Adrenal Cortex and Medulla in Preclinical Toxicology

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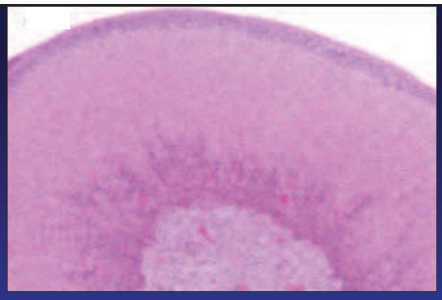
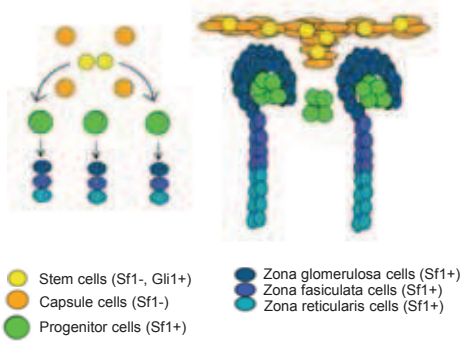
Adrenal Glands

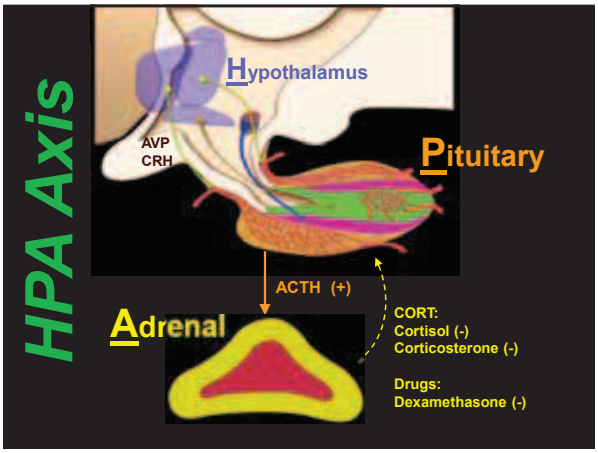
- Most common endocrine organ with chemically-induced lesions
- Understanding structure and function is important for interpreting the mechanisms and significance of chemically-induced lesions



Rat Adrenal Cortex
Zona Glomerulosa (Arcuata) 20%
Zona Fasciculata 65%
Zone Reticularis 15%

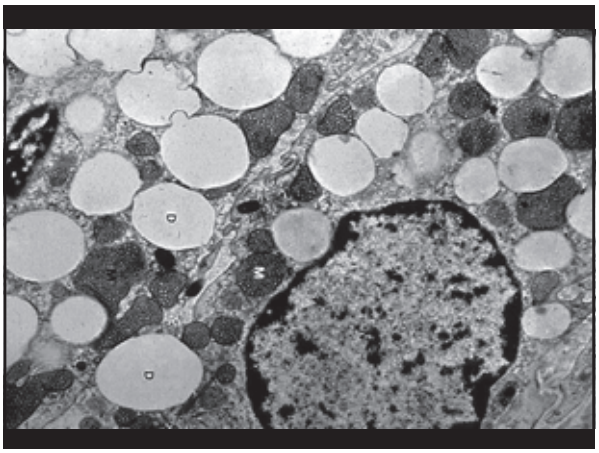
Homeostatic Model of Adrenocortical Growth





Subcellular Structure/Function Relationships

- Lipid droplets (cholesterol esters)
- Smooth endoplasmic reticulum
- Mitochondria
- Minimal storage of hormone
- Steroids are hydrophobic molecules diffuse out of cells



Steroidogenesis

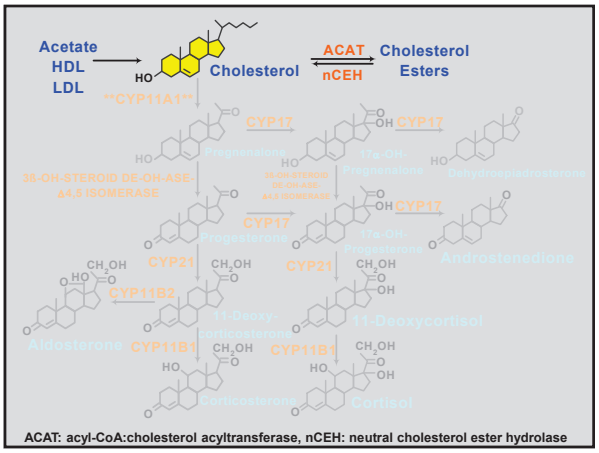
- ACTH (cAMP second messenger)
  - ↑StAR protein (steroidogenic acute regulator protein)
    - Moves cholesterol to inner mitochondrial membrane
  - ↑Cyp11A1
- Mitochondria
  - Side chain cleavage (Cyp11A1)
  - Hydroxylation to Pregnenalone
- SER
  - Converted to 11-Deoxycorticosterone
- Mitochondria
  - Hydroxylated to Corticosterone or Cortisol

Adrenal Cortex: Steroid Hormones

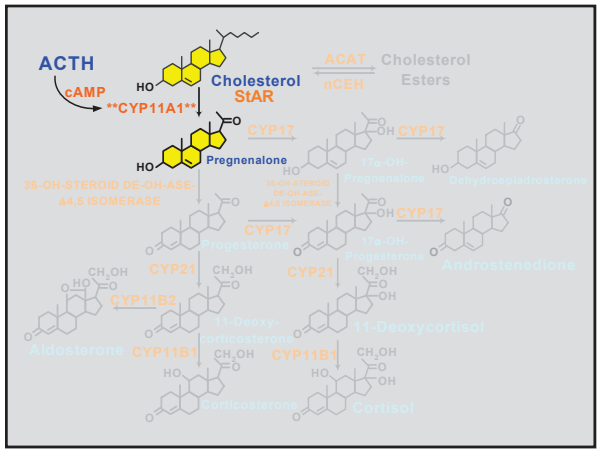
- Cholesterol is precursor
- Cytochrome P450 enzymes
  - Mitochondria, SER, Shuttling
- Secretion
  - Circadian rhythm
  - Nocturnal animals (rats, mice, cats): High at night
  - Daytime animals (dogs, humans): High in morning
  - Decreased secretion with age
- Bound in serum (90%) to CBG (transcortin)
- Metabolized in liver (hydroxylated and conjugated)

