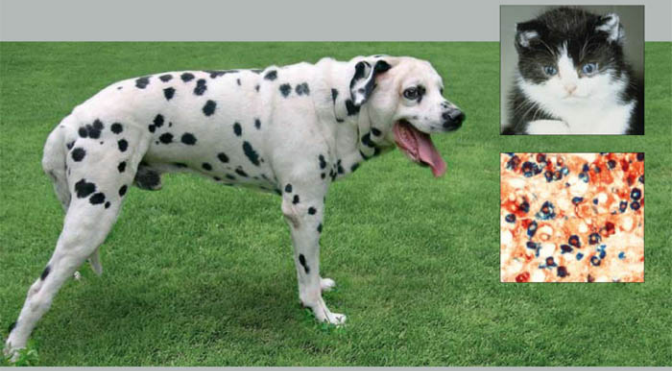
Ad Rijnberk · Hans S. Kooistra (eds.)

Clinical Endocrinology of Dogs and Cats

An Illustrated Text



Second, revised and extended edition

schlütersche

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Second, revised and extended edition

schlütersche

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Abbreviations

ACE	Angiotensin-converting enzyme
ACTH	Adrenocorticotropic hormone
ADH	Antidiuretic hormone
AL	Anterior lobe (pituitary)
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AMH	Anti-Müllerian hormone
ANP	Atrial natriuretic peptide
APUD	Amine precursor uptake and decarboxylation
AQP	Aquaporin
AR	Androgen receptor
ARR	Aldosterone:renin ratio (PAC:PRA)
ATR	Angiotensin receptor
AVP	Arginine-vasopressin
BGC	Blood glucose curve
BNP	Brain natriuretic peptide
cAMP	Cyclic adenosine monophosphate
CBG	Corticosteroid-binding globulin
CDI	Central diabetes insipidus
CEH	Cystic endometrial hyperplasia
CIRCI	Critical illness-related corticosteroid insufficiency
CGRP	Calcitonin gene-related peptide
CLIP	Corticotropin-like intermediate lobe peptide
C-PTH	Carboxy-terminal fragments of PTH
CRH	Corticotropin-releasing hormone
CT	Calcitonin
DDAVP	1-deamino,9-D-arginine vasopressin
DHEA	Dehydroepiandrosterone
DHT	Dihydrotestosterone
DIT	Diiodotyrosine
DKA	Diabetic ketoacidosis
DLA	Dog lymphocyte antigen
DNES	Diffuse neuroendocrine system
DOC	Desoxycorticosterone
DOPA	Dihydroxyphenylalanine
EHTT	Ectopic hyperfunctioning thyroid tissue
β-END	β-Endorphin
Epo	Erythropoietin
ER	Endoplasmatic reticulum
FNA	Fine-needle aspiration
FSH	Follicle-stimulating hormone
fT_4	Free thyroxine
GH	Growth hormone
GHRH	Growth hormone-releasing hormone
GIP	Gastric inhibitory polypeptide
GLP	Glucagon-like peptide
GLUT	Glucose transporter protein
GnRH	Gonadotropin-releasing hormone
GR	Glucocorticoid-preferring receptor
HDDST	High-dose dexamethasone suppression test
HDL	High density lipoproteins
HHS	Hyperglycemic hyperosmolar state
HM	Home monitoring (blood glucose)
HSD	Hydroxysteroid dehydrogenase
IAPP	Islet amyloid polypeptide
IGF	Insulin-like growth factor
IGF-BP	IGF-binding protein
IL	Interleukin
Insl3	Insulin-like peptide 3
LDDST	Low-dose dexamethasone suppression test
LDL	Low density lipoproteins
LH	Luteinizing hormone
MIT	Monoiodotyrosine
MPA	Medroxyprogesterone acetate
MR	Mineralocorticoid-preferring receptor
α-MSH	α-melanocyte-stimulating hormone
NDI	Nephrogenic diabetes insipidus
NFA	Non-functional adenoma
NEFA	Nonesterified fatty acids
NF-κB	Nuclear factor kappa B
NIS	Sodium iodide symporter
NSH	Nutritional secondary hyperparathyroidism
O,p'-DDD	2,4'-Dichlorodiphenyldichloroethane
OPG	Osteoprotegerin
PAC	Plasma aldosterone concentration
PBGM	Portable blood glucose meter
PET	Pancreatic endocrine tumor
PI	Pars intermedia (pituitary)
PIF	Prolactin-inhibiting factor
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
PGFM	13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$
PL	Posterior lobe or neurohypophysis
PMDS	Persistent Müllerian duct syndrome
PNMT	Phenylethanolamine N-methyl transferase
POMC	Pro-opiomelanocortin
Posm	Plasma osmolality

PP	Pancreatic polypeptide
PPAR	Perioxisome proliferator-activated receptor
PRA	Plasma renin activity
PrRP	Prolactin-releasing peptide
PRL	Prolactin
PTH	Parathyroid hormone
PTHrP	Parathyroid hormone-related peptide
PU/PD	Polyuria/polydipsia
RANKL	Receptor activator of nuclear factor-kappa β ligand
RAS	Renin-angiotensin system
rT_3	Reverse triiodothyronine
SIAD	Syndrome of inappropriate antidiuresis
SPECT	Single photon emission computed tomography
SRIF	Somatostatin-release inhibiting factor
SRS	Somatostatin receptor scintigraphy
SRY gene	Sex-determining region of the Y chromosome
SS	Somatostatin
SSTR	Somatostatin receptor
T_3	Triiodothyronine
T_4	Thyroxine
TBG	Thyroid hormone binding globulin
Tg	Thyroglobulin
TGF	Transforming growth factor
TLI	Trypsin-like immunoreactivity
TNFα	Tumor necrosis factor α
TPO	Thyroid peroxidase
TRH	Thyrotropin-releasing hormone
TSH	Thyroid-stimulating hormone
TR	Thyroid hormone receptor
TT_4	Total thyroxine
UACR	Urinary aldosterone:creatinine ratio
UCCR	Urinary corticoid:creatinine ratio
Uosm	Urine osmolality
UTR	Untranslated region (DNA)
VLDL	Very-low density lipoproteins
VP	Vasopressin
XY SRS	XY Sex reversal syndrome
ZFY	Zinc finger protein, Y-linked

Preface to the first edition

Endocrinology is one of the disciplines concerned with communications and controls within the organism by means of chemical messengers. The whole of intercellular communication is covered in large part by three systems: (1) the nervous system, (2) the endocrine system, and (3) the immune system. Over the past few decades it has become apparent that the separation of these systems is artificial, in that they share many common features. The nervous system elaborates compounds that can act as local mediators or true circulating hormones, while several hormones can act as neurogenic mediators within the central nervous system. Moreover, at the level of the hypothalamus and pituitary there is an intimate link between the nervous system and the endocrine system, thereby integrating the two into one control unit. The immune system is now also recognized as a regulatory system subject to endocrine control. It in turn exerts a reciprocal controlling effect on neuroendocrine systems.

Within this wide spectrum of communication in the living animal there are messenger substances which conform to the classic characteristics of hormones, i.e., products of endocrine glands which are transported by the blood to some distant site of action. Most of the endocrine diseases known to occur in dogs and cats are the result of dysfunction of one or more of these glands and hence this book concentrates on the disorders of these glands.

Most of the chapters deal with separate endocrine glands. For each gland there is an introductory section on the relevant morphology and physiology, followed by descriptions of the disorders of the gland. Because the clinician's suspicion of the presence of an endocrine disease is largely based upon pattern recognition, in which the physical changes play an important role, many illustrations have been included. The features of some endocrine diseases differ in the dog and the cat to such an extent that separate descriptions are needed. Chapters on diagnostic and therapeutic protocols are included at the end of the book to provide a quick reference for both students and practitioners. These will suffice in many cases, but at some time the help of a specialist may be required.

Clinical endocrinology has at least four fascinating characteristics. First, hormones and thus endocrine glands are involved in the regulation of the function of almost every organ system. Therefore the study of this discipline requires the challenging combination of broad pathophysiological interest and specific expertise in the field of endocrinology. Second, endocrinology itself occupies a common ground between biochemistry, physiology, and clinical medicine. Third, in part because of the first two features, clinical endocrinology is a discipline of contemplation, reflection, and stimulating discussion. Fourth, it is very fortunate that many endocrine disorders are amenable to treatment.

The authors hope that this book will serve as a helpful guide to veterinary clinicians in this fascinating field.

Utrecht, December 1994
Ad Rijnberk

Preface to the second edition

As we complete the manuscripts and illustrations for the second edition of this book, we pause to reflect upon the journey from the first to the second edition. As for the first edition, we hope that a brief general description of the multifaceted field of endocrinology has a place and is worth continuing. The changes with this edition are in the addition of newly recognized disease entities, further elucidation of mechanisms of disease, and progress in diagnosis and treatment.

In this second edition the information on basic and clinical endocrinology has been updated and ranges from molecular biology to the clinical approach to the patient. All of the chapters have been completely rewritten and new illustrations have been included. The information on calciotropic hormones is no longer distributed over three chapters but integrated into a single chapter.

We are pleased that most of the authors and contributors for the first edition also helped in preparing the second edition. At the same time, we are grateful that new authors with specific knowledge were willing to join in. The expertise and critical attitude of the coauthors and contributors was vital for the writing process and occurred in a very pleasant atmosphere.

Dr. Bruce E. Belshaw, with whom the editors and several of the authors have had the pleasure of working in endocrinology, has helped us in editing the English language of this book. He has done so with helpful insight and sympathy.

For many years Mrs. Yvonne Pollak has contributed to our work in clinical endocrinology in several ways. In the 1960s she enthusiastically began assisting in studies of iodine metabolism and thyroid disease and thereafter became increasingly involved in the wide range of diagnostic techniques that nuclear medicine can offer, maintaining a special interest in the applications to clinical endocrinology. In addition, she applied her varied talents to preparing the drawings for the first edition and with the same dedication, accuracy, and skill she has prepared the drawings for this edition. Mr. Joop Fama made several of the new photographs presented in this book and also digitalized and improved many of the older pictures. Together with the drawings these photographs are very essential for this illustrated text. His input is highly appreciated.

The editors hope that this new edition will serve as an up-to-date guide to veterinary clinicians in the rapidly developing field of clinical endocrinology of companion animals, and that the book may stimulate students to study this fascinating discipline.

Utrecht, April 2009
Ad Rijnberk
Hans S. Kooistra

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Clinical Endocrinology



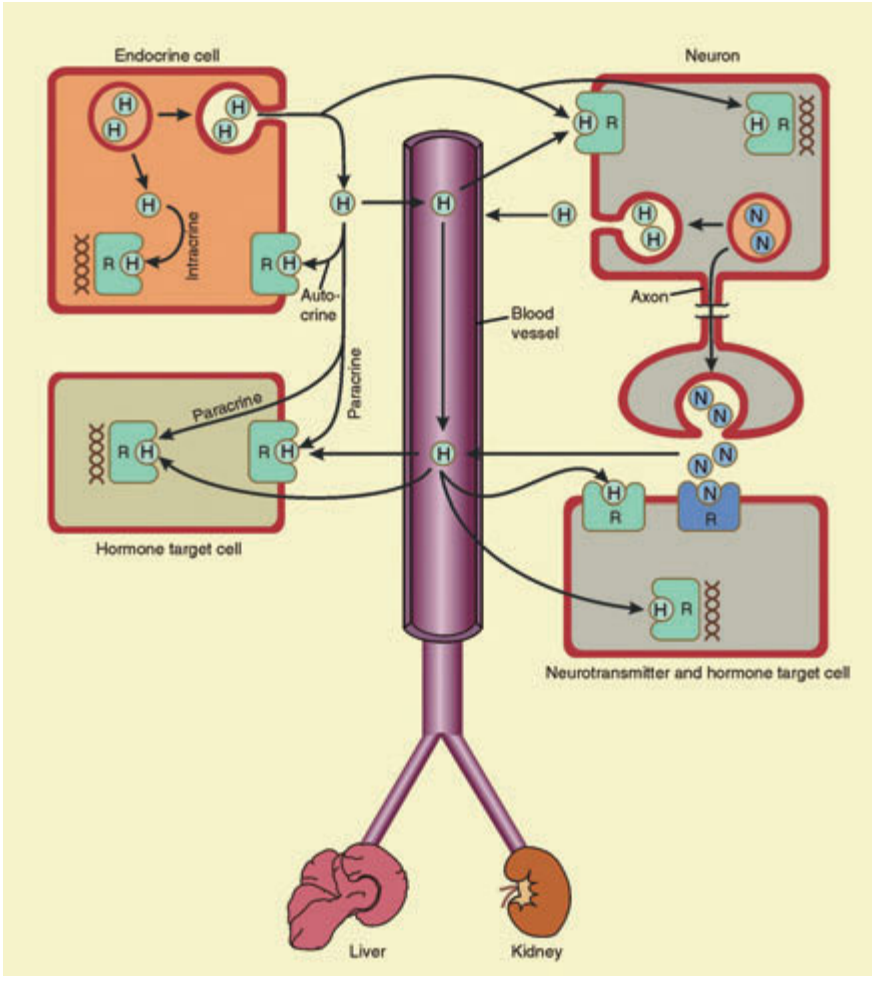


Figure 1.1:
Chemical communication involves hormones (H) and neurotransmitters (N), acting on target cells via receptors (R). Hormones may reach the target cells through the circulation (endocrine), or act on neighboring cells (paracrine), or on receptors in the same cell (autocrine), or act inside the cell without being released (intracrine). Neurons release neurotransmitters from nerve terminals. The same neurotransmitters can be released to act as hormones via the synaptic junctions or by direct release as hormones by the neuron. The liver and kidney serve as major sites for metabolism and excretion of hormones. (Modified from Webb and Baxter, 2007).[4]

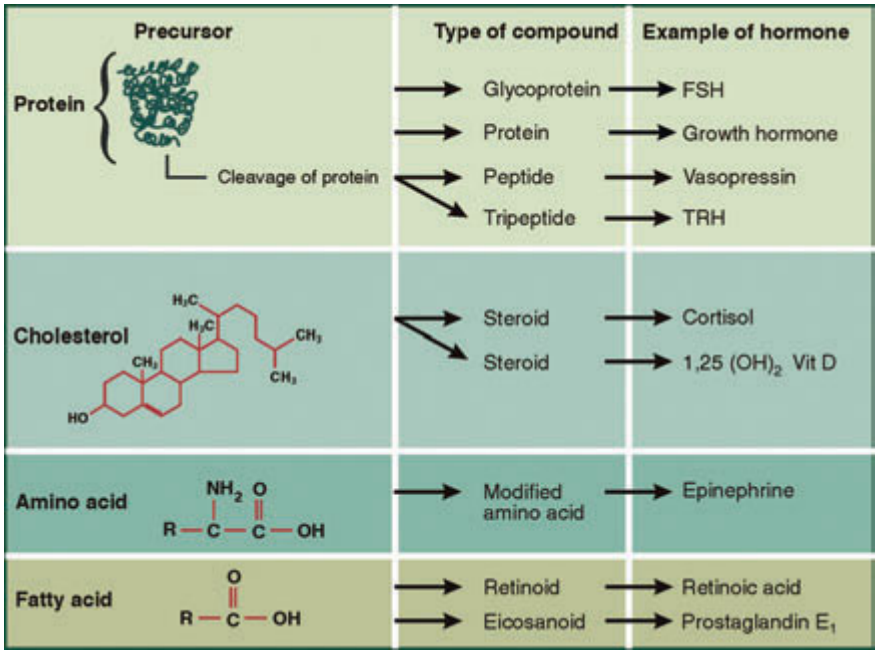


Figure 1.2:
Sources of the major hormones, with examples of each chemical type. (Modified from Webb and Baxter, 2007).[4]

1 Introduction

Ad Rijnberk
Jan A. Mol

1.1 Hormones

A little more than 100 years ago the term hormone was coined by Ernest Henry Starling, Professor of Physiology at University College, London.[1] During a conversation at a dinner with the distinguished biologist William Hardy, the two decided that they needed a word for an agent released into the bloodstream that stimulated activity in a different part of the body. They turned to a classical colleague, who produced the Greek verb for »excite« or »arouse« (ormao).[2] At the same time, the word »endocrine« appeared, to contrast the actions of substances secreted internally into the bloodstream with those secreted externally (exocrine) into ducts such as the lumen of the gastrointestinal tract.

Since 1905, the science concerned with hormones, endocrinology, has enormously increased our understanding of physiological processes in health and disease. Clinical endocrinology, progressing parallel to laboratory-based endocrine research, has led to important discoveries having significant impact on many disease states.

The traditional and still major part of clinical endocrinology deals with the glands that produce hormones and in particular with the circulating concentrations of hormones to which cells expressing specific receptors for hormones are exposed. Glandular biosynthesis and secretion, the way in which hormone is transported to target cells, and metabolic inactivation all determine the effective hormone concentration.

The capacity to form hormones is not limited to endocrine glands. In recent years the traditional view of the endocrine system's glandular nature has broadened to include production of hormones in specialized endocrine cells scattered in organs whose primary function is not endocrine, such as the stomach, the small intestine, the heart, and adipose tissue (see also chapter 10). Hormones may also be activated outside the endocrine organs, by proteolytic cleavage of protein prohormones (e.g., in the vascular bed). Others, such as dihydrotestosterone, triiodothyronine, and estradiol, are in part secreted by endocrine glands and in part formed in peripheral tissues from circulating precursors.

Endocrinology also includes messengers that circulate primarily in restricted compartments such as the hypothalamic-pituitary portal system, as well as messengers that act on adjacent cells (paracrine), on the cell of origin (autocrine), and within the secretory cell (intracrine) (fig. 1.1). Many hormones, of which insulin and dihydrotestosterone are examples, have both paracrine actions in the tissues in which they are formed and classical endocrine actions at peripheral sites. Other forms of intercellular communication studied by endocrinologists include exocrine secretion (e.g., in milk and semen) and the release of pheromones (in air or water).[3]

There are strong similarities in signaling mechanisms between the endocrine and nervous systems. The same molecule can be both a hormone and a neurotransmitter. For example, catecholamines are hormones when released by the adrenal medulla and neurotransmitters when released by nerve terminals. Thyrotropin-releasing hormone (TRH) is a hormone when produced by the hypothalamus, but has several neurotransmitter actions in the central nervous system.

1.1.1 Chemical nature of hormones

Chemically, hormones are derived from the major classes of biological molecules, i.e., they can be proteins (including glycoproteins), peptides or peptide derivatives, amino acid analogues, cholesterol derivatives, or lipids (fig. 1.2).

Polypeptide hormones are direct translation products of specific mRNAs, cleavage products of larger precursor proteins, or modified peptides. They can be as small as TRH (three amino acids) or as large and complex as growth hormone (GH) and follicle-stimulating hormone (FSH), which have about 200 amino acid residues and molecular weights in the range of 22 000–32 000.

Catecholamines, such as norepinephrine, epinephrine, and dopamine, are derived from a single amino acid, tyrosine.

Steroid hormones are derived from cholesterol and are of two types: (1) those in which the steroid nucleus is intact, such as the gonadal and adrenal steroids, and (2) those in which the B ring is open, such as vitamin D and its metabolites.

Retinoids are derived from carotenoids (vitamin A) in food. As for steroid hormones, the active products of vitamins act through nuclear hormone receptors.

Eicosanoids, including prostaglandins, leukotrienes, and thromboxanes, are derived from fatty acids (figs. 1.2, 1.3). They are produced by most cells and released with little storage, cleared rapidly from the circulation, and act via both cell surface and nuclear receptors.

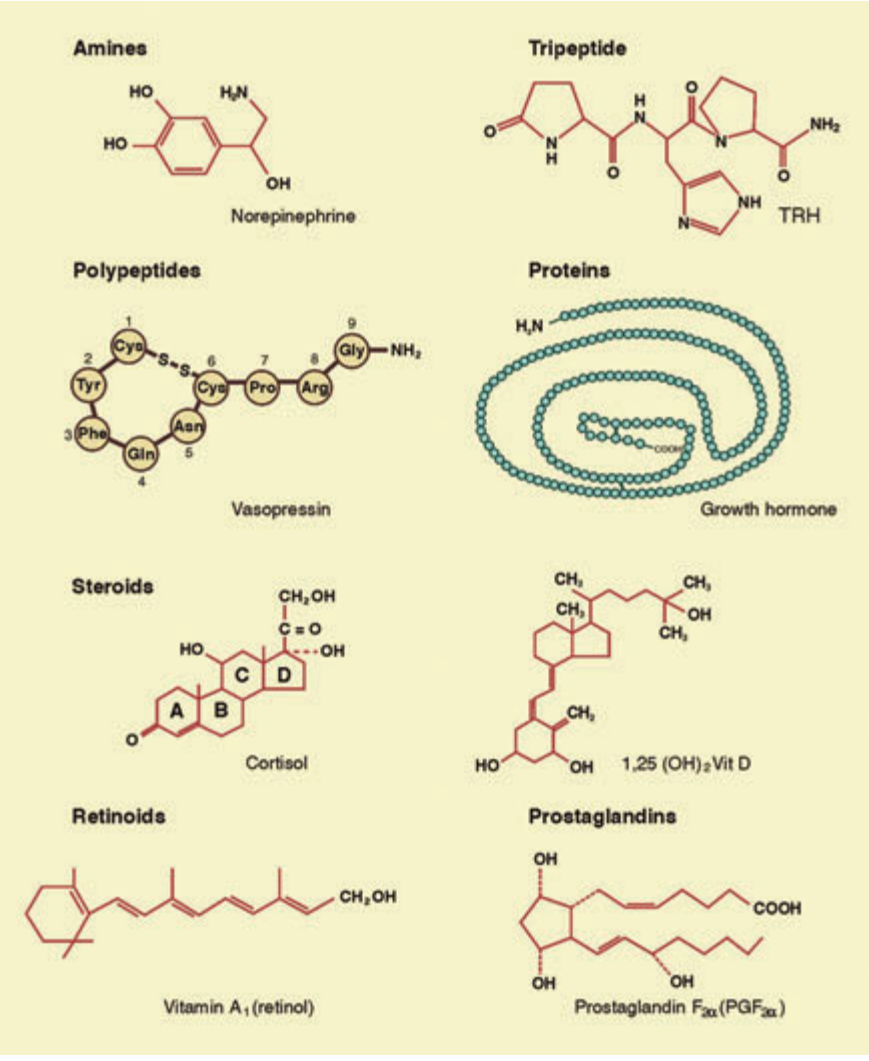


Figure 1.3:
Examples of different types of hormones. Each circle in the protein hormone represents an amino acid, just as shown for the polypeptide hormone.

1.1.2 Storage, release, and transport

Most endocrine cells have a limited capacity to store the final product. Even in cells with well-developed organelles for storing hormone, such as the Golgi apparatus, the amount of hormone stored is usually very small. The major exceptions are thyroglobulin, the precursor of thyroid hormones that is stored in the thyroid follicles, and the intermediate forms of vitamin D stored in adipose tissue.

The release process may involve freeing soluble derivatives from precursors by proteolysis (thyroid hormones from thyroglobulin), exocytosis of storage granules (peptide hormones), or passive diffusion of newly synthesized molecules (steroid hormones). In many instances the rate of hormone release fluctuates, synthesis and release being tightly linked. Many hormones, the pituitary hormones being prominent examples, are released in a pulsatile fashion.[5,6]

The majority of water-soluble hormones such as proteins and peptides are transported in blood without binding to specific proteins. This explains the half-life of only a few minutes in plasma of most of the nonglycosylated peptide hormones. The more insoluble a hormone is in water, the more important is the role of transport proteins. Thyroid and steroid hormones are largely transported bound to proteins. Protein-bound hormones cannot per se enter cells but serve as a reservoir from which free hormone is liberated for cellular uptake.

The distribution between bound and free hormone in plasma is determined by the amount of hormone and the amount and affinity of the proteins that bind it. The free hormone enters and interacts with its specific receptor in target cells and participates in the regulatory feedback mechanisms. Hence, changes in the amount of transport protein can cause considerable changes in hormone concentrations in plasma

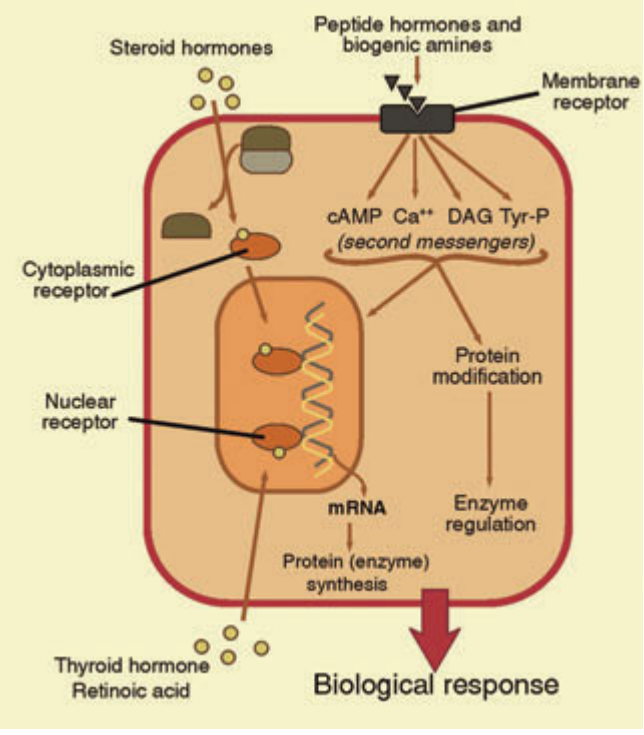


Figure 1.4:
Classical schematic model of hormone action. Steroid hormones bind to cytoplasmic or nuclear receptors. The hormone-receptor complex then binds to specific regions of DNA, resulting in activation or repression of a restricted number of genes. Peptide hormones and catecholamines bind to specific receptors in the cell membrane. This ligand-receptor interaction causes the generation of a second messenger. Many of the actions of second messengers (e.g., on gluconeogenesis and lipolysis) occur outside the nucleus, but they may also influence gene transcription.

without producing symptoms and signs of hormone deficiency or excess. If the regulatory feedback mechanisms that control hormone synthesis are intact, they maintain the amount of free hormone within a fixed (normal) range.

1.1.3 Action, metabolism, and elimination

Hormones exert their effects by binding to specific receptors, which can be on the cell membrane or intracellular. Most membrane receptors are complex protein structures with intracellular and extracellular domains. Intracellular receptors are proteins with similar overall structures and functions. Each is composed of three domains that can act somewhat independently: (1) the amino terminal domain that mediates effects on transcription, (2) the DNA-binding domain, and (3) the carboxyl terminal domain that mediates ligand binding, dimerization, and effects on transcription.

Peptide hormones and catecholamines operate via receptors located in the cell membrane, with the recognition / binding site exposed on the cell surface. Activated cell surface receptors use a variety of strategies to transduce signal information, thereby activating second messengers (fig. 1.4), which amplify and pass on the molecular information. Many peptide hormones ultimately signal via regulation of protein phosphorylation. In this most common process, through which proteins are covalently modified, a phosphate group is donated to the protein by nucleotide triphosphates. This allows peptide hormones to rapidly change their conformation and thus the function of existing cellular enzymes [enzyme activation or inactivation]. It also allows somewhat slower changes involving the transcription of genes coding for enzyme proteins and thus influencing the concentration of cellular enzymes [enzyme induction].

Steroid hormones and thyroid hormones act via structurally related intracellular receptors. These hormones are transported in plasma mainly bound to carrier proteins. The small amounts of free hormones are transported into the cytosol and bind to specific receptor proteins to form a hormone-receptor complex. This complex can bind to specific regulatory sequences – the positive and negative response elements – in promoter regions of genes in the chromosomal DNA. Thereby it acts as a regulator of gene transcription. As a result, the formation of messenger RNA is increased or decreased and thus the synthesis and secretion of proteins (enzymes, hormones) is enhanced or suppressed (fig. 1.4).

In recent years it has become clear that apart from this classical genomic mechanism of steroid action, steroids can also mediate rapid effects by nongenomic mechanisms. For glucocorticoids three different mechanisms have been proposed: (1) nonspecific interactions with cellular membranes which change their physicochemical properties and the activities of membrane-associated proteins, (2) specific interactions with a membrane-bound glucocorticoid receptor, and (3) nongenomic effects mediated by the cytosolic glucocorticoid receptor. In the latter concept, the cytosolic glucocorticoid receptor not only mediates the well-known genomic actions but is also involved in rapid direct effects in the cytosol.[7]

Degradation and inactivation of hormone takes place in target tissues as well as in nontarget tissues such as liver and kidney. Peptide hormones are mostly inactivated in target tissues by proteases. Steroid and thyroid hormones are largely metabolized and also largely conjugated, which makes them soluble, in the liver and kidney and then excreted via the bile and urine.

A change in the rate of hormone degradation does not influence the steady state as long as the feedback control of synthesis and release is intact, but if the control mechanism

is defective, changing the rate of hormone degradation may have clinical consequences. As an example, the degradation of glucocorticoids is enhanced in hyperthyroidism[8,9] and glucocorticoid insufficiency will ensue if the increased breakdown is not compensated by increased adrenocorticotropin-stimulated production of cortisol.

1.2 Genes encoding hormones

Proteins play a pivotal role in the synthesis and action of hormones. This concerns not only the synthesis of protein hormones but also enzyme proteins for steroid synthesis and for processes such as post-translational modification of peptide hormones.

1.2.1 DNA regions

Genes encoding proteins consist of several components. Exons are the regions of the gene that are transcribed into messenger RNA (mRNA), which is single stranded and has a sequence that corresponds to the coding (or sense) strand of DNA. During transcription it is synthesized in the 5' to 3' direction by a transcriptional apparatus that »reads« the complementary (or antisense) strand of DNA. Exons are usually interspersed with introns. These sequences are spliced out of the primary transcript before it leaves the nucleus (fig. 1.5).[10,11]

Exons consist of coding sequences that are translated into protein and untranslated regions (UTRs) at both ends of the gene (5'– and 3'-UTR). Transcription of genes is mediated by the interaction of many proteins with defined regulatory regions (or *cis* regulatory elements) present in the promoter region located upstream from the transcription start site, or present within intron areas, or in the 3'-UTR of the gene[10]:

Promoters

Transcription is invariably controlled at least in part by sequences located in the 5' flanking region of the gene before (5' or upstream from) the start of transcription. One element of the promoter is the binding site for RNA polymerase II. In many genes this region includes a short nucleotide sequence known as a TATA box (TATAAA or related sequence), approximately 30 bases upstream from the site at which transcription begins.

Enhancers and silencers

The cis regulatory elements that increase transcription independently of their position and orientation are called enhancers, and those that decrease transcription are called silencers. Such elements can be located within a gene itself, usually in an intron, or at some distance (up to thousands of nucleotides) away from it.

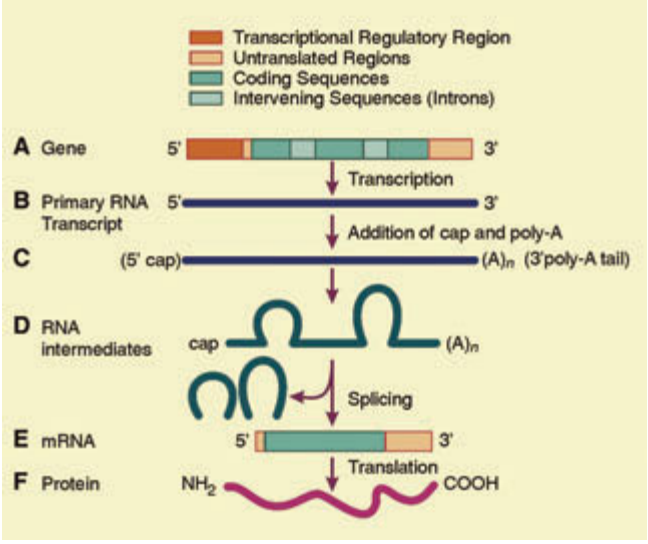


Figure 1.5:
Schematic illustration of the steps involved in the gene-encoded synthesis of a protein. The different regions of a generic gene are shown in A. The same color scheme is used in E but omitted in B–D, which illustrate RNA processing. (Adapted from White, 2004.)[10]

Locus control regions

These regions are required to establish a tissue-specific open chromatin domain (see chapter 1.2.2) in the vicinity of a particular locus and thus permit appropriate tissue-specific expression.

CpG islands

Cytosine methylation by a DNA methylase (DNA methylation) gives rise to the formation of CpG (cytosine-guanine) islands and is associated with inactivation of gene expression. This minimizes expression of permanently inactivated genes when differentiated cells divide. Conversely, hypomethylation is associated with active transcription.

1.2.2 Protein factors

Histones

Within chromosomes the DNA is organized into nucleosomes, each consisting of eight positively charged histone molecules. Higher-order winding organizes nucleosomes into chromatin. This organization renders DNA relatively inaccessible to transcription factors. Transcription can be enhanced by remodeling of nucleosomes to permit assembly of transcription complexes.[10] In this way the information potential of the genome is extended beyond the limitations of the genomic code, i.e., cell specificity is achieved without expansion of the genomic code.

General transcription factors

The promoter of a gene is bound by general transcription factors to form a transcription initiation complex that ultimately has a molecular weight of greater than 2 million Da. A part of this complex separates the DNA strands and allows binding adjacent to the TATA box. This is followed by binding of other protein complexes and RNA polymerase II.[10]

Transcriptional regulatory factors

Each of these factors consists of a DNA-binding domain and at least one activation domain that interacts with elements of the transcriptional apparatus. Almost all DNA-binding domains include an α-helical protein segment that fits into the major groove between two turns of the DNA helix. Many of these domains (including those of the intracellular hormone receptors) are stabilized by chelated zinc atoms and are called *zinc fingers*. The domains called *home domains* are 60 amino acid motifs that are most often found in transcription factors regulating embryonic development. An example of such a factor is Pit 1, which plays a role in the morphogenesis of the pituitary gland (see chapter 2.1, fig. 2.5).

1.2.3 RNA processing

The primary RNA transcript of a gene is modified in several ways in the nucleus before being exported as mRNA to the cytoplasm, where it is translated into protein (fig. 1.5)[10]:

Cap structure

The first posttranscriptional event during the course of RNA maturation in the nucleus is the addition of a cap. The cap is formed by addition of a guanosine to the 5' end of the mRNA and methylation of this guanosine and subsequent methylation of the adjacent nucleotide(s). This structure is required for the export of mRNA from the nucleus, and it also facilitates the binding of RNA to ribosomes and thus enhances the initiation of translation.

Poly(A)tail

In the nucleus most transcripts are clipped 12–16 bases downstream from a consensus poly(A) addition site, AAUAA or AUUAAA. Then a nucleotide sequence consisting entirely of repeated adenosines is added to the 3' end of the RNA. These poly(A)tails generally range between 50 and 250 bases and may play a role in RNA stability.

Splicing of introns

An important aspect of the maturation of RNA is the removal of introns by splicing. This process is mediated by spliceosomes, which are large complexes of small RNA molecules and proteins named snRNPs (small nuclear ribonucleoproteins, and pronounced »snerps«). The reason for the presence of interruptive introns in genes has not been established. When genes encoding the same protein are compared among different species, the nucleotide sequences within introns are found to be much less similar than the coding sequences. This suggests that the exact sequence of an intron is relatively unimportant except for sequences involved in splicing and regulation of gene expression.

MicroRNAs

In the complex interplay of several factors influencing the generation and expression of mRNA, small RNA sequences can also play a critical role. These microRNAs (miRNAs) of 20–22 nucleotides can silence gene expression after transcription. This class of regulators contains suppressors of tumor progression and metastasis.[12]

1.2.4 Translation

Within the nucleotide sequence of the mature mRNA transcript there is an open reading frame which is translated into protein by the ribosomal protein synthesis apparatus that reads the mRNA nucleotide sequence in triplets or codons (fig. 1.6). The ribosome reads the sequence from the start codon AUG that encodes a methionine residue until it reaches a stop codon (UAA, UGA, or UAG), at which point the ribosome dissociates from the mRNA.

Codons are actually read by small transfer RNA (tRNA) molecules that are specific for each amino acid. A tRNA molecule has a nucleotide triplet (called an anticodon) that is complementary to a mRNA codon. A tRNA is charged with the appropriate amino acid at its 3' end by a specific aminoacyl tRNA synthase.[10]

1.2.5 Posttranslational processing

As mentioned in the introduction of chapter 1.2, secretory and cell surface proteins play a pivotal role in endocrinology. These proteins are synthesized on ribosomes bound to the endoplasmic reticulum (ER) and undergo posttranslational processing. All of these proteins contain an N-terminal segment called the signal peptide (see for example fig. 4.4). It consists of approximately 20 amino acids, most of which are hydrophobic. The N terminus is bound by a ribonucleoprotein complex, the signal recognition particle (SRP). This is then bound by the SRP receptor, which is inserted in the membrane of the ER and recruits specific proteins to form a transmembrane channel to begin transporting the protein across the ER membrane after its synthesis.

The nascent protein is transported across the ER membrane in an unfolded state and must then adopt the correct conformation. This often requires interactions with chaperone proteins, the formation of disulfide bonds, and glycosylation.[10] In addition to its contribution to proper folding or stability of the protein, glycosylation may also be required for

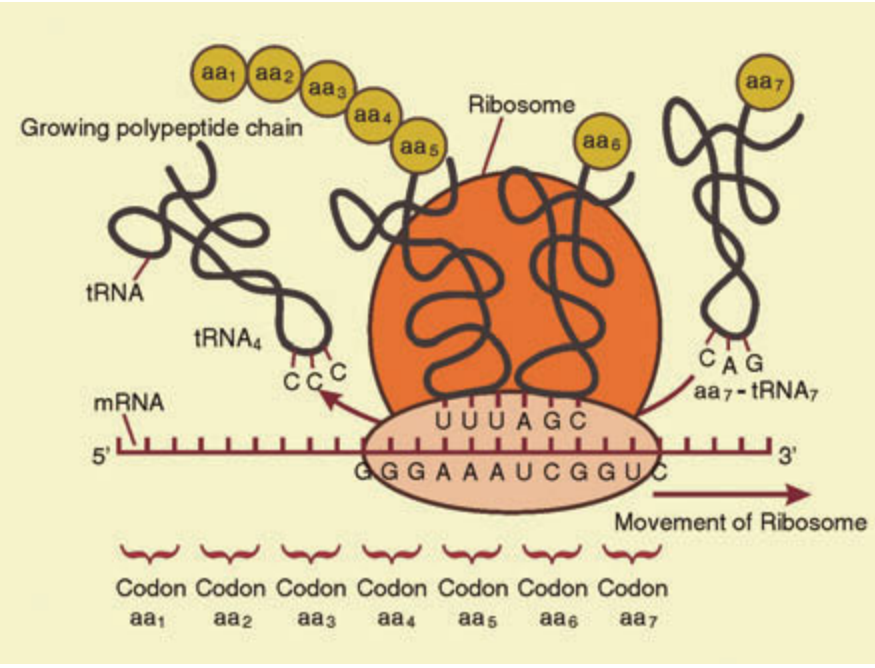


Figure 1.6:
Ribosomal protein synthesis. A, C, G, and U are nucleotides in RNA. They are illustrated in mRNA only in the region in contact with the ribosome, and only in transfer RNA (tRNA) in the region of the anticodon that interacts with mRNA through complementary base pairing. aa_{1-7} represent successive amino acids in the nascent polypeptide. (Adapted from White, 2004.)[10]

proper targeting to subcellular organelles such as lysosomes (see for example fig. 3.3).

Secretory and cell surface proteins are transported in specific vesicles to the Golgi apparatus, where they may undergo additional processing. They are sorted within the Golgi apparatus into vesicles containing proteins destined for the cell surface (receptors), and those for proteins that are secreted in a regulated manner (hormones). Some peptide hormones, such as parathyroid hormone (chapter 9.1.1), are synthesized as preprohormones. They require additional proteolytic steps that usually take place within secretory vesicles. Some preprohormones contain multiple peptide hormones within their primary sequence, such as proopiomelanocortin (fig. 4.5).

Certain proteins, particularly enzymes such as cytochrome P-450 (see for example chapter 4.1.1), are synthesized as apoproteins that require the addition of functional groups such as heme before they are active. This occurs at the site at which the enzyme is to function (e.g., mitochondria). There are many additional types of posttranslational processing, including phosphorylation, binding of lipids, and chemical modification of amino acids.[10]

1.3 Endocrine disorders

Endocrine disorders occurring in the dog and the cat can be divided into the following six broad categories, most of which can be further subdivided:

Deficient hormone production

Endocrine glands may be injured or destroyed by autoimmune disorders or by neoplasia and theoretically also by infection or hemorrhage, and the resulting hypofunction is said to be primary. Primary hypofunction may also be due to agenesia of an endocrine gland or it may be iatrogenic (e.g., due to castration). Hypofunction can also be due to inadequate stimulation of the gland and is then said to be secondary. These principles as well as the ones to follow are illustrated by drawings depicting a generalized hypothalamic-pituitary system in relation to a peripheral endocrine gland (fig. 1.7).

In hypofunction of a pituitary-dependent endocrine gland, pituitary cells can adapt via the classical feed-back concept, i.e., increased secretion of the corresponding pituitary hormone and increased numbers of specific pituitary cells, according to the one-cell-one-hormone concept. According to this concept each adenohypophyseal cell type produces a single hormone, which is secreted upon stimulation by a particular hypothalamic releasing hormone. However, in recent years it has become clear that cells of one cell line may be transformed into another to satisfy the demand for a specific pituitary hormone. Thus, contrary to the restrictive one-cell-

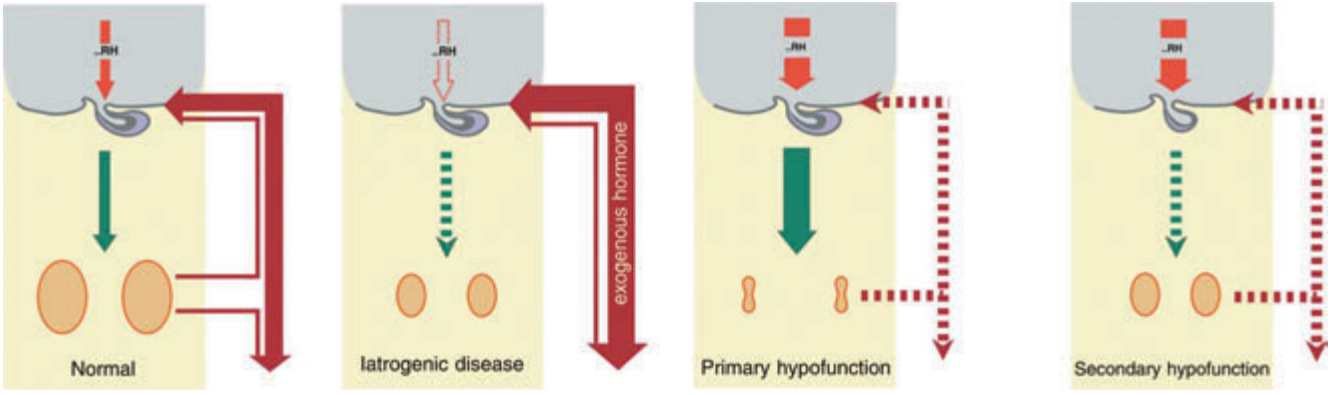


Figure 1.7:
Left: Generalized hypothalamic-pituitary system and a related endocrine gland under normal conditions and as influenced by administration of a hormone produced by the peripheral gland. The hormone secreted by the peripheral gland is partitioned in the circulation between a small free fraction (open parts of arrows) and a large fraction bound to carrier proteins (dark parts of arrows). The differences in hormone production are indicated by differences in thickness and continuity of lines and arrows. Right: Illustration of primary and secondary (pituitary) hormone deficiency states.

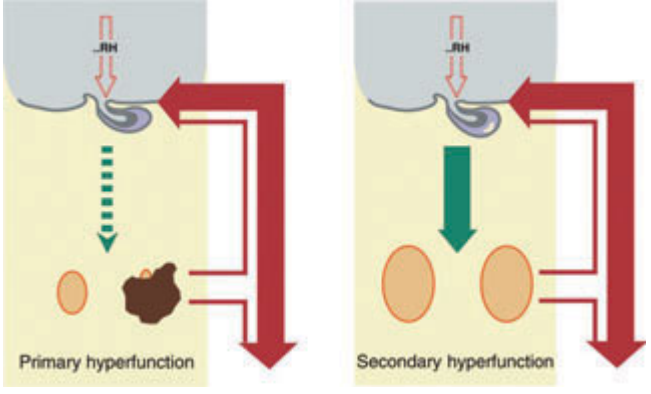


Figure 1.8:
Schematic illustration of two different forms of hormone excess: (1) tumor in a peripheral endocrine gland (left), and (2) hormonally active lesion in the pituitary gland (right). For explanation, see legend of fig. 1.7.

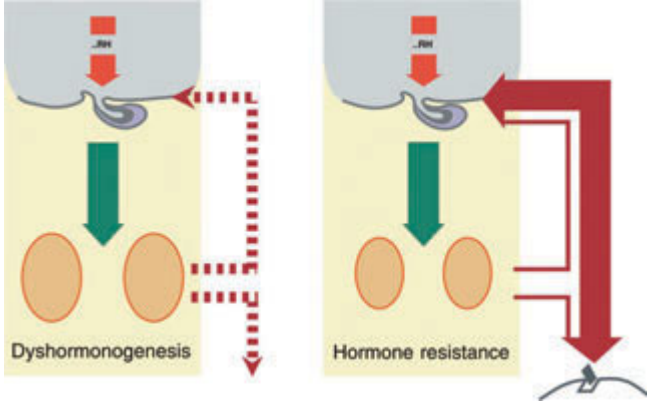


Figure 1.9:
Schematic illustration of altered feedback control in situations of (1) defective hormone synthesis in a peripheral endocrine gland (left), and (2) resistance to hormone action due to a receptor defect (right). For explanation, see legend of fig. 1.7.

one-hormone concept, adenohypophyseal cells are not irreversibly monohormonal but may become polyhormonal. This alteration of the morphologic features and the secretory capacity of mature cell types without cell division is called transdifferentiation (chapter 3.3.1).[13]

Excessive hormone production

The most frequent causes of hormone excess syndromes are hypersecretion of hormone by a tumor of the endocrine gland (primary hyperfunction) and hypersecretion due to hyperstimulation of the endocrine gland, of which there may be several causes (secondary hyperfunction) (fig. 1.8). Excessive hormone production may also be traced to cells that are not normally the primary source of circulating hormone (ectopic hormone production, see for example chapter 4.3.4). Rarely, hormone hypersecretion is the result of expression or activation of receptors in an endocrine gland that does not normally harbor functional receptors of this type. For example, the adrenal cortex may express aberrant receptors such as luteinizing hormone receptors (chapter 4.3.5). When hormones are used to treat nonendocrine diseases or when hormone replacement for an endocrine deficiency is excessive, the resulting syndrome of hormone excess is said to be iatrogenic.

Defective hormone synthesis

Genetic defects can cause abnormalities in hormone synthesis. Sometimes this leads not only to hormone deficiency but also to manifestations of a compensatory adaptation, such as goiter resulting from defective thyroid hormone synthesis (fig. 1.9).

Resistance to hormone action

Hormone resistance is defined as a defect in the capacity of normal target tissues to respond to the hormone (fig. 1.9). It may be an inherited disorder involving one or more molecular abnormalities, including defects in receptors and in postreceptor mechanisms. Hormone resistance may also be acquired, as is insulin resistance in some forms of diabetes mellitus (chapter 5.2.1). A common feature of hormone resistance is an elevated concentration of the hormone in the circulation in the presence of diminished or absent hormone action.

Abnormalities in hormone transport

Feedback control of hormone production and release is mediated by the concentration of free hormone. Thus a change in the concentration of transport or carrier proteins in the plasma usually affects only the total hormone concentration in plasma but not hormone action.

Finally, endocrine glands may be affected by **abnormalities not impairing function**. These include tumors, cysts, and infiltrative diseases not leading to significant impairment of hormone secretion.

1.4 Clinical assessment

1.4.1 History and physical examination

The diagnostic process is hampered by the inaccessibility for physical examination of all of the endocrine glands except the thyroids, parathyroids, and testes. However, deranged hormone secretion has consequences for the function of other organ systems, usually leading to multiple abnormalities which often have a characteristic pattern. The diagnosis of an endocrine disease thus often begins with the recognition of a pattern of characteristics in the medical history and in the findings of the physical examination.[14]

Many forms of hormone excess or deficiency lead to manifestations that are readily apparent at the time of the initial presentation of the patient for examination. Especially now that the definitive diagnosis can often be secured by laboratory data, veterinary clinicians have learned to recognize the patterns of physical characteristics of endocrine syndromes. Nevertheless, in some cases the changes are very subtle and it is necessary to rely completely on laboratory testing. This is especially true when endocrine disease is being considered in the differential diagnosis of common problems such as weakness, lethargy, and weight loss or gain.

1.4.2 Laboratory testing

The development of techniques for the measurement of hormones in biological fluids has made it possible to assess endocrine function in quantitative terms by the following approaches:

Hormone concentrations in plasma

The total concentration of steroid and thyroid hormones in plasma ranges between 1 and 1000 nM, while that of peptide hormones is generally between 1 and 500 pM. The application of radioimmunoassay, radioreceptorassay, chromatography, and more recently molecular biological techniques has transformed endocrinology from a largely descriptive discipline to a more quantitative one. Yet there are only a few situations in which a single measurement of the concentration of a hormone in plasma provides a reliable assessment of hormone production. There are several reasons for caution in assessing isolated measurements of hormone concentration in plasma:

Several hormones are secreted in a pulsatile manner (fig. 1.10) and/or their concentrations may vary in a diurnal rhythmicity, as well as with the sexual cycle, and pregnancy.[5,6]

Steroid and thyroid hormones are transported in plasma largely bound to proteins. The low percentage ($<$ 1–10 % of the total) of unbound hormone exerts the biological effect. The total hormone level reflects the amount of free hormone only if the amount and the affinity of binding protein remain constant or fluctuate within narrow limits.

The range of reference values for most hormones is fairly broad. Thus it is possible for the level in an individual animal to double or to decrease by half and yet still be in the reference range.[15] For this reason it is sometimes useful to measure the concentrations of a related pair of hormones simultaneously (e.g., cortisol and adrenocorticotropin).[16]

Some messengers circulate only in restricted compartments, such as the hypothalamic-pituitary portal system, and do not reach the systemic circulation in appreciable quantities.

Paracrine and autocrine effects of hormones are usually not reflected by hormone concentrations in plasma.

Exocrine secretion of hormones and the release of pheromones cannot be determined by measuring the hormone concentration in plasma.

Urinary excretion

Measurements of urinary excretion of hormones have the advantage of reflecting average concentrations in plasma and hence average production rates over the time interval between collections. Certain limitations must be kept in mind:

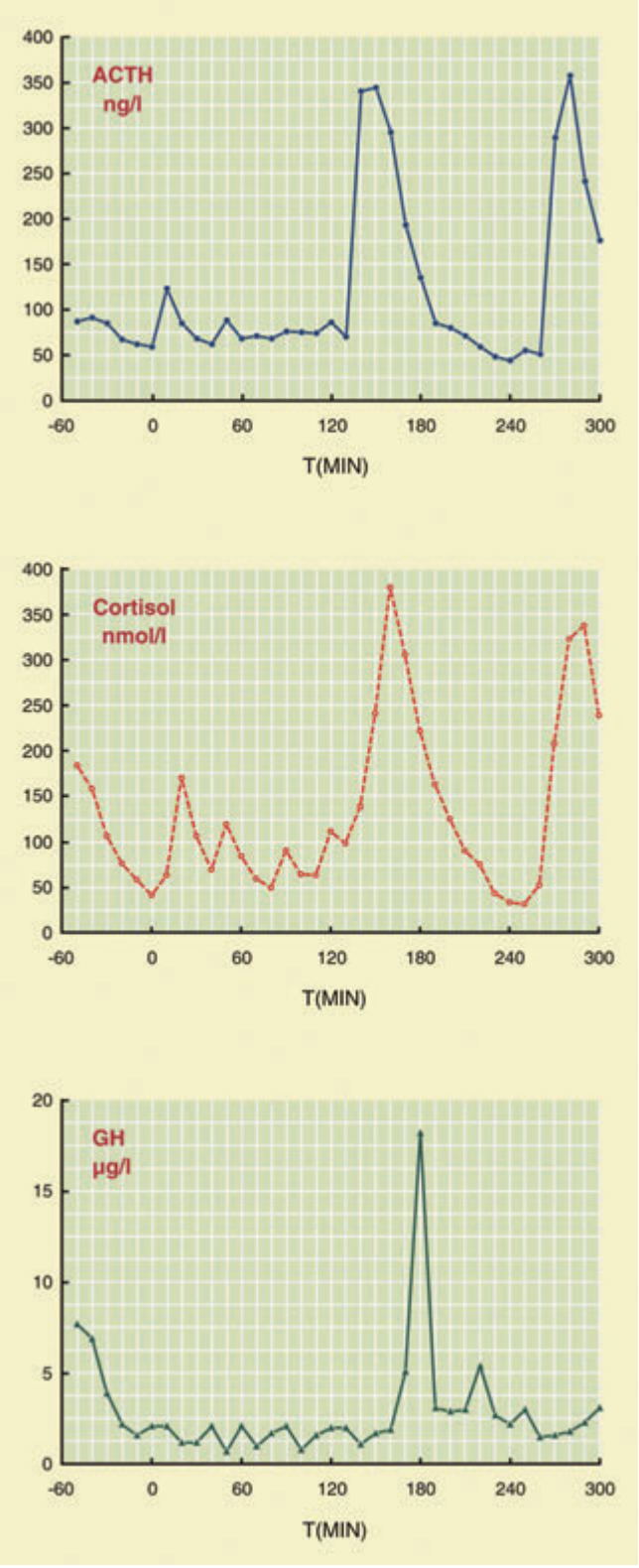


Figure 1.10:
Results of measurements of cortisol, adrenocorticotropin (ACTH), and growth hormone (GH) in frequently collected blood samples of a healthy adult dog. A meal was given at time 0'. The figure clearly illustrates the pulsatile character of hormone secretion.

- Collection of urine during a 24-hour period is a cumbersome procedure in most animals. It can be circumvented by relating the hormone concentration to the urinary creatinine concentration.
- The concentration of a hormone in urine is less meaningful if the hormone, such as thyroxine, is excreted in intact or conjugated form predominantly via the bile and only in very small amounts in the urine.
- There is considerable individual variation in the metabolism, and hence urinary excretion, of some of the peptide hormones.
- Changes in renal function may influence the rates of hormone excretion in the urine.

Production and secretion rates

These techniques can circumvent many of the problems associated with isolated measurements of hormones in plasma or urine, but they are difficult to perform and often require administration of radionuclides, for which reason they are not generally available.

Dynamic endocrine tests

Dynamic testing provides additional information. It involves either stimulation or suppression of endogenous hormone production. Stimulation tests are utilized most often when hypofunction of an endocrine organ is suspected. In the most commonly employed stimulation tests a tropic hormone is administered to test the capacity of a target gland to increase hormone production. The tropic hormone can be a hypothalamic releasing hormone such as corticotropin-releasing hormone (CRH), in which case the target gland is the pituitary and the measured response is the increment in the plasma concentration of ACTH, or it can be a pituitary hormone such as ACTH, with the adrenal cortex as the target gland being assessed by the measurement of the increment in the plasma concentration of cortisol. Suppression tests are utilized when endocrine hyperfunction is suspected. They are designed to determine whether negative feedback control is intact. A hormone or other regulatory substance is administered and the inhibition of endogenous hormone secretion is assessed.

Dynamic tests continue to be of importance in the diagnosis of certain disorders but in circumstances in which hormone pairs can be measured accurately (e.g., thyrotropin [TSH] and thyroxine; chapter 3.3.1) they are required less often.

Hormone receptors and antibodies

The measurement of hormone receptors in biopsy material from target tissues may become increasingly useful in companion animal endocrinology, especially in the diagnosis of hormone resistance. Measurement of antibodies to hormones or antigens in endocrine tissues may also be essential in order to characterize certain endocrine abnormalities such as autoimmune phenomena. Antibodies against hormones may also interfere with diagnostic procedures such as radioimmunoassays.[17]

1.4.3 Diagnostic imaging

The inaccessibility of most of the endocrine glands for direct physical examination has been progressively overcome during the past two decades by the use of diagnostic imaging techniques such as ultrasonography, scintigraphy, computed tomography (CT), and magnetic resonance imaging (MRI).[18] The former technique is relatively inexpensive but requires extensive operator experience, whereas the latter three may be easier to perform but require expensive equipment as well as immobilization which necessitates anesthesia.

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2 Hypothalamus-Pituitary System

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2.1 Introduction

The hypothalamus and pituitary form a complex functional unit that transcends the traditional boundary between neurology and endocrinology. Many key elements of this system are neither purely endocrine nor purely neural. There are three components:

(1) A neuroendocrine system connected to an endocrine system by a portal circulation. The neuroendocrine system consists of clusters of peptide- and monoamine-secreting cells in the anterior and middle portions of the ventral hypothalamus. Their products – releasing hormones and inhibiting factors – are transported by nerve fibers to terminals in the outer layer of the median eminence (fig. 2.1[1]). Here they are released into capillaries of the hypothalamic-hypophyseal portal system for transport to the anterior lobe (AL) of the pituitary, where they regulate hormone production and secretion (fig. 2.2 and table 2.1).

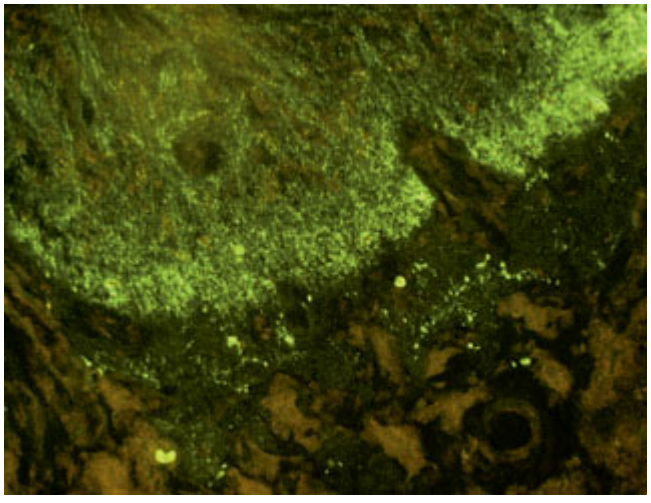


Figure 2.1:
Nerve fiber terminals containing corticotropin-releasing hormone (CRH) in the outer layer of the median eminence of a dog, visualized by indirect immunofluorescence. Note the presence of CRH-immunoreactive fibers outside the terminal zone in close proximity to the capillary system.[1]

Table 2.1:
Terminology for the parts of the hypophysis (glandula pituitaria) according to the Nomina Anatomica Veterinaria (N.A.V.) and the variants in the Nomina Histologica Veterinaria (N.H.V.), and Nomina Anatomica (N.A., for man)[2]

N.A.V.	N.H.V.	N.A.
Adenohypophysis (Lobus anterior)		
Pars infundibularis adenohypophysis	Pars proximalis adenohypophysis	Pars tuberalis
Pars intermedia adenohypophysis		Pars intermedia
Pars distalis adenohypophysis		Pars distalis
Neurohypophysis (Lobus posterior)		
Pars proximalis neurohypophysis (infundibulum)		Infundibulum
Pars distalis neurohypophysis		Lobus nervosus

For practical reasons the terminology in this book is confined to the three functional units: Anterior lobe (= Pars infundibularis and Pars distalis of the adenohypophysis), Pars intermedia, and Posterior lobe (see also fig. 2.2).

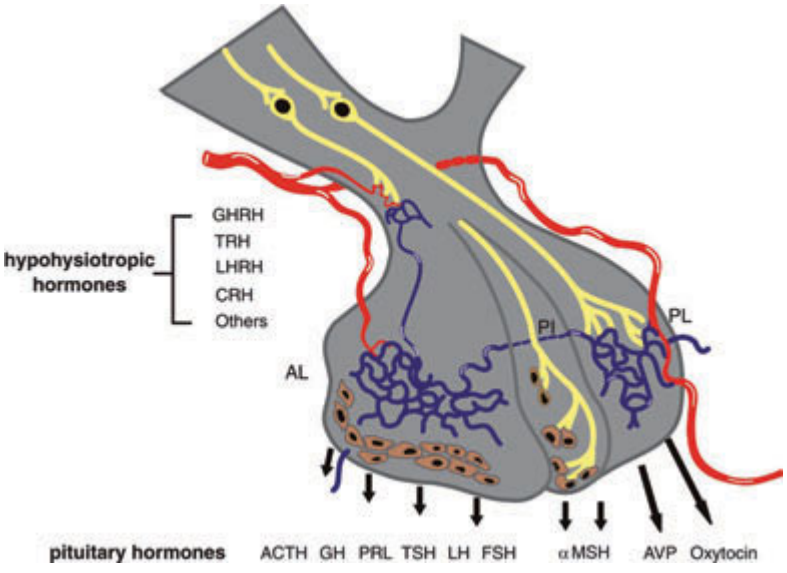


Figure 2.2:
Schematic representation of the relationship of the hypothalamus and pituitary. The hypothalamus exerts control over the anterior lobe (AL) through releasing and inhibiting factors that reach the AL cells via capillaries of the pituitary portal system. The posterior lobe (PL) of the pituitary is a downward projection of the hypothalamus. The pars intermedia (PI) is under direct neurotransmitter control.

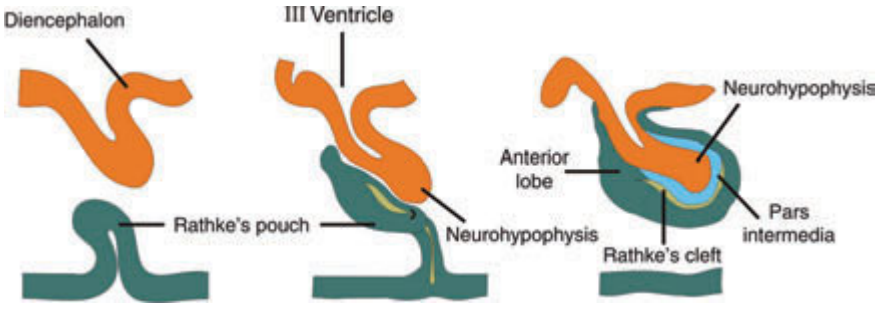


Figure 2.3:
Schematic representation of the ontogenesis of the pituitary gland.

(2) A neurosecretory pathway in which hormones are produced by neurons in the anterior hypothalamus and transported by nerve fibers that traverse the ventral hypothalamus and pituitary stalk to terminate on fenestrated blood vessels in the neurohypophysis or posterior lobe (PL) (fig. 2.2). The neurohypophyseal hormones are stored in secretory vesicles in the terminal ends of the nerve fibers and secreted into the systemic circulation in response to an appropriate stimulus.
(3) The pars intermedia (PI) is directly innervated by predominantly aminergic nerve fibers from the hypothalamus. This direct neural control is largely a tonic (dopaminergic) inhibitory influence.

During embryogenesis the adenohypophysis develops from Rathke's pouch, which arises from the roof of the primitive mouth in contact with the base of the brain. Rathke's pouch subsequently separates by constriction from the oral cavity. The anterior wall thickens and forms the pars distalis of the AL. The posterior wall of Rathke's pouch is closely apposed to the neural tissue of the PL to form the pars intermedia, remaining separated from the AL by the hypophyseal cleft or cavity, which was the lumen of Rathke's pouch. In the dog and the cat the adenohypophysis extends as a cuff or collar around the proximal neurohypophysis and even envelops part of the median eminence (figs. 2.3, 2.4).

Pituitary gland development is primarily the result of the interaction between neuroectodermal and oroectodermal tissues. In recent years several of the signaling molecules and transcription factors involved in this process have been identified (fig. 2.5).[3,4] The adenohypophyseal cells follow three main pathways of differentiation:
(1) Cells expressing pro-opiomelanocortin (POMC), leading to secretion of adrenocorticotropic hormone (ACTH) and α-melanocyte-stimulating hormone (α-MSH) by corticotrophs and melanotrophs, respectively
(2) Gonadotroph cells secreting follicle-stimulating hormone (FSH) and luteinizing hormone (LH)
(3) Pit1-dependent cell lines (somatotroph, lactotroph, and thyrotroph cells), leading to secretion of growth hormone (GH), prolactin (PRL), and thyroid-stimulating hormone (TSH).

Following proliferation of the progenitor cells, the different endocrine cell phenotypes arise in a distinct temporal fashion. As in other species, in the fetal dog adenohypophysis ACTH-immunoreactive cells are the first to differentiate from the pituitary progenitor cells.[5]

The rostral hypophyseal arteries form the uniquely organized capillary plexus of the median eminence that is in close proximity to nerve terminals of the hypophysiotropic neurons. The blood-brain barrier is incomplete in the area of the median eminence, permitting protein and peptide hormones and other charged particles to move to the intercapillary spaces and the nerve terminals contained therein. These terminals respond to humoral and neuronal stimuli by secreting releasing and inhibiting factors into the portal system. The portal capillaries coalesce into a series of vessels that descend through the pituitary stalk and form a second capillary plexus surrounding the AL cells (fig. 2.2).

Caudal hypophyseal arteries supply the PL. From the primary plexus of the PL blood flows not only to the systemic circulation but also to the AL and the hypothalamus. The intrapituitary vascularization involved in this has not been fully elucidated but there appears to be some degree of circulatory flow, from the AL to the PL, from there to the infundibulum, and then back to the AL. The vascularization of the PI is closely linked to that of the PL, but while the PL has a rich blood supply, the PI is poorly vascularized. Blood-borne factors play a relatively less significant role in control of PI function.

2.2 Anterior lobe

In agreement with the main pathways of cell differentiation (chapter 2.1), the peptide hormones secreted by the AL can be divided into three categories: (1) the somatomammotropic hormones GH and PRL, (2) the glycoprotein hormones TSH, FSH, and LH, and (3) the corticomelanotropins α-MSH, ACTH, β-endorphin (β-END), and β-lipotropin (β-LPH). The hormones of the third group are derived from the precursor POMC, which is synthesized not only in the

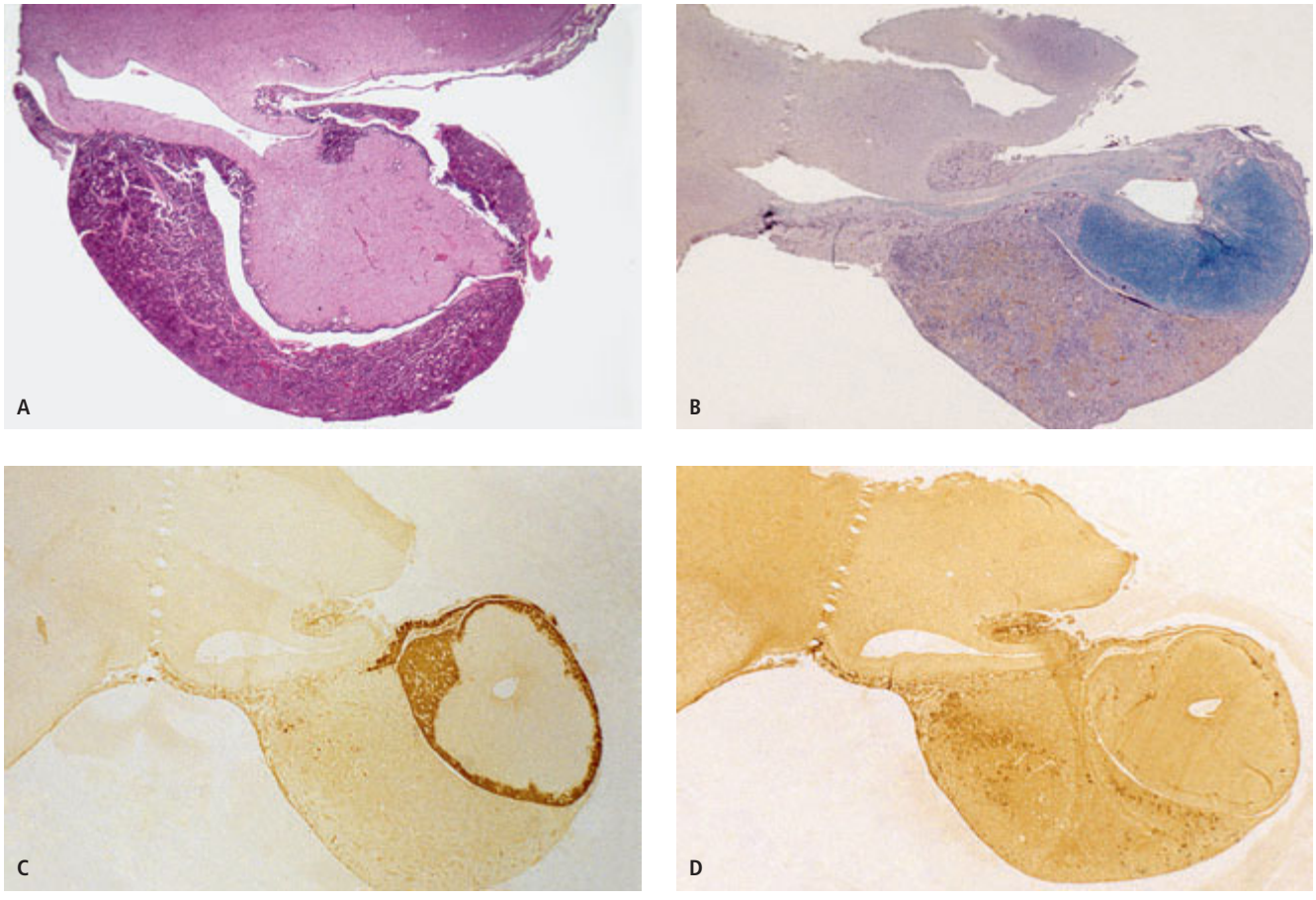


Figure 2.4:
(A) Sagittal section of a dog pituitary. The AL is separated from the PI and PL by the hypophyseal cavity and surrounds it up to the pituitary stalk and median eminence. The PI is a narrow zone around the periphery of the PL. H&E stain. (Courtesy of Dr. B. E. Belshaw.)
(B) PAS-Alcian Blue-orange G stain of a sagittal section of a cat pituitary. The third ventricle extends deeply into the PL (blue), which is surrounded by a thin rim of PI. Sections of a cat pituitary immunostained for α-MSH (C) and ACTH (D). The latter picture clearly illustrates that in the cat the AL also extends upward around the pituitary stalk. (Courtesy of Prof. Dr. H. J. Th. Goos and Mrs. A. Slob.)

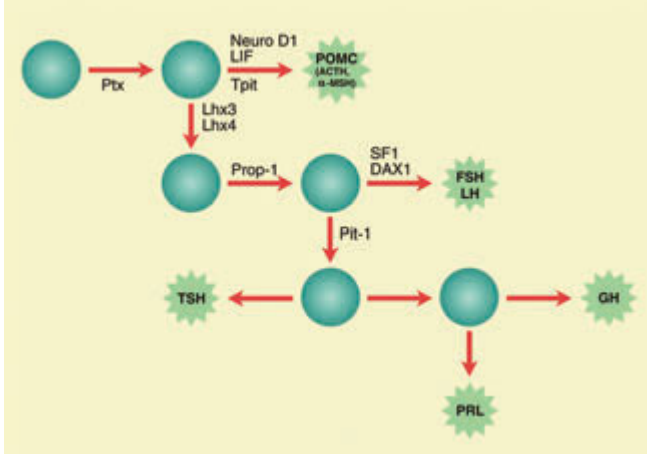


Figure 2.5:
Simplified model of the differentiation of AL cell lineages. Each type of endocrine cell is labeled with the hormone it synthesizes. Steps in precursor cell differentiation and some of the involved transcription factors are indicated.
Ptx1 = pituitary homeobox; Neuro D1 = neurogenic differentiation factor D1; LIF = leukemia inhibiting factor; Tpit = T-box pituitary transcription factor; Lhx3/4 = LIM-domain transcription factors 3 and 4; Prop1 = prophet of Pit1; Pit1 = pituitary transcription factor 1, also referred to as POU1F1; SF1 = steroidogenic factor 1; DAX1 = dosage sensitive sex-reversal-adrenal hypoplasia congenital critical region on the X chromosome 1.

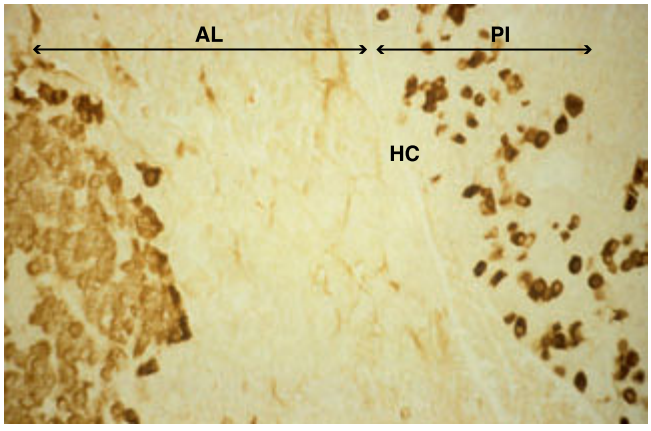


Figure 2.6:
Pituitary of a dog with pituitary-dependent hypercortisolism, immunostained with an antibody to ACTH. At the left is a nest of immunopositive hyperplastic corticotropic cells in the anterior lobe (AL). Excessive ACTH production by this microadenoma resulted in cortisol excess, which reduced immunoreactivity in the rest of the AL via negative feedback. In the pars intermedia (PI), on the other side of the hypophyseal cavity (HC), the persistence of immunoreactivity in corticotropic cells indicates their insensitivity to negative cortisol feedback.

corticotropic cells of the AL but also in cells of the pars intermedia (fig. 2.6). They will be discussed in more detail in chapter 4.

The hormone-producing cells of the AL are classified according to their specific secretory products: somatotrophs (secreting GH), lactotrophs (secreting PRL), thyrotrophs (secreting TSH), corticotrophs (secreting ACTH and related peptides), and gonadotrophs (secreting LH and FSH). The distribution of the various secretory cells of the AL is not random but has a topological and numeric organization, which is best known for the human pituitary gland but may also be true for the dog and cat. The AL consists of a central »mucoid« wedge containing thyrotrophs and corticotrophs and lateral wings containing somatotrophs and lactotrophs. The gonadotrophs are distributed diffusely throughout the gland. The distribution of cell types is roughly 15 % corticotrophs, 10 % thyrotrophs, 50 % somatotrophs, 15 % lactotrophs, and 10 % gonadotrophs.[6]

It is now clear that the classic concept that each cell type stores a single hormone, its secretion regulated by a specific hypothalamic releasing hormone (HRH), is no longer tenable. Some anterior pituitary cells are multifunctional and exhibit mixed phenotypes with multiple HRH-receptor expression and/or hormone storage. These multifunctional AL cells are involved in cell plasticity processes directed at increasing hormone production during demanding physiologic and pathophysiologic situations such as lactation, ovulation, hypothyroidism, and low temperatures.[7,8] For example, in dogs with primary hypothyroidism longstanding thyroid hormone deficiency may lead to AL cells staining for both GH and TSH, and so-called paradoxical secretion, i.e., GH release stimulated by thyrotropin-releasing hormone (TRH) (see also the section on diagnosis in chapter 3.3.1).

Under physiologic and most pathologic conditions the basal plasma concentration of each of the six major AL hormone systems (ACTH, LH and FSH, TSH, GH, and PRL) is regulated via a feedback (closed loop) system. Secretion of both AL hormones and hypophysiotropic hormones is suppressed by the products of target endocrine glands such as the thyroids, adrenals, and gonads (see also chapter 1.3). Apart from this long-loop feedback, some hormones such as PRL regulate their own secretion directly by acting on the hypothalamus (short-loop feedback). Upon this powerful feedback control with primary blood-borne signals, other signals are superimposed. These may originate within the central nervous system (open loop) and can be mediated by neurotransmitters and hypophysiotropic hormones (fig. 2.7). Thus influences are exerted that represent the environment (temperature, light-dark), stress (pain, fear), as well as intrinsic rhythmicity.

The releasing and inhibiting hormones are stored in nerve terminals in the median eminence in concentrations 10–100 times greater than elsewhere in the hypothalamus. The portal blood flow to the pituitary is not compartmentalized and thus the hypophysiotropic hormones secreted into it gain access to all types of cells in the AL. Specificity is achieved not by anatomic segregation but by the presence of specific receptors on individual types of AL cells.

These regulatory factors influence peptide synthesis and/or release in AL cells, where each of the steps in hormone synthesis and ultimate secretion represents a potential control point in the regulation of circulating hormone levels (see fig. 1.4). Modulation of the amount of mRNA, the efficiency of transcription and translation, the processing from prepro-hormone to hormone, and intracellular degradation of stored hormone determine, separately or jointly, the amount of hormone available for release.

The hypophysiotropic hormones whose structures have been elucidated are, with one exception, peptides with sequence lengths ranging from 3–44 amino acids (fig. 2.8). Species variation in amino acid sequences can occur with increasing length. Whereas the structures of TRH, GnRH, and somatostatin (three, ten, and 14 amino acids, respectively) are identical in all mammals studied, the structure of GHRH varies. Yet CRH, with 41 amino acids, is identical in man, dog, horse, and rat.[9]

The only nonpeptide hypophysiotropic hormone is dopamine. In addition to its major role as a neurotransmitter, it is the most important inhibitor of prolactin (PRL) secretion. The existence of a separate PRL-releasing hormone has long been a matter of debate. It has been concluded that regulation

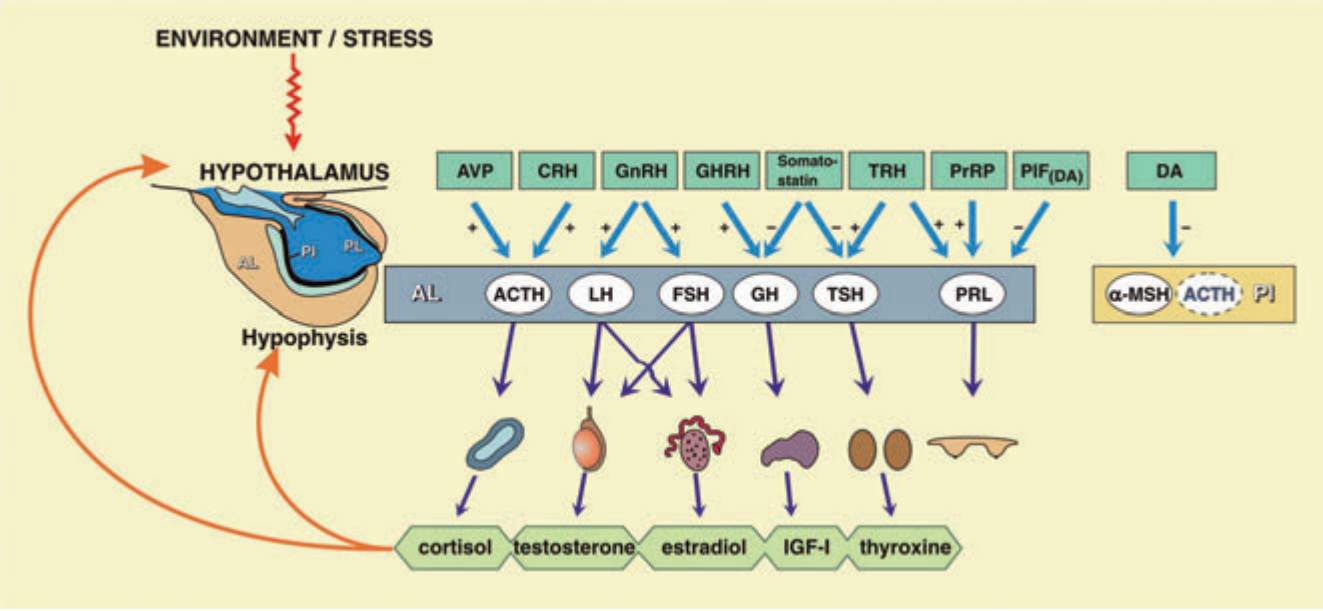


Figure 2.7:
Schematic illustration of the hypophysiotropic regulation of the secretion of hormones by the adenohypophysis.
AVP = arginine-vasopressin; CRH = corticotropin-releasing hormone; GnRH = gonadotropin-releasing hormone; GHRH = growth hormone-releasing hormone; TRH = thyrotropin-releasing hormone; PrRP = prolactin-releasing peptide; $PIF_{(DA)}$ = prolactin-inhibiting factor (dopamine); ACTH = adrenocorticotropic hormone; LH = lutein-izing hormone; FSH = follicle-stimulating hormone; GH = growth hormone; TSH = thyroid-stimulating hormone; PRL = prolactin; α-MSH = α-melanocyte-stimulating hormone; IGF-I = insulin-like growth factor-I.

hormone	*structure*	*main function*
TRH	pyro Glu His Pro NH_2	releases TSH and prolactin
GnRH	pyro Glu His Trp Ser Tyr Gly Leu Arg Pro Gly NH_2	releases LH and FSH
somatostatin	S S Ala Gly Cys Lys Asn Phe Phe Trp Lys Thr Phe Thr Ser Cys	inhibits GH and TSH release
CRH (canine)	Ser Glu Glu Pro Pro Ile Ser Leu Asp Leu Thr Phe His Leu Leu Gln Glu Ala Arg Ala Met Glu Leu Val Glu Arg Leu Ala Gln Gln Ala His Ser Asn Arg Lys Leu Met Glu Ile Ile NH_2	releases ACTH
GHRH (canine)	Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Arg Ser Met Ile Asp Gln Leu Leu Lys Arg Ala Ser Leu Gln Gln Gln Gly Glu Arg Asn Arg Glu Gln Gly Ala Lys Val Arg Leu NH_2	releases GH

Figure 2.8:
Structure and main function of hypothalamic hypophysiotropic hormones.

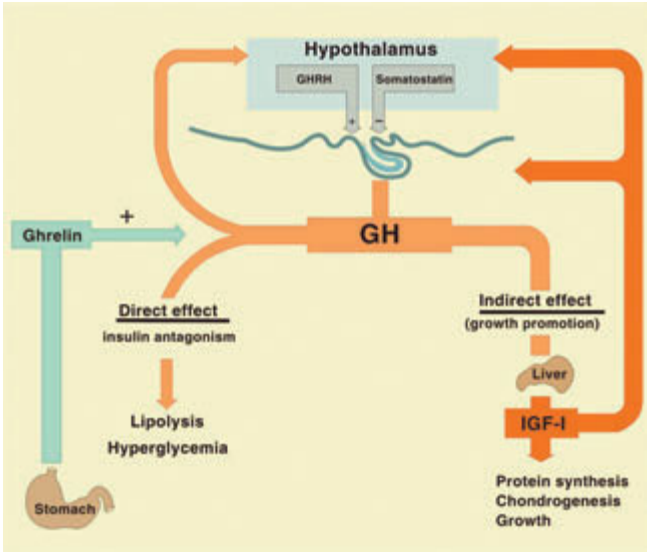


Figure 2.9:
The secretion of GH is under inhibitory (somatostatin) and stimulatory (GHRH) hypothalamic control and is also modulated by a long-loop feedback control by IGF-I, a peptide primarily formed in the liver under the influence of GH. GH itself exerts a short-loop negative feedback by activating somatostatin neurons. The gastric peptide ghrelin is the natural ligand for the GH secretagogue receptor that stimulates GH secretion at the pituitary level. The direct catabolic (diabetogenic) actions of GH are shown on the left side of the figure and the indirect anabolic actions on the right.

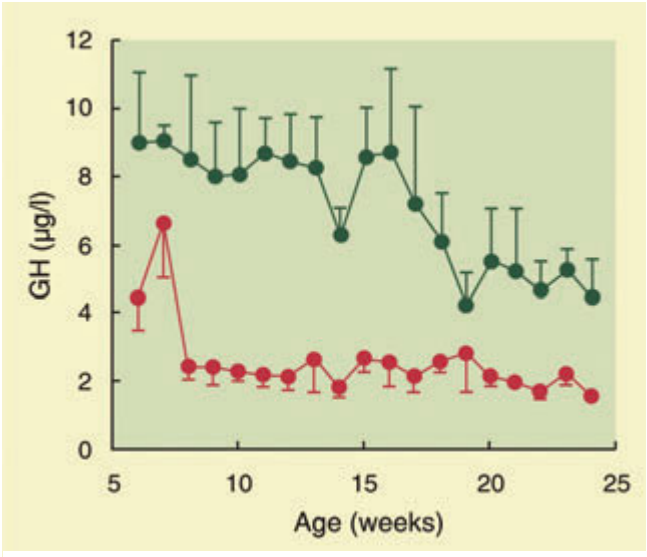


Figure 2.10:
Basal plasma GH concentration (mean ± SEM, n = 6) in beagles (red line) and Great Danes (green line) from six to 24 weeks of age.

of PRL release should be viewed as a fine balance between the action of dopamine as an inhibitor and several hypothalamic factors (mainly serotonin) as well as systemic and local factors, all acting as stimulators but none of which has yet emerged as the primary PRL-releasing hormone.[10] Several candidates have been proposed, including TRH and a PRL-releasing peptide (PrRP) from the hypothalamus (fig. 2.7). PrRP, a 31-amino-acid-peptide, increases plasma PRL concentration but by many times less than TRH. However, PrRP can increase PRL responses to TRH several-fold.[11] It is possible that PrRP primarily regulates food intake.[12]

2.2.1 Somatotropin and lactotropin

Only two of the six AL hormones are discussed here, the others being discussed in detail in other chapters. Somatotropin or growth hormone (GH) and lactotropin or prolactin (PRL) have similarities in amino acid composition and share some biological activities, and for these reasons they are often classified together as somatolactotropic hormones. They are rather large, single-chain polypeptides containing 190 (GH) and 199 (PRL) amino acids and having two (GH) or three (PRL) intrachain disulfide bridges (fig. 1.3). Their molecular weights are approximately 22 and 23 kDa, respectively. The amino acid sequences of canine and porcine GH are identical and differ by only one amino acid from that of the cat.[13–15] The amino acid sequences of canine PRL and feline PRL differ in eight amino acids.[16,17] In nonprimates a single gene encodes for GH, whereas in several species there is a large family of paralogous genes related to PRL.[18,19]

2.2.1.1 Pituitary growth hormone

GH release is characterized by rhythmic pulses and intervening troughs (fig. 1.10). The GH pulses predominantly reflect the pulsatile delivery of GHRH from the hypothalamus, whereas GH levels between pulses are primarily under the control of somatostatin (SS) or somatotropin-release inhibiting factor (SRIF) (fig. 2.9). Five SS receptors ($sst_{1–5}$) are expressed in the pituitary, sst_2 being the predominant subtype in the dog.[20]

Pituitary somatotrophs are not only stimulated via GHRH and its receptor (GHRH-R), but also via receptors different from GHRH-R. Several synthetic GH secretagogues (GHSs), both peptidergic and nonpeptidergic in structure, stimulate GH release via the GHS receptor.[21,22] The endogenous ligand for the GHS receptor, ghrelin, has also been identified.[23] This 28-amino-acid-peptide is primarily expressed in enteroendo-

crine cells of the gastric fundus. There is little structural heterogeneity among species. For example, human and dog ghrelin differ in only two amino acids.[24] Ghrelin not only stimulates GH release but also stimulates food intake, thereby increasing body weight, while reducing mobilization of adipose stores. In addition, ghrelin accelerates gastric and intestinal emptying.[25,26] In dogs and cats plasma ghrelin concentration has been reported to increase during fasting and to decrease after food intake, while ghrelin administration increases food intake (chapter 11.1.1).[27–29] In young dogs ghrelin is a more potent GH secretagogue than GHRH.[30]

Although in primates GH can bind to both the GH receptor (GHR) and the prolactin receptor, in nonprimate mammals such as the dog it can only bind to its specific receptor, GHR.[31] The coding sequence of canine GHR has extensive homology with that of several other species.[32] The effects of GH can be divided into two main categories: rapid or metabolic actions and slow or hypertrophic actions. The acute catabolic responses are due to direct interaction of GH with the target cell and result in enhanced lipolysis and restricted glucose transport across the cell membrane due to insulin antagonism. The slow anabolic effects are mediated via a growth factor primarily synthesized in the liver and known as insulin-like growth factor-I (IGF-I). In its chemical structure IGF-I has approximately 50 % sequence similarity with insulin (as does IGF-II), indicating that it evolved from a common ancestral molecule. Contrary to insulin, the IGFs are bound to carrier proteins in plasma, the IGF-binding proteins (IGF-BPs). This prolongs their half-life, consistent with their long-term growth-promoting action. Insulin and IGF seem to complement each other, insulin being the acute and IGF the long-term regulator of anabolic processes. The insulin receptor and the IGF-I receptor belong to the same subfamily of receptor tyrosine kinases.[33]

In adult dogs there is a strong linear correlation between plasma total IGF-I concentration and body size, while basal plasma GH concentrations are quite similar among various breeds. For example, plasma IGF-I concentrations have been found to be six times higher in standard poodles than in toy poodles.[34] In addition, a single IGF-I nucleotide polymorphism haplotype is common to all dogs of small breed and nearly absent in those of giant breeds.[35] Yet it may be questioned whether IGF-I is the main determinant of body size. Thus far only total IGF-I concentration has been measured. Without measurement of free IGF-I and/or IGF-BPs, no insight is gained into possible differences in IGF-receptor exposure among dogs of different body size. The six IGF-BPs are known as important modulators of IGF actions.[36] In addition, serial measurements of plasma GH concentration have revealed that the initially very high levels in Great Dane pups decrease to adult levels by about half a year of age. In miniature poodles the GH level does not change significantly with time and values in young animals are within the reference range for adult dogs.[37,38] Long-term infusion of IGF-I does not stimulate their growth, but GH administration does.[39,40] In a comparative study in both Great Dane and Beagle pups the nutritional conditions were such that the plasma IGF-I concentrations were not significantly different. In the Beagles GH secretion was high until the age of seven weeks, whereas in the Great Danes it remained high much longer (fig. 2.10).[41] These observations indicate that GH hypersecretion at a young age rather than IGF-I is the main determinant of body size.

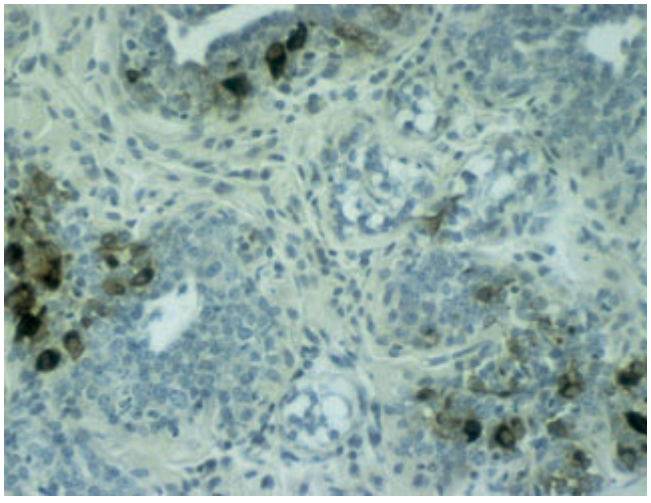


Figure 2.11:
Histologic section of the mammary gland of a progestin-treated dog, indirectly immunostained with monkey-anti-canine GH. The immunopositive staining is located in cells of hyperplastic ductular epithelium.

As a closing remark on the actions of GH it should be mentioned that the separation of the two opposing biological actions, as illustrated in fig. 2.9, is not as strict as suggested above. GH exerts its growth-promoting effect not only via IGF-I produced in the liver but also directly and by stimulation of local IGF-I secretion in several tissues. For example, in the growth plate GH stimulates cell differentiation directly and clonal expansion indirectly through the local production of IGF-I. In line with these growth-promoting effects of GH, the expression of the GH receptor increases locally during distraction-induced bone regeneration in dogs.[42]

2.2.1.2 Mammary growth hormone

In dogs circulating GH not only originates from the pituitary but can also be of mammary origin. In the 1970s and 1980s the administration of progestins to dogs was found to be the cause of elevated plasma GH levels and physical changes of growth hormone excess. Progestin-induced GH is not released in a pulsatile manner, does not respond to stimulation with GHRH, and is not inhibited by the administration of somatostatin.[43] This progestin-induced GH originates from foci of hyperplastic ductular epithelium in mammary tissue (fig. 2.11).[44] The gene encoding mammary GH is identical to

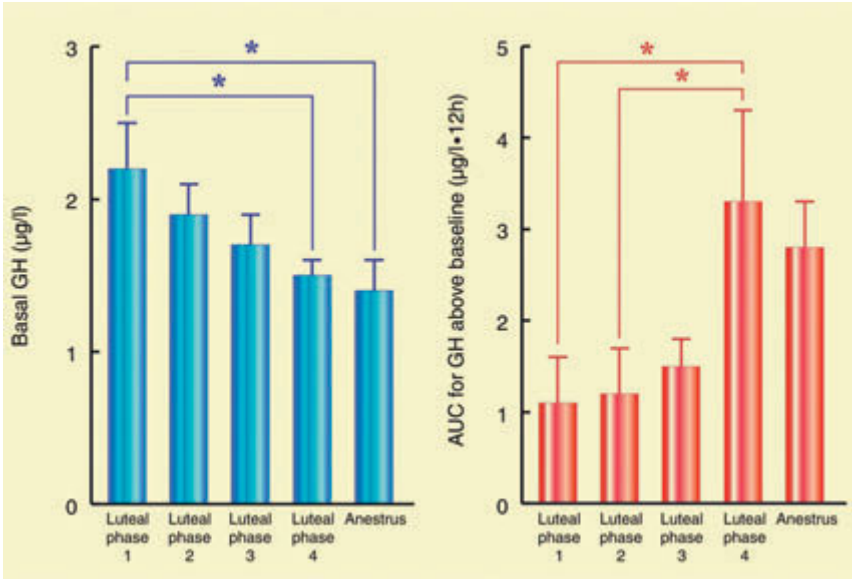


Figure 2.12:
Mean (± SEM) basal plasma GH concentration and mean (± SEM) area under the curve (AUC) for GH above the baseline in six Beagle bitches. Blood samples were collected at 10-min intervals for 12 h in the first, second, third, and fourth quarter of the luteal phase (luteal phases) 1–4 and during midanestrus. * Indicates significant difference.

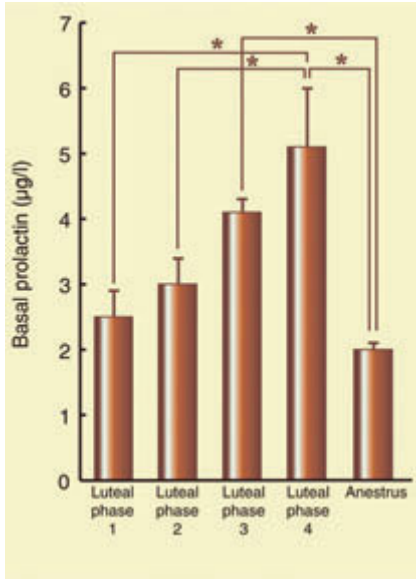


Figure 2.13:
Plasma PRL concentrations in six beagle bitches in four stages of the luteal phase and during midanestrus. See also legend to fig. 2.12.

that encoding GH in the pituitary gland.[45] Progestins stimulate GH promoter activity in the mammary gland indirectly rather than directly. In contrast to the adenohypophysis, the mammary gland lacks expression of the transcription factor Pit-1.[46]

Progesterone-induced release of mammary GH is a normal physiological process during the luteal phase of the estrous cycle, which has consequences for the pulsatile secretion pattern of pituitary GH. The plasma GH profile during the first half of the luteal phase is characterized by higher basal plasma GH levels and lower GH pulses than during anestrus (fig. 2.12).[47,48]

The local production of GH, the expression of the GH receptor, and the associated production of IGF and IGF-BPs appear to participate in the cyclic changes in the mammary gland. The presence of this highly proliferative environment may also enhance the risk of malignant transformation and promotion of tumor growth, with an associated inhibition of programmed cell death.[49,50] In both humans and dogs with mammary cancer there is evidence that locally produced GH enhances malignant transformation in an autocrine manner.[51,52] Although there are similarities between progestin-induced epithelial changes in the mammary gland and the uterus, it is now clear that progestin-induced GH in plasma does not originate from uterine epithelium and that mammary GH is not required for the development of progestin-induced cystic endometrial hyperplasia in the bitch.[53,54]

2.2.1.3 Prolactin

Under the influence of the above-mentioned hypothalamic inhibitory and stimulatory factors, PRL is also secreted in pulses. In addition, gonadal hormones modulate PRL secretion. In bitches plasma PRL concentration increases during the second part of the luteal phase (fig. 2.13).[48] The association of increasing PRL and declining plasma progesterone has been substantiated in pregnant bitches by the administration of a progesterone-receptor antagonist and by ovariectomy.[55,56] Both interventions caused plasma PRL concentration to rise. In male dogs castration does not affect plasma PRL concentration.[57]

Among the many functions ascribed to PRL, its involvement in reproduction is best known.[58] PRL is an essential luteotropic factor in canines (in contrast to humans) and thus mandatory for maintaining progesterone secretion during

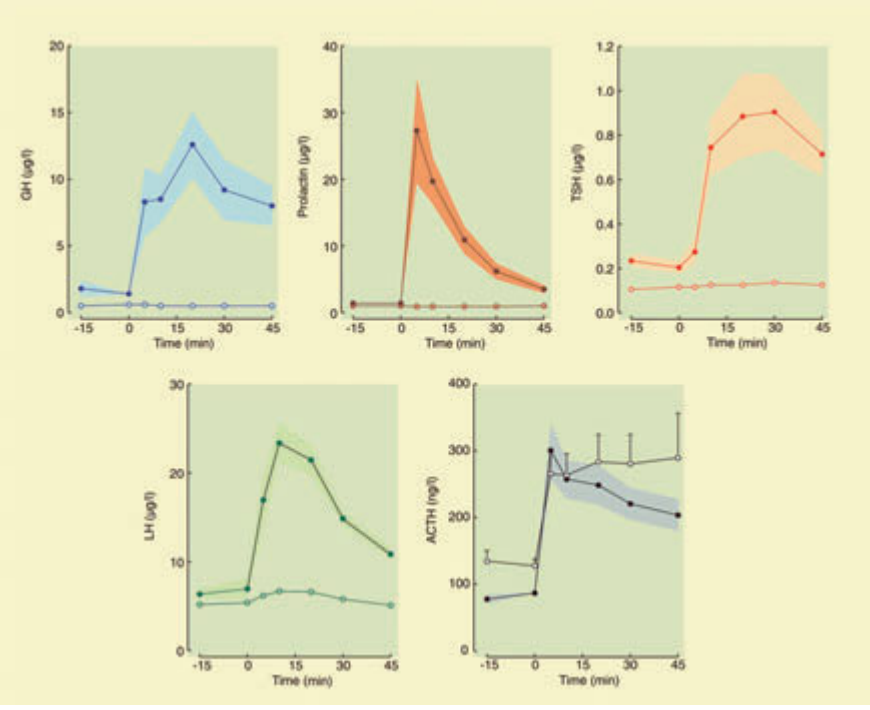


Figure 2.14:
Responses of plasma GH, PRL, TSH, LH, and ACTH to the combined injection of four hypothalamic releasing hormones (CRH, GHRH, TRH, and GnRH) in eight German shepherd dogs (-○-) with pituitary dwarfism (means ± SEM if exceeding the size of the symbols). The curves with shaded areas represent the responses (mean ± SEM) in healthy beagles (-●-)[72,73].

the normal lifespan of the corpora lutea. Hypophysectomy and treatment with dopamine agonists shorten the luteal phase.[59–61] Consistent with the secretion patterns of GH and PRL (figs. 2.12, 2.13), the GH-induced proliferation of mammary epithelium is followed by lobular-alveolar differentiation under the influence of PRL. Not only mammogenesis but also lactogenesis (acquisition of the ability to produce milk) and galactopoiesis (maintenance of milk secretion) depend on PRL.[58]

As discussed in more detail in chapter 2.2.5 and chapter 7, pregnant and nonpregnant bitches (but not queens) have similar luteal phases and similar changes in their mammary glands. Lactogenesis appears at the end of the luteal phase in nonpregnant bitches, allowing them to care for and nurse a litter.[62] As will be discussed in chapter 2.2.5, nonpregnant bitches not caring for pups may also undergo behavioral changes in this stage of the cycle.

2.2.2 Congenital growth hormone deficiency

Inadequate GH secretion early in life causes retardation of growth. Dwarfism due to GH deficiency is best known as a genetically transmitted condition (autosomal recessive inheritance) in German shepherd dogs and Carelian bear dogs.[63,64] German shepherd dogs with pituitary dwarfism have a combined deficiency of GH, TSH, and PRL, as well as impaired release of gonadotropins, whereas ACTH secretion is preserved (fig. 2.14).[65] Diagnostic imaging and histological examination often reveal cystic changes in the pituitary gland and hypoplasia of adenohypophyseal tissue.[66] The search for the causative gene defect has excluded transcription factors Prop-1, Pit-1, Lhx4, and the LIF-receptor gene (fig. 2.5) as candidates for pituitary dwarfism in German shepherds.[67–70] The gene encoding for Lhx3 appears to be the most likely site of the mutation.[71]



Figure 2.15:
(A) Four-month-old German shepherd dog with pituitary dwarfism. The woolly appearance of the coat is due to complete lack of development of primary guard hairs. (B) Dwarf German shepherd dog at one year of age, with the characteristic fox-like face and alopecia developing on the neck.

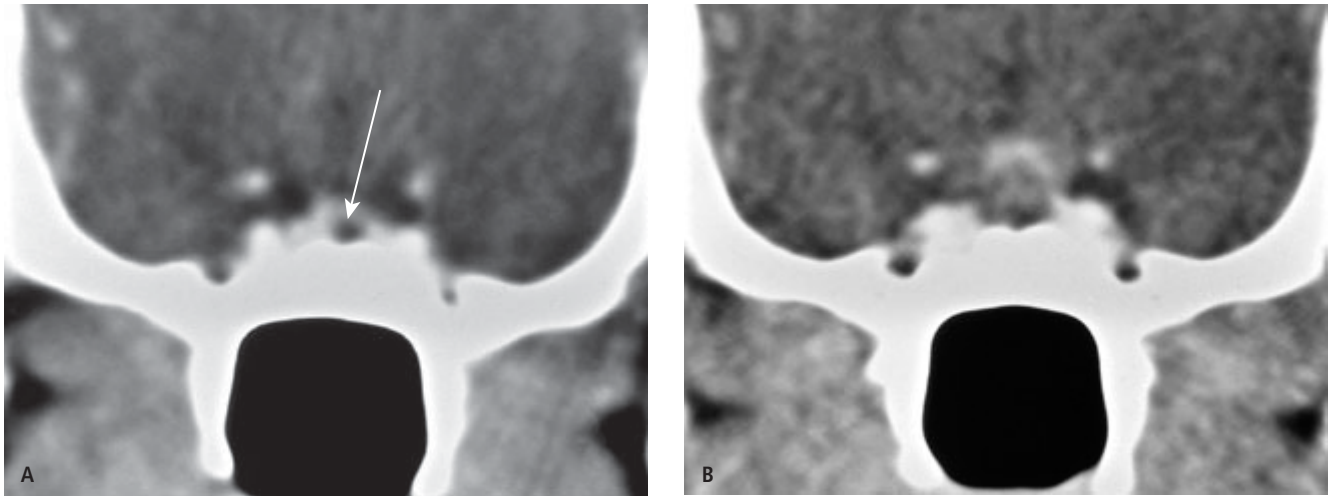


Figure 2.16:
Contrast-enhanced CT images of a six-month-old dwarf German shepherd dog (A) with a pituitary of normal size (height 3.6 mm; width 4.3 mm) but having a radiolucent area due to a cyst (arrow). At the age of three years (B) the pituitary is enlarged (height 6.5 mm; width 5.4 mm) and the greater part of it lacks contrast enhancement due to the cyst.

Clinical manifestations

Affected animals are usually presented at the age of two to five months because of poor growth and an abnormally soft and woolly hair coat (fig. 2.15). The latter is due to retention of lanugo or secondary hairs and lack of primary or guard hairs. This stagnant development of the skin and coat finally results in alopecia and a thin and grayish-brown-pigmented skin. As well as proportional growth retardation, the animals have a pointed muzzle, resembling that of a fox (fig. 2.15). There is usually no remarkable delay in dentition. Unilateral or bilateral cryptorchidism is common in males and the females often have frequent anovulatory estrous cycles.

Initially the dwarfed dogs are lively and alert – they can be amusing and even quite appealing – but eventually they become lethargic, lose appetite, and turn into thin, dull, almost hairless animals with a sad appearance. This stage usually appears by the age of two to three years and is commonly associated with severe secondary hypothyroidism and impaired renal function. The latter may have both a renal and a prerenal component, i.e., maldevelopment of glomeruli due to lack of GH and low filtration pressure due to lack of thyroid hormone.

Routine biochemical variables are usually not abnormal, except that plasma creatinine is elevated in most of the pituitary dwarfs. As can be expected in secondary hypothyroid-



Figure 2.17:
A female German shepherd dog with pituitary dwarfism before (A) and after two years of treatment with medroxyprogesterone acetate and l-thyroxine (B).

ism (fig. 2.14), plasma concentrations of T_4 and TSH are low. Mean plasma IGF-I concentration (± SEM) is lower (62 ± 10 µg/l) in pituitary dwarfs than in immature healthy German shepherd dogs (345 ± 50 µg/l).[65] GH deficiency has rarely been mentioned in cats but there has been a report of an undersized kitten with bilateral corneal opacity in which GH deficiency was diagnosed on the basis of a low plasma IGF-I concentration.[74]

Differential diagnosis

Congenital hypothyroidism may be the most important differential diagnosis, although it results in a quite different appearance (chapter 3.2). The possibility should also be considered that the apparently dwarfed animal is the result of an unexpected and perhaps unrecognized mating with a small sire, or is simply a small individual within the normal biological variation. Hypochondroplastic dwarfism in Irish setters has been reported to occur as result of a single autosomal recessive inheritance.[75] Retardation of growth can also be the result of undernutrition or congenital abnormalities of vital organs such as the heart, liver, and kidneys. Corticosteroid administration at an early age also quite rapidly retards growth (chapter 4.3.6).

Diagnosis

Although the medical history and the physical changes are usually highly suggestive of GH deficiency, a definitive diagnosis requires measurement of GH in plasma, employing a homologous radioimmunoassay. Since basal GH values may also be low in healthy animals, a stimulation test should be performed. To test GH secretion alone, GHRH, ghrelin, or α-adrenergic drugs such as clonidine and xylazine can be used (chapter 12.1.2). When insight in the secretory capacity of other pituitary hormones is required, the combined AL-stimulation test (fig. 2.14 and chapter 12.1.3) is to be preferred over repeated single stimulation tests.

The amino acid sequence of IGF-I is less species specific than that of growth hormone and therefore it can be measured in a heterologous assay. As mentioned above, plasma IGF-I concentration is usually low in German shepherd dwarfs, even when age and size are taken into account. However, interpretation of results must also take account of the possibility of a low caloric intake and particularly a low protein intake, which may also lower plasma IGF-I concentration.[37,76,77]

Diagnostic imaging (CT or MRI) at a young age often reveals small pituitary cysts. They may become larger as the animal grows (fig. 2.16), eventually becoming so large as to cause neurological symptoms (see also chapter 2.2.6). However, healthy dogs, particularly those that are brachycephalic, may also harbor small pituitary cysts.

Treatment

The lack of homologous GH for therapeutic use in dogs led to initial attempts at therapy with bovine and human GH. This was not very successful, in part because it resulted in antibodies to the heterologous GH.[78] The ability of progestins to induce expression of the GH gene in canine mammary tissue and the release of the resulting GH into the systemic circulation (fig. 2.11) offered an alternative approach to circumvent this problem. Subcutaneous injections of medroxyprogesterone acetate are given in doses of 2.5 to 5.0 mg/kg body weight, initially at three-week intervals and subsequently at six-week intervals. If the growth plates have not yet closed some increase in body size can be expected. The muzzle becomes less pointed and a complete adult hair coat with primary hair appears (fig. 2.17). In parallel with this physical improvements, plasma IGF-I concentration rises and plasma GH concentration usually increases without exceeding the reference range.[79] Proligestone has been reported to be similarly effective in a dose of 10 mg/kg every three weeks.[80]



Figure 2.18:
An eight-year-old male Pomeranian in which progressively increasing alopecia for 1 year was the only problem. This type of alopecia has been presumed to be due to GH deficiency, but is now known to be the result of mild hypercortisolism (chapter 4.3.1).

There are, however, some adverse effects, including recurrent periods of pruritic pyoderma and, infrequently, development of mammary tumors. Occasionally recovery is very rapid and continuing treatment becomes overtreatment, leading to acromegalic changes (chapter 2.2.4). This may be prevented by discontinuing treatment for a few month, certainly when the IGF-I concentration approaches 200 µg/l. In females, continuing administration of a progestin will lead with certainty to cystic endometrial hyperplasia (chapter 7.10), which can be avoided by ovariohysterectomy before the start of the treatment.

In recent years porcine GH, which is identical to canine GH (chapter 2.2.1), has become available for therapeutic use. It is administered in thrice weekly subcutaneous doses of 0.1–0.3 IU per kg body weight. Because this treatment can result in GH excess leading to diabetes mellitus, monitoring of plasma IGF-I and glucose, at least once every six weeks, is of the utmost importance. The lanugo hairs regrow but the growth of the guard hairs is variable.

Treatment with either progestins or pGH should be accompanied by thyroid hormone replacement, according to the principles in chapter 3.3.1.

Prognosis

Without treatment the prognosis for German shepherd dwarfs is usually poor. By the age of three to five years the animal has usually become bald, thin, and dull, in part due to impaired renal function and secondary hypothyroidism. Substitution therapy for the secondary hypothyroidism can partly correct this, but continuing expansion of the pituitary cyst may impair the function of adjacent brain tissues, thereby contributing to the animal's misery. At this stage owners usually request that the animal be euthanized, if they have not done so long before this.

Treatment with thyroxine and either progestins or growth hormone usually leads to a relatively healthy life for many years, provided that complications such as pyoderma can be managed and acromegaly due to overtreatment is avoided.

2.2.3 Acquired growth hormone deficiency

Hypophysectomy, as in the treatment of pituitary-dependent hypercortisolism (chapter 4.3.1), leads to a very low plasma GH level that does not respond to stimulation.[81] As was initially the case in humans, this intervention in dogs and cats is usually followed by long-term substitution with orally administered cortisol and thyroxine. Adult-onset growth hormone deficiency in humans produces a wide array of manifestations, from metabolic and cardiovascular complications to a reduced quality of life as a result of diminished physical and mental energy.[82] Some dogs do not regain their liveliness or their muscle mass and hair coat following hypophysectomy, even though the hypophysectomy has brought an end to the hypercortisolism and they are receiving appropriate supplementation with cortisol and thyroxine. When recurrence of hypercortisolism has been excluded and hypopituitarism with severe growth hormone deficiency has been demonstrated by the absence of GH response to stimulation, treatment with either pGH or progestins (chapter 2.2.2) can be expected to bring improvement.

Apart from growth hormone deficiency due to damage to the pituitary gland, for almost three decades there have been reports in the veterinary literature on the spontaneous occurrence of isolated growth hormone deficiency in mature dogs. It has been proposed that such a deficiency of GH may explain some forms of alopecia occurring in breeds such as the Pomeranian (fig. 2.18), miniature poodle, chow chow, and keeshond. The alopecia has been described in both sexes, at any age but usually beginning at one to three years of age, and mainly involving the trunk, the caudal surfaces of the thighs, the perineum, and the neck. The alopecia does not appear to be attributable to any of the endocrine diseases known to result in skin atrophy and hair loss (hypothyroidism, hypercortisolism, and hyperestrogenism due to testis tumor). Although treatment with heterologous GH has had poor to moderate results, the condition has been given names such as »adult-onset growth hormone deficiency« and »growth-hormone responsive dermatosis«. Uncertainty about the role of GH is illustrated by alternative names such as »castration-responsive alopecia«, »biopsy-responsive alopecia«, »congenital adrenal hyperplasia-like syndrome«, and »alopecia X«.[83]

The entity does not seem to be well defined, for in about one-third of the cases the GH response to stimulation has



Figure 2.19:
A male Dalmatian dog at five years of age (A) and at ten years of age after developing acromegaly (B). Note the overall increase in body size, the thick folds of skin on the head and neck, and the enlarged tongue.

been normal. Yet in some in which there was a normal response to stimulation, treatment with GH was reported to be effective. In others, seemingly unrelated measures such as castration or administration of testosterone were followed by the appearance of a new hair coat.[84] Furthermore, in Pomeranians both with and without alopecia, the mean circulating GH concentration did not increase significantly after stimulation in either group.[85] Thus the proposed relation between some forms of this adult-onset alopecia and decreased GH secretion is not on very solid ground. It is even unlikely that there is a true growth hormone deficiency, for when plasma IGF-I has been measured, it has invariably been within the reference range.[84]

The fact remains that in some mature dogs with alopecia there is no response or only a weak response of plasma GH to stimulation with either GHRH or α-adrenergic agonists such as clonidine or its structural analog xylazine (chapter 12.1.2). This lack of response is most likely a functional disturbance. A preliminary study in miniature poodles with alopecia has led to the proposal that mild cortisol excess may be responsible for the altered GH responses.[86] Glucocorticoids are well known to suppress the GH response to various stimuli in humans and dogs.[87–90] In dogs with pituitary-dependent hypercortisolism, GH release in pulses is impaired, probably as a result of alterations in pituitary somatotroph function and changes in suprapituitary regulation.[91]

The hypothesis that both the alopecia and the lack of growth hormone response to stimulation might be the result of mild hypercortisolism has recently been tested in alopecic Pomeranians and miniature poodles. Serial measurements of urinary corticoids with low-dose dexamethasone suppression tests satisfied two criteria of hypercortisolism in both groups, namely, increased cortisol production and decreased sensitivity to glucocorticoid feedback.[92,93] This form of hypercortisolism is discussed in more detail in chapter 4.3.1. Whether this type of alopecia is the result of hypercortisolism in other breeds as well remains to be determined.

2.2.4 Growth hormone excess

Hypersecretion of growth hormone in the adult results in a syndrome characterized by overgrowth of connective tissue, bone, and viscera. The pituitary origin of the disease in humans was recognized in 1886 by Pierre Marie, who derived its name from the Greek words *akron* (extremity) and *megas* (large) for the characteristic enlargement of the hands and feet. In dogs and cats, as in humans, the GH excess can be caused by a somatotroph adenoma of the pituitary gland. In addition, dogs can develop the syndrome from progestin-induced hypersecretion of GH in the mammary gland (chapter 2.2.1.2). Finally, some of the physical and biochemical changes in dogs with primary hypothyroidism may be caused by GH excess resulting from the adenohypophyseal changes brought about by deficiency of thyroid hormone. The latter form of GH excess is discussed in chapter 3.3.1.

2.2.4.1 Excessive pituitary growth hormone

Pituitary tumors that might have secreted excessive amounts of GH have been reported in dogs[94–96], but only recently has GH hypersecretion been confirmed in a dog with acromegaly and a somatotroph adenoma.[97] In cats the disease is less rare and probably underdiagnosed.[98,99]

Clinical manifestations

The recently described dog with acromegaly of pituitary origin had very pronounced characteristics of longstanding GH excess (fig. 2.19), and is used here as the prototype for

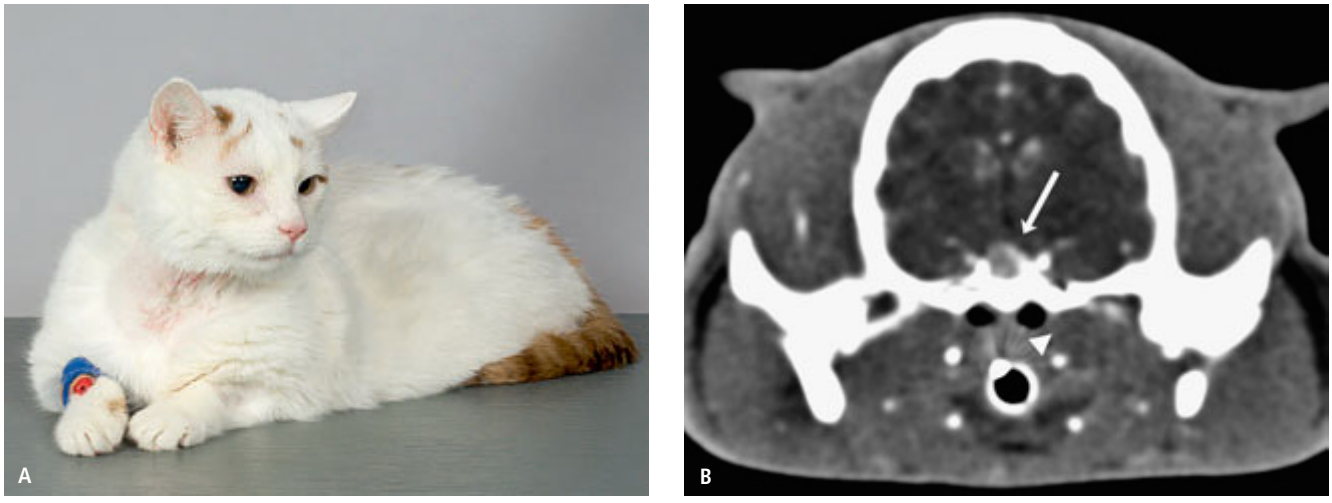


Figure 2.20:
(A) An eleven-year-old castrated male cat with acromegaly and diabetes mellitus requiring 25 IU of insulin four times daily. Basal plasma GH was 51 µg/l and IGF-I was 3871 µg/l. The cat has a sturdy physique and somewhat coarse facial features. The owner had noticed that it was becoming larger, with a heavy head.
(B) Contrast-enhanced CT image through the pituitary fossa revealed an enlarged pituitary gland, 4.5 mm in height and 4.2 mm in width (arrow). Also visible are thick mucosal folds of the palatum molle which almost completely obliterated the nasopharynx (arrowhead). Three weeks after transsphenoidal hypophysectomy, the cat no longer required insulin.

description of the condition.[97] The soft tissue overgrowth included thickening of the skin, particularly of the head and neck, and enlargement of the tongue with respiratory stridor. The osseous changes caused widening of the interdental spaces, increasing stiffness, difficulty in standing up, and neck rigidity – due to articular cartilage proliferation, periarticular periosteal reaction, and severe spondylosis deformans. Metabolic changes were manifested in polyphagia, weight gain, excessive panting, and polyuria and polydipsia. Laboratory examinations revealed normoglycemia with impaired glucose tolerance. The only other remarkable finding in routine blood examinations was mild anemia. Normochromic normocytic anemia has been found in dogs treated with pharmacological doses of porcine GH, and is associated with depletion of the erythroid cell series as well as cellular atrophy in the bone marrow. It is considered to be a species-specific effect.[100]

Now that more than 100 cases of acromegaly have been described in cats, it is a well-recognized syndrome. It is primarily a disease of castrated males, six to 15 years of age. In principle the physical changes are the same as in the dog but usually less pronounced (fig. 2.20). Almost all of the affected cats are presented because of poorly controllable diabetes mellitus, due to GH-induced insulin resistance. Initially, and probably prior to the development of the diabetes mellitus, the owner may have noticed polyphagia, weight gain, and polyuria and polydipsia. In the stage of insulin-resistant diabetes mellitus, some owners have noted lameness, increasing size of the paws, and broader facial features. In about half of the cats, physical examination reveals a heavy head, prognathia inferior, increased distance between upper and lower canine teeth, and stiffness and lameness. In some there is a systolic cardiac murmur and late in the course of the disease congestive heart failure may develop. Chronic GH excess leads to hypertrophy of the myocardium, with increased collagen content.[101] If the pituitary tumor is very large, it may cause impaired vision, mydriasis, and circling movements (chapter 2.2.6). Laboratory findings usually include hyperglycemia and glucosuria without ketonuria, and there may be elevated levels of hepatic enzymes secondary to the hepatic lipidosis, as well as mild hyperproteinemia and hyperphosphatemia.

Differential diagnosis

In cats the main differential diagnosis is hypercortisolism, which can also give rise to insulin resistance (chapter 4.3). Although GH excess and hypercortisolism lead to different physical changes, the difference is not always obvious and thus cats with insulin resistance are usually tested for both disorders. The simultaneous occurrence of both a somatotroph adenoma and a corticotroph adenoma (double adenoma) should also be considered.[102,103]

Diagnosis

In cats requiring lente insulin in doses $\geq$ 1.5 IU/kg body weight per injection and/or having physical signs of acromegaly, the finding of plasma GH $>$ 7.2 µg/l and IGF-I $>$ 590 µg/l is usually diagnostic.[103] Feline GH can be measured in heterologous radioimmunoassays, namely, the

species-specific assays for dogs and sheep.[104,105] Since a high value can be the fortuitous result of a secretory pulse in a non-acromegalic subject, it is advisable to collect three to five samples for GH assay at 10-min-intervals.

IGF-I is bound to a transport protein and is much less subject to fluctuation than is GH. Its amino acid sequence is less species specific than that of GH. Feline IGF-I can be measured in an assay for human IGF-I and because this is more readily available than suitable assays for GH, it is commonly used to diagnose acromegaly in diabetic cats.[99,106] However, the recommended cut-off value is high (1000 µg/l)[99], which may lead to underdiagnosis. Nonelevated IGF-I concentrations have been reported in cats with elevated plasma GH concentrations.[106,107] Particularly in acromegalic cats with untreated diabetes mellitus, false-negative IGF-I results can be expected.[108] On the other hand, increased IGF-I levels have been observed in nonacromegalic insulin-resistant diabetic cats, constituting false-positive results.[109,110] Some of these inconsistencies may be related to nutritional status, for studies in rats and humans have shown that when nutritional condition is poor, plasma IGF-I concentration may be lowered and plasma GH concentration increased.[111]

When the diagnosis of acromegaly has been confirmed, the pituitary gland should be visualized by computed tomography or magnetic resonance imaging (fig. 2.20).

Treatment

Although acromegaly is being recognized in cats with increasing frequency, there have been few reports of experience with treatment. In humans transsphenoidal adenomectomy is the treatment of choice. Transsphenoidal hypophysectomy in one cat led to reversal of insulin resistance and complete cessation of diabetes mellitus[102] and cryohypophysectomy in two others resulted in diminished insulin resistance and lowering of plasma IGF-I concentrations.[112,113]

The most frequently reported treatment for feline acromegaly has been radiation therapy. In five cases cobalt 60 (gamma) radiation lowered the insulin requirement transiently and reduced the size of the pituitary tumor.[114,115] In one cat in which linear accelerator (high-energy x-ray) radiation was used, insulin resistance was reduced but plasma IGF-I concentration remained elevated and the acromegaly continued as an active disease process.[116] Beta radiation reduced the insulin requirement only slightly in one cat but linear accelerator radiation reduced the insulin dose in another cat by half.[117] Possible adverse effects of radiation therapy are discussed in chapter 2.2.6.2.

Depending on the receptor profile of the tumor, somatostatin analogues are effective in a high percentage of humans with acromegaly, reducing both GH and IGF-I levels and the size of the tumor.[118] In one cat treated with the somatostatin analogue octreotide, plasma GH concentration was normalized[119] but in four others octreotide had little or no effect on serum GH levels.[114] A pre-entry test with a single intravenous injection of octreotide was introduced recently to evaluate the potential effectiveness of octreotide treatment in acromegalic cats. Those responding favorably might be candidates for long-acting release (LAR) octreotide treatment.[103]

The recently introduced GH-receptor antagonist pegvisomant has been reported to be effective, safe, and well-tolerated in humans with acromegaly.[120] As long as there are no species-specific antagonists, this approach is not an option for dogs and cats.

Prognosis

In acromegalic cats the short-term prognosis may be relatively good, as long as the insulin-resistant diabetes mellitus can be managed satisfactorily, although this usually requires large daily doses of insulin at considerable expense.[107] Complications such as congestive heart failure or an expanding pituitary tumor usually result in death or euthanasia within one to two years. The response to treatment of the somatotroph adenoma by surgery, radiation, and/or a somatostatin analogue can be monitored by measurements of plasma IGF-I. In humans IGF-I is considered to be the best biochemical marker for this purpose, although inconsistent results have been reported, i.e., elevated GH with normal IGF-I in 11 % of noncured patients, and elevated IGF-I with normal GH in 24 %.[121]

2.2.4.2 Excessive mammary growth hormone

As mentioned in chapter 2.2.1, the release of GH from mammary tissue is a normal physiological process in dogs during the luteal phase of the estrous cycle. In some middle-aged and older bitches sufficient amounts of GH may be released to result in acromegaly (and diabetes mellitus). Because progesterone levels in bitches during nonpregnant metestrus and pregnancy are similar (chapter 7.2.1), acromegaly can also be expected to occur during pregnancy, and recently the occurrence of this in two bitches was reported.[122] Administration of progestins may also give rise to GH excess and signs and symptoms of acromegaly.[123,124]

Progestins also induce GH expression in mammary tissue in cats[125], but the GH does not reach the systemic circulation[126] and consequently does not lead to acromegaly. It does, however, have a local proliferative effect that also involves IGF-I[127] and may result in marked enlargement of one, several, or all of the mammary glands. This fibroepithelial hyperplasia can occur in young queens at the time of their first estrus. It can also be caused by the administration of synthetic progestins such as megestrol acetate and medroxyprogesterone acetate.[128] It is discussed in more detail under the heading of estrus prevention (chapter 7.10).

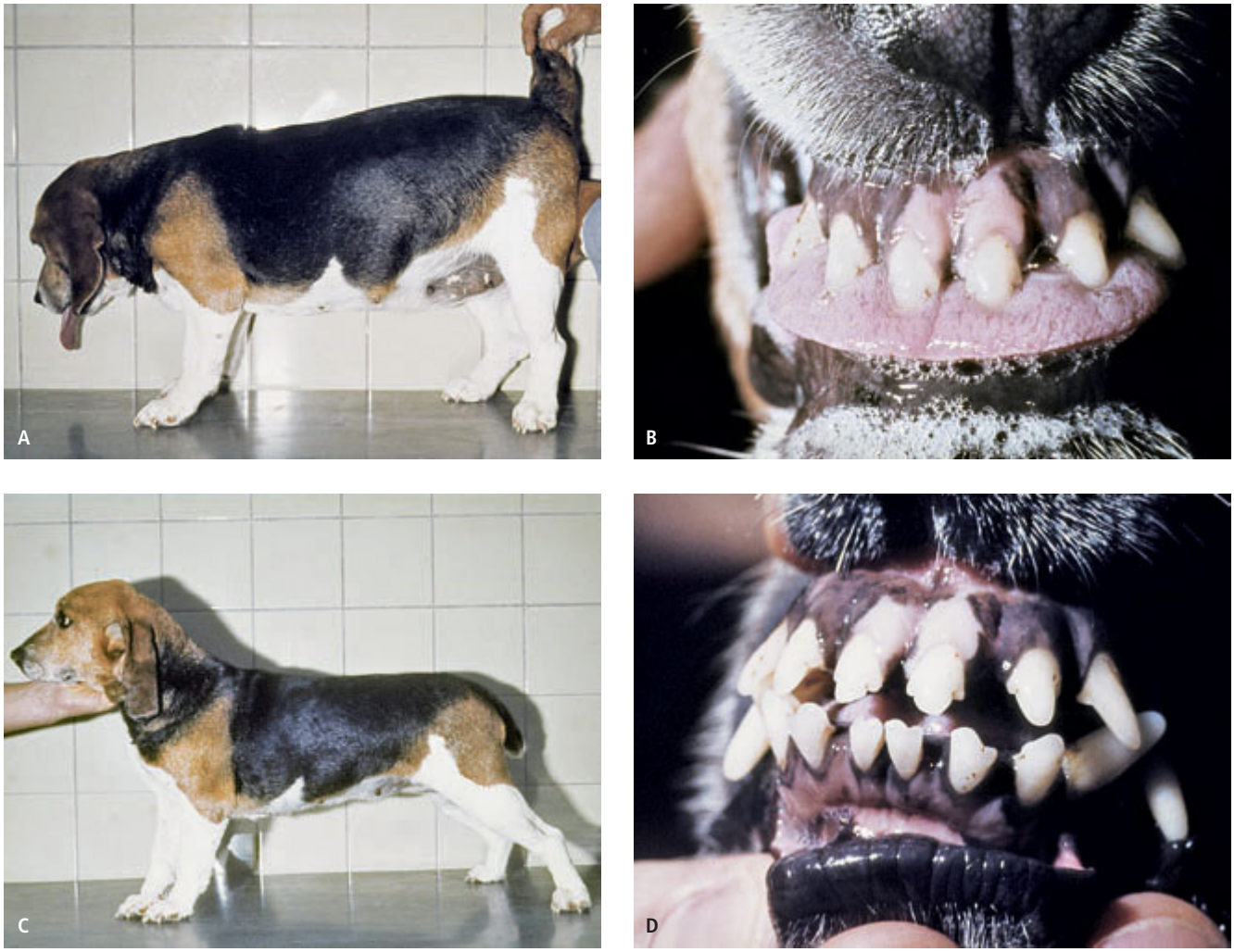


Figure 2.21:
(A, B) An eight-year-old female beagle with severe acromegaly and diabetes mellitus that developed during the current metestrus. Note the heavy body and the large tongue. During the two previous metestrus periods the owner had noticed polyuria, polyphagia, and excessive panting and snoring.
(C, D) The same dog, three months after ovariohysterectomy. The soft tissue overgrowth has regressed but the bony changes causing prognathism and widened interdental spaces remain. [129]

Clinical manifestations

Canine acromegaly due to mammary GH typically begins three to five weeks after estrus and produces the same signs and symptoms characteristic of excess pituitary GH described in chapter 2.2.4.1: thick folds of skin on the head and neck, fatigue, respiratory stridor, prognathism with widening of the interdental spaces, and abdominal enlargement due to visceromegaly (fig. 2.21). Initially, most of these changes regress following metestrus but with successive estrous cycles they become progressively more severe, until the full clinical picture develops. Early mild forms are usually primarily characterized by polyuria, polydipsia, sometimes polyphagia, and fatigue and snoring. The polyuria is without glucosuria unless diabetes mellitus also develops from the repeated exposure to GH excess.[129,130]

Progestins used for estrus prevention can produce similar changes, especially when given frequently and in relatively high doses (fig. 2.22). A comparative study of the effects of two progestins revealed that they resulted in similar plasma concentrations of GH and IGF-I, and similar degrees of insulin resistance.[131]

Laboratory studies often reveal hyperglycemia and increased plasma alkaline phosphatase. The latter may be due in part to the glucocorticoid activity which is intrinsic to progestins.[132,133]



Figure 2.22:
(A) A female mongrel Belgian shepherd dog at the age of three years.
(B) Two years later the dog was presented because of decreased endurance, intolerance to warmth (frequent panting, preference for cool places), exaggerated growth of the coat, increase in abdominal size, and inspiratory stridor. It had high plasma levels of GH (≥ 45 µg/l), induced by thrice yearly injections of medroxyprogesterone acetate for prevention of estrus.
(C) After the coat was clipped the physical changes were more prominent: heavy head, trunk and limbs, and thick folds of skin on the neck.
(D) Physical examination revealed prognathism, wide spacing of the teeth, and a relatively large tongue.[134]

Differential diagnosis

In pronounced cases the clinical features, including the specific medical history, are not easily confused with those of other diseases. However, in some dogs the metabolic changes lead to polyuria, polyphagia, and hyperglycemia which, together with the increase in abdominal size, may mimic the signs of hypercortisolism. Redundant folds of skin on the head and neck may also occur in primary hypothyroidism leading to GH excess (chapter 3.3.1).

Diagnosis

As in pituitary GH excess, measurement of plasma GH (at 10-min intervals) and of IGF-I will confirm the diagnosis. It is usually advisable not to delay treatment pending the laboratory results, for the sooner treatment is started, the greater the chance of preventing permanent diabetes mellitus (see below).

Treatment

Progestin-induced acromegaly can be treated effectively by withdrawal of exogenous progestins and/or by ovari(ohyster)-ectomy. The animal may then change dramatically (fig. 2.21), due to the reversal of the soft tissue changes. The size of the abdomen decreases, as does the thickening of oropharyngeal soft tissues and thus the associated snoring. The bony changes appear to be irreversible but do not appear to cause problems to the animal. In cases in which the GH excess did not lead to complete exhaustion of the pancreatic β cells, the elimination

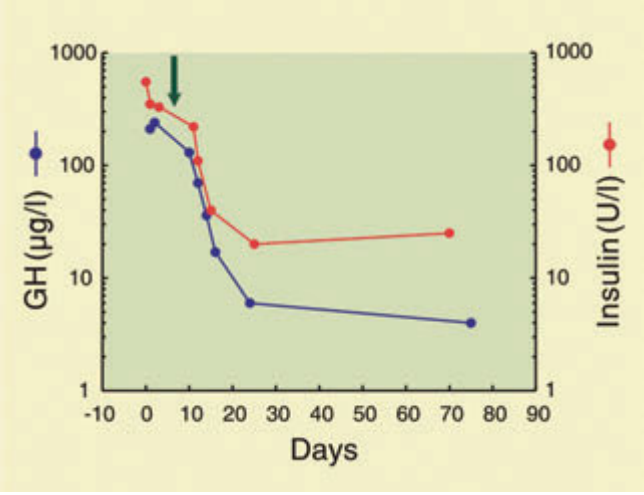


Figure 2.23:
Plasma GH and insulin concentrations (log scales!) in the dog shown in fig. 2.21, immediately before and after ovariohysterectomy (arrow). The dog was in the luteal phase of the estrous cycle and had developed persistent hyperglycemia. Following reversal of the insulin resistance caused by progesterone-induced GH excess, both the hyperinsulinemia and the hyperglycemia disappeared.

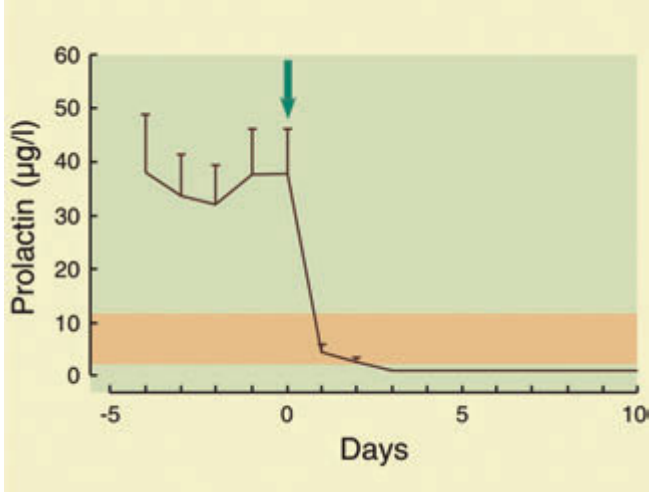


Figure 2.24:
Mean (± SEM) plasma concentration of PRL in six pseudopregnant Afghan Hounds before and during ten days of metergoline administration (2 mg twice daily). The arrow marks the start of treatment. The horizontal bar indicates reference ranges in anestrous bitches[137].

of the progesterone source by the ovari(ohyster)ectomy may prevent persistent diabetes mellitus (fig. 2.23).

Serious problems can arise in dogs in which the progestin causing the acromegaly has been administered only recently, for its action may persist for several months. Progesterone-receptor blockers may be helpful, as they are known to lower plasma GH and IGF-I concentrations in canine acromegaly[135], but there is as yet no long-term experience with their use.[136] Some caution seems warranted, for they also partially block glucocorticoid receptors.

Prognosis

Dogs with progestin-induced GH excess have a good prognosis following elimination of the progestin source. Diabetes mellitus resulting from the progesterone-induced GH excess is thereby also sometimes reversible.

2.2.5 Prolactin and pseudopregnancy in the dog

Pseudopregnancy is the syndrome that more or less accompanies the extended luteal phase of all nonpregnant ovarian cycles in the bitch. If its effects are mild it is generally referred to as a physiological or covert pseudopregnancy. In contrast, in overt or clinical pseudopregnancy, mammary development and/or behavioral changes are barely distinguishable from those of late pregnancy or lactation. Some breeds such as the Afghan hound and the basset hound appear to be especially predisposed to development of overt pseudopregnancy.[137]

Pathogenesis

In bitches (but not queens) the secretion of progesterone during the luteal phase is quite similar to that during pregnancy (chapter 7.2.1). It is therefore not surprising that the resulting effects can closely mimic pregnancy. Plasma PRL rises during the second half of pregnancy. In most nonpregnant bitches it rises only slightly during the luteal phase, from a mean around 2.5 µg/l to a mean around 5.0 µg/l[138], but in those with overt pseudopregnancy it rises to around 35 µg/l or higher.[137,139] This is primarily a consequence of a rapid decrease in progesterone secretion[48,56], but an abrupt decrease does not always lead to pseudopregnancy. Only in bitches predisposed to pseudopregnancy does it induce the substantial increase in PRL which in turn triggers the symptoms and signs of pseudopregnancy.[140]

Clinical manifestations

About four to eight weeks after estrus, bitches in pseudopregnancy may exhibit behavior which can be interpreted as nest building and caring for offspring. This can include reluctance to leave the home, aggression, digging, and the mothering of objects. Other signs are restlessness, loss of appetite, and frequent licking of the abdomen. The mammary glands can develop to such an extent that the body contour closely resembles that of late pregnancy or lactation. The mammary secretion varies from only a few drops of a clear or brownish fluid to considerable amounts of true milk.

Treatment and prognosis

In most dogs the symptoms of pseudopregnancy cease spontaneously after a couple of weeks, but sometimes the changes are so severe and long lasting that the owners cannot cope



Figure 2.25:
A nine-year-old male boxer dog with a large pituitary tumor and secondary hypothyroidism, only manifested by somnolence, slight alopecia in the groins and flanks, and a thin coat (A). There was marked atrophy of the testes (B). There were as yet no neurological symptoms.

with them and ask for treatment. For this purpose, prolactin can be suppressed and pseudopregnancy terminated by administration of:

1. Dopamine agonists such as bromocriptine (10 µg/kg twice daily for ten days) and cabergoline (5 µg/kg once daily for six days). Vomiting, which frequently occurs with bromocriptine, can be avoided by reducing the dose by half for the first four days and by administering the drug after meals. It has been reported that long-term administration (> 14 days) may lead to coat color changes.[141]
2. The serotonin antagonist metergoline (0.1 mg/kg twice daily for ten days). This drug lowers PRL release (fig. 2.24) without the risk of vomiting, but hyperexcitation, some increase in aggression, and frequent whimpering may occur.[137]

2.2.6 Pituitary tumors

Pituitary adenomas are considered to be benign, but in humans they can invade the adjacent dura mater, the cavernous sinus, and the sphenoid sinus. Microscopic examination has revealed dural invasion in as high as 45 % of cases.[142] Because of their extension and infiltration of regional structures these tumors have a high rate of recurrence after surgical resection. The diagnosis pituitary carcinoma is reserved for tumors with demonstrated metastatic dissemination, either systemic or within the central nervous system.[143,144] The diagnosis »invasive adenoma« is a contradiction in terms but for comparative purposes it can also be used in dogs and cats.[145,146]

Pituitary tumors have both endocrine and nonendocrine manifestations. Endocrine excess syndromes caused by corticotroph adenomas or somatotroph adenomas are discussed in chapter 4.3.1 and chapter 2.2.4.1, respectively. Prolactinomas occur in humans but have not been described in dogs and cats. Clinically nonfunctional adenomas (NFAs) constitute 50 % of all pituitary adenomas in humans and include gonadotroph (staining for FSH, LH, and α-subunit), thyrotroph (staining for TSH) and null cell (immunonegative) adenomas. The main consequences of NFAs are mass effects.[147] Their occurrence in dogs and cats has not been explicitly reported.

The nonendocrine manifestations of pituitary adenomas result from pressure by the tumor on adjacent brain structures. There may also be anterior pituitary failure, i.e., partial or complete anterior pituitary hormone deficiency. In principle, deficiency of all six major hormones (LH, FSH, GH, TSH, ACTH, and PRL) can occur. The interpretation of results of suprapituitary stimulation tests (chapter 12.1) may pose problems when there is also hormone excess that affects the secretion of other pituitary hormones.[148] The enlarging pituitary may also affect the function of the neurohypophysis (chapter 2.3.2).

2.2.6.1 Hormone deficiency

In adult animals, GH deficiency is not easily recognized as a distinct clinical syndrome, although longstanding GH deficiency leads to reduced physical activity, muscle atrophy, skin atrophy, and alopecia (chapter 2.2.3). Partial or total TSH deficiency is often a component of hypopituitarism and is discussed in chapter 3.3.2. Secondary adrenocortical failure as a result of ACTH deficiency may occur late in the development of large pituitary tumors. The resulting cortisol deficiency (chapter 4.2.2) contributes to gradual deterioration of the animal and a relatively trivial illness or anesthesia can precipitate vascular collapse. Gonadotropin deficiency in female dogs may remain unnoticed because of the naturally long interestrous interval. In male dogs continuing gonadotropin deficiency (chapter 8.2) results in testis atrophy (fig. 2.25). The



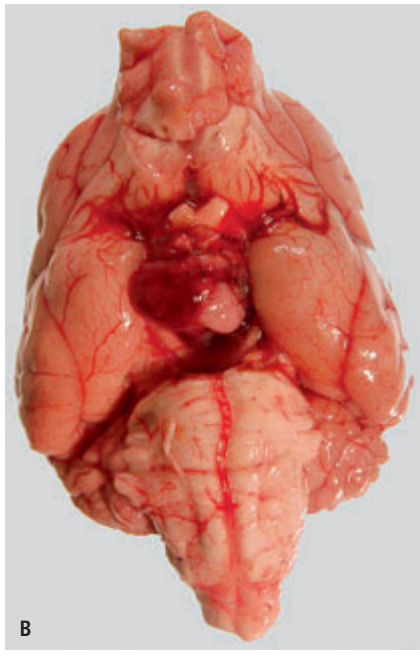


Figure 2.26:
(A) Gadolinium-enhanced axial MRI scan of an eleven-year-old female castrated Jack Russell terrier admitted as an emergency after the sudden onset of continuous panting, circling, and cycling movements in lateral recumbency, and a history of epileptiform seizures. The MRI scan revealed an irregular pituitary mass, 1.7 × 1.4 cm, compressing the surrounding brain. The mass contained cavities filled with fluid resembling blood.
(B) Necropsy revealed a necrotic and hemorrhagic pituitary corticotroph adenoma, originating from the pars intermedia.

testes become very small and soft and as a result the epididymis, which does not change, is more easily delineated. Neurohypophyseal dysfunction is unusual in anterior pituitary disease that remains restricted to the pituitary fossa, but more common when the suprasellar extension of large tumors compresses the hypothalamus (chapter 2.3.3).

2.2.6.2 Mass effects

Continued suprasellar expansion of the tumor exerts pressure on the diaphragma sellae, the hypothalamus and – if the expansion is sufficiently rostral – the optic chiasm. Lateral suprasellar extension of a pituitary tumor may impair oculomotor nerve function.[149] The expanding tumor can be expected to cause headache and visual field defects in the dog and cat, as in man, but because of the lack of an autoanamnesis, often the veterinarian must at first rely on rather vague and nonspecific symptoms. These include lethargy, a tendency to seek seclusion, and a decrease in appetite.[150,151] Suspicion of a mass effect from a pituitary tumor may be supported by the owner's description of the animal's tendency to lower its head to avoid being patted. Progressive enlargement of the mass may give rise to severe neurological abnormalities such as pacing, head pressing, circling, and continuous howling. Seizures usually do not occur. Very large pituitary tumors may cause pressure on the optic chiasm to such an extent that visual disturbances are noticed by the owner.[152]

Physical examination can reveal a variety of signs, including dullness, one or more of the above neurological signs, weight loss due to increasing anorexia, and occasionally mydriasis with or without anisocoria. Ophthalmoscopic examination rarely reveals edema of the papilla.

The mass effects may also appear rather suddenly. In humans this is known as pituitary apoplexy and it is characterized by peracute headache, vomiting, visual impairment, and loss of consciousness. It is caused by either hemorrhage or infarction within a pituitary tumor (fig. 2.26) or a nontumorous pituitary gland. This syndrome has now been described in five dogs. The three most severe cases were presented as emergencies with sudden collapse and severe depression; in two there was blindness and bilateral mydriasis. In four dogs there was a large corticotroph adenoma with hemorrhage. In the fifth dog the pituitary hemorrhage without tumor was probably part of the hemorrhagic diathesis due to idiopathic thrombocytopenia.[153,154]

Differential diagnosis

Due in part to the nonspecific character of the signs and symptoms, the differential diagnoses range from other neurologic diseases, such as parasellar lesions and increased intracranial pressure, to metabolic disorders such as hypothyroidism and hepatic encephalopathy.

Diagnosis

Laboratory findings indicating low basal function of peripheral endocrine glands, e.g., a low plasma thyroxine level and low urinary corticoid excretion, may raise suspicion of anterior pituitary failure, but the diagnosis of partial or total hypopituitarism should rest on direct evidence of deficiencies of the pituitary hormones themselves. This can be accomplished by stimulation tests with hypophysiotropic hormones such as GHRH, GnRH, CRH, and TRH. Measurements of the respective pituitary hormones – GH, LH, ACTH, and PRL – permit assessment of pituitary reserve capacity. The tests can in principle be performed in an outpatient setting,

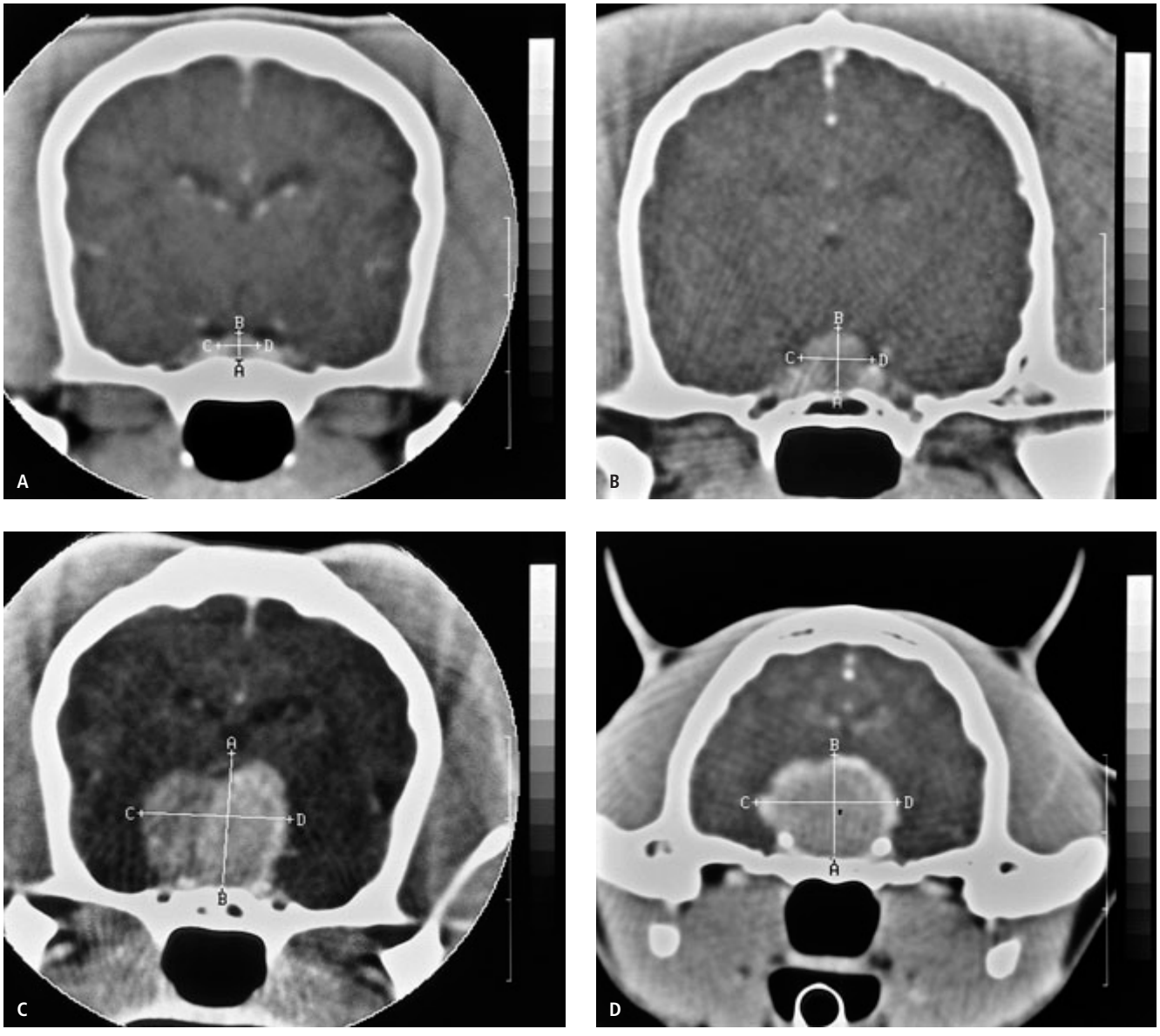


Figure 2.27:
Transverse CT images of skulls of three dogs and one cat.
(A) A healthy beagle. Contrast enhancement enables the visualization of a normal-sized pituitary, the margins of which are indicated by A–B (3.6 mm) and C–D (5.0 mm).
(B) A twelve-year-old female mongrel greyhound with pituitary-dependent hypercortisolism. Contrast enhancement reveals a definitely enlarged pituitary (A–B = 8.6 mm and C–D = 9.2 mm).
(C) A ten-year-old female Australian terrier with dexamethasone-resistant pituitary-dependent hypercortisolism without noticeable neurological symptoms. Contrast enhancement reveals a very large pituitary (A–B = 16.6 mm; C–D = 17.7 mm).
(D) A 14-year-old castrated male domestic shorthair cat presented with mild symptoms and signs of pituitary-dependent hypercortisolism and central blindness. A very large pituitary is revealed by contrast enhancement (A–B = 13.6 mm; C–D = 17.9 mm).

but it is cumbersome to perform them separately. Hence a combined anterior pituitary test has been developed, in which all four hypophysiotropic hormones are injected within 20 s and blood samples are collected for measurements of all four pituitary hormones in each sample (chapter 12.1.3). When pituitary apoplexy is suspected, blood should be collected immediately to determine whether interference with vasopressin release has led to hypernatremia.[153]

Contrast-enhanced helical CT (fig. 2.27) and MRI (fig. 2.28) provide imaging of the pituitary with high spatial and contrast resolution, revealing pituitary enlargement and its relation-

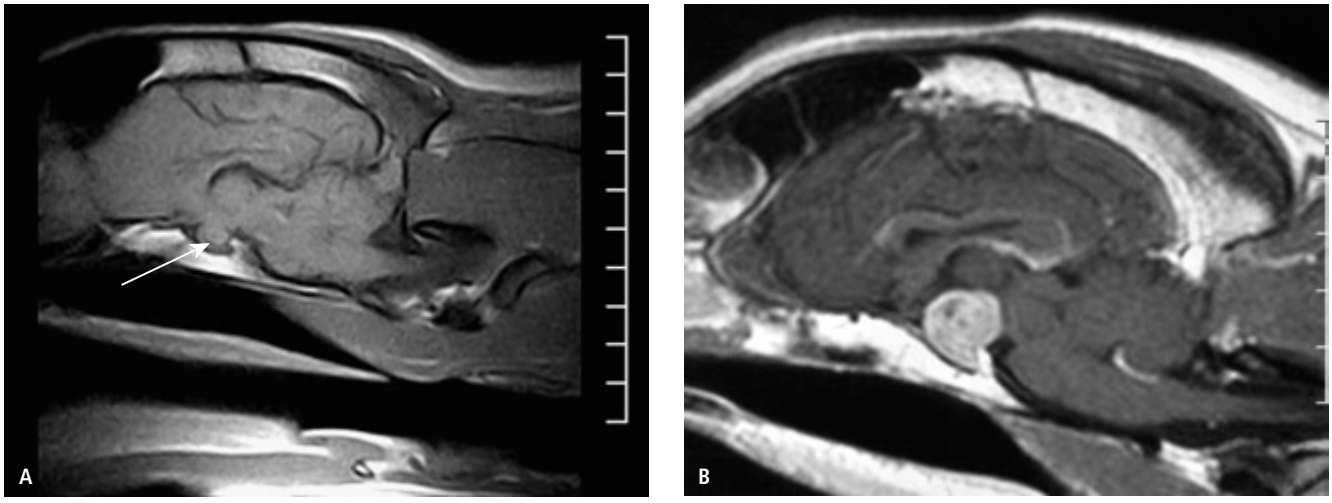


Figure 2.28:
Sagittal MR images of the skulls of a healthy dog (A) and a dog with pituitary-dependent hypercortisolism (B). In the healthy dog the hypophyseal cleft between the anterior lobe and the neurointermediate lobe can be distinguished (arrow). In the dog with pituitary-dependent hypercortisolism there is suprasellar extension of the pituitary mass.

ship to surrounding structures and bony anatomic landmarks for surgical intervention.[155] Dynamic helical CT and MRI also allow visualization of the posterior lobe. Its displacement may reveal the location of an adenoma or microadenoma in the anterior lobe, whereas the inability to visualize the posterior lobe in large pituitary tumors might be compatible with vasopressin insufficiency (chapter 2.3.3.1).[156]

Treatment

Anterior pituitary failure can be treated by substitution of the hormones inadequately produced by the target glands. Since gonadal hormones are not essential, this can be limited to oral administration of thyroxine (10–15 μg/kg twice daily) and cortisone (0.25–0.5 mg/kg twice daily). This results in some improvement in alertness and also in appetite if the animal had been anorectic. Especially when by virtue of its size the tumor has already had neurological effects, any improvement will be temporary. Immediate corticosteroid administration is indicated in cases suspected of pituitary apoplexy; in such a crisis the dose should be four to five times the long-term substitution dose (chapter 4.3.6).

In principle there are three options to reduce the size of the pituitary tumor: medical therapy, hypophysectomy, and radiation therapy (see also discussion on the treatment of pituitary GH excess in chapter 2.2.4.1). Most experience with medical treatment has been gained in dogs with pituitary-dependent hypercortisolism. Dopaminergic drugs such as bromocriptine do not effectively decrease cortisol production[157], but better results have been obtained with the dopamine D2 receptor agonist cabergoline. Cabergoline has a higher affinity for the D2 receptor and has a longer half-life than bromocriptine. Despite the fact that D2 receptors are only moderately expressed in tumorous and nontumorous dog pituitaries[20], in 17 out of 40 dogs with pituitary tumors, treatment with cabergoline decreased cortisol production and decreased tumor size. However, among the nonresponders were relatively many dogs with large pituitary tumors.[158]

Hypophysectomy is used successfully to treat pituitary-dependent hypercortisolism (chapter 4.3.1) and with increasing experience it has been used to remove pituitary tumors up to 2 cm in diameter. Total or subtotal removal of large pituitary tumors with mass effects gives immediate relief in the form of decreased neurological signs and return of appetite. The animal can resume a normal life for months to years after surgery. If the tumor recurs, transsphenoidal debulking can be considered.

Radiation therapy is indicated in both dogs and cats when a pituitary tumor is already causing neurological abnormalities.[159] It reduces the size of the tumor and thereby the neurological manifestations. The outcome is better in dogs with mild neurological signs than in those with severe signs or stupor.[160] Radiation therapy increases survival time over that in untreated dogs.[161] Median survival time has been reported to be 22.6 months in dogs[162] and 17.4 months in cats.[163] Radiation therapy does not cause a prompt change pituitary hypersecretion and thus dogs with pituitary-dependent hypercortisolism may require continued medical treatment. Cats with a macrotumor and diabetes mellitus may not require insulin treatment after the completion of a series of fractionated radiation therapy treatments (chapter 2.2.4.1).[164]

Acute side effects of radiation treatment include local skin changes (erythema, hair loss, leukotrichia), pharyngeal mucositis, and mild otitis externa. The risk of late side effects (hearing impairment, brain necrosis / fibrosis) depends on the volume of brain tissue treated and the daily and the total dose of radiation administered.[159]

2.3 Posterior lobe

As illustrated in fig. 2.2, the posterior lobe or neurohypophysis is an extension of the ventral hypothalamus. The two neurohypophyseal hormones are synthesized in the supraoptic and the paraventricular nuclei in the hypothalamus, from which axons extend through the pituitary stalk to the posterior pituitary. The hormones vasopressin and oxytocin are formed by separate neurons and migrate down the axons incorporated in precursor proteins. They are stored in secretory granules within the nerve terminals in the neurohypophysis and are released by exocytosis into the bloodstream in response to appropriate stimuli. The nonapeptides oxytocin and vasopressin contain internal disulfide bonds linking cystine residues at positions one and six. They are synthesized as part of a large precursor molecule composed of a signal peptide, the hormone, and a carrier protein termed neurophysin and (for vasopressin only) a glycopeptide. Oxytocin differs from vasopressin only at positions three and eight, i.e., it contains the amino acids isoleucine and leucine, respectively, instead of phenylalanine and arginine (fig. 1.3).

