COMPARATIVE HEMATOLOGY OF LABORATORY ANIMALS

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BLOOD COLLECTION

- Collection Site/Method
  - Cardiac
    - Elevated phosphorus and potassium
    - Increased muscle enzymes
  - Standardize the site
  - Anesthetics
    - Isoflurane minimally stressful
    - Generally CO₂ and pentobarbital stressful


BLOOD COLLECTION

- Anesthesia method
  - CO₂ administration in rodents
    - Increased potassium – due to metabolic acidosis
    - Increased lactate
    - Increased pCO₂
    - Decreased pH

Orb sin

COLLECTION SITE


TERMINOLOGY

- Submandibular vein
  - Facial vein
- Retro-orbital venous plexus
  - Retro-orbital sinus

Clinical Biochemistry Parameters in C57BL/6J Mice after Blood Collection from the Submandibular Vein and Retroorbital Plexus


"Using Monika Anatomica Veterinaria as a guide, there is no submandibular vein in the mouse."
JAALAS, 49:4, p.400, 2010

Jerry Silverman

BRAINTREE SCIENTIFIC
Blood Collection for Facial Vein (Submandibular) Blood Samples

MEDIPOND, INC.
The Facial Vein and the Submandibular Vein meet at the rear end of the Mandibular Bone, providing a convenient and consistent source of blood.
Evaluation of Saphenous Venipuncture and Modified Tail-clip Blood Collection in Mice


BLOOD COLLECTION

- Hemolysis
  - Mild hemolysis (1+)
  - Generally non-significant
  - Very common in rodent samples
  - Moderate to Severe (2+ to 4+)
  - Potassium and bilirubin elevated along with other analytes

BLOOD COLLECTION

- Tube Selection and Proper filling
  - Standardize for comparison between studies
  - Lithium heparin
  - EDTA
  - Sodium citrate
    - 3.2% vs 3.8%
    - Avoid underfilling

EDTA

- Advantages
  - Ideal for cellular morphology
  - Inhibits bacterial proliferation
- Disadvantages
  - Cannot be used for some chemistry analytes
  - Underfilled tubes – RBCs shrink due to hypertonicity
- Types
  - K2EDTA (Sprayed on the side)
    - Ideal of routine CBC
    - Will not dilute the sample
  - K3EDTA (Liquid)
    - Causes sample dilution and affect RBC size

LITHIUM HEPARIN

- Advantages
  - Can also be used for clinical chemistry
  - For small animals with limited blood, collection into a single tube minimizes loss
  - Do not have to wait for sample to clot
- Disadvantages
  - Morphology not ideal
  - Interferes with some immunoassays

SODIUM CITRATE

- 3.2% vs 3.8%
  - Both can be used, however
  - The milder chelation of 3.2% provides greater accuracy of coagulation results
  - Cannot compare data if collected into different %
BLOOD COLLECTION

- **Processing Times**
  - Serum must sit for 30 minutes, minimum to allow to fully clot
  - Delayed processing alters analytes
    - e.g. Decreased glucose
- **Freezing and Thawing**

RODENT ERYTHROCYTES

- **As compared to Dogs**
  - More polychromasia (1-6% circulating RBCs or 100,000-350,000/µL)
  - More anisocytosis
  - Occasional Howell Jolly bodies

INTERPRETING DECREASED RBC PRODUCTION IN RODENTS

- **Most species**
  - Indices not changed as few reticulocytes normally in circulation
- **Mice/rats**
  - Decreased MCV
  - Decreased RDW
  - Increased MCHC
- **Short RBC life span**
  - Anemia of chronic disease develops more quickly

ERYTHROCYTE LIFE SPANS

- **Dogs** 100-115 days (17,000-79,000/µL)
- **Rats** 45-50 days (135,000-250,000/µL)
- **Mice** 43 days (221,000-370,000/µL)

RODENT – ERYTHROCYTE PARASITES

- **Plasmodium berghei**
  - Many experimental mouse models
  - Young Wistar rats highly susceptible
- **Mycoplasma coccoides (Eperythrozoon coccoides)**
  - Mice and rats
  - May cause anemia

MOUSE NEUTROPHIL

- nuclei display more hypersegmentation
- even small changes may be significant
- older mice may have a marked neutrophilia due to long-standing inflammation
- low number of ring forms normal, increase with neutrophilia
Mouse Eosinophil
- Low numbers in peripheral blood
- Band-shaped nucleus that can form a ring

