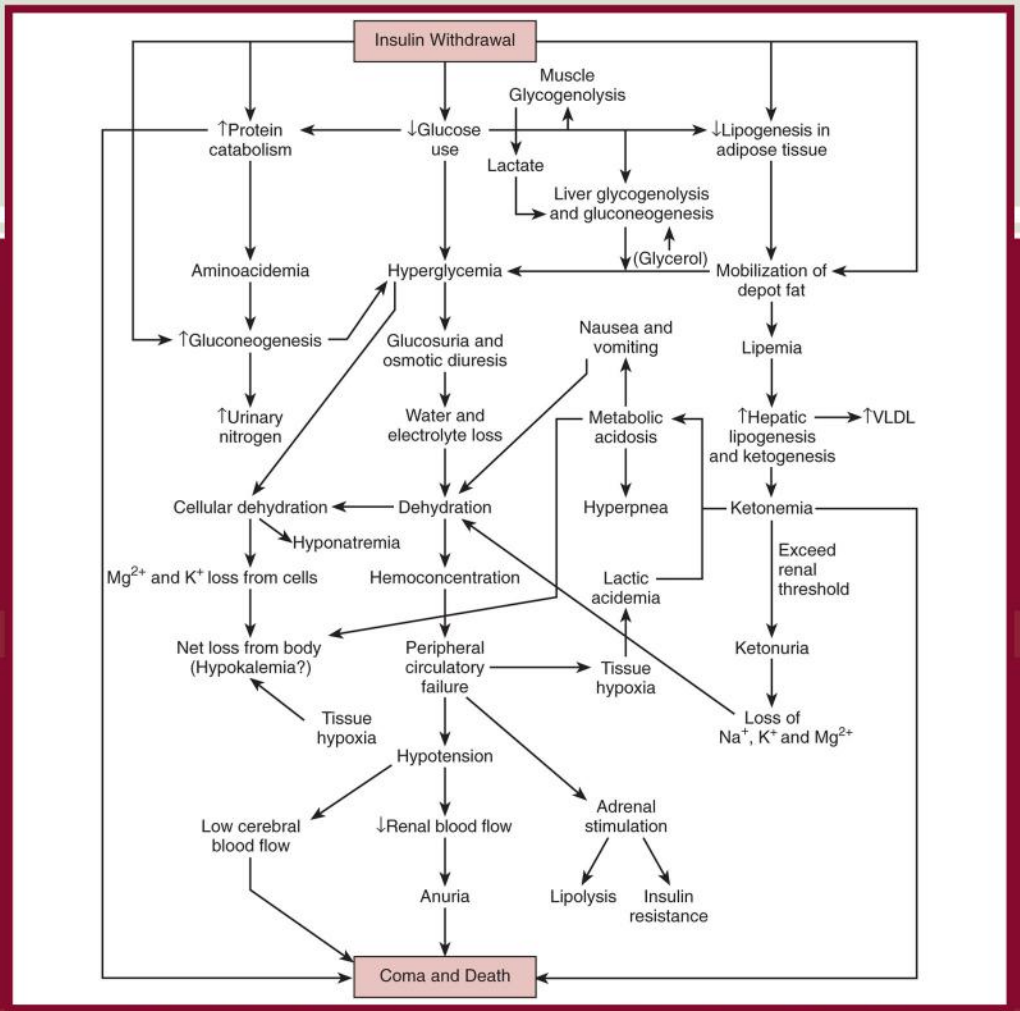
Quick Look Series IN VETERINARY MEDICINE

Metabolic and Endocrine Physiology

Third Edition



Larry R Engelking

Quick Look Series IN VETERINARY MEDICINE

METABOLIC AND ENDOCRINE PHYSIOLOGY

THIRD EDITION

Larry R Engelking, PhD
Professor of Physiology
Department of Biomedical Sciences
Cummings School of Veterinary Medicine
Tufts University



Jackson, Wyoming

Teton NewMedia
Teton NewMedia
90 East Simpson, Suite 110
Jackson, WY 83001

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Exclusive worldwide distribution by CRC Press an imprint of Taylor & Francis Group, an Informa business
Version Date: 20140205

International Standard Book Number-13: 978-1-4822-4121-1 (eBook - PDF)

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Preface to the First Edition

Metabolic and Endocrine Physiology, the first published volume in the QUICK LOOK SERIES *in Veterinary Medicine, has been written primarily for veterinary students who wish to organize their thinking in endocrinology, interns and residents preparing for their specialty board exams, animal science and graduate students in physiology, and practicing veterinarians who wish to update their general knowledge of endocrinology. Emphasis has been placed on instructional figures, flow diagrams, and tables, while text material has been held to a minimum. Over 200 multiple choice questions have been included to gauge the reader's capacity to effectively deal with the subject matter.*

This "quick look" is not intended to replace the excellent detailed manuscripts, reviews, and textbooks of endocrinology available, many of which were consulted during its preparation. Care has been taken to present relevant information in an up-to-date, accurate, and reliable fashion; however, all authors are fallible, this one being no exception, and if a reader detects errors, or if clarity of presentation can be improved, feedback would be genuinely appreciated.

Only those who have tried to encompass the vast science of veterinary endocrinology into one instructional review know how difficult is the problem of organizing the material, and how impossible is the achievement of a complete, concise, consistent, and logical sequence. In general, the endocrine system is first defined and described, and then each endocrine gland is discussed separately. Where appropriate, common endocrine disorders have also been included.

The study of metabolism and endocrinology is distinguished from other basic health science disciplines by its steadfast concern with "integrative" mechanisms that control and fine tune virtually all tissues and organ systems. In the healthy animal, physiologic variables such as blood volume and pressure, body temperature, ionic composition of the extracellular fluid compartment, general anabolism vs. catabolism, metabolic rate, the onset of reproductive cycles, and the blood glucose concentration must be controlled and maintained within narrow limits, even in the face of significant environmental challenges. A primary goal in the preparation of this text has been to concisely elucidate the endocrine mechanisms responsible for maintaining homeostatic control of those and other important physiologic variables, and to assist the reader in understanding common pathophysiologic deviations from normal. I hope you will find this "quick look" at metabolic and endocrine physiology to be informative, inspirational, challenging, and relevant to your educational needs.

Larry Rex Engelking

Preface to the Second Edition

Six years have passed since publication of the first edition. All chapters have been updated, and three have been added; two on ***Neonatal Physiology****, and one more on* ***Mineral Imbalances****.*

This "quick look" at metabolic and endocrine physiology was conceived and written to provide readers with a reasonably thorough, understandable, and current review of this complex discipline. Readers are encouraged to learn through examination of fundamental metabolic concepts to better understand how the endocrine system operates, and to use logic and integration of ideas to better understand common deviations from normal. Much of the science of endocrinology pertains to integrative control systems, and endocrine pathophysiology is most often related to control systems gone awry. Thus, the basic integrative endocrine control systems that operate in animals have been given primary consideration.

Attempts have been made to convey information in a concise, logically-sequenced, well-illustrated, and reliable fashion. However, if readers detect errors, or if clarity of presentation should be improved, constructive feedback would be appreciated. It is hoped that readers will again find this text to be practical, informative, and relevant to their scholastic needs.

Larry Rex Engelking

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Preface to the Third Edition

Six years have passed since publication of the second edition. All chapters have been updated, and the text has been reorganized into non-reproductive and reproductive endocrinology sections. Overviews at the head of each chapter have been replaced by learning objectives, which can be found with 368 new study questions in the back of the text. Six new chapters have been added, as well as an appendix.

Metabolic and Endocrine Physiology offers highly illustrative and succinct figures and tables to assist readers in understanding essential concepts. Attempts have been made to keep the writing concise, relevant, and up-to-date, but if clarity of presentation should be improved, constructive feedback would again be appreciated.

Larry Rex Engelking

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Acknowledgments

First Edition

I am grateful to Jim and Matt Harris for their support and encouragement during the planning and preparation of the first edition of this text. I also wish to thank Karen Feeney, Kevin Sullivan, and Jan Cocker for their editorial assistance, patience, and attention to detail during that time. Carroll Cann, Cindy Roantree, and Susan Hunsberger of Teton NewMedia were also instrumental in bringing the first edition to fruition.

Second Edition

I am indebted to members of the V'04 through V'09 classes of the Cummings School of Veterinary Medicine for their conscientious efforts in detecting minor errors and inconsistencies in the first edition, and for suggesting avenues for improvement. Dr. Elizabeth Allegretto, V'09, was particularly helpful with suggested improvements to chapters dealing with steroid biochemistry. During preparation of the second edition, Carroll Cann and Susan Hunsberger of Teton NewMedia were joined by Sue Haun and Mike Albiniak, who spent many hours reproducing, altering and adding to the figures and text. Thanks are extended to those professionals and for their expert assistance.

Third Edition

Members of the V'10 through V'14 classes of the Cummings School of Veterinary medicine have been helpful in suggesting improvements to the second edition, and I am grateful for their input. Additionally, John Spahr, Carroll Cann, Mike Albiniak and Sue Haun of Teton NewMedia spent many months working on this project, and I again appreciate their dedicated assistance.

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Quick Look Series IN VETERINARY MEDICINE

METABOLIC AND ENDOCRINE PHYSIOLOGY

THIRD EDITION

Ch 1 Endocrine System (Overview)

A

Chemical class	Hormone	Major source
Amino acid derivatives		
Biogenic amines	Dopamine (DA)	CNS
	Norepinephrine (NE)	CNS, ANS, adrenal medulla
	Epinephrine (Epi)	CNS, adrenal medulla
	Histamine (His)	CNS, mast cells, GI tract
	Serotonin (5-HT)	Pineal, blood platelets, GI tract
Iodothyronines	Thyroxine (T_4)	Thyroid, peripheral tissues
	Triiodothyronine (T_3)	Thyroid, liver, peripheral tissues
	Reverse T_3 (rT_3)	Thyroid, liver, peripheral tissues
Small peptides (< 50 amino acids)	Vasopressin (ADH)	Posterior pituitary
	Oxytocin (Oxy)	Posterior pituitary
	Melanocyte-stimulating hormone (MSH)	Pars intermedia, anterior pituitary
	Thyrotropin-releasing hormone (TRH)	Hypothalamus, CNS
	Gonadotropin-releasing hormone (GnRH or LHRH)	Hypothalamus, CNS
	Somatocrinin (GHRH)	Hypothalamus, CNS
	Somatostatin (SS, GHIH or SRIH)	Hypothalamus, CNS, stomach, pancreas, intestine
	Melanocyte-releasing hormone (MRH)	Hypothalamus, CNS
	Melanocyte release-inhibiting hormone (MRIH)	Hypothalamus, CNS
	Adrenocorticotropic hormone (ACTH)	Anterior pituitary, pars intermedia
	ACTH -releasing hormone (CRH)	Hypothalamus, CNS
	Angiotensins (A-II, A-III)	Plasma, CNS
	Opioid peptides (e.g., Enk, Endor)	CNS, other tissues
	Secretin	GI tract, CNS
	Cholecystokinin (CCK)	GI tract, CNS
	Gastrin (G)	GI tract, CNS, pancreas
	Gastric inhibitory polypeptide (GIP)	GI tract
	Vasoactive intestinal polypeptide (VIP)	GI tract, other tissues
	Glucagon	GI tract, pancreas
	Glucagon-like peptide (GLP)	GI tract
	Ghrelin	GI tract
	Obestatin	GI tract
	Gastrin-releasing peptide (GRP)	GI tract, CNS
	Motilin	GI tract, CNS
	Neurotensin	GI tract, CNS
	Substance P	GI tract, CNS
	Guanylin	GI tract, CNS
	Pancreatic polypeptide (PP)	GI tract, pancreas, CNS
	Atrial natriuretic peptide (ANP)	Heart
	Brain natriuretic peptide (BNP)	CNS, Heart
	CNS natriuretic peptide (CNP)	CNS, Vasculature
	Urodilatin	Kidney
Proteins (> 50 amino acids)	Calcitonin (TCT or CT)	Thyroid, other tissues
	Insulin	Pancreas
	Growth hormone (GH or somatotropin)	Anterior pituitary
	Thyroid-stimulating hormone (TSH)	Anterior pituitary
	Prolactin (PRL)	Anterior pituitary
	Follicle-stimulating hormone (FSH)	Anterior pituitary
	Luteinizing hormone (LH or ICSH)	Anterior pituitary
	Parathyroid hormone (PTH)	Parathyroids
	Erythropoietin (EPO)	Kidney
	Placental lactogen (PL)	Placenta
	Chorionic gonadotropin (CG)	Placenta
	Inhibin (I)	Gonads
	Activin	Gonads
	Relaxin	Gonads
	Somatomedins (e.g., IGF-1)	Liver
	Leptin	Adipocytes

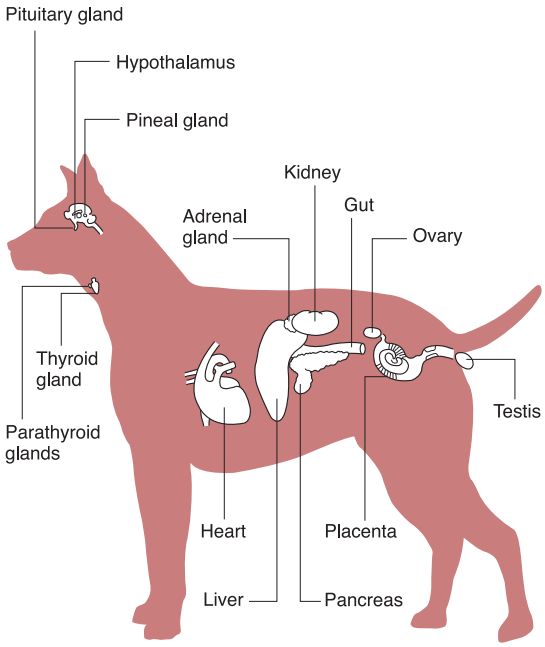
A *(continued)*

Chemical class	Hormone	Major source
Steroids	Progesterone (Prog)	Corpus luteum, placenta, testes, adrenal cortex
	Testosterone (T)	Testes, ovaries, adrenal cortex, placenta
	Estrogens (E_1, E_2, E_3)	Ovaries, adrenal cortex, testes, placenta
	Dihydrotestosterone (DHT)	Testosterone-sensitive tissues
	Glucocorticoids (e.g., cortisol)	Adrenal cortex
	Mineralocorticoids (e.g., aldosterone)	Adrenal cortex
	Ercalcitriol and Calcitriol ($(1,25(OH)_2\ D_2$ and $1,25(OH)_2\ D_3$)	Skin, liver, kidney
Fatty acid derivatives (Eicosanoids)	Prostaglandins (PGs)	Most tissues
	Leukotrienes (LTs)	White blood cells
	Thromboxanes (TXs)	Platelets, placenta

LHRH = leuteinizing hormone–releasing hormone; GHRH = GH-releasing hormone; GHIH = GH-release–inhibiting hormone; SRIH = somatotropin release–inhibiting hormone; ENK = enkephalin; Endor = endorphin; ICSH = interstitial cell–stimulating hormone; IGF-1 = insulin-like growth factor 1.

The Endocrine Organs Assist in Maintaining Homeostasis

B



"The coordinated physiological processes which maintain most of the steady states in the organism are so complex and so peculiar to living beings that I have suggested a special designation for these states, **homeostasis.** The word does not imply something immobile, a stagnation; it means a condition which may vary, but which is relatively constant."
(Quote from Cannon WB: The wisdom of the body. New York, NY: WW Norton, 1932)

The endocrine system can be described using two words of Greek origin: **endocrine** and **hormone.** The term **endocrine** means **"to separate within,"** which refers to wide separation of endocrine tissues throughout the body, while the term **hormone** means **"I excite."**

Nonreproductive veterinary endocrinology has been based largely on studies in dogs, however, the increasing popularity of cats and other companion animals has resulted in studies that have increased awareness of their endocrine disorders as well. These disorders appear to be involved in about **10%** to **20%** of reported medical diseases in dogs, and less than **5%** in cats. **Reproductive veterinary endocrinology,** on the other hand, has largely been based on studies in large animal species.

Hormones are synthesized in a variety of cell types and are secreted and transported to various target tissues, where they affect diverse metabolic functions by regulating rates of specific reactions without themselves contributing energy or initiating the process. Many hormones (e.g., insulin, adrenocorticotropic hormone, somatostatin, or their structurally similar progenitors) that were originally thought to have developed with complex multicellular and multitissue higher animals, have been found in single cell organisms, indicating an early role in intercellular communication (Ch. 72).

Because many hormones are secreted into blood prior to use (**endocrine action**), circulating levels can give some indication of endocrine gland activity and target organ exposure. Because of the small amounts of hormones required, blood levels can be extremely low. For example, circulating levels of **protein hormones** normally range from **10^{-10}** to **10^{-12} Mol,** and circulating levels of **thyroid** and **steroid hormones** normally range from **10^{-6}** to **10^{-9} Mol.** Hormones include **proteins** and **glycoproteins** (often with molecular weights of 30 kd or less), **smaller polypeptides** (<50 amino acids), **amino acid derivatives** (the biogenic amines and iodothyronines), and **steroids.** Sometimes **fatty acid derivatives** (the **eicosanoids:** prostaglandins, leukotrienes, and thromboxanes) are also classified as hormones (**Part A**).

Endocrine Glands

The **central nervous system (CNS)** is a major component of the endocrine system. Many hormones, particularly small polypeptides such as those found in the gut, are also important **CNS neurotransmitters** (Chs. 47-51). Large amounts of these compounds and small amounts of insulin and adrenocorticotropic hormone (ACTH), as well as their respective receptors, have been found in the brain. There they appear to exhibit broad, although ill-defined actions on pain sensitivity, as well as sexual, feeding, and other behavioral phenomena. Also, the synthesis, secretion, and action of neurotransmitters involve processes similar to those of hormones. Thus, the distinction between a hormone and a neurotransmitter is becoming increasingly difficult to make. Specialized nerve cells in a part of the brain known as the **hypothalamus** synthesize hormones that are either stored in the **posterior pituitary** (e.g., oxytocin and antidiuretic hormone), or transported by portal blood to the **anterior pituitary** (immediately below the hypothalamus; Ch. 7). Through its ability to release additional hormones from the pituitary, the hypothalamus can control salt and water balance, reproductive function, growth, skin darkening, lactation, and the body's response to stress. The **pineal gland** is a part of the CNS, and has been viewed historically as either the "seat of the soul", or "a third eye" (Chs. 60 and 61). This gland produces **melatonin,** a biogenic amine that is involved with, among other things, photoperiodic regulation of reproductive events in seasonal breeders.

The **thyroid gland** produces iodothyronines (from iodine and the amino acid, tyrosine), namely **tetraiodothyronine** (**thyroxine, T_4**), **triiodothyronine** (**T_3**), and **reverse T_3** (**rT_3**) (Chs. 36-38). Triiodothyronine is the active metabolite of **T_4** that increases oxygen utilization, and therefore the basal metabolic rate of many tissues. Parafollicular cells of the thyroid produce **calcitonin** (**TCT** or **CT**), a protein hormone involved with calcium homeostasis (Chs. 16 and 17).

The **parathyroid glands,** which are embedded in the thyroid just in front of the trachea (and behind the larynx), produce a protein hormone known as **parathyroid hormone (parathormone or PTH),** which plays an important role in maintaining optimal blood levels of calcium and phosphate (Chs. 16 and 17).

The **adrenal glands** are situated immediately above the kidneys, and are composed of an **outer cortex** and an **inner medulla.** The cortex produces **corticosteroids** (e.g., cortisol, aldosterone, and small amounts of the sex steroids), and the medulla produces **catecholamines** (e.g., epinephrine and norepinephrine). Cortisol is a **glucocorticoid** involved in glucose homeostasis, while aldosterone is a **mineralocorticoid** involved with electrolyte balance. The **catecholamines** help to produce the "fight or flight" response (Chs. 32-35).

The **gastrointestinal tract** is the largest endocrine organ system of the body, producing several **neurocrine, paracrine,** and **endocrine mediators.** Those such as cholecystokinin (CCK), secretin, and gastrin primarily regulate gastrointestinal physiology (i.e., motility, secretion, and digestive action). However, others may be involved with the release of hormones such as insulin (e.g., gastric inhibitory polypeptide, GIP), and therefore become involved with energy balance (Chs. 47-51).

The **endocrine pancreas** (which lies adjacent to the stomach) consists of islet tissue scattered throughout the larger exocrine portion of the gland. The endocrine pancreas produces insulin, glucagon, and somatostatin, and it is integrally involved with carbohydrate, protein, and lipid metabolism (Chs. 40-46).

The **kidney** is a regulatory and excretory organ, filtering and secreting waste products and drugs from the circulation into urine. It also produces hormones involved in the control of blood pressure (renin and urodilatin), erythropoiesis (erythropoietin), and calcium/phosphate homeostasis (hydroxylation of vitamin D) (Chs. 27-31).

The **gonads** (ovaries and testes) produce the sex steroids (e.g., progesterone, testosterone, and estrogen), as well as several protein hormones also involved with reproductive function (e.g., relaxin, inhibin, and activin; Chs. 52-59).

The **placenta,** a primary organ of pregnancy serving the fetus, produces several hormones, many of which are also produced by other glands (Ch. 62). Two hormones produced only by the placenta are **placental lactogen (PL)** and **chorionic gonadotropin (CG).**

Part B is a diagrammatic representation of primary hormone-secreting tissues.

The action of a hormone at its target site is dependent upon nine general factors:

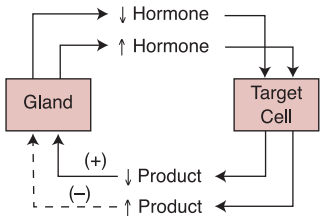
1. The rate of synthesis and/or secretion of the hormone from the endocrine gland of origin.
2. Specific (liver-derived) transport proteins in plasma (steroid and thyroid hormones).
3. Hormone concentrations in blood, interstitial fluids, and/or the intracellular environment.
4. Conversion to a more or less active form in target tissues (e.g., T_4 to T_3 or rT_3).
5. Duration of and intervals between hormone exposures.
6. The number and/or activity of hormone-specific receptors on or in respective target cells.
7. Intracellular activities/concentrations of enzymes, cofactors and substrates affected by the hormone.
8. Concurrent effects of antagonistic or synergistic hormones.
9. Degradation, conjugation, and/or excretion of the hormone (largely by the liver and kidneys).

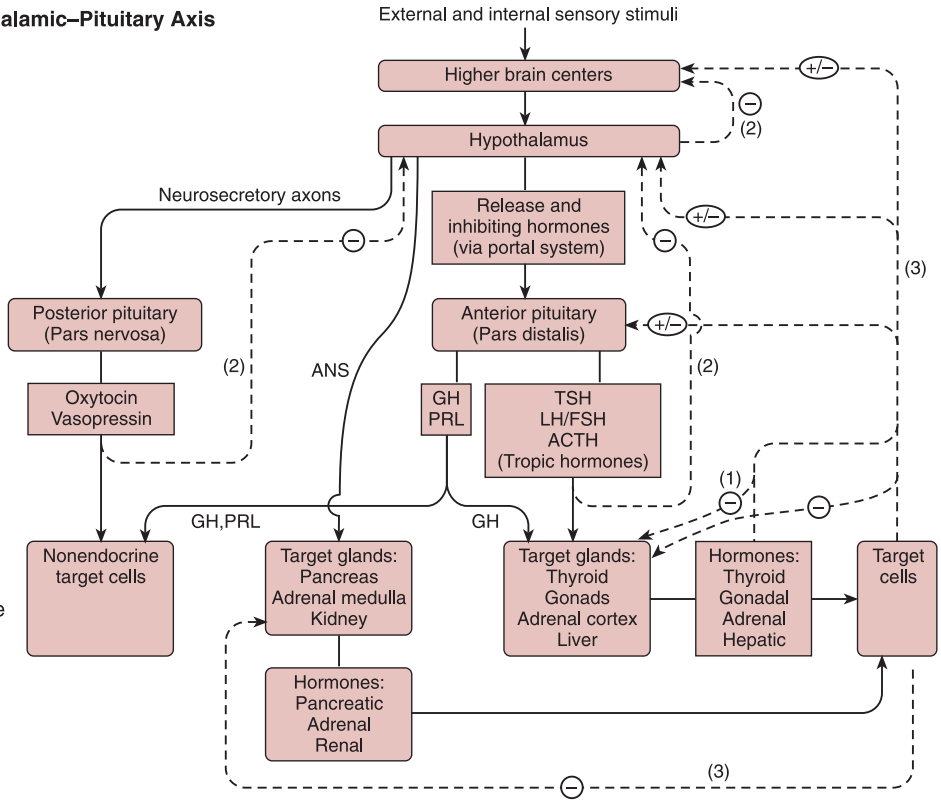
The exquisiteness with which hormonal effects can be modulated becomes important in achieving a primary objective of endocrine regulation: **metabolic stability.**

Ch2 Endocrine Secretory Control

(Autocrine, Paracrine, and Endocrine Mediators)

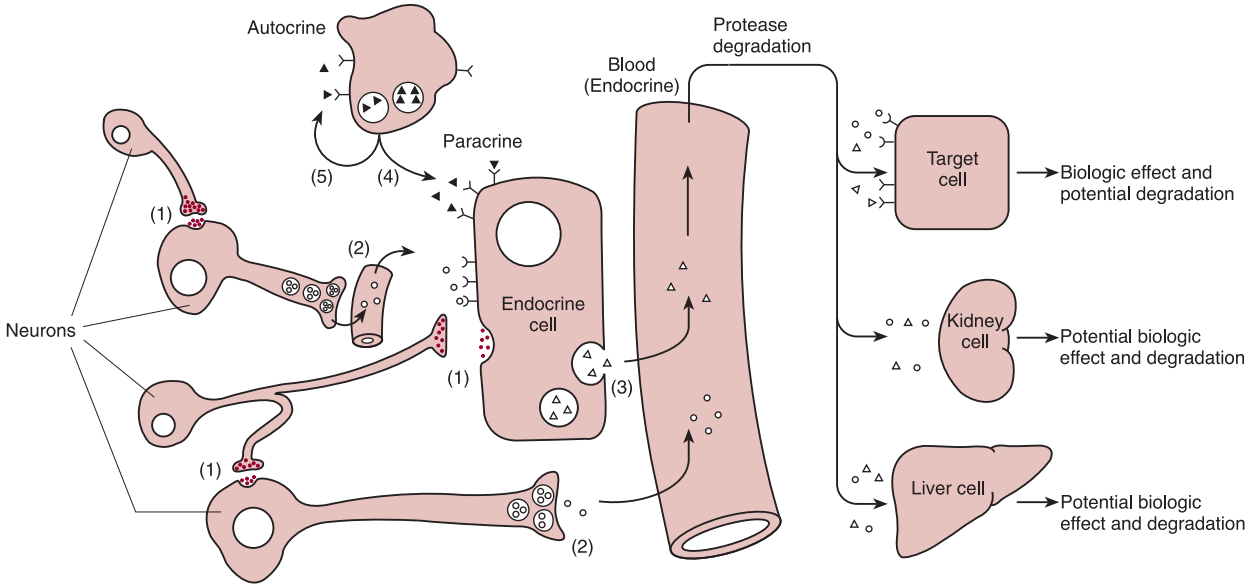
A Feedback Control of the Hypothalamic–Pituitary Axis





The hypothalamic-pituitary axis is controlled through direct and indirect feedback from many sources, including the axis itself (short-loop feedback), higher brain centers, and both endocrine and nonendocrine products from several target tissues (long-loop feedback). The autonomic nervous system (ANS) augments functional expression of the endocrine components of this axis.

B Variations in Chemical Communication Within an Animal



Source: Part B modified from Norris DO: Vertebrate endocrinology. 3rd ed., San Diego, CA: Academic Press, 1997:4.

Chemical regulatory mechanisms are the bases for controlling all physiology, and it is through these mechanisms that homeostatic balance and survival occurs. A simple hormonal regulatory mechanism involves receptor identification of the hormone, then adjustment of the target cell to a preprogrammed set point. This cell sends a message to the same or a different receptor indicating that an appropriate response has been instituted (i.e., **negative feedback**). **Positive feedback** is sometimes invoked when rapid change or short-term adaptation is required to drive a system to a higher level than the preprogrammed set point. Long term positive feedback is generally detrimental, and can lead to death. Long-term negative feedback is generally advantageous to survival.

There are about 200 types of differentiated cells in animals; only a few produce hormones, but virtually all of the 35 trillion canine cells are targets for one or more of the over 50 known hormones. We know that a given hormone can affect several different cell types; that more than one hormone can affect a given cell type; and that hormones can exert many different effects in one cell, or in different cells. A **target cell** is any cell in which a hormone (ligand) binds to its receptor, whether or not a biochemical/physiologic response has been determined from that binding.

Feedback Control

It is characteristic of the endocrine system that a balanced state of feedback regulation be maintained among the various glands in order to maintain **homeostasis**. This is particularly notable with respect to **release** and **inhibiting hormones** (or factors) from the hypothalamus, which regulate synthesis and secretion of anterior pituitary hormones. **Tropic** (**or trophic**) **anterior pituitary hormones,** in turn, promote hormone secretion from various target endocrine glands. Characteristically, elevated hormone concentrations result in both **direct** and **indirect feedback control** of their production by the originating gland (autocrine control), anterior pituitary, hypothalamus, and higher brain centers. **Part A** depicts control via **ultra-short-loop** negative feedback (process **1** in **Part A**), **short-loop** feedback (process **2** in **Part A**), and **long-loop** feedback (process **3** in **Part A**). Target tissues may also produce nonendocrine products that either inhibit or stimulate further endocrine secretion, and sensory input (i.e., sight, sound, touch, taste, and smell) may also stimulate or inhibit hypothalamic activity, and thus endocrine secretion (**Part A**). The **autonomic nervous system** (**ANS**), also controlled by the **hypothalamus**, influences certain endocrine gland secretions as well (e.g., pancreatic insulin and glucagon release, adrenal medullary catecholamine release, and renal renin release).

Categories of Chemical Regulators

Both classic endocrine and nerve cells synthesize and release chemical messengers. These messengers may then act on the same cell in which they are produced (**autocrine**) (process **5** in **Part B**), on other target cells in their vicinity without entering the circulation (**paracrine**) (process **4** in **Part B**), or they may go to distant target cells through the circulation (**endocrine**) (process **3** in **Part B**). Nerve cells produce neurotransmitters that are released at nerve terminals. These neurotransmitters can be released into blood to act as endocrine agents (**neurocrine** or **neuroendocrine**) (process **2** in **Part B**), or they can be released to act directly on a target cell in a **paracrine** fashion (e.g., another nerve, muscle, or endocrine cell) (process **1** in **Part B**).

Prostaglandins sometimes act in an autocrine fashion, and somatostatin acts in the stomach and endocrine pancreas as a paracrine agent (Chs. 43, 47, and 48). Entrance of insulin into the circulation in search of its distant target cells (e.g., muscle and adipose tissue) is an example of endocrine secretion.

Exocrine glands, on the other hand, secrete their products into **ducts** through which they are conveyed to their sites of action in such places as the digestive tract or the body's surface. Exocrine secretions include saliva, sweat, milk, urine, pancreatic and biliary secretions. **Pheromones** are specialized chemical agents secreted through exocrine glands to the body surface for interorganismal communication, namely to other members of the same species.

Evolutionary Considerations

Hormones, hormone precursors, and several agents that mediate or modulate hormone action (e.g., receptors, second messengers, prostaglandins, etc.) have been found in bacteria, worms, insects, and plants. *E. coli.*, for example, contain a molecule that cross-reacts with anti-insulin antibodies, and stimulates glucose oxidation in isolated fat cells. Its function in *E. coli* remains unknown. Most chemical regulators, such as biogenic amines, small peptides or proteins, are at home in aqueous media. Other chemical regulators like steroids, thyroid hormones and eicosanoids have low aqueous solubility, and, unlike peptides, readily pass through cell membranes. Chemical regulation of the internal environment is thought to have evolved from **paracrine-** and **autocrine-type** secretions as seen in primitive multicellular organisms (Ch. 72). As more sophisticated cardiovascular and nervous systems evolved, the same primitive messengers continued to appear in **endocrine**, **neural**, and **neuroendocrine** secretions. There is considerable structural conservation of chemical messengers across animal species. There are profound differences, however, in target organ responses to those messengers. For example, **prolactin** causes milk secretion in mammals, yet in fish and amphibians it is involved with water balance. It is generally believed that the functional adaptation of hormones evolved to fit the nutritional uniqueness of each animal species. Although chemical messengers and physiologic functions may not have changed throughout evolution, the means of fulfilling those functions did.

Higher animals (i.e., mammalian vertebrates) have well-developed **nervous systems** for **immediate** physiologic responses. Although their **endocrine systems** are **slower** acting, the physiologic responses they provoke generally last longer. Mammalian vertebrates also have well-integrated neural and endocrine regulatory systems, and in many cases these two systems are difficult to separate (e.g., the adrenal medulla, hypothalamic nuclei, the posterior pituitary, and neurocrine regulation of gut function). In contrast, invertebrates are more dependent on paracrine and autocrine regulation because they possess primitive nervous systems.

Endocrine Disrupters

Comparative endocrinologists have been concerned with possible disrupters of endocrine functions from natural ecosystems that might affect animal populations adversely. These **endocrine disrupters** may be chemicals produced by human activities (anthropogenic), that mimic natural chemical regulators or prevent their actions. They might induce metabolic events at the wrong time, or prevent a critical event from occurring on schedule. For example, the pesticide **dichlorodiphenyltrichloroethane** (**DDT**) has estrogenic effects in vertebrates, whereas one of its metabolites, **dichlorodiphenyldichloroethylene** (**DDE**) acts as an antiandrogen. **Phytoestrogens** are estrogenic compounds produced by certain plants, and their consumption has been shown to disrupt reproduction and other metabolic processes in herbivores. Wastes from wood pulp mills may also be disruptive of reproductive process in fishes. One of the actions of **polychlorinated biphenyls** (**PCBs**) is to block the synthesis of serotonin, thereby altering gonadal function through effects at the pineal, hypothalamic, or pituitary level (Chs. 56, 60, and 68). The PCBs may also compete with thyroid hormones for their receptors, induce hypothyroidism, and prevent normal development of the nervous system. The **cadmium ion,** a common aquatic pollutant, is a potential inorganic disrupter known to enhance release of reproductive hormones. Significant reductions in human sperm counts worldwide over the last three generations, and a marked increase in testicular cancer, are considered by some to be consequences of endocrine disrupters. Some researchers also propose a link that may exist between endocrine disrupters and the worldwide decline we are observing among amphibian populations.

Degradation and Elimination of Hormones

Metabolism of hormones and their precursors is utilized to generate more or less active forms from precursors, and to degrade hormones to inactive forms. Most hormone elimination occurs through degradation. Some metabolites of degraded hormones are biologically active, others inactive. Degradation of hormones may be carried out by **serum proteases**, by **peripheral target cells**, or more frequently by the **liver** or **kidneys** (**Part B**). Some hormones are eliminated unchanged into bile or urine.

There are also differences in a hormone's degradation and elimination in different animal species. Glucocorticoids, for example, are usually degraded by the liver to inactive metabolites that can eventually be eliminated in urine; however, dogs reduce these metabolites to a greater extent than do primates, and cats are thought to eliminate glucocorticoids primarily in bile.

Ch3 Hormone Disposition, Measurement and Secretion

(Production & Distribution Volume, Clearance & Immunoassay)

A

Plasma Clearance (CL_p) = (mg/min Removed) / (mg/ml Plasma) = ml/min

$CL_{systemic}$ = CL_{renal} + $CL_{hepatic}$ + CL_{other} (e.g., saliva, sweat, gut or metabolism at other sites)

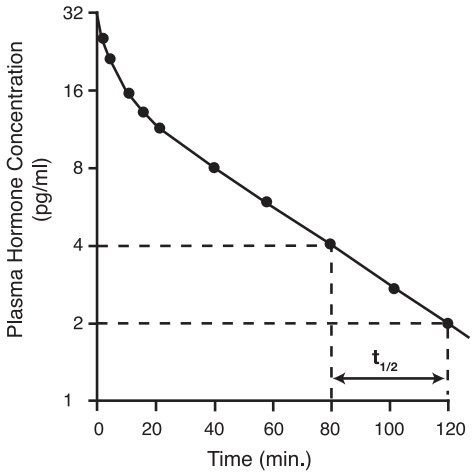
Production Rate (PR) = Plasma Conc. (P_c; mg/ml) x CL_p (ml/min) = mg/min

Excretion Rate (ER) = Urine and/or Bile Flow (ml/min) x H. Conc. (mg/ml) = mg/min

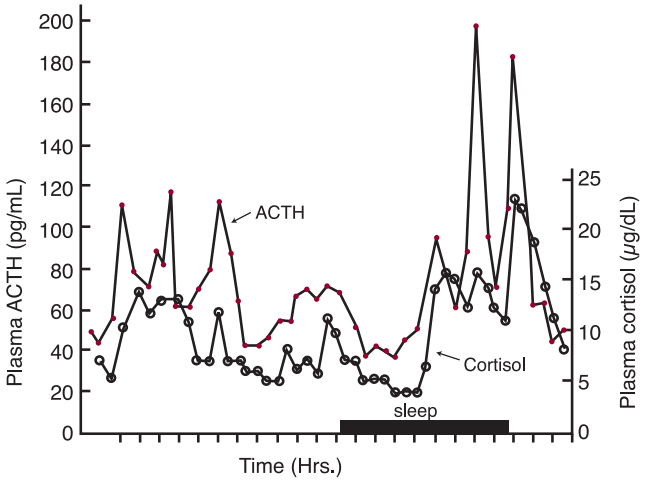
At Steady State, PR = ER

Dist. Vol. (V_d) = Amt. of H. in Body (mg) / Conc. in Blood or Plasma (mg/ml) = ml

Plasma Half-Life ($t_{1/2}$) = 0.693 x V_d / CL_p = min



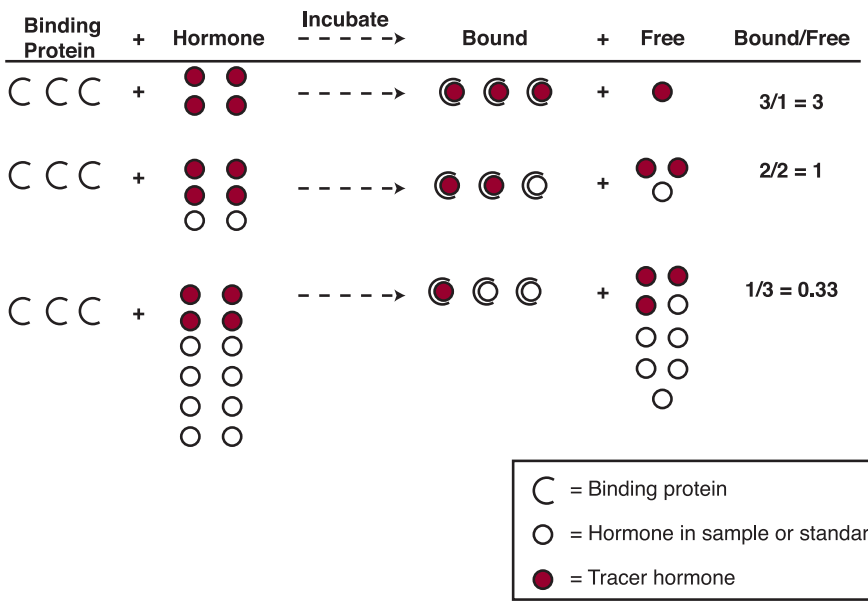
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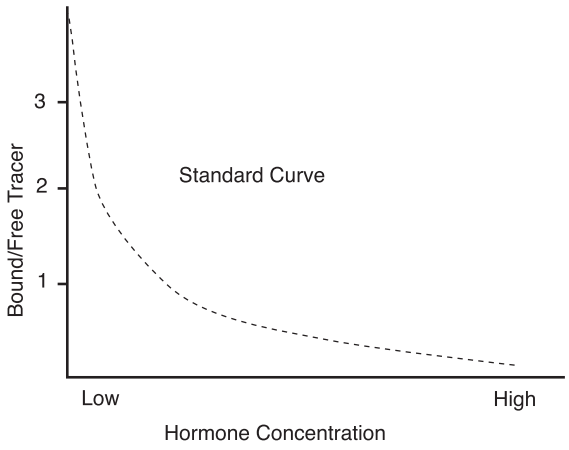


ACTH is secreted in irregular bursts throughout the day, and plasma cortisol tends to rise and fall in response to those bursts. Additionally, a diurnal (circadian) rhythm, controlled by the suprachiasmatic nucleus of the hypothalamus (Ch. 60), appears to help control ACTH and cortisol release, allowing secretion to increase during sleep and the early morning hours (Ch. 22).

B

Principle of Radioimmunoassay





Sources: Part A modified from Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Goodman Gilman A [ed]: Goodman & Gilman's pharmacological basis of therapeutics. 9th ed, New York, NY: McGraw-Hill, 1996:21. **Part B** modified from Berne RM, Levy MN: Physiology. 3rd ed, St Louis, MO: Mosby, 1993:829. **Part C** modified from Ganong WF: Review of medical physiology, 22nd ed, New York, NY: McGraw-Hill Medical, 2005:373.

Disposition

Net irreversible removal of a **hormone** (**H.**) from plasma is generally the result of target cell uptake and degradation, metabolism by the kidneys and/or liver, and urinary or biliary excretion (Ch. 2). The disappearance of a hormone from plasma is referred to as **plasma clearance** (CL_p) (i.e., ***the volume of plasma irreversibly removed of a given substance (e.g., hormone) per unit time***). More specifically, this is equivalent to the mass removed/unit time divided by the circulating mass/unit volume (**Part A**), and it carries the units **ml/min** (not mg/min). Plasma clearance is a useful pharmacokinetic concept because it is reasonably constant over the range of concentrations clinically encountered in healthy animals, and it can be normalized between animals of varying size by dividing through by the body weight (**kg**). Systems for hormone or drug elimination are not usually saturated and, thus, the absolute rate of elimination is a linear function of its plasma concentration. Elimination of hormones and drugs from the body usually conforms to first-order kinetic principles. Clearance by means of multiple organs of elimination is additive, and these separate clearances will equal total **systemic clearance** ($CL_{systemic}$; **Part A**).

The amount of hormone eliminated from the body in bile and/or urine (i.e., the **excretion rate** (**ER**; **mg/min**)), is equal to the bile and/or urine flow rate (**ml/min**) times the hormone concentration (**mg/ml**) in that fluid. At **steady state** (**SS**), ***it must equal out***, therefore **production rate** (**PR**) and/or **secretion rate** will equal **ER** (i.e., total body elimination).

Volume is another fundamental parameter that is useful in assessing processes of hormone disposition. The **distribution volume** (V_d) relates the amount of hormone in the body to its concentration (**C**) in blood or plasma, depending on the fluid being measured (**Part A**). This volume does not necessarily refer to an indentifiable physiologic volume, but merely to the fluid volume that would be required to contain all of the hormone in the body at the same concentration as that in blood or plasma. V_d may vary widely depending on the degree of plasma protein binding, the partition coefficient of the hormone in fat and the degree of binding to other tissues. V_d for a given hormone can change as a function of a patient's age, species, physiologic or pathophysiologic state, and body composition.

The **plasma half-life** ($t_{1/2}$) of a hormone or drug is the time it takes for the plasma concentration (or the amount of substance in the body) to be reduced by **50%**. The $t_{1/2}$ computation includes the rate constant **0.693**, which is used for first-order process elimination from a single compartment (e.g., plasma). As indicated in the **Part A** figure, plasma concentrations of an administered hormone (or drug) often follow a multiexponential (i.e., curvalinear) pattern of decline, therefore, two or more half-life terms may be calculated.

Early studies of pharmacokinetic properties of hormones and drugs in disease states were compromised by their reliance on **plasma $t_{1/2}$** as a sole measure of alterations in disposition. In recent years it has been appreciated that $t_{1/2}$ is a derived parameter that changes as a function of both CL_p and V_d (**Part A**). Although CL_p is a useful measure of the body's ability to eliminate a hormone (or drug), the organs of elimination can only clear those substances from the blood or plasma with which they are in direct contact. As CL_p **decreases**, due to disease processes, for example, $t_{1/2}$ would be expected to **increase**. However, this reciprocal relationship is met only when the disease or physiologic state does not change V_d. Changes in protein binding of a hormone affect CL_p as well as V_d, leading to unpredictable changes in $t_{1/2}$ as a function of physiologic state (e.g., pregnancy), age, or disease. Hepatic disease can decrease the plasma protein concentration, and plasma hormone-protein binding, which might increase CL_p and decrease the $t_{1/2}$ of certain protein-bound hormones because higher concentrations of the free hormones would be present and available for degradation and elimination. This might be true if V_d remained unaltered. However, in other instances CL_p might decrease in liver disease and $t_{1/2}$ increase if the hormone is primarily metabolized, conjugated and/or eliminated by hepatobiliary processes (e.g., many steroid hormones).

Measurement

Immunoassay is the most common means of assessing hormone concentration. Monoclonal antibodies, produced in animals, react with small peptide, protein, steroid or thyroid hormone molecules, and they can react with these substances at concentrations in the picomolar range. They are commonly obtained by injecting the antigen (i.e., hormone to be measured) into a mouse or rat, or by incubating it with cells *in vitro*. The animal spleen or cells incubated *in vitro* are immortalized by fusing them to myeloma cells or transforming them with tumor viruses, which produce a number of clones of antibody-producing cells. These clones are then screened with the hormone antigen until a suitable antibody-producing clone is found. Although radiolabeled hormones (usually ^{131}I) have been traditionally employed, linking the antigen to an enzyme, fluorescent label, chemiluminescent label, or latex particle that can be aggultinated with the antigen, are alternative immunoassay techniques in wide use.

The principle of **radioimmunoassay** is dipicted in **Part B**. The sample, which may be plasma, urine, CSF or a tissue extract, is incubated with a fixed amount of radioactively labeled hormone (the tracer), and a fixed amount of a specific hormone-binding protein, most often an antibody. Nonradioactive hormone molecules compete with tracer molecules for binding sites on the protein, and the concentration of binding sites is fixed and limiting. Therefore, progressive increases in the number of nonradioactive hormone molecules in the sample will displace more and more tracer molecules from binding sites. At the end of incubation bound tracer hormone molecules are separated from those that are free. Radioactivity in individual bound and free tracer fractions is counted, and if hormone concentrations in the samples are high, the percentage of radioactivity remaining in the bound fractions will be low, and vice versa. The absolute amount of hormone in the sample is determined through comparison with a standard curve generated by incubating varying amounts of known hormone with tracer and binding protein. A typical standard curve is exponential (**Part B**), however it can be rendered linear through reciprocal or logarithmic transformation of the data.

Immunoassays may not always distinguish completely or sufficiently between similar hormones secreted by the same gland (e.g., two adrenal steroid hormones), between a peptide hormone and its prohormone, or between a hormone and its metabolic products. This holds potential for experimental or clinical misinterpretations unless the specificity of each assay is carefully documented.

Nonimmunologic assays include chemical assays, which take advantage of chemically reactive groups in the molecule; bioassays, which assess the activity of the hormone incubated with cells or tissues *in vitro* or injected into an animal; and receptor-binding and other assays, which exploit the high affinity of the hormone for receptors or other molecules such as plasma-binding proteins. These assays, however, are infrequently employed since **immunoassays** have far greater **sensitivity** and **specificity**.

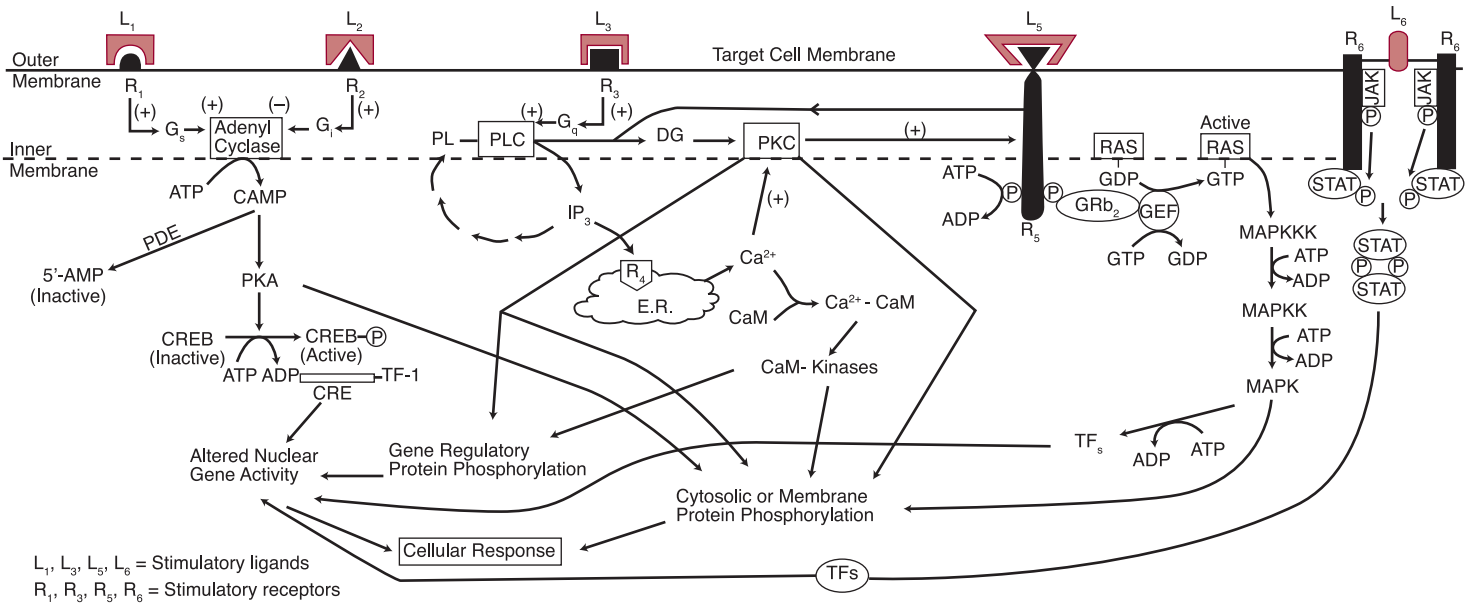
Hormone Secretion

Quantitation of hormone output by an endocrine gland can only be accomplished accurately *in vivo* through catheterization of blood supply to and from that gland. Urinary and/or biliary excretion are cumbersome because they require bladder and/or common bile duct catheterization. However, a simple plasma concentration can provide a useful index of hormone **PR** when CL_p is within normal limits, and can be taken as a constant (i.e., since $PR = P_c \times CL_p$, **PR** is proportional to P_c if CL_p is constant; **Part A**). This is the theoretical basis for employing plasma hormone measurements alone as an index of endocrine gland activity. Hormone release is typically characterized by **episodic bursts**, and in some cases **diurnal variation** (**Part C**). It is hazardous to conclude too much from a single plasma value. Multiple measurements taken at different times of the day are usually needed. To reduce the number of laboratory analyses, however, multiple samples of equal volume can be pooled, with a single careful measurement of this pool yielding the average plasma concentration over time.

Ch4 Mechanisms of Catecholamine and Polypeptide Hormone Action: I

(Receptors and Second Messengers)

A



L_1, L_3, L_5, L_6 = Stimulatory ligands
R_1, R_3, R_5, R_6 = Stimulatory receptors
L_2 = Inhibitory ligand
R_2 = Inhibitory receptor
R_4 = IP_3 Stimulatory receptor
R_5 = Receptor Tyrosine Kinase
R_6 = Receptor JAK Kinase
E.R. = Endoplasmic Reticulum
GRB_2 = Ras activator controller
GEF = Ras guanine exchange factor

B

Ligands Working through the Four Primary Intracellular Messenger Pathways

L_1 (G_s (↑cAMP))
β-Adrenergics
Secretin
Glucagon
Calcitonin (CT)
Follicle-stimulating hormone (FSH)
Luteinizing hormone (LH)
Chorionic gonadotropin (CG)
Melanocyte-stimulating hormone (MSH)
Parathormone (PTH)
Thyroid-stimulating hormone (TSH)
TSH-releasing hormone (TRH)
Antidiuretic hormone (ADH on V_2 receptors)
Adrenocorticotropic hormone (ACTH)
ACTH-releasing Hormone (CRH)
Histamine (H_2 receptors)
Others

L_2 (G_i(↓cAMP))
α_2-Adrenergics
Prostaglandins (PGs)
Acetylcholine (ACh on M_2 receptors)
Opiate-like peptides
Angiotensin II
Somatostatin (GHIH or SS)
Others

L_3 (G_q (Ca^{2+}/DG))
Vasopressin (ADH on V_{1A} & V_{1B} receptors)
Acetylcholine (ACh on M_1, M_3 & M_5 receptors)
Angiotensin II
α_1-Adrenergics
Oxytocin (Oxy)º
Gastrin (G)
Cholecystokinin (CCK)
Others

L_5 (MAP K)
Insulin
Relaxin
Nerve growth factor (NGF)
Insulin-like growth factor (IGF-1)
Epidermal growth factor (EGF)
Platelet-derived growth factor (PDGF)
Fibroblast growth factor (FGF)
Hepatocyte growth factor (HGF)
Colony-stimulating factor-1 (CSF-1)
Bone morphogenic proteins (BMPs)
Others

L_6 (JAK-STAT)
Growth hormone (GH)
Prolactin (PRL)
Placental lactogen (PL)
Erythropoietin (EPO)
Colony-stimulating factors (CSFs)
Interferon
Cytokines
Others

Target cells have hormone-specific receptors capable of recognizing and binding hormones and neurotransmitters; only those cells respond to the presence of these ligands. This binding, in turn, initiates intracellular events leading to a final cellular response. Receptors are usually hormone specific, but to a limited extent other hormones or xenobiotics with similar structure may bind to them.

Receptors

Hormone receptors occur in different cell locations, depending on the type of hormone they bind. Those for catecholamine and polypeptide hormones are found on the target cell surface (**Part A**), while steroid and thyroid hormone receptors are intracellular (cytoplasm or nucleus, Ch. 6). Receptor number/target cell ranges from about **2,000** to **100,000**, varying with physiologic conditions.

Because of the small number of hormone molecules present, **receptors** should have **high affinity**, or a strong tendency to bind with the hormone, **high specificity** with little tendency to bind other molecules, and **low capacity** so that all receptor sites can be occupied (i.e., saturated) at relatively low hormone concentrations. Receptor **distribution** should correspond to known hormone target cells, and correlate with anticipated physiologic effects.

Hormone receptor affinity and number are not static. Genetics, age, cell cycle stage, degree of differentiation, and the physiologic or pathophysiologic state are important variables. For example, ionic balance and temperature can affect receptor concentration and affinity, as can antibodies against the receptor or the concentration of homologous and heterologous hormones. The number of functional receptors is modulated up or down regularly, permitting target cells to respond optimally to small changes in hormone concentrations. Prolonged hormone exposure usually results in reduced target cell functional expression, since cells become desensitized. **Down-regulated** receptors can **1**) be destroyed after endocytosis, **2**) be internalized and remain stored in intracellular vesicles, **3**) remain on the cell surface but bind hormones inappropriately, or **4**) form receptor-hormone complexes that induce sub-optimal responses. Conversely, in developmental conditions, the first contact of hormones with their target cells usually results in receptor **up-regulation**, with accelerated binding and functional expression within target cells.

Second Messengers

Four primary intracellular second messenger pathways that respond to the presence of cell surface nerurotransmitter or catecholamine/polypeptide hormone binding are the **cAMP, Ca^{2+}/DG, MAP K,** and **JAK-STAT** pathways (**Part A**), and they are not necessarily unrelated.

The cAMP Messenger System

Hormones or neurotransmitters affecting cell metabolism via **cyclic adenosine monophosphate** (**cAMP**) are bound on the cell surface to specific receptors. This binding results in either activation or inhibition of the enzyme **adenyl cyclase** (also called **adenylate** or **adenylyl cyclase**), which is responsible for **cAMP** formation from **adenosine triphosphate** (**ATP**). Transfer of a signal from an occupied receptor on the membrane's outer face to adenyl cyclase, located on the cytoplasmic side of the membrane, occurs via **guanosine triphosphate** (**GTP**)**-binding proteins** [(**G_s** (**stimulatory**) or **G_i** (**inhibitory**) **proteins**]. Some hormones that activate adenyl cyclase (**Part B**) have a common five amino acid sequence that binds to gangliosides on the plasma membrane. This same amino acid sequence is found in the structures of the plant toxins abrin and ricin, as well as cholera and diphtheria toxins.

cAMP is referred to as a second messenger, since the hormone stimulating its production is the first. The intracellular cAMP concentration in the absence of stimulatory signals is about 50-100 nM (0.5-1 x 10^{-7} M), and more than 2 μM (1 x 10^{-6} M) following stimulation. It stimulates activation of **cAMP-dependent protein kinase** (i.e., **protein kinase A** (**PKA**)), which facilitates phosphorylation of various protein substrate serine/threonine residues within the target cell. **cAMP-dependent phosphodiesterase** (**PDE**), which **inactivates cAMP** to **5 -AMP**, can be activated by various ligands (e.g., insulin in adipocytes, muscle and liver cells).

PKA is capable of activating a number of other intracellular enzymes by phosphorylating their kinases, leading to a stimulatory cellular response. The effects of simulatory kinases are usually balanced or antagonized by **phosphatases**, enzymes that remove phosphate groups added to substrates by kinases. Alternatively, cAMP-mediated phosphorylation can deactivate other enzymes. After a hormone binds to its receptor (e.g., epinephrine binding to a β-adrenergic receptor), the cAMP messenger system generates a cascade of effects that ultimately alters metabolite flux within the cell. For example, inactivation/activation of reciprocal pathways within responsive cells can inhibit metabolite release, while stimulating storage.

cAMP can also act as a hormone second messenger by **altering gene expression**. Target DNA molecules are known to possess a **cAMP regulatory element** (**CRE**) that binds a **transcription factor** (**TF**) known as **cAMP response element binding protein** (**CREB**). Following cAMP activation of PKA, a catalytic subunit is freed for translocation into the nucleus where it phosphorylates CREB. This protein becomes capable of complexing with CRE and another TF (**activated TF-1**). The result of this complex series of interactions is the stimulation or inhibition of **RNA polymerase** and **transcription** of the target gene, and stimulation or inhibition of protein synthesis.

Actions of cAMP are terminated when it is hydrolyzed by **PDE**. Because PDE activity is also modulated by hormones via a G-protein (not shown), cAMP levels inside cells are under dual control. Two hormones can function antagonistically if one stimulates adenyl cyclase, and the other cAMP-dependent PDE (e.g., glucagon & insulin, respectively, on hepatocytes).

In **primitive life forms** such as the slime mold, which is an aggregation of cells that were once dispersed as individual amoebae, **cAMP** appears to be a primary aggregating stimulus, and is secreted into the medium when nutrients are in short supply. Similarly, in glucose-deprived *E. coli*, cAMP causes derepression of the lac operon, which enables this organism to metabolize galactose.

The MAP K and JAK-STAT Messenger Pathways

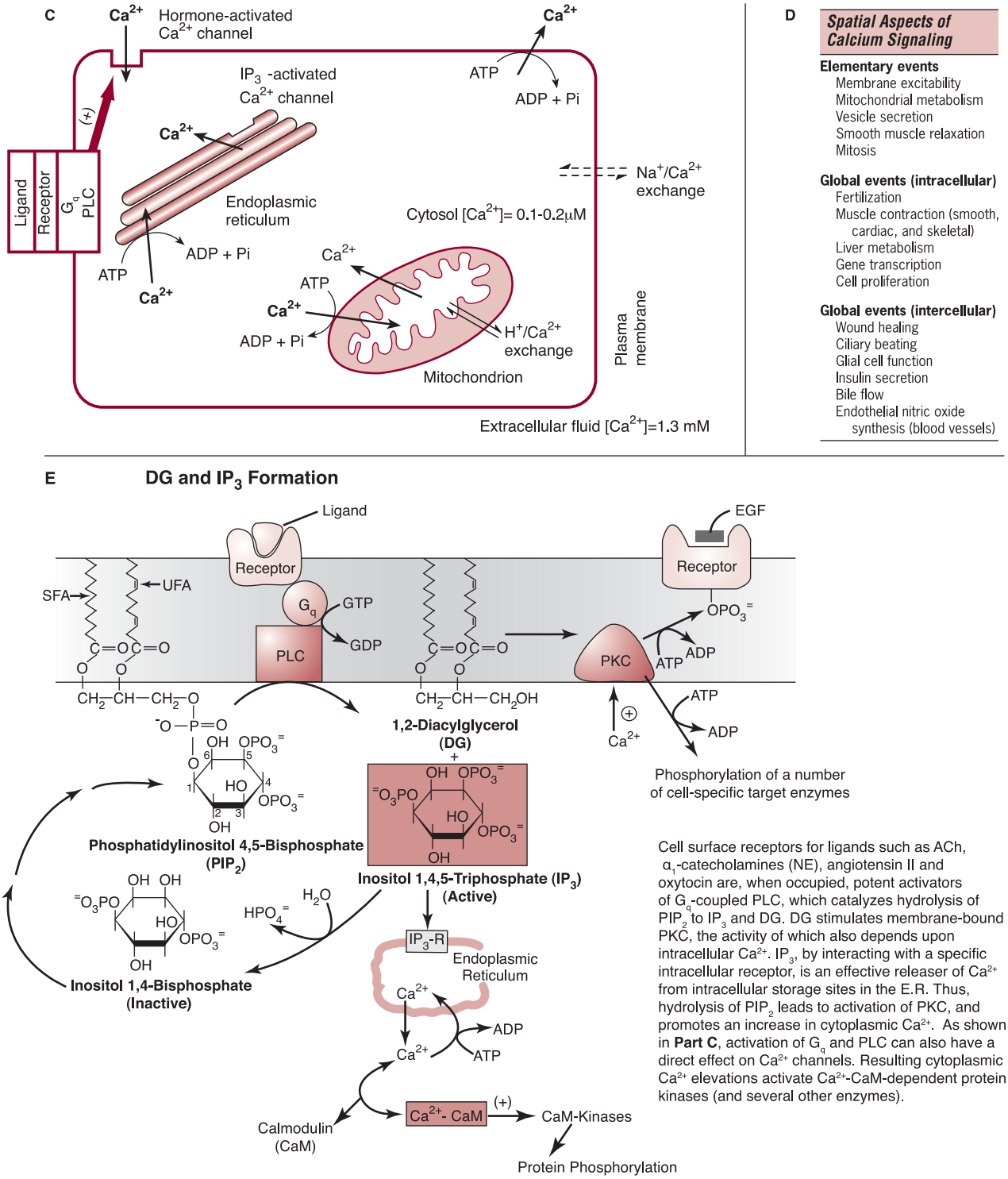
Insulin and certain **growth factors** (**GFs**) have receptors with both extracellular domains, and intracellular **tyrosine kinase** (**TK**) domains (**Parts A & B,** Ch. 42). Binding of insulin or a GF ligand (**L_5**) to its receptor causes it to **autophosphorylate**. One pathway activated through this phosphorylation leads (through the membrane-bound **Ras monomeric GTP-binding regulatory protein**) to stimulation of the **m**itogen-**a**ctivated **p**rotein **k**inase cascade (or **MAP K cascade**). When activated, terminal **MAP Ks** phosphorylate multiple target proteins in the cytosol, membrane and nucleus, including transcription factors (**TFs**) that regulate expression of genes required for cell division, survival, or phenotypic differentiation.