2.3.1 Oxytocin

Oxytocin stimulates milk ejection by contraction of the myoepithelial cells surrounding the alveoli and ducts in the mammary gland. The release of oxytocin is brought about through a neuroendocrine reflex. Suckling of the nipple sends neural impulses to the brain that reach the hypothalamus and direct the release from the neurohypophysis. It also stimulates rhythmic myometrial contractions in the uterus, aiding in expulsion of the fetus. In dogs plasma oxytocin concentration increases during late pregnancy and further increases during the expulsive stage of parturition.[165,166] Primary uterine inertia in bitches is associated with low plasma oxytocin concentrations.[167] Therapeutically, oxytocin is widely used to sustain uterine contractions. Oxytocin has an essential role in activating maternal behavior. Apart from its role in parental care, oxytocin also plays a role in social attachments and affiliations. Positive interactions between humans and dogs are associated with several neurohumoral changes in both species, including an increase in plasma oxytocin.[168] Results of recent behavioral studies indicate that oxytocin also increases trust among humans.[169]

2.3.2 Vasopressin

As in most mammals, in dogs and cats arginine vasopressin ((A)VP) or antidiuretic hormone (ADH) (in pigs: lysine vasopressin) plays a vital role in water conservation. VP like other pituitary hormones is secreted in a pulsatile fashion (fig. 2.28).[170] The major determinant of its release is plasma osmolality. Specialized neurons called osmoreceptors are concentrated in the anterior hypothalamus, which is near but separate from the supraoptic nuclei. This area is supplied with blood by small perforating branches of the anterior cerebral arteries. Osmotic stimulation increases both basal and pulsatile VP secretion (fig. 2.29).[170] Significant changes in circulating blood volume and blood pressure may also influence VP release and the setting of osmoregulation. Significantly elevated plasma VP concentrations have been found in dogs with dilated cardiomyopathy.[171] In addition, the stress of fear and the administration of preanesthetic or anesthetic agents may increase plasma VP concentration.[172,173] The opioid methadone in particular has a strong stimulatory effect on VP release, considered to be a direct effect.[174]

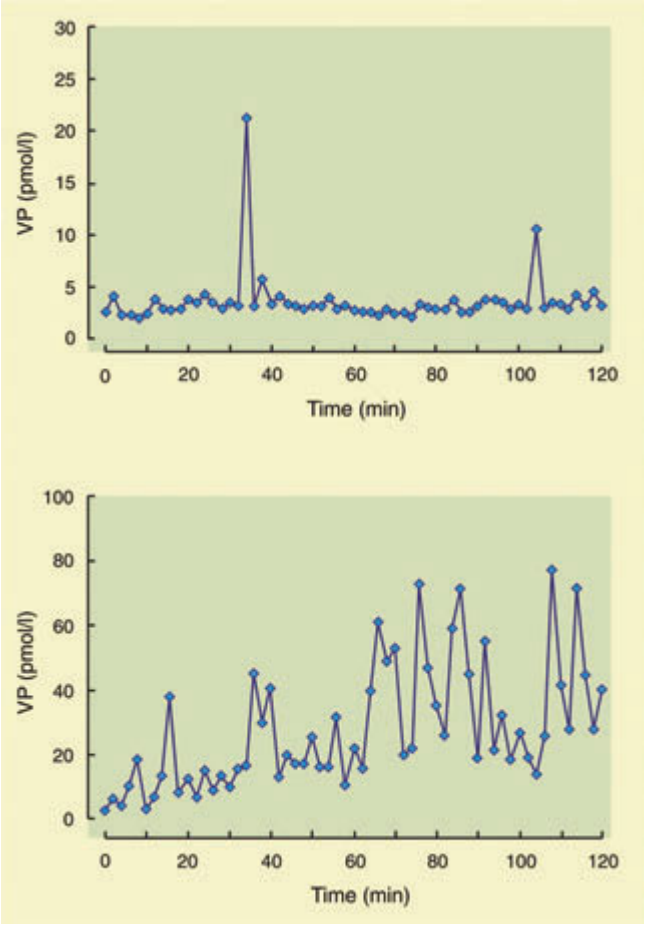


Figure 2.29:
Plasma vasopressin (VP) concentration in blood samples collected every 2 min for 2 h in a five-year-old beagle under basal conditions (upper panel), and during osmotic stimulation (lower panel) with hypertonic saline (infusion of 20 % NaCl at 0.03 ml/kg/min for 1 h). Note the differences in scale of the y-axis. Volume and electrolyte losses due to blood sampling were corrected by intravenous infusion of lactated Ringer's solution.

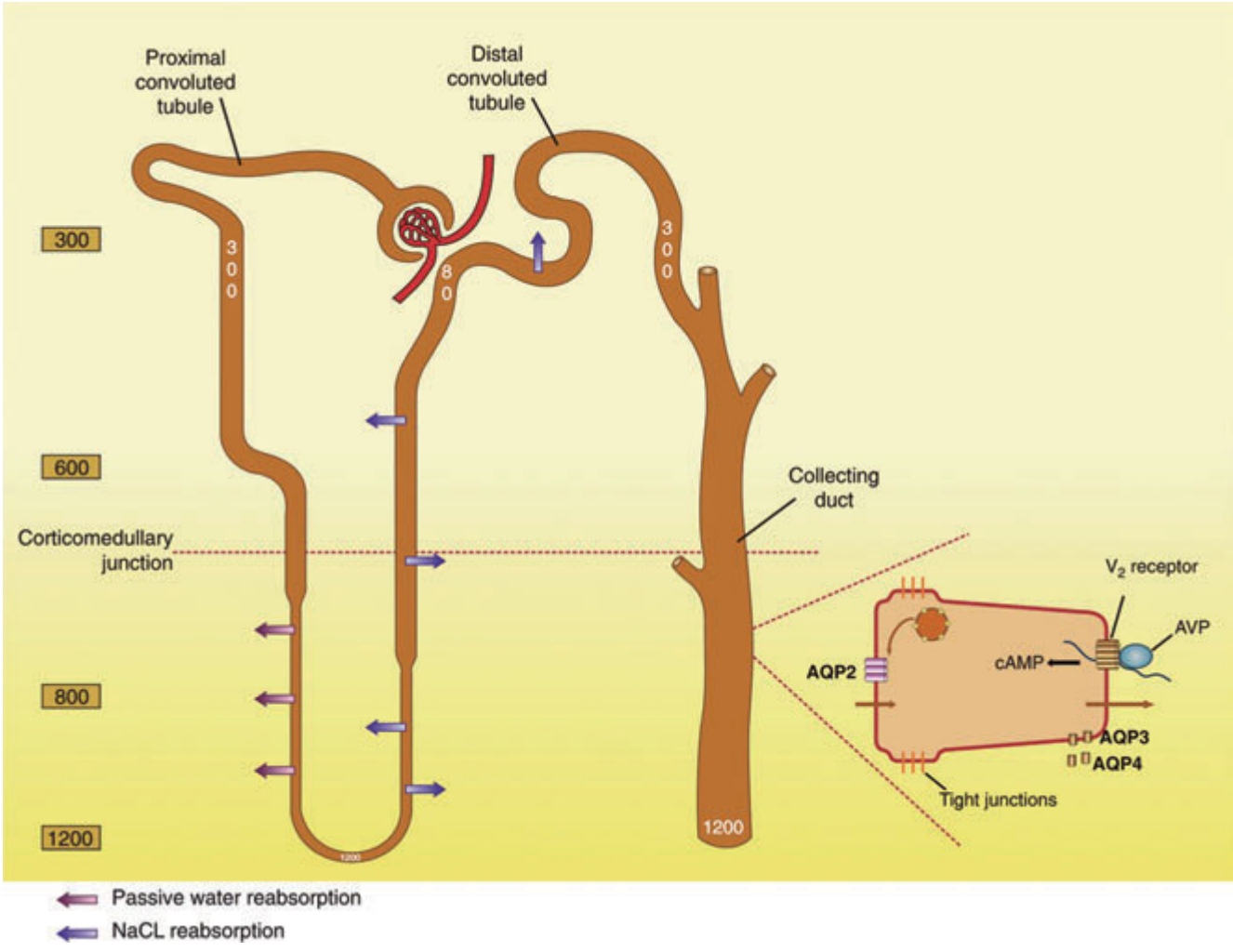


Figure 2.30:
In a medium- to large-size dog the kidneys filter about 90 liters of plasma daily. About 75 % of this is passively reabsorbed in the proximal convoluted tubule together with the active transport of solutes such as sodium, potassium, bicarbonate, amino acids, and glucose. Following this isotonic reabsorption, an additional 5 % of the water is withdrawn from the descending limb of Henle's loop (without solute) by the hypertonic interstitium. The remainder is diluted to an osmolality of about 80 mOsm/kg by selective reabsorption of sodium and chloride in the ascending limb of Henle's loop and the distal convoluted tubule. In the absence of VP urine passes largely unmodified through the distal tubules and collecting ducts, resulting in maximal water diuresis. In presence of VP, solute-free water is reabsorbed osmotically through the cells of the collecting ducts, resulting in the excretion of small volumes of concentrated urine. This antidiuretic effect is mediated via a G-protein-coupled V_2 receptor that induces (via cyclic AMP) translocation of aquaporin (AQP2) water channels into the apical membrane. Tight junctions on the lateral surface of the cells prevent unregulated water flow.

The anterior hypothalamus not only contains osmoreceptors regulating VP secretion but also thirst osmoreceptors. The control of vasopressin secretion and thirst sensation are partially interwoven, in that drinking not only leads to satiation of thirst but also to cessation of vasopressin secretion. The osmotic threshold for VP secretion is slightly lower than that for thirst perception. Under physiologic conditions water balance is accomplished more by free water excretion regulated by VP than by water intake regulated by thirst.[175]

In addition to systemic signals – primarily plasma osmolality (Posm) and blood volume – influencing VP secretion and water intake, fluid balance is regulated by presystemic signals. In dogs drinking induces volume-dependent oropharyngeal signals that inhibit thirst and vasopressin secretion, well before the ingested water has left the stomach. In dehydrated dogs drinking decreases vasopressin secretion within minutes, while Posm is still elevated.[176,177]

The effects of VP are mediated by three receptor subtypes: V_1 receptors on blood vessels, V_2 receptors on renal collecting duct epithelial cells, and V_3 receptors mediating ACTH secretion from the adenohypophysis (chapter 4.1). The adjustments of water reabsorption needed to maintain water and electrolyte balance occur in the distal convoluted tubules and in the collecting ducts (fig. 2.30), and depend on the hor-

mones aldosterone and VP. Aldosterone stimulates sodium and water reabsorption and potassium excretion (chapter 4.1). VP facilitates the diffusion of water from the collecting ducts into the hypertonic renal medulla. The cellular mechanism of VP activity in the renal tubule involves binding to specific contraluminal V_2-receptor sites on the serosal surface of the cell, an adenylate cyclase response, and phosphorylation of proteins that lead to transient insertion of water channels (aquaporins) in the luminal membrane of the cell.

Several different aquaporins (AQPs) in the kidney have been characterized, each correlating with well-defined segmental permeabilities in the nephron. AQP-3 and AQP-4 are localized in the basolateral membrane of the collecting duct and permit water to pass from the cell to the interstitium. AQP-2 is the major VP-regulated water channel.[178] Within a few minutes VP can increase water permeability of the cells of the collecting ducts by stimulating translocation of AQP-2 from an intracellular reservoir to the apical plasma membrane (fig. 2.30). After VP withdrawal, AQP-2 is redistributed into the cell by endocytosis, and water permeability decreases. During stimulation by VP a small percentage of AQP-2 is excreted into the urine. This urinary AQP-2 excretion closely reflects changes in VP exposure and has been proposed as a marker for collecting duct responsiveness in polyuric dogs.[179]

Cations, drugs, and hormones can influence the action of VP, thereby causing polyuria. In hypercalcemia AQP-2 downregulation and reduced apical plasma membrane delivery of AQP-2 play important roles in the development of polyuria.[180] Glucocorticoids also interfere with the action of VP, although in dogs loss of reactivity of the osmoreceptor system also seems to contribute to the corticosteroid-induced polyuria (fig. 2.31).[181] Even physiological increases in cortisol inhibit basal vasopressin release in dogs.[182]

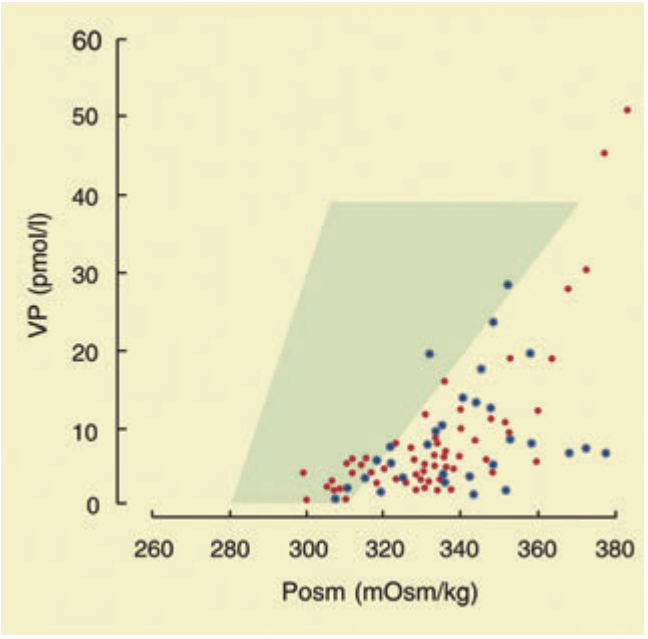


Figure 2.31:
Relation of plasma vasopressin (VP) concentration to plasma osmolality in nine dogs with pituitary-dependent hypercortisolism (red dots) and six dogs with hypercortisolism due to an adrenocortical tumor (blue asterisks) during hypertonic saline infusion. The green area represents the range in healthy dogs.

2.3.3 Diabetes insipidus

The term diabetes insipidus is derived from the Greek *diabainein* (passing through) and the Latin *insipidus* (without taste). It is characterized by large volumes of urine with an osmolality lower than that of blood plasma and so dilute that it is almost tasteless. In fact, the term diabetes insipidus (DI) only denotes polyuria. When diabetes mellitus has been excluded, DI and polyuria can be regarded as synonymous. From a pathophysiological point of view three fundamentally different pathogenetic categories can be distinguished:

- A disturbance of the hypothalamic-pituitary system causing insufficient VP release (central diabetes insipidus).
- A disease or functional change of the kidney, leading to insufficient response to VP (nephrogenic diabetes insipidus).
- Sustained and excessive drinking (primary polydipsia), resulting in a tendency to plasma hypotonicity and consequently little or no stimulation of VP release.

2.3.3.1 Central diabetes insipidus

The disease is characterized by three primary findings: (1) dilute urine in spite of strong osmotic stimuli for VP secretion, (2) absence of renal disease, (3) a rise in urine osmolality following the administration of vasopressin.

Pathogenesis

Both complete and partial central diabetes insipidus (CDI) have been recognized in dogs and cats. In complete CDI there is very little increase in urine osmolality with increasing plasma osmolality. The animal is essentially devoid of releasable VP (fig. 2.32). In partial CDI there is release of VP with increasing plasma osmolality but in subnormal amounts (fig. 2.33).

Among the possible causes of impaired VP release, an intracranial tumor is likely in middle-aged and elderly animals, most often a primary pituitary neoplasm.[183–185] There are two possible mechanisms for the impaired release. (1) The enlarging pituitary adenoma in the anterior lobe increasingly compresses the posterior lobe in the restricted space of the pituitary fossa, resulting in pressure atrophy of the posterior lobe and diminished VP release. During dynamic computed tomography the normally characteristic and distinct contrast enhancement of the neurohypophysis (neurohypophyseal flush) is less pronounced or absent.[186,187] (2) Large pituitary tumors with suprasellar extension can compress the hypothalamic nu-

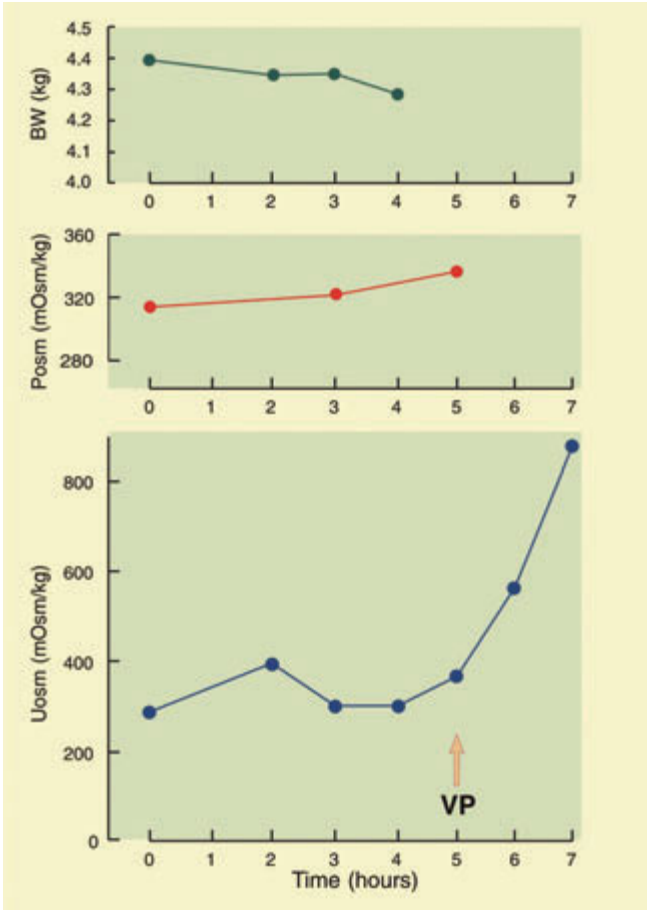


Figure 2.32:
The effect of water deprivation on body weight, plasma osmolality (Posm), and urine osmolality (Uosm) in a four-year-old castrated male cat with polyuria and polydipsia following head trauma. The arrow indicates the time of injection of vasopressin (VP). The dehydration-induced rise in Posm did not result in a sustained rise in Uosm. This, in combination with the sharp rise following vasopressin administration, provides the diagnosis of complete central diabetes inspidus.

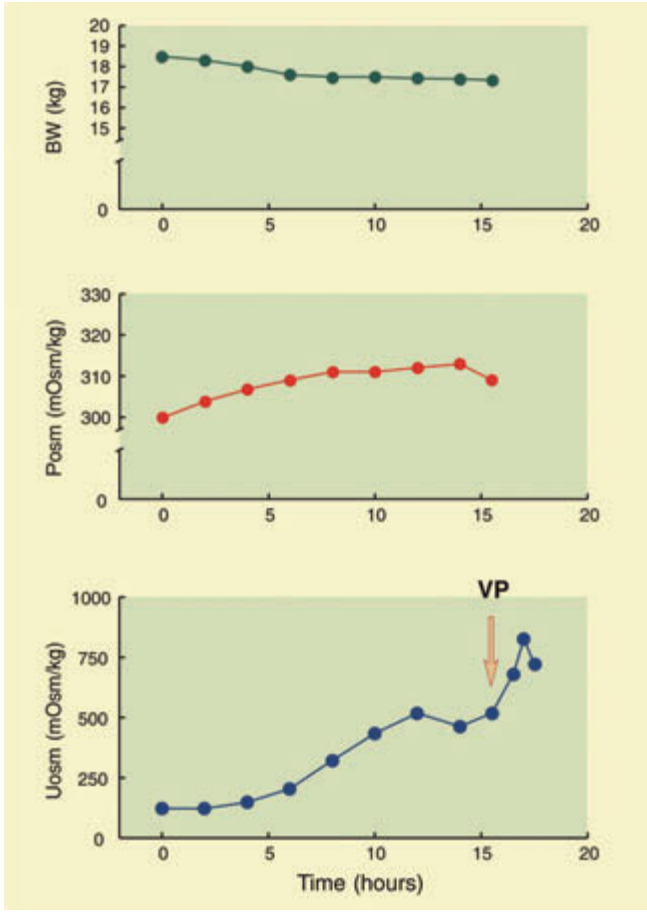


Figure 2.33:
In a five-month-old mongrel dog with polyuria, water deprivation led to a slow, subnormal rise in urine osmolality (Uosm). After this reached a definite plateau, the administration of vasopressin (VP) caused a further 60 % increase. These findings are compatible with partial central diabetes insipidus.

clei, impairing VP synthesis probably via degeneration of the hypothalamic neurons. Both mechanisms can contribute to VP deficiency and inability to react adequately to osmotic stimuli. It may be difficult to diagnose CDI in patients in which a pituitary corticotroph adenoma has caused hypercortisolism (chapter 4.3.1).[188] CDI may be overlooked because of the polyuria caused by the glucocorticoid excess (fig. 2.31). CDI may become apparent when the hypercortisolism has been eliminated by treatment.[183]

Neoplastic nonpituitary lesions reported to cause CDI include meningioma and malignant lymphoma.[185,189] A nonneoplastic cause of CDI is the trauma and subsequent inflammation of larva migrans.[190] CDI has also been described in association with congenital pituitary anomalies[191,192], although in one of these cases the pathogenetic role of an early head trauma remained unclear.[192] Severe head injury is known to be a cause of CDI, particularly in condition, and there are now several reports of this cats.[193–196] There may be spontaneous remission, probably by regeneration of disrupted axons in the pituitary stalk.

CDI can also occur as a complication of pituitary surgery, most often performed to treat pituitary-dependent hypercortisolism (chapter 4.3.1). Diabetes insipidus appears immediately following surgery[197] and often disappears spontaneously after days to months. If the pituitary stalk is sectioned so high as to induce retrograde degeneration of the hypothalamic neurons, the CDI can be permanent. An immunohistochemical study of the hypothalamic paraventricular and supraoptic nuclei in healthy dogs revealed that VP-positive cells tend to decrease after hypophysectomy.[198] The incidence of

prolonged and permanent CDI after hypophysectomy in dogs with pituitary-dependent hypercortisolism is correlated with pituitary tumor size, i.e., the risk is higher in dogs with large pituitary tumors.[199–201] Apparently the magnocellular neurons do not resume function after being compressed for a long time.

There remains the possibility of the so-called idiopathic form of CDI. This term is used in cases in which there is no demonstrable lesion in the hypothalamus or pituitary. This may be the case especially in young animals, although the subsequent course of the disease, diagnostic imaging, or autopsy may eventually reveal a lesion that could not be identified initially.[202]

Clinical manifestations

The major manifestations are polyuria, polydipsia, and a near-continuous demand for water. In severe cases water intake and urine volume may be immense, requiring micturition almost every hour throughout day and night. Although in partial CDI water intake and urine volume may be only moderately increased, in severe cases of complete CDI water intake may be so enormous as to interfere with food intake and thus result in weight loss. In animals in which a large neoplasm is the underlying cause, there may be additional neurological symptoms and endocrine deficiencies (chapter 2.2.6). CDI caused by head trauma may not only be associated with soft tissue and skeletal lesions, but damage to the hypothalamus-pituitary region may cause additional hormone deficiencies, such as secondary hypothyroidism.[193,194]

Both urine specific gravity (Usg) and urine osmolality (Uosm) will be below that of plasma: Usg < 1.010 and Uosm < 290 mOsm/kg, although in mild cases Uosm may be up to 600 mOsm/kg. Blood examination usually reveals no abnormalities except for slight hypernatremia due to inadequate replenishment of the excreted water. If water is withheld from an animal with complete CDI, life-threatening hypertonic encephalopathy occurs within a few hours (PNa^+ > 170 mmol/l; Posm > 375 mOsm/kg), initially manifested by ataxia and sopor. This can also occur when the causative lesion extends to the thirst center and adipsia develops.[203]

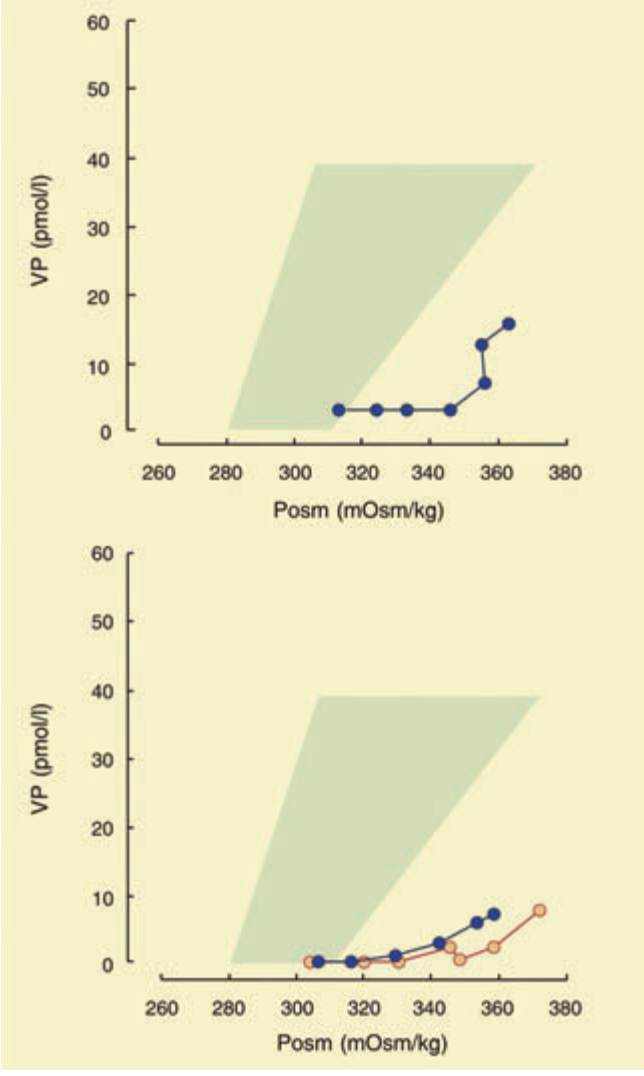


Figure 2.34:
Relation of plasma vasopressin (VP) concentration to plasma osmolality (Posm) during hypertonic saline infusion in three dogs with polyuria. Upper panel: ten-year-old castrated male German pointer with primary hyperaldosteronism. Lower panel: nine-year-old castrated male Labrador retriever (-●-), and 9.5-year-old castrated male Old English sheepdog (-○-) with polycythemia due to renal neoplasia. The green areas represent the range in healthy dogs.

Differential diagnosis

Apart from central diabetes insipidus there are in principal only two basic disorders which can account for the polyuria: nephrogenic diabetes insipidus and primary polydipsia (chapter 2.3.3.2 and chapter 2.3.3.3), both of which are infrequent, but a wide variety of conditions cause polyuria. Young animals may have congenital kidney disease, and at all ages acquired kidney disease may cause polyuria. Especially in middle-aged and elderly animals, endocrine conditions such as diabetes mellitus, hypercortisolism, hyperaldosteronism, hyperthyroidism, pyometra, progestin-induced (luteal phase) GH excess, hyperparathyroidism, and hypercalcemia of malignancy must be considered. Other conditions such as hepatoencephalopathy and polycythemia may also be associated with polyuria. In several of these conditions impaired VP release and/or interference with its action has been documented. These include hypercortisolism (fig. 2.31), hyperaldosteronism[204], GH excess[205], pyometra[206], hepatoencephalopathy[207], and polycythemia (fig. 2.34).[208]

It can be assumed that in some of these conditions the hormonally induced changes (corticosteroid-induced sodium retention) may cause hypervolemia and thereby lead to an altered setting of the osmoreceptor system and consequently to delayed and decreased VP responsiveness to osmotic stimu-

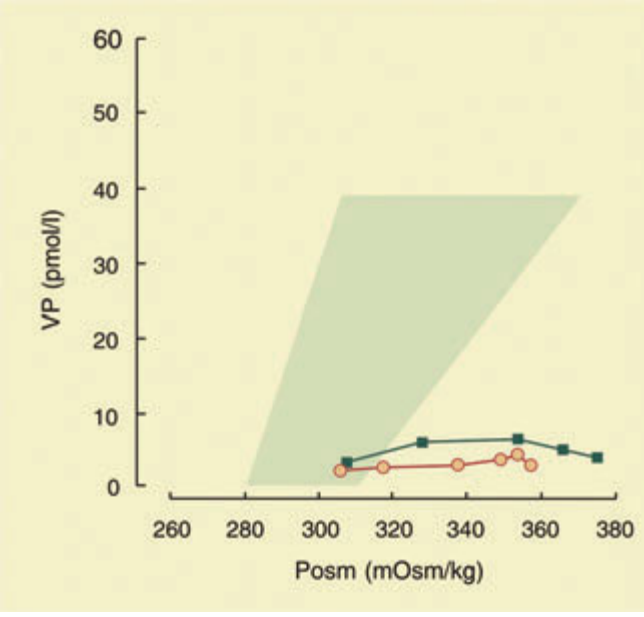


Figure 2.35:
Relation of plasma vasopressin (VP) concentration with plasma osmolality during hypertonic saline infusion in two dogs with central diabetes insipidus caused by a pituitary tumor.[209] See also legend to fig. 2.34.

lation. In a similar manner polycythemia may impair VP release. The polyuria in these conditions will at least in part be the result of disturbed VP secretion. As indicated at the end of chapter 2.3.2, interference of cations such as Ca^{2+} and hormones such as corticosteroids with the action of VP can also contribute to the polyuria.

Diagnosis

For some time the water deprivation test combined with vasopressin administration, as shown in the figures 2.32 and 2.33 and described in detail in chapter 12.2.2, has been used for the differential diagnosis of polyuria. However, the test is difficult to perform correctly, is unpleasant for the animal, relies heavily on the emptying of the bladder at each collection, and is indirect because changes in urine concentration are used as an index of VP release. Furthermore, the stimulus to VP release is a combination of hypertonicity and hypovolemia, especially near the end of the period of dehydration.

A more direct way to diagnose CDI is by measuring plasma VP during osmotic provocation by hypertonic saline infusion (fig 2.35 and chapter 12.2.3) or water restriction.[210] In severe CDI the water deprivation test gives the correct diagnosis, but in all other categories of polyuria, in which there is variable concentration of urine during dehydration, it may be less reliable. However, as mentioned above, polyurias due to other diseases may also be associated with disturbed VP release. In addition, hyporesponsiveness of VP to a hypertonic stimulus has been observed in polyuric dogs which otherwise meet criteria of primary polydipsia.[211] Also in humans it has been demonstrated that the chronic overhydration of primary polydipsia can downregulate VP release in response to hypertonicity.[212] Thus in some dogs it may remain a thorny problem to distinguish between polyuric entities.

An overall diagnostic approach to the polyuric dog is presented in chapter 2.3.3.4. As discussed there, increasingly the diagnostic procedure starts with serial Uosm measurements in urine samples collected by the owner at home. The owner then administers desmopressin (DDAVP) for four to five days and collects another series of urine samples during the last day of desmopressin treatment. In both complete and partial central diabetes insipidus, polyuria and polydipsia cease after administration of desmopressin and Uosm rises from low values to $>$ 1000 mOsm/kg. If Uosm remains $<$ 1000 mOsm/kg, central diabetes insipidus is very unlikely and instead there is primary polydipsia or functional nephrogenic diabetes insipidus.

When there is a history of head trauma or suspicion of a pituitary lesion / tumor that might cause additional pituitary deficiencies, adenohypophyseal function should be studied (chapter 12.1) and the pituitary gland should be visualized by CT and / or MRI. The neurohypophysis can be visualized with dynamic CT.[213] In the presence of a pituitary adenoma the neurohypophysis can be displaced or no longer be visible (chapter 4.3.1).[187] However, in only a small proportion of cases does a pituitary tumor interfere with VP release sufficiently to cause CDI.

Treatment

The vasopressin analogue desmopressin or DDAVP (1-deamino, 9-D-arginine vasopressin [Minrin®, Ferring AB, Malmö, Sweden]) is the drug most commonly used for treatment. It is available for use in humans in ampoules for parenteral injection (4 µg per ampoule), intranasal solution (100 µg per ml), and oral (tablets with 0.1, 0.2, and 0.4 mg). One drop of the intranasal solution (= 1.5–4 µg DDAVP) administered in the conjunctival sac twice daily sufficiently controls the polyuria in most dogs with central diabetes insipidus. With the administration of three drops / day urine production usually returns to normal, but some owners (in part for financial reasons) administer it only twice daily. In cats in which conjunctival administration is difficult, the injectable form (1 ampoule) can be given once or twice daily.[214] In a series of five cats with CDI the oral administration of ¼ to ½ tablet of 0.1 mg two to three times daily has been reported to be an effective alternative.[195]

In dogs and cats undergoing hypophysectomy the administration of desmopressin is started immediately after surgery. In healthy dogs this prevents postoperative hypernatremia.[197] In both dogs and cats undergoing hypophysectomy for pituitary-dependent hypercortisolism, mild hypernatremia can occur in the first 24 h after surgery, despite prophylactic administration of desmopressin.[215,216] This is probably in part related to the fact that the hypercortisolism-induced vasopressin resistance

is insufficiently compensated in the postoperative period by infusions and water intake. Treatment with desmopressin is continued for three weeks, for it is uncertain whether sufficient hypothalamic VP will reach the systemic circulation, and if so, how long the recovery from the surgical damage to the pituitary stalk will take. Total hypophysectomy deprives the animal of the neurohypophyseal storage of VP and the ability to release it after stimulation. Usually the pituitary stalk is sectioned low enough to preclude retrograde degeneration of the supraoptic and paraventricular neurons and there is sufficient leakage of VP from the stalk to prevent CDI.[217] In time the axons of the magnocellular neurons may regenerate to establish new neurohemal connections to form a substitute for the posterior pituitary. If polyuria recurs when desmopressin is discontinued, it is resumed for as long as needed.

If additional pituitary deficiencies have been revealed as a result of head trauma, pituitary tumor, or hypophysectomy, they should be treated as required with thyroxine and/or cortisol (chapter 3.3.2 and chapter 13.1.1).

Prognosis

In the absence of a tumor the long-term prospects are good. Appropriate treatment relieves the symptoms. Untreated animals with the complete form are always at risk of life-threatening dehydration if they do not have access to water for more than a few hours. Those with CDI caused by a pituitary tumor may lead acceptable lives for many months, until the tumor begins to cause mass effects (chapter 2.2.6.2). As mentioned under pathogenesis, the persistence of CDI after hypophysectomy in dogs with pituitary-dependent hypercortisolism depends on the size of the pituitary tumor.

2.3.3.2 Nephrogenic diabetes insipidus

In nephrogenic diabetes insipidus (NDI) the kidneys are unable to concentrate urine despite adequate plasma levels of VP. The condition may be congenital or acquired but the congenital form appears to be extremely rare. Among the few case reports[218,219] is one in which necropsy revealed a portoazygos shunt and renal medullary lesions.[218] In humans mutations of the genes encoding for the VP receptor and the AQP-2 water channels have been identified in families of NDI patients.[220,221] In dogs familial occurrence of NDI has been documented in huskies, in which the defect was ascribed to a low affinity of the V_2 receptors for VP.[222]

In contrast, acquired or secondary NDI is the most common cause of polyuria in dogs and cats and may be caused by a wide range of endocrine and metabolic disorders. Several of these have already been mentioned in the section on differential diagnoses of CDI (chapter 2.3.3.1). As discussed at the end of chapter 2.3.2, in some of these conditions the polyuria may be the result of downregulation and reduced apical plasma membrane delivery of AQP-2. In a dog that had partial NDI that disappeared upon removal of an intestinal leiomyosarcoma, it was hypothesized that the polyuria was due to tumor-associated (i.e., paraneoplastic) interference with the production or function of AQP-2.[223]

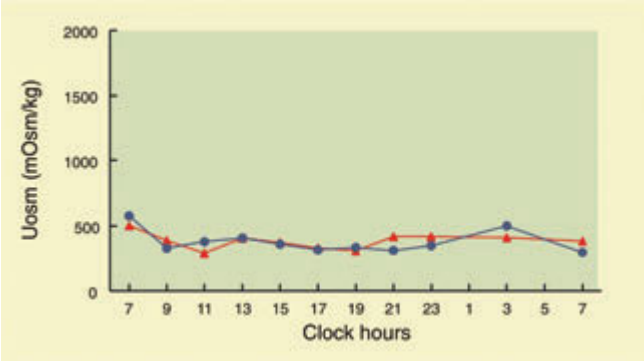


Figure 2.36:
Results of serial measurements of urine osmolality (Uosm) before (blue line) and during desmopressin administration (red line) in a dog with primary hyperaldosteronism (see also upper panel of fig. 2.34).

Chronic renal failure in adult animals leads to isosthenuria (Usg 1.008–1.012), but the polyuria may not be the iatrotropic problem that causes the owner to consult the veterinarian. However, there are reports of nephropathies in which polyuria was the main presenting problem, e.g., in a dog with extensive cryptococcal lesions in the renal medulla.[224]

Juvenile-onset renal diseases have been reported to occur as familial diseases but also as isolated cases of renal failure. Various underlying abnormalities have been reported in familial renal disease, such as amyloidosis, glomerular basement disorders, and polycystic kidney disease.[225] These animals usually develop chronic renal insufficiency and consequently may be presented with polyuria due to the isosthenuria, although initially the presenting problem may have been poor physical condition.[226] Particularly with tubular changes and fibrosis in the renal medulla, Usg may be below that of isosthenuria.

Diagnosis

Serial measurements of Uosm reveal it to be low with only minor fluctuations. Desmopressin has little or no effect, but this does not necessarily mean that the primary abnormality is renal, for poor response to desmopressin may also occur in conditions causing severe vasopressin resistance (fig. 2.36).

In the rare patient with severe congenital NDI, urine osmolality is not increased by water deprivation in the modified water deprivation test (chapter 12.2), nor by administration of VP. In several of the partial and acquired forms of NDI, water deprivation produces some urine concentration and VP also has some effect. Plasma VP measurements during the water deprivation test and/or during hypertonic saline infusion may help to differentiate among CDI, NDI, and primary polydipsia. These approaches are discussed in more detail in chapter 2.3.3.4.

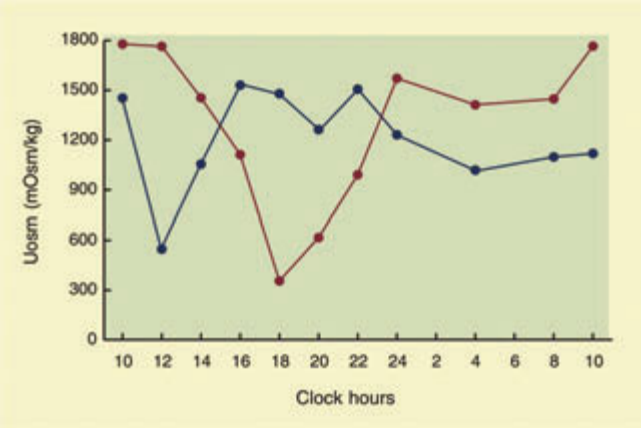


Figure 2.37:
Fluctuations of urine osmolality (Uosm) in samples collected at home in two healthy dogs with, according to the owners, unremarkable drinking and micturition behavior: a 9.5-year-old castrated male schnauzer (blue line) and a 2.5-year-old male Border collie (red line).[208]

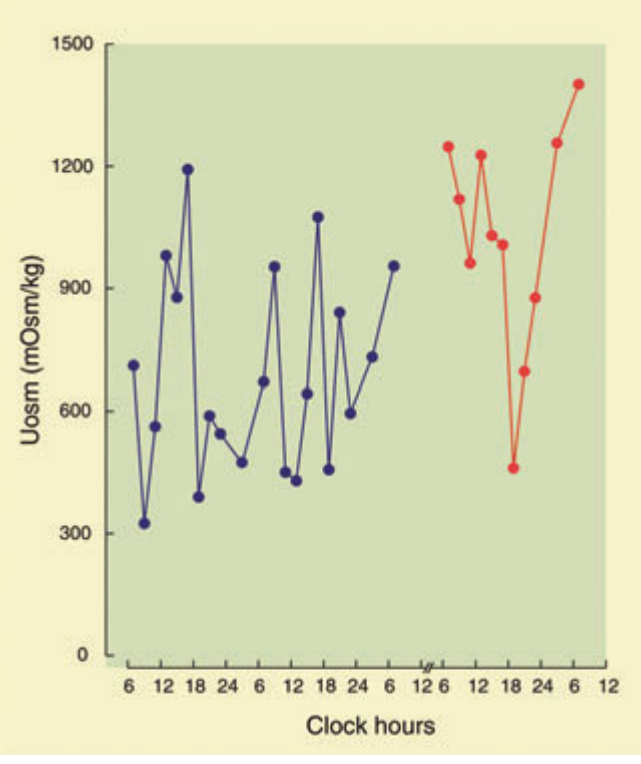


Figure 2.38:
Urine osmolality (Uosm) in two series samples collected at 2 h-intervals at home from a six-year-old West Highland white terrier that had polydipsia and polyuria for nine months. The history and physical examination revealed no other symptom or sign and urine specific gravity (Usg) was 1.025 in the morning urine sample brought by the owner. The dog had developed the habit of beginning to drink excessively at around 17.30 h, when the owner was expected home from work. This resulted in a marked decrease in Uosm by 18.00 h. but Uosm also decreased sharply earlier in the day when his wife prepared to take the dog for a walk.

Treatment

Oral administration of hydrochlorothiazide (Esidrex®, Novartis; other brand names: Microzide®, Hydrodiuril®, Oretic®) (2–4 mg/kg twice daily) and a low-sodium diet can decrease urine volume[219], albeit probably without a significant change in urine osmolality. It has been proposed that the thiazide diuretic and a low sodium diet cause extracellular volume contraction. As a result, the glomerular filtration rate decreases and proximal tubular reabsorption of sodium and water increases. Consequently, less sodium and water are delivered to the collecting tubules and urinary volume is reduced, but not normalized.[227] However, there is increasing evidence that sodium depletion and increased proximal tubular water reabsorption do not solely account for the antidiuretic effect. Hydrochlorothiazide can also directly increase water permeability in the medullary collecting ducts, probably by inducing AQP-2 expression independent of VP.[228]

2.3.3.3 Primary polydipsia

Primary polydipsia occurs primarily in dogs and is characterized by a marked increase in water intake that cannot be explained as a compensatory mechanism for excessive fluid loss. There is no urinary concentrating defect and at various times during the day the dog may produce either highly diluted urine or concentrated urine. Marked fluctuations in Uosm may also occur even though the owner observes nothing remarkable in the dog's drinking behavior (fig. 2.37). In a series of 89 healthy pet dogs, Uosm in morning urines ranged from 273 to 2620 mOsm/kg (Usg 1.009 to > 1.050) with a mean (± SD) of 1541 ± 527 mOsm/kg (Usg 1.035 ± 0.010).[229] In none of these dogs was the sometimes marked variation in water intake and urine volume considered by the owners to be associated with abnormal drinking.

Dogs with more pronounced primary polydipsia exceed the iatrotropic threshold and are presented to the veterinarian because of polyuria and polydipsia. This is said to be the case in hyperactive young dogs that are left alone for many hours during the day or have undergone important changes in their environment. Placing the dog in a completely different environment, as during hospitalization, has sometimes stopped the problem. The problem may also develop later in life, such as in the excitement of anticipated pleasure of going for a walk (fig. 2.38). Transient primary polydipsia has also been reported to occur in association with gastrointestinal disease, in which case water intake was in excess of the fluid losses via diarrhea and vomiting.[230]

As mentioned in chapter 2.3.2 satiation of thirst occurs in dogs during drinking, before any detectable change in plasma osmolality, plasma volume, or blood pressure as a result of absorption of the water. Oropharyngeal signals inhibiting thirst normally prevent the dog from drinking an amount of water in excess of its physiological need. Nevertheless, water consumption was shown to fluctuate with food intake and exercise: dogs consumed 40 % of their total daily water intake

during 2 h after eating dry food, and after treadmill running for 30 min water intake was higher than the water losses during the exercise.[231] These factors together with other environmental factors and/or moments of intense interactions in some dogs seem to bring about a complex of signals that override the oropharyngeal and osmotic signals that normally result in appropriate water intake.

The results of routine blood examinations are usually unremarkable, except for Posm and plasma sodium concentration, which are often at or just below the lower limit of their respective reference ranges, although values at the upper limits of the reference ranges have also been reported.[232] Both hyponatremia and normonatremia have also been reported in humans with primary polydipsia.[233]

Diagnosis

Marked fluctuations in Uosm in serial urine samples collected at frequent intervals and some values $>$ 1000 mOsm/kg (fig. 2.38 and chapter 12.2) provide the diagnosis of primary polydipsia. However, the fluctuations are not so pronounced in all dogs with primary polydipsia and in some Uosm fluctuates between about 200 and 600 mOsm/kg. In such cases a water deprivation test (chapter 12.2.2) can be very helpful. Within 8 h of water deprivation Uosm should exceed 1000 mOsm/kg with weight loss $\leq$ 3 % and only slight increases in Posm and plasma sodium concentration.[232]

Plasma VP measured during the water deprivation test in dogs with primary polydipsia remains low[232], in agreement with the earlier observation that production of highly concentrated urine can occur with relatively low VP concentrations.[234] During hypertonic saline infusion abnormalities in VP release may include episodes of hypersecretion as well as delayed and low responses to plasma hypertonicity. It is not clear whether the latter indicates a primary disturbance in osmoreceptor function and regulation of VP secretion, or that it is the result of chronic overhydration downregulating VP release in response to hypertonicity.[212,232] The occasionally observed early »hyperresponses« might represent erratic secretory bursts, but might also reflect the pulsatile release pattern induced by the hypertonicity (figs. 2.29 and 2.39).[211]

Treatment

Dogs with strong fluctuations in Uosm at set times during the day are the least difficult to treat. The water bowl can be removed during periods when excessive water intake can be expected and the conditioned behavior can be discouraged. In dogs with less pronounced spontaneous fluctuations in Uosm at low values, but in which water deprivation leads to Uosm $>$ 1000 mOsm/kg in $<$ 8 h, some caution is warranted. In some of these dogs Posm may not be low and water restriction, although effective, may increase Posm and lead to severe sensations of thirst. Although the current criteria for primary polydipsia appear to be fulfilled, there may be an as yet ill-defined disturbance in osmoreceptor function and regulation of VP secretion (see above).

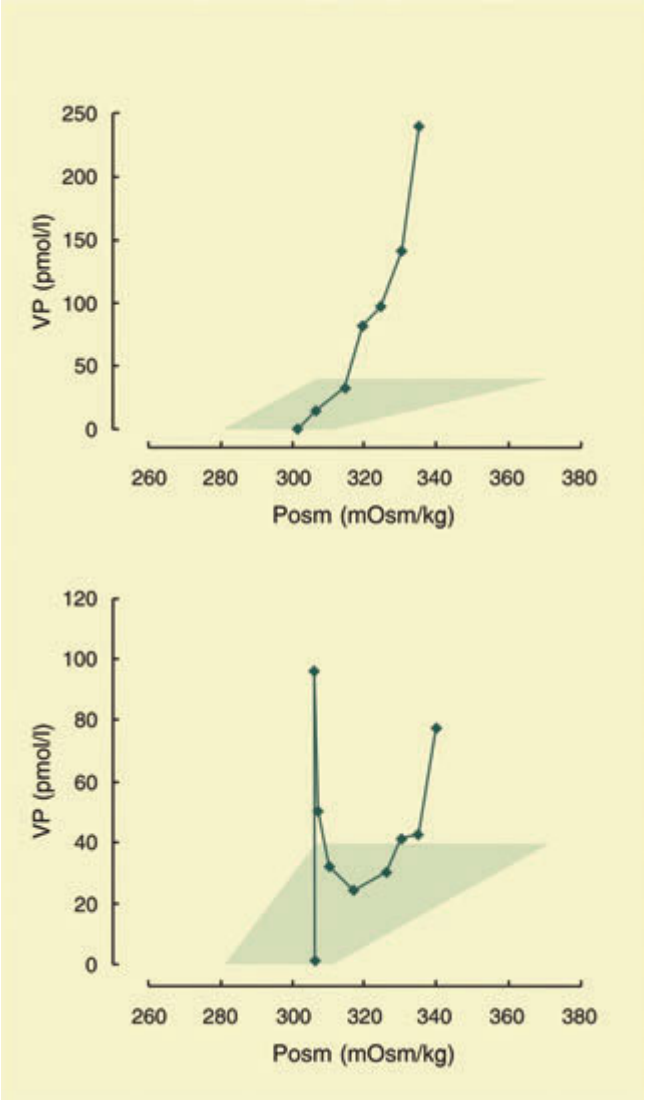


Figure 2.39:
Plasma vasopressin (VP) concentration during hypertonic saline infusion in a six-month-old Jack Russell terrier (upper panel) and a two-year-old Maltese dog (lower panel). The results of serial measurements of Uosm were characteristic of primary polydipsia, 223–1658 mOsm/kg in the former and 88–1387 mOsm/kg in the latter. Basal plasma osmolality (299 and 306 mOsm/kg) and basal plasma sodium (143 and 146 mmol/l) were near or below the lower limit of the reference ranges for Posm (303–320) and for PNa^+ (141–150). In the Jack Russell terrier the effect of hypertonic stimulation on plasma VP was interpreted as a hyperresponse. In the Maltese dog the VP response also appeared unrelated to the gradual rise in Posm. [211] See also legend to fig. 2.34.

Prognosis

While the owner may report that the dog's drinking behavior has improved and that polyuria and urinating in the house have ceased, follow-up measurements of Uosm may reveal the continuation of marked fluctuations (e.g., 345 to 1804 mOsm/kg); the owners may have learned to live with the abnormality.[232] In most cases the excessive drinking persists, but with the measures described above an acceptable situation can be achieved.

2.3.3.4 Algorithm for polyuria / polydipsia

In this section the diagnostic approaches discussed in the three previous sections are integrated with the help of an algorithm (chapter 14.2). This is primarily for dogs, in which the problem is much more frequent than in cats. In such a schematic approach the nuances of the previous sections are omitted, but by virtue of its simplification it may be helpful as an initial guide. The algorithm begins with the problem presented in the medical history: polyuria / polydipsia (pu / pd). Then it gives attention to relevant points in the signalment, history, and physical examination[235], such as possible causes of hypercalcemia (lymphoma, anal sac tumor).

Laboratory examination begins with checking urine glucose and specific gravity. It may happen that an animal presented with a seemingly convincing history of pu / pd has only increased water intake because the owner has changed the food to dry food. The blood tests relate to the differential diagnoses listed in chapter 2.3.3.1.

If laboratory examinations do not provide a diagnosis, further examination outside the neurohypophyseal system may include diagnostic imaging of the abdomen. If ultrasonography reveals a lesion in the kidneys or adrenals, for example, the imaging may be expanded to CT and / or MRI. If no abnormality is found, the approach proceeds to the last part of the algorithm.

The last part of the algorithm includes three test procedures that may provide a definite choice among the three differential diagnoses discussed in the previous sections: central diabetes insipidus, nephrogenic diabetes insipidus, and primary polydipsia. These tests are described in detail in chapter 12.2, where attention is also given to the relation between Uosm and Usg.

2.3.4 Vasopressin excess; Syndrome of inappropriate antidiuresis (SIAD)

Elevated or normal VP secretion is inappropriate in the presence of low plasma osmolality. Reduced suppressibility of VP causes water retention and may lower plasma osmolality to the extent of causing clinical manifestations of cellular overhydration. In principle this condition is associated with highly concentrated urine but in humans there is also a variant with resetting of the osmostat in which VP secretion can be fully suppressed, resulting in dilute urine at low plasma sodium concentrations.[236] Only a few cases of the syndrome have been described in dogs but there also appear to be two forms, with and without polyuria.

Pathogenesis

Of the three reported cases of SIAD in dogs without polyuria, one was considered to be idiopathic[237], the second was due to encephalitis[238], and the third was attributed to *Dirofilaria immitis* infestation, aggravated by a low-sodium diet.[239] Of the three reported cases of SIAD with polyuria as the presenting symptom, two were considered to be idiopathic[240] and the third was associated with a tumor in the thalamus and dorsal hypothalamus.[241]

SIAD is also known to occur in humans as a complication of transsphenoidal resection of pituitary adenomas.[242] The syndrome may arise as a consequence of uncontrolled VP release from surgically damaged hypothalamic centers in combination with underestimated perioperative blood loss. Hyponatremia due to SIAD has also been reported after hypophysectomy in dogs with pituitary-dependent hypercortisolism and was attributed to hypothalamic damage and severe intraoperative arterial hemorrhage.[215]

In addition to endogenous VP excess, it is possible that similar abnormalities are produced by excessive administration of vasopressin. Particularly dogs with primary polydipsia incorrectly treated with DDAVP seem to be at risk of developing the hypotonicity syndrome.

Clinical manifestations

In principle both defective water excretion and increased water intake may lead to plasma hypotonicity, which is essentially hyponatremia. In hyponatremia, two-thirds of the relative water surplus is intracellular, making generalized cellular edema the hallmark of acute hypotonia. Unlike extracranial tissues that can expand freely, the distending brain is compressed against the unyielding cranium, provoking the syndrome of cerebral edema. This syndrome includes weakness, lethargy, and nausea, and may culminate in resting tremor, generalized seizures, and coma.

Brain cells may adapt to chronic hypotonicity by extruding electrolytes, but if hypotonicity is severe the adaptive losses may be insufficient to prevent clinical manifestations. Disturbed brain function usually prevents the animal from continued drinking, which allows Posm to rise and leads to recovery.

In addition to neurological manifestations, some of the reported cases included polyuria, which seems paradoxical in presumed VP excess. The subject of polyuria and abnormal-

ities in VP release was discussed in chapter 2.3.3.3 and in reports on dogs with primary polydipsia.[211,232] Healthy medium-sized to large dogs have sufficient diluting capacity to excrete up to 5–8 l of free water per day. When intake exceeds this amount, dilutional hypotonicity occurs and neurological symptoms may develop. If primary polydipsia is associated with an abnormality in renal free water clearance such as SIAD, plasma hypotonicity may develop at relatively low intakes. In humans SIAD has been implicated as contributing to hypotonicity in schizophrenic patients with primary polydipsia.[243] As discussed in chapter 2.3.3.3, VP hyperresponsiveness to osmotic stimulation has also been reported in dogs with primary polydipsia and thus a similar combination could be present. However, these high responses might in large part have been reflections of the strong pulsatile nature of VP release in stimulated conditions (fig. 2.29).[170]

Diagnosis

The diagnosis of SIAD begins with exclusion of other potential causes of hypotonicity such as hypoadrenocorticism, hypothyroidism, recent diuretic use, and hospital-acquired fluid imbalance. Then the following criteria should be fulfilled:

- Plasma hypotonicity (Posm < 280 mOsm/kg).
- Inappropriately high urine concentration in the presence of plasma hypotonicity.
- Plasma VP concentration inappropriately high relative to Posm.
- Improvement after fluid restriction.

As mentioned above, the diagnosis of SIAD in dogs with polyuria can be questioned. Measurements of urinary AQP-2 may help to unravel the role of VP in these conditions.[179]

Treatment

In the event of hyponatremia due to SIAD after hypophysectomy in dogs, treatment consists of restriction of fluid intake, close monitoring of plasma sodium concentration, and immediate cessation of desmopressin.[215,216] In chronic SIAD, fluid restriction should also be effective. Continuing urinary and insensible fluid losses then induce a negative water balance and fluid volume is restored. This may not be very effective in cases in which there is high urine concentration.[237]

Receptor-specific VP(V_2) antagonists, so-called aquaretic agents, may block the action of VP in the collecting duct cells and thus promote water excretion specifically. Although initially not very effective[244], more recent nonpeptide V_2-receptor antagonists are effective and can be administered orally.[245,246] One has been used in one dog with SIAD with good results.[237] Administration of 3 mg/kg every 12 h resulted in marked aquaresis. The dog recovered from the neurological symptoms, although hyponatremia persisted. A higher dose rate may be required to achieve long-term resolution of hyponatremia.[237]

Prognosis

In what has been described as the idiopathic form of SIAD with polyuria, water restriction may allow the animals to live an almost normal life for several years. As in primary polydipsia the animal's behavior will indicate continuing thirst but the neurological signs will only reappear when too much water is given accidentally. If the disease is caused by a tumor or comparable lesion, it will determine the prognosis.

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3 Thyroids

Ad Rijnberk
Hans S. Kooistra

3.1 Introduction

In the dog and the cat the thyroid glands are separate lobes lying beside the trachea from about the third to the eighth tracheal ring. They are covered ventrally by the sternohyoid and sternothyroid muscles. The major blood supply is via the cranial thyroid artery, a branch of the common carotid, and the principal venous drainage is via the caudal thyroid vein, which enters the internal jugular vein. Normal thyroid glands are not palpable.

The thyroids are assembled from two different embryologic structures, reflecting their dual endocrine function. The thyroglobulin-producing follicular cells originate from a midline evagination of the pharyngeal epithelium. The calcitonin-producing cells – parafollicular or C cells – are derived from the neural crest, originating from the fourth pharyngeal pouch. The thyroid primordium begins descending toward its final position while still connected to the floor of the pharynx by a narrow channel, the thyroglossal duct and during the descent remnants of tissue may be left along the tract. In addition, in their development the thyroids are intimately related to the aortic sac, which leads to the frequent occurrence of accessory thyroid tissue in the mediastinum of the adult animal. Rarely, such accessory tissue is the sole functioning thyroid tissue and its secretion may be insufficient to maintain a normal metabolic (euthyroid) state (chapter 3.2.2). Several of the genes involved in the early and later stages of thyroid morphogenesis have been identified.[1]

The basic functional unit of the thyroid is the follicle, a hollow sphere 30–300 μm in diameter. Its wall is a single layer of thyroid epithelial cells which are cuboidal or flat when quiescent (fig. 3.1) and columnar when active. The lumen is filled with a colloid containing a large (~660 kDa) glycoprotein called thyroglobulin (Tg)[2] that is unique to the thyroid and within the sequence of which the thyroid hormones are synthesized and stored. The C cells are largely located in the interfollicular spaces (fig. 3.1).

3.1.1 Hormone synthesis and secretion

The main hormonal secretory product of the thyroid gland is 3,5,3',5'-L-tetraiodothyronine or L-thyroxine (T_4). The other thyroid hormone, 3,5,3'-L-triiodothyronine (T_3), is secreted in much smaller quantities (about 20 % of that of T_4). Most of the circulating T_3 is produced in peripheral tissues by deiodination of the outer ring of T_4. Inner ring deiodination

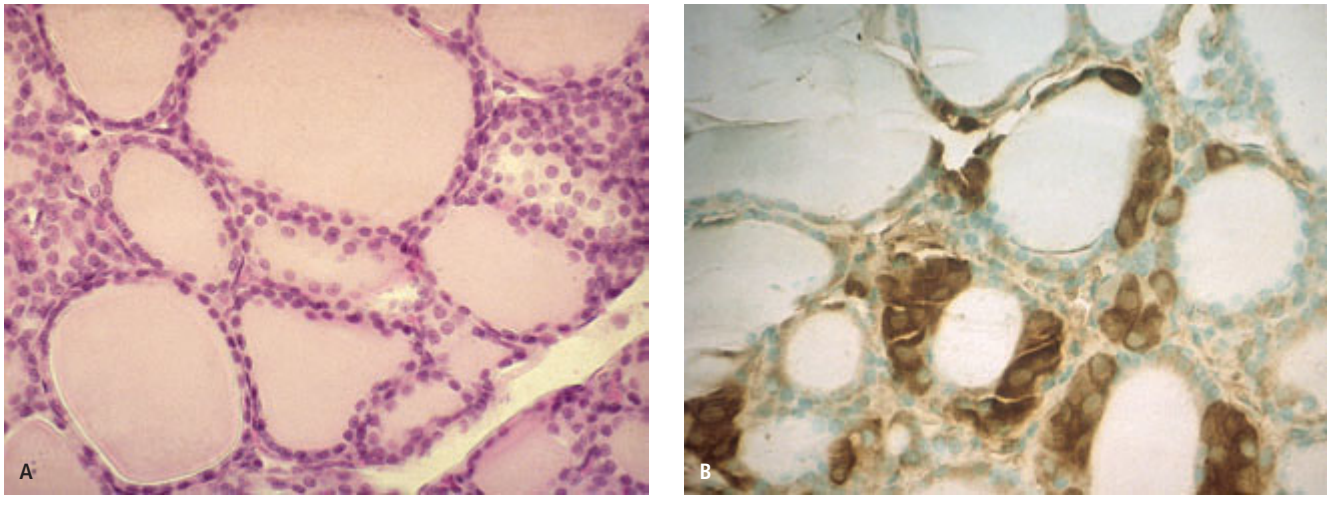


Figure 3.1:
(A) Photomicrograph of the thyroid gland of a healthy adult dog, illustrating the variable size of the thyroid follicles.
(B) Immunoperoxidase stain for the calcitonin-secreting C cells or parafollicular cells in a healthy adult dog.

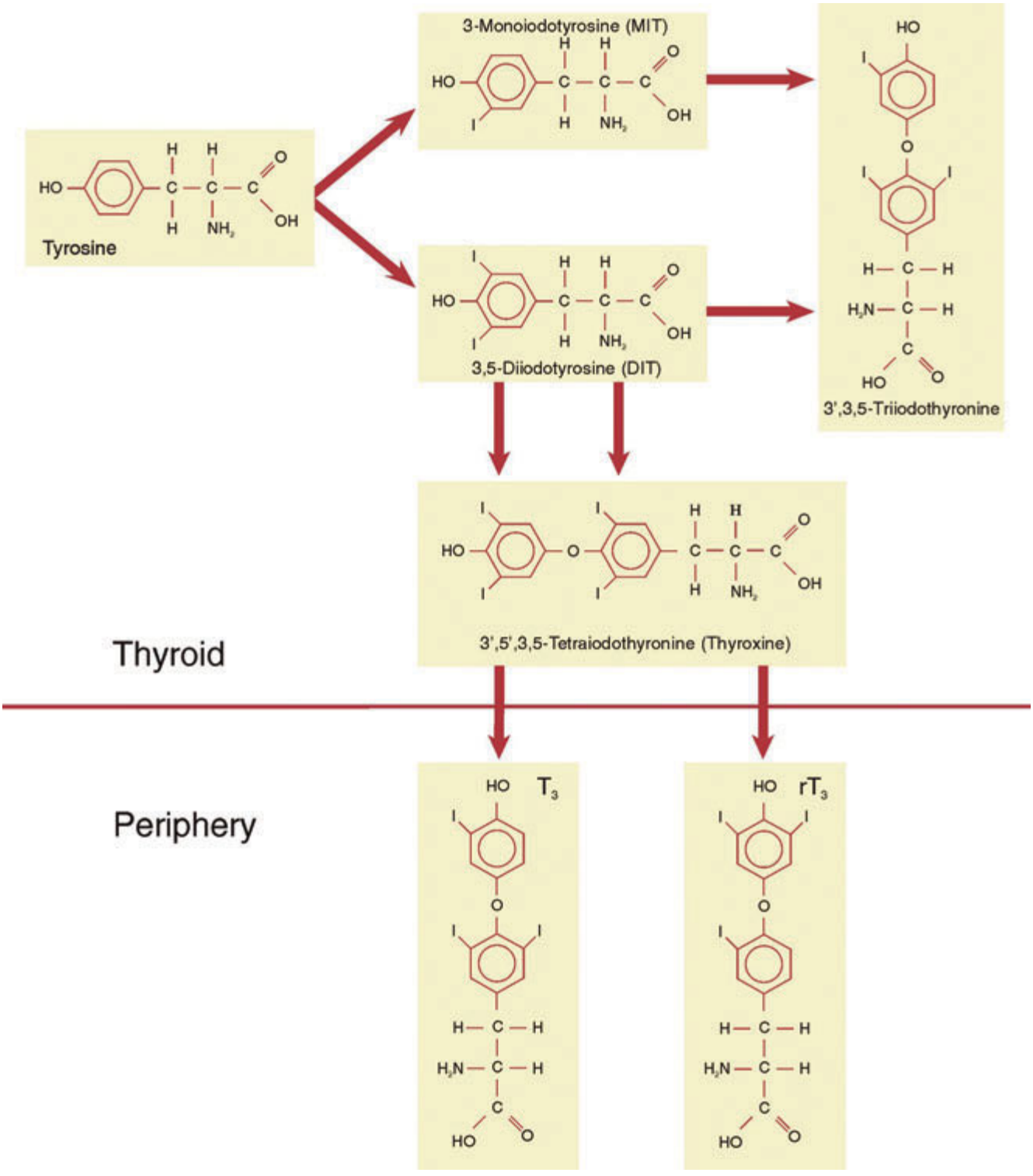


Figure 3.2:
Chemical structures of the amino acid tyrosine, intrathyroidally formed iodotyrosines (MIT and DIT) and iodothyronines (T_4 and T_3), and two products of the peripheral deiodination of T_4, namely, T_3 and reverse T_3 (3',5',3-triiodothyronine).

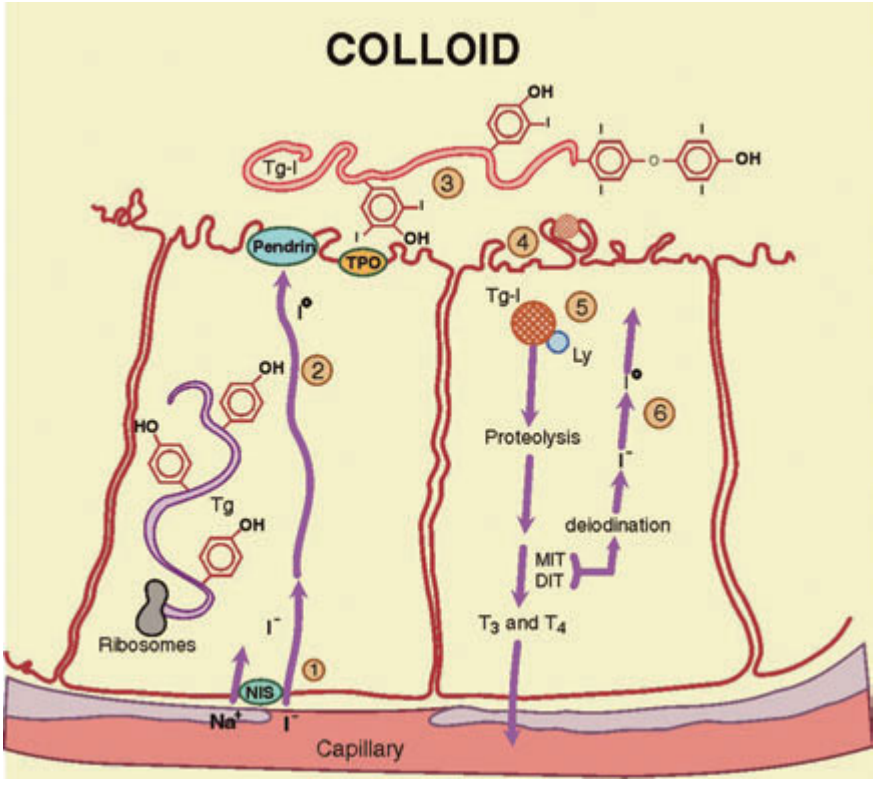


Figure 3.3:
Two follicular cells, representing thyroid hormone biosynthesis (left) and secretion (right): (1) Active transport of iodide from the blood into the thyroid cell via sodium iodide symporter (NIS), (2) oxidation of the iodide by thyroid peroxidase (TPO) and transfer of the oxidized iodide to tyrosine residues of thyroglobulin (Tg), (3) coupling of two DIT molecules to form T_4 or MIT + DIT to form T_3 (see also fig. 3.2), (4) endocytosis or pinocytosis of colloid droplets, (5) fusion of colloid droplets with lysosomes (Ly) and subsequent hydrolysis of Tg with release of T_3 and T_4, (6) deiodination of free iodotyrosines and intrathyroidal reutilization of iodide.

results in the metabolically inactive 3,3',5'-triiodothyronine (reverse T_3, rT_3) (fig. 3.2).

Iodide, the main building block of the thyroid hormones, is actively transported (»trapped«) from the extracellular fluid into the thyroid follicular cells by an active, saturable, energy-dependent process, which derives its energy from Na^+-K^+-ATPase. This iodide carrier is a large (> 600 amino acids) transport protein called *sodium iodide symporter* (NIS), located at the basal membrane of the thyrocyte (fig. 3.3). The resulting thyroid:plasma iodide ratio is ~25. An additional thyroid cell protein, called pendrin, is thought to facilitate the apical transfer of iodide into the follicular lumen.[3]

The gastric mucosa, salivary glands, and choroid plexus are also able to concentrate iodide via NIS but in contrast to the thyroid, they do not bind it organically. These tissues as well as the thyroid also concentrate structurally-related monovalent anions such as thiocyanate (SCN^-), perchlorate (ClO_4^-), and pertechnetate (TcO_4^-). However, unlike iodide, these ions are also not organically bound in the thyroid and hence their duration within the thyroid is short. This property together with a short physical half-life makes the radioisotope of pertechnetate ($^{99m}TcO_4^-$) a valuable radionuclide for imaging the thyroid by scintillation scanning.

Once within the thyroid cell, inorganic iodide is rapidly oxidized in the presence of hydrogen peroxide (H_2O_2) to a reactive intermediate that is then incorporated into tyrosine residues of acceptor proteins, mainly thyroglobulin (Tg). The iodination is catalyzed by thyroid peroxidase (TPO), a membrane-bound heme-protein enzyme. Studies in dog thyroid cells have shown that the regulatory cascade controlling H_2O_2 generation in thyrocytes is different from that of the O_2-generating system of macrophages and leukocytes.[4]

Iodination of the tyrosine residues of Tg results in the formation of monoiodotyrosine (MIT) and diiodotyrosine (DIT). MIT and DIT then undergo oxidative coupling to form the iodothyronines, which remain bound to Tg until secreted (fig. 3.2). This coupling reaction occurs separately from iodination but is also catalyzed by TPO. The thiocarbamide drugs, including propylthiouracil, methimazole, and carbimazole, are competitive inhibitors of TPO.[5] Their resulting ability to block thyroid hormone synthesis makes them useful in the treatment of hyperthyroidism (chapter 3.4.1). Tg is iodinated at the apical (follicular) border of the cell and is then moved into the colloid by exocytosis (fig. 3.3).

Secretion of thyroid hormones requires that Tg be taken back into the thyroid cell via pinocytosis (fig. 3.3). Pseudopods from the apical plasma membrane surround a portion of the colloid to form an intracellular colloid droplet.[6] Each droplet is enclosed in a membrane derived from the apical border and is combined with a lysosome. This phagolysosome moves toward the basal aspect of the cell and becomes smaller and

more dense with progression of the hydrolysis of Tg by the lysosomal proteases (fig. 3.3). This digestion of Tg releases T_4 and T_3, as well as the inactive iodotyrosines, peptides, and individual amino acids. The biologically active thyroid hormones T_4 and T_3 diffuse from the cell into the circulation, whereas MIT and DIT are largely prevented from release into the circulation by the action of intracellular deiodinase (fig. 3.3). Tg itself is normally not released into the circulation in significant quantities and in healthy dogs only very small quantities can be measured in the peripheral blood by a sensitive homologous immunoassay.[7]

3.1.2 Hormone transport, tissue delivery, and metabolism

Plasma T_4 and T_3 are largely bound to protein. Less than 0.05 % of T_4 and less than 0.5 % of T_3 circulate as »free« or unbound hormone, but it is the free hormone concentration that is maintained constant by the feedback regulatory system and that appears to parallel the rate of cellular uptake of these hormones. Thus it is the free hormone concentration that determines thyroid status irrespective of the total hormone concentration in the plasma. Dogs have a high-affinity thyroid hormone binding globulin (TBG), in addition to which albumin and prealbumin bind thyroid hormones with low affinity. Cats do not appear to have a high-affinity TBG, only prealbumin acts as a thyroid hormone binding protein.[8] In addition to these binding proteins, a minor part of the thyroid hormones can be bound to lipoproteins and also to transthyretin, which in part exists as a complex with retinol (vitamin A)-binding protein.[3]

The concentration and/or capacity of circulating binding compounds may be changed by a variety of diseases and pharmacologic agents, thereby affecting primarily the plasma total thyroxine (TT_4) concentration. Glucocorticoids and acetylsalicylic acid are known to lower plasma TT_4 concentration without affecting the concentration of free T_4.[9,10] Breed differences may also account for deviations from established reference ranges for TT_4 for the total dog population. In general, dogs of small breeds tend to have somewhat higher plasma TT_4 concentrations than do those of large breeds. Low TT_4 concentrations have been reported in whippets, and in sled dogs and greyhounds both TT_4 and free T_4 concentrations have been reported to be relatively low.[11–13]

In recent years several plasma membrane carriers for transport of both T_4 and T_3 to intracellular sites of action and metabolism have been identified. There is increasing evidence for tissue-specific as well as generalized transporters belonging to a number of different transporter protein families. Each of these families has many members, with small variations in structure that alter the specificity of the target substance. Mutations in one of these carriers in humans have been found to be associated with severe psychomotor retardation and greatly elevated plasma T_3 levels.[14]

Deiodination is the most significant metabolic transformation of the thyroid hormones. About 80 % of the secreted T_4 is deiodinated to form T_3 and rT_3, predominantly in the liver and kidney. T_3 has a higher binding affinity for nuclear T_3 receptors than does T_4 and therefore outer ring monodeiodination generates a more biologically active iodothyronine. T_3 has approximately three to four times the metabolic potency of T_4, which means that almost all thyroid hormone metabolic action can be ascribed to the action of T_3. T_4 and T_3 are inactivated by inner ring deiodination to rT_3 and 3,3'-diiodothyronine (3,3'-T_2), respectively. The three deiodinase enzymes (D1, D2, and D3) that catalyze these reactions differ in tissue localization, substrate specificity, and physiologic and pathophysiologic modulation. Thus the biological activity of thyroid hormone is additionally regulated locally by tissue-specific deiodinases.[15] The notion that the thyroid itself contributes little to the T_3 pool does not apply to states of hyperfunction, for then the T_3:T_4 ratio in the thyroid secretion increases.

Factors that impair T_3 formation, such as fasting and nonthyroidal disease, almost invariably increase plasma rT_3 concentration. There is evidence that illness leads to increased activity of type 3 deiodinase (D3), which primarily deiodinates the inner ring. It decreases the T_3:rT_3 ratio in two ways: it prevents conversion of T_4 to T_3 by instead catalyzing the conversion of T_4 to rT_3, and it also catalyzes the degradation of T_3 to 3,3'-T_2.[15] There is no convincing evidence that low circulating T_3 concentrations in illness are associated with inadequate thyroid hormone effect at the tissue level. Indeed, the impaired conversion of T_4 to T_3 is probably beneficial in sparing protein catabolism.[16]

As mentioned above, T_4 binds more tightly to binding proteins in plasma than does T_3, which results in T_4 having a lower metabolic clearance rate and longer half-life. Overall, the kinetics of thyroid hormone distribution and turnover are much more rapid in dogs than in humans, in part because of the lower binding of both T_4 and T_3 in canine plasma.[17,18] The plasma half-life of T_4 is about 0.6 days in dogs, compared to about seven days in humans.

3.1.3 Regulation of thyroid function

Thyroid function is mainly regulated by thyrotropin (thyroid-stimulating hormone, TSH), a 28 kD glycoprotein secreted by the anterior lobe of the pituitary. The TSH molecule consists of an α- and αβ-subunit. The α-subunit is identical to that of gonadotropins, whereas the β-subunit is distinct and confers on the TSH molecule its biological activity. In both the dog and the cat the genes encoding the β-subunit of TSH have been cloned and sequenced.[19,20] Like all pituitary hor-

mones, TSH is released in a pulsatile fashion (fig. 1.8), albeit that the fluctuations in its plasma concentration are very small, particularly in the euthyroid state.[21]

TSH stimulates the thyroids by interacting with specific cell surface (G-protein-linked) receptors on thyroid follicular cells to enhance the activity of adenylcyclase. It thus stimulates the generation of cyclic adenosine monophosphate (cAMP) as a second messenger inside the cell (fig. 1.4). TSH rapidly promotes pinocytosis at the apical border of the follicular cell, thereby accelerating resorption of Tg and subsequent hormone release. Long-term TSH stimulation leads to thyroid hypertrophy and hyperplasia, and thyroid enlargement may ensue to the extent that the glands become palpable (goiter). The mitogenic action of TSH in dog thyroid is entirely mediated by cAMP.[22]

The regulation of TSH secretion is primarily under the dual control of the hypothalamic TSH-releasing hormone (TRH) and thyroid hormones. TRH interacts with specific receptors on pituitary thyrotropic cells to release TSH and on lactotropic cells to release prolactin (fig. 2.7). TSH secretion is inhibited primarily by T_3, which is produced locally by 5'-deiodination (D2), and also by T_3 derived from the systemic pool of free T_3 (fig. 3.4). Negative feedback by T_3, formed by D2, also occurs at the paraventricular nucleus of the hypothalamus. Somatostatin and possibly other neuropeptides exert an inhibitory influence on TSH release (figs. 2.7, 3.4).

There is also an intrathyroidal regulation of thyroid function which is especially important in the presence of either insufficient or excessive iodine supply. This autoregulation enables immediate adaptation to acute iodide excess (e.g., from disinfection of a large area of skin with iodine) that might otherwise lead to hyperthyroidism, primarily by lowering the expression of the genes encoding TPO and NIS.[23] On the other hand, in iodine deficiency thyroid function is increased long before the thyroidal organic iodine stores (Tg) are exhausted. The thyroid also adapts to low intake of iodine by preferential synthesis of T_3 rather than T_4.

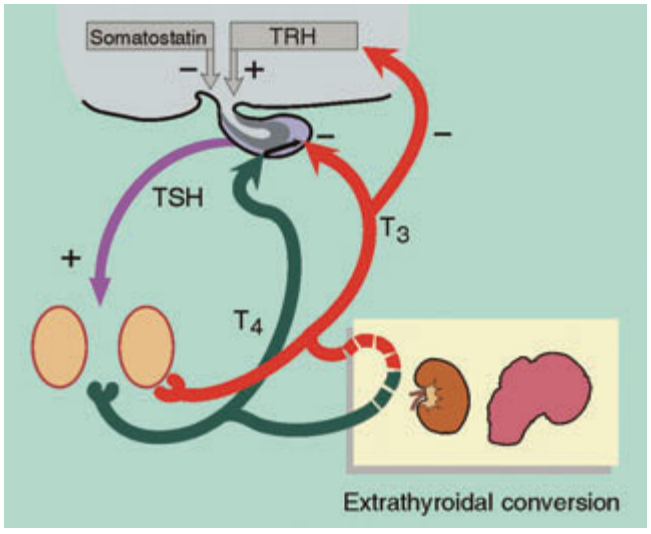


Figure 3.4:
The hypothalamic-pituitary-thyroid axis. Hypothalamic TRH reaches the thyrotropic cells in the anterior lobe of the pituitary via the local portal vessels and enhances TSH secretion. Thyroid hormones, particularly systemically and locally produced T_3, exert negative feedback at the pituitary and hypothalamic levels.

3.1.4 Thyroid hormone action

Most of the effects of thyroid hormones are thought to be mediated by an interaction of T_3 with a specific nuclear receptor, quite similar to that of steroid hormones (fig. 1.4). This nuclear thyroid hormone receptor (TR) has a high affinity for T_3, 15-fold greater than its affinity for T_4. The TR belongs to the family of steroid-thyroid-retinoid receptors. There are multiple TRs, divided into α- and β-forms on the basis of sequence similarities and chromosomal location. In several species each of the two TR genes yields at least two differentially spliced products, and this seems to hold true for the dog as well.[24]

In the past decade this classical or genomic mechanism has been complemented by reports on thyroid hormone action involving novel extranuclear (nongenomic) mechanisms. At initiation these nongenomic mechanisms do not depend upon intranuclear complexing of TR and thyroid hormone but some of them require T_4 and are insensitive to T_3. Recently a cell surface receptor for iodothyronines was discovered to occur on a structural protein (integrin) of the plasma membrane of virtually all cells.[25] The normally stable ambient concentrations of thyroid hormone have led to the suggestion that these nongenomic actions contribute to a basal setting of cellular functions. For example, this receptor mediates actions of thyroid hormone on intracellular protein trafficking and on plasma membrane ion pumps.[26]

There is characteristically a lag time of hours or days before thyroid hormones reach their full physiological effects, but they have effects in almost all tissues of the body. In many respects thyroid hormones can be viewed as tissue growth factors, this being best exemplified by the consequences of thyroid hormone deficiency at a young age (chapter 3.2). The earliest recognized physiological effect of thyroid hormones is stimulation of the basal metabolic rate or calorigenesis. Animals deficient in thyroid hormones have difficulty in maintaining body temperature and may be unable to survive in a cold environment. The ability of thyroid hormones to affect the genes encoding for proteins such as Na^+-K^+-ATPase and Ca^{2+}-ATPase accounts for a large fraction of the effects on calorigenesis. Na^+-K^+-ATPase concentration in muscles is much lower in dogs with spontaneous hypothyroidism than in those that are euthyroid.[27]

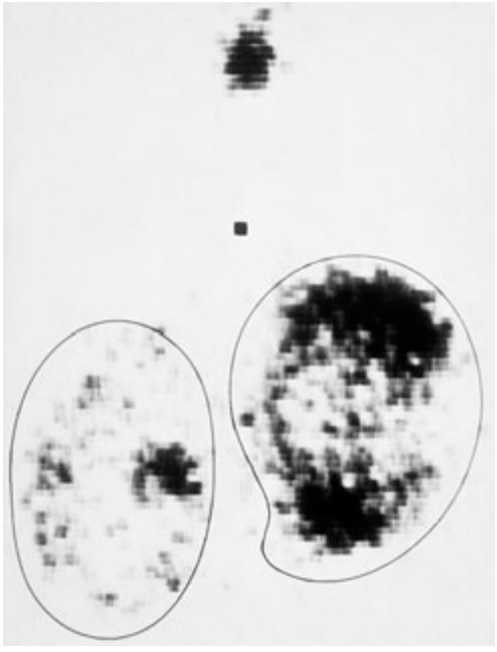


Figure 3.5:
Scintiscan of a dog with a bilateral thyroid tumor (palpated outlines indicated by solid lines). The patchy distribution of the radioactivity is compatible with the heterogeneous character of the tumor: Areas lacking the capacity to trap radioioidide (anaplastic tumor, necrosis, and/or hemorrhage) are intermingled with areas that do accumulate it (predominantly follicular tumor tissue). Cranial to the reference mark (square dot) on the midline over the cricoid cartilage there is an accumulation of radioactivity in a thyroglossal duct remnant (at the level of the lingual bone).

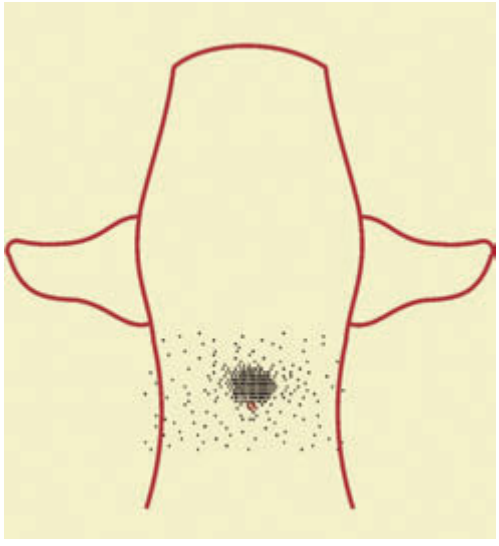


Figure 3.6:
Rectilinear ^{131}I-scintiscan of a four-year-old female German Pointer weighing 18 kg. The dog was presented because of longstanding symmetrical areas of alopecia on the flanks. The dog's growth had been retarded and it had disproportionately short legs. There were no symptoms of reduced mental or physical activity. The scan reveals only one small area of ^{131}I accumulation, in the midline, cranial to the normal site of the thyroid glands. Apparently this small remnant from the thyroglossal duct was insufficient to maintain euthyroidism. Substitution therapy with l-thyroxine was followed by regrowth of hair.

3.2 Hypothyroidism in young animals

Early in life the presence of thyroid hormones is crucial for growth and development of all body tissues and particularly the skeleton.[28] Hence disproportionate dwarfism may be a prominent sign of congenital or juvenile-onset hypothyroidism, in addition to the signs also seen in adult-onset hypothyroidism (chapter 3.3).

3.2.1 Acquired juvenile hypothyroidism

Iodine deficiency is the classic cause of acquired juvenile hypothyroidism. It occurred in times when owners took too literally the notion that dogs and cats are carnivores. A diet consisting of meat alone is deficient in many respects and certainly in iodine. The lack of this essential ingredient of the thyroid hormones results in TSH-induced thyroid hyperplasia. In mild deficiencies the increased capacity for hormone production compensates sufficiently and euthyroidism is maintained. However, in severe iodine deficiency there is insufficient production of thyroid hormone despite the compensatory thyroid hyperplasia. Animals with severe iodine deficiency are presented with the combination of large goiters and signs of hypothyroidism such as sluggishness and retarded growth.[29,30] This entity is no longer seen in countries in which it is customary to feed manufactured diets, which are rather rich in iodine.

Antimicrobial sulfonamides are known to inhibit TPO in a reversible, dose-dependent, and duration-dependent manner.[31,32] There have been reports of dogs in which treatment with sulfonamides for several weeks led not only to low plasma TT_4 concentrations, but also to clinical manifestations of hypothyroidism. Particularly in young dogs, increased TSH secretion (via negative feedback, fig. 1.9) can result in palpable thyroid lobes.[33,34]

Another, very rare, cause of acquired juvenile-onset hypothyroidism is lymphocytic thyroiditis. It has been reported in a breeding line in a closed colony of cats, with symptoms such as lethargy and a dull hair coat already present at the age of seven weeks.[35] Lymphocytic thyroiditis is the common cause of primary hypothyroidism in adult dogs. Rarely the process

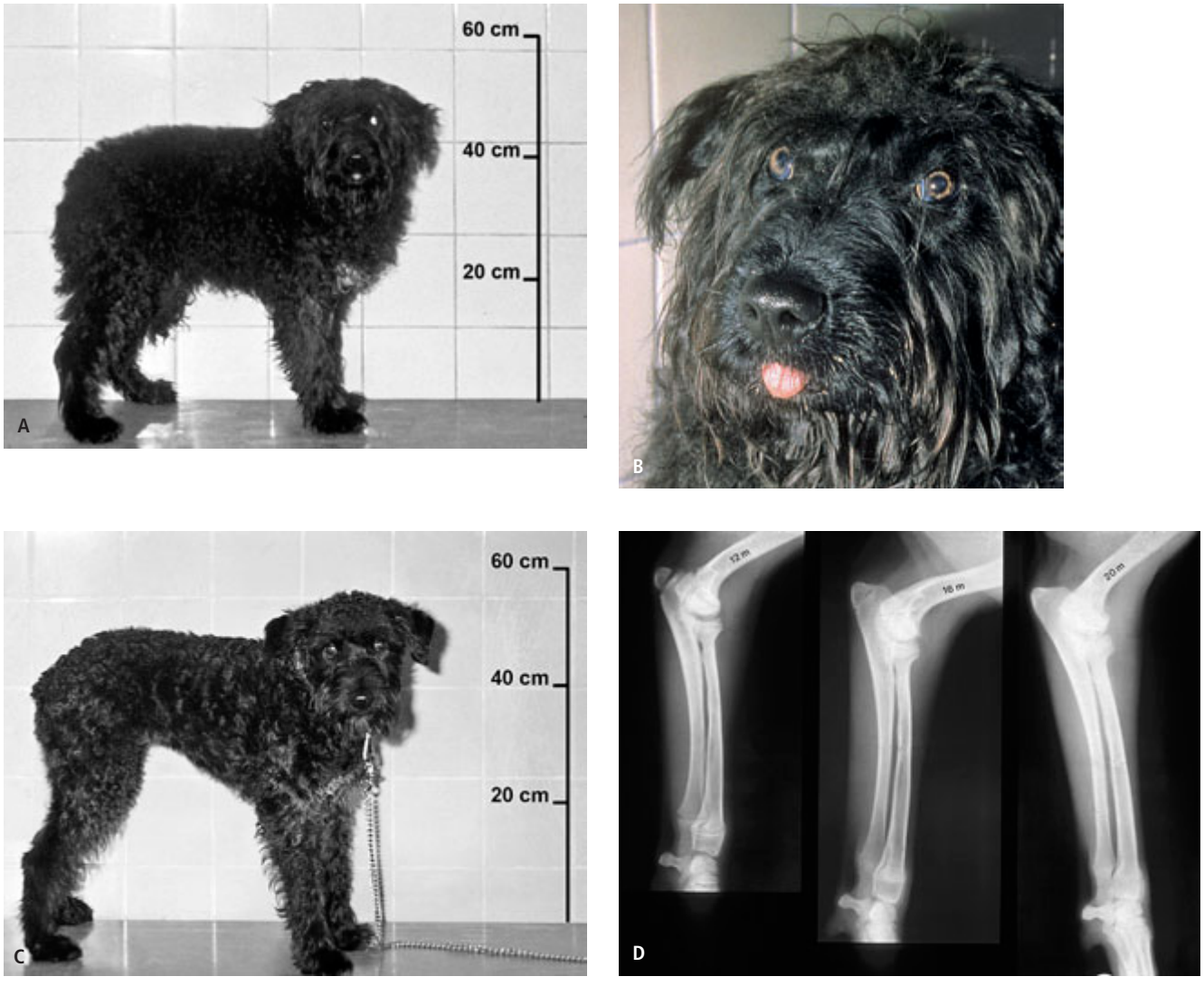


Figure 3.7:
(A, B) A female Bouvier des Flandres presented at the age of one year for retarded growth and sluggishness. The dog was in good nutritional condition, but weighed only 13 kg. It had disproportionately short legs, a dull facial expression, and a large tongue. Radioiodine scintigraphy revealed complete athyreosis.
(C, D) The same dog after four months of oral substitution with l-thyroxine. Note the more alert expression and the growth in height. Probably related to the rapidly ensuing sexual maturation (the dog came into estrus after two months of treatment), the growth plates closed and there was no further growth in height. The age in months is indicated on each radiograph.

of autoimmune destruction of the thyroid glands occurs during adolescence and as a consequence the dog's growth can be retarded, in addition to its developing the signs of hypothyroidism of the adult.

3.2.2 Thyroid dysgenesis

Ectopia of thyroid tissue is common in the dog and is also known to occur in cats.[36,37] In most cases it is the result of the descent of primitive thyroid tissue together with the aortic sac during embryonic life. In about 50 % of adult dogs, accessory thyroid tissue can be found embedded in the fat on the intrapericardial aorta. Accessory thyroid tissue may also lie cranial to the thyroid glands as a remnant of the thyroglossal duct. It may be detected because it gives rise to a neoplasm (chapter 3.4.2) or it may be an incidental finding during scanning for other reasons (fig. 3.5). It may also be associated with the absence of normal thyroid glands and yet its function may be insufficient to prevent hypothyroidism (fig. 3.6). Complete athyreosis has also been found (fig. 3.7). The search for the etiology requires molecular studies of the genes involved in the differentiation, migration, and growth of the thyroid gland. In humans mutations have been found in the genes encoding transcription factors and the TSH receptor, although their involvement in the general population of patients with thyroid dysgenesis has been questioned.[38–40]



Figure 3.8:
Enlarged thyroid glands of an eleven-month-old male Pomeranian. The goitrous glands were first noticed when the dog was five months old. There was a defect in the organification of iodide in the thyroid. The animal was of about normal size but had a thin hair coat and retention of deciduous teeth after eruption of the permanent teeth.

Clinical manifestations

The manifestations of hypothyroidism due to thyroid dysgenesis vary according to the duration and severity of the disease before therapy is instituted. In complete athyreosis, symptoms are noticed during the second or third month of life, although some animals may not reach this age. Abnormalities in the newborn that may suggest hypothyroidism include a large fontanel (which should be closed at birth in dogs but not in cats), hypothermia, hypoactivity, suckling difficulties, and abdominal distension.

As the hypothyroid puppy or kitten grows older, its head becomes relatively large and broad, the facial features become puffy, and the tongue becomes broad and thick (fig. 3.7). Growth in height is slow and the affected animal engages in little physical activity in comparison with littermates. Mental development appears to be retarded. The coat may be thin and lacking guard hairs.[41] Deciduous teeth persist into adulthood, but are shed when treatment with thyroid hormone is given.[42]

Radiography of the spine and long bones reveals delayed skeletal maturation and abnormally short vertebral bodies that may even give rise to spinal cord compression. In the long bones the appearance of ossification centers is delayed and physeal growth is retarded. The epiphyseal dysgenesis may also be associated with scattered foci of ossification, giving the epiphyses a granular appearance.[28] When the disease remains unrecognized, the physes of the vertebral bodies and the long bones are still open at the age of three or four years.[43,44]

Diagnosis

Measurements of plasma T_4 concentration before and after stimulation with TSH (chapter 12.3.1) will confirm the diagnosis of primary hypothyroidism. Thyroid scintiscanning may reveal the cause to be ventral midline ectopia or complete athyreosis.

Treatment

As soon as the condition is diagnosed, treatment should be started with l-thyroxine (10 µg l-thyroxine per kg body weight, twice daily). The animal will become much more lively and will develop a normal hair coat. When hypothyroidism is not detected early enough during skeletal maturation, the additional growth may be marginal because administration of thyroxine will also lead to closure of the growth plates (fig. 3.7). The mental sluggishness disappears, however, and usually there is little evidence of persisting mental retardation, a dreaded complication of late detection of congenital hypothyroidism in children.

3.2.3 Defective thyroid hormone synthesis

Congenital hypothyroidism may also occur because of an enzyme deficiency that prevents synthesis of thyroid hormones. Such congenital defects are rare and although in principle any step in thyroid hormone synthesis may be affected, only unresponsiveness to TSH and defective peroxidase activity have been found thus far in the dog and the cat.[45–47] Of these the latter seems to be the least rare form. Animals with this so-called organification defect concentrate iodide in the thyroid but have limited ability to utilize the iodide in thyroid hormone synthesis. The disorder appears to be heterogeneous, for in some animals the defect is complete and no TPO activity can be demonstrated, while in others it is partial. In the latter case the defect may be due to an abnormal localization of the enzyme within the thyroid cell, i.e., insufficiently migrated to the plasma membrane.[48,49]

Recently the familial occurrence of congenital hypothyroidism with goiter due to an organification defect has been described in toy fox terriers and rat terriers. In the affected dogs of both breeds the same mutation was found in the gene encoding TPO. It is suggested that this mutation was crossed into rat terriers from toy fox terriers.[50,51] A DNA-based test has been developed for detection of carriers of this autosomal recessively inherited defect.[50]



Figure 3.9:
Two eight-week-old littermate kittens. In comparison with the healthy kitten (A), the hypothyroid kitten (B) has a more infantile appearance with its round head and small ears and also its blue irises, while those of the healthy kitten have changed to the yellow of adulthood. The thyroid glands could not be palpated. The hypothyroidism was caused by the lack of organification of iodide by the thyroids (fig. 3.10).

Clinical manifestations

The clinical hallmark of these defects is the combination of goiter and hypothyroidism (fig. 3.8). The severity of both the goiter and the hypothyroidism may vary considerably and it may also be difficult to palpate a goiter in a very young animal (fig. 3.9). The clinical features of the hypothyroidism do not differ from those in thyroid dysgenesis (chapter 3.2.2).

Diagnosis

The diagnosis of hypothyroidism can be confirmed by measuring the plasma T_4 concentration. When a goiter is detected, stimulation with TSH is redundant, as the goiter is already evidence of increased endogenous TSH secretion.

The diagnostic challenge is the elucidation of the defect in thyroid hormone synthesis that is causing the increased TSH secretion. This requires in vivo studies with radioiodine. If there is an organification defect, the uptake of radioiodide by the thyroid is elevated but the iodide is not organically bound, as is readily demonstrated by the precipitous discharge of the accumulated radioactivity from the thyroids when an ion that competes for uptake, such as perchlorate or thiocyanate, is given (fig. 3.10).

Treatment

As in all forms of hypothyroidism except that caused by iodine deficiency, treatment consists of oral administration of l-thyroxine (chapter 3.2.1). This will lower TSH secretion and as a result the goiter will shrink.

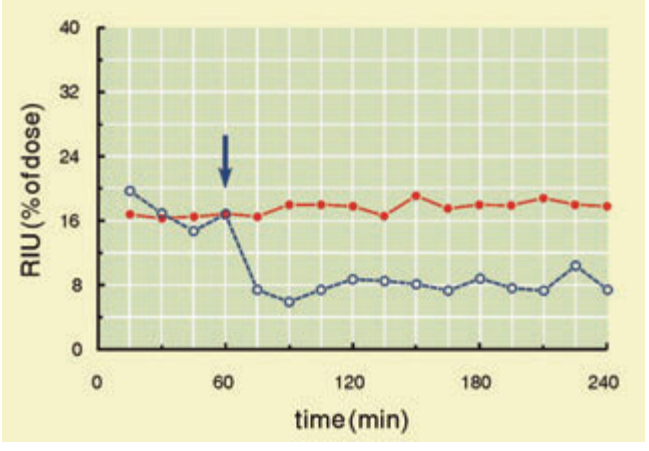


Figure 3.10:
Measurements of thyroidal radioiodide uptake (RIU) at 15-min intervals (red line) in a cat with defective organification. The iodide accumulated very rapidly in the thyroid and remained at a constant level of about 17 % of the administered dose, due to release and rapid reuptake. The latter was demonstrated in a repeat test (blue line) by intravenous administration of the competing ion perchlorate (arrow), which caused an abrupt discharge of radioactivity.

3.2.4 Central hypothyroidism

Central hypothyroidism is due to TSH deficiency. This can be classified as pituitary (secondary hypothyroidism) or hypothalamic (tertiary hypothyroidism), but the distinction is not necessary in the initial differentiation between primary and central hypothyroidism. Hyposecretion of TSH is usually accompanied by decreased secretion of other pituitary hormones. The best known example of secondary hypothyroidism at an early age is that of pituitary dwarfism in German Shepherd puppies that is characterized by combined deficiency of adenohypophyseal hormones. In these animals the TSH deficiency is associated with absolute deficiencies of growth hormone and prolactin, while secretion of luteinizing hormone and follicle-stimulating hormone is less severely impaired (chapter 2.2.2).[52,53] In these dogs manifestations of hypothyroidism are overshadowed by those of growth hormone deficiency, in part because a small but significant fraction of thyroid gland function (~10–15 %) is independent of TSH; hypothyroidism due to central causes is less severe than primary hypothyroidism.[54]

Isolated TSH deficiency (monotropic deficiency) has been suggested as the most likely abnormality in a family of giant schnauzers with dwarfism.[55] It has been questioned whether the presumed TSH deficiency was secondary or tertiary.[56] In a young boxer with congenital hypothyroidism supposedly of central origin, plasma growth hormone concentration was elevated,[57] but this is now known to be associated with primary hypothyroidism (chapter 3.3.1).

3.3 Hypothyroidism in adult animals

Hypothyroidism is the clinical syndrome resulting from deficient production of thyroid hormone. In about 95 % of cases of adult onset it is a primary thyroid disorder and in 5 % or less it is due to TSH deficiency (pituitary or hypothalamic).

3.3.1 Primary hypothyroidism

Pathogenesis

In the spontaneous form a progressive autoimmune process leads to lymphocytic infiltration and disappearance of thyroid follicles. So-called idiopathic forms, in which there is thyroid atrophy without inflammatory infiltrate, are also thought to be the end result of an autoimmune disorder.[58] The immunologic and molecular pathogenesis of autoimmune thyroiditis has not yet been elucidated. It has been reported that the development of canine hypothyroidism is associated with loss of self-tolerance in lymphocytes ($CD4^+$ T cells) to Tg,[59] but it is not clear whether this is cause or effect. In studies of the possible involvement of dog leukocyte antigen (DLA), an association was found in several breeds between canine hypothyroidism and a DLA-allele.[60,61] Antibodies against TPO seem to play little or no role in thyroiditis in dogs,[58,62] in contrast to thyroiditis in humans. The immune-mediated destruction is a slow process and clinical manifestations of thyroid hormone deficiency only become evident after destruction of > 75 % of the thyroid follicles.

Although they may not be of great pathogenetic importance, autoantibodies against Tg may serve as markers of autoimmune thyroiditis.[63] Circulating antibodies against Tg are detected in over 50 % of hypothyroid dogs. As the autoimmune destruction progresses, thyroid follicles are replaced by fibrous and adipose tissue and the inflammatory cells disappear, resulting in the histological appearance of noninflammatory atrophy. The absence of inflammation is likely to result in the disappearance of antibodies from the circulation over time.[58]

Antibodies against Tg form a heterogeneous group directed at several epitopes. When an epitope includes a hormonogenic site, an antibody can be directed against a fragment that contains T_4 or T_3. These Tg antibodies occasionally interfere with immunoassays used to measure the plasma concentrations of thyroid hormones, especially T_3. Depending on the type of assay, antibodies recognizing epitopes of a thyroid hormone may cause either falsely elevated or lowered values. Although antibodies against thyroid hormones are not uncommon, it should be noted that they rarely affect the results of the immunoassays to the extent that the reference range is exceeded.[58] This is especially true for T_4.

The immunologic damage may also involve one or more other endocrine glands and lead to multiple endocrine deficiencies, known as the polyglandular failure syndrome. The combination of hypothyroidism and hypoadrenocorticism is known as Schmidt's syndrome.[64,65] In a large retrospective study of dogs with primary hypoadrenocorticism, about 5 % had concurrent endocrine gland failure, hypothyroidism being the most frequent and diabetes mellitus and hypoparathyroidism occurring less frequently.[66]

Hypothyroidism can also be iatrogenic, especially in cats treated for hyperthyroidism, which occurs frequently in this species (chapter 3.4.1). The hypothyroidism may be an adverse effect of radioiodine therapy or bilateral surgical thyroidectomy. Hypothyroidism has also been reported in a dog following external radiation therapy for a functional thyroid carcinoma.[67]

Clinical manifestations

Thyroiditis usually remains unnoticed, although very rarely transient signs of hyperthyroidism (mainly characterized by polyuria) have been observed. This is probably due to release of thyroid hormone into the circulation during an acute phase of destructive thyroiditis. Eventually most dogs with thyroiditis probably develop signs of thyroid hormone deficiency.

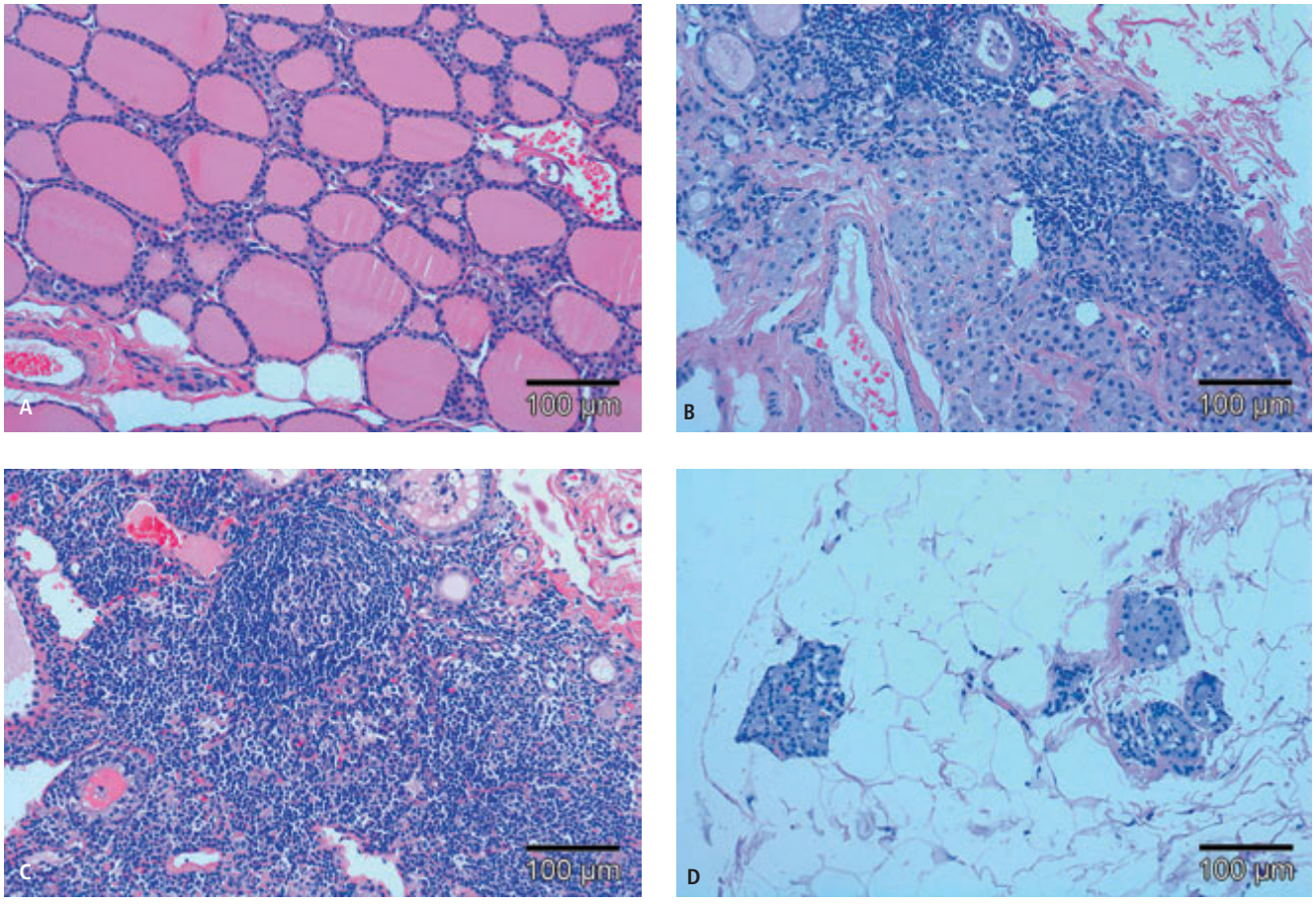


Figure 3.11:
HE-stained sections of the thyroid gland of a healthy dog (A), and of thyroid biopsies (B–D) from dogs with primary hypothyroidism in different stages of loss of thyroid epithelium:
(A) Thyroid follicles lined by low cuboidal epithelial cells and filled with colloid. Small groups of pale C cells lie between the follicles.
(B) Thyroid follicles with high cuboidal epithelium and almost no colloid. Diffuse, slight to moderate lymphocytic infiltration.
(C) Severe lymphocytic infiltration and loss of follicles. A few follicles of different sizes can still be recognized, often containing lymphocytes.
(D) Adipose tissue with small clusters of thyroid follicular cells and small aggregates of C cells.

Acquired primary hypothyroidism is mainly a condition of young-adult and middle-aged dogs. Although dogs of large breeds may be affected more frequently than those of small breeds, there is no pronounced breed predisposition. The incidence is equally distributed between males and females.[68] There has been only one convincing description of spontaneous primary hypothyroidism in an adult cat, a five-year-old spayed female domestic shorthair cat.[69]

Thyroid hormones influence the function of almost all tissues of the body and thus the classical clinical picture of overt hypothyroidism involves manifestations from nearly all organ systems. There may be concurrent effects of growth hormone excess (see chapter 2 and the section on diagnosis below).[70] The time required for clinically appreciable effects differs considerably: lethargy may be noticed within a few months but skin changes can take almost a year.[71]

Central to the clinical manifestations is usually a history of slowing of mental and physical activities. Most hypothyroid dogs have some degree of mental dullness, lethargy, and disinclination to exercise (fig. 3.12). These symptoms are gradual in onset, often subtle, and sometimes unrecognized by the owner until after treatment has been started. Among the observable changes in the hair and skin are alopecia (often with pigmentation), thick folding of the skin, and a puffy facial appearance. The thickening and puffiness are evidence of cutaneous mucinosis or myxedema, which is accumulation in the dermis of glycosaminoglycans and hyaluronic acid with associated edema.[72] It may be due to both hypothyroidism and growth hormone excess (figs. 3.12–3.16).[70,73] Occasionally, hypothyroidism is associated with secondary skin infections, including Malassezia infections.[74,75]



Figure 3.12:
A four-year-old male mongrel shepherd dog with primary hypothyroidism. The dog's lethargic appearance is quite apparent. In addition, its coat is thin and there is alopecia and pigmentation of the skin of the flanks, groin, and nose.



Figure 3.13:
A four-year-old male boxer with primary hypothyroidism. The skin was thick and inelastic, most noticeably in the thick folds on the shoulders and lower parts of the forelegs, and above the eyes. The latter together with drooping of the upper eyelids gave the dog a somewhat tragic facial expression. The stiff gait had caused abnormal wearing of the nails of the front feet.

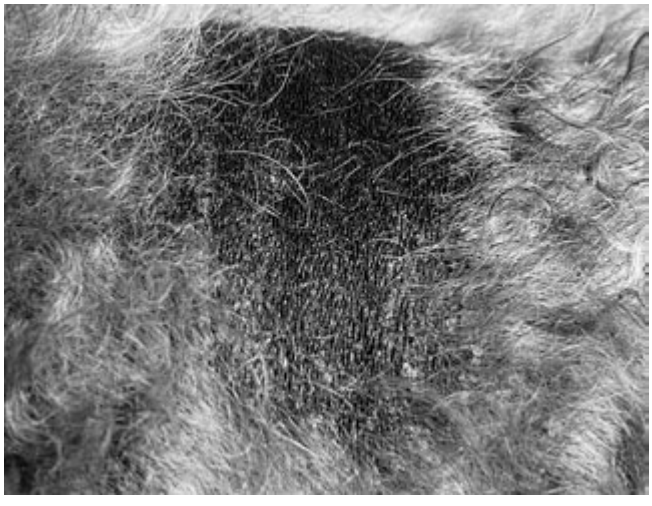


Figure 3.14:
Skin of a six-year-old female poodle with primary hypothyroidism, showing dark pigmentation and a somewhat roughened surface resembling emery paper.

Table 3.1: Clinical manifestations of primary hypothyroidism in adult dogs

System	Common	Less common or rare
Metabolism	Weight gain Appetite unchanged or reduced Cold intolerance	Low body temperature
Skin and Hair	Coat coarse and scanty Nonpruritic truncal alopecia starting over points of wear Mucopolysaccharide thickening of skin (myxedema)	Hyperpigmentation Secondary pyoderma Seborrhea
Cardiovascular	Bradycardia, weak peripheral pulse and apex beat Low voltage ECG (fig. 3.17)	Poor peripheral circulation Cool skin
Reproductive and Endocrine	Persistent anestrus Loss of libido Testicular atrophy	Gynecomastia Galactorrhea Polyglandular deficiency (Schmidt's syndrome)
Neuromuscular	Lethargy and somnolence Stiff gait	Vestibular ataxia Head tilt (fig. 3.18) Facial nerve paralysis Lameness
Gastrointestinal		Diarrhea
Hematological	Nonregenerative anemia	
Biochemical	Hypercholesterolemia Hypertriglyceridemia Mild hyperglycemia	Elevated creatinine kinase Hyponatremia Hyperkalemia



Figure 3.15:
(A) A four-year-old female German shepherd with primary hypothyroidism. The puffy appearance due to myxedema produces a lethargic or tragic facial expression. The blepharoptosis contributes to this appearance.
(B) These changes were especially appreciated in retrospect, when the dog was reexamined after four months of substitution therapy with l-thyroxine.



Figure 3.16:
(A) A two-year-old female Leonberger in which primary hypothyroidism caused marked loss of hair, leaving a sparse, coarse, and short coat.
(B) There was an impressive regrowth of hair after seven months of substitution therapy with l-thyroxine.

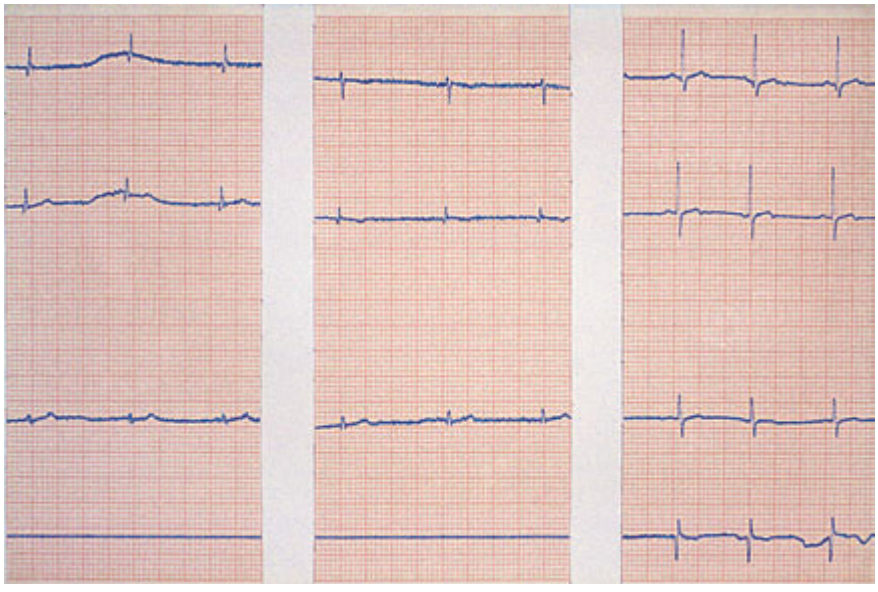


Figure 3.17:
ECG recording from a four-year-old male boxer with pronounced hypothyroidism (calibration: 1 cm = 1 mV; paper speed 25 mm/s). Left: Leads I, II, and III. Middle: Leads aVR, a VL, and aVF. Right precordial leads CV_6LU, CV_6LL, CV_6RL, and V_{10}. There is low voltage of the deflections in all leads. In less pronounced (= less long-standing) cases the ECG changes may be less remarkable or even absent.



Figure 3.18:
A five-year-old female boxer with primary hypothyroidism and signs of vestibular disease manifested by a head tilt. There was also facial nerve palsy. These features are regarded as manifestations of a more generalized polyneuropathy,[78,79] with hyperlipidemia as a serious predisposing factor.[85]

Table 3.1 lists the clinical manifestations by organ system, of which some changes in the cardiovascular and nervous systems are illustrated in figs. 3.17 and 3.18. Changes in a single organ system sometimes dominate to the extent of obscuring the causative disease.[76] This can occur with persisting galactorrhea,[77] vestibular disease,[78,79] and locomotor disturbances. With regard to the latter, generalized locomotor problems can be explained by a severe reduction in Na^+-K^+-ATPase capacity in the skeletal muscles,[27] while hypothyroidism has also been reported to be associated with generalized myopathy.[80,81] Rarely, a hypothyroid dog is presented as an emergency in a comatose state. Low ambient temperatures can cause decomposition of hypothyroidism into myxedema coma with severe hypothermia.[82,83]

Routine laboratory examinations can reveal several hematological and biochemical abnormalities (table 3.1). Possible consequences of severe hyperlipidemia include neurological signs due to atherosclerosis and thromboembolic events.[84,85] Both the nonregenerative anemia (see also chapter 10.3) and the hyperglycemia are usually mild.

Differential diagnosis

Because the presenting symptoms of hypothyroidism can vary widely, a common pitfall in diagnosis is simply to overlook the possibility that the presented problems could be due to hypothyroidism. For example, it is not uncommon for dogs with hypothyroidism to be presented for attention to cardiopulmonology (lethargy misinterpreted as exercise intolerance) or orthopedics (locomotor disturbance). Lethargy, the most common sign of hypothyroidism, may be mistaken for metabolic (hepatoencephalopathy) or cerebrocortical disease (encephalitis, hydrocephalus). The atrophy of the skin and its adnexa must take into consideration such conditions as estrogen excess (chapter 8.4) and hypercortisolism (chapter 4.3).

Diagnosis

As a measure of thyroid function, T_4 has to be preferred over T_3 because it is produced exclusively by the thyroid gland while T_3 in plasma is largely derived by peripheral conversion (chapter 3.1). In most dogs with primary hypothyroidism, plasma concentrations of TT_4 and free T_4 (fT_4) are below the reference range. However, they can also be decreased in dogs without a thyroid disorder because of drugs or illness (chapter 3.1.2). The terms nonthyroidal illness and sick euthyroid syndrome have been introduced for this derangement of thyroid homeostasis. Illness in this context comprises virtually all nonthyroidal diseases, surgical and nonsurgical trauma, and inadequate caloric intake. Consequently, the finding of a low basal plasma thyroid hormone concentration is of little diagnostic value.[86,87] For this reason stimulation tests using either TSH or TRH have been advocated. The TRH-stimulation test using measurement of plasma TT_4 concentration does not distinguish with sufficient accuracy between

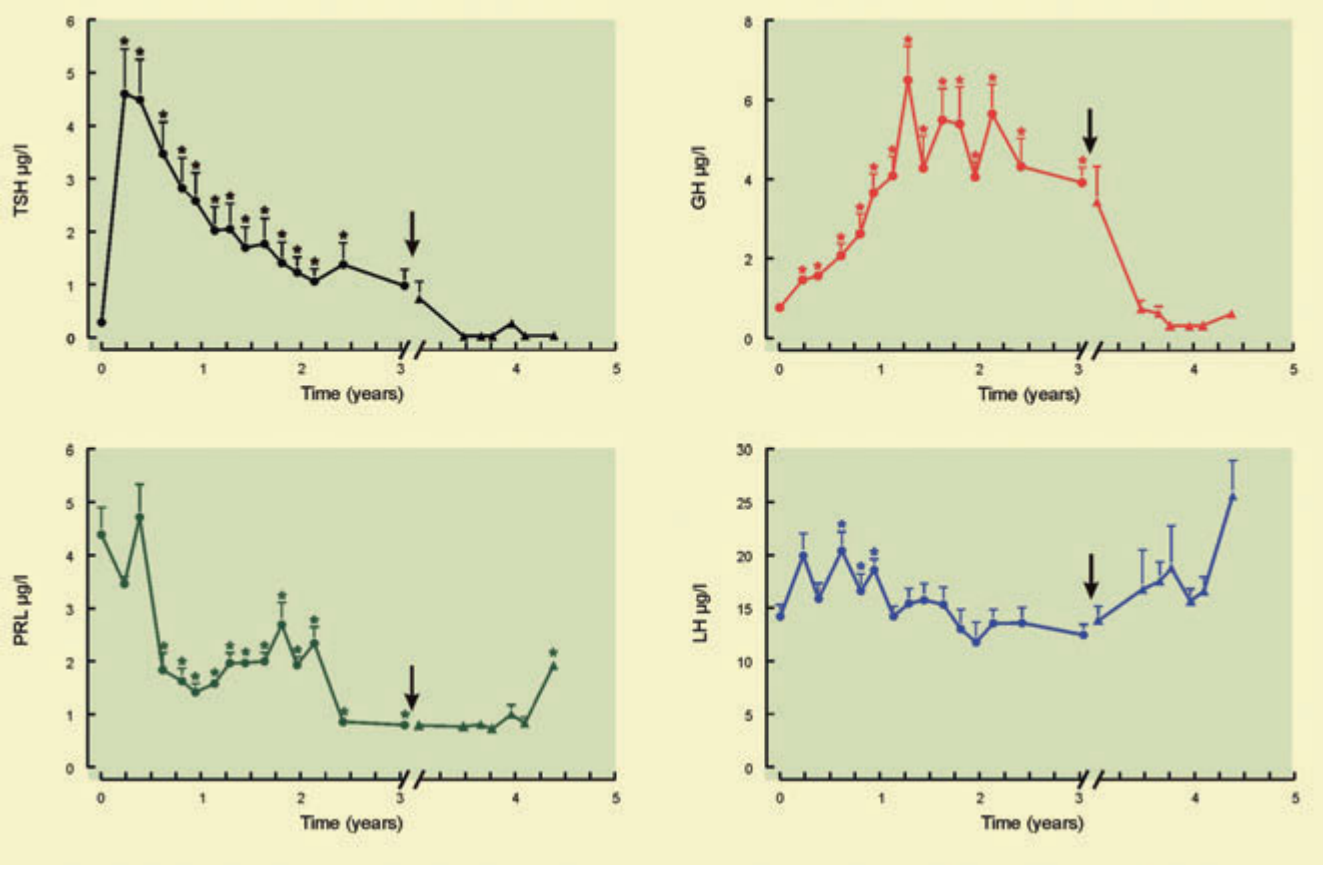


Figure 3.19:
Mean (± SEM) basal plasma concentrations of TSH, GH, PRL, and LH measured at two-month intervals in seven spayed beagles with induced hypothyroidism at time point 0. Three of these dogs were followed up for 1.5 years while receiving l-thyroxine substitution (beginning of substitution marked by arrow). Asterisks indicate statistically significant difference from value at time zero.[97]

dogs with hypothyroidism and those with nonthyroidal illness.[88] Until the end of the last century, primary hypothyroidism in dogs was diagnosed by the finding of a low plasma TT_4 (and/or fT_4) concentration insufficiently responsive to stimulation with bovine TSH (bTSH).[89,90]

It was expected that introduction of a homologous immunoassays for plasma TSH in dogs would greatly aid and simplify assessment of the canine pituitary-thyroid axis by the paired measurement of T_4 and TSH. It was hoped that a single blood sample would suffice to confirm the diagnosis of primary hypothyroidism by revealing a low T_4 concentration in the presence of a high TSH concentration. However, using the TSH-stimulation test as the gold standard, it was found that in as many as one-third of dogs with primary hypothyroidism, plasma TSH concentration was not elevated.[86,87,91] Frustration with the limitations of the available endogenous canine TSH assay caused most clinicians to resume using the TSH-stimulation test,[92] albeit now usually employing recombinant human (rh)TSH instead of bTSH.[93–95] Meanwhile, the gold-standard status of the TSH-stimulation test has been questioned.[96]

Strategies for modification of the TSH assay have been suggested to improve the diagnostic value of TSH measurements.[92] However, there is now experimental evidence that it may not be so much the assay but rather the changes in pituitary function with time that can explain the low TSH values found in some dogs with primary hypothyroidism. As illustrated in fig. 3.19, the induction of primary hypothyroidism causes an initial increase in plasma TSH concentration but this is followed by a gradual loss of the feedback response of TSH to low plasma T_4 concentrations. This is accompanied by hypersecretion of GH and hyposecretion of PRL. The associated pituitary enlargement is characterized by thyrotrope hyperplasia, large vacuolated thyroid deficiency cells,

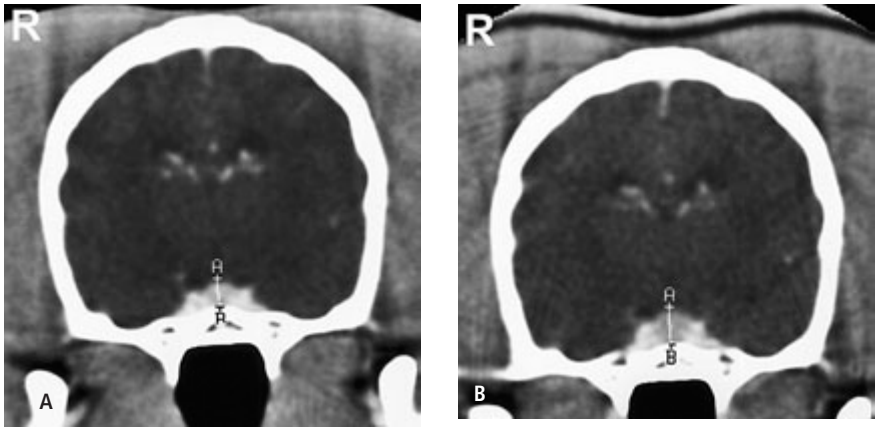


Figure 3.20:
Transverse CT images of the skull of a beagle prior to induction of hypothyroidism (A), and three years after thyroidectomy (B). Contrast enhancement reveals the normal size of the pituitary gland before thyroidectomy and its enlargement after the induction of hypothyroidism.

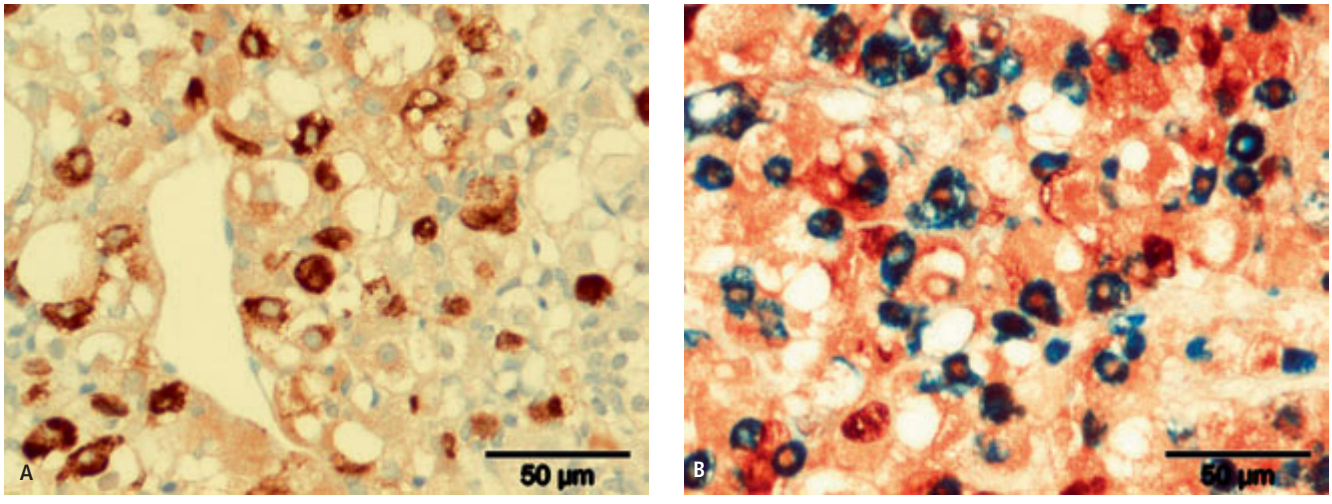


Figure 3.21:
Sections of the pituitary gland of a hypothyroid dog: (A) stained with an antibody against GH (brown) and (B) with antibodies for both GH (blue) and TSH (orange). Both large and normal-sized cells stain positively for GH. Several cells are positive for both GH and TSH.

and double-staining cells, indicative of transdifferentiation (figs. 3.20 and 3.21). The latter is associated with the development of thyrosomatotropic cells and paradoxical GH-responsiveness to stimulation with TRH.[97,98] The pituitary enlargement and the functional changes are found to be reversible by substitution with l-thyroxine.[97] Similar changes are observed in dogs with spontaneous hypothyroidism, with the omission of hypoprolactinemia in intact males and females. On the contrary, plasma prolactin concentration may be elevated in intact females that have recently entered an estrous cycle and the hypothyroidism may even be associated with galactorrhea.[99]

The results of these studies on the adenohypophyseal changes in primary hypothyroidism provide an explanation for the low plasma TSH concentrations that have been observed but do not resolve the diagnostic dilemma. In dogs with clinical signs of hypothyroidism, the combination of a low plasma TT_4 and an elevated plasma TSH concentration is diagnostic for primary hypothyroidism. When TT_4 is low but TSH is within the reference range, a TSH-stimulation test can be performed (chapter 12.3.1), although the result may not be conclusive.[96] If they are not, methods not involving biochemical assessment of the pituitary-thyroid axis – such as a radionuclide scan or thyroid uptake measurement with $^{99m}TcO_4^-$, high-resolution ultrasonography, or even a thyroid biopsy – seem to be the most reliable for diagnosing primary hypothyroidism in dogs.[96,100] In a study of $^{99m}TcO_4^-$ uptake in dogs with primary hypothyroidism and nonthyroidal illness, there was no overlap in thyroid uptake at 45–120 min after injection (fig. 3.22).[96] In high-resolution ultrasonography of the thyroid glands, loss of echogenicity, homogeneity, and fusiform shape are particularly characteristic of primary hypothyroidism.[101,102] Demonstration of circulating antibodies to Tg indicates the presence of thyroiditis but provides no information about thyroid function. As indicated in the section on pathogenesis, the absence of antibodies against Tg does not exclude hypothyroidism. In addition, dogs with anti-

bodies against Tg may have thyroiditis that has not yet resulted in hypothyroidism.

Treatment

Although T_3 is the metabolically active thyroid hormone, it is not the supplement of choice. A primary advantage of providing the »prohormone« T_4 is that the body is given the opportunity to regulate the amount of T_3 generated by normal physiologic mechanisms. Appropriate T_4 therapy results in normal levels of both T_4 and T_3.

Both T_4 production rates and parenteral l-T_4 replacement doses required to maintain euthyroidism are around 5 µg per kg body weight per day.[103] However, when T_4 is administered orally its bioavailability is low and variable, due to incomplete and variable gastrointestinal absorption. Most commonly oral supplementation with tablets of synthetic l-thyroxine is started at a dose rate of 10 µg/kg twice daily. Plasma T_4 concentration increases following oral administration, peaks around 4–6 h later, and then declines until the following dose is given.[104] A follow-up examination is made after two months. When blood is collected at 10–12 h after the last dose, plasma T_4 concentration should be above the lower limit of the reference range for the type of dog (chapter 3.1.2). If it is not, the dose should be adjusted. Because of the individual variation in intestinal absorption of T_4, further follow-up examinations and adjustments may be needed.

A recently introduced solution of l-T_4 for oral administration has a higher bioavailability than the tablet formulation, especially when the solution is given without food.[105] According to the authors, the pharmacokinetic properties of liquid l-T_4 support the use of a dose of 20 µg/kg once daily.

Prognosis

Hypothyroidism is one of the most gratifying diseases to treat, because of the ease and completeness with which it responds to treatment. With appropriate treatment and follow-up examinations every half year, usually all of the alterations associated with hypothyroidism are reversible. The long-term prognosis is excellent.

3.3.2 Central hypothyroidism

(see also chapter 3.2.4)

In central hypothyroidism the thyroids are not affected primarily but are deprived of stimulation by TSH. Histological examination reveals no loss of follicles but rather the characteristics of inactivity (fig. 3.23). The condition is rare compared with primary thyroid failure. Spontaneous causes include tumor of the pituitary or adjacent regions and head trauma.[106] Tertiary hypothyroidism has been documented in a dog with a large pituitary tumor and effacement of the overlying hypothalamus.[107] Central hypothyroidism can also result from surgical removal of a pituitary tumor.[108]

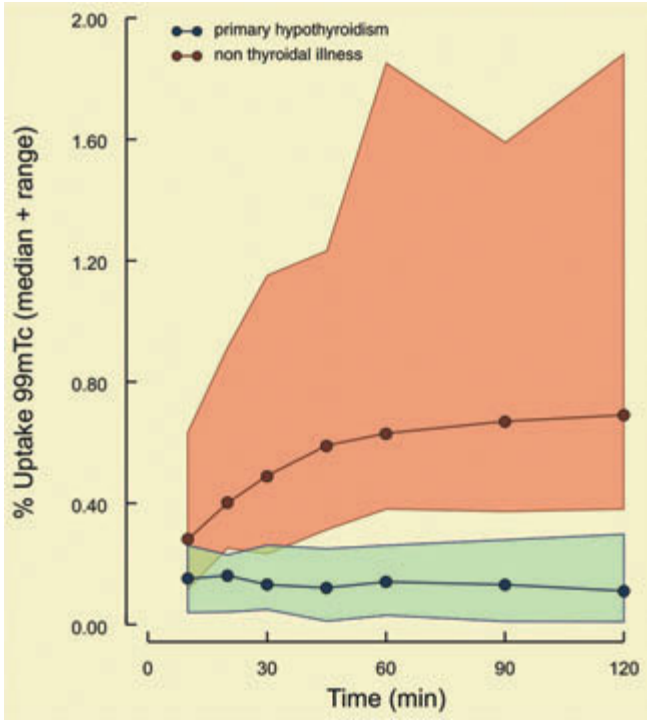


Figure 3.22:
Median values and ranges for thyroidal uptake of $^{99m}TcO_4^-$, expressed as percent uptake of the injected dose, in 14 dogs with primary hypothyroidism (green) and 13 with nonthyroidal illness (orange).

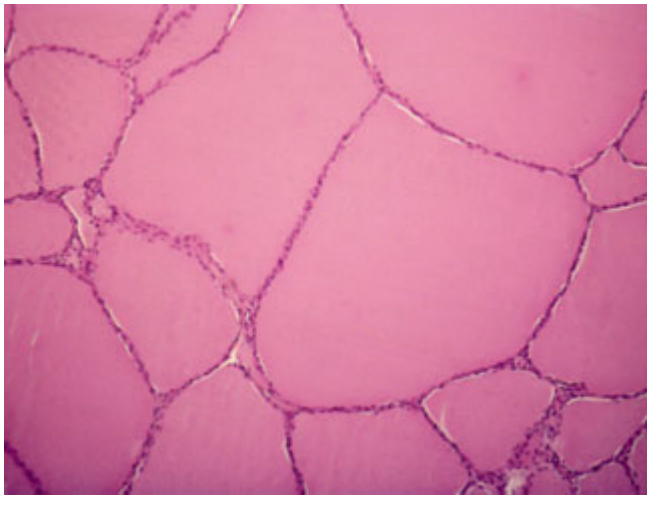


Figure 3.23:
H&E-stained section of the thyroid of a nine-year-old long-haired German pointer with secondary hypothyroidism. Note the large follicles and the flat, inactive epithelium (compare with fig. 3.1).

Clinical manifestations

The clinical picture is similar to that of primary hypothyroidism, although generally less pronounced. There may be lethargy and alopecia, but thickening of the skin is less pronounced (fig. 2.25). As described in the previous section, the thickening that occurs in primary hypothyroidism is partly a consequence of the associated growth hormone excess. In central hypothyroidism the persisting negative feedback on TSH secretion that is responsible for this is lacking. On the contrary, there is often impaired secretion of other pituitary hormones such as growth hormone and gonadotropins (fig. 2.25).

Not uncommonly, the lesion causing reduced TSH secretion is a hormone-secreting tumor, such as a corticotrope adenoma that is hypersecreting ACTH. The symptoms and signs arising from such a pituitary tumor may precede, accompany, and even obscure the manifestations of pituitary failure. In the presence of an ACTH-secreting tumor, central hypothyroidism may only become manifest after reversal of the associated hypercortisolism (chapter 4.3.1).

Diagnosis

The diagnosis of central hypothyroidism should be based on the demonstration of low concentrations of T_4 and TSH in plasma. In secondary hypothyroidism, plasma T_4 concentration increases in a TSH-stimulation test, although repeated stimulation may be necessary (chapter 12.3.1). A TRH-stimulation test can be used if there is reason to suspect tertiary hypothyroidism. A prerequisite for correct interpretation of these tests is the certainty that the low T_4 (and TSH) concentrations are not caused by illness or drugs.

In addition, diagnostic assessment should include (1) the secretion of other pituitary hormones (see also chapters 2.2.6 and 12.1), and (2) the morphology of the pituitary and adjacent areas by diagnostic imaging (chapter 2.2.6).

Treatment

Treatment with l-thyroxine is the same as in primary hypothyroidism (chapter 3.3.1). Hypofunction of any other endocrine glands resulting from pituitary hormone deficiencies should also be corrected. In practice this is usually confined to treatment for a coexisting ACTH deficiency. It is even advisable to assess pituitary-adrenocortical function and to treat an eventual deficiency by cortisol supplementation (chapter 4.2.2) before T_4 therapy is begun. This will avoid the risk of precipitating a crisis due to glucocorticoid deficiency.

Prognosis

In the spontaneous forms the prognosis is completely dependent upon the course of the causative lesion in the hypothalamus-pituitary area. In the iatrogenic form following hypophysectomy, supplementation with l-thyroxine (and glucocorticoids!) enables the animal to live a healthy life for many years (chapter 4.3.1).

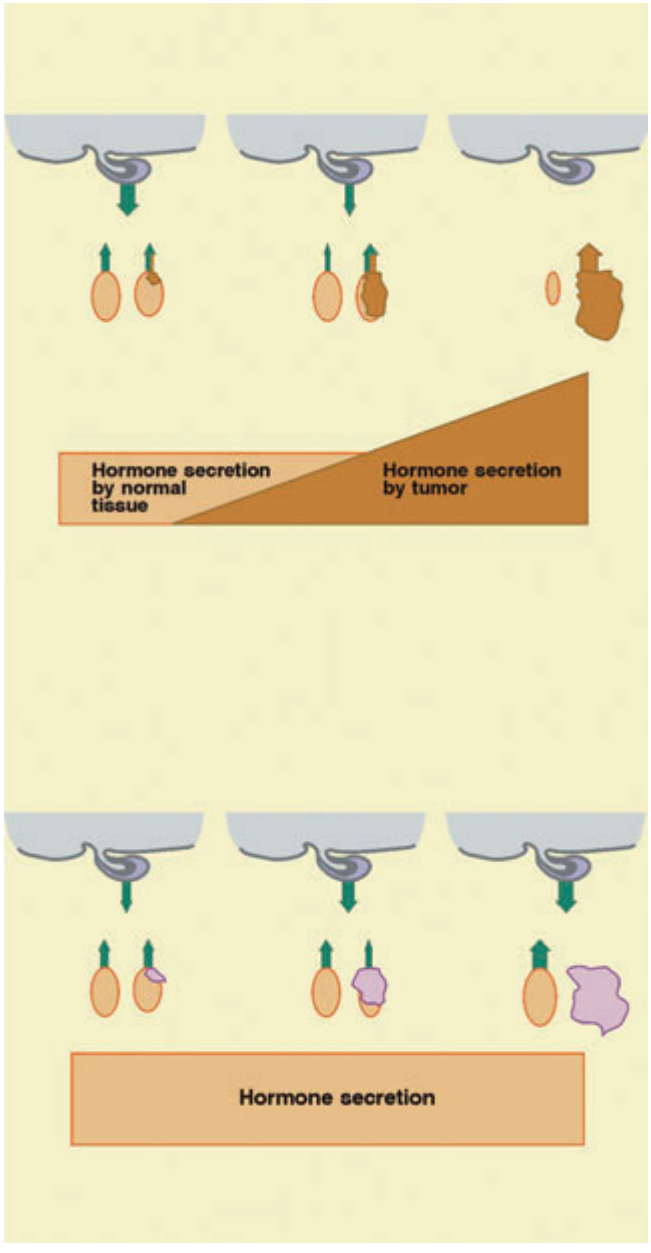


Figure 3.24:
Progression of a functional thyroid tumor to a state of hyperthyroidism (upper figure). As hypersecretion of thyroid hormone progresses, TSH release successively declines and the unaffected thyroid tissue becomes inactive. During development of a nonfunctional destructive thyroid tumor (lower figure), thyroid hormone secretion is sustained via the feedback-controlled increased secretion from the contralateral unaffected lobe.



Figure 3.25:
This twelve-year-old castrated male cat was presented for weight loss and extreme restlessness. Its nutritional condition was poor and its behavior was frantic (A). The hypermetabolic state caused panting (B), which the owner also observed when the cat was at rest.

3.4 Hyperthyroidism and thyroid tumors

Neoplastic transformation of the thyroid may come to attention in two ways. In dogs, most commonly it is the physical presence of the tumor that is first detected by the owner. However, if the tumor produces thyroid hormone, it may with increasing size produce such an excess (fig. 3.24) that the animal develops symptoms of hyperthyroidism. This is almost invariably the case in cats and is only occasionally seen in dogs.

A disease entity comparable to Graves' disease in humans, in which TSH-receptor antibodies stimulate the thyroid, has not been observed in dogs or cats. Because the clinical aspects of thyroid neoplasia differ considerably between dogs and cats, they are discussed separately in the following sections.

3.4.1 Hyperthyroidism in cats

Feline hyperthyroidism is a relatively common disease of middle-aged and elderly cats, with a mean age of twelve to 13 years. There is no breed or sex predilection. The thyroid hormone excess is produced by thyroid adenomatous hyperplasia or adenoma, involving one or, more often, both thyroid lobes. Thyroid carcinoma, which is the main cause of hyperthyroidism in dogs, accounts for only 3 % of cases in cats.[109]

The pathogenesis of adenomatous thyroid hyperplasia in cats is not clear. The condition resembles toxic nodular goiter (Plummer's disease) in humans. The thyroids of hyperthyroid cats contain multiple hyperplastic nodules surrounded by inactive follicular tissue. Experimental transplantation of the adenomatous tissue into nude mice has shown that its growth does not depend upon extrathyroidal humoral stimulation.[110] Instead, intrinsic cell abnormalities must be responsible for its unregulated growth and function.[111] The most likely candidates are thought to be mutation of the TSH receptor or mutation of its associated G proteins.[112,113]

Clinical manifestations

The adenomatous glands tend not to become very large, so rarely is veterinary help sought because of a mass detected by the owner. Thus it is the signs and symptoms due to effects of thyroid hormone excess on organ systems that lead to veterinary examination. The classic presentation of a hyperthyroid cat is that of a skinny, restless, elderly cat with an increased appetite and polyuria (fig. 3.25). It is likely to give the impression of a tense and anxious animal with an impaired tolerance for any stress, such as restraint.[114] Many organ systems can be affected and the associated signs and symptoms are listed in table 3.2. This full spectrum is less likely to be present now that the disease is usually recognized in an early stage. In an elderly cat, weight loss – often together with increased appetite – may be sufficient reason to suspect hyperthyroidism.

In about 10 % of cases the clinical picture may be quite different. In these cats weight loss remains an important feature, but there is lethargy and anorexia rather than hyperactivity and increased appetite. This form, called »apathetic hyperthyroidism«, may represent an end-stage of the disease and may

Table 3.2: Clinical manifestations of hyperthyroidism in cats

System	Common	Less common or rare
Metabolism	Weight loss in spite of polyphagia	Mild hyperthermia Anorexia
Respiratory	Panting	Dyspnea
Cardiovascular	Tachycardia (gallop rhythm) Pounding heart beat Left ventricular hypertrophy (echocardiography)	Cardiac murmur Cardiac arrhythmias Congestive heart failure
Neuromuscular	Restlessness (irritability)	Weakness Muscle wasting
Renal	Polyuria (low urine s.g.)	Mild elevation of plasma urea and creatinine concentrations*
Gastrointestinal	Increased fecal volume	Diarrhea and vomiting
Skin and Hair	Unkempt hair coat	
Hematological	Neutrophilic leukocytosis with eosinopenia and lymphopenia (= stress leukogram?)	Hematocrit elevated
Biochemical	Elevated plasma ALT, AP, LDH Urinary corticoid:creatinine ratio elevated Hypokalemia	Mild hyperphosphatemia*

* May be found, but probably not a direct manifestation of hyperthyroidism.

also be associated with cardiac disorders (see also table 3.2). This severe form of feline hyperthyroidism has also been called »thyroid storm«, a term used for a rare clinical entity in humans. Radioactive iodine therapy, thyroid surgery, vigorous thyroid palpation, and stress may cause acute elevation of plasma thyroid hormone concentration and have been implicated as possible precipitating factors for »thyroid storm«.[115] A wide range of clinical features has been associated with this form of the disease, including arterial hypertension and hypokalemic myopathy. It is not clear whether in these cases possible coexisting conditions such as hyperaldosteronism (chapter 4.4) may play a role.

The multisystemic effects of thyroid hormone excess not only lead to a variety of physical changes but may also give rise to several biochemical abnormalities (table 3.2). Most of these are reversed with treatment, including elevated plasma concentrations of liver enzymes and increased urinary corticoid:creatinine ratios.[116,117] The hemodynamic alterations of hyperthyroidism are responsible for marked increases in the glomerular filtration rate. The often observed mild proteinuria is regarded as a reflection of glomerular hypertension and hyperfiltration, and is also resolved with treatment.[118] Of more concern has been the increase in the plasma creatinine concentration after treatment of hyperthyroidism, although it is often still within the reference range. Although considered to be the unmasking of preexisting chronic kidney disease, it seems to have little clinical significance. The survival of treated hyperthyroid cats does not seem to be affected by post-treatment azotemia.[119] Studies of calcium homeostasis in feline hyperthyroidism have revealed several alterations.[120] Although these abnormalities have not been associated with any symptom or sign, there has been one report of a hyperthyroid cat with hyperphosphatemia and calcification of its paws that resolved with return to the euthyroid state.[121] Consistent with the effect of thyroid hormone on Na^+-K^+-ATPase (chapter 3.1.4), hypokalemia may be found, whereby the possibility of coexisting hyperaldosteronism (chapter 4.4) should be considered.

Differential diagnosis

There are at least two nonthyroidal disorders that may simulate certain aspects of the syndrome. First, the weight loss in combination with increased appetite and large volumes of somewhat fatty feces may be mistaken for pancreatic insufficiency and less likely for gastrointestinal lymphoma, as in the latter case there will be inappetence. Weight loss in spite of increased appetite together with polyuria also raises the possibility of diabetes mellitus, but routine urinalysis will immediately resolve this.

Diagnosis

When hyperthyroidism is suspected, the first step should be a careful palpation of the neck area by gently sliding the thumb and index finger along the sides of the trachea. The thyroids are only loosely attached to the surrounding tissues and therefore enlargement usually causes descent along the trachea, sometimes even as far as the thoracic inlet. The thyroids are usually easily moved along the trachea. Enlargement of one or both lobes can be found by an experienced examiner in up to 90 % of cats with hyperthyroidism. However, it should be noted that occasionally thyroid enlargement is found without hyperthyroidism. In such cases the disease may develop with time. Rarely the thyroid enlargement arises from ectopic (sometimes intrathoracic) thyroid tissue.

The final diagnosis ought to rest on a direct measurement of thyroid function. For reasons explained above (chapters 3.1, 3.3.1), measurement of the plasma concentration of T_4 is of greater diagnostic value than that of T_3. In about 90 % of cats presented with the syndrome of hyperthyroidism, the T_4 concentration in plasma exceeds the upper limit of the reference range. Plasma T_4 concentration fluctuates over time and in cats with mild hyperthyroidism, T_4 values may be in the high-normal range. In addition, concomitant nonthyroidal disease may lower the value below the reference range.[122] When plasma T_4 concentration falls within the reference range and the animal is still suspected of hyperthyroidism, the measurement of T_4 can be repeated two to four weeks later.

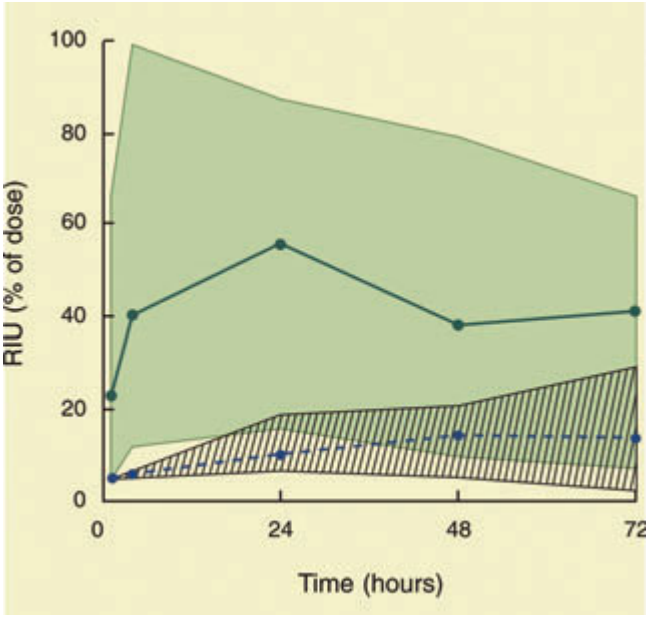


Figure 3.26:
Thyroidal radioiodine uptake (RIU) (median and range) in 20 hyperthyroid cats (green) and ten healthy house cats (hatched).[124]

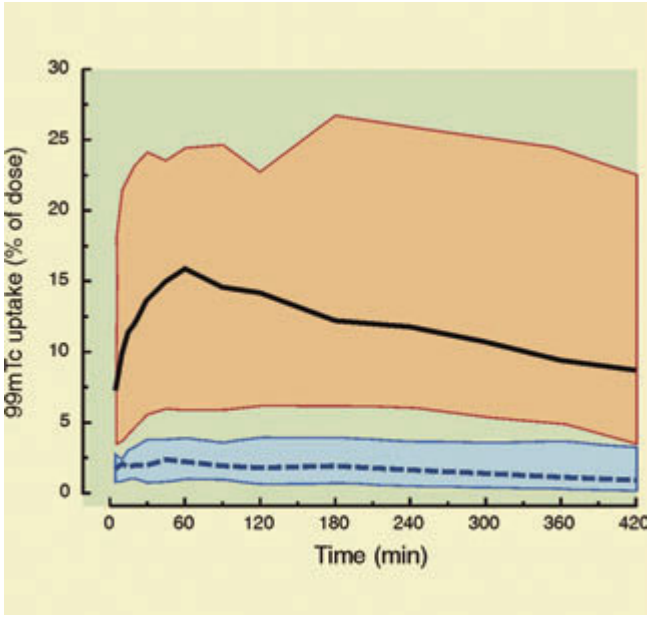


Figure 3.27:
Thyroidal $^{99m}TcO_4^-$ uptake (median and range) in 18 hyperthyroid cats (beige) and 13 healthy house cats (blue).[125]

In most cases measurement of fT_4 concentration by direct equilibrium dialysis adds little or no useful diagnostic information. Nonthyroidal disease may be associated with false positive results and therefore feline hyperthyroidism should not be diagnosed solely on the finding of a high fT_4 concentration.[122] Recently it was reported that cats with hyperthyroidism have plasma TSH concentrations (measured with an assay for canine TSH [Immulite canine TSH®, Diagnostic Products Corporation, DPC, Los Angeles, CA, USA]) below the limit of quantification (see also fig. 3.24). This offers an additional tool in the diagnostic approach to feline hyperthyroidism. Undetectable and low TSH concentrations have also been reported in cats with histological evidence of nodular thyroid disease, i.e., mild or subclinical hyperthyroidism.[123]

One can also consider testing the suppressibility of plasma T_4 concentration in a T_3-suppression test. Following seven eight-hourly oral doses of 15–25 µg T_3, the T_4 concentration in healthy cats is suppressed to low values. Due to the autonomous (TSH-independent) character of T_4 hypersecretion in hyperthyroid cats, T_4 concentration 2–4 h after the last dose of T_3 remains practically unchanged.[124]

Although not available in all clinics, radioiodine uptake studies with ^{131}I or ^{123}I may contribute to the diagnosis. In hyperthyroid cats there is rapid uptake of the tracer to higher values than in normal cats (fig. 3.26).[125] As explained in chapter 3.1 $^{99m}TcO_4^-$ is also taken up by the thyroid gland but not organically bound. Nevertheless, the measurement can be valuable because it is usually higher than in healthy cats (fig. 3.27).[126] The best correlation of $^{99m}TcO_4^-$ uptake with plasma T_4 concentration has been found to be the 20-min thyroid:salivary gland ratio (T:S ratio) using the more intense image of the two thyroid lobes.[127]

The uptake visualized in the head of the cat by routine thyroid scintigraphy is largely due to pertechnetate accumulation in the zygomatic and molar salivary glands. The uptake in the small molar glands may be superimposed over the zygomatic uptake on routine ventral planar images.[128] Different sedative-anesthetic protocols influence thyroid and salivary gland uptake of $^{99m}TcO_4^-$ in different ways.[129,130] Another factor complicating the interpretation of the T:S ratio may be recent antithyroid medication. Enhanced thyroidal $^{99m}TcO_4^-$ uptake has been found following withdrawal, although the T:S ratio was significantly elevated only at 4 h after tracer injection.[131]

In hyperthyroid cats, scintiscanning with $^{99m}TcO_4^-$ reveals increased uptake in hyperplastic thyroid tissue and no uptake in the unaffected tissue, because TSH secretion is suppressed by

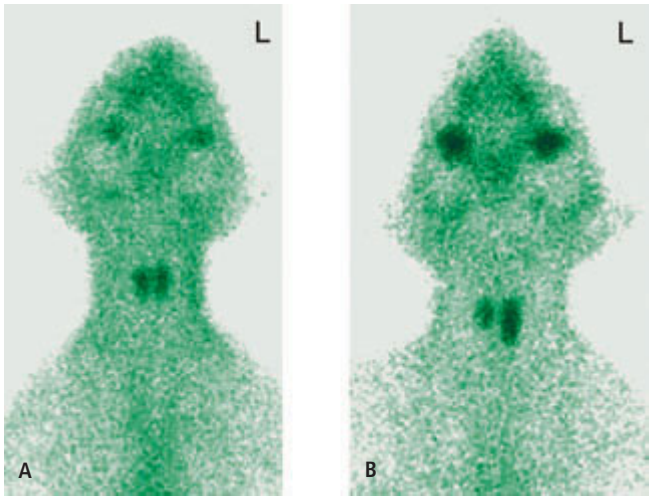


Figure 3.28:
Scintigraphic images 30 min after intravenous injection of 0.5–0.8 mCi (18.5–27.6 MBq) $^{99m}TcO_4^-$ in healthy cats (in dorsal recumbency).
(A) Symmetrical uptake in two normal thyroid lobes.
(B) Asymmetrical uptake in two normal lobes. In both images the focal uptake in the head is in salivary tissue.

the T_4 excess (figs. 3.24, 3.28, 3.29). Thyroid scintiscanning is especially useful in hyperthyroid cats in which no thyroid enlargement can be palpated, to determine whether one or both thyroid lobes are affected and whether ectopic hyperfunctioning thyroid tissue (EHTT) is present. The technique is also very useful in cases of recurrence of the disease following surgery (fig. 3.29), and when there is suspicion of distant metastases, although the latter is extremely rare. EHTT occurs in about 9 % of cases and has a significant effect on the rate of recurrence after surgery. Thyroid scintigraphy should be performed preoperatively in all cases.[109] Although $^{99m}TcO_4^-$ is usually administered intravenously, subcutaneous administration is safe and provides equivalent diagnostic images.[132]

Pertechnetate scintigrams have advantages over quantitative uptake measurements. Apart from its value in localizing thyroid lesions, visual inspection of a scan has equal or greater sensitivity for the diagnosis of hyperthyroidism than calculation of the T:S ratio.[127] Using a pinhole collimator, foci of higher uptake can be identified in the scan that may represent an early stage of hyperplasia.[127] Visual inspection may have lower specificity than the T:S ratio, since the observer may be misled by the asymmetry of the thyroid glands that occurs in some euthyroid cats.[133] In case of doubt, quantitative uptake measurements may be helpful if values can be compared with appropriately obtained reference values.

Treatment

There are three options for eliminating the excess production of T_4: (1) radioiodine ablation of the thyroid, (2) surgical thyroidectomy, and (3) inhibition of secretion by antithyroid drugs. When the facilities are not a limiting factor, the first option is to be preferred.

Thyroidectomy is performed by the modified intracapsular dissection technique. After incision on the ventral side of the gland, thyroid tissue is gently teased away from the capsule by blunt dissection with scissors and a moistened cotton-tipped swab. Following removal of the thyroid tissue the capsule is excised, preserving only a small cuff of the thyroid capsule and the blood supply to the parathyroid gland. It may be difficult to locate the parathyroid gland because of the anatomical changes caused by the thyroid nodule, and magnifying glasses should be used. With this approach either unilateral or bilateral thyroidectomy can be performed without a high incidence of hypoparathyroidism, depending on the skill and experience of the surgeon. EHTT in the ventral cervical or anterior mediastinal region are approached by a caudal cervical incision. By careful exploration through the thoracic inlet, the anterior mediastinum can be reached sufficiently to find and remove the lesion.[109,134]

The increase in cardiac output in hyperthyroidism may decompensate subclinical heart disease, although the prevalence of congestive heart failure is low. Preoperative treatment considerations are primarily centered on control of the hyperthyroidism rather than on its cardiovascular consequences.[115] Antithyroid drugs (see below) can be used to control the cardiovascular effects of hyperthyroidism before general anesthesia and surgery, but if these drugs cause serious side effects, beta blockers are a short-term alternative.[115] Systematic echocardiography revealed clinically relevant pretreatment abnormalities in less than 10 % of hyperthyroid cats and tachycardia ($>$ 220 bpm in a clinical setting) was cited as the main criterion for treatment with cardiac-related drugs.[135] Hypokalemia can be corrected preoperatively by administration of potassium orally (2 mmol KCl twice daily [Tumil-K®, Aesculaap, Boxtel, NL]) or by intravenous or subcutaneous injections (see also chapter 4.4).

