

Interpreting Histopathology in Juvenile Toxicity Studies

Thomas Steinbach, DVM, DACVP, DABT

STPI, Hyderabad, India

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Major Challenges in Juvenile Studies

- **Postnatal tissue development during study**
- **Absence of concurrent controls for unscheduled deaths**
- **Limited references or databases**

Presentation Outline

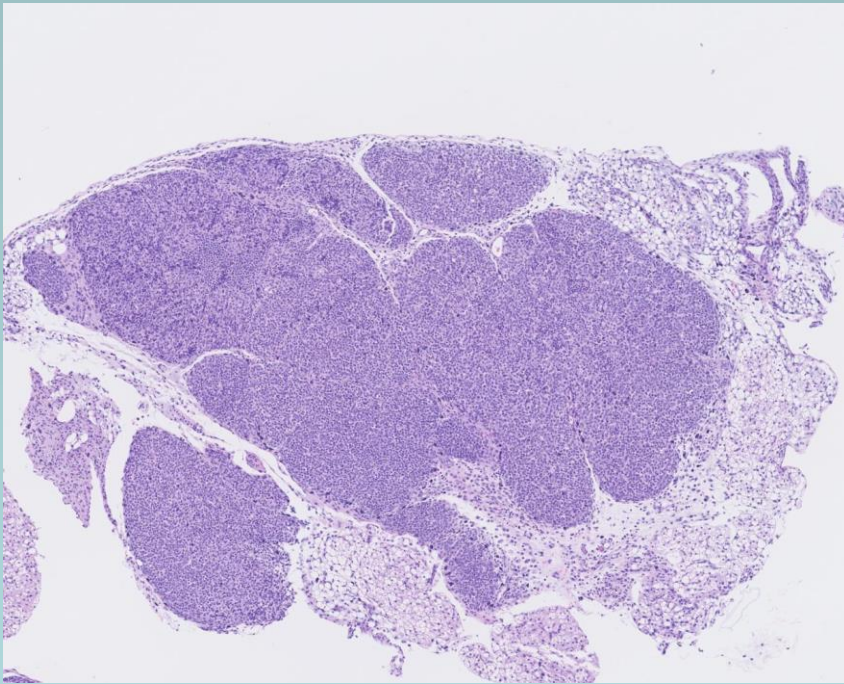
- **Real-life example**
- **Examples of postnatal histogenesis of the rat**
- **Acknowledgements:**
 - Case and photos from Dr. George Parker, CRL
 - Labeled images and tables from:
 - **Atlas of Histology of the Juvenile Rat, ed. George Parker and Catherine Picut, Academic Press, 2016**

Real-life example

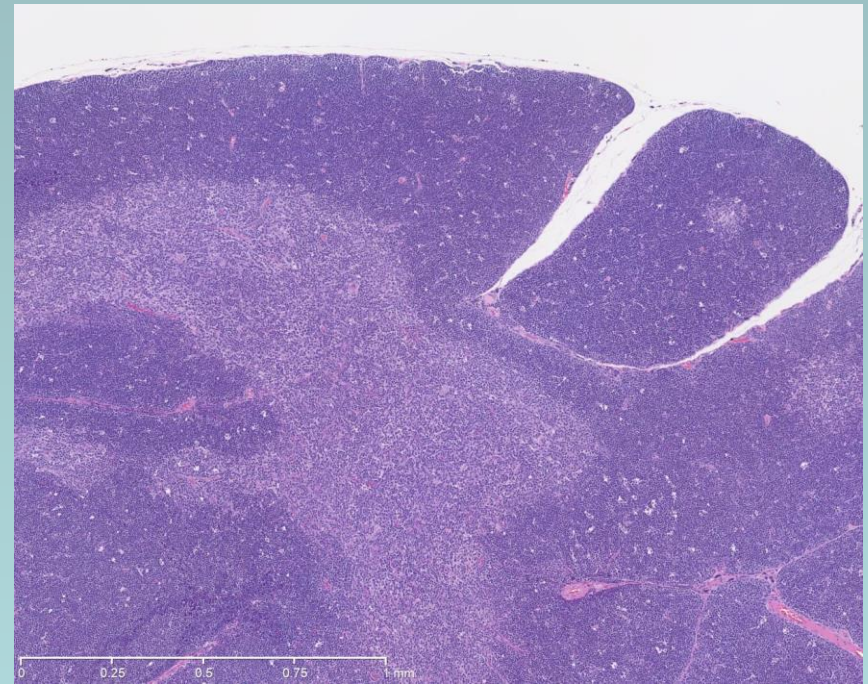
- **Study Design:**
 - Daily oral gavage PND1-21
 - 4 dose groups with recovery
 - Scheduled termination at PND22
 - Toxicity necessitated termination of high-dose group 4 at PND16

Thymus

4M PND12 FD 5x

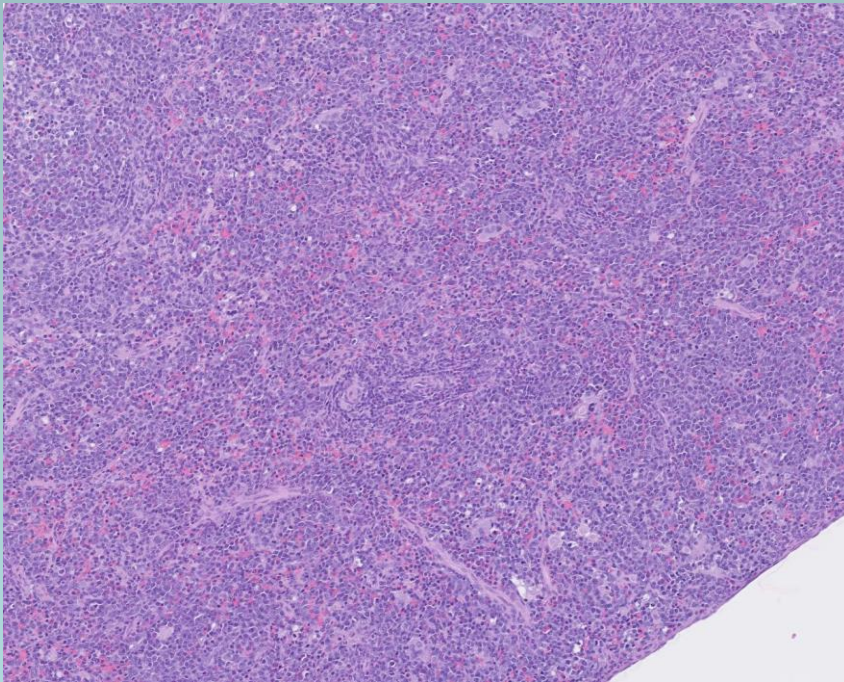


Ctrl PND42 5x

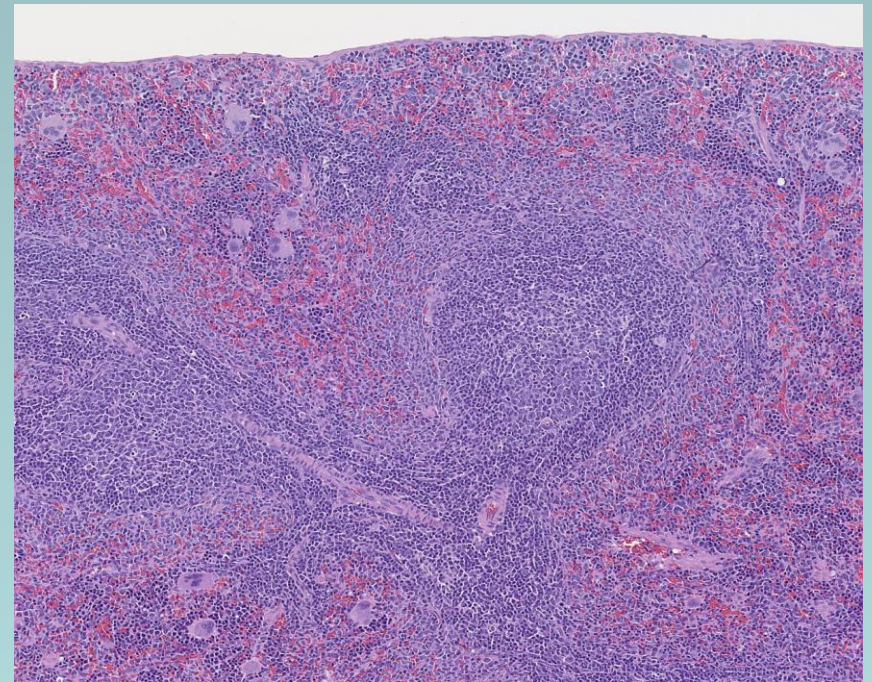


Spleen

4M FD PND12

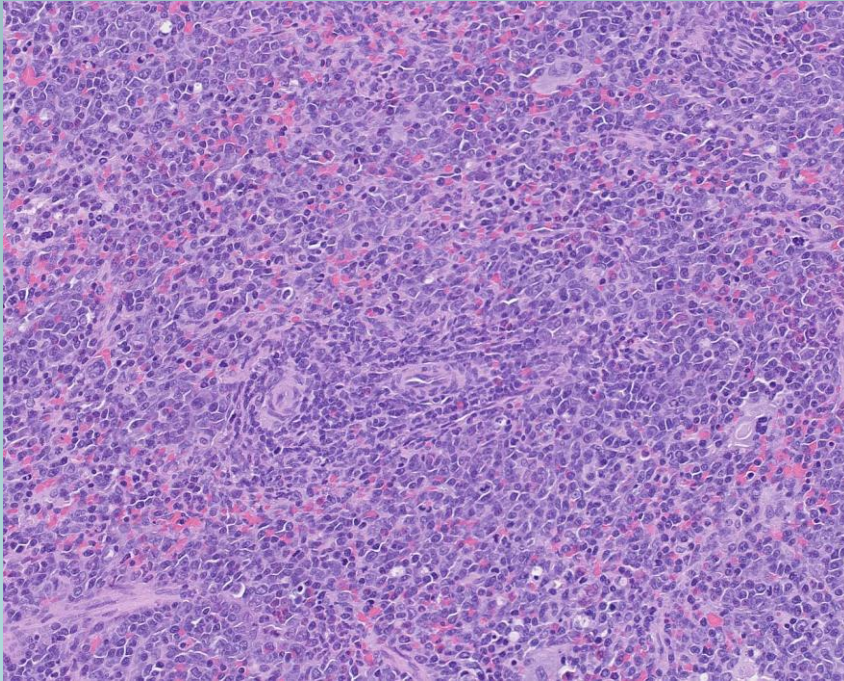


Ctrl rat PND42

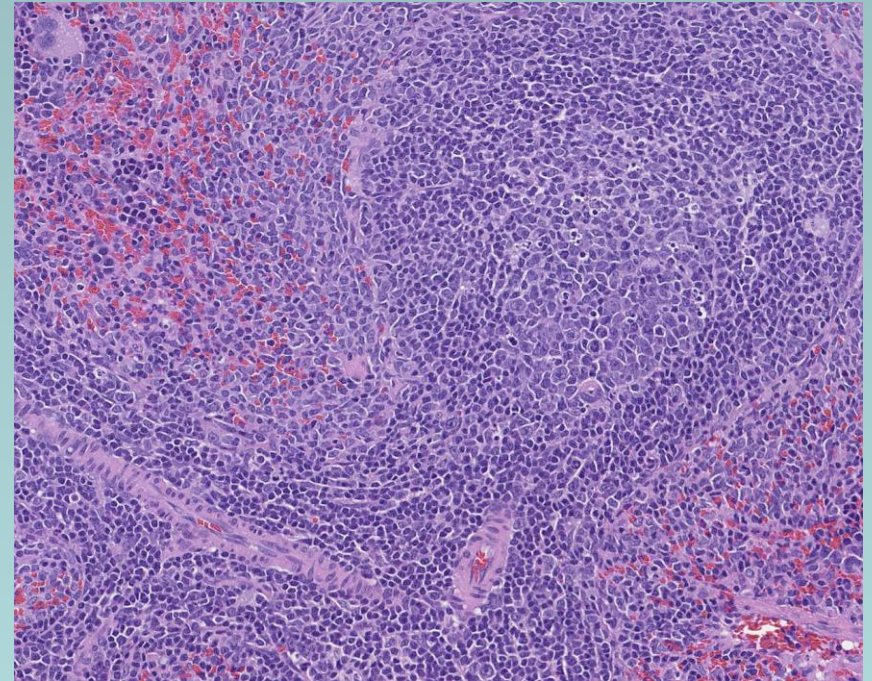


Spleen

4M FD PND12

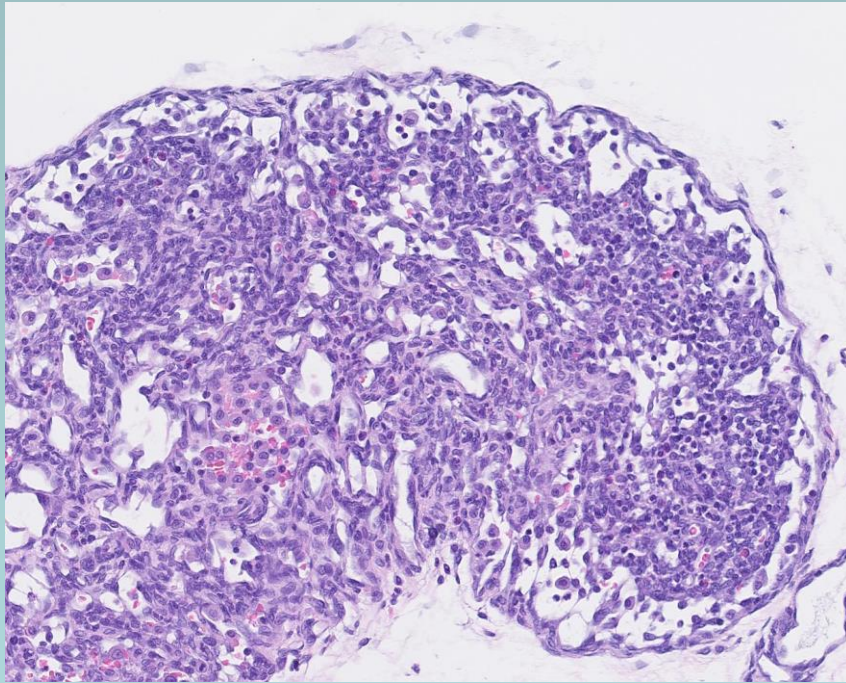


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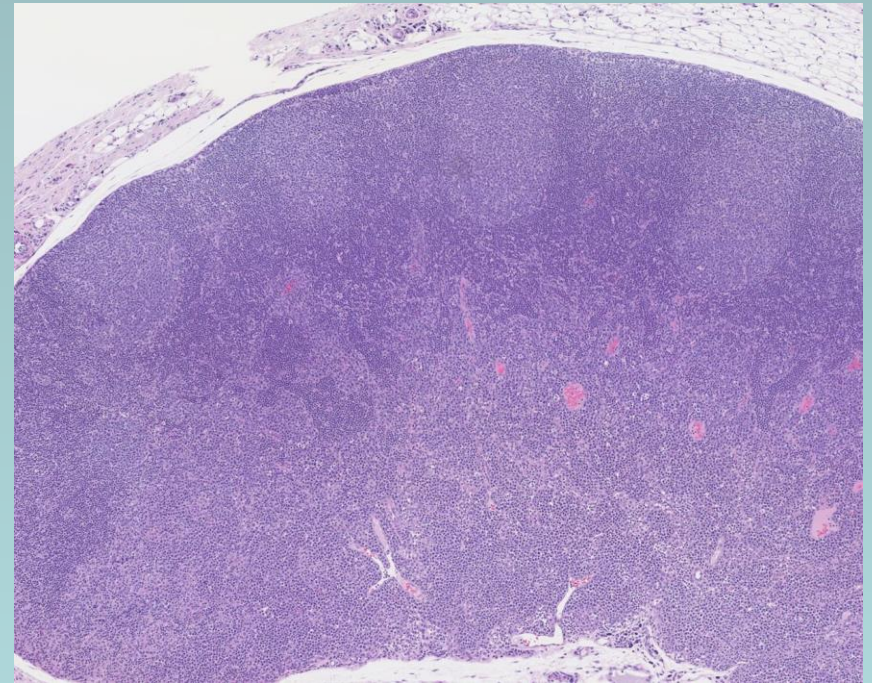


