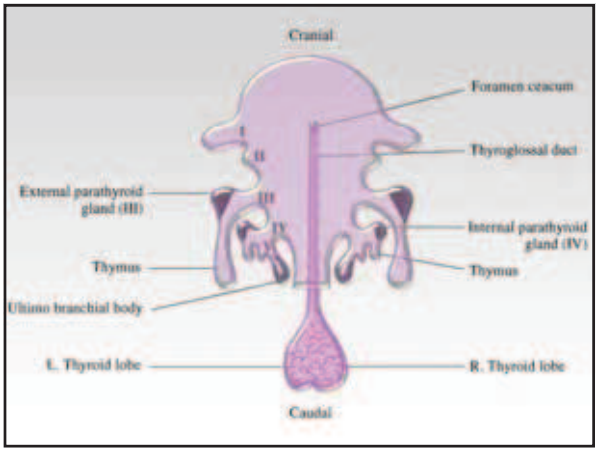
# Thyroid Follicular and C cells in Preclinical Toxicology

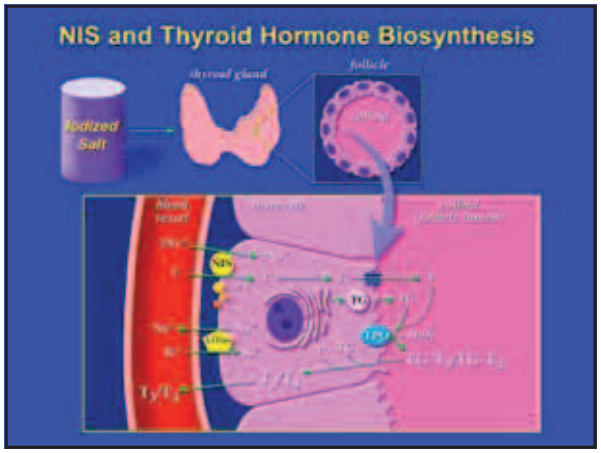
Thomas Rosol, DVM, PhD, DACVP
*Veterinary Biosciences*
*Ohio State University, Columbus, Ohio, USA*

01 November 2014
*STP-India Meeting*
*Bangalore*

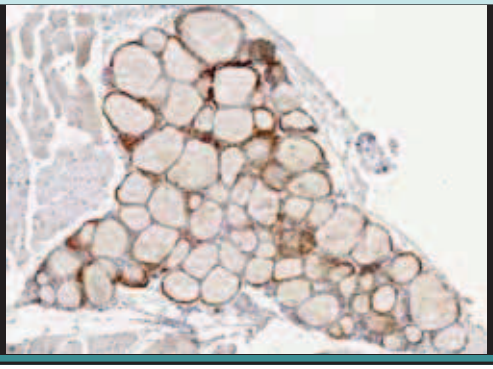
## Thyroid Gland

- Largest endocrine gland
- Devoted only to endocrine function
- Oldest gland phylogenically
  - All vertebrates, many invertebrates

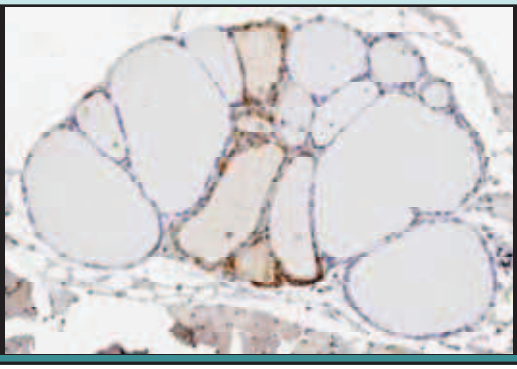


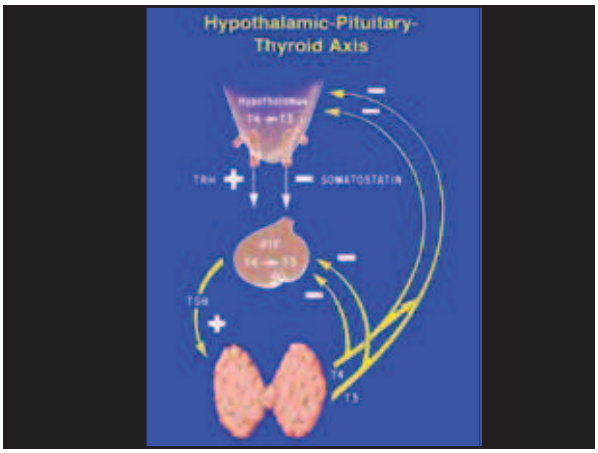



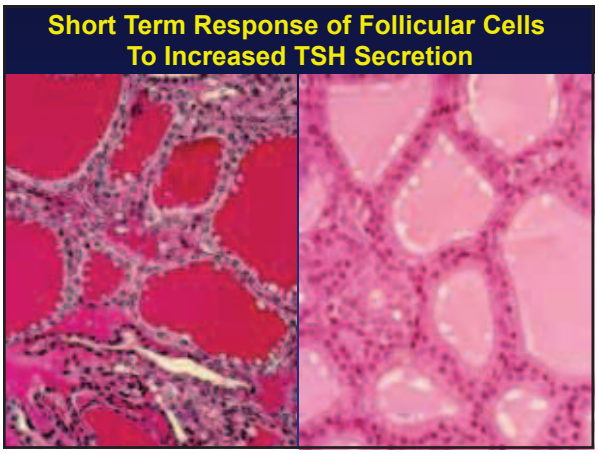

## NIS IHC: 2 mo. old Mouse

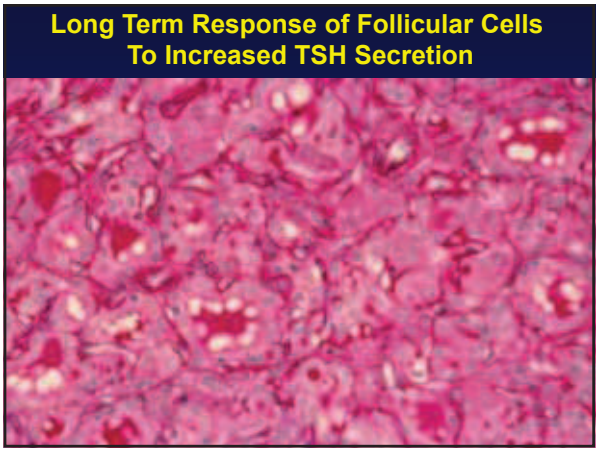


## NIS IHC: 9 mo. old Mouse






# Hypertrophy and Hyperplasia of Follicular cells

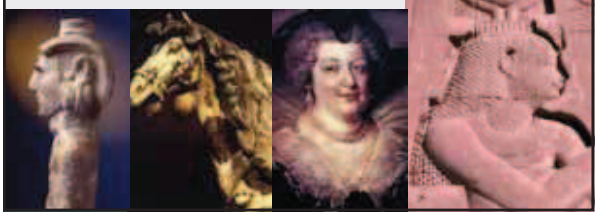
# (Goiter)