Receptors for prolactin, growth hormone and other ligands (**L_6**, **Part B**) are not themselves TKs, but are **receptor-associated TKs** since they initiate cytoplasmic TK activity when stimulated. They activate receptor-bound **Janus tyrosine kinases** (**JAKs**), which in turn phosphorylate **signal transducer and activator of transcription** (**STAT**) proteins. Phosphorylated STATs form homo- and heterodimers that move to the nucleus, where they act as TFs.

There is considerable **"cross-communication"** between the four primary intracellular second messenger pathways, thus providing cells with the ability to integrate signals from multiple extracellular stimuli into specific patterns of altered cellular response. Some cell-surface receptors are **inhibitory**, and actively stop or prevent a cell from responding to activation signals. Some recruit **phosphatases** that enzymatically remove phosphate groups from proteins that have been activated by one or more kinases. Target cells are like automobiles that have both gas pedals (activating receptors) and brakes (inhibitory receptors); they don't merely slow down by letting up on the gas. Cells that are chronically activated and run out of control may have one or more defects in these inhibitory signals (i.e., they may have lost their brakes).

Ch5 Mechanisms of Catecholamine and Polypeptide Hormone Action: II

(The Intracellular Ca^{2+}/DG Messenger System)



Cell surface receptors for ligands such as ACh, α_1-catecholamines (NE), angiotensin II and oxytocin are, when occupied, potent activators of G_q-coupled PLC, which catalyzes hydrolysis of PIP_2 to IP_3 and DG. DG stimulates membrane-bound PKC, the activity of which also depends upon intracellular Ca^{2+}. IP_3, by interacting with a specific intracellular receptor, is an effective releaser of Ca^{2+} from intracellular storage sites in the E.R. Thus, hydrolysis of PIP_2 leads to activation of PKC, and promotes an increase in cytoplasmic Ca^{2+}. As shown in **Part C**, activation of G_q and PLC can also have a direct effect on Ca^{2+} channels. Resulting cytoplasmic Ca^{2+} elevations activate Ca^{2+}-CaM-dependent protein kinases (and several other enzymes).

Use of the **cAMP, MAP K** and **JAK-STAT** second messenger pathways is widespread in animals, as is use of the **Ca^{2+}/DG** messenger system. The concentration of **free ionized calcium (Ca^{2+})** in the cytoplasm of most cells is usually kept below **0.2 μM**. Calcium ATPases pump **Ca^{2+}** across the plasma membrane to the cell exterior, into mitochondria, or into the lumen of the endoplasmic reticulum (**Part C**). A rise as small as **1μM** in the concentration of **cytosolic Ca^{2+}** can trigger many cellular responses. For example, in secretory cells such as the insulin-synthesizing β-cells of pancreatic islets (Chs. 40 and 41), a rise in cytosolic Ca^{2+} triggers exocytosis of secretory vesicles and release of insulin, and a rise in the cytosolic Ca^{2+} concentration of muscle cells triggers contraction. In both liver and muscle cells, an increase in the cytosolic Ca^{2+} concentration activates degradation of glycogen to glucose 1-phosphate.

We will now see how **Ca^{2+}** induces these and other varied metabolic responses, and how another second messenger, **inositol triphosphate (IP$_3$)**, which often mediates this rise in Ca^{2+}, is generated and functions. Still another second messenger, **diacylglycerol (DG)**, which is formed from the same precursors as IP_3, is used to regulate other cell functions through activation of **protein kinase C (PKC)**. All these second messengers interact in complex circuits to regulate crucial aspects of the growth, metabolism, and death of cells (Ch. 4).

The Calcium/Diacylglycerol Messenger System

The intracellular calcium/diacylglycerol (Ca^{2+}/DG) messenger system has a central role in mediating secretion of exocrine, endocrine, and neurocrine products, the metabolic processes of glycogenolysis and gluconeogenesis, the transport and secretion of fluids and electrolytes, the contraction of all forms of smooth muscle, and the birth, growth, and death (apoptosis) of cells (to name a few of its functions). It is a nearly universal means by which extracellular messengers (i.e., neurotransmitters and hormones) regulate cell function, and it is intimately related to the **arachidonic acid cascade,** and the **cAMP, MAP K** and **JAK-STAT** messenger systems.

Calcium is derived at the cellular level from both external and internal sources (**Part C**). It can enter from outside the cell by passing through **Ca^{2+}-specific voltage-** or **ligand-gated channels** that span the plasma membrane, or it can be released from internal Ca^{2+} stores in mitochondria and the endoplasmic reticulum (ER) (or sarcoplasmic reticulum; SR). When a Ca^{2+} channel opens, a concentrated plume of Ca^{2+} forms around its mouth, then dissipates rapidly by diffusion after the channel closes. Such localized signals, which can originate from channels in the plasma membrane or on internal organelles, represent the **elementary events** that occur in Ca^{2+} signaling (**Part D**). These elementary signals have two basic functions: they can activate highly localized cellular processes in the immediate vicinity of the channels primarily through enzyme phosphorylation, or, by recruiting channels throughout the cell, they can activate processes at a more global level. In smooth muscle, for example, Ca^{2+} increases that arise locally near the plasma membrane activate potassium (**K^+**) channels, thus causing muscle to relax. Yet when elementary release events deeper in the cell are coordinated to create a **global Ca^{2+} signal,** the muscle contracts. This is an example of how spatial organization enables Ca^{2+} to activate opposing cellular responses in the same cell. For sites of elementary Ca^{2+} release to produce global responses, individual channels must communicate with each other to set up Ca^{2+} waves. If cells are connected, such intracellular waves can spread into neighboring cells and become intercellular waves to cause responses within tissues.

Elementary calcium signaling begins when certain hormones or neurotransmitters interact with their plasma membrane receptors (e.g., catecholamines interacting with α_1-adrenergic receptors, or acetylcholine interacting with muscarinic receptors (**Part B**)). Activation of membrane-bound **phospholipase C (PLC**; through **G_q** protein) then catalyzes hydrolysis of **phosphatidylinositol 4,5-bisphosphate (PIP$_2$)** from the plasma membrane to produce **DG** and **inositol triphosphate (IP$_3$)** (**Part E**). Both **DG** and **IP$_3$** act as intracellular messengers: **DG** acts as a membrane-associated activator of **protein kinase C (PKC)**, and **IP$_3$** acts as a water-soluble inducer of **Ca^{2+}** release from mitochondria and the ER, thereby causing a transient rise in the **calcium–calmodulin (Ca^{2+}–CaM)** concentration of the cytosol. (**Calmodulin** is a protein that binds Ca^{2+} within the cytosol). These two events initiate further biologic effects specific for the cells in which they occur. The **IP$_3$** and **DG** may next be converted sequentially into intermediates that can be successively phosphorylated back into phospholipid (i.e., **PIP$_2$**) in the plasma membrane. The **DG** may be converted to phosphatidic acid, which can then enter the rephosphorylation pathway, or its unsaturated fatty acid in the 2 position (most likely **arachidonic acid**) can be hydrolyzed by **phospholipase A$_2$** and then used in the synthesis of **eicosanoids** (i.e., prostaglandins, thromboxanes, or leukotrienes). The **eicosanoids** are also capable of eliciting a biologic effect.

For the most part, however, **DG** is thought to remain embedded in the plasma membrane, where it helps activate **PKC**. Protein kinase C bound to DG is not fully active, however, until it also binds intracellular **Ca^{2+}** ions. Thus, the two arms of the DG/Ca^{2+} messenger system are coupled. Activated **PKC**, a serine/threonine kinase, phosphorylates a number of cell-specific target enzymes, including specific signal transduction and inhibitory enzymes. Properties of **PKC** also indicate that it plays a key role in several aspects of cell growth since activated PKC, a serine/threonine kinase, phosphorylates a number of cell specific targets, including **MAP K** (**Part A**). Some substances known as tumor promoters are potent and specific activators of this protein kinase. Tumor promoters – lipid-soluble chemicals isolated from several sources (mainly plants) – are thought to play a role in transforming a normal cell into a malignant cell capable of uncontrolled growth. Carcinogenesis generally requires both an initiator and a promoter (and most carcinogens are capable of acting as both).

Physiologic activators of **PKC** include some ligands that bind to **L$_5$** (**MAP K**) receptors (**Parts A** & **B**), including **EGF**. Phosphorylation of the EGF receptor by PKC decreases its affinity for EGF, moderating growth-stimulating activity. Overproduction of PKC in "normal" fibroblasts causes cells to grow unattached to an extracellular matrix, as do many tumor cells. (Normal cells grow only when attached to a matrix.) Clearly, **PKC** is of fundamental importance in controlling cell growth. The eventual understanding of its regulation should provide important insights into many aspects of normal cell metabolism and carcinogenesis.

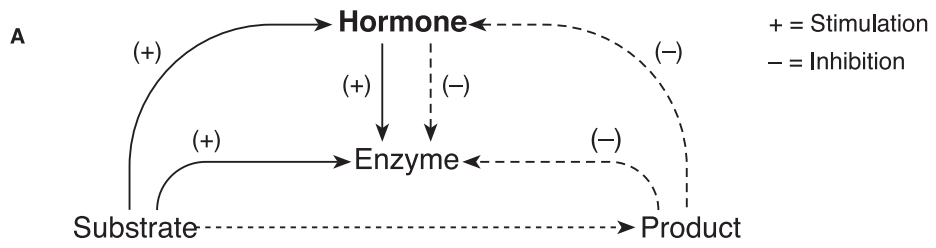
Summary

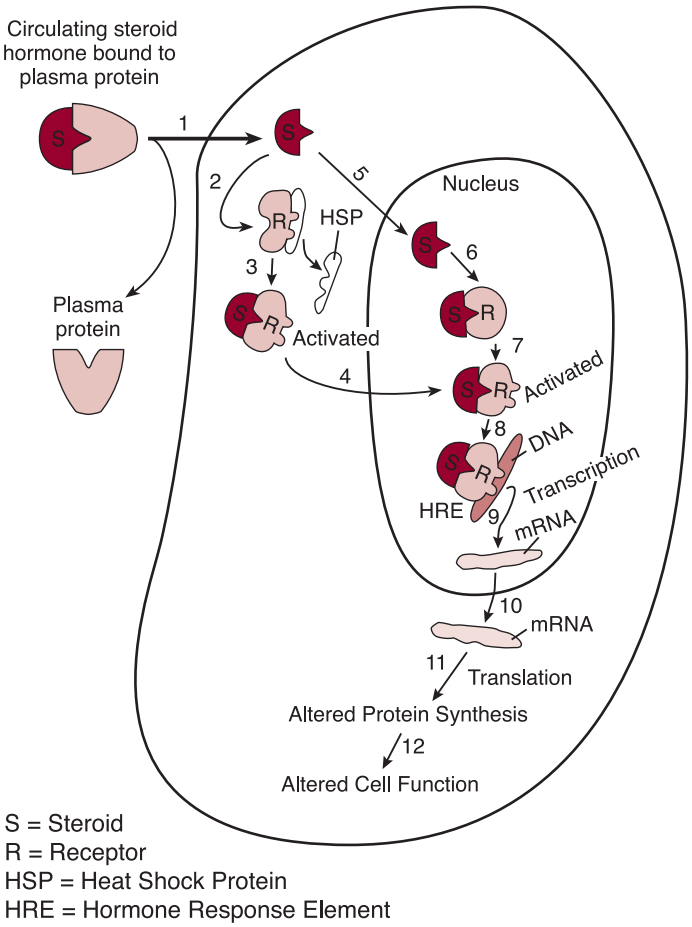
The principal mechanisms by which catecholamine and polypeptide hormones exert their intracellular effects are summarized in Chs. 4 and 5. Many extracellular ligands bind to receptors on the target cell surface, triggering release of intracellular mediators that initiate changes in cell function. Extracellular ligands are "first messengers," with the intracellular mediators being the "second." Second messengers bring about short-term changes in cell function by altering enzyme activity, and longer-term effects by altering transcription of various genes. Second messengers generally activate **protein kinases**, enzymes that catalyze phosphorylation of tyrosine or serine and threonine residues in proteins. **Phosphatases** are also important, since removal of a phosphate group inactivates some transport proteins or enzymes while activating others.

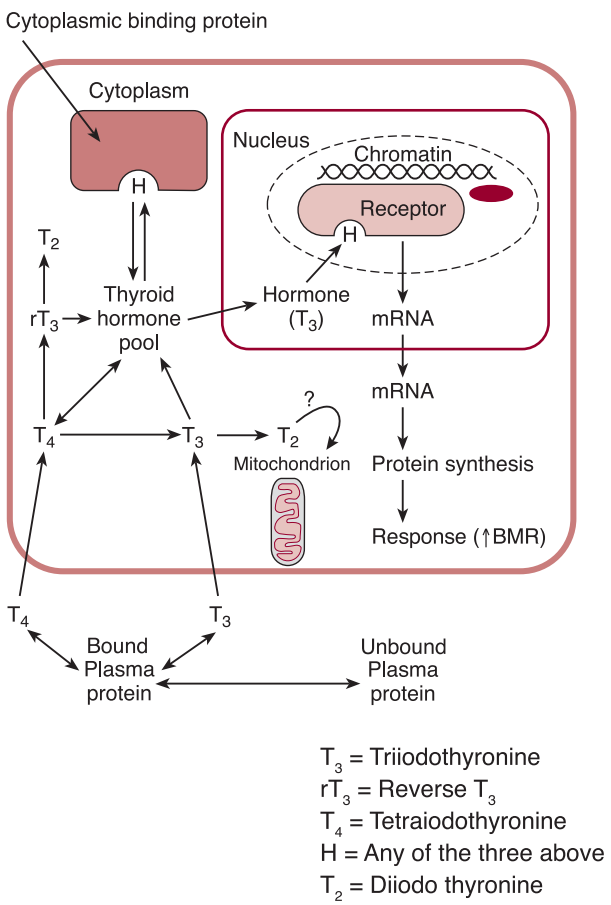
Clearly the **MAP K** and **JAK-STAT** pathways are complex, and there is considerable cross-talk between them and the **cAMP** and **PLC (Ca^{2+}/DG)** pathways. These second messenger systems are clearly interwoven, and difficult to separate. It is only possible in this ***Quick Look Series*** to emphasize major points, and present general concepts that will aid the reader in understanding the rest of endocrinology.

In comparing the **cAMP** to the **Ca^{2+}/DG** messenger system, the cAMP nucleotides may exert their effects in concert with or in opposition to those of Ca^{2+}–CaM and DG. Moreover, cAMP- and Ca^{2+}–CaM-dependent protein kinases may act on the same substrate that serves as a common effector of certain cellular processes. In controlling the metabolism and function of cAMP and Ca^{2+}, CaM integrates the two messenger systems on a molecular basis. Because the two systems are intertwined, cAMP may sometimes serve as a second messenger, and Ca^{2+} as the third messenger. At other times these roles may be reversed. Between the two systems, the response of the **Ca^{2+} pathway** appears to be inherently **faster**, partly because the availability of Ca^{2+} does not require enzymatic synthesis (as **cAMP** does). The Ca^{2+} system is also more **diversified**. Calmodulin is endowed with many receptor enzymes, including several protein kinases with different substrate specificities.

Ch6 Steroid and Thyroid Hormones
(Mechanisms of Action)







Hormones act by either increasing or decreasing protein (namely enzyme) activity and/or synthesis within target cells. Enzymes controlled by hormones are also generally under the influence of substrate induction and/or product negative feedback inhibition. Products produced by target cells can also return to further inhibit hormone secretion (e.g., glucose output by the liver feeds back negatively on glucagon secretion from the pancreas) (**Part A**). Other metabolites within target cells exert influences on the activity of specific enzymes (e.g., citrate inhibition of phosphofructokinase and the resulting anaerobic glycolysis, or second messengers generated in response to the presence of specific hormones on the outer plasma membrane).

Steroid Hormone Action

Unlike catecholamine and polypeptide hormones, **steroids**, which are lipophilic, are transported in plasma bound to carrier proteins (produced by the liver), and act in their target cells by either increasing or decreasing synthesis of specific proteins (e.g., **enzymes**). Steroids enter virtually all cells of the body, but bind only to specific receptor proteins in the cytoplasm and/or nucleus of target cells (i.e., gluco- and mineralocorticoid receptors are thought to be located in the cytoplasm, while $1,25(OH)_2D$ (a secosteroid), retinoic acid and sex steroid receptors are in the nucleus). In **Part B**, ***process 1*** shows a steroid hormone dissociating from a plasma transport protein and entering a target cell via diffusion through the lipid bilayer. Within the cytoplasm, unbound steroid receptors contain **heat shock protein** (**HSP**), which if translocated to the nucleus would mask the receptor DNA-binding domain. Binding of the cytoplasmic steroidal ligand with its receptor (***process 2***) promotes a conformational change (i.e.,**"activation"**) in the receptor protein itself (***process 3***), resulting in the release of **HSP**. The ligand-receptor complex translocates to the nucleus (***process 4***), binding to DNA (***process 8***). Some steroid hormones bypass ***processes 2-4***, moving directly into the nucleus (***process 5***), where they bind to nuclear receptors (***process 6***), thus activating/inactivating them (***process 7***). Once activated ligand-receptor complexes have bound to specific **hormone response elements** (**HREs**; ***process 8***), they function as regulators of gene transcription (***process 9***). New mRNAs are translocated to the cytoplasm (***process 10***) and assembled into translational complexes for the synthesis of proteins (***process 11***) that alter target cell function (***process 12***). Note that in some instances steroid receptor complexes actually repress, rather than induce, specific gene transcription.

At certain target sites (e.g., hepatocytes), **steroid**, **catecholamine** and/or **polypeptide hormones** can work in harmony, with the steroid increasing synthesis of enzymes that catecholamine and/or polypeptide hormones activate, thus creating a longer-lasting effect of greater metabolic magnitude. Cortisol (a glucocorticoid) increases synthesis of hepatic gluconeogenic enzymes that are stimulated by the other diabetogenic hormones, epinephrine, GH and glucagon. This coupling is an example of **metabolic** (**endocrine**) **synergism**.

Thyroid Hormone Action

Major effects of **thyroid hormones**, like those of steroid hormones, are produced via changes in the synthesis and/or activity of regulatory proteins in target cells, including key metabolic enzymes and receptors. Thyroid hormones, also being lipophilic, readily pass into a target cell's cytoplasm and nucleus to bind with receptors in the chromatin. The predominant nuclear receptor for thyroid hormones is specific for **triiodothyronine** ($\mathbf{T_3}$), the most active form. Following binding, regulation of gene expression occurs with subsequent induction of RNA synthesis (**Part C**).

Thyroid hormone receptors may bind to specific **thyroid hormone response element** (**TRE**) sites on DNA, even in the absence of T_3 (unlike steroid hormone receptors). The TREs are located near (generally upstream with respect to the start of transcription) to the promoters where transcription of specific thyroid hormone responsive genes is initiated. T_3 receptor binding results in stimulation, or in some cases inhibition, of gene transcription with consequent alterations in the levels of the mRNAs transcribed from them. Subsequent changes in mRNA levels alter the concentration of the protein products of these genes. These proteins then mediate the thyroid hormone response.

Most **tetraiodothyronine** ($\mathbf{T_4}$) presented to its target cell is deiodinated to $\mathbf{T_3}$ (or in some cases **reverse** $\mathbf{T_3}$ [$\mathbf{rT_3}$], the inactive form) before nuclear binding. Deiodinases and their rate of activity differ markedly in different tissues, and in different metabolic situations (Ch. 39). The deiodinases appear to be responsible for maintaining differences in thyroid hormone ratios (e.g., $\mathbf{T_3{:}T_4}$ or $\mathbf{rT_3{:}T_4}$) in various body tissues. The thyroid hormone pool (T_4, T_3, and rT_3) within the cytoplasm of target cells is complexed with cytoplasmic protein binders (Ch. 37).

Specific responses to thyroid hormones may be quite individual, and vary between species and tissues. The cell functions affected are often under multihormonal regulation, and therefore the direction metabolic pathways take under thyroid hormone stimulation may depend on the presence or absence of other hormones. In general, thyroid hormones (i.e., T_4 and T_3) increase the metabolic rate of their target cells by increasing **oxygen consumption**.

Until recently, $\mathbf{T_2}$ (3,5-diiodothyronine; formed from T_3 deiodination), because of its low affinity for nuclear T_3 receptors, was considered to be an inactive thyroid hormone metabolite. Some investigators now believe that in certain cell types $\mathbf{T_2}$ may stimulate mitochondrial respiration through a nuclear-independent pathway. Others suggest that $\mathbf{T_2}$ may also affect various carriers, ion-exchangers and enzymes outside mitochondria.

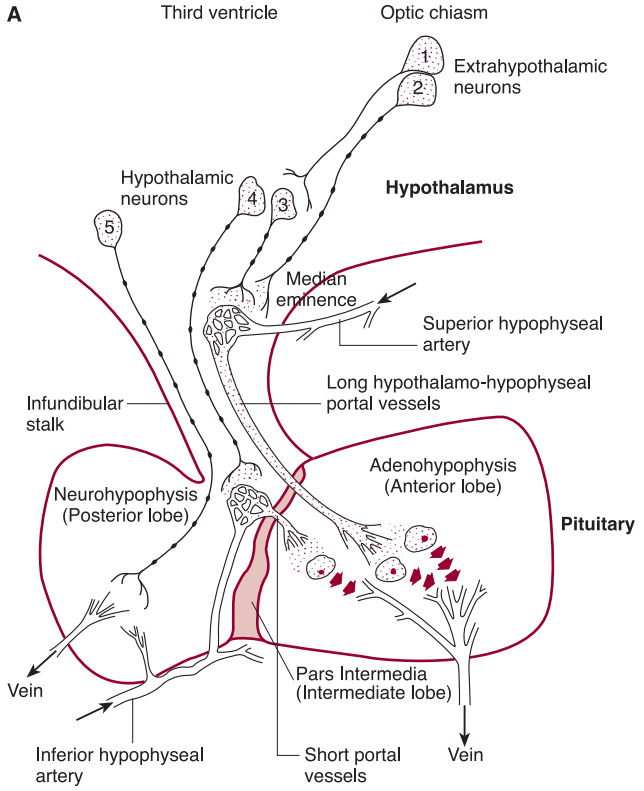
Up- and Down-Regulation

With intracellular receptors, up- and down-regulation generally means increased or decreased receptor gene expression with an increase or decrease in the concentration of receptor molecules. As with polypeptide hormone receptors, **down-regulation** of steroid and thyroid hormone receptors by their own ligands plays an important physiologic role by desensitizing target cells, preventing overstimulation. Although down-regulation of steroid receptors by their cognate hormones appears to be a common form of autoregulation, it is not detected in all target cells, for glucocorticoid-mediated up-regulation of its own receptors (**homologous up-regulation**) has been reported in a number of responsive cells. The ability of estrogen to increase the concentration of uterine progesterone receptors (i.e., estrogen priming) is an example of **heterologous up-regulation** (Ch. 56).

In summary, **steroid** and **thyroid** hormones are thought to regulate **about 1%** of all genes expressed by responsive cells. The proteins whose synthesis is either increased or decreased by these hormones may be **enzymes**, **structural proteins**, **receptor proteins**, **transcriptional proteins** that regulate expression of other genes, or **proteins that are exported by cells** (e.g., liver cells). Through this mode of action the response of metabolic pathways is either retarded or accelerated. Other consequences of steroid and thyroid hormone action include alterations in the processing of the primary RNA product, in the turnover of messenger RNA molecules, or in post-translational modification of proteins. This explains why **hours** are usually required for the biological effects of these hormones to become evident.

Some steroid and thyroid hormone actions are much more rapid, indicating that there may be some **nongenomic actions** of these hormones mediated via. **"cross-talk"** with the classic second messenger systems (Ch. 5).

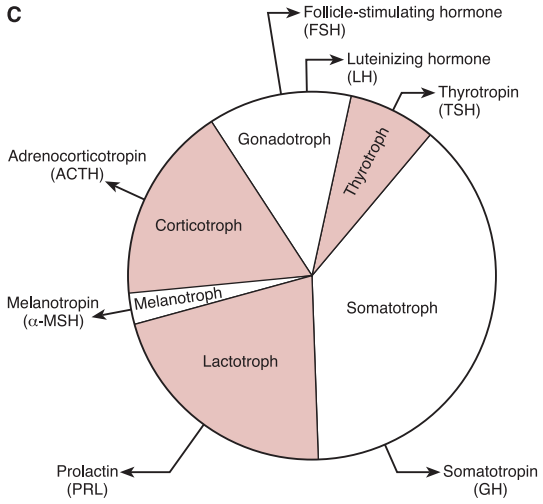
Ch 7 Hypothalamus and Pituitary
(Directors of the Endocrine Orchestra)

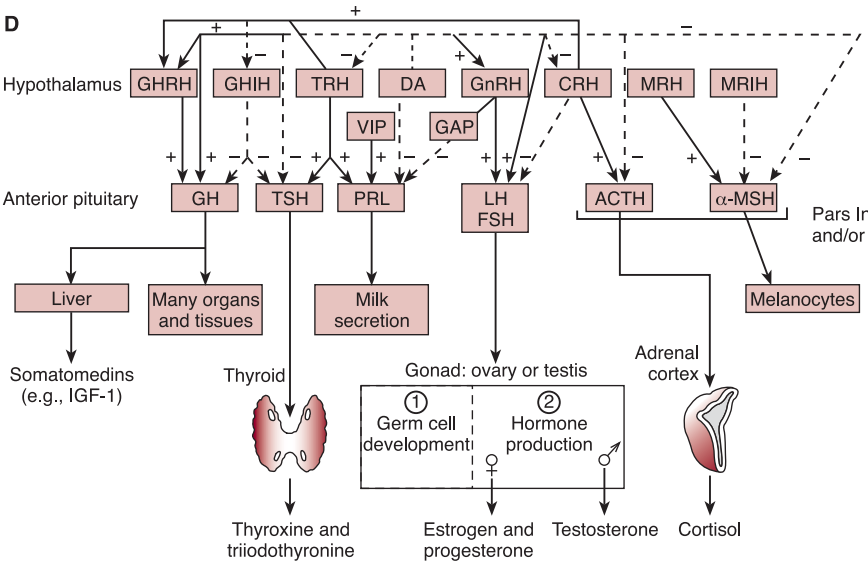


B

Agonist	GH	TSH	LH and FSH	ACTH	PRL	α-MSH
Norepinephrine	+	+	+	–	–	–
Acetylcholine	?	?	+	+	+	?
Dopamine	+	–	+	–	–	–
Serotonin	+	–/+	–	+	–	–
γ-Aminobutyric acid	+	–	–	–	–	?
Endorphin	+	?	–	?	+	?

+ =stimulatory; – =inhibitory





GHRH = Somatocrinin
GHIH = Somatostatin
GH = Somatotropin
TRH = Thyrotropin-Releasing Hormone
TSH = Thyrotropin
DA = Dopamine
VIP = Vasoactive Intestinal Polypeptide
PRL = Prolactin
GnRH = Gonadotropin-Releasing Hormone
GAP = GnRH-Assocaited Peptide
LH = Luteinizing Hormone
FSH = Follicle-Stimulating Hormone
CRH = ACTH-Releasing Hormone
ACTH = Adrenocorticotropin
MRH = Melanocyte-Releasing Hormone
MRIH = Melanocyte-Release Inhibiting Hormone
α-MSH = Melanocyte Stimulating Hormone
IGF-1 = Insulin-Like Growth Factor I

Source: Part A modified and redrawn from Gay VL. Fertil Steril 1972; 23:50. **Part C** modified from Berne RM, Levy MN. Principles of Physiology, 1st ed. St Louis: Mosby, 1990:537.

Removal of the canine pituitary (**hypophysectomy**) was proven to be **nonfatal** earlier this century. Nonetheless, the pituitary gland (particularly the anterior lobe) has been shown to be important in regulating secretory activity of other endocrine glands (with the notable exceptions of the pancreas and parathyroids). Only the gut and the outermost portion of the adrenal gland (i.e., the cortex) can match the complexity of the pituitary in the number of hormones produced. Primary differences are that the **gut** secretes **small peptide** hormones, the **adrenal cortex** secretes closely related **steroid** hormones, whereas the **pituitary** secretes numerous **polypeptide** hormones, many being quite different in chemical structure and size. These vary from **nonapeptides**, which are produced by the **neurohypophysis** (posterior pituitary; Ch. 12), to **adrenocorticotropin** (**ACTH**), composed of **39 amino acids** and produced by the **adenohypophysis** (anterior pituitary), or, where present, the **pars intermedia** (intermediate lobe of the pituitary). Other adenohypophyseal polypeptides are actually larger than ACTH (e.g., **GH**, **TSH**, **LH** and **FSH**), and are cosidered to be true **proteins** (>50 amino acids; **Part A**, Ch. 1).

The pituitary gland is formed by the confluence of two primary embryonic rudiments, one of which originates from an outpouching of neural tissue from the brain's third ventricle (the neurohypophysis), the other from ectoderm of the oral cavity (the adenohypophysis). The **anterior lobe** comprises about **80%** of pituitary weight in most species. The pituitary stalk extends to the hypothalamus through a dural reflection, and an **intermediate lobe** located between the anterior and posterior lobes is present in certain species and during fetal development, but is vestigial in primates. This lobe (the **pars intermedia**) is formed embryologically from the dorsal half of **Rathke's pouch**, an evagination of the roof of the pharynx, but is closely adherent to the posterior lobe in adult animals. It is separated from the anterior lobe by the remains of the cavity in Rathke's pouch, the **residual cleft**. The pars intermedia of the dog and horse is a significant source of **ACTH**, with tumors therein leading to spontaneous **pituitary-dependent hyperadrenocorticism** (**PDH**; Ch. 25). Because surrounding structures are vital, expansion of the pituitary gland due to tumor formation can result in superior extension with compression of the optic chiasm, and loss of vision.

Anatomic connections between the hypothalamus and pituitary are shown schematically in **Part A.** Note that neurosecretory cells are present in certain **hypothalamic nuclei** (neurons **3, 4,** and **5** in **Part A**). Some secretory axons from these nuclei pass down the infundibular stalk and terminate near blood vessels in the neurohypophysis (neurons **4** and **5**), while others terminate near capillary loops of the **median eminence** (neuron **3**). Hormones of the **neurohypophysis** (ADH and oxytocin) are products of **hypothalamic neurosecretory cells** [**supraoptic** (neuron **5**) and **paraventricular** (neuron **4**) **nuclei**, respectively], and are stored and released from the **pars nervosa** (neurohypophysis). The **hypothalamo-hypophyseal portal system** starts as a primary plexus in the **median eminence**, and conveys blood downward to sinusoids (i.e., capillaries) of the **anterior lobe**. This anatomical arrangement fits the true classification of a portal system (i.e., one that begins and ends in capillaries). Hypothalamic axons of the median eminence (e.g., neuron **3**) liberate multiple release and/or inhibiting factors (or hormones) into the portal system, and these short neural peptides in turn become involved with regulation of anterior pituitary function (by either stimulating or inhibiting release of anterior pituitary hormones). Although the anterior lobe does not appear to have nerve fibers like the posterior lobe, it does have limited vasoregulatory sympathetic innervation. It is generally believed, however, that there are no direct regulatory nerve fibers to the anterior lobe that involve selective endocrine secretory function.

Part A also depicts a somewhat **hypothetical anatomical relationship** between the hypothalamic area and the pituitary. Both long portal vessels (originating from the superior hypophyseal artery), and short portal vessels (originating from the inferior hypophyseal artery), may provide a means for communication between hypothalamic neurons and hormone-secreting cells of the adenohypophysis. Several types of neural stimuli are thought to bring about secretion of releasing hormones:

- Extrahypothalamic neurons (neuron **1**) may stimulate hypothalamic neurons (neuron **3**) to secrete releasing hormones. For example, norepinephrine (from neuron **1**) may stimulate secretion of gonadotropin-releasing hormone (GnRH) from neuron **3**.
- Neurons that have cell bodies located in higher brain centers (neuron **2**) may also secrete releasing hormones. For example, dopamine (DA), secreted from neuron **2** and subsequently entering the hypothalamo-hypophyseal portal system, may inhibit secretion of prolactin (PRL), thyroid-stimulating hormone (TSH), melanocyte-stimulating hormone (melanotropin; α-MSH), and ACTH from the adenohypophysis (and/or pars intermedia).
- The pathway depicted by neuron **4** indicates transport of hormones (e.g., oxytocin and/or ADH from hypothalamic nuclei) into a capillary bed that drains into the short portal vascular network servicing the periphery of the adenohypophysis. In this way, oxytocin could promote secretion of PRL, and ADH secretion of ACTH from the adenohypophysis. Both oxytocin and PRL are needed to initiate and maintain lactation, and ADH is a known stimulator of ACTH release.

In addition, it should be noted that neurotransmitters from higher brain centers may also be exerting control over the secretion of pituitary hormones (**Part B**).

Anterior Pituitary Cell Types

The **adenohypophysis** contains six major **endocrine-secreting cell types,** as well as some **null cells** that have all the cytoplasmic organelles needed for protein hormone synthesis, but contain few secretory granules. Their products, if any, have yet to be identified. The six major mammalian endocrine-secreting cell types, their relative proportions, and their major secretory products are depicted in **Part C.** Although they are known to aggregate to some extent, they do not form enclaves, but rather are interspersed among each other. They vary somewhat in size and in the characteristics of their secretory granules, but they can be identified with certainty by immunohistochemical staining of the hormones within.

Each anterior pituitary cell is regulated by one or more hypothalamic neurohormones that reach them through the hypothalamo-hypophyseal portal system (**Part D**). Three cell types produce classic **tropic hormones** that stimulate hormone secretion from the thyroid gland (**TSH**), the adrenal cortex (**ACTH**), or the gonads (follicle-stimulating hormone, **FSH**, and luteinizing hormone, **LH**). Growth hormone (**GH**), **α-MSH**, and **PRL** are **not** true tropic hormones because they do not directly stimulate secretion of other hormones (unless one recognizes somatomedin secretion from the liver (Ch. 11) as such in response to **GH**). Although **α-MSH** is found in the adenohypophyses of all vertebrates examined, in some mammalian species (e.g., rat, rabbit, dog, horse, sheep, and cattle) the pars intermedia is a well-defined structure, and contains large amounts of α-MSH (hence justifying the other name for α-MSH, **intermedin**).

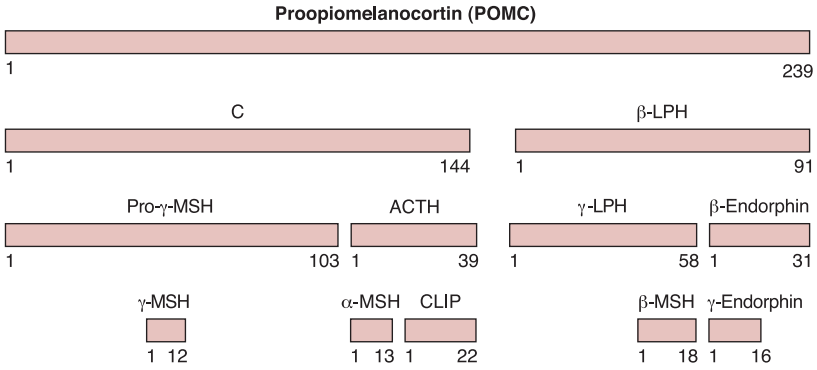
Circumventricular Organs

Four small areas near the brainstem appear to be **independent of the blood brain barrier** (**BBB**), and can sense plasma components that may not otherwise cross this barrier: **1**) the neurohypophysis and adjacent parts of the hypothalamic median eminence, **2**) the area postrema, **3**) the organum vasulosum of the lamina terminalis, and **4**) the subfornical organ. Control of the autonomic nervous system, ADH and oxytocin release, and the release of hypophysiotropic hormones (release and inhibiting hormones) controlling anterior pituitary function seem appropriately designed for this section of the CNS.

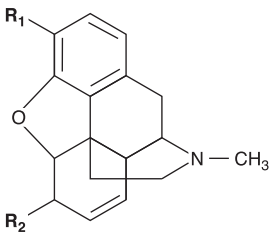
Ch8 Proopiomelanocortin and Related Peptides: I

(Endogenous Opiate-Like Peptides)

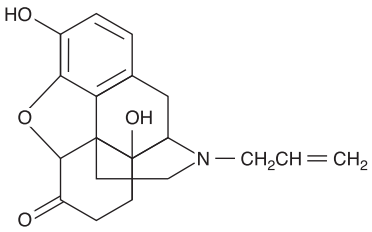
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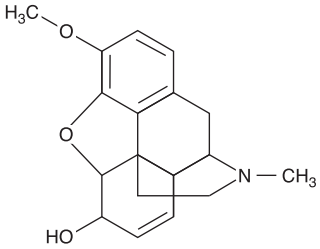
B



Complex carbon skeleton of the opiate drugs



Naloxone
(Opiate antagonist)



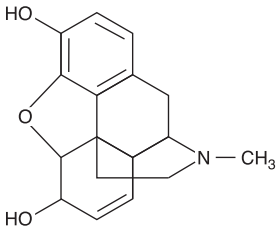
Codeine

CNS Effects of Morphine

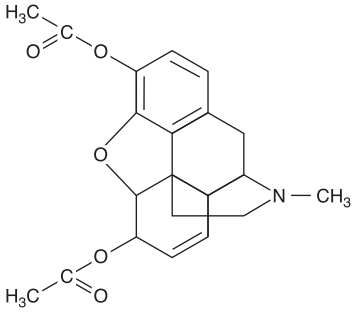
Increase ADH release
Analgesia
Convulsions
Cough suppression
Nausea & vomiting
Respiratory depression
Sedation (or excitation)
Thermoregulatory changes

Peripheral Effects of Morphine

Changes in blood pressure
Constipation
Eye (miosis/mydriasis)



Morphine



Heroin

C

POMC - - ➤ Tyr - Gly - Gly - Phe - Met - Thr - Ser - Glu - Lys - Ser - Gln - Thr - Pro - Leu - Val - Thr - Leu - Phe - Lys - Asn - Ala - Ile - Ile - Lys - Asn - Ala - Tyr - Lys - Lys - Gly - Glu
(β-Endorphin)

Prodynorphin - - ➤ Tyr - Gly - Gly - Phe - Leu - Arg - Arg - Ile - Arg - Pro - Lys - Leu - Lys - Trp - Asp - Asn - Gln
(Dynorphin A)

Proenkephalin - - ➤ Tyr - Gly - Gly - Phe - Met **(Met-Enkephalin)**
- - ➤ Tyr - Gly - Gly - Phe - Leu **(Leu-Enkephalin)**

The activity of neurons in both the peripheral and central nervous system (CNS) is affected by several **neurohormones** that act on cells quite distant from their site of origin. Neurohormones can modify (or modulate) the ability of nerve cells to respond to synaptic neurotransmitters. Several small polypeptides with profound effects on the nervous system have been discovered; examples are **Met-enkephalin**, **Leu-enkephalin**, **dynorphin A**, and **β-endorphin**, which carry a common terminal amino acid sequence essential to their function (see below). These and other peptide hormones probably act as neurotransmitters in selected cell types, and also have profound effects on general life events like mood, sleep, and body growth. Perhaps more importantly, these peptides function as **natural pain killers**, thus decreasing pain perception in the CNS.

Enkephalins were discovered during studies in the early 1970s that were focused on the mechanism of opium addiction. Several groups discovered that brain plasma membranes contain high-affinity binding sites for purified opiates, such as the alkaloid **morphine** (a narcotic, analgesic drug). Since such receptors exist in the brains of all vertebrates, from sharks to primates, the question was raised why vertebrates should have highly specific receptors for alkaloids produced by opium poppy plants, and why these receptors should have survived evolution. Since none of the neurotransmitters and peptides then known were structurally similar to morphine, nor could they serve as agonists for these receptors, a search was begun for natural compounds that could. This led to the discovery of two pentapeptides, **Met-enkephalin** and **Leu-enkephalin**, both of which bind to **"opiate" receptors** in the brain, and have the same effect as morphine when injected into the ventricles (cavities) of brains in experimental animals. **Enkephalins**, **dynorphins**, and **endorphins** appear to act by inhibiting neurons that transmit or perceive pain impulses: presumably these neurons contain abundant **"opiate peptide"** receptors.

Proopiomelanocortin

A large precursor glycoprotein known as **proopiomelanocortin** (**POMC**) is synthesized by specialized basophils of the pituitary (**Part A**), with the POMC gene expressed by both the **anterior** and **intermediate lobes**. POMC or related products are found in several other vertebrate tissues, including the **brain**, **placenta**, **gastrointestinal tract**, **reproductive tract**, **lung** and **lymphocytes**. This prohormone is the precursor of several important polypeptides, including **ACTH**, **α-MSH**, and the opioid peptide, **β-endorphin**. Although initial processing of the POMC protein in peripheral tissues (e.g., gut, placenta and male reproductive tract) resembles that in the pituitary, polypeptides derived from POMC may be precursors to other substances with important physiologic actions, for additional tissue-specific modifications of these peptides include phosphorylation, acetylation, glycosylation and amidation. In the CNS, POMC products appear to be important in areas where electrical stimulation can relieve pain.

POMC is cleaved in the pituitary to produce **ACTH** (39 amino acids), an **N**-terminal 103-amino-acid fragment with little known biologic activity (pro-γ-MSH), and a **C**-terminal 91-amino-acid fragment known as **β-lipotropin** (**β-LPH**). In turn, β-LPH may be cleaved to a 58-amino-acid fragment known as γ-LPH, and the 31-amino-acid fragment, **β-endorphin** (residues 61-91). In melanotropes of the adenohypophysis and pars intermedia, **ACTH** is further cleaved to yield **α-MSH** (residues 1-13 of ACTH), and a 22-amino-acid fragment called **corticotropin-like intermediate peptide** (**CLIP,** residues 18-39), which is thought to have little, if any, biologic activity.

Endogenous Opiate-Like Peptides

Morphine is a nonpeptide exogenous opiate analgesic (pain-killing) drug that binds specific receptors in the CNS (**μ**, **κ**, and **δ** receptors). Three common opiate drugs, **morphine, heroin,** and **codeine,** differ according to the groups attached to their complex carbon skeleton at $\mathbf{R_1}$ and $\mathbf{R_2}$ (**Part B**). The action of morphine is **blocked** by closely related molecules such as **naloxone**.

As stated above, researchers postulated that there are endogenous compounds that produce analgesic opiate-like properties, and, although their structures may differ from those of morphine, three distinct families of endogenous opiate-like peptides have been identified: **endorphins, dynorphins,** and **enkephalins**. All three have the same **N**-terminal 4- or 5-amino-acid sequence that allows them to bind the same receptors (**Part C**). Opiate effects of these peptides are also blocked by naloxone, indicating that exogenous opiate drugs and the endogenous opiate-like peptides bind the same **μ**, **κ**, and **δ** receptors. **Endorphins** arise from **POMC**, and **dynorphins** from **prodynorphin**. Although **β-endorphin** contains the 5-amino-acid sequence for metenkephalin at its amino terminus, it is **not** converted to this peptide. Instead, **enkephalins** are derived from **proenkephalin** (see **Part B**).

Endorphins are mainly found in the pituitary, pancreatic islets, and CNS, with high levels in the arcuate nucleus. Peptides from proenkephalin (meaning **"in the head"**) and prodynorphin are distributed widely throughout the CNS. Although each peptide family is usually located in different groups of neurons, occasionally more than one family is found within the same neuron. Proenkephalin peptides are present in areas of the CNS related to pain perception, modulation of affective behavior (e.g., eating, drinking, and sexual behavior), motor control, and regulation of the ANS and neuroendocrine system (i.e., the median eminence). Peptides from proenkephalin are also found in the adrenal medulla, and in nerve plexuses and exocrine glands of the stomach and intestine.

In addition to peptides, it now appears that morphine, codeine, and related compounds might occur naturally in mammalian tissues, as hepatic metabolic pathways that could synthesize these drugs have been described.

The opiate-like peptides are, mole for mole, as potent analgesics as morphine, and β-endorphin is actually five to ten times more potent. Since these compounds do not easily penetrate the blood-brain barrier, their effects in animals have been described only following injection into the CNS. Discovery of these compounds led to a new theory of the mechanism of pain perception in which **"nonpain"** is perceived as an equilibrium between incoming pain signals, and tonic **"antipain"** signals generated by mechanisms involving the endogenous opiate-like peptides. It is interesting, for example, that the analgesic, "antipain" effect of **acupuncture** can apparently be blocked by naloxone.

The opiate-like peptides also modulate secretion of certain pituitary hormones. For example, they are involved with **decreasing GnRH** output, and therefore **LH** and **FSH** release from the pituitary, yet they **facilitate GH** and **PRL** release (probably at the hypothalamic level) (**Part B**, Ch.7).

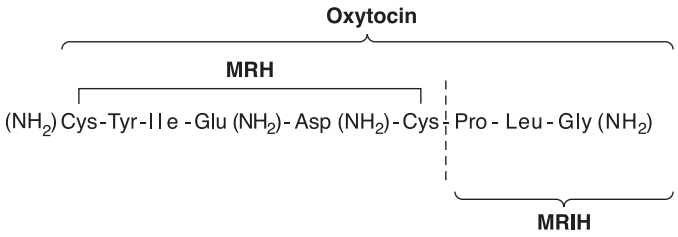
Terminology

Opium contains more than 20 distinct **alkaloids. Opiates** are drugs derived from opium, and include morphine, codeine, heroin, and a wide variety of semisynthetic compounds derived from them. The term **opioid** is more inclusive, applying to all agonists and antagonists with morphine-like activity, as well as to naturally occurring and synthetic **opioid peptides.** Although dynorphin, Met- and Leu-enkephalin have different origins from β-endorphin, the term **endorphin** has become generic, and is used today to refer to all three families of endogenous opioid peptides (enkephalins, dynorphins, and β-endorphins). The term **narcotic** was derived from the Greek work for **stupor**. Although at one time it referred to any drug that induced sleep, it is used today in a legal context to refer to a wide variety of abused substances. Although the term is not likely to disappear, it has lost its physiologic usefulness.

Ch9 Proopiomelanocortin and Related Peptides: II

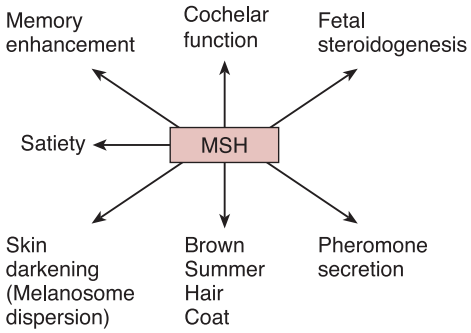
(Melanocyte-Stimulating Hormone)

D



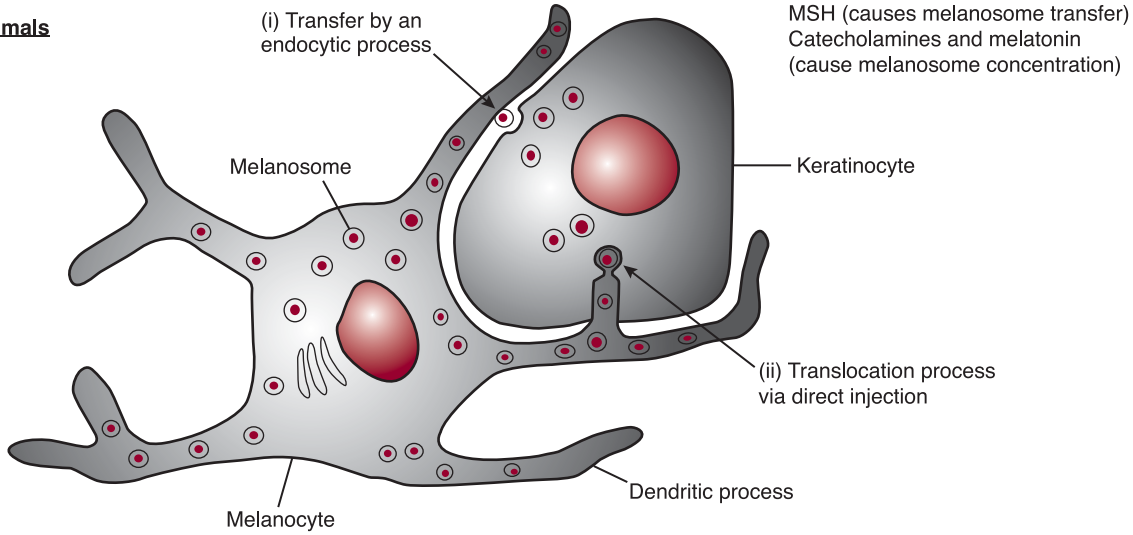
Both MRH and MRIH may have evolved from the oxytocin molecule.

F



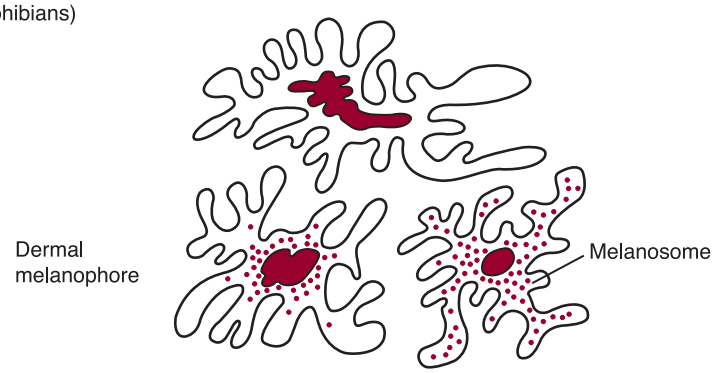
E

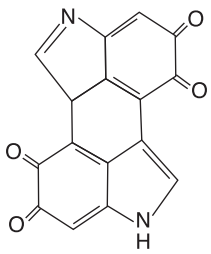
Mammals



The epidermal-melanin unit is composed of 1 melanocyte and approximately 36 neighboring keratinocytes working in synchrony to produce and distribute melanin. Melanosomes synthesize melanin, transfer it to dendritic processes, and then translocate it into keratinocytes. Although cellular mechanisms involved are incompletely understood, suggested mechanisms include (i) melanosome release and endocytosis, and (ii) direct inoculation ("injection"), perhaps secondary to keratinocyte-melanocyte membrane fusion.

Lower Vertebrates
(fishes & amphibians)





Structure of a eumelanin

Melanocyte-Stimulating Hormone

The darkening effect in amphibian skin caused by extracts of the pars intermedia was described earlier last century, with the putative causative agent called **intermedin.** Today, this agent is called **melanocyte-stimulating hormone** (**MSH**). In many mammalian species (e.g., rat, dog, horse, cat, rabbit, ox, etc), the pars intermedia is well-defined and contains large amounts of α-MSH, but in other mammals (and birds) it is practically vestigial, and so α-MSH is thought to originate from the adenohypophysis (anterior pituitary).

The release of MSH from the pars intermedia or the anterior pituitary is controlled by **MSH-releasing hormone** (**MRH**), **MSH release-inhibiting hormone** (**MRIH**), and **dopamine** (Ch. 7). The ring structure of **oxytocin**, which is produced by paraventricular nuclei in the hypothalamus and stored in the neurohypophysis, may be the source of **MRH**, and the tripeptide side chain the source of **MRIH** (**Part D**). There does not appear to be any direct feedback effect on MSH release from target tissues, since stimulation of melanocytes by α-MSH does not release any possible feedback candidates into the circulation. Perhaps the strong two- or three-way control of MSH release by the hypothalamus precludes a biologic requirement for target tissue negative feedback.

There are five known substances with **MSH activity**: **α-MSH**, **β-MSH**, **γ-MSH**, **ACTH**, and **β-LPH**. All are derived from **POMC**. The following **heptapeptide,** which appears in the five substances enumerated above, is apparently responsible for MSH activity: **Met-Glu(or Gly)-His-Phe-Arg-Trp-Gly**. All five molecules above can be extracted from mammalian pituitaries, however, only **ACTH** and **α-MSH** appear to be released from **POMC** *in vivo* to appear at high enough concentrations in the circulation to promote skin darkening.

β-Melanocyte-stimulating hormone (**β-MSH**) is believed to be an artifactual breakdown product of γ-LPH (see **Part A**). While MSH is approximately 30 times more potent than ACTH as a skin-darkening agent, sufficient amounts of **ACTH** can account for **hyperpigmentation** (e.g., **Cushing's-like** and **Addison's-like diseases**). Whether γ-MSH is physiologically significant is unknown.

In addition to its role as a precursor for β-MSH, γ-LPH, β- and γ-endorphin, **β-lipotropin** (**β-LPH**, **Part A**) has been proposed as an adenohypophysial hormone that stimulates lipolysis in adipose tissue (i.e., hydrolysis of stored triglycerides to free fatty acids and glycerol). A standardized bioassay for **β-LPH** activity involves culturing mouse or rabbit epididymal (testis) fat pads, adding **β-LPH** and measuring subsequent release of glycerol and/or free fatty acids into the culture medium. An additional bioassay technique employs measurement of the inhibition of ^{14}C-labeled acetate incorporation into lipid following addition of **β-LPH** to the culture medium. This procedure is, in effect, a measurement of lipogenesis (or lack thereof), which should be inversely correlated with lipolysis. β-Lipotropin, presumably of pituitary origin, has been identified in the systemic circulation of a number of mammalian species; however, concentrations observed have not apparently been high enough to significantly stimulate lipolysis. This brings into question the physiologic significance of **β-LPH** in mammalian lipid metabolism.

At one stage of evolution, **MSH** apparently mediated a protective adaptation (i.e., camouflage in the dark) (Ch. 72). The major bioassay for MSH, which is capable of detecting MSH with great precision over a range of 20 to 50 pg, is based on the darkening of amphibian skin under standardized conditions.

Animals possess a variety of specialized pigmented cells known as **chromatophores**, with the **melanophores** of lower vertebrates (fishes and amphibians) being perhaps the most recognized. Melanophores, containing melanin pigment, are derived from the neural crest, as are the **melanocytes** of higher vertebrates which are largely found in the epidermal stratum germinativum and in hair follicles. Melanophores differ from melanocytes in the manner by which they transfer melanin pigment to adjacent areas (**Part E**). Melanophores undergo rapid color change via intracellular displacement (migration) of melanosomes, while melanocytes transfer melanosomes to adjacent **keratinocytes** of the basal dermal layer via dendritic processes, thus protecting deeper layers of the skin from UV radiation. **Melanin** granules containing brownish-black **eumelanins** and yellow/reddish-brown **phaeomelanins** are concentrated in melanosomes. Melanocytes, unlike keratinocytes, are a stable cell population, normally living many years without undergoing cell division, while keratinocytes divide actively and live only a few days. If melanocytes begin dividing, the consequences can be fatal.

A major target of **α-MSH** in mammals is the **dermal melanocyte**, and in lower vertebrates the **dermal melanophore**. α-MSH stimulation causes **melanosome transfer**, while **melatonin** (in amphibians) and **catecholamines** (in mammals) cause skin pallor (**melanosome concentration**). The term **melanoderma** refers to increased melanin pigmentation of the skin, whereas the term **leukoderma** refers to a loss of this pigmentation.

Since MSH plays only a minor camouflage role in mammals, it is possible that this highly conserved peptide was put to different uses as the evolutionary process continued. Animals that change from a white **"winter coat"** to a brown **"summer coat"** employ the services of **MSH** to stimulate melanin production for the summer coat (**Part F**). Hypophysectomy of the short-tailed weasel during the winter causes the summer coat to be white, and treatment with either MSH or ACTH is sufficient to cause regrowth of the normal brown summer coat. The term **melanotrichia** refers to increased melanin hair pigmentation, whereas the terms **leukotrichia** or **poliosis** refer to a loss of hair pigmentation.

It has been postulated that, in hairy mammals, **MSH** may stimulate modified **sebaceous gland** activity containing pheromones (i.e., sexual attractants secreted to the outside of the body). This function may be important in species that rely heavily on olfaction when participating in reproductive events.

There is an established link between the **white hair coat** of **cats** (and various other animals), **blue eyes** and **deafness**. Neural crest-derived melanocytes are contained within the blood vessel-rich zone of the **cochlea** known as the **stria vascularis**. This structure is responsible for generation of the K^+-rich, Na^+-poor **endolymph** contained in the **scala media**, needed for maintenance of the **endocochlear potential** (critical for hair cell depolarization and auditory nerve electrical signal transduction). Cochlear melanocyte deficiencies are associated with the correspondingly low K^+ composition of endolymph, and subsequent **sensorineural deafness**.

A **melanin-concentrating hormone** (**MCH**; 17 amino acids) has been isolated from the pituitaries of fishes, where it appears to be involved in control of skin color (Ch. 72). MCH blocks MSH and ACTH release in teleosts, and in mammals (where its mRNA can be found in the lateral hypothalamus), it increases food intake (where **α-MSH** has been found to have the opposite effect).

The prominence of a pars intermedia in the primate fetus, coupled with the observation that the **N**-terminal pro-MSH peptide stimulates release of glucocorticoids and aldosterone, indicates to some that this hormone may have a fetal **steroidogenic effect.** Finally, MSH, which is distributed in the brain, has figured prominently in studies on the **enhancement of memory**.

The physiologic roles of α-MSH are not known in **fishes**, where most pigmentary changes apparently are under MCH and ANS control. In **birds**, the fact that feather pigments (including melanin) are under the control of gonadal, thyroidal, and gonadotropic hormones seems to be related to the loss of the pars intermedia. Black feathers, however, reportedly develop in birds treated with either MSH or ACTH (Ch. 72).

Ch10 Growth Hormone: I
(Actions and Secretory Control)

F

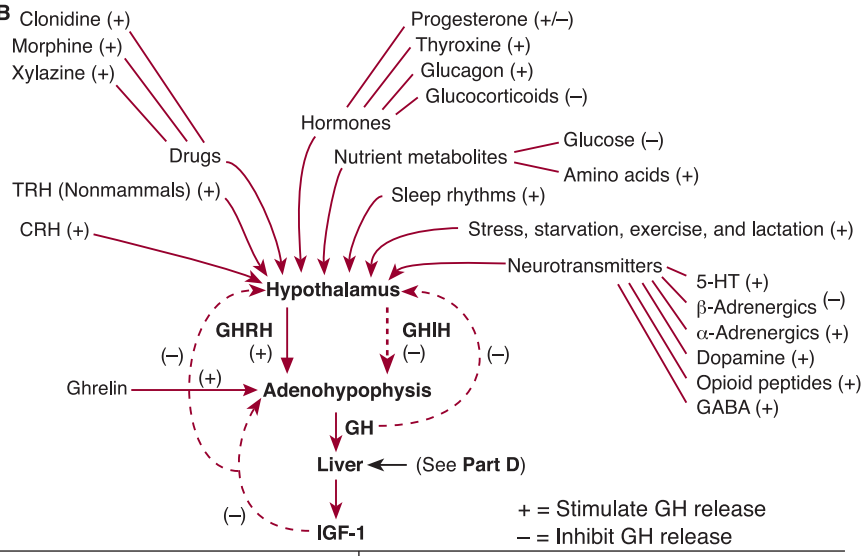
Protein meal = ↑GH, ↑Insulin, ↑ IGF-1

Carbohydrate meal = ↓GH, ↑Insulin, ↓IGF-1

Starvation = ↑GH, ↓Insulin, ↓IGF-1

Pituitary GH is released in response to falling plasma glucose levels, and a high dietary carbohydrate diet reduces GH output. Its role in counteracting hypoglycemia involves interference with insulin' s action on the liver, muscle and adipose tissue. Direct catabolic actions of GH are pronounced during starvation, when insulin levels are low.

B



A

Metabolic Effects of Growth Hormone

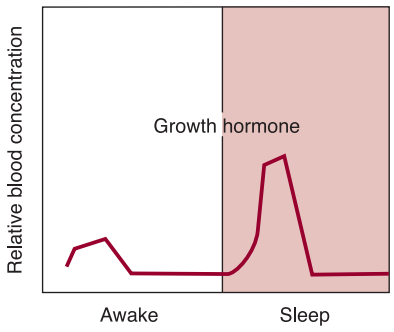
Indirect Growth-Promoting Actions in concert with IGF-1

Accelerates chondrogenesis in epiphyseal plates
Stimulates soluble collagen synthesis and increases urinary hydroxyproline excretion
Stimulates erythropoiesis, and proliferation of lymphoid tissue
Increases intestinal Ca^{2+} absorption & renal Ca^{2+} reabsorption (minor role)
Enhances skeletal maturation
Facilitates amino acid, K^+ & PO_4^{3-} entry into cells, and decreases the BUN
Sensitizes pancreatic β-cells to glucose and arginine
(without itself stimulating insulin release)
Enhances milk synthesis and secretion (ruminants)

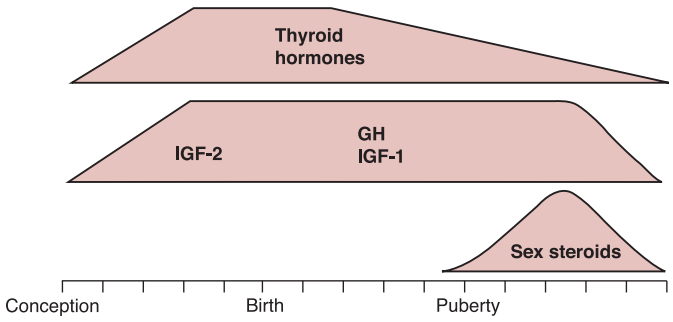
Direct Anti-Insulin Actions

Stimulates lipolysis
Increases hepatic gluconeogenesis
Decreases muscle and adipose hexokinase activity
Spares breakdown of muscle protein
Enhances FFA oxidation by muscle tissue

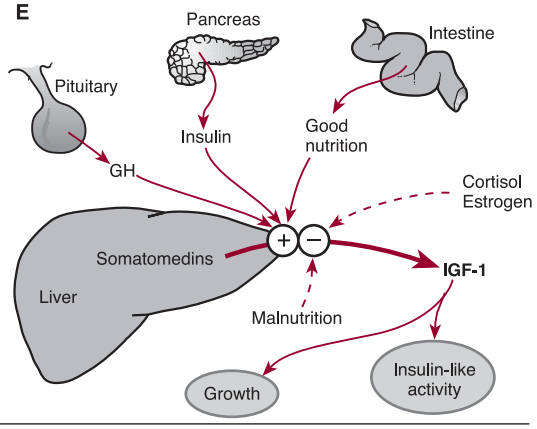
C



D



E



Source: Part D modified from Ganong WF. Review of medical physiology. 18th ed. Stamford, CT: Appleton & Lange, 1997: 382.