Mandibular lymph node

4M FD PND12

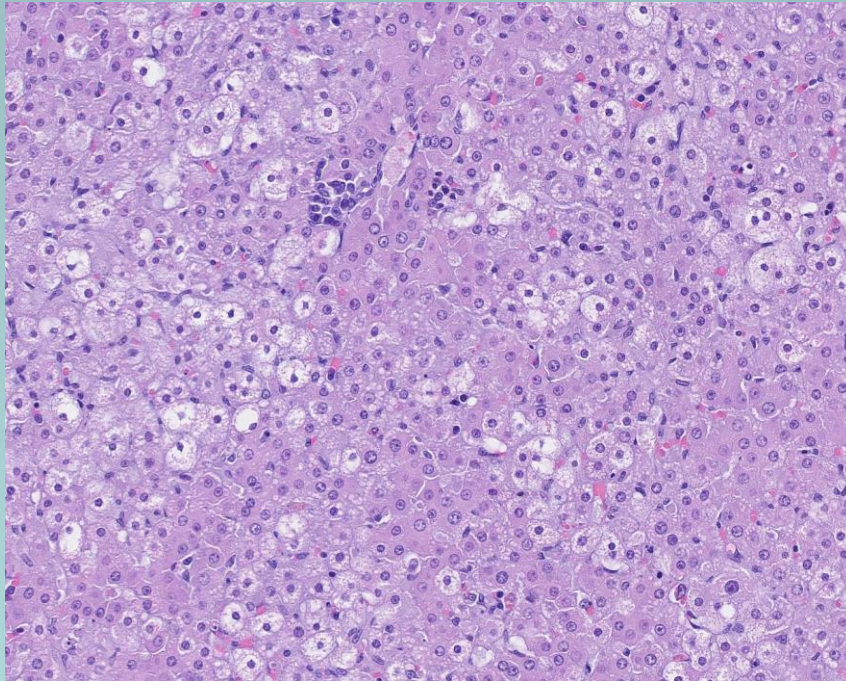


Ctrl rat PND42

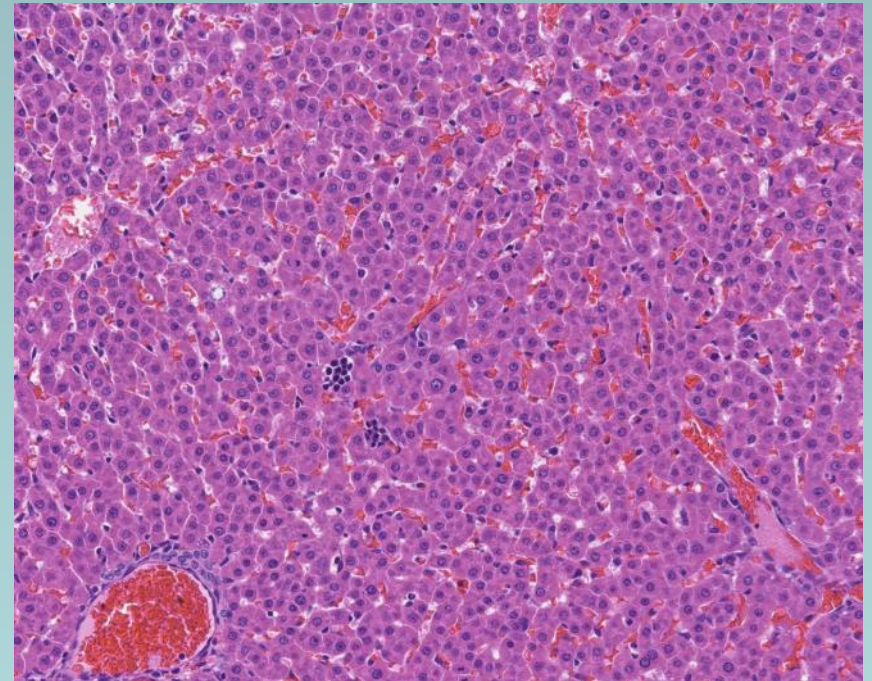


Liver

4M FD PND12



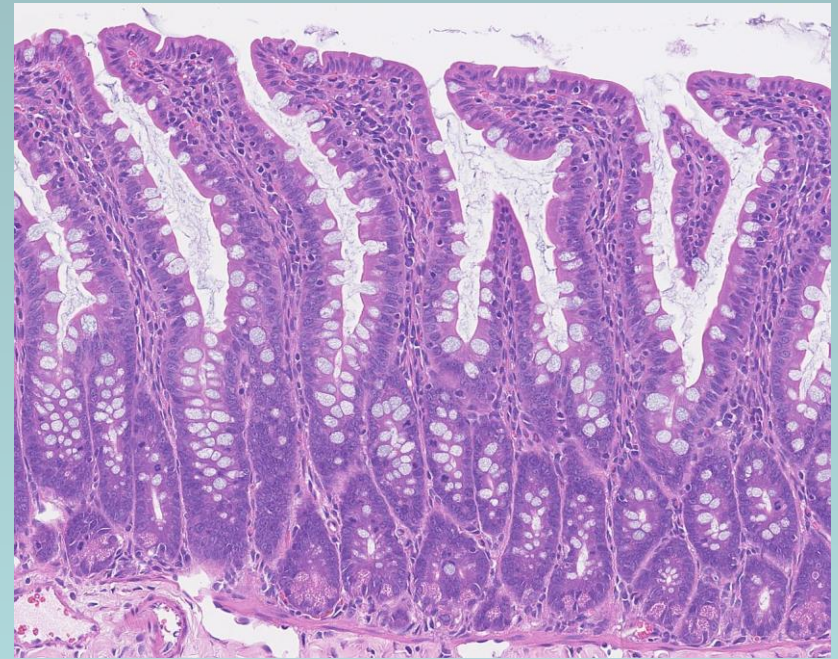
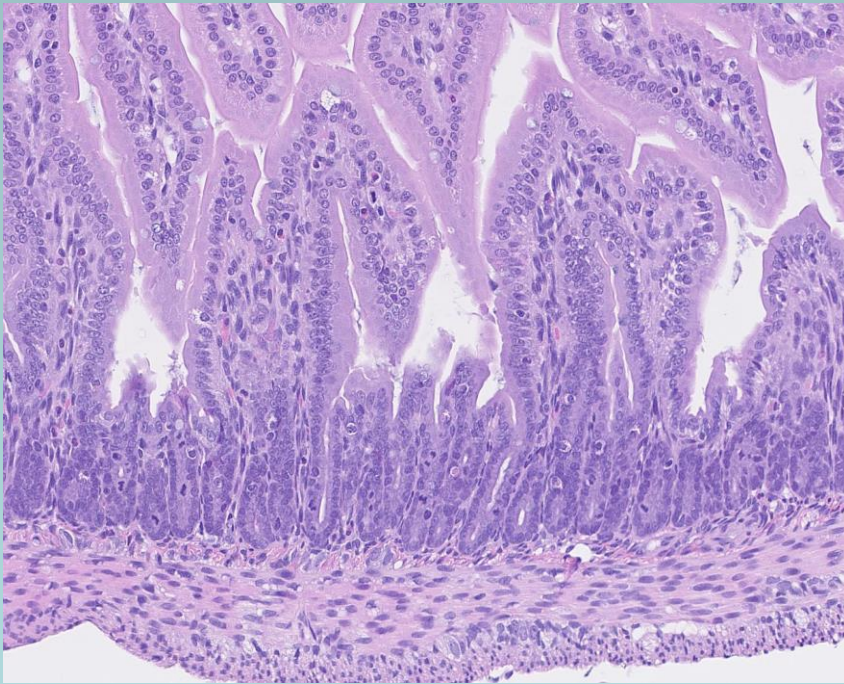
Ctrl rat PND42



Small intestine

4M UD PND16

Ctrl PND42



Postnatal development in the rat

TABLE 1 Postnatal Developmental Stages in Rat and Human

Stage Name in Rat/Human	Rat (Male)	Rat (Female)	Human
Neonatal/newborn	0–7 days	0–7 days	0–28 days
Infantile/infant	8–20 days	8–20 days	1–23 months
Juvenile/child	21–32 days	21–32 days	2–12 years
Peripubertal/adolescent	33–55 days	33–37 days	12–16 years
Late puberty/adolescent	56–70 days	38–46 days	

Adapted from Barrow (2011), Ojeda and Skinner (2006).

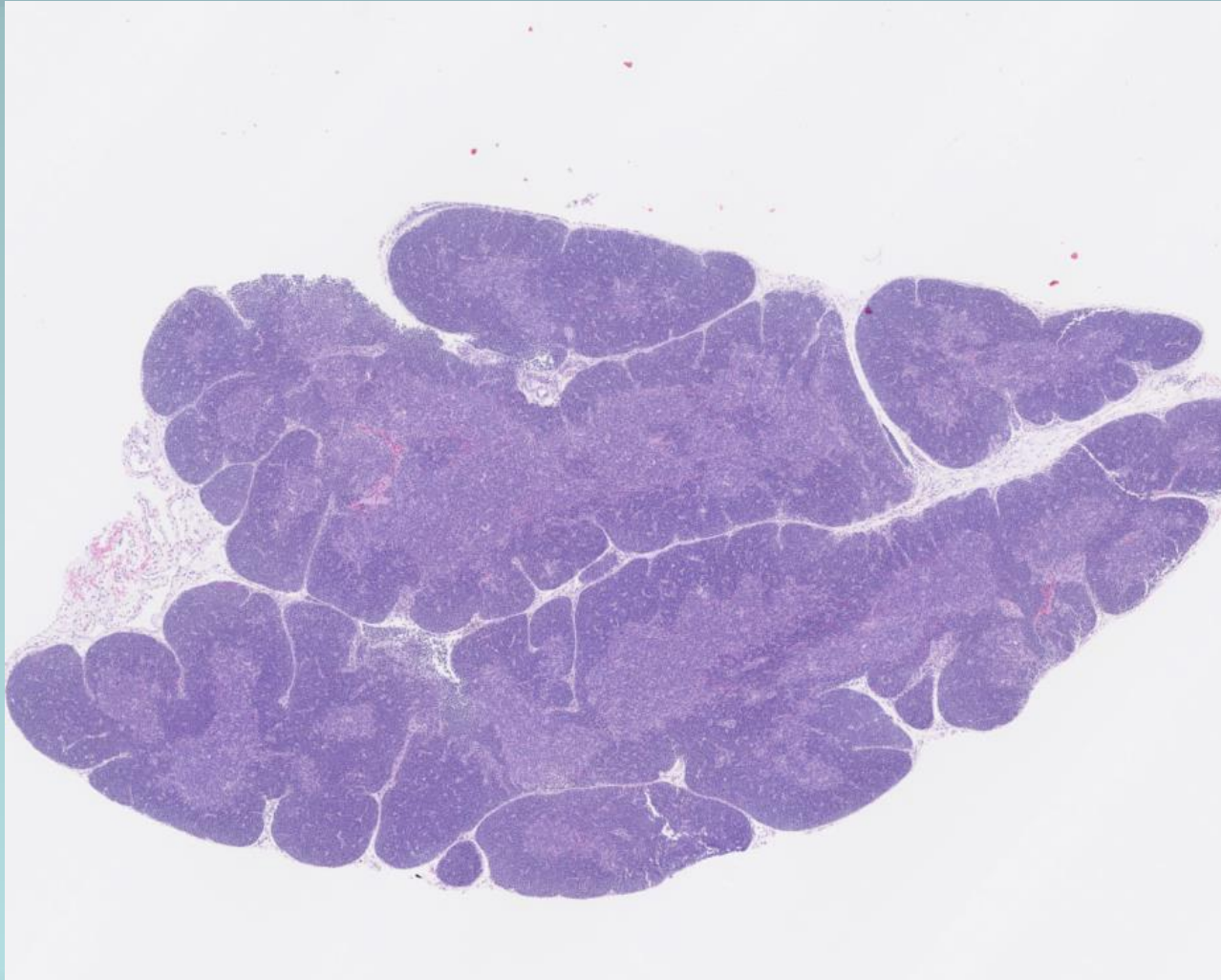
Examples of histogenesis in rat

- **Thymus**
- **Spleen**
- **Lymph node**
- **GI tract**
- **Liver**
- **Bone marrow**
- **Kidney**

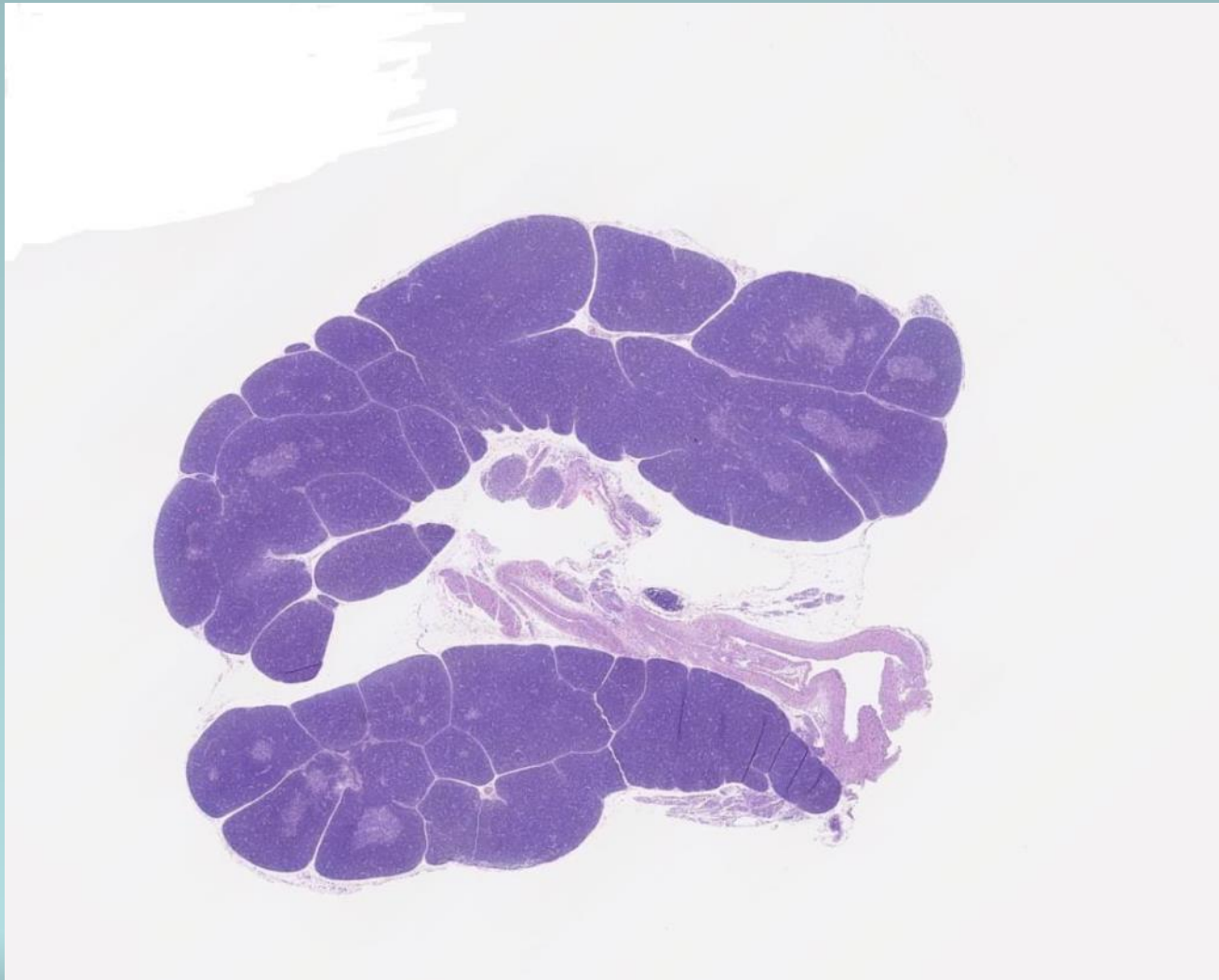
Thymus

- **Primary site to T-cell development, selection, and maturation.**
- **Stroma develops from epithelial cells**
- **In rat, Hassal's corpuscles are rare**