## Goiter

- Major human health problem
- Predominantly due to iodine deficiency
- Estimated over 200 million people affected
  - Adults
- Iodine deficient regions
  - E.g., Great Lakes in USA

## Goiter

- Ancient disease
  - Described for over 5000 years
- Historical interpretation
  - Sign of beauty
  - Punishment of gods



## Goiter: Outcome

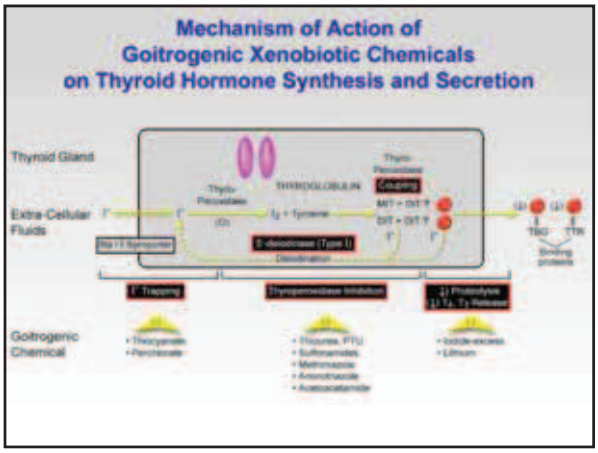
- Human
  - Diffuse Hyperplasia
- Rat
  - Diffuse hyperplasia
  - Focal hyperplasia
  - Neoplasia
- Dog
  - Diffuse hyperplasia

## Goiter: Causes

- Deficient iodine intake
- Goitrogenic chemicals
- Genetic enzyme defects
- Iodine excess

## Rat Thyroid Gland Tumorigenesis
### *Mechanisms to Disrupt Thyroid Function*

- Direct Thyroid Effect
  - Inhibit Hormone Synthesis
    - Inhibit iodide uptake; inhibit TPO
  - Inhibit Hormone Secretion
  - Follicular Cell Cytotoxicity
- Peripheral Effect
  - Competition of thyroid hormone binding proteins
  - Inhibition of $T_4$ deiodination
  - Increased metabolism and clearance of $T_4$ or $T_3$




## Thyroid Follicular Neoplasia
### *Humans*

- Thyroid cancer: Most common endocrine malignancy
  - Incidence has risen in past 4 decades
  - Uncommon deaths
- Thyroid nodules are common
  - Palpable: 4-7% of adults
  - Ultrasound: up to 67%, usually women
  - Most are benign
  - 5-15% are malignant

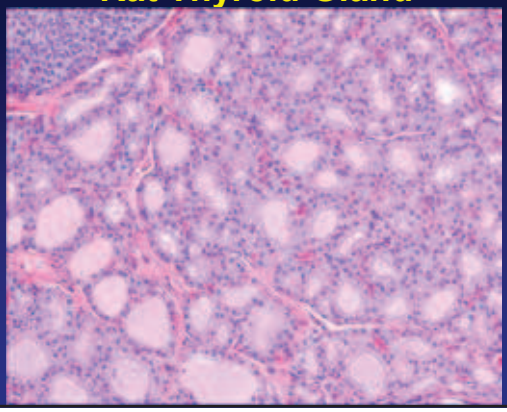
## Human Relevance Framework
### *Rat Thyroid Follicular Tumors*

- Fundamental differences in thyroid hormone economy in rats
  - Rapid half-life of $T_4$
  - Lack of thyroid binding globulin
  - High TSH concentrations (greater in males)
  - Low secretion rate of $T_4$ (inherently less able to make $T_4$ compared to humans)
  - Sensitive to the tumorigenic effects of drugs that decrease $T_4$ or $T_3$
  - Robust TSH response decreased $T_4$ or $T_3$

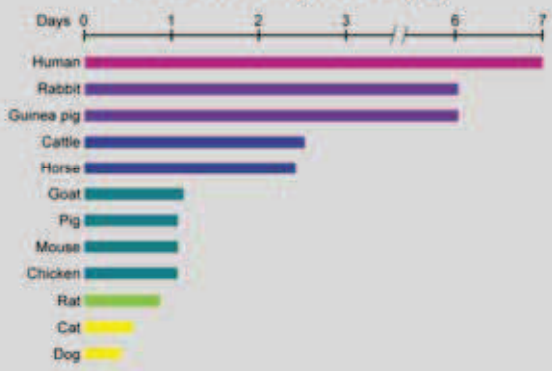
## Human Relevancy Framework
### *Thyroid Hormone Economy*

- Rats (esp. males) have increased incidence of proliferative lesions compared to humans likely due to increased TSH concentrations
  - Male rats have higher TSH compared to females
- Rats have shorter half-life of $T_4$ (12-24 h) vs. 5-9 days in humans
  - Due to less high affinity binding globulin, TBG, in rats
- Rats require greater $T_4$ (20 µg/kg) compared to humans (2.2 µg/kg) to substitute for the thyroid gland

## Rat Thyroid Gland



## Half-Life of Thyroxine ($T_4$)




## Thyroxine ($T_4$) Serum Protein Binding

| Species | TBG | Postalbumin | Albumin | Prealbumin |
|---|---|---|---|---|
| Human | ++ | - | ++ | + |
| Monkey | ++ | - | ++ | + |
| Dog | ++ | - | ++ | - |
| Mouse | - | ++ | ++ | - |
| Rat | - | + | ++ | + |
| Chicken | - | - | ++ | - |