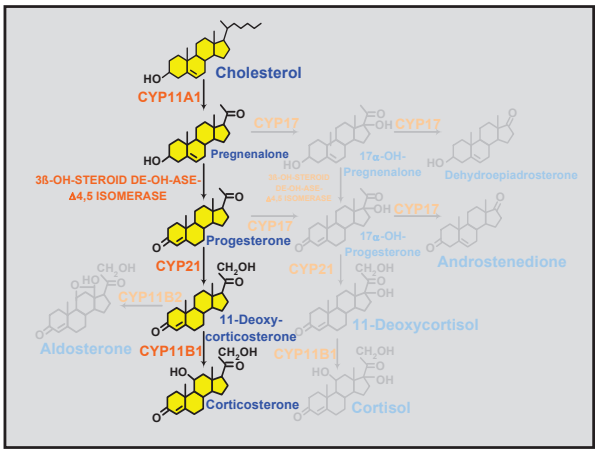
Major Glucocorticoids

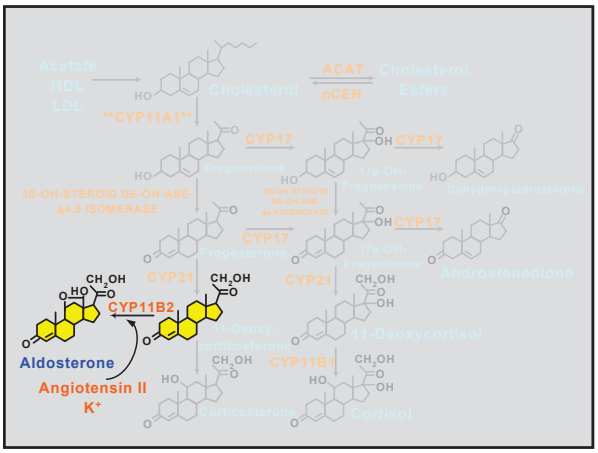
- Cortisol
  - Fish (teleosts)
  - Hamsters
  - Dogs (similar to humans)
  - Cats
  - Nonhuman primates
  - Humans
- Corticosterone
  - Amphibians
  - Reptiles
  - Birds
  - Rats
  - Mice
  - Rabbits (also have cortisol, increases during stress)

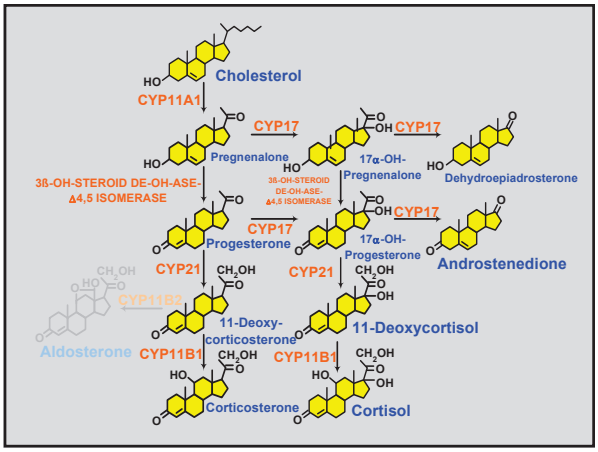


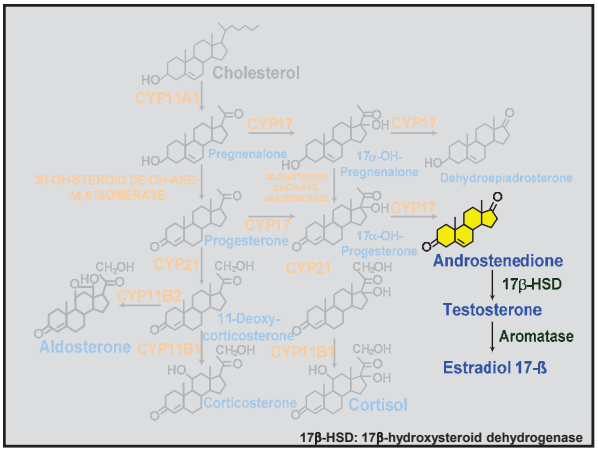
ACAT: acyl-CoA:cholesterol acyltransferase, nCEH: neutral cholesterol ester hydrolase

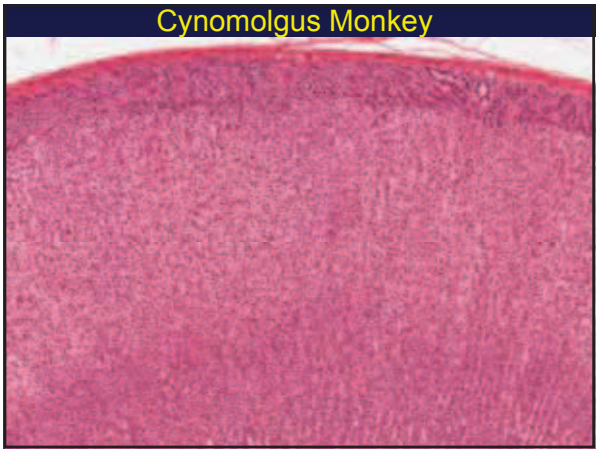


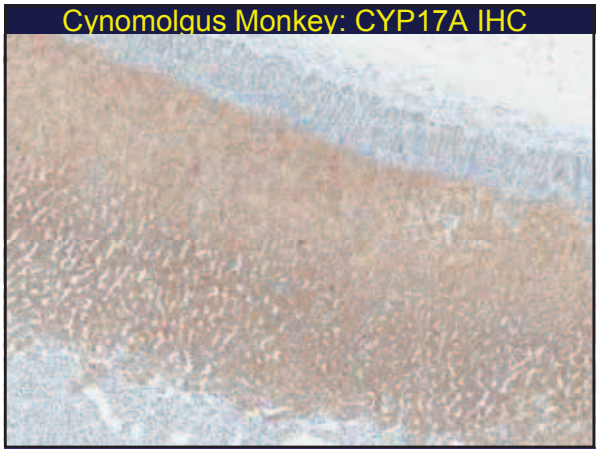












Cynomolgus Monkey: CYP11B2 IHC

Cynomolgus Monkey: Ki-67

Factors Contributing to Chemical Injury of the Adrenal Cortex

- Rich vascular supply
- High lipid content (steroidogenesis)
- Bioactivation by cytochrome P450 enzyme systems to reactive toxic forms
- Limited mechanisms of detoxification

Species Variations

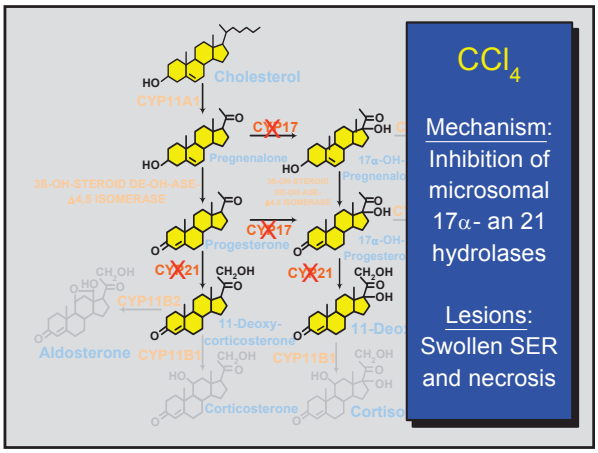
- Sensitivity varies according to species
- Variation in pathways of steroid metabolism
- Variation in xenobiotic metabolism
  - Dogs and humans are similar (e.g. *o,p'*-DDD)
  - Rats susceptible to DMBA

Ultrastructural Lesions

- May be more useful than histopathology
  - Correlates with mechanism of toxicity
  - Organelle-specific enzymes