Rat thymus-PND0



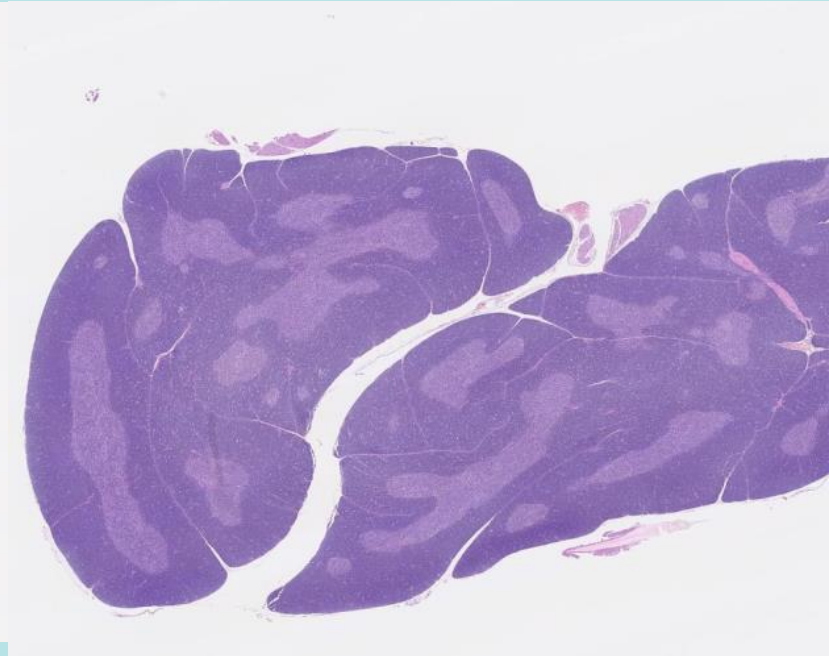
Rat thymus-PND7



Rat thymus-PND14

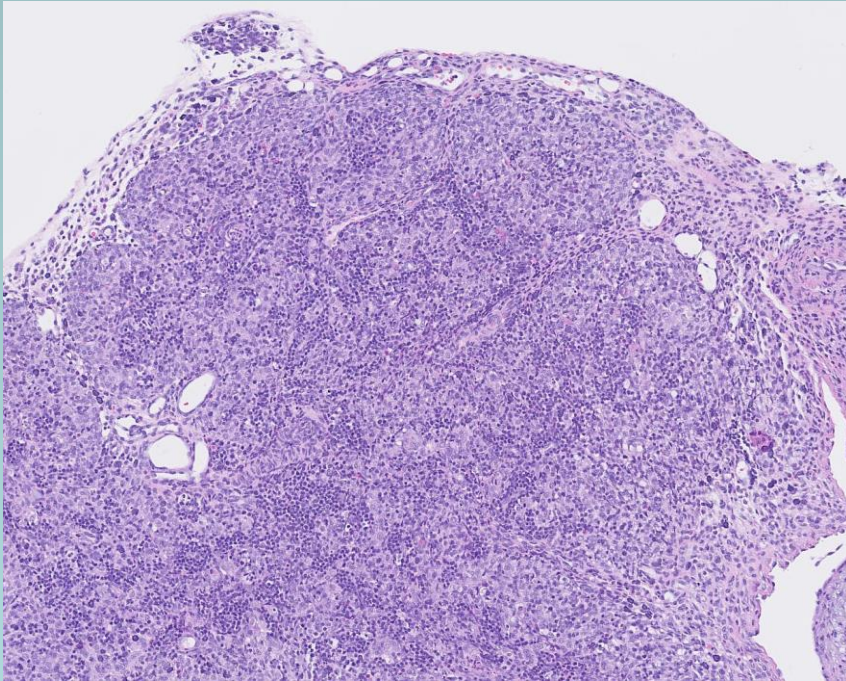


Rat thymus-PND42

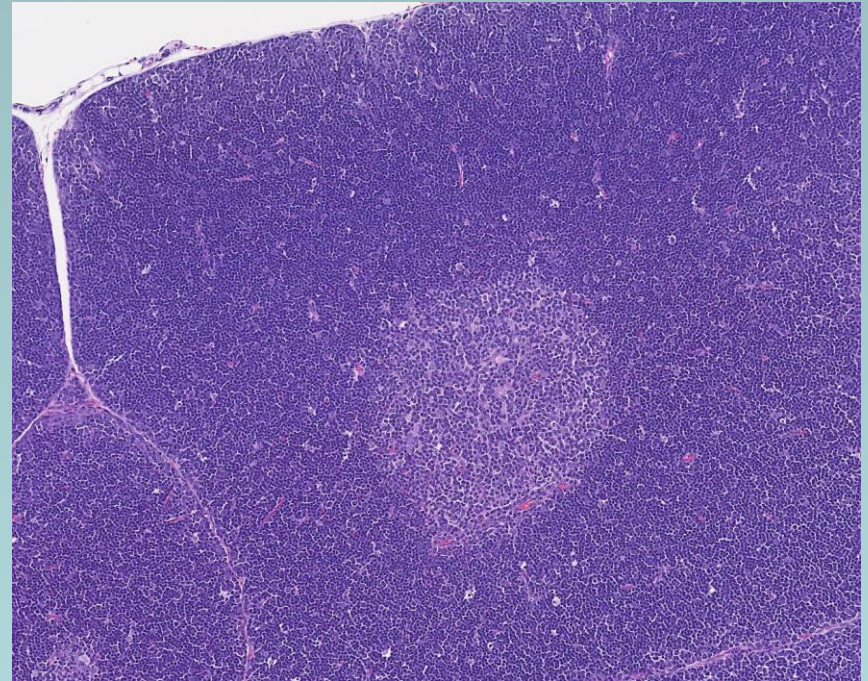


Rat thymus

4M FD PND12



Ctrl PND14



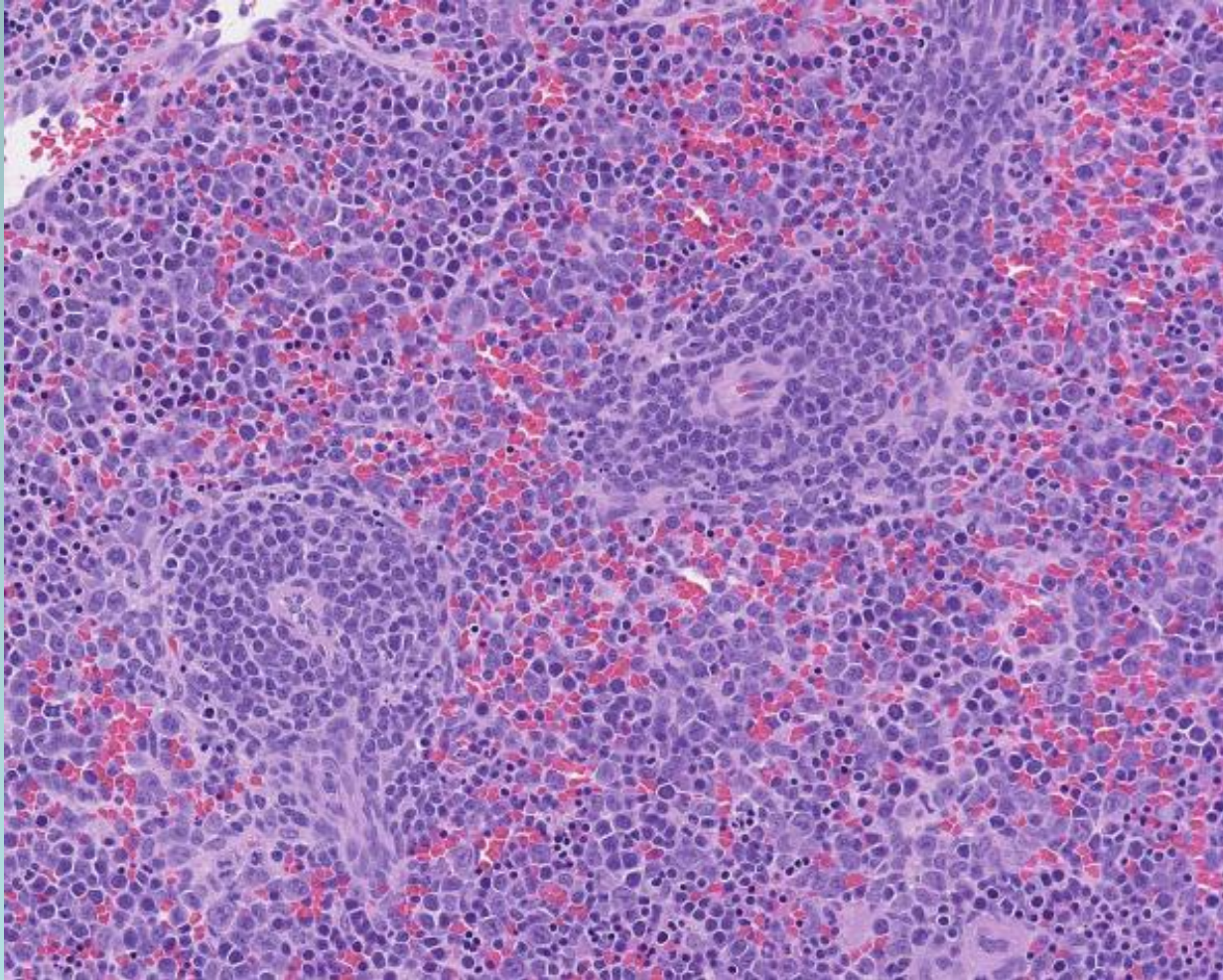
Spleen

- **Functions in immunology (white pulp) and blood storage (red pulp)**
- **White pulp consists of periarteriolar lymphoid sheaths (PALS), follicles, and marginal zones.**
- **PALS- T-cells; Follicles – B-cells; MZ- B-cells and macrophages**

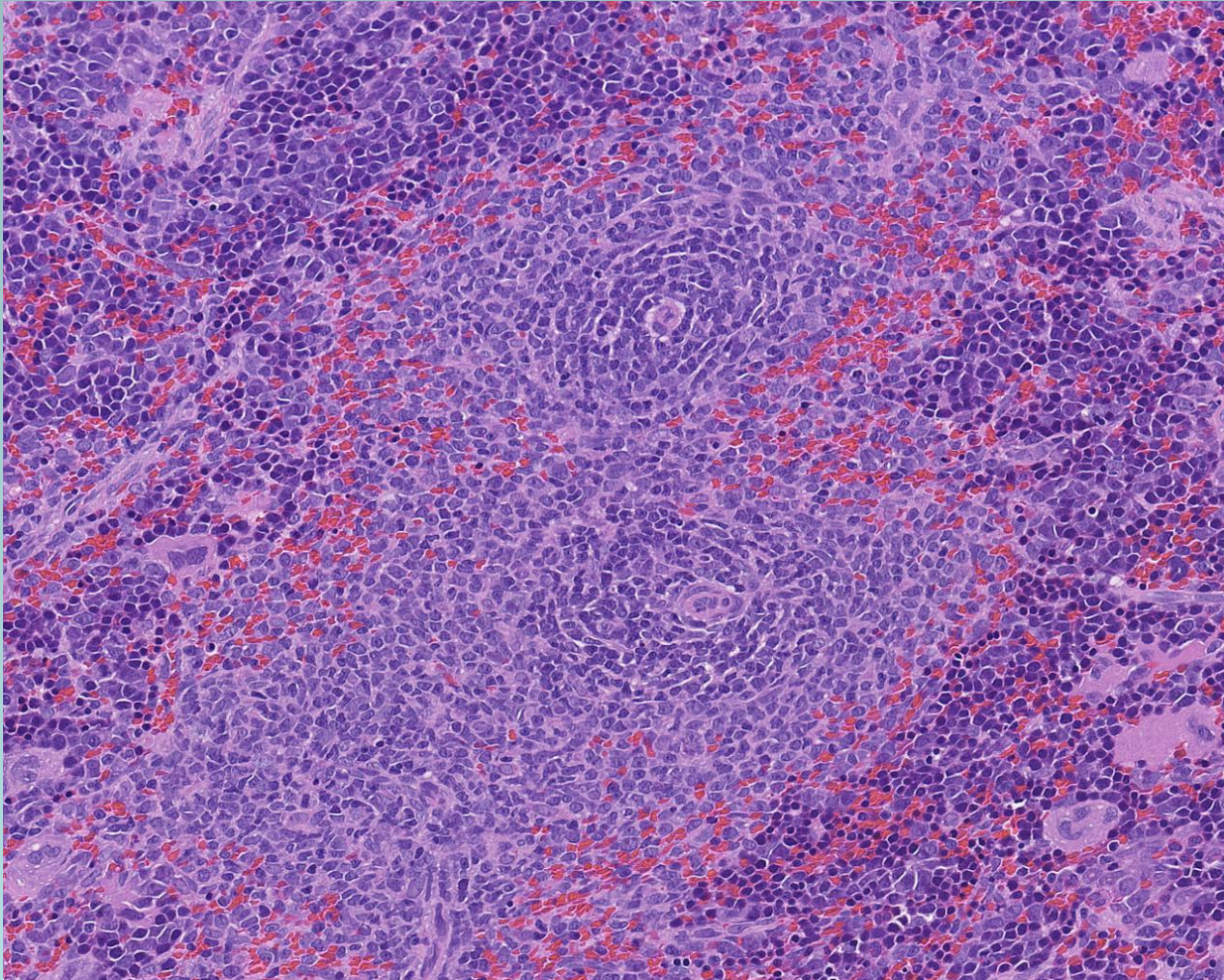
Spleen – red pulp

- Hematopoiesis in rat PND 0 mostly in liver and spleen
- PND 7 femur hematopoiesis dramatically increases
- Splenic EMH continues to PND 14 and gradually decreases to adult levels
- PND 0 – erythroid and myeloid precursors
- PND 7 – megakaryocytes become prominent

