Thyroid Disorders in Animals

Charles C. Capen

THYROID DISEASES IN ANIMALS

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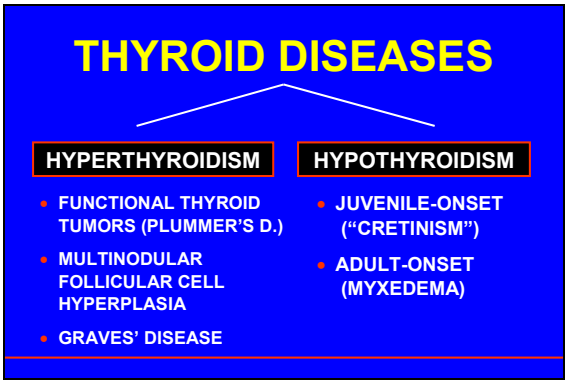
INTRODUCTION

Thyroid diseases are important clinical problems in a number of domestic and laboratory animal species. Examples of selected disorders of the thyroid gland have been chosen to illustrate the spectrum disease processes that occur in animals.



HYPERTHYROIDISM

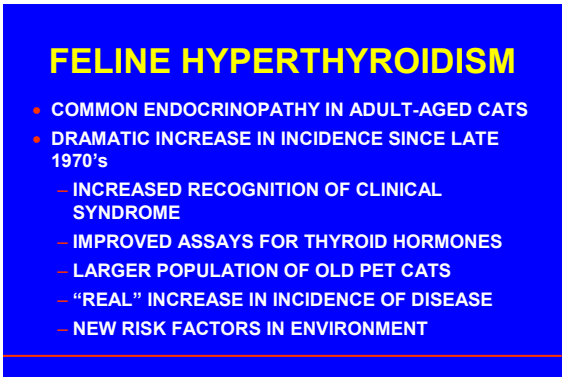
A. Introduction

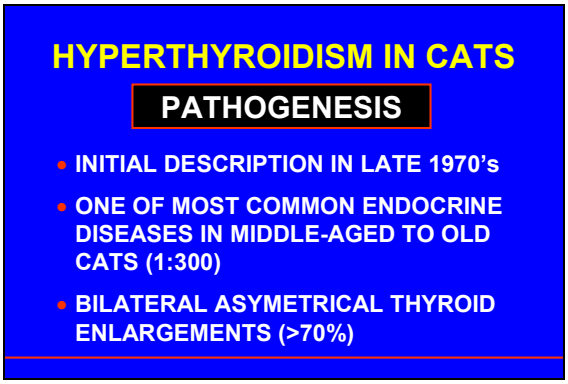
Among domestic animal species, disturbances of growth resulting from the production of excess thyroid hormone is most common in adult cats and often related to adenomas composed of hyperactive follicular cells. These neoplastic cells release both thyroxine (T_4) and triiodothyronine (T_3) at an uncontrolled rate resulting in markedly elevated blood levels of both hormones. Cats with hyperthyroidism have elevated levels of total serum T_4 and T_3. Normal serum levels of T_4 in cats, as measured by radioimmunoassay, are approximately 1.5 to 4.5 µg/dl and serum T_3 levels are 60 to 100 ng/dl. In hyperthyroid cats the total levels of T_4 in the serum range from 5.0 to over 50 µg/dl and total levels of T_3 in the serum range from 100 to 1,000 ng/dl. Hyperthyroidism is associated with weight loss in spite of a normal or increased appetite and

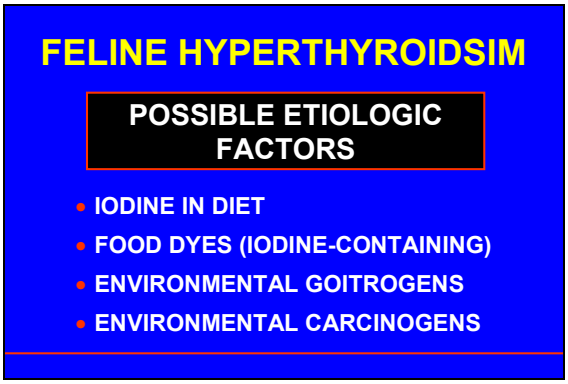
with restlessness and increased activity. There has been a dramatic increase in the incidence in feline hyperthyroidism since the late 1970s, and now it is one of the two most common endocrine diseases in adult-age cats.

Dogs have a very efficient enterohepatic excretory mechanism for thyroid hormones that is difficult to overload, either from endogenous production by a tumor or by exogenous administration of thyroid hormones. Hence, thyroid tumors in the dog only occasionally secrete sufficient amounts of thyroid hormone to overload the highly efficient enterohepatic excretory pathways for thyroid hormones and produce clinical signs of hyperthyroidism. The clinical signs of hyperthyroidism in dogs with functional thyroid tumors include polyuria and polydipsia and weight loss, despite increased appetite and polyphagia, leading to muscle atrophy and weakness. The levels of T_3 and T_4 in the serum of dogs with clinical hyperthyroidism are only mildly elevated: 300-400 ng/dl and 5-7 µg/dl, respectively. As compared to dogs, cats are very sensitive to phenol and phenol-derivatives. They have a poor ability to conjugate phenolic compounds such as T_4 with glucuronic acid and to excrete the T_4-glucuronide into the bile. In cats, the capacity for conjugation of T_3 with sulfate is also limited and can easily be overloaded.

B. Epidemiology







C. Thyroid Pathology

HYPERTHYROIDISM IN CATS

THYROID LESION

- FOLLICULAR CELL ADENOMA
- MULTINODULAR HYPERPLASIA OF FOLLICULAR CELLS ("ADENOMATOUS GOITER")
- FOLLICULAR CELL ADENOCARCINOMA

HYPERTHYROIDISM IN CATS

LESION IN CONTRALATERAL THYROID LOBE

- MULTINODULAR "ADENOMATOUS" HYPERPLASIA OF FOLLICULAR CELLS
- COLLOID INVOLUTION OF NORMAL FOLLICLES
- BILATERAL ASYMETRICAL INVOLVEMENT >70% OF CASES
- PRENEOPLASTIC AUTONOMOUS LESION

FELINE HYPERTHYROIDISM

- THYROID LESION IS AUTONOMOUS
  - GROWS IN SUBCUTIS OF NUDE MICE
- NO INCREASE IN THYROID STIMULATING IMMUNOGLOBULINS (e.g. LATS)
- MILD INCREASE IN THYROID GROWTH STIMULATING IMMUNOGLOBULINS
- ANALOGOUS TO TOXIC NODULAR GOITER IN HUMAN PATIENTS

