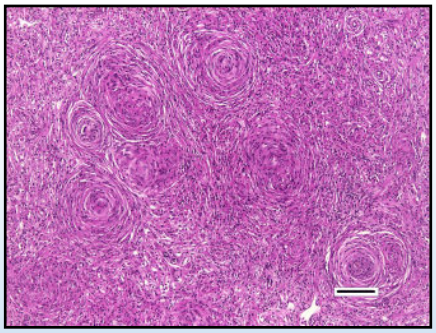
Slide 1 of 6

Case 3: What's Your Diagnosis?



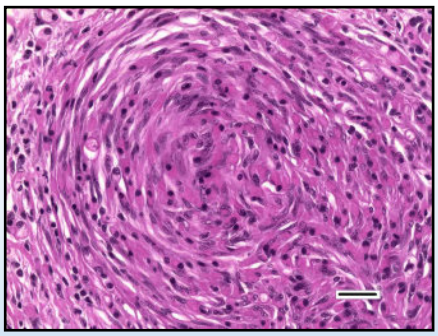
Slide 2 of 6

Case 3: What's Your Diagnosis?



Slide 3 of 6

Case 3: What's Your Diagnosis?



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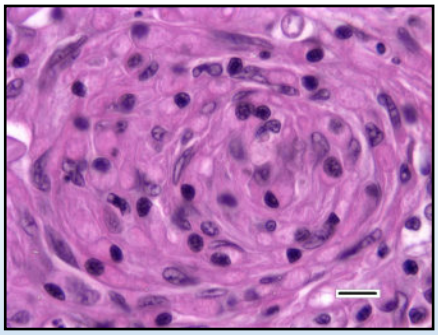

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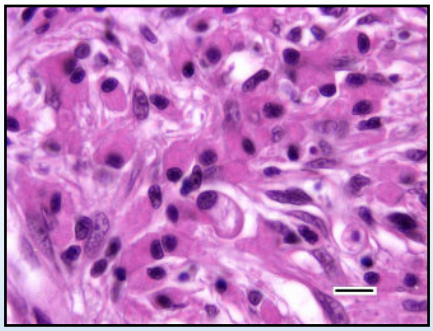

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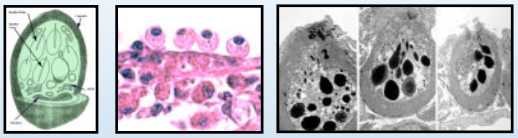
Discussion: Rodlet Cells

- The development, essential nature, and function of these enigmatic cells all remain undetermined
- Found in > 100 freshwater, brackish, and marine fish species to date
- Found in a wide variety of tissue types, especially epithelial mucosae and vascular endothelium
- Rodlets are cytoplasmic spicule-like structures

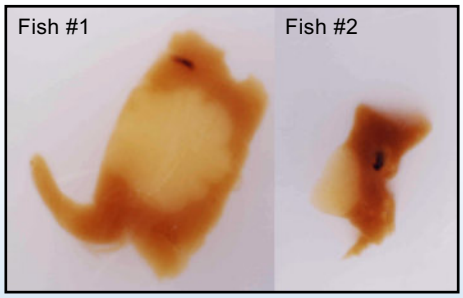


All images courtesy of SA Smith, VMRCVM

Discussion: Rodlet Cells

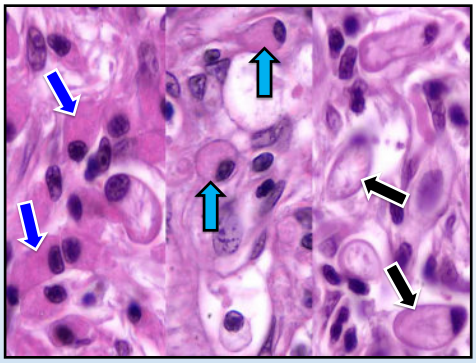
- First described as parasites (Thélohan, 1892)
- Later reported as a secretory cell (Leino, 1974, and others)
- Now widely thought to be of host origin
- Many authors consider these to be non-phagocytic leukocytes that represent a component of the innate immune system
- However, anti-pathogen activity has not been convincingly demonstrated
  - Increased numbers can be found in association with inflammation
  - But can also be abundant for no obvious reason