Chemical-Induced Injury of the Adrenal Cortex

- **Selected Examples of Mechanisms:**
  - **Inhibition of microsomal enzymes**



Chemical-Induced Injury of the Adrenal Cortex

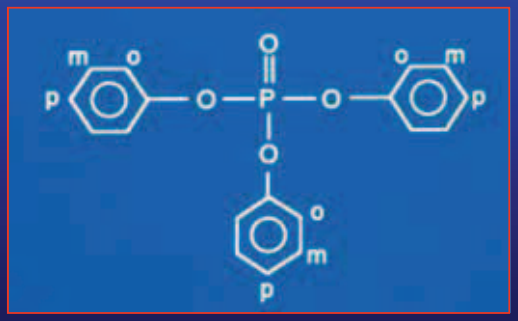
- **Selected Examples of Mechanisms:**
  - **Inhibition of neutral cholesterol ester hydrolase**

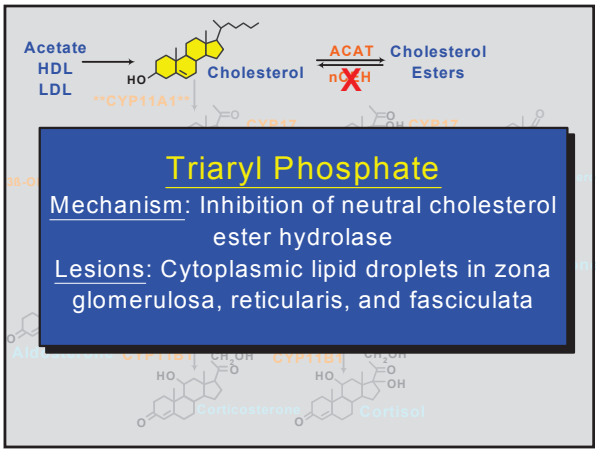
Inhibition of Neutral Cholesterol Hydrolase

Triaryl Phosphate

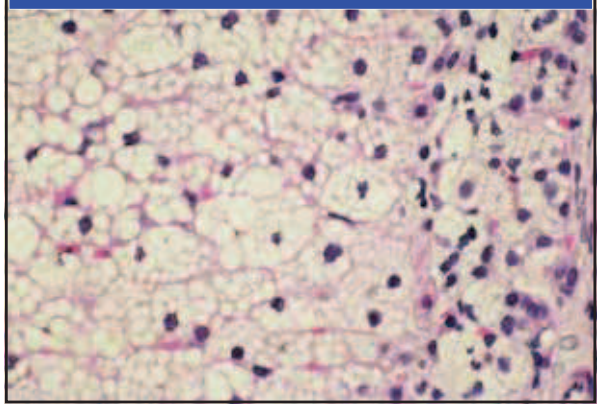
- Cholesterol lipidosis
- Adrenal cortical and ovarian interstitial cells

TRIARYL PHOSPHATES

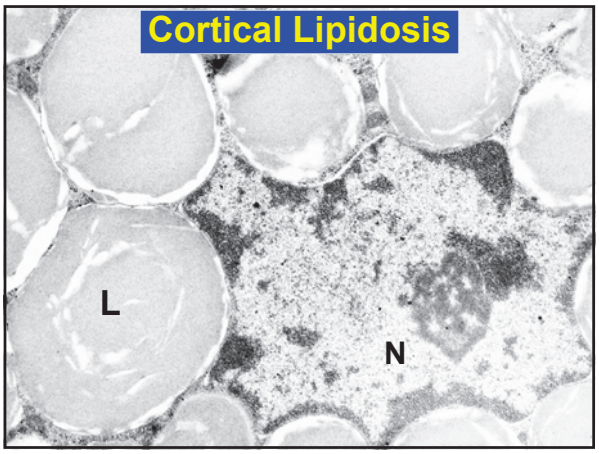




CORTICAL LIPIDOSIS: 3 WEEKS



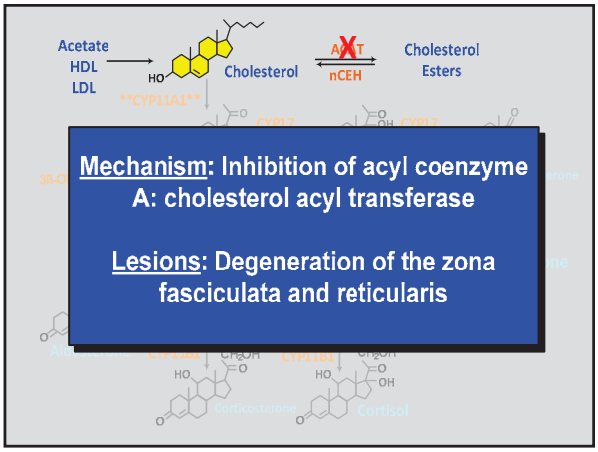
Cortical Lipidosis



Chemical-Induced Injury of the Adrenal Cortex

- **Selected Examples of Mechanisms:**
  - **Inhibition of ACAT* Enzyme in Cortical Cells**

*Acyl Coenzyme: Cholesterol Acyl Transferase



ACAT-Inhibiting Compounds

- **Degeneration of zonae fasciculata And reticularis**
  - **(↓) Mitochondria and SER**
- **Direct cytotoxic effect**
- **Species sensitivity:**
  - **Dog > Guinea Pig > Rabbit > Monkey > Rat**

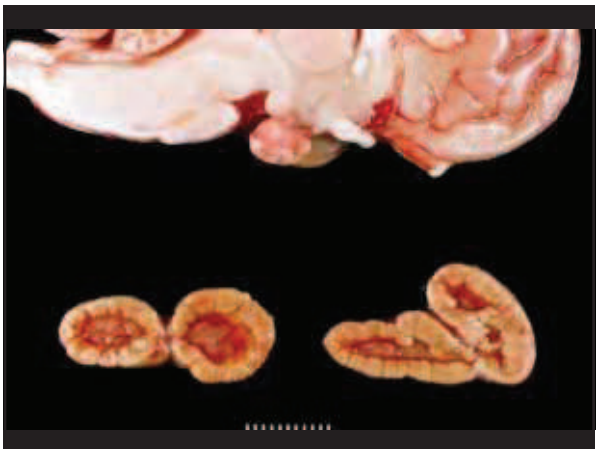
Chemical-Induced Injury of the Adrenal Cortex

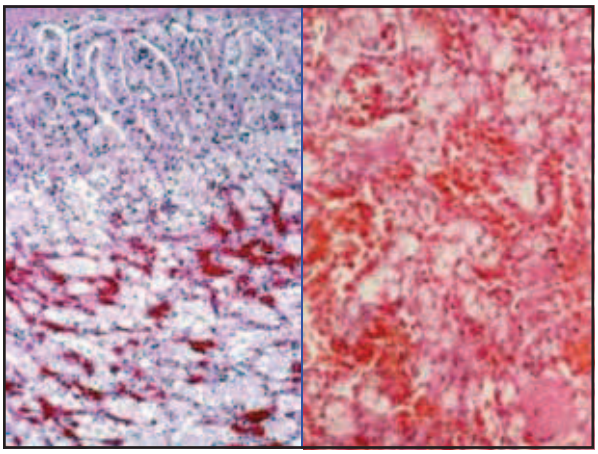
- **Selected Examples of Mechanisms:**
  - Selective mitochondrial degeneration