THYROID NEOPLASMS AND DEVELOPMENT OF CLINICAL HYPERTHYROIDISM

- ABILITY OF TUMOR CELLS TO SYNTHESIZE T_4/T_3
  - FOLLICULAR PATTERN
  - COLLOID FORMATION
- DEGREE OF ELEVATION OF BLOOD T_4/T_3

RATE OF SECRETION | RATE OF DEGRADATION

D. Species Differences in Degree of Elevation of Thyroid Hormone Levels

HYPERTHYROIDISM

SPECIES DIFFERENCES IN THYROID HORMONE LEVELS

CAT	DOG
• MARKED ELEVATION	• MILD ELEVATION

FELINE HYPERTHYROIDISM

HIGH BLOOD LEVELS OF THYROID HORMONE (T_4/T_3)

- CAT VERY SENSITIVE TO PHENOL AND PHENOL DERIVATIVES
- CAT HAS POOR ABILITY TO CONJUGATE PHENOLIC COMPOUNDS (T_4) WITH GLUCURONIC ACID
- SULFATE CONJUGATION (T_3) CAPACITY LIMITED AND EASILY OVERLOADED

E. Clinical Signs/Functional Disturbances

HYPERTHYROIDISM

- CATS
- DOGS

HYPERTHYROIDISM IN CATS

CLINICAL SIGNS

- HYPERACTIVITY
- WEIGHT LOSS DESPITE INCREASED APPETITE
- POLYDIPSIA, POLYURIA
- MODERATE HYPERTHERMIA
- TACHYCARDIA, CARDIOMEGALY

OCCULT HYPERTHYROIDISM

CATS

- SUPPRESSION OF SERUM T_4 INTO NORMAL RANGE BY NON-THYROIDAL DISEASES:
  - DIABETES MELLITUS
  - CHRONIC RENAL FAILURE
  - SYSTEMIC NEOPLASIA
  - LIVER DISEASE

HYPERTHYROIDISM IN DOGS

- FUNCTIONAL ADENOMA/ ADENOCARCINOMA DERIVED FROM THYROID FOLLICULAR CELLS
- RESEMBLES "PLUMMER'S DISEASE" IN HUMAN PATIENTS

HYPERTHYROIDISM IN DOGS

CLINICAL SIGNS

- INCREASED WATER TURNOVER
  - POLYURIA
  - INCREASED GFR (+ HEART RATE)
  - INCREASED SOLUTE LOAD
- WEIGHT LOSS DESPITE (↑) APPETITE
- MUSCLE ATROPHY AND WEAKNESS
- DECREASED EXERCISE TOLERANCE

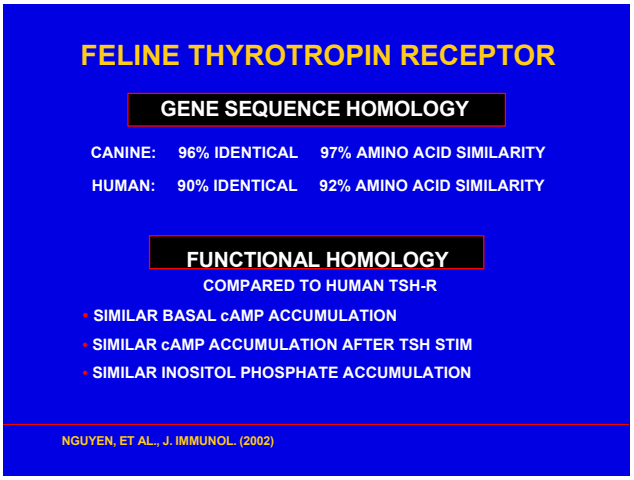
HYPERTHYROIDISM IN DOGS

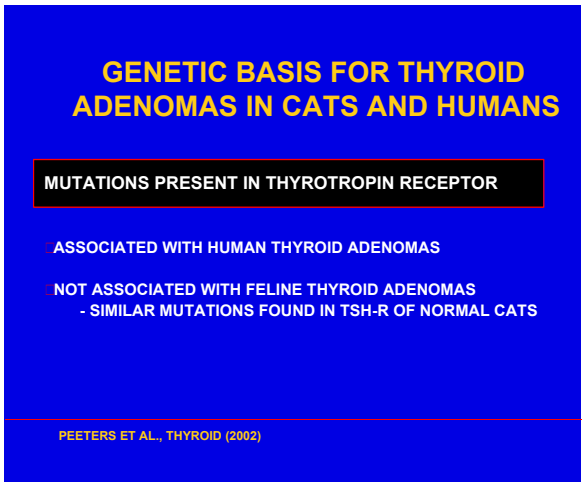
CLINICAL SIGNS

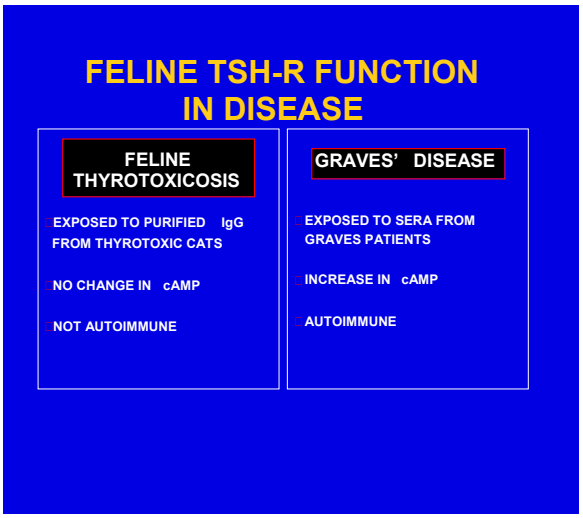
- INTOLERANCE TO HEAT
  - CONTINUAL PANTING
  - SEEK COOL PLACES
- NERVOUS, RESTLESS
  - ANXIOUS BEHAVIOR
  - WALKS CONTINUOUSLY
- TACHYCARDIA
- DIARRHEA
  - INCREASED GI MOTILITY