TBG: Thyroxine ($T_4$) Binding Globulin
Prealbumin (Transthyretin, TTR)

## Drug-Induced Tumors in Rodents
### *(order of prevalence)*

| RATS | MICE |
|---|---|
| • Thyroid | • Liver |
| • Liver | • Lung |
| • Testis | • Mammary Gland |
| • Mammary Gland | • Blood |
| • Adrenal | • Ovary |
| • Pituitary | |

## Tumorigenic Drugs in Rats (1)

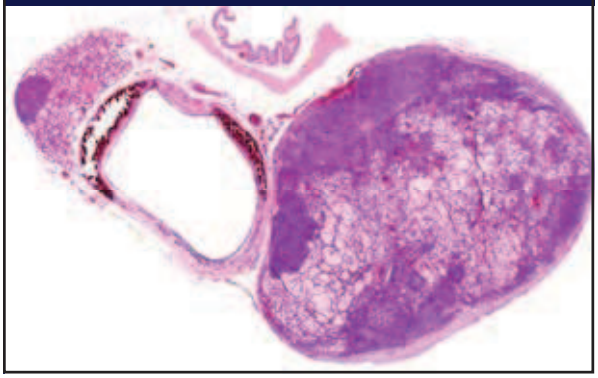
| Drug | Product Class |
|---|---|
| Amiodarone | Antiarrhythmic |
| Atenolol | β-Adrenergic Blocker |
| Bepridil | Ca-Channel Blocker |
| Dapsone | Antineoplastic |
| Griseofulvin | Antibiotic |
| Iodinated Glycerol | Expectorant |
| Methimazole | Anti-Thyroid |
| Midazolam | Sedative |

## Tumorigenic Drugs in Rats (2)

| Drug | Product Class |
|---|---|
| Phenobarbital | Antiepileptic |
| Minocycline | Antibiotic |
| Oxazepam | Antianxiety |
| Nicardipine | Ca-Channel Blocker |
| Sertraline | Antidepressant |
| Simvastatin | Hypolipidemic |
| Spironolactone | Diuretic |
| Vidarabine | Antiviral |

## Follicular Adenoma: Rat

## Follicular Carcinoma: Rat



## Inhibition of Hormone Synthesis
### *Inhibition of Iodide Uptake*

- Competition for NaI Symporter (NIS)
  - Thiocyanate
  - Perchlorate ($ClO_4^-$)
    - Rats more sensitive than humans, mice, rabbits

## Inhibition of Hormone Synthesis
### *Inhibition of Thyroperoxidase (TPO)*

- Organification of $I_2$ to tyrosine and coupling of iodotyrosines
  - Thiourea: reduces $I_2$ to $I^-$
- Inhibition of TPO
  - Thioamides
    - Propylthiouracil
    - Mercaptoimidazole
    - Methimazole, carbimazole, aminotriazole
    - Sulfonamides, such as sulfamethazine
  - Sulfonylureas (antidiabetic drugs)
    - 1st generation: acetohexamide, chlorpropamide, tolbutamide, tolazamide
  - Substituted phenols
    - Resorcinol, salicylamide

## Species Sensitivity to TPO inhibition by Sulfonamides

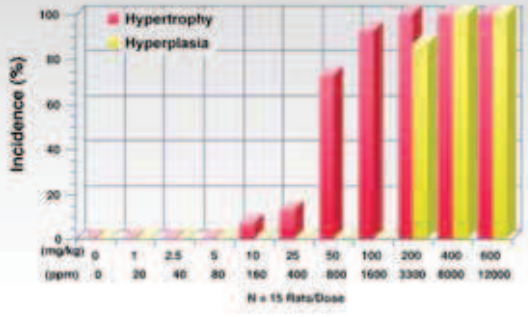
**Sensitive Species**

- Rat
- Mouse
- Dog
- Pig

**Resistant Species**

- Humans
- Primates
- Guinea pig
- Chicken

## Follicular Cell Hypertrophy and Hyperplasia in Male Sprague-Dawley (CD:RD) Rats Administered Sulfamethazine for 4 Weeks



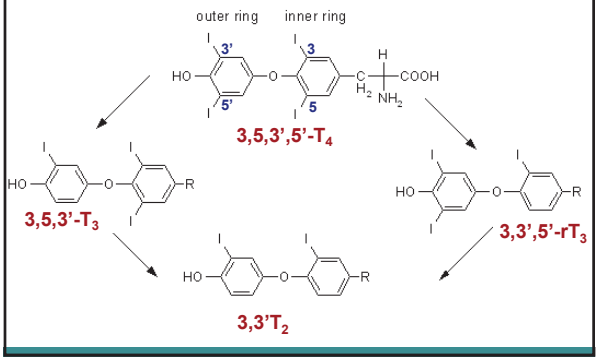

## Inhibition of Hormone Secretion
### *Excess of Iodide, Lithium*

- Excess of iodide
  - Decreased lysosomal proteases (humans)
  - Inhibition of colloid droplet formation (rats, mice)
  - Inhibition of TSH-mediated cAMP (dogs)
  - Excessive maternal intake of iodine
    - Goiter in offspring not adult
- Lithium
  - Inhibits colloid droplet formation by cAMP
  - Inhibits hormone release

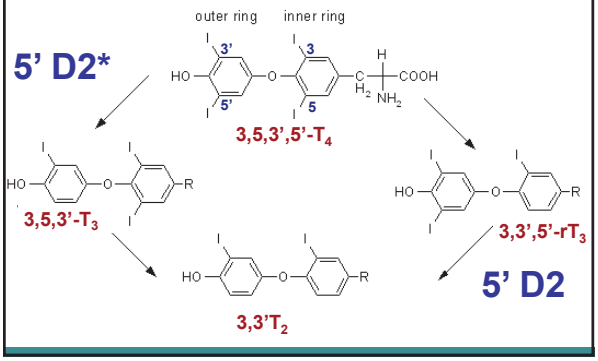
## Competition for Thyroid Hormone Binding Proteins