Mouse Basophil
- Long propagated myth that mice do not have basophils
- NOT TRUE!
- Very rare in circulation

Mouse – Bone Marrow
Neutrophilic ring forms

Rodent Platelets
- “Sticky” and clump easily

Rodent Leukocytes
- Rodent WBCs
  - Mostly lymphocytes
  - Fewer neutrophils; mouse < rat
  - Very few monocytes, eosinophils
  - Basophils extremely rare

Normal Leukocytes in Rodents
Mouse < Rat and Rabbit < Dog and Monkey

Leukocyte Counts in Rodents
- Less robust in as compared to large animals
  - Magnitude of changes (dog > rat > mouse)
  - Qualitatively similar effects with excitement and stress
- Inflammation in rodents
  - Increased neuts and lymphs, +/- monocytes
  - Left shifts in rodents less common than in larger animals but morphology of the shift when present is similar
- Decreased neutrophils
  - Difficult to evaluate when resting count is so low
SPLEEN

- Output is about 50% of the bone marrow under normal conditions
- Reservoir for hematopoiesis
- Megakaryocytes normally in clusters in the red pulp

HISTOPATHOLOGY - SPLEEN

RABBITS

- Heterophils
- 2-4% polychromasia
- 5-30% basophils
- Lymphocytes and neutrophils approximately equal in %
- Leukocytosis often absent in bacterial infections

GUINEA PIGS

- Heterophils
- Lymphocytes predominate
- Kurloff cells
- More common in females
- Presumed to be an NK cell

HAMSTER

- Very similar to mouse and rat
- Hibernation
  - Normal RBC lifespan ~ 50-70 days —Increases to 160 days
  - RBC counts and hemoglobin levels higher
  - WBC counts decrease

CASE: 2 MO, SYRIAN HAMSTER

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<th>Units</th>
<th>Results</th>
<th>Ref Interval</th>
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<td>Hemoglobin g/dL</td>
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<td>13.0-19.0</td>
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<td>Hematocrit %</td>
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<td>39-59</td>
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<td>MCV fl</td>
<td>91</td>
<td>68-75</td>
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<td>MCHC g/dL</td>
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<td>WBC x10⁹/µL</td>
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<td>Lymphocytes x10⁹/µL</td>
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<tr>
<td>Platelets x10⁹/µL</td>
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<td>Plasma Mod hemolysis</td>
<td>Mod hemolysis</td>
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</table>
Hamsters have been used experimentally to study Babesia spp. and as a tool for diagnosing human babesiosis (Babesia microti) through inoculation.

**PIG**
- Rouleaux
- Artifactual crenation
- High sedimentation rates
- Highly susceptible to hemolysis by hypotonic saline
- Little central pallor
- Band cells in normal pigs

**SHEEP**
- Small erythrocytes
- Small platelets
- Some hematology analyzers cannot distinguish platelets from erythrocytes

**DEER**
- Sickled erythrocytes
  - Non-pathogenic
  - Common

**RHESUS MONKEY**
- Very similar to humans
- Neutrophils have visible granules (light blue to magenta depending on the stain)
- Varied reports of neutrophil:lymphocyte ratios, probably due to restraint, anesthesia, subspecies, etc.
HEMATOLOGY CASE #1

- 7-month old male rhesus
- Pale haircoat
- Alopecia
- Seemed to be less active
- PCV = 5%
  - Non-regenerative
  - Normocytic, normochromic
  - Morphology - unremarkable

MACAQUE

Plasmodium inui

RING TROPHOZITES

Hemazoin - malaria pigment

WHITE MONKEY DISEASE

- Cage treated for rust several months prior
- Zinc toxicosis
- Decreased copper
- Severe, non-regenerative anemia
- Hairloss and depigmentation
- Baboons, macaques – young animals only

SQUIRREL MONKEY

- Blood smear morphology similar to macaques
- Lymphocytes reportedly predominate, but neutrophils can be seen as primary cell
- Occasional nRBCs - normal

OWL MONKEY

Aotus nancymae
- Most common species
- Blood smear morphology similar to macaques
- Lymphocytes predominate
- Occasional nRBCs - normal

