Kidney Overview

Nephron development continues into postnatal period
  New nephrons formed in nephrogenic zone through PND 4
  Nephron maturation continues through PND 15
  Full function at PND 30

Centrifugal development of cortex
  Cortex expands outward as new nephrons are formed

4 stages of nephron formation
  Stage 1- ellipsoids
  Stage 2- comma- or S-shaped structures
  Stage 3- glomeruli have cuboidal podocytes
  Stage 4- glomeruli have flattened podocytes

Renal papilla and collecting ducts are the most mature structures at birth
Kidney- PND 1
Kidney- PND 1
9 stages (#0-8) of hair follicle development in neonate
   Hair germ
   Hair peg
   Elongation and differentiation
   Final stage resembles anagen phase of adult

Fixed number of follicles
   Very crowded in neonates, but separate as the rat grows

Domains of follicles in neonates cycle synchronously
   Most follicles in histologic section may be in same phase

Synchronous cycling diminishes with successive cycles
   Multiple phases represented in histologic section
Skin Overview-2

PND0
Epidermis thicker than adult, with 5-6 cell layers and thick keratin layer
Follicles range from hair pegs to early anagen
A few sebocytes are present

PND 1-21
Epidermis becomes thinner, follicles develop and project downward, and sebaceous glands develop

PND 21
Follicles reach down to panniculus carnosus, transition from anagen to catagen stage (vacuolated cells)

PND 28
Catagen stage follicles regress into mid-level dermis

PND 35
Second anagen phase
PND 21- Skin
PND 35- Skin
Mammary Gland Overview

Branched ducts and alveoli in 6 pairs of MG
Terminal end buds (TEB) form at ends of ducts
   TEB start to appear at PND 14, maximal number at PND 21-28
Rapid hormone-induced MG growth ~ PND23
PND 21 – 84:
   TEB form additional alveolar buds with each estrus cycle
   Estrogen cycling promotes duct elongation and branching
MG fully developed by ~PND 84
PND 35  Female (left), Male (right)
PND 42  Female (left), Male (right)
PND 70  Female (left), Male (right)
Retina Overview

PND 1
Retina consists of nerve fiber, ganglion cell, and inner plexiform layers plus a thick, mitotically active, basally located neuroblastic monolayer

PND 5
Monolayer starts to separate into inner and outer nuclear layers

PND 7
All layers present, but proportions are different from adult histology
Development progresses from central to peripheral areas of retina

PND 14
All retinal cells are post-mitotic (no neuroblasts remain)

PND 21
All retinal layers have adult histomorphology
PND 1 retina
PND 14 Retina

NG
IP
IN
OP
ON
B

S
PND 1 hyaloid vessels
PND 7 eyelids starting to split
PND 14 Eyelids separate
Stomach Overview-1

Major developmental considerations relative to toxicologic pathology:

- Evolving gastric acidity in postnatal period
- Presence of transient villi in early postnatal period
- “Hyperplastic” appearance at PND 21
- Stress-associated glucocorticoids may hasten gastric maturation
Stomach Overview-2

PND 0 – 14 - “Bland” stage
- Gastric pH = 7
- Protein/lipid absorption by small intestine
- Little digestion
- Study design implications (TA bioavailability)
- Milk lymphocyte transfer

PND 15 – 21- “Transition stage”
- Glucocorticoid and thyroxin surge triggers trophic effect (upregulates gastrin and gastrin receptors)
- Parietal cells produce HCL
- Chief cells produce pepsinogen

PND > 21 – “Acidic stage”
- Gastric pH at adult levels (pH = 2) by PND 40
PND1 Stomach, glandular 40x  pH=7

No mucous cells
PND14 Stomach, glandular 40x  pH=6
PND 21 Stomach, glandular 30x  pH=4
PND 28 Stomach, glandular  pH= 3
Small Intestine Overview

Major developmental considerations relative to toxicologic pathology:

Nutrient resorption vacuoles may resemble toxic effects

- Resorption of lipid in duodenum at PND 0 -1 (base of villi), and then > PND 10 (tips of villi)
- Protein resorption in duodenum/jejunum (receptor mediated)
- Protein resorption in ileum (non-specific endocytosis)

At PND 21 the intestine appears “hyperplastic”.
PND 1 Duodenum, post-suckle

MP
PND7 Jejunum – protein resorption
PND21 Ileum-No resorption vacuoles
Large Intestine Overview

Major developmental considerations relative to toxicologic pathology:

Transient villi in cecum and colon

Nonspecific protein endocytosis up to PND 14

~PND 21 the large intestine appears “hyperplastic”
PND 1- Proximal Colon
PND 7- Proximal Colon
PND 21- Colon
Hematopoiesis Overview

Center of hematopoiesis moves as fetus develops
  yolk sac > aorta-gonad-mesonephros (AGM) > liver > bone marrow
GD15: liver is the primary hematopoietic organ
GD20: bones start to mineralize, forming marrow cavities
GD20-PND10: migration of hematopoiesis from liver to marrow
  Liver hematopoiesis dissipates dramatically by PND10
  Minor persistence of liver hematopoiesis through PND 42
PND0 Liver-rat
Rat Liver

PND3

PND10
GD20 Humerus
PND0 Sternum
PND14 Sternum
Lymphoid Organ Overview

Bone marrow, thymus, spleen, lymph nodes, Peyer’s patches, BALT, NALT

All immune system organs have postnatal development in rats

Primary organs (marrow & thymus) develop first

Postnatal development is independent of antigen

Secondary organs develop later

Postnatal development depends partially on stimulation

Gut-associated lymphoid organs mature earlier
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PND0 Thymus-rat
PND7 Thymus-rat
GD20 Spleen
PND7 Spleen-rat
PND14 Spleen-rat
PND42 Spleen-rat CD45RA IHC
PND0 Mandibular lymph node
PND7 Mandibular lymph node
PND14 Mandibular lymph node
PND42 Mandibular lymph node-rat, CD45RA IHC
PND0 Peyer’s patches
PND28 Peyer’s patches
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 (Gp 4) at PND16
Unscheduled Death Challenges

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

4M PND12 FD

Ctrl PND14
Spleen

4M PND 12 FD

Ctrl PND14
Mandibular lymph node

4M PND12 FD 20x  
Ctrl PND14 10x
Liver

4M PND12 FD

Ctrl PND10 liver
Kidney

4M PND12 FD

Ctrl PND 14
Proposals

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

Collect tissue specimens from discarded rats from DART studies
- Assemble a library of reference slides
- Digital scans for sharing?

Base interpretations on objective comparisons
Collaborators

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Shameless Commerce