- Less important in species with TBG
- Binding to prealbumin (transthyretin)
  - Chlorophenols, chlorophenoxy acids, nitrophenols
- Decreased $T_4$ in rats
  - Pentachlorophenol, 2,4-dichlorophenoxyacetic acid (2,4-D), dinoseb, and bromoxynil
- Decreased $T_3$ in rats
  - Bromoxynil
- Decreased $T_4$ and $T_3$ in rats
  - 2,4-D

## Thyroid Hormone Deiodination
### *Activation and Metabolism*




## Thyroid Hormone Deiodination
### *5' (outer ring) - Deiodinase 2*

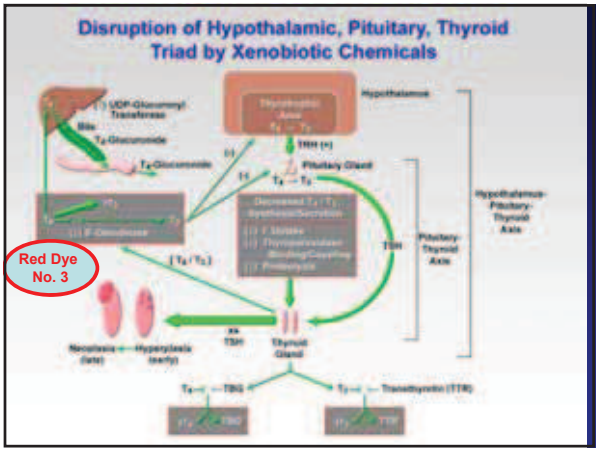


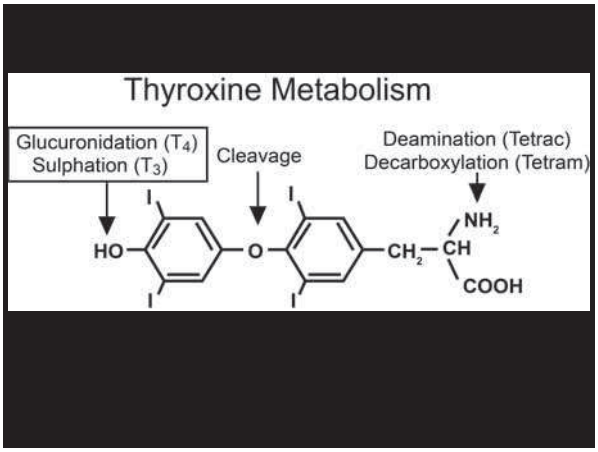
## Thyroid Hormone Deiodination
### *Activation and Metabolism*

- Deiodinase 1 (liver, kidney, thyroid)
  - Outer and inner ring deiodination
  - Substrates: $rT_3$>>$T_4$, $T_3$
  - Inhibited by propylthiouracil
  - Stimulated by $T_3$
- (5')-Deiodinase 2 (brain, pituitary, placenta, thyroid, skeletal muscle, brown fat)
  - Outer ring deiodination only
  - Major activating enzyme
  - Substrates: $T_4$ > $rT_3$
- Deiodinase 3 (brain, pregnant uterus, fetus, placenta)
  - Inner ring deiodination
  - Substrates: $T_3$ > $T_4$

## Inhibition of Thyroxine ($T_4$) Deiodination

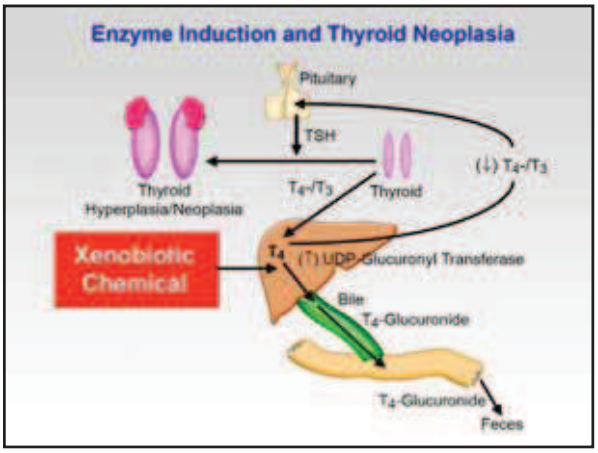
- $T_4$ functions as a prohormone
- Selenium deficiency
  - Se: cofactor for type I 5'-monodeoidinase
  - Lack of Se leads to decreased $T_3$ and increased $T_4$
- FD&C Red No. 3 and iopanoic acid
  - Inhibits type I 5'-monodeiodinase
  - Rats: Increased $T_4$, decreased $T_3$, increased reverse $T_3$, increased TSH
- Lipid peroxidation
  - Type I 5'-monodeiodinase




## UDP-GT
### *Uridine 5'-diphospho-glucuronosyltransferase*

- Important Phase II conjugative enzyme
  - Elimination of drugs and foreign chemicals
  - Not present in cats
- Transfers gluronosyl from uridine 5'-diphospho-glucuronic acid to lipophilic substrates with O, N, S, or carboxyl groups
- Increases water solubility for renal excretion



## Inducers of UDP-GT
***Examples***

- Phenobarbital (PB)
- Pregnenolone-16$\alpha$-carbonitrile (PCN)
- 3-methylcholanthrene (3MC)
- Arochlor 1254 (PCB)

## Effects of Microsomal Enzyme Inducers in Rats

| | PB | PCN | 3MC | PCB |
|---|---|---|---|---|
| $T_4$-UDP-GT | ↑ | ↑↑ | ↑↑ | ↑↑ |
| $T_3$-UDP-GT | ↑ | ↑ | ↔ | ↔ |
| Serum $T_4$ | ↓ | ↓↓ | ↓↓ | ↓↓↓ |
| Serum $T_3$ | ↔ | ↔ | ↔ | ↔ |
| Serum TSH | ↑ | ↑↑ | ↔ | ↔ |
| Thyroid Cell Proliferation | ↑ | ↑↑ | ↔ | ↔ |