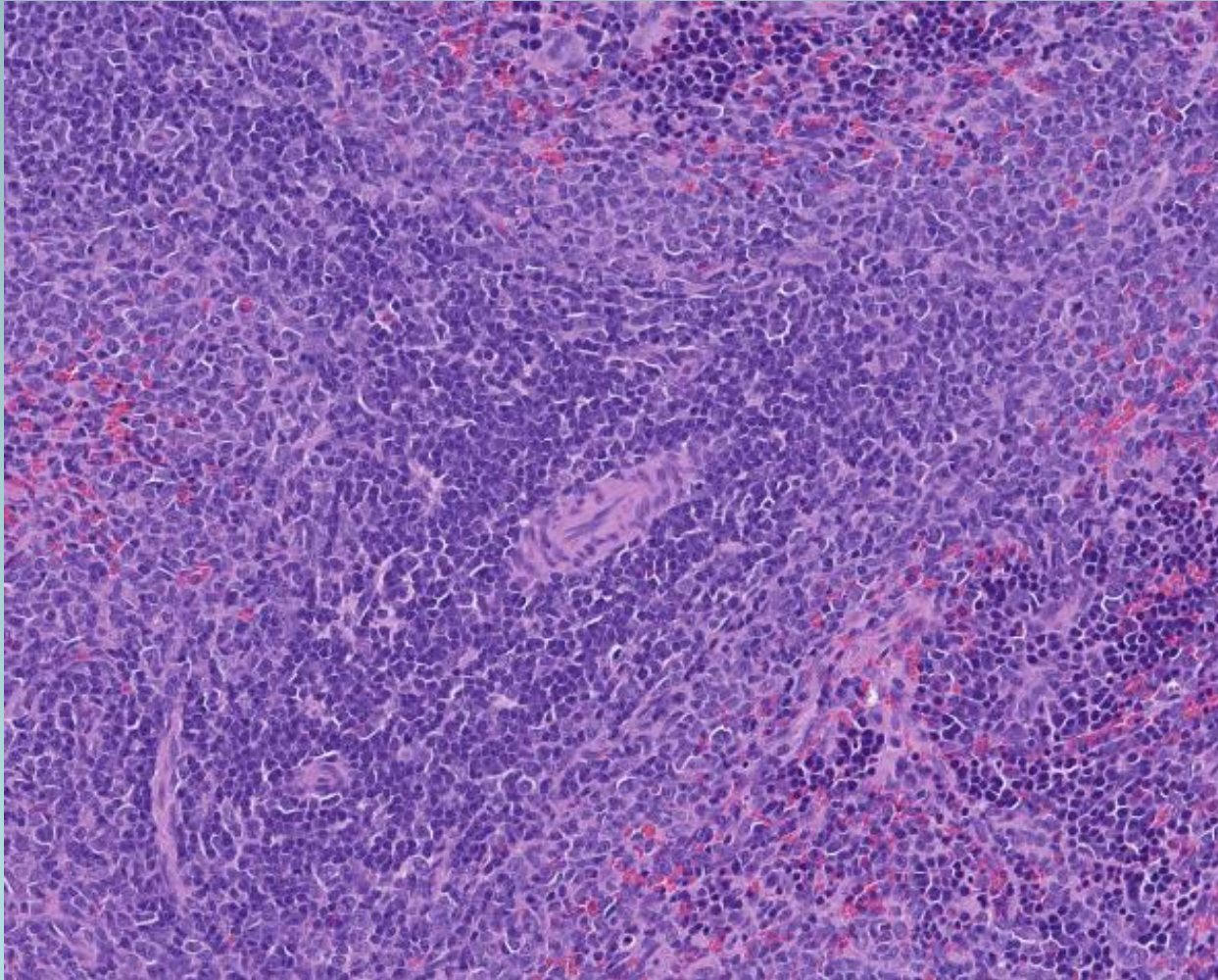
Rat spleen-PND0



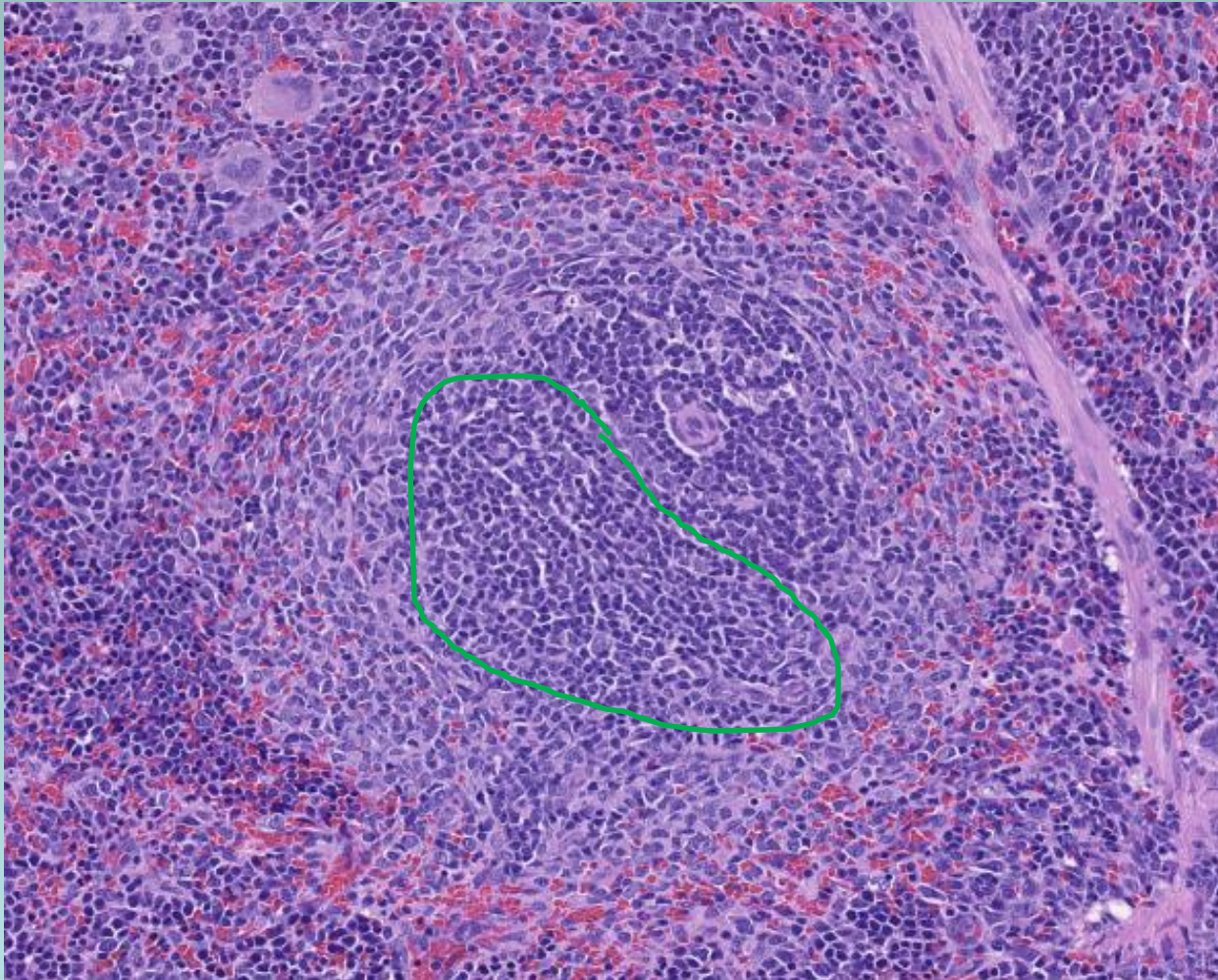
Rat spleen-PND7



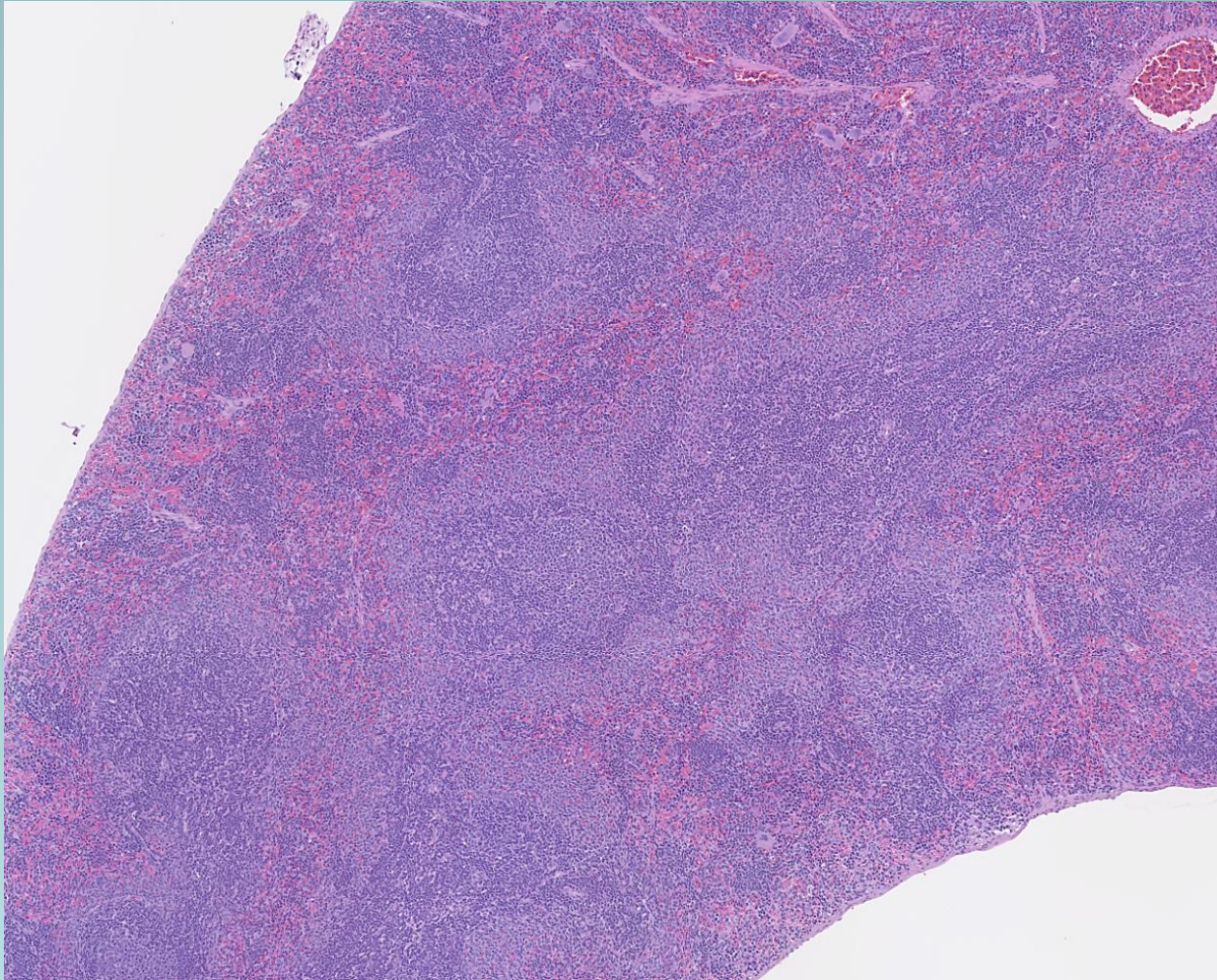
Rat spleen-PND14



Rat spleen-PND28

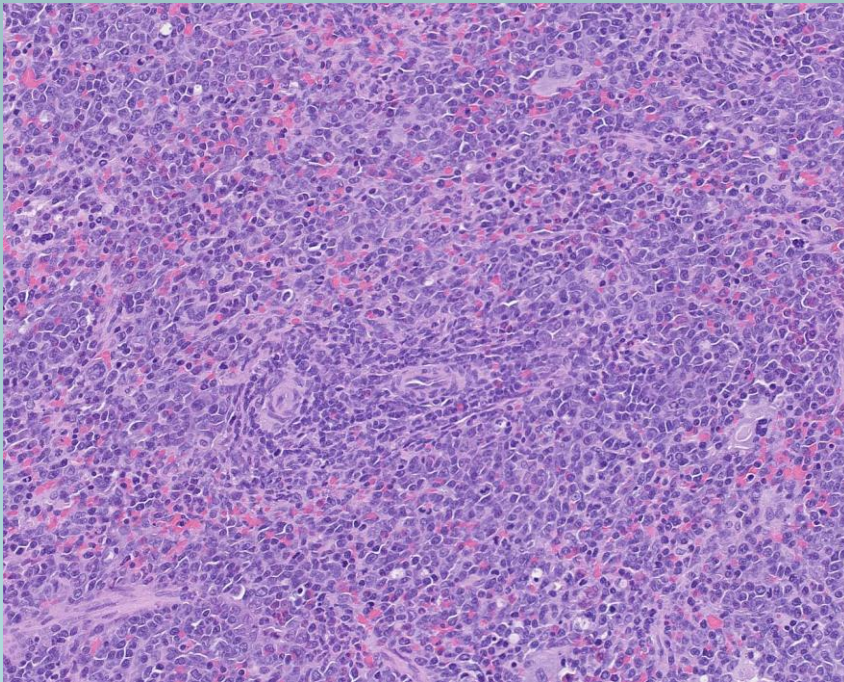


Rat spleen-PND35

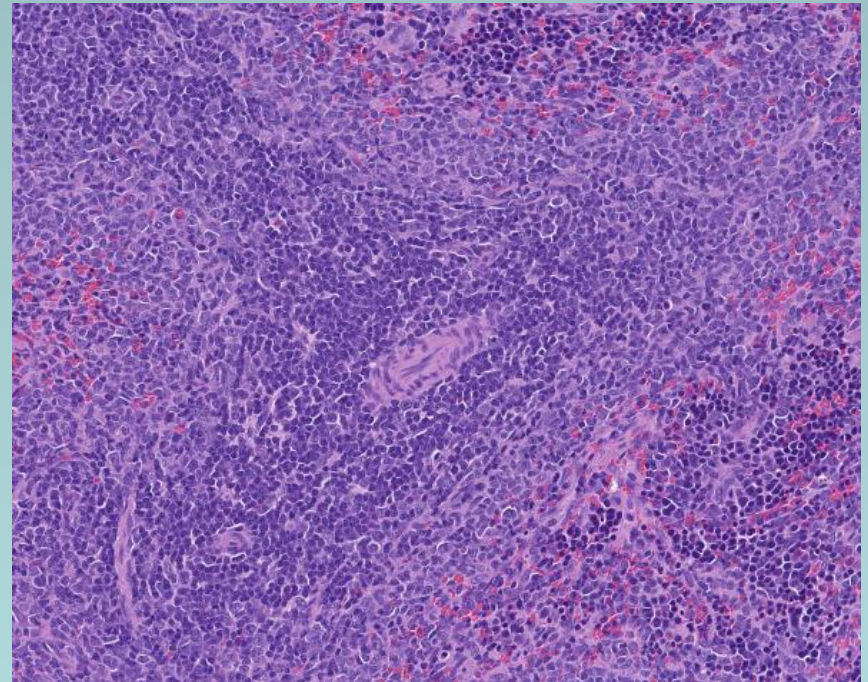


Spleen

4M PND 12 FD



Ctrl PND14



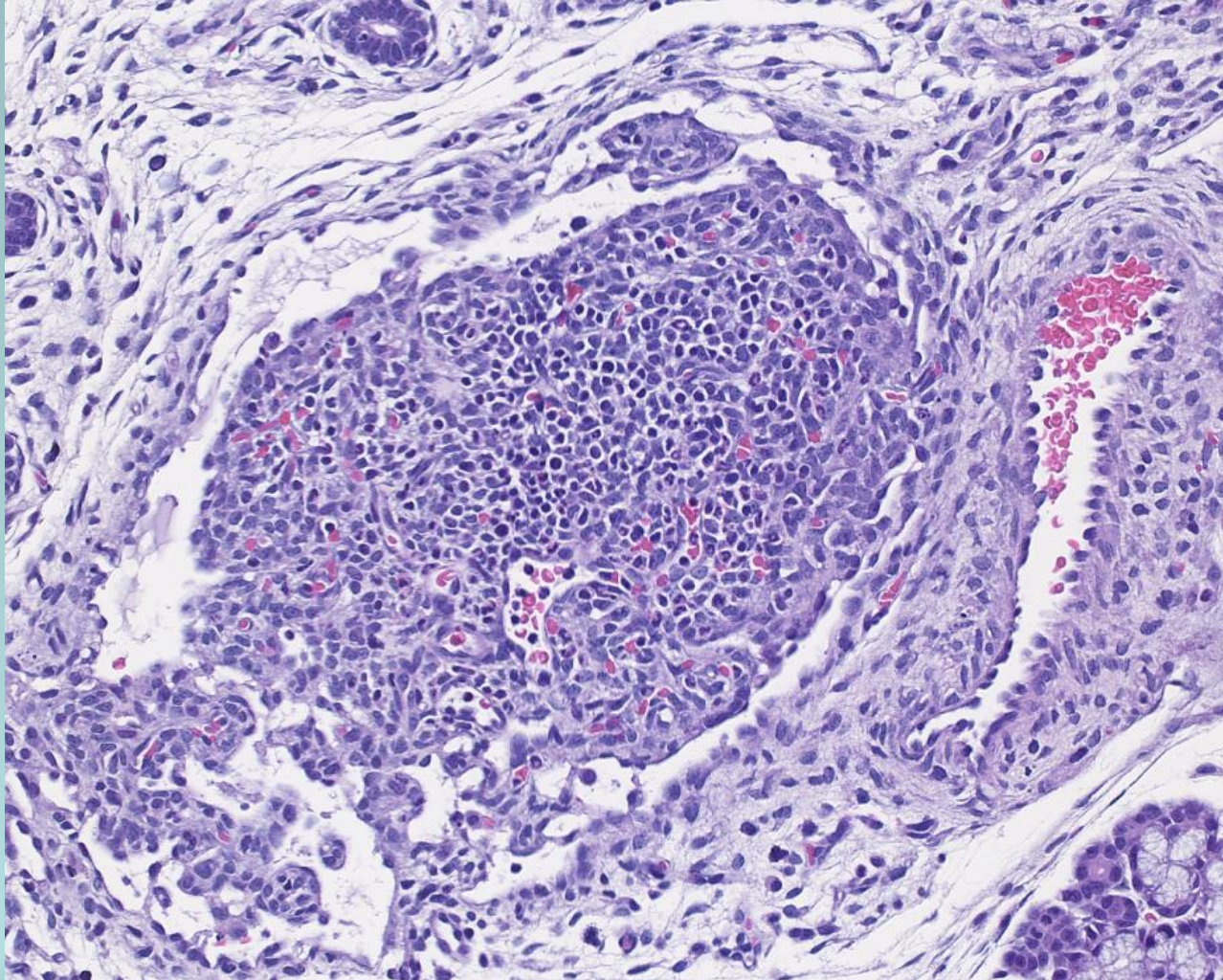
Lymph node

- **Lymph node development depends on antigenic stimulation, which depends on location**
 - Mesenteric LN mature by PND 28
 - Mandibular LN mature by PND 35
 - Axillary LN mature at PND 42
- **Postnatal development occurs later than thymus or bone marrow**

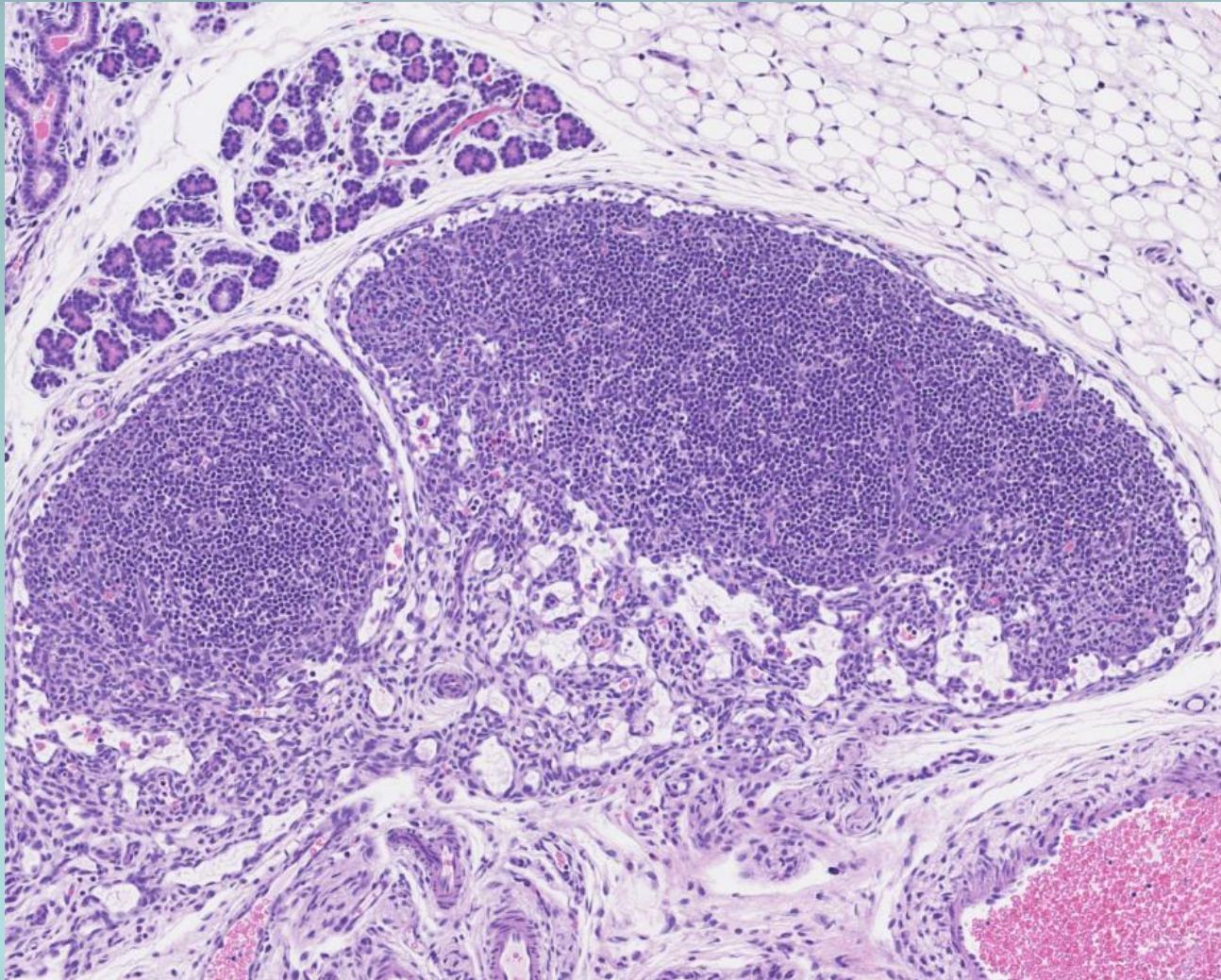
Lymph node

- **PND 0, LN are poorly developed**
 - No follicles, germinal centers, paracortex or plasma cell accumulations
- **By PND 42, most lymph nodes have follicles, germinal centers**

