The AFIP's Department of Veterinary Pathology & the American Registry of Pathology dedicate this Pathology of Laboratory Animals syllabus to COL (Ret.) William Inskeep II.

COL (Ret.) William Inskeep II
10 October 1949 – 2 July 2005

COL Inskeep entered active military service in 1971, with a Reserve Officer Training Corps commission in the US Air Force. He served five years as a Minuteman Missile Launch Officer at Francis E. Warren Air Force Base, Cheyenne, Wyoming. He was a graduate of Colorado State University and entered the US Army Veterinary Corps in June 1980. COL Inskeep was a Diplomate of the American College of Veterinary Pathologists and held the Surgeon General's "A" Proficiency Designator in the field of Veterinary Pathology. He was also a member of the Order of Military Medical Merit.

COL Inskeep had many diversified and challenging assignments as a Veterinary Corps Officer, his most recent being the Chair, Department of Veterinary Pathology, AFIP, 1997-2003. He also served as Veterinary Pathology Consultant to the Army Surgeon General, 2000-2003, as AFIP Deputy Director Army, 1998-2001, and as DoD Liaison Officer to the US Department of Agriculture, 1996-2000.

COL Inskeep was a graduate of the Army War College. His military awards include Defense Superior Service Medal, Legion of Merit, Meritorious Service Medal with oak leaf cluster, Joint Service Commendation Medal, Army and Air Force Commendation Medals, and Army Staff Badge.

COL Inskeep had many accomplishments over his 28 year military career. However, we will remember him most as a dedicated, enthusiastic mentor and teacher of many residents at the AFIP. We pay tribute to our long-time colleague and friend, and will greatly miss him.

# 49th Pathology of Laboratory Animals

**2 – 5 August 2005**

## Faculty Disclosure Information

At the time of printing this syllabus the following faculty had nothing to disclose:

Nancy E. Everds, DVM, DACVP
Martha A. Hanes, DVM, DACVP, DACLAM
Kenneth S. Latimer, DVM, PhD, DACVP
James B. Nold, DVM, PhD, DACVP
Jo Lynne Raymond, LTC, VC, USA, DACVP
Bruce H. Williams, DVM, DACVP

The following faculty member has not yet returned faculty disclosure statements:

Steven W. Barthold, DVM, PhD, DACVP
Gary B. Baskin, DVM, DACVP, DACLAM
Robert B. Moeller, Jr., DVM, DACVP

### Disclosure of Faculty Financial Affiliations

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**FACULTY**

Steven W. Barthold, DVM, PhD, DACVP
Center for Comparative Medicine
School of Medicine and Veterinary Medicine
University of California, Davis, Davis, California

Gary B. Baskin, DVM, DACVP, DACLAM
Sierra Biomedical
A Charles River Company
Mandeville, Louisiana

Nancy E. Everds, DVM, DACVP
DuPont Haskell Laboratory for Health and
Environmental Sciences
Newark, Delaware

Martha A. Hanes, DVM, DACVP, DACLAM
Integrated Laboratory Systems
Research Triangle Park
North Carolina

Kenneth S. Latimer, DVM, PhD, DACVP
Department of Veterinary Pathology
College of Veterinary Medicine
University of Georgia
Athens, Georgia

Robert B. Moeller, Jr, DVM, DACVP
California Animal Health and Food Safety Laboratory
Tulare, California

James B. Nold, DVM, PhD, DACVP
Safety Assessment, GlaxoSmithKline
Research Triangle Park
North Carolina

Jo Lynne Raymond, LTC, VC, USA, DACVP
US Army Medical Research Institute of
Infectious Diseases
Fort Detrick, Maryland

Bruce H. Williams, DVM, DACVP
Department of Telemedicine
Armed Forces Institute of Pathology
Washington, DC

**PROGRAM**
Tuesday, 2 August 2005

| | |
|---|---|
| 7:30 - 8:15AM | Registration |
| 8:15 - 8:30 | Welcome and Announcements - Dale G. Dunn, COL, VC, USA<br>Chair, Department of Veterinary Pathology |
| 8:30 - 9:30 | Diseases of Mice - Dr. Barthold |
| 9:30 - 9:45 | BREAK |
| 9:45 -10:45 | Diseases of Mice - Dr. Barthold |
| 10:45-11:00 | BREAK |
| 11:00-12:00PM | Diseases of Mice - Dr. Barthold |
| 12:00 -1:00 | LUNCH BREAK |
| 1:00 - 2:00 | Diseases of Rats - Dr. Barthold |
| 2:00 - 2:15 | BREAK |
| 2:15 - 3:15 | Diseases of Ferrets - Dr. Williams |
| 3:15 - 3:30 | BREAK |
| 3:30 - 4:30 | Diseases of Ferrets Dr. Williams |

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Wednesday, 3 August 2005

| | |
|---|---|
| 8:00 - 9:00AM | Clinical Pathology - Dr. Latimer |
| 9:00 - 9:15 | BREAK |
| 9:15 -10:15 | Clinical Pathology - Dr. Latimer |
| 10:15-10:30 | BREAK |
| 10:30-11:30 | Clinical Pathology - Dr. Latimer |
| 11:30-12:30PM | LUNCH BREAK |
| 12:30 - 1:30 | Clinical Pathology - Dr. Everds |
| 1:30 - 1:45 | BREAK |
| 1:45 - 2:45 | Clinical Pathology - Dr. Everds |
| 2:45 - 3:00 | BREAK |
| 3:00 - 4:00 | Clinical Pathology - Dr. Everds |
| 6:00 - 8:00 | Reception, National Museum of Health and Medicine, AFIP |

Thursday, 4 August 2005

| | |
|---|---|
| 8:00 - 9:00AM | Diseases of Primates - Dr. Baskin |
| 9:00 - 9:15 | BREAK |
| 9:15 -10:15 | Diseases of Primates - Dr. Baskin |
| 10:15-10:30 | BREAK |
| 10:30-11:30 | Diseases of Primates - Dr. Baskin |
| 11:30-12:30PM | LUNCH BREAK |
| 12:30 - 1:30 | Diseases of Hamsters, Guinea Pigs, and Gerbils - Dr. Hanes |
| 1:30 - 1:45 | BREAK |
| 1:45 - 2:45 | Diseases of Hamsters, Guinea Pigs, and Gerbils - Dr. Hanes |
| 2:45 - 3:00 | BREAK |
| 3:00 - 4:00 | Diseases of Rabbits - LTC Raymond |

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Friday, 5 August 2005

| | |
|---|---|
| 8:00 - 9:00AM | Diseases and Neoplasms of the Aging Rat - Dr. Nold |
| 9:00 - 9:15 | BREAK |
| 9:15 -10:15 | Diseases and Neoplasms of the Aging Rat - Dr. Nold |
| 10:15-10:30 | BREAK |
| 10:30-11:30 | Diseases and Neoplasms of the Aging Rat - Dr. Nold |
| 11:30-12:30PM | LUNCH BREAK |
| 12:30 - 1:30 | Diseases of Fish - Dr. Moeller |
| 1:30 - 1:45 | BREAK |
| 1:45 - 2:45 | Diseases of Fish - Dr. Moeller |
| 2:45 - 3:00 | Closing Remarks - COL Dunn |

# PATHOLOGY

# OF LABORATORY MICE

**Stephen W. Barthold, DVM, PhD**

Professor and Director
Center for Comparative Medicine
Schools of Medicine and Veterinary Medicine
University of California, Davis
One Shields Ave.
Davis, CA 95616

swbarthold@ucdavis.edu
(530) 752-1245

Director
UC Davis Mouse Biology Program
http://ccm.ucdavis.edu/mbp/
(530) 754-MOUSE
mbp@ucdavis.edu

# THE LABORATORY MOUSE

***Mus musculus* sensu lato**

**(*Mus domesticus, castaneus, musculus, et al.*)**

## Normal features:

Social structure:
- Deme – dominance heirarchy allows mature males to exist within the population (unusual for rodents)
- Pheromones

Physiology:
- Heterothermic: not quite homeothermic, not quite poikilothermic. Wide adaptive environmental range, but intolerant to sudden temperature variations. Death @ >80 degrees F if sudden.

Digestive system:
- Incisive foramen
- Continuously growing incisors, rooted molars
- Gastric yeast (*Torulopsis spp.*)
- Simple intestine
- Sexual dimorphism of submaxillary salivary gland serous cells
- Paneth cells in crypts - very prominent granules
- Ileal filamentous bacteria
- Absorption vacuoles in infant small intestine
- Physiologic hyperplasia in lactating, pregnant females
- Very short rectum - descending colon enveloped in serosa almost to anus, thereby making mouse susceptible to rectal prolapse
- Variable liver lobation
- Certain strains (BALB) prone to normal background hepatocellular fatty change. Exacerbated in disease.
- Hepatocellular polykarya, megalokarya (polyploidy)
- Hepatocellular intranuclear cytoplasmic invagination
- Giant pancreatic islets
- Hyalinosis and crystals in biliary and gall bladder epithelium (B6, 129)

Respiratory system:
- Obligate nasal breathers
- Prominent vomeronasal organs
- Single left lung lobe, four right lobes.
- No intrapulmonary bronchi (no cartilage)
- Cardiac muscle extends around large pulmonary veins
- Hyalinosis in respiratory, especially nasal epithelium (B6, 129); crystals in lung

Hematopoeitic / lymphoid system:
- Extramedullary hematopoeisis in liver, spleen (sometimes adrenal) in infant mice through weaning age. Continues in spleen throughout life, particularly in pregnancy. Disease states readily induce EMH in liver of adults.
- No tonsils
- Thymus does not completely involute
- Indistinct Hassel's corpuscles in thymus
- Ectopic thymus in thyroid, parathyroid and parathyroid in thymus
- Accessory spleens
- "Patches" of lymphoid cells on visceral pleura of lung, peritoneum (B1 cells)
- Mast cells can be common in spleen of some strains (i.e. A)
- Peyer's patches in both small and large intestine
- Short life span of erythrocytes (42 days compared to approx. 100 in humans), resulting in anisocytosis, polychromasia, Howell-Jolly bodies
- Peripheral blood granulocytosis in adult males

- “Doughnut” granulocytes, or ring forms especially in tissues
- Hemosiderin pigment accumulates in spleen, particularly multiparous females

Endocrine system:

- Accessory adrenals common
- No discernable zona reticularis
- X zone: basophilic cells surrounding medulla around 10 days of age, then disappear as mice mature. Swollen, pigmented residual cells
- Adrenal subcapsular spindle cells
- Epithelial-lined cysts in thyroid

Genitourinary system:

- Females have large “genital papillus” (clitoris) with urethral opening near end
- Single renal papillus extends into upper ureter
- Sexual dimorphism of Bowman’s capsular epithelium
- Numerous glomeruli/volume of cortex
- Copulatory plug - agonal event. Often found in urinary bladder, urethra as incidental finding, but can cause antemortem obstruction (obstructive uropathy)
- Redundant testes that are freely retractable into abdomen
- Proteinuria in adult males
- Mouse urinary protein highly antigenic. Produced in liver, excreted in copious amounts (pheromone signaling)
- Prominent accessory sex glands: prepucial glands, seminal vesicles

Skeletal:

- No Haversian bone
- Open (but inactive) epiphyses in many long bones of adults
- Hematopoeisis remains active in long bones throughout life

Cardiovascular:

- Bone/cartilage at aortic root - variable, and usually cartilagenous

Misc.

- Melanosis in B6 mice - meninges of olfactory bulbs, optic nerves, heart valves, endothelium of aorta, parathyroids, capsule and trabeculae of spleen

# Spontaneous (non-infectious) Diseases

## Amyloidosis

Amyloid is extracellular fibrillar protein with a high content of beta-pleated sheets that is deposited extracellularly in various tissues. Two types of amyloid occur in mice: AA, and AapoAII. AA amyloid is associated with an increase in serum precursor apoSAA, which is induced in hepatocytes in response to cytokines produced during inflammatory and neoplastic disease. AA fibril formation and deposition involves partial degradation of apoSAA by macrophages. AA amyloidosis can be induced with repeated injections of casein and other inflammatory stimuli (therefore also referred to as secondary amyloidosis). AapoAII amyloid deposits consist of intact apoAII proteins (no degradation). The precursor, apoAII, is also produced by the liver (also termed primary amyloidosis). Naturally occurring amyloidosis in aging mice generally consists of a mixture of AA and AapoAII amyloid. AA amyloid tends to be deposited in spleen, liver, kidneys, and many other organs. AapoAII amyloid tends to be less severe in liver and spleen, with more deposition in adrenals, intestine, heart, lungs, thyroid, ovaries and testes. In spite of these features, the patterns of amyloid deposition varies markedly among different genotypes of mice. Difficult to distinguish primary from secondary amyloidosis, but secondary (due to inflammatory disease) tends to prefer liver and spleen, whereas these sites are minimally involved with primary amyloidosis. Chronic antigenic exposure of infectious and parasitic (especially mites) disease accelerates amyloidosis, with earlier onset in strains like B6.

Amyloidosis is a singularly important spontaneous disease of mice. It is common in both domestic and wild mice. High prevalence and early onset of AapoII amyloidosis in A, SJL; high prevalence, late onset of amyloidosis (probably mixed amyloid) in B6 and B10 mice; rare in BALB, C3H, DBA. Common in aging Swiss mice, which are prone to AapoII amyloidosis. More common in male mice due to fighting. Tissue distribution patterns vary by genotype, but most frequent sites are renal glomeruli, renal interstitium, lamina propria of small and large intestine, myocardium, parotid salivary gland, thyroid, adrenal cortex, perifollicular areas of spleen, pulmonary alveolar septa, periportal liver, tongue, testis, ovary, myometrium, aorta, pancreas, etc. Associated with cardiac atrial thrombosis, renal papillary necrosis. Deposition of amyloid-like hyaline material is common in nasal submucosa near the ventral septum, but does not stain with Congo Red. Looks like amyloid, but biochemical composition has not been determined. Tumor-associated amyloid is also seen, particularly bronchioloalveolar adenomas in A and BALB (BALBs seldom develop multisystemic amyloidosis). Localized amyloid in corpora lutea in CBA and DBA (which seldom develop multisystemic amyloidosis). Congo Red/polarized light variable result: works well in some tissues, poorly in others of same mouse.

Dehydration:

Mice require large amounts of water. Dehydrate rapidly when water bottle or automatic water is not functional. Death within 24 hours.

Hypothermia /hyperthermia:

Mice susceptible to sudden extremes in temperature. More commonly, water bottle accidents flood cage, making mice wet, hypothermic. High mortality.

Integument:

Barbering: genotype-specific patterns
Trichotillomania: genotype-specific (B6)
Pugilism: genotype-specific patterns (male BALBs)
Nasal alopecia: barbering, trichotillomania, or mechanical abrasion
Ringtail: low humidity, preweanling mice.
Gangrene: low humidity, cold air in hairless mice. Cotton bedding infants

Nervous system:

Hydrocephalus (B6 mice)
Hypoplasia/aplasia of corpus callosum (incomplete penetrance, ca. 70% in 129, BALB. Incidence higher in litters born to lactating dams.
Brain sand
Vacuolation of neuropil (aging artifact)
Retinal degeneration: common in C3H, CBA and Swiss; A, AKR, BALB, C57BL and DBA. Seen in wild mice. Multigenic, but begins prior to weaning and is nearly complete by weaning.
Microophthalmia in Black mice (B6, B10, etc.) typically unilateral
Laminar necrosis of neurons in FVB mice due to seizures

Reyes-like syndrome: high morbidity and mortality among BALB/cBy-types

Gastrointestinal:

Malocclusion, incisor overgrowth
Tooth decay: acidified water
Foreign body gingivitis
Gastric mucosal hyperplasia in immunodeficient mice
Salivary ductal cysts
Duodenal and pyloric stress ulcers
Mesenteric disease in aged mice: enlarged, atrophic mesenteric lymph nodes
Eosinophilic secretory inclusions in biliary and gall bladder epithelium (B6)
Adynamic ileus associated with i.p. Avidin (chloral hydrate) anesthetic

Respiratory:

Crystal pneumonia in B6, motheaten (B6 mutant) and 129 mice
Eosinophilic secretory inclusions (hyalinosis) in respiratory epithelium (B6, 129)
Nasal obstruction - bedding
Inhalation pneumonia - bedding, food

Hematopoeitic / lymphoid:

Periarterial and periductal lymphocytic infiltrates: salivary gland, lung, kidney, etc.

Genitourinary:

Obstructive uropathy due to copulatory plugs
Glomerular hyalinosis (basement membrane thickening) in aged mice (AKR, BALB, CBA)
Glomerulonephritis in some strains, like NZBNZWF1, BALB
Glomerular amyloidosis and interstitial amyloidosis
Hydronephrosis
Polycystic kidneys (BALB)
Chloroform toxicity: exquisite susceptibility of DBA, C3H
Hyaline droplets in tubular epithelium associated with histiocytic sarcoma
Arteritis/segmental infarction
Megalokaryosis in male ductal epithelium of aged mice
Muco(hydro)metra: imperforate vagina
Cystic endometrial hyperplasia
Enlarged seminal vesicles in B6 mice

Musculoskeletal:

Sternal necrosis
Fibro-osseous proliferation (female mice)
Skeletal muscle mineralization (C3H)

Cardiovascular:

Atrial thrombosis (see amyloidosis)
Epicardial mineralization (BALB, DBA)
Myocardial mineralization (C3H)
Idiopathic polyarteritis: segmental infarcts in kidneys, vestibular disease, coronary artery, ovary

Misc.

Obesity: central pattern
Pregnancy toxemia: rare. BALB

**Neoplasia**

Lymphoid / myeloid tumors. Retroviruses (both MuLV and MMTV) significant factor

AKR: thymic
BALB: peripheral lymph nodes, generalized
SJL: mesenteric nodes, generalized

Mammary tumors. Retroviruses significant factor

C3H: high prevalence
BALB, C57BL: low prevalence

Bronchioloalveolar adenomas: BALB, A, CR common. C57BL rare.

Trichoepitheliomas

Hepatocellular tumors: DBA, A strain common

Eosinophilic, basophilic, clear cell foci are considered “preneoplastic”

Harderian gland adenomas/carcinomas

Myoepitheliomas

Common in BALB. Submaxillary and parotid salivary glands, but also mammary, prepucial and Harderian. Concomitant myeloid hyperplasia.

Misc:
Islet cell tumors
Pituitary adenomas (FVB mice)
Zymbal gland tumors
Sarcomas (lymphangio, fibro, myo, osteo, etc.): transgenic mice
Teratomas (129 and KO mice)

## PSEUDOPATHOGENS

Esophageal G+ cocci
Gastric yeast (*Torulopsis spp.)*
Ileal filamentous bacteria
*Entameba muris*
*Tritrichomonas muris, Trichomonas minuta & T. wenyoni, Chilomastix bettencourti, Octomitus pulcher*

## INFECTIOUS AGENTS OF MICE

### 2. VIRUSES

**A. DNA**

| | |
|---|---|
| Adenovirus | MAd-1 (FL), MAd-2 (K87) |
| Herpesvirus | mouse cytomegalovirus (MCMV) |
| | mouse thymic virus (MTV) |
| Papovavirus | K virus |
| | polyoma virus |
| Parvovirus | minute virus of mice (MVM) |
| | mouse parvovirus (MPV) |
| Poxvirus | ectromelia virus |

**B. RNA**

| | |
|---|---|
| Arenavirus | lymphocytic choriomeningitis virus (LCMV) |
| Arterivirus | lactate dehydrogenase–elevating virus (LDV) |
| Bunyavirus | hanta-like virus |
| Calicivirus | murine norovirus (MNV-1) |
| Coronavirus | mouse hepatitis virus (MHV) |
| Paramyxovirus | pneumonia virus of mice (PVM) |
| | Sendai virus |
| Picornavirus | mouse encephalomyelitis virus (MEV) |
| Reovirus | epizootic diarrhea of infant mice (EDIM) virus |
| | reovirus 1, 2, 3 |
| Retrovirus | murine leukemia virus (MuLV) |
| | murine mammary tumor virus (MMTV) |

**Adenoviridae**

**Murine Adenovirus 1 and 2**

MAd- 1 (FL)
MAd-2 (K87)

1. Prevalence: MAd-1 rare or nonexistent in laboratory mice. MAd-2 moderate.
2. Diagnosis: Serology, lesions (intranuclear inclusions)
3. Disease: MAd-1 (FL, ala Friend leukemia) causes multisystemic infection with viruria in young and immunodeficient mice. Lethal in suckling and SCID mice. Subclinical in older mice, including athymic nude mice. Type A intranuclear inclusions, especially in renal tubules, adrenal cortex. Animal model for adrenal necrosis. Infection of SCID and nude mice associated with enteritis, inclusions in enterocytes. Intestinal component has not been carefully examined. MAd-2 (K87) is strictly enterotropic and less virulent, even in infant and immunodefieient mice. Inclusions in enterocytes. Usually subclinical, but can cause runting and low mortality in suckling mice.
4. Transmission: nose, urine, feces.
5. Duration: acute to chronic
6. Comment: MAd-1 probably extinct. MAd-1 antigen does not cross-react with MAd-2 antibody, but MAd-2 antigen will react with MAd-1 antibody.

**Herpesviridae**

**Murine Cytomegalovirus (MCMV); Murid Herpesvirus 1 (MuHV-1)**

1. Prevalence: rare in laboratory mice; common in wild mice.
2. Diagnosis: serology (not generally used); salivary gland lesions
3. Disease: Natural infections and experimental infections in adult immunocompetent mice are subclinical and limited to salivary glands. Submaxillary glands preferentially involved, with intranuclear and intracytoplasmic inclusions in tubular epithelium and nonsuppurative interstitial inflammation. Disseminated infection in experimentally inoculated infant mice and immunosuppressed mice, including

SCID mice.
4. Transmission: saliva, tears, urine. In utero transmission rare, even in experimentally inoculated mice.
*5.* Duration: chronic and latent (only latent virus of mice)
6. Comment: Subfamily Beta-herpesvirinae, Genus Muromegalovirus. Studied as model for human disease, but does not mimic human disease or vertical transmission very well.

**Herpesviridae**
**Mouse Thymic Virus (MTV); Murid herpesvirus 3 (MuHV-3);** thymic necrosis virus; thymic agent

1. Prevalence: nonexistent or rare in laboratory mice; common in wild mice
2. Diagnosis: serology (not generally used); mouse bioassay (thymic necrosis in neonates)
3. Disease: Natural infection and infection in adult mice subclinical. Experimental inoculation of neonates causes thymic necrosis and generalized lymphoid (T cell) necrosis, with intranuclear inclusions. Infection of older mice largely restricted to salivary glands, but no lesions.
4. Transmission: saliva.
*5.* Duration: chronic
6. Comment: Unclassified herpesvirus. Because of salivary tropism, MTV (not to be confused with MTV of mammary tumor virus) is a frequent contaminant of MCMV stocks. International Union of Microbiological Societies 2000 has renamed the virus MuHV-3, which further confuses with MHV-3. Furthermore, MuHV-2 is not even a herpesvirus of mice, but rather voles!

**Papovaviridae**
**K Virus; Murine Pneumotropic Virus (MPtV)**

1. Prevalence: rare or nonexistent
2. Diagnosis: serology
3. Disease: Subclinical in adult mice. Necrosis, intranuclear inclusions in endothelial cells of villus lamina propria. Neonates develop viremic dissemination to pulmonary and hepatic vascular fields. Pulmonary endothelial damage with hemorrhage, edema, death. Viremia blocked in older mice by early neutralizing antibody response.
4. Transmission: fecal
*5.* Duration: acute to chronic
6. Comment: Polyomavirus Genus of Papovaviridae. Primary site of virus excretion is renal tubules, with minimal lesions. Therefore, biologically similar to other polyomaviruses. Due to rarity of this virus, natural infection of immunodeficient mice has not been reported, but consequences can be predicted. International Union of Microbiological Societies 2000 has renamed the virus MPtV, even though the virus is not primarily pneumotropic!

**Papovaviridae**
**Polyoma Virus; Murine Polyomavirus (MPyV);** parotid tumor agent; salivary gland tumor virus

1. Prevalence: rare as natural infection in laboratory mice. Common in wild mice.
2. Diagnosis: serology
3. Disease: Usually subclinical. Experimental inoculation of neonates with high doses of oncogenic strains results in multisystemic infection, followed by multiple hyperplastic and neoplastic foci in a variety of tissues (poly-oma). Natural infection in young mice multisystemic, but tumors are exceedingly unlikely. Renal tubules important target with viruria, as with many polyomaviruses of other species. Mild necrosis, inclusions, interstitial inflammation. Older mice clear infection rapidly, with no virus shedding. Natural and experimental infection of athymic nude mice resulted in chronic infection and posterior paresis due to vertebral tumors and progressive multifocal leukoencephalopathy (PML), as seen with polyomaviruses in primates. Vertebral tumors noted in beta2 microglobulin KO mice.
4. Transmission: urine, contamination of environment (wild mouse nests)
*5.* Duration: acute to chronic
6. Comment: laboratory animal husbandry practices preclude the inefficient life cycle of this virus. Polyoma virus is the type species of Polyomavirus Genus of Papoviridae.

**Parvoviridae**
**Minute Virus of Mice (MVM); Mice Minute Virus (MMV)**

1. Prevalence: common
2. Diagnosis: serology (HAI, ELISA, IFA)
3. Disease: Subclinical. Experimental infection of neonates multisystemic, with cerebellar hypoplasia, renal infarcts, anemia, etc. Virus infection requires receptor. Replication (& cytolysis) requires S phase in dividing cells. Natural disease with hematopoietic dyscrasia has been observed in immunodeficient mice (SCID, NOD, other KOs). Experimental infection with MVMi results in disrupted hematopoiesis in C3H mice.
4. Transmission: Contact. Vertical transmission shown experimentally
*5.* Duration: variable; chronic, possibly latent. Experimental studies in immunocompetent mice suggest acute infection with recovery.
6. Comment: 2 named strains, MVM-p (prototype) and MVM-i (immunosuppressive). Lymphoid tissues common target, with immunomodulation. New standards for nomenclature dictate that this virus now be called "mice minute virus (MMV).

**Parvoviridae**
**Mouse Parvovirus (MPV); <u>Mouse Parvovirus (MPV-1)</u>**

1. Prevalence: common
2. Diagnosis: serology (IFA, ELISA). Recombinant non-structural antigen now used (NS-1) as antigen. False positive reactions may rarely occur due to cross-reactivity with baculovirus components, resulting in low positive reaction to recombinant antigen, but negative to whole virus.
3. Disease: Natural infections subclinical, but immunomodulation common (T cell tropic).
4. Transmission: fecal?
*5.* Duration: chronic, possibly latent. MPV infects, and persists in, adult mice.
6. Comment: Does not share virus structural antigens with MVM. HAI and MVM-based ELISA therefore inaccurate. Shares common non-structural antigens with other parvoviruses, including MVM. Thus IFA with MVM-infected cells as antigen works. Differs from rat parvovirus. Number of strains unknown. Experimental infection induces allograft rejection. New nomenclature is MPV-1 (not to be confused with MMV-1).

**Poxviridae**
**Ectromelia Virus**

1. Prevalence: rare. Unknown natural origin.
2. Diagnosis: serology, lesions.
3. Disease: natural disease varies from subclinical to high mortality, depending upon mouse genotype, age, virus strain. Resistant adult mice recover early, with minimal virus shedding. Susceptible mice develop disseminated disease, with multifocal necrosis of liver, lymphoid tissue, intestine, spleen, integument, etc. Fulminant infection may result in death with minimal virus excretion. Semi-susceptible mice develop disseminated infection with full manifestations, including rash and ectromelia (shortening of extremities due to dry gangrene). These mice serve as the major source of virus excretion. Pox inclusions (Cowdry A & B) in skin and mucosal epithelium.
4. Transmission: skin, respiratory, urinary, fecal, etc.
*5.* Duration: acute
6. Comment: Genus Orthopox, Family Poxviridae. Several strains, including NIH-79, Wash-U, Moscow, Hampstead, St. Louis-69, Beijing-70, Ishibashi I-III, which vary in virulence. First description in England. Disease called mouse pox, virus called ectromelia virus…?????. Vaccine available (IHD-T), but modified live virus, prevents mortality but not infection with street virus, and causes seroconversion. Thus, not recommended except to protect valuable mice in imminent danger of exposure. Beware of mouse serum that is commercially available. Latest incidence of laboratory mice developing mouse pox due to such serum occurred in 2003.

**Arenaviridae**
**Lymphocytic Choriomeningitis Virus (LCMV)**

1. Prevalence: rare in laboratory mice, common in wild mice throughout the world, but focal distribution.
2. Diagnosis: Serology, but mice tend to mount poor antibody responses, or antibody is complexed with antigen. Bioassay (intracerebral inoculation of infant vs. adult mice), MAP test or PCR. Enzootically (in utero) infected mice are likely to be seronegative due to antibody complexed to high levels of antigen.

Seroconversion of sentinels placed in the enzootically infected room is therefore needed for screening, but antibody titers are often low. PCR is useful in such situations.
3. Disease: LCMV is non-cytolytic and does not directly cause disease. Disease associated with LCMV is immune-mediated, either through cytotoxic T lymphocytes (CTLs) or deposition of immune complexes. LCMV is maintained in naturally infected mouse populations through vertical transmission in utero from the dam to the early embryo. Pups infected in utero or in the first few hours of life have disseminated infections, including the thymus, so that T cells that traffic through the thymus become tolerant to the virus. Tolerance is LCMV specific, with normal immune response to other antigens and viruses. These mice are typically infected for life, and have CTL tolerance, but develop non-neutralizing antibody that is complexed with excess antigen. As mice age, they develop "late disease" with immune complex arteritis and glomerulonephritis, as well as diffuse lymphocytic infiltrations in various tissues. Mice infected as adults by natural routes recover from infection due to a vigorous CTL immune response, but with low or undetectable antibody. Experimental inoculation of adult mice intracerebrally results in lymphocytic choriomeningitis. Mice die at around 5-6 days from seizures. Disease is due to CTL-mediated pathology. Inoculation of mice intraperitoneally or other routes results in CTL-mediated hepatitis, and lymphocytic infiltration of multiple tissues. Inoculation of mice with immunosuppressive strains of the virus results in early infection of dendritic cells (DCs), which express high levels of the viral receptor, alpha-dystroglycan. DCs in the marginal zones of the spleen and lymph nodes serve as a conduit of virus into the white pulp or T cell regions of the lymph nodes. CTLs attack DCs and T cells, resulting in severe lymphocytic depletion with global immunological exhaustion (not tolerance), and persistence of virus. In contrast, prenatally infected mice have selective tolerance to the virus. These mice also develop lymphocytic infiltrates in tissues and immune-complex arteritis and glomerulonephritis as they age. Adoptive transfer of CTLs from immune mice to mice infected in utero leads to disease (lymphocytic choriomeningitis and hepatitis) and death, or recovery if they survive, whereas adoptive transfer of CTLs into mice infected as adults results in CTL exhaustion. Endocrinopathy also reported in mice infected prenatally or neonatally (growth hormone deficiency, thyroid deficiency, diabetes). Serositis is also a common lesion, regardless of route of inoculation.
4. Transmission: Early in utero transmission is important in the natural cycle of infection. Ova, placenta and early embryos are infected. Virus transmitted via multiple routes, including mouse biological products.
*5.* Duration: acute to chronic, depending on age, tolerance, exhaustion, and immune status.
6. Comment: Mouse is natural reservoir host. Zoonotic agent. Hamsters pose a serious risk, as they develop persistent infections following natural exposure as adults (unlike mice). Zoonotic infections have been reported in association with nude mice. Common laboratory strains of LCMV include Armstrong, Traub, WE, UBC, and Pasteur, and various substrains thereof. Virus strains are classified as "docile" or "aggressive" and "neurotropic' or "viscerotropic" for experimental purposes. "Docile" and "viscerotropic" strains are actually more virulent, but cause no overt disease because they infect DCs and T cells, with severe immunodeficiency; whereas "aggressive" and "neurotropic" strains induce mild infections resulting in T cell-mediated disease. Intermediate scenarios also occur between the prenatal tolerant and the adult resistance forms of infection. Natural infection of nude mice reported.

**Arteriviridae**

**Lactate Dehydrogenase-Elevating Virus (LDV)**

1. Prevalence: rare in laboratory mice, common in wild mice
2. Diagnosis: persistent enzyme elevation in blood. Serology reported to be poor because of high avidity antigen-antibody complexes in serum.
3. Disease: Subclinical. Late onset demyelination in immunosuppressed C58 and AKR mice, but disease is associated with N-ecotropic retrovirus. Ecotropic retrovirus infects glial cells of these strains, making the neurons susceptible to LDV through still undetermined mechanism. Susceptibility of mouse strain to neurologic disease is linked to the *fv-1*$^{n}$ allele.
4. Transmission: Inefficiently transmitted among mice. Natural salivary transmission via bite wounds, and possible sexual transmission.
*5.* Duration: persistent
6. Comment: select subpopulations of differentiated, but not precursor, macrophages and monocytes are the target of virus. Thus, persistent infection is maintained through continuous production of susceptible cells by the host. LDV has a single neutralizing epitope that undergoes variation as a means of evading host immunity. LDV can contaminate transplantable tumor lines, but virus can be eliminated by transient passage through rats. This virus is an arterivirus related to equine arteritis virus and simian hemorrhagic

fever virus. It belongs within the Order Nidovirales, which contains the Coronaviridae and Arteriviridae families.

**Bunyaviridae (?)**
**Hanta-like Virus**

1. Prevalence: unknown
2. Diagnosis: no available diagnostic method
3. Disease: Subclinical. Interstitial and perivascular nonsuppurative inflammatory lesions found in lungs of rats and mice.
4. Transmission: unknown
*5.* Duration: unknown
6. Comment: newly discovered agent in laboratory rodents. Most significant in rats, with pulmonary lesions, but experimental assays (PCR, serology.) suggest that laboratory mice are also naturally infected. Much to be learned. Probably not a Hanta virus. People working with this agent seroconvert.

**Caliciviridae**
**Murine Norovirus-1 (MNV-1)**

1. Prevalence: according to RADIL, nearly 30% seroconversion among mouse samples tested
2. Diagnosis: serology
3. Disease: Not well characterized. A single report of sporadic mortality among STAT1 and RAG2/STAT1 KO mice. Intracerebral inoculation of virus resulted in encephalitis, vasculitis, meningitis, hepatitis, and pneumonia. "Postage stamp" pathology figures in the single report preclude evaluation of lesions. RAG1 and RAG2 KO mice inoculated i.c., p.o. or i.n. were not susceptible to mortality, but were persistently infected. IFN alpha/beta receptor, IFN gamma receptor, PKR or iNOS KO mice were not susceptible to mortality, but IFN apha/beta/gamma KOs were more susceptible to lethal infection. STAT1, STAT1/PKR and STAT1/RAG were all susceptible, indicating importance of STAT1 innate immunity. Noroviruses in humans cause enteritis with villus blunting, nonsuppurative inflammation of lamina propria, but intestine not examined in mouse.
4. Transmission: oro-fecal (presumptive, based upon other noroviruses).
5. Duration: unknown
6. Comment: Studies needed. The mouse agent seems to "cluster" genetically differently from other noroviruses. Cattle and pigs are also hosts to noroviruses, in addition to humans (Norwalk and other agents).

**Coronaviridae**
**Mouse Hepatitis Virus (MHV); Murine Hepatitis Virus**

1. Prevalence: **VERY** common
2. Diagnosis: serology, lesions
3. Disease: epizootic - high mortality among neonates to subclinical among adults. Enzootic - usually subclinical in all ages, unless immunodeficient. Many virus strains, indistinguishable antigenically, but represented by 2 biotypes: respiratory and enteric. Respiratory strains replicate in nasal epithelium (minimal visible lesions in nose) and disseminate to varying degrees to secondary target organs, including lung, liver, lymphoid tissues, serosa, etc. in susceptible hosts. Focal necrosis with syncytia. Enteric strains largely restricted to bowel mucosa, with minimal dissemination. Ascending colon and cecum are major targets, with hallmark syncytia. Infant mice rapidly develop severe enteritis; adults infected and shed copious virus, but subclinical. Enteric disease is age-dependent, even in immunodeficient mice (related to mucosal kinetics, not immune response). Immunosuppression or co-infection with other agents increases susceptibility to MHV, but only during active infection. Immunodeficient mice cannot clear infection. Novel manifestations of infection are emerging in immunodeficient mice, such as granulomatous serositis (akin to feline infectious peritonitis?) in gamma interferon knock out mice.
4. Transmission: orofecal. In utero possible, but not likely.
*5.* Duration: acute in immunocompetent mice, but there are growing numbers of reports in which infection may be persistent in a variety of genetically altered mice. B cell deficient mice may have mild or no clinical disease, but shed virus persistently. Regardless of immune status, genetic alteration, strain, etc. MHV infection is often subclinical but NOT LATENT!!!!

6. Comment: Coronaviruses of mice are antigenically related to rat coronaviruses, but biologically different. Immunity to MHV is virus strain-specific and short-lived. Coronaviruses are highly mutable, particularly in the S genes, which determine strain specificity. Thus, mice can be repeatedly infected, which has led to much confusion about the issue of latency. Coronaviruses readily mutate and recombine, with evolution of new strains and repeated infection features of enzootic infection.

**Paramyxoviridae**
**Pneumonia Virus of Mice (PVM); Murine Pneumonia Virus (MPV)**

1. Prevalence: common
2. Diagnosis: serology; lesions in immunodeficient mice
3. Disease: subclinical upper respiratory infection in immunocompetent mice. Can cause lower respiratory disease in experimentally infected mice. Nude and SCID mice develop wasting disease due to progressive interstitial pneumonia (bronchiolar epithelium and type 2 pneumocytes). Virus targets respiratory epithelial cells and type 2 pneumocytes.
4. Transmission: respiratory
*5.* Duration: acute (except immunodeficient mice)
6. Comment: Genus Pneumovirus; Family Paramyxoviridae. The International Union of Microbiological Societies 2000 has renamed PVM to MPV, which is confusing with mouse parvovirus (MPV-1)

**Paramyxoviridae**
**Sendai Virus (SeV)**

1. Prevalence: recently common, but now rare in US. Still out there, though.
2. Diagnosis: serology, lesions
3. Disease: Sendai virus is the most clinically significant virus infection in laboratory mice because of its contagiousness and likelihood to cause clinical disease in immunocompetent mice of all ages. Lesions are necrotizing rhinitis, tracheobronchitis, bronchiolitis and interstitial pneumonia. Target cells are respiratory epithelium and type 2 pneumocytes. Recovery phase characterized by hyperplasia and squamous metaplasia of respiratory epithelium, cuboidal metaplasia of alveoli, focal fibrosis. Virus is not cytolytic. Disease due to host immune attack on infected cells. Infection of athymic and SCID mice results in proliferative (rather than necrotizing) bronchiolitis and interstitial pneumonia. Marked variation in genetic susceptibility related to kinetics of immune response and/or mucociliary clearance, depending upon mouse strain. Predisposes to bacterial respiratory and ear infections.
4. Transmission: respiratory/aerosol
5. Duration: acute (except immunodeficient mice)
6. Comment: Genus Respirovirus; Family Paramyxoviridae. Closely related to parainfluenza viruses. Recent studies suggest that Sendai virus is likely to be zoonotic.

**Picornaviridae**
**Mouse Encephalomyelitis Virus (MEV)**; **Theiler's Murine Encephalomyelitis Virus (TMEV)**; mouse polio, GD VII

1. Prevalence: common
2. Diagnosis: serology, lesions (when present)
3. Disease: majority of infections are subclinical. CNS signs include convulsions, posterior paresis (flaccid) in a small fraction of mice. Infects enterocytes with minimal effect. CNS infection due to dissemination with acute encephalomyelitis (neuronolysis) and demyelination (primary and immune-mediated attack on infected oligodendroglia). Severity of CNS disease is virus strain-, host age- and genotype-dependent.
4. Transmission: orofecal
5. Duration: variable with intermittent shedding. Must be considered persistent.
6. Comment: Genus Cardiovirus; Family Picornaviridae. Related antigenically to encephalomyocarditis (EMC) virus. Two serogroups: GD VII (GD VII, FA) and TO (TO, DA, BeAn 8386). TO group less virulent. Inefficiently transmitted…test and cull at the cage level can eliminate from colony if use microisolator caging and proper changing technique. International Union of Microbiological Societies 2000 has

renamed the entire virus group TMEV, although Theiler's virus is only one strain!

**Reoviridae**
**Epizootic Diarrhea of Infant Mice (EDIM) Virus; Murine Rotavirus-A/EDIM (MuRV-A/EDIM)**

1. Prevalence: common
2. Diagnosis: serology, lesions (if present), antigen in feces, E.M. of feces (many virions)
3. Disease: Lesions and diarrhea in mice infected at 12 or less days of age. All ages are susceptible to infection. Hydropic swelling of villus tip epithelium, malabsorption, diarrhea, runting. EDIM is a disease of intestinal proliferative kinetics, with viral tropism for terminally differentiated enterocytes, which are in abundance in neonates. Diarrhea is not simply due to epithelial damage. Neuroendocrine effectors, viral enterotoxin secretagogue, and disruption of disaccharidases (with malabsorption) all factors, with *E. coli* overgrowth. Maternal immunity abrogates disease in enzootic infection.
4. Transmission: orofecal
5. Duration: acute, but not fully characterized
6. Comment: Genus Rotavirus; Family Reoviridae. Typical rotavirus (type A), antigenically related to type A rotaviruses of other species. Commercially available ELISA for fecal antigen is useful, but false-positive reactions can occur with some feeds. Disease related to mucosal epithelial kinetics, not immune status. Adult SCID mice, like immunocompetent mice, do not develop lesions. International Union of Microbiological Societies 2000 has renamed MuRV-A/EDIM.

**Reoviridae**
**Reovirus 1,2,3; Murine Reovirus (MRV)**

1. Prevalence: moderate
2. Diagnosis: serology
3. Disease: Usually subclinical. Historic literature describes CNS disease, hepatitis, diarrhea with steatorrhea and "oily hair effect (OHE)." Experimental inoculation of neonatal mice with prototype Reovirus 1 and 3 results in myocarditis, encephalitis and hepatitis, with virus replication in many tissues. Reovirus 2 causes EDIM-like lesions.
4. Transmission: orofecal
5. Duration: acute
6. Comment: Genus Orthoreovirus; Family Reoviridae. Mammalian reoviruses are divided into 3 serotypes: 1, 2 and 3, which represent many different strains. Reovirus 3 only serotype reported to cause natural disease in mice, but mice are susceptible to infection by all. Cross-react serologically, so cannot accurately distinguish infecting serotype. International Union of Microbiological Societies 2000 has renamed the virus "Murine Reovirus (MRV)", as if only mice are the only natural host and only mice are susceptible!

**Retroviridae**

There are literally thousands of copies of retrovirus-like sequences scattered throughout the genome of the mouse. These include IAP's (intracisternal A particles), VL30's (virus like 30S RNA sequences), MuRRS's (murine retrovirus-related DNA sequences), GLN's (tRNA glutathione like sequences), MuRVY's (murine repeated virus sequences on the Y chromosome), and ETn's (early transposons). These are ancient acquisitions within the mouse genome, but remain active as retrotransposons, and contribute to spontaneous mutations and subline divergence. More recent acquisitions that are included within the murine genome are endogenous MuLVs and MMTVs. Exogenous MuLVs and MMTVs are horizontally transmissible, and not incorporated within the genome.

**A. Murine Leukemia Virus (MuLV)**

1. Prevalence: 100%. No laboratory or wild *Mus musculus* has been found without MuLV provirus within its genome.
2. Diagnosis: not done
3. Disease: variable, depending upon genotype of mouse. Disease manifestations may include neoplasia, demyelination, dilute color (DBA mice), hairlessness (HR mice), greyness, etc. Most MuLVs are not oncogenic.
4. Transmission: horizontal (exogenous) or vertical (endogenous). Endogenous proviruses inherited as

Mendelian genetic characteristics (up to 70 copies of ecotropic MuLVs are scattered throughout the genome in strain-specific patterns).
5. Duration: persistent, chronic and latent
6. Comment: Exogenous viruses are horizontally transmitted (through saliva, milk, semen) like conventional viruses, but no longer exist in laboratory mice. Wild mice have exogenous retroviruses and these are the historic source of some laboratory strains, including Friend, Moloney and Rauscher (FMR) viruses. Endogenous viruses are part of the mouse genome and all mouse strains carry endogenous provirus sequences, most of which are defective or incomplete. Each mouse strain has its own endogenous MuLV gene patterns, inserted in different multiple sites within the genome. Because these proviruses are "genes," they are given gene designations (for example, AKR mice carry multiple copies of the same endogenous provirus gene *akv-1, akv-2, akv-3, akv-4*). Provirus sequences encode both replication competent (few) and replication-defective (many) viruses. These are major determinants of strain-specific characteristics. For example, MuLV germ line insertions result in dilute coat color of DBA mice and lack of hair in hairless mice. Ecotropic (primarily endogenous viruses), xenotropic (primarily endogenous viruses), amphotropic (exogenous viruses in wild mice only), and polytropic (usually recombinants with different receptor tropism than amphotropic) behavior and host determinants (restriction genes *fv-1, -4*) and receptors are important in evolution of recombinant viruses which are pathogenic. Non-replicating, endogenous MuLV sequences can recombine with replication-competent MuLVs to produce oncogenic recombinants, called mink cell focus (MCF) forming viruses that are capable of circumventing host receptor restriction (interference) as polytropic viruses with alternate receptors. Important host determinant is *fv-1*, with *B* (BALB) and *N* (NIH) alleles that determine specificity of Friend virus (Fv), and other ecotropic MuLVs. Expression of non-oncogenic endogenous MuLVs occurs at different times and in different tissues, with evolution of recombinant, oncogenic variants (sometimes). MuLVs are often called "acute" or "non-acute," referring to rate of cancer induction. Acute viruses are largely experimental anomalies that have transposed host proto-oncogenes that have direct and immediate effects upon host cell division and are "defective." Defective viruses need "non-acute" ecotropic virus to complete replication ("helper" virus). Acute viruses are often associated with sarcomas…thus once called "sarcoma" viruses. Non-acute MuLVs occur naturally, and are represented by the endogenous, replication-competent forms. These viruses have longer latent periods for induction of disease, and generally work by genomic insertion (in contrast to proto-oncogene effects of acute viruses).

**B. Murine Mammary Tumor Virus (MMTV)**

I. Prevalence: nearly 100% among laboratory strains of mice, although there is a line of "Lake Casitas" mice that was found without endogenous *mtv.*
2. Diagnosis: not done
3. Disease: variable expression of mammary neoplasia and lymphoproliferative disease, depending upon mouse strain.
4. Transmission: same as MuLV. Exogenous (Bittner milk agent or MMTV-S) transmitted via milk, saliva & semen. Endogenous provirus (0 to 4 copies) in genome of most strains, with gene designations…*mtv-1, mtv-3,* etc. Previously named MMTV-O, -P, -L, -X, etc.
*5.* Duration: persistent
6. Comment: Exogenous viruses eliminated by foster nursing or rederivation. Bittner agent intentionally maintained in some populations of C3H mice as a model system (C3H-MMTV+). SJL mice develop B cell lymphoproliferative disease due to expression of MMTV T cell superantigen (*Sag).* B cells express *Sag*, which acts as a T cell mitogen which stimulates T cells to elaborate lymphokines in excess, resulting in B cell proliferation. B cells that express virus home to mammary gland, allowing infection of mammary tissue and subsequent virus excretion in milk. *Ecotropic* MMTV is also responsible for thymic leukemia in GR mice. Thus, not all MMTVs produce mammary tumors, and some produce leukemias. Unlike MuLVs, MMTVs do not depend upon recombination for oncogenesis, but rather direct insertional activation of proto-oncogenes. The LTR region of MMTV is used as a mammary gland specific promoter in transgene constructs. Although other species, including humans, have mammary tumor virus sequences within their genome, the mouse is the only species that is known to have replication-competent mammary tumor virus.

*** International Union of Microbiological Societies (2000). Virus Taxonomy. Seventh Report of the International Committee on Taxonomy of Viruses. MHV van Regenmortel, et al. (Eds), Academic**

**Press, San Diego, pp. 311-323.**

## II. BACTERIA

*Burkholderia gladioli*
*Chlamydia muridarum*
*Chlamydophila psittaci*
Cilia-Associated Respiratory (CAR) Bacillus
*Citrobacter rodentium*
*Clostridium difficile*
*Clostridium perfringens*
*Clostridium piliforme* (aka *Bacillus piliformis*)
*Corynebacterium bovis*
*Corynebacterium hoffmani*
*Corynebacterium kutscheri*
*Eperythrozoon coccoides*
*Escherichia coli*
*Helicobacter spp.*
*Klebsiella oxytoca*
*Lawsonia intracellularis*
*Leptospira ballum*
*Mycobacterium avium-intracellulare*
*Mycobacterium chelonae*
*Mycoplasma spp.*
*Pasteurella pneumotropica*
*Proteus mirabilis*
*Pseudomonas aeruginosa*
*Salmonella enterica*
*Staphylococcus aureus*
*Staphylococcus xylosus*
*Streptobaeillus moniliformis*
*Streptococcus spp.*

### *Burkholderia gladioli*

1. Prevalence: uncommon
2. Diagnosis: lesions, culture
3. Disease: otitis media in athymic nude mice
4. Transmission: orofecal (presumptive)
5. Duration: unknown
6. Comment: Single case report of outbreak among nude mice. Bacterium isolated from environment, feces, sipper tubes, drinking water, and soiled bedding. Consistently isolated from oropharynx.

### *Chlamydia muridarum*

1. Prevalence: unknown, but probably rare
2. Diagnosis: lesions, isolation (cell culture, egg inoculation, elementary bodies in impression smears, serology-IFA)
3. Disease: no natural disease reported, except after serial mouse-mouse passage of lung tissue from subclinically infected mice. Eventually causes interstitial pneumonia, with dyspnea.
4. Transmission: probably respiratory.
5. Duration: probably persistent.
6. Comment: “Nigg” agent. Clara Nigg discovered it during attempts to isolate influenza virus from human throat washings by intranasal inoculation of mice. Also called “grey lung disease” agent and mouse pneumonitis agent. Mouse biovar (MoPn) was once thought to be C. trachomatis, but it does not share type-specific antigens with 2 human strains of *C. trachomatis* (bivars trachoma and lymphogranuloma venereum). Recent evaluation of 16S rRNA sequences have reclassified the Chlamyidae into two major phylogenetic groups: Chlamydophila, which branches into three clusters, including *C. pneumoniae*, *C. pecorum*, and a third group which includes *C. psittaci*, *C. abortus*, *C. caviae*, and *C. felis*; and Chlamydia, which branches into two major groups of *C. suis* and a branch including *C. trachomatis* and *C. muridarum*. Thus, *C. muridarum* is now considered closely related to *C. trachomatis*, but genetically

distinct. MoPn is increasing in popularity as an experimental respiratory infection model.

***Chlamydophila psittaci***

1. Prevalence: sporadic
2. Diagnosis: elementary bodies in impression smears, egg isolation.
3. Disease: pulmonary lesions in mice following serial intranasal passage of lung tissue; splenomegaly, hepatomegaly, serofibrinous peritonitis with ascites in mice following i.p. passage of liver/spleen tissue. Natural infection apparently subclinical and persistent.
4. Transmission: probably respiratory.
5. Duration: probably persistent.
6. Comment: little is known about natural *C. psittaci* infection in mice, but it has been documented at least twice in the 1970's in conventional mice following mouse-mouse passage of mouse tissue. See Comments under Chlamydia, above.

**Cilia-Associated Respiratory (CAR) Bacillus**

1. Prevalence: common
2. Diagnosis: serology available, but not used much in US. PCR increasing. Although apparent on H&E to the trained observer, silver stains reveal organisms among cilia of respiratory epithelium.
3. Disease: None to chronic suppurative pneumonia. Experimental infection results in peribronchiolar lymphoplasmacytic infiltrates, similar to Mycoplasma. Probably also a B cell mitogen. Experimental studies reveal lesions to be more severe in BALB mice vs. B6 mice. Mice are more susceptible to experimental infection than rats, but natural disease is more prevalent in rats. Can be a co-infection with *Mycoplasma pulmonis,* Sendai virus, etc.
4. Transmission: direct contact
5. Duration: chronic
6. Comment: Rats, rabbits, humans also infected with CAR bacilli, but with antigenically diverse members of the group. CAR Bacillus is an unclassified agent related to *Flexibacter* and *Flavobacterium spp.* (gliding bacteria group).

***Citrobacter rodentium*** (Transmissible Murine Colonic Hyperplasia)

1. Prevalence: rare
2. Diagnosis: culture, lesions. Must culture multiple mice in early stage of infection, as agent is often absent when clinical signs and lesions are overt.
3. Disease: rectal prolapse, sticky feces. Thickening of the descending colon and cecum due to mucosal hyperplasia. Inflammation and erosion variable, but tend to occur most in young mice. Genotype, age and diet are factors. Agent attaches to surface mucosa, but disappears at the time of peak disease (around 2 weeks). Immunodeficient mice cannot eliminate organisms, but develop mild, transient lesions. Thus, acquired immune responses appear to play a role in pathogenesis.
4. Transmission: direct contact, inefficient
5. Duration: acute (around 2 weeks) with no carrier state
6. Comment: Old name was *Citrobacter freundii*; pathogenic strain (4280). Agent is non-motile with characteristic sugar fermentation profile. Not all Citrobacters are pathogenic in mice and *C. rodentium* does not cause disease in other species. Eliminate by improving husbandry. This agent is currently popular as a model bacterium for studying attaching and effacing enteropathogenic *E. coli* (EPEC) and enterhemorrhagic *E. coli* (EHEC). *C. rodentium*, EPEC and EHEC share a similar pathogenicity island called locus of enterocyte attachment (LEE) that carries the determinants for type III secretion system. Type III secretion system allows the bacteria to secrete proteins into target cells, which then facilitate attachment.

***Clostridium difficile***

1. Prevalence: unknown, but common in some sources of mice
3. Diagnosis: lesions, culture, toxin assay.
3. Disease: thrombosis and necrosis of duodenal villus tips, mucosal edema, enterocyte necrosis and exfoliation with mild hyperplasia in large intestine.
7. Transmission: orofecal

8. Duration: unknown, but possibly transient as with other Clostridia.
6. Comment: Clostridial overgrowth with enteropathy is symptomatic of another underlying factor, such as antibiotic "toxicity", dysbiosis, stress, diet change, etc. Mice can develop enteric lesions with overgrowth of *C. perfringens*, *C. difficile*, and possibly *C. spiroforme.*

***Clostridium perfringens***

1. Prevalence: rare
2. Diagnosis: culture, toxin assay, lesions
3. Disease: sporadic outbreaks of necrotizing enterocolitis in post-weaning mice and lactating mice. Lesions consist of mucosal necrosis, hyperplasia and edema.
4. Transmission: orofecal
5. Duration: unknown
6. Comment: Clostridial overgrowth with enteropathy is symptomatic of another underlying factor, such as antibiotic "toxicity", dysbiosis, stress, diet change, etc. Mice can develop enteric lesions with overgrowth of *C. perfringens*, *C. difficile*, and possibly *C. spiroforme*.

***Clostridium piliforme*** (Tyzzer's Disease)

1. Prevalence: rare (once common)
2. Diagnosis: PCR of feces or tissues. Lesions are diagnostic, with the use of special stains to reveal typical intracytoplasmic organisms. Serology developed but often not used in US.
3. Disease: Sudden death with or without diarrhea. Colitis and/or typhlitis with dissemination to liver (focal hepatitis) and occasionally heart (myocarditis). Special stains (silver, Giemsa, PAS) reveal intracytoplasmic fascicles of bacteria. Enteric lesions have intracellular fascicles of organisms in enterocytes, and smooth muscle necrosis (with fewer organisms) is frequent. Immunosuppression exacerbates disease in carrier mice and stress predisposes to disease. Resistance to disease has been reported to be B-cell mediated. In experimental studies, nude mice (T cell deficient) were no more susceptible to disease than immunocompetent mice, but in other studies, the converse seemed to be true.
4. Transmission: Orofecal via spores. Spores survive for > 1 year. Recent experience suggests that transmission appears to be mediated by direct contact and the agent may not be highly contagious.
*5.* Duration: unknown
6. Comment: Old name was *Bacillus piliformis*. Cannot be grown in cell-free medium, but can be grown in cell culture. Partial species-specificity of isolates. Depopulate. In a recent outbreak, T cell receptor beta knock out mice had clinical disease, but other triggering events seem to be needed, even in SCID, RAG and NUDE mice. Probable triggering mechanism is weaning, intestinal dysbiosis, and other stressors.

***Corynebacterium bovis*** (***pseudodiphtheriticum***)

1. Prevalence: rare
2. Diagnosis: culture, lesions, Gram stain of lesions.
3. Disease: orthokeratotic dermatitis in nude mice. High mortality in suckling mice, transient disease in weanlings, asymptomatic in adults.
4. Transmission: contact
5. Duration: unknown
6. Comment: sporadic problem in nude mouse colonies

***Corynebacterium hoffmani***

1. Prevalence: common
2. Diagnosis: culture, lesions
3. Disease: conjunctivitis, especially in BALB mice
4. Transmission: direct contact
*5.* Duration: unknown, chronic
6. Comment: frequently associated with conjunctivitis, but cause-and-effect not fully established. Probably opportunistic pathogen.

***Corynebacterium kutscheri*** (Pseudotuberculosis)

1. Prevalence: rare (once common)
2. Diagnosis: culture, Gram stain of lesions, serology (not used)
3. Disease: Caseopurulent abscesses containing prominent colonies of Gram-positive bacilli (Chinese letter configurations) at edge of lesions. Lesions in liver, kidney, lung and other sites. Cervical lymph nodes may be enlarged, but not abscessed. Subclinical carrier state common. Immunosuppression and other stressors cause exacerbation of disease.
4. Transmission: direct contact
5. Duration: chronic, but probably not latent
6. Comment: Pathogenesis not fully understood in mouse (see rat). Depopulate.

***Eperythrozoon coccoides***

1. Prevalence: rare (once common)
2. Diagnosis: blood smear, Giemsa stain; splenectomy/immunosuppression
3. Disease: subclinical or anemia, splenomegaly, RE proliferation. Intra- and extracellular forms
4. Transmission: via blood; *Polyplax serrata* vector
5. Duration: chronic, with eventual recovery
6. Comment: potentiates MHV, LCMV, LDHV. Occasional contaminant of biological products. No longer considered Rickettsiales; reclassified as a member of Class Mollicutes, Order Mycoplasmatales (related to Mycoplasma).

***Escherichia coli***

1. Prevalence: common intestinal inhabitant, disease variable
2. Diagnosis: culture, lesions
3. Disease: hyperplastic typhlocolitis in immunodeficient mice (SCID and multiple deficient, but not nude). Overgrowth in infant mice with viral enteritis.
4. Transmission: orofecal
*5.* Duration: chronic
6. Comment: Immunodeficient mice infected with an otherwise non-pathogenic, nonlactose fermenting *E. coli*. Some of these infections may be mis-diagnosed *Citrobacter rodentium*, but others seem to be *E. coli* (author's experience). Disease requires multiple immune deficiencies, and may be complicated by Helicobacter. Reports of this agent/disease were before awareness of Helicobacter, which produces similar lesions. Other typical *E. coli* strains may overgrow in infant mice with viral enteritis.

***Helicobacter hepaticus***
***H. bilis***
***H. muridarum***
***H. rodentium***
***H. typhlonius***
***H. ganmani***
***et al.***

1. Prevalence: presumably common, but unknown
2. Diagnosis: lesions, silver stain, culture (requires special medium, microaerophilic conditions); PCR; membrane antigen extract ELISA (has partial species specificity). Recombinant protein antigens tend to be sensitive and specific, but not useful for detecting cross-reactive antibody against all species of Helicobacter.
3. Disease: acute to chronic hepatitis, which may be associated with increased incidence of hepatic neoplasia; chronic typhlocolitis, proliferative typhlocolitis
4. Transmission: oro-fecal
5. Duration: chronic
6. Comment: There are 27 named species of Helicobacter, and over 35 yet to be named. They infect a wide variety of host species, and appear to have only weak host species specificity. Mice can be naturally infected with *H. hepaticus*, *H. bilis*, *H. muridarum*, *H. (Flexispira) rappini*, *H. rodentium*, *H. typhlonius*, *H. ganmani* and probably other unnamed species. Mice can be experimentally infected with an even longer list, including *H. trognontum* (isolated from rats). *H. muridarum* may be associated with

chronic gastritis. *H. hepatiticus* and *H. bilis*, separately or in combination and possibly other species, are associated with chronic typhlocolitis in immunodeficient mice. Infection with one may modify disease caused by another, such as the protective effect of *H. hepaticus* that has been demonstrated in mice experimentally infected with *H. bilis*. Infection of mice with Helicobacter has been shown to abrogate sensitivity to experimental allergic encephalomyelitis in some strains of mice. As awareness of these syndromes increases, typhlocolitis has been found in immunosufficient mice that are naturally infected with *H. hepaticus*, including A/J, BALB/c, Swiss Webster and ICR mice. There is a curious gender pattern of disease susceptibility to *H. hepaticus*, with male mice being more susceptible to hepatitis, and females to enterocolitis. There is also a mouse genotype-related susceptibility to *H. hepaticus* disease, with A/JCr mice noted to be susceptible and C57BL mice resistant, and this is reflected in antibody titers. *Flexispira rappini* is *Helicobacter*, based upon 16S ribosomal DNA gene sequence, but sequence data also reveal that *H. rappini* is a mixture of *Helicobacter* species. Helicobacter-infected mice may be bacteremic, as evidenced by contamination of transplantable xenografts in SCID mice.

***Klebsiella oxytoca***

1. Prevalence: low
2. Diagnosis: culture
3. Disease: unusually high prevalence of suppurative female reproductive tract lesions reported in an infected colony of aging mice. Also associated with ascending urinary tract infections and otitis media.
4. Transmission: contact
*5.* Duration: chronic
6. Comment: this bacterium is basically an opportunistic bacterium, similar to other gram-negative bacteria in the mouse.

***Lawsonia intracellularis***

1. Prevalence: low
2. Diagnosis: lesions, PCR, and silver stains reveal typical coccobacillary forms in apical cytoplasm of enterocytes.
3. Disease: proliferative bowel lesions.
4. Transmission: orofecal (presumptive)
*5.* Duration: chronic (presumptive)
6. Comment: *Lawsonia intracellularis* is a single species of bacterium with no known genetic, biologic, or serologic differences detectable among isolates derived from birds and a wide variety of animals. Thus, this pathogen has a wide host range, and since it commonly infects laboratory hamsters, rats, rabbits, and other species, the opportunity for mouse exposure is there. Mouse infection is not reported in literature, but it has been observed and verified by PCR.

***Leptospira ballum***

1. Prevalence: rare in laboratory mice, common in wild mice. Not tested for in laboratory mice, so true prevalence not known.
2. Diagnosis: culture
3. Disease: none in mice.
4. Transmission: urine
5. Duration: chronic
6. Comment: *L. ballum* is the most common leptospire in wild mice and mouse-associated zoonoses. Mice do not generally develop lesions.

***Mycobacterium avium-intracellulare***

1. Prevalence: rare
2. Diagnosis: lesions, Acid-fast stains, culture
3. Disease: granulomata in lungs, liver, mesenteric lymph nodes reported in C57BL/6 mice.
4. Transmission: water?
5. Duration: chronic
6. Comment: single report. C3H/HeN and B6C3FI mice, F344 rats negative.

***Mycobacterium chelonae***

1. Prevalence: rare
2. Diagnosis: lesions, Acid-fast stains, culture
3. Disease: granulomatous inflammation of the tail in immunodeficient mice
4. Transmission: contact?
5. Duration: chronic
6. Comment: single report

***Mycoplasma pulmonis***
***M. arthritidis***
***M. neurolyticum***
***M. collis***

1. Prevalence: *M. pulmonis* moderate. *M. arthritidis* less common and others rare or nonexistent.
2. Diagnosis: culture, serology, lesions (if present), Serology utilizes *M. pulmonis* as antigen, with variable cross-reactivity among different strains. *M. arthritidis* infection can cause seroconversion to *M. pulmonis*.
3. Disease: Mycoplasmosis. *M. pulmonis* is associated with chronic respiratory disease. It tends to produce chronic suppurative rhinitis, otitis and bronchopneumonia in mice, often in concert with other agents (Sendai virus, CAR bacillus, etc.). Mycoplasmas attach to apical cell membranes of mucosal epithelial cells. *M. pulmonis*, *M. arthritidis* and *M. neurolyticum* inhabit the upper respiratory tract and *M. collis* inhabits the genital tract. Only *M. pulmonis* is a significant natural pathogen. Infections are often subclinical, but disease can be precipitated by viral infections. *M. arthritidis* and *M. pulmonis* cause arthritis when given intravenously and *M. neurolyticum* elicits an exotoxin causing neurolytic "rolling disease" when given intracerebrally. B6 mice are disease-resistant.
4. Transmission: respiratory, other
5. Duration: chronic
6. Comment: depopulate. Test and cull can be effective. Mycoplasma is a strong B cell mitogen, resulting in typical lymphoplasmacytic infiltrates in peribronchiolar regions of infected rodents. Recently, *M. arginni*, a contaminant of a human cell line, was found to be associated with polyarthritis in SCID mice inoculated with contaminated cultured human cells.

***Pasteurella pneumotropica***

1. Prevalence: high prevalence of infection, sporadic disease
2. Diagnosis: culture, lesions
3. Disease: opportunistic organism. Normal gut microflora. High frequency of isolation from nasopharynx and gut of normal mice. Associated with conjunctivitis, ophthalmitis, periorbital abscesses, otitis, pneumonia, cystitis, prepucial gland abscesses, subcutaneous abscesses, pyometra, etc. Dermatitis in nude mice.
4. Transmission: normal flora
5. Duration: chronic
6. Comment: emerging disease is suppurative bronchopneumonia in partially immunodeficient mice co-infected with *Pneumocystis murina*. Opportunistic pathogen.

***Proteus mirabilis***

1. Prevalence: agent common, disease sporadic
2. Diagnosis: culture, lesions
3. Disease: genitourinary infections, otitis. Septicemia reported in immunodeficient mice: fibrinopurulent peritonitis, hepatitis, pneumonitis, splenomegaly and significant mortality noted in SCID mice.
4. Transmission: contact, environmental
5. Duration: unknown

***Pseudomonas aeruginosa***

1. Prevalence: agent common, disease conditional
2. Diagnosis: culture, clinical disease associated with neutropenia

3. Disease: Opportunistic. Mice that are exposed to organism while neutropenic (x-irradiation, cytoxan) develop bacteremia and high mortality. Pseudomonas does not permanently colonize mice, but passes transiently through GI tract following exposure from water bottle sipper tubes. Organisms invade naso-squamous junction in nose.
4. Transmission: water bottle (sipper tube) contamination
5. Duration: transient but repeated infection
6. Comment: control with water treatment (acidification or chlorination). Water treatment has become an industry standard, but may cause more morbidity (dental erosion) than Pseudomonas.

***Salmonella enterica***

1. Prevalence: rare (once common)
2. Diagnosis: culture, lesions. Mesenteric lymph nodes are most consistent site for culture.
3. Disease: Salmonellosis. Normal microflora contribute resistance. Bacteria invade Peyer's patches of ileum to mesenteric lymph nodes to liver, spleen and blood, bile ducts, intestine (enterohepatic cycle). Disease dependent upon host/agent factors. Lesions include enteritis (terminal small intestine and cecum), mesenteric lymphadenopathy, Peyer's patch hyperplasia, hepatic granulomas, splenomegaly. Infection is often subclinical.
4. Transmission: intermittent fecal shedding
5. Duration: usually less than 1 month. Approximately 5% carrier rate among mice in an infected population.
6. Comment: Salmonella classification continues to change. Mammalian Salmonellas are now grouped under *S. enterica*, with over 2500 named serovars All serovars are potentially pathogenic, but serovar typhimurium is most common in naturally infected mice. Zoonotic agent. Depopulate.

***Staphylococcus aureus***

1. Prevalence: common
2. Diagnosis: culture, lesions
3. Disease: Abscesses, especially cervical lymph nodes with botryomycotic features in immunocompetent mice. Furunculosis and cervical lymph node abscesses in nude mice. Ulcerative dermatitis, often secondary to hypersensitivity dermatitis due to acariasis. Lesions feature prominent colonies of bacteria in surface exudate, with coagulation necrosis to varying depths of the skin. Skin lesions are reminiscent of burns associated with production epidermolytic exotoxins (analogous to Staphylococcal Scalded Skin Syndrome in humans). Epidermolysins are plasmid-encoded, with evidence of transfer to *Streptococcus mutans*. Also a factor in *Staphylococcus hyicus* dermatitis in pigs.
4. Transmission: environmental
5. Duration: chronic

***Staphylococcus xylosus***

1. Prevalence: common
2. Diagnosis: culture, lesions
3. Disease: Lesions on the tail of SJL mice reported, with most severely affected having sloughing and necrosis of tail. Also report of ulcerative dermatitis in nude mice. Microscopic features similar to *Staphylococcus aureus* burn-like lesions.
4. Transmission: environmental
5. Duration: chronic

***Streptobacillus moniliformis***

1. Prevalence: rare in laboratory mice
2. Diagnosis: culture - pleomorphic, non-motile filamentous rods
3. Disease: septicemia, diarrhea, conjunctivitis, hepatitis, serosal hemorrhages, and suppurative arthritis.
4. Transmission: carried and introduced by rats, then mouse-to-mouse contact transmission
*5.* Duration: chronic
6. Comment: carried in oropharynx of rats. Cause of rat-bite fever in humans (also *Spirillum minus*)

***Streptococcus spp.***

1. Prevalence: low
2. Diagnosis: culture, lesions
3. Disease: outbreaks of high incidence ulcerative dermatitis reported with beta hemolytic Streptococcus. Alpha hemolytic Streptococcus also reported in SCID mouse septicemia, Group B Streptococcus reported to cause rhinitis, meningitis and encephalomyelitis in nude mice. Endocarditis sporadic. Recent reports of dermatitis associated with *Streptococcus zooepidemicus*.
4. Transmission: contact
5. Duration: chronic
6. Comment: mice resistant to *Streptococcus pneumoniae* (Diplococcus)

## 7. FUNGI

*Aspergillus sp.*
*Encephalitozoon cuniculi*
*Paecilomyces variotii*
*Trichophyton mentagrophytes*
*Pneumocystis murina*

***Aspergillus sp.***

1. Prevalence: rare disease
2. Diagnosis: lesions, culture
3. Disease: pulmonary granulomas due to *Aspergillus terreus* in immunodeficient genetically altered mice (gp91 phox null) maintained on corncob bedding.
4. Transmission: inhalation from contaminated corncob bedding
5. Duration: chronic
6. Comment: opportunistic fungus: see Paecilomyces, below.

***Encephalitozoon cuniculi*** (formerly *Nosema muris*, *E. muris*, *E. rabii*, *N. cuniculi*)

1. Prevalence: rare
2. Diagnosis: lesions (rare), serology (not usually done), Gram stain for spores
3. Disease: Clinical signs in immunocompetent mice are negligible. Ascites and retardation of transplantable tumor growth have been reported. Immunodeficient mice (cortisone-treated, nudes, etc.) develop lethal disease, with ascites, wasting syndrome, enteritis, encephalitis, hepatitis, interstitial nephritis, splenomegaly. Microscopic findings include necrosis and nonsuppurative inflammation of multiple tissues, with numerous organisms.
4. Transmission: urine, transplantable tumors.
5. Duration: persistent
9. Comment: *E. cuniculi* is a common microsporidian parasite that infects a wide variety of mammals, including rabbits, guinea pigs, rats, mice, horses, foxes, cats, dogs, muskrats, leopards, baboons, and humans. There are three major genotypes, among which rabbit agent is genotype I, most human isolates are genotype III, and mouse isolates appear to be genotype II. Host species specificity may not be strict, as there are also group I human isolates. Mice are natural host for *E. cuniculi* only. Most isolates from other species are generally not available for genotyping, or have not been genotyped. Spore infects target cell through extrusion of polar tube, with injection of sporoplasm, which proliferates into many plasmodial cells (aka meronts) within parasitophorus vacuole. Develop into sporonts which undergo binary fission to sporoblasts, then spores, which are infectious for adjacent cells. Spores shed into environment via urine. Now considered to be a member of Kingdom Fungi.

***Paecilomyces variotii***

1. Prevalence: rare disease, commonly isolated from respiratory tract of rodents
2. Diagnosis: lesions, culture

3. Disease: infection usually subclinical. Pulmonary abscesses in NADPH oxidase deficient gp91phox null mice. Note similarity to *Aspergillus* (above)…opportunistic.
4. Transmission: contact.
5. Duration: chronic
6. Comment: opportunistic fungus with similar opportunistic behavior in immunodeficient humans.

***Pneumocystis murina sp. nov.***

Formerly *P. carinii* f. *sp. muris*

1. Prevalence: high rate of infection; disease rate low, except in immunodeficient mice (common)
2. Diagnosis: silver stain of lung lesions for cysts
3. Disease: not pathogenic for immunocompetent host. Steroids/low protein diet and immunodeficient genotypes allow overgrowth. Lungs firm, pale, mottled and do not collapse. Alveoli contain eosinophilic proteinaeceous exudate containing numerous trophic forms and cysts visible with silver stain. Trophic forms attach to type II pneumocytes. Interstitial pneumonia to varying degrees, depending upon host.
4. Transmission: contact aerosol. Immunocompetent mice can serve as carriers and transmit infection.
5. Duration: chronic, mediated by major surface glycoprotein (MSG) antigenic variation.
6. Comment: Rederive or replace immunodeficient mice. Serious life-limiting disease in nude, SCID and other immunologically deficient mice. Disease features vary with immune defect and exacerbated by virus infection, particularly PVM. Marginally immunodeficient, genetically modified mice may manifest atypical, mild forms of Pneumocystis pneumonia. Mouse, rat, human Pneumocystis differ genetically, based upon 18S rRNA gene sequence. Human agent is now *P. jirovecii*; rat agents are *P. carinii* and *P. wakefieldiae*.

***Trichophyton mentagrophytes***

1. Prevalence: rare disease
2. Diagnosis: lesions, culture, histochemistry
3. Disease: infection usually subclinical. Alopecia, focal crusts, especially on head. Favus in severe cases (yellow crusts on muzzle, head, ears, tail, extremities). Hair invasion is not a feature of mouse dermatomycosis.
4. Transmission: contact. Non-selective host range, including humans
5. Duration: unknown
6. Comment: nearly non-pathogenic in mouse. Disease/lesions exceedingly rare

## IV. PROTOZOA

*Cryptosporidium muris, parvum*
*Eimeria spp.*
*Giardia muris*
*Klossiella muris*
*Sarcocystis muris*
*Spironucleus muris*

***Cryptosporidium muris,***
***C. parvum***

1. Prevalence: rare
2. Diagnosis: histology, cysts in feces
3. Disease: both agents are mildly pathogenic and opportunistic, associated with enteritides of various primary origin, malnutrition, and immunodeficiency. Runting in infant mice. Sticky feces and weight loss in immunodeficient mice. Attach to brush border of surface epithelium of stomach (*C. muris*) or small intestine (*C. parvum*). Cholangitis with focal hepatic necrosis observed in nude mice infected with *C. parvum*. Lymphoplasmacytic infiltration of lamina propria, mild mucosal hyperplasia.
4. Transmission: orofecal, oocysts
5. Duration: unknown
6. Comment: opportunistic overgrowth. Treat primary problem. BOTH *C. muris* and *C. parvum* are zoonotic. Sporozoites invade epithelium, develop within parasitophorus vacuole into type I merozoites,

which replicate asexually with rupture of host cell, reinvasion of other epithelial cells with development into type I or type II merozoites. Type II merozoites develop into micro- and macrogametes, which form oocysts. Apical complex provides intimate association with host cell. Unique intracellular, extracytoplasmic location.

***Eimeria spp.***

1. Prevalence: rare (common in wild mice)
2. Diagnosis: oocysts in feces, lesions
3. Disease: enteritis, typhlitis, colitis with diarrhea, blood. Especially found in young (weanling) mice. *E. falciformis* in cecum and colon; *E. vermiformis* in distal small intestine; *E. papillata* in distal small intestine; *E. ferrisi* in cecum and colon.
4. Transmission: orofecal (oocysts)
5. Duration: moderate chronicity with recovery and strong immunity
6. Comment: Eighteen species of *Eimeria* have been described in *Mus musculus*, of which 4 species are pathogenic (*E. falciformis, E. vermiformis, E. papillata, and E. ferrisi*). Meronts, microgametes, macrogametes and oocysts.

***Giardia muris***

1. Prevalence: low (common in wild mice)
2. Diagnosis: cysts in feces; trophozoites in small intestine (wet mounts or histology)
3. Disease: often subclinical. Abdominal distention, yellow-white watery digesta in small intestine. Trophozoites aligned along brush border of villi. Lymphoplasmacytic infiltrates of duodenal and jejunal lamina propria. Opportunistic pathogen in mice, usually associated with other primary disease or immunodeficiency. Nude mice develop chronic enteritis, weight loss.
4. Transmission: orofecal (cysts)
5. Duration: approximately 1 month, except in immunodeficient mice
6. Comment: treatment controls disease. Hamsters frequently infected and infections are chronic with chronic malabsorption, enteritis, diarrhea, weight loss.

***Klossiella muris***

1. Prevalence: rare, except in wild mice (very common)
2. Diagnosis: histology, oocysts in urine
3. Disease: usually subclinical. Key target is kidney. Sporozoites enter portal bloodstream after ingestion of sporocysts, spread throughout the body. Schizogony in glomerular endothelium (usually not apparent); gametogeny and sporogony in tubular epithelium is obvious feature, with nonsuppurative interstitial nephritis.
4. Transmission: urine (sporocysts)
5. Duration: chronic.
6. Comment: depopulate or rederive

***Sarcocystis muris***

1. Prevalence: rare, except in wild mice. Reports in laboratory mice seem to be on the rise.
2. Diagnosis: histology, cysts in skeletal and heart muscle.
3. Disease: Sarcocysts filled with bradyzoites in myofibers of skeletal muscle and heart (less common). Incidental finding.
4. Transmission: feed contaminated with cat feces, or cannibalism of other infected mice. Cockroaches exposed to cat feces can transmit to mice.
5. Duration: chronic.
6. Comment: depopulate, rederive and/or break infectious cycle. Cat is definitive host.

***Spironucleus muris* (formerly *Hexamita muris*)**

1. Prevalence: common
2. Diagnosis: cysts in feces (Easter eggs); trophozoites in small intestine (wet mounts, histology)

3. Disease: usually subclinical. Opportunistic overgrowth with immunosuppression, immunodeficiency, enteritides of various origin, especially enterotropic MHV. Watery fluid and gas in upper small intestine. Trophozoites seek refugfe in crypts and may distend them. Mucosa hyperplastic and trophozoites may invade lamina propria. Nude mice develop chronic hyperplastic enteritis. Trophozoites in crypt lumina of distal small intestine. Occasional invasion of lamina propria.
4. Transmission: orofecal (cysts)
5. Duration: chronic
6. Comment: treatment controls disease. Eliminate primary pathogen. The syndrome of spironucleosis is rare and if present, other pathogens (especially enterotropic MHV) should be sought. Spironucleosis can also be seen in immunodeficient mice without other pathogens. *Spironucleus muris* in hamsters and rats may be subspecies, with relative host specificity.

***Toxoplasma gondii***

1. Prevalence: rare
2. Diagnosis: histology. Cysts containing PAS-positive bradyzoites or clusters of tachyzoites in muscle and heart.
3. Disease: Usually subclinical. Early infection may have enlarged, edematous mesenteric lymph nodes with necrosis of ileal lamina propria, focal hepatitis, myocarditis, interstitial pneumonia, and later brain lesions (gliosis, perivasculitis, meningitis). Tachyzoites in intestinal tissue, mesenteric lymph nodes, lung, spleen, brain, skeletal muscle etc. during early infection. Cysts in central nervous system, skeletal muscle and cardiac muscle in chronic infection.
4. Transmission: orofecal (cat feces containing oocysts). Also cannibalism and vertical (in utero) transmission.
5. Duration: chronic
6. Comment: *T. gondii* has a complex life cycle. Oocysts shed in feces of cats, sporulate in the environment; sporozoites released after ingestion and invade intestine of intermediate host (mouse). After invading cell, sporozoites differentiate into tachyzoites and replicate multiple generations. Can infect virtually any cell type. Immune response stimulates tachyzoites to differentiate into bradyzoites with a protective cyst wall. Ingestion of tissue cysts by cat results in release of tachyzoites, invasion of intestinal epithelium, and differentiation into sexually replicating forms (microgametes and macrogametes), with formation of oocysts.

## V. HELMINTHS

A. NEMATODES

*Aspicularis tetraptera*
*Syphacia obvelata*
*(Syphacia muris)*

B. CESTODES

*Cysticercus fasciolaris* (*Taenia taeniaeformis*)
*Rodentolepis nana, diminuta, microstoma*

***Aspicularis tetraptera,***
***Syphacia obvelata*** (Pinworms, oxyuriasis)

1. Prevalence: high
2. Diagnosis: ova in feces. Scotch-tape test for *Syphacia*. Adults in gut lumen. *Aspicularis* ova are symmetrical, *Syphacia* asymetrical.
3. Disease: minimal or none. Mucosal invasion with focal inflammation can be seen in immunodeficient mice. Rectal prolapse attributed to heavy infestations. *Syphacia* deposits eggs around anus, *Aspicularis* does not.
4. Transmission: orofecal. Ova very resistant to dessication and drift in air & dust. Pinworms are often the first to contaminate barrier-maintained mice.

5. Duration: chronic. Immunity develops with age
6. Comment: treatment controls, but seldom eradicates worms. Immune effects reported. *Syphacia muris* (rat pinworm) has been found in a colony of Stat6 KO mice, but other knock out mice in colony (IL4 alpha, IFN-gamma, IL-10) were not affected. Affected mice hosted very heavy parasite burdens in ceca.

***Cysticercus fasciolaris* (*Taenia taeniaeformis*)**

1. Prevalence: rare
2. Diagnosis: gross, microscopic lesions
3. Disease: strobilocercus in liver incidental finding in asymptomatic host
4. Transmission: contamination of food and bedding with cat feces
5. Duration: chronic
6. Comment: rodent is intermediate host for cat tapeworm

***Rodentolepis nana***
***R. diminuta***
***R. microstoma***

1. Prevalence: low in laboratory mice. Common in wild mice. Order of prevalence in wild mice listed above.
2. Diagnosis: ova in feces, adults in intestine
3. Disease: usually subclinical. Local enteritis or pancreatitis. *R. nana* and *R. diminuta* in intestine. *R. microstoma* in bile/pancreatic ducts and duodenum.
4. Transmission: All have arthropod intermediate host. Intermediate host for *H. nana* optional.
5. Duration: chronic
6. Comment: Formerly *Hymenolepis.* *R. diminuta* and *R. microstoma* ova have polar filaments. *R. nana* adult is smallest of the three (dwarf tapeworm). Zoonotic hazard, especially with *R. nana*, as no intermediate host required (superinfection with local mucosal cycle).

## VI. ARTHROPODS

A. MITES

*Demodex musculi*
*Myobia musculi*
*Myocoptes musculinis*
*Radfordia affinis*

*Psorergates simplex*
*Ornithonyssus bacoti*

B. LICE

*Polyplax serrata*

C. FLEAS

*Xenopsylla cheopsis*
*Nasopsyllus fasciatus*
*Leptopsylla segnis*

***Demodex musculi***

1. Prevalence: rare
2. Diagnosis: skin scrapings and histosections
3. Disease: usually subclinical, but dermatitis is being noted in various genetically altered mice, most recently auricular dermatitis in BALB-IL-13 KO mice. Mites in follicular openings of the superficial dermis of dorsal thorax, often with no inflammation.
4. Transmission: contact
5. Duration: chronic

6. Comment: not reported in laboratory mice since 1917 until 1997, when it was found in a colony of transgenic mice with NK and T cell deficiency. Recognition of infestation is on the rise among genetically altered mice.
Has been recently seen in other immunodeficient mice on both east and west coasts. Affected mice harbor few mites, but more severely immunodeficient mice may harbor higher parasite numbers.

***Myobia musculi***

1. Prevalence: common
2. Diagnosis: mites in fur, prefers fur of dorsal neck, shoulders. Single empodial claw on second pair of legs.
3. Disease: often subclinical. Hypersensitivity can occur with ulcerative dermatitiis, pruritis, trauma and erosion of pinnae. Genotype-dependent (C57BL)
4. Transmission: contact
5. Duration: chronic, with immune-mediated equilibrium
6. Comment: usually mixed infestations with other fur mites. Malnutrition, disease predisposes to overgrowth.

***Myocoptes musculinis***

I. Prevalence: common
2. Diagnosis: mites in fur, generalized distribution. Suckers on feet and pigmented third and fourth pair of legs.
3. Disease: usually inapparent, but mild alopecia can occur.
4. Transmission: contact
5. Duration: chronic
6. Comment: usually mixed infestations with other fur mites. Myocoptes dominates and is most common fur mite in laboratory mice.

***Radfordi affinis***

1. Prevalence: common
2. Diagnosis: double embodial claws on second pair of legs.
3. Disease: subclinical
4. Transmission: contact
5. Duration: chronic
6. Comment: usually mixed infestation with other fur mites.

***Psorergates simplex***

1. Prevalence: rare in laboratory mice, common in wild mice
2. Diagnosis: 1 mm white nodules visible on underside of skin around head and neck
3. Disease: follicular cysts filled with keratinized epithelium and mites at the epidermal junction.
4. Transmission: contact
5. Duration: chronic
6. Comment: once common, now rare

***Ornithonyssus bacoti***

1. Prevalence: rare
2. Diagnosis: typical appearing lice in fur and environment
3. Disease: anemia, debilitation and death
4. Transmission: Ornithonyssus lives off host, thus diagnosis may require careful examination of the environment.
5. Duration: intermittent
6. Comment: non-selective host range, including humans. Animal handlers may manifest pruritic dermatitis.

***Polyplax serrata***

1.. Prevalence: rare
2. Diagnosis: lice in fur, nits
3. Disease: irritabilitiy, pruritis, anemia, dermatitis
4. Transmission: contact
5. Duration: chronic, with developing immunity
6. Comment: sucking lice. Vector for *E. coccoides*.

**Fleas** Rare. *Xenopsylla* most common in laboratory mouse colonies. *Leptopsylla* can serve as intermediate host for *Hymenolepis*.

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# Big Steve's Mouse Glossary

Stephen W. Barthold, DVM, PhD
Center for Comparative Medicine
University of California, Davis

**AAALAC International.** Association for Assessment and Accreditation of Laboratory Animal Care International. A voluntary accreditation program for laboratory animal facilities and programs.
**AALAS.** American Association for Laboratory Animal Science.
**Acetylation.** Biochemical process involving addition of acetyl groups to DNA, favoring gene expression.
**Acrocentric chromosome.** Chromosome pattern in which the centromere is assymetrically toward one end and the telomeres are at the ends (in contrast to telocentric and metacentric). Mice do not have acrocentric chromosomes.
**Acrosome.** The cap-like structure structure investing the anterior portions of the head of spermatozoa.
**Agouti**. Pelage characteristics composed of black hairs with subapical yellow bands.
**Allele.** Alternate forms of an autosomal gene or locus situated at the same locus on the maternal or paternal chromosomes. A randomly inserted transgene is not an allele. A targeted mutation of the endogenous locus is an allele.
**Alleleic series.** An array of possible mutant forms of a gene, which usually cause multiple phenotypes.
**Allogeneic**. Strains or individuals that express different alleles of the same gene among 2 otherwise genetically similar mice.
**Antimorph.** A mutant allele that antagonizes the function of another gene and acts in a semi-dominant manner.
**Autosomes.** All chromosomes other than the sex (X and Y) chromosomes.
**Axenic.** Not contaminated by or associated with any foreign organisms (excepting endogenous retroviruses). A.k.a. germfree.
**BAC.** Bacterial artificial chromosome. A vector for cloning large segments of genome.
**Backcross**. Cross of a hybrid (F1) mouse to one of its homozygous parents. Also the cross of an F1 heterozygote with a partner that has the same genotype as one of its parents (when working with non-inbred systems).
**Barrier reared.** Population maintained behind a microbiological barrier. Does not necessarily mean that the animals behind the barrier are pathogen free. Read fine print.
**Base pair.** A pair of nitrogenous bases (usually one purine and one pyrimidine) held together by hydrogen bonds in a double-stranded region of nucleic acid molecule. Commonly used interchangeably with *nucleotide pair*. Normal base pairs in DNA are A-T and G-C; in RNA, A-U and G-C.
**Blastocyst.** The spherical structure produced by cleavage of the fertilized ovum, consisting of a single layer of cells (blastoderm) surrounding a fluid-filled cavity (blastocoele).
**Bruce effect.** The pheromone-driven effect of male urine (especially that of a dominant stranger) on breeding females, in which early pregancy is blocked by fetal resorption and estrus resumes.

**Carrier.** Heterozygote for a recessive allele.
**Centimorgan**. Metric used to describe linkage distances. 1 cM = 1% chance that a marker at one genetic locus will be separated from a marker at a second locus due to crossing over in a single generation. In humans, 1 cM=1,000,000 bp. See linkage map.
**Centromere.** The clear region where the arms of the chromosomes meet. They are highly specialized structural elements that function to segregate eukaryotic chromosomes during mitosis and meiosis.
**Chiasma.** The cross-shaped exchange configuration between non sister chromatids of homologous chromosomes that is visible during prophase I of meiosis.
**Chimera**. An individual mouse composed of distinct cell populations derived from genetically different mice, usually via injection of embryonic stem cells into a recipient blastocyst.
**Chromatid.** Either of the longitudinal subunits produced by chromosomal replication.
**Chromatin.** The aggregate DNA and histone proteins that makes up a eukaryotic chromosome.
**Chromosome.** A unit of genetic material (chromatin) in which the genome is arranged. The mouse has 20 pairs of chromosomes, including 19 autosomal pairs and the X and Y chromosomes.
**Chromosome map.** A diagragm showing the locations and relative spacing of genes along a chromosome.
**Chromosome painting.** Use of differentially labeled, chromosome-specific DNA strands for hybridization with chromosomes to label each chromosome with a different color.
***Cis* configuration.** The arrangement of linked genes in a double heterozygote in which both mutations are present on the same chromosome.
**Cistron.** A nucleotide sequence coding for a single polypeptide.
**Clone.** Organisms derived from a single parent and genetically identical to the parent. The term is also used in genetic engineering, meaning the linking of a specific gene or DNA fragment to a replicable DNA molecule, such as a plasmid or phage DNA.
**Cloned gene or DNA fragment.** A DNA sequence incorporated into a vector and transformed into a host organism.
**Codon.** A sequence of three adjacent nucleotides in an mRNA molecule, specifying either an amino acid or a stop signal in protein synthesis.
**Coisogenic**. Genetically identical, except for a difference at a single locus. More precise than congenic, referring to a strain that differs from another by a single gene mutation from the control strain.
**Commensal.** Populations of house mice that depend upon human-built habitats or food production for survival.
**Compensatory genes.** One or more genes that compensate for gain or loss of function of another gene, including transgene, thereby influencing phenotype.
**Complementation.** When two mutations are combined in an organism and the phenotype is wild type, the mutations are said to complement each other.
**Complex trait.** A multifactorial trait (phenotype) influenced by multiple genetic and environmental factors, each of relatively small effect, and their interactions.
**Conditional mutant**. Conditional expression of genes created by transgenic insertion of genetic material under control of tissue specific promotors.

**Congenic**. Strain that differs from another in the region of a single gene locus. Produced by > 10 successive backcrosses or intercrosses to the control strain.
**Conplastic.** Mice in which the mitochondrial genome from one strain is transferred onto a different genetic background so that the two genetically different strains have identical mitochondrial genomes.
**Consomic.** Strain that differs from another in an entire chromosome.
**Contig.** A set of contiguous, overlapping genomic clones that span a larger region of the genome.
**Coprophagy.** Ingestion of feces. Nearly 1/3 of the mouse's diet is re-ingested feces, allowing acquisition of B vitamins that are generated in the hind gut by microflora to be absorbed in the small intestine.
**Copulatory plug.** Male ejaculate that is a coagulum resulting from mixing of seminal vesicle secretions with coagulating gland secretions. The coagulum is retained in the vagina of females for several hours after intercourse, and can be used as a marker for successful breeding and timed pregnancy.
**Copy number.** The number of transgenes incorporated into the genome.
**CRE-*LoxP*.** Phage-derived CRE recombinase targets specific sites that have been flanked with *LoxP*, allowing genomic excision (gene knock outs). This is usually achieved by crossing a CRE transgenic mouse with a mouse containing a targeted gene that has been "flanked" with *LoxP* sites.
**Cross.** Matings among unlike (genetically distinct) homozygotes.
**Crossing over.** Breaking during meiosis of one maternal and one paternal chromosome, the exchange of corresponding sections of DNA, and the rejoining of the chromosomes. Can result in exchange of alleles between chromosomes.
**Cytoplast.** Enucleated oocyte or zygote (fertilized embryo) used as a nuclear transfer recipient (as in cloning).
**Delila effect.** Trichotillomania, or hair plucking behavior.
**Deme.** A social unit. Can represent an entire genetically-related wild population or a population within a single cage.
**Differential segment.** The region of donor flanking genetic material surrounding a selected locus in a congenic mouse that has been transferred by selective backcrossing onto a recipient mouse.
**Diploid.** A cell or organism with two complete sets of homologous chromosomes.
**Dominant.** Nature of inheritance of a phenotype, not a gene. A dominant phenotype is detectable when only one variant allele can be detected. If both alleles are detected, then co-dominant.
**Ecotropic retrovirus.** Retrovirus, either leukemia virus or mammary tumor virus, with tropism for mouse cells. The most notable ecotropic leukemia virus is the AKR provirus, with homologues and multiple copies among many of the inbred strains.
**Embryonic carcinoma cells (EC cells).** Cells derived from embryonic carcinoma (teratoma) of 129 mice. Generally replaced by embryonic stem cells.
**Embryonic stem cells (ES cells).** Early embryo cells that can replicate indefinitely and which can differentiate into other cells (pluripotential). 129 strain generally used because ES cells of this strain are conducive to manipulation.
**Endogenous provirus.** Retrovirus sequences, entire or partial, occult or expressed, that are inserted within the genome and transmitted as Mendelian characteristics. Some are

replication-competent, others are defective. Refers to both mammary tumor virus and leukemia virus sequences.

**Enhancer element.** A base sequence that increases the rate of transcription of nearby genes; the element need not be adjacent to the transcribed gene and enhancing activity is independent of orientation with respect to the gene.

**ENU.** Chemical mutagen, N-ethyl-N-nitrosourea.

**Epigenetic.** Inherited changes in gene expression resulting from altered chromatin structure or DNA modification (usually methylation) rather than from changes in DNA sequence.

**Epistasis.** Interactions between non-allelic genes in their effects on a trait. Any type of interaction in which the genotype at one locus affects the phenotypic expression of a genotype at another locus.

**ETN's.** Endogenous replication-incompetent murine retrovirus early transposon elements.

**Euchromatin.** Regions of chromosomes that have normal staining characteristics and undergo a normal cycle of condensation; relatively uncoiled regions of chromatin in the interphase nucleus and contains most of the functional genes.

**Exogenous retrovirus.** Retrovirus that is transmitted in a conventional, horizontal (contagious) means.

**Exon.** Sequences in the gene that are retained in the messenger RNA after the introns are removed from the primary transcript.

**Expressed sequence tag (EST).** Partial or complete cDNA sequence.

**Expressivity.** The observed quantitative differences in the expression of a phenotype among individuals that have the same mutant genotype. When quantitative differences are observed, the phenotype has variable expressivity.

**F generation.** Filial generation (generations of brother x sister matings)

**F1 hybrid.** First generation of a hybrid cross between 2 different parental inbred strains. Genetically identical mice that are heterozygous at all loci.

**F2 hybrid.** Progeny of F1 b x s matings. Genetically dissimilar.

**F3, etc.** Subsequent b x s hybrid matings.

**Feral.** Wild populations of commensal species (mice) that have become independent of human habitats or human food.

**Fixation.** A genetic term in which a low frequency of heterozygosity is attained at a given locus through repeated incrossing.

**Flanking genes.** Genes that flank a specific region of interest, such as a transgene. Important issue in incompletely backcrossed mice derived from two parental strains.

**Forward genomics.** Genomic approaches in which the outcome is predicted from the alteration or introduction of a known gene.

**Founder**. First generation mice derived from genetically altered eggs or embryos.

***Fv-1*.** A host resistance gene, with different alleles, that regulates intracellular retrovirus integration and replication. Different alleles (b and n) determine host tropism of ecotropic retroviruses.

***Fv-4***. A defective (replication-incompetent) endogenous retrovirus envelope gene (provirus) that is expressed on the cell surface and blocks entry of other ecotropic retroviruses. Thus, expression of *Fv-4*, in combination with other ecotropic retroviruses, is a major determinant of retrovirus-related phenotype (neoplasia, etc.).

**G.** Generation: G1, G2, G3, etc.
**Gene.** If you don't know this, you shouldn't be reading this glossary. A functional unit encoding protein or RNA whose inheritance can be assayed in genetic crosses, mapping, or inference through sequence.
**Gene dosage.** The number of genes. Many genes are duplicated within the genome, and transgenes often have tandem repeats.
**Gene targeting**. Creation of a null or mutant allele by homologous recombination or targeted gene placement.
**Genetic code.** The set of 64 triplets of bases (codons) that correspond to the 20 amino acids in proteins and the signals for initiation and termination of peptide synthesis.
**Gene trapping.** A mutation strategy that uses random integration of a reporter gene construct into the genome such that productive integration events bring the reporter gene under the transcriptional regulation of an endogenous gene. The inserted sequence acts as a tag from which to clone the trapped or mutated (if insertional mutagenesis occurs) gene.
**Genome.** The entire genetic composition of an organism.
**Genomic library.** A collection of genomic clones that collectively represent the entire genome. Any sequence of interest within the genome is likely to be present in at least one member of the library.
**Genotype.** Genetic composition of the mouse, usually in terms of particular alleles, genes, or loci. Can refer to single genes or loci, or to many.
**Germfree.** Born and raised in a sterile environment free of microbes. No mouse is technically germ free, as all laboratory mice have vertically transmitted retroviruses.
**Germline mutation.** A mutation that takes place in a reproductive cell, and thereby is genetically transmitted as a Mendelian characteristic.
**GLN's.** Endogenous replication-incompetent retrovirus tRNA glutathione like sequences.
**Gnotobiotic**. Population associated and maintained with known microflora.
**H-2 complex.** The major histocompatibility complex (MHC) of the mouse, located on chromosome 17.
**Haploid.** A cell or organism containing the set of chromosomes normally found in gametes.
**Haplotype.** A number of functionally related loci that are closely linked genetically. Alternative sates of such complexes are termed haplotypes.
**Hemizygous**. Contains 1 unique genetic determinant, introduced as a transgene.
**Heterochromatin.** Chromatin that remains condensed and heavily stained during interphase; commonly present adjacent to centromere and telomeres.
**Heterosis.** The superiority of hybrids over either inbred parent; a.k.a. hybrid vigor.
**Heterozygous**. Contains 2 different alleles of the same gene(s).
**Histone.** Small basic proteins that bind to DNA in chromatin.
**Homeobox.** A DNA sequence motif found in the coding region of many regulatory genes; the encoded amino acid structure has a helix-loop-helix structure.
**Homeotic (HOX) gene.** Any of a group of genes in which mutation results in the replacement of one body structure by another body structure.
**Homolog.** Genes are homologous if they evolved from a common ancestor. There is NO DEGREE of homology.

**Homozygous**. Contains 2 identical alleles of the same gene(s).
**Hotspot.** A site in DNA molecule at which the mutation rate is much higher than the rate for most other sites.
**Housekeeping gene.** A gene that is expressed at the same level in all cells and whose product participates in basic metabolic processes.
**Hybrid strain**. Derived by mating 2 inbred strains together.
**Hybrid vigor.** Heterosis.
**Hypomorph.** A mutant allele that does not eliminate the wild-type function of a gene and may give a less severe phenotype than a loss-of-function mutant.
**IAP's.** Endogenous replication-incompetent retrovirus-like elements that form intracisternal A particles.
**ICSI (intracytoplasmic sperm injection).** Microinjection of an ovum with spermatozoa or precursors as a means of fertilization. Can be accomplished with non-viable spermatozoa.
**ICNI (intracytoplasmic nucleus injection).** Microinjection of an ovum with a somatic or germ cell nucleus (karyoplast), a method used for cloning.
**ILAR.** Institute of Laboratory Animal Resources. ILAR is a branch of the National Science Foundation of the National Academy of Sciences which acts as an advisor to the federal government on policies relating to laboratory animals.
**Imprinting.** The expression or repression of genes under male or female parental control that vary from standard genetic mechanisms (dominance and recessive).
**Incipient congenic.** A new term devised by Jax labs for transgenic and targeted mutations in the process of backcrossing onto an inbred background strain, but with generations less than N10.
**Incross.** Matings among like homozygotes.
**Inbred strain.** Derived by b x s matings for >20 consecutive generations and maintained by continuous b x s matings. Parent x offspring may be substituted for b x s matings if in consecutive matings to younger of the 2 parents. There are over 2,400 inbred strains of mice.
**Induced mutant stocks/strains.** Stocks/strains carrying targeted mutations (knock outs, knock ins), retroviral or chemically induced mutations.
**Inducible mutant.** Inducible expression of genes created by transgenic insertion of genetic material under control of tetracycline, neomycin or other regulators. Inducers inactivate a repressor, usually by binding to it and thereby altering the ability of a repressor to bind to an operator.
**Inner cell mass .** The mass of cells within the blastocyst which develop into the corpus of the embryo, including embryonic stem cells. Foreign ES cells are microinjected into the blastocyst, and become incorporated into the inner cell mass, creating a chimera.
**Insertional mutagenesis.** Alteration (mutagenesis) of the genome by insertion of foreign DNA so that the genome is disrupted by homologous recombination or random insertion.
**Intercross.** Matings among two identically heterozygous individuals (F1 hybrids).
**Interspecific cross.** A cross between mice from two different species, such as between *M. musculus* and *M. spretus*, for the purpose of linkage analysis (in order to obtain high level of polymorphism between two parents).

**Intersubspecific cross.** A cross between mice from two different subspecies, such as between *M. musculus domesticus* and *M. musculus castaneus* (a natural intersubspecific cross is *M. molossinus*).
**Intron.** A noncoding DNA sequence in a gene that is transcribed but is then excised from the primary transcript in forming a mature mRNA molecule.
**Isogenic.** Genetically identical.
**IVF.** In vitro fertilization.
**Karyotype.** The number of chromosomes present in a given genome and the morphologic form that they assume (banding patterns, etc.) under microscopic examination. The laboratory mouse has 20 pairs of chromosomes (19 autosomal pairs and the X and Y sex chromosomes).
**Karyoplast.** Isolated donor nucleus, together with its nuclear membrane and envelope of cytoplasm (as in cloning).
**Kilobase (kb).** Unit of length of a duplex DNA molecule equal to 1,000 base pairs.
**Knock out.** A mouse with a null mutant gene created by homologous recombination.
**Knock in.** A mouse with a functional, usually mutated, gene placed into its genome by homologous recombination.
**Laboratory mouse.** Domesticated *Mus* of mixed genetic heritage. Genome heavily *Mus domesticus,* with varying degrees of *M. mollosinus, M. musculus, M. castaneus,* and possibly others, depending upon genotype. Cannot be called *M. domesticus* (sensu stricto) so must use *M. musculus,* the sensu lato designation for the *M. musculus* complex.
**Lee-Boot effect.** The hormonal effect within a group of females maintained in the absence of males, leading to pseudopregnancy and anestrus.
**Ligand.** The molecule that binds to a specific receptor.
**LINE element.** A type of transposable element that lacks long terminal repeats and undergoes transposition via an RNA intermediate; long interspersed element.
**Linkage**. Proximity of 2 or more markers with high probability of being inherited together. The chances of recombination between them is significantly less than 50%.
**Linkage group.** The set of genes present together in a chromosome.
**Linkage map.** Map of relative positions of genetic loci on a chromosome, determined on the basis of how often loci are inherited together. Distance between loci measured in centimorgans (cM).
**Linkage group.** A set of loci in which all members are linked to all other members of the group.
**Locus.** Position on a chromosome of a gene or other chromosome marker (or DNA at that position).
**LTR.** Long terminal repeat region of retroviruses which encode promoter, enhancer and other biologic functions used in genomic constructs and vectors.
**LTR retrotransposon.** A type of transposable element that transposes via an RNA intermediate and that has long terminal repeats in direct orientation at its ends.
**Map units.** Centimorgans.
**Marker**. An identifiable physical location on a chromosome (restriction enzyme site, gene, etc.) whose inheritance can be monitored by an assay such as enzyme activity, protein band, or DNA fragment.
**Megabase (Mb).** Unit of length of a duplex DNA molecule equal to 1 million base pairs.

**Meiosis.** The process by which diploid germ cells segregate their chromosomes into haploid nuclei within eggs and sperm. One replication of the chromosomes is followed by two successive divisions of the nucleus to produce four haploid nuclei.
**Mendelian genetics.** The mechanism of inheritance in which the statistical relations betweent he distribution of traits in successive generations result from genes, random union of gametes, and segregation of hereditary determinants in the reproductive cells.
**Metacentric chromosome.** Chromosome pattern in which the centromere is in the middle of the chromosome and the telomeres are at the ends. Metacentric chromosomes occur in mice with Robertsonian translocations.
**Methylation.** Biochemical process involving addition of methyl groups to DNA, silencing a gene or cluster of genes.
**Microinjection.** The process of inserting a fine needle into the pronucleus of a fertilized ovum for the purpose of transferring foreign DNA.
**Microsatellite markers.** Highly polymorphic, genetically stable 2-4 base pair nucleotide repeats (n=10-60) that are scattered throughout the genome. Length variation of these regions can be detected by PCR, allowing pedigree analysis due to polymorphism among different inbred strains of mice. A.k.a. SSLPs.
**Mitochondrial heteroplasmy.** Presence of more than one genetic origin of mitochondrial DNA within a cell.
**Mitosis.** The process of nuclear division in which the replicated chromosomes divide and the daughter nuclei have the same chromosome number and genetic composition as the parent nucleus.
**MMTV.** Murine mammary tumor virus.
**Mobile DNA.** Transposable elements.
**Modifier genes.** One or more genes of the genome which influence the expression of a gene, including transgenes, thereby modifying phenotype.
**Morula**. The solid mass of blastomeres formed by cleavage of a fertilized ovum, filling all the space occupied by the ovum before cleavage.
**Morula aggregation.** A process for creation of chimeras, in which cells are mixed in the morula stage.
**Mosiac (mosaism).** An organism composed of two or more genetically different types of cells. Specifically used to refer to a mouse consisting of cells with and without a gene alteration, arising from delayed integration of a transgene after the embryonic 2-cell stage.
**MuLV.** Murine leukemia virus.
**Murine**. Relating to rat or mouse.
**MuRRS.** Endogenous replication-incompetent murine retrovirus-related DNA sequences.
**MuRVY.** Endogenous retrovirus replication-incompetent murine repeated virus sequences on the Y chromosome.
**Mutagenesis.** Intentional induction of gene mutations with a mutagen, such as a chemical (such as ENU) or radiation treatment of the germline.
**Mutant.** Any heritable feature that differs from the wild type. Used in context of mutant DNA, allele, gene, chromosome, cell, organism, or phenotype.
**Mutation.** A new allele that arises in an individual that is not present in the genome of either of its parents. Somatic or germ line.

**N.** Backcross generation: N1, N2, N3, etc.
**N1 hybrid.** Backcross of F1 hybrid to one of the parental strains.
**NOD.** Non-obese diabetic mouse strain, which develops diabetes due to immune-mediated islet cell destruction.
**Non-LTR retrotransposon.** A type of transposable element that transposes via an RNA intermediate and that lacks terminal repeats at its ends, such as LINE elements.
**Nuclear transfer.** Process by which a donor nucleus (karyplast) is inserted into an enucleated egg or zygote (cytoplast).
**Null mutation.** A mutation that results in loss of gene function.
**OLAW.** Office of Laboratory Animal Welfare, National Institutes of Health. Develops and monitors, enforces compliance, and provides oversight of Public Health Service policies relating to laboratory animal welfare.
**Oncogene**. A gain-of-function mutation in a cellular gene called a proto-oncogene, whose normal function is to promote cellular proliferation or inhibit apoptosis; oncogenes are often associated with tumor formation and progression.
**Open reading frame (ORF).** A region of DNA or mRNA containing a series of codons uninterrupted by stop codons and therefore capable of coding for a polypeptide chain.
**Ooplasmic transfer.** Process by which healthy cytoplasm from a donor egg is used to enhance the cytoplasmic environment of a recipient egg.
**Operator.** A regulatory region in DNA that interacts with a specific repressor protein in controlling the transcription of adjacent structural genes.
**Operon.** A collection of adjacent structural genes regulated by an operator and a repressor.
**Ortholog.** Genes in two species that have evolved from a single common ancestral gene.
**Outbred**. Stocks of mice maintained by nonstandardized matings. Outbred mice have considerable genetic variability. The term is often used synonymously for randombred.
**P.** Parental generation
**Paralog.** Genes within the same species that have arisen from a common ancestor by duplication and subsequent divergence.
**Penetrance.** The degree to which a mutant genotype is expressed as a phenotype. Incomplete penetrance is usually due to modifiers in the genetic background.
**Phenotype**. Observable characteristics of a cell or organism.
**Pleiotropic effect.** Any phenotype that is a secondary manifestation of a mutant gene. Pleiotropy is the condition in which a single mutant gene affects two or more distinct and seemingly unrelated traits.
**Polygenic.** A genetic characteristic (phenotype) that results from interactions among products of two or more genes with alternative alleles.
**Polymorphism.** Differences among alleles, microsatellites, or SNPs that define different strains of mice.
**Polytropic retrovirus.** Retrovirus, either leukemia virus or mammary tumor virus, with tropism for mouse cells and cells from other species.
**Promoter**. A DNA sequence at which RNA polymerase binds and initiates transcription. Transgene which promotes the expression of a native gene or transgene, either within the context of specific tissues, or ubiquitously.
**Pronucleus**. Either of the 2 haploid gamete nuclei just pror to fusion into a fertilized ovum. Transgenics are generated by microinjection of the transgene into the pronucleus.

**Proteome.** The set of all proteins encoded in a genome.
**Proto-oncogene.** A eukaryotic gene that functions to promote cellular proliferation or inhibit apoptosis, in which gain-of-function mutations (oncogenes) are associated with cancer.
**Provirus.** The genetic sequence of a retrovirus that has been incorporated in the mouse genome.
**Pseudogene.** A gene or sequence that is derived from, and therefore partially homologous to a normal gene, but is not transcribed and does not create a functional protein.
**Pseudopregnant**. Females mated to vasectomized males. Ability to maintain eggs transferred to them without uterine competition from naturally ovulated/fertilized eggs.
**Quantitative trait.** A trait that is usually measured on a continuous scale that results from the combined action of several or many genes in conjunction with environmental factors.
**QTL.** Quantitative trait loci, in which linkage of a particular phenotype is quantatively and statistically determined.
**Randombred**. Stocks of mice maintained by systematic scheme of randomization. Randombred mice are genetically heterogeneous, but genetically stable compared to outbred mice.
**Recessive trait.** Nature of inheritance of a phenotype, not a gene. A recessive phenotype is detectable when both alleles have a particular variant or mutation and can be detected.
**Recipient**. Surrogate dam by embryo transfer. Does not influence the genetic makeup of the introduced embryos. Mated with vasectomized males. Chose strains of mice prone to large litters and with good fostering skills.
**Recombinant congenic strains.** Inbred strain derived by backcrossing one inbred strain onto another for 4 generations, then intercrossing filial generations to create recombinant inbred lines that are dominated by the initial backcrossed recipient line.
**Recombinant inbred strain**. Inbred strain derived by mating pairs of F2 hybrid mice (derived from 2 parental inbred strains), then continuously (>20) b x s mating their descendants to create "recombinant" inbred lines.
**Repressor.** A protein that binds specifically to a regulatory sequence adjacent to a gene and blocks transcription of the gene.
**Reprogramming.** "Resetting" of genetic expression program of donor nucleus so as to assume a program typical of the zygote genome (undoing methylation, imprinting, etc.).
**Reporter gene.** A gene sequence that is introduced into a transgenic construct that "reports" the integration of the contruct within the genome and its subsequence expression, such as LacZ, green fluorescence protein (GFP) etc.
**Restriction fragment.** A segment of duplex DNA produced by cleavage of a larger DNA molecule by a restriction endonuclease enzyme.
**Restriction fragment length polymorphism (RFLP).** Genetic variation in a population associated with the size of restriction fragments remaining after cutting with restriction enzymes and that contain sequences homologous to a particular probe DNA; the polymorphism results from the positions of restriction sites flanking the probe, and each variant represents a different allele. RFLPs are used to determine allelic variation in a gene or locus.

**Restriction map.** A diagram of a DNA molecule showing the positions of cleavage by one or more restriction enzymes.
**Restriction site.** The base sequence at which a particular restriction enzyme makes a cut.
**Retrotransposon.** An inserted genomic element that originated from reverse transcribed mRNA produced from another region of the genome. Proviral sequences can function as retrotransposons, thereby contributing to genetic drift.
**Reverse genomics.** Genomic approaches in which the outcome is not predicted, such as chemical mutagenesis. Phenotypes are identified, then genes are subsequently incriminated. Also a procedure in which mutations are made in cloned genes, then introduced back into the genome to determine effect.
**Robertsonian translocation.** A fusion between the centromeres of two chromosomes to produce a single chromosome (whole arm translocations). Some wild populations of *M.domesticus poschiavinus*, for example, have karyotypes with only 13 sets of chromosomes, seven of which are metacentric. Strains of inbred mice with single Robertsonian translocations are available for experimental use.
**SCID.** Severe combined immunodeficiency. SCID mice have a spontaneous mutation of the *Prk* gene (therefore an allelic variant), resulting in global immunodeficiency. SCID is NOT a strain; it is a mutation with a phenotype. SCID mice are available on various strain genetic backgrounds.
**Segregation.** Separation of pairs of alleles into different gametes during meisosis.
**Selfish DNA.** Transcribable elements that do not benefit the organism, and whose sole function appears to be self-propagation. These sequences do not contribute to the fitness of an organism, and are maintained by their ability to replicate and transpose.
**Sib (sibling).** A brother or sister, each having the same parents. In mice, sibs can occur among multiple litters of the same parents.
**SINE element.** A type of transposable element lacking long terminal repeats that undergoes transposition via an RNA intermediate; short interspersed element (in contrast to LINE elements, long interspersed elements).
**Sister chromatids.** Chromatids produced by replication of a single chromosome.
**SNPs**. Often referred to as "snips," single nucleotide polymorphisms, like microsatellites, can be used for pedigree analysis. SNPs represent minor, but stable variations in nucleotide sequences.
**Somatic cell.** Any cell of a multicellular organism other than the gametes and germ cells from which gametes develop.
**Somatic mutation.** Mutation in the genome of a somatic cell. Somatic mutations are not germ-line transmitted, but are responsible for genetic events within a cell population, such as cancer cells.
**Specific pathogen free. SPF.** Population free of a specific list of pathogens. Does not necessarily mean that all pathogens are on the list. Read the fine print!
**Speed congenics.** A method for accelerating the process of backcrossing of one inbred strain onto another by purposely selecting for maximal recipient strain genetic characteristics using microsatellite or SNP markers.
**Splice acceptor and donor.** The 5' and 3' ends of an exon, respectively.
**SSLPs.** Simple sequence length polymorphisms. A.k.a. microsatellites.
**Start codon.** An mRNA codon, usually AUG, at which polypeptide synthesis begins.
**Stereotypy.** Non-purposeful, repetitive acts of behavior.

**Stock**. Outbred or randombred line of mice. Genetically heterogeneous.
**Stop codon.** One of three mRNA codons…UAG< UAA, and UGA…at which polypeptide synthesis stops.
**Stereotypy.** Repetitive sequences of motor behavior that are topographically and morphologically invariant, often rhythmical, and apparently purposeless.
**Substrain.** An inbred strain that was separated from an inbred ancestor before F40, or separated by greater than 100 generations from a common inbred ancestor, or possesses a known genetic characteristic (mutation or contamination) that distinguishes the substrain from the parental inbred strain.
**Superovulation.** Above-normal ovulation induced by administration of gonadotropins to females prior to mating.
**Swiss mouse.** Any mouse that is genetically derived from a progenitor pool of two female and seven male mice obtained from Andre de Coulon in Lausanne, Switzerland by Clara Lynch at the Rockefeller Institute in 1926. Many Swiss mice are outbred stocks (CD-1, CF-1, CFW, NIH, etc.), but others are inbred (SJL, SWR, FVB, NFS, etc.).
**Sympatric.** Closely related species that have overlapping natural ranges, but do not interbreed, such as *Mus domesticus* and *Mus spretus*.
**Syngeneic**. Genetically identical at every locus. Identical genotypes.
**Syntenic (synteny).** Two loci known to be in the same linkage group. Conserved synteny is a situation in which two linked loci in one species have homologs that are also linked in another species.
**Targeted genomics.** A process of introducing foreign genetic material into targeted locations within the genome by homologous recombination for the purposes of introducing a gain- or loss-of-function mutation of a particular allele.
**TATA box.** The base sequence of 5'-TATA-3' in the DNA of a promoter region. TATA box binding proteins bind in this region.
**Telocentric.** The chromosome pattern in which the centromere is at one end and the telomere is at the other. All autosomal chromosomes and the X chromosome of the mouse are telocentric.
**Telomere.** "Caps" of repeated DNA sequences that protect the ends of chromosomes from degradation. Telomeres shorten at each round of cell division. Mouse telomeres are very long, relative to other species, such as humans.
**Tg.** Abbreviation for "transgenic" used in mouse nomenclature. Refers to random insertion of foreign transgene.
**Tm.** Abbreviation for "targeted mutation" used in mouse nomenclature. Refers to insertion of foreign genetic material by homologous recombination.
**Trait.** Any aspect of the appearance, behavior, development, biochemistry, or other feature of an organism. Phenotype.
***Trans* configuration.** The arrangement in linked inheritance in which a genotype that is heterozygous for two mutant sites has received one from each parent.
**Transcription.** The process by which the information encoded in a template strand of DNA is copied into a single stranded RNA of complementary base sequence.
**Transcriptional activator protein.** Positive control element that stimulates transcription by binding with particular sites in DNA.
**Transfection**. The uptake, incorporation and expression of recombinant DNA by eukaryotic cells.

**Transgene**. A foreign gene introduced by transfection.
**Transgenic**. Organism with genes from another organism placed into its genome through recombinant techniques, usually by microinjection of the pronucleus of fertilized eggs. DNA integration is random.
**Transgenic line.** A transgenic mouse strain in which the transgene has been stably integrated into the germline and inherited as a Mendelian characteristic.
**Translation.** The process by which the amino acid sequence of a polypeptide is synthesized on a ribosome according to the nucleotide sequence of an mRNA molecule.
**Translocation.** Interchange of parts between nonhomologous chromosomes.
**Transposable element.** A DNA sequence capable of moving (transposing ) from one location to another in the genome.
**Transomic.** Transgenic mouse created by microinjection of chromosome fragments into the embryonic nucleus (transfer of intact gene clusters).
**Tumor suppressor gene.** A gene that normally controls cell proliferation or that activates the apoptotic pathway, in which loss-of-function mutations are associated with cancer progression.
**Variegation.** Variable expression of a transgene in the same cell type or tissue within a mouse.
**Vector.** A DNA molecule, capable of replication, into which a gene or DNA segment can be inserted by recombinant DNA techniques; a cloning vehicle.
**VL30.** Replication-incompetent retrovirus-like 30S RNA sequences.
***vSAg.*** MMTV superantigen gene.
**Whitten effect.** The pheromone-related effect of male urine on breeding females, in which the estrus cycle is shortened, cycles among females are synchronized, and onset of puberty in young females is accelerated. Used to advantage for timed breeding.
**Wildtype.** The most common phenotype or genotype in a natural population; a phenotype or genotype arbitrarily designated as a standard for comparison. Wildtype can refer to single alleles or whole genomes.
**Xenotropic retrovirus.** Retrovirus, either leukemia virus or mammary tumor virus, with tropism for cells from other species, but not mouse.
**YAC.** Yeast artificial chromosome. A cloning vector that can accept very large genomic inserts; a chromosome introduced to yeast derived from such a vector and containing DNA from another organism.
**Zygote.** The fertilized egg containing both sire and dam pronuclei; a fertilized egg.

March, 2003

**THE LABORATORY RAT**
***Rattus norvegicus***

**Normal features:**

Gastrointestinal:
- Continuously growing incisors, rooted molars
- Simple intestine
- Sexual dimorphism of salivary glands
- Paneth cells in small intestine - not as prominent as mice
- Ileal filamentous bacteria
- Consistent liver lobation (unlike mouse)
- No gall bladder
- Hepatocytic polykarya, but not as striking as mouse

Respiratory:
- Obligate nasal breathers
- Prominent vomeronasal organs
- Single left pulmonary lobe, four right lobes
- No intrapulmonary bronchi
- Cardiac muscle extends around large pulmonary vessels
- Serous cells in respiratory epithelium (unique to rat)

Hematopoeitic / lymphoid tissue:
- Extramedullary hematopoeisis in liver, spleen of infants. EMH can be in spleens of adults, but not to the extent of mice. EMH in liver is common with chronic suppurative diseases.
- No tonsils
- Thymus involutes in adults, but present in young adults
- Accessory spleens
- Peripheral blood leukocytes consist of 80% lymphocytes
- Eosinophils have doughnut nuclei without lobation
- Hemosiderin accumulation in spleens of multiparous females

Genitourinary
- Anatomically similar to mouse
- Proteinuria in males (tubular alpha globulin)
- Adult females have cyclic eosinophil infiltration of endometrium

Skeletal:
- No Haversian bone
- Epiphyses close, but some not until late adulthood.
- Hematopoeisis remains active in long bones

Cardiovascular:
- os cordis-like structure in aortic root

Misc.
- Exobital lacrimal glands have striking karyomegaly, anisocytosis
- Prominent pineal gland

**Spontaneous (non-infectious) Diseases**

Integument
- Yellowing of albinos, especially males
- Ringtail: low humidity in pre-weanlings

Gastrointestinal
- Malocclusion
- Chloral hydrate ileus
- Many liver lesions in aging rat
  - polyploidy
  - spongiosis

peliosis
foci of hepatocellular alteration
bile ductular proliferation and fibrosis - cirrhotic lesions
extramedullary hematopoeisis

Respiratory:
Inhalation pneumonia - bedding, food
Alveolar histiocytosis

Genitourinary
Chronic Progressive Nephropathy
Single most life-limiting disease of aging rats. Accelerated development in males, and other factors, including high protein diets. Especially severe in Sprague-Dawley rats. None in GF rats
Nephrocalcinosis
Hydronephrosis
Urinary calculi: copulatory plugs, mixed mineral calculi
Cystic ovaries/endometrial hypertrophy, hyperestrogenism - constant light cycle for as little as 3 days
Seminal vesicular atrophy
Testicular atrophy
Hematuria

Musculoskeletal:
Degenerative osteoarthritis
Auricular chondritis: nodular inflammatory lesions on pinnae. SD, WI

Cardiovascular:
Myocardial degeneration and fibrosis - SD rats
Polyarteritis nodosa
associated with hypertension in hypertensive (SHR) strains, renal disease (with hypertension) in aging (male) SD rats

Nervous system:
Brain spongiform change - aging
Radiculoneuropathy - cauda equina of aging rats. Posterior paresis.
Hydrocephalus

Misc.
Light-induced retinal degeneration, cataracts in albino rats
Genetic retinal degeneration
Porphyrin staining around eyes - lacrimation, dehydration, etc.
Hyperthermia: very prone - can't sweat. Infertility in males. Dehydration: rapid with water bottle problems

**Neoplasia**

Mammary fibroadenoma: Common in aged female SD rats, sometimes males. Prolactin levels elevated.
Large granular cell leukemia: NK cells. F344, WAG. Primary site spleen. Splenomegaly. Leukemia, infiltration of lung, liver, lymph nodes. Associated with DIC syndrome with hemorrhage.
Pituitary adenomas: SD, WI
Leydig tumors: very common in F344. Hypercalcemia may be present.
Fibroma
Zymbol's gland tumors. Adenosebaceous, with transformation to squamous cell CA.
Pheochromocytoma
Thyroid carcinoma
Parafollicular cell carcinoma
Lymphomas / leukemias: rare
Mammary carcinomas: rare
Hepatoma
Mesothelioma

Glial tumors

# INFECTIOUS AGENTS OF RATS

## I. VIRUSES

### A. DNA

| | |
|---|---|
| Adenovirus | rat adenovirus |
| Herpesvirus | rat cytomegalovirus (RCMV) |
| Papovavirus | rat polyomavirus |
| Parvovirus | rat virus (RV) |
| | H-1 virus (H-1) |
| | rat parvovirus (RPV) |
| Poxvirus | cowpox virus |

### B. RNA

| | |
|---|---|
| Bunyavirus | Hanta virus |
| Coronavirus | sialodacryoadenitis virus (SDAV) |
| Paramyxovirus | pneumonia virus of mice (PVM) |
| | Sendai virus |
| Picornavirus | mouse encephalomyelitis virus (MEV) |
| Reovirus | infectious diarrhea of infant rats (IDIR) virus |
| | reovirus 1, 2, 3 |
| Retrovirus | rat leukemia virus (RLV) |

**Rat adenovirus**

1. Prevalence: common
2. Diagnosis: serology, inclusions. Cross-reacts antigenically with mouse MAd-2
3. Disease: subclinical. Intranuclear inclusions in enterocytes of small intestine found incidentally. Inclusions more common in young rats.
4. Transmission: orofecal
5. Duration: unknown
6. Comment: high prevalence of seroconversion with MAd-2 (K87) antigen. Rats not susceptible to MAd-1 or -2, so probably separate, but related viruses. Enteric inclusions found only occasionally, but unknown if this is the only adenovirus of rats. Isolation attempts have failed.

**Rat cytomegalovirus** (RCMV)

1. Prevalence: rare or non-existent in laboratory rats, common in wild rats
2. Diagnosis: histology. Serology, but seldom used
3. Disease: cytomegaly, inclusions in acinar and duct epithelium of salivary and lacrimal glands, especially submandibular gland. Mild nonsuppurative interstitial inflammation. Do not confuse with normal exorbital gland morphology.
4. Transmission: unknown (salivary?)
5. Duration: chronic

**Rat polyomavirus**

i. Prevalence: unknown
2. Diagnosis: histology

3. Disease: sialoadenitis noted in a colony of mu/mu (athymic) rats with wasting syndrome. Parotid gland duct epithelial cells had intranuclear inclusions. Antigen also in laryngeal and bronchiolar epithelium and kidney.
4. Transmission: unknown
5. Duration: unknown
6. Comment: seen only in NIH mu/mu rats. Does not cross-react with K or polyoma virus of mice. Probably more widespread, but only apparent in athymic rats. Agent not characterized.

**Rat parvoviruses**

Rat Virus
H-1 Virus
Rat Parvovirus (RPV)

1. Prevalence: common (all 3)
2. Diagnosis: serology, lesions (RV). As with MPV, RPV does not share virus structural antigens with RV, H-1. Rat and mouse parvoviruses are different viruses.
3. Disease: RV associated with natural disease. Others subclinical. Most RV infections are subclinical, but can cause fetal resorption, neonatal cerebellar hypoplasia with ataxia, hepatitis, jaundice, steatorrhea. Hemorrhagic disease in adults, especially when stressed or immunosuppressed. Hemorrhage in CNS and peritesticular reported.
4. Transmission: various routes of excretion, in utero possible but not likely
5. Duration: acute to chronic (latent?)
6. Comment: RV and H-1 antigenically cross-reactive, but serologically distinct. RPV not cross-reactive, but shares non-structural antigens. IFA serology, using infected cells therefore most useful seroassay.

**Cowpox virus**

1. Prevalence: rare
2. Diagnosis: lesions
3. Disease: subclinical. Necrotizing rhinitis.
4. Transmission: unknown
5. Duration: unknown
6. Comment: Soviet satellite Cosmos 1129

**Hanta virus**

1. Prevalence: widespread in wild Norway rats. Rare in laboratory rats.
2. Diagnosis: serology
3. Disease: none reported in rats, but not thoroughly evaluated
4. Transmission: urine, saliva, respiratory
5. Duration: chronic
6. Comment: Zoonotic. Synonyms: Korean hemorrhagic fever, muroid virus nephropathy, Hantaan virus (single virus), Hanta virus, hemorrhagic fever with renal syndrome (HFRS) virus, Hanta Pulmonary Syndrome (HPS) virus. Asian isolates associated with hemorrhage fever with renal syndrome: proteinuria, azotemia, petechiae, hemoconcentration, hypotension, renal failure, etc. in humans. American strains associated with acute pulmonary edema (radiologic white out) in humans. Navajo outbreak (Four Corners, Sin Nombre virus) and sporadic illness in *Northeast* where virus is enzootic. *Peromyscus spp*. major reservoir host in U.S. Recovery usual, but several fatal cases recently reported in associated with Peromyscus mouse reservoir. California has the most cases in US.

**Rat coronaviruses**

1. Prevalence: common
2. Diagnosis: serology, lesions

3. Disease: cervical edema, nasal/ocular discharge, photophobia, keratitis, megaloglobus, anestrus, fetal resorption. Virus replicates in upper respiratory mucosa (necrotizing rhinitis, tracheitis, bronchitis), with secondary involvement of salivary, lacrimal and lower respiratory tissues (sialodacryoadenitis and interstitial pneumonia). Sublingual salivary gland usually spared. Lacrimal gland dysfunction results in secondary keratitis sicca, ulcers, uveitis, megaloglobus, etc. Chronic wasting disease in athymic rats due to progressive pneumonia and salivary gland lesions. Olfactory mucosal dysfunction in sucklings may lead to failure to nurse, mortality. Pneumonia is common in infant rats.
4. Transmission: respiratory
5. Duration: acute (except in athymic rats)
6. Comment: many strains (like MHV), including 'Parker's Rat Coronavirus"

**Pneumonia virus of mice** (PVM)

i. Prevalence: common
2. Diagnosis: serology, lesions
3. Disease: subclinical. Lungs develop perivascular and focal interstitial lymphocytic infiltrates in seropositive (recovered) rats. Lesions persist after virus is cleared. Athymic rats likely to have persistent infections with chronic lung disease, but never reported.
4. Transmission: respiratory
5. Duration: acute
6. Comment: PVM more apt to produce lung lesions in naturally infected rats than mice.

**Sendai virus**

1. Prevalence: recently common; now rare
2. Diagnosis: serology, lesions
3. Disease: subclinical or mild in rats. Infant mortality. Disease similar to Sendai virus infection in genetically resistant mice (mild). Chronic wasting syndrome/pneumonitis in rnu/rnu (nude) rats.
4. Transmission: respiratory
5. Duration: acute

**Mouse encephalomyelitis virus** (MEV)

1. Prevalence: seroconversion to MEV (mouse) rare to moderate
2. Diagnosis: serology
3. Disease: none
4. Transmission: orofecal
5. Duration: unknown
6. Comment: MHG virus isolated from rats. Antigenically cross-reactive with MEV of mice. Probably mouse origin.

**Infectious diarrhea of rats (IDIR) virus**

1. Prevalence: mare
2. Diagnosis: lesions. Serology available, but not used.
3. Disease: suckling mats (less than i2 days of age) develop diarrhea, erythema of anus, runting. Malabsorption with watery digesta. Villus attenuation, necrosis, enterocytic syncytia and intracytoplasmic inclusions, especially distal small intestine.
4. Transmission: orofecal
5. Duration: acute
6. Comment: antigenically distinct rotavimus (separate from typical or type 1 rotaviruses). Pathogenesis similar to EDIM in mice, with age-dependent susceptibility to disease but not infection. Infectious to humans and humans probable source of infection in laboratory rats.

**Reovirus**

i. Prevalence: moderate
2. Diagnosis: serology
3. Disease: none
4. Transmission: omofecal, respiratory
5. Duration: unknown
6. Comment: see mouse. No disease reported in naturally or experimentally infected rats.

**Rat leukemia virus** (RLV)

1. Prevalence: 100 %
2. Diagnosis: not done
3. Disease: none
4. Transmission: germ line, endogenous provirus
5. Duration: chronic, latent
6. Comment: minimal significance in rat. RLV sequences have been combined with MuLV or other RLVs to form experimental rat sarcoma viruses (Kirsten, Harvey, etc.)

## INFECTIOUS AGENTS OF RATS

## II. BACTERIA

*Bordetella bronchiseptica*
*Campylobacter spp.*
Cilia-Associated Respiratory (CAR) Bacillus
*Clostridium piliforme* (*Bacillus piliformis*)
*Corynebacterium kutscheri*
*Erysipelas rhusiopathiae*
*Helicobacter spp.*
*Hemobartonella muris*
*Hemophilus spp.*
*Klebsiella pneumoniae*
*Lawsonia intracelluaris*
*Leptospira icterohemorrhagiae*
*Mycoplasma spp.*
*Pasteurella pneumotropica*
*Pseudomonas aeruginosa*
*Salmonella enterica*
*Spirillum minus*
*Staphylococcus spp.*
*Streptobacillus moniliformis*
*Streptococcus pneumoniae*
*Streptococcus spp.*

### *Bordetella bronchiseptica*

1. Prevalence: rare, depending on population
2. Diagnosis: culture
3. Disease: may be associated with respiratory disease, but probably in association with primary pathogen, such as Mycoplasma. Rhinitis, otitis, lower respiratory disease.
4. Transmission: contact
5. Duration: unknown
6. Comment: minor primary significance in rats

### *Campylobacter spp.*

1. Prevalence: variable
2. Diagnosis: culture, silver stains of lesions
3. Disease: there is a single report of intracytoplasmic organisms in apical cytoplasm of hyperplastic/neoplastic enterocytes with colonic adenocarcinoma. Agent not characterized, but morphologically reminiscent of *Lawsonia intracellularis* in hyperplastic enteritides of a variety of species (rabbit, guinea pig, hamster, ferret, lamb, calf, piglet, etc.). *Campylobacter jejuni* can also be isolated from mats with soft feces. This is probably common and agent can be cultured.
4. Transmission: orofecal
5. Duration: unknown

### Cilia-Associated Respiratory (CAR) Bacillus

i. Prevalence: common
2. Diagnosis: silver stain, lesions, serology developed but not used
3. Disease: subclinical to chronic respiratory disease similar to mycoplasmosis. Organisms on apical membrane among cilia of respiratory epithelium. Often coinfection with Mycoplasma.