Selective Mitochondrial Degeneration with Vacuolation

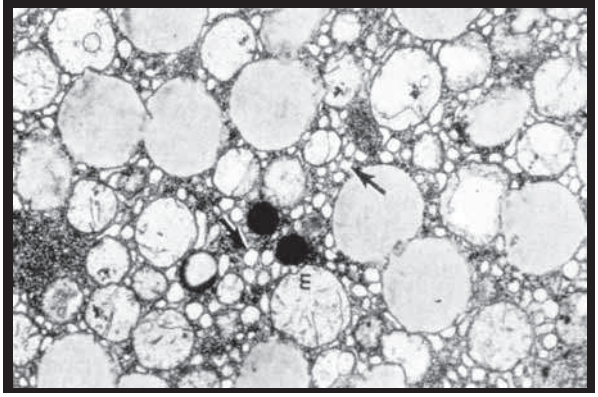
ORTHO, PARA, PRIME DDD (O,P'-DDD)

- **Zonae fasciculata and reticularis**
- **Zona glomerulosa much less sensitive**
- **Species susceptibility:**
  - **Dog unusually sensitive**

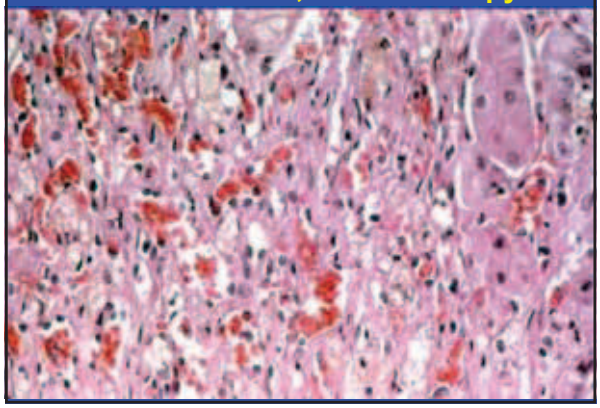




Vacuolation of Mitochondria



Cessation of O,P'DDD Therapy



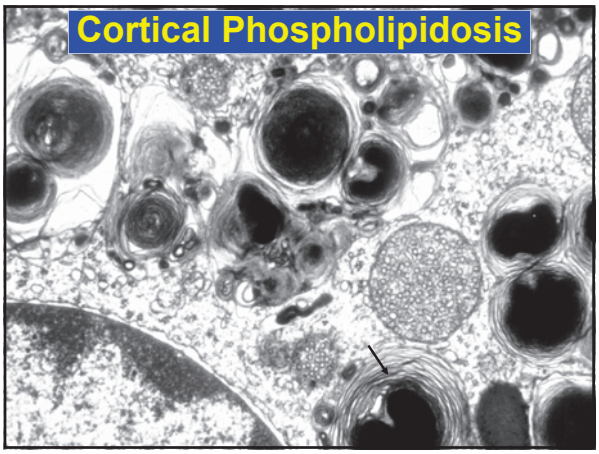
Chemical-Induced Injury of the Adrenal Cortex

- **Selected Examples of Mechanisms:**
  - Disruption of organellar membrane turnover

Disruption of Organellar Membrane Turnover

- Examples:
  - Cationic Amphiphilic Compounds (Chloroquine, Triparanol, Chlorophentermine)
  - Toxin activation of CYP P450 enzymes
- Lesion: Phospholipidosis of cortical cells (vacuolation, need EM)

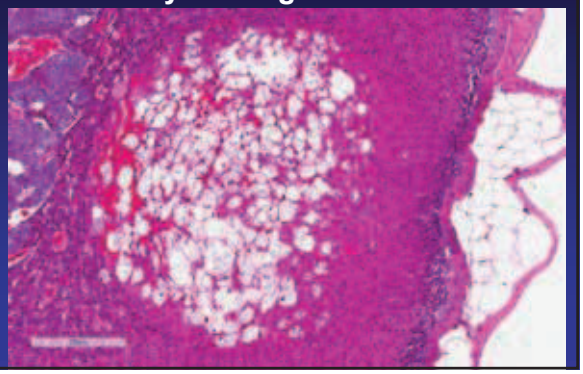
Cortical Phospholipidosis



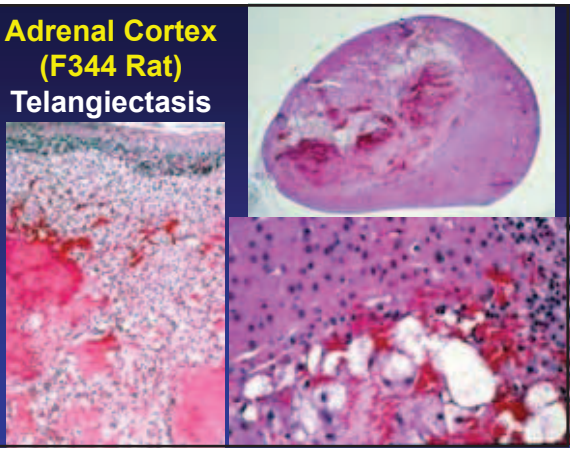
Spontaneous Degenerative Lesions of Adrenal Cortex

- Examples in Laboratory Rodents

Adrenal Cortex (Rat)
Cystic Degeneration



Adrenal Cortex (F344 Rat)
Telangiectasis

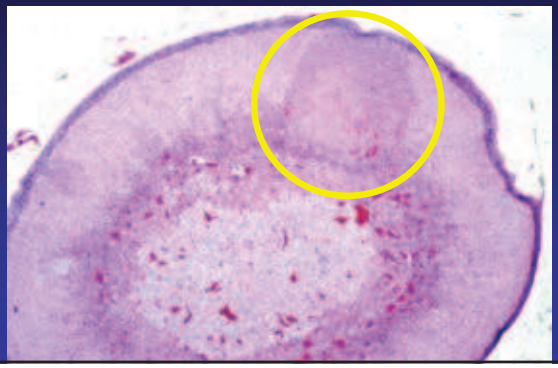


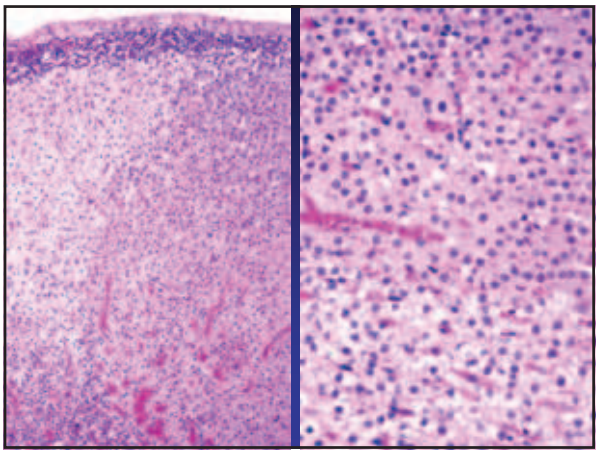
Proliferative Lesions of Adrenal Cortex

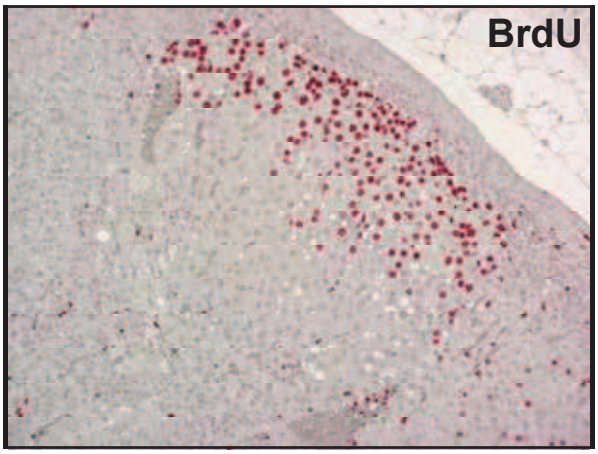
HYPERPLASIA

- Spindle Cell (Mice)
- Focal (Rats)
- Extracortical Nodular (Dog)