Aotus azarae
- Normally have elevated eosinophil counts
- Heightened eosinophilopoiesis
- Decreased recruitment kinetics

CHIMPANZEE

- Blood smear morphology similar to rhesus monkeys
- Have less platelets than other NHP species
NON-HUMAN PRIMATE BONE MARROW

HEMATOLOGY CASE #2

History
Day 1
Diarrhea 7 days post-weaning
Tx: Erythromycin PO BID

Day 4
Found recumbent in cage
Hypothermic (90°F)
Weak, staring into space
Tx: SQ fluids, oral dextrose, heat lamp, responded rapidly

CBC

PCV = 26 (35-43)
MCV = 34.8 (65.6-74.8)
MCHC = 28.5 (32-34)
Retic Count = 0.2% (>1% = regen)

Microcytic, hypochromic non-regenerative anemia

INITIAL BLOOD SMEAR

ASSESSMENT

Differential Primary Diagnoses
- Iron deficiency
  * Iron levels normal to elevated
- Inherited hemoglobinopathy
  * Never reported in any domestic species
- Beta- or alpha-thalassemia
  * Never reported in any domestic species

Secondary Considerations
- Vascular Injury
- Immune-mediated RBC destruction
- Infection

Schistocyte
Hypochromic
Acanthocyte
Ovalocyte
CLINICAL PROGRESSION

Day 7
Tx: Prednisolone 4mg IM BID
Procrit SQ

Day 9 “thrashing around”
Not using left arm or left leg
Difficulty holding up his head
No cranial nerve deficits noted
Pale
TX: Heparin 300U SQ

LACK OF COORDINATION

CLINICAL PROGRESS (CONT)

Day 11 – Blood Transfusion
Pre PCV = 21, Post PCV = 32
Femoral transfusion
TX: Rocephin 60mg IM BID
Discontinue erythromycin

REVERSE-PHASE HPLC

POSSIBLE DIAGNOSES

- Not a lack of iron availability
  … RBC iron utilization problem?
- Not a heritable form of hemoglobinopathy or thalassemia
  … Acquired form?
- Something else?

MICROCYTOSIS OF YOUNG RHESUS

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<th>Sex</th>
<th>Age</th>
<th>Diagnosis</th>
<th>WBC</th>
<th>RBC</th>
<th>HGB</th>
<th>HCT</th>
<th>MCV</th>
<th>MCHC</th>
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<td>30.5</td>
<td>18.4</td>
<td>721</td>
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Normal MCV = 65-73
DAY 23

DAY 67

DAY 103 NEUROLOGIC STATUS

OUTCOME

~200 days
... Clinically normal
Developed into a young male adult
Not used for breeding

COAGULATION IN RODENTS

Problems and Pitfalls
... Rodents are resistant to thrombus formation
... Standard coagulation biomarkers not very sensitive or specific
... Endpoints not standard in early screening studies
... Abnormalities in longer term studies often subtle
... Histopathology not sensitive
... Results easily altered by collection techniques

COAGULATION PANEL - RODENTS

Routine
... Absolute Platelet count
... Prothrombin time (PT)
... Activated partial thromboplastin time (APTT)
Variable
... Fibrinogen
... MPV
... Bleeding time
Distal tail incision, normal less than 60 seconds
D-dimer, ATIII can be helpful but not well characterized
Spontaneous Coagulopathy in Inbred WAG/RijYcb Rats

- Elevated APTT
- Normal PT
- Normal platelet

Hereditary defect in the intrinsic pathway

COAGULATION – PREGNANT RAT

- Suitable model for humans in late pregnancy
  - Increased fibrinogen
  - Increased platelet counts
  - Decreased PT
  - Overall activity of vitamin-K-dependent coagulation factors
  - Increased APTT
  - Increased ATIII

KNOCK-OUT MOUSE
FVB BACKGROUND

- No spontaneous hemorrhage
- Routine tail clip for genotyping
- Uncontrolled bleeding leading to death

Gene deletion results in an intrinsic pathway defect

CLINICAL CHEMISTRY OF LABORATORY ANIMALS

Kirstin Barnhart, DVM, PhD, DACVP
M. D. Anderson Cancer Center

GLUCOSE

- Mice
  - Higher in cardiac puncture as compared to jugular
  - Anesthetic method has been reported to affected results
  - Normally higher than large mammals
  - Average between 125 and 250 mg/dl after 4hr fast
  - Diabetes – defined as greater than 300 mg/dl
  - Must be persistent!
  - Common to have elevations greater than this with stress
- Rats
  - Lower average fasted levels, around 100 mg/dl