4. Transmission: respiratory, presumed
5. Duration: chronic
6. Comment: does not grow in cell-free medium. Found in multiple species. Experimental infection of SPF rats with CAR bacillus will induce chronic respiratory disease and natural infections manifesting as chronic respiratory disease have CAR bacillus in the absence of Mycoplasma, but natural infections are usually mixed.

***Clostridium piliforme* (*Bacillus piliformis*; Tyzzer's disease, megaloileitis)**

1. Prevalence: rare
2. Diagnosis: lesions, silver stains, serology developed but not used
3. Disease: ileitis with dissemination to liver and heart, especially in postweaning rats. Adynamic ileus can occur, resulting in megaloileitis, but not a constant feature. Silver stains reveal characteristic intracellular bacteria.
4. Transmission: spores, orofecal. In utero transmission possible in immunosuppressed rats, but not likely to be significant means of transmission.
5. Duration: unknown
6. Comment: see mouse.

***Corynebacterium kutscheri* (pseudotubemculosis)**

1. Prevalence: variable
2. Diagnosis: culture of lesions, oral washes, cervical lymph nodes. Lesions with characteristic Gram + colonies
3. Disease: rats may carry organisms in oral cavity and cervical lymph nodes without disease. Infected cervical lymph nodes may be slightly enlarged (reactive) without abscessation. Stress, immunosuppression, other unknown factors may precipitate bacteremic dissemination to lungs, kidneys, liver, etc. with formation of abscesses. Active lesions possess prominent colonies of Gram positive bacilli at periphery, but eventually scar over and resolve.
4. Transmission: direct contact
5. Duration: chronic
6. Comment: pathogenesis may differ from mice. Different organ distribution. Depopulate.

***Erysipelas rhusiopathiae***

1. Prevalence: rare
2. Diagnosis: culture lesions
3. Disease: a single report from Scandinavia with chronic fibrinopurulent polyarthritis, myocarditis and endocamditis. Organism isolated from lesions.
4. Transmission: unknown
5. Duration: unknown

***Helicobacter spp.***

1. Prevalence: rare
2. Diagnosis: culture, lesions, PCR
3. Disease: Proliferative and ulcerative typhlitis in athymic nude (rnu) rats infected with *H. bilis*. Reproduced experimentally in nude, but not immunocompetent rats. *H. trogontum* isolated from large intestine of Wistar and Holtzman rats, without lesions.
4. Transmission: orofecal
5. Duration: chronic

***Hemobartonella muris***

1. Prevalence: rare
2. Diagnosis: organisms on surface of emythrocytes; splenectomy of carriers

3. Disease: usually subclinical. Transient parasitemia, anemia, splenomegaly/
4. Transmission: *Polyplax spinulosa*
5. Duration: chronic, but recovery in some
6. Comment: severe anemia, parasitemia within 2 weeks of splenectomy Steroids do not activate subclinical infection.

***Hemophilus spp****.*

1. Prevalence: rare
2. Diagnosis: culture
3. Disease: mild inflammation of lower respiratory tract. Isolation from nose, trachea, lung and female genital tract.
4. Transmission: unknown
5. Duration: unknown
6. Comment: unclassified species. Single report, but a high prevalence of infection within the colony.

***Klebsiella pneumoniae***

1. Prevalence: infection common, disease rare
2. Diagnosis: culture
3. Disease: opportunistic gut microflora, but can be associated with respiratory disease (mild rhinitis) and abscesses.
4. Transmission: orofecal, contact
5. Duration: unknown, probably normal microflora

***Leptospira icterohemorrhagiae***

i. Prevalence: rare in laboratory rats, common in wild rats
2. Diagnosis: culture (see mouse)
3. Disease: subclinical
4. Transmission: urinary, contact, copulation, skin wounds
5. Duration: chronic
6. Comment: most common *L. icterohemorrhagiae* in rats, but other species too. Human infection often associated with rat origin.

***Mycoplasma spp****.* (chronic respiratory disease, murine respiratory mycoplasmosis)

- ***M. pulmonis***
- ***M. arthritidis***
- ***M. neurolyticum***
- ***M. collis***

1. Prevalence: common
2. Diagnosis: serology, culture, lesions. Infected rats may be seronegative for months.
3. Disease: often subclinical. Sneezing and snorting characteristic. *M. pulmonis* only significant natural pathogen, causing rhinitis, otitis, laryngitis, tracheitis, bronchiolitis, bronchopneumonia with pronounced bmonchiolectasis, bronchiolar lymphoid hyperplasia (strong B cell mitogen) and abscessation. *M. pulmonis* can also cause perioophoritis, salpingitis. Chronic respiratory disease associated with CAR bacillus and exacerbated by ammonia, Sendai virus, SDAV, etc. Other mycoplasmas inhabit respiratory or genital tract without disease. *M. arthritidis* infection can result in seroconversion to *M. pulmonis.*
4. Transmission: respiratory, contact
5. Duration: chronic
6. Comment: important rat pathogen

***Pasteurella pneumotropica***

1. Prevalence: common

2. Diagnosis: culture
3. Disease: opportunistic organism. Associated with respiratory, ear, reproductive, mammary gland, conjunctival and skin lesions, abortion. Co-pathogen with Sendai virus, Mycoplasma
4. Transmission: orofecal
5. Duration: chronic
6. Comment: inhabits respiratory, intestinal tracts as normal microflora

***Pseudomonas aemuginosa* - see mouse**

***Salmonella enterica***

1. Prevalence: low
2. Diagnosis: culture (especially mesenteric lymph nodes)
3. Disease: similar to mouse. Often subclinical. Starry coat, soft to fluid feces. Chronic cecal ulcers can occur in some rats.
4. Transmission: omofecal
5. Duration: acute to chronic. Approximately 60% of rats are carriers with intermittent shedding.
6. Comment: infection in rats is currently more common than in mice. Depopulate. Serovar enteritidis most common.

***Spirillum minus* - see *Streptobacillus moniliformis***

***Staphylococcus spp.***

1. Prevalence: moderate
2. Diagnosis: culture, lesions
3. Disease: ulcerative dermatitis over nape of neck, shoulders. Prominent colonies of Staphylococcus on surface. Mastititis.
4. Transmission: environmental
5. Duration: chronic
6. Comment: ulcerative dermatitis probably associated with epidermolytic strains of bacteria, but Koch's postulates not fulfilled.

***Streptobacillus moniliformis***

1. Prevalence: common in wild rats, rare in laboratory rats
2. Diagnosis: culture
3. Disease: commensal in rats. Disease minimal or absent. Inhabits oropharynx. Can be transmitted by bite, causing abscesses in rats, septicemia in mice (see mice) and rat-bite fever (Haverhill fever) in humans (Haverhill, MA 1926).
4. Transmission: rat bites, milk, contact
5. Duration: chronic carriers
6. Comment: *Spirillum minus* also a cause of rat-bite fever, but not found in laboratory rats.

***Streptococcus pneumoniae* (Diplococcus, Pneumococcus)**

i. Prevalence: low
2. Diagnosis: culture lesions, Gram stain exudate
3. Disease: subclinical carriers common (80 %). Rhinitis, fibrinopurulent pleuritis, pericarditis, peritonitis, periorchitis, meningitis, otitis and pneumonia. Abscesses.
4. Transmission: respiratory, contact
5. Duration: chronic carriers/acute disease
6. Comment: pathogenicity varies with serotype. Although mice are susceptible to infection, natural infection and disease is rare in mice. Outbreaks of disease can occur among rats.

***Streptococcus spp.***

1. Prevalence: organism(s) frequently isolated, but specific disease(s) rare
2. Diagnosis: culture, lesions
3. Disease: high morbitity epizootics with 50 % mortality among suckling rats. Runting, distended abdomens and fecal staining of perineum. Large numbers of Gram positive cocci on surface of normal appearing villi with minimal inflammation. *Enterococcus faecium-durans-2* and *Enterococcus hirae* identified in 2 outbreaks. *Streptococcus spp*. also an opportunistic pathogen.
4. Transmission: orofecal
5. Duration: unknown
6. Comment: likely to be encountered, but sporadic outbreaks

## INFECTIOUS AGENTS OF RATS

### III. FUNGUS

*Aspergillus fumigatus*
*Trichophyton mentagrophytes*
*Pneumocystis carinii*
*Pneumocystis wakefieldii*

#### *Aspergillus fumigatus*

1. Prevalence: rare
2. Diagnosis: conidia in tissue sections, culture
3. Disease: chronic suppurative rhinitis
4. Transmission: aerosol
5. Duration: chronic
6. Comment: over 20% prevalence of rhinitis found in two separate chronic (24 month) studies in Wistar rats.

#### *Trichophyton mentagrophytes*

1. Prevalence: rare in laboratory mats, can be common in wild rats
2. Diagnosis: skin scrapings/KOH of lesions, histology with methenamine silver
3. Disease: subclinical to florid ringworm on neck, back and base of tail. Patchy hair loss, hyperkeratosis, erythema. Arthrospores infesting keratin of hair follicles.
4. Transmission: contact.
5. Duration: chronic
6. Comment: zoonotic

#### *Pneumocystis carinii*
#### *Pneumocystis wakefieldii*

1. Prevalence: infection frequent, disease rare
2. Diagnosis: lesions, silver stain for cysts
3. Disease: pulmonary disease can be induced in naturally exposed young rats by low protein diet combined with steroids. Difficult to induce in older rats. Spontaneous disease not seen in immunocompetent rats and not reported as a problem in athymic rats.
4. Transmission: unknown
5. Duration: chronic
6. Comment: carriers very common in conventional, “dirty” mats, especially rats from colonies infected with multiple viruses (probably a reflection of overall dirtiness)

## INFECTIOUS AGENTS OF RATS

## IV. PROTOZOA

*Cryptosporidium parvum*
*Eimeria spp.*
*Giardia muris*
*Spironucleus muris*

### *Cryptosporidium parvum*

1. Prevalence: unknown
2. Diagnosis: organisms on brush border of villi
3. Disease: probably opportunistic or marginally pathogenic. Outbreaks of diarrhea with high mortality among infant rats has been reported. Surviving pups runted. Hyperplastic enteritis, especially jejunum, with organisms toward villus tips.
4. Transmission: orofecal, cysts
5. Duration: unknown
6. Comment: Experimental infection in rats is mild and transient, except in immunosuppressed or athymic mats.

### *Eimeria spp.*

1. Prevalence: common in wild rats, nonexistent in laboratory mats
2. Diagnosis: intestinal lesions, oocysts in feces
3. Disease: hyperplastic enteritis, especially in young rats.
4. Transmission: orofecal, oocysts
5. Duration: chronic
6. Comment: see mouse. Rat has several of its own species.

### *Giardia muris*

1. Prevalence: infrequent
2. Diagnosis: histology, cysts in feces
3. Disease: subclinical or diarrhea. Low-grade chronic enteritis with trophozoites aligned along brush border of villi.
4. Transmission: orofecal
5. Duration: chronic
6. Comment: see mouse

### *Spironucleus muris*

1. Prevalence: high
2. Diagnosis: microscopy of intestine, wet mounts of small intestinal digesta, cysts in feces
3. Disease: none or rare in rats. If disease present, probably opportunistic.
4. Transmission: orofecal, cysts
5. Duration: chronic
6. Comment: probably not pathogenic in rats

## INFECTIOUS AGENTS OF RATS

## V. HELMINTHS

A. NEMATODES
*Aspicularis tetraptera*

*Syphacia muris*

*Trichosomoides crassicauda*

B. CESTODES
*Cysticercus fasciolaris* (*Taenia taeniaeformis*)

*Rodentolepis nana, diminuta*

### ***Aspicularis tetraptera, Syphacia muris*** **(pinworms)**

1. Prevalence: common
2. Diagnosis: ova in feces. Scotch tape test for Syphacia, adults in lumen
3. Disease: none. Syphacia is only species that deposits ova on anus.
4. Transmission: orofecal. Ova resistant.
5. Duration: chronic
6. Comment: see mouse. *Syphacia obvelata* and *Syphacia muris* may be same species.

### ***Trichosomoides crassicauda*** **(bladder thread worm)**

1. Prevalence: infrequent
2. Diagnosis: bipolar ova in urine, worms in bladder, ureters and renal pelves
3. Disease: eggs hatch, penetrate stomach wall, migrate through lungs and other viscera and seek urinary tract epithelium. Migrating larvae incite eosinophilia and granulomata, especially in lungs. Adult females burrow in transitional epithelium, smaller males live in lumen or inside female uterus. Reaction minimal, but dead worms may serve as nidus for calculus formation.
4. Transmission: urine. Egg masses stick to environmental surfaces, resistant to dessication
5. Duration: chronic
6. Comment: Invermectin treatment can be effective

### ***Cysticercus fasciolaris*** **(*Taenia taeniaefommis*)**

1. Prevalence: rare
2. Diagnosis: cysts in liver
3. Disease: incidental finding of strobilocerci, usually in liver. Rats respond with fibroplasia. Sarcomas can develop from reactive zone. Studied as a model for parasite-induced neoplasia.
4. Transmission: contamination of feed and bedding with cat feces
5. Duration: chronic
6. Comment: intermediate state of cat tapeworm.

### ***Rodentolepis nana, diminuta*** **- see mouse**

## INFECTIOUS AGENTS OF RATS

# VI. ARTHROPODS

## A. MITES

*Demodex spp.*
*Laelaps echidninus* (spiny rat mite)
*Notoedres muris*
*Ornithonyssus bacoti* (tropical rat mite)
*Radfordia ensifera*

## B. LICE

*Polyplax spinulosa* (spined rat louse)
*Hoplopleura pacifica* (tropical rat louse)

## C. FLEAS

*Leptopsylla spp.*
*Nosopsyllus spp.*
*Xenopsylla spp.*

**Rat arthropods**

Arthropods are rare in laboratory rats, except Radfordia fur mites. Radfordia can be common in some colonies. Non-pathogenic fur mite. Demodex reported in laboratory rats as an incidental finding, Laelaps transmits Hepatozoon, but requires absolutely filthy conditions to survive. Notoedres is ear mange mite. Burrows in cornified epithelium, causing pruritis and self-trauma. Extremely rare in laboratory rats, but recently quite common in European pet rats. Ornithonyssus lives off host and is non-selective in host range, including humans. Can cause anemia, debilitation and death in rats. Pruritis in humans. Lice can also serve as vectors of disease, including Polyplax (Hemobartonella) and trypanosomes. Fleas can serve as intermediate hosts for Hymenolepis and transmit other agents.

# Pathology of the Domestic Ferret
# (*Mustela putorius furo*)

Bruce H. Williams, DVM, DACVP
Department of Telemedicine and Distance Learning
Armed Forces Institute of Pathology
Email: Williamsb@afip.osd.mil

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## Nervous System

### Canine Distemper

Synopsis: Canine distemper is the most serious disease in ferrets. Essentially 100% fatal, the morbillivirus that causes canine distemper results in an accelerated syndrome that closely mimics signs seen in canids and other susceptible species. Disease progression ranges from 12 days in ferret-adapted strains to approximately 42 in wild canine strains. The disease is profoundly immunosuppressive, with animals that survive this stage of the disease succumbing to neurologic dysfunction within several weeks. This disease n the U.S. is primarily seen in young kits from pet stores. Treatment is not recommended. Currently, there is one approved distemper vaccine for ferrets (Fervac-D, United Vaccines); however, many commercial modified live canine vaccines are used in ferrets. Recombinant vaccines using a canarypox vector are being developed for use in ferrets and exotic mammals.

Gross lesions. Similar to those seen in the dog. Photophobia, oculonasal discharge, hyperkeratosis of the planum nasale and footpads, a papular rash beginning on the chin and progressing to a generalized form, bronchopneumonia.

Microscopic lesions. Brightly eosinophilic, 2-5 um intracytoplasmic and intranuclear inclusions may be seen in a wide variety of epithelial cells, neurons, and occasionally in white blood cells and megakaryocytes. (The urinary bladder, renal pelvis, and biliary epithelium in my places are the most productive places to look for inclusions.) Additionally, multinucleate cells may be found in any of these sites. A non-suppurative encephalitis with demyelination may be seen in animals with neurologic disease. The presence of suppurative bronchopneumonia in a young ferret is suggestive of this disease.

Additional references.

Davidson, M. Canine distemper virus infection in the domestic ferret. *Comp Cont Educ Pract Vet* 8(7):448-453, 1986.

Hoover JP et al. Serologic response of domestic ferrets to canine distemper and rabies virus vaccines. *JAVMA* 194:234-238, 1989.

Kauffman CA et al. distemper virus infection in ferrets: an animal model of measles-induced immunosuppression. *Clin Exp Immunol* 47:617-625, 1982.

Williams ES et al. Canine distemper in black-footed ferrets (Mustela nigripes) from Wyoming. *J Wildl Dis* 24:385-398, 1988.

**Rabies**.

Synopsis: Ferrets, as well as any other mammal, are susceptible to rabies. Ferrets, however, have a low recorded incidence of rabies, with less than 25 confirmed cases since 1954. The disease can result in both furious (less common) and dumb forms, and often presents as a progressive hindlimb paralysis. Researchers have shown that ferrets inoculated IM with virulent rabies virus do not secrete the virus in their saliva. Currently, there is one approved killed rabies vaccine available for use in the ferret (Imrab, Rhone-Merieux).

Gross lesions. None.

Microscopic lesions. Intracytoplasmic eosinophilic viral inclusions (Negri bodies) may be demonstrated on HE stains or on standard fluorescent antibody tests.

Additional references:

Blanc J, Albert MR, Artois M. Rage experimentae du ferret. Rev. Med Vet, 133:553, 1982.

Hoover JP et al. Serologic response of domestic ferrets to canine distemper and rabies virus vaccines. *JAVMA* 194:234-238, 1989.

Mainka C. Rabies antibody production in ferrets after immunization with four different rabies vaccines. *Zentralbl Veterinarmed (B)* 41:574-579, 1995.

Niezgoda M, et al. Pathogenesis of experimentally induced rabies in domestic ferrets. *Am J Vet Res*. 1997 Nov; 58(11): 1327-1331.

Niezgoda M, et al. Viral excretion in domestic ferrets inoculated with a raccoon rabies isolate. *Am J Vet Res* 59(12) 1629-1632.

Rupprecht CE et al. Evaluation of an inactivated rabies virus vaccine in domestic ferrets. *JAVMA* 196:1614-1616, 1990.

**Neural Tube Defects**

Synopsis: NTD's are one of the most common birth defects in ferret kits. They may range from simple cranioschisis (external opening of the skull), to spina bifida, to craniorachischisis (opening of the skull and vertebral column with loss of cerebral tissue). Many variants are seen. Additionally, growth retardation and other birth defects (kidney defects appear commonly) may be seen in the fetus.

Gross lesions. Agenesis of skin and musculature overlying various segments of the skull and/or spinal cord, with variable loss os neural tissue.

Microscopic lesions. Additionally, there may be fusion or other deformation of the vertebrae. With cranioschisis or craniorachischisis, there is often agenesis of the cerebrum and cerebellum, with a rudimentary medulla (cerebrovasculosa) remaining.

Additional references.

Williams BH et al.. Iniencephaly and other neural tube defects in a litter of ferrets (Mustela putorius furo). *Vet Pathol* 31(2): 260-262, 1994.

## Gastrointestinal System

**Dental Disease**

Synopsis: Broken teeth are common in older ferrets, most commonly affected are the upper canines. While few broken teeth result in clinical debility, exposure of the pulp requires extraction or root canal procedures. Accumulation of dental calculi is common in older ferrets on semi-moist or moist diets. Tooth root abscesses are occasionally seen in ferrets. Dental malformations, including supernumerary teeth or decrease numbers of adult teeth have also been documented.

Gross lesions. Discoloration of broken teeth suggests devitalization. Draining tracts may be seen, especially in the area of the zygomatic arch with tooth root abscesses.

Microscopic lesions: N/A

Additional references:

Andrews PL, Illman O. Some observations of anatomical abnormalities and disease states in a population of 350 ferrets (Mustela furo). IZ. VersuchsteirkdI 21:346, 1979.

Berkovitz BK. Supernumerary deciduous incisors and the order of eruption of the incisor teeth in the albino ferret. *J. Zool*., 155:445, 1968

Verstraete FJM. Advances in Diagnosis and Treatment of Small Exotic Mammal Dental Disease. *Sem In Avian and Exot Pet Med* 12(1):37-48, 2003.

**Megaesophagus**

Synopsis: The cause of megaesophagus is currently unknown in the ferret. It presents similarly to megaesophagus in the dog and cat. Occasionally, secondary *Candida* infections may be seen. The condition occurs in middle-aged to older ferrets, and treatment is usually ineffective.

Gross lesions: Marked dilation of the intrathoracic esophagus. Ulcerations may be present anywhere along the length. Evidence of bronchopneumonia may be present due to aspiration.

Microscopic lesions. Often none. In chronic cases, there may be discernable atrophy of the muscular layers. In other cases, there may be hyperkeratosis of the lining epithelium, and the presence of numerous yeast within the mucosa, inciting a lymphocytic and neutrophilic inflammatory response.

Additional references:

Blanco MC et al. Megaesophagus in nine ferrets. *JAVMA* 205:444-447, 1995.

**Helicobacter mustelae**

Synopsis. This bacterium, recently discovered by James Fox et al. at MIT, causes disease in significant numbers of ferrets over the age of four years. The bacterium causes gastric disease via two mechanisms - a) the stimulation of a marked lymphoplasmacytic inflammatory response, resulting in loss of glandular epithelium, most prominently in the pylorus, and 2) the ability to increase the pH of the stomach. Animals over the age of 3 years rarely do not show evidence of *Helicobacter* infection.

Gastric ulcers are also commonly seen in animals with severe Helicobacter infection. (see below) Recent evidence that *H. mustelae*-infected ferrets have elevated levels of gastrin suggests a possible relationship with peptic ulcer disease.

Gross lesions: There are often no gross lesions in uncomplicated cases of gastric *Helicobacter*. Advanced cases may be coupled with gastric ulcers. In these cases, the gastric mucosa is often lined by moderate amounts of digested blood; gastric ulcers are often fine bleeding points concentrated in the pylorus.

Microscopic lesions: Warthin-Starry 4.0 is the stain of choice to demonstrate the presence of the bacteria in the superficial mucus and in extracellular locations within the gastric

glands. The pyloric stomach is the preferred biopsy site, although low numbers of bacilli may also be seen in the fundus and duodenum in severely infected animals.

Additional references.

Batchelder M, et al. Natural and experimental Helicobacter mustelae reinfection following successful antimicrobial eradication in ferrets. *Helicobacter*. 1996 Mar; 1(1): 34-42.

Fox JG, et al. *Helicobacter mustelae*-associated gastritis in ferrets. An animal model of *Helicobacter pylori* gastritis in humans. *Gastroenterology* 99:352-361-1990.

Fox JG et al. Gastric colonization of the ferret with *Helicobacter* species: Natural and experimental infections. *Rev Infect Dis 13(suppl 8)*:S671-680, 1991.

Fox JG et al. Role of gastric H in isolation of *Helicobacter mustelae* from the feces of ferrets. *Gastroenterology* 104:86-92, 1993.

Gottfried MR et al. *Helicobacter pylori*-like microorganisms and chronic active gastritis in ferrets. *Am J Gastroenterol* 85:813-818, 1990.

Otto G et al. Eradication of *Helicobacter mustelae* from the ferret stomach: an animal model of *Helicobacter pylori* chemotherapy. *Antimicrob Agents Chemother* 34:1232-1236, 1990.

Perkins SE et al. Helicobacter mustelae-associated hypergastrinemia in ferrets. AJVR 57(2):147-150, 1996.

**Gastric ulcers**

Synopsis. Ferret, like other mustelids, are extremely susceptible to stress-related gastric ulcers. This is a common finding in animals with other systemic diseases and often contribute to debility in older animals. They are often seen in association with gastric *Helicobacter mustelae* infection, however, a definitive cause-and-effect relationship has not been proven in this species.
vessels.

Gross lesions: Two distinct forms of gastric ulceration may be seen in the ferret. The most common form is the presence of digested blood within the stomach lumen. Ulcers are pinpoint, extremely difficult to see, and are present in the highest numbers in the pyloric region of the stomach. The second, less common form, is he presence of a single, focally extensive, ulcer in the pyloric stomach. These large ulcers may result in sudden death due to erosion into the submucosal blood vessels.

Microscopic lesions. Microscopically, ulcers appear as full-thickness areas of glandular necrosis and loss which are well-demarcated from the surrounding tissue. Bleeding ulcers may be covered with a layer of brown hemoglobin pigment.

Additional references.

Hudson M et al. A ferret model of acute multifocal gastrointestinal infarction. *Gastroenterology* 102:1591-1596.

**Inflammatory Bowel Disease**

Synopsis. Inflammatory bowel disease is extremely common in middle aged and older ferrets. IBD in the ferret generally falls into one of two categories: the lymphocytic/plasmacytic form, and the eosinophilic form (also known as eosinophilic gastroenteritis.

The cause of inflammatory bowel disease in the ferret, as in other species is mulitfactorial. The nature of the antigens preceipitating a chronic, uncontrolled inflammatory reaction in the intestine is largely unknown in the ferrets, but strong evidence exists that infection by *Helicobacter mustelae* and ferret coronavirus may eventually result in the development of this condition. Dietary antigens have not been investigated in this condition, although dietary modification commonly is effective in amerliorating clinical signs.

The lymphocytic form is more commonly seen and presents with milder clinical signs. The severity of histologic lesions, however, rarely equates with the severity of clinical disease. The more severe form, eosinophilic gastroenteritis was first described by James Fox et. al of MIT in 1992. Although the etiology of this disease is likewise unknown, presumed cases have been treated successfully with ivermectin, suggesting some form of parasitic origin. The wasting disease is most commonly seen in young male ferrets under 14 months of age. Peripheral eosinophilia may be seen in affected animals. Unlike the lymphocytic form, lesions of eosiniphilic IBD may be present in a wide range of abdominal organs, including the liver, spleen, and mesenteric lymph nodes.

Gross lesions: None.

Microscopic lesions. Small to moderate numbers of of lymphocytes, plasma cells, and eosinophils are commonly seen in the small intestine of the ferret. Lymphocytic forms of inflammatory bowel disease are associated with intramucosal lymphocytes (intraepithelial lymphocytes- IELs), and evidence villar atrophy, blunting, and fusion.

With EE, Eosinophilic infiltrates may be seen in the small intestine - a diffuse mucosal infiltrate and an eosinophilic vasculitis may be present. Additionally, prominent eosinophilic infiltrates may be seen in the mesenteric lymph nodes, liver, pancreas, or any other abdominal organ (and have rarely been reported in the thorax as well.). Aggregates of Splendore-Hoeppli material may be seen within the lymph nodes and rarely in the liver in areas of accumulated eosinophils, but are rarely seen in the gut.

Additional references.

Blomme, EA et al. Hypereosinophilic Syndrome with Hodgkin's-like Lymphoma in the Ferret. *J Comp Pathol* 120:211-217, 1999.

Fox JG et. al. Eosinophilic gastroenteritis with Splendore-Hoeppli material in the ferret (Mustela putorius furo). *Vet Pathol* 29:21-26, 1992.

Palley LS, Fox JG. Eosinophilic gastroenteritis in the ferret. *In* Kirk RW, Bonagura JD (eds.): Current Veterinary Therapy XI. Philadelphia, WB Saunders, 1992, pp. 1182-1184.

**Proliferative colitis**

Synopsis. Proliferative colitis is an uncommon disease which is usually seen in male ferrets under one year of age. The disease is sporadic, with only one or two animals in a large colony being affected. Clinical signs include tenesmus and production of small, frequent bowel movements which often contain frank blood and mucus. The disease is caused by a campylobacter-like organism (recently reclassified as a species of *Desulfovibrio*) which results in asymmetrical proliferation of immature epithelium, causing marked thickening of the wall. This condition is subject to periodic periods of recrudescence, often during times of stress. If untreated, it may be fatal.

Gross lesions. There is noticeable thickening of the colonic wall, which becomes opaque (normally you can see fecal material through the colonic wall).. The mucosa is prominently "cobblestoned."

Microscopic lesions. The mucosa is multifocally thickened up to five times normal by a proliferation of immature epithelial cells with vesicular nuclei and a moderate amount of basophilic cytoplasm Scattered islands of normal goblet cell may be present, but there is an overall marked decrease in goblet cells. Silver stains will demonstrate the presence of the bacteria in the apical cytoplasm of epithelial cells.

Additional references:

Finkler MR. Ferret colitis. *In* Kirk RW et al. (eds.). Current Veterinary Therapy XI. Philadelphia, WB Saunders, 1992, pp. 1180-1181.

Fox JG et al. Proliferative colitis in ferrets. *AM J Vet Res* 43:858-864, 1982

Fox JG, Lawson GH. Campylobacter-like omega intracellular antigen in proliferative colitis of ferrets. *Lab Anim Sci* 38:34-36, 1988.

Fox JG et al. Proliferative colitis in ferrets: Epithelial dysplasia and translocation. *Vet Pathol* 26:5150517, 1989.

Fox JG et al. Intracellular Campylobacter-like organism from ferrets and hamsters with proliferative bowel disease is a Desulfovibrio sp. *J Clin Microbiol* 32:1229-1237, 1994.

Krueger KL et al. Treatment of proliferative colitis in ferrets. *JAVMA* 194:1435-1436, 1989.

**Intestinal parasites**

Synopsis. With the exception of coccidia, intestinal parasites are uncommon in ferrets. *Toxocara cati, Toxascaris leonina, Ancylostoma* sp., *Dipylidium caninum, and Giardia* sp. have all been reported in ferrets. Three species of coccidia have been seen in ferrets: *Eimeria furo, Eimeria ictidea,* and *Isospora laidlawii.* While most coccidial infections are subclinical, lethal coccidial infections are occasionally seen in young kits. Ferrets have been experimentally infected with a number of intestinal parasites, including *Strongyloides stercoralis*

Gross lesions. Generally none, although digested blood may be present in the GI tract of kits severely affected with coccidial infections.

Microscopic lesions. Numbers of parasites range from very low to extremely high in severe infections where almost every enterocyte contains merozoites. All stages of the parasite, including micro- and macrogametocytes can be seen. Meronts contain up to 16 merozoites. Coccidial infections have also been seen in the hepatobiliary system.

Bell, JA. Parasites of domesticated pet ferrets. *Comp Cont Educ Pract Vet* 16(5):617-622, 1994.

Williams, BH et al. Biliary coccidiosis in a ferret (Mustela putorius furo). *Vet Pathol* 33(4):437-439, 1996.

**Epizootic catarrhal enteritis**

Synopsis. ECE is a coronaviral disease of ferrets which causes epizootics of high morbidity (up to 100%), but low mortality. The diarrhea is rapidly dehydrating and most mortalities occur in older animals with concurrent illness. Symptoms include vomiting and passage of a dark green stool with abundant mucus. During the recovery phase, stools assume a "birdseed" like appearance. Gross lesions. Generally none. The intestine may be flaccid with a moderate amount of watery ingesta.

Microscopic lesions. Sections should be taken from 3-4 different areas of the jejunum, as well as the remainder of the gastrointestinal tract. Early lesions include vacuolar degeneration and necrosis of apical enterocytes, with resultant marked villar atrophy, fusion and blunting. Later in the course of disease, there is a marked lymphocytic enteritis with large numbers of lymphocytes among mucosal epithelial cells.

Williams BH, Kiupel M, West KH, Raymond JT, Grant CK, Glickman LT: Coronavirus-associated epizootic catarrhal enteritis in ferrets. J Am Vet Med Assoc 217(4):526-30, 2000

**Gastrointestinal foreign bodies**

Synopsis. Gastrointestinal foreign bodies are commonly seen in young or bored, cage-bound ferrets. Ferrets commonly ingest latex, plastic, and foam rubber. Ferrets may also ingest towels or other forms of bedding. Anorexia and passage of abnormal stools are common presenting signs; abdominal pain is not commonly seen.

Gross lesions. A focal area of intestinal distention with or without hemorrhage may be seen. In many cases, the wall of the intestine at the site of the blockage is thinner than that of the adjacent intestine due to continuous peristaltic movements at the site of blockage. Intestinal perforation may rarely be seen.

Microscopic lesions. Ulceration, necrosis and thinning of the muscular layers at the site of blockage. Marked attenuation of villi and granulation tissue may be seen in longstanding blockages.

Additional references.

Mullen HS et al. Gastrointestinal foreign body in ferrets: 25 cases (1986-1990) *J Amer Anim Hosp Assoc* 28:13-19, 1992.

**Clostridium perfringens**

Synopsis. Clostridium perfringens type A has been reported in black-footed ferret kits.

Gross lesions: Gastric bloat, multifocal intestinal hemorrhage.

Microscopic lesions. Typical of clostridial infections. Marked coagulative necrosis of the intestinal mucosa with numerous adherent 2X6-8 um bacilli.

Additional references.

Schulman FY et al. Gastroenteritis associated with Clostridium perfringens type A in black-footed ferrets (Mustela nigripes). *Vet Pathol* 30:308-310, 1993.

**Mycobacterium avium-intracellulare infection**

Synopsis: This is a rare condition in ferrets which is most commonly seen in the gastrointestinal tract and mesenteric lymph nodes, although accumulation of macrophages containing the organism may be seen in any organ.

Gross lesions. Mesenteric lymphadenopathy is the most common gross lesion.

Microscopic lesion. The presence of large foamy macrophages with a grayish granular cytoplasm are suggestive of this disease - acid-fast stains reveal numerous bacilli within macrophages.

Additional references.

Schultheiss PC, Dolginow SZ. Granulomatous enteritis caused by *Mycobacterium avium* in a ferret. *JAVMA* 204:1217-1218, 1994.

Cryptosporidiosis

**Neoplasia**

Synopsis. The most common gastrointestinal neoplasm is, as in several other organ systems, lymphosarcoma. The lymphoblastic form of lymphosarcoma is the most common form in the intestine. (See hematopoietic system for a more detailed description of this condition.)

Additional references.

Fox JG, et al. Helicobacter mustelae-associated gastric adenocarcinoma in ferrets (Mustela putorius furo). Vet Pathol. 1997 May; 34(3): 225-229.

## Endocrine System

### Islet cell tumors

Islet cell neoplasms are the most common neoplasm of this species. These neoplasms generally result in hypoglycemia as a result of inappropriate secretion of insulin. Clinical signs include lethargy, stupor, ptyalism, and ataxia, and may progress to coma and death. Non-functional islet cell tumors are commonly seen in older animals at necropsy. While all islet cell tumors are potentially malignant, metastasis is rare, as opposed to islet cell neoplasms in the dog and cat.

Gross lesions. Islet cell tumors are reddish-brown, well-defined nodules which range in size from 2mm-1 cm. They are firmer than the surrounding pancreatic tissue and may be multiple. These neoplasms must be differentiated grossly from foci of pancreatic exocrine hyperplasia, a common benign age-related finding. (Foci of exocrine hyperplasia are generally the same color and consistency of the surrounding tissue, and may be numerous). Small reddish brown nodules may also be present in the mesentery adjacent to the pancreas.

Microscopic lesions. Similar to islet cell neoplasms in other species. These tumors are most commonly unencapsulated, and resemble normal, albeit greatly enlarged islets of Langerhans. Identical foci may be present in the surrounding mesentery. Metastasis to visceral organs is rare. These neoplasms stain strongly for insulin with scattered glucagon staining.

Additional references.

Andrews GA, et al. Immunohistochemistry of pancreatic islet cell tumors in the ferret (Mustela putorius furo). Vet Pathol. 1997 Sep; 34(5): 387-393.

Caplan ER, et al. Diagnosis and treatment of insulin-secreting pancreatic islet cell tumors in ferrets: 57 cases (1986-1994). J Am Vet Med Assoc. 1996 Nov 15; 209(10): 1741-1745.

Fix AS, Harms CA. Immunocytochemistry of pancreatic endocrine tumors in three domestic ferrets. *Vet Pathol* 27:199-201, 1990.

Marini RP et al. Functional islet cell tumor in six ferrets. *JAVMA* 202:430-433, 1993.

Weiss CA et al. Insulinoma in the ferret: clinical findings and treatment comparison of 66 cases. *JAAHA* 34(6):471-475, 1998.

### Adrenal-associated endocrinopathy

Synopsis. AAE is a common endocrine disorder of middle aged to older ferrets. The syndrome is the result of proliferative lesions in the adrenal cortex which secrete excess amounts of estrogenic hormones. As a result of this excess estrogens, affected ferrets exhibit a range of cutaneous, behavioral, and reproductive signs. While technically a form of hyperadrenocorticism,

AAE should not be confused with Cushing's disease, or hypercortisolism. Only rarely are cortisol levels elevated in these patients. Interestingly, unlike dogs and cats, metastasis occurs extremely late in the course of disease with adrenocortical carcinoma, and early removal of affected adrenals carries a fair prognosis.

Gross lesions. Bilaterally symmetrical alopecia beginning over the tailhead and progressing forwards over the flanks and abdomen is strongly suggestive of AAE. Additionally, the presence of an enlarged vulva in a spayed female also strongly suggests AAE. These clinical signs may be the result of any of the three types of proliferative adrenocortical lesions - hyperplasia, adenoma, or carcinoma. The normal length of the ferrets adrenal gland ranges from 3-5 mm; glands exceeding 5 mm often contain proliferative lesions. Diameters exceeding 1 cm is highly suggestive of adrenocortical carcinoma in the ferret.

Microscopic lesions. Proliferative lesions of the ferret adrenal cortex fall into three categories - hyperplasia, adenoma, and carcinoma. In a recent retrospective of 104 proliferative adrenocortical lesions archived at the AFIP, hyperplasia and carcinoma were present in 45% of cases each, while adenoma was present in 10%. The presence of necrosis, cellular atypia, and a mitotic rate greater than 1/10 hpf are strong indicators of malignancy. The presence of a single nodule in the adrenal cortex without factors associated with malignancy indicates adenoma, while the presence of multiple nodules is evidence of nodular cortical hyperplasia. Many neoplasms have a prominent spindle cell component which is primarily a proliferation of smooth muscle and has no prognostic significance. Extracapsular extension of proliferative cortical tissue may be seen in all three lesions, and does not occur indicate one lesion over another. Anaplastic carcinomas with large lakes of mucin have been identified as a distinct variant (2003) and are considered to be an aggressive form with the highest metastatic potential.

Additional references:

Gliatto JM et al. A light microscopical, ultrastructural and immunohistochemical study of spindle-cell adrenocortical tumors of ferrets. J Comp Pathol 113(2) 175-183, 1995

Gould WJ et al. Evaluation of urinary cortisol:creatinine ratios for the diagnosis of hyperadrenocorticism associated with adrenal gland tumors in ferrets. *JAVMA* 206:42-46, 1995.

Lawrence, HJ et cal. Unilateral adrenalectomy as a treatment for adrenocortical tumors in ferrets: five cases (1990-1992). *JAVMA* 203:271-275, 1993.

Lipman NS et al. Estradiol-17 beta-secreting adrenocortical tumor in a ferret. *JAVMA* 203:1552-1555, 1993.

Neuwirth L, et al. Adrenal ultrasonography correlated with histopathology in ferrets. Vet Radiol Ultrasound. 1997 Jan; 38(1): 69-74.

Peterson RA 2nd, Kiupel M, Capen CC. Adrenal cortical carcinomas with myxoid differentiation in the domestic ferret (Mustela putorius furo). Vet Pathol.;40(2):136-42, 2003.

Peterson RA 2nd, Kiupel M, Bielinska M, Kiiveri S, Heikinheimo M, Capen CC, Wilson DB. Transcription factor GATA-4 is a marker of anaplasia in adrenocortical neoplasms of the domestic ferret (Mustela putorius furo). Vet Pathol. 2004 Jul;41(4):446-9.

Rosenthal KL: Hyperadrenocorticism associated with adrenocortical tumor or nodular hyperplasia in ferrets: 50 cases (1987-1991). *JAVMA* 203:271-275, 1993.

Rosenthal KL et al. Questions about assays used for estradiol 1-17 beta (letter). *JAVMA* 204:1001-1002, 1994.

Rosenthal KL. Adrenal gland disease in ferrets. Vet Clin North Am Small Anim Pract. 1997 Mar; 27(2): 401-418.

Scott DW et al. Figurate erythema resembling erythema annulare centrifugum in a ferret with adrenocortical adenocarcinoma-associated alopecia. *Vet Dermatol* 5:111-115, 1994.

Wagner RA, Dorn DP. Evaluation of serum estradiol concentrations in alopecic ferrets with adrenal gland tumors. *JAVMA* 205:703-707, 1994.

Weiss CA, et al. Clinical aspects and surgical treatment of hyperadrenocorticism in the domestic ferret: 94 cases (1994-1996). *J Am Anim Hosp Assoc*. 1997 Nov; 33(6): 487-493.

Wheler CL, et al. Ferret adrenal-associated endocrinopathy. *Can Vet J*. 1998 Mar; 39(3): 175-176.

**Diabetes mellitus**

Synopsis. Diabetes mellitus, is a poorly-defined, uncommon disease which has been reported in both the domestic and the black-footed ferret. Blood glucose levels in affected ferrets generally range into the 500's, but levels as high as 725 g/dl have been reported. Polydipsia, polyuria, glucosuria, and loss of body condition have been reported in affected ferrets.

Gross lesions. None.

Microscopic lesions. Glycogenic vacuolation of the islets of Langerhans is the most consistent and noteworthy histologic lesion. Glycogen accumulation may also be seen in renal tubular epithelium. In several cases in the AFIP archive, lenticular cataracts have been noted.

**Thyroid Disease**

Synopsis. Thyroid abnormalities are extremely rare in the ferret. One case of thyroid adenocarcinoma has been documented in the ferret. In over 2500 cases on archive in the Registry of Veterinary Pathology at the AFIP, not one thyroid lesion has been catalogued.

A single case report of pseudohypoparathyroidism has been published (2003). The disease manifested initially as a seizure disorder, and lab tests showed low serum calcium, high serum phosphorus, and extremely high serum parathyroid hormone concentrations. The animal improved after treatment with dihydrotachuysterol, a Vitamin D analog.

Additional references:

Heard, DJ et al. Thyroid and adrenal function tests in adult male ferrets. AJVR 51(1):32-35, 1990.

Wilson GH, Greene CE, Greenacre CB: Suspected pseudohypoparathyroidism in a domestic ferret. J Am Vet Med Assoc.: 222(8):1093-6, 2003

## Hematolymphatic System

### Splenomegaly

Synopsis. The cause of this extremely common finding in ferrets is yet unknown; many theories abound. This condition is most commonly seen in middle-aged to older ferrets, but may be seen in ferrets as young as six months. As the incidence of neoplasia in enlarged spleens is somewhat less than 10%, this change most likely represents a response to chronic inflammatory disease (Bruce Williams, personal opinion). The previously reported syndrome of hypersplenism in a ferret is most likely not a distinct entity in this species. Marked enlargement of the spleen for any reason increases the spleen's phagocytic capability, resulting in increased RBC breakdown. Additionally, anemia of chronic disease may complicate many cases of splenomegaly. Lymphosarcoma is by far the most common splenic neoplasm, with hemangiosarcoma being rarely seen.

Gross lesions. Enlarged spleens may range up to 10 cm. in length. While most spleens are diffusely enlarged, a small percentage of spleens will contain single or multiple discrete nodules, which are more likely to represent splenic neoplasms.

Microscopic lesions. 95% of cases consist of a combination of marked congestion and extramedullary hematopoiesis, representing erythrocytic, leukocytic, and megakaryocytic lines. Florid EMH may resemble lymphosarcoma in that a large percentage of the cells within the red pulp may have a markedly increased nuclear/cytoplasmic ratio and a high mitotic rate, but represent the immature forms of the various cell lines. The marked variation in cell size, and the presence of islands of erythrocytic precursors and megakaryocytes contrasts well with the monomorphic population of cells seen in most cases of lymphosarcoma.

Large areas of coagulative necrosis, often bordered by a combination of viable and degenerate neutrophils and various amounts of granulation tissue may be seen in grossly enlarged spleens. As enlarged spleens are prone to rupture, various signs of splenic trauma, including hematoma, siderotic plaques, and large areas of parenchymal fibrosis are commonly seen.

Additional references.

Ferguson DC. Idiopathic hypersplenism in a ferret. *JAVMA* 186:693-695, 1985.

**Lymphosarcoma**

Synopsis. Lymphosarcoma is the most common malignancy in the domestic ferret. These neoplasms most commonly arise spontaneously, however, a recent article documents horizontal transmission of malignant lymphoma in ferrets using cell or cell-free inoculum. This finding, coupled with the occasionally clustering of lymphomas in a single facility, has prompted speculation that lymphosarcoma in the ferret may be the result of a retroviral infection. A viral agent has not, as of yet, been isolated from cases of lymphosarcoma in the ferret.

Several variants of lymphoma exist in the ferret. The most commonly seen form, in which the neoplastic cell is a mature, well-differentiated lymphocyte occurs in older ferrets, primarily resulting in peripheral lymphadenopathy, with visceral spread and subsequent organ failure late in the course of disease. A second form occurs primarily in young ferrets less than two years of age. This form, in which the neoplastic cell is a large blastic lymphocyte, is characterized by early visceral neoplasms, often with the production of a large thymic mass. An enlarging thymic neoplasm often results in compression of the lung lobes, dyspnea, and pleural effusion, and may often be misdiagnosed as pneumonia or heart disease by veterinarians with little experience in this species.. A third, uncommon form, in which combinations of peripheral lymphadenopathy and visceral neoplasms and numerous bizarre lymphoblasts may be seen, is known as the immunoblastic polymorphous variant.

Gross lesions. Adult (lymphocytic) form - diffuse lymphadenopathy. Splenic white pulp may be greatly expanded and grossly visible on cut section. In later stages, firm white nodules may be seen in a number of visceral organs, including the liver and kidney, and the spleen may be diffuse enlarged. Juvenile (lymphoblastic) form - The presence of a thymic mass is strongly suggestive of this condition. Diffuse hepatosplenomegaly is often seen due to massive infiltration of these organs also. Neoplastic cells may be seen in any organ, including the bone marrow.

Microscopic lesions. In the adult form, biopsy of peripheral lymph nodes reveals effacement of the normal architecture by an infiltrate of small non-cleaved lymphocytes which breach the capsule and extend into the surrounding tissue. (However, extension into surrounding tissue may also be seen in cortical hyperplasia of the mesenteric nodes due to the attenuated and occasionally absent capsule seen in these nodes.) The presence of tingible body macrophages scattered throughout the node ("starry-sky" effect) is commonly seen in this form. In the liver, neoplastic infiltrates are primarily seen extending from portal areas, which in the spleen, the earliest sign of lymphosarcoma is an expansion of the well-differentiated lymphocytes in the mantle of the periarteriolar lymphoid sheaths. Mitotic rates generally average 1-2/hpf.

In the juvenile form, examination of infiltrated organs often reveals effacement of normal architecture by a monomorphic population of large cleaved and non-cleaved lymphoblasts, which may be admixed with smaller, more well-differentiated cells. In the liver, neoplastic cells are more commonly seen as discrete nodules distending sinusoids and replacing hepatocytes, while in the spleen, the periarteriolar lymphoid sheath is totally replaced and expanded by a monomorphic lymphoblast population. Discrete nodules of blastic lymphocytes may be seen in any visceral organ; infiltration of lymph nodes is a late finding. The mitotic rate of the lymphoblastic cells is generally high, ranging up to 6/hpf. A recent immunophenotypic

characterization of thymic lymphomas of young ferrets revealed that 9/10 were C3+ (T cell origin) and 1/10 was CD 79+ (B cell origin).

Finally, the distribution of the immunoblastic polymorphous variant resembles that of the lymphocytic form. However, scattered through infiltrated nodes is a subpopulation of atypical large cleaved, often multinucleate lymphocytes which may range up to 50 or 60 um in diameter. Occasionally, Reed-Sternberg-like cells may be present. Bizarre-looking lymphocytes in this condition may be misinterpreted as megakaryocytes, however, use of immunohistochemical techniques such as Factor VII antigen, CD3 and BLA-36 (a lymphocyte marker) may be used to distinguish between the two cell lines in the spleen and bone marrow. The mitotic index in this form of lymphoma is also high.

A common request for pathologists working with ferrets is evaluation of splenic aspirates from animals with enlarged spleens. This task is fraught with pitfalls. As a general rule: extramedullary hematopoiesis will be seen in the VAST majority of cases. Evidence of erythrocytic precursors and abundant peripheral blood should lead the prudent pathologist to a diagnosis of EMH. Cases of splenic lymphosarcoma may be identified on splenic cytology by the presence of a monomorphic population of cells with large nuclei, prominent nucleoli, an absence of erythrocytic precursors, and minimal blood elements. Additionally, mitotic figures should be present.

Additional references.

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Li X, et al. Cutaneous lymphoma in a ferret (Mustela putorius furo). *Vet Pathol* 32:55-56, 1995.

Rosenbaum MR, et al. Cutaneous epitheliotropic lymphoma in a ferret. *J Am Vet Med Assoc.* 1996 Oct 15; 209(8): 1441-1444.

**Aleutian Disease**

Synopsis. Aleutian disease is caused by the same parvovirus that causes Aleutian disease in mink; however, the disease is quite different between these two species. In mink, AD results in rapidly life-threatening immune- mediated glomerulonephritis, vasculitis, and

hypergammaglobulinemia. In ferrets, there are notable similarities, including a hypergammaglobulinemia, and in late stages of the disease, an immune complex glomerulonephritis; however, the disease is much more insidious, with a progression of as long as 2 years. Ferrets in the late stages of disease will be hyperproteinemic ( 8-9 mg/dl, with >20% of this total being comprised of gammaglobulins. Serologic testing is available through United Vaccines (Madison, WI) or the Research Animal Diagnostic Laboratory in the Department of Comparative Medicine, Massachusetts Institute of Technology.

Gross lesions. Gross lesions are seen only late in the course of disease. Splenomegaly and lymphadenopathy are the most common gross lesions with this disease; splenic infarction as a result of marked splenomegaly may complicate the clinical and pathologic picture.. Enlarged, brown-tan kidneys may be present. In terminal cases, clotting abnormalities resulting from vasculitis and the marked hypergammaglobulinemia may result in petechial hemorrhage and hematuria.

Microscopic lesions. Several characteristic microscopic findings are seen in ferret AD as well as in the mink disease. Prominent plasmacytic infiltrates are seen in numerous organs, most prominently in the renal interstitium, hepatic portal areas, and in the splenic red pulp, where an almost pure population of plasma cells expands the red pulp. Additionally, there may be marked plasmacytosis of numerous lymph nodes and the bone marrow.. In most cases, there will be marked membranous glomerulonephritis and numerous ectatic protein-filled tubules as a result. (Note: Glomerulosclerosis is commonly seen in chronic interstitial nephritis in this species - but there is little evidence of tubular protein casts or plasmacytic infiltrate in uncomplicated CIN). Vasculitis may be seen in almost any organ.

Additional references:

Alexandersen S et al. Acute interstitial pneumonia in mink kits inoculated with defined isolates of Aleutian mink disease parvovirus. *Vet Pathol* 31:216-228, 1994.

Daoust PY, Hunter DB. Spontaneous Aleutian disease in ferrets. *Can Vet J* 19:133-135, 1978.

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Welchman E, et al. Aleutian disease in domestic ferrets: diagnostic findings and survey results. *Vet Rec* 132:479-484, 1993.

Wolfensohn SE, Lloyd MH. Aleutian disease in laboratory ferrets (letter). *Vet Rec* 134:1001, 1995.

## Urinary System

### Bacterial Urinary Tract Infections

Synopsis. Bacterial urinary tract infections are commonly seen in female ferrets, and uncommonly seen in male ferrets. The most common causative agent in the ferret is *E. coli*, with *Staphylococcus aureus* being isolated out of a significant number of cases. Bladder infections are often subclinical in female ferrets, and ascending infections resulting in pyelonephritis are not uncommon. Renal failure may result from severe pyelonephritis in this species.

Gross lesions. Often none. Hydronephrosis and hydroureter may be present in long-standing or resolved infections.

Microscopic lesions. Ulcerative cystitis and/or a suppurative tubulointerstitial nephritis. Bacteria are rarely seen.

**Prostatic Squamous Metaplasia**

Synopsis. Squamous metaplasia of the prostate has only recently been recognized as a common cause of dysuria and urethral blockage in the ferret. The squamous change in the prostate is the result of excess estrogens liberated from proliferative adrenal lesions (see adrenal-associated endocrinopathy, above). Accumulation of secretory material and lamellated keratin results in the formation of multiple prostatic cysts. Impingement of the prostatic cysts upon the prostatic urethra results in dysuria, and finally complete urinary blockage in male ferrets. The bladder of blocked ferrets may be manually expressed, but ferrets cannot void on their own. In earlier literature, due to the close association with the bladder, the condition was referred to as the "triple bladder syndrome". Surgery is directed toward removal of prostatic cysts and the affected adrenal.

Gross lesions. Single to multiple, variably-sized fluctuant cysts are present near the bladder trigone. The cysts are thick-walled, and firm on palpation. Identification of an enlarged adrenal gland or an adrenal neoplasm is often possible in these animals.

Microscopic lesions. Multiple cysts or fragments of cysts are often available for examination. Atrophic prostate glands (as a result of the effects of circulating estrogens) are often present at the periphery of the cysts, although in advanced cases, they may be lined by squamous, rather than glandular epithelium). The wall consists of multiple layers of squamous epithelium, surrounded by variable amounts of immature fibrous connective tissue. The lumenal contents of the cyst may vary from lamellated keratin and keratin debris, to abundant purulent inflammation (in which case there is often a combination of chronic-active inflammation and granulation tissue in the cyst wall and prostate (overeager manual expression of the bladder?).

Additional references:

Coleman GD et al. Cystic prostatic disease associated with adrenocortical lesions in the ferret (Mustela putorius furo). Vet Pathol, 35(6):547-549, 1998.

Nolte DM, Carberry CA, Gannon KM, Boren FC: Temporary tube cystostomy as a treatment for urinary obstruction secondary to adrenal disease in four ferrets. J Am Anim Hosp Assoc. 38(6):527-32, 2002.

**Urolithiasis**

Synopsis. Numerous references refer to the formation of struvite uroliths in ferrets; however, the actual incidence is probably overestimated, especially in light of recent findings of prostatic squamous metaplasia. Male ferret are more likely to develop uroliths than females; however, the syndrome has not been well characterized, and dietary influences have not been explored, although high ash cat foods are frequently blamed. Clinical signs include frequent licking of the genital area, dysuria, anuria, and occasionally, hematuria. Reportedly, pregnancy may increase the incidence of urolithiasis in pregnant jills due to the effects of estrogen on the ferret's handling of calcium and phosphorus. Cystine crystals have also been reported.

Gross lesions. Struvite uroliths often have a corrugated surface. Single or multiple uroliths may be present in the bladder, or rarely in the renal pelvis. Reports of struvite "sand" as may be seen in the feline urologic syndrome" are anecdotal.

Microscopic lesions. Similar to that seen in urolithiasis in other animals.

Additional references.

Dutton, MA. Treatment of cystine bladder urolith in a ferret (Mustela putorius furo). *Exotic Pet Pract* 1(8):7, 1996.

Nguyen HT et al. Urolithiasis in ferrets (Mustela putorius furo). *Lab Anim Sci* 29:243-245, 1979.

Palmore WP, Bartos KD. Food intake and struvite crystalluria in ferrets. *Vet Res Commun* 11:519-526, 1987.

**Renal Cysts**

Synopsis. Renal cysts are common incidental findings in the ferret. Although often submitted for histologic evaluation, they are of little clinical significance and have no effect on renal function. Rare cases of true polycystic disease may be seen in this species. Polycystic kidneys are enlarged, may be felt on external palpation, and may cause renal failure.

Gross lesions. Single or multiple cysts may be present in the cortex of one or both kidneys. When viewed from the capsular surface, they are thin, bulge slightly, and are fluid filled. Cysts may range up to 1 centimeter in diameter. Polycystic kidneys may be markedly enlarged and fill the posterior abdomen. They are composed of variable numbers of cysts with little intervening fibrous connective tissue.

Microscopic lesions. In benign cysts, there may be little or no fibrosis surrounding the cyst, or the cyst may have a thick wall of fibrous connective tissue throughout which are scattered numerous atrophic glomeruli and tubules. In a reported case of polycystic disease in a ferret, the kidney contained multiple fluid-filled cysts in both the cortex and medulla which were lined by cuboidal epithelium. The cysts were separated by abundant fibrous connective tissue which contained moderate numbers of lymphocytes.

Additional references.

Dillberger JE. Polycystic kidneys in a ferret. *JAVMA* 186:74, 1985.

**Chronic Interstitial Nephritis**

Synopsis. Chronic interstitial nephritis is a common finding in ferrets. Early lesions can be seen as early as 2 years, and advanced cases resulting in renal failure may occur as early as 4.5

years. The progression of the disease is most akin to that seen in older cats. Ferrets are generally maintained on a high protein diet with protein levels in excess of 34%. This is generally accomplished by feeding premium kitten chows or specially formulated ferret chows. Due to the prevalence of chronic interstitial nephritis in older ferrets, lowering of protein levels after three years of age is reached is generally advocated by most practitioners.

Gross lesions. Kidneys are generally pitted and large focal depressions may be seen in the outer cortex as a result of scarring. "Peeling" the renal capsule is recommended during the ferret necropsy. Severely affected kidneys may be asymmetric with respect to size.

Microscopic lesions. The pattern of microscopic changes associated with chronic interstitial nephritis in the ferret is unique. At low magnification, there are linear bands of fibrosis which extend from the capsule inward. Glomerular and tubular changes are most commonly seen in these areas of fibrosis. There is periglomerular and glomerular fibrosis resulting in glomerulosclerosis. The interstitium is expanded by fibrous connective tissue throughout which is scattered moderate numbers of lymphocytes and plasma cells. Tubules within these radiating streaks of fibrosis exhibit variable degrees of atrophy. Pathologists with little experience with ferret tissues may be tempted to diagnose chronic infarction. As the disease progresses, there is a diffuse glomerulosclerosis throughout the cortex, as glomeruli outside of the areas of interstitial fibrosis are affected. Areas of fibrosis tend to coalesce into large areas devoid of functional glomeruli and tubules.

## Reproductive System

**Estrus-associated Aplastic Anemia**

Synopsis. Ferrets are induced ovulators - intact females remain in estrus until mated, spayed, or are cycled out by injections of human chorionic gonadotropin. 50% of unmated jills will develop marked bone marrow suppression as a result of high levels of circulating estrogens. All three bone marrow cell lines are affected - erythrocytes, leukocytes, and megakaryocytes. Initially, there is a mild thrombocytosis and leukocytosis, but the condition soon progresses to a non-regenerative anemia, leukopenia, and thrombocytopenia. The anemia may remain non-

regenerative anemia up to 4 months past ovariohysterectomy in affected animals. In addition to thrombocytopenia, a liver-associated clotting abnormality may also be present. Hemorrhage is reported to be the most common cause of death. Similar signs may be caused by exogenous estrogen administration, but are not seen in cases of adrenal-associated endocrinopathy.

Gross lesions. Female ferrets in estrus have prominently swollen vulvas. Signs of hyperestrogenism include pale mucus membranes, alopecia, melena, thin watery blood, hemorrhages throughout the body, hematuria, pyometra, bronchopneumonia, and vaginitis.

Microscopic lesions. Diagnosis of aplastic anemia is most commonly made on the combination of a low PCV (<20%) in a jill in estrus. The most characteristic lesion in affected jills is hypocellularity of the bone marrow. There is also no evidence of splenic hematopoiesis; small amounts of EMH may be seen in the liver. There may be evidence of hemorrhage (hemosiderin-laden macrophages, erythrophagocytosis) in lymph nodes and the spleen. Suppurative metritis or pneumonia may be seen as a result of the marked leukopenia.

Additional references.

Bernard SL et al. Estrogen-induced bone marrow depression in ferrets. *AJVR* 44: 657-661, 1982.

Manning D, Bell J. Lack of detectable blood groups in domestic ferrets: Implications for transfusion. *JAVMA* 197:84-86, 1990.

Mead RA et al. Optimal dose of human chorionic gonadotrophin for inducing ovulation in the ferret. *Zoo Biol* 7:263-267, 1988.

**Mastitis**

Synopsis. Mastitis is occasionally seen in pregnant jills in the first few weeks of lactation. Hemolytic *E. coli* is the most commonly isolated organism, and results in a syndrome of gangrenous mastitis. If untreated, jills rapidly become septic and/or endotoxemic. *Staph aureus* is occasionally cultured from cases of mastitis and produces a more suppurative, less necrotic form of mastitis.

Gross lesions. Affected teats are swollen, necrotic, black, firm, and non-painful. In *Staph aureus* mastitis, the mammary glands are hot, painful, and reddish in color; purulent exudate may be expressed from the lactiferous ducts.

Microscopic lesions. The primary lesion in *E. coli* mastitis is diffuse severe coagulative necrosis which extends into the adjacent adipose tissue and muscle. There are large pockets of hemorrhage and edema in the affected glands; numerous bacteria may be seen. Areas of infarctions are well-demarcated by a line of degenerate neutrophils and cellular debris, and vascular thrombosis may be seen. Other signs of sepsis, or endotoxemia, including margination of neutrophils in the pulmonary capillaries and hypertrophy of Kupffer cells in the hepatic sinusoids may be seen, as well as colonies of gram-negative bacilli in numerous tissues.

In staphyloccoccal mastitis, there is less evidence of infarction. A purulent galactophoritis and mastitis is present. Staphylococci are often prominent.

Additional references.

Liberson AJ et al. Mastitis caused by hemolytic *Escherichia coli* in the ferret. *JAVMA* 183:1179-1181, 1983.

## Cardiovascular System

### Cardiomyopathy

Synopsis. Cardiomyopathy is a common disease in the American lines of ferrets, which has a presumed genetic basis. Several forms of this condition may be seen - dilatative, hypertrophic, and a restrictive form in which there is marked replacement of myocardium by fibrous connective tissue, with minimal change in chamber area. Signs of cardiomyopathy may be seen as early as 1 year of age in severely affected animals, but are more common between 5 and 7 years of age.

Gross lesions. Gross lesions are similar to those seen in other domestic species. In subclinical cases, a congested, occasionally nodular liver may be the only gross lesion as a result of chronic passive congestion in this organ. The heart may appear enlarged, and the right ventricle may appear thin or flabby. With progressively severe cases, there is often an accumulation of a serosanguinous ascitic transudate in the abdominal cavity, the pleural cavity, or both. In severe cases, the lungs are atelectatic and compressed by the presence of a globose heart and abundant pleural effusion. In cases in which the heart is not enlarged, examination of the left ventricular free wall and the interventricular septum may reveal marked thickening and impingement upon the ventricular lumen. Rarely, the presence of fibrous connective tissue may be seen upon close inspection of the cardiac wall, and occasionally, due a previous ischemic event, a focally extensive area of the ventricular wall may be translucent and paper thin as a result of total loss of myocytes in this area ad replacement by fibrous connective tissue.

Microscopic lesions. Early lesions consist of an increase in fibrous connective tissue around myocardial vessels which extends into the interstitium. As the condition progresses, there is atrophy and loss of myocytes. Focal areas of myocyte degeneration may be present, with an infiltrate of moderate numbers of macrophages, lymphocytes, plasma cells, and rare neutrophils. In some cases of cardiomyopathy, there may be marked focal malalignment of myocytes, suggesting orientation in several different planes.

Centrilobular fibrosis, edema, micronodular hemosiderosis, and loss of subcapsular hepatocytes with resulting fibrosis all attest to chronic hepatic congestion, which is a common finding in cardiac disease in the ferret. In contrast, the presence of chronic signs of left-sided heart failure are relatively uncommon. In terminal stages of the disease, there may be necrosis of centrilobular hepatocytes due to stasis and hypoxia. The presence of marked myocardial fibrosis with or without inflammation, and evidence of chronic systemic congestion are highly suggestive of cardiomyopathy in this species.

Additional references.

Greenlee PG, Stephens E. Meningeal cryptococcosis and congestive cardiomyopathy in a ferret. *JAVMA* 184:840-841, 1984.

Lipman NS et al. Clinical, functional, and pathologic changes associated with a case of dilatative cardiomyopathy in a ferret. *Lab Anim Sci* 37:210-212, 1987.

**Dirofilariasis**

Synopsis. Ferrets are also susceptible to heartworm infection, but due to the fact that most ferrets are kept indoors, cases are still uncommon. Ferrets in heartworm endemic areas are usually maintained on monthly ivermectin at approximately 0.2 mg/kg. (Note: in my experience, the vast majority of cases of dirofilariasis in ferrets come from Florida.) Due to the small size of the ferret heart, as few as two heartworms may result in fatal cardiac insufficiency. The small numbers of heartworms in these animals also necessitates the use of occult heartworm tests due to the low levels of circulating microfilaremia.

Gross lesions. Lesions of heartworm disease in the ferret are essentially the same as cardiomyopathy (see above), as infection commonly results in heart failure in this species. Aberrant cerebral heartworm migration has been noted in this species. The presence of heartworms within the right ventricles and pulmonary artery can be construed as the cause of death in any ferret in which it is observed.

Microscopic lesions. Microscopic lesions are as expected with heart failure (see above).

Additional references.

McCall JW. Dirofilariasis in the domestic ferret. *Clin Tech Small Anim Pract* 13(2):109-112, 1998.

Moreland AF et al. Dirofilariasis in a ferret. *JAVMA* 188:864, 1986.

## Respiratory System

**Endogenous lipid pneumonia**

Synopsis. This condition, also known as "foam cell foci" or "subpleural histiocytosis" is a common incidental finding in mustelids at necropsy and is of no clinical significance. It is often mistaken at necropsy by practitioners as a dissemination neoplasm. The cause of this finding, and the origin of the lipid, is not known.

Gross lesions. Multiple to coalescing white to yellow foci are present within the subpleural pulmonary parenchyma. A transverse cut through one of these foci will reveal its superficial nature.

Microscopic lesions. The basic lesion is simply an aggregate of lipid-laden macrophages in the alveoli immediately subjacent to the pleura. As the lesion increases in size, it may include moderate numbers of lymphocytes and cholesterol clefts.

**Aspiration pneumonia**

Synopsis. By far, the most common cause of pneumonia in the ferret is aspiration, either of orally administered medicants or of vomitus. Ferrets often resist liquid oral medication by fighting and squirming during administration, and often involuntarily inhale part of the medication.

Gross lesions. In cases of aspiration pneumonia, there may be consolidation of the cranioventral lung lobes, either unilaterally or bilaterally. The severity of lesions seen with aspiration of vomitus is proportionate to the length of time since the event. In most cases, aspiration occurs as a terminal event, so minimal gross lesions are seen. In long-standing cases, gangrenous, cavitated lesions may be seen in the pulmonary parenchyma.

Microscopic findings. The primary lesion in aspiration pneumonia is in the small airways. Bronchioles contain a mixture of viable and degenerate neutrophils, sloughed epithelial cells, and variable amounts of eosinophilic proteinaceous material (which may be admixed with food particles when vomitus is aspirated). Often, there is an accumulation of foamy macrophages in the surrounding alveoli. In long-standing cases, there may be a pronounced granulomatous response, with numerous foreign body and multinucleate giant cells admixed with lymphocytes, plasma cells, and cholesterol clefts. Occasionally, you may find eosinophilic crystalline proteins within the cytoplasm of macrophages. In cases of aspiration of vomitus, the lesion is characterized by extensive necrosis of the airway and surrounding alveoli, with sloughing of the bronchiolar epithelium and coagulative necrosis of the adjacent alveolar septa. Colonies of gram-negative bacilli or mixed colonies may be seen in cases of aspiration of vomitus.

**Influenza**

Synopsis. Ferrets are the only domestic animal species which is susceptible to the human influenza viruses. For this reason, they are a) often used as animal models in influenza research, and b) often infected by their human owners. The disease is quite similar to that in humans, with clinical signs being photophobia, a catarrhal nasal discharge, sneezing, coughing, pyrexia, anorexia, and malaise.

Gross lesions. Lesions are generally minimal, with congestion and exudation of the nasal mucosa and mild reddening of the tracheal mucosa.

Microscopic lesions. There is mild subacute inflammation and occasional necrosis of the nasal mucosa. A mild subacute interstitial pneumonia may be present. Because the disease is so rarely fatal, there is often little opportunity to examine tissues form infected animals.

Additional references.

Glathe, H. Enteral influenza infection of ferret. Archiv fur experimentelle Veterinarmedizin 38(5):771-777, 1984.

Renegar KB: Influenza virus infections and immunity: A review of human and animal models. *Lab Anim Sci* 42:222-232, 1992.

Smith H, Sweet C. Lessons for human influenza from pathogenicity studies in ferrets. *Rev Infect Dis* 10:56-75, 1988.

## Musculoskeletal System

### Chordoma

Synopsis. Chordomas are the most common neoplasm of the musculoskeletal system of the ferret. They arise in or adjacent to vertebra from remnants of primitive notochord, and are most commonly seen at the tip of the tail. Chordomas have also been documented in cervical spine. Early reports mischaracterized this neoplasm as a chondrosarcoma, and this mistake is still repeated by pathologists who are unfamiliar with ferret tissue. Chordomas are considered potentially malignant, however, metastasis has not been seen in neoplasms arising in the tail. Cutaneous metastasis was reported in one chordoma from the cervical spine.

Gross lesions. Chordomas are most commonly seen as club-like swellings at the tip o the tail which involve the last caudal vertebra. Cervical chordomas present as lytic neoplasms in the neck of animals with posterior paresis. Physical exam shows a markedly decreased range of motion and pain upon movement of the neck.

Microscopic lesions. Chordomas are locally aggressive neoplasms which often infiltrate vertebral bodies. The neoplasm is composed of foamy "physaliferous cells" which are separated by a moderate amount of myxomatous matrix. There are multifocal areas of well-differentiated cartilage and bone within these neoplasms.

Additional references.

Allison N, Rakich P. Chordoma in two ferrets. *J Comp Pathol* 98:371-374, 1988.

Dunn DG et al. A histomorphologic and immunohistochemical study of chordoma in twenty ferrets (Mustela putorius furo). *Vet Pathol* 28:467-473, 1991.

Williams BH, Eighmy JE, Dunn, DG."Cervical Chordomas in Two Ferrets" *Veterinary Pathol* 26(2) 186-189, 1993.

Ferret Polymyositis Syndrome.

This recently discovered disease of ferrets has been the subject of intesnse scrutiny for the last two years. This syndrome primarily affects ferrets less than one year of age. Clinical signs are non-descript and include persistent high fever, leukocytosis (which is occasionally extreme), hindlimb weakness, paresthesia, occasional abscessation of one or more peripheral nodes (often in the hindlegs), wasting, difficulty swallowing, and ultimately death. While

morbidity is low in affected facilities, mortality of this condition is high. At necropsy, suppuriatve inflammation of the thorax, centering on the esophagus is the primary finding. Special stains have failed to reveal an etiologic agent

Gross lesions: Gross lesions are minimal in most cases. Evidence of chronic wasting and generalized muscle loss is often seen due to the long duration of most cases.Mottling and multifocal esophageal hemorrhage may be seen. Acute cases may show swelling of peripheral lymph nodes, most commonly the popliteal nodes.

Microscopic lesions: The syndrome histologically resembles a bacterial infection of the thorax, with large numbers of neutrophils infiltrating the pleura, thoracic lymph nodes, and esophagus. The esophageal lesion is particular striking, as neutrophils infiltrate the serosal and muscular layers, eventually resulting in widespread necrosis and loss of muscle tissue, dysphagia, and death. Occasionally, suppurative inflammation of the peripheral lymph nodes and surrouing muscle and fat may be seen. Special stains have to date failed to reveal an etiologic agent; the possibility of an automimmune etiology has been

References:

None (yet.)

## Integumentary System

### Neoplasia

Synopsis. By far, the most common skin problem in ferrets is neoplasia. The incidence of cutaneous neoplasia increases with age in this species. While there are a wide range of cutaneous neoplasms that have been documented in the ferret, the two most common types of neoplasms seen in the skin of the ferret are 1) sebaceous epithelioma and 2) mast cell tumor.

Sebaceous epitheliomas appear as warty, verrucous lesions which may arise anywhere on the animals body, but have a predilection for the head and neck. Microscopic examination reveals an unencapsulated neoplasm composed of basal cells, of which a small percentage exhibit sebaceous and or squamous differentiation. Although early reports referred to these neoplasms as "basosquamosebaceous carcinomas", they possess no features of malignancy, and evidence of metastasis has not been seen.

Mast cell tumors are also common skin tumors in ferrets. Gross, they most often appear as flat, alopecic, hyperkeratotic plaques which are variably pruritic. Microscopic examination reveals a well-demarcated, unencapsulated neoplasm which is generally confined to the superficial dermis, and is composed of well-differentiated mast cells. Low numbers of eosinophils are scattered through the neoplasm, but vasculitis and collagen degradation is hardly

ever seen. Metachromatic stains such as toluidine blue or Giemsa reveal few cytoplasmic granules, so the diagnosis is primarily made (and rightly so) on the HE section.

Dermal leiomyomas/leiomyosarcomas are commonly seen in the skin of ferrets, and most commonly arise from smooth muscle associated with hair follicles (piloleimyomas). The skin of the back asnd neck are most commonly affected, but they may be seen anywhere on the body. These neoplasms are well encapsulated and have no metastatic potential; although cellular atypia and a moderate mitotic rate may be seen. Surgical excision is generally curative.

Vaccination-site fibrosarcomas have been recently described in the ferrets (2003). The presence of macrophages containing basophilic granular material (interpreted as vaccine material) or peripheral lymphocytic aggregates were described in these tumors and may vbe valuable in differentiating them from piloleiomyomas.

Additional references.

Parker GA, Picut CA. Histopathologic features and post-surgical sequelae of 57 cutaneous neoplasms in ferrets (Mustela putorius furo). *Vet Pathol* 30:499-504, 1993.

Stauber E, et al. Mast cell tumors in three ferrets. *JAVMA* 196:766-767, 1990.

Mikaelian I, Garner MM: solitary dermal leiomyosarcomas in 12 ferrets. *J Vet Diagn Invest* 14: 262-265, 2002.

Munday JS, Stedman NL, Richey LJ: Histology and immunohistochemistry of seven ferret vaccination-site fibrosarcomas. Vet Pathol. 2003 May;40(3):288-93.

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Tunev SS, Wells MG: Cutaneous melanoma in a ferret (Mustela putorius furo). Vet Pathol 39(1):141-3, 2002

**Dermatomycosis**

Synopsis. This is an uncommon disease in ferrets, but is occasionally seen in mink. Most cases occur either in very young animals kept in poor conditions, or in older, immunosuppressed animals. Both *Microsporum canis* and *Trichophyton mentagrophytes* have been seen in ferrets.

Gross lesions. Dermatophytosis is similar to that seen in other domestic species - animals have areas of crusting alopecia with brittle hair and numerous broken hair shafts. In immunosuppressed animals, the rash can become generalized (at which time it must be differentiated from that seen with canine distemper infection).

Microscopic lesions. Biopsies from affected sites are generally covered with a thick layer of keratin debris, degenerate neutrophils, and entrapped fungal arthrospores and hyphae. There is ulceration of the skin, and follicles often contain numerous fungal arthrospores which occasionally invade the hair shaft. Many follicles may not contain a hair shaft, only lamellar keratin debris. There is generally a neutrophilic or lymphoplasmacytic dermal infiltrate in perivascular and periadnexal areas.

Additional references:

Hagen KW et al. Dermatoycoses in fur animals: chinchila, ferret, mink, and rabbit. Vet Med Small Anim Clin 67(1): 43-48, 1972.

**Ectoparasites**

Synopsis. Ferrets are commonly infected with two types of ectoparasites: ear mites (*Otodectes cynotis*) and fleas (*Ctenocephalides sp.*) Most young ferrets and many older ones have clinical cases of ear mite infection which require periodic treatment. Grossly, ferrets with ear mites have copious amounts of a thick brown-black wax. However, swabs from the ears should be examined microscopically for the presence of adult mites or their eggs, as ferrets without mites may also have large accumulations of wax due to neglectful owners.

Sarcoptic mange has been reported in ferrets. This disease comes in two distinct forms in ferrets - a very pruritic whole-body form, and a variably pruritic form localized to the feet. Grossly this form is characterized by swollen feet, evident of self-mutilation, and nail loss. Histologically, the disease is similar to that in the dog, with marked ulceration and hyperkeratosis of the skin and a few cross sections of mites in the epidermis or deep under the overlying crust.

Demodectic mange is generally seen in older or immunosuppressed ferrets. Skin scrapings may demonstrate the presence of nymphs or adults. Skin biopsies reveal moderate hyperkeratosis and the presence of a few cigar-shaped mites within the hair follicles.

Additional references: Oxenham M. Flea control in ferrets. *Vet Rec* 138(15):372, 1996.

**Miscellaneous skin disorders**

1. Bacterial skin disease. Due to the nature of ferret skin, bacterial skin disease is fairly uncommon. Traumatic wounds, or poor husbandry are generally required for bacterial skin disease to occur in this species. King et al. described a case of superficial spreading pyoderma in the ferret following fire ant bites.
2. Pemphigus foliaceous. A recent submission to the AFIP (1999) for consultation of skin from a ferret with generalized eruptions revealed classic vesicular lesions consistent with pemphigus foliaceous in other species; i.e., intracorneal pustules containing rafts of acantholytic cells, a thickened epidermis, and prominent superficial lymphocytic and eosinophilic dermatitis. This disease has not been previously described in the ferret literature.

Additional references.

King, WW. Superficial spreading pyoderma and ulcerative dermatitis in a ferret. *Veterinary Dermatology* 7(1):43-47, 1996.

## Special Senses

### Cataracts

Synopsis. There are several reports of cataracts in individual animals and breeding colonies. While many causes have been postulated, no definitive cause has been isolated. Cases in individual animals are considered to be spontaneous. Cataractous change may also be seen in the lenses of diabetic animals, however, since the lifespan of diabetic ferrets is generally short, grossly visible cataracts generally have not formed.

Gross lesions. Cataracts in ferrets generally involve both the cortex and nucleus of the lens.

Microscopic lesions. The microscopic appearance e of cataracts in ferrets is similar to that in other domestic species, with formation of balloon cells in the outer cortex, initially, progressing toward the nucleus. Morgagnian change has not been described in ferrets.

Additional references.

Miller PE et al. Cataracts in a Laboratory Colony of Ferrets. *Lab Anim. Sci* 43:562-566, 1993.

## Neoplasia (other than previously described)

**Synopsis**. Numerous neoplasms have been described in the ferret, most of which are similar both grossly and histologically to those seen in other animals. Neoplasms easily represent up to 60% of total surgical biopsies of ferrets, with the balance being islet cell tumors, adrenal neoplasms, chordomas, and the skin tumors already mentioned. While the following is by no means an exhaustive list of the remaining, it represents those which I personally, feel are most commonly seen and most significant in this species.

**Reproductive**. Tumors of smooth muscle are the most common neoplasm of this system, and are also seen in the endocrine system (generally arising in the adrenal gland) and rarely in the gastrointestinal system and subcutaneous tissue. Low grade leiomyosarcomas, demonstrating an infiltrative nature, moderate atypia, and a moderate mitotic rate are more common than leiomyomas in this species. Additionally, leiomyosarcomas have been reported as occurring "free-floating" in the abdomen. As the majority of these tumors are attached to the adrenal gland, ovary, or testis, and are removed due to the organomegaly that they cause, the prognosis is generally good. Metastasis of leiomyosarcoma has not been seen.

Testicular neoplasms - Interstitial cell tumors are the most common neoplasm of the ferret testicle, but combinations of two or more neoplasms are not uncommon. (Indeed, one of my own ferrets, obtained as a 4-year-old cryptorchid had **FOUR** neoplasms in the same testicle - interstitial cell tumor, seminoma, Sertoli cell tumor, and a carcinoma of the rete testis). This illustrates the importance of removing cryptorchid testicles in this species - you can always find at least one neoplasm and often more in retained testicles.

Ovarian neoplasms - Tumors of germ cell or stromal cell origins are most commonly seen, epithelial neoplasms are rare. One teratoma has been reported.

**Gastrointestinal system.** The second most commonly seen neoplasm of the gastrointestinal system (after lymphosarcoma) are tumors of smooth muscle origin, arising from the muscular layers of the GI tract. Low-grade leiomyosarcomas are most commonly seen. Mesotheliomas are occasionally seen in the peritoneum and serosal surfaces of ferrets. They are locally aggressive, result in marked abdominal effusion, and warrant a poor prognosis. Pancreatic exocrine adenocarcinomas are occasionally seen in the pancreas - these neoplasms are locally aggressive with a moderate metastatic potential, most commonly to the liver. Intestinal adenocarcinomas are rare locally aggressive neoplasms Gastric carcinoma has been experimentally reproduced in the presence of *Helicobacter mustelae* with a carcinogenic compound.

**Musculoskeletal system** - Osteomas are generally seen arising from flat bones. They are expansile neoplasms composed of trabecular of well-differentiated bone lined by osteoblasts and a few osteoclasts. The trabeculae are wide and there is little intervening space. Marrow is not seen. Osteosarcomas are far less commonly seen, and appear to be equally divided between long bones and flat bones. Rhabdomyosarcomas are also uncommon neoplasms of ferrets.

**Integumentary system**. Apocrine cysts are a common finding in ferrets. They most commonly occur around the head, neck, prepuce, and vulva, due to the large numbers of scent glands in these regions. Apocrine gland cystadenomas and carcinomas are not uncommon and have a similar distribution. Apocrine gland carcinomas are locally aggressive neoplasms with a moderate potential for metastasis. Hemangiomas and low-grade hemangiosarcomas are occasionally seen; metastasis has not been reported. Squamous cell carcinoma has been reported several times in the ferret and has a predilection for the face, where it is locally destructive with a low metastatic potential.

**Urinary system**. Transitional cell carcinoma has been reported in the ferret.

**Hematolymphatic system**. Cranial mediastinal thymomas were resported in 2 5-year-old ferrets and should be considered in the differential diagnosis for thoracic neoplasia.

**Nervous system**. One case of a granular cell tumour has been reported in the prosencephalon of a ferret, and one granular cell tumor has been submitted to the AFIP. In this case, the granular cells stained positively for glial fibrillary acidic protein, suggesting astrocytic origin.

Additional references.

Beach JE, Greenwood B. Spontaneous neoplasia in the ferret (Mustela putorius furo). *J Comp Pathol* 108:133-147, 1993.

Bell RC, Moeller RB. Transitional cell carcinoma of the renal pelvis in a ferret. *Lab Anim. Sci* 40:537-538, 1990.

Brunnert SR et al. Leiomyosarcoma in a domestic ferret: Morphologic and immunocytochemical diagnosis. *Lab Anim. Sci* 41:180-182, 1991.

Cotchin E. Smooth muscle hyperplasia and neoplasia in the ovaries of domestic ferrets (Mustela putorius furo). *J Pathol* 130:169-171, 1980.

Fuentealba C et al. Liposarcoma arising from the madibular bone marrow in a ferret. C*an Vet J* 36(12): 779-780, 1995.

Fox JG et al. MNNG-induced gastric carcinoma in ferrets infected with *Helicobacter mustelae*. *Carcinogenesis* 14:1957-1961, 1993.

Hamilton TA, Morrison VT. Bleomycin chemotherapy for metastatic squamous cell carcinoma in a ferret. *JAVMA* 198:107-108, 1991.

Lloyd MH, et al. Synovial sarcoma in a ferret. Vet Rec. 1996 Dec 21; 139(25): 627-628.

Miller TA et al. Recurrent adenocarcinoma in a ferret. *JAVMA* 187:839-841, 1985.

Parker GA, Picut CA. Histopathologic features and post-surgical sequelae of 57 cutaneous neoplasms in ferrets (Mustela putorius furo). *Vet Pathol* 30:499-504, 1993.

Rice LE et al. Pyloric adenocarcinoma in a ferret. *JAVMA* 200:1117-1118, 1992.

Sakai H, Maruyama M, Hirata A, Yonemaru K, Yanai T, Masegi T: Rhabdomyosarcoma in a ferret (Mustela putorius furo). J Vet Med Sci. Jan;66(1):95-6, 2004.

Sleeman JM et al. Granular cell tumor in the central nervous system of a ferret (Mustela putorius furo). Vet Rec 138(3):65-66, 1996.

Taylor, TG et al. Thymoma in two ferrets. Lab Anim. Sci 445(4):363-365, 1995.

Williams BH et al. Peritoneal mesothelioma in two ferrets (Mustela putorius furo). *J Zoo Wildl Med* , 25:590-594,1994.

# *Leukocyte Interpretation*

*POLA COURSE August 2005*

Nancy Everds, DVM
Diplomate, ACVP
Haskell Laboratory for Health and Environmental Sciences
Newark, DE
nancy.e.everds@usa.dupont.com

# *Outline of Talk*

- General Comments
- Counting and Classifying Leukocytes
- Leukogram Interpretation
- Individual Cell Types
- Species Differences
- Hemoparasites
- Neoplasia

# *Absoluto, täydellinen, kamili, absolut!*

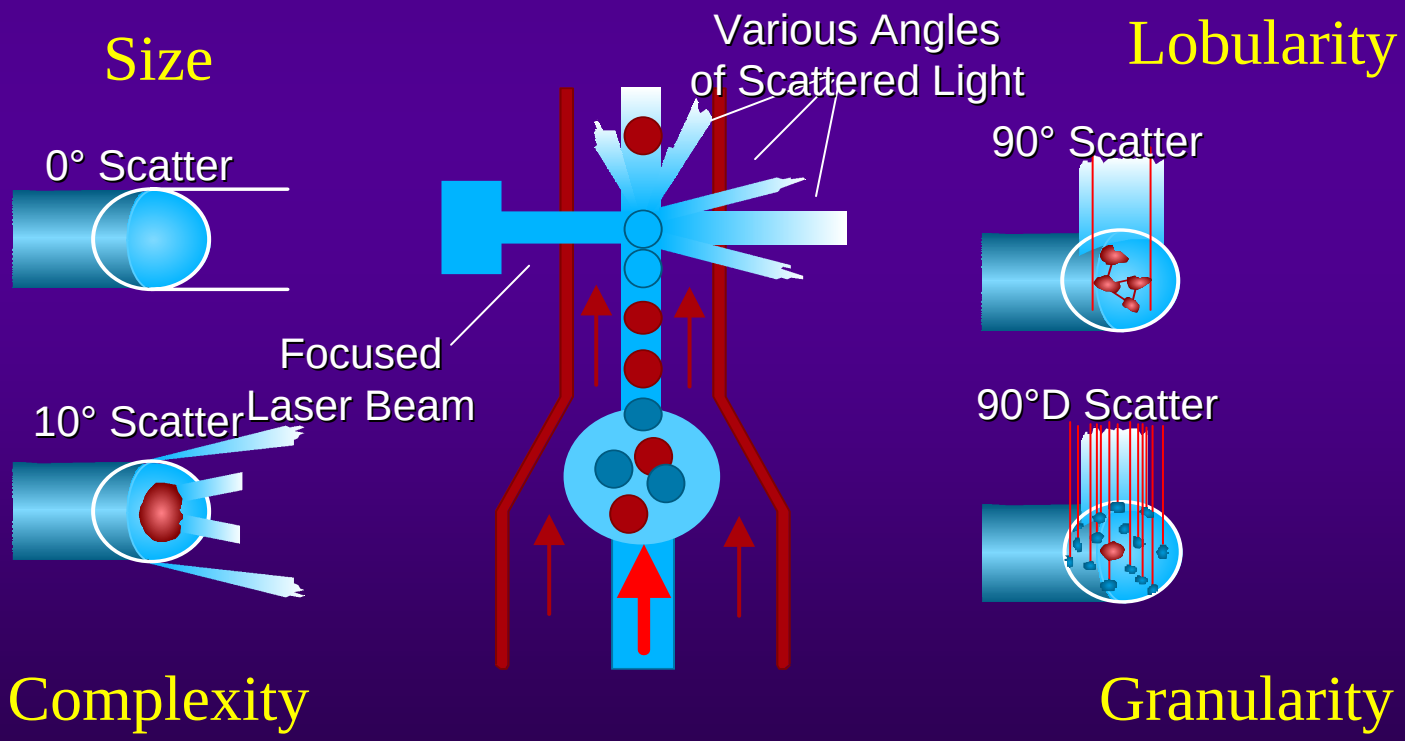
## *Use Absolute Counts*

| Tx | WBC (/uL) | SEG (%) | SEG (/uL) | LYM (%) | LYM (/uL) |
|---|---|---|---|---|---|
| Ref Range | 7,000-14,000 | NA | 500-1500 | NA | 5,000-14,000 |
| 1 | 10,000 | 10% | 1,000 | 90% | 9,000 |
| 2 | 50,000 | 5% | 2,500 | 95% | 47,500 |
| 3 | 50,000 | 40% | 20,000 | 60% | 30,000 |
| 4 | 2000 | 50% | 1,000 | 50% | 1,000 |

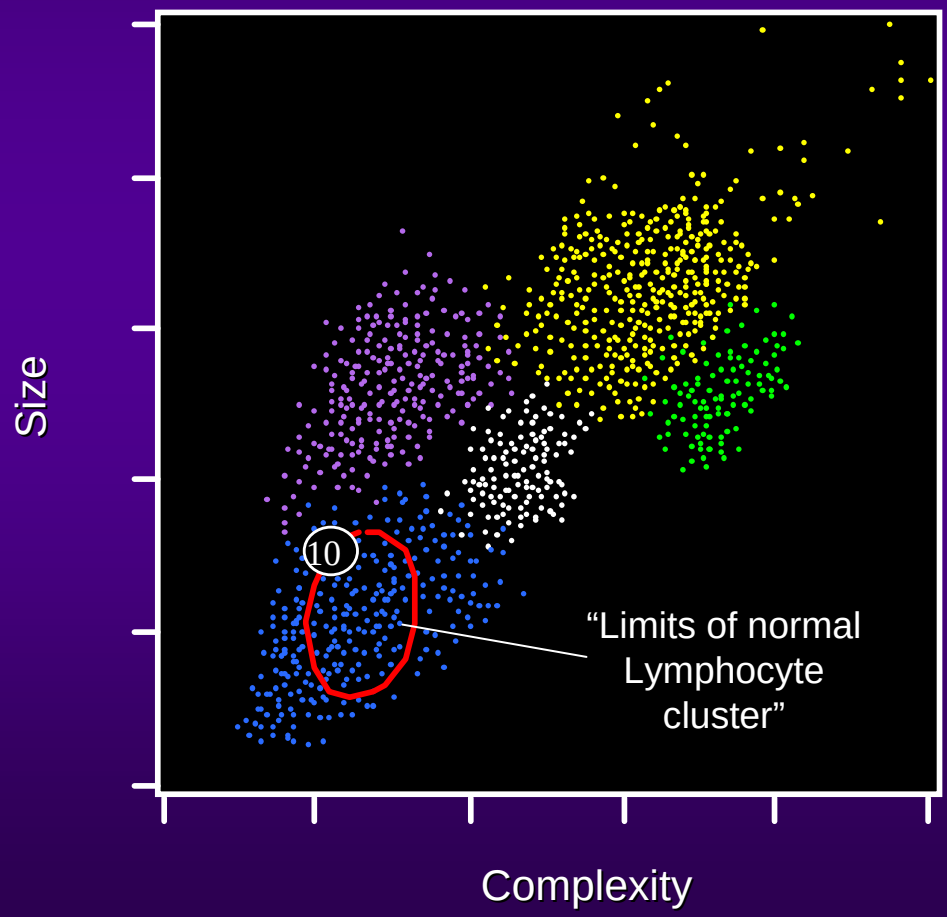
# *Counting/Classifying Leukocytes*

- Abbott CellDyn Instruments
  - Optical count
  - Impedance count
- Bayer Technicon/Advia Instruments
  - Two optical counts
    - Peroxidase stain (perox channel)
    - Nuclear complexity (baso channel)

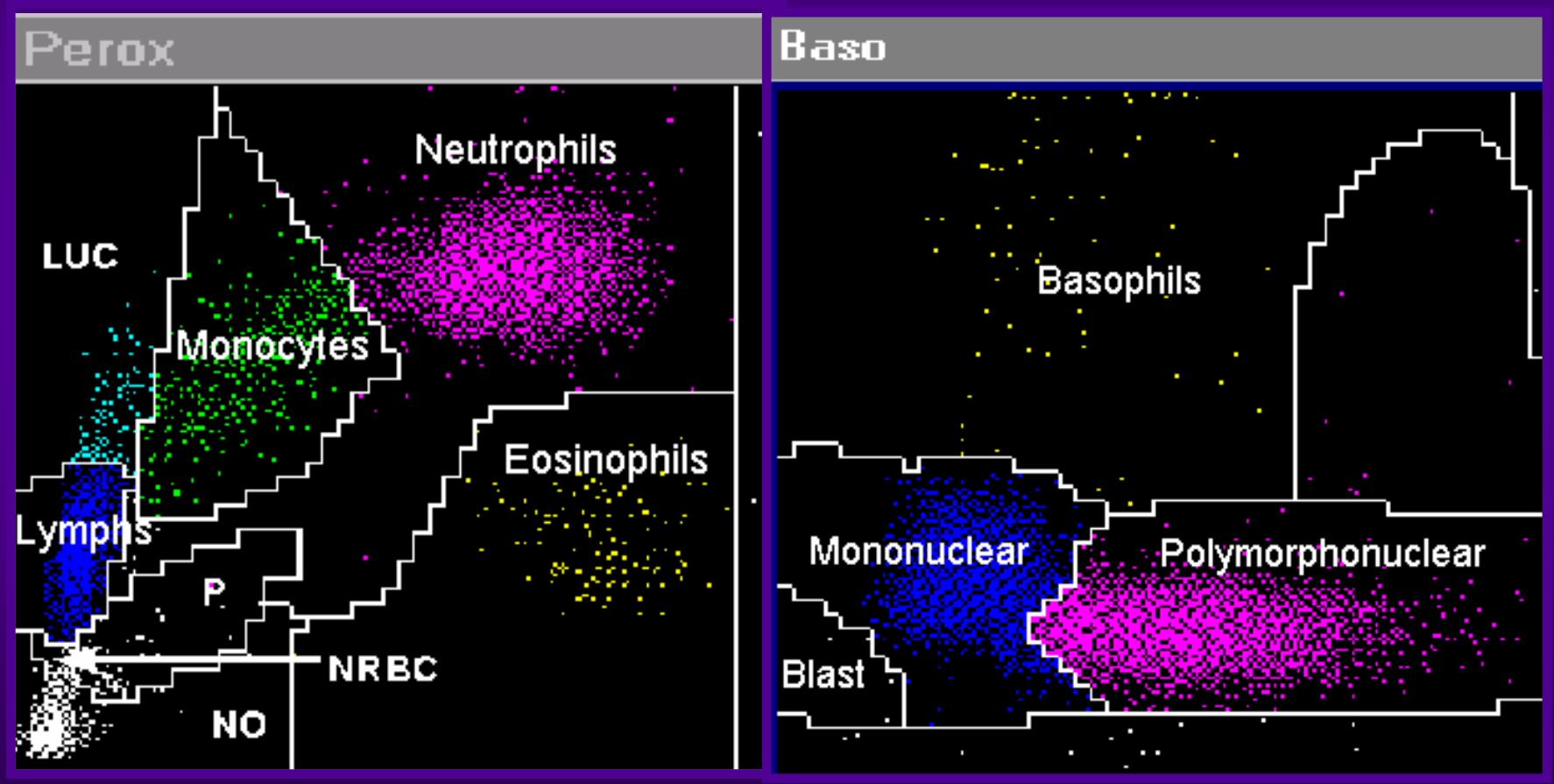
# *Counting/Classifying Leukocytes: CellDyn*



# *Counting/Classifying Leukocytes: CellDyn*



# *Counting/Classifying Leukocytes: Advia*



# *Hematology Analyzers with Automated Differentials*

- Species-specific, best for healthy animals
- “Flagging” poor for animals
- Monocytes, eosinophils, and basophils: poor correlation
- Review smear for morphology

# *Leukocyte Counts*

- Normal WBC counts:  mouse < rat, rabbit <dog, monkey
- Dependent on sampling site, anesthesia, etc.
  - Central site: lower counts

# *Species Differences: Lymphocyte/ Neutrophil (heterophil) Ratio*

- Lymphocyte > Neutrophils (heterophils)
  - Young humans, most nonhuman primates, rats, mice, cows (except young), gerbils, guinea pigs, hamsters, fish, some birds
- Lymphocytes = Neutrophils (heterophils)
  - Rabbits, most ferrets, some primates
- Lymphocytes < Neutrophils (heterophils)
  - Non-young humans, dogs, cats, horses, some ferrets

# *Leukocyte kinetics in health*

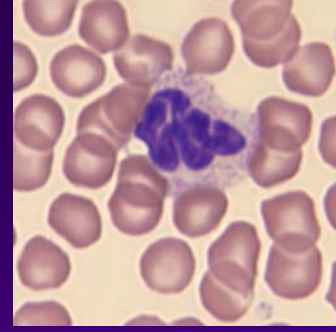
| Pool | Neuts | Lymphs | Monos | Eos | Baso |
|---|---|---|---|---|---|
| Marrow Storage | Y | N | N | Y | Minimal |
| Recirculation | N | Y | N | ? | N |
| Marginal and circulating pool | Y | Y | Y | Y | ? |
| Blood transit time | 10 hrs | Hours-years | 18-23 hrs | Minutes | 6 hrs |
| Tissue half-life | 1-2 days | Hours-years | Differ-entiate | Unknown | ? up to 2 weeks |

# *Major Circulating Leukocytes*

- Neutrophils
- Lymphocytes
- Monocytes
- Eosinophils
- Basophils

- Origin
- Growth factors
- Function
- Morphology
- Changes in health and disease

# *Neutrophil Origins*



Pluripotent stem cell

SCF, IL-3, IL-6, IL-11

Progenitor Cell
CFU-GM

IL-3, G-CSF, GM-CSF

Maturing Neutrophil Precursor

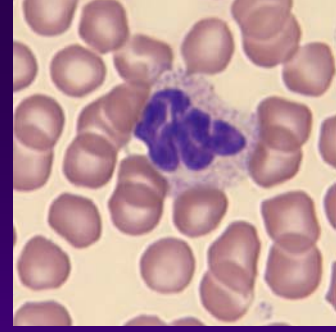
G-CSF, GM-CSF

Segmented Neutrophil

Supporting cells (monocytes, NK cells, T cells, stromal cells)

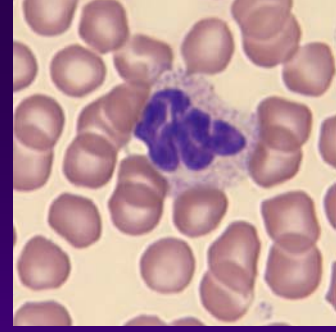
Neutrophils can be distinguished from eos and basos by the promyelocyte stage

# *Neutrophil Stages*



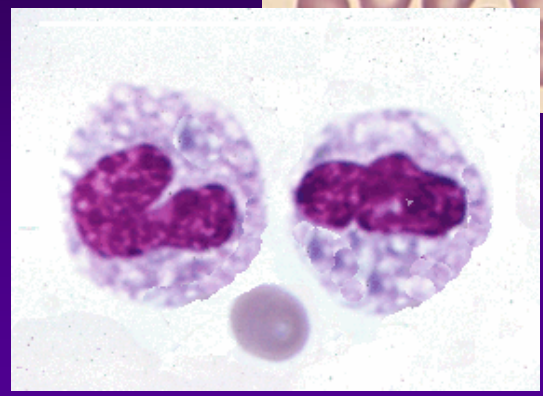
- Proliferating Stages
  - Myeloblast → Promyelocyte → Myelocyte
- Maturation Stages
  - Metamyelocyte → Band Neutrophil → Segmented Neutrophil
- Under increased demand, most mature cells are released first

# *Neutrophil Functions*



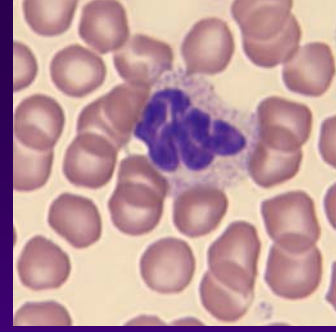
- Host defense
  - Adherence, emigration
  - Chemotaxis: N-formylated peptides, C5a, LTB4, RANTES, IL-8
  - Opsonization, phagocytosis
  - Killing of bacteria, other microbes (respiratory burst)
- Secretion of proinflammatory mediators

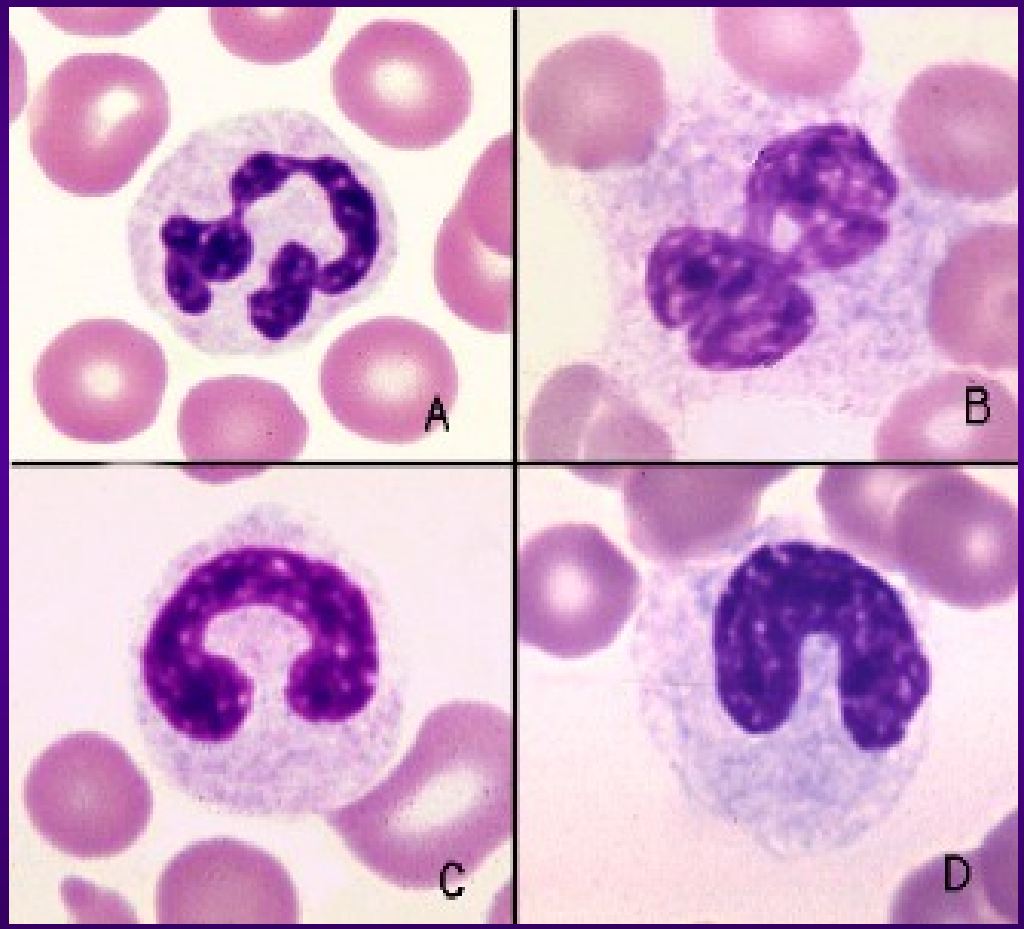
# *Neutrophil Morphology*



- Band neutrophils
- "Toxic" neutrophils
  - Caused by accelerated production
    - See toxic neutrophils after G-CSF administration
  - Basophilia, vacuolation, Döhle bodies, toxic granulation
- Ring-form nuclei (rodents, other lab animals)
- Heterophils (functionally similar to neutrophils)
  - Pink-staining granules
  - Rabbits, birds, reptiles, Guinea pits, hamsters, some fish

http://web.vet.cornell.edu/public/popmed/clinpath/CPmodules/heme1/toxic.htm





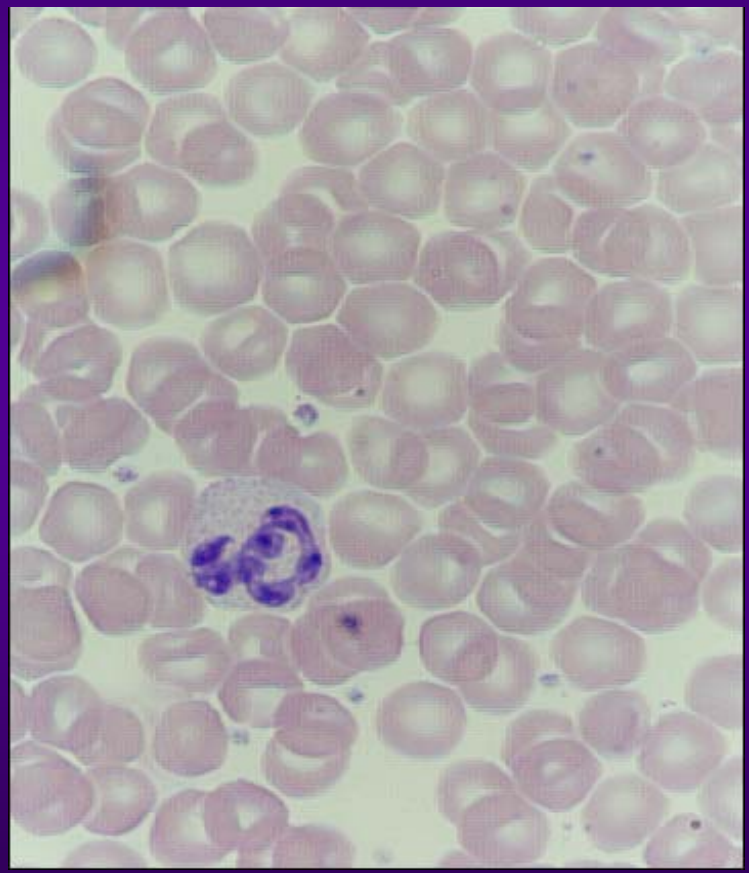

A: Segmented neutrophil

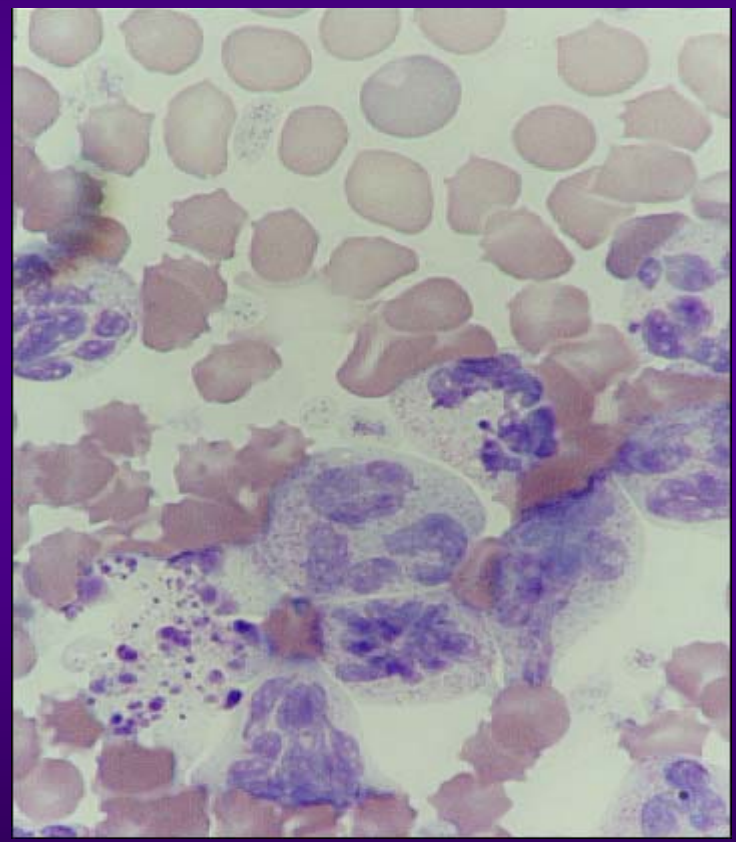
B: Toxic neutrophil

C: Normal late band neutrophil

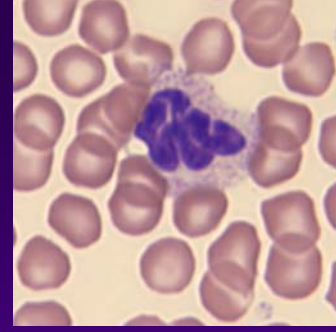
D: Toxic band neutrophil

# *Rat neutrophils rHu-GCSF*



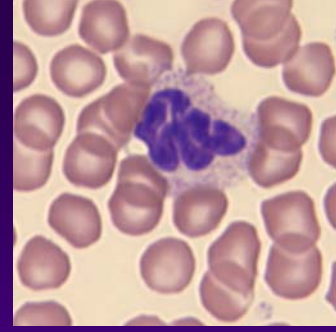


# *Increased Neutrophils-1*



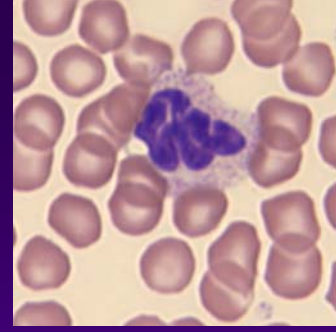
- Increased release and decreased egress (glucocorticoids): hours/days
  - Hypersegmented neutrophils
  - Endogenous or exogenous
  - ↓Lymphocytes, eosinophils
- Decreased margination (epinephrine): minutes
  - Shift to circulating pool (neuts and lymphs)
  - “Physiologic neutrophilia”
  - Cats, monkeys, mice, rats, dogs, birds
  - ↑Lymphocytes

# *Increased Neutrophils-2*



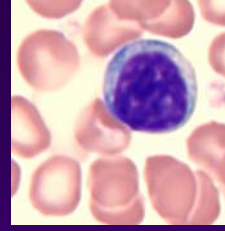
- Increased production and release from storage pool
  - Administration of CSFs
  - Inflammation (inflammatory cytokines)
    - Monkeys and dogs: bands or toxic neutrophils
    - Rodents: rare to see toxic neutrophils
    - Rodents: also have increased lymphs with inflammation
    - All species: +/- increased monocytes
- Neoplasia

# *Decreased Neutrophils*



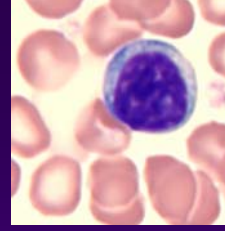
- Decreased production/release (bone marrow)
- Shift from central to marginal pool (shock)
- Increased destruction
  - Drug-induced neutropenia
  - Anti-neutrophil antibodies
- Increased egress (inflammation)
  - Degenerative left shift if early forms outweigh mature neutrophils
- How low is too low?
  - Normal mice
  - Dogs in controlled environment

# *Lymphocyte Origins*



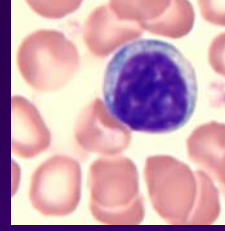
- Embryological origins
  - T-cells: Thymus
  - B-cells: Bone Marrow, Bursa of Fabricius
- Postnatal origins
  - Secondary lymphoid organs
- Recirculating and dividing cells
- Most peripheral blood lymphocytes are T-cells

# *Lymphocyte Functions*

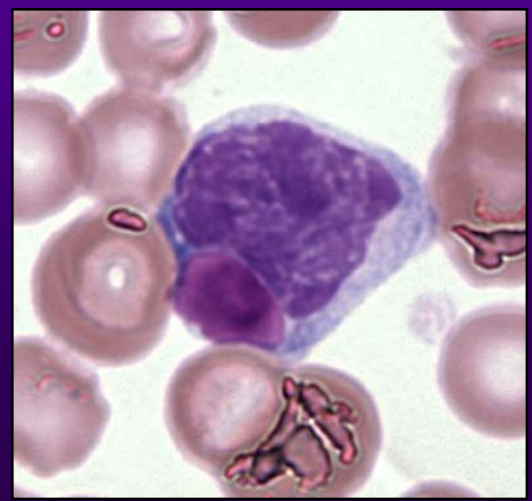


- Regulation of immune response (CD4+ T cells; Th1 and Th2)
- Cytotoxicity (CD8+ T cells, NK cells)
- Antibody production (B cells, plasma cells)
- Antigenic memory (T and B cells)

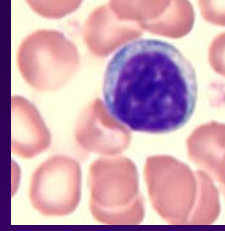
# *Lymphocyte Morphology*



- Mature lymphocytes (resting)
- Reactive lymphocytes (basophilic cytoplasm)
- Plasma cells (rare in peripheral blood)
  - Russell Bodies
- Kurloff Bodies
  - Guinea pigs and less frequently in capybaras
  - MPS inclusions in mononuclear cells
  - Increase with estrogen and pregnancy
  - 3-4% of WBCs

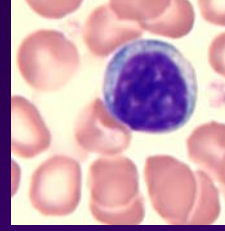


# *Increased Lymphocytes*



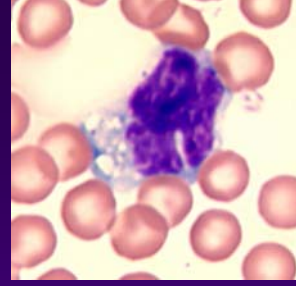
- Altered traffic patterns
- Demargination (excitement--epinephrine)
  - Associated with increased neutrophils
  - May not see neutrophil effect in lab animal species with lymphocyte predominance
- Increased production
  - Antigenic stimulation (viral, rickettsial, etc)
  - Neoplasia
- Inflammation
  - Rodents, occasionally with antigenic stimulation in other species
  - Persistent lymphocytosis (Bovine Leukosis Virus)

# *Decreased Lymphocytes*



- SCID mice, horses (Arabian), humans
  - Lack functional B and T lymphocytes
- Glucocorticoid-related
  - Redistribution of lymphocytes
  - May not see neutrophil effect in lab animal species with lymphocyte predominance
- Lympholysis (pharmacologic doses)
- Interference with lymph circulation
- Infection

# *Monocyte/Macrophage Origins*



Pluripotent stem cell

SCF, IL-3, IL-6, IL-11

Progenitor Cell
CFU-GM

IL-3, M-CSF, GM-CSF

Maturing Monocyte Precursor

IL-3, M-CSF, GM-CSF

Monocyte

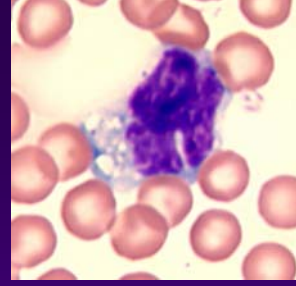
Inflammatory Mediators

Tissue Macrophage

Supporting cells (NK cells, T cells, stromal cells)

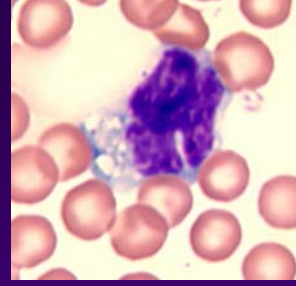
Promoncytes and monocytes are infrequently observed in bone marrow of most species (3 day transit time)

# *Monocyte/Macrophage Functions*

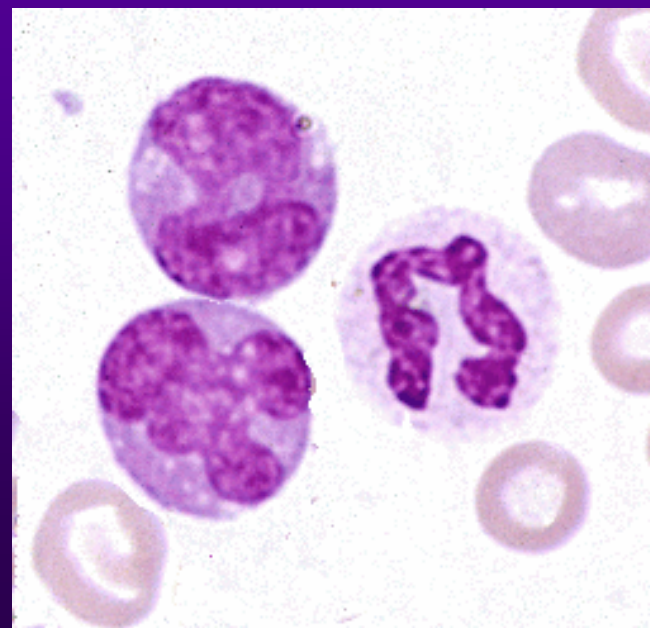


- Host defense
  - Chemotaxis
  - Activation
    - Especially by IFNγ
    - Enhanced antimicrobial properties
  - Antigen presentation, antibody-dependent cytotoxicity
  - Phagocytosis of microbes, foreign material, dead cells
- Secretion of proinflammatory mediators (CSFs, cytokines)
- Catabolism of serum proteins

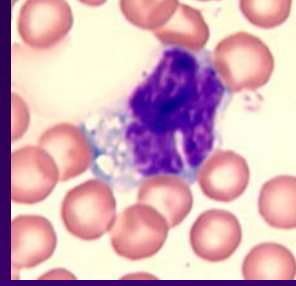
# *Monocyte Morphology*



- Largest leukocyte on peripheral smears
- Irregular cytoplasmic border
- Gray-blue cytoplasm +/- fine granulation
- Pleomorphic nucleus

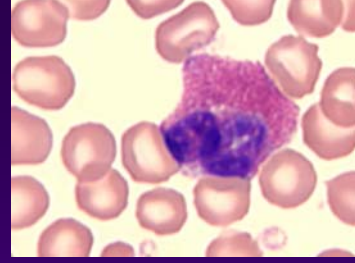


# *Monocyte Counts*



- Increased
  - Corticosteroids (dogs)
  - Inflammation (inconsistent response)
  - Recovery from inflammation
  - Leukemia
- Decreased
  - Not clinically recognized
  - Observed in conjunction with other cytopenias

# *Eosinophil Origins*



Pluripotent stem cell

GM-CSF, IL-3, IL-5

Progenitor Cell
CFU-E

GM-CSF, IL-3, IL-5

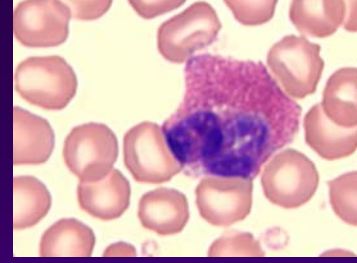
Maturing Eosinophil Precursor

IL-5

Mature Eosinophil

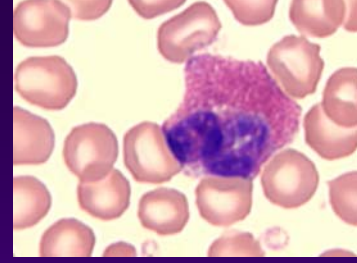
Eosinophils can be distinguished from neuts and basos by the promyelocyte stage

# *Eosinophil Functions-1*



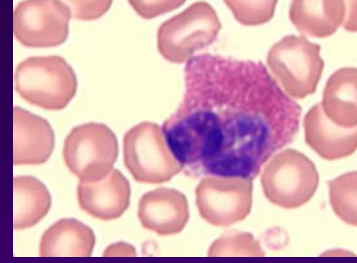
- Defense against helminth parasites
  - Secrete mediators into milieu
  - Enhanced by IL-5
- Phagocytosis of microbes (less effective than neutrophils)
- Participate in hypersensitivity reactions
- Anti-tumor activities (cytotoxicity?)