The most serious postoperative complication is hypocalcemia, signs of which appear within 24–72 h after bilateral thyroidectomy. They range from lethargy, anorexia, reluctance to move, and muscle tremors (face, ears) to tetany and convulsions. Tetany may be provoked by handling the cat. Treatment should be given promptly by intravenous administration of 0.5 mmol Ca^{2+}/kg body weight as calcium gluconate. It is better to avoid this dramatic event by routinely measuring plasma calcium concentration at about 20 h after surgery. If plasma calcium is $<$ 2.0 mmol/l or 10 % below the preoperative value, calcium borogluconate is given subcutaneously in a dose of 1–2 ml/kg, diluted with at least an equal volume of Ringer's solution. Oral supplementation with calcium carbonate, 15–20 mg/kg per meal, is started as soon as the cat

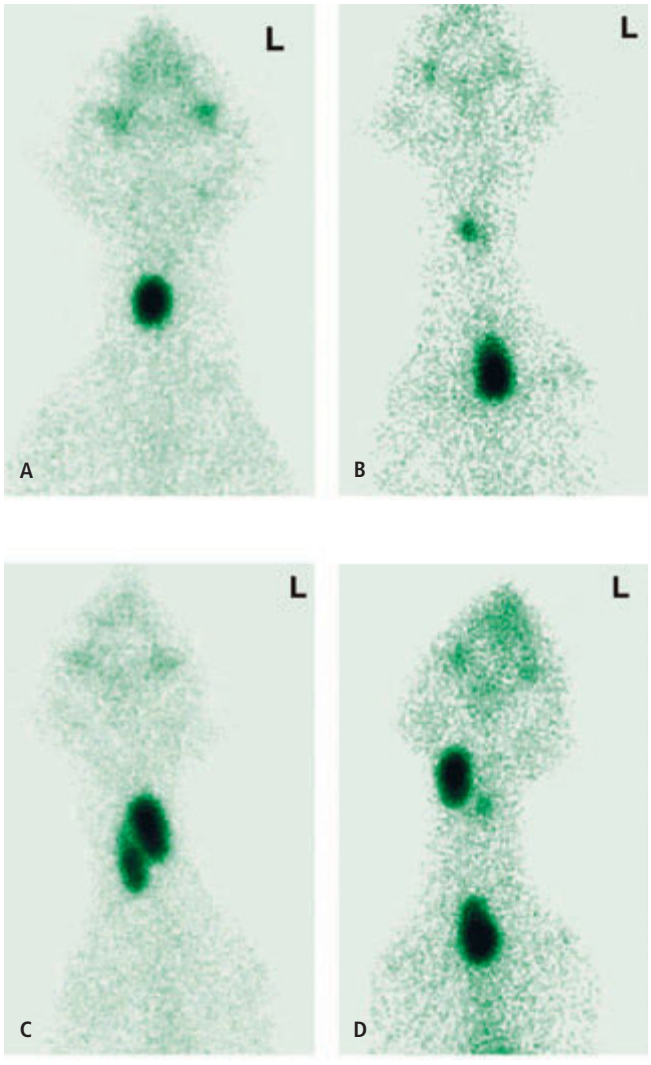


Figure 3.29:
Thyroid scintiscans.
(A) An eleven-year-old castrated male cat with signs and symptoms of hyperthyroidism (weight loss, polyuria, and anxious behavior) and unilateral thyroid enlargement. There is high uptake in the nodule and no visualization of the nonaffected lobe.
(B) A twelve-year-old neutered female cat with persistent weight loss, increased appetite, vomiting of fluid and food, and irritable behavior after bilateral thyroid surgery. There is high uptake at the location of the right thyroid and at the thoracic inlet.
(C) An eight-year-old castrated male cat with persistence of hyperthyroidism after thyroid surgery. There is high uptake near the thoracic inlet.
(D) A 13-year-old castrated male cat with weight loss and polyphagia. There is high uptake at the location of the right thyroid and at the thoracic inlet and lower uptake at the location of the left thyroid.

resumes eating. In addition, dihydrotachysterol [Dihydral®, Solvay Pharmaceuticals, Weesp, NL] is given in a dose of 0.05 mg once daily for three days and then lowered to 0.025 mg once daily. Plasma calcium concentration is measured at least twice daily, gradually decreasing to once weekly. The doses of dihydrotachysterol and calcium carbonate are adjusted to maintain plasma calcium within the reference range.

With an experienced surgeon, hypocalcemia occurs only temporarily in about 5 % of cases. However, if there is parathyroid damage, recovery can take weeks to months.[109] Parathyroid autotransplantation has been proposed as a treatment for accidental removal or devascularization of all parathyroid glands. The parathyroid gland is cut into small pieces and inserted into a small pocket made by blunt dissection in one of the sternohyoideus muscles. Resumption of parathyroid function may result, thereby decreasing the severity and duration of postoperative hypocalcemia. Careful postoperative monitoring of plasma calcium must be continued until this is ascertained.[136]

Oral substitution with l-thyroxine is started in a dose of 50 µg twice daily on the fourth day after bilateral thyroidectomy. Plasma T_4 concentration is measured after four weeks and then every six months. The dose is adjusted as needed to maintain plasma T_4 concentration within the reference range.

Radioiodine (^{131}I) by its β-radiation selectively destroys hyperfunctioning thyroid cells while sparing the suppressed normal thyroid tissue and the parathyroid glands. The normal follicles gradually resume function and there is usually no need for administration of thyroxine. Subcutaneous administration of the radioiodide is preferred, but it can also be administered

intravenously or orally.[137,138] The dose can be determined by a scoring system that takes account of the severity of the signs and symptoms, the size of the thyroid gland(s) (by palpation and/or imaging), and the plasma T_4 concentration. Using this scoring system ^{131}I dose is 3.0–6.0 mCi.[138] It has also been shown that a fixed dose of 4 mCi is effective and that the timing of discontinuation of antithyroid medication with methimazole does not affect the response to radioiodide therapy.[139]

From a medical point of view, radioiodine therapy is certainly the most attractive option. Complete cure is achieved by a noninvasive procedure without complications. Higher doses are often needed for destruction of all malignant tissue in cats with thyroid carcinoma.[140] With exclusion of preexisting renal disease, the survival time has been reported to be significantly longer in cats treated with ^{131}I than in those treated with the antithyroid drug methimazole.[141]

Facilities for radioiodine treatment are only available in licensed hospitals or clinics. Apart from specific equipment, radiation safety precautions are required and the animals must be hospitalized in a nuclear medicine isolation ward for at least one week. Caretakers are exposed to radiation while in close proximity to the cat and, during the first week following ^{131}I treatment, also to the radioactivity in urine and in saliva accumulated on the cat's coat.[142] The cat is discharged from the hospital when the radiation dose has decreased to a safe level as determined by the local radiation control authority. When the cat has returned home the owners must also follow certain safety precautions.

Approximately 5 % of treated cats fail to respond completely. If the hyperthyroid state persists for longer than three months after the initial treatment, retreatment should be considered, for it is curative in virtually all cases.[137] In less than 5 % cats treated with radioiodide, permanent hypothyroidism develops within a few months, characterized by symptoms such as lethargy, nonpruritic seborrhea sicca, matting of hair, and marked weight gain. The diagnosis is confirmed by the finding of a low plasma T_4 concentration with a high plasma TSH concentration, or by a TSH-stimulation test (chapter 12.3.1). Life-long supplementation with thyroxine (50 µg twice daily) is generally needed.[137] With long-term follow-up the percentage of cats developing hypothyroidism may rise to 30 %. Particularly those in which pretreatment scintigraphy revealed bilateral hyperactivity are at risk of developing a low plasma T_4 concentration. It has been questioned whether this is associated with clinical manifestations of hypothyroidism.[143,144]

Relapse as a result of newly developed nodular hyperplasia in the remaining unaffected thyroid tissue is very uncommon. The time between treatment with radioiodide and relapse is generally three years or more. Since both hypothyroidism and relapse can occur after treatment with radioiodide, it is advisable to test thyroid function at least once a year.[137]

Of the available **antithyroid drugs** the imidazole derivative methimazole is most commonly used. It exerts its effect by inhibiting TPO (chapter 3.1.1). The related compound carbimazole is converted to methimazole but yields only half the plasma methimazole concentration as the same dose of methimazole.[145] The doses needed to control hyperthyroidism in cats differ accordingly. The starting dose of methimazole is 1.25–2.5 mg per cat twice daily. This can be increased if the response after two to four weeks is inadequate. For carbimazole the starting dose is 2.5–5 mg per cat twice daily.[146] In cats that tolerate methimazole without side effects, its efficacy is greater than 90 %.[145]

Side effects have been reported in 18 % of cats treated with methimazole and include blood dyscrasias (neutropenia and/or thrombocytopenia), facial excoriation, hepatotoxicity, and gastrointestinal upsets (anorexia, vomiting). Cats with methimazole-induced blood dyscrasias usually recover within a week of discontinuing the drug. Continued methimazole administration in the presence of thrombocytopenia has led to hemorrhages, including epistaxis and oral bleeding.[147] There have been anecdotal reports that side effects are less common with carbimazole than with methimazole, but this has not been substantiated.[146]

In keeping with the possibility of these adverse reactions to methimazole, the treatment protocol should include control examinations at two, four, and six weeks with measurement of hematocrit, leukocyte and thrombocyte counts, and plasma concentrations of liver enzymes, creatinine, and T_4. This work-up should also be performed if a cat becomes ill during methimazole treatment, to differentiate between a »simple« gastrointestinal disturbance, for which lowering of the dose may be adequate, and blood dyscrasias or hepatopathy, in which case methimazole should be discontinued.[146]

When oral administration poses problems, methimazole can be administered in transdermal formulations in which pluronic lecithin organogel acts as a permeation enhancer to facilitate drug absorption across the epidermis. Chronic transdermal methimazole dosing (2.5 mg twice daily) is effective in lowering plasma T_4 concentration in hyperthyroid cats.[148,149] Administration of carbimazole in ointment form is equally effective (5 mg once daily for one week and then twice daily).[150] The ointment is applied to the inner surface of the pinna, alternating ears with each dose. The owner is instructed to wear gloves or finger cots for the procedure and to remove crusted material with moistened cotton before applying the ointment.

Although fewer gastrointestinal side effects have been reported with transdermal treatment, it is not convincingly less often associated with serious side effects than oral treatment. Using the same dose, it has lower efficacy than oral methimazole, probably because of lower bioavailability.[149]



Figure 3.30:
A nine-year-old male boxer in a very poor nutritional condition as a result of hyperthyroidism (A). Removal of a small thyroid adenoma resulted in resolution of the symptoms and signs, including the severe polyuria. By the time of a follow-up examination five months later (B), the dog had gained 5 kg in body weight. It had also become so lively and strong again, that it was difficult to keep on the table for the photograph.

Percutaneous ethanol injection (PEI) under ultrasonographic guidance is an alternative treatment in humans.[151] Injection of 96 % ethanol into the thyroid lesion causes hemorrhagic necrosis and fibrosis.[152] PEI is regarded as the first-line treatment for recurrent thyroid cysts and as an alternative to follow-up alone for small autonomously functioning nodules in humans who refuse ^{131}I therapy.[153] There has been one report on the use of PEI for solitary nodules in four hyperthyroid cats. Plasma T_4 concentration decreased and the clinical features of hyperthyroidism resolved. The disease did not recur in the twelve-month follow-up period. There were no adverse effects other than mild dysphonia.[154] The results in seven cats with bilateral thyroid lesions were less satisfactory: euthyroidism lasted less than six months and there was a high incidence of laryngeal paralysis and Horner's syndrome.[155]

Percutaneous ultrasound-guided radiofrequency heat ablation, performed in nine cats, also lowered plasma T_4 concentration only transiently, with a mean duration of euthyroidism of four months.[156]

Prognosis

In cats without severe complicating cardiac or kidney disease, the prognosis for restoration of health is excellent after successful surgery. There may be recurrence months or years after thyroidectomy; usually due to adenomatous hyperplasia in the contralateral lobe or ectopic tissue. After radioiodine treatment the prognosis is as good or better, for even with bilateral involvement or the presence of ectopic thyroid tissue, there is no risk of hypoparathyroidism and seldom need for supplementation with thyroid hormone. In the great majority of hyperthyroid cats either methimazole or carbimazole is effective, but the prognosis depends in part on whether there are adverse reactions to the drug.

3.4.2 Thyroid tumors and hyperthyroidism in dogs

Thyroid neoplasia accounts for about 2 % of all canine tumors. Most of the benign tumors (adenomas) are small and commonly not detected during life. They only very occasionally become cystic and thereby large enough to be detected by the owner.[157] A benign thyroid tumor may also be detected because of symptoms suggesting hyperthyroidism (fig. 3.30). Careful palpation of the neck may reveal a slightly enlarged thyroid. Over 85 % of the canine thyroid tumors discovered clinically are rather large (diameter $>$ 3 cm), solid, and malignant. Their malignant nature may already be evident during physical examination, because of changes such as attachment to adjacent structures and metastasis to regional lymph nodes.

Microscopic examination reveals most tumors to consist of both solid and follicular tissue, while some largely consist of one type or the other. Among thyroid cancers of domestic animals, that of the dog – particularly the follicular type – most closely resembles human follicular carcinoma. The similarities include not only the clinical behavior of the tumor but also the pattern of circulating thyroglobulin levels and the conservation of TSH receptors in the primary tumors (much less in metastases).[158,159] An intriguing difference is observed in DNA ploidy, there being a high incidence of hypodiploidy in canine tumors.[160] Mutations in tumor suppressor gene p53 seem to occur infrequently in dogs with thyroid carcinoma.[161]

Of the possible risk factors contributing to the development of thyroid cancer, the influence of iodine in the canine diet is unclear,[162] although in one study a high prevalence of thyroid tumors in necropsy material was ascribed to insufficient

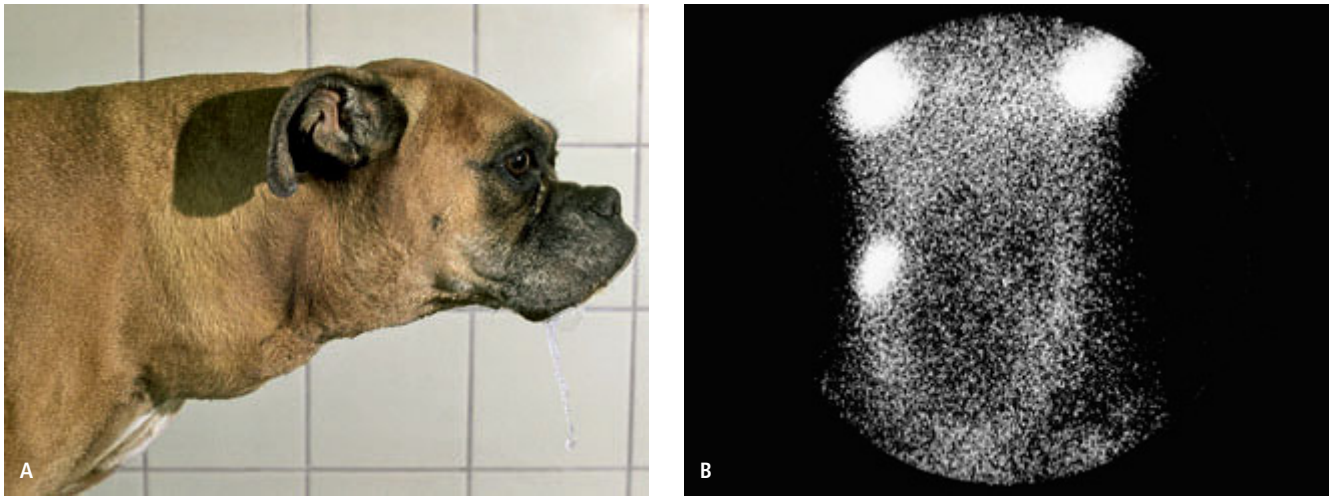


Figure 3.31:
A nine-year-old female boxer (A) with an enormous thyroid tumor causing tracheal obstruction and dysphagia (note the salivation). The pertechnetate scan (B) shows it to be functionally inactive, not concentrating pertechnetate. Such thyroid tumors are referred to as being »cold«. The large size of the tumor causes lateral displacement of the nonaffected thyroid in which pertechnetate uptake is normal. The uptake by the parotid salivary glands (at the top of the scan) is normal.

iodine intake.[163] Hypothyroidism due to lymphocytic thyroiditis was found to be associated with a high incidence of thyroid tumors in a colony of beagles. This also points to a possible role of chronic TSH exposure in promoting neoplastic growth of residual follicular epithelium.[164]

Thyroid tumors arise not only from follicular epithelium but also from the parafollicular C cells (fig. 3.1). These so-called medullary thyroid tumors are relatively rare in dogs.[157] It has been suggested that they may be more prevalent than previously thought and are of lower malignancy than the carcinomas arising from follicular cells.[165] Recently the familial occurrence of medullary thyroid carcinoma in Alaskan malamute-cross dogs was reported, but a predisposing gene defect has not yet been identified in this pedigree.[166] Medullary thyroid carcinoma in dogs does not seem to be associated with activating mutations in the RET proto-oncogene, as it is in humans.[166,167] Thyroid carcinosarcomas, consisting of both malignant epithelial (follicular) and mesenchymal (usually osteogenic or cartilaginous or both) elements, are extremely rare.[168]

Metastasis of canine epithelial thyroid carcinomas is relatively common, most often to the lungs and regional lymph nodes.[157,163] Lymph drains from the canine thyroid primarily via the upper pole lymphatics in the cranial direction, to the deep cervical lymph nodes.[169] Metastasis occurs to many other organs, including the pituitary gland.[170] While metastasis of thyroid carcinoma to bone is not uncommon in humans, it is rare in dogs.[157]

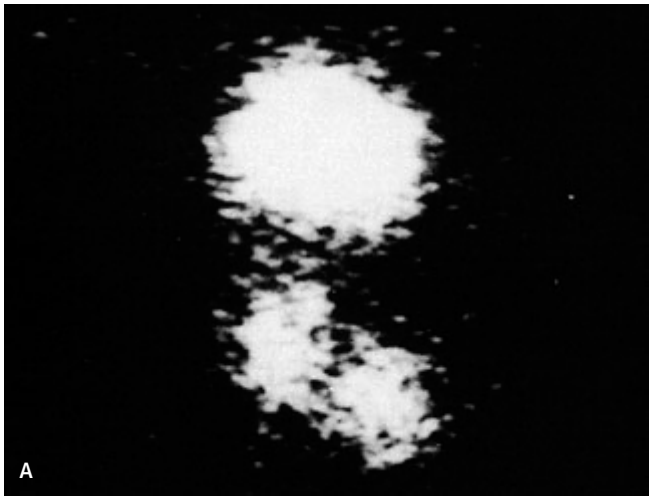
Clinical features

The mean age of dogs presented with thyroid tumors is nine years (range 5–15 years) and boxers are overrepresented. There is no sex predilection.[157] The signs and symptoms are due to: (1) thyroid enlargement and (2) hypersecretion of thyroid hormones.

Most thyroid tumors are discovered by the owners as a painless mass in the midcervical or ventrocervical region that causes no discomfort. However, as the tumors increase in size they may cause pressure symptoms such as dysphagia, hoarseness, and tracheal obstruction (fig. 3.31; table 3.3). A large and invasive tumor may even damage the cervical sympathetic trunk, causing Horner's syndrome.[171] Arterial invasion may cause an emergency situation of rapidly increasing swelling in

Table 3.3: Manifestations of nonhyperfunctioning thyroid tumors in dogs

System or organ	Common	Less common or rare
Thyroid	Unilateral tumor Usually large	Bilateral tumor Irregular shape Enlarged regional lymph nodes
Metabolism		Weight loss
Respiratory system		Respiratory distress
Gastrointestinal system		Dysphagia Anorexia
Neuromuscular system		Painful neck Horner's syndrome



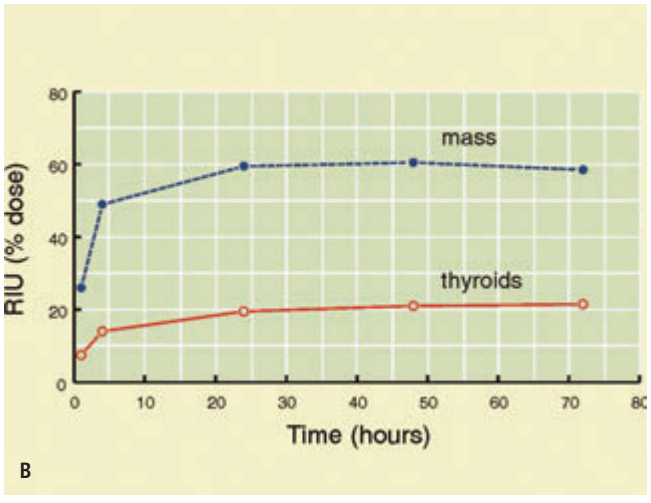


Figure 3.32:
(A) Scintiscan of a nine-year-old female miniature poodle with a midline cervical mass at the level of the hyoid bone, 48 h after intravenous administration of 3.7 MBq (100 µCi) $^{131}I^-$. There is normal uptake in both thyroids and even higher uptake in the mass. (B) radioiodide uptake in the thyroids and the mass. The mass did not produce excessive thyroid hormone, for plasma TT_4 was 46 nmol/l and uptake by the thyroids was not suppressed. Biochemical studies in similar cases have revealed that such tumors produce an iodoprotein similar to albumin and almost no Tg. In this dog, the administration of 740 GBq (20 mCi) $^{131}I^-$ intravenously produced complete and permanent ablation of the tumor.

the ventral cervical region due to hemorrhage.[172] Tumors arising from thyroglossal duct remnants develop in the ventral midline cranial to the larynx and may involve the base of the tongue and the hyoid bones (fig. 3.32). Tumors originating from ectopic thyroid tissue at the base of the heart may cause arrhythmias, pericardial effusion, and anterior cervical edema.[173]

Hypersecretion of thyroid hormone occurs in about 10 % of cases of thyroid tumor in dogs.[157,174] It may result in the syndrome of hyperthyroidism, very similar to that cats but often less severe (table 3.4). Occasionally there are symptoms of hyperthyroidism without palpable thyroid enlargement, in which case an intrathoracic hyperfunctioning tumor (fig. 3.33) in ectopic thyroid tissue should be considered.[174,175]

Medullary thyroid carcinomas in humans may express genes that are not normally expressed, or only at low levels, in normal C cells. The protein products of these genes include somatostatin, proopiomelanocortin, vasoactive intestinal peptide, and gastrin-releasing peptide, in some patients causing profuse, watery diarrhea.[176,177] Such systemic effects also occur in dogs: an otherwise unstoppable diarrhea in a seven-year-old collie ceased immediately after removal of a medullary thyroid carcinoma.[157]

Differential diagnosis

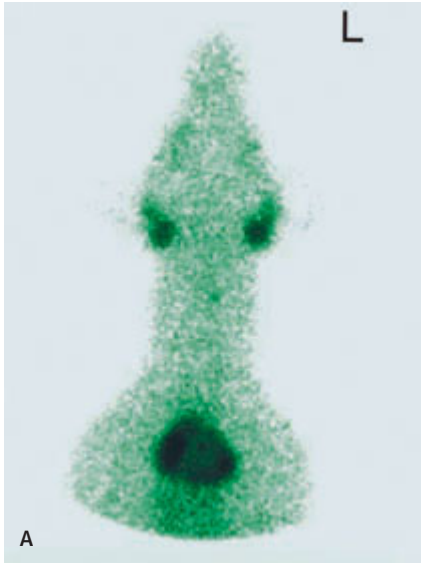
The differential diagnosis for a large cervical mass includes inflammation (pharyngeal penetration by a foreign body), hematoma, lymphoma, lipoma, and other tumors. Thyroid tumors also very rarely infiltrate the skin, mimicking inflammation with abundant granulation tissue.

Diagnosis and staging

The location and extent of the mass is determined by careful palpation of the underside of the neck while the animal is sitting in a relaxed position with its head lifted and tilted slightly backward. Small to medium-sized tumors are usually easy to move along the trachea, but palpation may also reveal attachment of the tumor to adjacent structures and enlargement of the deeply located cranial cervical lymph nodes. Functional status can be tested by measuring plasma concentrations of T_4 and TSH. A low plasma T_4 and high plasma TSH, indicating hypofunction, can be found in dogs in which the normal thyroid tissue is replaced by bilateral thyroid carcinoma or pre-existing thyroiditis. Hyperfunctioning thyroid tumors result in a high plasma T_4 and low plasma TSH (fig. 3.33).

Table 3.4: Manifestations of hyperfunctioning thyroid tumors in dogs

System or organ	Common	Less common or rare
Thyroid	Unilateral tumor, small or medium-sized	
Metabolism	Weight loss in spite of good appetite	Intolerance to hot environment
Respiratory system	Panting	
Cardiovascular system		Tachycardia Forceful heart beat
Renal system	Polydipsia and polyuria	
Gastrointestinal system		Diarrhea
Neuromuscular system	Weakness Fatigue and lethargy	Restlessness Muscle atrophy



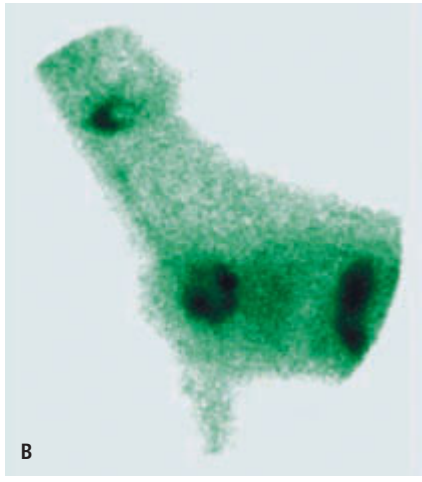
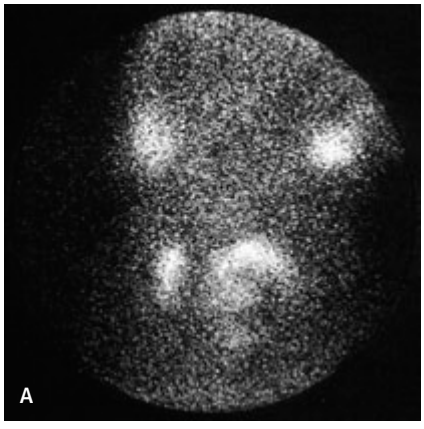


Figure 3.33:
Scintiscans 45 min after intravenous injection of 74 MBq $^{99m}TcO_4^-$ in an eleven-year-old, neutered male Jack Russell terrier presented for gradually increasing polyuria and polydipsia. There is normal distribution of radioactivity in the salivary glands and gastric mucosa (B), but almost none in the thyroid glands (A). The high uptake in the cranial portion of the thorax is due an autonomous hyperfunctioning thyroid tumor in the cranial mediastinum.[175] Plasma T_4 was 62 nmol/l and TSH was $<$ 0.02 µg/l.



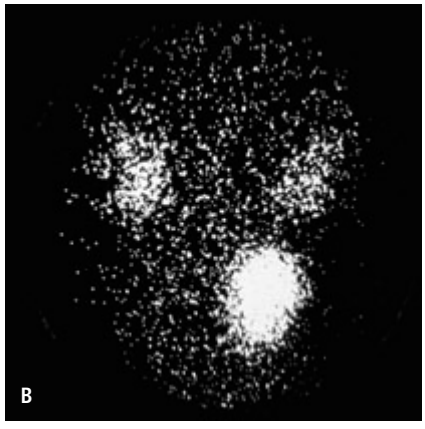


Figure 3.34:
(A) Scintiscan of a dog with a nonhyperfunctioning (also called »nontoxic«) thyroid tumor. The distribution of radioactivity in the tumor is irregular (see also fig. 3.5). Uptake in the contralateral lobe is not suppressed.
(B) Scintiscan of the boxer of fig. 3.30, showing a small hyperfunctioning (»toxic«) tumor of the left thyroid and no visualization of the right thyroid due to feedback suppression of pituitary TSH secretion.

Diagnostic imaging techniques such as ultrasonography, computed tomography, and magnetic resonance imaging can be of great help in identifying cysts, regional lymph node metastases, hemorrhage, necrosis, calcification, vascular displacement, and invasion.[178] Doubt as to whether a mass is of thyroidal origin can usually be resolved by a pertechnetate or iodide scintiscan (figs. 3.33–3.36). Pulmonary metastases can be detected by radiography and, if necessary, by computed tomography. These techniques are more sensitive for this purpose than scintigraphy because the metastases, particularly when solid or anaplastic, may not trap pertechnetate.[178]

Cytological examination of fine needle biopsies may reveal the identity of the mass, although it may be difficult to obtain aspirates without excessive blood and cystic tumors often contain a mixture of bloody fluid and degenerated tumor cells.[179] Blood contamination may be avoided by using a small needle ($<$ 22 G), inserting it into the tumor in only one direction, and aspirating with a syringe no larger than 5 ml.[162]

Staging of the tumor can be performed according to the standardized scheme of the World Health Organization (WHO).[180] In this T(tumor), N(regional lymph node), and M(distant metastasis) classification, T_0–T_3 represents the range of tumor size (0, $<$ 2 cm, 2–5 cm, and $>$ 5 cm diameter), subdivided into »a« (tumor freely movable) and »b« (tumor fixed to surrounding structures). N_0–N_2 represents the range of lymph node involvement from none to bilateral involvement, with the substages »a« (lymph node freely movable) and »b« (lymph node fixed). M_0 and M_1 indicate whether or not distant metastases have been detected. Using these indicators, four main staging groups can be distinguished (table 3.5).[180]

Treatment

As the great majority of the clinically detected tumors are malignant, the mass should be surgically removed without delay, provided it is resectable. The **surgical excision** of well-encapsulated and freely-movable thyroid carcinomas is often

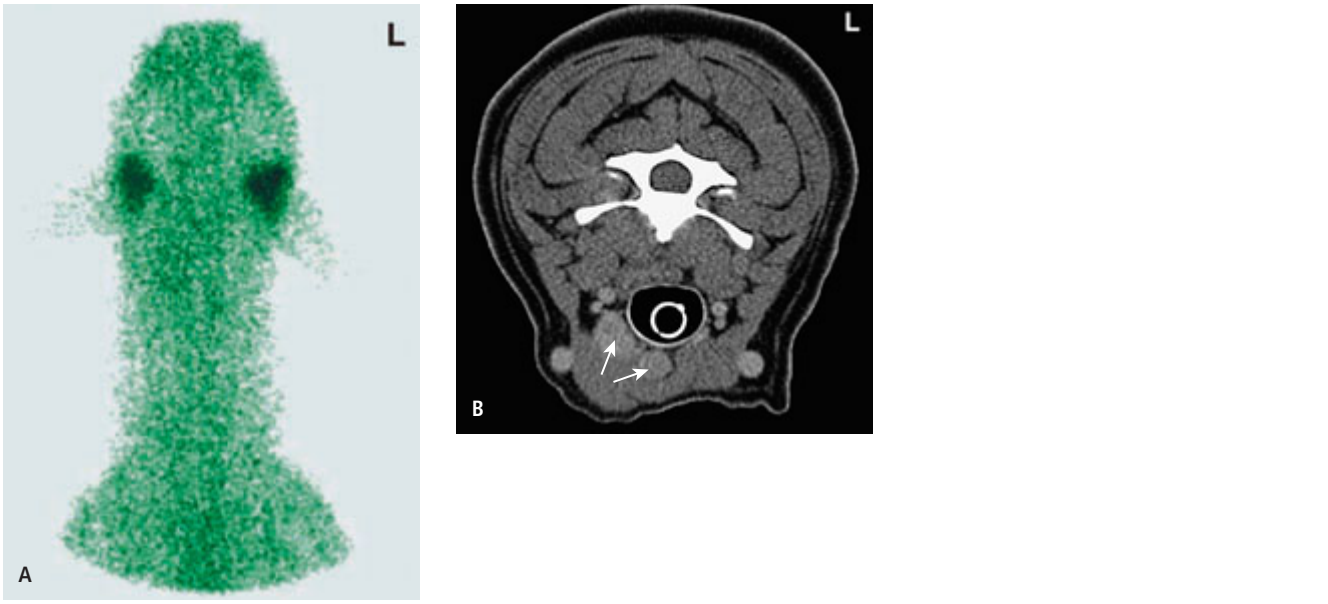


Figure 3.35:
A 13-year-old female Husky that had undergone surgery for thyroid carcinoma two years before. Recurrence of the tumor was visible in the neck for a few months.
(A) A pertechnetate scintiscan reveals no uptake by the tumor.
(B) Computed tomography (CT) reveals the mass to the right of the trachea (7.0 × 2.8 × 3.9 cm) at the level of the 2nd cervical vertebra (arrows). It appears to accumulate contrast medium.

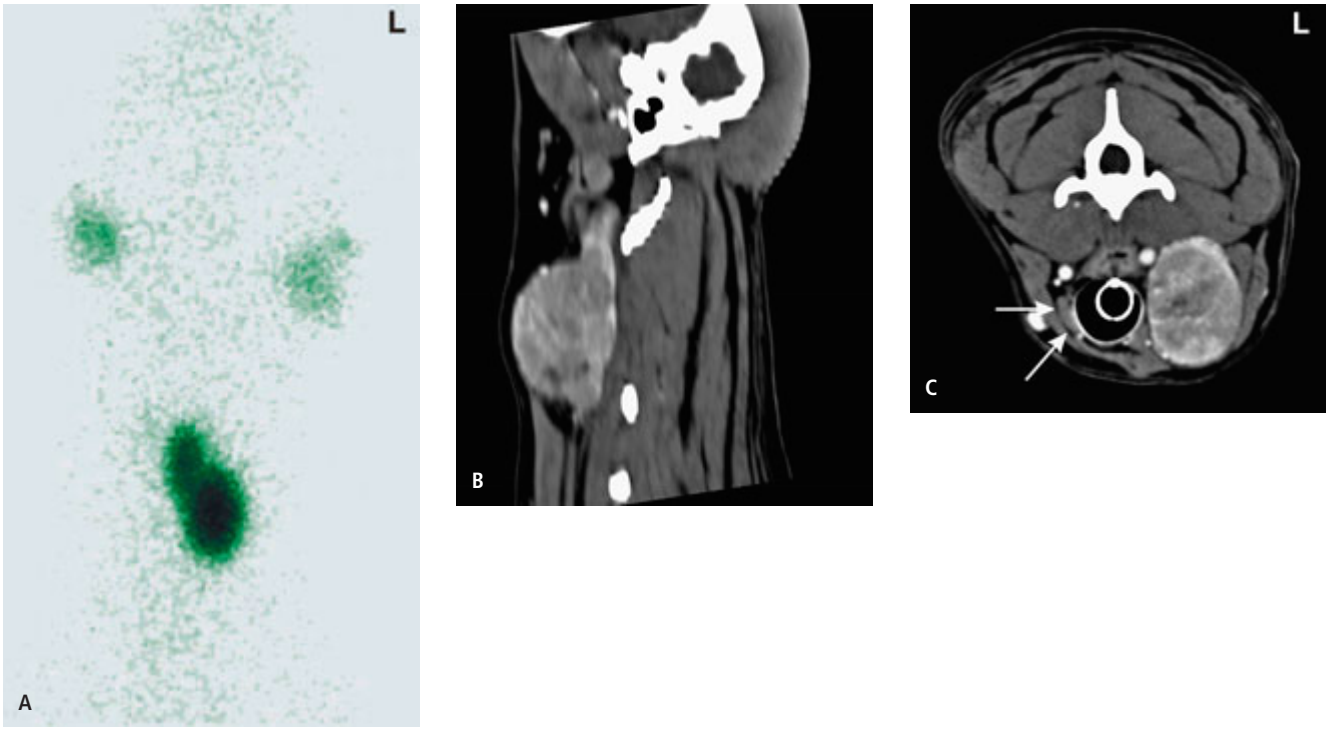


Figure 3.36:
A ten-year-old female West Highland white terrier with hyperthyroidism (plasma TT_4: 150 nmol/l) and a palpable mass in the neck suggesting bilateral thyroid tumor.
(A) A pertechnetate scintiscan also gives the impression of bilateral hyperfunctioning thyroid tumor.
(B, C) The CT scan reveals instead a single tumor on the left and atrophy of the thyroid on the right (arrows).

Table 3.5: Clinical staging of canine thyroid tumors[180]

Staging group	Primary tumor	Regional lymph nodes	Distant metastases
I	T_1 a,b	N_0	M_0
II	T_0	N_1	M_0
	T_1 a,b	N_1	M_0
	T_2 a,b	N_0 or N_1 a	M_0
III	T_3	Any N	M_0
	Any T	N_1 b or N_2 b	M_0
IV	Any T	Any N	M_1

curative. Symptoms and signs of hyperthyroidism disappear (fig. 3.29).[181] Excision of movable thyroid carcinomas in staging groups II (T_2a, N_0, M_0) and III (T_3a, N_0, M_0) resulted in long-term survival in the majority of the dogs.[182] Medullary thyroid carcinomas tend to be well circumscribed and resectable.[165] When there are bilateral tumors, an attempt should be made to spare one of the parathyroid glands, although this is only be possible if the tumor is well circumscribed and an external parathyroid can still be identified. If no parathyroid tissue can be preserved, treatment of hypoparathyroidism (chapter 9.2) will be necessary in addition to thyroxine replacement (chapter 3.3.1). Surgical excision of ectopic carcinomas at the base of the tongue poses a challenge because of their close attachments to the hyoid apparatus and the tongue, and because of abundant neovascularization.[183] Ectopic tumors arising from intrathoracic thyroid tissue may be resectable.[173]

Dogs with large, invasive tumors, particularly if they are bilateral or ectopic, are often poor surgical candidates and other options should be considered. In principle, administration of **radioiodide** is an attractive alternative (fig. 3.32). Particularly in dogs with hypersecreting tumors the high uptake and complete organification of ^{131}I should result in a high radionuclide concentration within the tumor, yielding a high effective dose of radiation. There have been studies in which ^{131}I therapy – irrespective of thyroid hormone status – extended survival time, even though in some cases there was little or no reduction in tumor mass.[184,185] Median survival time was significantly greater for dogs with local or regional tumors (stage II or III) than for those with stage IV tumors.[185] Myelosuppression has been recognized as a complication of high-dose ^{131}I therapy.[185,186] The stringent regulatory requirements regarding radionuclide use, the need for relatively large and repeated doses, and prolonged hospitalization limit the availability of this treatment option.

External beam radiation therapy with a linear accelerator or a cobalt therapy machine is indicated when complete excision of the tumor is not possible and radioiodide therapy is unlikely to be effective. Radiation protocols employing twelve treatments (4 Gy three times weekly), including the primary tumor and regional lymph nodes in the treatment field, may lead to considerable reduction in tumor volume, even to a clinically undetectable level. It may take 8–22 months to achieve the maximum reduction in tumor size.[187,188] Palliative treatment can be considered in dogs that are not candidates for full-course radiation therapy, such as those with distant metastases and discomfort caused by the primary tumor. The administration of four once-weekly fractions of 9 Gy was reported to halt tumor growth in all 13 dogs studied and to result in tumor regression in most. Tumor growth rate rather than the presence of lung metastases was an important determinant of survival time.[189] Full-course radiation therapy leads to acute side effects in the skin (moist skin desquamation and hair loss) and in the mucosa of the larynx, trachea, and esophagus (mucositis causing dysphagia, hoarseness, and cough). Pain is managed by application of anti-inflammatory drugs, opioids, and supportive care (e.g., soft and highly palatable food). In most cases the acute side effects are resolved in 3–4 weeks. Permanent alopecia and change in hair color and skin pigmentation are common after radiation treatment.[190] Hypothyroidism can be a late effect of irradiation of thyroid tumors.[67,187]

Chemotherapy with either doxorubicin or cisplatin may be considered in dogs with a high risk of developing metastases, namely those with large and bilateral thyroid carcinoma.[191] Partial remissions have been reported but there are no reports on improved (progression-free) survival time.[162]

Prognosis

The histological grade of malignancy, taking into account cellular and nuclear polymorphism, capsular and vascular invasion, and the frequency of mitoses, appears to be the most important prognostic factor for canine thyroid tumors treated by thyroidectomy.[192] In addition, the size of the tumor and bilateral occurrence are critical factors.[157,187] In other words, in dogs with medium-sized or small well-encapsulated carcinomas, surgical resection carries a good prognosis.

Thyroid-cell proliferation is TSH dependent (chapter 3.1.3) and since carcinomatous thyrocytes do have TSH receptors,[159] it can be assumed that the prognosis can be influenced favorably by TSH-suppressive treatment with l-thyroxine. Indeed, in humans it has been reported that tumor recurrence rates can be lowered if l-thyroxine is given after surgery to patients with nonmetastasized differentiated thyroid carcinoma.[193] This treatment has two objectives: (1) hormone replacement (correction of induced hypothyroidism) and (2) hormone suppression (reduction of plasma TSH levels that might stimulate growth of persistent or recurrent neoplastic tissue). In low-risk patients l-thyroxine is given to return TSH levels to within the reference range. Patients with high-risk thyroid cancer receive higher doses to achieve complete TSH suppression, which implies a state of subclinical hyperthyroidism that will need careful monitoring for cardiovascular disease.[194,195]

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4 Adrenals

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4.1 Introduction

The adrenals are paired glands situated craniomedial to the kidneys. Each consists of two functionally distinct endocrine glands of different embryological origin. The medulla of each gland consists of coalesced chromaffin cells of neuroectodermal origin that secrete epinephrine and norepinephrine. The surrounding cortex arises from mesoderm and histologically three zones can be distinguished: (1) zona glomerulosa (or arcuata), (2) zona fasciculata, and (3) zona reticularis (fig. 4.1).

In recent years several factors involved in adrenal development have been identified, but it remains unknown which factors are responsible for the differentiation of adrenal stem cells into cells of specific zones of the fetal adrenal cortex. In all mammalian species the growth and function of the fetal adrenal cortex are influenced by adrenocorticotropic hormone (ACTH) secreted by the pituitary gland. As ACTH is not a growth factor *per se*, at least some of its trophic actions are modulated by locally expressed growth factors such as basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), insulin-like growth factor (IGF)-II, and transforming growth factors.[1] Some of the genes encoding these growth factors (particularly IGF-II) are similarly overexpressed in fetal adrenals and adrenocortical carcinomas of humans.[2,3]

The zona fasciculata is the thickest layer. It consists of columns of cells extending from the zona reticularis to the zona glomerulosa. The cells are relatively large and contain much cytoplasmic lipid. This is lost during processing of histologic sections, giving the cells a vacuolated appearance for which they are called »clear cells«. In this zone glucocorticoids (cortisol and corticosterone) and androgens are produced.

The cells of the zona reticularis form anastomosing columns. They do not have significant lipid content but have densely granular cytoplasm, for which they are called »compact cells«. This zone produces androgens such as androstenedione, but also glucocorticoids. It functions together with the zona fasciculata as a single unit.

The zona glomerulosa lacks a well-defined structure. The small, lipid-poor cells are scattered beneath the adrenal capsule. They produce mineralocorticoids (primarily aldosterone) and are deficient in 17α-hydroxylase activity (see below) and therefore cannot produce cortisol or androgens.

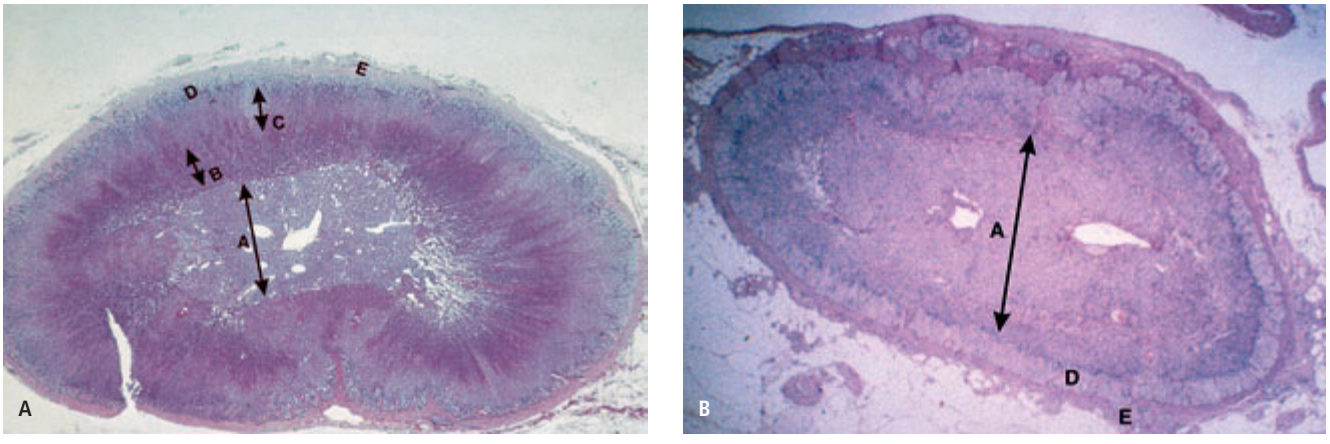


Figure 4.1:
(A) Histological section of the adrenal gland of a healthy dog: A = medulla; B = zona reticularis; C = zona fasciculata; D = zona glomerulosa; E = capsule.
(B) Similar section from a dog that received injections of progestagens. Their intrinsic glucocorticoid effect suppressed endogenous ACTH secretion, resulting in complete atrophy of both the zona fasciculata and the zona reticularis, while the zona glomerulosa remained intact.

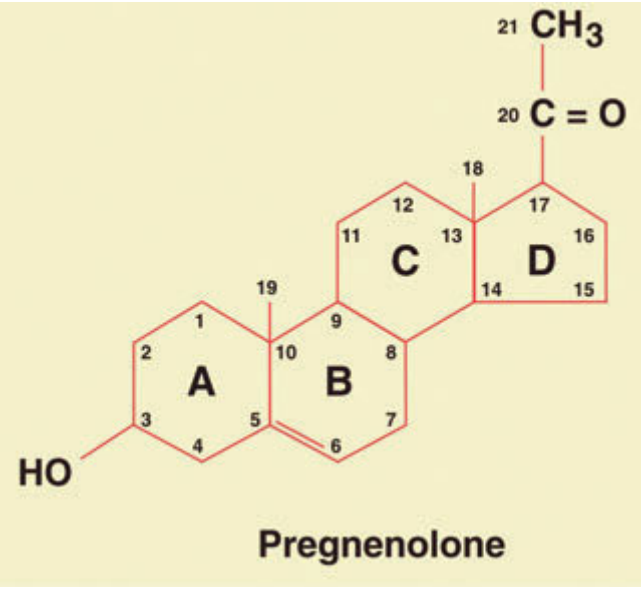


Figure 4.2:
Basic structure of adrenocortical steroids. In this pregnenolone molecule the four rings are identified by letters. Individual carbon atoms are numbered. (Recommendation of the International Union of Pure and Applied Chemistry, IUPAC-IUB 1967).[4]

4.1.1 Synthesis and secretion of corticosteroids

The adrenal cortex is rich in receptors that internalize low density lipoproteins (LDL). Free cholesterol liberated from the LDL serves as the starting compound in steroidogenesis, although cholesterol is also synthesized from acetate within the gland (fig. 4.2, table 4.1, and fig. 4.3). Cytochrome P-450 enzymes are responsible for most of the enzymatic conversions from cholesterol to steroid hormones. These enzymes are membrane-bound hemoproteins that catalyze oxidation, including oxidative cleavage of the precursor molecule. They are named for the ability of their heme group to absorb light at a wavelength of 450 nm after reduction.

Table 4.1: Nomenclature for adrenal steroidogenic enzymes and their genes

Enzyme name	Gene
Cholesterol side-chain cleavage (SCC) (desmolase)	CYP11A1
3β-Hydroxysteroid dehydrogenase (3β-HSD) (type II isoenzyme)	HSD3B2
17α-Hydroxylase / 17,20 lyase	CYP17
21-Hydroxylase	CYP21A2
11β-Hydroxylase	CYP11B1
Aldosterone synthase	CYP11B2

The difference in hormone production between zones is related to differences in two cytochrome P-450 enzymes. The mitochondrial cytochrome P-450 enzyme aldosterone synthase, which converts deoxycorticosterone via corticosterone to aldosterone, is only found in the zona glomerulosa. The characteristic enzyme in the other two zones is the microsomal cytochrome P-450$_{c17}$ (17α-hydroxylase/17,20-lyase), which catalyzes the 17α-hydroxylation of pregnenolone and progesterone as well as the side-chain cleavage at C_{17} of 17-α-hydroxy C_{21} steroids. The other steroidogenic enzymes occur in all three zones.

Steroidogenic cells cannot store the hormones, which are therefore secreted immediately after biosynthesis. Cortisol, 11-deoxycortisol, corticosterone, 11-deoxycorticosterone, and aldosterone are derived entirely from adrenocortical secretion, whereas the other steroids are derived from a combination of adrenocortical and gonadal sources. In dogs and cats the cortisol:corticosterone ratio in adrenal venous blood range from about 3:1 to 7:1.

4.1.2 Transport and metabolism

Following secretion, the adrenocortical hormones are largely bound to plasma proteins. Approximately 75 % of cortisol in plasma is bound with high affinity to corticosteroid-binding globulin (CBG). An additional 12 % of total cortisol in blood is bound with low affinity to albumin and erythrocytes. Only the free fraction, in the dog estimated to range from 6 to 14 %,[5–7] is biologically active. However, the amount of hormone that is potentially available to tissues is determined by the combination of free and bound fractions, because these fractions are in equilibrium. The CBG-binding capacity is higher in female dogs than in males.[8] The cortisol-binding capacity of CBG is diminished in dogs with portosystemic encephalopathy, probably as result of decreased CBG synthesis in the compromised liver.[9] Androgens and aldosterone are predominantly bound with low affinity to albumin. This explains the low plasma concentrations of these hormones.

The physiological role of the circulating binding proteins is most probably buffering, which prevents rapid variations in plasma cortisol concentration. They restrain the flux of active cortisol to the target organ and also protect it from rapid metabolic breakdown and excretion.

Unbound steroids readily diffuse into the salivary glands and the cortisol concentration in canine saliva is equivalent to 7–12 % of the total blood cortisol concentration, similar to the free fraction.[10] Measurement of salivary cortisol is increasingly used as noninvasive technique to investigate stress responses in studies of welfare and of human-dog interactions.[11–13] Up to four minutes can be taken to collect a saliva sample from a dog without the effect of handling being reflected in its cortisol concentration.[14] Of the devices tested, a

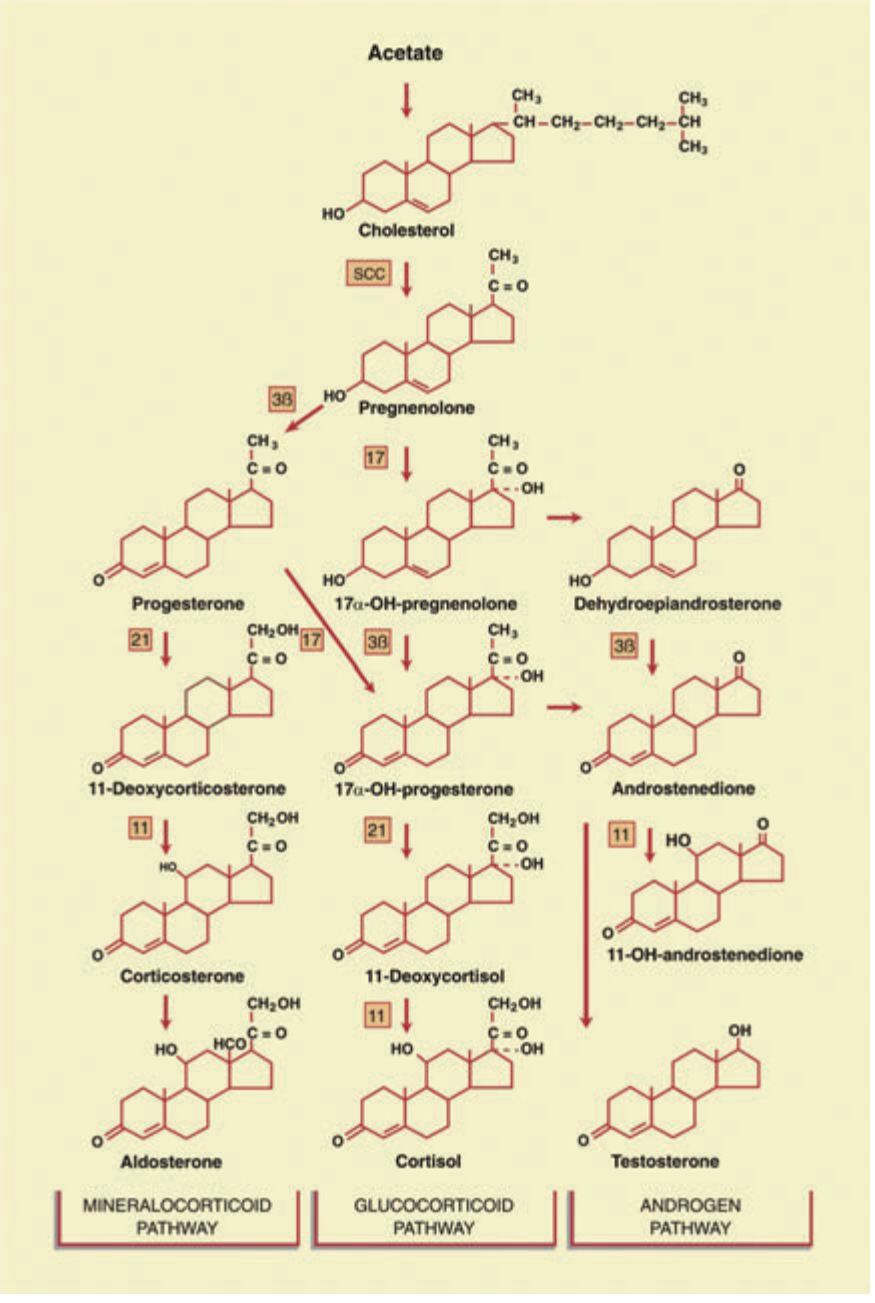


Figure 4.3:
Major biosynthetic pathways of adrenocortical steroid biosynthesis.
scc = cholesterol side-chain cleavage; 3β = 3β-hydroxysteroid dehydrogenase; 11 = 11β-hydroxylase; 17 = 17α-hydroxylase / 17; 20 lyase; 21 = 21-hydroxylase.

hydrocellulose eye sponge seems to be the best material for collection of canine saliva.[15,16]

The liver and the kidney are the major sites of corticosteroid metabolism, which inactivates them and increases their water solubility, as does subsequent conjugation with glucuronide or sulfate groups. The conversion of cortisol to the inactive cortisone by 11β-hydroxysteroid dehydrogenase (11β-HSD) is the most important pathway quantitatively. In several species including the dog, most of the inactivated and conjugated metabolites are readily excreted as glucuronides by the kidney, whereas in the cat the excretion is largely as sulfates via the bile.[17,18] One to two per cent of the total cortisol secretion is excreted unaltered in the urine. Measurement of this urinary »free« cortisol gives an integrated reflection of cortisol production (chapter 12.4.4).

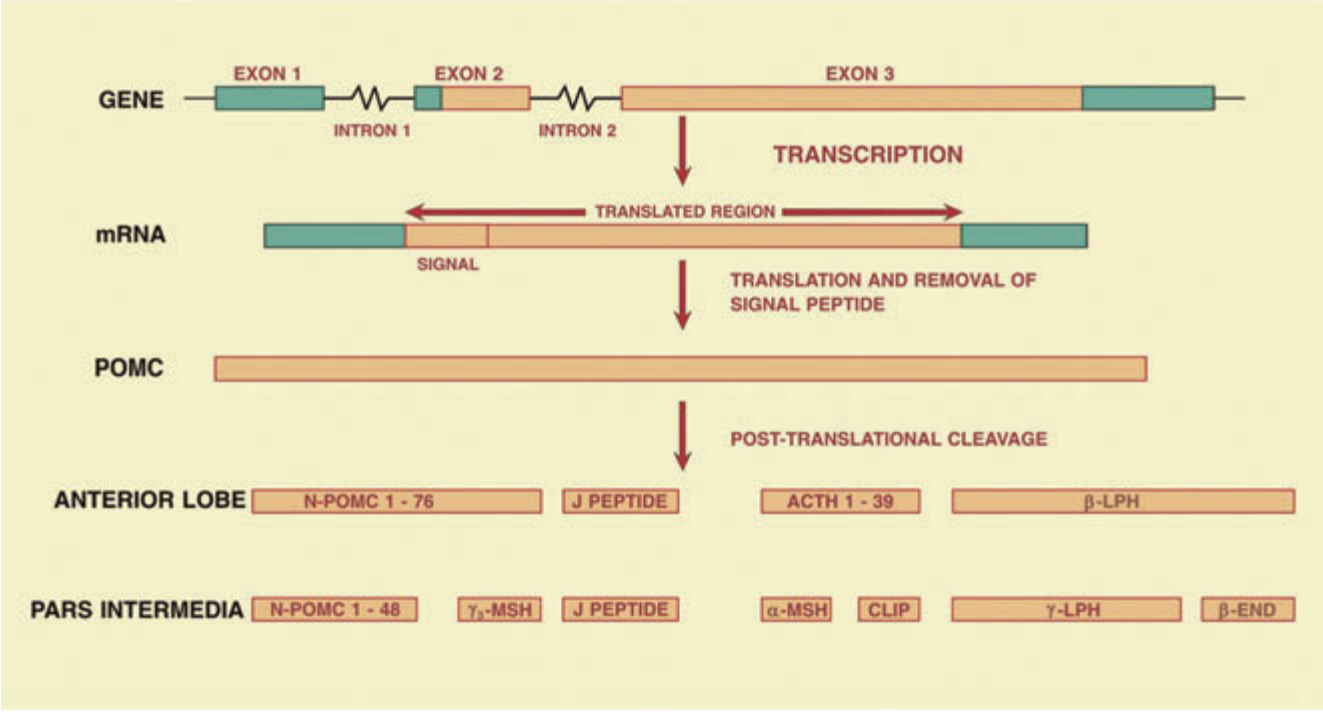


Figure 4.4:
Structure of the canine proopiomelanocortin (POMC) gene, its mRNA, and the processing of POMC in the anterior lobe and pars intermedia of the pituitary.
ACTH = adrenocorticotropic hormone; J PEPTIDE = joining peptide; β-LPH = β-lipoprotein; MSH = melanocyte-stimulating hormone; CLIP = corticotropin-like intermediate lobe peptide; β-END = β-endorphin.

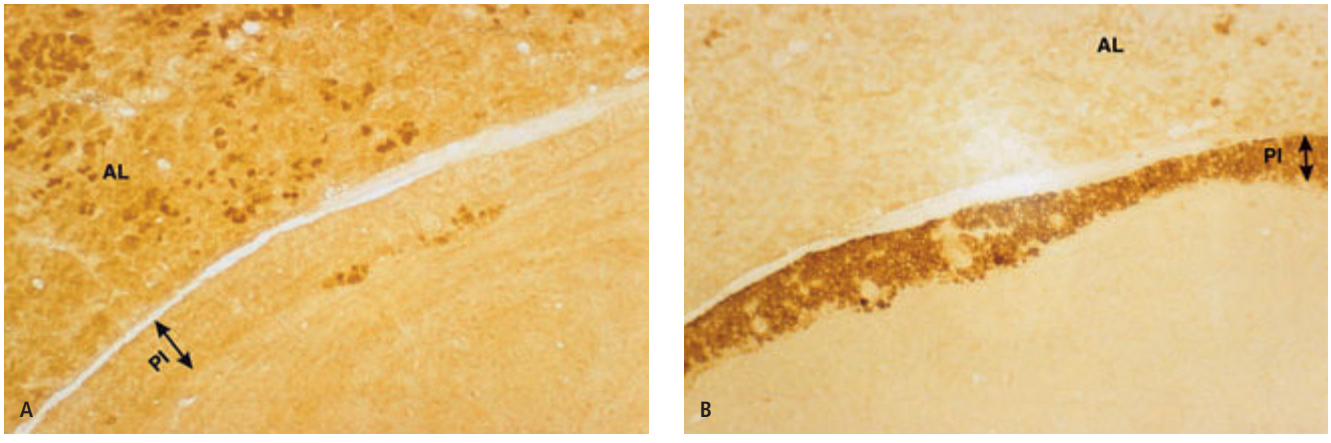


Figure 4.5:
Sections of the pituitary gland of a cat immunostained with anti-ACTH (A) and anti-α-MSH (B). Compared with the anterior lobe (AL) and pars intermedia (PI) of the dog (see fig. 2.6), there are few ACTH-positive cells in the PI but there are abundant MSH-positive cells.

4.1.3 Regulation of glucocorticoid secretion

Synthesis and release of glucocorticoids and androgens by the middle and inner zones of the adrenal cortex are almost exclusively controlled by the plasma concentration of ACTH (see also fig. 1.10). ACTH is a single-chain peptide of 39 amino acid residues. It is synthesized in the anterior lobe of the pituitary gland from the precursor molecule proopiomelanocortin (POMC), together with several peptides that are released together with ACTH (fig. 4.4). There is considerable amino acid sequence homology of ACTH between species and canine

ACTH differs from that of other species by only one amino acid in the carboxy-terminal part of the molecule.[19]

In dogs and cats the PI contains two types of cells that can also synthesize POMC.[20] One is similar to the corticotropic cells of the anterior lobe, in that it reacts with anti-ACTH (fig. 2.6). In the other, ACTH is cleaved into $ACTH_{1–14}$ (precursor of α-MSH) and corticotropin-like intermediate-lobe peptide ($ACTH_{18–39}$ or CLIP) (figs 4.4, 4.5). As for all adenohypophyseal hormones in the dog, the release of α-MSH is pulsatile, albeit in only a few pulses per 24 h (fig. 4.6).[21,22] PI secretory activity is under almost permanent inhibitory control by dopamine (fig. 4.7). In contrast, the PI in cats is actively secreting, responsive to the stress of physical restraint and β-adrenergic stimulants (fig. 4.8).[23,24]

α-MSH regulates the activity of tyrosinase, the rate-limiting melanocyte enzyme necessary for the synthesis of the two coat pigments, eumelanin (brown / black color) and pheomelanin (yellow / red color). A loss-of-function mutation in the receptor (MC1R) to which α-MSH binds on the plasma melanocyte membrane is responsible for the coat color of the yellow Labrador retriever and the golden retriever.[25,26] Melanocyte function does not seem to be fully dependent upon α-MSH of PI origin, for complete hypophysectomy does not lead to striking coat color changes (chapter 4.3.1). Administration of an α-MSH analogue leads to darkening of the coat color.[27] Apart from its classic role in pigment regulation, α-MSH is now known to also have several other biological activities, including control of body weight and anti-inflammatory effects.[28]

ACTH secretion by the AL is regulated by the hypothalamus and central nervous system via neurotransmitters that release the hypophysiotropic hormones corticotropin-releasing hormone (CRH) and arginine-vasopressin (VP) (fig. 4.7). The VP in portal blood is derived primarily from CRH-containing parvocellular neurons that originate in the paraventricular nucleus and project to the median eminence, thereby being fully separated from the VP involved in water homeostasis (chapter 2.3). In this neuroendocrine control four mechanisms can be distinguished: (1) episodic secretion, (2) response to stress, (3) feedback inhibition by cortisol, and (4) immunological factors (fig. 4.7).[29]

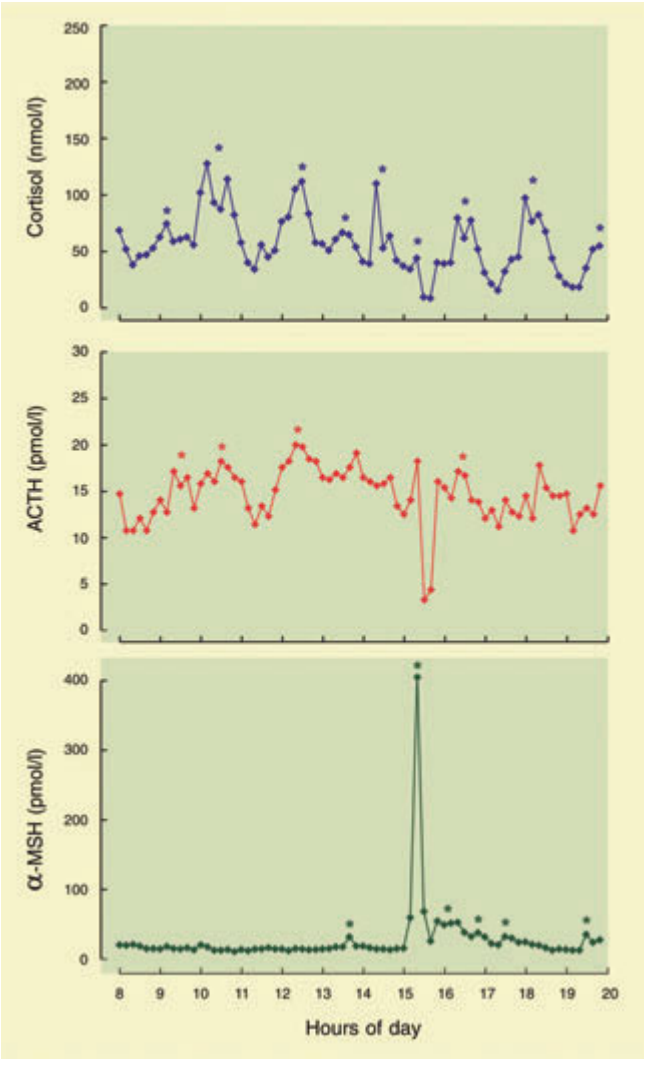


Figure 4.6:
Secretory profiles of α-MSH, ACTH, and cortisol, in a 1.5-year-old healthy beagle. Blood samples were collected at 10 min intervals for twelve hours. Significant pulses are indicated by asterisks.[21]

Central nervous system events regulate both the number and magnitude of ACTH bursts, ranging in the dog from six to twelve per 24 h period.[21,30] The episodic secretion in dogs and cats does not seem to increase in the early morning hours to the extent of a demonstrable circadian rhythm of cortisol concentration in plasma or saliva, as occurs in humans.[31,32]

ACTH and cortisol are secreted within minutes following the onset of stress such as anesthesia and surgery.[33,34] Stress responses originate in the central nervous system and increase the release of hypothalamic hypophysiotropic hormones such as CRH and VP. Dogs and cats seem to differ in their responses to stress. In laboratory dogs several emotional or neurogenic stresses did not stimulate secretion of ACTH or α-MSH[13] and only profound stress such as long-term immobilization consistently resulted in elevations of plasma cortisol.[14] Among privately-owned dogs, only those known to be afraid of gunshots responded to this noise by an increase in plasma cortisol.[35] However, using urinary cortisol as a measure of integrated cortisol production, the stress of introduction into a novel kennel or exposure to veterinary procedures is reflected in elevated urinary corticoid:creatinine ratios.[36–38] In cats, on the other hand, mild stress such as handling and intradermal skin testing causes impressive increases in

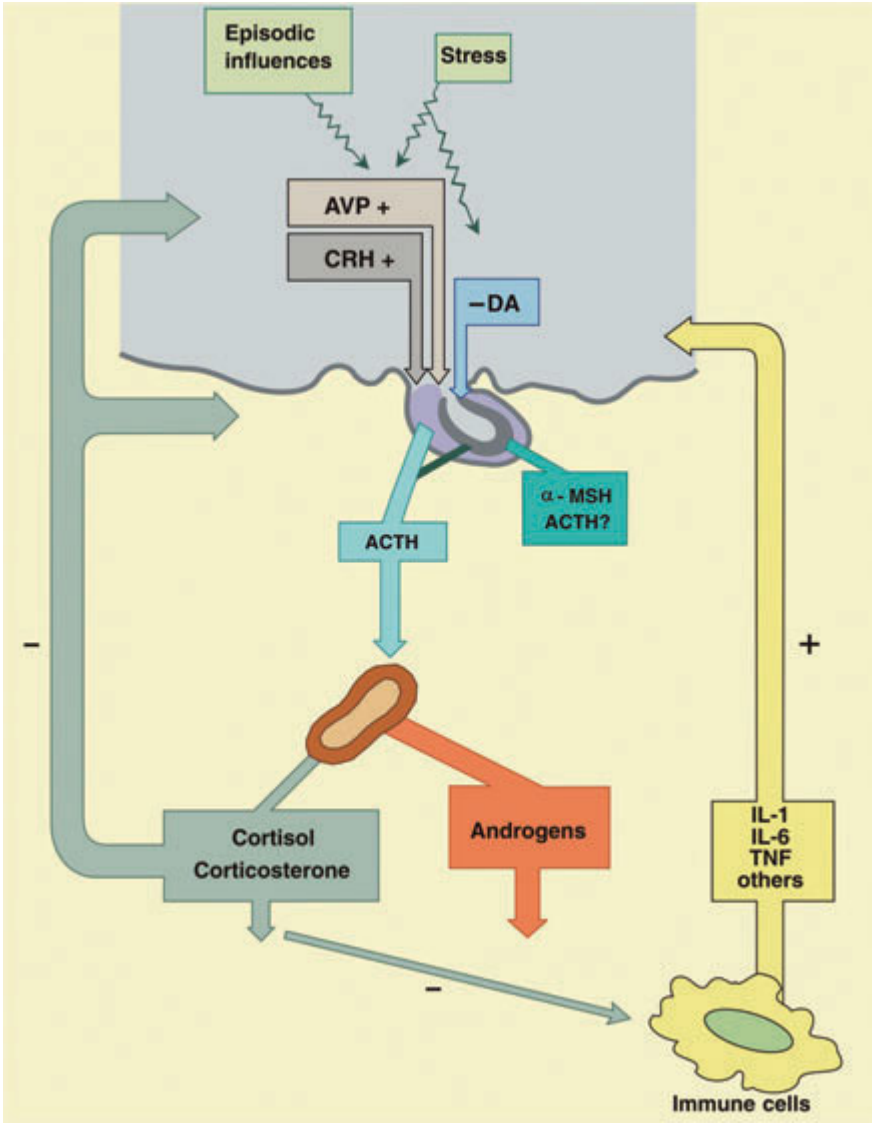


Figure 4.7:
Regulation of adrenocortical secretion of glucocorticoids and androgens. Central nervous system afferents (episodic influences and stress) are mediated by hypophysiotropic hormones such as CRH and AVP to stimulate ACTH release from the anterior lobe of the pituitary. ACTH stimulates the cells of the middle and inner zones of the adrenal cortex to produce chiefly cortisol, which inhibits the secretion and influence of the hypophysiotropic hormones on the corticotropic cells of the anterior pituitary. The melanotropic and corticotropic cells of the pars intermedia are largely under dopaminergic (DA) inhibitory control. The activation of the hypothalamic-pituitary-adrenocortical axis as evoked by challenges to the immune system is shown on the right.

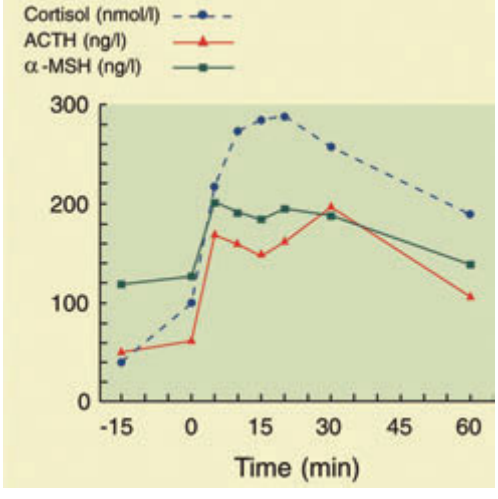


Figure 4.8:
Plasma concentrations of cortisol, ACTH, and α-MSH in six cats after intradermal skin testing between t_0 and t_5 and reading of the skin reactions at t_{15}. Blood was collected via previously placed jugular catheters. (Adapted from Willemse et al., 1993).[23]

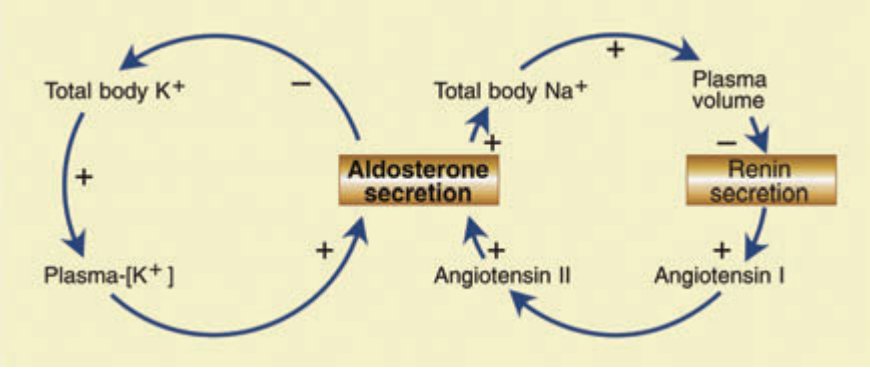


Figure 4.9:
Regulation of aldosterone secretion by the zona glomerulosa of the adrenal cortex. The two main regulators are angiotensin-II and potassium (K^+).

the plasma concentrations of cortisol, ACTH, and α-MSH (fig. 4.8).[23] Corticoid:creatinine ratios in urines collected in a clinic were considerably higher than those in urines collected at home (chapter 12.2.4).[39]

The third major regulator of ACTH and cortisol secretion is feedback inhibition. The inhibitory action of glucocorticoids is exerted at multiple target sites, of which two have been unequivocally identified being the neurons in the hypothalamus that produce corticotropin-releasing factors (CRH and AVP) and the corticotropic cells in the anterior lobe. The feedback actions of glucocorticoids are exerted through at least two structurally different receptor molecules, i.e., a mineralocorticoid-preferring receptor (MR) and a glucocorticoid-preferring receptor (GR). The MR has a 20-fold higher affinity than the GR for cortisol. Inhibition of basal secretion of ACTH by glucocorticoids appears to be mediated via occupancy of the MR. The dog brain and pituitary contain very high levels of MR, the highest being in the septohippocampal complex and the anterior lobe of the pituitary.[40] The GR is more evenly distributed in the brain, the amounts in the anterior lobe being about twice as high. The latter GR is mainly involved in the feedback effect of glucocorticoids released as a result of stress-induced ACTH secretion.

Challenges to the immune system by infections invariably activate the hypothalamic-pituitary-adrenocortical axis. These responses are mediated by proinflammatory cytokines, a group of polypeptides released from colonies of activated immune cells. Although other cytokines, such as interleukin(IL)-6 and tumor necrosis factor α (TNFα), are also associated with the responsiveness to stress, IL-1 particularly activates the hypothalamic-pituitary-adrenocortical axis.[41] It is released from activated macrophages in the periphery and also produced in the brain.[42] The regulatory actions of the cytokines are exerted predominantly at the level of the hypothalamus, where CRH is the major mediator of the hypothalamic response. These cytokine-mediated activations of the hypothalamic-pituitary-adrenocortical axis are also subject to feedback regulation by glucocorticoids, which not only impair the hypothalamic response to cytokine activation but also block cytokine production in macrophages (fig. 4.7). Thus a bidirectional communication exits between the neuroendocrine system and the immune system.[43]

In recent years it has become clear that apart from these four ACTH-dependent mechanisms, ACTH-independent mechanisms also have a role in fine tuning and modulating the response of the highly sensitive adrenocortical stress system appropriately to physiological needs. Studies of pulsatility and hormone kinetics have revealed asynchrony in ACTH and cortisol responses, indicating that signals other than ACTH influence cortisol secretion (fig. 4.6).[44] Multiple systemically-derived factors (neuropeptides, neurotransmitters, growth factors, cytokines, adipokines) and intra-adrenal paracrine regulation can influence release of corticosteroids. Adrenocortical cells express a great variety of receptors for these factors, enabling direct effects on cortisol release in health and disease. In several disease states, including critical illness, septicemia, and inflammation, there may be disorderly basal cortisol release independent of ACTH.[45] Overexpression of receptors for neuropeptides, neurotransmitters, hormones, or cytokines may give rise to hypercortisolism with suppressed plasma ACTH concentrations (chapter 4.2.3)

4.1.4 Regulation of mineralocorticoid secretion

The two primary mechanisms controlling aldosterone release are the renin-angiotensin system (RAS) and potassium. The RAS keeps the circulatory blood volume constant by promoting aldosterone-induced sodium retention during periods of hypovolemia and by decreasing aldosterone-dependent sodium retention during hypervolemia (fig. 4.9). Potassium

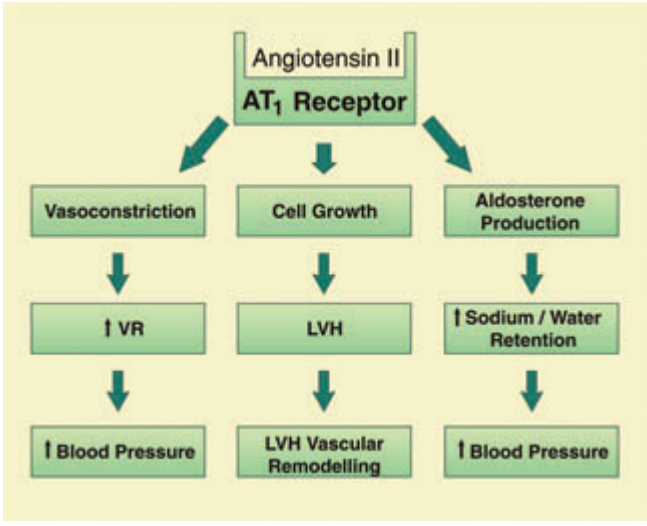


Figure 4.10:
Three major pathways of interaction of angiotensin-II with one of its receptors (AT1 receptor).
VR = vascular resistance; LVH = left ventricular hypertrophy. (Modified after Williams, 2005.)[46]

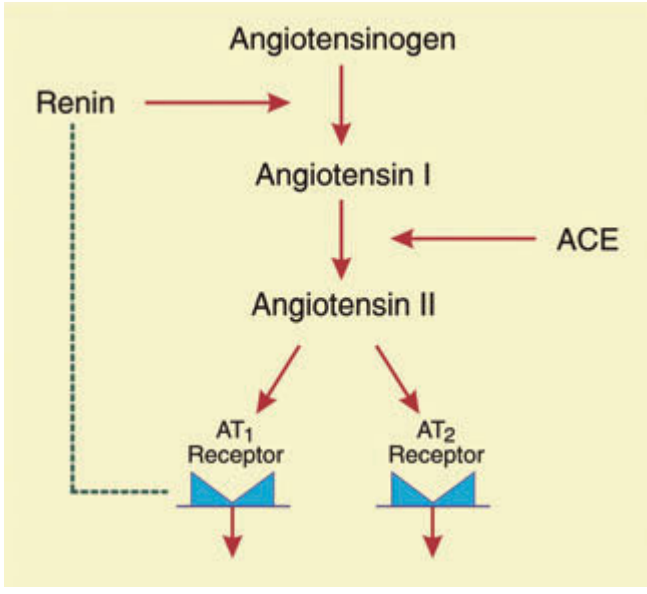


Figure 4.11:
Angiotensin-II synthesis and its interaction with two receptor subtypes, AT_1R and AT_2R.
ACE = angiotensin converting enzyme.

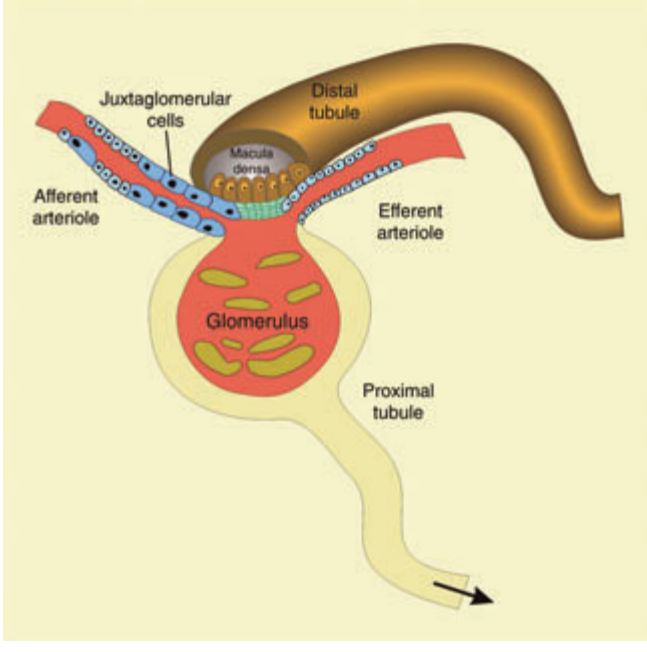


Figure 4.12:
Regulation of renin release from the juxtaglomerular cells of the kidney. Vascular receptors in the afferent arteriole stimulate renin secretion in response to reduced renal perfusion pressure. The macula densa in the distal tubule, adjacent to the afferent arteriole, senses distal tubular Na^+ delivery.

ions directly regulate aldosterone secretion, independently of the RAS. Hyperkalemia stimulates aldosterone secretion by depolarization, and hypokalemia inhibits it by repolarization, of the membranes of the zona glomerulosa cells. Thus aldosterone secretion is regulated by negative feedback loops for both potassium and the RAS.

In addition to these two regulatory mechanisms, aldosterone secretion is influenced by several other factors (ACTH, natriuretic peptides, and a variety of neurotransmitters), none of which is directly or indirectly connected to a negative feedback loop. They also have the common feature of usually responding to stress. ACTH is the classic representative of the group. While it is a very potent acute aldosterone secretagogue, its action is not sustained and it is not necessary to maintain normal glomerulosa cell function.[46]

The vast majority of the physiological actions of the RAS are mediated by angiotensin-II and one of its receptors (AT_1R). They include arteriolar vasoconstriction, cell growth, and aldosterone production (fig. 4.10). Angiotensin-II elevates vascular resistance and blood pressure, this being partially counteracted by the direct inhibitory action of AT_1Rs on renin biosynthesis and secretion (fig. 4.11). Angiotensin-II regulates the glomerular filtration rate and renal blood flow by constricting the efferent and afferent glomerular arterioles. Angiotensin-II has multiple effects on cardiac tissue (fig. 4.10). The actions of angiotensin II mediated by AT_2R are less well understood, but AT_2Rs may have a counterregu-

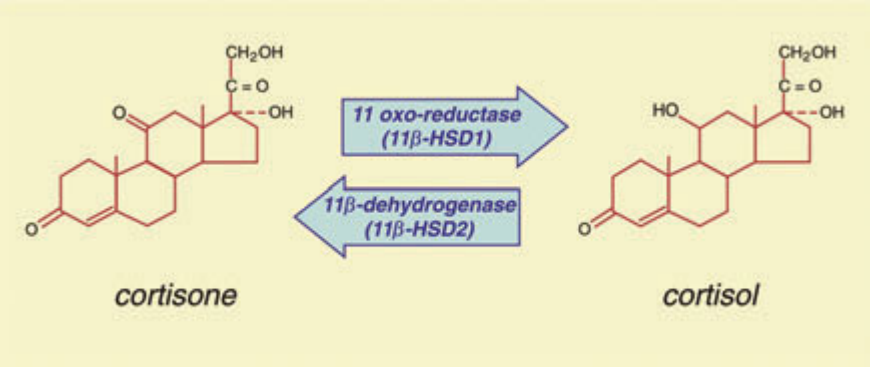


Figure 4.13:
Bidirectional conversion of cortisol and cortisone by isoenzymes (type 1 and type 2) of 11β-hydroxysteroid dehydrogenase (11β-HSD).

latory role opposing AT_1R-mediated vasoconstriction. In addition, activation of AT_2R leads to suppression of renin biosynthesis and release.[47,48]

Angiotensinogen is the precursor of several angiotensin peptides, including angiotensin-II. Angiotensinogen is produced mainly in the liver from its precursor preproangiotensinogen. In the circulation angiotensinogen is cleaved by renin and other enzymes to release angiotensin-I. The angiotensin-converting enzyme (ACE) converts the inactive decapeptide angiotensin-I to the active octapeptide angiotensin-II (fig. 4.11). ACE-inhibiting compounds are used clinically to disrupt the RAS, as in the treatment of heart failure.[49]

The proteolytic enzyme renin is synthesized in the juxtaglomerular cells of the kidney. Stimulation of renal baroreceptors is the most potent mechanism for its release. These stretch receptors in the afferent arteriole stimulate renin release in response to reduced renal perfusion pressure. Additional regulation is provided by the macula densa, a group of modified cells of the distal tubule near the end of the loop of Henle and intimately associated with the juxtaglomerular cells (fig. 4.12). Sodium concentration in the tubular lumen is monitored by the cells of the macula densa and low sodium levels trigger communication between the macula densa and the juxtaglomerular cells, resulting in renin release.

4.1.5 Glucocorticoid action

Tissue-specific actions of glucocorticoids are not only determined by their production rates and the activation of glucocorticoid receptors (GRs). In peripheral tissues, cortisol is metabolized at a prereceptor level by the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD). This enzyme occurs in two isoforms. Type 1 is widely distributed in many tissues, including liver, gonad, and adipose tissue. In vivo it acts predominantly as a reductase, generating active cortisol from the inactive glucocorticoid cortisone (fig. 4.13). The type 2 enzyme is predominantly expressed in mineralocorticoid target tissues such as the kidney (chapter 4.1.6). Expression of both isoenzymes of 11β-HSD is important in controlling tissue-specific action of glucocorticoids. Studies in humans suggest that 11β-HSD1 can facilitate glucocorticoid action by generating cortisol from inactive cortisone in, for example, adipose tissue.[50] Among the species studied, feline 11β-HSDs have the highest homology with the comparable enzymes in humans.[51] In dogs the tissue distribution of both 11β-HSDs is similar to that in humans and rodents.[52] In a study of the species-specific variability of the catalytic efficiency in the reduction of cortisone, the dog was found to have the lowest activity.[53]

The cortisol-activated receptor interacts with specific DNA sequences on target genes, resulting in changes in mRNA synthesis and subsequent synthesis of specific proteins. The transcription of target genes is also influenced by transcriptional coactivators and corepressors recruited by the GR. Inhibition of gene expression is a key component of glucocorticoid action. For example, in immune cells GR inhibits the action of nuclear factor-kappa B (NF-κB), a transcription factor that regulates the expression of several cytokine pathways, thereby exerting multifaceted effects to inhibit the immune response (chapter 4.3.6). These mechanisms together with the presence of GR splice variants and tissue-specific posttranslational modifications (phosphorylation, ubiquitination) are thought to account for the wide array of actions of cortisol.[54] In recent years insight into this diversity of actions has been further extended by the notion that glucocorticoids not only exert genomic effects, but also direct nongenomic effects (chapter 1.1.3).

Central to the metabolic effects of glucocorticoids is the synthesis of mRNAs which lead to synthesis of key enzymes in gluconeogenesis, such as pyruvate carboxylase, fructose-1,6-diphosphatase, and fructose-6-phosphatase. Especially in the fasted state, glucocorticoids contribute to the mainten-

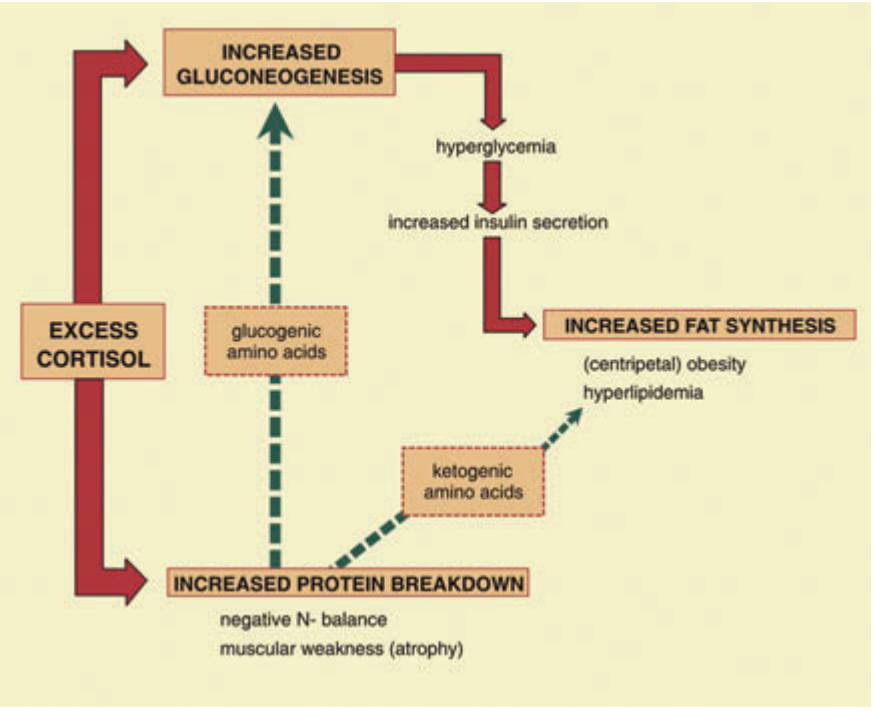


Figure 4.14:
Effects of cortisol excess on intermediary metabolism. Increased gluconeogenesis leads to hyperglycemia, which is controlled initially by increased insulin secretion. This in turn causes increased lipogenesis. Thus the end result of glucocorticoid excess is the catabolism of peripheral tissues such as muscle and skin to deliver the substrate for increased gluconeogenesis and lipogenesis.

ance of normoglycemia by gluconeogenesis and by the peripheral release of substrate. The latter is achieved via decreased glucose uptake and metabolism and decreased protein synthesis leading to increased release of amino acids. In addition, lipolysis is stimulated in adipose tissue. However, in situations of glucocorticoid excess the latter may be overruled by the hyperglycemia-induced hyperinsulinemia that promotes the opposite, i.e., lipogenesis and fat deposition (fig. 4.14).

Through these effects on intermediary metabolism and other effects, glucocorticoids affect almost all tissues and many processes, including blood cells and immunologic functions. Most of these effects are clinically relevant and will be discussed in sections on adrenocortical disease.

4.1.6 Mineralocorticoid action

The widespread mineralocorticoid receptors (MR) have equal affinity for aldosterone and the glucocorticoids cortisol and corticosterone, but the latter two hormones circulate at much higher concentrations than that of aldosterone. This has raised the question how the MR is protected from activation by cortisol. In the classic aldosterone targets (kidney, colon, salivary gland) this is accomplished by the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), which converts cortisol and corticosterone, but not aldosterone, to their 11-keto analogs (chapter 4.1.5, fig. 4.13). These analogs cannot bind to MR, thereby enabling aldosterone to occupy this receptor.[55]

As the major mineralocorticoid, aldosterone has two important actions: (1) it regulates extracellular fluid volume and (2) it is a major determinant of potassium homeostasis. These effects are mediated by the binding of aldosterone and/or deoxycorticosterone (DOC) to the mineralocorticoid receptor in the cytosol of epithelial cells, predominantly in the kidney. Aldosterone and DOC have approximately equal affinities for the mineralocorticoid receptor and circulate at roughly similar concentrations, but aldosterone is quantitatively more important because much more of it circulates as free hormone (chapter 4.1.2). In the distal convoluted tubule aldosterone and DOC increase the reabsorption of sodium and the excretion of potassium.

Once the hormone-receptor complex has reached the nucleus, it initiates a sequence of events leading to activation of amiloride-sensitive epithelial sodium channels in the apical membrane. Thereafter, increased sodium influx stimulates the Na^+K^+-ATPase in the basolateral membrane. As aldosterone increases active sodium reabsorption, an electrochemical gradient is established that facilitates the passive transfer of potassium from tubular cells into urine. Thus potassium is not

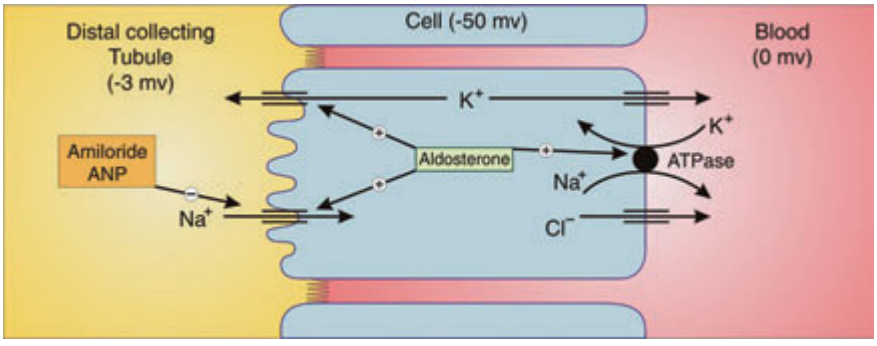


Figure 4.15:
Electrolyte transport in the distal renal tubule. Na^+,K^+-ATPase in the basolateral membrane is a major driving force for electroneutral cotransport by keeping intracellular Na^+ low and the cell interior negative. Potassium leaves the cell through conductance channels, driven by a concentration gradient. Aldosterone activates sodium channels, which can be inhibited by thiazide diuretics, amiloride, and atrial natriuretic peptide (ANP). Aldosterone also activates potassium channels and Na^+,K^+ATPase.

excreted in direct exchange for sodium, but rather in a manner that depends directly on the active reabsorption of sodium (fig. 4.15). If almost all sodium is reabsorbed more proximally in the nephron, as in the presence of severe volume depletion, little sodium reaches the distal reabsorptive site. Hence, despite high levels of aldosterone, there is minimal potassium excretion in the absence of sodium delivery to the distal tubule. Conversely, a high sodium intake will increase potassium excretion. This is particularly true if the animal is receiving a diuretic that blocks part of the proximal reabsorption of sodium, causing even more sodium to reach the distal reabsorptive site.[56]

In recent years it has become clear that the classical characterization of aldosterone as an electrolyte-regulating hormone is too narrow. In addition to its effects on classic epithelial targets such as kidney, colon, and salivary gland, aldosterone has major actions on other epithelial and nonepithelial tissues. Actions of aldosterone, probably in part nongenomic, on endothelial cells and on cardiac tissue contribute to blood pressure homeostasis.[57] It appears that aldosterone may increase blood pressure through two main mechanisms: (1) mineralocorticoid-induced expansion of plasma and extracellular fluid volume and (2) increased total peripheral resistance. With regard to the nonepithelial actions, it should be added that long-term mineralocorticoid excess may lead to microangiopathies with fibrosis and proliferation of endothelial and smooth muscle cells, in tissues such as heart and kidney (see also chapter 4.4.1).[58]

4.1.7 Adrenal androgens

ACTH stimulates the secretion of the adrenocortical androgens, dehydroepiandrosterone (DHEA) and androstenedione (fig. 4.3). Discrepancies between adrenal androgen and glucocorticoid secretion have led to the proposal of an additional »cortical androgen-stimulating hormone« (CASH). Many putative CASHs have been proposed, including POMC derivatives, prolactin, and IGF-I, but definite proof is lacking.[59]

In the absence of gonads, adrenocortical androgen production does not meet physiological requirements (chapter 8.2). In contrast to humans, dogs and cats with increased androgen secretion accompanying ACTH-dependent hypercortisolism do not develop dermal or behavioral symptoms of androgen excess. Their clinical manifestations are primarily determined by the glucocorticoid excess. However, occasionally sex steroid production by an adrenocortical tumor leads to physical and behavioral changes due to androgen excess (see also chapter 4.3.3).

4.2 Adrenocortical insufficiency

The term adrenocortical insufficiency includes all conditions in which the secretion of adrenal steroid hormones falls below the requirement of the animal. Its two major forms are: (1) primary adrenocortical insufficiency due to lesions or disease processes in the adrenal cortices and (2) secondary adrenocortical insufficiency due to insufficient ACTH release by the pituitary. In addition to these conditions of absolute hormone deficits, there can be relative adrenocortical insufficiency.

4.2.1 Primary adrenocortical insufficiency

Pathogenesis

Primary hypoadrenocorticism results from progressive destruction of the adrenal cortices, which must involve 90 % or more of the adrenocortical tissue before it causes symp-



Figure 4.16:
Cross-section of an adrenal of a healthy dog (A) and a dog with Addison's disease (B) in which the adrenal medulla is only surrounded by the capsule.

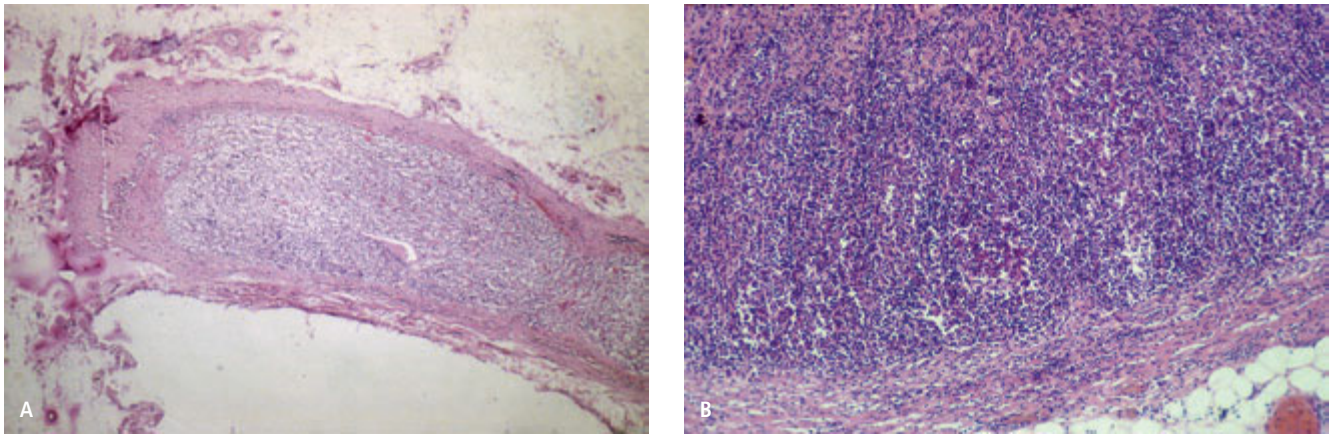


Figure 4.17:
(A) Section of an adrenal of a dog with primary adrenocortical insufficiency. The adrenal medulla is only surrounded by the fibrous capsule. All three zones of the cortex have completely disappeared.
(B) Lymphocytic adrenalitis throughout the cortex (HE, x10). Lymphocytic adrenalitis is probably an immune-mediated process that destroys the adrenal cortex with the end result as shown on the left.

toms and signs (fig. 4.16). The atrophy that is often found (fig. 4.17) is probably the end result of immune-mediated destruction. The condition is also termed Addison's disease, after Thomas Addison, a physician who in 1855 first described the syndrome in man, which at that time was usually the result of tuberculosis. Adrenocortical autoantibodies have been reported in most human patients with nontuberculous Addison's disease. The major autoantigens involved in the reaction with the adrenocortical autoantibodies include 21-hydroxylase, 17α-hydroxylase/17,20-lyase, and cholesterol side-chain cleavage enzyme, with 21-hydroxylase being the most common.[60] Primary hypoadrenocorticism in dogs was first described in 1953 by Hadlow.[61]

The immune-mediated destruction typically terminates in absolute deficiencies of glucocorticoids and mineralocorticoids, together with high plasma levels of ACTH due to pronounced negative feedback to the hypothalamus and pituitary (fig. 1.8). The destruction may also be confined to the middle and inner zones of the adrenal cortex, resulting in what is known as atypical primary hypoadrenocorticism. This may be more common than is generally appreciated, for it is easily overlooked because of the absence of mineralocorticoid deficiency, the main determinant of the symptoms and signs of typical or classic primary hypoadrenocorticism.[62] In a minority of cases, atypical primary hypoadrenocorticism progresses to include mineralocorticoid deficiency within months after the initial diagnosis.[62] There has also been one reported case of isolated hyperreninemic hypoaldosteronism in a dog.[63]

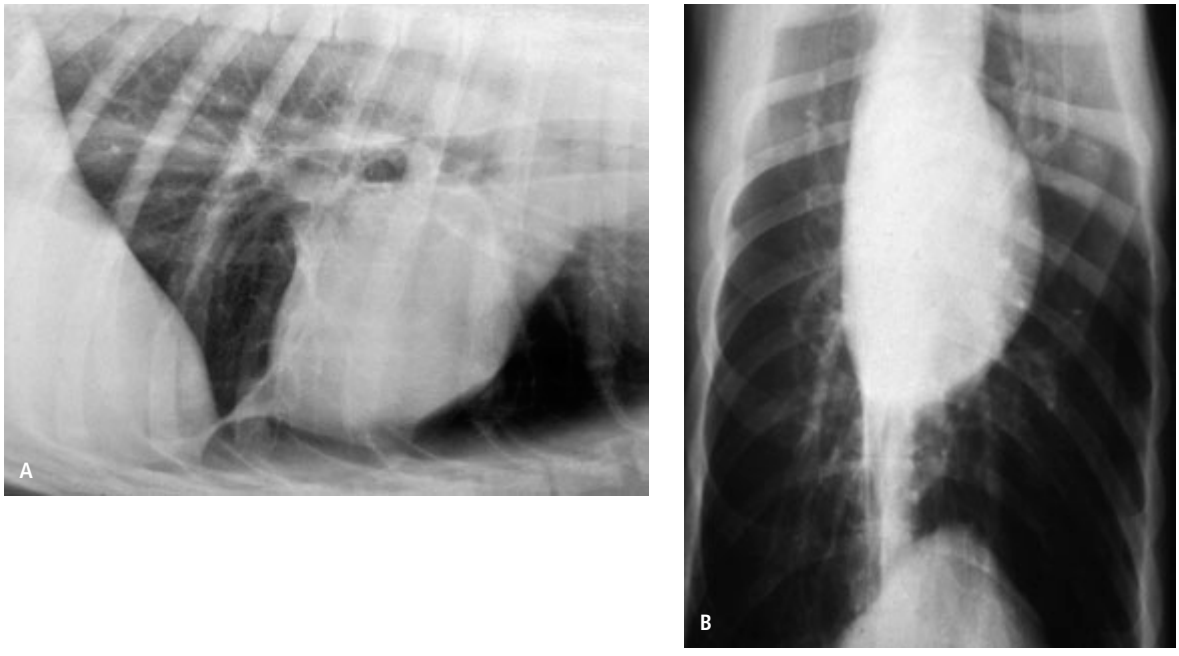


Figure 4.18:
Lateral (A) and dorsoventral (B) radiographs of a two-year-old male dog that arrived in a hypovolemic crisis due to primary hypoadrenocorticism. Hypovolemia is clearly evident in the microcardia and the poor filling of the caudal vena cava and pulmonary vessels.

As mentioned in chapter 3.3.1, primary hypoadrenocorticism may be part of a polyglandular deficiency syndrome.[64] Concurrent endocrine gland failure may include primary hypothyroidism, type I diabetes mellitus, and primary hypoparathyroidism.

Other possible causes of primary adrenocortical insufficiency include adrenocortical hemorrhage, fungal infection, and metastatic disease,[65] but they appear to be rare. Finally, treatment of hypercortisolism with o,p'-DDD or trilostane may deliberately or unintentionally destroy the adrenal cortices to the extent that iatrogenic hypoadrenocorticism ensues (chapter 4.3.1).

Clinical manifestations

Primary hypoadrenocorticism is an uncommon disease of primarily young to middle-aged dogs (mean four years) with a predilection for females.[64] The disorder has been documented in dogs as young as eight weeks.[66] Great Danes, Portuguese water dogs, Rottweilers, standard poodles, West Highland white terriers, bearded collies, Leonbergers, Nova Scotia duck tolling retrievers, and soft coated wheaten terriers have a higher relative risk of developing hypoadrenocorticism than dogs of other breeds. Moreover, familial occurrence has been documented.[67–69] Despite the breed predisposition and occurrence in certain families, the mode of inheritance of hypoadrenocorticism is undetermined in most breeds. Genetic studies have shown that in Portuguese water dogs, standard poodles, and Nova Scotia duck tolling retrievers it is an inherited disorder under the control of a single autosomal recessive locus.[66,70,71]

In cats hypoadrenocorticism is also a disease of young to middle-aged animals but it appears to be very rare in this species.[72,73] In the limited number of cases reported thus far, no sex predilection has been observed. There have been two reported cases of primary hypoadrenocorticism in cats due to infiltration of the adrenals by malignant lymphoma.[74]

As the disease is usually caused by gradual autoimmune destruction of the adrenal cortices, one might expect an insidious onset of slowly progressive weakness, fatigue, anorexia, and vomiting. Although this can be the case, frequently the animal is presented as an emergency in a state of severe depression, weakness, and hypotonic dehydration (fig. 4.18). The initial symptoms may have been very mild or scarcely recognized by the owner except in retrospect. Apparently the animal has been able to cope with the hormone deficits until a critical threshold in the maintenance of fluid and electrolyte homeostasis has been passed.

Although glucocorticoid deficiency may cause some lethargy, weakness, gastrointestinal disturbances, and mild nonregenerative anemia, all of which will certainly contribute to the

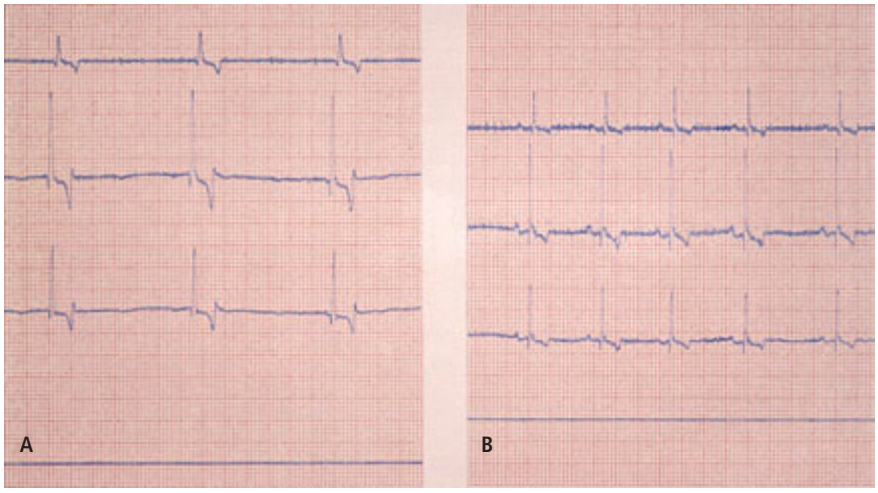


Figure 4.19:
ECG recordings (leads I, II, and III) of a four-year-old dog with primary hypoadrenocorticism (calibration: 1 cm = 1 mV; paper speed 25 mm/s).
(A) Before treatment (Na^+ = 131 mmol/l; K^+ = 8.7 mmol/l) there was extreme bradycardia and no P-waves.
(B) Treatment more than doubled the heart rate and P-waves reappeared.

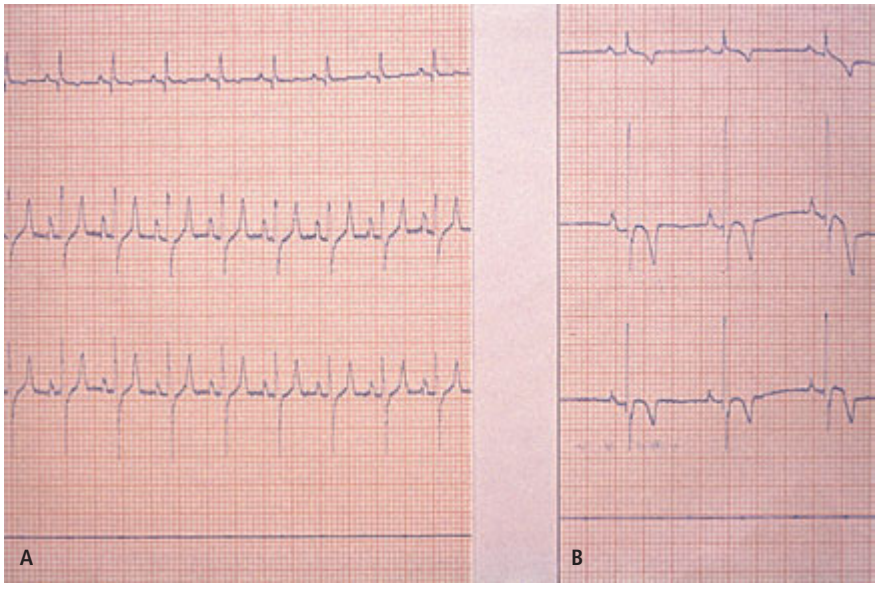


Figure 4.20:
ECG recordings (leads I, II, and III) of a three-year-old female beagle with primary hypoadrenocorticism (calibration: 1 cm = 1 mV; paper speed 25 mm/s).
(A) Before treatment (Na^+ = 137 mmol/l; K^+ = 6.8 mmol./l) the R-waves (lead II) were low and the T-waves were high and spiked.
(B) After treatment the R-waves became normal and the polarity of T-waves was reversed.

clinical manifestations,[75] the manifestations are primarily caused by mineralocorticoid deficiency. Many of the symptoms and signs (table 4.2) can be related to hypotonic dehydration due to the loss of sodium (fig. 4.18). Hyperkalemia contributes to the problems by affecting neuromuscular function, particularly leading to cardiac conduction disturbances. A low heart rate that is inappropriate for the physical condition of the patient should alert the clinician for the possibility of hyperkalemia (fig. 4.19), but the heart rate may not be very low if plasma potassium is not high enough to cause bradycardia and/or the heart rate is increased by the sympathetic drive resulting from the hypovolemic shock (fig. 4.20).

Differential diagnosis

The early symptoms and signs are often vague and mimic those of other diseases, but the cardinal features of the advanced stage of the disease – rapidly worsening depression, weakness, anorexia, and vomiting – evoke only a few differential considerations: ileus, renal insufficiency, acute gastroenteritis, or acute pancreatitis. Initially the differentiation may pose problems, as these conditions are occasionally also associated with electrolyte disturbances, but, further diagnostic work-up and especially the prompt response to treatment usually supports the suspicion of hypoadrenocorticism.

Table 4.2: Clinical manifestations of primary hypoadrenocorticism

System	Common	Less common
Metabolic	Poor appetite / anorexia, weight loss	Hypothermia
Neuromuscular	Lethargy / depression, weakness	Shaking / shivering, fascicular muscle contractions, restlessness, megaesophagus
Cardiovascular	Dehydration / hypovolemia (10–15 % of body weight), hypotonic veins, weak pulse. ECG: wide or absent P wave, wide QRS complex, low R wave, and high T wave	First-, second-, or third-degree atrioventricular block
Gastrointestinal	Anorexia, vomiting, diarrhea	Melena, abdominal pain
Renal & plasma biochemistry	Prerenal azotemia, hyponatremia, hyperkalemia, hyperphosphatemia, acidosis	Inappropriately low urine SG, hypoglycemia, hypercalcemia
Hematological	Hypoplastic anemia (usually masked by hemoconcentration due to dehydration)	Lymphocytosis, eosinophilia

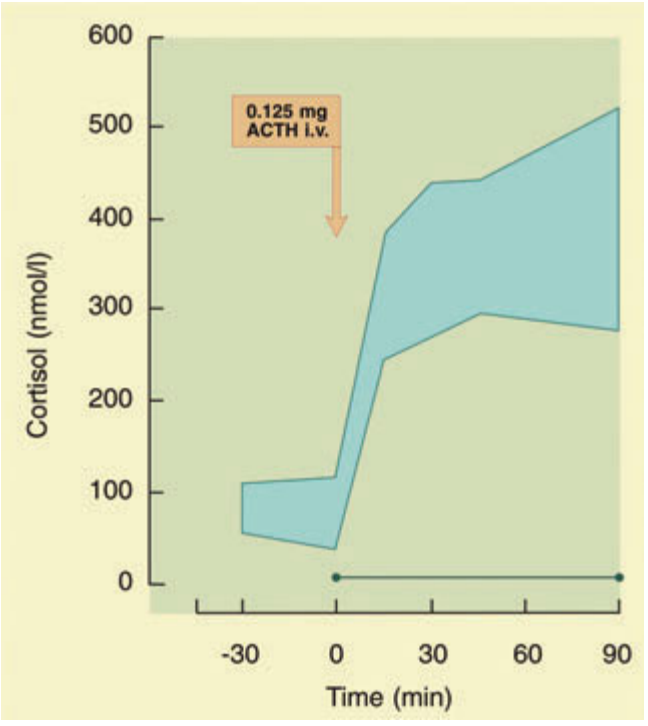


Figure 4.21:
Results of an ACTH-stimulation test in healthy cats (blue area) and in a cat with primary hypoadrenocorticism (solid line).

Diagnosis

From a pathophysiologic point of view, a Na:K ratio < 27 may be regarded as pathognomonic for typical primary hypoadrenocorticism.[64,76] However, such a low ratio may be found in several other conditions, including renal insufficiency, diabetes mellitus, and gastrointestinal disease, and it can also be caused by EDTA contamination of the sample.[77–79]

Given the characteristic biochemical findings of prerenal azotemia, hyponatremia, and hyperkalemia, together with a good response to treatment, there may be little doubt about the diagnosis. However, its consequence is lifelong treatment and therefore it should always be secured by a confirmative test. Basal levels of cortisol in urine and plasma are low in primary hypoadrenocorticism,[80] but they may also be low for other reasons (chapters 4.2.2, 4.3.6). Similarly, basal plasma aldosterone concentration (PAC) is low in dogs with complete primary hypoadrenocorticism, but may also be low in dogs without hypoadrenocorticism.[81,82] Therefore a test of adrenocortical reserve capacity is necessary to establish the diagnosis, i.e., the ACTH-stimulation test (fig. 4.21).

In the ACTH-stimulation test, synthetic ACTH (cosyntropin or tetracosactrin) is administered intravenously or intramuscularly and blood is collected immediately before and at 60 min after the injection for measurement of plasma cortisol. In healthy dogs, plasma cortisol concentration rises to 270–690 nmol/l after ACTH. In dogs with primary adrenocortical insufficiency it usually increases < 50 nmol/l above the low basal value (chapter 12.4.1). In dogs with typical primary hypoadrenocorticism there is also no significant rise in PAC following ACTH administration.[81,82]

In some cases the results of an ACTH-stimulation test using measurements of plasma cortisol may lead to erroneous conclusions. Chronic ACTH deficiency, such as after long-term glucocorticoid therapy or pituitary disease, may lead to severe atrophy of the glucocorticoid-producing zones of the adrenal cortices and consequently to hyporesponsiveness to ACTH administration. Furthermore, the ACTH-stimulation test usually does not include measurements of PAC. For these reasons and because of concerns about the availability and high cost of injectable ACTH,[83] alternatives have been developed. These are based on changes in the relations of the relevant endogenous hormones, i.e., the ACTH:cortisol ratio and the aldosterone:renin ratio. A recent study found that these ratios in dogs with Addison's disease did not overlap those in healthy dogs (figs. 4.22, 4.23).[84] Measurement of these ratios in a single blood sample tests two specific diagnoses: primary hypocortisolism and primary hypoaldosteronism.

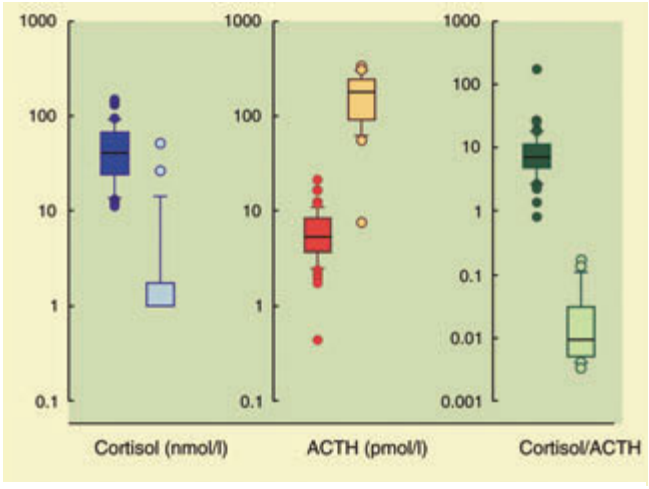


Figure 4.22:
Box-and-whisker plots of plasma cortisol and ACTH concentrations and the cortisol:ACTH ratio in 60 healthy dogs and 22 dogs with primary hypoadrenocorticism. The box represents the interquartile range from the 25th to 75th percentile. The horizontal bar through the box indicates the median, and the whiskers represent the main body of data. Outlying data points are shown by dots and open circles.

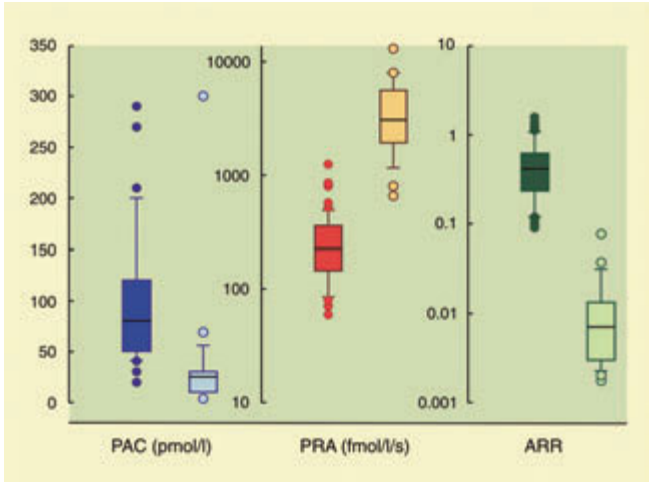


Figure 4.23:
Box-and-whisker plots of plasma aldosterone concentration (PAC), plasma renin activity (PRA), and the aldosterone:renin ratio (ARR) in 60 healthy dogs and 22 dogs with primary hypoadrenocorticism. See also legend for fig. 4.22.

The atrophy of the adrenal cortices reduces the length and thickness of the adrenal glands as determined by ultrasonography, but dimensions in dogs with hypoadrenocorticism overlap those in healthy dogs.[76,85]

Treatment

Animals presented in hypovolemic shock and suspected of having primary hypoadrenocorticism are treated without waiting for laboratory results. The aim is to correct the hypovolemia and electrolyte imbalance by fluid therapy and corticosteroid administration (fig. 4.24). Just prior to starting fluid administration, blood and urine are collected for routine laboratory analyses (table 4.2). If later the suspicion of hypoadrenocorticism is removed, it is reassuring to know that the core of the protocol is the correction of hypovolemia and that this and the corticosteroids will not be harmful in hypovolemic shock due to other causes.

The initial treatment scheme for an acute crisis of suspected primary hypoadrenocorticism consists of fluid therapy and parenteral administration of a glucocorticoid and a mineralocorticoid (see chapter 13.2.1). If the hyponatremia is severe, plasma sodium should be monitored during initial treatment to avoid a too rapid increase that can damage the CNS.[86–88] Most dogs and cats with primary hypoadrenocorticism improve rapidly after treatment is started. Usually dogs begin to eat on the following day, so that oral maintenance therapy can be started. In cats the signs of weakness, lethargy, and anorexia may persist for three to five days despite treatment.[72] The oral maintenance therapy (see also chapter 13.2.1) consists of a glucocorticoid, a mineralocorticoid, and salt (mixed with the food). If the salt causes vomiting directly after the meal, it can instead be added to the drinking water or given in tablet form. Including salt in the treatment provides flexibility in the adjustment of the mineralocorticoid dose based on plasma electrolyte values (see below). However, it has been reported that dogs do well without the addition of salt to glucocorticoid and mineralocorticoid substitution.[89]

Client instruction and follow-up

At discharge the importance of accuracy in administering the substitution therapy is explained to the owner. The first follow-up examination is made two to three weeks later. Plasma sodium and potassium concentrations are measured to determine whether adjustments are needed in the doses of mineralocorticoid and salt. These adjustments are made as follows:

- A slight increase or decrease in sodium combined with a normal potassium is corrected by adjusting the dose of salt alone.
- If sodium is low and potassium is high, or vice versa, only the dose of fludrocortisone is changed.
- If sodium is normal and potassium is abnormal, the dose of fludrocortisone is changed and the measurements are repeated in two to three weeks to determine whether the dose of salt must also be changed.

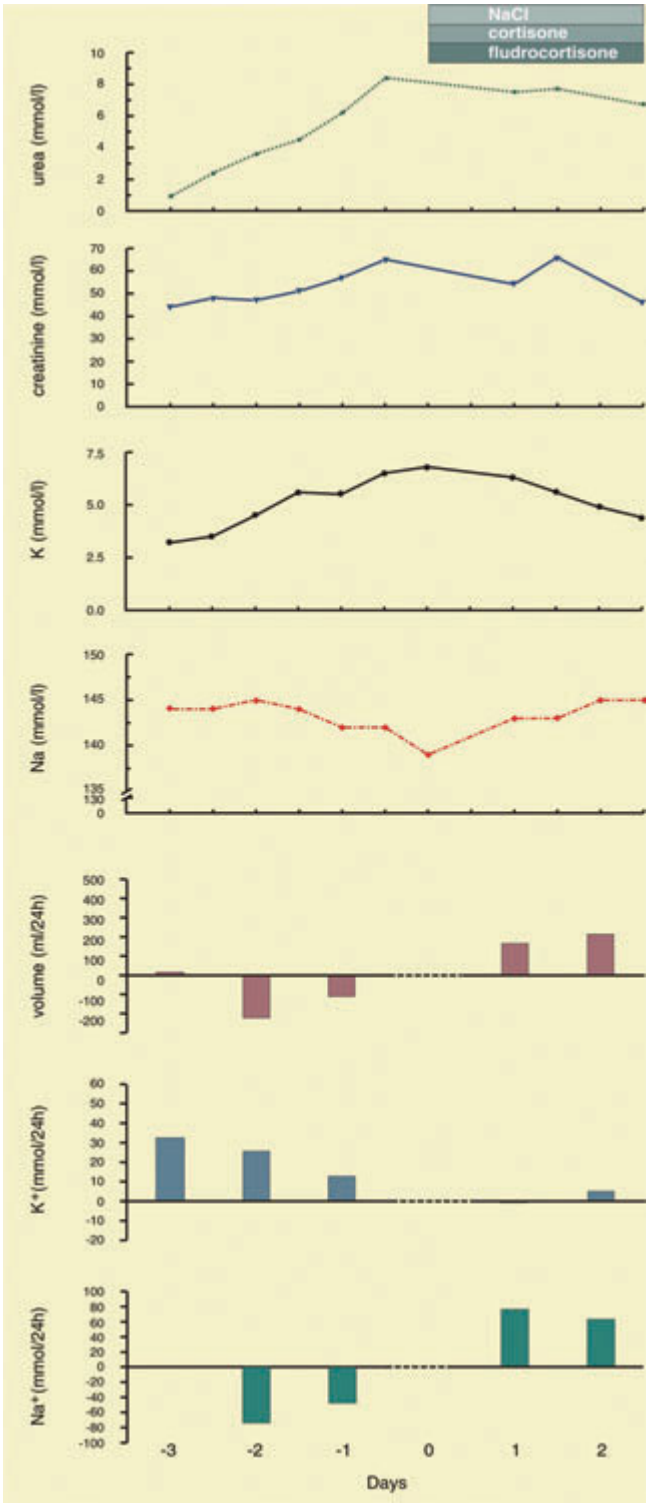


Figure 4.24:
Plasma urea, creatinine, sodium, and potassium concentrations and fluid and electrolyte balance in a six-year-old cocker spaniel that recovered from unrecognized primary hypoadrenocorticism with fluid therapy alone. No treatment was given from day –3 to day 0. The losses of sodium and fluid and the retention of potassium were compatible with primary hypoadrenocorticism and were reversed by treatment on days 1 and 2.

Adjustment of the dose of glucocorticoid is mainly guided by the history at follow-up. The dose is increased if there are symptoms and signs of hypocortisolism (lethargy, inappetence), and decreased if there are signs of hypercortisolism (polyuria, polyphagia).

The dose of glucocorticoid is increased during situations of stress such as fever, surgical procedures, injuries, or gastroenteritis with fluid loss. A good rule is to double the dose during periods of minor illness and to increase it by two to four times during periods of major stress, such as after intra-abdominal surgery or major trauma.

If the animal is unable to take the medications orally (vomiting, anesthesia), it may become necessary to give them by injection. The owner is provided with an injectable glucocorticoid preparation and if available also an injectable mineralocorticoid preparation, together with appropriate syringes and needles (chapter 13.2.1). If an injectable mineralocorticoid is not available, increasing the cortisone dose by four to six times may provide sufficient mineralocorticoid activity. It should be emphasized to the owner that the injectable medications should definitely be started when two successive oral doses have been missed.

Prognosis

With satisfactory replacement therapy, primary hypoadrenocorticism has an excellent prognosis in both dogs and cats.[89] Once therapy is stabilized, follow-up examinations are made twice yearly.

4.2.2 Secondary adrenocortical insufficiency

In secondary adrenocortical insufficiency there is hyposecretion by the middle and inner zones of the adrenal cortices as a result of ACTH deficiency (fig. 1.8).[64] In its spontaneous and complete form the condition is rare. It may be caused by a large pituitary tumor, which usually gives rise to multiple pituitary hormone deficiencies (chapters 2.2.6, 3.3.2). Secondary hypoadrenocorticism may also be associated with craniocerebral trauma.[90] Isolated ACTH deficiency due to an autoimmune hypophysitis, as described in man,[91] has not yet been reported in dogs or cats.

The iatrogenic form of secondary adrenocortical insufficiency due to long-term corticosteroid therapy is much more common than the spontaneous disease. Via negative feedback this therapy causes chronic suppression of CRH and ACTH synthesis and secretion, and as a consequence atrophy of the zona fasciculata and zona reticularis (fig. 4.1). If the exogenous steroids are discontinued for any reason, a period of relative or absolute hypocortisolism will ensue. After corticosteroid withdrawal several months may be required for full recovery of adrenocortical responsiveness to ACTH and re-

covery of pituitary ACTH release. The likelihood of adrenocortical insufficiency, its magnitude, and its duration all depend on the dose of the corticosteroid that has been given, its intrinsic glucocorticoid activity, and the schedule and duration of its administration. The condition is also be discussed in chapter 4.3.6.

Another iatrogenic form of the disorder is ACTH deficiency due to hypophysectomy (chapters 4.3.1, 13.1.1).

Clinical manifestations

In secondary adrenocortical insufficiency mineralocorticoid production is virtually unaffected, as it is primarily regulated by extrapituitary mechanisms (chapter 4.1.4). Hence there is not the tendency to hypotension and shock that gives primary adrenocortical insufficiency its dramatic features. On the contrary, although glucocorticoid deficiency may result in slight depression, anorexia, gastrointestinal disturbances, and mild nonregenerative anemia, the condition may escape attention for a long time. Nevertheless, it must be regarded as potentially dangerous because of the animal's inability to cope with stress by activating the pituitary-adrenocortical system. Major surgery or trauma might cause a crisis and/or failure to recover from anesthesia unless glucocorticoid supplementation is given (see also chapter 4.2.1). In addition, hypocortisolism may give rise to severe chronic hypoglycemia.[92]

Thus it may happen that the condition is recognized more or less incidentally during routine endocrine studies for problems such as lethargy or alopecia, or that a pituitary tumor has been diagnosed and subsequent studies of pituitary function reveal ACTH deficiency.

Diagnosis

Suspicion of secondary adrenocortical insufficiency is raised by finding a low urinary corticoid:creatinine ratio (chapter 12.4.4) in the absence of hyponatremia and hyperkalemia. In an ACTH-stimulation test (chapter 12.4.1), the basal plasma cortisol level will be low and the response to ACTH will be (1) normal or somewhat impaired or (2) absent. The former response excludes primary hypoadrenocorticism but not secondary hypoadrenocorticism, for a response might still be present soon after onset of the condition. The absence of a response can be the result of a longstanding ACTH deficiency. However, there remains the possibility of primary adrenocortical insufficiency with selective atrophy of the zona fasciculata and zona reticularis but with little or no involvement of the zona glomerulosa.[62,75] For differentiation between these possibilities further studies are required, including measurements of plasma ACTH and a CRH-stimulation test (chapter 12.1.1). In dogs with primary adrenocortical insufficiency, basal plasma ACTH concentration is high and there is an exaggerated response to CRH. In dogs with secondary adrenocortical insufficiency, ACTH levels are low and nonresponsive to stimulation with CRH.[93]

Once there is biochemical certainty about the presence of spontaneous secondary hypoadrenocorticism, the pituitary area should be visualized to search for a lesion causing the ACTH deficiency (chapters 2.2.6, 3.3.2).

Treatment

Although dogs seem to be able to live reasonably well in spite of cortisol deficiency, oral glucocorticoid administration increases activity and alertness. Cortisone acetate is given in a daily dose of 0.5–1.0 mg/kg or prednisolone acetate is given in a daily dose of 0.1–0.15 mg/kg. In addition to a glucocorticoid, treatment of other deficiencies (see chapters 2.2.6, 3.3.2) may be required. The animals are especially at risk during stress, and in those situations the glucocorticoid dose should be increased to prevent a crisis (chapter 4.2.1).

Prognosis

As in secondary hypothyroidism (chapter 3.3.2), the prognosis is highly dependent upon the development of the causative lesion.

4.2.3 Relative adrenocortical insufficiency

Several factors, such as trauma, surgery, and challenges to the immune system by infections, activate the hypothalamic-pituitary-adrenocortical axis. The resulting hypercortisolemia is an essential part of the stress response required for adequate adaptation to these noxious stimuli in order to restore homeostasis and enhance survival (chapter 4.1.3, fig. 4.7). An inadequate response is potentially fatal. In critically ill humans the secretory capacity of the adrenal cortices is commonly insufficient to compensate for the increased demand for cortisol.[94] Because it is not an absolute deficiency of cortisol but rather an imbalance between adrenal output and cortisol demand, this disorder is called relative adrenocortical insufficiency or critical illness-related corticosteroid insufficiency (CIRCI). CIRCI is defined as inadequate corticosteroid activity for the severity of the illness of a patient.[95]

Pathogenesis

The underlying mechanisms of relative adrenocortical insufficiency are largely unknown. It is characterized by insufficient corticosteroid-mediated down-regulation of inflammatory transcription factors. Comparable to diabetes mellitus type 2, it is a consequence of both inadequate circulating glucocorticoid and resistance to glucocorticoids at the tissue level.[96]

Cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1 have been shown to be involved in the development of resistance to glucocorticoids at the tissue level.[97] These cytokines have also been implicated in the reversible dysfunction of the hypothalamic-pituitary-adrenocortical axis during critical illness. TNF-α impairs CRH-stimulated

ACTH release, and studies in humans and dogs have revealed inappropriately low plasma ACTH levels in some patients with critical illness.[98–102] In addition, TNF-α has been shown to reduce cortisol synthesis by inhibiting the stimulatory actions of ACTH on adrenocortical cells.[103] Adrenal hypoperfusion and microvascular disease resulting from disseminated intravascular coagulation may also contribute, and may even result in long-term adrenal dysfunction.

Clinical manifestations

Systemic hypotension refractory to fluid loading and requiring vasopressors is a common manifestation of relative adrenocortical insufficiency in humans and dogs with critical illness.[100,102,104] The systemic hypotension may be due to down-regulation of smooth muscle adrenergic receptors; the expression of these receptors is modulated by glucocorticoids. In addition, the relative glucocorticoid deficiency may interfere with catecholamine production.

Diagnosis

Unlike patients with classic hypoadrenocorticism, those with relative adrenocortical insufficiency generally have normal to elevated plasma cortisol concentrations, but a blunted response in an ACTH-stimulation test. However, there is much controversy concerning the appropriate dose of synthetic ACTH and interpretation of the test results.[105] Several studies in humans have used an intravenous dose of 250 μg, whereas others have used a total dose of only 1 μg per adult human. In dogs the ACTH dose has ranged from 5 μg/kg to 250 μg/dog.[100–102] With regard to interpretation, what constitutes a normal adrenal response to critical illness is unknown, as is the amount of cortisol that is required or is optimal for a given critical illness in an individual patient. The latter is especially hindered by the lack of a test that quantifies glucocorticoid activity at the tissue level. Consequently, the endocrine diagnosis of relative adrenocortical insufficiency remains somewhat elusive at this time.

Results of two recent studies indicate that relative adrenocortical insufficiency is common in critically-ill dogs with sepsis, severe trauma, or gastric dilatation-volvulus. An increment of $<$ 83 nmol/l in the plasma cortisol concentration after synthetic ACTH administration was associated with increased incidence of systemic hypotension, higher likelihood to require vasopressor treatment, and decreased survival.[100,102] Relative adrenocortical insufficiency could not be demonstrated in dogs with critical illness due to canine babesiosis, although the increment in plasma cortisol after ACTH administration tended to be lower than in control dogs. However, dogs with babesiosis having an increment in plasma cortisol $<$ 83 nmol/l had a significantly higher cortisol:ACTH ratio than those with an increment $>$ 83 nmol/l, indicating that delta cortisol concentrations as sole variable to assess the secretory capacity of the adrenal cortices should be viewed with caution.[101]

Treatment

Routine administration of pharmacological doses of corticosteroids to patients with critical illness is inadvisable, because it does not improve outcome and enhances the risk of complications associated with the use of steroids.[106] The risk:benefit ratio of corticosteroid administration should therefore be assessed in each patient. It seems reasonable to initiate treatment with corticosteroids in critically-ill patients with systemic hypotension refractory to fluid loading and a subnormal response to ACTH administration. In these cases, the corticosteroids should ideally be administered in a physiological stress-dose, i.e., a dose sufficient to suppress the proinflammatory response without causing excessive immune paresis. Low doses of hydrocortisone have been reported to improve pressor responsiveness and survival in septic humans with relative adrenocortical insufficiency.[107] There are no reports of studies on the effects of low doses of corticosteroids in companion animal patients with critical illness. The duration of corticosteroid therapy should be guided by the duration of the underlying systemic inflammation.

Prognosis

Following recovery from the critical illness the dysfunction of the hypothalamic-pituitary-adrenocortical axis generally resolves spontaneously.

4.3 Glucocorticoid excess

Cortisol is the principal glucocorticoid released by the adrenals in dogs and cats (chapter 4.1.1). Thus endogenous glucocorticoid excess is essentially hypercortisolism. Prolonged exposure to inappropriately elevated plasma concentrations of free cortisol leads to symptoms and signs often referred to as Cushing's syndrome, after Harvey Cushing, the neurosurgeon who in 1932 first described the syndrome in man. Identical symptoms and signs are elicited by exogenous glucocorticoids in long-term therapy (chapter 4.3.6).

In about 80 % of cases of spontaneous hypercortisolism in both dogs and cats the disease is the result of excessive ACTH secretion by a pituitary adenoma (chapter 4.3.1). In most other cases the disease is ACTH-independent, due to hypersecretion by adrenocortical tumor (chapter 4.3.2). There have been case reports of two other forms of hypercortisolism, one ACTH dependent (chapter 4.3.4) and the other ACTH independent (chapter 4.3.5). The discussion of these different disease entities is preceded by a description of the common denominator of the clinical manifestations, glucocorticoid excess.



Figure 4.25:
A ten-year-old female mongrel dog with classic signs of hypercortisolism: alopecia and truncal obesity, particularly of the abdomen.



Figure 4.26:
A nine-year-old female mongrel dog with severe manifestations of glucocorticoid excess. In addition to the generalized alopecia and calcinosis cutis on the neck and shoulder, there is atrophy of the temporal muscles and muscles of the shoulder, arm, back, and thighs, and lordosis accentuating the pendulous abdomen (see also fig. 4.28).





Figure 4.27:
A nine-year-old female dachshund with hypercortisolism.
(A) The coat on the enlarged abdomen is thin and the atrophic skin readily bunches up into thin folds.
(B) The skin around two nipples showing keratin accumulation in atrophic hair follicles.

Clinical manifestations

Many of the symptoms and signs can be related to the actions of glucocorticoids presented in chapter 4.1.5 and fig. 4.14, namely, increased gluconeogenesis and lipogenesis at the expense of protein. In dogs the cardinal physical features are central obesity and atrophy of muscles and skin (table 4.3, figs. 4.25–4.28). Polyuria and polyphagia are also frequently dominating features.

The abdominal fat accumulation has been related to overexpression of 11β-HSD1 (chapter 4.1.5) in visceral fat, but in Cushing's syndrome due to adrenocortical tumor the expression of this enzyme is not increased in omental adipose tissue, as it is in human obesity.[108] It is also questionable whether this concept holds true for the dog, in which most, if not all, splanchnic cortisol production occurs in the liver.[109] An alternative explanation for the abdominal fat accumulation might be in the autonomic nervous system,

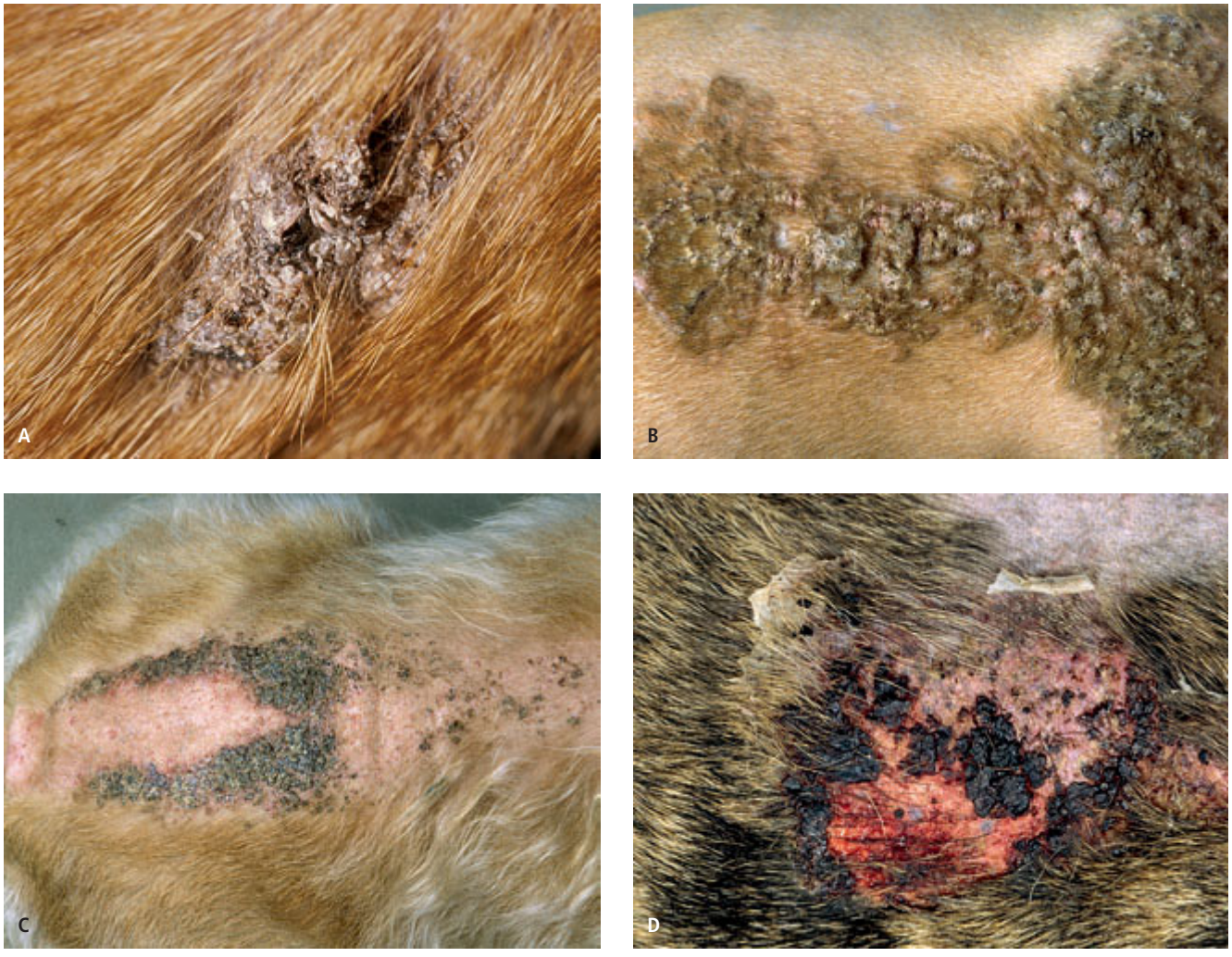


Figure 4.28:
Various manifestations of calcinosis cutis in dogs with hypercortisolism.
(A) Calcium deposits in the skin on the dorsal midline above the shoulder of an eight-year-old female boxer. Palpation revealed irregular firm plaques extending caudally to the lumbar area.
(B) Close-up above the shoulder of the dog in fig. 4.26.
(C) Erythema and calcinosis cutis in the lumbosacral area of a nine-year-old male mongrel dog.
(D) Gray plaques of calcinosis cutis in areas of skin easily traumatized and bleeding in an eleven-year-old male boxer. Calcinosis cutis occurs not only on the dorsal midline but also on the ventral abdomen and inguinal areas.

which is known to modulate lipolysis, lipogenesis, and fat cell number in a compartment-specific manner.[110] This metabolic puzzle may have been largely resolved by recent observations in rodents and humans that glucocorticoid excess changes the activity of AMP-activated protein kinase (AMPK), a sensor of cellular energy status and regulator of enzymes in lipid metabolism, in a tissue-specific manner. Glucocorticoid excess causes inhibition of adipose tissue AMPK, which may explain the accumulation of lipids in visceral fat tissue and, together with the abnormal hepatic AMPK activity, contributes to the development of fatty liver, dyslipidemia, and insulin resistance. In the hypothalamus glucocorticoids increase the AMPK activity, which leads to increased hunger.[111,112]

Glucocorticoid excess leads to muscle atrophy, primarily by inhibiting protein synthesis, to which the suppression of growth hormone secretion must contribute (see also

Table 4.3: Clinical manifestations of glucocorticoid excess in dogs and cats

System	Common	Less common
Metabolic	Polyphagia, weight gain, hepatomegaly, abdominal enlargement	Weight loss (muscle wasting), intolerance to hot environment
Skin and hair	Thin coat, alopecia, thin skin with keratin plugs in atrophic hair follicles	Hyperpigmentation, calcinosis cutis, full thickness skin defects (cats)
Respiratory / Cardiovascular	Panting at rest	Congestive heart failure Pulmonary embolism
Urinary	Polyuria and polydipsia Glucosuria (cats) Proteinuria (usually mild)	Urinary tract infection Glucosuria (dogs)
Neuromuscular	Lethargy, muscular weakness, muscular atrophy	Myotonia
Reproductive	Absence of estrus	Testicular atrophy
Hematology and biochemistry	Eosinopenia, lymphopenia, hyperglycemia (cats), elevated alkaline phosphatase (isoenzyme in dogs), increased ALT, low thyroxine (dogs), hypercholesterolemia, hyperlipidemia	Elevated hematocrit value, hyperglycemia (dogs), hypernatremia, hypokalemia



Figure 4.29:
Glucocorticoid excess usually results in muscle weakness (decreasing ability to climb, jump, and walk) and muscle atrophy. Very rarely there is hypertrophy due to myotonia (persistent muscle contraction) resulting from a degenerative myopathy. Affected dogs walk stiffly, particularly in the hind legs, this eight-year-old female poodle being a severe example. The continuous overextension makes walking very difficult.

fig. 4.29).[113,114] The decreased exercise tolerance and inability to climb stairs and to jump into a car, well-known symptoms of hypercortisolism in dogs, are also due to a generalized decrease in skeletal muscle Na^+K^+-ATPase.[115] The effects of glucocorticoid excess on the skin, hair follicles, and connective tissue include reduced proliferation of keratinocytes and fibroblasts, disturbed metabolism of extracellular matrix proteins, and disturbed synthesis of skin lipids.[116] Depending on the duration of glucocorticoid excess, the changes in dogs range from cessation of shedding, lack of regrowth of clipped hair, and some thinning of the coat to alopecia and a thin and easily-wrinkled skin (fig. 4.27). Probably related to the glucocorticoid-induced alterations in bone metabolism (chapter 9.7), calcium can be deposited in the dermis, causing skin lesions (fig. 4.28). Skin atrophy and immune suppression increase susceptibility to skin lesions and skin infections such as mycobacterial panniculitis and demodicosis.[117,118] It is no exaggeration to say that an adult animal with demodicosis should be suspected of hypercortisolism or hypothyroidism (see also chapter 3.3.1).

In dogs the polyuria of glucocorticoid excess is known to be due to both impaired osmoregulation of vasopressin release and interference with the action of vasopressin (chapter 2.3.2, fig. 2.31). Urinary tract infections, detected by positive urine cultures, are common in dogs with hypercortisolism. However, symptoms are rare and the urinalysis may be normal.[119]

The situation in cats is somewhat different from that in dogs. The cutaneous manifestations may initially give the impression of being less pronounced than in dogs (fig. 4.30). However, in some cases the skin is very fragile and tears during routine handling, leaving the cat with a full thickness skin defect.[120] Furthermore, glucocorticoid excess results in polyuria / polydipsia much less readily than in dogs and may only become obvious when diabetes mellitus develops. Cats are more susceptible than dogs to the diabetogenic effects of glucocorticoids and diabetes mellitus has been present in most of the reported cases of hypercortisolism in cats. Suspicion of hypercortisolism has often arisen specifically because of insulin resistance encountered in the treatment of diabetes mellitus.[121] Only about 10 % of dogs with hypercortisolism develop overt diabetes mellitus.

The disease usually begins insidiously and progresses slowly until the combination of symptoms and signs can be recognized as the syndrome of glucocorticoid excess. However, especially in the beginning, there may be only one or two symptoms (fig. 4.31). Very rarely dogs with glucocorticoid excess are presented as an emergency in respiratory distress. This might be due to the combination of intolerance to a hot environment and impaired ventilatory mechanics because of the physical changes (muscle wasting and enlarged abdomen). However, in such a patient it is also possible that the hypercortisolism is complicated by pulmonary embolism. This state of hypercoagulability is in part due to elevation of procoagu-



Figure 4.30:
A 17-year-old castrated male cat, referred because of problems in controlling its diabetes mellitus. In addition to polyuria, polydipsia, and weight loss, there was alopecia and muscular weakness in the hind legs. Basal UCCRs on two consecutive days (73 and 88 × 10^{-6}) were above the upper limit of the reference range (42 × 10^{-6}).[122] After three oral doses of 0.1 mg dexamethasone per kg body weight the UCCR decreased to 9 × 10^{-6}. CT revealed the pituitary to be moderately enlarged (4 mm wide).



Figure 4.31:
As in most textbooks, illustrations are included in this book to depict pronounced features. However, most diseases begin as only slight deviations in health and it may take several months before the classic changes affecting muscle and skin become apparent. For example, this nine-year-old male boxer had hypercortisolism due to an adrenocortical tumor but was presented only because of polyuria of four weeks duration, without physical changes.

lant factors and a decrease in the naturally occurring anticoagulant factor antithrombin.[123] Glucocorticoid excess has also been reported as a factor predisposing for the rarely occurring aortic / iliac thrombosis in dogs.[124,125]

Endogenous and exogenous glucocorticoid excess increases blood pressure and the highest values are found in dogs with severe hypercortisolism.[126,127] This hypertension is mediated by a variety of mechanisms involving the kidneys and vasculature, and including substrate saturation of 11β-HSD2. In severe hypercortisolism all available cortisol cannot be inactivated to cortisone and thus spills over onto the MR, to cause mineralocorticoid hypertension (see also chapter 4.4).[128] This may be particularly important when renal function is impaired, for in humans with renal disease 11β-HSD2 expression is decreased.[129] In principle hypertension is a risk factor for congestive heart failure, but this complication is rare in dogs with hypercortisolism.

Among the routine laboratory data (table 4.3) a consistent finding is elevation of plasma alkaline phosphatase (AP).[130] In dogs this is mainly due to the induction of an isoenzyme having greater stability at 65 °C than other AP-isoenzymes and therefore easily measured by a routine laboratory procedure. In most dogs with hypercortisolism plasma T_4 is decreased as a consequence of altered transport, distribution, and metabolism of T_4, rather than due to hyposecretion (chapter 3.1.2).

Diagnostic imaging may help to complete the picture of the physical changes that can be associated with glucocorticoid excess. On a lateral radiograph of the abdomen, which is often distended, there is usually good contrast due to the abdominal fat. In addition, hepatomegaly and a distended bladder may be seen, but abdominal radiography is of little use in the diagnostic work-up of dogs suspected of hypercortisolism.[131] Thoracic radiographic abnormalities may include bronchial and interstitial mineralization, particularly in dogs with hypoxemia.[132] Dystrophic calcifications in the skin and subcutis may also be visualized in the areas of predilection for calcinosis cutis. In summary, radiography can help to paint the picture, but is often superfluous. Ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI) are the imaging techniques now most frequently used, especially in the search for the location and characterization of the source of the hormone excess.

Differential diagnosis

For the differential diagnoses concerning the two main clinical features, i.e., polyuria and alopecia, the reader is referred to chapter 14, where algorithms for these problems are presented. Anticonvulsant therapy with phenobarbital may cause symptoms mimicking those of mild hypercortisolism, namely, polyphagia, polyuria, and a slight gain in weight. In contrast to tests of thyroid function (chapter 3.1.2), tests of adrenocortical function in dogs have not been reported to be affected by this treatment.[133,134] In humans phenobarbital induces liver

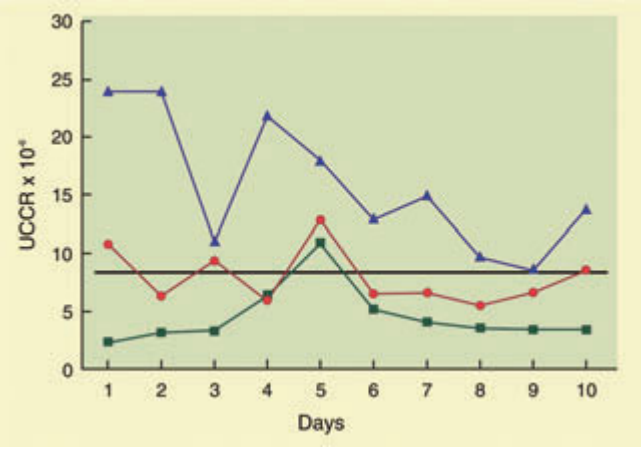


Figure 4.32:
The urinary corticoid:creatinine ratio (UCCR) in three Pomeranians (see also fig. 2.18) with mild hypercortisolism. In one dog (green line) the mean UCCR was 4.7×10^{-6} and only one value exceeded the upper limit of the reference range (8.3×10^{-6}) found in 88 healthy pet dogs (horizontal line).[139] In another dog (blue line) all values were above the reference range (mean UCCR 16.0×10^{-6}), and in the third dog (red line) the UCCRs fluctuated around the upper limit of the reference range (mean UCCR 8.1×10^{-6}).

P-450 cytochrome enzymes, leading to increased steroid clearance and falsely positive dexamethasone suppression tests in patients with Cushing's syndrome.[135]

Diagnosis

The biochemical diagnosis of hypercortisolism depends on the demonstration of two principal characteristics of all forms of the condition: (1) increased production of cortisol, and (2) decreased sensitivity to glucocorticoid feedback.[136] Measurement of the urinary corticoid:creatinine ratio (UCCR) provides an integrated assessment of the secretion of cortisol over a period of time and adjusts for fluctuations in plasma levels caused by the pulsatile release of cortisol (fig. 4.6). For the routine test the owner collects a morning urine sample on two consecutive days and the UCCRs in these two samples are averaged (chapter 12.4.4). In dogs the predictive value of a positive test result is 0.88 and that of a negative test result is 0.98.[137] In some dogs there is considerable day-to-day variation in the UCCR, which in mild forms of hypercortisolism occasionally leads to UCCRs just within the reference range, whereas collections on other days might have revealed one or two elevated UCCRs. The uncertainty can be resolved by measuring the UCCR in urine samples collected on ten consecutive days (fig. 4.32).[138]

The sensitivity of the pituitary-adrenocortical system to suppression is tested by administering a synthetic glucocorticoid in a dose that discriminates between healthy animals and animals with hypercortisolism. A potent glucocorticoid such as dexamethasone is used so that the dose will be too small to contribute significantly to the laboratory measurement. In this dexamethasone screening test or low-dose dexamethasone suppression test (iv-LDDST), 0.01 mg dexamethasone per kg body weight is administered intravenously in the morning. Blood for measurement of cortisol is collected 8 h later. In healthy animals plasma cortisol concentration is still suppressed at this time, whereas in dogs and cats with hypercortisolism it remains high or has escaped from initial suppression (chapter 12.4.2). The predictive value of a positive test result (plasma cortisol $\geq$ 40 nmol/l at 8 h) is 0.92 and that of a negative test result is 0.59.[137]

The iv-LDDST can have a false positive result due to the stress of the hospital visit and the blood collection (chapter 12.4.2). This can be avoided by the use of UCCRs and oral administration of dexamethasone.[140] In this o-LDDST the entire protocol is carried out by the owner at home (chapter 12.4.5).

When hypercortisolism has been confirmed it is necessary to distinguish between the different forms of the disease. This is discussed in the following sections.

4.3.1. Pituitary-dependent hypercortisolism

In both dogs and cats pituitary-dependent hypercortisolism is a disease of middle-aged and older animals, although it can occur in dogs as young as one year. In dogs there is no pronounced sex predilection, but in cats most reported cases have been in females.[141] It occurs in all dog breeds with possibly a slight predilection for small breeds such as dachshunds and miniature poodles. The incidence is much higher in dogs than in humans and has been reported to be one to two cases per 1000 dogs per year.[142] In cats the disease is rare.

The physical changes and the routine laboratory findings are those of glucocorticoid excess, as described in the previous section. Clinical manifestations that it is of pituitary origin are only observed when a pituitary tumor becomes large enough to cause neurological symptoms. These are often vague,[143] consisting of lethargy, inappetence, and mental dullness (see also chapter 2.2.6.2).

The pituitary lesions producing excess ACTH range from small nests of hyperplastic corticotroph (or melanotroph) cells (fig. 2.6) to adenomas (fig. 4.33) and large tumors (figs. 2.20, 4.34).[144] As discussed in chapter 2.2.6, some pituitary adenomas infiltrate surrounding tissues such as the cavernous sinus, dura mater, brain, and rarely the sphenoid bone. These are called »invasive adenomas«, whereas only the exceptional tumors with extracranial metastasis are considered to be carcinomas.[145,146] Corticotroph adenomas may coexist with somatotroph adenomas (chapter 2.2.4.1). The combined occurrence of pituitary-dependent hypercortisolism and cor-

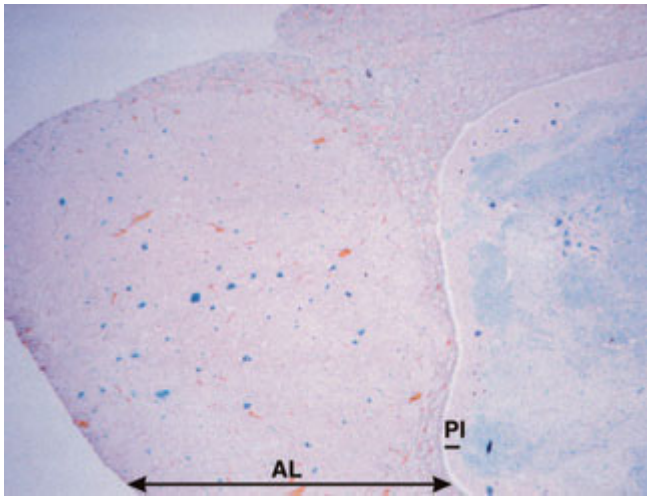


Figure 4.33:
Histological section of the pituitary of an eight-year-old female miniature poodle with pituitary-dependent hypercortisolism due to an adenoma (on the left) in the anterior lobe. On the right, separated by the hypophyseal cleft, is the neurointermediate lobe (PAS-Alcian blue orange-G stain).



Figure 4.34:
Cross section of the ventral two-thirds of the brain of a nine-year-old male boxer with pituitary-dependent hypercortisolism. The enlarged pituitary compresses the hypothalamus but not sufficiently to cause neurological symptoms.

tisol-producing adrenocortical tumor has also been reported, as has the combination with pheochromocytoma (fig. 4.67).[147,148] Pituitary-dependent hypercortisolism may also be a component of a syndrome of multiple endocrine neoplasia.[149,150]

As with several other tumors, the development of pituitary tumors from corticotroph or melanotroph cells is regarded a multistep process requiring more than one mutation in the proto-oncogenes involved in hormone production and/or cell proliferation and possibly also in tumor suppressor genes. An inherited aberration may be the earliest step.[151,152] Expression and mutation analysis has been performed in dogs with pituitary-dependent hypercortisolism for factors involved in pituitary organogenesis and corticotroph differentiation, such as Tpit (see fig. 2.5), and for *ras* proto-oncogenes.[153,154] In addition, the possible role of hypothalamic hormones and intrapituitary growth factors has been investigated.[155,156] These studies have not provided conclusive insight into the molecular pathogenesis of the formation of corticotroph adenomas in dogs. There is now evidence that the hallmark of pituitary-dependent hypercortisolism – resistance to glucocorticoid feedback regulation of the POMC gene by the GR – is caused by loss of nuclear proteins involved in transcriptional repression. These deficiencies may also contribute to tumorigenesis.[157]

In chapter 4.1 it was explained that in dogs and cats both the pituitary anterior lobe (AL) and pars intermedia (PI) have cells that can synthesize POMC, albeit with different post-translational processing. Thus ACTH excess may originate in both the AL and the PI. In about one-fourth to one-fifth of cases there is an adenoma in the PI, but tumors may also occur in both lobes.[158,159] This is of clinical interest not only because the PI tumors tend to be larger than the AL tumors,[144] but also because of the specific hypothalamic control of hormone synthesis in the PI. As mentioned briefly in chapter 2.1, the PI is under direct neural control, principally tonic dopaminergic inhibition,[160] which suppresses the expression of glucocorticoid receptors. This explains why pituitary-dependent hypercortisolism of PI origin is resistant to suppression by dexamethasone.[161]

However, this is not an absolute difference from AL lesions, as pituitary lesions causing hypercortisolism do not maintain the regulation characteristics of the lobe of origin.[162] Corticotroph adenomas in the AL become less sensitive than normal corticotroph cells to the suppressive effect of glucocorticoids. As mentioned in chapters 4.3 and 12.4 this is the functional hallmark of pituitary-dependent hypercortisolism that is used to differentiate normal animals from those with hypercortisolism in the low-dose dexamethasone suppression test (LDDST). This loss of suppressibility can be thought of as being on a sliding scale in both dogs and cats, resistance to glucocorticoid feedback ranging from scarcely demonstrable, in the LDDST, to complete resistance even to high doses of dexamethasone, in the high-dose dexamethasone suppression test (HDDST, chapter 12.4).[163,164]

Resistance to glucocorticoid feedback is significantly correlated with the size of the pituitary (fig. 4.35).[165] Not only do large tumors tend to be more resistant to the suppressive

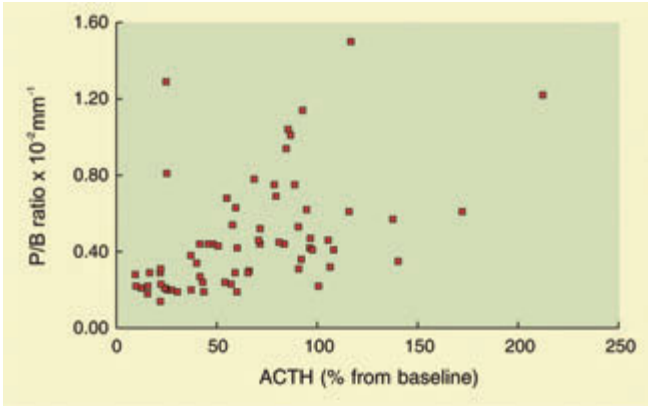


Figure 4.35:
Significant correlation (r = 0.72; P = 0.001) of the pituitary height/brain ratio (P/B) and the percentage of dexamethasone resistance of the plasma ACTH concentrations (ACTH, % from baseline) in 67 dogs with pituitary-dependent hypercortisolism.[163] ACTH (% of baseline) represents the plasma ACTH concentration 4 h after intravenous administration of 0.1 mg dexamethasone per kg body weight as percentage of the plasma ACTH concentration before dexamethasone administration.