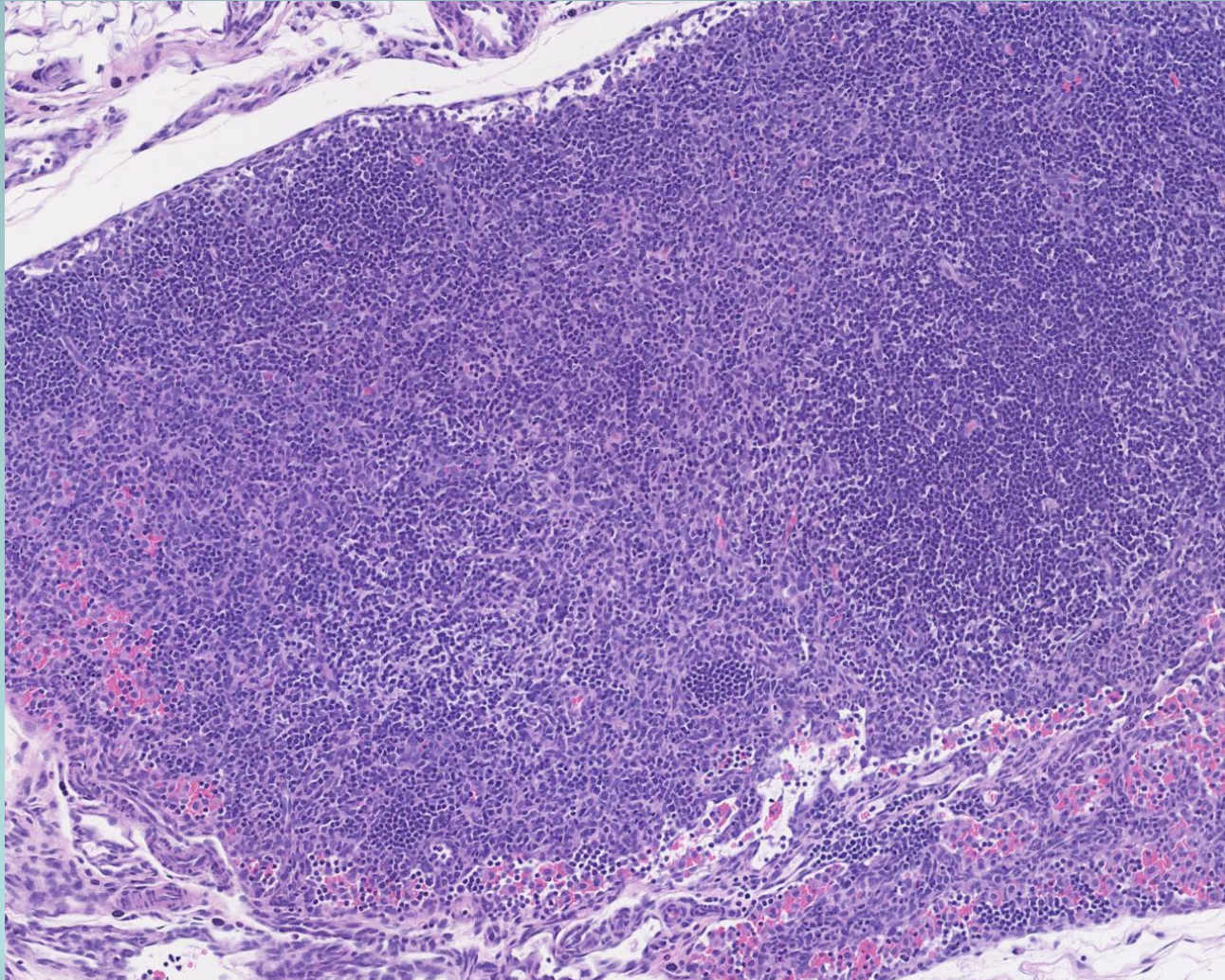
Rat mandibular lymph node-PND0



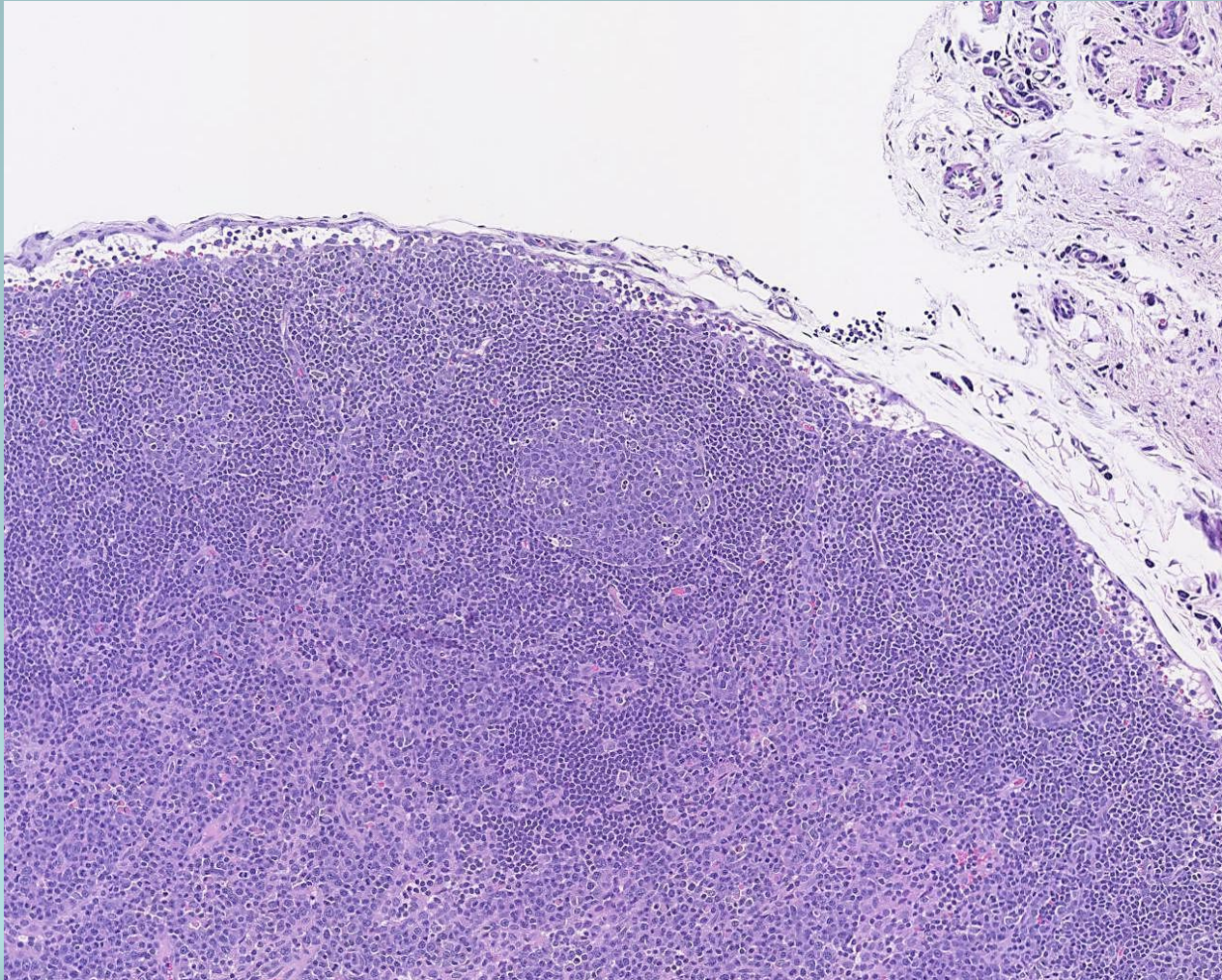
Rat mandibular lymph node-PND7



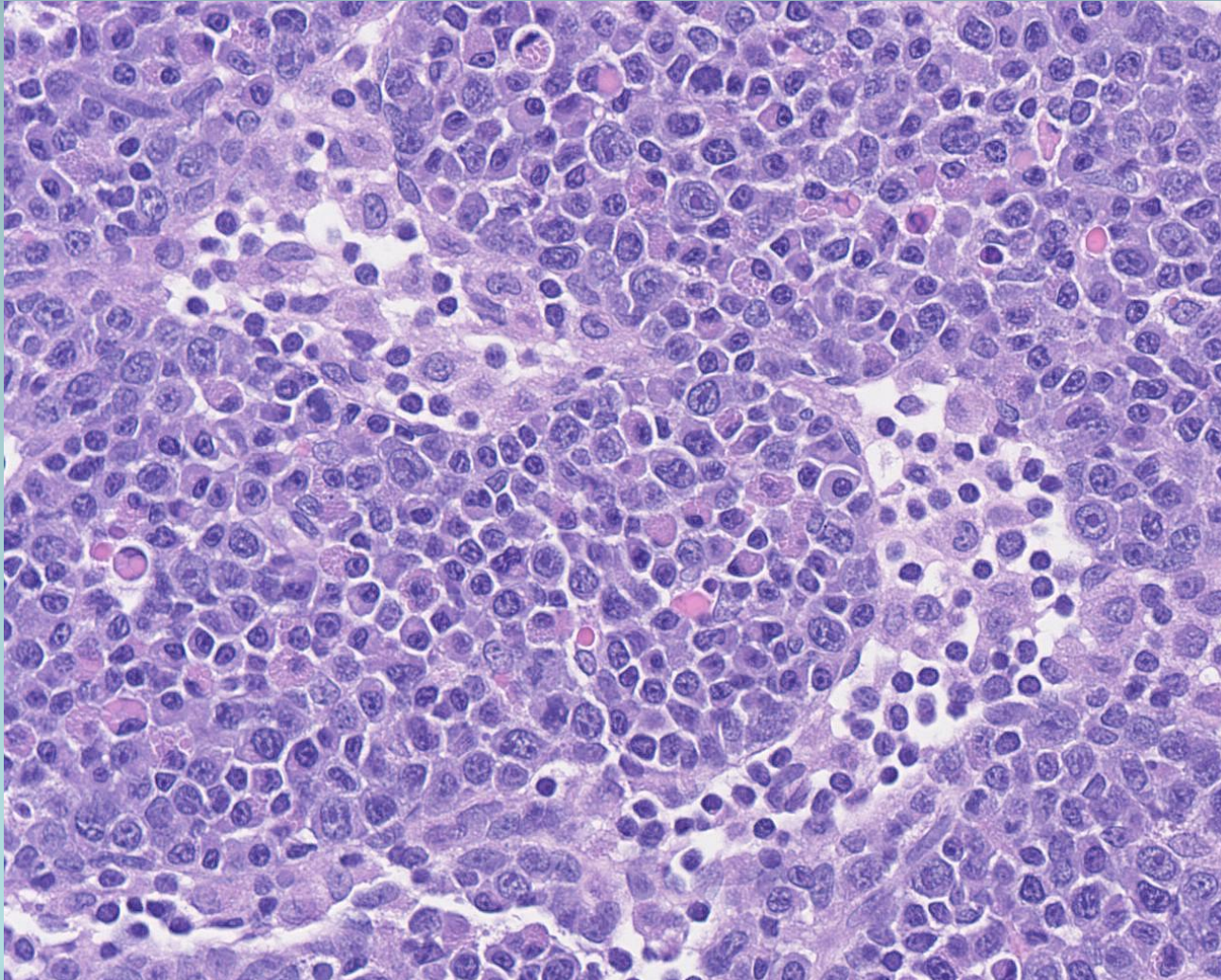
Rat mandibular lymph node- PND14



Rat mandibular lymph node- PND35

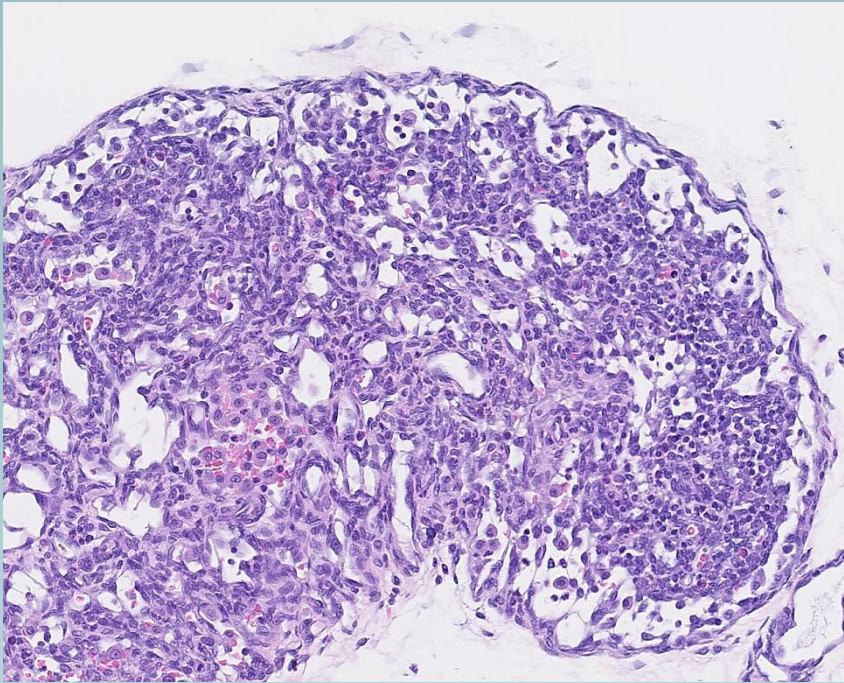


Rat mandibular lymph node- PND35

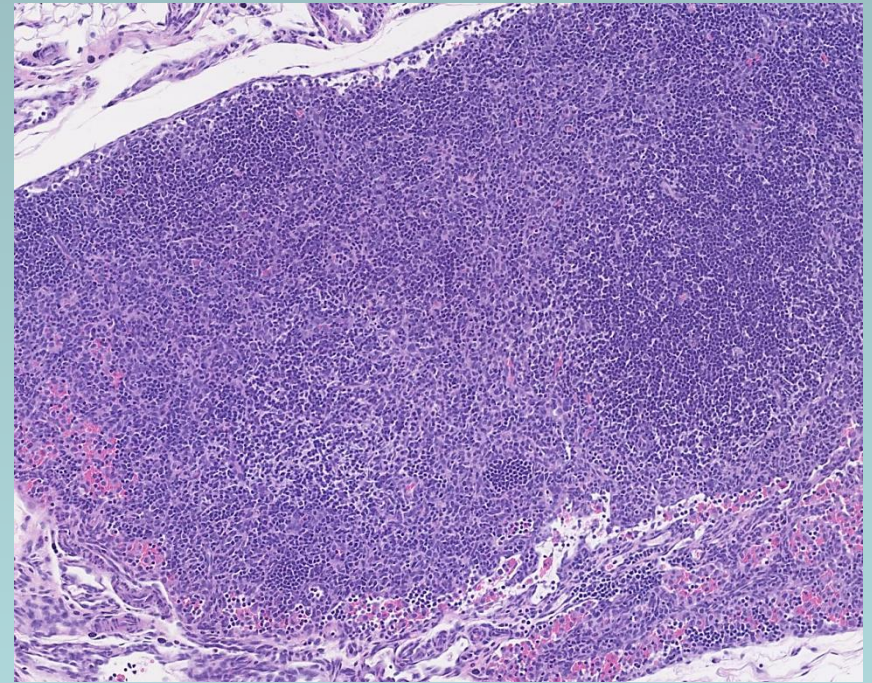


Mandibular lymph node

4M PND12 FD 20x



Ctrl PND14 10x



Gastrointestinal tract

- **Proximal portions (tongue, esophagus, nonglandular stomach) mature faster – PND 21 compared to more distal segments – PND 28**
- **Proximal portions functional at birth**

Gastrointestinal tract

- **Many factors influence development**
 - Glucocorticoids and thyroxine most important
- **Pups ingest milk for 2 weeks**
- **PND 16 begin solid matter**
- **PND 21 weaned**
- **Diet changes from high fat to low fat/high carb (rodent chow)**
 - Brush border enzymes in SI change from lactase to maltase/sucrase – (due to GC surge)
 - Occur even if weaning prevented – not influenced by environment

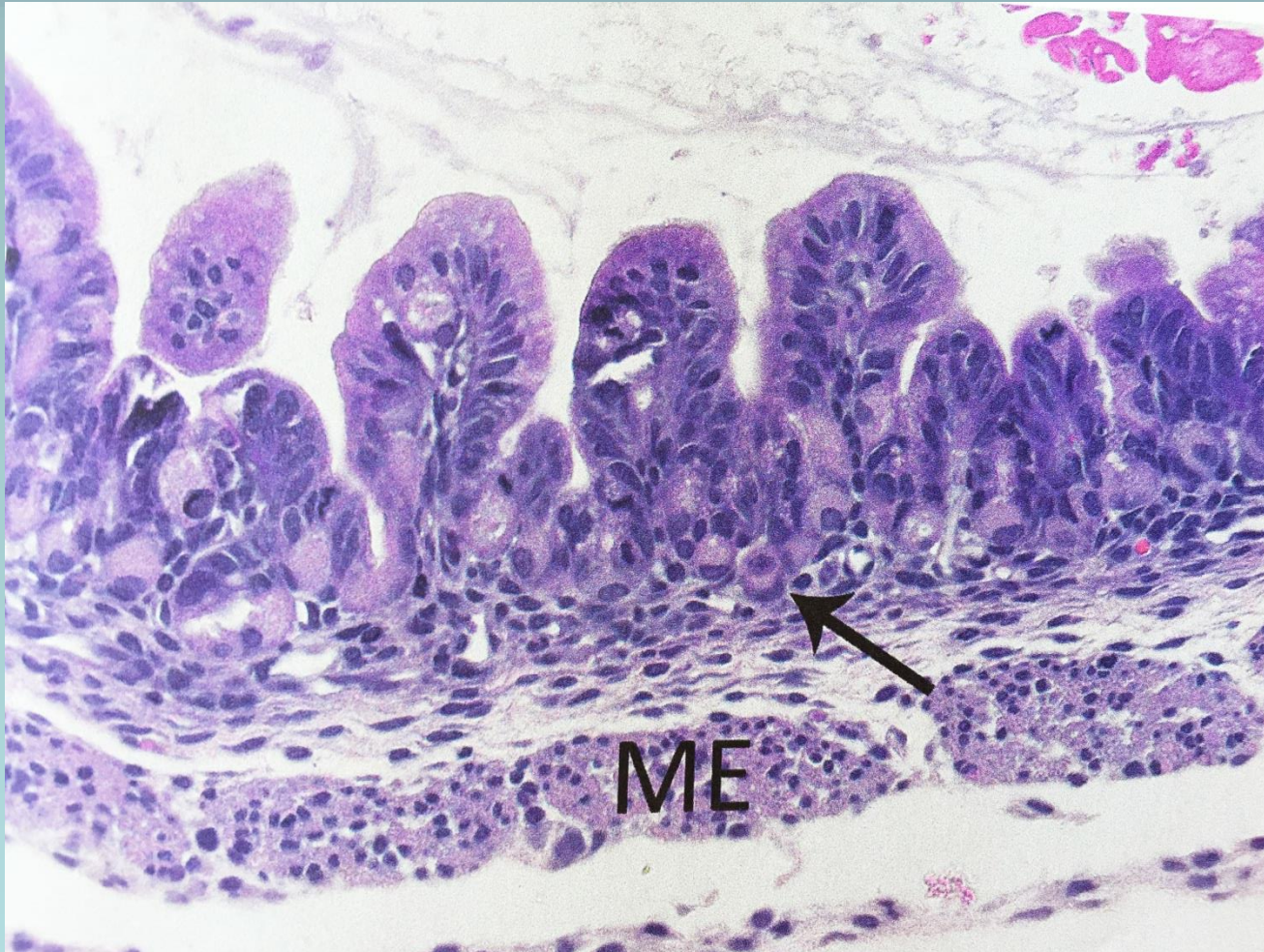
Noteworthy structures in GI tract

- **Transient villi in glandular stomach and colon**
 - Increase absorption
- **Vacuolated epithelium of small intestine**
 - Lipid/protein absorption
- **Glandular stomach and intestines deeply basophilic and appear hyperplastic**
 - Physiologic changes due to weaning