# *Eosinophil Functions-2*

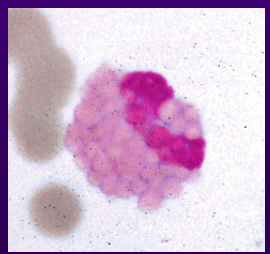


- Modulation of inflammation
  - Secrete cytokines
  - Modulate actions of basophils and mast cells
    - Inactivate mediators (histaminase, others)
    - Inhibit degranulation (release of $PGE_1$ and $PGE_2$)
  - Phagocytosis of immune complexes, anti-body coated RBCs, mast cell granules, inert particles

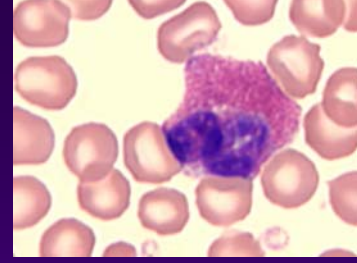
# *Eosinophil Morphology*



- Less segmented nucleus than neutrophils
- Markedly variable granule size, shape, and number across species
  - Main type: specific granule
  - Also: microgranule, primary, and small dense granule
- Heterophils vs. eosinophils
  - Eosinophil granules: round red-orange granules
  - Heterophil granules: smaller (rabbit) or needle shaped (bird)

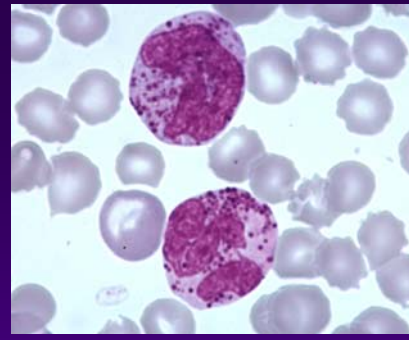


# *Eosinophil Counts*



- Increased: almost always mediated by release of IL-5 from T-cells
  - Inflammatory conditions
    - Hypersensitivity
    - Chronic inflammation of tissues rich in mast cells (skin, lung, GI, uterus)
    - Parasitism
    - Eosinophilic “itises”
  - Mast cell or eosinophil neoplasia
  - Idiopathic (“syndromes” and “complexes”)
- Decreased (automated cell counters)
  - Corticosteroids

# *Basophil Origins*



Pluripotent stem cell

GM-CSF, IL-3, IL-5

Progenitor Cell
CFU-Mast

Mostly IL-3 (also IL-5, GM-CSF

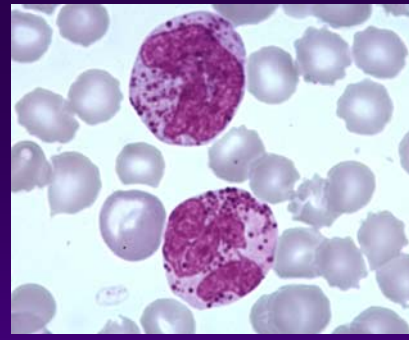
Maturing Basophil Precursor

IL-3

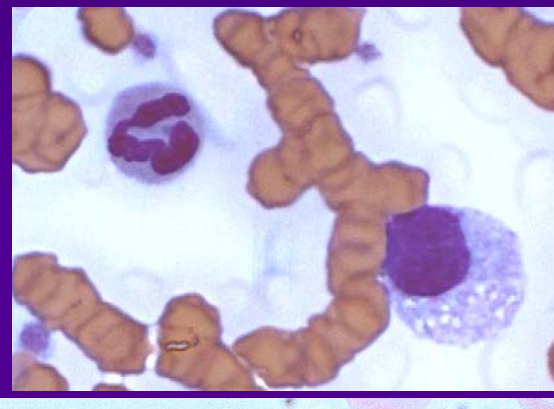
Mature Basophil

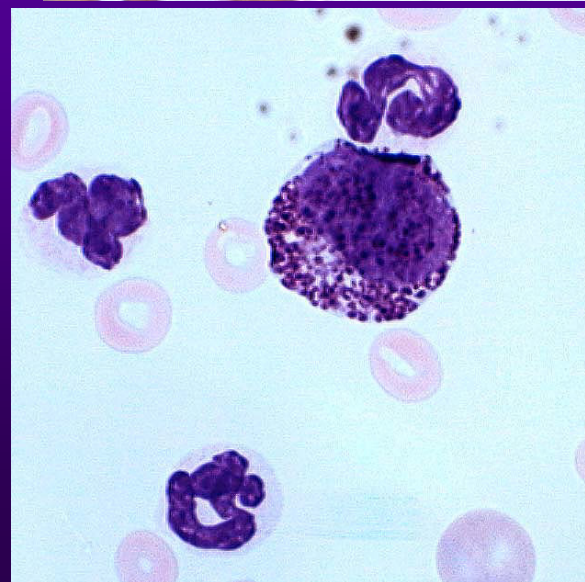
Basophils can be distinguished from eos and neuts by the promyelocyte stage

# *Basophil Morphology*

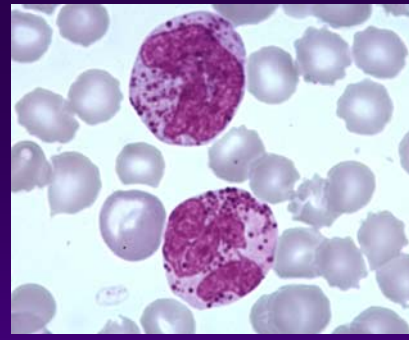


- Larger than neutrophils
- Less nuclear segmentation than neutrophils
  - Nucleus sometimes obscured by granules
- Round or oval granules
  - Variable in size and number with species
  - Stain bright purple
  - Dogs: granules are pale gray or non-staining
  - Degranulation in blood smear (birds, ferrets)
  - Tissue sections: use alcohol fixative
- Basos > Eos (birds)
- Large number of basophils (turtles)



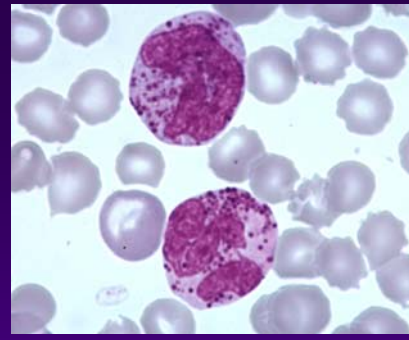


# *Basophil Functions*



- Functions
  - Recruitment of eosinophils (proinflammatory)
  - Modulation of hemostasis

# *Basophil Counts*



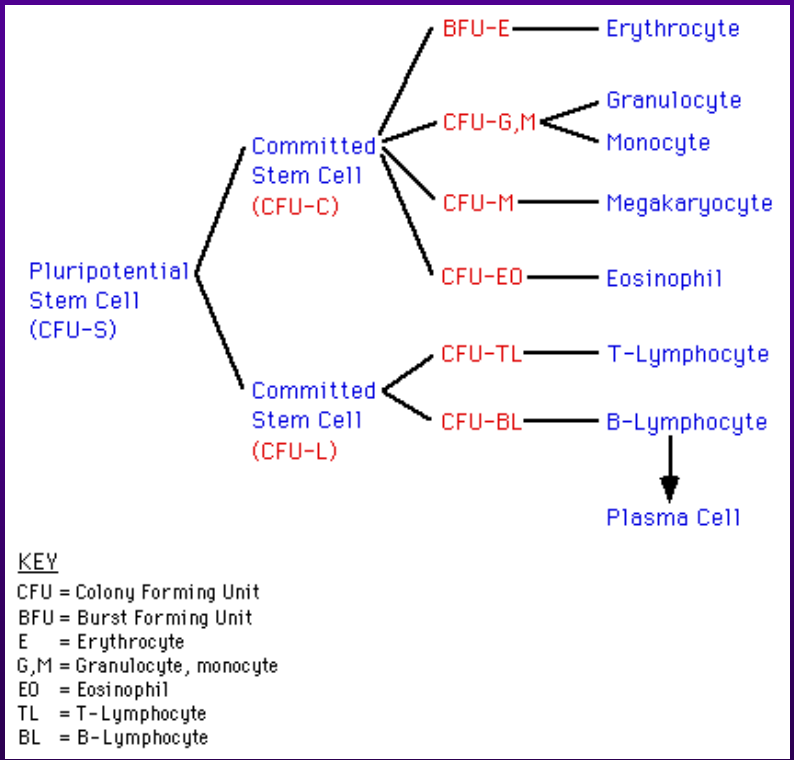
- Automated counters may be inaccurate!
- Increased basophils
  - Allergic disesase (food, stings, inhalant)
  - Parasitic diseases (heartworm disease, ectoparasites, gastrointestinal parasites)
  - Associated with increased eosinophils
  - Neoplasia
    - Primary basophil leukemia
    - Mast cell neoplasia
- Decreased basophils
  - Not clinically recognized in most species
  - Pharmacologic administration of corticosteroids

# *Other Causes of Increased Leukocytes*

- Decreased egress
  - Glucocorticoids
  - Pharmacologic block of recruitment or activation
  - Selectin deficiency (P-/E- mice)
    - Decreased rolling/adhesions
  - BLAD, Irish Setters, other
  - See hypersegmented neutrophils

# *Other Causes of Decreased Leukocyte Counts*

- If erythrocytes and platelets are affected, consider bone marrow effects



# *Leukocytes: Lab Animal Differences*

- Neutrophilia
  - Very subtle in rodents compared to other species
- Lymphocytosis
  - Consistently observed with inflammation in rodents
  - Markedly increased lymphocytosis in young cyno monkeys (40-80,000/uL)
- Neutropenia due to chemotherapeutic agents
  - May be difficult to determine (mice, rats)
- Effects of catecholamines and glucocorticoids
  - More pronounced in dogs & monkeys than rodents

# *Rat and Mouse Leukocytes*

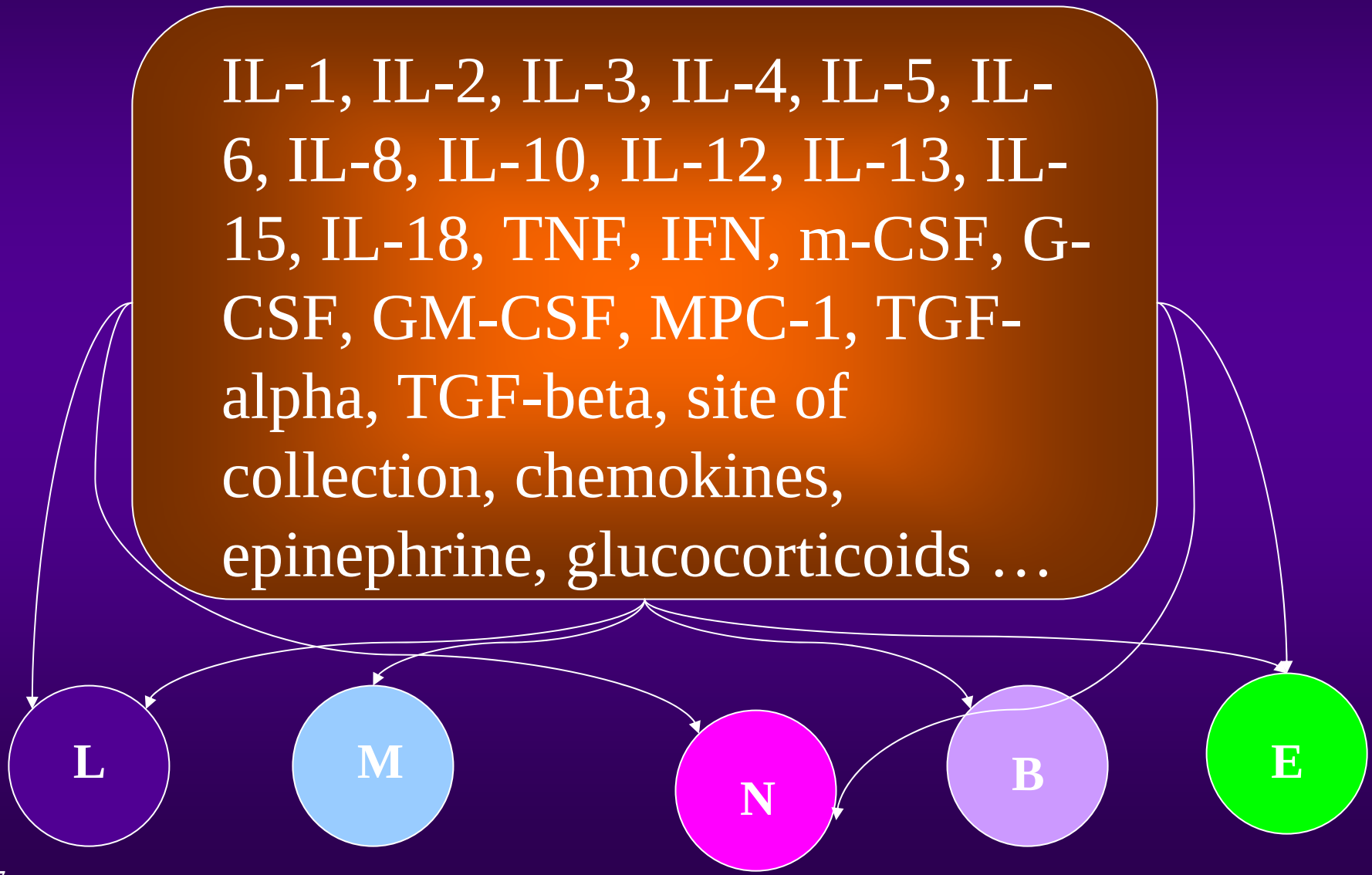
- Ring shaped nuclei
  - Neutrophils (heterophils) and eosinophils of rats,  mice, and gerbils
  - Observed in bone marrow and peripheral blood
  - More prevalent with accelerated granulopoiesis

# *Non-human Primates: Leukocyte Counts*

- Very sensitive to extra-treatment effects
- More problematic because fewer animals/group
- Common confounders
  - Excitement (epinephrine): minutes
  - Stress (glucocorticoids): hours/days
  - Intercurrent disease resulting in inflammation
    - Increased neutrophils and/or lymphocytes
    - May occur concurrently with stress
    - Lymphocytes up to 50,000-70,000/uL

# *Monkeys and Dogs: Leukocyte Counts*

- Two pretest samples increase confidence in interpretation of data
- Reference ranges can be useful for historical perspective
  - Usefulness of reference range depends on its partitioning
    - Age, sex, supplier, diet, vehicle, collection, housing, route of administration, etc.
    - Only appropriate ranges are useful


IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-15, IL-18, TNF, IFN, m-CSF, G-CSF, GM-CSF, MPC-1, TGF-alpha, TGF-beta, site of collection, chemokines, epinephrine, glucocorticoids …
L
M
N
B
E

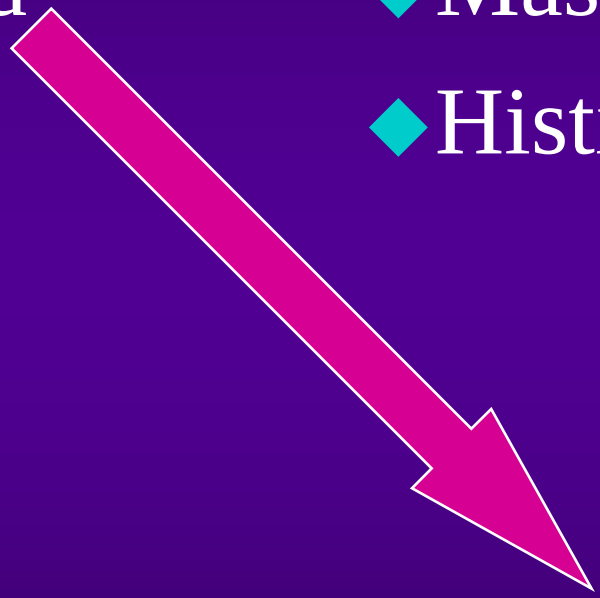
# *Hemoparasites*

- Trypanasoma
- Hemobartonella
- Eperythrozoon
- Plasmodia
- Etc…

http://www.cvm.missouri.edu/cvm/courses/vm556/Byhost/Labanim.htm

# *Hematopoietic Neoplasia of Mice and Rats*

- Lymphoid
- Myeloid
- Erythroid
- Mast Cell
- Histiocytic



Many subtypes…

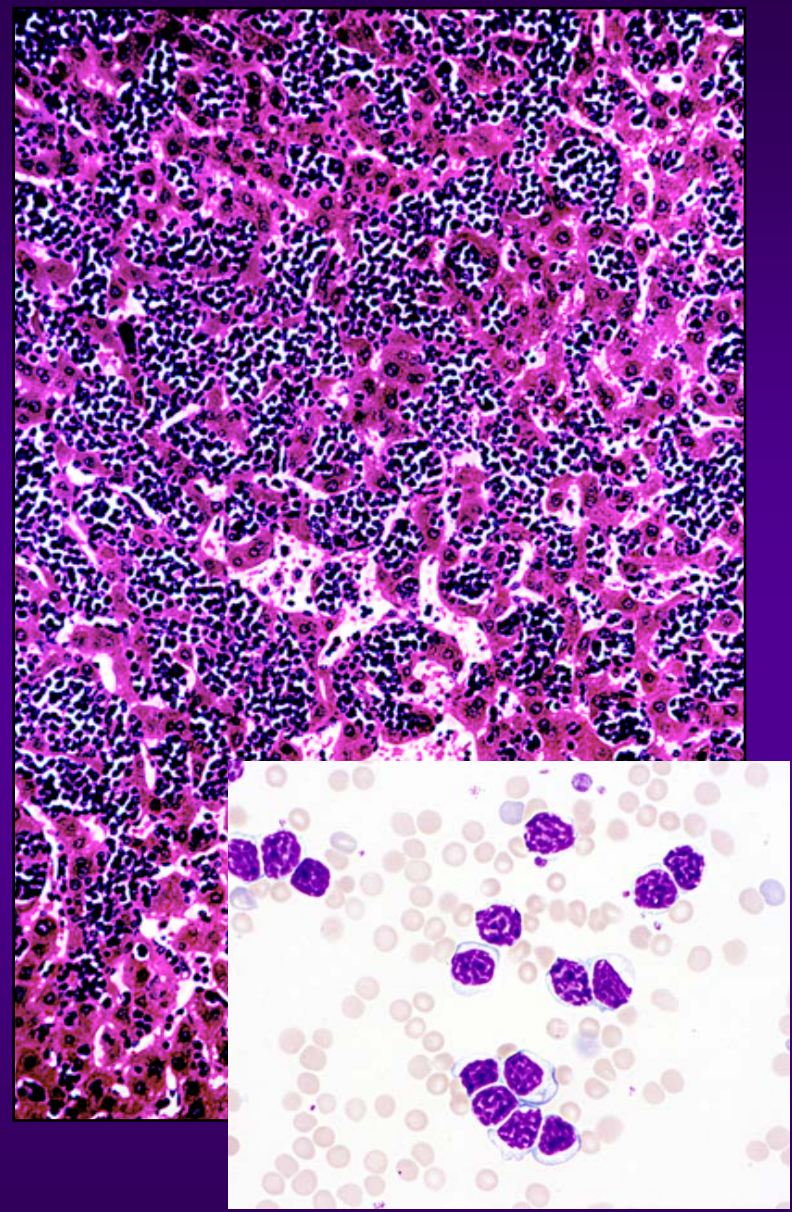
Many naming schemes

# *Lymphomas of Rodents*

- Small Lymphocyte Lymphoma
- Lymphoblastic (lymphocytic) Lymphoma
- Follicular Center Lymphoma
- Plasmacytic Lymphoma
- Immunoblastic Lymphoma
- Large Granular Lymphocytic (LGL) Lymphoma

# *Small Lymphocyte*

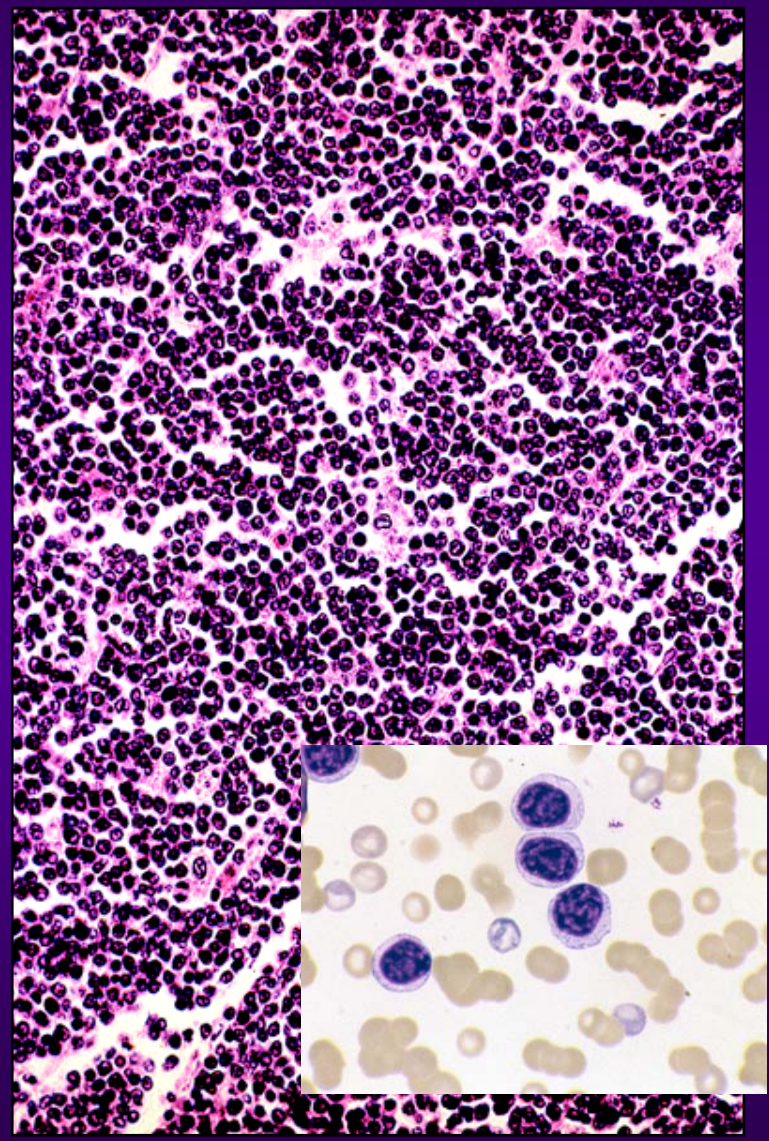
- Small to medium sized, well-differentiated lymphocytes
- Uniform size, noncohesive
- Chromatin densely clumped, with narrow rim of cytoplasm
- Differ little, if at all, from small circulating lymphocytes



Liver, Small Lymphocyte Lymphoma, Mouse

# *Small Lymphocyte (continued)*

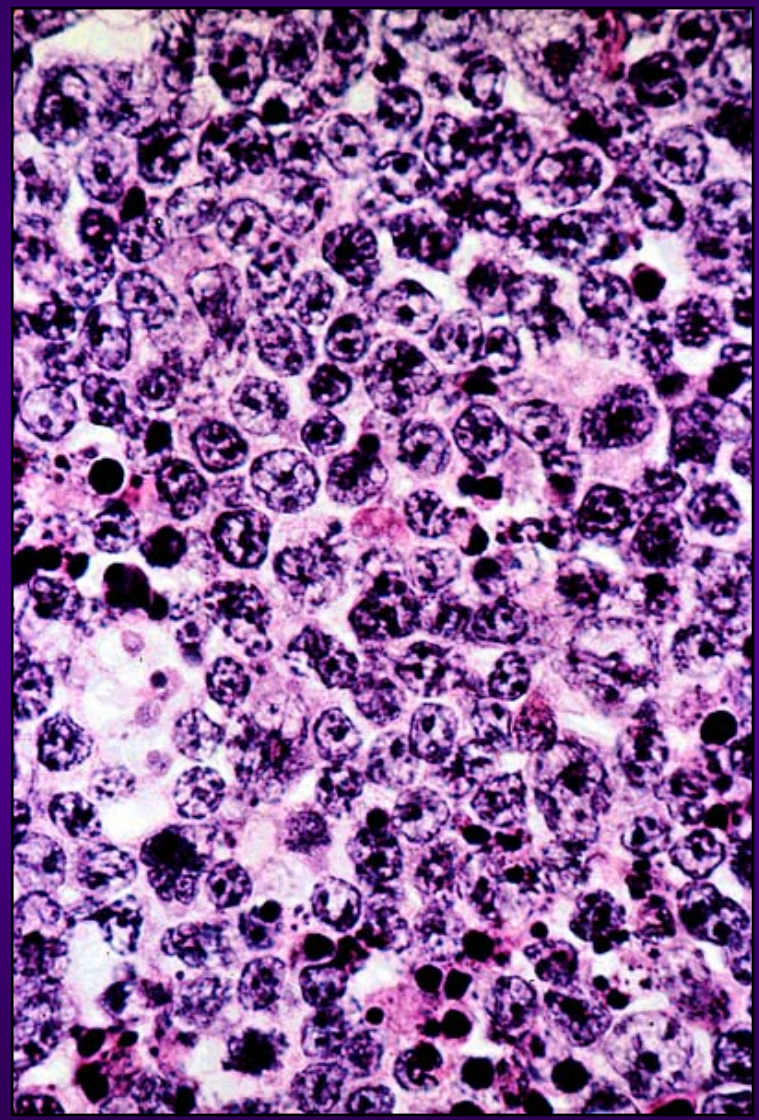
- May contain some slightly larger cells with larger irregular nuclei (small follicular center cells)
- Low mitotic index
- No tingible body macrophages
- B or T cell origin
- Spleen, liver, lymph nodes, thymus, bone marrow



Lymph Node, Small Lymphocyte Lymphoma, Mouse

# *Lymphoblastic (Lymphocytic)*

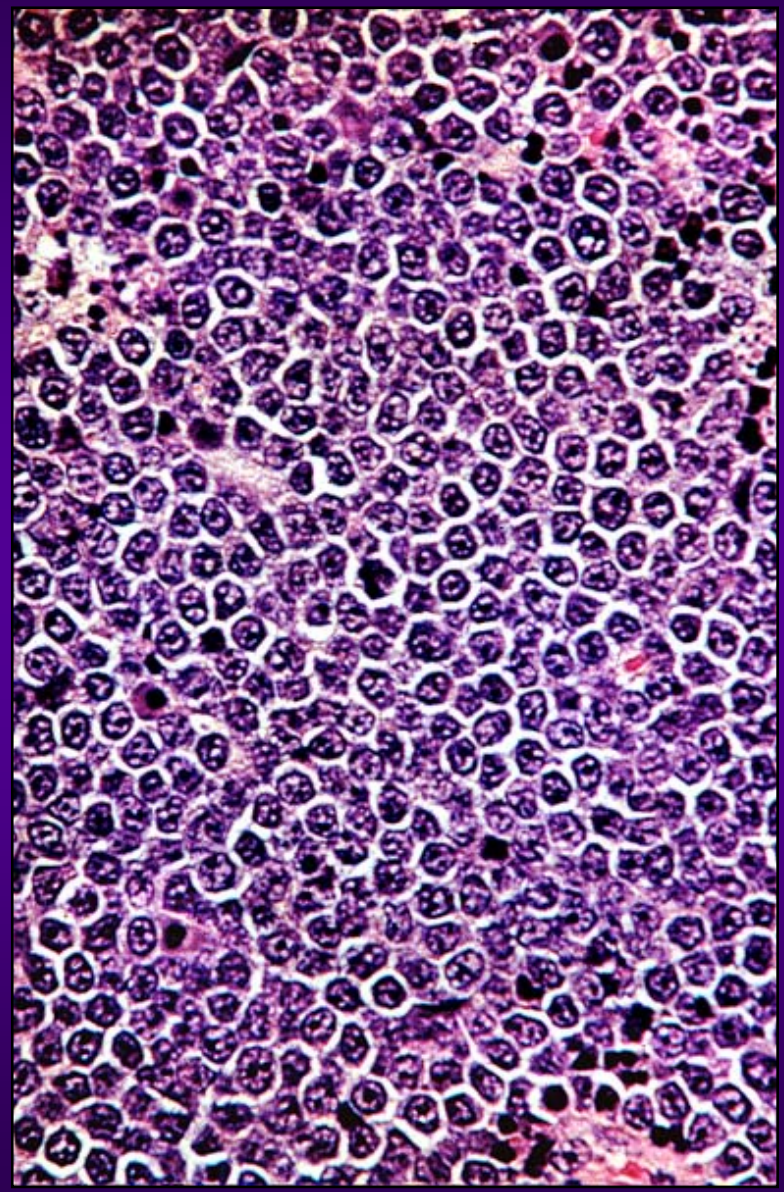
- Medium lymphoblasts with a high nuclear/cytoplasmic ratio
- Noncohesive, form homogenous sheets
- Nucleus round; prominent nucleolus
- Slight amount of basophilic cytoplasm, may be vacuolated
- High mitotic index
- May be leukemic, aggressive



Thymus, Lymphoblastic Lymphoma, Mouse

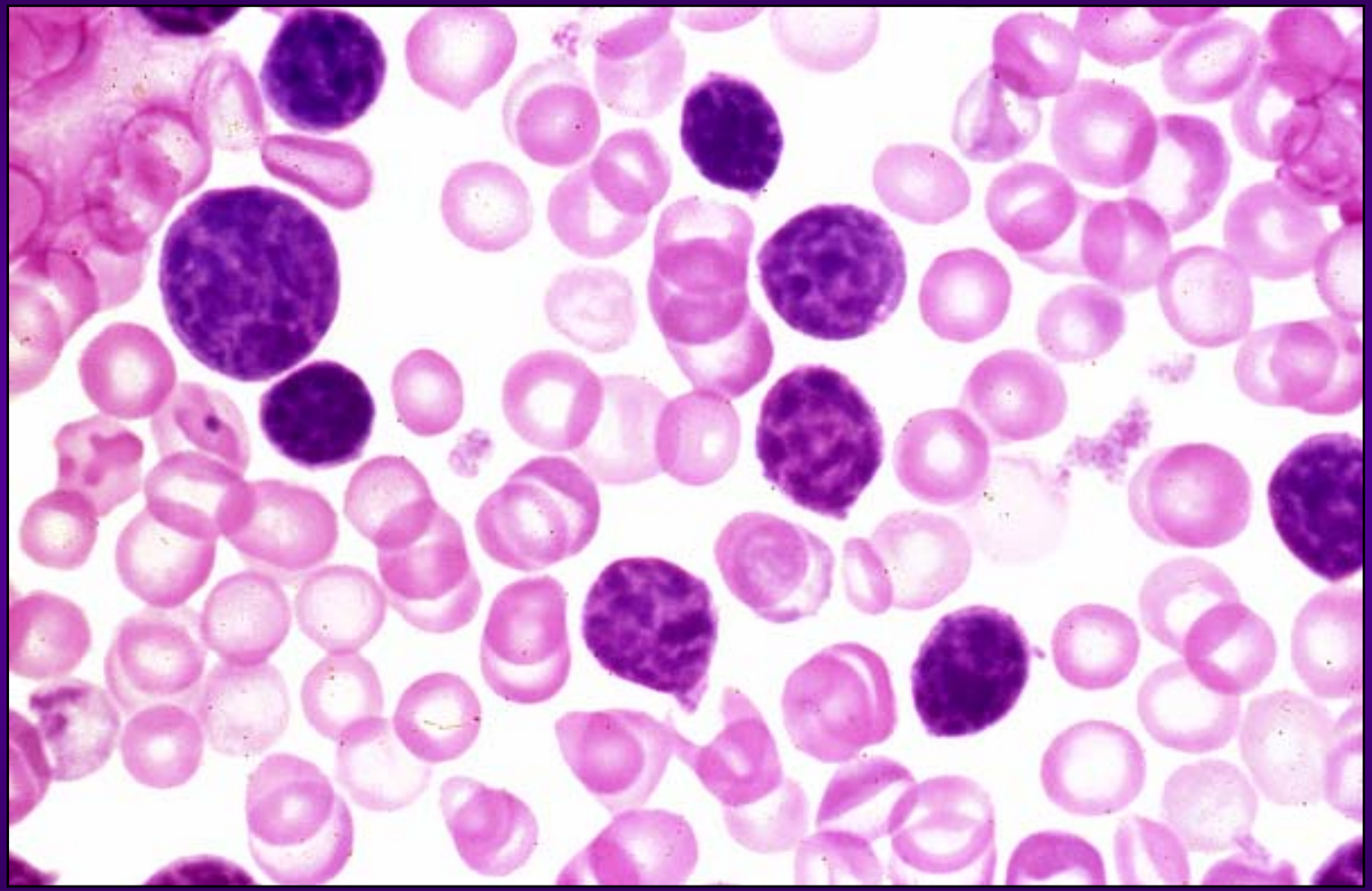
# *Lymphoblastic (Lymphocytic) (Continued)*

- Spleen, lymph nodes, thymus, liver, kidney, bone marrow, ovary, lung, CNS
- Typically induced by viruses, chemicals
- Starry sky appearance – TBM, apoptosis
- B or T cells, usually T cell in thymus
- Relatively rare in rats



Lymph Node, Lymphoblastic Lymphoma, Rat

# Peripheral blood, Lymphoblastic (Lymphocytic) Leukemia

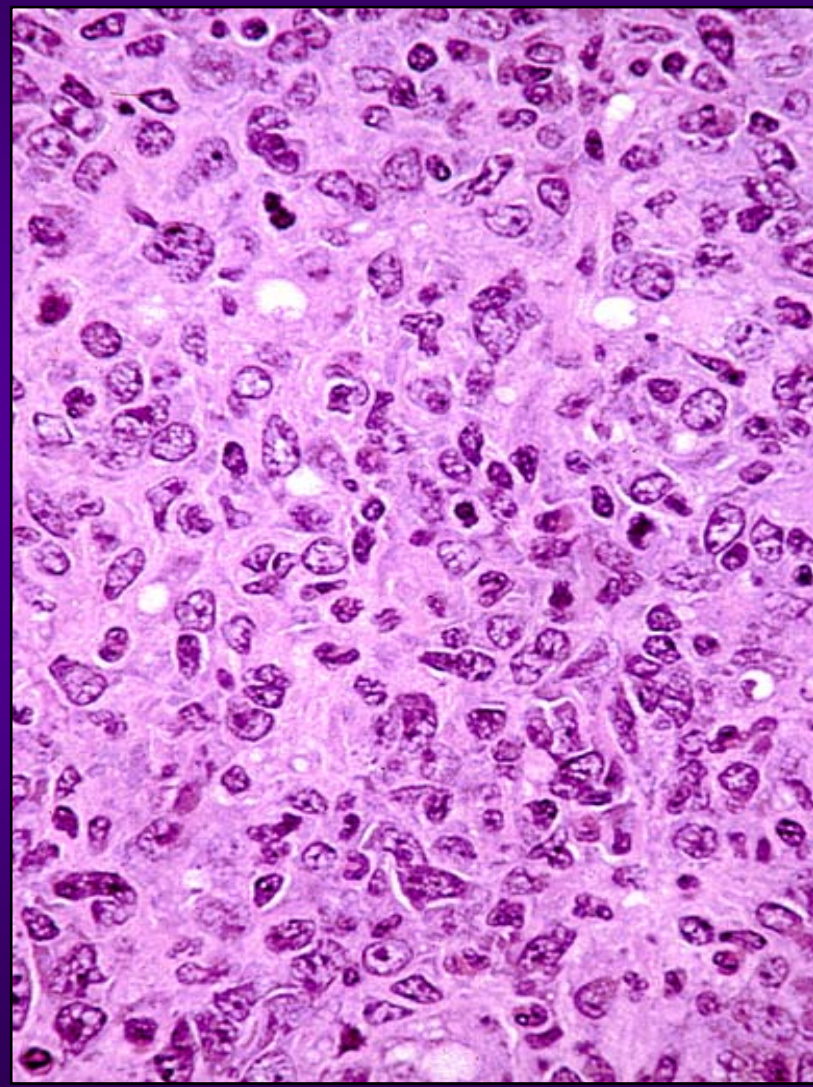


# *Follicular Center*

- Also known as…
  - Mixed
  - Pleomorphic
  - Centroblastic
  - Centrocytic
  - Centrocytic/centroblastic

# *Follicular Center (continued)*

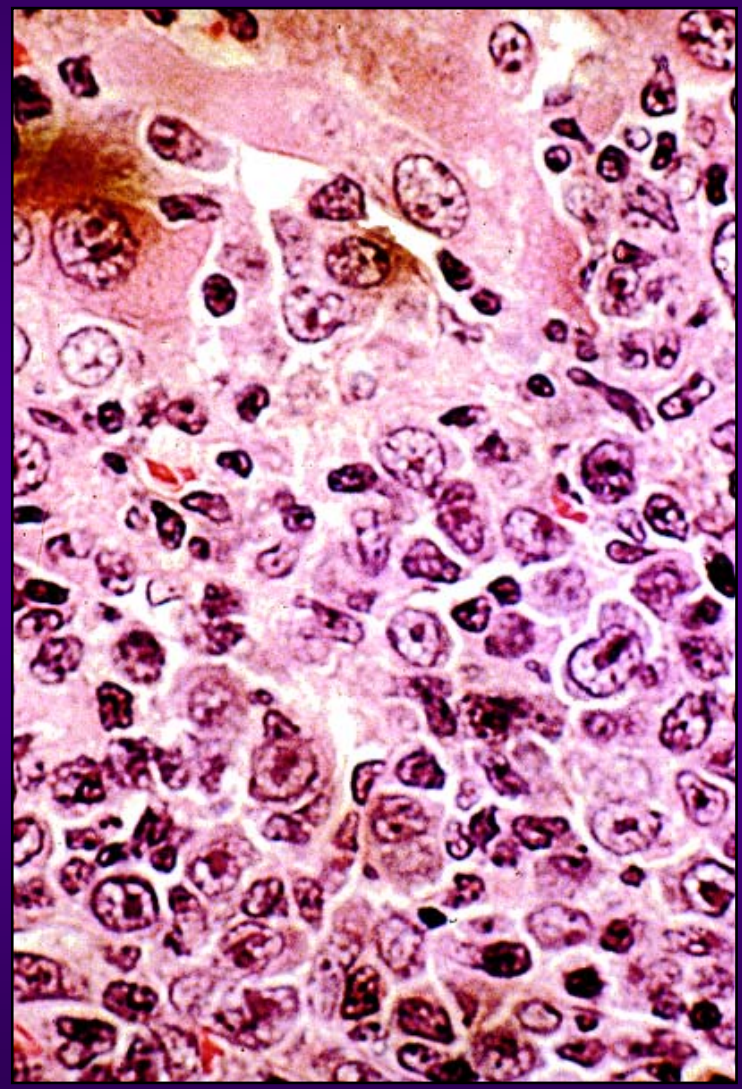
- Mice older than 1 year
- Occasionally in rats
- Medium to large sized cells
- Pleomorphic – small lymphocytes, small and large follicular center cells (centrocytes and centroblasts), macrophages and immunoblasts
- Proportion of each cell types varies by lymphoma and even by site



Spleen, FCC Lymphoma, Mouse

# *Follicular Center (continued)*

- Prominent nucleoli
- Occasionally multinucleated
- Cleaved or noncleaved nucleus
- Larger cells have vesicular nuclei
- Mitotic index low
- Early in progression: single or multiple sites, often in splenic white pulp

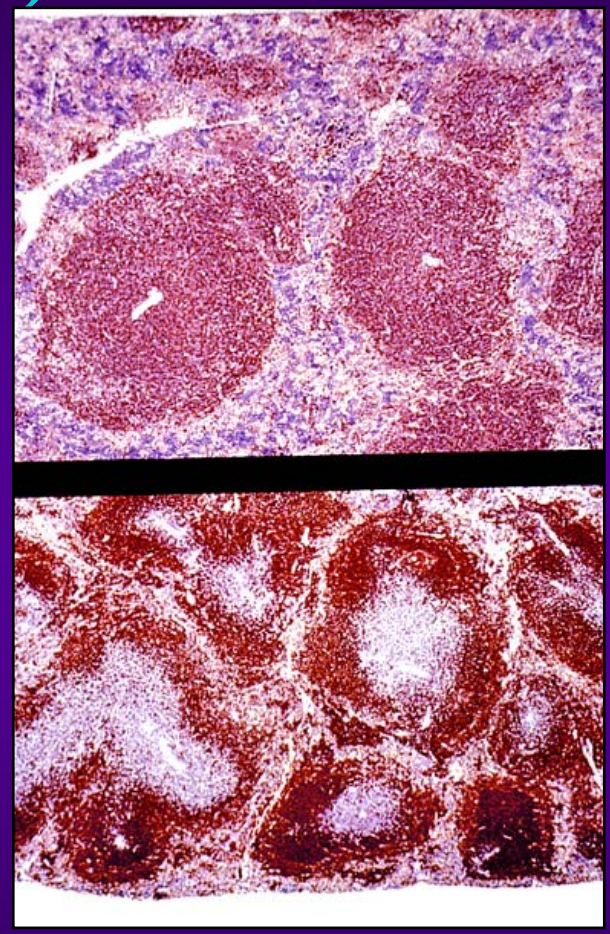


Liver, FCC Lymphoma, Mouse

# *Follicular Center (continued)*

- Follicular pattern--several splenic follicles may be involved
- Enlarged spleen – follicular or diffuse
- B cell origin; CD4+ (helper cells) admixed
- Spleen, liver, lymph nodes
- Immunohistochemistry – cytoplasmic immunoglobins

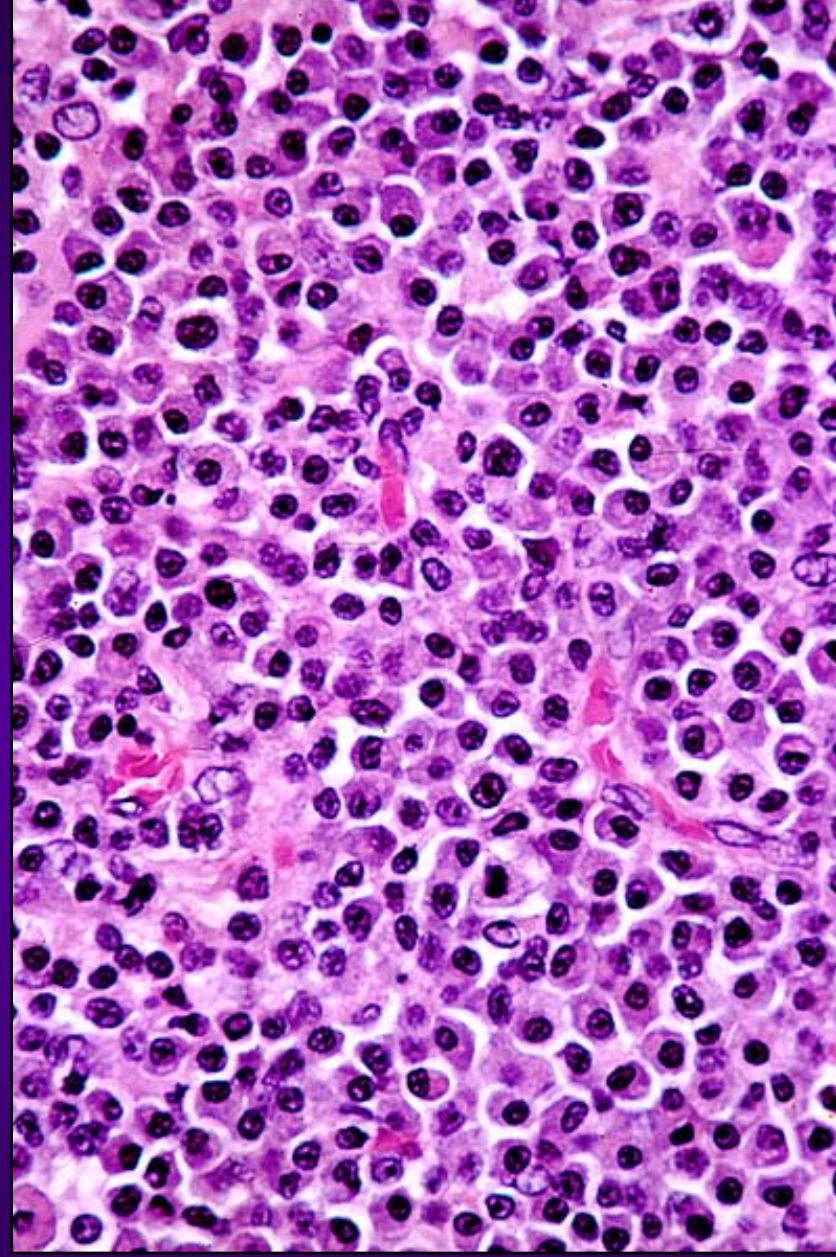
Normal Spleen B220



FCC Lymphoma B220

# *Plasmacytic*

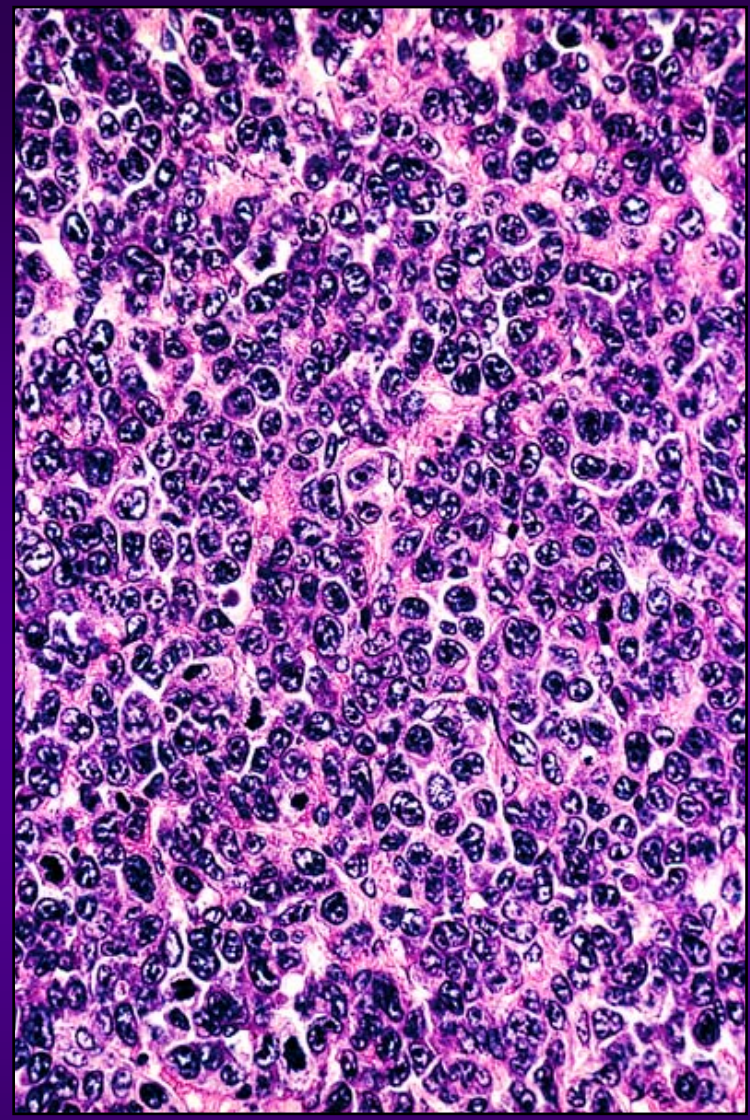
- Mature plasma cells or cells with plasmacytoid differentiation
- Nuclei round; cartwheel appearance
- Cytoplasm basophilic and pyroninophilic, small perinuclear halo may be present (Golgi)
- Low mitotic index
- Arise from immunoblasts
- Immunohistochemistry – cytoplasmic immunoglobins



Lymph Node,
Plasma Cell Lymphoma, Mouse

# *Immunoblastic*

- Large cells, conspicuous amphophilic cytoplasm
- Noncohesive, fairly monotypic
- Nuclei large, vesicular, one large central nucleolus; mitotic index high
- Plasma cells may be present
- Spleen, liver, lymph nodes
- More aggressive than follicular center lymphomas
- Rare in most strains



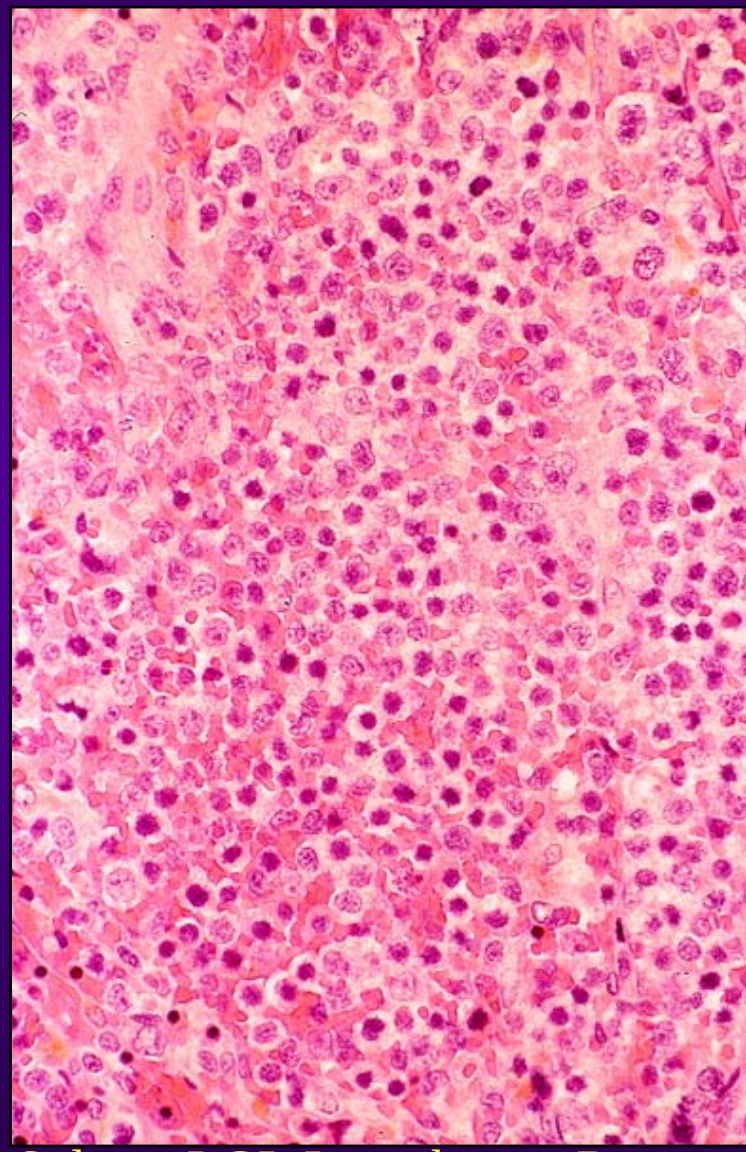
Immunoblastic Lymphoma, Mouse

# *Large Granular Lymphocyte (LGL) Lymphoma*

- Most common lymphoid neoplasia in Fischer (F344) and Wistar/Furth rats
- Incidence at 2 years: >50% in F344, 2% in Wistar, 0.5% in Sprague Dawley rats
- Gross appearance—splenomegaly
- LGL is the effector cell of NK activity in rat

# *LGL Lymphoma (continued)*

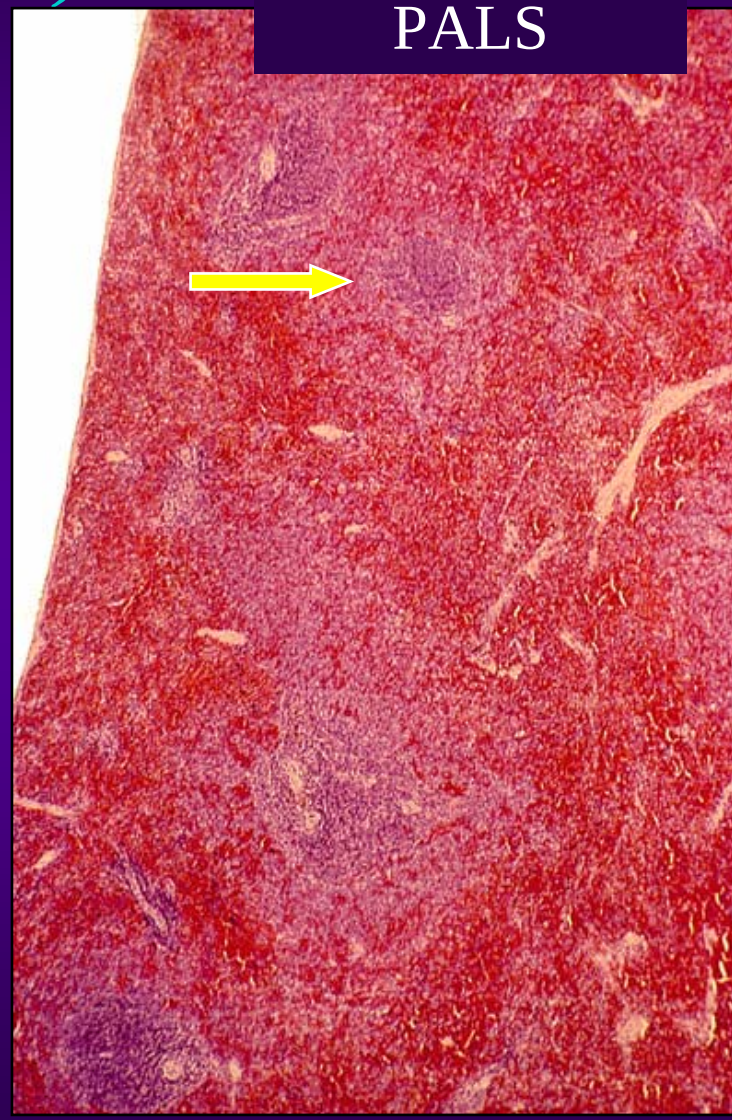
- Cells small, pleomorphic, scant cytoplasm, noncohesive
- Nuclei irregular, round to reniform, often eccentric, sparse chromatin, small nucleoli
- Mitotic rate low
- OX-8 immunoreactive, antibodies to serine esterase demonstrate granules



Spleen, LGL Lymphoma, Rat

# *LGL Lymphoma (continued)*

- Granules do not stain with H&E
- Neoplasm originates in marginal zone of spleen
- Starts with marked depletion of lymphocytes in PALS with apparent decrease in normal EMH and hemosiderin
- Most consistent: severely congested spleen, diffuse infiltration of red pulp by sheets of neoplastic mononuclear cells

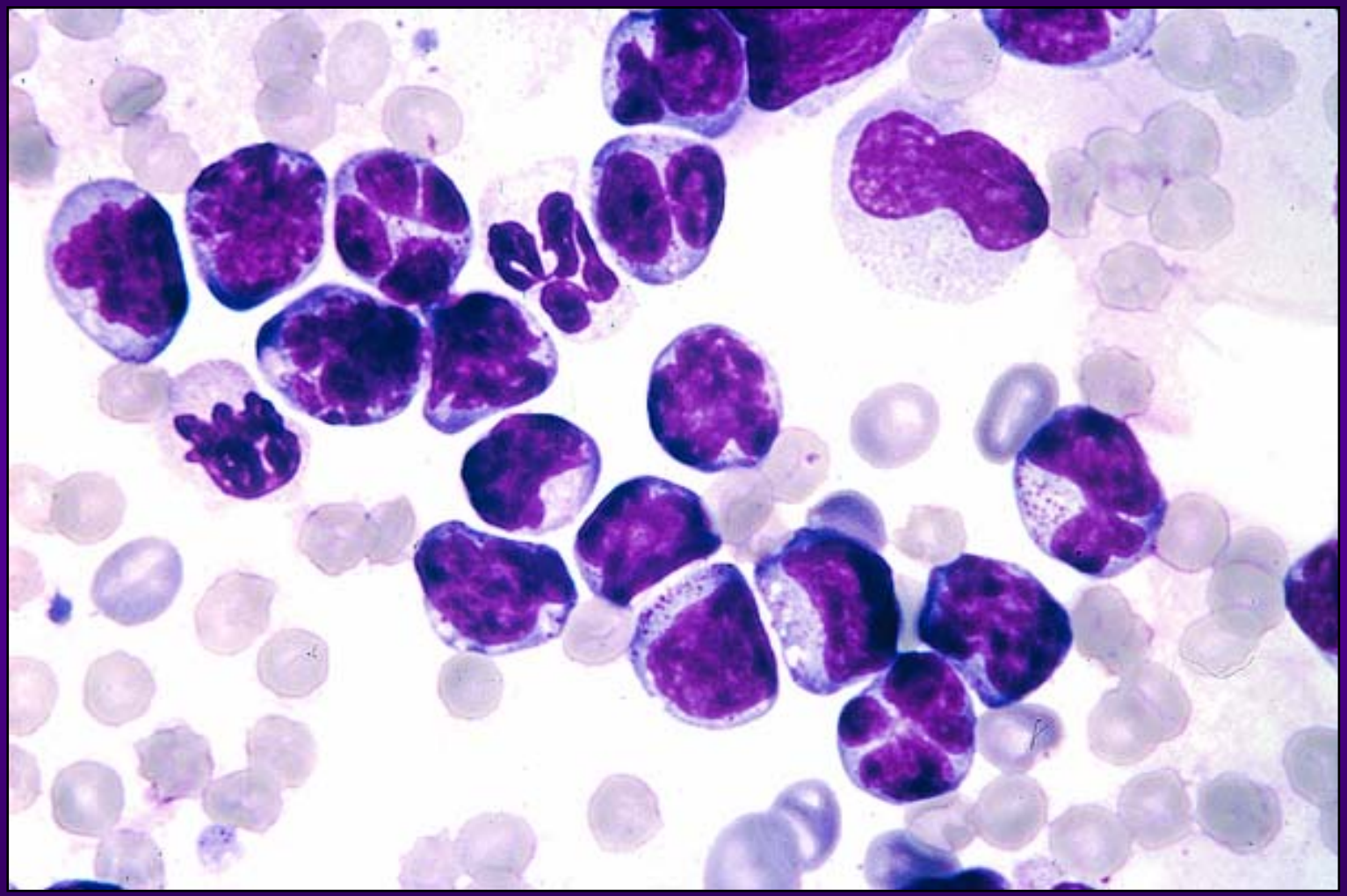



Spleen, LGL Lymphoma, Early, Rat

# *LGL Lymphoma (continued)*

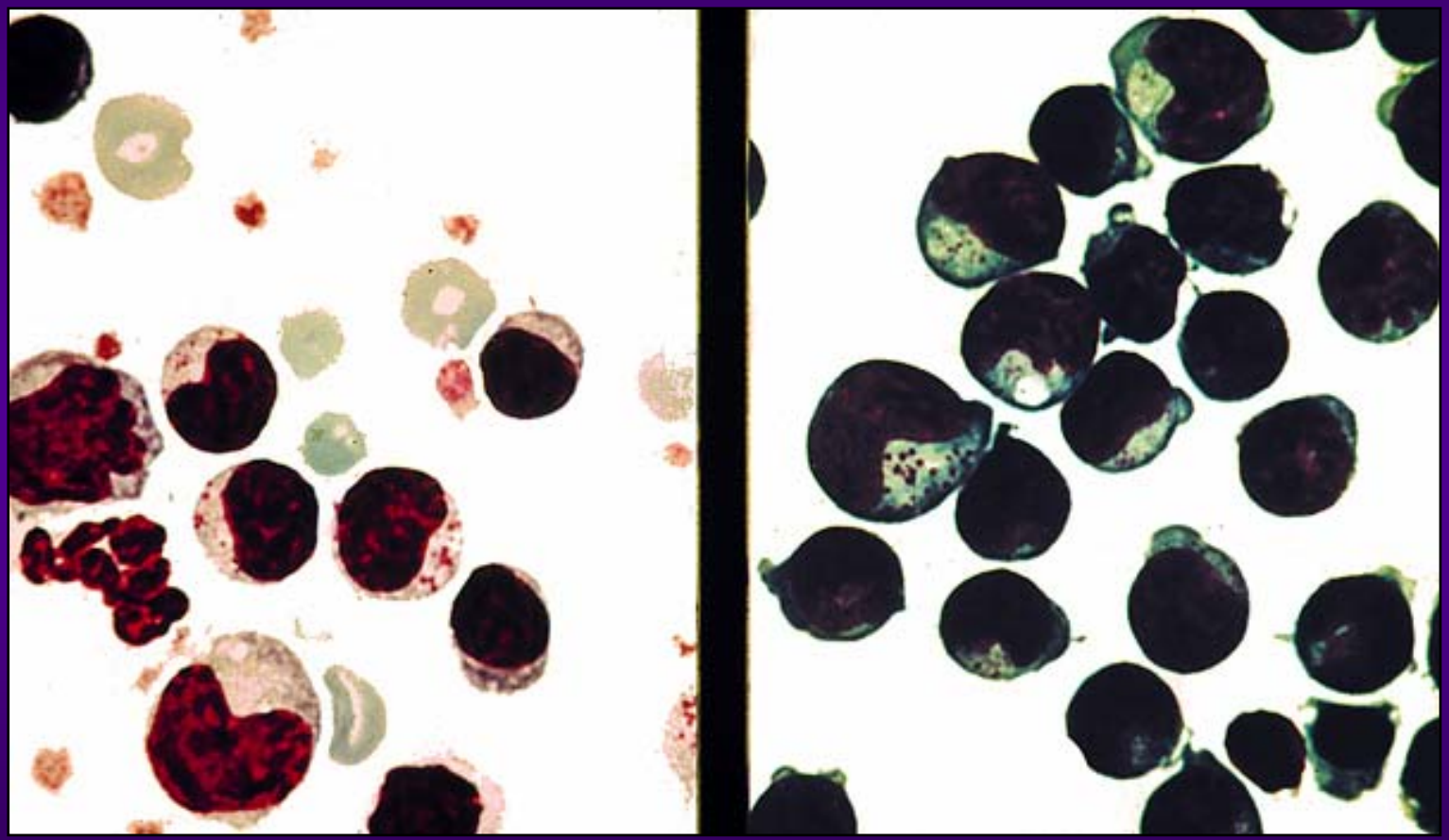
- Rapidly spreads to other organs
- Leukemia a common feature
- Liver - isolated cells in sinusoids (early); resembles lymphoblastic lymphoma (late)
- Lung, bone marrow, mesenteric lymph node, adrenal, mandibular lymph node, kidney

# Peripheral blood, LGL Lymphoma, Rat



Pink to purple cytoplasmic granules

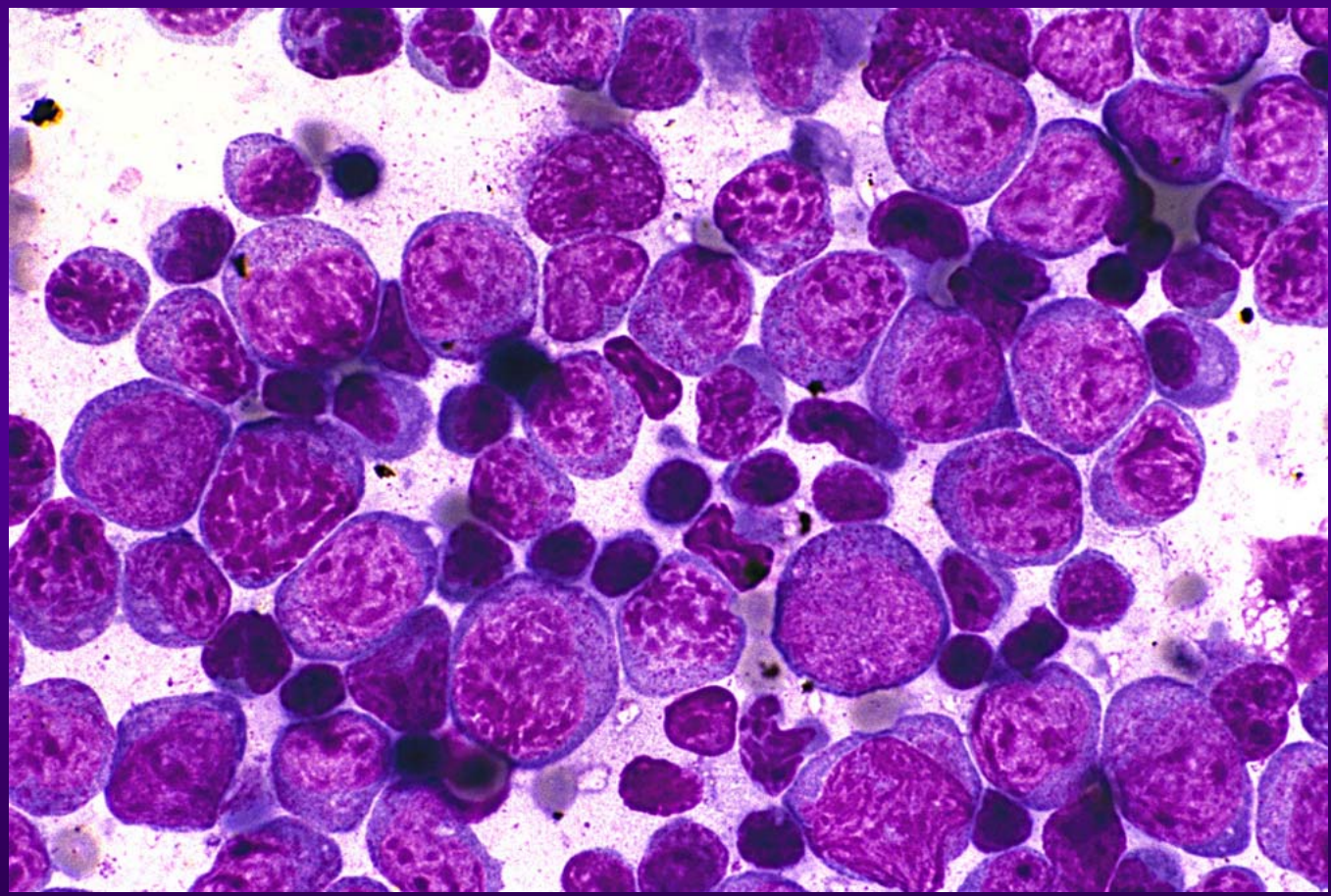
# Impression Smears, Large Granular Lymphocyte (LGL) Lymphoma, Rat



Blood (Wright's stain)

Spleen (special stain)

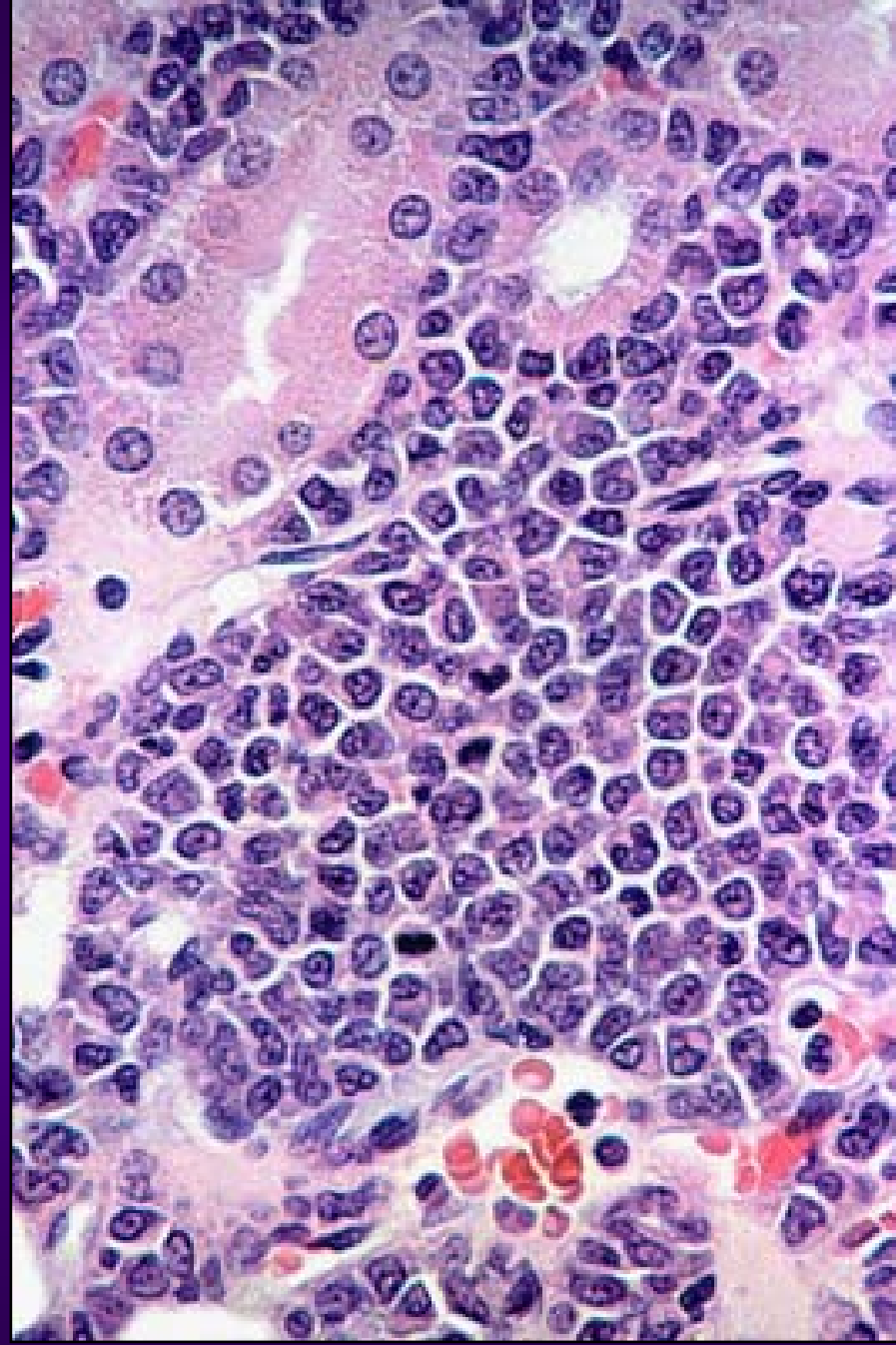
# *Myelogenous Leukemia*



Bone Marrow Smear

# *Granulocytic Leukemia*

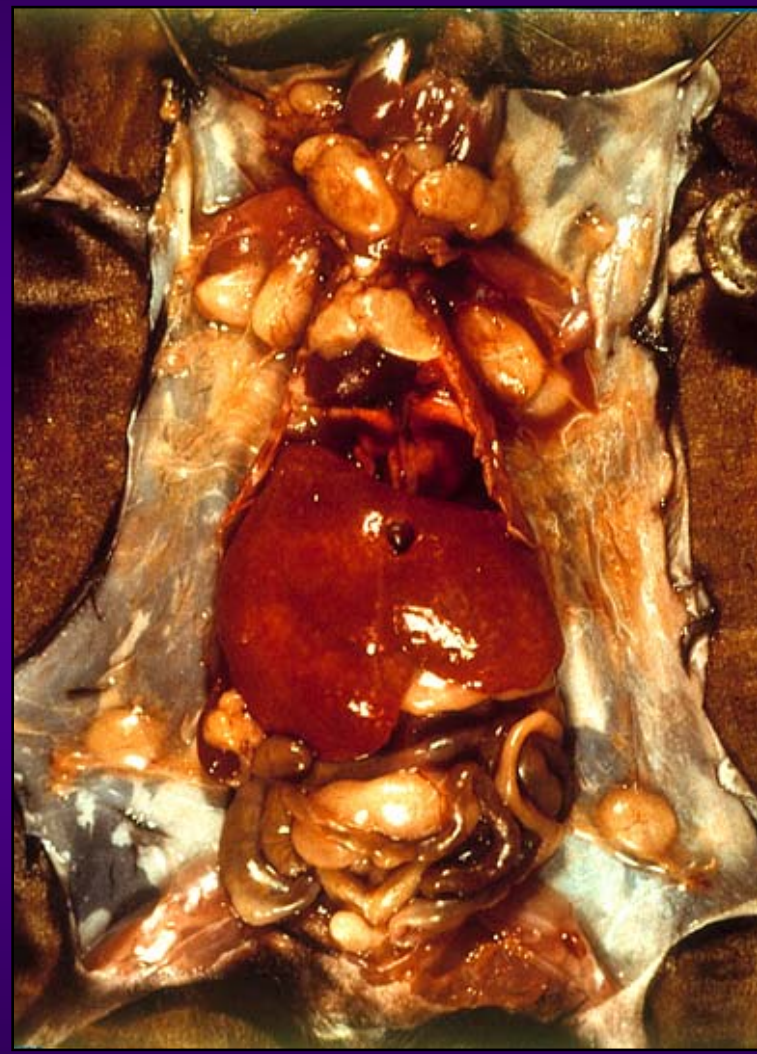
- Cells exhibit varying degrees of maturation, differentiation
- Nuclei have prominent nucleoli
- Azurophilic granules not visible in H&E (myeloblasts)
- Positive for lysosome markers, peroxidase, chloracetate esterase, granulocytic antigen markers



Kidney, Granulocytic Leukemia, Rat

# *Granulocytic Leukemia (continued)*

- Cell type may vary from immature (poorly differentiated) to mature
- Most cases resemble chronic myelogenous leukemia in humans with mainly mature forms
- Mostly leukemic, but may produce tissue masses
- 1$^{o}$ Spleen, 2$^{o}$ in liver; both very large, dull red color
- Also lymph nodes, bone marrow, kidney, testicle, ovary
- Liver - periportal infiltration, often with well-differentiated forms

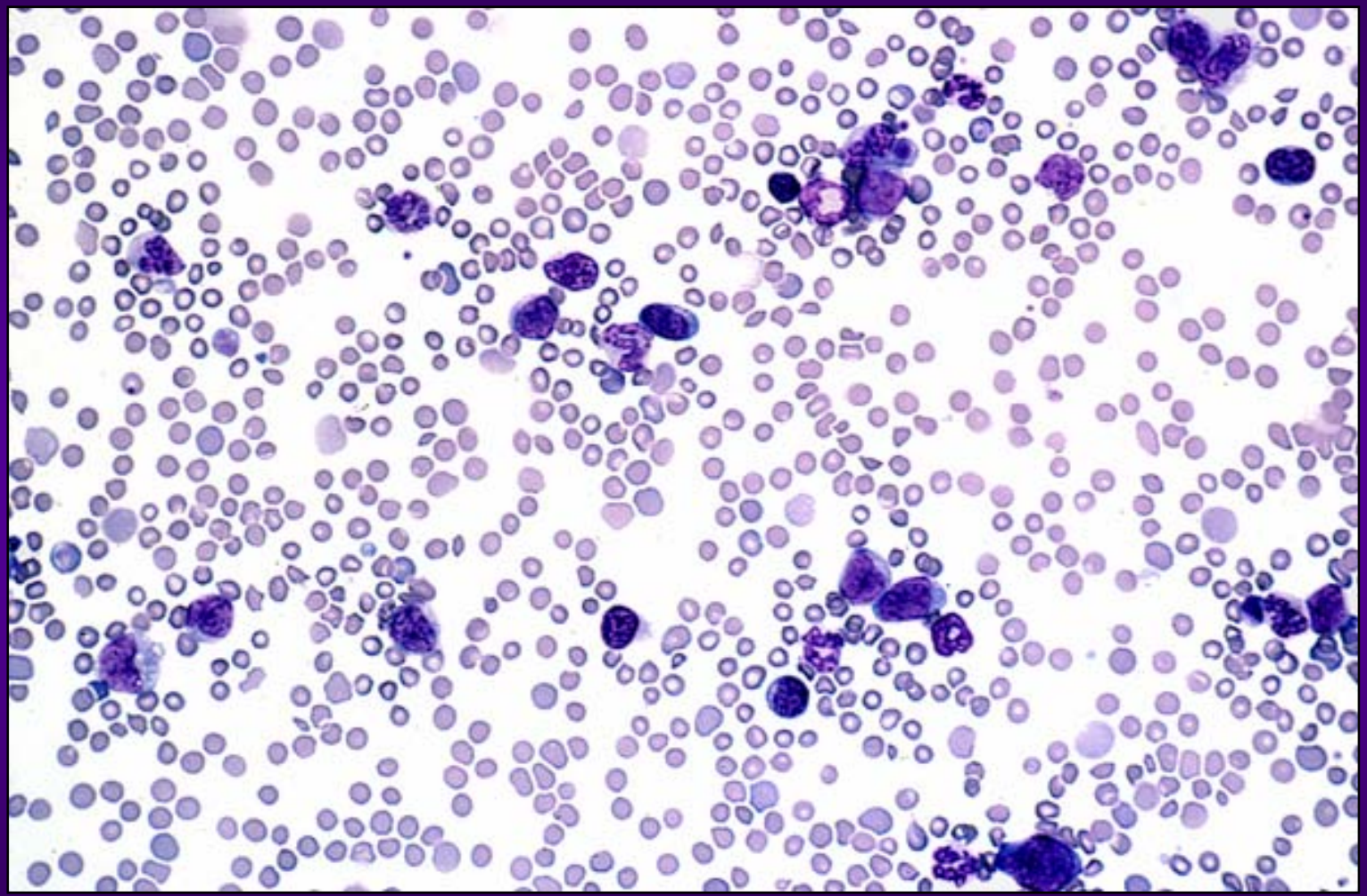


Granulocytic Leukemia, Mouse

# *Granulocytic Leukemia (continued)*

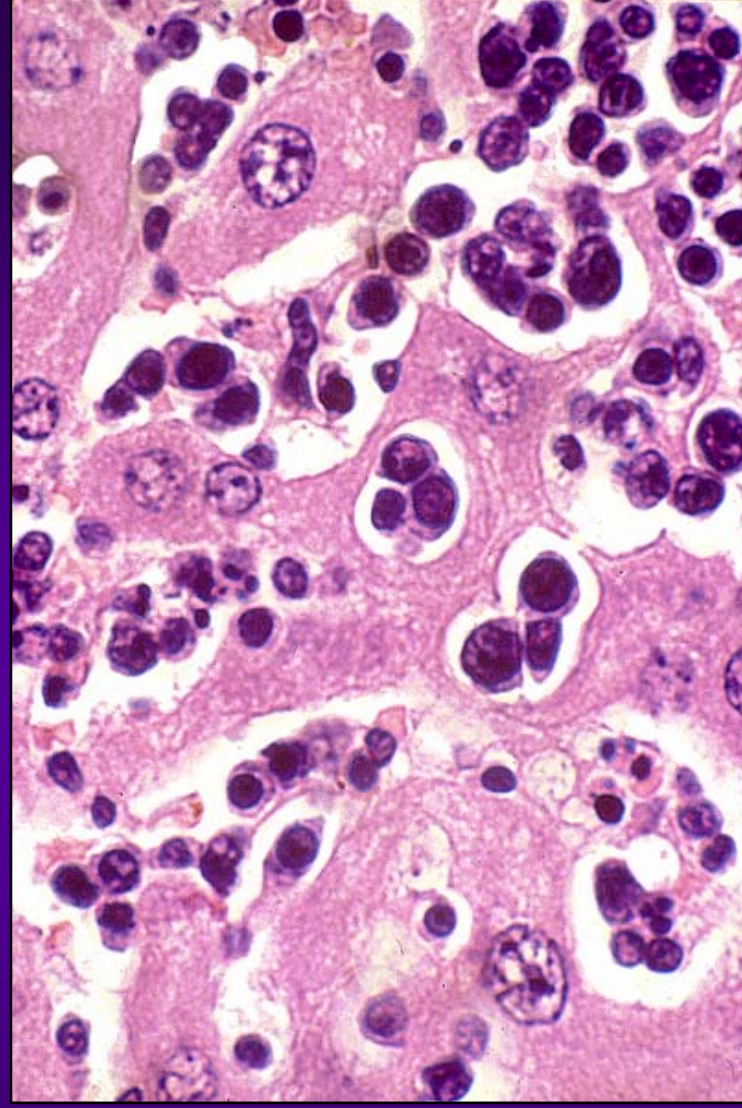
- Modifiers based on tinctorial characteristics of secondary granules
  - Basophilic
  - Eosinophilic
  - Neutrophilic
- Rare as spontaneous disease
- Induced in rats by irradiation, Mg deficiency, carcinogens (eg 7, 12-dimethyl (a)anthracene -- DMBA)

# Blood, Granulocytic Leukemia, Mouse



# *Erythroid Leukemia*

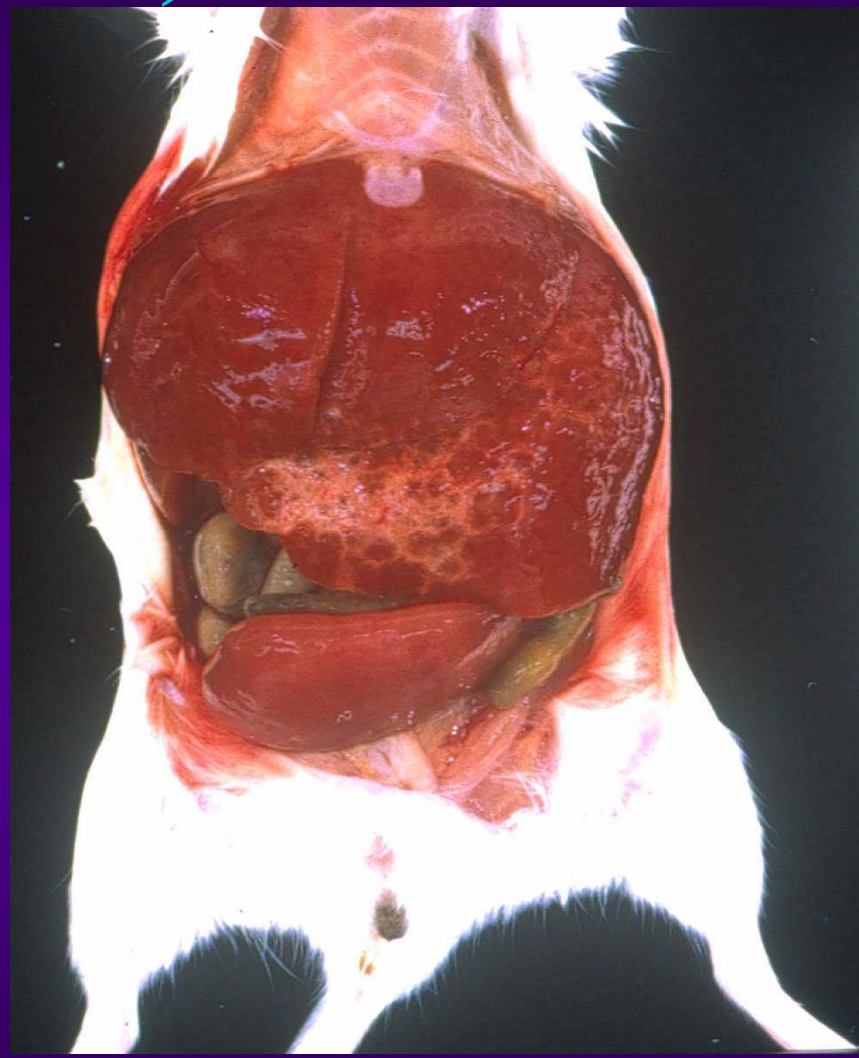
- Excessive proliferation of erythroblastic cells, possibly also granulocytes
- Dark cytoplasm, round to oval nuclei with thick coarse chromatin, large nucleoli
- Spleen, liver, bone marrow, thymus, kidney, lymph nodes
- Arises in splenic red pulp; white pulp compressed, atrophic
- Spreads first to liver sinusoids



Liver, Erythrocytic Leukemia, Rat

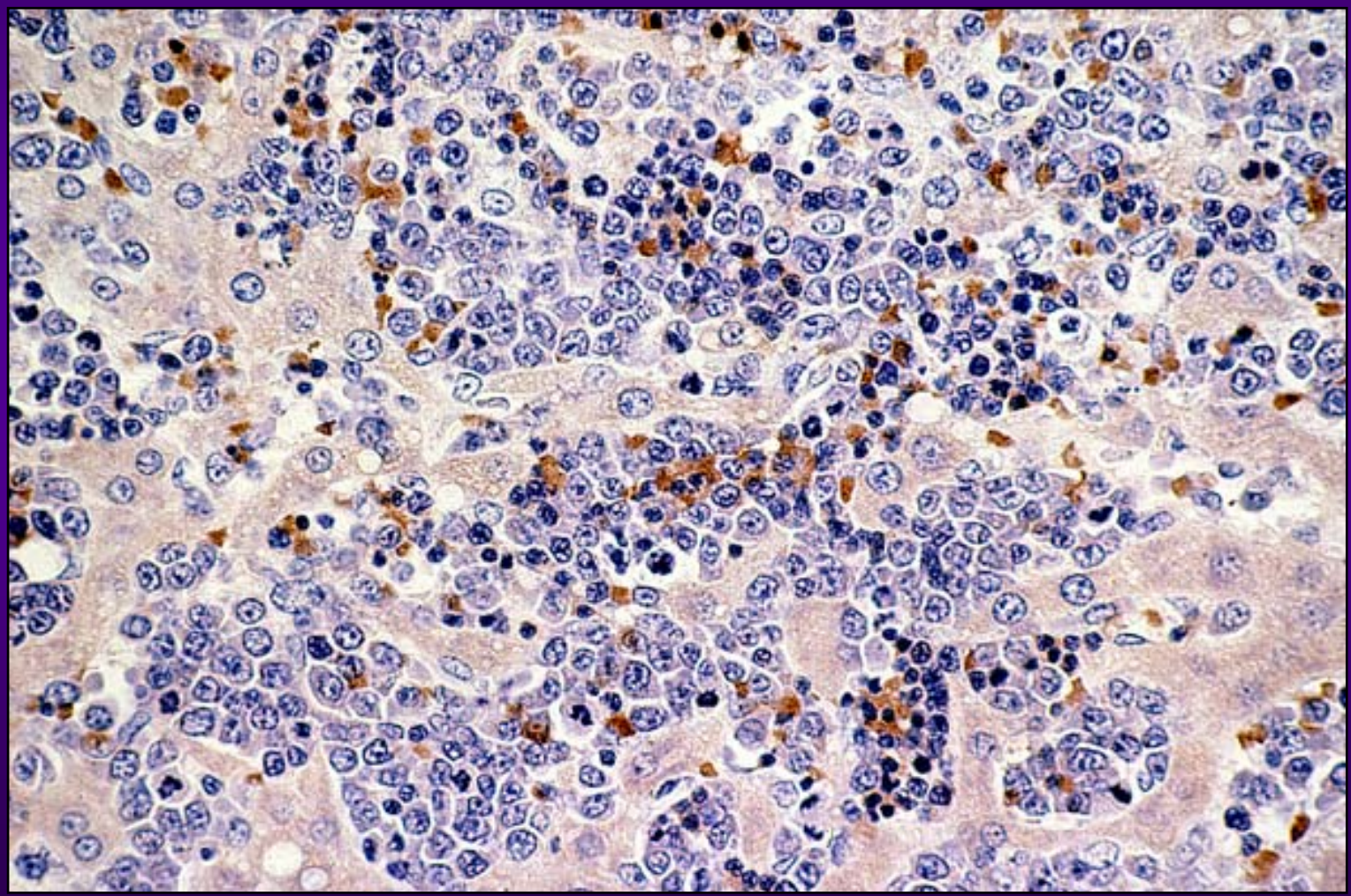
# *Erythroid Leukemia (continued)*

- Severe splenomegaly and hepatomegaly
  - Subcapsular hematomas may contribute
- Leukemia with immature erythroid precursors (erythroblasts, normoblasts)
- Rare spontaneous change in rats and mice
- Irradiation, carcinogens, viruses in mice
- Tg.Ac transgenic mice

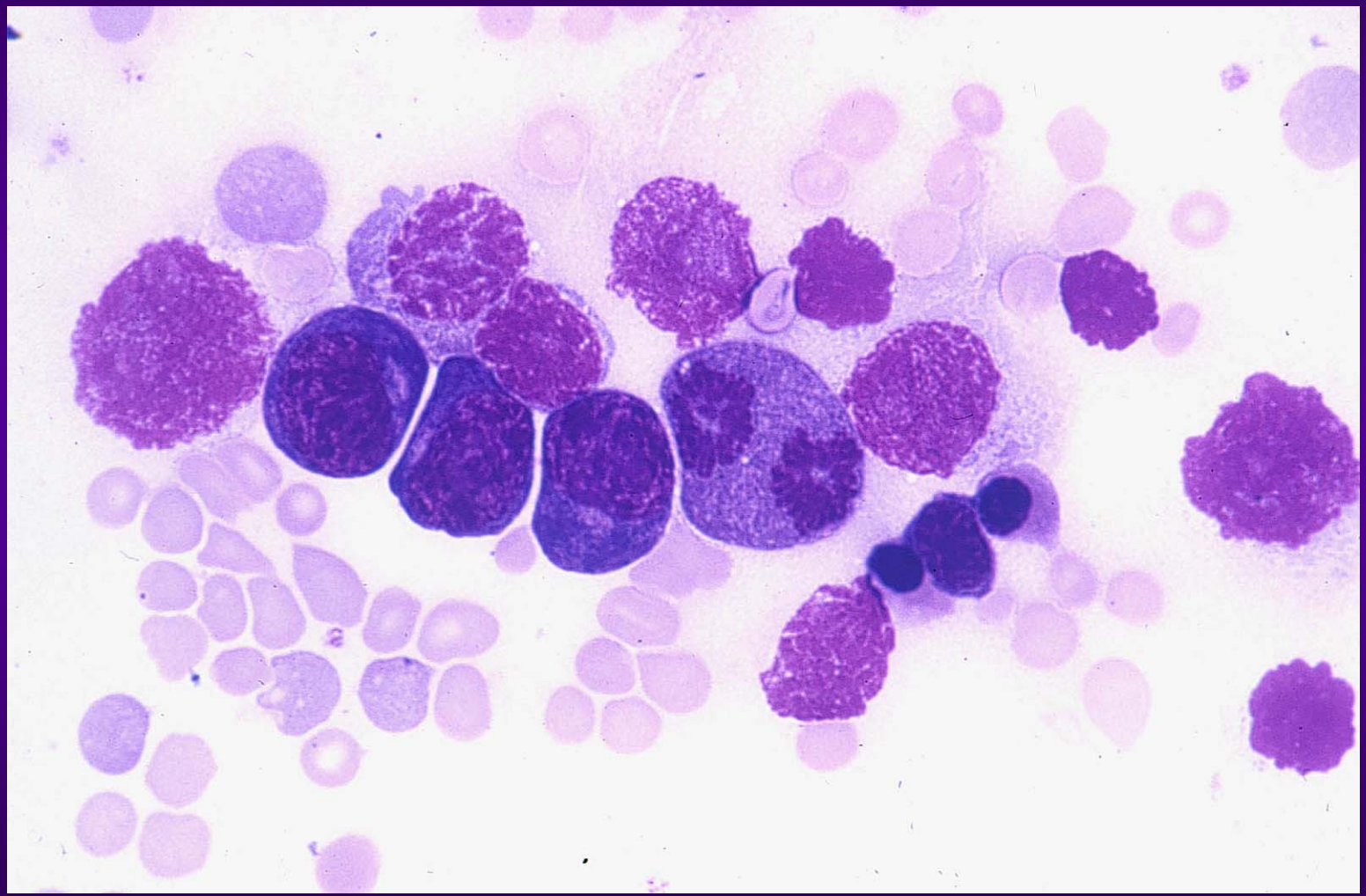


Erythrocytic Leukemia, Tg.Ac Mouse

# Liver, Erythrocytic Leukemia, Hemoglobin Stain, Rat

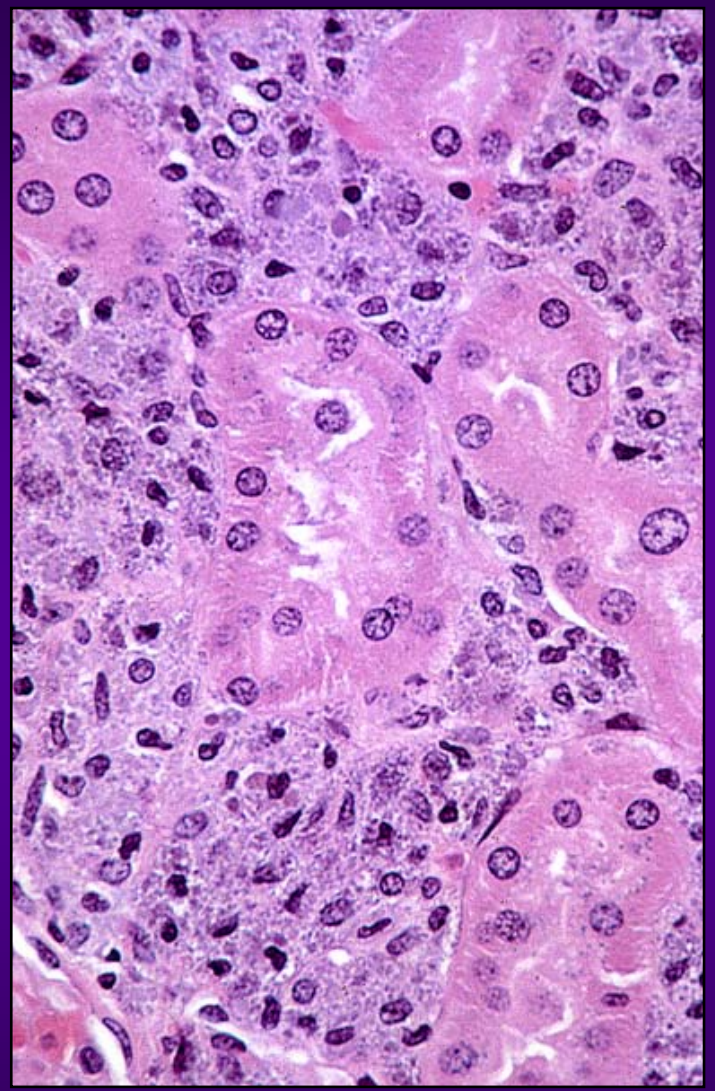


# Peripheral Blood, Erythrocytic Leukemia, Wright Stain, Rat

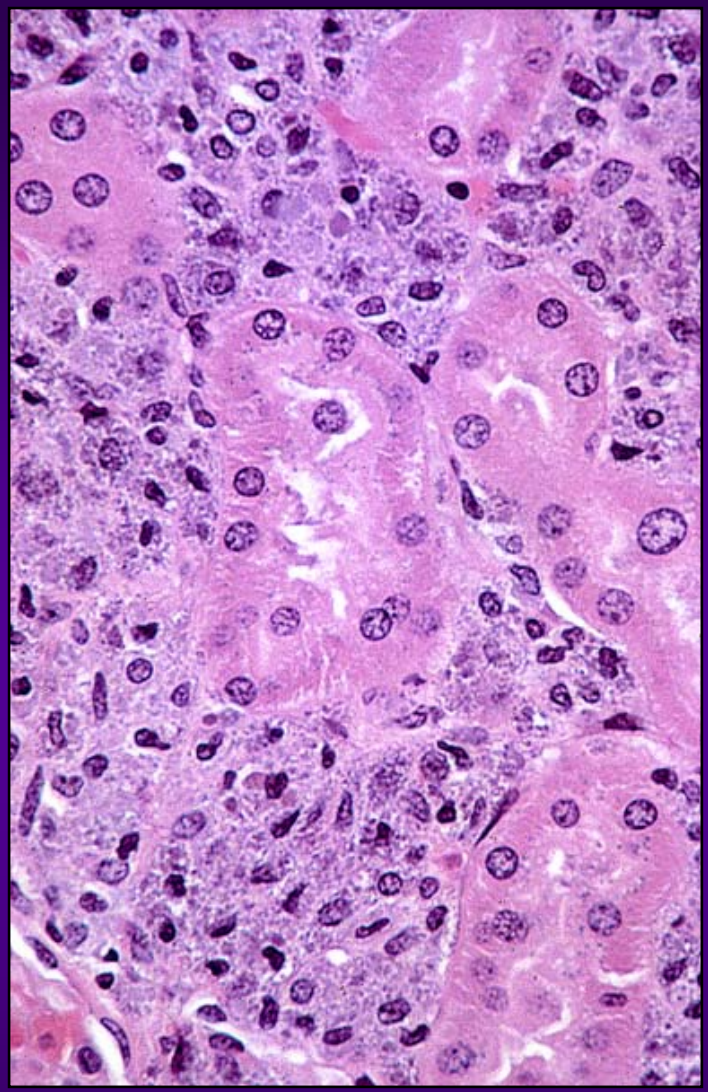


# *Mast Cell Tumor*

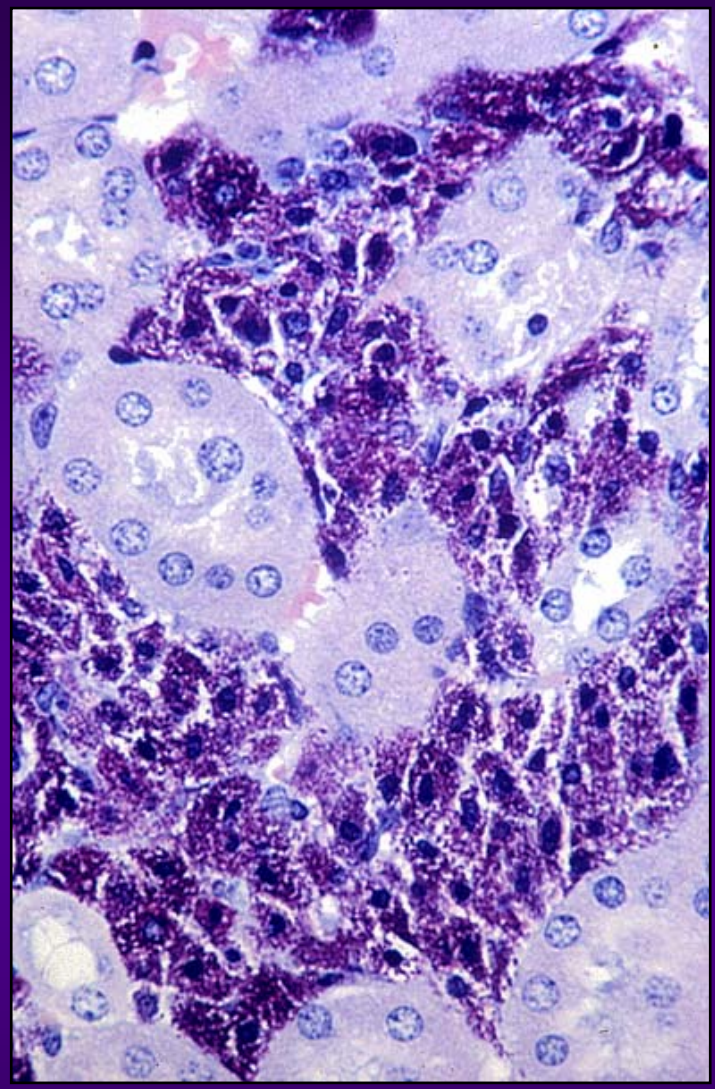
- Composed of well differentiated mast cells with metachromatic granules - often not visible on H&E
- Granules stain with Giemsa, Toluidine blue, etc.
- Uniform large round to oval nuclei, abundant granular slightly basophilic cytoplasm
- Nodules - widely distributed in lymph nodes, other organs
- Locally infiltrative growth pattern
- Generally no leukemia



Kidney, Mast Cell Tumor, Mouse



H&E stain

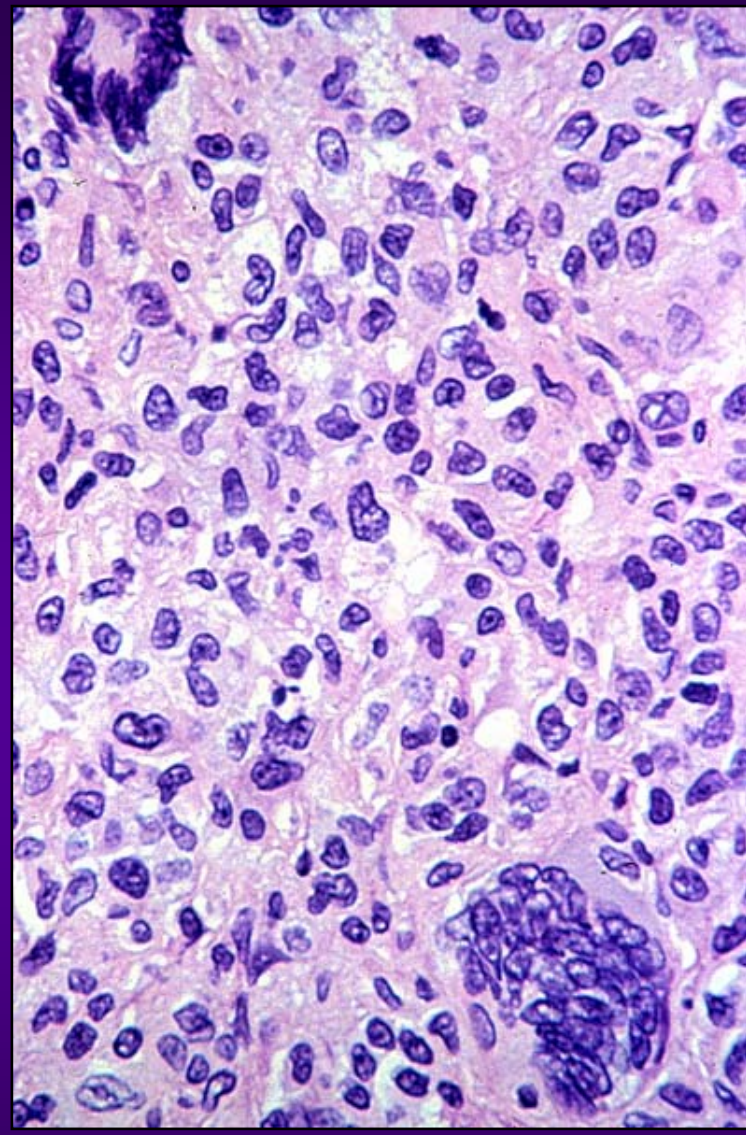


Giemsa stain

Kidney, Mast Cell Tumor, Mouse

# *Histiocytic Sarcoma*

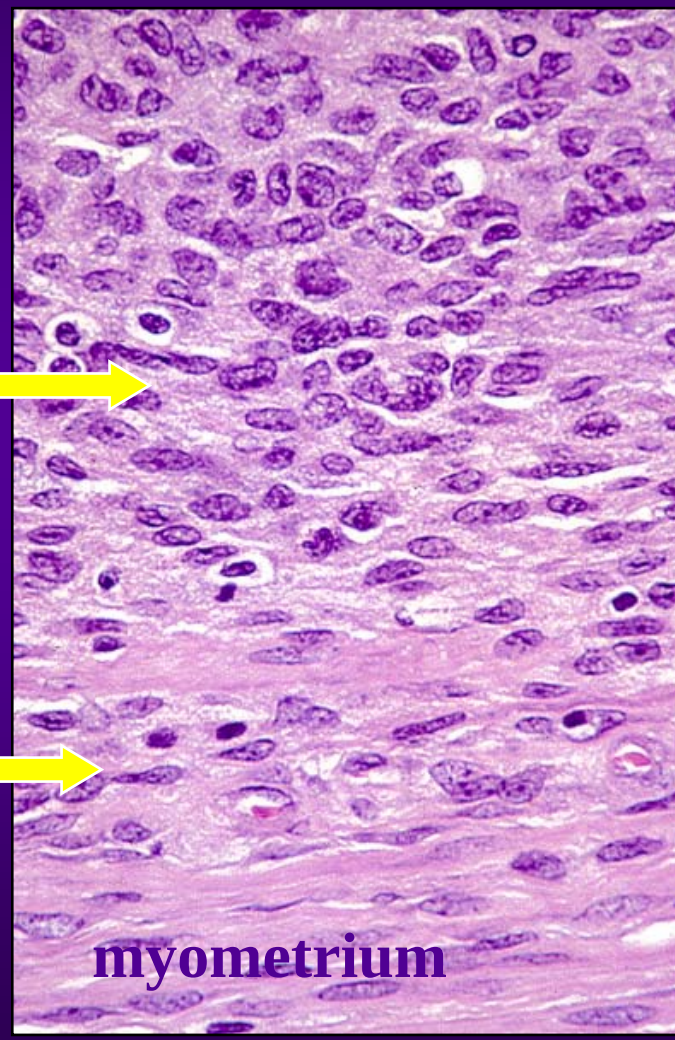
- Uniform round to oval cells with large amount of eosinophilic cytoplasm
- Nuclei elongated or folded, pleomorphic
- Areas of necrosis surrounded by pallisaded tumor cells (rat)
- Liver - generally diffuse involvement of sinusoids with numerous neoplastic cells (or may be focal)
- Multinucleated tumor giant cells often present



Skin, Histiocytic Sarcoma, Rat

# *Histiocytic Sarcoma (continued)*

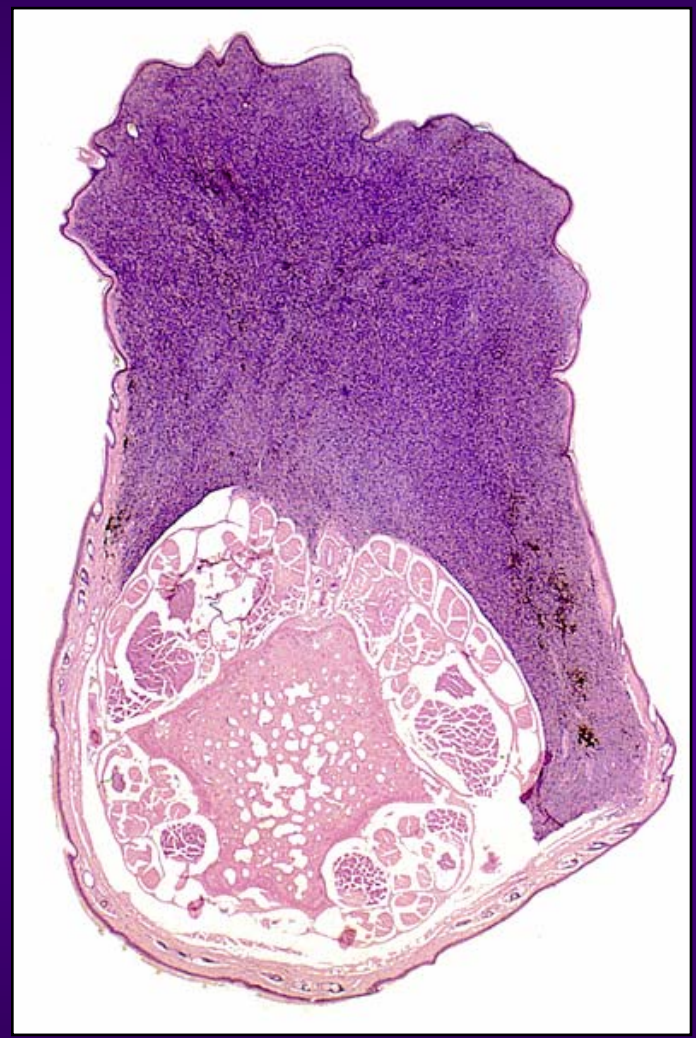
- Mitotic figures common in mouse, less in rat
- Marked variation: size, shape of cells and nucleus, nuclear/cytoplasmic ratio
- Mouse uterus - spindle shaped or fusiform, and fibroblast-like (may resemble malignant schwannoma)
- Metastases and spreads commonly along serosal surfaces and in vascular spaces
- Metastases found in vessels of lungs
- Erythrophagocytosis may be present.




Uterus, Histiocytic Sarcoma, Mouse

# *Histiocytic Sarcoma (continued)*

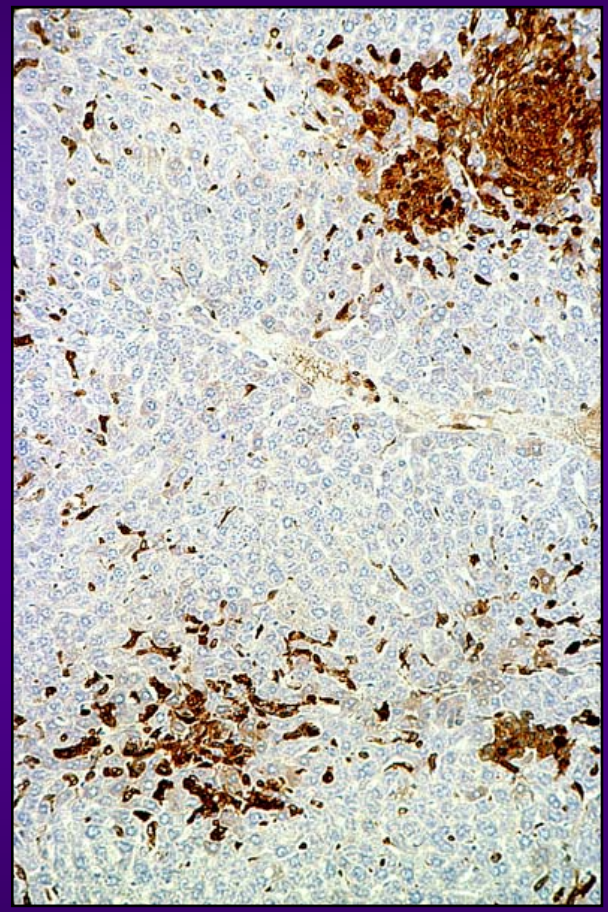
- Mice
  - Males - liver
  - Females - liver, uterus, vagina
  - Skin and other organs
- Rat
  - Liver (diffuse), skin, subcutaneous tissue, lung
  - Peritoneum, kidney, lymph nodes, bone marrow, ovary



Skin, Histiocytic Sarcoma, Mouse

# *Histiocytic Sarcoma (continued)*

- Most frequent non-lymphoid hematopoietic tumor in Sprague-Dawley rats
- Neoplastic cells are immunoreative for histiocyte markers such as lysozyme, ED-1,  MAC2
- Renal proximal tubules may have hyaline droplets containing lysozyme



Histiocytic Sarcoma, MAC-2, Mouse

# *Megakaryocytic/blastic Leukemia*

- Marked increase in large mononuclear cells including megakaryocytic cells in hematopoietic tissues
- Bone marrow, spleen, liver, kidney
- Lymph nodes not always involved, megakaryocytes may be present in medullary areas

# *Thymoma*

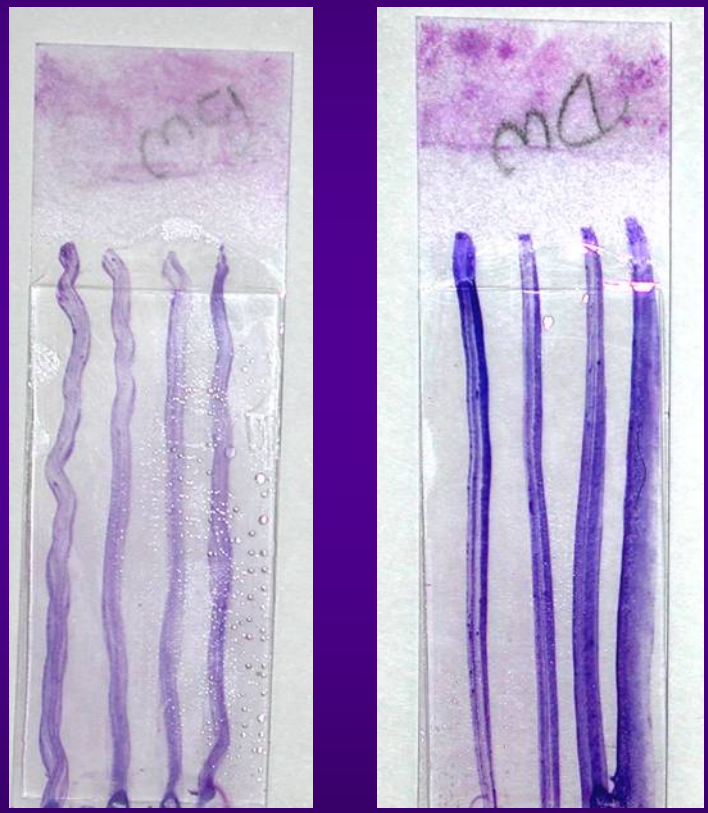
- Neoplastic cell is the thymic epithelial cell
- Localized tumors well encapsulated, may show slight local invasiveness
- Rarely metastasizes in rats
- Range from tumors with
  - Predominantly normal thymic structure with medullary differentiation to
  - Loss of normal thymic medullary structure and composed of mixture of neoplastic epithelial cells and lymphocytes

# *Thymoma (continued)*

- Relative proportions of lymphocytes and neoplastic epithelial cells may vary from case to case and within single tumor
- Inbred Wistar/Neuherberg rats have spontaneous thymomas – 97% in females, 36% in males. Cause dyspnea and occasionally death

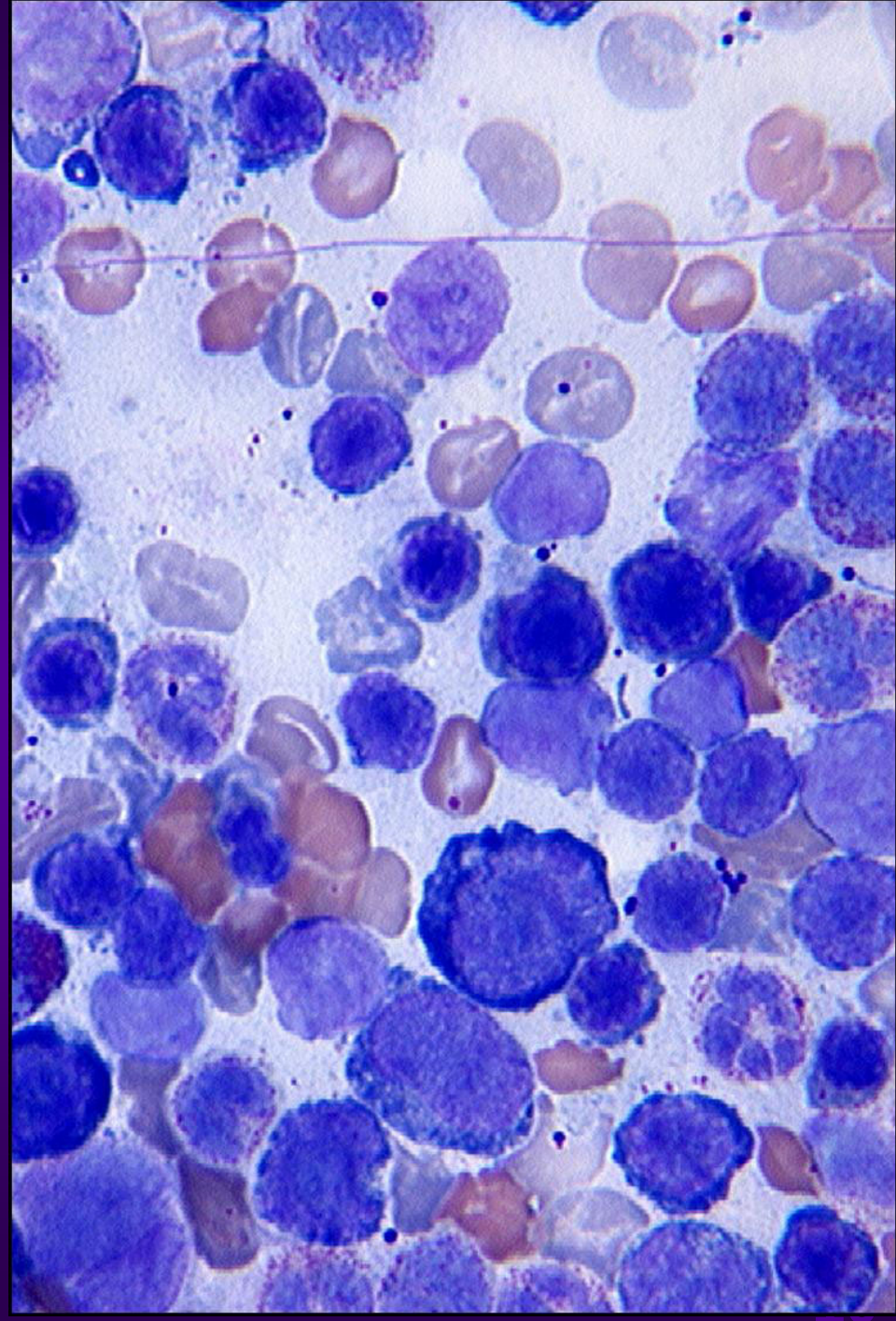
# *Bone Marrow Smears*


# *Bone Marrow Smears*



# What should trigger bone marrow review?

- Unexplained cytopenias
  - Decreased RBC mass with regeneration is *not* reason for review
- Unexplained increases in cell types
  - Thrombocytosis
- Other techniques
  - Flow cytometry
  - Cytochemistry



# *Acknowledgements*


- Perry Bain
- Ken Latimer
- Jerry Ward
- Peter Mann
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- Beth Wilkinson
- Rachel Cushwa

# *Erythrocyte Interpretation*

## *POLA COURSE August 2005*

Nancy Everds, DVM
Diplomate, ACVP
Haskell Laboratory for Health and Environmental Sciences
Newark, DE
nancy.e.everds@usa.dupont.com

# *POLA August 2005 Clinical Pathology*

- Introduction (Everds)
- Erythrocytes (Everds)
- Leukocytes (Everds)
- Platelets and Hemostasis (Latimer)
- Liver (Latimer)
- Renal/Acid-Base/Urinalysis (Everds)

# *Other…*

- Abbreviations (see handout)
- Veterinary Schools with good websites and example problems
  - Cornell
  - Univ of GA
  - Auburn
  - Purdue

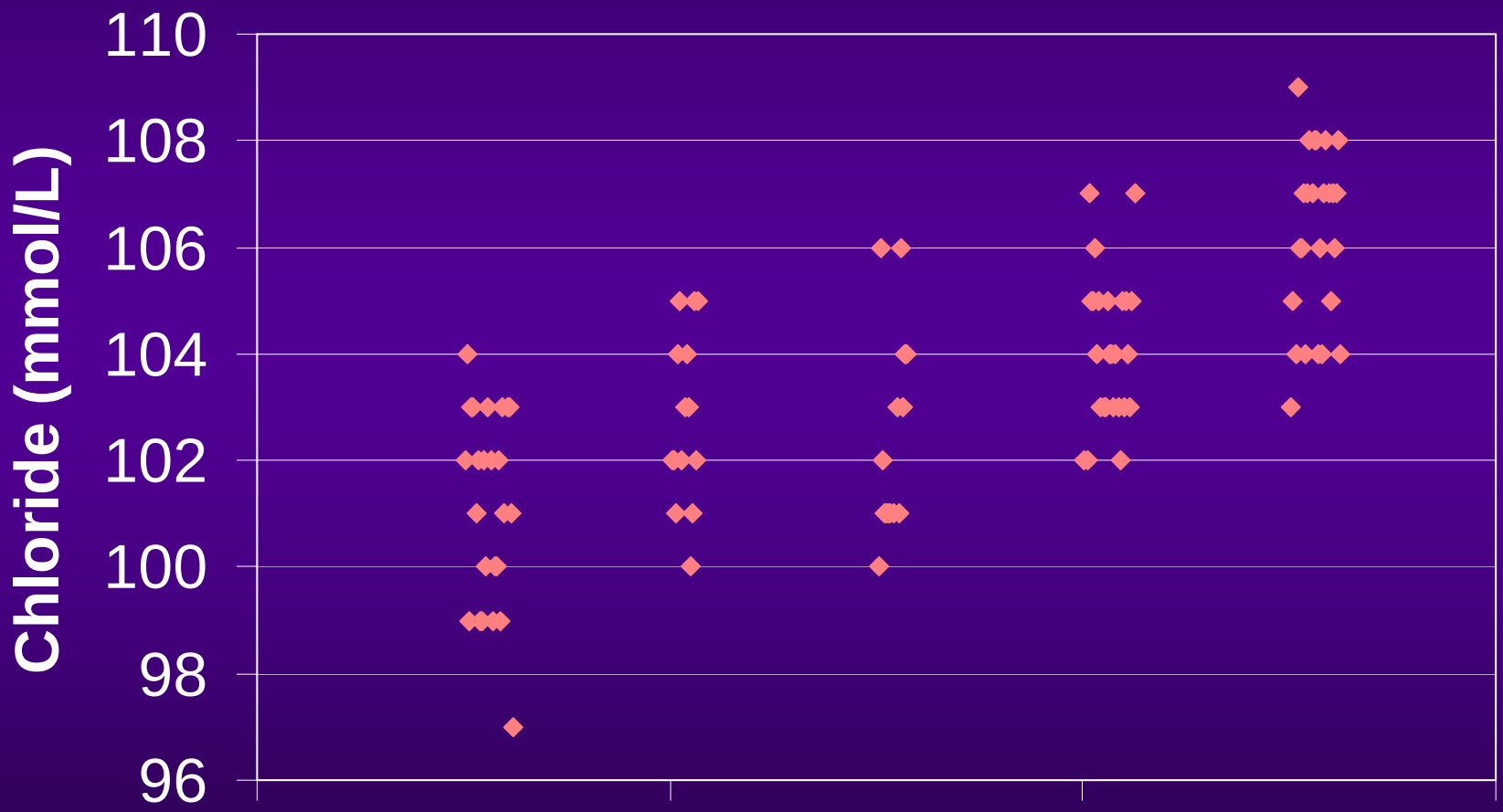
# *Collection of blood*

- *Quality of sample depends on proficiency of collector*
- Small animals (e.g. rodents, hamsters)
  - Retro-orbital sinus
  - Cardiac puncture, descending aorta, caudal vena cava
  - Tail vein
- Larger animals
  - Rabbits: lateral auricular vein, central auricular artery
  - Dogs: jugular, cephalic, femoral, also catheter
  - Monkeys: femoral, jugular, saphenous, also catheter

# *Variables Affecting Results*

- Sex
- Supplier
- Age
- Housing/Bedding
- Diet
- Fasting status
- Site of collection
- Anesthesia
- Anticoagulant
- Sample matrix

# *The Great Chloride Shift*



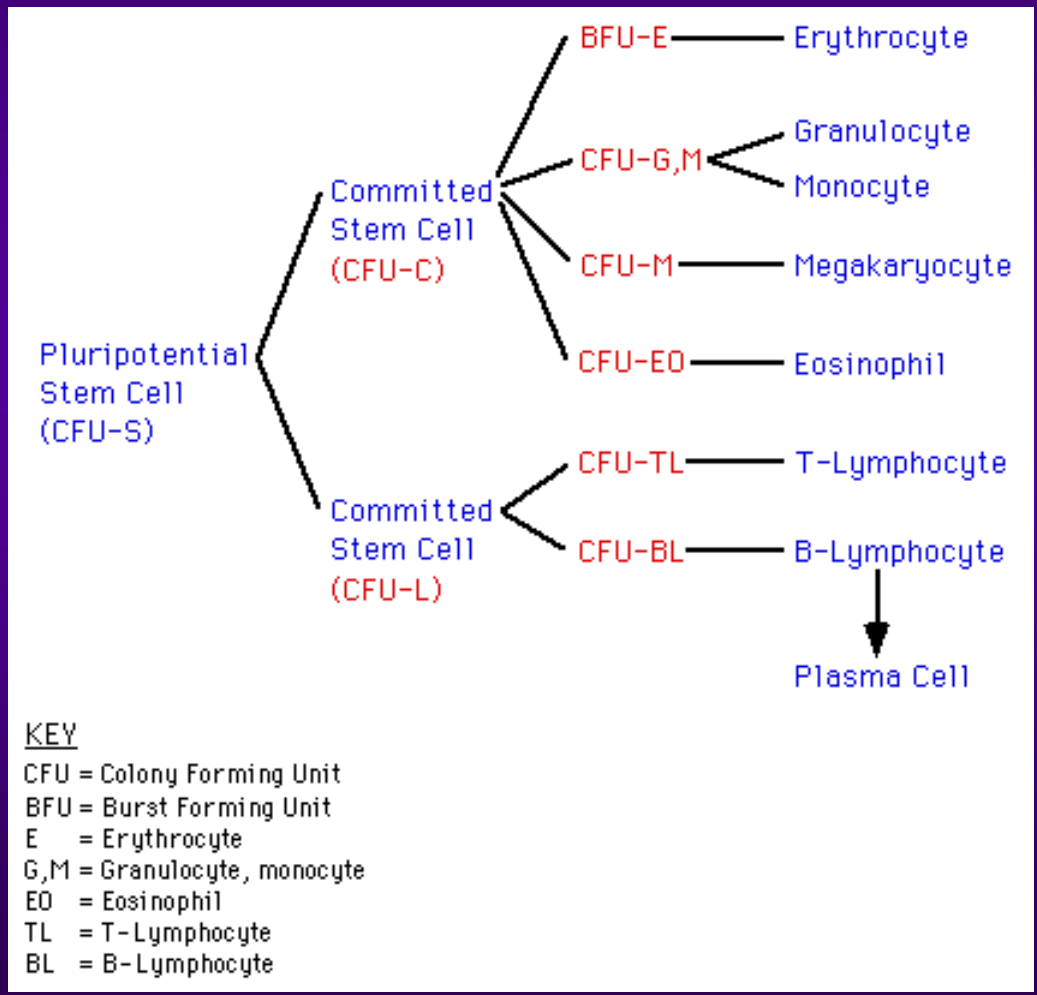
# *Reference Intervals*

- Important to use matched reference intervals when analyzing data
- Best comparison: Concurrent matched (age, sex, housing, etc) control group

# *Mammalian Hematopoiesis*

- Hematopoietic stem cells
  - Morphologically similar to small lymphocytes
  - Self-renew and are pluripotent
- Progenitors
  - Do not self-renew, but proliferate
  - CFU-E vs. CFU-GEMM
- Precursors
  - Mature and proliferate (early stages)

# *Mammalian Hematopoiesis*



# *Mammalian Hematopoiesis: Regulation of cell counts*

- Normal conditions
  - Most hematopoietic stem cells and progenitors are in $G_0$ phase
  - Mature progenitors are proliferating
  - Apoptosis in progenitor and mature cells
- Stress (bleeding, infection, etc)
  - Release of stored pools (bone marrow)
  - Decreased rate of apoptosis
  - Quiescent stem cells and progenitors recruited to differentiate

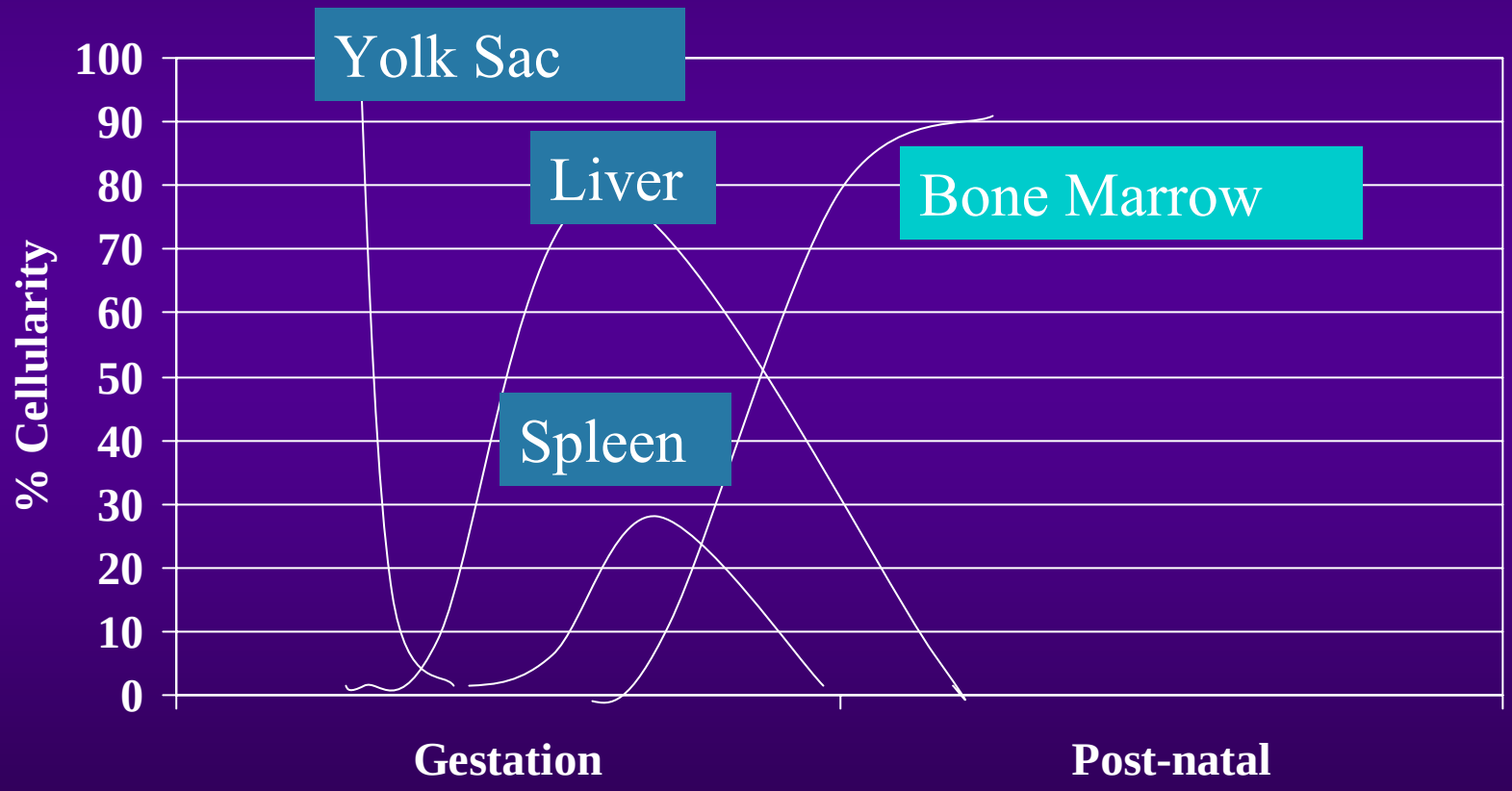
# *Mammalian Hematopoiesis: Regulation*

- Cytokines
  - CSFs, interleukins, flt-3 and kit ligands, TGF-β, TNF-α, Wnt, EPO, TPO, etc.
- Chemokines
- Extracellular matrix components
- Stromal cells

# *Mammalian Hematopoiesis*

- Embryo -- yolk sac > stem cells > tissues
- Fetus
  - Liver > spleen > bone marrow
  - Lymphoid tissues
- Neonate
  - Marrow of almost all bones
  - Lymphoid tissues

# *Mammalian Hematopoiesis*



# *Mammalian Hematopoiesis*

- Normal postnatal production
  - Bone marrow (long bones, axial skeleton)
    - Spleen, liver (rodents)
  - Lymphoid tissue (lymphocytes)

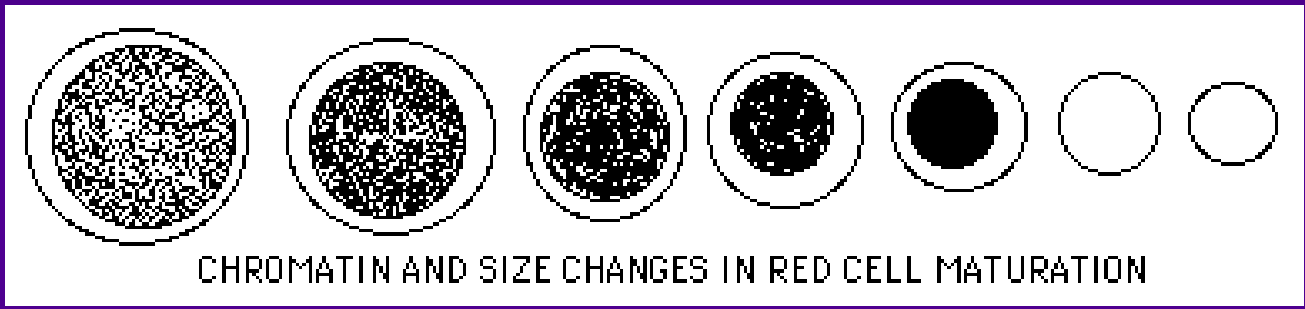
# *Instrumentation*

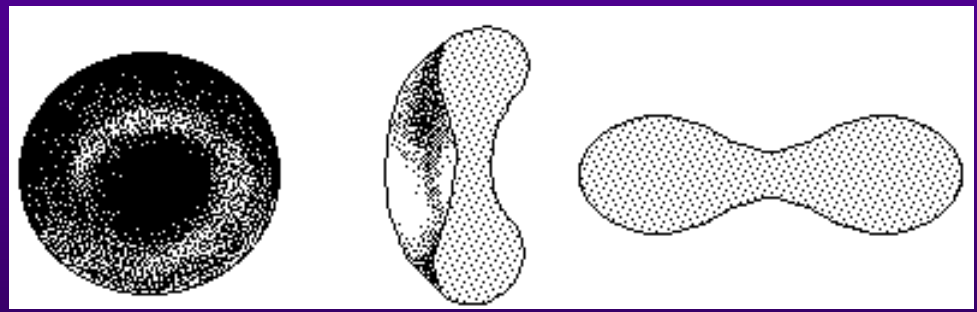
- Very important in hematology
- Instrumentation affects
  - Accuracy of results
  - Types of artifacts

# *Instrumentation*

- Instruments with animal-specific applications
  - Bayer Advia 120 (Technicon H-1E)
  - Abbott CellDyn series (3500, etc)
- Other instruments also appropriate
  - Coulter S+IV
  - Serono-Baker
  - Sysmex

# *Erythrocytes*



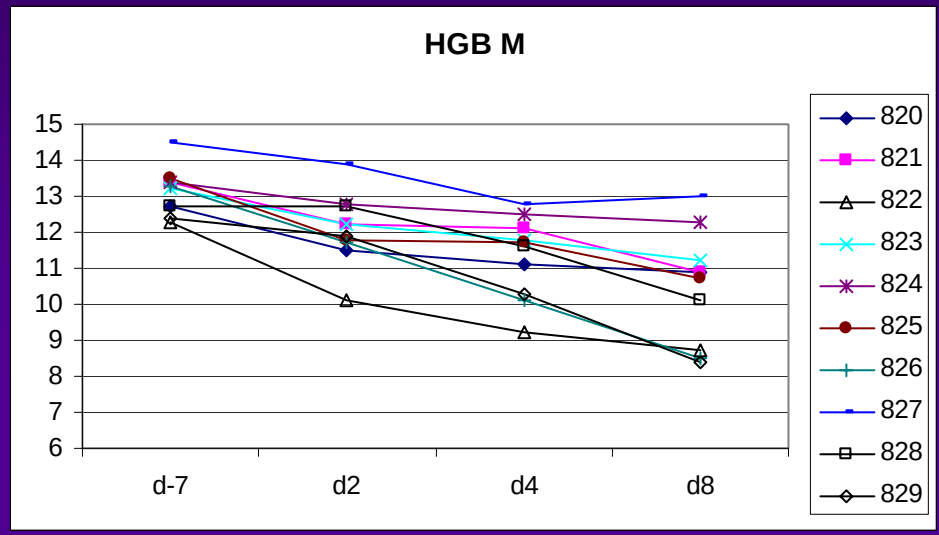



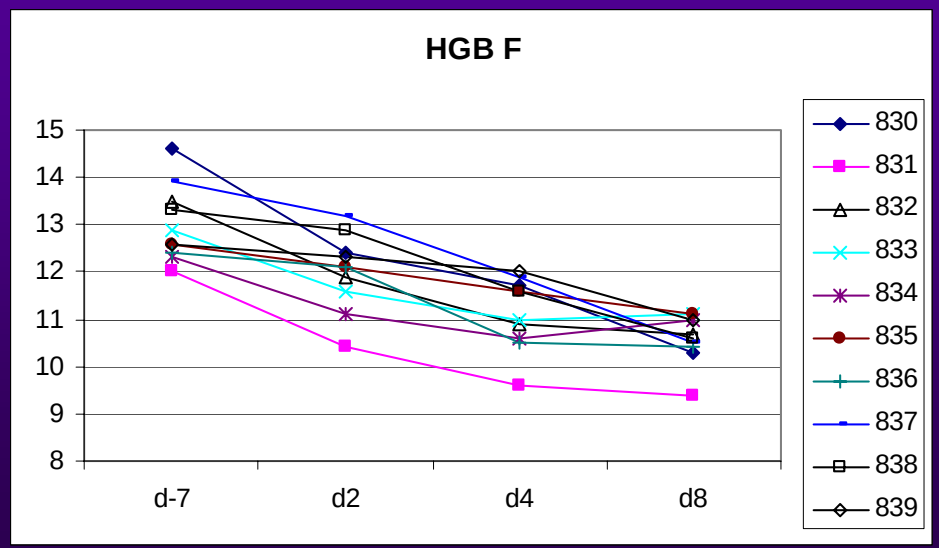
# *Outline of Talk: Erythrocytes*

- Determination of RBC parameters
- Red cell mass effects
  - Increased RBC mass
  - Decreased RBC mass
    - Hemorrhage
    - Destruction
    - Decreased production

# *Example 1: Monkeys- Change in Red Cell Mass Over Time*

- Hemoglobin concentration decreased consistently over time
- Same effects with other red cell mass parameters



# *Example 1 (Conclusions): Red Cell Mass Effects*

- Red cell mass decrements
  - Occurred across groups, including control
  - Unrelated to dose
  - Progressed during study
- Effects could be due to:
  - Amount of blood collected for clinpath and other tests (plasma concentrations of drugs or metabolites)
  - Vehicle effects

# *Hematology Tests*

- Hematology tests are interrelated
- Instrument and microscopic data should be consistent
- Interpret results as related parameters

# *Hematology Tests**

- Complete blood count* and preparation/ evaluation of blood smear
- Additional appropriate tests

Complete blood count includes:
RBC, HGB, HCT
MCV, MCH, MCHC, RDW
+/- retics
WBC count and absolute differential counts
PLT +/- MPV

*Weingand et al., Fundamental and Applied Toxicology, 29:198-201, 1996. "Harmonization of animal clinical pathology testing in toxicity and safety studies"

# *Functional Classification Of Parameters*

| RBC Mass | Descriptive |
| --- | --- |
| RBC | MCV |
| HGB | MCH |
| HCT | MCHC |
| | RETIC |
| | RDW |

# *Hemoglobin Concentration*

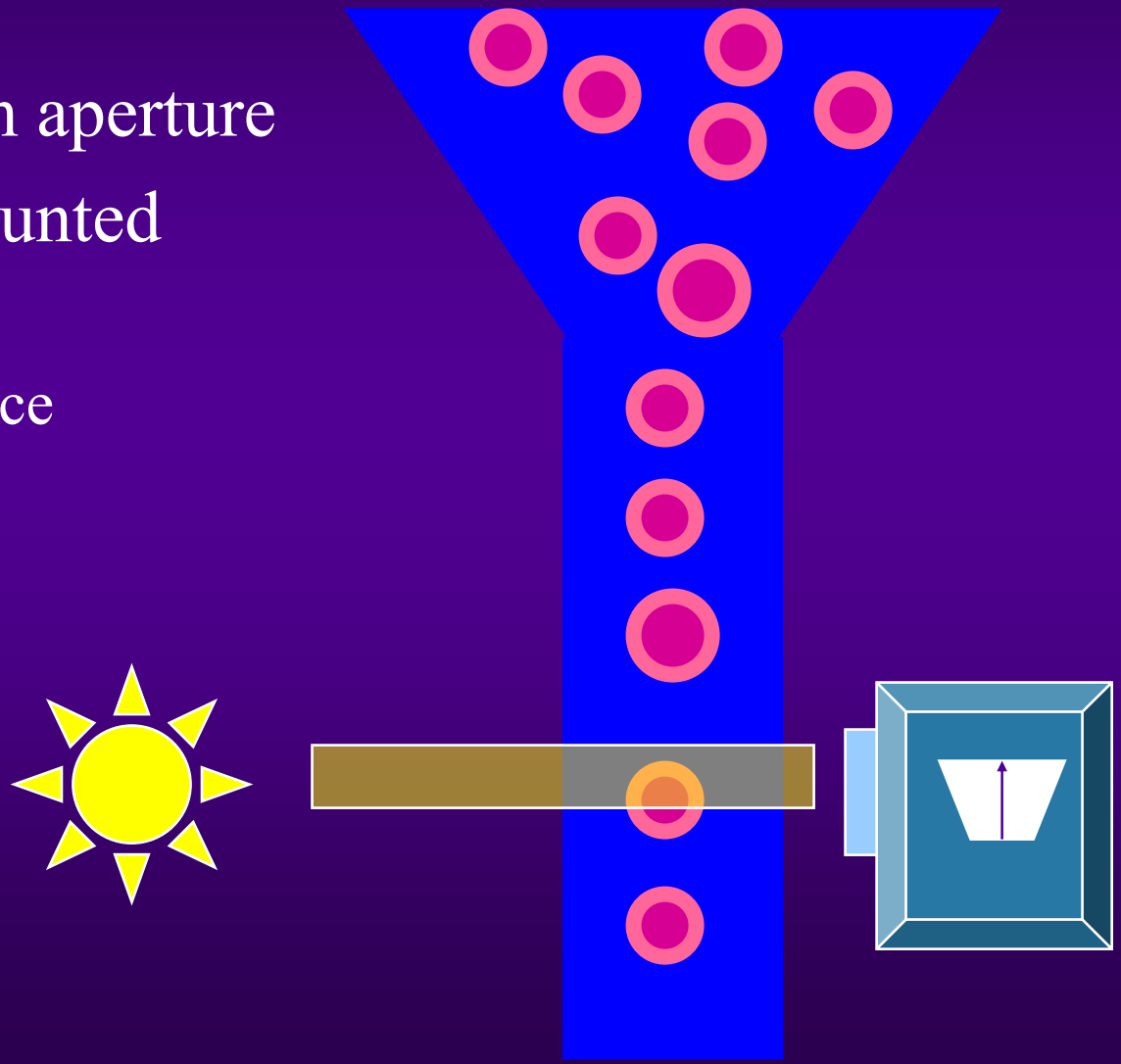
| Measured | Calculated |
|---|---|
| HGB* | HCT |
| RBC | MCH |
| MCV | MCHC |
| RETIC | RDW |

*Hemoglobin is measured after RBCs are lysed:

$$\text{Hemoglobin} \xrightarrow{K_3Fe(CN)_6} \text{MetHb} \xrightarrow{KCN} \text{Cyanmethemoglobin}$$

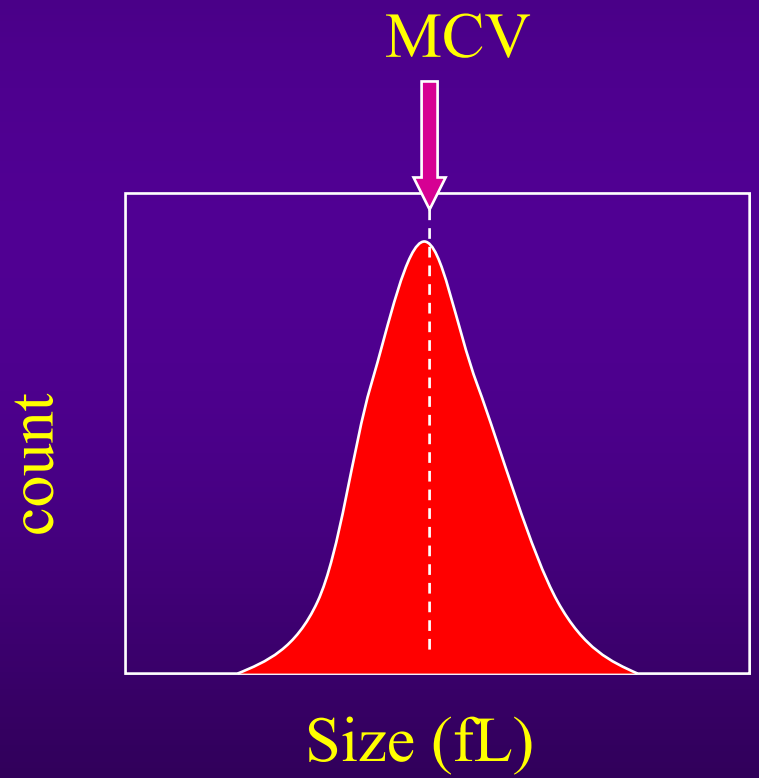
# *Red Cell Counting/Sizing*

- RBCs flow through aperture
- RBCs sized and counted
  - Optical
  - Electrical impedance



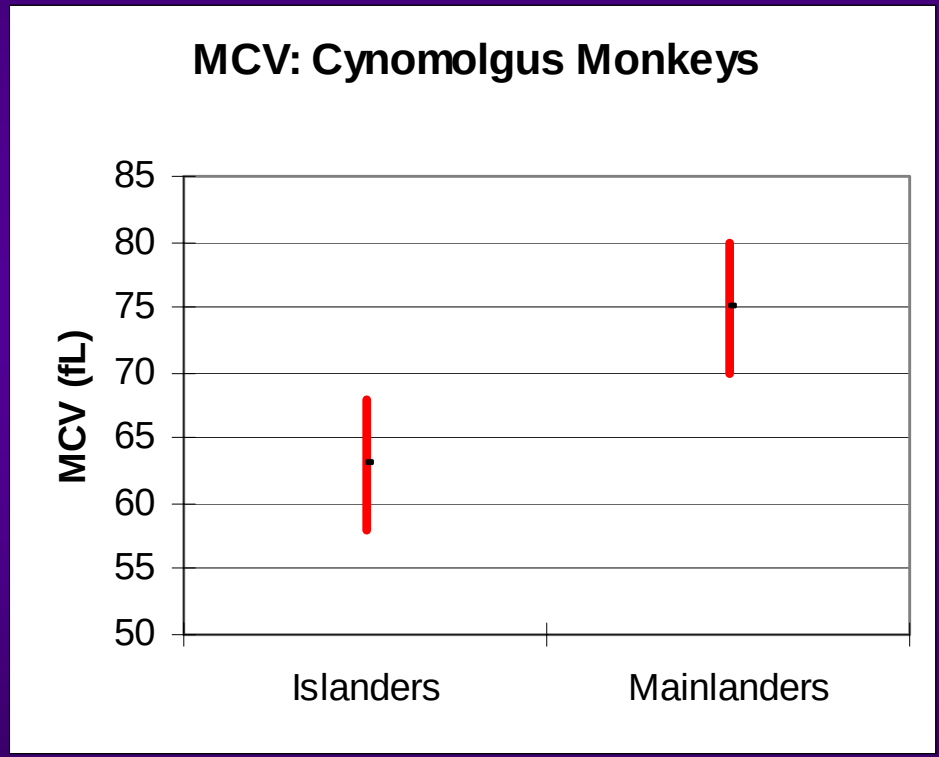
# *Red Cell Counting/Sizing*

- RBCs sized and counted
  - RBC count
  - Mean cell volume



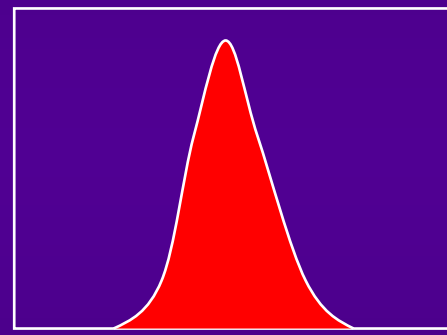
# *MCV and Monkey Source (Cynomolgus)*

- China
  - 70-80 fL
- Mauritius
  - 58-68 fL
- Use one source if possible
- Compare to pretest only



# *Red Cell Counting/Sizing*

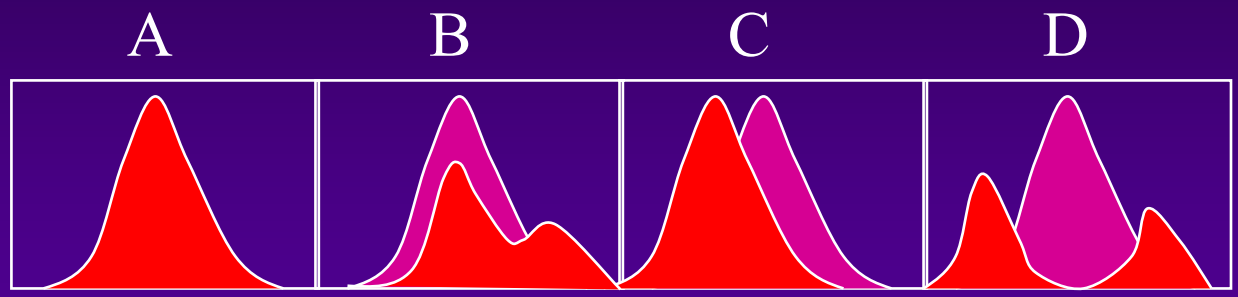
| Measured | Calculated |
|---|---|
| HGB | HCT* |
| RBC | MCH |
| MCV | MCHC |
| RETIC | RDW** |



* Hematocrit = Mean Cell Volume x Red Cell Count

**RDW = Coefficient of variation of RBC volume distribution
(RDW = [Std Dev x 100]/mean MCV)

# *Red Cell Counting/Sizing*




| | | | | |
|---|---|---|---|---|
| **MCV (fL)** | 61.2 | 73.6↑ | 58.2 ↓ | 61.0 |
| **RDW (%)** | 12.1 | 15.8↑ | 12.0 | 24.2↑ |
| **Description** | Normal | Macrocytic Anisocytosis | Microcytic | 2 populations of RBC Anisocytosis |