F. Recent Mechanistic Studies in the Etiopathogenesis in Cats

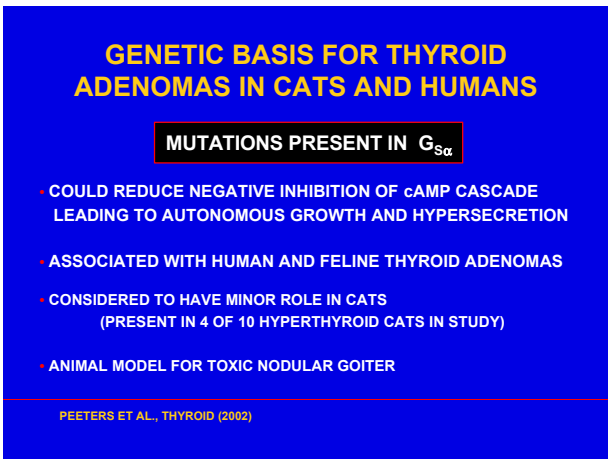
1) Absence of Stimulating Mutations of Thyrotropin Receptor Gene



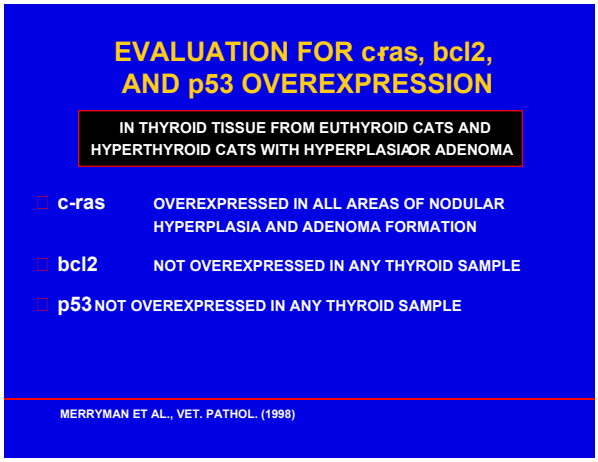


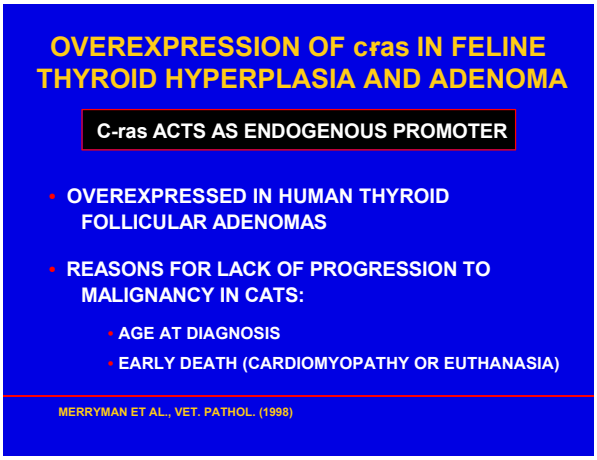


2) Mutation in the Gsα Protein Gene in Some Cats with Hyperthyroidism



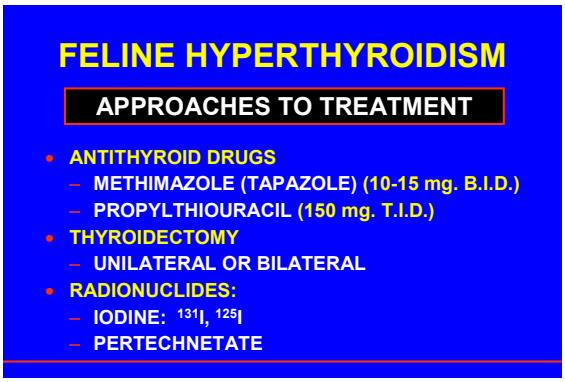
3) Overexpression of c-ras Oncogenes in Focal Thyroid Hyperplasia and Adenomas