Produced by somatotropes of the adenohypophysis (Ch. 7), **somatotropin** (also called **growth hormone** (**GH**)) is a protein of 191 amino acid residues, with 2 to 4 disulfide bridges. Its structure, which is similar to that of **prolactin** (**PRL**) (Ch. 68) and **placental lactogen** (**PL**), varies enough among different animal species that GH from one species may not exhibit GH-like effects in another. Porcine and primate GHs exhibit only transient effects in the guinea pig, and bovine and porcine GHs are thought (by some) to have insignificant endocrine effects in primates. The primary physiologic actions of GH are to promote growth in developing, well-fed animals, and provide a ready source of energy (e.g., glucose and long-chain free fatty acids (FFAs)) during starvation (**Part A**). The indirect **anabolic actions** of GH are mediated largely via other polypeptides known as **somatomedins** (namely **insulin-like growth factor 1**, **IGF-1**), whereas the anti-insulin, catabolic actions during starvation result from its direct effects on target cells in the absence of IGF-1. The somatomedins were so named because they mediate the anabolic actions of somatotropin.

Control of GH Secretion

Growth hormone is secreted from the adenohypophysis in a pulsatile, episodic fashion regulated largely by hypothalamic **GH-releaseing hormone** (**GHRH** or **somatocrinin**), **ghrelin**, and **GH release-inhibiting hormone** (**GHIH** or **somatostatin**), and negative feedback from **GH**, and **IGF-1** (**Part B**). GH secretion undergoes marked and rapid fluctuations in young animals and adults before it declines with old age. It is not surprising that it is under hypothalamic control.

Somatostatin is distributed throughout the nervous system, and in extraneural tissues of the stomach, pancreas, and intestine (Chs. 40, 48 & 51). The main site of **ghrelin** biosynthesis and secretion is the stomach, but it is also produced by the hypothalamus, and has marked GH-stimulating activity. It appears to be involved in the regulation of food intake (Ch. 50).

Approximately one-half of GH secretion occurs during **deep sleep** (**Part C**), with timing during the day and night hours shifted accordingly in nocturnal animals. The episodic pattern of GH release is important in modulating its metabolic activities, because the nearly complete absence of GH effects during trough periods is vital in maintaining its anabolic versus catabolic actions on target tissues. The plasma half-life of GH is about **20 min.**, whereas that of IGF-1 is much longer.

Factors known to **stimulate GHRH** and therefore **GH** secretion include various neurotransmitters and drugs, the hormones ghrelin, progesterone (in dogs), glucagon, thyroxine, ACTH-releasing hormone (CRH), and thyrotropin-releasing hormone (TRH; in nonmammals), as well as stress, exercise, lactation, sleep rhythms, and certain amino acids (e.g., Arg). Factors associated with a **decrease** in **GH** output include β-adrenergics, hyperglycemia, glucocorticoids, synthetic progestins (in primates), and GH and IGF-1 negative feedback (**Part B**).

Differences regarding the effects of the various stimuli above have been reported between species. For example, stress, which is associated with elevating ACTH and thus glucocorticoid levels, generally increases GH secretion in primates and inhibits it in rodents, but has no effect on the GH secretion of domestic ungulates (hoofed mammals). Starvation and lactation increase GH output in primates and domestic ungulates, whereas moderate exercise, amino acids (e.g., Arg), and hypoglycemia give inconsistent results in dogs. Synthetic progestins such as **megestrol acetate** (**MA**), sometimes used for estrus prevention, stimulate GH output in dogs, yet decrease it in primates. MA does not apparently affect GH output in cats, but continued use may lead to diabetes mellitus because of its glucocorticoid activity.

Indirect Growth-Promoting Effects

During fetal and adolescent life, **thyroxine**, **GH**, and the **somatomedins** exert profound synergistic effects on growth and development (**Part D**). **IGF-2** is largely independent of **GH**, and plays an important role in fetal development. In fetuses in which it is overexpressed, growth of organs, especially the tongue, skeletal muscles, kidneys, heart and liver, is disproportionate. Animals with **hyposomatotropism** and/or reduced **IGF-2** levels are born as **pituitary dwarfs**, while those with inadequate fetal **thyroxine** are born as **cretins** (Ch. 38). Secretion of **IGF-1** is largely independent of **GH** before birth, but is stimulated by **GH** after birth, having pronounced growth-stimulating activity. Its concentration rises in plasma during adolescence, peaking at puberty, then (like **GH**) declines with old age. IGF-2 levels are rather constant throughout postnatal growth.

Following the onset of puberty, the **sex steroids** (androgens and estrogens, which are secreted in low amounts throughout adolescence) assume a larger role in modulating growth and development than thyroxine. Although sex steroids initially stimulate pubertal growth, they ultimately terminate it by causing the epiphyses of long bones to fuse, halting linear body growth.

Secretion of **IGF-1** (also known as **somatomedin C**) from the liver and other tissues of young animals, is increased by **good nutrition**, **GH**, and **insulin** (**Part E**). The food supply is the most important extrinsic factor affecting growth, for the diet must be adequate not only in protein content, but also essential vitamins, minerals and calories so that ingested protein is not solely burned for energy purposes.

The IGF-1 peptide structurally resembles proinsulin, and binds to both insulin and IGF-1 receptors. Interestingly, mean IGF-1 (but not GH) concentrations in dogs are reported to vary according to breed, with smaller breeds exhibiting lower concentrations of IGF-1 than larger breeds. A close correlation appears to exist between body size and IGF-1 blood levels. Factors inhibiting release of IGF-1 include the steroid hormones **cortisol** and **estrogen**, as well as **malnutrition**. In animals fed a **protein meal** (e.g., carnivores), blood levels of GH, insulin, and IGF-1 rise (**Part F**). In animals fed a **carbohydrate meal only**, GH and IGF-1 levels fall, yet insulin levels rise. During **starvation**, GH levels rise, yet insulin and IGF-1 levels fall.

GH, **IGF-1** and **IGF-2** stimulate different receptors. **Growth factors** (**GFs**) and **insulin** exert their actions largely through the **MAP K messenger pathway**, while **GH** (**PRL** and **PL**) work through the **JAK-STAT pathway** (Ch. 4). There is remarkable cross-talk between these pathways, thus allowing cells to integrate signals from multiple extracellular stimuli into specific patterns of altered cellular response. For example, **GH** may act on cartilage to convert stem cells into cells that respond to **IGF-1**, allowing locally produced and circulating IGF-1 to make cartilage grow.

The growth-promoting actions of GH (mediated largely through the somatomedins) include enhanced amino acid entry into cells, enhanced Ca^{2+} absorption from the intestinal tract, K^+ and PO_4^{3-} retention by tissues, proliferation of lymphoid tissue, enhanced skeletal growth, and generalized extraskeletal cell proliferation (e.g., muscle, lung, kidney, pancreas, intestine, islets, parathyroids, skin, connective tissue and mammary glands; **Part A**). All of these **anabolic** actions are enhanced by the concurrent presence of thyroid hormones (**T_4** and **T_3**). As chondrogenesis is stimulated, the cartilaginous epiphyseal plates widen and lay down more bone matrix at the ends of long bones. In this manner stature is increased. GH hypersecretion or prolonged treatment with synthetic GH in adolescent animals leads to **gigantism** (Ch. 11).

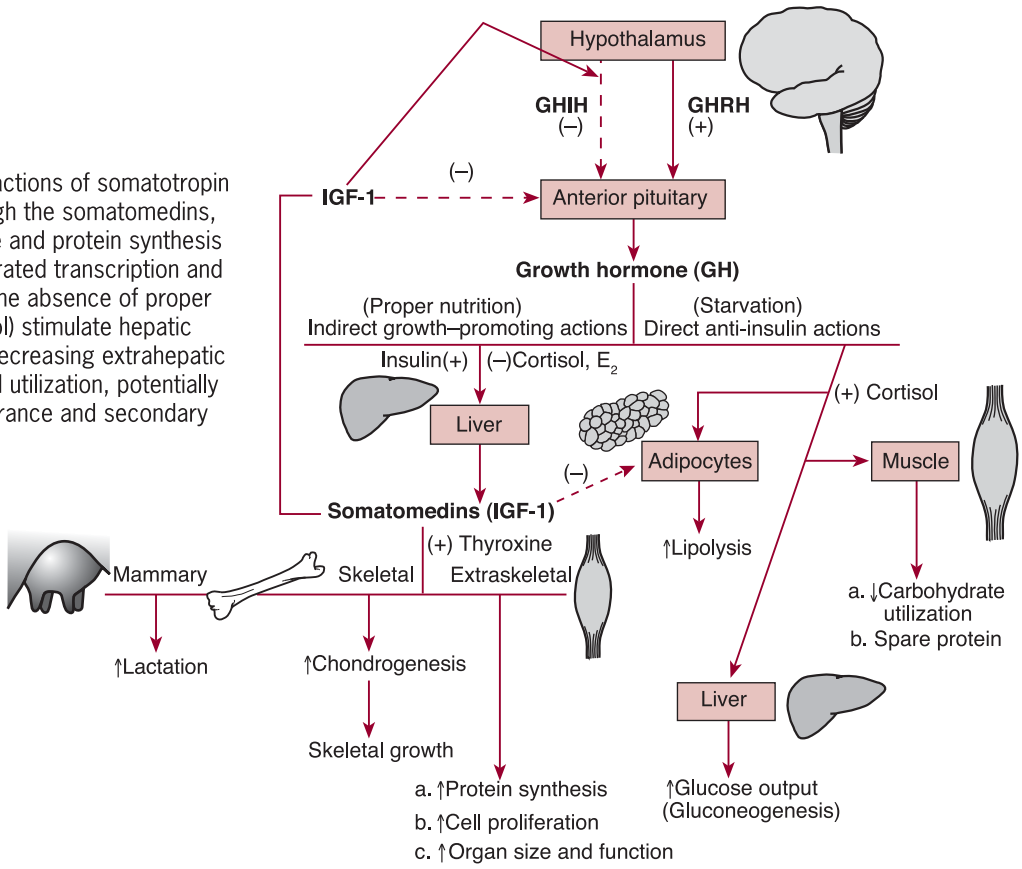
Direct Catabolic Effects

The direct **anti-insulin actions** of **GH** are manifested primarily through carbohydrate and lipid metabolism, and interference with insulin's action on the liver, muscle and adipose tissue. Cortisol and GH (in the absence of insulin) directly stimulate hepatic gluconeogenesis and adipose tissue lipolysis (via activation of hormone-sensitive lipase in adipocytes), while decreasing carbohydrate utilization by muscle and adipose tissue (via suppression of hexokinase activity; **Part A**). GH (but not cortisol) also spares breakdown of muscle protein (while encouraging muscle to extract FFAs from the circulation for energy). Direct **catabolic actions** of **GH** are pronounced during starvation (or hibernation), when insulin levels are low. In abnormal situations, the net effect (if GH levels remain high) could be hyperglycemia, ketonemia, and excessive insulin antagonism. **Diabetes mellitus** and/or **acromegaly** may develop following prolonged increases in the serum GH levels of adult animals, due either to hyperadenohypophysism, to over-use of drugs that stimulate GH secretion, or to over-use of GH obtained from recombinant DNA technology.

Ch 11 Growth Hormone: II
(Hypo- and Hypersomatotropism)

G

Most growth-promoting actions of somatotropin (GH) are mediated through the somatomedins, where amino acid uptake and protein synthesis are enhanced via accelerated transcription and translation of mRNA. In the absence of proper nutrition, GH (and cortisol) stimulate hepatic gluconeogenesis while decreasing extrahepatic carbohydrate uptake and utilization, potentially leading to glucose intolerance and secondary hyperinsulinism.



H

Signs of Hyposomatotropism

Small (proportional) stature
Soft puppy haircoat
Symmetric alopecia
Truncal alopecia
Cutaneous hyperpigmentation
Aggression (e.g., fear biting)
Delayed dental eruption
Short mandible
Suppressed immune responses
Cardiac disorders
Cryptorchidism
Megaesophagus
Testicular atrophy (infantile genitalia)
Estrual abnormalities
Delayed epiphyseal closure
Normal to subnormal mentality
Secondary adrenal insufficiency

I

Signs of Hypersomatotropism

Exercise intolerant
Enlargement of:
- Pharyngeal and laryngeal soft tissues
- Head
- Extremities
- Viscera (cardiomyopathy)

Hyperglycemia
Hypercholesterolemia
Myxedema and excessive skin folds
Broad face
Lower jaw protrusion (prognatism)
Organomegaly
Increased interdental spaces
Rapid toenail growth
PU/PD
Diabetes mellitus (secondary)
Cardiomyopathy/congestive heart failure

Part G summarizes the secretory control and actions of **growth hormone** (**GH**) and **insulin-like growth factor I** (**IGF-1**). Growth is a complex phenomenon that is affected endocrinologically by GH and the somatomedins (IGF-2 and IGF-1), and by thyroid hormones, androgens, estrogens, glucocorticoids, and insulin (Ch. 10). The food supply is the most important factor affecting growth, but various other factors can be associated with **small stature** in domestic animals (e.g., gastrointestinal diseases, portosystemic shunt, glycogen storage diseases, renal and cardiovascular abnormalities, hydrocephalus, skeletal dysplasia, hypothyroidism, hypo- or hyperadrenocorticism, diabetes mellitus, and hypopituitarism). This chapter will be limited to a short discussion of the pathophysiologic effects of **hypo-** and **hypersomatotropism**.

Pituitary Dwarfism

Pituitary dwarfism is largely a result of **hyposomatotropism**, and may be an inherited condition in some dogs and cats. Additional deficiencies in other adenohypophyseal hormones may lead to various degrees of secondary hypogonadism, hypoadrenocorticism, and hypothyroidism (which also limit growth). The most commonly reported cause of pituitary dwarfism in prepubertal dogs is a **cystic Rathke's pouch,** a condition first reported in Germany around 1940. Most domestic animals with primary hyposomatotropism are reportedly detected by 2 to 3 months of age, and are found to grow slowly with near-normal body proportions.

Pituitary dwarfism is sometimes encountered as a simple, autosomal recessive abnormality in the **German shepherd**, where a combined deficiency of GH, TSH, PRL, LH and FSH exists. Pituitary ACTH output is preserved in these animals, which argues against a cystic Rathke's pouch in this breed.

Insensitivity to GH, as seen in the pygmies of Central Africa, may also cause pituitary dwarfism. Circulating levels of **GH** are apparently increased; however, **IGF-1** levels are deficient. This disorder is reportedly associated with absent or defective GH receptors. Insensitivity to GH may also arise from abnormalities in GH structure or lack of responsiveness to IGF-1. Although these secondary causes of pituitary dwarfism may well exist in domestic animals, they have yet to be convincingly described. **All cases of pituitary dwarfism described in dogs and cats to date show low to undetectable GH and IGF-1 concentrations.**

Part H lists several abnormalities associated with pituitary dwarfism. Significant growth takes place only if treatment with GH occurs before epiphyseal closure. Otherwise, response to therapy is reportedly limited. The long-term prognosis for pituitary dwarfism is said to be poor in domestic animals.

Acquired Hyposomatotropism

Hyposomatotropism may also develop in **adult animals** following destruction of the pituitary by inflammatory, traumatic, vascular, or neoplastic conditions. **Panhypopituitarism,** a deficiency of all pituitary hormones due largely to decreased output of hypothalamic releasing hormones, may also occur.

Because **glucocorticoids** suppress GH secretion, prolonged or excessive administration of glucocorticoids, or Cushing's-like syndrome, may cause hyposomatotropism. Signs of acquired hyposomatotropism in dogs include **alopecia** and **hyperpigmentation** (i.e., **adult-onset**, **GH-responsive dermatosis**).

Gigantism and Acromegaly (Hypersomatotropism)

Excess GH causes **gigantism** if it is present before the epiphyses of long bones close at puberty. **Acromegaly** is caused by high circulating titers of GH in the adult, and is commonly associated with pituitary adenoma in older cats, excessive exogenous or endogenous progesterone in female dogs, and GH-induced diabetes mellitus (DM).

In fact, signs of DM may be observed as the initial consequences of acromegaly (e.g., hyperphosphatemia without azotemia, hypercholesterolemia, PU/PD, polyphagia, and a net weight gain of the lean body mass (Chs. 44 and 45)). Although severe insulin resistance and hyperglycemia usually develop, ketosis is reportedly rare in acromegalic animals.

Saucerotte first described acromegaly in 1772 as a condition where patients exhibited excessive growth, and where body proportions became **distorted** because linear growth had ceased and could not be reinitiated. Cartilage tends to proliferate in the joints of patients with acromegaly, resulting in abnormally proportioned extremities and an elongated jaw.

Cats reportedly manifest hypersomatotropism and acromegaly at 8 to 14 years of age, and exhibit many of the signs listed in **Part I.** Polyuria and polydipsia (PU/PD) may result from renal hypertrophy or glucouria (i.e., GH-induced DM). Acromegaly in **dogs** reportedly develops following prolonged administration of **progestins** for estrus suppression (megestrol or medroxyprogesterone acetate), which may cause hypertrophy and hyperplasia of pituitary somatotrophs, or more likely, induced expression of the GH gene in mammary glands, with subsequent release of GH into the systemic circulation. This condition may also evolve in untreated dogs during the **diestrual** phase of the estrous cycle, particularly in intact, older bitches. Pregnancy, which is also associated with prolonged, elevated progesterone levels, is not associated with hypersomatotropism, and progesterone-induced acromegaly has not been described in cats. Progesterone-induced acromegaly has also been associated with the development of canine mammary tumors.

Since GH is a strong diabetogenic hormone, promoting hepatic gluconeogenesis and insulin resistance in peripheral tissues, patients with gigantism or acromegaly are susceptible to **GH-induced DM** (Ch. 44).

Synthetic GH

Mammalian nonprimate GH, previously used to treat dwarfism in young animals and acquired hyposomatotropism in adult dogs and cats, has been replaced by **synthetic human GH** (**hGH**), manufactured by recombinant DNA technology. Human GH is similar to mammalian nonprimate GH immunologically, and it appears to be active in the dog and possibly the cat. **Recombinant bovine somatotropin** (**bST or rbST**) also appears to be biologically active in dogs. **Canine GH** is not currently available for therapeutic use, and reports of treating dogs with **porcine GH**, **hGH** or **bST** have not necessarily been good, largely due to antibody formation. Since **progestins** induce expression of the GH gene in canine mammary glands with subsequent release of GH into blood, this therapy has apparently been somewhat successful. Numerous side effects, however, have been reported.

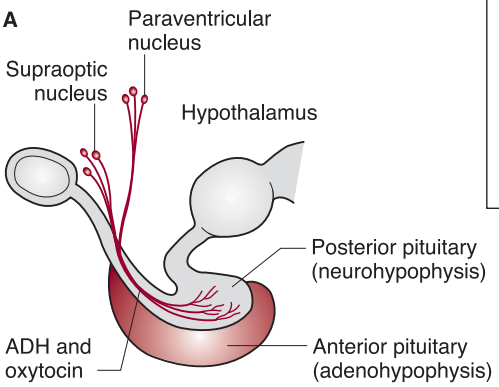
Recombinant bovine somatotropin has made possible the manipulation of bovine lactational physiology. It appears that **bST** (working through **IGF-1**) can increase milk production in dairy cows by **5%** to **25%** after the first two to three months of lactation, with feed efficiency increasing from **5%** to **15%**. Given daily injections of bST (or use of sustained-release preparations), this increased yield has been reported to persist throughout the remainder of lactation. An increase in the average number of services (from about 2.0 to 2.5), however, is apparently required to achieve conception, which causes cows to remain open (unbred) for about 21 days longer.

bST stimulates hepatic gluconeogenesis and mimics PRL activity on the mammary glands by enhancing their ability to synthesize milk components. It reportedly partitions nutrients to these tissues while taking them away from other organs. Pharmacologic doses of somatotropin injected into growing **pigs** and **lambs** reportedly increase nitrogen retention, improve feed efficiency, increase muscle mass, reduce carcass lipid content, and increase carcass protein content.

GH, **IGF-1**, **PTH-related peptide** (**PTH_{rp}**), and **PRL** have all been detected in milk (Ch. 67).

Ch12 Antidiuretic Hormone (Vasopressin)

A



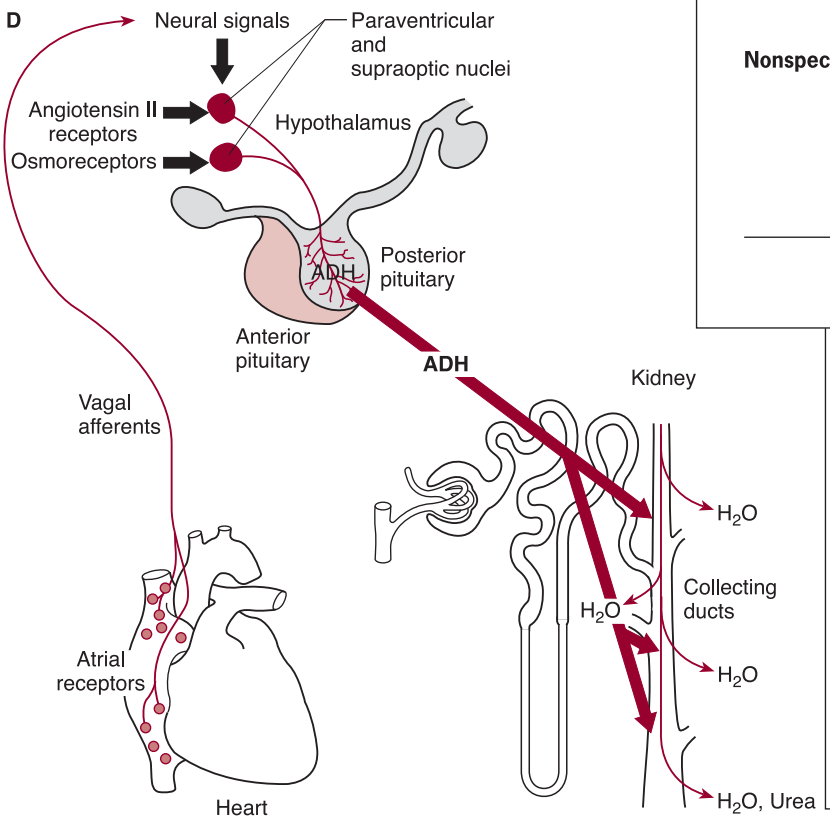
B

	Oxytocin	ADH	AVT	DDAVP
	NH_2	NH_2	NH_2	
1.	Cysteine ----S	Cysteine -------S	Cysteine ----S	Cysteine ------S
2.	Tyrosine	Tyrosine	Tyrosine	Tyrosine
3.	Isoleucine	*Phenylalanine	Isoleucine	Phenylalanine
4.	Glutamine	Glutamine	Glutamine	Glutamine
5.	Asparagine	Asparagine	Asparagine	Asparagine
6.	Cysteine ----S	Cysteine -------S	Cysteine ----S	Cysteine ------S
7.	Proline	Proline	Proline	Proline
8.	*Leucine	Arginine (Lys in pigs)	Arginine	D-Arginine
9.	Glycinamide	Glycinamide	Glycinamide	Glycinamide

C

	Stimulate ADH secretion	Inhibit ADH secretion
Specific	Hypovolemia Hypernatremia Angiotensin II	Hypervolemia Hyponatremia
Nonspecific	Insulin β-Adrenergics Morphine Hypoglycemia Stress, exercise, pain, trauma, nausea Lactation	α-Adrenergics Opiate antagonists Ethanol Glucocorticoids

D



E

ADH Receptor	Second Messenger	Function
V_{1A}	↑IP_3, Ca^{2+}	Vasoconstriction, Hepatic glycogenolysis, CNS transmitter
V_{1B}	↑IP_3, Ca^{2+}	ACTH secretion
V_2	↑cAMP	Renal water retention

Source: **Part A** modified from Chastain CB, Ganjam VK. Clinical endocrinology of companion animals. 1st ed. Philadelphia, PA: Lea & Febiger, 1986:47. **Part E** modified from Berne RM, Levy MN. Principles of physiology. 1st. ed. St. Louis: Mosby, 1990:447

Antidiuretic hormone (**ADH or arginine vasopressin, AVP**), a nonapeptide secreted by the posterior pituitary, inhibits diuresis and increases urinary osmolarity by increasing permeability of collecting ducts of the kidneys so that water is reabsorbed into the hypertonic interstitium. Without the effects of ADH, the urine produced is voluminous and hypotonic to plasma (**hyposthenuric polyuria**).

Neuropeptides of the Posterior Pituitary

Although the posterior pituitary contains several neuropeptides that may possess important physiologic actions (Ch. 72), the best characterized are **oxytocin** (Ch. 69) and **ADH**. Both are neurohormones synthesized by paraventricular and supraoptic nuclei of the hypothalamus (**Part A**), and each has a characteristic **neurophysin** associated with it as it passes down axons to be stored in granules of the **neurohypophysis** (**posterior pituitary**). Both the neuropeptide and its neurophysin are exocytosed into the general circulation following an action potential; however, they quickly dissociate in plasma. Neurophysins were originally thought to be binding polypeptides, but it now appears they may be parts of their precursor.

In hippopotamuses and most pigs, **arginine** of the more typical mammalian AVP molecule is replaced by **lysine** to form **lysine vasopressin** (**Part B**). The posterior pituitary of some species of pigs and marsupials contains a mixture of arginine and lysine vasopressin. Each is a nonapeptide consisting of a six-member disulfide-containing ring. The structures of ADH and oxytocin differ by only two amino acids, and both are derived from a single precursor present in non-mammalian vertebrates, **arginine vasotocin** (**AVT**) (see **Part B**), that exhibits similar physiologic properties. The arginine in position 8 of ADH is critical to its **pressor** activity, but not its **antidiuretic** action. Substitution of D-arginine in this position, along with removal of the terminal amino group of cysteine, produces a highly potent and long-acting antidiuretic peptide possessing virtually no pressor activity, namely **1-desamino-8-D-arginine vasopressin** (**DDAVP**) (see **Part B**). This agent is used to treat **DI** (Ch. 13).

Factors Regulating ADH Secretion

Stimuli and inhibitors of ADH secretion are presented in **Part C.** Secretion of ADH is regulated mainly by the **osmolarity of plasma** and changes in **blood volume** and/or **pressure**. Hypothalamic nuclei that synthesize ADH (or some closely related nuclei) act as **osmoreceptors**, sensing modest changes in plasma osmolarity (namely the Na^+ concentration). An increase in plasma osmolarity of only **1%** causes these osmoreceptors to shrink, and to initiate nerve impulses that release **ADH**. Conversely, when plasma osmolarity is reduced, secretion is inhibited. Because ADH is rapidly degraded in plasma (biologic **t½** is approximately **5 to 10 minutes**), circulating levels can be reduced to zero within minutes after secretion is inhibited. As a result, the ADH system can respond rapidly to fluctuations in plasma osmolarity.

A decrease in blood volume or pressure is sensed by low-pressure stretch receptors (i.e., **baroreceptors**) in the left atrium, and high-pressure receptors in the aortic arch and carotid sinus. Signals from these receptors are relayed to ADH secretory neurons via afferent fibers in the vagus nerve. The sensitivity of this baroreceptor system is less than that of the osmoreceptors, and a **5%** to **10%** change in volume is required to alter ADH secretion.

Decreases in blood volume and/or pressure are also sensed by **juxtaglomerular** (**JG**) **cells** in the kidney that synthesize and secrete **renin**. As pressure declines, renin levels increase, which in turn elevates plasma levels of **angiotensin II** (Chs. 27 and 28). Angiotensin II is a potent vasoconstrictor, as well as a stimulus for thirst, aldosterone and ADH release. **Part D** depicts the regulation of ADH secretion and its effect on water retention in the kidney. During periods of dehydration, **increased plasma osmolarity** provides about **70%** of the increased thirst drive, the remaining **30%** is due to **hypovolemia**.

Dehydrated animals (e.g., dogs, cats, camels and others) sometimes rapidly drink just enough water to compensate their water deficit. They usually stop drinking before the water is fully absorbed, and while their plasma is still hypertonic. It has been hypothesized that some pharyngeal "metering" takes place, but it is not well described. **Water intoxication** is not uncommon in ruminants deprived of water. If excessive, extra- and intracellular fluid compartments rapidly expand, hemolysis can occur with normal cellular metabolic processes disrupted, sometimes causing death. Similar symptoms can occur with the **syndrome of inappropriate ADH** (**SIADH**) **secretion**, often due to noxious stimuli, various drugs or excessive ectopic secretion of ADH by certain tumors. When dilution develops slowly, marked degrees of hypoosmolarity may occur with few symptoms, although the danger of water intoxication remains.

Primary Actions of ADH

Blood pressure is one factor that determines the renal glomerular filtration rate (GFR). The filtrate normally lacks plasma proteins and cellular components of blood, and the initial concentrations of solutes like Na^+, glucose, and amino acids are identical to those in plasma. Numerous mechanisms operate to return solutes and most of the water back into blood; however, if something interferes with these reabsorptive processes, a larger than normal amount of urine will be produced (i.e., **diuresis**). Should more reabsorption occur than normal, more fluid is reabsorbed and **antidiuresis** results. Regulation of water reabsorption in the kidney is essential in the maintenance of a normal blood volume and pressure.

As previously stated, an increase in the osmolarity of plasma or a decrease in blood pressure triggers release of **ADH**, which in turn causes increased water reabsorption in the kidneys, **antidiuresis** and a concentrated urine. Similarly, an increase in blood pressure and/or a decrease in the osmolarity of plasma represses ADH release, and causes diuresis with a corresponding drop in blood volume and pressure. Thus, ADH is important in the **minute-to-minute** control of blood volume and pressure because it is stored by the posterior pituitary, and can be released quickly upon demand. Another important action of ADH occurs in the brain, where it stimulates **thirst** (like angiotensin II; Ch. 27). Consumption of water will also add fluid to the vascular system, and will thus increase blood pressure.

Antidiuretic hormone acts in the kidney to increase permeability of the **collecting ducts** to water. A simplified model of this action is shown in **Part C**, Ch. 13. ADH binds to a $\mathbf{V_2}$ receptor on the basolateral membrane of renal target cells. Binding to this receptor, which is coupled to adenyl cyclase, increases intracellular levels of **cAMP**, which in turn activates one or more protein kinases. Phosphorylated proteins resulting from this next act to insert water channels (**aquaporins**) into the apical (i.e., luminal) membrane. Aquaporins are found not only in collecting ducts of the kidney, but also in the brain, salivary and lacrimal glands, and respiratory tract.

There are at least three types of ADH receptors: $\mathbf{V_{1A}}$, $\mathbf{V_{1B}}$, and $\mathbf{V_2}$, and all are G protein–coupled (**Part E**).

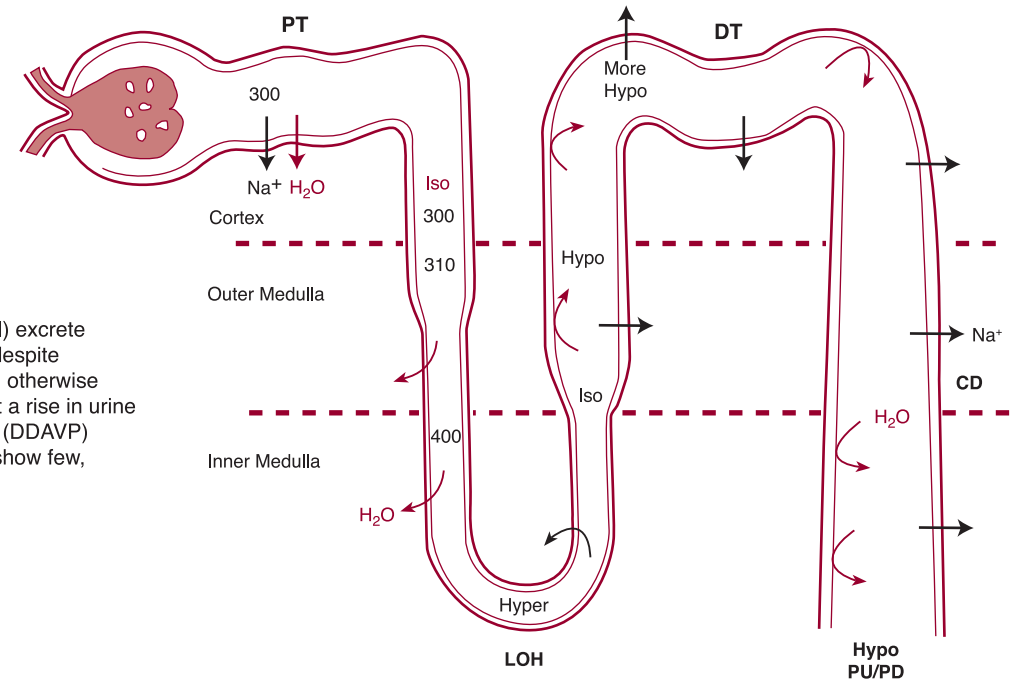
Secondary Actions of ADH

The **pressor actions of ADH** include vasoconstriction of systemic, coronary, and pulmonary blood vessels, and dilation of cerebral and renal vessels largely through $\mathbf{V_{1A}}$ receptor stimulation. The dose of ADH that produces pressor activity is about **100 times** the dose that provides its antidiuretic action; therefore, ADH is probably not a physiologic pressor agent in mammals. However, high doses of ADH can cause constriction of arteriolar smooth muscle and elevate the blood pressure. This may increase the GFR enough to override the antidiuretic action of ADH and, therefore, produce a net **diuresis**.

In large amounts, ADH also stimulates secretion of **ACTH**. An ADH response test using changes produced in serum **cortisol** levels has been recommended as an indicator of hypothalamic–pituitary function. An increase in serum cortisol after administration of ADH would indicate normal adenohypophyseal function. Conversely, **high circulating levels of cortisol inhibit ADH release**, as well as exerting separate actions on the kidney (**Part C;** Chs. 22 and 24).

Ch13 Diabetes Insipidus
(Central and Nephrogenic)

A



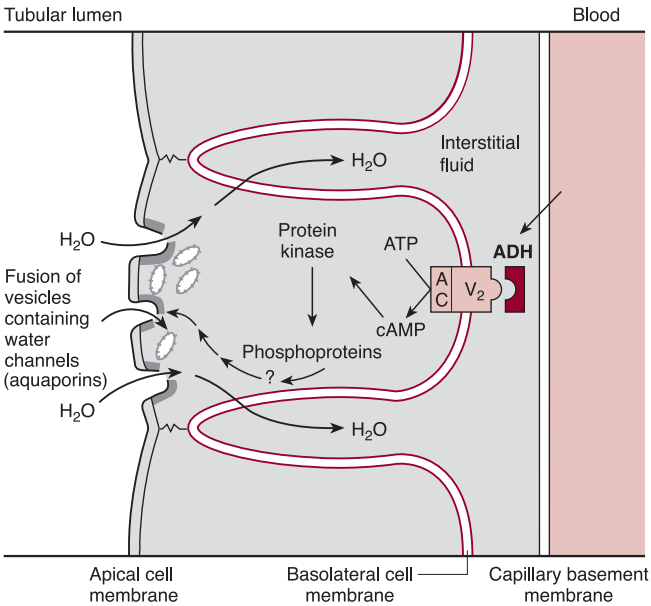
Patients with diabetes insipidus (DI) excrete large volumes of hypotonic urine despite a high serum osmolarity that would otherwise promote ADH release. They exhibit a rise in urine osmolarity following desmopressin (DDAVP) administration, and they typically show few, if any, signs of kidney disease.

B

Causes of Diabetes Insipidus

Central (CDI)		CDI may result from any condition that damages the neurohypophyseal system (e.g., trauma, neoplasia, infections, vascular (aneurysms), cysts, autoimmune hypothalamitis). Permanent CDI usually requires an injury causing bilateral neuronal degeneration of supraoptic and paraventricular nuclei.
Nephrogenic (NDI)		
Primary (familial)		Congenital defect involving impaired renal responsiveness to ADH
Secondary (acquired)	**Pyometra:**	*Escherichia coli*-associated pyometra can cause reversible renal tubular insensitivity to ADH.
	Hypercalcemia:	Damages renal tubular ADH receptors and inactivates adenyl cyclase.
	Hepatic insufficiency:	Decreased urea production decreases the renal medullary concentration gradient.
	Hyperadrenocorticism:	Cortisol interferes with ADH action.
	Hypoadrenocorticism:	Aldosterone deficiency causes renal medullary solute washout, and loss of the medullary concentration gradient. Cortisol deficiency leads to hypercalcemia.
	Pyelonephritis:	Inflammation of the renal pelvis.
	Hypokalemia:	Collecting ducts are less responsive to ADH.
	Hyperthyroidism:	Mechanism unclear.

C Medullary Collecting Duct of the Kidney



The binding of **ADH** to V_2 receptors on basolateral membranes of epithelial cells in the collecting ducts increases intracellular **cAMP**, which in turn initiates cellular events leading to the insertion of **aquaporins** into apical membranes. This allows H_2O of the filtrate to be reabsorbed into the hypertonic medullary interstitium, thus **concentrating the urine.**

Patients with **diabetes insipidus** (**DI**) produce rather large volumes of hypotonic, tasteless (i.e., insipid) urine, and therefore exhibit dire thirst (**Part A**). Although several factors can cause **polyuria/polydipsia** (**PU/PD**) in domestic animals, **DI** is suspected when water consumption is greater than 100 ml/kg/day, and urine production (**without glucose**) is greater than 50 ml/kg/day. The high fluid consumption may interfere with food intake in severe cases, resulting in weight loss.

DI is primarily a water abnormality (**water out** > **water in**), and serum profiles may not reveal abnormalities other than hypernatremia (& hyperosmolarity). If water is withheld from these patients, they can develop life-threatening hypertonic encephalopathies (serum [Na^+] > 170 mEq/L; serum osmolarity > 375 mOsm) quickly. The causes of DI are subdivided into those of **central** or **nephrogenic** origin, as follows.

Central Diabetes Insipidus (CDI)

Central diabetes insipidus results from the destruction of ADH production sites in the hypothalamus (the supraoptic and paraventricular nuclei), loss of major axons that carry ADH to its storage sites in the posterior pituitary, or disruption of the ability to release stored ADH (**Part B**). Animals with **CDI** reportedly have difficulty increasing their urine osmolarity, even with water restriction. Consequently, the specific gravity of their urine can remain **hyposthenuric** (**≤ 1.006**).

Although CDI may have multiple etiologies, the most common identifiable cause appears to be **head trauma**. Metastatic tumors that develop in the hypothalamus or posterior pituitary can also cause CDI, as can infections, aneurysms and cysts. Although an autoimmune hypothalamitis leading to CDI has been described in humans, where ADH cell antibodies are present, this form of CDI has not yet been reported in animals.

CDI is reported to temporarily follow pituitary surgery, but if damage to the pituitary stalk induces retrograde degeneration of hypothalamic neurons, CDI can apparently become permanent.

An **absolute primary polycythemia** due to EPO excess leads to an increased blood volume, hyperviscosity of blood, and impaired microcirculation of the brain. ADH release is impaired, with resultant PU/PD. Neurological symptoms include ataxia, seizures, blindness, tremor, and alterations of behavior.

Nephrogenic Diabetes Insipidus (NDI)

Diabetes insipidus of renal origin (**i.e., NDI**), can be caused by a number of etiologies that result in the inability of the kidneys to respond to ADH. Plasma ADH concentrations may be normal to elevated; however, a partial or nearly complete lack of renal responsiveness has been noted. **Primary NDI** is characterized by an inability of the renal aquaporin mechanism to allow water reabsorption from collecting ducts, and **secondary** (**or acquired**) **NDI** is associated with several disorders that interfere with normal interactions between ADH and its renal receptors. These are referred to as secondary because ADH, its receptor sites and postreceptor mechanisms are apparently present, however, less responsive (**Part C**).

Female dogs and cats sometimes develop **pyometra** due to the presence of *E. coli*-associated endotoxins. Renal concentrating mechanisms in the kidneys become compromised due to developing ADH insensitivity, and PU/PD develops. When the pyometra is successfully treated, this type of acquired NDI can disappear within a matter of days.

Hypercalcemia can also cause secondary NDI by damaging ADH receptors on cell membranes of medullary collecting ducts, and inactivating the postreceptor cAMP mechanism. PU/PD is a common early symptom of hypercalcemia, which has many etiologies (see succeeding Chapters).

Hepatic insufficiency may cause PU/PD if urea output is compromised. Urea generally accounts for about 25% of the renal medullary concentration gradient, and, when deficient, is associated with increased urine output and compensatory polydipsia.

Cushing's-like syndrome and disease (**hyperadrenocorticism**), due to several etiologies (Ch. 25), is also associated with secondary DI. **Glucocorticoids** may interfere with the release of ADH from the posterior pituitary or the actions of this hormone on collecting ducts, and they also have a tendency to increase the glomerular filtration rate (GFR). In some instances (e.g., pituitary ACTH-secreting tumors), the tumor itself may cause compression of neurosecretory cells in the posterior pituitary, thus reducing ADH output.

Hypoadrenocorticism (**Addison's-like disease**) can result in both a mineralocorticoid (i.e., **aldosterone**) and glucocorticoid (i.e., **cortisol**) deficiency (Ch. 29). The aldosterone deficiency causes natriuresis, hyperkalemia, eventual renal medullary solute washout, and diuresis, while the cortisol deficiency leads to (among other things) a hypercalcemia due to excessive Ca^{2+} reabsorption throughout the nephron. The hypercalcemia, in turn, decreases renal responsiveness to ADH (see above).

Pyelonephritis, an infection and inflammation of the renal pelvis, affects the active concentrating mechanism in the ascending thick limb of the loop of Henle, which eventually leads to loss of the medullary concentration gradient. Secondary NDI associated with pyelonephritis can apparently progress to renal failure.

Hypokalemia, like hypercalcemia, is thought to cause the collecting ducts to become less responsive to ADH, and it may also reduce ADH release from the posterior pituitary. Hypokalemia is associated with reduced aldosterone release from the adrenal cortex, which in turn causes less Na^+ to be reabsorbed into the renal interstitium. Hypokalemia is also associated with a decrease in neuromuscular irritability (Ch. 19), which is a more common symptom than PU/PD.

PU/PD is a common finding in animals with **hyperthyroidism** (Ch. 39). Although the mechanism is unclear, it is believed that the increased medullary blood flow associated with this disease may cause solute washout from the *vasa recta*, thus decreasing medullary hypertonicity. The GFR is also increased in hyperthyroid animals, and high titers of thyroid hormones may have a direct inhibitory affect on the concentrating abilities of the nephron.

Many patients with DI are reportedly classified as being **idiopathic** (i.e., denoting that they possess a disease of unknown origin), and exhibit no other evidence of neuroendocrine dysfunction. **Excessive water intake** can also give rise to polyuria, and, therefore, is sometimes classified as a type of DI. Excessive drinking may be a behavioral problem (i.e., psychogenic polydipsia), or it may result from a malfunction in the thirst mechanism (dipsogenic DI).

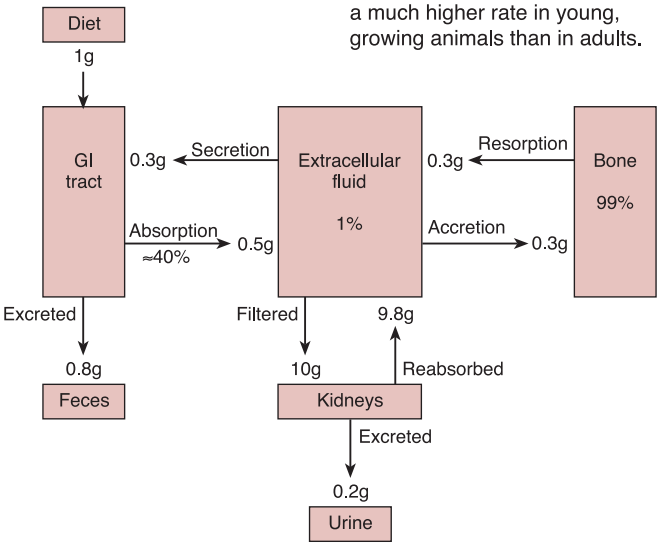
Desmopressin (1-desamino-8-D-arginine vasopressin, DDAVP, **Part B**, Ch. 12) is a synthetic analog of the naturally occurring ADH, and is the drug of choice for treating DI. Desmopressin not only has **antidiuretic** properties, but also **hemostatic** properties. It causes platelet aggregation and the release of hemostatic factors in dogs, and, therefore, is sometimes used in the treatment of bleeding disorders. The biological half-life of DDAVP is reported to be 4 to 8 times longer than the natural hormone, and is sometimes administered intranasally, instilled into the conjunctival sac, injected subcutaneously, or in tablet form. Because most peptides are digested in and by the intestine, orally administered vasopressin is less effective. Although patients with idiopathic DI have been reported to be favorably treated, patients with nephrogenic DI and those with hypothalamic or pituitary tumors apparently have a less favorable prognosis, particularly if neurologic signs are evident. Central DI due to head trauma has a variable prognosis, with some patients recovering satisfactorily.

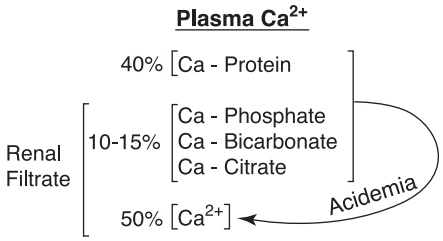
Sensitive radioimmunoassays for ADH are available that allow plasma ADH to be measured. Although random plasma samples are of little value, ADH concentrations can be determined as a part of "dynamic" testing, either during H_2O deprivation or with infusions of a hypertonic NaCl solution.

Ch 14 Calcium (Physiologic Actions)

B Calcium Homeostasis

The Ca^{2+} in bone turns over at a much higher rate in young, growing animals than in adults.



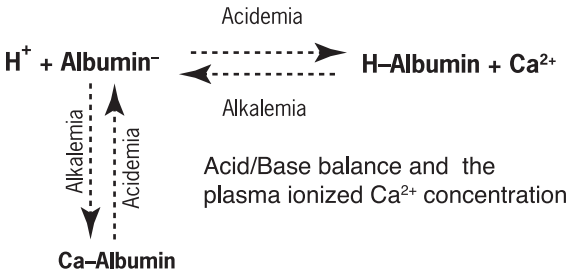


A

Primary Functions of Ca^{2+}

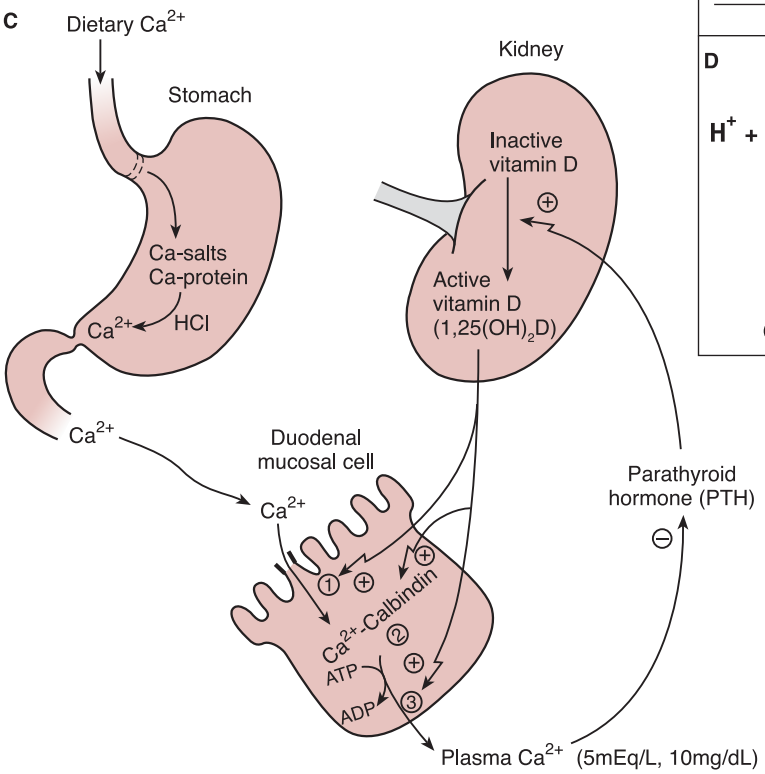
Neurotransmitter release	Blood coagulation
Membrane permeability	Bone formation
Muscle contraction	Cell motility
Enzyme activation	Cell secretion
Intracellular second messenger	Cellular differentiation
Hormone release	Cell death (apoptosis)

D



Acid/Base balance and the plasma ionized Ca^{2+} concentration

C



Ca^{2+} metabolism in the rabbit differs in several respects from that in other mammalian species (see Ch. 72). The amount absorbed by the intestine appears to increase in direct proportion to the amount consumed, being relatively independent of $1,25(OH)_2D$. Consequently, increased amounts normally appear in urine.

Serum **calcium** (**Ca^{2+}**), **magnesium** (**Mg^{2+}**), and **phosphate** (**PO_4^{3-}**) levels are closely regulated in all domestic animal species by the actions of **vitamin D**, **PTH**, and **calcitonin** on the **GI tract**, **bone**, and **kidneys**. The active form of **vitamin D** (**ercalcitriol or calcitriol ($1,25(OH)_2D$)**) and **PTH** tend to raise serum Ca^{2+} levels, an action counterbalanced by **calcitonin**. Other hormones such as **glucocorticoids**, **estrogens**, **glucagon**, and **growth hormone** normally play minor regulatory roles in Ca^{2+} homeostasis.

Calcium

The **calcium** concentration of extracellular fluid and that in the cytoplasm of cells must be regulated within narrow limits if normal body functions are to be maintained. Calcium ions serve many important roles in the body (**Part A**). They are components of bones and teeth, they are responsible for excitation and contraction of muscle cells, as well as the induction of spontaneous excitations of cardiac pacemaker cells (Ch. 19). Calcium ions are essential for exocytosis of secretion granules in neurons and glandular cells, and they serve as second messengers in many target cells (Ch. 5). Certain key metabolic enzymes are activated by intracellular Ca^{2+}, and Ca^{2+} serves as a co-factor for several important blood-clotting proteins (factors VII, IX, and X).

The lipid bilayer of cell membranes normally exhibits a low permeability to Ca^{2+}; therefore, influx of Ca^{2+} into responsive cells is controlled by a heterogeneous group of Ca^{2+} channels regulated by membrane potential, intracellular messengers, and ligands targeting cell membrane receptors. These receptors are coupled to G-protein, and are found on cardiac and smooth muscle, on parathyroid chief cells, thyroid C-cells, renal tubular epithelial cells, and the placenta (among other tissues). The Ca^{2+} receptor is responsible for sensing the ionized Ca^{2+} concentration. Influx of Ca^{2+} into responsive cells is known to regulate cell function by interactions with intracellular Ca^{2+}-binding proteins (e.g., calmodulin), and calcium-sensitive protein kinases (Ch. 5).

Calcium is the **fifth most abundant element in the body**. Nearly **99%** is found in the skeletal system, where calcium, together with phosphate, is essential for bone strength and serves as a storehouse to replenish serum deficits. However, less than **1%** of skeletal Ca^{2+} is available normally in adult animals for free exchange with extracellular fluid (ECF) (**Part B**).

The normal ECF concentration, which is carefully regulated at about **10 mg/dL** (**5 mEq/L**), changes little over a lifetime despite major fluctuations in dietary Ca^{2+}, Ca^{2+} entering and leaving bone, renal Ca^{2+} excretion, and the additional demands of pregnancy or lactation. Intracellular Ca^{2+} is compartmentalized, yet the free cytoplasmic concentration (normally about 10,000 times lower than the ECF concentration), can change dramatically as a result of release from intracellular stores and influx from the ECF.

An ordinary 75-kg adult mammal ingests about **1 g/day** of calcium in food and liquid (as shown in **Part B**). Calcium also enters the gut in digestive secretions and as part of desquamated mucosal cells. Also, during active glucose absorption intercellular spaces between mucosal cells become swollen, and Ca^{2+} gets secreted into the lumen through tight junctions. Total Ca^{2+} entering the lumen by these means (less ingestion) amounts to about **0.3 g/day**. Consequently, about **1.3 g/day** is available for absorption. Approximately **0.5 g** is absorbed (**≈ 40%** of that available), and **0.8 g** is excreted in the stool. Although **0.5 g/day** is absorbed, net absorption amounts to only **0.2 g/day**. In the normal, non-pregnant adult animal, renal Ca^{2+} excretion is generally balanced by intestinal absorption. If the plasma Ca^{2+} concentration declines, **intestinal Ca^{2+} absorption**, **bone resorption**, and **renal tubular Ca^{2+} reabsorption** increase in an attempt to return plasma concentrations to normal levels. During growth and pregnancy, intestinal absorption normally exceeds urinary excretion, and Ca^{2+} accumulates in newly formed fetal tissues and bone. Metabolic bone diseases (MBDs) that promote reductions in bone mass (osteopenia), and metabolic abnormalities that reduce lean body mass usually increase urinary Ca^{2+} excretion without altering intestinal absorption (Chs. 18 and 72).

Active Ca^{2+} absorption occurs primarily in the **upper small intestine** (i.e., the duodenum) by a three-step mechanism (**Part C**). Calcium is not ionized at neutral pH; instead, gastric **HCl** solubilizes calcium salts and frees Ca^{2+} from dietary protein, thus permitting small intestinal absorption. The active form of **vitamin D** (**$1,25(OH)_2D$**) plays a critical role in duodenal Ca^{2+} absorption by opening Ca^{2+} channels in mucosal cell membranes, and stimulating transcription of specific proteins, including Ca^{2+}-ATPase and calbindin, a cytoplasmic Ca^{2+}-binding protein. Through these actions vitamin D can increase the efficiency of intestinal Ca^{2+} absorption from **40-70%**. By **opening Ca^{2+} channels** in mucosal cell membranes (step **1** in **Part C**, an effect independent of transcription), vitamin D helps to increase Ca^{2+} influx from the lumen. This phenomenon, known as **transcaltachia**, occurs over a period of seconds to minutes, whereas the effects on transcription take hours. The gradient for Ca^{2+} influx is secondarily maintained by the buffer action of **calbindin** (step **2** in **Part C**), which as a Ca^{2+}-binding protein exhibits significant homology with calmodulin, and myosin light chain. Step **3**, the active pumping of Ca^{2+} out of mucosal cells, is facilitated by **Ca^{2+}-ATPase**. In addition to intestinal Ca^{2+} absorption, vitamin D may also facilitate the active absorption of **PO_4^{3-}** and **Mg^{2+}**. The mechanisms for these events are independent of and less well defined than those in the absorption of Ca^{2+}.

Serum Ca^{2+} exists in more than one form, yet about **50% is ionized** (**Part B**). Ionized Ca^{2+} is the only form that influences secretion of PTH, and can be used for the maintenance of neuromuscular excitability and blood coagulation. Approximately **10% to 15%** of serum calcium is complexed with **phosphate**, **citrate**, or **bicarbonate**, while **40%** is **protein bound** to albumin and, to a much lesser extent, α- and β-globulins. Complexed and protein-bound forms are circulating storage forms from which Ca^{2+} can be readily released.

Plasma proteins like albumin are more ionized (**$albumin^-$**) when the plasma pH is high (**alkalemia**), providing more protein anion to bind with Ca^{2+} (**Part D**). Conversely, in **acidemia** plasma proteins become less ionized, thereby giving up their bound Ca^{2+}. Hypercalcemia, which can result from acidosis, also occurs with excessive resorption of bone or increased absorption from the GI tract. Symptomatic **hypercalcemia** generally results in decreased neuromuscular excitability (as well as "bones, groans, and stones"), while **hypocalcemia** results in increased neuromuscular excitability (and tetany) (Chs. 18 and 19).

About two-thirds of the bone mass consists of inorganic minerals, especially **hydroxyapatite** (**$Ca_{10}(PO_4)_6(OH)_2$**), but also **brushite** (**$CaHPO_4$**), and the **Na^+**, **K^+**, and **Ca^{2+} salts of carbonate** (**CO_3^{2-}**). Thus, bone contains a number of proton acceptors, including PO_4^{3-}, HPO_4^{2-}, OH^-, and CO_3^{2-}, and bone dissolution can help mitigate a fall in pH. During the acute, uncompensated phase of metabolic acidosis, CO_3^{2-} on the bone surface acts as a proton acceptor. An important mechanism appears to be the chemical exchange of free protons for Ca^{2+}, Na^+, and K^+ bound to the carbonate, with the general buffering action as follows:

$$2H^+ + CO_3^{2-} \dashrightarrow CO_2 + H_2O$$

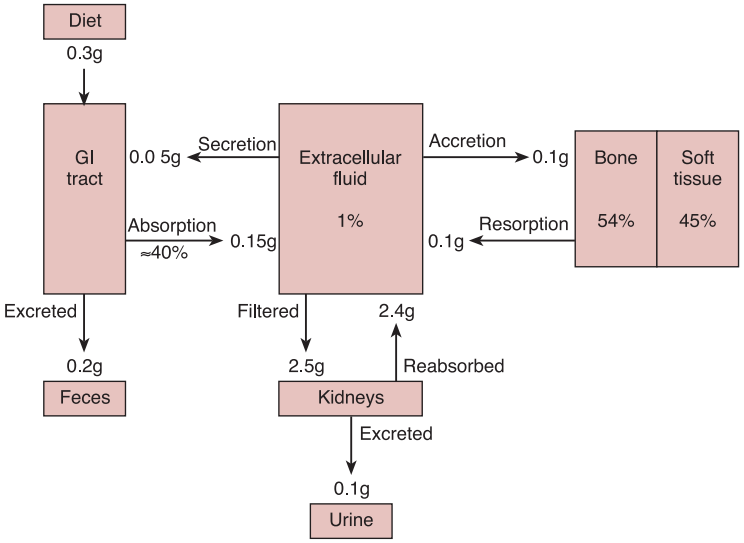
During the more chronic, compensated phase of metabolic acidosis, such as occurs with renal failure, buffering takes place through a combined exchange with bone carbonate cations, as occurs during the acute phase, and increased activity of osteoclasts, which help to mobilize additional bone mineral (**$Ca_{10}(PO_4)_6(OH)_2$** and **$CaHPO_4$**). Both acute and chronic bone buffering lead to enhanced urinary excretion of bone Ca^{2+}, which can reduce bone mass (osteopenia) and cause renal Ca^{2+} stone formation. Additionally, as stated above, **metabolic acidosis reduces the charge equivalency on albumin**, thus reducing the amount of Ca^{2+} bound to protein in plasma. This also increases the filtered load of Ca^{2+}, which in turn increases urinary Ca^{2+} excretion.

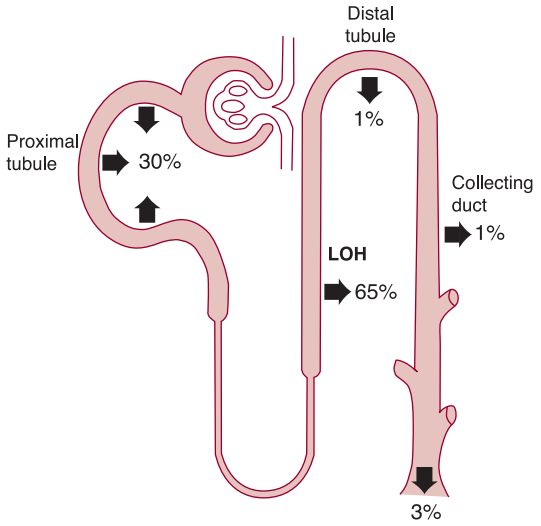
Ch15 Magnesium and Phosphate (Physiologic Actions)

A

Primary Functions of Mg^{2+}	
Enzyme activation (Cofactor for various kinases)	Regulation of protein synthesis. Complexes with ADP and ATP (holds ATP in position so that ATPase can attach)
Suppression of Ca^{2+} release from the sarcoplasmic reticulum	Structural component of bones and teeth

B Magnesium Homeostasis

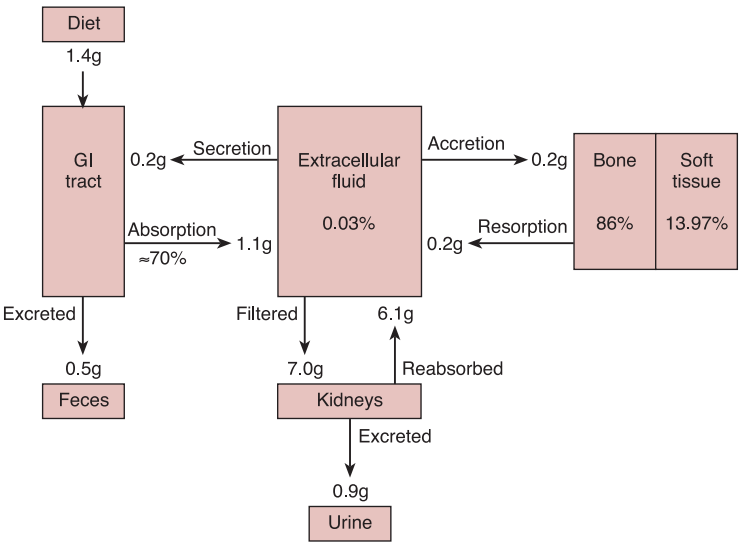


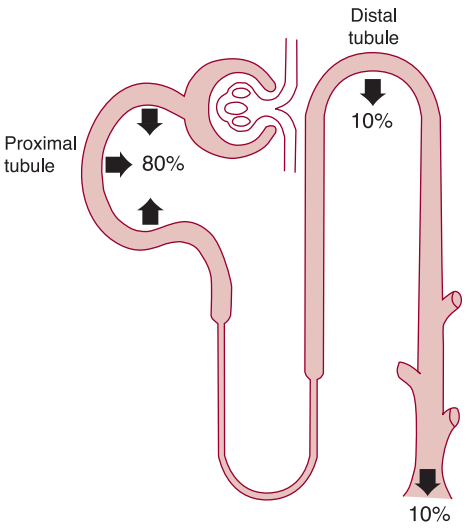


C

Primary Functions of PO_4^{3-}					
Major intracellular anion	Component of ADP and ATP RNA and DNA Metabolic intermediates	Component of all membranes (i.e., phospholipids)	Regulates enzyme action and protein function	Structural component of bones and teeth	Urinary buffer

D Phosphate Homeostasis





Magnesium

Magnesium, the second most abundant intracellular cation, has several roles including enzyme activation, regulation of protein synthesis, and suppression of Ca^{2+} release from the sarcoplasmic reticulum (**Part A**). Magnesium binds at the center of the **plant chlorophyll** molecule, and it also complexes with **ATP** inside animal cells. Enzymes requiring ATP are inactive in the absence of Mg^{2+} (or Mn^{2+}, a trace element substitute).