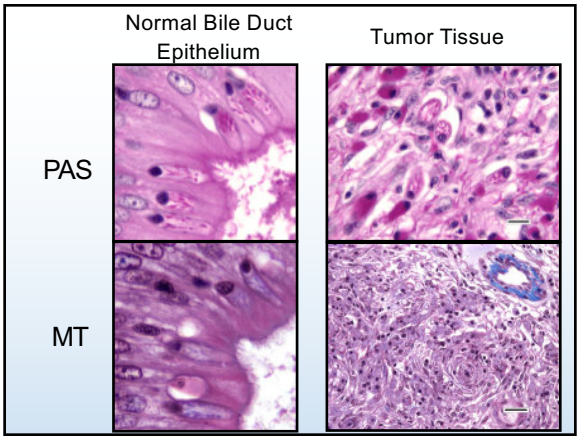
Diagnostics: Rodlet Cell Tumors



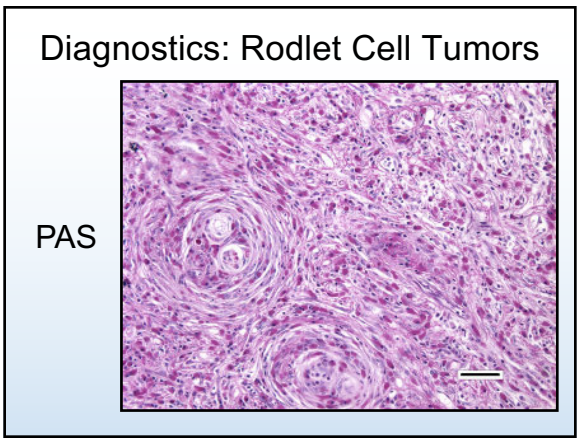
Appearance of each tumor in its paraffin block

Diagnostics: Rodlet Cell Tumors

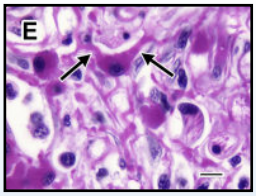




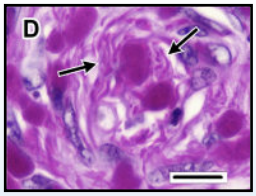
Diagnostics: Rodlet Cell Tumors



Diagnostics: Rodlet Cell Tumors

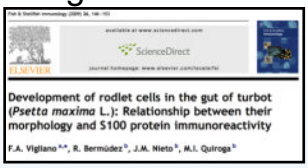


Cytoplasmic processes (PAS)

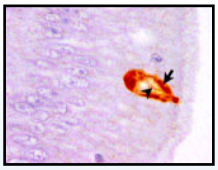


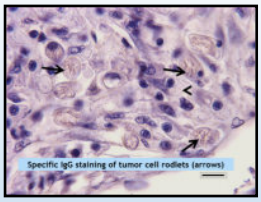
Rodlet-like material (PAS)

Diagnostics: Rodlet Cell Tumors


Development of rodlet cells in the gut of turbot (*Psetta maxima* L.): Relationship between their morphology and S100 protein immunoreactivity

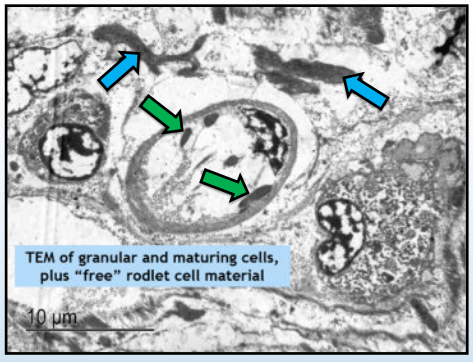
F.A. Vigliano [a,*], R. Bermúdez [b], J.M. Nieto [b], M.I. Quiroga [b]