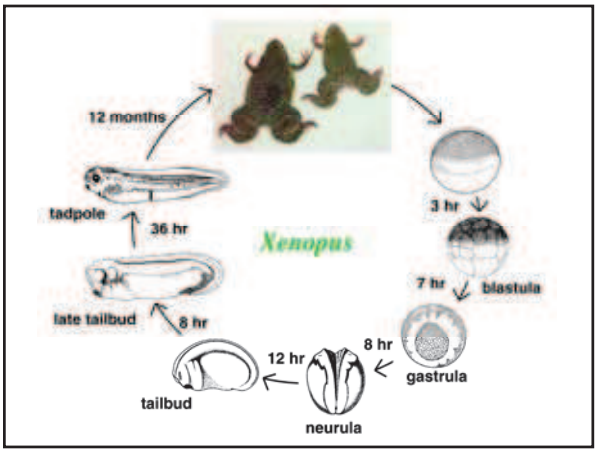
*CD Klaassen, Tox. Pathol., 2001*

## Endocrine Disrupters (EDCs)
***Thyroid Gland***

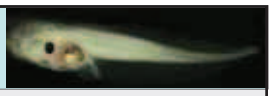
- Endocrine Disrupter Chemicals
  - (anti)-estrogenic compounds
  - (anti)-androgenic compounds
  - Interference with steroidogenesis
  - Dysregulation of thyroid hormones
    - Similar mechanisms as preclinical drug toxicity in rats
    - Assays
      - Rats (28-day, pubertal, adult male)
      - Amphibian Metamorphosis Assay

## Amphibian Metamorphosis Assay

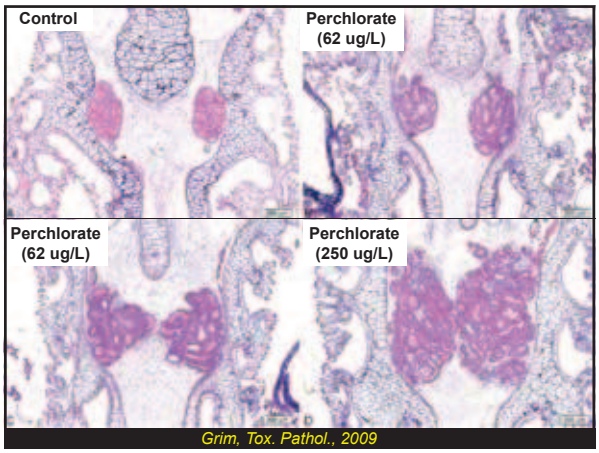
- Detect substances that interact with hypothalamic-pituitary-axis during development
  - Conserved thyroid structure in vertebrates
- Metamorphosis of *Xenopus Laevis* (African Clawed Frog) tadpoles
- Test for Endocrine Active Substances (EAS)
- OECD (Org. Economic Co-operation & Development guidelines (www.oecd.org/dataoecd/44/52/40909207.pdf)



## Nieuwkoop and Faber (N&F) Stages



- 21-day test
  - Hind limb length, development stage, thyroid histology
- Nieuwkoop and Faber (N&F) staging
  - 45-49: First form tadpole
  - 49-56: Second form tadpole
  - 56-60+: Third form tadpole




*Grim, Tox. Pathol., 2009*


*Grim. Tox. Pathol., 2009*

## Human Relevance Framework

***Thyroid Follicular Tumors: MOA & Key Events***

↓ T4 or T3 (many mechanisms)

↓ Inhibition of thyrotropes in pituitary

↑ TSH secretion, thyrotrope hyperplasia

Thyroid follicular cell hypertrophy

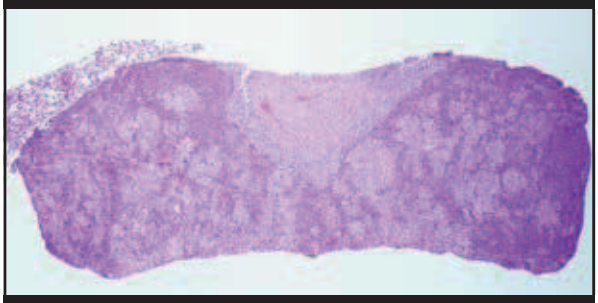
Colloid depletion

Increased cell proliferation

Hyperplasia

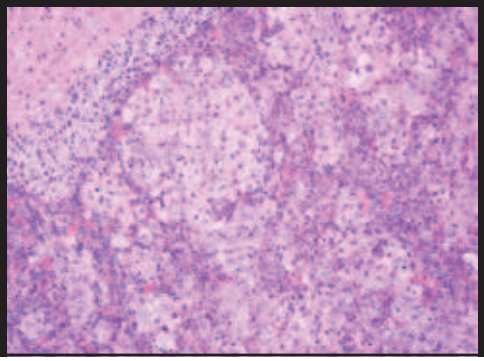
## Mouse: TSH Cell Hyperplasia

(chemical inhibition of thyroxine synthesis)



## Mouse: TSH Cell Hyperplasia

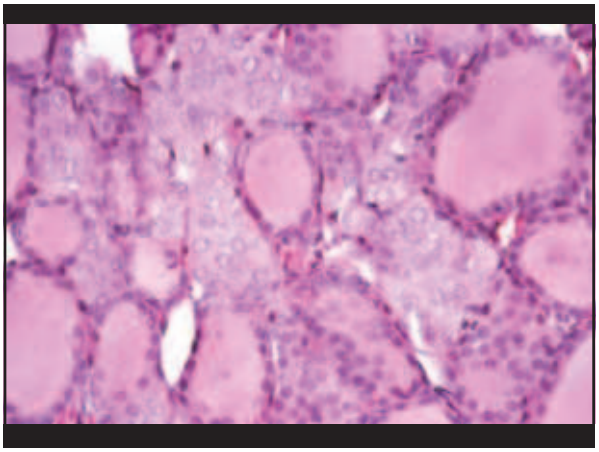
(chemical inhibition of thyroxine synthesis)