About **54%** of **Mg^{2+}** is found in **bone**, **45%** is in the **intracellular fluid of soft tissue**, and only **1%** is in **ECF** (**Part B**). Effects of Mg^{2+} on central and peripheral nerves mimic those of Ca^{2+}; that is, Mg^{2+} enhances excitation when deficiencies exist, and depresses it when excesses occur (Ch. 19). The synthesis, release, and target cell effects of **PTH** also require Mg^{2+}, but excessive levels inhibit PTH secretion and possibly its action on target cells.

Normal plasma Mg^{2+} concentrations are about **1.5-2.5 mEq/L**, with about 30% of plasma Mg^{2+} being protein bound. Free plasma Mg^{2+} that is filtered by the kidneys consists of an ionized fraction (55% of total), and a non-ionized component (15% of total) that is complexed to HCO_3^-, citrate, HPO_4^{2-}, and SO_4^{2-}. Mg^{2+} is extensively reabsorbed by the kidneys, with urinary excretion (**≈ 3%** of the filtered load) and keeps pace with intestinal absorption. PTH helps to facilitate Mg^{2+} reabsorption along the functional nephron, with the majority (**65%**) occurring in the ascending thick limb of the loop of Henle (**LOH; Part B**). Serum Mg^{2+} concentrations generally decrease in PTH deficiency as urinary excretion increases.

Magnesium is not efficiently absorbed across the GI tract, and it is of variable bioavailability, depending on dietary composition. Fecal concentrations (**≈ 45 mEq/L**) are normally much higher than those in plasma; however, absorption generally depends upon load, and when intake is restricted, intestinal absorption rises. The active form of vitamin D (**$1,25(OH)_2D$**) is thought to be responsible for this increase (Ch. 14). Magnesium, like Ca^{2+}, is wasted by the kidneys in acidosis, and an alkaline pH in the GI tract impairs absorption. Serious Mg^{2+} depletion can occur from **intestinal malabsorption** or **loop diuretic overuse** (which impairs renal reabsorption in the ascending thick limb of the LOH), and dangerous ventricular arrhythmias are reported.

Insulin stimulates the translocation of **Mg^{2+}**, **K^+**, **PO_4^{3-}**, **amino acids**, and **nucleosides** into cells independent of its actions on **glucose** uptake (Ch. 42). Therefore, insulin, secreted largely in response to a carbohydrate load, lowers serum Mg^{2+}, K^+, and PO_4^{3-}, and is considered to be an important regulator of K^+ balance [along with aldosterone and epinephrine (Ch. 26)]. **Part B** shows the processes affecting Mg^{2+} homeostasis (based on a normal dietary intake of **0.3 g/day** by a 75-kg mammal).

Phosphate

Calcium and **PO_4^{3-}** concentrations in the body are regulated in such a way that the product of the free ionized plasma concentrations of each equals a constant (**k**):

$$\mathbf{k = [Ca^{2+}][PO_4^{3-}]}$$

This constant, however, will change according to different physiologic states or pathophysiologic conditions. For example, **k** is greater in growing than in adult animals. This relationship implies that if there is an increase in the **[Ca^{2+}]** of extracellular fluid (ECF), a corresponding decrease in the **[PO_4^{3-}]** will occur. Likewise, an increase in the **[PO_4^{3-}]** of ECF should cause a decrease in the **[Ca^{2+}]**. This generalization is useful for illustrating some of the relationships that exist between the regulatory mechanisms governing these two ions. Minute-to-minute adjustments in their ECF concentrations are accomplished primarily through a combination of bone destruction (**resorption**) or formation (**accretion**), an increase in the efficiency of absorption of these ions from the **small intestine**, and/or alterations in their **renal** excretion.

Phosphate is concentrated in cells, and exists in both inorganic form, primarily as **HPO_4^{2-}** and **$H_2PO_4^-$**, and in organic compounds such as **nucleic acids**, **phospholipids**, **phosphoproteins**, **ATP**, and **intermediates** of several metabolic pathways (**Part C**). In primate erythrocytes, for example, **2,3-diphosphoglycerate** (**2,3-DPG**: also called **2,3-bisphosphoglycerate, 2,3-BPG**), may account for over **50%** of available phosphate, and it is about equimolar in quantity with **hemoglobin**. However, in several animal species studied, erythrocytic 2,3-DPG concentrations are known to vary considerably.

Along with **Ca^{2+}**, **PO_4^{3-}** is a major constituent of **bone** (i.e., **hydroxyapatite** (**$Ca_{10}(PO_4)_6(OH)_2$**, and **brushite ($CaHPO_4$)**. **Eighty-six percent** of PO_4^{3-} normally appears in bone, **13.97%** in intracellular fluid of soft tissues, and only **0.03%** is in ECF (**Part D**). The plasma phosphate concentration is normally about the same as that of magnesium (**≈ 2 mEq/L**), with **two-thirds** of this total existing as organic compounds, and **one-third** as inorganic compounds. The dibasic form of inorganic phosphate (**HPO_4^{2-}**) accounts for about **80%**, and the monobasic form (**$H_2PO_4^-$**) **20%**. Di- and monobasic phosphates circulate as free anions, partially bound to Na^+, Ca^{2+}, and Mg^{2+}. Release of PO_4^{3-} from bone is stimulated by the same factors that release Ca^{2+}, namely PTH and $1,25(OH)_2D$, and its entry into cells is stimulated by **insulin**. **Part D** shows processes affecting phosphate homeostasis (based on a normal dietary intake of **1.4 g/day** by a 75-kg mammal).

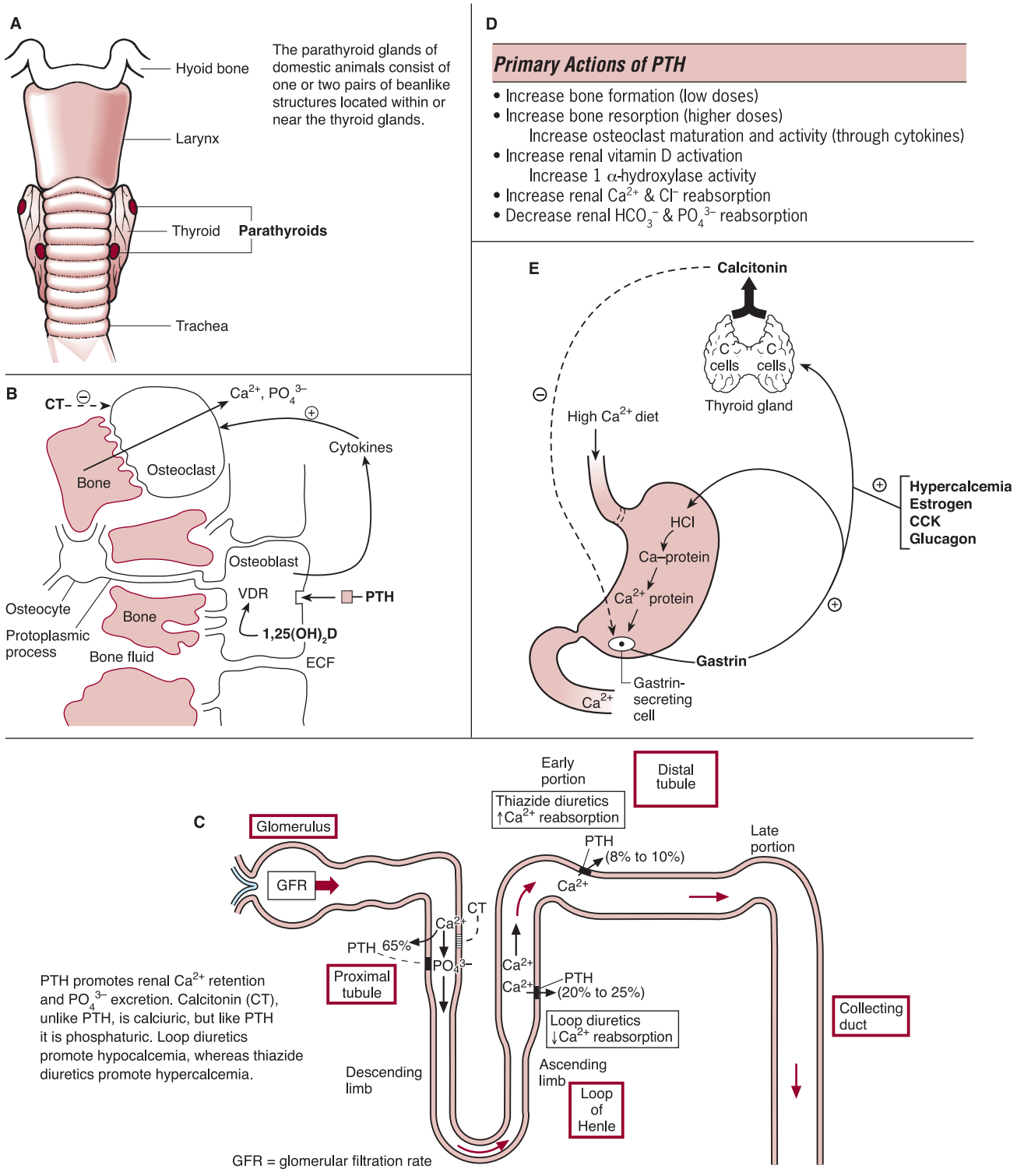
Phosphate absorption from the digestive tract increases as dietary PO_4^{3-} rises, and is stimulated by **$1,25(OH)_2D$** (like Ca^{2+} and Mg^{2+}). Approximately **60-70%** of dietary PO_4^{3-} is absorbed by active transport utilizing a $Na^+/(HPO_4^{2-}/H_2PO_4^-)$ cotransporter, and by passive diffusion. Absorption is thought to take place principally in the forestomaches of ruminants, from the large intestine of horses, and from the duodenum and jejunum of most other animal species. Parotid saliva in ruminant animals contains high levels of inorganic phosphate, which buffers protons dissociated from volatile fatty acids. High fiber diets typically increase, while concentrated rations decrease salivary phosphate secretion.

Unless otherwise needed for growth or lactation, the amount of phosphate excreted in urine generally equals the net amount absorbed by the digestive tract. Thus, the kidney plays a vital role in phosphate homeostasis. Unlike Ca^{2+} and Mg^{2+}, serum $HPO_4^{2-}/H_2PO_4^-$ is not protein bound, thus it readily passes through glomeruli of the kidney. Renal $HPO_4^{2-}/H_2PO_4^-$ excretion is regulated by the GFR as well as the maximal rate of tubular reabsorption. Usually, about **80%** of phosphate in the glomerular filtrate is reabsorbed by the $Na^+/(HPO_4^{2-}/H_2PO_4^-)$ cotransporter in the **proximal tubule**, with another **10%** reabsorbed by the **distal nephron**. This reabsorption process is **inhibited** by expansion of the ECF volume, hypercalcemia, and the hormones **PTH**, **PTH_{rp}**, **ANP**, **calcitonin**, and **cortisol** (all are **phosphaturic**). Elevated serum inorganic phosphates tend to enhance the secretion of PTH indirectly by binding with Ca^{2+}, thereby suppressing the free ionized Ca^{2+} concentration. Hormones which **enhance** renal phosphate reabsorption include **insulin**, **IGF-I** and **IGF-II**, **GH**, **PRL**, and **PL**. Renal phosphate reabsorption is thus increased with growth, pregnancy, lactation, and low phosphate diets, and is decreased during periods of slow growth, renal failure, or excess intake of dietary phosphates. Renal diseases affecting the **proximal tubules** have pronounced affects on PO_4^{3-} homeostasis, since the bulk of the PO_4^{3-} filtered load (**≈ 80%**) is normally reabsorbed in the proximal nephron (**Part D**).

The **PO_4^{3-} intestinal absorption efficiency** (**≈ 70%**) is normally much higher than that for **Mg^{2+}** (**≈ 40%**), making urinary phosphate excretion higher. Phosphate, unlike Mg^{2+}, is an important buffer inside cells and in the renal tubular filtrate (where it gives rise to **urinary titratable acidity; $HPO_4^{2-}/H_2PO_4^-$**). Plasma $HPO_4^{2-}/H_2PO_4^-$ concentrations are normally too low (compared to HCO_3^-) to make the phosphate buffer system quantitatively significant in blood.

Ch16 Parathormone, Calcitonin, and Vitamin D: I

(PTH, PTH_{rp}, and CT)



Source: **Part A** modified from Chastain CB, Ganjam VK. Clinical endocrinology of companion animals, 1st ed. Philadelphia, PA: Lea & Febiger, 1986:180.

Parathormone (**PTH**), a protein hormone of 84 amino acid residues, is the only known endocrine secretion of the parathyroid glands. The biologic activity of PTH resides in the first 34 amino acids, and it interacts with **calcitonin** and **vitamin D** to maintain calcium (**Ca^{2+}**), phosphate (**PO_4^{3-}**), and magnesium (**Mg^{2+}**) homeostasis.

Parathyroid Glands and PTH

The **parathyroid glands** are found in all air-breathing vertebrates, and appear first in amphibians coincident with their transition to terrestrial life (Ch.72). Investigators have suggested that the appearance of parathyroid glands may have arisen from a need to protect against the development of hypocalcemia, as well as the necessity to maintain skeletal integrity. Terrestrial animals, compared to their aquatic cousins, live in a relatively low calcium, high phosphate environment.

The parathyroids may be embedded within the thyroid glands (as in mice, dogs, and cats), or lie separately near the thyroids (as in goats and rabbits) (**Part A**). Consequently, thyroidectomy may result in loss of PTH and a lowered plasma Ca^{2+} concentration because of simultaneous removal of parathyroid tissue.

The parathyroids have two types of cells: **chief cells** (or "principal" cells), and **oxyphil cells.** Chief cells have a clear cytoplasm and are the PTH-secreting cells. They are also part of the amine precursor uptake and decarboxylation (APUD) series of cells (Ch. 51). Oxyphils, which represent only 1% of total parathyroid tissue, are apparently inactive. The number of oxyphils is reported to increase with age, and these cells are more rare in some species than in others (e.g., horses have comparatively more oxyphils than dogs and cats).

A low serum ionized Ca^{2+} concentration (not protein-bound Ca^{2+}) **has the greatest positive influence on PTH release**. Although **Mg^{2+}** is only one-half to one-third as potent as **Ca^{2+}** on PTH release, it appears that a basal level of Mg^{2+} is needed for optimal secretion, as wide fluctuations in the serum Mg^{2+} concentration (>↑↓Mg^{2+}) inhibit PTH release. Also, chief cells are known to contain **β-adrenergic** and **glucocorticoid receptors**, indicating that **"physiologic stress"** may also increase PTH release. **High serum ionized Ca^{2+}** levels and/or **$1,25(OH)_2D$** feedback negatively on the parathyroids, and in cases of **renal secondary hyperparathyroidism**, where circulating levels of $1,25(OH)_2D$ are reduced, intestinal Ca^{2+} absorption and serum Ca^{2+} levels are also reduced, and PTH secretion rises (Ch. 18).

PTH is degraded by its target cells. Metabolic degradation by the kidney, for example, involves specific proteases on the surface of renal tubular epithelial cells, as well as lysosomal enzymes within those cells. The circulating **half-life of PTH** in mammals is generally less than **10 minutes**.

Actions of **PTH on bone** are both direct and indirect, some considered anabolic and others catabolic. When administered in low, intermittent doses, PTH **stimulates** bone formation, and has hence been used to treat osteoporosis. Plasma levels of alkaline phosphatase, an osteoblastic enzyme whose activity parallels bone formation, can be increased with low doses of PTH. This **anabolic** action is thought to be mediated by increases in **insulin-like growth factors** (Ch. 10), the synthesis of which PTH stimulates. Wheather total skeletal mass increases or decreases under the influence of PTH depends on dose as well as on concomitant factors that affect bone remodeling, including age, mechanical stress (i.e., exercise), availability of Ca^{2+}, PO_4^{3-}, vitamin D, and other factors with endocrine or paracrine influences.

Both **PTH** and **vitamin D** (**$1,25(OH)_2D$**) have receptors on/in mature **osteoblasts,** but not on/in **osteoclasts**. Receptors for $1,25(OH)_2D$ are intracellular, while those for PTH are on the plasma membrane (**Part B**). At higher doses, such as in **hyperparathyroidism**, PTH (like $1,25(OH)_2D$) indirectly promotes osteoclast activation while inhibiting osteoblast formation. These two hormones stimulate osteoblasts to produce **cytokines** that accelerate maturation of osteoclasts in a paracrine fashion. Local release of lysosomal enzymes from osteoclasts and end products of glycolysis then create an environment that favors **bone dissolution**. **Osteoclasts** appear to phagocytize bone, digesting it in their cytoplasm. This is why bone around an active osteoclast has a ruffled or "chewed-out" edge. Alone, the effects of PTH on bone cannot account for its overall action of increasing the plasma-ionized Ca^{2+} concentration. The PO_4^{3-} also released from bone complexes with Ca^{2+} in ECF, thus limiting the rise in the plasma ionized Ca^{2+} concentration. Thus, an additional renal mechanism must coordinate with the bone effect to cause the plasma ionized Ca^{2+} concentration to increase.

PTH promotes renal Ca^{2+} reabsorption in the ascending thick limb of the loop of Henle (LOH), and the distal tuble (DT) (**Part C**). It also **decreases PO_4^{3-} reabsorption** in the proximal tubule, where the bulk of PO_4^{3-} reabsorption normally occurs. Thus, by favoring renal Ca^{2+} reabsorption while promoting renal PO_4^{3-} excretion (the **phosphaturic** effect), PTH elevates the plasma ionized Ca^{2+} concentration. **Loop diuretics**, which inhibit electrolyte reabsorption in the ascending thick limb of the LOH, decrease Ca^{2+} reabsorption, whereas **thiazide diuretics**, which act similarly on the early portion of the DT, enhance it. Consequently, thiazide diuretics are sometimes used to treat patients with Ca^{2+}-containing renal stones. Additional renal effects include **enhancing renal Cl^- retention and HCO_3^- excretion**. Thus, in **hyperparathyroidism**, hormone-induced bicarbonaturia and hyperchloremia produce acidemia, which dissociates Ca^{2+} from albumin. The primary actions of PTH are summarized in **Part D**.

Parathormone-Related Protein

Parathyroid hormone-related protein or **peptide** (**PTH_{rp} or PTHrP**) was originally identified as a product of nonparathyroid tumors of squamous cell origin that resulted in severe, if not fatal, hypercalcemia (i.e., **pseudohyperparathyroidism**). It is now known to be produced by many different tissue types. It has 140 amino acid residues (PTH has 84). Several amino acids at the active terminal are similar to those in PTH, so both can compete for the same receptor. Although many physiologic actions of these two hormones are similar, some are quite different. PTH_{rp} is primarily a tissue hormone or factor, acting where it is produced, and it may be that circulating PTH cannot reach these sites, making the actions of circulating PTH somewhat dissimilar from those of locally produced PTH_{rp}.

PTH_{rp} has a marked effect on growth and development of **cartilage** *in utero*, acting through a protein called **Indian hedgehog**. It is also expressed in the **brain**, where evidence indicates that it inhibits excitotoxic damage to developing neurons. A different segment of PTH_{rp}, that between amino acids 75 and 84, uniquely stimulates active **placental** Ca^{2+} transport from dam to fetus (Ch. 64). Some hypothesize that the PTH_{rp} in **mammary** tissue may aid in the uptake of Ca^{2+} into milk, and that presence in **milk** may aid Ca^{2+} absorption in the neonatal GI tract. PTH_{rp} is found in **skin keratinocytes**, **smooth muscle**, and **teeth**, where it is present in the enamel epithelium that caps each tooth. In it's absence, teeth will not erupt. PTH_{rp} is also found in the **avian shell gland**, where it aids in shell calcification and relaxes oviductal smooth muscle (Ch. 72).

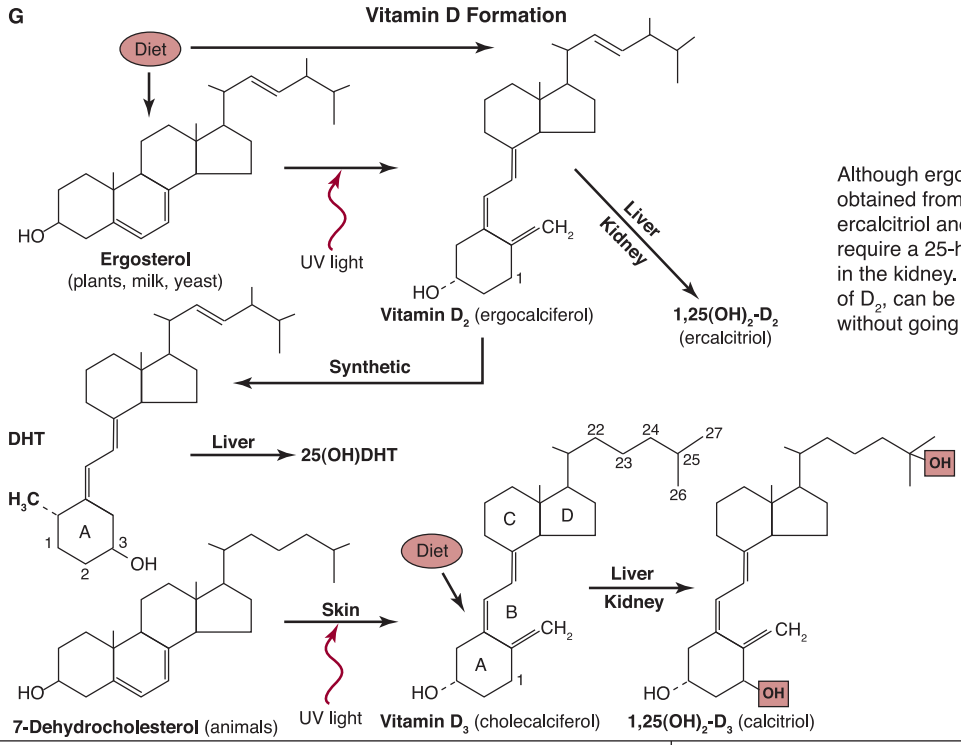
Calcitonin

In nonmammalian vertebrates, the source of **calcitonin** (**CT**; a 32-amino-acid peptide with a **five-minute biologic half-life**) is the ultimobranchial bodies, a pair of glands derived embryologically from the neural crest. These bodies are incorporated into the **thyroid gland** of mammals, where ultimobranchial tissue is found as **parafollicular cells,** also known as C ("clear") cells. Total thyroidectomy does not, however, reduce circulating CT levels to zero, indicating that this hormone also originates from other tissues (e.g., pituitary, thymus, lung, gut, liver, bladder, etc.). Like chief cells of the parathyroids, mammalian C cells exhibit APUD characteristics.

The exact physiologic role of CT is unknown. There is general agreement that **PTH has a far greater impact on Ca^{2+} homeostasis than does CT,** and although some patients with medullary carcinoma of the thyroid have been reported to have high circulating titers of CT and watery diarrhea, most apparently have no abnormalities in Ca^{2+} homeostasis. Similarly, a syndrome due to CT deficiency has not been described.

Ch17 Parathormone, Calcitonin, and Vitamin D: II (CT and $1,25(OH)_2D$)

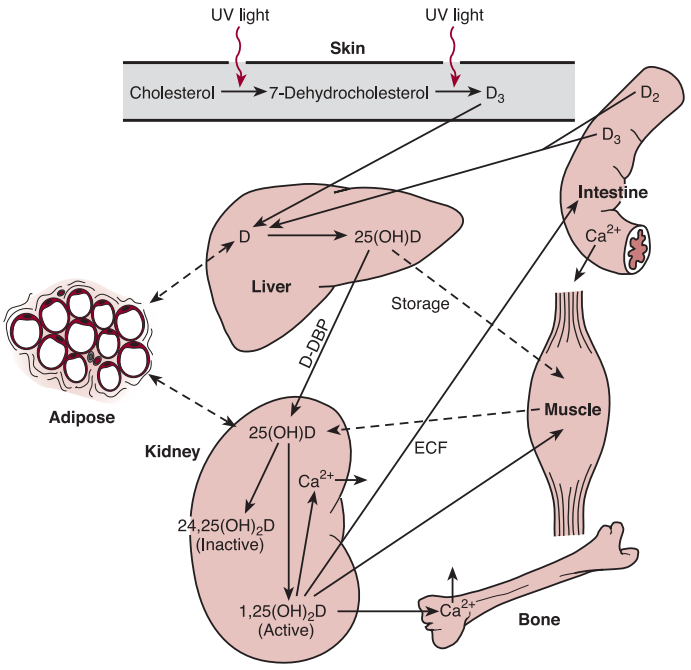
G



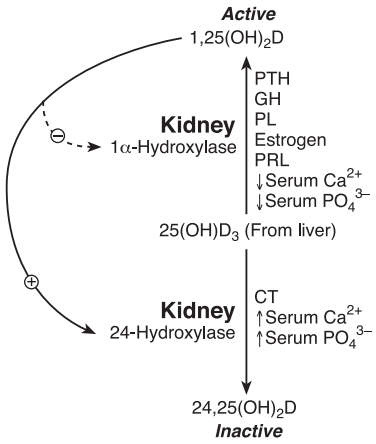
Although ergocalciferol and/or cholecalciferol can be obtained from the diet, or from biosynthesis in the skin (D_3), ercalcitriol and calcitriol formation (the active forms) each require a 25-hydroxylation in the liver, and a 1-hydroxylation in the kidney. Dihydrotachysterol (DHT), a synthetic analog of D_2, can be activated via a 25-hydroxylation in the liver without going through a second renal hydroxylation.

F

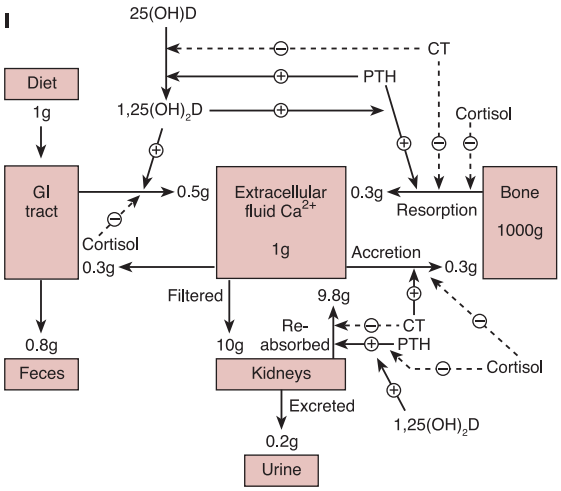
The various metabolites of vitamin D can be stored in the skin, in muscle and adipose tissue, and in the liver and kidneys. The active forms of this vitamin, which can be considered hormones, are carried in plasma bound to a vitamin D binding protein (D-DBP). Dietary sources are absorbed from the intestinal tract via facilitated diffusion, and can be found (with other fat-soluble vitamins) in circulating chylomicrons.



H



I



Calcitonin (**CT**) may be more active in young than in adult animals, and may play a role in skeletal development. It has been suggested that CT protects against postprandial hypercalcemia, and it may protect the bones of the mother from excess Ca^{2+} loss during pregnancy. Fetal bone formation and lactation can be major drains on Ca^{2+} reserves, and plasma concentrations of **1,25(OH)$_2$D** and **CT** are both elevated during pregnancy. Besides **hypercalcemia**, **estrogens** also enhance **CT** release, as do the gut hormones **cholecystokinin** (**CCK**), **glucagon**, and **gastrin** (**Part E**). Glucagon release from a necrotic pancreas may stimulate excessive CT release, thus leading to hypocalcemia. It appears that a high-Ca^{2+} diet releases gastrin, which in turn acts as an anticipatory hormone causing CT release. Gastrin also increases gastric HCl secretion, which releases dietary Ca^{2+} bound to protein (a prerequisite for absorption). Calcitonin, in turn, feeds back negatively on gastrin.

Calcitonin apparently prevents bone resorption by inhibiting osteoclasts, causing them to shrink and withdraw their bone-resorbing processes. Calcitonin also promotes the urinary excretion of Ca^{2+}, PO_4^{3-}, and Mg^{2+} by reducing their reabsorption in the proximal tubule (Ch. 16, **Part C**). The physiologic actions of **CT** can be summarized as follows:

Primary
- Prevent hypercalcemia during rapid, postprandial absorption of Ca^{2+}
- Protect maternal skeleton during pregnancy
- Antiosteoclastic

Secondary
- Decrease renal Ca^{2+}, PO_4^{3-}, and Mg^{2+} reabsorption
- Renal inactivation of vitamin D

Vitamin D

The actions of **PTH** are enhanced by the active form of **vitamin D** (**Parts D-I**). Whether as a **hormone** produced in the skin, or as a dietary **vitamin**, it must be chemically modified by 2 hydroxylations, which occur sequentially in the **liver** and **kidneys**, before it can play an active role in **Ca^{2+}** homeostasis. Discovery of the necessity for hepatic and renal hydroxylations of **cholecalciferol** (**D_3**) and/or **ergocalciferol** (**D_2**) explained a number of clinical conditions, and lead to more satisfactory treatments.

D_2 and **D_3** are seco-steroids in which the **B rings** have been broken by fission of a carbon-carbon bond (**Part G**). In this case fission is caused by **UV light** on **ergosterol** of plants producing D_2, and on **7-dehydrocholesterol** in the epidermis producing D_3. Irradiation of milk and yeast is a commercial means of producing D_2 from ergosterol. Sunlight-activated epidermal D_3 formation is probably the largest source of this vitamin for omnivores and herbivores, but carnivores derive most of their vitamin D from the diet. Although UV light is partially filtered out by hair coat, pigmented layers of the skin, and window glass, it is hypothesized that the nose of hairy mammals may be an important location for D_3 formation.

Once D_2 and/or D_3 reach the liver, the first step in sequential activation occurs, namely an **unregulated hydroxylation** by a monooxygenase (hydroxylase) to produce **25(OH)D_2** and/or **25(OH)D_3**, inactive forms. These compounds, which can be stored in the liver, muscle and adipose tissue (**Part F**), are transported (bound to a liver-derived plasma vitamin D binding protein (**D-DBP**)) to the **kidneys** where further **controlled hydroxylation** occurs in the **1-position** of each to produce the active hormones (**1,25(OH)$_2$D$_2$** and/or **1,25(OH)$_2$D$_3$**), or in the **24-position** of each to produce inactive forms (**24,25(OH)$_2$D$_2$** and/or **24,25(OH)$_2$D$_3$**) (**Part H**). Hypocalcemia, hypophosphatemia, PTH and the hormones of growth, pregnancy and lactation stimulate activity of renal **1α-hydroxylase**. During pregnancy the **placenta** may also synthesize 1,25(OH)$_2$D, which can augment intestinal Ca^{2+} absorption in the dam. In normal adult animals the relative plasma concentrations of the 3 hydroxylated forms (**25-hydroxy-, 24,25-dihydroxy-,** and **1,25-dihyroxy-**) are about **100:10:1**.

1,25(OH)$_2$D serves to regulate serum Ca^{2+} and PO_4^{3-} levels through **facilitating intestinal Ca^{2+}** and **PO_4^{3-} absorption**, and through **promoting the actions of PTH on bones and kidneys**. Since most of the actions of 1,25(OH)$_2$D and PTH are similar, vitamin D is used to treat patients with **PTH deficiency** (note: PTH is a protein, and when given orally is digested by exocrine pancreatic proteases). **Vitamin D deficiency** causes **rickets** in the young, and **osteomalacia** in adults, and in rabbits, rats and humans there is hypertension from lack of negative feedback on the renin-angiotensin system, and impaired insulin secretion. Hypovitaminosis D has also been associated with certain cancers. Since several forms of vitamin D are therapeutically available (e.g., D_2; 25(OH)D_2; 1,25(OH)$_2D_2$; reduced D_2 (DHT); 25(OH)DHT; D_3; 25(OH)D_3; and 1,25(OH)$_2D_3$ (1,25-DHC)), consideration of normal vs. abnormal hepatic and renal function should be given before proceeding with the administration of the more potent, active forms of the vitamin.

As with vitamin A, **vitamin D excess** can result in symptoms of toxicity. Hypercalcemia and hyperphosphatemia, excessive Ca^{2+} deposition in soft tissues (especially the kidneys, heart, lungs and vasculature), hypercalciuria and kidney stones have been described. Reptile pets seem to be particularly susceptible. Vitamin D toxicity can also occur, for example, when **cholecalciferol rodenticides** have been consumed by dogs and cats (who have feasted on poisoned rodents), or when too much vitamin D has been administered to animals with hypoparathyroidism. Glucocorticoids and CT have been administered to reverse symptoms of vitamin D toxicity, for glucocorticoids interfere with the mechanism of 1,25(OH)$_2$D action on the small intestine and kidneys, and CT generally exerts opposite actions to both PTH and 1,25(OH)$_2$D, including activation of renal 24-hydroxylase (**Part H**).

25(OH)DHT, a synthetic analog of **D_2** (**Part G**), appears to be active in the intestine and bone of nephrectomized rats. Its A ring is rotated so as to place the 3-hydroxyl group in approximately the same geometrical position as the 1-hydroxyl group of 1,25(OH)$_2$D, allowing it to interact with receptor sites without undergoing renal 1-hydroxylation. This compound may be considered when treating vitamin D deficient animals with renal disease.

To **summarize** the effects of other hormones on Ca^{2+} homeostasis (besides **PTH**, **CT** and **1,25(OH)$_2$D**), **estrogens** counter the action of PTH on bone, yet stimulate CT secretion and renal vitamin D activation (Chs. 20 and 59). **GH** stimulates bone and cartilage growth, helps to facilitate intestinal Ca^{2+} absorption, and modestly inhibits renal Ca^{2+} reabsorption. Many anabolic actions of GH are facilitated through the **somatomedins** (**IGFs;** Ch. 10). **Glucocorticoids** (**e.g., cortisol**) are largely **anti-calcium**, allowing bone resorption to overcome accretion. They also counter PTH actions in the kidneys, and 1,25(OH)$_2$D actions in the intestine (Ch. 23).

Nonmammalian Vertebrates

Although **fish** lack parathyroid glands, Ca^{2+} regulation appears to be accomplished by a hypercalcemic pituitary factor (**PRL** or **hypercalcin**), and by a hypocalcemic factor (**hypocalcin**) from the corpuscles of Stannius embedded in the kidneys (Ch. 72). Scales are important Ca^{2+} stores. The hypercalcemic factor appears to be important to freshwater fish, and the hypocalcemic factor to saltwater fish. **Salmon CT**, which is used clinically, is a more potent hypocalcemic factor in mammals than is **mammalian CT**. This is apparently due to the relative resistance of mammals to clear salmon CT from blood, thereby prolonging its half-life.

Parathyroid glands are present in birds and herptiles, with the effects of PTH and parathyroidectomy similar to those observed in mammals.

Ch18 Disorders of Calcium Homeostasis

(Hyper- and Hypocalcemia)

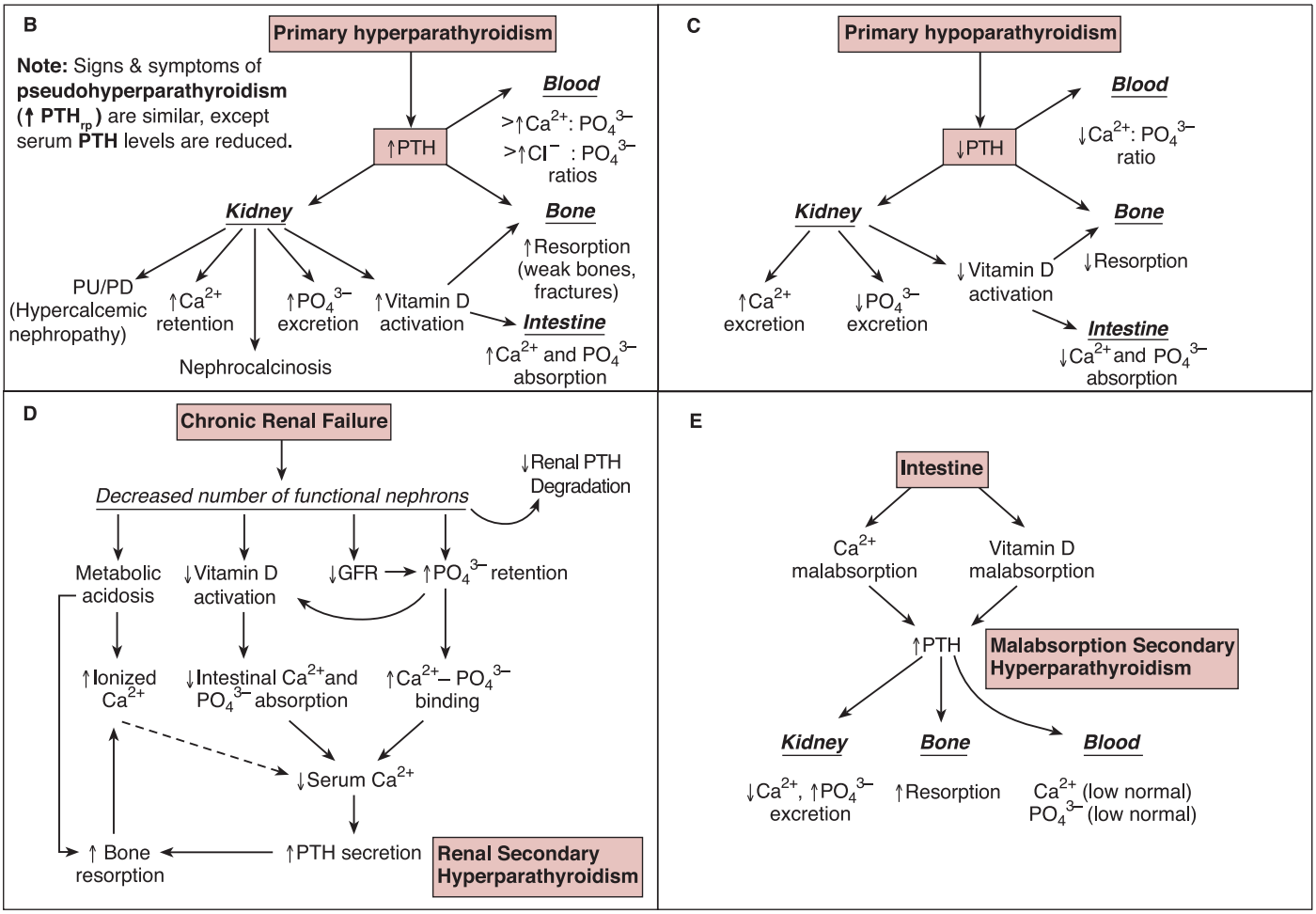
A

Causes

Hypercalcemia	*Hypocalcemia*
Primary hyperparathyroidism	Primary hypoparathyroidism
Thiazide diuretic therapy	Secondary hyperparathyroidisms
Lithium salts	Hypomagnesemia
Vitamin A toxicity (Rodenticide toxicosis)	Anticonvulsant therapy (Phenobarbital & phenytoin)
Excessive Ca^{2+} ingestion	Ethylene glycol toxicity
Renal failure	PO_4^{3-}-containing enemas
Prolonged immobilization	Accidental EDTA-containing transfusion
Hibernation	Osteoblastic metastases
Lymphosarcoma	Excessive sweating
Squamous cell carcinoma	Onset of lactation
Thyroid adenocarcinoma	Renal failure
Hypoadrenocorticism (↓ cortisol)	Vitamin D deficiency
	Oxalate toxicity
Apocrine cell adenocarcinoma of the anal sac	Hypercalcitoninism
	Loop diuretic overuse
Vitamin D toxicity	

Symptoms

Hypercalcemia		*Hypocalcemia*
Decreased neuromuscular excitability		Increased neuromuscular excitability
Bones:	dissolution of bone, pain, and fractures	Muscle cramps and pain
		Irritability
		Impaired mentation
Groans:	constipation, anorexia, dyspepsia (hypergastrinemia)	Seizures
		Prolonged Q-T intervals (ECG)
		Congestive heart failure
Stones:	nephrocalcinosis, kidney stones, PU/PD, metabolic acidosis	Laryngospasm
		Bronchospasm
		Tetany
Moans:	fatigue, myalgia, muscle weakness, joint pain, decreased Q-T intervals (ECG)	Cataracts
		Coagulopathies
		Milk fever
Overtones:	depression, memory loss, confusion, lethargy, and coma	



When intricate control mechanisms maintaining the normal plasma free ionized Ca^{2+} concentration fail, the result is either hyper- or hypocalcemia. **Hyperparathyroidism** indicates overproduction of PTH; however, this is not always associated with **hypercalcemia.** The term **primary hyperparathyroidism** is used to describe excess parathyroid tissue secreting PTH that has not been suppressed by a high plasma Ca^{2+} concentration. **Secondary hyperparathyroidism** (of various causes) refers to parathyroid hyperplasia and high circulating PTH levels that are an appropriate response to prolonged **hypocalcemia. Pseudohyperparathyroidism** is used to describe any hypercalcemia resulting from malignancy (not caused by bone metastasis).

Hypercalcemia

Primary Hyperparathyroidism

Signs of primary hyperparathyroidism are produced by **hypercalcemia**, **bone resorption**, and **calcium nephropathy** ("bones, groans, stones, moans, and overtones") resulting from **excessive PTH secretion** (**Part A**). Most cases have been associated with polyuria/polydipsia (**PU/PD**) from hypercalcemic nephropathy. Except for rare carcinomas, parathyroid tumors are not apparently palpable.

Symptoms include hypercalcemia, hypercalciuria, hypophosphatemia, hyperphosphaturia, and reduced creatinine clearance. Whether **hypercalcemia** is the **cause** or **consequence** of renal disease should be carefully determined. Because PTH decreases renal reabsorption of PO_4^{3-}, the plasma **Ca^{2+}-to-PO_4^{3-}** ratio significantly increases. Additionally, PTH also suppresses renal HCO_3^- reabsorption, yet favors Cl^- reabsorption. Urinary loss of HCO_3^- and PO_4^{3-} leads to **hyperchloremia** in an attempt to retain electroneutrality, and thus an increase in the serum **Cl^--to-PO_4^{3-}** ratio also occurs. **Part B** shows the pathogenesis of primary hyperparathyroidism.

Pseudohyperparathyroidism

Hypercalcemia resulting from malignancy has been associated with squamous cell carcinoma and lymphosarcoma in dogs and cats, probably due to these tumors producing high levels of **PTH_{rp}** or a lymphokine (osteoclast-activating factor). The second most reported cause of hypercalcemia in dogs is apocrine gland adenocarcinomas of the anal sacs. Symptoms are apparently similar to primary hyperparathyroidism; however, PTH levels are low (because normal parathyroids are being suppressed by high titers of Ca^{2+}). As hypercalcemia of malignancy can be a near terminal event in some patients, treatment is usually based on relieving the biochemical effects of PTH_{rp}, and lowering serum Ca^{2+} levels.

Vitamin D Toxicosis

Hypercalcemia and **hyperphosphatemia** are anticipated signs in animals with vitamin D toxicity. Increased bone resorption and increased intestinal absorption of Ca^{2+} and PO_4^{3-} are the causes. **Cholecalciferol rodenticide toxicosis** in dogs and cats reportedly causes weakness, lethargy, and anorexia within two days of exposure. Diffuse gastric and intestinal hemorrhage may follow. Excessive administration of vitamin D to hypoparathyroid cats that have been surgically treated for hyperthyroidism is an additional concern. Some house plants also contain vitamin D (e.g., *Cestrum diurnum, Solanum mafacoxyton, and Trisetum flavescens*), and consumption can also cause vitamin D toxicosis.

Other Causes of Hypercalcemia

These include hydrochlorothiazide diuretics (increase renal Ca^{2+} reabsorption), lithium (causes excessive PTH secretion), vitamin A toxicity (causes excessive osteoblast activation through up-regulation of $1,25(OH)_2D$ receptors), excessive Ca^{2+} ingestion, acute or chronic renal failure, and hibernation or prolonged immobilization (osteoporosis) (**Part A** and Ch. 20).

Hypocalcemia

Primary Hypoparathyroidism

Although some animals reportedly exhibit naturally occurring **hypoparathyroidism**, a more common cause of PTH deficiency appears to be surgical damage or removal of parathyroid tissue during thyroid surgery. Severe hypomagnesemia also causes hypocalcemia because optimal amounts of Mg^{2+} are required for PTH synthesis and secretion. Parathyroid hypofunction leads to declining Ca^{2+} and increasing PO_4^{3-} levels in plasma. Urinary Ca^{2+} excretion rises acutely, then declines with plasma concentrations. Urinary PO_4^{3-} excretion also diminishes. All of these alterations are explained by loss of PTH effects on: **1**) bone resorption, **2**) renal retention of Ca^{2+} and excretion of PO_4^{3-}, and **3**) intestinal absorption of both Ca^{2+} and PO_4^{3-} (**Part C**). Other pathophysiologic effects of hypocalcemia are listed in **Part A.**

Detection of antibodies against parathyroid tissue in human patients has led to speculation that some forms of primary hypoparathyroidism in animals may be caused by autoimmune processes.

Pseudohypoparathyroidism

Pseudohypoparathyroidism is a rare disorder characterized by target tissue resistance to PTH. Although it has been described in humans, reports of domestic animals with this specific disease are lacking. Symptoms in humans are hypocalcemia, increased serum concentrations of PTH (a form of secondary hyperparathyroidism), and a variety of skeletal development defects. Renal tubular resistance to PTH causes hypercalciuria and diminished phosphaturia.

Renal Secondary Hyperparathyroidism

Patients with this syndrome reportedly exhibit **modest hypocalcemia** and a more pronounced **hyperphosphatemia**. Like primary hyperparathyroidism, renal secondary hyperparathyroidism is characterized by **excessive PTH secretion** leading to excessive bone resorption (**Part D**). The driving force for PTH secretion is a low (normal) plasma Ca^{2+} concentration. Chronic renal insufficiency can result from several different causes in older animals, but when it progresses to the point where the number of functional nephrons is significantly reduced, hyperphosphatemia and azotemia develop. Although serum PO_4^{3-} has no direct regulatory influence on PTH secretion, it may, when elevated, contribute to parathyroid stimulation by virtue of its ability to bind Ca^{2+} and thus lower the plasma free ionized Ca^{2+} concentration. Intestinal Ca^{2+} and PO_4^{3-} absorption may also be impaired by a defect in renal vitamin D hydroxylation. However, renal disease also causes **metabolic acidosis**, and this apparently has a tendency to offset reductions in the free ionized Ca^{2+} concentration.

Malabsorption Secondary Hyperparathyroidism

Decreased intestinal absorption of dietary Ca^{2+} and increased fecal excretion lead to this syndrome. The degree of **Ca^{2+} malabsorption** is reportedly proportional to the extent of small bowel disease. An additional factor is **inadequate absorption of vitamin D**. As in pancreatitis or liver disease, signs of malabsorption/maldigestion are generally gastrointestinal. Consequences of Ca^{2+} and vitamin D malabsorption are secondary hyperparathyroidism leading to bone loss, modest hypocalcemia, hypophosphatemia, hypocalciuria, and hyperphosphaturia (**Part E**).

Nutritional Secondary Hyperparathyroidism

Animals fed **all-meat diets** with **low Ca^{2+}-to-PO_4^{3-}** ratios can develop a secondary hyperparathyroidism that leads to skeletal abnormalities. Although serum Ca^{2+} and PO_4^{3-} concentrations may remain within the normal range, these animals apparently exhibit hypocalciuria and hyperphosphaturia (Ch. 72).

Other Causes of Hypocalcemia

These include long-term anticonvulsant therapy (phenobarbital and phenytoin), loop diuretic overuse, ethylene glycol toxicity (acute renal failure with severe hyperphosphatemia), PO_4^{3-}-containing enemas (hyperphosphatemia), accidental transfusion with citrated or ethylenediaminetetraacetic acid (EDTA)-containing blood (calcium chelators or laboratory error because EDTA was used), and osteoblastic metastases. Loss of Ca^{2+} in sweat can lead to hypocalcemia in endurance horses, and parturient hypocalcemia occurs in several animal species (**Part A**).

Ch19 Mineral Imbalances: I

(Na^+, K^+, PO_4^{3-}, and Mg^{2+})

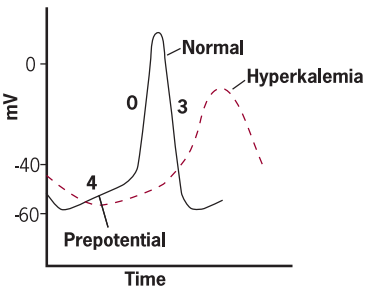
A Pathophysiologic effects associated with Na^+ imbalances

Hypernatremia	
↑ECF volume *(Hypertension;* ***e.g., NaCl engorgement****)*	***↓ECF volume*** *(e.g., diabetes insipidus,* ***excessive free water loss****)*
Increased cardiac output	Concentrated serum electrolytes
Increased renal blood flow	Concentration alkalosis
Decreased aldosterone and ADH	Increased aldosterone and ADH
Cellular dehydration	Cellular dehydration (nonketotic DM)
Increased neuromuscular irritability	

Hyponatremia	
↓ECF volume (Hypotension; e.g., diarrhea, sweating and diabetes mellitus)	***↑ECF volume (e.g., water intoxication)***
Decreased cardiac output	Dilute serum electrolytes
Decreased renal blood flow	Dilutional acidosis
Increased aldosterone	Decreased aldosterone and ADH
Increased intracellular fluid volume (hypotonic hypovolemia with cellular hydration)	Increased intracellular fluid volume

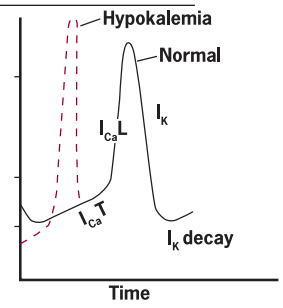
B Pathophysiologic effects associated with K^+ imbalances

Hyperkalemia	*Hypokalemia*
Increased neuromuscular excitability	Decreased neuromuscular excitability
Cardiac toxicity (arrhythmia,weak contractions, and bradycardia)	Tachycardia

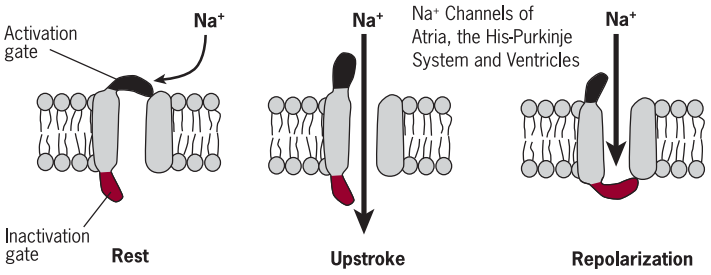


Depolarize Pacemaker Cells
- ↓ Rate of rise of phase 4
- ↓ Ca^{++} conductance
- ↓ Amplitude of phase 0
- ↑ Length of pacemaker AP
- ↓ Heart rate

Cardiac arrest in diastole



Hyperpolarize Pacemaker Cells
- ↑ Rate of rise of phase 4
- ↓ K^+ conductance of phase 4
- ↑ Ca^{++} conductance
- ↑ Amplitude of phase 0
- ↓ Length of pacemaker AP
- ↑ Heart rate

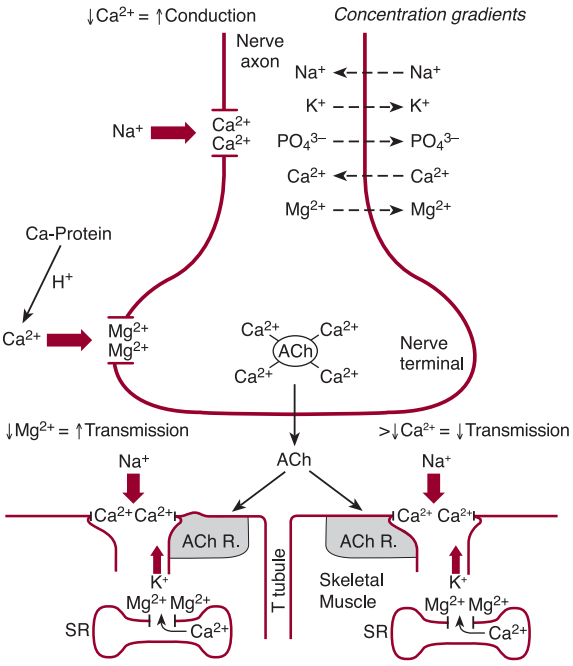


At rest the voltage activation gate on the Na^+ channel is closed, and the inactivation gate is normally open. During the upstroke of the AP, both gates are open, and Na^+ flows into the cell, down its electrochemical gradient. During repolarization, the activation gate remains open, but the inactivation gate is closed. In hypokalemia a greater proportion of inactivation gates remain open (because of hyperpolarization), thereby making it easier to fire rapid APs. In hyperkalemia, a greater proportion of inactivation gates remain closed, thereby reducing the amplitude, and slowing the development of APs.

C Pathophysiologic effects associated with PO_4^{3-} imbalances

Hyperphosphatemia	*Hypophosphatemia*
Increased neuromuscular excitability	Decreased neuromuscular excitability
Hypocalcemia (↑Ca^{2+} binding)	Aciduria
	Rickets and osteomalacia
	Hemolysis

D



Intracellular Mg^{2+} also dampens release of Ca^{2+} from the sarcoplasmic reticulum (SR). Severe hypocalcemia, as occurs with milk fever, will decrease transmission of action potentials (because Ca^{2+} entry into nerve terminals is required for exocytosis of neurotransmitters).

E Pathophysiologic effects associated with Mg^{2+} imbalances

Hypermagnesemia	*Hypomagnesemia*
Decreased neuromuscular excitability	Increased neuromuscular excitability
Depressed respiration	Cardiac arrhythmia
Depressed deep tendon reflexes	Generalized tremors
Depressed sinoatrial node and cardiac conducting system	Grass tetany (or wheat pasture poisoning) in ruminants
Decreased vascular smooth muscle contraction, decreased blood pressure	Kaliuresis

Note: The effect of hyper- or hypokalemia on cardiac rhythm (**Part B**) is complex, and several different arrhythmias may be seen. The rate of change appears to influence the type of arrhythmia produced. For example, a slow elevation in K^+ produces widespread block and depressed automaticity, while more rapid elevations may produce ventricular ectopic rhythms and, terminally, ventricular fibrillation. Obviously, care must be exercised in the administration of K^+-containing fluids to patients.

All organ systems are dependent upon normal plasma concentrations of calcium (Ca^{2+}), phosphate (PO_4^{3-}), sodium (Na^+) potassium (K^+), and magnesium (Mg^{2+}). These concentrations, in turn, largely depend on dietary intake, acid/base balance, and renal, gastrointestinal, and endocrine function. The physiologic processes most affected by these minerals are neuromuscular function and blood volume/pressure homeostasis.

The effects of **PTH**, **1,25-DHC**, and **CT** on Ca^{2+} and PO_4^{3-} balance were discussed in previous chapters, and the effects of **aldosterone** on Na^+ and K^+ balance are discussed in Chs. 26-28. Other important interactions between these minerals will be the subject of this chapter.

The effects of changes in the extracellular fluid (ECF) concentrations of these minerals (and H^+) on **neuromuscular irritability (NI)** can be depicted in the following expression:

$$\textbf{NI} \propto (Na^+ \bullet K^+ \bullet PO_4^{3-})/(Ca^{2+} \bullet H^+ \bullet Mg^{2+})$$

Plasma K^+, Mg^{2+} and Ca^{2+} concentrations are **inversely** correlated with **pH** (i.e., acidosis tends to displace K^+ and Mg^{2+} from cells and liberate ionized Ca^{2+} from plasma protein binding sites, while alkalosis has the opposite effects). Because respiratory changes develop more rapidly than renal changes, the free ionized plasma Ca^{2+} concentration is more commonly influenced by respiratory alterations in pH. Hypocalcemia, for example, sometimes becomes symptomatic by reducing serum ionized Ca^{2+} levels through hyperventilation (as caused by heat or pain). Also, rapid correction of acidemia can sometimes dangerously decrease serum K^+ by encouraging K^+ reentry into cells in exchange for buffered H^+.

The most potent influence on blood volume and pressure is Na^+, along with its accompanying anions, Cl^- and HCO_3^-. Together these ions account for about **93%** of **ECF tonicity**. The Na^+ **concentration** generally affects ECF volume in two ways: **1**) it helps maintain ECF volume when more water than electrolyte is lost (largely by pulling water out of intracellular fluid sites), and **2**) it helps deplete ECF volume when more Na^+ than water is lost (by allowing water to move into intracellular fluid sites) (**Part A**). When isotonic fluids are lost (e.g., by hemorrhage), Na^+ has no direct affect on ECF volume; however, through the renin-angiotensin system (Chs. 27 and 28), aldosterone-mediated renal Na^+ retention contributes to restoration of plasma volume (at the expense of K^+ depletion). Increasing the ECF Na^+ concentration increases its concentration gradient into excitable tissue, which tends to be depolarizing. For this reason, Na^+ appears in the numerator of the **NI equation**.

When the extracellular K^+ **concentration** rises, its diffusion gradient out of cells decreases; therefore, it has less of a hyperpolarizing effect (and more of a depolarizing effect on excitable tissue). The depolarizing nature of **hyperkalemia** can also be depicted by the **Nernst equation: $E_{K^+} = 61.5 \log ([K^+_E]/[K^+_I])$**. If normal K^+ concentrations for extracellular ($[K^+_E]$) and intracellular fluid ($[K^+_I]$) are given as **5.5** and **150 mEq/L**, respectively, then the equilibrium potential for K^+ (E_{K^+}) is **–90 mV**. When $[K^+_E]$ increases to **6.5 mEq/L**, for example, E_{K^+} becomes **–84 mV** (i.e., a depolarizing effect).

Modest increases in extracellular K^+ have a tendency to depolarize pacemaker cells of the heart and, thus, decrease intracellular negativity. This decreases Ca^{2+} influx into pacemaker cells of the SA and AV nodes through both **T** (**transient**) and **L** (**long-lasting**) channels, effectively decreasing rate of rise of the **prepotential** (**phase 4**), and decreasing **amplitude** of **phase 0** (**Part B**). This decreased Ca^{2+} **conductance** prolongs the pacemaker action potential and slows heart rate. **Hyperkalemic depolarization** also causes closure of voltage inactivation gates on His-Purkinje and myocytic Na^+ **channels**. Consequently, myocytes become less capable of firing action potentials when activation gates open, and the QRS complex of the ECG lengthens. Cardiac arrest (i.e., hyperkalemic paralysis (**cardioplegia**)) may occur in diastole. Hyperkalemia (a 20-30 mM K^+ buffer solution) closes nearly all Na^+ inactivation gates, and is sometimes employed in coronary artery bypass surgery to temporarily stop the beating heart and facilitate attachment of vessels to the myocardial surface.

During the early part of the SA node prepotential (**phase 4**), K^+ conductance may be contributing more to the pacemaker potential than Ca^{2+}. However, in **hypokalemia**, the K^+ current (I_K) rapidly decays, and that of Ca^{2+} ($I_{Ca}T$) substantially increases, leading to an increased slope of phase 4. Ca^{2+} current through the L channels ($I_{Ca}L$) is increased, and tachycardia ensues.

Because **phosphate** is concentrated within cells, and is a part of membrane-bound phospholipids, ATP, DNA, and other important compounds, **hypophosphatemia** is associated with a decrease in **NI**. Additionally, phosphate is an important buffer in the renal filtrate, therefore hypophosphatemia is associated with **aciduria**. Severe hypophosphatemia (<1.5 mg/dl) will sometimes cause **hemolysis** with hemoglobinuria. The glycolytic pathway in erythrocytes is impaired due to ATP depletion, thus enhancing RBC fragility. Conversely, **hyperphosphatemia**, which is more common in domestic animals, increases **NI**, partially because PO_4^{3-} binds Ca^{2+} and creates a **secondary hypocalcemia** (**Part C**).

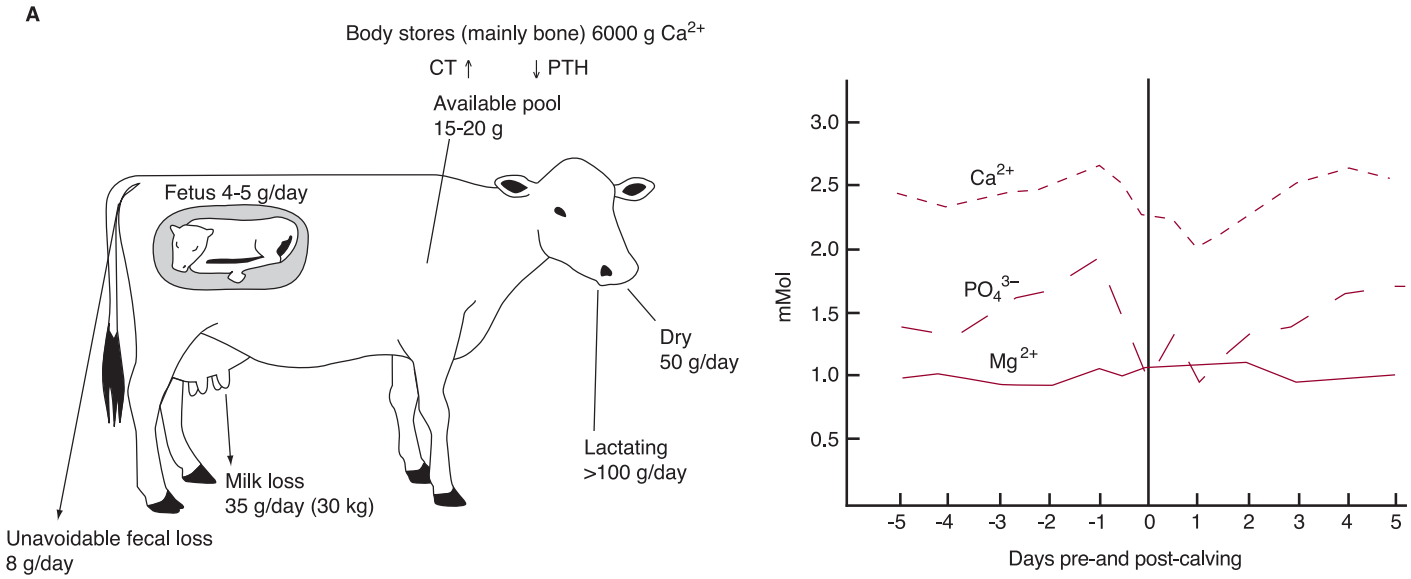
As seen in **Part D**, Ca^{2+} has a tendency to align itself in Na^+ neuronal channels. Thus, when the Ca^{2+} **concentration** rises, Na^+ has a more difficult time entering neurons (i.e., paresis), and when it falls nerve depolarization increases (hypocalcemic tetany). Potassium administration, because it is depolarizing, would obviously aggravate symptoms of hypocalcemic tetany. However, when severe decreases in the plasma Ca^{2+} concentration occur, as in **milk fever** of dairy cows, transmission of action potentials can be compromised, with reduced acetylcholine (ACh) release at neuromuscular junctions (i.e., paresis). Most animals that experience milk fever, however, first experience a short period of enhanced excitability before becoming **"downer cows"** (Ch. 20).