# *Calculated Red Cell Indices*

| Measured | Calculated |
|---|---|
| HGB | HCT |
| RBC | MCH* |
| MCV | MCHC** |
| RETIC | RDW |

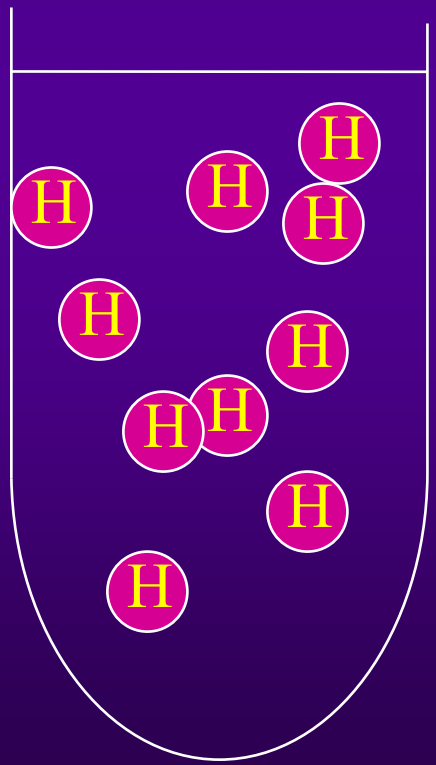
* MCH = Average weight of hemoglobin in each red cell (HGB/RBC)

**MCHC = Average weight of hemoglobin as a function of total red cell mass (HGB/HCT, or HGB/MCVxRBC)

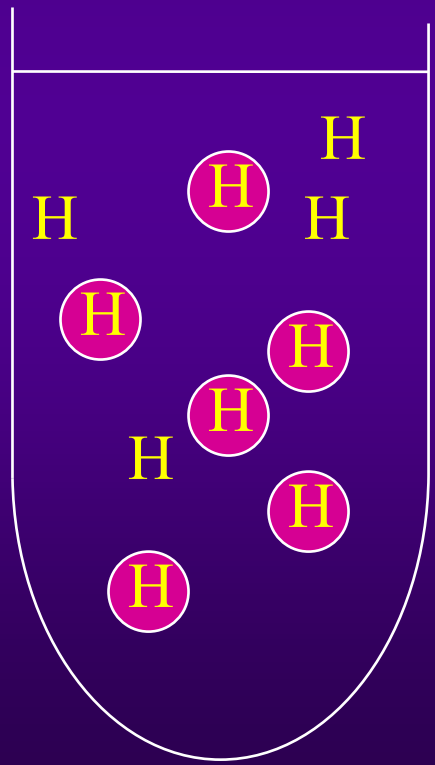
# *Calculated Red Cell Indices*

MCHC = HGB/(MCV x RBC)

Normal



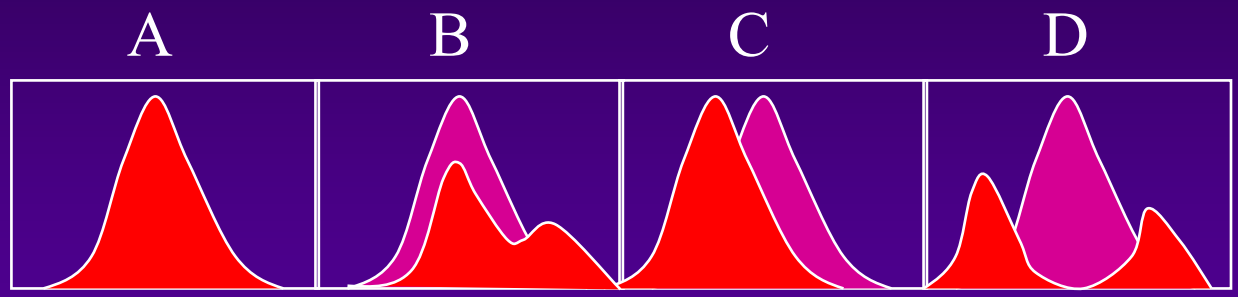

Extracellular HGB



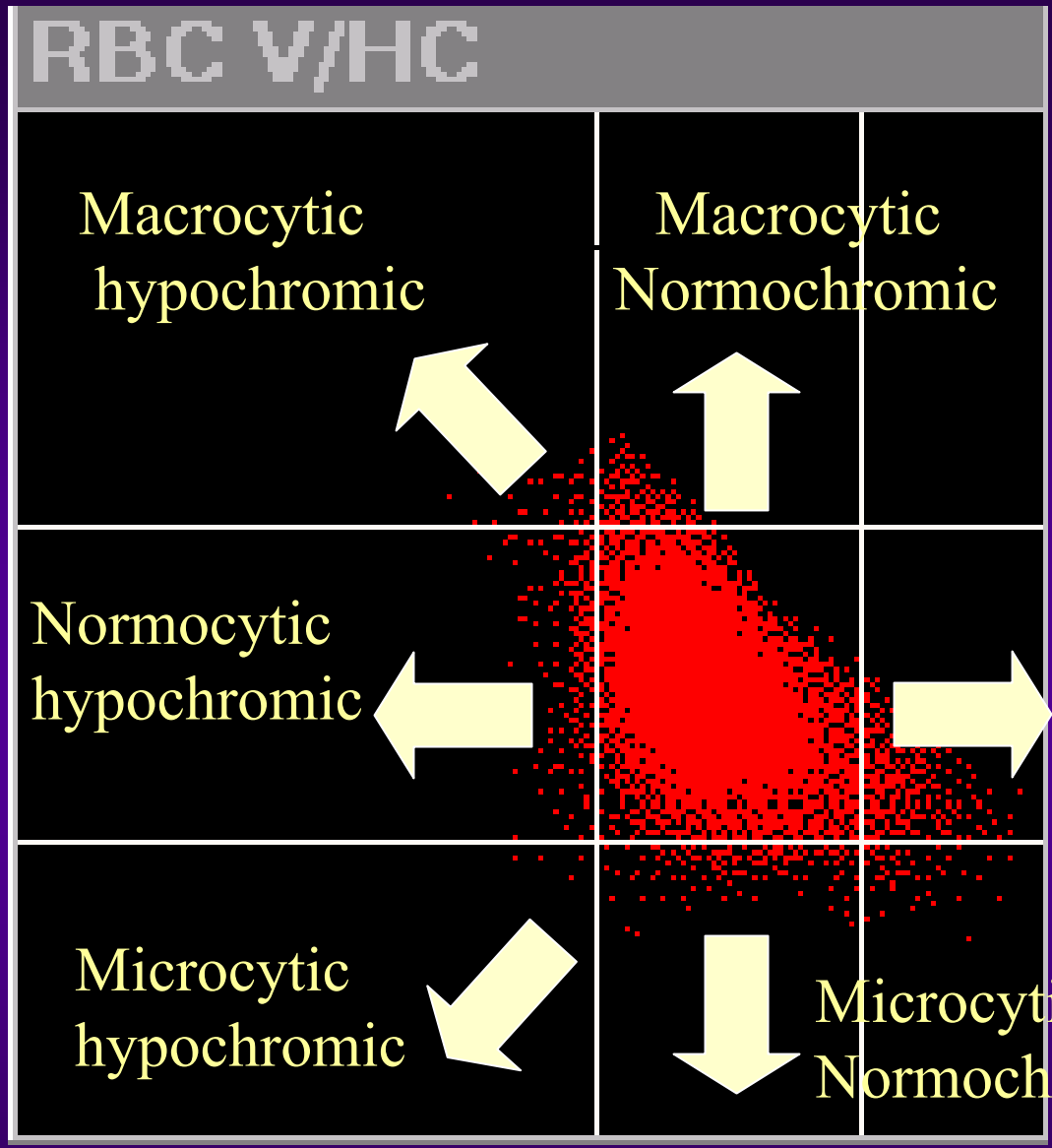

RBC decreased

No change in hemoglobin concentration

# *Calculated Red Cell Indices*




| | | | | |
|---|---|---|---|---|
| **MCH (pg)** | Not useful | - | - | - |
| **MCHC g/dL** | 29.5 | 27.5 ↓ | 29.5 | 27.1 ↓ |
| **Process** | Normal | Hypochromic | Normochromic | Hypochromic |

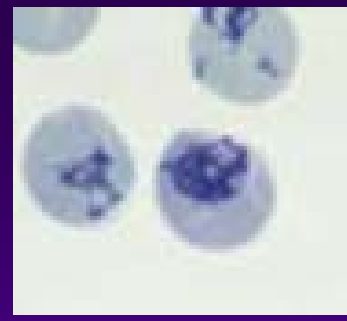



RBC Volume (fL)

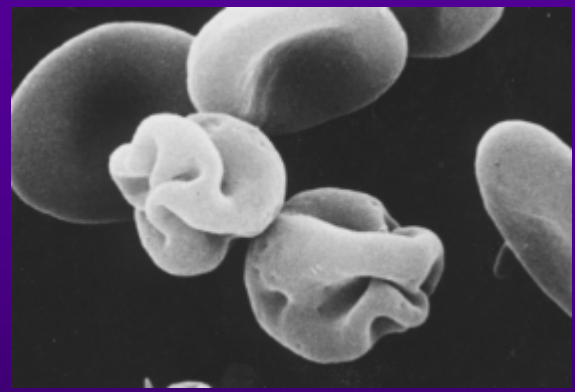
**Red Cell Hemoglobin Concentration (g/dL)**

From MHG - Dr. T. Skelton AACC 2001

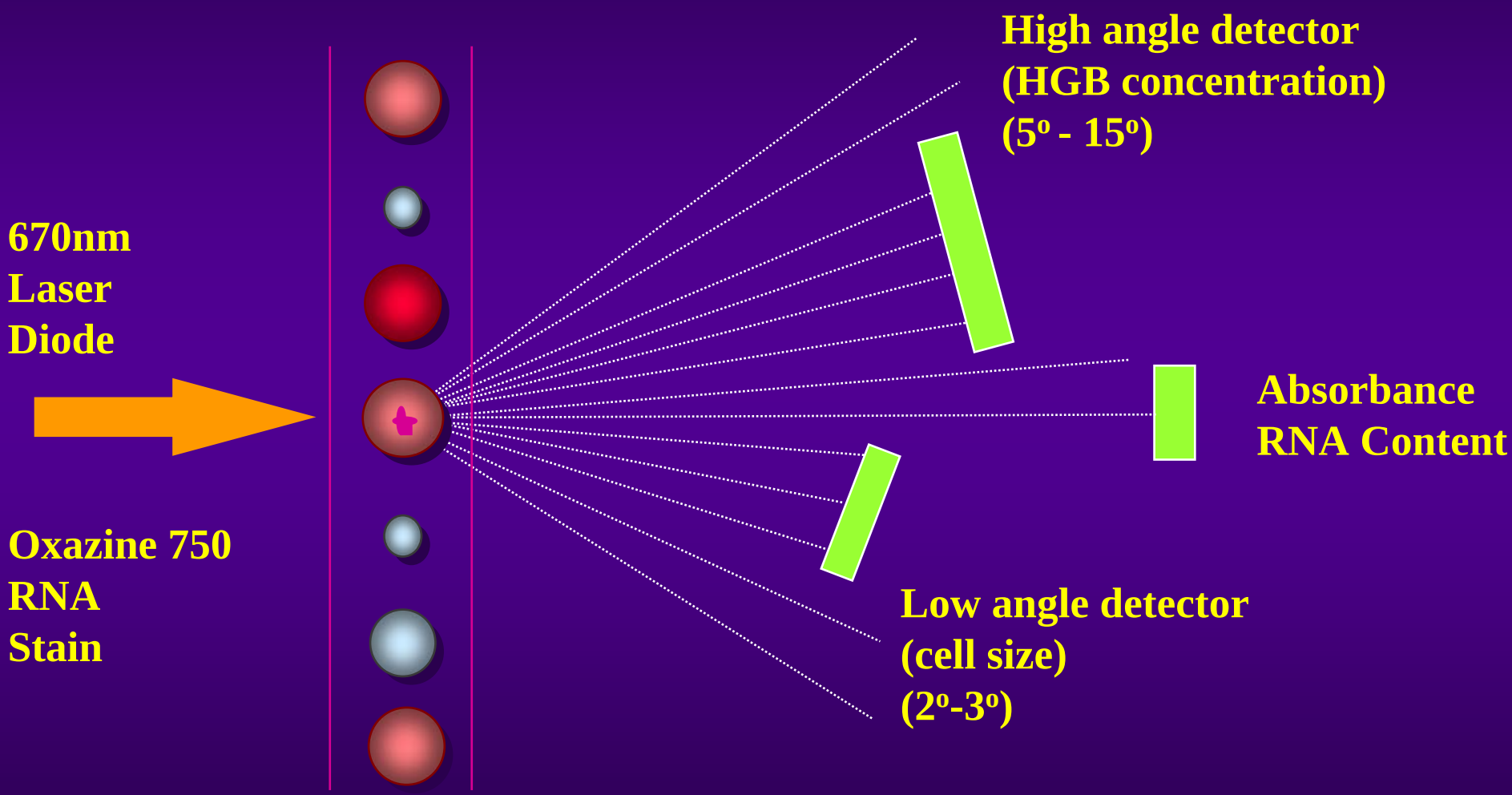
# *Reticulocyte counting*



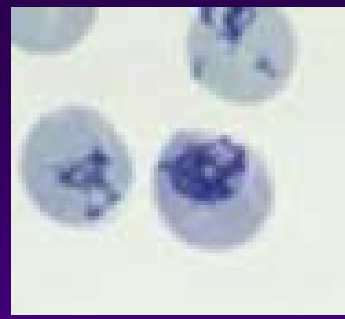
| Measured | Calculated |
|---|---|
| HGB | HCT |
| RBC | MCH |
| MCV | MCHC |
| RETIC | RDW |



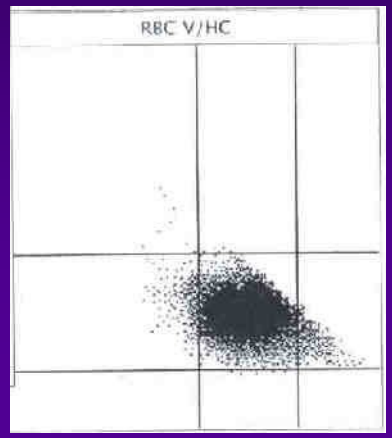
# *RBC/Reticulocyte Analysis*

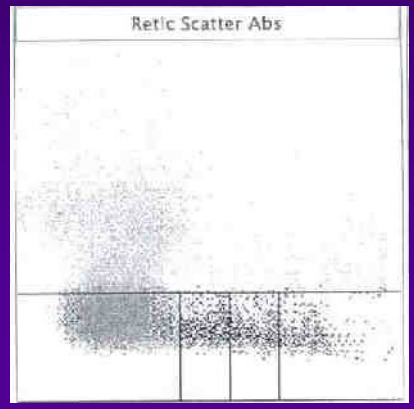


# *Reticulocyte Counting*

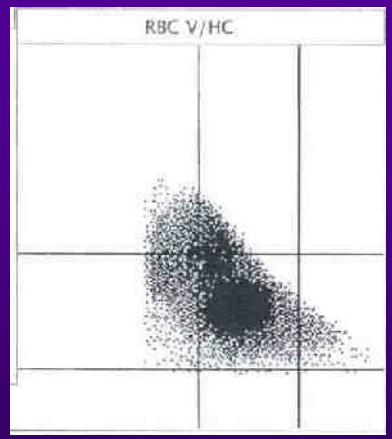


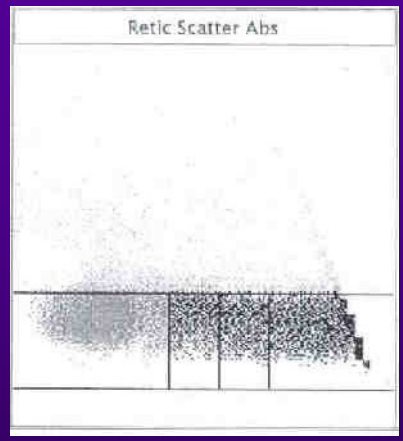
Normal



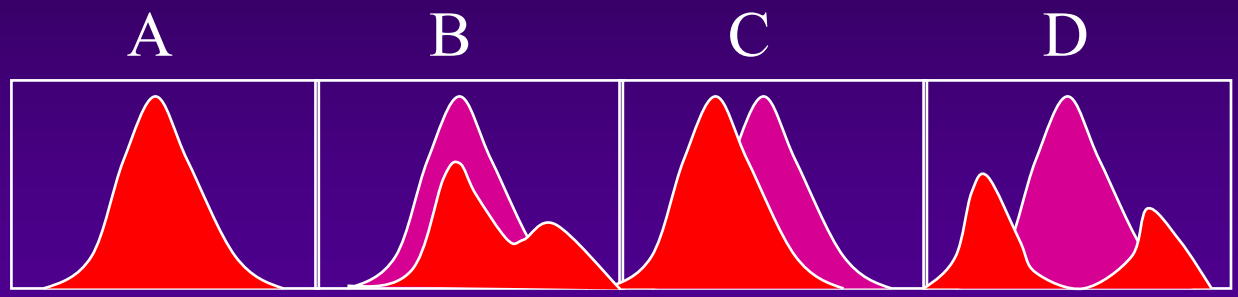




Increased Reticulocytes



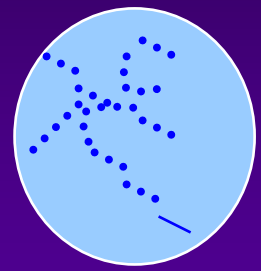
# *Reticulocytes*

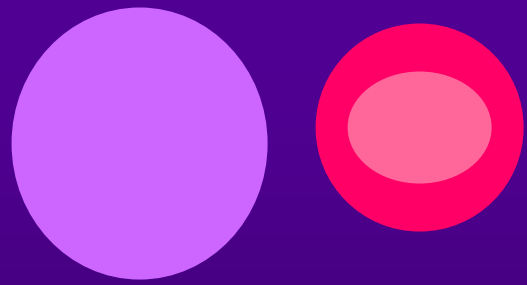


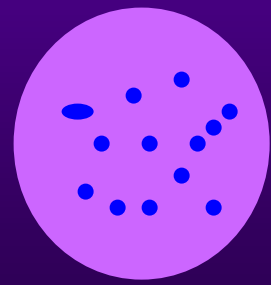

| | | | | |
|---|---|---|---|---|
| **HGB (g/dL)** | 16.5 | 14.2 ↓ | 12.4 ↓ | 13.8 ↓ |
| **Retic count (x$10^3$/uL)** | 189 | 325 ↑ | 187 | 289 ↑ |
| **Process** | Normal | Regenerative | Non-regenerative | Regenerative |

# *Characteristics of Young Red Cells*

- Stained reticulum (reticulocytes)
  - With new methylene blue stain
- Less than full hemoglobin content
  - Decreased MCHC
- Larger (increased MCV)
- Polychromasia (bluish)
  - With Wright's stain
- Basophilic stippling
  - Ruminants, rodents, gerbils, others
  - With Wright's stain



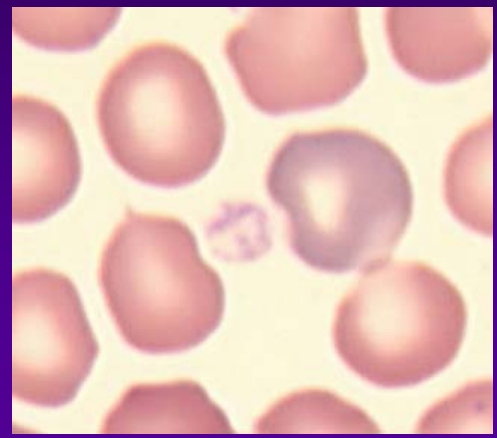




# *Effects of Red Cell Lifespan: Reticulocytes*

- Rodents vs. larger animals: shorter RBC lifespan, higher retic counts
  - RBC lifespan (retic counts x$10^3$ cells/µL)
    - Humans 120 days (27-125)
    - Dogs 100-115 days (17-79)
    - Rats 45-50 days (135-250)
    - Mice 43 days (221-370)
- **Rodents: more polychromasia and anisocytosis**

# *Evaluate blood smear*



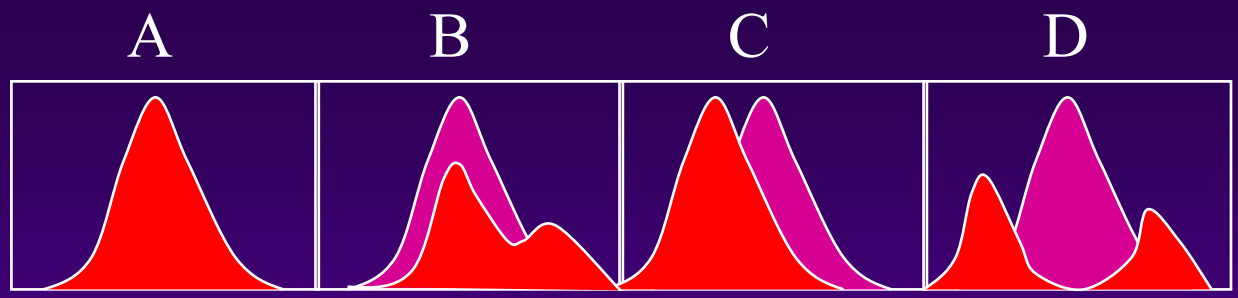
- Confirm instrument data
  - RBC density (RBC, HGB, HCT)
  - Size (MCV, RDW)
  - Hemoglobin content (MCHC)
  - Maturity of cells (RETIC count)
- Evaluate any other morphologic changes
  - Rouleaux, schistocytes, keratocytes, dacryocytes, Howell-Jolly bodies, acanthocytes, nucleated red cells, etc etc

# *Correlation of RBC data*

- Instrument data should make sense
  - Samples with increased MCV and decreased MCHC should have increased reticulocytes
- Microscopic morphologic data should match instrument data
  - Polychromasia if reticulocytes increased
  - Microcytes if MCV decreased
  - Anisocytosis if RDW increased

# *Interpretation of RBC Data*

- RBC mass: increased, normal, or decreased
- Size (MCV): macro, normo, or microcytic
- Hemoglobin content (MCHC): hyper, normo, hypochromic
- Regeneration (retic and associated parameters)




| | A | B | C | D |
|---|---|---|---|---|
| **MCV** | 61.2 | 73.6 ↑ | 58.2 ↓ | 61.0 |
| **RDW** | 12.1 | 15.8 ↑ | 12.0 | 24.2 ↑ |
| **MCHC** | 29.5 | 27.5 ↓ | 29.5 | 27.1 ↓ |
| **HGB** | 16.5 | 14.2 ↓ | 12.4 ↓ | 13.8 ↓ |
| **Retic count** | 189 | 325 ↑ | 187 | 289 ↑ |
| **Description** | Normal | Macrocytic Hypochromic Regenerative Anemia | Microcytic, Normochromic Non-regenerative Anemia | Normocytic (2 populations) Hypochromic, Regenerative Anemia |

# *Increased Red Cell Mass (HGB, HCT, RBC) (polycythemia)*

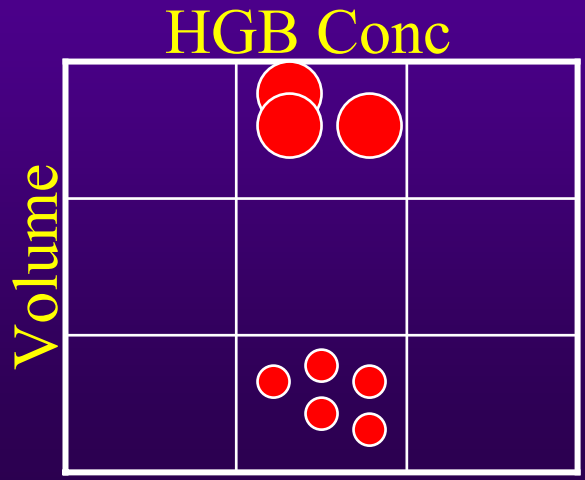
- Relative increase in red cell mass
  - Dehydration
- Absolute increase in red cell mass
  - Splenic contraction (cats>>dogs>>rodents)
  - Excess EPO (exogenous or endogenous)
  - Activating mutations of EPO receptor
  - Decreased oxygenation ($\downarrow PaO_2$)
    - Abnormal hemoglobin (MetHb, etc)
    - Abnormal oxygenation (pulmonary, cardiovascular)
  - Polycythemia vera

# *Increased Red Cell Mass (Polycythemia)*

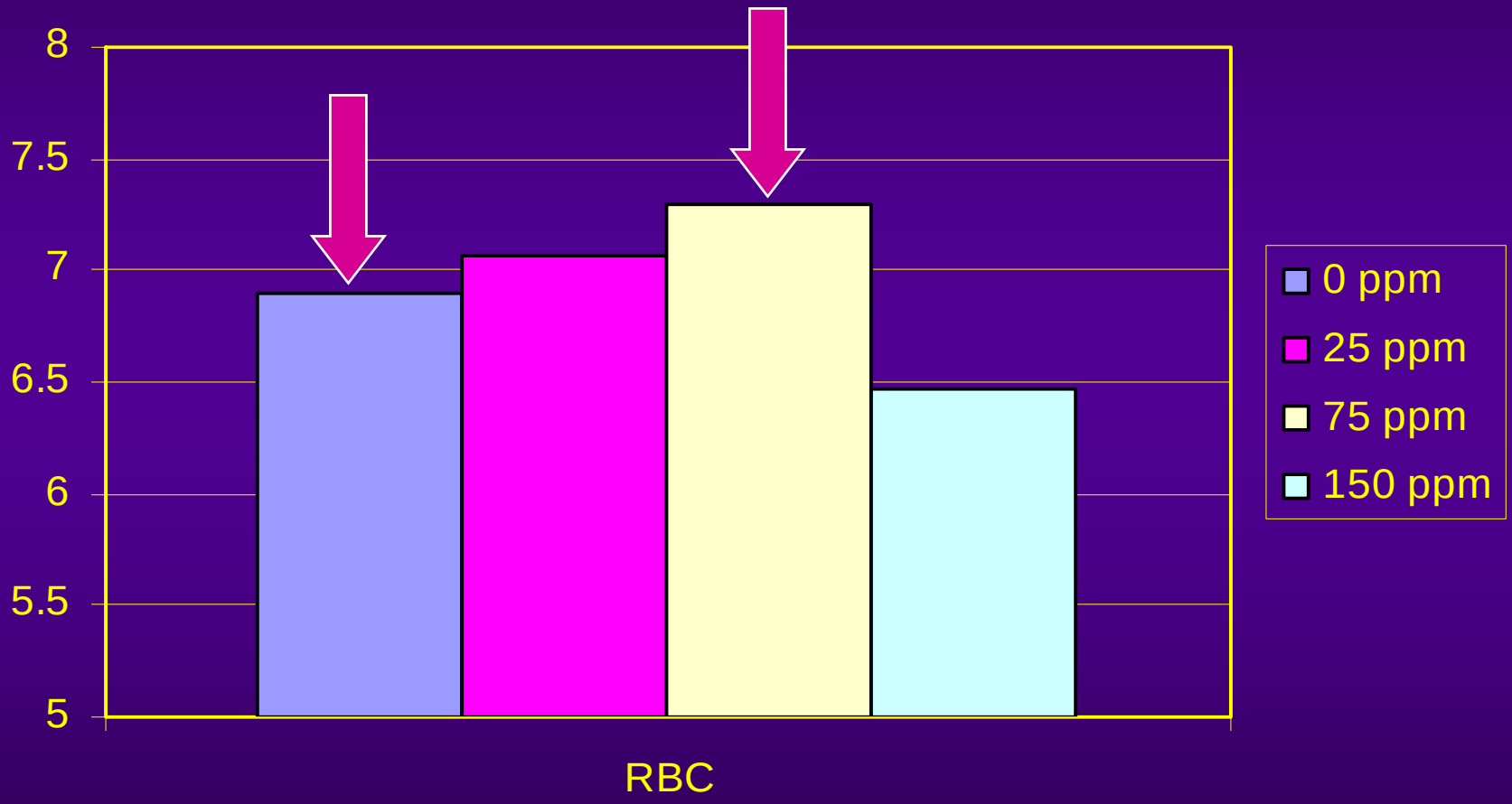
- Relative polycythemia
  - Dehydration
  - Splenic contraction
- Absolute polycythemia
  - Primary: Polycythemia vera (myeloproliferative disorder)
  - Appropriate
    - Decreased oxygenation: high altitude, cardiopulmonary disease
  - Inappropriate
    - Hydronephrosis or renal cysts, activating mutations, EPO-secreting neoplasms

# *Change in RBC Counts With/Without Change in HGB or HCT*

- Increased RBC with microcytosis
  - Breed idiosyncrasy, Fe deficiency, portosystemic shunts)
- Decreased RBC with macrocytosis
  - Altered nucleic acid synthesis (reverse transcriptase inhibitors, B12 deficiency, FeLV infection)



# ↑ RBC with ↓ Red Cell Mass



# *Decreased Red Cell Mass*

- Relative decreased red cell mass
  - Plasma volume expansion (rare)
  - Excess fluids, pregnancy, neonates
- Absolute decreased red cell mass
- Definitions of anemia
    - RBC mass below reference range
    - Decreased oxygen-carrying capacity of blood
      - Some species: 2,3-DPG favors release of O2 to tissues (dog, horse, pig)

# *Decreased Red Cell Mass: Causes*

- Loss (hemorrhage)
- Destruction (hemolysis or decreased half-life)
- Decreased production (bone marrow effects)

# *Red Cell Loss (hemorrhage): Causes*

- Iatrogenic due to excessive blood collection
  - Over-collection: effect of experiment in anemic animals
  - Collect consistent and reasonable amount of blood from all groups
- Obvious hemorrhage
- Occult loss (gastrointestinal, urinary)

# *Red Cell Loss (hemorrhage): Chronic*

- Generally associated with some RBC regeneration in laboratory animals
- Diagnose via clinical signs, rather than specific hematologic changes (GI, UA)
- Long term loss may lead to classic iron-deficiency (non-regenerative)
  - Rare in laboratory animal facility
  - Ulcerated masses (older rodents)
  - Wasting disease (marmosets)

# *Red Cell Loss (hemorrhage): Acute*

- Clinical evidence
- Release of RBCs by splenic contraction
- Associated with ↑ neutrophils and platelets
- Recovery by 2 weeks in most species
- Maximum allowable volume of collection
  - Set by IACUCs
  - May still have marked effect on experimental outcome
  - Keep sample collected to a minimum

# *Red Cell Destruction (hemolysis): Causes*

- Infection (bacterial, parasitic)
- Trauma to RBCs (fragmentation)
- Immune-mediated
- Altered metabolism (toxicants, mutations)
- Altered RBC membrane

# *Red Cell Destruction: Extravascular vs. Intravascular*

Extravascular

- Antibody/Complement-mediated
- Altered cell membrane
- Decreased glycolysis
- Increased macrophage activity

Intravascular

- Complement-mediated
- Altered cell membrane
- Trauma
- Osmotic lysis

# *Red Cell Destruction: Extravascular vs. Intravascular*

## Extravascular

- Slower onset
- No free HGB in plasma or urine
- ±Hyperbilirubinemia
- May be non-anemic "compensated hemolytic anemia"

## Intravascular

- Acute disease
- Free hemoglobin in plasma (↑MCHC)
- ±Hemoglobinuria
- ±Hyperbilirubinuria
- Other morphologic findings

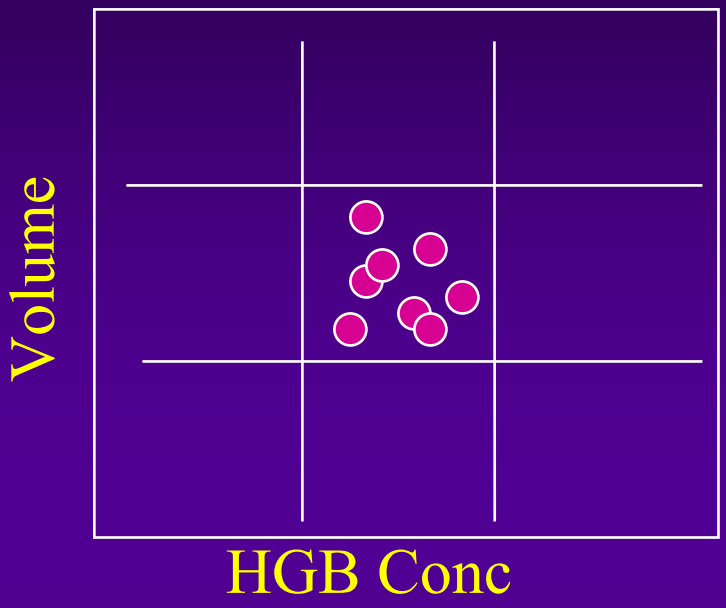
# *Red Cell Destruction: Responses*

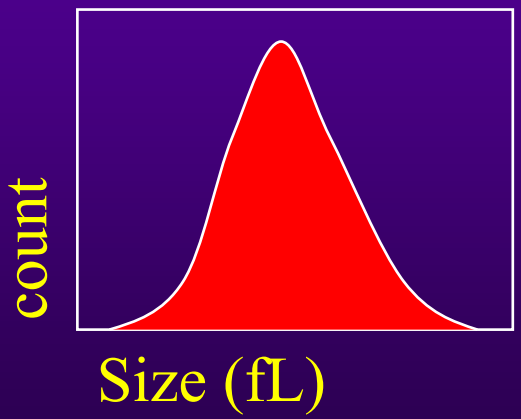
- Increased production of RBCs
- Bone marrow hyperplasia
  - Other bones recruited
- Extramedullary hematopoiesis
  - Spleen, liver, lymph nodes, other organs
  - ↑spleen weights: mouse>rat>other species

# *Red Cell Destruction: Responses*

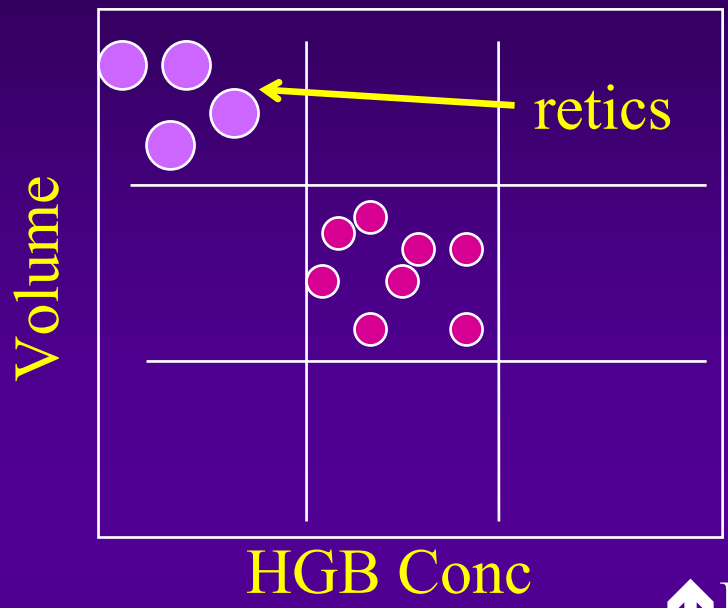
- Peripheral blood changes
  - Increased reticulocytes and polychromasia
    - Dogs >80,000/uL; Cats >60,000/uL)
  - Possible morphologic changes
    - Spherocytes (remodeling)
    - Schistocytes (fragmentation)

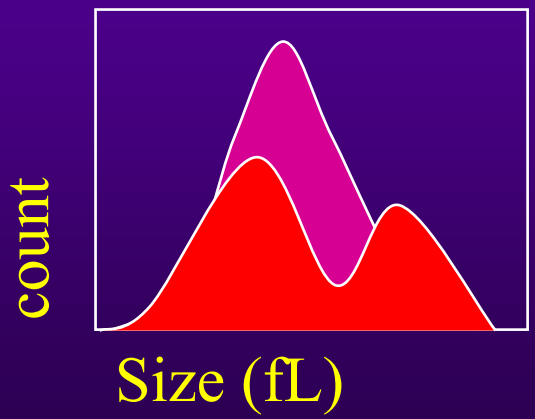
## Normal hemogram



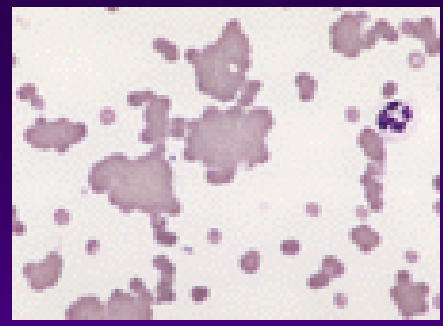

## Regeneration




↑RETIC
↑ MCV
↓MCHC
↑ RDW

# *Coombs' Test*



- Species-specific antibodies
- Detects immune-mediated hemolysis
- Endpoint: agglutination

# *Coombs' Test*

Positive Result

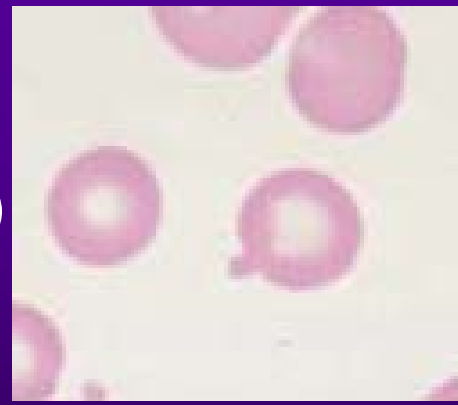
- AIHA present
- Hapten
- Infection
- Non-specific binding

Negative Result

- No AIHA
- Prozone
- Corticosteroids
- Below limit of detection
- Post-transfusion

# *Red Cell Oxidation Injury*

- Drugs, chemicals, metals, plants, food
- Methemoglobin
  - MetHb reductase: rodents>>dogs, people
  - MetHb cannot carry $O_2$
- Other results of oxidative injury
  - Heinz bodies (denatured hemoglobin)
    - NMB stained slides
  - Eccentrocytes



# *Effects of Red Cell Lifespan: Reticulocyte Response*

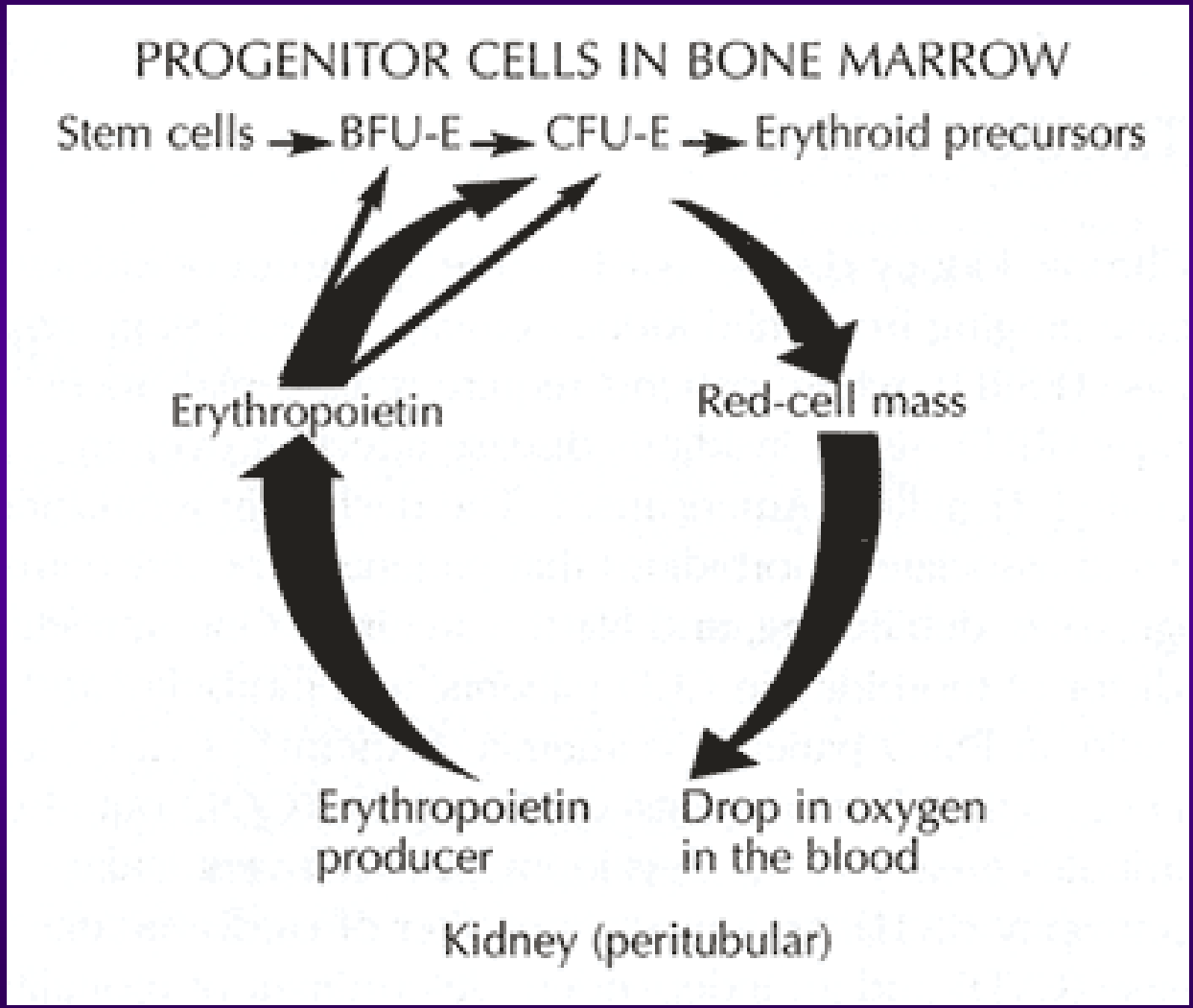
- Rodents vs. larger animals: shorter RBC lifespan, higher retic counts
  - RBC lifespan (retic counts x$10^3$ cells/μL)
    - Humans 120 days (27-125)
    - Dogs 100-115 days (17-79)
    - Rats 45-50 days (135-250)
    - Mice 43 days (221-370)
- **Reticulocyte response is more exuberant in rodents**
- **Changes in reticulocyte counts occur faster in rodents**

# *Decreased Red Cell Production: Causes*

- Decreased erythropoietin
  - Renal disease
  - Decreased EPO (antibodies, decreased production)
  - Abnormal EPO receptors (mutant mice)
- Direct bone marrow effect
  - Cytotoxic effects (precursors or stroma)
  - Defects in hemoglobin or nucleic acid synthesis
  - Abnormal maturation/maturation arrest

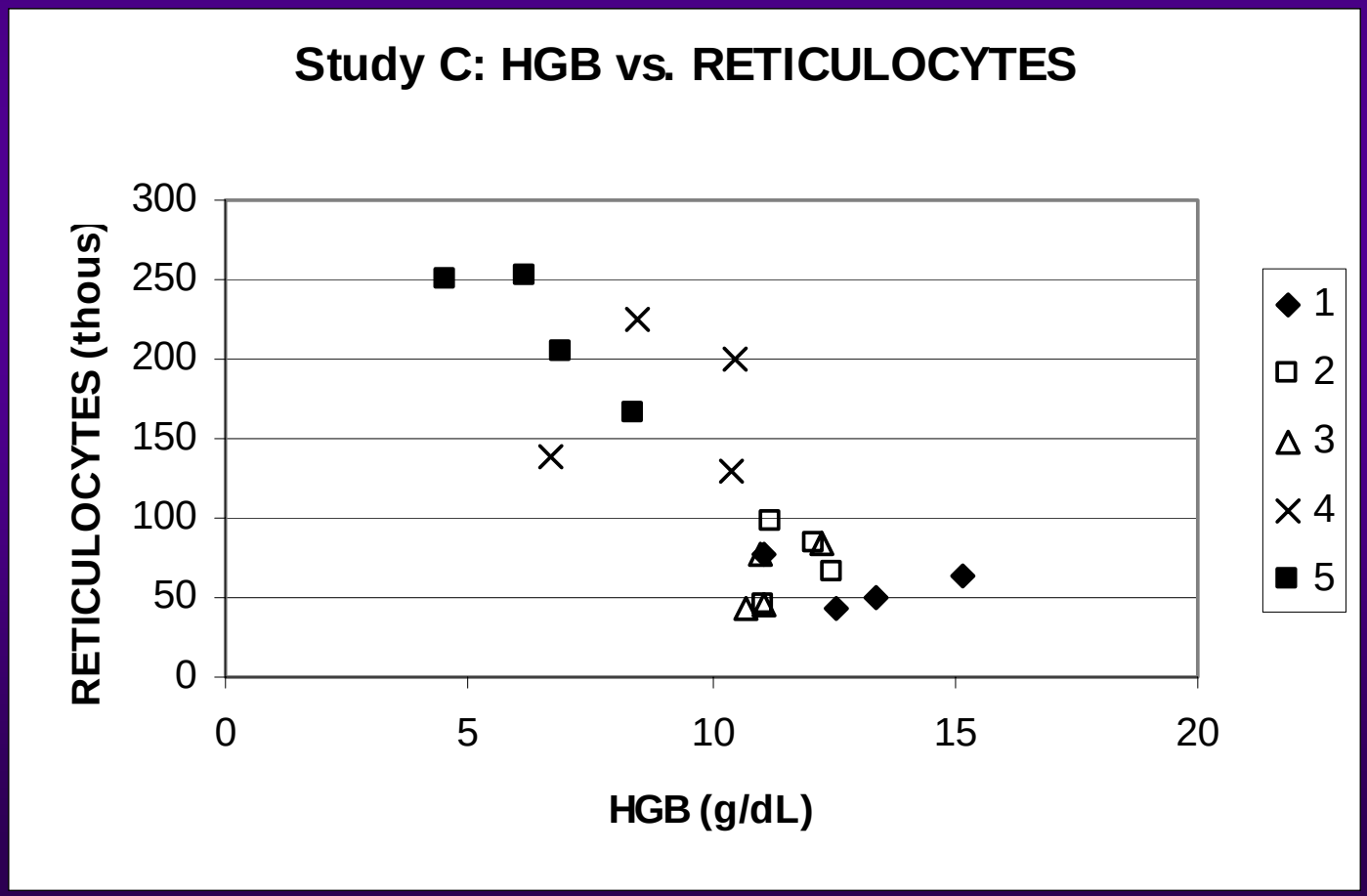
# *Red Cell Mass Decrements and Stimulation of Erythropoiesis*

- Decreased red cell mass
- Hypoxia at peritubular kidney cells
- Increased erythropoietin (EPO)
- EPO causes increased red cell production
  - Stimulates proliferation of primitive and mature red cell progenitor cells
  - Stimulates proliferation and survival of more mature red cell precursors
  - Prevents apoptosis of late stage red cell precursors
  - Causes release of more immature reticulocytes



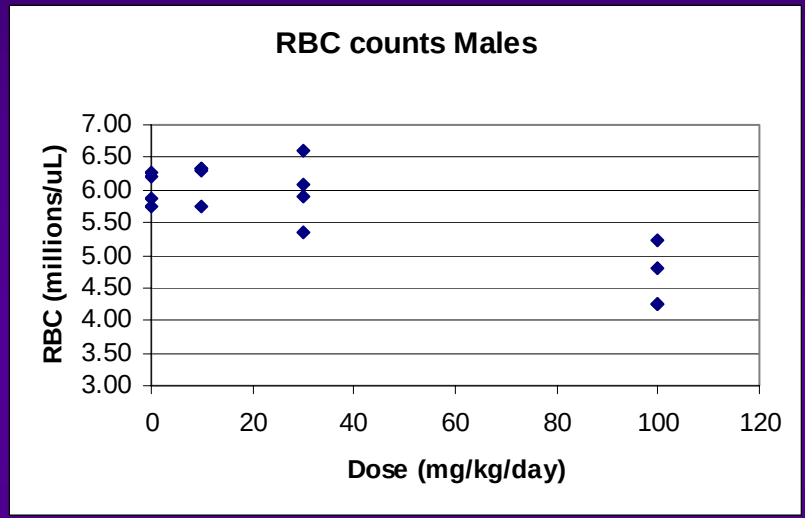

**http://www.powerpak.com/CE/ckd/pharmacy/figures.cfm#figure1**

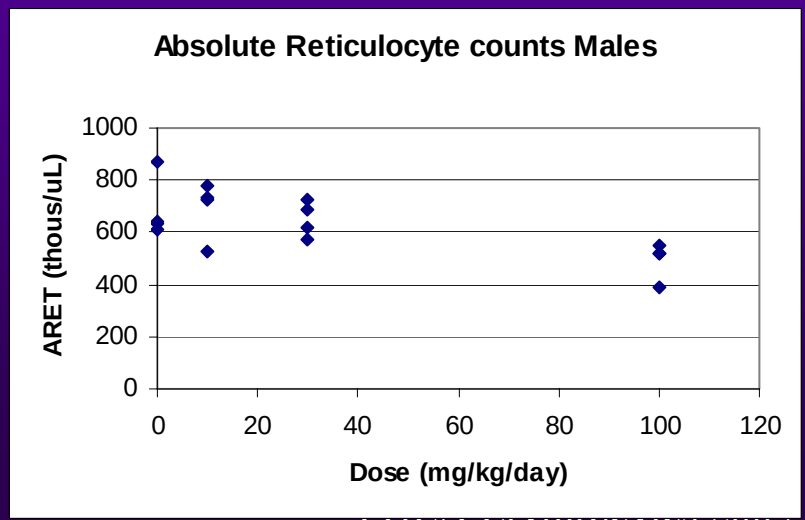
# *Appropriate Reticulocyte Responses (Monkey)*



# *Inappropriate Reticulocyte Responses (Rat)*

- 5-day Rat Toxicology Study with Novel Anti-inflammatory Compound
- Decreased RBC counts at 100 mg/kg/day
- Decreased absolute retic counts
- **Conclusion:**
  - Inappropriate reticulocyte count in light of red cell changes



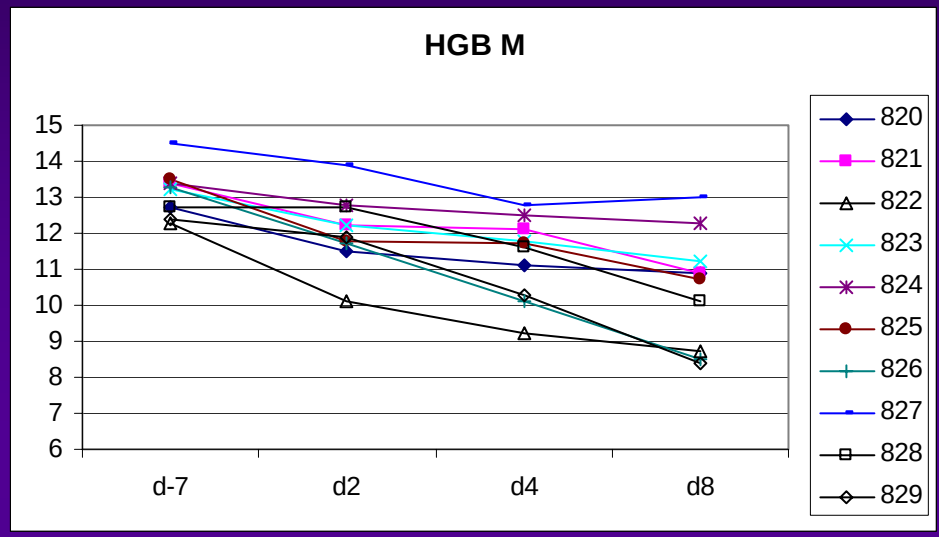



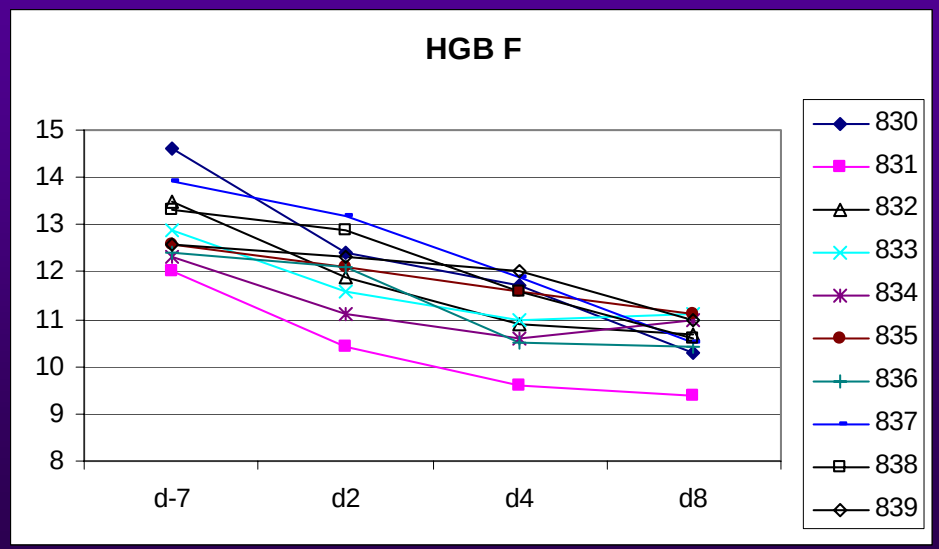
# *Interpretation of Reticulocyte Responses*

- Decreased reticulocytes: ***Always inappropriate in the face of decreased red cell mass***
  - Bone marrow effects
  - Effects of other disease processes (anemia of chronic disease, renal insufficiency)
- Even normal or increased reticulocyte responses may be inadequate
  - Always need to compare to red cell mass effects
  - Increased retics may be inappropriate for degree of decreased red cell mass
- Species differences need to be considered in evaluating reticulocytes

# *Example 1: Monkey Study Revisited*

- Decreased RBC mass over time
- Not related to dose of test compound
- Retics
  - Most: ↑↑ compared to pretest
  - High dose: Similar to pretest



# *Example 1: Monkey Study Revisited: Conclusions*

- In light of decrements in RBC mass, reticulocyte response was:
  - Appropriate in control and low dose monkeys
  - Inappropriate in high dose monkeys
- Further information
  - High dose monkeys were clinically dehydrated
  - Therefore, red cell mass was probably actually lower than that of control and low dose monkeys.

# *Decreased Red Cell Production: Characteristics*

- Mild-moderate non-progressive anemia
- Absent or inadequate reticulocyte response
  - Inappropriate for decreased red cell mass
  - Rodents: decreased RDW, increased MCHC
  - Other peripheral blood changes
- Bone marrow
  - Abnormal proportions or morphology of red cell precursors

# *Effects of Red Cell Lifespan: Decreased Red Cell Production*

- Rodents vs. larger animals: shorter RBC lifespan, higher retic counts
  - RBC lifespan (retic counts x$10^3$ cells/μL)
    - Humans 120 days (27-125)
    - Dogs 100-115 days (17-79)
    - Rats 45-50 days (135-250)
    - Mice 43 days (221-370)
- **Peripheral blood RBC mass decreases quicker in rodents when bone marrow is affected**

# *Decreased RBC Production*

- Bone Marrow Effects
- Destruction or inhibition of precursors

| | Regen Anemia (all) | Non-regen dog | Non-regen rodent |
|---|---|---|---|
| RETIC | ↑ | | |
| MCV | ↑ | | |
| RDW | ↑ | | |
| MCHC | ↓ | | |
| Polychr | ↑ | | |

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- Bone Marrow Effects
- Destruction or inhibition of precursors

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|---|---|---|---|
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| MCHC | ↓ | → | |
| Polychr | ↑ | → | |

# *Decreased RBC Production*

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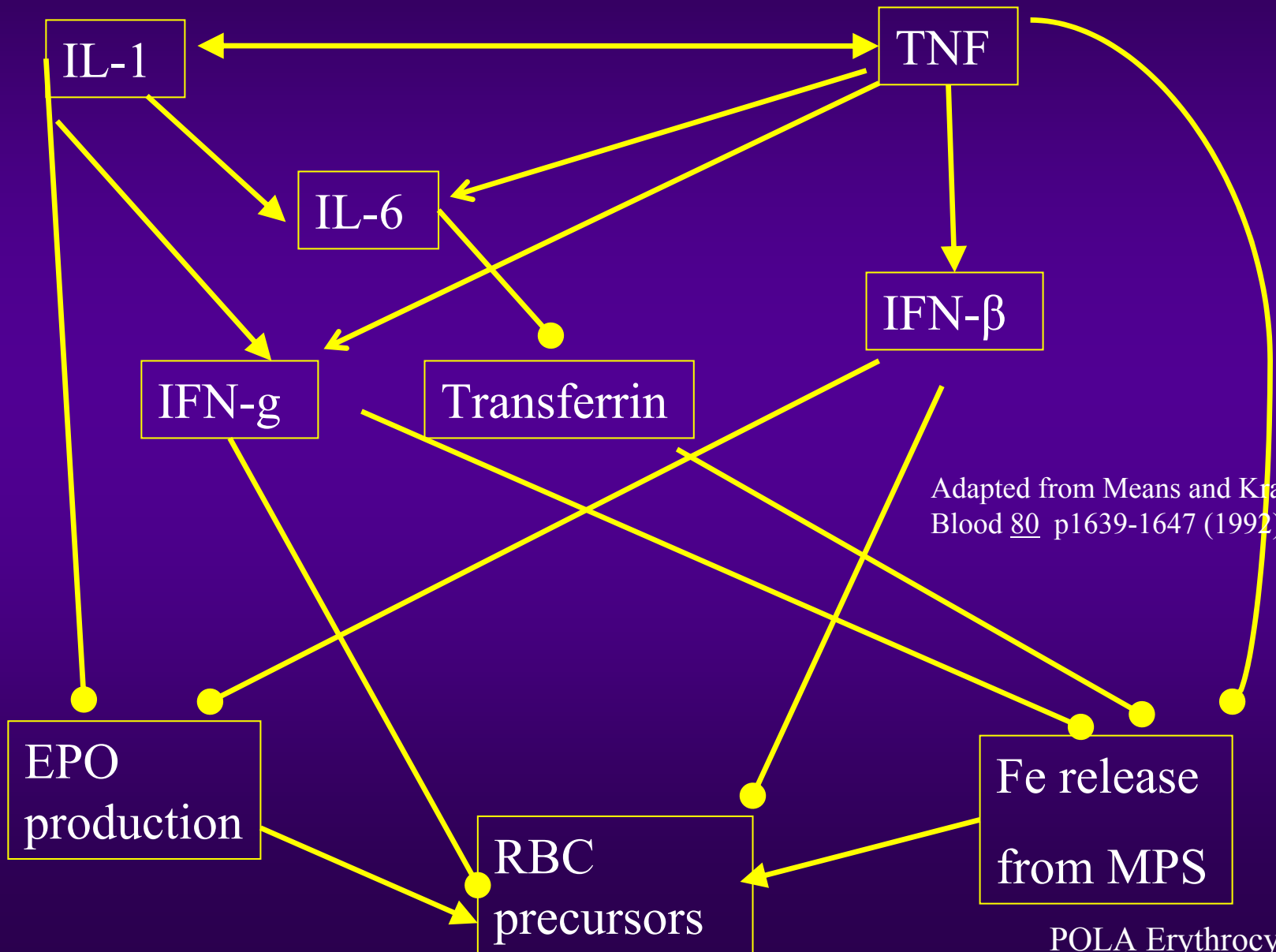
| RDW | ↑ | → | ↓ |
| MCHC | ↓ | → | ↑ |
| Polychr | ↑ | → | ↓ |

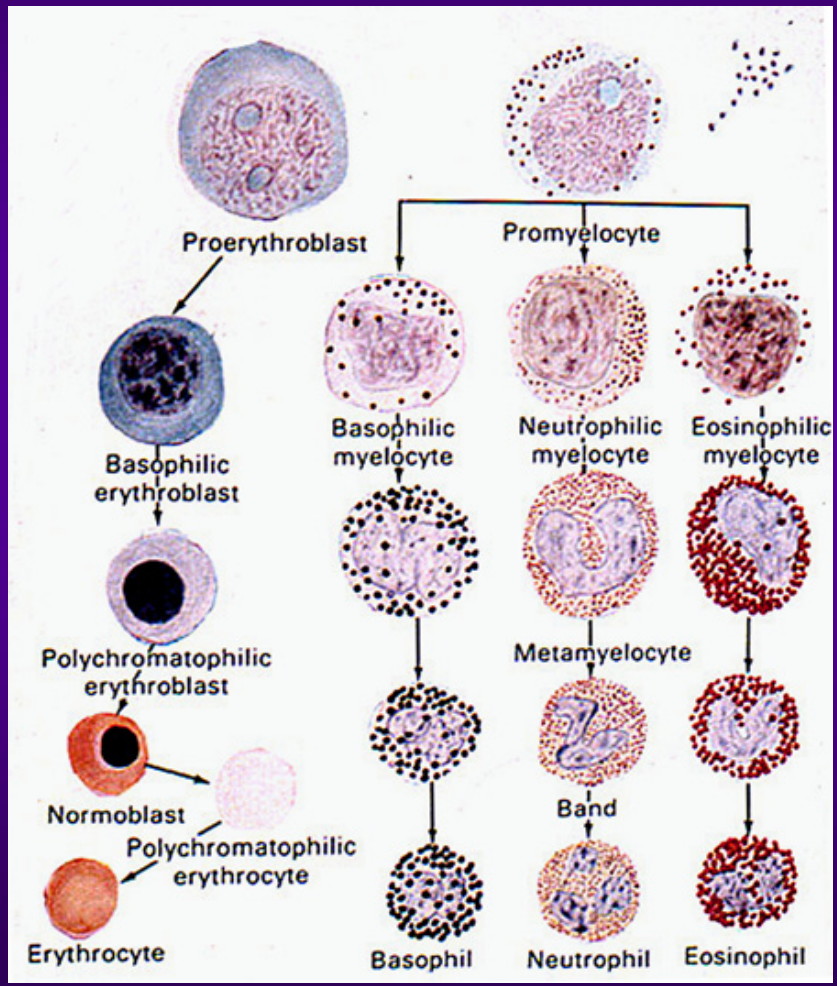
# *"Anemia of Chronic Disease"*

- Most common cause of decreased red cell mass
- Secondary to many underlying processes
  - Inflammation and endocrine most common
  - Also "poor performing" animals
- Cause of anemia
  - ↓ RBC production
  - ↑ RBC destruction (hemolysis)
  - Sequestration of iron by MΦs

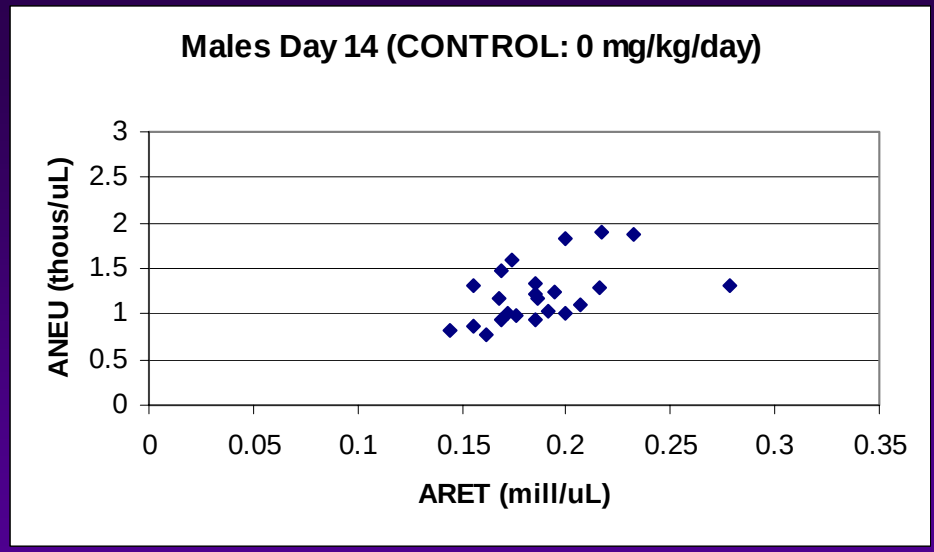
# *Anemia of "chronic disease"*

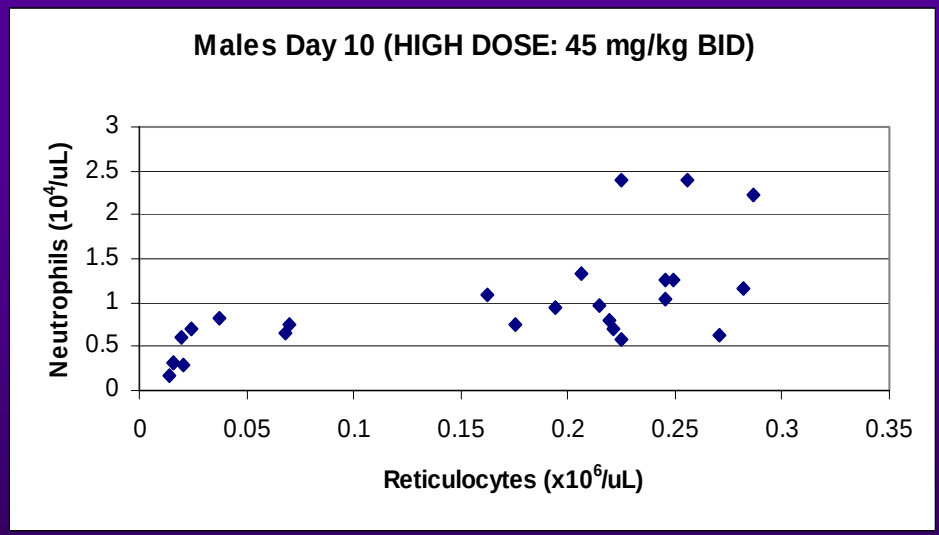


# *Decreased Red Cell Production: Characteristics*

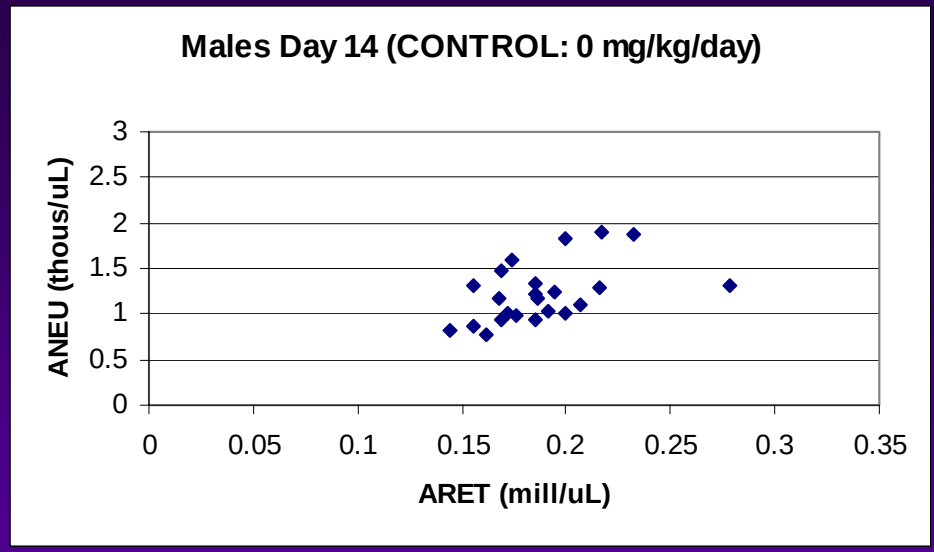


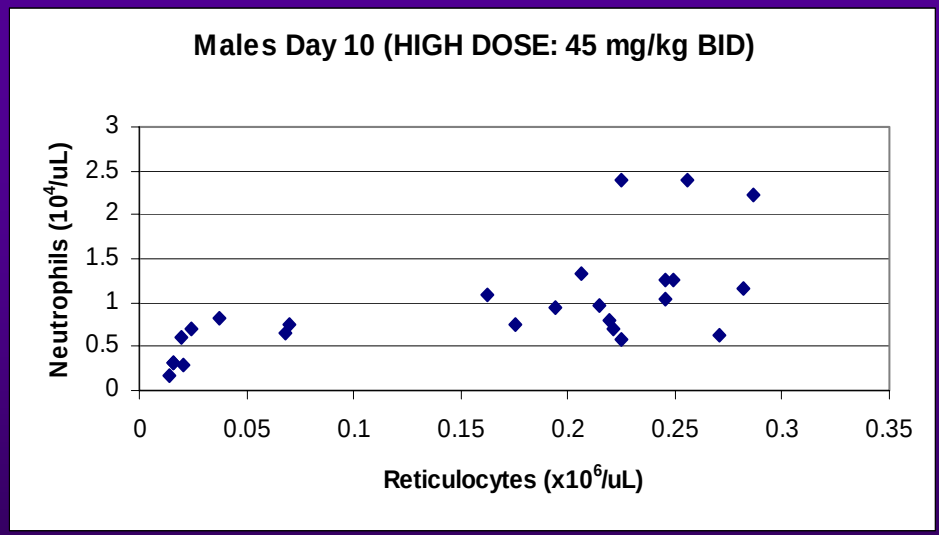
- High dose rats removed from study for humane reasons on Day 10
  - GI (diarrhea)
  - Clinical dehydration
  - RBC mass w/in normal limits
- What's going on?



- Two Issues
  - Dehydration
    - Expect increased RBC mass
  - Low reticulocytes
    - Not appropriate
    - Lower than basal counts
- Other findings
  - Neutrophils
    - Same rats have decreased neuts and retics
- Common mechanism for GI, red cell, and neutrophil effects?



# *Aplastic Anemia*

- Decreases of all three cell types
  - Erythrocytes, leukocytes, and platelets
  - Decrease in relationship to lifespan
- Processes affecting rapidly dividing cells
  - Ionizing radiation
  - Parvoviruses
  - Chemotherapy
- Other processes implicated in aplastic anemia
  - Predictable and idiosyncratic drug reactions
  - Infections (viral, parasitic)

# *Summary: Interpretation of Red Cell Effects*

- Evaluate red cell mass parameters
  - RBC, HGB, HCT
- Evaluate size, shape, and hemoglobin content
  - MCV, MCHC (MCH), RDW
- Evaluate smear for confirmation of instrument data and other morphology

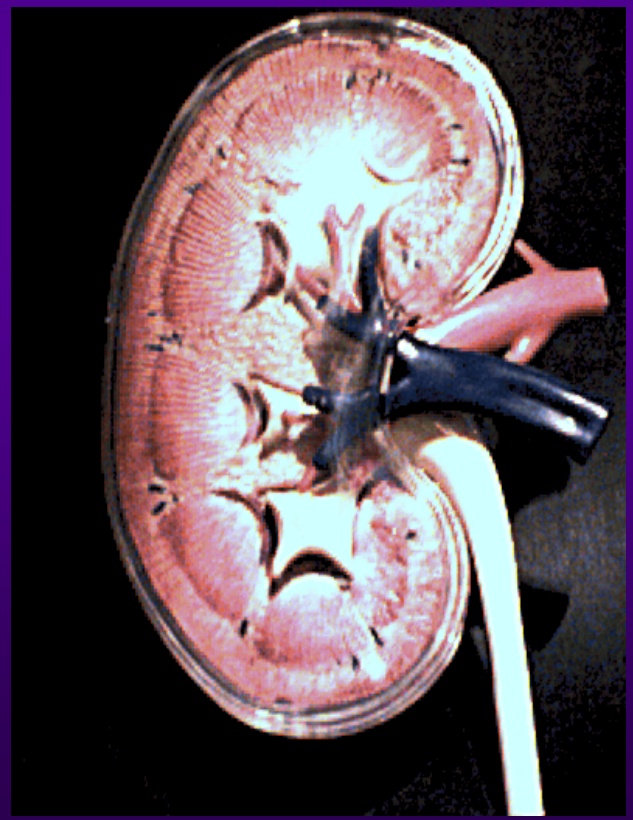
# *Kidney, Urinalysis, and Acid-Base*

*POLA COURSE August 2005*

Nancy Everds, DVM
Diplomate, ACVP
Haskell Laboratory for Health and Environmental Sciences
Newark, DE
nancy.e.everds@usa.dupont.com

# *Outline*

- Urea nitrogen and creatinine
- Urinalysis
- Acid-base



# *Urea Nitrogen*

- Majority synthesized in liver from ammonia
  - Product of protein catabolism
- Passive filtration into urine
- Reabsorption from tubules
  - Proportional to urine flow rate

# *Creatinine*

- Most produced from endogenous phosphocreatine (stored in skeletal muscle)
- Relatively constant production
  - Proportional to muscle mass
- Distributed throughout body water
- Freely filtered by glomerulus
  - Small amount reabsorbed in tubules (male dogs)
  - More accurate than urea nitrogen because no tubular reabsorption and minimal tubular secretion

# *Increased Urea Nitrogen and Creatinine Concentrations*

- Decreased glomerular filtration rate
  - Prerenal
  - Renal
  - Postrenal

# *Increased Urea Nitrogen and Creatinine Concentrations: Prerenal*

- Decreased GFR due to…
  - Decreased renal perfusion
  - Most commonly dehydration, also shock
- Usually urea nitrogen 30-80 mg/dL and creatinine <3 mg/dL

# *Increased Urea Nitrogen and Creatinine Concentrations: Renal*

- Not very sensitive
  - Non-functional nephrons (~3/4)
- Increases usually occur after loss of concentrating ability (dilute urine)
  - Cats often maintain concentrating ability
- Phosphorus changes similar to urea nitrogen and creatinine

# *Increased Urea Nitrogen and Creatinine Concentrations: Postrenal*

- Oliguria or anuria
- Obstruction or leakage
  - Physical exam/imaging useful
  - Rapid recovery after relief of obstruction/leak

# *Extrarenal influences On Urea Nitrogen and Creatinine*

- Dehydration (especially mice)
  - Urinalysis helpful
- Urea nitrogen only
  - Increased protein catabolism (small bowel hemorrhage, starvation, fever, corticosteroids)
  - High protein diets or post-prandial
- Creatinine only
  - Noncreatinine chromogens
  - Rhabdomyolysis, physical conditioning
  - Quickly cleared if normal kidney function

# *Other Indicators of Renal Function*

- Clearance of exogenous chemicals
- Cystatin C

# *Urea Nitrogen and Creatinine*

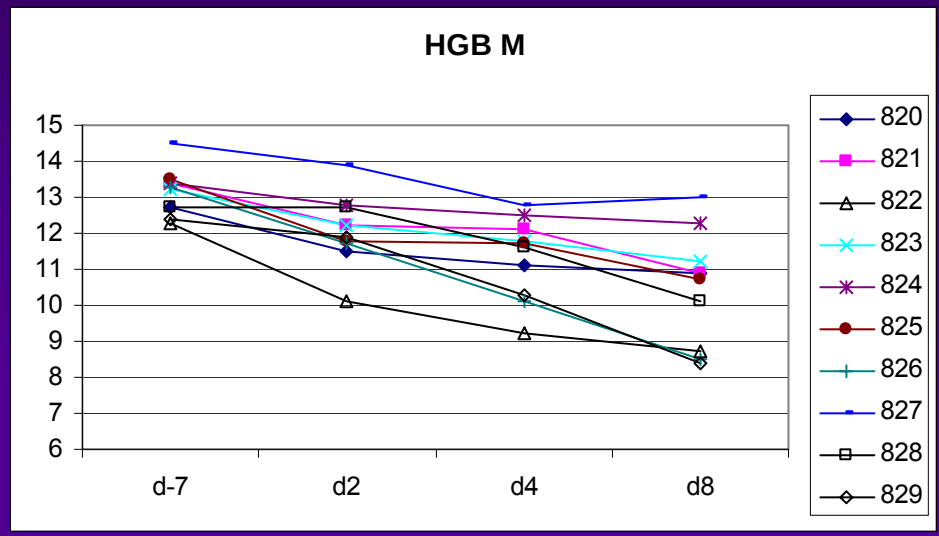
- Very insensitive under clinical conditions
- More sensitive under controlled conditions in laboratory animals
- Rats and dogs
  - Tight ranges
- Mice and monkeys
  - Broad ranges

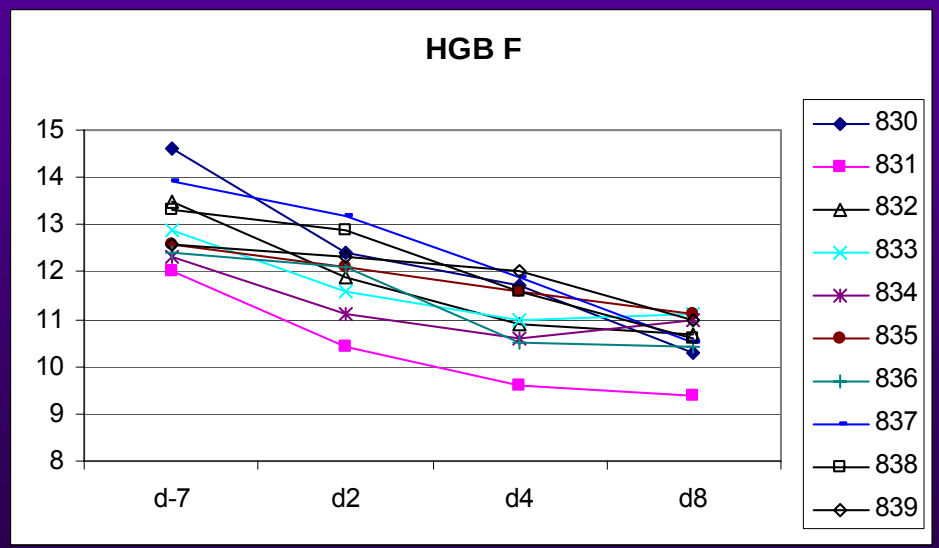
# *Reasons for Decreased Urea Nitrogen and/or Creatinine*

- Decreased urea nitrogen
  - Hepatic insufficiency
  - Low protein diets
  - Anabolic steriods
  - Increased fluid intake/urine output
- Decreased creatinine
  - Decreased muscle mass
  - Generalized wasting

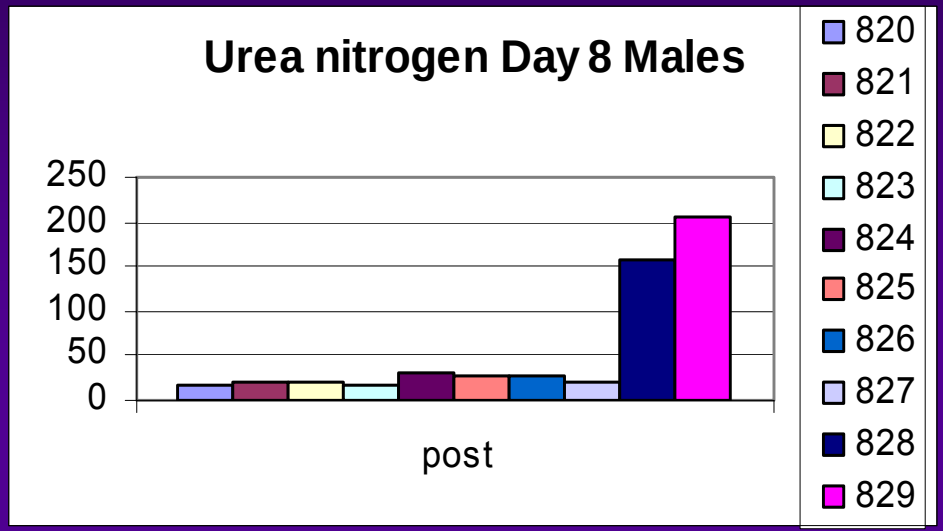
# *Example 1: Monkey Study Revisited*

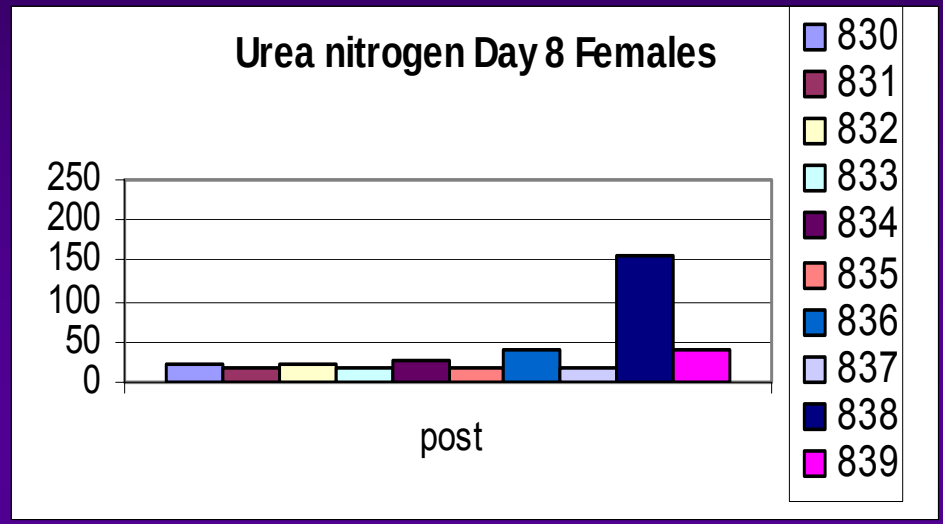
- Decreased RBC mass over time
- Not related to dose of test compound
- Retics
  - Most: ↑↑ compared to pretest
  - High dose: Similar to pretest

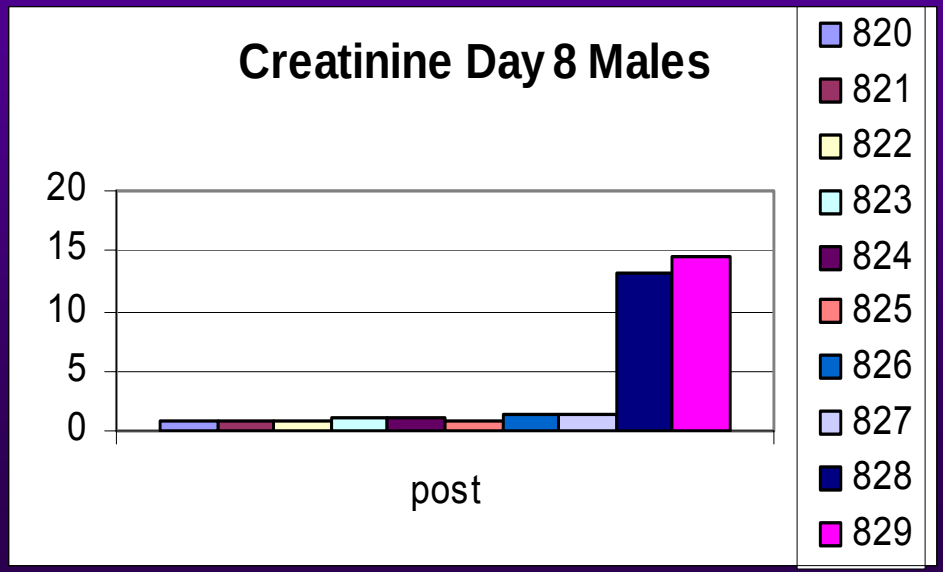


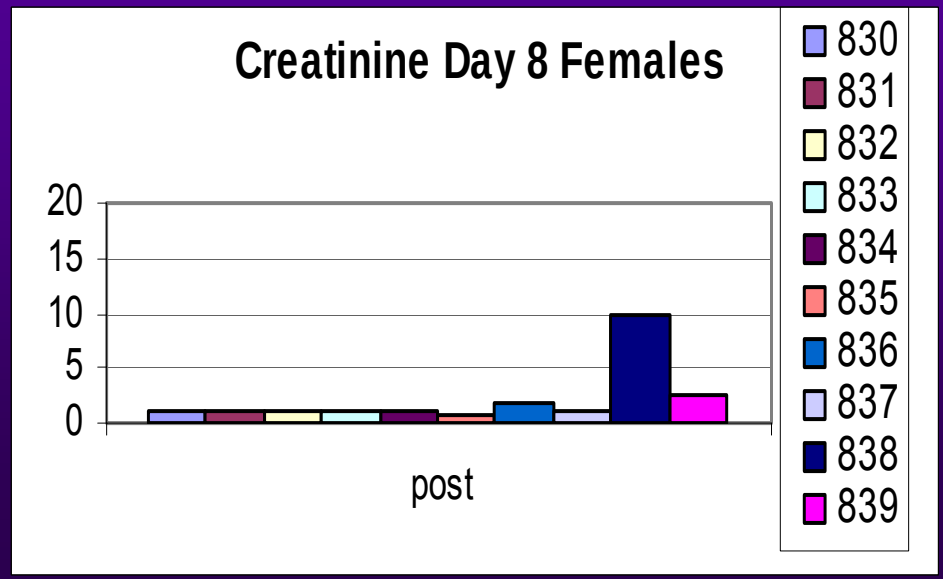



# *Monkey: Urea Nitrogen and Creatinine (Males and Females)*



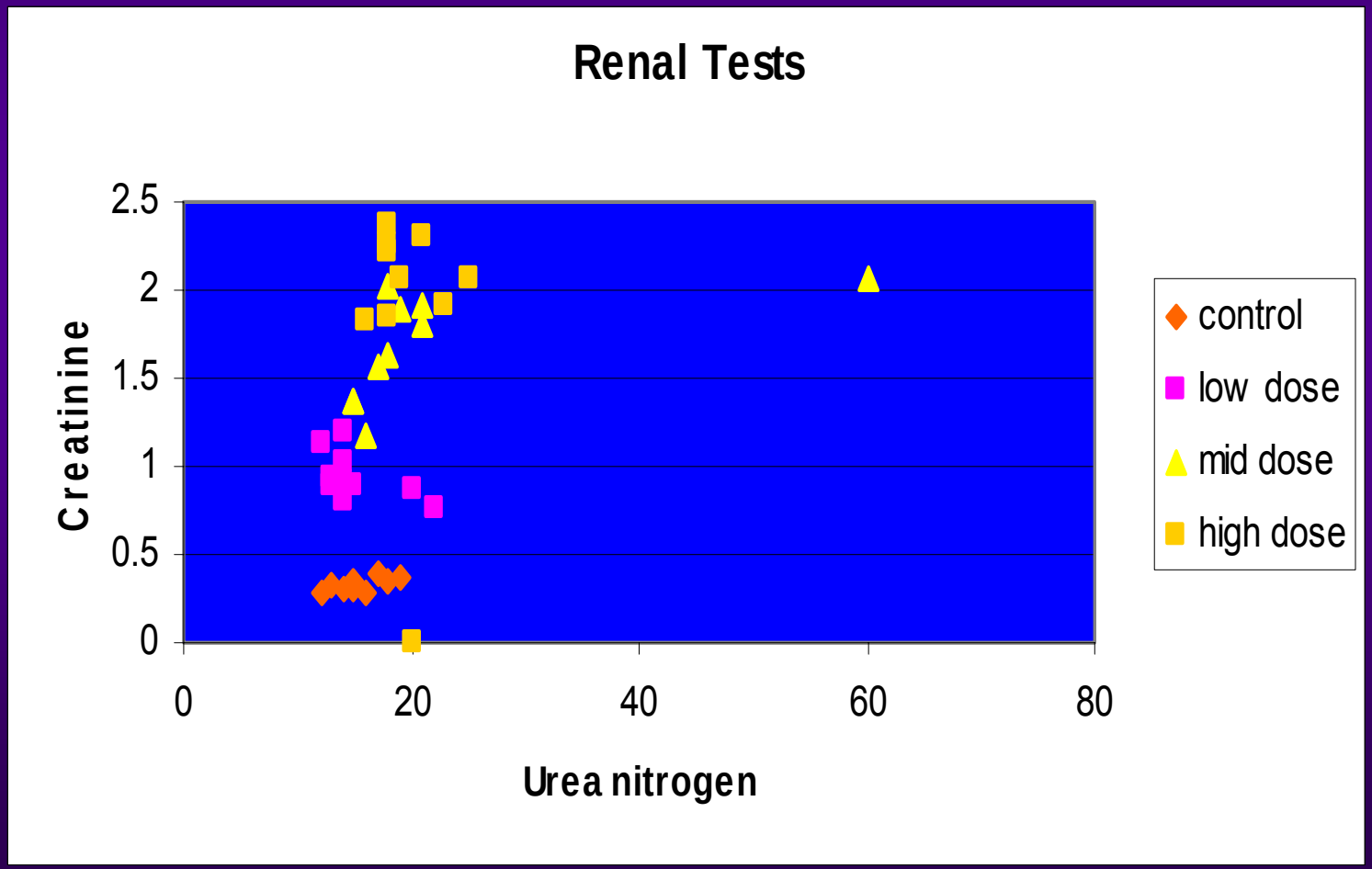



# *Urea Nitrogen and Creatinine: Interpretation*

- Need to know hydration status
  - Urine concentration +/- volume
  - Clinical information also helpful
    - Vomiting/diarrhea/excess urination/decreased water consumption?
    - Clinical assessment?
- Dehydration affects interpretation of other parameters
  - Compound-related decrease in RBC mass?
  - Effects on proteins?

# *Renal parameters*

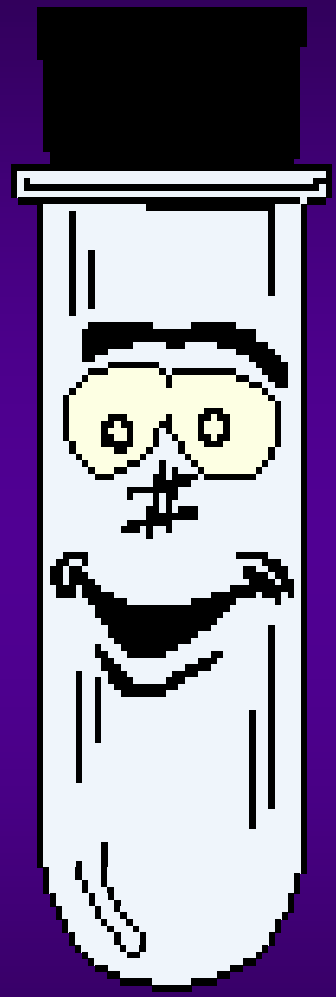


# *Urinalysis*

Volume, Appearance, Specific Gravity, Dipstick, Microscopic, Quantitative Tests

# *Urinalysis*

- Overnight urine collection
  - Very insensitive
  - Sample handling problems
- Free catch urine
  - Spot-measurements of parameters

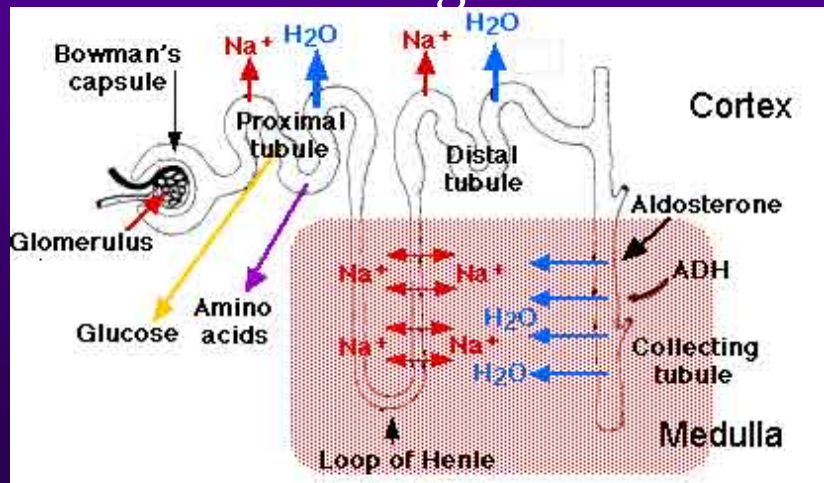


# *Urinalysis: Color and Transparency*

- Color
  - Yellow-amber
  - Abnormal color can affect dipstick results
- Transparency
  - Species differences (cloudy urine in rabbits and horses)

# *Urinalysis: Volume*

- Osmolality during formation of urine
  - Osmolality of glomerular filtrate similar to plasma
  - Obligatory reabsorption of water in the PT
  - Osmolality increases in descending loop of Henle
  - Osmolality decreases in the ascending loop of Henle
  - Osmolality increases in the distal and collecting tubules (ADH effects)




http://www.columbia.edu/cu/biology/courses/w2501/kidney_notes.html

# *Urine Volume and Specific Gravity*

- Generally urine volume and specific gravity are inversely proportional
- Increased urine volume: need to identify primary (underlying) cause
  - Increased water consumption
  - Increased urination
  - Inability to concentrate (diuretic effect)
- Decreased urine volume
  - Decreased water consumption
  - Increased fluid loss (other sites)
  - Obstruction to outflow (check urinary bladder)

# *Dipstick Tests: Protein*

- Protein test
  - Measures mostly albumin
  - False + in alkaline urine
- Proteinuria
  - Hemorrhage (+ blood test)
  - Inflammation (leukocytes microscopically)
  - Renal disease (glomerular disease, tubular disease)

# *Dipstick Tests: Bilirubin*

- Conjugated bilirubin: passes through the glomerulus
- Unconjugated bilirubin is bound to albumin and does not reach filtrate
- Low renal threshold for bilirubin
  - Bilirubinuria occurs before bilirubinemia
  - Threshold especially low for male dogs
- Bilirubinuria indicates presence of conjugated bilirubin in blood (obstruction)

# *Dipstick Tests: Glucose*

- Freely filtered by glomerulus
- Completely resorbed in the PCTs unless transport maximum exceeded
- Glucosuria
  - Hyperglycemia: exceeding transport maximum
    - Excited cats
    - Diabetics
  - Normoglycemia: tubular disease
    - Fanconi's syndrome (transport defect)
    - Other renal tubular damage)

# *Inaccurate Urine Dipstick Tests*

- Urine leukocyte test
  - Literature says specific, not sensitive
  - Unpublished data in dogs: sensitive, not specific
- Nitrites
  - Test for bacteria
  - Not useful in laboratory animals due to collection methods
- Urine specific gravity
  - Use refractometer method, not dipstick

# *Microscopic Examination of Urine*

- Epithelial cells
- Erythrocytes
- Leukocytes
- Casts
- Bacteria
- Crystals
- Sperm

# *Serum/Plasma Electrolytes (Na+, K+, Cl-, TCO2)*

- Spurious increases
  - Small sample volume (evaporation)
  - Incorrect anticoagulant
- Increased K+
  - Hemolysis (rats, mice, humans, but not dogs)
  - Thrombocytosis (serum vs. plasma)
- Dehydration
  - Increased or decreased Na, Cl

www.istat.com

# *Electrolytes (Na+, K+, Cl-, TCO2)*

- Individual animal patterns important for acid/base
- Correlative changes in clinical signs
  - Gastric distension, diarrhea

# *Determination of Proteins*

- Total protein (measured)
  - Albumin (measured)
    - Synthesized by liver
    - Carrier molecule, oncotic pressure
  - Globulins (calculated)
    - Synthesized by liver (fibrinogen, other acute phase and carrier proteins)
    - Synthesized by lymphocytes (immunoglobulins)

# *Proteins*

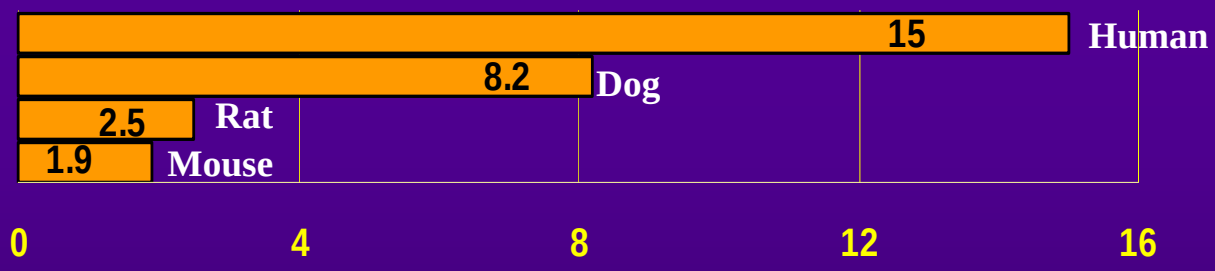
- Changes in protein concentrations can be due to changes in
  - Absorption (malabsorption)
  - Change in synthesis (hepatic disease, acute phase response)
  - Change in half-life (acute phase response)
  - Consumption (clotting cascade)
  - Loss (hemorrhage, renal, GI, third space)

# *Proteins*

- Albumin and globulin regulated individually
  - Albumin: decreases with inflammation
  - Globulin: increases with inflammation
- Important to interpret the changes separately

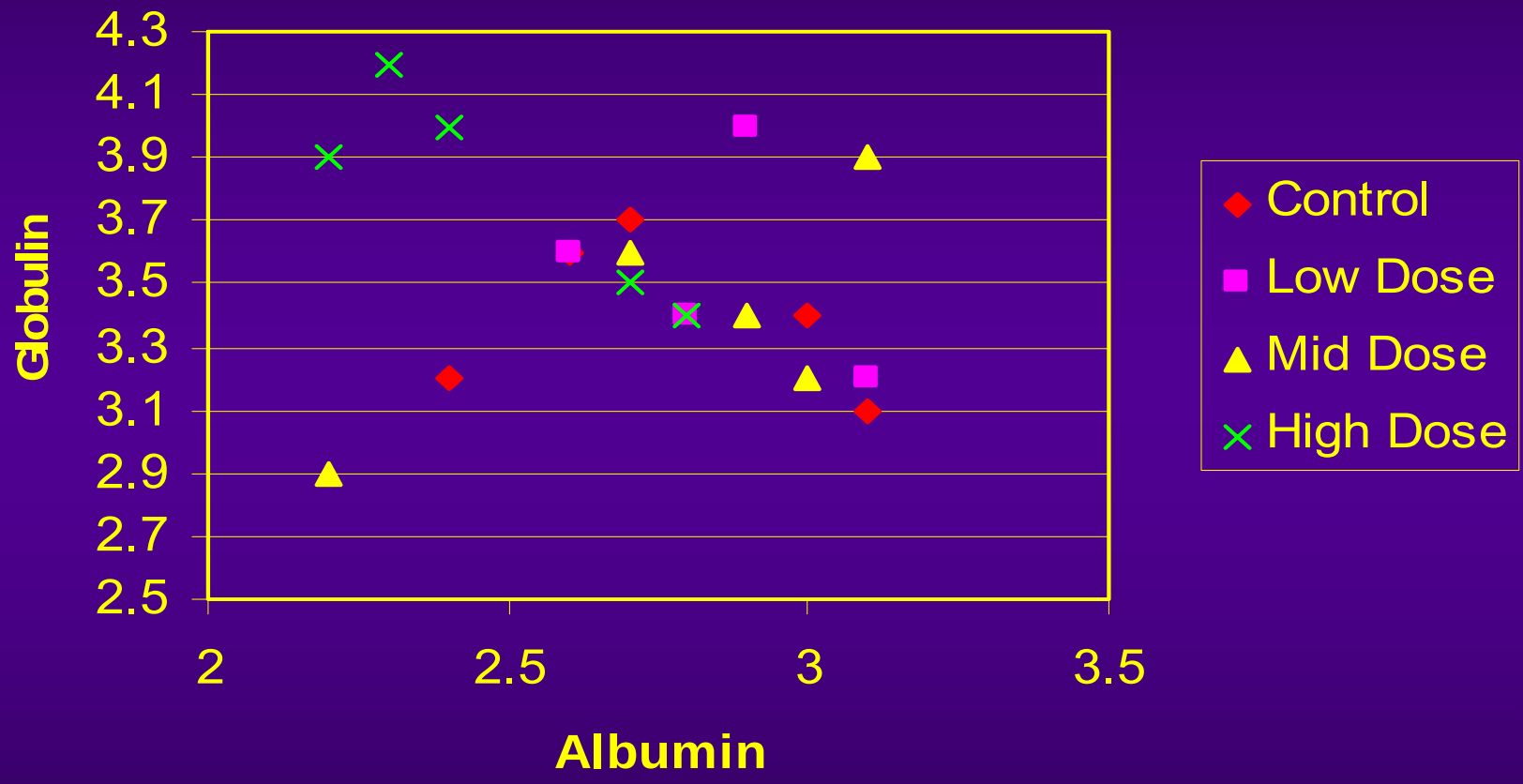
# *Total Protein, Albumin, and Globulin*

- Short half-life: more rapid effects
- Analagous to RBCs and retics



| Process | Total Protein | Albumin | Globulin |
|---|---|---|---|
| Dehydration | ↑ | ↑ | ↑ |
| Loss of high-protein fluid (GI tract, kidneys) | ↓ | ↓ | ↓ |
| Dehydration and loss | ↑or ↓ | ↑or ↓ | ↑or ↓ |
| Inflammation | Usu. ↑ | ↓ | ↑ |
| Inflammation and dehydration | ↑ | N or↑ | ↑ |
| Inflammation and loss | ↑or ↓ | ↓ | N or↓ |
| Inflammation, dehydration, and loss | ? | ? | ? |
| Other additional processes: Decreased synthesis (hepatic), selective loss (glom disease) | ? | ? | ? |

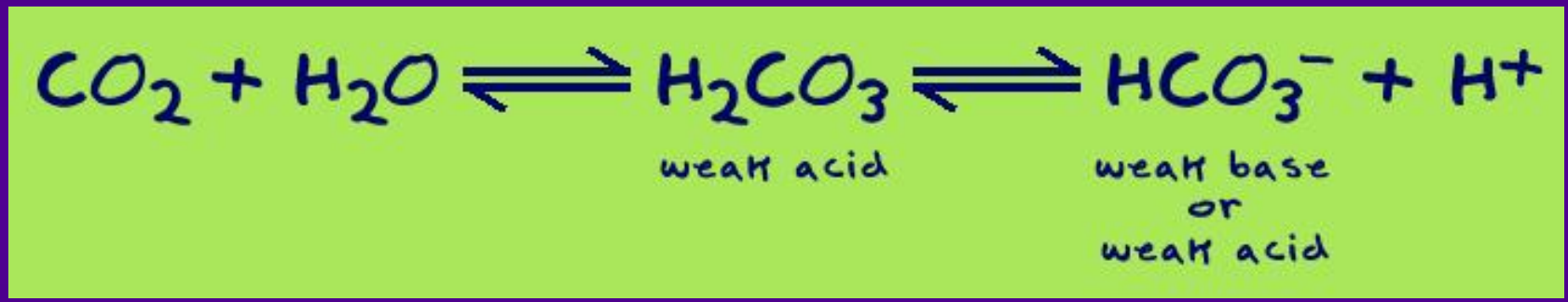
# *Albumin vs. Globulin*



# *Phosphorus*

- Increased serum phosphorus
  - Increased intake
  - Maldistribution (dextrose infusion, tissue injury, metabolic acidosis)
  - Decreased renal excretion (renal failure)
  - Hemolysis
  - Osteolytic lesions
- Decreased serum phosphorus
  - Decreased intake or loss (diet, vomiting, malabsorption)
  - Maldistribution (parenteral feeding, recovery from starvation)
  - Decreased renal loss (decreased reabsorption, proximal diuretics, renal tubular disorders)

# *Acid-Base*



# *Acid Base*

- $TCO_2$ or $HCO_3$-
- Cl-
- Na+
- K+
- Anion Gap
  - Cations (Na+ and K+) minus anions (HCO3- and Cl-)

# *Acidemia/Alkalemia vs. Acidosis/Alkalosis*

- Acidemia: decreased blood pH
- Alkalemia: increased blood pH
- Acidosis and alkalosis: physiologic processes that may result in blood pH changes

# How To Interpret Acid Base Problems

# *General Approach to Uncomplicated Acid-Base Problems*

- Evaluate TCO2 (or $HCO_3$-)
- Evaluate Cl-
- Evaluate Anion Gap

# *Evaluate: TCO2 (or $HCO_3$-)*

- **Condition: Decreased $HCO_3$-**
  - Metabolic acidosis
- Why is $HCO_3$- low?
  - Loss (saliva, intestinal secretions, urine rich in $HCO_3$)
    - Must be compensated by increased Cl- (electroneutrality)
    - Low $HCO_3$-, high-normal to high Cl-
  - Titration (excess of organic ions: ketosis, uremic acids, lactic acidosis, ethylene glycol)
    - Anion Gap is increased because of increased organic anions
    - Normal Cl- but increased Anion Gap

# *Evaluate: TCO2 (or $HCO_3$-)*

- **Condition: Increased $HCO_3$-**
  - Metabolic alkalosis
- Why is $HCO_3$- increased?
  - Loss of HCl (gastric, abomasal)
    - $NaCl + H_2CO_3 >> HCL$ (secreted)$+ NaHCO_3$
    - Vomiting (stomach contents) in monogastric animals
    - Reflux of abomasal contents into rumen
    - Increased $HCO_3$- and decreased Cl-

# *Summary: Metabolic Acidosis/ Alkalosis*

- $HCO_3$- decreased: metabolic acidosis
  - High-normal to high Cl-: loss of $HCO_3$-
  - Normal Cl- and increased AG: titrational (organic acid excess)
- $HCO_3$- increased: metabolic alkalosis
  - Decreased Cl- (loss of HCl-rich fluids)

- $HCO_3$- decreased: metabolic acidosis
  - Cl- high-normal to high: loss of $HCO_3$- rich fluids
  - Cl- normal and AG increased: titrational (organic acid excess)
- $HCO_3$- increased: metabolic alkalosis
  - Cl decreased (loss of HCl-rich fluids)

- Mixed metabolic acidosis/alkalosis
  - Loss of HCl-rich fluids>>hypovolemia and increased $HCO_3$- (alkalosis): low Cl-
  - Hypovolemia>>increased lactic acid
  - Lactic acid buffers $HCO_3$->>normal or slightly increased $HCO_3$-
  - Organic anions>>high AG

# *Take Home Lessons*

- Renal/UA
  - Evaluate renal tests for GFR along with urine volume/concentration/hydration status
- Acid Base
  - Evaluate TCO2 (or $HCO_3$-)
  - Evaluate Cl-
  - Evaluate Anion Gap

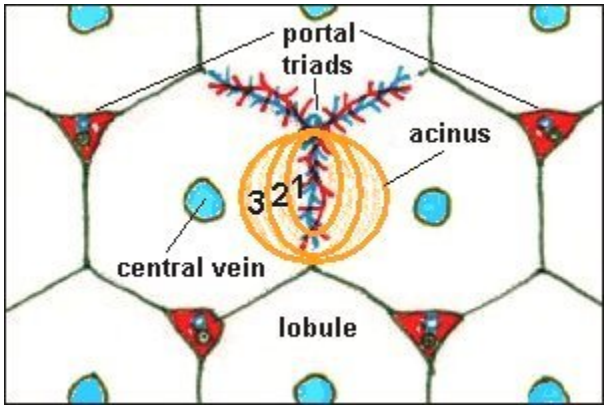
**Topics in Clinical Pathology**
49th Pathology of Laboratory Animals

Dr. Ken Latimer
Department of Pathology
College of Veterinary Medicine
The University of Georgia
Athens, GA 30602-7388

Telephone: (706) 542-5844
E-mail: latimer@vet.uga.edu

## Liver

I. General comments.
   A. Liver performs many important life-sustaining functions.
      1. Hematologic (*e.g.,* lymph formation, coagulation factor synthesis, iron metabolism, recycle RBC products).
      2. Immunologic (*e.g.,* Kupffer cells and interleukin production).
      3. Carbohydrate metabolism (*e.g.,* glycogen storage and catabolism, glucose homeostasis).
      4. Lipid metabolism (*e.g.,* bile acid synthesis and recycling, cholesterol metabolism, fat storage and metabolism, ketones as by products of lipid catabolism.
      5. Protein metabolism (*e.g.,* albumin, acute phase proteins, coagulation factors).
      6. Vitamin activation, storage, and synthesis.
      7. Endocrine hormone metabolism.
      8. Storage function (*e.g.,* glucose, fat, vitamins, minerals/metals).
      9. Digestive function (*e.g.,* bile acids).
      10. Detoxification and excretion (*e.g.,* bilirubin and copper excretion, metabolism of ammonia, cholesterol, hormones, drugs, and toxins).
   B. Hepatic acinus is basic functional unit.
      1. Centered around portal triad.
      2. Blood flows from portal vein and hepatic artery through sinusoids to central vein.
      3. Parenchyma designated as zones 1, 2, and 3.
         a. Zone 1 closest to blood supply (direct-acting toxins).
         b. Zone 3 distant from blood supply (hypoxia, toxic metabolites)
      4. Bile flows through canaliculi in opposite direction to bile duct in portal triad.




C. No pathognomonic signs of liver disease (depression, weight loss, anorexia, vomiting).
D. Goals of laboratory testing.
    1. Identify abnormalities related to hepatobiliary system or its function.
    2. Attempt to classify disease as hepatocellular and/or biliary origin.
    3. Monitor response to treatment or progression of disease.
    4. Offer a clinical prognosis.
E. Liver biopsy may be necessary for definitive diagnosis.

II. Hepatic abnormalities detected by laboratory tests.
A. Hepatocellular leakage or necrosis.
B. Decreased hepatic functional mass (hepatic insufficiency, ~70% of functional hepatocytes lost).
C. Cholestasis.
D. Alterations in portal blood flow.

III. Laboratory tests of hepatobiliary integrity.
A. Common enzymatic assays.
    1. Alanine aminotransferase (ALT).
    2. Aspartate aminotransferase (AST).
    3. Sorbitol dehydrogenase (SDH).
    4. Alkaline phosphatase (ALP, Alk Phos, SAP).
    5. Gamma-glutamyltransferase (GGT, γGT).
B. Hepatic uptake, conjugation, and secretion.
    1. Bilirubin.
    2. Bile acids.
    3. Exogenous anionic dyes (not used very much).
        a. Sulfobromophthalein (BSP) excretion (hard to obtain dye).
        b. Indocyanine green (ICG) excretion (very expensive).
C. Tests of portal blood clearance.
    1. Ammonia (labile).
    2. Bile acids (very stable).
    3. Globulin concentration (intestinal antigens).
D. Evaluation of hepatocellular synthesis.
    1. Glucose.

2. Albumin and A/G ratio.
3. Clotting tests.
4. Bile acids.
5. Cholesterol.

IV. Concepts of basic enzymology.
A. Measure enzymatic activity, NOT enzyme concentration.
B. Report enzyme activity in mU/ml or U/L.
1. Amount of enzymatic activity to catalyze conversion of 1 mmol of substrate or produce 1 mmol of product / minute *under specified conditions*.
a. Temperature (30°C or 37°C).
b. Specific substrate.
c. Ionic strength of buffers, etc.
2. Need reference intervals for specific laboratory!
C. Isoenzymes occasionally exist (ALP, LDH, CK).
1. Multiple forms of an enzyme that have same catalytic specificity but vary in other properties.
a. Heat inactivation.
b. Chemical inhibition.
1). Levamisole and canine ALP.
c. Electrophoretic mobility.
D. Increased enzymatic activity in serum from one or more of the following:
1. Increased cell membrane permeability > leakage.
2. Induce cellular enzyme production.
3. Influence of enzyme ½-life.
4. Decreased enzyme inhibition, catabolism, or removal.
E. Importance of enzyme ½-life in plasma or serum.
1. Enzymes with short ½-life are most sensitive indicators of liver damage and recovery.
a. Enzymes with short ½-life.
1). ALP in cat (6 hours).
2). SDH (<12 hours).
3). Creatine kinase (CK, muscle damage).
b. Enzymes with moderate ½-life.
1). AST (20-36 hours).
2). ALT (40-60 hours).
c. Enzymes with long ½-life.
1). ALP in dog (72 hours).
2). GGT (96 hours).

V. Leakage vs induction of hepatic diagnostic enzymes.
A. "Leakage" enzymes (ALT, AST, SDH).
1. These enzymes are NOT membrane bound.
2. Location within cell determines "leakage" effect.
a. Cytosol (rapid leakage).

1). Alanine aminotransferase (ALT).
b. Cytosol and mitochondria (less rapid leakage).
1). Aspartate aminotransferase (AST).
2). Sorbitol dehydrogenase (SDH).
3. ↑ intracellular enzyme concentration = ↑serum activity.
a. Concentration gradient effect.
4. ↑ number of enzyme-producing cells = ↑ serum activity.
B. "Induction" enzymes are membrane bound (ALP, GGT).
1. Often induced by drugs, especially corticosteroids and anticonvulsants.
2. Induction occurs rapidly (days), peaks over time (3-4 weeks), and declines slowly to baseline (weeks to months).

VI. Specific characteristics of "leakage" and "induction" enzymes.
A. Alanine aminotransferase (ALT, SGPT, leakage).
1. Located in cytosol > leaks readily.
2. Liver specific in dog and cat.
3. [↓] in hepatocytes of other species limits use.
4. ↑ activity correlates with # cells damaged.
a. Doesn't differentiate focal or diffuse liver lesions.
b. Doesn't differentiate reversible vs. irreversible liver damage.
c. Doesn't correlate w/ hepatic insufficiency:
1). ↓ ALT in fibrosis when function tests are abnormal.
2). ↑ ALT in acute injury when function tests may be normal.
5. Serum ½-life = 40-60 hours.
6. Hepatic injury > peaks (1-2 days) > return to baseline (2-3 weeks).
7. Serum enzyme activity varies w/ disease state.
a. 100x w/ toxic insult, severe trauma.
b. 40x w/ bile duct obstruction.
c. 4x w/ anticonvulsants, corticosteroids.
d. ↑ ALT mild to absent w/ fatty liver.
e. Variable ↑ w/ trauma.
8. Prognostic considerations.
a. 50% ↓ in ALT over 1-2 days after acute injury = favorable prognosis.
b. ↓ ALT may indicate unfavorable prognosis in.
1). Massive necrosis w/ exhausted ALT supply.
2). Chronic hepatic fibrosis w/ loss of functional hepatocytes.
B. Aspartate aminotransferase (AST, SGOT, leakage).
1. Located in cytosol and mitochondria.
a. More severe damage to release mitochondrial form.
2. Similar considerations as ALT.
3. Not liver specific.
a. Cardiac muscle.
b. Skeletal muscle.

c. RBCs (hemolyzed blood sample).

4. Use in conjunction w/ CK in large animals.
    a. Hepatic vs muscular origin of enzymatic activity.
5. More sensitive than ALT but lacks specificity.

C. Sorbitol dehydrogenase (SDH, leakage).
1. Cytosol and mitochondria.
2. Liver specific.
    a. Used in large animals and rodents.
    b. No advantage over ALT in small domestic animals.
3. Short ½-life (<12 hours).
    a. Can't mail samples to D-Lab for analysis.
4. Returns to baseline rapidly after injury (4-5 days).
    a. Normalizes before AST.
    b. May normalize before animal is presented.

D. Alkaline phosphatase (ALP, SAP, Alk Phos, induction).
1. Membrane bound.
2. High sensitivity but low specificity.
3. Broad reference interval makes ALP useless in large animals.
4. Various isoenzymes produced.
    a. Hepatic.
        1). Most of serum activity.
        2). INHIBITED BY LEVAMISOLE (>90%).
        3). Induced by the following:
            a). Cholestasis (50-100x).
            b). Anticonvulsants (4-6x).
            c). Corticosteroids (early).
        4). Adequate marker of cholestasis in FASTED rats.
    b. Steroidal.
        1). ONLY IN DOG.
        2). Endogenous and exogenous corticosteroids.
        3). Appears ≈ 30 days w/ constant corticosteroid exposure.
        4). NOT INHIBITED BY LEVAMISOLE (<10%).
    c. Bone.
        1). ↑ osteoblastic activity.
        2). Young growing animals (3-6x).
        3). Metabolic bone disease.
        4). Bone neoplasms.
        5). INHIBITED BY LEVAMISOLE
    d. Intestinal.
        1). Short ½-life (3-6 minutes).
        2). Major source of serum ALP in nonfasted rats.
    e. Renal cortex.
        1). Short ½-life (3-6 minutes).
    f. Placenta.
        1). Third trimester of pregnancy.

E. Gamma-glutamyltransferase (GGT, induction).

1. "Normal" serum activity from liver.
2. Similar to ALP in small animals.
    a. Induced by corticosteroids (9x w/in 15 days).
3. Used as marker of cholestasis in large animals.
    a. Narrower reference interval.
4. Transferred via colostrum to pups and calves.
    a. ↑ GGT activity w/ colostrum ingestion (1,000x).
5. Renal tubular lesions.
    a. Increased GGT activity in URINE.
    b. $U_{GGT}/U_{Creatinine} > 25$ .

VII. Tests of hepatic uptake, conjugation, and secretion.

A. Bilirubin (losing popularity).

1. Suspicion of hyperbilirubinemia.
    a. Clinical jaundice or icterus (2-3 mg/dl).
    b. Yellow plasma (1.5 mg/dl).
        1). ß carotenes in adult horses & ruminants.
    c. Biliprotein or δ-bilirubin causes persistence of hyperbilirubinemia because bilirubin irreversibly bound to albumin.
2. Methods of measurement.
    a. Serum (mg/dl) measurement is quantitative.
        1). Conjugated (direct) bilirubin measured first.
        2). Total bilirubin measured after alcohol added.
        3). (T-bili) - (C-bili) = Unconjugated (or indirect) bilirubin.
    b. Urine measurement is qualitative.
        1). Bilirubin (conjugated).
    c. Exposure to light ↓ [bilirubin] 50% / hour.

3. Overview of bilirubin metabolism in health and disease.

a. Bilirubin production and excretion in health

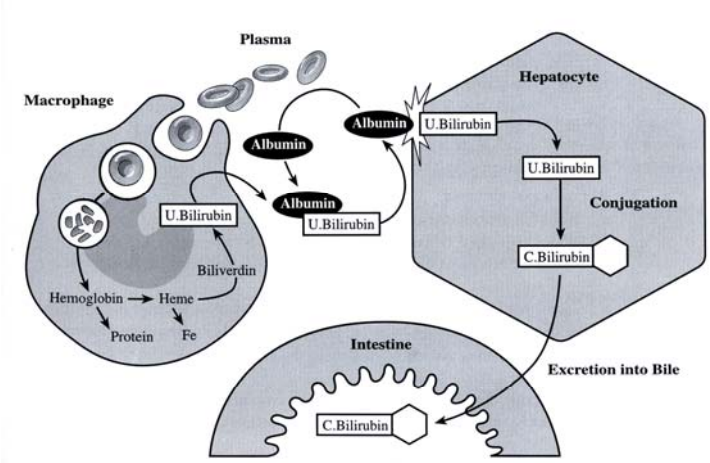



**Fig. 7.1.** Bilirubin metabolism. Bilirubin is produced as a breakdown product of hemoglobin. The unconjugated bilirubin (U. Bilirubin) is transported in the blood (ionically bound to albumin) to the liver. Unconjugated bilirubin is taken up by hepatocytes, conjugated (C. Bilirubin), and excreted into bile.

b. Bilirubin excretion in health and disease.

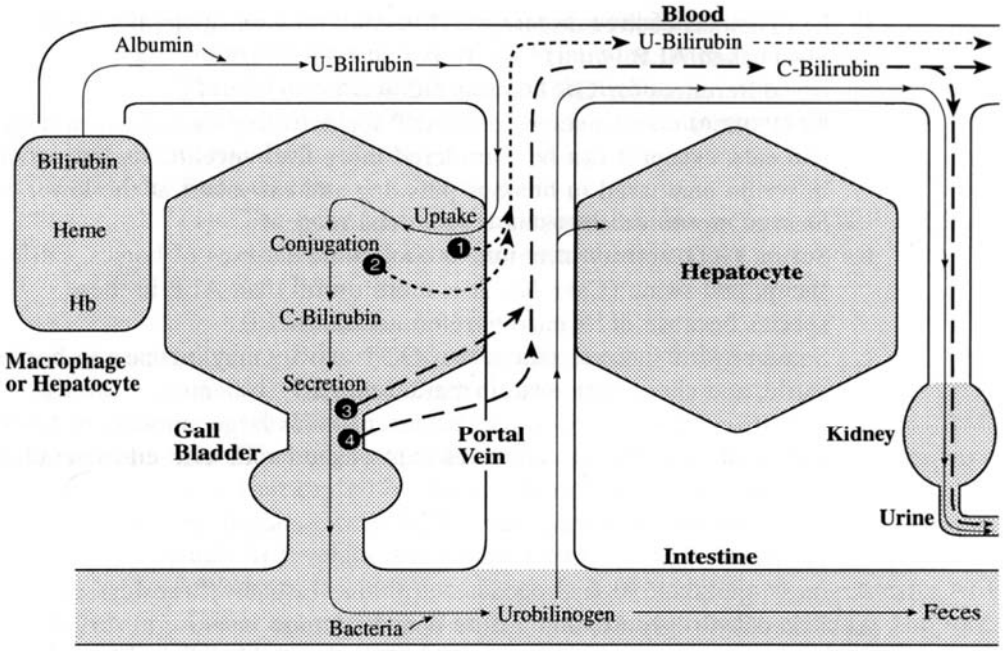



**Fig. 7.1. Bilirubin excretion. Small solid lines depict the normal excretion pathway. Figures in black circles indicate abnormalities in excretion: (1) failure of uptake, (2) failure of conjugation, (3) failure of secretion, and (4) cholestasis. The small dashed line indicates unconjugated hyperbilirubinemia (U-bilirubin), and the large dashed line indicates conjugated hyperbilirubinemia (C-bilirubin) and bilirubinuria.**

4. Mechanisms of hyperbilirubinemia.
    a. Retention of bilirubin.
        1). ↑ production.
        2). ↓ hepatocellular uptake.
        3). ↓ hepatocellular conjugation.
    b. Cholestasis or "regurgitation."
        1). Obstruction of bile flow.
            a). Extrahepatic obstruction.
            b). Intrahepatic obstruction.
5. Clinical interpretation of bilirubin data.
    a. Unconjugated hyperbilirubinemia.
        1). EARLY hemolysis or massive internal bleeding.
        2). Normal ALP, GGT.
        4). Lack of bilirubinuria.
        5). U-Bili predominates in horses and ruminants no matter what the cause of disease.
        6). Anorexia in horses causes hyperbilirubinemia.

    b. Conjugated hyperbilirubinemia.
        1). COMMON in hepatocellular disease and obstruction of bile flow (except large animals).
        2). ↑ ALP, GGT.
        3). C-Bili water soluble > urine.
            a). Bilirubinuria precedes hyperbilirubinemia.
            b). Trace bilirubin in concentrated dog urine.
            c). Bilirubinuria abnormal in other species.
    c. U-Bili and C-Bili cannot reliably distinguish hemolysis from intrahepatic and extrahepatic diseases!
        1). Limits usefulness of bilirubin determination.
        2). Use Hct, ALP, GGT, and other liver function tests.

B. Bile acids (very popular).
    1. General comments.
        a. Bile acids are stable compounds.
        b. Good test for practitioner.
        c. Results easier to interpret than bilirubin.
    2. Test method and procedure.
        a. Spectrophotometric assay most popular.
        b. Collect fasting and 2-hour postprandial blood specimens.

3. Overview of bile acid metabolism and enterohepatic recirculation.

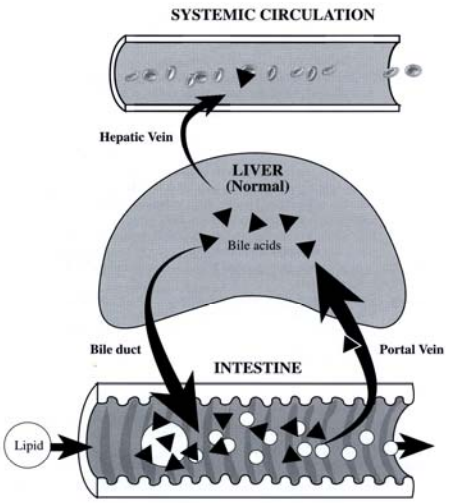



**Fig. 7.2a.** Bile acid metabolism. (a) Bile acids are produced by hepatocytes and delivered via the bile duct to the intestine, where they aid in lipid digestion. Most of the bile acids are subsequently absorbed from the intestine by the portal circulation. In normal animals, most of the portal blood bile acids (approximately 70–95%) are removed by hepatocytes and re-excreted into bile (enterohepatic recirculation).

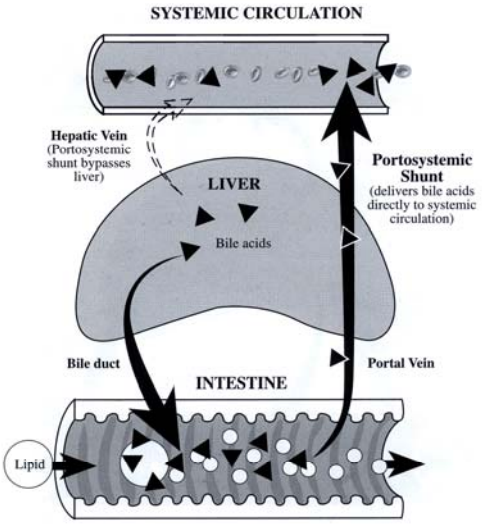



**Fig. 7.2b.** Bile acid metabolism (continued). (b) Portosystemic shunts bypass the liver and deliver bile acids directly from the portal blood to the systemic circulation. Bile acid removal by hepatocytes from the systemic circulation is less efficient than uptake from the portal circulation, and serum bile acid levels increase.

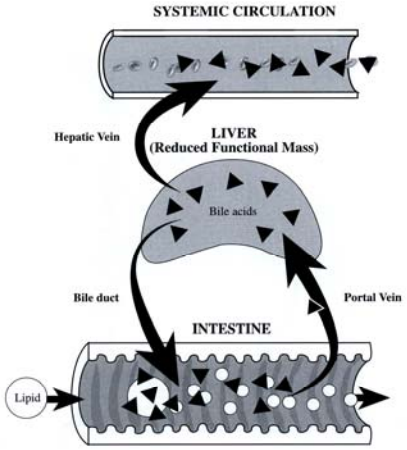



**Fig. 7.2c.** Bile acid metabolism (continued). (c) With reduced hepatic functional mass, removal of bile acids from portal blood is less efficient, resulting in increased serum bile acid levels.

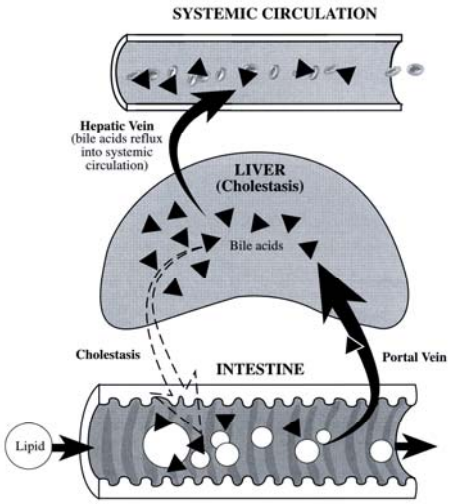



**Fig. 7.2d.** Bile acid metabolism (continued). (d) In cholestatic disease, bile acids reflux into the systemic circulation, increasing serum bile acid levels.

4. Interpretation of bile acid test results.
    a. Increased bile acid concentration.
        1). ↓ hepatic uptake (↓ functional mass).
        2). Failure of bile acid conjugation.
        3). ↓ bile acid secretion and cholestasis.
        4). Intra- or extrahepatic shunting of portal blood > bypasses hepatocytes.
    b. Decreased bile acid concentration.
        1). Difficult to detect b/c recycled.
    c. Dehydration, hypovolemia, and chronic passive congestion have little effect on [SBA].

C. Cholephilic dye excretion tests (BSP, ICG).

1. General comments.
    a. Not performed very frequently.
    b. BSP difficult to obtain.
    c. ICG extremely expensive.
    d. Technique measures hepatic dye uptake.
2. Test procedures.
    a. Measure dye retention after 30 min (dog and cat).
    b. Measure half-time for disappearance ($T_{½}$) (large animals).
3. Problems in interpreting dye retention or clearance.
    a. Anasarca, ascites, obesity = ↓ retention.
    b. Hypoalbuminemia = ↓ retention.
    c. Decreased hepatic blood flow = ↑ retention.
    d. Decreased functional mass (55%) = ↑retention.
    e. Hyperbilirubinemia = ↑ retention.
    f. Albumin-binding drugs = ↑ retention.
    g. Portosystemic shunt = no effect unless hepatic atrophy.
    h. Too cumbersome for routine clinical use!

VIII. Tests for portal blood clearance.

A. Ammonia (cerebral toxin that can be quantitated).

1. Largely replaced by serum bile acid measurement.
2. Clinical suspicion of hepatic encephalopathy.
    a. Excitability, tremors.
    b. Compulsive walking, head pressing, blindness.
    c. Coma, convulsions, stupor.
    d. $NH_3$ toxic, $NH_4^+$ nontoxic (not absorbed).
3. Test method (8-12 hour fast).
    a. Baseline blood ammonia concentration.
        1). Ammonia-free heparin.
        2). Separate plasma from cells immediately.
        3). Store on ice & test w/in 1 hour.
        4). Freeze @ -20°C, test w/in 48 hours.
        5). Venous occlusion = ↑ $NH_3$.
        6). Difficult to perform in private practice.
    b. Ammonia tolerance test.
        1). NOT INDICATED W/ HIGH BASELINE AMMONIA!
        2). Give $NH_4Cl$ > collect sample in 30 minutes.
        3). 3-10x increase over baseline.

4. Overview of ammonia metabolism.

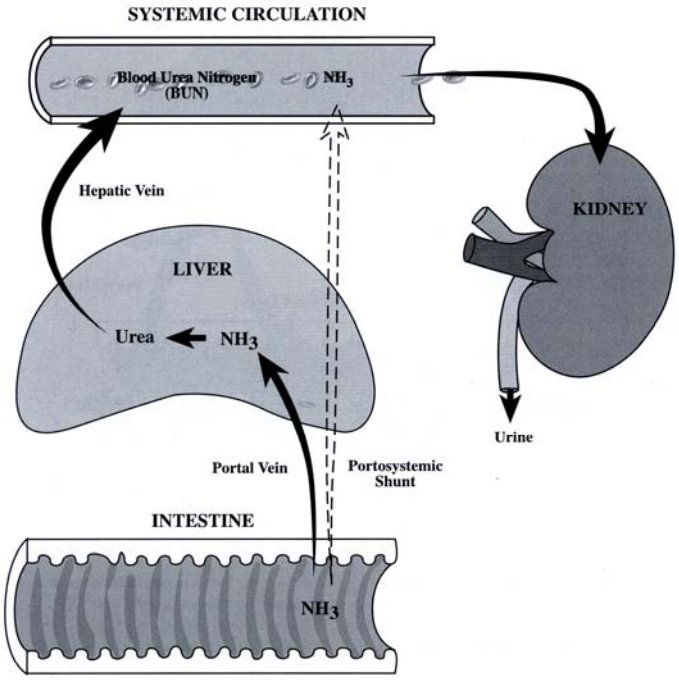



**Fig. 7.3.** Ammonia metabolism. Ammonia is produced in the gastrointestinal tract by enteric microflora (as well as amino acid metabolism in cells) and transported to the liver by the portal circulation. Most of the ammonia is converted to urea in the liver. The urea enters the systemic circulation (blood urea nitrogen or BUN) and is excreted in urine. With portosystemic shunting of blood, ammonia bypasses the liver and enters the systemic circulation, resulting in hyperammonemia (dashed arrow).

5. Interpretation of ↑ plasma ammonia.
    a. Hepatic insufficiency (COMMON).
        1). ↓ BUN may be present.
        2). 60-70% ↓ in hepatic functional mass.
    b. Portosystemic shunts (COMMON).
        1). Congenital or acquired.
        2). Lack of hepatotrophic factors > hepatic atrophy and insufficiency.
        3) Ammonium biurate crystals in urine sediment.
    c. Congenital urea cycle enzyme deficiency (RARE).
    d. Urea toxicosis of ruminants (INFREQUENT).

B. Increased globulin concentration (polyclonal).
1. ↓ Kupffer cell mass.
2. Enteric antigens to systemic circulation.
3. Increased globulin production (β-γ bridging).

IX. Tests of hepatic synthesis.
- A. Glucose.
  - a. > 80% liver nonfunctional.
  - b. Fasting hypoglycemia.
  - c. Prolonged postprandial hyperglycemia.
- B. Albumin and A/G ratio.
  - a. ≈ 80% loss of functional hepatic mass.
  - b. Seen in chronic (not acute) liver disease.
- C. Clotting tests.
  - a. Hepatic synthesis of factors II, VII, IX, and X.
  - b. Vitamin K-dependent carboxylation of glutamic acid resides for activity.
  - c. 30% decrease in [factor] = abnormal clotting.
- D. Bile acids.
  - a. Enterohepatic recirculation > recycled.
- E. Cholesterol.
  - a. Decreased synthesis.

## Simplified Approach to Hemostasis

I. Adequate hemostasis depends upon the following.
- A. Vascular integrity.
- B. Platelet number and function.
- C. Clotting factors (including accelerators and inhibitors).

II. Vascular injury = lack of integrity.
- A. Apparent vascular injury.
  1. Trauma (accidental, elective surgery).
  2. Local injury (gastric ulcer) vs widespread injury (septicemia).
  3. Diseased vessels (vasculitis, necrosis)
- B. Inapparent vessel injury (purpura).
  1. Vascular purpura
     - a. Scurvy = abnormal collagen cross-linking (guinea pigs)
     - b. Equine purpura hemorrhagica
  2. Platelet deficiency.
     - a. Number (thrombocytopenia).
     - b. Function.
  3. Coagulation factor deficiencies.
     - a. Hereditary (usually a single factor).
     - b. Acquired (often multiple factors).
- C. Post thrombosis.
  1. Thromboembolism.
  2. Vascular parasites (filarids).
  3. Amyloidosis
  4. Sepsis.
  5. Neoplasia.

III. Megakaryocytes and platelets.
- A. Adequate number and function for clotting.
- B. Megakaryocytes.
  1. Originate from CD 34+ myeloid cells.
  2. Express gycoprotein IIb/IIIa, glycoprotein Ib, and acetylcholinesterase.
  3. Growth supported by thrombopoietin, stem cell factor, IL-3, IL-6, IL-11, and leukemia inhibitory factor.
  4. Contain α-granules (precursors of dense granules), microfilament network, and microtubular network.
  5. Production of platelets regulated by platelet mass (not platelet number).
- C. Platelets.
  1. Lifespan varies from 3 to 10 days.
  2. 25% to 30% of platelets reside in splenic red pulp.
  3. Young ("shift") platelets have a larger mean platelet volume.
- D. Thrombocytes.
  1. Nucleated cells with platelet and phagocytic functions.
  2. Found in birds, reptiles, amphibians, and fishes.
- D. Mechanisms of thrombocytopenia include the following:
  1. Artifactual (sample drawn from IV line or aggregation from poor venipuncture).
  2. Decreased production (bone marrow lacks megakaryocytes).
  3. Increased consumption (plugging leaks in vasculature).
  4. Increased destruction (immune-mediated thrombocytopenia).
  5. Increased sequestration in spleen (difficult to prove).