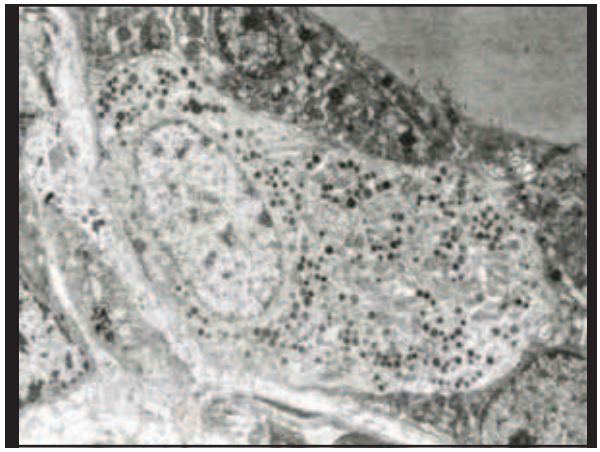


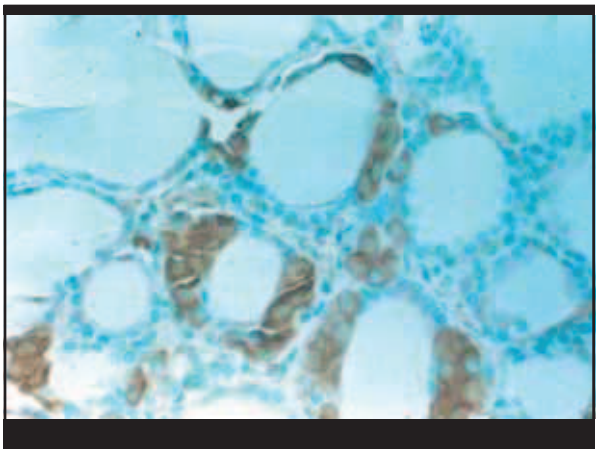
## Human Relevance Framework

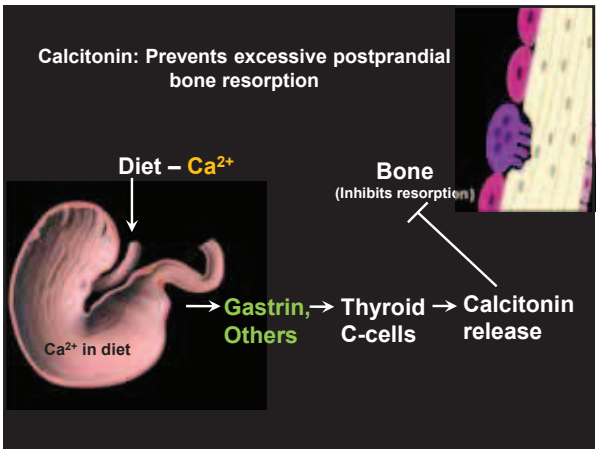
*Thyroid Follicular Tumors: MOA & Key Events*

- Hyperplasia
- Adenoma
- Carcinoma (death)
- Metastasis (death)








## Pathophysiology of Calcitonin *Mammals*

- No clinical conditions associated with calcitonin excess or deficiency
- Biomarker: C-cell hyperplasia, tumors
- Drug Tx: Hypercalcemia, osteoporosis

## Calcitonin Content in Endocrine Glands

| Gland | Calcitonin Activity (MRC units/mg) |
|---|---|
| • Utimobranchial | 4000-6000 |
| • Salmon | |
| • Chicken | |
| • Thyroid | 100-200 |
| • Pig | |
| • Sheep | |
| • Human | |

## Therapeutic Uses of Calcitonin

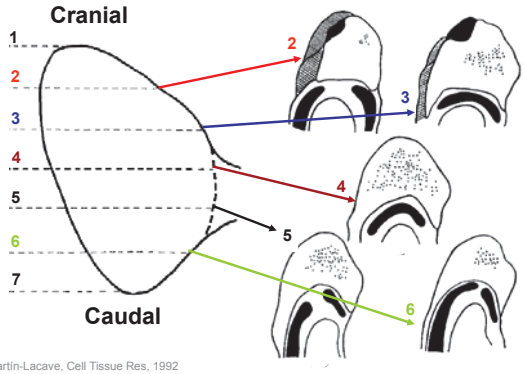
- Osteoporosis
- Analgesia in vertebral fractures
- Hypercalcemia - short lived effect

Salmon calcitonin
100 times more potent than human calcitonin

## C-Cell Numbers

- Humans
  - < 1% of thyroid cells
  - May decrease with age
- Rats
  - 5% of thyroid cells
  - Increase to 10% after 120 days
  - Individual cells (intrafollicular and parafollicular)
  - Interfollicular clusters
    - increase in number and size with age

## C-Cell Distribution: Rat Thyroid Gland




Martín-Lacave, Cell Tissue Res, 1992

## Thyroid C-Cell Tumors
### *Species Occurrence*

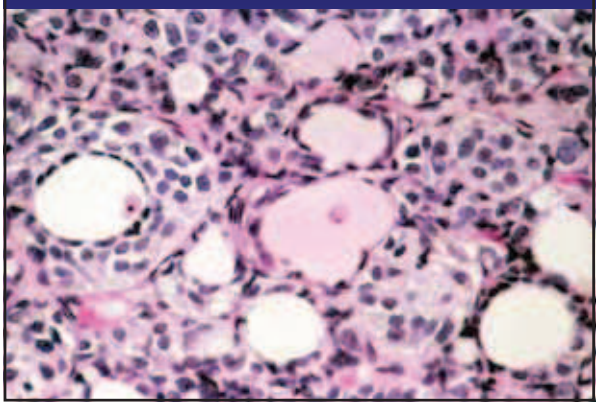
- Rat (F344, SD, *WAG/Rij* )
  - Higher incidence in females (Wistar)
    - OVX decreases CT synthesis and secretion
- Bull (ultimobranchial)
- Dog
- Mouse
- Horse
- Ferret (with islet cell tumors)
- Mouflon (sheep)
- Zebrafish (ultimobranchial)

## Factors Influencing Development

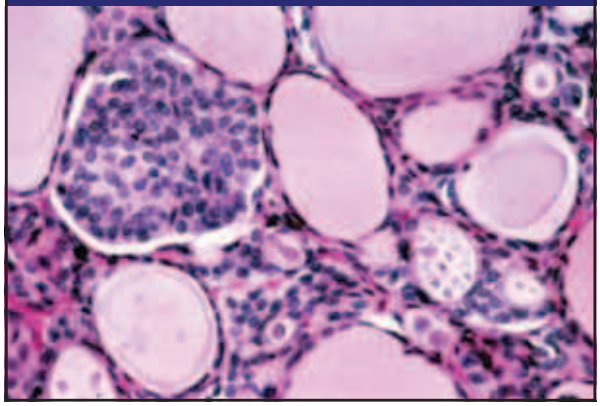
### Thyroid C-Cell Proliferative Lesions