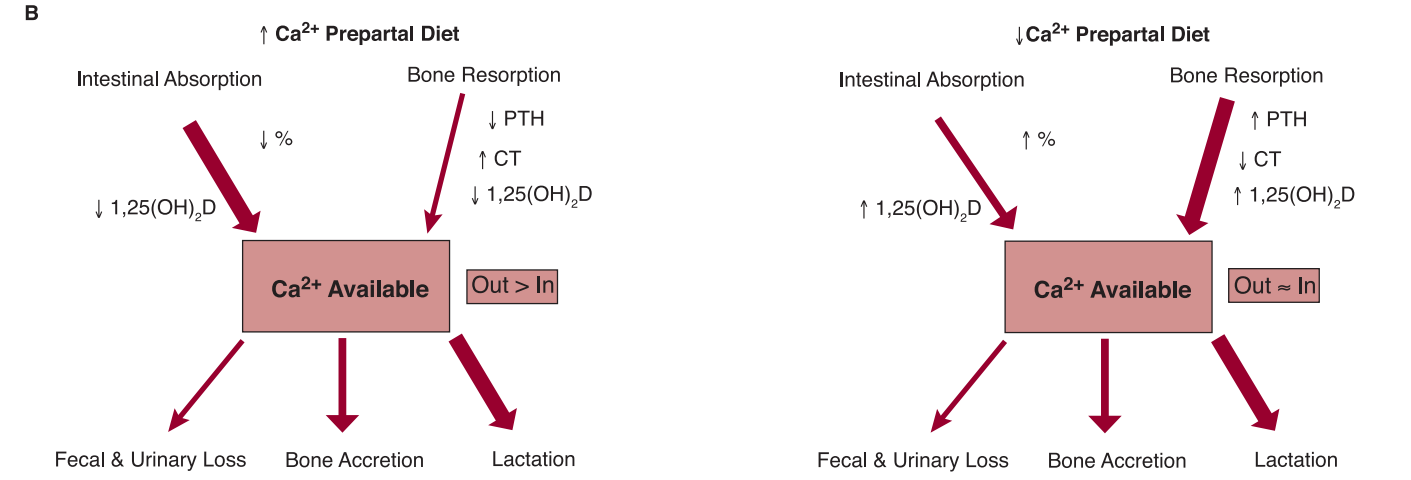
The Mg^{2+} ion is an important cofactor inside cells. Kinetic studies reveal that enzymes having ATP or other nucleoside triphosphates as a substrate are essentially inactive in the absence of Mg^{2+} (or Mn^{2+}). Mg^{2+}, the second most prevalent intracellular cation, helps hold **Na^+-K^+ ATPase** in place so that ATP can attach. Movement of K^+ into tissue cells from interstitial fluid,and from the glomerular filtrate into epithelial cells of the ascending limb of the loop of Henle, becomes impaired, leading to **kaliuresis**. Because of this, hypomagnesemia eventually leads to **hypokalemia**. **Hypomagnesemia** is a common electrolyte disorder in critically ill animals, which predisposes them to a variety of neuromuscular, cardiovascular, and metabolic complications (**Part E**). Magnesium deficiency reduces **PTH** secretion from the parathyroids, which also contributes to a hypocalcemia. ECG alterations include a widened QRS complex, a prolonged PR interval, a depressed ST segment, and peaked T waves. Hypomagnesemia also predisposes animals receiving digitalis to arrhythmias. Common causes of **hypomagnesemia** include disorders leading to small intestine malassimilation, excessive loop diuretic therapy, and diabetic ketoacidosis. Factors that usually cause a shift of K^+ from intra- to extracellular fluid sites also cause a shift in Mg^{2+} as well. Conversely, hypomagnesemia can develop due to the dilutional effects of fluid therapy and the extra- to intracellular Mg^{2+} shift that occurs with **insulin administration** (Ch. 42).

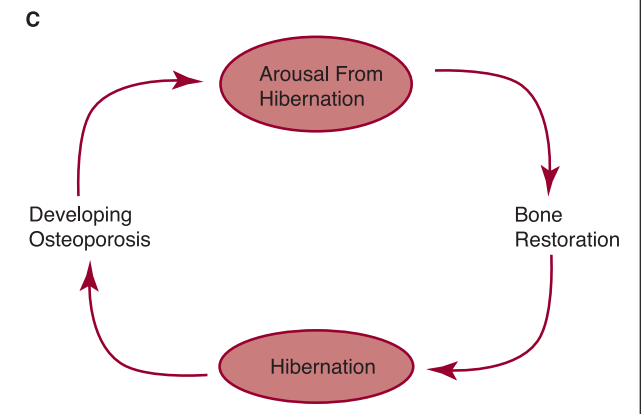
The Mg^{2+} **ion** has a tendency to block Ca^{2+} channels (similar to the way Ca^{2+} blocks Na^+ channels; see **Part D**). **Hypermagnesemia** will therefore decrease Ca^{2+} entry into nerve terminals (i.e., cause decreased transmission and paresis), as well as Ca^{2+} movement into the cytoplasm of cardiac and smooth muscle cells (from both inside and outside the cell) (**Part E**). Although Mg^{2+} administration would aggravate hypocalcemic paresis in dairy cows, it helps to alleviate symptoms of hypocalcemic tetany in other animals (because the release of **ACh** is inversely related to the ECF Mg^{2+} concentration).

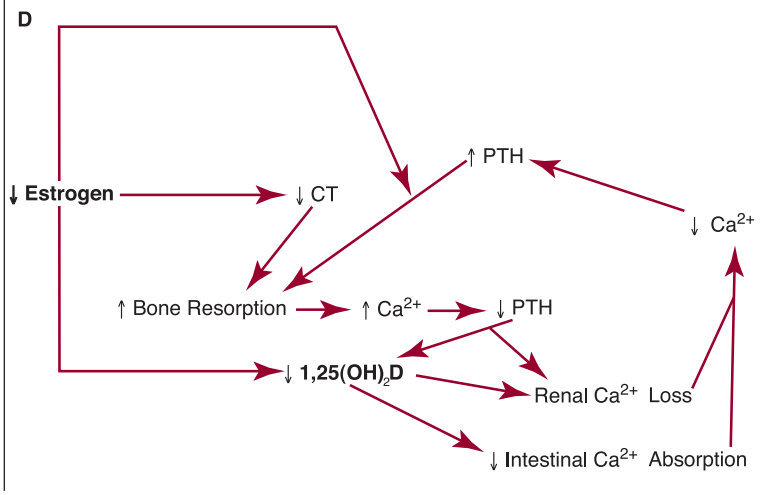
Ch20 Mineral Imbalances: II

(Parturient Hypocalcemia and Osteoporosis)









Parturient Hypocalcemia in Cattle

Parturient hypocalcemia (**or milk fever**) is one of the more costly production diseases of cattle. More than **90%** of cases occur between **24 hrs before** and **48 hrs after parturition**. Analysis of the incidence of this disease shows that it occurs in both dairy and beef cattle, but that in the former the disease is predominantly a **Ca^{2+}** and **PO_4^{3-}** imbalance (**Part A**), whereas in the latter the **hypomagnesemic** condition prevails.

To understand the range of pathophysiologic symptoms observed with milk fever, one must be aware of the physiologic actions dependent upon **Ca^{2+}**, **Mg^{2+}**, and **PO_4^{3-}** (see previous chapters). As previously discussed, **Ca^{2+}**, **Mg^{2+}**, and **PO_4^{3-}** are normally present in extracellular fluids (ECFs) in relatively small amounts compared with **Na^+**, **Cl^-**, and **HCO_3^-**. The plasma concentrations of these electrolytes are more likely to change with acute additions or deletions from the system.

A typical case of milk fever is accompanied by dullness, dilated pupils, cold ears, a constricted anal sphincter, hemoglobinuria, and a disinclination to eat. **A period of hyperexcitability usually precedes collapse**, and narcosis may follow in which the neck may be kinked. Finally, if animals are left untreated coma supervenes, with the head turned onto the flank.

Acute hypophosphatemia frequently accompanies **hypocalcemia** in cases of milk fever. The low blood **PO_4^{3-}** concentration is correlated with muscle weakness, hemoglobinuria, and a disinclination to rise (**Part C**). Intravenous phosphate injections may retard the hemolysis, and in many cases **calcium borogluconate** injections allay other acute signs of this disease. In many instances untreated animals remain recumbent for several days, with damage to nerves and muscles occurring due to the pressure of recumbency.

Cows fed a **high Ca^{2+} prepartal diet** become dependent upon intestinal **Ca^{2+}** absorption, and are therefore more susceptible to the development of this condition (**Part B**). Their rate of bone resorption is low, and parathyroid glands remain relatively inactive. Anorexia and intestinal stasis that often occur near parturition interrupt the major inflow of **Ca^{2+}** and **PO_4^{3-}** into the body, making it difficult to meet demands of lactation. Conversely, **Ca^{2+}** and **PO_4^{3-}** homeostasis may be different in cows fed a **low Ca^{2+} prepartal diet**, where the parathyroids remain active and bone resorption keeps pace with the initial mineral demands of lactation. Once intestinal function returns, the efficiency of **Ca^{2+}** absorption will increase (**↑%**) because of **PTH-induced activation of vitamin D**.

An additional approach to treating dairy cows prone to developing milk fever is based upon the principle of **strong ion difference** (**SID**; also called **dietary cation-anion difference** (**DCAD**), or **fixed ion difference** (**FID**)). **Anionic salts** (e.g., **$CaCl_2$**, **NH_4Cl**, or **$CaSO_4$**) are added to feed to produce a diet that is negatively charged (i.e., one that contains more ions of **Cl^-** or **SO_4^{2-}** than of **Na^+**). These acidifying salts effectively mobilize **Ca^{2+}** and **PO_4^{3-}** from bone. As **plasma pH decreases**, the **HCO_3^-** buffer equation is shifted to the left, the blood and urinary **HCO_3^-** concentrations decrease, as does **urinary pH**. The efficacy of this dietary strategy has been followed through cow-side testing of urinary pH, with values between **5.5 - 6.5** yielding the best results.

Parturient Hypocalcemia in Dogs and Cats

Parturient hypocalcemia in bitches and queens can be a life-threatening condition (not to be confused with eclampsia (or preeclampsia) in women, where the condition is characterized by hypertension prior to delivery). The cause of the hypocalcemia may be similar to that described above for dairy cows (i.e., maternal **Ca^{2+}** loss to the fetal skeleton and to milk production, or to excessive dietary **Ca^{2+}** supplementation during pregnancy and to parathyroid gland atrophy). Signs and symptoms of parturient hypocalcemia in bitches and queens reportedly develop during peak lactation periods rather than immediately following parturition.

Osteoporosis

All normal animals gain bone early in life, during growth, and after a plateau begin to lose bone as they age. When this loss is accelerated or exaggerated, as it is in human **osteoporosis**, it can lead to fractures, including compression of vertebral bodies with resultant kyphosis. Fractures associated with human osteoporosis have a high morbidity and mortality rate.

In animals who have been **immobilized** for any reason (e.g., diseased or tightly confined), and in those who are **hibernating**, bone resorption generally exceeds bone formation and **disuse osteoporosis** develops (**Part C**). In animals used to study the mechanisms of space osteoporosis, net bone loss during flight was found to be more a consequence of reduced bone formation than to accelerated bone resorption due to the loss of gravity. Animals with chronically elevated **glucocorticoid** levels (i.e., those with **Cushing's-Like Syndrome** or **Disease**), may develop osteoporosis due to reduced intestinal **Ca^{2+}** absorption and excessive renal **Ca^{2+}** loss (Ch. 25).

In developing osteoporosis, the plasma **Ca^{2+}** concentration vacillates between being slightly elevated, and slightly reduced (**Part D**). As plasma **Ca^{2+}** levels rise due to elevated bone resorption, **PTH** secretion is reduced, **$1,25(OH)_2D$** activation is similarly reduced, renal **Ca^{2+}** loss increases, and intestinal **Ca^{2+}** absorption decreases. These changes promote a decline in the plasma **Ca^{2+}** concentration, which then promotes more **PTH** release, and more bone resorption.

One cause of bone loss after menopause in humans is **estrogen deficiency** (note: animals usually don't live long enough to experience menopause). Estrogen promotes **calcitonin** (**CT**) release, suppresses the action of **PTH** on osteoblasts, and promotes the renal hydroxylation of **vitamin D**. With declining estrogen levels, these actions are reduced, bone resorption is accelerated, and osteoporosis develops. Osteoporosis can be delayed and/or prevented by weight-bearing exercise, and in some cases by vitamin D supplementation.

In summary, all organ systems are dependent on normal plasma concentrations of **Ca^{2+}**, **PO_4^{3-}**, **Mg^{2+}**, **Na^+**, **H^+**, and **K^+**, which in turn are dependent upon dietary intake, acid/base balance, and normal renal, hepatic, gastrointestinal, and endocrine activity. Changes in the ECF concentrations of these ions have profound effects on **neuromuscular irritability** (**NI**), and other physiologic activities.

Hypercalcemia may develop from primary hyperparathyroidism, pseudohyperparathyroidism, vitamin D toxicosis, excessive thiazide diuretic therapy, vitamin A toxicity, excessive Ca^{2+} ingestion, acute or chronic renal failure, or to disuse osteoporosis. Signs and symptoms of hypercalcemia include "bones, groans, stones, moans, and overtones". **Hypocalcemia** develops from primary hypoparathyroidism, pseudohypoparathyroidism, or the renal, malabsorption, and nutritional secondary hyperparathyroidisms. Parturient hypocalcemia is a serious condition in both large and small animal species, and calcium imbalances have profound affects on the ECG, smooth muscle contraction, NI, blood coagulation, and bone health.

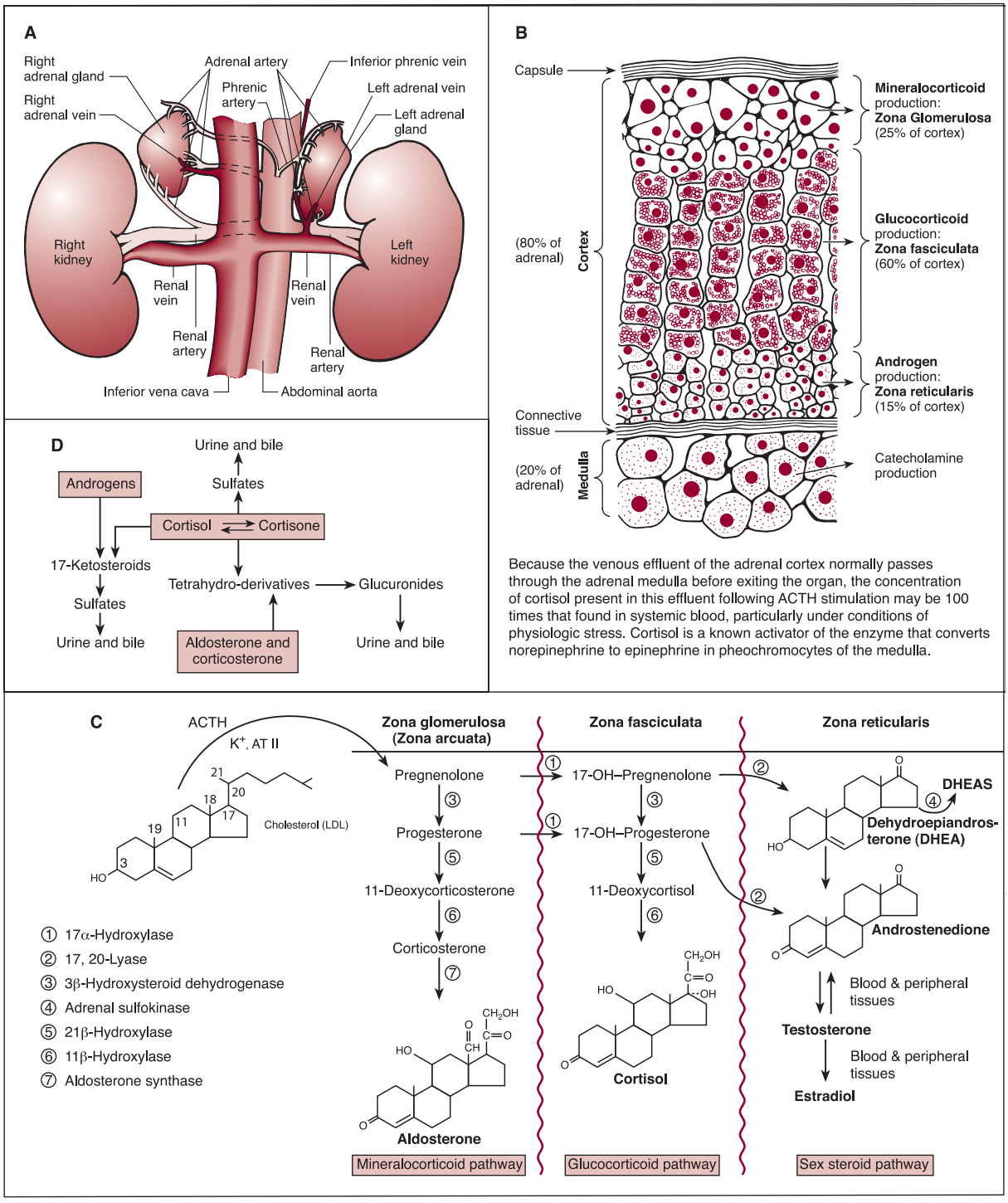
Sodium and its accompanying anions (**Cl^-** and **HCO_3^-**) have profound influences on ECF volume, and thus blood pressure. Together these ions normally account for about **93%** of **ECF tonicity**. When the ECF volume is expanded, **aldosterone** and **ADH** secretion are normally reduced. Water intoxication and NaCl engorgement are associated with an expanded ECF volume, while diabetes, diarrhea, vomiting, and excessive sweating are associated with a reduced ECF volume. Modest extracellular increases in either the **Na^+** or **K^+** concentrations tend to depolarize excitable cells.

Hypophosphatemia is associated with hemolysis, aciduria, and a decrease in **NI**, whereas **hyperphosphatemia**, which is more common in domestic animals, increases **NI**. Since **PO_4^{3-}** binds **Ca^{2+}** in the circulation, hyperphosphatemia can reduce the serum ionized **Ca^{2+}** concentration.

The **Mg^{2+} ion** is involved with **Na^+/K^+-ATPase** activity, and thus **hypomagnesemia** leads to excessive **K^+** loss from the body. Hypomagnesemia also reduces **PTH** secretion, which contributes to hypocalcemia. Since **Mg^{2+}** has a tendency to block **Ca^{2+}** channels, **hypermagnesemia** decreases **Ca^{2+}** entry into nerve terminals, thus reducing **NI**. Factors causing a shift of **K^+** from intra- to extracellular sites (e.g., extracellular hyperosmolarity and acidosis), generally affect **Mg^{2+}** similarly. Conversely, the dilutional effects of fluid therapy and **insulin** administration tend to promote hypomagnesemia.

Ch21 ACTH and Glucocorticoids: I

(Corticosteroidogenesis and Degradation)



The **adrenal glands** of birds and mammals are small oval structures located bilaterally at the anterior aspects of the kidneys (**Part A**). Each gland consists of two primary endocrine segments: an outer adrenal **cortex** that secretes **steroid hormones**, and a smaller inner **medulla** that secretes the **catecholamines**, norepinephrine and epinephrine [from the Greek words *epi* (the top) and *nephros* (the kidney)]. In birds, medullary tissue is mixed with cortical tissue (Ch. 72). In mammals, however, the dark red medulla is distinct from the pale yellow cortex. **Medullary tissue** is composed of a spongework of **chromaffin cells** (or **pheochromocytes**). In addition, small groups of nerve cells (paraganglia) are scattered throughout the medulla, which can be regarded as a specialized ganglion of the sympathetic nervous system (because secretory cells are richly innervated by cholinergic preganglionic fibers from lower thoracic segments of the spinal cord). Postganglionic neurons of the adrenal medulla have become secretory cells that add catecholamines directly to blood.

The **cortex**, which normally accounts for about 80% of adrenal tissue, consists of cells with well-marked nuclei aligned in three distinct functional zones (**Part B**). The outermost zone in most mammals is called the **zona glomerulosa** (because cells form clusters resembling glomeruli in the kidney). In dogs and cats, the arc-like configuration of this zone causes it to sometimes be referred to as the **zona arcuata**. The second zone inward from the capsule is called the **zona fasciculata**, and the innermost zone the **zona reticularis** (sometimes referred to as the cortical graveyard).

The adrenal medulla does not regenerate following injury, but when the inner two zones of the cortex are removed, they are regenerated from glomerulosa cells. Following hypophysectomy (i.e., loss of ACTH), the zona fasciculata and zona reticularis atrophy, whereas the zona glomerulosa remains almost unchanged because nonpituitary factors (e.g., angiotensin II and K^+) maintain aldosterone synthesis and secretion. Conversely, injections of ACTH cause hypertrophy of the inner two zones, but do not affect the size of the zona glomerulosa.

Biosynthesis of Adrenal Steroids

Although several different steroids have been isolated from adrenal tissue, only **cortisol** (**hydrocortisone**), **corticosterone**, **deoxycorticosterone**, **aldosterone**, and the **androgens** (**dehydroepiandrosterone sulfate** (**DHEAS**) and **androstenedione**), are secreted in physiologically significant amounts (**Part C** and Ch. 22, **Part E**). Birds, mice and rats secrete more corticosterone than cortisol; cats, sheep and primates secrete more cortisol than corticosterone; and dogs secrete approximately equal amounts of each. Corticosterone exhibits both gluco- and mineralocorticoid activity (see Ch. 22, **Part E**), and when the **zona fasciculata** fails (the source of cortisol), corticosterone production by the **zona glomerulosa** can partially compensate diminished glucocorticoid activity.

Cholesterol (**CH**) is the starting material from which all steroid hormones are produced, and at the adrenals it is largely derived from circulating **low-density lipoprotein** (**LDL**). If necessary, adrenocortical cells can also synthesize CH from its primary precursor, acetate. The first step in steroid hormone biosynthesis is to form the **progestogens** (or progestins, namely pregnenolone and progesterone) from CH, which then function as precursors to all other corticosteroids. In the presence of angiotensin II (AT II) and/or hyperkalemia, enzymes of the mineralocorticoid pathway in the **zona glomerulosa** are expressed (particularly aldosterone synthase), and in the presence of ACTH enzymes of the glucocorticoid pathway in the zona fasciculata are expressed (particularly 17α-hydroxylase). The **zona glomerulosa** cannot produce cortisol because it lacks 17α-hydroxylase, and the **zona fasciculata** cannot produce androgens because it lacks 17, 20-lyase. Most testosterone and estrogen not formed in the gonads (testes and ovaries) are produced in peripheral tissues and in the circulation from adrenal androstenedione. Secretion of adrenal androgens is controlled by ACTH (not pituitary gonadotropins), and there appears to be no sex difference in secretion (i.e., it is low and normally the same in both males and females). Adrenal androgen production, however, is thought to be more important in fetal than in adult life (Ch. 63).

Aldosterone is a mineralocorticoid that has little direct effect on general metabolism, but does affect ionic equilibrium by controlling renal Na^+ and K^+ excretion. In contrast, **cortisol** exhibits pronounced effects on carbohydrate, protein, and lipid metabolism. **Cortisone** is a less active glucocorticoid formed from cortisol through the activity of **11β-hydroxysteroid dehydrogenase-2** in the liver and kidneys, and little (if any) is secreted by the adrenals (Ch. 22).

Adrenocortical Enzyme Imbalances

Partial deficiencies in any one of the adrenal enzymes depicted in **Part C** can lead to overproduction of some corticosteroids, and underproduction of others. An **11β-hydroxylase** deficiency, for example, would lead to overproduction of 11β-deoxycortisol and 11-deoxycorticosterone, to underproduction of cortisol and aldosterone, and to overproduction of androgens. A **21β-hydroxylase deficiency** would have similar effects. Deficiencies in these enzymes would cause symptoms of glucocorticoid and mineralocorticoid deficiency, and ACTH secretion would increase. Elevated ACTH would lead to adrenocortical hyperplasia and accumulation of precursor steroids (i.e., 17-OH-progesterone and 17-OH-pregnenolone), which would then be shunted into further adrenal androgen formation. Animals that exhibit partial deficiencies in these enzymes may develop a dermatosis due to developing hyperprogestinism and hyperandrogenism.

Tumors of the **zona reticularis** may cause it to partially transform itself into the **fasciculata** cell type. When this occurs, features of androgen excess as well as glucocorticoid excess may appear. Additionally, androgen excess is known to reduce the activity of 11β-hydroxylase and aldosterone synthase, causing increased 11-deoxycorticosterone metabolites to appear in urine. As 11-deoxycorticosterone concentrations increase in blood, symptoms of mineralocorticoid excess may also develop.

Drugs such as **metyrapone** and **ketoconazole** block corticosteroidogenesis. Metyrapone inhibits 11β-hydroxylase, and ketoconazole inhibits several enzymes, including cholesterol desmolase, which converts CH to pregnenolone.

Catabolism of Adrenal Steroids

Most cortisol is bound to transcortin (CBG) in blood, and to a lesser degree, albumin (Ch. 22). **Unbound cortisol** appears in saliva and milk (Ch. 67), and that filtered by the kidneys is mostly (80%) reabsorbed. Free cortisol levels in milk and saliva have been used as indirect indicators of that in blood.

Adrenal steroids are catabolized and inactivated principally by the liver and kidneys (**Part D**). **Tetrahydroglucuronides** of aldosterone, cortisol, cortisone, and corticosterone are freely soluble in water; about 15% of these metabolites appear in feces, with the remainder appearing in urine. Biliary excretion of adrenal steroids allows for much of what is excreted to be reabsorbed by the intestine and returned to the liver via portal blood, thus remaining within the **enterohepatic circulation** (**EHC**).

A smaller portion of cortisol (but not corticosterone) is converted in the liver to **17-ketosteroid** derivatives of cortisol and cortisone that are conjugated to sulfate and excreted in urine and bile (**Part D**). DHEA and androstenedione secreted by the adrenal cortex and gonads have a keto group in the 17 position; therefore, they are also found in urine. The daily urinary 17-ketosteroid excretion in males is normally greater than that in females.

The rate of hepatic inactivation of steroid hormones, including the sex steroids, is decreased in liver disease, and therefore their concentrations in blood remain elevated over time. During surgery and other stresses, glucocorticoid levels in plasma sometimes rise higher than they do with maximal ACTH stimulation (in the absence of stress).

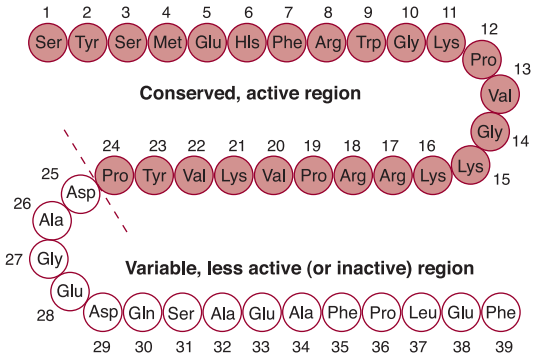
Ch22 ACTH and Glucocorticoids: II
(ACTH Secretion and Action)

E

Steroid	Glucocorticoid activity	Mineralocorticoid activity	Approximate normal plasma concentration (μg/dL)
Naturally occurring			
Cortisol	1.0	1.0	10
Cortisone	0.8	0.8	—
Corticosterone	0.5	15	1
Deoxycorticosterone	0.01	100	0.07
Aldosterone	0.3	3000	0.009
Synthetic			
Prednisone	4	<0.1	—
6α-Methylprednisone (Medrol)	5	<0.1	—
9α-Fluoro-16α-OH-prednisolone (triamcinolone)	5	<0.1	—
9α-Fluoro-16α-Methylprednisolone (dexamethasone)	30	<0.1	—
9α-Fluorocortisol	10	500	—

All potencies are relative to the glucocorticoid and mineralocorticoid activity of cortisol (liver glycogen deposition and renal Na^+/K^+ balance, respectively), which have been assigned the arbitrary value of 1.0. (Data from various sources.)

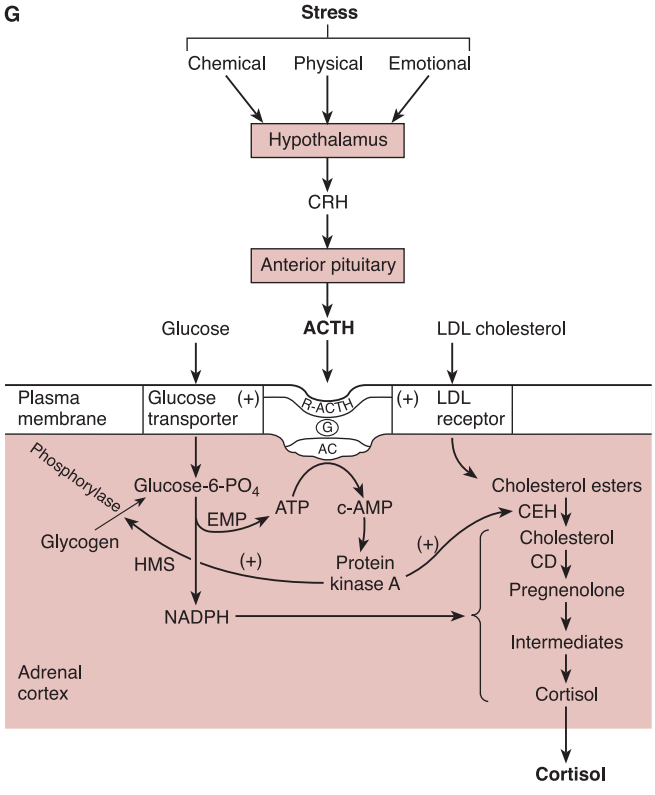
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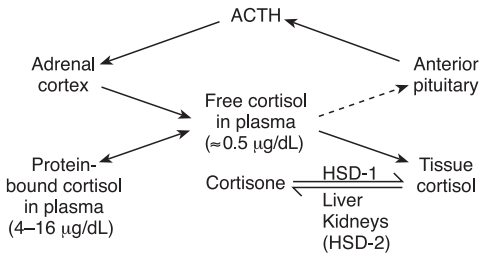
ACTH

ACTH is a peptide chain containing 39 amino acid residues, with its origin from pituitary POMC being discussed in Ch. 8. The first 24 amino acids generally constitute an active core, while amino acids 25-39 constitute a stabilizing tail that varies slightly in composition between animal species. The circulating t½ of ACTH is about 10 minutes, with a large part of an injected dose appearing in the kidneys. However, the true site of ACTH inactivation remains unknown.

G

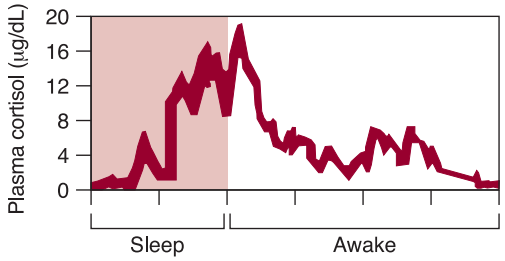


H



I

The biologic clock located in suprachiasmatic nuclei of the hypothalamus (Ch. 60) is responsible for the diurnal ACTH rhythm that regulates circadian cortisol release.



Sources: Part F modified from Murray RK, Granner DK, Mayes PA, Rodwell VW [eds]: Harper's illustrated biochemistry. 27th ed, New York, NY: McGraw-Hill Medical, 2006:447. **Part H** modified from Ganong WF: Review of medical physiology. 17th ed, Stamford, CT: Appleton & Lange, 1995:227. **Part I** redrawn from Weitzman ED, et al: J Clin Endocrinol Metab 33:14, 1971. The Endocrine Society.

Adrenal Steroid Potency

Relative potencies of naturally occurring and synthetic corticosteroids are shown in **Part E**. **Corticosterone** and **deoxycorticosterone** have more mineralocorticoid activity than cortisol, and **cortisone** is a weaker glucocorticoid than cortisol. Normal plasma concentrations of cortisol are approximately 1100 times > aldosterone, yet aldosterone has a mineralocorticoid potency that is 3000 times > cortisol. Although both **cortisol** and **aldosterone** bind with equal affinity to renal mineralocorticoid receptors (MRs), cortisol does not normally activate those receptors since the kidney expresses **11β-hydroxysteroid dehydrogenase-2** (**HSD-2**) activity (which converts cortisol to the less active cortisone, which binds MRs weakly). Cortisone is only active in tissues expressing **HSD-1** activity, which converts cortisone to cortisol.

Corticosteroid activity can be altered through structure modification. Of the synthetic corticosteroids, **prednisone** and **prednisolone** possess primary glucocorticoid activity, while **9α-fluorocortisol** exhibits both gluco- and mineralocorticoid activity. Synthetic corticosteroids are highly effective because they exhibit specificity, and long biologic half-lives.

ACTH Secretion

The primary regulator of adrenal cortical activity is the anterior pituitary hormone, **ACTH**. It controls growth of the inner two zones of the cortex, maintains structural and functional integrity of all cells in the cortex, and stimulates secretory activity, particularly of **cortisol**. Large doses of other naturally occurring substances, including ADH, serotonin (5-HT), and VIP are capable of stimulating adrenal activity, but there is no firm evidence that these agents play a role in the physiologic regulation of cortisol release. ACTH increases sensitivity of the adrenal glands to its subsequent presence. However, the naturally occurring and synthetic glucocorticoids eventually **feedback negatively** on the adrenal glands, hypothalamus and pituitary, decreasing responsiveness of the adrenal glands to ACTH and inhibiting further ACTH release (Chs. 2 and 25).

Mammalian ACTH has been purified from several sources (e.g., the bovine, porcine, ovine, and primate), and in all of these species it has 39 amino acid residues in a single polypeptide chain, with a molecular weight of about 4500. The 1-24 amino acid fragment possess full potential for stimulating adrenal cortical activity, fragment 1-19 stimulates only 80% activity, and the 1-16 fragment possesses little activity for stimulating cortisol secretion. Amino acids 25-39 appear to lie outside of the region responsible for stimulating adrenal cortical activity (**Part F**).

A classic bioassay for ACTH had been an adrenal ascorbic acid depletion (**AAAD**) test, as **vitamin C** is needed for steroid hydroxylation reactions. However, the AAAD test has been largely replaced today by **immunoassay** (Ch. 3).

Many adverse conditions cause secretion of **ACTH-releasing hormone** (**CRH**) from the hypothalamus (which in turn causes **ACTH** release from the anterior pituitary). Such conditions include chemical, physical, and emotional **stress** (e.g., overcrowding, extreme external cold or heat, severe exercise, traumatic shock, toxins, hemorrhage, infections, starvation, hypoglycemia, etc.) (**Part G**). **CRH** is a polypeptide containing 41 amino acid residues, with a plasma half-life of approximately 1 hour. Both **ADH** and **angiotensin II** potentiate the actions of **CRH** on pituitary **ACTH** release, which is thought to aid in the phasic response to trauma (Chs. 12, 27, and 35). In rats, CRH and ADH have been colocalized in the same neurons. In contrast, oxytocin may reduce CRH-mediated ACTH secretion in some animals.

CRH has also been shown to enhance release of **GH** from the anterior pituitary (Ch. 7), an action independent of that of the opiate peptides. CRH apparently facilitates release of **GHRH** from the hypothalamus in response to stress while inhibiting somatostatin (**GHIH**) release (Ch. 10). Another secondary action of CRH on the anterior pituitary involves the sex hormone axis. **CRH** has been shown to inhibit **LH** and **FSH** release (Ch. 7), which may help to explain why the estrous cycles of some animals tend to shut down during periods of stress. Although an ACTH-inhibiting factor may exist in mammals, it has not been confirmed.

Mechanism of ACTH Action

The mechanism of action of **ACTH** on adrenal cortical cells in the inner two zones is as follows: When ACTH binds to its receptor (**R-ACTH**), adenyl cyclase (**AC**) is activated through a guanosine-binding protein (**G**). In addition, binding of ACTH to its receptor favors transport of **glucose** into cortical cells (as well as **cholesterol** from **LDL**). Glucose is oxidized via the Embden-Meyerhoff pathway (EMP). This yields **ATP**, as well as **NADPH** via the hexose monophosphate shunt (HMS). Through activation of AC, ATP is converted to **cAMP**, which in turn activates protein kinase A. Protein kinase A activates a phosphorylase needed for glycogenolysis, and also phosphorylates cholesterol ester hydrolase (**CEH**), thus increasing its activity. Consequently, more free cholesterol is formed and converted to pregnenolone via CH desmolase (**CD**)-stimulated side chain cleavage, and then to intermediates of cortisol biosynthesis. Note that NADPH derived from the HMS is required for steroid biosynthesis.

Unlike other glandular cells, steroid-producing cells do not store their hormones, but rather synthesize them on demand. Stimulation by ACTH increases cortisol secretion in dogs within **1 to 2 minutes**, with peak rates occurring in about **15 minutes**. ACTH also exerts minor actions on cortical aldosterone and medullary catecholamine biosynthesis (Chs. 26 and 32).

Cortisol Transport

Cortisol is bound in plasma to an α-globulin called **transcortin** (or **corticosteroid-binding globulin, CBG**), and to a lesser extent to **albumin**. The plasma half-life for cortisol is about **60 to 90 minutes**. Bound cortisol is inactive, yet it serves as a circulating reservoir of the hormone. At normal plasma concentrations of cortisol and transcortin, there is little free cortisol available to tissues (**Part H**). When binding sites on CBG become saturated (cortisol concentrations >20 μg/dL), however, free and tissue cortisol levels rise. Transcortin is synthesized in the **liver**, and its production is increased by **estrogen** (e.g., during pregnancy) and decreased by liver disease (e.g., cirrhosis). Some patients with nephrosis (loss of CBG in urine) have low total plasma cortisol, yet exhibit few symptoms of glucocorticoid deficiency because free cortisol levels change minimally.

Cortisol Circadian Rhythmicity

Plasma concentrations of cortisol vary within a normal range in mammals of about 4 to 16 μg/dL, and show circadian rhythmicity, with normal concentrations rising during the sleeping hours (**Part I**). Because of this circadian rhythmicity, the time of blood sampling should be taken into account when interpreting clinical concentrations of plasma cortisol (Ch. 3).

In summary, the endogenous adrenal corticosteroids possess overlapping potencies, however, cortisol, corticosterone and cortisone are primarily glucocorticoids, while aldosterone and deoxycorticosterone are primarily mineralocorticolds. The chief stimulator of **cortisol** release is pituitary **ACTH**, which in turn is regulated by **CRH** from the hypothalamus. Both ADH and angiotensin II appear to potentiate the actions of CRH on both pituitary ACTH and GH release during stress. Conversely, CRH appears to inhibit pituitary LH and FSH release (Ch. 7).

Unlike other endocrine cells, steroid-producing cells synthesize and secrete upon demand. Therefore, adrenocortical steroidogenesis is a comparatively slow process. Cortisol is bound in plasma by **transcortin**, a protein synthesized by the liver. The plasma half-life of cortisol is dependent upon several factors, to include the ability of liver cells to conjugate it. Plasma cortisol concentrations may vary widely, and typically exhibit circadian rhythmicity.

Ch23 ACTH and Glucocorticoids: III

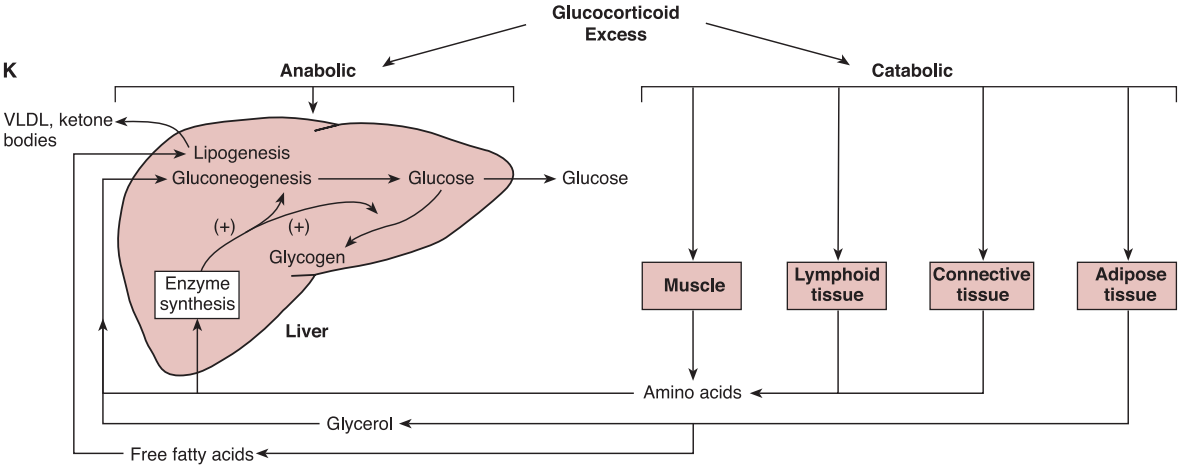
(Organ and Tissue Effects)

J

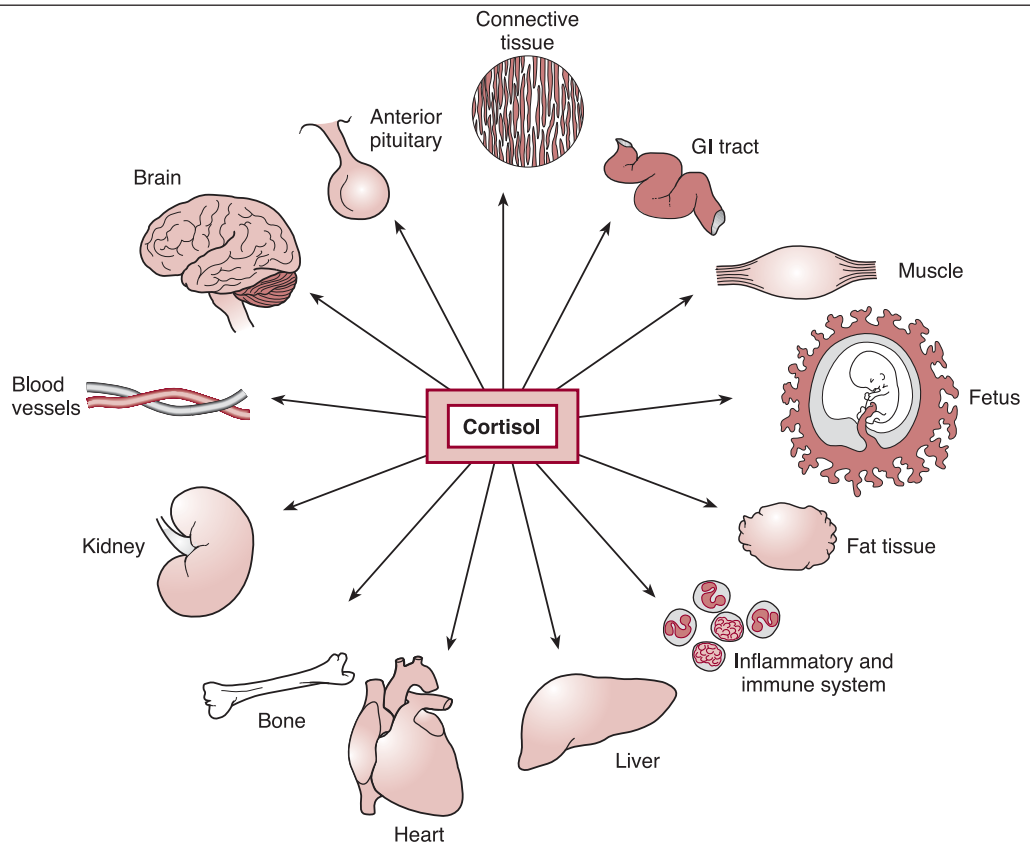
Hormone	Protein binding (%)	Plasma t½ (days)	Metabolic clearance (ml/min)
T_4	99.97	6	0.7
T_3	99.7	1	18
Cortisol	94	0.07	140
Testosterone	89	0.04	860
Aldosterone	15	0.016	1100
TSH	None	0.034	50
Insulin	None	0.006	800

Data from Berne RM, Levy MN: Physiology. 4th ed, St Louis, MO: Mosby, 1998.

K



L



Metabolic Clearance

Hormone plasma half-life (t½) is directly correlated with the % of protein binding (**Part J**). Thyroxine (T_4), is tightly protein bound, whereas aldosterone is only 15% bound, and has a plasma t½ of about 25 mins. Larger protein hormones have longer half-lives than smaller proteins and peptides, and hormone exit from plasma is not necessarily unidirectional. Sometimes a hormone returns from other compartments, possibly following dissociation from receptors, or exhibits a delayed return via lymphatic channels.

Although <1% of secreted **cortisol** normally appears unchanged in urine, about 30% of cortisol metabolites are excreted by canine kidneys (less in felines), because they are variably protein-bound. Peptide and smaller protein hormones are filtered to some degree by glomeruli, but they usually undergo tubular reabsorption and degradation within proximal tubular epithelial cells, so that only a small fraction finally appears in urine.

Glucocorticoids and Intermediary Metabolism

Primary effects of glucocorticoids on carbohydrate metabolism are increased hepatic gluconeogenesis and glycogenesis, and decreased glucose utilization in extrahepatic insulin-sensitive tissues (**Part K**). Free fatty acid mobilization from adipocytes also occurs, with conversion of the fatty acids in the liver to triglycerides and ketone bodies. Protein synthesis is reduced, and there is a generalized breakdown of muscle protein into amino acids for hepatic gluconeogenesis. **In general, the actions of glucocorticoids on carbohydrate, fat, and protein metabolism are opposite to those of insulin.**

In large amounts, glucocorticoids inhibit several steps associated with the inflammatory and immune responses evoked by, for example, tissue trauma, chemical irritants, infection, or foreign proteins. Therefore, they are frequently used therapeutically.

In this chapter we will begin to examine the physiologic and supraphysiologic (i.e., pharmacologic) actions of the glucocorticoids (illustrated schematically in **Part L**, and summarized in Ch. 24, **Part M**).

Starvation

Cortisol acts **permissively** to **facilitate fuel mobilization** in times of need. The nocturnal surge of cortisol discussed in Ch. 22 supports the enhanced gluconeogenesis and lipolysis necessary for overnight metabolic stability and, if food continues to be unavailable, for longer periods of time. The rate of amino acid mobilization from muscle is noticeably increased during starvation. Therefore, when liver glycogen stores have been depleted (by exercise, for example), deficient gluconeogenesis from amino acids may lead to death from hypoglycemia if glucocorticoid secretion is also deficient. Cortisol also antagonizes the actions of insulin on glucose metabolism, thereby inhibiting insulin-stimulated glucose uptake into muscle, lymphoid, connective, and adipose tissue (which leaves more glucose in blood to go to insulin-insensitive tissues such as nerves) (**Part K**). Cortisol also increases appetite.

In short, **cortisol** is an important diabetogenic, anti-insulin hormone during starvation. Its primary hyperglycemic and lipolytic actions and secondary proteolytic and ketogenic actions are usually exhibited only when its secretion is greatly stimulated by metabolic stress. Cortisol then potentiates and extends the duration of **hyperglycemia** evoked by **glucagon**, **epinephrine**, and **growth hormone**, and accentuates loss of body protein.

Effects on Muscle

At low concentrations, cortisol has an **inotropic** effect on skeletal muscle that may be exerted via an increase in acetylcholine synthesis at the neuromuscular junction. Cortisol also increases **β-adrenergic receptor** synthesis (like thyroxine and progesterone). Cortisol excess, however, causes insulin insensitivity (decreased glucose uptake), muscle proteolysis, and consequently reduced muscle mass and strength (particularly of slow oxidative type I muscle fibers).

Effects on Fat Tissue

Cortisol enhances the synthesis of **adipolytic triglyceride lipase** (i.e., **hormone-sensitive lipase**), thereby enhancing the actions of other lipolytic hormones, and decreases glucose uptake into adipocytes, thereby reducing triglyceride deposition (because glucose is required for adipocyte glycerol formation). However, when food intake is increased, glucocorticoid excess induces fat redistribution to centripetal areas, with deposits occurring in the face ("moonface") and supra-scapular regions ("buffalo hump"). Excessive fat distribution can lead to a **pendulous abdomen** in animals.

Hyperlipidemic Action

As previously stated, cortisol increases **lipolysis** by epinephrine, ACTH, and GH during starvation by decreasing glucose entry into adipocytes and increasing hormone-sensitive lipase synthesis. Free fatty acids enter the circulation and are removed into **liver** cells, where they are repackaged into triglyceride and exocytosed into the circulation as very-low-density lipoprotein (**VLDL**), thereby creating a **hypertriglyceridemia**. Cortisol also decreases sensitivity of the anterior pituitary to **TRH**, thereby reducing TSH output. The relative hypothyroid state that follows can cause **hypercholesterolemia**, as T_4 is required to maintain LDL receptor synthesis in liver cells (Ch. 37).

Effects on the Liver

As indicated above, glucocorticoids **enhance hepatic glucose production** by **1**) increasing the delivery of amino acids and glycerol (gluconeogenic substrates) from peripheral tissues to the liver, and by **2**) increasing the rate of gluconeogenesis through increasing the amount (and activity) of the key gluconeogenic enzymes (i.e., pyruvate carboxylase, PEP carboxykinase, fructose 1,6-bisphosphatase, and glucose 6-phosphatase). Glucocorticoids may also increase **hepatic glycogen deposition** in the resting state by providing, through gluconeogenesis, more available glucose 1-phosphate. Through the actions of glucocorticoids on important hepatic enzymes controlling glucose homeostasis, the gluconeogenic effects of glucagon, GH, and epinephrine are enhanced.

Glucocorticoids also promote the hepatic resynthesis of triglyceride from incoming long-chain fatty acids, and facilitate VLDL formation and exocytosis. When long-chain free fatty acids are in excess, cortisol may facilitate their hepatic conversion to **ketone bodies** (**Part K**). By increasing hepatic **angiotensinogen** production, cortisol excess may also lead to hypertension (Chs. 25 and 27).

Effects on Bone

Glucocorticolds, in general, have a tendency to **reduce Ca^{2+} input into the body, and enhance urinary Ca^{2+} excretion**. Therefore, they are usually associated with lowering serum Ca^{2+} levels, and are sometimes used to depress the hypercalcemia of vitamin D toxicity.

Glucocorticoid excess, however, leads to a decrease in bone formation, and an increase In bone resorption. These steroids decrease bone formation by inhibiting protein synthesis in osteoblasts, and over time promote the osteoporotic process (Ch. 20). Glucocorticoids also reduce Ca^{2+} and PO_4^{3-} availability to bone by: **1**) opposing the actions of vitamin D in the small intestine, thus decreasing Ca^{2+}, Mg^{2+}, and PO_4^{3-}- absorption, and **2**) decreasing Ca^{2+} and PO_4^{3-} reabsorption in the nephron, thus promoting their excretion into urine (Ch. 17). The decrease in the plasma Ca^{2+} concentration that follows increases PTH release from the parathyroids, which then promotes further bone loss. Cortisol also reduces the synthesis of type I collagen, a fundamental component of the bone matrix.

Ch24 ACTH and Glucocorticoids: IV
(Physiologic and Pharmacologic Actions)

Tissue Responses to the Glucocorticoids

M

Effector	Response
Blood	Hypertriglyceridemia* Hypercholesterolemia* Hyperglycemia* ↑Erythrocytes, platelets, neutrophils* ↓Lymphocytes, eosinophils, basophils* ↓Antibodies
Bone	↑Resorption + ↓Accretion = ↓Bone mass* ↓Type I collagen*
Connective Tissue	↓Collagen synthesis* ↓Fibroblast activity*
CNS	↑Appetite ↓Sensory acuity ↓REM sleep* Insomnia* Mood swings* ↓Seizure threshold* ↓CRH secretion
Fat Tissue	↑Hormone-sensitive lipase synthesis ↑Lipolysis ↓Glucose uptake Fat redistribution (facial, suprascapular, abdominal)
Muscle	Inotropic ↑β-Adrenergic receptor synthesis ↑Proteolysis* ↓Glucose uptake (insulin insensitivity)*
Growth and Development	Maturation of: CNS Retina GI tract and liver enzymes Lungs ↑Surfactant synthesis ↓Growth of young animals*
GI Tract	Stomach (ulcerogenic)*: ↑HCl secretion ↓HCO_3^- secretion ↓Prostaglandin production Duodenum: ↓Ca^{2+} absorption
Permissive	↑Catecholamine actions ↑Glucagon actions

*Actions occurring when plasma titers of cortisol are substantially elevated (e.g., during therapeutic application, Cushing's-like syndrome or disease, or severe chemical, physical, or emotional stress).

Effector	Response
Immune System	↓Immune response ↓Macrophage interleukin-1 release ↓T-cell interleukin-2 and -6 release ↓Tumor necrosis factor α ↓T-cell proliferation ↓B-cell proliferation ↓Antibody production
Inflammatory System	↓Inflammatory Response* ↓Phospholipase A_2 ↓Arachidonic acid ↓Leukotrienes ↓Neutrophil function ↓Bacterial killing ↓Cyclooxygenase (COX–2) ↓Prostaglandins ↓Thromboxanes Vasodilation ↓Platelet-activating factor ↓Nitric oxide
Kidneys	↑Excretion of H_2O load Maintain GFR (↓Cortisol → ↓Urine volume) Blunt ADH release and renal effects* PU/PD* ↓Ca^{2+} and PO_4^{3-} reabsorption* Glutamine → NH_4^+ + glucose
Liver	↑Gluconeogenesis ↑Glycogenesis ↑Lipogenesis ↑Ketogenesis ↑Angiotensinogen
Pituitary	↓ACTH secretion (acute) and synthesis (chronic) ↓TSH → ↓T_4 → ↓LDL receptors → ↑Cholesterol ↓GH ↓ADH
Cardiovascular	Assist in maintaining normal blood volume and pressure: ↑Myocandial performance Permit normal responsiveness of arterioles to NE and Ang. II ↓Pdn. of vasodilator PGs ↓Permeability of vascular endothelium

Effects on Connective Tissue

Cortisol excess inhibits collagen synthesis and fibroblast activity, producing **thinning of skin** and the walls of capillaries (which can result in capillary fragility, rupture, and intracutaneous hemorrhage). Loss of connective tissue leads to poor wound healing.

Effects on the Cardiovascular System

Cortisol helps to maintain normal blood pressure and volume by: 1) sustaining myocardial performance and increasing cardiac output, **2**) permitting normal responsiveness of arterioles to the constrictive actions of catecholamines and angiotensin II, **3**) decreasing production of vasodilator prostaglandins from the vascular endothelium, and **4**) decreasing permeability of the vascular endothelium. In adrenally insufficient animals, vascular smooth muscle becomes less responsive to the vasoconstrictive effects of norepinephrine and angiotensin II, capillaries dilate and, terminally, become permeable to colloids. Failure to respond to norepinephrine impairs vascular compensation for the hypovolemia of adrenal insufficiency, and severe hypotension with vascular collapse can occur (Ch. 29).

Effects on the Kidneys

Cortisol aids in the excretion of a water load by: 1) helping to maintain the glomerular filtration rate, **2**) suppressing ADH release, and **3**) decreasing ADH effects on collecting tubules. With cortisol excess, **PU/ PD** is evident. Cortisol is also required for generation of NH_4^+ from glutamine, and aids in the renal gluconeogenic conversion of glutamine hydrocarbons to glucose. As mentioned above, cortisol reduces renal Ca^{2+} and PO_4^{3-} reabsorption.

Effects on the GI Tract

Although cortisol **facilitates maturation of the fetal GI tract**, glucocorticoid excess in adult animals increases parietal cell HCl secretion and decreases epithelial neck cell HCO_3^- and prostaglandin production in the stomach, thereby **reducing the gastric mucosal barrier** and favoring peptic ulcer formation. Cortisol also blunts the action of $1,25(OH)_2D$ on the small intestine, thereby **reducing Ca^{2+} absorption**.

Effects on the CNS and Pituitary

Cortisol increases appetite, decreases rapid eye movement (REM) sleep, and modulates excitability, mood, and behavior. In excess, cortisol can cause insomnia, elevate or depress moods, decrease memory, and lower the threshold for seizure activity. Cortisol excess also decreases the ability to detect a salty taste and dampens acuity to sensory stimuli. **Glucocorticoid therapy** can depress CRH and ACTH output, thereby making it more difficult for the pituitary to rebound once therapy has been discontinued. High levels of cortisol also suppress ADH, TSH, and GH release from the pituitary.

Cortisol reduces glucose utilization by insulin-sensitive tissues (e.g., muscle and adipose tissue), thereby sparing this important nutrient for use in the CNS.

Effects on Growth and Development

Glucocorticoids accelerate development of several **organ systems** in differentiating and fetal tissues. Although mechanisms are unclear, these effects are thought to be due to interactions with several growth factors. Examples are increased lung surfactant production in the fetus and neonate, and accelerated development of hepatic and GI enzyme systems at the time of parturition. Maturation of the retina and CNS is partially dependent on glucocorticoids. In excess, glucocorticoids can inhibit the growth of young animals, which is potentially a major complication of therapy. This may be a direct effect on bone cells, although decreased GH secretion and somatomedin generation are also thought to contribute (Ch. 10).

Effects on Formed Elements of Blood

Cortisol **increases** the number of circulating **erythrocytes** by stimulating their production and decreasing their destruction, and increases the number of circulating **platelets** and **neutrophils** by increasing their release from bone marrow and decreasing their removal from the circulation (i.e., inhibiting diapedesis). Since neutrophils are normally 88% of the white blood count, excessive cortisol causes a **leukocytosis**.

Cortisol **decreases** the number of circulating **lymphocytes**, **eosinophils**, and **basophils** four to six hours following a dose by redistributing them away from the periphery rather than by increasing their destruction. Because cortisol decreases the mass of lymphoid tissue by directly inhibiting mitosis, it can be used therapeutically to treat lymphomas and lymphocyte leukemia. Involution of lymph nodes, the thymus, and the spleen leads to decreased antibody production, which may aid in the reduction of an immune response by an organ transplant recipient; however, antibiotics would be necessary adjunct therapy in order to counteract possible infections.

Effects on Inflammatory and Immune Responses

Although cortisol is required for survival of stressed, traumatized, or infected animals, many defense mechanisms engaged in the response to these insults appear to be inhibited by high titers of glucocorticoids. This apparent paradox can be explained by the following: Basal or modestly elevated levels of cortisol may be beneficial during the initial metabolic, inflammatory, and immune responses; however, when local inflammatory reactions become more intense and spread to adjacent uninjured sites, higher titers of glucocorticoids may be required to limit inflammatory and immune responses so that they do not destroy normal tissues.

High levels of cortisol **inhibit phospholipase A_2** and **cyclooxygenase**, thereby reducing eicosanoid biosynthesis (i.e., prostaglandins, thromboxanes, and leukotrienes) from arachidonic acid. Cortisol also inhibits synthesis of **nitric oxide** and **platelet-activating factor**, thereby impairing the vascular component of inflammation. Inhibition of **leukotriene** synthesis impairs neutrophil phagocytosis and the bactericidal abilities of the organism. Further inhibition of antigen presentation and macrophage **lymphokine** release (i.e., interleukin-1) impairs proliferation and further cytokine release from **T cells**. Ultimately, **B-cell** function is reduced, **antibody** production declines, and both cellular and humoral immunity are affected.

Glucocorticoids also interfere with the elaboration of **histamine** or with its actions in mediating the inflammatory response, which includes local hyperemia and resultant edema. One postulated mechanism for this effect is the glucocorticold-induced inhibition of the kallikreins, enzymes that catalyze formation of kinins from a plasma precursor protein. Kinins induce inflammation by causing release of histamine normally observed following the combination of antigen and antibody. Glucocorticoids also **stabilize lysosomal membranes**, thereby reducing release of hydrolytic enzymes following cell injury. This reduces spread of the inflammatory reaction.

Although the antiinflammatory and immunosuppressive effects of glucocorticoids may be useful in treating various disease states, **when these hormones are administered therapeutically over long periods of time, they may: 1**) increase susceptibility to bacterial, fungal, and viral infections and allow their dissemination; **2**) delay wound healing; **3**) exacerbate the symptoms of diabetes mellitus, osteoporosis, and psychiatric disorders; and **4**) seriously retard anterior pituitary release of ACTH and adrenal release of cortisol once discontinued. Therefore, **judicious care must be exercised in their use.**

Permissive Effects

Small amounts of glucocorticoids need to be present for catecholamines to promote bronchodilation, and also exert their calorigenic, lipolytic and pressor actions.