GLUCOSE

- Non-human primates
  - Chimpanzees
    - Similar to humans
  - Old World monkeys
    - 20-30 mg/dl lower than humans
    - 100-126 mg/dl likely to be overt diabetes
- New World monkeys
  - Many species are similar to rodents and have marked elevations due to stress
  - Glucose greater than 200 mg/dl is common in squirrel and owl monkeys
DIABETES MODELS

- C57bl/6J ob/ob
  - Obese, hyperglycemic, hyperinsulinemic
- Zucker fatty rat (fa/fa)
  - Obese, hyperinsulinemic, normoglycemic
- Rhesus and cynomolgus monkeys
  - Insulin resistant
  - Overt type II diabetes

DIABETES TESTING

- OGTT
- IVGTT
- Euglycemic clamp

OGTT
- Better than a fasting blood glucose
- Best used as a screening test
- Plagued by many factors that cause poor reproducibility
- 20% non-diagnostic
- Must be confirmed with a repeat test

IVGTT
- Indirect measure of insulin sensitivity
- Calculates a glucose disappearance rate (KG)
- Using differential equations (Minimal model) can measure insulin sensitivity/resistance

EUGLYCEMIC CLAMP

- Insulin prime (10 minutes)
- Insulin steady state (40mU/m2/min)
  - Hyperinsulinemic (at this point)
- 20% dextrose infusion
- Glucose testing every 5 minutes
- Adjust glucose infusion rate to maintain euglycemia (80-90 mg/dl) – this is the “clamp”
- Measure insulin every 10 minutes, every 30 minutes measures Potassium

  High Glucose infusion rate - SENSITIVE
  Low Glucose infusion rate - RESISTANT

Advantages
- Widely accepted as the reference standard to determining insulin sensitivity in humans
- Directly measures whole body glucose disposal at a given level of insulinemia
- Excellent test characteristics

Disadvantages
- Time Consuming
- Labor intensive
- Expensive
- Experienced Operator to manage technical difficulties
PROCEDURE

- Place 3 catheters
  - 1 Peripherally inserted central catheter (PICC)
  - 1 18g peripheral, 1 22g peripheral
- Two infusion pumps
- Bedside analyzer of glucose (Istat)

PROPER LENGTH

INTRODUCTION

Hyperinsulinemic-euglycemic clamp studies in ob/ob mice treated with PBA or TUDCA.

U Özcan et al. Science 2006;313:1137-1140
Published by AAAS
**IVGTT RESULTS**

**Normal**

**Insulin Resistant**

**GLUCOCORTICOIDs**

- **Mice/rats**
  - Corticosterone predominates, 99% circulates bound to cortisol-binding protein and albumin
- **Squirrel monkeys**
  - Extremely high circulating levels
  - 1/3 of cortisol circulates unbound
  - Impaired clearance, decreased receptor affinity
  - High levels of ACTH

**HEPATIC ENZYMES - MOUSE**

- **Hepatocellular Injury**
  - ALT
    - 1° liver but also cardiac and testicular injury
    - 11,000% increase with mouse hepatitis virus
    - Significant elevations due to *Helicobacter hepaticus*
  - AST
    - Very non-specific
    - Primarily in periportal hepatocytes
- **Cholestasis**
  - Alkaline phosphatase
    - Highest levels in intestine and kidney
  - GGT
    - Very insensitive, normal levels very low

**HEPATIC ENZYMES - RATS**

- **Hepatocellular Injury**
  - ALT
  - AST
  - Sorbitol Dehydrogenase (SDH)
    - Frequently the most sensitive and specific
    - Requires rapid processing
  - Glutamate Dehydrogenase (GLDH)
    - Not affected by muscle
- **Cholestasis**
  - Alkaline phosphatase
  - GGT
    - Very insensitive, normal levels very low

**HEPATIC ENZYMES - RABBIT**

- **Hepatocellular Injury**
  - ALT
    - Half-life 5 hours
    - Liver activity similar to heart muscle
  - Alkaline phosphatase
    - Also less specificity
  - GGT
    - Very insensitive, normal levels very low
- **Cholestasis**
  - Hyperbilirubinemia
  - ALP