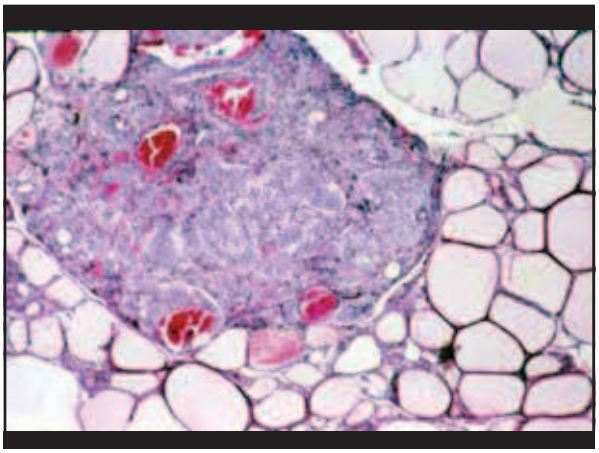
- **Irradiation**
- **Vitamin D**
- **High Dietary Calcium**

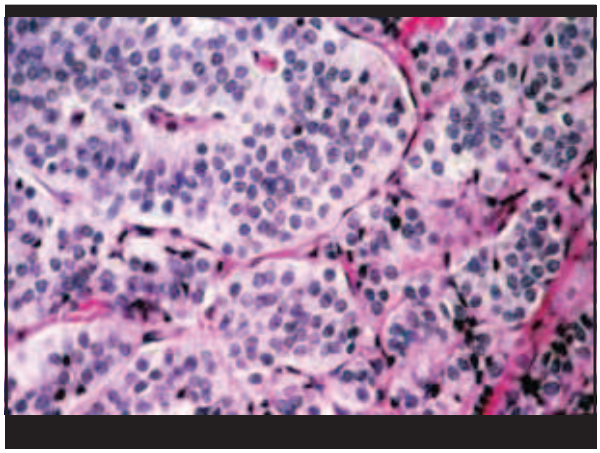
**Diffuse C-Cell Hyperplasia**

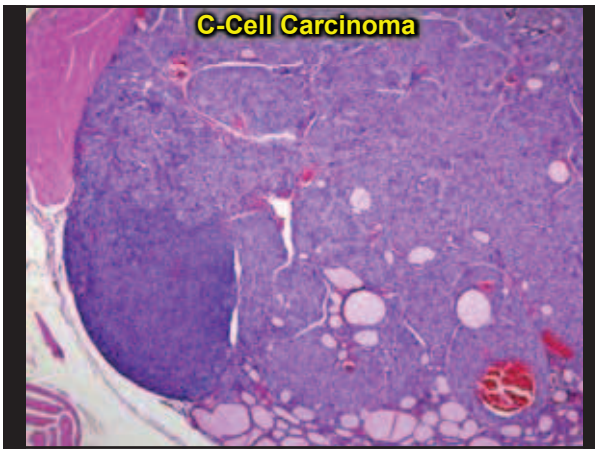


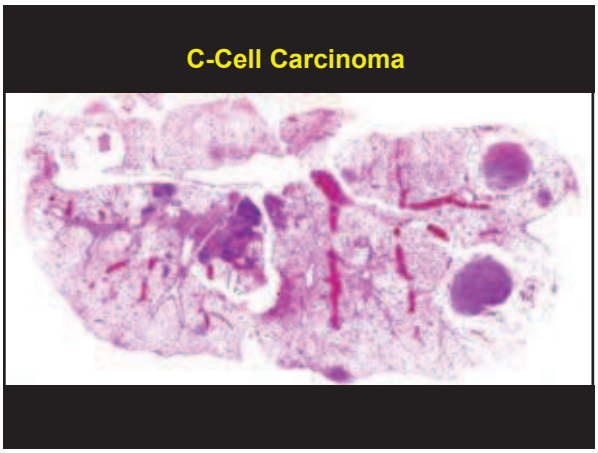
**Focal C-Cell Hyperplasia**

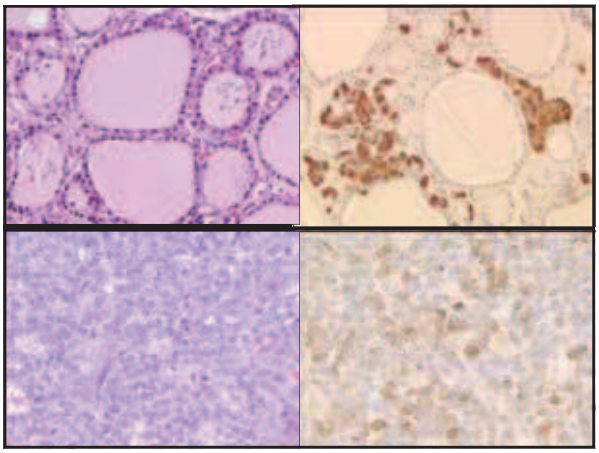






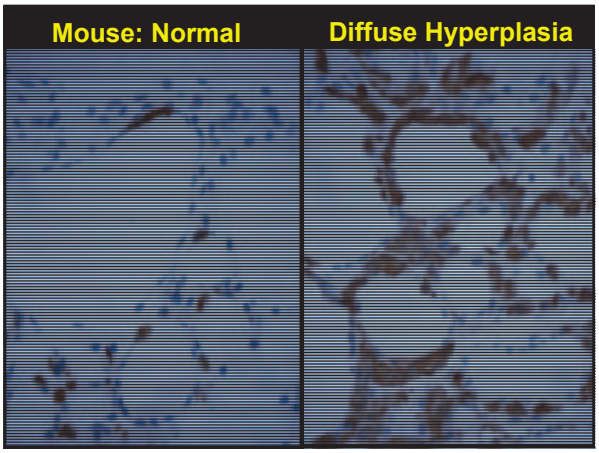


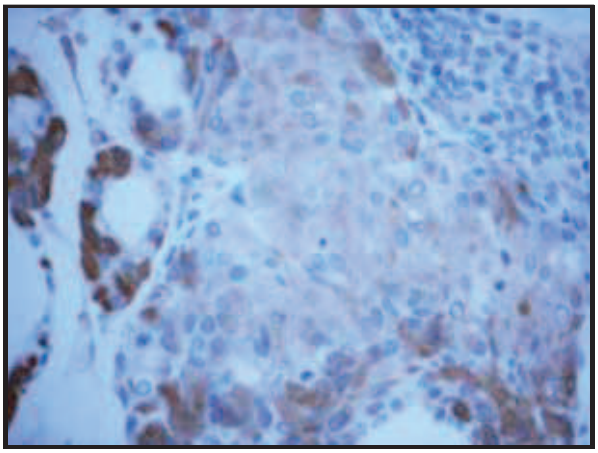



## C-Cell Histopathology: Mice

- Use immunohistochemistry
  - Normal C-cells
  - Diffuse hyperplasia
  - Early focal hyperplasia
- Can only see focal hyperplasia and tumors in routine histopathology