Ch25 Cushing's-Like Syndrome and Disease (Glucocorticoid Excess)

A

Signs and Symptoms of Cushing's-Like Syndrome or Disease (PDH)

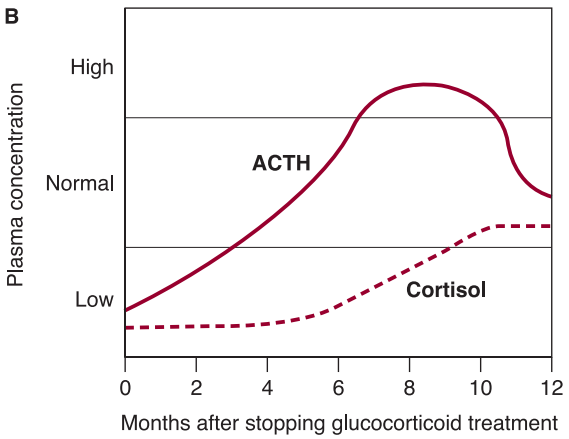
Physical
Cutaneous hyperpigmentation
Short stature and immature hair coat in young animals
PU/PD
Polyphagia
Abdominal enlargement
Thin skin (bilateral alopecia in dogs)
Bruising
Hirsutism (horses)
Exercise intolerance (muscle weakness and wasting)
Increased respiratory rate
Lethargy and obesity
Cold intolerance
Reduced acuity to sensory stimuli
Skin infections
Hypothyroidism
Calcinosis cutis
Exophthalmos
Anestrus, clitoral hypertrophy
Testicular atrophy
Osteoporosis
Hepatomegaly
Peptic ulcers
Hypertension, congestive heart failure

CBC
Mature leukocytosis
Neutrophilia
Lymphopenia
Eosinopenia
Erythrocytosis (mild)

Plasma Profile
↑Alkaline phosphatase, ↑alanine aminotransferase (ALT)
↑Cholesterol
Hypertriglyceridemia (↑VLDL)
Hyperglycemia, ↑insulin
↓Blood urea nitrogen (BUN)
↓Creatinine
↓T_4, T_3, TSH, GH, FSH, LH

Urinalysis
↓Specific gravity (hyposthenuria)
Urinary tract infection
Glucosuria, proteinuria
Urinary calculi (calcium phosphate or calcium oxalate)
↑Cortisol: Creatinine ratio

B



Glucocorticoids inhibit hypothalamic CRH and pituitary ACTH secretion, as well as adrenal cortisol production, with the degree of inhibition being proportionate to the circulating glucocorticoid level. Prolonged glucocorticoid therapy thus causes adrenal atrophy and reduced pituitary ACTH release. When treatment is stopped, a slow rise in ACTH to supranormal levels may be observed before normal adrenal cortisol output is restored. During this period of time, the animal is in a state of **secondary hypoadrenocorticism** (**Addison's-like disease**; see Chapter 29).

Source: Part B modified from Ganong WF. Review of medical physiology. 18th ed. Stamford, CT: Appleton & Lange, 1997: 352.

In 1932, **Harvey Cushing** published his observations that basophilic tumors of the pituitary were associated with secondary adrenocortical hyperplasia. The collective condition, including tumors of the pituitary with associated hyperadrenocorticism, was thus called **Cushing's disease** in humans, and **Cushing's-like disease** in animals. The term **Cushing's syndrome** was originally used to denote primary hypersecreting tumors of adrenal cortices (in order to differentiate them from ACTH-secreting tumors of the pituitary). However, today Cushing's syndrome (or Cushing's-like syndrome) is used as a general term to denote the clinical and pathophysiologic manifestations resulting from chronic exposure to excessive amounts of glucocorticoids. Therefore, Cushing's syndrome can have several origins (e.g., pituitary-dependent hyperadrenocorticism, PDH; ectopic ACTH-secreting tumors; primary hyperadrenocorticism; or chronic excesses of exogenously administered ACTH or glucocorticoids). The term Cushing's disease is still sometimes applied to those cases of Cushing's syndrome resulting from PDH, which accounts for the majority of cases in domestic animals.

Cushing's-Like Disease, or PDH

Pituitary-dependent hyperadrenocorticism (**PDH**) in animals is caused by excessive **ACTH** secretion, which results in excess **cortisol** secretion and, eventually, bilateral adrenocortical hyperplasia. Although ectopic ACTH-secreting tumors have been described in humans, they are less commonly reported in animals.

Pituitary-dependent hyperadrenocorticism is characterized by a lack of glucocorticoid negative feedback on ACTH secretion, and loss of hypothalamic control. Possible causes include pituitary hyperplasia, pituitary adenoma, pituitary carcinoma, and CNS dysfunction resulting in excessive stimulation of pituitary corticotrophs by CRH. Most animals with PDH apparently have a **pituitary tumor** originating from either the pars distalis, or pars intermedia (particularly dogs and horses). **Mineralocorticoid overproduction does not seem to occur** in animals with **PDH**, and TRH appears to increase ACTH release in horses with this disease (Ch. 72).

The most common cause of hyperadrenocorticism in domestic animals is PDH. ACTH secretory bursts are chronically excessive, which result in excessive episodic secretory activity of the adrenal **zona fasciculata** and sometimes **zona reticularis**. However, plasma concentrations of ACTH and cortisol sometimes fall within normal reference ranges because of the relatively short physiologic half-lives of these hormones. Urinary cortisol conjugate excretion over a 24-hour period, however, is generally elevated, as evidenced by increased urinary cortisol/creatinine ratios. In dogs, PDH results in bilateral alopecia, whereas in horses excessive hair growth (hirsutism) occurs. This latter effect may be due to excessive androgen production by the **zona reticularis**, or to failure of seasonal hair shedding.

Because **ACTH** shares a common amino acid sequence with **α-MSH** (Chs. 8 and 9), patients with PDH would be expected to exhibit hyperpigmentation. Although hyperpigmentation may be evident, not all animals with PDH reportedly exhibit this sign. Also, hyperpigmentation has been described in animals with either pituitary or adrenal causes for Cushing's-like syndrome.

Primary Hyperadrenocorticism

Functional adrenocortical tumors (adenomas or carcinomas) causing signs and symptoms of Cushing's-like syndrome can apparently be identified by ultrasonography. These tumors function independent of ACTH control, and both **CRH** and **ACTH** blood levels are reportedly low or undetectable. If the tumor is unilateral, cortical atrophy of the uninvolved adrenal will also be evident. Histologically, the **zona reticularis** is apparently reduced, the **zona fasciculata** enlarged, and the **zona glomerulosa** may be near normal.

Hyperadrenocorticism is a common endocrinopathy in dogs, with about **80-85%** of cases resulting from **PDH**. The other **15-20%** are apparently due to **adrenocortical tumors**. Adrenocortical carcinoma and adenoma occur with about equal frequency. Bilateral tumors occur in dogs, however, they reportedly occur with far less frequency than unilateral tumors. Hyperadrenocorticism is occasionally seen in cats, with most associations described here applying to this species as well. Hyperadrenocorticism is rare in horses and other domestic animal species, however, **iatrogenic Cushing's-like syndrome** can occur in any animal chronically exposed to excessive amounts of exogenous glucocorticoids.

Mitotane (o,p'-DDD; Ortho,para'-ODD; **Lysodren**; Bristol Myers Oncology) is a common drug used in the treatment of adrenocortical tumors. Although closely related to the insecticides DDD and DDT, mitotane is the prototype of a drug with selective antitumor activity against adrenocortical carcinoma; however, it is also toxic to normal adrenal cortical cells. As most adrenal tumors are handled surgically, this drug is used almost exclusively for adrenal hyperplasia.

Iatrogenic Cushing's-Like Syndrome

Chronic exposure to excessive amounts of exogenous glucocorticoids may also result in the development of the classic signs and symptoms of Cushing's-like syndrome (**Part A**). Reports of chronic exposure to excessive amounts of injectable, oral, topical, or ophthalmic glucocorticoids indicate the importance of this source of the syndrome. Iatrogenic Cushing's like syndrome is sometimes referred to (incorrectly) as "iatrogenic hyperadrenocorticism." Since the pituitary and adrenal glands actually become downregulated, and adrenal cortisol output becomes reduced during exogenous glucocorticoid therapy, this should be referred to as "iatrogenic secondary hypoadrenocorticism." **Due to the sustained negative feedback effects of long-term exposure to exogenous glucocorticoids, the pituitary and adrenal glands may take weeks-months to recover before a normal ACTH:cortisol balance is restored following discontinuation of therapy** (**Part B**).

Signs and Symptoms of Glucocorticoid Excess

Chronically excessive glucocorticoid levels, whether caused by hyperadrenocorticism or iatrogenic Cushing's-like syndrome, have adverse effects on the metabolism of many tissues, including the brain, muscles, skin, vasculature, kidney, liver, and skeleton (**Part A**). Although hypophysectomy may alleviate symptoms in cases of PDH, this procedure necessitates extensive replacement therapies since all of the tropic hormones from the pituitary are removed. Adrenalectomy requires only corticosteroid therapy.

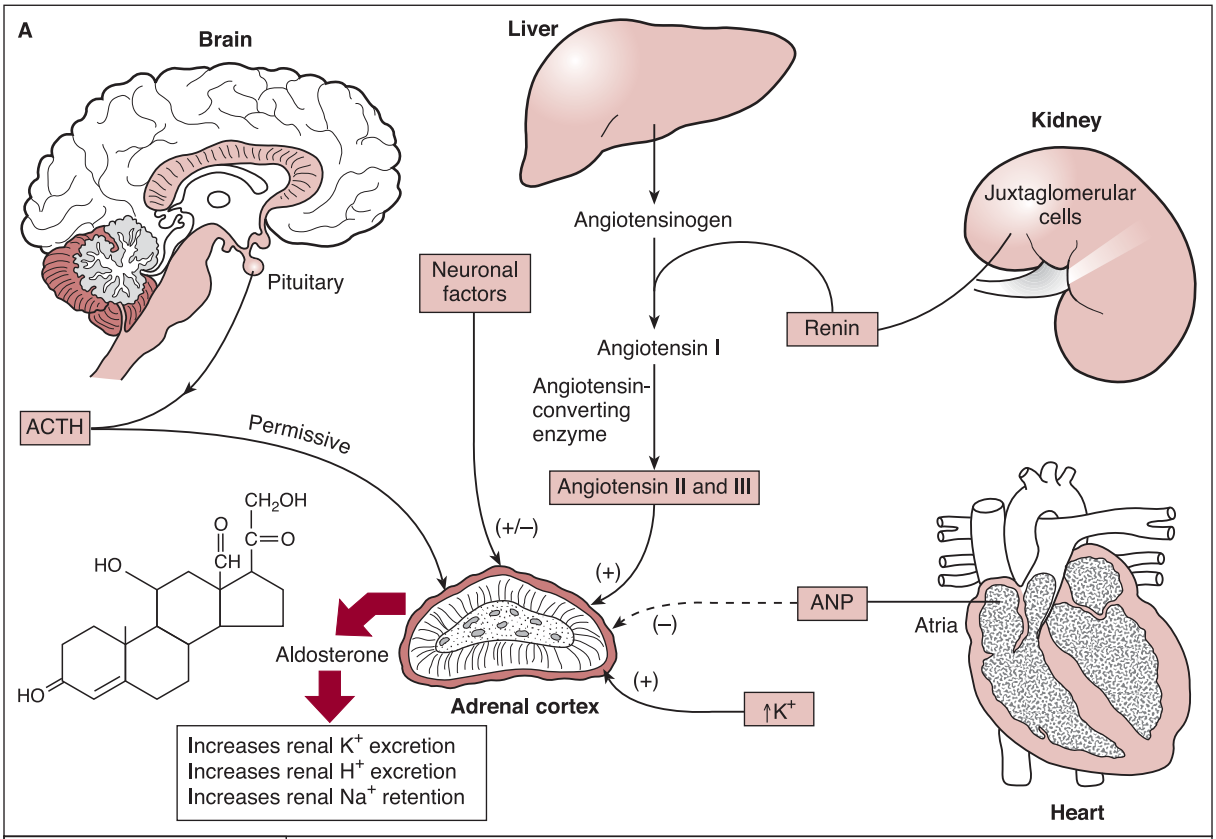
Common signs and symptoms of **glucocorticoid excess** include polyuria/polydipsia (PU/PD), bilateral alopecia in dogs, polyphagia, abdominal enlargement, enhanced respiratory rate, muscle weakness, and lethargy (among others). Since cortisol enhances insulin resistance, glucocorticoid excess also leads to a **secondary diabetes mellitus**, with most of the attending clinical signs. Unless pancreatic β-cells have been exhausted, insulin levels may be elevated.

Glucocorticoid excess causes a **steroid hepatopathy**, with hepatomegaly and excessive glycogen deposition (vacuolar hepatopathy) in dogs. Plasma elevations of alkaline phosphatase, alanine aminotransferase, and cholesterol appear to be good indicators of hyperadrenocorticism, particularly when plasma bilirubin concentrations remain within the normal range. The urine specific gravity is typically low (hyposthenuria) in hyperadrenocorticism, owing to the effects of glucocorticoids on the GFR and on renal responsiveness to ADH (Ch. 24). Glucosuria and proteinuria may also be evident, along with symptoms consistent with urinary tract infection. The proteinuria may evolve from glucocorticoid-induced hypertension. Urine protein/creatinine ratios, like urine cortisol/creatinine ratios discussed above, may be well above normal. Since cortisol reduces renal Ca^{2+} reabsorptlon, urinary calculi may also be evident.

Glucocorticoid excess can also inhibit normal pituitary and hypothalamic function, resulting in a secondary hypothyroidism (decreased TSH), testicular atrophy and anestrus (decreased gonadotropins), and short stature among young animals (decreased GH).

Ch26 Mineralocorticoids

(Effects on Na^+, K^+ and H^+)



The **zona glomerulosa** of the adrenal cortex secretes **aldosterone**, its major mineralocorticoid, independent of direct pituitary control. **Corticosterone** and **11-deoxycorticosterone** are additional corticosteroids secreted by the adrenal cortex; however, they possess less than 3% of the activity of aldosterone (Ch. 22). Recent studies indicate that small amounts of aldosterone may be produced by cardiac and vascular cells as well.

Although **ACTH** plays an important **permissive** role in maintaining biosynthetic stability and responsiveness of the **zona glomerulosa** to other controlling factors (e.g., increased serum **K^+** and the **angiotensins**; **Part A**), it plays a minor facilitory role in aldosterone release. The major target sites for aldosterone are the **distal convoluted tubules** and **cortical collecting ducts** of the **kidneys**, where it facilitates **Na^+ reabsorption** and **K^+** and **H^+ secretion**.

The mineralocorticoid receptor (MR) has a higher affinity for cortisol *in vitro* than the glucocorticoid receptor, and cortisol is present in larger amounts *in vivo* than aldosterone (Ch. 22). This raises the question as to why cortisol does not activate renal MRs. The answer is that the kidneys (and other mineralocorticoid-sensitive tissues) contain **11β-hydroxysteroid dehydrogenase type 2** (**HSD-2**), which leaves aldosterone untouched while converting cortisol to cortisone, and corticosterone to its 11-oxy derivative (Chs. 21 and 22). These products bind MRs weakly.

Factors Stimulating Aldosterone Release

A 1% increase in the plasma K^+ concentration (<0.1 mEq/L) is a potent stimulus for **aldosterone** release from the **zona glomerulosa**. Aldosterone secretion is also increased in the hyponatremic, volume-depleted patient (**Part B**). This is largely due to the presence of **angiotensin II** and **III**, which are formed in blood after a drop in blood pressure or volume (Ch. 27). The angiotensins and K^+ stimulate conversion of cholesterol to pregnenolone, and corticosterone to aldosterone in the **zona glomerulosa** (Ch. 21). They do not, however, increase secretion of 11-deoxycorticosterone. Although ACTH increases cAMP levels in adrenocortical cells, the angiotensins increase diacylglycerol levels and the activity of protein kinase C. Also, K^+ apparently depolarizes cells of the **zona glomerulosa**, opening voltage-gated **Ca^{2+} channels** and thus increasing intracellular Ca^{2+} levels (Chs. 4 and 5).

Chronically elevated blood volume or pressure stretches atria of the heart, causing release of **atrial natriuretic peptide** (**ANP**), which in turn **inhibits aldosterone release** from the adrenal cortex (**Part A**) and stimulates renal Na^+ excretion. This peptide also inhibits ADH release from the posterior pituitary and renin release from juxtaglomerular cells of the kidney (Chs. 27, 28, and 31).

The adrenal cortex is also supplied with neurons that secrete a variety of products. Serotonin, norepinephrine, acetylcholine, vasoactive intestinal polypeptide, vasopressin, and prostaglandins have all been found in the adrenal cortex, and all can stimulate aldosterone release. Somatostatin may also be produced locally, and is known to inhibit angiotensin II-induced aldosterone release. Although neuronal regulation of aldosterone release may occur, it is not considered a primary physiologic control mechanism.

In normal animals, plasma aldosterone secretion increases during the active portion of the day. It is secreted at a rate 100 times slower than cortisol, and about 15% of aldosterone is bound in plasma to **albumin**. Its biologic half-life is about **25 minutes** (Ch. 23).

Mineralocorticoid Target Sites

Aldosterone and other mineralocorticoids increase **Na^+ reabsorption** and **K^+ and H^+ secretion** predominantly in principal (or primary) cells of the **distal nephron** and **cortical collecting ducts**. To a lesser extent, Na^+/K^+ ATPase activity is also increased in sweat and salivary glands, the gastric mucosa, and the large intestine. Aldosterone has also been shown to stimulate K^+ uptake by muscle, liver, adipose and nerve tissue.

Aldosterone and Renal Na^+ Reabsorption

The primary effect of aldosterone on Na^+ transport in the distal nephron is to increase its movement from tubular lumen to interstitium, and then to blood. Because H_2O is passively reabsorbed with Na^+, there is little increase in the plasma Na^+ concentration, and ECF volume expands in an isotonic fashion. Although only **3% of total renal Na^+ reabsorption is regulated by aldosterone**, deficiency of this hormone produces a significantly negative Na^+ balance, while excess produces **hypertension**.

Aldosterone, like other steroid hormones, binds to nuclear receptors and enhances DNA-dependent mRNA synthesis in its target cells (**Part C**). The mRNA, in turn, stimulates protein synthesis at the ribosomal level.

The functions of aldosterone-induced protein synthesis remain unsettled; however, three hypotheses have evolved (see numbered processes in **Part C**):

1. **Metabolic hypothesis:** Aldosterone increases mitochondrial oxidation of substrates (perhaps free fatty acids) in its target cells, thus providing reduced nicotinamide adenine dinucleotide (NADH) for ATP production.
2. **Permease hypothesis:** Aldosterone increases (passive) permeability of apical (luminal) membranes to Na^+.
3. **Na^+ pump hypothesis:** Aldosterone increases synthesis of Na^+/K^+ ATPase, and when these pump molecules are inserted into basal (plasma) membranes, Na^+ extrusion is increased.

Although all three hypotheses may be correct, the third appears to be the most widely accepted.

Aldosterone fails to exert any effect on Na^+ reabsorption for 10 to 30 minutes or more when injected directly into the renal artery, and for **1 to 2 hours** when secreted endogenously. This latent period represents the time needed to increase protein synthesis within its target cells.

Aldosterone and Renal K^+ Secretion

As most filtered K^+ is reabsorbed in the proximal nephron, urinary K^+ excretion largely reflects the quantity secreted from epithelial cells of the **distal tubules** and **cortical collecting ducts**.

Aldosterone-stimulated Na^+ reabsorption leaves the relatively impermeable chloride anion behind in the tubular filtrate, thus increasing the **transepithelial potential difference.** This relatively high negativity of the tubular filtrate (about –50 mV; **Part C**) allows K^+ to diffuse down its concentration gradient into the tubular lumen. As aldosterone also increases Na^+/K^+ ATPase activity in basal membranes, more K^+ is available for secretion. Chronically elevated levels of aldosterone will thus result in **hypokalemia**.

Aldosterone and Renal H^+ Secretion

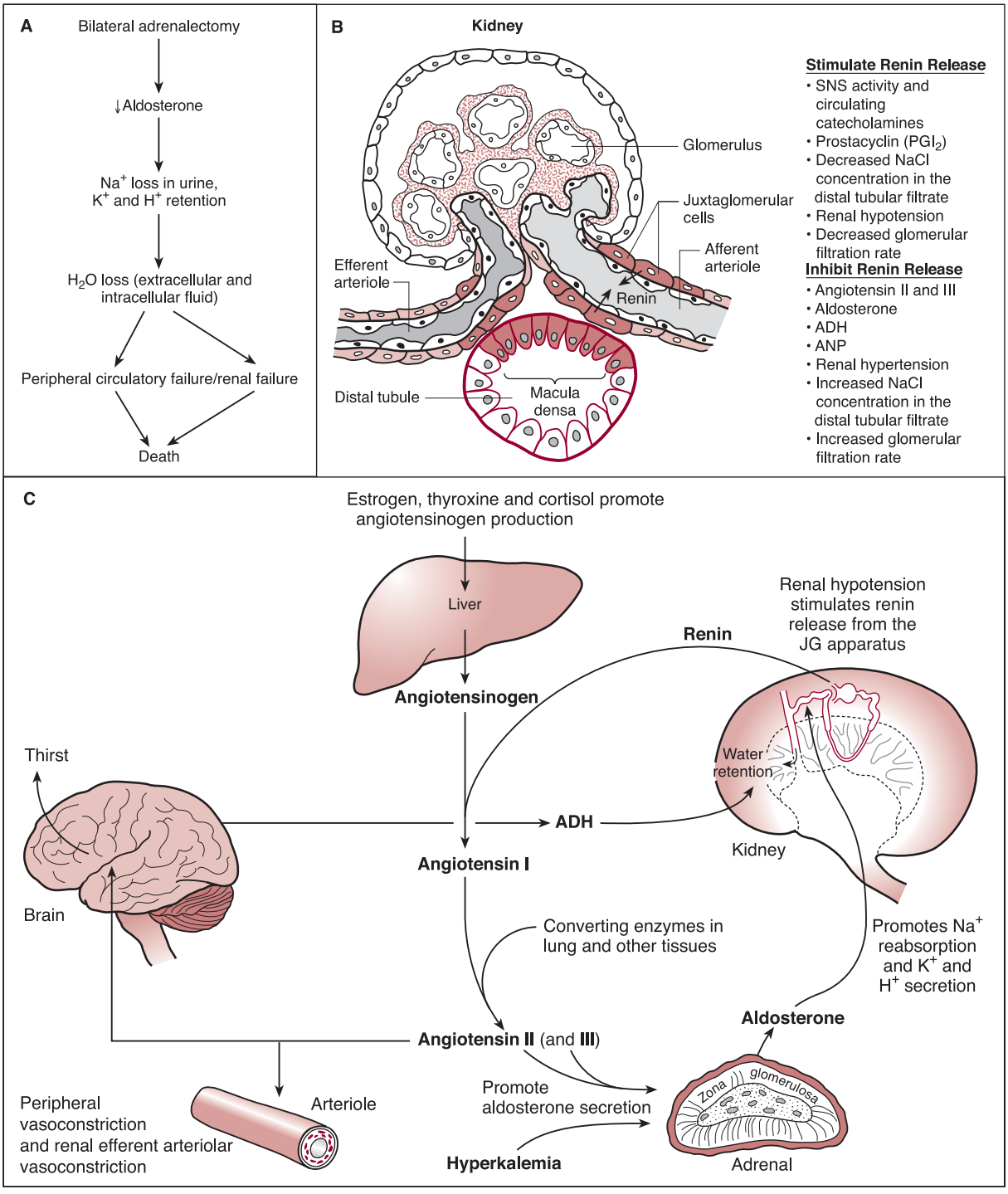
Aldosterone also enhances distal tubular secretion of H^+ as the transepithelial potential difference increases. Aldosterone excess causes hypokalemia, which eventually leads to an increase in the intracellular H^+ concentration of distal tubular epithelial cells (relative to K^+). This favors distal tubular secretion of H^+, thus leading to the development of **metabolic alkalosis**. In contrast, aldosterone deficiency produces hyperkalemia and metabolic acidosis. If acidemia is the primary originating event, there is cellular exchange of K^+ for H^+ (**Part C**), leading to increased aldosterone-stimulated renal H^+ secretion and hyperkalemia.

Hypothyroidism

Aldosterone-stimulated renal Na^+/K^+ ATPase activity is dependent on adequate titers of the thyroid hormones (**T_3** and **T_4**). With **thyroid deficiency**, the aldosterone Na^+ conservation mechanism is impaired and renal Na^+ excretion is enhanced (Ch. 36). This leads to osmotic dilution of body fluids (i.e., hyponatremia), and hypotension (Ch. 38). Additionally, renin substrate levels may be reduced.

Ch27 Renin-Angiotensin System: I

(The Juxtaglomerular Apparatus)



Source: **Part B** modified from Ham AW. Histology. 7th ed. Philadelphia: JB Lippincott, 1974:753, and Ganong WF: The renin-angiotensin system and the central nervous system. Fed Proc 1977;36:1771.

Although hypophysectomy is not life-threatening, **bilateral adrenalectomy** is (**Part A**). The life-maintaining principles supplied by the adrenal cortices are cortisol, a glucocorticoid, and perhaps more importantly the renal Na^+-retaining and K^+-secreting mineralocorticoid, **aldosterone**, which is produced by cells of the **zona glomerulosa** (Ch. 26). Aldosterone deficiency, whether it occurs in an experimental animal or in a patient, results in hyperkalemia, metabolic acidosis, hyponatremia, peripheral circulatory failure, renal failure, and inexorably, **death**. Aldosterone secretion is only one of many factors affecting **urinary Na^+ excretion**. Other factors include the **glomerular filtration rate** (**GFR**), which directly affects the amount of time functional nephrons have to reabsorb Na^+; the **natriuretic peptides** (i.e., ANP, BNP, CNP and urodilatin; Ch. 31); the presence or absence of **osmotic diuresis**; and changes in **tubular Na^+ reabsorption** independent of aldosterone, for this steroid controls only **3%** of renal Na^+ reabsorption. It should also be noted that although aldosterone is an important hormone in the control of Na^+ balance, an acute reduction in plasma Na^+ of about 20 mEq/L is needed to stimulate aldosterone release, and changes of this magnitude are rare. However, the plasma K^+ concentration need increase only 1 mEq/L to stimulate aldosterone release, and transient increases of this magnitude may be expected following a K^+-rich meal.

Another factor controlling aldosterone release is the **renin–angiotensin system**, a multifactorial physiologic control system working to control blood pressure and volume. A major component of the renin–angiotensin system is the **juxtaglomerular** (**JG**) **apparatus** of the kidney. The JG apparatus is a combination of specialized vascular and tubular cells located near the glomerulus, where afferent and efferent arterioles come into close contact with the distal tubule (**Part B**). The JG cells are specialized myoepithelial cells of the afferent arteriole that synthesize, store, and secrete into blood a proteolytic enzyme called **renin** (not to be confused with **rennin**, a milk clotting enzyme secreted by the stomach's of young animals). **Macula densa** cells are specialized distal renal tubular epithelial cells that sense the low NaCl concentration of the filtrate, and directly signal **JG cells** to secrete renin into blood. When glomerular filtration is reduced (e.g., following blood loss), there is more time for NaCl reabsorption in the proximal nephron, and therefore the filtrate in the distal tubule will have a reduced NaCl concentration. Other factors that promote and inhibit renin release are listed in **Part B.** The circulating half-life of this polypeptide in plasma is about **15 minutes**.

Part C depicts the processes involved in the renin–angiotensin system. Circulating renin splits the end off a liver-derived plasma protein called **angiotensinogen** (or **renin substrate**), thus generating the decapeptide **angiotensin I.** Within a few seconds, two additional amino acids are split off angiotensin I to form **angiotensin II.** This conversion occurs mainly in pulmonary capillary endothelial cells through the activity of dipeptidyl carboxypeptidase, otherwise known as **angiotensin-converting enzyme** (**ACE**). This enzyme is found to a lesser degree in blood plasma and renal tissue. **Angiotensin II** persists in blood for approximately **1 minute**, but it is rapidly inactivated by a number of different blood and tissue enzymes collectively called **angiotensinase** (**Part D**, Ch. 28). While active in blood, angiotensin II stimulates aldosterone synthesis and release from the adrenal cortex, among other actions.

The Angiotensins

Angiotensin II is one of the most potent known **vasoconstrictors**. It promotes norepinephrine release from sympathetic nerve endings (Ch. 33), as well as epinephrine and norepinephrine release from the adrenal medulla. It also vasoconstricts peripheral arterioles, efferent arterioles of the kidney, and to a lesser extent, veins. Primary stimuli for angiotensin generation are a **decrease in blood volume and/or pressure** (e.g., hemorrhage), and a **decrease in the glomerular filtration rate** (**GFR**).

Arteriolar constriction increases peripheral resistance, thereby raising arterial pressure back toward normal. Also, mild constriction of veins increases mean circulatory filling pressure, sometimes by as much as 20%, which promotes an increased tendency for venous return of blood to the heart (i.e., "preload"), helping it to pump against the extra pressure load.

Other effects of angiotensin II are primarily related to more long-term body fluid volume restoration: **1**) it has a direct effect on proximal tubules of the kidneys to enhance NaCl reabsorption; **2**) it stimulates aldosterone secretion; **3**) it stimulates thirst; and **4**) it promotes ADH and ACTH secretion. Angiotensin II also decreases sensitivity of the baroreceptor reflex, thus potentiating its pressor effects.

A metabolic product of angiotensin II, des-Asp-angiotensin II or **angiotensin III,** is as potent as angiotensin II in releasing aldosterone but is a less effective pressor agent. It is important in rats, where it accounts for almost 60% of angiotensin activity. In humans and dogs, only about 10% of angiotensin activity is attributable to angiotensin III. Further catabolism of angiotensin III produces a hexapeptide known as **angiotensin IV**, which is thought to have little biologic activity.

Angiotensin II Receptors

There appears to be two major classes of **angiotensin II receptors** (**AT_1** and **AT_2**) on plasma membranes of target cells, with the AT_1 class being further subdivided into **AT_{1A}** and **AT_{1B}** receptors. The AT_1 receptors are coupled to a G-stimulatory (G_s) protein, which activates phospholipase C (PLC) and catalyzes hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) from the plasma membrane to produce diacylglycerol (DG) and inositol triphosphate (IP_3; Ch. 5). The IP_3, in turn, promotes Ca^{2+} release into the cytoplasm. The AT_2 receptors also act via a G_s protein, however they activate various phosphatases within cells, which in turn antagonize growth effects and open K^+ channels. Additionally, AT_2 receptor activation increases nitric oxide (NO) production, which in turn acts through cGMP.

The **AT_2** receptors are more plentiful in the fetus and neonate, where they may be assisting in maintaining a rather **low** vascular resistance. However, they apparently persist in the brain and other organs of adult animals. The AT_{1A} subtype is found in blood vessel walls, in the brain, and in several other organs, and appears to help mediate many of the known effects of angiotensin II. High levels of angiotensin II down-regulate AT_{1A} receptors, while AT_{1B} receptors are up-regulated. The AT_{1B} receptors are found primarily in the pituitary and adrenal cortex, where high circulating levels of angiotensin II can help to assure adequate ADH, ACTH, and aldosterone output.

Independent Renin-Angiotensin Systems

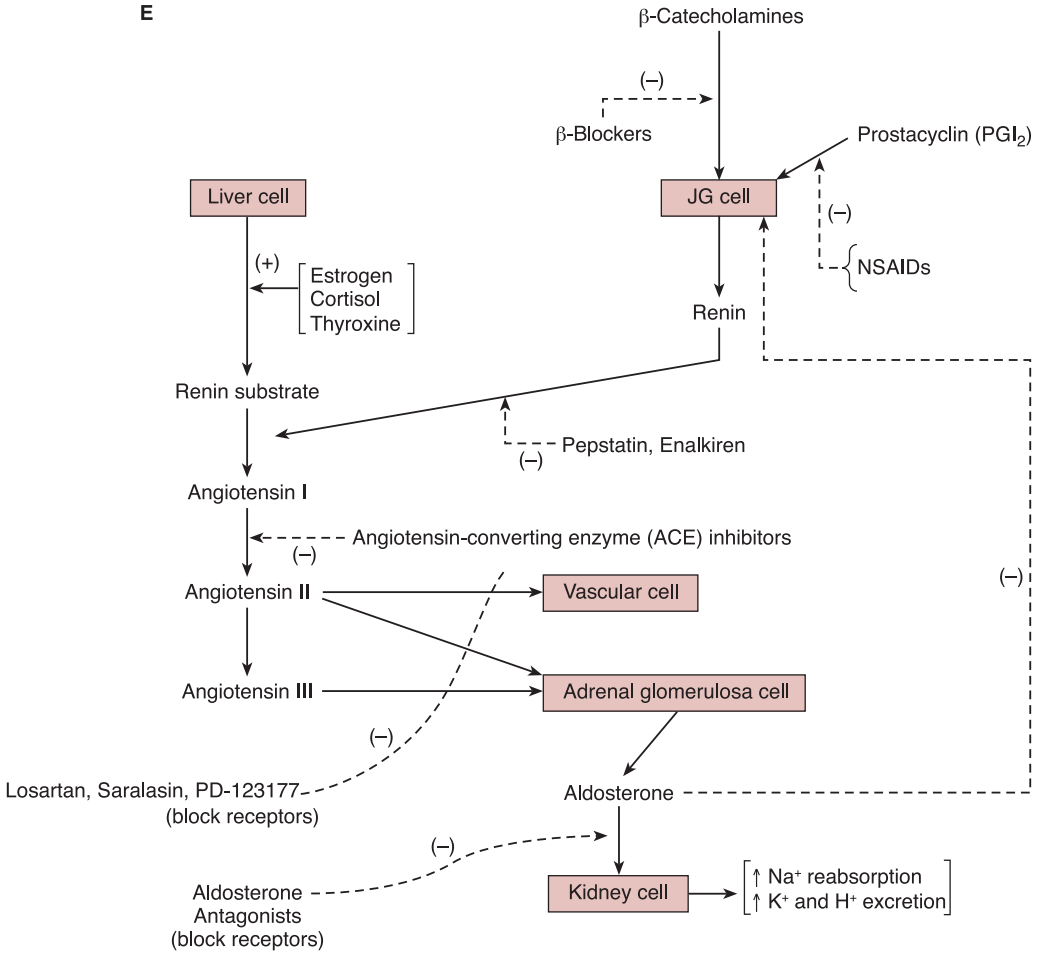
In addition to the classic multiorgan system described above that generates circulating angiotensin II, several different tissues of the body appear to contain independent renin–angiotensin systems that can generate this polypeptide, apparently for **local use**. Components of this system, for example, are found in walls of blood vessels, in the uterus and placenta, and in fetal membranes, and prorenin is found in amniotic fluid. In addition, components of this system have also been identified in the eyes, exocrine pancreas, heart, adrenal cortex, testes, ovaries, anterior pituitary, pineal, and brain.

Although these local systems do not appear to contribute significantly to circulating renin or angiotensin levels under normal conditions, they may do so with malignancy. For example, certain renin-secreting ovarian tumors have been known to cause hypertension.

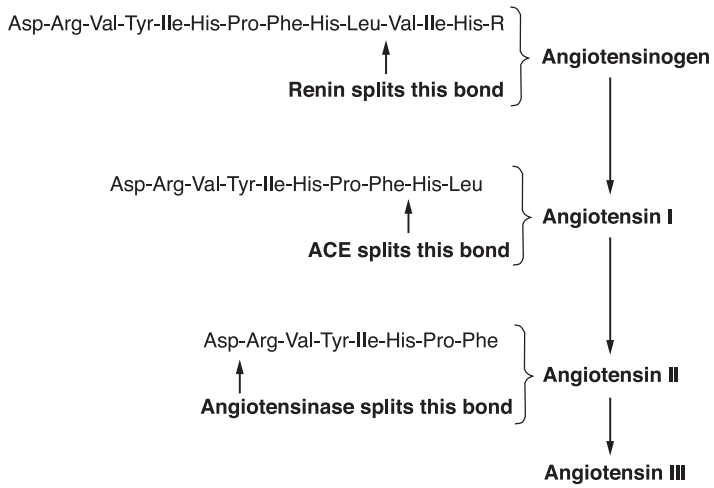
Angiotensin II does not cross the BBB, but it affects circumventricular organs of the CNS (i.e., the subfornical organ, organum vasculosum of the lamina terminalis, and area postrema that lack a classical BBB). Through these organs it can produce neurally-mediated increases in blood pressure, and increase water intake.

Ch28 Renin-Angiotensin System: II (Hypertension)

E



D



Structure of the amino terminal end of angiotensinogen and angiotensins **I**, **II**, and **III**. R = remainder of the protein. Following removal of a 24-amino acid leader, angiotensinogen contains 453 amino acid residues. The structure of angiotensin **II** in dogs, rats and many other mammals is the same as that in primates, but cattle and sheep possess Val instead of Ile at position 5. (Modified from Ganong WF, Review of Medical Physiology, 22nd ed, McGraw-Hill; pg. 455, 2005).

F

Examples of Disorders Associated with Secondary Hypertension in Animals

Adrenal
Hyperadrenocorticism (↑ cortisol, ↑ aldosterone)
Pheochromocytoma (↑ catecholamines)

Renal
Vascular, glomerular, and tubular diseases

Thyroid
Hyper- or hypothyroidism

Liver Disease

Diabetes Mellitus

Hyperestrogenism (↑ hepatic angiotensinogen production)

Hypercalcemia

Anemia and **polycythemia** (hyperkinetic heart syndrome)

Iatrogenic
Vasoconstricting drugs
Excess glucocorticoid therapy

Factors Regulating Renin Secretion

Renin secretion is regulated by several variables (Ch. 27, **Part B**). The sympathetic nervous system (SNS) plays a role in stimulating renin release via renal sympathetic nerves and circulating catecholamines. Adrenergic effects on JG cells are exerted via **β_1-adrenergic receptors** (and intracellular cAMP), and are thus enhanced by **isoproterenol**, a β-agonist, and attenuated by **propranolol**, a β-blocker (**Part E**). Although renal innervation is not a requisite for renin release, the SNS can modulate the secretion of renin, and thus have an indirect effect on aldosterone release.

Locally produced prostaglandins (mainly prostacyclin, **PGI_2**) stimulate renin release, apparently by a direct action on JG cells.

Intrarenal arteriolar pressure is monitored by **stretch receptors** (i.e., high pressure baroreceptors) in the JG body (i.e., afferent arterioles). When blood pressure falls, renin is released. In addition, a drop in GFR due to pressure and volume depletion reduces the filtered load and increases time for NaCl reabsorption in the proximal nephron. These combined events reduce delivery of NaCl to cells of the **macula densa**, which in turn sense the reduced NaCl concentration of the filtrate and signal adjacent JG cells to secrete renin into the circulation.

Negative feedback on renin release is exerted by angiotensin II and III, aldosterone, ADH, ANP, volume repletion, and restoration of the GFR. Evidence indicates that atrial stretching, volume expansion, and high-Na^+ states elicit elaboration of **ANP** into the circulation. At the renal level, ANP reduces renin release and causes renal vasodilation, natriuresis, and diuresis. It also inhibits aldosterone release from the adrenal cortex (Ch. 31).

Renin Substrate

Angiotensinogen, also called **renin substrate**, is an α_2-globulin synthesized in the liver. It is the prohormone of the angiotensins and appears in higher concentrations in plasma as a result of hepatic **glucocorticoid**, **thyroxine**, or **estrogen** stimulation. Although the amount of available renin substrate is not rate-limiting under ordinary circumstances, an increase in its concentration may lead to the production of inappropriately high amounts of **angiotensin**, and thus **aldosterone**. Increases in plasma renin substrate concentrations during pregnancy inevitably lead to increases in angiotensin and aldosterone production. In spite of these dramatic changes, however, normal pregnancy elicits few signs of hyperaldosteronism. There is no tendency toward hypokalemia or hypernatremia, and blood pressure at mid-pregnancy, when changes in the renin–angiotensin system are maximal, tends to be lower than in the nonpregnant state. It has been postulated that edema of late pregnancy is due to these changes, but hyperaldosteronism of nonpregnancy leads to hypertension, not edema (Ch. 65). Estrogen therapy, pharmacologic amounts of glucocorticoids and hyperthyroidism also elevate renin substrate levels. In severe liver disease, adrenocortical insufficiency, or hypothyroidism, plasma renin substrate levels may be low.

Hypertension

Hypertension (**HT**) involves abnormally sustained elevations in **arterial blood pressure** (**BP**), which are associated with a high incidence of morbidity and mortality in animals. Since **BP** is the product of cardiac output (**CO**) and total peripheral resistance (TPR), it is a function of three variables; heart rate (**HR**), stroke volume (**SV**), and **TPR**:

$$\mathbf{BP = (HR \times SV) \times TPR}$$

Therefore, pathophysiologic processes elevating one or more of these variables over time can cause **HT**. Antihypertensive therapies are usually designed to target one or more of these variables.

BP measurements and detection of HT in reasonably calm animals are routine today, and some define HT in dogs and cats as a **systolic blood pressure** (**SBP**) **> 160 mmHg**, and/or a **diastolic blood pressure** (**DBP**) **> 100 mmHg** in repeated, reliable measurements. **Essential HT**, which is common in humans, is defined as persistently elevated BP with no identifiable cause, whereas **secondary HT**, which is more common in animals, is generally associated with an identifiable pathophysiologic process (**Part F**).

Increased sympathetic nervous system responsiveness can occur secondary to a pheochromocytoma (Ch. 34), and plasma volume expansion will occur secondary to increased Na^+ retention (renal failure or hyperaldosteronism). Cushing's-like syndrome also causes HT (Ch. 25), partially due to glucocorticoid stimulatory effects on renin substrate release (**Part E**). Activation of the renin-angiotensin system with subsequent renal Na^+ retention and peripheral arteriolar vasoconstriction are common results of intrarenal diseases affecting the glomerulus and/or vasculature. Since hypothyroidism can lead to atherosclerosis (Ch. 38), this endocrine disorder is sometimes associated with HT. Hyperthyroidism, due to β-adrenergic stimulatory effects, can lead to increased renin release and cardiac output (Ch. 39). Since volume overload occurs, if peripheral resistance does not remain low, HT can ensue. Renal sympathetic nerve ablation has been employed to treat HT.

Organs particularly vulnerable to the pathophysiologic effects of HT are the heart, kidneys, eyes, and brain. Increased afterload stress on the heart causes left ventricular hypertrophy, and renal vascular damage from HT may result in glomerulosclerosis. Although pressure diuresis can occur, as renal function continues to deteriorate there are usually further increases in peripheral resistance (which makes HT itself a self-perpetuating disease). Retinal hemorrhages and detachment also occur, with blindness reportedly being the presenting complaint. Cerebrovascular accidents, seizures and syncope have also been reported.

Basic Renin–Angiotensin System Pharmacology

When the design of a complex physiologic regulatory system becomes apparent, it is sometimes possible to modify that system at several points with drugs. The renin–angiotensin system is a good example (**Part E**).

Pharmacologic intervention into this system can be beneficial. In some instances, however, the system may be adversely affected by drugs that are being administered for purposes unrelated to blood volume and pressure regulation.

Because the JG cell is stimulated by catecholamine β-agonists, its secretion can be reduced by β-blocking agents such as **propranolol**. These β-blockers are sometimes used to treat **high-renin hypertension**. Additionally, aspirin, indomethacin, and other nonsteroidal anti-inflammatory drugs (**NSAIDs**) reduce cyclooxygenase activity and, therefore, inhibit local prostacyclin release.

Renin's action on angiotensinogen can be blocked by **pepstatin** or **enalkiren**, and although angiotensin I is biologically inactive, the effects of excessive production of renin can be additionally neutralized through administration of **ACE inhibitors**, which reduce angiotensin II and III biosynthesis via converting enzyme, largely in the pulmonary vasculature. **Saralasin**, **losartan** and **PD-123177** are drugs that bind to stereospecific plasma membrane receptors for angiotensin II (AT_1 and AT_2), preventing them from illiciting physiologic responses.

Drugs that inhibit renin secretion and reduce production of the angiotensins or prevent their actions can also be useful diagnostic agents in characterizing the contribution of the renin–angiotensin system to the hypertension of individual patients. For example, hypertensive patients with aldosterone-secreting tumors would be expected to exhibit **low-renin hypertension**; therefore β-blockers, NSAIDs, ACE inhibitors, and saralasin would have minimal effects in normalizing blood pressure and volume in these patients. However, the **spirolactones**, which are structural analogues of aldosterone, compete with aldosterone for its receptors and thus reduce its effects in the kidney. These can be effective therapeutic agents for treating low-renin hypertension, as well as edematous states.

Calcium channel antagonists (**CCAs**) have been used to reduce **BP** through reducing **TPR**, however they tend to vasodilate afferent arterioles. Therefore they may increase intraglomerular pressure in certain animals.

Ch29 Addison's-Like Disease (Hypoadrenocorticism)

A

Causes of Addison's-Like Disease

Primary Hypoadrenocorticism

Idiopathic
- Autoimmune-mediated destruction of the Adrenal Cortex*

Spontaneous
- Infections
- Hemorrhage
- Neoplasms
- Trauma
- Amyloidosis

Iatrogenic (adrenal suppressive therapeutic agents)
- Cytotoxic (e.g., from Mitotane* (o,p'DDD)
- Ketoconazole (blocks ACTH actions)
- Megestrol acetate (a synthetic progestin in cats)

Secondary Hypoadrenocorticism

Naturally occurring
- ↓CRH or ↓ACTH (hypothalamic or pituitary failure); from inflammation, tumors, trauma, or congenital defects

Iatrogenic
- Prolonged glucocorticoid administration*

Hyporeninemic Hypoaldosteronism
- Renal disease

Pseudohypoaldosteronism
- ↑Aldosterone resistance

o,p'DDD = Mitotane (Ortho para'DDD); ACTH = Adrenocorticotropic Hormone; CRH = ACTH Releasing Hormone
*Most common causes

B

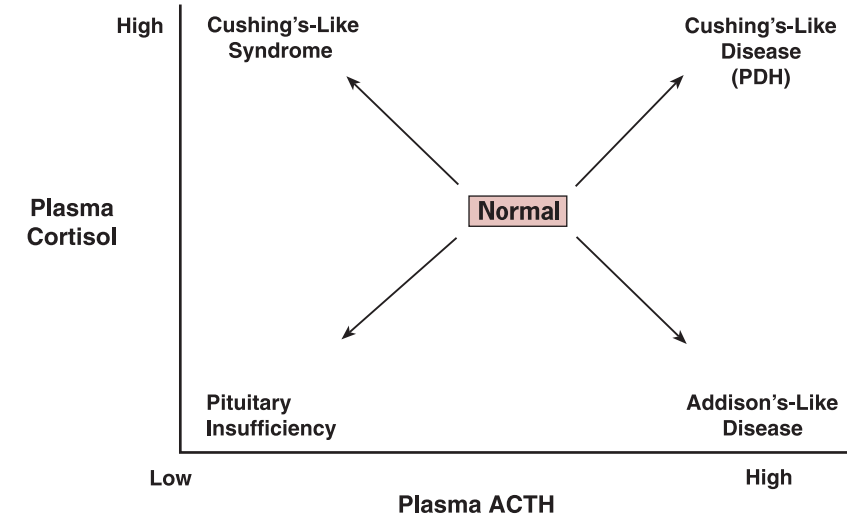
Signs and Symptoms of Addison's-Like Disease

Physical
- Anorexia; thin and weak
- Ectomorphy
- Vomiting, diarrhea (bloody), and dehydration
- PU/PD
- Weak pulses and bradycardia
- Small heart (↓work load)
- Painful abdomen
- Hypotension

Urine
- ↓Specific gravity

Blood
- CBC (normocytic, normochromic anemia)
  - ↓–↑ Hematocrit
  - ↓(Mild) erythrocytes, platelets, and neutrophils
  - ↑(Mild) lymphocytes, eosinophils, and basophils
- Hyponatremia
- Hyperkalemia
- ↓Na^+/K^+ ratio
- Hypochloremia
- Hypercalcemia
- Hypoglycemia
- ↑BUN, ↑ Creatinine (Prerenal Azotemia)
- ↑ACTH, ↓Cortisol, ↓Aldosterone
- Acidemia

C



Thomas Addison described the signs and symptoms of human adrenocortical insufficiency in 1855. Naturally occurring adrenocortical insufficiency in animals, however, was not reported until the 1950s. Hypoadrenocorticism is referred to today as Addison's disease in humans, and either Addison's disease or Addison's-like disease in animals. It appears to be more frequent in young to middle-aged females.

Causes of Hypoadrenocorticism

The causes of Addison's-like disease are listed in **Part A. Idiopathic primary hypoadrenocorticism** is reportedly bilateral, and apparently has an immune-mediated basis in animals, as in humans. Animals with this disease sometimes exhibit additional endocrinopathies (e.g., hypothyroidism, diabetes mellitus, or hypogonadism). **Iatrogenic primary hypoadrenocorticism** may follow administration of the adrenocorticolytic drug **mitotane** (o,p -DDD, Ortho para DDD, **Lysodren**) for the treatment of Cushing's-like syndrome (Ch. 25).

Iatrogenic secondary hypoadrenocorticism is the most common cause of adrenal insufficiency in animals, and occurs following prolonged administration of glucocorticoids. Exogenous glucocorticoids (i.e., oral, injectable, or topical) inhibit ACTH release, and can cause adrenal atrophy. **Depending on the amount and length of glucocorticoid administration, a return to normal pituitary–adrenal function can take weeks to months following steroid withdrawal** (Ch. 25).

Aldosterone deficiency can also occur secondary to renal disease (**hyporeninemic hypoaldosteronism**), and increased resistance to the actions of aldosterone can produce a syndrome known as **pseudohypoaldosteronism**. Animals with these conditions would be expected to exhibit a marked hyperkalemia, metabolic acidosis, NaCl wasting and hypotension (see below).

Signs and Symptoms of Hypoadrenocorticism

The signs and symptoms of this disease are first reflected in a **glucocorticoid deficiency**, and later in both a glucocorticoid and a **mineralocorticoid deficiency** (Chs. 21 and 22, and **Part B**). However, more than 90% of the adrenal cortex is reportedly destroyed before clinical signs and symptoms become obvious. As animals encounter stressful situations (e.g., trauma, surgery, or kennel boarding), they apparently have more difficulty recovering, and short-term starvation can lead to a fatal hypoglycemia.

As **physical signs** of this disease can mimic those of others (e.g., GI, hepatic, or renal disorders), a definitive diagnosis requires a judicious physical exam combined with a CBC, serum chemistry profile, and urinalysis. An additional assessment of adrenal reserve capacity through an ACTH stimulation test has also be employed.

In severe cases, **ectomorphy**, in which tissues derived from ectoderm predominate, may be evident. There may be a preponderance of linearity and fragility, a large surface area with thin muscles and subcutaneous tissue, and slightly developed digestive viscera (as contrasted with endomorphy and mesomorphy).

Although changes in the CBC are generally secondary to glucocorticoid deficiency (Chs. 23 and 24), dehydration due to mineralocorticoid deficiency can apparently complicate interpretation of the **hemogram** until patients become rehydrated. An unchanged hemogram in an ill, stressed patient, however, would be abnormal.

Hyponatremia, hypochloremia, hyperkalemia, and a Na^+/K^+ ratio less than 20:1 are not reportedly uncommon in patients with hypoadrenocorticism, and the hyperkalemia may require therapy to prevent cardiac arrhythmia (Ch. 19). Although other causes of a **low Na^+/K^+ ratio** exist (e.g., renal failure or severe GI disorders), hypoadrenocorticism is apparently indicated when the low ratio is associated with other signs and symptoms listed in **Part B.** Because hypoaldosteronism also impairs distal renal tubular H^+ secretion, a **metabolic acidosis** may also develop. Hypotension and decreased tissue perfusion will exacerbate the acidemia.

Hypercalcemia associated with hypoadrenocorticism most likely develops because of a decrease in urinary Ca^{2+} excretion. Glucocorticoids normally suppress renal Ca^{2+} reabsorption, and in their absence excessive reabsorption is thought to occur.

Glucocorticoids are required to maintain the plasma glucose concentration between meals by stimulating hepatic gluconeogenesis and decreasing insulin sensitivity in peripheral tissues. Therefore, their absence due to hypoadrenocorticism may result in **hypoglycemia**. It should be noted, however, that apparently not all animals with hypoadrenocorticism exhibit hypoglycemla.

Since glucocorticoids permit normal responsiveness of arterioles to the constrictive actions of catecholamines and angioteinsin II, and decrease permeability of the vascular endothelium (Ch. 24), vascular smooth muscle becomes unresponsive in their absence to these circulating vasoconstrictors, capillaries dilate and become quite permeable. These effects impair vascular compensation for the hypovolemia caused by mineralocorticoid deficiency, and vascular collapse occurs. Gastrointestinal ulcers develop, bleeding frequently occurs, and Addisonian-like patients may exhibit **bloody diarrhea**.

Prerenal azotemia would also be expected due largely to dehydration and ECF volume contraction. The hyponatremia that develops in this disease due to hypoaldosteronism would eventually lead to renal medullary solute washout, diuresis, and a decreased urine specific gravity.

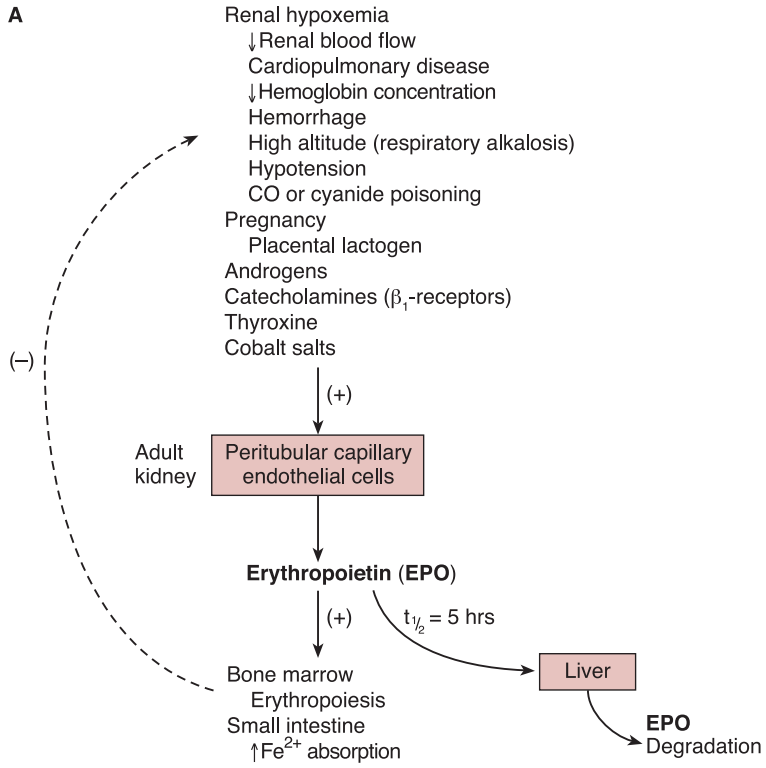
Cutaneous hyperpigmentation would also be expected in patients with primary hypoadrenocorticism, because deficient adrenal cortices remove inhibitory feedback control on the hypothalamus and anterior pituitary. The resulting high titer of ACTH, and potentially α-MSH, would stimulate dermal melanophores, because both hormones contain the polypeptide chain that is responsible for melanosome dispersion (Ch. 9). For unknown reasons, however, some animals reportedly fail to exhibit this melanophore response in spite of high liters of ACTH.

In summary, primary hypoadrenocorticism (Addison's-like disease) first exhibits itself as a glucocorticoid deficiency, and then as a mineralocorticoid deficiency. Due to lack of glucocorticoid negative feedback inhibition on the hypothalamic-pituitary axis, plasma ACTH levels are elevated (**Part C**). Patients with pituitary insufficiency would be expected to exhibit a secondary hypoadrenocorticism due to lack of adrenal stimulation by ACTH. Pituitary or hypothalamic tumors, trauma, inflammation or congenital defects have been associated with this metabolic disorder (**Part A**). In contrast, Cushing's-like disease (also known as pituitary-dependent hyperadrenocorticism (PDH)) results in high circulating titers of ACTH, with eventual bilateral adrenocortical hyperplasia. Therefore, blood levels of both ACTH and cortisol would be elevated (**Part C**). Functional adrenocortical tumors (adenomas or carcinomas) cause signs and symptoms of Cushing's-like syndrome, and ACTH blood levels are usually low. Chronic exposure to excessive amounts of exogenous glucocorticolds initially results in the development of the classic signs and symptoms of Cushing's-Like syndrome, and when long-term therapy is discontinued, iatrogenic secondary hypoadrenocorticism may follow.

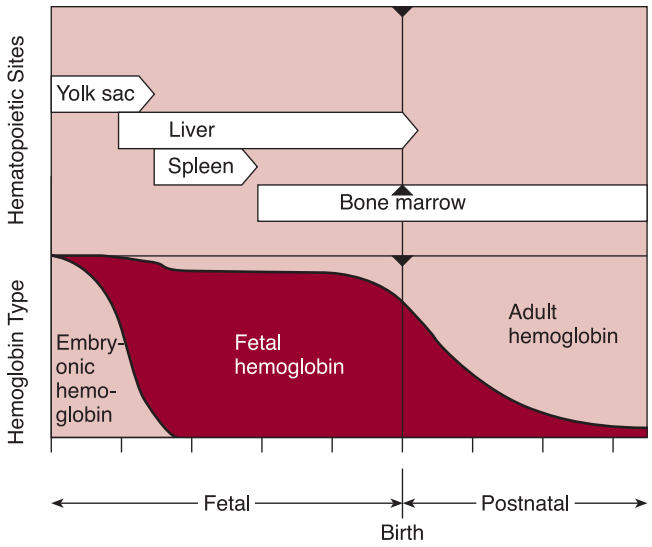
Since death from hypoadrenocorticism is most often attributed to **vascular collapse** and **hypovolemic shock** (rather than hyperkalemia), rapid correction of hypovolemia has been a major priority in an **Addisonian crisis**. Intravenous infusion of a normal saline solution with glucose can be used to correct the hypovolemia and hypoglycemia, and the hyperkalemia will be reduced by simple dilution. Glucocorticoid and mineralocorticoid therapy also apparently become important in the long-term management of this condition.

Ch30 Erythropoietin (Production and Action)

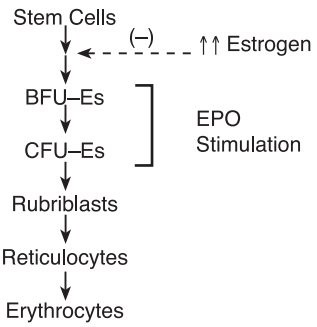
A



B



C



D

EPO-Secreting Tumors

- Renal carcinomas
- Hepatocellular carcinomas
- Cerebellar hemangioblastomas

Excess EPO can promote an **absolute polycythemia**, leading to an increase in blood viscosity, decreased cerebral perfusion, and potentially heart failure. Most neurologic symptoms reported in animals with an absolute polycythemia (e.g., seizures, ataxia, blindness, tremor & behavioral changes) are associated with impaired microcirculation of the brain.

The glycoprotein hormone **erythropoietin** (**EPO**) is produced by the adult kidneys in response largely to **renal hypoxemia**. Erythropoietin, containing 166 amino acid residues, stimulates erythropoiesis in bone marrow and Fe^{2+} uptake by the small intestine (**Part A**). Iron is an essential component of the heme fraction of hemoglobin.

As the kidneys maintain a rather constant blood flow (compared with other tissues and organs of the body), it seems appropriate that nature selected them as major sites for EPO production in adult mammals.

Fetal Versus Adult Hemoglobin

In many mammals, the fetus has a different hemoglobin from that of the adult. **Fetal hemoglobin** (**HbF**) does not bind erythrocytic 2,3-bisphosphoglycerate (2,3-BPG; also called 2,3-diphosphoglycerate (2,3-DPG)) with as great an affinity as **adult hemoglobin** (**HbA**), thus giving HbF a greater oxygen-binding affinity. Theoretically, this helps to enhance the oxygen diffusion gradient from maternal to fetal blood. Fetal and adult hemoglobins can be distinguished from one another by their mobility when subjected to electrophoresis, and by amino acid analysis, ultraviolet absorption spectra, oxygen dissociation curves, and other tests.

Erythropoiesis (and thus Hb production) occurs in the embryonic yolk sac, then subsequently in the fetal liver and spleen. Bone marrow does not normally establish full erythropoietic potential until the second half of pregnancy, when changeover to the adult type of hemoglobin begins. This changeover continues on into neonatal life, when HbA quickly becomes predominant (**Part B**). In sheep, HbF has been detected up to 35 days of age, but this is thought to be a residue from that made before birth. Fetal hemoglobin in calves has been reported to account for 41% to 100% of total hemoglobin at birth, yet diminishes rapidly as it is replaced with HbA at 2 to 3 months of age. By the end of the first year, HbF is typically only 1% of total hemoglobin. On the other hand, in pigs and horses, the hemoglobin of the fetus is indistinguishable from that of the adult, and replacement of fetal blood by adult blood in humans does not seem to compromise survival of the fetus. Thus, **HbF may be more of a physiologic luxury than a necessity in mammals.**

In addition to variations in the hemoglobin molecule of individual animals (HbF versus HbA), there are also variations between species in the protein (globin) part of the molecule, but not the heme fraction.

Fetal Versus Adult Erythropoietin

During **fetal** life, the major site for **EPO production** (as for erythropoiesis) is **perivenous hepatocytes**. As animals mature, EPO production is taken over by **interstitial cells** of the inner renal cortex, lying close to peritubular capillaries surrounding the **proximal tubules**. Although the adult liver is thought to retain a capacity for producing about **15%** of EPO, when renal function and/or mass is significantly reduced by disease the liver cannot adequately compensate, and EPO production falls. A small amount of EPO is also produced in the **brain**, where it is said to exert a protective effect against excitotoxic damage triggered by hypoxia. Small amounts are also produced by the **uterus** and **oviducts** in response to estrogen, where EPO appears to help mediate estrogen-dependent angiogenesis (Ch. 56).

Although EPO-releasing tumors that result in **polycythemia** have been reported in animals (**Part D**), the majority of problems related to EPO and erythropoiesis are associated with **anemia** from **decreased renal EPO production**.

Control of Erythropoietin Production

The major factor controlling EPO production and secretion is the **Po_2 of blood perfusing the kidneys**. Decreased renal blood flow, cardiopulmonary disease, decreased hemoglobin production, hemorrhage, high altitude, and hypotension are all examples of factors that cause renal hypoxemia (see **Part A**). Anemia due to decreased EPO production is therefore a major factor to consider in patients with renal failure or any of the above conditions. Placental lactogen and testosterone are also known to be physiologic stimuli for EPO production, and catecholamine-producing tumors (i.e., pheochromocytomas) and thyroxine-producing tumors are also known to stimulate EPO release (Chs. 34 and 39, respectively). Besides the presence of EPO, erythropoiesis also requires appropriate levels of growth hormone, thyroxine, and cortisol (in both males and females).

In addition to the low Po_2 of high altitude, animals develop **respiratory alkalosis** through hyperventilation, which becomes an additional stimulus for EPO release and erythrocytic 2,3-BPG formation. Like renin secretion, renal EPO secretion is facilitated by catecholamines via a **β_1-adrenergic mechanism**, although the renin-angiotensin system (Chs 27 & 28) is separate from the EPO system.

The principal site for **EPO inactivation** is the liver, and the hormone has a circulating half-life of about **five hours**. However, the increase in circulating red cells that it triggers takes about **two to three days** to appear (because red cell maturation is a relatively slow process).