IV. Coagulation factor deficiencies.
- A. Acquired.
  1. Multiple factors involved.
  2. Especially disseminated intravascular coagulation/fibrinolysis and rodenticide ingestion..
- B. Hereditary.
  1. Single factor involved.
  2. Lack of production or nonfunctional protein.

V. Nomenclature of clotting factors.

| | |
|---|---|
| I | Fibrinogen. |
| II | Prothrombin. |
| III | Tissue thromboplastin / tissue factor. |
| IV | Calcium. |
| V | Proaccelerin. |
| VI | Doesn't exist. |
| VII | Proconvertin. |
| VIII | Hemophilia A (antihemophilic factor). |
| IX | Hemophilia B (Christmas factor). |
| X | Stuart-Prower factor. |

XI Plasma thromboplastin antecedent (PTA).
XII Hageman factor.
XIII Fibrin stabilizing factor.
--- Prekallikrein / Fletcher factor.
--- High molecular weight kininogen (HMWK) / Fitzgerald factor.

VI. Guide to commonly used coagulation tests.

A. Bleeding time.
1. Time (min) for bleeding to cease from standardized incision.
2. Evaluates formation of platelet plug and depends upon:
a. Adequate number of platelets.
1). Prolonged if < 75,000 platelets / μl.
b. Adequate function of platelets.
1). Prolonged with aspirin, NSAIDs.
2). Prolonged with ↓ von Willebrand's factor.
3). Prolonged with ↑ FDPs.
4). Prolonged with uremia.
c. Normal vessel wall structure.
1). Prolonged with vitamin C deficiency (*e.g*, scurvy in guinea pigs).

B. Activated clotting time (ACT).
1. Time (sec) for whole blood to clot in glass tube w/ contact activator at 37°C.
2. Evaluate intrinsic and common systems.
3. Platelets provide phospholipid.
a. Prolonged ACT if < 10,000 platelets /μl.
4. Relatively insensitive - detects 5% or less factor activity.
a. Prolonged ACT w/ intrinsic or common system factor deficiency.

C. Activated partial thromboplastin time (APTT).
1. Time (sec) for clot formation in citrated plasma after addition of contact activator, phospholipid and calcium.
a. Platelets have no effect on test.
2. Evaluate intrinsic and common systems.
3. More sensitive than ACT.
a. Detects 30% or less factor activity in intrinsic and common systems.
b. Can use modification of APTT to document specific factor deficiency.

D. One-stage prothrombin test (OSPT).
1. Time (sec) for clot formation in citrated plasma after addition of thromboplastin and calcium.
a. Platelets have no effect on test.
2. Evaluate intrinsic and common systems.
3. Detects 30% or less factor activity in extrinsic and common systems.
4. *Factor VII has shortest half life.*

E. Thrombin clotting time (TCT).
1. Time (sec) for clot formation in citrated plasma after addition of thrombin and calcium.
2. Most sensitve measure of fibrinogen concentration.

a. Evaluates last step in common system.

3. Prolonged TCT with.
   a. Hypofibrinogenemia (DIC).
   b. Increased FDPs (compete w/ fibrinogen for thrombin binding).
   c. Heparin treatment.

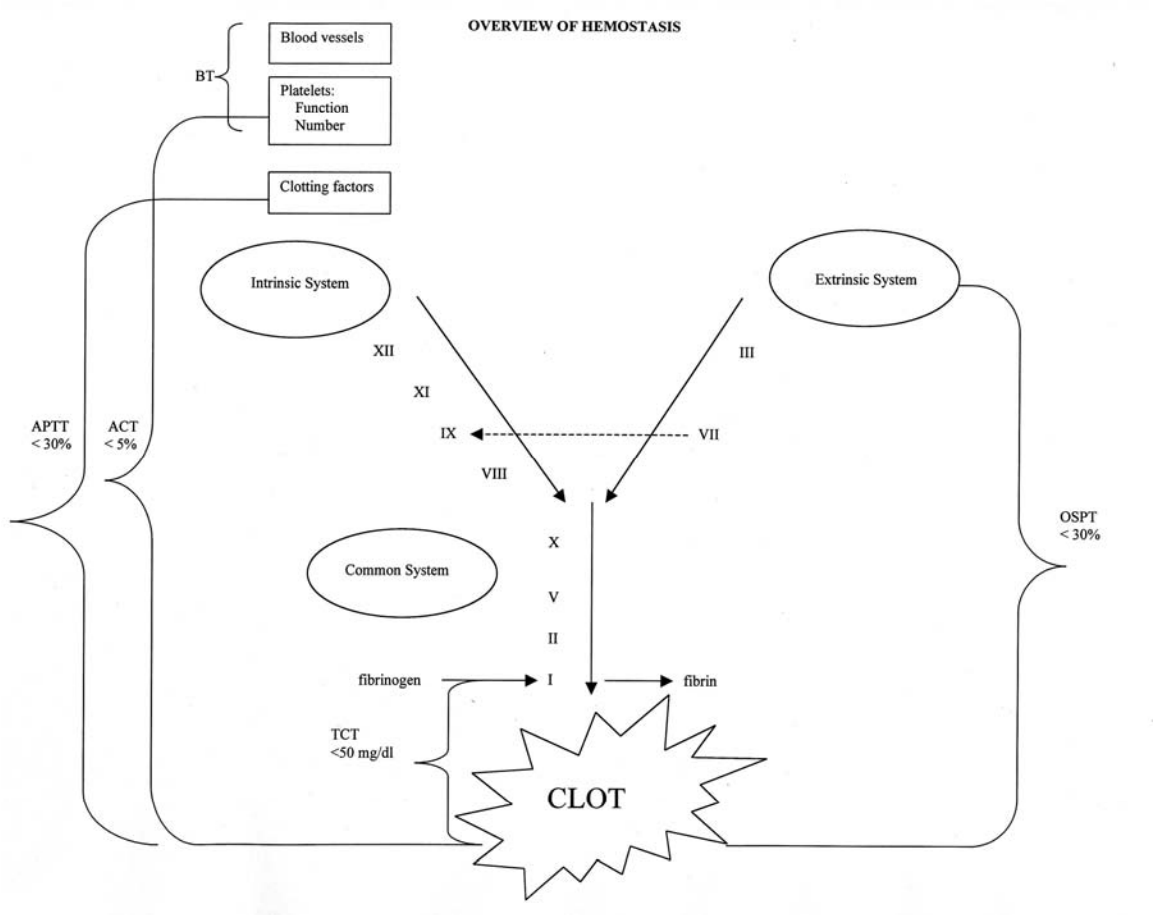
F. Fibrin(ogen) degradation products (FDPs) and D-dimers.

1. FDPs may be derived from fibrin clot lysis and/or fibrinogen cleavage.
2. D-dimers are derived from lysis of cross-linked fibrin clots.

G. PIVKA

1. Proteins induced by vitamin K absence or antagonism.
2. Inactive proteins, antigenically similar to factors II, VII, IX, and X.
3. Increased PIVKA before prolonged PT or APTT.
4. Vitamin K epoxide reductase > inhibited > vit K epoxide concentration Increases.
5. Direct ELISA measurement of PIVKA II (decarboxyprothrombin).
6. Indirect measurement of PIVKA using OSPT and APTT with a 30:1 dilution of citrated plasma to increase test sensitivity.
7. Rodenticide toxicosis (animals) or monitor coumarin therapy (people).

VII. Simplified coagulation cascade.




APTT = activated partial thromboplastin time.
ACT = activated clotting time.
OSPT = one-stage prothrombin time.
TCT = thrombin clotting time.

VIII. Expected hemostasis results in various conditions.

| **Condition** | **Plts** | **BT** | **ACT** | **APTT** | **OSPT** | **TCT** | **Fib** | **FDP's** |
|---|---|---|---|---|---|---|---|---|
| Hemophilia A | N | N | N,↑ | ↑ | N | N | N | N,↑ |
| Hemophilia carrier | N | N | N | N | N | N | N | N |
| Von Willebrand's | N | ↑ | N,↑ | ↑ | N | N | N | N |
| DIC | ↓ | ↑ | ↑ | ↑ | ↑ | ↑ | ↓ | ↑ |
| Early Vit K inhib | N | N | N | N | ↑ | N | N | N |
| Late Vit K inhib | N | N | ↑ | ↑ | ↑ | N | N | N |
| Severe TCP | ↓ | ↑ | ↑ | N | N | N | N | N |
| Heparin treatment | N | N | ↑ | ↑ | ↑ | ↑ | N | N |

Plts = platelet count.
BT = bleeding time.
ACT = activated clotting time.
APTT = activated partial thromboplastin time.
OSPT = one-stage prothrombin time.
TCT = thrombin clotting time.
Fib = fibrinogen.
FDP's = fibrin / fibrinogen degradation products.

IX. Tips on interpreting hemostasis profiles.

A. Vascular abnormalities most common with the following:
1. Trauma.
2. Endothelial damage.
3. Guinea pigs with scurvy.

B. Prolonged bleeding time most common with the following:
1. Altered platelet function.
a. Aspirin, NSAIDs.
b. von Willebrand's disease (lack vWF and factor VIII).
2. Thrombocytopenia.

C. Activated bleeding time (ACT) commonly prolonged with the following:
1. Thrombocytopenia (< 75,000 platelets / µl).
2. Severe coagulation factor deficiency (<5% activity remaining).

D. Hereditary factor deficiencies with clinical bleeding.
1. Usually lack one factor, frequently factor VIII (hemophilia A, von Willebrand's) or factor IX (hemophilia B).

E. Acquired factor deficiencies with clinical bleeding.
1. Usually are multiple factor deficiencies.
a. DIC.
1). Consumption of nonenzymatic factors V , VIII, and fibrinogen (factor I).
2). Consumption of platelets (thrombocytopenia).
3). Production of FDP's.
b. Rodenticide toxicosis (warfarin, diphacinone).
1). Factors II, VII, IX, and X not functional.
2). OSPT abnormal first b/c factor VII has shortest ½ life.
3). OSPT and APTT prolonged in established toxicosis.
4). TCT normal because add thrombin (factor IIa) during test.
5). PIVKA test may detect before clotting abnormalities.

X. More recent hereditary abnormalities of hemostasis in laboratory animals.

A. RIIIS/J inbred mice.
1. von Willebrand's disease Type IA (Blood. 1990;76:2258-2265).
a. Prolonged bleeding time w/ normal platelet count.
b. Normal platelet aggregation/agglutination with collagen and

ristocetin.

B. Wistar and Wistar Furth rats.
    1. Alpha granule defect (gray platelet syndrome) in WF rats (Blood. 1998;91:1599-1608).
        a. Platelets and megakaryocytes have defect.
        b. Prolonged bleeding time (>30 minutes) with defective clot formation.
        c. Hereditary macrothrombocytopenia common.
    2. Decreased glycoprotein IIb/IIIa (GPIIb/IIIa) antigen in Wistar rats (J Lab Clin Med. 1996;128:601-611).
        a. Prolonged bleeding time.
        b. Absence of platelet aggregation ADP and thrombin.
        c. Probable autosomal dominant inheritance.
        d. Unique thrombopathy with some characteristics of variant Glanzmann's thrombasthenia.
    3. Macrothrombocytopenia in Wistar Furth rats (Blood. 1988;71:1676-1686).
        a. Average platelet count is approximately one-third that of other rat strains.
        b. Average megakaryocyte concentration was 30% lower
        c. Recessive inheritance pattern.

C. Primates
    1. Severe type-3 vWD (Comp Med. 52:368-371, 2002).
        a. Young male rhesus monkey (*Macaca mulatta*).
        b. Excessive bleeding from minor wounds.
        c. No detectable or functional vWF.
        d. Low factor VIII activity.
        e. Moderate prolongation of APTT.
        f. Whole blood transfusion to control hemorrhage.

# PATHOLOGY OF NONHUMAN PRIMATES

Gary B. Baskin, DVM
Veterinary Pathologist
Sierra Biomedical – A Charles River Company
gbaskin@sbi.criver.com

## COMMON BACKGROUND CHANGES IN MACAQUES

Mineralization in the brain (corpora amylacea)
Protein inclusions in transitional epithelium (cytokeratin)
Macrophages in intestinal villi
Mineralization of adrenal corticomedullary zone
Multinucleated cells in renal pelvis
Mineralization in renal papilla
Lymphoid nodules in bone marrow
Lymphoid infiltrates in salivary glands, brain, prostate, etc.
Herniation of intestinal glands into GALT
Reticuloendothelial hyperplasia in spleen

## BACTERIAL DISEASES

### TUBERCULOSIS

Etiology: Mycobacterium tuberculosis, M. bovis, atypical mycobacteria
Transmission: Respiratory, oral. M. tuberculosis and M. bovis are typically acquired from infected humans or ruminants in the country of origin. Tuberculosis is rare in wild populations.
Clinical: Tuberculosis in monkeys, especially rhesus, is a rapidly progressive disease that seldom becomes arrested, as it does in humans. New World monkeys are generally more resistant than OWM. Often there are no clinical signs in caged monkeys. Severely affected monkeys may show coughing, wasting, enlarged lymph nodes, splenomegaly, and hepatomegaly. With modern management, most cases are likely to be diagnosed by tuberculin testing, following CDC guidelines for testing and diagnosis in quarantine (MMWR 42/#29, 1993). For testing, use Mammalian Old Tuberculin, 1,500 units (some recommend 3,000 units) intradermally in upper eyelid. Read at 24, 48, & 72 hrs. and look for swelling. Monkeys that have been inoculated with Freund's Complete Adjuvant are often tuberculin-positive. Orangutans have a high incidence of false positive tuberculin reactions.
Pathology: M. tuberculosis and M. bovis cause disseminated yellow-white granulomas in the lung, lymph nodes, spleen, liver, and other organs. Typical lesions are tuberculoid granulomas characterized by caseous centers, giant cells, lymphocytes and epithelioid cells. AFB may be difficult to find and are best sought in the caseous center. Often many sections must be examined to confirm the diagnosis. The auramine-rhodamine fluorescent stain is very useful if AFB are sparse. Culture or PCR is necessary to identify the species of mycobacteria.

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MYCOBACTERIUM AVIUM-INTRACELLULARE INFECTION

Etiology: M. avium-intracellulare complex, M. paratuberculosis

Transmission: M. avium is a natural pathogen of birds and M. intracellulare is a common environmental saprophyte. Water supplies and soil are frequently contaminated with MAI.

Clinical: Infection with MAI occurs in a setting of immunodeficiency, usually due to SIV or SRV in macaques. These monkeys usually have chronic fluid diarrhea. Monkeys with M. avium-intracellulare may be weakly positive with OT, but are usually more strongly positive when tested with tuberculin from M. avium.

M. paratuberculosis infection has only been confirmed in Macaca arctoides. These animals have diarrhea and wasting.

Pathology: M. avium and M. paratuberculosis typically cause intestinal lesions characterized by a firm, thickened mucosa due to a diffuse histiocytic infiltrate in the

lamina propria of the small and large intestines. The histiocytes are filled with abundant AFB. Mesenteric lymph nodes are enlarged and yellow-white. Epithelioid cell change, caseation, and giant cells are not usually features of lesions caused by these bacteria, although tubercles have rarely been reported. M. avium-intracellulare infections are associated with immunodeficiency and are seen in acquired immunodeficiency syndromes in macaques infected with SRV and SIV.

References:

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LEPROSY

Etiology: Mycobacterium leprae

Transmission: Respiratory, skin?

Clinical: Clinical findings include nodular thickening of skin and peripheral nerve and paralytic deformity of hands and feet. Natural infections have occurred in chimpanzees and sooty mangabeys (Cercocebus torquatus atys).

Pathology: Leprosy is a pathologically complex disease that has a spectrum of lesions that depend on the degree of cell-mediated immunity the host is able to mount against M. leprae. In lepromatous leprosy, the host mounts no CMI, while in tuberculoid leprosy, the host has a vigorous CMI respone. Borderline leprosy occurs when the host mounts an

intermediate level of CMI. Natural infections in nonhuman primates have taken the lepromatous form, indicating no CMI. Lesions occur predominantly in the skin and peripheral nerves, particularly in cooler areas (ears, tail, scrotum). The nasal mucosa and testicles are also frequently involved. In lepromatous leprosy, focal or diffuse histiocytic infiltrates with variable numbers of lymphocytes and plasma cells occur. Large numbers of acid-fast bacilli are demonstrable with Fite-Faraco acid-fast stain. Nerve lesions are pathognomonic. In polar tuberculoid leprosy, granulomas that resemble tuberculoid granulomas form in the skin and nerves. Acid-fast bacilli are rare in tuberculoid leprosy. Borderline leprosy shares features of lepromatous and tuberculoid leprosy.
References:

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SHIGELLOSIS
Etiology: Shigella flexneri, S. sonnei, others are less common
Transmission: Fecal-oral
Clinical: Variable. Asymptomatic carriers are common. Infected monkeys may have soft stool, fluid diarrhea, or more commonly, the bloody mucoid diarrhea of classical dysentery. Monkeys with colitis due to Shigella will rapidly dehydrate and die unless treated promptly and vigorously. Shigella affects only primates. Clinical disease in carriers is often precipitated by stress.
Pathology: The lesions of shigellosis are limited to the colon, may be focal or diffuse, and are characterized by edema, hemorrhage, erosion and ulceration, and pseudomembrane formation. Shigellosis does not typically cause septicemia. Microscopically the lesion is purulent, necrotizing colitis, often with crypt abscesses. Shigella occasionally causes periodontitis in monkeys. The diagnosis must be confirmed by culture.
References:

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SALMONELLOSIS

Etiology: Salmonella enteritidis, S. typhimurium

Transmission: Fecal-oral. Rodent feces are the most common source.

Clinical: Salmonella can be carried asymptomatically and may occur sporadically or as an epizootic. Watery to bloody mucoid diarrhea is the most common clinical sign. Monkeys frequently become moribund and die.

Pathology: Necrotizing, suppurative enterocolitis. Salmonellosis may cause septicemia, resulting in pyogranulomas in the liver and other organs. Salmonellosis may resemble shigellosis, but shigellosis does not cause septicemia and does not affect the small intestine.

References:

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CAMPYLOBACTERIOSIS

Etiology: Campylobacter (Vibrio) fetus ss, C. jejuni, C. coli

Transmission: Oral

Clinical: Asymptomatic carriers are common. Diseased monkeys have fluid, sometimes bloody diarrhea and dehydration. Campylobacter has been associated with abortions in primates. Isolation requires special media and atmosphere.

Pathology: The small intestine and colon appear reddened, roughened, and edematous. The colonic lesions can be similar to shigellosis, but are usually much less severe and the small intestine can also be affected. The colonic mucosa is sometimes hyperplastic. Silver stains demonstrate spiral bacteria.

References:

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HELICOBACTERIOSIS

Etiology: Helicobacter pylori

Transmission: Oral

Clinical: Helicobacter pylori usually causes no clinical symptoms, except for occasional vomiting, but is prevalent in the rhesus stomach.

Pathology: Lesions are seldom grossly apparent, but sometimes focal reddening or erosions of the gastric mucosa can be seen. Mononuclear inflammatory cell infiltrates in the lamina propria of the stomach, superficial erosions, and epithelial hyperplasia may be noted. The organism can be seen with H&E, but Giemsa or silver stains will more readily demonstrate the slightly curved, rod-shaped, gull-wing, or loosely coiled organisms, 1-4 μm long, associated with gastric epithelium in the antral mucosa. It is best to culture a biopsy rather than a swab. Since H. pylori is urease positive, a rapid urease test can be used rather than culture. Helicobacter pylori is most common and severe in the antrum.

References:

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GASTROSPIRILLUM HOMINIS-LIKE ORGANISMS (GHLO), H. HEILMANNII

Etiology: Gastrospirillum hominis-like organisms, also called Helicobacter heilmannii

Pathology: GHLO are nearly ubiquitous in rhesus monkeys, mainly in the fundus of the stomach. Organisms are 3.5-10 μm long, tightly coiled (6-8 coils per cell), spiral bacteria with bipolar flagella. They are located in the surface mucus, lumens of gastric pits, and in parietal cells.

References:

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STREPTOCOCCUS PNEUMONIAE (DIPLOCOCCUS)

Etiology: Streptococcus (Diplococcus) pneumoniae

Transmission: Respiratory

Clinical: Streptococcus pneumoniae tends to occur in small focal outbreaks. Animals, though often found dead, may have signs of pneumonia, meningitis, arthritis, depression, or dehydration. Growth in culture is inhibited by optochin (ethyl hydrocuprein hydrochloride).
Pathology: S. pneumoniae causes fibrinopurulent serositis affecting meninges, pleura, peritoneum, and/or joints. Often associated with severe fibrinopurulent pneumonia. Sometimes only septicemia may be noted, especially if the animal is splenectomized. Numerous thrombi and infarcts can result in permanent CNS damage if the animal survives. Diplococci are easily seen on a gram-stained impression smear of exudates.
References:

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YERSINIOSIS
Etiology: Yersinia pseudotuberculosis, Y. enterocolitica
Transmission: Wild birds and rodents are the reservoir hosts. Transmission is by ingestion of feed contaminated by feces of infected vermin.
Clinical: Affected monkeys are often found dead, but sometimes show diarrhea, depression, and dehydration. Yersinia is occasionally associated with abortions and stillbirths.
Pathology: The infection begins as a focal necrotizing enteritis and mesenteric lymphadenitis, which rapidly becomes septicemic, resulting in necropurulent hepatitis, splenitis, and myelitis. Large colonies of gram-negative bacteria in necrotic centers are nearly always diagnostic.
References:

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Chang J, et al. Fatal Yersinia pseudotuberculosis infection in captive bushbabies. J Am Vet Med Assoc 177:820-821, 1980.

Kageyama T, et al. Yersinia pseudotuberculosis infection in breeding monkeys: detection and analysis of strain diversity by PCR. J Med Primatol 31:129-135, 2002.

LISTERIOSIS

Etiology: Listeria monocytogenes

Transmission: Listeria is widespread in the environment. Oral transmission occurs from contaminated food, while Listeria can also be transmitted transplacentally.

Clinical: Disease occurs in stillborn and neonatal infants. Abortion, intrauterine death, neonatal sepsis, and meningoencephalitis occur in infants, although the mother is clinically normal.

Pathology: Purulent placental villitis (hematogenous pattern), purulent meningoencephalitis, intrauterine pneumonia, and focal necrosis in the liver and other organs can be found, along with gram-positive rods in the tissues.

References:

McClure HM, et al. Perinatal listeric septicemia in a Celebese black ape. JAVMA 167:637-638, 1975.

Tribe GW. Listeria monocytogenes associated with abortion in cynomolgus monkeys. Primate Supply 7:9-13, 1983.

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BORDETELLOSIS

Etiology: Bordetella bronchiseptica

Transmission: Respiratory

Clinical: Animals are often asymptomatic. Mucopurulent nasal discharge, dyspnea, and death have been reported.

Pathology: Fibrinopurulent hemorrhagic bronchopneumonia with fibroplasia around the bronchioles is the most common pathological finding.

References:

Graves IL. Bordetella bronchiseptica isolated from a fatal case of bronchopneumonia in an African green monkey. Lab Anim Care 18:405-406, 1968.

Kohn DF, et al. Bordetella bronchiseptica infection in the lesser bushbaby (Galago senegalensis). Lab Anim Sci 27:279-280, 1977.

Seibold HR, et al. Pneumonia associated with Bordetella bronchiseptica in Callicebus species primates. Lab Anim Care 20:456-461, 1970.

MORAXELLA (BRANHAMELLA) CATARRHALIS

Etiology: Moraxella (Branhamella) catarrhalis

Transmission: Aerosol

Clinical: The "bloody nose syndrome" is found in cynomolgus macaques. The clinical signs consist of epistaxis and periorbital edema. Some clinicians believe the bloody nose syndrome is associated with low humidity, with or without Moraxella.
Pathology: Mucohemorrhagic rhinitis. Gram-negative diplococci can be found in the exudate.
References:

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Olson LC, et al. Epistaxis and bullae in cynomolgus macaques. Lab Anim Sci 33:377-379, 1983.

TETANUS
Etiology: Clostridium tetani
Transmission: C. tetani is a soil organism and an obligate anaerobe that can contaminate wounds and cause postpartum infections.
Clinical: Tetanus is a nonimmunizing disease, usually fatal in 1-10 days due to respiratory paralysis and exhaustion. Multiple episodes are possible. A deliberate, stiff gait, trismus, extensor rigidity, and opisthotonos are usually seen. The tail and ears are often erect, and the forearms are often flexed. The syndrome begins in the upper limbs and then moves to the lower. Tetanus toxin is extremely potent, and only small amounts are required to produce tetanus. Antibody is not usually detectable in affected animals.
Pathology: None. Tetanus must be diagnosed clinically.
References:

Rawlins RG, et al. A five-year study of tetanus in the Cayo Santiago rhesus monkey colony: behavioral description and epizootiology. Am J Primatol 3:23-39, 1982.

Kessler MJ, et al. Clinical description of tetanus in squirrel monkeys. Lab Anim Sci 29:240-242, 1979.

Goodwin WJ, et al. Tetanus in baboons of a corral breeding colony. Lab Anim Sci 37:231-232, 1987.

STAPHYLOCOCCUS
Etiology: Staphylococcus aureus
Transmission: Staphylococcus is commonly carried asymptomatically in the nose and throat, but occasionally infects breaks in the skin and may invade the bloodstream.
Clinical: Pustular dermatitis is seen in young animals. Breaks in skin become infected, resulting in cellulitis, abscesses, and lymphadenitis. Bacteremia often develops, leading to visceral abscesses, endocarditis, and septic shock. Vegetative valvulitis may cause septic emboli and infarcts in various organs. Indwelling catheters are a common source of infection. The source of infection is usually clinically obvious.
Pathology: Cellulitis, abscesses filled with thick creamy pus, fibrinous pericarditis, vegetative valvulitis, thrombosis and infarction are found in infected animals. Histologic lesions consist of fibrinopurulent exudate with masses of gram-positive cocci. Monkeys sometimes develop secondary immune complex glomerulonephritis.

References:

Taylor WM, et al. Catheter-tract infections in rhesus macaques (Macaca mulatta) with indwelling intravenous catheters. Lab Anim Sci 48:448-454, 1998.

KLEBSIELLA

Etiology: Klebsiella pneumoniae

Transmission: Respiratory, most commonly carried in nose and throat.

Clinical: Klebsiella may cause nasal discharge, pneumonia, meningitis, or serositis.

Pathology: Fibrinopurulent pneumonia, serositis, and septicemia. Abundant gram-negative bacteria with prominent capsules are seen in exudates. Macrophages often predominate in pulmonary lesions. Exudates sometimes have a gelatinous consistency.

References:

Snyder SB, et al. A study of Klebsiella infections in owl monkeys. JAVMA 157:1935-1939, 1970.

Hunt DE, et al. Control of an acute Klebsiella pneumoniae infection in a rhesus monkey colony. Lab Anim Care 18:182-185, 1968.

Fox JG, et al. Meningitis caused by Klebsiella sp in two rhesus monkeys. JAVMA 167:634-636, 1975.

Gozalo A, et al. Klebsiella pneumoniae infection in a new world nonhuman primate center. Lab Primate Newsletter 30:13-15, 1991.

ESCHERICHIA COLI

Etiology: E. coli

Transmission: Fecal-oral

Clinical: Pneumonia, meningitis, and diarrhea are the most common clinical signs.

Pathology: Fibrinopurulent pneumonia and serositis, pyelonephritis, or hemorrhagic gastroenteritis, ulcerative colitis. Pyelonephritis in macaques is nearly always due to E. coli.

References:

McClure HM, et al. Enteropathogenic Escherichia coli infection in anthropoid apes. JAVMA 161:687-689, 1972.

Mansfield KG, et al. Identification of enteropathogenic Escherichia coli in Simian Immunodeficiency Virus-infected infant and adult rhesus macaques. J Clin Microbiol 39:971-976, 2001.

PSEUDOMONAS spp.

Etiology: Pseudomonas aeruginosa and P. pseudomallei (melioidosis)

Transmission: P. aeruginosa is ubiquitous in moist environments worldwide. P. pseudomallei is an environmental saprophyte in SE Asia.

Clinical: P. aeruginosa is predominantly a problem in debilitated, burned, immunocompromised, and neutropenic patients. Infections are common in animals that have been immunosuppressed with steroids or whole body irradiation. P. pseudomallei may infect animals and man in SE Asia and can remain clinically latent for years.

Pathology: P. aeruginosa can infect many tissues, but the pathological hallmark is a vasculitis without thrombosis. Bacilli are seen in the vessel wall. There is severe

necrosis usually, but neutrophils are often sparse. P. pseudomallei causes melioidosis, which may include pneumonia, abscesses, and granulomas.
References:

Fritz PE, et al. Naturally occurring melioidosis in a colonized rhesus monkey. Lab Anim 20:281-285, 1986.

Mutalib AR, et al. Melioidosis in a banded leaf-monkey (Presbytis melalophos). Vet Rec 115:438-439, 1984.

Bodey GP, et al. Infections caused by Pseudomonas aeruginosa. Rev Inf Dis 5:279-313, 1983.

NOCARDIOSIS
Etiology: Nocardia asteroides
Transmission: Organisms are common in soil and organic material. Inhalation and ingestion are the usual modes of transmission.
Clinical: Nocardia asteroides infection is often associated with defects in cellular immunity.
Pathology: Infections are often predominant in the lungs, but may disseminate. Mixed inflammatory infiltrates, abscesses, and granulomas are found. The organism is gram-positive, filamentous, branching, often beaded, and variably acid-fast.
References:

Liebenberg SP, et al. Disseminated nocardiosis in three macaque monkeys. Lab Anim Sci 35:162-166, 1985.

Sakakibara I, et al. Spontaneous nocardiosis with brain abscess caused by Nocardia asteroides in a cynomolgus monkey. J Med Primatol 13:89-95, 1984.

## VIRAL DISEASES

HERPESVIRUS B
Etiology: Herpesvirus simiae (B virus), cercopithecine herpesvirus 1
Transmission: Bites, scratches, venereal, ocular, and possibly aerosol modes of transmission are suspected. Virus is shed in oral and genital secretions, as well as vesicular fluid. Viremia is rare, but does occur. There is no vertical transmission. The virus is latent in sensory ganglia. Humans have become infected from monkey cell cultures. About half of human infections have been found in animal handlers, with the other half being found in laboratory workers.
Clinical: Herpes simiae is the macaque homologue of herpes simplex 1 in humans. The rate of seropositivity in conventional captive adult macaques is 73-100%. Herpes B causes a lifelong infection with intermittent reactivation and virus shedding in saliva or genital secretions. In macaques, lesions consist of vesicles and ulcers in the oral cavity and lips and conjunctivitis. Disseminated infections occur rarely, especially in young and debilitated animals. Latent infection is common. Epizootic disease has been reported in M. radiata. Severe disease has been reported in colobus and DeBrazza's monkeys. Asymptomatic macaques can shed virus!! There have been about 50 human cases reported, 29 of which were fatal. In humans, vesicles at the site of inoculation, conjunctivitis, flu-like symptoms, and severe, often fatal, encephalomyelitis have been

reported. There is no evidence of asymptomatic human infection. Antibody titers to herpes simplex virus are not protective in humans.

Pathology: In monkeys, vesicles or ulcers on oral mucous membranes and esophagus, and focal necrosis in various organs, if generalized, are seen. Intranuclear inclusion bodies and syncytial cells are associated with the lesions. In humans, conjunctivitis, vesicles at site of bite or scratch, and necrosis of CNS are noted.

Virus Detection: Most infected animals are seropositive, but a small percentage can be seronegative. Culture and PCR can be used to detect the virus, but are only useful if the animal is actively shedding virus. The virus is usually shed only intermittently and briefly. PCR of cranial and dorsal root ganglia provides the best evidence of viral status, but is not possible in living animals.

Colony Management: One should assume that all macaques are shedding B virus. Monkeys should be handled by properly trained personnel using proper protection (gloves and full face protection at least).

Handling of unanesthetized monkeys should be avoided.

Management programs must be tailored to individual circumstances. One should consider serological screening of all macaques. If circumstances allow, seropositive and seronegative animals could be separated into clean and infected colonies. Colonies in the USA vary between 10-90% seropositive. The percentage of seropositive monkeys that are shedding virus at any one time is unknown, but is probably very small. Some monkeys shed virus consistently and others intermittently. One could consider viral culture on seropositive monkeys, depending on the degree of human contact. Virus shedders that come into contact with humans should be eliminated, if possible. Even in SPF colonies, the risk is not zero, because rare seronegative monkeys are actually infected.

Herpesvirus B can infect and cause fatal disease in owl monkeys, marmosets, African green monkeys, gibbons, and patas monkeys. Do not mix species!

Management of Bites: Wounds should be cleaned immediately. Wound excision should be considered if surgical expertise is immediately available. Draw blood from the monkey and the human victim immediately for serological testing. Culture the monkey (buccal and conjunctival swabs) immediately. Do follow-up cultures and consider placing the victim on acyclovir if the monkey was shedding virus at the time of the bite. Acyclovir appears to be helpful if given before neurological damage occurs. Physicians should consult Dr. Louisa Chapman, Centers for Disease Control (404-639-3747).

Serology and Viral Culture: Samples for serology or viral culture should be sent to the NIH B Virus Resource Laboratory, Viral Immunology Center, Georgia State University, 50 Decatur Street, Atlanta, GA 30303. Information and submission forms can be obtained from Dr. Richard D. Henkel, NIH B Virus Reference Laboratory, Georgia State University, PO Box 4118, Atlanta, GA 30302-4118 (404-651-0808; biordh@panther.gsu.edu).

References:

Perkins FT, et al. Precautions against B virus infection. Brit Med J 1:899-901, 1966.

Keeble SA, et al. Natural virus-B infection in rhesus monkeys. J Path Bacteriol 76:189-199.

Loomis MR, et al. Fatal herpesvirus infection in patas monkeys and a black and white colobus monkey. J Am Vet Med Assoc 179:1236-1239, 1981.

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Katz D, et al. ELISA for detection of group-common and virus-specific antibodies in human and simian sera induced by herpes simplex and related simian viruses. J Virol Methods 14:99-109, 1986.

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Palmer AE. B virus, Herpesvirus simiae: historical perspective. J Med Primatol 16:99-130, 1987.

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Weigler BJ. Biology of B-virus in macaque and human hosts: a review. Clin Inf Dis 14:2, 1992.

Chellman GJ, et al. Activation of B virus (Herpesvirus simiae) in chronically immunosuppressed cynomolgus monkeys. Lab Anim Sci 42:146-151, 1992

Sauber JJ, et al. An attempt to eradicate Herpesvirus simiae from a rhesus monkey breeding colony. Lab Anim Sci 42:458-462, 1992.

Wells DL, et al. Herpesvirus simiae contamination of primary rhesus monkey kidney cell cultures. Diagn Microbiol Infect Dis 12:333-336, 1989.

Artenstein AW, et al. Human infection with B virus following a needlestick injury. Rev Infect Dis 13:288-291, 1991.

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Hunt RD, et al. Herpesvirus B infection. In: Jones TC, et al. (eds). Monographs on Pathology of Laboratory Animals: Nonhuman Primates I. Springer-Verlag, 1993, 78-81.

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Anderson DC, et al. Primary Herpesvirus simiae (B-virus) infection in infant macaques. Lab Anim Sci 44:526-530, 1994.

Ward JA, et al. B virus-specific pathogen-free (SPF) breeding colonies of macaques: issues, surveillance, and results in 1992. Lab Anim Sci 44:222-228, 1994.

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Davenport DS, et al. Diagnosis and management of human B virus (Herpesvirus simiae) infections in Michigan. Clin Inf Dis 19:33-41, 1994.

Carlson CS, et al. Fatal disseminated cercopithecine herpesvirus 1 (herpes B) infection in cynomolgus monkeys (Macaca fascicularis). Vet Pathol 34:405-414, 1997.

Ostrowski SR, et al. B-virus from pet macaque monkeys: an emerging threat in the United States? Emerg Inf Dis 4: , 1998.

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Ohsawa K, et al. Herpesvirus papio 2: alternative antigen for use in monkey B virus diagnostic assays. Lab Anim Sci 49:605-616, 1999.

Hilliard JK, et al. B-virus specific-pathogen-free breeding colonies of macaques (Macaca mulatta): retrospective study of seven years of testing. Lab Anim Sci 49:144-148, 1999.

Ward JA, et al. Herpes B-virus specific-pathogen free breeding colonies of macaques (Macaca mulatta): diagnostic testing before and after elimination of the infection. Comp Med (Lab Anim Sci) 50:317-322, 2000.

Thompson SA, et al. Retrospective analysis of an outbreak of B virus infection in a colony of DeBrazza's monkeys (Cercopithecus neglectus). Comp Med 50:649-657, 2000.

Hirano M, et al. One-step PCR to distinguish B virus from related primate alphaherpesviruses. Clin Diagn Lab Immunol 9:716-719, 2002.

SIMIAN AGENT 8 (SA8)

Etiology: Alphaherpesvirus related to h. simiae, herpesvirus papio 2, HSV-1, HSV-2.

Transmission: SA8 is endemic in African green monkeys. No human infections have been reported. Sooty mangabeys carry a similar virus.

Pathology: Lesions are rarely reported in cercopithecoids. Lesions in baboons previously attributed to SA8 were probably due to herpesvirus papio 2.

References:

Malherbe H, et al. Neurotropic virus in African monkeys. Lancet ii:530, 1958.

Henkel RD, et al. Serological evidence of alphaherpesvirus infection in sooty mangabeys. J Med Primatol 31:120-128, 2002.

HERPESVIRUS PAPIO 2

Etiology: Alphaherpesvirus related to h. simiae and SA8. Previously identified as SA8.
Transmission: Endemic in baboons. Venereal and oral transmission.
Pathology: Oral, genital, and cutaneous vesicular, papillomatous, or ulcerative lesions are seen in baboons. Inguinal lymphadenopathy has also been seen. Lesions usually resolve spontaneously, but may recur. Severe permanent damage to the female reproductive tract may occur. This could be a good model for h. simplex 2 in humans.
References:

Levin JL, et al. A naturally occurring epizootic of simian agent 8 in the baboon. Lab Anim Sci 38:394-397, 1988.

Eberle R, et al. Herpesvirus papio 2, an SA8-like herpesvirus of baboons. Arch Virol 140:529-545, 1995.

Martino MA, et al. Clinical disease associated with simian agent 8 infection in the baboon. Lab Anim Sci 48:18-22, 1998.

Eberle R, et al. Shedding and transmission of baboon herpesvirus papio 2 (HVP2) in a breeding colony. Lab Anim Sci 48:23-28, 1998.

SIMIAN VARICELLA VIRUS

Etiology: This is a group of closely-related herpesviruses including deltaherpesvirus, Medical Lake macaque virus, Liverpool vervet monkey virus, and others. All are antigenically related to human varicella-zoster virus.
Transmission: Respiratory. Latency is common and the origin of some outbreaks is unexplained.
Clinical: Herpetic rash, depression, respiratory difficulty, affecting patas, African green monkeys, macaques.
Pathology: Vesicles on skin, oral mucous membranes, and esophagus are commonly seen. Focal necrosis in the lungs, liver, spleen, lymph nodes, adrenal, bone marrow, and intestinal tract have been noted. Intranuclear inclusion bodies are present. Simian varicella becomes latent in ganglia.
References:

Bladely GA, et al. A varicella-like disease in macaque monkeys. J Infect Dis 127:617-625, 1973.

Iltis JP, et al. Simian varicella virus (deltaherpesvirus) infection of Patas monkeys leading to pneumonia and encephalitis. Soc Exp Biol Med 169:266-279, 1982.

Schmidt NJ, et al. Serological investigation of an outbreak of simian varicella in Erythrocebus patas monkeys. J Clin Microbiol 18:901-904, 1983.

Roberts ED, et al. Pathologic changes of experimental simian varicella (Delta herpesvirus) infection in African green monkeys. Am J Vet Res 45:523-530, 1984.

White RJ, et al. Chickenpox in young anthropoid apes. Clinical and laboratory findings. J Am Vet Med Assoc 161:690-692, 1972.

Mahalingam R, et al. Prevalence and distribution of latent simian varicella virus DNA in monkey ganglia. Virol 188:193-197, 1992.

Roberts ED. Simian varicella. In: Jones TC, et al. (eds). Monographs on Pathology of Laboratory Animals: Nonhuman Primates I. Springer-Verlag, 1993, 93-100.

Gray WL, et al. Rapid diagnosis of simian varicella using the polymerase chain reaction. Lab Anim Sci 48:45-49, 1998.

Gray WL, et al. Experimental simian varicella virus infection of St. Kitts vervet monkeys. J Med Primatol 27:177-183, 1998.

HERPESVIRUS SAIMIRI

Etiology: Gammaherpesvirus (cebid herpesvirus 2)

Transmission: Oral

Clinical: The squirrel monkey is the natural host and there is a high incidence of natural infection. Herpes saimiri procuces no disease in squirrel monkeys, but causes lymphomas in marmosets, owl monkeys, African green monkeys, howler monkeys and spider monkeys. Lymphadenopathy, hepatomegaly, splenomegaly, and leukemia are seen clinically.

Pathology: There are no lesions in squirrel monkeys, despite a lifelong latent infection of T cells. In tumorigenic hosts, leukemic infiltrates of immature lymphocytes occur in the liver, kidney, spleen, lymph nodes, adrenals, and other organs. Focal necrosis in the liver, spleen, kidney, adrenal cortex, lymph nodes, thymus, and bone marrow may also be evident. There are no inclusion bodies.

References:

Hunt RD, et al. Morphology of a disease with features of malignant lymphoma in marmosets and owl monkeys inoculated with Herpesvirus saimiri. J Natl Cancer Inst 44:447-465, 1970.

Melendez LV, et al. Herpes saimiri II. Experimentally induced malignant lymphoma in primates. Lab Anim Care 19:378-386, 1969.

Falk LA, et al. Oral excretion of Herpesvirus saimiri in captive squirrel monkeys and incidence of infection in feral squirrel monkeys. J Natl Cancer Inst 51:1987-1989, 1973.

Hunt RD, et al. Herpesvirus saimiri and Herpesvirus ateles infection. In: Jones TC, et al. (eds). Monographs on Pathology of Laboratory Animals: Nonhuman Primates I. Springer-Verlag, 1993, 87-93.

HERPESVIRUS TAMARINUS

Etiology: Alphaherpesvirus (cebid herpesvirus 1)

Clinical: Squirrel monkeys are the natural hosts and have a high incidence of natural infection. Infections are usually inapparent, but oral vesicles or ulcers similar to h. simplex in man and h. simiae in macaques may occur. Herpes T produces a fatal generalized disease in owl monkeys and marmosets characterized by a vesicular rash and oral vesicles and ulcers.

Pathology: Sometimes oral vesicles can be seen in squirrel monkeys. In owl monkeys, tamarins and marmosets, a typical generalized herpesvirus infection with vesicles and ulcers on skin and oral mucous membranes, ulcers in GI tract, focal necrosis in liver, adrenal, spleen, lung, and lymph nodes occurs. Occasional syncytial cells are seen, as

well as eosinophilic intranuclear inclusion bodies. In colonies, there is often high morbidity and mortality.

References:

Melnick JL, et al. A new member of the herpesvirus group isolated from South American marmosets. J Immun 92:596-601, 1964.

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Hunt RD, et al. A pathologic study of herpes-T in the owl monkey (Aotus trivirgatus). Path Vet 3:1-26, 1966.

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Hunt RD, et al. Herpesvirus platyrrhinae infection. In: Jones TC, et al. (eds). Monographs on Pathology of Laboratory Animals: Nonhuman Primates I. Springer-Verlag, 1993, 100-103.

HERPESVIRUS ATELES

Etiology: Herpesvirus

Clinical: The natural host is the spider monkey (Ateles geofroyii), although no disease is apparent in spider monkeys. Herpesvirus ateles induces lymphomas in marmosets and owl monkeys. Lymphadenopathy, hepatomegaly, splenomegaly are the clinical features.

Pathology: Malignant lymphoma in lymph nodes, liver, spleen, kidney, adrenal, bone marrow and other tissues.

References:

Hunt RD, et al. Pathologic features of Herpesvirus ateles lymphoma in cotton-topped marmoset (Saguinus oedipus). J Natl Cancer Inst 49:1631, 1972.

Rangan SRS, et al. Tumors and viruses in nonhuman primates. Adv Virus Res 24:1, 1979.

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HERPES SIMPLEX VIRUS

Etiology: Herpes simplex virus

Transmission: Latent or active infections are found in many humans, which are the natural reservoir. Human-to-monkey and monkey-to-monkey transmission have been described.

Clinical: Lesions may be local or generalized. Oral vesicles and ulcers, conjunctivitis, encephalitis, and death may occur. Owl monkeys, tree shrews, lemurs, marmosets, and tamarins are susceptible to generalized disease. Chimpanzees and gibbons can be infected, but usually the infection remains confined to skin, oral cavity, external genitalia, and conjunctiva.

Pathology: Oral, lingual, labial, or genital vesicles and ulcers assosicated with conjunctivitis and keratitis are seen. Necrotizing meningoencephalitis may occur and focal necrosis can be found in the visceral organs. Multinucleated cells and intranuclear inclusion bodies can be seen.

References:

Smith PC, et al. The gibbon (Hylobates lar): a new primate host for herpesvirus hominus. I. A new natural epizootic in a laboratory colony. J Infect Dis 120:292-297, 1969.

Melendez LV, et al. Natural herpes simplex infection in the owl monkey (Aotus trivirgatus). Lab Anim Care 19:38-45, 1969.

McClure HM, et al. Viral diseases noted in the Yerkes Primate Center colony. Lab Anim Sci 21:1002-1010, 1971.

McClure HM, et al. Natural genital herpesvirus hominis infection in chimpanzees (Pan troglodytes and Pan paniscus). Lab Anim Sci 30:895-901, 1980.

Heldstab A, et al. Spontaneous generalized herpesvirus hominis infection of a lowland gorilla (Gorilla gorilla gorilla). J Med Primatol 10:129-135, 1981.

Hunt RD. Herpesvirus simplex infection. In: Jones TC, et al. (eds). Monographs on Pathology of Laboratory Animals: Nonhuman Primates I. Springer-Verlag, 1993, 82-86.

Huemer HP, et al. Fatal infection of a pet monkey with human herpesvirus 1. Emerg Inf Dis 8: , 2002.

CYTOMEGALOVIRUS

Etiology: Betaherpesvirus

Transmission: CMV is shed in the urine and may also be acquired transplacentally. CMV is highly species-specific.

Clinical: There are usually no clinical signs. In macaques, widespread latent infection exists, with most monkeys seroconverting during the first year of life. Disease is produced only in fetuses and immunodeficient individuals. CNS and respiratory tract signs may be noted. CMV is a common opportunistic infection in SIV- and SRV-infected macaques.

Pathology: In immunodeficient animals, generalized infections with necrotizing meningitis and neuritis, interstitial pneumonia, arteritis, enterocolitis, orchitis, and focal necrosis in liver and spleen are apparent. Infected cells are often enlarged and may contain characteristic large basophilic intranuclear inclusion bodies and granular eosinophilic cytoplasmic inclusion bodies. These occur in mesenchymal cells rather than the surface epithelium, as with other herpesviruses.

References:

Baskin GB. Disseminated cytomegalovirus infection in immunodeficient rhesus monkeys. Am J Pathol 129:345-352, 1987.

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Asher DM, et al. Persistent shedding of cytomegalovirus in the urine of healthy rhesus monkeys. Proc Soc Exp Biol Med 145:794-801, 1974.

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Kuhn E-M, et al. Immunohistochemical studies of productive rhesus cytomegalovirus infection in rhesus monkeys (Macaca mulatta) infected with Simian Immunodeficiency Virus. Vet Pathol 36:51-56, 1999.

RHESUS LYMPHOCRYPTOVIRUS (EPSTEIN-BARR-LIKE VIRUSES)
Etiology: Nonhuman primate EBV-related gammaherpesviruses, HVMF1 in Macaca fascicularis
Transmission: Contact
Clinical: Most infections are latent. In immunodeficient animals, lymphocryptoviruses have been associated with lymphoma and with squamous epithelial proliferative lesions on the skin and mucous membranes.
Pathology: Extranodal systemic B-cell lymphoma and focal squamous cell proliferations resembling oral hairy leukoplakia on oral, genital, and cutaneous surfaces in immunodeficient animals. Intranuclear inclusions are present in epithelial lesions.
References:

Rangan SRS, et al. Epstein-Barr virus-related herpesvirus from a rhesus monkey (Macaca mulatta) with malignant lymphoma. Int J Cancer 38:425-432, 1986.

Landon JC, et al. Seroepidemiologic studies of Epstein-Barr virus antibody in monkeys. J Natl Cancer Inst 46:881-884, 1971.

Ishida T, et al. Survey of nonhuman primates for antibodies reactive with Epstein-Barr virus antigens and susceptibility of their lymphocytes for immortalization with EBV. J Med Primatol 16:359-371, 1987.

Bocker JF, et al. Characterization of an EBV-like virus from African green monkey lymphoblasts. Virol 101:291-295, 1980.

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Hayashi K, et al. An animal model for Epstein-Barr virus (EBV)-associated lymphomagenesis in the human: malignant lymphoma induction of rabbits by EBV-related herpesvirus from cynomolgus monkeys. Pathol Int 50:85-97, 2000.

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Schmidtko J, et al. Posttransplant lymphoproliferative disorder associated with an Epstein-Barr-related virus in cynomolgus monkeys. Transplantation 73:1431-1439, 2002.

MARMOSET LYMPHOCRYPTOVIRUS

Etiology: Gammaherpesvirus, lymphocryptovirus

Clinical: Lymphocryptovirus can cause fatal lymphoproliferative disease in marmosets. Weight loss, inappetence, diarrhea, and abdominal masses may be observed. Infected monkeys are seropositive for EBV.

Pathology: Lymphoproliferative disease/lymphosarcoma, primarily involving the gastrointestinal tract and mesenteric lymph nodes.

References:

Ramer JC, et al. Fatal lymphoproliferative disease associated with a novel gammaherpesvirus in a captive population of common marmosets. Comp Med (Lab Anim Sci) 50:59-68, 2000.

RHESUS RHADINOVIRUS

Etiology: Gammaherpesvirus closely related to human herpesvirus 8 (KSHV). Also called RFHVMn and RFHVMm.

Clinical: There is a high incidence of seropositivity in some research colonies of Macaca mulatta and M. nemestrina. An association with retroperitoneal fibromatosis has been proposed.

Pathology: Lymphoid hyperplasia, possibly driven by the viral homologue of IL-6, has been described.

References:

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Mansfield KG, et al. Experimental infection of rhesus and pig-tailed macaques with macaque rhadinoviruses. J Virol 73:10320-10328, 1999.

ENCEPHALOMYOCARDITIS VIRUS

Etiology: Encephalomyocarditis virus (picornavirus)

Transmission: Oral, possibly other routes. Rodents are reservoir hosts.

Clinical: Encephalomyocarditis virus often causes sudden death in monkeys. It can cause myocarditis in nonhuman primates, pigs, elephants, and some other species. EMCV is probably not a significant human pathogen, although some people are seropositive.

Pathology: With EMCV there is pericardial effusion with pale areas in the myocardium. Myofiber necrosis with inflammation and edema is also seen, along with secondary

lesions of acute heart failure. Extensive myocardial scarring has been seen in animals that survive acute infection. Some strains of EMCV cause necrosis of the exocrine pancreas in some species.

References:

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Blanchard JL, et al. Encephalomyocarditis virus infection in African green and squirrel monkeys: comparison of pathologic effects. Lab Anim Sci 37:635-639, 1987.

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Baskin GB. Encephalomyocarditis virus infection, nonhuman primates. In: Jones TC, et al. (eds). Monographs on Pathology of Laboratory Animals: Nonhuman Primates I. Springer-Verlag, 1993, 104-107.

Emerson CL, et al. Antibody responses to two encephalomyocarditis virus vaccines in rhesus macaques (Macaca mulatta). J Med Primatol 25:42-45, 1996.

Huneke RB, et al. Antibody response in baboons (Papio cynocephalus anubis) to a commercially available encephalomyocarditis virus vaccine. Lab Anim Sci 48:526-528, 1998.

MONKEYPOX

Etiology: Orthopoxvirus that is immunologically related to smallpox and vaccinia

Transmission: Monkeypox is a zoonotic disease of monkeys and humans in tropical rain forests of western and central Africa. Old and New World monkeys, as well as apes, are susceptible. The animal reservoir remains unknown, but is possibly squirrels and probably is not monkeys. The disease occurs sporadically, not epidemically.

Clinical: Vaccinia is protective, but hasn't been used since 1980. Monkeypox in children resembles discrete ordinary smallpox, except lymphadenopathy occurs commonly in monkeypox. Human-to-human transmission has occurred. In monkeys, the disease may be mild to fatal. Usually, cutaneous papules 1 to 4 mm in diameter become pustules and then crust over and drop off, leaving small scars. In more severe disease, facial edema, dyspnea, oral ulcers, and lymphadenopathy are seen.

Pathology: Hyperplasia and necrosis of epidermis, with swelling of keratinocytes and large eosinophilic intracytoplasmic inclusions point to monkeypox. Visceral lesions can occur.

References:

Von Magnus P, et al. A pox-like disease in cynomolgus monkeys. Acta Pathol Microbiol Scand 46:156-176, 1959.

Prier JE, et al. A pox disease of monkeys. Ann NY Acad Sci

Gispen R, et al. Histopathological and virological studies on monkeypox. Arch Ges Virusforsch 21:205-216, 1967.

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Centers for Disease Control. Human monkeypox - Kasai Oriental, Zaire, 1996-1997. MMWR 46:304-307, 1997.

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YABAPOX

Etiology: Poxvirus

Transmission: The mosquito is the vector.

Clinical: Natural infections have occurred in rhesus and baboons. Humans are also susceptible. Rapidly growing subcutaneous nodules up to 4 cm in diameter appear on the head and limbs. These nodules spontaneously slough and heal in 6 to 12 weeks.

Pathology: Unlike other poxviruses, yabapoxvirus infects histiocytes rather than epithelial cells. Yabapoxvirus induces subcutaneous proliferation of round to polygonal histiocytes which often contain eosinophilic cytoplasmic inclusions. These are usually described as benign histiocytomas, similar to lumpy skin disease of cattle.

References:

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BENIGN EPIDERMAL MONKEYPOX (TANAPOX, OrTeCaPOX)

Etiology: Poxvirus unrelated to smallpox

Clinical: This disease infects macaques and humans. Multiple crusted macules, which heal in 3 to 4 weeks,

appear on the face and arms.
Pathology: Epidermal hyperplasia and necrosis are evident, with the epithelial cells swollen and containing eosinophilic cytoplasmic inclusion bodies.
References:

Hall AS, et al. A contagious pox disease in monkeys. J Am Vet Med Assn 151:833-838, 1967.

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Downie AW, et al. Tanapox: a new disease caused by a poxvirus. Brit Med J 1:363-368, 1971.

MOLLUSCUM CONTAGIOSUM
Etiology: Poxvirus unrelated to smallpox
Clinical: Smooth-surfaced, hemispheric, waxy, umbilicated epithelial papules, 3-8 mm in diameter, may appear anywhere on skin, but especially on the eyelid and groin in humans and chimpanzees.
Pathology: There is marked acanthosis with large basophilic intracytoplasmic inclusion bodies that become more prominent towards the skin surface.
References:

Douglas JD, et al. Molluscum contagiosum in chimpanzees. J Am Vet Med Assn 151:901-904, 1967.

MEASLES
Etiology: Human measles virus (Paramyxoviridae:Morbillivirus). Some species of NHP may also be susceptible to some strains of canine distemper virus, which is closely related to measles.
Transmission: Respiratory. Measles is a human virus and humans are the reservoir. Measles is not a natural disease of nonhuman primates, but is acquired through contact with humans.
Clinical: Measles can affect apes, macaques, baboons, African green monkeys, marmosets, and squirrel monkeys, and probably others. The disease may be subclinical or may consist of a maculopapular rash, conjunctivitis, facial erythema, respiratory difficulty, and diarrhea (especially in marmosets and owl monkeys). Measles virus infection causes temporary immunosuppression, and may affect research results. Human measles vaccine and veterinary canine distemper/measles vaccines will protect nonhuman primates from disease.
Pathology: Focal necrosis on oral mucous membranes (Köplik's spots), maculopapular to vesicular rash, interstitial pneumonia, and gastroenterocolitis. Syncytial cells and intranuclear and intracytoplasmic inclusion bodies can be seen. In marmosets and owl monkeys measles is an often fatal gastroenterocolitis rather than a predominantly respiratory infection. Measles virus is immunosuppressive.
References:

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Lowenstine LJ. Measles virus infection, nonhuman primates. In: Jones TC, et al. (eds). Monographs on Pathology of Laboratory Animals: Nonhuman Primates I. Springer-Verlag, 1993, 108-118.

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CALLITRICHID HEPATITIS

Etiology: Lymphocytic choriomeningitis virus (callitrichid hepatitis virus). Arenavirus.
Transmission: LCM virus is endemic in mice worldwide. LCM may be spread to callitrichids by feeding pinkies or through contact with mouse urine or oral secretions.
Clinical: LCM affects several species of tamarins and marmosets (Callitrichidae), which may be found dead or may die after showing weakness and anorexia for several days. They may develop seizures and respiratory distress.
Pathology: Jaundice, subcutaneous and intramuscular hemorrhage, hepatosplenomegaly, and pleuropericardial effusions are seen grossly. Microscopic changes consist of hepatocellular swelling and necrosis, lymphocytic and neutrophilic infiltrates, acidophilic bodies, and portal phlebitis. Other possible lesions include meningitis, encephalitis, gliosis, necrosis in the spleen and lymph nodes, and interstitial pneumonia. No inclusion bodies are present. Enveloped virus-like particles 85-105 nm in diameter can be demonstrated in the RER and Golgi of degenerated hepatocytes by electron microscopy.
References:

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Montali RH, et al. Pathology and immunohistochemistry of callitrichid hepatitis, an emerging disease of captive New World primates caused by lymphocytic choriomeningitis virus. Am J Pathol 147:1441-1449, 1995.

ADENOVIRUS
Etiology: Adenovirus (numerous)
Clinical: There are usually no clinical signs, although adenovirus can frequently be isolated from the intestine and lung of healthy animals. In some cases, conjunctivitis and respiratory infections, diarrhea, and pancreatitis can be seen in rhesus monkeys. Severe infections develop in immunodeficient animals.
Pathology: In immunodeficient monkeys, necrotizing alveolitis and bronchiolitis, pneumonia, necrotizing pancreatitis, and enteritis due to adenovirus may be found at necropsy. Intranuclear inclusions vary from small and eosinophilic to large, basophilic, and "smudgy." Intranuclear inclusions may be found in gastrointestinal epithelial cells, unassociated with other lesions.
References:

Boyce JT, et al. Simian adenoviral pneumonia. Am J Pathol 91:259-276, 1978.

Chandler FW, et al. Pancreatitis associated with an adenovirus in a rhesus monkey. Vet Pathol 11:165-171, 1974.

Baskin GB, et al. Adenovirus enteritis in SIV-infected rhesus monkeys. J Inf Dis 160:905-907, 1989.

Umemura T, et al. Aspiration pneumonia with adenovirus infection in a Japanese macaque (Macaca fuscata fuscata). Lab Anim 19:39-41, 1985.

Landon JC, et al. Viral induced simian conjunctivitis. Nature 222:683-684, 1969.

Martin BJ, et al. Pancreatitis associated with simian adenovirus 23 in a rhesus monkey. Lab Anim Sci 41:382-384, 1991.

PAPILLOMAVIRUS
Etiology: Papillomavirus
Clinical: Papillomas appear on the skin and oral or genital mucosa.
Pathology: Focal hyperkeratosis, parakeratosis, and acanthosis occur. Papillomavirus antigens can be demonstrated by immunohistochemistry and virions by TEM.
References:

Sundberg JP, et al. Papillomavirus infections. In: Jones TC, et al. (eds). Nonhuman Primates II, Monographs on Pathology of Laboratory Animals, ILSI, Springer-Verlag, New York, 1993, 1-8.

FOCAL EPITHELIAL HYPERPLASIA
Etiology: Papovavirus (papillomavirus)
Clinical: Circumscribed, soft elevations of the oral mucosa of the lips, tongue, and gingiva occur in otherwise healthy animals. This is usually a benign condition that may persist for years or may spontaneously regress. Fairly common in chimpanzees, but also reported in howler monkeys (Alouatta fusca). A similar condition occurs in humans.
Pathology: Focal acanthosis with koilocytosis and mild chronic inflammation are seen. Virions can be demonstrated by TEM in about 50% of cases.
References:

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SV40

Etiology: Papovavirus (polyomavirus subgroup)

Transmission: SV40 is shed in urine.

Clinical: SV40 is a natural virus of rhesus monkeys. There are usually no clinical signs, but widespread latent infection in wild and captive macaques has been reported. SV40 can cause CNS and respiratory signs in immunodeficient animals.

Pathology: SV40 does not cause lesions in immunocompetent animals, but interstitial pneumonia, renal tubular necrosis, encephalitis, and demyelination similar to progressive multifocal leukoencephalopathy can be found in immunocompromised monkeys. PML probably represents a reactivated latent infection, whereas pneumonia, nephritis, and meningeoencepalitis may represent primary infections. Lesions in the brain may have the typical distribution of PML or may be around ventricles (particularly in brainstem) and in the superficial cortex. Astrocytes and oligodendrocytes are infected. Large basophilic intranuclear inclusions can be seen in lung, oligodendroglia, and renal tubular epithelium.

References:

Gribble DH, et al. Spontaneous progressive multifocal leukoencephalopathy (PML) in macaques. Nature 254:602-604, 1975.

Holmberg CA, et al. Isolation of simian virus 40 from rhesus monkeys (Macaca mulatta) with spontaneous progressive multifocal leukoencephalopathy. J Inf Dis 136:593-596, 1977.

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Lednicky JA, et al. Natural isolates of simian virus 40 from immunocompromised monkeys display extensive genetic heterogeneity: new implications for polyomavirus disease. J Virol 72:3980-3990, 1998.

Simon MA, et al. Association of simian virus 40 with a central nervous system lesion distinct from progressive multifocal leukoencephalopathy in macaques with AIDS. Am J Pathol 154:437-446, 1999.

SIMIAN HEMORRHAGIC FEVER VIRUS

Etiology: Arterivirus

Transmission: Simian hemorrhagic fever virus is endemic in some wild Patas monkeys (Erythrocebus patas) and possibly other African species (African green monkeys, baboons), which remain persistently viremic, but asymptomatic for life. Animals may be viremic without antibody. Transmission from Patas to macaques appears to require parenteral exposure to blood or body fluids. The virus spreads much more readily among macaques by contact or aerosol.

Clinical: SHFV causes explosive epizootics with nearly 100% mortality in macaques. Clinical signs in macaques include fever, anorexia, depression, facial edema, epistaxis, and cutaneous and subcutaneous hemorrhage. Severely elevated LDH, disseminated intravascular coagulation, and thrombocytopenia usually occur. Some asymptomatic macaques from southeast Asia have antibodies to SHF, suggesting there may be additional less pathogenic viral strains.

Any epizootic of hemorrhagic disease should be reported to the Special Pathogens Branch, Centers for Disease Control, Atlanta, GA.

Pathology: Gross lesions are variable, may be absent, and are seen only in the final stage of disease. Petechial hemorrhage on mucosal and serosal surfaces, hemorrhage and necrosis of the mucosa of the proximal duodenum, splenomegaly, and splenic lymphoid follicles ringed with a zone of bright red hemorrhage may be found. Microscopic changes consist of lymphoid necrosis, vasculitis, hemorrhage, and intravascular fibrin deposition (DIC). Large amounts of fibrin are present in splenic cords. Lymphohistiocytic meningoencephalitis is occasionally present. Hepatic necrosis with Councilman's bodies is not a feature of simian hemorrhagic fever, unlike other hemorrhagic fevers. Additionally, in SHF, aspartate aminotransferase is found in greater amounts than alanine aminotransferase, while the reverse is true in the other hemorrhagic fevers.

References:

Palmer AE, et al. Simian hemorrhagic fever. I. Clinical and epizootiologic aspects of an outbreak among quarantined monkeys. Am J Trop Med Hyg 17:404-412, 1968.

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Smith SL, et al. Sequence of the 3' end of the simian hemorrhagic fever virus genome. Gene 191:205-210, 1997.

EBOLA VIRUS

Etiology: Mononegavirales:Filoviridae:Filovirus:Ebola. Subtypes Sudan, Zaire, Ivory Coast, Reston. Closely related to Marburg virus. The Asian origin of Ebola-Reston is unclear. Ebola is a BSL-4 agent.

Transmission: The natural reservoir host of filoviruses is unknown. Monkeys are probably not a primary virus reservoir. Most human infections have occurred via contact with blood and body fluids. The virus is shed in the urine and oronasal secretions. Aerosol transmission has been documented in monkeys.

Clinical: African strains of Ebola cause fatal hemorrhagic fever in humans and chimpanzees. Humans can become infected with Ebola-Reston, but do not become ill. Ebola-Reston causes a fatal disease in Philippine cynomolgus monkeys, and 5-10% of rhesus, African green, and cynomolgus monkeys imported from Africa and Asia (Philippines, Indonesia, Mauritius, China) are seropositive. Testing must be done with paired serum—single specimens are not useful because low IFA titers are uninterpretable. The incubation period in monkeys is about 2-14 days. Fever, weight loss, anorexia, lethargy, coma, hemorrhage, rash, and diarrhea are common symptoms. Severely elevated LDH and thrombocytopenia have been noted. Survivors clear virus in about 3 weeks. There is no evidence of persistent infections in monkeys. Healthy monkeys with low titers are probably not infected. African filoviruses (Ebola-Zaire>Ebola-Sudan) are more pathogenic than Asian (Ebola-Reston). African green monkeys are more resistant to disease than macaques. Wild chimpanzees have had fatal infections with African Ebola virus.

Pathology: Maculopapular rash, splenomegaly, widespread petechial hemorrhages, hemorrhage in proximal duodenum, and interstitial pneumonia are seen. Lymphoid necrosis, massive fibrin deposition in the spleen, hepatic necrosis, necrosis of the adrenal cortex and pulmonary bronchiolar and alveolar epithelium, and interstitial nephritis may be observed. Amphophilic cytoplasmic inclusion bodies may be seen in many tissues including the liver, adrenal gland, and spleen. Extensive viral replication in tissue

macrophages and interstitial fibroblasts is evident. Much of the necrosis may be secondary to ischemia. Viral antigen or RNA can be detected in tissues to confirm the diagnosis. The presence of hepatocellular necrosis, necrosis of the zona glomerulosa of the adrenal cortex, and interstitial pneumonia may help differentiate Ebola and Marburg from simian hemorrhagic fever.

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MARBURG

Etiology: Marburg virus (filovirus)

References:

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Johnson BK, et al. Marburg, Ebola, and Rift Valley fever virus antibodies in East African primates. Trans R Soc Trop Med hyg 76:307-310, 1982.

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Schou S, et al. Marburg and Ebola virus infections in laboratory nonhuman primates: a literature review. Comp Med (Lab Anim Sci) 50:108-123, 2000.

HEPATITIS A VIRUS

Etiology: Picornavirus

Transmission: Fecal-oral

Clinical: Humans, chimpanzees, marmosets, owl monkeys, macaques, and African green monkeys are susceptible to infection. Seroconversion and elevation of transaminases are usually the only clinical evidence of infection. Some HAV isolates may be unique to

nonhuman primates. There is a zoonotic potential. A vaccine (Havrix) is available. HAV is common in cynos imported for the pharmaceutical industry.
Pathology: Periportal and parenchymal mononuclear inflammation, slight focal hepatocellular degeneration and necrosis, acidophilic bodies, and Kupffer cell hyperplasia are common findings. Hepatic enzymes are often elevated, which may compromise toxicology studies.
References:

Hinthorn DR, et al. An outbreak of chimpanzee-associated hepatitis. J Occupational Med 16:388-391, 1974.

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HEPATITIS B VIRUS
Etiology: Hepadnavirus. Chimpanzees, gibbons, orangutans, and woolly monkeys probably have species-specific variants.
Transmission: Hepatitis B may be transmitted through infected blood, saliva, or semen. Parenteral inoculation or intimate contact is required.
Clinical: HBV infects humans, chimpanzees, gibbons, gorillas, and possibly cynomolgus monkeys (Macaca fascicularis). Usually there are no clinical signs other than seroconversion and elevated transaminases. A vaccine is available.
Pathology: Chronic periportal inflammation and focal hepatocellular necrosis are found in the liver.
References:

Kornegay RW, et al. Subacute nonsuppurative hepatitis associated with hepatitis B virus infection in two cynomolgus monkeys. Lab Anim Sci 35:400-404, 1985.

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Popper H, et al. The pathology of viral hepatitis in chimpanzees. Virchows Arch A Path Anat Histol 387:91-106, 1980.

Animal Models of Hepatitis. ILAR Journal. 42:73-177, 2001. Entire issue devoted to this subject.

MacDonald DM, et al. Detection of hepatitis B virus infection in wild-born chimpanzees (Pan troglodytes verus): phylogenetic relationships with human and other primate genotypes. J Virol 74:4253-4262, 2000.

HEPATITIS C VIRUS

Etiology: Hepatitis C virus

Clinical: Only humans and chimpanzees are known to be susceptible.

References:

Abe K, et al. Three different patterns of hepatitis C virus infection in chimpanzees. Hepatol 15:690-695, 1992.

Abe K, et al. Lack of susceptibility of various primates and woodchucks to hepatitis C virus. J Med Primatol 22:433-434, 1993.

Animal Models of Hepatitis. ILAR Journal. 42:73-177, 2001. Entire issue devoted to this subject.

ORTHOREOVIRUS

Etiology: Orthoreovirus

Clinical: Sporadically affects baboons. Affected animals show disorientation, ataxia, and paresis.

Pathology: Lesions consist of lymphoplasmacytic perivascular cuffing in brain, spinal cord, and meninges as well as microglial nodules, demyelination, axonal degeneration, vacuolization, and hemorrhage in the CNS.

References:

Leland MM, et al. Outbreak of orthoreovirus-induced meningoencephalomyelitis in baboons. Comp Med (Lab Anim Sci) 50:199-205, 2000.

SIMIAN PARVOVIRUS

Etiology: A macaque parvovirus related to human B19 parvovirus.

Clinical: Simian parvovirus can cause transient anemia when experimentally inoculated into immunocompetent macaques. Progressive anemia has been observed in naturally infected macaques made immunodeficient by SRV or SIV.

Pathology: Dyserythropoiesis and intranuclear inclusion bodies are seen in bone marrow.

References:

O'Sullivan MG, et al. Identification of a novel simian parvovirus in cynomolgus monkeys with severe anemia. J Clin Invest 93:1571-1576, 1994.

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Foresman L, et al. Progressive anemia in a pig-tail macaque with AIDS. Contemp Top 38:20-22, 1999.

RABIES
Etiology: Rabies virus (rhabdovirus)
Clinical: Only 1 spontaneous case of rabies has been reported in nonhuman primates.
References:

Boulger LR. Natural rabies in a laboratory monkey. Lancet 1(444):941-943, 1966.

Karasszon D. Experimental infection of rhesus monkeys with Hogyes' fixed strain of rabies virus. Acta Vet Acad Scien Hungar 19:299-306, 1969.

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Smith RE, et al. Rabies vaccination of captive white-handed gibbons potentially exposed to wild rabies virus. Lab Anim Sci 37:668-669, 1987.

## PRIMATE RETROVIRUSES

### CLASSIFICATION OF RETROVIRUSES

FAMILY: RETROVIRIDAE

| | | |
|---|---|---|
| SUBFAMILY: ONCOVIRINAE | EXAMPLES | |
| GROUP: | | |
| TYPE A | | Murine intracisternal Type A |
| TYPE B | | Mouse mammary tumor virus |
| TYPE C | | Avian subgroup: Avian leukosis<br>Mammalian subgroup: murine leukemia<br>BLV, HTLV-1&2, STLV-1 (have tat gene) |
| TYPE D | | MPMV, SAIDS retrovirus, SMRV, PO-1-Lu |
| SUBFAMILY: LENTIVIRINAE | | |
| | | Visna, Maedi, Ovine Progressive Pneumonia |
| | | Caprine Arthritis Encephalitis Virus<br>Equine Infectious Anemia Virus |

Feline T-lymphotropic Virus

Bovine Immunodeficiency-like Virus

Simian Immunodeficiency Virus

Human Immunodeficiency Virus

SUBFAMILY: SPUMAVIRINAE

Foamy viruses

PRIMATE ONCOVIRUSES

STLV-1 (Retroviridae:oncovirinae:Type C)

Simian T-cell leukemia virus (STLV-1) is closely related (90-95% homologous) to the human T-cell leukemia virus type 1 (HTLV-1), the etiologic agent of adult T-cell leukemia/lymphoma, tropical spastic paraparesis, and HTLV-associated myelopathy. There is a high incidence of natural infection in many wild and captive Old World monkeys, including baboons, African green monkeys, Patas monkeys, various macaques, and chimpanzees. The incidence of infection correlates with age, reaching a peak in animals over 16 years old, and is higher in females than males. Transmission occurs by sexual contact or parenteral inoculation. Neonatal transmission is probably unusual. Persistent infection without seroconversion has not been observed. STLV-1 typically infects CD4+ T-cells in macaques and CD8+ T-cells in African monkeys, but some infected T-cell lines express neither marker.

Although most infected animals remain latently infected and asymptomatic for life, STLV-1 has been associated with lymphoma/leukemia in baboons, African green monkeys, and macaques by seroepidemiology or molecular biological techniques. Most investigators believe STLV-1 is not pathogenic in Asian monkeys. HTLV-1/STLV-1 does not contain recognized oncogenes and integrates monoclonally into the tumor-cell DNA of individuals, but randomly between individuals. Tumorigenesis has been linked to tax, a nonstructural viral gene that activates cellular genes such as the IL-2 receptor (IL-2R). The relationship between cellular activation and tumorigenesis is poorly understood, making STLV-1 in African species an important model for studying the pathogenesis of HTLV-1 associated diseases in man. STLV-1 appears to be nonpathogenic in Asian primates.

There is some evidence that STLV-1 may alter macaque cell surface markers and cytokine profiles.

There are at least 4 molecular subtypes of HTLV-1. Each probably arose from a separate interspecies transfer from monkeys to humans.

References:

Tsujimoto H, et al. Isolation of simian retroviruses closely related to human T-cell leukemia virus by establishment of lymphoid cell lines from various non-human primates. Int J Cancer 35:377-384, 1985.

Ishida T, et al. A field study of infection with human T-cell leukemia virus among Asian primates. Microbiol Immunol 29:839-846, 1985.

Mone J, et al. Simian T-cell leukemia virus type 1 infection in captive baboons. AIDS Res Hum Retrovir 8:1653-1661, 1992.

Saksena NK, et al. Sequence and phylogenetic analyses of a new STLV-1 from a naturally infected tantalus monkey from central Africa. Virol 192:312-320, 1993.

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Homma T, et al. Lymphoma in macaques: association with virus of human T lymphotrophic family. Science 225:716-718, 1984.

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Traina-Dorge V, et al. Immunodeficiency and lymphoproliferative disease in an African green monkey dually infected with SIV and STLV-1. AIDS Res Hum Retrovir 8:97-100, 1992.

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Saksena NK, et al. Seroepidemiologic, molecular, and phylogenetic analyses of simian T-cell leukemia viruses (STLV-1) from various naturally infected monkey species from central and western Africa. Virol 198:1, 1994.

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Vandamme A-M, et al. The simian origins of the pathogenic human T-cell lymphotropic virus type 1. Trends in Microbiology 6:477-483, 1998.

HTLV-1

References:

Kazanji M. HTLV type 1 infection in squirrel monkeys (Saimiri sciureus): a promising animal model for HTLV type 1 human infection. AIDS Res Hum Retrovir 16:1741-1746, 2000.

SIMIAN TYPE-D RETROVIRUSES (Retroviridae:oncovirinae:Type D)

SRV Serogroups:

SRV-1: SAIDS/CA, SAIDS/NE

SRV-2: SAIDS/WA, SAIDS/OR (rhesus and Celebes variants)
SRV-3: Mason-Pfizer monkey virus
SRV-4: SRV from cynos at Berkley, CA
SRV-5: SRV from Chinese rhesus

SRV-Pc: SRV from baboons (Papio cynocephalus)

Squirrel monkey endogenous virus
Langur Endogenous Virus (PO-1-Lu)

Type D retrovirus from talapoins (Miopithecus sp)

All type D viruses to date are of primate origin. SRV-1 is more common in rhesus, while SRV-2 is more common in cynos and pigtails. The preferred nomenclature is D/serotype/laboratory/species. Endogenous type D viruses occur in squirrel monkeys and langurs and related proviral sequences have been identified in African and Asian colobines. The endogenous viruses appear to be nonpathogenic. The exogenous viruses infect many species of macaques, naturally occurring infection in the wild being demonstrated in M. fascicularis (Indonesia, but not those from the Philippines or Seychelles Islands), M. nemestrina (Indonesia), M. radiata (India), M. tonkeana (Sulawesi), and M. mulatta (China). The incidence in captive colonies varies from colony to colony, but can be quite high. Virus can be isolated from peripheral blood mononuclear cells by coculture on Raji cells.

Type D viruses infect B cells, T cells (CD4+ and CD8+), macrophages, epithelial cells (salivary gland, intestine, oral Langerhans cells), and choroid plexus. Vertically infected viremic animals have more widespread provirus than those infected horizontally. The virus is shed in saliva and transmission requires direct physical contact or contact with fomites. Biting, licking, and grooming are probably the usual modes of transmission, although vertical transmission also occurs. Some monkeys (probably infected near birth) become persistently infected but antibody-negative, serving as healthy carriers. Because of this, animals must be screened repeatedly by ELISA and PCR (or culture) to ensure they are virus-free. Experimental formalin-killed whole virus and recombinant vaccines have been used successfully. Strict husbandry procedures to prevent spread by fomites is essential to any eradication program.

Type D viruses induce an immunosuppressive disease in macaques which may be epizootic in previously naive populations or may be enzootic. Exposed animals may develop an antibody response that clears the infection (although virus-negative, antibody-positive animals may still harbor virus in bone marrow or gut), or become intermittently virus-positive with or without antibodies. They may develop an acute or protracted immunodeficiency disorder with or without fibroproliferative lesions. Retroperitoneal fibromatosis and subcutaneous fibrosarcomas have been associated with SRV-2. These may be derived from vascular smooth muscle, and contain virus. Neutropenia, anemia, and terminal lymphopenia are common. Some animals develop persistent generalized lymphadenopathy. Most eventually develop diarrhea, weight loss, bacterial infections, and/or opportunistic infections (CMV, Cryptosporidium, Candida, noma). A few B-cell lymphomas have occurred in cynos, but not in rhesus. The clinical outcome in an

infected individual is related to the antibody (Ab) response. Monkeys that die early in the course of infection have no Ab and high levels of circulating viral antigen (Ag). Monkeys that survive with persistent viremia make intermediate levels of Ab and have intermediate levels of Ag. Monkeys that clear the infection have high levels of Ab and no Ag. Some animals apparently recover from infection. Neutralizing antibody is thus important in protection against SRV. Lesions that appear to be caused directly by type D viruses include lymphoid hyperplasia, which evolves into atrophy, nonsuppurative enteritis, sialoadenitis, and myositis

SRV-Pc is not known to be pathogenic. SRV-1, SRV-2, and MPMV have been cloned and sequenced and several clones are infectious and pathogenic.

Type D virus has very rarely been detected in humans, but the significance of this is unclear. Type D virus infection of humans is very rare to nonexistent.

SRV has compromised many research studies by causing anemia, altered immune responses, altered expression of cell surface markers, altered cytokine profiles, and nonspecific histological changes. In cynos (but not rhesus), the extensive lymphoid hyperplasia induced by SRV may progress to lymphoma.

References:

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Marx PA, et al. Isolation of a new serotype of simian acquired immune deficiency syndrome type D retrovirus from Celebes black macaques (Macaca nigra) with immune deficiency and retroperitoneal fibromatosis.

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Hu S-L, et al. Protection of macaques against simian AIDS by immunization with a recombinant vaccinia virus expressing the envelope glycoproteins of simian type D retrovirus. Proc Natl Acad Sci USA 86:7213-7217, 1989.

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PRIMATE LENTIVIRUSES (Retroviridae:lentivirinae)

HUMAN LENTIVIRUSES

| | |
|---|---|
| HIV-1 | Worldwide |
| HIV-2 | West Africa |

NONHUMAN PRIMATE LENTIVIRUSES

SIVmac
Macaca mulatta (rhesus)
SIVsmm
Cercocebus torquatus atys (sooty mangabey)
SIVmne
Macaca nemestrina (pigtailed macaque)
SIVagm/gri, SIVagm/tan, SIVagm/ver
Cercopithecus sp. (African green monkey)
SIVmnd
Papio sphinx (mandrill)
SIVstm
Macaca arctoides (stump-tailed macaque)
SIVcyn
Macaca fascicularis (cynomolgus monkey)
SIVcpz
Pan troglodytes (chimpanzee)
SIVWCM
Cercocebus torquatus lunulatus (white-crowned mangabey)
SIVSYK
Cercopithecus mitis (Sykes monkey)
SIVHU
Cercocebus MacacaHomo

All retroviruses contain env, pol, and gag genes. Lentiviruses have additional regulatory genes: HIV-1/SIVCPZ contain vif, vpu, vpr, tat, rev, nef; HIV-2/SIVSMM/SIVMAC/SIVMNE/SIVSTM contain vif, vpx, vpr, tat, rev, nef; SIVAGM contains vif, vpx, tat, rev, nef; SIVMND contains vif, vpr, tat, rev, nef.

Human Immunodeficiency Virus type 1 (HIV-1) is the cause of the acquired immunodeficiency syndrome (AIDS) in humans worldwide. HIV-1 probably originated in chimpanzees and has apparently been transmitted to humans on several different occasions. The closest known virus is SIVcpz. The other SIVs are much more closely

related to HIV-2. HIV-2 probably originated from sooty mangabeys. Both sooty mangabeys and chimpanzees are kept as pets and hunted for food in West Africa.

Pigtailed macaques (M. nemestrina) can be acutely infected with high doses of HIV-1 (based on ability to reisolate virus in culture, detection of HIV-1 gag DNA in PBMC, and persistent seroconversion), but do not become viremic or antigenemic. It is difficult to reisolate HIV-1 from PBMC after 8 weeks, although viral DNA can be demonstrated. No virus or antibody can be detected in the CSF. Inoculated animals have not become immunodeficient. The M. nemestrina/HIV-1 model has not proven to be very useful.

Gibbons become persistently infected with HIV-1IIIB, but do not develop disease. Gibbons are endangered and no research colonies exist, making them useless as an animal model.

Chimpanzees are easily infected with small amounts of HIV-1, seroconvert, and virus can be reisolated from serum for a few weeks and from PBMC persistently. Chimps, unlike humans, develop a broad-spectrum antibody response (including antibody-dependent complement-mediated lysis). They do not develop changes in CD4+ lymphocytes or immune function, and do not develop opportunistic infections or other signs of disease. Chimpanzee monocytes/macrophages are resistant to HIV infection, although this can be overcome by in vivo passage. Although chimp CD4+ lymphocytes are readily infected, they produce less virus than human cells. Chimps have more CD8+ suppressor cells than humans. There is not an increased level of apoptosis (programmed cell death) in lymphocytes from HIV-infected chimps and their TH cells are not susceptible to gp120-induced anergy. Chimpanzees have strong lymphokine-activated killer (LAK) cell activity, which humans do not. Chimps do not demonstrate gp120-specific antibody-dependent cellular cytotoxicity (ADCC). They do not show cytotoxic T-lymphocyte (CTL) activity. HIV infection does not appear to cause chronic lymphocyte activation in chimps. Only HIVLai(IIIb) has been titrated in chimpanzees. Additional titrated stocks are needed for challenge studies. About 150 chimps have been infected. A few HIV-1 infected chimpanzees do show signs of progression to AIDS. Provisions for retirement of infected animals must be made.

References:

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Human Immunodeficiency Virus type 2 (HIV-2) (previously HTLV-IV) is the cause of AIDS, or "slim" disease, in western Africa, with little international spread. HIV-2 is apparently less pathogenic than HIV-1. Viral load is lower in HIV-2-infected people than in HIV-1-infected people, until immunosuppression is severe. This may be the reason heterosexual and perinatal infection with HIV-2 is less efficient until the terminal stages of AIDS. HIV-2 is closely related to SIVsm, and may belong to a single, highly diverse group that cannot be separated into distinct phylogenetic lineages by species of origin. The sooty mangabey is probably the natural reservoir and human infection may be a zoonosis. Macaques and baboons can be infected, but HIV-2 infrequently induces disease in macaques and disease does not develop at all in baboons.

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Simian immunodeficiency viruses (SIV) (previously STLV-III) are comprised of at least 13 groups (HIV-2/SIVSMM/SIVMAC/SIVSTM/SIVMNE; HIV-1/SIVCPZ; SIVAGM/SIVWCM; SIVMND1; SIVMND2 ; SIVRCM and SIVSYK; SIVdeb; SIVcol; SIVmon; SIVgsn; SIVmus; SIVtal; SIVlhoest/sun) of related viruses that occur naturally and are indigenous in at least 30 African primates, including Cercopithecus sp (African green monkeys, vervets, grivets, tantalus monkeys, Sykes monkeys), Papio sp (mandrills and anubis), Cercocebus sp (sooty, red capped, and white crowned mangabeys), and Pan troglodytes (chimpanzees). Other, presently uncharacterized, SIVs are known to exist in other species. These animals are persistently infected, but appear to remain

asymptomatic for life. SIVAGM is the most genetically diverse group and has co-evolved with the 4 geographically separate subspecies of African green monkeys (vervets, grivets, tantalus, sabaeus), indicating SIV has been present in this species for a very long time. The natural route of transmission is unknown. Persistent infection without seroconversion has been observed in sooty mangabeys.

Why the natural hosts remain asymptomatic while heterologous hosts develop fatal immunodeficiency is an area of active research. Disease progression in all species appears to be related to virus load. Viral replication occurs throughout the clinically latent period of infection. Humans typically initially respond to HIV with a vigorous cytotoxic T -ymphocyte (CTL) and antibody-dependent cellular cytotoxicity (ADCC) response, but with weak neutralizing antibody and little complement-activating antibody. SIV-infected African green monkeys and sooty mangabeys (the natural hosts) also respond with little neutralizing- or complement-activating antibody. The antibody response is primarily anti-env, with little anti-gag. Viral load in African green monkeys is comparable to that in asymptomatic HIV-1-infected people, while sooty mangabeys carry higher viral loads. Such studies indicate that pathogenicity does not lie in the virus alone, or in a particular gene, but in the virus/host system. HIV-1/SIVMAC and HIV-1/SIVAGM chimeras have been constructed and are being used to understand the molecular functions of each virus.

SIV has also been isolated from several species of macaques (M. mulatta, M. nemestrina, M. fascicularis, M. arctoides) housed in laboratories. SIV does not infect Asian monkeys in the wild. SIVMAC is closely related to SIVSMM and probably represents a cross-species infection that occurred in captivity. SIVSMM is probably the progenitor of SIVMAC, SIVMNE, SIVSTM, and HIV-2. SIVMAC and SIVSMM are highly pathogenic to macaques, producing an immunodeficiency disease. SIVAGM is much less pathogenic in macaques, although disease has been produced in M. nemestrina.

The immunodeficiency disease produced in macaques by SIV from sooty mangabeys or macaques has many features similar to human AIDS. SIV is CD4+ lymphocyte and macrophage tropic, causing depletion of CD4+CD29+ T cells (helper/inducer cells). Some isolates (SIVSMM(PBj)) are highly pathogenic, causing death within days, while others (SIVSMM(Delta/D915) are attenuated. Most isolates commonly used in laboratories cause fatal immunodeficiency within a few months to a few years in most inoculated animals. Experimentally infected macaques initially have a rash and develop lymphadenopathy. This phase persists for a variable time, but eventually lymphoid tissues become depleted and opportunistic infections occur. The most common are CMV, Candida, M. avium-intracellulare, Cryptosporidium, Pneumocystis, Trichomonas, Plasmodium, adenovirus, lymphocryptovirus, and SV40. Lesions thought to be directly caused by SIV include rash, lymphoid hyperplasia, lymphoid depletion, retroviral pneumonia, retroviral encephalitis, giant cell disease, aseptic thrombosis, and glomerulosclerosis. Weight loss, diarrhea, and anemia/thrombocytopenia are common clinical findings. SIV is not oncogenic, but lymphoid neoplasms are common and have been associated with lymphocryptovirus.

Because of the similarities to human AIDS, the SIV-infected macaque has been widely used as an animal model to study pathogenesis, immunoprophylaxis, therapy, and vertical transmission. As with any model, however, there are some dissimilarities and other factors to be aware of when interpreting research results. As examples, retroviral

syncytial cells are seen commonly only in the CNS of AIDS patients, whereas they are frequently widespread in terminally ill SIV-infected macaques. The entire spectrum of neurological lesions seen in AIDS encephalitis has not been reproduced with SIV. SIV has no variability in the V3 loop of the envelope protein, whereas HIV-1 V3 is hypervariable and contains the principal neutralizing domain. This may be important for env-based vaccines and immune recognition of env products may be very important in low-dose mucosal exposures. SIV grown on human cell lines acquires human cellular antigens as it buds through the cell membrane. When such virus is used in vaccine preparations or challenge inocula, the macaque immune system responds to these, providing some protection against infection. This situation has caused the success of early vaccine trials to be re-evaluated.

Zoonotic transfer of SIV from chimpanzees and sooty mangabeys to humans has been documented on at least 8 occasions. SIV-infected monkeys are important in the bushmeat and pet trade in Africa. A few laboratory workers have seroconverted to SIV and virus (SIVHU) has been isolated from one. To date, viral load is low and the worker is asymptomatic with normal immune function. The natural history of SIV infection in man remains unknown. SIV is a zoonotic agent!

From Peeters, et al. Emerg Inf Dis, 2002.

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SPUMAVIRUSES

The foamy viruses are nonpathogenic, but are prevalent and widespread in the tissues of macaques and other primates. They cause life-long infections in monkeys and some are transmissible through sexual contact, blood, or breast-feeding. Humans have become infected with nonhuman primate spumaviruses.

Foamy viruses frequently contaminate primary cell cultures, particularly kidney and lymphocyte, and should not be mistaken for other agents. Foamy virus produces vacuolated multinucleated cells in many cultures, mimicking the CPE of other viruses. The RT characteristics are similar to lentiviruses. By TEM, spumaviruses are

morphologically distinct from other retroviruses because they are decorated with prominent radially arranged spikes.

References:

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## PRION DISEASES/SPONGIFORM ENCEPHALOPATHY

References:

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# FUNGAL DISEASES

## CANDIDIASIS

Etiology: Candida albicans

Transmission: Candida albicans is ubiquitous.

Clinical: Candida albicans infection (as opposed to colonization) occurs in immunodeficient or debilitated animals or animals on long-term antibiotic therapy. Dysphagia associated with white pseudomembranes on oral mucous membranes occurs.

Pathology: Pathological findings consist of white pseudomembrane on oral and esophageal mucous membranes. The underlying tissue may be ulcerated. Organisms can be seen on H&E, but are best studied with PAS or GMS stains. Septate pseudohyphae and oval budding blastospores are seen in the superficial epithelium, but rarely invade past the basement membrane.

References:

Migaki G, et al. Mycotic infections of the alimentary tract of nonhuman primates: a review. Vet Pathol 19(Suppl 7):93-103, 1982.

McCullough B, et al. Multifocal candidiasis in a capuchin monkey (Cebus apella). J Med Primatol 6:186-191, 1977.

PNEUMOCYSTIS
Etiology: Pneumocystis carinii (there may be species-specific variants)
Transmission: Aerosol
Clinical: Clinically apparent disease occurs only in immunodeficient animals and may be horizontally acquired or reactivated latent infections. Fever, dyspnea, and cough are seen clinically.
Pathology: Lesions are usually restricted to the lung, but generalized infections have been described in severely immunodeficient humans. Foamy eosinophilic intra-alveolar exudate mixed with alveolar macrophages, interstitial lymphocytic infiltration, and hypertrophy of alveolar lining cells have been noted. The organisms are found in the exudate and along alveolar walls. Cysts are easily demonstrable with GMS stain.
References:

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HISTOPLASMOSIS
Etiology: Histoplasma capsulatum var capsulatum, Histoplasma capsulatum var duboisii (African histoplasmosis)
Transmission: Inhalation of spores of H. capsulatum from soil rich in bird or bat excreta is the most common means of transmission, although H. duboisii may be spread by dermal contact and may have a long incubation period.
Clinical: H. capsulatum infection is usually latent. Clinical disease can occur with heavy exposure or may be associated with pre-existing lung lesions. Disseminated disease can result from immunodeficiency. H. capsulatum is rare in monkeys.

H. duboisii has been reported only from Africa and only in humans and baboons. It affects skin, lymph node, and bone and is much larger than H. capsulatum.
Pathology: Granulomatous inflammation in affected organs is seen on necropsy. Histiocytes are often filled with the yeast form of the organism. H. capsulatum 2-4 μm, H. duboisii 7-15 μm.
References:

Baskin GB. Disseminated histoplasmosis in an SIV-infected rhesus monkey. J Med Primatol 20:251-253, 1991.

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BLASTOMYCOSIS

Etiology: Blastomyces dermatiditis

Transmission: Blastomyces dermatiditis is common in soil and is transmitted by inhalation.

Clinical: Papules or draining abscesses are found on the skin. Blastomycosis has occasionally been seen in monkeys housed outdoors in the US.

Pathology: Granulomatous nodules with necropurulent centers in lung, skin and bone are found microscopically. Yeasts, found in the exudate, are 8-25 μm, have single broad-based buds, and are visible with PAS & GMS. In South American monkeys, it may be necessary to differentiate Blastomyces dermatiditis from Paracoccidioides brasiliensis, which has multiple buds.

COCCIDIOIDOMYCOSIS

Etiology: Coccidioides immitis

Transmission: Inhalation of spores.

Clinical: The disease is limited to arid regions of North and South America. Infection is usually subclinical, but respiratory disease or dissemination, particularly to bone, may occur.

Pathology: Coccidioides induces necropurulent granulomatous inflammation which contains the organism. Double contoured 20-80 μm spherules that may contain smaller endospores are seen.

References:

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CRYPTOCOCCOSIS

Etiology: Cryptococcus neoformans

Transmission: Inhalation

Clinical: CNS and ocular abnormalities are the usual clinical signs.

Pathology: Gelatinous nodules or cystic areas are seen grossly, especially on the meninges. Sparse granulomatous inflammation surrounds abundant yeasts. The organism is 5-10 μm, has single buds, and is surrounded by a thick mucin-positive capsule.
References:

Garner FM, et al. A systemic cryptococcosis in 2 monkeys. J Am Vet Med Assn 155:1163-1168, 1969.

Sly DL, et al. Disseminated cryptococcosis in a patas monkey (Erythrocebus patas). Lab Anim Sci 27:694-699, 1977.

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## SYSTEMIC PATHOLOGY

### PERIODONTAL DISEASE

Etiology: Although the etiology is unknown, Shigella sp has occasionally been found in culture. Noma is associated with SRV infection.
Clinical: Animals will usually continue to eat. Gingival redness is often associated with dental calculus. Squirrel monkeys are particularly susceptible to calculus.
Pathology: Lesions vary from slight reddening of the gums to necrotizing ulcerative gingivitis. Gingival bleeding is common. Interproximal craters with alveolar bone destruction are seen in severe cases. Spirochetes can be demonstrated in gingival connective tissue by silver staining. Shigella flexneri, serotype 4, is sometimes present.

Gangrenous necrosis of bone and overlying soft tissues can be found in noma (Cancrum oris), which is associated with type D retrovirus infection.

References:

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Schou S, et al. Nonhuman primates used in studies of periodontal disease pathogenesis: A review of the literature. J Periodontol 64:497-508, 1993.

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## FIBROUS GINGIVAL HYPERPLASIA

Etiology: The etiology is unknown, but gingival hyperplasia may be a sequela to chronic gingivitis. There is a possibility that it is familial in macaques. Phenytoin may also produce gingival enlargement.

Clinical: There are usually no clinical signs.

Pathology: One can find mild to marked firm enlargement of the marginal and alveolar gingiva, including the interdental papillae. The hyperplastic tissue is normal in color and may completely cover the teeth. Microscopically, the tissue is dense collagen with little inflammation.

References:

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## ACUTE GASTRIC DILATATION (BLOAT)

Etiology: Bloat is thought to be multifactorial, including food restriction, overeating, and anesthesia. Usually it occurs in caged monkeys. Clostridium perfringens type A can be isolated in large numbers and may be responsible for gas production.

Clinical: Both OWM and NWM are usually found dead with their abdomens enlarged and taut.

Pathology: The stomach is markedly distended with gas and brown watery fluid. The intestine is congested. Subcutaneous emphysema occurs if the stomach ruptures. Affected monkeys die of respiratory insufficiency, impaired venous return, and shock. The large amount of fluid and ingesta differentiates bloat from postmortem distension.

References:

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Newton WM, et al. Acute bloat syndrome in stumptailed macaques: a report of four cases. Lab Anim Sci 21:193-196, 1971.

DIVERTICULOSIS

Etiology: Diverticulosis could possibly be congenital and could be the result of spastic contractions of the colon.

Clinical: Usually, there are no overt symptoms. Abdominal pain is sometimes noted.

Pathology: Saccular protrusions along taenia coli and muscular hypertrophy have been seen. The colon may become impacted and inflamed.

CARDIOMYOPATHY

Etiology: The cause is unknown in NWM. Cardiomyopathy is associated with aging in all species. In great apes, it is associated with obesity.

Clinical: Ascites, congestive heart failure, and posterior paralysis have been seen in squirrel monkeys and marmosets. In the Great Apes, cardiomyopathy is common from middle age onward and is associated with obesity. In some cases there may be no clinical signs. Sudden death, especially during anesthesia, is not unusual. Cardiomyopathy is common in old macaques, but seldom causes clinical disease.

Pathology: Cardiomegaly, myocardial hypertrophy, fibrosis, atrial thrombosis, saddle thrombus in the aorta, ascites, and pulmonary edema are seen in NWM. In apes, myocardial fibrosis and hypertrophy is most often seen.

References:

Goxalo A, et al. Spontaneous cardiomyopathy and nephropathy in the owl monkey (Aotus sp.) in captivity. J Med Primatol 21:279-284, 1992.

Schulman FY, et al. Fibrosing cardiomyopathy in captive western lowland gorillas (Gorilla gorilla gorilla) in the United States: a retrospective study. J Zoo Wildlife Med 26:43-51, 1995.

Tolwani RJ, et al. Dilative cardiomyopathy leading to congestive heart failure in a male squirrel monkey (Saimiri sciureus). J Med Primatol 29:42-45, 2000.

AMYLOIDOSIS

Etiology: The cause of amyloidosis is unknown.

Clinical: Rhesus and pigtailed macaques, baboons, and chimpanzees develop chronic diarrhea, weight loss, and hepatomegaly, often associated with osteoarthritis.

Pathology: Amyloid is deposited in the spleen, lymph nodes, liver, and lamina propria of the gastrointestinal tract. Often, nonspecific chronic inflammation is seen in the colon. Lymphoid atrophy is seen in some cases.
References:

Gribble DH. Granulomatous enteritis and intestinal amyloidosis in nonhuman primates. Path Vet 9:81-82, 1972.

Casey HW, et al. Generalized amyloidosis in a rhesus monkey. Lab Anim Sci 22:587-593, 1972.

Chapman WL, et al. Amyloidosis in rhesus monkeys with rheumatoid arthritis and enterocolitis. JAVMA 171:855-858, 1977.

Benditt EP, et al. Chemical characteristics of the substance of typical amyloidosis in monkeys. Acta Path Microbiol Scand 80: suppl 233:103-108, 1972.

Blanchard J, et al. Amyloidosis in rhesus monkeys. Vet Pathol 23:425-430, 1986.

Slattum MM, et al. Amyloidosis in pigtailed macaques (Macaca nemestrina): pathologic aspects. Lab Anim Sci 39:567-570, 1989.

Ellsworth L, et al. Factors associated with intestinal amyloidosis in pigtailed macaques (Macaca nemestrina). Lab Anim Sci 42:352-355, 1992.

Blanchard JL. Generalized amyloidosis, nonhuman primates. Monographs on Pathology of Laboratory Animals: Nonhuman Primates I, Springer-Verlag, 1993, 194-197.

INSULAR AMYLOIDOSIS
Etiology: Unknown
Clinical: Diabetes mellitus often develops. Macaca nigra and Macaca fascicularis have a higher incidence than other species. Also common in baboons.
Pathology: Amyloidosis of the islets of Langerhans is seen, but is not associated with amyloid deposition in other organs.
References:

Palotay JL, et al. Insular amyloidosis in spontaneously diabetic nonhuman primates. Vet Pathol 19(Suppl 7):181-192, 1982.

Cromeens DM, et al. Insular amyloidosis and diabetes mellitus in a crab-eating macaque (Macaca fascicularis). Lab Anim Sci 35:642-645, 1985.

Howard CF. The insular amyloidotic lesion and its relationship to diabetes mellitus, Macaca nigra. In: Jones TC, et al. (eds). Monographs on Pathology of Laboratory Animals: Nonhuman Primates II. Springer-Verlag, 1993, 197-202.

Wagner JD, et al. Diabetes mellitus and islet amyloidosis in cynomolgus monkeys. Lab Anim Sci 46:36-41, 1996.

Wagner JD, et al. Naturally occurring and experimental diabetes in cynomolgus monkeys: a comparison of carbohydrate and lipid metabolism and islet pathology. Toxicol Pathol 29:142-148, 2001.

Hubbard GB, et al. Spontaneous pancreatic islet amyloidosis in 40 baboons. J Med Primatol 31:84-90, 2002.

KAOLIN GRANULOMA

Etiology: Kaolin-containing antidiarrheals aspirated or delivered subcutaneously or peritracheally.
Clinical: Pneumonia and swelling in throat area.
Pathology: Macrophages filled with birefringent crystals of kaolin are found in the lung or peritracheal tissues.

PROLIFERATIVE ARTERIOPATHY
Etiology: Unknown
Clinical: The lesion has been observed in rhesus and stumptails (M. arctoides), but is not associated with clinical signs.
Pathology: Irregular eccentric nodular thickenings composed of proliferating cells in the tunica media are found in the walls of medium-sized arteries of the renal cortex. They sometimes occur in whorls, with gaps in the internal elastic membrane. Mononuclear inflammatory cells are in the adventitia. There are no other associated lesions.
References:

Beach JE, et al. An unusual form of proliferative arteriopathy in macaque monkeys. Exp Molec Pathol 21:322-338, 1974.

INTESTINAL ADENOCARCINOMA IN MACAQUES
Etiology: Unknown
Clinical: Affects older (over 15 years) rhesus monkeys with fairly high incidence. Affected animals usually have diarrhea and weight loss. The only distinguishing feature from other causes of this syndrome is a palpable abdominal mass. This is the only neoplasm that regularly affects immunocompetent macaques.
Pathology: Mucinous adenocarcinoma, most frequently located at the ileocecal-colic junction. These tumors are locally invasive and some metastasize.
References:

Plentl AA, et al. Adenocarcinoma of the large intestine in a pregnant rhesus monkey (Macaca mulatta). Report of a case. Folia Primatol (Basel) 8:307-13, 1968.

Fanton JW, et al. Adenocarcinoma of the small intestine in two rhesus monkey. J Am Vet Med Assoc 185(11):1377-8, 1984.

Lembo TM, et al. Stenosing colonic adenocarcinoma in a female rhesus monkey. J Med Primatol 26:229-32, 1997.

Johnson EH, et al. Colonic adenocarcinoma in a rhesus macaque (Macaca mulatta). J Med Primatol 25:435-8, 1996.

Uno H, et al. Colon cancer in aged captive rhesus monkeys (Macaca mulatta). J Med Primatol 44:19-27, 1998.

Kerrick GP, et al. Metastatic large intestinal adenocarcinoma in two rhesus macaques (Macaca mulatta). Contemp Topics 39:40-42, 2000.

Rodriguez NA, et al. Clinical and histopathological evaluation of 13 cases of adenocarcinoma in aged rhesus macaques (Macaca mulatta). J Med Primatol 31:74-83, 2002.

COLON CARCINOMA OF TAMARINS
Etiology: Unknown

Clinical: There is a high incidence in cotton-top tamarins (Saguinus oedipus oedipus), which develop diarrhea, weight loss, intestinal obstruction, and palpable abdominal masses.
Pathology: Carcinomas arise in association with preexisting chronic ulcerative colitis. Poorly differentiated mucinous carcinomas, usually in structureless masses, have been noted. Early lesions are best demonstrated with PAS. Colon carcinomas are often multiple and often metastasize.
References:

Chalifoux LV, et al. An analysis of the association of gastroenteric lesions with chronic wasting syndrome of marmosets. Vet Pathol 19:141-162, 1982.

Chalifoux LV, et al. Colonic adenocarcinoma associated with chronic colitis in cotton-top marmosets. Gastroenterology 80:942-946, 1981.

Lushbaugh CC, et al. Spontaneous colonic adenocarcinoma in marmosets. Prim Med 10:119-134, 1978 (Karger, Basel).

Dufrain RJ. Is cancer of the colon familial in cotton-top tamarins? Cancer Genetics and Cytogenetics l4:83-87, 1985.

Lushbaugh C, et al. Histology of colon cancer in Saguinus oedipus oedipus. Digestive Dis Sci 30:119s-125s, 1985.

Lushbaugh C, et al. Histology of colitis: Saguinus oedipus oedipus and other marmosets. Digestive Dis Sci 30:45s-51s, 1985.

Russel RG, et al. Coronavirus-like particles and Campylobacter in marmosets with diarrhea and colitis. Digestive Dis Sci 30: 72s-77s, 1985.

Estes MK. Evaluating viral agents in marmoset colitis. Digestive Dis Sci 30:80s-81s, 1985.

Clapp NK, et al. Natural history and pathology of colon cancer in Saguinus oedipus. Digestive Dis Sci 30:107s-113s, 1985.

Yardley JH. Comments on comparative pathology of colonic neoplasia in cotton-top marmosets. Digestive Dis Sci 30:126s-133s, 1985.

Kirkwood JK, et al. Adenocarcinoma of the large bowel and colitis in captive cotton-top tamarins. J Comp Pathol 96:507-515, 1986.

Chalifoux LV, et al. Adenocarcinoma, colon, cotton-top tamarin. In: Jones TC, et al. (eds). Monographs on Pathology of Laboratory Animals: Nonhuman Primates II. Springer-Verlag, 1993, 87-94.

Johnson LD, et al. A prospective study of the epidemiology of colitis and colon cancer in cotton-topped tamarins. Gastroenterology 110:102-115, 1996.

Wood JD, et al. Colitis and colon cancer in cotton-top tamarins (Saguinus oedipus oedipus) living wild in their natural habitat. Digest Dis Sci 43:1443-1453, 1998.

Bertone ER, et al. Family history as a risk factor for ulcerative colitis-associated colon cancer in cotton-top tamarin. Gastroenterology 114:669-674, 1998.

Brack M. Gastrointestinal tumors observed in nonhuman primates at the German primate center. J Med Primatol 27:319-324, 1998.

Gore MA, et al. Callitrichid nutrition and food sensitivity. J Med Primatol 30:179-184, 2001.

ENDOMETRIOSIS

Etiology: Endometriosis results from the implantation of normal endometrial tissue in ectopic locations. This is thought to occur through retrograde menstruation through the fallopian tube.
Clinical: Endometriosis occurs only in species with a true menstrual cycle (OWM, apes, humans). Depending on the site of implantation and of associated adhesions, abdominal swelling, constipation, and/or uremia may result.
Pathology: Multiple cysts that contain a chocolate-brown fluid, extensive fibrosis, hemosiderosis, and adhesions are found in the lower abdominal and pelvic organs of adult females. The cysts are often adhered into a single mass. Endometriosis can cause obstruction of the intestine and ureters. Histologically, endometrial epithelium and stroma must be present to differentiate endometriosis from endometrial carcinoma. Dense fibrosis and hemosiderosis are usually present.
References:

Bertens APMG, et al. Endometriosis in rhesus monkeys. Lab Anim 16:281-284, 1982.

McCann TO, et al. Endometriosis in rhesus monkeys. Am J Obstet Gynecol 106:516-523,1970.

Splitter GA, et al. Endometriosis in four irradiated rhesus monkeys. Vet Pathol 9:249-262, 1972.

Schenken RS, et al. Etiology of infertility in monkeys with endometriosis: measurement of peritoneal fluid prostaglandins. Am J Obstet Gynecol 150:349-353, 1984.

Lindberg BS, et al. Endometriosis in rhesus monkeys. Upsala J Med Sci 89:129-134, 1984.

Fanton JW, et al. Endometriosis: Clinical and pathologic findings in 70 rhesus monkeys. Am J Vet Res 47:1537-1541, 1986.

Fanton JW, et al. Surgical treatment of endometriosis in 50 rhesus monkeys. Am J Vet Res 47:1602-1604, 1986.

INVOLUTIONAL CHANGE
Etiology: Involutional change is a normal repair process following pregnancy. During pregnancy, fetal trophoblasts invade and remodel endometrial arteries. This process resolves after parturition, but leaves permanent changes around vessels.
Pathology: Endometrial vessels are very prominent grossly. Histologically, large pale cells and extracellular matrix material surround the vessels in the endometrium and myometrium. Sometimes thrombosis, calcification, and hemosiderin are present. These changes are good markers of previous pregnancy.
References:

Bronson R, et al. Involution of placental site and corpus luteum in the monkey. Am J Obstet Gynecol 113:70-75, 1972.

Hayama S-I, et al. Pregnancy-induced sclerosis in the myometrial vessels of cynomolgus monkeys (Macaca fascicularis). Primates 31:427-429, 1990.

Blankenship TN, et al. Trophoblastic invasion and modification of uterine veins during placental development in macaques. Cell Tissue Res 274:135-144, 1993.

Cline JM, et al. Uterine vascular changes indicating prior pregnancy in macaques. Vet Pathol 32:585, 1995.

Blankenship TN, et al. Trophoblastic invasion and the development of uteroplacental arteries in the macaque: immunohistochemical localization of cytokeratins, desmin, type IV collagen, laminin, and fibronectin. Cell Tissue Res 272:227-236, 1993.

ARTHRITIS

Etiology: Arthritis is associated with calcium pyrophosphate crystal deposition on the articular surface of cartilage in joints. The underlying metabolic defect in chondrocytes is unknown.

Clinical: Enlarged joints, muscle atrophy and contracture, and wasting are the most prominent signs. Arthritis is common in rhesus.

Pathology: Arthritis can affect any joint. Interphalangeal joints and knees are most obviously affected. Reduced synovial fluid, cartilage erosion and ulceration, synovial hyperplasia, and neutrophilic infiltrates are also found. Calcium pyrophosphate crystals can be observed in articular surfaces by SEM in many cases. Arthritis may be a form of CPDD (pseudogout).

References:

Roberts ED, et al. Calcium pyrophosphate deposition disease (CPDD) in nonhuman primates. Am J Path 116:359-361, 1984.

Roberts ED, et al. Calcium pyrophosphate deposition in nonhuman primates. Vet Pathol 21:592-596, 1984.

Kandel RA, et al. Calcium pyrophosphate dihydrate crystal deposition disease with concurrent vertebral hyperostosis in a Barbary ape. Arth Rheum 26:682-687, 1983.

Kessler MJ, et al. Reduction of passive extension and radiographic evidence of degenerative joint disease in caged and free-ranging aged rhesus monkeys (Macaca mulatta). J Med Primatol 15:1-9, 1986.

Renlund RC, et al. Rhesus monkeys as a model for calcium pyrophosphate dihydrate crystal deposition disease. J Med Primatol 15:11-16, 1986.

Roberts ED. Pyrophosphate arthropathy, Macaca mulatta. In: Jones TC, et al. (eds). Monographs on Pathology of Laboratory Animals: Nonhuman Primates II. Springer-Verlag, 1993, 138-142.

Cerroni AM, et al. Bone mineral density, osteopenia, and osteoporosis in the rhesus macaques of Cayo Santiago. Am J Phys Anthropol 113:389-410, 2000.

PHYSICAL AGENTS

HYPERTHERMIA

Etiology: High environmental temperature, inadequate conditioning, insufficient fluids, and exertion (often following fighting) cause hyperthermia. Conditioning is important– don't move animals abruptly from air-conditioned quarters to outside quarters in extreme heat.

Clinical: Markedly elevated temperature, disorientation, dehydration, and coma are signs of hyperthermia.

Pathology: Tissues often appear autolyzed out of proportion to the time after death. Petechial hemorrhages on the epicardium and other serosal surfaces, congested lungs, and

muscles that appear "boiled" are seen. Histologically there is often extensive rhabdomyolysis. Epicardial hemorrhage is the most consistent finding.

HYPOTHERMIA
Etiology: Low environmental temperature, such as following heating system failures, leads to hypothermia. Susceptibility varies with species.
Clinical: The body temperture drops below normal. Subcutaneous edema, muscular stiffness and feeble movements are signs of hypothermia. Necrosis of extremities is seen if frostbite occurs. Animals housed alone in metal cages are particularly susceptible.
Pathology: There are no specific findings for hypothermia. Necrosis of extremities, especially the tail and ears, is seen with frostbite.
References:

Laber-Laird K, et al. Unexpected frostbite in cynomolgus macaques after a short exposure to snow. Lab Anim Sci 38:325-326, 1988.

TRAUMA DUE TO FIGHTING
Etiology: The propensity for intraspecies aggression is part of the spectrum of normal primate behavior and varies greatly with species and social grouping. Rhesus monkeys are probably the most aggressive. Monkeys will attack and kill strangers and will fight to establish dominance hierarchies. Rhesus monkey females will kill entire martilines during a matriline overthrow. Rhesus monkeys will also kill young males if they are not removed from enclosures at the appropriate age. Pigtailed macaque males that are newly introduced into a group will kill infants that they did not father. It is important to remember that monkeys are captive wild animals with complex behavior and social structure which must be understood and incorporated into management decisions.
Clinical: Lacerations, bruises, abrasions, punctures, and crush injuries of the face and distal extremities are commonly seen. Injuries to underlying soft tissue are often more extensive than is apparent from the appearance of the skin lesions. Gangrene of distal extremities can develop due to extensive crushing and bacterial contamination. Males induce lacerations, due to the prominent canine teeth. Females induce extensive crushing injuries.
Pathology: Extensive muscle necrosis, gangrene, myoglobinuric nephrosis, and hyperkalemia are often seen during necropsy. There is often associated exertional rhabdomyolysis, especially of the pectoral muscles.
References:

Reuter JD, et al. Review of exertional rhabdomyolysis and a case in a rhesus monkey (Macaca mulatta). J Med Primatol 27:303-309, 1998.

HEAD TRAUMA IN INFANT SQUIRREL MONKEYS
Etiology: Injury occurs when a mother jumps to the floor while carrying an infant on her chest.
Clinical: This type of trauma is usually fatal. Healed fractures are seldom observed.
Pathology: Skull fractures with subdural, parenchymal, and ventricular hemorrhage are found at necropsy.
References:

Lee KJ, et al. Skull fractures in infant squirrel monkeys (Saimiri sciureus). Lab Anim Sci 30:1006-1008, 1980.

## NUTRITIONAL/METABOLIC/MISCELLANEOUS DISEASES

### VITAMIN C DEFICIENCY (Scurvy)

Etiology: A deficiency of vitamin C in the diet causes scurvy. Primates cannot synthesize vitamin C and therefore require an adequate dietary source. Vitamin C has a limited shelf-life in feed. It is often sprayed on the surface of pelleted feeds during manufacturing, and can be washed off if feed is soaked to soften it.
Clinical: In squirrel monkeys, cephalohematomas are the most characteristic lesions. In Old World monkeys, epiphyseal fractures, loose teeth, and anemia are commonly seen. Affected animals respond rapidly to vitamin C (7.5-10 mg/kg).
Pathology: Those species requiring vitamin C in the diet lack L-glulono-g-lactone oxidase, an enzyme necessary to form ascorbic acid. The basic defect is an inability to form normal collagen. This is manifested by subperiosteal hemorrhage, epiphyseal fractures, and abnormal ossification of bones. In bones, there is a widened zone of calcified but unossified cartilage.
References:

De Klerk WA, et al. Vitamin C requirements of the vervet monkey (Cercopithecus aethiops) under experimental conditions. S African Med J 47:705-708, 1973.

Demaray SY, et al. Suspected ascorbic acid deficiency in a colony of squirrel monkeys (Saimiri sciureus). Lab Anim Sci 28:457-460, 1978.

Blackwell CA, et al. Cranial hyperostosis of squirrel monkeys (Saimiri sciureus). Lab Anim Sci 24:541-544, 1974.

Ratterree MS, et al. Vitamin C deficiency in captive nonhuman primates fed commercial primate diet. Lab Anim Sci 40:165-168, 1990.

Eisele PH, et al. Skeletal lesions and anemia associated with ascorbic acid deficiency in juvenile rhesus macaques. Lab Anim Sci 42:245-249, 1992.

Roberts ED. Vitamin C deficiency, Old and New World monkeys. Monographs on Pathology of Laboratory Animals: Nonhuman Primates I, Springer-Verlag, 1993, 202-206.

Borda JT, et al. Ascorbic acid deficiency in Cebus apella. Lab Primate Newsletter 35:5-6, 1996.

### ABDOMINAL FAT NECROSIS AND SAPONIFICATION

Etiology: Torsions within the omentum presumably cause nodular fat necrosis, while terminal starvation leads to saponification of subcutaneous and abdominal fat.
Clinical: There are no clinical findings.
Pathology: In nodular fat necrosis, firm yellow nodules are found in mesenteric fat. Histologically, these consist of granulomatous fat necrosis and mineralization. This is an incidental finding, especially in rhesus. Saponification consists of innumerable pinpoint white spots in the subcutaneous and visceral fat and is a terminal event. The lesions are due to soap formation and are not associated with inflammation.

FATAL FASTING SYNDROME OF OBESE MACAQUES

Etiology: Anorexia and acute weight loss from any cause lead to this syndrome in obese macaques.

Clinical: Anorexia, lethargy and death occur in obese macaques. Azotemia is often present.

Pathology: Affected animals are usually still fat, even though there has been recent weight loss.

Fatty change is seen in the liver and kidney, along with small foci of necrosis and areas of ectatic acinii in the pancreas. The exact metabolic mechanism is unknown.

References:

Bronson RT, et al. Fatal fasting syndrome of obese macaques. Lab Anim Sci 32:187-192, 1982.

Laber-Laird KE, et al. Fatal fatty liver-kidney syndrome in obese monkeys. Lab Anim Sci 37:205-209, 1987.

Gliatto JM, et al. Fatal fasting syndrome of obese macaques. Monographs on Pathology of Laboratory Animals: Nonhuman Primates I, Springer-Verlag, 1993, 198-202.

Christe KL, et al. The use of a percutaneous endoscopic gastrotomy (PEG) tube to reverse fatal fasting syndrome in a cynomolgus macaque (Macaca fascicularis). Contemp Top 38:12-15, 1999

SIMIAN BONE DISEASE (VITAMIN D3 DEFICIENCY IN NWM)

Etiology: Diets deficient in vitamin D3 cause bone disease in New Morld monkeys. NWM cannot utilize vitamin D2. A Ca/P imbalance from feeding excessive fruit may also contribute.

Clinical: Distorted limbs, kyphosis, pathologic fractures, and decreased bone radiodensity are seen.

Pathology: Simian bone disease is a nutritional secondary hyperparathyroidism that results in bone resorption and fibrous replacement (fibrous osteodystrophy).

References:

Hunt RD, et al. A comparison of vitamin D2 and D3 in New World primates. I. Production and regression of osteodystrophia fibrosa. Lab Anim Care 17:222-234, 1967.

Duboulay GH, et al. Nutritional bone disease in captive primates. Symp Zool Soc London 21:223, 1968.

Krook L, et al. Simian bone disease: a secondary hyperparathyroidism. Cornell Vet 52:459, 1962.

ANEMIA OF OWL MONKEYS

Etiology: The etiology is unknown, but the anemia responds to parenteral vitamin E and selenium, even though diet and serum levels seem adequate. Some of the 11 distinct karyotypes of owl monkeys are susceptible, while others are not. Enteropathy and colitis (common in owl monkeys) may cause malabsorption of vitamin E.

Clinical: Pallor, icterus, and lethargy are commonly seen.

Pathology: Pallor of mucous membranes and other tissues, icterus, petechiae in the CNS, anemia, circulating nucleated RBCs, centrilobular necrosis in liver, EMH in liver and

spleen, hemosiderosis, microinfarcts in brain, edema, ascites, pulmonary edema, cardiomegaly and muscle necrosis can be observed.
References:

Sehgal RC, et al. Therapeutic efficacy of vitamin E and selenium in treating hemolytic anemia of owl monkeys. Lab Anim Sci 30:92-98, 1980.

Bronson RT, et al. Morphology and morphogenesis of cerebral ring hemorrhages in anemic monkeys. Acta Neuropathol 51:155-160, 1980.

Bronson RT. Necrotizing myopathy associated with hemolytic anemia in owl monkeys. J Neurological Sci 47:105-109, 1980.

King NW. Vitamin E-responsive hemolytic anemia and necrotizing myopathy, owl monkeys. In: Jones TC, et al. (eds). Monographs on Pathology of Laboratory Animals: Nonhuman Primates II. Springer-Verlag, 1993, 226-232.

CHRONIC COLITIS OF RHESUS MONKEYS

Etiology: The etiology is unknown but is possibly an immune reaction to bacterial lipopolysaccharides.
Transmission: This condition is endemic in most rhesus colonies. It is not clear whether or not it is transmissible.
Clinical: Chronic nonresponsive watery diarrhea, wasting, and joint contracture with or without arthritis are usually seen. Appetite remains good while fecal cultures and parasitic exams are negative.
Pathology: Diffuse lymphoplasmacytic colitis that is often associated with amyloidosis and arthritis is seen on necropsy.
References:

Hird DW, et al. Diarrhea in nonhuman primates: a survey of primate colonies for incidence rates and clinical opinion. Lab Anim Sci 34:465-470, 1984.

Wright GJ, et al. Electrolyte abnormalities associated with diarrhea in rhesus monkeys: 100 cases (1986-1987). J Am Vet Med Assoc 196:1654-1658, 1990.

Elmore DB, et al. Diarrhea rates and risk factors for developing chronic diarrhea in infant and juvenile rhesus monkeys. Lab Anim Sci 42:356-359, 1992.

Munoz-Zanzi CA, et al. Effect of weaning time and associated management practices on postweaning chronic diarrhea in captive rhesus monkeys (Macaca mulatta). Lab Anim Sci 49:617-621, 1999.

CHRONIC COLITIS OF TAMARINS

Etiology: Unknown. Diet, environment, genetics, viruses, and bacteria have all been proposed as factors.
Clinical: Diarrhea and weight loss. Associated with colonic carcinoma.
Pathology: Diffuse chronic active colitis with irregularity of the surface and crypts.
References:

Chalifoux LV, et al. An analysis of the association of gastroenteric lesions with chronic wasting syndrome of marmosets. Vet Pathol 19:141-162, 1982.

Chalifoux LV, et al. Colonic adenocarcinoma associated with chronic colitis in cotton-top marmosets. Gastroenterology 80:942-946, 1981.

Lushbaugh C, et al. Histology of colitis: Saguinus oedipus oedipus and other marmosets. Digestive Dis Sci 30:45s-51s, 1985.

Russel RG, et al. Coronavirus-like particles and Campylobacter in marmosets with diarrhea and colitis. Digestive Dis Sci 30:72s-77s, 1985.

Estes MK. Evaluating viral agents in marmoset colitis. Digestive Dis Sci 30:80s-81s, 1985.

Stonebrook MJ, et al. Temperature-metabolism relations in the cotton-top tamarin (Saguinus oedipus) model for ulcerative colitis. J Med Primatol 23:16-22, 1994.

Johnson LD, et al. A prospective study of the epidemiology of colitis and colon cancer in cotton-topped tamarins. Gastroenterology 110:102-115, 1996.

Wood JD, et al. Colitis and colon cancer in cotton-top tamarins (Saguinus oedipus oedipus) living wild in their natural habitat. Digest Dis Sci 43:1443-1453, 1998.

Bertone ER, et al. Family history as a risk factor for ulcerative colitis-associated colon cancer in cotton-top tamarin. Gastroenterology 114:669-674, 1998.

Saunders KE, et al. Novel intestinal Helicobacter species isolated from cotton-top tamarins (Saguinus oedipus) with chronic colitis. J Clin Microbiol 37:146-151, 1999.

## PROTOZOAN PARASITES

### HEPATOCYSTIS

Etiology: Hepatocystis kochi, H. simiae (African), H. taiwanensis, H. semnopitheci (Asian).

Transmission: Midges (culicoides)

Clinical: Although both African and Asian monkeys develop hepatocystosis, there are usually no clinical symptoms.

Pathology: 2-4 mm opaque cysts appear in the liver. These rupture to release merozoites, which infect RBCs. Trophozoites develop in the RBCs, while schizogeny occurs in the liver. Since schizogony occurs in the liver, there are no malarial symptoms. Heavy parasitemia can alter hematology results because infected RBCs do not lyse and automated counters therefore count them as nucleated cells. Ruptured cysts result in eosinophilic granulomas that resolve into small circular scars in the liver.

References:

Turner TR, et al. Hepatocystis parasitemia in wild Kenya vervet monkeys. J Med Primatol 11:19 1-194, 1982.

Leathers CW. The prevalence of Hepatocystis kochi in African green monkeys. Lab Anim Sci 28:186-189, 1978.

Vickers J. Hepatocystis kochi in Cercopithecus monkeys. JAVMA 149:906-908, 1966.

Nakazawa M, et al. Histopathology of hepatocystosis in cynomolgus monkeys imported from southeast Asia. Jap J Med Sci 49:295-301, 1987.

Ryan MJ, et al. Diagnostic exercise: hepatic granulomas in a cynomolgus monkey. Lab Anim Sci 36:56-58, 1986.

### MALARIA

Etiology: Plasmodium cynomolgi, P. inui, P. knowlesi, P. gonderi, P. brasilianum, and numerous others naturally infect Asian, African, and South American nonhuman primates.

Transmission: Mosquitoes transmit these agents in nature.
Clinical: OWM, NWM and apes contract Plasmodium infections, but they are usually subclinical unless the animal is splenectomized or immunosuppressed. Some species will cause disease characterized by jaundice, anorexia, listlessness, fever, anemia, and splenomegaly. The periodic release of organisms from RBCs causes these clinical signs. The length of the cycle is determined by the periodicity of the particular Plasmodium. Mixed infections are common.
Pathology: Grossly, the lungs, liver, and spleen are gray and the blood is thin. Histologically, tissue macrophages are filled with malarial pigment, and there are hemodsiderosis and parasitized RBCs in tissues. Intravascular clotting with thrombi and parasitized RBCs is common. Often there is pulmonary and cerebral edema.
References:

Bray R. The malaria parasites of anthropoid apes. J Parasitol 49:888-891, 1963.

Eyles DE. The species of simian malaria: taxonomy, morphology, life cycle, and geographical distribution of the monkey species. J Parasitol 49:866-887, 1963.

Stokes WS, et al. Acute clinical malaria in a cynomolgus monkey. Lab Anim Sci 33:81-85, 1983.

Donovan JD, et al. Hematologic characterization of naturally occurring malaria in cynomolgus monkeys. Lab Anim Sci 33:86-89, 1983.

McWilson W. Simian and anthropoid malarias: their role in human disease. Lab Anim Care 20:368-376, 1970.

Young MD. Natural and induced malarias in Western Hemisphere monkeys. Lab Anim Care 20:361-367, 1970.

Schofield LD, et al. An outbreak of Plasmodium inui malaria in a colony of diabetic rhesus monkeys. Lab Anim Sci 35:167-168, 1985.

Schmidt LH, et al. The characteristics of Plasmodium cynomolgi infections in various Old World primates. Am J Trop Med Hyg 26:356-372, 1977.

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BABESIOSIS
Etiology: Babesia entopolypoides (Entopolypoides macaci), other Babesia sp.
Transmission: Hard ticks (Ixodidae)
Clinical: Babesiosis is endemic in several domestic baboon colonies. Many African and Asian species carry Babesia, which can remain latent for long periods. Antibody can be detected by IFA. Usually asymptomatic unless immunosuppressed or splenectomized. Can see high parasitemia and mild anemia.
Pathology: Usually none. Babesia must be differentiated from Plasmodium on blood smears and in tissue sections. Babesia do not produce pigment like Plasmodium do.

References:

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Ruebush TK, et al. Thick transmission of Babesia microti to rhesus monkeys (Macaca mulatta). Am J Trop Med Hyg 30:555-559, 1981.

Ruebush TK, et al. Experimental Babesia microti infections in Macaca mulatta: recurrent parasitemia before and after splenectomy. Am J Trop Med Hyg 30:304-307, 1981.

Chin W, et al. Plasmodium inui and Babesia microti infections in the squirrel monkey, Saimiri sciureus. Am J Trop Med Hyg 32:691-693, 1983.

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AMEBIASIS

Etiology: Entamoeba histolytica and Balamuthia mandrillaris have been reported in nonhuman primates.

Transmission: Oral

Clinical: Asymptomatic carriers are common, but amebae can cause diarrhea. OWM, NWM and apes are affected.

Pathology: Amebae are often found in the mucosa without associated lesions or clinical signs. Amebae can cause mild to severe colitis, however. Chronic low-grade colitis or, more commonly, classical flask-shaped ulcers occur. Trophozoites are seen in the mucosa, submucosa, and sometimes in the muscularis. Trophozoites sometimes invade lymphatics or blood vessels and spread to form amebic abscesses in the lung, liver, or brain. In leaf-eating monkeys (ie, colobus), the stomach is the primary site of infection, and organisms may not be found in the feces. Balamuthia mandrillaris, a free-living ameba, has caused fatal meningeoencephalitis in nonhuman primates.

References:

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Johnson CM. Observations on natural infections of Entamoeba histolytica in Ateles and rhesus monkeys. Am J Trop Med 21:49-61, 1941.

Beaver PC, et al. Invasive amebiasis in naturally infected New World and Old World monkeys with and without clinical disease. Am J Trop Med Hyg 39:343-352, 1988.

Loomis MR, et al. Hepatic and gastric amebiasis in black and white colobus monkeys. JAVMA 183:1188-1191, 1983.

Palmieri JR., et al. Gastric amebiasis in a silvered leaf monkey. JAVMA 185:1374-1375, 1984.

Amyx HL, et al. Hepatic amebiasis in spider monkeys. Am J Trop Med Hyg 27:888-891, 1978

Rideout BA, et al. Fatal infections with Balamuthia mandrillaris (a free-living amoeba) in gorillas and other Old World primates. Vet Pathol 34:15-22, 1997.

BALANTIDIASIS
Etiology: Balantidium coli
Transmission: Oral
Clinical: Balantidium coli has been found in OWM, NWM and apes. Many animals have B. coli in the colon without showing any clinical signs. It is not clear whether Balantidium is a primary pathogen or a secondary invader associated with other pathogenic organisms. There is some evidence it may be a primary pathogen in apes and pigtailed macaques. Balantidium is sometimes associated with diarrhea.
Pathology: Organisms are often found in the lumen of the colon of normal animals and in ulcerative lesions, mucosa, capillaries, lymphatics, and mesenteric lymph nodes of animals with colitis. It may be a primary pathogen in great apes and pigtailed macaques, but is usually associated with some other pathogen in other species.
References:

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Teare JA, et al. Balantidiasis (epizootic) in gorillas. JAVMA 181:1345-1347, 1982.

Lee RV, et al. Typhlitis due to Balantidium coli in captive lowland gorillas. Rev Inf Dis 12:1052-1059, 1990.

ENCEPHALITOZOONOSIS
Etiology: Encephalitozoon cuniculi
Transmission: Encephalitozoon is shed in urine and then transmitted orally. It can cross the placenta and be transmitted to the fetus.
Clinical: NWM infected with E. cuniculi usually show no clinical signs, but it has been associated with stillbirths and abortions in squirrel monkeys.
Pathology: In adults, focal microgranulomas are found in the brain and kidney. In fetuses and infants, disseminated granulomas are found in the lung, aorta, pulmonary arteries, adrenal, liver, placenta, and other tissues. Vasculitis and aortitis are common. Gram staining identifies the gram-positive organisms in cysts and lesions. Cysts are not usually associated with inflammation.
References:

Brown RJ, et al. Nosematosis in a squirrel monkey. Naval Aerospace Medical Research Laboratory Special Report 72-1, 1972.

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Wasson K, et al. Mammalian micorsporidiosis. Vet Pathol 37:113-128, 2000.

ENTEROCYTOZOONOSIS

Etiology: Enterocytozoon bieneusi

Transmission: Organisms are ubiquitous in nature, but the means of transmission is unkown.

Clinical: Enterocytozoon bieneusi can be carried asymptomatically in the hepatobiliary tree of normal rhesus monkeys. Infected animals may have elevated alkaline phosphatase. Enterocytozoon occurs as an opportunistic infection in SIV-infected macaques. Clinical signs specific for this organism have not been reported, but it has been associated with chronic diarrhea.

Pathology: Immunocompetent monkeys may have mild lymphoplasmacytic choledochitis and cholecystitis. Organisms are most common in the gallbladder and bile ducts, but are also found in the small intestinal epithelium and pancreatic duct in immunodeficient animals. Spores are 4-12 μm and are perinuclear. They stain with Ziehl-Neelsen, Brown-Hopps, Weber's modified trichrome, and methenamine-silver stains. Lesions consist of proliferation of the biliary epithelium, fibrosis, and infiltration with lymphocytes and plasma cells. Extrusion of individual biliary epithelial cells with organisms can be seen. Within the liver, bridging portal fibrosis and marked bile ductular hyperplasia with a lymphoplasmacytic infiltrate may occur.

References:

Mansfield KG, et al. Identification of an Enterocytozoon bieneusi-like microsporidian parasite in simian-immunodeficiency-virus-inoculated macaques with hepatobiliary disease. Am J Pathol 150:1395-1405, 1997.

Tzipori S, et al. Transmission and establishment of a persistent infection of Enterocytozoon bieneusi, derived from a human with AIDS, in simian immunodeficiency virus-infected rhesus monkeys. J Infect Dis 175:1016-1020, 1997.