**CHEMISTRY CASE - RABBIT**

**Clinical Pathology**
- ALP – marked increase
- ALT- marked increase
- Hyperbilirubinemia
- Hyperproteinemia

**Differentials**
- Intestinal obstruction
- Tyzzer’s disease
- Hepatic coccidiosis
- Hepatic lipidosis
HEPATIC COCCIDIOSIS

*Eimeria stiedae* oocyst

HEPATIC ENZYMES - PIG

**Hepatocellular Injury**

- **ALT**
  - Half-life 50 hours
  - Skeletal and cardiac muscle have higher activity
- **Sorbitol dehydrogenase**
  - Half-life ~ 2 hours
  - Best for acute injury
- **AST**
  - An indicator of non-specific organ damage

**Cholestasis**

- **ALP**
  - Decreases related to food intake
  - Some utility as a hepatic marker
- **GGT**

HEPATIC ENZYMES - NHP

- **ALT/AST**
  - Magnitude of the elevations not as dramatic as some species
  - Normal 10-50 IU/L
  - With liver disease 50-500 IU/L
  - Muscle Contribution
    - Often greater than liver
    - Look at the ALT/AST together with LDH and CK

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<td>33</td>
<td>803</td>
<td>2,440</td>
<td>16,780</td>
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GAMMA GLUTAMYLTRANSFERASE - NHP

- Distribution studied in macaques and baboons
- Liver, pancreas, kidney
- More sensitive marker of biliary disease than ALP
- Higher in young animals and decrease with age
- Higher in Mauritian cynos as compared to Southeast Asian
- Can be significantly elevated with no change in ALP or bilirubin

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<th>Chimp 3</th>
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CREATINE KINASE ISOENZYMES

- Electrophoretic separation of isoenzymes
- Isoenzymes, rat 1,2
  - CK-MM - skeletal muscle and heart
  - CK-MB - 1° heart but also skeletal muscle
  - CK-BB - 1° brain but also spleen, kidney, liver, heart and skeletal muscle
- Rat heart - 69.9 % MM, 24.43% MB, 6.38% BB
- Rat skeletal muscle - 93.6% MM, 6.09% MB, 5.3% BB
- Of limited usefulness for cardiac diagnosis
- Many better tests available

LACTATE DEHYDROGENASE

- Elevates with skeletal and cardiac muscle injury
- Very non-specific
- Mice – Lactate dehydrogenase elevating virus
- Unexplained elevations can be associated with tumors

MYOglobin VS HEMOGLOBIN

- Both will cause a positive urine dipstick reaction for blood
- Both may or may not be associated with intact RBCs
- Hemoglobin quickly bound to haptoglobin and retained in serum
- Myoglobin unbound and clears quickly
- Ammonium precipitate test can confirm

CARDIAC BIOMARKERS

- Troponins (I and T)
- BNP and NT-proBNP
- Atrial natriuretic peptide (ANP)

TROponins

- Protein present in the thin myofilaments
  - Involved in regulation of muscle contraction
- Different isoforms present in skeletal and cardiac muscle
  - Troponin I
    - More widely available assays
    - Most commonly used
  - Troponin T
    - Sensitivity and specificity equal to troponin I
    - Not as many assays available

TROPoNINS (CONT)

- Indicate cardiac ischemia/injury
- Assays have been validated for a wide variety of species
- Interpret with caution in the face of renal disease
- Pick either I or T but do not need to run both

BNP AND NT-PRO

- BNP is produced by the ventricles in response to cardiomyocyte stretching
- NT-proBNP is the cleaved portion of the hormone, may be less affected by degradation
- Strong indicator of heart failure
- Can be elevated by renal disease
- Species
  - Dogs – works well
  - Primates – works well
  - Rats – some assays have not cross-reacted well
BILIRUBIN

- Rodents
  - May be used for cholestasis
  - Heritable deficiency in bilirubin UDP-glucuronosyltransferase
    - Gunn rat – severe unconjugated hyperbilirubinemia
    - Models of Gilbert syndrome
- Rabbits
  - Total activity is normally low
  - Elevations rarely seen

BILIRUBIN

Adult primates - uncommon
- ... Leptospirosis
- ... Hepatocellular carcinoma
- ... Right sided heart failure