The factors stimulating erythropoietin production, as well as the primary effects of the hormone in bone marrow and the small intestine, are shown in **Part A**. **Hyperestrogenism,** which can occur from estrogen over-administration or from increased endogenous secretion, interferes with stem cell differentiation into erythrocytic precursors (**Part C**). Sustained elevations in circulating estrogen eventually lead to pancytopenia and aplastic anemia. This condition has been reported in male dogs with testicular **Sertoli cell tumors**, and in female dogs with **ovarian granulosa cell tumors** (Ch. 57).

Mechanism of Erythropoietin Action

Erythropoietin acts on bone marrow to increase the production of **rubriblasts**, which are the earliest morphologically recognizable erythrocytic precursors. It does this at normal concentrations by stimulating development of stem cells into relatively mature progenitors known as erythroid colony-forming units (**CFU-Es**), and at higher concentrations the more immature erythroid burst-forming units (**BFU-Es**) may develop. When EPO concentrations are elevated, young enucleated, but not fully mature erythrocytes (**reticulocytes**) may be released from bone marrow into the circulation.

Whenever the concentration of EPO rises, bone marrow increases its rate of erythrocyte production primarily by increasing stem cell and committed progenitor cell input rather than by shortening the maturation time of erythrocytes. This helps to make the erythropoietic process more highly efficient in meeting homeostatic needs of the organism. On average, **about 1% of circulating erythrocytes are replaced daily**, with smaller animals being associated with shorter erythrocytic life-spans. The number of times any given erythrocyte traverses the splenic circulation is directly proportional to its longevity.

Synthetic Erythropoietin

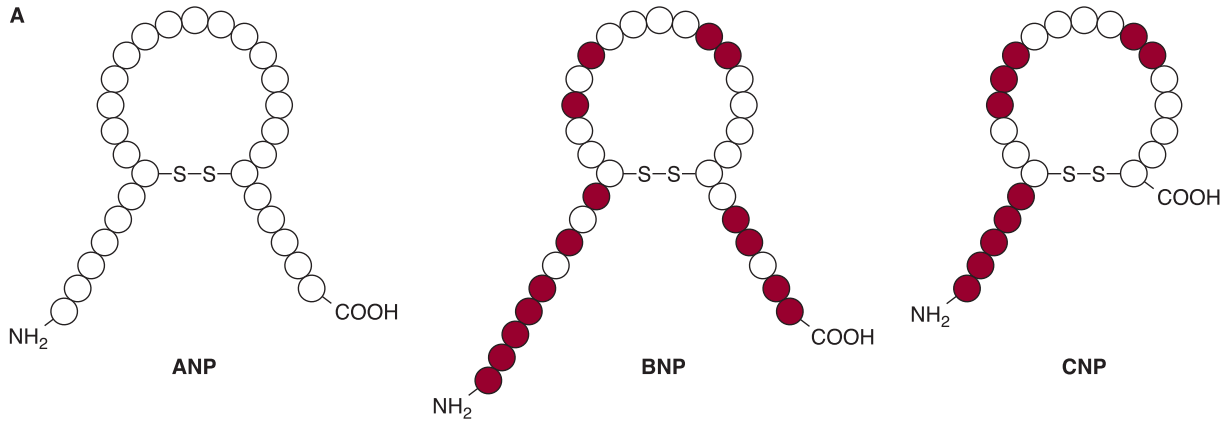
The gene for erythropoietin has been cloned (like that for somatotropin), and recombinant EPO produced in culture is now therapeutically available. As the structure for EPO has apparently been fairly well conserved across species, either intravenous or subcutaneous **recombinant human erythropoietin alfa** (**rHuEPO alfa** or **epogen**) has been reported to be effective in the treatment of anemic domestic animals. However, some dogs and cats with chronic renal failure have been reported to develop **anti-rHuEPO antibodies**, and therefore exhibit a blunted response to therapy. Anti-rHuEPO antibody production has not been reported in humans.

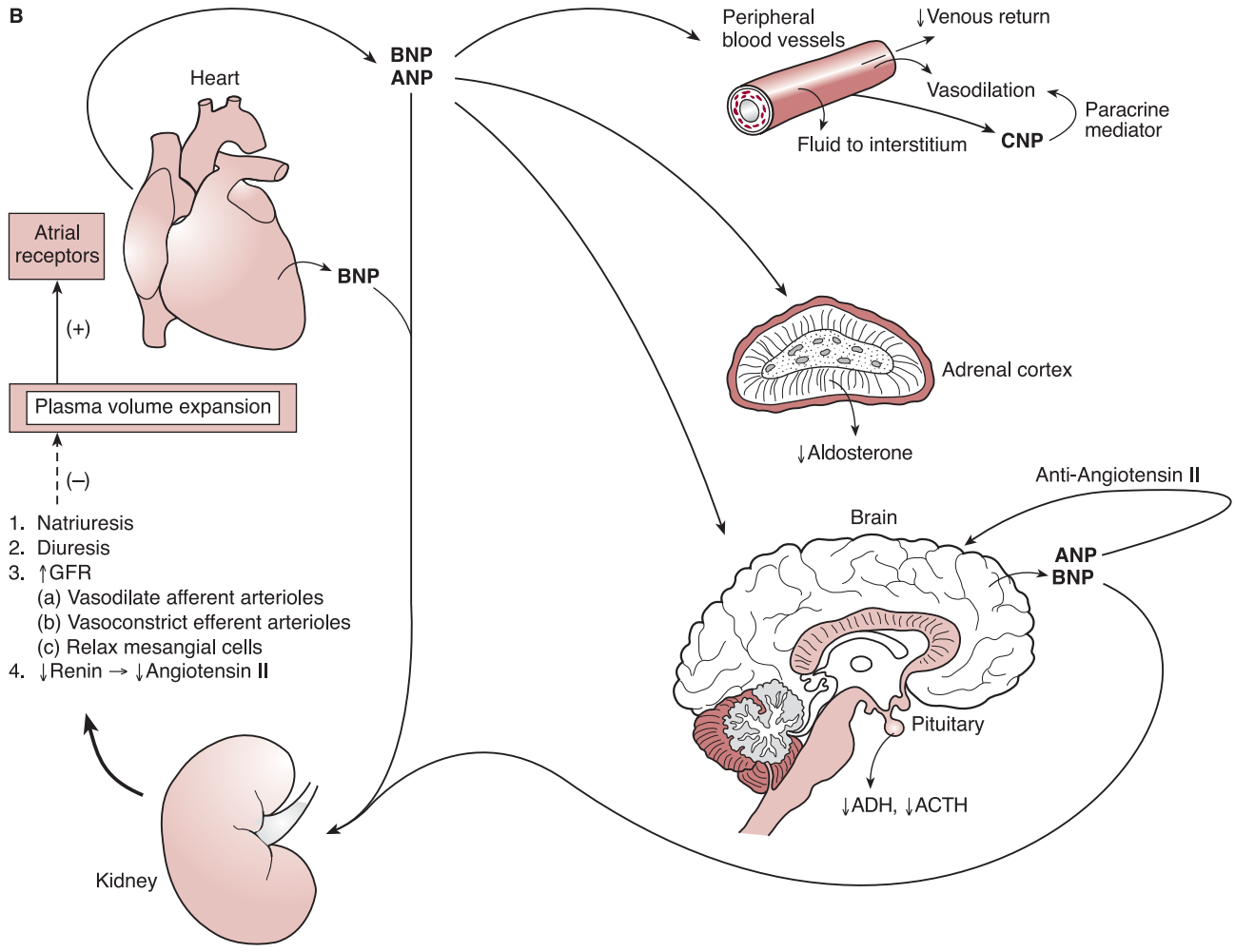
Erythropoietin-Secreting Tumors

One to 3% of renal carcinomas, 5% of hepatocellular carcinomas, and 10% of cerebellar hemangioblastomas are reportedly associated with **erythrocytosis**, probably due to excessive EPO secretion (**Part D**).

Ch31 Natriuretic Peptides

(ANP, BNP, CNP, and Urodilatin)





Mechanical stretch of the atrial wall caused by blood volume expansion is known to result in the secretion of a peptide hormone into blood that reduces vascular volume. This hormone, produced in atrial myocytes and known as **atrial natriuretic peptide** (**ANP**), is 28 amino acids in length, has a characteristic 17-amino-acid ring formed by a disulfide bond between two cysteines (**Part A**), and is cleaved from a storage precursor molecule that has 151 amino acid residues, including a 24-amino-acid signal peptide. It was first reported in 1981 by **DeBold** and coworkers, who found that intravenous administration of atrial extracts to rats caused natriuresis and diuresis, and it has been found in all vertebrates examined to date (Ch. 72).

Ultrastructurally, myocytes of mammalian atria (but not those of ventricles) resemble typical protein secretory cells. They possess secretory-like granules that increase in number in animals undergoing water deprivation and sodium deficiency. Because atria also appear to possess fluid volume receptors, they are an ideal site for the synthesis and release of a substance that can participate in fluid volume regulation.

Atrial stretch is directly correlated with a positive Na^+ balance and, hence, volume expansion. In order to counteract these effects, **ANP** reduces both Na^+ and fluid levels in blood through its actions on **blood vessels**, the **hypothalamus**, the **adrenal cortices**, and the **kidneys** (**Part B**). In general, **ANP acts through antagonizing the actions of aldosterone, ADH, and angiotensin II,** as well as other components of the renin–angiotensin system.

Physiologic Actions of ANP

Renal actions of **ANP** include increasing the glomerular filtration rate (GFR) and, therefore, the filtered load of Na^+. It does this by vasodilating afferent arterioles, vasoconstricting efferent arterioles, and possibly increasing glomerular membrane permeability. Receptors for ANP are found on glomerular **mesangial cells**, where ANP-stimulated relaxation presumably increases the effective surface area for filtration. Atrial natriuretic peptide also decreases renin secretion by juxtaglomerular (JG) cells of afferent arterioles, which has the indirect effects of decreasing plasma angiotensin II and III concentrations, reducing peripheral resistance, and decreasing aldosterone secretion. Inhibition of NaCl reabsorption by the collecting ducts also occurs due to the presence of ANP. Although this effect may be augmented by reduced aldosterone levels, ANP has been found to act directly on cells of medullary collecting ducts through its second messenger, **cyclic guanosine monophosphate** (**cGMP**). Through cGMP, ANP apparently inhibits Na^+ channels from opening in apical membranes, thereby reducing NaCl reabsorption. Atrial natriuretic peptide also reduces the ability of ADH to act on medullary collecting ducts. Through these combined influences on renal function, **ANP causes natriuresis and diuresis.**

At the level of the hypothalamus and pituitary, ANP inhibits ADH secretion from the neurohypophysis, and ACTH secretion from the adenohypophysis. Atrial natriuretic peptide also decreases the responsiveness of the adrenocortical **zona glomerulosa** to stimuli that normally increase aldosterone release (e.g., increased K^+ and angiotensin II).

This peptide also causes relaxation of vascular smooth muscle, which in turn causes a decline in arterial blood pressure. There is a reduction in venous return to the heart and, therefore, a reduction in cardiac output following ANP release. The ability of ANP to decrease intravascular volume is achieved not only through its renal effects, but also through its ability to facilitate transport of intravascular fluid into interstitial fluid spaces. Through arteriolar vasodilation, an increase in capillary hydrostatic pressure occurs, thus allowing filtration pressure to overcome reabsorption pressure. The actions of ANP on vascular smooth muscle occur in large part through its ability to decrease responsiveness of vascular smooth muscle tissue to various vasoconstrictor substances, particularly angiotensin II.

Part B summarizes the physiologic actions of ANP.

Other Natriuretic Peptides

Natriuretic peptides have been isolated from other tissues (e.g., kidneys, vascular endothelium, smooth muscle, adrenals, and CNS). **Brain natriuretic peptide** (**BNP**; also known as **B-type natriuretic peptide**) was initially isolated from the porcine brain, and later found in higher concentrations in the heart. BNP has 32 rather than 28 amino acid residues in dogs, pigs and primates (**Part A**: dark circles represent amino acids different from those in ANP), but 35 amino acid residues in cats. In the CNS, BNP may be concerned with neural regulation of blood pressure, and **systemically** it appears to act **similarly to ANP**. BNP is normally produced in cardiac atria, but in conditions leading to **ventricular hypertrophy** ventricular myocytes become a major source. Since **ANP** originates primarily from **atria**, and **BNP** from **enlarged ventricles**, increased circulating levels of these peptides may reflect different cardiac abnormalities. Plasma levels of both hormones are elevated in congestive heart failure, and their measurement in blood is seeing increased use in cardiology.

ANP is also found in neurons of the brain, and an ANP-containing neural pathway projects from the hypothalamus to areas of the lower brainstem concerned with neural control of the CV system. It exists in two forms within the CNS that are smaller than circulating ANP from the heart. The effects of **BNP** and **ANP** in the **brain** are generally **opposite to those of angiotensin II**.

Another **C-type natriuretic peptide** (**CNP**; because it was the third in the sequence to be isolated), is produced by vascular endothelial cells, with lesser amounts found in the pituitary, kidneys and brain. Little is present in the heart and circulation, and it appears to be primarily a **paracrine mediator**. CNP has local vasodilatory and antiproliferative properties (**Part B**), and it contains 22 amino acid residues (**Part A**). **Dendroaspis nartiuretic peptides** (**DNPs**) are found in primate plasma, and the venom of the green mamba snake. Little is known about their physiologic properties.

Urodilatin (**kidney natriuretic peptide**), is a 32 amino acid natriuretic peptide derived from the same prohormone as ANP. It is synthesized primarily by intercalated cells of the cortical collecting ducts, and secreted directly into the **tubular lumen**. It inhibits Na^+, water and urea reabsorption in medullary regions of the collecting ducts, and differs slightly in structure from ANP, BNP and CNP. It is **not found in systemic blood**, and only appears to influence renal reabsorptive functions. Like other natriuretic peptides, urodilatin is secreted in response to a rise in blood pressure, and an increase in the effective circulating volume (ECV). Circulating ANP, BNP and CNP have short plasma half-lives, and are degraded by **neutral endopeptidase** (**NEP**; that is inhibited by thiorphan). Urodilatin escapes NEP degradation since it does not circulate in blood, and appears to be a more potent and effective natriuretic and diuretic peptide.

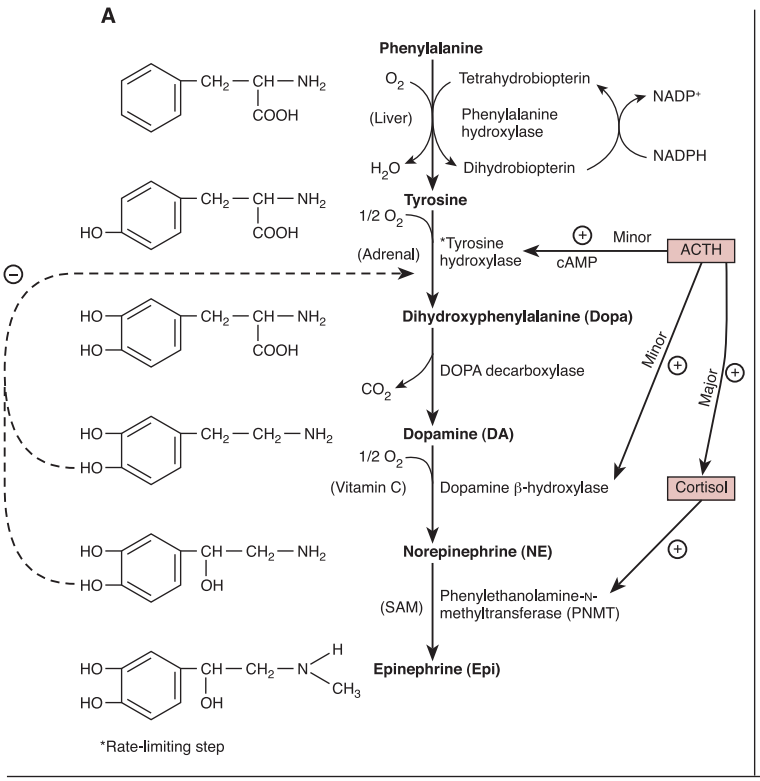
Guanylin is a polypeptide hormone produced by the small intestine in response to NaCl ingestion. It activates guanylyl cyclase to produce cGMP as a second messenger, inducing intestinal Cl^- secretion and natriuresis (Ch. 50).

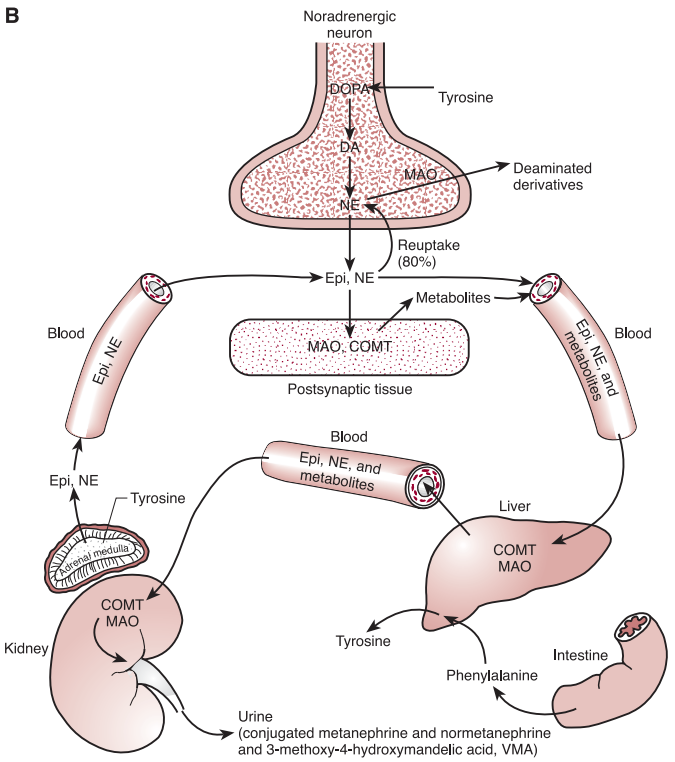
Natriuretic Peptides in Nonmammalian Vertebrates

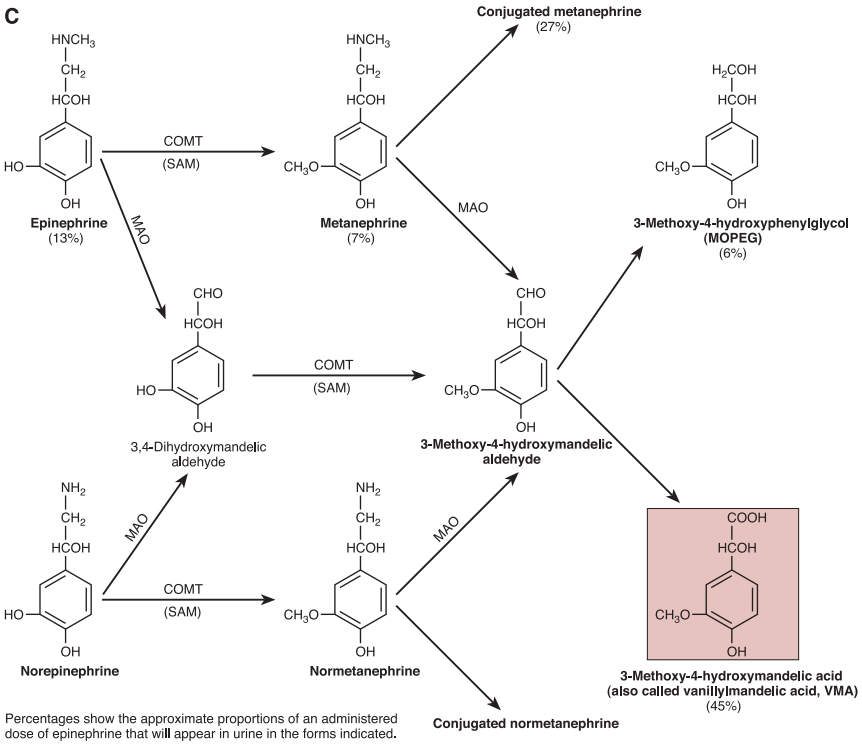
Bioassayable ANP-like activity has been demonstrated in atria of teleosts (bony fishes), birds, amphibians, and reptiles (snakes, lizards, and turtles). Although natriuretic peptide–like activity was not found in the brains of rainbow trout, it was found in the brains of two marine species. Avian and amphibian ANP-like peptides are known to **decrease aldosterone release**. It is tempting to predict that this peptide system for reducing blood pressure and volume has been conserved across species lines. However, the role of these peptides in fishes appears to be **stimulation of corticosteroid secretion**, which is opposite to its role in amphibians, most birds, and mammals (Ch. 72).

Ch32 Adrenal Medulla: I

(Catecholamine Biosynthesis and Degradation)







Two enzymes are involved in degrading the catecholamines, **monoamine oxidase** (**MAO**), and **catecholamine-O-methyltransferase** (**COMT**). MAO removes the amine group, and COMT methylates the 3-OH group. MAO is localized in mitochondria both pre- and postsynaptically, whereas COMT is localized only in the cytoplasm postsynaptically. At NE synapses postsynaptic COMT-containing cells are the smooth muscle, glandular cells and other nonneuronal tissues receiving sympathetic innervation. In the CNS, on the other hand, most of the COMT is localized in glial cells rather than in postsynaptic target neurons.

Uptake of NE into presynaptic postganglionic sympathetic neurons via a specific membrane transporter rapidly removes much of the catecholamine released into the synapse (up to 80%; **Part B**). Once inside the presynaptic neuron, the transmitter enters synaptic vesicles and is made available for recycling, or it is degraded by **MAO-A**. A second uptake mechanism is localized in target cells (e.g., smooth muscle, cardiac muscle and glandular cells), the liver and kidneys where both NE and Epi can be removed by facilitated diffusion and degraded enzymatically by both **MAO-B** and **COMT**. **Progesterone** has a tendency to increase MAO activity, whereas **estrogens** inhibit it.

The medulla of the mammalian adrenal gland is a modified sympathetic ganglion. It consists of cholinergic sympathetic preganglionic nerve endings, as well as modified chromaffin cells, that are homologous to postganglionic sympathetic adrenergic neurons. Chromaffin cells (pheochromocytes) secrete **norepinephrine** (**NE**) and/or **epinephrine** (**Epi**) directly into blood when the sympathetic nervous system is activated. Although the adrenal medullae are not essential to life, the cortices are (because they produce **aldosterone** and **cortisol**). Therefore, the medullae may be regarded as endocrine luxuries for animals, available to reinforce the effects of generalized sympathetic nervous system discharge.

Adrenal glands of nonmammals fail to exhibit the anatomical medullary-to-cortical relationships characteristic of mammals. Avian adrenal glands, for example, consist of a mixture of cortical and chromaffin cells with no distinct cortex and medulla found, and cardiac chromaffin cells have been claimed to represent the homologue of the adrenal medulla in lungfishes. Nonetheless, both NE and Epi are produced by pheochromocyters of nonmammals, and their physiologic actions are thought to be similar to those described for mammals (Ch. 72).

Fetal and neonatal adrenals secrete predominantly **NE**, followed by a gradual increase in the proportion of **Epi** secreted. The percentage of adrenal catecholamine secreted as **NE** in mature dogs and primates is reported as being 0% to 20%; rodents, 2% to 50%; rabbits, 8% to 13%; carnivores (i.e., cats), 27% to 60%; ungulates (hoofed mammals), 15% to 50%; and whales, 83%.

Pheochromocytes also contain and secrete **opioid peptides** (Ch. 8). Most circulating **metenkephalin** originates from the adrenal medullae, but circulating opioid peptides do not cross the blood-brain barrier to any degree, and their function in blood is unknown. **Adrenomedullin** (**AM**), a circulating polypeptide, has been isolated from pheochromocytes. It appears to reduce adrenal aldosterone secretion but increases vascular nitric oxide (NO) production. AM's overall role in cardiovascular control is still being investigated.

Catecholamine Biosynthesis

Catecholamines are produced largely through hydroxylation and decarboxylation of the amino acids **phenylalanine** and **tyrosine,** respectively (**Part A**). Phenylalanine, an essential amino acid, is converted to tyrosine through a physiologically irreversible reaction catalyzed by the enzyme **phenylalanine hydroxylase.** Thus, whereas phenylalanine is a nutritionally essential amino acid for catecholamine biosynthesis, tyrosine is not provided the diet contains adequate amounts of phenylalanine. The phenylalanine hydroxylase complex is a mixed-function oxygenase present in the mammalian **liver,** but absent from other tissues. Thus, patients with liver disease may have difficulty hydroxylating phenylalanine to tyrosine. Cofactor requirements for this hydroxylation are O_2 and tetrahydrobiopterin, a relative of folic acid. This pteridine is reconstituted in hepatocytes by the reduction of dihydrobiopterin by NADPH.

Following release from liver tissue, **tyrosine** may be removed from the circulation by catecholamine-producing tissues (e.g.,neurons and the adrenal medulla) as a substrate for biogenic amine biosynthesis. It is first converted to **dihydroxyphenylalanine** (**DOPA**) by the rate-limiting, iron-containing enzyme, **tyrosine hydroxylase.** This enzyme, which is **cyclic-AMP** (**cAMP**)-dependent, functions as an oxidoreductase, with tetrahydropteridine again acting as cofactor (not shown). Dihydroxyphenylalanine is next decarboxylated to **dopamine** (**DA**) by **DOPA decarboxylase,** and the DA thus formed is transported into granular vesicles where it is converted into **NE** by the copper-containing and vitamin C-dependent enzyme, **dopamine β-hydroxylase.** Both tyrosine hydroxylase and dopamine β-hydroxylase can be activated by the pituitary "stress" hormone, **ACTH**, which primarily controls cortisol production in the adrenal cortex (Chs. 21 and 22). Product negative feedback regulation of tyrosine hydroxylase is thought to be exerted by both **DA** and **NE**.

Methylation of **NE** to form **Epi** occurs through the action of **phenylethanolamine-N-methyltransferase** (**PNMT**), using S-adenosylmethionine (**SAM**) as the methyl donor. Because the venous effluent of the adrenal cortex normally passes through the adrenal medulla, the concentration of glucocorticoids (e.g., **cortisol**) present in this effluent may be 100 times that found in systemic arterial blood, particularly under conditions of physiologic stress. **Cortisol is a known activator of PNMT**, which enhances conversion of **NE** to **Epi**, thus helping to assure adequate levels of this humoral mediator during times of stress.

Although cholinergic neurons and the adrenal medulla lack a reuptake mechanism for secreted products, sympathetic postganglionic neurons exhibit this activity. Once released into synaptic space, up to **80%** of secreted **NE** can reenter **neurons** through an active **reuptake** mechanism (**Part B**). This process appears to be important for conserving NE, and for quickly terminating it's activity. Additionally, as NE accumulates in synaptic space, it stimulates presynaptic **α_2-adrenergic receptors**, which **reduce** cAMP levels and thus tyrosine hydroxylase activity (Ch. 33). This negative feedback mechanism helps to modulate further neuronal NE output. Studies have shown that dopamine β-hydroxylase is sometimes released from the adrenal medulla and from nerve endings along with NE, but (unlike NE) it cannot reenter nerve terminals via the active reuptake mechanism.

Catecholamine Degradation

Circulating **NE** and **Epi** are **inactivated** by the enzymes **catecholamine-0-methyltransferase** (**COMT**) and **monoamine oxidase** (**MAO**), predominantly in the liver (**Part B**). Catecholamine-0-methyltransferase is a cytosolic enzyme found in many tissues of the body, which catalyzes addition of a methyl group, usually in the 3 position (meta) on the benzene ring of the catecholamines, with SAM again acting as the methyl donor. Monoamine oxidase is an oxidoreductase that deaminates monoamines. It is located in mitochondria of many tissues, but occurs in highest concentrations in the liver, stomach, kidney, and intestine. Two isozymes of MAO have been described. The **MAO-A** isozyme is found in neural tissue, while the **MAO-B** isozyme is found in extraneural tissues. Inhibitors of MAO are effective in treating depression.

Catecholamines circulate in plasma in loose association with albumin, which accounts for about 60% of plasma protein. They exhibit a short physiologic half-life in most animals of about **1-2 minutes**.

Although a number of catecholamine metabolites have been found in blood and urine, only a few have diagnostic significance because they are found in readily measurable amounts. **Metanephrines** represent the methoxy derivatives of epinephrine and norepinephrine, and small amounts of these are normally conjugated to **sulfates** and **glucuronides** by the liver, returned to the circulation and excreted in urine (**Part C**). The *0*-methylated deaminated product of the metanephrines is 3-methoxy-4-hydroxymandelic acid (also called **vanillylmandelic acid, VMA**), which is generally the most plentiful catecholamine metabolite found in urine. Urinary levels of metanephrine, normetanephrine, their sulfate and glucuronide conjugates and more importantly, **VMA** are useful in screening patients for **pheochromocytoma**, a usually benign, well-encapsulated, intermittently-secreting vascular tumor of adrenomedullary chromaffin tissue (Ch. 34).

Dopamine is also similarly acted upon by **MAO** and **COMT**, yielding dihydroxyphenylacetic acid and 3-methoxytyramine, respectively, as degradative intermediates (not shown in **Part C**). Further action by these enzymes (i.e., COMT acting upon dihydroxyphenylacetic acid and MAO acting upon 3-methoxytyramine) yields **homovanillic acid** as a final degradative product.

Ch33 Adrenal Medulla: II

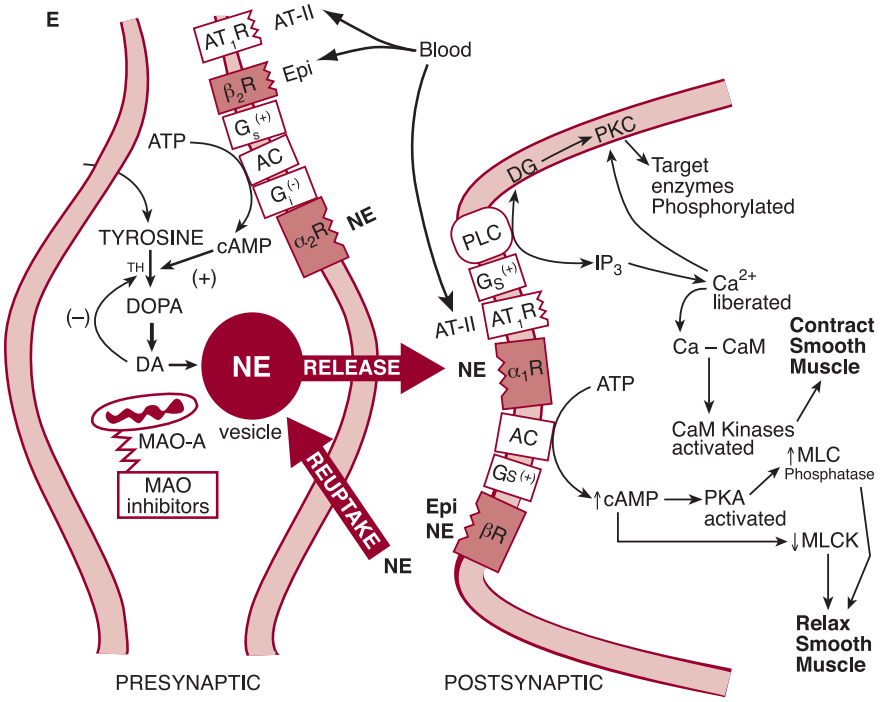
(Adrenergic Receptors and The Sympathoadrenal Response)

D

Pharmacologic Agent	Adrenergic receptor subtype α_1	α_2	β_1	β_2
Antagonists				
Propranolol	–	–	+	+
Terazosin	+	–	–	–
Prazosin	+	–	–	–
Atenolol	–	–	+	–
Esmolol	–	–	+	–
Metoprolol	–	–	+	–
Timolol	–	–	–	+
Butoxamine	–	–	–	+
Yohimbine	+	±	–	–
Atipamezole	–	+	–	–
Tolazine	–	+	–	–
Phentolamine	±	+	–	–
Agonists				
Clonidine	–	+	–	–
α-Methyl-norepinephrine	–	+	–	–
Phenylephrine	+	–	–	–
Methoxamine	+	–	–	–
Dobutamine	–	–	+	–
Prenalterol	–	–	+	–
Isoproterenol	–	–	+	+
Epinephrine	±	±	+	+
Norepinephrine	+	+	+	±
Fenoterol	–	–	–	+
Albuterol	–	–	–	+
Terbutaline	–	–	–	+
Metaproterenol	–	–	–	+

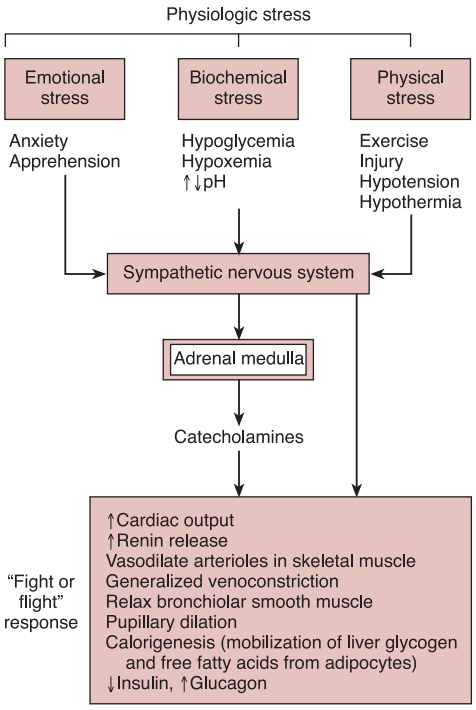
– = no binding; ± = weak binding; + = major binding

E



R = receptor
G_S = stimulatory G-protein
PLC = phospholipase C
AC = adenyl cyclase
DG = diacylglycerol
TH = tyrosine hydroxylase
AT-II = angiotensin II
G_i = inhibitory G-protein
PKC = protein kinase C
PKA = protein kinase A
AT_1R = angiotensin II receptor subtype
MAO = monoamine oxidase
IP_3 = inositol triphosphate
Ca-CaM = calcium calmodulin
MLCK = myosin light chain kinase

F



G

Organ or Tissue	Adrenergic Receptor	Responses or Effect
Adipose tissue	β_1, β_3	Increased lipolysis
Blood vessels	α_1, α_2	Vasoconstriction
	β_2	Vasodilation
Bronchioles	β_2	Dilation
Eye		
Iris radial muscle	α_1	Constriction (mydriasis—dilated pupil)
Ciliary muscle	β_2	Relaxation for far vision
GI tract		
Motility and tone	α, β_2	Decreased
Sphincters	α_1	Contraction
Heart	β_1	Increased force and rate of contraction
Kidney		
Juxtaglomerular and interstitial cells	β_1	Increased renin release and EPO release
Bladder		
Detrusor	β_2	Relaxation
Sphincter	α_1	Contraction
Ureter	α_1	Increased motility and tone
Liver	α_1, β_2	Increased glycogenolysis and gluconeogenesis
Most other tissues	β_2	Increased calorigenesis and K^+ uptake
	α_1	Cellular K^+ loss
Pancreas		
Acini	α	Decreased secretion
Islets	α_2	Decreased insulin* and glucagon secretion
	β_2	Increased insulin and glucagon* secretion
Pineal gland	β_2	Increased melatonin synthesis and secretion
Skin (apocrine glands)	α_1	Increased sweating
Spleen capsule	α_1	Contraction
	β_2	Relaxation
Uterus	α_1	Contraction of myometrial smooth muscle
	β_2	Relaxation of myometrial smooth muscle

* Primary effect in pancreatic islet tissue.

Adrenergic Receptors

The response of effector organs and tissues to adrenergic nerve stimulation or to circulating **catecholamines** depends on the type of plasma membrane receptor stimulated. Generally, there are two basic types of adrenergic receptors: **alpha** (α) and **beta** (β). The α-adrenergic receptors are further subdivided into α_1 and α_2 receptors, and the β receptors into (β_1, β_2 and β_3). Both α_1 and α_2 receptors respond more to NE than to Epi, β_2 and β_3 receptors respond more to Epi than NE, and β_1 receptors respond to NE and Epi about equally. Various pharmacologic agonists and antagonists of the adrenergic receptors are presented in **Part D.**

Since **β-adrenergic receptors** are coupled to $G_s^{(+)}$ protein, their activation by either Epi or NE results in an increase in **AC** activity, **cAMP** generation, and subsequent activation of **PKA** (**Part E**). In contrast, postsynaptic **α_1-adrenergic receptors** mediate their intracellular effects by activating membrane-bound **PLC** (also through a $G_s^{(+)}$ protein), which generates **DG** and **IP_3** as second messengers (Chs. 4 and 5). In turn, IP_3 liberates **Ca^{2+}** from intracellular stores, and DG and Ca^{2+} activate membrane-bound **PKC**. In smooth muscle cells (e.g., arterioles), this intracellular mechanism is required for contraction. Cytoplasmic **Ca^{2+}** binds to calmodulin (**CaM**), calmodulin-dependent myosin light chain kinase (**MLCK**) is activated, myosin becomes phosphorylated, and increased myosin ATPase activity leads to **contraction**. Conversely, smooth muscle cells **relax** when intracellular **cAMP** levels rise (due to **β_2-adrenergic receptor** stimulation), since activated PKA **decreases MLCK** activity, but increases that of **MLC phosphatase** (which dephosphorylates myosin). In **cardiac myocytes**, **cAMP** generation and subsequent **PKA** activation from **β_1-receptor** stimulation leads to **phosphorylation of Ca^{2+} L-channel proteins** in plasma membranes, allowing extracellular Ca^{2+} to diffuse into cells in support of enhanced **contractility**. In **SA** and **AV nodes** of the heart, **β_1-receptor** stimulation leads to increased Ca^{2+} conductance through both **Ca^{2+} T** and **L channels**, increasing rapidity of depolarization and **heart rate** (Ch. 19).

As **catecholamines** and **AT-II** accumulate in the synaptic space, negative (or positive) feedback signals can also be transmitted to sympathetic nerve terminals. Through the binding of accumulated NE to **presynaptic α_2-adrenergic receptors**, a $G_i^{(-)}$ protein is stimulated, which **decreases AC activity** (thus lowering cAMP levels in the nerve terminal). Since cAMP is needed for **TH** activity (Ch. 32), NE biosynthesis is reduced in a negative feedback fashion. Conversely, when the sympathetic nervous system is discharging rapidly (e.g., in hypovolemic or hypoglycemic shock), adrenal **Epi** secretion and **AT-II** generation will be enhanced, and circulating Epi and AT-II levels will be increased. In order to augment discharge from postganglionic sympathetic neurons, **Epi** stimulates presynaptic **β_2-adrenergic receptors**, which through a $G_s^{(+)}$ protein **elevates cAMP** levels in the nerve terminal. This, in turn, increases NE synthesis and release. Circulating **AT-II** has a similar effect of augmenting NE release from postganglionic sympathetic neurons, however, the presynaptic mechanism is less clear. Angiotensin II may be working through one of the **AT_1** receptor subtypes (Ch. 27). At critical postsynaptic sites (e.g., peripheral arterioles, efferent arterioles of the kidneys, and to a lesser extent, veins), **AT-II** becomes a potent vasoconstrictor. It works through the same postsynaptic mechanism as **NE** (**Part E**), activating membrane-bound **PLC** thus generating **DG** and **IP_3** as second messengers.

Factors Stimulating Adrenomedullary Catecholamine Release

Adrenomedullary catecholamine release is brought about through generalized discharge of the sympathetic nervous system in response to emergency or stressful situations. Physiologic stress can be conveniently subclassified as either emotional, biochemical, or physical (**Part F**). Anxiety and apprehension, for example, bring about **emotional stress;** acute hypoglycemia, hypoxemia, and derangements in acid-base balance can bring about **biochemical stress;** and certainly injury, exercise, hypotension, and hypothermia can induce **physical stress.**

The Sympathoadrenal Response

When the sympathetic nervous system is activated, the combined sympathoadrenal responses resemble those of severe stress and anger. They generally increase the ability to fight or avoid a predator, and in so doing utilize primary catabolic pathways (i.e., glycogenolysis, lipolysis, etc.). The collective response, therefore, is frequently referred to as the **"fight or flight" response** (depicted in **Part F**), and animals may generally look angry or frightened.

One easy way to mimic various physiologic aspects of the fight or flight response is to inject Epi directly into the circulation. Epinephrine, when administered **intravenously** at physiologic levels, produces a marked tachycardia with an increase in cardiac output and an overall fall in peripheral vascular resistance. This latter effect varies in different segments of the circulation. For example, in the skin and splanchnic vascular beds (where α_1-adrenergic receptors predominate), Epi constricts arterioles, but it dilates arterioles in skeletal and cardiac muscle (where β_2-adrenergic receptors predominate). Epinephrine dilates the pupil and relaxes ciliary muscle of the eye (which favors far vision), relaxes bronchiolar smooth muscle (making it easier to breathe), and increases the rate and depth of respiration (a central effect that increases minute volume, thus leading to a fall in alveolar CO_2). Epinephrine also relaxes visceral smooth muscle of the gut, with the exception of sphincters, which are constricted. Both actions tend to delay passage of intestinal contents (**Part G**).

Epinephrine stimulates cellular metabolism, and has a strong **calorigenic effect**. In general, mammalian (white) adipose tissue is unresponsive to most lipolytic hormones apart from the **catecholamines**, **thyroxine**, and **cortisol**. Under normal physiologic conditions, it is likely that Epi is the main lipolytic stimulus, providing needed energy to blood in the form of free fatty acids. Brown adipose tissue is characterized by a well-developed blood supply and a high content of mitochondria and cytochromes. Metabolic emphasis is placed on oxidation of both glucose and fatty acids, which is important because brown adipose tissue is a site of heat production in newborn animals. Epinephrine is an important inducer of nonshivering thermogenesis in brown adipose tissue, perhaps acting through **β_3 receptors**.

Another important function of the **adrenal gland** is to help maintain the **blood glucose concentration** by secreting both **cortisol** and **catecholamines** (Chs. 21-24). When the blood glucose concentration is lowered acutely (e.g., in hypoglycemic shock), Epi is released to force breakdown of liver glycogen in order to restore the blood glucose concentration. Both Epi and cortisol also stimulate hepatic gluconeogenesis. This is particularly important to ruminant animals and carnivores, which store only modest amounts of hepatic glycogen. In muscle tissue, the glycogenolysis stimulated by Epi results in an increase in lactate formation; glucose formation is not stimulated, because muscle tissue lacks the enzyme glucose-6-phosphatase. The lactate, however, can be used by liver tissue as a gluconeogenic substrate.

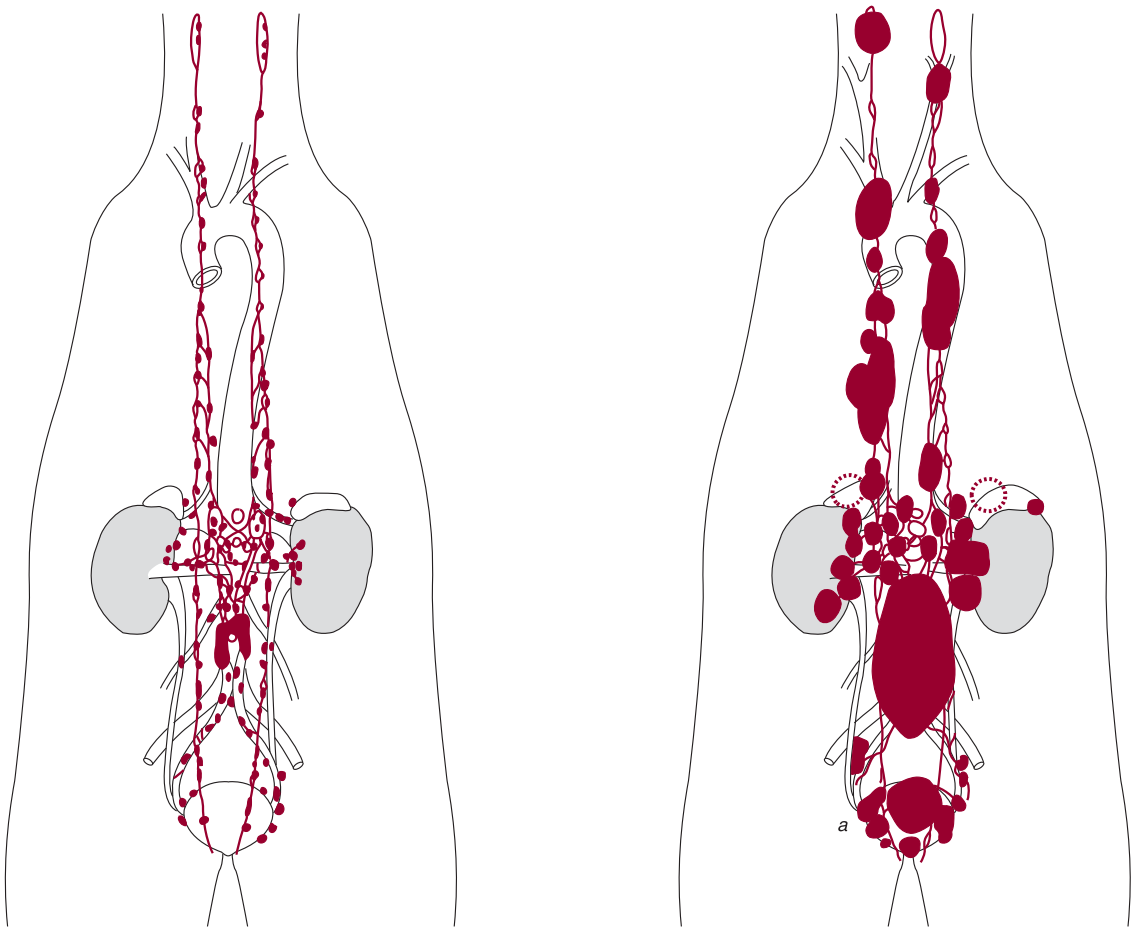
Catecholamines normally reduce pancreatic insulin output, yet increase glucagon secretion (Chs. 40-43). Glucagon, like the catecholamines, is a potent stimulator of hepatic glycogenolysis and gluconeogenesis.

Juxtaglomerular (**JG**) **cells** of the kidney are stimulated by catecholamines to produce **renin**, which initiates the renin–angiotensin system (Chs. 27 and 28). In response to hypovolemic shock, the catecholamines thus help to restore blood pressure and volume. Catecholamines stimulate **EPO** release from interstitial cells of the inner renal cortex (Ch. 30).

Additional actions of the catecholamines are included in **Part G.**

Ch34 Pheochromocytoma
(Catecholamine-Secreting Tumor)

A



B

Stimuli Causing Paroxysmal Catecholamine Release from a Pheochromocytoma

- Activity
  - Postural change
  - Exertion
  - Mating
- Eating
- Urination and defecation
- Emotional stress
- Trauma and pain
- General anesthesia and barbiturates
- Hormones/drugs (e.g., glucagon, ACTH, and histamine)

C

Signs and Symptoms of Pheochromocytoma

Physical

- Generalized weakness
- Tremor
- Anxiety and nervousness
- Panting
- Tachycardia
- Mydriasis
- Pale mucous membranes
- Muscle wasting
- Nausea, vomiting, and abdominal pain
- Weak bowel sounds
- ↑BMR
- Hypertension (sustained or paroxysmal)
  - ↑Systolic and diastolic blood pressure

Blood

- ↑Serum alkaline phosphatase (SAP)
- ↑Alanine aminotransferase (ALT)
- Hypercholesterolemia
- Hyperglycemia
- Ketoacidosis
- ↑Red blood cell (RBC) mass

Urine

- PU/PD
- Proteinuria
- ↑VMA excretion
- ↑Normetanephrine and metanephrine excretion
- Glucosuria
- Ketonuria

Source: Part A modified from Page LB, Copeland RB. In: Dowling HF, et al., eds. Disease-a-month (January). St. Louis: Year Book, 1968:7.

Adrenal tumors may be functional (i.e., producing and secreting one or more hormones), or nonfunctional. Excess secretion of progesterone, cortisol, aldosterone, and/or their precursor steroids have all been documented in animals (Chs. 21-29), as has excess secretion of adrenal (or extra-adrenal) catecholamines. The most common functional adrenal tumors in animals secrete either **cortisol** or **catecholamines**. Aldosterone-secreting adrenal tumors (Conn's-like syndrome) are uncommonly encountered in domestic animals, as are tumors expressing sex steroids from the **zona reticularis**.

A **pheochromocytoma** is a **catecholamine-secreting tumor** arising from chromaffin cells (pheochromocytes) of the sympathoadrenal system. Over **90%** are located in the abdomen, and **90%** of those arise within the adrenal glands. The other **10%** of abdominal pheochromocytomas are extra-adrenal. Although this tumor type reportedly occurs with a higher incidence in older dogs, there appears to be no breed or gender predisposition.

Most mammalian **chromaffin cells** are localized within the adrenal medulla and arise from neuroectoderm. Those few that are not associated with the adrenal gland are generally associated with sympathetic ganglia, and normally regress early in postnatal development. However, if they do not regress, they sometimes become sites of tumor formation (i.e., **paragangliomas**). **Part A** shows the anatomic distribution of chromaffin tissue (paraganglia) in the newborn **(left),** as well as reported locations of paragangliomas in animals **(right),** with the most common locations being the aortic bifurcation and the bladder wall.

Pheochromocytoma most often leads to **secondary hypertension** in animals, but if diagnosed properly it can be effectively treated by resection of the tumor. If left undetected, however, this condition is potentially fatal. Unfortunately, pheochromocytoma is reportedly diagnosed during life in fewer than half of the animal patients in whom it is later found at necropsy, and in 30% to 60% of human patients in whom it is found at autopsy.

Most pheochromocytomas are slow maturing and somewhat independent of physiologic stress. Therefore, they tend to release catecholamines into the circulation in a **sporadic**, uncontrolled fashion. A high incidence of metastasis at the time of necropsy has been reported in dogs, along with entrapment and compression of major blood vessels. Pheochromocytomas frequently invade or extend into the lumen of the adjacent vena cava, or entrap and compress the caudal vena cava. Luminal narrowing of the aorta, renal, adrenal, and hepatic vessels may also occur. Other sites of metastasis include the lungs, regional lymph nodes, spleen, heart, bones, pancreas, and central nervous system. Therefore, pheochromocytomas typically occur in conjunction with other, often serious disorders, which can hinder their detection.

Catecholamine Release

Although the precise mechanism of catecholamine release from a pheochromocytoma remains largely undefined, it is apparent that most are **noninnervated**; therefore, catecholamine release may not be initiated by neural impulses. Rather, alterations in blood flow to the tumor site, various physical activities, direct pressure, and/or a variety of chemicals or drugs may initiate secretion (**Part B**). While some tumors tend to secrete excessive amounts of catecholamines (both norepinephrine and epinephrine) continuously, others do so **episodically**.

Signs and Symptoms

Although many of the signs and symptoms reported for **pheochromocytoma** are associated with other, more common disorders (e.g., **hyperthyroidism**), several are specifically predictable, and result from a lack of normal feedback control on catecholamine release (**Part C;** Chs. 32 and 33). Findings on physical exam are reportedly variable, however, and directly related to tumor activity.

An important action of thyroxine is to augment the response of adrenergic effectors to the catecholamines. An increased **basal metabolic rate** (**BMR**) accords with hyperthyroidism, but could also be a manifestation of the calorigenic action of excess catecholamines. Generalized hypermetabolism produced by excess thyroxine causes autoregulatory vasodilation to increase blood flow to tissues, producing a **lowered diastolic pressure**, an increased cardiac output, and a wide pulse pressure. Patients with pheochromocytoma, in contrast, would be expected to have **elevated diastolic pressures**.

Increases in **red blood cell mass** may be due to catecholamine-stimulated erythropoietin release from kidneys, or an erythropoietin-like peptide produced by the tumor itself. High catecholamine levels suppress insulin release and stimulate hepatic glycogenolysis and gluconeogenesis. The resulting **hyperglycemia** may result in glucosuria.

Catecholamine inhibition of pituitary vasopressin release via a non-pressor interaction with arterial baroreceptors has been described, and could help explain the polyuria and polydipsia that develops in these patients. Biochemical confirmation of excessive catecholamine production is generally found in either plasma or urine via assay for norepinephrine, epinephrine, normetanephrine, metanephrine, total urinary catecholamines (free plus conjugated), and/or **vanillylmandelic acid** (**VMA**) (Chs. 32 and 33).

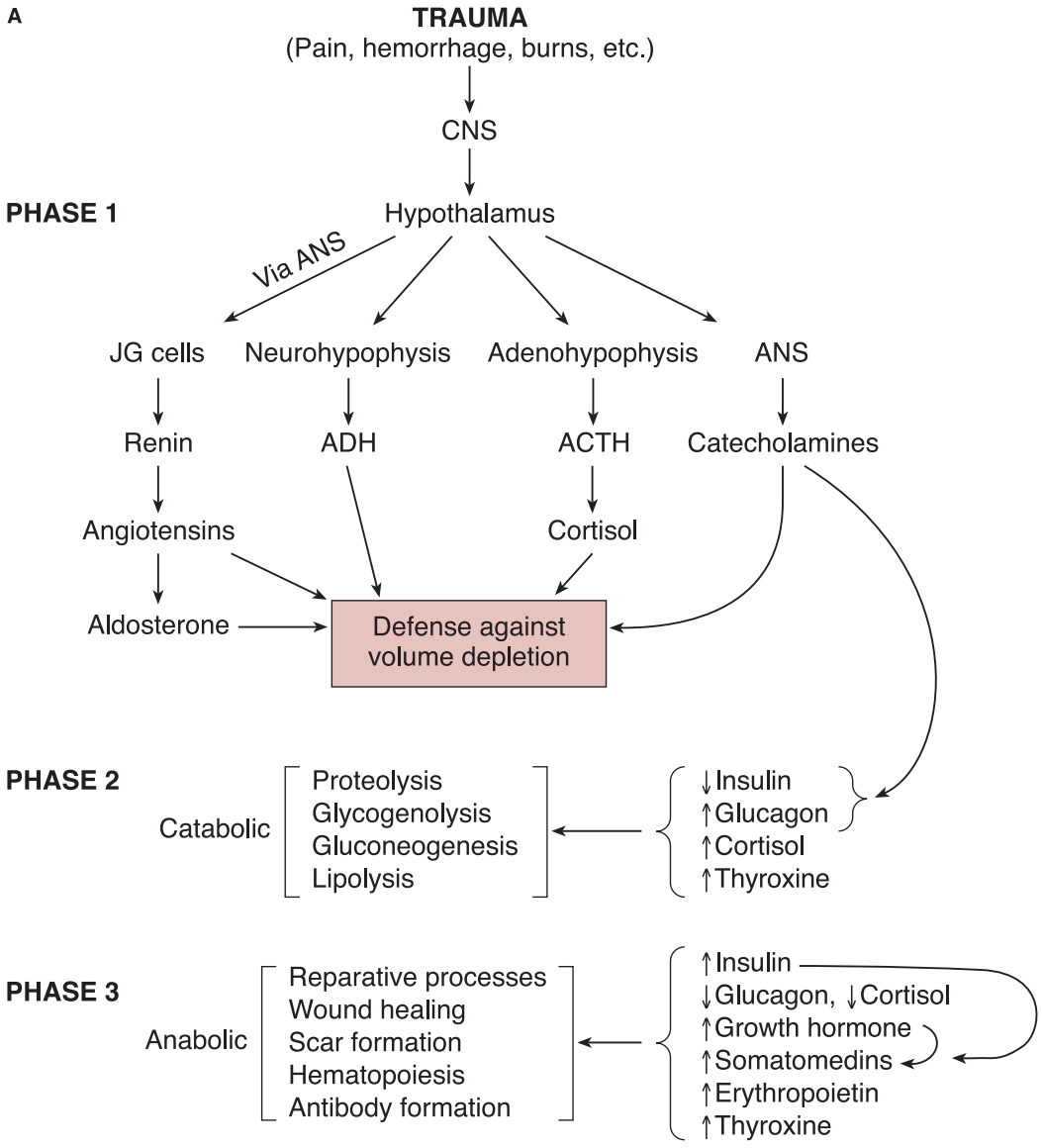
A **clonidine** suppression test may also be useful in diagnosing this disease. Clonidine is an **α_2-agonist** that works presynaptically to inhibit norepinephrine release (Ch. 33). In animals with increased catecholamine release due to neurogenic stimulation rather than a tumor, clonidine should reduce plasma catecholamine levels. Patients with pheochromocytoma, on the other hand, should fail to respond to this suppression test.

Phentolamine can also be used in a similar fashion. This drug blocks α-adrenergic receptors, and therefore lowers arterial blood pressure by blocking α-mediated vasoconstriction. A patient should be hypertensive, however, before using this test, and it runs the risk of inducing serious hypotension.

A **glucagon** stimulation test may also be useful in patients who have infrequent symptoms and signs. Following intravenous injection, patients with pheochromocytoma should manifest a substantial increase in plasma catecholamines, and a rise in blood pressure (which could be hazardous). Imaging studies may also be required to determine whether the tumor is adrenal or extra-adrenal.

In summary, the adrenal medulla is a specialized ganglion in the sympathetic division of the ANS, with cell bodies of its preganglionic neurons located in the thoracic spinal cord. Axons of these preganglionic fibers travel in the greater splanchnic nerve to the adrenal medullae (pl.), where they synapse on chromaffin cells and release ACh, which activates nicotinic receptors. Chromaffin cells of the adrenal medullae release catecholamines (**norepinephrine**, **epinephrine** and small amounts of **dopamine**) into the general circulation, which exert a wide range of physiologic actions largely through stimulation of various α and β-adrenergic receptors. Some norepinephrine reaches blood from adrenergic nerve endings elsewhere in the body. Only the adrenal medullae release epinephrine into blood since they contain **PNMT**, which is usually up-regulated by **cortisol** (Ch. 32). Pheochromocytomas that lie away from the adrenal glands release mainly norepinephrine, since they are too far away to receive the high concentrations of cortisol required to activate PNMT. Although physiologic actions of circulating **dopamine** are not well described, injection of this agent promotes **renal vasodilation** and **enhanced cardiac contractility**, which can be useful in treatment of traumatic and cardiogenic shock. Catecholamines have a circulating t½ of about **1-2 minutes**, and their activity is short-lived. They are ultimately methoxylated, oxidized to 3-methoxy-4-hydroxymandelic acid (**vanillylmandelic acid, VMA**), and excreted in urine.

Ch35 Phasic Responses to Trauma
(Immediate, Catabolic, and Anabolic)



Physiologic trauma (i.e., hemorrhagic shock, pain, burns, severe infections, hypoxemia, invasive surgery, extreme cold exposure, etc.) evokes compensatory responses in the organism that involve several endocrine glands. One of the earliest detectable changes following trauma is a depletion of cholesterol and ascorbic acid in the adrenal glands, which is associated with increased output of adrenal hormones (i.e., cortisol, aldosterone, norepinephrine, and epinephrine). A **negative nitrogen balance** is characteristic of the early response, which can sometimes be reversed by force-feeding. The sequence of compensatory physiologic events following trauma cannot occur in the absence of the hypophysis (pituitary); therefore, hypophysectomized or adrenalectomized animals have been found to be notoriously vulnerable to traumatic stresses such as those enumerated above.

It is not surprising that the pituitary–adrenal axis is activated by such a wide variety of potentially lethal stimuli. From a teleologic perspective, it seems likely that the general reaction by which fat and labile protein are catabolized as a result of injury (or even in anticipation of injury) provides energy and amino acids for the healing process. It also appears to this author that the pituitary–adrenal response to trauma may be primitive and independent of nutrition, as a wounded animal is necessarily reduced in its capacity to feed itself.

Part A summarizes endocinologic aspects of the phasic response to trauma.

Phase 1

Immediate responses to trauma (pain, hemorrhage, burns, etc.) are mediated primarily through actions initiated by the hypothalamic-pituitary-adrenal and the sympathoadrenal axes. These responses are largely concerned with ensuring **continued blood supply to vital organs**, particularly the heart, lungs, and brain. Other tissues such as skeletal muscle (approximately 50% of the body weight), the gastrointestinal tract, kidneys, and reproductive organs, are not accorded the same high priority. Major compensatory efforts appear to be a defense against fluid volume depletion and hypotension, and in this exercise the CRH-ACTH-adrenal and sympathoadrenal axes collaborate with ADH, the renin–angiotensin system, and norepinephrine secreted from sympathetic postganglionic nerve endings to restore and maintain blood pressure and volume compatible with life. Over a relatively short period of time following hemorrhage (minutes-hours), these autonomic and endocrine control systems stimulate **lifesaving compensatory fluid shifts**. If mean arterial blood pressure falls below 50 mmHg, reduced blood flow to the vasomotor center will also elicit a powerful **CNS ischemic response** through additional sympathetic output. When this occurs, systemic arterial pressure often rises to a level as high as the heart can possibly create. This vasomotor effect is thought to be a result of failure of the circulatory system to carry CO_2 and lactate away from the lower brain stem.

At the level of the anterior pituitary, both **ADH** and **angiotensin II** potentiate the actions of **CRH** on pituitary **ACTH** release, while CRH reduces pituitary LH and FSH release (Ch. 22). **Cortisol** helps to restore blood pressure and volume by **1**) sustaining myocardial performance, by **2**) maintaining normal responsiveness of arterioles to the constrictive actions of angiotensin II and norepinephrine, and by **3**) decreasing permeability of the vasculature as well as the production of vasodilator prostaglandins by vascular endothelial cells (Ch. 24).

Catecholamine release follows hypothalamic sympathetic nervous system (SNS) activation, both from the adrenal medulla and from sympathetic postganglionic nerve endings. In addition to promoting **renin** release from JG cells of afferent arterioles (Chs. 27 and 28), **norepinephrine** (and **angiotensin II**) vasoconstrict peripheral arterioles, thus changing the balance of Starling's forces in capillary beds to favor movement of water from intra- and interstitial fluid sites into the vasculature. This **capillary fluid shift** has the potential for restoring about 70% of the fluid lost through hemorrhage, and also has a tendency to modestly decrease hematocrit and the plasma protein concentration. **Aldosterone** and **ADH**, respectively, act mainly at the renal level to conserve Na^+ and reduce urinary fluid loss (Chs. 12 and 26).

Responses of the **SNS** and **adrenal medullae** are not necessarily parallel, and each traumatic stress is unique. The **SNS** is most important in response to cold stress, exercise, and in counteracting hypotension (e.g., hypovolemic shock). **Epinephrine** secretion is elicited more by anxiety, hypoxia, and hypoglycemia. During physiologic starvation (but perhaps not cachexia) adrenal medullary secretion (mainly of epinephrine) is increased, and activity of the SNS (which is mainly responsible for thermogenesis) is depressed. This is an admirable arrangement, becuause epinephrine is useful for blood glucose homeostasis, and suppression of a tonic thermogenic influence conserves calories. The nervous system is organized so that individual compoents of the SNS, including those responsible for controlling secretory activity of adrenal chromaffin cells, can be called on singly or in any appropriate combination during physiologic stress. During fetal life there is high norepinephrine production, with epinephrine production increasing following birth (Ch. 72).