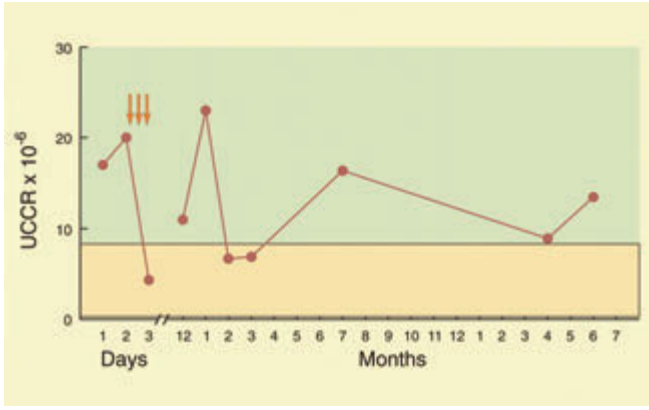


Figure 4.36:
Three daily UCCRs in a 13-year-old female poodle are shown at the left. After the second urine collection the owner administered three doses of 0.1 mg dexamethasone per kg body weight at 8 h intervals. The horizontal band is the reference range for basal UCCRs measured in 88 healthy pet dogs (0.3–8.3 × 10^{-6}).[139] The two basal UCCRs are elevated and the UCCR is then suppressed by more than

effect of dexamethasone, they also release ACTH precursors (POMC, pro-ACTH; fig. 4.35) more often than do small corticotroph adenomas.[166,167] Dogs with high plasma levels of the PI-peptide α-MSH have higher plasma levels of the precursors than do those in which plasma α-MSH is not elevated.[166] The release of incompletely processed or unprocessed POMC by dedifferentiated corticotroph macroadenomas may result in high plasma levels of POMC peptides without excess ACTH and consequently without hypercortisolism.[168] A cat with a melanotroph PI adenoma and extremely high plasma concentrations of α-MSH was found to have no evidence of ACTH-dependent hypercortisolism.[146]

Diagnosis

When hypercortisolism has been confirmed it is necessary to distinguish between pituitary-dependent hypercortisolism and other forms. Despite decreased sensitivity to suppression by glucocorticoids, ACTH secretion in most animals with pituitary-dependent hypercortisolism due to a corticotroph adenoma in the AL can be suppressed by a ten-fold higher dose of dexamethasone, resulting in decreased secretion of cortisol. In the other forms of glucocorticoid excess the hypersecretion of cortisol is not dependent on pituitary ACTH and is therefore not influenced by the high dose of dexamethasone (see also fig. 1.9). Two procedures are used, one employing plasma cortisol and the other employing the UCCR (chapters 12.4.3, 12.4.4). In both, a decrease of > 50 % from baseline values confirms pituitary-dependent hypercortisolism. Often the test for diagnosing cortisol excess and for the differentiation between different forms is combined in one test using UCCRs and oral dexamethasone administration (fig. 4.36).

When there is < 50 % suppression, the hypercortisolism may still be pituitary dependent, due to a pituitary ACTH excess that is extremely resistant to dexamethasone suppression. Further differentiation requires measurements of plasma ACTH. In animals with hypersecreting adrenocortical tumors, basal ACTH concentration is usually suppressed. If interpretation of ACTH values is uncertain, as may occur with the simultaneous occurrence of both entities, further studies are required: a CRH-stimulation test (chapter 12.1.1) and visualization of the adrenals and the pituitary. It may also be helpful to measure plasma α-MSH; high values occur especially with PI tumors, which are often dexamethasone resistant and rather large (chapter 4.3 and fig. 4.37).

As mentioned in chapter 2.2.3, dogs with skin atrophy in breeds such as the miniature poodle and Pomeranian have been found to satisfy two criteria of hypercortisolism: increased cortisol production and decreased sensitivity to glucocorticoid feedback.[138] The routine tests for hypercortisolism (chapters 12.4.2, 12.4.4) are often negative, but serial measurements of the UCCR for ten days may demonstrate the presence of mild and fluctuating hypercortisolism (figs. 4.32, 4.38). Following treatment for hypercortisolism the hair coat returns (fig. 4.39).[169]

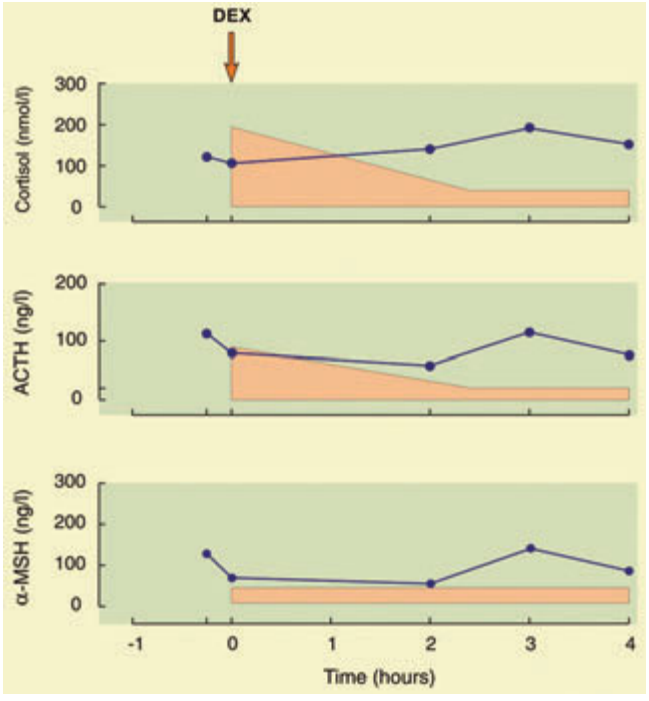


Figure 4.37:
Results of an iv-HDDST test (chapter 12.4.3) in a ten-year-old female standard schnauzer. Dexamethasone-resistant hypercortisolism was indicated by UCCR values (basal 39 and 66 × 10^{-6} and after dexamethasone 31 × 10^{-6}). Plasma concentrations of cortisol and ACTH did not decrease in the iv-HDDST, which together with elevated plasma α-MSH levels, was compatible with a pituitary tumor originating in the PI. Diagnostic imaging revealed both a pituitary tumor and bilateral adrenal tumors.[149]

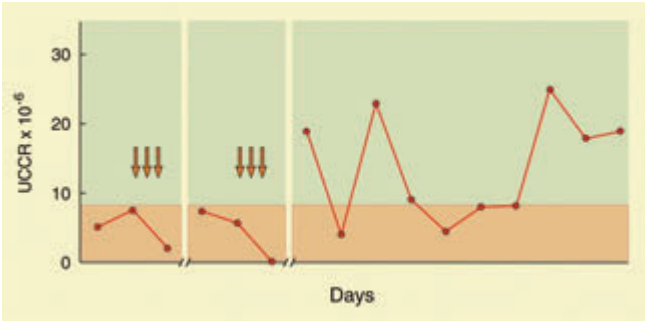


Figure 4.38:
Two dexamethasone suppression tests using UCCRs, in a seven-year-old male miniature poodle with longstanding and gradually progressing alopecia; they were interpreted as indicating suppressible normocorticism. However, when the UCCR was measured daily for ten days, it was found to fluctuate between normal and elevated values (see also fig. 4.32 and legend to fig. 4.36).





Figure 4.39:
A seven-year-old male miniature poodle with mild pituitary-dependent hypercortisolism (fig. 4.38), only manifested by gradually progressing alopecia, before (A) and seven months after destruction of the adrenal cortices with o,p'-DDD (B).

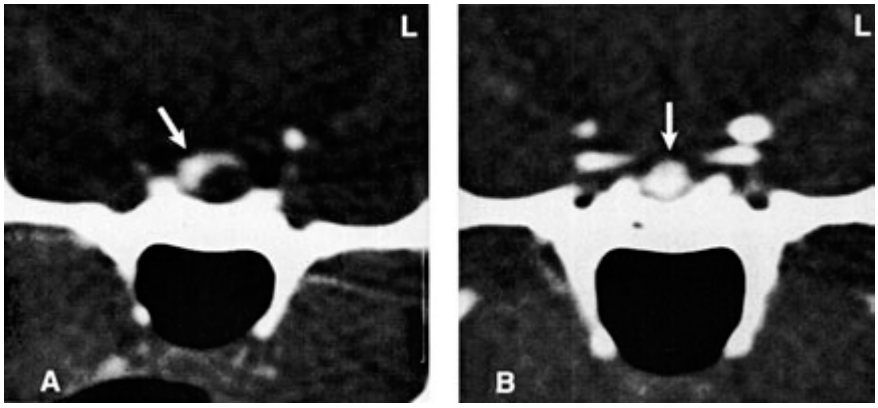


Figure 4.40:
Transverse dynamic CT image through the pituitary fossa at the moment of maximal contrast enhancement of the arterial cerebral circle in a 6-year-old Yorkshire terrier (A) and a 7-year-old Maltese dog (B) with pituitary-dependent hypercortisolism. (A) The pituitary is not enlarged and the pituitary flush (arrow) is displaced dorsally and to the right indicating an adenoma ventrally and to the left. (B) The pituitary gland is not enlarged.

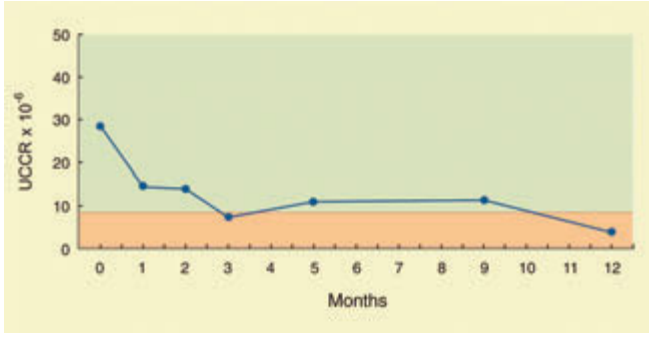


Figure 4.41:
UCCRs (averaged duplicates on two consecutive days) in a seven-year-old castrated male dachshund with alopecia, lethargy, and weight gain due to pituitary-dependent hypercortisolism. Especially because the symptoms and signs were mild, the owners decided to postpone treatment and to follow the course of the disease by UCCR measurements. The dog gradually recovered, became more lively, and lost weight. After about twelve months the hair coat had fully regrown. Such exceptional cases have also been observed in man and have been ascribed to spontaneous necrosis of a pituitary corticotroph adenoma.[172] See also legend to fig. 4.36.

When biochemical findings confirm pituitary-dependent hypercortisolism, the pituitary is visualized by computed tomography (CT) or nuclear magnetic resonance imaging (MRI) (figs. 2.27, 2.28). This visualization is imperative if either hypophysectomy or pituitary irradiation is to be used for treatment.[170] The surgical landmarks for hypophysectomy are best visualized by CT while the zones for intense pituitary radiation with a linear accelerator must be outlined by MRI. Dynamic contrast-enhanced CT facilitates contrast enhancement of the neurohypophysis and the adenohypophysis. Absence of the pituitary flush indicates atrophy of the neurohypophysis due to compression by a pituitary tumor. Displacement or distortion of the pituitary flush in the early phase of dynamic CT can be used to identify and localize microadenomas originating from the AL or PI in dogs (fig. 4.40).[171]

Treatment at the pituitary level

Spontaneous recovery is rare (fig. 4.41) and life expectancy in severe cases is usually less than one year if the disease is left untreated. Death may ensue as a result of complications such as heart failure, thromboembolism, or diabetes mellitus. In mild cases with apparently little progression the course of the disease can be followed by measurements of the UCCR (fig. 4.36).

The treatment of pituitary-dependent hypercortisolism should be directed at eliminating the stimulus for cortisol production, i.e., the pituitary lesion causing excessive ACTH secretion. In the last decade experience has been gained with microsurgical transsphenoidal hypophysectomy in dogs and cats with pituitary-dependent hypercortisolism (fig. 4.42).[173,174] With appropriate short-term and long-term substitution therapy (chapter 13.1.1) this is an effective treatment (fig. 4.43). It can only be performed in specialized institutions with intensive perioperative care, and where imaging techniques such as CT and MRI can be used to define the location and size of the pituitary prior to surgery.

When the surgeon has acquired the necessary experience, the results compare favorably with those of chemotherapy with o,p'-DDD. The main advantage for long-term survival, compared with therapy at the adrenal level (discussed below), is in avoiding the neurological problems that could eventually occur as a result of an expanding pituitary tumor.[175] Survival and disease-free fractions after hypophysectomy are higher in dogs with nonenlarged pituitaries than in dogs with enlarged pituitaries. Also, prolonged central diabetes insipidus is a more frequent complication after hypophysectomy in dogs with enlarged pituitaries than in those with nonenlarged pituitaries.[176] UCCRs higher than 5×10^{-6} and the presence of pulses in plasma ACTH at six to ten weeks after surgery are risk factors for recurrence.[177,178]

Several attempts have been made to reduce pituitary hypersecretion of ACTH medically, but now that the disease is known to be of primary pituitary origin it is understandable

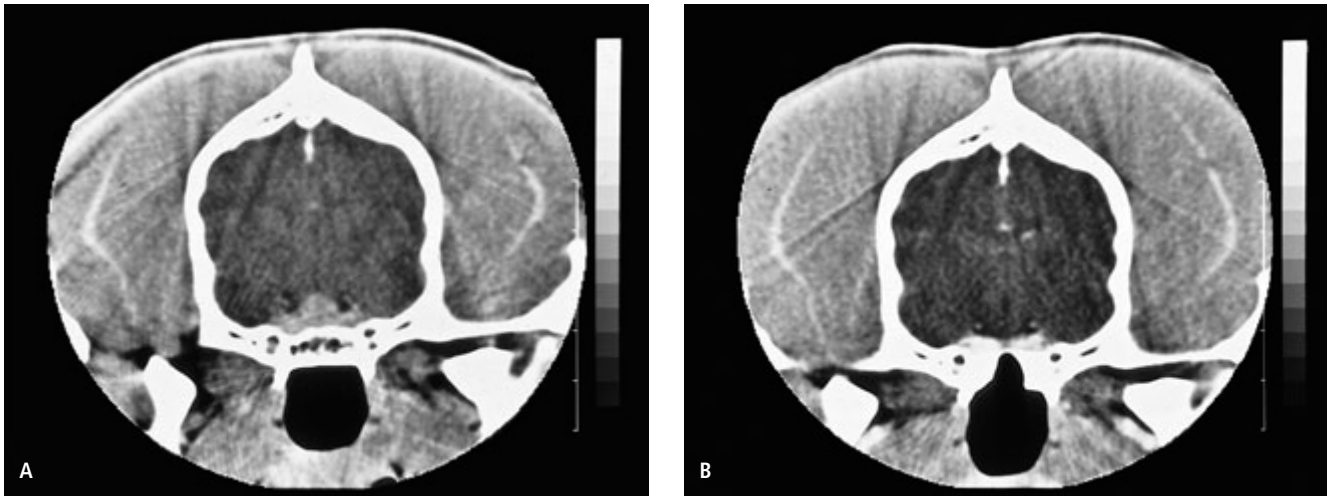


Figure 4.42:
Transverse CT images of the head of a nine-year-old female Bouvier-cross with pituitary-dependent hypercortisolism, before (A) and three months after hypophysectomy (B). Prior to surgery contrast enhancement revealed a pituitary tumor 7.3 mm high and 8.3 mm wide, but no pituitary tissue could be visualized after surgery. In this dog the hypercortisolism was characterized as dexamethasone-resistant because the UCCR after dexamethasone suppression (23×10^{-6}) was $>$ 50 % of the average of the two basal UCCRs (33×10^{-6}). The high basal plasma ACTH (238 and 240 ng/l) and α-MSH (185 and 235 ng/l) concentrations suggested that the tumor originated from melanotroph cells of the pars intermedia. After surgery the UCCR on two consecutive days was $<$ 0.5 and 1.1×10^{-6}. The dog lived for five more years and died from an unrelated condition at the age of 14 years.



Figure 4.43:
(A) Six-year-old castrated male affenpinscher with signs of glucocorticoid excess (polyphagia, alopecia, weight gain, and lethargy) and elevated UCCRs (25 and 13×10^{-6}; ref. range: $0.3–8.3 \times 10^{-6}$) and basal plasma ACTH (56 and 50 pmol/l; ref. range: 0.4–21 pmol/l). CT revealed an enlarged pituitary and dynamic CT revealed a pituitary adenoma (see fig. 4.40). Four months after hypophysectomy (B) there was good regrowth of the hair coat and UCCRs were 0.5 and 0.4×10^{-6}.



Figure 4.44:
An eight-year-old male miniature poodle with pituitary-dependent hypercortisolism and diabetes mellitus before (A) and six months after (B) destruction of the adrenal cortices with o,p'-DDD. In addition to the recovery from hypercortisolism, the insulin demand decreased considerably and remained stable and low.



Fig. 4.45:
A nine-year-old castrated male dachshund with pituitary-dependent hypercortisolism (basal UCCRs 42 and 48 × 10^{-6}; after three oral doses of 0.1 mg dexamethasone/kg: 6 × 10^{-6}). The dog's ravenous appetite was of greatest concern to the owner, illustrated by the empty can which the dog had tried to eat (A). Following destruction of the adrenal cortices with o,p'-DDD and replacement therapy the dog and owner resumed a normal life (B, photograph seven months after initiation of treatment).

that neuropharmacological approaches with an antiserotoninergic drug and a monoamine-oxidase inhibitor were unsuccessful.[179–181] The medical treatment of pituitary-dependent hypercortisolism of PI origin, characterized by high plasma α-MSH concentrations, was aimed at increasing dopaminergic inhibitory tone with the dopamine-agonist bromocriptine. Although a short-term effect was observed, the drug did not prove to be efficacious in lowering UCCRs.[182]

In the interests of new medical therapies the expression of somatostatin receptor subtypes (mainly subtype sst_2) and dopamine receptor subtypes (subtype D_2 modestly expressed) has been identified on canine corticotroph adenomas.[183] The D_2-agonist cabergoline has been reported to decrease plasma ACTH and α-MSH concentrations and UCCRs in slightly less than half of dogs with pituitary-dependent hypercortisolism.[184] Investigators in the same clinic also tested retinoic acid, a ligand for the nuclear receptor peroxisome proliferator-activated receptor-g (PPAR-g), that arrests pituitary tumor growth in a nude mouse model. They observed improvement in both the physical changes and the endocrine variables in all dogs treated.[185] In both studies it is difficult to evaluate the reported recovery, for the UCCRs were lowered but remained around the relatively high upper limit of their reference range and the reduction in size of the pituitary tumor was not completely convincing.

As discussed in chapter 2.2.6.2, the main indication for radiotherapy is to reduce the size of a pituitary tumor that is compressing the brain. Since it usually does not reduce sufficiently the hypersecretion of ACTH, additional therapy at the adrenal level (see below) is required.

Treatment at the adrenal level

This consists of eliminating the glucocorticoid excess by bilateral adrenalectomy or by medical therapy. Total adrenalectomy achieves a complete cure of the hypercortisolism and the prognosis with glucocorticoid and mineralocorticoid replacement (chapter 4.2.1) is good unless or until expansion of the pituitary tumor causes neurological problems (chapter 2.2.6.2). The perioperative and postoperative medication is described in chapter 4.3.2. In the absence of alternatives, bilateral adrenalectomy has also been used in cats, but with complications such as sepsis, thromboembolism, and poor wound healing.[186,187] Presurgical treatment with metyrapone, an inhibitor of steroid synthesis (see below), together with perioperative administration of antimicrobials and heparin can aid in preventing these complications.[188,189]

For many years the most common form of treatment of pituitary-dependent hypercortisolism in dogs has been use of the adrenocorticolytic drug o,p'-DDD. Some treatment schedules aim at selective destruction of the zona fasciculata and zona reticularis, sparing the zona glomerulosa. However, in 5–6 % of the dogs in which this is attempted, the zona glomerulosa is also destroyed to such an extent that iatrogenic hypoadrenocorticism develops. Also, in more than half of the cases in which selective destruction is the aim, there are one or more relapses of hypercortisolism during treatment.[190] In order to circumvent these complications a treatment schedule has been devised with the aim of complete destruction of the adrenal cortices and substitution for the induced hypoadrenocorticism (figs. 4.44, 4.45).[191,192] This nonselective destruction has been reported to be associated with fewer recurrences than with selective destruction.[193] Since the introduction of trilostane for the medical management of pituitary-dependent hypercortisolism, o,p'-DDD is seldom used for this purpose. Its main use now is for the treatment of adrenocortical tumors (chapter 4.3.2).

Trilostane is a competitive inhibitor of the 3β-hydroxysteroid dehydrogenase / isomerase system which is essential for the synthesis of cortisol, aldosterone, progesterone, and androstenedione (fig. 4.3). Trilostane also inhibits other enzymes involved in steroid biosynthesis, such as 11β-hydroxylase and possibly 11β-hydroxysteroid dehydrogenase.[194,195]

In dogs with pituitary-dependent hypercortisolism (PDH), trilostane has the potential of significantly reducing basal and ACTH-stimulated plasma cortisol concentrations.[196–201] The resulting loss of negative feedback, leads to increased plasma ACTH levels.[197,202,203] Very high plasma ACTH may indicate trilostane overdosage.[203]

Trilostane treatment also causes a slight decrease in plasma aldosterone concentration and although it usually remains within the reference range,[197,199] the decrease leads to hypovolemia and activation of the RAS (chapter 4.1.4, fig. 4.9), often with significant increases in plasma renin activity.[203]

Trilostane is absorbed rapidly from the gastrointestinal tract. Administration with food significantly increases the rate and extent of absorption. There is marked variation in the optimal dose and to avoid adverse effects due to overdosage, treatment is started at a relatively low oral dose of 2 mg/kg once daily. The dose is then adjusted according to the clinical response and the results of ACTH-stimulation tests (chapter 13.2.2). The efficacy of treatment is also monitored by clinical signs and measurements of plasma sodium, potassium, urea, creatinine, liver enzymes, and ACTH.[203]

It has been reported that the UCCR cannot be used as an alternative to the ACTH-stimulation test to determine the optimal dose of trilostane.[198,204] In more than half of the dogs with pituitary-dependent hypercortisolism in a recent study the UCCR did not decline below the upper limit of the reference range within two months after the dose of trilostane was considered to be satisfactory. However, in those that developed hypocortisolism, based on clinical manifestations and an ACTH-stimulation test, the UCCR was below the upper limit of the reference range several weeks before hypocortisolism was diagnosed. Consequently, in long-term follow-up the UCCR may serve as an early indicator of hypocortisolism.[204]



Figure 4.46:
(A) An eight-year-old male dachshund with polyphagia, polydipsia, polyuria, and alopecia. The basal UCCRs were 47 and 44 × 10^{-6} and the UCCR was reduced to 13 × 10^{-6} after high oral doses of dexamethasone. CT revealed mild contrast enhancement in a normal-size pituitary. Both adrenals were slightly enlarged.
(B) Treatment with trilostane 30 mg once daily resulted in complete recovery.

Within about a week on an appropriate dose of trilostane there is a clear reduction in water intake, urine output, and appetite, followed by improvement in the coat and skin, reduction of central obesity, and increased physical activity (fig. 4.46). Trilostane's inhibiting effect on aldosterone secretion may cause plasma potassium to increase slightly.[196,197,199,201] Its short duration of action may be responsible for the lack of improvement in some hyperadrenocorticoid dogs.[200,205] This may be remedied by twice daily administration, beginning at 1 mg/kg per dose.

Trilostane can be used in cases of hypercortisolism due to functional adrenocortical tumors if neither adrenalectomy nor destruction of adrenocortical tissue with o,p'-DDD (chapter 4.3.2) is an option.[206] It can also be used as palliative treatment in cases of metastasis of a functional adrenocortical tumor.[207] It holds promise for cats with pituitary-dependent hypercortisolism,[208,209] but there is as yet little actual experience with its use in cats and more studies are needed before this can be generally recommended.[209]

Treatment of pituitary-dependent hypercortisolism with trilostane may produce distinct changes in the ultrasonographic appearance of the adrenal glands. In most trilostane-treated dogs there is a clear increase in the thickness of the adrenal glands, due to the continuing stimulation by ACTH. Long-term trilostane treatment may result in adrenal glands with an irregular shape and a nodular appearance.[197,210,211]

Overdosage of trilostane results in cortisol deficiency and sometimes even mineralocorticoid deficiency.[201,203,212,213] In addition, necrosis, apoptosis, and hemorrhage in the zona fasciculata and zona reticularis may cause life-threatening hypocortisolism.[211] If hypoadrenocorticism occurs trilostane must be stopped immediately and corticosteroid substitution started (chapter 13.2.1). In most cases adrenocortical function recovers sufficiently within a few weeks and substitution can be stopped, but some dogs require long-term substitution therapy.[201,203]

The median survival time for treatment with trilostane once daily (662 days) is similar to that for selective adrenocorticolysis with o,p'-DDD (708 days).[214] The median survival time for treatment with trilostane twice daily (900 days) is also comparable to that for nonselective adrenocorticolysis with o,p'-DDD (720 days).[193] In both studies, body weight and age at diagnosis were negatively correlated with survival.

Another therapeutic option could be the inhibition of adrenocortical steroidogenesis by ketoconazole, a synthetic imidazole analogue used as a broad-spectrum antifungal agent resulting from its binding to yeast and fungal cytochrome P-450. At high concentrations, ketoconazole also affects certain cytochrome P-450 enzymes in microsomal and mitochondrial fractions of mammalian cells.[215] It has been used in dogs in the treatment of both pituitary-dependent hypercortisolism and hypercortisolism due to adrenocortical tumor. The initial dose is 5 mg/kg twice daily for seven days and



Figure 4.47:
Cut surface of a small adrenocortical tumor in the cranial pole of the left adrenal. The tumor was surgically removed from a ten-year-old female miniature schnauzer with hypercortisolism. The atrophic adrenal cortex is visible as a small rim surrounding the medulla at the caudal pole.



Figure 4.48:
Large adrenocortical tumor removed at autopsy from a nine-year-old male boxer with hypercortisolism. Tumor tissue protrudes into the longitudinally opened vena cava.

then 10 mg/kg twice daily. Some dogs require 15 mg/kg twice daily to control hypercortisolism, but this may have adverse effects such as anorexia, vomiting, diarrhea, and icterus. These may be resolved by administering ketoconazole with food and temporarily reducing the dose.[216] The major limitations in using ketoconazole in dogs are adverse effects and failure of some dogs to respond.[217] In some countries ketoconazole is the only legally available drug for veterinary use.

Aminoglutethimide, another inhibitor of steroidogenesis, has been used in dogs with pituitary-dependent hypercortisolism, but low efficacy and adverse effects limit its use.[218] Metyrapone reduces cortisol synthesis by blocking the conversion of 11-deoxycortisol to cortisol (fig. 4.3). As mentioned above, it has been used for controlling the harmful effects of hypercortisolemia prior to bilateral adrenalectomy.[120]

Prognosis

With the above methods for either destruction of the adrenal cortices or inhibition of steroidogenesis, hypercortisolism can be satisfactory controlled. Most animals can continue satisfactorily for several years (figs 4.44–4.46), provided that the pituitary lesion does not expand to cause neurological signs. Because of this possibility hypophysectomy is preferred where possible.

4.3.2. Hypercortisolism due to adrenocortical tumor

Histologically adrenocortical tumors can be divided into adenomas (fig. 4.47) and carcinomas (fig. 4.48), a distinction that is by no means always straightforward.[219] Microscopic examination of a seemingly benign tumor may reveal its expansion into blood vessels.[147] Whether adrenocortical carcinoma develops from adrenocortical adenoma or occurs as a separate entity has yet to be determined, but there are indications that in humans adrenal tumorigenesis is a multistep process progressing from normal to adenomatous cells and ultimately to malignant cells.[220] Increased mRNA expression of IGF-II is one of the dominant transcriptional changes in human adrenocortical carcinoma.[221] Data on the expression of genes involved in adrenal tumorigenesis in dogs and cats are still lacking.

Adrenocortical tumors can be either endocrinologically silent or hormonally active. Silent tumors may be found during diagnostic imaging of the abdomen for other purposes. An adrenal tumor discovered incidentally during diagnostic imaging for reasons unrelated to adrenal pathology is referred to as an incidentaloma.[222] Adrenocortical tumors causing hypercortisolism occur in both dogs and cats in middle and old age with no definite sex predilection.[147,223] Most adrenocortical tumors are unilateral solitary lesions, the two glands being affected about equally, but bilateral tumors occur in about 10 % of cases.[147,224,225] The clinical findings are those of

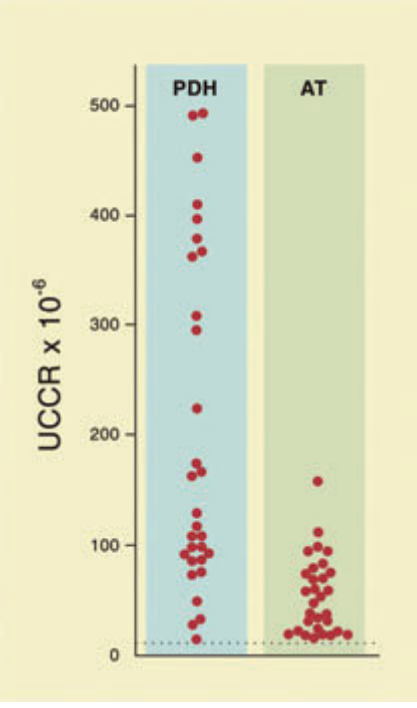


Figure 4.49:
Basal urinary corticoid:creatinine ratios (UCCR) in dogs with hypercortisolism and resistance to suppression of these values (< 50 % suppression) by three eight-hourly administrations of 0.1 mg dexamethasone/kg body weight. The diagnoses of pituitary-dependent hypercortisolism (PDH) and adrenocortical tumor (AT) were based upon measurements of plasma ACTH and visualization of the adrenals. Note that in several cases of AT the UCCR were only moderately elevated and that the highest ratios were found in dogs with PDH.

glucocorticoid excess (chapter 4.3). There may also be mass-related symptoms and signs caused by metastases or non-specific features of malignancy such as weight loss and anorexia. A palpable abdominal mass, vascular obstruction by tumor thrombi of the caudal vena cava (fig. 4.48),[226] or hemo(retro)peritoneum secondary to rupture of an adrenal tumor are rare consequences of adrenocortical tumor.[227–229]

In addition to cortisol, adrenocortical tumors may also produce other adrenocortical hormones in excess. Hypersecretion of adrenal sex hormones by cortisol-secreting adrenocortical tumors has been reported to be quite common.[230,231] Androgen hypersecretion may reflect dedifferentiation of adrenocortical tumors, with steroidogenesis proceeding to its final product, cortisol, in hyperplastic and well-differentiated benign adrenocortical tissue but dedifferentiated adrenocortical tumors being unable to carry steroidogenesis efficiently to term.[232] Mixed cortisol- and aldosterone-producing adrenocortical tumors have also been reported in dogs.[233–236]

Another interesting feature of adrenocortical tumors is that they may occur together with pheochromocytoma (chapter 4.5).[148,149,237]

Diagnosis

Some dogs with adrenocortical tumor have only moderate cortisol excess and thus moderate symptoms and signs. In these cases the UCCR is often around the upper limit of the reference range, but suspicion is aroused by the finding that it is not suppressed by dexamethasone. Although adrenocortical tumors usually greatly exceed the size of the normal gland, the tumor tissue is often only moderately active, i.e., the neoplastic transformation results in lower function per unit of volume (fig. 4.49).

Hypersecretion of cortisol by adrenocortical tumors cannot be suppressed by administration of dexamethasone (fig. 1.9). As measured by either plasma cortisol concentration or the UCCR (chapter 12.4), resistance to suppression by a high dose of dexamethasone is with about equal probability due to adrenocortical tumor or dexamethasone-resistant pituitary-dependent hypercortisolism.[238] In some dogs with a cortisol-secreting adrenocortical tumor, dexamethasone administration causes a paradoxical rise in both the UCCR and plasma cortisol.

Hypercortisolism due to adrenocortical tumor can be differentiated from nonsuppressible forms of pituitary-dependent hypercortisolism by measuring plasma ACTH (chapter 4.3.1). In addition, an adrenocortical tumor is often readily detected by ultrasonography. Hence it is common practice in cases of nonsuppressible hypercortisolism to measure plasma ACTH and perform ultrasonography of the adrenals. If an adrenocortical tumor is found it is still useful to have ACTH measurements, because plasma ACTH should be low and if it is not, further studies are warranted to determine whether there is also pituitary-dependent hypercortisolism.[239]

The preferred procedures for visualization of the adrenals are magnetic resonance imaging (MRI) and computed tomography (CT) (fig. 4.50).[240] Ultrasonography is less expensive, requires less time, and does not require anesthesia, and so it is often used first even though it is more difficult to perform and to interpret than CT or MRI. It provides a good estimate of the size of the tumor and may reveal information about its expansion (fig. 4.51).[224,241] It is sometimes difficult to distinguish between macronodular hyperplasia and adrenocortical tumor by ultrasonography and so CT or MRI may also be needed. Whatever is used, the findings should be interpreted in conjunction with those of biochemical studies,[242] i.e., basal plasma ACTH and if necessary a CRH-stimulation test (chapter 12.1.1).

When the presence of an adrenocortical tumor has been confirmed, the possibility of distant metastases should be considered. During abdominal ultrasonography for identification of the adrenals the liver should also be examined for meta-

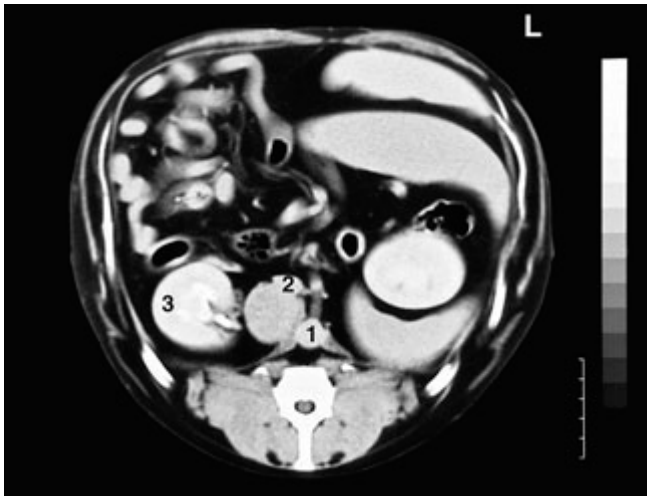


Figure 4.50:
Contrast-enhanced CT image of the abdomen of a nine-year-old male German shepherd dog with a well-demarcated mass between the aorta (1), the caudal vena cava (2), and the right kidney (3), consistent with an adrenal tumor.

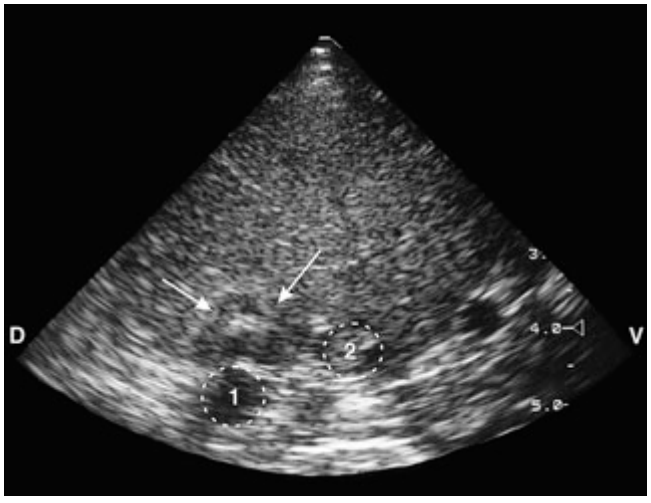


Figure 4.51:
Transverse ultrasonogram from the right lateral intercostal region, immediately cranial to the right kidney, of an eight-year-old miniature poodle (D = dorsal; V = ventral). Lateral to the aorta (1) and dorsal to the caudal vena cava (2) an adrenocortical tumor is visualized (arrows). The lumen of the caudal vena cava is echogenic due to the presence of a tumor thrombus.

stases. If possible metastases are found, ultrasound-guided biopsy can be performed. Thoracic radiographs or a CT scan of the thorax should be made to exclude metastases in the lungs.

Treatment

Treatment has two objectives: removal of the adrenocortical tumor and containment of hypercortisolism. When diagnostic imaging has revealed no metastases and it is likely that there is a resectable unilateral tumor, it should be removed by surgery. Successful removal of the affected adrenal will result in complete recovery without the need for lifelong medication. Adrenalectomy can be performed via a ventral midline celiotomy, with a paracostal extension of the incision when needed, or via a paracostal approach.[147,243–246] In humans adrenalectomy is now often performed by laparoscopy, with lower perioperative morbidity and mortality than by open transabdominal surgery.[247] Laparoscopic adrenalectomy may also become the surgical procedure of choice in veterinary medicine,[248] but most surgeons still prefer transabdominal access because it provides maximal exposure of the tumor and vessels, and in particular of tumor thrombi in the caudal vena cava, thereby minimizing the chance of tumor spillage.

Because of the atrophy of the nontumorous adrenocortical tissue due to the longstanding glucocorticoid excess, glucocorticoid substitution is needed initially. At the time of anesthesia, when intravenous fluid administration is started, 5 mg hydrocortisone/kg body weight is added to the first bottle for administration over a period of 6 h. Subsequently 0.5 mg hydrocortisone/kg is administered subcutaneously at 6 h intervals until oral medication is possible (chapter 13.2.1). This will consist of 1 mg cortisone acetate/kg body weight twice daily, gradually reduced and then stopped six to eight weeks after surgery.[147] After bilateral adrenalectomy lifelong substitution with a glucocorticoid and a mineralocorticoid is required, according to the treatment protocol for primary hypoadrenocorticism (chapter 4.2.1).

Hypercortisolism due to adrenocortical tumor can also be treated medically. Drugs for this purpose are classified as adrenocorticolytic or adrenocorticostatic. Adrenocorticolytic drugs destroy adrenocortical cells and thereby reduce steroid synthesis, whereas adrenocorticostatic drugs interfere with steroidogenesis without cell damage.

Administration of the adrenocorticolytic drug o,p'-DDD is often the treatment of choice in dogs in which tumor tissue cannot be completely removed surgically or when the disease recurs after adrenalectomy. It is also used in cases of metastasized adrenocortical tumor. Because of the potential of toxic effects of o,p'-DDD in both humans and animals, owners must be given careful instructions on how to recognize and respond to them. o,p'-DDD should preferably not be used in a household in which there is a pregnant woman or young child. Although the hypercortisolism per se due to adrenocortical tumor may be treated successfully by selective destruction (chapter 4.3.1),[249] the aim of o,p'-DDD treatment should be complete destruction of all adrenocortical cells and substitution therapy for the induced adrenocortical insufficiency. The treatment protocol for complete adrenocortical destruction consists of 25 days of oral administration of 50–75 mg o,p'-DDD/kg body weight per day.[191] In dogs of low body weight o,p'-DDD doses up to 100 mg/kg per day

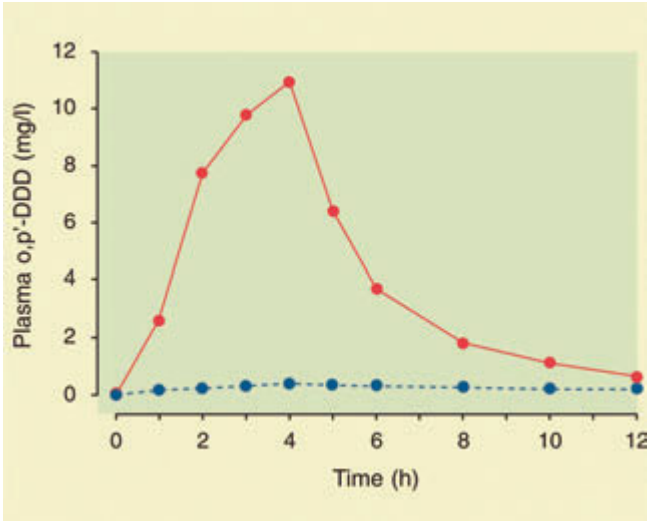


Figure 4.52:
Mean o,p'-DDD concentrations in plasma of six dogs given the drug as intact tablets without food (blue line) or with food (red line). The systemic availability of this lipophylic drug is very poor when intact tablets are given without food but ordinary dog food seems to contain sufficient fat to facilitate good absorption.

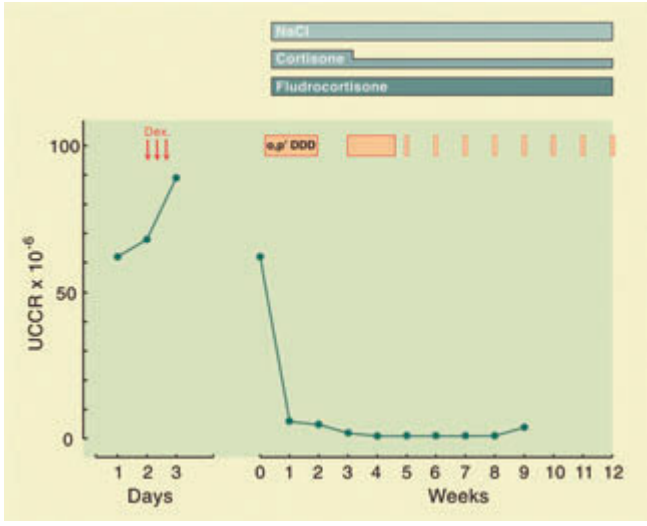


Figure 4.53:
UCCRs in an eleven-year-old female mongrel dog weighing 24.8 kg. On the left are the values on two control days and after three oral doses of dexamethasone, 0.1 mg/kg. Treatment with 500 mg o,p'-DDD three times daily was monitored by weekly measurements of the UCCR after cortisone and fludrocortisone were omitted on the preceding evening. Treatment was discontinued for a few days because of the dog's inappetence and was then resumed once weekly for three months. Two years after the start of o,p'-DDD therapy there were no signs of recurrence of hypercortisolism.

may be required for complete destruction. o,p'-DDD is given daily for the first five days, thereafter on alternate days. The daily dose is divided into three or four portions and administered with food (fig. 4.52). On the third day, substitution therapy is begun with cortisone acetate (2 mg/kg per day), fludrocortisone acetate (0.0125 mg/kg per day), and sodium chloride (0.1 g/kg per day), all divided into at least two portions. If for any reason the dog cannot take or retain the tablets and salt two times in succession, injectable medications should be started (chapter 13.2.1). A written instruction for owners is presented at the end of chapter 13.

After 25 days of o,p'-DDD administration, a follow-up examination is made. The cortisone dose is reduced to 0.5–1.0 mg/kg per day, but is always doubled for one or two days in the event of anesthesia, severe physical stress, or injury. Complete adrenocortical destruction results in very low UCCRs in morning urine samples collected after omitting the cortisone and fludrocortisone administration on the preceding evening. The doses of fludrocortisone and salt are adjusted by measurements of plasma sodium and potassium (see also chapter 4.2.1). o,p'-DDD is then continued for at least three months at the same dose once weekly (fig. 4.53).

Owner compliance is essential for successful chemotherapy with o,p'-DDD. During the first month the owner reports at least once weekly and whenever questions or problems arise. The owner is also instructed very clearly to stop giving o,p'-DDD if partial or complete inappetence develops, but, with equal emphasis, to continue adrenocortical hormone substitution and to contact the veterinarian, who may increase the cortisone substitution temporarily. If a loss of appetite is ignored and o,p'-DDD is continued, the dog may begin to vomit, refuse substitution therapy, and develop a hypoadrenocorticoid crisis. However, with good instructions this is rare and usually the o,p'-DDD administration can be resumed after a few days without further problems.

Despite this treatment with o,p'-DDD, there are recurrences, causing the owner to contact the veterinarian because the animal's appetite and water intake have increased. Omitting the cortisone substitution may ameliorate the symptoms temporarily, but possible recurrence should be investigated by repeating UCCR measurements. Two morning urine samples are collected at an interval of four to five days, each time omitting cortisone and fludrocortisone on the preceding evening. UCCRs exceeding the upper limit of the reference range indicate glucocorticoid excess and o,p'-DDD is again given daily for 25 days and then once weekly for at least half a year or even lifelong.

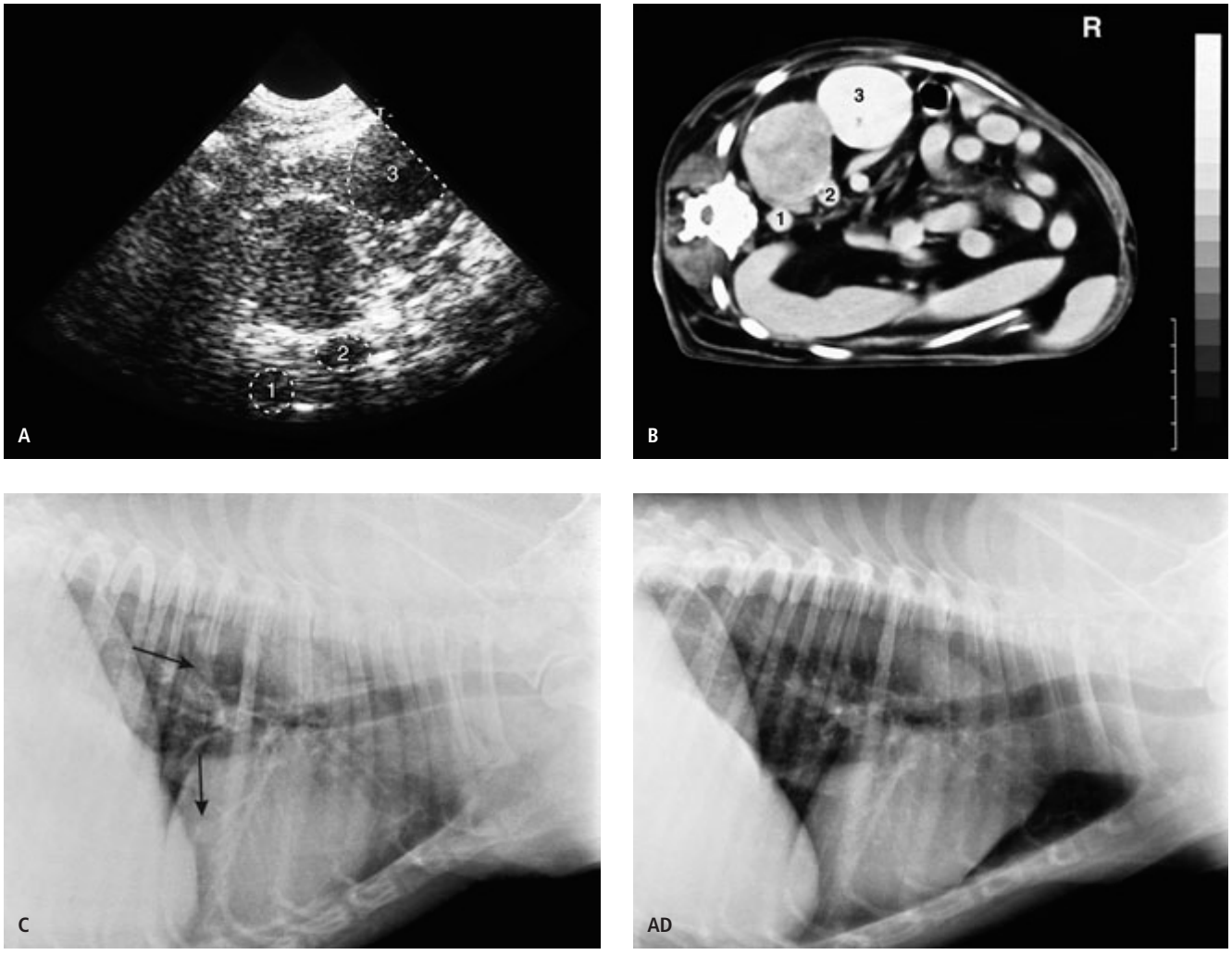


Figure 4.54:
Diagnostic images in a ten-year-old castrated female miniature pinscher of 8 kg with hypercortisolism due to a tumor of the right adrenal cortex. The abdominal ultrasonogram (A) can be compared with the CT image (B) in lateral recumbency. A large tumor of the right adrenal gland is shown between the aorta (1), caudal vena cava (2), and right kidney (3). One year after surgical removal of the tumor, in which there was microscopic expansion into blood vessels, the hypercortisolism had recurred. The expiratory radiograph of the thorax of this obese dog (C) revealed several nodular densities (arrows) consistent with pulmonary metastases. The dog was given 125 mg o,p'-DDD four times daily for 35 days and corticosteroid replacement was started. o,p'-DDD was continued once weekly for 1.5 years and two years after the start of o,p'-DDD there was no evidence of recurrence of hypercortisolism or lung metastases (D).

If adrenalectomy or adrenocortical destruction with o,p'-DDD is not an option, the adrenocorticostatic drug trilostane can be used. It has been used successfully in a dog with hypercortisolism due to a functional adrenocortical tumor[206] and can also be used as palliative treatment in case of metastases of a functional adrenocortical tumor.[207]

Prognosis

The prognosis is excellent after complete surgical resection of adrenocortical tumor that has not metastasized. This is true for bilateral as well as unilateral tumors, although maintenance therapy for induced hypoadrenocorticism is required after bilateral adrenal resection. Dogs with irresectable adrenocortical tumor or recurrence after resection can be treated with o,p'-DDD according to the above schedule. This often leads to complete and permanent remission of the hypercortisolism (fig. 4.53) and ultrasonographic examinations may reveal that the size of the tumor has decreased considerably.[250] Even lung metastases may disappear (fig. 4.54).

4.3.3. Hypersecretion of sex hormones by adrenocortical tumor

Adrenocortical tumors can produce various hormones other than cortisol or aldosterone. This is most pronounced in neutered pet ferrets, in which excessive secretion of sex hormones by unilateral or bilateral adrenocortical tumors is the most common form of hyperadrenocorticism. Plasma concentrations of cortisol and ACTH are usually not affected.[251] In this species, the neoplastic adrenocortical tissue expresses functional LH receptors. Activation of these receptors by the high plasma LH concentrations due to the neutering causes excessive secretion of androstenedione, 17α-hydroxyprogesterone, and/or estradiol, leading to vulvar swelling in neutered female ferrets, recurrence of sexual behavior in neutered male ferrets, and symmetrical alopecia.[252]

Increased secretion of progesterone or other sex hormones from noncortisol-secreting adrenocortical tumor has also been reported in cats[253–257] and dogs,[231,232] but seems to be rare in both species. A sex steroid hormone-secreting adrenocortical tumor should be considered in neutered animals with newly developed physical and behavioral sexual changes such as urine spraying and aggression in neutered male cats. The castrated male cat develops spines on the penis (fig 8.5) whereas the castrated female develops hyperplasia of the vulva. Hypersecretion of progesterone by a well-differentiated adrenocortical carcinoma in a castrated male Himalayan cat was associated with bilateral alopecia.[253] Endocrine testing may reveal elevated plasma concentrations of androstenedione, testosterone, estradiol, 17-hydroxyprogesterone, and/or progesterone, and these values may increase following stimulation with ACTH.[231] Information about the size of the tumor, its expansion, and the presence of metastases can be obtained by ultrasonography, CT, or MRI (chapter 4.3.2).

Adrenalectomy is the treatment of choice and usually results in resolution of clinical manifestations, including regression of penile spines.

4.3.4 Ectopic ACTH syndrome

In about 15 % of humans with Cushing's syndrome, the glucocorticoid excess is the result of ACTH secretion by nonpituitary tumors. These are often malignant tumors originating from cells of the diffuse neuroendocrine system (chapter 10.1), and include thymic, pancreatic, and gastrointestinal tumors. They may be small and therefore difficult to locate. Plasma ACTH concentrations and cortisol secretion rates can be extremely high. Consequently the clinical manifestations can be very pronounced, including hypokalemia due to the severe cortisol excess exceeding the capacity of 11β-HSD2 (chapter 4.1.6).[258]

This condition has been documented in an eight-year-old German shepherd dog. The UCCRs (236 and 350 × 10^{-6}) and plasma ACTH concentrations (159 and 188 ng/l) were very high and not suppressible with dexamethasone. These findings were initially interpreted as being consistent with pituitary-dependent hypercortisolism. However, histological examination of the tissue removed by transsphenoidal hypophysectomy revealed no adenoma. The clinical manifestations exacerbated, including severe hypokalemia (2.2 mmol/l). Both the UCCR (1518 and 2176 × 10^{-6}) and plasma ACTH (281 ng/l) were further increased. CT of the abdomen revealed a tumor in the region of the pancreas and laparotomy revealed a 5 mm nodule in the pancreas, a 3 cm metastasis in an adjacent lymph node, and metastases in the liver. Partial pancreatectomy and extirpation of the lymph node were performed and histological examination revealed a neuroendocrine tumor with metastasis in the lymph node. The second surgical intervention did not alter the course of the disease, probably because of additional metastatic tumor tissue that was not discovered. Nevertheless, the dog did well for more than two years on treatment with trilostane.[259]

Thus ectopic ACTH secretion should be suspected when there is very severe hypercortisolism and highly elevated plasma ACTH concentrations that are not suppressible with high doses of dexamethasone and in the absence of a demonstrable pituitary tumor. Diagnostic imaging may reveal a neuroendocrine tumor. The condition may not be extremely rare, as there have been two more reports of individual cases in which this diagnosis has been proposed. In another German shepherd dog a primary hepatic carcinoid was held responsible for severe hypercortisolism with persistent hypokalemia.[260] In a dachshund with hypokalemia an extrapituitary ACTH-producing microadenoma was considered, but no tumor was found and there was some suppression of the plasma cortisol concentrations in the LDDST.[261]

4.3.5 Food-dependent glucocorticoid excess

In addition to autonomous cortisol secretion by adrenocortical tumors (chapter 4.3.2), ACTH-independent hypercortisolism may be due to expression of ectopic or hyperactive eutopic hormone receptors. In humans, various adrenocortical membrane-bound receptors functionally coupled to steroidogenesis have been reported, including gastric inhibitory polypeptide (GIP), catecholamine, vasopressin, serotonin, and LH receptors.[262,263] As mentioned in chapter 4.3.3, activated LH receptors on adrenocortical tumor cells in ferrets cause excessive secretion of androstenedione, 17α-hydroxyprogesterone, and/or estradiol[252] (chapter 4.3.3) and in exceptional cases also cause hypercortisolism.[264]

Food-dependent hypercortisolism, presumably due to adrenocortical expression of functional GIP receptors, was reported recently in a six-year-old vizsla.[265] In this dog with clinical manifestations of hypercortisolism and slightly elevated UCCRs, basal and CRH-stimulated plasma ACTH concentrations were low, but diagnostic imaging revealed no adrenocortical tumor. Ingestion of a meal resulted in significant increases in plasma cortisol concentration and the UCCR. Consistent with the diagnostic criteria for food-dependent hypercortisolism in humans,[262,266] administration of 3 µg octreotide per kg body weight completely prevented the meal-induced hypercortisolemia. The dog was treated successfully with trilostane, administered two hours before meals.

4.3.6 Iatrogenic hypercorticism and iatrogenic secondary hypoadrenocorticism

Alterations in the chemical structure of glucocorticoids have resulted in synthetic compounds with greater glucocorticoid activity than the natural hormones cortisol, cortisone, and corticosterone (fig. 4.55). The increased glucocorticoid activity is due to increased affinity for the GR and delayed plasma clearance of the hormone, which increases tissue exposure. In addition, the pharmaceutical formulation of injectable preparations plays a role. Esterified microcrystalline suspensions are slowly absorbed from the subcutaneous or intramuscular injection site. Many of these synthetic glucocorticoids have negligible mineralocorticoid effects and thus do not result in sodium retention and hypokalemia (table 4.4).

The duration of action of a glucocorticoid is not solely determined by its presence in the circulation. Binding to a receptor protein (chapter 4.1.5) produces a glucocorticoid-receptor complex that modifies the process of DNA transcription, thereby altering – via RNA translation – the rate of synthesis of specific proteins. By this modification of the phenotypical expression of the genetic information the glucocorticoid may continue to exert an effect after it has disappeared from the circulation.[267]

Hydroxylation at C-11 is required for glucocorticoid activity (figs. 4.2, 4.3). Cortisone and prednisone are 11-ketocompounds (fig. 4.56) and therefore must be converted to cortisol and prednisolone, respectively, for glucocorticoid activity. This conversion by 11β-HSD1 occurs predominantly in the liver (fig. 4.13) and is only moderately impaired by liver disease. Thus topically applied prednisolone is effective but topically applied prednisone is not. Cortisone and prednisone can be used for systemic, not topical, therapy. All glucocorticoid preparations marketed for topical use are 11β-hydroxylcompounds, obviating the need for biotransformation.[267]

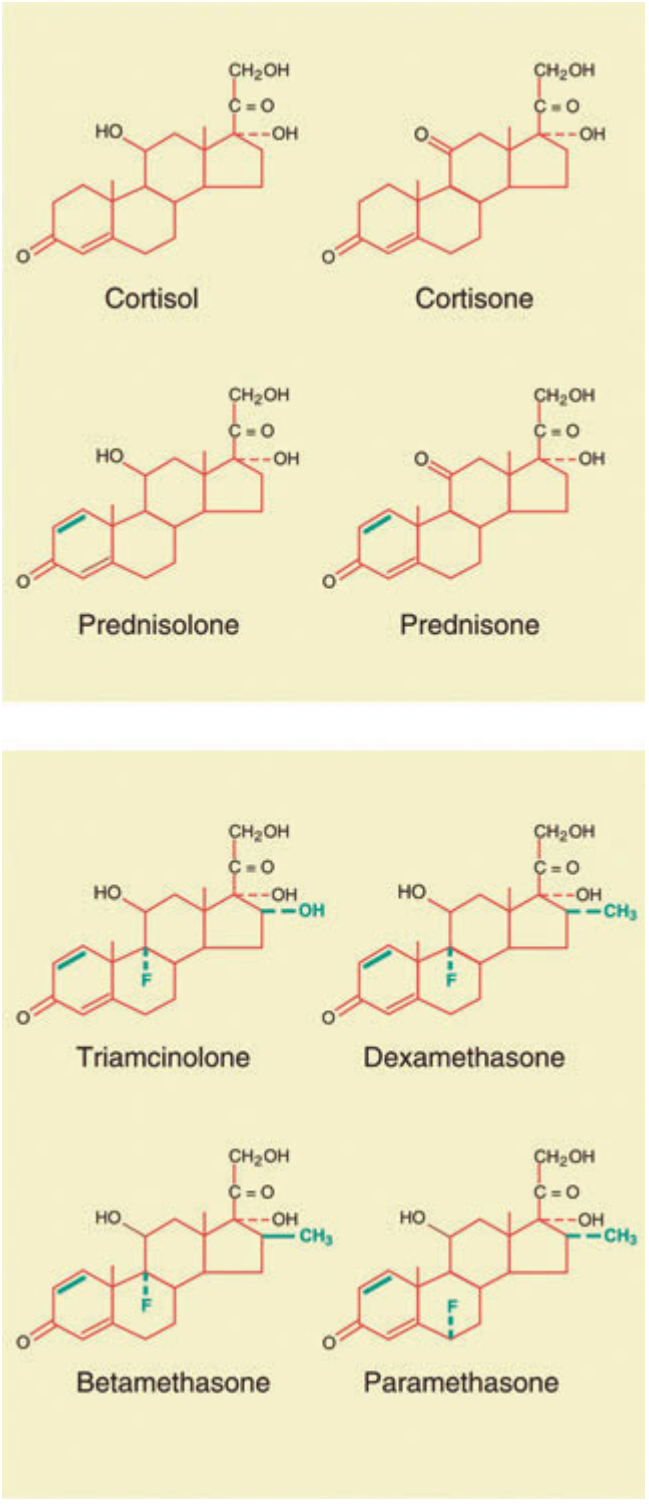


Figure 4.55:
Structures of commonly used glucocorticoids. The chemical modifications introduced to enhance glucocorticoid activity are shown in green.

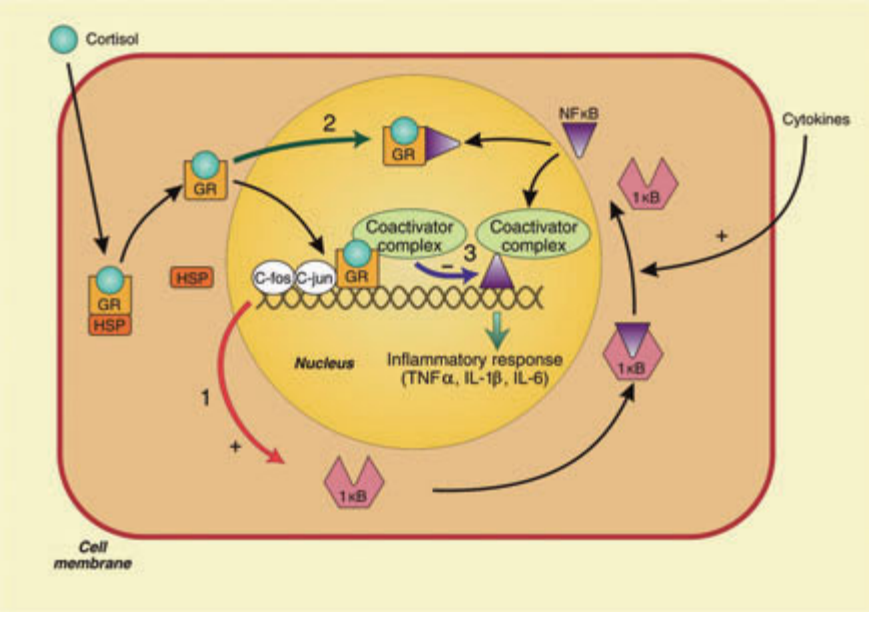


Figure 4.56:
The anti-inflammatory action of glucocorticoids. Cortisol binds to the cytoplasmic glucocorticoid receptor (GR). Conformational changes in the receptor-ligand complex result in dissociation from heat shock proteins (HSPs) and migration to the nucleus. There it binds to specific glucocorticoid-response elements in association with the activator protein-1 (AP-1), comprising c-fos and c-jun. The anti-inflammatory effects of glucocorticoids are mediated via (1) Induction of the inhibitory protein 1κB, which binds and inactivates the transcription factor NF-κB, (2) binding of the GR-glucocorticoid complex to NF-κB, thus preventing initiation of an inflammatory process, and (3) competition of both GR and NF-κB for the limited availability of coactivators. (Modified from Stewart, 2008).[54]

4.3.6.1 Glucocorticoids as pharmacological agents

Glucocorticoids are used for substitution in adrenocortical insufficiency (chapter 4.2.1) and for the diagnosis and differential diagnosis of hypercortisolism (chapter 4.3). However, this constitutes only a small part of their application in practice, where they are widely used for the treatment of various allergic, autoimmune, inflammatory, and neoplastic diseases.

There is no simple mechanism of action underlying the many effects of glucocorticoids on inflammatory and immune responses. Many hundred glucocorticoid-responsive genes have been identified (chapter 4.1.5). Two particular transcription factors seem to be important in mediating anti-inflammatory effects of glucocorticoids. Activator protein-1 (AP-1) is a proinflammatory transcription factor induced by cytokines. The GR-ligand complex can prevent interaction with AP-1, thereby mediating inhibitory effects of glucocorticoids. Similarly, functional antagonism exists between the GR and nuclear factor kappa B (NF-κB). NF-κB is a widely expressed transcription factor that activates a series of genes involved in lymphocyte development, inflammatory response, host defense, and apoptosis.[54]

4.3.6.2 Iatrogenic hypercorticism

As in spontaneous hypercortisolism, the development of signs and symptoms of glucocorticoid excess depends on the severity and duration of the exposure. The effects vary among individual animals and initially seem to be less pronounced in cats. Within days after the start of glucocorticoid administration polyuria / polydipsia and polyphagia develop. After several weeks of glucocorticoid therapy, the classic physical changes such as centripetal obesity, muscular weakness, and skin atrophy develop (fig. 4.57).

The side effects of glucocorticoid therapy are not confined to the manifestations of glucocorticoid excess, which may include diabetes mellitus.[268,269] Suppression of the immune response may precipitate fatal infections.[270] In addition, there is increased risk of complications such as pancreatitis, and gastrointestinal hemorrhage, ulceration, and perforation.[271]

4.3.6.3 Iatrogenic secondary hypoadrenocorticism

Both systemic and topically applied corticosteroids cause prompt and sustained suppression of the hypothalamic-pituitary-adrenocortical axis (chapter 4.2.2).[272–274] Depending on the dose, the continuity, the duration, and the preparation or formulation, this suppression may continue for weeks or months after cessation of corticosteroid administration (fig. 4.58).[275]

An animal may appear to be healthy during corticosteroid therapy, but nevertheless it lacks the ability to increase cortisol secretion sufficiently in response to stress. If stressed, it may develop signs of acute adrenocortical insufficiency, such as hypotension, weakness, anorexia, and vomiting. It may not recover from surgery without additional glucocorticoid supplementation. Similar long-lasting suppression of the hypothalamic-pituitary-adrenocortical system occurs in dogs treated with progestins.[276] Also in cats, where progestins are used in the treatment of various dermatologic and behavioral disorders, the affinity of the GR for these compounds may cause a similar suppression of the pituitary-adrenocortical system.[277]

During prolonged glucocorticoid treatment, tests of pituitary-adrenocortical reserve function (chapters 4.2.2, 1.2.4.1, fig. 4.58) are not needed. A test is indicated when the gluco-



Figure 4.57:
(A) A three-year-old female mongrel dog that was treated for six months with injections of 9F,16-methylprednisolone and 6-methylprednisolone for pruritus due to an underestimated flea infestation. Note the obesity and the thin coat.
(B) With antiparasitic treatment and omission of the corticosteroids the dog regained its normal shape and a thick hair coat.

corticoid administration has been reduced to replacement levels or stopped, and the recovery of the integrity of the system is questionable. This applies especially to animals that need an increase in the corticosteroid dose to cover stressful events such as general anesthesia and surgery. When secondary hypoadrenocorticism is to be expected or has been demonstrated and the animal is at risk, a glucocorticoid should be given at four times the maintenance dose (chapter 4.2.1), i.e., 1 mg cortisone/kg body weight four times daily or an equivalent dose of another glucocorticoid (table 4.4).

Table 4.4: Actions of commonly used glucocorticoid preparations (the glucocorticoid potency of cortisol is set at 1 for comparison)

Name and duration of action	Glucocorticoid potency	Mineralocorticoid activity
Short acting		
Cortisol (hydrocortisone)	1	Yes
Cortisone	0.8	Yes
Prednisone	4	No
Prednisolone	4	No
Intermediate acting		
Methylprednisolone	5	No
Triamcinolone	5	No
Long acting		
Bethamethasone	25	No
Dexamethasone	30	No

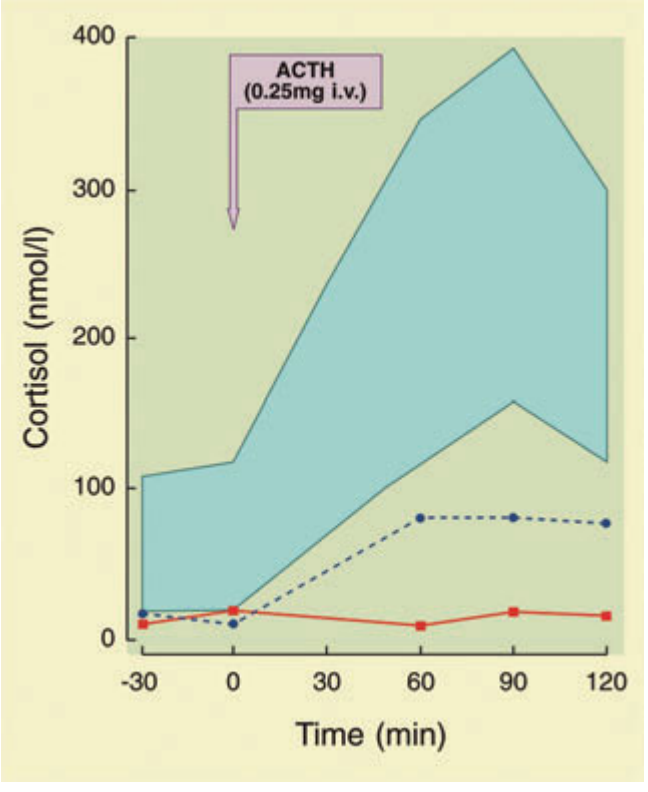


Figure 4.58:
ACTH-stimulation test results in a reference population of dogs (blue area) and in the dog in fig. 4.57 at first admission (red line) and three weeks after stopping the prolonged glucocorticoid treatment (blue line).

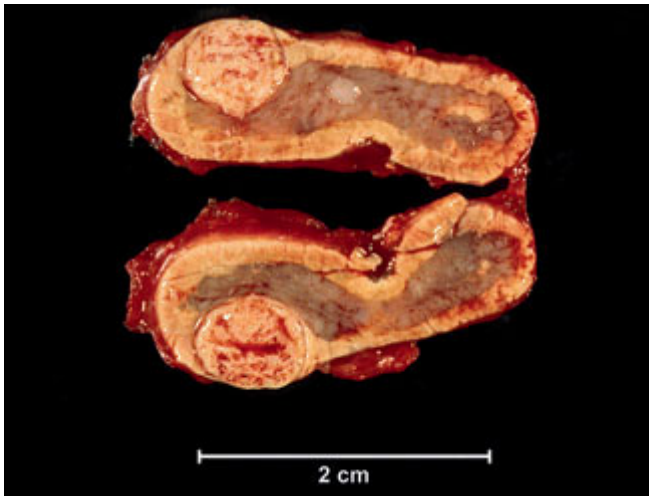


Figure 4.59:
Longitudinal section of the left adrenal of a ten-year-old castrated male German shorthaired pointer with primary hyperaldosteronism. At the cranial end (left) there is an aldosteronoma about 7 mm in diameter.[279]

4.3.6.4 Withdrawal from glucocorticoids

Discontinuance of glucocorticoid therapy may not only result in exacerbation of the disease that is being treated but also in symptoms and signs of the corticosteroid withdrawal syndrome. As mentioned above, the patient may even develop secondary adrenocortical insufficiency.

The cardinal features of glucocorticoid withdrawal are anorexia, lethargy, and weight loss. The lethargy may be the result of what humans experience following glucocorticoid withdrawal: myalgia, arthralgia, headache, and postural hypotension. These symptoms occur in patients in whom the dose has been tapered to a normal glucocorticoid maintenance dose and are due to the sudden cessation of the glucocorticoid-induced inhibition of prostaglandin production. Many of the features of the corticosteroid-withdrawal syndrome can be produced by prostaglandins.[267]

The dose should therefore be reduced gradually, as in the transition from spontaneous hypercortisolism to normocorticism (chapter 4.3.2), in which initially at least twice the maintenance dose is given. The recovery of pituitary-adrenocortical function is not promoted by administering ACTH. It is not the ACTH secretion but rather the hypothalamic hypophysiotropic stimulation that recovers last and administering ACTH will only retard this recovery and that of the pituitary corticotroph cells.[278]

4.3.6.5 Alternate-day glucocorticoid therapy

In alternate-day glucocorticoid therapy a short-acting glucocorticoid (prednisone or prednisolone) is given once every 48 h. The aim is to retain the therapeutic benefits while minimizing the adverse effects. Thus it is an attempt to prevent the development of Cushing's syndrome and secondary hypoadrenocorticism. Although it is not known whether alternate-day administration definitely yields a better overall risk:benefit ratio than a once-daily dose, it is common practice to use the alternate-day schedule when glucocorticoids are administered over a long period.

To induce remission of a fulminant autoimmune or immune-mediated inflammatory process, treatment is begun by administering the glucocorticoid once daily. When there are signs of improvement an attempt is made to reduce the dose. The following schedule is an example for oral administration of prednisolone:

- Days 1–3: 2–4 mg/kg once daily.
- Days 4–6: 1–2 mg/kg once daily.
- Days 7–14: 1–2 mg/kg on alternate days.

The dose is lowered further at weekly intervals if there are no exacerbations of the disease. Usually the final dose cannot be lower than about 0.5 mg/kg every 48 h. In some diseases it may be necessary to administer a higher dose or even to resume full daily doses temporarily.

4.4 Mineralocorticoid excess

Reducing the effective arterial blood volume activates the renin-angiotensin system (RAS), which in turn persistently stimulates aldosterone synthesis. Conditions in which this occurs include chronic edematous diseases such as heart failure, and hypoproteinemia due to hepatic cirrhosis, the nephrotic syndrome, and protein-loosing enteropathy. Despite the high levels of renin and angiotensin and the secondarily increased plasma aldosterone concentration, fluid volume remains reduced and blood pressure is low-normal. The effect of aldosterone can be blocked by administering spironolactone, a nonmineralocorticoid steroid that competes directly with aldosterone for binding to the mineralocorticoid receptor.

This pathophysiological mechanism that is activated in response to hypovolemia is called **secondary hyperaldosteronism**; i.e., high-renin hyperaldosteronism. In primary mineralcorticoid excess there is low-renin hyperaldosteronism due to autonomous hypersecretion of aldosterone by tumorous or nontumorous adrenals.

4.4.1 Primary mineralocorticoid excess

In dogs and cats excessive activation of mineralocorticoid receptors can be the result of hypersecretion of aldosterone by an adrenocortical tumor. In cats hyperaldosteronism due to nontumorous adrenocortical hypersecretion has also been re-

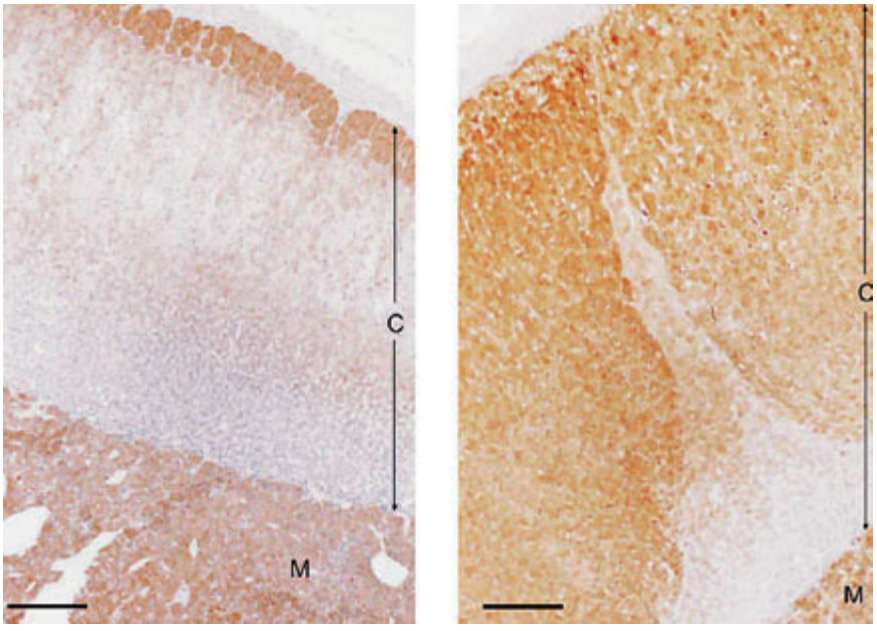


Figure 4.60:
Histological sections of adrenals stained with neuron-specific enolase (NSE). In the healthy cat (left), the staining of the cortex (C) is confined to the zona glomerulosa with only slight staining of the outer part of the zona fasciculata. In the cat with primary hyperaldosteronism (right), the cortex consists of multiple hyperplastic nodules, staining positively for NSE. Staining of the adrenal medulla (M) is similar in the two sections. Bar = 200 µm.

ported. In addition, adrenocortical tumors secreting the mineralocorticoid deoxycorticosterone (DOC) have been reported in dogs and cats.

There have been two case reports of primary hyperaldosteronism in dogs, one with a small aldosteronoma (fig. 4.59) and the other with a large adrenocortical carcinoma and hepatic metastases.[279,280] The occurance of primary hyperaldosteronism has also been mentioned for in three other dogs, one with an adenoma and two with adenocarcinomas.[281] Symptoms and signs that might be compatible with primary hyperaldosteronism were attributed to bilateral adrenocortical hyperplasia in another dog.[282] Plasma aldosterone and renin are suppressed by elevated levels of DOC, as observed in a dog with hypersecretion of DOC by an adrenocortical carcinoma.[283] Dogs with physical and biochemical features of both glucocorticoid and mineralocorticoid excess have in all reported cases been found to have adrenocortical carcinoma.[284–286]

Primary hyperaldosteronism seems to be less rare in cats than in dogs. About 20 cases have been reported in which the disease was due to usually unilateral adrenocortical tumors of varying degrees of malignancy, ranging from well-capsulated adenomas to carcinomas with growth into the caudal vena cava and distant metastasis.[287–291] Not only plasma aldosterone may be elevated but also some of the precursors, such as progesterone.[292] In addition to cases due to adrenocortical tumor, there has been a report of eleven cats with »idiopathic« primary hyperaldosteronism caused by bilateral adrenocortical hyperplasia (fig. 4.60).[293]

Clinical manifestations

As noted in chapter 4.1.6, mineralocorticoid excess causes two abnormalities: (1) increased sodium retention, and (2) increased potassium excretion. The initial sodium retention is followed by natriuresis, so sodium balance is reestablished and edema does not develop. This is called the »escape phenomenon«, meaning the escape by the renal tubules from the sodium-retaining action of aldosterone. Natriuretic peptides (chapter 10.2) play an important role in this phenomenon.[294,295]

Nevertheless, mineralocorticoid excess tends to be associated with extracellular fluid expansion, hypertension, and increased cardiac output.[296] This is probably responsible in part for the main presenting symptoms of hyperaldosteronism in dogs: polyuria and polydipsia. In canine hyperaldosteronism the release of vasopressin following an osmotic stimulus is delayed, and there is resistance to the action of vasopressin (figs. 2.34, 2.36), similar to that in hypercortisolism (chapters 2.3.2, 4.3).

The progressive depletion of potassium and the development of hypokalemia affect several organ systems, but become particularly manifest in the neuromuscular system by affecting the polarization of nerve and muscle membranes. Muscle weakness is likely to occur at plasma potassium concentrations around 2.5 mmol/l, and areflexic paralysis may develop with more severe hypokalemia.

As in dogs, mineralocorticoid excess in cats occurs in middle and old age. The main presenting symptoms are changes in neuromuscular function. Affected cats have episodic weakness

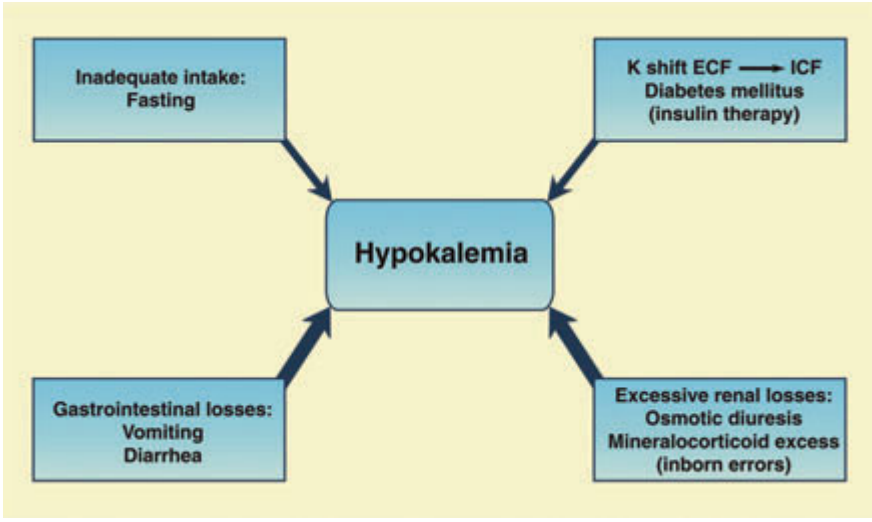


Figure 4.61:
The main routes for development of hypokalemia.

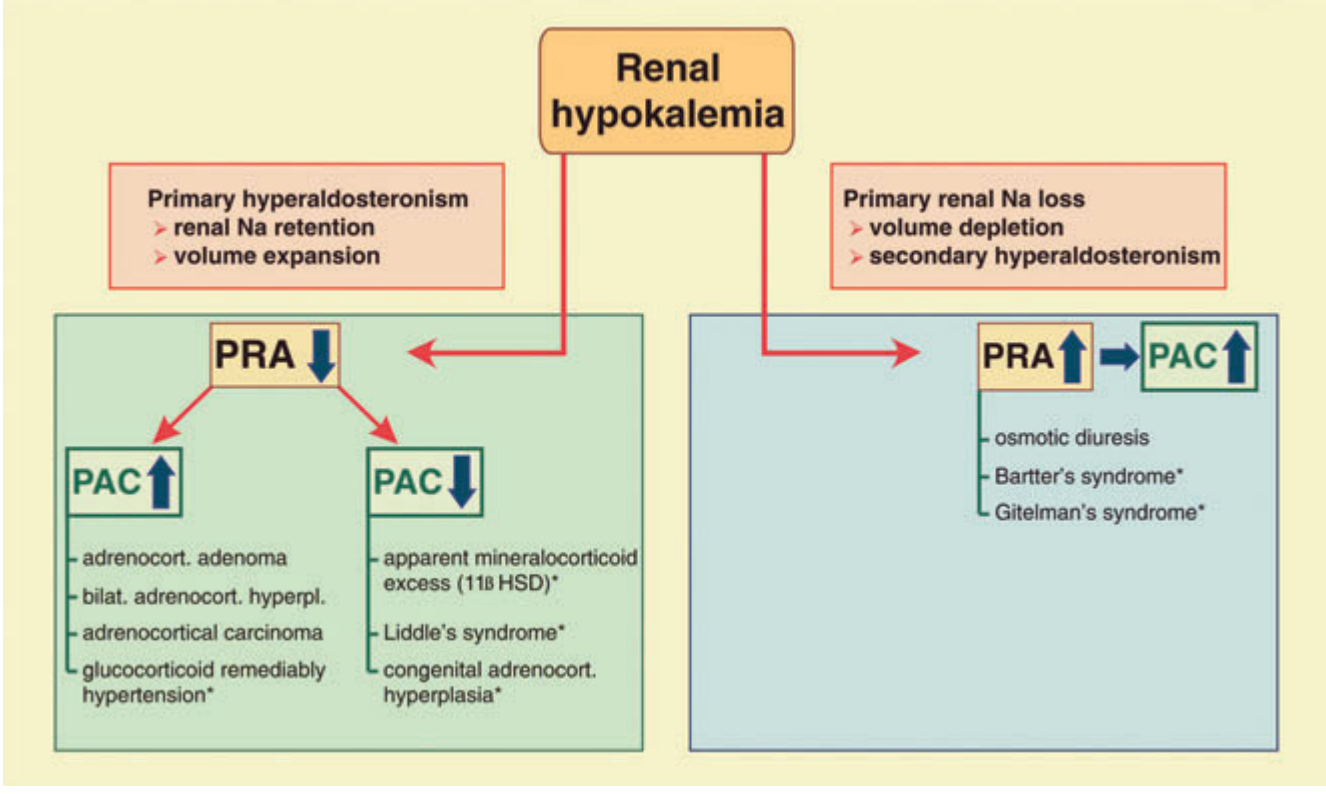


Figure 4.62:
Changes in plasma renin activity (PRA) and plasma aldosterone concentration (PAC) that can occur in hypokalemia developed via the renal route. The congenital conditions described in humans but not (yet) in dogs or cats are marked with an asterisk.

and a characteristic ventroflexion of the neck, in some cases leading to flaccid paresis with hyporeflexia and muscle hypotonia. In other cats the presenting physical features are dominated by signs of arterial hypertension, i.e., loss of vision due to retinal detachment and retinal and intravitreal hemorrhages.

The most consistent routine laboratory finding is hypokalemia. Mineralocorticoid excess also favors increased acid secretion by a variety of mechanisms, leading to (usually mild) hypokalemic metabolic alkalosis.[297] In addition there may be hypophosphatemia and hypomagnesemia, as well as elevation of plasma alkaline phosphatase (in dogs) and creatine kinase. Particularly in cats idiopathic hyperaldosteronism is often associated with slowly progressing renal insufficiency, probably due to aldosterone-induced arteriolar and glomerular sclerosis, tubular atrophy, and interstitial fibrosis (see also chapter 4.1.6). Even in end-stage renal failure, there is a tendency to hypophosphatemia rather than to hyperphosphatemia.[293]

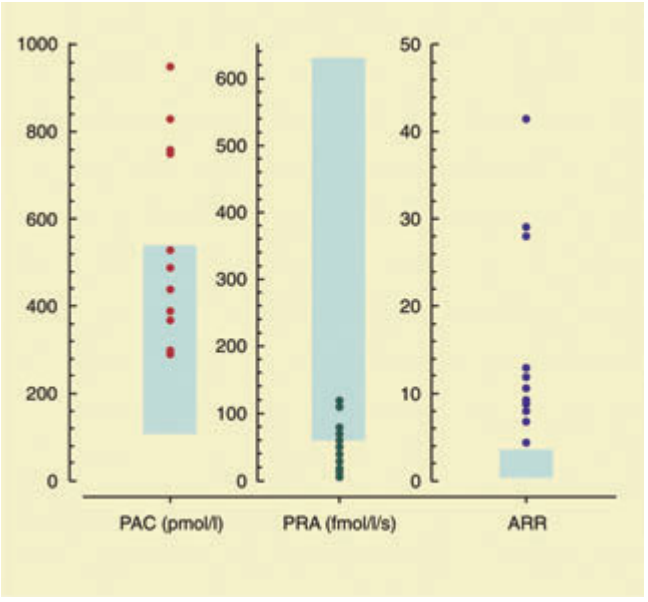


Figure 4.63:
Plasma aldosterone concentration (PAC), plasma renin activity (PRA), and the PAC:PRA ratio (ARR) in eleven cats with nontumorous (idiopathic) primary hyperaldosteronism. Grey areas represent reference values in healthy cats.[293]

Differential diagnosis

For polyuria in dogs there is the well-known list of differential diagnoses given in chapter 2.3.3.4 (for the algorithm see chapter 14.2). The main routes for development of hypokalemia are given in fig. 4.61. The possibilities for the renal route are specified in more detail in fig. 4.62.

Diagnosis

In primary mineralocorticoid excess, the plasma concentration of aldosterone (or DOC) is characteristically high and plasma renin activity (PRA) is immeasurably low. In hyperaldosteronism due to adrenocortical tumor plasma aldosterone concentration (PAC) is usually highly elevated. In cats with idiopathic hyperaldosteronism PAC is usually only slightly elevated or within the upper limit of the reference range. As hypokalemia is a predominant factor in lowering PAC,[298] in the presence of hypokalemia moderately elevated aldosterone values can be regarded as inappropriately high. The PRA must also be taken into account. The combination of a high-normal or elevated PAC and low PRA indicates persistent aldosterone synthesis in the presence of little or no stimulation by the renin-angiotensin system. In humans the PAC:PRA ratio (ARR) is considered to be a very useful aid in diagnosing primary hyperaldosteronism. This also seems to be true for cats with idiopathic hyperaldosteronism (fig. 4.63).[293]

The ARR is elevated in 10–20 % of human patients with arterial hypertension and most of these have excess aldosterone production from both adrenal cortices.[299] The diagnostic value of the ARR is principally determined by the sensitivity of the renin assay and interpretation should rest upon comparison with an appropriate control population. The ARR is currently regarded as the most reliable means of detecting primary hyperaldosteronism, but the measurements should be repeated if the initial result is inconclusive or difficult to interpret because of suboptimal sampling conditions.[300]

Control populations of both dogs and cats have been studied. In dogs the ARR ranged from 0.1 to 1.5, and both PAC and the ARR were slightly lower in spayed than in intact female dogs. In cats the ARR was 0.3–3.8, being somewhat higher in neutered than in intact cats. The ARR was higher in cats $\geq$ 5 years of age than in younger cats. Blood samples were collected with the animals in various positions and sampling was associated with a wide variety of stress responses. Nevertheless, the reference ranges were similar to the relatively narrow range obtained in humans under standardized conditions. PRA and PAC are much higher in blood collected from humans in the upright rather than in the supine position. This physiological response to rapid pooling of blood in the lower extremities and to shifts in plasma fluid in surrounding tissues is a less important factor in small quadrupeds such as cats.[301,302]

An alternative diagnostic approach may be measurement of the urinary aldosterone:creatinine ratio (UACR). Cats excrete smaller quantities of aldosterone and its 18-glucuronidated metabolite in urine than do humans or dogs, but nevertheless the UACR can be determined.[303] This would allow the development of a dynamic test, such as employing a suppressive agent that would reduce the UACR in healthy individuals but have little or no effect in those with primary hyperaldosteronism. In 42 healthy cats the upper limit for the UACR was 46.5×10^{-9}. The administration of sodium chloride did not significantly lower the UACR but administration of fludrocortisone (0.05 mg/kg body weight) reduced it by

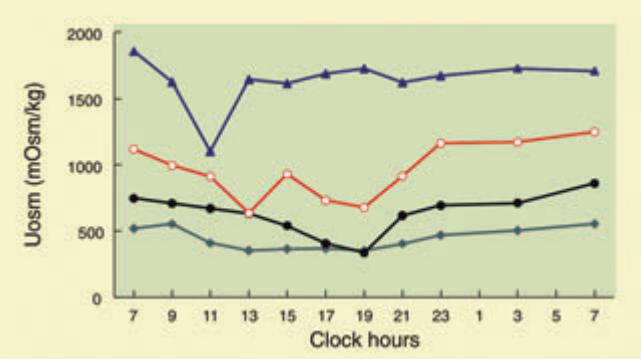


Figure 4.64:
Serial measurements of urine osmolality (Uosm, see also chapter 12.2.1) in a ten-year-old castrated male German shorthaired pointer with primary hyperaldosteronism (see also fig. 4.59) during the administration of three different doses of the mineralocorticoid-receptor antagonist spironolactone: 25 mg thrice daily (♦), 50 mg twice daily (●), and 50 mg thrice daily (○). The dose was increased at monthly intervals. The line at the top (▲) depicts Uosm values after left-sided adrenalectomy.

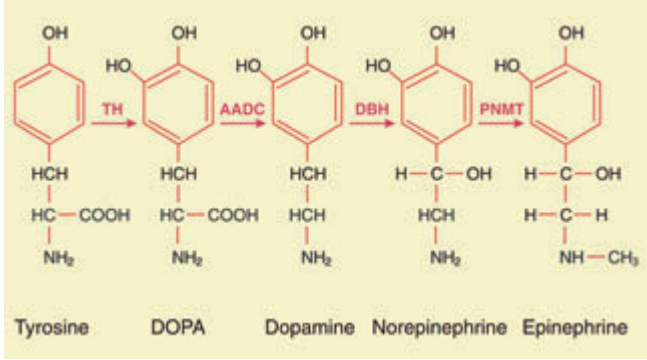


Figure 4.65:
Biosynthesis of catecholamines. The conversion of tyrosine to DOPA (dihydroxyphenylalanine) by tyrosine hydroxylase (TH) is the rate-limiting step. Aromatic L-amino acid decarboxylase (AADC) converts DOPA to dopamine. Dopamine is hydroxylated to norepinephrine by dopamine β-hydroxylase (DBH). The enzyme phenylethanolamine N-methyl transferase (PNMT) catalyzes the conversion of norepinephrine to epinephrine. Glucocorticoids enhance the expression of the gene encoding PMNT.

44–97 % (median 78 %). In a cat with an aldosterone-producing adrenocortical carcinoma the UACR was within the reference range and was not lowered by fludrocortisone administration.[304] This test may prove to be a practical noninvasive diagnostic tool, but further evaluation is required, particularly with regard to its discriminatory power in diagnosing idiopathic hyperaldosteronism.

Subtype classification – differentiating between tumorous and nontumorous mineralocorticoid excess – requires diagnostic imaging. Ultrasonography and computed tomography have been used in dogs and cats to identify and characterize adrenal tumors.[279,305] As in humans the findings are not always immediately conclusive.[306] The visualization of a small aldosteronoma may pose problems while nodular hyperplasia might be interpreted as microadenoma.[279,293]

Treatment

Unilateral adrenalectomy is the treatment of choice for confirmed unilateral primary hyperaldosteronism. There have been several reports of successful surgical treatment,[279,288,291] including the successful excision of an adrenocortical tumor and the associated caval thrombus.[307] Preoperatively and perioperatively hypokalemia should be controlled as well as possible, by oral and intravenous supplementation. Postoperative intravenous fluids can be confined to 0.9 % sodium chloride solution without potassium chloride, unless plasma potassium remains below 3.0 mmol/l. In principle during the first few weeks after surgery a generous dietary intake of sodium can be provided to avoid hyperkalemia that could develop from hypoaldosteronism due to chronic contralateral adrenocortical suppression. Analogous to the postoperative management of hypercortisolemia due to adrenocortical tumor (chapter 4.3.2), temporary fludrocortisone therapy could also be considered. However, in the reported cases such postsurgical measures have not been necessary and their omission does not seem to have had deleterious effects.

If surgery is not possible or if the adrenocortical disease is bilateral, medical treatment is possible with the mineralocorticoid-receptor antagonist spironolactone and oral supplementation with potassium gluconate. The initial doses are 2 mg spironolactone/kg and 0.5 mmol potassium gluconate/kg, twice daily. Persistent arterial hypertension can be treated with the calcium blocker amlodipine (1–2 mg/kg). In cases of adrenocortical tumor medical treatment may lead to resolution of symptoms and signs such as the myopathy in cats and the polyuria in dogs, but complete normalization may not be achieved (fig. 4.64).[279,291] Particularly plasma potassium tends to remain below the reference range, despite increasing doses of both spironolactone and potassium. Doses of spironolactone > 4 mg/kg may cause anorexia, diarrhea, and vomiting. These side effects may be due to interference by spironolactone with aldosterone action on transepithelial electrolyte transport in the distal colon.[308]

Experience is very limited, but medical treatment appears to be preferable in cats with hyperaldosteronism due to bilateral adrenocortical hyperplasia. The hyperaldosteronism is usually somewhat milder than in cases due to tumor and normokalemia may be maintained for a long period with spironolactone alone or together with low doses of potassium.[293]

Prognosis

After complete removal of a unilateral nonmetastasized mineralocorticoid-producing tumor, the prognosis can be excellent, without any medication. In both forms the disease may be associated with renal insufficiency.[291,293] Successful removal of the tumor will probably prevent further progression of aldosterone-induced arteriolar sclerosis and interstitial fibrosis in the kidneys (chapter 4.4.1). The prognosis may not be as favorable in cats with idiopathic hyperaldosteronism treated with spironolactone, for this treatment will not abolish the mineralocorticoid excess as definitely as surgery may do.

4.5 Adrenal medulla

4.5.1 Introduction

The adrenal medulla, which comprises approximately one-fourth of the adrenal mass, develops during fetal life as part of the autonomic nervous system. The cells of the adrenal medulla, called pheochromocytes or chromaffin cells, can be regarded as modified postganglionic sympathetic neurons lacking axons. They are innervated by preganglionic fibers of the sympathetic nervous system that induce the release of catecholamines into the bloodstream. Some extra-adrenal chromaffin tissue is also present adjacent to the aorta, in the carotid bodies, in viscera, and within sympathetic ganglia.[309,310]

Most of the blood supply of the adrenal medulla is via a portal system from the adrenal cortex, so that the medulla receives high concentrations of glucocorticoids. These induce the enzyme phenylethanolamine N-methyl transferase (PNMT) that is responsible for the conversion of norepinephrine to epinephrine (fig. 4.65). Some of the chromaffin cells, however, receive direct arterial blood supply bypassing the adrenal cortex. These cells contain predominantly norepinephrine.[310,311]

Catecholamines include epinephrine (adrenaline), norepinephrine (noradrenaline), and dopamine. In contrast to corticosteroid production by the adrenal cortex, adrenal medullary catecholamine synthesis is not essential for survival, i.e., after bilateral adrenalectomy extra-adrenally produced catecholamines fill the need. Catecholamines are synthesized from tyrosine by a process of hydroxylation and decarboxylation (fig. 4.65). With these features the adrenal medulla belongs to a system previously called APUD system (**a**mine **p**recursor **u**ptake **d**ecarboxylase system; see also chapter 10). Catecholamines are stored within the chromaffin cells in cytoplasmic vesicles, together with various other substances such as chromogranin-A, somatostatin, enkephalins, synapophysin, vasoactive intestinal polypeptide, ACTH, and CRH.[310] All of the epinephrine in the circulation is derived from the adrenal medulla, whereas circulating norepinephrine is mostly from postganglionic sympathetic neurons and only to a small extent from the adrenal medulla.

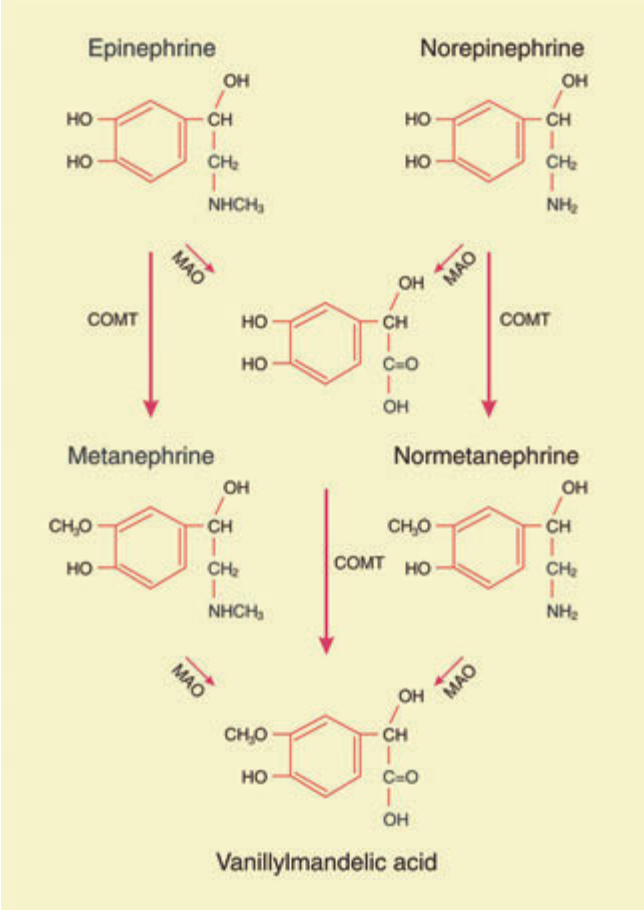


Figure 4.66:
Metabolism of catecholamines. Two enzyme systems are involved: COMT (catechol-O-methyl transferase) and MAO (monoamine oxidase).

Secretion of catecholamines is part of the activation of the sympathetic nervous system. Examples for stimuli are exercise, perceived danger, surgery, hypovolemia, hypotension, and hypoglycemia. The plasma half-life of catecholamines is very short (1–3 min). They are metabolized to the inactive compounds normetanephrine, metanephrine, and vanillylmandelic acid. They may also be inactivated in the liver by conjugation with sulfate or glucuronide. Excretion is via the urine (fig. 4.66).

Catecholamines bind to receptors in the plasma membrane, from which signal transduction to intracellular sites takes place via G-proteins. Adrenergic receptors are of two broad categories: α- and β-receptors, which are further divided into subgroups (α_1, α_2, β_1, β_2, β_3). The α-receptors have about the same affinity for norepinephrine and epinephrine, whereas β-receptors (in particular β_2-receptors) have a much higher affinity for epinephrine. The effects of catecholamines de-

Table 4.5: Catecholamine receptor types and subtypes

Organ / tissue	Receptor type	Effect
Cardiovascular system	β1	Increase in heart rate, increase in contractility
	α2	Vasoconstriction
	β2	Vasodilatation in skeletal muscle arterioles, coronary arteries, and all veins
Bronchial muscles	β2	Relaxation
Gastrointestinal tract	β2	Decrease in motility
Pancreatic islets	α2	Decrease in insulin and glucagon secretion
	β2	Increase in insulin and glucagon secretion
Liver	β2	Increase in glycogenolysis and gluconeogenesis
Adipose tissue	β2	Increase in lipolysis
Urinary bladder	α2	Increase in sphincter tone
	β2	Relaxation of M. detrusor
Eye	α1	Mydriasis

pend on the density of the different subtypes of receptors on specific organs and on the relative concentrations of epinephrine and norepinephrine (table 4.5). These effects are modulated by reflex mechanisms, e.g., as the blood pressure increases the heart rate is slowed and cardiac output tends to decrease. Additionally, the central nervous system (CNS) plays an important integrative role, so that one vascular bed may be dilated while others remain unchanged.[309,310,312]

4.5.2 Pheochromocytoma

Pheochromocytomas are catecholamine-producing neuroendocrine tumors arising from either chromaffin cells of the adrenal medulla or extra-adrenal paraganglia. The latter are referred to as extra-adrenal pheochromocytoma or paragangliomas.[313] Most tumors are derived from the adrenal medulla; paragangliomas have thus far been described in only a few case reports. Pheochromocytoma is considered to be rare in dogs and even less frequent in cats. However, due to the difficulties in diagnosing pheochromocytoma, quite a few may be overlooked and therefore the prevalence may be higher than generally assumed. Most tumors are unilateral; only occasionally are both adrenal glands affected. Pheochromocytomas may coexist with glucocorticoid-producing adrenocortical tumors, ACTH-producing pituitary tumors (fig. 4.67), or other endocrine tumors and as such be part of a multiple endocrine neoplasia syndrome.[148,149,314] Inherited multiple endocrine neoplasia syndromes (MENs) known to occur in humans[315] have thus far not been identified in dogs or cats.

Although pheochromocytomas tend to grow slowly, they should be considered potentially malignant tumors. In up to 50 % of cases the tumor is locally invasive and extends into the lumen of adjacent vessels and other tissues. Pheochromocytomas may metastasize to lymph nodes, spleen, liver, kidney, pancreas, lung, heart, bone, and CNS. Extraluminal compression of vessels by large tumors also occurs.[314,316,317]

Clinical manifestations

Pheochromocytomas occur most often in older dogs. There is no apparent sex or breed predilection. Symptoms and signs result from secretion of excessive amounts of catecholamines, or, infrequently, from the space-occupying or invasive nature of the tumor. Hormone secretion is sporadic and unpredictable and the clinical presentation is highly variable. Symptoms are often episodic and may only recur after weeks or months or may appear several times per day. They may be dramatic and life-threatening or they may be unapparent.

The symptoms and signs can be categorized as:

- Nonspecific: anorexia, weight loss, lethargy.
- Related to the cardiorespiratory system and / or to hypertension: tachypnea, panting, tachycardia, arrhythmias, collapse, pale mucous membranes, nasal-, gingival-, ocular hemorrhage, acute blindness.
- Related to the neuromuscular system: weakness, anxiety, pacing, muscle tremors, seizures.
- Miscellaneous: polyuria / polydipsia, vomiting, diarrhea, abdominal pain.

Large tumors may cause abdominal distension, ascites, and hind-limb edema, or rarely intra-abdominal or retroperitoneal hemorrhage due to tumor rupture.[314, 316–321]

Diagnosis

Since clinical manifestations are nonspecific, variable, and easily explained by disturbances of other organ systems, diagnosis of pheochromocytoma is challenging. There are no consistent abnormalities in routine hematology, blood biochemistry, or the urinalysis. There may be anemia, neutrophilia, increased liver enzymes, azotemia, and hypoalbuminemia. Although arterial hypertension is one of the hallmarks of the disease, it is detected in only approximately 50 % of dogs by the time of examination. Due to its episodic nature, hypertension might be detected in a higher percentage of patients by repetitive blood pressure measurements, but even so it is not pathognomonic for pheochromocytoma.

Tumor size varies greatly, from a diameter of a few millimeters and > 10 cm. In most dogs the pheochromocytoma is of sufficient size to be visualized by ultrasonography. Ultrasonography also enables identification of tumor invasion of surrounding tissue and vessels. However, no pattern of echogenicity or architecture is specific for pheochromocytoma (fig. 4.68).[322,323] The differential diagnoses for an adrenal mass include nonfunctional lesions such as myelolipoma, cyst, abscess, hematoma, and metastasis, and hypersecretory tumors,

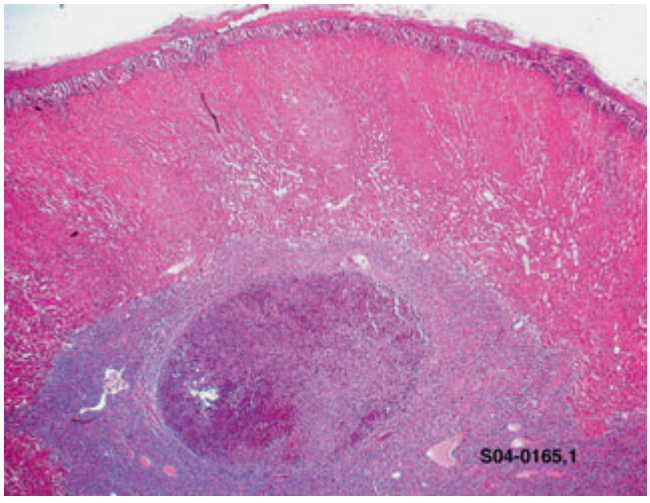


Figure 4.67:
Histological section of an adrenal gland from a dog with both pituitary-dependent hypercortisolism and pheochromocytoma. The adrenal cortex is moderately hyperplastic and there is a pheochromocytoma in the adrenal medulla. (Courtesy of Prof. Dr. Andreas Pospischil, Institute of Pathology, Vetsuisse Faculty, University of Zurich.)

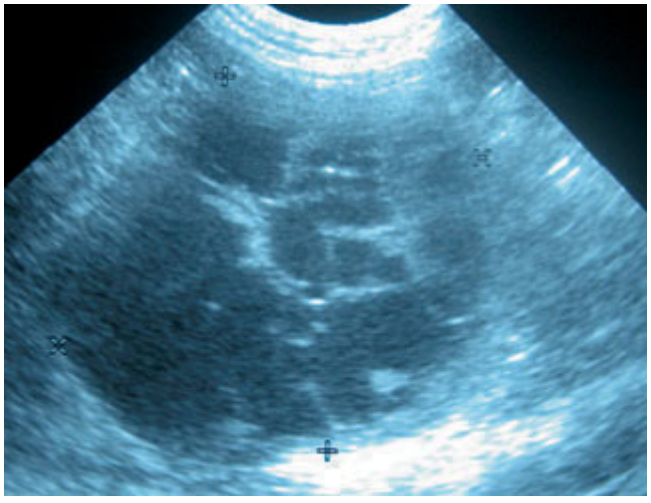


Figure 4.68:
Ultrasonographic image of a pheochromocytoma. The parenchyma is irregular due to various hypo- to anechoic areas. The largest diameter of the mass was 5.4 cm.

producing cortisol or a cortisol precursor, pheochromocytoma, and aldosteronoma. In dogs cortisol-producing tumors are by far the most common hypersecretory adrenal tumors and the clinical manifestations may be similar to those of pheochromocytoma. Hence it may be necessary to rule out hypercortisolism due to an adrenocortical tumor in some cases. On rare occasions both diseases occur simultaneously, further complicating the work-up.

CT and MRI are more sensitive than ultrasonography in identifying adrenal masses and characterizing the extent of local invasion. However, they do not provide a definitive diagnosis. Anesthesia and contrast media may provoke a hypertensive crisis and arrhythmias. Other advanced diagnostic imaging procedures such as scintigraphy with ^{123}I-labeled metaiodobenzylguanidine (^{123}I-MIBG) and positron emission tomography with *p*-[18F]fluorobenzylguanidine ([18F]MFBG) take advantage of the fact that these radiopharmaceuticals have similarities to norepinephrine and accumulate in the adrenal medulla. These techniques may therefore be more specific for the diagnosis of pheochromocytoma, but they have only been described in a small number of dogs and no data on sensitivity, specificity, and predictive values are available.[324,325] Similarly, information on the diagnostic value of fine-needle aspiration (FNA) is scarce. The risks and disadvantages (hypertensive crisis, arrhythmias, nondiagnostic samples, misinterpretation) of FNA have to be carefully weighed against the potential benefits.

The work-up of human patients with a suspected pheochromocytoma routinely includes biochemical testing, i.e., measurement of urinary catecholamines and their metabolites metanephrine, normetanephrine, and vanillylmandelic acid. Measurement of free metanephrines in plasma and urine is a more recent test. Measurements of free metanephrines in plasma and 24 h urine are reported to be more sensitive than measurements of plasma or 24 h urinary catecholamines. This higher sensitivity may be explained by the fact that although pheochromocytomas produce catecholamines they do not always release them but rather their metabolites. There is some controversy concerning the preferability of testing blood or urine. Plasma metanephrine measurements may have a higher sensitivity than measurements of 24 h urinary metanephrines, but their specificity may be lower.[325,326]

Evaluation of these variables in veterinary medicine has just begun. In a preliminary study the urinary concentrations of dopamine, norepinephrine, epinephrine, normetanephrine, and metanephrine, all related to creatinine concentration, were determined in healthy dogs and in dogs with pheochromocytoma. The normetanephrine:creatinine ratio had the highest discriminating power (fig. 4.69).[327] This may be surprising in light of the fact that epinephrine (which is metabolized to metanephrine) and not norepinephrine (which is metabolized to normetanephrine) is the main secretory product of the adrenal medulla. However, in dogs with pheochromocytoma the situation may be similar to that in humans, in which most tumors contain less epinephrine than the normal medulla, or even none.[328] Stress associated with the hospital

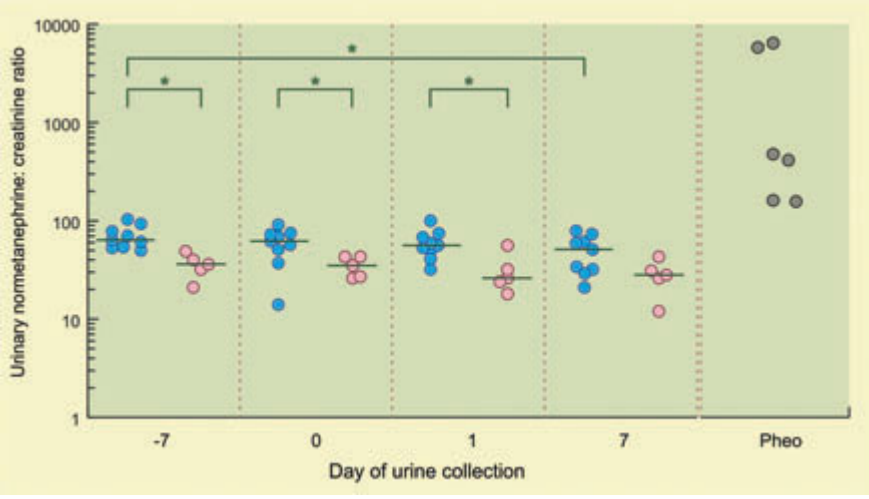


Figure 4.69:
Urinary normetanephrine:creatinine ratios in healthy dogs and in six dogs with pheochromocytoma. In the healthy dogs urine was collected at different times: day 0, in the hospital following the physical examination, and day –7, day 1, and day 7, at home seven days prior to and one and seven days after the hospital visit. Blue circles = dogs of clients; pink circles = dogs of staff. In the dogs with pheochromocytoma (Pheo) urine was collected once. * Indicates significant difference.

visit and the urine sampling increases urine catecholamine excretion. Urine collection should therefore take place at home after adaptation to the procedure.[327] Sample collection and urine processing require certain conditions, including acidification, avoidance of light, and cooled or frozen storage. Close collaboration with the laboratory is necessary for provision of reference ranges and technical assistance. No studies of plasma metanephrine measurements in dogs have been published and it may well be that they are less suitable in dogs because of the adverse influence of hospital-associated stress.

Treatment

Adrenalectomy is the treatment of choice and should be performed as soon as possible. If the tumor has invaded adjacent vessels and other tissues, the surgery can be extremely demanding and should be performed by an experienced surgeon. The patients carry a high anesthetic risk due to potential hypertensive crisis and arrhythmias requiring professional perioperative monitoring. An α-adrenergic blocker should be started immediately after diagnosis and given for at least one to two weeks before adrenalectomy.[314] The aim is to reverse the effects of excessive adrenergic stimulation (hypertension, hypovolemia) and to minimize perioperative complications. Phenoxybenzamine is used most often. The initial dose of 0.25 mg/kg BID should be gradually increased every few days until signs of hypotension or adverse drug reactions such as vomiting occur or the maximal dose of 2.5 mg/kg is reached.

Potential complications after surgery include hemorrhage, hypotension, hypertension, arrhythmias and tumor recurrence. Perioperative mortality is approximately 20–30 %. Dogs pretreated with phenoxybenzamine have a decreased mortality rate compared with untreated dogs.[314] Animals surviving this initial period may live for several years, even with advanced stage disease.[329–331]

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5 Endocrine Pancreas

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5.1 Introduction

5.1.1 The endocrine pancreas

The pancreas is an essential organ, responsible for both digestion and glucose homeostasis. It is located in the epigastric and mesogastric segments of the abdominal cavity and consists of a thin, slender right (duodenal) lobe and a shorter, thicker left (splenic) lobe, which are united at the pancreatic body. The form is that of a V, the apex of which lies caudomedial to the pylorus (fig. 5.1).

In most dogs the pancreas has two excretory ducts, in conformity with its origin from two different primordia, whereas in most cats only one duct persists. There is great variation in the pattern of the duct system within and between species. Blood is supplied by branches of the celiac and cranial mesenteric arteries; venous drainage is by vessels that terminate in the portal vein.

The endocrine function of the pancreas is provided by clusters of cells known as the islets of Langerhans. In the adult animal they constitute roughly 1–2 % of the total pancreatic mass and are scattered irregularly throughout the exocrine tissue. There are four major types of cells in the islets: β-cells (by far the most abundant) that produce insulin and amylin, α-cells that produce glucagon, δ-cells that produce somatostatin, and PP-cells that produce pancreatic polypeptide.[1,2] Most textbooks state that β-cells are located in the center of the islet but several studies have shown that the distribution differs between species and that in dogs and cats β-cells are often located in the periphery of the islet (fig. 5.2).[3,4] Several other peptides and hormones have been identified in the islets by the use of immunostaining techniques including TRH, ACTH, calcitonin gene-related peptide, cholecystokinin, gastrin, and pancreastatin. Although some of these appear to participate in the regulation of islet-cell function, their relevance is largely unknown.[5]

The islets are highly vascularized and their capillaries are fenestrated, increasing permeability. An islet-acinar portal system communicates between the endocrine and exocrine pancreatic tissue. It is assumed that blood coming from the islets flows into the acinar capillaries before leaving the pancreas and that islet hormones have a role in regulating the exocrine pancreas.[6] The islets are innervated by sympathetic and parasympathetic fibers which influence the release of pancreatic hormones.

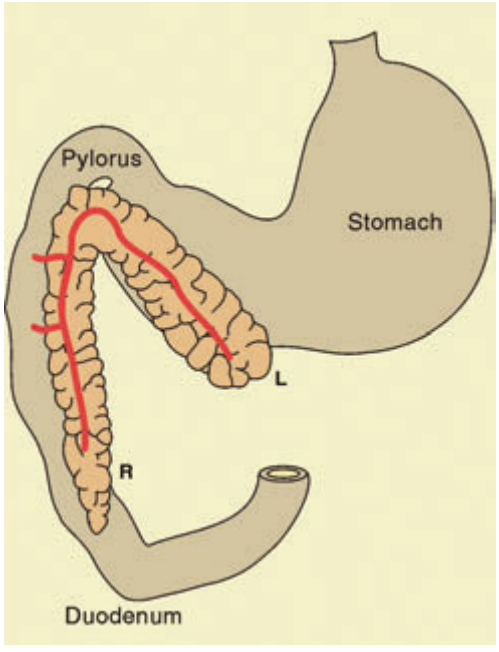


Figure 5.1:
Schematic drawing of the ventral aspect of the pancreas, showing its left (L) and right (R) lobes.

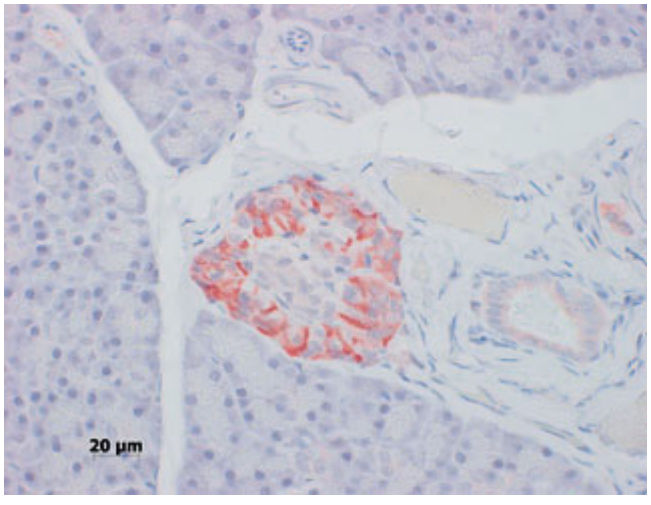


Figure 5.2:
Histologic section of the pancreas of a healthy cat, showing an islet of Langerhans surrounded by exocrine tissue. β-cells (red) are shown by immunohistochemical staining for amylin.

5.1.2 Insulin synthesis and structure

Glucose homeostasis is maintained by a complex system of regulating and modulating hormones and factors, of which insulin is the most important. Insulin is the only hormone that decreases blood glucose concentration.

The synthesis of insulin begins in the rough endoplasmic reticulum with the formation of preproinsulin, which is converted to proinsulin by removal of a small peptide fragment. Proinsulin is further processed to insulin by removal of another peptide, called C-peptide (connecting peptide) (fig. 5.3). Insulin and C-peptide are packaged and stored in secretory granules and released in equimolar amounts by the process of exocytosis. Within the granules insulin coprecipitates with zinc ions to form hexamers and microcrystals, but in the circulation it is a monomer.

The concentration of C-peptide in plasma is an indicator of β-cell function, but its measurement is mainly used in human medicine and for research purposes. Proinsulin is largely converted before secretion, so it does not appear in the circulation in appreciable quantities. There is some uncertainty whether elevated fasting proinsulin levels and a change in the proinsulin:insulin or proinsulin:C-peptide ratio are early indicators of β-cell damage.[7]

Insulin consists of two polypeptide chains, an A chain of 21 amino acids and a B chain of 30 amino acids, connected by two disulfide bridges (fig. 5.3). The insulin molecule has been highly conserved during evolution and the differences between species are small. Canine insulin is identical to porcine insulin and differs in just one amino acid from human insulin. Feline insulin is most similar to bovine insulin, also differing in only one amino acid, while differing from canine insulin at three positions (table 5.1). Circulating insulin is almost entirely unbound, has a half-life of 5–8 min, and is metabolized mainly in the liver and kidney.

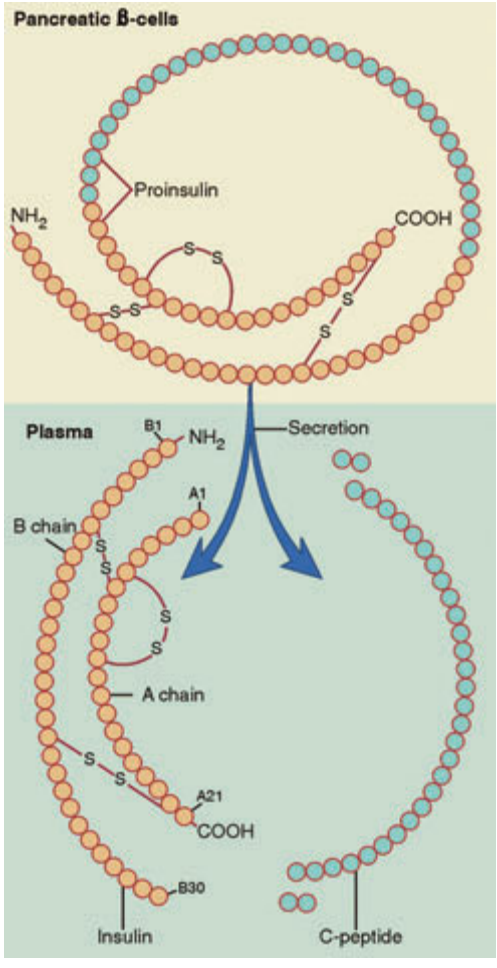


Figure 5.3:
Synthesis and secretion of insulin. Proinsulin is processed in the β-cells to insulin by removal of a peptide fragment called C-peptide (connecting peptide). Insulin consists of an A chain of 21 amino acids and a B chain of 30 amino acids, connected by two disulfide bridges.

5.1.3 Regulation of insulin secretion

Continuous availability and moment-to-moment adjustment of insulin is essential for the normal control of carbohydrate, protein, and lipid metabolism. The body has complex mechanisms to ensure adequate basal insulin secretion between meals as well as increased insulin secretion following meals. The most important regulator is the concentration of glucose in the blood and there is a positive feedback relation between blood glucose concentration and the insulin secretion rate (fig. 5.4).

Glucose is transported into β-cells via the glucose transporter protein GLUT 2 (chapter 5.1.4), which allows rapid equilibration between extracellular and intracellular glucose concentrations. Within the β-cells glucose is metabolized (phosphorylation by glucokinase and production of pyruvate) to produce ATP. The increase in the ATP:ADP ratio is followed by closure of ATP-sensitive potassium channels in the β-cell membrane, preventing potassium ions from leaving the β-cell. This in turn causes membrane depolarization and opening of voltage-dependent calcium channels in the membrane. The increase in cytosolic calcium then triggers insulin release.[7]

The secretion of insulin is biphasic following intravenous injection of a bolus of glucose. The first phase starts within a few minutes, lasts 5–10 min, and involves exocytosis of preformed insulin that is readily released from secretion granules. It is followed by a slowly increasing second phase that is directly related to the level to which glucose is elevated

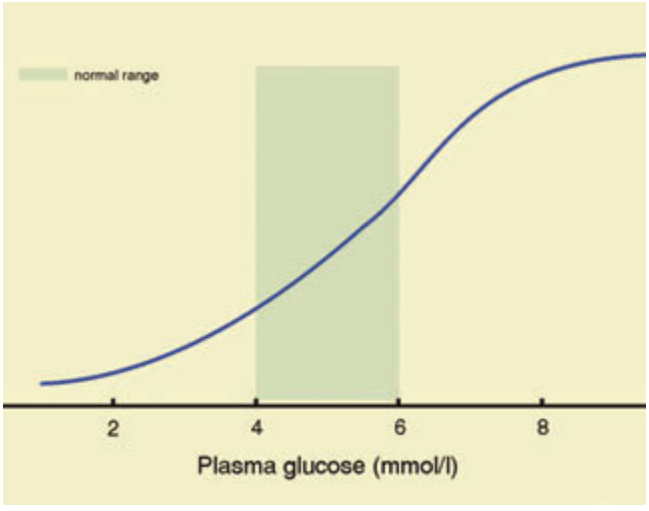


Figure 5.4:
Relation between insulin and glucose: insulin secretion is stimulated by an elevated glucose concentration and inhibited by a low glucose concentration.

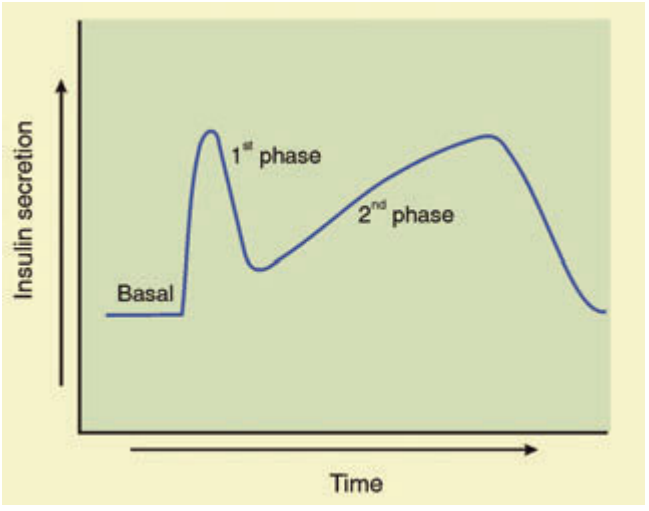


Figure 5.5:
Biphasic insulin response to an intravenous glucose injection.

(fig. 5.5). Orally administered glucose triggers more pronounced insulin secretion than does glucose given intravenously. This phenomenon is due to the actions of so-called incretin hormones, the most important being glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide, also called gastric inhibitory polypeptide (GIP). Incretins are secreted by endocrine cells in the gastrointestinal tract in response to nutrients and are then carried in the bloodstream to the pancreatic islets, where they interact with their receptors on β-cells to amplify insulin secretion. In several species GLP-1 has additional effects, such as reduction of glucagon secretion and stimulation of β-cell differentiation and proliferation, but it is not known whether these also occur in dogs and cats. In addition to glucose and other sugars, amino acids and fatty acids also stimulate insulin secretion. Stimulation can be direct or potentiated by incretins. The autonomous nerve system also exerts a modulating influence on islet hormone release, but its importance is still unclear. In general terms, insulin secretion is stimulated by vagal nerve fibers and inhibited by sympathetic nerve fibers (table 5.2).

Several other pancreatic hormones influence insulin secretion directly or indirectly. Amylin (islet amyloid polypeptide, IAPP) is a single-chain 37-amino-acid peptide cosecreted with insulin. Several effects of amylin, which have been demonstrated in humans and rodents, are of physiological relevance and contribute to the regulation of nutrient metabolism. They include inhibition of food intake, modulation of glucagon release, and delay of gastric emptying. Amylin and its metabolic effects may play a role in the development of human and feline type 2 diabetes mellitus.[8]

Table 5.1: Species differences in the amino acid sequence of insulin

	A8	A10	A18	B30
Human	Thr	Ile	Asn	Thr
Porcine	Thr	Ile	Asn	Ala
Canine	Thr	Ile	Asn	Ala
Bovine	Ala	Val	Asn	Ala
Feline	Ala	Val	His	Ala

Table 5.2: Factors influencing insulin secretion

Stimulants of insulin secretion	Inhibitors of insulin secretion
Glucose	Somatostatin
Several other sugars (e.g., xylitol, sorbitol)	Epinephrine, norepinephrine
Amino acids	
Fatty acids	
Incretins (e.g., GLP-1, GIP)	
Other intestinal hormones (gastrin, cholecystokinin)	
Glucagon	
Keto acids	
Acetylcholine	

Glucagon, a single-chain peptide of 29 amino acids, has long been a »neglected« hormone, but there is increasing evidence that glucagon disturbances play an important role in diabetes mellitus. It is a major catabolic hormone acting in concert with insulin to maintain normal blood glucose concentration by opposing many of the key metabolic effects of insulin. After food intake, insulin secretion increases to conserve energy and to prevent hyperglycemia. As the interval after food intake increases and blood glucose begins to decrease, glucagon is secreted to prevent hypoglycemia and to mobilize energy stores. Changes in the ratio of insulin to glucagon are largely controlled by the blood glucose concentration and to a lesser extent by the concentration of amino acids. There is paracrine signaling between insulin and glucagon, such that insulin inhibits glucagon secretion and glucagon stimulates release of insulin.

Somatostatin is a 14-amino-acid peptide that has been identified in many tissues. Pancreatic somatostatin has an inhibitory effect on absorption and digestion and on motility of the gastrointestinal tract. It is a potentially important paracrine inhibitor of insulin and glucagon secretion.

The hormones mentioned here have additional effects and interrelations and there are certainly other hormones and effects which are not yet known. In short, however, it is obvious that the pancreatic islets finely tune metabolism during times of feeding as well as during food deprivation.

5.1.4 Actions of insulin

Insulin regulates numerous metabolic processes through binding to high-affinity cell surface receptors. These receptors are widely distributed throughout the body and are found in tissues in which insulin mediates glucose uptake (such as muscle and adipose tissue) as well as in those in which it does not (such as liver, brain, kidneys, and erythrocytes).

Like the receptors for other protein hormones, the receptor for insulin is embedded in the plasma membrane. It is a tetrameric protein, composed of two α-subunits and two β-subunits linked by disulfide bonds. The α-subunits are extracellular and contain insulin binding domains, while the β-subunits penetrate through the cell membrane (fig. 5.6). The insulin receptor belongs to the large group of tyrosine kinase receptors. They mediate their activity by transferring phosphate groups to tyrosine residues on intracellular target proteins.

Binding of insulin to the α-subunits triggers the tyrosine kinase activity of the β-subunits, leading to autophosphorylation which activates the catalytic activity of the receptor. The »substrate« proteins phosphorylated by the insulin receptor are called insulin-receptor substrate (IRS) molecules. They are key mediators in the insulin signaling pathway and act as docking proteins between the insulin receptor and a complex network of intracellular molecules. How the intracellular signals lead to the final biological effects of insulin is the focus of very active research. Dysregulation within the signaling cascade may lead to insulin resistance, in which IRS molecules seem to play a major role.

Within seconds after insulin binds to its receptor, the so-called rapid insulin actions lead to the cellular uptake of glucose, amino acids, potassium, and phosphate. Intermediate actions occur within a few minutes, mainly affecting protein and glucose metabolism, followed several hours later by delayed actions which mainly concern lipid metabolism.

Glucose is a polar molecule and cannot diffuse across cell membranes. Its transport is facilitated in several tissues by a family of glucose transporter (GLUT) proteins or (in the intestine and kidney) by active transport with sodium. At least 14 different GLUT proteins have been identified in humans, named in order of their discovery, GLUT 1–14. Each appears to have evolved for a specific task. GLUT 4 is the major insulin-responsive transporter and is found almost exclusively in muscle and adipose tissue. Insulin stimulates glucose transport in these two tissues by causing the translocation of GLUT 4 molecules from the cytosol to the cell membrane, with which they fuse and function as pores for glucose entry (fig. 5.6). When insulin levels decrease, the GLUT 4 molecules are removed from the cell membrane. In various other tissues such as brain, liver, kidney, and intestinal tract, glucose uptake is insulin-independent and occurs via other GLUT proteins.[1]

Insulin is the most important anabolic hormone in the body and prevents catabolism of nutrient stores. Its main function is to ensure storage of glucose as glycogen, amino acids as protein, and fatty acids as fat. The main target tissues for insulin are liver, muscle, and adipose tissue (fig 5.7). Insulin facilitates the oxidation of glucose to pyruvate and lactate by the induction of enzymes such as glucokinase, phosphofructokinase, and pyruvate kinase. Insulin promotes glycogen synthesis in liver and muscle by increasing glycogen synthetase activity. Gluconeogenesis is decreased by insulin because insulin promotes protein synthesis in peripheral tissues, decreasing the availability of amino acids for gluconeogenesis. Additionally, insulin decreases the activity of hepatic enzymes involved in the conversion of amino acids to glucose.

In adipose tissue insulin promotes the synthesis of lipids and inhibits their degradation. Insulin activates the enzymes pyruvate dehydrogenase and acetyl-CoA carboxylase, which promote the synthesis of fatty acids from acetyl-CoA. Insulin also increases the activity of lipoprotein lipase, an enzyme located in the endothelium of capillaries of extrahepatic tissues, which promotes the entry of fatty acids into adipose tissue. Inhibition of lipolysis is mediated by inhibition of hormone-sensitive lipase.

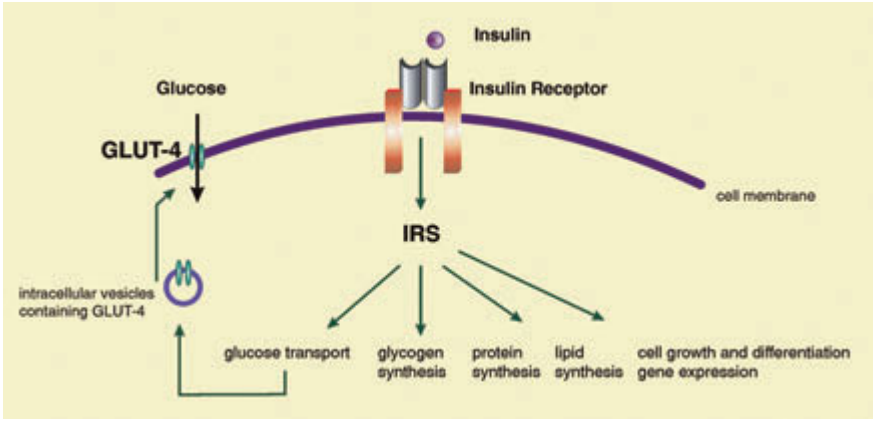


Figure 5.6:
Simplified scheme of insulin action. Glucose binding to its receptor protein initiates activation cascades that result in translocation of GLUT 4 to the cell membrane. This facilitates glucose influx and the synthesis of glycogen, protein, and lipid, as well as regulation of cell growth and expression of various genes. IRS (insulin receptor substrate) acts as a docking protein between the receptor and a complex network of intracellular signaling molecules.

Insulin stimulates protein synthesis and inhibits protein degradation and thus promotes a positive nitrogen balance.[9] The main antagonist of insulin is glucagon. Glucagon acts predominantly on the liver, increasing gluconeogenesis and glycogenolysis and decreasing glycogen synthesis. It is also a ketogenic hormone, due to its ability to enhance lipolysis. Insulin and glucagon act in concert following ingestion of protein. Both are released when amino acids increase in the plasma. Insulin causes a decrease in blood glucose and amino acids, while glucagon counters the decrease in glucose by stimulating hepatic gluconeogenesis. This interaction allows growth and survival on diets of almost exclusively protein and fat.

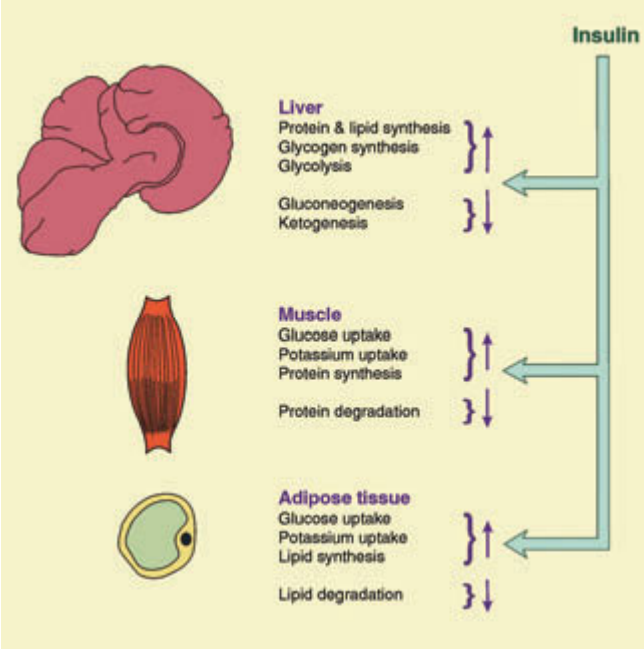


Figure 5.7:
Effects of insulin in liver, muscle, and adipose tissue.

5.2 Diabetes mellitus

5.2.1 Classification

Traditionally, diabetes mellitus in dogs and cats has been categorized more or less according to the scheme used in human medicine. However, it has long been uncertain whether this was justifiable, because of the scarcity of knowledge about the etiopathogenesis of diabetes in pet animals. Recent studies have provided evidence of the similarity of diabetes in humans, dogs, and cats. Although there is still much to unravel, the human classification may be used in order to facilitate recognition and differentiation of the various forms of the disease. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus of the American Diabetes Association, working in close collaboration with the WHO, defines diabetes mellitus in their latest report (2008) as »a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both«.[10]

The same committee has abandoned the long-used terms insulin-dependent and non-insulin-dependent diabetes mellitus (IDDM, NIDDM), since they were based on treatment rather than etiology and were therefore regarded as more confusing than helpful. The vast majority of human cases of diabetes fall into two broad categories, now named type 1 and type 2. It is currently assumed that diabetes in dogs is usually similar to type 1 and diabetes in cats closely resembles type 2.

In type 1 diabetes, which accounts for about 10 % of cases in humans, the cause is an absolute deficiency of insulin secretion due to T-cell mediated autoimmune destruction of the β-cells. A marker of the disease is the presence of circulating islet-related autoantibodies such as islet-cell autoantibodies (ICA), insulin antibodies (IA), glutamic acid decarboxylase (GAD) autoantibodies, and tyrosine phosphatase IA-2 antibodies. There is a genetic contribution and the autoimmune process is triggered by environmental factors that are still poorly defined. The disease is typically diagnosed in children and adolescents, but it may also have a slow and initially mild course with manifestation later in life (latent autoimmune diabetes in adults, LADA). There is a subgroup of type 1 diabetes, termed idiopathic, which is strongly inherited with no evidence for autoimmunity.

Type 2, which is largely identical to the previous NIDDM, accounts for up to 90 % of human cases. It is characterized by two defects: insulin resistance and β-cell dysfunction. Both are usually present at the time of diagnosis, although it is uncertain which is primary. The main sites of insulin resistance are liver, muscle, and adipose tissue. Insulin resistance in humans has a strong genetic basis and is promoted by obesity, physical inactivity, certain drugs, and high glucose levels. Obesity is of great importance, as reflected by the worldwide recognition that the increasing prevalence of obesity parallels the increasing prevalence of type 2 diabetes. Obesity is a subject of intense research, in particular since the discovery that adipose tissue is an active organ releasing large amounts of nonesterified fatty acids (NEFA) and various proteins, called adipokines. Some of the latter, such as adiponectin and possibly leptin, may improve insulin sensitivity, but others, such as NEFAs and proinflammtory cytokines, such as TNF-α and IL-6, induce or worsen insulin resistance (see also chapter 11).

Dysfunction of β-cells is essential for the development of type 2 diabetes. A characteristic feature is loss of first-phase glucose-induced insulin secretion. The second phase is also impaired, but to a lesser extent. The reasons for β-cell failure are largely unknown. Hyperglycemia and hyperlipidemia can be damaging (referred to as glucose toxicity and lipotoxicity). Another suggested factor is the deposition of amyloid in the islets due to polymerization of amylin. In contrast to type 1 diabetes, type 2 can usually be managed without insulin administration, at least for several years. Also, the risk of ketoacidosis is much higher in type 1.

The third category of diabetes, »Other specific types«, refers to diabetes that develops in association with diseases or factors other than defined as type 1 or type 2. Some of these are also relevant in dogs and cats. Diabetes can develop secondary to disorders of the exocrine pancreas (pancreatitis, pancreatic carcinoma), hypersecretion of counterregulatory hormones (hypersomatotropism, hypercortisolism, hyperthyroidism), and administration of glucocorticoids or progestins. A number of genetic syndromes, not yet described in animals, are also included in this category. The extent of glucose intolerance varies widely and insulin therapy may or may not be required; overt diabetes only develops in those individuals having a susceptibility to the disease. These disease associations should be differentiated from the coexistence of type 1 diabetes with endocrine diseases that result from common autoimmune destructive processes, e.g., diabetes with hypothyroidism (chapter 3.3.1) or Addison's disease (chapter 4.2.1).

The fourth category in humans, gestational diabetes, is of little importance in dogs and cats, but the diabetes associated with diestrus in dogs can be considered its equivalent (chapter 2.2.4.2).

5.2.2 Metabolic disturbances

Hyperglycemia develops when insulin secretion is absent or is inadequate for the degree of insulin resistance. Initially, insulin resistance may be compensated by increased insulin secretion, but eventually this is no longer possible. Absolute or relative lack of insulin has profound effects on carbohydrate, fat, and protein metabolism (fig. 5.8). Hyperglycemia results in part from the reduced entry of glucose into muscle and adipose tissue. Intestinal absorption of glucose is unaffected, as is entry of glucose into brain, kidney, and erythrocytes. The second and potentially more important cause of hyperglycemia is the unopposed production of glucose in the liver via gluconeogenesis and glycogenolysis. Glucagon contributes to the increased production of glucose, as do other stress hormones. When the renal capacity for glucose reabsorption is exceeded, glucose is lost in the urine. The resulting osmotic diuresis is compensated by increased water intake and the polydipsia may become severe. The loss of energy via glucosuria is compensated by increased food intake. Appetite stimulation by the hypothalamus, due to deficient glucose utilization, and various other mechanisms play a role.

Derangement of lipid metabolism plays a major role in the development of diabetes and its complications, and diabetes is sometimes regarded as a disease of lipid rather than of carbohydrate metabolism. The intracellular deficits of glucose and the lack of insulin lead to acceleration of lipid catabolism. The increased availability of glycerol accelerates hepatic gluconeogenesis. The increased levels of NEFA are also transported to the liver. There they undergo β-oxidation to acetyl CoA, the amount of which may exceed the need for ATP production by further oxidation in the Krebs cycle. This causes a shift to ketone body production, which can result in ketoacidosis. Increased hepatic concentration of fatty acids also results in enhanced hepatic synthesis of triglycerides and very-low density lipoproteins (VLDL). The consequences are hepatic steatosis and hyperlipidemia.

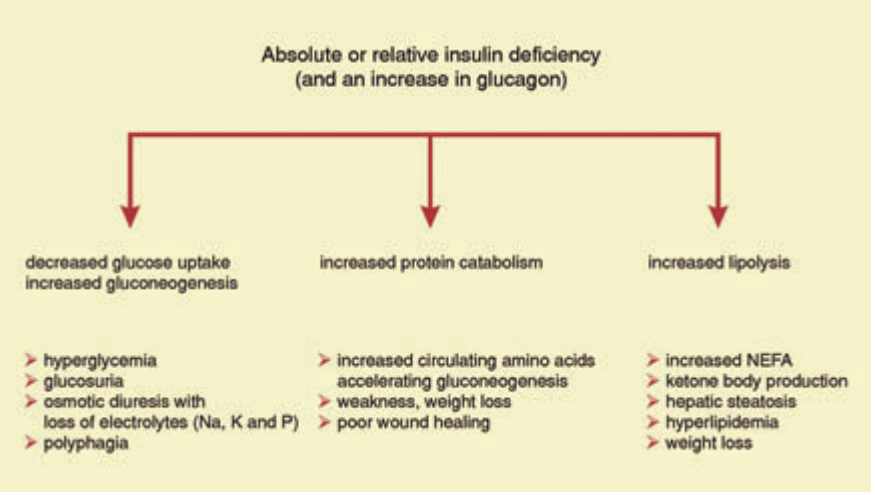


Figure 5.8:
Overview of the effects of insulin deficiency.

Protein metabolism shifts toward decreased protein synthesis and increased proteolysis. The increased availability of amino acids further accelerates hepatic gluconeogenesis. The consequences are negative nitrogen balance, loss of muscle mass, and possibly cachexia.

In diabetic humans there are chronic complications that severely affect the quality of life and life expectancy. These involve the vascular system (microvascular and macrovascular disease) and the nervous system, skin, and lenses. The hypotheses that have been proposed to explain these include increased activity of the polyol pathway with accumulation of sorbitol, increased formation of advanced glycation end products, and decreased antioxidant status.

The serious chronic complications in humans – nephropathy and cardiovascular disease – are rare in diabetic dogs and cats, most probably because of the shorter lifespan. The most common diabetic complication in dogs is cataract. Recent studies have shown that the activity of the enzyme aldose reductase in the lens is increased, which leads to accumulation of sorbitol. Because sorbitol is hyperosmotic, there is an influx of water, swelling and rupture of lens fibers, and altered membrane permeability. Aldose reductase activity is low in the lenses of older cats, which may in part explain why cats have much less serious diabetic cataracts.[11]

Electron microscopic studies of peripheral nerves have revealed that more than 90 % of diabetic dogs and cats have neuropathies similar to those in diabetic humans.[12,13] Although neuropathy is rarely recognized clinically in dogs, it is a common problem in cats. The reasons for this difference and the underlying mechanism are unknown.

5.2.3 Diabetes mellitus in dogs

Diabetes mellitus is one of the most common endocrine disorders in dogs, having a prevalence of 0.3–0.6 %.[14,15] In many dogs the disease is similar to human type 1 diabetes, which is caused by autoimmune destruction of β-cells in genetically predisposed individuals. Antibodies against β-cells and several islet components (insulin, GAD65, IA2) have been demonstrated in the serum of dogs with newly diagnosed diabetes, suggesting that these antigens are involved in the autoimmune process.[16,17] The observation that certain breeds of dogs are predisposed to diabetes[18] recently led to genetic studies. The risk of diabetes was shown to be associated with certain dog leukocyte antigen (DLA) haplotypes. Since most dogs are middle-aged to elderly at the time of diagnosis, canine type 1 diabetes seems to correspond best to the subgroup of type 1 diabetes termed latent autoimmune diabetes in adults (LADA).[19–24]

Dogs with diabetes may have concurrent endocrine diseases of possible autoimmune etiology (such as hypothyroidism and Addison's disease), a combination which may be equivalent to human autoimmune polyendocrine syndrome type 2. Humans who carry a particular HLA genotype are at a higher risk of diabetes, a situation similar to the high risk DLA haplotype in dogs.[25]

Diabetes mellitus occurs occasionally in dogs less than twelve months of age, most likely not due to autoimmune destruction but to β-cell aplasia or abiotrophy. There has been no evidence that dogs develop an equivalent of human type 2 diabetes. Other forms of diabetes (a category previously called secondary diabetes) include pancreatic destruction due to acute or chronic pancreatitis or pancreatic neoplasia, and insulin resistance due to other diseases or factors. Evidence for acute or chronic pancreatitis was reported in 13 % of dogs



Figure 5.9:
Bilateral cataract in a dog with diabetes mellitus.

with diabetes mellitus in one study[26] and 28 % of those in another.[27] However, a cause and effect relation is not yet clear, and while diabetes is a known risk factor for pancreatitis, pancreatitis may also cause such destruction of β-cells that diabetes ensues. It has also been hypothesized that β-cell antigens released in the inflammatory process could stimulate an immune reaction that exacerbates the destruction. Exocrine pancreatic insufficiency can also be a sequela of pancreatitis and is occasionally seen in dogs with diabetes.

The increase in progesterone levels during diestrus in intact bitches results in a rise in circulating levels of growth hormone (GH) originating from the mammary gland.[28,29] This is in principle a physiological event, but some dogs develop diabetes during this phase of the cycle due to the diabetogenic actions of GH. Acromegalic features caused by the growth-promoting effects of GH may also be obvious (chapter 2.2.4.2). Before the onset of overt diabetes, there may have been mild symptoms in preceding diestrus phases that were overlooked. Remission of diabetes is possible, provided that castration is performed promptly and that there is still sufficient β-cell function. Glucose intolerance and diabetes may also be induced by glucocorticoids. In most dogs with hypercortisolism, however, blood glucose concentration is normal or only slightly elevated. Overt diabetes develops in only about 10 % of the cases. Administered progestins and/or glucocorticoids may also induce diabetes, more often in cats than in dogs.

Signalment and clinical manifestations

Diabetes typically occurs in middle-aged to elderly dogs, most being five or more years of age, and rarely occurs in dogs < 12 months of age. The proportion of females has decreased from more than 70 % to around 55 %, most probably because of more frequent early neutering and the consequent decrease in diestrus-associated diabetes (chapter 2.2.4.2).[15,19] Samoyeds, various terrier breeds (Australian, Tibetan, cairn, West Highland white), miniature schnauzers, beagles, and poodles (miniature and toy) are at increased risk for diabetes. The boxer, German shepherd dog, and golden retriever appear to be at low risk[18]. Preliminary genetic studies have suggested a genetic component for both susceptibility and resistance to diabetes.[23,25]

The four typical symptoms of diabetes mellitus are polyuria, polydipsia, polyphagia, and weight loss. These are sometimes unnoticed until the dog develops blindness due to diabetic cataracts (fig. 5.9). About 50 % of diabetic dogs develop cataract within the first six months and about 80 % within 16 months after the diagnosis of diabetes.[30] Due to the potential danger of lens-induced uveitis, the eyes should be monitored closely during the course of diabetes. The risk of capsule rupture appears to be particularly high in dogs with rapidly progressing cataracts.[31] The prognosis following early surgical intervention is usually good.

Symptoms and signs other than cataract depend on the duration and severity of the diabetes and possible concurrent disease such as pancreatitis or infections. The diabetic dog may be obese, of normal weight, or underweight. Its haircoat may be dull and hepatomegaly may be palpable. Otherwise, the dog with so-called uncomplicated diabetes is usually in relatively good physical condition. In contrast, dogs with diabetes complicated by ketoacidosis or the hyperosmolar nonketotic syndrome are usually presented with symptoms of lethargy, anorexia, reduced water intake, and vomiting (see below).

Diagnosis and workup

Diabetes is diagnosed on the basis of appropriate symptoms and signs, persistent hyperglycemia, and glucosuria. There are no diagnostic criteria for diabetes in dogs, as there are in humans, and thus the blood glucose level signifying diabetes is imprecise. Most diabetic dogs are not presented for veterinary examination until blood glucose concentration exceeds the renal capacity for glucose reabsorption (~10 mmol/l) and hence polyuria and polydipsia develop. Stress hyperglycemia is not a relevant differential diagnosis in dogs, as it is in cats. Blood glucose concentration may also be increased by anxiety and by other diseases, but this hyperglycemia is either mild or its cause (e.g., head trauma[32] or seizures) is readily apparent. If mild hyperglycemia (blood glucose 7–9 mmol/l) persists in an unstressed and otherwise unremarkable dog, a search for diseases causing insulin resistance, such as hypercortisolism, may be warranted. Glucosuria alone is insufficient for the diagnosis of diabetes, since it may also by caused by renal defects

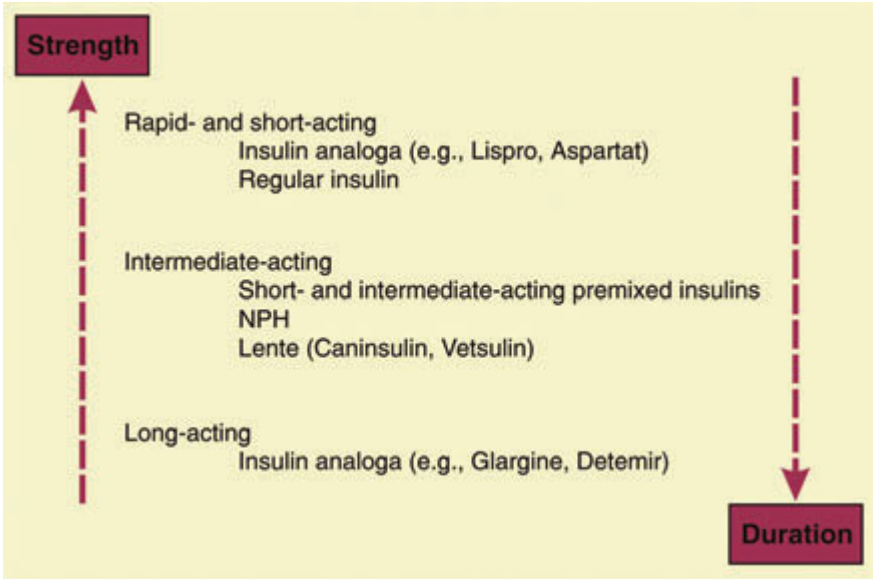


Figure 5.10:
Strength and duration of action of short-, intermediate-, and long-acting insulin.

and certain drugs.[33] Measurement of fructosamine is not required for the diagnosis per se in dogs, but it is useful in long-term management and an initial measurement provides a reference point and is therefore recommended.[34]

Fructosamine is the product of an irreversible reaction between glucose and amino groups on plasma proteins and it reflects the mean blood glucose concentration in the preceding one to two weeks. It is not affected by short-term changes in blood glucose concentration. Reference ranges differ slightly between laboratories, but are usually about 200–350 µmol/l. It is unusual for a diabetic dog or cat to have a normal fructosamine level at the time of diagnosis but diabetes of very short duration (< 5 days) or hypoproteinemia are possible reasons.[35] In a newly diagnosed diabetic dog, fructosamine is usually > 400 µmol/l and may be > 1000 µmol/l. Glycated hemoglobin is another indicator of long-term metabolic control, but for technical reasons it is rarely used in veterinary medicine.

Further workup should answer the following questions:
- How severe is the disease, i.e., is diabetic ketoacidosis present?
- Are there concurrent diseases such as stomatitis / gingivitis or urinary tract infection, which could hinder management of the diabetes?
- Is there evidence for underlying disease / factors which could have caused the diabetes, such as pancreatitis, hypercortisolism, diestrus, or diabetogenic drugs?

Routine hematology, plasma or serum biochemistry, urinalysis, and urine culture should be performed. Typical findings include a stress leukogram, hyperlipidemia, slight to moderate elevation of alanine aminotransferase (ALT) and alkaline phosphatase (ALP), urine specific gravity > 1.020 despite polyuria, and glucosuria, proteinuria, and bacteriuria with or without pyuria. There may be a trace of ketone bodies in the urine even in uncomplicated diabetes. Additional diagnostic procedures that may be indicated include radiographs, abdominal ultrasonography, measurement of trypsin-like immunoreactivity (TLI), and canine pancreatic lipase immunoreactivity (cPLI). Testing for hypercortisolism should be delayed until treatment of the diabetes is stabilized. Measurement of circulating insulin concentration is not helpful in most cases.

Treatment

The aims of therapy are to eliminate the symptoms and signs of diabetes mellitus and prevent short-term complications (hypoglycemia and ketoacidosis), thereby enabling the animal to have a good quality of life. It is not necessary to maintain normal or near normal blood glucose levels, as is the aim in humans, for most diabetic dogs and cats appear to do well when the blood glucose ranges between 15 mmol/l before insulin administration and 5 mmol/l at the time of the glucose nadir (= lowest plasma glucose concentration).

Successful treatment requires that the owner be highly motivated and work in close collaboration with the veterinarian, who follows a strict protocol (see chapter 13.3.1). Treatment consists of insulin therapy, dietary management, body weight reduction if the dog is overweight, daily exercise, cessation of diabetogenic drugs, and control of concurrent or underlying problems. All dogs with diabetes should be treated with insu-

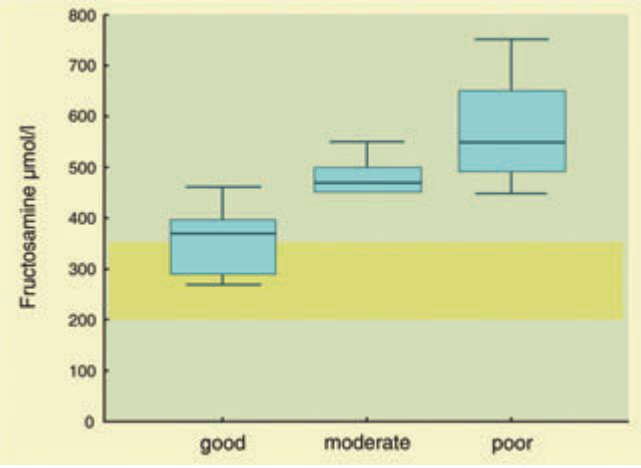


Figure 5.11:
Fructosamine concentrations in plasma of diabetic dogs with good, moderate, and poor metabolic control. In these box-and-whisker plots, the box represents the 25th to 75th percentile (= middle half of the data). The horizontal bar through the box is the median and the whiskers extending above and below the box represent the main body of data, which in most cases is equal to the range. The reference range (200–350 µmol/l) is shown in yellow.

lin. Oral hypoglycemic drugs are ineffective for metabolic control, even though α-glucosidase inhibitors or chromium may have slight auxiliary effects.