- Neonatal rhesus monkeys
  - Normal destruction of fetal hemoglobin
  - Immature liver

RENAL FUNCTION - RATS

- BUN/creatinine
- Uniquely susceptible to nephropathy from α2-urinary globulin
  - ... Contributes to strange odor of rat and mouse urine
  - ... Persistence in proximal tubule of rats leads to cytotoxicity of tubular epithelium

Reference Values for Serum Proteins of Common Laboratory Rodent Strains

Commercially available agarose system

URINE PROTEINS (RAT)

- Alphaα2u – globulin
  - ... Predominant urinary protein in adult male rat
  - ... Synthesized in liver under influence of androgens
  - ... Low levels in juvenile and senescent males
  - ... Minimal in females
  - ... LMW (16-20 kDa) lipocalin freely filtered at glomerulus
    - ... Associated with chemical-induced male rat specific renal tumor formation
    - ... Various exogenous chemicals bind α2u – globulin and prevent lysosomal degradation
    - ... Epithelial cell degeneration and renal tumor formation through increased cellular proliferation

URINE PROTEINS (RATS)

- Major constituents of urinary protein in rats (cont.)
  - ... Albumin
    - ... Urine albumin ~1mg/24hr in young adult male rat
    - ... Increased amounts excreted with age
  - ... Tamm-Horsfall glycoprotein
    - ... Wide range of normal values in literature
    - ... ↑ Day-to-day variability in urinary excretion
  - ... Diet influences protein excretion
    - ... High-protein diet → increased α2u – globulin excretion
    - ... Protein-deficient diet → decreases in both albumin and α2u – globulin
URINE PROTEINS

- **Mouse**
  - Proteinuria is common in mice
  - Small quantities of α- and β- globulin
  - Prealbumins known as mouse urinary protein (MUP) – “uromucoid”
  - LMW lipocalin synthesized in liver
  - Structurally similar to α2u – globulin
  - Urine concentration very high in healthy mice (1.060-1.080)

URINALYSIS

- **Rabbits**
  - Urine is major route of calcium excretion – leads to cloudy, turbid urine that will become pasty if fed a high calcium diet
  - Calcium oxalate, calcium carbonate, struvite

URINALYSIS - NHP

- **Specific gravity**
  - Typical range is 1.003 – 1.020
  - No defined isosthenuric range
- **Urinary crystals common in macaques**
  - Calcium oxalate, amorphous phosphate, struvite
- **Ketonuria**
  - Can be seen due to negative food intake
  - Fecal contamination can cause a false positive

CALCIUM OXALATE

- Can be normal in many domestic and lab animal species
- Pathogenic
  - Increased calcium excretion
  - Oxalates
  - Ethylene glycol

CALCIUM CARBONATE

- Rabbits
- Horses
- Guinea pigs
- Goats

URINARY BIOMARKERS – RATS MODEL OF POLYCYSTIC KIDNEY DISEASE

- Proximal tubular dysfunction
  - N-acetyl-β-D-glucosaminidase
- Distal tubular dysfunction
  - S-transferase Yb1

Wiedmeyer C.E., and Royal, A. B. Comp Med, 60:6, 448-454, 2010

- Glomerular lesions
- Collagen IV
- Glomerular and proximal tubular
  - Albumin
**CALCIUM**

- Rabbits often have high circulating levels
  - Absorb very efficiently from the gut independent of need or vitamin D levels
  - Up to 16 mg/dl
  - Can make interpretation of high calcium difficult

**LIPIDS**

- Mice
  - HDL predominant
  - No spontaneous atherosclerosis due to low levels of LDL and CETP
- Rats
  - HDL predominant
  - Overnight fast, ~30% will have hyperlipemia, but cholesterol unchanged
  - Study of gerbils, hamsters, guinea pigs
    - Guinea pig best natural model
  - Rabbits
    - Easier to induce hypercholesterolemia than the rat
    - To be effective most rodent models need to be genetically modified

**CHOLESTEROL DIETS**

- Induce hemolytic anemia in the guinea pig
- Induce hemolytic anemia in the rat
  - Proposed mechanism is decreased phospholipid in RBC membrane causing decreased deformability

**SD RATS, 7 WEEKS, NON-FASTED**

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**HYDRAZINE ADMINISTRATION**