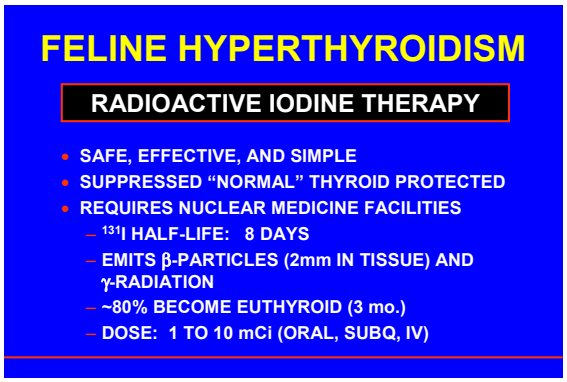




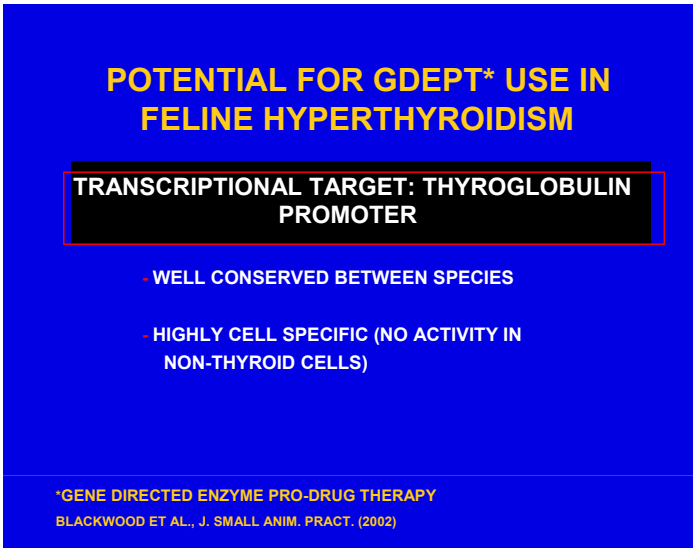
G. Approaches to Therapy

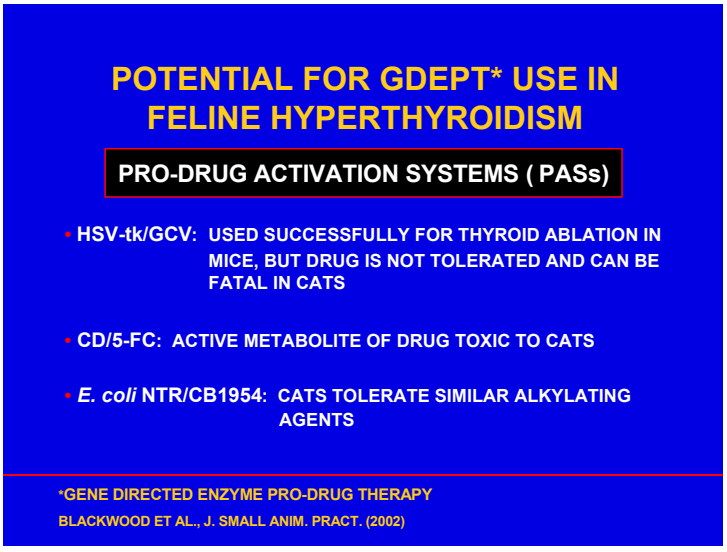
1) Routine Therapy





2) Transcriptionally Targeted Somatic Gene Therapy





HYPOTHYROIDISM

A. Introduction

Hypothyroidism is a well-recognized clinical entity in dogs. Although the disease may occur in many purebred and mixed breed dogs, certain breeds (Doberman Pinschers, Golden Retrievers, Beagles) appear to be more commonly affected than other breeds. Clinical hypothyroidism usually is the result of primary diseases of the thyroid gland, especially idiopathic follicular atrophy also termed "follicular collapse" and lymphocytic thyroiditis. In cases of "follicular collapse" there is a progressive loss of follicular cells and replacement by adipose connective tissue with a minimal inflammatory response.

Lymphocytic thyroiditis in dogs closely resembles Hashimoto's disease in humans and appears to be genetically conditioned, at least in certain breeds. Though the mechanisms for the disease are not well-established for the dog, it seems that a polygenic pattern of inheritance similar to that observed in the human disease also occurs in dogs. The immunologic basis for the development of chronic lymphocytic thyroiditis in both man and dog appears to be through the production of autoantibodies primarily directed against thyroglobulin and a microsomal antigen (thyroperoxidase). In cases of lymphocytic thyroiditis, the thyroid gland consists of either a diffuse or nodular infiltration of lymphocytes, plasma cells, and macrophages. Many of the remaining thyroid follicles are small and lined by tall columnar follicular cells, reflecting the long-standing stimulation of the thyroid gland by TSH; an attempt to compensate for the low blood levels of thyroid hormones. Ultrastructurally, numerous lymphocytes and macrophages are observed within the follicular basement membrane extending between follicular cells into the lumens of follicles.

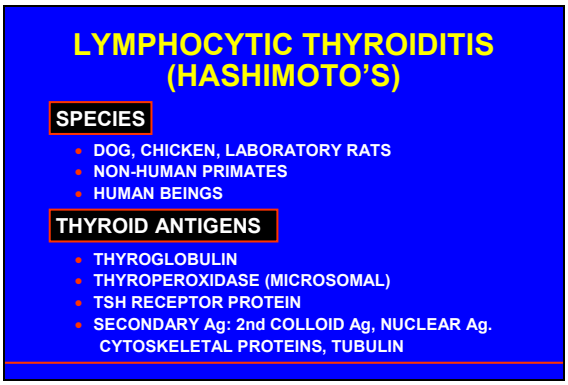
Clinical disturbances associated with hypothyroidism vary among affected animals and not every sign is seen in each animal patient. Many clinical signs associated with hypothyroidism are due to a reduction in basal metabolic rate. A gain in body weight without an associated change in appetite occurs frequently. The weight gain may vary from slight to striking obesity. The animal usually is less active and the owner may observe a reluctance to play or take walks. The inactivity also contributes to the weight gain. Dogs with hypothyroidism may have difficulty in maintaining normal body temperature and are often "heat seekers." They will lie on or near sources of heat, such as registers, radiators, and electric blankets, and be reluctant to venture outdoors in cold weather. Excessive shivering may be observed and the skin frequently feels cool.

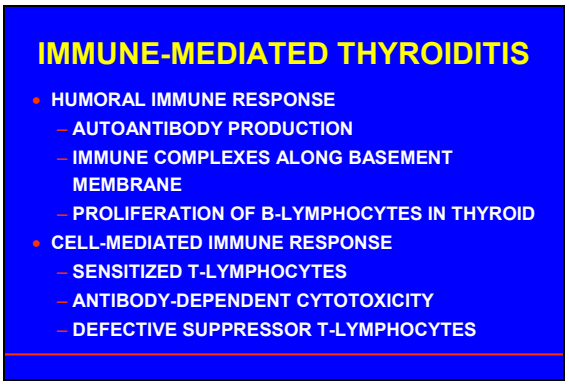
In long-standing and severe hypothyroidism in dogs, myxedema may develop and produce a characteristic appearance. There is accumulation of mucin and acid mucopolysaccharides in the dermis and subcutis. This material binds considerable amounts of water and produces a marked thickening of the skin. Myxedema is obvious around the face and head where accentuation of the normal skin folds causes a sad or "tragic appearance." Other manifestations of hypothyroidism are the failure of hair regrowth after clipping for either cosmetic or therapeutic purposes and abnormalities in reproduction including lack of libido and reduction in sperm count in males or abnormal or absent estrous cycles with reduced conception rates in

females. In addition, a change in attitude often is observed by the owner. The affected animal often appears dull and less active.

Hypothyroidism is encountered only sporadically in other animal species. Hypothyroidism is reported infrequently in cats and the clinical signs include prolonged periods of apathy, poor hair growth, severe seborrhea, and myxedema of the face with a blunted serum T_4 increase in response to exogenous TSH administration. Hypothyroid chickens are small, moderately obese with increased accumulations of abdominal fat, have a small, dry comb, and an abnormally silky plumage. Feathers from chickens with hypothyroidism are long, silky, and with reduced numbers of hooklets on the distal barbules to grasp the proximal barbule necessary for normal feather structure.