Insulin preparations are categorized according to duration of action as short-, intermediate-, and long-acting (fig. 5.10). In dogs with uncomplicated diabetes, treatment is started with an intermediate-acting insulin, which is porcine-derived, *lente*-type insulin (Caninsulin® / Vetsulin®, Intervet) licensed for use in dogs. In some countries, other insulins (Insuvet® Lente, Insuvet® PZI, both Schering Plough; PZIVet®, IDEXX) are also available for veterinary use. Lente insulin is a mixture of 30 % short-acting amorphous and 70 % long-acting crystalline insulin. The starting dose is 0.25–0.5 U/kg, administered twice daily. Once-daily administration of a higher dose is not recommended because it increases the risk of hypoglycemia.[36,37] The diabetic patient should receive meals of constant composition and caloric content, fed at the same times each day, just before each dose of insulin. A high-fiber diet (> 8 % fiber on a dry-matter basis) is preferred.[38,39] To simplify treatment, dogs are fed two meals of equal size. In those that are obese, the meals should be reduced to achieve a 1 % reduction in weight per week. Concurrent severe disease such as pancreatitis or renal failure usually requires a different dietary regimen, which has priority over the dietary management of diabetes.

Intact bitches that have developed diabetes during diestrus should be castrated as soon as possible, eventually after one to three days of stabilization with insulin. Most remain hyperglycemic after castration and require insulin treatment, but insulin resistance can gradually decrease during days to weeks of treatment and complete remission of the diabetes may be achieved by close monitoring and appropriate adjustment of the dose of insulin. All intact bitches with diabetes should be castrated, even if there has been no obvious temporal relation betweens diestrus and the onset of diabetes. Even though remission of the diabetes is not usually achieved by castration, it is necessary to prevent progesterone-induced hypersecretion of mammary-derived GH during subsequent diestrus and the resulting insulin resistance and disruption of treatment.

In the rare instance in which castration is not possible, use of the progesterone receptor antagonist aglepristone is a reasonable alternative. Aglepristone may also be given to dogs which developed diabetes during progestin treatment.

After diagnosis of diabetes the dog may be kept in the hospital for one to two days to begin insulin therapy and to complete the workup. During this time blood glucose concentration should be measured three to four times over the day and the dose of insulin reduced if blood glucose falls below 5 mmol/l. It is not necessary to increase the dose of insulin if blood glucose remains high, because full action of insulin develops over a few days (so-called equilibration). The initial workup and beginning of treatment may also be on an outpatient basis.

One of the most important periods in the owner's care of a diabetic pet is the time during which the veterinarian or the nurse teaches the technical aspects of the treatment. The owner must be able to mix the insulin correctly (gentle rolling, not shaking), load a syringe without air bubbles, administer an injection subcutaneously on the lateral wall of the chest, know how to deal with such problems as injection pain or bleeding and injection into the fur rather than the subcutis. The owner must recognize the symptoms of hypoglycemia, recurrence of polyuria and polydipsia, and symptoms of diabetic ketoacidosis, and understand that these require consultation with the hospital. The owner should also know that the insulin should be stored in the refrigerator in the up-right position and that Caninsulin is a U-40 insulin, in contrast to U-100 insulins for humans, and that only a U-40 syringe should be used.

It usually takes two to three months for reasonable glycemic control to be achieved, but lifelong supervision and periodic adjustment of therapy is usually needed. Follow-up examinations should be made at one, three, six to eight, and ten to twelve weeks after diagnosis, and then approximately every four months. The examination includes assessment of the owner's observations of symptoms, measurement of body weight, and measurement of blood glucose and fructosamine concentrations. The presence or absence of polyuria, polydipsia, polyphagia, lethargy, and weight loss are used to assess the quality of metabolic control.[40]

Fructosamine concentration increases when glycemic control worsens and decreases when glycemic control improves. Since even well-controlled diabetic dogs are slightly to moderately hyperglycemic throughout the day, fructosamine does not usually become completely normal during therapy. Thus the finding of a normal fructosamine concentration (especially in the lower half of the reference range) should raise concern about the possibility of prolonged periods of hypoglycemia due to insulin overdosage. Fructosamine levels of 350–450 µmol/l indicate good metabolic control, levels of 450–550 µmol/l indicate moderate control, and those above 550 µmol/l indicate poor control (fig. 5.11). High fructosamine levels indicate poor control but do not help to identify the cause and thus all possibilities must be considered: insulin underdosage, short duration of insulin effect, diseases causing insulin resistance, and the Somogyi effect.

Glucose measurements are required to characterize the problem and evaluate the action of insulin. Single measurements are sufficient when symptoms of diabetes have been resolved and blood glucose around the time of insulin administration is found to be 10–15 mmol/l and fructosamine is 350–450 µmol/l. This indicates satisfactory control and additional blood glucose measurements are unnecessary. In contrast, serial blood glucose curves (BGC), for which glucose is measured every 1–2 h, should be obtained in animals with persistence of polyuria, polydipsia, and weight loss, and fructosamine levels above 550 µmol/l. Insulin and food are given at home and the BGC measurements are begun as soon as possible thereafter. The most important variables evaluated by the BGC are insulin efficacy, the glucose nadir, and the duration of effect. Insulin efficacy (= difference between the highest and the lowest glucose concentration) is interpreted with reference to the highest blood glucose concentration and the insulin dose. A small difference (e.g., 3 mmol/l) is acceptable if the highest blood glucose is $<$ 12 mmol/l but not acceptable if it is $>$ 17 mmol/l. A difference of 6 mmol/l would indicate satisfactory insulin efficacy in an animal receiving an insulin dose of $<$ 0.5 U/kg but insulin resistance if the dose is $>$ 1.5 U/kg. In the latter case, technical problems and the counterregulatory phase of the Somogyi effect must also be considered.

The glucose nadir, which should be interpreted next, is ideally 5–8 mmol/l. A lower nadir can be caused by an insulin dose that is too high, excessive overlap of insulin actions, lack of food intake, and strenuous exercise. A glucose nadir $>$ 9 mmol/l can be the result of an insulin dose that is too low, stress, the counterregulatory phase of the Somogyi effect, and technical problems of the owners (fig. 5.12). In an animal already being treated with high doses, insulin resistance is also possible. It is very important to identify the cause, for it is necessary in order to correct the treatment.

The duration of insulin effect is defined as the time from the injection through the glucose nadir until the glucose concentration returns to 12–15 mmol/l. If the duration is less than

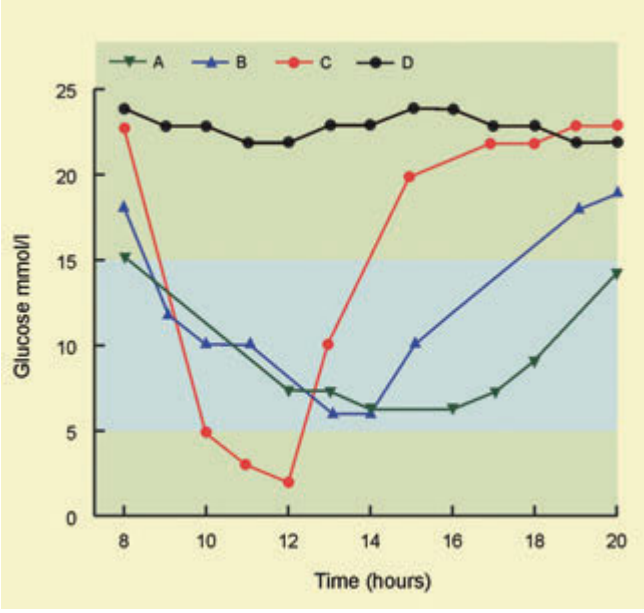


Figure 5.12:
Representative blood glucose curves in animals treated with an intermediate-acting insulin BID, at 8 a.m. and 8 p.m. The blue area is the preferred range of blood glucose concentration in treated diabetic dogs and cats (15–5 mmol/l).
(A) Ideal curve.
(B) Short duration of insulin effect.
(C) Somogyi effect with counterregulation after rapid decrease in blood glucose concentration.
(D) Poor response due to technical problems, the counterregulatory phase of the Somogyi effect, insulin resistance, poor insulin absorption, or insulin antibodies.

8–10 h, there is usually polyuria, polydipsia, and other symptoms of diabetes and if more than 14 h there is a risk of hypoglycemia or the Somogyi effect. It may be possible to improve the duration of action by manipulating the diet, but if not, changing to an insulin with a different action profile is indicated.

Depending on the results of the BGC, a change in the insulin dose and sometimes a change in the insulin preparation is required. As a rule of thumb, changes in the dose should be of the order of 10–25 %, but following hypoglycemia or the Somogyi effect, the dose should be decreased by at least 50 %. Changes should not be made more frequently than every five to seven days, except in case of repeated hypoglycemia.

In the past, BGCs were almost always performed in veterinary hospitals, because most owners are unable to collect venous blood samples. Even so, that approach is time-consuming and expensive and therefore probably not performed as often as it should be. In addition, the results of such BGCs can be influenced by stress, lack of exercise, and differences in the feeding routine. Fortunately, methods are now available that enable the owner to measure blood glucose at home. Capillary



Figure 5.13:
Obtaining a blood drop from the inner surface of the pinna of a diabetic dog, using the slight suction created by a lancing device.

blood is obtained from the inner aspect of the dog's pinna by means of a lancing device (fig. 5.13) and blood glucose concentration is measured with a portable blood glucose meter (PBGM). Alternatively, some owners can be trained to collect blood from a peripheral vein with a needle and syringe. In either case, home monitoring of blood glucose (HM) can be an imposing challenge for the owner and every effort should be made to minimize the technical difficulties. The owner should be provided with a PBGM that is simple to operate and should have ready access to veterinary support whenever required.

In the Clinic of Small Animal Internal Medicine of the University of Zurich, HM is not begun before the third week of treatment. This allows the owner to become familiar with the disease and to gain experience with the injection of insulin. Once the owner is comfortable with the procedure, he or she measures the animal's fasting blood glucose concentration twice weekly and a BGC once monthly. The former serves to detect morning hypoglycemia, in which case the owner is instructed to contact the clinic.[41,42]

For determination of the BGC, blood glucose concentration is measured before insulin injection (fasting) and then every 2 h until the next injection. Interpretation of the BGC follows the same rules as used in the hospital. There may be considerable day-to-day variability of blood glucose concentration[43] due to difference in insulin absorption and different

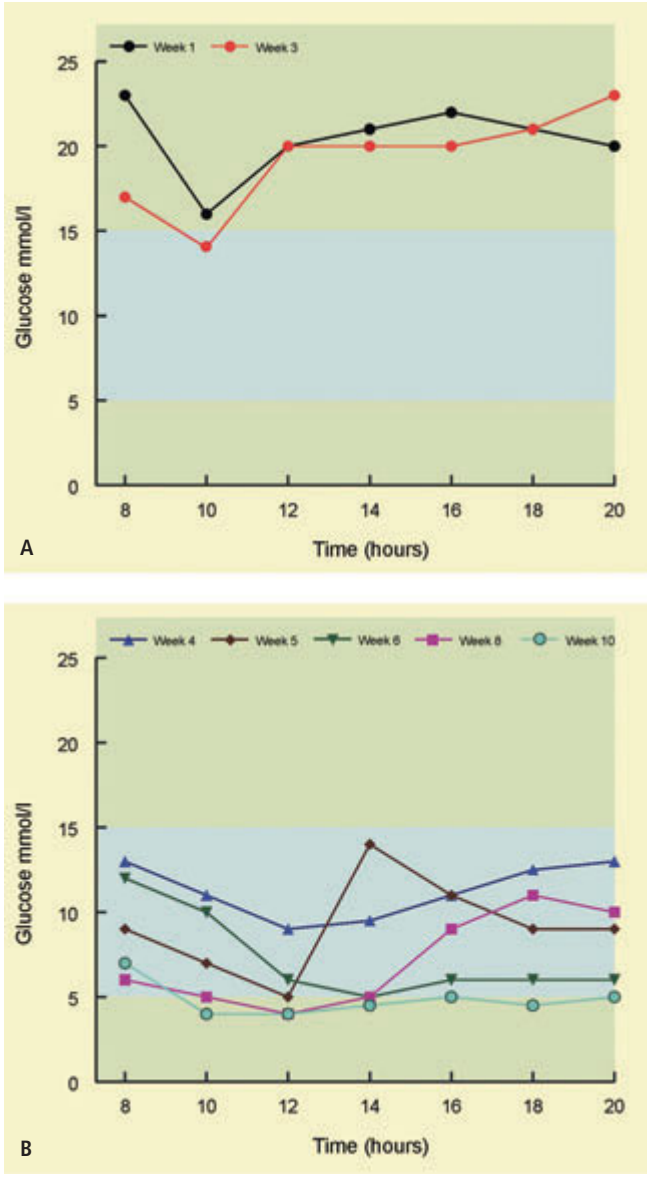


Figure 5.14:
Blood glucose concentrations in a collie bitch that developed diabetes during diestrus and was castrated immediately after diagnosis. The dog remained hyperglycemic postoperatively and was discharged on 0.5 U/kg lente insulin BID.
(A) Blood glucose curves determined in the hospital at one and three weeks after castration. After each curve, the dose of insulin was increased by 25 %.
(B) Blood glucose curves determined at home by the owner at 4, 5, 6, 8, and 10 weeks after castration. Blood glucose levels decreased progressively and the dose of insulin was reduced after each curve. At week 10, insulin was stopped and the dog remained in remission thereafter.
The blue area is the preferred range of blood glucose concentration in treated diabetic dogs and cats (15–5 mmol/l).

levels of stress and exercise. Individual curves may thus not reflect the true glycemic situation, regardless of whether they are obtained in the hospital or at home. However, one of the major advantages of HM is that it enables the BGC to be measured frequently, which may be of particular importance in animals that are difficult to regulate or in which insulin resistance is likely to decrease and needs close supervision (fig. 5.14).

5.2.4 Diabetes mellitus in cats

Diabetes mellitus is a common endocrine disease in cats. It was recently reported that in the USA there was an increase in its prevalence in veterinary teaching hospitals from 0.08 % in 1970 to 1.2 % in 1999.[44] In Australia the current hospital prevalence is 0.56 % and in the UK the prevalence in an insured cat population is 0.43 %.[45,46] Type 1 diabetes appears to be very rare in cats, in contrast to dogs. Antibodies against β-cells and insulin have not been found in cats and lymphocytic infiltration, a marker of immune-mediated destruction, has only been described in a small number of cases.[47,48]

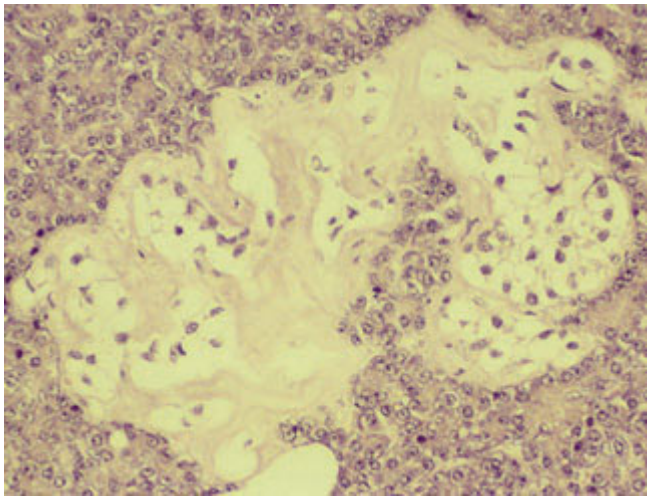


Figure 5.15:
Pancreatic islet of a cat with diabetes mellitus (H&E, 250x). There are massive amorphous deposits of amyloid (pink material), together with hydropic degeneration of islet cells.

It is currently assumed that in approximately 80 % of diabetic cats the disease resembles type 2 diabetes, based on clinical characteristics and islet histology.[21] Type 2 diabetes is a heterogeneous disease involving a combination of impaired insulin action (insulin resistance) and β-cell failure. Environmental and genetic factors play a role in the development of both factors, but the genetic factors have not yet been characterized in cats. The most convincing arguments for the existence of genetic factors have been derived from studies in Australia and the UK in Burmese cats, in which the frequency of diabetes was shown to be about four times higher than in domestic cats.[45,46] Additional risk factors include increasing age, male gender, being neutered, physical inactivity, glucocorticoid and progestin administration, and obesity.[44,46,49] As in humans, the most important risk factor in cats is obesity and it has been shown that obese cats are 3.9 times more likely to develop diabetes than those of optimal weight.[50] In healthy experimental cats an average weight gain of 1.9 kg during a feeding trial was associated with a more than 50 % decrease in insulin sensitivity. Male cats tended to have lower insulin sensitivity prior to the trial and gained more weight than did female cats, which might explain their greater risk for diabetes.[51]

In humans it is now well accepted that adipose tissue is an important endocrine organ, producing various factors collectively termed adipokines that influence insulin sensitivity. Among them are leptin, adiponectin, and proinflammatory cytokines such as TNF-α and IL-6 (see also chapter 5.2.1).[52] Preliminary studies in obese cats have shown that, as in humans, adiponectin levels decrease in obesity while leptin and TNF-α levels increase.[53] It is important to note that although obesity induces insulin resistance, not all obese cats develop diabetes. When β-cells are healthy, the adaptive response to obesity and insulin resistance is an increase in insulin secretion, so that normal glucose tolerance is maintained. However, when there is β-cell dysfunction, glucose tolerance is impaired and eventually type 2 diabetes results. Initially, the first phase of insulin release is markedly reduced, whereas the second phase is delayed and often exaggerated. This is the threshold situation before the development of overt hyperglycemia and symptoms of diabetes, and it occurs when insulin secretion capacity is reduced by 80–90 %.[54,55]

It is not yet known what is responsible for the reduction in insulin secretion and the progression to diabetes: amyloid deposition, glucotoxicity, and/or lipotoxicity? Islet amyloid is derived from amylin (also called islet amyloid polypeptide), a hormone cosecreted with insulin from β-cells. Cats are among the few species in which the amino acid sequence of amylin predisposes it to fold into β-pleated sheets. These are deposited as amyloid in the islets, leading to loss of β-cells (fig. 5.15). It is assumed that amyloid deposition is accelerated in a state of insulin resistance, which leads to increased cosecretion of insulin and amylin. Amyloid deposition is found in about 90 % of cats with diabetes, but it is also a frequent finding in older healthy cats[56] and hence it probably should be regarded as a contributing factor and not the primary cause of β-cell failure.

Glucose toxicity is the concept that prolonged hyperglycemia impairs insulin secretion by the β-cells. The phenomenon can be nicely demonstrated in healthy cats in which insulin secretion ceases after three to five days of continuous induction of high blood glucose levels.[57] Initially, the suppression of insulin secretion is reversible, but eventually the β-cell damage be-



Figure 5.16:
Overweight cat (10 kg) with diabetes mellitus.



Figure 5.17:
Cat with plantigrade posture due to diabetic neuropathy.

comes permanent. Lipotoxicity is the analogous effect of excessive fatty acids on the β-cells, although the damage has not been shown as convincingly as with glucose. These are very important concepts because immediate treatment of diabetes may reverse the adverse effects of glucose toxicity and increases the probability of complete remission of the diabetes. Due to glucose toxicity, circulating insulin concentration at the time of diagnosis is usually low[58] and thus measuring insulin does not help to predict whether remission is possible.

Other specific types of diabetes (formerly called secondary diabetes) in the cat account for approximately 20 % of cases. The causes include pancreatitis, hypercortisolism, hypersomatotropism (acromegaly), and exposure to diabetogenic hormones (progestins, glucocorticoids). Pancreatic lesions are often identified by ultrasonography or by islet histopathology,[59] but they are often mild and thus probably not the initiating cause of diabetes. Some cats, however, have serious pancreatitis, which could be the factor that triggers diabetic ketoacidosis. It is generally difficult to decide which of the two – diabetes or pancreatitis – is the cause and which is the effect (see also chapter 5.2.3). Glucocorticoids and growth hormone have strong diabetogenic actions, and approximately 80 % of cats with hypercortisolism and presumably 100 % of those with hypersomatotropism are diabetic.

Signalment and clinical manifestations

Diabetes occurs most often in middle-aged to elderly cats, more than 95 % being older than five years. There is a strong sex predilection, approximately 70 % being male. Burmese cats are at risk, but no other breed has been reported to be. Approximately 60 % of diabetic cats are overweight (fig. 5.16), 35 % are of normal weight, and 5 % are underweight.

Most diabetic cats have classical symptoms of diabetes: polyuria, polydipsia, polyphagia, and weight loss. About 10 % have overt signs of diabetic neuropathy, such as hind limb weakness, decreased ability to jump, and plantigrade posture (fig. 5.17). There is rarely weakness of the front legs as well. Lethargy and a dry, unkempt haircoat are common. Physical examination often reveals hepatomegaly and neurological abnormalities consistent with peripheral neuropathy. Cats have long been assumed not to develop diabetic cataracts, but a recent study of 50 diabetic cats showed that almost all had lens opacities that were more pronounced than in nondiabetic cats. They were much less severe than in diabetic dogs, being detected only by ophthalmic examination, and none of the cats was blind.[60]

In cats with concurrent disease such as pancreatitis, hypercortisolism, or hypersomatotropism, other symptoms and signs may be more prominent. Those with diabetes complicated by ketoacidosis or hyperosmolar nonketotic syndrome are usually presented with lethargy, anorexia, reduced water intake, and vomiting (see below).

Diagnosis and workup

The diagnosis and workup are generally similar for dogs and cats but a few differences should be noted. First, the renal threshold is higher in cats than in dogs (cats ~15 mmol/l, dogs ~10 mmol/l) and thus glucosuria does not occur until blood glucose reaches a higher level. Second, cats are prone to stress-induced hyperglycemia that may be difficult to differentiate from diabetes; it can be mild but concentrations $>$ 15 mmol/l are not exceptional and thus glucosuria may also be present.[61,62] Stress hyperglycemia may be recognized when repeated blood glucose measurements also reveal normal values, but some cats have stress hyperglycemia during their entire stay in the hospital. This can be resolved by measuring fructosamine, which is above 400 µmol/l in diabetic cats and may be as high as 1500 µmol/l, but is not elevated in cats with stress hyperglycemia. Fructosamine concentration may also be normal when diabetes is of very recent

Table 5.3: Oral agents used in type 2 diabetes in humans

	Action (simplified)	Predomination site of action
Approved classes in humans		
Sulfonylureas	Increase insulin secretion	β-cells
Meglitinides	Increase insulin secretion, in particular first-phase	β-cells
Biguanides	Reduce hepatic gluconeogenesis, increase insulin sensitivity	Liver, muscle, adipose tissue
Thiazolidinediones	Improve insulin sensitivity	Muscle, adipose tissue
α-glucosidase inhibitors	Delay absorption of carbohydrates	Intestinal tract
Other agents (eclectic)		
Transition metals (vanadium, chromium)	Various, may improve insulin action	Largely unresolved
Incretin mimetics (e.g., GLP-1 analogs)	Stimulate glucose dependent insulin secretion, inhibit glucagon secretion	Islets
Amylin analogs	Delay gastric emptying, inhibit glucagon secretion, increase satiety	Brain, islets

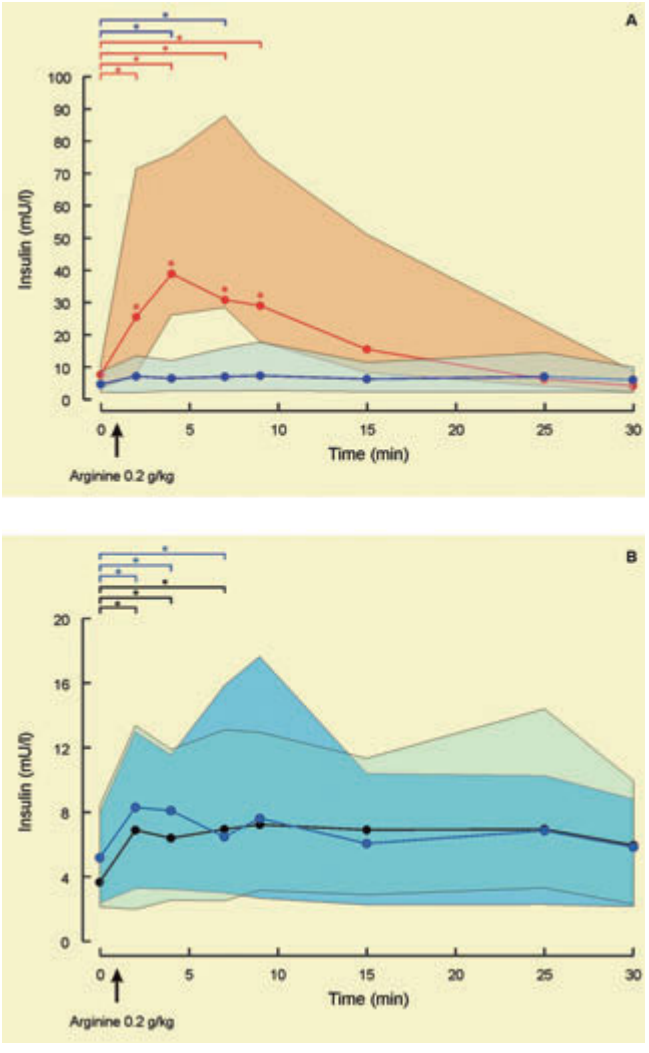


Figure 5.18:
Insulin concentration before and at 2, 4, 7, 9, 15, 25, and 30 minutes after administration of arginine 0.2 g/kg.
(A) Insulin concentration (range and median values) in healthy cats (red line) and in cats with newly diagnosed diabetes mellitus (blue line). At most intervals healthy cats had significantly higher insulin concentrations, but the baseline concentrations were not different.
(B) Insulin concentration in newly diagnosed diabetic cats: those in which remission occurred during the first four months of therapy (blue line) and those in which there was no remission (black line). The difference between the two groups was not significant.

onset and when there is concurrent hyperthyroidism or hypoproteinemia.[35,63] As in dogs, further workup should clarify the severity of diabetes and the presence of concurrent disease or other contributing factors. Routine hematology, plasma or serum biochemistry, urinalysis, and urine culture should be performed, as well as radiography and ultrasonography, if indicated.

Measurement of plasma insulin concentration (baseline or after injection of an insulin secretogogue) does not help to identify the type of diabetes or to predict whether there is sufficient residual β-cell function for eventual remission of the disease (fig. 5.18). The plasma insulin concentration is usually low at the time of diagnosis, regardless of whether remission is possible or not. Glucose toxicity contributes to the low release of insulin, due to the loss of β-cell function. Insulin therapy may reverse glucose toxicity, leading to partial or complete recovery of β-cell function.[58,64]

Treatment

The aim of therapy is identical in dogs and cats, namely, good control of the clinical features. This is usually achieved if blood glucose is maintained between 15 and 5 mmol/l throughout the day. Cats can be more difficult for the owner to treat and it is very important to provide sufficient information on all relevant aspects of the disease as well as ready access to veterinary support when needed. Treatment should follow a precise and easily understood protocol (see chapter 13.3.1), with written instructions for the owner.

Since 80 % of diabetic cats have type 2 diabetes, oral hypoglycemic drugs may in theory be used. Five classes of these drugs have been approved for treatment of type 2 diabetes in humans and others are under investigation (table 5.3). Except for sulfonylureas, they have either not been investigated in diabetic cats (meglitinide, thiazolidinediones) or have been found unsuitable for use as the sole agent (biguanide, α-glucosidase inhibitors). Sulfonylureas stimulate insulin secretion and thus some residual β-cell function is required for them to

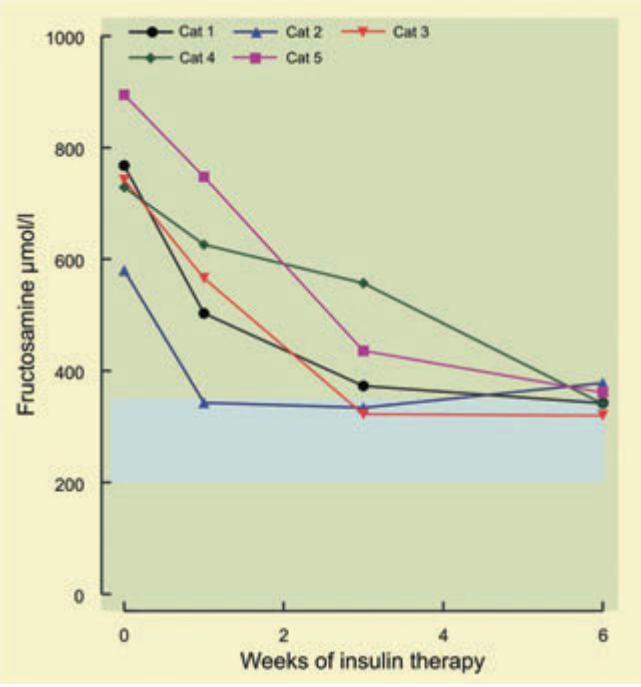


Figure 5.19:
Decrease in fructosamine concentration in five cats in which remission of diabetes was achieved during the first two months of insulin therapy. The reference range is shown in blue (200–350 µmol/l).

be effective. Glipizide is the member of this class that has been used most often in cats. It should only be used in diabetic cats that are in good physical condition, are not ketotic, and have only moderate symptoms and signs of diabetes. The initial dose is 2.5 mg twice daily, increased to 5 mg twice daily after two weeks if there are no adverse effects and hyperglycemia is still present. The disadvantages of glipizide are that treatment is successful in only 30 % of diabetic cats[65] and that the drug may have negative effects on islets and accelerate β-cell loss. Increased amyloid deposits have been observed in cats receiving glipizide in experimental trials, probably because glipizide stimulates the secretion of amylin as well as insulin.[54] An analogous limitation of sulfonylureas was found in recent studies on human β-cell culture, in which sulfonylureas increased β-cell apoptosis.[66] Since glipizide offers no medical advantage over insulin, it should only be used in cases in which the owner is unable to inject insulin.

Treatment of diabetes mellitus should be started as soon as possible after the diagnosis is confirmed. Good glycemic control reverses the effects of glucose toxicity and increases the probability of remission. The mainstays of treatment are administration of insulin and management of the diet.

Intermediate-acting insulins are preferred in cats with uncomplicated diabetes. A porcine derived, lente-type insulin (Caninsulin® / Vetsulin®, Intervet) is licensed for use in cats in many countries and is probably the insulin most widely used. There are additional preparations for veterinary use (Insuvet® Lente, Insuvet® PZI, both Schering Plough; PZIVet®, IDEXX) in some countries.[67,68]

The initial dose of lente insulin is 1 U/cat twice daily for cats weighing < 4 kg and 1.5–2.0 U twice daily for those weighing > 4 kg. If blood glucose is < 20 mmol/l at the time of diagnosis, the initial dose is no more than 1 U twice daily, independent of body weight.

The cat may be hospitalized for one to two days until the workup is completed. Blood glucose is measured three to four times over the day and the dose of insulin is reduced if glucose is found to be < 5 mmol/l. The twice-daily dose is increased in increments of 0.5–1.0 U at intervals of five days. Satisfactory regulation is usually achieved in one to three months. The initial workup and onset of treatment can also be managed on an outpatient basis.

In some cats the duration of insulin action is < 12 h. This problem is well recognized and applies not only to lente insulins, but to other types as well. For example, long-acting insulin analoga, although designed for once-daily administration, usually have to be given twice daily to cats. Another problem is inconsistent absorption of insulin, causing erratic blood glucose levels. In humans this problem has led to the recent development of insulin analogs, of which insulin glargine (Lantus®, Aventis) is currently the most frequently used long-acting insulin analog. Two basic amino acids are substituted on the C-terminal portion of the B chain to alter the isoelectric point. This makes the analog soluble for injection at a slightly acidic pH but after injection small crystals precipitate at the pH of the subcutaneous tissue. Another amino acid substitution at the end of the A chain improves the cohesion between insulin molecules. In humans insulin glargine is thought to be absorbed steadily without peaks and to have a duration of action > 24 h.[69] Glargine has recently gained popularity among owners of diabetic cats, who are often well informed via the internet and discussion forums. In cats the duration of action of glargine is longer than that of lente insulins and it has been reported that once-daily injection of glargine treatment is as successful as twice-daily injection of lente insulin.[70] In our experience the duration of action of glargine in cats is usually < 24 h and glycemic control is better with twice-daily than with once-daily injection. Glargine may be a suitable alternative for cats in which duration of action of lente insulin is too short for metabolic control.

It has been postulated that the remission rate is higher in cats treated with glargine than with other types of insulin but the number of published cases[71,72] is still too small to allow a definitive conclusion.

Opinions on diets for diabetic dogs and cats have changed in recent years. The cat is a true carnivore, which distinguishes it clearly from the omnivorous dog. The natural diet of wild fe-

lids, such as mice and birds, contains less than 10 % carbohydrate on a dry-matter basis. This is very different from many of the commonly used manufactured cat foods, in which the carbohydrate content is up to 50 %. Cats have a high protein requirement and the activity of hepatic enzymes responsible for phosphorylation of glucose for subsequent oxidation or storage is lower in cats than in omnivores. Cats are metabolically adapted to utilize primarily protein and fat, and diets high in carbohydrate appear to be unfavorable. In diabetic cats fed a diet low in carbohydrate and high in protein, clinical control was better and there was a higher rate of remission of the diabetes.[73] The previous remission rate of ~25 % may be increased to ~50 % when insulin therapy is combined with a high-protein, low-carbohydrate diet.

It is likely that the composition of manufactured cat food[74] and physical inactivity contribute to the high prevalence of obesity in cats. Obesity decreases insulin sensitivity and is a major risk factor for diabetes. Since obesity-induced insulin resistance is nearly completely reversible and even slight to moderate weight loss improves metabolic control, weight reduction should be strongly encouraged in overweight cats (approximately 1 % per week).

The timing of feeding relative to insulin administration does not seem to play an important role. The quality of metabolic control in cats receiving their meal at the same time as the insulin injection was not different from that in cats fed 45 min after the injection.[75] The feeding schedule should, however, be consistent from day to day: either two meals of equal size around the time of insulin administration or free access to food day and night.

Follow-up examinations are essential during long-term management. In cats close supervision is of particular importance during the first months, because remission of diabetes may occur and if unnoticed and insulin administration is not terminated, serious hypoglycemia may occur. Most cats go into remission during the first three months of therapy (fig. 5.19), but remission can occur after one year or more. Scheduling of follow-up examinations, interpretation of blood glucose values, and the aims of therapy are the same as in dogs (see chapters 5.2.3, 13.3.1). Blood glucose measurements in the hospital are even more difficult to interpret in cats than in dogs, due to cats' susceptibility to stress hyperglycemia. Cat owners are introduced to HM in the same way as owners of diabetic dogs. We recommend measuring fasting blood glucose twice weekly, both to keep in practice and to detect hypoglycemia, and a BGC for 12 h at least once a month. About 70 % of cat owners are able and willing to perform HM on a long-term basis. It should be stressed that variability is high even in BGCs performed at home and therefore a single curve may be misleading. In complicated cases, more than one curve can be obtained at home before a treatment decision is made (fig. 5.20).[76,77]

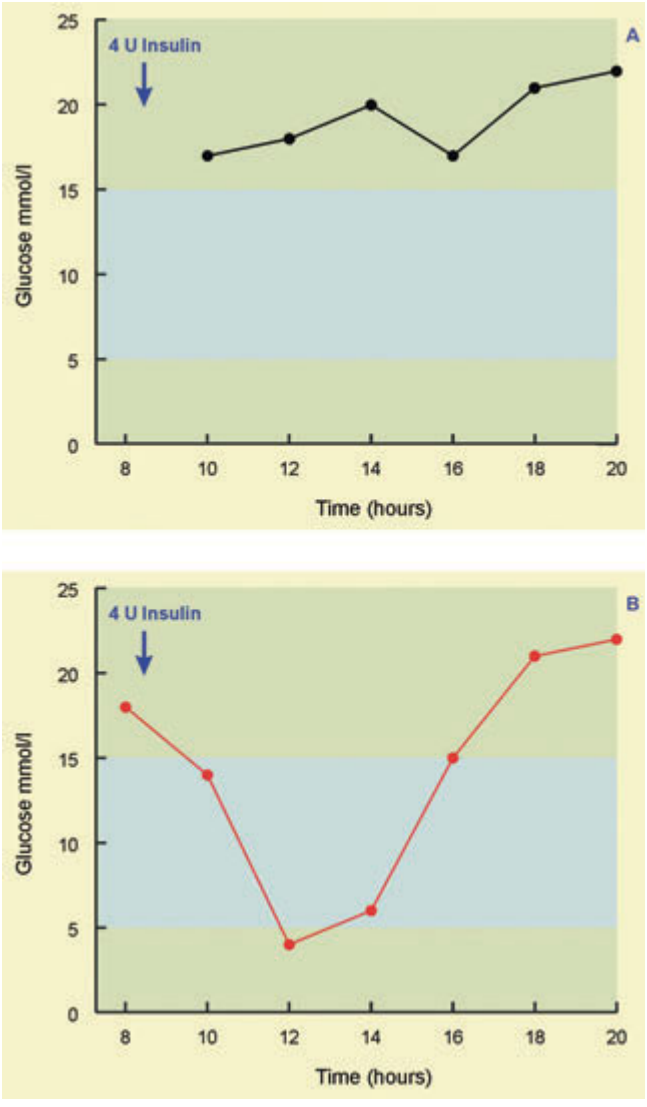


Figure 5.20:
Blood glucose concentrations in a diabetic cat in which the disease was waxing and waning. The cat weighed 6 kg and received 4 U lente insulin BID at the time of referral.
(A) Blood glucose curve obtained in the clinic on the day of admission. The values are highly elevated and the differential diagnosis was: technical problems, stress hyperglycemia, insulin underdosage, counterregulatory phase of the Somogyi effect, insulin resistance, lack of insulin absorption, and interference by insulin antibodies.
(B) Blood glucose curve obtained at home a few days later with the same dose of insulin, showing the Somogyi effect. It is likely that the curve obtained in the hospital reflected the late counterregulatory phase of the Somogyi effect. The Somogyi effect represents the physiological response to hypoglycemia induced by insulin overdosage. Counterregulatory hormones, glucagon and epinephrine being most important, raise blood glucose concentration so vigorously that marked hyperglycemia can occur for up to 72 h. Reduction of the insulin dose resolves the problem. The preferred range for blood glucose concentration in treated diabetic dogs and cats is shown in blue (15–5 mmol/l).

5.2.5 Problems associated with the regulation of diabetes in dogs and cats

Most animals can be adequately stabilized within the first three months of therapy, but periodic adjustments continue to be needed, as in the case of further loss of β-cells or a change in insulin sensitivity due to other disease. If symptoms persist in spite of insulin therapy, the following stepwise approach can be used.

First step. Confirm that the initial workup and treatment thus far have been according to the protocol in chapter 13.3.1. Then increase the dose of lente insulin every five to seven days until it reaches 1.0–1.5 U/kg twice daily.

Second step. Confirm that the insulin used by owner is not outdated, has not been diluted, frozen, or heated, and is mixed correctly before being drawn into the syringe. Confirm that the syringe is for U-40 insulin and not U-100. Observe the owner's method of mixing, drawing up, and injecting the insulin. Review the diet and exercise regimen.

This second step in problem-solving is often neglected, but the technical errors it covers are frequent causes of problems in regulation.

Third step. Perform a BGC to determine whether there may be a Somogyi effect or short duration of insulin effect. Blood glucose should be measured at home every 1–2 h for at least 12 h.

Fourth step. If no explanation for the problem has been identified, diseases causing insulin resistance should be considered. In principle, any concurrent disease – inflammatory, infectious, or neoplastic – may cause insulin resistance. The most relevant possibilities are pancreatitis, pancreatic neoplasia, hypercortisolism, hypersomatotropism (cat), diestrus (dog), infection of oral cavity or urinary tract, chronic renal failure, and obesity.

Poor absorption of insulin can be considered in cats receiving PZI insulin and can be evaluated by changing to lente insulin. Circulating insulin antibodies can also be considered and it may be worth trying insulin of a different species.

Hypoglycemia is always a potential problem during insulin therapy. It may be the result of decreasing insulin resistance, remission of the diabetes, twice daily administration of a long-acting insulin, inappetence, or vomiting.

5.2.6 Diabetic ketoacidosis (DKA) and hyperglycemic hyperosmolar state (HHS)

DKA and HHS are the two most serious complications of diabetes mellitus. Both are potentially life threatening and require immediate intense therapy.

DKA is defined as hyperglycemia, metabolic acidosis, and hyperketonemia (with ketonuria). It is frequently the initial presenting manifestation of diabetes, but it may also occur at any time during treatment. It results from a relative or absolute decrease in insulin together with an increase in glucagon and other stress hormones. In at least two-thirds of cases there is a concurrent disease, such as urinary tract infection or pancreatitis, which may increase stress hormone release and trigger DKA.[78,79]

Insulin deficiency and stress hormone excess cause the release of large amounts of free fatty acids from adipose tissue, which are then transported to the liver. Hepatic reesterification of the fatty acids is impaired, in favor of their entry into mitochondria and oxidation to ketone bodies (acetoacetate, β-hydroxybutyrate, and acetone). Acetoacetate and β-hydroxybutyrate are acids that cause metabolic acidosis. They are eliminated via the kidney, which exacerbates osmotic diuresis, dehydration, and electrolyte loss. Additionally, hepatic gluconeogenesis and glycogenolysis are enhanced and peripheral glucose utilization is reduced, which causes hyperglycemia, osmotic diuresis, and volume depletion.

The symptoms depend on the stage at the time of presentation. Classical symptoms of diabetes (polydipsia, polyuria, polyphagia, weight loss) have usually occurred previously but have been unnoticed or disregarded by the owner. As the metabolic situation deteriorates, lethargy, anorexia, vomiting, abdominal pain, dehydration, weakness, collapse, and mental dullness usually develop. Mental depression (sopor, stupor, or even coma) can be due to dehydration, shock, severe acidosis, and hyperglycemia / hyperosmolality. Dehydration results from osmotic diuresis together with insufficient water intake due to anorexia and / or vomiting. Kussmaul respiration (a slightly increased, deep breathing pattern) can be observed in severe cases due to respiratory compensation of metabolic acidosis. The patient's breath can have a fruity or acetone odor. Abdominal palpation may reveal hepatomegaly. Icterus is a frequent presenting sign in cats with DKA, due to severe hepatic lipidosis, pancreatitis, or pancreatic neoplasia causing extrahepatic cholestasis. Signs of a concurrent disease may also be present.

Typical laboratory findings are ketonuria and metabolic acidosis (reduced blood levels of bicarbonate and total carbon dioxide, TCO_2), together with hyperglycemia, glucosuria, and elevated plasma fructosamine. Increased plasma levels of liver enzymes are also common. Hypovolemia can cause an elevated hematocrit, prerenal azotemia, elevated total protein and albumin, and lactic acidosis. As a result of the osmotic

diuresis and acid-base disturbances, hyponatremia, hypokalemia, and hypomagnesemia may be present. Hypophosphatemia is also possible which, especially in cats, may cause an acute hemolytic crisis (plasma phosphate concentration often < 0.5 mmol/l).

β-hydroxybutyrate is the most abundant ketone body in DKA but it is not detected in urine by most test strips for ketones. Hence the test for ketonuria may be only moderately positive in an animal with DKA.

DKA is one of the most complex metabolic emergencies and its treatment is demanding. It requires 24 h surveillance with frequent reevaluation of clinical and laboratory parameters and appropriate adjustments of therapy (see also protocol in chapter 13.3.2). Rehydration should be started immediately with a balanced electrolyte solution at a rate that will normalize hydration in ~12 h. In most cases, correction of hypovolemia will also restore the acid-base balance quickly and additional treatment with bicarbonate to correct metabolic acidosis is often unnecessary and can even be detrimental. Potassium deficits may be severe, although the initially measured value may be normal, and correction must be started before treatment with regular insulin is begun. Since plasma phosphate levels may also be low or may decrease quickly with fluid therapy, phosphate may also be supplemented. Concomitant potassium supplementation should be reduced if potassium phosphate is used for phosphate supplementation. Initially, electrolytes should be reevaluated every 4–6 h. Regular insulin therapy should be started ~4 h after the beginning of fluid therapy and correction of electrolytes. Intermittent IM injection of insulin is used most often but constant IV infusion is also a good option (see chapter 13.3.2). Fluid therapy can be tapered off and lente insulin can be started when the animal is stable, eats and drinks, and does not vomit. The prognosis is guarded and ~25 % of patients with DKA die or are euthanized.

HHS is much less common than DKA. Patients with HHS have severe hyperglycemia (> 30 mmol/l), severe hyperosmolality (> 340 mOsm/kg), and profound dehydration, without acidosis or ketonuria. The pathogenesis of HHS is similar to that of DKA, but why some diabetic patients DKA and others develop HHS is not known. In most cases, serious concurrent diseases contribute to the development of HHS, renal failure being particularly common. The principles of treatment are identical to those of DKA. The prognosis is guarded to poor and most animals die or are euthanized.[80]

5.3 The hypoglycemic syndrome

The hypoglycemic syndrome is primarily characterized by a low circulating glucose concentration. Values below 2.8 mmol/l are often accompanied by symptoms, but values just below the lower limit of the reference range may not be. Hence the hypoglycemic syndrome is not defined by hypoglycemia alone, but – according to Whipple's triad – hypoglycemia accompanied by symptoms that are relieved by administration of glucose (or feeding).[81]

A low blood glucose value, especially if unexpected, may be an artifact (table 5.4). PBGM devices measure glucose quickly and conveniently, but are less accurate than measurements in an accredited veterinary laboratory. To exclude artifact as the cause of a low glucose values, an accurate measurement should be made in two or more separately-collected blood samples before undertaking an extensive diagnostic workup.

The symptoms of hypoglycemia are due to activation of the autonomic nervous system, i.e., neuronally-released transmitters as well as epinephrine and norepinephrine released by the adrenal medulla, and the lack of an energy substrate available to the central nervous system (neuroglycopenia) (table 5.5). The

Table 5.4: Causes of artifactual hypoglycemia

A. Collection and handling of the blood sample

Prolonged storage of blood (> 1 h) before separation of serum / plasma from the cell component, particularly if blood is not collected in a sodium fluoride-coated tube. (Sodium fluoride inhibits glucose metabolism by blood cells.)

Hemolysis of the blood sample can interfere with the measurement technique. Especially in sodiumfluoride-coated tubes hemolysis can be severe (centrifuge within 30 min after collection).

Plasma or serum samples older than 24–48 h.

B. Measurement

Portable blood glucose meters (developed for human diabetes mellitus patients) can give erroneously low glucose concentrations due to

- insufficient application of blood, despite »beep« given by device as an indication of the opposite,
- tendency of these devices to give lower than actual blood glucose values*,
- blood samples with a high hematocrit value.

Incorrect use of other laboratory devices to measure glucose.

Error of other laboratory devices used to measure blood glucose.

* Note that glucose concentration measured by a PBGM is lower in venous blood than in capillary blood, which is lower than in arterial blood. The differences can be several tenths of a mmol per liter.

Table 5.5: Symptoms and signs of hypoglycemia

Autonomic symptoms	Neuroglycopenic symptoms
Adrenergic symptoms	Lethargy
Muscle twitching	Behavioral changes
Muscle tremors	Confusion
Anxiety	Generalized muscular weakness
Polyuria / polydipsia	Posterior paresis
Cholinergic symptoms	Visual impairment (»blindness«)
Hunger	Ataxia
Polyphagia	Seizures
	Loss of consciousness
	Death

severity of the symptoms and signs depends on the glucose nadir: convulsions and loss of consciousness often occur when plasma glucose concentration is < 2.8 mmol/l. The rate of decrease and the duration of the hypoglycemia also determine the severity of the symptoms and signs. The blood glucose threshold for symptoms of hypoglycemia also depends on individual variation and the underlying disease.

Glucose is the primary energy substrate for the brain. In contrast to other tissues, the brain cannot utilize free fatty acids as an energy source. In addition to glucose it can use ketone bodies, which are metabolites of free fatty acids, but they can only provide up to half of the energy requirement. Moreover, in adult dogs fasting leads to an appreciable ketosis after only days to weeks.[82] Thus preservation of the function of the central nervous system in postprandial or fasting states is mainly dependent upon increased production of glucose.

Initially the glucose is derived almost exclusively from hepatic glycogen (fig. 5.7), but glycogenolysis can only sustain the plasma glucose concentration for a short period and after about two days of fasting, liver glycogen stores are completely depleted.[83] Secondly, glucose production in the liver and kidneys is activated. The precursors for hepatic glucose synthesis are glycerol released from adipose tissue and lactate / pyruvate and amino acids derived from muscle. In the adult dog the catabolic state of fasting is primarily the result of a decrease in insulin release; secretion of the counterregulatory hormones glucagon and growth hormone does not change significantly.[82]

When these corrective mechanisms do not compensate increased peripheral glucose utilization (demand-side hypoglycemia) or decreased availability of glucose (supply-side hypoglycemia), the syndrome of hypoglycemia may occur. There are several, often critical, illnesses in which the hypoglycemia is not severe (> 3.0 mmol/l) and symptoms do not occur. In diseases such as sepsis, severe parenchymal liver disease, or hypoadrenocorticism, hypoglycemia is often an incidental finding and the clinical manifestations of the disease are not related to it. In addition, symptoms and signs that may be ascribed to hypoglycemia, such as lethargy, muscle weakness, and confusion, may also be related to other aspects of the disease. Long-term starvation (especially in young individuals), portosystemic shunting, hypoadrenocorticism (chapter 4.2), and polycythemia are examples of disorders that rarely present with symptoms related to hypoglycemia alone.[84] An example in which the symptoms are solely due to hypoglycemia is increased utilization of glucose due to an overdose of exogenous insulin or oral hypoglycemic drugs such as sulfonylurea derivatives (chapter 5.2). The following discussion is confined to disorders that are mainly characterized by symptoms and signs of the hypoglycemic syndrome: insulinoma, nonpancreatic tumors associated with hypoglycemia, and juvenile hypoglycemia.

5.3.1 Insulinoma

Insulin-secreting pancreatic endocrine tumors (PETs), more commonly known as insulinomas (fig. 5.21), continue to produce insulin despite the hypoglycemia they provoke. Immunohistochemical staining of these insulin-secreting PETs often reveals that they are also positive for somatostatin, glucagon, gastrin, pancreatic polypeptide, and / or growth hormone.[85–87] In addition, IAPP immunoreactivity and IAPP-derived amyloid deposits have been found in 25 % of primary PETs.[88]

The first case of insulinoma in a dog was reported in 1935 by Slye and Wells.[89] Since then, insulin-secreting PETs have been diagnosed in many dog breeds, especially medium-to-large breeds, and rarely in small breeds, such as the West Highland white terrier.[90,91] There is no pronounced breed or sex predisposition. At the time of diagnosis the dogs' ages vary between 4 and 13 years, with an average of about 8½ years.[92] Insulinomas are rare in cats; reports are confined to single cases.[93–95]

Canine insulinomas are often solitary (~ 90 %) and usually < 2.5 cm in diameter. Ten to 14 % of insulinomas are multiple or grow diffusely.[96,97] In the dog they are often malignant (> 95 %) and there are macroscopically visible metastases, primarily in regional lymph nodes and the liver, in 40–50 % of cases at the time of surgery.

Clinical manifestations

Symptoms related to insulinoma are almost always the result of hypoglycemia (see table 5.5) and only rarely due to mass effects. Initially, the changes in locomotion and behavior due to hypoglycemia are often subtle and are commonly disregarded by the owner. The symptoms occur intermittently and can frequently be related to fasting, excitement, or exercise. There may be weight gain if the owner has responded to the animal's increased appetite. This is an important clue, for insulinoma is one of the few diseases that can cause an increase in body weight. In most dogs the diagnosis is made within five months of the onset of symptoms. Apart from occasional obesity, no abnormalities are found by physical examination. Peripheral neuropathy is a rare occurrence with insulinoma. The associated proprioception deficits and depressed spinal reflexes are the result of degenerative changes in the radial and ischiadic nerves.[98,99] Apart from the hypoglycemia, results of routine laboratory investigation are usually unremarkable.

Differential diagnosis

In middle-aged and elderly dogs, other causes of the hypoglycemic syndrome are limited to nonpancreatic tumor, portosystemic shunting, hypoadrenocorticism, and polycythemia. However, in the latter disorders there are rarely symptoms of hypoglycemia.

Diagnosis

When a presumptive diagnosis has been made on the basis of the signalment and a detailed medical history but the plasma glucose concentration is not low, it should be measured on two or more occasions before feeding in the morning, after fasting overnight. If hypoglycemia is not found but there have been convincing symptoms of it, a supervised fast can be undertaken. Fasting for 24 h is in most cases sufficient to reveal hypoglycemia, but if not, fasting is prolonged for up to 72 h, with repeated measurements of blood glucose. The chronic nature of this disease often results in few or no symptoms at plasma glucose concentrations even < 2.8 mmol/l. For glucose to pass the blood-brain barrier, it requires a carrier system consisting of membrane-associated glycoproteins, i.e., glucose transporters (GLUT) (see chapter 5.1.4). It has been postulated that patients with insulinoma have increased uptake of glucose by the brain via changes in the setup of these glucose transporters.[100,101] Hence requiring Whipple's triad to be fulfilled by the presence of symptoms in order to confirm the diagnosis of the hypoglycemic syndrome could be hazardous. However, the medical history and a low plasma glucose concentration are often sufficient, so that it is not necessary to provoke signs of hypoglycemia. Plasma fructosamine or glycosylated hemoglobin concentrations can be measured as complementary investigations; low plasma concentrations of these may be indicative of prolonged hypoglycemia in dogs with insulinoma.[102,103]

The hallmark of the diagnosis is the association of persistent hypoglycemia and inappropriately high plasma insulin concentrations. Circulating insulin concentrations are typically within the reference range or higher despite hypoglycemia. The simultaneous occurrence of blood glucose < 3.5 mmol/l and plasma insulin > 10 mU/l (70 pmol/l) is diagnostic.[104] Insulin:glucose ratios, such as the amended insulin-to-glucose ratio (AIGR), have been advocated to improve the diagnostic value of glucose and insulin measurements. However, there are two major reasons that limit the presumed additional value of these ratios:

- Reference ranges vary between laboratories and assay methods. More modern monoclonal antibody-based assays, such as the immunoradiometric assay (IRMA), measure lower plasma insulin immunoreactivity than the outdated polyclonal antibody-based assay or radioimmunoassays (RIA) in dogs with insulinoma.[105]
- There are very erratic oscillations in plasma insulin concentration in human patents with insulinoma.[106] The action of insulin on the liver and peripheral tissues, and thus indirectly on plasma glucose concentration, may persist for 40 min or longer.[107] Hence measuring glucose and insulin in the same sample will not necessarily reveal a direct causative relationship.



Figure 5.21:
Insulinoma of a ten-year-old male Malinese shepherd during surgery.

Measurements of circulating C-peptide and proinsulin concentrations (chapter 5.1.2), which are used in the diagnosis of insulinoma in humans, have not been developed for dogs, but could support the presumptive diagnosis and differentiate exogenous hyperinsulinism.[108,109] Provocation tests, such as the intravenous glucose tolerance test and glucagon tolerance test, have been used in dogs with insulinoma.[104,110] However, as in humans, the value of these tests has been too limited to justify their routine use and, in addition, they may provoke severe hypoglycemia.[96,97]

The survival time and quality of life of dogs with insulinoma treated surgically may be longer and better than that of dogs treated medically.[111,112] Accurate detection, localization, and staging of the primary tumor and metastases are essential for the selection of appropriate candidates for surgery. A few reports have described the use of transabdominal ultrasonography, with varying results in detecting the primary pancreatic tumor (36 % and 75 %).[113–115] However, transabdominal ultrasonography may be useful in detecting lesions in the liver or peripancreatic tissues (regional lymph nodes) suggestive of metastatic disease or neoplasia of nonpancreatic origin. In a recent comparative study of three diagnostic imaging techniques [abdominal ultrasonography, computed tomography (CT), and somatostatin receptor scintigraphy (SRS)], CT was best in detecting and localizing the primary tumor but often failed to identify metastatic lesions correctly (fig. 5.22).[115]

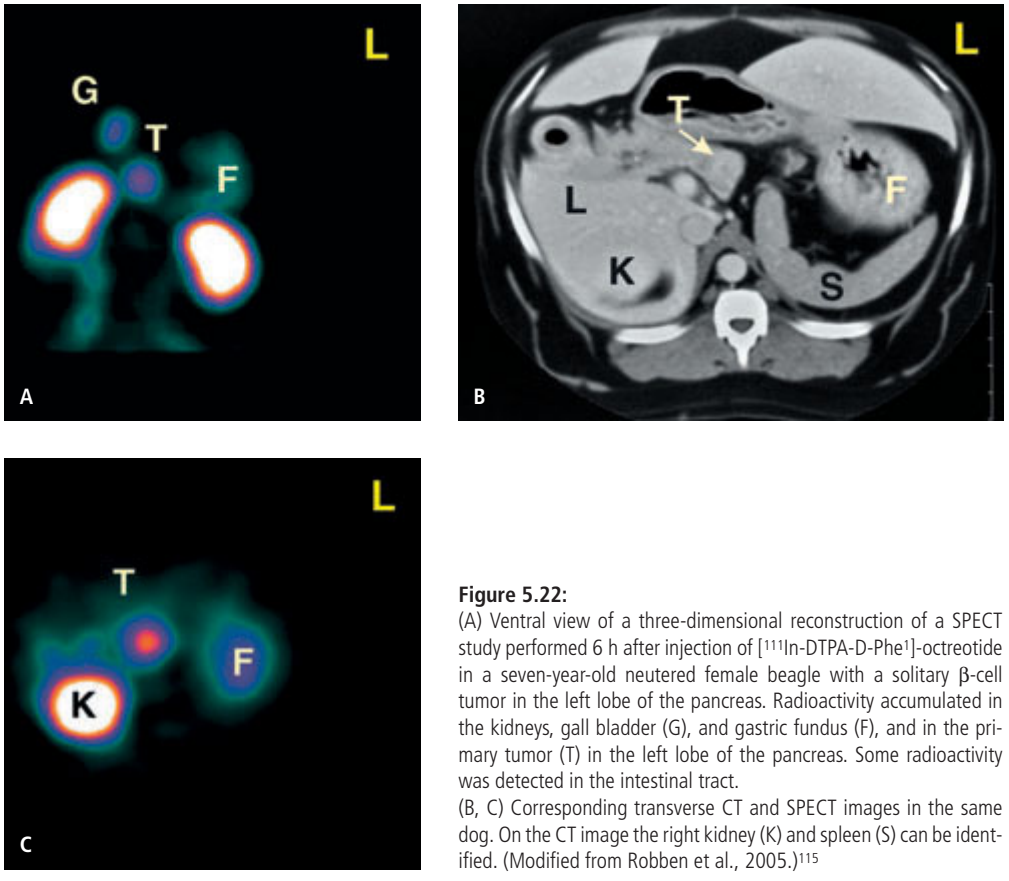


Figure 5.22:
(A) Ventral view of a three-dimensional reconstruction of a SPECT study performed 6 h after injection of [^{111}In-DTPA-D-Phe^{1}]-octreotide in a seven-year-old neutered female beagle with a solitary β-cell tumor in the left lobe of the pancreas. Radioactivity accumulated in the kidneys, gall bladder (G), and gastric fundus (F), and in the primary tumor (T) in the left lobe of the pancreas. Some radioactivity was detected in the intestinal tract.
(B, C) Corresponding transverse CT and SPECT images in the same dog. On the CT image the right kidney (K) and spleen (S) can be identified. (Modified from Robben et al., 2005.)[115]

Transabdominal ultrasonography and CT provide information on anatomical relations and the localization of lesions. SRS provides more information on the nature of the lesion. In vitro and in vivo studies have demonstrated that canine insulinoma tissues express somatostatin receptors.[114,116] At the mRNA level, expression of four somatostatin receptor subtypes (SSTR1, 2, 3, and 5) was demonstrated in canine insulinoma tissues.[117] SRS uses the ^{111}In-labelled somatostatin analogue octreotide that binds with high affinity to somatostatin receptors, especially SSTR2 and to a lesser extent to SSTR5. The thus concentrated radionuclide can be visualized with regular scintigraphy and even better with single photon emission computed tomography (SPECT) (fig. 5.22).[115–117] These scan results also could have predictive value for the effectiveness of treatment with octreotide or octreotide-based radiotherapy.[118] Currently, the described diagnostic imaging techniques have a modest accuracy in detecting canine insulinomas. A better understanding of the use of CT and SRS in insulinoma could improve their accuracy, as could the combination of different imaging techniques.[115,119] Also, other currently available techniques – endoscopic and intraoperative ultrasonography – could prove useful in insulinoma detection.[120] To date, the intraoperative localization and staging of canine insulinoma by inspection and palpation of the organs of interest or by use of intravenous methylene blue infusion remain the standard for localization of primary tumors, and to a lesser extent, metastases.[121]

Treatment

Treatment of hypoglycemia due to insulinoma consists of lifestyle changes, medical therapy, and/or surgery. Whenever possible, surgery is the treatment of choice, because it is the only option that can result in complete remission of the hypoglycemic syndrome. Owners should be informed that dogs with insulinoma often have micrometastases and that the hypoglycemic syndrome frequently recurs after surgery because of growth of these functional metastases.

The goal of therapy should be alleviation of symptoms and not normalization of plasma glucose concentration per se. Most dogs with insulinoma appear to be comfortable even with subnormal plasma glucose concentrations. Physical exercise should be limited and excitement avoided to reduce the risk of a hypoglycemic crisis. A third important initial step is to divide the dog's food over five to eight meals per day, thereby shortening the intervals between meals. Changes in the diet are not advised, for changes in diet composition have not been proved to be beneficial and they carry the risk of

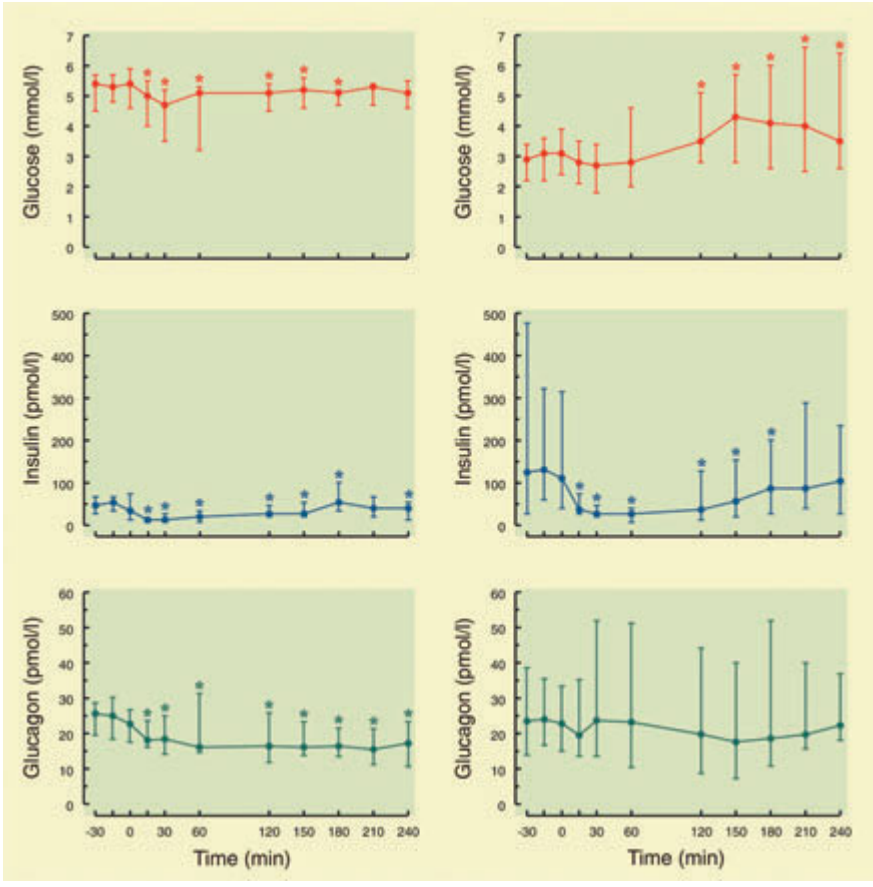


Figure 5.23:
Plasma concentrations (median and range) of glucose, insulin, and glucagon after a single subcutaneous injection of 50 µg octreotide at T = 0 min. Left panels: healthy dogs, fasted overnight. Right panels: dogs with insulinoma, without food for 4–6 h. Note the difference in response to octreotide (thus far unexplained): In the healthy dogs a decrease in plasma insulin and glucagon concentrations coincides with a minor decrease in plasma glucose concentration. In contrast, in the dogs with insulinoma a decrease in insulin concentration without a significant effect on plasma glucagon concentration coincides with a significant increase in plasma glucose concentration. Also note the wide range of basal plasma insulin concentrations in the dogs with insulinoma, in comparison with the healthy dogs.
* Significantly different from baseline values. (Adapted from Robben et al., 2006.)[105]

gastrointestinal disturbances that could increase the risk of a hypoglycemic crisis. If symptoms of hypoglycemia persist in spite of these measures, the total amount of food fed over the day could be increased, even though this could lead to weight gain. These simple measures may stabilize the dog for months and should not be underestimated.

If these measures do not suffice, or no longer do so, and surgery is not an option, medical treatment can be undertaken to control hypoglycemia. Glucocorticoids interfere with the action of insulin and promote gluconeogenesis. The initial daily dose of prednisolone is 0.5–1.0 mg/kg divided in two to three doses; this can be increased gradually if needed. Often high doses are needed, which frequently give rise to the side effects of iatrogenic hypercorticism. As an alternative, treatment can be started with diazoxide (Proglicem®, Schering-Plough, 100 mg diazoxide/capsule). This is a benzothiadiazide diuretic that inhibits insulin secretion. It also stimulates hepatic gluconeogenesis and glycogenolysis, and inhibits peripheral use of glucose. The initial dose is 10 mg/kg body weight divided in two daily doses. Doses as high as 60 mg/kg/day may be necessary to prevent symptoms of hypoglycemia Adverse reactions may be prevented or postponed by slowly increasing the dose to effect and by administering it with food. If side effects develop (ptyalism, anorexia, vomiting, and diarrhea), they can be stopped by reducing or temporarily stopping the drug. However, in a dog with insulinoma, necessitating a continuous intake of food, even these side effects can be hazardous. Hence lower doses of diazoxide can be combined with glucocorticoid therapy, which will also reduce the costs of therapy. Bone marrow depression and diabetes mellitus are rare side effects of diazoxide.

If these measures fail to prevent hypoglycemic symptoms, alternative medical therapies can be considered. Chemotherapy with alloxan and streptozotocin has been tried to treat insulinoma, but the clinical results have been variable and protocols to reduce the risk of nephrotoxicity have not been well established.[122] The somatostatin analogue octreotide (Sandostatin®, Sandoz, 50, 100 or 200 µg octreotide/ml) inhibits the secretion of insulin by unaffected and neoplastic β-cells. The effect of a single dose of octreotide on plasma insulin and glucose concentrations has been reported in dogs with insulinoma (fig. 5.23).[105] In contrast to humans, all dogs responded to a single subcutaneous dose of 50 µg octreotide. In humans, the absence of high affinity somatostatin receptors can cause worsening of hypoglycemia due to inhibition of the release of the counterregulatory hormones glucagon and growth hor-

mone.[123] The suppressive effect of octreotide on plasma insulin concentration in dogs lasts only 3–4 h, which could explain treatment failures in dogs with insulinoma. The effect of a slow-release formulation of octreotide has not yet been studied in dogs.[124,125]

If despite treatment such serious hypoglycemic effects as ataxia, convulsions, or even coma, develop, an emergency protocol should be started (see protocol in chapter 13.3.3). This includes glucose administration, but should be followed as soon as possible by additional measures to maintain an adequate plasma glucose concentration. If octreotide is being considered, one should be aware that the delayed rise in plasma glucose concentration in some dogs can be preceded by an initial decrease (fig. 5.23).[105] Hence it is preferable to combine octreotide with other emergency measures. Furthermore, it is important to note that somatostatin has been shown to prevent the diazoxide-induced hyperglycemia in healthy dogs.[126]

Surgery is undertaken not only to remove the tumor tissue, if possible, by partial pancreatectomy, but also for thorough inspection of the abdomen for metastases, which may be of great prognostic importance. Depending on the findings during surgery, lymph node excision and partial hepatectomy may also be necessary. A major concern is the perioperative control of plasma glucose concentration. Medical treatment is started preoperatively and if liquid diets are used in the final 12–24 h before surgery, fasting can be reduced to the final 6 h. The use of α_2-agonists during surgery may be beneficial to control plasma glucose concentration. They inhibit insulin release by postsynaptic α_2-adrenoreceptor stimulation of pancreatic β-cells, although they may also activate hepatic glycogenolysis and stimulate growth hormone release.[127] Plasma glucose concentration is checked and corrected by infusion of glucose, if necessary. Postoperatively, plasma glucose concentration is monitored closely and if there is euglycemia or hyperglycemia, any glucose infusion is gradually stopped. Small amounts of food and water are offered as soon as the dog is able to accept them. Postoperative hypoglycemia is usually the result of incomplete removal of the tumor and/or metastases. Successful surgery is often followed by hyperglycemia for days or weeks, until the normal β-cells recover from suppression. Only rarely is insulin therapy needed to bridge over this temporary deficiency. Pancreatitis can complicate postoperative stabilization. Depending on its severity and the associated vomiting and abdominal pain, and whether there is persisting hypoglycemia, intravenous glucose and medications (see above) will be necessary.

Prognosis

In about 40 % of cases there are macroscopically visible metastases at the time of surgery. With regard to control of hypoglycemia, the success of surgery depends not on the amount of tumor removed but rather on the amount remaining. During or shortly after surgery, the animal may die from pancreatitis or from neurological effects of uncontrolled hypoglycemia. These complications and/or nonresectable tumor mass may be reason for euthanasia.

In most dogs, hypoglycemia due to insulin-secreting PETs recurs after surgery, which suggests that most have metastasized before they are diagnosed and surgery is attempted. For those that benefit from surgery, the mean survival time without symptoms or the need for medication is 1–1.5 years, which can be extended (in some cases to three years or more) by resuming the dietary measures and medication with diazoxide or prednisolone or both.[112]

5.3.2 Nonpancreatic tumors associated with hypoglycemia

Pathogenesis

Hypoglycemia may also result from a variety of tumors of both epithelial and mesenchymal origin, most often the latter. The most frequent nonpancreatic tumors are leiomyoma, leiomyosarcoma, hepatoma, hepatocellular carcinoma, and tumors with extensive hepatic metastases.[128–130] In the past, several mechanisms have been suggested to explain the hypoglycemia: deranged tumor metabolism with excessive utilization of glucose, parenchymal liver destruction with failure of gluconeogenesis and glycogenolysis, ectopic insulin production, and inhibition of glucagon release. Now there is convincing evidence that incompletely processed insulin-like growth factors (pro-IGF-II and IGF-I) cause the hypoglycemia in humans,[131,132] a mechanism that has also has been documented in a few cases of hypoglycemia in dogs (see also chapter 10.1).[130,133]

Clinical manifestations and diagnosis

The symptoms can be the result of the underlying tumor disease or the hypoglycemia (chapters 5.3, 5.3.1). The combination of a low plasma glucose concentration and a nonpancreatic tumor makes a paraneoplastic syndrome likely. The presumptive diagnosis can be strengthened by exclusion of other differential diagnoses. Finding a low plasma glucose concentration together with a low plasma insulin concentration can help to exclude insulinoma (chapter 5.3.1). Finding increased plasma levels of insulin-like growth factors is one of the few options to support the cause-effect relation between the tumor disease and the occurrence of hypoglycemia. Resolution of hypoglycemia after successful treatment of the tumor disease also supports the diagnosis of this paraneoplastic syndrome.

Treatment and prognosis

The hypoglycemia can usually be treated by adjusting the feeding regime (see chapter 5.3.1); drugs such as glucocorticoids are rarely necessary. The prognosis is mainly determined by the underlying tumor.

5.3.3 Juvenile hypoglycemia

In puppies of miniature breeds such as the Yorkshire terrier and the Chihuahua, insufficient food supply of any cause (starvation, gastrointestinal disturbances, and inactivity due to cold) may cause hypoglycemia. Similar forms of juvenile hypoglycemia have not been well documented in kittens. Hypoglycemia due to a glycogen storage disease with an autosomal recessive inheritance pattern has been found in a family of Norwegian forest cats. It leads to death as a result of perinatal hypoglycemic collapse or late-juvenile-onset neuromuscular degeneration.[134]

Pathogenesis

Puppies have relatively high rates of glucose utilization, disproportionately large brains, and relatively limited stores of gluconeogenic substrate. During fasting the hepatic glycogen stores are rapidly depleted and the possibly still immature gluconeogenesis cannot supply the large amounts of glucose needed. Puppies of small breeds develop hypoglycemia within 24 h of fasting. This leads to hypoinsulinemia and hyperglucagonemia, i.e., a ketogenic endocrine setting.[135] Underlying diseases such as portosystemic shunting that cause anorexia or impaired liver function may contribute to the precipitation of the condition.

Clinical manifestations and diagnosis

The symptoms of juvenile hypoglycemia are not dissimilar to those of other forms of hypoglycemia, although some of the symptoms may in part be due to the ketosis. The animals are usually presented with lethargy or in coma. There may also be muscular weakness, muscle twitching, and generalized convulsions. At admission most are in a good nutritional state and physical examination reveals no remarkable abnormalities.[136] In some there are symptoms of an underlying disease such as congenital portosystemic shunt or severe intestinal parasitism. The blood glucose concentration may be extremely low, even < 2.0 mmol/l.

Treatment and prognosis

Intravenous administration of a 20 % or 50 % glucose solution (0.8 and 0.2 ml/100 g body weight, respectively) is indicated if there are neurological signs, even mild muscle spasms.[136] If the pup can take the glucose solution orally, this is administered at regular intervals until the appetite returns. Then small amounts of food are given at intervals of 2 h. If tube feeding is needed, oral rehydration is guided by blood electrolyte measurements.

The prognosis is good if hypoglycemia is corrected before brain damage occurs. The risk of developing the syndrome of hypoglycemia decreases with increasing age and body weight.[135]

5.4 Other endocrine tumors associated with the pancreas

Pancreatic endocrine tumors can secrete any of the hormones produced by the α-, β-, δ-, and PP cells under physiological conditions (chapter 5.1). In addition to the insulin-secreting PETs, there have been reports in dogs of PETs that secrete glucagon, and rarely somatostatin or pancreatic polypeptide.[137–139] Furthermore, PETs can produce hormones that are not released under physiological conditions by α-, β-, δ-, or PP-cells, gastrin being the one reported most frequently in companion animals.[140]

5.4.1 Gastrinoma

In 1955 Zollinger and Ellison were the first to describe a syndrome in humans associated with hypersecretion of gastrin by pancreatic tumors. Gastrin comprises three biologically active peptides, ranging in size from 14 to 34 amino acids. Gastrin is secreted by G-cells in the gastric and duodenal mucosa and does not occur in appreciable amounts in the normal pancreas. Nevertheless, more than 70 % of dogs with a gastroenteric gastrin-secreting tumor, called gastrinoma, have a tumor mass in the pancreas.[141] Gastrinomas are usually malignant and metastases can be found in more than 70 % of cases during surgery.[140,141] The syndrome occurs in middle-aged and elderly dogs with a mean age of about nine years. There appears to be no pronounced breed or sex predisposition.[140] Gastrinomas are rare in cats, reports being confined to single cases.[142]

Clinical manifestations

The clinical features can be traced back to the main biologic actions of gastrin, i.e., directly stimulating hydrochloric acid secretion by gastric parietal cells and indirectly by releasing histamine from fundic enterochromaffin-like cells, and trophic effects on the gastric mucosa. The hypersecretion of hydrochloric acid and the hypertrophic gastritis resulting from gastrin hypersecretion lead to anorexia, vomiting, and weight loss. Intermittent diarrhea is caused by maldigestion secondary to enzyme inactivation.[140] The development of erosive esophagitis and gastroduodenal ulcers can lead to hematemesis and melena. In addition, there may be polydipsia.

The animals are usually lethargic and in poor nutritional condition. Some have symptoms of abdominal pain. Perforating ulcers lead to symptoms and signs of acute abdomen and septic shock. Laboratory examination may reveal regenerative anemia and hypoproteinemia as a result of blood loss. Profuse vomiting can lead to hypokalemia, hypochloremia, and metabolic alkalosis. Leukocytosis probably reflects gastrointestinal erosion and inflammation.

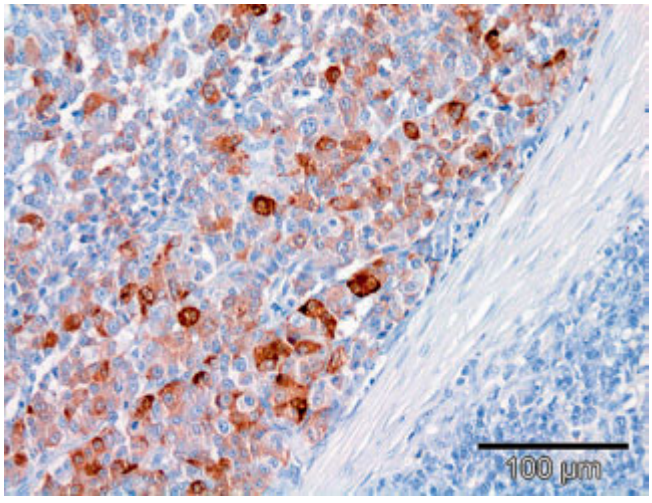


Fig. 5.24:
Glucagonoma in the pancreas of a dog. Immunohistochemical staining for glucagon. Note cytoplasmic staining for glucagon in tumor cells (left). The tumor is surrounded by a capsule. Normal exocrine pancreas at the lower right. (Courtesy of Dr. J.J. van der Lugt.)

Diagnosis

Suspicion may arise when endoscopy reveals esophagitis, hypertrophic gastritis, and gastric and/or duodenal ulceration. A pH < 1.5 of endoscopically collected unstimulated gastric juice can be indicative of acid hypersecretion.[140] A presumptive diagnosis of gastrinoma is based on the clinical findings and elevated circulating gastrin concentration in the absence of other causes of hypergastrinemia. These include chronic renal failure, administration of H_2-receptor antagonists, gastric outlet obstruction, chronic gastritis, liver disease, and Basenji enteropathy. In the reported cases of gastrinoma, circulating gastrin concentrations were 1.5–100× the upper limit of the reference range. In cases in which gastrin is < 10× the upper limit of the reference range, a secretin provocative test can be used, but reference values have not yet been established. The diagnosis can be confirmed by finding a gastrinoma, although it has been suggested recently that immunocytochemistry is necessary to confirm the relation between hypergastrinemia and an endocrine tumor in the pancreas.[139,141]

As for insulinomas, accurate detection, localization, and staging of the primary tumor and metastases are essential to select appropriate candidates for surgery. Ultrasonography, CT, and magnetic resonance tomography have not been evaluated for this purpose but the small size of these tumors would appear to limit their usefulness. Nevertheless, abdominal ultrasonography may be used to detect possible metastases. Furthermore, gastric wall thickening and large ulcers may also be examined. SRS has been used in veterinary medicine to detect gastrinomas,[140] but it seems that intraoperative inspection and palpation of the organs of interest remains the standard for localization and staging of gastrinomas.

Treatment and prognosis

The ideal treatment of gastrinoma is surgical resection, but this is rarely curative because of unresectable metastases. Dogs undergoing surgery should receive medical therapy perioperatively. Even without complete surgical resection of tumor tissue, medical therapy can be beneficial.

Symptomatic measures concentrate on restoration of the fluid and electrolyte balance, treatment of gastrointestinal ulceration with sucralfate (Ulcogant®, Merck, 250 ml suspension [0.2 g/ml], 0.5–1 g every 8 h), and inhibition of gastric acid secretion. Therapeutic control of gastric acid secretion can be achieved by use of specific antagonists of the regulators involved. The histamine H_2-receptor antagonists cimetidine (Zitac®, Intervet, 100 and 200 mg cimetidine/tablet, 5–10 mg/kg every 6 h) and ranitidine (Zantac, GlaxoSmithKline, 150 and 300 mg ranitidine/tablet, 2 mg/kg every 8 h) may have little or no beneficial effect. Famotidine (Pepsid, Pfizer, 10 mg famotidine/tablet, 0.5–1.0 mg/kg every 12 h) is a more potent H_2-receptor antagonist, but a parietal cell Na/K-ATPase or proton pump inhibitor such as omeprazole (Losec®, Astra Zeneca, 10, 20, and 40 mg omeprazole/tablet, 0.7 mg/kg orally once daily) may be more effective.[140,141] The long-acting somatostatin analogue octreotide binds to somatostatin receptors on the tumor cells and thereby interferes with gastrin release. Furthermore, it directly decreases gastric acid secretion stimulated by gastrin and other secretagogues. Combination therapy appears to have the additional benefit that antisecretory drugs inhibit gastric acid secretion via a different mechanism.[140] Receptor-mediated radiotherapy of tumors with radiolabeled somatostatin derivatives such as octreotide holds some promise for treatment of metastatic gastrinoma.[141]

The high grade of malignancy of gastrinomas makes the long-term prognosis poor.

5.4.2 Glucagonoma

Glucagon-secreting PETs or glucagonomas have rarely been described in dogs.[138] The syndrome produced by glucagonomas is characterized by lethargy, anorexia, weight loss, skin rash (necrolytic migratory erythema), stomatitis, mild anemia, hyperglycemia (mild diabetes mellitus), hypoaminoacidemia, and hyperglucagonemia. In humans, the reduction in plasma amino acid levels is held responsible for the skin lesions: intravenous amino acid infusion can resolve the erythema. It is noteworthy that superficial necrolytic dermatitis is more often observed with diabetes mellitus and severe liver failure such as hepatic cirrhosis. This has led to the suggestion that the common denominator for the skin lesions is the liver failure leading to a deficiency of essential nutrients for the skin.[143,144]

A presumptive diagnosis can be confirmed by finding an elevated plasma glucagon concentration in the absence of hypoglycemia. Presurgical diagnostic imaging and exploratory laparotomy can help to localize the primary pancreatic tumor and any metastases. As with other PETs, immunohistochemistry supports a definitive diagnosis (fig. 5.24).

Surgical resection is the first treatment option. Medical therapy with somatostatin analogues might be an option. Corticosteroids should be avoided, as development of diabetes mellitus worsens the situation.[143] The long-term prognosis is poor, because most glucagonomas are malignant and dogs with this tumor are often seriously debilitated by the time of diagnosis.

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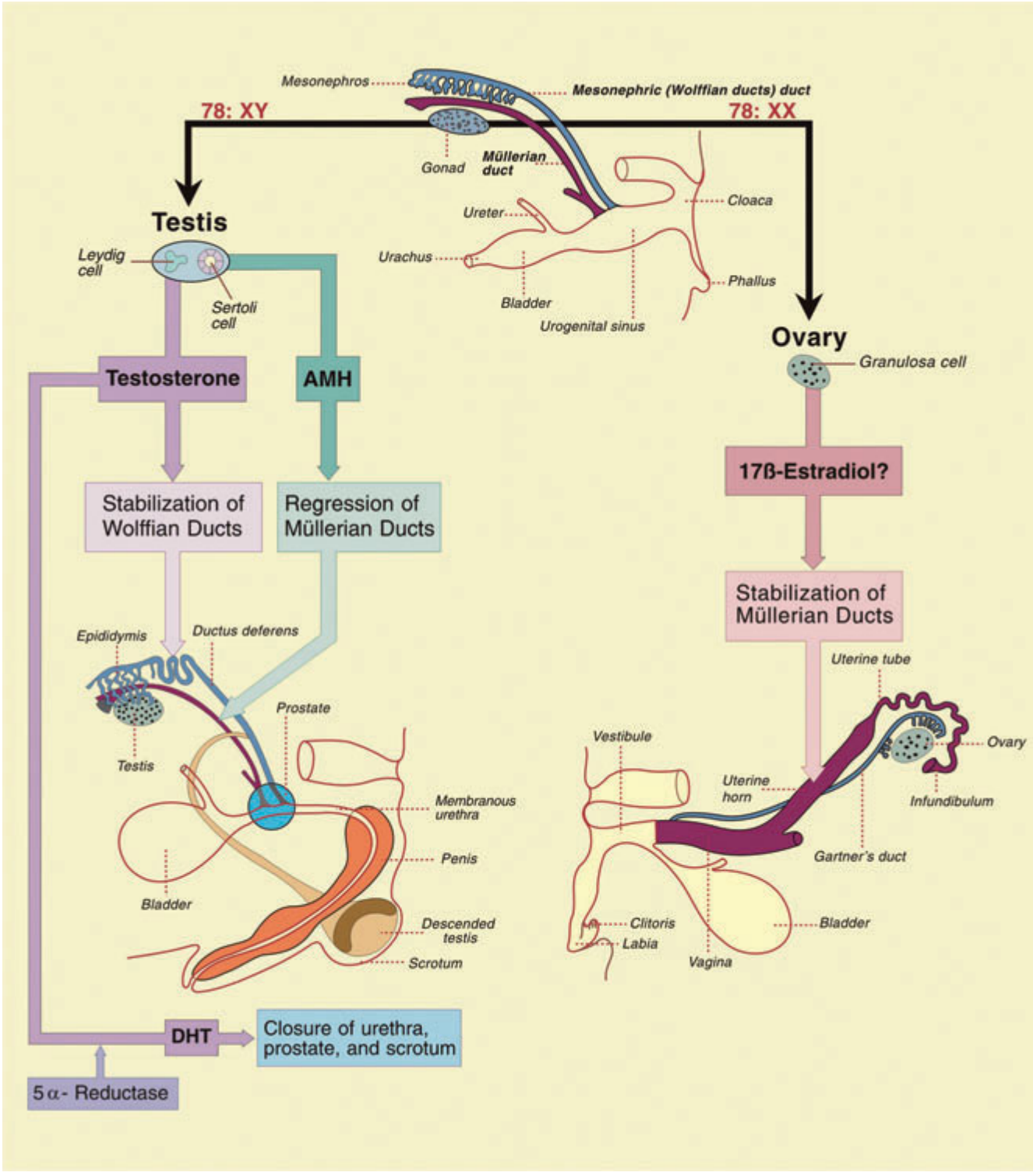


Figure 6.1:
Schematic representation of male and female differentiation and development from the undifferentiated state under stimulation and inhibition of sex steroids and regulatory peptides. The presence of a Y chromosome leads to male differentiation of the gonad with subsequent secretion of testosterone and Antimüllerian Hormone (AMH). Testosterone stabilizes the former Wolffian (or mesonephric) duct. Dihydrotestosterone (DHT) is required for complete development of the external male genitalia and closure of the urethra. Secretion of AMH by the fetal Sertoli cells is necessary to inhibit the growth and development of the Müllerian ducts into female internal genitalia.

6 Gonadal Development and Disorders of Sexual Differentiation

Heidi J. Kuiper

6.1 Introduction

Sexual differentiation is dependent on the expression of sex determining genes and the production of hormones in the fetal gonads. Normal mammalian sexual development is a complex process that relies on successful completion of successive steps that determine chromosomal sex and the development of gonadal sex and phenotypic sex (fig. 6.1).

6.1.1 Establishment of the chromosomal sex

The complete chromosomal complement of the dog consists of 39 chromosome pairs (78 chromosomes). Thirty-eight pairs are autosomes and one is the sex chromosomes (gonosomes). The cat has 38 chromosomes (19 pairs), comprising 18 pairs of autosomes and one pair of gonosomes. The chromosomal sex of the conceptus is determined at the time of fertilization. Because the ovum produced by the female always has an X chromosome and the sperm has either an X or a Y, it is the gonosome of the sperm that determines the chromosomal sex of the zygote after conception. The zygote will have the gonosomes of either a female (XX) or a male (XY).

6.1.2 Establishment of the gonadal sex

Prior to sex differentiation, male and female gonads cannot be distinguished and are therefore termed bipotential or indifferent. They have bipotential genital ridges, Wolffian and Müllerian ducts, a urogenital sinus, a genital tubercle, and genital swellings (fig. 6.1).

The bipotential gonads arise from the urogenital ridge, a region adjacent to the mesonephros that ultimately determines the cell lineages of the adrenal cortex, gonad, and kidney. The testes and ovaries have functional counterparts with corresponding functions in reproduction. These counterparts include the Leydig and theca cells, which comprise the steroidogenic compartment; the Sertoli and granulosa cells, which support germ cell maturation; the germ cells (spermatocytes and oocytes); and the peritubular myoid and stroma cells, which form the connective tissue of the gonads.[1,2]

6.1.2.1 Genes essential for gonadal development

In the male, more than in the female, sex differentiation requires not only the participation of a large number of genes at different loci but also the correct timing and adequate levels of expression of these genes. Essential in the development of the male gonadal sex in mammals is the expression of a dominant genetic factor localized on the Y chromosome: the SRY gene (sex-determining region of the Y chromosome). The product of the SRY gene is a transcription factor (often called testes determining factor) that is thought to play a key role in initiating the cascade of gene regulations resulting in testicular induction.[3–5] In the presence of a Y chromosome, containing an SRY gene, undifferentiated gonads always develop into testes, while in the absence of the SRY gene or its gene product the gonads develop into ovaries (fig. 6.2). Consequently, normal development of the reproductive organs results in XY individuals having testicles and XX individuals having ovaries. Although the importance of the SRY gene for sex determination is unquestionable, the exact mechanism by which it induces male gonadal sex differentiation is still unknown. The structural homology of SRY to transcriptional regulators of the high-mobility-group family led to the hypothesis that SRY activates downstream genes, which mediate the conversion of the bipotential gonad into a testis.[1] SRY might very well initiate testis development by upregulating SOX9 (sex-determining region Y-box 9) expression because SRY expression begins at CS (Carnegie Stages) 16 in the dogs testis, followed by upregulation of SOX9 expression at CS 17.[6] The SOX9 structure is typical for a transcription factor and upregulation of SOX9 is essential for testis development. Furthermore, in birds and reptiles, which do not have SRY, induction of testes is only associated with SOX9 expression.

Recent studies have identified several other transcription factors that are expressed in the gonadal ridge and are required for gonadal development and sexual differentiation – such as Wilms' tumor related 1 (WT1), steroidogenic factor-1 (SF-1), and GATA4 – presumably because they activate the expression of essential target genes. WT1 is involved in urogenital development and has been suggested to regulate the expression of target genes essential for both male and female gonadogenesis. SF-1 is a member of the nuclear hormone receptor family and the onset of SF-1 expression signifies the onset of the sex determination period.[7] WT1 may increase the levels of SF-1. Another gene that interacts with SF-1 in sex determination and steroidogenesis is DAX-1 (dosage-sensitive sex reversal-adrenal hypoplasia congenita critical region

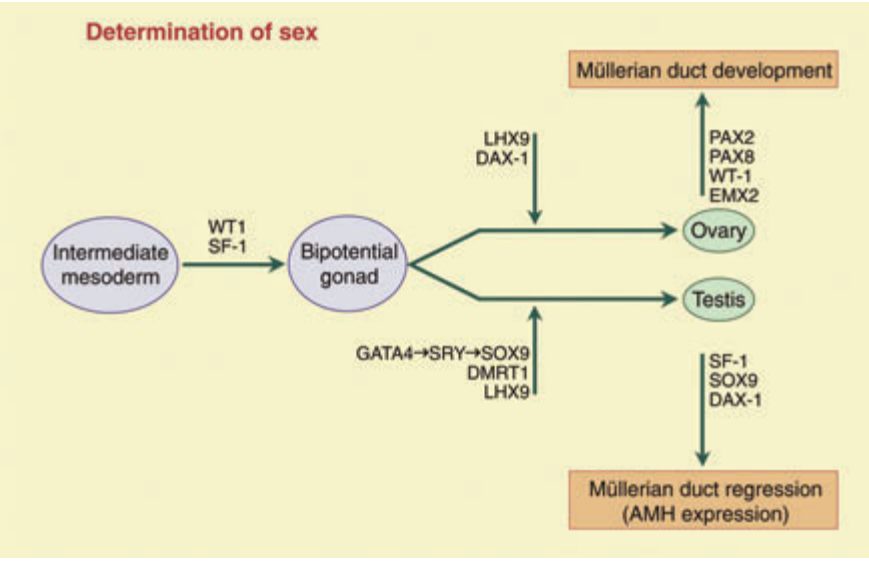


Figure 6.2:
Molecular events in mammalian sex determination. Genes believed to have key functions are depicted: WT1 = Wilms' tumor gene; SF-1 = steroidogenic factor 1 gene; LHX9 = LIM homeobox protein 9 gene; DAX-1 = dosage-sensitive sex reversal-adrenal hypoplasia congenita critical region on the X chromosome; GATA4 = GATA binding protein 4 gene; SRY = sex determining region of the Y chromosome; SOX9 = sex determining region Y-box 9 gene; DMRT1 = doublesex and mab-3 related transcription factor 1 gene; PAX2 = paired box gene 2; PAX8 = paired box 8 gene; EMX2 = empty spiracles homeobox 2 gene.

on the X chromosome, gene 1), a transcriptional regulator that inhibits target genes.[8] GATA4 encodes a transcription factor that promotes SRY expression in the XY gonad.[7] The LHX9 (LIM-homeobox 9) gene, encodes a homeodomain transcription factor that has been described as essential for gonad formation in mice and gives rise to the development of both Sertoli and granulosa cells.[9] DMRT1, the doublesex and mab-3 related transcription factor 1, is conserved among vertebrates, is involved in testis differentiation in mammals, birds, reptiles, amphibians, and fish, and is associated with Sertoli cell maturation.

6.1.3 Development of the Wolffian and Müllerian ducts

The internal genitalia derive from the genitourinary tract, which is initially identical in male and female embryos (fig. 6.1). At the indifferent stage, male and female embryos have two identical sets of paired ducts: the Müllerian (paramesonephric) ducts and the Wolffian (mesonephric) ducts.[1] Whether there is development of Wolffian ducts for the male or the Müllerian ducts for the female depends on autosomal genes that permit or prevent the production of AMH (anti-Müllerian hormone or Müllerian inhibiting substance (MIS)) in the Sertoli cells of the fetal gonad. AMH is the first secretory product of the fetal testis and thus marks the end of the testis induction period and the beginning of testis function.[6] Müllerian duct regression in male dogs begins by day 36 of gestation and is completed at day 46.[10]

When the Leydig cells in the male gonad begin to produce testosterone, it determines the further development of the male ducts. The proximal part of the Wolffian duct coils and forms the epididymis and the distal part forms the vas deferens. The seminal vesicles develop from lateral outgrowths of the caudal end of the vas deferens.

In the absence of AMH and testosterone, female organogenesis proceeds by regression of the mesonephric Wolffian ducts and stabilization of the Müllerian ducts (fig. 6.1). Development of the Müllerian ducts takes place in the craniocaudal direction, to give rise to the female internal genitalia. The cranial portion of the Müllerian duct gives rise to the oviducts. The middle portion gives rise to the uterine horns, which fuse caudally to form the body of the uterus. The caudal portion gives rise to the uterovaginal plate with the participation of both Müllerian and Wolffian duct components, to form the cervix and cranial vagina. The urogenital tubercle of the female undergoes limited growth and remains exposed as a cleft into which the vagina and urethra open.[11,12] The Wolffian ducts recede in female mammals but remnants may be present in the form of an appendix vesiculosa, epoophoron, paroophoron, or duct of Gartner.[13]

6.1.3.1 Genes essential for development of Wolffian and Müllerian ducts

Several genes are involved in the initial development of the Wolffian and Müllerian ducts. Thus, contrary to the older view that fetal ovarian development is passive, female germ cell differentiation and normal fetal ovarian morphogenesis require the expression and collaboration of various genes. PAX2 (paired box gene 2), a transcriptional regulator of the paired-box family, is expressed in the epithelium of the mesonephric tubules as well as in the Wolffian and Müllerian ducts. PAX8 is coexpressed with PAX2 and has additional

functions in urogenital development. WT1 is required for the formation of the caudal mesonephric tubules, but not the cranial tubules that later form the efferent ducts.[13] The LHX1 (LIM-homeobox 1) gene is expressed in epithelium of the mesonephric tubules of the Wolffian and Müllerian ducts and is presumed to play a role in the formation or very early differentiation of both Wolffian und Müllerian ducts. EMX2 (empty spiracles homeobox 2) is also required for the formation of both pairs of genital ducts.[13]

The gene encoding the AMH glycoprotein is transcriptionally regulated by several genes, including SF-1 and SOX9. These work synergistically and stimulate AMH transcription in the gonad, while DAX-1 is inhibitory (fig. 6.2).[14]

Androgen-mediated differentiation of the duct system and external genitalia also depends on the functional integrity of the androgen receptor (AR) gene located on the X chromosome. The AR protein is a ligand-dependent transcription factor that regulates the transcription of specific genes by binding androgen-AR complexes to regulatory DNA sequences close to target genes. Epithelial cells of the skin, the urethral plate in the glans, and the stroma and epithelium of the tubular urethra of the penile shaft are known to be strongly AR positive in man and are presumed to be so in other mammals, also.[11]

6.1.4 Establishment of the phenotypic sex

Like the internal genitalia, the external genitalia derive from structures initially found in both sexes, including the genital tubercle, urethral folds, the urethral groove, and the genital swellings. While the urethral groove in females is open, part of the urogenital sinus forms the vestibule. The labioscrotal folds form the vulva. In contrast to male sex development, formation of a female phenotype does not require the presence of gonads and the hormones they produce.[15] If the gonads are removed from an embryo that is still sexually indifferent, a female phenotype nevertheless develops.[16]

In males androgens are critical for virilization.[1] Testosterone, secreted by the Leydig cells through the activation of the 3β-HSD gene, is required for differentiation of the male duct system. Dihydrotestosterone, a potent androgen produced from testosterone by one of the 5α-reductase enzymes within the target cells of the urogenital sinus, is required for differentiation of the external genitalia. This includes closure of the urogenital sinus, elaboration of the urethral and prostate glands, elongation of the genital tubercle and fusion of the urethral folds over the urethral groove to form the penis and penile urethra, and relocation of the genital swellings posterior to the genital tubercle prior to their fusion to form the scrotum (fig. 6.1).[11,17]

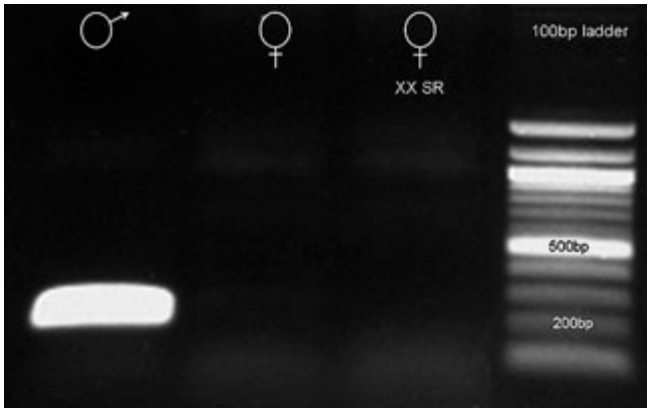


Figure 6.3:
Detection of a 201 bp product specific for the canine SRY gene using PCR and the primers Dog_SRY_F 5'-AAG CGA CCC ATG AAC GCA TT-3' and Dog_SRY_R 5'-TTC GGG TAT TTC TCT CTG TG-3' (EMBL Accession No. L77494). The product is present in the reference male (left) but absent in both the female (middle) and the XX sex-reversed patient (right). A 100 bp ladder is shown as reference for the size of the PCR products.

In dogs and cats, the fetal testes migrate from the posterior poles of the kidneys and pass through the abdominal wall to reach the scrotum. The descent is completed a few weeks after birth in these species (see also chapter 8).

6.2 Abnormal sexual differentiation

Disorders in male or female development may result in an intersex individual or hermaphrodite. The latter term is derived from Greek mythology, referring to Hermaphroditus, the issue of Hermes and Aphrodite, who was neither female nor male but at the same time both. Intersexuality or hermaphroditism may be manifested in a variety of phenotypes, ranging from mild forms of genital malformation to ambiguous external genitalia with complete sterility, depending on the specific stage at which sex differentiation was disrupted. Individuals with both ovarian and testicular tissue are called true hermaphrodites and must be differentiated from pseudohermaphrodites, in which chromosomal and gonadal sex agree and yet external appearance is that of the opposite sex.

Intersexuality can originate from disorders of chromosomal sex, disorders of gonadal development, or disorders of phenotypic sex. Hence, correct classification of intersexuality requires identification of the subject's chromosomal sex, gonadal sex, and phenotype.

Identification of chromosomal sex requires cytogenetic examination, but the polymerase chain reaction (PCR) can also be used to investigate specific regions of the Y chromosome for the SRY gene (fig. 6.3) or the ZFY (zinc finger pro-



Figure 6.4:
Four male cats with the tortoiseshell coat color indicating a chromosomal anomaly. The two at the top of the picture had the XXY syndrome and the two at the bottom were XX/XY chimeras. The latter two were presumed to be fertile because spermatogenesis was observed in some seminiferous tubules.

tein, Y-linked) gene. Gonadal sex should be determined by histological examination of the gonads by a person experienced in this field; ultrasonographic examination is not conclusive. Phenotypic sex can be determined by physical examination, diagnostic imaging of the abdomen, and hormone measurements.

6.2.1 Disorders of chromosomal sex

6.2.1.1 Chimerism and mosaicism of sex chromosomes

Errors in the constitution of the sex chromosomes can influence gonadal differentiation. The majority of animals with sex chromosome abnormalities have few symptoms, the most common being primary anestrus in phenotypic females and infertility in phenotypic males.[18] In some cases ambiguous genitalia provide an impetus for further investigation.[19]

In both chimerism and mosaicism of the sex chromosomes the animal has two or more genetically different cell lines. Chimeras arise from fusion of two or more zygotes after conception, while mosaics originate from a single zygote and the chromosome abnormality usually results from a mitotic nondisjunction. Neither disorder is considered to be inherited.

XX / XY chimeras have been described in several dog breeds. A uterus, cervix, and ovaries with follicles were found in a dachshund with a small prepuce and a scrotum lacking testes. The penis could not be extruded from the prepuce.[20] An XX / XY karyotype was also demonstrated in a Belgian shepherd dog with male behavior, abdominal testes, and a uterus.[21] Ovotestes and a uterus were present in a schipperke with an enlarged clitoris.[22] An abnormal phenotype has been found in all of the reported cases of an XX / XY karyotype in dogs. Cases of 78,XX / XY chimerism with both ovarian and testicular tissue are termed true hermaphrodites.

Chimerism of sex chromosomes is also known in cats, occurring most often in fertile tortoiseshell (calico) colored tom cats (fig. 6.4). Most of these have both a 38,XX and a 38,XY cell line.[23–25] Among 38 tortoiseshell colored tom cats, 7 had the XX / XY karyotype.[26] Some were fertile males or pre-

sumed to be fertile. A 38,XX/38,XY chimera with ovotestes was reported by Leaman et al.[27] The presence of an ovotestis is often seen in chimeras in other species, but this is the only reported case in cats.

Chimerism in cats and dogs is usually whole body, i.e., in all tissues. Blood chimeras, well known in freemartin cows, have not been reported in cats or dogs and are unlikely because of the difference in placental structure.

The only reported case of mosaicism involving the sex chromosomes in a dog was an infertile female toy poodle with a 77,X0/78,XX karyotype and no signs of intersexuality.[28] As in the X0 syndrome (see below), there was dysgenesis of both gonads. In addition to small ovaries, there was a relatively small uterus without a functional body.

Karyotyping is necessary for the diagnosis of chimerism or mosaicism in order to define the sex chromosome error. This can be performed on peripheral blood lymphocytes or cultured fibroblasts.

Gonadectomy has been recommended when there is intra-abdominal testicular tissue, since this is associated with an increased risk of Sertoli cell neoplasia (see also chapter 8). Hysterectomy has been recommended in true hermaphrodites because of the risk of endometritis. These risks must be weighed against the morbidity and mortality associated with abdominal surgery.

6.2.1.2 XO syndrome (gonadal dysgenesis)

In gonadal dysgenesis or the X0 syndrome the second sex chromosome is missing. Most often the paternal X chromosome is lost during spermatogenesis or after fertilization. Germ cells are usually absent and the gonads consist mainly of fibrous tissue (streak gonads). In the absence of germ cells there is no gonadal steroid stimulation of the female genitalia and the individual is infertile. In humans the syndrome is termed Turner's syndrome, but in mammals the term X0 syndrome should be used.

There have been only a few cases reported in dogs. One affected bitch had a paradoxical pattern of persistent proestrus.[29] One was presented because of primary anestrus and a small body size, and appeared to have small ovaries.[28] Another had facial deformities.[30] A six-month-old Doberman with this syndrome had ambiguous genitalia.[31] The syndrome has also been reported in two three-day-old kittens, of which one was found dead[32] and the other was euthanized because of spina bifida.[33] The X0 syndrome was also diagnosed in a 2.5-year-old Burmese cat which was smaller than its littermates; it had primary anestrus and its ovaries did not respond to gonadotropin stimulation and contained inactive germinal epithelium.[34]

The most prominent manifestation of gonadal dysgenesis is primary anestrus. The onset of puberty occurs at six to 23 months of age in the normal bitch and at four to 21 months of age in the queen, and in both species the diagnosis of primary anestrus requires the absence of pubertal estrus by 24 month of age.[35] For the diagnosis of gonadal dysgenesis other possible causes of primary anestrus must be excluded. These include mosaicism, chimerism, XX- or XY-sex reversal syndrome, male or female pseudohermaphroditism, oophoritis, and hypothyroidism. The plasma concentrations of LH and FSH will be elevated in the absence of ovarian tissue. The final diagnosis should rest upon the cytogenetic demonstration of X-monosomy. In addition, histological examination of gonadal tissue obtained by laparoscopy or at laparotomy can confirm the diagnosis of gonadal dysgenesis.

In an unusual case in an Eskimo dog, there was persistent proestrus which necessitated ovariohysterectomy,[29] but in most cases no therapy is required for there are no physical changes that interfere with the health of the animal.

6.2.1.3 XXY syndrome

Another chromosomal anomaly resulting in abnormal sexual development is the XXY syndrome (Klinefelter's syndrome in humans), which is known to occur in almost all domestic animals. The presence of the Y chromosome may lead to male gonadal differentiation with subsequent AMH and testosterone production, so that the individual is phenotypically male. The presence of an extra X chromosome (or several X chromosomes) causes atrophy and hyalinization of the seminiferous tubules together with Leydig cell abnormalities and decreased steroid secretion by the Leydig cells.[36]

Most humans with Klinefelter's syndrome are described as having atrophic testes, gynecomastia, and a hypoplastic penis. There have been only three reported cases in dogs. One of these had a normal male phenotype, small testes, and no spermatogenesis.[37] The second was initially thought to be a male pseudohermaphrodite, for there was a bicornuate uterus and testes, one of which had descended.[38] The third dog was a one-year-old poodle with bilateral cryptorchidism and a 78,XY/79,XXY mosaicism demonstrated in lymphocytes and in gonadal tissue cultures.[39]

Although the true incidence of the XXY syndrome in cats is unknown, it is the most commonly reported sex chromosome abnormality in this species. It is known to occur in several breeds of cats and in almost all reported cases it is associated with a tortoiseshell or calico coat color (fig. 6.4).[40,41] In cats the genes for orange and nonorange are X-linked alleles at the Orange locus. The random inactivation of one X chromosome in all somatic cells during embryogenesis in all XX females is then visible as either an orange or a non-orange coat color. Females heterozygous at the Orange locus develop random patches of tortoiseshell or calico color because only one

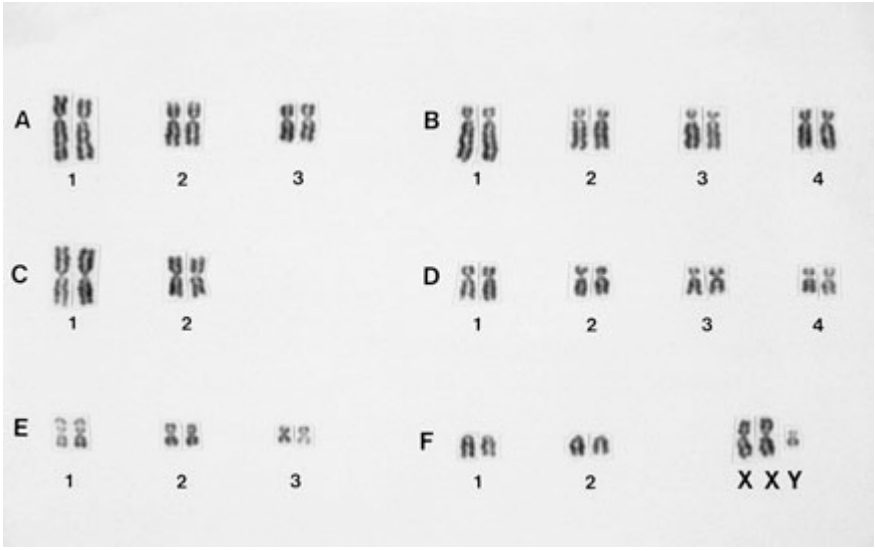


Figure 6.5:
Karyotype of a cat with two X chromosomes and a Y chromosome (similar to Klinefelter's syndrome in man). Chromosomes are arranged according to a standardized system developed for the domestic cat. (Courtesy of Dr. A.A. Bosma, Department of Functional Morphology, Faculty of Veterinary Medicine, Utrecht University.)

allele is expressed. The same situation occurs in tom cats with the XXY syndrome. Tortoiseshell tom cats often have a 39,XXY karyotype (fig. 6.5), but other observed karyotypes include 38,XX/39,XXY, 38,XX/57,XXY, 38,XY/57,XXY, 38,XY/39,XXY/40,XXYY, and 38,XX/38,XY/39,XXY/40,XXYY.[26,42–45] Not all cats with the XXY syndrome have the tortoiseshell coat color.[46] They can also have a single coat color, but it is the tortoiseshell or calico coat that is usually the reason for cytogenetic investigation. The tortoiseshell or calico coat color occurs not only in male cats with the XXY syndrome, but also in XX/XY or XY/XY chimerism.

All XXY cats are infertile. The testes descend but are small and lack spermatogenesis. The cats have a normal male phenotype but are somewhat small. Most have normal male behavior.

The diagnosis should be based on cytogenetic examination. In the reported cases of XXY in cats no therapy was necessary. In XXY dogs with a uterus, gonadectomy and hysterectomy may be required.

6.2.1.4 XXX syndrome (X trisomy, triple X syndrome)

This is a rare chromosomal anomaly that is probably the result of meiotic nondisjunction. Three cases have been reported in dogs: (1) an infertile four-year-old Airedale terrier bitch with a small uterus, female phenotype, and ovaries without follicles, (2) an infertile five-year-old mixbred bitch having ovaries of normal size, shape, and histological structure with corpora lutea and primary follicles, and (3) an infertile two-year-old Labrador retriever bitch with anestrus.[47–49] The XXX syndrome has not been reported in cats, but one case of 37,X0/39,XXX mosaicism has been reported in a pregnant cat with one normal ovary containing follicles and one dysgenetic ovary lacking corpora lutea or developing follicles.[50]

The reported dogs with XXX syndrome were examined because of infertility. There are several acquired conditions leading to infertility in dogs, such as cystic endometrial hyperplasia and hypothyroidism. In addition, mosaicism, chimerism, XX or XY sex reversal syndrome, and male pseudohermaphroditism can also result in an almost normal female phenotype with infertility. The final diagnosis should rest on cytogenetic demonstration of X trisomy. Patients with XXX syndrome require no special treatment, for the physical changes do not interfere with general health.

6.2.2 Disorders of gonadal sex

Disorders of gonadal differentiation can result in a phenotype that is the opposite of the chromosomal sex. Individuals whose intersex stems from disorders of gonadal differentiation are termed »sex reversed«. In affected dogs the sex of the gonads does not, or only partially, agree with the chromosomal sex. Animals with the XY sex reversal syndrome develop ovarian tissue despite the fact that they carry a Y chromosome. Animals with the XX sex reversal syndrome have testicular tissue despite the absence of a Y chromosome. Presumably due to mutated genes in the cascade of gene

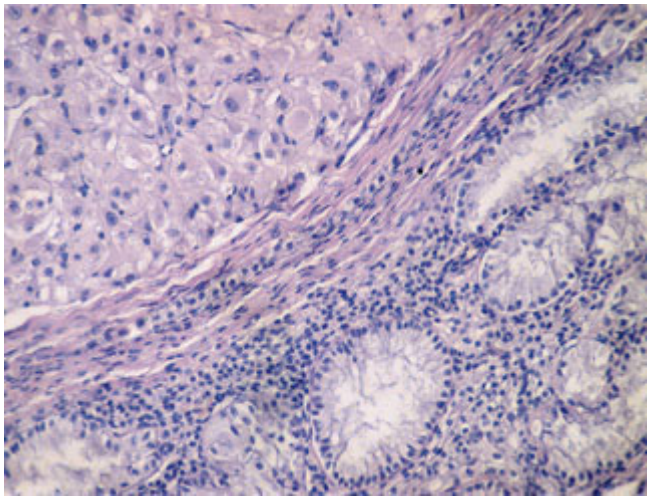


Figure 6.6:
Histological section of an ovotestis from a true hermaphrodite dog. There are seminiferous tubules (lower right) as well as ovarian tissue with a corpus luteum (upper left).



Figure 6.7:
Genital tract removed from an XX male dog. Although the gonads are in the normal position of ovaries and connected to a juvenile uterus, histological examination revealed their exclusively masculine composition, although lacking spermatogenetic elements.

regulations that results in testicular induction, gonadal development is started or stopped independent of the presence of an SRY gene. In XY sex-reversed individuals the cascade stops even though testicular induction began in the presence of a Y chromosome, while in XX-sex reversed individuals testicular induction begins even though no Y chromosome is present.

6.2.2.1 XY sex reversal syndrome (XY SRS)

The XY sex reversal syndrome has not been reported in cats, and only once in a dog. This three-year-old Yorkshire terrier with an enlarged clitoris, bilateral ovotestes, epididymis, and uterus had a male chromosome complement. There were two types of X chromosome, one cell line being normal and the other having a translocation involving the X chromosome and an autosome. Thus the dog was presumably a mosaic with a karyotype of 78,XY/78,XYrcp(X;autosome) and not strictly sex reversed.[51]

In humans with XY SRS, both mutations in the sex-determining SRY gene[52] and mutations in other autosomal genes of the cascade, such as in SF-1, WT1, and SOX9, have been reported to be responsible for the XY sex reversal syndrome.[1,53] The mutations are presumed to interrupt the cascade required for testes development.

6.2.2.2 XX sex reversal syndrome (XX SRS)

The XX sex reversal syndrome occurs frequently in dogs but has not been reported in cats. This congenital anomaly is of special interest to dog breeders because monogenic autosomal recessive inheritance has been demonstrated in the American cocker spaniel and is most likely to occur in other affected breeds.[54] The anomaly is known in several dog breeds, including the beagle, Chinese pug, Kerry blue terrier, Weimaraner, German shorthaired pointer, West Highland white terrier, basset hound, Doberman, viszla, Walker hound, soft-coated wheaten terrier, Norwegian elkhound, Jack Russell terrier, German pinscher, cocker spaniel, and Komondor.[55–59]

Even though there is no Y chromosome but – as in a female – two X chromosomes, one or both gonads contain testicular tissue. The most frequent combination in XX sex-reversed dogs is bilateral ovotestes (fig. 6.6). Less frequent are one ovotestis and one ovary, one ovotestis and one testicle, or both gonads completely developed to cryptorchid testicles. Rarely there is a testicle on one side and an ovary on the other. If both ovarian and testicular tissue are present, the individual is a true hermaphrodite (hermaphroditismus verus). If only testicular tissue is present, the individual is called an XX male (fig. 6.7). The underlying defect is a single etiologic form of XX sex reversal in which the degree of gonadal masculinization may be partial or complete. The reason why some XX sex-reversed individuals develop into true hermaphrodites and others become XX males is not known. Although in those with much testicular tissue the oviducts can be absent, the uterus is always present.[60] The external appearance of XX sex-reversed dogs can be ambiguous. In a female phenotype there may be an enlarged clitoris or abnormally large vulva, or the anogenital distance can be altered. The degree of masculinization in true hermaphrodites depends directly on the



Figure 6.8:
Empty scrotum and hypoplastic prepuce and penis of a true hermaphrodite cocker spaniel. The skin irritation has been caused by urinary incontinence.

amount of testicular tissue in the gonads (fig. 6.8). In most true hermaphrodites there is no visible difference in the phenotype. For example, Meyers-Wallen and Patterson found the external phenotype in 20 of 22 true hermaphrodites to be indistinguishable from normal females.[54] These animals are fertile and mostly remain undiscovered in the dog population.

In humans a translocation of the SRY gene to an autosome is often responsible for the XX sex reversal syndrome, the individual being termed SRY-positive. However, all of the reported XX-SRS dogs have no SRY sequence and are thus SRY-negative.[56] In dogs mutations in several of the autosomal genes leading to activation of the cascade of testis differentiation have been suggested to result in SRY-negative SRS and attempts have been made to identify the mutation resulting in XX-SRS in the American cocker spaniel. Candidate genes in humans and goats – such as FOXL2, PISRT1, WT1, GATA1, FOG2, Lhx1, SF-1, SOX9, and Lhx9 – have been shown not to be responsible for the XX-SRS in the American cocker spaniel population.[7,9,61–64] This supports the notion that there are still unknown genes in the cascade responsible for testis differentiation.

Clinical manifestations

Several true hermaphrodites and XX males have a female phenotype with some degree of masculinization, ranging from a small clitoric protuberance with a small os penis up to a hypoplastic penis (fig. 6.9). Depending on the amount of ovarian tissue, affected dogs can have normal estrous cycles, be fertile (always as females), and be able to deliver normal litters.[65] The symptoms and signs may include infertility, primary anestrus, irregular estrous cycles, and urinary incontinence. Some develop endometritis or pyometra and in the gonads neoplasms can develop. Hematuria, genital swelling, and attraction of male dogs may occur, presumably due to cyclic activity of the ovaries.

Differential diagnosis

A female phenotype with masculinization also occurs in male and female pseudohermaphrodites, chimeras, mosaics, and possibly XY sex-reversed dogs. A female phenotype without overt masculinization can also occur in X monosomy, trisomy, cystic endometrial hyperplasia, and hypothyroidism (see also chapter 7).

Diagnosis

Elevation of the plasma testosterone concentration after stimulation with hCG or GnRH provides a presumptive diagnosis (chapter 12.5.1). Ultrasonography and retrograde contrast radiography can reveal female internal genitalia in dogs with a male appearance (fig. 6.10). Affected dogs cannot usually be differentiated from normal females during gonadectomy, because testicular tissue is usually in the center of the gonad and therefore not visible. A definitive diagnosis is based upon histological examination of the gonads by a pathologist who is familiar with XX-SRS and upon cytogenetic demonstration of a female XX karyotype. Affected dogs have a complete uterus and many have epididymes adjacent to the ovotestes or testes.[56]

Treatment

Vulvar irritation caused by a protruding clitoris can be resolved by resection of the os clitoris. Gonadectomy has been recommended when there is intra-abdominal testicular tissue, which carries an increased risk of Sertoli cell neoplasia (see also chapter 8). Hysterectomy has been recommended in true hermaphrodites because of the risk of endometritis. These risks must be weighed against the associated morbidity and mortality of abdominal surgery.

Prognosis

Local irritation of the vulva usually resolves after removal of the enlarged clitoris or the os clitoris. XX-SRS is an inherited disorder and breeding should be discouraged to prevent the homozygous fertile hermaphrodite from transmitting the recessive trait. Heterozygous carriers resemble normal males and females and at present there is no practical means of identifying them. Because XX SRS is presumably a monogenic, autosomal recessive inherited disorder, it is likely that the male and female siblings of an XX sex-reversed dog are carriers of the disease allele or that the females may be true hermaphrodites.

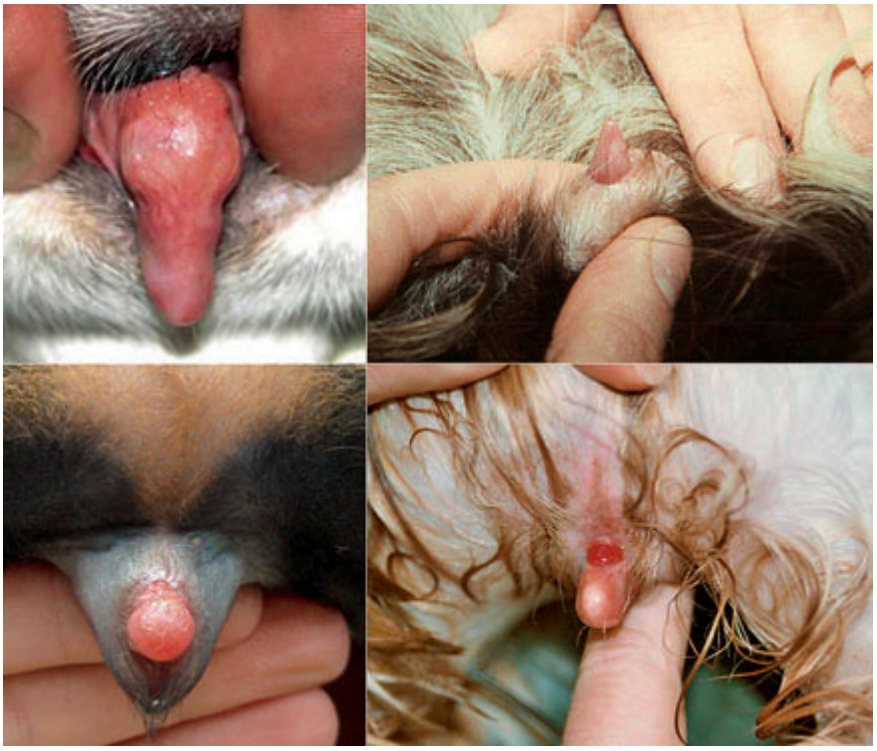


Figure 6.9:
Rudimentary male genitalia of four unrelated XX sex reversed dogs of different breeds (Komondor, mixbred, German pinscher, and American cocker spaniel).

6.2.3 Disorders of phenotypic sex

XY and XX sex reversal must be differentiated from pseudohermaphroditism, in which chromosomal and gonadal sex always agree but the phenotype is that of the opposite sex.[56] There are male and female forms of pseudohermaphroditism.

6.2.3.1 Female pseudohermaphroditism (pseudohermaphroditismus femininus)

Masculinization of androgen-sensitive tissues in individuals having ovaries and an XX-karyotype is referred to as female pseudohermaphroditism. The masculinization ranges from an enlarged clitoris to male external genitalia (fig. 6.11) with internal parts of a prostate gland, but the Fallopian tubes, uterus, and cranial part of the vagina are not visibly altered.[56]

Female pseudohermaphroditism was found less frequently than other forms of intersexuality in a survey of 52 canine cases.[66] It develops as a result of exposure to exogenous or endogenous androgens. All of the few reported cases in dogs have apparently been the result of administration of methyl testosterone orally or testosterone propionate parenterally during gestation.[67–70] The administration of androgens to intact female dogs should therefore be discouraged.[71] Ovotestes were found in the six female offspring of an American Staffordshire Terrier bitch treated with oestradiol benzoate and



Figure 6.10:
Retrograde cystourethrography in a true hermaphrodite cocker spaniel with hypoplastic male external genitalia. Note the male urethra and the accumulation of contrast material in the female genitalia.



Figure 6.11:
Female pseudohermaphroditism in a dog as the result of administration of anabolic steroids to the dam during pregnancy.

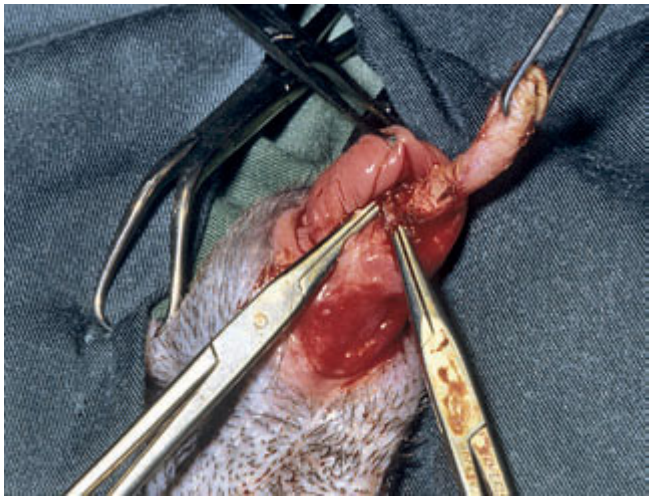


Figure 6.12:
Surgical removal of the os clitoris from a male pseudohermaphrodite dog with female external genitalia.

synthetic androgens during pregnancy and thus they were considered to be true hermaphrodites, but the reason why they developed testicular tissue is unknown.[72]

Congenital adrenocortical hyperplasia due to 11β-hydroxylase deficiency, which results in endogenous androgen exposure, has only been described in one cat, a female pseudohermaphrodite.[73] This cat had a calico-colored coat and an XX karyotype, and a fully formed penis, prepuce, and scrotum, but no palpable testes. Laparotomy revealed two ovaries, two uterine horns, and a uterine body. Congenital adrenocortical hyperplasia is the most common cause of ambiguous genitalia in children, in which it is inherited as an autosomal recessive disorder resulting in a deficiency of either 21-hydroxylase or 11β-hydroxylase required for adrenocortical synthesis of cortisol and aldosterone. The low secretion of cortisol results in high ACTH release and consequently increased secretion of adrenal androgens.

The clinical manifestations depend on the duration and amount of androgen exposure. Like male pseudohermaphrodites, female pseudohermaphrodites may be presented with symptoms suggesting lower urinary tract disease and endometritis. In less severe cases the irritation caused by the enlarged clitoris may require surgery (fig. 6.12).

A female phenotype with masculinization is also seen in sex reversed dogs, male pseudohermaphrodites, chimeras, and mosaics. Low or undetectable plasma testosterone concentrations before and after stimulation with hCG or GnRH indicate the absence of testicular tissue (fig. 6.13).

If there is endometritis, ovariohysterectomy is the treatment of choice. An enlarged clitoris or os clitoris may be removed surgically if it causes irritation. Less severe cases of masculinization due to administration of androgens during gestation may not require treatment. In congenital adrenocortical hyperplasia, administration of a glucocorticoid will reduce pituitary ACTH release and consequently the excessive production of sex steroids in the adrenal glands.

6.2.3.2 Male pseudohermaphroditism (pseudohermaphroditismus masculinus)

Male pseudohermaphrodites have a male karyotype (XY) and two testes, but the genital ducts and/or external genitalia are incompletely masculinized, leading to internal and/or external parts of the female genital tract. Male pseudohermaphrodites can be classified as having: (1) defective regression of the Müllerian ducts, or (2) disturbances in androgen-dependent masculinization. In principle, these maldevelopments can be the result of: (1) defective testicular differentiation,(2) an error in the release or action of AMH, (3) an error in the synthesis of testosterone, or (4) defects in the androgen-dependent target tissues such as 5α-reductase deficiency and low or absent androgen receptor activity.

The **persistent Müllerian duct syndrome (PMDS)** is the most common form of male pseudohermaphroditism in dogs. A defect in AMH (MIS)-induced Müllerian duct regression is responsible for the presence of oviducts, Fallopian tubes, uterus, cervix, and cranial vagina in otherwise completely normal male dogs (fig. 6.14). These dogs have a normally developed penis with a prepuce and scrotum.[38,56,74] Half of the

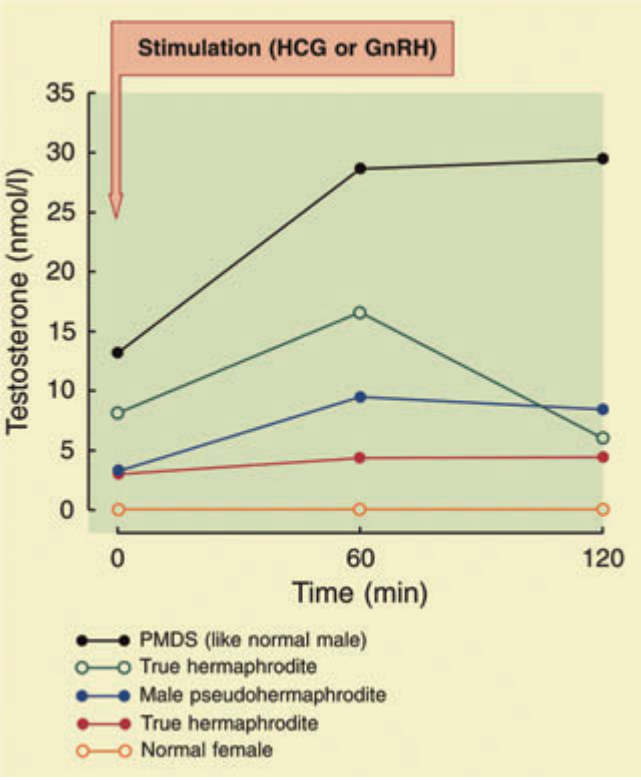


Figure 6.13:
Schematic illustration of plasma testosterone concentrations before and after stimulation with hCG or GnRH (chapter 12.5.1). Both basal testosterone concentration and the response to stimulation depend on the amount of functional testicular tissue, as shown by the different values in the two cases of true hermaphroditism.

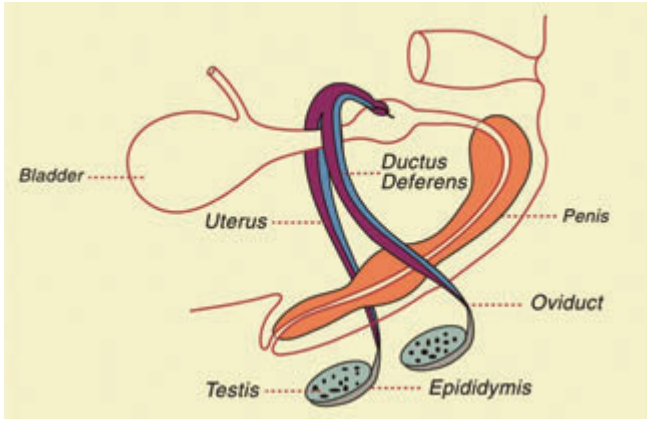


Figure 6.14:
Schematic representation of persistent Müllerian ducts in a male dog. Note that the vasa deferentia terminate in the wall of the uterus.

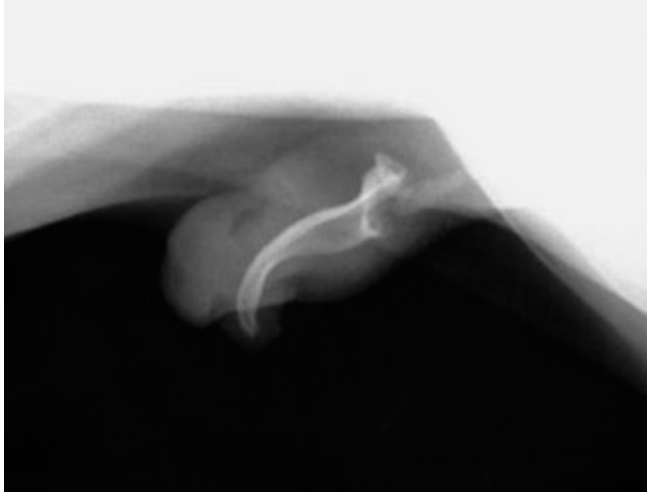


Figure 6.15:
Radiograph of a two-year-old mixbred cryptorchid PMDS dog with malformation of the os penis.

affected dogs have scrotal testes, while the other half are unilaterally or bilaterally cryptorchid. Most of the affected dogs are fertile.

PMDS was first described in the miniature schnauzer and an autosomal recessive mode of inheritance in this breed has been proved by breeding experiments.[74,75] A single case in this breed was diagnosed in Germany.[76] PMDS has also been found in other dog breeds, including the basset hound[77] and poodle,[78] and in two cocker spaniels with an enlarged clitoris and a scrotum with undescended testes.[59] PMDS was also suspected in a dachshund bitch with an enlarged clitoris and abdominal testes.[79] Moreover, PMDS has been reported in a two-year-old mixbred cryptorchid dog with an underdeveloped penis, a hypoplastic uterus, and hypospadia of the glans penis, in which radiographic examination revealed a malformed os penis (fig 6.15).[80] Studies in miniature schnauzers and basset hounds demonstrated that AMH is produced and is bioactive in the critical period of Müllerian duct regression.[81] Thus defects at the receptor or postreceptor level, as demonstrated in comparable cases in humans, are the most likely explanation.[74] PMDS has not been reported in cats.

Dogs with PMDS can be fertile if the testes have descended and the epididymis is not affected by inflammatory changes. Animals with PMDS are often presented with symptoms suggesting lower urinary tract disease. Endometritis is probably the most common problem and may result in hematuria, abdominal pain, and systemic illness. Symptoms suggesting lower urinary tract disease can be so prominent that the underlying condition is overlooked, for the veterinarian is unlikely to think of endometritis (or even pyometra) in a dog that appears to be male. This may cause severe delay in correct diagnosis. In miniature schnauzers the associated high incidence of cryptorchidism may give rise to Sertoli cell neoplasia.[38,56]

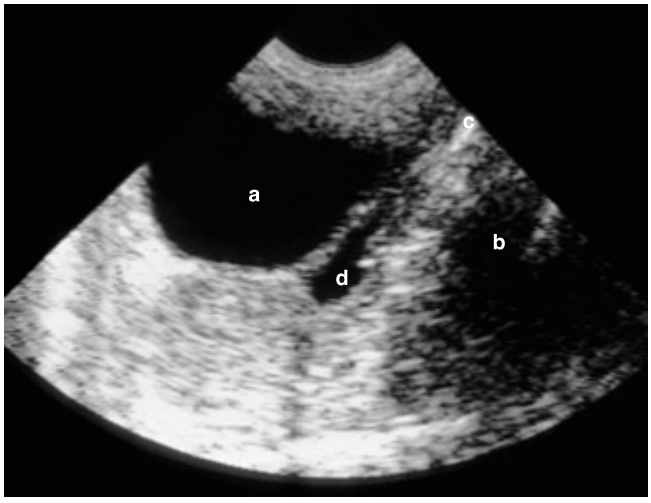


Figure 6.16:
Longitudinal ultrasonogram from the ventral abdominal wall of a male basset hound with persistent Müllerian duct syndrome (PMDS). Dorsal to the bladder (a) and craniodorsal to the prostate (b) and cranial urethra (c), the persistent Müllerian duct (d) is visualized.

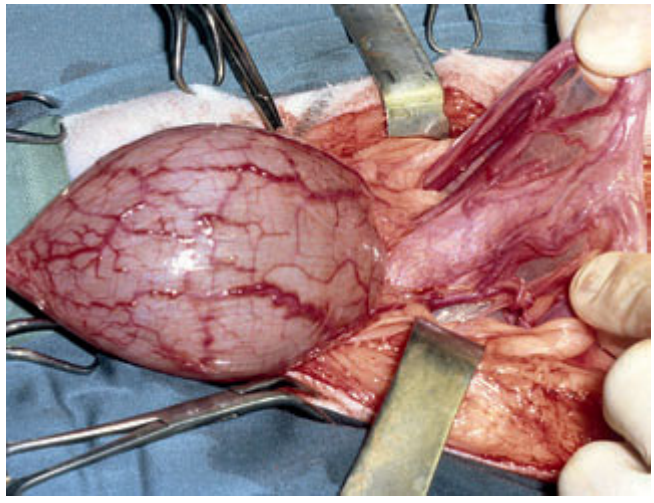


Figure 6.17:
Persistent Müllerian ducts in a male basset hound as seen at laparotomy. The bladder is retracted caudally to reveal the uterus and uterine horns (between the fingers of the surgeon).

Abdominal radiography and retrograde contrast radiography may reveal female internal genitalia in dogs with a male appearance. However, in some patients with PMDS the internal female genitalia cannot be detected by radiographic examination although they can easily be found by ultrasonography (fig. 6.16), CT, or MRI. In dogs with descended testes, normal male external genitalia, and an XY karyotype, the finding of a uterus provides the diagnosis of PMDS.

PMDS dogs with endometritis can be treated successfully by hysterectomy (fig. 6.17). Most also require orchidectomy because of abnormalities of the epididymis or testis. Selective hysterectomy or vasectomy can be performed in dogs with PMDS having unaffected testes and epididymes.

Breeding of dogs with PMDS should be discouraged. As both parents of affected animals are carriers, the veterinarian should inform the breeder about the inheritance and the fact that affected dogs with or without unilateral cryptorchidism contribute to spreading of the defective allele in the dog population (fig. 6.18).

In addition to defective regression of the Müllerian ducts, in rare cases defective androgen-dependent masculinization can also result in male pseudohermaphroditism. Affected dogs have testes and female-appearing external genitalia with a cul-de-sac caudal vagina. Under the influence of AMH the Müllerian ducts regress and so that there is no uterus or cranial vagina, but the genital duct and/or external genitalia are incompletely masculinized.[56] The resulting phenotype can vary from complete (severe) to incomplete (mild), depending on the primary defect. This varying phenotype can be the result of: (1) defects in the production of luteinizing hormone (LH) or its receptor, (2) defects in androgen production, (3) partial or complete absence of androgen receptor activity, or (4) defective conversion of testosterone to dihydrotestosterone by 5α-reductase.[82,56] Indeed, defects in LH synthesis and in the LH receptor as well as in androgen production and androgen dismantling are known in humans and some animal species, but not as yet in the dog. It has been suggested that in the absence of dihydrotestosterone the labioscrotal folds fail to fuse and the urogenital sinus fails to close, resulting in periscrotal hypospadias and the blind pouch that resembles a vagina.[83]

Male pseudohermaphroditism due to a failure of target organ response to androgens is referred to as testicular feminization.[56] A defect in the androgen receptor gene results in partial or complete absence of androgen-dependent masculinization. Less severe mutations cause compromised masculinization, while severe gene mutations cause complete androgen insensitivity. The physical result ranges from ambivalent appearance to phenotypic male – but sterile – dogs. Since they have bilateral testes and secrete normal amounts of testosterone and anti-Müllerian hormone, no Müllerian duct derivates are present. In all animal species this is assumed to be an X-chromosomal recessive trait, but complete testicular feminization in the dog has not yet been reported. One dog with incomplete testicular feminization had a female phenotype with testes bilateral to the vulva and no uterus. Studies with fibroblast cultures suggested that the androgen receptor was nonfunctional. The testicular feminization was incom-

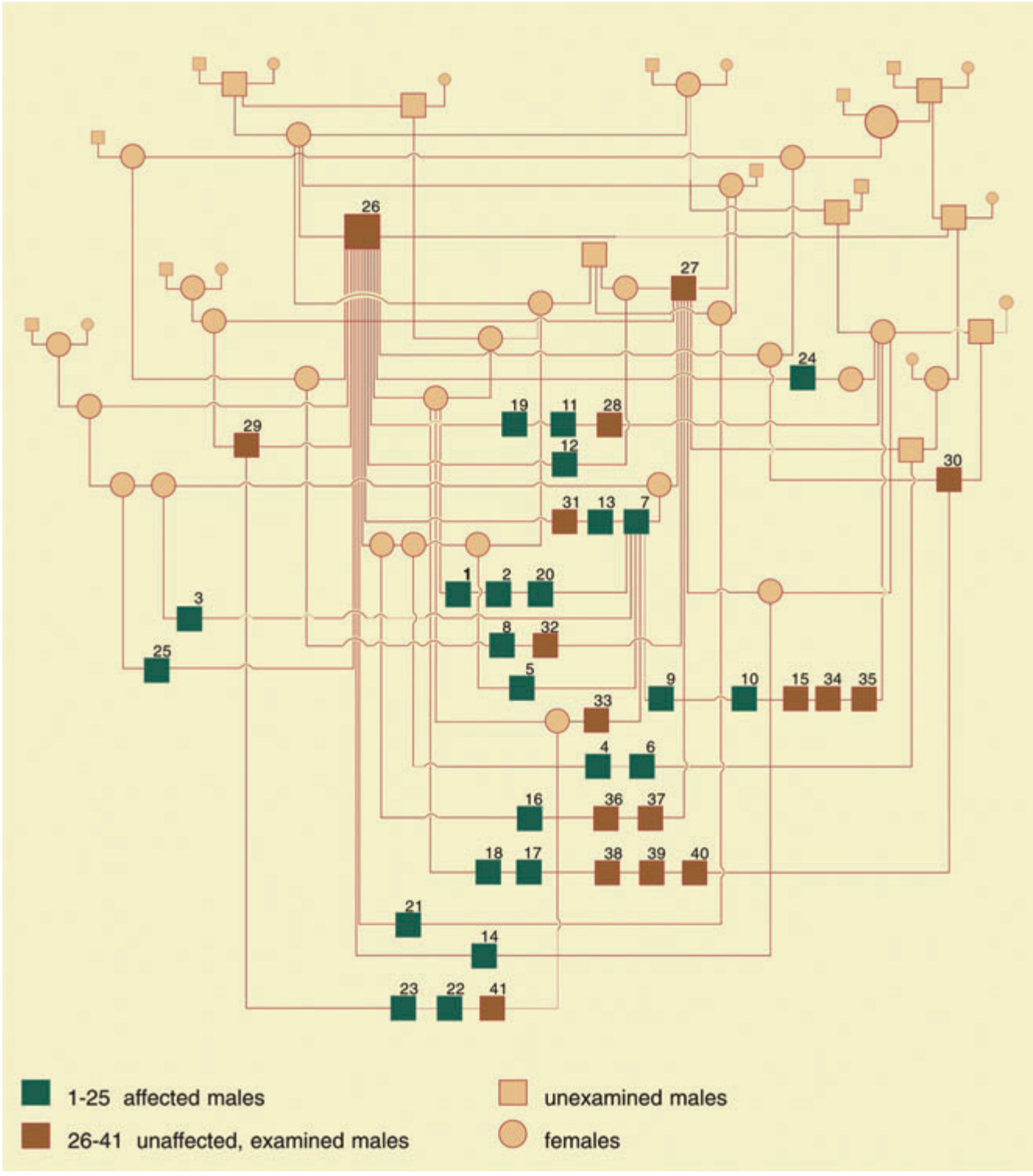


Figure 6.18:
Schematic representation of familial relations in basset hounds with persistent Müllerian duct syndrome (PMDS). Mating of assumed male carriers nos. 26 and 27 with related females resulted in affected offspring such as nos. 14 and 21. Offspring of affected male no. 7 include even more affected littermates, supporting an autosomal recessive mode of inheritance: (Courtesy of Dr. R.F. Nickel.)

plete, for an epididymis and partially developed ductus deferens were present as Wolffian duct derivates.[82]

There have been two reported cases of male pseudohermaphroditism in cats due to testicular feminization. One cat had a vulva and clitoris of normal size and shape, no uterus, but two abdominal testes at the caudal poles of the kidneys. The chromosome complement was 38,XY and the cat was thought to be a case of complete testicular feminization.[84] The other case consisted of a Himalayan cat with testes in a blind scrotum, an enlarged clitoris protruding from a vulva-like structure, and no Müllerian duct derivates.[85]

In dogs and cats with a female phenotype the finding of elevated plasma testosterone concentrations after stimulation with hCG or GnRH can prove the presence of testicular tissue (chapter 12.5.1). Without karyotyping, chimerism or mosaicism cannot be distinguished from a disorder of androgen-dependent masculinization.

Resection of an os clitoris stops vulvar irritation. If necessary, a complete clitoridectomy can be performed. Orchidectomy may be necessary in some cases. In all cases of testicular feminization the breeder should be informed of the X-recessive inheritance of the trait in humans.

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7 Ovaries

Auke C. Schaefers-Okkens
Hans S. Kooistra

7.1 Introduction

The ovaries lie caudal to the kidneys, at the level of the third or fourth lumbar vertebra. They are attached by the broad ligaments to the dorsolateral wall of the abdominal cavity and by suspensory ligaments to the middle and ventral thirds of the last one or two ribs (dog) or to the diaphragm (cat). The ovaries are connected to the cranial ends of the uterine horns by the proper ligaments of the ovary (fig. 7.1). The ovaries of the dog are enclosed completely, and those of the cat partially, in a peritoneal pouch, the ovarian bursa (fig. 7.2). The bursa contains the uterine tubes and is usually opaque in the dog

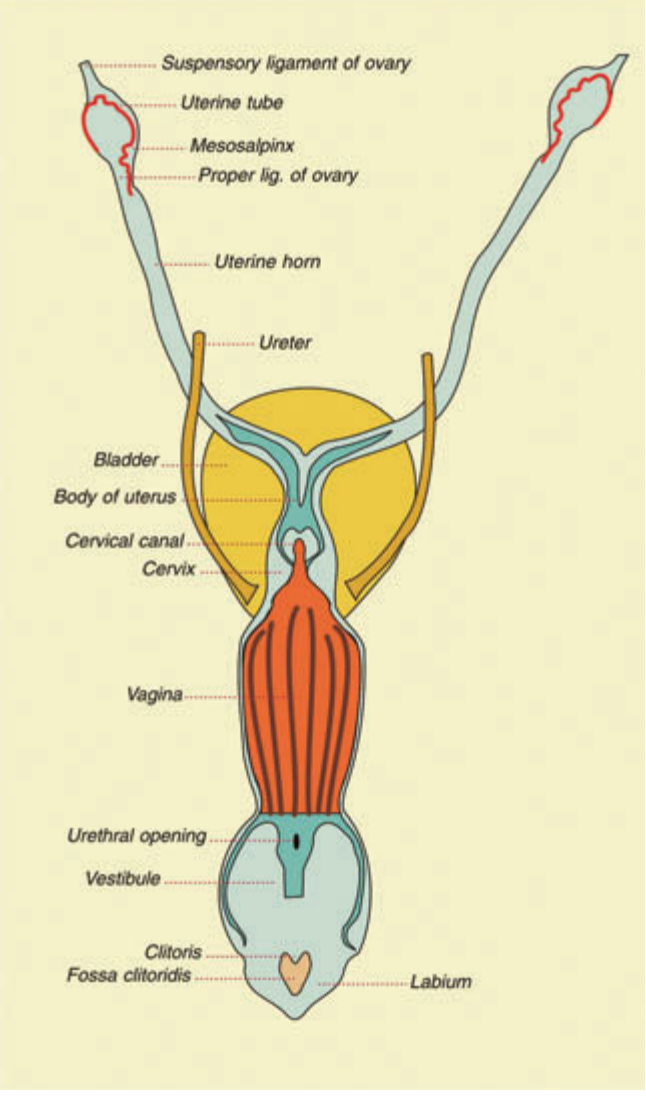


Figure 7.1:
Dorsal view of the genitalia of the bitch, partially opened on the midline. (Modified from Evans and Christensen, 1993.)[1]

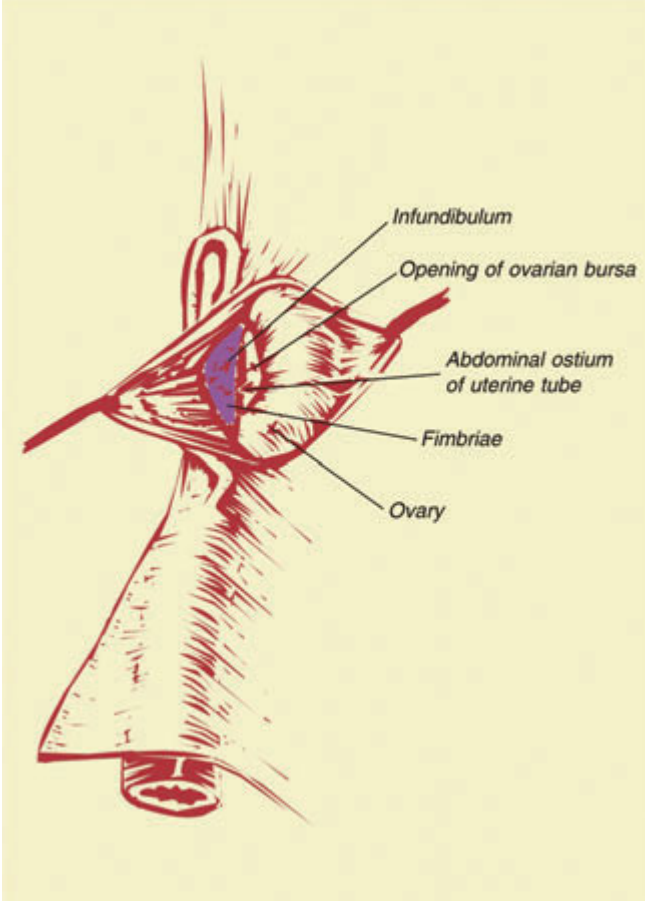


Figure 7.2:
Lateral aspect of the left ovary, with opened ovarian bursa. (Modified from Evans and Christensen, 1993.)[1]

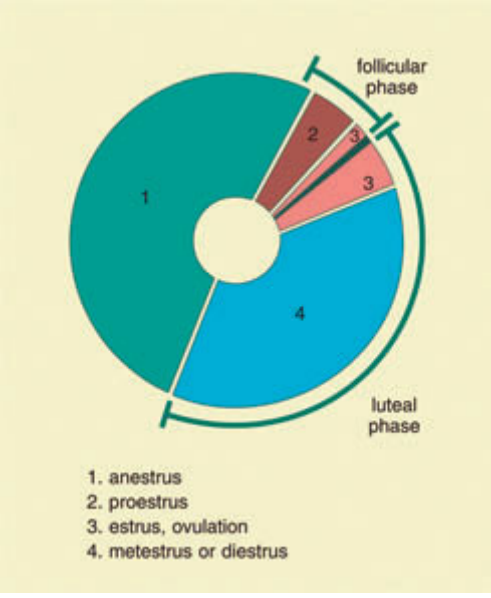


Figure 7.3:
Schematic representation of the estrous cycle and anestrus in the dog.

due to its fat content. The surface of the ovary is covered by the germinal epithelium of the cortex and is free of serosa. Germ cells growing inward from the cortex give rise to follicles, many of which degenerate and become atretic. Tertiary follicles develop during the follicular phase and become visible at the surface of the ovary due to the considerable increase in the amount of follicular fluid they contain. The infundibula are open lateral to the ovaries to collect the ova following ovulation. In the dog, the fimbriated extremities lie mainly within the bursa but a portion generally protrudes through the slit-like opening of the bursa.

7.2 Estrous cycle, anestrus, pregnancy, and parturition

7.2.1 Estrous cycle, anestrus, pregnancy, and parturition in the dog

In the healthy bitch the onset of puberty occurs at six to 18 months of age. Each estrous cycle, which lasts about three months, is followed by an anestrus of variable duration. The mean interval between estrous cycles is about seven months, with a range of four to twelve months. The interestrous interval may be regular or variable in individual bitches.

7.2.1.1 Estrous cycle

The stages of the canine estrous cycle are proestrus, estrus, and metestrus (diestrus) (fig. 7.3). The average duration of proestrus is nine days, with a range of three to 17 days. Proestrus is defined as the period from onset of sanguineous vaginal discharge and vulvar swelling until the first willingness to accept mating. Estrus has an average duration of nine days, with a range of three to 21 days. During estrus the bitch accepts mating and the vulva begins to shrink and soften. The discharge usually persists and may remain sanguineous or turn straw-colored. Metestrus (diestrus) begins when the bitch no longer accepts mating. It has an average duration of about 70 days if we assume that it ends when plasma progesterone concentration declines for the first time to < 3 nmol/l.

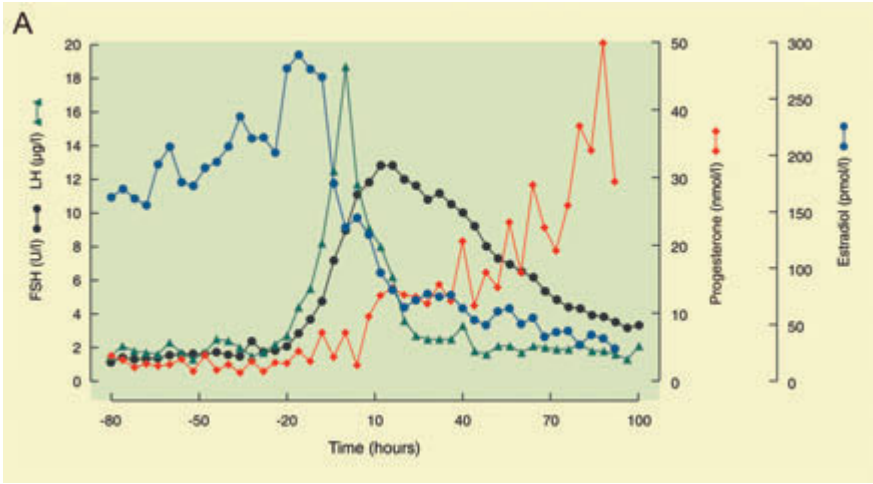
In addition to this behavior-oriented classification, the estrous cycle can be classified according to ovarian function, as the follicular phase, the phase of preovulatory luteinization and ovulation, and the luteal phase (fig. 7.3).

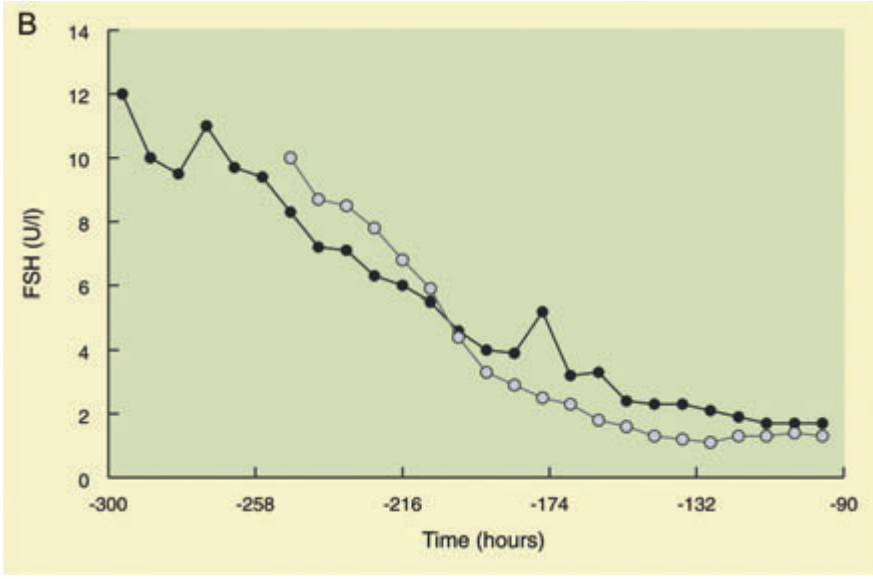
7.2.1.2 Follicular phase

As tertiary follicles develop in the ovaries they produce estradiol-17β. The plasma estradiol-17β concentration increases gradually during the early follicular phase, leading either to a plateau interval or a sharp increase just before the beginning of the preovulatory luteinizing hormone (LH) surge, with peak concentrations of approximately 300–350 pmol/l about 1–2 days before the preovulatory LH surge (fig. 7.4).[2] Then plasma estradiol-17β concentration decreases to basal values of approximately 35 pmol/l about 80 h after the preovulatory LH surge.

Follicle development is not readily apparent during laparoscopic examination because the ovary is hidden in the ovarian bursa and because the follicles remain below the ovarian surface until just prior to ovulation.