- Oral gavage for 7 days
- Decreased ALT, lipids and globulins
- Hepatic lipidosis
- Inhibits pyridoxal-dependent enzymes (e.g. AST and ALT)

**ELEVATED TRIGLYCERIDES WITH NON-LIPEMIC SERUM**

- Common in NHPs
- Elevations as high as 2500
- Always in non-lipemic serum,
  - Generally, animals are fasted for 12 hours because anesthesia required for blood collection
  - May be anorexic and on a negative energy plane
GLYCEROL
- Total triglycerides contains 5-10 mg/dl glycerol
- Free glycerol included in total triglycerides
  - Measured without lipase for selective ID
- Hypertriglycerolemia in humans
  - Glycerol kinase deficiency
  - Diabetes, metabolic syndrome
  - Martin and Rand, 2007 Vet Record (Feline diabetes)

SERUM AMYLOID
- Acute Phase Reactant
  - Produced at high levels by the NHP liver
- Contributes to reactive amyloidosis
  - Common problem in NHP colonies
  - Frequently liver only
  - Can be systemic
- Hamsters also have frequent amyloidosis

HEPATIC AMYLOIDOSIS - RHESUS

EVALUATING INFLAMMATION IN PRIMATES
- WBC
  - Much smaller elevations than dogs
  - Even a mild left shift is very significant
- Sedimentation Rate
  - Very valuable in chimpanzees
  - Rarely helpful in macaques or new world monkeys
- SAA
  - Strongly associated with hepatic amyloidosis in chimpanzees and rhesus macaques

EVALUATING INFLAMMATION IN PRIMATES
- Fibrinogen
  - Strongly associated with acute inflammation in both chimpanzees and rhesus macaques
- Iron/TIBC/% Saturation
  - Acute, significant decrease in serum
  - Prevalent in many NHP species

ACUTE PHASE PROTEINS - RODENTS
- Rats
  - Fibrinogen
  - α2-macroglobulin
  - Total α- and β-globulins
  - Erythrocytes
  - Albumin
  - Serum iron
- Mice
  - Fibrinogen
  - Ceruloplasmin
  - C-reactive protein
  - Serum amyloid P
  - Serum amyloid A
  - α1-fetoprotein
WASTING SYNDROMES IN NEW WORLD MONKEYS

Marmosets

Owl Monkeys

INDEX CASE – ADULT FEMALE OWL MONKEY

1/25/2010

Clinical signs
- Emaciated
- Ravenous
- Bright and alert
- Symmetrical, non-pruritic alopecia

Pancytopenia
- Severe neutropenia
- Severe lymphopenia
- Moderate anemia
- Borderline regenerative
- Moderate thrombocytopenia

BLOOD SMEAR - 1/25/2010

- RBC
  ... 2+ anisocytosis
  ... 2+ poikilocytosis
  ... 1+ hypochromasia
  ... Rare schistocytes

- WBC
  ... NSF

CBC – 1/29/2010

- Pancytopenia
  ... Severe neutropenia
  ... Mild left shift
  ... Severe lymphopenia
  ... Severe anemia
  ... Mild regeneration, exaggerated nRBC increase
  ... Moderate thrombocytopenia

BLOOD SMEAR - 1/29/2010

- RBC
  ... 3+ anisocytosis
  ... 1+ poikilocytosis
  ... 2+ hypochromasia

- WBC
  ... Moderate Dohle bodies
  ... Mild toxic change
Anemia

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Mean Platelet Volume

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BONE MARROW EVALUATION

- 18 gauge intraosseous catheter
  - Left femur
    - Dry tap
  - Right femur
    - No blood return into the syringe.
    - Small fibrous clumps of marrow pushed out with the stylet
    - Impression smears made

ERYTHROID DYSPLASIA

MYELOID DYSPLASIA
MEGAKARYOCYTIC DYSPLASIA

- Approximately 25 animals affected
- Consistent clinical pathology findings
  - Pancytopenia
  - Profound panhypoproteinemia
  - Elevated GGT
- Intestinal histopathology
  - Villous blunting and fusion

TDX: Celiac disease d/t dietary intolerance

WASTING SYNDROME TO DATE

MARMOSET WASTING SYNDROME

- Some similarities with owl monkey
  - Emaciation despite strong appetite
  - Vitamin D deficiency
  - Alopecia
- Differences with owl monkey
  - Severe diarrhea
  - Paralysis and muscular weakness
  - Lethargy