## Tumorigenic Drugs in Rats

| Drug | Product Class |
|---|---|
| Exenatide | GLP-1R agonist (F) |
| Liraglutide | GLP-1R agonist (M/F) |
| Aledronate | Bisphosphonate (M) |
| Arformoterol | $\beta_2$ receptor agonist (F) |
| Atenonol | $\beta_2$ receptor agonist (M) |
| Colesevelam | Bile acid sequestrant (F) |
| Naratriptan | 5-$HT_{1D/1B}$ receptor antagonist (M/F) |
| Palonosetron | 5-$HT_3$ receptor antagonist (F) |

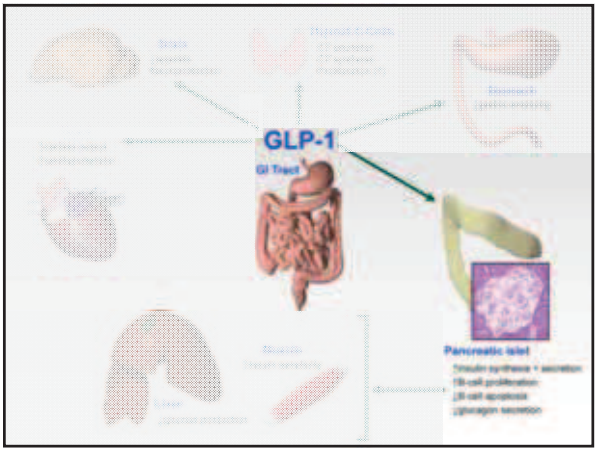
## Tumorigenic Drugs in Mice

| Drug | Product Class |
|---|---|
| Liraglutide (likely class effect) | GLP-1R Agonist (M/F) |

## Glucose Homeostasis

*Fasting vs. Postprandial*

- Fasting glucose level
  - Insulin
  - Glucagon
- Postprandial glucose level
  - Incretin (GI) hormones
    - GLP-1 (glucagon-like peptide-1)
    - GIP (gastric inhibitory polypeptide; glucose-dependent insulinotropic polypeptide)
    - Only GLP-1 increases insulin in diabetics

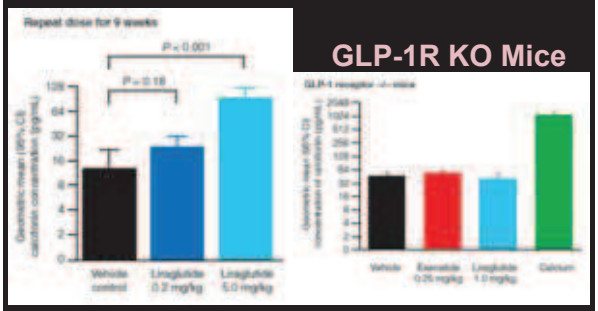



## GLP-1

*Very Short Half-Life in Blood*

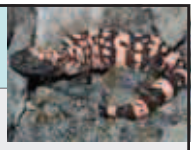
- Half-life: 1-5 minutes
- GLP-1 (7-36) amide and 7-37 forms
- Degraded by plasma DPP-4 (dipeptidyl peptidase-4)
  - DPP-4 inhibitors: Limited ability to increase GLP-1

## Calcitonin Secretion in Mice




Knudsen et al, Endocrinology, 2010

## Exenatide (Byetta®)



- First GLP-1 agonist (2005)
- Synthetic form of exendin-4
  - Isolated from Gila monster salivary glands
  - 50% homology to human GLP-1
  - Longer half-life in humans
- Adjunctive therapy for DM
- Twice daily injections

## Long-Acting GLP-1 Agonists

- Liraglutide (Victoza®)
  - Modified rGLP-7-37 with palmitic acid moeity
  - Once daily injection
- Bydureon®
  - Exenetide and microsphere formulation
  - Once weekly injection
- Dulaglutide (once weekly)
  - GLP (7-37) linked to Fc IgG fragment
- Albiglutide (once weekly or biweekly)
  - GLP-1 dimer fused to albumin
- Lixisenatide (once daily)

## C-cells and GLP-1 agonists

***Rats and Mice***

- C-cell hyperplasia
  - Diffuse
  - Focal
- C-cell adenomas
- C-cell carcinomas
- Rats are more sensitive than mice

## Human Relevance Framework

***C-cell Tumors in Rodents: Mode of Action & Key Events***

Drug binding to GLP-1R on C-cells

↑ Cytoplasmic cAMP & CT secretion

↑ C-cell proliferation

↑ C-cell diffuse hyperplasia

↑ C-cell focal hyperplasia

↑ C-cell adenomas

↑ C-cell carcinomas

## GLP-1R in Rodent C-cells

***Weight of Evidence Approach***

- Immunohistochemistry
- In situ hybridization
- Receptor binding in vivo
- Downstream effects in rodent C-cell lines and not in human C-cell lines
- Lack of findings in dogs and NHP
- Lack of downstrean and proliferative effects in GLP-1R KO mice

## GLP-1 Agonist-Induced C-cell Proliferation in Rodents

- Rodent-specific effect (?)
- Receptor expression greatest in rodents
- GLP-1R KO mice confirm role of receptor
  - Physiologic role in rodents
- No proliferation in dogs and NHP
- No increase in calcitonin in dogs and NHP
- Equivocal or no increase in calcitonin in humans
- *Long-term effect in humans need to be monitored*