The external signs of proestrus, such as hyperemia and edema of the vulva and bloody vaginal discharge, are related to the high estradiol-17β concentration (fig. 7.5). The hormonal changes are also associated with lengthening and hyperemia of the uterine horns, enlargement of the cervix, and thickening of the vaginal wall. The percentage of superficial cells in the vaginal smear increases and the percentage of parabasal and small intermediate cells decreases (fig. 7.6). Superficial cells dominate as the follicular phase progresses (fig. 7.7). However, although vaginal cytology gives an indication of the stage of the estrous cycle, it is not a reliable indicator of the preovulatory LH surge or of ovulation. Vaginoscopy will reveal that the vaginal mucosal folds are swollen, very pale, and have a smoothly rounded (balloon-like) surface during the follicular phase (fig. 7.8). At the end of the follicular phase, i.e., during the decline in estradiol-17β and the rise in progesterone concentrations in plasma, shrinkage begins in response to reduced estradiol-dependent water retention. These cyclic changes are





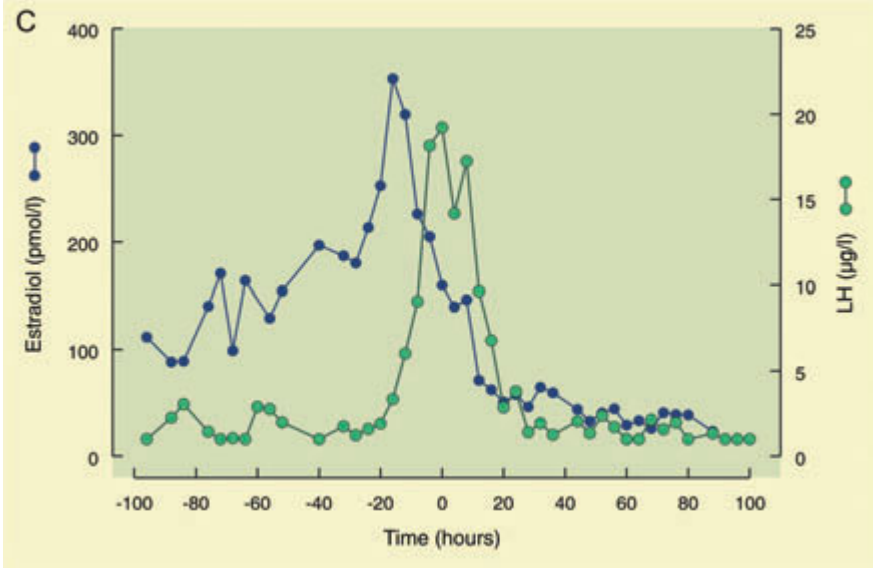


Figure 7.4:
(A) Mean plasma concentrations of LH, FSH, estradiol, and progesterone before and after the preovulatory LH surge (at time = 0), i.e., during the follicular phase, ovulation, and the fertilization phase, in 6 bitches.
(B) Plasma FSH concentration in two beagle bitches during the follicular phase (until 100 h before the preovulatory LH surge). Note the decrease in plasma FSH in the early follicular phase.
(C) Plasma concentrations of LH and estradiol from 100 h before until 100 h after the preovulatory LH surge in a beagle bitch with a preovulatory surge in plasma estradiol coinciding with the start of the preovulatory LH surge. Note the bifurcated preovulatory LH surge. (Modified from De Gier et al., 2006.)[2]



Figure 7.5:
The vulva of a Beagle bitch during anestrus (A) and proestrus/estrus (B).

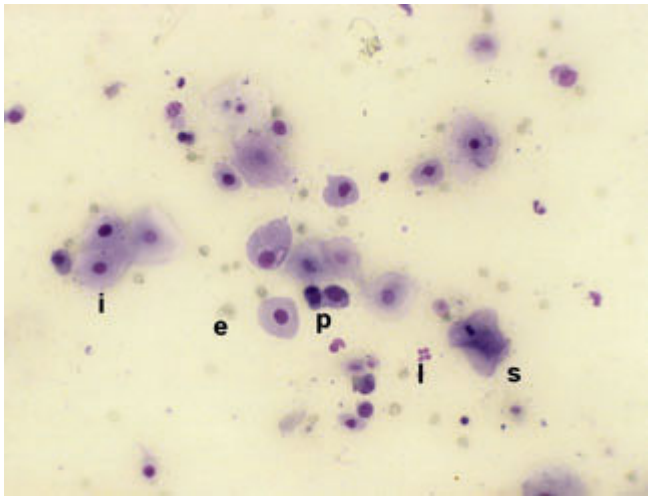


Figure 7.6:
Vaginal cytology in the bitch at the onset of the follicular phase, showing primarily intermediate (i) cells, some superficial (s) and parabasal (p) cells, erythrocytes (e), and leukocytes (l). (May-Grünwald Giemsa stain, x200).

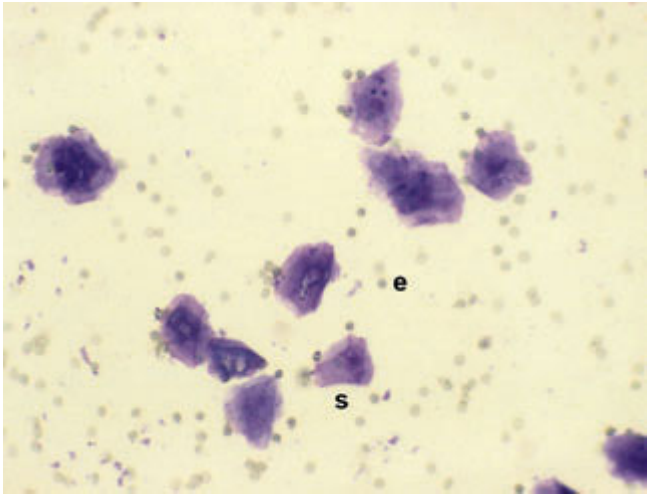


Figure 7.7:
Vaginal cytology in the bitch during the second half of the follicular phase, at ovulation, and at the onset of the luteal phase. The smear shows superficial cells (s) and erythrocytes (e). (May-Grünwald Giemsa stain, x200).

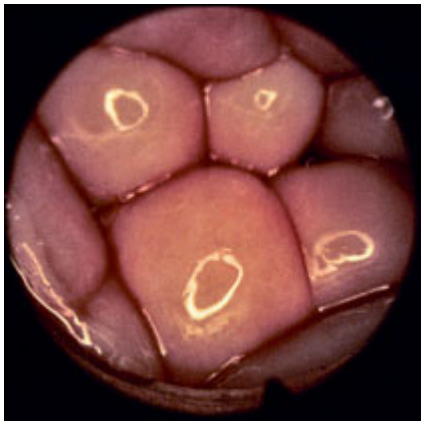


Figure 7.8:
Vaginoscopic view in the bitch at the onset of the follicular phase. Note the swollen, pale mucosal folds with a smoothly rounded surface (balloons) and the bloody secretion between the folds.

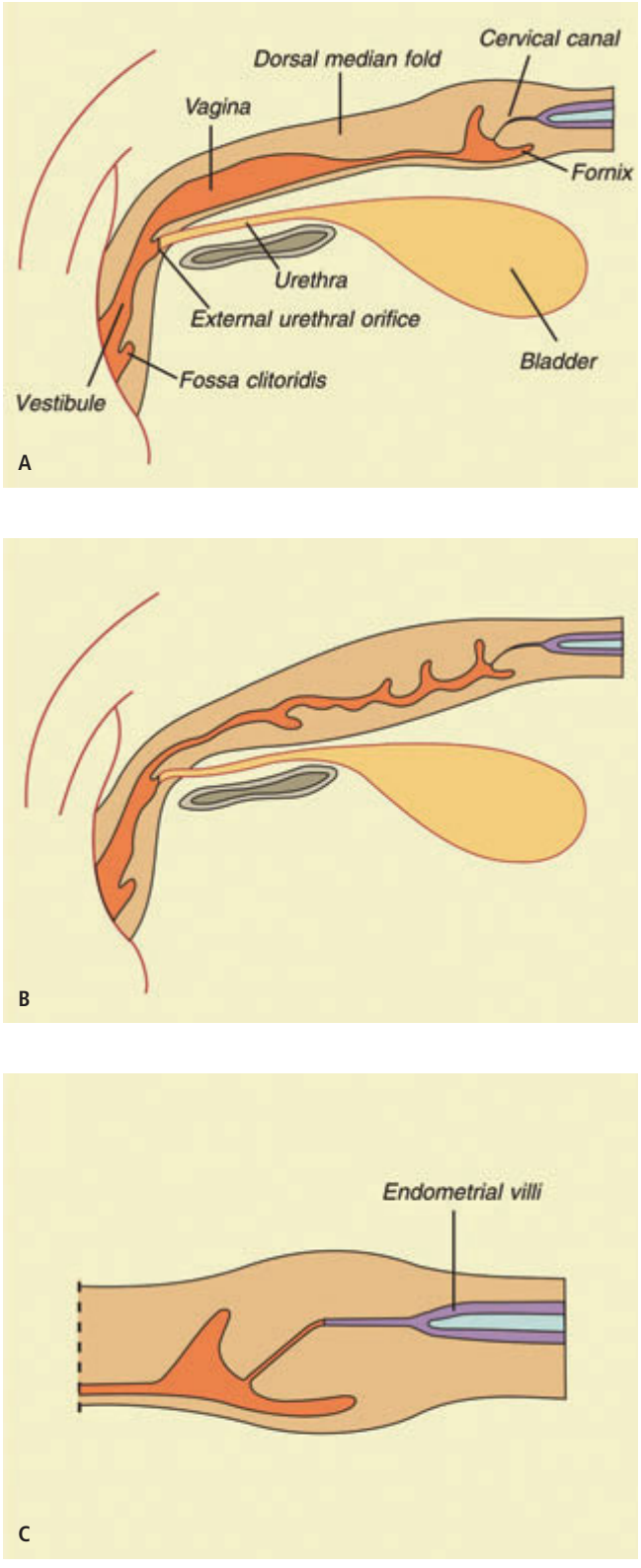


Figure 7.9:
Schematic illustration of a sagittal section through the vestibule, vagina, and cervix of a bitch (A) during anestrus and (B) during proestrus/estrus. In the latter the vaginal wall is extremely folded. (C) Close-up view of the cervix and cranial vagina during anestrus. Note the very short cervical canal.

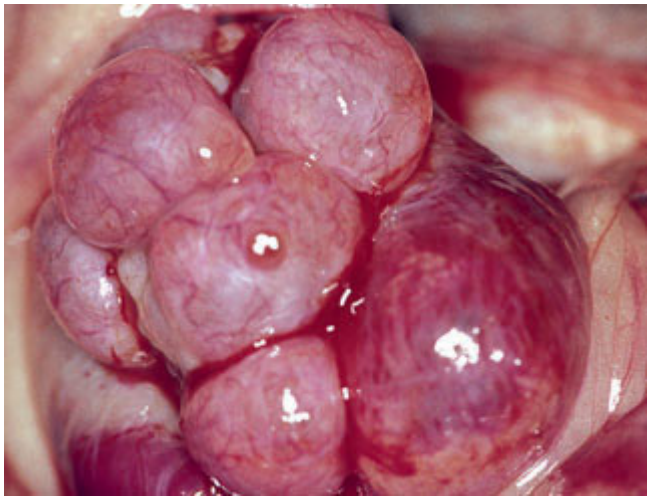


Figure 7.10:
Ovary of the bitch at the time of ovulation. The bursa which normally encloses the ovary has been removed.

most marked in the dorsal median fold and precede those of the midvaginal mucosa (fig. 7.9A–C).

Plasma LH concentration is low during the follicular phase, with frequent increases of short duration.[2,3] The plasma concentration of follicle-stimulating hormone (FSH) is relatively high at the beginning of the follicular phase, similar to the concentration in late anestrus, but declines to low levels during the progression of the follicular phase (fig. 7.4B).[2] Plasma progesterone concentration fluctuates at low levels. During the second part of the follicular phase plasma progesterone concentration can increase slightly, probably as a result of partial luteinization of granulosa cells.

7.2.1.3 Preovulatory luteinization and ovulation

The average duration of the preovulatory LH surge is 36 h. The mean plasma LH concentrations before and after the surge do not differ. The LH surge is frequently bifurcated (fig. 7.4C) and coincides with a rising plasma progesterone concentration.[2] The preovulatory FSH surge begins concomitantly or a few hours prior to the preovulatory LH surge, and is not bifurcated. Plasma FSH concentration is lower before the surge than after (fig. 7.4A).[2]

There is rapid and extensive luteinization during the preovulatory LH surge. Ruptured follicles have several characteristics of rapidly developing corpora lutea (fig. 7.10). In the dog most ova are released in an immature state as primary oocytes. The first meiotic division and the extrusion of the first polar body are not completed until at least 48 h after ovulation. Total maturation after ovulation requires two to three days before fertilization can occur. A recent study showed that

fertilization usually occurs 90 h or more after ovulation in metaphase II oocytes.[4] There was no significant influence of breed or age on ovulation rate, maturation, and developmental kinetics. The most peculiar aspect in the canine species is oocyte meiotic maturation, while fertilization follows the same pattern as in other mammals.[4]

Plasma progesterone concentration is around 6–13 nmol/l at the time of the LH surge and 15–25 nmol/l at the time of ovulation, 36–48 h later. The start of estrus behavior is usually synchronous with the preovulatory LH surge, but in some bitches it begins days before the LH surge and others not until days thereafter or never. Shrinkage of the vaginal mucosa starts about midway through the follicular phase and continues through the phase of preovulatory luteinization and ovulation, whereby many longitudinal folds can be observed (fig. 7.11).

7.2.1.4 Luteal phase

Plasma estradiol-17β concentrations are significantly higher throughout the luteal phase than at four to nine days after the LH surge.[5] The concentration of progesterone, coming from the corpora lutea, increases in the peripheral blood during the remainder of estrus and the onset of metestrus (diestrus). Thus estrus behavior is observed in the bitch during the period of increasing progesterone concentration. This reaches a plateau from about day 10 to day 30 after the LH surge. In nonpregnant bitches it then declines slowly to a basal level of 3 nmol/l for the first time about 75 days after the onset of the luteal phase (fig. 7.12). What initiates regression of the corpus luteum in the bitch remains unknown. It is not prostaglandin $F_{2\alpha}$ from the endometrium, as in the cow and ewe, for hysterectomy does not influence the length of the luteal phase in the bitch.[6] During the first half of the luteal phase the canine corpus luteum functions independent of pituitary support.[7] Thereafter experimentally induced inhibition of prolactin secretion causes a sharp decline in progesterone secretion (fig. 7.13), which has led to the assumption that prolactin acts as a luteotropic factor in the second half of the luteal phase.[8,9] There are no strong indications that LH has luteotropic properties in the bitch.

The secretion patterns of prolactin, growth hormone (GH), FSH, and LH are characterized by a fluctuating baseline with occasional distinct elevations, indicating pulsatile secretion.[3,10–12] The mean plasma LH concentration scarcely changes during the luteal phase, except for a slight increase in the second half of the luteal phase. The mean plasma prolactin concentration increases slightly but significantly during the progression of the luteal phase (figs. 7.12, 7.13).[11] In overtly pseudopregnant bitches (see chapter 2.2.5) and in pregnant bitches the rise in plasma prolactin concentration is much greater.[13,14] Plasma GH concentration is higher in the first part of the luteal phase than in the second half of the luteal phase and anestrus (fig 2.12).[10] Pulsatile secretion of GH also changes during the luteal phase, there being greater basal GH secretion and less GH secreted in pulses during stages having a high plasma progesterone concentration, i.e., during the first part of the luteal phase (fig 2.12).[10] This is most likely the result of partial suppression of pituitary GH release by progesterone-induced GH production in the mammary gland (see also chapter 2.2.1).[15]

The pattern of secretion of progesterone thus influences the pattern of secretion of both GH and prolactin in the bitch.[11] High plasma progesterone concentrations during the first half of the luteal phase induce elevated plasma GH concentrations and the decline in the progesterone concentration during the second half of the luteal phase increases prolactin release. These hormonal changes may promote the physiological proliferation and differentiation of mammary gland tissue during the luteal phase in the bitch. Growth hormone, in concert with insulin-like growth factor (IGF)-I, IGF-II, and IGF-binding proteins, initiates mammary proliferation,[16] whereas prolactin promotes final mammary differentiation, i.e., lobuloalveolar development.[17]

Progesterone-induced GH production may also have an effect on uterine epithelium. During each luteal phase, regardless of whether or not the bitch is pregnant, progesterone-dependent uterine epithelial changes occur. In progestagen-treated dogs the hyperplastic changes in the uterine epithelium are associated with the intracellular presence of immunoreactive GH.[18] Progesterone-induced GH production may also have metabolic effects. Progestagen-induced GH excess leads to insulin resistance.[15] The exposure to progesterone-induced elevated plasma GH concentrations during the first half of the luteal phase may also cause some insulin resistance. For an animal such as the dog, evolving from predators, there may have been evolutionary advantages in this temporary insulin resistance during the first half of pregnancy. Especially during long periods between catches of prey, insulin resistance may be a safeguard against hypoglycemia.[19] It could serve to maintain blood glucose concentration immediately after the intake of a low carbohydrate meal (a prey), while insulin is secreted in response to other food components such as amino acids. Finally, high GH concentrations have been demonstrated in canine mammary gland secretions and particularly in colostrum, through which GH may promote gastric and intestinal development in the newborn.[20]

The transition from estrus to metestrus (diestrus) occurs during the initial part of the luteal phase. In this period the cytology of the vaginal mucosa changes from chiefly superficial cells to chiefly intermediate and parabasal cells and leukocytes (fig. 7.14). This is an indication that the fertile period is over. At the time of the maturation of the oocytes, the shrinkage of the vaginal mucosa continues and increasing numbers of sharp-edged summit profiles appear in the vagina. In the transition period from estrus to metestrus, the mucosa thins and profiles become round. In the beginning of metestrus there is a visible patchwork of red and white areas (fig. 7.15).

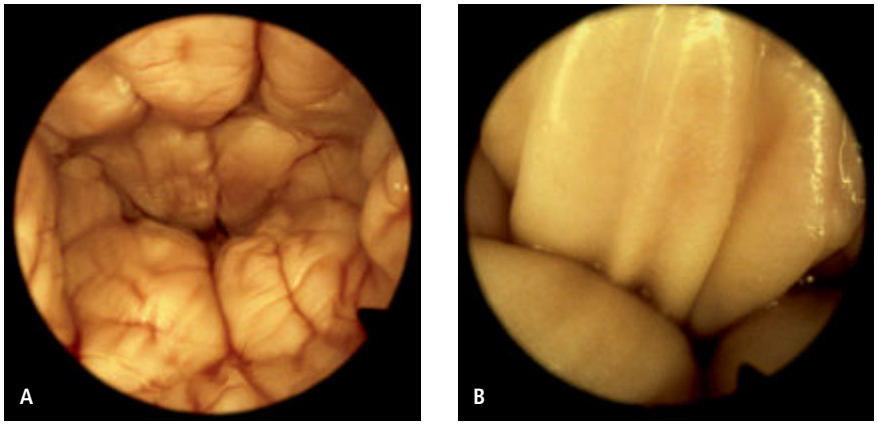


Figure 7.11:
Vaginoscopic view at the time of ovulation. Plasma progesterone concentration was 22 nmol/l.
(A) The mucosal shrinkage has resulted in longitudinal folds.
(B) Close-up showing shrinkage of the longitudinal folds of the dorsal median fold in the cranial vagina.

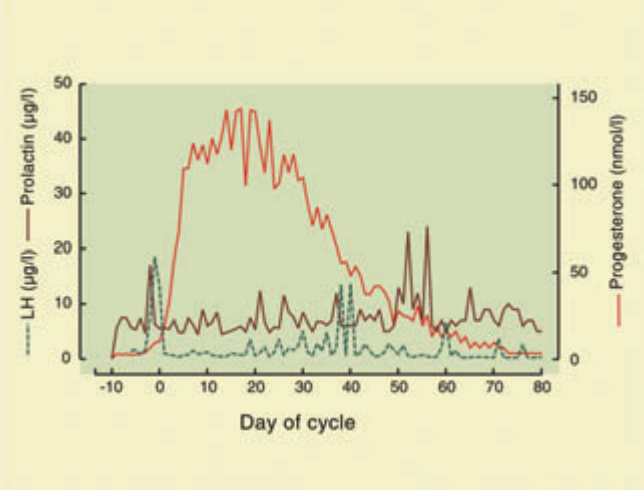


Figure 7.12:
Mean plasma concentrations of LH, progesterone, and prolactin in three dogs during the follicular and luteal phases. The data have been synchronized to day 1, the day after the onset of the follicular phase on which plasma progesterone concentration reached 16 nmol/l. (Modified from Okkens et al., 1990.) [8]

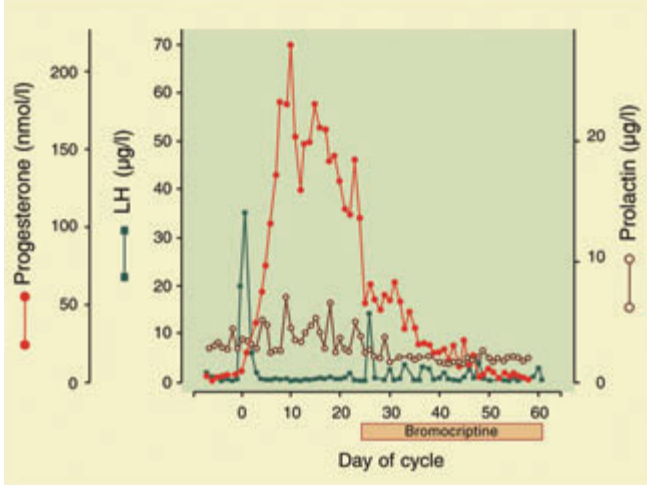


Figure 7.13:
Mean plasma concentrations of progesterone, prolactin, and LH of four dogs treated with the dopamine agonist bromocriptine (20 µg/kg body weight, twice daily, orally) from day 20–24 after the onset of the luteal phase until the end of the luteal phase (bar). The data have been synchronized to day 1, the day after the onset of the follicular phase on which plasma progesterone concentration reached 16 nmol/l. (Modified from Okkens et al., 1990.) [8]

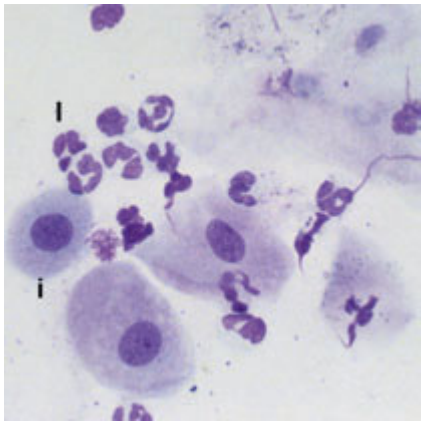


Figure 7.14:
Vaginal cytology during metestrus, which begins six to ten days after the preovulatory LH surge. This smear shows intermediate cells (i) and leucocytes (l).



Figure 7.15:
Vaginoscopic view during metestrus. The profiles are rounded and there is a patchwork of red and white areas.

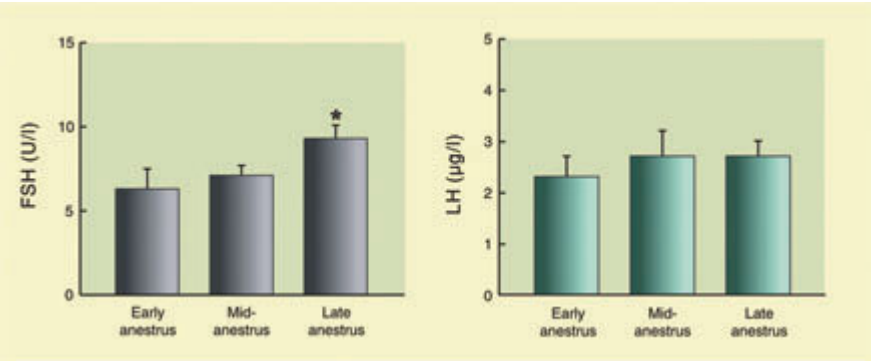


Figure 7.16:
Mean (± SEM) basal plasma concentrations of FSH and LH in six beagle bitches during early, mid-, and late anestrus.
* Significantly different from early anestrus. Progression of anestrus is associated with a significant rise in plasma FSH concentration but no significant change in plasma LH concentration. (Modified from Kooistra et al., 1999.)[3]

7.2.1.5 Anestrus

The time of onset of anestrus depends on the criterion used to define the end of the luteal phase, i.e., when mammary development subsides after two to three months, or when plasma progesterone concentration falls below 3 nmol/l, or when the influence of progesterone on the endometrium is no longer evident. In any case, the transition from the luteal phase to anestrus is gradual and varies considerably among bitches. The estrous cycle can begin at any time throughout the year and there appears to be little, if any, seasonal influence. Variation in mean interestrous intervals may be breed related and there may also be differences among strains within breeds. In the collie, for example, the mean interval is 36 weeks and in the German shepherd dog it is 20–22 weeks. The basenji and the Tibetan mastiff both have a single annual estrous cycle, which may be influenced by the photoperiod. Other environmental factors can also affect the interestrous interval; placing an anestrous bitch in close proximity to a bitch in estrus may cause the onset of proestrus to be advanced by several weeks. Moreover, bitches housed together often have synchronous cycles.

The endocrine changes that lead to termination of anestrus, and thus to the start of a new estrous cycle, are not completely understood in the bitch. The increase in basal plasma FSH concentration which occurs during the progression of anestrus is critical in the initiation of folliculogenesis (fig. 7.16).[3,21] The progression from early to late anestrus is also characterized by a greater number and greater amplitude of gonadotropin-releasing hormone (GnRH) pulses.[22] In addition, there is increased pituitary sensitivity to GnRH and increased ovarian responsiveness to gonadotropins from early to late anestrus[23,24] and there is increased LH pulsatility shortly before the onset of proestrus.[3,25,26] There is some evidence that factors that decrease opioidergic activity promote LH release and the termination of anestrus.[25] Finally, during the course of anestrus in the bitch, there is an increase in hypothalamic mRNA encoding for the estrogen receptor and in the expression of the gene encoding for P450 aromatase, which catalyzes estrogen biosynthesis.[27,28] Although there are sporadic elevations, plasma estradiol concentration is usually low and does not begin to rise until late anestrus.

Apart from changes in the hypothalamic-pituitary-ovarian axis, there is involvement of dopaminergic influences in the initiation of a new follicular phase in the bitch. Administration of dopamine-2 agonists, such as bromocriptine and cabergoline, shortens anestrus and is associated with a lowering of plasma prolactin concentration (fig. 7.17). Consequently, it has been suggested that the shortening of anestrus by dopamine agonists is the result of suppression of prolactin secretion, as prolactin may inhibit gonadotropin release.[29,30] However, although low dosage metergoline decreases prolactin release via a serotonin-antagonistic pathway, it does not shorten anestrus, indicating that it is not the decrease in plasma prolactin concentration but another dopamine-agonistic influence that is responsible for the transition to a new follicular phase.[31] Also, administration of bromocriptine in a dose too low to decrease plasma prolactin concentration nevertheless induces a premature new follicular phase (fig. 7.18).[32] Finally, under physiological conditions plasma prolactin concentration is low during anestrus and does not change during the transition from anestrus to the next follicular phase.[33]

Bromocriptine-induced shortening of anestrus is associated with a prompt rise in basal plasma FSH concentration without a concomitant increase in basal plasma LH concentration,[12] similar to what occurs during normal late anestrus (fig. 7.16). This further supports the notion that in the bitch an increase in the circulating plasma FSH concentration is a critical event in the initiation of ovarian folliculogenesis.[3,12]

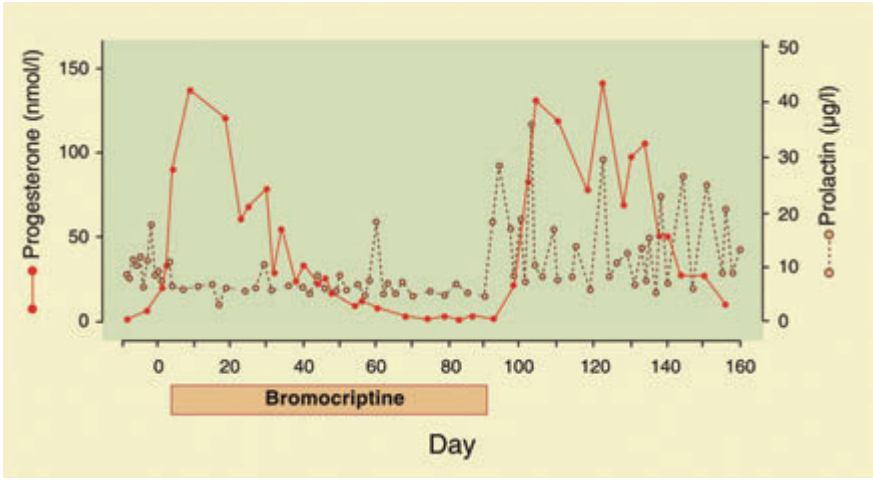


Figure 7.17:
Plasma concentrations of progesterone and prolactin in a bitch treated with the dopamine agonist bromocriptine (bar), from ovulation in the first estrous cycle to the onset of the next follicular phase. The luteal phase and especially anestrus are considerably shortened. (Modified from Okkens et al., 1985.)[29]

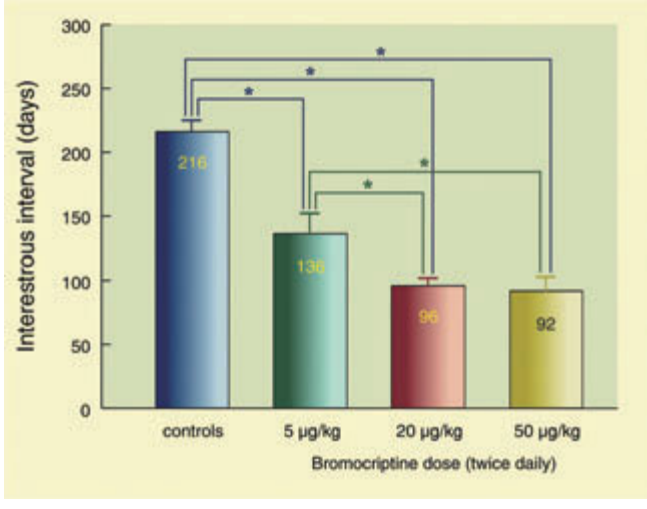


Figure 7.18:
Mean (±SEM) interestrous interval in control bitches and in bitches receiving the dopamine agonist bromocriptine in oral doses of 5 (n = 60), 20 (n = 6), and 50 (n = 8) µg/kg body weight twice daily starting 28 days after ovulation and continuing until the next ovulation. * Indicates significant difference. In the bitches receiving 5 µg/kg twice daily, the difference in mean plasma prolactin concentration before and during treatment was not significant but the interestrous interval was significantly shorter than that of the control bitches. In the bitches receiving 20 or 50 µg/kg twice daily, plasma prolactin concentration was significantly lower during treatment than before treatment. (Modified from Beijerink et al., 2003.) [32]

7.2.1.6 Pregnancy and parturition

The length of gestation varies greatly in dogs. In dogs of various breeds the mean gestational period was 62.0 days (n = 184) with a variation of 24 days (54–77).[34] The length of gestation and litter size were negatively correlated. In a beagle colony the mean gestational period was 65.3 days (n = 290), with a variation of 16 days (57–72).[35] The variation was, however, reduced to three days (64–66) (n = 54) when gestation was calculated as the interval from the preovulatory LH surge to parturition. In another study the length of gestation was calculated in bitches of six breeds (n = 113) that had been mated at a fixed time after ovulation.[36] The optimal time for mating was based on the rapid increase in plasma progesterone concentration, which strongly correlates with the preovulatory LH surge. The gestational period was 61.4 days, with a variation of 8 days (58–65). The variation in the length of gestation within any one of the six breeds was low, with a range of four to seven days, or even less when litters of one pup were excluded. There were one to 15 pups per litter, with a median of eight pups. The length of gestation correlated negatively with litter size for litters of 13 or fewer pups. However, within an individual breed the number of pups had no influence on the length of gestation. This study indicates that breed is a major determinant of the length of gestation in the bitch and that this is coupled to breed-related differences in litter size.[36]

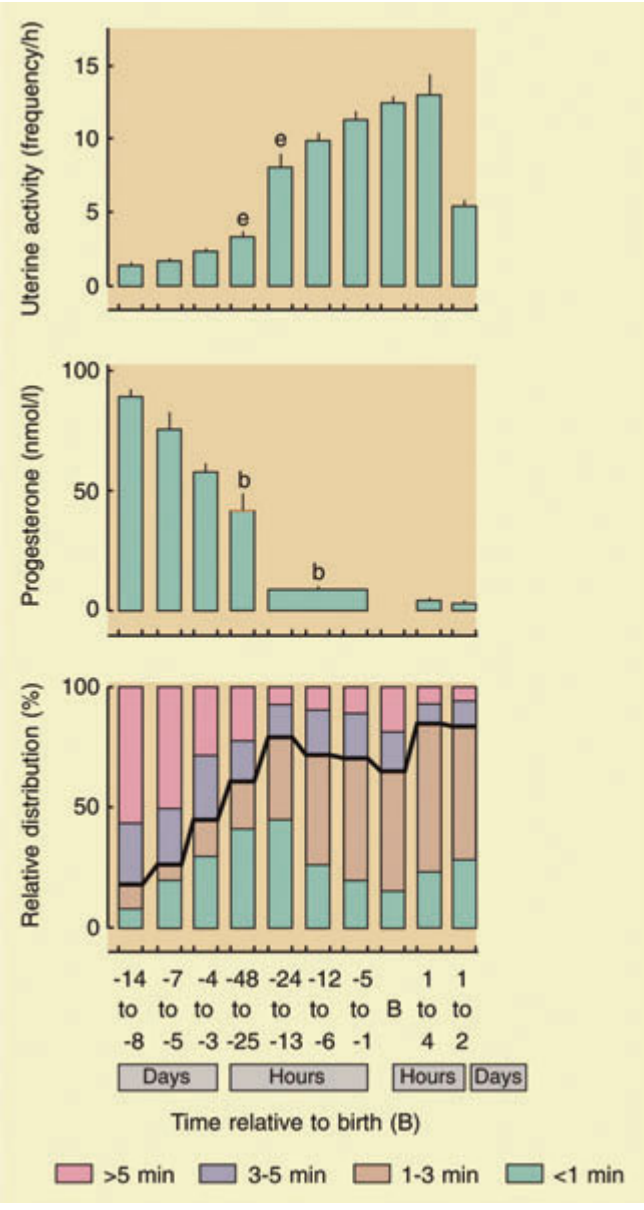


Figure 7.19:
Mean values for uterine activity (burst frequency/h), plasma progesterone concentration, and the relative distribution (%) of the duration of individual bursts of EMG activity for various periods around spontaneous parturition in five dogs. Differences between columns with a similar superscript are significant ($P < 0.001$). Birth (B) = period between birth of the first and the last pups. (Modified from Van Der Weyden et al., 1989.) [37]

Progesterone is the hormone responsible for the maintenance of pregnancy in the dog. Ovariectomy during pregnancy results in either resorption of the fetuses or abortion. Progesterone promotes endometrial gland growth, stimulates uterine secretions, promotes placental integrity, and inhibits uterine motility. Although progesterone is secreted by the corpora lutea, the plasma progesterone concentration is not overtly influenced by the number of corpora lutea.

In the bitch the duration of pregnancy is equal to or somewhat shorter than the luteal phase. During pregnancy the plasma hormone profiles are very similar to those described for the luteal phase of the estrous cycle, with the exception of relaxin, which circulates in elevated concentrations after three to four weeks of pregnancy, and the hormonal changes during the last days of pregnancy and during parturition. During pregnancy, progesterone concentration in plasma fluctuates in a manner similar to that during the estrous cycle, until it declines to a plateau at 16–48 nmol/l. It is maintained at this level for one to two weeks, then falls rapidly to 3–6 nmol/l just before parturition. The decrease in progesterone concentration is essential for the onset of parturition and is negatively correlated with a progressive qualitative change in the pattern of uterine activity (fig. 7.19).[37] During prepartum luteolysis and parturition, the plasma concentration of 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (PGFM), a fairly stable metabolite of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) originating from the fetal-placental unit, is high (fig. 7.20).[14,38] $PGF_{2\alpha}$ is important for prepartum luteolysis. Nevertheless, several injections of it must be given to induce parturition and even after induction a normal course of parturition is not certain.

The mean plasma cortisol concentration increases significantly prior to parturition.[14] No data are available on fetal and placental cortisol secretion in dogs. The prepartum increase in plasma cortisol concentration in the bitch is probably related to the physical and emotional stress caused by enhanced uterine activity and labor pains (fig. 7.21).[14,39]

Plasma prolactin concentration rises during pregnancy. During the rapid decrease in circulating progesterone concentration prior to parturition, there is a large, transient surge in prolactin secretion (fig. 7.21).[14,39] Just as it is in the estrous cycle, prolactin is a luteotropic factor. Suppression of prolactin secretion by dopamine agonists, such as bromocriptine and cabergoline, causes abortion in the second half of pregnancy.[40]

Plasma LH and FSH concentrations decrease between late gestation and the 30 h period prior to parturition. After parturition, plasma LH and FSH concentrations are lower than in the late gestational period.[14]

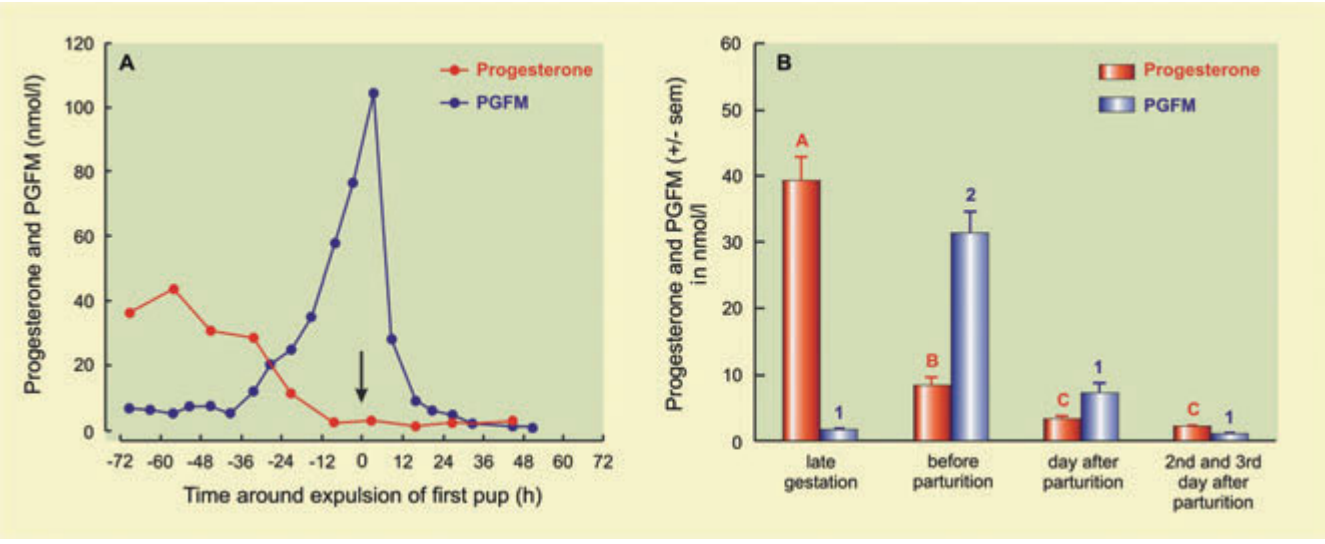


Figure 7.20:
(A) Plasma concentrations of progesterone and prostaglandin $F_{2\alpha}$ metabolite (PGFM) at 12 h (P4) and 6 h (PGFM) intervals around the time of expulsion of the first pup (t = 0) in a three-year-old beagle bitch.
(B) Mean (± SEM) plasma progesterone (red bars) and PGFM concentrations (blue bars) in six bitches during late gestation (days 54–58 of pregnancy), before parturition (30–0 h before expulsion of the first pup), the day after parturition (0–24 h after expulsion of the last pup), and the 2nd and 3rd days after parturition (24–72 h after expulsion of the last pup). Different superscripts A,B,C and 1,2 denote significant differences. (Modified from Baan et al., 2008.)[14]

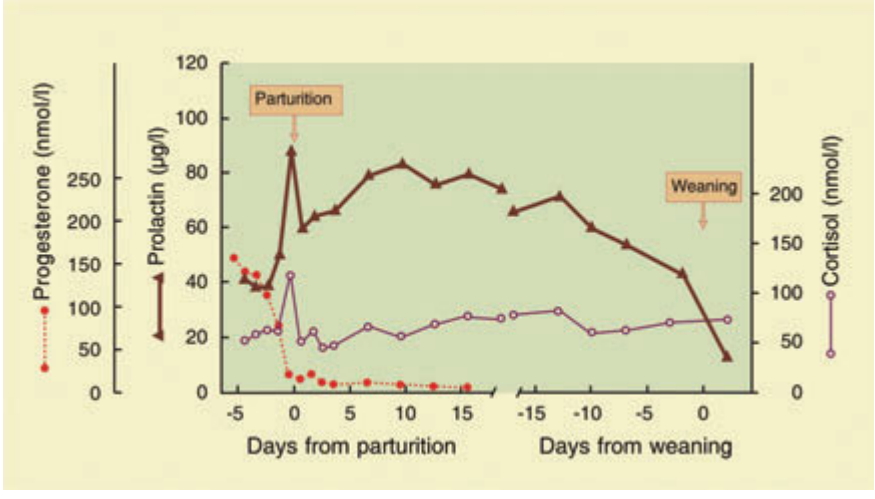


Figure 7.21:
Mean serum concentrations of progesterone, cortisol, and prolactin in the period around parturition and lactation in a group of six beagle bitches. (Modified from Concannon et al., 1978.)[39]

7.2.2 Estrous cycle, anestrus, pregnancy, and parturition in the cat

Puberty occurs at between four and 18 months of age in the queen and its onset is influenced by the season of the year. It often occurs when the hours of daylight are increasing. Physical condition is also an important factor, for puberty does not usually occur before body weight reaches about 2.5 kg. Puberty may occur earlier in short-haired breeds than in long-haired breeds.

Queens can go through several periods of estrus per season (seasonally polyestrous). Cats kept in a common household can become nonseasonal breeders as a result of night-time illumination.

Queens are induced ovulators. Copulation, vaginal stimulation, and administration of gonadotropin or GnRH analogues induce ovulation within 24–48 h.[41] It is likely that ovulation can also be induced by external stimuli such as stroking. Although considered to be induced ovulators, as many as 60 % of unmated female domestic cats ovulate without known external provocation.[42]

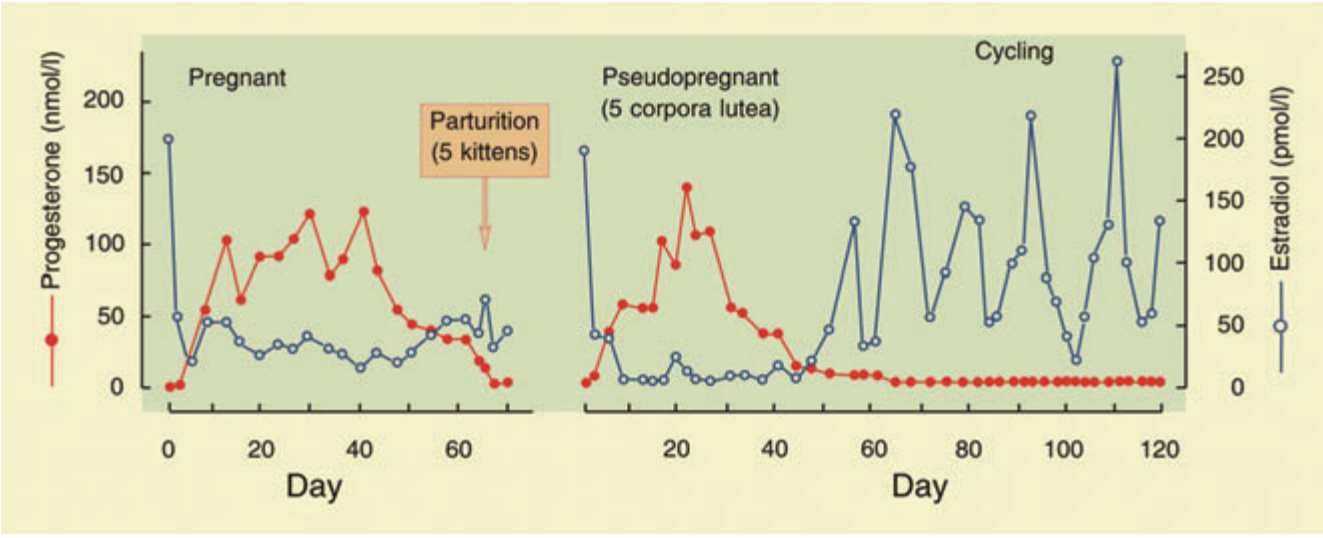


Figure 7.22:
Plasma estradiol and progesterone concentrations during pregnancy, pseudopregnancy, and polyestrus in the cat. (Modified from Verhage et al., 1976.)[43]

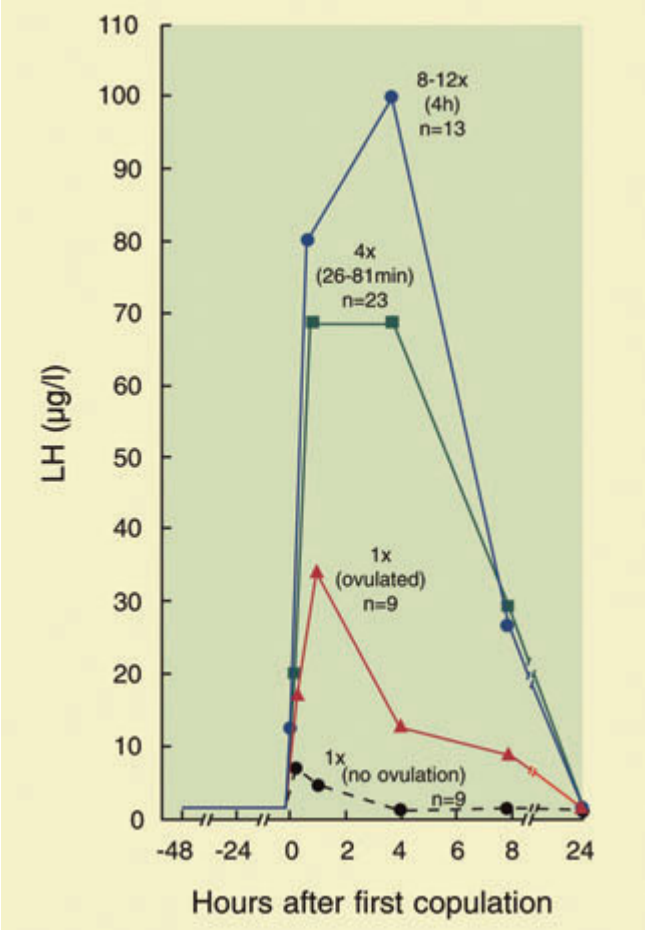


Figure 7.23:
Mean serum LH concentration in cats confirmed to have ovulated following one copulation (red line), four copulations within 26–81 min (green line), or 8–12 copulations during 4 h (blue line), and in cats which did not ovulate following a single copulation (black interrupted line). All copulations were on the third day of estrus. (Modified from Concannon et al., 1980.)[44]

7.2.2.1 Estrous cycle and anestrus

The stages of the estrous cycle of the queen include proestrus, estrus, postestrus, and metestrus (diestrus). Proestrus is characterized by rubbing the head and neck against objects but not permitting breeding by the male. It is observed in only a minority of the estrous cycles and lasts for about one to two days. Estrus, the phase in which mating is allowed, lasts seven to nine days. Estrus behavior includes crouching with the forequarters pressed to the ground and the pelvis elevated, rhythmic stamping of the hind legs, frequent vocalizing, and restlessness. Estrus occurs during maximal follicular activity and estradiol secretion; plasma estradiol concentration rises to 184–257 nmol/l and then decreases within five to seven days after copulation (fig. 7.22).[43] The external genitalia are slightly swollen and some clear secretion appears. The absence of cellular debris in the vaginal smear is the earliest sign of follicular activity. There is a distinct increase in anuclear cells and a slight increase in partially cornified superficial cells. Intermediate cells decrease during the follicular phase and parabasal cells are absent in the second half of the follicular phase.

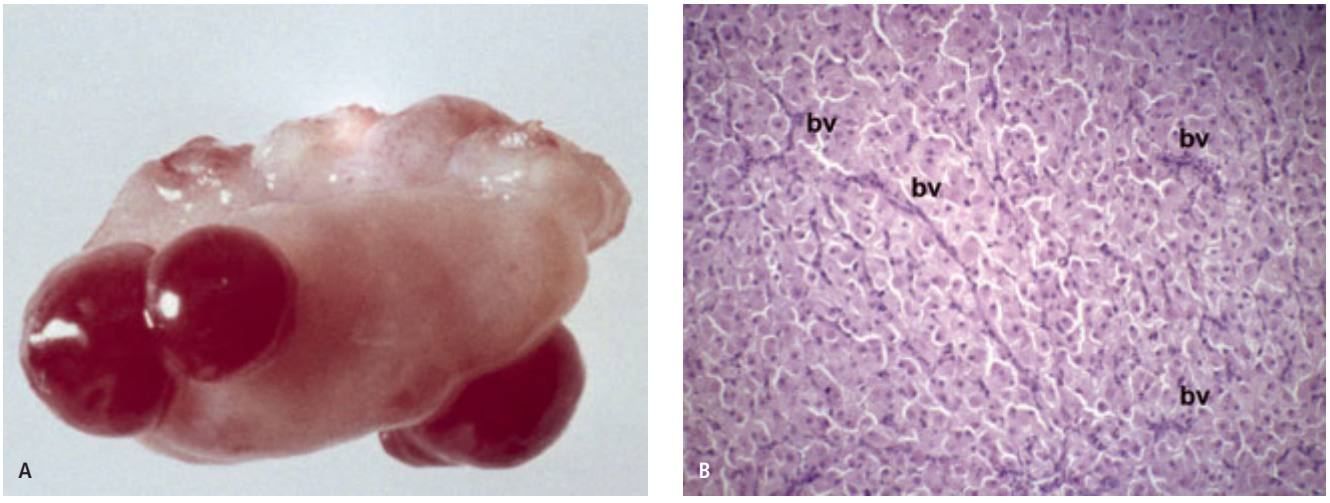


Figure 7.24:
(A) The ovary of a queen six days after mating, with luteal tissue called corpora rubra because of its red color.
(B) Microscopic section of a corpus luteum of a queen 21 days after mating. It consists primarily of large luteal cells and blood vessels (bv). (H&E stain, x475).

If breeding is permitted, LH release begins within minutes after copulation, peaks within 2–4 h, and returns to the basal level within 24 h. In the early follicular phase there may be a period of refractoriness to this copulation-induced LH release. A rise in plasma LH concentration does not always occur following a single mating, but the LH peak is higher and more prolonged when multiple matings are permitted (fig. 7.23).[44] The increase in LH release due to multiple matings is, however, not indefinite and the LH response declines after a certain number of matings. The duration of estrus appears to be similar in queens regardless of whether there is coitus with ovulation, coital contact without ovulation, or no coital contact. An estrus in which the queen has not been induced to ovulate is followed by a postestrus period with an average duration of eight to ten days, after which the next estrus begins. Plasma progesterone concentration is at its basal level during the postestrus period.

Ovulation usually occurs 24–48 h after copulation and the occurrence of the LH peak, but it can be delayed for up to 90 h. Ovulation is followed by pregnancy or a luteal phase without pregnancy (called »pseudopregnancy«). Pseudopregnancy in the queen does not give rise to signs and symptoms and is thus not comparable to that in the bitch (chapter 2.2.5). In both pregnancy and pseudopregnancy, plasma progesterone concentration begins to rise 24–48 h after ovulation, accompanied by the development of luteal tissue. The luteal tissue is initially red and therefore sometimes referred to as corpora rubra, but it subsequently develops into yellow corpora lutea (fig. 7.24). The progesterone-dominated phase lasts about 38 days in the pseudopregnant queen and approximately 60 days in the pregnant queen. Plasma progesterone concentrations in pseudopregnancy and pregnancy are similar until day 21. Thereafter plasma progesterone concentration is lower in pseudopregnancy than in pregnancy (fig. 7.22). The interestrous interval for a pseudopregnant queen is approximately seven weeks. During the progesterone-dominated phase, particularly at the end of this phase, there can be follicle growth (and regression) which causes elevations in plasma estradiol concentration.

Anestrus is a period without cycle activity. Plasma estradiol and progesterone concentrations are at baseline levels. In the northern hemisphere this phase occurs during late autumn and the onset of winter (October, November, December) in queens exposed to natural daylight.

Photoperiods influence the reproductive processes via the pineal gland and its principal hormone, melatonin, which affects the hypothalamic-pituitary-ovarian axis. Plasma melatonin and prolactin concentrations change congruently with photoperiod changes and are highest during periods of darkness (fig. 7.25).[45] Folliculogenesis and estradiol secretion are stimulated during days with 14 h of light, leading to an estrous cycle frequency of two per month.[46] Estrus can be induced with as little as 12 h of light if a social stimulus such as the presence of a tomcat or a queen in estrus is introduced three weeks after an increase to 12 h of light.[47] Estrous activity ceases immediately and estradiol concentration decreases rapidly after a change from 14 h to 8 h of light (fig. 7.26). Although gonadotropin secretion may be decreased during a short light period, continuous exposure to light does not ap-

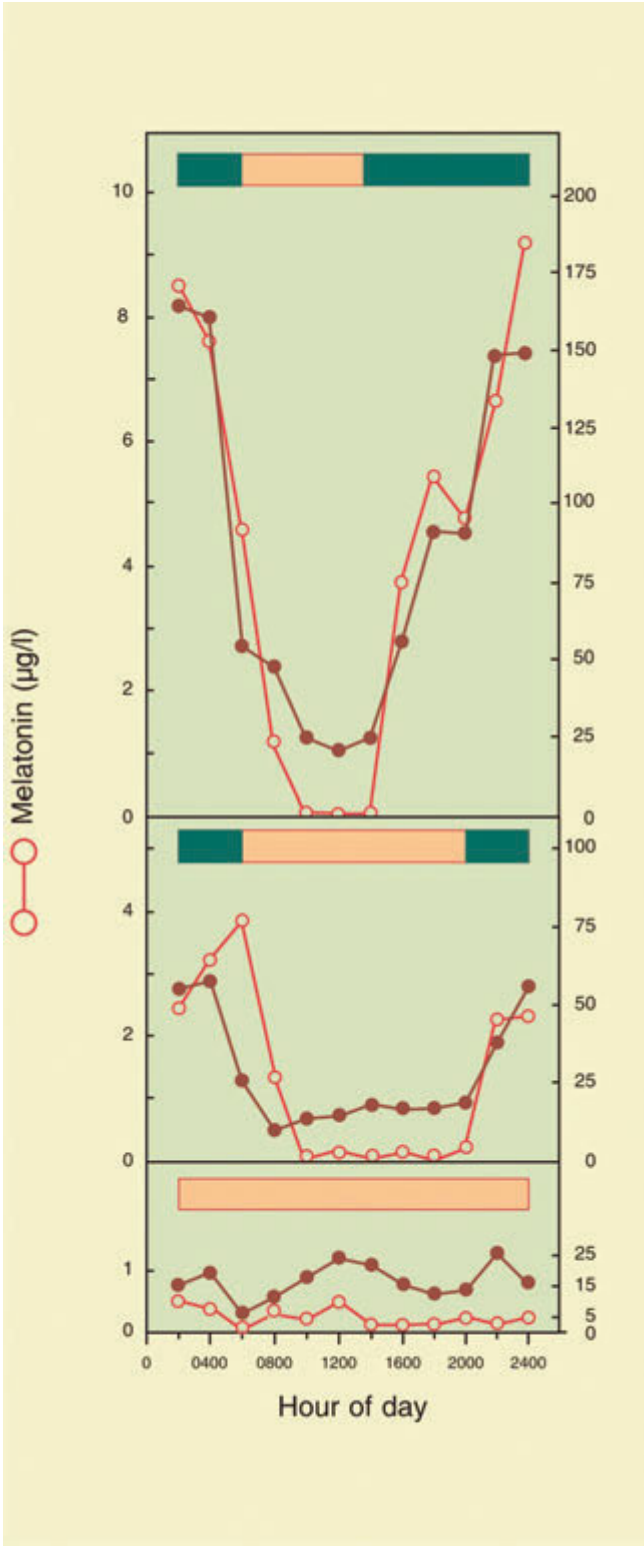


Figure 7.25:
The effects of three different photoperiods on mean plasma concentrations of melatonin and prolactin in four cats, measured at 2 h intervals. Horizontal bars indicate the timing of each lighting regimen; lower panel: 24 h of light, middle panel 14 h of light and 10 h of darkness; upper panel 8 h of light and 16 h of darkness. (Modified from Leyva et al., 1984.)[45]

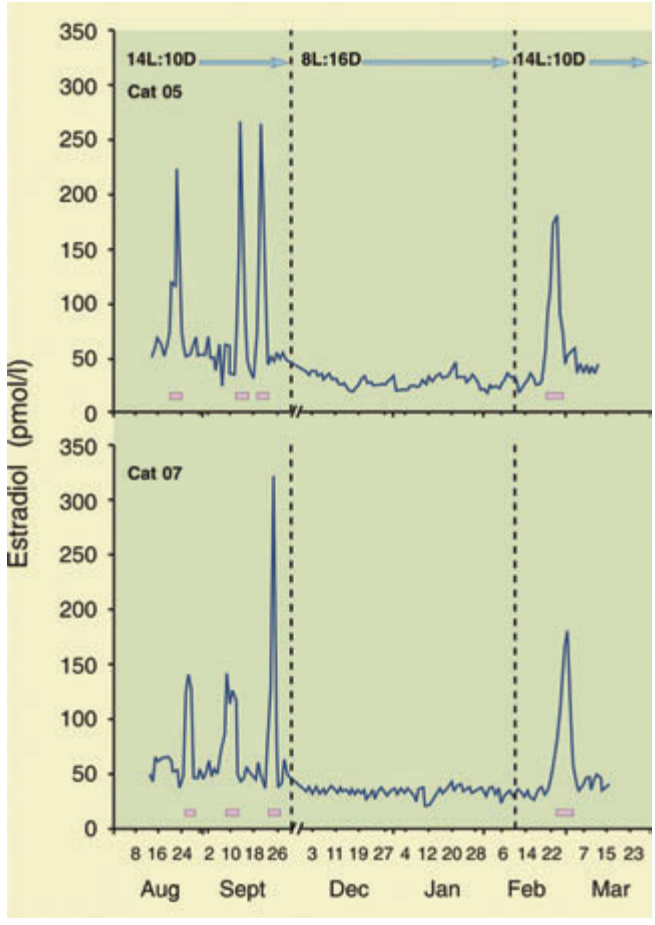


Figure 7.26:
Plasma estradiol concentration in two cats during a photoperiod regimen of 14 h of light, then 8 h of light, and then 14 h of light again. The purple horizontal bars indicate periods of sexual receptivity. (Modified from Leyva et al., 1989.)[46]

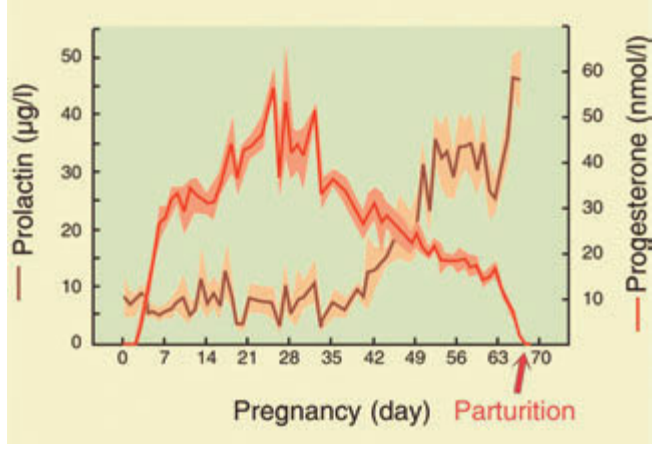


Figure 7.27:
Mean (± SEM) plasma concentrations of prolactin and progesterone in eight queens during gestation. Day 0 is the day of copulation. (Modified from Banks et al., 1983.) [49]

pear to be optimal. Cycle frequency decreases to one per month with exposure to 24 h of light. Estradiol secretion during estrus under exposure to 24 h of light appears to be approximately twice that observed under exposure to 14 h of light, while the number of large antral follicles doubles about 45 days after the onset of continuous light. This may cause a depletion of the tertiary follicle population, after which a long interval is necessary for tertiary follicle restoration.[46]

7.2.2.2 Pregnancy and parturition

In the cat, progesterone, produced throughout the entire pregnancy by the corpora lutea, is probably responsible for maintaining pregnancy. The placenta either does not secrete progesterone or secretes it in amounts insufficient to maintain pregnancy. Plasma progesterone concentration increases continuously through days 25–30, then slowly declines during the second half of pregnancy (fig. 7.27).[48]

It is not yet clear why there is a difference between pseudopregnant and pregnant animals in the functional activity of corpora lutea. Pregnancy involves pregnancy-specific secretion of luteotropic hormones of placental or pituitary origin, of which prolactin appears to be important. Prolactin secretion in the pregnant queen begins to increase around day 35, reaches a plateau at about day 50, and increases again just before delivery (fig. 7.27).[49] If prolactin secretion is suppressed by treatment with the dopamine agonist cabergoline, progesterone secretion decreases and abortion may follow. Prolactin secretion does not increase in the pseudopregnant queen, which may be the cause of the early regression of the corpora lutea.

Fertilization of oocytes by spermatozoa of different males (superfecundation) is common in domestic cats. Fertilization and subsequent development of an ovum when a fetus is already present in the uterus (superfetation) has, however, never been proved. The explanation for fetuses of different ages could be arrested development.

The first estrus after parturition can be expected within one to 21 weeks. Little is known about fertility during this estrus, but if it occurs during lactation, which is not uncommon, fertility may be lower than normal.

7.3 Medical pregnancy termination

For many decades unwanted pregnancy in dogs has been avoided early in gestation by administering relatively large doses of estrogens. This prolongs the transport time in the oviduct and tightens the utero-tubular junction, resulting in failure of implantation and hence embryonic death.[50] However, the use of estrogens can result in side effects such as cystic endometrial hyperplasia, pyometra, and bone marrow suppression,[51] although using low doses of estradiol benzoate (three, five and seven days after mating) decreases the incidence of these adverse effects.[52]

Maintenance of pregnancy in the bitch depends on ovarian secretion of progesterone by the corpus luteum throughout gestation (chapter 7.2.1). During the second part of the luteal phase, luteotropic factors from the pituitary, such as prolactin, are essential for maintenance of the corpus luteum.[8,53] Consequently, unwanted pregnancy in dogs can be terminated by pharmacological agents that suppress prolactin secretion (during midgestation) or interfere with the synthesis or action of progesterone. The use of many of the abortifacients is accompanied by unwanted and sometimes severe side effects.

Repeated administration of prostaglandin $F_{2\alpha}$ or its analogues during midterm pregnancy in the bitch results in luteolysis.[54] The narrow margin between a lethal dose (LD_{50}) and a therapeutic dose, side effects (vomiting, diarrhea, hyperpnea, and ataxia), and the need for repeated administration are important factors limiting the use of prostaglandins in veterinary practice. Dopamine agonists such as cabergoline, which is better tolerated than bromocriptine, cause luteolysis by suppressing pituitary secretion of prolactin. Reports on the efficacy of the dopamine agonists differ, depending on the dose and the day of pregnancy on which administration is begun. The combined use of cabergoline and prostaglandins has also been reported.[55] When used in combination, they are generally effective at lower doses than with single administration and there are fewer side effects.

Because of the undesirable side effects of the above mentioned drugs, attention has been given to agents that prevent the action of progesterone, i.e., progesterone receptor antagonists. Progesterone receptor blockers (antiprogestins), such as mifepristone, registered for use in humans, and aglepristone, have a chemical structure related to that of progesterone, but they carry a **p**-(dimethylamino) phenyl group at the 11β-position of the steroidal skeleton. Antiprogestins reversibly bind to the progesterone receptor, thereby preventing endogenous progesterone from occupying its binding site, which limits its biological activity. Aglepristone has a binding affinity for the progesterone receptor that is probably three times greater than that of the native hormone. The mean residence time for a single administration of 20 mg/kg body weight or 10 mg/kg body weight administered twice with a 24 h interval is six days. This rather long mean residence time is due to both slow absorption from the injection site and slow excretion. Around 60 % of the administered dose is excreted within the first 10 days and a total of around 80 % in 24 days. Excretion is essentially via the feces. Antiprogestins may also interact with the glucocorticoid receptor, different antiprogestins having different binding affinities for the progesterone and glucocorticoid receptors.

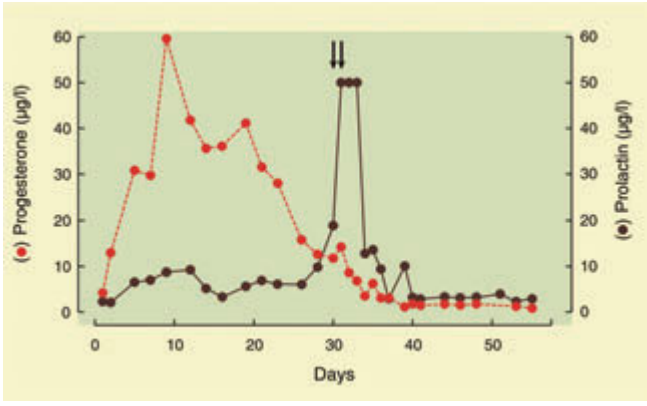


Figure 7.28:
Plasma concentrations of prolactin and progesterone in a four-year-old beagle bitch, from the day of ovulation (Day 1) to the end of the luteal phase. On Days 30 and 31 (arrows), the bitch was treated with aglepristone in a subcutaneous dose of 10 mg/kg body weight. (Modified from Galac et al., 2000.)[57]

Aglepristone was effective in terminating pregnancy in 66 of 69 dogs in which pregnancy had been confirmed by ultrasonography.[56] In another study, all pregnancies (n = 6) confirmed by ultrasonography were terminated within four to seven days after administration of 10 mg aglepristone per kg body weight on two consecutive days between 27 and 31 days following mating.[57] Only a small amount of mucoid vaginal discharge was observed and ultrasonographic examination indicated that pregnancy was terminated by abortion and not by fetal resorption.

Plasma progesterone concentration does not change significantly during aglepristone treatment and pregnancy is terminated despite a high concentration.[57,58] After aglepristone treatment in midgestation, plasma progesterone declines to less than 3 nmol/l within eight to 34 days,[57] leading to a significantly shorter luteal phase than in control dogs. This premature luteolysis is probably caused by increased $PGF_{2\alpha}$ secretion. In line with this supposition, increases in circulating concentrations of PGFM, the main metabolite of $PGF_{2\alpha}$, have been reported after pregnancy termination by the progesterone receptor blocker mifepristone.[59]

Plasma prolactin concentration increases within 24 h after aglepristone treatment in midgestation and returns to basal levels in two to four days (fig. 7.28).[57] This increase in plasma prolactin concentration is probably due to occupation of the central progesterone receptors by the progesterone receptor blocker, which mimics a sudden decline in progesterone concentration. A similar increase in plasma prolactin concentration is observed after ovariectomy in the luteal phase. Consistent with the substantial increase in plasma prolactin concentration, symptoms of pseudopregnancy, including moderate anorexia and lethargy, have been reported in some of the dogs treated with aglepristone in midgestation.

Shortening of the interestrous interval, due to shortening of both the luteal phase and anestrus, is commonly observed after aglepristone treatment in midgestation.[57,58] The interestrous interval is also shortened when aglepristone is administered either to bitches in early gestation or to nonpregnant bitches in the first half of the luteal phase. In these cases only anestrus is shortened, not the luteal phase.[58,60]

It can be concluded that aglepristone is suitable for pregnancy termination in the dog. Mucoid vaginal discharge is the only gynecologic sign if aglepristone is administered around day 28 of pregnancy; no other symptoms nor signs of parturition are observed during this stage of gestation. However, if the drug is administered after day 40 of gestation, signs of parturition such as uterine contractions and straining may be observed. According to the instructions of the manufacturer, aglepristone can be used for abortion until day 45 of pregnancy. Day 45 has probably been chosen because thereafter expulsion is a premature parturition rather than an abortion.

There remains the question whether it is advisable to treat unintentionally mated bitches just after mating or when pregnancy has been diagnosed unequivocally, around day 26. A good reason to treat only after pregnancy confirmation is the fact that after a misalliance less than 40 % of bitches become pregnant[61] and thus early treatment will result in 60 % being treated unnecessarily.

Aglepristone is also useful for pregnancy termination in the queen, although its efficacy in the queen seems to be less than in the dog.[62] The advised treatment for pregnancy termination in queens is 15 mg aglepristone per kg body weight on two consecutive days. It is probably better not to administer aglepristone in the first half of pregnancy, because of the risk for the development of endometritis. When administered in the second half of pregnancy, there should be careful control to confirm the expected abortion. Observations of pregnant cats treated with aglepristone for fibroadenomatous hyperplasia of mammary gland tissue indicate that there is a risk for endometritis, probably due to incomplete abortion.[63] If abortion is not complete, oxytocin should be given and its effect monitored.

Because not all bitches and queens abort completely after one treatment, examination by palpation and ultrasonography after one week is necessary. After repeated administration of aglepristone, several examinations are necessary for possible incomplete abortion or ongoing pregnancy. In addition, abortion in the second half of pregnancy may cause serious obstetrical problems, if not properly guided.



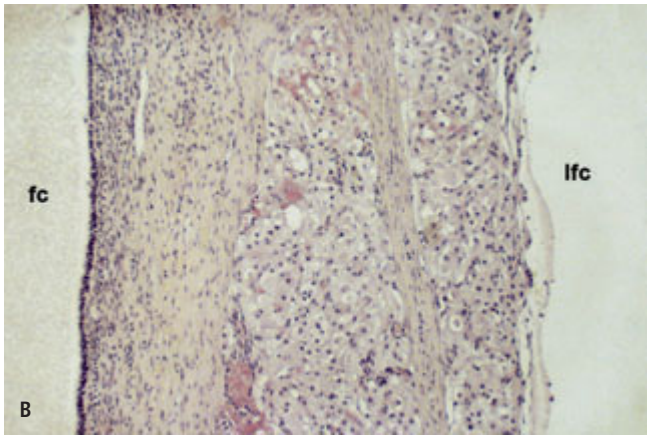


Figure 7.29:
(A) Follicular cyst (fc) and luteinized follicular cysts (lfc) in a four-year-old bitch with shortened interestrous intervals and persistent estrus symptoms. During these estrus periods the measured plasma progesterone concentration did not reach levels normally observed at the time of ovulation.
(B) Close-up, showing the wall of the follicular cyst (fc) and the wall of a luteinized follicular cyst (lfc). Note the luteinized cells bordering the luteinized follicular cyst. (H&E stain).

7.4 Induction of parturition

The progesterone receptor blocker aglepristone is an effective drug for induction of parturition in the bitch. In a study of this the course of parturition, pup survival, and growth rates were recorded in six bitches that received aglepristone and in six control bitches.[64] Aglepristone (30 mg/kg body weight) was administered on day 58 of pregnancy. If parturition did not proceed a standard obstetric protocol was employed. Expulsion of the first pup occurred between 32 and 56 h after treatment with aglepristone, at which time plasma progesterone concentration was still elevated. The length of gestation in the bitches in which parturition was induced (59.5 ± 0.2 days) was significantly shorter than in those that whelped spontaneously (62.2 ± 0.5 days). The duration of the expulsion phase, the intervals between pups, the number stillborn, and the number of clinical interventions needed during parturition did not differ significantly between the two groups. Pup survival and mean birth weights also did not differ significantly between the two groups and aglepristone treatment had no significant influence on growth rates.

Parturition has also been induced by aglepristone at a lower dose (15 mg/kg body weight), followed after 24 h by administration of either the $PGF_{2\alpha}$ analogue alfaprostol (0.08 mg/kg body weight) of or by oxytocin (0.15 IU per kg body weight) every 2 h until expulsion of the last pup.[65] In the latter study, the bitches whelped within 27 to 38 h after treatment was started.

7.5 Persistent estrus

The bitch is considered to have a persistent estrus if ovulation has not occurred within 25–30 days from the onset of proestrus, while estrus symptoms, such as sanguineous discharge and estrus behavior, are still present. In addition, the vaginal smear contains a majority of superficial cells. Continuous or persistent estrus can also occur in the queen.

Pathogenesis

Ovarian cysts and ovarian tumors can cause persistent estrus in dogs and cats. Ovarian cysts and symptoms of persistent estrus are known to occur in bitches that have undergone incomplete ovariectomy. Cystic follicles and luteinized follicular cysts may synthesize and secrete estrogens and progesterone, the latter depending on the degree of luteinization (fig. 7.29). Follicles normally undergo preovulatory luteinization, after which ovulation occurs and corpora lutea are formed. Luteinized follicular cysts, however, fail to ovulate.

There is probably a difference in the pathogenesis of persistent estrus between young dogs during their first and second estrous cycle and older dogs. In young dogs, persistent estrus is not uncommon and is probably the result of a failure of folliculogenesis and subsequent ovulation. This usually occurs without the formation of cysts, as opposed to older dogs in which persistent estrus is commonly associated with cysts.[66]

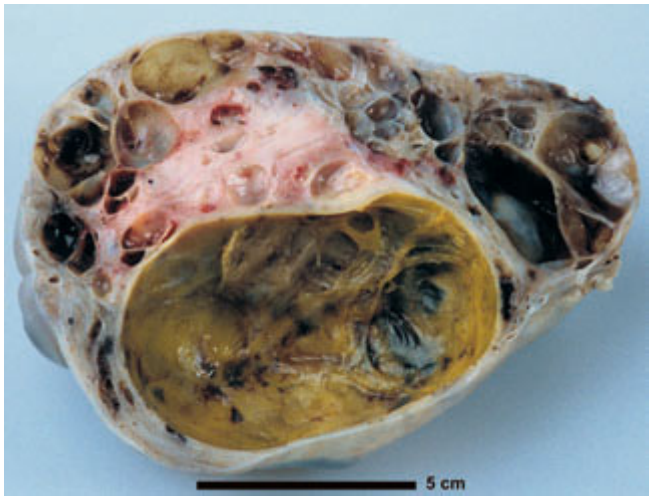


Figure 7.30:
Granulosa cell tumor of a nine-year-old Belgian shepherd dog that had estrus behavior for four months. The plasma concentrations of progesterone and estradiol-17β were 7 nmol/l and 270 pmol/l, respectively. The estradiol-17β concentration in the cyst fluid was 1195 pmol/l.

Young dogs generally respond well to treatment and luteinization follows, and a normal follicular phase and ovulation can be expected during their next cycle. In contrast, the problem in older dogs is often recurrent. Ovarian follicular cysts producing estrogens are common in queens. They may arise from mature or atretic follicles and their occurrence may increase with age.

Functional, hormone-producing, ovarian tumors, which frequently originate from sex cord stroma, are the other important cause of persistent estrus (fig. 7.30). They occur mainly in older dogs and cats, but are sometimes observed in young bitches or in bitches with ovarian tissue left in situ as a result of incomplete ovariectomy. This functional tumor is usually a granulosa cell tumor.

Furthermore, estrogens administered to terminate an unwanted pregnancy occasionally cause persistent estrus, possibly by inducing ovarian cysts. Rarely, liver disease is the cause of persistent estrus, supposedly because of defective hepatic metabolism of reproductive steroid hormones.[66]

Diagnosis

The diagnosis is based on the persistence of sanguineous discharge, vaginal cornification, estrus behavior, vaginoscopic findings, and the plasma concentrations of progesterone and estradiol. Plasma progesterone concentration is lower than 16 nmol/l, but plasma estradiol concentration is not consistently elevated. A history of incomplete ovariectomy or hormone therapy can contribute to the diagnosis. Abdominal palpation can be helpful in ruling out a tumor, although the size and consistency of these tumors vary considerably. Ultrasonography is very valuable in diagnosing ovarian cysts and cystic tumors (fig. 7.31). When interpreting ultrasonographic findings it is important to realize that in the dog the morphology of cysts may mimic that of vesicular follicles and antra in young developing corpora lutea. Computed tomography provides better spatial resolution than ultrasonography, and is easier to perform and to interpret (fig. 7.32).

Differential diagnosis

A split heat is a heat that stops before ovulation and starts again after an interval of days to weeks. A split heat may be difficult to distinguish from persistent estrus if the interval is very short or unobserved.

Therapy

Cysts can be treated by giving GnRH, such as gonadorelin or buserelin (repeated subcutaneous doses of 0.1 ml/kg body weight), but this does not always resolve the problem. If luteinization of cystic follicles or further luteinization of luteinized cysts takes place, estrus will stop, plasma progesterone concentration will increase, and the vaginal smear will contain mainly intermediate and parabasal cells and leukocytes. If the problem persists estrus can be stopped by oral administration of low, once daily, doses of megestrol acetate (first week: 0.1 mg/kg body weight; second week: 0.05 mg/kg body weight). Ovarian tumors should be removed.

7.6 Split heat

As mentioned above, a split heat is a heat which stops before ovulation and starts again after days or weeks. The vaginal discharge changes from red to brown and the vaginal smear contains intermediate cells, parabasal cells, and leukocytes. Vaginoscopy reveals that the swelling of the vaginal mucosal folds is diminishing. Split heat is observed fairly often in both young and older bitches and especially in certain breeds, such as the German shepherd dog. It is probably caused by prematurely regressing follicles. Ovulation usually occurs if proestrus returns. Treatment is usually not necessary but close monitoring of the estrous cycle is essential to determine the appropriate mating period.

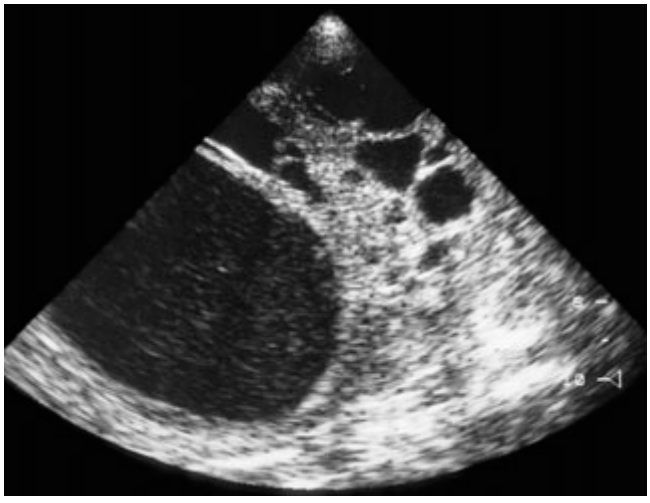


Figure 7.31:
Longitudinal ultrasonogram of the abdomen of the bitch of fig. 7.30, revealing small and large cysts in the tumor.

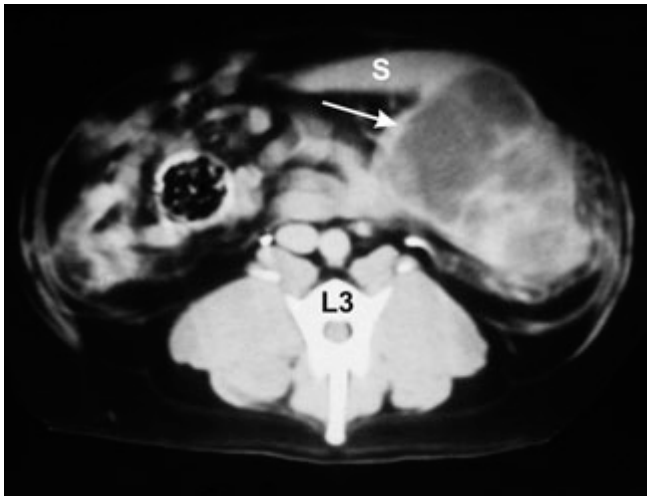


Figure 7.32:
Contrast-enhanced CT image of the abdomen at the level of the third lumbar vertebra (L3) of a four-year-old collie with persistent estrus. Originating from the left ovary there is a large, cystic, space-occupying lesion (arrow), in close contact with the ventral extremity of the spleen (S).

7.7 Hypoluteoidism

Progesterone, secreted by the corpora lutea, is necessary for maintenance of pregnancy. Although hypoluteoidism can lead to infertility, it appears to be rare. For the diagnosis plasma progesterone concentration must be measured accurately by radioimmunoassay because the commercial ELISA kits are not reliable in the critical range of 3–16 nmol/l. Furthermore, it is essential that any progestagen used to prevent abortion is not detected by the radioimmunoassay for progesterone. A 2.5-year-old Bernese mountain dog was reported to have had two previous pregnancies ending in abortion around day 50 of pregnancy. On day 42, ultrasonography revealed living fetuses but plasma progesterone concentration had decreased to 8.3 nmol/l, which is just above the threshold necessary to maintain a vital pregnancy. Oral treatment with medroxyprogesterone acetate was started and continued until day 58, in order to prevent abortion due to progesterone deficiency. During medroxyprogesterone acetate treatment the plasma concentration of endogenous progesterone decreased further. On day 59 a cesarean section was performed because of dystocia, and four living and one dead pup were delivered.[67] Because of the potential side effects of progestagens, such as CEH-pyometra (chapter 7.11), bitches should not be treated with progestagens after ovulation unless primary hypoluteoidism has been proved.

7.8 Prolonged anestrus

A bitch which has not been in estrus by 18–24 months of age is considered to have primary anestrus. A major cause of primary anestrus is true hermaphroditism or pseudohermaphroditism (chapter 6.2.3). If a bitch has been in estrus and its interestrous interval is now more than twelve months or twice its usual interestrous interval, this anestrus is considered to be prolonged. One of the reasons for prolonged anestrus is hypothyroidism. However, hypothyroidism may also be associated with prolonged or abbreviated proestrus or mild estrus symptoms. Prolonged anestrus may also be induced by drugs such as progestagens or glucocorticoids. In the latter case and in spontaneous hypercortisolism anestrus is probably the result of a decreased circulating level of gonadotropic hormones.[68] Lymphocytic oophoritis, most likely an immune-mediated disorder, also has been reported to cause prolonged anestrus.[69] On the other hand, oophoritis has also been described in a bitch which had estrous cycles with very short luteal phases and short interestrous intervals.[70] The duration and frequency of estrous cycles become more irregular with ageing and the interestrous interval increases after eight years of age. An apparent prolonged anestrus can also be the result of a silent estrus or the owner's failure to detect estrus.

Diagnosis

A general physical and gynecological examination[71] should be performed, after which one or more of the following tests may be useful:

- Measurement of the plasma progesterone concentration. If $>$ 3 nmol/l, the bitch was probably in estrus and either the owner did not notice it or the bitch had a silent estrus.

- Measurement of plasma concentrations of thyroxine and TSH. If the results are inconclusive and hypothyroidism is still suspected, thyroid scintigraphy and/or a TSH-stimulation test may be performed (chapter 3.3.1).
- Measurement of plasma concentrations of LH and FSH. High FSH and LH values indicate gonadal absence (aplasia, ovariectomy) or failure. Although not essential for diagnosis, it is of interest that an elevated LH, but not of FSH, can be further stimulated with GnRH.[72]
- A GnRH-stimulation test with measurement of plasma testosterone concentrations (chapter 12.5.1): This test can confirm male pseudohermaphroditism or true hermaphroditism in a phenotypically female dog.
- Determination of the karyotype. Abnormalities in sexual differentiation may present as primary anestrus in phenotypically female dogs. The abnormalities may include the presence of abnormal complements of sex chromosomes as well as sex chromosome complements that do not match the animal's phenotype (chapter 6.2.1).[69]
- Laparoscopy or laparotomy, to examine the genital tract and collect tissue for histological examination.
- Abdominal ultrasonography usually does not reveal the cause of prolonged anestrus.

Treatment

Treatment depends on the cause of the prolonged anestrus. Hypothyroidism is treated with l-thyroxine (chapter 3.3.1). If the animal has silent heats, estrus can be detected by cytological examinations at regular intervals and close visual examination of the vulva. The optimal mating period can be determined by measuring progesterone. In most cases of true hermaphroditism or pseudohermaphroditism, treatment is not possible. If no specific cause for the prolonged anestrus is found, estrus may be induced (chapter 7.9).

7.9 Estrus induction

Induction of a follicular phase can be achieved by several methods, including the use of synthetic estrogens, GnRH agonists, exogenous gonadotropins (LH, FSH, human Chorionic Gonadotropin, equine Chorionic Gonadotropin, and human Menopausal Gonadotropin), dopamine agonists, and opiate antagonists (naloxone). These methods vary widely in their efficacy of inducing estrus as well as the resulting fertility of the induced estrus.[41] In a study in which GnRH was administered in pulses of 15–500 ng/kg body weight every 90 min for seven to nine days to 36 anestrous bitches, treatment resulted in proestrus in 26, estrus in 20, ovulation in 16, and pregnancy in 12 bitches. Efficacy was dose-dependent.[73] A fertile estrus could also be induced by administering a timed-release GnRH agonist, followed by a GnRH analogue on the first day of induced estrus.[74] Anestrus in the bitch can also be terminated by administering LH. In one study proestrus was induced by porcine LH in all of 16 bitches, of which twelve came into estrus and seven ovulated. Those in which proestrus occurred but not estrus had all been treated in early anestrus.[75] The rapid increase of plasma estradiol concentration that is observed after LH treatment suggests that an increase in follicular steroidogenesis is a primary effect of LH. The insufficient response to porcine LH in bitches in early anestrus may be due to lack of FSH or follicular FSH receptors in this stage of anestrus. Follicular aromatase in rats and most other species studied appears to be primarily under up-regulation control by FSH.[76]

Shortening of anestrus and thus stimulation of folliculogenesis can also be induced by administration of dopamine agonists such as bromocriptine and cabergoline.[25,29,32] The result of treatment with dopamine agonists depends on the dose of the administered dopamine agonist and the period in the estrous cycle or anestrus in which treatment is started. When bromocriptine was started during the luteal phase, in an oral dose of 20 mg/kg twice daily, the mean interestrous interval was shortened from 216 to 96 days (fig. 7.18).[32] When it was started in the same dose during anestrus, 100 days after ovulation, the next proestrus appeared after a mean interval of about 45 days.[77] The fertility of estrus initiated by bromocriptine treatment appears to be normal.

7.10 Estrus prevention

Estrus can be prevented medically or surgically. Ovariectomy has certain advantages. It is effective after a single procedure. It considerably lowers the risk for mammary cancer if performed before or after the first luteal phase but in any case before about 2.5 years of age. It also prevents the development of pyometra and progesterone-induced GH excess (chapter 2.2.4.2). There are, however, several disadvantages, such as the risk of anesthesia and surgery, and the irreversibility of the procedure. There are also possibile side effects, such as urinary incontinence or unwanted changes in the hair coat. Early-age gonadectomy is associated with an increased incidence of cystitis.[78] The risk of urinary incontinence is greater if the intervention is carried out prior to the first estrus (see also chapter 8.2).[78] Urinary incontinence occurs mainly in dogs of large breeds. The boxer, Doberman, Bouvier des Flandres, giant schnauzer, Irish setter, Old English sheepdog, Weimaraner, and Rottweiler appear to be especially at risk for developing urinary incontinence.

In the cat ovariectomy is the treatment of choice. It does not lead to urinary incontinence. Furthermore, endogenous progesterone and progestagens are, as in the bitch, tumorigenic and mammary tumors in the cat are quite often malignant.[79,80]

Medical prevention of estrus can be accomplished with several types of drugs, not all of which can be used in every country. Progestagens are the most important among them but androgens can also be used, primarily for short-term prevention.

Androgens probably inhibit pituitary gonadotropin release, thus preventing follicular development. One orally administered synthetic androgen, mibolerone, is also anabolic. It has no progestational or estrogenic activity and its advantage therefore lies in its minimal influence on the endometrium. Thickening of the myometrium may occur but only when excessive doses are used. Although subsequent fertility in bitches treated with this drug appears to be good, it is not recommended in the U.S.A. for use in breeding bitches or in bitches prior to the first estrus. Androgens may also have side effects, including clitoral hypertrophy, vaginal discharge, liver dysfunction, and weight gain. Androgens are contraindicated in bitches with a liver or kidney disease. Furthermore, androgens can induce development of mammary tumors. If administered to a pregnant dog, androgens may cause defects in the urogenital tract of female puppies. In addition, androgens may cause an increase in aggressiveness and a change in micturition behavior. Bitches may begin to urinate like a male dog and queens may develop urine spraying behavior.

GnRH agonists administered at high doses over a long period of time also prevent estrus by down-regulation of GnRH receptors on pituitary gonadotropes. However, the early stimulatory effect of GnRH analogues, which causes estrus if they are administered in anestrus and sometimes also if administered in the luteal phase, make them less suitable for clinical use.[81] In one study, GnRH agonist implants, applied before puberty (mean age: 4.9 ± 0.3 months), prevented reproductive function for one year. Following removal of the implant estrus occurred naturally in seven of ten bitches and could be induced in the other three after 1.2–14.3 months.[82] The age at puberty of the treated bitches was 25.5 ± 5 (18–31) months. Long-acting GnRH antagonists, suitable for use in clinical practice, have not yet been marketed.

Progestagens. The mechanism of the contraceptive action of progestagens is still unclear. In some species there is evidence that contraceptive progestagens inhibit gonadotropin release. However, high doses of medroxyprogesterone acetate administered to beagle bitches for several months did not reduce the increased circulating LH concentration in ovariectomized bitches nor did it lower LH concentration in intact bitches.[83] In another study, high contraceptive doses of megestrol acetate did not suppress basal gonadotropin secretion during anestrus, nor was the hypersecretion of LH and FSH that occurs in ovariectomized bitches suppressed.[84] Chronic medroxyprogesterone acetate (MPA) treatment did not affect FSH secretion, except for an increase two months after the start of treatment, and did not affect LH secretion (fig. 7.33).[85] Pulsatile FSH and LH release is maintained during MPA treatment, but there are indications of changes in the pulsatile secretion pattern of the gonadotropins. In general, LH pulses coincide with an FSH pulse, but during MPA treatment LH pulses coincided with small and sometimes insignificant FSH pulses (fig. 7.33).[86] The results of this study also suggest that there may be a direct negative effect of medroxyprogesterone acetate on follicle development in the ovary.[86]

The progestagens most frequently used for estrus prevention in the dog are proligestone and MPA. The single subcutaneous injection dose recommended by the manufacturer for proligestone ranges from 10 mg/kg for a dog of about 60 kg, to 30 mg/kg for a dog of 3 kg, and for medroxyprogesterone acetate the single subcutaneous injection dose is 2 mg/kg (with a maximum dose of 60 mg). They should be administered during anestrus, about one month before the expected follicular phase (fig. 7.34). In most bitches the first estrus after injection of proligestone can be expected within nine to twelve months; after injection of MPA it may be up to two to three years. MPA can also be administered orally, 5 mg once daily (10 mg for large dogs during the first five days) for as long as estrus prevention is wanted or for a maximum of 21 days. Estrus recurs after two to nine months. In the U.S.A. the advised dose of megestrol acetate, a progestagen which probably has a stronger progestagenic effect than MPA, is 0.5 mg/kg orally once daily for 32 days starting in anestrus, or 2 mg/kg for eight days starting at the onset of proestrus. Considering the results which are obtained with lower doses of MPA, this recommended dose seems quite high.

In the usual household the queen is not affected by photoperiod influences and may cycle throughout the year. This can be prevented by oral administration of 5 mg MPA or 2 mg megestrol acetate once weekly. Alternatively, owners who can detect the symptoms that precede estrus can administer these drugs only when these symptoms occur. The side effects of oral administration appear to be less serious than those accompanying injections. In addition, should the queen unexpectedly be found to be pregnant, the oral medication can be stopped and parturition allowed to occur normally. Another option is to reduce estrus frequency in the queen by inducing ovulation. This can be accomplished by mechanical stimulation of the vagina (touching the vestibulum / vagina with a cotton probe) or by treatment with a gonadotropic hormone or GnRH during estrus. The induced pseudopregnancy delays the recurrence of estrus.

Side effects associated with the use of progestagens for estrus prevention:

- Development of cystic endometrial hyperplasia (chapter 7.11).
- Prolonged pregnancy. This occurs if progestagens are administered subcutaneously at the onset of the follicular phase and the bitch is mated. The gestation will be prolonged and a caesarian section may be needed.
- Hypersecretion of mammary GH (chapter 2.2.4.2).
- Diabetes mellitus. In cats, this is usually caused by the glucocorticoid effects inherent in progestagens.[87,88] In dogs, however, apart from glucocorticoid effects, diabetes mellitus is largely due to GH excess.[15] The hypersecretion of GH caused by progestagen administration can be treated successfully by the progesterone-receptor blocker aglepristone.[89]
- Increased risk of neoplastic transformation of mammary tissue. This ranges from hyperplasia to adenomas and

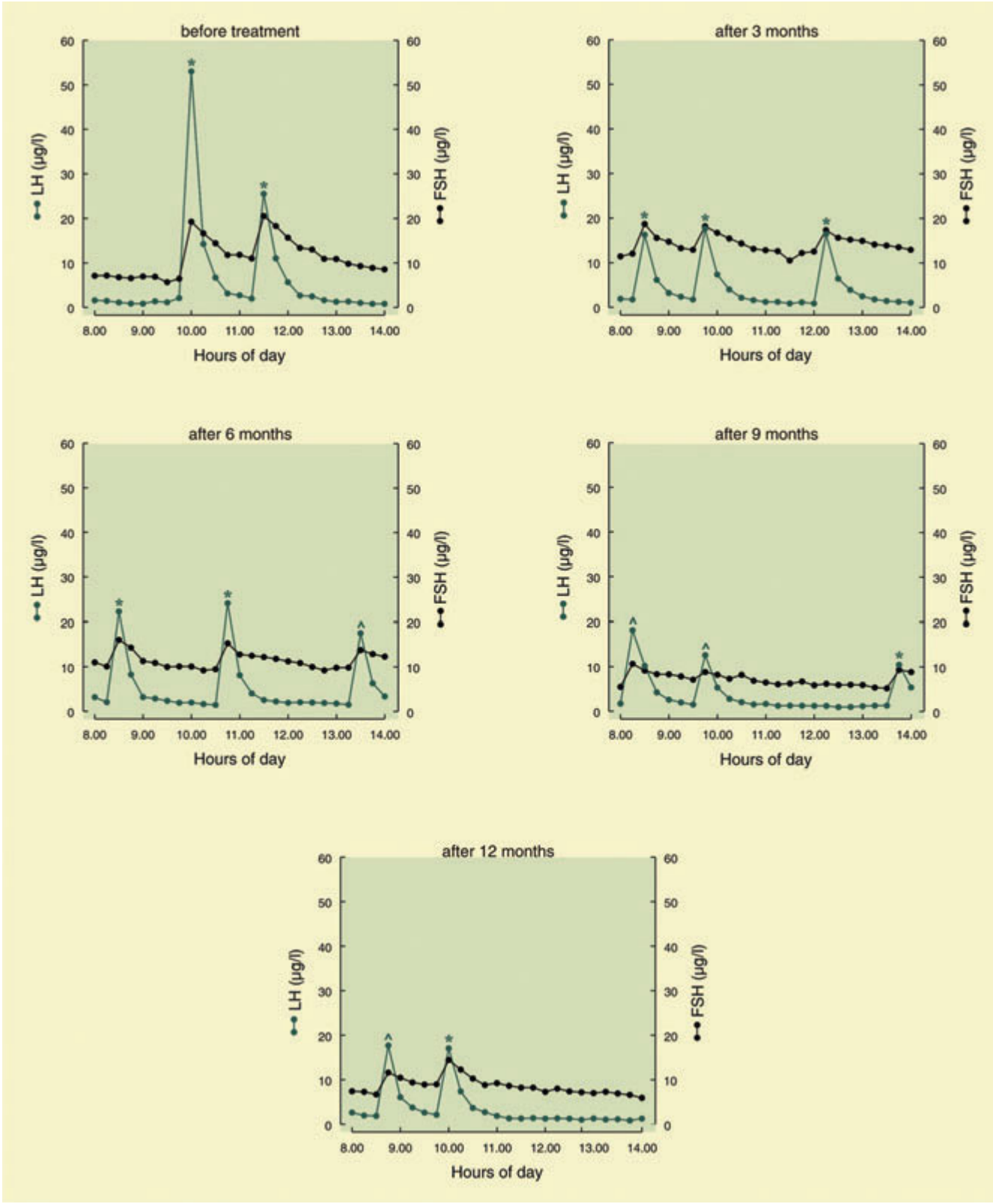


Figure 7.33:
Six-hour plasma profiles of FSH and LH in a three-year-old beagle bitch before and three, six, nine and twelve months after the start of treatment with medroxyprogesterone acetate (10 mg/kg, every four weeks).
* Significant pulses of both FSH and LH. ^ Significant LH pulse without significant increase in FSH. (Modified after Beijerink et al., 2008.)[86]

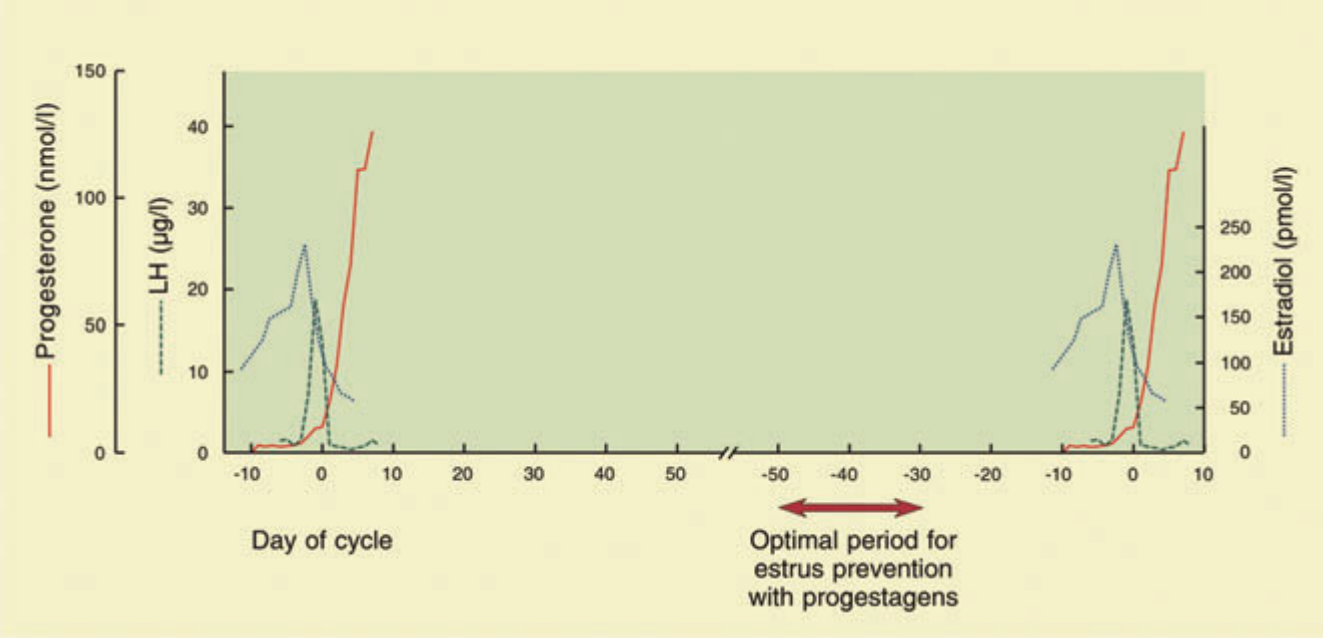


Figure 7.34:
Optimal period for progestagen treatment for estrus prevention in the bitch.

malignant tumors. The progestagen-induced neoplastic transformation of mammary tissue starts with proliferation of undifferentiated terminal ductal structures, so-called terminal end buds.[90] This proliferation increases the susceptibility of the mammary tissue to malignant transformation. However, the hyperplasia itself may also give rise to problems, especially in the queen. In young queens exogenous progestagens (and also endogenous progesterone!) may cause extensive proliferation of mammary duct epithelium and stroma, leading to a very large fibroadenomatous hyperplasia (fig. 7.35). The latter disorder can be treated effectively with the progesterone receptor blocker aglepristone.[63]

The occurrence of these side effects, with the exception of »prolonged pregnancy«, is largely dependent upon total progestagen exposure. With the advised dosage regimens the exposure may be higher with MPA and megestrol acetate than with proligestone, the latter being a rather weak progestagen.



Figure 7.35:
A ten-month-old queen with fibroadenomatous hyperplasia of the mammary glands. Following her first estrus she had been treated with 2 mg megestrol acetate once weekly for three weeks.

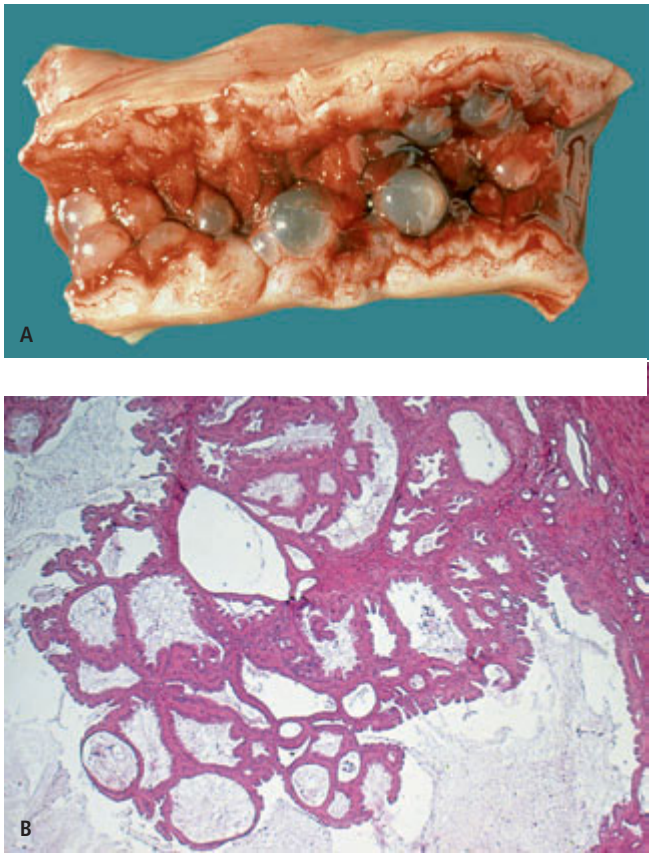


Figure 7.36:
(A) Cystic endometrial hyperplasia in an eight-year-old bitch. The lumen of the uterus is filled with aggregates of bulging cysts arising from the endometrium.
(B) Multicystic proliferation in the bitch due to cystic endometrial hyperplasia with papillary overgrowth of the endometrium, which is mainly composed of epithelial tissue with scant connective tissue. (H&E stain, x40).

7.11 Cystic endometrial hyperplasia-endometritis

Pathogenesis and pathology

Cystic endometrial hyperplasia (CEH) is a common disorder of the uterus of the bitch and the queen. If the endometrial hyperplasia is accompanied by inflammation the condition is called CEH-endometritis. CEH can develop either as a consequence of repeated endogenous progesterone influence during successive luteal phases or as a consequence of exogenous progestagens. It is therefore a common disorder in older bitches, which have completed several luteal phases. It is not the result of »retained« corpora lutea.

GH has been demonstrated by immunohistochemistry in the hyperplastic glandular epithelial cells of the uterus of progestagen-treated dogs. Although endogenous progesterone and exogenous progestagens may induce both development of CEH and hypersecretion of GH in mammary tissue, the latter does not seem to play a role in the pathogenesis of CEH.[18]

In the queen CEH is mainly a disease of older animals but occasionally it occurs at an early age, especially after administration of progestagens. Queens with CEH-endometritis often have corpora lutea without a history of mating,[42] which may be due to the fact that queens do not always require coital contact to induce ovulation (chapter 7.2.2). This probably means that several ovulations have occurred unnoticed and consequently the animal has been repeatedly under progesterone influence. CEH-endometritis has also been observed in ovariectomized queens that have been treated with progestagens.

CEH-associated alterations of the uterine glandular epithelium first occur close to the uterine lumen (fig. 7.36), but note that accessory glands can also be present in the myometrium (adenomyosis) or even in the serosa (endometriosis). CEH is usually diffuse, but it can be limited to only parts of the uterus. If the cervix is closed, which is often the case under progesterone influence, mucometra develops (fig. 7.37). In CEH without infection there are no inflammatory cells, while infection results in neutrophils and plasma cells.

Clinical manifestations

Bitches and queens with uncomplicated CEH do not exhibit signs of systemic disease. Infertility due to failure of implantation or to fetal resorption can, however, be observed. If infection is also present the signs and symptoms are often dependent upon cervical patency. The systemic disease is usually milder when the cervix is open than when it is closed. Massive quantities of pus may be found in the lumen of the uterus, especially if the cervix is closed (pyometra). In this situation the animal is lethargic and may be anorectic. The enlarged uterus may cause abdominal distention. With an open cervix vaginal discharge ranges from yellow to chocolate or red, depending on the presence or absence of blood. The bacterial infection may cause deposition of immune complexes in the glomerular capillary walls. This may cause proteinuria, but it does not usually lead to permanent renal failure. The elevated plasma urea and creatinine concentrations are generally prerenal in origin, i.e., due to hypovolemia. Vomiting associated with the uremia may be an aggravating factor. One must also be alert to the possibility of peritonitis due to a perforated uterus.

The bacterial infection and more specifically *E. coli* antigens may cause loss of medullary hypertonicity.[91] In addition, a decrease in renal sensitivity to vasopressin has been demonstrated in dogs with pyometra.[92] These changes may lead to a decreased ability to concentrate urine. The associated polyuria and polydipsia are common in dogs with CEH-endometritis, but rare in cats.

Anemia is present in about 40 % of bitches with CEH-endometritis. This may be the result of blood loss in the uterus, but the inflammatory process can also lead to decreased erythropoiesis.

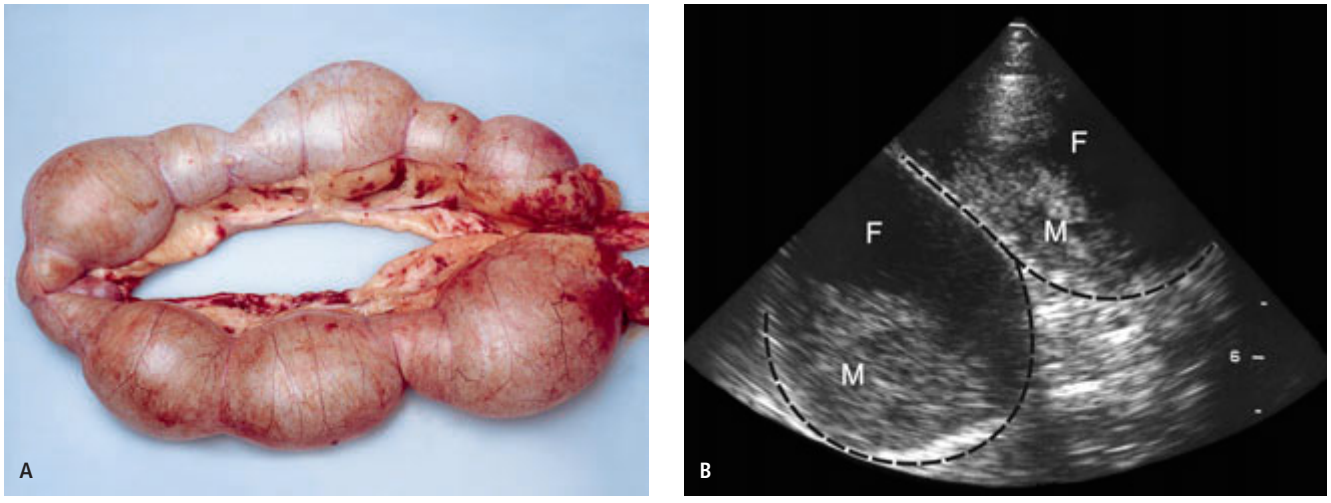


Figure 7.37:
(A) Mucometra with a thin uterine wall in a seven-year-old Bouvier des Flandres, treated for several years with high doses of progestagens.
(B) Transverse ultrasonogram of the abdomen of the same bitch. The uterus is severely dilated (delineated by interrupted line) and filled with fluid (F). Inspissated mucus (M) causes amorphous echogenicity in the dependent part of the uterine horns.



Figure 7.38:
Lateral radiograph of the abdomen of a seven-year-old mixbred dog with pyometra. The dilated, fluid-filled uterus causes displacement of other viscera.



Figure 7.39:
Ultrasonogram of the abdomen of a seven-year-old bitch with cystic endometrial hyperplasia. The uterus is slightly dilated, fluid-filled, and has an irregularly thickened wall with small cysts.

Diagnosis

CEH-endometritis is usually a disease of the middle-aged or elderly bitch or queen. It occurs during the luteal phase of the estrous cycle or under influence of exogenous progestagens. In cases of endometritis with an open cervix the discharge can be observed during vaginoscopy. Culture of the discharge usually reveals *E. coli*, and sporadically other bacteria. Routine laboratory examinations often reveal neutrophilia, anemia, and hyperproteinemia. The enlarged uterus can be palpated or visualized by radiography (fig. 7.38) or, preferably, ultrasonography (fig. 7.39). In cases of CEH without accumulation of fluid, visualization is only possible by ultrasonography.

Differential diagnosis

Several of the signs and symptoms of CEH-endometritis can also be associated with pregnancy, vaginal tumors causing discharge, or vaginitis. For other causes of polyuria / polydipsia such as progestagen-induced GH excess, diabetes mellitus, hyperthyroidism, and hypercortisolism, the reader is referred to the relevant chapters, including chapter 2.3.3.4.

Treatment

Ovariohysterectomy is the treatment of choice for CEH-endometritis. If the affected bitch or queen is young and the owner wishes to breed it, medical therapy can be started. Medical treatment should include administration for at least two weeks of an antibacterial agent, selected by means of bac-



Figure 7.40:
Vaginal septum between the vestibule and the vagina in a bitch, observed during anestrus.
U = urethral orifice.

teriological culture and an antibiogram. Additional administration of the progesterone receptor blocker aglepristone, in a dose of 10 mg/kg body weight on days 1, 2, 8, and 15 improves the outcome.[93] The combination of antibiotics, aglepristone, and $PGF_{2\alpha}$ may further improve the results of treatment. Prostaglandins may be administered as the $PGF_{2\alpha}$ salt dinoprost tromethamine in a dose of 100–150 µg/kg body weight twice or thrice daily for four days, or as cloprostenol in a subcutaneous dose of 1 µg/kg on days 3, 5, 8 10, 12, and 15.[94] $PGF_{2\alpha}$ causes premature regression of the corpora lutea if it is administered repeatedly in the second half of the luteal phase. Uterine contractions, cervix dilatation, and evacuation of the uterine contents can be expected. Side effects, observed mainly at the onset of $PGF_{2\alpha}$ therapy, may include salivation, vomiting, diarrhea, hyperpnea, ataxia, restlessness, and pupillary dilatation within minutes after administration. Walking the dog during this time diminishes the side effects, as does a lower dose administered more frequently. There is a risk of uterine perforation during this medical therapy and the risk is greater if the cervix is closed at the onset of therapy. CEH-endometritis may recur with the next estrous cycle.

Treatment in the queen is as described for the bitch. In addition, it is sometimes possible to pass a tomcat catheter through the cervix in order to deposit a water-soluble antibiotic, such as 100 mg ampicillin in 5 ml water, within the uterus. Estrus generally follows soon after completion of the treatment.

Prognosis

The prognosis after medical treatment alone is often uncertain, but probably improved since a progesterone receptor blocker in combination with $PGF_{2\alpha}$ can be used. Endometritis in the bitch after a mismating treatment with estrogens has a fairly good prognosis, as opposed to that for medical treatment of severe CEH in the elderly bitch. In cats the prognosis after medical treatment is much better than in dogs; many cats later conceive and deliver normal litters.

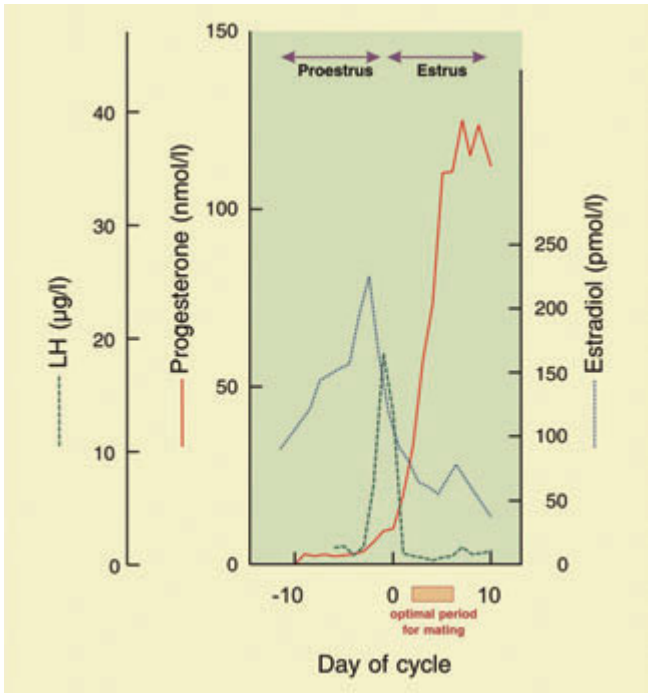


Figure 7.41:
The optimal time of mating related to plasma concentrations of estradiol, LH, and progesterone.

7.12 Fertility disorders in the bitch due to breeding management problems

Fertility disorders may be related to (1) abnormalities of the estrous cycle, such as split heat, persistent estrus, and hypoluteoidism, (2) anatomical abnormalities, such as strictures and septa (fig. 7.40), (3) cystic endometrial hyperplasia, or (4) infectious diseases, such as canine brucellosis or herpesvirus infection. Fertility problems may arise during any stage of the reproductive cycle. They may result in a failure to mate, a missed conception, or premature termination of pregnancy (see also previous sections). Many of the observed fertility problems are the result of inappropriate management of the bitch and can be solved if a proper breeding program is introduced (chapter 14.3). This section will therefore focus on fertility disorders related to breeding management problems.

Knowledge of reproductive physiology is indispensable for good breeding management. As mentioned in chapter 7.2.1, the length of proestrus is usually nine days, but it can be as few as three to as many as 17 days. The length of estrus is usually nine days but may range from three to 21 days. The onset of estrus is usually synchronous with the preovulatory LH surge, but occasionally it is as early as two to three days before or as

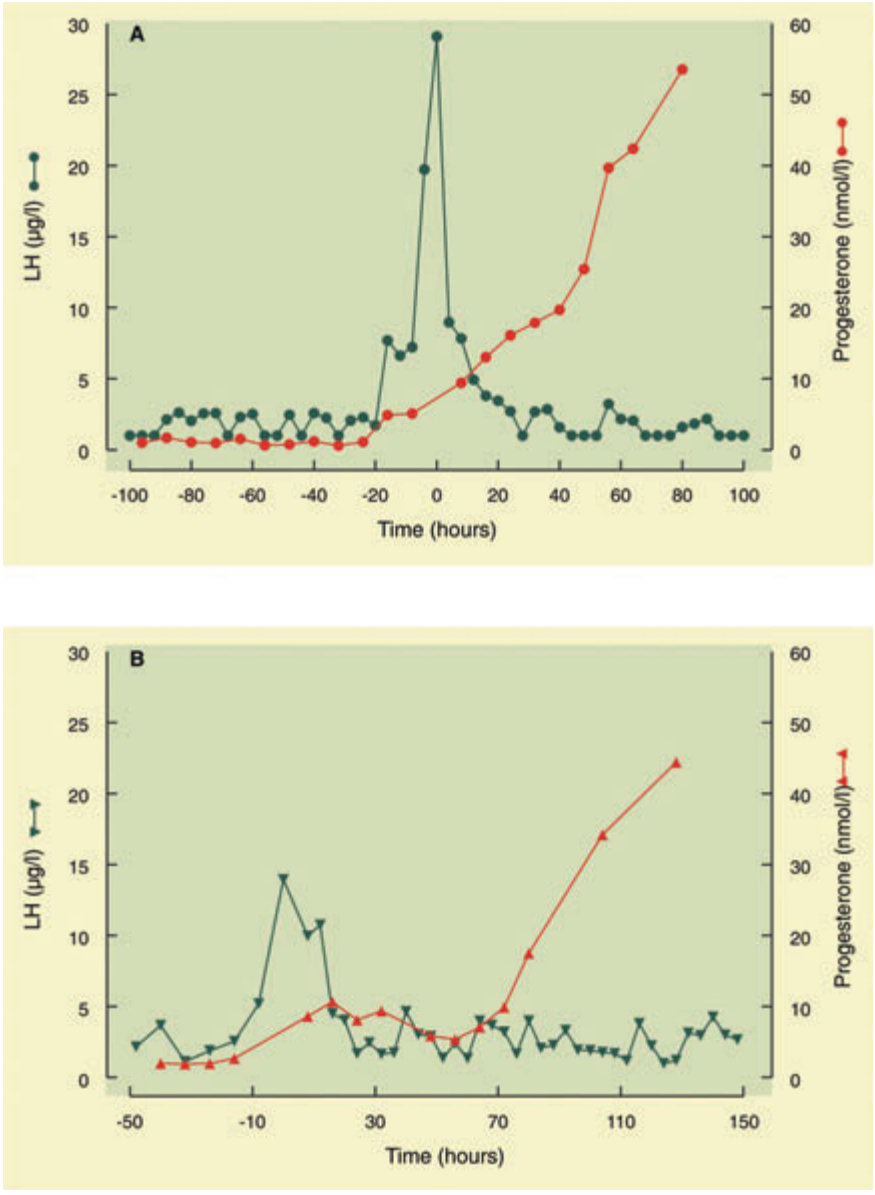


Figure 7.42:
Plasma concentrations of LH and progesterone during the periovulatory period (LH surge at time = 0) in two bitches: Lower panel: six-year-old beagle; after the initial increase, plasma progesterone concentration remains stable for three days. Upper panel: five-year-old beagle; plasma progesterone concentration increases markedly within 24 h. Also note the bifurcated LH surges. (Modified from De Gier et al., 2006.)[2]

late as four to five days after the LH surge. Moreover, some bitches never exhibit estrus behavior. Hence it is clear that breeding a bitch on a standard day in the cycle will usually give poor results. Breeding according to estrus behavior will give better results, but some bitches will still be bred too early and others too late. Determination of the ovulation period is therefore of the utmost importance. Several methods have been described to determine the ovulation period and the optimal time for mating. The primary methods are measurement of plasma progesterone and vaginoscopy.

The ovulation period can be defined satisfactorily by thrice-weekly measurements of plasma progesterone. The concentration increases slightly at the time of the preovulatory LH surge and then rapidly at the onset of ovulation, thereby exceeding 16 nmol/l. The optimal time for mating begins 24 h later (fig. 7.41) and is based on the time needed for maturation of the oocytes and capacitation of the sperm. The latter requires at least 7 h. By determining the optimal time for mating using a rapid radioimmunoassay for plasma progesterone, pregnancy was achieved in 105 of 112 (94 %) bitches with normal fertility and 81 of 104 (78 %) of those with suboptimal fertility.[95] In the latter group only 23 % of previous matings had been successful.

Determining the preovulatory LH surge would also be suitable for estimating the time of ovulation. Rapid radioimmunoassays for determination of the plasma LH concentration are not yet available, but in-hospital ELISA LH kits are available. However, more frequent blood sampling would be required than for progesterone because of the risk of missing the preovulatory LH surge. More importantly, the time between the preovulatory LH surge and the rapid rise in plasma progesterone concentration (indicating ovulation and formation of corpora lutea) varies (fig. 7.42). Hence plasma progesterone concentration is the preferred variable for estimating the ovulation period.

Vaginoscopy can also be used to attempt to determine the ovulation period (chapter 7.2.1). The mucosal changes are, however, a response to hormone-controlled alterations and are therefore secondary changes. Interpretation of the changes is also subjective. Vaginoscopy is thus a less reliable method for estimating the ovulation period than measuring plasma progesterone. For the experienced veterinarian it is a useful tool for monitoring the stages of the estrous cycle, but mating advice based on vaginoscopy should include the recommendation to mate at least twice, with an interval of 48 h.

Vaginal cytology is very useful in diagnosing early proestrus, progressing proestrus-estrus, or metestrus (chapter 7.2.1). There are, however, no reliable changes in the smear indicative of the preovulatory LH surge or of ovulation. During the transition from estrus to metestrus the percentage of round cells increases rapidly and leukocytes reappear. However, an early metestrus smear can easily be confused with an early proestrus smear. Hence the use of vaginal cytology is not suitable for determining the appropriate period for mating the bitch.

Also ultrasonography can be used for ovulation determination, but because both pre-ovulatory follicles and post-ovulatory corpora lutea have cavities, examination must be performed by experienced persons with excellent equipment, preferably twice a day. This method appears to be less practical than detection of ovulation via determination of the plasma progesterone concentration.[96,97]

In spite of correctly timed breeding, some bitches will refuse the dog or other mating problems may arise. Some breeds, such as English and French bulldogs and the Newfoundland dog, are especially prone to mating problems. The cause of the mating problem can be related to the dog (abnormal anatomy, inexperience, behavioral problems), the bitch (behavioral problems, vaginal abnormalities), or the owner (inexperience). With due regard for possible hereditary consequences, artificial insemination can be used.

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8 Testes

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Frederik J. van Sluijs

8.1 Introduction

In the dog the testes lie obliquely within the scrotum, their long axis directed caudodorsally. The epididymis, which is relatively large in dogs, is attached along the dorsolateral border of the testis and consists of a head, body, and tail. The head arises from the testis cranially and is the thickest part. The body or middle part is slightly smaller and the tail is attached to the caudal end of the testis and is continuous with the ductus deferens. In the cat the testes are located closer to the anus and their long axis is directed caudoventrally.

Tubules with seminiferous epithelium make up about 80 % of the testis. They are composed of supporting cells and spermatogenic cells (fig. 8.1). Seminiferous tubules are the site of spermatogenesis, i.e., where spermatogonia develop into spermatozoa. This occurs in three distinct phases: (1) the proliferation phase, in which undifferentiated spermatogonia undergo rapid cell multiplication by mitotic divisions, (2) the meiotic phase, in which spermatocytes develop, and (3) the differentiation phase or spermiogenesis, in which spermatids are transformed into spermatozoa. After completion of spermatogenesis, the spermatozoa are released into the lumen of the seminiferous tubules, a process referred to as spermiation.[1] With increasing age there is neither deterioration in spermatogenesis nor a change in tubular diameter in healthy canine testes. However, aged dogs often have testicular tumors which affect spermatogenesis and are often not clinically detectable.[2]

Sertoli cells, which line the seminiferous tubules, have an important supportive function during spermatogenesis. They express androgen receptors and receptors for follicle-stimulating hormone (FSH) and are thought to regulate development of the germ cells via the synthesis and secretion of molecules which act upon the surrounding germ cells. Androgens and androgen receptors are essential for maintenance of spermatogenesis, whereas males are still fertile without the influence of FSH. [3]

In the basal region of the seminiferous epithelium the plasma membranes of adjacent Sertoli cells form specialized junctional complexes which constitute the structural basis of the Sertoli cell barrier. The primary function of this barrier, previously known as the blood-testis barrier, is probably to insure proper conditions for germ cell development in the tubules. Some molecules enter the tubules nearly instantaneously, while others are almost completely excluded. For example, testosterone and glucose appear to have accelerated entry rates, while peptide hormones (including the gonadotropins) are generally excluded. Peptide hormones produced or secreted into the tubular lumina are retained there by the barrier and probably do not function as endocrine factors

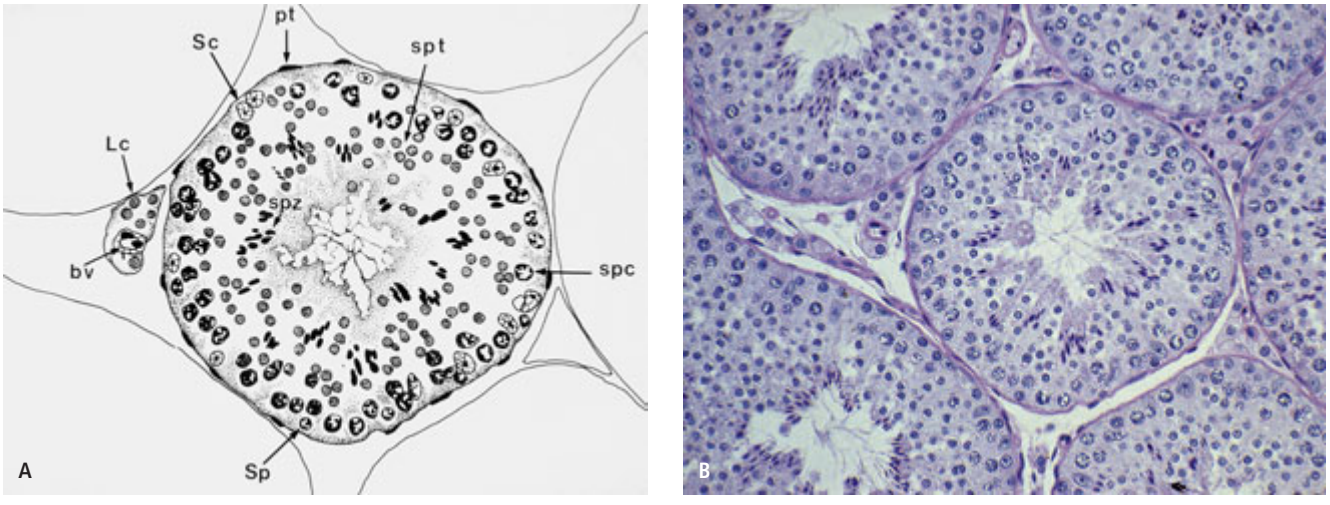


Figure 8.1:
Cross section of a seminiferous tubule in the dog. Sc = Sertoli cells; spc = spermatocytes; spt = spermatids; spz = spermatozoa; sp = spermatogonia; pt = peritubular cells; Lc = Leydig cells; bv = blood vessel ([A] schematic, [B] PAS-hematoxylin stain, x475). (Courtesy of Dr. K.J. Teerds, drawing by H. Halsema.)

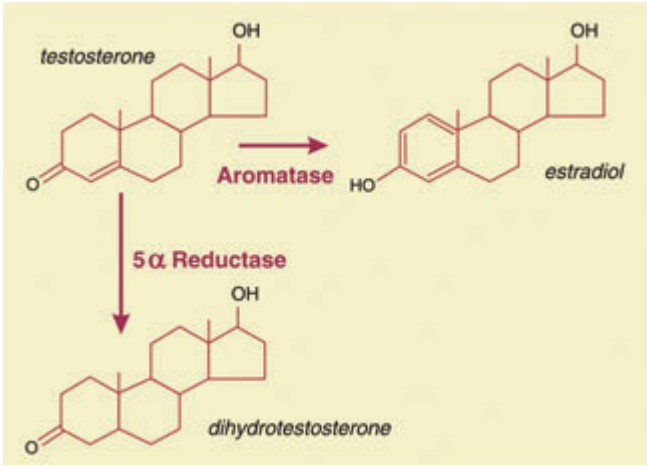


Figure 8.2:
Schematic illustration of the conversion of testosterone to dihydrotestosterone and estradiol, catalyzed by 5α-reductase and aromatase, respectively.

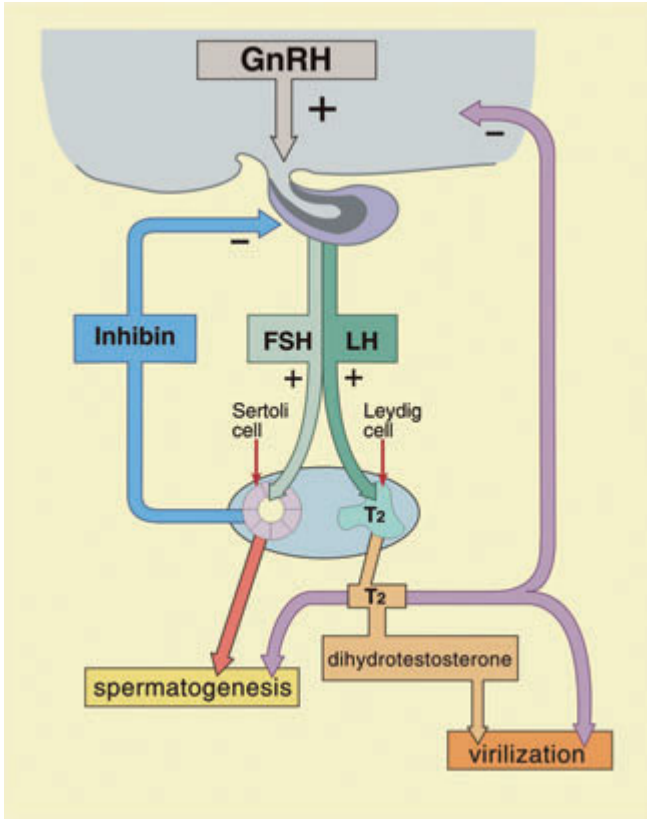


Figure 8.3:
Endocrine control of testicular function. Androgen secretion is regulated by LH, with feedback from testosterone (T_2). Spermatogenesis is controlled by FSH, with feedback from inhibin. Testosterone is converted to dihydrotestosterone in several target tissues.

outside the testis. Sertoli cells also produce androgen-binding protein, which is required to maintain the high concentrations of testosterone in the tubular compartment needed for spermatogenesis.[4]

Between the seminiferous tubules lie groups of interstitial or Leydig cells. They are the main constituent of the endocrine portion of the testis and produce the androgens that drive the spermatogenic process.[3,4]

8.1.1 Hormone synthesis and secretion

The main hormones secreted by the testes are androgens and estrogens. Androgens are produced by the interstitial or Leydig cells, which are stimulated by luteinizing hormone (LH). The primary androgen is testosterone. Like other steroid hormones it is produced from cholesterol, which is converted in the mitochondria to pregnenolone. Pregnenolone is further metabolized outside the mitochondria to several other steroids via various pathways (see also fig. 4.3). Apart from direct interaction of testosterone with the androgen receptor, many effects are exerted after its conversion to dihydrotestosterone, which has a higher affinity for the receptor. The conversion is effected by an NADPH-dependent 5α-reductase (fig. 8.2), which is not present in the tubular compartment of the testes.[4] Testosterone can also be converted to estradiol by the aromatizing enzyme system that is present in mammalian testes as well as in adipose tissue, prostate, and bone.[3] The peripheral contribution to total estradiol production appears to be large (of the order of 75–80 %) as compared to the testicular contribution. Testicular steroids are secreted into blood, lymph, and tubular fluid. Blood is quantitatively the most important effluent system because the flow rate is 20 times that of lymph or tubular fluid.

Another hormone secreted by the testis is inhibin, a glycoprotein hormone produced primarily by the Sertoli cells (fig. 8.3). It consists of two dissimilar, disulphide-linked subunits termed α and either β_A or β_B. The β subunit of inhibin shares a sequence homology with members of the transforming growth factor β family such as TGFβ, activin, and anti-Müllerian hormone (AMH).

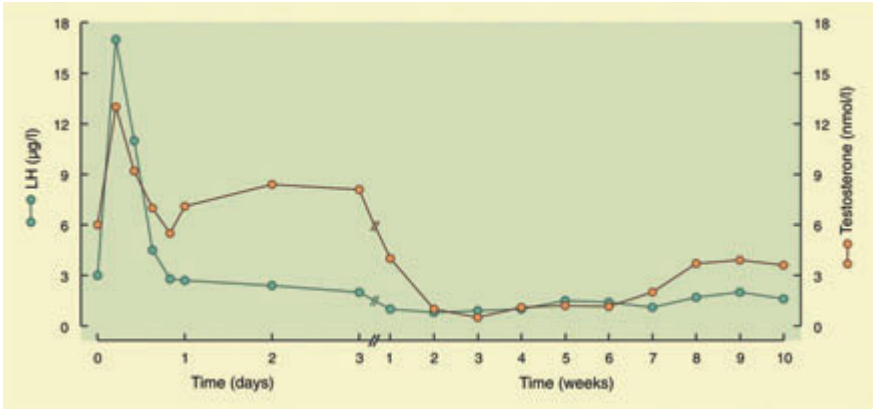


Figure 8.4:
Plasma concentrations of LH and testosterone in a dog after administration on day 0 of an implant that slowly releases the GnRH agonist deslorelin.

8.1.2 Regulation of testis function

Testicular function is controlled by the gonadotropins. Androgen secretion is regulated by LH and spermatogenesis is controlled by FSH and locally produced androgens (fig. 8.3).

LH is secreted by the hypophysis in a pulsatile pattern with a frequency of approximately 4.5 pulses every 6 h. LH pulses are usually followed by a testosterone pulse within 60 min.[5] Diurnal rhythmicity has been described, with lowest levels in the morning and peak levels in the afternoon (LH) or evening (testosterone).[6] FSH is also secreted in a pulsatile fashion, but synthesis and secretion of LH and FSH are differentially regulated by the frequency of GnRH pulses from the hypothalamus. Pituitary LH and FSH secretion are under negative feedback control by testosterone. In addition, pituitary FSH secretion is inhibited specifically by inhibin.[7]

Within the testis, androgens mainly act as paracrine agonists. Together with other locally produced factors, such as endogenous opioids and proteins produced by the peritubular cells (P-Mod-S), they regulate Sertoli cell function and thereby indirectly the process of spermatogenesis.[8]

8.2 Hypogonadism

Male hypogonadism refers to all forms of endocrine and secretory hypofunction of the testes. The term hypogenitalism is used for underdeveloped external genitalia. Two forms of hypogonadism are recognized: (1) primary or hypergonadotropic hypogonadism, and (2) secondary or hypogonadotropic hypogonadism. Primary hypogonadism – atrophy of the testes in the presence of normal or increased plasma concentrations of gonadotropins – may result from many diseases, such as orchitis of either infectious (*Brucella canis*) or autoimmune etiology, trauma, and testicular torsion. In rare cases it may be due to a chromosomal defect, such as in male tricolor cats with a 39,XXY karyotype (chapter 6.2.1).[9]

The ultimate form of primary hypogonadism is that resulting from castration. It is very common in dogs and cats, although its prevalence varies markedly from country to country, depending on cultural factors, the urban or rural location of the household, and the species: male cats are more likely to be castrated than male dogs. The behavior of most sexually intact male cats makes them undesirable as pets.[10]

Secondary hypogonadism occurs rarely, as a consequence of low concentrations of gonadotropins due to a pituitary tumor. Isolated gonadotropin deficiency has not yet been described in dogs or cats. Antiandrogens such as cyproterone acetate may act as progestagens and their therapeutic use may inhibit gonadotropin secretion, with reversible secondary hypogonadism. The same holds true for corticosteroids. Both endogenous and exogenous corticosteroids reduce the plasma LH concentration.[11] Administration of supraphysiological doses of androgens also results in hypogonadotropic hypogonadism.[4,12] The high plasma androgen concentration suppresses pituitary gonadotropin secretion and consequently testicular testosterone secretion. Because the testosterone concentration in testicular tissue is normally much higher (25–100×) than in plasma, the LH-dependent testicular testosterone concentration will become too low to stimulate testicular function.

A more recently introduced iatrogenic cause of secondary hypogonadism in dogs is the use of implants of slow-release GnRH-agonists, such as deslorelin. These causes plasma LH and testosterone concentrations to first rise and then decline to low values for prolonged periods of time (fig. 8.4), resulting in temporary loss of fertility, as testosterone is vital for spermatogenesis.[13–15] This opposite effect is due to GnRH-receptor down-regulation, internalization, and signal uncoupling after sustained exposure to the GnRH analogue.[15]

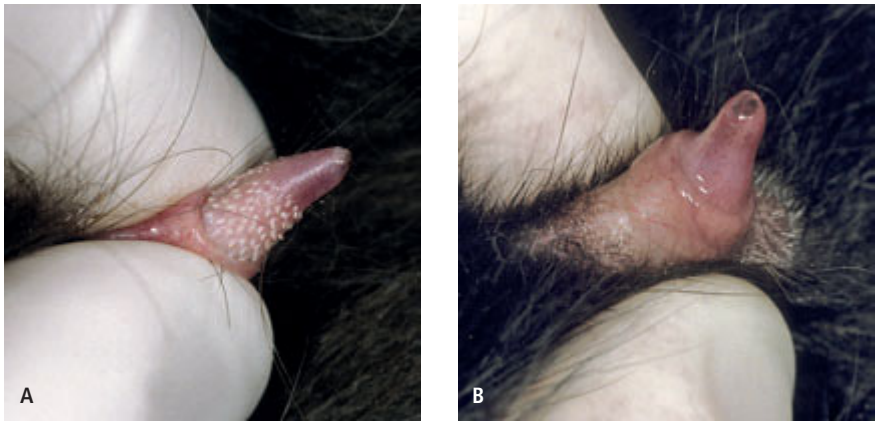


Figure 8.5:
Penis of an intact (A) and castrated (B) tomcat. The typical barbs on the penis of the intact male are absent in the castrated male.

Clinical manifestations

Testis atrophy is characterized by small and soft testes. The atrophy does not affect the epididymis, which is relatively large and firm in comparison with the adjacent testis. Secondary reversible hypogonadism induced by implants releasing GnRH agonists also induces transient testicular atrophy, which can be used clinically to assess the duration of action of an implant.

If testis atrophy occurs at an early age the androgen deficiency may result in underdevelopment of secondary sexual characteristics, i.e., hypogenitalism. Affected tomcats do not have a typical feline masculine appearance and the prepuce and penis remain underdeveloped. The penis lacks the barbs that are typical of male felidae (fig. 8.5). Hypogonadism also affects male behavior, lessening the tendency to marking and roaming as well as aggressive behavior toward other males.[16]

Gonadectomy is often carried out before the cat reaches puberty and initially leads to no serious physical or behavioral problems.[10,17] When performed prior to physeal closure, it delays that closure and leads to significant, although not readily visible, lengthening of long bones (see also chapter 9.7 and table 9.2).[18] In cats, spontaneous femoral capital physeal fractures, with histological signs of necrosis of the epiphysis, have been reported to be associated with prepubertal gonadectomy.[19–22] The vast majority of the affected cats were overweight, neutered males.

With time the main changes after gonadectomy seem to be consequences of increased appetite, in cats leading to increased body weight and increased plasma concentrations of insulin, leptin, IGF-I, and prolactin.[23,24] The increase in food intake, body weight, and body fat mass can be prevented almost completely by treatment with estradiol, which is consistent with studies in rodents demonstrating the importance of gonadal estrogen in increasing the satiating potency of cholecystokinin released in response to ingested lipid.[25,26] The possible clinical consequences of overweight are covered in chapters 5.2.4 and 11.

In male dogs gonadectomy does not result in hyperprolactinemia, but LH concentrations are high because of the absence of androgen feedback.[27] Gonadectomy does not generally affect thyroid or adrenocortical function, although slight differences have been found between gonadectomized and intact dogs in some test results.[27,28] Decreased plasma androgen concentration following gonadectomy in dogs is accompanied by loss of bone volume and increased plasma PTH concentration,[29] but not to the extent that it leads to clinically noticeable problems.

Sex steroid deprivation and persistently high plasma gonadotropin concentrations also affect the biology of collagen and muscle. Gonadectomy leads to a slightly increased occurrence of injuries of the anterior cruciate ligament.[30] It also alters the proportion of collagen and muscle fibers along the lower urinary tract. Regardless of gender, there is a larger proportion of collagen in gonadectomized dogs than in intact dogs.[31] The resulting decrease in tissue elasticity has an adverse effect on the collecting phase of micturition and on bladder contraction, but in contrast to female dogs (chapter 7.10), male dogs rarely develop neutering-induced urinary incontinence.



Figure 8.6:
Calipers for measuring testicular size.

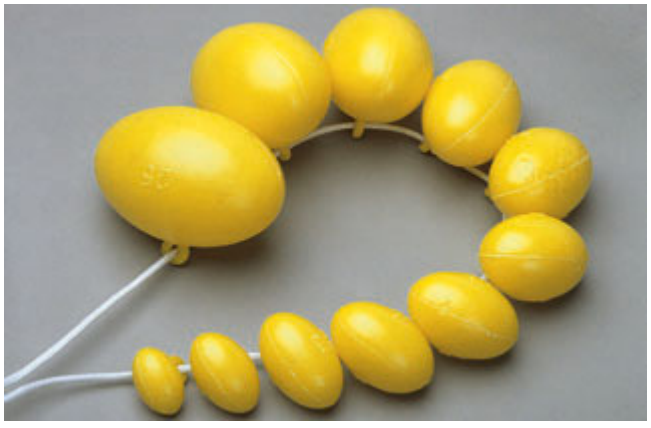


Figure 8.7:
Prader's orchidometer. The volume of the testis, excluding the epididymis, is estimated by comparison with the ellipsoids, whose size is marked in ml.

Differential diagnosis

Hypogonadism (including the result of castration) should be differentiated from cryptorchidism. Ectopic testes are difficult to detect by palpation in obese animals and in abdominal cryptorchids. In tomcats the presence of barbs on the penis (fig. 8.5) indicates secretion of androgens by testicular Leydig cells. The presence of an endocrinologically functional testis can be demonstrated unequivocally by a GnRH-stimulation test (chapter 12.5.1).

Diagnosis

The consistency of the testes is determined by palpation. Their size can be measured with calipers (fig. 8.6) or estimated with Prader's orchidometer (fig. 8.7). In the dog the dimensions of the testes depend on the body mass.[32] They range from 1.5 × 1.5 × 2 cm in toy breeds to 3 × 3 × 5 cm in large breeds. In the cat the testes have a diameter of approximately 1 cm.

Treatment

The most frequent cause of hypogonadism is castration. This has usually been elected by the owner and requires no treatment. In the rare cases in which treatment of hypogonadism is requested, androgen replacement therapy may be given. The treatment of obese orchiectomized cats with estradiol has not been investigated in long-term studies.

Prognosis

Primary hypogonadism is usually incurable but testosterone replacement can be given lifelong. The prognosis in secondary hypogonadism depends on the course of the primary disease (chapter 2.2.6).

8.3 Cryptorchidism

Cryptorchidism is a developmental defect in which there is failure of complete descent of one or both testes into the scrotum. The reported incidence in dogs varies from 1.2 % to 9.7 %,[33,34] depending on the population studied. It is a congenital disease and is considered to be a sex-limited inherited trait in dogs.[35] Cryptorchidism occurs more often in purebred than in crossbred dogs and bilateral cryptorchid dogs are reported to be more inbred than unilateral cryptorchids. Although a single autosomal recessive allele has been cited as a probable cause, transmission of the defect is probably due to more than one gene. Cryptorchid dogs are considered to be homozygous for the defect and their removal from the breeding line generally causes a decrease in frequency of the abnormal allele. Because cryptorchidism is a sex-limited trait that can only be detected in males, the genotype of the carrier female can only be assessed by progeny testing. This requires large numbers of puppies and makes the condition difficult to eliminate from a canine population. Cryptorchidism has been found in at least 68 canine breeds.[35] A retrospective study[36] of 2912 dogs identified 14 breeds with a significantly increased risk: toy poodle, Pomeranian, Yorkshire terrier, miniature dachshund, Cairn terrier, Chihuahua, Maltese, boxer, Pekingese, English bulldog, Old English sheepdog, miniature poodle, miniature schnauzer, and Shetland sheepdog. The incidence of cryptorchidism in the cat has been reported to vary from 1.7 % to 3.8 %.[37,38] Persian cats were overrepresented in both studies.

Normal testicular descent can be divided into three phases:

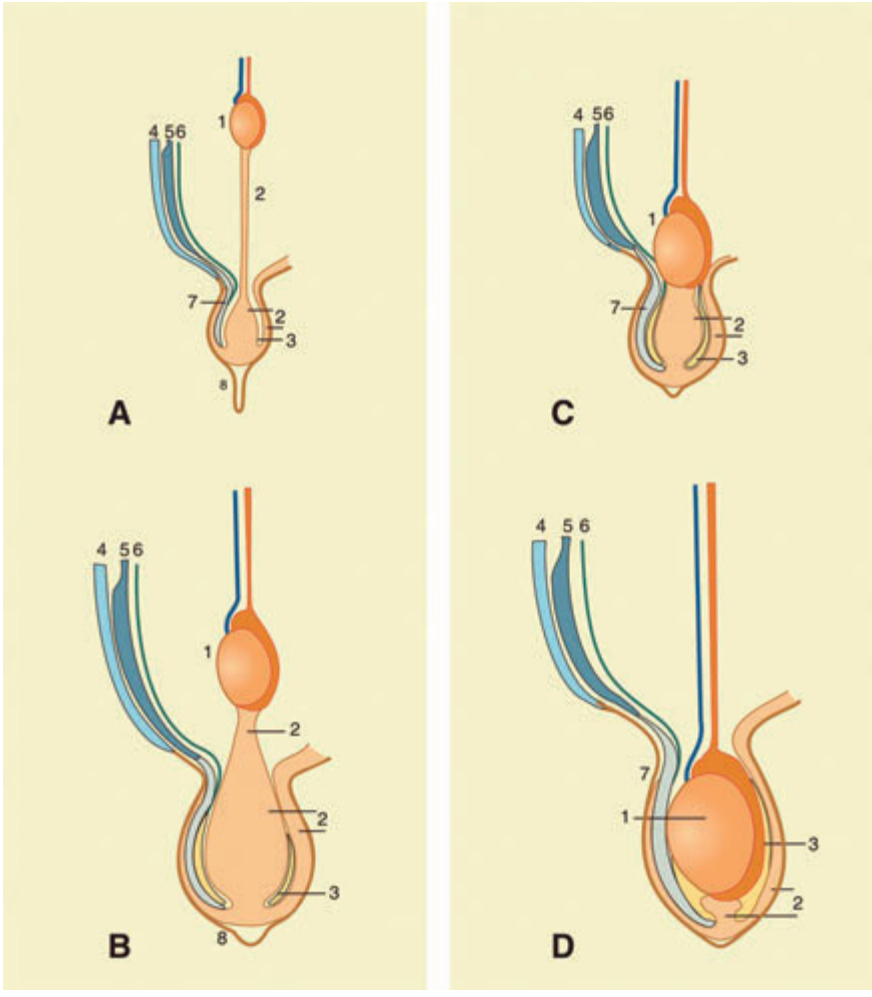


Figure 8.8:
Schematic representation of the normal descent of the testis (A → D). 1 = testis; 2 = gubernaculum; 3 = vaginal process; 4 = external oblique abdominal muscle; 5 = internal oblique abdominal muscle; 6 = peritoneum; 7 = cremaster muscle; 8 = external spermatic fascia. (Modified from Wensing, 1980.)[104]

(1) abdominal testis translocation, specifically retention near the neck of the developing bladder as the abdominal cavity enlarges, followed by slight testis relocation to the future inguinal ring; (2) transinguinal migration of the testis, moving the cauda epididymis and testis through the abdominal wall; and (3) inguinoscrotal migration of the testis, from a subcutaneous location outside the inguinal canal to the final position in the bottom of the scrotum.[39] The process of descent is controlled by the gubernaculum testis (fig. 8.8). This is a mesenchymal cord that extends from the caudal pole of the testis to the inguinal canal. During the process of descent, the gubernaculum increases in size just distal to the external opening of the inguinal canal. At the same time, the cranial suspensory ligament between the cranial pole of the testis and the abdominal wall close to the diaphragm regresses. The enlargement or outgrowth of the gubernaculum exerts traction upon the intra-abdominal part of the gubernaculum and this pulls the testis and epididymis distally through the abdomen toward the inguinal area and then through the inguinal canal. These steps constitute abdominal translocation and transinguinal migration. After completion of the outgrowth, the gubernaculum regresses and pulls the testis further caudally. This is the inguinoscrotal migration that moves the testis into the scrotum. Complete absence of the outgrowth reaction has not been observed, but substantial underdevelopment does occur with low frequency. In these cases there is a partial migration of the testis from its original position just caudal to the kidney to the vicinity of the internal inguinal opening. The final result in such cases is either permanent low abdominal cryptorchidism or delayed testicular descent. Abnormal location of the gubernaculum can take three forms (fig. 8.9). First, the extra-abdominal part of the gubernaculum does not expand beyond the inguinal canal but, instead, thrusts back into the abdominal cavity (reversed outgrowth). The traction normally developed by the outgrowth is absent, and the testis fails to leave its original position caudal to the kidney. This results

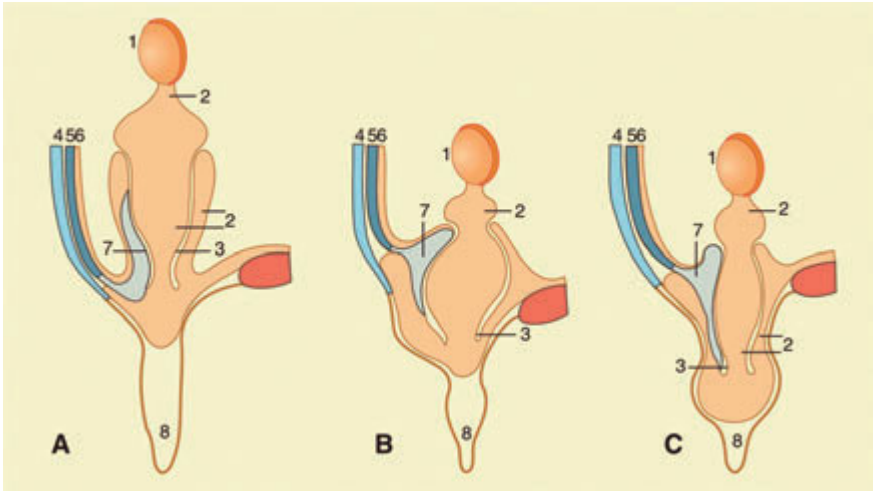


Figure 8.9:
Schematic representation of three forms of abnormal descent of the testis. (A) Reversed outgrowth of the gubernaculum. (B) Outgrowth of the gubernaculum partly in the abdomen. (C) Outgrowth of the gubernaculum partly outside the abdomen. The numbers refer to the same structures as in fig. 8.8. (Modified from Wensing, 1980.) [104]

in high abdominal cryptorchidism. Secondly, the outgrowth occurs partly in the inguinal canal and partly within the abdomen. Only slight displacement of the testis in the direction of the internal inguinal opening will then occur. Thirdly, the outgrowth reaction is partly outside the abdomen, in which case descent will progress further and the testis may even reach the internal inguinal opening. The final outcome is difficult to predict, but low abdominal or inguinal cryptorchidism is the most likely result.

Abdominal translocation of testes is dependent on insulin-like peptide 3 (Insl3), produced by the fetal Leydig cells. Insl3 stimulates growth of the gubernaculum to form an anchoring structure. Directional guidance for inguinoscrotal testis migration is provided by calcitonin gene-related peptide (CGRP) released from the genitofemoral nerve, descending down with the developing gubernaculum. Testosterone stimulates production or release of CGRP, which acts as a chemoattractant and induces the developing tip of the gubernaculum to grow toward the source of CGRP.[40–42] Testosterone and AMH are not obligatory for the thinning and elongation of the cranial suspensory ligament and the expansion of the gubernaculum.[39] In most species abdominal translocation is the longest phase of testicular descent, but in the dog inguinoscrotal migration requires a similar interval as the abdominal translocation. Transit through the inguinal canal is rapid, requiring less than two to four days.[39]

Several possible etiologies for cryptorchidism have been suggested, such as abnormal testicular differentiation, deficient androgen production, deficient production/action of anti-Müllerian hormone (AMH), and deficient action of Insl3. But in most cases the etiology is unknown, albeit that in dogs predisposing factors such as familial occurrence, litter size, and sex ratio in the litter have been documented.[43,44] In humans cryptorchidism is associated with impaired germ cell development, and altered plasma concentrations of gonadotropins and inhibin, which has led to the suggestion that there may be primary developmental disorders in cryptorchid testes.[45] However, it is not clear whether these abnormalities are a cause or a consequence of cryptorchidism.[46]

Clinical manifestations

The most striking abnormality is the absence of one or both testes from the scrotum. Dogs with bilateral cryptorchidism are considered to be infertile. Dogs with unilateral cryptorchidism are generally regarded as being potentially fertile, but their fertility is probably lower than that of normal dogs.[35] Plasma concentrations of testosterone and estradiol in dogs with unilateral inguinal or abdominal cryptorchidism do not differ from those in normal dogs.[47] Cats with unilateral cryptorchidism, in which the scrotal testis has been removed, have the behavioral characteristic of intact males.[38]

There is an increased risk of neoplasia in cryptorchid testes and some types of testicular neoplasms may cause feminization and blood dyscrasias (chapter 8.4).

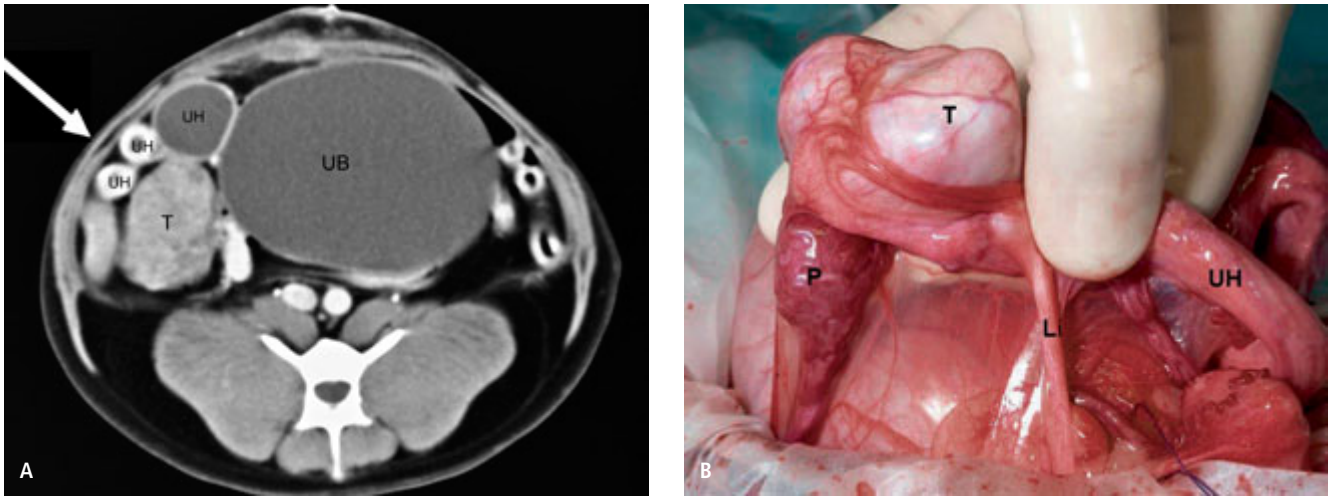


Figure 8.10:
(A) Contrast-enhanced transverse CT image of the abdomen of a five-year-old male miniature schnauzer with persistent Müllerian duct syndrome (PMDS), presented with unilateral cryptorchidism and signs of feminization, showing an intra-abdominal neoplastic testis (T). In addition, a fluid-filled uterus (UB, uterine body) and uterine horns (UH, arrow) can be identified.
(B) The neoplastic cryptorchid testis (T) was in close proximity to the uterine horn (UH). It contained a Sertoli cell tumor. Li = ligament; P = pampiniform plexus.

8

Differential diagnosis

Unilateral cryptorchidism should be differentiated from monorchism, in which no testicular tissue is present. Monorchism has been described in two cats.[38]

Diagnosis

Cryptorchidism is diagnosed by inspection and palpation. Cryptorchid testes may be present in the abdomen, at the inguinal ring, or in the inguinal canal. Abdominal testes cannot be palpated. Those in the inguinal area can sometimes be palpated, but in young animals it is difficult to determine the position of the testes reliably because of their small size during the first weeks of life. In addition, the cremaster muscle may hold immature testes in the inguinal canal or retract them from the scrotum when the animal is exposed to stress during physical examination. Cats have large inguinal fat pads which make inguinal testes extremely difficult to palpate.

Bilateral cryptorchidism in cats can be suspected by the presence of barbs on the penis (fig. 8.5). In dogs rectal palpation of the prostate gland may provide evidence for the presence or absence of circulating testosterone. Diagnostic imaging of the inguinal region and the abdomen by ultrasonography or computed tomography (fig. 8.10) often reveals a cryptorchid testis. If palpation and diagnostic imaging are inconclusive, basal and GnRH-stimulated plasma testosterone concentrations may distinguish between animals without testes and those with one or two cryptorchid testes (chapter 12.5.1).

There is disagreement in the literature about the time of testicular descent in dogs and cats. Detailed data have been published only for beagle and mongrel puppies.[48] In these dogs the testes reached their final position in the scrotum at 35 and 40 days postpartum. Based on these findings, puppies should be examined at six to twelve weeks of age. If the testes have not descended by eight weeks of age, cryptorchidism may be diagnosed tentatively. However, testicular descent has been reported to be complete as late as six months of age in some dogs.[49,50] Thus periodic reexaminations should be performed until six months of age.

Treatment

Human chorionic gonadotropin (hCG) and gonadotropin-releasing hormone (GnRH) have been tried and reported anecdotically to be effective.[51–53] The scientific basis for this form of treatment is not clear, since there is no evidence that testicular descent is controlled by gonadotropins. As the inguinal canal is usually closed in abdominal cryptorchids, success can only be expected in inguinal cryptorchidism. Testosterone has been tried as a therapy for cryptorchidism with little or no success.[53] Surgical placement of the retained testis in the scrotum (orchidopexy) has been shown to improve testicular function and may even result in normal fertility.[54,55] However, it is generally considered to be unethical because it conceals a congenital abnormality and promotes spread of the defect in the population. Surgical removal of the retained testis or castration are frequently advised because this eliminates the risk of developing testicular neoplasms and pre-

vents spread of the defect within the population. Although there is a higher risk of developing Sertoli cell tumor and seminoma in cryptorchid testes than in scrotal testes, the risk of fatal complications such as pancytopenia or metastasis is still very low. A decision analysis has shown that the risk of tumor-related mortality and morbidity is of the same order of magnitude as the risk of mortality and morbidity due to anesthesiological or surgical complications.[56] Based on these findings there is no persuasive reason to advise castration of cryptorchid dogs.