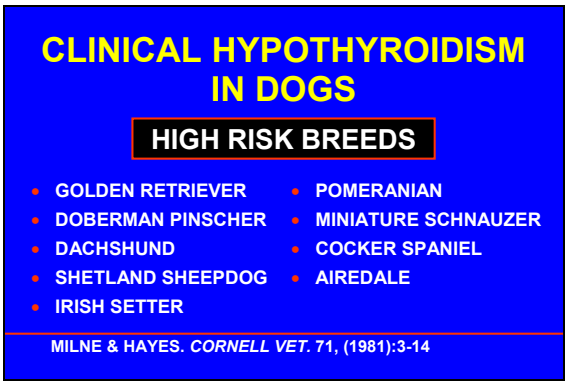
B. Thyroid Pathology and Pathogenic Mechanisms

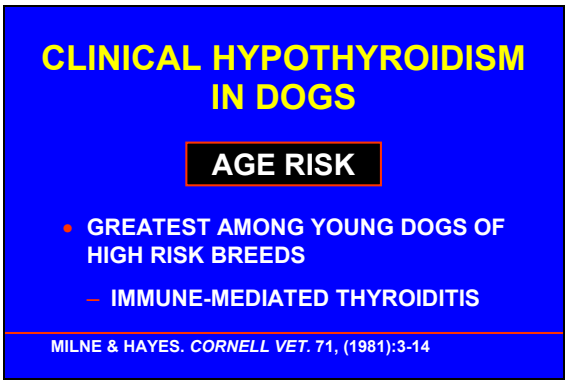


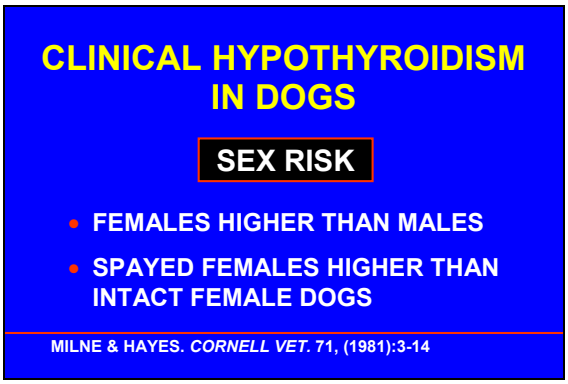


1) Primary *vs*. Secondary/Tertiary

C. Clinical Signs/Functional Disturbances







D. Approaches to Therapy

CONGENITAL DYSHORMONOGENIC GOITER

A. Introduction

Congenital dyshormonogenic goiter has been studied in several animal species and is characterized by the inability of the animal to synthesize and secrete adequate amounts of thyroid hormones prior to or at birth. Congenital goiter is inherited as an autosomal recessive gene in sheep, Afrikander cattle, and Saanen dwarf goats. Clinical signs of hypothyroidism include a subnormal growth rate, absence of normal wool development or a rough, sparse, hair coat, myxedematous swellings of the subcutis, weakness, and sluggish behavior. Most lambs with congenital goiter die shortly after birth or are sensitive to the effects of adverse environmental conditions.

Although thyroidal uptake and turnover of ^{131}I are greatly increased compared with euthyroid controls, circulating levels of T_4 and T_3 are consistently low in this animals. The levels of protein-bound iodine in animals with inherited congenital goiter are often markedly elevated. This appears to be the result of the iodination of albumin and other plasma proteins by the thyroid gland in response to long-term stimulation by TSH. The lack of a defect in the mechanisms related to iodide transport, iodide organification or the dehalogenation of thyroxine, the absence of normal 19S thyroglobulin in animals with goitrous thyroids, and relatively minute amounts of thyroglobulin-related antigens (0.01% of normal), suggests an impairment of thyroglobulin biosynthesis in animals with congenital goiter. The concentration of thyroglobulin-mRNA sequences in thyroid tissue from affected animals is considerably reduced (1-2% of normal).

B. Thyroid Pathology and Pathogenic Mechanisms

CONGENITAL GOITER "DYSHORMONOGENESIS"

SPECIES — SHEEP, GOATS, CATTLE

DEFECT — IMPAIRED THYROGLOBULIN SYNTHESIS

CONGENITAL (DYSHORMONOGENIC) GOITER

SHEEP

- INHERITED DEFECT (AUTOSOMAL RECESSIVE)
  - INITIALLY IN MERINO BREED
- IMPAIRED THYROGLOBULIN BIOSYNTHESIS
  - DECREASED SERUM T_4 AND T_3
  - INCREASED THYROIDAL ^{131}I UPTAKE
  - IODINATION OF ALBUMIN AND OTHER PROTEINS

FAILURE OF HORMONE SYNTHESIS BY THYROID FOLLICULAR CELLS

MOLECULAR DEFECT

- DEFECTIVE PROCESSING OF PRIMARY TRANSCRIPTS FOR THYROGLOBULIN mRNA
- ABERRANT TRANSPORT OF THYROGLOBULIN mRNA FROM NUCLEUS TO RIBOSOMES

SUBCELLULAR DISTRIBUTION OF THYROGLOBULIN mRNA (Tg mRNA) SEQUENCES IN GOATS WITH CONGENITAL GOITER WITH INABILITY TO SYNTHESIZE THYROGLOBULIN

Tg mRNA	DISTRIBUTION OF Tg mRNA (% RECOVERED SEQUENCES) NORMAL	GOITER	CONCENTRATION OF Tg mRNA SEQUENCES IN GOITER (% OF NORMAL)
NUCLEAR	7	35	42
CYTOPLASM	25	38	7
MEMBRANE-BOUND	70	27	1-2

van HERLE et al. NEW ENGL. J. MED. 301:1979, 239 & 307

C. Clinical Signs/Functional Disturbances

CONGENITAL (DYSHORMONOGENIC) GOITER

SHEEP

- THYROID GLANDS UNIFORMLY ENLARGED
  - >225 gm (1.5 gm = NORMAL)
  - DARK RED (INCREASED BLOOD SUPPLY)
  - TSH-MEDIATED FC HYPERTROPHY / HYPERPLASIA
- CLINICAL EVIDENCE OF HYPOTHYROIDISM
  - STILLBORN OR WEAK, GROW SLOWLY
  - SILKY WOOL COAT
  - LOW LONG-TERM SURVIVAL

THYROID NEOPLASIA IN ANIMALS

A. Bulls (Not) Cows C-Cell Tumors Related to High Calcium Intake

- Frequently Metastasize to Regional Lymph Nodes
- Amyloid Accumulation in Interstitium of Tumor

B. Dogs: Follicular Cell Adenocarcinomas

- Compact Cellular/Follicular Histologic Pattern Most Common
- Hyperthyroidism Uncommon Due to Efficient Hepatic Degradation Mechanisms
- Frequently Metastasize to Distant Sites
- Poor Prognosis

C. Cats: Follicular Cell Adenomas and Adenomatous Hyperplasia

- Consistent Follicular Pattern with Colloid Production
- Hyperthyroidism Common Due to Well-differentiated Proliferative Lesions and Low Capacity Hepatic Degradation Mechanism
- Rarely (<5%) Malignant with Metastasis to Distant Sites