- Unable to get specific S100 staining of non-neoplastic or neoplastic rodlet cells
- However, rodlets stained with IgG used as isotype control

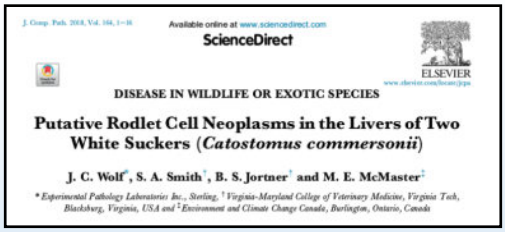
Diagnostics: Rodlet Cell Tumors



Basis for Diagnosis: Rodlet Cell Tumors

- **Neoplastic** vs. **Inflammatory**
  - Expansile and infiltrative behavior at margins of lesion
  - Tendrils of tissue encircle intact cords of healthy-appearing hepatocytes
  - Little or no inflammatory cell component and no fibrosis
- **Rodlet cell tumor** vs. **Rodlet cell-rich sarcoma**
  - The swirling tumor stroma appears to be derived from cytoplasmic processes of the granular tumor cells
  - There are no additional structurally-relevant cell types evident at high magnification
  - The granular cells, intermediate cells, and rodlet cells appear to represent a continuum, in which the granular and intermediate cells may be RC precursors

Publication: Rodlet Cell Tumors



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DISEASE IN WILDLIFE OR EXOTIC SPECIES

Putative Rodlet Cell Neoplasms in the Livers of Two White Suckers (*Catostomus commersonii*)

J. C. Wolf*, S. A. Smith†, B. S. Jortner‡ and M. E. McMaster‡

*Experimental Pathology Laboratories Inc., Sterling, †Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia, USA and ‡Environment and Climate Change Canada, Burlington, Ontario, Canada

INHAND Non-Rodent Fish Working Group

- ✓ Species Introduction
- ✓ Cardiovascular
- ✓ Endocrine
- ✓ Female Reproductive
- ✓ Gastrointestinal
- ✓ Hematopoietic
- ✓ Hepatobiliary
- ✓ Integumentary
- ✓ Male Reproductive
- ✓ Neurologic
- ✓ Respiratory
- ✓ Skeletal
- ✓ Soft Tissue
- ✓ Special Senses
- ✓ Urinary

Current Status

- Began January, 2017
- Draft nomenclature now established and chapters written for most systems
- Currently, DropBox library contains 592 photomicrographic images
- Manuscript destined for publication in JSTP's Journal of Toxicologic Pathology

Acknowledgements: Fish INHAND

- James Baily (Charles River Laboratories, Edinburgh, UK)
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- Stephen Feist (Cefas, Weymouth, UK)
- Satoshi Furukawa (Nissan Chemical Ind., Japan)
- Yuuki Katou (Shinogi & Co., Osaka, Japan)
- Heike Schmidt-Posthaus (University of Bern, Switzerland)
- Christine Ruehl-Fehlert (Bayer AG, Wuppertal, Germany)
- Helmut Segner (University of Bern, Switzerland)
- Shiva Kumar Shanmukhappa (Novartis, Cambridge, MA)
- Jan Spitsbergen (Oregon State U, Corvallis, OR)
- Jeff Wolf (EPL, Inc., Sterling, VA) **-- Chair**

References

- Peterson TS, Spitsbergen JM, Feist SW, Kent ML. 2011. Luna stain, an improved selective stain for detection of microsporidian spores in histologic sections. Dis Aquat Organ, 95(2):175-180.
- Vigliano FA, Bermúdez R, Nieto JM, Quiroga MI. 2009. Development of rodlet cells in the gut of turbot (*Psetta maxima* L.): relationship between their morphology and S100 protein immunoreactivity. Fish Shellfish Immunol, 26(1):146-153.
- Wolf JC, Smith SA, Jortner BS, McMaster ME. 2018. Putative rodlet cell neoplasms in the livers of two white suckers (*Catostomus commersonii*). J Comp Pathol, 164:1-16.