Stomach

- **Three phases**
 - Bland phase < 2 weeks
 - Transitional phase PND 14-20
 - Acidic phase – PND 21
- **Bland – no HCl or pepsinogen, colonized by bacteria**
- **Transitional – pH drops, parietal & chief cells appear**
- **Acidic – HCl/pepsinogen increase dramatically**

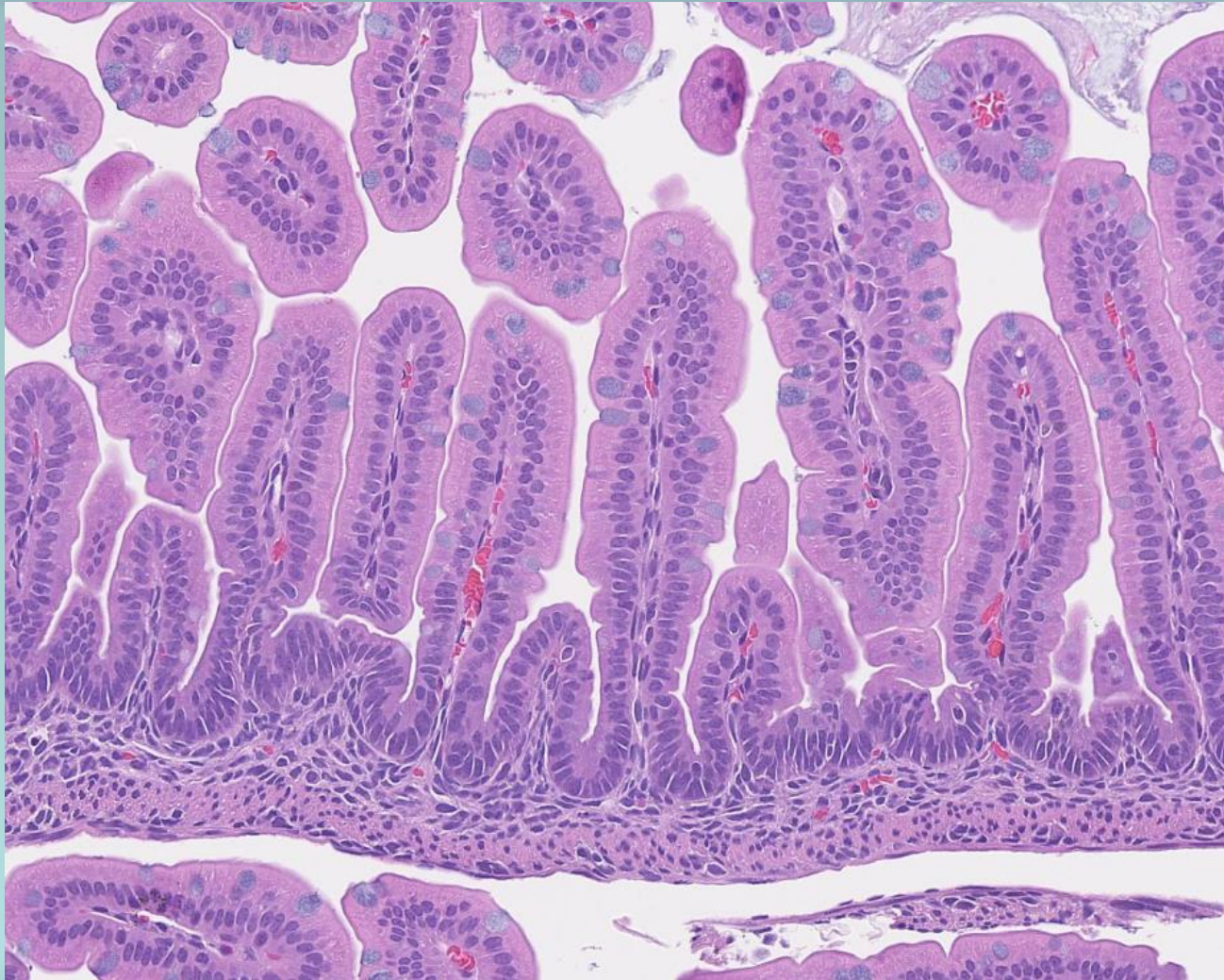
Stomach – distal fundus



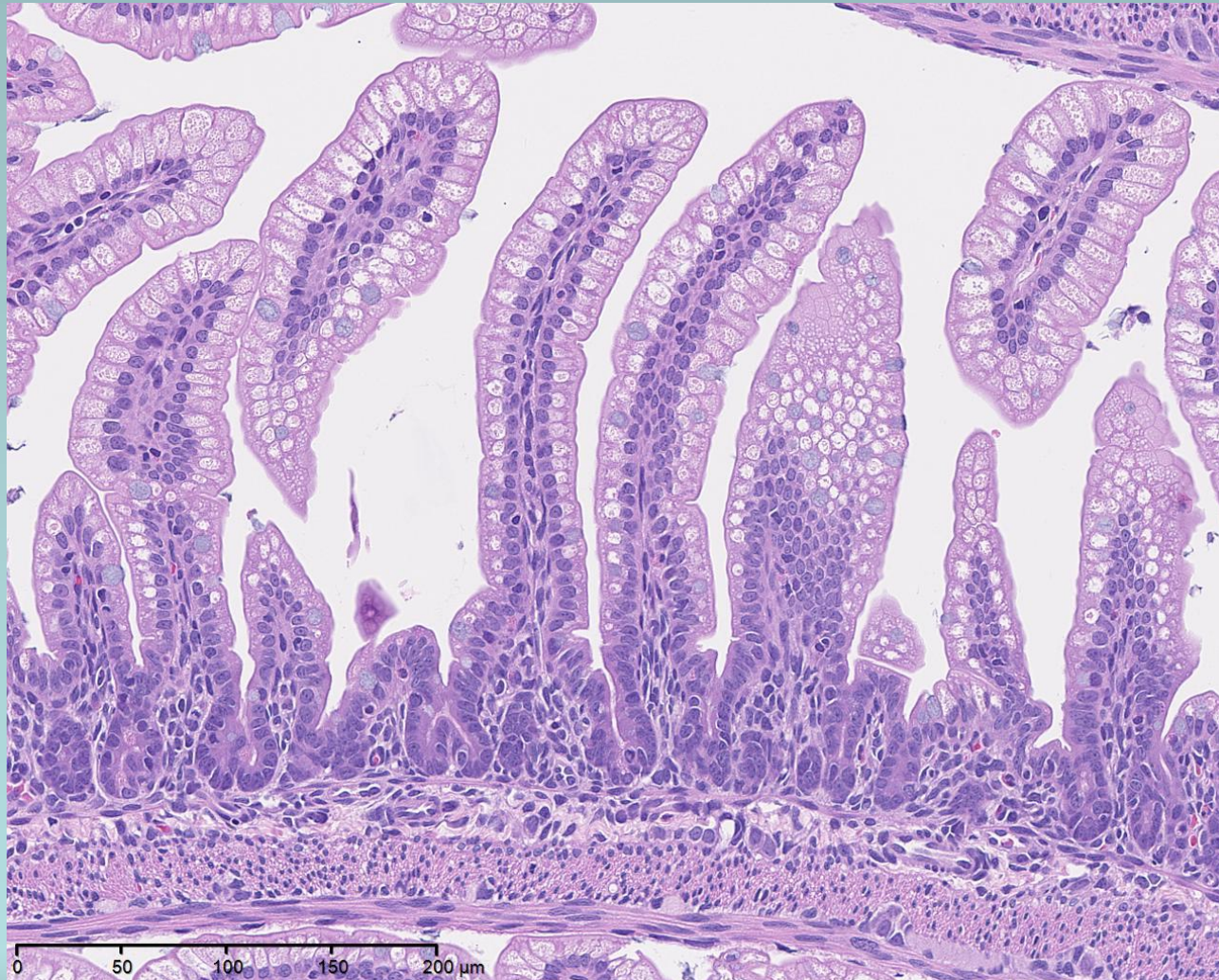
Small Intestine

- PND 0-14 – villi and crypt sizes don't change
- PND 15-21 – dramatic increase in height of villi and depth of crypts
- PND 21 – epithelium appears hyperplastic

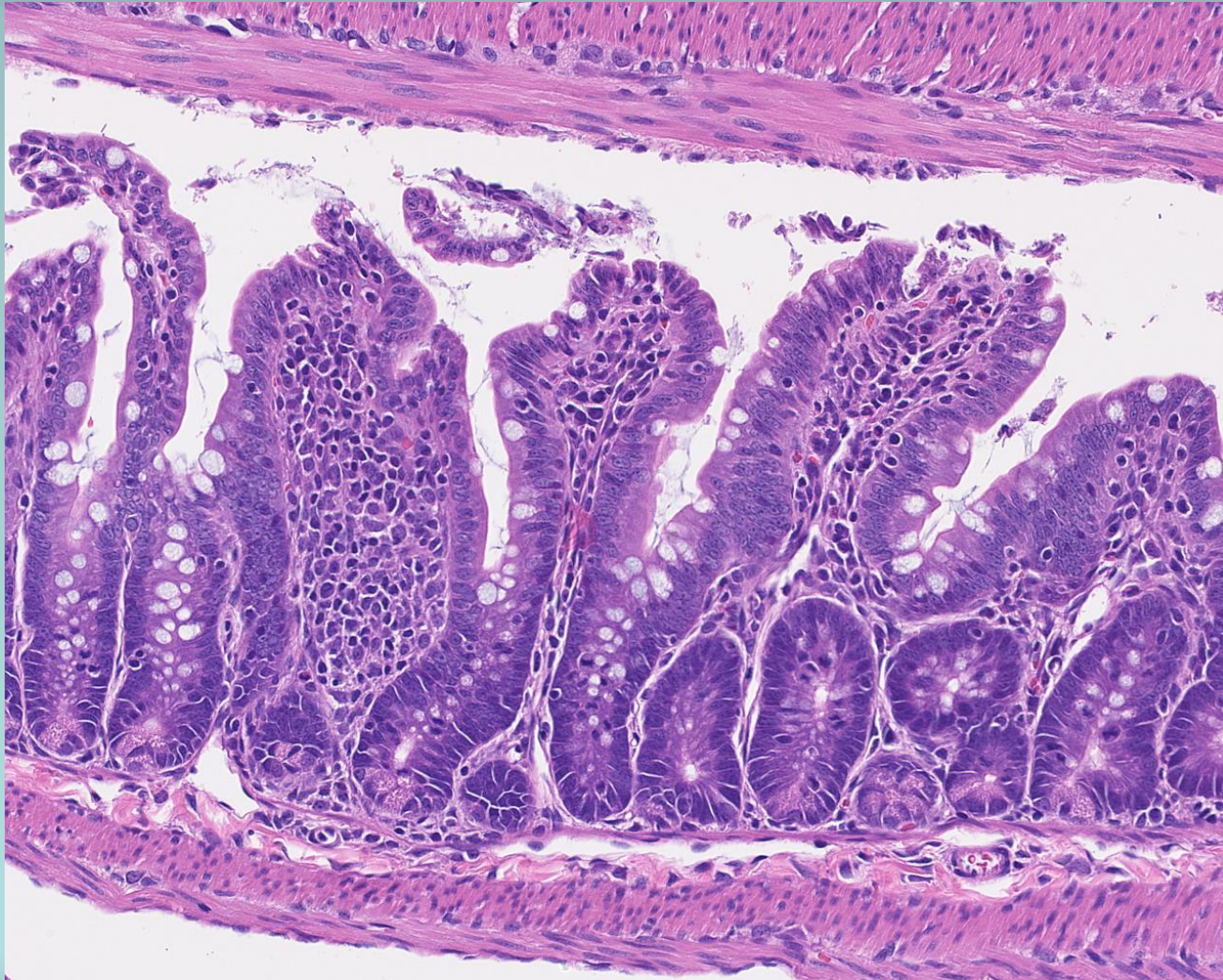
Ctrl rat small intestine-PND0



Ctrl rat small intestine-PND14



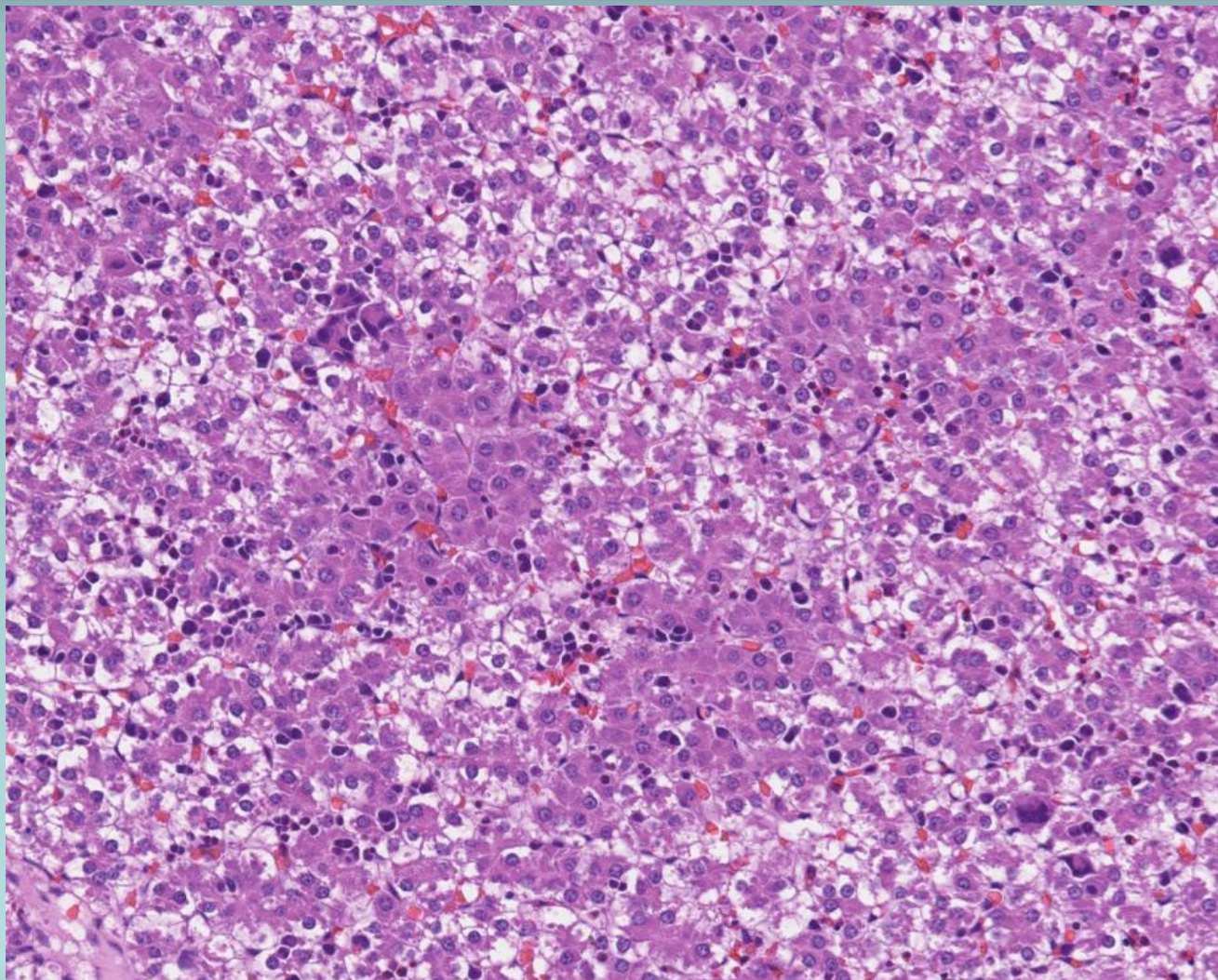
Ctrl rat small intestine-PND28



Liver

- **Hepatic plates loosely arranged at birth, gradually reduce to single cell thickness**
- **Glycogen levels high at birth, disappear in first day**
- **EMH present at birth until PND 7, then decreases rapidly. By PND 14 it is rare**