COMPARATIVE PATHOBIOLOGY OF THE THYROID GLAND

A. Introduction

The basic functions of the hypothalamic-pituitary-thyroid axis are similar in animals and humans. However, species differences must be considered before attempting to use animal data related to the effects of drugs and chemicals on thyroid function for assessment of human risk. Long-term alterations of the pituitary-thyroid axis by various xenobiotic chemicals or pathophysiological disturbances, such as those caused by iodine deficiency and partial thyroidectomy, are more likely to predispose laboratory rodents, rats and mice to a higher

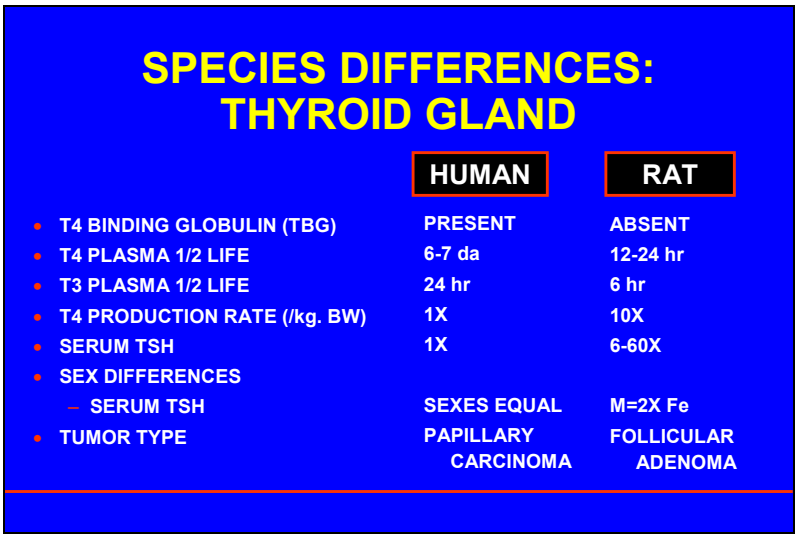
incidence of hyperplasia and tumors of follicular cells than in the human thyroid. This appears to be particularly true for male rats which have higher circulating levels of TSH than females.

The plasma half-life of T_4 is shorter in rats (12-24 hours) than in humans and monkeys (5-9 days). This is related, in part, to differences between these species in the transport proteins for T_4 and T_3 because rodents, birds, amphibians, or fish do not synthesize the high affinity protein (thyroxine-binding globulin [TBG]) which binds T_4.

Many xenobiotic chemicals and drugs disrupt one or more steps in the synthesis and secretion of thyroid hormones or enhance the catabolism of thyroid hormones, especially those which increase the cytochrome p450 thyroxine-metabolizing enzymes of the liver. In long-term studies using laboratory rodents, this results in subnormal levels of T_4 and T_3 which are associated with a compensatory increase in the secretion of pituitary TSH. Rats and mice are particularly sensitive to the decreased availability of T_4 and T_3 and respond with hypertrophy and hyperplasia of follicular cells and in long-term studies there is increased incidence of thyroid tumors. These tumors develop as an indirect response to the hormonal imbalance.

In the indirect or secondary mechanism of thyroid oncogenesis in rodents, the specific xenobiotic chemical or physiologic perturbation evokes a stimulus, such as the chronic hypersecretion of TSH, that promotes the development of nodular proliferative lesions derived from follicular cells. Compounds acting by this indirect mechanism usually have little or no evidence for mutagenicity or for producing DNA damage.

By comparison, there is little, if any, increase in the incidence of thyroid cancer in humans who have markedly altered changes in thyroid function and elevated TSH levels. The relative resistance to the development of thyroid cancer in humans with elevated plasma TSH levels is in marked contrast to the response of the thyroid gland of rats or mice to chronic TSH stimulation. Hence, laboratory rodents are not good models for safety assessment of a new drug or chemical projected for use in humans, particularly when the compound affects thyroid function.



B. New Drug Development and Testing for Carcinogenicity in Rodents

MARKETED DRUGS WITH A TUMORIGENIC RESPONSE IN RODENTS BY TARGET ORGANS

RATS	MICE
THYROID (15)	LIVER (18)
LIVER (13)	LUNG (17)
TESTIS (13)	MAMMARY GLAND (6)
MAMMARY GLAND (10)	BLOOD (6)
ADRENAL (9)	OVARY (5)
PITUITARY (8)	ALL OTHERS (3)
ALL OTHERS (4)	

U.S. PHYSICIAN'S DESK REFERENCE (1994)
DAVIES AND MONRO. J. AM. COLL. TOXICOL. 14:90-107, 1995
( ) No. OF STUDIES

PROLIFERATIVE LESIONS THYROID FOLLICULAR CELLS IN RODENTS

MORPHOLOGIC CONTINUUM

NORMAL

HYPERPLASIA

ADENOMA

CARCINOMA

SIGNIFICANCE IN RISK ASSESSMENT

EXAMPLES OF MARKETED DRUGS WITH A TUMORIGENIC RESPONSE

THYROID GLAND

DRUG	PRODUCT CLASS	SPECIES
MINOCYCLINE	ANTIBIOTIC	R
OXAZEPAM	ANTIANXIETY	R
NICARDIPINE	Ca-CHANNEL BLOCKER	R
SERTRALINE	ANTIDEPRESSENT	R
SIMVASTATIN	HYPOLIPIDEMIC	R
SPIRONOLACTONE	DIURETIC	R
VIDARABINE	ANTIVIRAL	R

U.S. PHYSICIANS' DESK REFERENCE (1994)
DAVIES AND MONRO. *J. AM. COLL. TOXICOL.* 14:90-107, 1995

EXAMPLES OF MARKETED DRUGS WITH A TUMORIGENIC RESPONSE

THYROID GLAND

DRUG	PRODUCT CLASS	SPECIES
AMIODARONE	ANTIARRHYTHMIC	R
ATENOLOL	β-ADRENERGIC BLOCKER	R
BEPRIDIL	Ca-CHANNEL BLOCKER	R
DAPSONE	ANTINEOPLASTIC	R
GRISEOFLUVIN	ANTIBIOTIC	R
IODINATED GLYCEROL	EXPECTORANT	R
METHINAZOLE	ANTI-THYROID	R
MIDAZOLAM	SEDATIVE	R

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DAVIES AND MONRO. *J. AM. COLL. TOXICOL.* 14:90-107, 1995