8.4 Testicular neoplasia

Testicular tumors are relatively common in dogs. Their estimated incidence is 67.8 per 100000 male dogs,[57] representing 5–15 % of all neoplasms in this species.[58] There are three major types of testicular neoplasms in the dog: Sertoli cell tumor, seminoma, and Leydig cell tumor, and they occur with approximately equal frequency. Cryptorchidism is an important risk factor for testicular neoplasms. In cryptorchid dogs, the incidence of Sertoli cell tumor is 23 times higher and that of seminoma 16 times higher than in dogs with scrotal testes. The incidence of Leydig cell tumors is similar in cryptorchid and scrotal testes.[36,58–61] Other tumors (gonadoblastoma, rete testis mucinous adenocarcinoma, leiomyoma of the tunica vaginalis, schwannoma, and undifferentiated sarcoma/carcinoma) have been described in individual dogs,[62–65] but these are exceptional cases. Bilateral tumors and the occurrence of more than one type of tumor in a single dog or even in a single testis are not uncommon.[66–69] Using antibodies against the LH receptor and 3β-HSD to identify Leydig cells and against vimentin to identify Sertoli cells, 13 of 86 canine testicular tumors were found to contain tumor cells of more than one type.[70] Testicular neoplasms are reported rarely in cats. None were present in 1,567 feline tumors (from both sexes), but single case reports have included Sertoli cell tumors,[71,72] Leydig cell tumors, and other types such as teratoma[73] and androblastoma[74]. Mixed tumors have also been described in cats.[72,74] A striking finding in cats is the relatively large number of tumors in ectopic testicular tissue.[75,76] The common practice of castrating male cats at an early age may contribute to the low incidence of testis tumors in this species.

Tumor size, hormone secretion, and the incidence of metastasis vary with the histological type. Sertoli cell tumors and seminomas may become quite large, especially in cryptorchid testes. Leydig cell tumors are the smallest and may be an incidental finding at necropsy. Approximately 8–39 % of the Sertoli tumors in dogs are associated with feminization.[34,69,77] Feminization has also been reported in a dog with a seminoma and in a limited number of dogs with Leydig cell tumors, but these are exceptional cases and may represent undetected mixed tumors. Feminization in dogs with testicular tumor may be associated with blood dyscrasias.[77–82] Feminization and blood dyscrasias have been attributed to increased secretion of estrogens by the tumor, but this has been investigated in only a small number of dogs.[80,83,84] In one study the plasma concentration of estradiol was elevated in three of ten dogs.[80] In another study the difference in plasma estradiol concentration between tumor bearing and healthy control dogs was not significant,[83] but determinations with a different estradiol radioimmunoassay revealed elevated plasma concentrations of estradiol before and after stimulation with the GnRH-analogue busereline in five dogs with feminizing testicular tumors compared with five healthy control dogs (fig. 8.11). These findings indicate that feminization in dogs with testicular tumors is probably caused by increased secretion of estrogens by the tumor. It was also found that Sertoli cell tumors secrete increased amounts of bioactive inhibin,[83] but the significance of this finding is unclear at present.

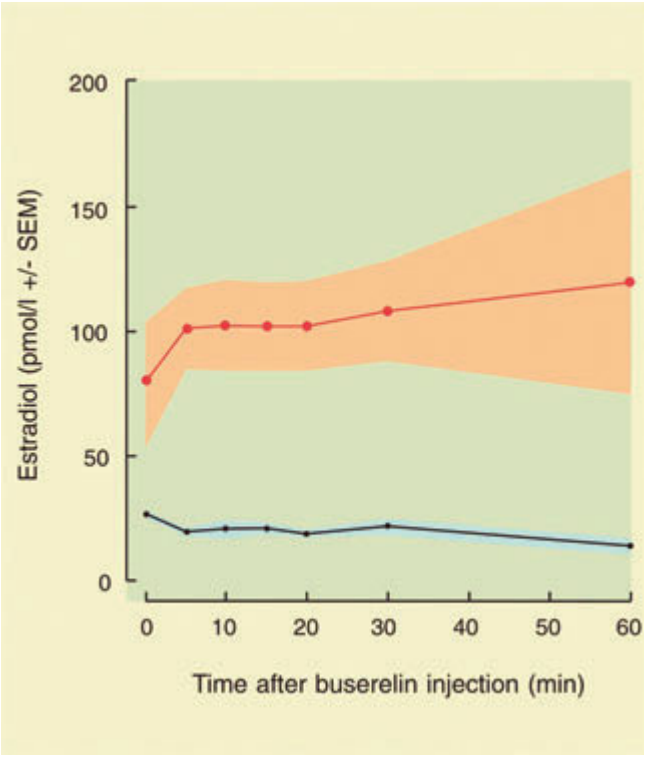


Figure 8.11:
Plasma concentration of estradiol in five control male dogs (blue) and five with Sertoli cell tumor (beige) at various times after IV administration of 0.5 µg buserelin per kg body weight.

Clinical manifestations

Testicular tumors cause noticeable testicular enlargement. In cryptorchid dogs this may result in a palpable abdominal mass. Dogs with testicular neoplasia may have bilaterally symmetri-

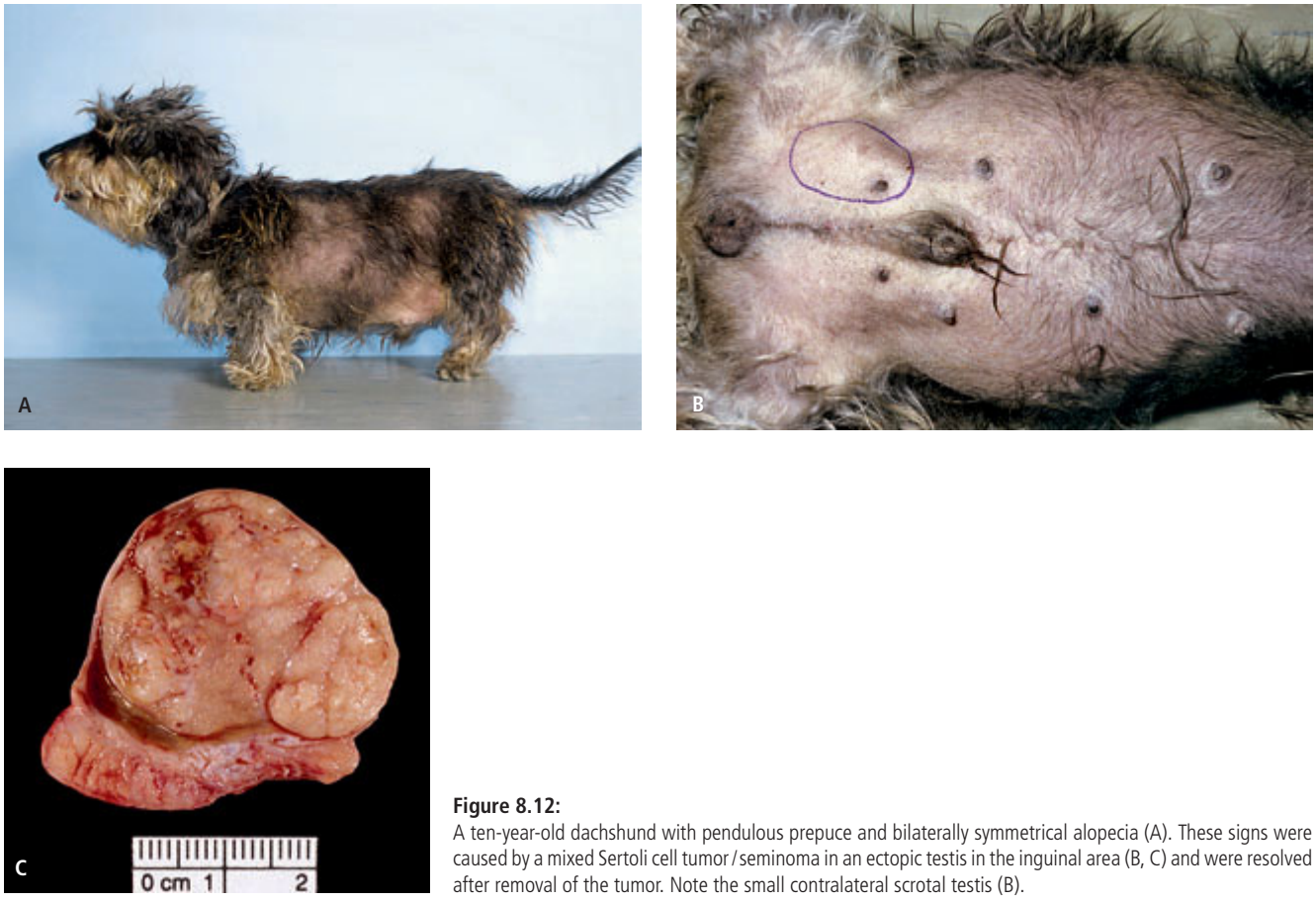


Figure 8.12:
A ten-year-old dachshund with pendulous prepuce and bilaterally symmetrical alopecia (A). These signs were caused by a mixed Sertoli cell tumor / seminoma in an ectopic testis in the inguinal area (B, C) and were resolved after removal of the tumor. Note the small contralateral scrotal testis (B).

cal alopecia (fig. 8.12), atrophy and pigmentation of the skin, and signs of feminization such as gynecomastia (fig. 8.13), a pendulous prepuce (fig. 8.12), atrophy of the prepuce, and atrophy of the contralateral testis, and they may be attractive to other male dogs. There may be blood dyscrasias varying from thrombocytopenia to pancytopenia. In severe cases this may lead to hemorrhagic diathesis and anemia (fig. 8.14).

Occasionally dogs with an intra-abdominal testis tumor are presented as an emergency, due to testicular torsion.[85,86] Anorexia and lethargy may be accompanied by swelling of the scrotal and inguinal areas and a stiff gait. Physical examination reveals a painful abdominal mass. It should be added that non-neoplastic abdominal testes may also undergo torsion.[87] Scrotal testicular torsion is very rare in dogs.[88]

Differential diagnosis

Testicular enlargement by tumor should be differentiated from orchitis and testicular torsion. The skin disorders may mimic other endocrine diseases such as hypothyroidism (chapter 3.3), hypercortisolism (chapter 4.3), and possibly growth hormone deficiency (chapter 2.2.2). Blood dyscrasias may also be caused by other conditions, such as idiopathic or immune-mediated thrombocytopenia, myeloproliferative disorders, and aplastic anemia. Symptoms and signs of abdominal testicular torsion are nonspecific and other causes of »acute abdomen« must be considered as possible differential diagnoses.

Diagnosis

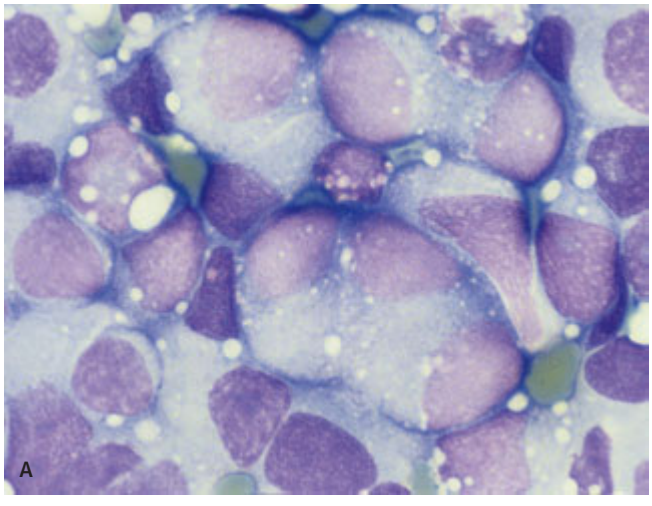
Testicular neoplasia in dogs and cats is diagnosed by the finding of a palpable mass in a scrotal or ectopic testis. The consistency is usually firm and these tumors are rarely found painful by palpation. In dogs with testicular enlargement due to orchitis or testicular torsion the swelling is mostly soft and painful. In cryptorchid dogs, testicular tumors may not be noticed unless skin disorders or signs of feminization develop. Cytological examination of a fine-needle aspiration biopsy may reveal the type of testicular neoplasm (fig. 8.15). Ultrasonography of scrotal testes may be used to detect small neoplasms in the testis that otherwise may be missed by palpation.

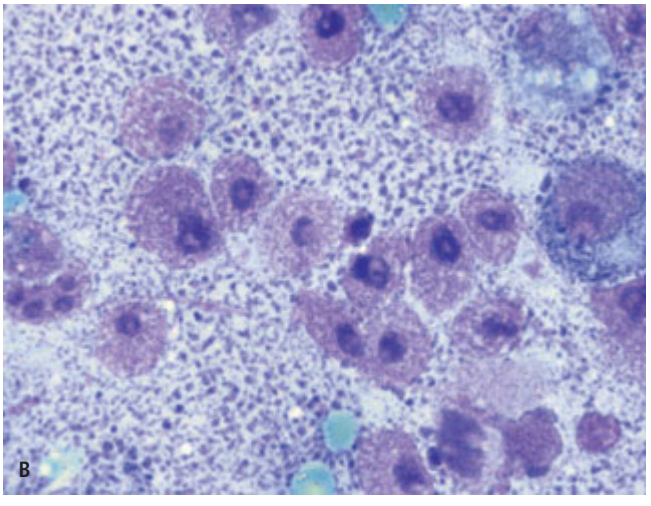


Figure 8.13:
Gynecomastia in a seven-year-old Bouvier with a Sertoli cell tumor in an abdominal testis.



Figure 8.14:
Petechia on the penis of a dog with thrombocytopenia, which can occur as a result of estrogen-induced bone marrow depression.





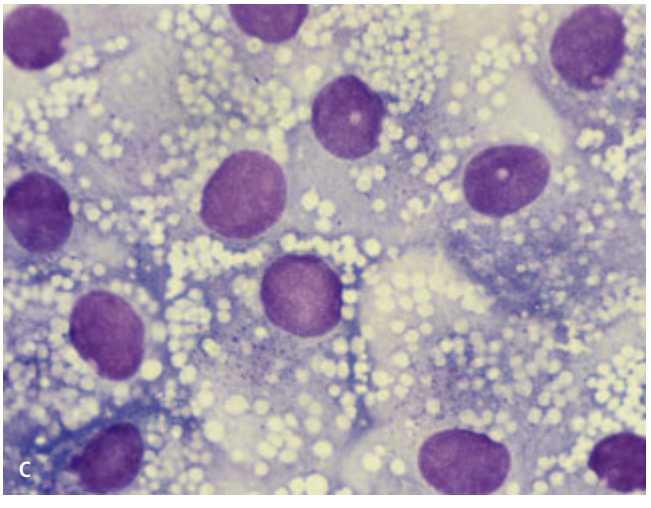


Figure 8.15:
Fine-needle aspiration biopsies of canine testes.
(A) Sertoli cell tumor. There is a uniform population of pleomorphic cells. Note the marked variability in nuclear size. The nuclei are generally round to oval and have a finely-clumped chromatin pattern with prominent and occasional multiple nucleoli. There are variable degrees of cytoplasmic vacuolization.
(B) Seminoma. Note the marked variations in cell and nuclear size. Nuclei have coarsely-clumped chromatin and usually contain a single, large, irregularly-shaped nucleolus. There is often a high mitotic index. Cytoplasm is lightly basophilic and granular.
(C) Leydig cell tumor. There is a uniform population of cells with abundant cytoplasm and numerous small cytoplasmic vacuoles containing cholesterol (May-Grünwald Giemsa stain, x1000).

This technique may also help in the search for the presence of an ectopic testis tumor (see also chapter 8.3).

Treatment

Testicular tumors are treated by orchidectomy. Removal of the tumor is usually simple, but blood transfusions may be necessary in patients with severe blood dyscrasias. If both testes are tumorous, both should be removed. In cases of unilateral testis tumor the contralateral scrotal testis, which may be atrophic due to suppression of GnRH secretion by feedback of the autonomously hypersecreting tumor, can be left in place. An ectopic contralateral testis is best removed because of the high incidence of Sertoli cell tumors in nonscrotal testes.

Prognosis

The prognosis after surgical removal of the affected testis depends on the type of tumor but is usually good. Associated skin disorders and signs of feminization are reversible, but more severe forms of blood dyscrasia are not amenable to treatment and can result in fatal complications. Metastases are uncommon but may occur with all types of testicular tumors. The reported incidence is 1–10 % for Sertoli cell tumors, 3 % for seminomas, and 2–3 % for Leydig cell tumors.[34,59–61,67]

8.5 Male infertility

Infertility in the male dog or cat may be congenital (thus no offspring) or acquired (may have sired offspring). Possible causes of congenital infertility include an abnormal hypothalamic-pituitary-gonadal axis, chromosomal and/or sexual differentiation abnormalities (see chapter 6), segmental aplasia of the ducts, cryptorchidism (chapter 8.3), and defects in spermatogenesis. Acquired fertility disorders may be caused by testicular hyperthermia due to inflammation or environmental factors, testicular neoplasia (chapter 8.4), infections of the reproductive tract, endocrine disorders, exposure to toxins, medication, or may be idiopathic. Idiopathic infertility is the most common form in men (~50 %) and it is assumed that a large proportion of these have a genetic origin.[89] A similar high incidence of idiopathic infertility is suspected in the dog. Endocrine disorders associated with infertility are hypothyroidism and hypercortisolism. Hypothyroidism caused by lymphocytic thyroiditis was shown to be related in incidence to lymphocytic orchitis and reduced fertility in a colony of beagles.[90] However, hypothyroidism induced by ^{131}I did not change reproductive function in male dogs.[91] Exogenous glucocorticoid excess in dogs was found to exert negative feedback on the secretion of LH by the pituitary, resulting in decreased secretion of testosterone by the Leydig cells.[11] Basal plasma LH concentration in dogs with spontaneous hypercorticolism did not differ from that in healthy dogs, but the response to suprapituitary stimulation tended to be lower than in healthy dogs.[92]

Clinical manifestations

Male infertility ranges from complete absence of libido to the inability to sire offspring in spite of normal mating. Depending on the cause there may be other signs that are characteristic of the underlying condition.

Diagnosis

Diagnosis of male infertility is based on a Breeding Soundness Evaluation (BSE), which consists of a medical and reproductive history, a complete physical examination, semen collection for semen analysis, testing for *Brucella canis*, and ultrasound examination of the testes, epididymes, and prostate. Endocrine testing of the hypothalamic-pituitary-gonadal axis by a GnRH-stimulation test may be necessary (chapter 12.5.1). Particular attention should be paid to endocrine diseases such as hypothyroidism and hypercortisolism. Testicular biopsy is performed only if the results of all less invasive methods are inconclusive.

Possible results of semen analysis include oligozoospermia (< 200 million sperms in the entire ejaculate, providing that the ejaculate was collected in a representative way); teratozoospermia (< 70 % of sperm cells with normal morphology); asthenozoospermia (< 50 % progressively forward motility); leukozoospermia (> 2000 white blood cells per µl in the ejaculate); azoospermia (no sperm observed in the ejaculate); and hemozoospermia (blood seen grossly or in cytological smears). More than one abnormality may be present in a single sample (fig. 8.16).

Testicular biopsy is indicated in dogs which are persistently azoospermic or severely oligospermic. A wedge biopsy is preferred over a percutaneous needle biopsy because specimens obtained with needle biopsies contain insufficient tubules in circular cross section to allow detailed histomorphometric analysis of spermatogenesis.[93] Testicular biopsy is not entirely harmless and should be undertaken with care. However, if superficial avascular areas are biopsied the method can be considered safe.[94] Antisperm antibodies induced by Trucut testicular biopsies were found to be transient and nonpredictive of changes in the total number of morphologically normal motile sperm cells.[95]

Leukozoospermia indicates prostatitis (with or without benign prostatic hyperplasia), orchitis, epididymitis, and/or urinary tract disease. Orchitis and epididymitis are diagnosed by ultrasonography and fine needle aspiration biopsy. The latter method should be used with care. Epididymal aspiration may cause hematoma, fibrosis, or sperm granuloma, which could result in obstruction.[96] Diagnosis of infection of the reproductive organs requires bacteriological culture of the ejaculate. Mycoplasma and *E. coli* are the infective organisms cultured most frequently.[97]

Teratozoospermia may be caused by insufficient testosterone production, hyperthermia, reproductive tract infection, or genetic or familial disorders. It is often observed in combination with leukozoospermia and infection. Asthenozoospermia may be caused by ciliary dyskinesia, antisperm antibodies, benign prostatic hyperplasia, reproductive tract infection, or improper collection or handling of the sample. Oligozoospermia may be caused by toxins, medication (sex steroids, anabolic steroids, glucocorticoids, ketoconazole, cimetidine, and chemotherapeutic agents), reproductive tract infection or obstruction, and benign prostatic hyperplasia. It may also be due to incomplete ejaculation.

Azoospermia may be the result of a congenital defect or epididymal blockage. As in oligozoospermia it can be due to incomplete ejaculation. When it is suspected, semen should be collected several times under different circumstances to ensure that a full ejaculate is obtained. Collection should be made at least three times at two month intervals before more invasive diagnostic procedures are attempted. Alkaline phosphatase (AP), which is secreted by the epididymis, can be measured in the seminal plasma of the first and second fractions of the ejaculate of oligozoospermic or azoospermic dogs. This may help to differentiate between complete and incomplete ejaculation, for in complete ejaculation AP is $>$ 5000 IU/l[98,99] while values $<$ 5000 IU/l in several ejaculates indicate ductal blockage rather than incomplete ejaculation. In such cases fine needle aspirates can be obtained from the epididymes, but with the risk of causing sperm granuloma and induction of antisperm antibodies. A search for chromosomal abnormalities should be started in dogs with a lifelong history of hypoplastic testes and no sperm.

Treatment

Treatment of male infertility depends on the underlying cause. Infections of the reproductive tract are treated with long-term (four to six weeks) appropriate antibiotic therapy. Antibiotics that penetrate and maintain therapeutic levels in the male reproductive tract are trimethoprim-sulfa and fluorinated quinolones. Acute orchitis and/or epididymitis require rapid diagnosis and treatment. Unilateral orchidectomy is successful in preserving a normal spermogram from the remaining testis in more than 75 % of the cases. Aggressive antibiotic therapy may also be successful, but sperm granulomas often form, leading to epididymal blockage. Blockage of the reproductive tract can be treated surgically, but the chance of success is small.

If the results of a GnRH-stimulation test reveal a high plasma level of estradiol-17β and relatively low testosterone, there may be hyperfunction of the aromatizing enzyme system and treatment with aromatase inhibitors can be considered. These drugs inhibit the enzymatic transformation of testosterone to estradiol-17β and as a result plasma estradiol decreases and plasma testosterone increases.[100] In several selected clinical cases this therapy has improved semen quality in dogs.[100]

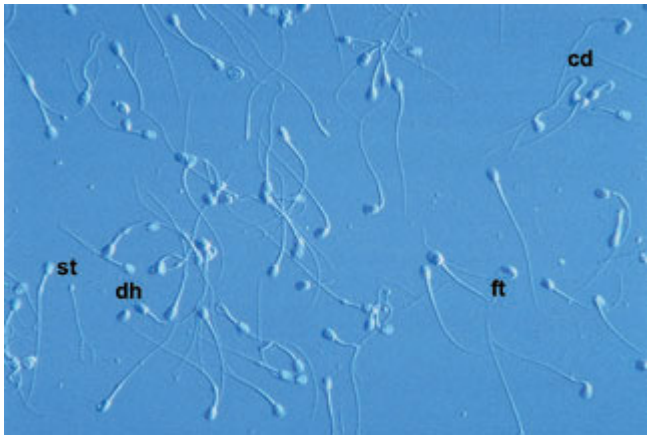


Figure 8.16:
Differential interference contrast photograph (x300) of semen from a healthy dog with 85 % of normal spermatozoa. Abnormalities include detached head (dh), sperm tail without head (st), cytoplasmic droplet (cd), and folded tail (ft). (Courtesy of Prof. Dr. B. Colenbrander.)

Treatment of infertility of male dogs with GnRH agonists, dopamine agonists, and neutriceuticals such as glycosaminoglycans and antioxidants, have not been well evaluated and should therefore be used with caution and only after a thorough diagnostic work-up.[101,102]

Owners of treated animals should be informed that the spermatic cycle in the dog requires approximately 62 days plus an additional 15 days for sperm transport through the epididymis, during which time the sperm cells mature. Response to treatment may require several cycles of spermatogenesis and thus several months may be needed for regeneration and improvement. In all cases in which male infertility is likely to be a hereditary disorder, treatment should not be offered and the dog should be withdrawn from breeding.

Prognosis

Leukozoospermia due to infection of the reproductive tract has a guarded prognosis because there is considerable risk of epididymal blockage by scar tissue. Teratozoospermia has a guarded prognosis, but exceptionally well-planned matings may be successful. Abnormal sperm morphology in men is sometimes correlated with a high incidence of chromosomal abnormalities of the affected sperm, which may lead to aneuploidy in embryos resulting from in vitro fertilization or intracytoplasmic sperm injection.[103] There has been no reported correlation of teratozoospermia with birth defects in puppies after normal matings. Asthenozoospermia has a guarded prognosis, but in some cases sperm motility can be improved considerably by extending the semen with an extender. Oligozoospermia and azoospermia generally have a poor prognosis, but semen quality may improve if there are underlying causes that can be treated successfully.

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9 Calciotropic Hormones

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9.1 Introduction

Calcium is the most abundant mineral in mammals. It is an essential structural component of the skeleton and contributes to many important physiological functions, such as nerve conduction, muscle contraction, enzyme activity, and blood coagulation. About half of the circulating calcium is loosely bound to plasma proteins (mostly albumin). Ten percent is bound to other ions and the remainder comprises the biologically significant ionically active fraction. It is essential that the concentration of calcium remains constant despite the variations in its intake and excretion. In healthy states the total plasma calcium concentration varies within narrow limits and is fairly constant even under extreme dietary variations (fig. 9.1). Calcium homeostasis is maintained by direct mechanisms and by calciotropic hormones. Three organs are especially involved in maintenance of the calcium homeostasis: the gut, the kidney, and the skeleton.

Direct regulation

When calcium is absorbed from the intestine, it tends to raise the plasma calcium concentration. Independent of hormonal control some calcium is deposited in bone and less is dissolved from the soluble phase into the circulation. In addition, more calcium is filtered by the glomeruli and excreted. When calcium concentration decreases, more calcium enters from the labile pool into the circulation and less is lost via the kidneys (fig. 9.2). In both situations endogenous fecal excretion does not seem to be much influenced.

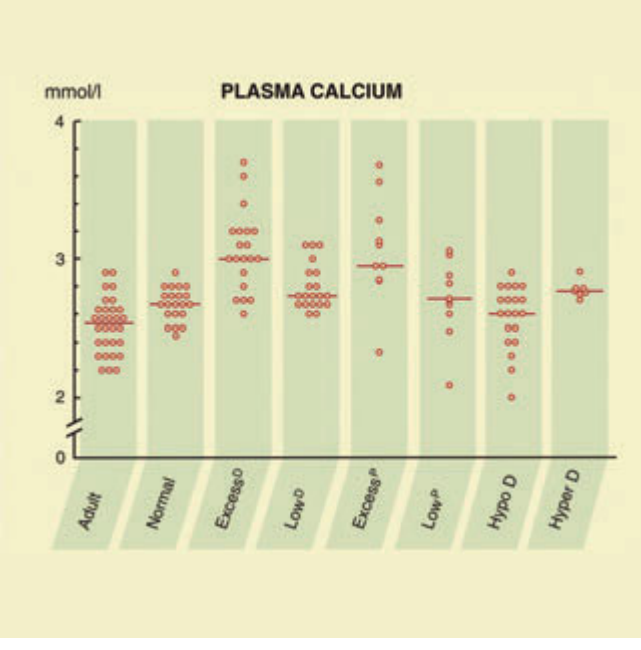


Figure 9.1:
Plasma calcium concentrations (with median values) are given for adult dogs and young dogs (all younger than six months) receiving food containing 1.1 % calcium and 12.5 µg vitamin D/kg diet (Normal), young Great Danes[1] fed 1.5 months a diet with 3.3 % Ca (Excess^{D}) or 0.55 % Ca (Low^{D}), young poodles[2] fed 1.5 months a diet with 3.3 % Ca (Excess^{P}) or 0.33 % Ca (Low^{P}), mongrel dogs on standard food only without vitamin D (Hypo D), and young Great Danes fed 1.5 months a diet containing 100 µg vitamin D/kg diet (Hyper D).[3] Despite 6–10x difference in daily calcium or vitamin D intake, the median plasma calcium concentrations vary within narrow limits.

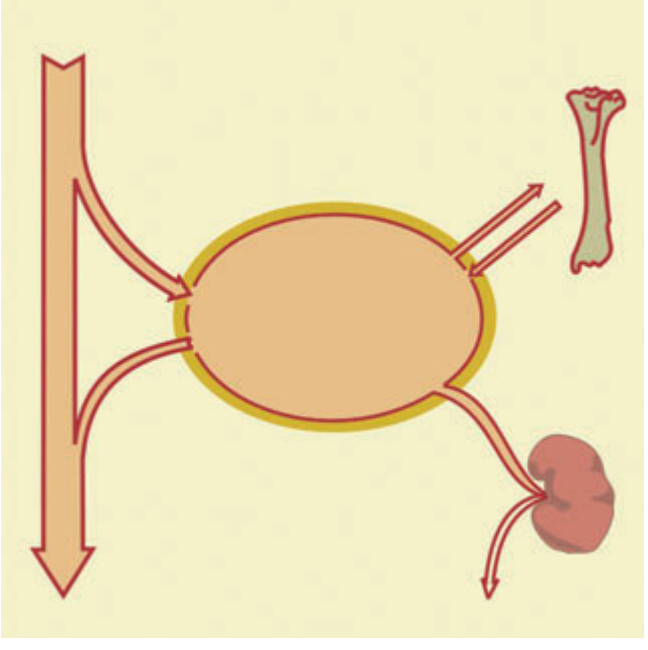


Figure 9.2:
Three organs are especially involved in calcium homeostasis: intestine, kidney, and skeleton. When calcium is absorbed from the intestine, the calcium concentration in the extracellular fluid will tend to increase. Due to direct regulation, more calcium will be stored in the labile phase of the skeleton and more calcium will be filtered in the glomeruli, which contributes to the normalization of the calcium concentration in the extracellular fluid.

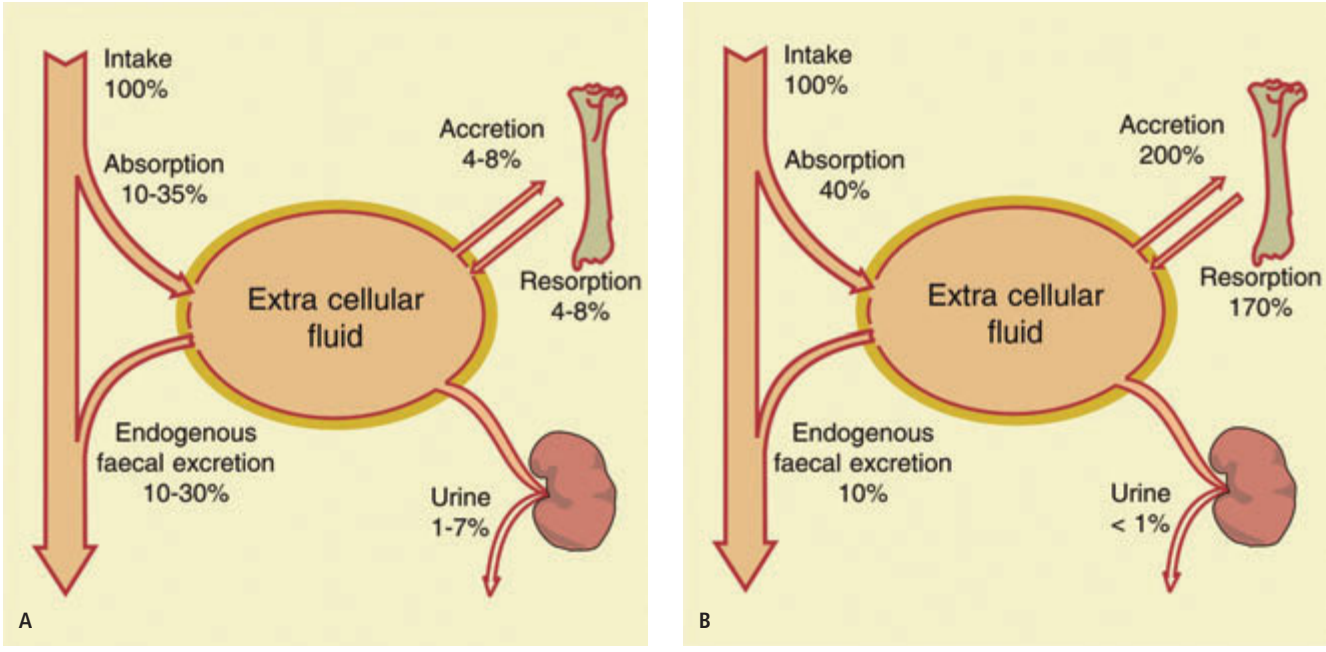


Figure 9.3:
The relative calcium fluxes in adult and young growing dogs.
(A) In adult dogs a calcium intake of 100 mg per kg body weight per day covers all losses.
(B) In young dogs calcium metabolism is characterized by high calcium turnover in the skeleton and more efficient absorption; the requirements in absolute amounts depend on the size and growth rate of the dog, and may vary from 50–350 mg/kg body weight.

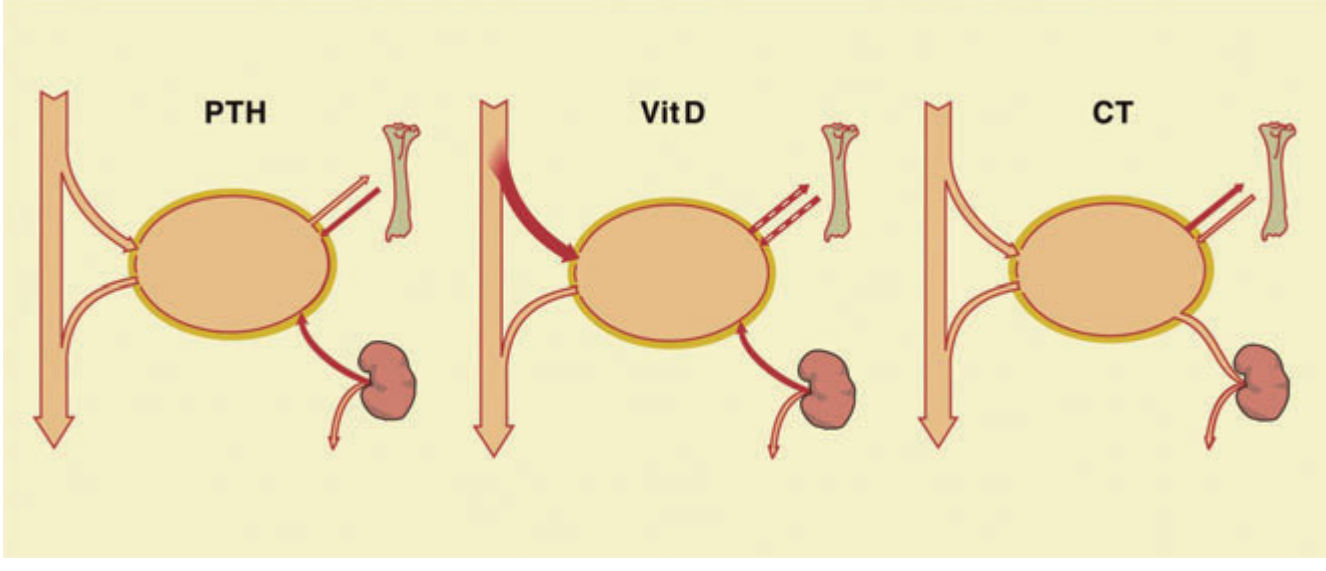


Figure 9.4:
Influences of calciotropic hormones on calcium metabolism. PTH increases osteoclasia and calcium reabsorption in the renal tubules. Vitamin D metabolites increase active calcium absorption in the intestine and renal reabsorption; in addition they activate osteoclasia and osteoid and cartilage mineralization. CT decreases osteoclastic activity and thus increases bone mineralization.

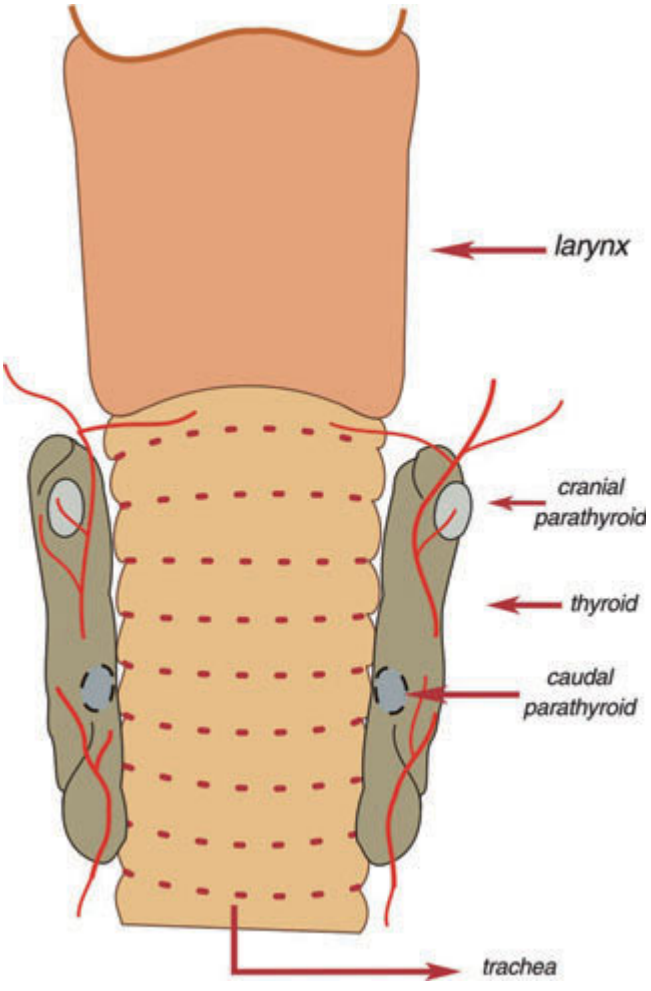


Figure 9.5:
The location of the parathyroid glands. The cranial or »external« parathyroids are loosely attached to the thyroid capsule. The caudal or »internal« parathyroids are subcapsular and usually embedded in thyroid tissue.

Hormonal control

There is an efficient hormonally controlled system that helps to retain calcium in animals living in a calcium-deficient environment and eating food with a low calcium content (table 9.1), such as carnivores may eat when bones are not part of the meal. In adult animals, a low calcium intake may be sufficient to replace the losses in urine and feces (fig. 9.3A). However, growth presents a formidable challenge for maintaining plasma calcium concentration in the normal range, since large amounts of calcium are transferred to the growing skeleton (fig. 9.3B). This is especially so in young dogs of large breeds.[4] In conditions in which calcium homeostasis is under stress (such as rapid growth, over- or under supplementation, or pregnancy and lactation) calcium metabolism is regulated by the calciotropic hormones: parathyroid hormone (PTH), vitamin D, and calcitonin (CT) (fig. 9.4). Synthesis and release of these hormones are mainly triggered by variations in plasma calcium concentration.

9.1.1 Parathyroid hormone

9.1.1.1 Development of the parathyroid glands

Developmentally, the parathyroid glands arise from the third and fourth endodermal pharyngeal pouches. Studies in mice have demonstrated that the transcription factor encoded by Gcm-2 is a key regulator of parathyroid gland development. The expression of this gene is restricted to the chief cells, and if this gene is mutated the parathyroid glands fail to form.[5] The parathyroid glands generally consist of four small oval disks with a diameter of 1–4 mm. The two largest parathyroids arise from the fourth branchial pouches and remain almost stationary during embryonic development, accounting for their final location at the cranial pole of the thyroid (fig. 9.5). Two smaller parathyroids are usually located beneath the thyroid capsule, embedded at various depths near the caudal thyroid pole. They develop from the third branchial pouches in association with the thymus; migration with the descent of the thymus may give rise to ectopic parathyroid tissue.

9.1.1.2 PTH synthesis and secretion

The major cell of the parathyroids is the chief cell. It has clear or slightly eosinophilic cytoplasm, depending on the amounts of intracellular fat and glycogen (fig. 9.6). The cytoplasm of active chief cells has a higher density due to the abundance of

Table 9.1: Analysis of foodstuffs for carnivores

	Dry matter*	Calcium*	Phosphorus*	Vitamin D+
Horse meat	25.5	0.03	0.18	4 IU
Heart	24.8	0.01	0.20	4 IU
Rumen	23.3	0.11	0.14	n. k.
Liver	27.1	0.01	0.36	80 IU
Poultry by-products	30.1	0.02	0.20	n. k.
Egg	25	0.04	0.15	100 IU
Catfish	20.0	0.02	0.18	20 IU
Minimal requirement	100	0.8		
Recommended allowance for growth (NRC 2006)	100	1.2	1.0	55 IU

* = grams per 100 g product with dietary energy density of 4000 kcal ME/kg
+ = IU per 100 g product (1 IU vitamin D = 0.025 µg)
n. k. = not known
Absolutely and relative to phosphorus, the calcium content of animal foodstuff is too low to fulfill the recommendations.

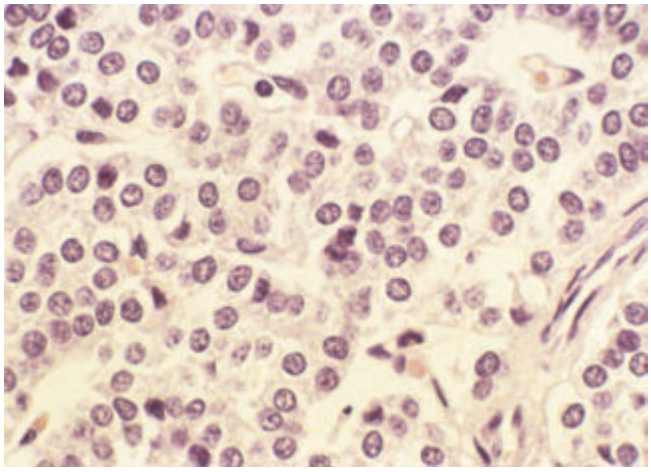


Figure 9.6:
Histological section of the parathyroid gland of a dog with renal secondary hyperparathyroidism; note the large pale (= active) chief cells (H&E, x600).

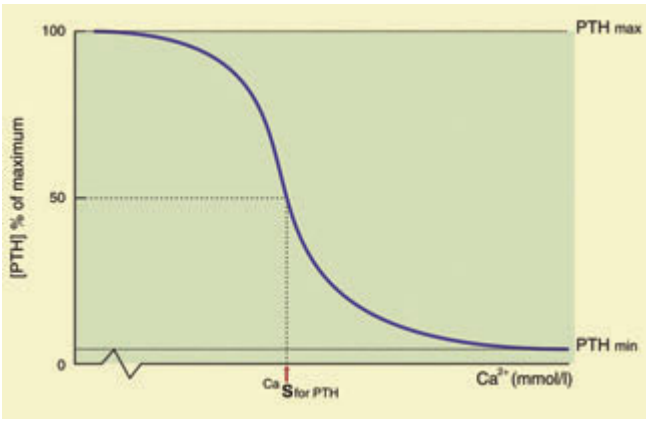


Figure 9.7:
Inverse sigmoidal relationship between the extracellular ionized calcium concentration and PTH secretion. $Ca_{S\ for\ PTH}$ indicates the Ca^{2+} setpoint for PTH secretion, i.e., the extracellular ionized calcium concentration suppressing the plasma PTH concentration to 50 % of its maximum. Also note that there is a nonsuppressible element to PTH secretion even at very high calcium concentrations.

9

organelles and membrane-bound secretion granules as well as to the loss of glycogen and lipid. PTH is an 84 amino acid, single chain polypeptide synthesized by proteolytic cleavage of a preprohormone (chapter 1.2.5). The amino acid sequences of canine and feline PTH are highly homologous with the sequence of this peptide in other mammalian species.[6,7] The intact 1–84 molecule is the major circulating form. The full biologic activity of the intact hormone resides within the amino-terminal 1–34 fragment.

In the absence of a stimulus for PTH release, as in hypercalcemia, there is increased degradation of intact PTH causing the release of carboxy-terminal fragments (C-PTH). Since C-PTH fragments do not bind to the receptor of PTH, they have long been considered to be biologically inactive. However, recent findings suggest that C-PTH fragments may exert effects opposite to those of PTH(1–34).[8] Clinically, C-PTH fragments may cause PTH resistance in renal failure patients (see also chapter 9.3.2).[8] In situations of hypocalcemia, degradation of PTH within the parathyroid cell is minimal, and the major product released is intact bioactive PTH(1–84). Other adaptive mechanisms of the parathyroid cell to sustained hypocalcemia are increased PTH gene expression and proliferation of chief cells.

Circulating C-PTH fragments may also be derived from peripheral (largely renal and hepatic) breakdown of the intact hormone, whereas their renal excretion is decreased. This may result in high immunoreactive PTH concentrations not related to the concentrations of bioactive PTH, especially when radioimmunoassay are used that recognize only the carboxy-terminal parts of PTH.

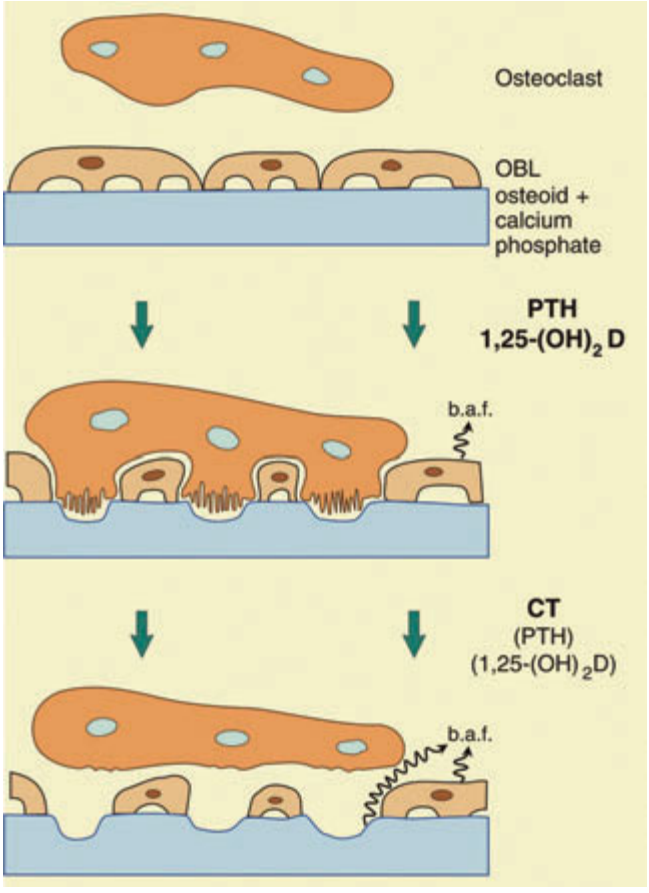


Figure 9.8:
Bone lining cells, osteoblasts (OBL), separate bone from nonresorbing osteoclasts. PTH and calcitriol ($1,25(OH)_2D$) change the shape of the OBL, allowing osteoclasts to resorb bone. CT prevents bone resorption by promoting the retraction of the brush border of the osteoclasts; this occurs even in the presence of PTH and/or $1,25(OH)_2D$. Biologically active factors (b.a.f.), released by OBL and from the bone during resorption, have chemotactic and mitogenic actions on bone cells.

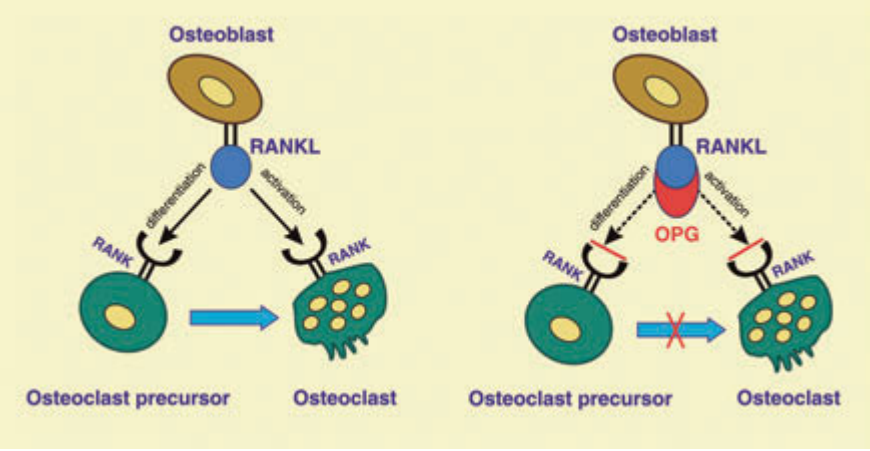


Figure 9.9:
Osteoclast differentiation and activation. In normal physiological bone remodeling the osteoblast plays a central role. Left: The ligand of the receptor activator of nuclear factor -κβ (RANKL) is produced by osteoblasts and stroma cells and binds to the receptor RANK present on osteoclasts. Thereby bone resorption is stimulated. The production of RANKL is under the influence of the calciotropic hormones PTH and calcitriol. Right: On the contrary, osteoblasts produce osteoprotegerin (OPG) that acts as a decoy receptor and blocks the RANKL binding to RANK. Thereby osteoclast production and activation is blocked resulting in inhibition of bone resorption.

9.1.1.3 Regulation of PTH secretion

The ionized fraction of blood calcium is the most important determinant of PTH secretion. PTH secretion is regulated at a setpoint that maintains the concentration of plasma ionized calcium within narrow limits. Concentrations below the setpoint stimulate and those above it inhibit hormone secretion (fig. 9.7).The PTH response to similar reductions in calcium concentration may be less for slow than for fast reductions in calcium concentration.[9] In addition to the ionized calcium concentration, calcitriol (1,25-$(OH)_2$D; a metabolite of vitamin D) and phosphate have significant roles in regulating PTH secretion.

The effects of changes in plasma ionized calcium concentration on PTH secretion occur within minutes. The molecular mechanism underlying ionized calcium-regulated PTH secretion involves activation of a cell surface calcium-sensing receptor. In this context it should be mentioned that often total (= bound and ionized), rather than ionized, calcium is measured. Therefore one should be aware of factors that may influence the fraction of plasma calcium that is ionized. Of these, the circulating albumin concentration is of greatest relevance, since it is the main calcium-binding protein. When in patients with hypoalbuminemia a »normal« plasma calcium concentration is found, there may actually be elevated levels of ionized calcium. Acid-base status also influences the protein binding of calcium; alkalosis decreases and acidosis increases the ionized calcium concentration.

9.1.1.4 PTH action

Binding of PTH to a plasma membrane receptor, the PTH / PTHrP receptor, causes a rise in cyclic 3',5'-adenosine monophosphate (cAMP) and possibly other second messengers (see also fig. 1.4) in cells of the main target organs, i.e., kidney and bone. In the kidney, PTH enhances reabsorption of calcium from the glomerular filtrate and increases excretion of phosphate. When plasma PTH levels are persistently increased, PTH also stimulates the renal production of calcitriol. The effects of PTH on bone can be either catabolic or anabolic, depending on the mode of secretion. High concentrations of PTH cause catabolic actions: osteoblasts shrink and change their shape, allowing osteoclasts to come into contact with bone-matrix surface and to resorb bone. Osteoclasts are recruited and activated by local biologically active factors (figs. 9.8, 9.9) originating from osteoblasts and resolved from bone matrix. Thereby, PTH causes the release of calcium and phosphate into the extracellular fluid. Intermittent low doses of PTH cause anabolic actions in bone, with an increase in the number of osteoblasts, the alkaline phosphatase concentration, and collagen synthesis. The plasma PTH concentration in dogs decreases during the first months of life (fig. 9.10) and thereby parallels bone cell activity during skeletal growth.

Overall, the combination of calcium mobilization from bone and retention of calcium by the kidneys causes the plasma calcium concentration to rise. In addition, PTH contributes indirectly to the maintenance of normocalcemia by stimulating the formation of calcitriol, which in turn enhances intestinal calcium and phosphate absorption and calcium reabsorption and mobilization from bone. The phosphatemic effect of PTH and calcitriol tends to blunt the hypercalcemic effect of PTH owing to the formation of calcium phosphate complexes, but this is counteracted by the phosphaturic action of PTH.

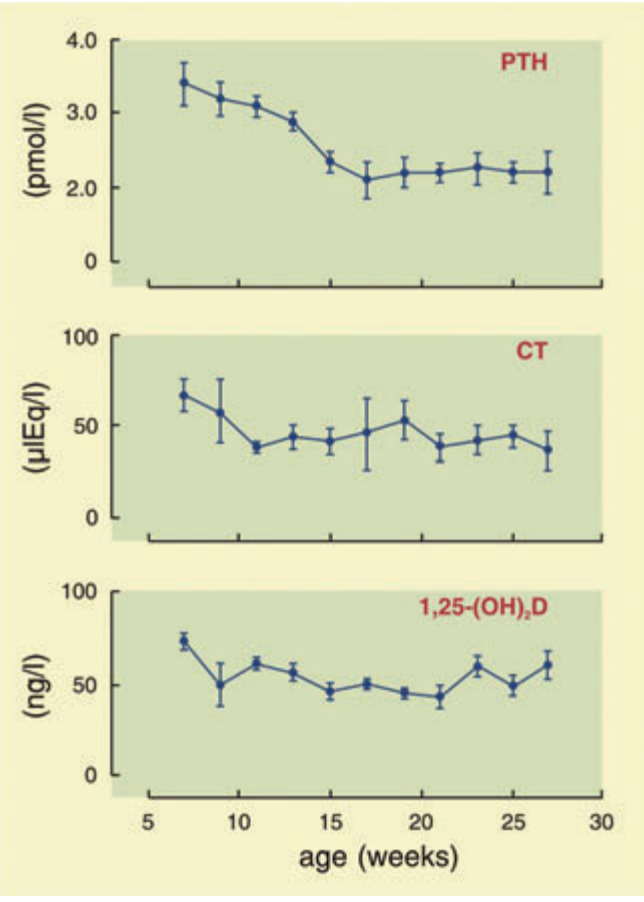


Figure 9.10:
Plasma concentrations (mean ± SEM) of immunoreactive PTH, CT, and 1,25-$(OH)_2$D in growing Great Danes from six to 26 weeks of age. Both PTH and CT, but not 1,25-$(OH)_2$D, are significantly negatively correlated with age.

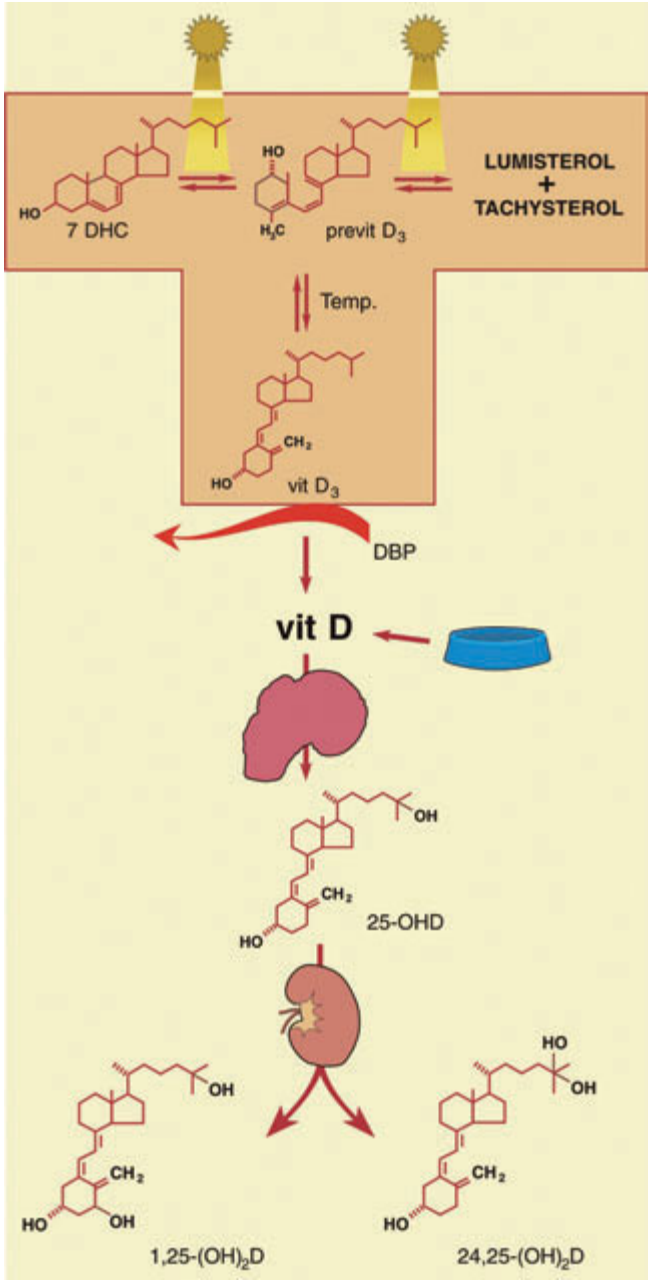


Figure 9.11:
In the skin (beige area) of most mammals,[11] but not the dog and cat,[10] dehydrocholesterol (7 DHC) is photosynthesized under the influence of sunlight (UV-B) into provitamin D_3, followed by a temperature-dependent isomerization into vitamin D_3. Other isomers including lumisterol and tachysterol can be formed under prolonged radiation. When synthesized or absorbed with the food, vitamin D is bound to vitamin D-binding proteins (DBP) and transported to the liver for its first hydroxylation by 25-hydroxylase into 25-OHD, followed by a second hydroxylation in the kidney into 24,25-$(OH)_2$D and the biologically most active metabolite, 1,25-$(OH)_2$D by 24- and 1α-hydroxylase, respectively. (Modified from How et al., 1994.)[11]

9.1.2 Vitamin D

9.1.2.1 Vitamin D sources and synthesis

There are two forms of vitamin D: ergocalciferol (vitamin D_2) that is naturally occurring in plants and cholecalciferol (vitamin D_3) that is synthesized by vertebrates. Amphibians, reptiles, birds, omnivores, and herbivores synthesize vitamin D_3 in the skin under the influence of UV light. However, dogs and cats are not capable of synthesizing sufficient vitamin D_3 in the skin (fig. 9.11).[10] This is due to low levels of 7-dehydrocholesterol (7-DHC) in the skin[11] caused by high degradation of 7-DHC by a reductase.[12] Thus, vitamin D is an essential vitamin for dogs and cats, as they are solely dependent on dietary resources to meet their vitamin D_3 requirement.

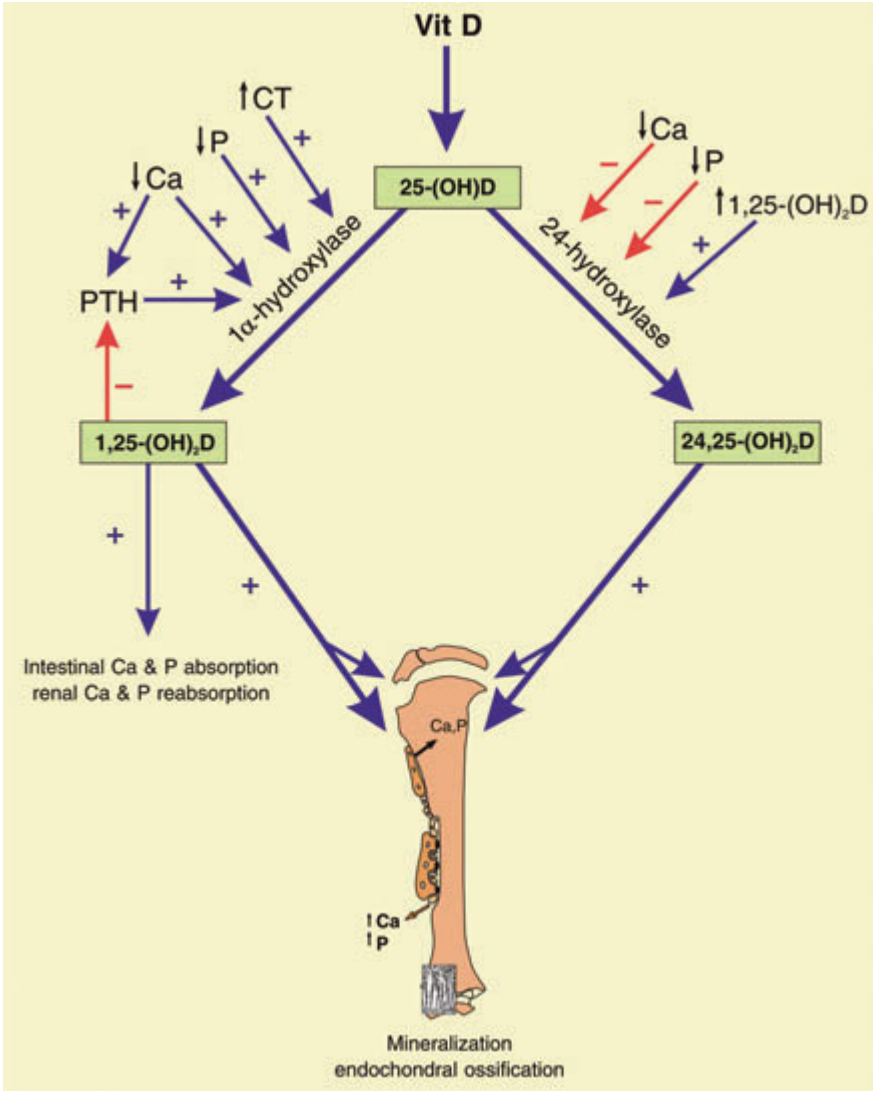


Figure 9.12:
General diagram of vitamin D metabolism and catabolism describing the regulation of the major vitamin D metabolites. (Modified from Hazewinkel and Tryfonidou, 2002.)[102]

9.1.2.2 Vitamin D metabolism

Vitamin D must be metabolically activated before it can produce its known physiological actions in target organs. Vitamin D is hydroxylated by 25-hydroxylase in the liver to 25-hydroxyvitamin D (25-OHD). The second, most important, step in the bioactivation of vitamin D is the formation of 1,25-dihydroxycholecalciferol (1,25-$(OH)_2$D = calcitriol), the biologically active vitamin D metabolite in target organs related to calcium homeostasis. In addition to calcitriol, another metabolite is produced in the kidney, i.e., 24,25-$(OH)_2$D (fig. 9.11). This metabolite was first considered to be a product in the catabolic pathway of vitamin D with no biological action. However, 24,25-$(OH)_2$D is now considered to have biologic activity mainly in bone.[13,14] The enzymes responsible for the production of 1,25-$(OH)_2$D and 24,25-$(OH)_2$D in the kidney are 1α-hydroxylase and 24-hydroxylase, respectively. The catabolism of both vitamin D metabolites is mediated by 24-hydroxylase distributed in various tissues (fig. 9.12).

9.1.2.3 Regulation of vitamin D metabolites

The plasma level of all vitamin D metabolites is a function of production and metabolism/catabolism. 25-OHD and 24,25-$(OH)_2$D circulate in levels of nmol/l, whereas calcitriol circulates in levels of pmol/l. Plasma levels of 25-OHD reflect the vitamin D status (e.g., deficiency or intoxication). The

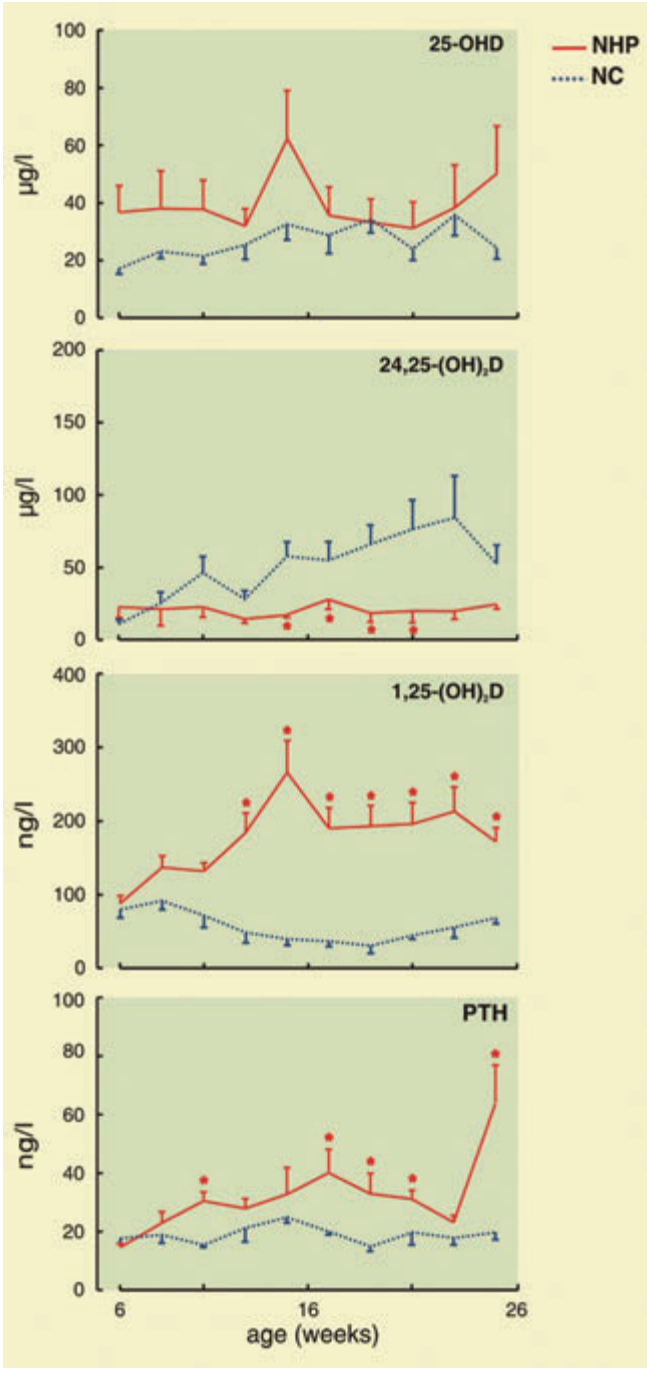


Figure 9.13:
Plasma concentrations of the vitamin D metabolites and of PTH in poodles with nutritional hyperparathyroidism (NHP) compared with normally fed dogs (NC) (0.05 % and 1.1 % Ca, respectively). The vitamin D content of the food was the same for both groups, reflected in no differences in 25-OHD concentrations in the plasma of both groups. In NHP, PTH increases $1,25(OH)_2D$ synthesis at the expense of hydroxylation into $24,25(OH)_2D$. This illustrates the reciprocal relationship between the synthesis of these metabolites (Modified from Nap, 1993.)[2] (* $p < 0.05$)

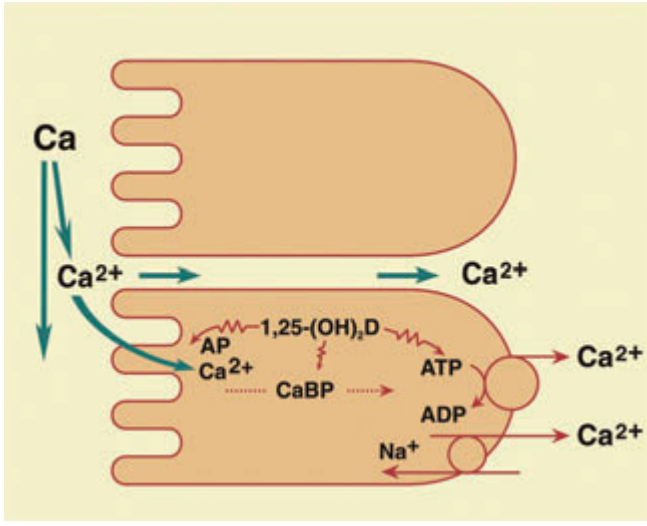


Figure 9.14:
Intestinal calcium absorption is the sum of passive and active absorption. Passive paracellular calcium absorption occurs under the influence of the concentration gradient between the intestinal lumen and the interstitium. Transcellular active absorption is influenced by $1,25\text{-}(OH)_2D$. In the intestinal cell synthesis of alkaline phosphatase (AP), calcium binding protein (CaBP), and ATP-ase are stimulated and thereby cellular absorption, transport, and expulsion of calcium.

plasma levels of calcitriol are a function of production and catabolism and are under hormonal and mineral influences, resulting in tight regulation of the plasma $1,25\text{-}(OH)_2D$ concentration (fig. 9.12). Renal synthesis of calcitriol is directly responsive to plasma concentrations of calcium, phosphate, PTH, and calcitonin. The catabolism of calcitriol is reciprocally related to the synthesis of calcitriol. For example, calcitonin decreases calcitriol formation but stimulates 24-hydroxylation, whereas PTH has the opposite effects (fig. 9.13). Calcitriol also regulates its own catabolism by induction of 24-hydroxylase activity at the level of the target organs and thereby regulates its own biological activity.

Plasma levels of the vitamin D metabolites differ between small and large breed dogs raised on the same balanced diet with sufficient vitamin D. In young large breed dogs plasma calcitriol concentrations ($\pm$ 300 pmol/l) are two times higher than in small breed dogs. This can be explained by the fact that the catabolism of calcitriol is lower in large breed dogs than in small breed dogs, in keeping with the high demands in the rapidly-growing skeleton of these dogs with juvenile gigantism.[15,16]

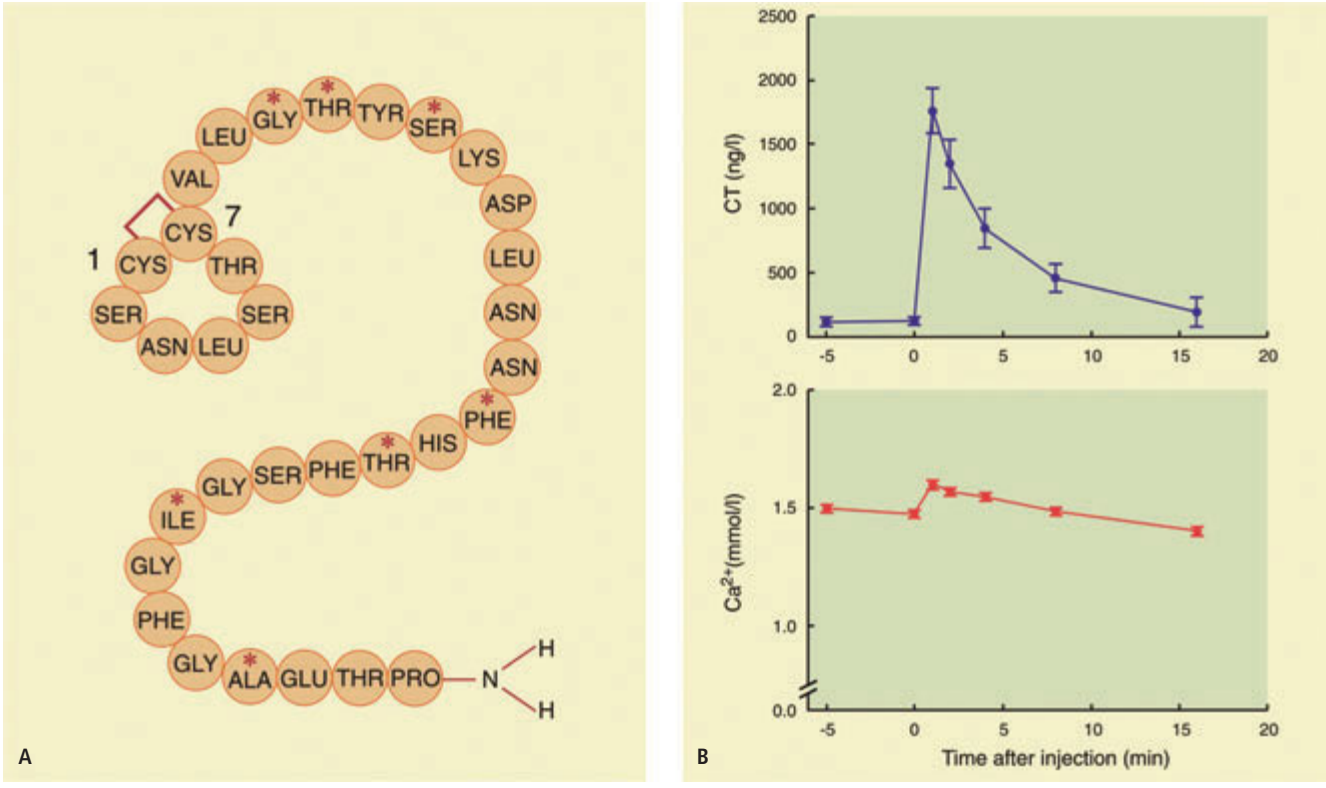


Figure 9.15:
(A) canine calcitonin (CT) consists of 32 amino acids with a disulfide bridge between the cysteines at positions 1 and 7, and only differing in seven amino acids (*) from bovine CT.[19] (B) Effects of the infusion of 1 mg calcium per kg body weight on plasma ionized calcium and CT concentrations of a healthy dog.

9.1.2.4 Vitamin D action

Calcitriol exerts its genomic effects through the nuclear vitamin D receptor in the three main target organs: bone, kidney, and intestine. These effects take ten to 14 days to be expressed. The main effects of calcitriol on bone include (1) an increase in the number of osteoclasts and their activity, and (2) a permissive role for PTH action on osteoblasts. 24,25-$(OH)_2$D mainly stimulates bone formation without a concomitant increase in bone resorption.[13,14] The effects of calcitriol on the kidney include increased reabsorption of calcium, phosphate, and sodium, and the feedback control of its own synthesis (closed feedback loop). In the mucosal cells of the proximal small intestine, calcitriol stimulates the uptake, transport, and extrusion of calcium (fig. 9.14). In the distal part of the small intestine, phosphate absorption is promoted similarly, although independent of calcium absorption.

In addition to its genomic effects, calcitriol also has effects that are too rapid (within minutes) to involve changes in gene expression, i.e., nongenomic pathways. These effects are mediated by a membrane receptor. The majority of the nongenomic actions of calcitriol have an uncertain role; it is speculated that they modulate the genomic actions of calcitriol.[17]

9.1.3 Calcitonin

The thyroid glands produce thyroid hormones and calcitonin (CT) in two distinct cell types, the thyroid follicular cells and the parafollicular or C cells, respectively (chapter 3.1). The C cells originate from the ultimobranchial bodies. These are a pair of transient embryonic structures derived from the fourth pharyngeal pouch and located symmetrically on the sides of the developing neck. The C cell precursors migrate from the neural crest bilaterally to the fourth pharyngeal pouches and become localized in the thyroid gland.[18] Within the thyroids the C cells are scattered in the interfollicular space, mostly in a parafollicular position (fig. 3.1).

9.1.3.1 CT synthesis and action

Both synthesis and secretion of CT are stimulated by calcium infusion as well as by calcium ingestion (fig. 9.15).[4] The molecular mechanism underlying the stimulatory action of a high ionized calcium concentration on CT secretion involves activation of a calcium-sensing receptor on the cell surface of the C cells, the same receptor that leads to decreased PTH secretion from the chief cells. The amino acid sequence of canine

calcitonin (cCT) has been elucidated (fig. 9.15) and this has allowed the development of a homologous radioimmunoassay for CT in the dog.[19,20] The circulating concentrations of CT decrease during the first three months of life in the dog (fig. 9.10).

During calcium ingestion, the plasma CT concentration is raised directly (by calcium) and indirectly (e.g., by gastrin), causing osteoclasts to retract their brush border and to decrease lysosomal enzyme secretion (fig. 9.8). As a consequence, the plasma calcium concentration is prevented from rising (and therefore the PTH concentration does not fall) and thus calcium is routed to the bone and not lost via the kidneys (fig. 9.4). CT has no direct effects on the intestine or the kidney in the dog, but influences the hypothalamic satiety center and influences 1,25-$(OH)_2$D synthesis (fig. 9.12).[21]

9.1.4 Calciotropic hormones and bone metabolism

Functionally the skeleton can be considered as two organs: (1) a supporting and protecting framework and (2) a reservoir of minerals. Each has its own regulatory mechanism with consequences for skeletal integrity, involving the same cellular structures. Since most cellular activity occurs during skeletal growth, most derangements of skeletal integrity are observed in dogs and cats during early life.

Growth in width of the long bones starts when the periosteum, surrounding the cartilaginous template, forms primitive (i.e., woven) bone which organizes itself into highly organized lamellar bone (fig. 9.16). Growth in length of the long bones is limited to those places in which cartilage remains during the adolescent life, i.e., the physeal growth plates (fig. 9.16). The cartilage also extends to the epiphyseal ends of the long bone, allowing for the proportional growth of the epiphyses. This proportional growth and longitudinal growth occurs via the process of endochondral ossification (fig. 9.16).[22,23]

In adulthood, about one quarter of bone is organic material (of which 90 % is collagen) and about three quarters is inorganic material. The latter is initially a poorly crystallized calcium phosphate and later crystalline hydroxyapatite (HA). For mineralization of bone, calcium- and phosphate-rich vesicles are extruded from osteoblasts into the extracellular matrix. In addition to this cellular regulation of mineralization, physicochemical processes of direct formation of crystalline HA and growth of HA crystals play a role in tissue mineralization. Pyrophosphate, two phosphate molecules linked through an oxygen molecule, inhibits calcium phosphate crystallization in soft tissues and body fluids by binding to the surface of calcium phosphate and blocking the formation and growth of HA crystals. Enzymatic degradation of pyrophosphate by alkaline phosphatase, produced by osteoblasts, can raise the local Ca^{2+} and PO_4^{3-} concentration to a point where HA precipitation begins.

Diphosphonates (not normally present in biological systems and with phosphate-oxygen replaced by a phosphate-carbon binding) have the same binding and mineralization inhibiting properties as pyrophosphates and are completely stabile in an aqueous biological environment. They are used as a coating of implants such as heart valve replacements to prevent their mineralization and they are used as a marker of tissue mineralization. By labeling diphosphonates with ^{99m}technetium, increased radionuclide accumulation can be found at skeletal sites with increased (physiological or pathological) mineralization (figs. 9.17, 9.18).

Osteoblasts covering bone surface, the so-called bone lining cells, separate multinucleated osteoclasts from bone matrix. The osteoclasts are able to resorb mineralized bone at their brush border with the aid of acid phosphatase (fig. 9.8) and are mainly found in metaphyseal areas, where they shape the funnel, as well as on the inner surface of the diaphysis at the endosteal side, where they adapt the medulla to hemopoetic and mechanical demands (fig. 9.16).

The osteocyte is the most abundant cell type of bone. There are approximately ten times as many osteocytes as osteoblasts, and the number of osteoclasts is only a fraction of the number of osteoblasts. The osteocytes communicate with neighboring osteocytes and surface osteoblasts by cytoplasmic extensions running through caniculi. The role of osteocytes can be considered as the mechanosensory cells of bone, and the caniculi network as the structure that mediates mechanosensing. Loading of the bone may cause a flow of interstitial fluid through this network which will mechanically activate osteocytes as well as ensure transport of cell signaling molecules, explaining the communication between osteocytes and osteoblasts.[24] In addition, electrical potentials can change the chemical concentration and composition of this interstitial fluid. Electrical potentials can originate from loading of bone crystals (HA) by the piezoelectric effect or can be applied by special medical equipment to stimulate fracture healing or endosseous new bone formation.[25] Thus, an increase in pressure on porous bone will cause fluid flow by compression or by electrical induction and as a result new bone will be formed normalizing the pressure. This is a hormone-independent regulatory process of bone formation. The clinical relevance of this regulatory mechanism is illustrated in fig. 9.19.

In physiological states including growth, osteoblast and osteoclast activity is coupled (fig. 9.8). In addition to the hormone-independent regulation of bone remodeling, an increasing number of substances is being recognized that influences bone metabolism. One of the most important signaling pathways of osteoclast differentiation and activation is the receptor activator of nuclear factor -κβ (RANK) pathway (fig. 9.9).[26]

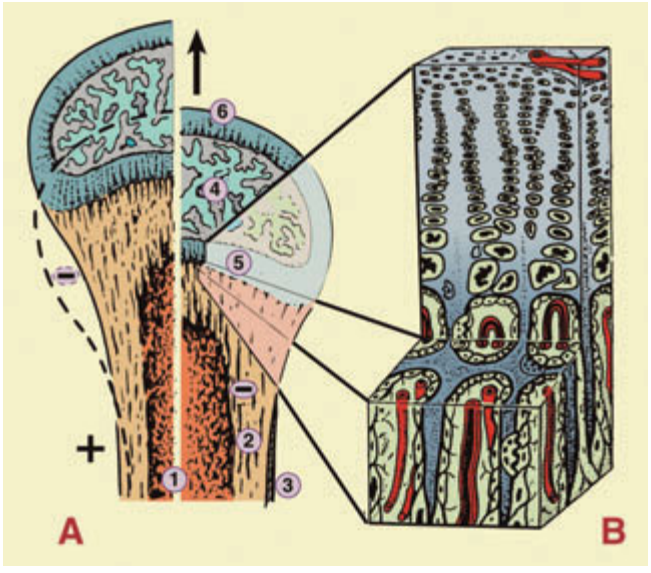


Figure 9.16:
(A) Schematic representation of the proximal end of a long bone with (1) medullary cavity, (2) diaphysis, (3) periosteum, (4) secondary ossification center (epiphysis), (5) physeal growth plate, (6) epiphyseal cartilage. During longitudinal growth (↑) periosteal bone formation (+) and bone resorption (–) in the medulla and at metaphyseal sides, maintain the bone's characteristic form as part of the remodeling process.
(B) The inset shows the process of endochondral ossification: chondrocytes are orientated in rows and while dividing and enlarging, they move away from their nutrient vessel. The intercellular substance mineralizes and consequently seals off the chondrocytes from nutrition, causing death of chondrocytes in their lacunae. Metaphyseal vessels grow into the empty lacunae, introducing osteoblasts which cover the mineralized cartilage with osteoid that will be bone after its mineralization. Multinucleated chondroclasts remove the remnants of mineralized cartilage to complete the process of endochondral ossification. (Modified from Nap et al., 1994.)[22]

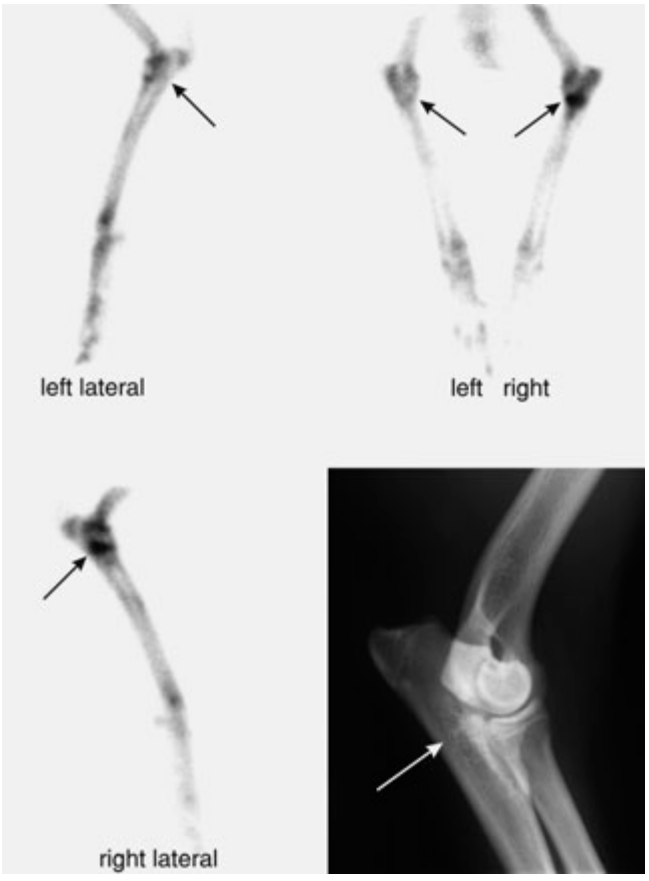


Figure 9.17:
A 2.5-year-old Labrador retriever with lameness of the right front leg for four months. The radiograph of the right elbow revealed only minor sclerosis at the base of the medial coronoid (arrow). The bone scintigraphy scans, using diphosphonates labeled with $^{99m}TcO_4^-$, clearly demonstrated increased bone cell activity in the area of the right medial coronoid, in comparison with the left side. This is indicative for a fragmented coronoid process.

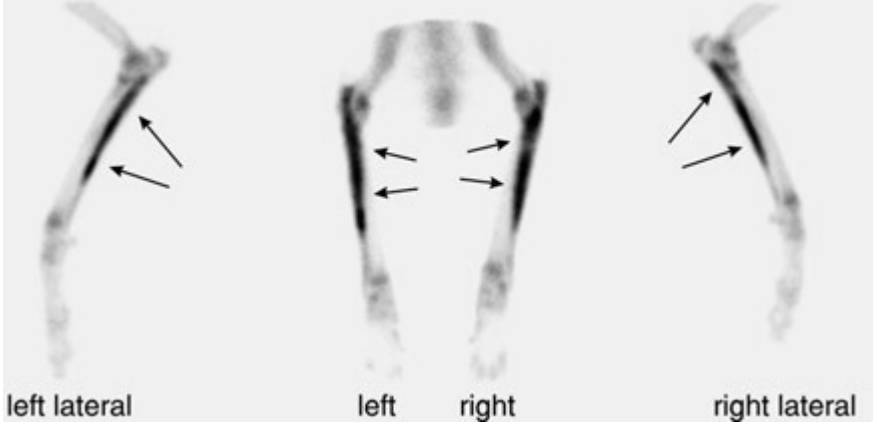


Figure 9.18:
Bone scintigraphy scans, using diphosphonates labeled with $^{99m}TcO_4^-$, of a 1.5-year-old Labrador retriever with shifting lameness and bone pain without fever, revealing increased bone cell activity in the medullary cavity (arrows) of the left and right ulna, typical of enostosis.

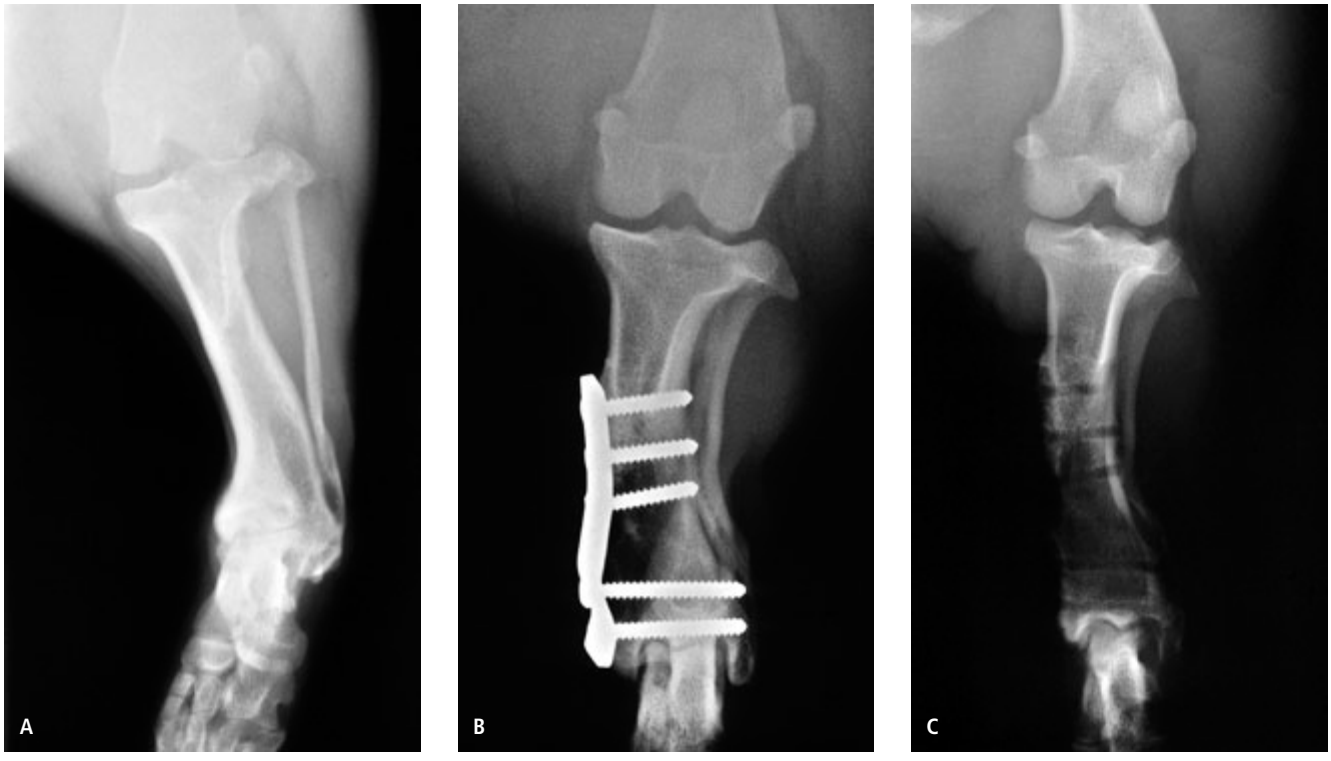


Figure 9.19:
The clinical relevance of the hormone-independent processes of bone remodeling is demonstrated with a radiograph of the tibia of a ten-month-old dachshund with severe varus deformity and thickening of the concave cortex (A). Following corrective osteotomy (B), fixation with a bone plate was performed, which neutralized the forces acting on the bone. The radiograph after plate removal six months later (C) revealed disuse osteoporosis, i.e., osteoporosis due to lack of external forces.

9.2 Hypoparathyroidism

Hypoparathyroidism is the state of deficient PTH secretion or action. The latter may be the result of the release of biologically ineffective hormone or target cell resistance to PTH (pseudohypoparathyroidism), but so far these abnormalities have not been observed in dogs and cats. Thus for the time being for these species the definition of the disease may be confined to deficient secretion of PTH. As with other endocrine glands, theoretically a primary form and a secondary form can be distinguished. Secondary hypoparathyroidism is encountered in situations of hypercalcemia, which has an inhibitory influence on PTH release (chapter 9.1.1). However, because of the causative hypercalcemia, the hypofunction will not become manifest as such. In contrast, primary hypoparathyroidism has serious clinical consequences.

Pathogenesis

From a pathogenetic point of view there are two main causes of primary PTH deficiency: (1) neck surgery and (2) idiopathic disease. The former type is especially encountered following surgical treatment of hyperthyroidism or primary hyperparathyroidism. It may be a transient or a permanent hormone deficiency, depending on the viability of the tissue left in situ at the time of surgery (see also chapter 3.4).

This section will concentrate on the second form. In this spontaneous disease the few histological studies available have revealed parathyroid atrophy, i.e., no parathyroid tissue may be found on surgical exploration.[27] In addition lymphocytic infiltrations have been found in some cases, suggesting an immune mediated cause of the atrophy.[28,29]

Clinical manifestations

In both the dog and the cat spontaneous hypoparathyroidism is rare. The disease may occur at almost any age but the occurrence appears to be highest in young adults (one to four years of age).

The presenting signs and symptoms are directly attributable to the decreased concentration of extracellular ionized calcium. The rate of decrease in the plasma calcium concentration is an important determinant in the development of neuromuscular manifestations. For example, signs of hypocalcemic tetany may occur in dogs after bilateral thyroidectomy when calcium values are still higher (e.g., 1.8 mmol/l) than might be found in cases of spontaneous PTH deficiency, in which a plasma calcium concentration of 1.3 mmol/l may not be associated with clinical manifestations of tetany.

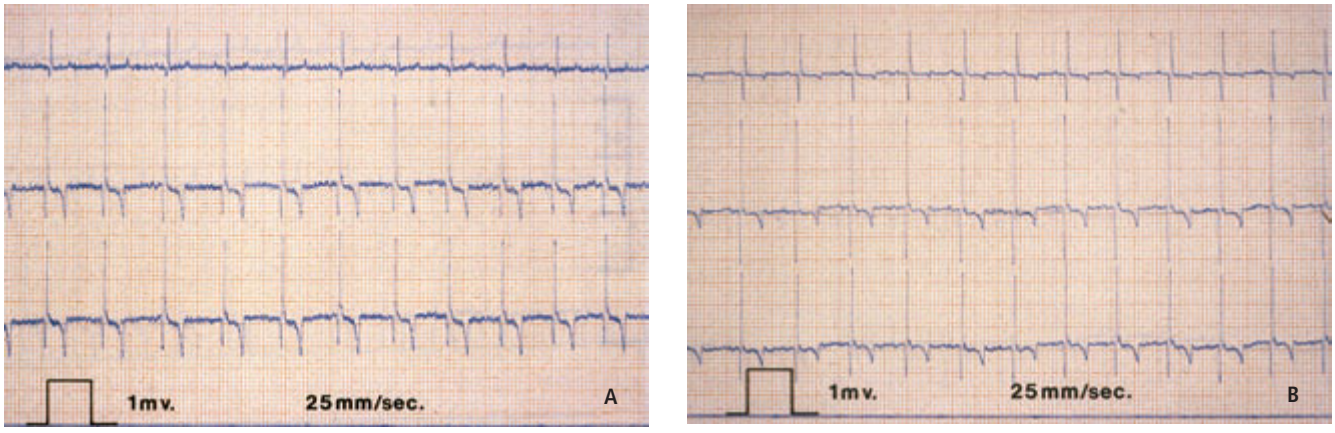


Figure 9.20:
ECG recordings (leads I, II, and III) of a two-year-old female German shepherd dog with primary hypoparathyroidism (calibration: 1 cm = 1 mV; paper speed 25 mm/s). (A) On admission (total plasma calcium 1.0 mmol/l) the recordings were disturbed by muscle twitching, and the T waves were deep and wide. (B) During administration of calcium these ECG changes disappeared; at the time of this recording total plasma calcium had only increased to 1.35 mmol/l. (Courtesy of Drs. J.J. van Nes and A.A. Stokhof).

Neuromuscular signs may include focal muscle twitching, rear limb cramping, stiff gait, generalized muscle spasms, and convulsions.[30] The onset of these neuromuscular signs is often during exercise, excitement, or stress. In some cases intense facial rubbing and licking and biting of the legs may be seen, which can be interpreted as paresthesias due to increased sensory excitability, known from the disease in humans.[31] In addition there may be lethargy and anorexia. On the other hand, once tetany occurs there may be an alarm reaction giving rise to restlessness and panting.

Examination often reveals a somewhat anxious and panting animal that may have a stiff gait, muscle rigidity, and muscle fasciculations. The increased muscle tone may lead to hyperthermia. The cardiac manifestations of hypocalcemia may include a weak femoral pulse. In the ECG prolongation of the QT interval and T wave changes such as peaking and inversion may be seen (fig. 9.20). Several dogs and cats with primary hypoparathyroidism have bilateral lenticular cataract.[30] The mechanism is still obscure, but these cataracts are not attributed to the hypocalcemia per se, but rather to the (local) calcium-phosphate product.[32]

Differential diagnosis

Although not completely identical, similar neuromuscular features may be observed in hypoglycemia (chapter 5.3), epilepsy, and possibly tetanus. Occasionally severe hyperkalemia may also give rise to muscle twitching (chapter 4.2.1). As to the cause of hypocalcemia, in principle conditions such as renal failure, puerperal tetany, ethylene glycol (antifreeze) poisoning, acute pancreatitis, and hypoalbuminemia may also be considered, but usually associated symptoms and signs point to the underlying disease so that there is little chance of confusion.

Diagnosis

In the absence of renal failure, the diagnosis of hypoparathyroidism is virtually certain if hypocalcemia and hyperphosphatemia are found. The diagnosis may be further supported by measurement of the plasma PTH concentration. An inappropriately low plasma PTH concentration while there is hypocalcemia confirms the diagnosis, provided that the assay used is sensitive enough to measure plasma PTH in healthy animals. Commercially available assays for intact human PTH have been validated for use in dogs and cats.[33–36]

Treatment

Emergency treatment of hypocalcemic tetany, requires slow (5–10 min) intravenous injection of calcium in a dose of 0.5–1.0 mmol Ca^{2+}/kg body weight (= 20–40 mg Ca^{2+}/kg) as calcium gluconate. Once the signs of hypocalcemia are controlled, the calcium gluconate can be administered subcutaneously (1:4 diluted with 0.9 % NaCl) every 6 h until oral medication can be started. Dilution of the calcium gluconate solution and caution in giving repeated subcutaneous administrations is advised, as these injections may lead to calcinosis cutis and skin necrosis.[37,38]

Oral maintenance therapy comprises supplementation with a vitamin D compound and calcium lactate or carbonate. Vitamin D may be administered as calcitriol, 1α-hydroxycholecalciferol, or dihydrotachysterol. The latter two vitamin D compounds require 25-hydroxylation in the liver, which is PTH-independent. Calcitriol and 1α-hydroxycholecalciferol have a more rapid onset of action but a shorter half-life than dihydrotachysterol. In addition, calcitriol and 1α-hydroxycholecalciferol may be difficult to administer to small animals because of the small volumes required from the available preparations.

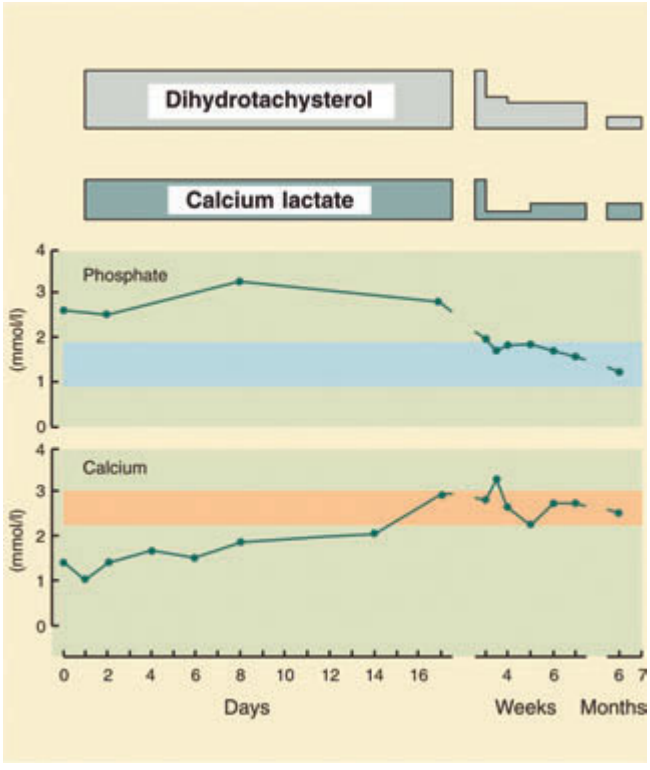


Figure 9.21:
The dog described in the legend of fig. 9.20 was treated initially with 500 µg dihydrotachysterol and 2.5 g calcium lactate twice daily. This caused the plasma calcium concentration to gradually rise until it was within the reference range (zone). When hypercalcemia developed the doses were lowered. The dog did very well for many years on twice daily 100 µg dihydrotachysterol and twice daily 1 g calcium lactate as a supplement to a balanced commercial dog food. (Courtesy of Dr. J.J. van Nes.)

Dihydrotachysterol is given initially in a dose of 20–30 µg/kg body weight, together with calcium lactate (25–100 mg/kg). After about two to three weeks dihydrotachysterol reaches its maximal effect and the dose has to be lowered to prevent hypercalcemia (fig. 9.21). In the long run it is often possible to omit supplementation with calcium; the calcium supply via commercially manufactured foods will be sufficient.

Hypercalcemia may be suggested by polyuria and when confirmed by measurements of plasma calcium, supplementation should be stopped to minimize the risk of renal insufficiency due to nephrocalcinosis. With discontinuation of the administration of dihydrotachysterol there is no immediate risk of hypocalcemia, as the effect of the drug continues for several days.

Prognosis

With adequate monitoring of the plasma calcium concentration the prognosis is excellent. Initially the calcium should be measured daily and when less critical, weekly. Once the dog or cat is stable on maintenance therapy, two to four follow-up examinations per year are usually sufficient. With proper guidance the life expectancy is not different from that of a healthy dog.

9.3 Hyperparathyroidism

Hyperparathyroidism can be primary or secondary. Primary hyperparathyroidism is the state of autonomous hypersecretion of PTH, most commonly by an adenoma of the chief cells. Secondary hyperparathyroidism is an adaptive increase in PTH secretion, unrelated to intrinsic disease of the parathyroids. In the latter, the increased PTH secretion is the result of chronic decreases in the concentration of ionized calcium in plasma. Several conditions may lead to these events, but in dogs and cats there are only two in which secondary hyperparathyroidism produces clinically significant manifestations: chronic renal failure (chapter 9.3.2) and calcium deficiency during growth (chapter 9.3.3).

9.3.1 Primary hyperparathyroidism

Pathogenesis

A small solitary parathyroid adenoma (fig. 9.22) is the most common cause of primary hyperparathyroidism in both the dog and cat.[39,40] At surgery the other glands may appear normal or atrophied. The PTH excess may also be caused by an adenoma of more than one gland or by one or more minimally enlarged glands with multiple hyperplastic nodules.[41] Very rarely the is disease caused by a parathyroid carcinoma.[39,42]

Differentiation of parathyroid adenoma from primary hyperplasia is troublesome. Both parathyroid adenoma and multiple nodular hyperplasia have characteristics of intrinsic autonomy, i.e., suppression of the remaining parathyroid cells, suggesting that there is no functional difference between the two abnormalities.[43] In addition, it may be expected that primary hyperplasia would be characterized by polyclonal expansion, but in man monoclonality has been demonstrated in some cases of primary parathyroid gland hyperplasia, blurring further the distinction between hyperplasia and neoplasia.[44] It may therefore be argued that there is a continuum of morphological structures with focal hyperplasia at one end and adenoma at the other, indicating that multinodular hyperplasia is a multiple form of parathyroid adenoma.

Clinical manifestations

Primary hyperparathyroidism is an uncommon disease of older dogs (≥ 6 years) and there is no pronounced sex predilection.[45,46] Keeshonds are overrepresented in case series of canine primary hyperparathyroidism and in this breed the dis-



Figure 9.22:
Surgical specimen following unilateral thyroparathyroidectomy in a nine-year-old male Malinese shepherd dog with primary hyperparathyroidism. Note the parathyroid adenoma originating from the parathyroid tissue at the cranial pole (top) of the thyroid gland.

order follows an autosomal dominant mode of inheritance with possibly age-dependent penetrance.[46–48] In cats the disease is even less frequent and occurs in the same age range, possibly with a predilection for females and Siamese cats.[40,49]

The disease may be asymptomatic or there may be mild or severe systemic illness. Roughly three categories or stages of presentation can be distinguished. In the mildest form there may be no symptoms or signs and the disease is discovered because hypercalcemia is found by a routine laboratory examination. In the second form polyuria, probably resulting from decreased vasopressin-regulated expression of aquaporin-2 in the kidney collecting ducts,[50] develops insidiously in an otherwise healthy dog; in cats polyuria is less common.[41] In the third and thus far most common form the disease period may be rather short and the animals are presented with polyuria / polydipsia (dogs!) and lethargy, anorexia, vomiting, weakness, and weight loss. Especially in cats the manifestations may be rather nonspecific and can be confined to anorexia and malaise. When presented, cases in the third category are usually characterized by weakness and lethargy (fig. 9.23). The hypercalcemia is frequently associated with urolithiasis and secondary urinary tract infection.[45]



Figure 9.23:
A nine-year-old male Malinese shepherd dog with emaciation, dehydration, and weight loss due to primary hyperparathyroidism.

Differential diagnosis

The main problem in the differential diagnosis of primary hyperparathyroidism is distinguishing it from other conditions associated with hypercalcemia and specifically hypercalcemia of malignancy (chapter 9.4). Other causes of hypercalcemia such as hypervitaminosis D (chapter 9.5.2), acute renal failure, and primary hypoadrenocorticism (chapter 4.2.1) pose less of a diagnostic problem because of the changes associated with the primary disease.

Moderate hypercalcemia with no obvious identifiable cause is seen regularly in cats.[51] Longhaired cats seem to be predisposed and diet history may reveal that acidifying diets have been fed. This idiopathic hypercalcemia in cats may be associated with calcium oxalate urolithiasis.

Diagnosis

The presence of hypercalcemia is established when three measurements of total and ionized plasma calcium concentration reveal values exceeding the reference range. This, in combination with normo- or hypophosphatemia and the appropriate signs, may give rise to the suspicion of primary hyperparathyroidism. Nevertheless, the approach should be to exclude hypercalcemia of malignancy, which is more common than hypercalcemia of parathyroid origin. The exclusion procedures include careful inspection of the perianal region, thoracic radiography, and cytological examination of aspirates from lymph node(s) and / or bone marrow (see also chapter 9.4).

Because of the small size of the parathyroid lesions, they are rarely palpable in dogs. In cats it is sometimes possible to palpate enlarged parathyroid glands in a manner similar to the palpation of enlarged thyroid glands.[40] Radiography and routine laboratory data (other than hypercalcemia, hypophosphatemia, and elevated levels of alkaline phosphatase) are usually unremarkable, unless the disease is complicated by another disorder such as renal failure.

The parathyroid glands may be visualized by ultrasonographic examination as round or oval structures that are anechoic or hypoechoic compared with surrounding thyroid parenchyma, but due to their small size parathyroid glands are not routinely seen on ultrasonographic examination.[52–54] Parathyroid glands exceeding 4 mm in diameter are highly suspicious for parathyroid pathology. In humans, parathyroid scintigraphy using ^{99m}technetium-sestamibi has been proven useful in identifying parathyroid tumors. However, ^{99m}technetium-sestamibi scintigraphy has a poor sensitivity and specificity when used in hypercalcemic dogs for the detection of morphological changes of the parathyroid glands.[55]

Definite differentiation between parathyroid and nonparathyroid causes of hypercalcemia may rely on measurement of the plasma PTH concentration. As discussed in chapter 9.1, this is best performed with the two-site type of assay that measures intact PTH and is unaffected by renal function. In the absence of renal failure (see chapter 9.3.2), an elevated PTH level confirms the diagnosis of primary hyperparathyroidism. But a plasma PTH concentration within the reference range, occurring in approximately 70 % of dogs with primary hyperparathyroidism,[45] also confirms the diagnosis, as in hypercalcemia of nonparathyroid origin PTH concentrations should be low as a result of the inhibitory effect of the high plasma calcium concentration on PTH release. A serious diagnostic problem may arise when it is suspected that primary hyperparathyroidism is complicated by renal failure.

Dogs with hypercortisolism may have elevated plasma PTH concentrations, which may be associated with abnormalities in calcium and phosphate metabolism in these dogs.[56] The elevated plasma PTH concentrations in dogs with hypercortisolism have been reported to reduce significantly with trilostane treatment.[57]

Treatment

Surgical resection of abnormal parathyroid tissue has long been the treatment of choice. However, dogs with primary hyperparathyroidism have also been treated using percutaneous ultrasonographically guided techniques of chemical ablation (injection of ethanol)[58] or radiofrequency heat ablation[59]. A retrospective study indicated that 45 of 48 parathyroidectomies, 13 of 18 percutaneous ultrasound-guided ethanol ablation procedures, and 45 of 49 percutaneous ultrasound-guided heat ablation treatments resulted in control of hypercalcemia for a median of more than 500 days.[60] The results of another retrospective study indicated, however, that ultrasound-guided ethanol ablation had limited effect.[46] During ultrasound-guided ethanol or heat ablation of a parathyroid tumor it is often necessary to redirect the needle several times to ablate all abnormal tissue and the operator needs to go through a learning curve.[60] Leakage of the ethanol or extension of thermal necrosis into the surrounding tissues may cause damage to structures such as the recurrent laryngeal nerve. In human medicine the sonographically guided percutaneous injection of ethanol is considered an alternative to surgery in patients who are not suited for surgical intervention.[61] The efficacy of percutaneous ethanol injection for treatment of primary hyperparathyroidism in humans does not approach that of surgery, and post-ablation periglandular fibrosis can make future surgery or ablation difficult.[62] Calcimimetic compounds that stimulate the calcium-sensing receptor on the surface of the chief cells and thereby decrease PTH secretion may hold promises for the medical treatment of primary hyperparathyroidism in the near future.

Surgical removal of a parathyroid adenoma results in a rapid decline, i.e., usually within 48 h, in plasma calcium concentration and a rise (if lowered) in plasma phosphate concentration (fig. 9.24). When an adenoma is not identified immediately, all four parathyroid glands should be inspected carefully for the presence of nodular hyperplasia. Macroscopically suspected glands are removed, leaving at least one parathyroid gland in situ. Especially in critically hypercalcemic cases, perioperative measures to reduce the hypercalcemia should be directed at increasing urinary calcium excretion by volume expansion, i.e., intravenous therapy with isotonic saline.

Following surgical removal of the parathyroid mass(es) or after ethanol or heat ablation, there is a rapid decline in the circulating PTH concentration, while the unaffected parathyroids are still suppressed from the long-term hypercalcemia. This together with the elevated bone turnover and thus high calcium accretion (»bone hunger«) may lead to postoperative hypocalcemia. Therefore plasma calcium concentration should be monitored carefully after the treatment (fig. 9.24). In order to prevent signs of hypocalcemia, administration of vitamin D and calcium (see chapter 9.2) should be started when the plasma calcium concentration declines to the lower limit of the reference range. If signs of tetany have already occurred, calcium gluconate can be given intravenously and/or subcutaneously (see chapter 9.2). The aim is to maintain the plasma concentration in the lower part of the normal range, so that there is sufficient stimulus for restoration of the function of the remaining parathyroid tissue. It may be necessary to continue this substitution for several weeks. Once the plasma calcium concentration is stable, withdrawal of the vitamin D can be attempted gradually by first giving it every other day and then increasing the number of days between administrations. When the hypocalcemia does not recur, the calcium supplementation can also be lowered gradually. One should be careful not to induce hypercalcemia, as this is now a more serious risk than in primary hyperparathyroidism; vitamin D induces not only hypercalcemia but also a tendency to hyperphosphatemia, which combination much more easily leads to nephrocalcinosis than hypercalcemia per se.

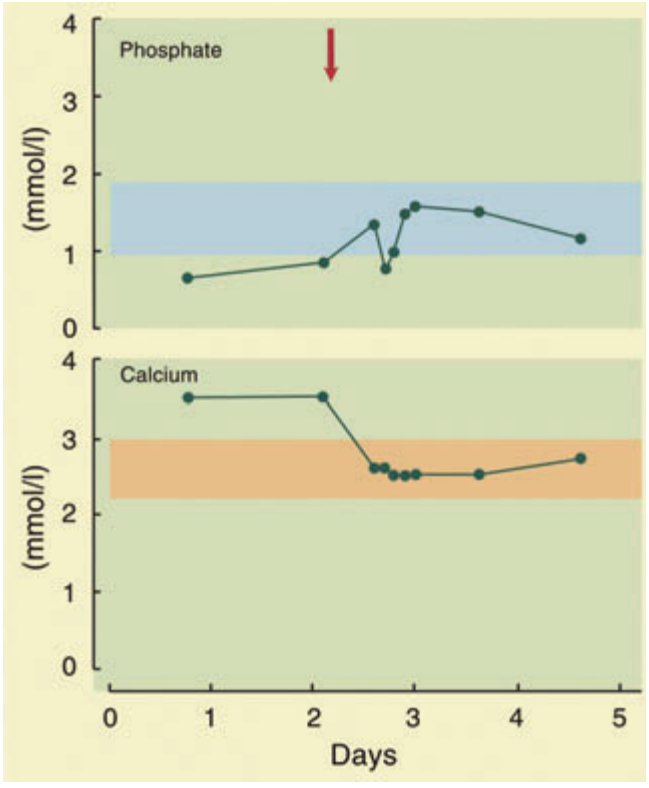


Figure 9.24:
Plasma calcium and phosphate concentrations in a seven-year-old castrated female Airedale terrier with primary hyperparathyroidism before and after removal (arrow) of a solitary parathyroid adenoma measuring 7 × 5 × 4 mm. Plasma PTH concentration ranged from 15–22 ng/l. In this dog the disease was rather mild and of short duration (polyuria lasting three to four weeks), and apparently had not yet caused suppression of the nonaffected parathyroid tissue to the extent that postsurgical hypocalcemia developed.

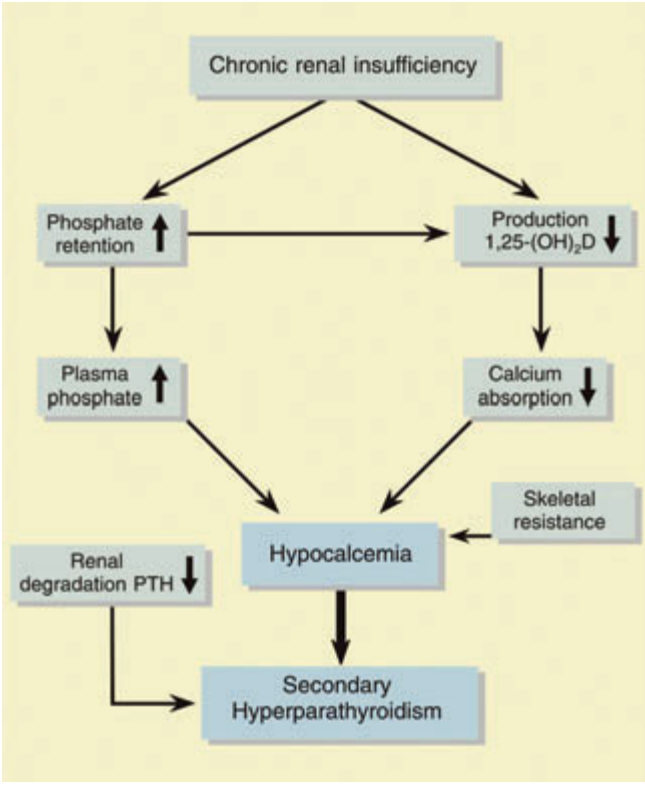


Figure 9.25:
Principal factors involved in the pathogenesis of secondary hyperparathyroidism due to chronic renal insufficiency. The main stimuli are (1) renal retention of phosphate, which causes precipitation of calcium in soft tissues, and (2) decreased production of 1,25-$(OH)_2$D.

Prognosis

When the source of the PTH excess can be removed or destroyed successfully and the posttreatment period can be overcome adequately, the prognosis is excellent.

9.3.2 Renal secondary hyperparathyroidism

Pathogenesis

Several factors are involved in the pathogenesis of secondary hyperparathyroidism in animals with chronic renal failure (fig. 9.25). The initial stimulus appears to be chronic reduction in circulating ionized calcium because of renal retention of phosphate. High plasma concentrations of phosphate may precipitate calcium in soft tissues and also seem to decrease the release of calcium from bone.[63] In addition, decreased production of 1,25-$(OH)_2$D in the kidney causes a reduction in the intestinal absorption of calcium. A further contributing factor to the hypocalcemia is the relative skeletal resistance to PTH. The concerted actions of these factors lead to hypocalcemia, which stimulates PTH secretion and results in hypertrophy of all parathyroid glands. The renal insufficiency contributes to the increase in PTH levels because it is associated with a decreased rate of removal of the hormone from the circulation. As discussed in chapter 9.1 the increase in biologically inactive C-PTH fragments may cause PTH resistance in renal failure patients.[8]



Figure 9.26:
Demineralization of all bones of the skull and mandible of a dog with advanced secondary renal hyperparathyroidism. Due to subperiosteal bone resorption the contours of the bone are hardly visible. The teeth have maintained a normal density, causing an increased contrast between teeth and bone.

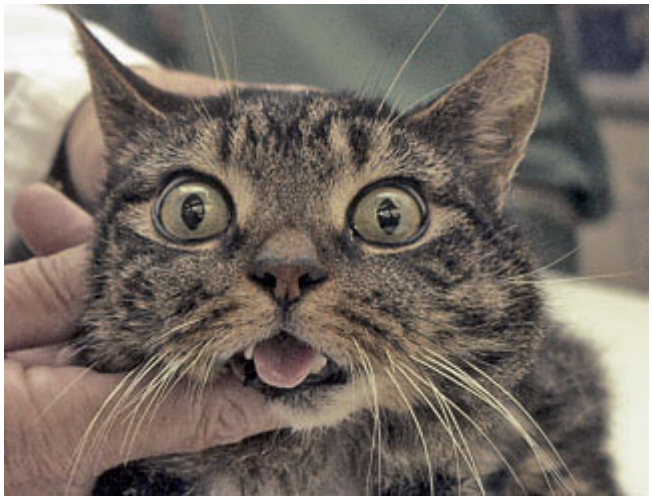


Figure 9.27:
A five-year-old cat with chronic renal insufficiency. The associated secondary renal hyperparathyroidism (plasma PTH = 882 ng/l) had caused severe bone demineralization with a so-called »rubber jaw« and the inability to close the mouth.

Clinical manifestations

The animal may be presented with the classic signs of renal insufficiency, such as anorexia, vomiting, polydipsia, polyuria, and depression, but in some cases these features may be mild or only intermittent. In longstanding cases signs of secondary hyperparathyroidism may develop. Although not common, symptoms of neuromuscular irritability and tetany similar to those of hypoparathyroidism may occur. The skeletal changes range from mild to severe forms of fibrous osteodystrophy. In older dogs the volume of bone is usually not affected and the changes are most prominent in the skull with loss of teeth and hypoostotic osteodystrophy (fig. 9.26). As a result of the accelerated bone resorption the mandibles may become pliable, for which the term »rubber jaw« is used. The jaws may fail to close properly (fig. 9.27).

When renal insufficiency develops before maturation of the skeleton the repair by proliferation of connective tissue may exceed the rate of bone resorption. This results in an increase in bone volume. This hyperostotic osteodystrophy results in facial swelling (fig. 9.28).

The laboratory findings are usually dominated by the abnormalities associated with the renal insufficiency, such as elevated plasma concentrations of urea, creatinine, and phosphate. Despite the often low normal plasma calcium concentrations, PTH secretion increases and gradually causes the skeletal changes indicated above.

Treatment

The aim of the treatment is to reduce the plasma PTH concentration below a »toxic« level in order to improve survival and quality of life.[64,65] The most important step in the prevention and treatment of renal osteodystrophy is the restriction of dietary phosphorus. Restriction of dietary proteins has not been proved to have a beneficial effect.[66] The phosphate restriction may be reinforced by administering aluminum-containing antacids that prevent phosphate absorption. In cases in which there is a tendency to hypocalcemia this approach may be extended by supplementation with calcium and vitamin D sterols (chapter 9.2). Supplementation with low daily doses of calcitriol (2.5–5.0 ng/kg, PO, q 24 h) can control renal hyperparathyroidism[67] but may induce hypercalcemia on the long term. Analogues of calcitriol, such as 22-oxacalcitriol, have been experimentally proved to be effective in decreasing plasma PTH concentrations without causing hypercalcemia.[68] Their clinical application needs further evaluation.



Figure 9.28:
A seven-month-old male Great Dane with renal insufficiency. In this young dog the secondary renal hyperparathyroidism caused hyperostotic osteodystrophy, which led to facial swelling (A). Lifting of the upper lip (B) revealed that the facial swelling was due to increased volume of the maxilla.

9.3.3 Nutritional secondary hyperparathyroidism

In growing dogs, especially of the larger breeds, and cats a substantial amount of calcium is laid down as calcium phosphates in newly-formed osteoid and cartilage. If insufficient calcium is available in the food, the calcium concentration in plasma will tend to decrease, initiating hyperparathyroidism. Since in carnivores nutritional secondary hyperparathyroidism (NSH) is especially seen in animals fed an unbalanced food mainly based on meat or meat by-products (table 9.1), this entity is also known as the **all meat syndrome**.

In NSH, PTH production and secretion increase, leading to increases in calcium reabsorption in the kidney, osteoclasia, and 1,25-$(OH)_2$D synthesis. The former two effects result in a rapid normalization of the plasma calcium concentration (fig. 9.4), whereas the latter effect requires a few days but eventually will lead to an augmentation of the intestinal absorption efficiency of calcium and phosphate (fig. 9.14). The circulating phosphate concentration will increase due to augmented intestinal phosphate absorption and increased bone resorption with liberation of phosphate. Concomitantly and due to the hyperparathyroidism, the tubular maximum for phosphate will decrease, causing hyperphosphaturia and preventing further elevation of the plasma phosphate concentration. Depending on the growth velocity of the animal (and thereby its calcium requirement) and the severity of the calcium deficiency, the increased bone resorption will cause clinical problems within one to three months.[69]

Clinical manifestations

Cancellous bone in the epiphyseal and metaphyseal areas may become so thin that spiculae will collapse, causing compression fractures. Osteoclasts at the endosteal side of long bones will remove cortical bone to such an extent that the cortex will bend under the influence of body weight and muscle tone, causing folding (greenstick) fractures and deformed skeletal protuberances.

On presentation, the patient will be alert and have a good hair coat, and a disproportionally enlarged abdomen due to the fact that the growth of the skeleton lags behind that of the soft tissues (fig. 9.29). The animal will be reluctant to walk due to bone pain and pathological fractures. There may be fractures and abnormal alignment of bones, and bones may be painful upon palpation. In severe cases there may be paresis posterior due to compression fractures of vertebrae (fig. 9.29). As explained earlier, plasma calcium concentration is very effectively regulated and its measurement does not contribute to the diagnosis (fig. 9.1). Plasma and urinary concentrations of phosphate may be elevated. Due to high bone turn-over, the plasma level of alkaline phosphatase will be increased.

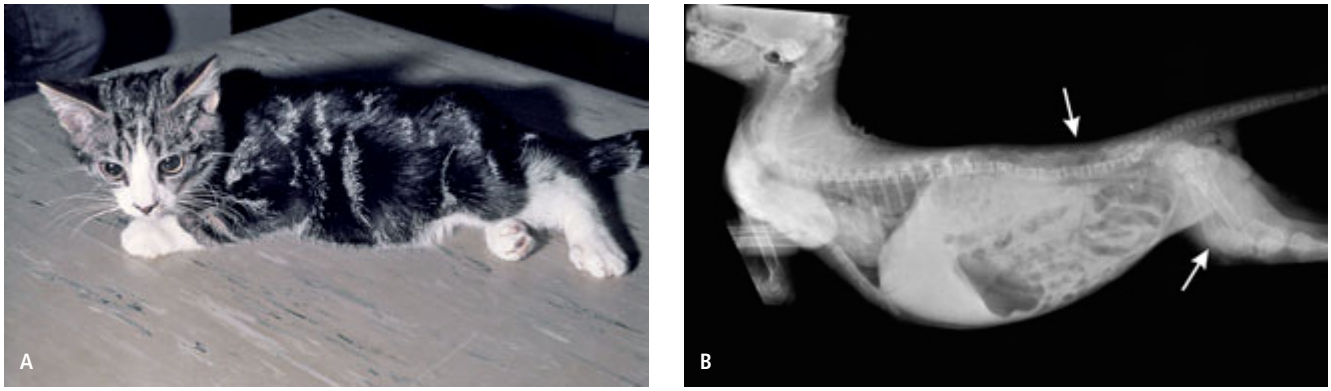


Figure 9.29:
(A) Kitten, three months of age and fed chicken meat almost exclusively, was in good general condition but unable to stand.
(B) The radiograph revealed the disproportionally enlarged abdomen, thin cortices and wide medullae of the long bones, pathological fractures of both femurs, and compression fractures of vertebrae (arrows).

In adult animals the calcium requirement is lower than in young growing animals. Nevertheless, very prolonged dietary calcium deficiency may cause problems that become manifest by loosening of teeth due to alveolar resorption.

Diagnosis

PTH and 1,25-$(OH)_2$D concentrations will be elevated (fig. 9.13), but these measurements are not readily available. The most practical way to make a diagnosis is the combination of a carefully taken history, focused on dietary composition, and radiographs of the affected sites. The most characteristic features are thin cortices, a wide medullary cavity, pathological fractures, bending of protuberances (including calcaneus and ischiatic tuberosity), and growth plates of normal width bordered by a well-mineralized metaphysis (fig. 9.29). Bone biopsies reveal mineralized osteoid with massive osteoclasia.

Differential diagnosis

Hypervitaminosis A (chapter 9.7) and hypovitaminosis D (chapter 9.5.1) should be considered, as well as inborn metabolic disorders such as osteogenesis imperfecta. In adult dogs, renal secondary hyperparathyroidism (chapter 9.3.2) and parodontal diseases should be taken into account.

Treatment

In the severe stage of NSH the pathological fractures of the long bones can neither be treated by splinting, since the bone will break just proximal to the splint, nor by osteosynthesis, because of the weakened nature of the bones. Therapy is limited to good nursing to prevent additional damage to the skeleton, especially the vertebrae, and food with a normal calcium content (i.e., 1.2 % on a dry matter basis).[69] This will improve skeletal mineralization in three weeks. Extra calcium as calcium carbonate (50 mg Ca/kg body weight per day) can be prescribed during this period. Since the endogenous 1,25-$(OH)_2$D concentration in the plasma (fig. 9.13), and thus its effects on intestines and bone cells, is highly increased (fig. 9.4), additional administration of vitamin D is contraindicated.

Prognosis

The prognosis depends on the severity and the extent of pathological fractures. Compression fractures of vertebrae can, but not necessarily, have a bad prognosis. Healed greenstick fractures and bent long bones will not always cause locomotion disturbances. Narrowing of the pelvis may cause recurring constipation although in less severe cases, in which treatment is begun soon enough, constipation may not remain a problem (fig. 9.29).

9.4 Hypercalcemia of malignancy

Parathyroid hormone-related protein (PTHrP) was initially identified as the protein responsible for humoral hypercalcemia of malignancy (see also chapter 10.1). Later it became apparent that PTH and PTHrP genes have arisen from a common ancestral gene and represent two members of a small gene family. PTHrP is larger than PTH (139–177 versus 84 amino acids), but shares 70 % sequence homology with PTH in the N-terminal region. The posttranslational processing of PTHrP is extremely complex and appears to be analogous to that of proopiomelanocortin (fig. 4.4), in that it is processed into a series of peptides with potentially different functions.[70] Peptides containing the first 34 amino acids of both PTH and PTHrP bind with equal affinity to PTH / PTHrP receptor. The PTH / PTHrP receptor is expressed in many tissues and its transcription is tissue specific. PTH functions mainly in an endocrine manner to regulate extracellular calcium concentration, whereas PTHrP mainly acts in an autocrine or paracrine manner to modulate a range of physiological and developmental responses.[71] PTHrP is synthesized

at one time or another during fetal life in virtually every tissue.

Although awareness of the physiological roles of PTHrP is of recent origin, it was already speculated in the 1940s that in man certain tumors might produce a substance similar to PTH and this condition was called pseudohyperparathyroidism. Malignant tissues often revert to a fetal pattern of gene expression; synthesis of PTHrP may be part of this pattern. In dogs and cats, humoral hypercalcemia of malignancy was first described in malignant lymphoma in the 1970s.[72,73] In addition, the condition was found to be associated with adenocarcinomas originating from apocrine glands of the anal sac region in dogs.[74,75] Hypercalcemia of malignancy associated with elevated circulating PTHrP concentrations has also been described in cases of malignancies originating from the mammary gland[76] and the ovary[77], as well as in malignant melanoma[78] and multiple myeloma.[79]

Pathogenesis

In principle, malignancy-associated hypercalcemia may arise through: (1) local osteolysis due to bone metastasis, (2) secretion of PTHrP, and (3) production of calcitriol. Other mechanisms of humoral hypercalcemia of malignancy include tumor production of various substances that stimulate bone resorption, including cytokines (notably interleukin-1) and growth factors such as transforming growth factor beta (TGF-β). An important humoral pathway for hypercalcemia of malignancy involves the production of soluble forms of receptor activator of nuclear factor-kappa β ligand (RANKL), a newly discovered member of the tumor necrosis factor superfamily and a critical regulator in bone metabolism, which is capable of stimulating the activity of osteoclasts by binding to a receptor (RANK) on their surface.[80]

Local osteolysis may be expected especially in hematological malignancies that produce substances that act locally in the bone marrow to mobilize calcium and phosphate.[81] Tumor-derived PTHrP activates osteoclasts systemically through the RANK / RANKL / osteoprotegerin triad (fig. 9.9), i.e., by increasing RANKL expression and decreasing the production of osteoprotegerin (OPG), the endogenous RANKL inhibitor.[82] The plasma PTHrP concentrations are elevated in most hypercalcemic dogs with adenocarcinomas derived from apocrine glands of the anal sac.[83,84] In dogs with malignant lymphoma the plasma PTHrP concentration by itself may not be high enough to cause hypercalcemia and thus other factors, such as the production of calcitriol, may interact synergistically or additively. [83–85]

In hypercalcemic cats, increased plasma PTHrP concentrations have been found to be associated mainly with malignant lymphoma and carcinomas.[35] In squamous cell carcinomas, hypercalcemia of malignancy was most often detected in cases involving the mandible with radiographic evidence of bone lysis.[86]



Figure 9.30:
Perineum of a twelve-year-old female cocker spaniel with a large adenocarcinoma of the apocrine glands of the anal sac region, which caused hypercalcemia.

Clinical manifestations

Hypercalcemia of malignancy is diagnosed in 57–67 % of hypercalcemic dogs.[83,87] In contrast, neoplasia is diagnosed in only 30 % of hypercalcemic cats. [86] In 10–40 % of dogs with malignant lymphoma there is hypercalcemia, with a marked overrepresentation of boxers.[88] Most canine malignant lymphomas that are associated with hypercalcemia belong to the T-cell subclass.[88] Multiple myeloma is associated with hypercalcemia in 15–20 % of cases.[79,89]

Adenocarcinomas of apocrine glands of the anal sac region occur mainly in older (≥ 9 years) dogs. There is some contradictory information regarding gender predisposition. In the early reports the dogs were almost exclusively female.[74,90,91] In more recent reports on retrospective studies, with the histopathology being an important inclusion criterion, the sex distribution was about equal.[92–94] It has been suggested that this change could be a reflection of an increased frequency and earlier age of neutering in recent years.[92] The patient groups seem to differ also with regard to the prevalence of hypercalcemia. In the early case series almost all dogs were hypercalcemic, whereas in the recent retrospective studies hypercalcemia is reported in about half of the dogs diagnosed with anal sac gland carcinoma. Dogs with adenocarcinoma of apocrine glands of the anal sac may be presented for the signs of hypercalcemia or because of a swelling in the perineum. This swelling has an intact overlying skin that is usually not attached to the tumor. Only occasionally they are so large at the time of presentation that there are problems with defecation (fig. 9.30). When a

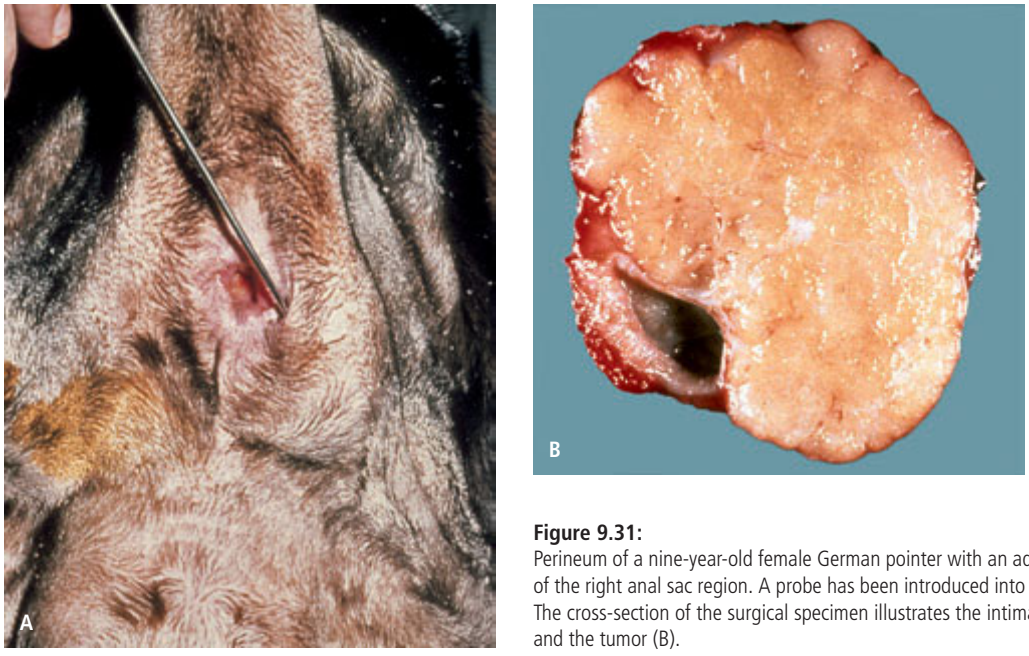


Figure 9.31:
Perineum of a nine-year-old female German pointer with an adenocarcinoma of the apocrine glands of the right anal sac region. A probe has been introduced into the natural orifice of the anal sac (A). The cross-section of the surgical specimen illustrates the intimate relationship between the anal sac and the tumor (B).

probe is introduced into the orifice of the corresponding anal sac, it appears to enter into the mass (fig. 9.31). The tumors are invariably malignant[95] and by the time of the first examination there may already be metastasis to regional lymph nodes (internal iliac / lumbar) or to distant sites (e.g., liver, lung, kidney).

As in primary hyperparathyroidism, the hypercalcemia of malignancy gives rise to polyuria, polydipsia, anorexia, weight loss, and lethargy in dogs. In keeping with the concept that the hypercalcemia associated with adenocarcinoma of the anal sac is mostly due to an excess of PTHrP, laboratory examination often reveals the combination of hypercalcemia and hypophosphatemia. As mentioned above, in malignant lymphoma most probably also other factors contribute to the hypercalcemia. As a consequence hypophosphatemia is found less often, which may be the reason that malignant lymphoma in a high frequency is associated with nephrocalcinosis and renal insufficiency.

In cats, the malignancy-induced hypercalcemia also causes anorexia and malaise, but the polyuria and polydipsia are less pronounced than in dogs. Signs of lower urinary tract disease have been observed in about 25 % of hypercalcemic cats, in some cases associated with calcium oxalate urolithiasis.[86] There are two case reports on anal sac adenocarcinoma in the cat, but hypercalcemia was not a feature.[96,97]

Differential diagnosis

The differential diagnosis of hypercalcemia has been discussed briefly in chapter 9.3.1.

Diagnosis

The cause of the hypercalcemia may be apparent because there is malignant lymphoma or an anal sac tumor. However, it may happen that hypercalcemia is found and that only with procedures such as thoracic radiography, abdominal ultrasonography, and / or cytological examination of aspirates from lymph nodes or bone marrow the diagnosis malignant lymphoma can be secured.

In cases in which nonparathyroid malignancy is suspected but cannot be proved, an increase in the plasma PTHrP concentration may indicate whether the hypercalcemia is caused by an underlying malignancy.[84]

A modified clinical stage scheme, based on the TNM system (see also chapter 3.4.2) has been developed for dogs with anal sac gland carcinoma. This scheme permits categorization that is useful in decisions on treatment and communication on prognosis.[94]

Treatment

Surgical removal of an adenocarcinoma of the anal sac may abolish hypercalcemia if there are no metastases or there are metastases that have lost the capacity to produce PTHrP (fig. 9.32). This decrease in circulating calcium concentration is associated with a decrease in plasma PTHrP concentration.[83] Chemotherapy for malignant lymphoma may also decrease both calcium and PTHrP concentrations in plasma.[83] In dogs with anal sac gland carcinoma various chemotherapeutic agents have been used. In one study this did not result in significantly longer median survival time.[92] In

another study there were some indications that platinum chemotherapy (cisplatin and carboplatin) may have some antitumor activity.[93] Successful treatment of hypercalcemia of malignancy may result in transient hypoparathyroidism and hypocalcemia.[98]

Especially if there is dehydration, plasma calcium concentration may reach critically high values. Therefore volume expansion with fluid therapy is an important supportive measure before treatment can be started that will result in elimination of the cause(s) of the increased bone resorption. The first goal of fluid therapy is to restore normal hydration, as the hypovolemia decreases glomerular filtration and thus calcium clearance. For this purpose 0.9 % NaCl (10–15 % of body weight) is administered intravenously over a period of ~6 h. Thereafter the rate of administration is gradually reduced to 2 ml/kg/h, while the plasma calcium concentration is monitored. Furosemide may be added to the infusion so that it is administered at a rate of 1 mg/kg/h. However, this drug should not be given until rehydration is complete, because it may further reduce glomerular filtration rate and thereby reduce the filtered load of calcium. Medical treatment for hypercalcemia may include glucocorticoid therapy (see also chapter 4.3.6). Glucocorticoids are primarily used to reduce bone resorption from malignant lymphomas. Biphosphonates may also be safe and effective in the treatment of dogs and cats with hypercalcemia associated with increased bone resorption. Intravenous administration of 1–2 mg pamidronate disodium per kg body weight rapidly decreases circulating total and ionized calcium concentrations without evident toxicosis.[99,100]

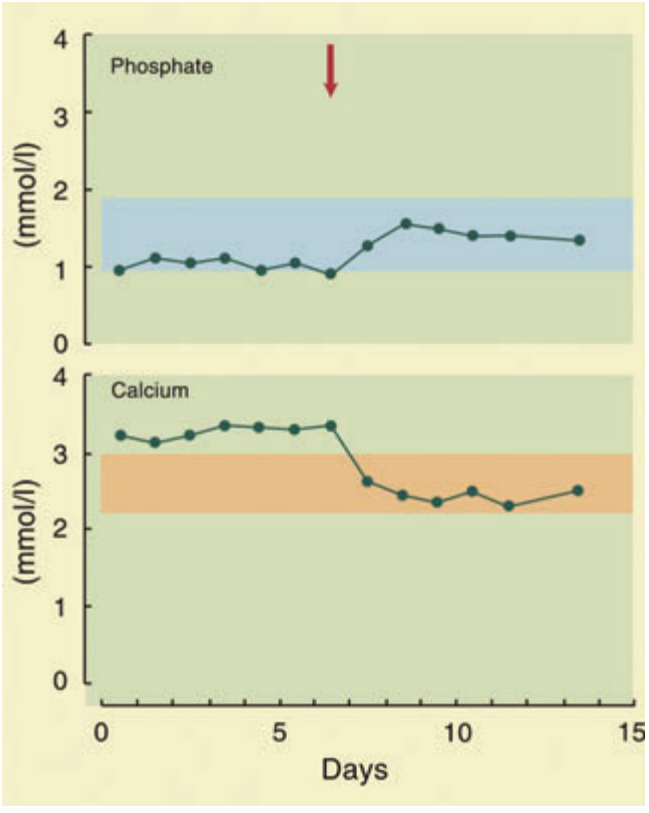


Figure 9.32:
Plasma calcium and phosphate concentrations in a twelve-year-old female longhaired German pointer. Removal of an anal sac tumor led to normal calcium and phosphate concentrations.

Prognosis

In dogs with malignant lymphoma that is associated with hypercalcemia, the prognosis for response and survival with chemotherapy is worse than in dogs with malignant lymphoma and normocalcemia. This may be related to the fact that hypercalcemia is especially encountered among lymphomas of the T-cell immunophenotype, which have a much less favorable prognosis than the B-cell lymphomas.[101]

In the absence of metastases the prognosis after surgical removal of an adenocarcinoma of the anal sac is excellent. However, there are often metastases to regional lymph nodes; resection of these iliac / lumbar lymph nodes may have a positive effect on prognosis. Hypercalcemia, however, is a negative predictor for survival.[94]

9.5 Vitamin D-related disorders

9.5.1 Hypovitaminosis D

Dogs and cats are dependent on the dietary vitamin D content to fulfill their requirement.[102,103] Prey, home-made diets containing animal fat, and commercial pet foods contain sufficient vitamin D (table 9.1). Only when extremely deficient diets are fed (i.e., only lean meat or only vegetables) vitamin D deficiency may develop, and then especially in pups or kittens which have not had the chance to store enough vitamin D in their body fat.[69] Hypovitaminosis D in young animals (*rickets*) occurs rarely, but may be mentioned by the owner because it is a classic bone disease. In adult dogs and cats hypovitaminosis D (*osteomalacia*) does not cause clinically relevant disturbances in bone metabolism. This section will therefore focus on rickets.

When there is a low vitamin D intake, insufficient calcitriol is formed. This leads to insufficient calcium and phosphate absorption from the intestine, low osteoclastic activity, and insufficient renal reabsorption of calcium and phosphate. As a result, the plasma calcium concentration tends to decrease. This in turn stimulates the parathyroid glands to hypersecrete PTH, thereby increasing calcium reabsorption and osteoclastic activity (fig. 9.4) and decreasing the renal tubular maximum for phosphate (causing hypophosphatemia). Due to hypovitaminosis D, newly formed osteoid is not mineralized. The mineralized bone is therefore sealed off eventually by nonmineralized osteoid, making it inaccessible to the os-





Figure 9.33:
(A) Young mixbred dog with clearly noticeable bulging metaphyseal areas of the distal radius and ulna, as well as pronounced palpable areas near the growth plates of all ribs.
(B) The radiograph of the radius and ulna revealed thin cortices, wide medullary cavities, and increased width of the growth plates with a mushroom appearance, typical for hypovitaminosis D.



Figure 9.34:
Radiograph of a four-month-old boxer with hypertrophic osteodystrophy. A pathognomonic radiolucent area (arrow) parallels the growth plates and is separated from it by a thin mineralized area. In addition, the well-mineralized cortex differs considerably from what is seen in hypovitaminosis D (see for comparison fig. 9.33).

teoclasts for resorption and remodeling. The newly-formed cartilage will not mineralize and this prevents completion of the cascade of events in endochondral ossification (fig. 9.16).

Clinical manifestations

The animal is alert, its coat may be in poor condition, and its body conformation may be disproportional due to the fact that growth of bones lags behind that of the soft tissues. The animal is reluctant to walk and palpation of the bones causes pain. The legs are bent and the metaphyseal areas of long bones and ribs are enlarged (fig. 9.33). The plasma calcium concentration is low to normal (fig. 9.1), whereas the phosphate concentration is low in plasma ($<$ 1 mmol/l) and high in urine ($>$ 20 mmol/l), the latter due to the concomitant hyperparathyroidism.

On radiographic examination the cortex of the long bones is thin and may be folded or there may be pathological fractures. The growth plates are extremely wide for the chronological age of the animal (fig. 9.33).

Diagnosis

The plasma concentrations of 25-OHD and 24,25-$(OH)_2$D are very low and the concentration of calcitriol is low to normal. The radiological abnormalities are quite typical for hypovitaminosis D (fig. 9.33). A biopsy of the greater tubercle, to obtain cancellous bone and growth plate cartilage without disturbing growth in length, will reveal osteoid seams covering poorly-mineralized trabeculae and an extremely wide growth plate.

Differential diagnosis

This entity can be confused with or be complicated by nutritional secondary hyperparathyroidism, depending on the mineral content of the food. However, the plasma concentration of vitamin D metabolites and the radiological appear-

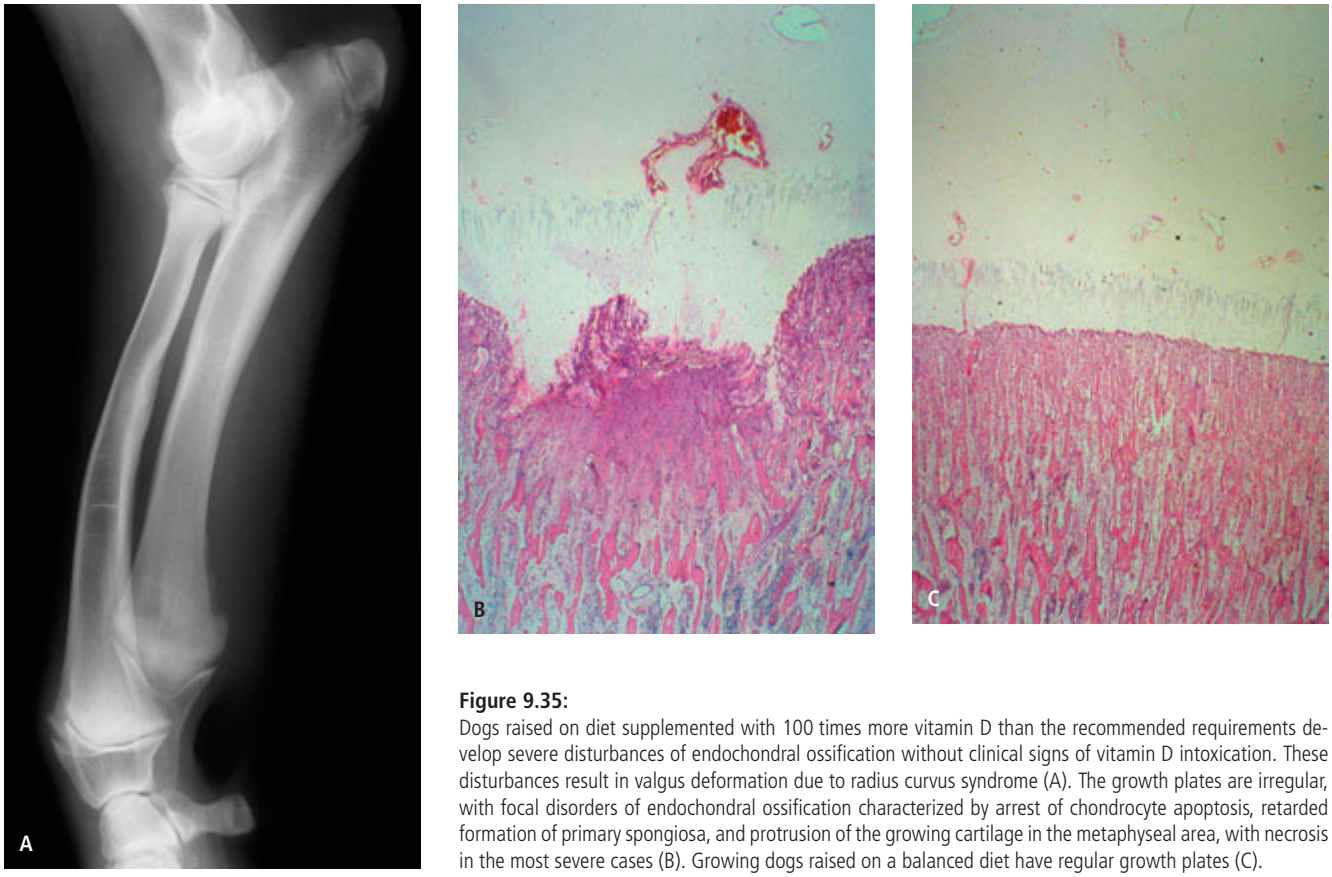


Figure 9.35:
Dogs raised on diet supplemented with 100 times more vitamin D than the recommended requirements develop severe disturbances of endochondral ossification without clinical signs of vitamin D intoxication. These disturbances result in valgus deformation due to radius curvus syndrome (A). The growth plates are irregular, with focal disorders of endochondral ossification characterized by arrest of chondrocyte apoptosis, retarded formation of primary spongiosa, and protrusion of the growing cartilage in the metaphyseal area, with necrosis in the most severe cases (B). Growing dogs raised on a balanced diet have regular growth plates (C).

ance of growth plates will be different. With regard to the latter, hypertrophic osteodystrophy (fig. 9.34) and congenital disorders such as chondrodysplasia[104] have to be considered.

Treatment

The dog or cat must be fed a normal food, containing 400 IE vitamin D per kg, as soon as possible.[69] Within four weeks, mineralization of cortices, growth plates, and callus will occur to such an extent that corrective orthopedic surgery can be performed if necessary.

Prognosis

The prognosis for mineralization of bone and cartilage is good, but functional recovery depends on the severity of the skeletal abnormalities.

9.5.2 Hypervitaminosis D and vitamin D intoxication

Hypervitaminosis D usually results from excessive supplementation of vitamin D in the diet.[105] Granulomatous disease in humans is also associated with increased production of 1,25-$(OH)_2$D by activated macrophages in the granulomas. Hypercalcemia combined with elevated plasma 24,25-$(OH)_2$D has been reported in a dog with granulomatous lymphadenitis[106] and in dogs with granulomas due to angiostrongylosis.[107]

Vitamin D intoxication may result from overdosage of vitamin D in the treatment of hypoparathyroidism or from intoxication with cholecalciferol-containing rodenticides.[108–110] Vitamin D intoxication leads to increased formation of 25-OHD, augmented calcium and phosphate absorption from the intestine, and increased calcium and phosphate reabsorption in the kidneys. The resulting hypercalcemia as well as the direct feedback effect of vitamin D on the activity of the chief cells in the parathyroid glands (fig. 9.12) causes hypoparathyroidism, which increases the tubular maximum for phosphate. The elevated plasma concentrations of calcium and phosphate lead to increased urinary excretion of both elements. Eventually calcification of soft tissues will occur, including vessel walls and heart valves, as well as kidney tubules with renal failure as a consequence.

In contrast to vitamin D intoxication, hypervitaminosis D is characterized by normocalcemia, normophosphatemia, and no clinical signs of soft tissue calcification, but with disturbances of endochondral ossification (fig. 9.35).[111] In hyper-

vitaminosis D effective counterbalance is provided by low plasma PTH levels, high calcitonin levels, and the effectiveness of 24-hydroxylase, resulting in lower plasma calcitriol levels than in controls.[112] Commercially available dry dog food may exceed by four to 13 times the recommended requirements of vitamin D. It has been shown that a tenfold increased vitamin D intake during growth is sufficiently counterregulated by the calciotropic hormones, resulting in maintenance of calcium homeostasis and only minimal microscopic changes in endochondral ossification.[3]

Clinical manifestations

The symptoms and signs of vitamin D intoxication may be dominated by one or more of the signs of hypercalcemia, such as polydipsia / polyuria, dehydration, weakness, and anorexia.[108] If complicated by renal insufficiency, there may be vomiting and other signs of azotemia. Routine laboratory investigations will reveal that calcium and phosphate concentrations in plasma and urine are elevated. The circulating concentration of PTH is low and that of 25-OHD is high, whereas the plasma calcitriol concentration is low to normal (except when calcitriol has been administered and is the cause of intoxication).

Diagnosis

The diagnosis can be made on the basis of the history and the finding of elevated concentrations of calcium and phosphate in plasma and urine. Especially hypercalcemia of malignancy (chapter 9.4) and hyperphosphatemia due to primary renal disease (chapter 9.3.2) should be ruled out. For the differential diagnosis of hypercalcemia, the reader is referred to chapter 9.3.1.

Treatment

The aim of the treatment is to minimize nephrocalcinosis by increasing renal calcium excretion and by decreasing intestinal calcium absorption. In mild cases glucocorticoids can be prescribed to reduce intestinal absorption and increase renal excretion of calcium. In addition, a diet without calcium should be given to minimize intestinal calcium absorption. In cases with severe hypercalcemia ($>$ 4.0 mmol/l), general weakness, and anorexia, fluid therapy should be given, since dehydration contributes to the increased plasma calcium concentration. Mild volume expansion together with furosemide will promote calciuria. The treatment with glucocorticoids, furosemide, and the special diet should be continued for at least one month, since the release of the vitamin D stores in body fat may take several weeks. [108]

Treatment of the hypercalcemia with injections of calcitonin in order to reduce calcium release from the bone by osteoclasts has been recommended.[108] However, osteoclasia is not the main cause of hypercalcemia in hypervitaminosis D. In addition, the use of heterologous calcitonin may cause antibody formation and contribute to the feeling of illness. The use of substances that inhibit bone resorption, such as the biphosphonates widely used for the treatment of osteoporosis in humans, has given promising results in experimental settings and in several disease processes in dogs and cats.[100,113,114]

Prognosis

Neuromuscular disturbances and encephalopathy due to rapid development of severe hypercalcemia may occur and death may ensue. If there is renal damage, the prognosis is guarded. In milder cases, treatment can be successful.[108]

9.6 Calcitonin-related disorders

9.6.1 Nutritional secondary hypercalcitoninism

Supplementation of balanced commercial foods and the use of home-made unbalanced diets are common errors. Especially young dogs of large breeds are often given extra mineral and vitamin mixtures. Studies in giant and miniature dogs have revealed that in large-breed dogs overfeeding of a balanced diet or supplementing an otherwise balanced diet with calcium or vitamin D causes hypercalcitoninism, with severe consequences for skeletal development.[111,115]

During calcium ingestion, the plasma CT concentration is raised directly (by calcium) and indirectly (e.g., by gastrin), causing osteoclasts to retract their brush border (fig. 9.8). As a consequence, the plasma calcium concentration is prevented from rising (and therefore the PTH concentration does not fall) and thus calcium is not lost via the kidneys but routed primarily to the bone, ready to be used at a later stage or added to the mineral content of the bone (fig. 9.4).

Chronic high intake of calcium causes C-cell hyperplasia in young dogs.[4,116] Persistent hypercalcitoninism causes decreased osteoclastic activity and hypermineralization of the skeleton. The imbalance of the calciotropic hormones and the (direct or indirect) effect of calcium on chondrocytes may lead to disturbed endochondral ossification. In this situation the chondrocytes do not mature, the intercellular substance does not mineralize, and the chondrocytes continue to live and prevent blood vessels from invading. The disturbed cartilage maturation is characterized by thickened cartilage and is known as osteochondrosis.[22,117]

The consequences of decreased osteoclasia with hypermineralization as well as signs of osteochondrosis may be observed in the same patient in varying gradations. However, in some cases one of these disturbances may dominate the clinical features. Therefore each entity will be discussed separately.

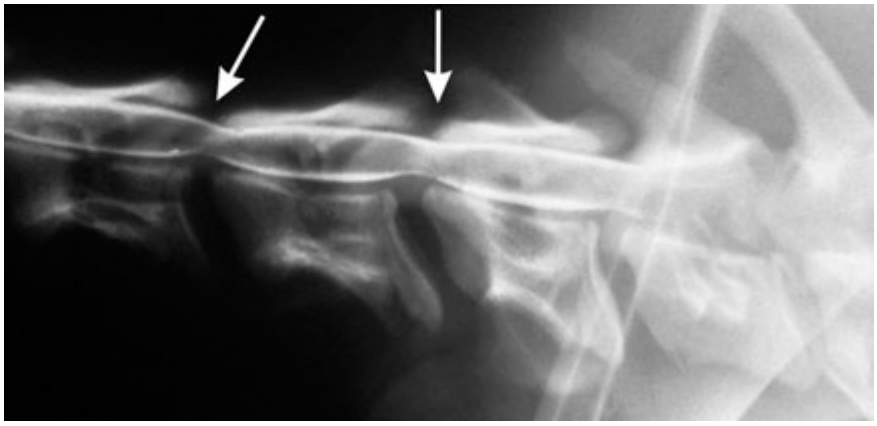


Figure 9.36:
Myelogram of the cervical region of a six-month-old Great Dane with an uncoordinated gait, pain reaction upon hyperextension of the neck, and positive crossed extensor reflexes of the rear limbs. The radiograph reveals impingement of the spinal cord at the cranial orifices of the 5th and 6th cervical vertebrae (arrows), typical of the canine wobbler syndrome.

9.6.1.1 Decreased osteoclasia

Chronic excessive calcium intake (with or without a constant ratio to phosphorus) causes hypercalcitoninism, which induces decreased osteoclastic skeletal remodeling (fig. 9.4). Especially foramina, which do not widen in proportion to soft tissue growth, may cause noticeable hindrance to both nervous structures and blood vessels, which may lead to cervical spondylomyelopathy and enostosis, respectively.

9.6.1.1.1 Cervical spondylomyelopathy

Retarded skeletal remodeling of the spinal canal at the cranial vertebral orifice may cause irreversible damage to the spinal cord. This occurs especially in the cervical region and may give rise to ataxia (uncoordinated gait), thereby being one of the causes of the so-called *canine wobbler syndrome*.

Diagnosis

The clinical findings include uncoordinated gait in young dogs (approximately six months of age) of a giant breed (e.g., Great Dane), with pain in response to extension of the neck, hyperactivity of the reflexes of the pelvic limbs, and positive crossed extensor reflexes of the pelvic limbs. Radiographs of the cervical vertebrae may reveal narrowing of the cranial orifices of the fourth, fifth, and sixth cervical vertebrae and myelography will reveal impingement on the spinal cord at these locations (fig 9.36).

Differential diagnosis

Discospondylitis, inflammatory (infectious) meningitis, and traumatic or congenital abnormalities should be considered in young dogs with these neurological signs. Cervical disc protrusion and the vertebral instability as seen in older dogs (approximately six years of age) of large breeds (e.g., Doberman) can give identical clinical signs.

Treatment

The spinal cord may be so seriously damaged that the lesion is irreversible and any treatment unsuccessful. In milder cases correction of the diet, glucocorticoid therapy, and avoidance of microtrauma caused by pulling on the collar may lead to clinical improvement. Surgical decompression is indicated in young dogs with progressive signs. [118]

Prognosis

In mild cases improvement will follow after four weeks of conservative treatment, but the prognosis in more severe cases with multiple compressions is guarded.