Carville A, et al. Development and application of genetic probes for detection of Enterocytozoon bieneusi in formalin-fixed stools and in intestinal biopsy specimens from infected patients.

Chalifoux LV, et al. Ultrastructural morphology of Enterocytozoon bieneusi in biliary epithelium of rhesus macaques (Macaca mulatta). Vet Pathol 35:292-296, 1998.

Mansfield KG, et al. Localization of persistent Enterocytozoon bieneusi infection in normal rhesus macaques (Macaca mulatta) to the hepatobiliary tree. J Clin Microbiol 36:2336-2338, 1998.

SARCOCYSTOSIS
Etiology: Sarcocystis sp
Transmission: Fecal-oral. Primates are intermediate hosts with the final host being a carnivore.
Clinical: Sarcocystis infects many species of wild-caught monkeys, but there are usually no symptoms. In extremely heavy infections, myositis may occur, resulting in muscle pain and reluctance to move.
Pathology: The presence of characteristic cysts in skeletal muscle is the definitive diagnostic finding. Usually there is no inflammatory reaction. In very heavy infections, eosinophilic myositis may occur.
References:

Kuncl RW, et al. Prevalence and ultrastructure of Sarcocystis in rhesus monkeys. Jpn J Vet Sci 50:519-527, 1988.

Dubin IN, et al. Sarcocystis in Macaca mulatta. J Parasitol 33:515-553, 1947.

Karr SL, et al. A survey of sarcocystis in nonhuman primates. Lab Anim Sci 25:641-645, 1975.

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Lane JH, et al. Acute fulminant sarcocystosis in a captive-born rhesus macaque. Vet Pathol 35:499-505, 1998.

CRYPTOSPORIDIOSIS
Etiology: Cryptosporidium sp
Transmission: Fecal-oral
Clinical: Clinical disease occurs in young, debilitated, or immunosuppressed animals. Diarrhea, weight loss and anorexia usually resolve spontaneously in normal animals, but are untreatable in immunodeficient animals. Cryptosporidium is a common opportunistic infection in SRV- and SIV-infected macaques.
Pathology: Organisms are present on surface epithelium of intestinal villi. Villus atrophy and eosinophilic inflammation in lamina propria are often associated with the presence of the organisms. In immunodeficient monkeys, one can see generalized infection with organisms and eosinophilic inflammation in conjunctiva, trachea, bronchioles, bile ducts and gallbladder, and the pancreatic duct. There is often marked hyperplasia of biliary and pancreatic duct epithelium and periductal fibrosis.
References:

Miller RA, et al. Clinical and parasitologic aspects of cryptosporidiosis in nonhuman primates. Lab Anim Sci 40:42-46, 1990.

Blanchard JL, et al. Disseminated cryptosporidiosis in simian immunodeficiency virus/delta-infected rhesus monkeys. Vet Pathol 24:454-456, 1987.

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Baskin GB. Cryptosporidiosis of the conjunctiva in SIV-infected rhesus monkeys. J Parasitol 82:630-632,1996.

Yanai T, et al. Pulmonary cryptosporidiosis in simian immunodeficiency virus-infected rhesus macaques. Vet Pathol 37:472-475, 2000.

TRICHOMONIASIS

Etiology: Trichomonas spp

Transmission: Fecal-oral

Clinical: Trichomoniasis is a widespread, asymptomatic enteric infection. The numbers often increase in diarrhea, but a relationship to clinical signs has not been demonstrated.

Pathology: Trichomonas spp often fill crypts in large intestine, but may also invade the epithelium without inflammatory reaction. Occasional invasion of the stomach and colon, associated with inflammation, has been reported. Trichomonal gastritis occurs in SIV-infected rhesus monkeys.

References:

Migaki G, et al. Trichomonal granuloma of the pelvic cavity in a rhesus monkey. Vet Pathol 15:679-681, 1978.

Bunton TE, et al. Invasive trichomoniasis in a Callicebus moloch. Vet Pathol 20:491-494, 1983.

Pindak FF, et al. Detection and cultivation of intestinal trichomonads of squirrel monkeys (Saimiri sciureus). Am J Primatol 9:197-205, 1985.

Scimeca JM, et al. Intestinal trichomonads (Tritrichomonas mobilensis) in the natural host Saimiri sciureus and Saimiri boliviensis. Vet Pathol 26:144-147, 1989.

Blanchard JL, et al. Trichomonas gastritis in rhesus monkeys infected with the simian immunodeficiency virus. J Inf Dis 157:1092-1093, 1988.

Brady AG, et al. Enteric trichomonads of squirrel monkeys (Saimiri sp.): natural infestation and treatment. Am J Primatol 14:65-71, 1988.

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Brack M, et al. Intestinal trichomoniasis due to Tritrichomonas mobilensis in tree shrews (Tupaia belangeri). Lab Anim Sci 45:533-537, 1995.

GIARDIASIS

Etiology: Giardia lamblia

Transmission: Fecal-oral

Clinical: Asymptomatic carriers are common. Giardia may be associated with self-limiting diarrhea and malabsorption and can be a serious opportunistic infection in SIV-infected macaques.
Pathology: The organisms are present on the epithelial surface of the small intestine and are most commonly found in the middle of the jejunum. The mucosa may be normal or may have nonspecific villous atrophy and inflammation of the lamina propria.
References:

Hamlen HJ, et al. Giardiasis in laboratory-housed squirrel monkeys: A retrospective study. Lab Anim Sci 44:235-239, 1994.

TRYPANOSOMIASIS
Etiology: Trypanosoma cruzi
Transmission: The insect Triatoma (reduviid "kissing bug") is the vector, with the reservoir being many different mammals.
Clinical: Infected animals may be asymptomatic or may show lymphadenopathy, hepatosplenomegaly, eyelid edema, drowsiness (sleeping sickness-like), heart failure, and sudden death.
Pathology: Trypanosomal forms are found in the blood. An enlarged, mottled heart is the most common finding at necropsy. Chronic myocarditis with cystic collections of leishmanial forms in muscle cells is characteristic. Skeletal and smooth muscle can be affected. Necrosis and glial nodules in brain have been seen. Trypanosoma is differentiated from Toxoplasma by the presence of a kinetoplast.
References:

Olson LC, et al. Encephalitis associated with Trypanosoma cruzi in a Celebes black macaque. Lab Anim Sci 36:667-670.

Seibold HR, et al. American trypanosomiasis (Chagas' disease) in Hylobates pileatus. Lab Anim Care 20:514-517, 1970.

Marinkelle CJ. The prevalence of Trypanosoma cruzi infection in Colombian monkeys and marmosets. Ann Trop Med Parasitol 76:121-124, 1982.

Kasa TJ, et al. An endemic focus of Trypanosoma cruzi infection in a subhuman primate research colony. J Am Vet Med Assn 171:850-854, 1977.

Gleiser CA, et al. Trypanosoma cruzi infection in a colony-born baboon. J Am Vet Med Assn 189:1225-1226, 1986.

Ndao M, et al. Trypanosoma cruzi infection of squirrel monkeys: comparison of blood smear examination, commercial enzyme-linked immunosorbent assay, and polymerase chain reaction analysis as screening tests for evaluation of monkey-related injuries. Comp Med 50:658-665, 2000.

TOXOPLASMOSIS
Etiology: Toxoplasma gondii
Transmission: Infection occurs by ingestion of food contaminated by cat feces containing oocysts or ingestion of raw meat containing cysts. Remember that many NHP will catch and eat rodents that may be infected. Primates in the wild are rarely seropositive. Epizootics have occurred in captive New World species.
Clinical: NWM are more susceptible than OWM, which are usually asymptomatic. Lethargy, CNS signs, and sudden death may occur.

Pathology: Pulmonary edema, lymphadenopathy, splenomegaly, and intestinal ulceration are common. Necrosis, with or without inflammation, can occur in the liver, spleen, lymph nodes, heart, lung, adrenal, intestinal muscle, and brain. Individual organisms and cysts are found in these tissues.
References:

McKissick GE, et al. Enzootic toxoplasmosis in caged squirrel monkeys Saimiri sciureus. Path Vet 5:538-560, 1968.

Wong MM, et al. Spontaneous toxoplasmosis in macaques: a report of four cases. Lab Anim Sci 24:273-278, 1974.

McConnell EE, et al. Toxoplasmosis in free-ranging chacma baboons (Papio ursinus) from the Kruger National Park. Trans Roy Soc Trop Med Hyg 67:851-855, 1973.

Hessler JR, et al. Lethal toxoplasmosis in a wooly monkey. J Am Vet Med Assn 159:1588-1594, 1971.

Dubey JP, et al. Acute death associated with Toxoplasma gondii in ring-tailed lemurs. J Am Vet Med Assn 187:1272-1273, 1985.

Dickson J, et al. Epidemic toxoplasmosis in captive squirrel monkeys (Saimiri sciureus). Vet Rec 112:302, 1983.

Schoondermark-Van de Ven E, et al. Congenital toxoplasmosis: an experimental study in rhesus monkeys for transmission and prenatal diagnosis. Exp Parasitol 77:200-211, 1993.

Anderson DC, et al. Toxoplasmosis. In: Jones TC, et al. (eds.). Monographs on Pathology of Laboratory Animals: Nonhuman Primates I, Springer-Verlag, 1993, 63-70.

Dietz HH, et al. Toxoplasmosis in a colony of New World monkeys. Vet Parasitol 68:299-304, 1997.

## METAZOAN PARASITES

### LICE

Etiology: Several species of biting and sucking lice have been described in nonhuman primates.
Clinical: Lice are usually seen on debilitated animals caged alone. Normal grooming behavior in socially housed monkeys probably controls lice. Pediculosis seldom causes significant cutaneous lesions.
Pathology: Lice and eggs (nits) are observed on the hair or skin.
References:

Winsatt JH, et al. An infestation of sucking lice in a juvenile rhesus macaque. Lab Anim Sci 38:203, 1988.

Mader DR, et al. Management of an infestation of sucking lice in a colony of rhesus macaques. Lab Anim Sci 39:252-255, 1989.

### RESPIRATORY MITES

Etiology: Pneumonyssus and Pneumonyssoides are found in the lungs, while Rhinophaga is found in the nasal cavity.
Transmission: Unknown, but close contact is required.

Clinical: Pneumonyssus simicola was once the most important, with nearly 100% incidence in rhesus monkeys. Since the advent of routine treatment with ivermectin, the incidence of pulmonary acariasis in research colonies has declined significantly. Usually there are no clinical signs. Radiographs are of little use in diagnosis. Rarely, monkeys will present in acute respiratory distress due to pneumothorax caused by rupture of a "mite house." Lung mites can cause serious clinical disease in langurs and proboscis monkeys.
Pathology: Focal, yellow, air-filled cysts 1-10 mm in diameter are found throughout the pulmonary parenchyma. The cysts are raised if they occur on the pleural surface. In severe cases, delicate fibrous adhesions develop between the visceral and parietal pleura. Bronchial lymph nodes are deeply pigmented due to the deposition of mite pigment. Microscopic lesions consist of chronic bronchiolitis with bronchiectasis and eosinophilic granulomatous inflammation, cross sections of mites, and golden brown refractile pigment.
References:

Innes JRM, et al. Pulmonary acariasis as an enzootic disease caused by Pneumonyssus simicola in imported monkeys. Am J Pathol 30:813-827, 1954.

Hull WB. Respiratory mite parasites in nonhuman primates. Lab Anim Care 20:402-406, 1970.

Brack M. Histochemistry of the lung mite pigment in infections of Pneumonyssus sp in nonhuman primates. Parasitol 64:47-52, 1972.

Robinson PT, et al. Clinical and pathologic aspects of pulmonary acariasis in Duoc langur and proboscis monkeys. Zool Garten 51:161-169, 1981.

Rawlings CA, et al. Pneumothorax associated with lung mite lesions in a rhesus monkey. Lab Anim Sci 23:259-261, 1973.

Joseph BE, et al. Treatment of pulmonary acariasis in rhesus macaques with ivermectin. Lab Anim Sci 34:360-364, 1984.

CUTANEOUS MITES
Etiology: Sarcoptes sp, Demodex sp and Psorergates sp have all been found in nonhuman primates.
Transmission: Contact
Clinical: Sarcoptes and Demodex lesions in monkeys are similar to those in other animals. Psorergates causes focal nonpruritic plaques. Demodex is commonly found in the lips and brow of asymptomatic monkeys.
Pathology: Chronic dermatitis associated with cross-sections of mites in the characteristic location for the species is diagnostic. Demodex can be found incidentally in sections of lip and brow.
References:

Lebel RR, et al. Demodectic mites of subhuman primates. J Parasitol 59:719-722, 1973.

Lee KJ, et al. Psorergatic mange of the stumptail macaque. Lab Anim Sci 31:77-79, 1981.

Bowman TA, et al. Comparison of treatments for Psorergates mites in stumptailed macaques. Lab Anim Sci 37:100-102, 1987.

Goldman L, et al. Human infestation with scabies of monkeys. Arch Dermatol Syph 59:175-178, 1949.

Raulston GL. Psorergatic mites in patas monkeys. Lab Anim Sci 22:107-108, 1972.

Hickey TE, et al. Demodectic mange in a tamarin (Saguinus geoffroyi). Lab Anim Sci 33:192-193, 1983.

Seier JV. Psorergatic acariasis in vervet monkeys. Lab Anim 19:236-239, 1985.

Baskin GB. Cutaneous acariasis. In: Jones TC, et al. (eds.). Monographs on Pathology of Laboratory Animals: Nonhuman Primates II, Springer-Verlag, 1993, 23-26.

PENTASTOMES

Etiology: Armillifer and Porocephalus are found in OWM; Porocephalus is found in NWM.

Transmission: The parasite is transmitted orally, with monkeys being the intermediate hosts. The final host is a primate-eating snake, where the adult form is found in the lung.

Clinical: There are no clinical signs.

Pathology: Nymphs are found encysted in the pleural or peritoneal cavity. Nymphs are C-shaped, annulated and enclosed in a transparent cyst. There is no host reaction while the nymph is alive, but chronic inflammation if it dies.

References:

Cosgrove GE, et al. The pathology of pentastomid infection in primates. Lab Anim Care 20:354-360, 1970.

Lok JB, et al. Pentastomiasis in captive monkeys. Lab Anim Sci 37:496-496, 1987.

CESTODES

Etiology: Adult Cestodes: Cyclophyllidean tapeworms of the families Anaplocephalidae (esp. Bertiella), Davaineidae, Hymenolepiididae, and Dilepididae are found in the intestine.

Larval Cestodes: Pseudophyllidean cestodes in the family Diphyllobothriidae which form spargana (plerocercoid larvae), and Cyclophyllidean cestodes which form cysticercoid larvae, cysticercus larvae (Taenia), coenurus larvae (Multicepts), or hydatid larvae (Echinococcus) are found in various tissues.

Transmission: Ingestion of eggs or an infected intermediate host (often arthropod) is the mode of transmission.

Clinical: Usually no clinical symptoms are noted.

Pathology: Adults are found free within the intestinal lumen, where they do not produce any lesions. Larvae are more commonly found than adults in nonhuman primates. Larvae may be found in retroperitoneal, subcutaneous, or muscular tissues or in the abdominal, pleural, or cranial cavities. There is usually little host response. Specific diagnosis is based upon the characteristic morphology of the larvae.

References:

Guillot LM, et al. Pulmonary cestodiasis in a cynomolgus monkey (Macaca fascicularis). Lab Anim Sci 42:158-160, 1992.

Houser WD, et al. Hydatid disease in a macaque. J Am Vet Med Assn 159:1574-1577, 1971.

Hubbard GB, et al. Mesocestoides infection in captive olive baboons (Papio cynocephalus anubis). Lab Anim Sci 43:625-627, 1993.

Fincham JE, et al. Pleural Mesocestoides and cardiac shock in an obese vervet monkey (Cercopithecus aethiops). Vet Pathol 32:330-333, 1995.

Sasseville VG, et al. A case of pulmonary cestodiasis in a simian immunodeficiency virus-infected pigtailed macaque (Macaca nemestrina) in which virus-infected leukocytes are present within the lesion. J Med Primatol 25:251-256, 1996.

LUNGWORMS

Etiology: Filaroides sp and Filariopsis sp have been reported in nonhuman primates.
Transmission: Larvae are passed in feces, while the rest of the cycle is unknown.
Clinical: Lungworms are most common in NWM, but there are no associated clinical signs.
Pathology: 1-2-mm focal brown irregular spots are found on the pleural surface of the lung. The small slender adults are found in terminal bronchioles and alveoli. The females are viviparous. There is usually little inflammation.

FILARIDS

Etiology: Dipetalonema sp, Tetrapetalonema sp, Wuchereria sp, and Edesonfilaria sp are the most common, but there are over 40 species.
Transmission: Blood-sucking insects, such as the midge and mosquito, transmit filarids.
Clinical: There are no clinical signs. Though the parasites have been found in NWM and OWM, they are most common in NWM.
Pathology: The long slender adults are found in the peritoneal cavity (Dipetalonema sp), subcutis, or connective tissue, depending on the species. In heavy peritoneal infections, there may be a slight increase in peritoneal fluid and slight villous proliferation of the serosa. Microfilaria are found circulating in the blood and can be identified by the pattern of acid phophatase staining. Granulomas and arterial thickening in the spleen are associated with the microfilaria of Edesonfilaria in cynomolgus monkeys.
References:

Dunn F, et al. On some filarial parasites of South American primates with a description of Tetrapetalonema tamarinae from the Peruvian tamarin marmoset. J Helminthol 37:261-286, 1963.

Jacotot H, et al. Splenite chronique dans un cas de filariose chez un cynocephale. Bull Soc Pathol Exot 49:300-301, 1956.

McCoy OR. Filarial parasites of the monkeys of Panama. Am J Trop Med Hyg 16:383-403, 1936.

Webber WAF. The filarial parasites of primates: a review. I: Dirofilaria and Dipetalonema. Ann Trop Med Parasitol 49:123-141, 1955.

Narama I, et al. Microfilarial granulomas in the spleens of wild-caught cynomolgus monkeys. Vet Pathol 22:355-362, 1985.

Nonoyama T, et al. A pathological study in cynomolgus monkeys infected with Edesonfilaria malayensis. Lab Anim Sci 34:604-609, 1984.

Eberhard ML. Chemotherapy of filariasis in squirrel monkeys. Lab Anim Sci 32:397-400, 1982.

Chalifoux LV. Filariasis, New World primates. In: Jones TC, et al. (eds.). Monographs on Pathology of Laboratory Animals: Nonhuman Primates I, Springer-Verlag, 1993, 206-214.

STRONGYLOIDES

Etiology: Strongyloides cebus in NWM, S. fulleborni in OWM.

Transmission: Strongyloides have a complex life cycle, making it possible to enter the host both orally and by penatration of the skin.

Clinical: Infected monkeys are usually asymptomatic, but diarrhea can result from very heavy infections, especially due to hyperinfection. Coughing may be associated with larval migration.

Pathology: Adult females (only the females are parasitic) burrow into the mucosa of the proximal small intestine, forming tunnels in which ova are deposited. Larvae hatch and break out of the tunnels into the lumen. In hyperinfection, 1st-stage larvae develop rapidly to 3rd-stage and penetrate the bowel before being passed in the feces. This process may affect the full thickness of colon and sometimes the ileum. There is sometimes a severe inflammatory response to the larvae. Larvae have been seen in the lymphatics and lymph nodes. Hemorrhage in the lung is due to migrating larvae. In autoinfection, 3rd-stage larvae pass through the anus and penetrate the perianal skin. Strongyloidiasis may be particularly severe in apes.

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NOCHTIA

Etiology: Nochtia nochti

Transmission: Nochtia has a direct life cycle and is transmitted orally. It affects Asian macaques.

Clinical: There are no clinical symptoms.

Pathology: Gastric polyps or papillomas, which contain the embedded slender bright red parasite and eggs, occur at the junction of the fundic and pyloric regions of the stomach. Severe hyperplasia of the mucous neck cells of the gastric crypts and mucous metaplasia of the underlying fundic or pyloric glands may occur. The lesions do not become malignant. Physaloptera tumefaciens can produce a similar lesion, but is much larger and only the head is buried in the mucosa, with the body free in the gastric lumen.

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ANATRICHOSOMA
Etiology: Anatrichosoma cutaneum, A. cynomolgi
Transmission: The initial mode of transmission is unknown, but embryonated eggs are deposited in nasal or cutaneous stratified squamous epithelium and sloughed.
Clinical: Parasites in the nasal epithelium of OWM cause no clinical signs. In the skin, there may be a mild peeling of the epidermis of the palms of the hands and soles of the feet. It is possible to see serpentine tracks with intense inflammation.
Pathology: Worms and eggs can be found in cross sections of nasal epithelium or skin of the face, hands, and feet. There is usually little inflammation. Anatrichosoma is seldom seen in animals routinely treated with anthelminthics.
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OESOPHAGOSTOMUM (nodular worms)
Etiology: There are several species of Oesophagostomum, but they are not clearly defined.
Transmission: Oesophagostomum is transmitted orally and has a direct life cycle.
Clinical: Oesophagostomum is found in OWM and is usually asymptomatic. Heavy infections are associated with diarrhea and anemia.
Pathology: Adults live free in the lumen of the large intestine and cause no damage. Ingested larvae develop into 4th stage in the gut wall and then return to the lumen. Larvae cause the characteristic lesion that consists of 3-4 mm dark nodules in the submucosa and muscularis of the large intestine. Nodules contain brown exudate and small white larvae. Histologically, the nodules consist of a central abscess with necrosis and mixed inflammatory cells encircled by fibrosis. Nodules sometimes occur in ectopic sites such as the peritoneum, kidney, liver, lung, etc.
References:

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TRICHURIS (whipworms)
Etiology: Trichuris trichiura
Transmission: Oral. Trichuris has a direct life cycle.
Clinical: Both OWM and NWM are plagued by Trichuris but are usually asymptomatic.
Pathology: The adults are found with their anterior end embedded in the mucosa of the cecum and proximal colon, causing little host reaction. Heavy infestations can rarely be associated with intussusception.

PHYSALOPTERA
Etiology: Physaloptera tumefaciens and P. dilatata

Transmission: Ingestion of the intermediate host (cockroach) is the mode of transmission.
Clinical: Usually there are no clinical signs.
Pathology: Adults are found attached to the gastric mucosa.

CAPILLARIA HEPATICA
Etiology: Capillaria hepatica
Transmission: Adult lives in liver. Eggs become embedded in hepatic parenchyma. A susceptible host must ingest infected liver or food or water contaminated with eggs from a decomposed infected liver.
Clinical: C. hepatica is a common parasite of rats in many parts of the world, but also infects a variety of other species, including chimpanzees, New World monkeys, cynomolgus monkeys, and humans. Clinical signs include hepatomegaly and eosinophilia.
Pathology: Acute to chronic hepatitis with eosinophilia. Eggs embedded in hepatic parenchyma. Eggs measure about 60 by 30 μm, are thick-walled, pitted, and double operculated.

ENTEROBIUS
Etiology: Enterobius vermicularis, E. anthropopitheci
Transmission: Fecal-oral
Clinical: Common in chimpanzees. Rare fatal infections have been described.
Pathology: Usually incidental. Enterocolitis and typhlitis can occur.
References:
Hasegawa MK, et al. Fatal infection with human pinworm, Enterobius vermicularis, in a captive chimpanzee. J Med Primatol 31:104-108, 2002.

ACANTHOCEPHALA (thorny-headed worms)
Etiology: Prosthenorchis elegans is found in the cecum and colon. P. spirula is found in the terminal ileum.
Transmission: Ingestion of the intermediate host (cockroaches and beetles) is the mode of transmission.
Clinical: Nonspecific signs such as cachexia, intussusception, and rectal prolapse are seen in New World monkeys.
Pathology: Adults embed deeply into the intestinal mucosa, causing marked granulomatous inflammation and a nodule visible from the serosal surface. They often penetrate the mucosa and invade the muscle layers. Attachment sites may perforate and cause peritonitis. Heavy infections can cause mechanical blockage and intussusception.
References:
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ATHESMIA

Etiology: Athesmia foxi
Transmission: The mollusk is the intermediate host.
Clinical: Although common in NWM such as capuchin, squirrel monkey, marmosets and titi monkeys, there are usually no clinical signs.
Pathology: The small trematodes are found in the interlobular bile ducts of NWM. Their small size makes them easy to miss on gross examination.
References:

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GASTRODISCOIDES
Etiology: Gastrodiscoides hominis
Transmission: Gastrodiscoides affects Macaca spp and humans. Snails are the intermediate host. Macaques become infected by ingesting metacercariae encysted on vegetation.
Clinical: Gastrodiscoides infection is usually asymptomatic, although large numbers of organisms may cause mucoid diarrhea.
Pathology: Adults are found in the lumen of the cecum and colon.

SCHISTOSOMA
Etiology: Schistosoma mansoni, S. hematobium, S. japonicum
Transmission: The snail is the intermediate host.
Clinical: Usually there are no clinical signs.
Pathology: Adults are found in mesenteric (mansoni, japonicum) or pelvic and mesenteric (hematobium) veins of various wild-caught OWM and NWM. Phlebitis and thrombosis can occur. Black pigment can be seen in the liver and spleen, with granulomas containing eggs in many tissues, especially in the liver. Eggs may also be in vessels. Eggs are identified by the characteristic location of the spine.
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## PEDIATRIC PATHOLOGY OF NONHUMAN PRIMATES

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PRIMATE SERVICES

Genetics laboratory for typing Macaca mulatta: William H. Stone, PhD, Trinity University, San Antonio, TX 78284 (512-736-8347).

Genetic management of primate colonies: Bennett Dyke, PhD, Department of Genetics, Southwest Foundation for Biomedical Research, PO Box 760549, San Antonio, TX 78245-0549. (bdyke@darwin.sfbr.org)

Simian Virus Reference Laboratory: Dr. S.S. Kalter, Virus Reference Laboratory, Suite 202-203, 7540 Louis Pasteur, San Antonio, TX 78229, (512-696-5510).

Herpes B serology and virology: NIH B Virus Resource Laboratory, Viral Immunology Center, Georgia State University, 50 Decatur Street, Atlanta, GA 30303. Information and submission forms can be obtained from Dr. Richard D. Henkel, NIH B Virus Reference Laboratory, Georgia State University, PO Box 4118, Atlanta, GA 30302-4118. (404-651-0808; biordh@panther.gsu.edu) Julia Hilliard, PhD, Director, Office of Sponsored Programs, Georgia State University, PO Box 4118, Atlanta, GA 30302-4118.

Primate Information Center: Erik McArthur, BS or Cathy Johnson-Delaney, DVM, Primate Supply Information Clearinghouse, Washington Regional Primate Research Center, University of Washington, PO Box 357330, Seattle, WA 98195-7330, (206-543-5178; fax 206-616-1710; psic@bart.rprc.washington.edu).

Laboratory Primate Newsletter: Judith E. Schrier, Editor, Psychology Dept., Box 1853, Brown University, Providence, RI 20912.

Environmental Enrichment Services: J. Erwin, PhD, Director, Dept. Primate Ecology, Diagnon Corp., 15215 Shady Grove Rd., Ste 306, Rockville, MD 20850, (301-258-9477).

PRIMATE INFO NET WEB SITE

http://www.primate.wisc.edu/pin

# DISEASES OF HAMSTERS

***POLA - 2005***

Marti Hanes, DVM
Toxicologic Pathologist
Integrated Laboratory Systems, Inc
Box 13501 Research Triangle Park, NC
mhanes@ils-inc.com

**Syrian hamster** (*Mesocricetus auratus*) (a.k.a. Golden hamster), compact body, short legs, 4 front toes, 5 rear, 6 inches long, 120g, with a short tail, dark ears, and black eyes; may be any color, including Albino. 22 chromosome pairs, females are larger than males.
**Chinese hamster** (*Cricetulus griseus*) (a.k.a. gray hamster ) which is shorter, 30-35g, gray dorsal, and with a black stripe down its back. 11 chromosome pairs. Most often used are Syrian and Chinese hamsters

**European hamster** (*Cricetus cricetus*) (a.k.a. blackbellied hamster) 22 chromosome pairs
**Armenian hamster** (*Cricetulus migratorius*) (a.k.a. migratory hamster)
**Dzungarian hamster** (*Phodopus sungorus)* (a.k.a. Siberian, dwarf, striped hairyfooted hamster) 30-50g,

> Molting: After transfer into a short daylight regimen, the brownish summer pelage of the Djungarian hamster (Phodopus sungorus) changes into the whitish winter phenotype. Although changes in serum prolactin levels are identified as the initiating hormonal signal, morphological data about molting in that species are sparse. The aim of this study was to characterize in detail the summer and winter pelage of the Djungarian hamster and to analyze the alterations in the skin and pelage induced by photoperiodic changes. The main difference between summer and winter hair types is the pattern of pigmentation. In contrast to other mammalian species showing seasonal changes, the winter coat of the Djungarian hamster is not characterized by an increase in hair density. Molting patches were observed at all times, even in the winter coat, showing that the light regimen does not control the process of molting itself but the pattern of pigmentation and eventually the loss of hair during the single molting wave. **Kuhlmann MT, Clemen G, Schlatt S. Molting in the Djungarian hamster (Phodopus sungorus Pallas): seasonal or continuous process? :** J Exp Zoolog Part A Comp Exp Biol. 2003 Feb 1;295(2):160-71.

**South African hamster** (*Mystromys albicaudatus*) (a.k.a. white tailed rat)

**Specialized Anatomic and Unique features**

· **Cheek pouches** in hamster are well developed, are highly distensible evaginations of lateral buccal walls, are used to store and transport food, can be easily evaginated under anesthesia was a common site for experimental tumor implantation and vascular physiology studies; are immunologically privileged sites.
· **Hip or flank glands** in hamsters are sebaceous glands with pigmented cells and terminal hairs which secrete during sexual arousal in both sexes and are used for olfactory marking of territory.

**Gastrointestinal:** Long duodenum, long jejunum, short ileum, big cecum, long colon-

· **Stomach** of hamsters has a distinct constriction between the forestomach and glandular stomach and there is almost no lesser curvature resulting in two blind sacs. Pregastric fermentation
· **Cecum-** divided into apical and basal portions separated by a semilunar valve, series of 4 valves in the ileocecocolic area.

· **Kidneys** have extremely long papillae which extend into the ureters. For water conservation
· **Heart** -accessible Purkinje network in addition to S.A. node- useful for conduction experiments
· **Vaginal Discharge**-Post ovulatory, dimethyl disulfide (smells like rotten eggs), used as breeding tool, can be mistaken for inflammatory exudate. Duplex uterus, 7pr mammae. Females are hyperactive during estrus, and can travel considerable distance
· **Sexual dimorphism**: Male Syrian hamsters have bigger adrenals due to 3x thicker zona reticularis; in most rodents, females have bigger adrenals.
· **Rederivation**: Germfree derivation not practical, cross fostering doesn't work, infants are extremely immature when born, have sharp incisors at birth, and usually will reject others' infants
· **Liver- Intranuclear Inclusions**, Nonspecific- Cause not definitely known, but thought to be formed by invagination of nuclear membrane with incorporation of some cell cytoplasm. Inclusions are weakly eosinophilic with H&E, PAS-negative, 5-8 microns in diameter, homogeneous or granular, and usually eccentrically located within the nucleus. The nuclei containing them are frequently enlarged and have an irregular wrinkled profile. Intracytoplasmic Nonglycogenic Present in liver in normal circumstances, but more numerous if there is hepatic damage. Eosinophilic with H&E, strongly PAS-positive before and after diastase digestion, thus, indicating nonglycogenic character. Stain intensely with Sudan black, suggesting presence of bound lipids; are also acid-fast with Ziehl-Neelsen. Usually 2-30 microns in diameter,
· **Trophoblastic Giant Cells**. Are derived from the fetal placenta; specifically from the trophoblast ;the epithelial cell layer covering the blastocyst. The blastocyst erodes the uterine mucosa to establish the hemochorial placenta seen in hamsters, guinea pigs, other rodents, and primates; placenta in hamster called labyrinthine hemotrichorial. The trophoblastic giant cells are in direct contact with maternal bloodstream. These cells exhibit remarkable migratory activity, and frequently are found inside mesometrial uterine arteries and ovarian arteries; they apparently only migrate toward arterial blood. Can be seen three weeks postpartum.
· **Hematology**-polychromasia, anisocytosis, RBC 50-78days, heterophils, 60-70% lymphocytes
· **Immunologic tolerance**- lack of suppressor T cells, atypical cytotoxic T cells
· **Behavior-** Syrian hamsters are solitary creatures that don't enjoy each other's company except when breeding. They are easily disturbed and agitated. females are aggressive, especially when lactating or pregnant, prone to fight and kill others. Cannibalism is common, especially when stressed, primiparous females. Some neonates may survive with limb amputation. Chinese hamsters are more pugilistic. Hamsters are nocturnal, with high activity, several miles on a exercise wheel within 24 hrs. Active chewers and adept escape artists. Permissive rather than obligatory hibernators . Maybe induced with low temp, short days, solitude, adequate to abundant food stores and nest materials. High temperature low water may stimulate estivation, animals will loose weight and increase brown fat stores, reproductive activity ceases with atrophy, pseudohibernation-sensitive to touch

**Disease Differential List for Hamsters**

· **Enteritis**; wet tail, diarrhea, constipation-Tyzzer's, pathogenic E. coli, Clostridium difficile, Campylobacter Cryptosporidium, Salmonella, Giardia, Chlamydia, cecal mucosal hyperplasia, Proliferative ileitis, cestodiasis, gastric

hairballs, ingestion of bedding, intussusception, rectal prolapse, uterine prolapse, dietary change, water deprivation sub clinical-Spironeucleus, adenovirus

· **Respiratory** Disease, otitis, pneumonia- Sendai, Pasteurella pneumotropica, Streptococcus pneumonia, S. agalactiae, PVM, Mycoplasma pulmonis, congestive heart failure

· **Hepatitis**/Hepatic necrosis (white spots on liver) - Leptospirosis, Tularemia, Salmonella, E. coli, Tyzzer's, Taenia sp, Polycystic liver disease, hepatic cirrhosis, hamster papovavirus

· **Bumps**, subcutaneous nodules-normal-testes, cheek pouches, flank glands), abscesses, hernias, neoplasia, arthropathy, mastitis, impacted cheek pouch, granulomas in skin or lymph nodes

· **Dermatitis**- Demodicidosis, bedding associated, trauma, cannibalism, protein deficiency, Notodres, dermatophyte (/), bacterial-P. pneumotropica, Staph. aureus

· **Ocular** -LCM, bacteria, bedding/dust, bite wounds, tooth abscess, trauma

· **Teeth**- malocclusion, parvoviral infection, dental caries, periodontal disease

· **CNS**-torticollis-encephalitis or inner ear; incoordination-insecticide, LCM, tick paralysis, epilepsy; SHN

· **Infertility, infant loss**-seasonal quiescence, over use of males, dystocia, mastitis, pseudopregnancy, cannibalism, agalacia, immaturity, stress, reluctance to breed, senescence, starvation, Vit. E deficiency in females, cold, prolonged darkness, pair incompatibility, inadequate nesting materials, transparent cage, large fetal load

## BACTERIAL INFECTIONS

· **Proliferative Ileitis** (Transmissible Ileal Hyperplasia, "Wet-tail", proliferative regional enteritis, proliferative bowel disease, terminal ileitis, terminal enteritis, enzootic intestinal adenocarcinoma, atypical ileal hyperplasia, hamster enteritis.) Is common and important natural disease of hamsters; usually occurs as an epizootic disease of weaning animals with a morbidity of 20-60% and a mortality of affected animals reaching 90%; usually associated with stressful conditions such as transport, overcrowding, surgical procedures, diet, and experimental manipulations. The term wet tail should not be used as it includes all the diseases that cause diarrhea in hamsters, this is a significant cause of disease in pet store animals, and a potential complication to research. Incriminated agents include *E coli, Campylobacter sp, and Cryptosporidium, Chlamydia sp., and Campylobacter-like* organisms. Clinical signs include are usually confined to younger animals, esp. during the weaning process, and are resistant after 12 weeks. Include lethargy, anorexia, irritability, ruffled hair coat, huddling in corners, fetid watery diarrhea, dehydration, weight loss, drop in body temperature, abdominal distention, and occasionally convulsions. Rectal prolapse or intussusceptions frequently occur. Usually die within 48 hours of appearance of signs; some animals remaining alive become runted, emaciated and cachectic; with perineal soiling. Necropsy: Early see segmentally enlarged terminal ileum with roughened reddened mucosa and reddened serosa; flaccid cecum with fetid watery contents. Later see more thickening of ileum with small white spots and serosal nodules; then see markedly thickened, rigid, friable terminal ileum with white nodules on serosal side, occasional local fibrinous peritonitis with or without adhesions, occasional

intussusception of ileum into cecum, caseous material in the lumen, and a necrotic mucosa. The opened bowel reveals an abrupt transition of the cranial, normal ileum and caudal cecum with the affected, hyperplastic mucosa. Microscopically, hyperplasia of crypt epithelial cells, migration of mitotically active, immature epithelium onto the villi, with elongation, distortion, fusion and widening of villi in terminal ileum. This is followed by downward extension and penetration of crypts through lamina propria, muscularis mucosa, submucosa and Peyer's patches, muscularis externa, and the serosa with concomitant necrosis of epithelial cells and development of varying degrees of necrosis and hemorrhage, pyogranulomatous inflammation and crypt microabscesses. Chronic lesions include fibrous tissue proliferation around false diverticula (not true diverticula because muscle layers penetrated).With silver(Steiner's, Warthin-Starry) or PAS stains, numerous and characteristic small bacteria can bee seen in the apical cytoplasm of proliferation enterocytes. Macrophages in the lamina propria and submucosa contain abundant PAS+ material in the cytoplasm. Ultrastructurally, see slightly curved bacilli usually free in cytoplasm near apices of cells.

· *Campylobacter fetus ssp. jejuni* has been isolated from outbreaks of proliferative ileitis and from clinically normal animals. May have watery diarrhea. Hamsters have been somewhat resistant to experimental disease. Hamsters may shed the organism for several months. It is a zoonotic threat.

· *Escherichia coli* clinically similar to other causes of diarrhea. Isolates of strains 1056, 1126, 4165 from naturally occurring cases of enteritis were pathogenic when injected into susceptible recipients. Ligated loop test revealed changes in most weanlings and some adults. The small intestine may contain yellow to dark red fluid material. histopathological changes include blunting and fusion of villi degeneration and sloughing of enterocytes, PMNs in the lamina propria. But hyperplasia of the intestinal mucosa is not seen. The mesenteric lymph nodes may have lymphoid hyperplasia or PMN infiltration. There may be focal coagulative necrosis in the liver with PMNs, and gastric ulcers. Colitis, typhlitis, intussusception. Ultrastructurally the ileal enterocyte cytoplasm reveal bacilli. Enteropathogenic *E coli* may play a role in ileal hyperplasia.

· *Clostridium piliforme* (**Tyzzer's Disease**). (a.k.a. *Bacillus pilliformis*) Several epizootics have been observed. Gram negative spore forming, 16s ribosomal RNA indicates closed relation to *Clostridia sp* than *Bacillus sp.* Wide host range with interspecies transmission a possibility. Hamsters infected by contact with affected animals or bedding. Predisposing factors include intestinal parasitism, poor sanitation, inappropriate feeding. Animals infected with liver homogenates had detectable lesions in the intestine and liver in 3 days. In Syrian hamsters 28% of 64 weanlings became ill with huddled, sleepy appearance and staining of perineum with pale yellow feces; animals died or were moribund within 48 hours. Gross lesions were variable. The lesions may be confined to either the liver or the intestinal tract. Multifocal hepatic necrosis may be present. Intestinal lesion when viable

grossly consist of varying degrees of dilation of the large intestines and occasional reddening of the serosa of the ileum ; the intestines filled with foamy yellow material, gray plaques were occasionally seen in cecal or colonic mucosa. Several small white spots were occasionally in the liver. Microscopically, hepatic lesions were characterized by foci of coagulative necrosis with peripheral neutrophilic infiltration. Intracellular bundles of bacteria are best demonstrated at the periphery of hepatic lesions. Lesions in the intestinal tract consist of edema of the lamina propria with PMNs and effacement of the mucosal architecture. There may be extension of inflammation into the muscular tunics. Typical bacilli are demonstrable in the enterocytes adjacent to affected areas. Focal granulomatous myocarditis has been associated with Tyzzer's in hamsters. Bacilli are best seen with Warthin-Starry or Giemsa. Organisms are long, pleomorphic, and sometimes beaded bacilli; are not generally believed to grow in cell-free media; are Gram-negative and spore forming; and are cultured in chick embryos. Significant cause of morbidity and mortality with interspecies transmission.
· **Salmonellosis**. *S. enteritidis* serotypes typhimurium and enteritidis .
Hamsters are very susceptible and disease is frequently a cause of diarrhea. Transmission is probably ingestion of contaminated food and bedding. Explosive outbreaks of acute salmonellosis are characterized by depression, anorexia, dyspnea, ruffled coat and high mortality. At necropsy there are multifocal pinpoint areas in the liver, patchy pulmonary hemorrhage and red hilar lymph nodes. Microscopically foci of congestion, hemorrhage, interstitial pneumonia, and erosion and necrosis of the walls of veins and venules with formation of pulmonary phlebothrombosis (partially occluding septic thrombi). There was focal necrosis in the liver and spleen, and hepatic venous thrombosis. Embolic glomerular lesions and focal splenitis may occur. There were no enteric signs or lesions. Organism may be recovered from the blood, lung and other viscera. Danger of interspecies transmission. Lesions were reproduced in inoculated hamsters.

· **Antibiotic-associated Enterocolitis** (Clostridial Enteropathy)
Lincomycin, clindamycin, ampicillin, vancomycin, erythromycin, cephalosporins, gentamicin, and penicillin.
Profuse diarrhea, with high mortality, occurs within 2-10 days following the oral or parenteral administration of certain narrow spectrum antibiotics. The predominant bacterial microflora of the hamster intestine are Lactobacillus and Bacteriodes. Following therapy overgrowth with Clostridium difficile occurs, resulting in acute colitis, diarrhea and death. In animals treated with vancomycin 100% mortality, oral administration of cecal content from normal animals (yummy) provided some protection. This alteration of the inhibitory barrier of gram neg anaerobes and other Clostridia, may allow colonization of *C. difficile* and elaboration of toxin. The cecum is distended with fluid contents, with hemorrhage into the gut wall. Histopathology may reveal lesions from mild typhlitis to acute pseudomenbranous typhlitis. There is effacement of the mucosal epithelium, edema of the lamina propria, leukocyte infiltration, mucosal hyperplasia.

Terminal ileum and colon may be involved. Anaerobic culture should recover. *C difficile*, cytotoxicity by cell culture of mouse inoculation, cytotoxin neutralized by antitoxin.

· **Non-antibiotic associated Clostridial Enteropathy**- acute onset enteritis has been seen in hamsters w/o history of antibiotic administration. Necrotizing typhlitis with mucosal damage are characteristic. C. difficile cytotoxin may be demonstrated in cecal contents of affected hamsters.
· Cecal Mucosal Hyperplasia -Spontaneous cases of cecal hyperplasia have been observed in weanling and suckling hamsters. Diarrhea, runting, and high mortality were associated with disease. At necropsy, ceca are congested, contracted and opaque. Microscopic changes observed include increased mitotic activity and hyperplasia of enterocytes lining cecal crypts, and focal mucosal erosions. Bacterial cultures and EM have failed to id an agent.
· **Leptospirosis**- hamsters are susceptible to various species of *Leptospira sp*.. Severe hemolytic disease, jaundice, hemoglobinuria, nephritis, and hepatitis within 4-6 days.
· **Tularemia** (*Francisella tularensis* ) One descriptive report of an acute outbreak in a colony with 100% mortality. Animals had roughened haircoats, huddled, and died within 48 hours. Grossly, lungs were mottled with subpleural hemorrhages; livers were enlarged and pale; spleens were enlarged, one had white foci; the Peyer's patches were raised and chalky white; mesenteric lymph nodes were enlarged and were chalky white. Microscopically, there was necrosis of lymphoreticular tissues with variable hemorrhages and bacteremia. Source of infection not definitely known; contaminated fresh vegetables suspected.
· ***Yersinia pseudotuberculosis*** Infection is by fecal contamination of food and water by wild rodents and birds. Chronic emaciation with intermittent diarrhea. See caseous lesions in mesenteric lymph nodes, spleen, liver, lungs, gallbladder, and intestinal walls. See intermittent diarrhea.
· ***Streptococcus sp***. beta-hemolytic streptococci causing acute suppurative mastitis in two hamsters.
· ***Streptococcus (Diplococcus) pneumoniae*** Infections caused pneumonia in one colony.
· ***Pasteurella pneumotropica*** . causing mastitis, and dermal abscesses midway between the eyes and ears of 13 hamsters which had received whole body irradiation.
· ***Staphylococcus sp***. purulent exudate in focal lesions on the skin and feet. , lymphadenitis,
· ***Actinomyces bovis*** . Hamsters bearing tumor transplants in their cheek pouches and receiving long-term cortisone treatment developed abscesses on their lips which contained *Actinomyces sp* organisms. There is another descriptive report of *Actinomyces bovis* caused purulent pockets of material in a submaxillary gland in with sulfur granules.
· ***Mycoplasma pulmonis*** -Experimental and natural infections have been reported, but role as a pathogen not known.

· ***Corynebacterium kutcheri***--G+ diptheroid bacillus isolated from the oral flora , esophagus, cecal contents, submaxillary nodes and upper respiratory tract of normal adult Syrian hamsters, possible reservoir host. Giemsa of smear-"Chinese characters"

## VIRAL INFECTIONS

· **Lymphocytic Choriomeningitis (LCM)** (Arenaviridae, Genus Arenavirus). Wide host range (primates including man, rodents), natural reservoir is wild mouse. Infection exposure with urine or saliva from animals shedding virus, including oronasal or skin abrasions. cage to cage aerosol transmission not impatient part of transmission. Congenital infection is hamsters occurs, cell culture and transplantable tumors have been contaminated and are a laboratory source of importance. The disease depends on the age, strain and dose of virus and route. Experimentally, newborn hamsters have approximately half clear the virus with lymphocytic infiltration. The other half will be viremic for 3 mo, viuric for 6mo. At six months there is chronic wasting, lymphocytic infiltration in the liver, spleen, lung, meninges and brain. Vasculitis and glomerulitis with antigen and antibody complexes in the arterioles and glomerular basement membranes. LCM infected hamsters are the primary source of LCM in humans. Hamster source of infection for man.; infections were contracted from hamsters or infected tumor-cell lines passaged in hamsters. Sequelae post exposure may vary from subclinical infections to influenza like symptoms. Occasional viral meningitis or encephalomyelitis may occur. Diagnosis by serology, IFA , however, complement fixation tests may be confounded by high anticomplement antibodies in young hamsters.

· **Parvoviral Infection**- An epizootic of high mortality with malformed and missing incisors has been observed among sucking and weaning pups in a breeding colony of Syrian hamsters. Necrosis and inflammation of the dental pulp with mononuclear leukocytic infiltration of the dental lamina and osteoclasis of alveolar bone. Seroconversion to rat Toolan H-1, a parvovirus that has previously been shown to have similar effects in experimentally infected neonates.

· **Hamster Papovavirus-(HaPV)polyoma**- similar to but not identical with the polyoma virus of mice. It is the cause of Transmissible lymphoma, which can occur in epizootics among young hamsters, and keratinizing skin tumors of hair follicle origin or subclinical infections. IT is not a papilloma virus. HaPV is not common, but infection in both European and Syrian hamster colonies have been reported in the US and Europe. Theorized to have been introduced from wild European hamsters to Syrians. Spread by urine, causes a multisystemic infection with persistence in the kidney and shedding in the urine. Virus is oncogenic, but tumor formation is a side effect instead of being essential to the life cycle of the virus. Typical of polyoma viruses, the virus can infect cells lytically with virus replication, or transform cells without replication. Lymphomas do not have detectable virus, however HaPV epitheliomas have virus replication in the epithelium (similar to papilloma virus). Hamsters are susceptible to the oncogenic effects of the

virus beyond the neonatal period.
Affected hamsters are thin, with palpable abdominal masses. Lymphomas in the mesenteric lymph nodes, axillary and inguinal lymph nodes with out involvement of the spleen. Infiltration of the liver sinusoids, kidney, thymus and other organs occurs. Cytologically, the tumors are usually lymphoid, but erythroblastic, myeloid and reticulosarcomas have been seen. Tumors arising in the thymus are T-cell, mesentery are B-call. Variably differentiated from blastoid to plasmacytoid. mesenteric masses may involve the intestinal wall, and may exhibit central necrosis. Skin tumors are non-glaborous areas with keratinizing structures resembling trichofolliculomas.
Epizootic HaPV is unique, lymphoid tumors are otherwise rare in hamsters, and then only seen in aged animals. Trichoepithelioma have not been described in hamsters unless they were associate with HaPV. Crystalloids can be visualized in the keratinizing epithelium. No serological test., depopulate.
· **Adenovirus**- INIB have been seen in ileal enterocytes in tissues from young (<4wks) hamsters. Mouse Adenovirus K87 strain antibodies have been detected in hamsters. Large amphophilic intranuclear inclusions in villar enterocytes, goblet cells and rare cryptal epithelium. Asymptomatic, no inflammation. Virus viable on EM and serology, significance is unknown.
· **Cytomegalovirus (Salivary Gland Virus) (Herpesvirus**). Virus is host specific; produces subclinical disease in Chinese hamsters. Acinar cells of the submaxillary glands are more affected than ductal cells with intranuclear and occasionally cytoplasmic inclusions, megalocytes and lymphocytic infiltrations
· **Pneumonia Virus of Mice (PVM) (Paramyxovirus**). Natural hosts include hamsters and rats. Conventional colonies are seropositive without clinical disease. Old literature suggests it was the etiologic agent of interstitial pneumonitis with consolidation, however more temporal significance is unknown.
· **Sendai Virus (Paramyxovirus**).Sendai infections are widespread, confirmed clinical disease is scarce. There are reports of mortality in newborn Syrian and Chinese hamsters, and they are regarded as natural hosts. Mild necrotizing bronchiolitis and focal interstitial pneumonia can be seen.

**Papillomavirus** A combination of 9,10-dimethyl-1,2-benzanthracene (DMBA) application and excisional wounding on the lingual tips of Syrian Golden hamsters (Mesocricetus auratus) induces dysplastic and malignant mucosal lesions. Papillomavirus genus-specific antigen and viral particles, measuring 55 nm in diameter, were demonstrated in the nuclei of squamous cells of dysplastic lesions showing koilocytotic change. In this study, we cloned a circular genome at a single Kpnl site from one of these dysplastic lesions. The genomic sequence of this clone, consisting of 7647 bp, was shown to be that of a novel papillomavirus with a conserved genomic organization. We named the new virus hamster oral papillomavirus (HOPV). All dysplastic lesions induced by this combination of DMBA application and excisional wounding contained viral DNA. Although Southern blot hybridization analysis could not detect the HOPV genome, PCR analysis demonstrated the latent HOPV genome in the tongue and skin of an untreated hamster. These results suggest that latently present HOPV genome is reactivated by the DMBA/wounding procedures. Lingual HOPV infection may be an important model for gaining insight into the interactions between papillomavirus infection, chemical carcinogens and physical irritations in carcinogenesis or malignant transformation.
**Iwasaki T, Maeda H, Kameyama Y, Moriyama M, Kanai S, Kurata T. Presence of a novel hamster oral papillomavirus in dysplastic lesions of hamster lingual mucosa induced by application of dimethylbenzanthracene and excisional wounding: molecular cloning and complete nucleotide sequence.** J Gen Virol. 1997 May;78 ( Pt 5):1087-93.

· Other virus- Syrian hamsters will seroconvert to mouse

encephalomyelitis virus **(GDVII), Reo 3, SV5, paramyxovirus, and rat parvoviruses.** There is an endogenous oncovirus, as well as a in vivo sensitivity of newborn hamsters to oncogenic viruses.

MYCOTIC INFECTIONS

Spontaneous confirmed infection have not been reported.

**PROTOZOAN PARASITES**

· ***Spironucleus (Hexamita)*** muris.-Normal flora, found in small intestine and cecum transmit by ingestion. Non pathogenic to hamsters, dubious pathogenic to mice. Organisms have six anterior and two posterior flagella ; are pyriform 7-9 m long and 2-3 wide, normally feed on intestinal bacteria and usually viewed as incidental finding. However, under unusual circumstances may see mucosal damage and clinical signs. also may see the flagellates in the peripheral blood of hamsters with enteritis. Transmission to rats and mice. May alter macrophage activity and immune response in the mouse.

· ***Giardia muris , G . mesocricetus*** Common in some hamster colonies; found in small intestine, usually asymptomatic non-pathogenic however suggested to be responsible in part of chronic intestinal amyloidosis, with diffuse infiltration of the intestinal lamina propria with plasma cells and lymphocytes, and mural fibrosis. Causes enteritis in mice; transmit by ingestion. Wet mount from the duodenal region reveals pear shaped organisms with characteristic rolling, tumbling movement. H&E stained sections reveal pear shaped ellipsoidal organisms attached to the brush border of the enterocytes. Organisms may be seen I the inter villar spaces, crypts of the duodenum, extending to the villar tips. Has 8 flagella, is pyriform, has a large sucking disc on anterior ventral side, and has two nuclei. Cysts are thick walled ovoid and more easily visualized with Giemsa or phase contrast and have four nuclei. Unknown level of threat to humans., possible interspecies transmission.

· ***Encephalitozoon cuniculi*** infection described in a study of transplantable plasmacytoma of hamsters.

· ***Balantidium coli***. Rare in hamsters; found in cecum and colon, usually non-pathogenic but occasionally causes enteritis and diarrhea

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**HELMINTH PARASITES**

**Pinworms**-Diagnosis by perianal eggs retrieved from cellophane tape impression smears, fecal float, cecal smears at necropsy.

· ***Syphacia mesocriceti*** (Hamster pinworm) not as common as *S. oblevata*. differences are minor morphology

· ***Syphacia obvelata*** (Mouse pinworm), in cecum and lesser extent colon; direct L.C.; eggs laid around anus; autoinfection occurs; esophageal bulb round; eggs flat on one side; small cervical alae; mild enteritis with heavy infection

· ***Syphacia muris*** (Rat pinworm) infection was experimental;
· ***Aspiculuris tetraptera*** (Mouse pinworm). no documented evidence that hamsters are susceptible.
· ***Trichosomoides nasalis*** (Europe). In nasal mucosa;.

**Cestodes.**

· ***Hymenolepis sp***. Relatively common when compared to mice and rats., usually asymptomatic. Diagnosis is by identification of eggs in fecal samples, crush preps of intestinal contents, or histology
· ***H. nana*** (dwarf tapeworm); is the smaller even than *H. diminuta* found in lower small intestine. With direct life cycle get quick immunity because of tissue phase.
· ***Hymenolepis diminuta***. Not common; need intermediate host. (flour beetle, moth, flea); cause acute catarrhal enteritis or chronic enterocolitis. Is zoonotic if ingest Intermediate host.
· ***Hymenolepis microstoma***. Uncommon; found in duodenum, bile duct, gallbladder, and liver; can get inflammation and necrosis; intermediate host. are flour beetles and fleas.
· ***Cysticercus fasciolaris (Taenia taeniaeformis***). Larval stage of dog and cat tapeworm; see cyst in liver. Contaminated food with feces from definitive host.

## ARTHROPOD PARASITES

**ACARIASIS**

Male hamsters usually have a larger mite load. Skin scrapings cleared with 10%KOH or NaOH.
· ***Demodex sp.*** mites are common in animal facilities. Syrian hamsters born to infected suckling dams acquire the infection during the suckling period. Are generally of low pathogenicity, and clinical signs rarely occur. Older animals and those hamsters subjected to experimental stressors may develop alopecia, over the back neck and hindquarters. Denuded areas are nonpuritic, dry and scaling.
· ***D.criceti*** Found usually in the epidermal pits with sparing of the dermis. No tissue reaction or pigmentation;
· ***Demodex aurati*** Found in hair follicles and in pilosebaceous units. hair follicles may be dilated with mites.
· ***Sarcoptes scabei***. Itch mite, sarcoptic mange mite; rare in hamsters; only in epidermis; burrows in epidermis; papular dermatitis with pruritus and selfmutilation.
· ***Notoedres notodres***. Found in the stratum corneum, with scabby lesions of the ears, nose, feet, perineum.
· ***Notodres cati***-outbreak reported
· ***Speleorodens clethrionomys***- nasal mite infestation detected in three breeding colonies in Europe
· ***Ornithonyssus bacoti*** (tropical rat mite); troublesome pest of lab animals; can debilitate and cause death from chronic blood loss; can infect man; vectors of many microorganisms.

· ***Ornithonyssus sylvarium*** (northern fowl mite); a bloodsucker, usually on plumage of chickens; hamsters are incidental hosts; entire L.C. on host.

## MYIASIS

rare cases of *Wohlfahrtia vigil* (flesh fly), *Sarcophaga hemorrhoidalis* (flesh fly), *Musca domestica* (house fly). cause dermal myiasis; infestation with maggots if wild or when no screens. Flesh fly females are I and lay larvae in open wounds and fetid sores; larvae develop in 4-7 days, pupate for four days; full life cycle is two weeks; larvae are voracious and often actively invade healthy tissue.

## NEOPLASIA

Reported incidence of spontaneous malignant neoplasms in Syrian hamsters is about 4%, with marked variation in individual colonies, reflecting the influence of both genetic and environmental influences. Majority are endocrine or alimentary and benign.
**Adrenocortical adenomas** one of most frequent.
**Cutaneous lymphoma** (mycosis fungoides)-epidermotropic in adults, lethargy, weight loss, patch alopecia, exfoliative erythroderma. Dense infiltrates of neoplastic lymphocytes in the dermis with extention into the epidermis. The most common malignant tumor is the lymphosarcoma , often involving the thymus, thoracic lymph nodes, mesenteric lymph nodes, superficial lymph nodes, spleen, liver, and others. with variable cell types.
**Other reported tumors** have included: gastrointestinal tract, and skin appendages, glioblastoma, astrocytoma, medulloblastoma, ependymoma, pineocytoma mesothelioma induced by asbestos,.
Newborn hamsters commonly used in vivo to screen for potentially oncogenic viruses. syrian polyoma virus <30 days. Chinese hamsters have high incidence of endomyometrial neoplasms

## MISCELLANEOUS CONDITIONS

· **Pregnancy toxemia.** Reported to occur in some colonies; in one report said to resemble human eclampsia with associated renal cortical necrosis.
· **Diabetes mellitus.** Occurs spontaneously in some inbred strains of Chinese hamsters; inherited by recessive gene, Clinically hamsters demonstrate weight loss, glucose intolerance, mild to severe hyperglycemia, polydipsia, polyuria, hypoinsulinemia, ketonuria. Microscopically, many islets exhibit involution with progressively severe and widespread nuclear pyknotic , shrunken and more eosinophilic cytoplasm with cytoplasmic vacuoles and loss of granules.
· **Arteriolar Nephrosclerosis (Hamster Nephrosis)-** degenerative renal disease important disease of aging hamsters. More frequent in females. Similar to progressive glomerulonephropathy in aged rats. Animals experience weight loss and polyuria, polydipsia. A chronic viral association with LCM has been suggested due to the lymphoplasmocytic interstitial component. Renal vascular hypertension, and antigen antibody antigen complex deposition has also been proposed as causes .Amyloidosis may be concurrent. Affected kidneys are irregular, granular, rough, pate, and pitted. Glomerular changes are

characterized by segmental to diffuse thickening of basement membranes, with deposition of eosinophilic matrix, and frequently obliteration of normal structure. Degeneration of renal tubules is characterized by atrophy, flattening of cells or regeneration with poorly differentiated epithelium. Interstitial fibrosis, basement membrane thickening, tubular dilation with proteinaceous eosinophilic material and casts, fibrinoid change in renal arteries, mild inflammatory response. A significant case of death and morbidity in older hamsters.

· **Polycystic Disease (Polycystic liver disease).** Liver cysts are of varying size up to 2cm , single or multiple, subcapsular and parenchymal, have a thin wall, and contain light to straw colored fluid; similar cysts can occur in epididymus, seminal vesicles, pancreas, endometrium , ovaries and adrenals. Liver cysts are lined by cuboidal epithelium that becomes flattened in larger cysts; occasionally the epithelium is continuous with bile duct epithelium.; cysts maybe multiple and separated by fibrous bands. Adjacent tissues may demonstrate pressure atrophy, hemosiderin deposition, bile duct proliferation and lymphocytic inflammation. Considered to be congenital in origin due to either failure of fusion of interlobular and intralobular ducts, or failure of redundant ducts to atrophy. Usually incidental findings at necropsy

· **Amyloidosis** Commonly seen in normal aging hamsters (over 1 year of age) and in animals with chronic infections. The disease may vary in incidence among colonies, females most common, 5mo-15mo of age. Clinically a increase in serum albumin is accompanied by an increase in serum globulins. Can be experimentally caused by casein injections, and suppressed in females with injection of androgens. Kidneys, adrenals and liver when affected are enlarged and pale. Can also be seen in the lungs, spleen, stomach, intestines, ovaries, testes, and epididymides. Grossly appears white, with Lugol’s solution turns brown color, and with added dilute sulfuric acid turns a blue color. Deposition of eosinophilic homogeneous material in glomeruli of kidneys, around splenic lymphoid follicles, cortices of the adrenals, and around portal triads in the liver with variable involvement of the sinusoids. Microscopically, with H&E is amorphous, slightly pink material with “apple-green” birefringence when stained with Congo Red and is yellow with Thioflavin T. See massive deposition of amyloid with leishmanial infections, tuberculosis, treatment with DES and with certain tumors. Clinically similar to nephrosis, significant liver and adrenal function occur very late in the disease.

· **Atrial Thrombosis and congestive heart failure.** Occurs frequently in older female hamsters, and is often associated with amyloidosis. Thrombi most commonly seen in left atria only (87%). Changes in coagulation parameters and fibrinolytic factors are consistent with a consumptive coagulopathy, blood stasis due to myocardial insufficiency probably is a major contributor to the pathogenesis. Disease recognized by development of dyspnea, tachycardia, and cyanosis due to congestive heart failure. A firm to friable thrombus adherent to adjacent endocardium is present in the left atrium, with variable extention into other chambers of the heart. There is bilateral ventricular hypertrophy, with pulmonary edema and pleural effusion. Thrombi maybe

moderately organized. Myocardial changes when present are characterized by nuclear hypertrophy, vacuolation of the cytoplasm, fiber atrophy, interstitial fibrosis. Concurrent medial degeneration and calcification of coronary arteries. Valvular myxomatous or fibrotic change. Occasionally, some inflammation in heart wall but not significant

· **Age related vascular changes** - fibrinoid degeneration of arterioles, cerebral mineralization,

· **Spontaneous Hemorrhagic Necrosis of the CNS of Fetal Hamsters.** (SHN). Most likely due to Vitamin E deficiency in dams. Recognized in fetal hamsters during last trimester and newborn hamsters. Animals are stillborn, weak or cannibalized. Grossly, see hemorrhage or edema in calvarium and spinal canal Microscopic changes most extensive in the forebrain (proencephalon), with symmetrical subependymal vascular degeneration, and edema with hemorrhage in the adjacent neuropil. Intraventricular hemorrhage has been observed. Lesions may proceed posteriorly and deeper into the neuropil of the brain. When the process is severe, it may affect the spinal cord, but usually spares the cerebellum . There may be thinning and dissolution of developing cortical mantles and neuropil. Retina and internal ear may become edematous and exhibit some necrosis. Myopathy in some fetuses.

Cardiomyopathy in some dams. Appears that disorder of the capillary bed is primary lesion. Vitamin E reduces incidence and severity, and corn oil or linoleic acid increases incidence and severity. It is alleviated by Vit. E supplementation.

· **Hepatic Cirrhosis**. Incidence has been high in some colonies; up to 20% reported; seen in older animals and more in females. Grossly, see uniform nodularity in all lobes. Microscopically, see extensive periportal proliferation of hyalinized fibrous connective tissue with proliferation of bile ducts, nodular hepatocellular regeneration with concurrent degeneration and necrosis, and lymphocytes and neutrophils.

· **Bedding Associated Dermatitis**- Chinese and Syrian hamster leg lesions related to wood shavings. Foot pads with degeneration and atrophy of the digits. Necrotic areas with ulceration may spread to the shoulders. Microscopically, pieces of wood shavings are present in the lesions with pyogranulomatous inflammation. Mulitnucelated giant cells may be present around migrating wood particles. Doe not appear to be a problem with mice and rats, DDX: trauma, cannibalism

· **Dental disease- malocclusion** (molars are rooted, incisors misalign with trauma.) experimental models of periodontal disease, spontaneous is uncommon

## Animal models of human disease

National statistics on animal use in research can provide guidance in setting priorities for research into alternative methods, i.e., those methods that can replace, reduce, or refine animal-based procedures. All else being equal, fields of research causing the most suffering to the largest numbers of animals should be considered prime candidates for alternative research. We examined national statistics on animal use in research in the United States to determine the extent to which vaccine testing accounts for those animals that experience unrelieved pain and distress. During 1998, 96,536 regulated animals were reported to have experienced unrelieved pain and distress in research (laboratory-bred mice and rats, as well as all non-mammals, are excluded from the U.S. reporting system). Vaccine-related testing alone accounted for 61% of this total. Of the 58,820 animals used in such vaccine testing, nearly all were hamsters (68%) and guinea pigs (28%), at least 74% were used in potency tests, and at least 55% were used in testing of Leptospira vaccine. This analysis and an earlier one both underscore the need to develop and implement alternative methods in vaccine testing. **Stephens ML, Alvino GM, Branson JB. Animal pain and distress in vaccine testing in the United States.** Dev Biol (Basel). 2002;111:213-6.

# Experimental Infectious Disease

## Experimental Bacterial-

***Clostridium difficile:*** Studies suggest that asymptomatic colonization with Clostridium difficile (CD) decreases the risk of CD-associated disease (CDAD) in humans. A hamster model was used to test the efficacy of colonization with 3 nontoxigenic CD strains for preventing CDAD after exposure to toxigenic CD. Groups of 10 hamsters were given 10(6) nontoxigenic CD spores 2 days after receiving a single dose of clindamycin. Five days later, the hamsters were given 100 spores of 1 of 3 toxigenic CD strains previously shown to cause mortality within 48 h. Each nontoxigenic strain prevented disease in 87%-97% of hamsters that were challenged with toxigenic strains. Failure to prevent CDAD was associated with failure of colonization with nontoxigenic CD. Colonization with nontoxigenic CD strains is highly effective in preventing CDAD in hamsters challenged with toxigenic CD strains, which suggests that use of a probiotic strategy for CDAD prevention in humans receiving antibiotics might be beneficial. **Sambol SP, Merrigan MM, Tang JK, Johnson S, Gerding DN. Colonization for the prevention of Clostridium difficile disease in hamsters. J Infect Dis. 2002 Dec 15;186(12):1781-9. Epub 2002 Nov 22.**

***Other****: Mycobacterium tuberculosis, MB leprae, Campylobacter sp., Corynebacterium paulometabuleri*

## Experimental Viral

**LCM**-In adult Syrian golden hamsters (Mesocricetus auratus), intraperitoneal or footpad inoculation of the Lymphocytic Choriomeningitis Virus (LCMV) strains, WE or Armstrong (ARM), caused systemic infection and induced serum LCMV-antibody. Hamster and virus strain-dependent lethal disease also occurred. With WE, MHA and PD4 inbred hamsters failed to eliminate infection and died of wasting disease. LSH and CB inbred hamsters resisted lethal WE-disease and cleared infection. LVG hamsters and inbred LHC hamsters were intermediate in WE-susceptibility; some died of wasting, while others survived with little illness. Resistance to lethal WE-disease directly correlated with a delayed-type hypersensitivity (DTH) response to live-virus footpad inoculation. In WE-resistant LSH and CB hamsters, DTH-responses were induced by intraplantar WE-inoculation; footpad edema began by 5 days, reached maximum thickness by 7 to 9 days, and subsided thereafter. In the other hamster strains, DTH to WE could not be elicited. Unlike WE, ARM was hamster-avirulent; infections were self-limited and did not induce DTH. All survivors of primary LCMV (WE or ARM)-infection resisted secondary WE-challenge, and did not develop DTH to LCMV. Immunosuppressive treatments, abrogating DTH and antibody responses to LCMV, rendered all hamsters susceptible to lethal WE-infection. Hamster DTH most likely mediated resistance to virulent LCMV-infection. **Genovesi EV, Johnson AJ, Peters CJ.Delayed type-hypersensitivity response of inbred strains of Syrian golden hamsters (Mesocricetus auratus) to lethal or non-lethal lymphocytic choriomeningitis virus (LCMV) infections.** Microb Pathog. 1989 Nov;7(5):347-60.

**Sheep-associated Malignant Catarrhal Fever-**Lesions induced in hamsters by inoculation with the "sheep-associated" agents of malignant catarrhal fever (SA-MCF) isolated from a red deer (Cervus elaphus), designated D/1 and of bovine origin (C/2), are described. Clinical signs in hamsters inoculated with the D/1 isolate occurred as early as 13 days after infection although the mean incubation period in animals that developed signs was 27 days. Increased numbers of polymorphonuclear leucocytes were present in the blood of clinically affected hamsters. Gross lesions included erosions of epithelium in the buccal cavity, hemorrhage of the forestomach, dilated fluid-filled intestines and enlargement of the mesenteric lymph node. Microscopic lesions were widespread throughout the body but had a predilection for epithelial surfaces. They consisted of hyperplasia of certain lymph nodes, vasculitis and interstitial accumulations of mononuclear cells of lymphoid appearance in non-lymphoid tissues. Cytolysis was also seen. Lesions produced by the C/2 isolate were similar and both isolates produced disease comparable with that seen in naturally occurring cases in cattle and deer. It is suggested that disease might arise through a dysfunction of the immune system following infection of host large granular lymphocytes by the SA-MCF agent, in a way similar to that suggested for the rabbit.
**Buxton D, Jacoby RO, Reid HW, Goodall PA. The pathology of "sheep-associated" malignant catarrhal fever in the hamster.** J Comp Pathol. 1988 Feb;98(2):155-66.

**Coxsackie B3 virus** The hemodynamic changes of the left ventricle (LV) of golden hamsters surviving for 14 months after acute coxsackie B3 virus myocarditis were assessed with the use of a high fidelity micromanometer pressure system. Of 25 infected hamsters, 10 survived to the 14th month, and 4 of these had cardiomegaly. Body weight (BW) was 150.0 +/- 20.7 g (mean +/- SD) (controls, 164.5 +/- 20.1 g, NS); heart weight (HW), 0.499 +/- 0.084 g (controls, 0.448 +/- 0.035 g, NS); and HW/BW, 3.39 +/- 0.79 X 10(-3) (controls, 2.74 +/- 0.23 X 10(-3), p less than 0.05). The hemodynamic data under anesthesia were: HR, 378 +/- 42 (controls, 414 +/- 43, NS); LVSP, 108 +/- 16 mmHg (controls, 126 +/- 16, NS); LVDP, 4.0 +/- 4.8 mmHg (controls, 0.6 +/- 0.7, NS); LVEDP, 9.7 +/- 7.5 mmHg (controls, 3.4 +/- 1.4, NS); peak positive dp/dt, 4960 +/- 1431 mmHg/sec (controls, 6714 +/- 1326, p less than 0.05); (dp/dt)/DP40, 56.8 +/- 9.8 sec-1 (controls, 73.1 +/- 7.0, p less than 0.01); peak negative dp/dt, 3876 +/- 1072 mmHg/sec (controls, 4971 +/- 599, p less than 0.05); and time constant T of LV pressure fall, 7.7

+/- 1.3 msec (controls, 5.9 +/- 0.7, p less than 0.01). Five hamsters had congestion of the lungs and liver with or without an elevation of LVEDP. One of them had an organizing thrombus in the left atrium, and one had an aneurysm in the LV free wall. Though markedly varied in extent, residual myocardial fibrosis was always evident in the hearts in which isovolumic contractility and early diastolic relaxation of the LV were significantly impaired. In a clinical extension of these findings, it may be that some cases of dilated cardiomyopathy in man develop in a way similar to the pathological processes noted in this experiment.
**Morita H, Kitaura Y, Deguchi H, Kotaka M, Kawamura K. Experimental coxsackie B3 virus myocarditis in golden hamsters. II. Evaluation of left ventricular function in intact in situ heart 14 months after inoculation.** Jpn Circ J. 1984 Oct;48(10):1097-106.

**Yellow Fever (YF)** A hamster viscerotropic strain of yellow fever (YF) virus has been derived after serial passage of strain Asibi through hamsters. The parental Asibi/hamster p0 virus causes a mild and transient viremia in hamsters with no outward, clinical signs of illness. In contrast, the viscerotropic Asibi/hamster p7 virus causes a robust viremia, severe illness, and death in subadult hamsters. The genome of the hamster viscerotropic Asibi/hamster p7 virus has been sequenced and compared with the parental nonviscerotropic Asibi/hamster p0 virus identifying 14 nucleotide changes encoding only seven amino acid substitutions. The majority of these substitutions (five of seven) fall within the envelope (E) protein at positions Q27H, D28G, D155A, K323R, and K331R. These results support an important role for the E protein in determining YF virus viscerotropism. **McArthur MA, Suderman MT, Mutebi JP, Xiao SY, Barrett AD. Molecular characterization of a hamster viscerotropic strain of yellow fever virus.** J Virol. 2003 Jan;77(2):1462-8.

**West Nile** Results of experiments evaluating the efficacy of three immunization strategies for the prevention of West Nile virus (WNV) encephalitis are reported. Immunization strategies evaluated included a killed virus veterinary vaccine, a live attenuated chimeric virus vaccine candidate, and passive immunization with WNV-immune serum; all were tested by using a hamster model of the disease. Each product protected the animals from clinical illness and death when challenged with a hamster-virulent wild-type WNV strain 1 month after initial immunization. The live attenuated chimeric virus vaccine candidate induced the highest humoral antibody responses, as measured by hemagglutination inhibition, complement fixation, and plaque reduction neutralization tests. Although the duration of protective immunity was not determined in this study, our preliminary results and the cumulative experience of other virus vaccines suggest that the live attenuated chimeric virus provides the longest lasting immunity.
**Tesh RB, Arroyo J, Travassos Da Rosa AP, Guzman H, Xiao SY, Monath TP. Efficacy of killed virus vaccine, live attenuated chimeric virus vaccine, and passive immunization for prevention of West Nile virus encephalitis in hamster model.**Emerg Infect Dis. 2002 Dec;8(12):1392-7.

**Prion- Scrapie** Current detection of transmissible spongiform encephalopathy (TSE) relies on the proteolytic generation of a protease-resistant core from the scrapie isoform of prion protein (PrP(Sc)) followed by immunoblotting. This process is non-quantitative, time-consuming, and technically demanding. Recently, an alternative in vitro test for TSE based on the differential extraction of brain homogenates using guanidine hydrochloride followed by DELFIA (Dissociation Enhanced Lanthanide FluoroImmunoAssay) has been developed. In the present study, this approach was adopted using a panel of anti-PrP monoclonal antibodies (MAbs) in conventional sandwich enzyme-linked immunosorbent assay (ELISA) to investigate hamster and two distinct strains of mouse prion diseases. Although PrP species were present in both soluble and insoluble fractions from normal as well as TSE samples, only the PrP species in the insoluble fractions from the latter samples were protease-resistant. In addition, certain anti-PrP MAb pairs could distinguish the PrP species in infected brains from those in the normal samples. The ability to differentiate disease-associated PrP isoforms without proteinase K digestion could serve as a panacea for developing a reliable and rapid diagnostic test for prion diseases. **Kang SC, Li R, Wang C, Pan T, Liu T, Rubenstein R, Barnard G, Wong BS, Sy MS. Guanidine hydrochloride extraction and detection of prion proteins in mouse and hamster prion diseases by ELISA.** J Pathol. 2003 Apr;199(4):534-41.

# Experimental Parasitic Disease

## Filariasis

***Acanthocheilonma (Dipetalonema) viteae-***Three species of rodents were immunized with 50 irradiated (35 krad) stage-3 larvae (L3) of the filaria Acanthocheilonema viteae and challenged with an infection of normal L3. The immunization induced a significant reduction of the worm burden developing from the challenge infection in all host species, the jird (Meriones unguiculatus), the multimammate rat (Mastomys coucha) and the golden hamster (Mesocricetus auratus). The induced resistance was highest in jirds (92.5 +/- 9.7) followed by golden hamsters (59.4 +/- 26.6) and multimammate rats (55.1 +/- 40.4). The time course of antibody response against antigens of L3, adult worms and microfilariae, as studied by ELISA, showed quantitative and qualitative differences between the species. The antibody response against L3 antigens in immunoblots was similar in all species. Only one of the golden hamsters developed an antibody response against the surface of vector derived L3, while sera of jirds and multimammate rats did not react with L3 surface.
**Schrempf-Eppstein B, Kern A, Textor G, Lucius R. Acanthocheilonema viteae: vaccination with irradiated L3 induces resistance in three species of rodents (Meriones unguiculatus, Mastomys coucha, Mesocricetus auratus).** Trop Med Int Health. 1997 Jan;2(1):104-10.

***Brugia pahangi*** The susceptibility of Mongolian jirds, Meriones unguiculatus, and PD4 hamsters, Mesocricetus auratus, to Brugia pahangi was compared based on the percentage adult worm recoveries, mean microfilaremia levels, and adult worm lengths. Fourteen male jirds and seventeen male PD4 hamsters were each inoculated subcutaneously in the left inguinal region with 90-100 L3 of B. pahangi and necropsied 130-150 days after inoculation. There were no significant differences between jirds

and hamsters in mean adult worm recoveries (24.7 vs 25.4%) and prepatent periods (69.9 vs 77 days after inoculation). In hamsters, 85% of recovered worms were found in the heart and lungs and 15% were found in genital lymphatic vessels. In jirds, distribution of recovered worms was 66% in genital lymphatics, 23% in the heart and lungs, 8% in the peritoneal cavity, and 3% in lymphatic vessels in other sites. The mean microfilaremia level in jirds (16.5/20 microliter) was significantly higher than in hamsters (8.7/20 microliter. Female worms in the genital lymphatics of jirds were significantly longer than female worms in the genital lymphatics of PD4 hamsters (33.5 vs 27.3 mm). Lengths of worms in other locations were similar between the two species.
Carraway JH, Malone JB. Brugia pahangi: comparative susceptibility of the Mongolian jird, Meriones unguiculatus, and the PD4 inbred hamster, Mesocricetus auratus. Exp Parasitol. 1985 Feb;59(1):68-73.

# Experimental Trematodiasis

**Bilharzia-*Schistosoma mansoni*** The ability of carnosine to improve some liver disorders induced by Schistosoma mansoni parasite in hamsters (Mesocricetus auratus). Results indicate that parasitic infestation induced elevation in serum alkaline phosphatase, gamma-glutamyl transferase, aspartate aminotransferase and procollagen III peptide as a marker of liver fibrosis. Administration of carnosine (10 mg/day) for 15 days either concurrent with infection, 2 and 4 weeks post-infestation was effective in reducing differential worm burden. It was also effective in renormalizing blood glucose level depending on the time course. The most evident effect of carnosine was on serum procollagen III peptide level, which was lowered in infested groups treated with carnosine. Histopathological studies confirmed the potential use of carnosine for intervention in schistosomiasis. **Soliman KM, Abdel Aziz M, Nassar YH, Abdel-Sattar S, El-Ansary A. Effects of carnosine on bilharzial infestation in hamsters: biochemical and histochemical studies.** Comp Biochem Physiol B Biochem Mol Biol. 2002 Mar;131(3):535-42.

***Schistosma haematobium*** Schistosomiasis is a major health problem in many subtropical developing countries, causing a number of serious pathologies, including bladder cancer. Most of the toxic compounds formed as a result of these infestations are derived either exogenously or formed endogenously and can be conjugated with glutathione (GSH) via glutathione S-transferase (GST). The present study investigates the effect of Schistosma haematobium infection on the activity of GST and glutathione reductase (GR) and levels of glutathione and free radicals (measured as thiobarbituric acid reactive substances) in different organs of the male hamster. The total activity of GST was increased in several organs; in kidney by 50 and 46% at 6 and 10 weeks postinfection, respectively, and in bladder tissues by 169, 23, and 130% at 2, 4, and 6 weeks postinfection, respectively. In support of this, the expression of GST isozymes was also induced in kidney and bladder tissues at early stages (2, 4, and 6 weeks) and reduced at the later stages of infection (8 and 10 weeks). In contrast, the expression of these isozymes was decreased in the spleen and liver at 2, 4, 6, 8, and 10 weeks postinfection. Also, such activity was decreased in lungs by 74 and 78% and in bladders by 65 and 72% at 8 and 10 weeks postinfection, respectively. GSH levels increased in lungs by 95, 40, and 56% at 2, 4, and 6 weeks and in spleen by 26 and 74% at 4 and 6 weeks, respectively, but decreased at later stages of S. haematobium infection in these organs. The depletion of GSH levels also occurred in bladders by 72 and 54% at 8 and 10 weeks postinfection, respectively. The activity of GR was increased in the livers, lungs, and kidneys of the S. haematobium-infected hamster. TBARS also increased in the lung by 14, 65, 53, 828, and 624% and in the kidney by 64, 29, 87, 190, and 111%, and in the bladder by 216, 23, 1468, 528, and 1025% at 2, 4, 6, 8, and 10 weeks postinfection, respectively. This study indicates that low GST expression and high levels of free radicals could provide new evidence for damage to the bladder and other organs as a result of S. haematobium infection **Sheweita SA, Mostafa MH, Ebid F, El-Sayed W. Changes in expression and activity of glutathione S-transferase in different organs of schistosoma haematobium-infected hamster.** J Biochem Mol Toxicol. 2003;17(3):138-45.

***Echinostoma friedi*** intestinal flukes from SE Asia Viable eggs produced weekly per infective stage was used as a measure of the reproductive success of Echinostoma friedi during the first 12 weeks of infection in hamsters. The weekly reproductive success was not constant during the experiment in relation to the egg output and the proportion of viable eggs produced. The egg release started during week 2 post-inoculation, attaining a maximum during week 3. A decline in egg output was observed from week 9. Viable eggs were only produced from week 3 post-inoculation and a maximum was attained at week 4 of the experiment. A decline in egg viability was observed from week 9. Considering together the egg output and the egg viability, the maximum weekly reproductive success was obtained during week 4 post-inoculation. The changes in the weekly reproductive success were not reflected in variations in worm numbers and body sizes during the course of the infection. The humoral immune response of golden hamsters during the infection with E. friedi was determined. Increases of IgG levels against somatic and excretory/secretory products of E. friedi were detected coinciding with the reduction in the reproductive success. **Toledo R, Espert A, Carpena I, Munoz-Antoli C, Esteban JG. An experimental study of the reproductive success of Echinostoma friedi (Trematoda : Echinostomatidae) in the golden hamster.** Parasitology. 2003 May;126(Pt 5):433-41.

# Experimental Cestodiasis

***Taenia solium*** -Two groups of hamsters were infected with Taenia solium cysticerci, one of which was suppressed with methyl-prednisolone acetate on the day of infection and every 14 days thereafter. The other did not receive steroid treatment. Faecal and serum samples were taken prior to infection and then at weekly intervals. Parasite circulating- and coproantigens were detected by a capture ELISA with rabbit polyclonal antibodies against T. solium tapeworms. IgG antibodies in serum and in faecal supernatants were detected by ELISA with excretory-secretory products of T. solium adults recovered from hamsters. Infections remained up to 17 weeks in suppressed hamsters, but after week 11 no tapeworms were found in non-suppressed hosts. T. solium coproantigens in both groups of hamsters were positive from the 1st week post-infection (wpi) until the tapeworms were rejected. Circulating antigens were detected only in non-suppressed hamsters from the 3rd wpi until 1 week before T. solium was eliminated. All infected hamsters developed serum IgG antibodies against tapeworms which were detected from the 2nd wpi and decreased slowly after T. solium expulsion. Specific IgG in faecal supernatants was detected from the 3rd wpi only in non-suppressed hamsters. When suppression was stopped, coproantibodies could also be detected. The presence of IgG antibodies indicates that tapeworms induced an immune response in the experimental host and that when hamsters were suppressed with corticosteroids the immune response was impaired and did not allow the detection of IgG coproantibodies. This indicates, in addition, that the passage of T. solium antigens from the small intestine to the circulation was blocked. **Avila G, Benitez M, Aguilar-Vega L, Flisser A. Kinetics of Taenia solium antibodies and antigens in experimental taeniosis.** Parasitol Res. 2003 Mar;89(4):284-9. Epub 2002 Nov 06.

***Echinococcus multilocularis-*** Golden hamsters as alternative definitive hosts of Echinococcus multilocularis were used for coproantigen detection by means of sandwich ELISA. The test was performed in hamsters infected with approximately 20,000, 4,000, 500, 0 (control) and 100,000 (i.e., group I, II, III, IV and V respectively) protoscoleces. Comparison of mean OD values of each group showed significant differences depending on the number of protoscoleces administered and days postinfection. There was also a relatively high statistical correlation between the number of recovered worms and ELISA OD values (correlation coefficient = 0.699, $P < 0.05$), although accurate comparison of worm burdens among individual animals was difficult when numbers of infecting worms fell within the same range. **Sakai H, Furusawa R, Oku Y, Kamiya M.**
**Echinococcus multilocularis coproantigen detection in golden hamster, an alternative definitive host.** Exp Anim. 1996 Jul;45(3):275-8.

# Experimental Fungal Infection

***Paracoccidiodes brasilensis*** -In pathogenicity studies of 31 Paracoccidioides brasiliensis isolates preserved using Castellani's method we intraperitoneally inoculated 104 young adult hamsters and found laminated concentric structures and calcified appearance that resembled Schaumann bodies, in 43 of them, especially in animals with apparently good condition. We characterized these structures histologically and histochemically using different stains (PAS, Grocott, haematoxylin-eosin, Von Kossa). The Von Kossa staining revealed calcium in these structures. Similar structures have been described in patients with sarcoidosis and also in hamsters inoculated with P. brasiliensis. We found no correlation between the presence of these calcifications and serum calcium levels.
**Essayag SM, Landaeta ME, Hartung C, Magaldi S, Spencer L, Suarez R, Garcia F, Perez E. Histopathologic and histochemical characterization of calcified structures in hamsters inoculated with Paracoccidioides brasiliensis.** Mycoses. 2002 Nov;45(9-10):351-7.

## Experimental Protozoal Infection

***Leishmania infantum.*** Leishmaniasis is a common parasitic disease in Southern Europe, caused by Leishmania infantum. The failures of current treatment with pentavalent antimonials are partially attributable to the emergence of antimony-resistant Leishmania strains. This study analyses the in vitro susceptibility to pentavalent antimony of intracellular amastigotes from a range of L. infantum strains, derived from the same infected animal, during in vitro and in vivo passages and after host treatment with meglumine antimoniate. RESULTS: SbV-IC50 values for strains from two distinct isolates from the same host and one stock after two years of culture in NNN medium and posterior passage to hamster were similar (5.0 ± 0.2; 4.9 ± 0.2 and 4.4 ± 0.1 mgSbV/L, respectively). In contrast, a significant difference ($P < 0.01$, t test) was observed between the mean SbV-IC50 values in the stocks obtained before and after treatment of hosts with meglumine antimoniate (4.7 ± 0.4 mgSbV/L vs. 7.7 ± 1.5 mgSbV/L). Drug-resistance after drug pressure in experimentally infected dogs increased over repeated drug administration (6.4 ± 0.5 mgSbV/L after first treatment vs. 8.6 ± 1.4 mgSbV/L after the second) ($P < 0.01$, t test). CONCLUSIONS: These results confirm previous observations on strains from Leishmania/HIV co-infected patients and indicate the effect of the increasing use of antimony derivatives for treatment of canine leishmaniasis in endemic areas on the emergence of Leishmania antimony-resistant strains. **Carrio J, Portus M. In vitro susceptibility to pentavalent antimony in Leishmania infantum strains is not modified during in vitro or in vivo passages but is modified after host treatment with meglumine antimoniate.** BMC Pharmacol. 2002 May 2;2(1):11. Epub 2002 May 02.

***Entamoeba histolytica*** The protozoan parasite Entamoeba histolytica is the causative agent of amoebiasis, a human disease characterized by dysentery and liver abscess. The physiopathology of hepatic lesions can be satisfactorily reproduced in the hamster animal model by the administration of trophozoites through the portal vein route. Hamsters were infected with radioactively labeled amoebas for analysis of liver abscess establishment and progression. The radioimaging of material from

parasite origin and quantification of the number inflammation foci, with or without amoebas, described here provides the first detailed assessment of trophozoite survival and death during liver infection by E. histolytica. The massive death of trophozoites observed in the first hours postinfection correlates with the presence of a majority of inflammatory foci without parasites. A critical point for success of infection is reached after 12 h when the lowest number of trophozoites is observed. The process then enters a commitment phase during which parasites multiply and the size of the infection foci increases fast. The liver shows extensive areas of dead hepatocytes that are surrounded by a peripheral layer of parasites facing inflammatory cells leading to acute inflammation. Our results show that the host response promotes massive parasite death but also suggest also that this is a major contributor to the establishment of inflammation during development of liver abscess. **Rigothier MC, Khun H, Tavares P, Cardona A, Huerre M, Guillen N. Fate of Entamoeba histolytica during establishment of amoebic liver abscess analyzed by quantitative radioimaging and histology.** Infect Immun. 2002 Jun;70(6):3208-15.

***Babesia microti-***The presently used therapy for Babesia microti infections, a combination of quinine and clindamycin, does not always result in parasitologic cures. To identify possible alternative chemotherapeutic agents for such infections, we screened, in the hamster-B. microti system, 12 antiprotozoal drugs that have either recently been released for human use or were in experimental stages of development at the Walter Reed Army Institute of Research for the treatment of malaria and leishmaniasis. Several well-recognized antimalarial drugs, such as mefloquine, halofantrine, artesunate, and artelenic acid, exhibited little or no effect on parasitemia. Two 8-aminoquinolines, WR006026 [8-(6-diethylaminohexylamino)-6-methoxy-4-methylquinoline dihydrochloride] and WR238605 [8-[(4-amino-1-methylbutyl)amino]-2,6-dimethoxy-4-methyl-5 -(3-trifluoromethylphenoxy-7) quinoline succinate], produced clearance of patent parasitemia. Furthermore, blood from infected hamsters treated with WR238605 via an intramuscular injection failed to infect naive hamsters on subpassage, thus producing a parasitologic cure. These two compounds merit further screening in other systems and may prove useful in treating human babesiosis.
**Marley SE, Eberhard ML, Steurer FJ, Ellis WL, McGreevy PB, Ruebush TK 2nd. Evaluation of selected antiprotozoal drugs in the Babesia microti-hamster model.** Antimicrob Agents Chemother. 1997 Jan;41(1):91-4.

***Cryptospordia* sp.** Cryptosporidiosis, a parasitic disorder caused by Cryptosporidium parvum, is frequently a fulminating and life-threatening disease in immunocompromised hosts. The immune status of the host plays a critical role in determining the length and severity of the disease. Dehydroepiandrosterone (DHEA) is an immunomodulator that has been demonstrated to upregulate immune parameters. Ten aged (20-24 mo) Syrian golden hamsters were treated with DHEA for 7 days prior to intragastric inoculation with 1 x 10(6) C. parvum oocysts. An additional 10 aged hamsters were infected similarly but retained as untreated controls. The untreated hamsters presented with generalized infections as determined by oocyst shedding in the feces and parasite colonization of the small intestine. Hamsters treated with DHEA exhibited a significant reduction in cryptosporidial infection when compared to untreated hamsters. These results suggest that DHEA may be an effective prophylactic agent for cryptosporidiosis in immunocompromised patients. **Rasmussen KR, Healey MC. Dehydroepiandrosterone-induced reduction of Cryptosporidium parvum infections in aged Syrian golden hamsters.** J Parasitol. 1992 Jun;78(3):554-7.

**Neospora caninum** Djungarian hamsters were examined for the susceptibility to Neospora caninum infection. After 29 Djungarian hamsters were intraperitoneally inoculated with 5 x 10(6) N. caninum tachyzoites of JPA1 strain, some animals showed symptoms such as ataxia, and many tissue cysts were detected in the brain and a cyst in the muscular tunics of stomach. Especially, more than 100 cysts per head were observed after 5 weeks post inoculation. It is suggested that the Djungarian hamster is a model useful to examine neosporosis.**Uchida Y, Ike K, Kurotaki T, Takeshi M, Imai S. Susceptibility of Djungarian hamsters (Phodopus sungorus) to Neospora caninum infection.** J Vet Med Sci. 2003 Mar;65(3):401-3.

## Toxicology/ Carcinogenesis –

**Oral cavity cancers** represent 2.5% of the cancers that occur in the United States and are ranked sixth worldwide. Since current therapeutic protocols are relatively ineffective, alternative strategies for prevention need to be developed and tested in appropriate animal models. In the study reported herein, the hamster cheek pouch (HCP) was used to evaluate the ability of black raspberries to inhibit oral cavity tumors. Male Syrian Golden hamsters, 3-4 weeks of age, were fed 5% and 10% lyophilized black raspberries (LBR) in the diet for two weeks prior to treatment with 0.2% 7,12-dimethylbenz(a) anthracene in dimethylsulfoxide and for 10 weeks thereafter. HCPs were painted 3X/week for eight weeks. The animals were sacrificed 12-13 weeks from the beginning of DMBA treatment and the number and volume of tumors (mm3) determined. There was a significant difference ($p = 0.02$) in the number of tumors between the 5% LBR and control groups (27 tumors/14 animals and 48 tumors/15 animals, respectively) and an intermediate number of tumors in the 10% berry-treated animals (39 tumors/15 animals). These experiments support previous studies from our laboratories showing the chemopreventive activity of black raspberries and show, for the first time, that dietary black raspberries will inhibit tumor formation in the oral cavity. **Casto BC, Kresty LA, Kraly CL, Pearl DK, Knobloch TJ, Schut HA, Stoner GD, Mallery SR, Weghorst CM. Chemoprevention of oral cancer by black raspberries.** Anticancer Res. 2002 Nov-Dec;22(6C):4005-15.

**Benzanthracene induced tumor** The study examines the role of the pulsed-dye laser at 585 nm, coupled with retinoic acid at therapeutic dose of 5.0 mg/kg body weight, in inhibiting chemically induced tumor growth in the hamster cheek pouch to determine whether pulsed-dye laser therapy can inhibit tumor growth and whether a combination of pulsed-dye laser and retinoic acid has a synergic effect on treatment efficacy. STUDY DESIGN: Randomized, prospective study of hamster model. METHODS: Forty-eight male golden Syrian hamsters were painted with 0.5% solution of 9,10-dimethyl-1,2-benzanthracene in acetone for 6 weeks to induce dysplasia in both sides of the cheek pouches. The hamsters were then randomly divided into four groups of 12 hamsters each as follows: (1) control group, (2) pulsed-dye laser

treatment only (8.0 J/cm(2) and two pulses), (3) retinoic acid treatment only (5.0 mg/kg/d by intraperitoneal injection), and (4) combined pulsed-dye laser and retinoic acid treatment. The treatment period was 40 days. Tumors were measured throughout the study. RESULTS: The results indicated that retinoic acid and pulsed-dye laser each significantly delay tumor growth and reduce tumor volume when used alone. Tumor volumes were statistically different among the treatment groups. There was also a statistical difference in tumor volume between the retinoic acid treatment group and the combined pulsed-dye laser and retinoic acid treatment group. CONCLUSIONS: The study demonstrated the greater advantage of combining pulsed-dye laser with retinoic acid over using either retinoic acid or pulsed-dye laser alone for delay of oral cancer progression. Clinical trials are warranted to establish efficacy in humans.
**Polavaram R, Fuentes CF, Shapshay SM, Wang Z. The role of laser vascular targeting and retinoic acid in oral cancer inhibition** Laryngoscope. 2003 Apr;113(4):715-9.

**NKK-pulmonary adenocarcinoma-** Lung cancer is the leading cause of cancer death in industrialized countries. Pulmonary adenocarcinoma (PAC) is the most common histologic type of lung cancer, and it is reproducibly induced by the tobacco-specific nitrosamine 4-(methylnitrosamino)- 1-(3-pyridyl)-1-butanone (NNK) in laboratory rodents. We have recently shown that the growth of cell lines derived from human PACs is controlled by beta-adrenergic receptors, and that NNK is a high affinity agonist for this receptor family. DESIGN: In the current study, we have tested the relevance of these in in vitro findings for in vivo NNK-induced lung tumorigenesis, using a well-established hamster model of NNK-induced PAC. RESULTS: Our experiments demonstrate a significant increase in NNK-induced PAC multiplicity in animals chronically exposed to the beta-adrenergic agonist epinephrine or theophylline which causes intracellular accumulation of the beta-adrenergic second messenger cAMP. On the other hand, our data show that administration of the beta-adrenergic antagonist propranolol prior to each NNK injection significantly inhibited the development of PACs. DISCUSSION: Our findings support the hypothesis that the development of tobacco-associated PAC may be modulated by beta-adrenergic agents, and that the interaction of NNK with beta-adrenergic receptors contributes to the genesis of this histologic lung cancer type. **Schuller HM, Porter B, Riechert A. Beta-adrenergic modulation of NNK-induced lung carcinogenesis in hamsters. :** J Cancer Res Clin Oncol. 2000 Nov;126(11):624-30.

**Tumorigenesis-**DEN and cigarette smoke- In recent intervention studies, beta-carotene failed to reduce or even increased the incidence of lung cancers in smokers. In the present investigation, the modifying effects of beta-carotene at various doses on the development of upper respiratory tract tumors were investigated in Syrian hamsters treated with diethylnitrosamine (DEN) and cigarette smoke. A total of 120 male 5-week-old hamsters were divided into 4 groups, each consisting of 30 animals. After a single subcutaneous (s.c.) injection of 100 mg/kg DEN, hamsters in groups 1-4 were respectively administered diets supplemented with beta-carotene at doses of 0.5%, 0.05%, 0.005% or 0% during experimental weeks 1 to 13, and simultaneously exposed to cigarette smoke. The duration of cigarette smoke exposure was 9 min twice a day, 5 days a week. Because of a marked reduction of body weight in group 1, the highest dose of beta-carotene was changed to 0.25% after 10 days. In all groups, epithelial hyperplasias and/or papillomas were induced in the larynx and trachea. However, the incidence and multiplicity of papillomas in group 1 were significantly ($P < 0.05$) lower than the group 4 values. Moreover, the beta-carotene treatments significantly ($P < 0.05$ or 0.01) reduced both the incidence and multiplicity of hyperplasias in a dose-dependent manner. The levels of retinol and beta-carotene in the serum, and the retinol level in the liver, were also elevated with dose dependence. Our results thus indicate that beta-carotene inhibits tumorigenesis, even at the high dose of 0.25%, under the present experimental conditions. Furukawa F, Nishikawa A, Kasahara K, Lee IS, Wakabayashi K, Takahashi M, Hirose M. Inhibition by beta-carotene of upper respiratory tumorigenesis in hamsters receiving diethylnitrosamine followed by cigarette smoke exposure. Jpn J Cancer Res. 1999 Feb;90(2):154-61

**Oral hygiene-cheek pouch** Several dentifrices that contain hydrogen peroxide are currently being marketed. The increased use of bleaching agents containing (or generating) H2O2 prompted this review of the safety of H2O2 when used in oral hygiene. Daily exposure to the low levels of H2O2 present in dentifrices is much lower than that of bleaching agents that contain or produce high levels of H2O2 for an extended period of time. Hydrogen peroxide has been used in dentistry alone or in combination with salts for over 70 years. Studies in which 3% H2O2 or less were used daily for up to 6 years showed occasional transitory irritant effects only in a small number of subjects with preexisting ulceration, or when high levels of salt solutions were concurrently administered. In contrast, bleaching agents that employ or generate high levels of H2O2 or organic peroxides can produce localized oral toxicity following sustained exposure if mishandled. Potential health concerns related to prolonged hydrogen peroxide use have been raised, based on animal studies. From a single study using the hamster cheek pouch model, 30% H2O2 was referred to as a cocarcinogen in the oral mucosa. This (and later) studies have shown that at 3% or less, no cocarcinogenic activity or adverse effects were observed in the hamster cheek pouch following lengthy exposure to H2O2. In patients, prolonged use of hydrogen peroxide decreased plaque and gingivitis indices. However, therapeutic delivery of H2O2 to prevent periodontal disease required mechanical access to subgingival pockets. Furthermore, wound healing following gingival surgery was enhanced due to the antimicrobial effects of topically administered hydrogen peroxide. For most subjects, beneficial effects were seen with H2O2 levels above 1%. **Marshall MV, Cancro LP, Fischman SL. Hydrogen peroxide: a review of its use in dentistry. :** J Periodontol. 1995 Sep;66(9):786-96.

## Reproductive behavior /teratology/birth defects /spermatogenesis

**Penile growth-** Seasonal variation in prepubertal penile growth has not previously been studied. The present study assessed the influence of daylength and androgens on penile development in the Siberian hamster (Phodopus sungorus). Adult penile masses were achieved at 18 and 8 weeks of age in hamsters maintained from birth under short (10 h light:14 h dark) versus long (14 h light:10 h dark) daylengths, respectively. Insulin-like growth factor I concentrations, previously implicated in penile growth, did not differ between hamsters maintained in short versus long daylengths. Gonadectomized juvenile males maintained in short and long daylengths and administered testosterone attained adult penile masses well in advance of untreated gonad-intact males maintained in short daylengths. Hamsters from both photoperiods, castrated as juveniles and first treated with testosterone in adulthood, also achieved adult penile masses. The photoinhibited gonad is insufficient to promote penile growth, and prepubertal gonadal secretions during short daylengths are not necessary for eventual penile development. Among young born near the end of the mating season, onset of neuroendocrine refractoriness to short daylengths at about 100 days of age and subsequent gonadal development induces growth in all reproductive tissues. Timing of puberty and increased androgen secretion controlled by daylength are the primary determinants of postnatal penile growth, which may also be affected by prenatal and early postnatal organizational actions of androgens. **Park JH, Spencer EM, Place NJ, Jordan CL, Zucker I.Seasonal control of penile development of Siberian hamsters (Phodopus sungorus) by daylength and testicular hormones.** Reproduction. 2003 Mar;125(3):397-407.

**sexual dysfunction-** A high incidence of sexual dysfunction among women is reported in the clinical literature. Little experimental investigation has been initiated on the ability of phosphodiesterase (PDE) inhibitors to overcome deficits in sexual functioning because of selective serotonin reuptake inhibitors (SSRIs). The effects of fluoxetine, an SSRI, and zaprinast, a PDE-5 inhibitor, on the lateral displacement response (used as a measure of sensitivity to reproductively relevant stimuli) of hamsters in behavioral estrus were investigated. In Experiment 1, hamsters that were maximally sensitive to reproductively relevant stimuli because they were at the peak of behavioral estrus were administered fluoxetine (10 mg/kg, i.p.); they had significantly decreased lateral displacement responses compared to vehicle-administered hamsters. In Experiment 2, hamsters that were relatively less sensitive to sexual stimuli because they were at the termination of behavioral estrus were administered zaprinast (3 mg/kg; i.p.); they had significantly enhanced lateral displacement responses compared to responses seen following vehicle administration. In Experiment 3, fluoxetine-induced deficits in the lateral displacement of hamsters at the peak of behavioral estrus were overcome by the coadministration of zaprinast. These data confirm previous findings that sexual dysfunction can be induced by SSRIs and extend the current knowledge to suggest that administration of a PDE-5 inhibitor can override SSRI-induced deficits in sexual functioning. **Frye CA, Rhodes ME. Zaprinast, a phosphodiesterase 5 inhibitor, overcomes sexual dysfunction produced by fluoxetine, a selective serotonin reuptake inhibitor in hamsters.** Neuropsychopharmacology. 2003 Feb;28(2):310-6.

**Ethanol-**Golden hamsters voluntarily consume substantial amounts of ethanol without developing dependence, apparently because ethanol metabolism is rapid and efficient. Six adult female hamsters were given continuous free access to Purina chow, water and a 20% (v/v) ethanol solution before and during pregnancy and during lactation; six control females did not receive the ethanol solution. Intakes of food and water were not elevated during pregnancy, but increased markedly for both groups during lactation. Ethanol consumption remained substantial but unchanged throughout the experiment, with ethanol consumers taking an average of 3.8 g (4.4 kcal) of ethanol solution daily. No significant differences were observed in the size and weight of litters either at delivery or at 16 days postpartum.
**DiBattista D.Voluntary ethanol consumption during pregnancy and lactation in the golden hamster.** Physiol Behav. 1989 Oct;46(4):771-3.

## Environmental-Triphenyl tin, fly ash, magnetic fields

**Ultrafine particles-** Particulate air pollution is associated with cardiorespiratory effects and ultrafine particles (UFPs, diameter < 100 nm) are believed to play an important role. We studied the acute (1 h) effect of intratracheally instilled unmodified (60 nm), negatively charged carboxylate-modified (60 nm), or positively charged amine-modified (60 or 400 nm) polystyrene particles on bronchoalveolar lavage (BAL) indices and on peripheral thrombosis in hamster. The latter was assessed by measuring the extent of photochemically induced thrombosis in a femoral vein via transillumination. Unmodified and negative UFPs did not modify thrombosis and BAL indices. Positive UFPs increased thrombosis at 500 microg per animal (+ 341 +/- 96%) and at 50 microg per animal (+ 533 +/- 122%), but not at 5 microg per animal. Neutrophils, lactate dehydrogenase, and histamine were increased in BAL at all these doses but protein concentration was increased only at 500 microg per animal. Positive 400-nm particles (500 microg per animal) did not affect thrombosis, although they led to a neutrophil influx and an increase in BAL proteins and histamine. Using the Platelet Function Analyser (PFA-100), the platelets of hamsters were activated by the in vitro addition of positive UFPs and 400-nm particles to blood. We conclude that intratracheally administered positive ultrafine and 400-nm particles induce pulmonary inflammation within 1 h. Positive UFPs, but not the 400-nm particles enhance thrombosis. Hence, particle-induced lung inflammation and thrombogenesis can be partially uncoupled. **Nemmar A, Hoylaerts MF, Hoet PH, Vermylen J, Nemery B.**
**Size effect of intratracheally instilled particles on pulmonary inflammation and vascular thrombosis.** Toxicol Appl Pharmacol. 2003 Jan 1;186(1):38-45.

- Irritation-cheek pouch

**Dental caries** experimental with microbial and dietary induction

**Caries**-Several studies have suggested that green tea and Oolong tea extracts have antibacterial and anticariogenic properties in vitro and in vivo. The aim of the present study was to determine the effect of a standardized black tea extract (BTE) on caries formation in inbred hamsters on a regular and a cariogenic diet. Eighty hamsters were divided into four groups of 20 animals each. Two groups received a pelleted regular diet (LabChow) with water or BTE ad libitum. The other two groups received a powdered cariogenic diet (Diet 2000, containing 56% sucrose) with water or BTE ad libitum. The animals were kept for 3 months on their respective diets and then were sacrificed. The heads were retained, the jaws were prepared and stained using alizarin mordant red II, and were then scored for dental caries according to the Keyes method. This is the

first study indicating that BTE, as compared with water, significantly decreased caries formation by 56.6% in hamsters on a regular diet and by 63.7% in hamsters on a cariogenic diet ($P < 0.05$). In the cariogenic diet group BTE, reduced the mandibular caries score of the hamsters slightly more than the maxillary caries score. The fluoride content of the standardized BTE solution was frequently monitored during the experiment; the mean fluoride concentration was found to be 4.22 ppm. A frequent intake of black tea can significantly decrease caries formation, even in the presence of sugars in the diet. **Linke HA, LeGeros RZ. Black tea extract and dental caries formation in hamsters.** Int J Food Sci Nutr. 2003 Jan;54(1):89-95.

## · Neoplasia

**Estrogen induced-** Mechanisms of **estrogen-induced tumorigenesis** in the target organ are not well understood. It has been suggested that oxidative stress resulting from metabolic activation of carcinogenic estrogens plays a critical role in estrogen-induced carcinogenesis. We tested this hypothesis by using an estrogen-induced hamster renal tumor model, a well established animal model of hormonal carcinogenesis. Hamsters were implanted with 17beta-estradiol (betaE2), 17alpha-estradiol (alphaE2), 17alpha-ethinylestradiol (alphaEE), menadione, a combination of alphaE2 and alphaEE, or a combination of alphaEE and menadione for 7 months. The group treated with betaE2 developed target organ specific kidney tumors. The kidneys of hamsters treated with alphaE2, alphaEE, or menadione alone did not show any gross evidence of tumor. Kidneys of hamsters treated with a combination of alphaE2 and alphaEE showed early signs of proliferation in the interstitial cells. Kidneys of hamsters treated with a combination of menadione and alphaEE showed foci of tumor with congested tubules and atrophic glomeruli. betaE2-treated tumor-bearing kidneys showed >2-fold increase in 8-iso-prostaglandin F(2alpha) (8-iso-PGF(2alpha)) levels compared with untreated controls. Kidneys of hamsters treated with a combination of menadione and alphaEE showed increased 8-iso-PGF(2alpha) levels compared with untreated controls, whereas no increase in 8-iso-PGF(2alpha) was detected in kidneys of alphaEE-treated group. A chemical known to produce oxidative stress or a potent estrogen with poor ability to produce oxidative stress, were nontumorigenic in hamsters, when given as single agents, but induced renal tumors, when given together. Thus, these data provide evidence that oxidant stress plays a crucial role in estrogen-induced carcinogenesis. **Bhat HK, Calaf G, Hei TK, Loya T, Vadgama JV. Critical role of oxidative stress in estrogen-induced carcinogenesis.** Proc Natl Acad Sci U S A. 2003 Apr 1;100(7):3913-8. Epub 2003 Mar 24.

**Pancreatic induced-**The p16(INK4A)/CDKN2A tumor suppressor gene is known to be inactivated in up to 98% of human pancreatic cancer specimens and represents a potential target for novel therapeutic intervention. Chemically induced pancreatic tumors in Syrian golden hamsters have been demonstrated to share many morphologic and biological similarities with human pancreatic tumors and this model may be appropriate for studying therapies targeting p16(INK4A)/CDKN2A. The purpose of this study was to investigate the fundamental biochemistry of hamster P16 protein. Using both in vivo and in vitro approaches, the CDK4 binding affinity, kinase inhibitory activity, and thermodynamic stability of hamster and human P16 proteins were evaluated. Furthermore, a structural model of hamster P16 protein was generated. These studies demonstrate that hamster P16 protein is biochemically indistinguishable from human P16 protein. From a biochemical perspective, these data strongly support the study of p16-related pancreatic oncogenesis and cancer therapies in the hamster model. **Li J, Qin D, Knobloch TJ, Tsai MD, Weghorst CM, Melvin WS, Muscarella P.Expression and characterization of Syrian golden hamster p16, a homologue of human tumor suppressor p16 INK4A.** Biochem Biophys Res Commun. 2003 May 2;304(2):241-7.

**Pancreatic duct adenocarcinomas** Alteration of the Fhit gene was investigated in pancreatic duct adenocarcinomas induced by N-nitrosobis(2-oxopropyl)amine (BOP) in Syrian golden hamsters. The animals received 70 mg/kg BOP, followed by repeated exposure to an augmentation pressure regimen consisting of a choline-deficient diet combined with DL-ethionine and then L-methionine and administration of 20 mg/kg BOP. A total of 15 pancreatic duct adenocarcinomas were obtained 10 wk after the beginning of the experiment, and total RNAs were extracted from each for assessment of aberrant transcription of the Fhit gene by reverse transcription-polymerase chain reaction analysis. Aberrant transcripts lacking nucleotides in the regions of nt -75 to 348, nt -15 to 348, or nt -75 to 178 were detected in 11 adenocarcinomas (73.3%). Southern blot analysis of eight tumors did not show any evidence of gross rearrangement or deletion. These results indicated that changes in the Fhit gene occurred frequently and thus may hav
**Tsujiuchi T, Sasaki Y, Kubozoe T, Konishi Y, Tsutsumi M. Alterations in the Fhit gene in pancreatic duct adenocarcinomas induced by N-nitrosobis(2-oxopropyl)amine in hamsters.** Mol Carcinog. 2003 Feb;36(2):60-6.

**Matrix metalloproteinases (MMPs)** have been implicated as playing an important role in cancer invasion and metastasis. MMPs have been identified in various malignancies, including pancreatic duct adenocarcinomas. METHODS: We investigated the circulating level of MMP-2 and MMP-9 in sera from Syrian golden hamsters into which hamster pancreatic duct adenocarcinoma tissues had been transplanted subcutaneously (HPDt hamsters). Northern blot analysis and gelatin zymographic analysis were performed to detect the expression of MMPs and that of tissue inhibitors of metalloproteinases (TIMPs) in HPDt hamsters. RESULTS: Northern analysis revealed overexpression of MMP-2, MMP-9, and TIMP-2 mRNAs in subcutaneous tumors of HPDt hamsters as compared with normal pancreatic tissue. Sera from HPDt hamsters possessed significantly higher levels of serum MMP-2 and MMP-9 than control sera, as determined by gelatin zymographic analysis, and there was a significant correlation between tumor growth and serum MMP levels. CONCLUSIONS: These results indicate that overexpression of MMP mRNAs is involved in the progression of pancreatic duct adenocarcinomas, and that MMP protein expression in hamster sera is associated with the presence of pancreatic duct adenocarcinoma cells. The findings also suggest that serum MMPs could be useful markers for monitoring patients with pancreatic duct adenocarcinomas. **Iki K, Takeo T, Kubozoe T, Aoki S, Hayashi J, Tsunoda T. Detection of serum MMPs in tumor-bearing hamsters.** J Hepatobiliary Pancreat Surg. 2002;9(4):478-84.

**Transplantable melanoma** The activity of superoxide dismutase (SOD) and glutathione peroxidase (GSHPx), as well as the concentration of thiobarbituric acid reactive substances (TBARS) in tissues of transplantable melanoma in the golden hamster were measured and compared. Ten inbred male hamsters were used for the experiment. They were divided into two groups and were given Bomirski melanoma cells

subcutaneously. The first group was given melanotic (Ma) melanoma cells. The second group was given amelanotic (Ab) melanoma cells. Thirty days after the transplantation the hamsters were dissected and the tumor tissues were taken and homogenized. A statistically significantly higher activity of the measured antioxidant enzymes was found in homogenates of Ma tumor than in homogenates of the Ab tumor. Activity of SOD is 8% higher in melanotic melanoma, 24% higher in CAT, and 45% higher in GSHPx. Statistically significant differences between TBARS concentrations were not confirmed. The higher activity of antioxidant enzymes in the melanotic tumor is a result of increased generation of oxygen-derived free radicals. It is presumed that it is strictly connected with intensified production of quinone and semiquinone radicals in the process of melanogenesis. **Wozniak A, Drewa T, Drewa G, Wozniak B, Schachtschabel DO. Activity of antioxidant enzymes and concentrations of thiobarbituric acid reactive substances (TBARS) in melanotic and amelanotic Bomirski melanoma tissues in the golden hamster (Mesocricetus auratus, Waterhouse).** Neoplasma. 2002;49(6):401-4.

## · **Immunology**- MHCII, IgA, IgG, histocompatability

## Transplantation

Long-term xenograft survival can be achieved in hamster hearts transplanted into rats treated with cobra venom factor (CVF) and cyclosporine A (CsA). This phenomenon of "accommodation" is associated with expression of protective genes such as bcl-2, bcl-X(L), and heme-oxygenase-1. We examined whether accommodation could be induced in hamster-to-rat lung xenografts and whether the pattern of protective genes is similar to cardiac xenografts. METHODS: We used hamster-to-rat cardiac and lung xenotransplantation models. Cardiac xenotransplants were treated with CVF+CsA and compared with untreated controls. Lung xenotransplants were treated with either CVF+CsA or FK506 and cyclophosphamide (Cp) and compared with untreated controls. All recipients were killed by 21 days after transplantation. We examined graft survival and protein expression of protective genes, and we performed histologic and immunohistologic analyses. RESULTS: Rejection occurred rapidly in untreated rats. CVF+CsA or FK506+Cp treatment significantly influenced graft survival. Eight of 12 CVF+CsA-treated heart transplants survived 21 days. Seven of 16 CVF+CsA-treated lung grafts and five of 12 FK506+Cp-treated lung xenografts survived 21 days. We observed significant protein expression of bcl-2, bcl-X(L), and heme-oxygenase-1 in cardiac xenografts treated with CVF+CsA at 2, 14, and 21 days after transplantation, compared with normal hamster hearts. We also observed significant expression of these proteins in lung xenografts treated with either CVF+CsA or FK506+Cp at 21 days after transplantation, compared with normal lungs. CONCLUSIONS: Accommodation may be a general phenomenon for all organs, mediated through protective genes. Induction of accommodation does not require disruption of the complement system. **Tabata T, de Perrot M, Keshavjee S, Liu M, Downey GP, Waddell TK. Accommodation after lung xenografting from hamster to rat.** Transplantation. 2003 Mar 15;75(5):607-12.

## · Behavioral- Stress

Stress decreases sexual activity, but it is uncertain which aspects of stress are detrimental to reproduction. This study used an escapable/inescapable stress paradigm to attempt to dissociate physical from psychological components of stress, and assess each component's impact on reproductive behavior in the male Syrian hamster (Mesocricetus auratus). Two experiments were completed using this protocol where two animals receive the same physical stressor (an electric footshock) but differ in the psychological aspect of control. One group (executive) could terminate the shock for themselves as well as a second group (yoked) by pressing a bar. Experiment 1 demonstrated a significant increase in plasma glucocorticoids at the end of a single 90-min stress session with no difference in glucocorticoid levels between the executive and yoked groups at any time point. Experiment 2 quantified male reproductive behavior prior to and immediately following 12 days of escapable or inescapable stress in executive, yoked, and no-stress control hamsters (n = 12/group). Repeated-measures analysis of variance revealed a number of significant changes in reproductive behavior before and after stress in the three treatment groups. The most striking difference was a decrease in hit rate observed only in the animals that could not control their stress (yoked group). Hit rate in the executive males that received the exact same physical stressor but could terminate the shock by pressing a bar was nearly identical to control animals that never received any foot shock.Therefore, we conclude that coping or control can ameliorate the negative effects of stress on male reproductive behavior**. Holmer HK, Rodman JE, Helmreich DL, Parfitt DB. Differential effects of chronic escapable versus inescapable stress on male syrian hamster (Mesocricetus auratus) reproductive behavior.** Horm Behav. 2003 Mar;43(3):381-7.

## Circadian

The suprachiasmatic nuclei (SCN) contain the master circadian pacemaker in mammals. Generation and maintenance of circadian oscillations involve clock genes which interact to form transcriptional/translational loops and constitute the molecular basis of the clock. There is some evidence that the SCN clock can integrate variations in day length, i.e. photoperiod. However, the effects of photoperiod on clock-gene expression remain largely unknown. We here report the expression pattern of Period (Per) 1, Per2, Per3, Cryptochrome (Cry) 1, Cry2, Bmal1 and Clock genes in the SCN of Syrian hamsters when kept under long (LP) and short (SP) photoperiods. Our data show that photoperiod differentially affects the expression of all clock genes studied. Among the components of the negative limb of the feedback loop, Per1, Per2, Per3, Cry2 but not Cry1 genes show a shortened duration of their peak expression under SP compared with LP. Moreover, mRNA expression of Per1, Per3 and Cry1 are phase advanced in SP compared with LP. Per3 shows an mRNA peak of higher amplitude under SP conditions whereas Per1 and Per2 peak amplitudes are unaffected by photoperiod changes. Bmal1 expression is phase advanced without a change of duration in SP compared with LP. Furthermore, the expression of Clock is rhythmic under SP whereas no rhythm is observed under LP. These results, which provide further evidence that the core clock mechanisms of the SCN integrate photoperiod, are discussed in the context of the existing molecular model. **Tournier BB, Menet JS, Dardente H, Poirel VJ, Malan A, Masson-Pevet M, Pevet P, Vuillez P. Photoperiod differentially regulates clock genes' expression in the suprachiasmatic nucleus of Syrian hamster.** Neuroscience. 2003;118(2):317-22.

## Melatonin

Pineal melatonin synthesis is stimulated at night following an increase in arylalkylamine-N-acetyltransferase (AA-NAT) activity. Depending on the species, two mechanisms of enzyme activation have been described: a cAMP/phospho-cAMP response element-binding protein-dependent stimulation of Aa-nat gene transcription in the rat, presumed to occur in all rodents, or a posttranslational regulation of AA-NAT protein in ongulates. The present data obtained in the Syrian hamster indicate another route of AA-NAT regulation. Elevated nocturnal levels of Aa-nat

mRNA were strongly suppressed following light exposure or adrenergic antagonist administration, demonstrating the involvement of norepinephrine in the stimulation of melatonin synthesis. However, administration of adrenergic agonists during the day did not increase Aa-nat mRNA unless a protein synthesis inhibitor was given during the previous night. This indicates that an inhibitory protein, synthesized at night, prevents melatonin synthesis during the day. By contrast, a protein synthesis inhibitor given at the beginning of the night markedly reduced Aa-nat mRNA, suggesting that a stimulatory protein (transcription factor?) is necessary for Aa-nat gene transcription at night. Noteworthy, hamsters raised in long photoperiod were responsive to adrenergic agonist injection only in the first hour after light onset, a response that may be important in this photoperiodic species in which the melatonin peak extends into the morning hours in a short photoperiod.
**Garidou ML, Diaz E, Calgari C, Pevet P, Simonneaux V.**
**Transcription factors may frame Aa-nat gene expression and melatonin synthesis at night in the Syrian hamster pineal gland.** Endocrinology. 2003 Jun;144(6):2461-72.

## · **Nutritional and metabolic** - diabetes, glucose, **Lipid metabolism**

Seasonal mammals commonly exhibit robust annual cycles of adiposity, food intake and energy metabolism. These cycles are driven by changes in the external daylength signal, which generates a diurnal melatonin profile and acts on neuroendocrine pathways. The white adipose tissue hormone leptin reflects overall adiposity in seasonal mammals, and consequently undergoes significant seasonal fluctuations in secretion. The seasonally breeding Siberian (Djungarian) hamster is a convenient laboratory model to study the effect of a seasonal time-keeping clock on energy metabolism, appetite regulation and the control of adiposity. We have shown that administration of exogenous leptin at physiological doses induces significant loss of adipose tissue for short-day housed winter-like hamsters in which endogenous adipose tissue and leptin concentrations are already low. By contrast, long-day housed hamsters with high adipose tissue reserves are refractory to the effects of leptin. This phenomenon of seasonal leptin resistance appears to be a general feature of other seasonally breeding mammals, and may reflect the operation of an annual timer controlling leptin uptake and/or action on central nervous system signal transduction pathways. The mobilization of fat by leptin in short-day housed hamsters is not associated with changes in expression in either anorexic or anabolic peptides expressed in leptin-receptor rich structures in the arcuate region of the hypothalamus, and suggests that leptin may target other structures. These data contrast with studies, which show that homeostatic mechanisms in response to feed-restriction induce changes in hypothalamic peptides in a similar manner to nonphotoperiodic species. Thus, the long-term seasonal regulation of body weight set point and leptin feedback may operate through separate pathways to those responsible for acute responses to food restriction.
**Rousseau K, Atcha Z, Loudon AS. Leptin and seasonal mammals.** J Neuroendocrinol. 2003 Apr;15(4):409-14

## · Aging/Misc.- polymyopathy, goiter, athlerosclerosis,

**Beta-glucan fractions from barley and oats are similarly antiatherogenic in hypercholesterolemic Syrian golden hamsters.** J Nutr. 2003 Feb;133(2):468-75.**Delaney B, Nicolosi RJ, Wilson TA, Carlson T, Frazer S, Zheng GH, Hess R, Ostergren K, Haworth J, Knutson N.**

The cholesterol-lowering activities of oats and barley are commonly attributed to the beta-glucan fractions. Although beta-glucan is present in both grains and appears to be chemically similar, the effect of source on cholesterol-lowering activity has not been evaluated. In the present study, the antiatherogenic properties of beta-glucan concentrates from oats and barley were evaluated in Syrian golden F(1)B hamsters consuming a semipurified hypercholesterolemic diet (HCD) containing cholesterol (0.15 g/100 g), hydrogenated coconut oil (20 g/100 g) and cellulose (15 g/100 g). After a 2-wk lead-in period, control hamsters were fed the HCD, whereas experimental hamsters consumed HCD formulated to include beta-glucan (2, 4, or 8 g/100 g) by addition of beta-glucan concentrate prepared from oats or barley at the expense of cellulose. Compared with control hamsters, dose-dependent decreases that were similar in magnitude in plasma total and LDL cholesterol concentrations were observed in hamsters fed beta-glucan from either source at wk 3, 6 and 9. Compared with controls, liver cholesterol concentrations were also reduced ($P < 0.05$) in hamsters consuming 8 g/100 g oat or barley beta-glucan. In agreement with previously proposed mechanisms, total fecal neutral sterol concentrations were significantly increased ($P < 0.05$) in hamsters consuming 8 g/100 g barley or oat beta-glucan. Aortic cholesterol ester concentrations were significantly reduced ($P < 0.05$) in hamsters fed 8 g/100 g beta-glucan from barley or oats. Although aortic total cholesterol and cholesterol ester concentrations were significantly correlated with LDL cholesterol ($r = 0.565$, $P < 0.004$ and $r = 0.706$, $P < 0.0001$, respectively), this association could explain only half of the variability. This study demonstrated that the cholesterol-lowering potency of beta-glucan is approximately identical whether its origin was oats or barley.

**Shimizu T, Okamoto H, Chiba S, Matsui Y, Sugawara T, Akino M, Nan J, Kumamoto H, Onozuka H, Mikami T, Kitabatake A. VEGF-mediated angiogenesis is impaired by angiotensin type 1 receptor blockade in cardiomyopathic hamster hearts.** Cardiovasc Res. 2003 Apr 1;58(1):203-12.Coronary microcirculation plays an important role in the progression of cardiac remodeling. Among angiogenic factors, it has been reported that angiotensin II may contribute to neovascularization. However, it is unknown whether inhibition of the renin-angiotensin system suppresses angiogenesis, especially within the heart. Our aim was to evaluate the effects of the angiotensin-converting enzyme inhibitor enalapril and the angiotensin II receptor type I blocker valsartan on cardiac microvasculature, function, vascular endothelial growth factor (VEGF) expression, and survival in cardiomyopathic hamsters. METHODS: Male cardiomyopathic hamsters (BIO TO2) were administered either a placebo (group C), enalapril (30 mg/kg/day) (group E), or valsartan (40 mg/kg/day) (group V), starting at the age of 6 weeks. This continued until death. Hemodynamic study, histological analysis, and northern blot analysis were performed at 39 weeks. RESULTS: Group V showed significant increases in percent fibrosis, end diastolic pressure, and LV dP/dt min, and significant decreases in percent fractional shortening, LV dP/dt max, capillary density, and the level of mRNA expression of VEGF compared with group C. Group E showed significant increases in percent fractional shortening while the capillary density and level of mRNA expression of VEGF were unchanged. The 300-day survival rate was significantly lower in group V (25.0%) but higher in group E (100%) than that of group C (66.7%). CONCLUSIONS: Therapy with valsartan may have adverse effects on survival rate concomitant with the progression of cardiac remodeling owing to impaired VEGF-mediated angiogenesis. Therapy with enalapril has a neutral effect on VEGF-mediated angiogenesis, leading to the suppression of cardiac remodeling and an increase in life expectancy.

**Hoshino F, Urata H, Inoue Y, Saito Y, Yahiro E, Ideishi M, Arakawa K, Saku K. Chymase inhibitor improves survival in hamsters with myocardial infarction.** J Cardiovasc Pharmacol. 2003 Jan;41 Suppl 1:S11-8.The purpose of the present study was to assess the effects of chronic treatment with an orally active chymase inhibitor, 4-[1-(naphthylmethyl)benzimidazol-2-ylthio]butanoic acid (TEI-E548), in a hamster myocardial infarction model. In the first experiment, after confirming the biochemical inhibitory action of TEI-E548 on human and hamster chymases (Ki = 6.2 and 30.6 nM, respectively), the biological action of TEI-E548 in vivo was assessed by the inhibition of hamster chymase-induced microvascular leakage. In the second experiment, myocardial infarction was produced by coronary artery ligation in male Syrian hamsters. TEI-E548 (0.1% containing chow) was given 24 h after surgery and continued for 3 or 5 weeks, while the control and sham-operated groups were fed a standard chow. The survival rate was assessed in each group. At the end of each study period, blood pressure was measured at the left hind-limb, the heart rate and cardiac function were measured by echocardiography, the end-diastolic pressure by a direct catheterization, and organ weights and biochemical parameters, including plasma renin and angiotensin-converting enzyme activities and plasma angiotensin I and angiotensin II concentrations, were measured. In the first experiment, a standard chow containing 0.1% TEI-E548 completely inhibited the hamster chymase-induced microvascular leakage. In the second experiment, TEI-E548 treatment significantly increased the survival rate (37% versus control), and attenuated cardiac hypertrophy (13% versus control) and end-diastolic left ventricular pressure (34% versus control), but it did not decrease the infarction size nor improve the ejection fraction. The plasma angiotensin II concentration post-myocardial infarction was significantly suppressed by TEI-E548 throughout the study period. We conclude that TEI-E548 is an orally active useful chymase inhibitor and improves survival and cardiac hypertrophy of the post-myocardial infarction hamster.

## Strains-

- **Bio 2.4(agouti)-benign prostatic hypertrophy**
- **Bio 14.6-(acromelanic White) cardiomyopathy**

Myocardial fibrosis was evaluated with magnetic resonance (MR) imaging in Bio14.6 hamsters. MATERIALS AND METHODS: Gated gradient-echo T1-weighted images and spin-echo images with gadopentetate dimeglumine enhancement (0.2 mmol/kg) were obtained. RESULTS: Myocardial enhancement persisted for 13 minutes after administration of gadopentetate dimeglumine, and myocardial signal intensity peaked at 13 minutes on gradient-echo T1-weighted images. The enhanced areas were greater in Bio14.6 hamsters at 25-42 weeks than at 10 weeks. Pathologic data revealed enhancement with inflammation at 10 weeks and fibrosis with vessel proliferation at 25-42 weeks. Pathologic fibrotic change was greater at 32-42 weeks than at 10 weeks. The myocardium of 42-week-old Bio14.6 hamsters showed remarkable contrast enhancement, which continued for 13 minutes. There was no correlation between gadolinium enhancement and pathologic findings in the evaluation of myocardial degeneration and fibrosis. CONCLUSION: Gadolinium-enhanced MR imaging was useful for estimating myocardial fibrotic changes with vessel proliferation and myocardial damage. **Nanjo S, Yamazaki J, Yoshikawa K, Miura M, Seno A. Efficacy of contrast-enhanced MR imaging in cardiomyopathy: an experimental study using Bio14.6 hamsters.** Acad Radiol. 2002 Oct;9(10):1139-47.

- **Bio TO-2 dilated strain**

Chronic angiotensin I-converting enzyme inhibition can be associated with aldosterone escape. We investigated the effects of enalapril, spironolactone, and their combination on hemodynamics and cardiac remodeling in cardiomyopathic hamsters to determine whether these drugs could exert additive effects. Cardiomyopathic hamsters, Bio TO-2 dilated strain, were orally treated with enalapril (20 mg. kg. day ) and/or spironolactone (20 mg. kg. day ) according to a 2 x 2 factorial design from 120 days of age. Animals were investigated at 180 (10 animals per group) and 240 (16 animals per group) days of age. Compared with corresponding untreated groups, enalapril significantly decreased mean blood pressure (-18%); enalapril and spironolactone significantly increased cardiac output (+28%, +11%) and femoral blood flow (+10%, +12%) and significantly decreased systemic (-38%, -17%) and femoral (-26%, -13%) vascular resistances. Enalapril and spironolactone significantly decreased left ventricle cavity area (-21%, -26%) and left (-34%, -47%) and right (-37%, -48%) ventricle collagen density. Spironolactone significantly increased left ventricle wall thickness (+4%). There were significant enalapril x spironolactone interactions for most variables (compared with control group, +52%, +36%, +45% for cardiac output; +26%, +28%, +26% for femoral blood flow; -50%, -30%, -45% for systemic vascular resistance; -33%, -20%, -35% for femoral vascular resistance; -27%, -31%, -40% for left ventricle cavity area; and -46%, -58%, -60% for left and -39%, -50%, -66% for right ventricle collagen density in enalapril, spironolactone, and enalapril + spironolactone groups, respectively). In cardiomyopathic hamsters, enalapril and spironolactone in combination did not improve hemodynamics more than enalapril alone but induced stronger effects than each drug alone on cardiac remodeling. **Goineau S, Pape D, Guillo P, Ramee MP, Bellissant E. Combined effects of enalapril and spironolactone in hamsters with dilated cardiomyopathy. :** J Cardiovasc Pharmacol. 2003 Jan;41(1):49-59.

- **UM-X7.1 cardiomyopathic hamsters (CMH).**

In view of the potentially beneficial effect of GH on ventricular function of humans suffering from idiopathic dilated cardiomyopathy, we undertook a study to evaluate the optimal time to initiate treatment with GH and its duration in UM-X7.1 cardiomyopathic hamsters (CMH). GH (1 mg/kg.d) therapy was initiated either in the early or late (30 and 160 d old, respectively) phases of the disease and continued until death at 240 d of age. Age- and sex-matched Golden Syrian hamsters (GSH) were used as controls. Basal IGF-1 levels in serum were reduced by nearly half in CMH compared with GSH but were increased within a physiological range in male hamsters. In contrast, female hamsters presented elevated basal serum IGF-1 levels that were not further elevated by GH administration, as reported in experimental models and humans. Accordingly, the present study will focus on the effects of GH therapy on cardiac performance in male hamsters. GH did not improve ventricular function when starting at a late stage of the disease compared with CMH controls. Maximum rate of left ventricular pressure development decreased by approximately 64% in CMH treated early with recombinant bovine GH. Ventricular dysfunction was associated with morphologic indices of hypertrophy, ventricular dilatation, and extensive fibrosis. Mortality was strikingly increased in GH-treated CMH for 210 d (four males and eight females), as opposed to four females (and no male) in the vehicle-

treated group. These results suggest that chronic treatment with recombinant bovine GH in CMH, starting at an early stage of lesion development, is associated with a reduced cardiac performance at the terminal stage of the disease. **Marleau S, Lapointe N, Massicotte J, Cemeus C, Jasmin G, Dumont L, Sirois MG, Rouleau JL, Du Souich P, Ong H. Effect of chronic treatment with bovine recombinant growth hormone on cardiac dysfunction and lesion progression in UM-X7.1 cardiomyopathic hamsters.** Endocrinology. 2002 Dec;143(12):4846-55.