Adrenal Cortex (F344 Rat)
Focal (Eosinophilic) Hyperplasia

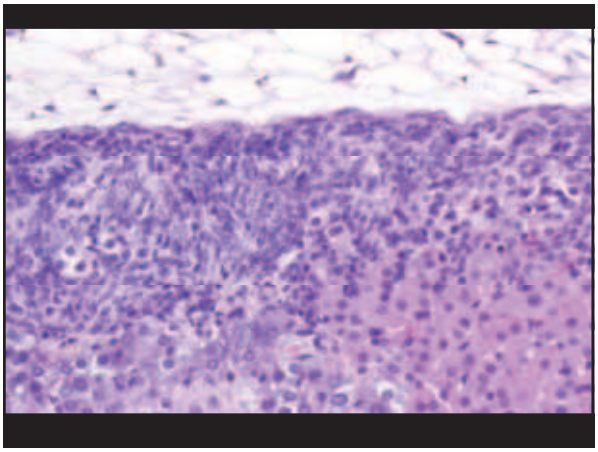






Subcapsular Spindle Cell Hyperplasia

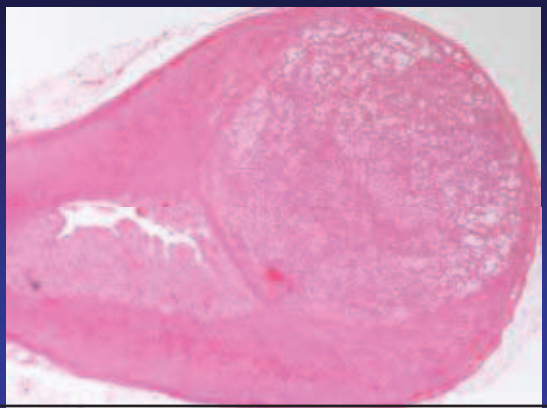
- Common in mice
- Infrequent in rats and hamsters



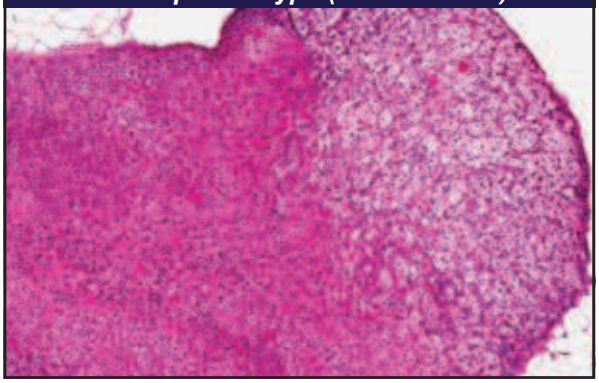
Proliferative Lesions of the Adrenal Cortex

NEOPLASIA

Adrenal Cortex Adenoma: Rat



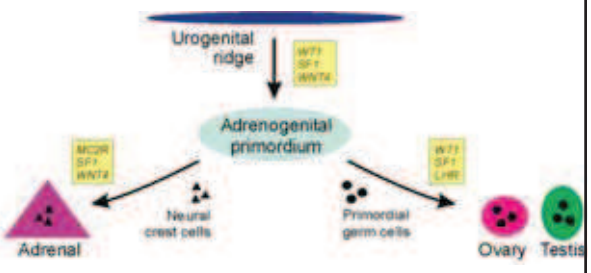
Adrenal Cortex Adenoma: Mouse
Subcapsular Type (A and B cells)



Mechanism of Induction of Cortical Neoplasia

- Hormonal Imbalance: (↑Gonadotropins, esp., ↑LH)
  - Gonadectomy
    - Species: Mice, Hamsters, Ferrets, Goats
  - Transgenic Mice Deficient in Inhibin
  - Estrogen Receptor Antagonists
  - Ionizing Radiation, Chemicals (DMBA)
    - Ovarian Follicle Destruction
    - (↓)E_2, (↑) LH

Development of the adrenal gland and gonads from the urogenital ridge



Bielinska M et al. Vet Pathol 2006;43:97-117

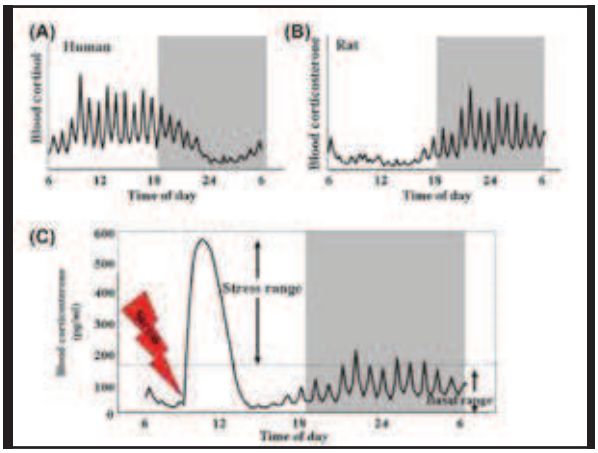
Adrenocortical Tumors
(Human Relevance)

- Humans
  - Adenomas: 5% of people over 50 years, incidentalomas, nonfunctional
    - Carcinoma is rare (500/yr in USA)
- Dogs: Similar to humans
  - Functional tumors more common
- Gonadectomy
  - Goats, ferrets, hamsters, and mice

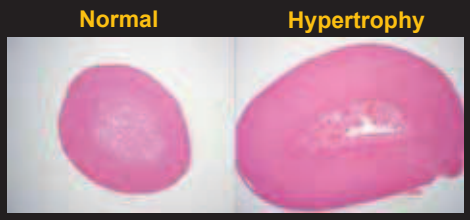
The Stress Response and the Adrenal Cortex

- Physiological response to an environmental change
- Activation of the HPA axis with increased glucocorticoids
- Maximizes survivability and suppresses nonessential functions
- Increased gluconeogenesis; decreased insulin sensitivity; diabetogenic
- Suppression of reproduction
- Regulation of immune function
- Increases activity and appetite

Interpreting Stress Responses during Routine Toxicity Studies:
A Review of the Biology, Impact, and Assessment



Rat Adrenal Glands



Chemicals as stressors

- The HPA response is not stressor-specific
- Chemicals can alter homeostatic balance
  - Organ effects
  - Metabolism effects
- High doses of test articles

Increased Adrenal Gland Weight (Adrenal Hypertrophy)