9.6.1.1.2 Enostosis

In dogs with enostosis (also known as canine panosteitis and eosinophilic panosteitis), a delay in remodeling of the nutritional foramen in the diaphyses of all long bones is present. Consequently, edema occurs in the medullary cavity and beneath the sensitive periosteum. Later there is extra bone formation, both in the medullary cavity on organized fibrous tissue and subperiosteally due to the elevation of the periosteum by the edema (fig. 9.37).[119]

Diagnosis

Dogs of larger breeds not over two years of age develop shifting lameness of varying severity.[120,121] Physical examination may reveal an elevated body temperature, severe lameness of one or more legs, and a painful reaction to deep palpation of the long bones. Routine laboratory investigations are inconclusive. In the subacute phase (at least three weeks after the start of the initial signs) radiographic examination of long bones may reveal medullary new bone formation (fig. 9.37). In the more severe cases there may be noticeable subperitoneal new bone. Other causes of lameness of one or more legs in these young dogs (including osteochondritis dissecans, fragmented coronoid process, ununited anconeal process) can occur solely or together with enostosis and may confuse the results of the physical examination. Bone scintigraphy (figs. 9.17, 9.18) and other imaging techniques may help to make the diagnosis and differentiate it from other developmental orthopedic disorders.

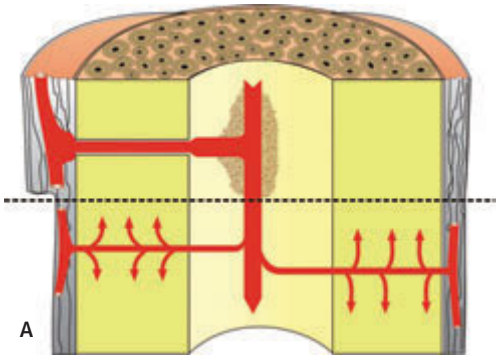




Figure 9.37:
(A) Schematic representation of the pathophysiologic mechanism of enostosis. The cortex receives its blood supply from the periosteal arteries (outer 1/3) and medullary vessels (inner 2/3). A relative delay in remodeling of the nutritional foramen causes impingement of the nutrient arteries, resulting in edema beneath the sensitive periosteum and the medullary cavity.
(B) German shepherd dog, nine months of age, suffering from enostosis with shifting lameness, pain upon palpation of the long bones, and radiopaque areas due to new bone formation in the medullary cavity. These confluent dense areas are first present near the nutritional foramina (arrow) of the long bones.

9

Differential diagnosis

Disturbances of skeletal mineralization including nutritional secondary hyperparathyroidism, painful conditions such as hypertrophic osteodystrophy, and even infectious diseases may be included in the list of differential diagnoses of shifting lameness with elevated temperature.

Treatment

The treatment should be directed at augmentation of osteoclastic activity by providing foods low in calcium, such as meat (Table 9.1). Although this might theoretically be logical, there have been no studies proving that this will have a beneficial effect. In periods of pain, the dog can be treated with nonsteroidal anti-inflammatory drugs or with low doses of glucocorticoids, provided that joint cartilage damage has been excluded.

Prognosis

The prognosis for enostosis is good in the long term, since periods of severe and shifting lameness disappear after the age of two years. Prior to that age these symptoms may recur repeatedly.

9.6.1.2 Osteochondrosis

Osteochondrosis is a disturbance of endochondral ossification. It can be localized at any site where growing cartilage is present during the growth period (fig. 9.16) but especially at sites and times of high growth velocity.[122] In particular, it can occur in the growth plate of the distal ulna (which accounts for 90 % of the growth in length of the ulna). It can be present temporarily in dogs of large breeds without becoming clinically significant.[123] When present to such an extent that it causes a decrease in the growth in length of the ulna, it also influences the growth in length of the radius, causing the *radius curvus syndrome* (fig. 9.38). When present in joint cartilage, microtrauma can cause fissure lines and eventually separation of diseased cartilage, called *osteochondritis dissecans*.

9.6.1.2.1 Radius curvus syndrome

This abnormal development of the front legs can develop in dogs with an age of four to six months and especially in dogs of large breeds, raised on excessive amounts of food or raised on lesser amounts of food but with excess calcium, with or without excess phosphate,[115,116,124] with or without excess vitamin D,[3,111] but not with excess protein.[125]



Figure 9.38:
(A) Deerhound, eight months of age, with bilateral valgus deformation due to radius curvus syndrome,[119] with a retained cartilage cone (arrow) in the distal ulnar metaphysis (B). (C) The radius may push the humerus proximally against the anconeal process, which breaks off in its growth plate, causing an ununited anconeal process.

Diagnosis

There will be bilateral valgus deformity with cranial bowing of the radius. Radiographs will demonstrate a cartilage cone at the distal growth plate of the ulna, together with a curvature of the radius and a thickened concave cortex (fig. 9.38) and an abnormal alignment of both the carpus and the elbow joint. Due to elbow incongruity, the anconeal process can be loosened in its growth plate (fig. 9.38).[122]

Differential diagnosis

Dogs with chondrodysplasia as prescribed in breed standards (such as the basset hound) or as in inherited disorders (as in the Alaskan malamute[104]) are physically similar in the front legs, but also short in the rear legs. Traumatic injury to the growth plates of the distal radius or ulna may cause early closure of the affected (part of the) growth plate and consequently valgus deformation; mostly this affects only one front leg and no cartilage cone is present.

Treatment

Restriction in food and calcium intake alone can lead to normalization of the endochondral ossification.[69,123] When the valgus deformity is severe, conservative treatment will not normalize the stance nor will it prevent secondary effects, such as incongruity of the elbow joint, detachment of the anconeal process, valgus deformity, and carpal abnormalities. Additional corrective surgery will be needed in these cases. [126]

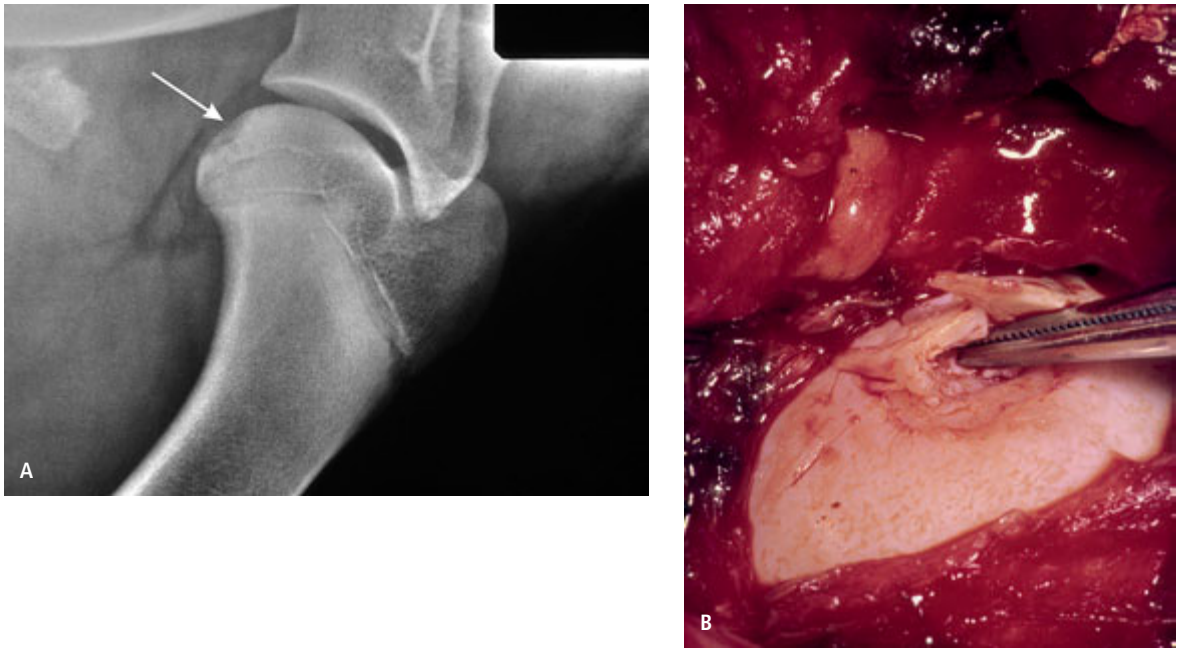


Figure 9.39:
(A) Radiograph of shoulder joint of a seven-month-old Bouvier de Flandres with lameness of both front legs and pain reaction on hyperflexion of the shoulder joint. There is an indentation of the contour of the subchondral bone at the caudal aspect of the humeral head (arrow), indicating osteochondrosis.
(B) Based on the concomitant clinical manifestations, arthrotomy was performed and revealed osteochondritis dissecans with a cartilage flap. Removal of the flap was followed by curettage of the cartilage defect.

9.6.1.2.2 Osteochondritis dissecans

Osteochondritis dissecans (OCD) designates osteochondrosis in joint cartilage whereby thickened cartilage is detached and the inflammation of subchondral bone and joint capsule causes pain.[117] It can occur in a variety of joints (i.e., shoulder, elbow, stifle, and tibiotarsal joint) and is very often bilateral. The genotype of the dog also plays an important role in the occurrence of this disorder. Of all environmental factors calcium intake is the most important.[127]

The dogs, of medium-sized or large breeds, being approximately half a year of age and often rapidly growing, are lame or have a stiff gait in one or more legs.[127] Joints are overfilled and painful upon hyperextension or hyperflexion, and crepitation may be present. With radiographs or other imaging techniques an indentation of the contour of the subchondral bone can be seen, or even a mineralized cartilage flap (fig. 9.39).

Diagnosis

Clinical and radiological investigation will help to make the diagnosis. Arthrography, computed tomography, and arthroscopy may precede arthrotomy.

Treatment

In mild cases no treatment may be needed or nonsteroidal anti-inflammatory drugs can be given when needed. Large cartilage flaps can be removed and lesions curetted to induce early healing (fig. 9.39). Thickened cartilage (i.e., osteochondrosis) in other joints can be prevented from detachment by decreasing overload (by reducing body weight and by rest) in order to diminish microtrauma of the unmineralized cartilage.

Prognosis

The prognosis depends on the severity of the lesion, the secondary arthrotic changes, and the joint affected. The lesion in the proximal shoulder can heal completely, whereas lesions in the talus may continue to interfere with joint stability and cause severe arthrosis. [128]

9.7 Miscellaneous

In addition to the calciotropic hormones, other hormones and nutritional factors may play a significant role in bone metabolism. Their influences on bone or cartilage cells are summarized as follows.

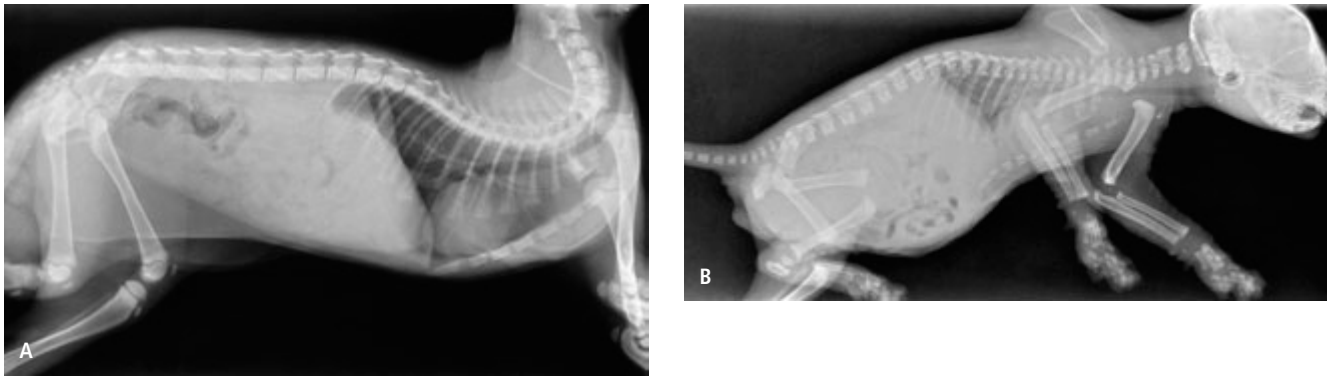


Figure 9.40:
Radiographs of two littermate tomcats (see also fig. 3.9) at eight weeks of age: (A) healthy and (B) congenitally hypothyroid, revealing retarded skeletal growth and development.

Growth hormone (GH) promotes the differentiation and (via IGF-I) the proliferation of the chondrocytes of the growth plates (fig. 9.16).[23,129] Deficiency of GH at a young age causes proportional dwarfism (fig. 2.14)

Thyroid hormone influences proliferation and maturation of the growth plate chondrocytes, and probably part of these effects is mediated by modulating local GH and/or IGF-I actions.[23,130] Thyroid hormone deficiency in young dogs (fig. 3.8) and cats leads to retardation of growth and maturation of the skeleton (figs. 3.10, 9.40).[131]

Glucocorticoids are known to impair chondrogenesis directly and indirectly, by interacting locally with the GH-IGF-I pathway and the effects of thyroid hormone.[23,132] This may lead to stunted growth in height when given for a prolonged period to immature animals. Since glucocorticoids increase PTH release and decrease calcium absorption from the intestine, their effect on bone is generalized osteoclasia, resulting in osteoporosis (fig. 9.41). However, chronic excess of either endogenous or exogenous glucocorticoids only rarely leads to pathological fractures in mature dogs and cats.

Testosterone causes an increase in bone growth, whereas **estrogens** accelerate skeletal maturation and thereby cause premature closure of the growth plate, resulting in decreased longitudinal growth.[23,129] Estrogens exert their effect directly through the estrogen receptor, as well as indirectly by interacting with the local GH-IGF-I axis. Castration of immature male dogs and cats results in greater height at the shoulder (table 9.2),[133] whereas exogenous gonadal steroids may stunt growth after an initial growth spurt.

Osteoporosis is a major problem in anestrogenic women. Cessation of ovarian function also causes bone loss in dogs,[134] but mostly not to the extent that it leads to clinical problems.

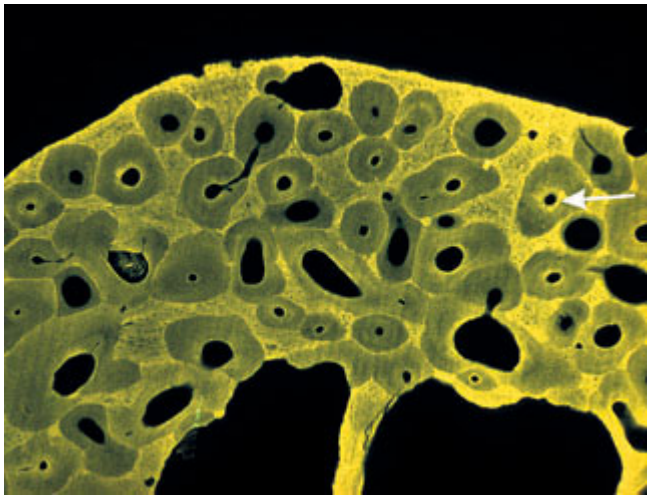


Figure 9.41:
Microradiograph of transverse section of the rib of a dog with hypercortisolism. Osteoporosis characterized by widened Haversian canals as compared to normal (arrow). (Courtesy of Department of Pathology, Free University, Berlin.)

Table 9.2: Effects of prepubertal gonadectomy on skeletal growth in dogs[133]

Age at gonadectomy	Number of dogs	Physeal closure of distal radius-ulna	Length of radius
Controls	10	41.6 ± 1.2 weeks	16.8 ± 0.9 cm
7 weeks	14	59.4 ± 3.1 weeks	18.6 ± 0.7 cm
7 months	8	54.6 ± 1.2 weeks	17.6 ± 1.0 cm

Early gonadectomy leads to enuchism: later physeal closure and taller stature.





Figure 9.42:
Hypervitaminosis A in a three-year-old cat, which was fed almost exclusively cat food and raw liver and was referred because of lameness of both front legs and an inability to groom itself. The radiographs revealed new bone formation without bone loss on the vertebrae (A) and around the elbow joint (B), causing ankylosis.

Vitamin A (or retinol) is formed in the gut of dogs by the reversible reduction of retinaldehyde originating from carotene. Cats require retinol (as present in a variety of foodstuffs), since cats lack carotenase in their intestinal mucosa.[135] Vitamin A is oxidized in its target cells to retinoic acid. Retinoic acid interacts via nuclear receptors with the genome to regulate cellular growth and differentiation.[136] Vitamin A is important for normal osteoblastic, chondroblastic, and osteoclastic activity. High doses of vitamin A inhibit chondrogenesis in growth plates and inhibit collagen synthesis by osteoblasts in both dogs and cats. Since cats are not able to form retinyl esters in order to excrete the excess of this fat-soluble vitamin, chronic vitamin A intoxication is more likely to be diagnosed in cats than in dogs.

Hypervitaminosis A in cats is characterized by new bone formation without osteolysis, starting at the points of insertion of ligaments, muscles, and joint capsules, which causes narrowing of the intervertebral foramina in the vertebral bodies and ankylosis of vertebrae and larger joints. This causes pain, lameness, and stiffness (fig. 9.42). The vitamin A concentration in plasma or in a liver biopsy (since the liver is the major organ in vitamin A storage) can support the diagnosis.[137,138] Although ankylosis is irreversible, the cat will improve with appropriate analgesia and feeding a low vitamin A-containing food for several weeks.

9.8 Puerperal tetany

At the peak of lactation, two to three weeks postpartum, hypocalcemia may occur in bitches and less often in queens. Puerperal tetany occurs mainly in bitches of small breeds with large litters. Little is known about the pathogenesis, but insufficient calcium supply during nursing may be a causative factor. In cats, preparturient hypocalcemia has also been reported.[139]

Clinical manifestations

Once the plasma calcium concentration has reached a critical level, the signs may proceed rapidly from restlessness, panting, and ataxia to tetany with tonic/clonic convulsions and opisthotonus. Examination usually reveals an anxious, restless animal with tachycardia and hyperthermia. In a very severely hypocalcemic (and hypomagnesemic) lactating bitch the typical muscle tremors and rigidity were not part of the clinical features. In this dog the clinical manifestations were dominated by atony, weakness and paresis.[140]

Diagnosis

The diagnosis is usually made by the recognition of the combination of a heavily lactating animal with signs of increased neuromuscular excitability. Laboratory examination will reveal hypocalcemia and usually also hypophosphatemia.

Treatment

The condition may be fatal if left untreated. Consequently, treatment is begun without delay, i.e., without laboratory confirmation. As in hypocalcemia of primary hypoparathy-

roidism, calcium gluconate is injected intravenously (see chapter 9.2). The signs of tetany usually disappear within a few minutes. In order to prevent rapid recurrence a similar dose is given subcutaneously (1:4 diluted with 0.9 % NaCl). Puppies or kittens should be removed to reduce the lactational calcium loss. When sufficiently mature, the puppies or kittens can be weaned. If not, they can be returned after 24 h and in the meantime fed a milk substitute.

Prognosis

With a nutritionally balanced diet and oral calcium supplementation (chapter 9.2) during the remainder of the lactation period, there are usually no recurrences. For the next pregnancy and lactation care should be given to supply the dam with a complete, well-balanced diet. Additional feeding of the litter as early as possible may also help to prevent tetany. There is no need to give extra calcium in excess of the normal requirements during pregnancy, and in line with experiences in other species it may even be contraindicated.

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10 Tissue Hormones and Humoral Manifestations of Cancer

Ad Rijnberk
Hans S. Kooistra

10.1 Introduction

As discussed in chapter 1.1 the capacity to synthesize and secrete hormones is not confined to endocrine glands. In the last two decades it has become clear that body functions are also strongly influenced by diffuse hormonal secretion emanating from many cellular sources. Initially it was thought that these cells, although occurring in different anatomical sites, shared a common embryologic origin and common functional properties. Because of some common biochemical characteristics the acronym APUD (**a**mine **p**recursor **u**ptake and **d**ecarboxylation) was coined for these cells, and because of the presumed common embryogenesis from the neural crest, the term neuroendocrine was introduced.

It is now known that not all of these cells originate from neural crest or ectoderm. For example, those producing gastrointestinal and pancreatic hormones are derived from endoderm. It has therefore been proposed to de-emphasize embryologic origin and instead to designate this widespread endocrine/paracrine/autocrine system as the »diffuse neuroendocrine system« or DNES, of which neuroendocrine cells with APUD characteristics are one constituent.[1] A preeminent example of this system has been presented in chapter 2.2.1, i.e., growth hormone (GH) producing cells in the mammary gland (fig. 2.11).

Part of the relevance of these tissue hormones or the DNES is in the recognition of the wide distribution of peptide-secreting cells that may exert autocrine and paracrine actions (fig. 1.1) for vital processes such as epithelial growth. In the gut there is a functional convergence of tissue hormones and the nervous system, in which DNES cells and local peptide-containing neuronal cells and ganglia coordinate local neuroendocrine regulatory functions.

In addition to their important roles in physiology, the cells of the DNES may be involved in excessive secretion under the influence of exogenous or endogenous stimulation such as in the case of progestin-induced GH excess (chapter 2.2.4.2). It can also result from neoplastic transformation of DNES cells. Hormone excess syndromes caused by tumors in tissues that do not normally secrete the hormone in significant amounts have been termed »paraneoplastic endocrine syndromes« or »ectopic hormonal syndromes«. Examples are hypercalcemia due to PTHrP secretion by a tumor originating from apocrine glands of the anal sac region (chapter 9.4), hypercortisolism due to ACTH excess produced by a pancreatic neuroendocrine tumor (chapter 4.3.4), and hypoglycemia due to gastric or hepatic tumors secreting incompletely processed insulin-like growth factors (chapter 5.3.2).

These ectopic hormone syndromes are not truly ectopic. Instead, they are the consequence of tumor-induced amplification of a property that is normally present in the cells from which the neoplasm originated.[2] A common feature in these syndromes is the elaboration of peptide hormones. In general, steroid synthesis by neoplasms depends on their origin in adrenal or gonadal tissue. Complete synthesis of steroid (or thyroid) hormones by tumors originating from nonendocrine tissue has not been described in dogs or cats and seems to be extremely rare in man.

This chapter concerns some peptides that have been studied to some extent in dogs and cats but have not been discussed in detail in previous chapters and/or are known to be associated with humoral manifestations of cancer.

10.2 Natriuretic peptides

Natriuretic peptides (NP) are structurally related peptides that play an important role in the integrated control of volume homeostasis (fig. 10.1). They are released into the circulation by stretching of the myocardium. The best known action of these peptides is that on the kidney, where they promote the excretion of sodium and water. These diuretic and natriuretic effects result from complex interactions with renal hemodynamics, tubular sodium handling, and modulation of a number of hormones and intrarenal paracrine factors, thereby opposing the actions of the renin-angiotensin system (chapter 4.4.1).[3]

Atrial natriuretic peptide (ANP) is a 28-amino acid peptide secreted from the cardiac atria in response to stretching of the atrium (figs. 10.2, 10.3). It is secreted in equimolar amounts with a physiologically inactive 98-amino acid N-terminal fragment of pro-ANP (NT-proANP). Canine NT-proANP is 87 per cent homologous with human NT-proANP, allowing measurements of NT-proANP in dogs with assays for human NT-proANP fragments. Brain natriuretic peptide (BNP) was initially purified from porcine brain extracts and hence the name, but it was subsequently found in much higher concen-

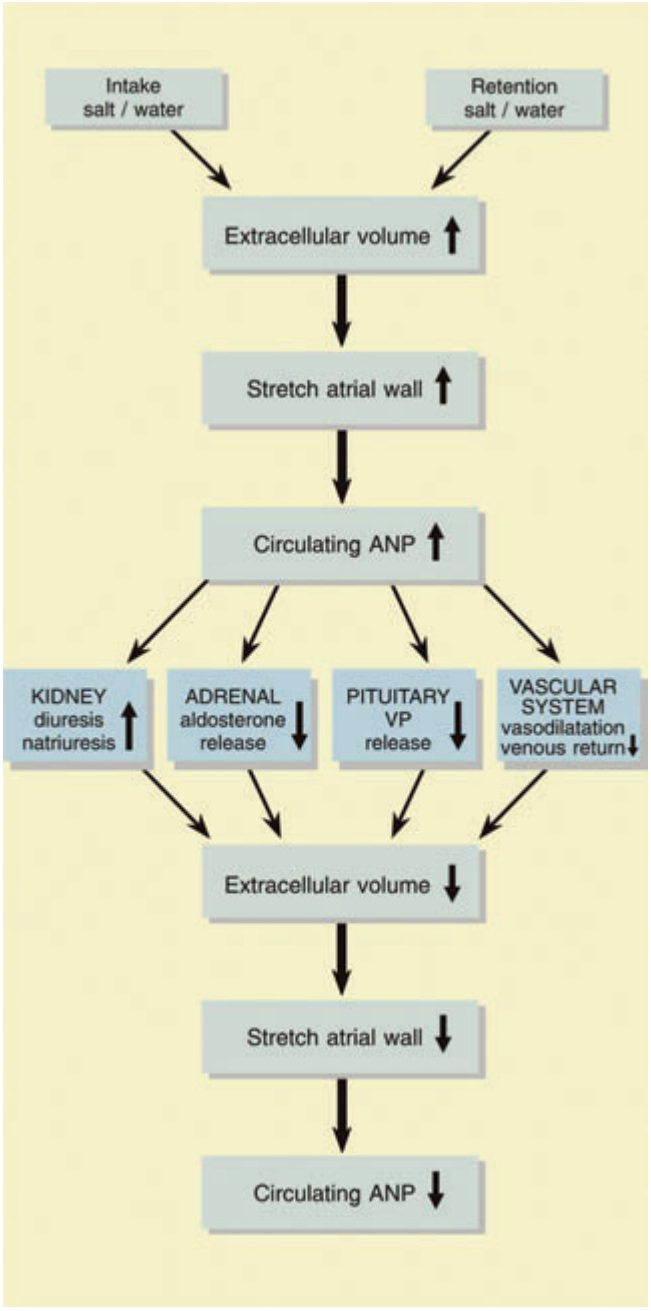


Figure 10.1:
Role of natriuretic peptides in volume homeostasis.
↑ = increase, ↓ = decrease

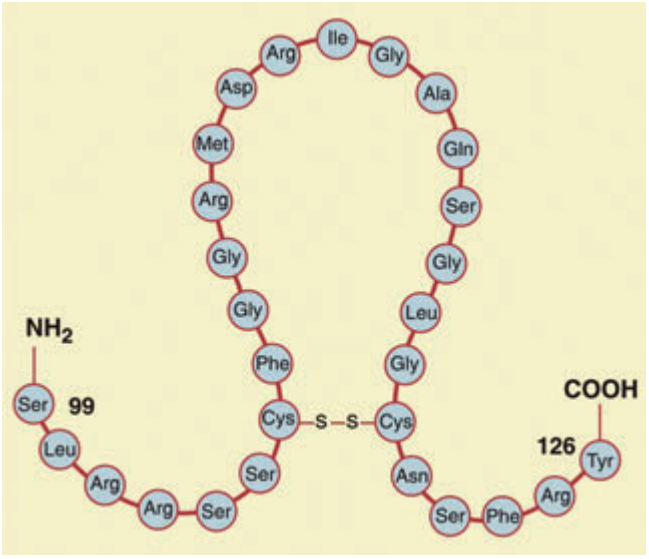


Figure 10.2:
The amino acid sequence of canine ANP. The disulfide bond is essential for biological activity.

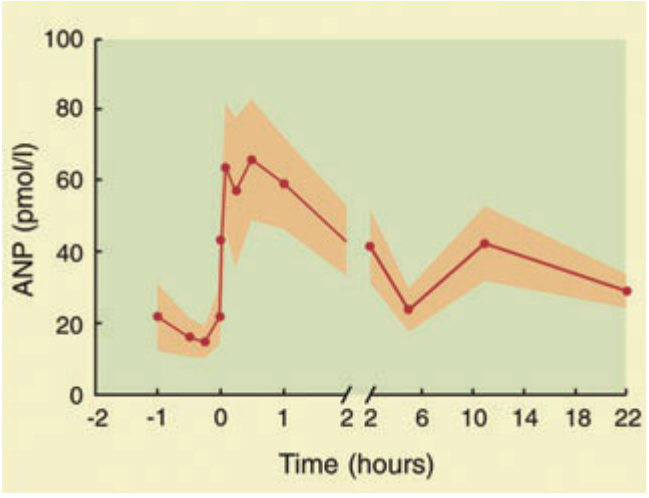


Figure 10.3:
Plasma ANP concentrations (mean ± SEM) in eleven dogs with pericardial effusion, as influenced by pericardiocentesis (at time zero). This illustrates that it is not pericardial or atrial pressure but rather atrial stretch that causes ANP release. (Adapted from Stokhof et al., 1994.)[5]

trations in cardiac ventricles. ProBNP undergoes posttranslational modification similar to that for proANP, resulting in BNP and NT-proBNP. Canine NT-proBNP shares only 45 per cent homology with human NT-proBNP. Recently assays have been introduced that enable measurements of concentrations of NT-proBNP in dog plasma.[4]

The half lives of both NT-proANP and NT-proBNP are longer than those of ANP and BNP, respectively. These physiologically inactive fragments are also more stable in the laboratory than the active hormones. These characteristics make measurements of the pro-fragments more suitable for clinical assessment of heart disease. Studies in dogs and cats

have revealed that measurements of plasma NT-proANP and NT-proBNP are useful adjuncts in diagnosing cardiac disease and in assessing the severity of the disease. These measurements are also helpful in distinguishing between animals with congestive heart failure and those with primary pulmonary disease.[6–9] Renal function must be taken into account when interpreting elevated concentrations.[4]

10.3 Erythropoietin

Erythropoietin (Epo) is a glycoprotein having a molecular mass of 34 kD and containing 165 amino acids. About 40 % of its mass is composed of carbohydrate. It is primarily produced in interstitial fibroblasts in the kidney. Extrarenal sources account for less than 10 % of the production, for which the liver is the main site. Epo exhibits a high degree of sequence homology among mammals. Human Epo is 85 % identical to feline and canine Epo.[10,11]

The release of Epo is regulated by classic feedback control. It is secreted in response to renal tissue hypoxia, whereas hyperoxia decreases its production. This includes not only systemic hypoxia, but also local changes in renal blood flow caused by renal cysts or tumors that compress the surrounding renal parenchyma. Other hormones may influence Epo secretion and thereby erythropoiesis. GH and IGF-I have been reported to decrease Epo secretion from rat kidneys.[12] Chronic GH administration to dogs causes a dose-related normochromic, normocytic, nonregenerative anemia leading to a 10 % decrement in the hematocrit.[13] This can explain why bitches that develop diabetes mellitus in the luteal phase of the estrous cycle, i.e., due to progesterone-induced GH excess of mammary origin (chapter 2.2.4.2), may have low hematocrit values. Glucocorticoids are important in promoting erythropoiesis directly in situations of hematological stress, such as in coping with hemorrhage or erythrolysis.[14,15] Thyroid hormone induces Epo gene expression and directly promotes differentiation and maturation of erythroid cells toward nucleated red blood cells.[16] In the absence of thyroid hormone, such as in primary hypothyroidism, hematocrit values are usually low (chapter 3.3.1).

With progressive loss of renal parenchyma, such as in chronic renal insufficiency, a relative deficiency of Epo may develop. Indeed, in dogs with chronic renal failure, circulating concentrations of Epo have been reported in the low normal range, despite mild to moderate anemia.[17] Recombinant human Epo (rhEpo) has been used to treat this anemia. In both dogs and cats rhEpo induces a rapid and substantial red cell response, but in several treated animals the effect was short-lived because of the development of antibodies against rhEpo.[18] Apparently there is sufficient structural difference between rhEpo and feline Epo (fEpo) or canine Epo (cEpo) to induce an immune response. The use of rcEpo has been reported to stimulate erythrocyte production in dogs with nonregenerative anemia secondary to renal failure without causing the profound erythroid hypoplasia that may occur in rhEpo-treated dogs.[19] Unfortunately this does not hold true for cats and even with use of rfEpo red-cell aplasia may develop.[20] This has also been reported to occur in cats in which fEpo was delivered via gene therapy.[21]



Figure 10.4:
Transverse CT of the thorax at the level of T-7 in a twelve-year-old male beagle with pituitary-dependent hypercortisolism and polycythemia. The right ventricle is enlarged and there is marked enlargement of the right caudal lobar pulmonary artery (arrow) caused by a thrombus.[28]

Increased production of Epo may lead to the syndrome of polycythemia, termed secondary polycythemia because it is secondary to excessive production of Epo or another erythroid-stimulatory substance. In this syndrome most symptoms and signs can be related to hyperviscosity. They include lethargy, disorientation, tremors, ataxia, episodic weakness, and seizures. The sludging of blood cells may result in thrombosis and hemorrhagic diathesis. In most of the reported cases the underlying cause was a renal carcinoma and its removal resolved the problems.[22,23] Polycythemia has also been observed in dogs with tumors of nonrenal origin, such as a cecal leiomyosarcoma and a cervical schwannoma.[24,25] Nonneoplastic renal disorders such as pyelonephritis have also been reported to cause secondary polycythemia.[26,27] In these cases the erythrocytosis has been suggested to be the result of circulatory disturbances in the kidney, sufficient to cause local tissue hypoxia without destruction of the cells responsible for Epo production. Consistent with the previously-mentioned effect of glucocorticoids on erythropoiesis, spontaneous hypercortisolism may also be accompanied by elevated hematocrit values (table 4.3). Polycythemia has been observed in a dog with pituitary-dependent hypercortisolism (fig. 10.4).[28]

In addition to these secondary forms of polycythemia there is polycythemia vera, in which the circulating concentration of

erythropoietin is low and excessive erythropoiesis is caused by a population of abnormally replicating erythroid progenitor cells that fail to respond to inhibitory signals. The mechanism or cause of this myeloproliferative disorder with characteristics of malignant transformation remains obscure.

Initial treatment consists of temporary alleviation by repeated phlebotomy and replacement of the removed volume by colloidal and electrolyte solutions.[29]

10.4 Humoral manifestations of cancer

As discussed in chapter 10.1, a wide range of DNES hyperplasias and tumors produce peptides causing hormonal syndromes of an »ectopic« nature. Measurements of the particular hormone can be used as an aid in diagnosis and in following the result of treatment. Many of these tumors can be visualized with labeled ligands to receptors on the DNES cells. For example, octreotide scintigraphy can visualize somatostatin-binding sites (see also chapter 5.3.1 and fig. 5.22). Furthermore, tumors with somatostatin receptors may respond to a somatostatin analogue such as octreotide by decreased peptide secretion and diminished growth.

The secretion of peptides by cancers is not confined to the well-known peptide hormones but may also include cytokines. Tumor stroma contains inflammatory cells such as macrophages, dendritic cells, and T-regulatory lymphocytes that are involved in the development and progression of cancer. In humans the systemic effects of pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) include fatigue, depression, anorexia, fever, and hyperalgesia.[30–32] This cancer cachexia often results in marked weight loss and particularly loss of muscle mass. The latter is due to increased degradation of myofibrillar protein, especially myosin heavy-chain, and sometimes also decreased protein synthesis. The enhanced protein degradation is mediated by the ubiquitin-dependent proteolytic system, which can be activated by cytokines such as TNF-α and IL-1.[33]

In addition to anorexia and protein loss, a hypermetabolic state also plays an important role in cancer cachexia. There is increased thermogenesis in brown adipose tissue caused by uncoupling of mitochondrial respiration from adenosine-5'-triphosphate (ATP) synthesis. There is experimental evidence that TNF-α can stimulate heat production in cancer patients indirectly by promoting the expression of uncoupling proteins, and also by direct uncoupling of mitochondrial respiration.[33]

Similar to neoplasms in humans, osteosarcomas in dogs increase resting energy expenditure and protein loss.[34] The syndrome of cancer cachexia has been studied in canine and feline patients by measuring weight loss and changes in body condition. Both weight loss and loss of lean body mass are more prevalent in cancer in cats than in dogs.[35,36]

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11 Obesity

Margarethe Hoenig

11.1 Introduction

Obesity is a pandemic in humans and pet animals. This most common nutritional disorder in cats and dogs occurs when energy input – food intake – is greater than energy output. The excess energy leads to deposition of triglycerides in adipose tissue. The oversupply of high-energy diets to a pet population now frequently leading a very sedentary lifestyle is the most likely cause.

Obesity is now thought to occur in one-third to one-half of the dog and cat populations.[1–3] It is a risk factor for several diseases, including arthritis, cardiovascular disease, respiratory conditions, dermatopathies, urinary tract disease, and cancer. In cats, it increases several-fold the risk of developing diabetes mellitus. Gender, neutering, and age are risk factors for obesity in both dogs and cats. In dogs, females are more prone to become obese, while in cats males are. An increased prevalence of obesity has been found in certain breeds of dogs, including the Labrador retriever, Cairn terrier, cocker spaniel, dachshund, and beagle. No breed prevalence has been documented in cats.[4–7]

As we increase our understanding of the physiology of dogs and cats and recognize obesity as a disease that should be recorded and monitored, we can expect to progress in defining the factors involved in its pathophysiology and unraveling its causative role in co-morbidities.

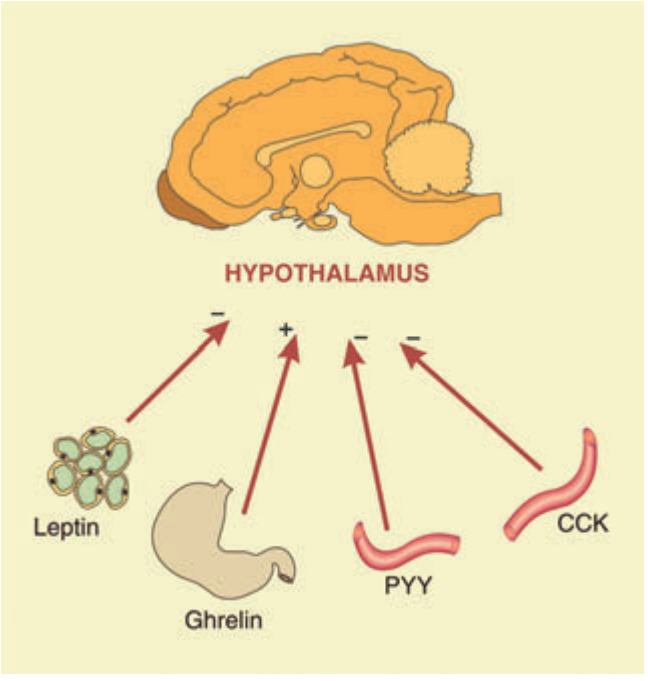


Figure 11.1:
Known regulators of appetite in dogs and cats.
CCK = cholescytokinin; PYY = peptide YY.

11.2 Pathophysiology

11.2.1 Appetite regulation

Understanding the regulation of appetite is an important part of understanding and treating obesity. Unfortunately, very few original data are available from dog and cat studies and most of what is known about appetite regulation stems from other species, notably humans and rodents. Appetite is regulated by several hormones and gastrointestinal factors as well the central and autonomic nervous systems (fig. 11.1). The main central regulator of appetite is the hypothalamus, which senses external stimuli. Few of the peripheral factors have been studied in dogs and cats; they include cholecystokinin, leptin, and ghrelin.

Cholecystokinin, which is secreted in the duodenum, is an appetite suppressant in both dogs and cats.[8,9] Leptin is a hormone synthesized primarily in differentiated adipocytes (fig. 11.1). In healthy subjects, leptin acts centrally, especially on the hypothalamus, inhibiting food intake and increasing energy expenditure. Leptin has been found to be high in obese dogs[10] and cats,[11] which indicates that obesity is a leptin-resistant state. Leptin is also highly correlated with fat mass and can be regarded as a marker of obesity. It decreases with weight loss.[11]

Ghrelin, often called the hunger hormone, is synthesized primarily by the acid-producing cells in the stomach (chapter 2.2.1) and its appetite-stimulating effect is thought to be mediated through neuropeptide Y and agouti gene-related peptide. In dogs, plasma ghrelin concentration has been shown to increase when food is withheld and to decrease after feeding.[12,13] It is increased in obese dogs.[14] In cats, ghrelin has been cloned and purified[15] and has been shown to increase with fasting, but there have been no published studies of ghrelin in obese cats.

Peptide YY (PYY) is thought to mediate the appetite-suppressing effect of dirlotapide (Slentrol®, Pfizer) and mitrata-

pide (Yarvitan®, Janssen), new agents used for the treatment of canine obesity. Dirlotapide and mitratapide inhibit microsomal triglyceride transfer protein, which leads to lipid accumulation in enterocytes. It has been proposed that this change triggers PYY release, which acts as a satiety signal.[16]

11.2.2 Hormonal and metabolic changes

The development of obesity leads to marked alterations in insulin secretion and a decrease in insulin action, i.e., an increase in insulin resistance (fig. 11.2). The mechanism by which an increase in fat mass causes these changes is not understood. It appears that insulin resistance precedes changes in insulin secretion, at least in cats.[17] A recent study revealed that each kg increase in weight led to approximately 30 % loss in insulin sensitivity and glucose effectiveness.[18] The decrease in insulin sensitivity is associated with increased lipolysis in adipose tissue and a rise in the concentration of nonesterified fatty acids in plasma. It is thought that this rise contributes to insulin resistance, not only by increasing glucose output from the liver but also by suppressing insulin-stimulated glucose transport through the insulin-sensitive glucose transporter GLUT 4. In obese cats, GLUT 4 expression was decreased when glycosylated hemoglobin values were still normal.[19] Changes in the subcellular distribution of GLUT 4 have also been observed in obese dogs.[20] Probably because of increased secretion of tumor necrosis factor α from large adipocytes, which regulates lipoprotein lipase, fatty acids are not only deposited in fat cells but are also shunted to muscle cells, where they are deposited.[17]

Obesity also leads to changes in insulin secretion. At a time when fasting blood glucose concentration is still maintained within the normal range, the insulin secretion pattern has already changed in obese cats and dogs compared with lean animals, primarily due to a large increase in second-phase release. The beta cell response is adequate to maintain fasting levels, but it is not adequate to maintain glucose tolerance in all cases. In dogs, glucose intolerance was related to the degree of obesity and was not seen until the dog exceeded its ideal body weight by approximately 70 %.[21] Glucose tolerance was still normal in approximately 30 % of obese cats with a similar degree of obesity.[17] The obese glucose-intolerant cats of that study had a significantly higher area under the curve of insulin concentration during the last 30 min of testing and lower glucose clearance when they were lean, compared with the obese cats having normal glucose tolerance. This suggests that abnormalities in insulin action rather than in insulin secretion were already present in the cats when lean and predisposed them to more severe changes when they became obese. Persistent insulin resistance in cats eventually leads to a decrease in the total insulin secretory capacity and overt diabetes mellitus. At that time insulin secretion is low and erratic.[22] Similar data are not available for dogs.

In humans, obesity increases secretion of both insulin and proinsulin and there is a marked increase in the proinsulin:insulin ratio when diabetes mellitus develops (chapter 5.2.1). This suggests that proinsulin can be used as an indicator and sensitive marker of beta cell dysfunction.[23,24] Proinsulin has been shown to be a marker for insulin resistance, and proinsulin levels in humans are related to atherosclerosis and cardiovascular disease.[25] Proinsulin secretion is also increased in obesity in cats[26] but it is not known whether there is a change in the proinsulin:insulin ratio with the development of diabetes mellitus and thus whether this ratio might be used as an indicator of the disease. Changes in proinsulin secretion in obesity have not been studied in dogs.

There are other hormonal changes in obese cats and dogs. As in humans, obese cats and dogs have low adiponectin concentrations.[11,27] Adiponectin is secreted from adipose tissue and modulates glucose and lipid metabolism. Its concentration is inversely related to body mass and positively correlated with insulin sensitivity.

Thyroid hormone changes are also seen in obesity. Thyroid hormones are involved in regulation of the resting (basal) metabolic rate, thermogenesis, and lipolysis (chapter 3.1). Free thyroxine (FT_4) concentration and the FT_4 fraction have been shown to be significantly higher in obese cats than in lean cats, although usually still within the normal range until development of the morbid obesity seen in clinical patients. In cats, FT_4 has the strongest positive correlation with the obesity indices body fat, body mass index, girth, nonesterified fatty acids, and leptin.[28] Energy expenditure is lower in obese than in lean cats and increases with administration of triiodothyronine (T_3), suggesting that thyroid hormone is partly involved in the low heat production.[29] Plasma total thyroxine (TT_4) and T_3 concentrations were higher in obese than in lean dogs, although still within the reference range.[30]

One of the main questions concerning the progression from compensated obesity to diabetes in both cats and humans concerns the role of islet amyloid polypeptide (IAPP), also called amylin, a hormone cosecreted with insulin. It is the precursor of islet amyloid, which is thought to be formed when the endoplasmic reticulum is unable to process amylin correctly. This leads to the formation of toxic fibrils and eventually amyloid deposition, with apoptosis of beta cells that exceeds the rate of their regeneration.[31] In obese cats the secretion of amylin follows the pattern of insulin secretion, i.e., there is hyperamylinemia.[32] Yet there is no evidence that this leads to amyloid formation in obese cats, which suggests that neither obesity nor hyperamylinemia per se is sufficient to cause amyloidosis in the presence of a normal beta cell mass.

Compared with lean cats, obese cats have lower expression of peroxisome proliferator-activated receptors (PPARs), which are transcription factors involved in carbohydrate, lipid, and protein metabolism, and in cell differentiation.[8] PPARα is in-

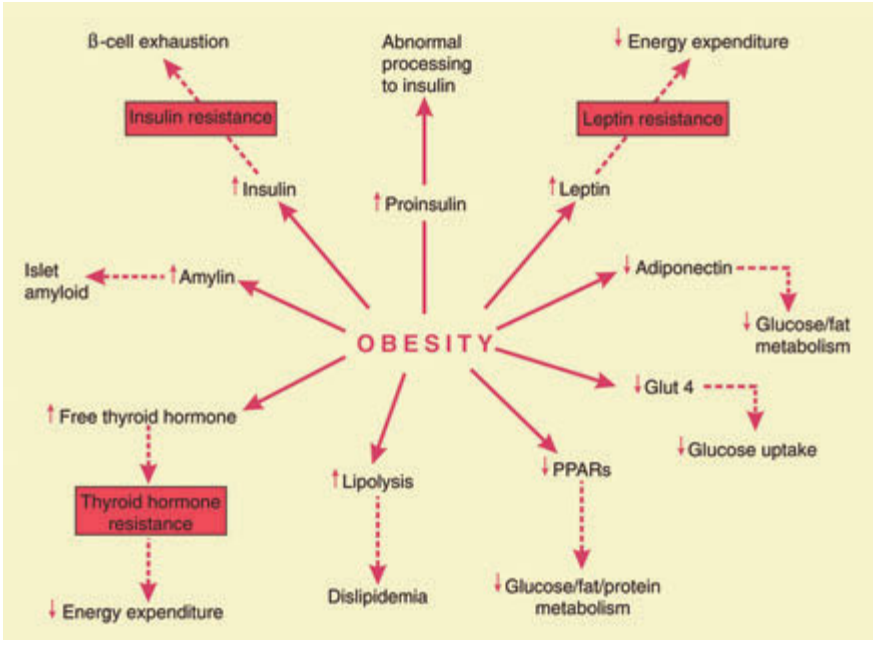


Figure 11.2:
Known hormonal and metabolic changes in obese dogs and cats.

volved in adipocyte mitochondrial biogenesis and the up-regulation of genes involved in fatty acid oxidation. The low expression suggests that fat cells of obese cats are less metabolically active than those of lean cats. PPARγ is highly expressed in adipose tissue and is involved in adipocyte differentiation.[33] It is activated by fatty acids and thiazolidinediones, drugs which increases insulin sensitivity in many species, including cats.[34] The low PPARγ concentration seen in obese cats supports the finding of marked insulin resistance. Information about PPAR expression in obese dogs is lacking, although it has been shown that the PPARα agonist, fenofibrate, lowers serum concentrations of triglyceride and cholesterol.[35]

Obesity is characterized in both dogs and cats by marked alterations in lipid metabolism and lipoproteins. There is an increase in the plasma concentration of nonesterified fatty acids (NEFAs), which is thought to increase VLDL synthesis.[35–38] Plasma triglycerides are increased, a change mostly due to an increase in the very low density lipoprotein (VLDL) fraction. Overproduction of VLDL in cats was associated with an increased number of large and medium-sized VLDL particles,[38] which have been associated with cardiovascular disease in humans.[39] Overproduction of VLDL has also been associated with decreased expression of PPARα. Atherogenesis and coronary artery disease are not features of feline obesity or diabetes mellitus, and diabetic cats are not prone to high blood pressure or such complications as hypertensive retinopathy or proteinuria.

There have been conflicting findings concerning cholesterol. In obese dogs, plasma cholesterol has been found to be either higher or not different from that in lean dogs and the high density lipoprotein (HDL) fraction, which represents cholesterol, has been reported to be either increased or decreased.[40,41] As in humans, hypertension and atherosclerosis have been reported in obese dogs on a high fat diet, suggesting that the dyslipidemia of obesity is more detrimental in dogs than in cats.[42] In newly obese cats, plasma cholesterol is increased by increased HDL cholesterol.[37] A significant decrease in HDL cholesterol is seen in long-term obesity in cats.[38] However, in contrast to humans, dogs and cats have much higher concentrations of HDL than LDL cholesterol and the former remains high in spite of any decrease with obesity.

The type of fat deposition has received great attention in human medicine because visceral fat (the android type of fat deposition) is thought to play a primary role in causing insulin resistance. Visceral fat is resistant to the antilipolytic effects of insulin and it leads to many of the metabolic abnormalities in obese humans, such as reduced hepatic insulin extraction, increased gluconeogenesis, and perturbed lipid metabolism.[43] However, abdominal fat deposition in cats is equally divided between subcutaneous and intra-abdominal, regardless of diet.[11] In insulin-resistant obese dogs fed a high-fat diet, abdominal subcutaneous fat increased more than visceral fat,[44] which argues against a primary role of visceral fat deposition in the change of insulin sensitivity in both species.

Diagnosis

There are subjective as well as objective methods to assess body composition. A nine-point body condition scoring system is probably the most frequently used method in private

practice, but has the drawback of being subjective.[45,46] It is also difficult to score animals that have lost weight recently or have long hair. In cats, a body condition score of 1–3 indicates an animal that is underfed and whose ribs are easily visible (1) or have minimal fat covering and are easily palpated (3). The ideal body condition is 5, which indicates a well-proportioned cat in which a waist is easily observed. Overfed cats are scored 7–9, depending on the amount of fat: 7 if the ribs are not easily palpated and 9 if they cannot be palpated at all because of heavy deposits of fat. In dogs, 1–3 indicates an underfed animal: 1 if there is loss of muscle and fat mass and 3 if the ribs are easily palpated. A score of 4 or 5 indicates an ideal body condition: the ribs are palpable and have some fat covering, the waist is easily noted, and the abdominal tuck is evident. Scores of 6–9 indicate that a dog is overfed: 6 if the ribs are difficult to palpate, 9 if they cannot be palpated, there is no observable waist or abdominal tuck, and there are fat deposits over the entire body.

There are also objective methods to judge obesity in dogs and cats; some require specialized equipment, others do not. Body mass index (BMI) is well known from human medicine, where it is widely used to assess adiposity. It has also been calculated in cats according to the following formula: BMI = body weight (kg)/body length (m) × height (m), where height is the distance from the point of the shoulder through the point of the elbow to the proximal boundary of the metacarpal pad and length is the distance from the point of the shoulder to the tuber ischium.[47] BMI correlates well with other indices of obesity, but only within well-defined populations, because of the great variation in size of the general pet population.

Obesity assessed by measuring the body circumference or girth immediately caudal to the last rib correlates well with measurements of body fat by dual energy x-ray absorptiometry (DEXA).[48] Girth and BMI measurements require no specialized equipment. Highly sophisticated assessment of adiposity is possible with DEXA and magnetic resonance imaging (MRI). MRI can provide exact quantification of specific adipose depots within the body.[11,44]

Treatment

Assuming that obesity is primary and not secondary to a disease such as hypothyroidism or hypercortisolism, the aim of treatment is to decrease energy input and increase energy output. This is accomplished through management of diet and lifestyle,[49] and recently therapeutic intervention has also become available for dogs. The diet should be high in protein, which increases loss of fat mass and preserves lean body mass in both dogs and cats.[11,50] Recently it was shown that a diet high in fiber and high in protein led to greater reduction in voluntary food intake than a diet with either moderate protein / high fiber content or high protein / moderate fiber content.[51] Because different fiber types and protein sources were used for all three diets in that study, it is not clear what effect can be ascribed to protein or to fiber. Energy restriction should proceed slowly in order to avoid the development of hepatic lipidosis, especially in cats. A 1–1.5 % weight loss per week has been reported to be safe. The value of added substances such as conjugated linoleic acid or carnitine needs to be examined in well-controlled studies. Several commercial weight-loss diets are available to dog and cat owners. These diets provide the necessary nutrients despite reduced caloric intake. There are also several computer programs that can help owners to design a weight-loss program for their pet. Owners of intact cats need to be aware that neutering decreases energy requirements[52] and increases appetite (see also chapter 8.2).[53]

Many pet owners find it easier to increase energy expenditure in a dog than in a cat, because dogs can be walked and many dogs enjoy other activities such as swimming. Indoor cats are more limited in activity but owners can also encourage exercise by providing toys and by placing small amounts of food around the house rather than providing it in one dish.

Recently, dirlotapide (Slentrol®, Pfizer) was approved by the Federal Drug Administration in the United States for the treatment of obesity in dogs and mitratapide (Yarvitan®, Janssen Animal Health, Belgium) was approved by the European Commission. These drugs decrease intestinal absorption of fat by inhibition of microsomal triglyceride transfer protein. The accumulation of lipids in enterocytes is thought to increase peptide YY concentrations in plasma, resulting in satiety (chapter 11.2.1). The dose of dirlotapide is titrated individually within a range of 0.01–0.2 ml/kg.[16] Mitratapide solution is provided in bottles of three sizes to facilitate administration of the dose in the food according to the dog's weight. Treatment is given for three weeks and then interrupted for two weeks to evaluate the dog's nutritional requirements. The diet is adjusted accordingly and mitratapide is resumed for an additional three weeks. There is no similar drug for use in cats at present.

Prognosis

In order for the treatment of obesity to be successful, it is important for the veterinarian and the owner to recognize that obesity is a disease and will have detrimental consequences for the pet if left untreated. The veterinarian needs to monitor and record indices of obesity at check-ups and provide achievable milestones for the owner during the course of the weight loss. Exercise is an important part of any program and will benefit both pet and owner.

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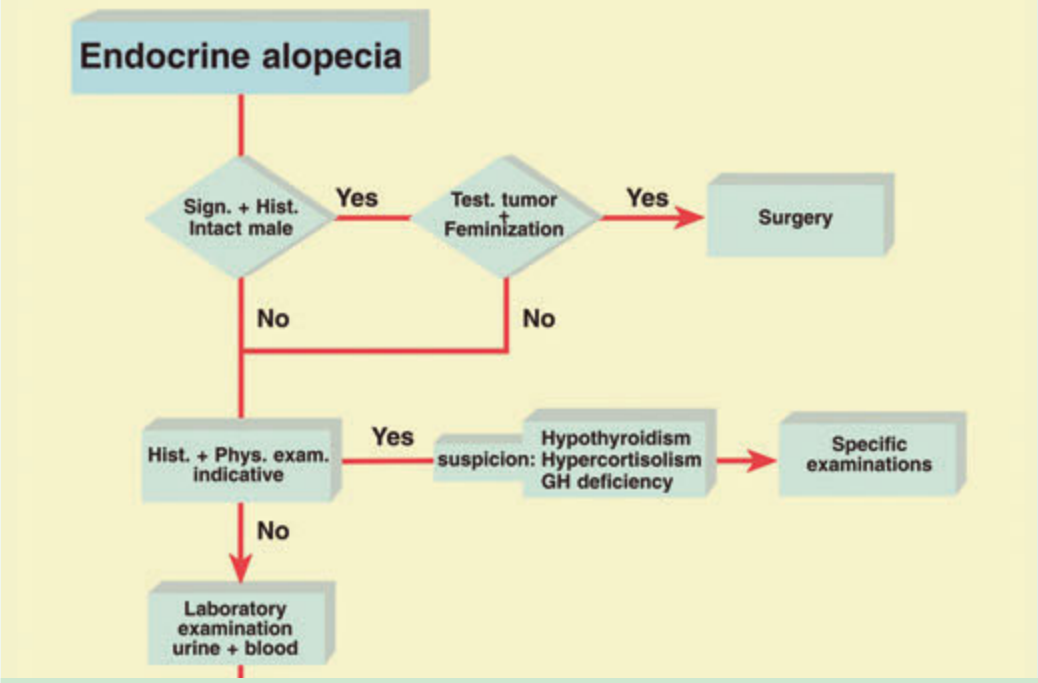
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Protocols and Algorithms



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12 Protocols for Function Tests

Ad Rijnberk
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12.1 Pituitary anterior lobe

12.1.1 CRH-stimulation test

Indication

Suspicion of decreased secretory capacity of corticotroph cells, due to (1) damage by tumor or surgery, or (2) suppression by exogenous or endogenous (adrenocortical tumor) glucocorticoid excess.

Performance

At –15, 0, 5, 10, 20, 30, and 45 min, 2–3 ml blood is collected in ice-chilled EDTA-coated tubes for measurements of ACTH and cortisol. At time zero, 1 μg oCRH/kg body weight is injected intravenously.

Interpretation

With a two-site immunoradiometric assay (IRMA), basal ACTH in control dogs was 4.0 ± 0.9 pmol/l (mean ± SEM) and CRH caused an increment of 68 ± 14 pmol/l with the peak occurring in 5–10 min. Basal cortisol was 48 ± 5 nmol/l and at the peak in 30–45 min the increment was 380 ± 39 nmol/l.[1] The values in a combined anterior pituitary function test (chapter 12.1.3) did not differ significantly from those in a CRH-stimulation test alone.[2]

CRH caused virtually no release of endogenous ACTH or cortisol in dogs with autonomously hypersecreting adrenocortical tumors[3] and caused no increase in ACTH in a dog with hypercortisolism due to ectopic ACTH secretion.[4]

Comment

Vasopressin can be used in place of CRH to stimulate the pituitary-adrenocortical axis (chapter 4.1), except for differentiating between pituitary-dependent hypercortisolism and adrenocortical tumor. Cortisol release from adrenocortical tumors can be stimulated directly by vasopressin, probably via expression of vasopressin receptors associated with the neoplastic transformation.[3] The vasopressin analogue desmopressin (see also chapter 2.3.3), with a strong selective affinity to the V_2 receptor, does not cause this release of cortisol from adrenocortical tumor, but there is also little or no response in some dogs with pituitary-dependent hypercortisolism, so it seems not reliable for differentiation.[5]

12.1.2 GHRH-stimulation test

Indication

Suspicion of decreased secretory capacity of somatotroph cells, due to (1) a pituitary lesion (congenital anomaly, tumor, surgery) or (2) progestin-induced GH secretion by the mammary gland.

Performance

Blood samples are collected before and after intravenous administration of 1 μg hGHRH/kg body weight, as in the CRH test (chapter 12.1.1).

Interpretation

In healthy anestrous dogs basal plasma GH between pulses was 1.3 ± 0.3 μg/l (mean ± SEM) and at 10–20 min after GHRH administration plasma GH ranged from 5–28 μg/l.[2,6]

In dogs with congenital GH deficiency there is no significant increase in plasma GH concentration following GHRH. In dogs with GH excess of mammary origin, GHRH administration results in almost no increase in the more or less elevated GH levels.[7,8] In dogs with hypercortisolism, including those erroneously thought to have acquired GH deficiency (chapter 2.2.3), the cortisol-induced increase in endogenous somatostatin tone inhibits the GH response to hGHRH.[9,10]

Comment

An α_2-agonist, either clonidine (10 μg/kg body weight) or xylazine (100 μg/kg body weight), can be used in place of GHRH. In healthy dogs the increase in GH tends to be somewhat higher than after 1 μg GHRH/kg.[10] Also, ghrelin can be used to stimulate endogenous GHRH release. An increase in plasma GH of $>$ 5 μg/l following intravenous administration of 2 μg ghrelin/kg body weight excludes congenital GH deficiency.[11]

12.1.3 Combined anterior pituitary function test

Indication

Suspicion of multiple anterior pituitary hormone deficiencies due to pituitary tumor, other (para)sellar tumors, pituitary cystic lesions, congenital anomaly (pituitary dwarfism), pituitary surgery, or pituitary irradiation.

Performance
Immediately after collection of the zero blood sample from the jugular vein, four releasing hormones are injected via a catheter in the cephalic vein within 30 seconds, in the following order and doses: 1 µg CRH/kg, 1 µg GHRH/kg, 10 µg GnRH/kg, and 10 µg TRH/kg. Blood samples are collected at the times given for the CRH test (chapter 12.1.1) for measurement of ACTH, cortisol, GH, PRL, TSH, and LH.[2,12]

Interpretation
The results are compared with those in healthy control dogs (fig. 2.14). Only the LH response is lower in the combined test than when its releasing hormone GnRH is administered alone.[2]

12.1.4 Sample handling

To prevent the rapid proteolytic degradation of some of the pituitary hormones, blood samples are collected in EDTA-coated ice-chilled tubes and are centrifuged in a cooled centrifuge. Heparin-coated tubes should be used for samples to be assayed in a one-step immunoassay based on a chemiluminiscent reaction (e.g., for T_4, cortisol, progesterone), because EDTA inhibits this reaction. Plasma should be stored at or below –20 °C. If the samples cannot be brought immediately to the laboratory where the assays are performed, they should be shipped on dry ice by an overnight mail service.

12.2 Pituitary posterior lobe

12.2.1 Serial measurements of urine osmolality

Indication
First step in differentiating central diabetes insipidus, nephrogenic diabetes insipidus, and primary polydipsia.

Principles
(1) In dogs with primary polydipsia, water intake can vary considerably over the day, resulting in marked variations in urine osmolality (Uosm). (2) If Uosm remains low with little variation, the response to a vasopressin analogue can be tested.

Performance
Urine samples are collected at home while water is available ad libitum. The owner is provided with written instructions and eleven small labeled tubes in a suitable box for mailing to the laboratory. Urine is collected at 2 h intervals during the day and at 4 h intervals during the night for a period of 24 hours and the samples are sent to the laboratory for measurement of Uosm.

If Uosm remains low throughout the first test, the owner receives a second set of tubes and a dropper bottle of desmopressin (DDAVP). The owner administers one drop of desmopressin in the conjunctival sac three times daily for four days and on the fourth day repeats the collection of the eleven urine samples at 2 h intervals.

Interpretation
Marked fluctuations in Uosm and any values higher than 1000 mOsm/kg indicate good renal concentrating ability, showing the polyuria to be the result of variations in water intake, i.e., primary polydipsia. If Uosm remains low and the measurements are repeated after desmopressin administration, central diabetes insipidus is indicated by prompt cessation of the polyuria and polydipsia and the increase in Uosm from low values to $>$ 1000 mOsm/kg. If Uosm does not exceed 1000 mOsm/kg after desmopressin, central diabetes insipidus is unlikely and there is either primary polydipsia or (functional) nephrogenic diabetes insipidus.

Comment
Urine specific gravity (Usg) measured by refractometry can be used in place of Uosm. They are well correlated ($r = 0.98$, $P < 0.001$) and their linear regression is $Usg = 1.0048 + 2.32 \times 10^{-5}$ Uosm.[13] This converts to 1.028 Usg = 1000 mOsm/kg. However, Uosm measurements are preferable because homeostatic mechanisms maintaining water and electrolyte balance are related to osmolality rather than to specific gravity. Also, the standard deviation of the Usg values has been found to be 0.004.[14] Uosm calculated from Usg has been reported to be within ± 200 mOsm/kg of the measured value in 84.5 % of samples (n = 181), but in 4.4 % the difference was more than 500 mOsm/kg.[15] Test strips are completely unsuited for estimating Usg.[13]

12.2.2 Modified water deprivation test

Indication
Differentiation of central diabetes insipidus, nephrogenic diabetes insipidus, and primary polydipsia.

Principle
In this indirect test of vasopressin secretory capacity, plasma osmolality (Posm) is increased by water deprivation to stimulate vasopressin release. The effect of endogenous vasopressin is determined indirectly by measurements of Uosm.

Performance
Following 12 h of fasting, water is withheld and then plasma and urine osmolality (Posm and Uosm) are measured every 2 h, or hourly if the polyuria is severe. When the loss of body weight, measured at each interval, approaches 5 % of initial body weight, the test is stopped. When Posm ≥ 310 mOsm/kg and there is $<$ 5 % increase in Uosm between consecutive collections but Uosm is still $<$ 1000 mOsm/kg, 2–4 µg of the va-

sopressin analogue DDAVP (see also treatment section of chapter 2.3.3.1) is administered intravenously. Uosm is measured again 1 h and 2 h later.

Interpretation
In primary polydipsia Uosm gradually rises to > 1000 mOsm/kg during water deprivation. In both nephrogenic diabetes insipidus and central diabetes insipidus, it remains low. In complete central diabetes insipidus it rises by 50 % or more following the administration of DDAVP and is > 650 mOsm/kg (~sg 1.020). In partial central diabetes insipidus the increase in Uosm is ≥ 15 %. In nephrogenic diabetes insipidus DDAVP causes little or no increase in Uosm (figs. 2.32, 2.33).[16] In part because of the indirect character of the test, the results are not always conclusive.[17]

Comments.
Measurement of Usg is suitable in the initial urine examination for polyuria, but not for the water deprivation test (chapter 12.2.1), in which decisions are made on the basis of changes of 5 %.

When urine volume is very large, there is usually complete emptying of the bladder at each micturition and it is usually not difficult to collect a sample during micturition. When urine volume is small, catheterization may be needed to obtain a sample and/or ascertain that the bladder is emptied. This may be necessary to determine whether water deprivation can and should be continued. In cats the use of an indwelling urinary catheter may be necessary.

12.2.3 Vasopressin measurements during hypertonic saline infusion

Indication
Suspicion of deficient vasopressin release or inappropriate vasopressin secretion.

Performance
The euhydrated and fasted animal is infused for 2 h via the jugular vein with 20 % NaCl solution at a rate of 0.03 ml/kg body weight per minute. Samples for plasma VP (Pvp) and Posm are obtained at 20 min intervals. Because there is the risk of inducing critical hypertonicity in a severely polyuric animal, Posm should be measured in the samples immediately and when it reaches ~350 mOsm/kg the hypertonic saline infusion should be stopped.

Interpretation
The slope of the regression line for Posm and Pvp is used as a measure of the sensitivity of the osmoregulatory system. In the nomogram developed by Biewenga et al., the 90 % range for sensitivity was 0.24–2.47 pmol/ml per mOsm/kg.[17] The 90 % range for the threshold of the system was 276–309 mOsm/kg. See also fig. 2.31, fig. 2.34, and fig. 2.35.

Comment
The test requires very close observation and monitoring of Posm. This, and the fact that vasopressin is very sensitive to proteolytic breakdown, makes it advisable that the test be performed in institutions having experience with it.

12.3 Thyroid

12.3.1 TSH-stimulation test

Indication
Suspicion of hypothyroidism, particularly when basal plasma T_4 and TSH concentrations are not conclusive (chapter 3.3.1).

Performance
Blood is collected for measurements of plasma T_4 immediately before and 4–6 h after the intravenous injection of 100 µg recombinant human TSH (rhTSH).

Interpretation
In healthy dogs plasma T_4 rises above 32 nmol/l and is at least 1.5x the basal T_4 concentration. Post-TSH T_4 values < 20 nmol/l and < 1.5 × the basal T_4 concentration are diagnostic of hypothyroidism. If post-TSH T_4 is between 20 and 32 nmol/l or is > 32 nmol/l but < 1.5 × the basal T_4, the diagnosis is unresolved. This can occur in severe systemic illness.[18] Quantitative measurement of thyroidal uptake of $^{99m}TcO_4^-$ can be helpful in resolving the diagnosis.[19]

Comments
The biological activities of rhTSH and bovine TSH (bTSH), which was used in the past, are similar.[20] Maximal stimulation of the thyroid is achieved with 100 µg or even 50 µg rhTSH per dog. [21] Higher doses do not increase the T_4 response and responses to a fixed dose do not vary with body weight.[18,20] One ampule of the highly purified recombinant form of human TSH contains 1100 µg of lyophilized rhTSH, of which less than $^1/_{10}$ is needed per dog. Fortunately, the reconstituted rhTSH can be stored at 4 °C for 4 weeks and at –20 °C for at least eight weeks without loss of biological activity, allowing for several TSH-stimulation tests per ampule.[22]

In secondary hypothyroidism it may be necessary to administer rhTSH on three consecutive days to produce an increase in plasma T_4 concentration.

12.3.2 TRH-stimulation test

Indication
Suspicion of deficient TSH secretion. The test is a component of the combined anterior pituitary function test (chapter 12.1.3) but can also be used to test for the paradoxi-

cal GH response that may occur in pituitary hyperplasia due to primary hypothyroidism (chapter 3.3.1).

Performance
TRH is administered intravenously in a dose of 10 µg per kg body weight and blood is collected at –15, 0, 5, 10, 20, 30, and 45 min. For sample handling see chapter 12.1.4.

Interpretation
In healthy dogs the maximum plasma TSH concentration (mean ± SEM; 1.26 ± 0.22 µg/l) occurs at 10 min after injection.[12] A low basal plasma TSH concentration and lack of increase after TRH administration is consistent with secondary hypothyroidism, as occurs after hypophysectomy.[23] However, in spontaneous primary hypothyroidism basal TSH concentration may also be below the upper limit of the reference range, and may not be increased by TRH (chapter 3.3.1).[19,24]

In contrast to healthy dogs, those with primary hypothyroidism respond to TRH administration by an increase in plasma GH concentration (mean ± SEM at 10 min: 11.9 ± 3.5 µg/l), as a result of the development of thyrosomatotroph pituitary cells (chapter 3.3.1).[25]

Comment
Studies have been undertaken to determine whether the plasma T4 response to TRH might be helpful in the diagnosis of primary hypothyroidism. However, contrary to expectations, there is often no significant increase in plasma T_4 after TRH administration in healthy dogs.[26] Although the low and variable responses to TRH make it of no value for identifying dogs with hypothyroidism,[27] a definite increase in T_4 after TRH might exclude hypothyroidism.

12.4 Adrenal cortex

12.4.1 ACTH-stimulation test

Indication
Suspicion of decreased adrenocortical reserve (primary or secondary adrenocortical insufficiency) and testing of adrenocortical reserve to guide treatment of hypercortisolism with trilostane (chapter 4.3.1).

Performance
Synthetic ACTH (cosyntropin or tetracosactrin) is administered intravenously and blood is collected immediately before and at 60 min after the injection for measurement of plasma cortisol. It was customary in the past to administer the full contents of a vial (0.25 mg) of synthetic ACTH, but the high cost prompted re-evaluation of the dose and 5 µg/kg was found to be sufficient for maximal adrenocortical stimulation.[28,29]

If treatment for adrenocortical insufficiency is already being given, the morning dose of cortisone is postponed on the test day until the test is completed. This is not necessary if dexamethasone is used rather than cortisone, because dexamethasone is not measured in cortisol assays. Glucocorticoid treatment for more than three days can by itself result in a subnormal response via induction of secondary hypocortisolism (chapters 4.2.2, 4.3.6).

Interpretation
In healthy dogs, plasma cortisol concentration rises to 270–690 nmol/l after ACTH. In primary adrenocortical insufficiency it usually increases < 50 nmol/l above the low basal value. In secondary adrenocortical insufficiency plasma cortisol is low and, depending on the severity and duration of the insufficiency, the increase after ACTH is subnormal or absent.

Comment
Differentiation between primary and secondary adrenocortical insufficiency can be confirmed by measuring plasma ACTH, which is extremely high in primary hypoadrenocorticism and below the level of detection in secondary hypoadrenocorticism. In healthy dogs the reference range for the cortisol/ACTH ratio (CAR), with cortisol in nmol/l and ACTH in pmol/l, has been reported to be 1–26.[30]

12.4.2 Low-dose dexamethasone suppression test (iv-LDDST)

Principle
The sensitivity of the hypothalamic-pituitary-adrenocortical axis to suppression by glucocorticoids is tested by administering a low dose of a potent glucocorticoid that causes suppression in healthy animals but not in those with hypercortisolism.

Indication
Suspicion of hypercortisolism.

Performance
Collect blood for cortisol assay immediately before and at 4 h and 8 h after administering 0.01 mg dexamethasone per kg body weight intravenously.

Interpretation
In dogs the finding of plasma cortisol > 40 nmol/l at 8 h after dexamethasone confirms hypercortisolism with a predictive value of a positive test result of 0.92 (confidence interval, CI: 0.85–0.96), and a predictive value of a negative test result of 0.59 (CI: 0.43–0.73).[31] The measurements at 0 and 4 h are not needed for the diagnosis per se but may be useful in the differential diagnosis. A high value at 8 h after a lower value at 4 h indicates escape from the suppression by dexamethasone.[32] If the values at either 4 h and/or 8 h are at least

50 % lower than the 0 h value, the hypercortisolism is pituitary dependent.

In cats there is much less experience with the iv-LDDST, because of the low incidence of hypercortisolism in this species. Nevertheless, in pituitary-dependent hypercortisolism in cats, as in dogs, resistance to glucocorticoid feedback ranges from slight to complete (see also chapter 12.4.3).[33] The same dose of dexamethasone is used and the same criteria are applied for the diagnosis. Increasing the dose of dexamethasone to 0.1 mg/kg has been proposed but would lead to an increase in false-negative test results.

Comment

The stress of disease and hospitalization can cause false-positive results.[34] Stress from other procedures such as ultrasonography performed during the test may also override the suppressive effect of dexamethasone.[35] Long-term anticonvulsant treatment with phenobarbital does not affect the results.[36,37]

12.4.3 High-dose dexamethasone suppression test (iv-HDDST)

Principle and indication

Although the iv-LDDST reveals the sensitivity of the pituitary-adrenocortical system to be decreased in most cases of hypercortisolism due to a functional corticotroph adenoma in the anterior lobe, the system is usually suppressible with a high dose of dexamethasone. Plasma cortisol levels in animals with functional adrenocortical tumors, corticotroph adenomas arising from the pars intermedia, or ectopic ACTH secretion usually cannot be suppressed with the high dose of dexamethasone. Consequently, the iv-HDDST is indicated after the diagnosis hypercortisolism has been established, to differentiate between a corticotroph adenoma of the anterior lobe of the pituitary and other causes of hypercortisolism. However, the impairment of glucocorticoid feedback varies considerably with the size of the pituitary and large corticotroph tumors may be associated with complete resistance to dexamethasone.[38]

Performance

Blood for cortisol assay is collected immediately before and 3–4 h after intravenous administration of 0.1 mg dexamethasone per kg body weight.

Interpretation

A decrease in plasma cortisol of $>$ 50 % in this test indicates that the hypercortisolism is pituitary dependent. A decrease of $<$ 50 % indicates that the hypercortisolism is ACTH independent (primarily adrenocortical tumor, chapter 4.3.2), or pituitary dependent but dexamethasone resistant (chapter 4.3.1), or due to ectopic ACTH excess (chapter 4.3.4).



Figure 12.1:
Box with cushion lining for sending three urine samples to the laboratory for cortisol assay. The tubes should be no more than half filled, so that the stopper is not expelled by freezing. The fourth tube contains the dexamethasone tablets for the suppression test.

12.4.4 Urinary corticoid:creatinine ratios with high-dose suppression test (UCCR + o-HDDST)

Principle

The cortisol in the morning urine reflects the production during ~8 h, integrating the fluctuations in plasma cortisol. Urinary cortisol is related to urinary creatinine to correct for differences in urine concentration. The test includes the oral administration of high doses of dexamethasone to assess the sensitivity to glucocorticoid feedback.

Indication

Suspicion of hypercortisolism and differentiation between hypercortisolism due to corticotroph adenoma of the anterior pituitary and dexamethasone-resistant forms of hypercortisolism.

Performance

The owner is provided with three tubes for urine samples and a one with dexamethasone tablets, in a cushioned box for mailing the urine tubes to the laboratory (fig. 12.1). The owner collects morning urine samples at home at the same time (e.g., 7 A.M.) on three consecutive days, after taking the dog for its last walk at the same time (e.g., 11 P.M.) on the preceding evening. After collecting the second urine sample, the owner gives the three oral doses of dexamethasone (0.1 mg per kg body weight) at 8 h intervals. An example of the instruction sheet for the owner is given as an annex to this chapter, together with directions for collecting urine at home from cats.

Interpretation

In our laboratory the basal UCCR in healthy pet dogs is $0.3–8.3 \times 10^{-6}$ [39] and in healthy cats it is $8.0–42.0 \times 10^{-6}$.[40] The reference values of the laboratory performing the assays should be used. The mean of the two basal UCCR values is calculated and values exceeding the upper limit in healthy dogs or cats provides the diagnosis of hypercortisolism. If the UCCR of the third urine sample is < 50 % of the mean of the first two the hypercortisolism is pituitary-dependent. For the interpretation of post-dexamethasone UCCRs > 50 % of the mean basal values, see chapter 12.4.3.

Comments

Stress (including hospitalization) during or prior to the urine collection should be avoided as much as possible, for it readily activates the pituitary-adrenocortical axis and thus elevates cortisol excretion. The urine samples should be collected by the owner at home under conditions free of stress.[41–43] The high sensitivity of the UCCR not only reveals stress responses but also increased cortisol production associated with diseases such as malignant lymphoma and hyperthyroidism.[40,44] From studies in such populations it has been concluded that the UCCR has high sensitivity but lacks specificity.[45] However, in the appropriate populations – animals suspected of hypercortisolism – the specificity of the LDDST and the UCCR is similar (0.73 and 0.77, respectively). More importantly, the predictive value of a positive UCCR (0.88; CI 0.80–0.93) and that of a negative UCCR (0.98; CI 0.88–1.00) compare well with these variables for the LDDST (chapter 12.4.2).[31]

The UCCR is also very useful for monitoring the result of treatment following hypophysectomy or adrenalectomy as well as treatment with o,p'-DDD.[46,47] The replacement glucocorticoid and/or mineralocorticoid should be omitted the night before the urine sample is collected and resumed directly after it is collected. Both complete adrenocortical ablation and hypophysectomy result in UCCRs $< 2.0 \times 10^{-6}$.

12.4.5 Urinary corticoid:creatinine ratios with low-dose suppression test (UCCR + o-LDDST)

Indication

Dogs in which results of the UCCR and/or the iv-LDDST have been inconclusive or negative but in which there is still suspicion of hypercortisolism.

Performance

The owner collects urine on two consecutive mornings at 8.00 h for measurement of the UCCR, without changing the feeding regimen. After collection of the second urine sample, the owner administers 0.01 mg dexamethasone per kg body weight orally. The dog is walked at 12.00 h and 14.00 h to empty its bladder and the third urine sample is collected at 16.00 h for measurement of UCCR.

Interpretation

In seven healthy pet dogs the UCCR at 16.00 h was $< 1.0 \times 10^{-6}$.[48] In dogs with mild pituitary-dependent hypercortisolism the UCCR following dexamethasone was $> 1.0 \times 10^{-6}$.[49]

Comments

The bioavailability of dexamethasone is lower after oral administration than after intravenous administration, but the oral dose of 0.01 mg dexamethasone per kg body weight is still sufficient to suppress the system in healthy pet dogs. This lower exposure to dexamethasone may give the o-LDDST a higher discriminatory power than the iv-LDDST, in addition to which the test is done at home, without the stresses of a hospital visit and an invasive test procedure.[49]

In both the iv and the oral LDDST the dosage of dexamethasone is critical. For the studies of the o-LDDST mentioned above dexamethasone was triturated with lactose and microcrystalline cellulose was used as a diluent. Capsules were prepared with the following doses: 0.25, 0.1, 0.05, 0.01, 0.005, and 0.001 mg dexamethasone. This series of capsules facilitated dosage with an accuracy of 0.1–0.2 kg body weight.

12.5 Ovary and Testis

12.5.1 GnRH-stimulation test

Indication

(1) Suspicion of decreased secretory capacity of gonadotroph cells. (2) Search for functional ovarian or testicular tissue, as in bitches with primary anestrus due to hermaphroditism[50] or incomplete ovariectomy. (3) Detection of hormonal abnormalities in the pituitary-gonadal axis in cases of infertility. (4) Suspicion of anorchism or cryptorchidism in male dogs.

Performance

Blood is collected before and after intravenous administration of 0.1 ml GnRH/kg body weight (Fertagyl® or Receptal®), in EDTA- or heparin-coated tubes (see also chapter 12.1.4) for measurement of LH, FSH, progesterone, estradiol, and/or testosterone. The sampling scheme depends on the hormones of interest. For LH and FSH, blood is collected at –40, 0, 5, 10, 20, 30, and 60 min. For progesterone one basal sample (–40 or 0 min) suffices. For estradiol, blood is collected at –40, 0, 60, and 120 min. For testosterone, blood is collected at 0, 60, and 90 min.

Interpretation

Plasma progesterone > 3 nmol/l indicates the presence of luteal (ovarian) tissue but values < 3 nmol/l do not exclude it.

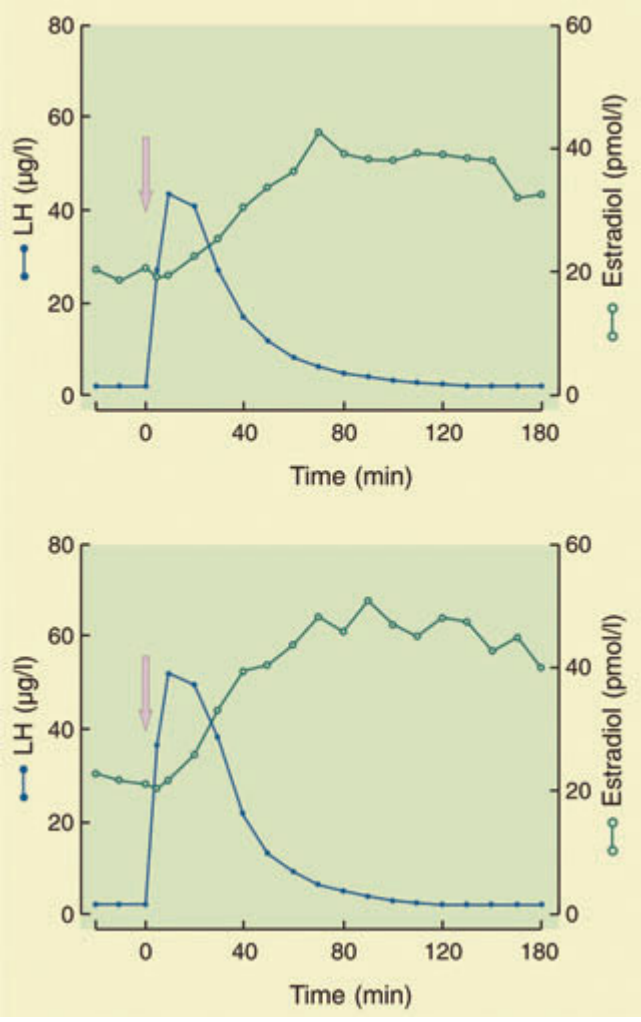


Figure 12.2:
Mean LH (blue) and estradiol (green) responses to GnRH (0.1 ml Fertagyl® per kg body weight) in six healthy female dogs during early (upper panel) and advanced (lower panel) anestrus. (Adapted from Van Haaften et al., 1994.)[51]

In six healthy female dogs mean basal plasma LH (~2.7 µg/l) did not change significantly during the progression of anestrus. In contrast, mean (± SEM) basal plasma FSH concentration increased significantly during progression of anestrus, with values of 6.3 ± 1.2 U/l in early anestrus, 7.1 ± 0.6 U/l in mid-anestrus, and 9.3 ± 0.8 U/l in late anestrus (see also fig. 7.16).[52] Following GnRH stimulation, peak LH concentrations of ~42 µg/l (early anestrus) and ~50 µg/l (advanced anestrus) occurred after 5–20 min (fig. 12.2). GnRH stimulation induced a moderate increase in plasma estradiol that did not return to pretreatment values during 160 min following stimulation (fig. 12.2). The LH and estradiol responses were greater in advanced anestrus than in early anestrus.[51] These findings indicate that the stage of anestrus must be taken into account in interpreting the results of the GnRH-stimulation test.

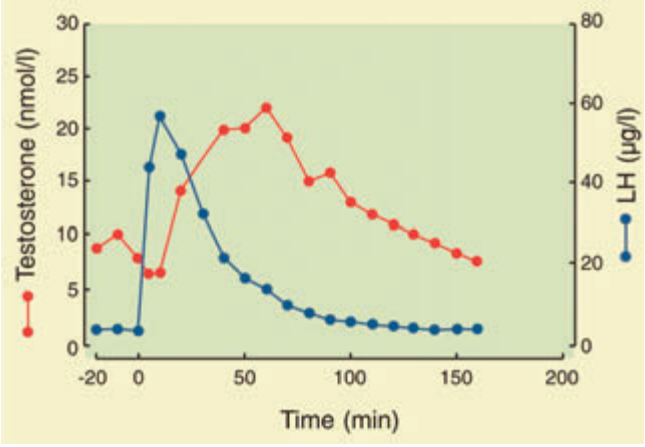


Figure 12.3:
Mean LH (blue) and testosterone (red) responses to GnRH (0.1 ml Fertagyl® per kg body weight) in six healthy male dogs. (Adapted from Knol et al., 1993.)[55]

In four healthy anestrous bitches the median basal FSH concentration was 5.6 µg/l (range 2.8–13.9 µg/l) and GnRH administration resulted in peak values ranging from 18 to 27 µg/l at 10 min. However, GnRH administration did not induce a significant rise in plasma FSH concentration in four ovariectomized bitches. Their basal plasma FSH concentrations (range 40–108 µg/l) did not overlap with the GnRH-induced FSH peak values in the anestrous bitches, suggesting that measurement of FSH in a single plasma sample may be sufficient to verify neuter status in bitches.[53]

Basal plasma estradiol concentration in ovariectomized bitches overlaps that in anestrous bitches, but GnRH administration only causes a significant rise in plasma estradiol concentration if ovarian tissue is present.[54]

In six healthy male dogs mean (± SEM) basal plasma LH and testosterone concentrations were 4.6 ± 0.5 µg/l and 9.7 ± 1.7 nmol/l, respectively. Following stimulation the mean (± SEM) plasma LH was 57 ± 13 µg/l at 20 min and the mean (± SEM) plasma testosterone was 16 ± 4 nmol/l at 40 min (fig. 12.3).[55] Sampling for testosterone may also be done at 60 and/or 90 min (fig. 12.3) as the concentration declines slowly.[56,57] In female dogs and castrated male dogs plasma testosterone concentration is low and does not change after GnRH administration.

Comment

If remnant ovarian tissue is suspected after ovariectomy of a queen, GnRH (0.1 ml/kg body weight of Fertagyl® or Receptal®) is injected during estrus behavior and plasma progesterone is measured seven to ten days later. A value > 3 nmol/l indicates the presence of ovarian tissue.

Client information for UCCR + o-HDDST

Your dog has symptoms and signs that could be caused by excessive production of the hormone cortisol from the adrenal cortex. In order to measure the production of cortisol, we need three morning urine samples.

Please collect a sample during the dog's first morning urination on three successive days. Take the dog out to urinate fairly late in the evening before each sample so that the morning sample is from urine produced during the night. Make the collection at about the same time each morning.

Place the urine sample in the tube numbered 1, 2 or 3, respectively. Fill the tube only half-full (below the mark) and then place it in the freezer or freezer compartment of the refrigerator until all three samples can be mailed or brought to the laboratory. The tubes can be filled to less than the mark, but please do not fill them above the mark because this can cause the stopper to be forced out when the urine is frozen. The samples should not be allowed to remain unrefrigerated for more than a day, and so should not be mailed just before the weekend.

Using the first two samples to measure cortisol production, we can then examine the control of the adrenal cortex in the third sample, after dexamethasone has been given. So you administer the enclosed tablets of dexamethasone after collecting the second urine sample, roughly according to the following schedule:

08.00 hours _____ tablet(s)

16.00 hours _____ tablet(s)

24.00 hours _____ tablet(s)

Hence the third morning urine sample will be collected about 8 hours after the last tablet(s). The tablets can be given with or without food (e.g., in a small piece of meat). It is likely that the dog will drink more and urinate more than usual for about a day following the tablets.

For cats, urine is collected by replacing the litter box for the night with one containing aquarium gravel that has been washed with tap water and dried. The gravel does not absorb cortisol from the urine, as litter does. The next morning the urine is taken up with a syringe or pipette and poured into the tube through gauze. In place of aquarium gravel, there is also a litter specifically designed for urine collection in cats.[58]

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13 Treatment Protocols

The preceding chapters have covered diagnostic procedures and modes of treatment for endocrine disturbances in dogs and cats. This chapter adds treatment protocols requiring further elaboration.

It concludes with the instructions for owners for dogs being treated for hypercortisolism with o,p'-DDD. These instructions have proved to be very useful to owners in carrying out this somewhat complicated protocol.

13.1 Pituitary

13.1.1 Hypophysectomy

Björn P. Meij

The total hypophysectomy that is performed in dogs and cats for the treatment of pituitary tumors leads to immediate cessation of the release of both adenohypophyseal and neurohypophyseal hormones. The resulting hypocortisolism and vasopressin deficiency would be life threatening if not correctly anticipated and treated. Particularly in animals that have been in a state of hypercortisolism for a long time, sudden cessation of cortisol excess would lead to collapse within hours after surgery and death within 24–36 h. Similarly, the abrupt cessation of vasopressin secretion would lead to severe plasma hypertonicity, causing brain damage. Consequently, the immediate postoperative treatment concentrates on fluid therapy, parenteral administration of supraphysiological doses of cortisol, and administration of a vasopressin analogue. The patients that undergo hypophysectomy greatly benefit from strict anesthesia and immediate postoperative care protocols that prevent mistakes that may have dramatic consequences. Close monitoring of these patients in an intensive care unit in the first 24–48 h after surgery is essential for a successful outcome. Once food intake is resumed, cortisol substitution is given orally and is gradually reduced to a physiological dose, and oral thyroxine substitution is also begun.

Treatment during hypophysectomy and the immediate postoperative period

- From the onset of the surgical procedure:
  - Intravenous infusion of a 0.45 % sodium chloride (NaCl) and 2.5 % glucose solution with 20 mmol/l potassium chloride (KCl) is started at maintenance rate (10 ml/kg/hour).
- Immediately following removal of the pituitary gland:
  - Hydrocortisone, 1 mg/kg every 6 h.
  - Desmopressin, 1 drop (~5 µg) in the conjunctival sac every 8 h.
- Analgesia with 0.3 µg buprenorphine per kg body weight every 8 h, as required.
- Antibiotic therapy consists of intravenous administration of 20 mg amoxycillin/clavulanic acid per kg body weight every 8 h.

Plasma concentrations of sodium, potassium, chloride, and glucose and plasma osmolality are measured before and immediately after surgery and then at 8, 24, and 48 h postoperatively. The results together with continuous monitoring of fluid balance are used to adjust fluid administration and electrolyte supplementation.

In case blood samples are collected for 4 h after hypophysectomy to monitor the immediate post-surgical decline of the plasma ACTH concentration (which is a prognosticator for long term remission),[1] intravenous administration of hydrocortisone is only started after the last blood sample has been collected.

Maintenance therapy

Drinking is allowed as soon as the animal is awake and when it begins to eat and drink, cortisone acetate 1 mg/kg is given orally every 12 h. After the dog is discharged, the dose of cortisone is gradually lowered over a period of four weeks to 0.25–0.5 mg/kg every 12 h. L-thyroxine is given orally in doses of 15 µg/kg twice daily. Desmopressin is administered in the conjunctival sac, 1 drop (~5 µg) three times daily, for two to three weeks. The dog's water intake is recorded by the owner for at least four weeks after surgery and if severe polyuria and polydipsia occur when desmopressin is stopped, it is resumed. The owner is instructed to try reducing the frequency of administration after several weeks. If it proves to be too difficult to administer desmopressin in the conjunctival sac, it can be given orally in the form of 0.1 mg tablets, one tablet three times daily. Antibiotics (amoxycillin/clavulanic acid) and analgesia (Tramadol) are continued orally for two weeks after surgery.

Comments

In healthy dogs this protocol prevents postoperative hypernatremia,[2] but mild and occasionally severe immediate postoperative hypernatremia may develop after hypophysectomy in dogs with pituitary-dependent hypercortisolism. This is most likely due to vasopressin resistance caused by hypercortisolism.[3,4]

At hospital discharge enough time is taken to inform the owner, with written instructions, on initial and lifelong medications. The owner is instructed to double the dose of cortisone during every period of moderate to severe stress, such as that caused by significant illness or severe excitement and anxiety, and to continue this for the duration of the stress. The owner must also be provided with injectable hydrocortisone and syringes and needles if traveling with the dog, in the event that the cortisone cannot be given orally for any reason.

Monitoring for possible recurrence of hypercortisolism is achieved by measuring UCCR in morning urine samples collected at home (chapter 12.4) at two and eight weeks after hypophysectomy and every six months thereafter. The evening dose of cortisone is omitted prior to each sample and the morning dose is delayed until after the sample is collected.

Follow-up examinations are made at eight weeks after hypophysectomy and then once yearly for the remainder of the dog's life. Each follow-up consists of physical examination, routine biochemistry, and measurement of plasma thyroxine.[4]

13.2 Adrenal cortex

13.2.1 Primary hypoadrenocorticism

Joris H. Robben
Hans S. Kooistra

Emergency treatment

The symptoms and signs in a dog with primary hypoadrenocorticism admitted as an emergency are primarily those of hypovolemic shock resulting from mineralocorticoid deficiency. The first and often life-saving step is correction of the dehydration – often amounting to 10–15 % of body weight – and electrolyte disturbances. After the initial resuscitation by intravenous or intraosseous administration of fluid (see below) in a dose of 100 ml/kg, the infusion is continued to provide the equivalent of 10–15 % of body weight during the first 4–8 h and 100 ml/kg/24 h thereafter. This is accompanied by monitoring of physical cardiovascular variables, urine production, and central venous pressure. The fluid therapy decreases hyperkalemia by (1) dilution, (2) movement of potassium into cells with restoration of metabolic acidosis, and (3) increased renal excretion of potassium. Plasma potassium should be monitored closely, for hypokalemia can develop during this phase.

Traditionally, 0.9 % NaCl has been used for initial fluid therapy because it provides the needed water and sodium but no potassium to exacerbate the hyperkalemia (see also chapter 4.1.6). However, raising plasma sodium concentration too rapidly in patients with severe hyponatremia may cause neurological disorders and thus fluid with a lower sodium concentration may be preferable. Furthermore, restoration of circulating volume and the consequent increase in glomerular filtration results in adequate kaliuresis, even if the infused fluid contains some potassium.

Hypocortisolism is corrected by adding hydrocortisone acetate (5 mg/kg) or prednisolone succinate (1 mg/kg) or dexamethasone phosphate (0.2 mg/kg) to the initial intravenous or intraosseous dose of fluid. Thereafter, hydrocortisone (1 mg/kg) or prednisolone (0.5 mg/kg) is administered subcutaneously every 6 h.

The hypoaldosteronism is corrected by subcutaneous administration of desoxycorticosterone pivalate (Percorten® V) in a dose of 2 mg/kg every three weeks or desoxycorticosterone acetate in oil in a dose of 0.1 mg/kg once daily. If neither of these mineralocorticoids is available but hydrocortisone is available for glucocorticoid replacement, the dose of hydrocortisone can be doubled to make use of its slight intrinsic mineralocorticoid activity (lacking in prednisolone and dexamethasone). However, this should be changed as soon as possible to the orally administered mineralocorticoid fludrocortisone with supplemental salt (see below).

Maintenance therapy

The following oral maintenance medication is divided into at least two portions per day:

- Glucocorticoid: Cortisone acetate in a daily dose of 0.5–1.0 mg/kg or prednisolone in a daily dose of 0.1–0.15 mg/kg.
- Mineralocorticoid: Fludrocortisone acetate in a daily dose of 0.0125–0.025 mg/kg is usually sufficient to maintain normal electrolyte concentrations. Alternatively, desoxycorticosterone pivalate can be given subcutaneously in a dose of 2 mg/kg every three weeks. The dose of the mineralocorticoid may have to be increased slightly over the years.
- Sodium chloride: Table salt is added to the dog's food in a dose of 0.1 g/kg body weight per day. It may provoke vomiting of the meal and the salt can then be given with the drinking water or as tablets.

13.2.2 Treatment of hypercortisolism with trilostane

Hans S. Kooistra

Treatment with trilostane is started at 2 mg/kg daily in one or two portions given with food. The dose is subsequently adjusted according to the clinical response and the results of ACTH-stimulation tests. In addition, the efficacy of treatment is monitored by clinical signs and measurements of plasma sodium, potassium, urea, creatinine, liver enzymes, and ACTH.[5]

The maximal lowering of plasma cortisol concentration occurs between 2 and 6 h after oral administration of trilostane.[6] Hence treatment is evaluated by ACTH-stimulation tests performed 2–3 h after a dose, the first test approximately two weeks after the start of the treatment. Adjustment of the dose is determined as follows:

- If clinical manifestations of hypercortisolism such as polyuria and polydipsia have ceased and the post-ACTH plasma cortisol concentration is 40–150 nmol/l, the dose of trilostane is left unchanged.
- If clinical manifestations of hypercortisolism have not decreased or ceased but the post-ACTH plasma cortisol concentration is 40–150 nmol/l, the dose of trilostane is increased slightly or given in two portions daily rather than one portion.
- If clinical manifestations of hypercortisolism have not ceased and the post-ACTH plasma cortisol concentration is > 150 nmol/l, the daily dose of trilostane is increased by ~1 mg/kg.
- If clinical manifestations of hypercortisolism have ceased but the post-ACTH plasma cortisol concentration is 150–250 nmol/l, the dose of trilostane is not changed but the dog is monitored closely for signs of possible recurrence of hypercortisolism.
- If clinical manifestations of hypercortisolism have ceased and the dog's condition is considered satisfactory but the post-ACTH plasma cortisol concentration is < 40 nmol/l, the dose of trilostane is a) not changed if plasma cortisol was increased by ACTH, or b) decreased by ~1 mg/kg if there was no response to ACTH. The dog is monitored closely for signs of hypocortisolism and reexamined at monthly intervals.
- If there are signs suggesting adrenocortical insufficiency, such as lethargy and anorexia, trilostane treatment is stopped immediately and an ACTH-stimulation test is performed. If necessary, intravenous fluid and corticosteroids are started (see chapter 13.2.1).

Follow-up examinations are repeated at intervals of two to three weeks and the dose is adjusted as required until the response is satisfactory. The dog is then reexamined one month later and at intervals of three to six months thereafter.

13.3 Endocrine pancreas

13.3.1 Treatment of diabetes mellitus in dogs and cats

Claudia E. Reusch

Initial presentation

- Diagnosis of diabetes mellitus (hyperglycemia, glucosuria, elevated fructosamine).
- Laboratory evaluation (routine hematology, blood biochemistry, urinalysis, urine culture).
- Radiographs, abdominal ultrasonography, TLI, cPLI if indicated.
- Cessation of diabetogenic drugs.
- Start intermediate-acting insulin (lente insulin; Caninsulin®, Vetsulin®, both Intervet/Schering Plough):
  - dogs: 0.25–0.5 IU/kg BID
  - cats: 1–2 IU/cat BID.
- Treatment may be started in the hospital for one to two days. Blood glucose is measured three to four times daily and the dose of insulin is decreased if blood glucose is < 5 mmol/l. Treatment may also be started without hospitalization.
- Begin treatment of concurrent problems (e.g., urinary tract infection, stomatitis/gingivitis).
- Castration if intact bitch. Consider administration of a progesterone receptor blocker such as aglepristone if the bitch cannot undergo surgery or has recently received progestins.
- Prescribe dietary management:
  - dogs: high-fiber diet
  - cats: high-protein, low-carbohydrate diet, provided that dietary management of another disease does not have priority
  - if overweight, aim for 1 % weight loss per week
  - give food just before insulin administration.
- Instruct the owner (duration at least 1 hour).
- Give the owner written instructions.

Reevaluation one week after diagnosis

- Owner gives food and insulin at home and then brings patient to the clinic as soon as possible.
- History, physical examination, body weight.
- Measure blood glucose concentration every 1–2 h for the remainder of the day.
- Measure blood fructosamine.
- Adjust insulin dose if required: in dogs increase by 10–25 %, in cats by 0.5 1.0 IU/injection.
- After Somogyi reaction or overt hypoglycemia, reduce dose by at least 50 %.

Reevaluation three weeks after diagnosis

- Repeat history, physical examination, body weight, blood glucose curve (BGC), fructosamine, and dose adjustment, as after one week.
- Introduce owner to home monitoring (HM) and instruct on all relevant technical aspects (duration at least 0.5 h)
- Owner measures fasting blood glucose twice weekly and performs a BGC once per month.

Reevaluation six to eight weeks after diagnosis

- Repeat history, physical examination, body weight, BGC, fructosamine, and dose adjustment, as after one week. BGC may not be required if animal appears to be doing well, blood glucose measured close to the time of insulin administration is 5–10 mmol/l, and fructosamine is 350–450 µmol/l.
- If owner performs HM, check his / her technique.

- Test for underlying disease (hypercortisolism, hypersomatotropism) if there are suggestive symptoms and/or signs.

Reevaluation ten to twelve weeks after diagnosis
- Repeat all measures as at six to eight weeks.

Continuing reevaluations every four months
- Repeat all measures as at six to eight weeks.
- Urine culture at least once yearly.

Goals of therapy
- Resolution of polyuria, polydipsia, and polyphagia and return to normal body weight.
- Maintenance of blood glucose between 15 mmol/l at the time of insulin administration and 5 mmol/l at the nadir.
- Maintenance of fructosamine at 350–450 µmol/l, although fructosamine is the least important variable for evaluation of metabolic control.

13.3.2 Management of diabetic ketoacidosis

Joris H. Robben
Claudia E. Reusch

The clinical manifestations of diabetic ketoacidosis depend on the stage at the time of presentation and usually include vomiting, polyuria, lethargy, and weight loss. The animals are often severely hypovolemic and may be presented in a state of sopor or stupor, or even in coma. Laboratory findings include hyperglycemia (sometimes severe), metabolic acidosis, ketonemia, and ketonuria. The fluid loss via osmotic diuresis, vomiting, and hyperventilation is usually equivalent to 10–12 % of body weight.

Diabetic ketoacidosis is one of the most complex metabolic emergencies and its treatment is demanding. It requires 24 h surveillance with frequent reevaluation of physical and laboratory variables and appropriate adjustments of therapy. Treatment consists of fluid therapy, electrolyte supplementation, correction of acid-base balance, and correction of the hyperglycemia.

Fluid therapy
Hypovolemia and shock should be treated as soon as possible with intravenous fluid (30–90 ml/h). Dehydration is usually resolved within 12–24 h. This requires (1) maintenance fluid (~2 ml/kg/h), (2) correction of the fluid deficit (= amount of dehydration), and (3) replacement of losses due to vomiting and polyuria. Fluid therapy is started with a solution having a sodium concentration of 140 mmol/l or slightly higher (154 mmol/l; NaCl 0.9 %), depending on the plasma sodium concentration and the state of hydration. The type of solution is adjusted according to plasma sodium concentration and fluid balance. Fluid balance is monitored closely; with a central venous catheter and a urine catheter in place.

Potassium supplementation
The potassium deficit can be severe even though plasma potassium is normal or even elevated. The lack of insulin contributes to the loss of intracellular potassium, aggravated by vomiting, osmotic diuresis, and secondary hyperaldosteronism due to the hypovolemia. Consequently, potassium must be supplemented in virtually all patients with diabetic ketoacidosis. The initial dose depends on the pretreatment plasma potassium concentration (table 13.1). Plasma potassium is measured again after 2 h of fluid therapy, since it is rapidly lowered by dilution and osmotic diuresis. In addition, potassium shifts from the extracellular to the intracellular space with correction of the metabolic acidosis. The cotransport of potassium with glucose is amplified by insulin therapy and if hypokalemia is severe, plasma potassium should be corrected to near normal before insulin therapy is started. Intravenous administration of potassium should not exceed 0.5 mmol/kg/h, to avoid cardiac arrhythmias.

Phosphate supplementation
Hypophosphatemia can develop during the first few days of treatment, especially if there is no food intake after initial treatment. Phosphate should be supplemented if plasma phosphate is < 0.5 mmol/l in dogs or < 0.8 mmol/l in cats. It is supplemented by continuous intravenous infusion at a rate of 0.03–0.06 mmol/kg/h for 6 h. Higher infusion rates, up to 0.12 mmol/kg/h, are sometimes necessary. The duration of infusion is determined by measuring plasma phosphate every 6 h. Simultaneous supplementation of potassium should be reduced if potassium phosphate is used for the infusion.

Bicarbonate supplementation
In most cases, correction of hypovolemia will also restore the acid-base balance quickly, so that administration of bicarbonate to correct metabolic acidosis is often unnecessary and can even be detrimental. Hence bicarbonate supplementation

Table 13.1: Potassium supplementation in diabetic ketoacidosis treated with 0.9 % NaCl*

Plasma potassium (mmol/l)	mmol KCl added per liter of 0.9 % NaCl	Maximum rate (ml/kg/h)
3.6–5.0	20	24
3.1–3.5	30	16
2.6–3.0	40	11
2.1–2.5	60	8
< 2.0	80	6

* If any other crystalloid solution is used, its potassium content must be taken into account.

should be considered only if blood gasses can be measured during treatment, the initial pH is < 7.1, and there is concomitant renal failure. The bicarbonate deficit in mmol can be calculated by:

$NaHCO_3$ = 0.3 × body weight (kg) × (24 – [bicarbonate (mmol/l)]).

The initial dose is one-fourth to one-half the calculated bicarbonate deficit, administered over ~6 h. Acid-base balance is determined at least every 3 h.

Insulin and glucose therapy

Insulin therapy should be postponed for 2–4 h in patients with severe diabetic ketoacidosis, hypovolemic shock, dehydration, hypokalemia (< 3.5 mmol/l), and hyperglycemia. The patient is first stabilized by administering intravenous fluids and potassium, which often lowers blood glucose by dilution, diuresis, and improved uptake of glucose in peripheral tissues.

Regular crystalline insulin can be administered intermittently or as a continuous rate infusion. The goal in either case is to reduce the hyperglycemia in a controlled manner, reduce osmotic diuresis, and improve acid-base status.

Intermittent, intramuscular (IM) technique

Start regular insulin if blood glucose is > 15 mmol/l:

- Administer 2 IU of regular insulin IM per cat or dog < 10 kg, and 0.25 IU/kg IM in dogs > 10 kg. Ideally, the blood glucose concentration should be decreased by 3 mmol/l/h, to avoid large shifts in osmolality which can have detrimental neurological effects.
- Measure blood glucose after 1 h.
- If blood glucose is still > 15 mmol/l, administer 1 IU of regular insulin IM per cat or dog < 10 kg, and 0.1 IU/kg IM in dogs > 10 kg.
- Repeat steps two and three until glucose is < 15 mmol/l.
- If blood glucose is < 8 mmol/l, administer glucose to prevent hypoglycemia, since most patients are anorexic at this stage. Add an amount of 50 % glucose to the maintenance fluid to result in a 5 % glucose solution. Do not administer insulin! Measure blood glucose every hour and adjust the glucose infusion rate accordingly. It may be more convenient to administer 20 % glucose, at one-fourth the maintenance rate, via a dedicated intravenous syringe pump and a central intravenous catheter. Total fluid administration should be calculated and infusion rates of other solutions reduced accordingly.
- When blood glucose reaches 8–15 mmol/l and the patient is rehydrated, give 0.5 IU of regular insulin/kg every 6–8 h SC instead of IM. If the patient is not yet rehydrated, continue IM administration of insulin.
- When regular insulin is given SC, determine its maximum effect by measuring blood glucose 2–3 h after administration. Adjust the dose in steps of 10–20 % to maintain blood glucose at 6–8 mmol/l. It is often helpful to plot blood glucose concentration measured hourly for 6–8 h after injection, to determine the duration of action.

Continuous rate infusion

- First administer regular insulin at a rate of 0.05 IU/kg/h (cat) or 0.1 IU/kg/h (dog) by means of a syringe pump. For this purpose, add the dose for 0.5 IU/kg to 50 ml of 0.9 % NaCl in a syringe or the dose for 5 IU/kg to a 500 ml flask or bag of 0.9 % NaCl. Administer at a rate of 5 ml/h in cats or 10 ml/h in dogs. Adding 2 ml of plasma to 50 ml of 0.9 % NaCl solution before adding insulin prevents adhesion of insulin to the synthetic material of the syringe and tubing. Alternatively, fill the syringe and tubing with insulin-containing solution, discard the solution after 1 h, and then refill the system with a fresh insulin-containing solution.
- The continuous rate infusion of glucose is started if blood glucose falls below 15 mmol/l (see step 5 of the intermittent technique), while adjustments are being made in the continuous rate infusion of regular insulin (table 13.2). Add the appropriate amount of 50 % glucose to the maintenance fluid to make a 5 % solution. Alternatively, 20 % glucose can be administered via a dedicated syringe pump and a central line at one-fourth the maintenance rate, which simplifies adjustments in glucose administration. Aim at a blood glucose concentration of 9–12 mmol/l.
- After the patient is stable and rehydrated, regular insulin can be administered SC three to four times/day (see steps 6 and 7 of the intermittent technique).

After the patient has been stabilized, its condition improved, and it has begun to eat, treatment with longer-acting insulin can be initiated.

Table 13.2: Glucose infusion adjustments with continuous rate infusion of regular insulin

Blood glucose (mmol/l)	Glucose administration	Regular crystalline insulin (ml/h)	
		Dog	Cat
> 15	Maintenance fluid	10	5
12–15	Maintenance fluid with 5 % glucose	7	3.5
9–12	Maintenance fluid with 5 % glucose	5	2.5
6–9	Maintenance fluid with 5 % glucose	5	2.5
< 6	Maintenance fluid with 5 % glucose	Stop insulin infusion	

13.3.3 Treatment of hypoglycemia

Joris H. Robben

- Patients with severe, acute symptoms and signs related to hypoglycemia should be treated immediately. Time required for measurement of blood glucose should never postpone emergency treatment, for the longer the period of hypoglycemia, the greater the risk of irreversible brain damage.
- If intravenous injection is not possible (e.g., because the patient is having seizures), apply glucose syrup to the oral mucosa. Do not pour syrup into the patient's mouth, for it may be aspirated.
- Serious neurological symptoms such as seizures should decrease within 1–2 min after giving glucose. If they do not, administer diazepam rectally (1 mg/kg), but continue trying to raise blood glucose concentration via the oral mucosa.
- As soon as intravenous injection is possible, slowly (5–10 min) administer a bolus of glucose: 6–12 ml of 20 % glucose or 2.5–5 ml of 50 % glucose for a cat or small dog, and 20–35 ml of 20 % glucose or 8–15 ml of 50 % glucose for a large dog. If blood glucose decreases 15–30 min after the bolus, immediately start a continuous rate infusion of 2.5–5 % glucose at 1.5 to two times the maintenance rate.
- Continuous rate infusion of 20 % or 50 % glucose should be via a central venous catheter, because these hyperosmolar solutions can cause phlebitis if administered in peripheral vessels. The rate of continuous infusion is adjusted by serial measurements of blood glucose every 20–60 min. If blood glucose falls below the reference range, the infusion rate is increased by 25–50 %. If blood glucose is very low, an additional bolus must be given. The target of treatment is a normal blood glucose concentration, not hyperglycemia.
- The patient must be fed as soon as possible after stabilization, if necessary by force feeding or enteral tube feeding. Parenteral feeding should be considered if the enteral route is not available.
- Diazepam (1 mg/kg) or propofol (2–6 mg/kg) can be given to effect if the patient still has seizures after normalization of the blood glucose concentration. These anticonvulsants have a relatively short half-life, so the neurological status of the patient can be examined shortly after stopping the medication.
- In animals with refractory or persistent hypoglycemia due to insulin overdosage or an insulin-secreting pancreatic tumor, the counterregulatory hormone glucagon can be administered. Start with a 50 ng/kg bolus followed by a continuous rate infusion at an initial rate of 5–10 ng/kg/min. Adjustment of the dose is based on measurements of blood glucose.
- Hyperglycemia may stimulate insulin release from an insulin-secreting pancreatic tumor (insulinoma), resulting in rebound hypoglycemia. Consequently, it is probably better to avoid high blood glucose concentrations in patients with a tentative diagnosis of insulinoma. Even normal blood glucose concentrations may not be needed to control symptoms: 2.8–3.5 mmol/l is often sufficient, because insulinoma patients have adapted to a chronic hypoglycemic state.
- Dexamethasone (0.5–1 mg/kg added to intravenous fluids and administered over 6 h) or diazoxide (5–30 mg/kg BID) can also be considered to control hypoglycemia. Both drugs have a slow onset of action and should be given as soon as possible. If the patient is unable or unwilling to swallow, diazoxide capsules can be opened and the powder can be dissolved in water and administered by gastric tube.

Client information for o,p'-DDD therapy in dog[7]

In your dog excessive amounts of the hormone cortisol are produced by adrenocortical tissue. The treatment with o,p'-DDD aims at complete destruction of all adrenocortical tissue (including adrenocortical tumor tissue). The requirement for the hormones normally produced by adrenocortical tissue is then provided by lifelong administration of replacement hormone tablets. It is very important that the instructions for the replacement hormone be followed carefully and completely, for deficiency of these hormones can result in a life-threatening crisis.

The initial treatment of your dog consists of: _____ tablets of o,p'-DDD (= Lysodren® or Mitotane®) _____ times daily for in total 25 days. The o,p'-DDD is given daily for the first five days and then every other day. For good absorption and to prevent vomiting, the tablets should always be given with food.

For the first two days only o,p'-DDD is given. On the third day the replacement of the adrenocortical hormones is begun with the addition of cortisone, fludrocortisone, and ordinary salt. To allow a more gradual change from the excessive hormone production, the dose of cortisone is kept higher than the normal requirement for the first week after o,p'-DDD therapy.

During the first two months your dog receives as replacement therapy:

Cortisone acetate: _____ × daily _____ tablets of _____ mg

Fludrocortisone acetate: _____ × daily _____ tablets of _____ mg

NaCl (salt): _____ × daily _____ gram

Follow-up

The first follow-up examination is at one month after the beginning of o,p'-DDD therapy. At this time the dose of cortisone is usually reduced by half. Results of blood examination will be used to determine whether the doses of fludrocortisone and salt need to be adjusted. After this, follow-up examinations are usually made once every six months. Their purpose is to be certain that the replacement doses of fludrocortisone and salt are correct. Sometimes, in spite of the destructive action of o,p'-DDD on adrenocortical tissue, symptoms of the disease reappear. This can occur after several months or even after four to five years. It is then necessary to repeat the treatment with o,p'-DDD.

The first signs of recovery are often already apparent during the o,p'-DDD therapy. The excessive thirst and hunger diminish and the dog's endurance increases. The recovery of the coat takes longer, but once this begins, after about two months, a very thick coat usually develops. The recovery of the skin and coat may be preceded by a short period of excessive scaling and some itching. This can be relieved by a treatment with shampoo once or twice a week.

Complications

With the above treatment instructions, most dogs recover without complications, but there can be complications associated with the o,p'-DDD or the replacement therapy. If you notify the veterinarian in time, the problems can usually be resolved without difficulty.

In the beginning of treatment there may be mild side effects from o,p'-DDD, such as nausea, incoordination, or slight disorientation. These signs usually disappear if administration is continued but simply spread out more over the day. If the dog refuses to eat or eats almost nothing, stop the o,p'-DDD completely, but be sure to continue the replacement medications, and notify the veterinarian.

A deficiency in replacement medications can lead to a life-threatening crisis and emergency treatment may be required. It is far better to contact the veterinarian before a crisis occurs. The first warning is often loss of appetite. Many dogs with the disease have an excessive appetite, and a decrease in appetite is an expected sign of recovery. However, an almost complete refusal to eat should be recognized as a warning. You should stop o,p'-DDD immediately, continue the replacement medications, and obtain the veterinarian's advice promptly.

Special circumstances in replacement therapy

It is extremely important to give the replacement medications without interruption. Yet there may be situations in which your dog cannot or will not take anything orally or cannot retain the medications because of vomiting. If for any reason your dog cannot take or retain the tablets and salt for two times in succession, injectable medications should be started. This also applies if your dog must be fasted before being brought to the veterinarian for treatment that requires anesthesia.

- The cortisone tablets are replaced by subcutaneous injections of hydrocortisone acetate (50 mg/ml) in a dose of _____ ml twice daily. The hydrocortisone injections are continued until the dog can again swallow and retain the cortisone tablets.

- The fludrocortisone tablets and salt are replaced by subcutaneous injections of desoxycorticosterone acetate (DOCA, 1 mg/ml) in a dose of _____ ml once daily or of desoxycorticosterone pivalate in a dosage of _____ ml once every three weeks. If neither DOCA nor DOC pivalate (chapter 13.2.1) is available, you should double the dose of hydrocortisone. The salt is not needed when DOC injections are used. The DOC injections are continued until the dog can again swallow and also retain the fludrocortisone and salt.

If you take your dog on vacation or on a trip away from home for more than one or two days, also take the injectable medications, syringes, and needles, and this instruction sheet, for not all veterinarians may have these medications at hand. If you leave the dog in the care of someone else, also make provision for the possible need for the injections, even if you have not yet had to use them yourself.

In cases of anesthesia, severe physical stress, or injury, the dose of cortisone should be doubled for one or two days. With these exceptions, the dose of cortisone remains unchanged for life, while the dose of fludrocortisone and salt may have to be adjusted by the veterinarian.

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14 Algorithms

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Ad Rijnberk

In these step-by-step procedures for problem solving the emphasis is on associated symptoms and signs that may point to an endocrine disturbance. The history and physical examination are aimed at the detection of endocrine disease, and the use of a standard form for these steps may be helpful.[1] When suspicion of an endocrine disturbance arises, it can be tested by specific examinations.

If the history and physical examination do not reveal clues suggesting an endocrine disturbance, the next step is laboratory examination of urine and blood. If the routine laboratory examinations reveal no abnormal values, diagnostic imaging and work-up at specialist level may be required.

14.1 Endocrine alopecia

Endocrine disturbances may cause atrophy of the skin and adnexa. Atrophy of hair follicles results in slow, abnormal (dull), or absent hair growth. Skin atrophy may also manifest itself by stagnant regrowth of hair after clipping. Depending upon the severity and duration of the endocrine disturbance, alopecia may develop. In the dog the classical causes of alopecia are hypothyroidism (section 3.3), hypercortisolism (section 4.3), and hyperestrogenism (section 8.4). Growth hormone deficiency may also cause alopecia (chapter 2.2.2), but the alopecias that have been ascribed to acquired growth hormone deficiency do not seem to fit in this category (see chapter 2.2.3).

14.2 Polyuria and polydipsia

In the first part of this algorithm the signalment, history, and physical examination may reveal a reason to suspect an endocrine disorder as the cause of the polyuria / polydipsia (PU / PD). The second step is a urinalysis. If an animal with a seemingly convincing history of PU / PD is found to produce urine with a high osmolality (Uosm) or specific gravity (Usg), indicating that the kidneys are able to concentrate urine, primary polydipsia must be considered. Alternatively, water consumption may be increased because the owner has changed the animal's diet to a dry food. A high Uosm or Usg in an animal with PU / PD may also be due to glucosuria.

Other than kidney disease, hepatic failure may also cause polyuria, especially when associated with hepatic encephalopathy. In this condition abnormal metabolism of amino acids gives rise to »false« neurotransmitters, which lead to increased secretion of ACTH and subsequently cortisol excess.[2] As parathyroid tumors are usually very small and a malignancy causing hypercalcemia may not have been detected by physical examination (section 9.3), measurement of plasma calcium and phosphate should always be included in the laboratory profile for PU / PD. Polycythemia and hyperaldosteronism may also cause PU / PD in dogs.[3,4]

If the routine laboratory examinations reveal no abnormal values suggesting the cause of the PU / PD, abdominal ultrasonography, serial measurements of urine osmolality (chapter 12.2.1), the modified water deprivation test (chapter 12.2.2), and vasopressin measurements during hypertonic saline infusion (chapter 12.2.3) may be required.

14.3 Breeding management of the bitch

If the general and gynecological examinations reveal no abnormalities, this algorithm can be used after the onset of proestrus, characterized by swelling of the vulva and bloody vaginal discharge.

The information needed for good management of breeding includes the appearance of the vaginal discharge, the vaginoscopic appearance of the vaginal mucosa, the cytological findings in a vaginal smear, and the plasma progesterone (P4) concentration.

The plasma P4 concentrations (1 nmol/l = 0.32 ng/ml) used in this algorithm are based on measurements by radioimmunoassay with tritium-labeled progesterone. The use of other reliable methods for the determination of the P4 concentration in peripheral blood may require working with slightly different P4 concentrations. For example, with a radioimmunoassay using radioiodine-labeled progesterone, ovulation is considered to occur at plasma P4 concentrations above 13 nmol/l and immediate mating is advised when plasma P4 reaches 26 nmol/l.[5] These differences may be due to differences in specificity of the antibodies in the two assays or in the assay standards used.

14.4 Weight loss in spite of good appetite

This algorithm can be used when an animal loses weight in the absence of other problems such as PU / PD, fever, or diar-

rhea. The first step is to carefully evaluate food intake. Some owners feed strictly according to the recommendations of the manufacturer or seller of the food, not taking account of energy expenditure.

Large and widespread malignancies such as malignant lymphoma increase energy demand, as do cardiac abnormalities that result in tachycardia. However, in patients with these disorders weight loss is rarely seen as the primary problem and in most of them the appetite is poor.

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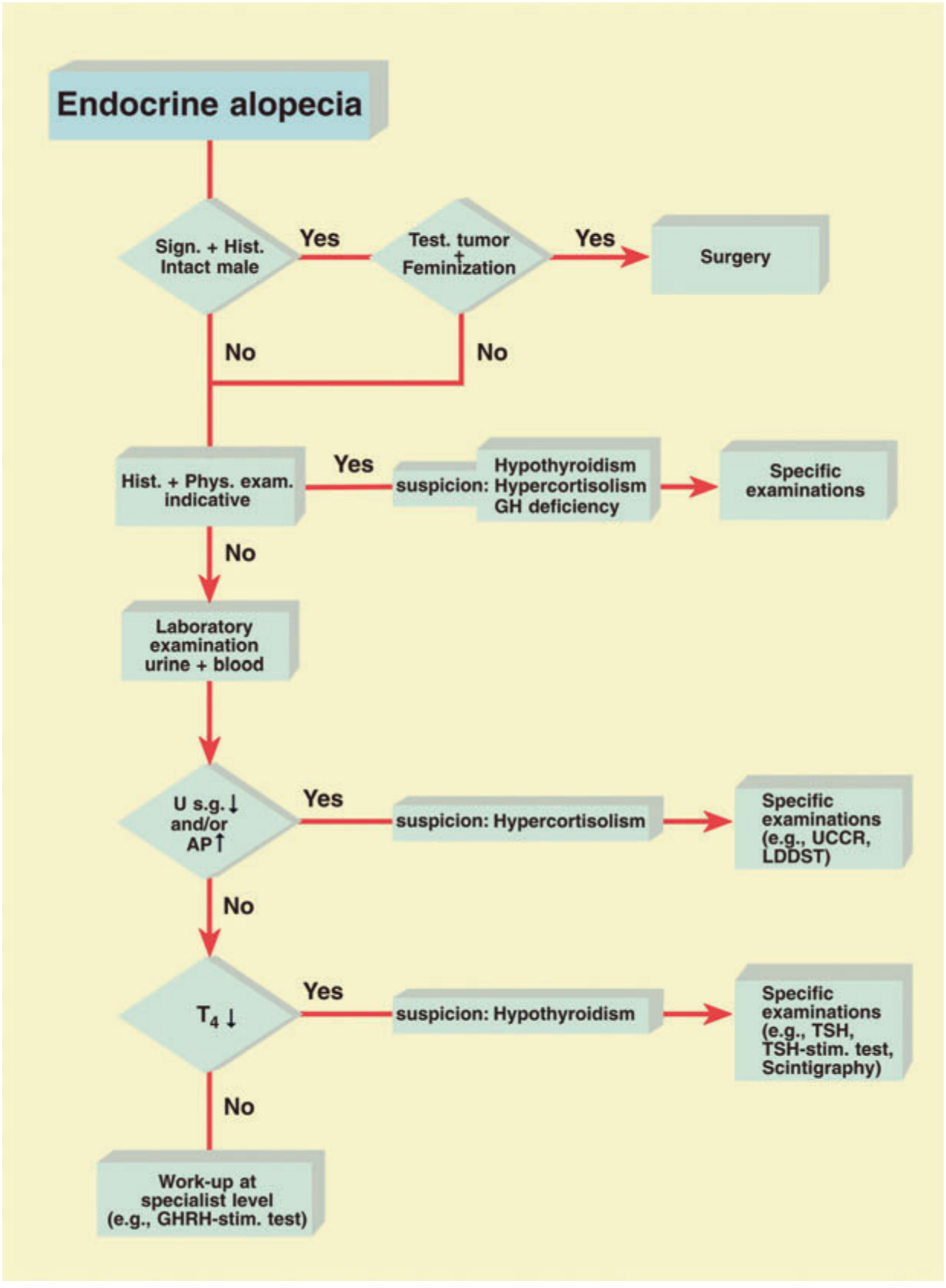

Endocrine alopecia
Sign. + Hist. Intact male
Yes
Test. tumor + Feminization
Yes
Surgery
No
No
Hist. + Phys. exam. indicative
Yes
suspicion: Hypothyroidism Hypercortisolism GH deficiency
Specific examinations
No
Laboratory examination urine + blood
U s.g.↓ and/or AP↑
Yes
suspicion: Hypercortisolism
Specific examinations (e.g., UCCR, LDDST)
No
T_4 ↓
Yes
suspicion: Hypothyroidism
Specific examinations (e.g., TSH, TSH-stim. test, Scintigraphy)
No
Work-up at specialist level (e.g., GHRH-stim. test)

Figure 14.1

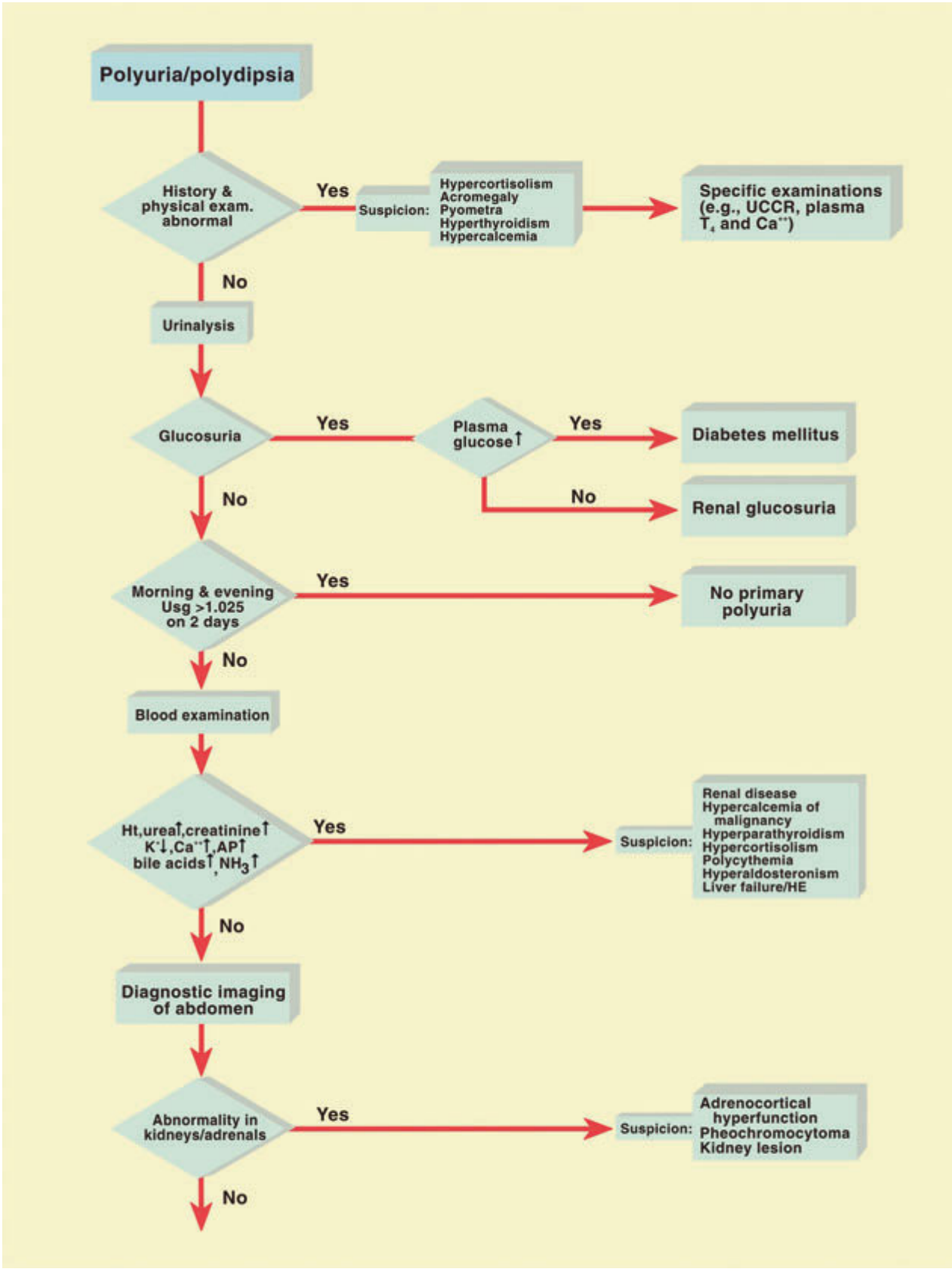

Polyuria/polydipsia
History & physical exam. abnormal
Yes
Suspicion:
Hypercortisolism
Acromegaly
Pyometra
Hyperthyroidism
Hypercalcemia
Specific examinations (e.g., UCCR, plasma T_4 and Ca^{++})
No
Urinalysis
Glucosuria
Yes
Plasma glucose↑
Yes
Diabetes mellitus
No
No
Renal glucosuria
Morning & evening Usg >1.025 on 2 days
Yes
No primary polyuria
No
Blood examination
Ht,urea↑,creatinine↑ K^+↓,Ca^{++}↑,AP↑ bile acids↑, NH_3↑
Yes
Suspicion:
Renal disease
Hypercalcemia of malignancy
Hyperparathyroidism
Hypercortisolism
Polycythemia
Hyperaldosteronism
Liver failure/HE
No
Diagnostic imaging of abdomen
Abnormality in kidneys/adrenals
Yes
Suspicion:
Adrenocortical hyperfunction
Pheochromocytoma
Kidney lesion
No

Figure 14.2a

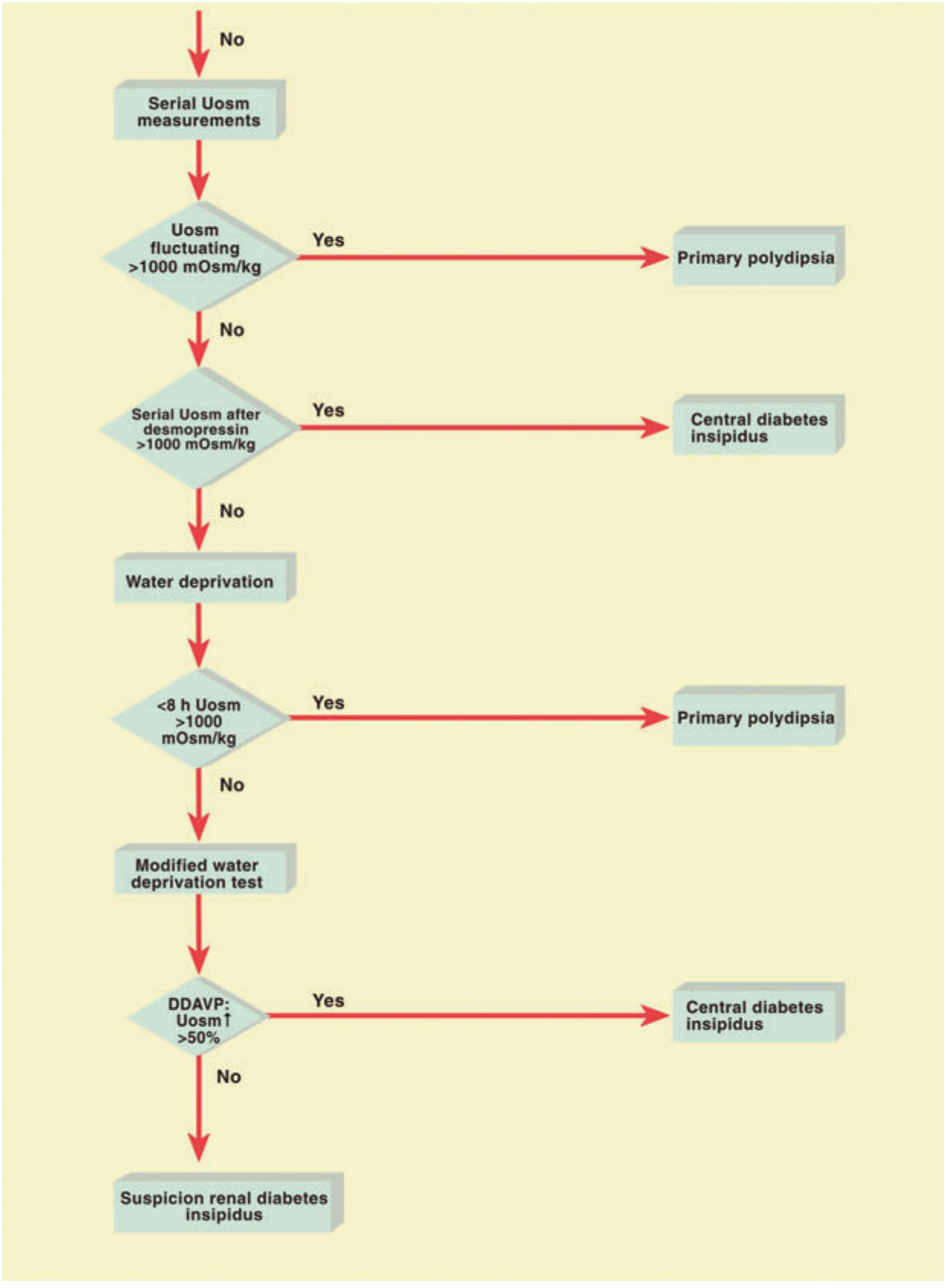

No
Serial Uosm measurements
Uosm fluctuating >1000 mOsm/kg
Yes
Primary polydipsia
No
Serial Uosm after desmopressin >1000 mOsm/kg
Yes
Central diabetes insipidus
No
Water deprivation
<8 h Uosm >1000 mOsm/kg
Yes
Primary polydipsia
No
Modified water deprivation test
DDAVP: Uosm↑ >50%
Yes
Central diabetes insipidus
No
Suspicion renal diabetes insipidus

Figure 14.2b

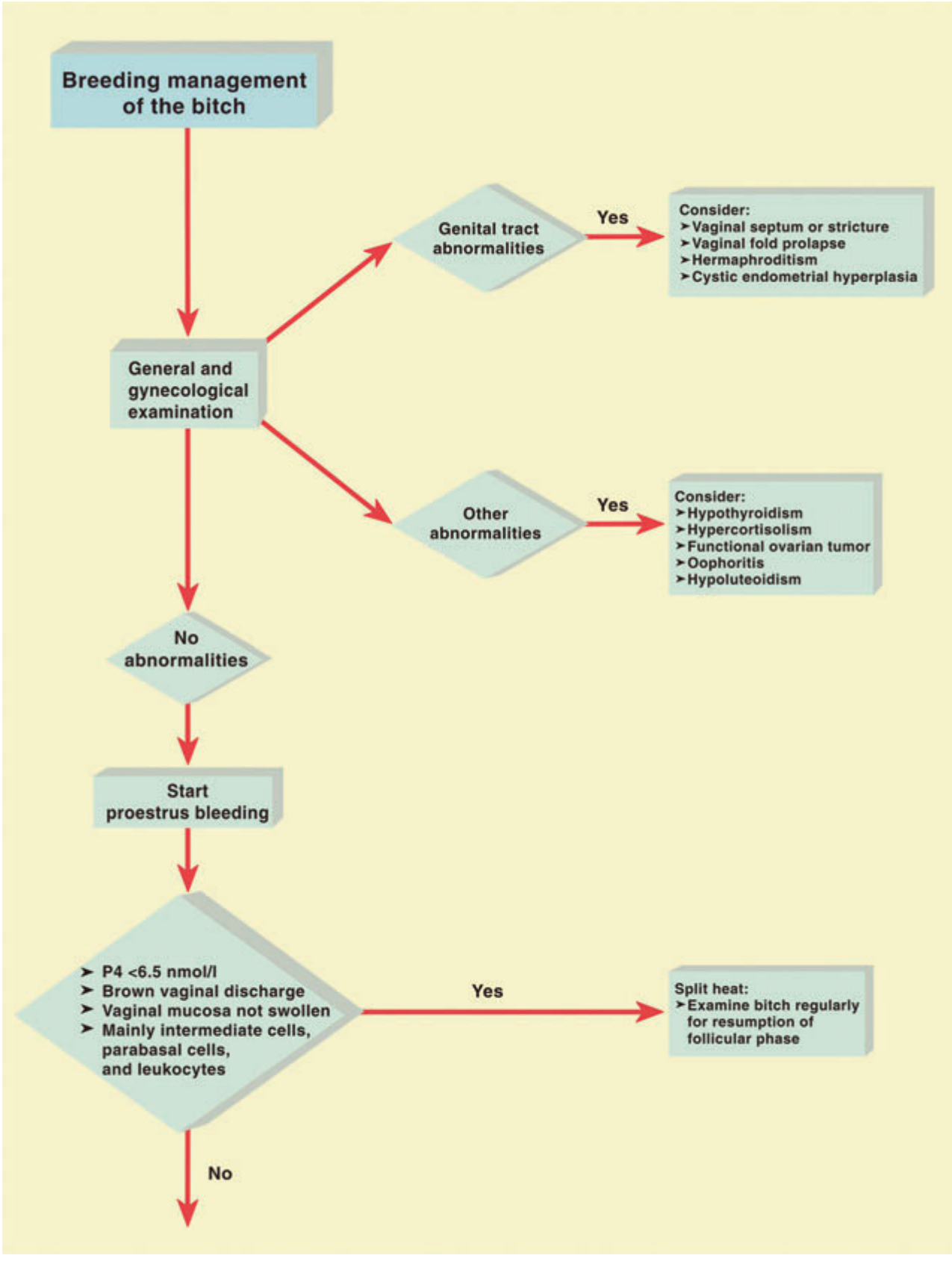

Breeding management of the bitch
General and gynecological examination
Genital tract abnormalities
Yes
Consider:
➤Vaginal septum or stricture
➤Vaginal fold prolapse
➤Hermaphroditism
➤Cystic endometrial hyperplasia
Other abnormalities
Yes
Consider:
➤Hypothyroidism
➤Hypercortisolism
➤Functional ovarian tumor
➤Oophoritis
➤Hypoluteoidism
No abnormalities
Start proestrus bleeding
➤ P4 <6.5 nmol/l
➤ Brown vaginal discharge
➤ Vaginal mucosa not swollen
➤ Mainly intermediate cells, parabasal cells, and leukocytes
Yes
Split heat:
➤Examine bitch regularly for resumption of follicular phase
No

Figure 14.3a

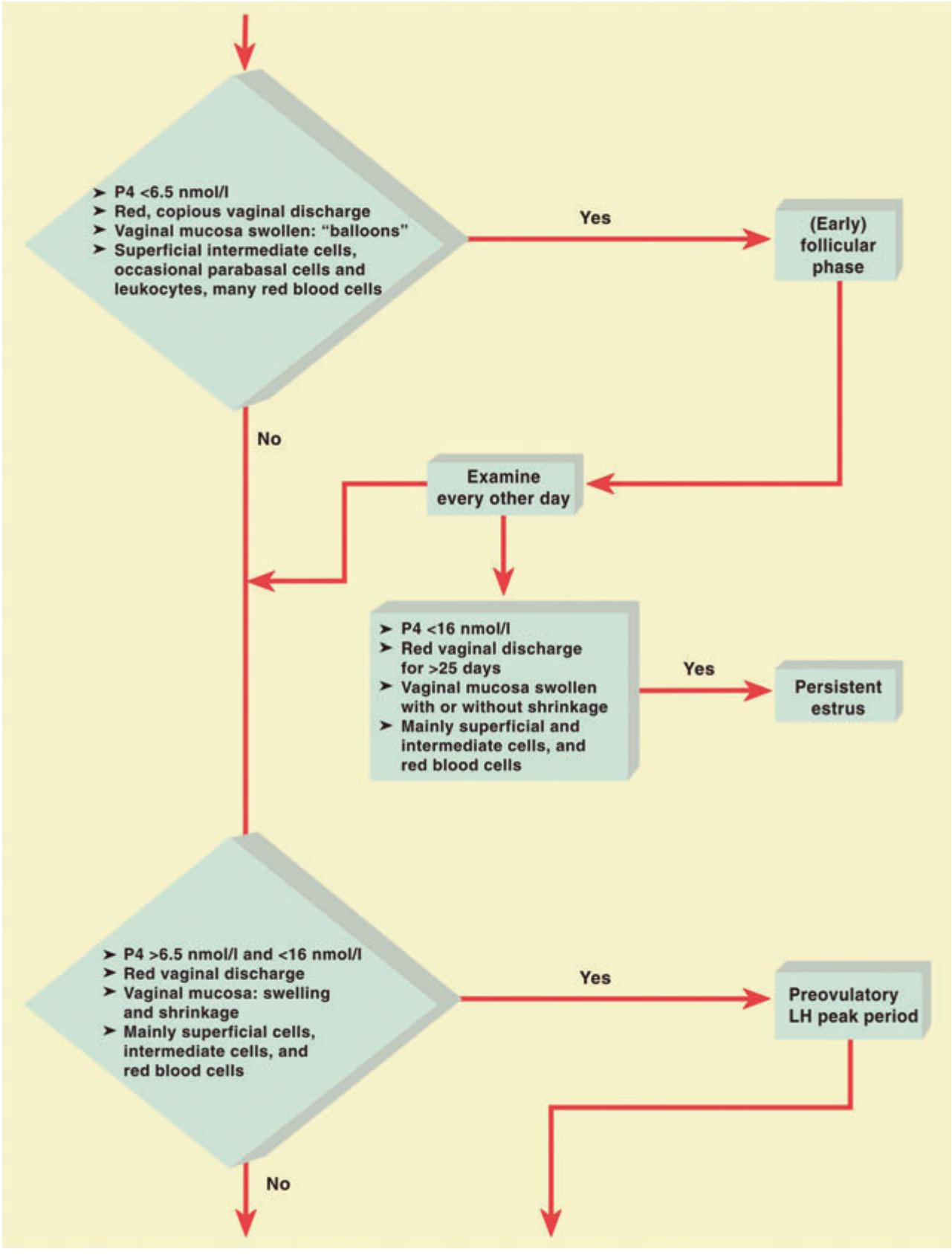

➤ P4 <6.5 nmol/l
➤ Red, copious vaginal discharge
➤ Vaginal mucosa swollen: "balloons"
➤ Superficial intermediate cells, occasional parabasal cells and leukocytes, many red blood cells
Yes
(Early) follicular phase
No
Examine every other day
➤ P4 <16 nmol/l
➤ Red vaginal discharge for >25 days
➤ Vaginal mucosa swollen with or without shrinkage
➤ Mainly superficial and intermediate cells, and red blood cells
Yes
Persistent estrus
➤ P4 >6.5 nmol/l and <16 nmol/l
➤ Red vaginal discharge
➤ Vaginal mucosa: swelling and shrinkage
➤ Mainly superficial cells, intermediate cells, and red blood cells
Yes
Preovulatory LH peak period
No

Figure 14.3b

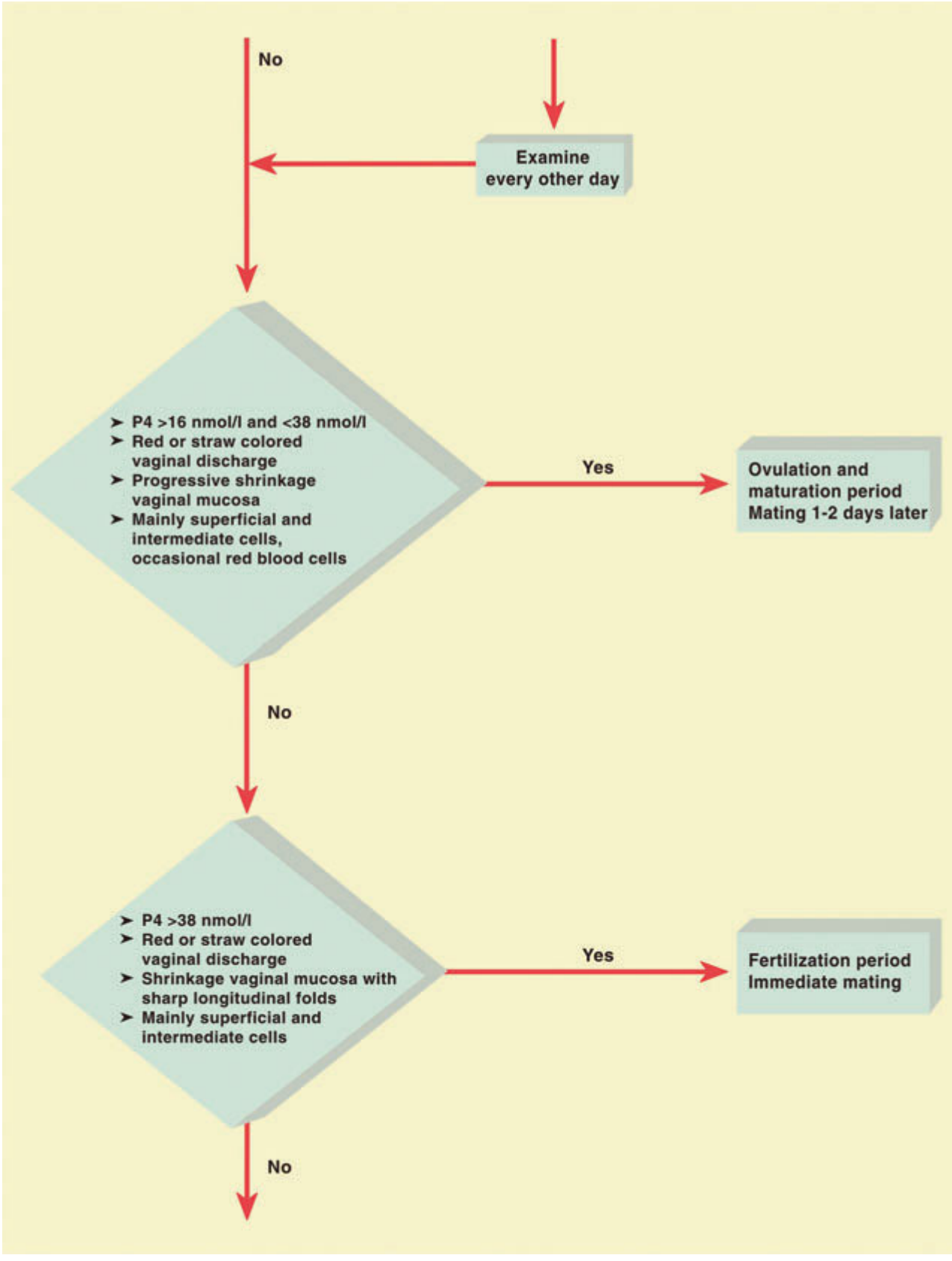

No
Examine every other day
➤ P4 >16 nmol/l and <38 nmol/l
➤ Red or straw colored vaginal discharge
➤ Progressive shrinkage vaginal mucosa
➤ Mainly superficial and intermediate cells, occasional red blood cells
Yes
Ovulation and maturation period
Mating 1-2 days later
No
➤ P4 >38 nmol/l
➤ Red or straw colored vaginal discharge
➤ Shrinkage vaginal mucosa with sharp longitudinal folds
➤ Mainly superficial and intermediate cells
Yes
Fertilization period
Immediate mating
No

Figure 14.3c

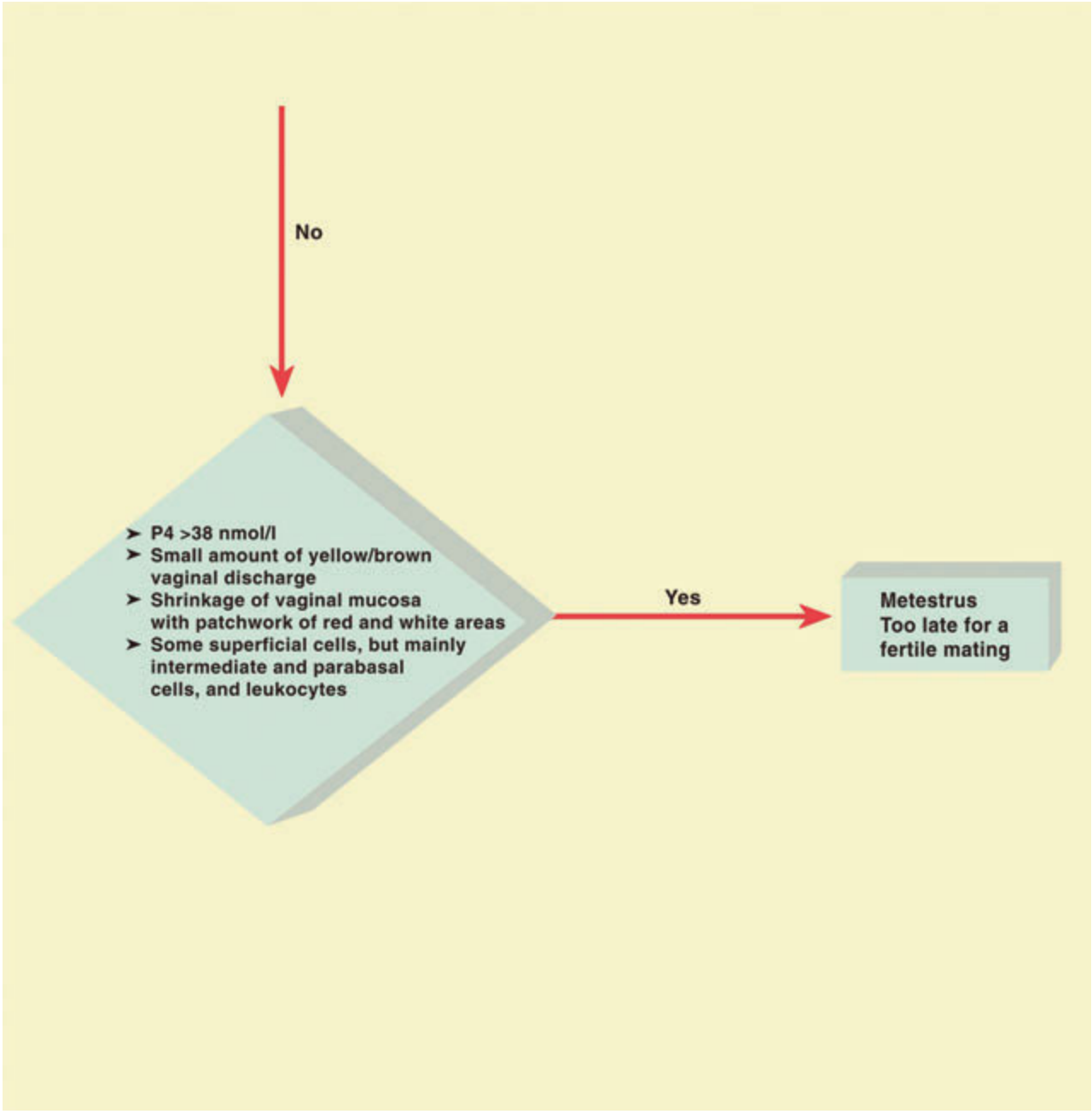

No
P4 >38 nmol/l
Small amount of yellow/brown vaginal discharge
Shrinkage of vaginal mucosa with patchwork of red and white areas
Some superficial cells, but mainly intermediate and parabasal cells, and leukocytes
Yes
Metestrus
Too late for a fertile mating

Figure 14.3d

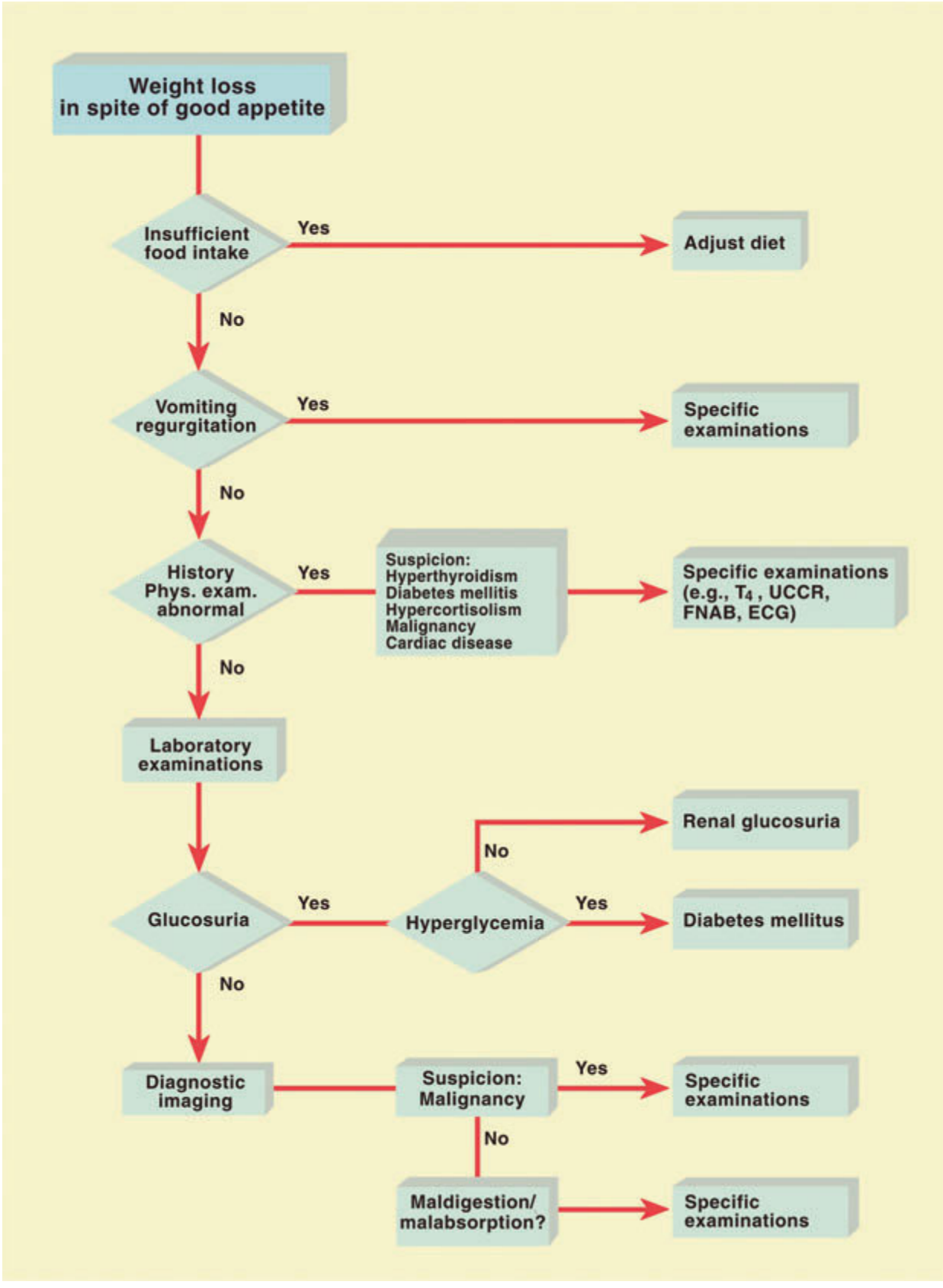

Weight loss in spite of good appetite
Insufficient food intake
Yes
Adjust diet
No
Vomiting regurgitation
Yes
Specific examinations
No
History Phys. exam. abnormal
Yes
Suspicion: Hyperthyroidism Diabetes mellitis Hypercortisolism Malignancy Cardiac disease
Specific examinations (e.g., T_4, UCCR, FNAB, ECG)
No
Laboratory examinations
Renal glucosuria
No
Glucosuria
Yes
Hyperglycemia
Yes
Diabetes mellitus
No
Diagnostic imaging
Suspicion: Malignancy
Yes
Specific examinations
No
Maldigestion/ malabsorption?
Specific examinations

Figure 14.4

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