- **LSH (golden)-gentle**
- **MHA (white)-dental caries**
- **PD4(white)-large, placid disposition**
- **DSNI-dominant spot normal inbred**
- **Bio F(1)B hydrid-hypercholesterolemia, atherosclerosis**

We have compared lipoprotein metabolism in, and susceptibility to atherosclerosis of, two strains of male Golden Syrian hamster, the Bio F(1)B hybrid and the dominant spot normal inbred (DSNI) strain. When fed a normal low-fat diet containing approximately 40 g fat and 0.3 g cholesterol/kg, triacylglycerol-rich lipoprotein (chylomicron+VLDL) and HDL-cholesterol were significantly higher (P<0.001) in Bio F(1)B hamsters than DSNI hamsters. When this diet was supplemented with 150 g coconut oil and either 0.5 or 5.0 g cholesterol/kg, significant differences were seen in response. In particular, the high-cholesterol diet produced significantly greater increases in plasma cholesterol and triacylglycerol in the Bio F(1)B compared with the DSNI animals (P=0.002 and P<0.001 for cholesterol and triacylglycerol, respectively). This was particularly dramatic in non-fasting animals, suggesting an accumulation of chylomicrons. In a second experiment, animals were fed 150 g coconut oil/kg and 5.0 g cholesterol/kg for 6 and 12 months. Again, the Bio F(1)B animals showed dramatic increases in plasma cholesterol and triacylglycerol, and this was confirmed as primarily due to a rise in chylomicron concentration. Post-heparin lipoprotein lipase activity was significantly reduced (P<0.001) in the Bio F(1)B compared with the DSNI animals at 6 months, and virtually absent at 12 months. Bio F(1)B animals were also shown to develop significantly more (P<0.001) atherosclerosis. These results indicate that, in the Bio F(1)B hybrid hamster, cholesterol feeding reduces lipoprotein lipase activity, thereby causing the accumulation of chylomicrons that may be associated with their increased susceptibility to atherosclerosis. **Dietary cholesterol reduces lipoprotein lipase activity in the atherosclerosis-susceptible Bio F(1)B hamster.** Br J Nutr. 2003 Mar;89(3):341-50.

- **APA strain-** glomerulosclerosis

Although it has been said that Syrian hamsters of the APA strain (APA hamsters) spontaneously develop glomerulosclerosis with age, more prominent and severe glomerulosclerosis with proteinuria as well as arteriosclerosis is induced in diabetic APA hamsters. In this study, in order to supply new information on APA hamsters, tests on renal function and histology were done on non-diabetic and streptozotocin (SZ)-induced diabetic APA hamsters (APA-N and APA-D, respectively), and the data were compared with those of normal Syrian (golden) hamsters (GOL). At 4, 8, 12, 20, and 32 weeks of age, the markers indicating renal function, serum urea nitrogen and creatinine levels and the urinary total protein level were measured and thereafter histological studies were done. Although there were no remarkable differences between APA-N and GOL in serum urea nitrogen and creatinine levels, APA-N excreted more urinary total protein from the early weeks of age. In APA-D, an apparent worsening in these markers indicating renal function was detected and diabetic nephropathy in this model was confirmed also in terms of renal function. In the histological studies, the major lesion observed in APA-D was diffuse glomerulosclerosis. This may mean that renal dysfunction in APA-D was mainly caused by the glomerular change and that it is similar to other experimental diabetic animals and human diabetic patients. These data show that the diabetic APA hamster is a desirable model of human diabetic nephropathy. **Inenaga T, Nishida E, Kawamura S, Yoshikawa Y. Renal function tests on diabetes-induced and non-induced APA hamsters.** Exp Anim. 2002 Oct;51(5):437-45.

- **dt(sz)** mutant-paroxysmal dystonia

Recent studies have shown beneficial effects of an adenosine A(2A) receptor agonist in dt(sz) mutant hamsters, an animal model of paroxysmal dystonia, in which stress and consumption of coffee can precipitate dystonic attacks. This prompted us to examine the effects of adenosine receptor agonists and antagonists on severity of dystonia in dt(sz) hamsters in more detail. 2. The non-selective adenosine A(1)/A(2A) receptor antagonists, caffeine (10 - 20 mg kg(-1) i.p.) and theophylline (10 - 30 mg kg(-1) s.c.), worsened the dystonia in dt(sz) hamsters. 3. Aggravation of dystonia was also caused by the selective adenosine A(1)/A(2A) antagonist CGS 15943 (9-chloro2-2-furyl)[1,2,4]triazolo[1,5-c]quinazolin-5-amine) at a dose of 30 mg kg(-1) i.p. and by the adenosine A(1) antagonist DPCPX (8-cyclopentyl-1,3-dipropylxanthine; 20 - 30 mg kg(-1) i.p.), while the A(2) antagonist DMPX (3,7-dimethyl-1-propargylxanthine; 2 - 4 mg kg(-1) i.p.) and the highly selective A(2A) antagonist ZM 241385 (4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-ylamino]ethyl)phenol; 2 - 5 mg kg(-1) i.p.) failed to exert any effects on dystonia. 4. In contrast to the antagonists, both the adenosine A(1) receptor agonist CPA (N(6)-cyclopentyladenosine; 0.1 - 1.0 mg kg(-1) i.p.) and the A(2A) agonist CGS 21680 (2p-(2carboxyethylphen-ethylamino-5'-N-ethylcarboxamindoadenosine; 0.1 - 2.0 mg kg(-1) i.p.) exerted a striking improvement of dystonia. 5. These data suggest that the precipitating effects of methylxanthines are, at least in part, related to their adenosine receptor antagonistic action. 6. Although adenosine receptor agonists can be regarded as interesting candidates for the therapy of paroxysmal dystonia, adverse effects may limit the therapeutic potential of adenosine A(1) agonists, while beneficial effects of the adenosine A(2A) agonist CGS 21680 were already found at well tolerated doses. **Richter A, Hamann M. Effects of adenosine receptor agonists and antagonists in a genetic animal model of primary paroxysmal dystonia.** Br J Pharmacol. 2001 Sep;134(2):343-52.

## Parasitology

**Ancylostoma ceylanicum** Syrian hamsters become anemic and exhibit delayed growth following oral infection with third-stage Ancylostoma ceylanicum hookworm larvae. Here we describe experiments designed to determine the feasibility of adult worm transfer (AWT) between hosts, a technique that would facilitate the specific study of bloodfeeding hookworms in vivo without prior exposure of the host to larva-specific antigens, permit the ex vivo manipulation of adult parasites prior to reimplantation, and also allow for cross-species transfer of worms.

Weanling hamsters given an oral AWT of 40 or 60 mixed-sex A. ceylanicum worms rapidly developed anemia; in the higher-dose group, hemoglobin levels declined from prechallenge levels by 44% within 4 days following AWT. Long-term survival of transferred worms was demonstrated by recovery of parasites from the intestines 42 days after AWT. AWT hamsters acquired humoral immune responses against soluble adult hookworm extracts and excretory-secretory products that were comparable in magnitude to those of animals given a typical infection with larvae. In AWT experiments employing the nonpermissive murine model, C57BL/6 mice given adult worms rapidly became anemic and lost weight in a manner similar to AWT hamsters. Infection of additional mouse strains demonstrated that while C57BL/10 and CD-1 mice also developed anemia following AWT, BALB/c mice were resistant. The technique of AWT to mice may further our understanding of hookworm pathogenesis by allowing the study of adult hookworm infections in a species with well-characterized genetics and an abundance of available reagents. **Bungiro RD Jr, Anderson BR, Cappello M. Oral transfer of adult Ancylostoma ceylanicum hookworms into permissive and nonpermissive host species.** Infect Immun. 2003 Apr;71(4):1880-6.

Zak, O Handbook of Animal Models of Infection. Academic Press. 1999
(hamster specific)
Amebic (*Entamoeba histolytica*) liver abscess model Pp 862-4
Hookworm (*Necator americanus, Ancylostoma ceylonicum*) model Pp890-891
Leishmania (*L. mexicana, L. panamensis, L. braziliensis*)model Pp775-9, 783-7
Lyme (*Borrelia burgdorferi sensu lato*)arthritis model 347-351
Meloidosis (*Burkholderia pseudomallei*)model 199-202
Mycoplasma pneumonia (*Mycoplasma pneumoniae*) model 527-31
Sporotrichosis (*Sporotrix schenckii*) model 749-53
Syphilis (*Treponema pallidum*) model 285-8
Trypanosomiasis (*Trypanosoma cruzi)* 802-3
Malaria (*Plasmodium berghei*) encephalitis 759

# DISEASES OF GUINEA PIGS

**POLA 2005**

Marti Hanes, DVM, DACVP, DACLAM, DABT
Veterinary Toxicologist
Integrated Laboratory Systems
Research Triangle Park, North Carolina
919-281-1110 x718
mhanes@ils-inc.com

## General Features of Guinea Pigs

- nervous, will go off feed with any changes
- long gestation, dystocia, breed all year, will nurse other infants- good for rederivation
- precocial birth, wean early eat solid food at 3-4 days
- few viral infections
- major diseases: scurvy, respiratory tract infections, and enteric disease
- male dominated hierarchy, males will groom infants
- bite wounds from aggressive males, barbering of subordinates
- diurnal, copraphagic, not cache food, eat frequently, require constant source of water, and need training on sipper tubes
- indiscriminate defecation- water bowls, food dishes
- responsive to sound, startle, freeze, stampede
- vocalize, complex communication

## Anatomic Features

- two inguinal nipples
- intact vaginal closure membrane
- large vesicular glands
- pubic symphysis under action of relaxin
- large adrenal glands
- long colon (60% length of rat)
- large tympanic bullae
- intracoronary collateral network
- open inguinal canals
- thick medial walls of pulmonary vessels
- cervical thymus
- The common alpha-subunit of glycoprotein hormones (CGalpha) is a core protein shared by follicle-stimulating hormone (FSH), luteinizing hormone (LH), and thyroid-stimulating hormone (TSH). In order to obtain a molecular basis for an efficient superovulation technique applicable to a wide range of animal species and to discuss the phylogenetic aspect based on molecules related to the reproductive system, we determined cDNA sequences of CGalpha in seven laboratory animals: the guinea pig, Mongolian gerbil, golden hamster, mastomys, Japanese field vole, the JF1 strain of Mus musculus molossinus, and rabbit. Comparison of the inferred CGalpha amino acid sequences of these animals and other mammals (human, mouse, rat, cow, pig, and sheep) showed that the signal peptides and the first ten residues at the N-terminus of the apoprotein were variable, while the rest of the apoproteins were highly conserved. In particular, all rodents had a leucine residue at the apoprotein N-terminus, except the guinea pig, which had a phenylalanine residue, as in the cow, pig, sheep,

and rabbit. Phylogenetic trees constructed from amino acid sequences suggest a closer relationship between the guinea pig and artiodactyls than to rodents, confirming the taxonomic peculiarity of the guinea pig. **Suzuki O, Mochida K, Yamamoto Y, Noguchi Y, Takano K, Matsuda J, Ogura A.**
**Comparison of glycoprotein hormone alpha-subunits of laboratory animals.** Mol Reprod Dev. 2002 Jul;62(3):335-42.

### Hematology

- heterophils
- lymphocytes-large and small predominate
- Kurloff cells

## Disease Differential List

- **Diarrhea** -Tyzzer's, colibacillosis, *salmonella*, coccidiosis, *Arizona sp, Citrobacter sp*, coronavirus, clostridial typhlitis, clostridial dysbiosis, cryptosporidiosis
- **Respiratory** -*Bordetella, S. pneumonia, S. zooepidemicus, Klebsiella, Pasteurella multocida, P. aeruginosa, C. freudii, S. aureus,* heat stress, diaphragmatic hernia, pregnancy toxemia, gastric torsion
- **Ptyalism** - Malocclusion, hypovitaminosis C, heat stress, fluorosis
- **SQ Swelling** - Abscess-(*S. zooepidemicus, S. aureus, Streptobacillus moniliformis, Y. pseudotuberculosis),* neoplasia (leukemia)
- **Dermatitis** - Bite wounds, *Trixascarus cavia, cryptococcosis, Staphylococcus sp.*, dermatophytosis, pediculosis, *Chirodiscoides, Psoroptes.*
- **Otitis- Otosclerosis, Otitis Media** - Often subclinical - caused by *S.pneumoniae, S. zooepidemicus, Bordetella sp, and Pseudomonas,*
- **Mastitis** - Sporadic, early lactation, not necessarily contagious. Blue breast is characterized by red to purple enlarged, firm, congested, edematous mammary glands. Lesions can vary from mild degeneration to necrosis of ductal epithelium with PMN infiltration in ducts and alveoli. Scattered cells occupy the interstitium. Chronic mononuclear cell infiltration with interstitial fibrosis and architectural obliteration will affect future usefulness of these animals as breeders. Isolates have included *E. Coli, Klebsiella and S, zooepidemicus*
- **Conjunctivitis** - Isolates have includes *Streptococcus*, coliforms, *Staphylococcus* and *Pasteurella multocida, Listeria.* Smears should be examined for *Chlamydia.*
- **Infertility** - Age, stress, flooring, estrogen in feed, bedding adherent to genitals, increased temp, nutritional deficiency, metritis, preputial dermatitis, segmental aplasia of uterus, cystic ovaries, perinatal death, abortion-stillbirth-(dystocia, *Bordetella, Salmonella, Strep*, CMV, birth asphyxiation, pregnancy toxemia).
- **Death** - Chilling, overheating, septicemia, toxemia, *Salmonella*, typhlitis, enteritis, pregnancy toxemia, antibiotic reaction, pneumonia, volvulus of cecum or stomach, dystocia, dehydration, stuck foot in grate, fractured limb.
- **Anorexia and/or Weight Loss** - Neophobia, water deprivation, temperature, change in food, unpalatable food/improperly compounded, toxin ingestion, malocclusion, oral laceration, obesity, metabolic disease, renal failure, Vitamin C deficiency, infection, neoplasia, loss of cage mate, mechanical failure, territorial behavior-dominant male, amyloidosis with pododermatitis, protein deficiency, metabolic calcification, ectoparasitism, Urolithiasis.
- **Reluctance to Move** - Hypo Vitamin C, *Bordetella, Salmonella*, malnutrition, Vitamin E deficiency, Osteoarthritis, spinal trauma/fracture, dystrophy/myopathy.

# I. BACTERIAL INFECTIONS

- ***Bordetella bronchiseptica*** - A small gram negative rod, important disease causing organism in the upper respiratory tract of several species with apparent interspecies transmission. Isolates do not appear to have differences in virulence. Affects guinea pigs of all ages, following intranasal inoculation. Severe disease and mortality are most common in young guinea pigs in the winter. Guinea Pigs may harbor inapparent infection. The number of subclinical nasal shedders may be quite high. Rabbits should not be housed with guinea pigs. The organism has an affinity from ciliated respiratory epithelium. Pregnant sows may abort, produce stillborn or die. At necropsy, there is mucopurulent to catarrhal exudate in the nasal passages, and trachea. Pulmonary consolidation is usually anterioventral and may involve all lobes. Mucopurulent exudate in the airways and tympanic bullae may occur. Pleuritis or pyosalpinx may be seen. Microscopically, acute to chronic suppurative bronchopneumonia with heterophilic infiltration, obliteration of the normal architecture and fibrinous exudate may be present. The organism can be cultured on blood agar from the respiratory tract, tympanic bullae or affected uterus. Differential diagnoses must include *Streptococcus, Klebsiella, Staphylococcus*, or previous Freud's adjuvant administration (chronic interstitial fibrosis and granulomatous pulmonary inflammation).
- **Salmonellosis** - *S. typhimurium, S. enteritidis***,** *S. dublin* are important and severe diseases of guinea pigs. Transmission is by ingestion of contaminated food, water or bedding. The conjunctiva is a portal of entry in guinea pigs. Salmonella are frequently in a carrier state and are intermittently shed in the feces, making elimination of the pathogen difficult. Predisposing factors include youth or old age, stress of pregnancy or weaning, nutritional deficiencies, disease, genetics, serotype, environment (winter) and experimental stress. Salmonellosis may be enteric, systemic, epizootic, enzootic and zoonotic. Sporadic outbreaks with high mortality in guinea pigs may approach 100%. Clinical signs are often non-specific: per acute-death, acute- depression, rapid deterioration, lethargy, dyspnea; in chronic disease see anorexia, weight loss, and rough hair coat, conjunctivitis, ocular discharge, general unthriftiness, small litters, abortion, and sporadic death. Diarrhea is variable. Lesions of acute salmonellosis are similar to other animals and involve the liver, spleen, lymphoid tissues and intestine (enlargement, congestion, focal necrosis), the spleen may be massive. Increased fluid contents in the gastrointestinal tract. Pregnant animals may have a purulent metritis. Lesions in subacute to chronic cases may demonstrate yellow necrotic foci in the liver and viscera with hyperemia. Peyer's patch hyperplasia and splenomegally. Microscopically, multifocal granulomatous hepatitis, splenitis and lymphadenitis with the areas of necrosis are surrounded by mononuclear cells and neutrophils (paratyphoid nodules). Diagnosis by culture of mesenteric lymph nodes, conjunctiva, feces, cecum, or aborted material (SS agar, MacConkey's or others). Isolation should be serotyped, because non-pathogenic species exist. Control is best depopulation, sanitation and restocking because of the carrier state. Guinea pigs do not respond to antibiotics well and may develop enterotoxemia. DDX: clostridium enterotoxemia, yersinosis, Tyzzer's and pneumococcal septicemia. Zoonotic and interspecies transmission potential is high.

- **Cervical Lymphadenitis** (*Streptococcus zooepidemicus*) - B-hemolytic, G+, encapsulated, Lancefield Group C. Transmission through skin wounds, aerosol or genital. Oral cavity abrasions caused by coarse plant feeds and conjunctiva are commonly implicated. After

penetration, the organism drains to the local lymph nodes. Uni-or bilateral swelling at angle of jaw is referred to as "lumps" or cervical lymphadenitis, but may be misleading because other lymph nodes may be affected and a similar appearance may be caused by agents, such as *Streptobacillus moniliformis*, *Yersinia pseudotuberculosis*, *Salmonellae (S. linate,*) Zygomycetes, and Cavian leukemia. Grossly, see abscessed ventral and cervical lymph nodes and abscesses in various organs. Septicemia and acute fatal pneumonia occur in the epizootic form. Torticollis may occur due to otitis media and otitis interna. Nasal Discharge, ocular discharge, dyspnea and cyanosis may develop. Chronic infections can be exacerbated by stressors. At necropsy, infection may vary from an acute fatal septicemia to a chronic suppurative process in the lymph nodes, thoracic and abdominal viscera, uterus, and ears. Microscopic evidence of pneumonia, pleuritis, myocarditis, pericarditis, and peritonitis, otitis media, nephritis, arthritis, and cellulitis will be seen characterized by necrotizing suppurative inflammation or fibrinosuppurative inflammation. Chains of gram positive cocci may be seen in direct smears or tissue sections. Culture on blood agar from abscesses, heart blood or lungs will yield small beta-hemolytic mucoid colonies. Systemic antibiotics such as enrofloxacin or chloramphenical are effective. Abscesses may be drained and flushed. Affected animals should be removed from the colony until the abscesses have drained and healed. In epizootic cases, depopulation is advised. Scratch injection with ATCC12960 has provided some immunity. Apparently S. zooepidemicus is strictly an animal pathogen.

- ***Streptococcus (Diplococcus*) Pneumonia** - Caused by a lancet-shaped, gram positive encapsulated cocci in chains and pairs. Capsular type 19 and less frequently type 4 have been isolated from guinea pigs. The disease seldom occurs in well managed facilities, and may be carried as an inapparent infection in the respiratory tract in up to 50%. Aerosol transmission, mostly during the winter months, with young and pregnant sows at greatest risk. Other predispositions include change in environmental temperature, poor husbandry, experimental procedures and inadequate nutrition. During outbreaks, death, stillbirths and abortions occur. Pneumococci may activate the alternative complement cascade, and are protected from phagocytosis through the polysaccharide capsules. At necropsy, fibrinopurulent pleuropneumonia, pericarditis and pulmonary consolidation are apparent. Histologically, an acute fibrinosuppurative bronchopneumonia is present with thrombosis of pulmonary vessels. Infiltrating cells may be elongate and form palisading patterns in affected airways. Splenitis, fibrinopurulent meningitis, metritis, lymphadenitis with focal hepatic and ovarian abscessation have been reported. Guinea pigs with borderline Vitamin C deficiency may develop *S. pneumoniae* associated suppurative arthritis and osteomyelitis. Direct smears will yield G+ diplococci, blood agar to culture, and is a little fastidious. DDX: *Bordetella sp., S. zooepidemicus*. Serotypes are same as seen in human, interspecies transmission is not proven.

- ***Staphylococcus sp.* -** Ulcerative pododermatitis and acute staphylococcal exfoliative dermatitis are associated with coagulase pos staphylococcus.

- **Pododermatitis** - Caused by infection subsequent to trauma, wire cage bottoms and lack of appropriate sanitation. The contact surfaces of the forefeet are swollen, painful and encrusted with necrotic tissue and clotted blood. Amyloidosis has been temporally associated with advanced cases. Isolated cases of pneumonia, mastitis and conjunctivitis have been seen.

- **Dermatitis** - Seen in strain 13 guinea pig, and seen in young pigs born to affected dams. It is characterized by alopecia, erythema of the ventrum, with exfoliation. Skin lesions regress in two weeks, with hair re-growth. At necropsy, there is erythema, scabbing, with cracks in the epidermis and hair loss. Histologically, there is marked epidermal cleavage parakeratotic hyperkeratosis and minimal inflammatory response. Possible interspecies transmission.
- ***Clostridium piliforme, Bacillus piliformis*** - Tyzzer's lesions are usually confined to the gastrointestinal tract in young guinea pigs. Large numbers of spirochetes were associated with clostridial organisms near lesions. Experimental inoculation of young animals produced lesions in intestine and liver by four days. Necrotizing ileitis and typhlitis with transmural involvement. Hepatic lesions when present are characterized by focal coagulative necrosis in periportal regions with variable numbers of PMN's. Warthin-Starry and Giemsa stains will demonstrate the organism. Interspecies transmission.
- ***Clostridium difficile*** **(Antibiotic toxicity and dysbacteriosis)** - A fatal enterocolitis caused by clostridial toxin after administration of narrow spectrum drugs. Clindomycin, lincomycin, erythromycin, penicillin, bacitracin, and ampicillin cause disease 1-5 days after administration. Profuse diarrhea with high mortality, after rapid kill-off of gram positive normal flora. For example, after IM injection of 50,000 IU penicillin there was a 100X decrease of G+ within 12 hours, followed by a 10,000,000X increase in G- bacteremia due to E. coli. In addition, some AB are excreted in the bile. This can be prevented by using a broader spectrum antibiotic. Oddly, C. difficile is usually susceptible to Pen in vitro. At necropsy, the cecal mucosa is edematous and hemorrhagic. The terminal ileum demonstrates hyperplasia of the mucosa, with mononuclear cell infiltrates in the lamina propria. Cecal epithelial degeneration, edema of the lamina propria, and leucocytic infiltration. Assay of cecal contents for C. difficile, this syndrome may occur in the absence of history of AB administration. Safer AB: chloramphenicol, enrofloxicin, trimethoprim-sulfa, and aminogycosides. Rule out other common causes of GIT disease.
- ***Klebsiella*** **pneumoniae** - Patterns of epizootic septicemia and pneumonia with pleuritis, pericarditis and splenic hyperplasia are reported. Culture of *Klebsiella sp* is diagnostic.
- ***Citrobacter freundii*** - An epizootic of *Citrobacter* septicemia with pneumonia, pleuritis, enteritis with isolation of the organism was reported.
- ***Clostridium perfringens*** - Acute fatal typhlitis, sporadic. The cecum contains fluid, gas and ingesta. Micro-degeneration and sloughing of enterocytes, necrosis of adjacent submucosa, C. perfringens, spirochetes and other bacteria have been isolated and causally associated.
- **CAR Bacillus** - "Cilia associated respiratory bacilli" "gliding bacteria" filamentous, 0.2u-6-8u long trilaminar cell wall, exacerbates other respiratory disease. Guinea pigs are not very susceptible. Direct contact. No gross lesions with rat origin bacteria.
- ***Campylobacter*****-like Organism** - Segmental epithelial hyperplasia of the duodenum was seen in steroid treaded guinea pigs. An outbreak with diarrhea, weight loss and mortality, yielded affected animals with adenomatous hyperplasia in the ileum and jejunum. Similar to the changes seen in hamster Campylobacter associated disease with organisms in the immature crypt epithelium on EM>
- ***Pseudomonas aeruginosa*** - Pulmonary botryomycosis with sulfur granules present in the focal suppurative lesions was reported.

- ***Corynebacterium sp.*** - C. kutcheri, one report, mostly a disease of mice and rats, possibly an interspecies exposure. Has caused chorioamnionitis in man.
- ***Yersinia pseudotuberculosis*** - Experimental disease can be produced, but spontaneous disease is rare. In the acute form, small cream colored nodules are seen in the terminal ileum and cecal intestinal wall with enteritis and ulceration of the mucosa. In the subacute to chronic forms, miliary to caseous lesions may be present in the mesenteric lymph nodes, spleen, liver and lung. Culture is diagnostic.
- ***Streptobacillus moniliformis*** - Similar to Streptococcal infections, suppurative creamy to caseous exudate, lesions culture positive for S. moniliformis include cervical lymphadenitis, abscessation, and pyogranulomatous pneumonia.
- **Listeriosis (*Listeria monocytogenes*)** - Unilateral or bilateral conjunctivitis with serous lacrimation to purulent ulcerative keratoconjunctivitis with neovascularization. Diffuse moderate to severe inflammatory infiltrate composed of neutrophils and few lymphocytes and monocytes expand the cornea and present in the superficial bulbar and palpebral conjunctiva. Lacrimal gland necrosis and inflammation, no other lesions noted. Listeria is a gram+ aerobic nonspore forming bacterium widely distributed in silage and hay.

## II. RICKETTSIAL/CHLAMYDIAL INFECTIONS

- ***Chlamydia psittici-Guinea Pig Inclusion Disease (GPIC)*** - Spontaneously occurring conjunctival infection due to Chlamydia psittici (member of the psitticosis-lymphogranuloma-trachoma group). It is widespread among conventional colonies, frequently asymptomatic, but may be demonstrated with conjunctival smear stained with Giemsa. Young, four-to-eight week old animals, are most susceptible with most adults seropositive. May also see rhinitis or genital tract infection. There may be abortions or respiratory tract infections with concurrent immunosuppression and/or Streptococcus or Bordetella. Transmission by direct contact and cervically by pregnant sows. Grossly, the conjunctiva may be reddened or exudative. Conjunctival smears contain sloughed cells, heterophils and lymphocytes. Antigen is demonstrable with specific antibody and IFA. Self-limiting disease (three-to-four weeks) with no residual damage. DDX: Streptococcus zooepidemicus, Coliforms, Staphylococcus sp. Pasteurella, Listeria.

## III. VIRAL INFECTIONS

- **Cytomegalovirus (Herpesvirus)** - Species specific. Natural infection in humans, primates, mice, rats and guinea pigs. Cells produce characteristic large intranuclear and intracytoplamic inclusion bodies. The infection may be latent or persistent. Natural infection of guinea pigs is characterized by lesions in the salivary glands, kidneys, and liver. Most will seroconvert within a few months. It is transmitted by exposure to infected saliva, urine or transplacentally. Pregnant guinea pigs and inoculated animals develop more extensive lesions. Lymphoproliferative (lymphoid hyperplasia), mononucleosis-like syndromes and lymphadenopathy occur in experimentally infected animals. Lesions are often found as incidental lesions at necropsy. Primarily seen in the salivary duct epithelium. Large eosinophilic intranuclear inclusions with marked karyomegaly and margination of nuclear chromatin in affected cells. Occasional intracytoplasmic inclusions are seen in ductal epithelial cells. There may be a concurrent mononuclear cell infiltrate around infected ducts.

In acute systemic infections, interstitial pneumonia with necrosis and inclusions can be seen in the liver, kidney, lung, spleen and lymph node. Useful animal model.

Human cytomegalovirus (HCMV) is the most common cause of congenital viral infection in the developed world, and can lead to significant morbidity. Animal models of HCMV infection are required for study of pathogenesis, because of the strict species-specificity of cytomegalovirus (CMV). Among the small animal CMV models, the guinea pig CMV (GPCMV) has unique advantages, in particular its propensity to cross the placenta, causing disease in utero. In order to develop quantitative endpoints for vaccine and antiviral therapeutic studies in the GPCMV model, a quantitative-competitive PCR (qcPCR) assay was developed, based on the GPCMV homolog of the HCMV UL83 gene, GP83. Optimal amplification of GPCMV DNA was observed using primers spanning a 248 base pair (bp) region of this gene. A 91 bp deletion of this cloned fragment was generated for use as an internal standard (IS) for PCR amplification. Standard curves based upon the fluorescent intensity of full-length external target to IS were compared with signal intensity of DNA extracted from blood and organs of experimentally infected guinea pigs in order to quantify viral load. Viral load in newborn guinea pigs infected transplacentally was determined and compared with that of pups infected with GPCMV as neonates. Viral loads were highest in pups infected as neonates. The most consistent isolation and highest quantities of viral DNA were observed in liver and spleen, although viral genome could be readily identified in brain, lung, and salivary gland. Viral load determination should be useful for monitoring outcomes following vaccine studies, as well as responses to experimental antiviral agents. **Schleiss MR, Bourne N, Bravo FJ, Jensen NJ, Bernstein DI. Quantitative-competitive PCR monitoring of viral load following experimental guinea pig cytomegalovirus infection.** J Virol Methods. 2003 Mar;108(1):103-10.

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- **Guinea Pig Adenovirus Infection -** Seen in the United States and Europe. Low morbidity but high mortality in infected animals. Usually related to experimental manipulation with impairment of the immune system. Grossly, consolidation of the cranial lobes of the lung is seen. Necrotizing bronchitis, bronchiolitis with desquamation of the epithelial lining and inflammatory cell infiltration. Some airways will be obliterated by cell debris, WBC's and fibrin. Nuclei often contain distinctive basophilic inclusions 7-15u in diameter. Electron microscopy reveals classic arrays. This virus has not been isolated; however, disease has been reproduced with cell-free filtrates.
- **Coronavirus-like Infection in Young Guinea Pigs** - Characterized by wasting, anorexia, diarrhea seen after arrival from vendor. Low morbidity and mortality. Acute to subacute necrotizing enteritis involving the distal ileum. Copious amounts of mucoid material present throughout the gastrointestinal tract. Blunting and fusion of the affected villi with synctial giant cells in the intestinal mucosa. Viral particles consistent with coronavirus seen on EM.
- **Guinea Pig Herpes-like Virus (Herpesvirus) (Caviid Herpesvirus 2)** - Isolated from primary kidney cell culture - does not produce disease in the natural host.
- **Guinea Pig X-Virus (GPXV) (Herpesvirus) (Caviid Herpesvirus 3) -** Originally isolated from leukocytes of Strain 2 pigs - experimental inoculation into Hartley Strain caused, viremia, focal hepatic necrosis and mortality - a possible complicating factor for research.
- **Lymphocytic Choriomeningitis (LCM) (Arenavirus)** - Rare disease in guinea pigs. Lymphocytic infiltrates in meninges, choroid plexus, ependyma in liver adrenal and lungs. Wide host range, exposure by inhalation, ingestion, or through intact skin. DX viral antigen in tissue or section. Complicates research and is transmissible to humans.
- **Cavian Leukemia (Retroviridae, Type C Oncovirus Group, Mammalian Group)** - Spontaneous disease in inbred and outbred strains. Cases in young adults. Leukocytes vary from 50,000-250,000. Can be produced with transplanted cells and cell free extracts. Preponderance of cells is lymphoblastic in blood smear. Large lymph nodes in the cervical,

axillary and inguinal areas are seen with splenomegally and hepatomegally. There is a moderate infiltration of leukemic cells in the spleen, liver, bone marrow, lung, thymus, GIT-GALT, heart, eyes and adrenals. Model of viral associated Neoplasia.

- Serologic evidence of subclinical exposure: Sendai, Murine Poliovirus, Reovirus3.

## IV. MYCOTIC INFECTIONS

Dermatomycoses are most common; usually caused by T. mentagrophytes. Systemic mycoses are relatively rare with only a few reports.

- ***Trichophyton mentagrophytes*** - Strain related susceptibility. Mortality rate in neonates approaches 100%. Spontaneous regression in adults, recurrence in sows at parturition. Related to environmental conditions: heat, humidity. Cutaneous lesions may first appear on the nose, other regions of the head and then on the sides and back. On gross examination, the lesions are circumscribed, erythematous, edematous, and scaly with alopecia. Pustules are usually due to secondary bacteria. On microscopic exam, there is hyperkeratosis, epidermal hyperplasia, PMN infiltration, pustules in the epidermis and hair follicles. Arthrospores seen in H&E, PAS or GMS. Wet preps with 10% KOH. Culture in Sabouraud's dextrose. Very zoonotic. Cull and slaughter is advisable. Systemic griseofulvin may cause teratogenisis. Useful animal model.

  -Although fungal disease is uncommon in rodents, dermatophytosis is the most common mycosis seen in clinical practice. T. mentagrophytes is the most common etiologic agent, and the guinea pig is the most common species affected, although there are reports in all pet and laboratory rodent species except the gerbil. Despite the low incidence of clinical disease, rodents are common asymptomatic carriers of dermatophytes, and ringworm is the most common zoonotic disease transmitted from rodents to people. **Pollock C.Fungal diseases of laboratory rodents.** Veterinary Clin North Am Exot Anim Pract. 2003 May;6(2):401-13.

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- Zygomycetes sp.
- Aspergillus sp.
- Histoplasma capsulatum.
- Cryptococcus neoformans.
- Torulopsis pintolopesii- gastric yeast, normal flora, unaffected by ketoconazole

## V. PROTOZOAN PARASITES

- ***Toxoplasma gondii*** - Naturally occurring disease is rare under current housing practices. Infections are asymptomatic, multifocal hepatitis and pneumonitis, cysts in the myocardium, CNS. Can be passed in the milk. Infected through ingestion of Felid oocysts in food or bedding need to differentiate from E. cunculi.
- ***Encephalitozoon cuniculi*** - Infectious, are usually asymptomatic. Similar to E. cuniculi infections in other animals, multifocal granulomatous encephalitis and interstitial nephritis. Glial nodules, perivascular lymphoplasmacytic cuffing in the neuropil and meninges. Obligate intracellular protozoa, incidence varies from 25-95%. Becomes clinical with stress or immunosuppression. Hematogenously spread via macrophages, selectively parasitizing vascular endothelium, especially brain and kidney life cycle 3-5 days. Gross-Kidney - variably severe multiple white pinpoint foci, indented gray area over cortical surface. Micro mild to moderate granulation to chronic interstitial nephritis and tubular dilation. The granulomas are principally in the epithelial cells of collecting tubules. Organisms are most abundant in early stages of disease within complicates research.

| | T. gondii | E. cuniculi |
|---|---|---|
| H&E | Moderate stain | Poorly stained |
| PAS | Cyst wall positive | Cyst wall negative |
| GRAM | Gram nothing | Gram positive |
| Pseudocyst | Small 60u | Large 120u |
| Spores | Not | Birefringent |
| Giemsa | Granulated cytoplasm | Light blue cytoplasm |
| Iron Stain | No stain | Stains black |
| Fatal to Mice | Is fatal | Not fatal |

- ***Klossiella cobayae*** - Infects kidneys of guinea pigs. Organisms form schizonts in glomerular endothelial cells that are 8-12u and multinucleated. Within the tubular epithelium at the corticomedullary junction and medulla are variably sized, irregularly round 12-75u diameter vacuoles that enlarge the cells and compromise the tubular lumen. Microgametes and macrogametes, sporoblasts and sporocysts are seen. Generally an incidental finding, tubular epithelial cell regeneration with variable amounts of lymphocytes and plasma cells in the interstitium.
- ***Giardia caviae*** - A flagellate in the small intestine. Adheres to surface of epithelium and apparently causes no clinical signs or lesions.
- ***Cryptosporidium wrairi*** - A recognized pathogen. In small intestine, clinical infection occurs in juveniles. Infection rate of 30-40% are typical. Causes diarrhea, weight loss, emaciation, morbidity may range from 0-50%. At necropsy, animals are thin and potbellied, with perineal fecal staining. Small intestine will contain watery material and little food. Acute lesions seen in the jejunum, ileum and cecum. Hyperplasia of the crypt epithelium, edema of the lamina propria and leucocytic infiltration. Necrosis and sloughing of enterocytes occur at the villar tips. In chronic lesions, there is villar atrophy, and flattening of enterocytes. Organisms are very small, round, intracellular, but extracytoplasmic with hold fasts, present within the brush border of enterocytes. They can be seen in H&E but PAS is popular. E. coli has been associated with clinical cases of C. wrairi.
- ***Eimeria caviae*** - Typical Eimerian life cycle. Should not be a problem in a well-managed colony. Following ingestion of sporulated oocysts, sporozoites penetrate the intestinal mucosa, schizogeny detectable in seven days, diarrhea at 10-13 days, severely affected will have diarrhea before oocytes sporulate. Weaning and seasonal fluctuation. At necropsy the colonic wall is hyperemic, the mucosa congested and edematous, with petechial hemorrhages, and gray-white nodules. Ingesta may be flecked with blood. Chronically, colonic hyperplasia, edema of the lamina propria, infiltration with PMN's and mononuclear cells, microgametes and macrogametes in large numbers. DX see organisms in mucosal scraping, fecal float, and histology. Rx: coccidiostats.
- ***Balantidium caviae*** - Ordinarily non-pathogenic but can be a secondary invader. Organisms are large, have cilia in variable number of rows, have a large ovoid to

ellipsoid macronucleus, have a smaller micronucleus, have one contractile vacuole, and have a bean-shaped cytostome.

## VI. HELMINTH PARASITES

- **Nematodes *Paraspidodera uncinata*** (Cecal Worm) - Found in cecum and colon; rarely produces clinical disease. Is not common. Is 2-5mm long and has a direct life cycle of 65 days, with no migration beyond the intestinal mucosa.
- **Trematodes *Fasciola sp***. - Has been reported several times and produced severe liver damage. Infection associated with feeding greens contaminated with metacercariae.

## VII. ARTHROPOD PARASITES

- ***Mallophagia*** - Large biting lice cause pediculosis, pruritis, rough hair coat, alopecia in heavy infections.
- ***Gyropus ovalis*** - The oval guinea pig louse (uncommon) wide head -1.0-1.2mm long.
- ***Gliricola porcelli***.- The slender guinea pig louse (common) thin head -1.0-1.5mm long.
- ***Chirodiscoides caviae*** - Relatively common in commercial supplies, lab facilities and pet animals; can bee seen moving around especially in the lumbar region, pretty harmless. Usually seen in a group of 3-2 immature males and one more mature female easily overlooked unless heavy infestation (200/cm3) when see pruritus with alopecia. Microscopic exam for diagnosis.
- ***Trixacarus caviae*** - Burrowing sarcoptic mange, intense pruritus, widespread alopecia, and hyperkeratosis. Severe dry, scaling, crusting, dermatitis, especially near shoulders, inner thighs, abdomen. Probably some historical confusion with Sarcoptes and Notodres. Self-mutilation, some mortality. Hematological changes associated with intense marked pruritis, include heterophilia, monocytosis, eosinophilia and basophilia. Flaccid paralysis and convulsions have been noted, vigorous scratching has lead to seizures. Microscopically-paraffin embedded specimen see epidermal hyperplasia, orthokeratosis, parakeratosis, irregular burrows in stratum corneum contains mites and eggs. Spongiosis, PMN infiltration, hair follicles not usual. 10% KOH mites and eggs. Human contacts may experience urticaria. TX: ivermectin.
- ***Psoroptes cuniculi*** - Rabbit ear mites may affect guinea pigs.
- ***Demodex caviae*** - One report; no clinical signs.
- **Mycoptes musculi** and **Notodres muris** - probable interspecies infection, not common.

## VIII. METABOLIC DISEASES/AGING DISEASE

- **Scurvy (Vitamin C Deficiency)** - Only primates, guinea pigs, red vented bulbul birds, channel catfish and Indian fruit-eating bats are known to require exogenous ascorbic acid. Ascorbic acid is essential in the hydrolase reaction for formation of hydroxyproline and hydroxylysine in the collagen molecule. Lack of l-gulonolactone oxidase in the glucose to Vitamin C pathway. Connective tissue cells don't produce collagen at a

normal rate, with deficient or defective production of interosseous matrix. Vitamin C is also necessary for the catabolism of cholesterol to bile acids. In scurvy, epiphyseal cartilage persists, bone formation is suppressed. The cartilage lattice lengthens but is not replaced by bone. The resultant structure is very susceptible to trauma, resulting in multiple microfractures. With increased capillary fragility, results from increased intercellular space between endothelial cells, vacuolar degeneration of endothelia and depletion of subendothelial collagen. Increased Prothrombin time, increased susceptibility to bacterial infection (especially Strep pneumoniae) impaired macrophage migration, decreased phagocytosis of PMN's. At necropsy, there is enlargement of the ostochondral junctions with hemorrhage into the soft tissues. Especially in periarticular regions of hind limbs. Animals are thin and unkempt, with variable diarrhea. Variable blood in feces, ecchymosis in the urinary bladder and adrenals enlarged. Microscopically persistence of irregular epiphyseal cartilage evident in young animals, microfractures of cartilaginous spicules and hemorrhage. Proliferation of mesenchymal cells in periosteal regions and medullary cavity with displacement of BM hematopoeitic cells. May be aggregates of pink material between cells. Dental abnormalities occur: fibrosis of the pulp and derangement of odontoblasts. Subclinical hemosiderin laden macrophages in the lamina propria of the intestine. Severe skeletal abnormalities create a locomotor problem. Requires 5mg/kg daily, pregnant sows require six times that.

- **Metastatic Calcification -** Seen most often in guinea pigs over one year of age. Muscle stiffness, unthriftiness, mineral deposition may be confined to soft tissues around elbows and ribs, or may be widespread and include mineralization of lungs, trachea, heart, aorta, liver, kidney, stomach, uterus, and sclera. Dietary factors, possibly low magnesium and high phosphorus. High Ca/PO4 appears to interfere with Mg absorption. Rabbit food contains an excess of Vitamin D. Guinea pigs require more folic acid than rabbits. Use von Kossa or Alizarin stain for mineral in tissues.

## Myopathies

- **Myopathy/Myositis** - Necrotizing with necrosis of myofibers and leucocytic infiltration, loss of cross striation, multinucleated strap cell formation, variable mononuclear cells.
- **Nutritional Muscular Dystrophy** - Associated with Vitamin E and Selenium deficiency. Depression, conjunctivitis, spontaneous hind limb weakness, marked decrease in fertility. May die within one week of onset. Elevation of CPK. Marked pathology - coagulative necrosis, hyalinization of myofibers, fragmentation, increased basophilia, rowing of nuclei (regeneration) not mineralization, testicular degeneration later with Vitamin E deficiency. RX: tocopherol.
- **Myocardial/Skeletal Muscular Dystrophy with Mineralization** - Poorly understood, multifocal mineralization, may be seen as an incidental finding, especially in hind legs. May be asymptomatic, multifocal mineral with minimal inflammatory response, myocardial degeneration with mineral occasionally seen. In chronic lesions, will get mineral and fibrosis. There may be a genetic component.

- **Pregnancy Toxemia -** Two different forms with similar clinical presentations. Both occur in advanced pregnancy with depression, acidosis, ketosis, proteinuria, ketonuria, and lowered urinary pH to 5-6. Pregnancy in guinea pigs has been referred to as a "parasitism of staggering proportions".
- **Fasting Metabolic Pregnancy Toxemia** - Nutritional - OBESE sows, last two weeks of pregnancy, especially second pregnancy. Uterine contents are frequently 50% of non-pregnant weight. Stress factors: shipping, change in feeding, cabbage deprivation. Low blood sugar, ketosis, hyperlipemia, comatose and die in 5-6 days. Necropsy: animals have abundant fat with marked fatty infiltration in the liver, kidneys, adrenals, even in vessels stained with fat stains. Caused by reduced carbohydrate intake and mobilization of fat as an energy source.
- **Circulatory/Toxic Pregnancy Toxemia (pre-eclampsia) -** Uteroplacental ischemia occurs due to compression of the aorta caudal to the renal vessels by the gravid uterus. This results in a significant reduction of blood pressure in the uterine vessels, with placental hemorrhage, necrosis, thrombocytopenia, ketosis, and death. On micro, there will be leucocytic infiltration, multifocal periportal liver necrosis, nephrosis, and adrenocortical hemorrhage. This has been reproduced experimentally. Animal model.
- **Diabetes Mellitus -** Spontaneous disease with no clinical signs early in the disease. Onset three months, hyperglycemia, glucouria, rarely ketonuria, reduced fertility. Frequently, animals introduced into the colony will become diabetic, suggesting an infectious agent, not identified. Micro, there is vacuolation and degeneration of the beta cells in the islets of Langerhan's with fatty infiltration of the exocrine pancreas and fibrosis of the vascular stroma. Glomerular tufts demonstrate thickened basement membranes with sclerosis in advanced cases.
- **Segmental Nephrosclerosis -** Common in aged (>1yr) guinea pigs, characterized by irregular, pitted renal cortices. Possibly related to autoimmune disease, infectious agents and vascular disease. Resulting from focal ischemia and fibrosis. Spontaneous deposits of IgG and complement (C3) were demonstrated along the mesenchymal and peripheral glomerular basement membrane. Accelerated disease is seen in animals fed high protein diets. Elevated blood pressure and renal hypertension has been noted. At necropsy there are multiple granular pitted areas on the renal surface. Pale streaks extend down the cortex to the medulla in advanced cases. Microscopically, segmental to diffuse interstitial fibrosis with distortion of the normal architecture is seen. Mostly, the convoluted tubules and loops of Henle are involved. Tubules are lined by poorly differentiated caboodle to squamous epithelium. Scattered tubules contain variable degrees of proteinaceous material and cell debris. There is senescence of individual glomeruli with fibrosis which may become extensive. There may be scattered foci of lymphoplasmacytic inflammation, medial hypertrophy of the renal vessels and endothelial hyperplasia. There may be increased BUN and Creatinine values with non-regenerative anemia and low urine specific gravity.

## IX. NEOPLASIA

True neoplastic lesions are rare, usually over three years of age, a serum factor, possibly asparaginase, has been demonstrated to have anti-tumor activity. Foa Kurloff cell inhibit transformed human epithelial cells in vitro.

- Lung-Pulmonary/bronchogenic papillary adenoma (35%): small white circumscribed, visible - papillary structures lined by a single layer of hyperchromatic cuboidal epithelium. Malignancy is rare.
- Skin and subcutis: trichofolliculoma, sebaceous adenoma, penile papilloma, lipoma, fibrosarcoma, fibroma, carcinoma.
- Uterus: leiomyoma (25%), fibroma, myxosarcoma, leiomyosarcoma.
- Ovaries: cystic rete ovarii (hormonal imbalance associated with leiomyosarcoma), teratoma.
- Endocrine: adrenocortical adenoma.
- Mammary: adenocarcinoma in males and females with mets to lymph nodes.
- Hematopoietic- Cavian Leukemia.

## X . UNIQUE AND MISCELLANEOUS CONDITIONS

- **Kurloff Body -** A cytoplasmic inclusion found in a mononuclear leukocyte called a Kurloff cell. It is unique to guinea pigs. In the non-pregnant guinea pig, the cells are located primarily in the sinusoids of the spleen, and in the stromal tissues of the bone marrow and thymus, and not normally in lymph nodes. The cells are rare in fetuses and neonates, but are common in adults, especially females. Their numbers increase during pregnancy and after exogenous estrogen treatment in both sexes. The bodies are round to oval, usually 1-8 microns in diameter, and are initially granular and later more finely fibrillar to homogeneous within a vacuole. They are PAS-positive, stain positive for fibrinoid material by the Lendrum stain and are thought to be secreted by the cell itself. It is interpreted to be composed primarily of mucopolysaccharides and glycoprotein associated with a protein polysaccharide material. On ultrastructure, the inclusions are membrane bound, and other cytoplasmic organelles in these cells are consistent with secretory activity. Large numbers of cells may be present in the placental labyrinth of pregnant sows. Kurloff cells have been shown to release inclusion material into the fetal endothelium and trophoblast. *In vitro,* the material has a toxic effect on macrophages. Kurloff cells may have a role in preventing maternal rejection of the fetal placenta, creating a barrier separating fetal antigens from immunologically competent maternal cells. These cells have been misinterpreted as lupus cells.
- **Rhabdomyomatosis** (Nodular Glycogen Infiltration) - Occasionally observed as an incidental finding in guinea pigs of various ages. In the past it was considered a degenerative lesion or a ”blastemoid” tissue malformation. However, it is now considered to be a congenital disease related to a disorder of glycogen metabolism. Occasionally, larger lesions may appear grossly as pale pink, poorly defined foci or

streaks. Rhabdomyomatosis may be seen most frequently in the left ventricle, but has been reported in any region. The lesion consists of a spongy network of enlarged, vacuolated myocardial cells supported by a loose network of delicate fibrils. The myocyte cytoplasm is abundant. The nucleus is central or peripheral. The cytoplasm may consist of pink fibrillar to granular material radiating from the nucleus (spider cells) or may have an abortive striated appearance. Vacuoles are round to polygonal and contain abundant glycogen (PAS positive) that is washed out during processing. Glycogen can be demonstrated in alcohol fixed specimens. There may be displacement and flattening of myocyte nuclei in affected fibers. It is considered an incidental finding and normally does not compromise cardiac function. This occurs also in swine, cattle, dogs, and man.

- **Perivascular Lymphoid Nodules in Lung -** Aggregates of lymphocytes in the adventitia of pulmonary vessel may be seen in a variety of stains and can be seen in young guinea pigs as early as five days. Very common. Usually seen around smaller branches of pulmonary arteries and veins. They appear to enlarge with age. Germ-free animals usually do not have these, at least up to three months of age. May be grossly visible as circumscribed pale, pinpoint up to 0.5mm subpleural foci. May be mistaken for granulomas created after SQ injection of Freud's adjuvant.
- **Osseous Metaplasia** - Occasionally seen in guinea pigs, hamsters and rats. No clinical significance. May occur anywhere, but lung is common site and less so in kidney. Have seen plates of bone with bone marrow in the eye. Spicules are composed of dense lamellar bone with varying degrees of calcification. There is usually no reaction in adjacent alveoli. Large numbers of foci with bone marrow have been seen in X-irradiated guinea pig. On microscopic, concentric to eccentric aggregates of small arteries and veins. There may be focal to diffuse infiltrates in the alveolar septa of some animals. Most animals are free of these lesions. Airways and alveoli are clear. On electron microscopy, the lymphocytes have normal morphology and no virus.
- **Alopecia -** Frequently see thinning of hair in young animals at time of weaning; is associated with the period of transition between loss of baby fur and appearance of more coarse guard hairs. Seen in sows in late pregnancy; is thought to be due to reduced anabolism of maternal skin associated with the rapid increase in fetal growth. The condition worsens with subsequent pregnancies. Nutritional (protein deficient <15% CP) and genetic factors are also involved and affect degree of alopecia. May also be caused by barbering, rough surfaces in cages or infections. Culling the most severely affected breeders reduces severity of alopecia.
- **Cystitis and Urolithiasis (urinary calculi)** - Stones occur anywhere in urinary tract and vary in size from sand to stones. Usually are calcium and magnesium carbonates and phosphates. Age, sex and immunosuppresion are related to development. E. coli cystitis in breeding females causes a thickening of the bladder mucosa, congestion, intramural/intralumenal hemorrhage, leukocytic infiltration in the submucosa, occasionally with desmoplasia, ulceration, PMN's. May be the initiating factor or secondary to uroliths in the urinary bladder.

- **Cystic Ovaries (cystic rete ovarii, serous cysts)** - Usually seen in sows over one year old. Thin walled, fluid filled, fluctuant cysts up to 2cm in diameter and that contain clear fluid. Variable size, lined by low cuboidal to columnar epithelial cells. Solitary cilia or tufts of cilia are present on the lumenal surface . There may be marked compression of ovarian tissue. Associated with cystic endometrial hyperplasia, mucometra, endometritis and fibroleiomyomas.
- **Trophoblastic Giant Cells** - Derived from the outermost layer of the fetal placenta (trophoblast) which is in direct contact with the maternal blood supply. (The guinea pig has a labyrinthine hemomonochorial placenta in which there is a single trophoblast layer forming a continuous syncytial layer.) These cells have remarkable migratory activity and can migrate out into the myometrium.
- **Embryonic Placentoma**- A multi-layered transitory growth of parthenogenic origin that occurs within the ovary of the young female, the placentoma is resolved by fibrosis.
- **Gastric Dilation**- Acute, occurs sporadically, frequently affected animals are found dead with previous indication of illness. Gastric volvulus will demonstrate 180 degree.
- **Cecal Torsion** - Acute death, displaced cecum distended with fluid and gas, wall may demonstrate edema and hemorrhage.
- **Colonic Intussusception -** Stress, dehydration, straining, hemorrhage protrusion of colon prolapsed through rectum. Reduction is unsuccessful.
- **Focal Hepatic Necrosis** - Multifocal coagulative necrosis, frequently subcapsular, with minimal or no inflammation. May be a terminal event due to portal blood flow changes.
- **Chronic Hepatopathy** - Characterized by variably severe periportal and interstitial fibrosis, hepatocellular degeneration, bile duct proliferation suggestive or an anoxic change.
- **Liver Contusion** - Fracture of the capsule of the liver with hemorrhage into the peritoneum. Caused by trauma, mishandling falls.
- **Foreign Body Pneumonitis** - Pneumoconiosis - focal pulmonary lesions associated with inhalation of food or bedding materials. Incidental finding with animals on wood chips or rice straw. At necropsy there may be areas of atelectasis, or circumscribed nodules in the parenchyma of the lung, usually not visible grossly. Recent lesions will have plant fibers lodged in small airways with PMN's and macrophages; lesions of longer duration will exhibit focal granulomatous bronchiolitis and alveolitis with macrophages and multinucleated giant cells. Plant fibers are birefringent; an incidental finding that may complicate research.
- **Freund's Adjuvant Granuloma -** After subcutaneous injection of Freund's, multifocal granulomatous response in lung.
- **Cataracts** - Autosomal dominant, possibly associated with IDDM or L-tryptophan deficiency. Will get lens desiccation with long-term anesthesia.

- **Pea Eye** - Hyperplastic nodule of lacrimal or zygomatic gland origin on inferior conjunctival sac.
- **Fatty Infiltration of the Pancreas** - The proportion of exocrine pancreas decreases with age with no apparent impairment of function. Histologically, there are large areas of adipose tissue between normal pancreatic tissue.
- **Thymus** - Degenerating thymocytes are frequently observed in close association with Hassell's corpuscles, especially in younger animals. They may evolve into thymic cysts.
- **Germfree Animals** - Germfree animals have a disproportionately large cecum. In conventional animals the cecum comprises about 10% of the body weight; whereas, in germfree it comprises about 25-30%. Predisposes animals to rupture, herniation, torsion, volvulus and uterine prolapse; Germfree animals also have hypoplastic lymph nodes and lymphatics along the gastrointestinal tract, lower WBC's, changes in WBC differentials, and serum protein concentration.
- **Hairless** - Euthymic strain available, useful in dermal research. Hair follicles form and produce rudimentary hair. Hair present on face and feet. Nonpigmented. Fragile.
- **Malocclusion of Teeth** - Open rooted maxillary premolar and molar teeth overgrow laterally to the labial mucosa. Mandibular premolar and molar teeth overgrow to the lingual side and occasionally incarcerate the tongue. Will see weight loss, anorexia, salivation, wasting. May see secondary malocclusion of the incisors. Cause varied; may be improper diet, a genetic predisposition (e.g., high incidence in strain 13 involving more than one gene with incomplete penetrance), one report of fluorosis (caused impairment of dentin and enamel formation). Guinea pig teeth grow continuously throughout life, at necropsy food particles will be trapped around cheek teeth. Cheek teeth will have irregular contours and sharp edges.

## Animal Models (Publications from last three years surveyed in Pub Med 5/03)

- **Toxicology**
  - lung (allergy-latex, dust, anaphylaxis, asthma, inhalation-leather conditioner, toluene)
  - skin (dermal, allergic, hypersensitivity, psoriasis, wound repair, thermal, poison ivy, military-mustard gas, euthymic hairless GP)
  - cardiac and vascular (tetrodotoxin, implants, septic shock, antiarrhythmics, vasoconstriction)
  - pharmaceutical( antifungal, antiviral, antibiotics, antianxiety, antihistamines, antioxidants)
  - reproductive (embryology, teratology, neonatology, infertility, hormones, ethanol)
  - intestinal (heavy metal, inflammation-ileitis, colitis)
  - hepatic/pancreatic (lipid, diabetes, anesthetics-halothane, bile-free radicals, gallstones)
  - ocular(myopia, conjunctivitis)
  - auditory(noise, antibiotics )
  - proprioception (balance, Meniere's disease)

    - nerve( amyotropic lateral sclerosis, EAE, spinal cord injury, epilepsy electrolytes, antinflammatories, MS)
- **Surgery** - Otolaryngology, cardiology, implants, mastectomy, transplantation, device infection
- **Bone/Cartilage Disease** –
- Osteoarthritis
    - A new image analysis system was employed to quantify the main histological parameters reflecting osteoarthritic features, at the cartilage and bone levels, in the meniscectomized guinea pig model of osteoarthritis (OA).Meniscectomized (MNX) and sham-operated (SH) guinea pigs were studied 1 and 3 months after partial meniscectomy at the medial side of the left knee (n=10 to 12 animals/group). The left proximal tibias were included in methylmethacrylate. Sections were cut and stained with safranin O or Goldner trichrome. Parameters were quantified using special programs of a Biocom image analyser. The following parameters were evaluated at the medial side of the tibia: cartilage thickness (CT); fibrillation index (FI); proteoglycan content ratio based on safranin O staining intensities (PC); chondrocyte density (CD); bone volume (BV) and subchondral bone plate thickness (SBPT). The degree of user interaction varied from manually tracing objects to almost complete computer automation. Meniscectomy resulted in significant variations of these reproducible histomorphometric parameters both after 1 month (FI: +522%, P<0.01) and 3 months (FI: +162%, P<0.001; PC: -36.7%, P<0.001; CD: -31.8%, P<0.001; SBPT: +8.7%, P<0.05) post-operation (results expressed as percentage variation of MNX vs SH). The linear correlation analysis including data from SH and/or MNX animals at the two grouped time points revealed significant r values, in particular between cartilage (CT) and subchondral bone parameters (SBPT) (r=-0.41, P<0.01).CONCLUSIONS: Contrary to scoring evaluation, this system allowed to show the time-dependent impact of the pathology with an early fibrillation of the medial tibial cartilage appearing as soon as 1 month post-surgery, and the close relationship between bone and cartilage parameters during the progression of OA. Pastoureau P, Leduc S, Chomel A, De Ceuninck F. Quantitative assessment of articular cartilage and subchondral bone histology in the meniscectomized guinea pig model of osteoarthritis. Osteoarthritis Cartilage. 2003 Jun;11(6):412-23.

    - Vitamins C, D, and A
        - BACKGROUND: On the basis of in vitro studies, the antioxidant nutrients vitamins E and C are postulated to interact in vivo. OBJECTIVE: We developed a guinea pig model to evaluate the combined deficiency of vitamins E and C in vivo. DESIGN: Weanling guinea pigs were fed a control diet or a vitamin E-deficient diet for 14 d, after which one-half of each group had vitamin C removed from their diet, thus creating 4 diet groups. Some animals were observed for clinical signs. Others were killed for evaluation. RESULTS: Of 21 guinea pigs that were observed after being fed the diet deficient in both vitamins, 8 died 9 +/- 2 d (+/- SD) after starting the diet. Eight additional guinea pigs developed a characteristic syndrome at 11 +/- 3 d. First, they became paralyzed in the hind limbs. Within a few hours, the paralysis progressed to include all 4 limbs and caused difficulty in breathing, which would have caused death had the animals not been euthanized. Histopathologic evaluation did not identify a lesion in the muscles or nervous system that could account for the paralysis. Biochemical measurements confirmed the deficiencies and indicated that the double deficiency caused lipid peroxidation in the central nervous system. CONCLUSIONS: A distinct clinical syndrome of combined vitamin E and vitamin C deficiency occurs in guinea pigs. This syndrome indicates that these antioxidant vitamins are related in vivo. We speculate that acute oxidative injury in the central nervous system underlies the clinical syndrome. Hill KE, Montine TJ, Motley AK, Li X, May JM, Burk RF. Combined deficiency of vitamins E and C causes paralysis and death in guinea pigs.Am J Clin Nutr. 2003 Jun;77(6):1484-8.

    - , rotator cuff, osteopenia

- **Immunology** - Viral, complement, IgE,
    - Asthma,

- The effects of ketotifen and lodoxamide on eosinophil infiltration were assessed in a guinea pig model of allergic conjunctivitis. The two active treatments were coded in this masked study in which 30 male guinea pigs, sensitized to chicken egg albumin (ovalbumin), were randomly assigned to one of three groups: Group 1, instillation of 0.9% NaCl into the conjunctival sac of both eyes; Group 2, instillation of 0.025% ketotifen into the left eye and 0.9% NaCl into the right eye; Group 3, instillation of 0.1% lodoxamide into the left eye and 0.9% NaCl into the right eye. Ovalbumin was administered topically to each eye, except in Group 1 where it was only applied to the left eye. (111)In-oxine labeled eosinophils were injected into the jugular vein of each guinea pig; the animals were sacrificed 17 hours after ovalbumin had been applied. The level of radioactivity in the ketotifen- and lodoxamide-treated eyes was approximately 60% of that in the saline-treated eyes. Moreover, the mean level of radioactivity in the ketotifen- and lodoxamide-treated eyes was comparable with the mean level of radioactivity in the saline-treated eye of Group 1, which had not been exposed to allergen. These results indicate that the therapeutic effects of ketotifen and lodoxamide in allergic conjunctivitis may be partly mediated by an inhibitory effect on eosinophils. Schoch C. Effects of ketotifen 0.025% and lodoxamide 0.1% on eosinophil infiltration into the Guinea pig conjunctiva in a model of allergic conjunctivitis. J Ocul Pharmacol Ther. 2003 Apr;19(2):153-9.
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- autoimmune encephalitis, DHT
- **Stress** - Adrenocortical function, corticosteroids, renal hypertension, thermoregulation
- **Hematology, Coagulation** - Thrombosis, complement, eosinophilia, PAF
- **Nutrition** - Fatty acids, vitamins

On the basis of in vitro studies, the antioxidant nutrients vitamins E and C are postulated to interact in vivo. OBJECTIVE: We developed a guinea pig model to evaluate the combined deficiency of vitamins E and C in vivo. DESIGN: Weanling guinea pigs were fed a control diet or a vitamin E-deficient diet for 14 d, after which one-half of each group had vitamin C removed from their diet, thus creating 4 diet groups. Some animals were observed for clinical signs. Others were killed for evaluation. RESULTS: Of 21 guinea pigs that were observed after being fed the diet deficient in both vitamins, 8 died 9 +/- 2 d (x +/- SD) after starting the diet. Eight additional guinea pigs developed a characteristic syndrome at 11 +/- 3 d. First, they became paralyzed in the hind limbs. Within a few hours, the paralysis progressed to include all 4 limbs and caused difficulty in breathing, which would have caused death had the animals not been euthanized. Histopathologic evaluation did not identify a lesion in the muscles or nervous system that could account for the paralysis. Biochemical measurements confirmed the deficiencies and indicated that the double deficiency caused lipid peroxidation in the central nervous system. CONCLUSIONS: A distinct clinical syndrome of combined vitamin E and vitamin C deficiency occurs in guinea pigs. This syndrome indicates that these antioxidant vitamins are related in vivo. We speculate that acute oxidative injury in the central nervous system underlies the clinical syndrome. **Hill KE, Montine TJ, Motley AK, Li X, May JM, Burk RF. Combined deficiency of vitamins E and C causes paralysis and death in guinea pigs.** Am J Clin Nutr. 2003 Jun;77(6):1484-8.

- **Neoplasia** -Carcinogens-aflatoxin (liver), bracken fern (urinary bladder), reproductive
- **Physiology** - Cardiac, hearing, nerve conduction
- **Infectious animal models**
  - Prions (Creutzfeldt-Jakob)
  - Viral(Marburg/Ebola, Herpes-genital, LCM, Cytomegalovirus, Respiratory Syncytial Virus, Foot and Mouth Disease, equine morbillivirus [Hendra, Nipah], poliovirus, PIV-3,Venezuelan HF-Guanarito, Adenovirus 5, Pichinde, HIV)

**Ebola virus** The filoviruses Ebola virus (EBOV) and Marburg virus (MARV) cause severe hemorrhagic fever in humans for which no vaccines are available. Previously, a priming dose of a DNA vaccine expressing the glycoprotein (GP) gene of MARV followed by boosting with recombinant baculovirus-derived GP protein was found to confer protective immunity to guinea pigs (Hevey et al., 2001. Vaccine 20, 568-593). To determine whether a similar prime-boost vaccine approach would be effective for EBOV, we generated and characterized recombinant baculoviruses expressing full-length EBOV GP (GP(1,2)) or a terminally-deleted GP (GPa-) and examined their immunogenicity in guinea pigs. As expected, cells infected with the GPa- recombinant secreted more GP(1) than those infected with the GP(1,2) recombinant. In lectin binding studies, the insect cell culture-derived GPs were found to differ from mammalian cell derived virion GP, in that they had no complex/hybrid N-linked glycans or glycans containing sialic acid. Despite these differences, the baculovirus-derived GPs were able to bind monoclonal antibodies to five distinct epitopes on EBOV GP, indicating that the antigenic structures of the proteins remain intact. As a measure of the ability

of the baculovirus-derived proteins to elicit cell-mediated immune responses, we evaluated the T-cell stimulatory capacity of the GPa- protein in cultured human dendritic cells. Increases in cytotoxicity as compared to controls suggest that the baculovirus proteins have the capacity to evoke cell-mediated immune responses. Guinea pigs vaccinated with the baculovirus-derived GPs alone, or in a DNA prime-baculovirus protein boost regimen developed antibody responses as measured by ELISA and plaque reduction neutralization assays; however, incomplete protection was achieved when the proteins were given alone or in combination with DNA vaccines. These data indicate that a vaccine approach that was effective for MARV is not effective for EBOV in guinea pigs. **Mellquist-Riemenschneider JL, Garrison AR, Geisbert JB, Saikh KU, Heidebrink KD, Jahrling PB, Ulrich RG, Schmaljohn CS. Comparison of the protective efficacy of DNA and baculovirus-derived protein vaccines for EBOLA virus in guinea pigs.** Virus Res. 2003 Apr;92(2):187-93.

Foot and mouth disease virus (FMDV) is the aetiological agent of a highly contagious vesicular disease of cloven-hooved animals. The gene coding for the capsid polyprotein (P1) of FMDV from serotype 'O' vaccine strain (O75Madras) was cloned and expressed in yeast Pichia pastoris. The expressed P1 protein was characterised by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Western Blot analysis. Immunisation of Guinea pigs with recombinant P1 induced FMDV type O specific immune response. The humoral response to vaccine was measured by indirect ELISA and a serum neutralisation test (SNT). The Guinea pig sera showed high titres both in ELISA and SNT. Upon challenge with virulent Guinea pig adapted homologous type 'O' virus, the animals showed a protective index of 2.52. This study shows that the yeast expressed FMDV P1 could be a safe vaccine in non-endemic countries and a cost-effective vaccine in endemic countries. This is the first report on the production of FMDV structural proteins in yeast and their application as a vaccine.**Balamurugan V, Renji R, Saha SN, Reddy GR, Gopalakrishna S, Suryanarayana VV. Protective immune response of the capsid precursor polypeptide (P1) of foot and mouth disease virus type 'O' produced in Pichia pastoris.** Virus Res. 2003 Apr;92(2):141-9.

**Herpes simplex virus (HSV)** In two recent clinical trials, a vaccine containing herpes simplex virus (HSV) type 2 glycoprotein D (gD2) and a novel adjuvant AS04 comprising alum (Al) and 3-deactylated monophosphoryl lipid A (3-dMPL) afforded HSV-seronegative women significant protection against HSV-2 genital disease (vaccine efficacy, 73% in study 1 and 74% in study 2) and limited protection against infection (46% in study 1 and 39% in study 2). In the present report, studies in the guinea pig model investigated the protection afforded by gD2/AS04 against HSV-1 and HSV-2 genital herpes and investigated whether immunization could prevent or reduce recurrent disease in guinea pigs that developed mucosal infection. Immunization with gD2/AS04 conveyed nearly complete protection against primary disease with either virus but did not prevent mucosal infection. Guinea pigs immunized with gD2/AS04 were significantly better protected against recurrent disease than were guinea pigs immunized with a gD2/Al vaccine, which suggests that inclusion of 3-dMPL improved protection against latent infection. **Bourne N, Bravo FJ, Francotte M, Bernstein DI, Myers MG, Slaoui M, Stanberry LR. Herpes simplex virus (HSV) type 2 glycoprotein D subunit vaccines and protection against genital HSV-1 or HSV-2 disease in guinea pigs.** J Infect Dis. 2003 Feb 15;187(4):542-9. Epub 2003 Feb 07.

- Bacterial (*Staphylococcus, Borrelia, Moraxella (Branhamella), Legionella, Helicobacter, Serpulina, Leptospirosis, Pseudomonas, Mycobacterium, Streptococcus, Treponema, Corynebacterium, Yersinia*)

**Tuberculosis (TB)** remains an enormous global health problem, and a new vaccine against TB more potent than the current inadequate vaccine, Mycobacterium bovis BCG, is urgently needed. We describe a recombinant BCG vaccine (rBCG30) expressing and secreting the 30-kDa major secretory protein of Mycobacterium tuberculosis, the primary causative agent of TB, that affords greater survival after challenge than parental BCG in the highly demanding guinea pig model of pulmonary TB. Animals immunized with rBCG30 and then challenged by aerosol with a highly virulent strain of M. tuberculosis survived significantly longer than animals immunized with conventional BCG. The parental and recombinant vaccine strains are comparably avirulent in guinea pigs, as they display a similar pattern of growth and clearance in the lung, spleen, and regional lymph nodes. The pMTB30 plasmid encoding the 30-kDa protein is neither self-transmissible nor mobilizable to other bacteria, including mycobacteria. The pMTB30 plasmid can be stably maintained in Escherichia coli but is expressed only in mycobacteria. The recombinant and parental strains are sensitive to the same antimycobacterial antibiotics. rBCG30, the first vaccine against TB more potent than nearly century-old BCG, is being readied for human clinical trials. **Horwitz MA, Harth G. A new vaccine against tuberculosis affords greater survival after challenge than the current vaccine in the guinea pig model of pulmonary tuberculosis.** Infect Immun. 2003 Apr;71(4):1672-9.

The search to identify Mycobacterium tuberculosis antigens capable of conferring protective immunity against tuberculosis has received a boost owing to the resurgence of tuberculosis over the past two decades. It has long been recognized that lymphoid cells are required for protection against M. tuberculosis. While traditionally the CD4(+) populations of T cells were believed to predominantly serve this protective function, a pivotal role for CD8(+) T cells in this task has been increasingly appreciated. We show that the 50- to 55-kDa Apa protein, specified by the Rv1860 gene of M. tuberculosis, can elicit both lymphoproliferative response and gamma interferon (IFN-gamma) production from peripheral blood mononuclear cells (PBMC) of purified protein derivative (PPD)-positive individuals, with significant differences recorded in the levels of responsiveness between PPD-positive healthy controls and pulmonary tuberculosis patients. Flow cytometric analysis of whole blood stimulated with the recombinant Apa protein revealed a sizeable proportion of CD8(+) T cells in addition to CD4(+) T cells contributing to IFN-gamma secretion. PBMC responding to the Apa protein produced no interleukin-4, revealing a Th1 phenotype. A DNA vaccine and a poxvirus recombinant expressing the Apa protein were constructed and tested for their ability to protect immunized guinea pigs against a challenge dose of virulent M. tuberculosis. Although the DNA vaccine afforded little protection, the poxvirus recombinant boost after DNA vaccine priming conferred a significant level of protective immunity, bringing about a considerable reduction in mycobacterial counts from the challenge bacilli in spleens of immunized guinea pigs, a result comparable to that achieved by BCG vaccination. **Kumar P, Amara RR, Challu VK, Chadda VK, Satchidanandam V. The Apa protein of Mycobacterium tuberculosis stimulates gamma interferon-secreting CD4+ and CD8+ T cells from purified protein derivative-positive individuals and affords protection in a guinea pig model.** Infect Immun. 2003 Apr;71(4):1929-37.

Quinolone in vivo bactericidal activity was investigated in a guinea pig pneumonia model using three **Streptococcus pneumoniae** strains with decreasing susceptibility to ciprofloxacin. Treatment regimens resulted in values of AUC(0-24 h) and C(30 min) similar to those of standard oral regimens in human serum. Efficacy was defined as a significant difference in number of viable bacteria in the lungs compared with the control. Ciprofloxacin, levofloxacin and gemifloxacin were effective against the levofloxacin-susceptible strain. Only gemifloxacin achieved a >/=99.9% reduction versus control against the levofloxacin intermediate-resistant strain. Gemifloxacin achieved a 99.69% reduction and was the only quinolone significantly different from the control (P<0.05) against the levofloxacin-resistant strain. Gemifloxacin offers in vivo activity against ciprofloxacin- to levofloxacin-resistant pneumococci. **Garci;a-Olmos M, Parra A, Garci;a-Calvo G, Ponte C, Gimenez MJ, Aguilar L, Soriano F. Efficacy and pharmacodynamics of gemifloxacin versus levofloxacin in guinea pig pneumococcal pneumonia induced by strains with decreased ciprofloxacin susceptibility.** Int J Antimicrob Agents. 2003 Jun;21(6):568-573.

The activity of ABT-773 was studied against extracellular and intracellular *Legionella pneumophila* and for the treatment of guinea pigs with **L. pneumophila pneumonia.** The ABT-773 MIC at which 50% of isolates are inhibited (MIC(50)) for 20 different Legionella sp. strains was 0.016 microg/ml, whereas the MIC(50)s of clarithromycin and erythromycin were 0.032 and 0.125 microg/ml, respectively. ABT-773 (1 microg/ml) was bactericidal for two L. pneumophila strains grown in guinea pig alveolar macrophages. In contrast, erythromycin and clarithromycin had easily reversible static activity only. Therapy studies of ABT-773 and erythromycin were performed with guinea pigs with L. pneumophila pneumonia. When ABT-773 was given to infected guinea pigs by the intraperitoneal route (10 mg/kg of body weight), mean peak levels in plasma were 0.49 microg/ml at 0.5 h and 0.30 microg/ml at 1 h postinjection. The terminal half-life phase of elimination from plasma was 0.55 h, and the area under the concentration-time curve from 0 to 24 h (AUC(0-24)) was 0.65 microg. h/ml. For the same drug dose, mean levels in the lung were 15.9 and 13.2 microg/g at 0.5 and 1 h, respectively, with a half-life of 0.68 h and an AUC(0-24) of 37.0 microg. h/ml. Ten of 15 L. pneumophila-infected guinea pigs treated with ABT-773 (15 mg/kg/dose given intraperitoneally once daily) for 5 days survived for 9 days post-antimicrobial therapy, as did 14 of 15 guinea pigs treated with erythromycin (30 mg/kg given intraperitoneally twice daily) for 5 days. All of the ABT-773-treated animals that died appeared to do so because of drug-induced peritonitis rather than overwhelming pneumonia. None of 12 animals treated with saline survived. ABT-773 is as effective as erythromycin against L. pneumophila in infected macrophages and in a guinea pig model of Legionnaires' disease. These data support studies of the clinical effectiveness of ABT-773 for the treatment of Legionnaires' disease. **Edelstein PH, Higa F, Edelstein MA. In vitro activity of ABT-773 against Legionella pneumophila, its pharmacokinetics in guinea pigs, and its use to treat guinea pigs with L. pneumophila pneumonia.** Antimicrob Agents Chemother. 2001 Oct;45(10):2685-90.

- Chlamydial/Rickettsial (*Coxiella, C. psittaci*-Inclusion conjunctivitis, urethritis)

It is well known that pathology caused by chlamydial infection is associated closely with the host response to the

organism and that both innate and adaptive host responses contribute to tissue damage. While it is likely that the organism itself initiates the acute inflammatory response by eliciting cytokine and chemokine production from the host cell, the adaptive response is the result of activation of the cell-mediated immune response. While there are several studies describing the nature of the pathologic response in primate, guinea pig, and murine models, there is less information on the kinetics of the CD4 and CD8 response following primary and challenge infections. In this study, we have quantified by flow cytometry the mononuclear cell response to genital infection with the agent of guinea pig inclusion conjunctivitis in the cervix, endometrium, and oviducts at various times following a primary intravaginal infection and after a challenge infection. Tissues from individual animals were assessed for cells expressing CD4, CD8, or Mac-1 and for B cells. Peak responses of each subset occurred 10 to 14 days after a primary infection. The number of Mac-1-expressing cells in each tissue site was found to be dependent on the size of the inoculating dose of chlamydiae. The responses of each cell type were generally stronger in the cervix than in the upper genital tract. In contrast to the murine model but consistent with the primate models, there were equal numbers of CD4 and CD8 cells present in the infiltrates. Twenty-one days after challenge infection, which was performed 50 days after the primary infection, there was a significant increase in the number of CD4, CD8, and B cells in the oviduct compared to the number of these cells at the same time after a primary infection, providing clear cellular evidence for a cell-mediated immune pathologic response. **Rank RG, Bowlin AK, Kelly KA. Characterization of lymphocyte response in the female genital tract during ascending Chlamydial genital infection in the guinea pig model.** Infect Immun. 2000 Sep;68(9):5293-8.

The ***Coxiella burnetii*** phase-I cellular vaccine is efficacious in humans, imparting nearly complete protection against Q fever. However, this vaccine can also induce sterile abscesses and granulomas at the inoculation site in humans previously sensitized by natural infection or vaccination. To decrease the possibility of vaccinating immune persons, vaccinees are currently screened by skin testing to detect pre-existing Q fever immunity. We developed a model of abscess hypersensitivity in Hartley guinea pigs to assess the likelihood that Q fever vaccines would induce adverse vaccination reactions in previously sensitized individuals. METHODS: Guinea pigs (4 to 6/group) were sensitized to C. burnetii by immunization and aerosol challenge, or by intraperitoneal inoculation. Eight weeks later, animals were then vaccinated SC with a Q fever cellular (WCI) or chloroform:methanol residue (CMR) vaccine. Development of adverse reactions at the vaccination site was assessed histologically and by observation of increases in erythema and/or induration. RESULTS: The WCI vaccine caused greater magnitude and duration of erythema and induration at the vaccination sites than did the CMR vaccine. In addition, non-immune guinea pigs developed induration when given WCI, but not CMR vaccine. CONCLUSIONS: The CMR vaccine may prove a safe alternative to WCI vaccines for use in individuals unscreened for prior immunity to C. burnetii.
**Wilhelmsen CL, Waag DM. Guinea pig abscess/hypersensitivity model for study of adverse vaccination reactions induced by use of Q fever vaccines.** Comp Med. 2000 Aug;50(4):374-8.

- Protozoal (*Trypanosoma, Cryptosporidia*)

Several studies have confirmed that epidermal Langerhans' cells (LC) play a central role in the induction of skin-related immunological events. In order to assess the role of LC in Chagas' disease, guinea-pigs were infected intradermally with ***Trypanosoma cruzi***, sacrificed at different time-points, and their tissues were processed for routine histology, electron microscopy and immunohistochemistry. Parasitaemia was observed earliest at day 6 p.i. with 2 peaks at days 9 and 28, and disappeared on day 56 p.i. Parasite-specific serum IgG and IgM were first detected on day 12 p.i. The level of IgG gradually increased by day 84 p.i. All the infected guinea-pigs showed significant alterations in the distribution and morphology of epidermal LC during parasitacmia. The number of LC had significantly decreased in the epidermis by day 3 p.i., only returning to normal levels by day 56 p.i., although the number of LC in the underlying dermis increased concomitantly. Parasites were carried to the regional lymph node, where clustering of parasite-laden dendritic cells (DC) with lymphocytes was seen by electron microscopy. This evidence suggests that LC might be involved in antigen presentation in Chagas' disease. **Nargis M, Chisty MM, Ihama Y, Sato H, Inaba T, Kamiya H. Kinetics of Trypanosoma cruzi infection in guinea-pigs, with special reference to the involvement of epidermal Langerhans' cells in the induction of immunity.** Parasitology. 2001 Oct;123(Pt 4):373-80.

Cryptosporidia from natural cryptosporidiosis in guinea pigs were experimentally transmitted to both adult and juvenile guinea pigs. Cryptosporidia were associated with the villi of the ileum, jejunum, and duodenum. Both juveniles and adults were equally susceptible to cryptosporidia, as determined by decreases in villus height, increases in crypt depth, and decreases in villus height/crypt depth ratios, when compared with uninoculated animals. When multiple paired comparisons were made between 2 and 10 days postinoculation, there were significant decreases in villus height/crypt depth ratios with time. A dose study showed that 6-week-old guinea pigs were all infected with doses as low as 325 oocysts per animal. When sampled at weekly intervals postinoculation, guinea pigs had significant evidence of infection up to 2 weeks but had recovered completely by 4 weeks. Guinea pigs mounted a specific humoral

immune response against cryptosporidia, as measured by an immunoperoxidase technique. Guinea pigs challenged by reinoculation with cryptosporidial oocysts were completely refractory to reinfection. These studies show that cryptosporidiosis in guinea pigs is a useful small animal model of this disease. **Chrisp CE, Reid WC, Rush HG, Suckow MA, Bush A, Thomann MJ. Cryptosporidiosis in guinea pigs: an animal model.** Infect Immun. 1990 Mar;58(3):674-9.

- Fungal/ Algae (dermatophytes- *Trichophyton*, pulmonary allergen-*Penicillium*, *Aspergillus fumigatus* , endocarditis)

***Trichophyton mentagrophytes*** is both zoophilic and a common causative organism in human dermatomycosis. Therefore this dermatophyte is widely used for experimental efficacy testing of antimycotic agents and their active ingredients. The use of the guinea pig as an animal model for dermatomycosis is based on the predisposition of this species to spontaneous dermal fungal infections. A previously described guinea pig model was modified according to the results of pilot experiments. The modification consists of 1) evaluation of the infectious activity of the primary mycotic tissue cultures obtained from patients and 2) the efficacy testing itself with treatment of the infected skin area including the continuous clinical observation for 28 days. At first the required duration of cultivation and the number of spores for a reproducible infection of all animals were determined. The following efficacy test consisted of four groups with ten animals each. Group I (control of infection) remained without further treatment after experimental infections, groups II-IV received a single treatment by spraying at the day of infection with isopropanol (70%) (negative control), water (mechanic control) and the antimycotic agent (treated group), respectively. After 28 days under continuous examination, clinical symptoms (scabs; reddening, scaliness) were statistically analyzed. The model takes into account the duration and severity of infection in order to evaluate the differences between the four groups. The experimental protocol presented allows the efficacy of antimycotic agents to be demonstrated by means of statistical analyses. As an example the results of a successful prophylactic treatment against T. mentagrophytes with the antimycotic prophylactic Laudamonium (1%) are presented. **Treiber A, Pittermann W, Schuppe HC. Efficacy testing of antimycotic prophylactics in an animal model.** Int J Hyg Environ Health. 2001 Dec;204(4):239-43.

The attempt to establish vaccination strategies against infections caused by ***Aspergillus fumigatus*** seems to be questionable. Invasive aspergilloses are opportunistic diseases of the immunocompromised host and only a passive immunization with immunoglobulins could be taken into consideration. Until now there have been no preclinical and/or clinical data available concerning the efficacy of specific immunoglobulins; animal experiments could offer an approach for the preclinical assessment of this topic. Generally, A. fumigatus is an opportunistic pathogen. Birds show a relatively high susceptibility to infections caused by A. fumigatus. In laboratory animal species, rabbits seem to have the highest susceptibility followed by mice, rats and guinea-pigs. Mice are easy to handle in all-day laboratory use, and infections are mostly established by the intravenous, intranasal or intraperitoneal route. The main target organs of infection are the kidneys by all three infection routes. Forty clinical isolates of A. fumigatus tested showed a comparable virulence in systemic infections in the intravenously infected mouse model. By using histopathological techniques, we also observed infectious lesions within the central nervous system in all cases. Only A. fumigatus strains lacking green pigmentation showed a significantly lower virulence. Histopathological examinations are of great benefit in the study of these animal models as they give detailed information about the infectious process. Measuring colony-forming units in tissues is only of minor use in prediction as it cannot discriminate between infective tissue lesions and cavity-infections/persistence, e.g. in the kidney pelvis. Quantitative methods for measuring fungal organ burdens, e.g. by chitin-enzyme-linked immunosorbent assays, have also been described and offer an alternative towards solely measuring colony-forming units in tissues. **Schmidt A. Animal models of aspergillosis - also useful for vaccination strategies?** Mycoses. 2002 Feb;45(1-2):38-40.

- Helminths(Trichostrongyles, *Onchocerca*, *Baylisascaris*, *Dictyocaulus*)

Four guinea pigs from a colony of approximately 50 animals were examined for progressive neurologic disease of 5 days' duration. Signs of neurologic dysfunction included cachexia, stupor, hyperexcitability, lateral recumbency, and opisthotonos. Results of gross pathologic, microbiologic, and serologic examinations were unremarkable. Histologic examination of cerebral and cerebellar sections revealed multifocal malacia and regions of eosinophilic granulomatous inflammation. Cross-sections of nematode larvae, identified as Baylisascaris sp., most likely B. procyonis, the raccoon ascarid, were seen in the brain of some affected animals. An intact Baylisascaris larva was recovered from a symptomatic animal when cerebral tissue was processed by the Baermann extraction technique. Results of further investigation indicated that wood shavings used for the guinea pigs had been contaminated by raccoon feces, some of which contained numerous B. procyonis eggs. The bedding source for this colony was changed and, to date, no new

cases of neurologic disease have been seen. This report emphasizes the potential insidious entrance of B. procyonis into well-managed laboratory animal facilities. **Van Andel RA, Franklin CL, Besch-Williford C, Riley LK, Hook RR Jr, Kazacos KR. Cerebrospinal larva migrans due to Baylisascaris procyonis in a guinea pig colony.** Lab Anim Sci. 1995 Feb;45(1):27-30.

Sclerosing keratitis is the predominant cause of blindness due to onchocerciasis which is a major human parasitic disease caused by the filarial parasite ***Onchocerca volvulus***. In the present investigation, native pathogenic antigens of O. volvulus which are particularly potent in causing interstitial keratitis were characterized utilizing a guinea pig model. Following demonstration of the protein nature of these antigens using pronase digestion, the crude O. volvulus antigen extract was subjected to stepwise procedures of protein purification. At each stage of purification, pooled antigen fractions were injected into one cornea of presensitized guinea pigs followed by clinical evaluation of stromal inflammation and vascularization at different intervals of time after intrastromal challenge. Initial purification of the pathogenic antigens was carried out in the following order: molecular sieve chromatography on Bio-gel A-5m. anion exchange chromatography on Mono Q followed by DEAE-Sepharose CL-6B and cation exchange chromatography on Mono S. Two out of six different pools from the Mono S column (pool a eluted unbound at 10 mM-NaCl and pool e eluted between 130 mM and 475 mM-NaCl) were found to be most pathogenic. Further purification of Mono S pool a and pool e separately by gel filtration chromatography using Superose 12 demonstrated that the fractions which were most potent in inducing interstitial keratitis contained proteins with approximate molecular masses between 100 and 200 kDa. These results show that minor subfractions of total crude antigens of O. volvulus are largely responsible for induction of experimental interstitial keratitis. We have demonstrated the presence of these antigens in O. volvulus microfilariae by their cross-reactivities with anti-microfilarial antibodies, and hence the relevance of the purified antigens to ocular onchocerciasis in man since sclerosing keratitis is associated with invasion of the cornea by O. volvulus microfilariae. Isolation of these two pathogenic antigen pools represents the practical limits of purification and subsequent animal experiments possible with the available amounts of native parasite material obtained from infected human individuals in the absence of a suitable non-human host or of an in vitro culture system for O. volvulus. **Chakravarti B, Lass JH, Diaconu E, Roy CE, Herring TA, Chakravarti DN, Greene BM. Characterization of native pathogenic antigens of Onchocerca volvulus: identification of high molecular mass protein antigens eliciting interstitial keratitis in a guinea pig model.** Exp Eye Res. 1995 Apr;60(4):347-58.

- Arthropods (*Triatoma*)

  The protective effect of experimental immunization was studied in guinea pigs exposed to vectorial infection by ***Trypanosoma cruzi***. Immunized animals received an inoculum of live-attenuated T. cruzi epimastigotes into a granuloma previously induced by Freund's complete adjuvant in the hind footpad. Seven days later, a delayed-type hypersensitivity reaction was triggered by reinjection of the parasites in the front footpad. The animals were then placed in Triatoma infestans-colonized corrals and exposed to vectorial T. cruzi transmission of the parasite for up to 200 days. The effectiveness of this immunizing protocol was controlled in terms of the number of bites necessary for infection (NBNI) in immunized as compared with control animals. Periodic entomological census allowed for the determination of vector biting and infection rates and the calculation of NBNI. Although this measurement was quite variable between yards, an overall average of 4,973 bites was enough to infect a control guinea pig in 4 separate experiments. The corresponding figure for the experimental group was 21,307 bites, implying that immunized animals could resist a 4.28-fold increase (range: 1.99-8.32) in the number of vector bites before becoming infected. **Basombrio MA, Nasser JR, Segura MA, Gomez LE. Trypanosoma cruzi: effect of immunization on the risk of vector-delivered infection in guinea pigs.** J Parasitol. 1997 Dec;83(6):1059-62.

# DISEASES OF THE RABBIT

Jo Lynne Raymond, DVM
Diplomate, ACVP
Assistant Director, Ultrastructural Pathology
United States Army Medical Research Institute of Infectious Diseases
Ft. Detrick, MD
(301)-619-6353 Email: JoLynne.Raymond@det.amedd.army.mil

## GENERAL INFORMATION

Rabbits are classified in the Order Lagomorpha. They differ from rodents because they possess an additional pair of incisor teeth directly behind the large incisors of the upper jaw.

There are over 100 different breeds of rabbits that are descendants of the European wild rabbit, *Oryctolagus cuniculus*. The majority of rabbits used in biomedical research are New Zealand white.

## CENTRAL NERVOUS SYSTEM

### Viral Diseases:

Rabbits are very sensitive to infection with **herpes simplex virus**. Affected rabbits exhibit typical neurologic signs and anorexia and weakness. There is a nonsuppurative, necrotizing meningoencephalitis with amphophilic intranuclear inclusion bodies. This is a zoonotic disease.

Rabbits are animal models for herpes simplex virus keratitis, malignant catarrhal fever, pseudorabies and bovine herpesvirus type 5.

**Ref: Gref, P; et al: Herpes simplex encephalitis in a domestic rabbit. J Comp Path 2002, Vol. 126, pp. 308-311.**

**Weissenbock, H; et al: Naturally occurring herpes simplex encephalitis in a domestic rabbit. Vet Pathol 34:44-7 (1997).**

Rabbits are susceptible to infection with **rabies virus**. All reports have been in states with enzootic raccoon rabies in rabbits that were housed outdoors with potential or reported contact with wildlife. The clinical disease is the paralytic form and affected rabbits die within 3 to 4 days. Diagnosis is by immunofluorescent testing of brain tissue.

**Bacterial Diseases:**

**Pasteurella multocida** causes an acute suppurative meningoencephalitis, and suppurative otitis media/interna.

**Listeria monocytogenes** causes a nonsuppurative meningoencephalitis.

**Protozoal Diseases:**

**Encephalitozoon cuniculi** is an oval, 1 x 2 μm Gram positive, obligate intracellular microsporidian parasite that is widespread in domestic rabbits and is probably the most common spontaneous microsporidian in animals. Infection in rabbits is usually subclinical, but can cause nervous system disease and death in heavy infections. Dwarf rabbits are highly susceptible to infection and development of disseminated disease. Clinical signs include head tilt, seizures, ataxia, paralysis and muscular weakness. Natural transmission is by ingestion of spore-contaminated urine and transplacental transmission also occurs from pregnant does to their feti. Spores can survive in the environment for at least 4 weeks at room temperature. The organism has a polar filament that it uses to penetrate host cell membranes to inject sporoplasm into the host cell. Spores are spread hematogenously via macrophages. There are no gross lesions in the CNS, but histologically, there is focal nonsuppurative to granulomatous meningoencephalitis with astrogliosis and perivascular lymphocytic cuffs. In dwarf rabbits, uveitis with cataractous change and disseminated disease has been reported.

**Ref: Baneux, P.J.R.; et al: *In utero* transmission of *Encephalitozoon cuniculi* strain type I in rabbits. Lab Animals (2003) 37, pp. 132-138.**

**Harcourt-Brown, F.M.; et al: *Encephalitozoon cuniculi* in pet rabbits. Vet Record, April 5, 2003, pp. 427-431.**

**Nast, R; et al: Generalized encephalitozoonosis in a Jersey wooly rabbit. Can Vet J, 37:303-5, 1996.**

**Snowden, KF; et al: Animal models of human microsporidial infections. Lab Anim Sci, 48:589-92, 1998.**

**Wasson, K and Peper RL: Mammalian microsporidiosis, Vet Path 37:113-128 (2000).**

**Toxoplasma gondii** rarely causes clinical disease in laboratory rabbits. In a recent study, brown hares and rabbits were experimentally infected with *Toxoplasma gondii*. The hares had fatal, disseminated disease, while the rabbits had mostly subclinical disease. Areas of multifocal necrosis, granulomatous meningoencephalitis and tachyzoites and cysts are present. This is a differential diagnosis for encephalitozoonosis.

**Ref: Sedlak K; et al: Fatal toxoplasmosis in brown hares (*Lepus europaeus*): possible reasons of their high susceptibility to the infection. Vet Parasitol 2000 Nov 1;93(1):13-28.**

**Parasitic Diseases:**

**Baylisascaris procyonis** causes necrotizing tracts/lesions mainly in rabbits housed outdoors that have consumed feed contaminated by raccoon feces. Clinically there is a syndrome of progressive neurological signs. Diagnosis is by finding the parasites in tissue sections. Visceral lesions may be present in the heart, liver, and kidney.

**Ref: Kazacos, KR; et al: Fatal cerebrospinal disease caused by *Baylisascaris procyonis* in domestic rabbits. J Am Vet Med Assoc, 183:967-71, 1983.**

**Sato, H; et al: First outbreak of *Baylisascaris procyonis* larva migrans in rabbits in Japan. Parasitol Intl 51 (2002) pp. 105-108.**

**Neoplastic Diseases:**

**Pituitary adenoma** occurs in aged New Zealand white rabbits. Some secrete prolactin that can cause mammary gland hyperplasia and dysplasia. Blood prolactin levels can be measured.

**Neurofibromas** and **neurofibrosarcomas** have been reported. As in other species, they are locally invasive and very difficult to completely surgically excise. Recurrence is common.

**Ref: Lipman, NS; et al: Prolactin-secreting pituitary adenomas with mammary dysplasia in New Zealand white rabbits, Lab Anim Sci, 44:114-20.**

**SPECIAL SENSES**

**Viral Diseases:**

There can be a mucopurulent conjunctivitis in myxomatosis.

**Bacterial Diseases:**

**Pasteurella multocida** causes conjunctivitis and otitis media. Always remember to check the tympanic bullae at necropsy. Grossly, there will be dull yellow to gray viscous exudate within the bullae. Histologically, there is squamous metaplasia of the lining epithelium with primarily heterophilic inflammation in the submucosa.

**Staphylococcus aureus** causes a suppurative conjunctivitis. Diagnosis is made by demonstration of Gram positive cocci in section and by positive bacterial culture.

**Parasitic Diseases:**

**Psoroptes cuniculi** is the ear mite of rabbits and is the most common and costliest ectoparasite infection of rabbits. Ear mites are transmitted by direct contact and spend their entire life span in the external ear. Mites can survive off the host in the crust material for up to 21 days. Severely affected ears may contain as many as 10,000 mites. The mites are nonburrowing and chew and pierce the epidermis of the external ear. This activity incites an inflammatory response that is similar to an IgE mediated type I hypersensitivity reaction. The clinical appearance is the presence of light brown, thick, crusty, foul smelling exudate in the external ear canal and pinnae. The skin beneath the crusts is alopecic and erythematous. Self-excoriation can lead to secondary bacterial infections. Histologically there is hyperkeratosis, heterophils, macrophages, eosinophils, parasites, and eggs. The mites are easily demonstrated on swabs. A recent study documented that moxidectin, an equine dewormer, was a safe and effective treatment for psoroptic mange in rabbits. Another paper found selamectin at a dose of 6 or 18 mg/kg was shown to eliminated mites from rabbits naturally infested with ***P. cuniculi***.

**Ref: Curis, SK; et al: Use of ivermectin for the treatment of ear mite infestations in rabbits, J Am Vet Med Assoc, 196:1139-40, 1990.**

**Rafferty, DE and Gray, JS: The feeding behavior of *Psoroptes* sp. mites on rabbits and sheep. J Parasitol, 73:901-6, 1987.**

**Wagner R and Wendlberger U: Field efficacy of moxidectin in dogs and rabbits naturally infested with *Sarcoptes* spp., *Demodex* spp., and *Psoroptes* spp. Mites. Vet Parasitol 2000 Nov 10:93(2):149-58.**

There is a report of ***Taenia serialis*** causing exophthalmos in a rabbit. It presented as a soft tissue swelling within the orbit of the right eye.

**Ref: O'Reilly, A; et al: *Taenia serialis* causing exophthalmos in a pet rabbit. Vet Ophthal (2002) 5, 3, pp. 227-230.**

**Congenital Diseases:**

Congenital glaucoma or buphthalmia is an autosomal recessive condition of New Zealand white rabbits. One or both eyes may be affected. The globe is enlarged due to increased intraocular pressure as a result of the absence or underdevelopment of the aqueous humor outflow channels with incomplete cleavage of the iridocorneal angles.

**Ref: Burrows, AM; et al: Development of ocular hypertension in congenitally buphthalmic rabbits. Lab Anim Sci, 45:443-4, 1995.**

## Miscellaneous:

Prolapse of the deep gland of the third eyelid has been recently reported and appears clinically as a protrusion of a large tissue mass from the medial canthus of the eye. Histologically, the mass is composed of bilobated glands arranged in an alveolar-like pattern without inflammation. The cause is proposed to be abnormal laxity of the supporting connective tissue.

**Ref: Janssens, G; et al: Bilateral prolapse of the deep gland of the third eyelid in a rabbit: diagnosis and treatment. Lab Anim Sci, 49:105-9, 1999.**

## INTEGUMENTARY SYSTEM

## Viral Diseases:

**Myxomatosis** is caused by a leporipoxvirus that is endemic in the wild rabbit population. Recently, the entire genetic sequence of this virus was determined and myxoma virus was found to have a conserved complement of poxvirus-specific core genes that each poxvirus has that confers the ability to counter the immune responses of the infected host. There are two subtypes of the virus: the south American type that is found in *Sylvilagus brasiliensis* (forest rabbits) and the Californian subtype found in brush rabbits (*S. bachmani*). Myxoma virus is transmitted by direct or indirect contact and arthropod vectors such as ticks and fleas also play a role. After infection, a primary subcutaneous myxoid mass develops within 3-4 days. At 6-8 days, mucopurulent conjunctivitis, subcutaneous edema and multiple subcutaneous masses develop. In peracute cases, rabbits die suddenly with only conjunctival erythema. The high mortality rates are the result of multiorgan dysfunction with secondary bacterial infections due to immunosuppression by the virus. Histologically, there is proliferation of large, stellate mesenchymal cells, which are called myxoma cells interspersed in a mucinous, homogenous matrix with few inflammatory cells. There is also hypertrophy and proliferation of endothelial cells and the overlying epidermis ranges from hyperplastic to degenerative. Intracytoplasmic inclusion bodies are often present.

**Ref: Best, SM; et al: Coevolution of host and virus: cellular location of virus in myxoma virus infection of resistant and susceptible European rabbits. Virology 277, 76-91, 2000.**

**Cameron, C; et al: The complete DNA sequence of myxoma virus. Virology 1999 Nov 25;264(2):298-318.**

**Sobey, WR; et al: Myxomatosis: the effect of age upon survival in wild and domestic rabbits. J Hyg Camb 68:137-49, 1970.**

**Shope fibroma** is also caused by a leporipoxvirus that is transmitted by fleas and ticks. Like myxoma virus, the genetic sequence for this virus has also been recently determined. Shope fibroma virus is the first mammalian virus potentially capable of

photoreactivating ultraviolet DNA damage due to its ability to encode a type II DNA photolyase. The Eastern cottontail rabbit is the natural host, but other species of rabbits are susceptible as well. The virus produces firm flattened subcutaneous, freely moveable, up to 7 cm diameter masses primarily on the legs and feet, and less commonly on the muzzle and in the periorbital and perineal areas. In newborn and immunocompromised adults, shope fibroma virus can cause a fatal disseminated disease similar to myxomatosis. Histologically, there is localized fibroblastic proliferation with infiltration by low to moderate numbers of mononuclear and polymorphonuclear cells. Fibroblasts are fusiform to polygonal and contain intracytoplasmic eosinophilic viral inclusion bodies.

**Ref: Willer, DO; et al: The complete genome sequence of shope (rabbit) fibroma virus. Virology 1999 Nov 25;264(2):319-43.**

**Rabbit pox** is caused by an orthopoxvirus that causes confluent papules in the skin, as well as lesions in the respiratory and digestive tracts. Infection occurs via the respiratory tract followed by viremia with replication in the lymphoid tissue and skin.

**Cutaneous papillomatosis** is typically a benign disease caused by a papilloma virus that is mechanically transmitted through insect vectors. The natural host is the cottontail rabbits. Clinically, the lesions are cornified, pedunculated masses with fleshy central areas. Histologically, these lesions have the typical appearance of a typical squamous papilloma with viral inclusion bodies. Although most lesions are benign and regress spontaneously, some remain persistent or become malignant. Regression is dependent on the host genetic makeup as well as the genetic variability of the virus. Papillomatosis has been studied in rabbits to learn more about the prevention of malignant progression of human papillomavirus-associated lesions in humans.

**Ref: Campo, M. S.: Animal models of papillomavirus pathogenesis. Vir Res 89 (2002), pp. 249-261.**

**DNA vaccination prevents and/or delays carcinoma development of papillomavirus-induced skin papillomas on rabbits. J of Virol, Oct. 2000, Vol 74, No. 20, p. 9712-9716.**

**Hu, J; et al: Large cutaneous rabbit Papillomas that persist during cyclosporin A Treatment can regress spontaneously after cessation of immunosuppression. J of Gen Vir (2005), Vol 86, pp. 55-63.**

**Bacterial Diseases:**

**Pasteurella multocida** causes mucopurulent dermatitis and subcutaneous abscesses.

**Staphylococcus aureus** causes suppurative and necrotizing dermatitis (pyoderma). Sorehock or pododermatitis is also caused by this bacterium. Overweight males housed

on wire-floor cages are predisposed to sorehock. A recent study showed that colonization capacity is an important virulence determinant in rabbit staphylococcosis.

**Ref: Hermans K; et al: Colonisation of rabbits with *Staphylococcus aureus* after experimental infection with high and low virulence strains. Vet Microbiol 2000 Mar 15;72(3-4):277-84.**

**Hermans, K; et al: Rabbit staphylococcosis: difficult solutions for serious problems. Vet Micro 91 (2003), pp. 57-64.**

Treponematosis caused by **Treponema cuniculi** is common in wild rabbits and is often referred to as rabbit syphilis or vent disease. The disease is transmitted venereally, although transmission through extragential contact can occur and the organism is able to penetrate intact mucous membranes. Susceptibility is age and breed dependent. Treponema cuniculi is a 5-20 μm Gram negative helical bacillus with tight or irregular spirals. Clinically, there is edema and erythema at the mucocutaneous junctions of the vulva, prepuce, anal region, muzzle, and periorbital area. Lesions are often crusty. Popliteal and inguinal lymph nodes may be enlarged. The histologic changes are confined to the epithelium and superficial dermis and are characterized by epidermal hyperplasia, epidermal cell necrosis, erosions, and ulcerations with infiltration by predominantly plasma cells and lymphocytes. The diagnosis can be made by making a wet preparation of skin scrapings and examining them under dark field microscopy. The organism can also be demonstrated in tissue sections with silver stains.

**Pseudomonas aeruginosa** causes a moist dermatitis with a characteristic green color.

**Arcanobacterium pyogenes** and **Fusobacterium necrophorum** have been reported to cause suppurative and ulcerative dermatitis.

**Parasitic Diseases:**

**Taenia serialis** can produce a coernus that would present as a subcutaneous swelling or mass. This is a problem of wild rabbits. Rabbits serve as the intermediate host for cestodes; the definitive host is usually a dog or cat.

Fur mites, **Cheyletiella parasitovorax**, can cause a mild alopecia without pruritis or no clinical signs. These mites are transmitted by direct contact and the entire life cycle is spent on the rabbit. Clinically, there is alopecia, scaliness and crusts, especially over the dorsal trunk and scapular areas. Histologically, there is mild hyperkeratosis with mononuclear cell infiltration. The diagnosis is confirmed by finding the mites in skin scrapings.

Sarcoptic mange due to **Sarcoptes scabei** and **S. cuniculi** causes alopecia and dermatitis involving the face, nose, lips, and external genitalia.

Notoedric mange due to **Notoedres cati** occurs rarely.

**Mycotic Diseases:**

**Ringworm** caused by Trichophyton mentagrophytes, Microsporum canis and M. gypseum is relatively uncommon. Rabbits may serve as inapparent carriers and they can transmit infection to other animals, as well as humans. The young and immunocompromised are most susceptible. Clinically, lesions are most commonly present on the head and ears and are raised, circumscribed, and erythematous. Histologically, there is hyperkeratosis, epidermal hyperplasia, folliculitis, and arthrospores. Diagnosis is by skin scrapings cleared in 10% potassium hydroxide solution. In tissue sections, the arthrospores are stained with methenamine silver and periodic acid schiff stains.

**Ref: Zrimsek, P; et al: Detection by ELISA of the humoral immune response in rabbits naturally infected with *Trichophyton mentagrophytes.* Vet Microbiol 1999 Oct;70(1-2):77-86.**

**Neoplastic Diseases:**

Fibroma, squamous cell carcinoma, sebaceous adenocarcinoma, trichoepithelioma and trichoblastoma have been reported.

**Miscellaneous:**

**Barbering** or hair chewing is most common in young and group housed rabbits. It is characterized by alopecia without dermatitis on the face and back. Boredom and low roughage diets are considered to be predisposing factors. The differential diagnosis for hair loss includes hair pulling for nests, behavioral problem, malnutrition, ectoparasites, dermatophytosis, bacterial infections, cage rubbing and seasonal molting.

**MUSCULOSKELETAL SYSTEM**

**General:**

The skeleton composes only 6-8% of the total body weight of the New Zealand white rabbit versus 12-13% of a cat's body weight. The bones of a rabbit are relatively fragile and fractures occur readily, especially with improper handling.

**Bacterial Diseases:**

**Pasteurella multocida** can cause muscular abscesses.

**Nutritional and Metabolic Diseases:**

Nutritional problems are rare due to quality control standards in the commercial feed industry. However, problems can arise especially with the use of individually formulated

diets. **Vitamin E deficiency** can result in muscular soreness and stiffness. Grossly, there are pale streaks in skeletal and cardiac muscle. Histologically, there is myofiber degeneration and necrosis with mineralization and histiocytic inflammation.

Rabbits are very sensitive to levels of **Vitamin D** in the diet. In fact, toxicity can result from levels as little as 5 times normal. Adults are more sensitive than younger rabbits. Clinical signs are nonspecific and include anorexia, weight loss and infertility. There is increased calcium absorption from the intestine, increased renal tubular resorption and increased resorption from bone. Histologically, there is calcification of the renal tubular epithelium and glomerular and tubular basement membranes, smooth muscle, myocardium, intima and media of larger arterioles and arteries, gastric mucosa, large intestine and lung. In the skeleton, there is osteodystrophy with osteoid dysplasia and osteosclerosis. There is excess production and deposition of an abnormal osteoid that is highly cellular with many active osteoblasts.

**Ref: Zimmerman, TE; et al: Soft tissue mineralization of rabbits fed a diet containing excess Vitamin D. Lab Anim Sci, 40:212-15, 1990.**

**Congenital Diseases:**

**Splayleg** is a descriptive term applied to a condition in which rabbits lack the ability to adduct one or all legs and come to a standing position. This condition may be due to inherited syringomelia, hypoplasia pelvis, femoral luxation and distal foreleg curvature.

**Miscellaneous:**

**Vertebral fracture** is caused by improper handling leading to sudden, unsupported movement of the hindlimbs that causes fracture and less commonly vertebral luxation. Most fractures occur in the lumbosacral region, cause spinal cord damage, and produce paralysis.

**Neoplastic Diseases:**

**Osteosarcoma** is rarely reported in the rabbit. It can occur in the mandible, ribs, long bones and extraskeletal locations. As in other animals, metastasis is common and is usually to the lungs and lymph nodes.

## CARDIOVASCULAR SYSTEM

**General:**

The chambers of the right side of the heart are relatively thin and frequently a quantity of clotted blood will be found in the right ventricle with no evidence of contraction. The right atrioventricular valve is bicuspid instead of tricuspid.

**Viral Diseases:**

**Herpesvirus sylvilagus** is a gamma herpesvirus found in wild rabbits. Juveniles are affected to a greater degree than adults. Myocarditis and sudden death has been reported.

Pleural effusion and cardiomyopathy due to **coronavirus** occurs in laboratory rabbits. This virus is antigenically related to human coronavirus strain 229E and it has been suggested that the rabbit virus is a human contaminant. Transmission is through direct contact and there are carrier animals. Gross lesions of pleural effusion disease: pleural effusion, pulmonary edema, right-sided cardiac dilatation, peritoneal effusion, mesenteric lymphadenopathy, necrosis of the liver, kidney and lung, iridocyclitis, and lymphoid depletion. In pleural effusion disease histologically, there is lymphoid depletion of splenic follicles, focal degenerative changes in the thymus and lymph nodes, proliferative changes in glomerular tufts, and uveitis. In the cardiomyopathy form, there is focal to diffuse myocardial degeneration and necrosis, pulmonary edema, lymphoid depletion to hyperplasia, and diaphragmatic muscular degeneration and necrosis. The differential diagnosis for myocardial necrosis in rabbits is hypovitaminosis E, salmonellosis, pasteurellosis, encephalitozoonosis, and detomidine-containing anesthetic agents.

**Ref: Fennestad, KL: Pathogenic observations on pleural effusion disease in rabbits. Arch Virol, 70:11-19, 1985.**

**Bacterial Diseases:**

**Clostridium piliforme,** the causative agent of Tyzzer's disease, can cause a myocarditis that appears grossly as pale gray to tan streaks in the myocardium. Histo: there is focal to segmental myocardial degeneration with mononuclear cell infiltration and bacilli within adjacent myofibers.

**Nutritional and Metabolic Diseases:**

The Watanabe rabbit has been used extensively as an animal model of natural endogenous **atherosclerosis.** This trait is due to a single-gene defect in the gene that codes for low density lipoprotein (LDL) receptors. These rabbits develop a fulminant hypercholesterolemia in the face of a low cholesterol diet. These rabbits have increased plasma LDL cholesterol concentrations and increased plasma concentrations of apolipoprotein E. These lesions are very similar to those in man, but in rabbits they do not progress to advanced or complicated lesions as in man. However, dietary modifications such as the addition of fats to the diet can produce lesions more similar to those in humans. Hypercholesterolemia can also be induced experimentally in rabbits by feeding a high cholesterol diet and/or producing arterial injury by balloon catheter.

**Ref: Garibaldi, BA and Pecquet Goad, ME: Lipid keratopathy in the Watanabe rabbit. Vet Path, 25:173-4, 1988.**

**Ngatia, TA; et al: Arteriosclerosis and related lesions in rabbits. J Comp Path, 101:279-86, 1989.**

**Shell, LG and Saunders, G: Arteriosclerosis in a rabbit. J Am Vet Med Assoc, 194:679-80, 1989.**

**Neoplastic Diseases:**

**Hemangioma** and **hemangiosarcoma** have been reported in the rabbit.

**Animal Models:**

A transgenic rabbit model has been developed for **human hypertrophic cardiomyopathy**. The gene mutation is a common point mutation in the beta-myosin heavy chain (MyHC) gene, R400Q. Rabbits that carry this gene have substantial myocyte disarray and a three-fold increase in interstitial collagen in the myocardium. Premature death in these rabbits is common.

**Ref: Marian, AJ; et al: A transgenic rabbit model for human hypertrophic cardiomyopathy. J Clin Invest 1999 Dec; 104(12):1683-92.**

**RESPIRATORY SYSTEM**

**Viral Diseases:**

**Rabbit pox**, an orthopoxvirus, causes infection of the respiratory tract followed by viremia and viral replication in the lymphoid tissue and skin. The virus is transmitted by direct contact through inhalation. Grossly, papular lesions are present in the oropharynx and respiratory tract and are characterized histologically by focal necrosis with leukocyte infiltration.

**Herpesvirus sylvilagus** causes an interstitial pneumonia with prominent lymphoid hyperplasia in the lung.

Laboratory rabbits are susceptible to experimental infection with **Sendai virus**, a paramyxovirus. Viral replication is confined to the upper respiratory tract and rabbits remain asymptomatic.

**Ref: Machii, K; et al: Infection of rabbits with Sendai virus. Lab Anim Sci, 39(4):334-37, 1989.**

**Bacterial Diseases:**

**Pasteurella multocida** is a Gram negative, bipolar staining bacillus that is the cause of probably the major disease in rabbits. In conventional rabbit colonies, the incidence may be near 50% and up to 70% of the animals may harbor the organism in the upper

respiratory tract and tympanic bullae. P. multocida causes a variety of lesions in the respiratory tract including chronic rhinitis (snuffles) and pneumonia. The agent is transmitted through direct contact with animals shedding the organism from nasal or vaginal secretions. Nursing rabbits can become infected within the first week of life from nursing carrier does. Aerosols do not appear to be an important means of spread and using a modified barrier system can prevent infection. Fomites may be involved in transmission; however, a large number of organisms is required for infection. Interspecies transmission has been experimentally reproduced. There seems to be a seasonal influence with infection because most problems occur in the spring and fall. Predisposing factors include increased atmospheric ammonia concentration, pregnancy, concomitant disease, environmental disturbances and experimental manipulation.

Once present in the body, the organism spreads to other tissues. For example, it can travel to the lower respiratory tract by aerogenous routes; to the middle ear via the eustachian tube; hematogenously; local extension; and to the genital tract by venereal spread or nasal inoculation. Some mucoid variants (possess a hyaluronic acid capsule) of serotype A have the ability to resist phagocytosis. Virulent type A strains also have the ability to adhere to mucosal epithelium, which is apparently mediated by fimbriae. Other mucoid variants as well as smooth variants (type D) are phagocytosed but resist killing. Some isolates of serotype D have been reported to produce a heat labile, dermonecrotic toxin, but the contribution of this toxin to the strain's virulence is not known.

Some of the gross lesions associated with infection of the respiratory tract by P. multocida are catarrhal to mucopurulent rhinitis, atrophic rhinitis, chronic pneumonia characterized by localized consolidation of the anteroventral lobes with atelectasis, acute fibrinous pneumonia with fibrinohemorrhagic lobar pneumonia and pleuritis with possible pericarditis and/or pyothorax, and pulmonary abscesses.
Histologically, the pneumonia may be characterized by chronic bronchitis with peribronchial lymphocytic infiltration to alveolitis with primarily heterophilic inflammation. In the acute necrotizing form, there is destruction of alveoli and small airways, alveolar flooding with fibrinous exudate and erythrocytes and infiltration by large numbers of heterophils. Multinucleate giant cells may be present in affected alveoli.

Some other bacteria that cause similar lesions in the lung of rabbits are Bordatella bronchiseptica, Staphylococcus aureus, and Klebsiella pneumoniae. The diagnosis should be confirmed with bacterial culture. For control of this important disease, infected rabbits should be culled and barrier housing with adequate ventilation should be used. One study effectively vaccinated rabbits with a commercial swine vaccine developed against the heat-labile toxin.

**Ref: Al-Lebban, ZS; et al: Rabbit pasteurellosis: induced disease and vaccination, Am J Vet Res, 49:312-16, 1988.**

**Di Giacomo, RF; et al: Atrophic rhinitis in New Zealand rabbits infected with *Pasteurella multocida*. Am J Vet Res, 50:1460-65, 1987.**

**Di Giacomo, RF; et al: Transmission of *Pasteurella multocida* in rabbits. Lab Anim Sci, 37:621-23, 1987.**

**Lukas, VS; et al: An ELISA to detect serum IgG to *Pasteurella multocida* in naturally and experimentally infected rabbits, Lab Anim Sci, 37:60-4, 1987.**

**Manning, PJ; et al: A dot immunobinding assay for the serodiagnosis of *Pasteurella multocida* infection in the laboratory, Lab Anim Sci, 37:615-20, 1987.**

**Richardson, M; et al: Increased expression of vascular cell adhesion molecule 1 by the aortic endothelium of rabbits with *Pasteurella multocida* pneumonia, Lab Anim Sci, 47:27-35, 1997.**

**Suckow, MA; et al: Protective immunity to *Pasteurella multocida* heat-labile toxin by intranasal immunization in rabbits. Lab Anim Sci, 45:526-32, 1995.**

**Suckow, MA: Immunization of rabbits against *Pasteurella multocida* using a commercial swine vaccine. Lab Anim 2000 Oct:34(4):403-8.**

**Staphylococcus aureus**, a Gram positive coccus can produce septicemia and purulent bronchopneumonia in rabbits. Most strains that cause disease in rabbits are hemolytic, coagulase-positive, type C. Outbreaks in commercial and laboratory facilities are sporadic. The organism is transmitted by direct contact via aerosol. Like Pasteurella multocida , carrier animals can harbor the bacterium in the upper respiratory tract. Umbilical vessels and skin abrasions are two other possible entry sites. After inoculation, Staphylococcus can spread hematogenously or via local extension. Grossly, the pneumonia is often very suppurative and is composed of large amounts of white purulent material. Histologically, there are focal suppurative necrotizing lesions with colonies of cocci. The bacteria are often present in section, but infections should always be confirmed with bacterial culture. The laboratory must determine whether the strain is a pathogenic one.

**Bordatella bronchiseptica** is often present together with Pasteurella multocida. Its role as a definitive cause of disease in the respiratory tract of rabbits has not yet been firmly established. The organism can be recovered from the upper and lower respiratory tract of healthy rabbits. It is transmitted by direct contact through aerosols. Suppurative bronchopneumonia can be produced experimentally by treating rabbits with corticosteroids, then infecting them. However, B. bronchiseptica has been isolated from natural cases of localized pneumonia. Histologically, there is a chronic interstitial pneumonia, chronic bronchiolitis and perivascular and peribronchial accumulations of lymphocytes, plasma cells, and macrophages.

**Ref: Deeb, BJ; et al: *Pasteurella multocida* and *Bordatella bronchiseptica* infections in rabbits. J Clin Micro, 28:70-75, 1990.**

**Glass, LS; et al: Infection with and response to *Pasteurella multocida* and *Bordatella bronchiseptica* in immature rabbits. Lab Anim Sci, 39:406-10.**

**Percy, DH; et al: Incidence of *Pasteurella* and *Bordatella* infections in fryer rabbits: an abattoir survey, J of Applied Rabbit Res, 11:245-6, 1988.**

**Cilia-Associated Respiratory (CAR) bacillus** is a Gram negative, 6-8 μm motile, non-sporeforming bacillus that causes generally subclinical infections in rabbits. CAR bacillus isolates that infect mice and rats are host specific and do not infect rabbits. The organism colonizes the ciliated epithelial cells lining the larynx, trachea, and bronchi. Histologically, there may be a chronic tracheitis with goblet cell hyperplasia. The organism can be demonstrated in section with silver stain.

**Ref: Cundiff, DD; et al: Characterization of cilia-associated respiratory bacillus in rabbits and analysis of the 16S rRNA gene sequence. Lab Anim Sci, 45:22-26, 1995.**

**Cundiff, DD; et al: Characterization of cilia-associated respiratory bacillus isolates from rats and rabbits. Lab Anim Sci, 44:305-12, 1994.**

**Kurisu; et al: cilia-associated respiratory bacillus infection in rabbits, Lab Anim Sci, 40:413-5, 1990.**

**Mycotic Diseases:**

Pulmonary aspergillosis due to **Aspergillus fumigatus** and **A. flavus** is occasionally found at necropsy, almost exclusively in wild rabbits. The lesions are circumscribed nodules with a central area of necrosis surrounded by neutrophils, lymphocytes, plasma cells, macrophages, and multinucleate giant cells. Fungal hyphae can be demonstrated with silver or PAS stains.

**Neoplastic Diseases:**

**Mesothelioma** has been reported in the rabbit.

**Ref: Lichtensteiger, CA; et al: Peritoneal mesothelioma in the rabbit, Vet Path, 24:464-6, 1987.**

## GASTROINTESTINAL SYSTEM

**General:**

Rabbits are hind gut fermenters with a large and complex digestive system. They practice cecotrophy, which is the ingestion of mucous-coated night feces, which occurs daily, and is a method of recycling cecotrophs that are rich in B vitamins and proteins. Cecotrophy is controlled by the adrenal glands, and therefore may be altered during periods of excessive stress.

Rabbits possess abundant gut associated lymphoid tissue located in the Peyer's patches, lymphoid appendix and sacculus rotundus. These structures comprise nearly 50% of the total mass of lymphoid tissue in the body.

**Viral Diseases:**

**Rabbit pox,** an orthopoxvirus, causes papular, necrotic lesions in the liver.

**Oral papillomatosis** is caused by a papillomavirus and usually occurs in young rabbits, 2-18 months of age. The condition is characterized by the presence of white, fleshy papillomatous masses along the ventral aspect of the tongue. The virus is spread by direct contact in areas where there has been injury to the oral mucosa. Rough or hard food, chewing on rough cage bars and or malocclusion may predispose animals to infection. The histologic appearance is a typical squamous papilloma with basophilic intranuclear inclusions in epithelial cells.

**Adenoviral** infections have been reported in commercial rabbit operations in Hungary and are characterized by enteritis with profuse diarrhea and dehydration in young rabbits with a low mortality. It is speculated that co-infections with Escherichia coli play a prominent role.

**Ref: Bondon L, et al; Isolation of an adenovirus from rabbits with diarrhea. Acta Veterinarian Academiae Scientiarum Hungariae, 28:247-55, 1980.**

**Parvovirus** has been isolated from large numbers of clinically normal rabbits in Japan and surveys of laboratory rabbits in the U.S. have revealed high antibody titers in relatively large numbers of rabbits. It may play a role in the enteritis complex. In experimental infections, it caused listlessness and anorexia for 4 to 6 days post infection. Histologically, there was mild to moderate catarrhal enteritis.

**Ref: Metcalf, JB; et al: Natural parvovirus infection in laboratory rabbits. Am J Vet Res, 50:1048-51, 1989.**

**Rotavirus** causes mild to severe diarrhea with high morbidity in suckling and weanling rabbits. The virus is transmitted by direct contact by ingestion and is endemic in many rabbitries. It can be found in normal rabbits free of disease and antibody titers in normal rabbits may also be present. Clinically, the rabbit is dehydrated and the cecum is distended, congested and filled with fluid contents. Histologically, in the small intestine there is villar atrophy, blunting, and fusion with vacuolation and flattening of apical enterocytes. In the cecum, there are focal areas of desquamation with basophilic debris in the cytoplasm of affected enterocytes. A recent paper noted that the diarrhea may precede mucosal damage and that there may be generalized inhibition of sodium solute transport mechanisms, and of water reabsorption. Diarrhea is more severe with coinfections such as those with *E. coli*.

**Ref: Di Giacomao, RF; et al: Epidemiology of naturally occurring rotavirus infection in rabbits. Lab Anim Sci, 36:153-56, 1986.**

**Halaihel, N: et al: Rotavirus infection impairs intestinal brush-border membrane Na(+)-solute cotransport activities in young rabbits. Am J Physiol Gastrointest Liver Physiol 2000 Sep; 279(3):G587-96.**

**Schoeb, TR; et al: Rotavirus-associated diarrhea in a commercial rabbitry. Lab Anim Sci, 36:149-52.**

**Coronavirus** can cause enteritis in young rabbits, 3-8 weeks of age. Clinically, the affected rabbits are thin and dehydrated with fecal staining in the perineal region. The cecum is distended and filled with watery, beige to tan fecal material. The histological appearance is similar to rotavirus with necrosis of villous epithelial cells and M-cell necrosis. Diagnosis is by finding typical viral particles in feces.

**Ref: Descoteaux JP; et al: An enteric coronavirus of the rabbit: detection by immunoelectronmicroscopy and identification of structural polypeptides. Arch Virol 84:241-50, 1985**.

**Rabbit viral hemorrhagic disease** is caused by a calicivirus and is considered a foreign animal disease. The International Committee on Taxonomy of Viruses has proposed a new genus of Lagovirus within the Calicivirus family for RVHD virus and European brown hare disease virus. This is the only reportable disease in rabbits in the U.S. It is a peracute disease of adult rabbits that results in hepatic, enteric, and lymphoid necrosis. The virus is transmitted by direct contact and by contaminated fomites. Carrier states are present and the virus is shed in the urine for up to 4 weeks and long term fecal shedding may be possible. This virus has a predilection for hepatocytes and macrophages, where it replicates. A recent study suggested there is an association between RHDV infection and apoptosis of hepatocytes. The clinical signs vary but include sudden death, fever, depression, CNS signs, and serosanguinous discharge. Necropsy findings include hepatomegaly, splenomegaly, and hemorrhage and serosal ecchymoses. Histologically, there is hepatic necrosis that begins in the periportal areas and spreads to involve the entire lobule. There is also heterophilic infiltration, cryptal necrosis, pulmonary edema, hemorrhage, and lymphocytolysis. There are fibrin thrombi in the small vessels throughout the body. There is a report of a vaccine based on a recombinant myxoma virus expressing the rabbit hemorrhagic disease virus capsid protein that protected rabbits against lethal challenge with both of these viral agents. In an outbreak in Illinois, clinical signs included depression, anorexia, fever, paddling, convulsions and sudden death. Diagnosis was made by hemagglutination assay and viral antigen-detection ELISA.

**Ref: Campagnolo, E.R.; et al: Outbreak of rabbit hemorrhagic disease in domestic lagomorphs. J Am Vet Med Assoc. 2003 Oct 15;223(8)1151-5.**

**Chasey D, et al; Development of a diagnostic approach to the identification of rabbit hemorrhagic disease. Vet Record, Aug 12, 1995, pp. 158-60.**

**Green, KY; et al: Taxonomy of the caliciviruses. J Infect Dis 2000 May;181 Suppl 2;S322-30.**

**Jung, JY; et al: Apoptosis in rabbit haemorrhagic disease. J Comp Pathol 2000 Aug-Oct;123(2-3):135-40.**

**Kimura T; et al: Distribution of rabbit haemorrhagic disease virus RNA in experimentally infected rabbits. J Comp Pathol 2001 Feb-Apr;124(2-3):134-41.**

**Kovaliski, J: Monitoring the spread of rabbit hemorrhagic disease virus as a new biological agent for control of wild European rabbits in Australia. J of Wild Dis, 34(3), 1998, p. 421-28.**

**Motha, MX; et al: Evaluation of three tests for the detection of rabbit haemorrhagic disease virus in wild rabbits, Vet Rec, Dec 5, 1998, p.627-29.**

**Ohlinger, VF; et al: Identification and characterization of the virus causing rabbit hemorrhagic disease. J Virol 64(7):3331-36, 19990.**

**Park, JH; et al: Pathogenesis of acute necrotic hepatitis in rabbit hemorrhagic disease. Lab Anim Sci, 45:445-9, 1995.**

**Schirrmeier, H; et al: Pathogenic, antigenic and molecular properties of rabbit hemorrhagic disease virus (RHDV) isolated from vaccinated rabbits: detection and characterization of antigenic variants. Arch of Virol, 1999, 144:719-35.**

**Torres, JM; et al: Safety evaluation of a recombinant myxoma-RHDV virus inducing horizontal transmissible protection against myxomatosis and rabbit haemorrhagic disease. Vaccine 19 (2001) 174-182.**

**European brown hare disease** is also caused by a calicivirus and is an acute, contagious, highly fatal disease of European hares that closely resembles rabbit viral hemorrhagic disease.

**Ref: Gavier-Widen, D: Morphologic and immunohistochemical characterization of the hepatic lesions associated with European brown hare syndrome. Vet Path 31:327-334, 1994.**

**Bacterial Diseases:**

**Tyzzer's disease** is caused by infection with **Clostridium piliforme**, a Gram negative, motile, filamentous, sporeforming bacillus. The bacterium causes an acute disease characterized by a sudden outbreak of profuse, watery diarrhea with a short course and high mortality rate. Predisposing factors are important in this condition and include poor sanitation, stress and sulfonamide therapy. Many other species of laboratory and

domestic animals are also infected, therefore interspecies transmission must be prevented. Survivors can become chronically infected and serve as carriers. C. piliforme is transmitted by direct contact through ingestion. The organism can survive in soiled bedding for up to one year. Clinically, there is profuse, watery diarrhea with anorexia, dehydration, and rapid death. At necropsy, there is a classic triad of lesions that include segmental necrosis, edema and hemorrhage of the intestine; multifocal hepatic necrosis, and myocardial necrosis. Histologically, there is necrosis, edema, and hemorrhage with intracellular bacterial present in enterocytes and hepatocytes. The bacilli can be readily demonstrated with Giemsa, PAS, and silver stains.

**Ref: Waggie, RS; et al: An enzyme-linked immunosorbent assay for detection of anti-*Bacillus piliformis* antibody in rabbits, Lab Anim Sci, 37:176-9, 1987.**

**Listeria monocytogenes** can cause hepatic necrosis which histologically appears as coagulative necrosis of hepatocytes with heterophilic infiltration.

**Salmonella typhimurium** and **S. enteriditis** are Gram negative bacilli that can cause rare infections in rabbits that result in septicemia, diarrhea, abortions and death. These bacteria are transmitted by the fecal-oral route and cause polyserositis, focal hepatic necrosis, splenomegaly, enteritis with fibrinous exudate and suppurative metritis.

**Yersinosis** due to **Yersinia pseudotuberculosis** occurs rarely in domestic rabbits and is an acute to chronic infection. The organism is transmitted by ingestion of contaminated food and water. Wild rodents and birds are carriers. The clinical signs are nonspecific and include poor condition and weight loss. Histologically there is necrosis in the liver, spleen, cecum, and lymph nodes and occasionally, the reproductive tract may be affected. There are large numbers of coccobacilli within necrotic areas.

Several conditions fall into the **enteritis complex**. These include **mucoid enteropathy, carbohydrate overload, clostridiosis, colibacillosis, vibriosis, and proliferative enteropathy.**

**Mucoid enteropathy** or **mucoid enteritis** is a major cause of disease and mortality in young rabbits. There are several factors involved that include bacterial infection, the presence of toxins, dietary irregularity and/or obstruction. The condition can be induced experimentally by ligating sections of the large intestine. Clinically, this is a subacute disease characterized by the passage of copious amounts of gelatinous mucus in feces. There is also anorexia, polydypsia and subnormal body temperature. Rabbits 7-10 weeks of age are most often affected. It is proposed that an alteration in the cecal environment results in the production of a goblet cell secretagogue that is absorbed through the cecal mucosa. It is then transported to the colon, where it causes goblet cell hyperplasia. At necropsy, there is gastric distention by fluid and gas, distention of the jejunum by translucent watery fluid, cecal impaction by dry contents and gas and distention of the sacculated colon by clear, gelatinous mucoid exudate. Histologically, there is striking goblet cell hyperplasia in the jejunal, ileal and colonic mucosa with little or no

inflammation. In the colon, the crypts and lumen are distended with mucus and mucus plugs. Goblet cell hyperplasia of the gallbladder has also been described.

**Ref: Hotchkiss, CE and Merritt, AM: Evaluation of cecal ligation as a model of mucoid enteropathy in specific-pathogen free rabbits, Lab Anim Sci, 46:174-8, 1996.**

**Hotchkiss, CE and Merritt, AM: Mucus secretagogue activity of cecal contents of rabbits with mucoid enteropathy. Lab Anim Sci, 46:179-85, 1996.**

**Itagaki, S; et al: Lectin histochemical changes of colon goblet cell mucin in rabbit mucoid enteropathy, Lab Anim Sci, 44:82-4, 1994.**

**Lelkes, L and Chang, CL: Microbial dysbiosis in rabbit mucoid enteropathy, Lab Anim Sci, 37:757-64, 1987.**

In **carbohydrate overload,** low fiber, high starch diets fed to young animals results in high concentrations of starches in the cecum and colon. This can lead to proliferation of E. coli, Clostridium perfringens, or C. spiroforme. During the fermentation of these starches, toxins are produced that may damage the mucosal surface and cause movement of water and electrolytes into the lumen.