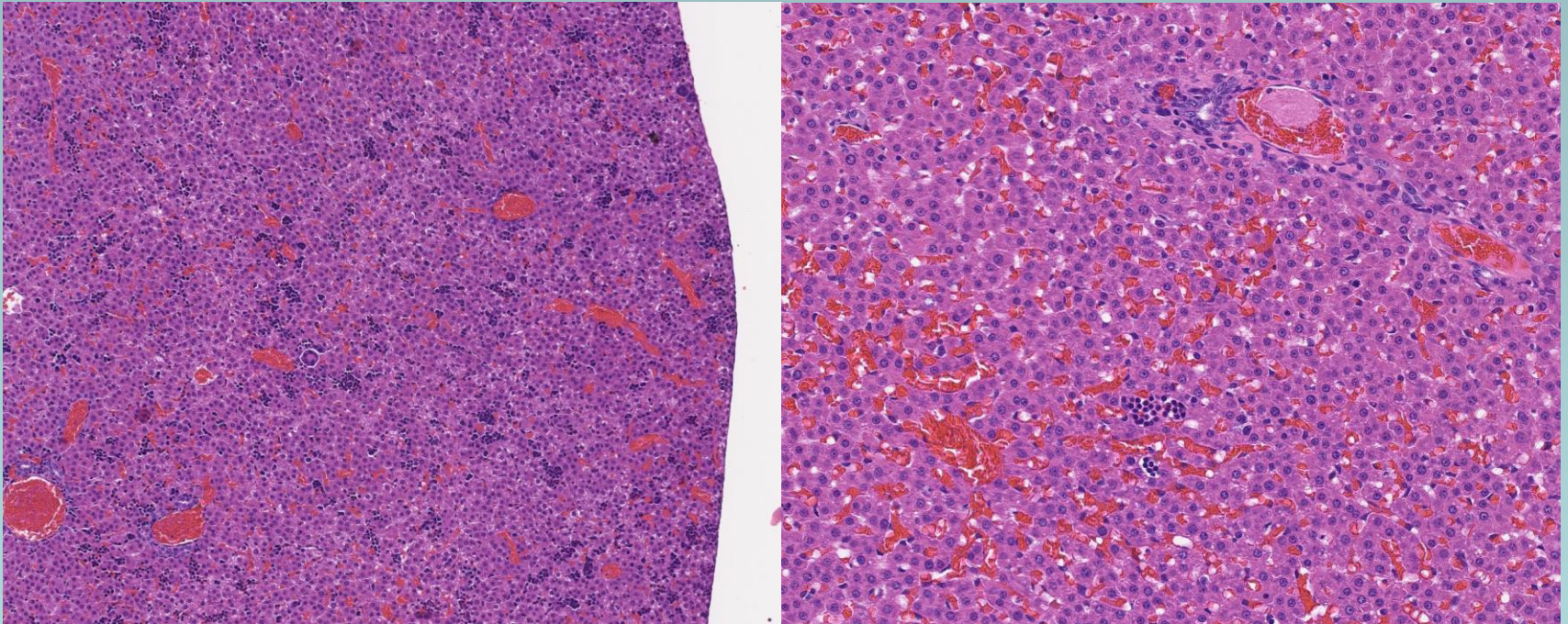
PND0 Liver-rat



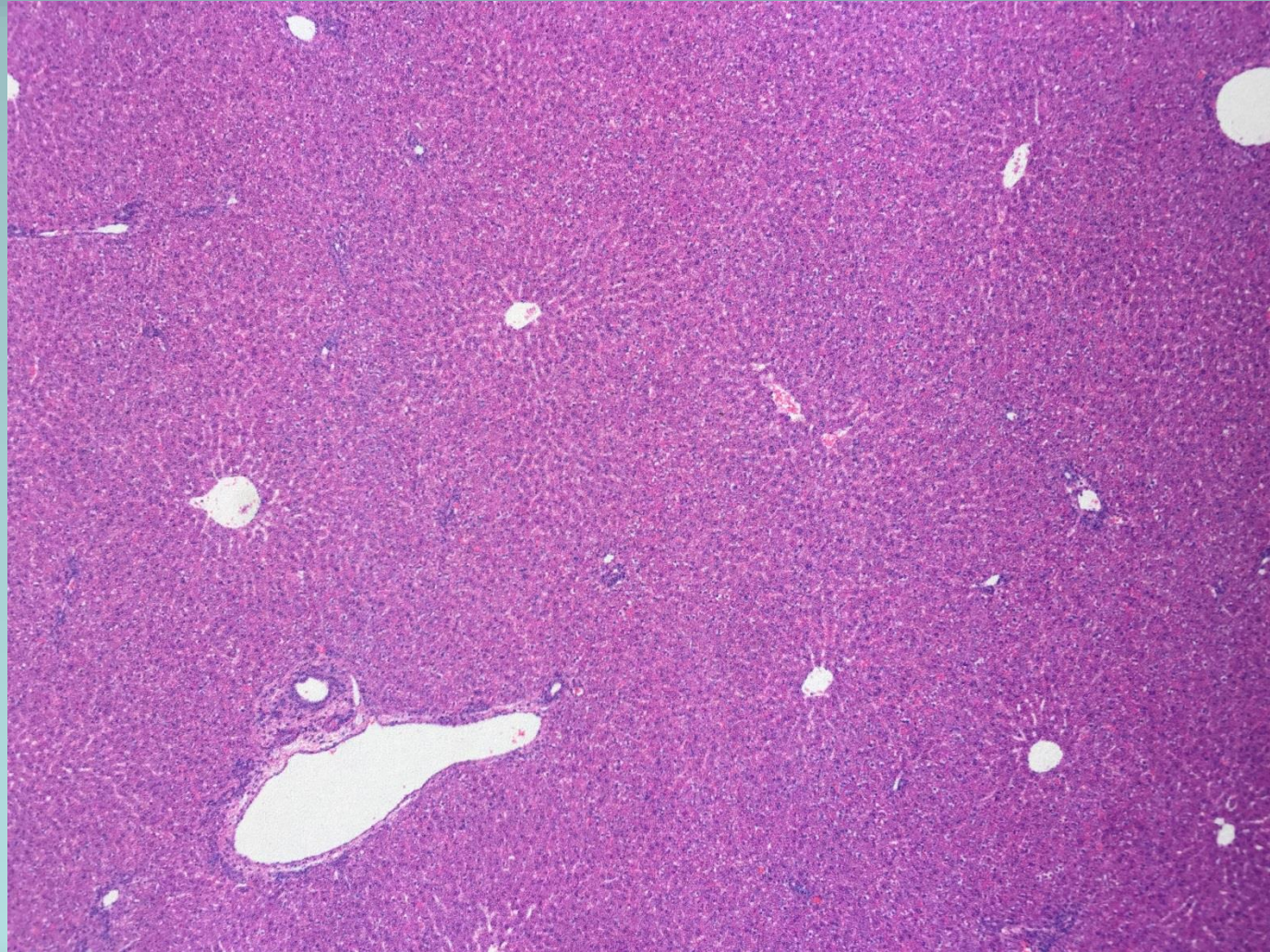
Rat Liver

PND3

PND10



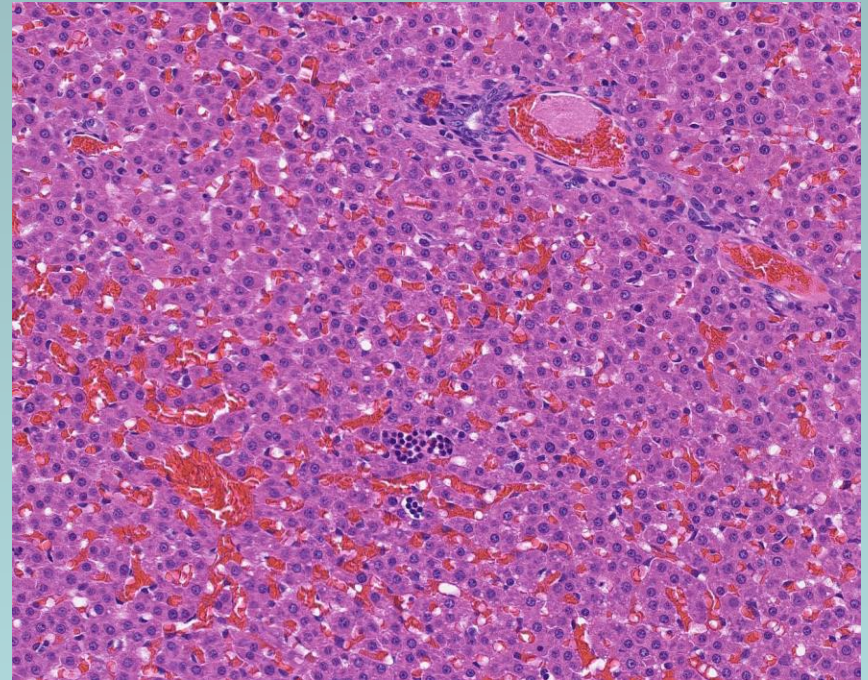
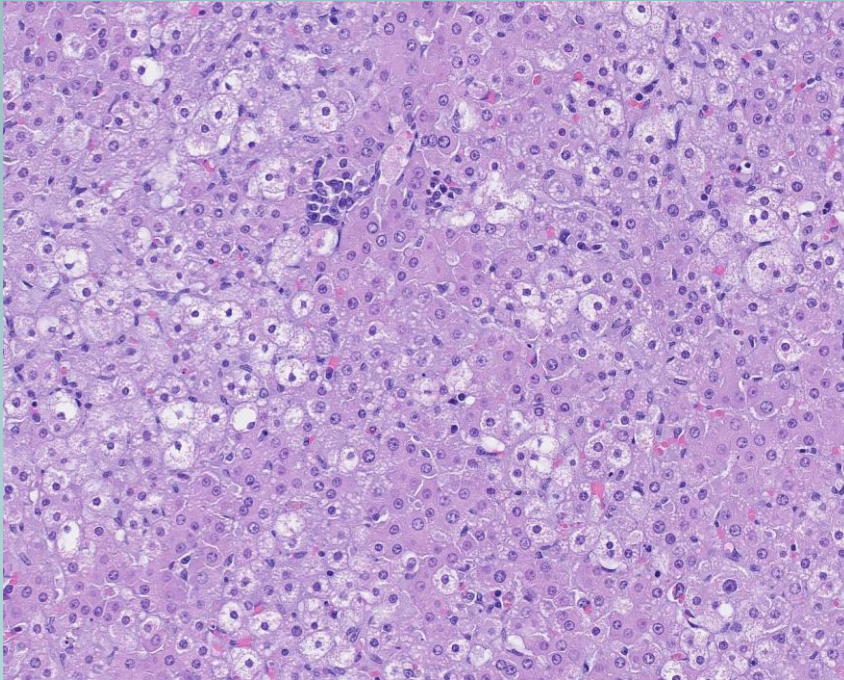
PND28 Liver-rat



Liver

4M PND12 FD 20x

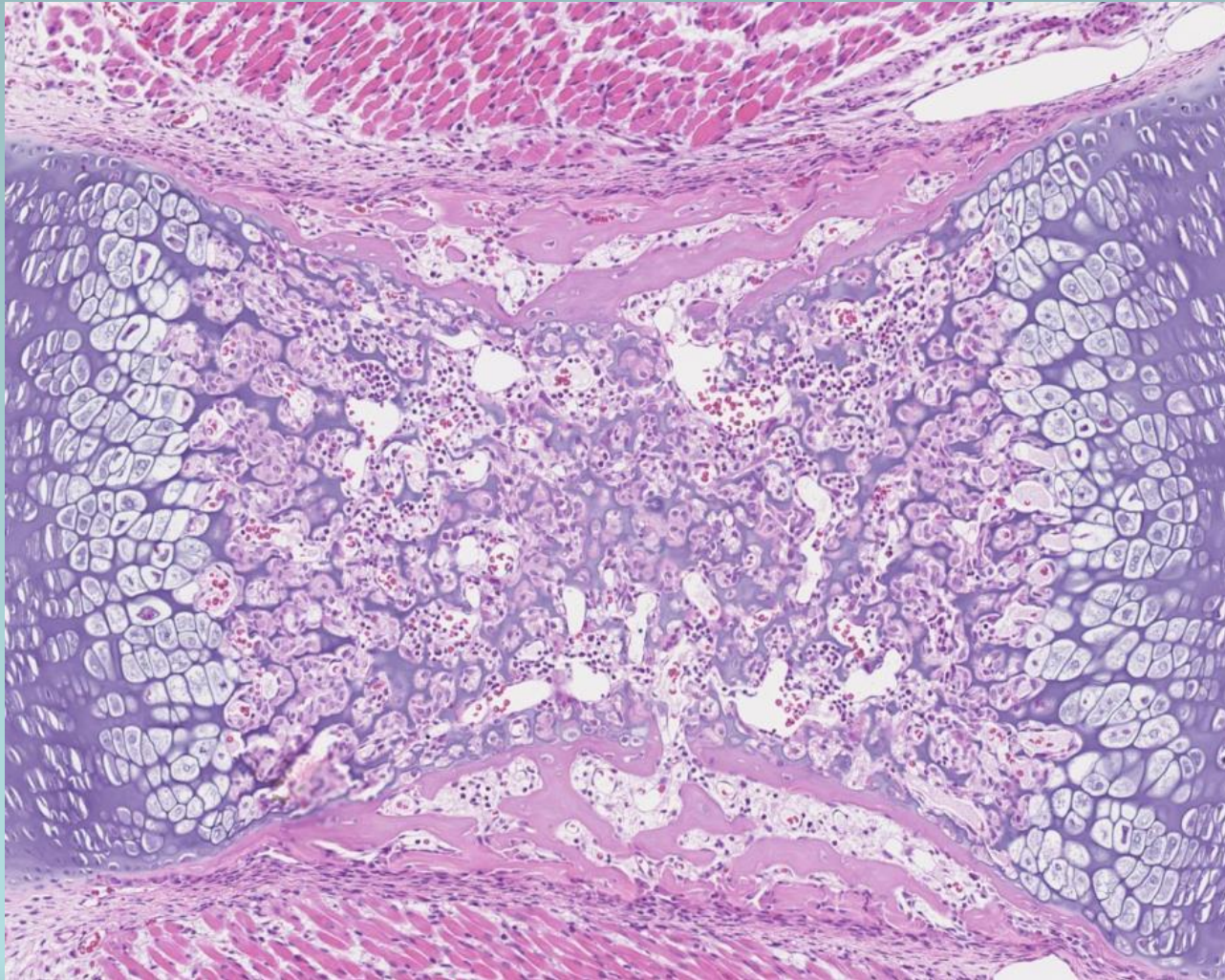
Ctrl PND10 liver 20x



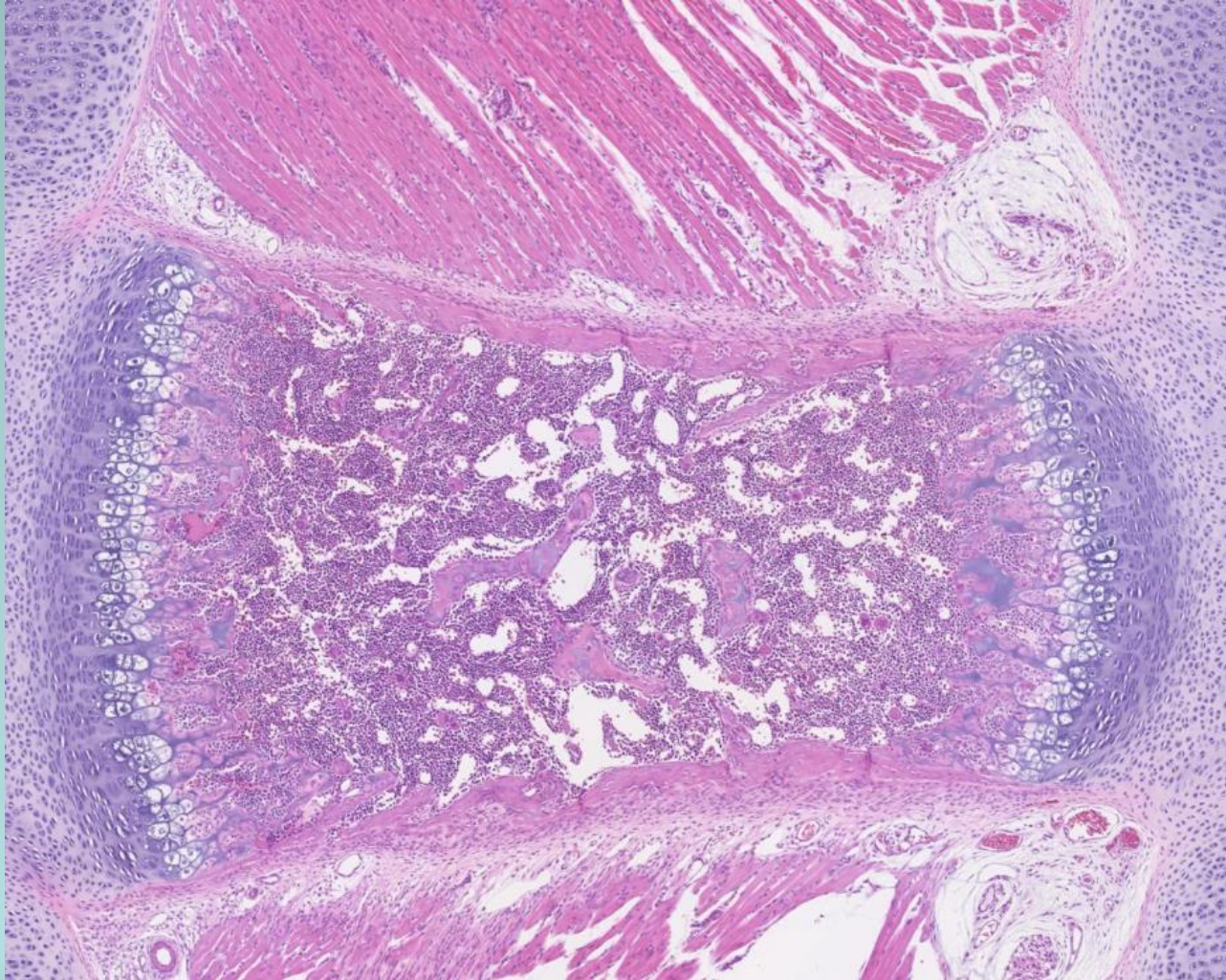
Bone marrow

- **Few hematopoietic cells at PND 0, femur > sternum**
- **PND 7 hematopoiesis increases dramatically**