- Stress
  - Known exposure to stress-inducing stimuli
  - Histopathology (Morphology)
  - Concurrence with other stress-related changes (e.g., thymic atrophy)
  - Clinical chemistry
    - Functional challenge
- Differential Diagnosis: Toxic Effect
  - Histopathology (Morphology)
  - Clinical chemistry
    - Functional challenge (functional suppression)
  - Mechanistic studies of adrenal cell function (steroidogenic enzymes)

Adrenal Hypertrophy: Significance

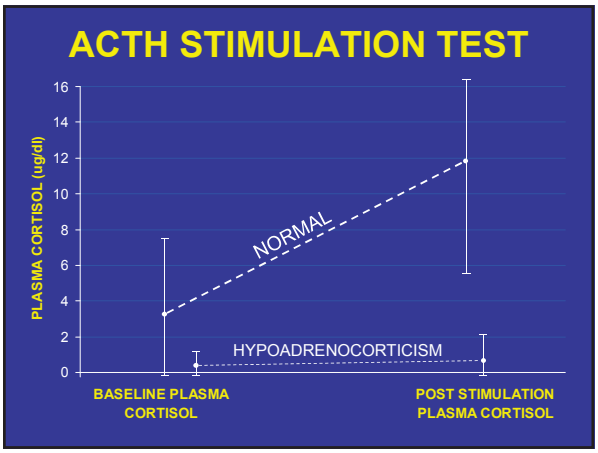
- Stress-Related
  - Primary and secondary stress-related effects
  - Less significant finding in toxicology study
  - Cause should be identified
  - Functional significance could be tested
- Test Compound-Related
  - Potentially serious
  - Understand mode of action
  - Human relevance
  - Species differences

Assessment of the HPA Axis

- ACTH challenge assay
  - Tests the integrity of the HPA axis through steroid hormone secretion
- Evaluation of *in vitro* mechanisms
  - H295R human adrenal cortical cells
    - Enzyme inhibition
      - CYP17, not well expressed in rodent adrenal glands
    - StAR function (steroidogenic acute regulatory protein; transport protein for cholesterol in mitochondria and rate limiting step in steroidogenesis)
    - Species differences in susceptibility to toxicants

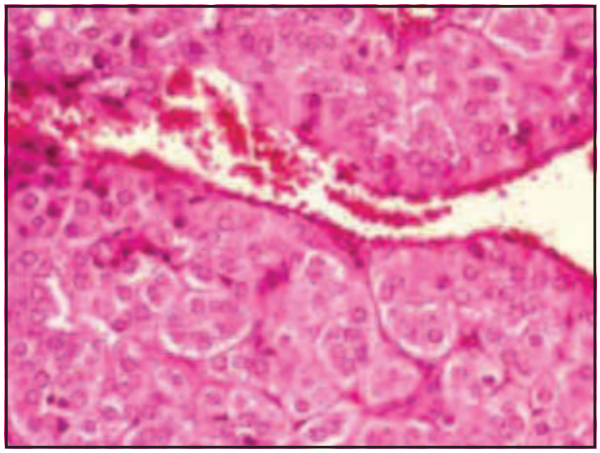
ACTH Stimulation Assay
Rodents

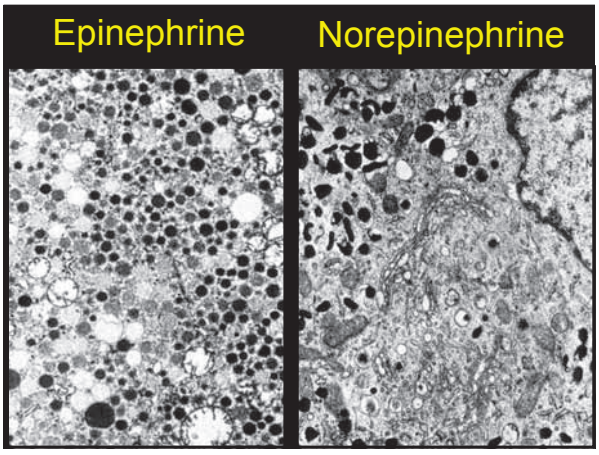
- Most sensitive time to measure corticosterone in rodents is about 7-10 a.m.
  - Trough of circadian rhythm
    - 12 hr light-dark cycle; starting at 6 a.m.
- Pre-test sample usually not collected (to avoid stress of blood collection)
- Post-ACTH sample at 0.5-2.0 hours

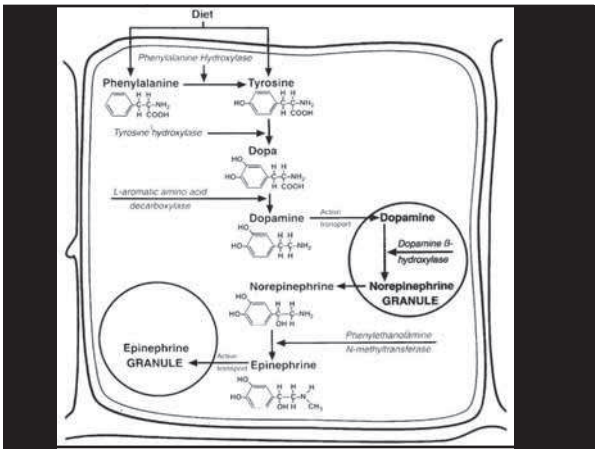


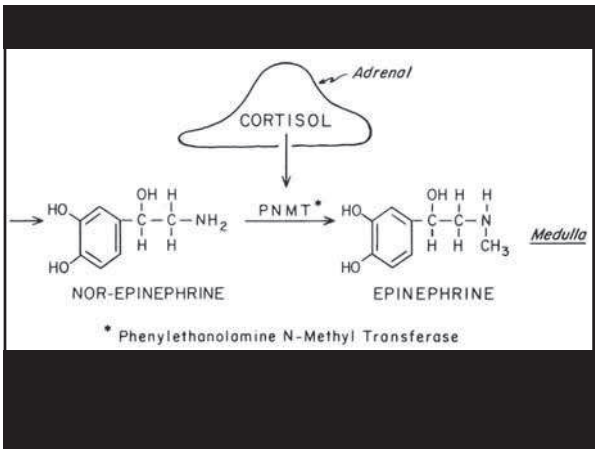
ADRENAL MEDULLA

- Less common site of toxic manifestations
- Proliferative lesions
  - Important in the rat
  - Less common in the mouse