IRON OVERLOAD IN NEW WORLD MONKEYS

INDEX CASE

- Squirrel monkey necropsy
  - Trauma
  - Poor body condition
  - Renal disease
  - Liver – ORANGE
- No clinical pathology available

LIVER – H&E STAINING
NORMAL HEPATIC IRON IN PRIMATES

LIVER – PRUSSIAN BLUE STAINING

IRON OVERLOAD

PREVIOUS REPORTS
- Lemurs
  - Identified in 1960s
  - 2006 – Am J Primatol, Glenn et al. - 50% incidence
- Callitrichidae
  - 1997 – Miller et al, Lab Anim Sci, common marmosets – 100% incidence
  - 2008 – Am J Primatol, Smith et al. – marmosets/tamarins – blood values correlate with liver histopathology

PREVALENCE
- 87 squirrel monkeys
  - 29 had serum iron >160 (33%)
- 67 owl monkeys
  - 23 had serum iron >160 (34%)
- Other findings
  - % saturation frequently approaching 100%
  - TIBC frequently mildly to markedly elevated

ASSOCIATED PATHOLOGY
- Concurrent disease in 50% of the animals with elevated iron
  - Weight loss, lethargy
  - Renal disease
  - Cardiac disease
  - Diarrhea
- Elevated GGT
CHALLENGING CASE – ADULT RHESUS MONKEY (H42)

- Moderate leukocytosis
- Moderate neutrophilia
- Severe left shift
- Slight monocytosis
- Hemoconcentration

WBC ct 16.30
Neutrophils 9.45
Bands 4.40
Metamyelocytes 0.31
Lymphocytes 0.99
Monocytes 0.99
Eosinophils 0.00

RBC ct 7.32
Hgb 16.7
HCT 50.7
MCV 69.2
MCH 22.7
MCHC 39.2
RDW 14
Platelet 556
MPV 9.76

CLINICAL CHEMISTRY – H42

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URINALYSIS – H42

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- Hematuria with hemoglobin casts
- Increased transitional epithelial cells
- Glucosuria
- Aciduria
- Proteinuria

H42 - COAGULATION

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NECROPSY - KIDNEY

KIDNEY – WARTHIN STARRY
CHALLENGING CASE – RHESUS MONKEY

- Juvenile, rhesus monkey
- Depressed
- Thin
- Prominent SQ pitting edema of dependent areas
- Marked lymphadenomegaly

CLINICAL PATHOLOGY DATA

- Serum biochemistry
  - Albumin: 1.5 g/dl (2.4-3.6 g/dl)
- CBC
  - PCV: 31.4% (35-45%)
  - WBC: 3600 cells/ul (5000-9000 cell/ul)
    - Moderate lymphopenia and monocytopenia
  - TP: 5.3 g/dl (6.8 g/dl)

CLINICAL PATHOLOGY DATA

- Urinalysis
  - Specific gravity: 1.026
  - 100 mg/dl protein
  - Urine protein:creatinine ratio = 0.8
    - 2 days later = 1.8

Lymph Node Cytology

NECROPSY: GROSS FINDINGS

- Mild peripheral SQ edema in extremities
- Moderate to marked peripheral and visceral lymphadenomegaly
- Multiple 1-2 mm nodules in the spleen

TDX: Leishmaniasis
ADDITIONAL DIAGNOSTICS

- **Serology:**
  - CDC: T. cruzi titer stronger than Leishmania titers
  - Heska Corp: Leishmania negative

- **Immunohistochemistry:**
  - Brazilian Leishmania specific assay:
    - Monkey - positive
    - Confirmed T. cruzi case - negative

---

**Lymph Node**

---

**Heart Pseudocyst**

---

**CELL CULTURE**

- T. cruzi epimastigotes 100x

---

**T. CRUZI IN MONKEYS**

- **KCCMR - Texas**
  - 50 adult male/female rhesus monkeys
  - Blood collected – sent to CDC for blood culture – gold standard for T. cruzi infection
  - 1/60 positive culture
  - 1 previous death confirmed on necropsy

- **Southwest Foundation – Texas**
  - 4 baboons (Williams et al, J Med Primatol, 38(2), 107-113, 2009)