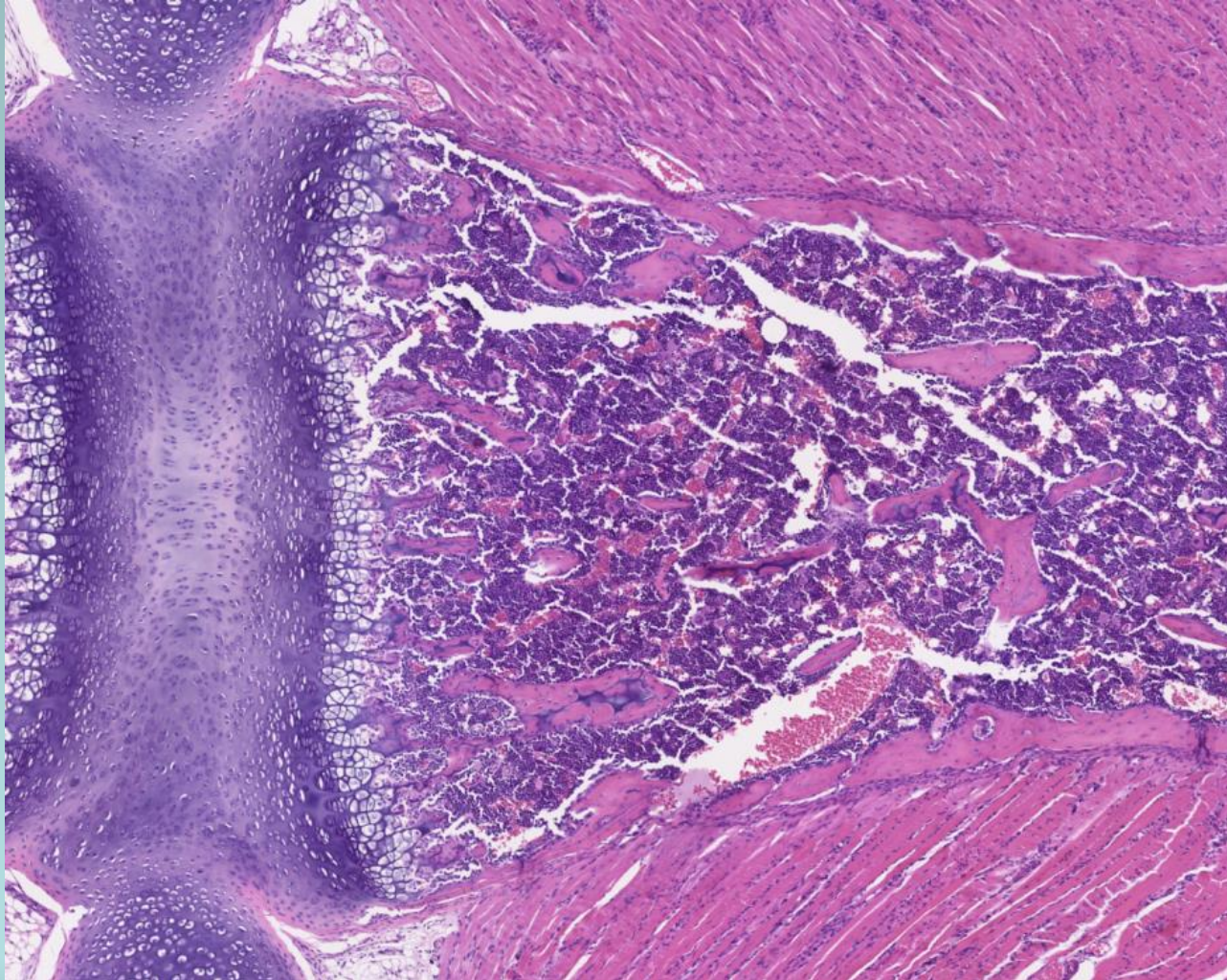
Rat sternum-PND0



Rat sternum-PND7



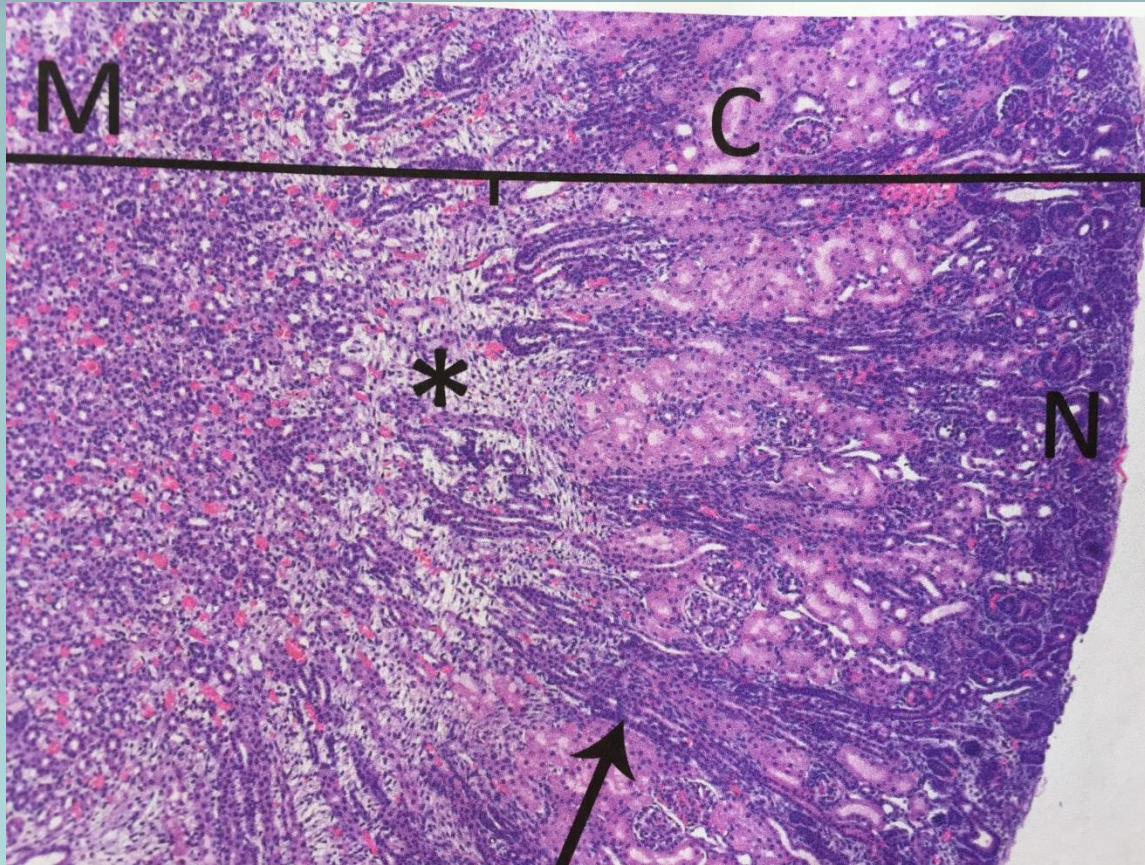
PND14 Rat sternum-PND14



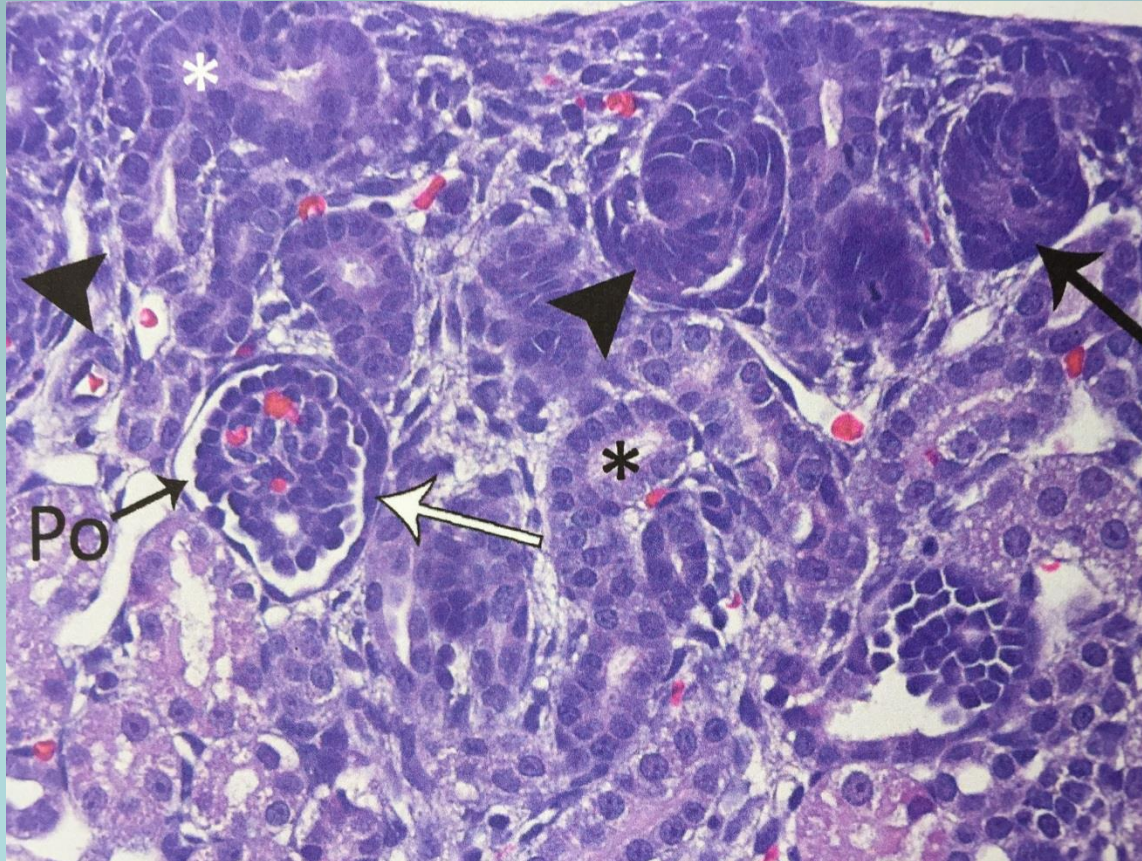
Kidney PND 0

- **N- nephrogenic zone – most immature nephrons**
- **Juxtamedullary zone – more mature nephrons**
- **Medulla – no distinction between IM and OM or inner and outer stripe**

Kidney PND 0



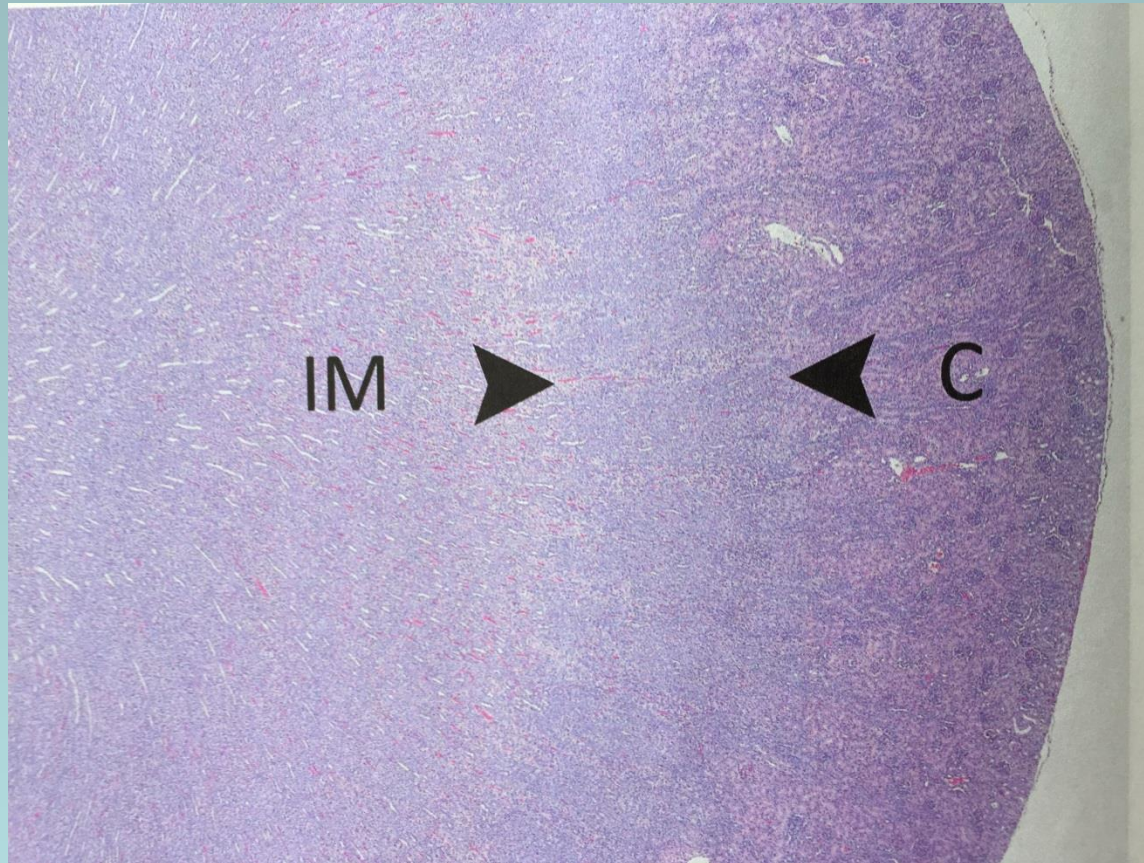
Kidney PND 0 Nephrogenic zone



Kidney PND 14

- **Nephrogenic zone is gone**
- **Outer zone of medulla appears**

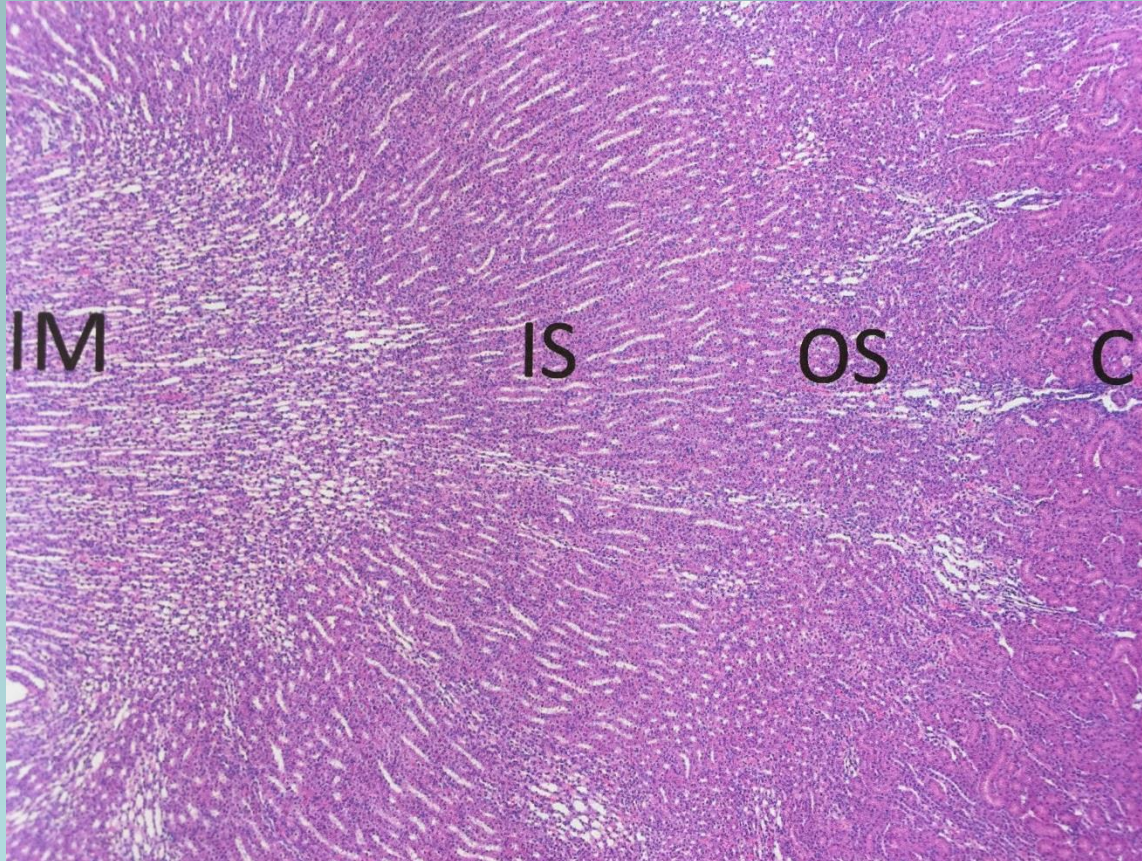
Kidney PND 14



Kidney PND 28

- **Morphologically mature**
- **Clear distinction between inner and outer medulla and inner and outer stripes of outer medulla**

Kidney PND 28



Unscheduled Death Challenges

- **Causes of histologic alteration in unscheduled deaths:**
 - Direct effect of test article
 - Indirect effect of test article
 - **inappetance, stress (immune system)**
 - Delayed development (e.g. endocrine disruption)
 - Normal postnatal histogenesis

A Modest Proposal

- Include additional non-dosed control animals in juvenile toxicology studies
- Sacrifice age-matched controls at time of unscheduled deaths
- Examine relevant tissues from non-dosed controls if questions arise during histopathology evaluation of unscheduled death animals

Questions?