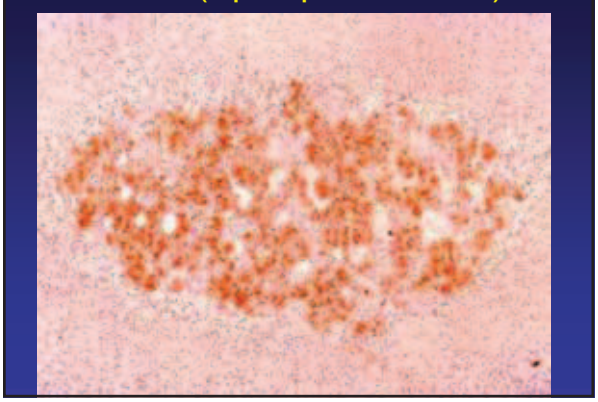


Immunohistochemistry

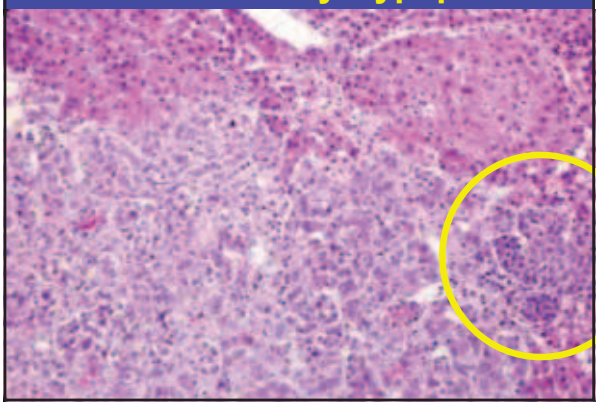
- Tyrosine hydroxylase: All chromaffin cells
  - Cytosolic (independent of granules)
  - All tumors produce this enzyme
- Chromogranin A: All chromaffin cells
  - Secretory Granules (variable in tumors)
- PNMT: Only Epinephrine cells
  - Cytoplasmic (variable in tumors)

Tyr Hydroxylase, Chromogranin A

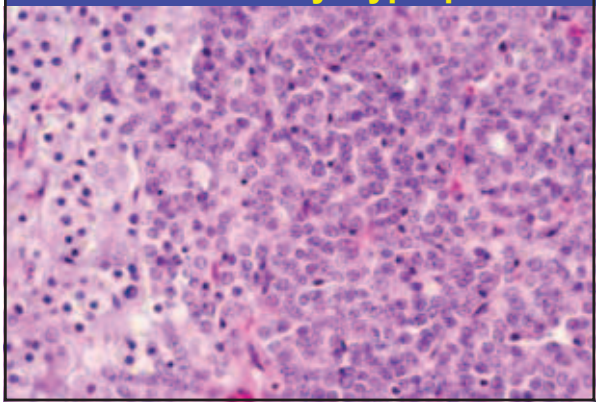
PNMT (Epinephrine Cells)



Focal Medullary Hypeplasia



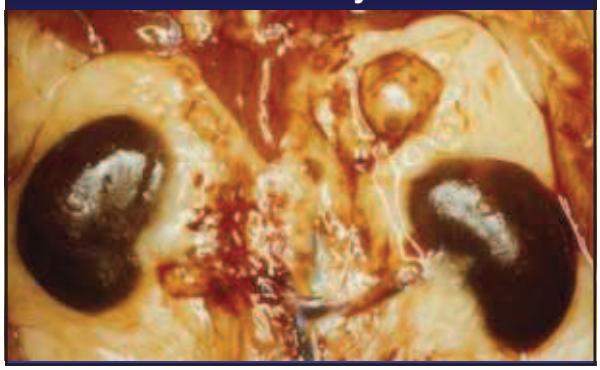
Focal Medullary Hyperplasia

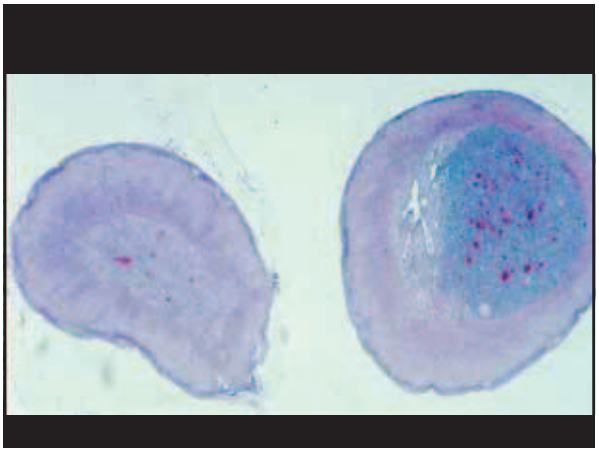


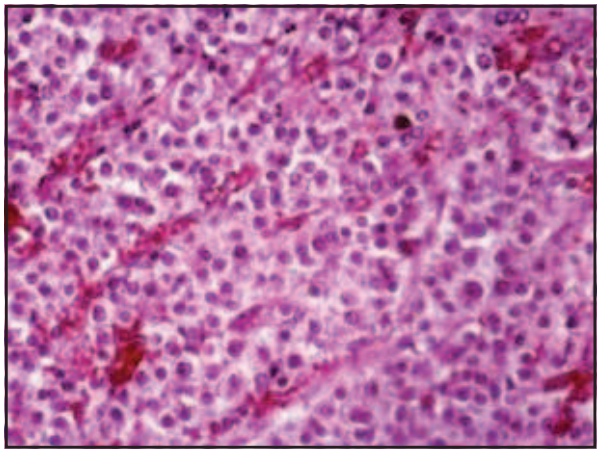
Adrenal Medulla: Neoplasms

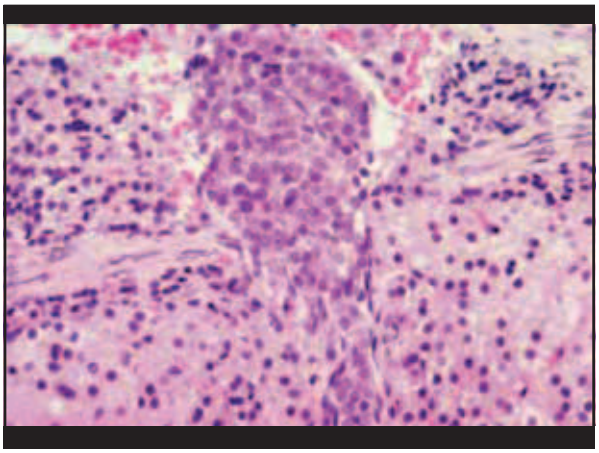
- Secretory Cells
  - Pheochromocytoma (benign)
    - May start as hyperplasia
    - Expandsile medullary mass
    - Well differentiated
    - Behavior
      - Space-occupying mass
      - Excess catecholamine secretion

Adrenal Medulla: F344 Rat
Pheochromocytoma









Interspecies Comparison of Pheochromocytomas

	RAT	MOUSE	HUMAN
• Lifetime Frequency	Up to 80%	<5%	<0.09%
• Sex Predilection	MALE	NONE	NONE
• Inducing Agents	HORMONES DRUGS, TOXINS DIETARY FACTORS	NONE	NONE
• Cell Type	NE	E+NE	E+NE

Factors Influencing Incidence of Pheochromocytomas in the Rat

- Age
- Strain
  - Holtzman 0.5%
  - Wistar 67%
- Sectioning technic
  - Single vs. serial
  - 7.5% of adrenal volume is medulla
- Chronic Stress, Ca^{2+}
  - Chronic renal and lung disease