RISK ASSESSMENT ISSUES AND THE ENDOCRINE SYSTEM

HORMONAL IMBALANCES

TISSUE		END POINT
THYROID FOLLICULAR CELLS	TSH	HYPERPLASIA, ADENOMA
LEYDIG (INTERSTITIAL) CELLS	LH	HYPERPLASIA, ADENOMA
OVARY (SURFACE EPITH./STROMA)	LH	TUBULO (STROMAL) ADENOMA
MAMMARY GLAND	PROLACTIN	HYPERPLASIA, NEOPLASIA

RISK ASSESSMENT ISSUES AND THE ENDOCRINE SYSTEM

HORMONAL IMBALANCES

TISSUE		END POINT
GLANDULAR STOMACH	GASTRIN	ECL HYPERPLASIA / NEOPLASIA
PANCREATIC ISLETS	GLUCOSE / INSULIN	HYPERPLASIA / ADENOMAS
ADRENAL MEDULLA	CALCIUM / VITAMIN D	HYPERPLASIA / NEOPLASIA
EXOCRINE PANCREAS	CCK	HYPERPLASIA / NEOPLASIA

C. Chemical Disruption of Thyroid Function

CHEMICAL DISRUPTION OF THYROID FUNCTION

DIRECT THYROID EFFECT

- HORMONE SYNTHESIS
- HORMONE SECRETION

INHIBITION OF IODINE TRANSPORT MECHANISM

- THIOCYNATES
  - PLANTS OF BRASSICAE FAMILY
  - VEGETABLES WITH HEAT-LABILE GLYCOSIDE
- PERCHLORATES
- TECHNETIUM

INHIBITION OF THYROPEROXIDASE (TPO)

- DECREASED IODINATION OF TYROSINE

$$2I^- \xrightarrow{TPO} I_2$$

- INHIBITION OF COUPLING OF IODOTYROSINES → IODOTHYRONINES

$$MIT + DIT \xrightarrow{TPO} T_3$$

$$DIT + DIT \xrightarrow{TPO} T_4$$

CHEMICALS DISRUPTING THYROID FUNCTION

THYROPEROXIDASE INHIBITORS

- THIOUREA
- PROPYLTHIOUREA (PTU)
- ANILINE DERIVATIVES: SULFONAMINDES
  - SULFAMETHAZINE
  - MANY OTHERS, NOT ALL
- METHIMAZOLE, CARBIMAZOLE
- AMINOTRIAZOLE
- ACETOACETAMIDE

SULFAMETHAZINE

- WIDELY USED SULFONAMIDE IN FOOD-PRODUCING ANIMALS
- CURRENT PERMISSIBLE RESIDUE LEVEL 100ppb
  - NEGLIGIBLE RESIDUE
- CARCINOGENICITY STUDIES AT NCTR
  - FISCHER 344 RATS
    - DOSE LEVELS (ppm): 0, 40, 600, 1200, 2400
  - $B_6C_3F_1$ MICE
    - DOSE LEVELS (ppm): 0, 300, 600, 2400, 4800

SULFONAMIDE - INHIBITION OF THYROPEROXIDASE

SENSITIVE SPECIES	RESISTANT SPECIES
RAT	HUMAN BEINGS
MOUSE	NONHUMAN PRIMATES
DOG	GUINEA PIG
PIG	CHICKEN

TAKAYAMA *et al.* TOXICOL. APPL. PHARM. 82:1986, 191-199.

INCIDENCE OF THYROID FOLLICULAR CELL NEOPLASIA (ADENOMA [AD] + ADENOCA [CA]) IN FISCHER 344 RATS ADMINISTERED SULFAMETHAZINE IN FEED FOR 2 YEARS

	DOSE ppm					
	0	10	40	600	1200	2400
MALES (%)	0	2	0	5	5	11*†
(N)	(0/118)	(2/87)	(0/66)	(4/88)	(4/83)	(10/87)
(AD/CA)	(0/0)	(0/2)	(0/0)	(2/2)	(2/2)	(3/7)
FEMALES (%)	4	0	1	5	10	9
(N)	(6/170)	(0/56)	(1/85)	(4/84)	(9/87)	(8/88)
(AD/CA)	(5/1)	(0/0)	(1/0)	(4/0)	(3/6)	(2/6)

*SIGNIFICANT TREND AT BONFERRONI COMPARED TO CONTROLS (0.001 LEVEL)
†OVERALL POSITIVE TREND AT 0.1% LEVEL (CHRONIC STATISTICAL PROCEDURE AFTER KODELL *et al.* [1983]).
LITTLEFIELD *et al.* Fd. Chem. Toxicol. 28:157-167, 1990.

INCIDENCE OF THYROID FOLLICULAR CELL NEOPLASIA (ADENOMA [AD] + ADENOCA [CA]) IN $B_6C_3F_1$ MICE ADMINISTERED SULFAMETHAZINE IN FEED FOR 2 YEARS

	DOSE ppm					
	0	300	600	1200	2400	4800
MALES (%)	1	0	1	5	4	33*†
(N)	(2/184)	(0/94)	(1/92)	(4/88)	(4/94)	(31/93)
(AD/CA)	(2/0)	(0/0)	(1/0)	(4/0)	(3/1)	(31/0)
FEMALES (%)	3	1	1	0	2	26*†
(N)	(5/180)	(1/91)	(1/93)	(0/94)	(2/94)	(23/89)
(AD/CA)	(5/0)	(1/0)	(0/1)	(0/0)	(2/0)	(22/1)

*SIGNIFICANT TREND AT P 0.05 (BONFERRONI CORRECTED FOR CONTROL VS. DOSE COMPARISON)
†OVERALL POSITIVE TREND AT 0.05 AND 0.001 LEVELS (CHRONIC STATISTICAL PROCEDURE AFTER KODELL *et al.* [1983])
LITTLEFIELD *et al.* Fd. Chem. Toxicol. 28:455-463, 1989.