**Clostridiosis** in rabbits has been attributed to infection with **Clostridium perfringens, C. difficile, and C. spiroforme. C. spiroforme** produces a type E iota toxin and is the most common of the clostridial bacteria associated with enteritis complex in juvenile rabbits. Infections in rabbitries are common and at necropsy of diarrheic rabbits, it is isolated from over 50% of the cases, and in one study, 90% of these strains were toxigenic. **C. perfringens** causes an enterotoxemia-like condition in young rabbits that results in cecal hemorrhage and edema. **C. difficile** causes colitis in rabbits after prolonged therapy with antibiotics in the penicillin family. Any disruption of the normal gastrointestinal flora due to feed changes, weaning, antibiotic therapy and concurrent infections (E. coli, Eimeria sp, Cryptosporidia, and rotavirus) allows colonization and proliferation of clostridial bacteria with subsequent toxin production. There are different clinical forms of the condition. In the peracute form, there is death with little or no premonitory signs. In the chronic condition, there is anorexia, wasting and intermittent diarrhea over several days. At necropsy, the body is in good nutritional condition and there is soiling of the perineal region with watery green to tarry brown feces. There is often a straw colored peritoneal effusion, ecchymoses on the cecal serosa, with occasional involvement of the distal ileum and proximal colon. There may be epicardial and thymic ecchymoses. The cecum and adjacent areas are frequently dilated and are filled with watery to mucoid, green to dark brown material and gas. Hemorrhage and ulceration and/or fibrin may markedly thicken these areas. Histologically, there is necrotizing typhlocolitis with effacement of the mucosal architecture, loss of epithelium, ulceration, fibrinous exudation, congestion, hemorrhage, and infiltration by primarily heterophils. Thrombi may be present on the mucosal surface. The diagnosis can be confirmed by anaerobic bacterial culture and the toxins can be identified.

**Ref: Butt, MT; et al: A cytotoxicity assay for *Clostridium spiroforme* enterotoxin in cecal fluid of rabbits. Lab Anim Sci, 44:52-54, 1994.**

**Perkins, SE; et al: Detection of *Clostridium difficile* toxins from the small intestine and cecum of rabbits with naturally acquired enterotoxemia, Lab Anim Sci, 45:379-84, 1994.**

The attaching and effacing (enteropathogenic) strains of **E. coli** cause colibacillosis in rabbits and are a major cause of enteritis in commercial rabbitries. The organism is not normally present or is present in small numbers within the gastrointestinal tract of suckling and weanling rabbits. There is a rapid proliferation of these bacteria with a change in intestinal pH due to such things as intestinal coccidiosis and diets that require a high HCl concentration for digestion. Some strains affect only suckling rabbits and attach to the full length of the small and large intestine, while other strains affect weanlings only and attach only to the ileum and large intestine. The organism attaches to the Peyer's patch dome epithelium and then later colonizes and attaches to enterocytes. At necropsy, the body is dehydrated, there is perineal staining with watery, yellow to brown fecal material, the cecum is distended with watery yellow to gray-brown contents and there may be serosal ecchymoses, edema in the cecal and colonic walls and enlarged mesenteric lymph nodes. The small intestine is usually grossly normal. Histologically, changes are most severe and extensive in weanling rabbits. Ileal villi are blunted with edema and heterophilic infiltration of the lamina propria. The enterocytes at the villar tips are swollen with attached bacilli.

**Ref: Thouless, ME; et al: The effect of combined rotavirus and *Escherichia coli* infections in rabbits. Lab Anim Sci, 46:381-85, 1996.**

**Peeters, JE; et al: Biotype, serotype and pathogenicity of attaching and effacing enteropathogenic *E. coli* strains isolated from diarrheic commercial rabbits, Infec and Immun, 56:1442-8, 1988.**

**Vibrio sp.** has been associated with typhlitis with degeneration and hyperplasia of cryptal epithelial cells. Organisms can be identified within epithelial cells by silver stains.

**Ref: Imia, LG; et al: Intraduodenal inoculation of adult rabbits for evaluating the immunogenicity of genetically attenuated *Vibrio cholerae* strains, Lab Anim Sci, 48:538-41, 1998.**

**Proliferative enteropathy** is caused by **Lawsonia intracellularis**, a slightly curved, Gram negative bacillus. The organism produces diarrhea in suckling, weanling, and adult rabbits. The disease in rabbits is similar to that in hamsters and pigs. At necropsy, there are semi-fluid, mucinous contents in a thickened colonic and rectal mucosa. Histologically, there is variable involvement in the terminal small intestine, cecum, and colon. Lesions vary from erosive and suppurative to proliferative. In the erosive form, there is focal to segmental loss of enterocytes with heterophilic infiltration. The proliferative form is characterized by multifocal to diffuse enterocyte hyperplasia and

hyperplasia of crypt and villar epithelium with infiltration by mononuclear inflammatory cells. Bacteria can be demonstrated within enterocytes with silver stains.

**Ref: Duhamel, GE; et al: Subclinical proliferative enteropathy in sentinel rabbits associated with *Lawsonia intracellularis*. Vet Path, 35:300-303. 1998.**

**Hotchkiss, CE; et al: Proliferative enteropathy of rabbits: the intracellular *Campylobacter*-like organism is closely related to *Lawsonia intracellularis*, Lab Anim Sci, 46:623-7, 1996.**

**Schoeb, TR; et al: Enterocolitis associated with intraepithelial *Campylobacter*-like bacteria in rabbits, Vet Path, 27:73-80, 1990.**

**Tularemia** caused by the Gram-negative, pleomorphic coccobacillus ***Francisella tularensis***. This is a disease of wild rabbits. Arthropods such as ticks, mosquitoes, flies and lice transmit the bacterium. Rabbits die of an acute fatal septicemia and the major gross lesions consist of pinpoint, pale foci in the liver, spleen and bone marrow.

**Protozoal Diseases:**

Intestinal coccidiosis is caused by numerous species in the genus **Eimeria**. The species considered to be most pathogenic in rabbits are **intestinalis and flavescens. Magna, irresidua,** and **piriformis** are considered to be intermediately pathogenic. Least pathogenic species include **perforans**, **neoleporis** and **media**. Coccidiosis is a common, widespread problem in commercial operations and research facilities. Coccidia may act as copathogens in other conditions and as with many of the conditions of the gastrointestinal tract in rabbits, changes in environment and management may predispose to infection. Coccidia are transmitted through fecal-oral contact. After passage in the feces, the oocysts require one or more days to sporulate. After the sporulated oocysts are ingested, sporozoites are released and then invade enterocytes and multiply by schizogony. One or more sexual cycles take place, then gametogony occurs and oocysts are formed and passed in the feces. Clinical disease occurs most frequently in weanlings. The sexual stage causes the most damage and results in extensive destruction of enterocytes and other cells within the lamina propria. Gross lesions include dark green to brown watery foul smelling exudate in the cecum and colon with edema and congestion of the mucosa. Where the lesion is located depends on the species involved. Histologically, there is destruction and necrosis of enterocytes, villar atrophy, marked heterophilic infiltration and presence of gametocytes and oocytes. The disease can be diagnosed by fecal flotation or by mucosal scrapings. Bacterial culture should also be performed, as there are often co-infections.

Hepatic coccidiosis occurs in both wild and domestic rabbits and is due to infection with **Eimeria stiedae**. Weanlings are most often affected; older rabbits develop immunity. The organism is transmitted through the ingestion of sporulated oocysts. After ingestion, the sporozoites penetrate the intestinal epithelium and are then transported to the liver, where there invade biliary epithelial cells and undergo schizogony. After, gametogony,

oocysts are released into bile ducts, pass to the intestinal tract via the bile and are then passed into the feces. Clinically, infections are often inapparent. However, anorexia, debilitation, constipation or diarrhea may be present in heavy infections. Other clinical signs may include enlarged liver, pendulous abdomen, and icterus. Liver enzymes and serum bilirubin may be elevated. At necropsy, there is hepatomegaly with multifocal, raised yellow to pearl gray, circumscribed foci that contain inspissated dark green to tan material. The gallbladder mucosa is thickened and filled with viscid green bile and debris. Histologically, there is bile duct hyperplasia with ectasia and papillary projections covered by reactive epithelial cells and gametocytes and oocysts. Periportal fibrosis and mixed inflammatory cell infiltrate may also be present. Oocysts are present in gallbladder aspirates or impression smears. Histopathology is pathognomic.

In **toxoplasmosis,** multifocal necrosis, granulomatous inflammation, tachyzoites, and tissue cysts may be present in the liver.

**Cryptosporidiosis** due to **Cryptosporidium parvum** is a rare primary cause of enteritis in young rabbits. It is usually identified as an incidental finding and causes villar blunting, a decrease in crypt-villous ratio, and edema.

**Parasitic Diseases:**

The pinworm of rabbits is **Passalurus ambiguus**. It is fairly common in rabbitries and causes occasional diarrhea. The parasite is transmitted through the fecal-oral route. Adult worms are present in the cecum and colon and larvae are present on the mucosa of the small intestine and cecum. Diagnosis is made by fecal flotation. Eggs are morulated and are slightly flattened on one side.

**Baylisascaris procyonis** can produce necrotic lesions (migration tracts) in the liver.

The **cysticercus** of **Taenia pisiformis** may be found in the liver and/or mesentery. Rabbits are the intermediate host for this cestode. In the Laboratory Animal Resources area at Colorado State University, they reported three cases within a daily census of 250 rabbits over 10 years, a <1% incidence.

**Owiny JR: Cysticercosis in laboratory rabbits. Contemp Top Lab Anim Sci 2001 Mar;40(2):45-8.**

**Obeliscoides cuniculi** is a trichostrongyle found in the stomach of rabbits that graze fresh grass or are fed fresh grass as feed. Most infections are asymptomatic, but in heavy infections anorexia, lethargy and weight loss may be seen. Treatment is ivermectin injected subcutaneously and then repeated 2 weeks later.

**Congenital Diseases:**

**Malocclusion** is the most common dental abnormality of rabbits and is inherited in an autosomal recessive pattern. It may also be caused by trauma, dietary problems and

neoplasia. In this condition, the mandible is abnormally long in relation to the maxilla, which results in failure of the incisors to wear normally and causes impaired mastication. The congenital form appears during 8-10 weeks of age. Clinical signs related to malocclusion include anorexia, dysphagia, bruxism, ptyalism, weight loss, and dental disease. Depending on the primary cause, treatment is corrective burring or extraction of affected teeth with supportive care and a change to a higher fiber diet.

**Miscellaneous Diseases:**

**Trichobezar** or "wool block" are masses of hair and ingesta in the stomach that result from excessive self-grooming. They are common and are usually an incidental finding. Predisposing factors may include low fiber diets, experimental manipulation and stress. Trichobezars may cause complete or partial obstruction with subsequent gastric rupture and peritonitis. Anorexia and fatty liver can also be seen. Gastrotomy to remove the blockage may be required as a life-saving measure.

**Ref: Haugh, PG: Hairballs in rabbits: an alternative treatment. Can Vet J, 28:280, 1987.**

**Turner, E: Furballs in rabbits. Can Vet J, 28:204, 1987.**

**Neoplastic Diseases**

**Bile duct adenoma** and **bile duct adenocarcinoma** have both been reported as incidental findings at necropsy. These neoplasms have minimal clinical significance.

## URINARY SYSTEM

**General:**

Rabbits have alkaline urine with dull yellow to brown calcium carbonate and triple phosphate crystals. Calcium and magnesium are excreted primarily via the urine. Urine may be pigmented dark red to orange which is an incidental finding and may indicate increased ingestion of dietary porphyrins or elevated urobilin.

**Viral Diseases:**

**Herpesvirus sylvilagus** can produce prominent lymphoid hyperplasia in the kidneys.

**Bacterial Diseases:**

Septic emboli can be found in the kidney with disseminated infections with **Staphylococcus sp.**

**Protozoal Diseases:**

The spores of **Encephalitozoon cuniculi** are spread in the urine. Infection begins with ingestion of contaminated urine and is spread within the body hematogenously within macrophages. The spores selectively parasitize vascular endothelium, especially in the brain and kidney, as well as renal tubular epithelium. Then the spores localize in the liver, lung, adrenal gland, spleen and other highly vascular organs. Within the cell, the spores are contained within a parasitophorous vacuole or pseudocyst and are called trophozoites or schizonts that multiply by ordinary fission or schizogony. When the trophozoites mature, they become sporonts, then sporoblasts, and then eventually spores. Gross lesions of the kidney include focal, irregular, depressed pale areas, 2-4 mm diameter on the cortical surface. Histologically, there is focal to segmental, granulomatous to chronic interstitial nephritis with tubular ectasia, and spores within tubular epithelial cells, macrophages, and in areas of inflammation. Spores can be demonstrated with Gram, Giemsa, and carbol fuchsin stains.

**Ref: Boot R; et al: Comparison of assays for antibodies to *Encephalitozoon cuniculi* in rabbits. Lab Anim 2000 Jul; 34(3):281-9.**

**Parasitic Diseases:**

Necrotic tracts due to **Baylisascaris procyonis** may be present in the kidney.

**Neoplastic Diseases:**

Renal cell carcinoma and nephroblastoma have been reported in the rabbits. Nephroblastoma or embryonal nephroma may be single or multiple and affect one or both kidneys. They are slow growing and are unlikely to metastasize.

**Miscellaneous:**

**Urine scalding** can occur as a result of the following problems: primary incontinence due to neurological conditions, conditions that prevent the rabbit from adopting a correct stance for urination, conditions that prevent normal grooming, calcium carbonate deposits in the bladder sediment, anatomical defects, poor husbandry and reproductive disease resulting in perineal inflammation. Secondary bacterial infection is common. Treatment is clipping and cleaning the affected area and antibiotics if needed.

**Ref: Donnelly, T.M.: Wet fur and dermatitis in a rabbit. Lab Animal, Vol 34, No. 2, pp. 23-25.**

**REPRODUCTIVE SYSTEM**

**General:**

Female rabbits are does; males are bucks. Bucks reach puberty at 6-10 months of age and does reach puberty at 4-9 months. The breeding lifespan of a doe is 3-4 years. The uterus has two horns and two separate cervices and placentation is hemochorial. Does are induced ovulators. Gestation lasts 25-29 days and does give birth to 4-10 kits. Following parturition, the kits nurse 1-2 times daily. The doe's milk is high in fat and protein and kits are weaned at 4-6 weeks of age.

**Bacterial Diseases:**

**Pasteurella multocida** can cause infections of the genital tract and produce lesions that include pyometra, suppurative orchitis, abscesses and suppurative mastitis. Histologically, there is transmural necrosis and heterophilic inflammation in the uterus and abscesses in the testes.

**Bacterial mastitis** has been attributed to the following organisms: **Staphylococcus, Pasteurella multocida,** and **Streptococcus**. In lay terms, it is called "blue breast" and occurs most commonly in recently kindled and heavily lactating does. Orphan young can spread disease to an unaffected doe and infection can spread to multiple mammary glands. Clinically, the skin overlying the mammary glands has a red to dark blue discoloration and the gland contains serous to purulent exudate. Nursing young may develop an acute fatal septicemia. Histologically, the lesions are suppurative with necrosis and often bacteria are present.

**Listeria monocytogenes** is a Gram positive, motile coccobacillus that causes fever, abortions, and sudden death in does in late gestation. This organism has a tropism for the uterus and placenta. Infected newborn kits may develop systemic disease, may have stunted growth an/or develop meningoencephalitis. The organism is transmitted through ingestion of contaminated feed and/or water and transplacentally.

**Nutritional and Metabolic Diseases:**

**Vitamin E deficiency** can cause neonatal mortality and infertility.

**Ref: Yamini, B; et al: Abortion, stillbirth, neonatal death, and nutritional myodegeneration in a rabbit breeding colony. J Am Vet Med Assoc, 194:561-2m 1989.**

**Hypo and hypervitaminosis A** produce similar clinical manifestations and include poor conception rates, congenital anomalies, fetal resorption, abortion, and birth of thin, weak kits.

**Pregnancy toxemia** occurs in does usually during the last week of pregnancy. Primiparous, obese animals on high planes of nutrition that suddenly go off feed are most at risk. Clinical pathological abnormalities include ketosis, hypocalcemia, hyperphosphatemia and fluctuating blood glucose. At necropsy, there are excessive body fat stores with fatty infiltration of the liver, kidney, and adrenal glands.

## Congenital Diseases:

**Endometrial venous aneurysms** are considered congenital defects characterized by multiple blood filled endometrial varices that are composed of dilated, thin walled veins that rupture and bleed periodically into the uterine lumen.

**Ref: Bray, MV; et al: Endometrial venous aneurysms in three New Zealand white rabbits. Lab Anim Sci, 42:360-2, 1992.**

## Neoplastic Diseases:

**Uterine adenocarcinoma** is the most common spontaneous neoplasm of the rabbit. The incidence increases with age and nearly all breeds are affected. The role of estrogens is equivocal. Grossly, there are multiple, nodular thickenings that protrude into the uterine lumen. Histologically, these thickenings are composed of acinar and tubular structures supported by a vascular myxoid stroma. There is serosal implantation and metastasis to the lung, liver, and regional lymph nodes.

**Leiomyoma/leiomyosarcoma** has been reported as incidental findings at necropsy.

**Mammary carcinoma** has been reported frequently in laboratory rabbits. Cystic hyperplasia progresses to benign neoplasia with progresses to invasive adenocarcinoma. Metastasis is to the lungs and regional lymph nodes.

In the testis, **interstitial cell tumor** is the most common neoplasm. Sertoli cell tumors and seminomas have also been reported.

**Brown, PJ; et al: A testicular seminoma in a rabbit. J Comp Path, 100:353-5, 1989.**

# HEMATOLYMPHATIC SYSTEM

## General:

Ear vessels are prominent and are readily accessible for blood collection. In the rabbit, the erythrocyte measures 6.5-7.5 μm in diameter. Polychromasia is a normal finding. Reticulocytes make up 2-5% of the red blood cell count and the life span of the red blood cell is 50 days. Heterophils are the counterpart of the neutrophil and measure 9-15μm and have distinct acidophilic granules. Eosinophils are 12-16 μm and have large cytoplasmic granules that stain dull pink-orange with conventional hematology stains. Lymphocytes are the predominant leukocyte in circulation. Small lymphocytes measure 7-10 μm and large lymphocytes are 10-15 μm. Lymphocytes normally contain a few azurophilic cytoplasmic granules. Basophils may be numerous and represent up to 30% of the circulating leukocyte population.

**Viral Diseases:**

Lymphoid necrosis in various lymphoid organs such as lymph nodes and spleen is due to **rabbit poxvirus.**

**Herpesvirus sylvilagus** causes leukocytosis, monocytosis, and lymphocytosis. There is prominent lymphoid hyperplasia of nonlymphoid organs.

**Ref: Hesselton, RM; et al: Pathogenesis of *Herpesvirus sylvilagus* infection in cottontail rabbits. Am J Path, Vol 133, No. 3, 639-47, 1988.**

In **rabbit viral hemorrhagic disease** there is splenomegaly and lymphocytolysis.

**Bacterial Diseases:**

Many of the previously mentioned bacteria including **Pasteurella multocida** and **Staphylococcus aureus** can produce septicemia.

**Neoplastic Diseases:**

**Lymphosarcoma** is most common in juvenile and young adult rabbits. It is typically the visceral form that involves the liver, spleen, and kidney, although rare localized forms have been reported. Occasionally, leukemia is present. At necropsy, there is lymphadenopathy, splenomegaly, and hepatomegaly with multiple, white circumscribed nodules. Histologically, the neoplastic cells are lymphoblastic. Both B-cell and T-cell types have been observed.

**Ref: Toth, LA; et al: Lymphocytic leukemia and lymphosarcoma in a rabbit. J Am Vet Med Assoc, 197:627-9, 1990.**

**Thymoma** occurs in rabbits at a low incidence and is usually diagnosed at necropsy as an incidental finding, however, radiographic examination often reveals an anterior mediastinal mass with enlarged cardiac silhouette with pleural effusion. It may cause coughing, tachypnea, dyspnea and exercise intolerance. Thymomas have been associated with paraneoplastic syndromes such as myasthenia gravis, autoimmune disease, and hypercalcemia of malignancy, etc. There is a report of a successful surgical removal of a thymoma in a rabbit.

**Ref: Clippinger, TL; et al: Removal of a thymoma via median sternotomy in a rabbit with recurrent appendicular neurofibrosarcoma, J Am Vet Med Assoc, 213:1140-3, 1998.**

**Animal Models:**

An animal model for **human Epstein-Barr virus-associated hemophagocytic syndrome** was recently developed in the rabbit using an EBV-related herpesvirus of

baboon (HVP). Affected rabbits have circulating atypical T lymphocytes that express HVP mRNA, hepatosplenomegaly, lymphadenopathy, and hemophagocytic histiocytosis in lymph nodes, spleen, bone marrow and thymus.

**Ref: Hayashi, K; et al: An animal model for human EBV-associated hemophagocytic syndrome: herpesvirus papio frequently induces fatal lymphoproliferative disorders with hemophagocytic syndrome in rabbits. Am J Pathol 2001 Apr:158(4):1533-42.**

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# DISEASES AND NEOPLASMS OF THE AGING RAT

James B. Nold, DVM, PhD, Diplomate, ACVP
GlaxoSmithKline, Research Triangle Park, North Carolina

General Comments

1. Limited mostly to Sprague-Dawley (CD) and Fischer 344 (F344) rats.
2. Based on information from chemical safety tests:
    a. Two-week repeated dose range-finding studies.
    b. 90-day to 120-day subchronic studies.
    c. 24-month to 30-month chronic toxicity/oncogenicity studies.
3. Rats
    a. Hsd:Sprague Dawley®SD® (Harlan Sprague Dawley, Inc.).
        • Direct descendants of original SD colony.
    b. Crl:CD®BR Rat (Charles River Laboratories).
        • Originated from the outbred SD® 40+ years ago.
    c. Fischer 344 (F344).
    d. Wistar.

I. Urinary System

A. Kidney

1. Chronic progressive nephropathy (CPN).
Synonyms: Spontaneous degenerative nephropathy
Rat nephropathy
Chronic nephrosis
Glomerulonephrosis

a. Most common renal disease of laboratory rats.

b. Proteinaceous casts, interstitial inflammation, fibrosis, thickened basement membranes, glomerulosclerosis, crescent formation.

c. Basophilic epithelium in early stages of disease. Seen in animals as young as 3-4 months.

d. Striking hyaline droplet degeneration.

e. More common and more severe in males than females.
Incidence in males is reduced if they are castrated.

f. Incidence is elevated in rats on a high protein diet.

g. Dietary caloric restriction markedly reduces the incidence and severity, regardless of protein content.

h. Commonly associated systemic lesions in advance CPN include multifocal mineralization (vessels, heart, lung) and fibrous osteodystrophy.

2. Acute hemorrhage in renal sinus

a. Acute hemorrhage in renal sinus is relatively common in sacrificed animals.

b. Rarely see hematuria in association with hemorrhage, therefore presumed to be an agonal change.

3. Hydronephrosis (pelvic dilatation)

    a. Relatively common in subchronic studies.

    b. Reported incidence of 2% in Sprague-Dawley breeding colonies.

    c. Selective breeding resulted in incidence of 33.6% in two generations.

    d. Right kidney is more commonly involved.

    e. An anatomic basis has been proposed in one strain of rats (Slonaker-Addis), but this was not confirmed by another study.

    f. Uroliths, pelvic inflammation, or pelvic mineralization may be present, but often the cause is not evident.

    g. Cortical tubular cysts commonly seen as a background lesion.

4. Microlithiasis (foci of tubular mineralization)

    a. Microscopically similar to "dehydration salts" in other species.

    b. Typically located in a band at the corticomedullary junction.

    c. More common in females.

    d. More common in animals that die.

5. Microcalculi in renal sinus

    a. Sometimes associated with hyperplasia of transitional epithelium of the renal sinus.

    b. Commonly associated with mild dilatation of the renal sinus.

6. Pigmentation of renal cortical epithelium

    a. Diffuse, particulate to amorphous, light brown cytoplasmic pigment is compatible with lipofuscin ("aging pigment").

    b. Globular, greenish brown pigment in some cases is hemosiderin.

7. Hyaline droplets

    a. Alpha-2µ-globulin.

    b. More common in males.

    c. Typically indicative of protein reabsorbed from the glomerular filtrate. Involves proximal convoluted tubule and/or pars recta, or collecting ducts.

    d. Hydrocarbon nephropathy (alpha-2µ-globulin), histiocytic sarcoma (lysozyme), Bence Jones protein, decahydronaphthalene (decalin).

8. Lipomatous foci

    a. Focal accumulation of lipocytes, typically in renal cortex.

    b. May be neoplasm or hamartoma.

9. Cystic/polycystic kidneys

    a. Congenital or acquired. Rare lesion, except in MRC/H strain.

    b. Chemically induced – diphyenylthiazole

    c. Congenital bile duct dysplasia, hepatic fibrosis and polycystic kidney (Caroli Syndrome)

10. Nephroblastoma (embryonal nephroma)

    a. Low incidence.

11. Tubular hyperplasia

    a. Simple hyperplasia. Increased number of cells, usually single layer, often basophilic, as in regenerative tubules of CPN. The “hyperplasias” of CPN usually called regenerative tubules.

b. Atypical hyperplasia. Dilated tubules two to five times normal diameter with lumens filled with clusters of tubular epithelial cells, which are somewhat pleomorphic, often with large and/or prominent nucleoli, and with cytoplasm that varies from eosinophilic to slightly basophilic. Usually recognized as a potentially preneoplastic lesion.

12. Oncocytic hyperplasia/oncocytoma

a. Tubular proliferation/mass composed of epithelial cells with abundant intensely eosinophilic granular cytoplasm. Cytoplasm contains numerous mitochondria and osmophilic bodies.

b. Arise from collecting ducts. Usually always benign.

c. Show increased incidence following administration of some compounds, but considered a distinct entity from proliferative cortical tubular lesions.

13. Tubular adenomas/adenocarcinomas

a. Consist of cellular lesions which, when small, resemble hyperplasias but
are generally larger and/or have amore complex structure. Generally are five or more times normal tubular diameters in size, and often consist of multiple, variably sized tubule-like structures or multiple solid clusters of cells separated by fine stromal bands.

b. Associated with hydrocarbon exposure.

(1) Tend to be located in the poles of the kidneys.

(2) Probably dependent on presence of alpha-2μ-globulin.

B. Urinary bladder

1. Papillary hyperplasia of urinary bladder epithelium.

a. Commonly associated with cystic calculi. Bladder may have been described as thickened at necropsy.

b. Calculi are not present in sections, therefore careful review of gross

necropsy observations is critical in diagnosis.

c. Hyperplasia typically is associated with some degree of inflammation, though often mild.

d. Nearly normal polarity and orderly maturation of epithelial cells is present in hyperplasia, in contrast to transitional cell carcinoma.

2. Lymphocytic infiltration in urinary bladder

a. Must be differentiated from mononuclear cell leukemia infiltrates in F344 rats.

b. The nonneoplastic infiltrates tend to be limited to the submucosa, whereas leukemic infiltrates are throughout the wall of the bladder.

c. Cell morphology on standard H&E sections isn't particularly helpful in differentiating between lymphocytic and leukemic infiltrates.

d. Multiple organs involvement is most important feature in distinguishing leukemia from inflammatory infiltrates.

3. Calculi

a. May be microscopic or macroscopic.

b. Usually associated with microcalculi in the renal pelvis.

4. Agonally, may see semen ejaculate in the urinary bladder or urethra. Not to be mistaken for a calculus or occlusion.

II. Respiratory System

A. Nasal cavity.

1. Incisive fissures (nasopalatine ducts) in nasal cavity.

a. Normal anatomic structure.

b. Provides direct communication between oral and nasal cavities.

c. Must be distinguished from cleft palate, particularly in teratology studies.

2. Maxillary "sinusitis"

a. Correct anatomic term is maxillary recess rather than maxillary sinus.

b. Commonly the site of mild suppurative inflammation, sometimes in association with similar inflammation in other parts of the nasal cavity.

3. Rhinitis

a. Typically involves neutrophilic infiltration, and sometimes includes erosion or ulceration of the nasal epithelium.

b. Etiology unknown.

c. Mycotic (aspergillus) rhinitis is seen sporadically.

B. Trachea

1. Mineralization of tracheal cartilage.

a. Very common as an aging change.

b. May represent normal anatomic progression with age.

C. Lung

1. Alveolar histiocytosis.

a. Multifocal aggregates of alveolar macrophages within alveoli and terminal airways.

b. May represent resolved inflammatory foci, or may represent a focal deficit in the pulmonary clearance mechanisms.

c. Sialoadenodacryoadenitis virus infection has an associated pneumonitis.

2. Mineralization of pulmonary arteries

a. Seen more commonly in F344 than in Sprague-Dawley rats.

b. Typically consists of focal or multifocal mineralization beneath the

intima or within the muscular wall of the arteries.

3. Hair shaft emboli in intravenous injection studies

4. Murine respiratory mycoplasmosis (*Mycoplasma pulmonis*)

   Synonym: Chronic respiratory disease (CRD)

   a. Used to be the most significant spontaneous lesion in most rat colonies.

   b. Principally an upper respiratory tract pathogen. Severe lung involvement is common only in advanced cases and/or aged animals.

   c. Earliest lesions in nares, larynx, and middle ear.

      (1) Epithelial hyperplasia and metaplasia.

      (2) Subepithelial lymphoid infiltration.

      (3) Purulent exudate.

   d. Lung involvement

      (1) Lymphoid hyperplasia and infiltration -- considered a characteristic lesion even in the absence of more advanced pathological changes. Note: the rat lung does normally contain some small bronchial associated lymphoid tissue.

      (2) Bronchiectasis -- large, distended airways filled with large quantities of purulent debris -- an advanced lesion.

   e. Urogenital

      (1) Mycoplasmosis also may infect the genital system, producing infertility and histologic changes in the ovaries and oviducts.

5. Eosinophilic perivascular infiltrates

      (1) Common incidental finding; unknown etiology.

6 Pulmonary neoplasms

a. Bronchiolo-alveolar adenoma/carcinoma.

b. Primary pulmonary neoplasms are rare spontaneous neoplasms in the rat.

7. New Viral Inflammatory Lesion

a. Perivascular and peribronchiolar lymphocytic infiltrates; inflammatory exudate of macrophages, neutrophils and lymphocytes; focal hyperplasia of alveolar Type 2 cells.

b. Reported in F344s in the USA and in several strains in European laboratories

c. Negative for Mycoplasma, Sendai virus, and other common murine respiratory pathogens.

d. New virus, as yet unclassified, to be called Rat Respiratory Virus (RRV)

III. Cardiovascular System

A. Heart

1. Cardiomyopathy

a. Consists of myofiber atrophy and interstitial fibrosis, most commonly beginning in the subintimal myocardium of the left ventricle.
b. More common and more severe in males than females.

2. Atrial thrombosis

a. May be so severe as to be grossly visible.

b. Typically has fibrosis and organization at the base, which continues into the atrial wall.

3. Myocardial mineralization

a. Most commonly is secondary to severe nephropathy.

4. Coronary arteriosclerosis

a. Uncommonly seen in the larger branches of the coronary arteries.

b. Resembles amyloid, but special staining attempts have been unsuccessful.

5. Endocardial hyperplasia/sarcoma (schwannoma)

a. Was called fibroelastosis.

b. Thickening and cellular proliferation in the subendocardium.

c. Infiltrates into the myocardium and expands into the ventricular lumen.

d. Size and extent of lesion will be a determining factor in whether to classify this as a hyperplasia or neoplasm.

6. Endocardiosis

a. Myxomatous thickening of the heart valves.

7. Atriocaval mesothelioma, described in F344 and S-D rats.

8. Mast cell accumulation, subepicardial in lymphatics – gee whizzer.

B. Vasculature

1. Polyangiitis (polyarteritis nodosa, panarteritis)

a. Grossly visible nodular thickening of mesenteric arteries. But, frequently may be diagnosed microscopically in absence of gross lesions.

b. Consists of fibrosis, inflammation and fibrinoid necrosis of muscular arteries in a variety of locations, including mesentery.

c. Mesenteric polyangiitis is commonly seen in sections of pancreas.

d. Commonly seen in testis and mesenteric arteries.

e. Fibrinoid necrosis of the muscular wall of arterioles, with surrounding mixed inflammatory cell infiltration.

2. Aortic mineralization

    a. Most commonly seen secondary to nephropathy.

    b. May be so severe as to be grossly noted ("brittle aorta").

3. Portal vein thrombosis

    a. Occasional incidental finding.

## IV. Gastrointestinal System

### A. Oral cavity

1. General information

    a. Dental formula: I 1/1, M 3/3.

    b. Root of upper incisor extends nearly to eye.

    c. Incisors grow continuously throughout life.

2. Broken teeth

    a. Incisors may be broken or trimmed too short by caretakers.

    b. Foreign material from feed may become impacted in pulp cavity and periodontal space.

    c. May result in endodontitis or periodontitis, sometimes with necrosis of tooth.

3. Odontodystrophy

    a. May be odontoma but, if neoplastic, does not appear to be an aggressive neoplasm.

    b. Incidence may approach 10%, though more typically is much lower.

c. Consists of a disorderly mass of tooth bud elements.

B. Salivary gland

1. Rat salivary glands

a. Submaxillary (submandibular) - mixed mucoserous.

b. Sublingual - mucous. Attached to anterior pole of submaxillary gland.

c. Parotid - primary serous. Plasmacytic infiltrates more common.

2. Acinar atrophy of salivary gland

a. Lobular atrophy of glandular cells with fibroplasia in the surrounding interstitium.

3. Focal tinctorial alteration

C. Liver

1. Hepatodiaphragmatic nodules in liver

a. Nodular protrusion on anterior surface of liver associated with small diaphragmatic hernia.

b. Microscopically consist of normal hepatic tissue, with a rounded outer contour.

c. More common in F344 rats than in S-Ds.

d. Diagnosis depends a great deal on necropsy observation, i.e., location of the nodule on the anterior surface of the liver and in association with a diaphragmatic hernia.

e. Must be carefully differentiated from true hepatic neoplasia.

2. Hepatic angiectasis (telangiectasia, peliosis hepatis)

a. Focal or multifocal sinusoidal dilatation, filled with blood.

b. Subcapsular foci may be associated with a depression in the surface

contour.

3. Cystic degeneration

    a. Consist of large vacuoles or cystic spaces between hepatocytes, variably filled with erythrocytes (resembles angiectasis), eosinophilic flocculent or fibrillar material, or eosinophilic proteinaceous fluid.

    b. May be found independently or within altered hepatocellular foci.

    c. Proliferation or hypertrophy of Ito cell (perisinusoidal fat-storing cells) may appear similar to cystic degeneration.

4. Mitotic figures and multinucleated hepatocytes

    a. Small number is normal.

    b. If elevated, may be test material induced.

5. Focal or multifocal inflammation in liver

    a. Possibly due to bacterial showering from gut.

    b. Bacteria or overt suppurative inflammation are rarely seen.

    c. May be acute, subacute or granulomatous.

6. Extramedullary hematopoiesis

    a. Common and may be normal, especially in younger rats.

    b. May reflect systemic hematopoietic demand.

7. Coagulative necrosis

    a. Sporadic small foci of coagulative necrosis are relatively common.

8. Periportal fatty change in liver

    a. Spontaneous lesion in older rats.
    b. May be more common in Sprague-Dawley than F344.

c. Must be carefully distinguished from compound-related fatty change.

9. Bile ductule hyperplasia in the liver

   a. Very common in F344 and Sprague-Dawley rats.

   b. Consist of hyperplasia and proliferation of bile ductule epithelium, sometimes surrounded by fibroplasia.

   c. The fibrotic variant has been called cholangiofibrosis. Probably is a chronic form of the purely hyperplastic lesion.

10. Paranuclear vacuoles in liver

   a. Usually subcapsular.

   b. Probably some type of artifact.

11. Torsion of liver lobes

   a. May be seen in any lobe, though the papillary process of the caudate lobe seems to be most prone to torsion.

   b. Acute torsion has hemorrhagic necrosis with variable neutrophilic infiltration.

   c. Chronic torsion has fibrosis and a variable degree of necrosis and neutrophilic infiltration. May also have nodular hepatocytic regeneration which must be distinguished from neoplasia.

12. Streamlining in liver

   a. Blood from different areas of the gastrointestinal tract are concentrated in different lobes of the liver due the phenomenon of streamlining.

   b. Hepatotoxins which are primarily absorbed from one region of the gut may cause lesions primarily in one lobe of the liver, thus it is essential to take multiple sections of liver on toxicologic pathology studies.

13. Tension lipidosis

    a. Grossly recognized as a circumscribed pale area.

    b. Usually occur at periphery of lobe near attachments to adjacent lobes or tissues.

14. Foci of cellular alteration

    a. Basophilic, eosinophilic, clear cell and mixed foci.

    b. Nomenclature based on tinctorial alteration of hepatocytic cytoplasm.

    c. Margins blend into the surrounding parenchyma.

    d. Some foci may compress adjacent parenchyma slightly.

    e. No disruption of hepatic lobular architecture.

    f. Hepatocytes within foci may be larger, smaller or same size as hepatocytes in surrounding parenchyma.

    g. Foci have been shown by special staining techniques to be metabolically different from the surrounding parenchyma. Glutathione S-transferase, placental form (GSTP) stains most foci, but should be used in conjunction with H&E staining. Other enzymatic stains include glycose-6-phosphatase, glucose-6-phosphate dehydrogenase, ATPase, and others.11

    h. Areas of cytoplasmic alteration are same as foci of cytoplasmic alteration, except for size.

15. Hepatocellular hypertrophy

    a. Occasionally a sporadic background finding, but usually a test material induced lesion.

    b. May be centrilobular, periportal or diffuse.

    c. Glycogen or lipid accumulation

d. Smooth endoplasmic reticulum increase (P450 enzyme induction).

e. Peroxisome proliferation.

16. Hepatocellular hyperplasia

a. Usually multifocal nodular lesion associated with previous or concurrent hepatic damage. Regenerative nodule.

b. Often accompanies mononuclear cell leukemia in F344. Not common in the S-D rat.

c. Spherical proliferation of hepatocytes without nuclear atypism.

d. May have cytoplasmic tinctorial variation, similar to altered foci.

e. Hepatic lobular architecture is evident but may be distorted. Portal triads often can be seen.

f. May reach a large size, i.e., several millimeters.

17. Hepatocellular adenoma

a. Nodular proliferations that are sharply demarcated by virtue of definite compression of surrounding parenchyma.

b. Usually have distinct cytoplasmic tinctorial changes.

c. Hepatic plates in adenoma are not continuous with plates of surrounding parenchyma, but impinge with them at a sharp angle.

d. Loss of normal lobular architecture.

e. May have increased mitotic rate or cellular atypism.

18. Hepatocellular carcinoma

a. Usually larger and more irregular than adenoma.

b. Compress or invade surrounding parenchyma.

c. Characterized by one or more of the following: cellular atypism, local invasion, haphazard arrangement of cells, broad sheets of cells, trabecular patterns, or glandular patterns.

19. Hepatoblastoma: Usually arise within a hepatocellular carcinoma. Rare in rats (more common in mice).

20. Cholangiocarcinoma

21. Hemangiosarcoma

a. Sinusoidal spaces lined by neoplastic endothelial cells.

b. May entrap hepatocytes.

D. Tongue

1. Papilloma

E. Pharynx

1. Papilloma

2. Squamous cell carcinoma

F. Stomach

1. General information

a. Nonglandular epithelium often is thickened at junction.

b. There often is a mild eosinophil infiltration in the submucosa beneath the junction.

2. Cystic dilatation of gastric glands

a. Consists of dilatation of the crypts of gastric glands.

b. No apparent inflammation or adverse effect is noted.

3. Gastric ulceration and erosion

a. Ulceration of the glandular or nonglandular mucosa is commonly seen in aged rats.

b. More common in gavage studies, probably due to trauma.

c. Edges of ulcers in nonglandular stomach have a variable degree of epithelial hyperplasia.

d. Erosion must be differentiated from multifocal autolysis of superficial mucosa. Inflammatory cell infiltration is critical feature of erosion.

4. Fibrosis

a. Hyaline appearing zone beneath lamina propria.

b. Old age change. Differential – amyloidosis.

G. Small intestine

1. Diverticulum

2. Adenoma (polyp)

3. Adenocarcinoma

H. Large intestine

1. Adenomatous polyp

2. Adenocarcinoma

3. Pinworms

I. Mesentery

1. Mesenteric fat necrosis

    a. Consist of nodular masses of necrotic, inflamed mesenteric fat. At necropsy is usually described as a mass in the peritoneal cavity or along the testes. This is a very common lesion

    b. Appears to be caused by torsion of mesenteric fat.

2. Mesothelioma

    a. Seen with moderate frequency, especially in F344.

    b. May consist of very small foci on surface of testis.

    c. Consist of fibrous tags covered by mesothelial cells.

    d. May see implants on serosal surface of various abdominal organs.

    e. Microscopic appearance of benign mesothelioma is similar to malignant mesothelioma. Presence of implants on abdominal organs is evidence of metastasis, therefore malignancy.

3. Hemangiosarcoma

J. Pancreas

1. Acinar atrophy of the pancreas

    a. Similar to acinar atrophy of salivary gland.
    b. Consists of atrophy of acinar elements with persistence of ducts and a variable degree of fibrosis.
    c. Islets are not affected.

2. Pancreatic vacuoles

    a. Reported to be an early manifestation of autolysis.

    b. Consists of clear vacuoles in acinar cells.

3. Hepatocytic islands in pancreas

a. Typically located around islets.

b. Consist of microscopically normal hepatocytes with Kupffer cells and bile ductules.

c. Appears to be functional liver. Toxic changes observed in the liver also are present in the hepatocytes in the pancreas.

4. Acinar cell hyperplasia

a. Focal or multifocal hyperplasia is relatively common.

b. Consists primarily of focus of tinctorial alteration.

5. Acinar cell adenoma

a. Less common than hyperplasia.

b. May be associated with corn oil gavage.

6. Tubular adenocarcinoma

7. Fatty infiltration

a. Common background finding

V. Endocrine System

A. Pancreatic islets

1. Islet cell hyperplasia or giant islets

a. Great variation in size of islets is commonly noted.

b. Probably is normal anatomic variation.

c. Background change called “hyperplasia”.

2. Islet cell adenoma/carcinoma

a. Adenomas are relatively common in laboratory rats.

b. Cell morphology is slightly altered, therefore diagnosis is based on size of lesion and evidence of compression or infiltration.

B. Pituitary

1. Pituitary cysts.

a. Very common.

b. Often have apical cilia on lining cells.

c. Probably represent cystic remnants of Rathke's pouch.

2. Focal hyperplasia of the pituitary

a. Very common.

b. Must be distinguished from adenoma.

3. Pituitary adenoma

a. Very common.

b. Incidence is 60% in males; 70% in females.

c. Chromophobe adenomas are most common.

d. Often have bizarre cellular features.

e. May cause compression of ventral aspect of brain. Incidence is lower in rats on low protein diet.

f. 81/83 stained for prolactin. 14/83 also stained for growth hormone.

g. Ovariectomy significantly decreases incidence of pituitary adenomas in female rats.

3. Pituicytoma

a. Occurs in the pars nervosa

b. Is an astrocytic tumor

C. Adrenal gland

1. Cortical vacuolation

a. Very common.

b. Focal/multifocal aggregations of adrenocortical cells which have clear cytoplasmic vacuoles.

2. Cystic degeneration, cortical

a. May be part of a continuum from cellular vacuolation to degeneration to large clear- or blood-filled spaces.

b. Focal to multifocal areas of degenerative, vacuolated cortical cells being replaced by larger clear-filled or blood-filled spaces.

c. More common in females.

d. Severely affected glands may grossly appear swollen, soft, dark red, or mass-like.

3. Angiectasis

a. Very common.

b. More common in cortex, but may also be in medulla.

c. Dilatation or coalescence of vascular spaces.

d. May be sequelae of cystic degeneration.

e. Combinations of cortical vacuolation, angiectasis, and cystic degeneration are very common. Consistency of diagnostic criteria and terminology is critical for purposes of tabulating these changes.

4. Extramedullary hematopoiesis

    a. Focal or multifocal aggregates of hematopoietic cells.

5. Ectopic adrenocortical tissue

    a. Most often present outside adrenal capsule. May be seen in kidney.

6. Adrenal medullary and cortical mixing

    a. Common to see clusters of cortical cells within the medulla.

    b. Depending on section, medullary tissue may extend out to the capsule. This must be differentiated from medullary hyperplasia.

7. Hyperplasia, cortical

    a. Distinct cortical foci, usually within the zona fasciculata and zona reticularis. Focal or multifocal.

    b. Cells may be variably sized, but are well-differentiated. May be vacuolated.

    c. May be associated with aforementioned degenerative changes.

    d. Common lesion, but not clear progression to neoplasm.

    e. Some call these foci of cellular alteration.

8. Hypertrophy, cortical

    a. Focal areas of enlarged cortical cells, may be vacuolated.

    b. No known progression to hyperplasia or neoplasia.

9. Adenoma, cortical

    a. Well-differentiated, but clearly expansile and compressive.

10. Carcinoma, cortical

a. Frequently metastasize.

11. Hyperplasia, medullary

a. Must be distinguished from pheochromocytoma.

b. Criteria for differentiation based on compression and size. Consistency within a study is important.

c. More common in males.

d. Basophilic clusters of medullary cells.

12. Pheochromocytoma

a. Common in aged rats, particularly males.

13. Ganglioneuroma of adrenal medulla (Complex Pheochromocytoma)

a. Have well differentiated ganglion cells surrounded by well differentiated neuropil.

b. Usually (always?) associated with a pheochromocytoma.

c. Not common, 28 in 60048 F344 rats from NTP program.

D. Thyroid

1. Ultimobranchial cysts or remnants

a. Cyst lined by squamous epithelial cells, filled with keratin and cellular debris.

b. May be very small, follicle-sized remnants with no cystic dilatation.

c. Most commonly seen in the central area of the thyroid.

2. Thyroid colloid concretions

a. Basophilic globules in colloid.

3. C-cell hyperplasia

4. C-cell adenoma

    a. C-cell masses greater than 5 average follicular diameters are classified as adenomas.

5. C-cell carcinoma

    a. Criteria for malignancy may include size, invasion, cellular pleomorphism, etc.

    b. May metastasize to lung.

6. Hyperplasia, follicular

7. Adenoma/carcinoma, follicular

E. Parathyroid

1. Fibrosis, parathyroid

    a. Thickening of fibrous trabeculae.

2. Ectopic parathyroid

    a. Common in mediastinum.

3. Hyperplasia

    a. Commonly secondary to spontaneous nephropathy.

    b. Usually diffuse within the gland.

4. Adenoma

    a. Uncommon.

    b. Distinguish from hyperplasia by unilateral distribution and remnants of

normal parathyroid tissue around the adenoma.

VI. Reproductive System and Mammary Gland

A. Testis

1. Normal rete testis

2. Squeeze artifact

3. Hypospermatogenesis or tubular atrophy

a. May occur as a primary (idiopathic) change or secondary to polyangiitis. Also seen near interstitial cell tumors.

4. Giant cell degeneration

a. Multinucleated giant cells in seminiferous tubules.

b. Often not associated with tubular atrophy, thus appears to be a distinct pathologic process.

5. Mineralization

a. May affect seminiferous tubules or blood vessels.

b. Appears grossly as white fibers visible from surface of testis.

6. Sperm granuloma

a. Tubule distended by a mass of spermatozoa.

b. Usually no associated inflammation.

c. May result in secondary tubular atrophy or hypospermatogenesis.

7. Interstitial cell hyperplasia

a. Very common in F344, where it is a precursor to interstitial cell tumor.

b. Difficult to establish criteria for differentiation between small interstitial cell tumors and large focal hyperplasia. Cellular morphology

is similar in both lesions.

c. In USA, hyperplasia is no greater than one seminiferous tubule diameter. In Europe, hyperplasia may be up to three (3) diameters.

8. Interstitial cell tumor (ICT)

a. Up to 95% incidence in 24-month-old F344 rats. Much lower incidence in Sprague-Dawley rats.

b. White to yellow foci visible through the tunica vaginalis of the testis.

c. Microscopically similar to ICT in other species.

9. Sertoli cell tumor

a. Rare, but have been reported in the rat testis.

10. Seminoma

B. Prostate

1. Normal prostate

a. Ventral prostate

b. Dorsolateral prostate

c. Ampullary gland

2. Coagulating gland (anterior prostate)

3. Senile atrophy and concretions

a. Epithelium is cuboidal or squamoid rather than columnar.

b. Lumina contain small, deeply basophilic concretions which are microscopically similar to the "corpora amylacia" of other species.

4. Chronic prostatitis

a. Very common in old rats.

b. Primarily an interstitial process, though some lumina may be filled with inflammatory cells and cellular debris.

5. Epithelial hyperplasia.

a. Seen with moderate frequency.

b. Focal or multifocal proliferation of epithelial cells, sometimes filling glandular lumen.

c. Physiologic versus atypical hyperplasia

6. Prostatic neoplasms

a. Uncommon as spontaneous entity in laboratory rats.

b. Spontaneous neoplasms occur more frequently in the ventral prostate, whereas chemically-induced neoplasms occur more frequently in the dorsal and lateral prostate.

c. Thus, consistent sectioning of the prostate is very important, and should include dorsal, lateral and ventral prostate.

C. Epididymis

1. Hypospermia

2. Atrophy

3. Sperm granuloma

D. Seminal vesicles

1. Inflammation

2. Glandular hyperplasia

E. Preputial and clitoral gland

1. General information

a. Are large organs in the rat, e.g. 10 mm long x 5 mm wide.

b. Consist of a large central duct surrounded by modified sebaceous cells.

c. Located on either side of prepuce or vulva.

2. Adenomas may be acinar or squamous

3. Preputial/clitoral gland adenocarcinomas

a. Occur in a low incidence as spontaneous entity.

b. A few are malignant, and metastasize to the lungs.

c. Must be distinguished from inguinal mammary gland neoplasms.

d. Most characteristic microscopic feature of acinar preputial/clitoral gland neoplasms is brightly eosinophilic cytoplasmic granules.

4. Duct ectasia

a. Consists of dilatation of the central lumen, sometimes with impacted secretory material. May be seen grossly as a discolored focus in the center of the gland.

b. Inflammation is variable.

F. Ovary

1. Atrophy

a. Present in virtually all rats at terminal sacrifice on chronic study.

b. Consists of reduction in number of follicles and corpora lutea with a variable amount of intracellular pigment in stromal cells.

2. Hyperplasia, interstitial gland (stromal)

a. Frequently in combination with atrophy will see hyperplasia of the interstitial glands.

3. Cysts

a. Parovarian cysts consist of dilatation of the ovarian bursa. May be difficult to detect on microscopic examination.

b. Intraovarian cysts. Exact genesis unknown. Are present in the ovarian parenchyma, therefore are microscopically distinct from parovarian cysts.

4. Papilloma of ovarian rete

a. Uncommon, but not rare.

b. Seen in the hilar region of the ovary.

c. Typical papilloma contained with a dilated tubular structure.

5. Teratoma

a. Uncommon, but not rare.

b. All three germ layers present. Epithelium and neural tissue are usually most prominent. The mesodermal element may be muscle, fat or other elements

6. Granulosa/theca cell tumors

a. Occur in low incidence.

b. Microscopically similar to other species.

7. Cystadenomas/adenocarcinomas

a. Uncommon.

8. Dysgerminoma

a. Not reported in the rat.

G. Uterus

1. Wall of uterus and cervix commonly has eosinophil infiltration

2. Mild dilatation is common in young rats

3. Cystic endometrial hyperplasia

   a. Not as common in rat as in mouse.

   b. In the rat is primarily cyst formation. The rat has less epithelial proliferation than the mouse.

4. Endometrial fibrosis

   a. May be normal for old rats.

5. Adenomyosis

6. Endometrial stromal polyp

   a. Very common in old rats.

   b. Consists of a polypoid mass of endometrial stroma and capillaries covered by epithelium.

   c. May be infarcted by torsion or other disruption of the stalk.

7. Endometrial stromal sarcoma

   a. The malignant counterpart of endometrial stroma polyp.

   b. Some degree of invasiveness is an important criterion in diagnosis.

8. Cystic dilatation of vaginal fornix

   a. Gross observation of mass near body of uterus or in cervix.

b. Lined by stratified squamous epithelium and filled with keratinaceous debris. May contain a neutrophilic infiltration, to the degree that they resemble abscess.

9. Granular cell lesions of the cervix and vagina

a. Usually within muscular wall or adventitial surface of cervix or vagina. Often not grossly visible.

b. Granular cells usually considered to be of Schwann cell origin.

c. Immunohistochemistry:

1) Positive for S100 protein, neuron-specific enolase (NSE), and Leu-7.

2) Negative for smooth muscle-specific actin.

d. Granular cell aggregates: lesions do not appear to be space-occupying and do not efface normal tissue architecture. Collagen is typically absent in the lesion. Scattered individual granular cells may be seen normally in these tissues or associated with regional nerves and ganglia.

e. Benign granular cell tumor: Space-occupying and expansile. Composed of typical granular cells and variable amounts of interstitial collagen.

f. Malignant granular cell tumors: Spindle cells with decreased granularity, pleomorphism and other features of malignancy. Metastases not reported.

H. Mammary gland

1. Sexual dimorphism

a. Mammary glands of males and females are microscopically similar at birth.

b. By 19 weeks, males and females are markedly different:

(1) Females have widely scattered tubuloacinar units.

(2) Males have more prominent glandular tissue, but not organized

into ductular units.

2. Senescent atrophy

    a. Decrease in glandular elements with intraluminal concretions and accumulations of pigment-laden cells.

3. Duct dilatation

    a. Moderately common in aged females.

    b. Cystic mammary ducts often called galactoceles.

4. Lobular hyperplasia

    a. Enlarged lobules with relatively normal appearing alveoli.

    b. Cystic alveoli and dilated ducts may be present.

    c. Lack a prominent collagenous stroma, which early fibroadenomas have.

5. Fibroadenoma

    a. Very common neoplasms in aged females, with a lower incidence in aged males.

    b. May be multiple, and may be nearly as large as the rat.

    c. Rats have mammary tissue on nearly the entire body, with the exception of the head, tail and distal extremities. Any subcutaneous neoplasm on the trunk of a rat may be a mammary neoplasm.

    d. Consist of epithelial elements surrounded by proliferating fibrous connective tissue.

    e. Some neoplasms consist almost entirely of fibrous connective tissue.

    f. Ovariectomy significantly decreases the incidence of mammary fibroadenomas in female rats.

6. Adenoma

7. Adenocarcinoma

VII. Lymphoreticular and Hematopoietic Systems

A. Spleen

1. Increased hemosiderin

a. Rat spleen normally has a moderate amount of hemosiderin. More in females than males.

b. May be markedly increased in some animals, due to diverse causes.

c. Difficult to establish criteria for distinguishing between normal and excessive hemosiderin.

2. Increased extramedullary hematopoiesis

a. Rat spleen normally has a small amount of EMH.

b. Chronic disease processes, e.g. neoplasms, commonly are associated with an increase in the level of splenic EMH.

c. Difficult to establish criteria for distinguishing between normal and excessive EMH.

3. Mesothelial cyst

a. Not uncommon.

b. Usually disrupted in processing, with only fibrous tags remaining on surface of spleen.

B. Lymph nodes

1. Cystic degeneration

a. Consists of cystic dilatation of sinusoids.

b. Often so prominent as to be noted at necropsy.

2. Plasmacytosis

a. Commonly seen in mandibular lymph nodes.

b. May be so prominent as to be noted grossly.

c. Medullary region consists of sheets of mature plasma cells.

d. Etiology and significance unknown. Seem to be more prominent in association with oral cavity lesions.

3. Senescent atrophy

a. Nearly ubiquitous in chronic studies.

b. Consist of reduction in lymphoid follicles with a moderate amount of intracellular pigment in medullary area.

4. Mast cells

a. Rats have numerous mast cells in many organs.

b. Are very obvious when in lymph nodes.

5. Mesenteric lymph node hemangioma

a. Uncommon, but not rare in mesenteric lymph nodes.

b. Rare in other lymph nodes.

C. Thymus

1. Epithelial remnants

a. Epithelial tubules or nests in medullary region of thymus of old rats.

b. Occasionally see neoplasms of these epithelial elements.

2. Thymic cysts

a. May be lined by squamoid cells or tall columnar cells with cilia.

3. Ectopic thymus in thyroid

a. Relatively common within or adjacent to the thyroid.

4. Atrophy/involution

a. Thymus often difficult to identify at necropsy in aged animals.

5. Thymoma

a. Consist of a mixture of "epithelial" and lymphoid elements, similar to other species.

b. May have epithelial tubules.

c. Often have a moderate population of eosinophils.

D. Bone Marrow

1. Fibrosis (myelofibrosis)

a. Usually a focal lesion. Occurs sporadically and spontaneously in aged rats.

b. Must be differentiated from fibrosis associated with fibrous osteodystrophy.

E. Systemic

1. Large Granular Lymphocyte (LGL) Lymphoma/Leukemia

a. Formerly called mononuclear cell leukemia (MCL).

b. Very common neoplasm in F344 rat. Rare, but occurs in Sprague-Dawley. Reported in Wistar and Wistar-Furth.

c. Splenic architecture is effaced by sheets of individualized neoplastic

cells.

d. Cell morphology is not specific on H&E sections.

e. Can involve virtually any organ in the body.

f. Early leukemic infiltrates are in the spleen and liver. Are more easily detected in the liver.

g. 30-50% of early deaths in 2-year F344 study are due to this disease.

h. Death apparently due to hemolytic anemia. Also thrombocytopenic.

i. Bone marrow involvement results in reduced bone formation.

j. Large granule lymphocytes are NK (natural killer) cells. Constitute 1-5% of normal rat peripheral leukocyte count.

k. Positive for OX-8 cell surface antigen.

l. Large granule lymphocytic leukemia has been reported in man and a cat.

2. Granulocytic leukemia (myelogenous leukemia)

a. Uncommon, incidence < 5%.

b. Distinct myelogenous differentiation of neoplastic cells. Granulocytic differentiation more common than erythroid or basophilic.

c. Cells immunocytochemically positive for lysozyme.

d. May be induced by ionizing radiation or some chemical agents.

3. Lymphoma (lymphosarcoma)

a. May be classified as follicular center cell, immunoblastic, plasma cell, or lymphoblastic, but usually not typed in routine carcinogenicity studies. Most are B cell origin.

b. Slight higher incidence than LGL or granulocytic leukemia,

approximately .5-.9% in our laboratory.

4. Histiocytic sarcoma

    a. May involve skin, subcutis, peritoneum, multiple organs.

    b. May be more fibrous in some tissues. Metastases to lung common.

    c. Immunocytochemically positive for lysozyme, vimentin, and ED-1.

    d. Secondary myeloid hyperplasia in the spleen common. Also hyaline droplets (lysozyme) common in the kidney.

VIII. Sense Organs

A. Eyes and Harderian glands

1. Cataracts and retinal degeneration

    a. Common in older rats.

    b. Retinal degeneration may be due to excessive light in animal rooms.

    c. Careful cage rotation schedule is necessary to prevent excessive incidence in one group.

    d. Microscopically similar to other species.

    e. Retinal degeneration often secondary to cataract, but can occur independently.

2. Phthisis bulbi

    a. Rupture of the eye due to trauma from cage wire or retrobulbar bleeding.

3. Scleral osseous metaplasia

    a. Common, more in F344 than in SD.

    b. Linear or ovoid foci of mineralization or ossification in the middle

layers of the sclera, most commonly in the middle or posterior thirds of the eyeball.

4. Keratitis

    a. Common in rats kept in wire cages and in gang-caged rats.

5. Corneal dystrophy/mineralization

    a. Common in F344.

6. Porphyrin accumulation in Harderian gland

    a. Cause of "red tears" or colored tears.

    b. Nonspecific change, indicating a sick rat.

7. Exorbital lacrimal gland

    a. Cellular pleomorphism is normal.

    b. Often get differentiation of Harderian gland type acini.

IX. Central Nervous System

A. Brain

1. Vacuoles and mucocytes

    a. Vacuoles commonly seen in otherwise normal brains.

    b. Mucocytes are vacuoles that contain amorphous gray material.

    c. Etiology and significance unknown. Probably both are artifacts.

    d. Mucocytes are reported to be more common in brains that are immersed in alcohol.

2. Lipofuscin pigmentation of neurons

    a. Microscopically similar to other species.

3. Compression of the brain stem

    a. Results from expansion of pituitary adenoma.

4. Astrocytoma

    a. Astrocytomas are fairly common in brain, and also occur in spinal cord. In rats, astrocytomas generally stain negatively for GFAP; thus, perhaps should be more correctly diagnosed as gliomas.

5. Oligodendroglioma

6. Granular cell tumor

    a. Usually in the superficial cortex, near the meninges.

    b. Distinctly bounded, as opposed to gliomas.

    c. Distinctly granular cytoplasm in polygonal neoplastic cells. Granularity is enhanced by PAS stain.

B. Spinal cord

1. Degenerative myelopathy

    a. Posterior paresis or ataxia.

    b. Lesions in the spinal cord and nerve roots, principally the ventral and lateral white tracts from $T_4$ - $L_4$ region and the ventral spinal roots from mid-thoracic area posteriorly.

    c. May occasionally see degenerative changes in the sciatic nerve.

    d. Characterized by demyelination, distended axon sheaths, swollen or absent axons, lipid filled macrophages, and reactive glial cells.

2. Chordoma

    a. Neoplasm derived from primitive notochord elements.

b. Characterized by large vacuolated "physaliphorous" cells.

X. Musculoskeletal System

A. Skeletal muscle

1. Atrophy of skeletal muscle

a. Relatively common, often secondary to degenerative myelopathy.

b. Reduction in cross-sectional area of myofibers, with apparent or real increase in endomysial connective tissue.

B. Bone

1. Fibrous osteodystrophy

a. Common in association with spontaneous nephropathy.

2. Degeneration of sternebral cartilage

a. Degenerative cleft formation in intersternebral cartilage is commonly seen.

b. Similar changes are reported in vertebra, but these bones are not as commonly sectioned in chronic studies.

3. Hyperostosis (osteopetrosis, osteosclerosis, trabecular hypertrophy)

a. Grossly, bones may be very hard and white.

b. Generalized increase in bone matrix.

c. More frequent in F344s, particularly old females.

4. Subarticular bone cyst

a. Normal finding. Seen subjacent to attachment of cruciate ligaments in the distal femur and proximal tibia.

XI. Integumentary System

A. Skin

1. Tarsal granulomas

a. Common in rats that are kept in wire cages, and especially in heavier animals. So, more common in males.

b. May be very large.

2. Keratoacanthoma

a. Common.

b. Must be differentiated from squamous papillomas and epidermal inclusion cysts.

3. Amelanotic melanoma (neural crest tumor)

a. Probably more common than incidence summaries show.

b. Solid sheets of spindled and interlacing cells, usually diagnosed as a neurofibroma/sarcoma or fibroma/sarcoma.

c. Frequently seen on the pinna of the ear or eyelids.

d. Electron microscopy shows characteristic premelanosomes.

4. Epidermal inclusion cyst

a. Very common, particularly on tail.

b. Consist of a cystic space filled with keratinaceous debris and lined by maturing stratified squamous epithelial cells.

c. Some may have a neck-like connection to the surface.

5. Chronic inflammation, tail

a. Focal to multifocal crusty lesions.

b. Chronic inflammation with acanthosis and hyperkeratosis.

c. Some lesions may be epidermal inclusion cysts or keratoacanthomas.

6. Auricular chondritis

a. Distortion and mineralization of the auricular cartilage.
b. Possibly associated with trauma.

c. Reported to be a heritable collagen disease in some strains.

B. Zymbal's gland

1. Large gland that lies around the external ear canal.

2. Very susceptible to carcinogenic influence.

3. Carcinomas may be very large. May have either sebaceous and squamous elements, or either element alone.

4. Rarely invade into the brain, presumably along cranial nerve tracts.

THE END

# DISEASES AND NEOPLASMS OF THE AGING RAT

JAMES B. NOLD, DVM, PHD, DIPLOMATE, ACVP
PATHOLOGY OF LABORATORY ANIMALS COURSE - AUGUST 2005

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DOCUMENT END

# DISEASES OF FISH

August, 2005

Robert B. Moeller Jr., DVM

California Animal Health and Food Safety Laboratory System

University of California

18830 Road 112

Tulare, California 93274

559-688-7543

rbmoeller@ucdavis.edu

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## BIOLOGY OF FISH

Fish have some unique anatomical and physical characteristics that are different from mammals; however, they still possess the same organ systems that are present in other animals. All fish are poikilothermic and must be able to adapt to changes in water temperature. Fish live in a variety of temperatures ranging from less than 0°C to hot geothermal springs. Yet, each species of fish must live in its particular specific temperature range. Abrupt temperature changes in the water can be lethal to fish.

Organ systems of fish vary to some extent from that of mammals due to the aquatic environment they live in. The following are some of the important differences.

### INTEGUMENT

Fish do not have a keratin layer over the epidermis. A cuticle composed of mucus, mucopolysaccharides, immunoglobulins and free fatty acids covers these animals. The epidermis is composed of a stratified squamous epithelium of variable thickness (4-20 cells thick). The outermost epidermal cells (Malpighian cell layer) retain the capacity to divide. Other cells present in the epidermis are goblet cells (responsible for secreting the cuticle), large eosinophilic club cells or alarm cells (present in most species of fish), eosinophilic granular cells (unknown function), leukocytes and macrophages.

The dermis is composed of an upper stratum spongiosum and a deeper stratum compactum. Numerous melanophores, xanthophores, and iridophores (give fish their silvery color) are observed scattered throughout the dermis. Scales are calcified plates originating in the dermis and covered by the epidermis. There are two types of scales: ctenoid scales and cycloid scales. Ctenoid scales of elasmobranchs have spicules extending from the external surface giving these fish a rough sandpaper-like texture. Cycloid scales of teleost fish have a smooth outer surface and are laid down in concentric a ring that makes them useful in determining the age of some fish. Scales also represent a source of calcium for fish; some fish will utilize the calcium in the scales in preference to the calcium in their skeleton during times of starvation or prespawning activity.

### RESPIRATORY

The gills consist of four holobranchs that form the sides of the pharynx. Each holobranch has two hemibranchs projecting from the gill arch. The hemibranch are composed of rows of long thin filament called primary lamella. The primary lamellas have their surface area increased further by the secondary lamella that are semilunar folds over the dorsal and ventral surface. Gas exchange takes place at the level of the secondary lamella. Epithelial cells bounded by pillar cells line the secondary lamella. A thin endothelial lined vascular channel lies between the pillar cells and is the site of gas exchange, removal of nitrogenous waste and some electrolyte exchange.

The pseudobranch lies under the dorsal operculum. This organ is a gill arch with a single row of filaments. The function of the pseudobranch is unknown, however it is believed that this structure supplies highly oxygenated blood to the optic choroid and retina and may have thermoregulation and baroreceptor functions.

## ENDOCRINE SYSTEM

### Adrenal Gland

There is no true adrenal gland present in most fish (exception is sculpins). The adrenal cortical tissue in most fish is represented by the interrenal cells. These cells are pale eosinophilic cuboidal cells associated with major blood vessels in the anterior kidney. Both glucocorticoid and mineralocorticoid are secreted.

The adrenal medullary cells (chromaffin cells) may vary in location. These cells are usually found with the sympathetic ganglia in clumps between the anterior kidney and spine or in the interrenal tissue.

### Thyroid Gland

The thyroid follicles are very similar to mammalian thyroid tissue. Thyroid follicles are distributed throughout the connective tissue of the pharyngeal area and may be observed around the eye, ventral aorta, hepatic veins and anterior kidney. It is important to realize that thyroid tissue can be widely distributed. Many times pathologist have erroneously considered this distribution of normal thyroid tissue to represent metastasis from a thyroid follicular cell tumor.

### Endocrine Pancreas

The endocrine pancreas is present in most fish as islet of Langerhans and is associated with the exocrine pancreas. In some species the islets are very large and may be grossly visible (Brockman bodies). During the spawning season the size and number of islet will increase in some fish. These should not be confused with an adenoma.

### Parathyroid Glands

The parathyroid glands are absent in fish; their function is taken over by other endocrine organs. (Corpuscles of Stannius)

### Ultimobranchial Gland

This gland lies ventral to the esophagus in the transverse septum separating the heart from the abdominal cavity. This organ secretes calcitonin (lowers serum calcium levels) that acts with hypocalcin (secreted by the corpuscles of Stannius) to regulate calcium metabolism.

**Corpuscles of Stannius**

These are islands of eosinophilic granular cells located in paired organs on the ventral surface of the kidney. This organ secretes a protein called hypocalcin (teleocalcin) that acts with calcitonin to regulate calcium metabolism.

**Urophysis**

This is a neurosecretory organ found on the ventral aspect of the distal end of the spinal cord. These bodies are composed of unmyelinated axons terminating on a capillary wall. The function of the urophysis is unknown.

**Pineal Gland**

The pineal gland is a light sensitive neuroendocrine structure that lies in the anterior brain and is a well-vascularized organ. This gland secretes melatonin that may play a role in controlling reproduction, growth, and migration.

## DIGESTIVE SYSTEM

The digestive system of fish is similar to the digestive tract of other animals. Carnivorous fish have short digestive tracts when compared to herbivorous fish. The stomach and intestines contain submucosal eosinophilic granular cells. The function of these cells is unknown. Some species of fish (Salmonids) have pyloric ceca, which are occasionally confused with parasites. These ceca secrete the digestive enzymes required to digest some food. Fish without the pyloric ceca have digestive enzyme production in the liver and pancreas. It is not possible to divide the intestine into large and small intestine.

The liver does not have the typical lobular architecture that is present in mammals. In many species of fish there are areas of exocrine pancreas (hepatopancreas) that are present near the small veins off the hepatic portal vein.

The pancreas is scattered in the mesentery, primarily near the pylorus.

## RETICULOENDOTHELIAL SYSTEM

Fish do not have lymph nodes. Phagocytic cells are present in the endothelial lining of the atrium of the heart and in the gill lamella. There are no phagocytic cells (Kupffer cells) in the liver. Melanomacrophage centers are present in the liver, kidney and spleen. Melanomacrophage centers increase in number during disease or stress.

The fish thymus is the central lymphoid organ. This organ is located subcutaneously in the dorsal commissure of the operculum.

Fish have the ability to produce specific immunoglobulins (IgM

only) and have both delayed and immediate hypersensitivity. Fish have the ability to produce virus neutralizing, agglutinating, and precipitating antibodies. Both B and T lymphocytes are present.

## CARDIOVASCULAR SYSTEM

The heart is composed of two chambers, one ventricle and one atrium. Some authors also describe the sinus venosus as the third chamber and bulbus arteriosus as the fourth chamber. Blood flows from the heart through the ventral aorta and the afferent branchial arteries, to the gills for oxygenation. Oxygenated blood returns via the efferent arteries to the dorsal aorta. The dorsal aorta then carries the oxygenated blood to the body. Some oxygenated blood also leaves the dorsal aorta and goes to the pseudobranch to be highly oxygenated and then is sent to the retina which has a high oxygen demand.

## URINARY SYSTEM

The kidneys of fish develop from the pronephros and mesonephros. The function of the kidney is osmoregulation. In freshwater fish, the kidney saves ions and excretes water. In saltwater fish, the kidney excretes ions and conserves water. The majority of nitrogenous waste is excreted through the gills. The other function of the kidney is hematopoiesis with hematopoietic tissue located in the interstitium of the kidney. This function is primarily in the anterior kidney but can be found throughout the entire kidney.

## SPECIAL SENSE ORGANS

### Lateral line system

There are two types of lateral line organs. These are the superficial neuromast and the two lateral line canal organs. There are two types of superficial neuromast; these are located in pits in the epidermis located primarily on the head. Their function is not completely known but is believed to aid in movement and orientation.

The second lateral line organ is the lateral line canal system that runs the entire length of the fish with continuous extensions over the head. This organ is sensitive to hydrostatic stimuli and sound.

## Necropsy and Biopsy Procedures

Fish, like other vertebrates, have a complex nervous system and when handled, can experience stress. When performing most diagnostic procedures, fish should be anesthetized before handling. If a fish is to be submitted for pathology, euthanasia should be done prior to placing the animal into 10% formalin or other fixative. It would be considered inhumane to place a fish in 10% neural buffered formalin or other fixative without euthanasia.

The 2000 AVMA Report of the AVMA Panel on Euthanasia states that acceptable anesthetics to be used for the euthanasia of fish are tricaine methane sulfonate (TMS, or MS222), benzocaine hydrochloride and barbiturates (Sodium pentobarbital 60 to 100 mg/kg). A conditionally acceptable method for euthanasia is a blow to the head followed by decapitation or pithing. Other commonly used methods are carbon dioxide (four alka-selzer tablets to 500 ml of water), electrocution. Hypothermia is not a recommended method for euthanasia. Other anesthetic agents are available for immobilizing fish for euthanasia; an excellent review of these agents and their mechanism of action can be found in Stoskopf's book, Fish Medicine.

The preferred method of euthanasia would be to anesthetize the fish to a deep plane of anesthesia (stage III or stage IV) and then sever the spinal cord just caudal to the brain. The most common and practical way to anesthetize a fish is to place the anesthetic agent in water. The fish will go through all four stages of anesthesia prior to death. The four stages of anesthesia and the clinical presentation of each are as follows:

Stage I; Induction and light sedation: The fish goes through an excitement phase with erratic swimming followed by reduced activity. The respiratory rate increases and there is a loss of some response to tactile stimulation.

STAGE II; Sedation: Fish swim slowly; have decreased gill movement (respiration), and a loss of equilibrium.

STAGE III; Anesthesia: Fish have a complete loss of equilibrium and are unable to swim. Gill movement (respiration) becomes very slow. The fish is unresponsive to external stimuli.

Stage IV; Anesthetic overdose: The fish has a total loss of gill movement and the opercules become distended. The fish goes into cardiac arrest.

## Collecting Samples for Bacteria and Fungi

Sampling for bacteria and fungi should be done on fish that are brought in for examination alive or from fresh fish that had died only recently (usually less than 6 hours).

Ideally, the fish is alive when sampling cutaneous lesions. The area to be cultured should not be handled prior to culturing. Like cutaneous smears, large ulcerated areas should be avoided and small developing lesions cultured. The sterile loop or culturette should be

rubbed into the lesion.

As with cutaneous lesions, gills from live fish are the best to culture. Gills are cultured by gently rubbing and rolling the sterile culturette through the gill arches. The culturette should pick up abundant mucus on the tip of the culturette. Since the gills and cutaneous lesions are exposed to the aqueous environment, a mixed bacterial culture should be expected. If a pure culture of potential pathologic bacteria is obtained, this should be considered a possible cause of disease in this fish.

The kidney is one of the most important internal organs to culture. There are two methods of culturing the kidney. The first method is to cut the dorsal fin off, sterilize the open area with heat, cut the vertebra with a sterile scissors or scalpel and snap the fish by bringing the head and tail together. This exposes the kidney for culturing. The second method, can allow for possible contamination of internal organs. Here the fish is dipped into 70% alcohol and the abdominal cavity opened aseptically to allow all organs to be exposed. Sterilize with heat, the desired internal organs to be cultured, cut open the organ with a sterile scalpel and culture.

Another method for culturing, particularly in cases where a bacterial septicemia is suspected, is to examine heart blood. Collection of heart blood from the atrium is most productive since the atrium contains phagocytic cells that assist in clearing bacteria from the blood.

**Biopsy Procedures**

For biopsy specimens and bacterial cultures, a fish does not need to be killed. The fish should be anesthetized prior to performing most clinical examinations and biopsies. Biopsy procedures on fish usually are cutaneous smears, fin biopsies and gill biopsies.

Cutaneous smears are done primarily for ectoparasites. Large ulcerated lesions should be avoided; Try to find smaller developing lesions for sampling. Prior to performing cutaneous smears, bacterial cultures should be taken. The procedure for cutaneous smears involves passing several clean microscope slides over the area of interest. Only light pressure on the glass slide needs to be used to remove some epidermis and mucus. On one slide, a drop of water is placed on the smear and a cover slip is placed on the slide for examination. The other slides should be air-dried or fixed in alcohol. These are stained with either new methyl blue stain or Diff-Quick stain.

A fin biopsy is accomplished by spreading out the fin and a triangular wedge shaped piece of tissue is cut between the rays of the fin. Place the fin biopsy on a slide with water and cover slip for examination.

A gill biopsy is performed by cutting a few tips of the primary lamella with the blades of the scissors. Place the lamellar tips on the slide with a drop of water, cover slip and examine. Both fin and gill biopsies should not cause undue harm to a fish.

## Necropsy Procedure

Ideally, the fish should be submitted alive for the post mortem examination. This gives the pathologist a chance to observe the fish prior to euthanasia and note any important clinical signs. Unfortunately, some situations do not allow the pathologist to evaluate the fish while they are alive. Fish should be dead less than 6 hours. Fish found floating in a tank longer than 6 hours are poor candidates for necropsying due to post mortem autolysis. Dead fish should be wrapped in paper or gauze and refrigerated. Do not freeze the fish.

Prior to performing the necropsy, insure that all necropsy tools, sterile loop or culturettes, glass slides for impression smears, 70% alcohol and 10% neutral buffered formalin or Bouin's solution (I prefer Formalin) are available. A systematic approach should be used when performing the necropsy. Evaluate the external surface and note the general body condition of the fish, identify and note lesions on the skin, fins, eyes, oral cavity and anus. Take cultures of the desired lesions.

After completion of the external examination, place the fish in lateral recumbency on a disposable towel. Remove the eyes and then the operculum with the pseudobranch; place these in formalin. Remove the second and third gill arches being careful not to crush the primary lamella. Take several primary lamella from one of the gill arches and place on the glass slide for parasitic examination. Place the remaining gills into the fixation solution.

Using aseptic techniques, open the abdominal cavity by cutting through the pectoral girdle to the spine and follow the abdominal cavity to the anus, extend this cut along the ventral midline from the gills to the anus and remove the body wall. Remove the body organs (heart, liver, intestines, spleen, gonads and swim bladder) for examination. When submitting the swim bladder for histopathology, insure that the red gas-forming organ is present. Sample both the anterior and posterior kidney in fish with both kidneys. In fish with fused kidneys, insure that anterior and posterior sections are submitted for examination.

Remove the brain by opening the skull just dorsal to the eyes and removing the bones over the brain. Sample all cutaneous lesions for histopathology. Insure that normal tissue from the margin of the lesion are submitted with the lesions. Cut into the skeletal muscle and look for parasitic cysts. Finally, open the stomach and intestine and examine food material.

If toxicology is desired, be sure to submit gills, kidney, liver, skeletal muscle, and fat. Toxicologic samples should be immediately frozen and stored in at -70 degrees C. Analysis of the tissue should occur as soon as possible after collection.

## Viral Diseases

1) **Lymphocystis Disease**

A) Iridovirus

B) Observed in most freshwater and saltwater species.

C) Clinically, fish are presented with variably sized white to yellow cauliflower-like growths on the skin, fins, and occasionally gills. Lymphocystis virus may go systemic with white nodules on the serosal surfaces of the mesentery and peritoneum.

D) Histopathology: Fibroblast undergoes cytomegaly with many basophilic cytoplasmic inclusion bodies and a thick outer hyalin capsule. The inflammatory response is variable but is usually a chronic lymphocytic inflammatory infiltrate.

E) The disease gains entry through epidermal abrasions. The virus infects dermal fibroblasts.

F) The disease is self-limiting and refractory to treatment. Nodules may last several months and cause infected fish to be susceptible to secondary bacterial infections. Reinfection can occur.

2) ***Herpesvirus salmonis*** **(Herpesvirus disease of Salmonids)**

A) Herpesvirus

B) Disease is observed primarily in rainbow trout fry.

C) Clinically the fish are lethargic with prominent gill pallor. Mucoid fecal casts are commonly observed trailing from vent.

D) Lesions:
1) Exophthalmos and ascites
2) Low hematocrit and numerous immature erythrocytes
3) Hemorrhage in eyes and base of fins

E) Histopathology:

1) Multifocal areas of necrosis involving the myocardium, liver, kidney, and posterior gut (leading to cast formation)

2) Syncytial cells involving the acinar cells of the pancreas is considered to be a pathognomonic sign.

F) Transmission of the virus is believed to be direct.

G) Control is by avoiding exposing susceptible trout to the virus. If the disease occurs, raising the water temperature to 15°C or more will minimize losses.

3) **Oncorhynchus masou virus (OMV, also known as Yamane tumor virus, Coho salmon tumor virus, Rainbow trout kidney virus)**

A) The etiologic agent is a herpesvirus (Salmonid herpes virus type 2). This virus is distinct from *Herpesvirus salmonis*.

B) The disease involves salmonids in Japan and Eastern Asia. The disease affects mostly rainbow trout and several species of salmon (Coho, chum, sockeye (Kokanee), and masou) with high mortality.

C) Clinical signs:
1. Exophthalmos, ascites, darkened skin
2. Pale gills due to anemia,
3. Hemorrhages over skin and internal organs.
4. Splenomegaly and pale swollen kidneys
5. Surviving fish may develop neoplasia over fins, opercula, body surface, cornea and oral cavity
6. Coho salmon develop skin ulcers, white spots on kidney and neoplasia in the oral cavity and skin

D) Histopathology: Acute stage sees multifocal necrosis of liver and kidney with necrosis of ellipsoids in the spleen. Neoplasia's are epithelial and my be either malignant or benign.

E) Surviving fish act as carriers and disease can survive for a prolonged time on fomites. Virus is shed in urine and feces. Virus can also be found in sperm and eggs at the time of spawning.

F) No treatment of infected fish. Eggs may be treated with a dilute iodine solution to sterilize them. Infected eggs this is probably of little help.

4) **Channel Catfish Virus**

A) Herpesvirus

B) Observed in fry or fingerling channel catfish (less than 10-gram weight) during the summer when water temperatures are above 22°C.

C) Clinically these fish usually show erratic swimming or spiraling followed by terminal lethargy. Mortality is very high.

D) Lesions: 1) Hemorrhage at the base of the fins and skins;
2) Ascites; exophthalmos; and pale gills;
3) Kidneys swollen and pale with hemorrhage;
4) Spleen is enlarged and dark red;

E) Histopathology: Multifocal areas of necrosis and hemorrhage are observed in the posterior kidney, liver, intestines, and spleen.

F) Infection is direct with transmission of the virus in the water or feed. Piscivorous birds, snakes, or turtles may mechanically carry the virus from pond to pond. Transovarian transmission has not been conclusively demonstrated but is suspected. Survivors are persistently infected and become carriers for life.

G) Control of the disease is by sanitation, purchasing of virus free brood stock and lowering water temperature to less than 19°C during an outbreak to lessen the mortality.

5) **Epithelioma papulosum (Fish (Carp) Pox)**

A) *Herpesvirus cyprini* (Cyprinid herpesvirus 1)

B) Non-fatal disease is observed in carp and other cyprinids

C) Lesions: Elevation of the epidermis with the formation of white to yellow plaques over the body of the fish. Healed lesions usually turn black.

D) Histopathology: There is epidermal hyperplasia with the epithelial cells occasionally demonstrating intranuclear inclusion bodies.

E) Transmission is unknown; however, it is probably direct.

6) **Infectious Haematopoietic Necrosis (IHN)**

A) Rhabdovirus

B) The disease is observed in the fry of trout (rainbow) and salmon (Chinook and sockeye) with mortality up to 100%.

C) Clinical signs and lesions:

1) Fish become lethargic or hyperactive.
2) The fish become dark due to increase in pigmentation.
3) Exophthalmus, abdominal distension, and fecal cast.
4) Hemorrhage on skin and viscera primarily at base of fins, behind the skull, and above the lateral line.
5) Anemia with pale gills.
6) Surviving fish may develop scoliosis.

D) Histopathology: There is prominent necrosis of hematopoietic tissue including melanomacrophages of the kidney, red pulp of the spleen and hepatic parenchyma. Necrosis of the submucosal eosinophilic granular cells is considered pathognomonic for IHN. (This lesion is observed in other systemic viral diseases.) Intranuclear and intracytoplasmic inclusions are occasionally observed in acinar and islet cells of pancreas.

E) The virus is transmitted by direct contact with infected survivors or by feeding contaminated feed. The virus is

probably shed in contaminated semen and eggs. The disease is most severe at 10°C and rare at temperatures above 15°C.

7) **Viral Hemorrhagic septicemia (VHS)**

A) Rhabdovirus

B) Widespread and very contagious viral disease of rainbow trout. This is a serious disease of trout in Europe. Affects both salmonids in fresh water and seawater. Disease occurs in temperatures below 14°C.

C) Two forms of the Disease --- Acute and Chronic

1) Acute disease: High mortality in affected fish. Fish have pale gills, dark body coloration, ascites, exophthalmus and erratic swimming behavior (spiraling). Hemorrhage is a common finding in the eyes, skin, serosal surfaces of the intestines and muscles. Necrosis of the hematopoietic and lymphoid elements of the anterior kidney and congestion and necrosis of the hepatic parenchyma are histopathologic findings.

2) Chronic disease: See a slower prolonged mortality. Fish become lethargic, have pale anemic gills, darken skin coloration, exophthalmus, and distention of the abdominal cavity. Internal organs are commonly involved with splenomegaly, hepatomegaly, and swollen kidneys.

D) Turbot, sea bass, and Atlantic salmon are commonly affected by similar viruses.

E) Transmission is believed to be direct with contact of carriers and contaminated water and feed. Vertical transmission via the egg is not reported.

8) **Spring Viremia of Carp (SVC) and Swim Bladder Infection virus (SBI)**

A) Caused by several subtypes of *Rhabdovirus carpio*.

B) Disease occurs in carp and other cyprinids.

C) Clinical Signs and Lesions:

1) Loss of coordination and equilibrium.
2) Exophthalmus and abdominal distension (ascites).
3) Inflamed and swollen vent.
4) Edema and hemorrhage in many organs.
5) In SBI see pronounced inflammation and hemorrhage of swim-bladder.

D) Often associated with secondary bacterial infections (Aeromonas salmonicida) leading to epithelial ulcerations (Carp Erythrodermatitis)

E) Transmission: Virus shed in feces and found in contaminated eggs.

9) **Infectious Pancreatic Necrosis (IPN)**

A) *Birnavirus*

B) Affects most salmonids; primarily rainbow trout and brook trout. IPN has also been implicated in disease among several nonsalmonid fish.

C) Clinical signs and lesions:

1) IPN is characterized by a sudden explosive outbreak with high mortality.
2) Affected fish become dark and rotate their bodies while swimming.
3) Diseased fish usually have distended abdomens and exophthalmus.
4) The presence of a gelatinous material in the stomach and anterior intestine is highly suggestive of IPN; mucoid fecal casts are common.
5) Infected fish commonly have a low hematocrit and hemorrhage in gut, primarily in the area of the pyloric ceca.

D) Histopathology:

Histologically, there is necrosis of the pancreatic acini, gut mucosa, and renal hematopoietic elements. A moderate inflammatory infiltrate is usually observed around the pancreatic acini. Hyalin degeneration of skeletal muscle is also observed.

E) Virus can be transmitted vertically in the eggs.

10) **Infectious Salmon Anemia (Hemorrhagic Kidney Syndrome)**

A) Orthomyxovirus

B) The disease was recognized in the 1980's and has been seen in Farmed raised Atlantic salmon in both North America and Europe. Atlantic salmon appear to be the fish most susceptible to the virus with mortality in infected stock being variable.

C) Clinical Signs and lesions:

1) Sudden death without any clinical signs

2) Lethargy, pale gills, and exophthalmos
3) Hemorrhage over the kidneys and other internal organs
4) Ascites, hepatomegaly, splenomegaly with congestion may also occur
5) Anemia (<10% hematocrit)

D) Histopathology:

The virus appears to be endotheliotropic. The posterior kidney is primarily affected. Hemorrhage, tubular degeneration and necrosis, and tubular casts are often seen in the kidney. Sinusoidal congestion, hemorrhage and necrosis of the liver is a common finding. The spleen has massive congestion with erythrophagia noted. Congestion and hemorrhage of other organs may also be seen.

E. Virus is transmitted by direct contact with infected fish. Contaminated equipment and water can also play a role in transmission of the virus. The salmon louse (*Lepeophtheirus salmonis*) is believed to play a significant role in the transmission of the virus. Brown trout, sea trout, and rainbow trout can become infected experimentally with the virus. Brown and rainbow trout demonstrate minimal lesions. It is unknown if these fish can act as carriers of the virus.

## Bacterial Disease

1) ***Aeromonas hydrophila* (Bacterial Hemorrhagic Septicemia)**

A) Gram negative motile rods

B) Effects many freshwater species and usually is associated with stress and overcrowding.

C) The clinical signs and lesions are variable. The most common finding is hemorrhage in skin, fins, oral cavity and muscles with superficial ulceration of the epidermis. Occasionally cavitary ulcers (similar to *A. salmonicida*) are observed. Exophthalmus and ascites are commonly observed. Splenomegaly and swollen kidneys are common. Histologically, multifocal areas of necrosis in the spleen, liver, kidney and heart with numerous rod shaped bacteria are observed.

D) Diagnosis is rendered by culturing the organism from affected animals: Remember this is a common water saprophyte with a great variation in virulence in serotypes.

E) Disease is transmitted via contaminated water or diseased fish.

2) ***Pseudomonas fluorescens* (Fin Rot)**

A) Short motile gram-negative rods with polar flagella.

B) Lesions similar to *Aeromonas hydrophila.* Fish often present with a hemorrhagic septicemia resulting in hemorrhage of the fins, skin, and tail. Later ulceration of the skin and fins is noted. *Pseudomonas fluorescens* is often pathogenic at low pond temperatures.

C) *Pseudomonas anquilliseptica* causes a serious problem in Japanese eels with a septicemia resulting in petechial hemorrhage on fins and tail and ulceration of the skin. This bacteria has also been known to cause similar lesions in trout and salmon.

3) **Vibriosis**

A) Gram negative rod, lives primarily in a marine environment

B) *Vibrio septicemia*: *V. alginolyticus* / *V. anquillarum* / *V. salmonicida*

1. Septicemia has similar lesions to Aeromonas hydrophila.

2. See hemorrhage in the skin of the tail and fins, ulceration of the skin, hemorrhage in the muscles and serosal surfaces. The spleen may be enlarged and bright red.

Histologically may see necrosis of the liver, kidney, spleen and occasionally the gut mucosa.

c) Ulcer Disease of Damselfish: *V. damsela*

1. Deep skin ulcers and necrotizing myositis.

2. Lesions similar to *Aeromonas salmonicida*.

E) *Vibrio salmonicida*: (Hitra disease or Cold water vibriosis)

1) Occurs primarily sea farmed Atlantic Salmon

2) Affected fish are usually fast growing.

3) Lesions similar to *Aeromonas* (Hemorrhage, skin ulcers, splenomegaly, hepatomegaly, and gut necrosis.

E) Organisms are ubiquitous in the marine environment often predisposing factors for infections are poor water quality, handling and other environmental stresses.

4) ***Edwardsiella tarda* (Edwardsiella septicemia)**

A) Gram negative motile pleomorphic curved rod

B) The disease affects primarily channel catfish (often at temperatures at or above 30°C but also observed in goldfish, golden shiners, largemouth bass, and the brown bullhead. This organism is the most serious bacterial disease involving the eel culture of Asia.

C) The lesions are similar to *A. hydrophila* with small cutaneous ulcers and hemorrhage observed both in the skin and muscle. Muscle lesions often develop into large gas filled (malodorous) cavities. Diseased fish lose control over the posterior half of their body yet continue to feed.

D) Edwardsiella is identified in many species of fish and other aquatic animals. This is probably not a problem unless the fish are stressed, overcrowded, or have poor water quality. These organisms are a potential zoonotic disease and an enteric disease of marine mammals.

**5) *Edwardsiella ictaluri* (Enteric septicemia of catfish)**

A. Gram negative motile pleomorphic curved rod

B. Disease affects primarily fingerlings and yearling catfish usually when temperature is between 24°C and 28°C.

C. Clinical signs of enteric septicemia of catfish closely

resembles those of other systemic bacterial infections. The most characteristic external lesion is the presence of a raised or open ulcer on the frontal bone of the skull between the eyes (**Hole in the head disease**).

D. Bacteria can survive in fish and aquatic environment.

6) ***Aeromonas salmonicida*** **(Furunculosis, Ulcerative disease of goldfish)**

A. Gram negative non-motile short rod

B. Bacteria affects primarily salmonids but other freshwater fish can be affected.

C. Clinically the disease may present as a septicemia with hemorrhage in the muscles, serosal surfaces, skin and fins. The major lesion is a subcutaneous swelling that often causes an ulcerative dermatitis. In chronic disease, these lesions may cavitate into the adjacent musculature. In the septicemic disease, there is splenomegaly, ascites, and swelling of the kidneys. Histologically, there is necrosis of the affected tissue with abundant colonies of bacteria and few inflammatory cells due to the bacteria's leukocytolytic exotoxin.

D. The disease is transmitted by contact with diseased fish, contaminated water, fomites, and infected eggs.

7) ***Yersinia ruckeri*** **(Enteric red-mouth)**

A. Gram-negative motile rod

B. The bacteria affect salmonids; rainbow trout are the most susceptible.

C. Clinically the disease manifest itself as a septicemia with exophthalmus, ascites, hemorrhage and ulceration of the jaw, palate, gills and operculum, musculature and serosal surfaces of the intestines, splenomegaly, and kidney swelling. Histologically numerous bacterial colonies admixed with inflammatory cells are observed in many areas of necrosis involving the liver, spleen and kidney.

D. The disease is transmitted by contact with diseased or carrier fish, and contaminated water. Bacteria persist in asymptomatic non-salmonid fish, in the aquatic environment and in some birds.

8) ***Streptococcus iniae***

A. Beta-hemolytic Streptococcus (Note: Beta hemolysin may not be present in culture media in all cases leading to the possible

believe that this bacteria is a non-pathogen.)

B. Disease of tilapia, hybrid striped bass and rainbow trout. Can be a major problem in raising tilapia and rainbow trout.

C. *Streptococcus iniae* presents either as an acute fulminating septicemia or a chronic form limited primarily to the central nervous system. The septicemic form may present with hemorrhage of the fins, skin, and serosal surfaces. Ulcers may appear. Microscopically, one observes a meningoencephalitis, polyserositis, epicarditis, myocarditis and/or cellulitis. Cocci/diplococci are present in the inflammation. In the chronic form, granulomas or granulomatous inflammation are evident in the liver, kidney, and brain (meningoencephalitis). In the chronic disease, the brain is the best organ to culture.

D. *Streptococcus iniae* is a problem primarily of closed recirculating culture system. Probably associated with overcrowding and poor water quality (high ammonia and nitrites). Depopulation, disinfection and restocking with disease free fish are the best means of elimination of the organism.

E. The bacteria are known to be a zoonotic agent. Individuals who have handled infected fish have developed cellulitis of the hands and endocarditis.

9) ***Flavobacterium columnare*** (***Flexibacter columnaris*****, Columnaris disease or Saddleback disease)**

A. Gram-negative slender rods (3-8 microns)

B. The disease is a serious disease of young salmonids, catfish and many other fish.

C. This is a highly communicable disease. Lesions usually first appear as small white spots on the caudal fin and progresses towards the head. The caudal fin and anal fins may become severely eroded. As the disease progresses, the skin is often involved with numerous gray-white ulcers. Gills are a common site of damage and may be the only affected area. The gill lesions are characterized by necrosis of the distal end of the gill filament that progresses basally to involve the entire filament. On wet mount preparations of gills, bacteria from aggregates of bacteria with a characteristic mounding or "Haystack" appearance.

D. *Flavobacterium columnare* infections are frequently associated with stress conditions. Predisposing factors for Columnaris disease are high water temperature (25°C-32°C.), crowding, injury, and poor water quality (<u>low</u> oxygen and increased concentrations of free ammonia).

*E. Flexibacter maritimus*: cause similar problems in salt-water

environment.

1. ***Flavobacterium psychrophilum* (old names: *Flexibacter psychrophilus and Cytophagia psychrophila*):** Cold Water Disease or Peduncle disease and Rainbow Trout Fry Anemia)

   A) **Cold Water Disease**: Tends to be in older fish. Fish develop dark skin, hemorrhage at the base of fins, and anemia with pale gills with increase mucus. Hemorrhage into the muscles is common. Periostitis of cranial and vertebral bones is common in chronic cases. A chronic meningoencephalitis occasionally is observed with abnormal and erratic swimming.

   B) **Rainbow Trout Fry Anemia (Old name: *Cytophaga psychrophila*):** Occurs primarily in rainbow trout fry. Fish develop abdominal distention, exophthalmos, increased pigmentation, lethargy, loss of balance, pale gills, and occasional cutaneous ulcers and necrosis of tail fins. Epidermal hyperemia and increase mucus secretions are common. Splenomegaly and hepatomegaly are common with multifocal necrosis of the liver spleen and kidney

   C) **Other *Flavobacterium*** sp.

      1) Usually a problem for individual fish. This disease is a cause of concern to primarily hobbyist and producers of ornamental fish. (Mollie granuloma, Mollie madness, Mollie popeye)

      2) Infected fish are usually emaciated and pale. Multifocal white nodules are observed in the visceral organs, the retina and choroid, and the brain. These nodules may be cystic or mineralized. Histologically the nodules are granulomas with a caseous center, a thin peripheral rim of macrophages and lymphocytes and a fibrous capsule (Must be differentiated from Mycobacterium).

   D) Overcrowding, accumulation of metabolite waste products (particularly ammonia), organic matter in the water, and an increase in water temperature may all be predisposing factors.

11) **Bacterial Gill Disease (*Flavobacterium branchiophilum* and others)**

   A. Bacterial gill disease is caused by a variety of bacteria. *Flavobacterium branchiophilum, Flavobacterium columnare, Flavobacterium psychrophilum,* and other various species of *Flavobacterium* (all are gram negative rods, *Myxobacter*) are the primary bacteria involved in this disease.

   B. Fry are the most susceptible to the disease, however, all ages may be affected. Clinically the fish become anorectic, and face the water current. Prominent hyperplasia (mucus and epithelial) of the gills is evident on gross and microscopic

examination. Microscopically, one observes proliferation of the epithelium that result in clubbing and fusion of the lamella. Necrosis of the gill lamella occurs in serious cases.

C. Overcrowding, accumulation of metabolite waste products (particularly ammonia), organic matter in the water, and an increase in water temperature may all be predisposing factors.

12) ***Renibacterium salmoninarum*** **(Bacterial Kidney Disease)**

A. Gram positive nonmotile diplobacillus.

B. This is a serious disease of salmonids. Brook trout are the most severely affected species.

C. The disease follows a slow course with clinical signs not present until the fish is well grown. The fish may exhibit exophthalmus, skin darkening, and hemorrhage at the base of the fins. Cutaneous vesicles and ulcers may develop in mature trout "spawning rash". Abscesses, cavitation and contraction of muscles is occasionally observed. Splenomegaly and swelling of the kidney and liver with abundant ascites fluid is commonly observed. The large swollen kidney and spleen have numerous white nodules visible in the parenchyma. Numerous granulomas (containing gram positive bacteria) are observed in the kidney and may be also present in the spleen, heart and liver.

D. Transmission of the disease is believed to be via direct contact with contaminated fish. It is believed that the organism enters through the epidermis and then becomes a systemic disease.

13) ***Mycobacterium*** **species (Picine Tuberculosis)**

A. Gram positive, acid fast rods (*M. marinum*, *M. chelonei* and *M. fortuitum* are the most common Mycobacterium species involved.)

B. All species of fish are affected. This disease affects both saltwater and freshwater aquariums as well as fish raised for food (up to 10 to 25% of pen raised fish).

C. Clinical signs of tuberculosis are quite variable. The most common signs are anorexia, emaciation, vertebral deformities, exophthalmus, and loss of normal coloration. Numerous variably sized granulomas are often observed in various organs throughout the body. Often numerous acid-fast bacteria are observed in the granulomas.

D. The aquatic environment is believed to be the source of initial infection with fish becoming infected by ingestion of bacterial contaminated feed or debris. Once an aquarium is

infected with this disease, it is difficult to remove except by depopulation of the aquarium and disinfecting the tank. Remember this is a zoonotic disease (atypical mycobacteriosis).

E. Atypical mycobacteriosis may manifest itself as a single cutaneous nodule on the hand or finger or may produce a regional granulomatous lymphadenitis of the lymphatics near the original nodule. Occasional local osteomyelitis and arthritis may also occur.

14) ***Nocardia* sp.**

A. Gram-positive filamentous rod (weakly acid-fast positive)

B. The organism is a problem with mostly aquarium fish. However, it is occasionally observed in cultured salmonids.

C. Clinically this is a chronic disease characterized by raised granulomatous masses in the mouth, jaw, gills and skin (The mouth and jaw are the most common sites). Dermal masses eventually ulcerate. Numerous white raised nodules (granulomas) are often observed in the viscera.

D. The exact route of transmission is unknown. However, it is felt that entry through wounds and abrasions is the most common source of infection. (Ingestion of the bacteria has been known to cause the disease.) Like Mycobacteriosis, once the aquatic environment is contaminated it is difficult to remove without depopulation and thorough cleaning of the environment.

15) **Epitheliocystis (Chlamydial infection)**

A. Obligated intercellular parasite. Organisms stain red with Macchiavello stain.

B. These organisms have been observed in many species of fresh water and marine fish. Mortality occurs most commonly in heavily infected juvenile fish.

C. Clinically infected fish may be asymptomatic or show respiratory distress or excessive mucus secretions. Multiple white cysts are observed on the gill lamella and skin. Histologically, the cyst consists of distended epithelial cells with numerous basophilic organisms.

D. The means of transmission is unknown.

## Mycotic Diseases

1) **Saprolegniasis**

A. Caused by various groups of aquatic fungi; primarily *Saprolegnia*, *Achlya*, and *Aphanomyces*.

B. Saprolegniasis affects all species and ages of freshwater and estuarine fish.

C. Clinically, affected fish develop white to brown cotton-like growths on skin, fins, gills and dead eggs. This organism is an opportunist that will usually grow over previous ulcers or lesions. Diagnosis is by finding broad nonseptate branching hyphae that produce motile flagellated zoospores in the terminal sporangia.

D. In the Atlantic menhaden, gizzard shad, and some other marine fishes, these fungi (primarily Aphanomyces), may present as an ulcerative mycosis that may progress to a deep necrotic lesion involving the muscle (**Ulcerative mycosis**). Histologically there is an intense granulomatous inflammation with broad (7 to 14 micron), nonseptate hyphae.

D. Most fish die due to osmotic or respiratory problems if the affected area of skin or gills is large.

E. The fungi are normal water inhabitants that invade the traumatized epidermis. Improper handling, bacterial or viral skin diseases, and trauma are the major causes of the disease. It is interesting to note that temperature has a significant effect on the development of infections. Most epizootics occur when temperatures are below the optimal temperature range for that species of fish.

2) **Branchiomycosis (Gill rot)**

A. Caused by two species: *Branchiomyces sanguinis* and *B. demigrans*.

B. Primarily a problem in carp, rainbow and brown trout, and eels.

C. Affected fish usually show respiratory distress. There is prominent gill necrosis caused by thrombosis of blood vessels in the gills. Histologically, the identification of nonseptate branching hyphae with an intrahyphal eosinophilic round body (apleospores) in and around blood vessels of the gill is diagnostic.

D. The disease occurs most commonly in overcrowded ponds with abundant organic matter and high ammonia levels. Usually warm water temperatures (20-25°C) bring about the disease.

3) **Ichthyosporidiosis**

A. *Ichthyophonus hoferi*; large 10-250 micron spores which may germinate to form large hyphae (similar to the hyphae of Saprolegnia).

B. This fungus infects all species of fish.

C. Clinically the fish are emaciated with small round occasionally ulcerated black granulomas in the skin. Scoliosis is occasionally observed. Internally, numerous granulomas are observed in many visceral organs. Microscopically, the lesion consists of granulomas with encysted large PAS-positive spores. Occasionally large irregular shaped hyphae are observed.

D. Transmission is unknown, but believed to be due to ingestion of contaminated feed.

4) **Exophiala sp.**

A. *Exophiala salmonis* and *E. psychrophila*; these fungal organisms have hyphae that are septated, irregular in width and branched.

B. This disease is observed in many species of fresh and saltwater fish. *E. salmonis* has become an organism of increased importance in caged cultured salmonids.

C. Clinically the fish become darker and lethargic, with erratic and whirling swimming behavior. Occasionally dermal nodules are present. Numerous round yellow to white granulomas are present in visceral organs (liver, kidney, spleen) with prominent enlargement of the posterior kidney common. Histologically, branched, irregular width, septated hyphae are present in the lesions.

D. Transmission is unknown.

## External Protozoal Diseases

1) ***Ichthyophthirius multifiliis*** **("Ich" or White Spot Disease)**

A. The largest protozoan parasite of fish. The trophozoite are up to 100 microns diameter, ciliated and contain an oval horseshoe shaped nucleus.

B. This is a disease of aquarium and hatchery reared fish.

C. Clinically fish become hyperactive with fish flashing and cutting against rocks or sides of aquariums. As the trophozoites enlarge they cause hyperplasia of the epidermis with white spots forming on the skin and gills. Severely infected fish may have respiratory problems and die. Histologically there is epidermal hyperplasia with the encysted trophozoite present in the epidermis.

D. The life cycle is direct. Encysted trophozoites (trophonts) leave the fish and settle to the bottom of the tank. The trophozoites (tomonts) divide into numerous tomites (theronts) that are released to infect the skin of the fish. The life cycle takes approximately 4 days to complete. However, it can be sped up by increasing the water temperature.

E. The only way to treat the disease is by interrupting the life cycle of the parasite. Removal of fish from the infected water for 3 days (25°C) will usually interrupt the life cycle (Tomites live only 48 hours at 26°C). One must treat the water to kill the tomites to prevent spread of the disease (Malachite green, formalin, methylene blue, or KMnO4). Remember, these treatments only kill the tomites and <u>not</u> the trophozoites that are encysted in the fish. Note that water treatments may alter the microflora of the tanks biofilter resulting in water quality issues.

F. *Cryptocaryon irritans* is the salt water equivalent to *Ichthyophthirius*.

2) ***Ichthyobodo necator*** **(Costiasis)**

A. Piriform shaped protozoa 6-12 microns long with two short and two long flagella. These are stalked protozoa that attach to the skin or gills.

B. This disease is observed in most aquariums and hatchery raised fish. This disease occurs primarily in cold waters (10°C) and affects very young fish when they are just beginning to eat food.

C. Clinically the fish may flash, produce abundant mucus over the skin (blue slime disease) and/or show respiratory distress (flaring of gills). Histologically the parasites are attached to the epithelial surface of the skin or gills.

D. Transmission of the parasite is by direct contact with the protozoa. This protozoon is a free swimmer so it can swim and then attach to the host where it undergoes binary fusion for reproduction.

3) ***Trichodina* sp. (Trichodiniasis)**

A. This disease is caused by a group of peritrichal ciliated protozoans. The organisms are saucer-shaped, 50 microns diameter, with rows of cilia at both ends and a macro and micronucleus. When viewed dorsoventrally, the parasite appears as an ornate disk with a characteristic ring of interlocking denticles forming a circle in the middle of the organism. (*Trichodina truttae* is considered to be a specific pathogen for salmonids).

B. These are observed on most fresh and saltwater fish. This protozoon is relatively common on many fish and is not always associated with disease.

C. Clinically fish usually exhibit flashing and become lethargic. There is an increase in mucus production causing a white to bluish haze on the skin. The skin may develop ulcers and the fins may fray. If the gills are involved, the fish may have severe respiratory distress. Histologically, masses of organisms are attached by adhesive discs and denticles of exoskeleton to the epidermis. The underlying epithelial cells undergo necrosis. There is secondary hyperplasia and hypertrophy of the gill epithelium.

D. Transmission is by direct contact with infected fish and or contaminated water.

4) ***Tetrahymena corlissi* and *Tetrahymena pyriformis***

A. Normally a free-living oval ciliated 50-70 micron long protozoa.

B. The organism has been known to affect the fry of various cultured fish (Guppy "Guppie killer" and Northern pike).

C. Clinically, one may observe necrosis and hemorrhage of the skin. In severe cases, the fish have rupture of the body walls and the fish eviscerate. Histologically one observes massive invasion of the musculature by this organism. The ventral abdominal wall is severely affected.)

C. This is a free-living protozoan that only becomes a problem at times of overcrowding and poor water quality (water having a high organic matter content).

5) **Dinoflagellates (Velvet disease, Coral fish disease)**

A. Dinoflagellate 100 microns diameter containing chromatophores and a single eccentric nucleus. When free swimming they are 20 microns diameter contain a transverse flagellum in the transverse furrow and a longitudinal flagellum in the longitudinal sulcus. Several species of dinoflagellate are involved:

1) *Piscinoodinum* (old name: *Oodinium*) - Velvet or gold dust disease
2) *Amyloodinium* - Coral fish disease

B. Problem in aquarium and cultured fish.

C. Clinically, fish flash in the water and become depressed with lateral opercular movement. A shimmering heavy yellow colored mucus secretion over the skin and gills is observed. Histologically, large oval organism (80 microns diameter) with multiple chromatophores and a single eccentric nucleus are attached to epithelial cells by pseudopodia.

D. Transmission is by direct contact of tomites with infected fish, and contaminated water. To treat this disease one must remove fish from their environment and raise the temperature in the tank to speed up the lifecycle of the organism. This cause the trophozoite (tomonts) to divide and release the tomites and dinospores which mature in the hot water and die (similar to Ich). Darkening the tank assists in killing the organisms since they have chloroplast which allows the organism to maintain itself for a long time without finding a host.

6) **Epistylis (*Heteropolaria* sp.; Red sore disease)**

A. Branched stalked ciliated protozoan (*Heteropolaria colisarum*).

B. Found primarily in wild populations of scaled fish.

C. Clinically, one observes ulcers or cotton-like growth on the skin, scales and spine resulting in a red-colored lesion. In catfish, the lesion involves the spines and bones that underlie the skin of the head and pectoral girdle. This protozoan parasite has also been observed on eggs.

D. This ciliated protozoan is primarily a free-living protozoan that lives on aquatic plants and is believed to be an opportunist. Outbreaks have occurred in catfish and salmon that have been maintained in water high in organic content.

7) **Glossatella (*Apiosoma*)**

A. This disease is caused by the ciliated protozoan *Apiosoma* that has a barrel-shaped body with cilia at the distal end

and a large rounded macronucleus.

B. This organism usually is not a problem but can affect many species of fish.

C. The organism can appear on the gills or skin causing increased mucus production and hyperplasia. Severe infections of the gills will cause respiratory problems.

D. This disease is a problem when fish are exposed to poor water quality.

**8) Amoebic gill disease**

A. *Neoparamoeba pemaquidensis and other free living amoeba. N. penaquedensis live in salt water.*

B. Sever problem in caged reared Atlantic Salmon and rainbow trout. Disease has been seen in Tasmania and US. An unidentified amoeba was been seen in Pallid sturgeon on the Missouri river with similar lesions.

C. Clinically see hyperplasia and fusion of gill lamellar epithelium creating white nodular mass on the gills. Histologically see epithelial hyperplasia with granulomatous inflammation. Amoebic organisms (trophozoites) are seen along the gill filaments or entrapped in cysts in the hyperplastic epithelium.

D. Probably a problem of overcrowding and poor water quality. Since *Neoparamoeba* are saltwater inhabitants the moving of these fish to fresh water often clears the problem. If problem is seen with fresh water fish place the fish in salt water may help diminish the problem.

## Internal Protozoal Diseases

1) **Henneguya (Blister disease, Myxosporidiosis)**

A. Myxosporidean parasite (6 Henneguya sp.) with two polar capsules and a long tail like extension of the spore shell. This parasite is believed to be a Myxosporidean in the fish and an *Aurantiactinomyxoa* in the mud worm.

B. Problem in many cultured freshwater fish; channel catfish can be heavily infected.

C. Clinically, fish are presented with numerous white cysts on the skin and gills. Cyst can become very large. Cysts may lead to gill epithelial hyperplasia leading to anoxia. Interlamellar forms may cause some necrosis of gills and occasional death. Treating affected fish with chemotherapeutic agents is usually ineffective and may cause more deaths.

D. The life cycle is unknown. It is felt that a mud worm (*Oligochaete* sp.) is involved in an indirect life cycle with asexual and sexual stages in the mud worm (*Aurantiactinomyxoa sp.*)and catfish (*Myxosporidean*).

E. *Henneguya exilis kudo* was once believed to be the cause of Proliferative Gill Disease. However, the evidence suggests that the interlamellar form of the parasite that evokes a serious inflammatory response is probably due to another Myxosporidean (*Aurantiactinomyxoa* sp. or the extrasporogenic stage of the myxozoan *Sphaerospora ictaluri*).

2) **Proliferative gill disease (Hamburger gill disease)**

A. Myxosporidean parasite; most likely an *Aurantiactinomyxoa* sp. (Triactinomyxid myxozoan). Note: some feel that this may represent the extrasporogenic stage of the myxozoan *Sphaerospora ictaluri*.

B. Problem in many cultured freshwater fish (primarily catfish) and usually involves new ponds.

C. Clinically there is rapid onset with the disease killing 10% to 95% of the fish. Water temperatures between 16 and 20 degrees centigrade favor optimal growth of the organism. Fish are presented in severe respiratory distress. Grossly there is intense granulomatous inflammation and swelling of the gills with epithelial hyperplasia and gill necrosis. Histologically, the cyst observed in the gill lamella cause necrosis of the cartilage, distortion of the gill lamella and an intense inflammatory response with numerous macrophages infiltrating the gill lamella around the cysts. Cyst have been observed in other organs (brain, spleen, liver, kidney).

D. The life cycle is unknown. The parasite is believed to maintain mild subclinical infections in some fish host or has an indirect life cycle involving a mud worm (*Oligochaete* of the *Duro* sp. (*Duro digitata*)). Infected oligochaetes release *Aurantiactinomyxoa* spores that infect more oligochaete and the channel catfish. Transmission of the spores from the fish to the oligochaete have not been observed. This suggests that the catfish may be an abnormal host for this parasite.

E. Survivors are believed to be resistant to reinfection.

3) ***Myxobolus cerebralis* (*Myxosoma cerebralis* or Whirling Disease)**

A. Myxosporidean parasite with a 10-micron oval spore with 2 piriform polar capsules.

B. Parasite affects primarily young salmonids (rainbow trout most susceptible; Brown trout and Coho salmon resistant).

C. Clinically, fish develop blackened tails and become deformed about the head and spine (scoliosis) with the fish swimming erratically (whirling). Histologically, there is necrosis of the cartilage, particularly of the head and spine, with numerous spores present in the area of inflammation. The necrosis of the cartilage is the cause of the deformation.

D. Transmission is believed to be by ingestion of spores or spore attachment and penetration. The life cycle of this organism is not completely known. A tubificid oligochaetes (tubifex mud worm, *Tubifex tubifex*) is an important intermediate or transport host. It is believed that the parasite undergoes sporulation in the tubifex worm were the organism takes on the form of a *Triactinomyxon* sp. It is believed that this parasite is then released from the tubifex worm and infects the trout. Tubifex worms are infected for life. Trout are believed to become infected by the ingestion of *Triactinomyxon* spores by eating the mud worms, by the ingestion of spores free in the water, or by free spores penetrating the epithelial surface of the fish. Released spores may attach and penetrate the epithelial surface of the fish (body, tail, gills, causal fin, or mouth). Spores develop into sporoplasms and invade epidermal cells (goblet or mucosal cells). These parasites then multiply and progressively migrate to the peripheral nerves by day 4-post infection. Later they migrate to the bone and cartilage. In the cartilage, the sporoplasms develop into trophozoites that undergo asexual mitosis forming numerous spores that infect the cartilage. Spore development is substantially influenced by temperature with lower temperatures causing spore development to take longer.

E. Spores are very resistant to environmental conditions and can with stand freezing and thawing, temperatures as high as 66°C, passing through the gut of birds and fish, and survive in sediment for up to 30 years. Control is done by removal of

all dead or infected fish and disinfecting the pond with calcium cyananide, lime, or chlorine. Decreasing the Oligocheate in the water can also be accomplished by concrete lining of ponds and raceways. Spores can be reduced in water by ultraviolet treatment of the water. Infected fish can be treated with Fumagillin in feed at 0.5g/kg of feed for two weeks.

4) **Microsporidians (*Glugea, Pleistophora, Loma*)**

A. Microsporidian parasites form cysts in various organs. The cysts are filled with small 1 to 2 micron spores. Parasitic cyst may induce hypertrophy of the infected cell (*Glugea*, *Loma*, *Spraguea*, and *Ichthyosporidium*) or does not cause hypertrophy of infected cells (*Pleistophora*).

B. Microsporidian parasites are found in numerous fresh and saltwater fish.

C. Clinically microsporidian present themselves as individual or multiple cysts that can become quite large and may give the appearance of neoplasms (xenomas). These cysts are filled with numerous refractile spores.

1) *Glugea* and *Loma*: Infect macrophages and other mesenchymal tissues which then undergo massive hypertrophy causing deformity of visceral organs (liver, gut, and ovaries) as well as infections in the muscle and subcutis.

2) *Pleistophora hyphessobryconis* (Neon tetra disease): This microsporidian infect the sarcoplasm of muscle fibers causing these fibers to be filled with these organisms. There is no inflammatory reaction around the cyst.

D. Transmission of the disease is most likely direct.

5) **Coccidiosis**

A. Primarily of the genus *Eimeria*. Various species of *Eimeria* are observed in the different fish.

B. Affects both fresh and saltwater fish. The coccidia not only infect the epithelium but also many other organs including the gonads. This is a very important problem in the carp and goldfish culture.

C. 1) *Eimeria subepithelialis*; carp: Nodular white raised areas in the middle and anterior gut.

2) *Eimeria carpelli*; carp: Ulcerative, hemorrhagic enteritis.

3) *Eimeria sardinae*; marine fish: Granulomatous reaction in the liver and testicles.

6) ***Hexamita* sp: (*Hexamita salmonis* and Hole *in* the Head Disease)**

A. Binucleated piriform protozoan with 6 anterior and 2 posterior flagella.

B. Infects many fish to include young salmonids (*Hexamita salmonis*), goldfish, and many other fish without apparent infections. Some fish, particularly angelfish, discus, and gouramis the infection can be severe.

C. Clinically, the young fish have anorexia, and become debilitated with reduced growth. The fish develop an acute enteritis with numerous organisms present in the feces.

D. In farmed Chinook and Atlantic salmon the disease can become systemic with fish becoming anemic with swollen kidneys and exophthalmos. Boils on the dorsal skin and numerous granulomas with organisms present have been observed. In angelfish, red Oscars and other cichlids, *Hexamita* (*Spironucleus*) infections have been associated with ulceration of the skin (particularly in the head region with erosion to the bone (Hole in the head disease in discus and gouramis). Affected fish may also develop gastroenteritis with organisms present, peritonitis and granulomas in multiple organs.

E. Transmission is by ingestion of infective cyst.

F. Disease is associated with poor water quality with multiple water changes often curative. A bath in metronidazole (5 ppm) has been known to help and appears to be nontoxic (up to 20 ppm). The drug appears to be absorbed by the gills and skin.

7) **Proliferative Kidney Disease (PKD, PKX, X Disease)**

A. Believed to be caused by a myxosporan parasite (*Sphaerospora* sp. or the prespore stage of *Tetracapsula byroslamonae*). However, the taxonomy of the parasite is not completely worked out.

B. Parasite causes a serious problem in cultured salmonids (Rainbow trout and salmon) in Europe and North America. Infected ponds can see a mortality between 10% and 95%. Outbreaks tend to occur in fingerlings with rising water temperatures. Water temperatures of 16 degrees centigrade seem to favor growth of the organism.

C. Clinically infected fish have a darker body pigmentation, exophthalmos, ascites and pale gills. Internally, the kidneys are swollen and have numerous grey white area of granulomatous inflammation scattered throughout. Diseased fish also develop anemia and hypoproteinemia. Histologically, the kidney has a granulomatous interstitial nephritis with macrophages and lymphocytes surrounding the amoeboid

parasites (15μ diameter and usually with multiple daughter cells). There is usually prominent tubular and hematopoietic tissue loss. The parasite may also be identified in the spleen, liver, muscle, gills and intestines.

D. The life cycle of the parasite is unknown. The marked inflammatory response observed in the infected fish and the lack of mature spores suggests that the fish may be an aberrant host.

8. **Cryptosporidiosis**

a. Intercellular extracytoplasmic protozoan

b. Cryptosporidium infects the intestine of several species of fish. (Carp; *Naso tang*, *Naso litatus*; tropical freshwater catfish, Plecostomus sp.; and cichlids)

C. The importance of cryptosporidiosis as a pathogen in fish is unknown. May cause some debilitation; believed to be a secondary invader after the immune system is depressed. Infected fish usually are presented emaciated and not doing well.

D. The importance of this organism as a reservoir for infection in other animals and man is unknown.

## Miscellaneous Parasites

### 1) *Lernea* - Anchor worm (Also *Salmincola* and *Lepeophtheirus* sp.)

A Copepod

B. Infects all freshwater fish and is a serious problem in cyprinids (bait minnows, goldfish, and carp).

C. Clinically, the parasite invades the skin, usually at the base of a fin. The head develops into an anchor that holds the female in place. The female then develops egg sacs (two finger like projections attached to the end of the body). The ulcers are slow to heal.

D. Other copepods such as Ergasilus sp. are found on the gills and cause serious gill damage.

E. *Lepeophtheirus salmonis* is incriminated as a carrier for infectious salmon anemia virus.

### 2) *Argulus* - Fish louse (*Branchiura*)

A. Parasite of the skin and occasionally the buccal cavity. Common in Koi; usually associated with overcrowding. Is a concern with farmed raised fish and the association with wild species.

B. Cutaneous ulcers due to piercing of epidermis by the retractile preoral stylet (a proboscis-like mouth) for sucking blood from the fish.

C. Transmission is by direct contact with eggs on vegetation or transfer from fish to fish.

### 3) *Gyrodactylus* sp.

A. Monogenetic trematode; flattened and leaf-like, no eyespot, cephalic end V shaped, has an attachment organ (haptor) and two large anchors with 16 marginal hooklets.

B. Affects most species of fish.

C. Fluke anchors itself to skin, fins, and gills that may cause excessive mucus secretions over gills and skin. Fish may undergo flashing and have fraying of fins. Severe infection (gills) may cause the fish to become dyspneic and die.

D. Life cycle is direct. The larvae are released and attach almost immediately to the host.

4) ***Dactylogyrus***

A. Monogenetic trematode; flattened and leaf-like, four anterior eyespots, cephalic end scalloped, ova present, has an attachment organ (haptor).

B. Affects most freshwater species, particularly carp and goldfish.

C. Fluke anchors to gills causing excessive mucous secretions, and frayed edges. Fish become anoxic with flaring of the gill opercula.

D. Life cycle is direct. The adults are oviparous and produce eggs with long filaments. The eggs are usually attached to the gills. The eggs develop into a onchomiricidium that then attaches to the fish.

5) ***Diplostomum spathaceum*** **(Eye fluke)**

A. Digenetic fluke; metacercaria is infective state in fish.

B. Gulls and pelicans are the definitive host. Snails (*Lymnaea* sp.) are the first intermediate host. Fish (salmonids) are the second intermediate host.

C. Clinically, the metacercaria are presented as white dots; later the eye becomes opaque. Blindness occurs in severe infections. The metacercaria are found in the anterior chamber, vitreous body, and lens causing cataracts.

6) ***Uvulifer ambloplitis*** **(Black spot disease)**

A. Digenetic fluke; metacercaria infect fish.

B. Herons and kingfishers are the definitive host; snails are the first intermediate host and fish are the second intermediate host.

C. Clinically, the fish have numerous black to brown spots up to 1 mm (dia) over the skin, gills and eyes. The spots contain a metacercaria surrounded by heavily pigmented fibrous connective tissue.

7) ***Acanthocephalus*** **(Thorny headed worm)**

A. *Pomphorhynchus* sp. and *Acanthocephalus* sp.

B. Acanthocephalans are observed in many species of fresh water and marine fish. Adult parasites live in the intestine. The larval second intermediate stage may encyst in the liver, spleen or mesentery.

C. Heavy infections are observed in feral fish. Infected fish

may not show signs. However, some fish are emaciated and have swollen abdomens. In heavy infections, raised subserosal nodules may be observed in the gut. These nodules may have the proboscis attached. Histologically, a severe granulomatous reaction is associated with the nodules. If the parasite penetrates the serosa, peritonitis may occur.

D. The life cycle is complex; an amphipod is the first intermediate host. In the amphipod, the acanthor develops into a cystacanth. Small fish are believed to be the second intermediate host (paratenic host) for the cystacanth. The life cycle is then completed with the ingestion of the cystacanth and development of the adult worm.

8) **Anisakis**

The parasite causes little problem in fish. However, in man, it can be a serious public health threat. Brown and white larvae (third stage) are observed in the viscera and musculature of fish. Many marine mammals are the definitive host with this nematode living in the stomach.

## Neoplasms

1) **Melanoma in Platyfish/Swordtail hybrids**

Unique invasive melanoma that occurs in the offspring from F1 hybrid platyfish/swordtail with the spotting traits that are crossed with swordtails. F1 hybrids with the spotting trait develop premelanosomes. F1 X swordtail cross will produce frank melanomas. The reason for these melanomas is believed to be due to enhancement of the macromelanophore gene due to a deficiency of modifier genes that leads first to melanosis and finally to invasive melanomas.

2) **Hepatoma and hepatocellular carcinoma in rainbow trout**

The fry of rainbow trout are very susceptible to aflatoxins in the feed. These hepatic neoplasms are associated with the ingestion of aflatoxins in the feed. Acute aflatoxicosis causes acute massive liver necrosis with bile duct proliferation.

3) **Stomatopapilloma of eels (Cauliflower disease)**

These are large firm cauliflower-like masses that are attached to the mouth. Tumors tend to proliferate in the summer and degenerate in the winter. A birnavirus, similar to infectious pancreatic necrosis virus, has been reported to have been isolated from the affected eel (*Anguilla anguilla*). However, initiation of the tumor with cell free extracts has been unsuccessful.

4) **Papilloma of the Brown bullhead**

Papillomas are common in the brown bullhead with occurrence on the head and lip. Viral particles have been observed ultrastructurally in the papillomas, but a virus has not been isolated. Some of these papillomas may progress and become locally invasive squamous cell carcinomas.

5) **Lip Fibroma (Fibropapilloma) of Angel Fish**

Tumor of the mucocutaneous junction of the lip near the midline. Adult female fish are the only effected fish. Tumors begin as small white vesicles that enlarge over several weeks. The tumors are firm, lobulated, and elevate the epidermis. On cut sections, the tumors are white with some having cavernous centers filled with clear fluid.

Histologically, the tumors consist of dense fibrovascular connective tissue arranged in whorls, streams and bundles and covered by a thick stratified squamous epithelium. Cause is unknown. A type "A" retrovirus has been isolated from

affected tissue. Laboratory transmission of the disease to other fish has not occurred.

6) **Dermal Fibrosarcomas of Walleye pike**

Fibrosarcomas are a common neoplasm affecting a large variety of fish. Dermal fibrosarcomas of Walleye pike arise in the dermis and cause multifocal nodules over the entire body. They can be very large and locally invasive. A type-C retrovirus has been associated with this disease. Occasionally, this neoplasm has also been associated with a herpesvirus induced epidermal hyperplasia or lymphocystis disease.

7) **Lymphosarcoma of Pike**

This is an epizootic condition in northern pike and muskellunge in certain regions (i.e. Lake Ontario). The lesion develops as a purple ulcerative cutaneous mass on the head, mouth and flank with invasion into the adjacent muscle and metastasis to spleen, liver and kidney. A type-C Retrovirus is believed to be the cause of this disease.

8) **Schwannoma/Neurofibromas of the bicolored damselfish (Damselfish Neurofibromatosis DNF)**

Neurofibromas have been reported in numerous species of fish. The bicolored damselfish has gained notoriety in that some of these fish develop multiple cutaneous schwannomas. This neoplasm is believed to possibly represent an animal model for von Recklinghausen Neurofibromatosis (NF type 1) in man. The similarities and differences between these two diseases are as follows: The primary lesion in both NF type 1 and DNF are neurofibromas, many of which are plexiform in nature. The fish tumors are often malignant. DNF the pigment lesions can be neoplastic and quite invasive, while the cafe-au-lait spots of NF type 1 are benign. NF type 1 appears to be genetically transmitted while DFN appears to be horizontally transmitted.

9) **Plasmacytoid Leukemia (Marine anemia) of Chinook salmon**

Plasmacytoid leukemia virus is observed in farmed raised Chinook salmon (Experimentally in Sockeye, Coho and Atlantic salmon). It is believed to be caused by a retrovirus (Salmon leukemia virus). Affected fish become lethargic, have dark skin, pale gills (anemia), and exophthalmus. The spleen, kidney, and retrobulbar tissues are enlarged and mottled. Petechial hemorrhage of the serosa is common. Infiltration of the liver, spleen, and kidneys with plasmablastic cells is noted. Plasmablast have a slightly lobulated nucleus with a central nucleoli.

## Nutritional Deficiencies

1) **Iodine Deficiency**

Iodine deficiency cause hyperplasia (goiter) of the thyroid tissue. The cause is not always known. Some goiters may be due to iodine deficiency (very difficult to produce). However, the most likely cause may be due to the affects of goitrogenic substances in the feed or due to the presence of goitrogenic pollutants in the water.

2) **Fatty Acid Deficiency (Linolenic and linoleic acid deficiency)**

Fish are capable of synthesizing most fatty acids but not the linolenic or linoleic acid series. Deficiencies of these fatty acids lead to depigmentation, fin erosion, cardiomyopathy, fatty infiltration of the liver, and myxomatous degeneration of fat.

3) **Vitamin C Deficiency**

Ascorbic acid is an essential vitamin of fish. Deficiencies of this vitamin lead to poor wound healing, ulceration of the skin on fins, hemorrhage, skeletal deformity and immune problems. The vitamin is needed for proper collagen synthesis which is important in wound healing, cartilage formation and ossification of cartilage to bone. Scoliosis may occur in affected fish. This vitamin is very temperature sensitive and oxidizes readily in stored feed.

4) **Vitamin E Deficiency**

Vitamin E deficiency is associated with necrosis and degeneration of skeletal and cardiac muscle, steatitis, and lipoidal liver disease.

5) **Pantothenic Acid Deficiency**

Pantothenic acid is a coenzyme needed in the metabolism of fats and carbohydrates. Deficiencies lead to anorexia due to hyperplasia of the gill lamellar epithelium and fusion of secondary lamella (nutritional gill disease). Anemia is usually associated with the disease.

6) **Methionine/Zinc and Cystine Deficiency**

Methionine deficiency (primarily in salmonids) leads to reduced growth rate with the development of bilateral cataracts. It is felt that deficiencies of vitamin A and riboflavin also play a role in this lesion. Zinc and cystine deficiencies can also cause cataracts.

7. **Nephrocalcinosis and Magnesium or Selenium toxicity**

Nephrocalcinosis seen in both wild and farm raises salmonids. The cause is unknown but believed to be due to magnesium deficiencies, selenium toxicity or prolonged exposure to elevated $CO_2$ in the water. See calcified nodules in enlarged swollen kidneys. Fish often present in poor body condition with abdominal swelling (ascites), darkened skin (fish that is in poor health) and irregular kidneys with the white calcified material present.

## Miscellaneous problems

1. **Gas bubble disease:**

A. A. Occurs when water becomes supersaturated with gasses (Not just oxygen)

B. Problem occurs under different conditions

1. When pressurized air injection into water is improperly applied (Usually the entering of air on the intake side of the pump).
2. Water has a rapid rise in temperature over a short period of time.
3. Large algeal blooms supersaturate the warmed water due to photosynthetic activity
4. Water from great depths is moved upward quickly (Deep wells or water release from dams).

C. Gas bubble disease is characterized by the formation of gas emboli filling capillaries and vessels in many organs. Affected fish become emphysematous due to gas bullae in the skin and fins. Often many small gas bubbles are seen in the skin, fins, and gills. Severely affected fish may develop exophthalmos due to gas build-up in the choroids gland in the posterior uvea. Fish frequently die due to asphyxiation.

E. To treat gas bubble disease one need to remove the source of the supersaturated water by fixing equipment or improving the water quality.

**2. Ammonia and nitrate toxicity**

a. These two substances go together and are influenced by bacteria in the aquatic environment. Ammonia and debris is broken down by *Nitrosomanas* bacteria in the water to toxic nitrite ($NO_2$) which is then converted to nontoxic nitrate ($NO_3$) by *Nitrobacter* bacteria. This process is often completed by the tanks filter system but can become a problem if these bacteria are not established in the tank or pond. This is often a new tank or pond problem.

b. Ammonia Toxicity:

1. Unionized ammonia ($NH_3$) is most toxic to fish with ionized $NH_4$ considered to be nontoxic. Water pH and to some extent water temperature play a major role in unionized ammonia levels in the water environment. Harmful ammonia levels vary with different species of fish. However levels at 0.05mg/l (0.05 ppm) $NH_3$ should be a concern.

2. Overcrowding (High stocking densities) and poor water quality (usually due to waste excretion and excess feed) due to a nutrient rich environment often leads to ammonia toxicity. Poor water filtration and bacterial degradation of nutrients can lead to rapid ammonia build

up and death.

3. Clinically fish often die peracutely; other fish may have excessive rapid gill movement with flared opercula, grasping near the water surface and erratic swimming. Since this is irritating, excessive mucus production on the gills and skin can be observed. Chronic ammonia toxicity often leads to gill epithelial hyperplasia. Resultant opportunistic bacterial infections involving the gills and skin are often observed later. Ammonia toxicity is often noted in ponds in the afternoon when the water pH and temperature are the highest.

4. To confirm ammonia toxicity one needs to evaluate ammonia concentrations in the water (TAN: Total Ammonia nitrogen) and determine the amount of unionized ammonia in the water based on pH and water temperature.

5. Treatment of affected tanks and ponds is by decreasing the feed given to the fish and water changes.

c. Nitrite toxicity ($NO_2$) (New fish tank disease or Brown blood disease)

1. Increase nitrite levels are often associated with poor water quality (excessive ammonia levels). Like ammonia, different fish species have different levels of tolerance to Nitrite.

2. Affected fish often die peracutely with no clinical signs or are seen gasping with flared opercula near the water surface. Chocolate brown colored blood and/or gills are often signs noted in affected fish (note: not all fish will have chocolate brown blood or gills).Check blood levels for methemoglobin concentrations is needed for a diagnosis (Normal methemoglobin concentrations vary with different species of fish). Fish chronically exposed to excessive nitrate levels can develop secondary bacterial infections.

3. Treatment: Most fish actively transport nitrite into the body by using the chloride cells in the gills. The addition of sodium chloride to water can aid in the treatment of nitrite toxicity by actively competing with nitrite ion in the water and preventing nitrite from being absorbed by fish. The addition of calcium to the water can also help prevent methemoglobinemia. Ascorbic acid in feed (catfish) also decreases methemoglobin formation.

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    d. Fish health issues; Fish health laboratory, Maine Department of inland fisheries and wildlife. www.state.me.us/ifw/fishlab/intro.htm
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