Agents associated with Pheochromocytomas in Rats

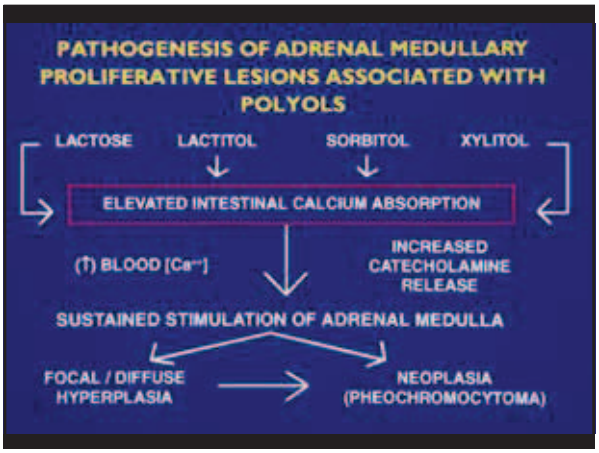
- Hypothalamic-Endocrine
  - Growth Hormone
  - Estrogen
  - Antithyroid Drugs
  - Alloxan
  - Neuroleptics
- Autonomic Nervous System
  - Nicotine
  - Reserpine

Agents associated with Pheochromocytomas in Rats

- Phosphodiesterase Inhibitors
  - Indolidan
  - Isomazole
- Dietary Factors
  - Excess Food
  - Excess Ca^{2+}, sugars, sugar alcohols

Agents associated with Pheochromocytomas in Rats

- Miscellaneous Factors
  - Gemfibrozil (Hypolidemic)
  - Zomepirone (Anti-inflammatory)
  - Diphenylamine (Anti-oxidant)
  - Retinol Acetate
  - 1,4, Dioxane
  - Ethylene Glycol Monoethyl Ether
  - P-chloroaniline
  - Radiation
  - Chronic Respiratory Disease

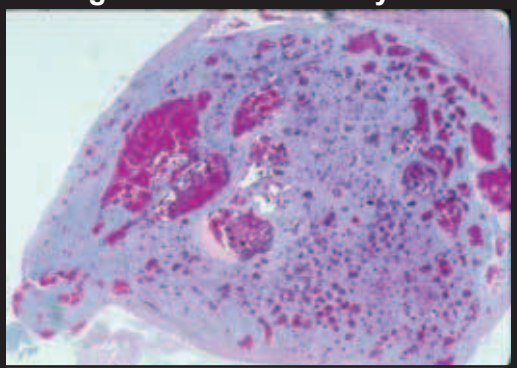


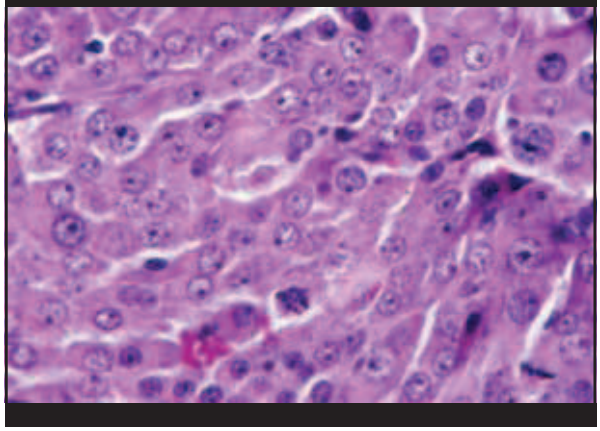
ADRENAL MEDULLA: NEOPLASMS

- Malignant Pheochromocytomas
  - Local invasion
  - Invasion into the posterior vena cava

Adrenal Medulla: F344 Rat

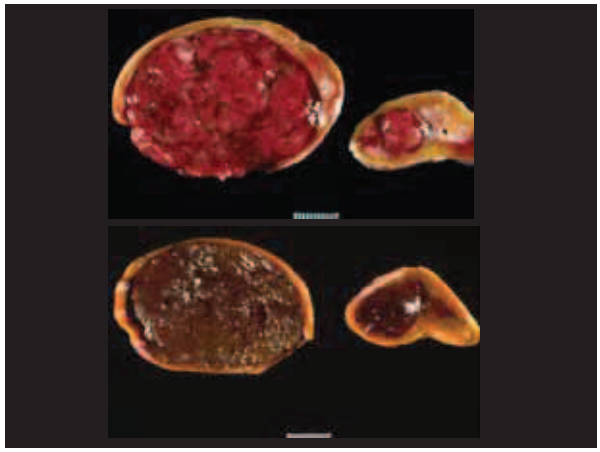
Malignant Pheochromocytoma

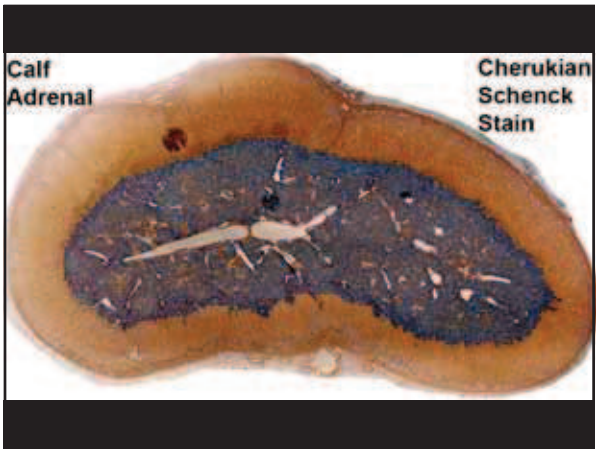




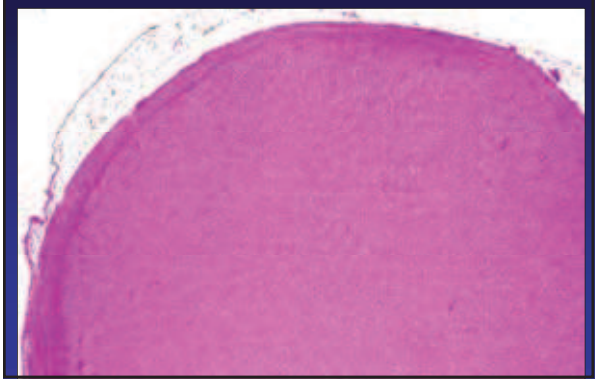
Differential Diagnosis of Adrenal Medullary and Cortical Tumors

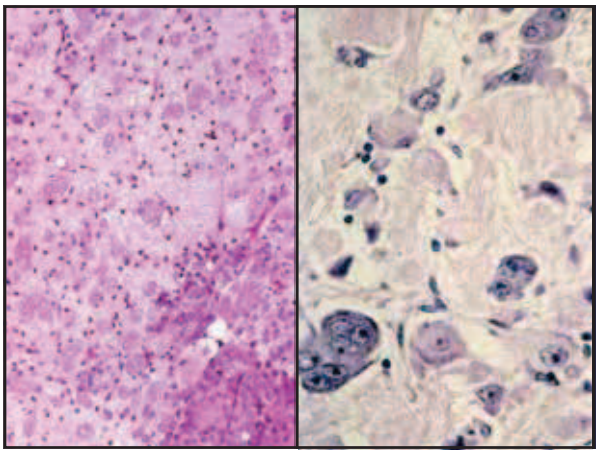
- Chromaffin Reaction
  - Dichromate fixative oxidizes catecholeamines to form brown-black pigment
  - Differentiate cortical and medullary neoplasms





Adrenal Medulla: Ganglioneuroma





Dual Differentiation ('Complex')
Pheochromocytoma & Ganglioneuroma