SULFAMETHAZINE (SM): MODE OF ACTION STUDIES

- SM SHOWN TO BE A POTENT INHIBITOR OF THYROPEROXIDASE IN RODENTS (IC_{50} = 1.2 X 10^{-6}M)
- MORPHOLOGIC EFFECTS ON THYROID (HYPERTROPHY / HYPERPLASIA) REVERSIBLE AFTER WITHDRAWAL OF SM
- SUPPLEMENTAL T_4 IN DIET (DOSES THAT NORMALIZED BUT DID NOT SUPPRESS TSH) INHIBITED DEVELOPMENT OF FUNCTIONAL AND MORPHOLOGIC CHANGES BY SM

SULFAMETHAZINE (SM): MODE OF ACTION STUDIES

- HYPOPHYSECTOMIZED RATS (NO TSH) ADMINISTERED SM DID NOT DEVELOP MORPHOLOGIC CHANGES IN THYROID FOLLICULAR CELLS
- *IN VITRO* SM DID NOT INCREASE THYROID CELL PROLIFERATION (FRTL-5) IN ABSENCE OF TSH
- NO EFFECT ON THYROID STRUCTURE / FUNCTION IN CYNOMOLOGUS MONKEYS (SM > 300 mg/kg)

CHEMICALS DISRUPTING THYROID FUNCTION

PERIPHERAL METABOLISM OF THYROID HORMONES

- INDUCTION OF HEPATIC MICROSOMAL ENZYMES
- INHIBITION OF 5'-DEIODINASE

XENOBIOTIC CHEMICALS INDUCING HEPATIC MICROSOMAL ENZYMES

CHARACTERISTICS

- NO MUTAGENICITY OR DNA DAMAGE
- HEPATIC TUMOR PROMOTION
  - CERTAIN MOUSE STRAINS
  - PROMOTION OF SPONTANEOUSLY INITIATED HEPATOCYTES
- THYROID TUMOR PROMOTION
  - RATS > MICE
  - MALES > FEMALES
  - UDP GLUCURONYL TRANSFERASE

HEPATIC MICROSOMAL ENZYME INDUCERS THAT DISRUPT THYROID FUNCTION IN RODENTS

- CNS-ACTING DRUGS
  - PHENOBARBITAL
  - BENZODIAZEPINES
- CALCIUM CHANNEL BLOCKERS
  - NICARDIPINE
  - NIFEDIPINE
- STEROIDS
  - SPIRONOLACTONE

HEPATIC MICROSOMAL ENZYME INDUCERS THAT DISRUPT THYROID FUNCTION IN RODENTS

- RETINOIDS
- CHLORINATED HYDROCARBONS
  - CHLORDANE, ALDRIN
  - DDT, TOXAPHENE, TCDD
- POLYHALOGENATED BIPHENYLS
  - PCB
  - PBB

CHEMICALS DISRUPTING THYROID FUNCTION

INHIBITORS OF 5'-DEIODINASE

T_4 —⊣ ⟶ T_3

- FD&C RED NO. 3 (ERYTHROSINE)
- AMIODARONE
- IOPANOIC ACID

FD&C RED NO. 3 (ERYTHROSINE)

HEALTH CONCERNS

- WIDELY USED COLORING AGENT IN FOODS, SOFT DRINKS, AND PHARMACEUTICALS
  - 2 mg/day ESTIMATED MEAN INTAKE OF COLOR IN U.S.A.
- HIGH IODINE CONTENT OF COLOR
  - 58% OF MOLECULAR WEIGHT
    (2',4',5',7'-TETRAIODOFLUORESCEIN)
- EFFECTS OF IODINATED COMPOUNDS ON PERIPHERAL METABOLISM OF IODOTHYRONINES

EFFECTS OF FD&C RED NO. 3 ON SERUM REVERSE TRIIODOTHYRONINE

MECHANISMS

- SUBSTRATE (T_4) ACCUMULATION DUE TO 5'-DEIODINASE INHIBITION

T_4 —⊣ ⟶ T_3

↘ rT_3

- TSH STIMULATION OF T_4 PRODUCTION
- rT_3 ACCUMULATION IN SERUM

INCIDENCE OF THYROID FOLLICLULAR CELL TUMORS IN MALE SPRAGUE-DAWLEY RATS ADMINISTERED FD&C RED NO. 3 IN FEED FOR 30 MONTHS*

	DOSE (% DIET)				
LESION	0	0.1	0.5	1.0	4.0
• ADENOMA (%)	0	0	2.5	1.5	22.0
• CARCINOMA (%)	0	4.5	1.5	4.4	4.4
• CYSTIC FOLLICULAR HYPERPLASIA (%)	2.2	12.0	16.0	7.3	23.0
• DIFFUSE F.C. HYPERPLASIA (%)	0.7	7.5	7.4	26.1	87.0

*PLUS *IN UTERO* EXPOSURE

SECONDARY MECHANISMS OF CARCINOGENESIS

HORMONAL IMBALANCES

- XENOBIOTIC CHEMICAL OR PHYSIOLOGIC PERTURBATION IS NOT A DIRECT ACTING CARCINOGEN ITSELF BUT EVOKES ANOTHER STIMULUS (CHRONIC TSH HYPERSECRETION) THAT PROMOTES THYROID TUMOR DEVELOPMENT IN SENSITIVE SPECIES (RODENTS)

SECONDAY MECHANISMS OF ONCOGENESIS

- THRESHOLD LEVEL CAN BE ESTABLISHED FOR THE CHEMICAL
- DOSES BELOW THIS LEVEL FAIL TO ELICIT HORMONAL IMBALANCES AND INCREASE TROPHIC HORMONE SECRETION
- ABSENCE OF ONCOGENIC EFFECT IN TARGET ENDOCRINE ORGAN
- NO-EFFECT LEVELS CAN BE ESTABLISHED FOR THE COMPOUND

PHYSIOLOGICAL PERTURBATIONS WITH HORMONAL IMBALANCES RESULTING IN THYROID TUMORS IN RODENTS

NO XENOBIOTIC CHEMICAL EXPOSURE

- IODINE-DEFICIENT DIET
- PITUITARY TUMOR (TSH) TRANSPLANT
- PARTIAL THYROIDECTOMY
- DIETS WITH NATURAL PLANT GOITROGENS

RODENT MODEL AS A PREDICTOR OF THYROID NEOPLASIA

- MARKED BIOCHEMICAL AND PHYSIOLOGICAL DIFFERENCES:

	RAT	MAN
ABSENT TBG	PA, A	TBG, A
T_4 HALFLIFE	24 HOUR	5-9 DAYS
TSH	300 ng/ml	2 ng/ml
NEOPLASIA	5%	2 X 10^{-3}%
TSH RESPONSE	SENSITIVE	NOT DETECTABLE

- RODENTS ARE AN INAPPROPRIATE MODEL FOR ASSESSING THYROID NEOPLASIA DUE TO HORMONE IMBALANCE
- RODENTS ARE VERY SENSITIVE TO DIRECT ACTING CARCINOGENS DUE TO STRONG PROMOTING EFFECT OF TSH

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