The **catcholamines** can be considered a first line of defense during **phase 1** of the response to trauma, with **ACTH**, **cortisol**, **ADH** and the **renin-angiotensin system** working over time.

Phase 2

The next phase in the physiologic response to trauma is largely **catabolic**, and involves redistribution of stubstrates for energy purposes. During this phase, **glucocorticoids** (e.g., cortisol) collaborate with hormones of the thyroid gland and endocrine pancreas (decreased insulin and increased glucagon), and with biogenic amines of the SNS and adrenal medulla to bring about decreased utilization of glucose yet increased utilization of fatty acids by the body. These hormones also cause mild muscle protein breakdown in order to provide the liver with needed gluconeogenic substrates and, therefore, a sustained output of glucose (through both glycogenolysis and gluconeogenesis). Adipose tissue lipolysis is driven during this phase largely by **cortisol**, **thyroxine**, and the **catecholamines**. **Insulin** secretion is inhibited by catecholamines (Ch. 42), and **glucagon** secretion is stimulated by both cortisol and the catecholamines (Ch. 43). As long as serum cortisol concentrations remain elevated, insulin responsiveness will be reduced, and phase 3 will be delayed.

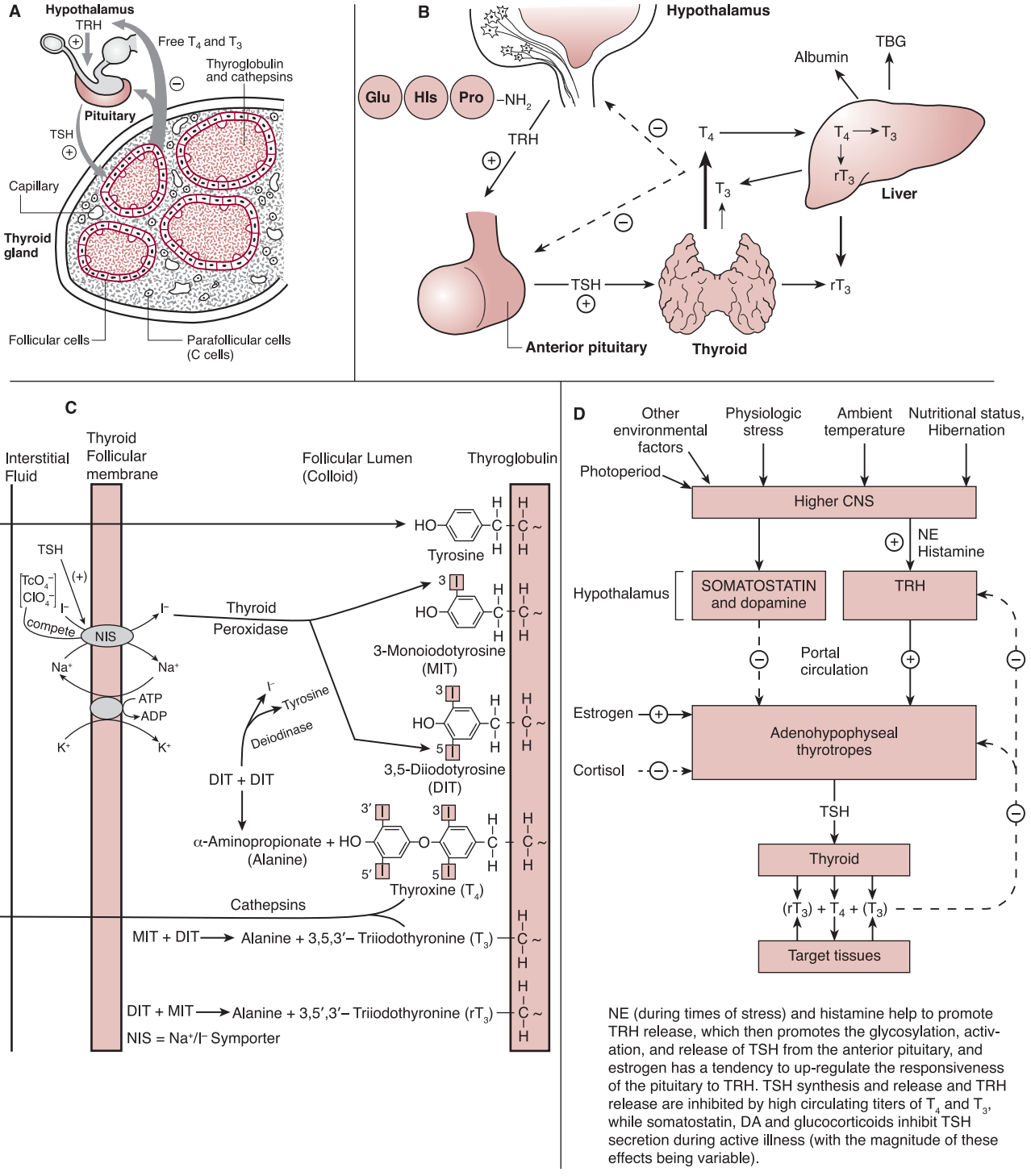
The duration of phase 2 is dependent on several factors (e.g., the type and severity of trauma, acute versus chronic injury, age, and nutritional status). The sooner an animal can be effectively and appropriately fed, and thus regain its nitrogen balance (i.e., move from a negative to a positive nitrogen balance), the sooner it will move into phase 3. Additionally, since pain is a driving force for elevated ACTH and thus glucocorticoid secretion, proper pain management becomes an important consideration for moving a patient from the largely catabolic phase 2 into the more anabolic, reparative phase 3.

Phase 3

Once metabolic stabilization has been achieved and the animal can begin effectively assimilating needed nutrients through either alimentation or parenteral feeding, the **anabolic reparative process** (phase 3) begins. The hormonal mix and the pool of energy-yielding substrates and protein precursors together provide a metabolic climate favorable to protein synthesis and cell proliferation. This is achieved by increasing the roles of **insulin**, **erythropoietin**, and **thyroid hormones** (T_4 and T_3), with important additional contributions from **growth hormone** and the **somatomedins**. Release of **IGF-1** from the liver is enhanced by good nutrition, GH and insulin, and since it structurally resembles proinsulin, it can bind to both insulin and IGF-1 receptors to help promote anabolism (Ch. 10).

Ch36 Thyroid: I

(Hormone Biosynthesis and Secretory Regulation)



NE (during times of stress) and histamine help to promote TRH release, which then promotes the glycosylation, activation, and release of TSH from the anterior pituitary, and estrogen has a tendency to up-regulate the responsiveness of the pituitary to TRH. TSH synthesis and release and TRH release are inhibited by high circulating titers of T_4 and T_3, while somatostatin, DA and glucocorticoids inhibit TSH secretion during active illness (with the magnitude of these effects being variable).

Source: **Part A** modified from Chastain CB, Ganjam VK. Clinical endocrinology of companion animals. 1st ed. Philadelphia, PA: Lea & Febiger, 1986:116.

The thyroid gland is present in all vertebrates, and is unique among endocrine glands in that it stores its secretory products (the thyroid hormones) extracellularly. It is among the most highly vascularized of endocrine glands in mammals, and appears to be one of the oldest phylogenetically (Ch. 72).

The thyroid gland is innervated by sympathetic, postganglionic nerve fibers and, therefore, responds to sympathetic activation. **Follicular cells** of the thyroid synthesize and secrete the thyroid hormones (**thyroxine, T_4; triiodothyronine, T_3; and reverse T_3, rT_3**) mainly in response to the presence (or absence) of **thyroid-stimulating hormone (TSH),** while **parafollicular cells** (otherwise known as "clear," or **C cells**), which are scattered in the interstitium, synthesize and secrete **thyrocalcitonin** (Chs. 16 and 17). **Thyroglobulin** is a glycoprotein synthesized by follicular cells and secreted into the colloid by exocytosis **(Part A).** It binds thyroid hormones until they are secreted. The step leading to liberation of thyroid hormones from thyroglobulin depends upon the activity of intracellular proteolytic enzymes known as **cathepsins,** which are required for normal thyroid function. When thyroid hormones are secreted, thyroglobulin will sometimes enter blood as well, for plasma levels have been found to increase in hyperthyroidism and some forms of thyroid cancer. However, the function of thyroglobulin (if any) in plasma remains obscure.

Biosynthesis of Thyroid Hormones

In response to physiologic need, hypothalamic **TSH-releasing hormone** (also called **thyrotopin-releasing hormone**, **TRH**) stimulates release of **TSH** from thyrotropes of the anterior pituitary (**Part B**). TSH is required for the uptake of **iodide** (I^-) used for hormone biosynthesis within thyroid follicular cells. **Iodine** (**I**) is converted to I^- before being absorbed from the small bowel, then I^- circulates in plasma, bound to plasma proteins (though some is free). Thyroid cell membranes contain a **Na^+/I^- symporter** (**NIS**) that transports Na^+ and I^- into cells, producing intracellular I^- concentrations 20-40 x > than plasma. Although the NIS is also found in salivary glands, the gastric mucosa, placenta, ciliary body, choroid plexus, and mammary glands, these latter tissues are not TSH-sensitive, and they do not organify or store I^-. Thyroid tissue ends up with > **90%** of the body I^- pool. Monovalent anions such as **perchlorate** (**ClO_4^-**), **pertechnetate** (**TcO_4^-**) , and **thiocyanate SCN**$^-$ compete with I^- for uptake, they are also concentrated by thyroid follicular cells, and technetium-labeled (**^{99m}Tc**) pertechnetate is used clinically in thyroid scans. **$KTcO_4$** and **KSCN** are also used to treat I^--induced hyperthyroidism.

In the process of hormone synthesis, **MIT** is produced, then **DIT** (**Part C**). Two DIT molecules condense to form **T_4 + alanine**, and an MIT and DIT condense to form **T_3 (or rT_3)**. The distribution of iodinated compounds in the mammalian thyroid is about **23% MIT**, **33% DIT**, **35% T_4,** and **7% T_3,** with only **trace amounts of rT_3**.

Although T_4 is the major thyroid hormone synthesized, it acts as a prehormone to **T_3**, the more active intranuclear form produced through **T_4 deiodination** in target tissues (Ch. 5). **rT_3** is the inactive form produced mainly by the liver and kidneys through T_4 deiodination (**Parts B** and **C**). Peripheral production of **T_3 decreases** and that of **rT_3 increases** during periods of sustained catabolism (e.g., starvation, anorexia, fever, burns, severe illness, or hibernation). This is thought to be a beneficial response to caloric restriction, serious illness, or stress that conserves energy through a **reduction in the BMR**.

Secretion, Plasma Transport, and Turnover of Thyroid Hormones

Thyroxine and (to a lesser extent) T_3 circulate bound to plasma proteins. The exact proteins and their binding affinities vary with species.

Thyroid hormone–binding globulin (**TBG**; see **Part B**), a glycoprotein synthesized by the liver, binds **T_4** with high affinity. It is present in relatively high amounts in primates and large domestic animals, but in lower amounts in dogs and cats. **Albumin** appears to be a more important plasma binding protein for small animals. During pregnancy, **estrogen** stimulates hepatic synthesis of **TBG** and decreases its clearance. Therefore, total plasma thyroid hormone levels are generally increased in the maternal circulation; however, free T_4 and T_3 levels usually remain unchanged until parturition, when the source of estrogen is removed (Chs. 37 and 65, **Part H**)

Although the ratio of **T_4-to-T_3** secretion from the thyroid of the dog is about **5:1**, circulating levels are about **20:1**. This is due to differences in serum binding (T_4 is more tightly protein bound), and perhaps a larger intracellular compartmentalization for T_3. Increased plasma protein binding of T_4 also means that the turnover rate of T_4 in dogs (circulating $t_{1/2} \approx$ 12-24 hrs.), is longer than that for T_3 ($t_{1/2} \approx$ 6 hrs.). Comparable circulating half-lives in primates are 7 days and 24 hrs., respectively, which is longer than for most hormones.

Thyroid hormones are lipophilic, metabolized primarily via deiodination or conjugation in the liver to sulfate and glucuronides, and excreted in bile. Approximately **45%** of T_4 is deiodinated to T_3 and rT_3 (**Part E**), and **55%** is excreted in bile. Lesser amounts are removed via deamination, decarboxylation, and urinary excretion. Most of the hormone that appears in the glomerular filtrate is reabsorbed by renal tubules.

Only about **15%** of **T_4** and **T_3** in the dog is reabsorbed from the gut following biliary excretion, compared to **79%** to **100%** in primates. This comparatively low reabsorption rate helps to explain why dogs have a higher production rate and replacement requirement for thyroxine than primates.

Regulation of Thyroid Hormone Secretion

Thyrotropin-releasing hormone (**TRH**) was the first releasing factor to be identified, purified, and synthesized. More than **80%** of **TRH** is extrahypothalamic, appearing in the higher brain centers, spinal cord, GI tract, retina, pancreatic islets, reproductive tract, and placenta. However, the highest single concentration is hypothalamic. **TRH** is a tripeptide whose release is inhibited in hot environments, and stimulated in cold environments. Physiologic stress, photoperiod, and nutritional status, among other factors, also indirectly control TRH release (**Part D**).

TSH is synthesized by basophilic thyrotropes of the adenohypophysis, and is a glycoprotein of 211 amino acids. It has a chain length similar to that of LH, FSH, and CG. TSH is usually not used clinically, because it is a protein and because T_4 administration is more effective. The plasma half-life of TSH is about **60 minutes**.

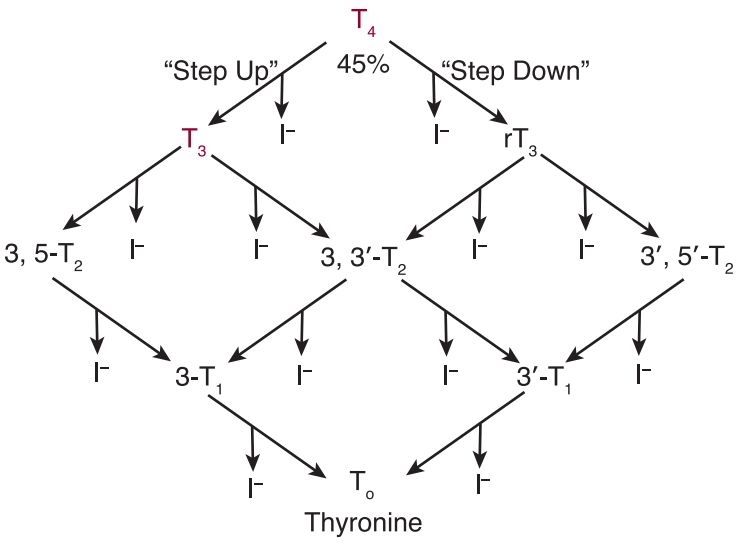
TSH maintains the structure, growth, and secretory activity of the thyroid by enhancing activity of the hexose monophosphate shunt (NADPH is needed for I^- reutilization), glycolysis, protein synthesis, tricarboxylic acid (TCA) cycle activity, and oxygen consumption. It also enhances thyroidal I^- uptake from plasma, thus increasing T_4 and T_3 synthesis. The response to TSH is reportedly greater in females than in males.

Thyroid hormone secretion is regulated via a classic physiologic negative feedback scheme involving the hypothalamic–pituitary–thyroid axis (**Part D**). There is also some intrathyroidal autoregulatory control. Note from **Part D** the negative feedback effects of T_4 and T_3 on hypothalamic TRH release and the release of TSH from adenohypophyseal thyrotropes. **Somatostatin** and **dopamine** (ostensibly from the hypothalamus) inhibit **TSH** release, while **norepinephrine** (released in response to stress) and **histamine** from higher brain centers stimulate **TRH** release. The pituitary response to TRH is further reduced by **cortisol**, and enhanced by **estrogen**.

Ch37 Thyroid: II

(Metabolic Effects of Thyroid Hormones)

E



F

Metabolic Effects of Thyroid Hormones

Calorigenic
General
- ↑O_2 consumption
- ↑BMR
- ↑Heat dissipation
  - Panting
  - Sweating
  - ↑β-adrenergic receptors
    - Cutaneous vasodilation
    - ↑Cardiac output

Body temperature, increased
Uncouple oxidation from phosphorylation in brown adipose tissue
- Neonates
- Arousal from hibernation

Protein synthesis
Stimulated
- Pre-adolescent growth and development

Inhibited
- Hyperthyroidism

Carbohydrate metabolism—provide more glucose
↑Effects of diabetogenic hormones (GH, cortisol, glucagon, and epinephrine)
↑Gluconeogenesis and glycogenolysis
↑Intestinal carbohydrate absorption

Lipid metabolism—provide more fatty acids
↑Lipolysis (synergistic with epinephrine)
↑β-Oxidation
- ↑Hepatic ketogenesis

↑Hepatic triglyceride synthesis
↑Hepatic LDL-receptor synthesis
- ↑Cholesterol clearance

↑Hepatic conversion of β-carotene to vitamin A

Interactions with catecholamines—↑β-adrenergic receptor synthesis
↓Diastolic pressure
↑Lipolysis in fat tissue

Skeletal muscle—maintain muscle protein synthesis
Muscle weakness occurs in both hyper- and hypothyroidism

Heart—↑β-adrenergic receptor synthesis
↑Chronotropic behavior
↑Ionotropic behavior
↑Na^+/K^+–ATPase
↑Ca^{2+}–ATPase

Skin
Maintain protein synthesis and turnover
Maintain hair coat and sebaceous gland activity

Bone
Maintain growth and epiphyseal closure

Erythrocytes
Support erythropoiesis
↑2,3-Diphosphoglycerate (2,3-DPG) synthesis

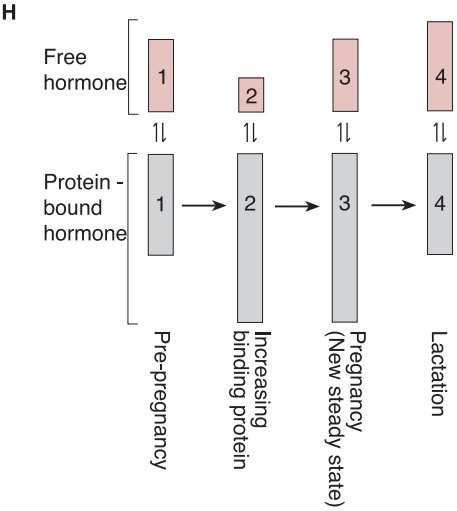
Brain
↑Fetal brain development, synapse formation, and myelination

Kidney—maintain renal response to aldosterone
↑ Na^+/K^+–ATPase activity in distal nephron

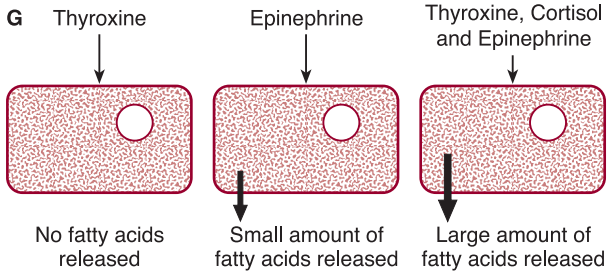
GI tract—maintain smooth muscle growth, development, and activity
Segmentation and peristalsis

Reproductive tract
Maintain gonadotropin output from the adenohypophysis

H



G



Source: Part G modified from Vander AJ, Sherman JH, Luciano DS. Human physiology, the mechanisms of body function. 3rd ed. New York: McGraw-Hill, 1980:200.

In addition to the classic hypothalamic-pituitary control system discussed in Ch. 36, it should be recognized that **growth factors** such as insulin, IGF-1, EGF and the autocrine actions of prostaglandins and cytokines may modify thyroid cell growth and hormone production. It is not clear how important these effects are clinically.

As discussed in Ch. 6, major effects of thyroid hormones are produced via changes in the synthesis and activity of **target cell** regulatory proteins, including key metabolic enzymes, hormones, and receptors. These occur via regulation of gene expression at the nuclear level, and subsequent induction of RNA synthesis. Nuclear activation occurs via binding of **T_3**, the most biologically active thyroid hormone, to specific high-affinity receptor sites.

The biologic activity of thyroid hormones is dependent on location of the iodine atoms (Ch. 39). Target tissue **deiodination** of the **T_4** *outer ring* (5'-deiodination) is a **"step up,"** producing **T_3** (**Part E**), which is 3-8 times more potent in target cells than **T_4**. **Deiodination** of the **T_4** *inner ring* is a **"step down,"** producing **rT_3**, which is metabolically inert. Further deiodination of **T_3** and/or **rT_3** results in iodothyronine derivatives (i.e., **T_2** & **T_1**) with no metabolic activity, then ultimately thyronine (**T_0**). Types, specificities and locations of the various deiodinases are discussed in Ch. 39.

Specific responses to thyroid hormones vary between species and tissues that are usually under multihormonal regulation, and may be affected by other factors such as environmental conditions, as well as carbohydrate, fat and protein intake.

Metabolic Effects of Thyroid Hormones

The metabolic effects of thyroid hormones are summarized in **Part F.**

Calorigenic Actions: General stimulation of oxygen consumption, which results in an increase in the **BMR**, may be the single most important effect of thyroid hormones. Thyroid hormones **increase oxygen consumption** in most tissues, with the notable exception of the brain, gonads, and spleen.

Enhanced mitochondrial oxidation results in energy, which is stored in the form of **ATP** or released as **heat** (thereby maintaining body temperature). Heat-dissipating mechanisms (such as panting and sweating) are also activated. Peripheral resistance is decreased due to cutaneous vasodilation brought about by an increase in **β-adrenergic receptor synthesis**. The enhanced cardiac output, which also occurs due to increased β-adrenergic receptor synthesis, also helps to dissipate heat.

Body Temperature: Body temperature is maintained via a variety of mechanisms. In nonprimate mammals, however, thyroid hormones play a key role in maintaining body temperature in cold environments. Under the stimulus of cold or a drop in core body temperature, hypothalamic **TRH**-secreting neurons secrete increased amounts of TRH, thus resulting in adenohypophyseal **TSH** release and subsequent thyroid hormone secretion from follicular cells of the thyroid gland (Ch. 36).

Thyroxine (**T_4**) also uncouples oxidation from phosphorylation in brown adipose tissue, which is important to newborn animals and those arousing from hibernation. The resulting nonshivering thermogenesis helps to assure survival.

Protein Synthesis: Nitrogen excretion is increased and weight is lost if food intake is not increased in hyperthyroidism. In the hypothyroid state, small doses of hormone will cause a positive nitrogen balance, while large doses may cause catabolism. Thyroid hormones stimulate the synthesis of many structural proteins, enzymes, and hormones and, therefore, play an important role (with **insulin**, **GH**, and the **somatomedins**) in pre-adolescent growth and development.

Carbohydrate Metabolism: In general, thyroid hormones act to **provide more glucose**, primarily via enhancement of the effects of the diabetogenic hormones (i.e., epinephrine, GH, cortisol, and glucagon). Hepatic glucose production is increased via gluconeogenesis and glycogenolysis, and carbohydrate absorption from the GI tract is also enhanced.

Lipid Metabolism: Thyroid hormones **provide more fatty acids** for oxidation and ketogenesis, an important component of their calorigenic action. Hepatic triglyceride synthesis is also stimulated following fatty acid and glycerol mobilization from adipose tissue. Thyroxine increases the sensitivity of hormone-sensitive lipase (also called adipolytic triglyceride lipase) to the lipolytic actions of cortisol and the catecholamines (i.e., epinephrine; see **Part G**), while decreasing sensitivity of this adipose tissue enzyme to the antilipolytic action of insulin. **Thyroxine**, like **estrogen** and **insulin**, also helps to maintain hepatic **low-density lipoprotein** (**LDL**)**-receptor synthesis** and, thus, removal of cholesterol from the circulation. It also assists in heptic conversion of β-carotene to vitamin A.

Interactions with Catecholamines: Thyroid hormones increase the **β:α-adrenergic receptor ratio** on muscle, adipose tissue and lymphocytes, therefore allowing catecholamines to increase the BMR in these tissues, albeit with decreased duration (Ch. 34). Thus, the catecholamine toxicity of hyperthyroidism can be reduced with β-adrenergic blocking agents (e.g., propranolol).

As hepatic TBG output increases with the estrogen-stimulation of pregnancy, free T_4 levels in blood are initially reduced, then return to a new steady state (**Part H**). Following removal of the placenta at the time of parturition, estrogen and TBG levels decline, and free T_4 levels rise in support of lactation.

Specific Organs and Tissues

Skeletal Muscle: Thyroid hormones are essential for normal muscle growth, skeletal maturation, and mental development. This is in part due to the effects of T_3 on protein synthesis and to its potentiation of the actions of growth hormone. **Muscle weakness** is present in both hypo- and hyperthyroidism.

Heart: As stated previously, **β-adrenergic receptors** are increased in hyperthyroidism, thus increasing sensitivity to the inotropic and chronotropic effects of the catecholamines.

Skin: Thyroid hormones maintain protein synthesis in skin, as well as patency of hair follicles and sebaceous glands.

Bone: In the absence of normal levels of thyroid hormones, bone growth is slowed and epiphyseal closure is delayed.

Erythrocytes: Thyroid hormones support **erythropoiesis**. There is a mild anemia in hypothyroidism and a slight polycythemia in hyperthyroidism due to related changes in protein turnover and oxygen needs. Thyroid hormones stimulate erythrocytic **2,3-diphosphoglycerate** (**2,3-DPG**) synthesis and activity, which decreases the affinity of hemoglobin for oxygen, and thus increases delivery of oxygen to tissues.

Brain: Slow mentation and reflexes occur in hypothyroidism, while irritability and restlessness occur in hyperthyroidism. Glucose and oxygen consumption do not seem to be involved. Adequate thyroid hormone levels are critical in the fetus for brain development, normal synapse formation, and myelination. Fetal hypothyroidism causes **cretinism** (Ch. 38).

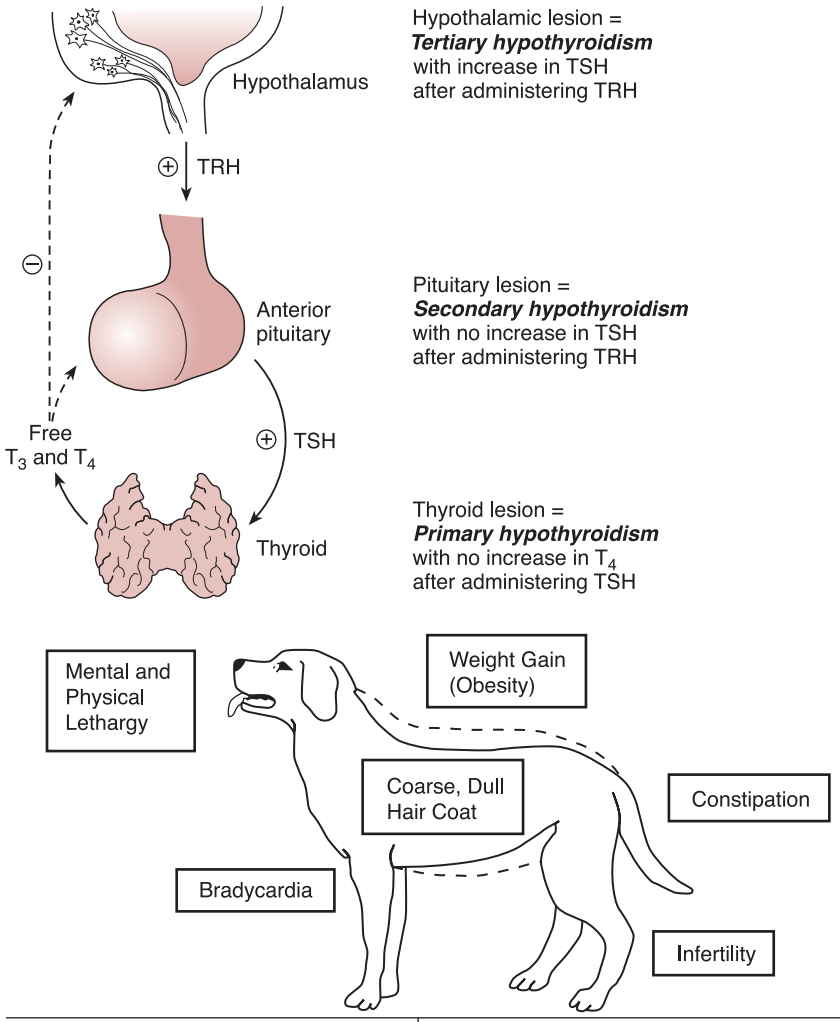
Kidney: Hypothyroidism leads to excessive renal Na^+ loss and hyponatremia. The renal response to **aldosterone** is depressed because thyroid hormones are needed to maintain Na^+/K^+ ATPase activity in distal renal tubular epithelial cells (Ch. 26).

GI Tract: Normal thyroid hormone levels are important for maintaining mucosal cell synthesis and smooth muscle activity of the GI tract. **Constipation** occurs in hypothyroidism (Ch. 38), and **diarrhea** in hyperthyroidism (Ch. 39).

Reproductive Tract: Thyroid hormones are required for normal reproductive function in both males and females. It is thought that they exert their effects at the hypothalamic level and, thus, affect **gonadotropin** release from the anterior pituitary. Thyroid function appears to have evolved hand-in-hand with reproductive endocrine function, and pituitary **thyrotropes** may have evolved from **gonadotropes** (Ch. 72).

Ch38 Hypothyroidism
(Signs and Symptoms)

A



B

Canine Breeds with a Predisposition to Hypothyroidism

Great Dane
Cocker Spaniel
Dachshund
Irish setter
Shetland sheep dog
Airedale
Boxer
Miniature schnauzer
Poodle
Pomeranian
*Golden retriever
*Doberman pincher

*Reportedly a higher incidence.

D

Goiters

Type I: Hypothyroid (primary)

Type II: Hyperthyroid (toxic and non-physiologic)

Type III: Hyperthyroid (physiologic)

Type IV: Normothyroid

An enlarged thyroid gland is referred to as a goiter, regardless of the cause of the enlargement.

C

Signs and Symptoms of Hypothyroidism

Metabolic rate
- ↓BMR
- Dullness
- Lethargy
- Unwillingness to exercise
- Cold intolerance
- Weight gain

Skin and hair coat
- Dry, scaly skin
- Seborrhea
- Coarse, dull hair coat
- Alopecia (bilateral)
- Changes in hair coat color
- Hyperpigmentation
- Myxedema

Eyes
- Corneal lipid deposits
- Corneal ulceration
- Uveitis

Skeletal (congenital hypothyroidism)
- Disproportionate dwarfism
- Short, broad skull
- Epiphyseal dysgenesis

CNS
- Mental retardation (cretinism)
  - decreased myelin formation
- Circling, head tilt
- Facial nerve paralysis, ↓facial sensation
- Ataxia
- Depression, irritability, seizures

Neuromuscular
- Lower motor neuron paresis/paralysis
- Decreased spinal reflexes
- Muscle weakness

Cardiovascular
- Bradycardia
- Decreased blood volume and pressure

Pulmonary
- Slow, shallow respirations
- Impaired response to hypercapnia

Gastrointestinal
- Constipation
- Loss of appetite
- Impaired absorptivity of certain nutrients

Renal
- Increased urinary Na^+ excretion
- ↓GFR, ↓Renal blood flow

Reproductive organs (female)
- Prolonged interestrus intervals
- Failure to cycle, infertility
- Galactorrhea
- Weak or still-born young

Reproductive organs (male)
- Low libido
- Testicular atrophy, infertility
- Hypospermia
- Azoospermia

Blood
- ↓T_4, ↓T_3
- Anemia
- Hypertriglyceridemia
- Hypercholesterolemia (and atherosclerosis)
- Hyponatremia

Hypothyroidism is common in dogs, yet comparatively rare in cats. Iatrogenic (i.e., veterinarian-induced) hypothyroidism reportedly occurs more often than naturally acquired hypothyroidism in cats, and is secondary to bilateral thyroidectomy for the treatment of hyperthyroidism, ablation of the thyroid with ^{131}I, or an overdosage of antithyroid drugs.

More than **95%** of canine cases are classified as **acquired primary hypothyroidism,** which results in compensatory increases in both TRH and TSH because the typical negative feedback of thyroid hormones on the hypothalamus and pituitary is lacking (**Part A**). Antibodies against the TSH receptor have been found in human patients, and lead to thyroid gland atrophy. As TRH is a stimulator of **prolactin** release from the anterior pituitary, primary hypothyroidism may lead to **galactorrhea** (i.e., excessive or spontaneous flow of milk irrespective of nursing) in sexually intact bitches. Destruction of the thyroid in animals has been reported to result from lymphocytic thyroiditis, idiopathic thyroid atrophy, or (rarely) neoplastic invasion. Lymphocytic thyroiditis may also be associated with the formation of antibodies to thyroglobulin, T_4 and T_3. **Secondary hypothyroidism** (decreased TSH) is seen in humans, but is uncommonly recognized in dogs (probably due to inadequate assay for TSH) (see **Part A**). Causes of acquired secondary hypothyroidism in dogs include pituitary neoplasia and pituitary malformations such as cystic Rathke's pouch, and hyperadrenocorticism. High levels of circulating glucocorticoids suppress the pituitary response to TRH (Ch. 36). **Tertiary hypothyroidism** (decreased TRH) has not been documented in dogs (see **Part A**).

Rare cases of hypothyroidism due to peripheral resistance to thyroid hormones have been decribed in humans. Affected individuals reportedly have abnormal thyroid hormone receptors or postreceptor defects. Consequences of hypothyroidism are most severe when the condition occurs in infancy. Untreated neonatal hypothyroidism results in irreversible **cretinism,** a congenital syndrome characterized by mental retardation and growth failure. Cretinism does occur in dogs, but it is rarely diagnosed because it usually results in early death. This syndrome is, unfortunately, remarkably common in humans, occurring once in every **8500** births. **Part B** lists canine breeds with a predisposition for hypothyroidism.

Signs and Symptoms

In spite of the wide spectrum of functional deficiencies associated with hypothyroidism, onset of the disease is reportedly slow and the manifestations are subtle, so that owners of domestic animals may not recognize the extent of illness until it reaches a rather advanced stage. Once thyroid hormone therapy is instituted, however, a dramatic improvement in overall physiologic status can be expected.

Characteristic signs of hypothyroidism include **loss of appetite, lethargy, obesity, constipation, hypercholesterolemia, bradycardia, anemia, coarse hair coat, and bilateral alopecia** (**Part C**). The **intestinal musculature** is among the many functional systems in which activity is conditioned by the presence of thyroid hormones. Hyperthyroidism is characterized by hypermotility and a tendency toward diarrhea, while hypothyroidism leads to decreased motility and constipation. Thyroid hormones are necessary for hepatic conversion of β-carotene to vitamin A, and the accumulation of β-carotene in blood (**β-carotenemia**) may occur in hypothyroid herbivores and omnivores.

The **anemia** that develops in hypothyroid patients is largely associated with impaired hemoglobin biosynthesis resulting directly from T_4 deficiency, but also from iron, folate, and B_{12} deficiency due to impaired intestinal absorption.

Myxedema, represented by the deposition of large amounts of mucopolysaccharides in the skin (particularly evident in the face and jowles), takes up Na^+ in the form of increased tissue fluid as well as Na^+ directly bound to the chondroitin sulfate in mucopolysaccharides. The loss of Na^+ would be compensated by renal conservation were it not for the fact that an additional route for Na^+ loss is the kidneys. Normal activity levels of the tubular Na^+ pumps and, more specifically, the active transport mechanism stimulated by aldosterone are dependent on adequate titers of thyroid hormones (Ch. 37). With thyroid deficiency, the **aldosterone** Na^+ conservation mechanism is impaired, and Na^+ wastage occurs. Renal loss of Na^+ which carries significant water osmotically, can substantially deplete the extracellular fluid volume (if left unchecked), including the blood volume. This lowered blood volume, together with a decreased response to the noradrenergic vasoconstrictor mechanism and the reduced hepatic angiotensinogen output, can bring about significant **hypotension**, and perhaps **coma**. Although the renal response to aldosterone is suppressed in hypothyroidism, the renal response to **ADH** is apparently maintained. **ANP** concentrations are usually low due to the hypotension. Renal blood flow and the glomerular filtration rate (**GFR**) are also reduced. Plasma **creatinine** levels usually remain within the normal range.

Hypothyroidism has a tendency to decrease the number of β-adrenergic receptors in heart muscle, as well as sarcolemmal calcium ATPase activity, Na^+/K^+ ATPase activity, and calcium channel function. Therefore, hypothyroidism causes **impaired myocardial conductivity and action**. Thyroid hormones, along with insulin and estrogen, also increase the number of hepatic LDL receptors. Therefore, hypothyroidism, like diabetes mellitus, results in a decreased ability to clear LDL and, therefore, cholesterol from the circulation, which can result in **atherosclerosis**. Impaired mental states associated with atherosclerosis and cerebral myxedema can be signs of hypothyroidism. Other effects of atherosclerosis including retinopathy and renal failure have been described in hypothyroid beagles.

Because thyroid hormones are essential for normal musculoskeletal growth and development, juvenile-onset hypothyroidism results in **stunted growth**. Pituitary **GH** secretion may be depressed because thyroxine augments hypothalamic GHRH output (Ch. 10). Hypothyroid animals demonstrate decreased widths of epiphysial growth plates and articular cartilages, and decreased volumes of epiphysial and metaphysial trabecular bones. These changes are not solely due to lack of pituitary GH, since administering exogenous GH fails to restore normal cartilage morphology or bone remodeling, whereas administering **T_4** succeeds.

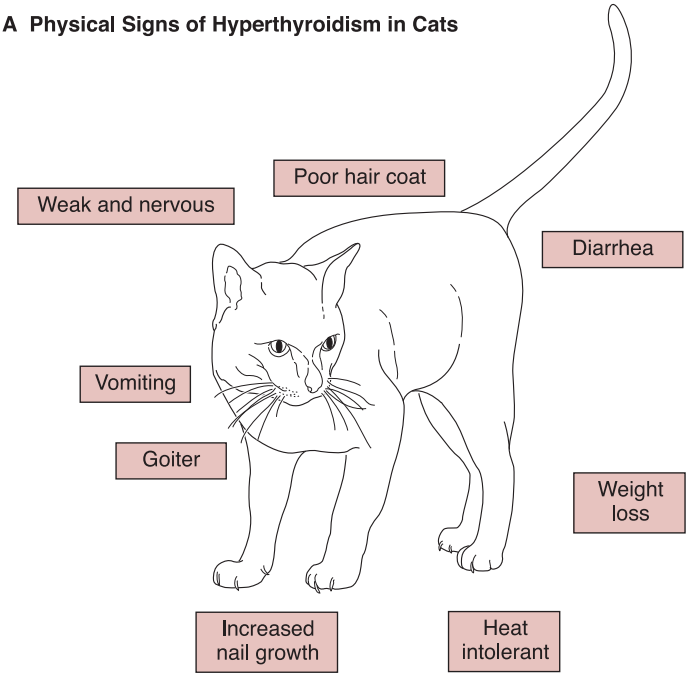
Thyroid hormone levels may also be reduced with a variety of infections, liver and renal diseases. Physiologic hypothyroidism occurs with starvation and hibernation, where significant reductions in BMR may occur to assure survival. Circulating T_3 levels have been found to be reduced in starved and hibernating animals, while **rT_3** levels are elevated. In general, these conditions are referred to as **"euthyroid sick syndrome."** When starved animals are appropriately refed, circulating proportions of the triiodothyronines are usually reversed.

Goiters

Any enlarged thyroid is referred to as a goiter, regardless of the cause or nature of the enlargement. There are four types considered to be significant in mammals, birds, and submammalian vertebrates (**Part D**). **Type I** goiters are caused by primary hypothyroidism. Circulating TSH levels are elevated due to reduced thyroid hormone negative feedback, and the excess TSH causes thyroid enlargement. Although iodide deficiency is a common cause of hypothyroidism, thyroid hyperplasia has been reported in foals of mares fed dry seaweed containing excessive iodide, which interferes with thyroxinogenesis, thus leading to lowered blood T_4 and compensatory increases in TSH. **Type II** and **III** goiters are usually hyperfunctioning. **Type II** is usually associated with low levels of TSH, and is referred to as diffuse thyrotoxic goiter or **Graves' disease** in humans. **Type III** goiters are best associated with pregnancy, where thyroxine-binding globulin synthesis and renal iodide clearance are elevated (Ch. 65). **Type IV** goiters develop in animals with otherwise normal thyroid function. Such enlargements may be caused by infiltration of the gland with bacteria, parasites, or by the presence of adenomas or carcinomas.

Ch39 Hyperthyroidism (Signs and Symptoms)

A Physical Signs of Hyperthyroidism in Cats



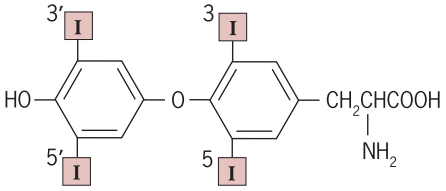
B

Signs and Symptoms of Hyperthyroidism

Physical	Blood
PU/PD	Erythrocytosis
↑BMR	Leukocytosis
Vomiting and diarrhea (dehydration)	Eosinopenia
Polyphagia (usually)	Lymphopenia
Weight loss	↑T_4 and T_3
Weakness (muscle wasting)	↓TSH
Reduction in dermal fat	↑ALT
Thin (and sometimes cachectic)	↑AST
Nervousness, restlessness, and aggressiveness	↑AP
Tachycardia, left ventricular hypertrophy	↑LDH
↑Cardiac output	↑Creatinine
Poor hair coat/alopecia (patchy)	↑BUN
Heat intolerant	Hyperglycemia
Respiratory alterations	Hypocholesterolemia
Panting and vocalization	↑Free fatty acids
Shivering	Hyperphosphatemia
Small goiters (cats), larger goiters (dogs)	Hypernatremia (mild) and hypokalemia
Increased nail growth	Hyperbilirubinemia

D

Thyroxine (T_4)



T_3 (3,5,3′-Triiodothyronine)
rT_3 (3,3′,5′-Triiodothyronine)
T_2 (3,5; 3,3′; or 3′,5′-Diiodothyronines)

Naturally Occurring Deiodinases

(D1) Type I: Thyroid, Liver, Kidney, Pituitary

$T_4 \dashrightarrow {}^*rT_3 \dashrightarrow T_2$

$T_4 \dashrightarrow T_3$

(D2) Type II: Several target tissues (e.g., Brain, Muscle, Pituitary, Placenta, Brown Adipose Tissue)

$^*T_4 \dashrightarrow T_2$

$rT_3 \dashrightarrow T_2$

(D3) Type III: Brain (Gilal Cells), Placenta, Intestine, Fetal Skin, Reproductive Tissues

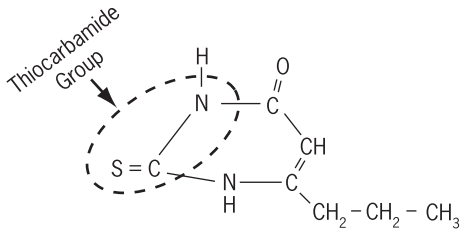
$^*T_3 \dashrightarrow T_2$

$T_4 \dashrightarrow rT_3$

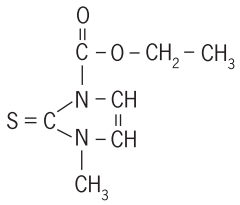
Note: $rT_3 \not\dashrightarrow T_3$ or T_4

*Preferred substrates

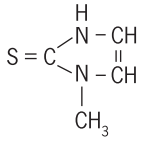
C Antithyroid Agents



Propylthiouracil (PTU)

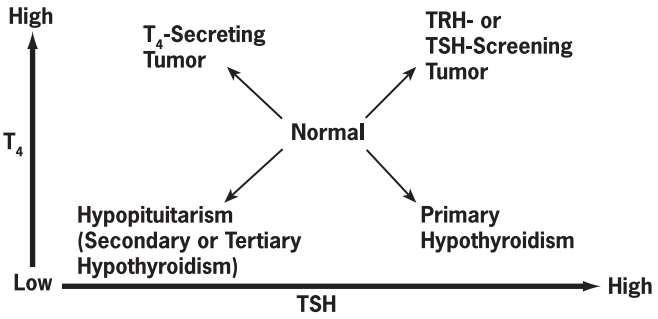


Carbimazole



Methimazole (Tapazole)

E



The term **thyrotoxicosis** refers to an excess of thyroid hormones, generally due to thyroid hypersecretion. Although TRH-secreting tumors of hypothalamic nuclei or TSH-secreting basophilic tumors of the anterior pituitary could conceivably cause thyrotoxicosis, most tumors in domestic animals are of primary thyroidal origin.

Hyperthyroidism, caused by autonomous growth and function of thyroid follicular cells, was first reported as a clinical entity in humans in 1913, and in **cats** in 1979. Most feline thyroid tumors are reportedly functional, noninvasive, and relatively small, while those in dogs are more often nonfunctional, invasive, and large. Although thyroid tumors account for only **1% to 4%** of canine neoplasms, more than **90%** are reportedly malignant. Only **10% to 20%** of thyroid tumors in dogs are reported to be hypersecreting.

Hyperthyroidism (i.e., the clinical entity resulting from excessive production and secretion of thyroid hormones) is therefore **more often seen in cats than in dogs, and it is postulated to be the most common endocrinopathy affecting older cats** (**Part A**).

Signs and Symptoms

The signs and symptoms of hyperthyroidism are listed in **Part B.** Disorders that can mimic hyperthyroidism include renal and cardiac failure, diabetes mellitus, liver disease, maldigestion/malabsorption, pheochromocytoma, and neoplasia.

Most animals with **thyrotoxicosis** show signs of weight loss and an unkept hair coat. They are generally restless, aggressive, and difficult to handle. Enlarged thyroid glands may be palpable, and the muscle wasting that accompanies this disease may eventually lead to periods of weakness. With an increased BMR, vitamin needs are increased, and therefore vitamin deficiency syndromes may be precipitated.

Increased calorie utilization generally leads to increased appetite and food intake. However, the **GI hypermotility** brought about by high titers of thyroid hormones frequently leads to vomiting and diarrhea. The glomerular filtration rate is reportedly increased in many hyperthyroid animals, and high titers of thyroid hormones may decrease the concentrating ability of the nephron. Therefore, medullary washout can occur, leading to polyuria and secondary polydipsia (**PU/PD**). Vomiting and diarrhea also cause dehydration, which again increases thirst. The cause of **azotemia** (increased BUN and serum creatinine) in animals with thyrotoxicosis is not as well understood. It has been postulated that increased protein catabolism may contribute. Azotemia is more likely to occur following treatment for the hyperthyroid state, and therefore renal function should be carefully assessed before, during, and following therapy.

Alterations in **respiratory function** (e.g., panting, decreased vital capacity and pulmonary compliance) are thought to result from a combined increase in CO_2 production, and decrease in respiratory muscle strength. An increase in β-adrenergic receptor synthesis results in reduced peripheral resistance (as does increased tissue metabolism), increased venous return to the heart, tachycardia, and an increase in **cardiac output**. Cardiac volume overload results in dilatation, and eventually left ventricular hypertrophy. Although hepatic angiotensinogen output is increased, diastolic pressure reportedly remains low.

The stress response to hyperthyroidism can be reflected in the complete blood count (**CBC**), which typically shows erythrocytosis, mature leukocytosis, eosinopenia, and lymphopenia. The erythrocytosis most likely results from both a direct effect of thyroid hormones on bone marrow function, and an increase in EPO secretion due to hypoxemia.

Animals with primary hyperthyroidism (i.e., resulting from an autonomous, hyperactive thyroid gland) are expected to have increased levels of both free and bound T_4 and T_3 in the circulation, and decreased levels of TSH. Increases in the plasma concentrations of several enzymes have been noted in cats (i.e., alanine aminotransferase, ALT; aspartate aminotransferase, AST; alkaline phosphatase, AP; and lactate dehydrogenase, LDH). Fatty liver infiltration, with some necrosis evident, is common in hyperthyroid cats, thus giving rise to an elevation in **liver enzymes** as well as a mild hyperbilirubinemia. However, as only ALT is liver-specific in cats, it is possible that other tissues may also be contributing to elevations in AP, LDH, and AST in this species (e.g., heart muscle, kidney, and bone tissue).

Because thyroid hormones increase the basal metabolic rate (BMR) and help to provide more glucose (Ch. 37), hyperthyroidism can result in heat intolerance and **hyperglycemia**. Hepatic LDL-receptor synthesis is increased by thyroid hormones, thereby making it easier for the liver to clear cholesterol from the circulation. Thus, **hypocholesterolemia** may be seen in hyperthyroidism. Because thyroid hormones also enhance the lipolytic effects of the catecholamines, fat depletion occurs and the **plasma free fatty acid level rises**.

Hyperthyroidism causes excessive **skeletal Ca^{2+} resorption**, which in turn decreases serum PTH and 1,25 $(OH)_2D$ levels (Chs. 16 and 17). As a result, Ca^{2+} absorption from the intestinal tract is compromised, as is its reabsorption from the renal filtrate. Consequently, excessive amounts of Ca^{2+} are lost in both urine and feces (although serum levels remain largely unchanged). With low serum PTH levels, however, renal tubular reabsorption of PO_4^{3-} is increased, resulting in excessive PO_4^{3-} retention (i.e., **hyperphosphatemia**).

As the renal response to **aldosterone** is enhanced by thyroid hormones, hyperthyroidism leads inexorably to increased Na^+ retention and renal K^+ excretion. The ensuing hypokalemia leads to a decrease in neuromuscular irritability (Ch. 19), and reportedly ventroflexion of the head.

Antithyroid Agents and Deiodinases

The thiocarbamide group of naturally occurring **goitrogens** exhibits antithyroid activity, and has been incorporated into drugs such as **methimazole** (**tapazole**), **carbimazole,** and **propylthiouracil** (**PTU; Part C**). These compounds are actively concentrated by the thyroid gland, where they inhibit thyroxinogenesis by interfering with the activity of thyroglobulin, by preventing the coupling of iodotyrosyl groups into T_4, and by strongly inhibiting the activity of **type I** deiodinase.

Three types of deiodinase are recognized in mammals (**Part D**). **Type I** is located primarily in thyroid, but also in liver, pituitary, and kidney tissue, where it deiodinates either the outer or inner ring of iodinated thyronines. It exhibits a strong preference for rT_3, which is rapidly deiodinated to diiodothyronine (T_2), but will also act on T_4 (which is converted to either T_3 or rT_3). **Type II** deiodinase prefers T_4 over rT_3 as substrate, and deiodinates only the outer ring. This deiodinase governs intracellular levels of T_3 in several target tissues. **Type III** deiodinase attacks only the inner ring, and prefers T_3 as substrate (although it can attack T_4 as well). Neither **type II** nor **type III** deiodinase is affected by PTU. It should also be noted that once rT_3 is formed, it can be deiodinated to T_2, but it cannot be upgraded to T_3 or T_4.

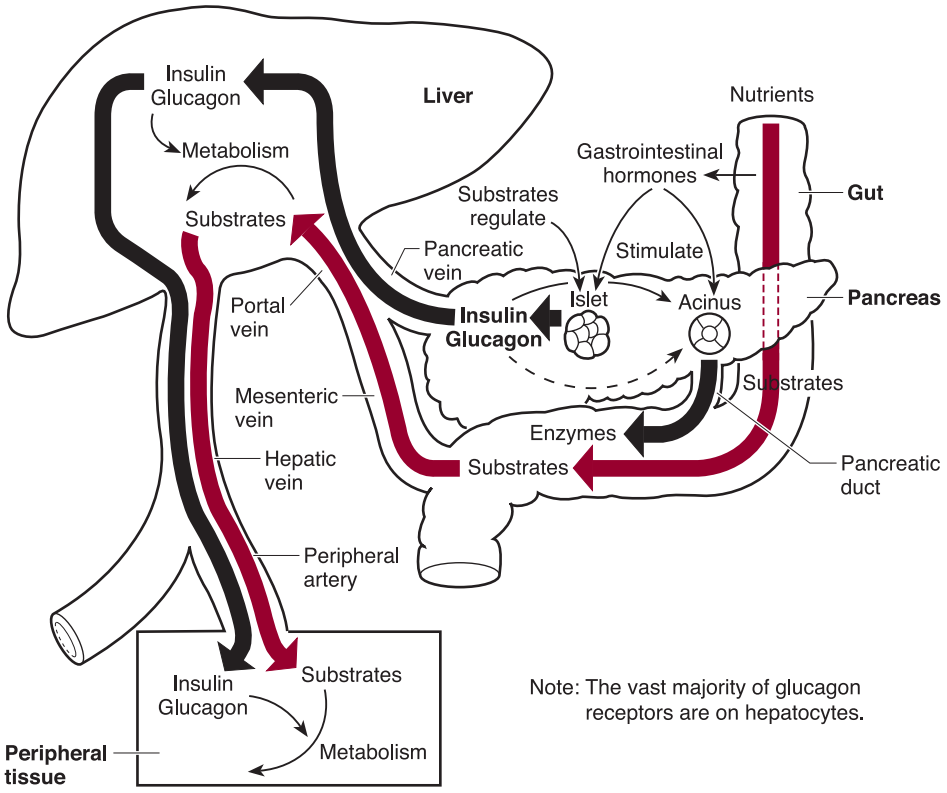
Deiodination rates differ markedly in different tissues, and therefore complicated models are sometimes needed to properly evaluate thyroid hormone kinetics.

Summary

Signs and symptoms of thyroid disease in animals are predictable consequences of the physiologic effects of thyroid hormones discussed in Chs. 36 and 37. Patients are usually encountered with one of two types of thyroid dysfunction: **1) hypothyroidism** caused by a T_4 deficiency, most notably in **dogs**, and **2) hyperthyroidism (thyrotoxicosis)** caused by a T_4 excess, most notably in **cats**. Acquired primary hypothyroidism promotes compensatory increases in both TRH and TSH secretion due to lack of T_4 negative feedback on the hypothalamic-pituitary axis. Secondary hypothyroidism is uncommon in dogs, but can be seen with pituitary neoplasia, pituitary malformations, or glucocorticoid excess. Consequences of hypothyroidism are most severe during fetal development. Although TRH- or TSH-secreting tumors could promote a thyrotoxicosis, most tumors in domestic animals are primarily of thyroidal origin, therefore mean plasma TSH levels are generally low (**Part E**). Animals with thyrotoxicosis reportedly show signs of weight loss, they are restless and aggressive, and difficult to handle.

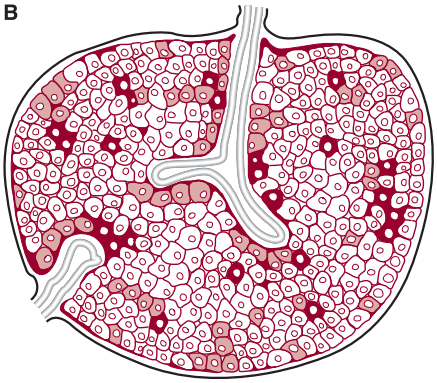
Ch40 Endocrine Pancreas: I
(The Islets of Langerhans)

A



Note: The vast majority of glucagon receptors are on hepatocytes.

B



Islet cell type	%	Hormone
α	20	Glucagon
β	80	Insulin
Δ	1–5	Somatostatin
PP	1–2	Pancreatic polypeptide

C

Regulators	*Insulin Release (β Cell)*	*Glucagon Release (α Cell)*	*Somatostatin Release (Δ Cell)*
Hormones			
Enteric (glucagon-like peptide-1 [GLP-1], gastric inhibitory polypeptide [GIP], gastrin, CCK)	↑	↓	↑
Insulin	↓	↓	—
Somatostatin (octreotide)	↓	↓	↓
Glucagon	↑	—	↑
Amylin and pancreastatin	↓	—	—
Cortisol	—	↑	—
Growth hormone	—	↑	—
GABA	—	↓	—
Catecholamines	↓ (α-Adrenergics)	↑ (β-Adrenergics)	—
Neural			
α-Adrenergic	↓↓	↓	—
β-Adrenergic	↑	↑↑	—
Vagal	↑↑	↑	—
Nutrients/Electrolytes			
Glucose	↑	↓	↑
Amino acids	↑	↑	↑
Free fatty acids	—	↓	—
Volatile fatty acids	↑ (ruminants)	—	—
Ketone bodies	↑	↓	—
K^+ (Nucleosides, Mg^{2+}, PO_4^{3-})	↑	—	—

Source: **Part A** modified from Berne RM, Levy MN. Principles of physiology. 1st ed. St. Louis: Mosby, 1990:505. **Part B** modified from Niewoehner CB. Endocrine pathophysiology. 1st ed. Madison, CT: Fence Creek, 1998:118, 119.

Multicellular organisms, unlike unicellular organisms, utilize a storage and release system for nutrients that permits intermittent rather than continual uptake. **Insulin**, secreted from specialized islet cells of the pancreas, is the primary hormone involved with energy storage, and its action is opposed by several **counter-regulatory hormones,** namely **glucagon**, **cortisol**, **epinephrine**, and **growth hormone**. In the absence of insulin, each acts on insulin-sensitive tissues including liver, muscle, and adipose tissue to release energy-rich nutrients in the form of glucose, lactate, amino acids, and/or free fatty acids (FFAs). Also, hormone-like products of islet cells (including **somatostatin**, **pancreatic polypeptide**, **pancreastatin**, and **amylin**) may play subsidiary roles in metabolic regulation (Ch. 41).

The liver plays an important role as a site for storage of glucose as glycogen, synthesis of triglyceride from fatty acids and glucose, and conversion of nutrients released from extrahepatic storage sites into forms more easily utilized by cells. Glucose can be oxidized by virtually all living cells, and is the primary source of energy for those lacking mitochondria (hence the inability of these cells to oxidize lipid). Although free fatty acids and ketone bodies may be oxidized by tissues containing mitochondria, the brain retains a strong requirement for glucose and fails to function properly when glucose supplies fall below **1 mg/kg/min**. The brain's dependency on glucose is protected by the counter-regulatory hormone system, which generally prevents glucose concentrations from falling below a critical limit, which would lead to serious brain dysfunction.

Ruminants are thought to be somewhat less dependent on the insulin–glucagon scheme than **carnivores** and **omnivores**, primarily because they are less dependent on tissue oxidation of glucose and more dependent on volatile fatty acids as a source of energy. Glucose accounts for only about **10%** of ruminant tissue carbon dioxide production, whereas in carnivores and omnivores, **20% to 50%** of carbon dioxide production is generally derived from carbohydrate. Nonetheless, blood glucose is important to all animals, and the physiologic roles of insulin and glucagon must be kept in mind when evaluating metabolic disorders.

Insulin and **glucagon** are secreted directly into portal blood; hence, the **liver** is normally the first organ subjected to the effects of these hormones before they are distributed to extrahepatic sites (**Part A**). This is quite different from the pharmacologic situation, in which insulin is injected into peripheral parts of the body and slowly finds its way to the liver in a diluted form.

Secretion of insulin and glucagon is coordinated with secretion of exocrine pancreatic enzymes, with secretion of both being stimulated by entry of nutrients into the GI tract, by GI hormones, and by the autonomic nervous system (ANS). Within the liver, these hormones, acting through second messengers that activate various hepatic enzymes, affect metabolism of ingested substrates. Islet hormones (particularly insulin) that pass through the liver with digested substrates affect disposition of these substrates by peripheral tissues. The substrates in turn feed back negatively on pancreatic islets to modulate further secretion of insulin and glucagon.

The blood glucose concentration, which can vary from as low as **37 mg/dL** in **cattle** to **160 mg/dL** in **pigeons**, is normally maintained within a fairly narrow range, with fluctuations kept to a minimum. In the **dog**, for example, a blood glucose concentration of around **100 mg/dL** is maintained, with daily fluctuations of only 10% to 30%. This minimal fluctuation could not be maintained if there were no precise control mechanisms, as the ingestion of a large carbohydrate meal (or strenuous exercise or starvation) would certainly cause greater deviations.

Islets of Langerhans

The endocrine pancreas is composed of nests of cells known as the **islets of Langerhans**, which are distributed throughout the exocrine pancreas (**Part B**). There are approximately one million islets in the porcine pancreas, many of which contain several hundred cells. The endocrine pancreas has great reserve capacity; however, like neurons, islet tissue has little regenerative capacity. Over 70% must usually be lost, however, before severe hyperglycemia develops.

There are four basic cell types within the islets, each producing a different primary secretory product. **Insulin-secreting B cells** (**β cells**) are located centrally, and normally comprise **80%** of total islet tissue. **Glucagon-secreting A cells** (**α cells**) comprise almost **20%** of islet tissue, and are located mainly in the periphery. **Somatostatin-secreting D cells** (**Δ cells**) are located between these two cell types, and are few in number. **Pancreatic polypeptide–secreting F cells** (**or PP cells**) are located mainly in islets of the posterior lobe, and receive a different blood supply.

The islets are more highly vascularized than exocrine pancreatic tissue, but comprise only **1-1.5%** of total pancreatic mass. The direction of **blood flow** within islet tissue is thought to play an important role in carrying insulin secreted from the islet central region to its peripheral zone, where it modulates and decreases glucagon release from α cells. Blood from the pancreas ultimately drains into the hepatic portal vein, where insulin and glucagon modulate liver function before insulin (particularly) moves into the systemic vasculature to affect muscle and adipose tissue. Islet tissue is also abundantly innervated by the **ANS**, where postganglionic parasympathetic and sympathetic axons either directly contact cells or terminate in interstitial spaces between them. **Parasympathetic** (**vagal**) activation increases insulin and glucagon release (insulin generally more than glucagon), while **sympathetic** activation increases glucagon but decreases insulin output (**Part C**). **Neural regulation of pancreatic islet cell hormone release**, both directly through sympathetic fibers and indirectly through stimulation of epinephrine release by adrenal medullae, plays a key role in glucose homeostasis during times of **stress** (Ch. 35).

Somatostatin and its secretagogues are discussed in Ch. 43. Somatostatin 14 and prosomatostatin (somatostatin 28) inhibit pancreatic insulin, glucagon, and PP release in a paracrine fashion. Somatostatin also inhibits gastrin release from G cells of the gastric antrum, and CCK release from duodenal I cells (Chs. 47-49). Patients with somatostatin-secreting pancreatic tumors thus develop hyperglycemia and other manifestations of diabetes that disappear when the tumor is removed. They also develop dyspepsia due to decreased gastrin and gastric HCl release, and sometimes gallstones, which are precipitated by decreased gallbladder contraction due to inhibition of CCK release.

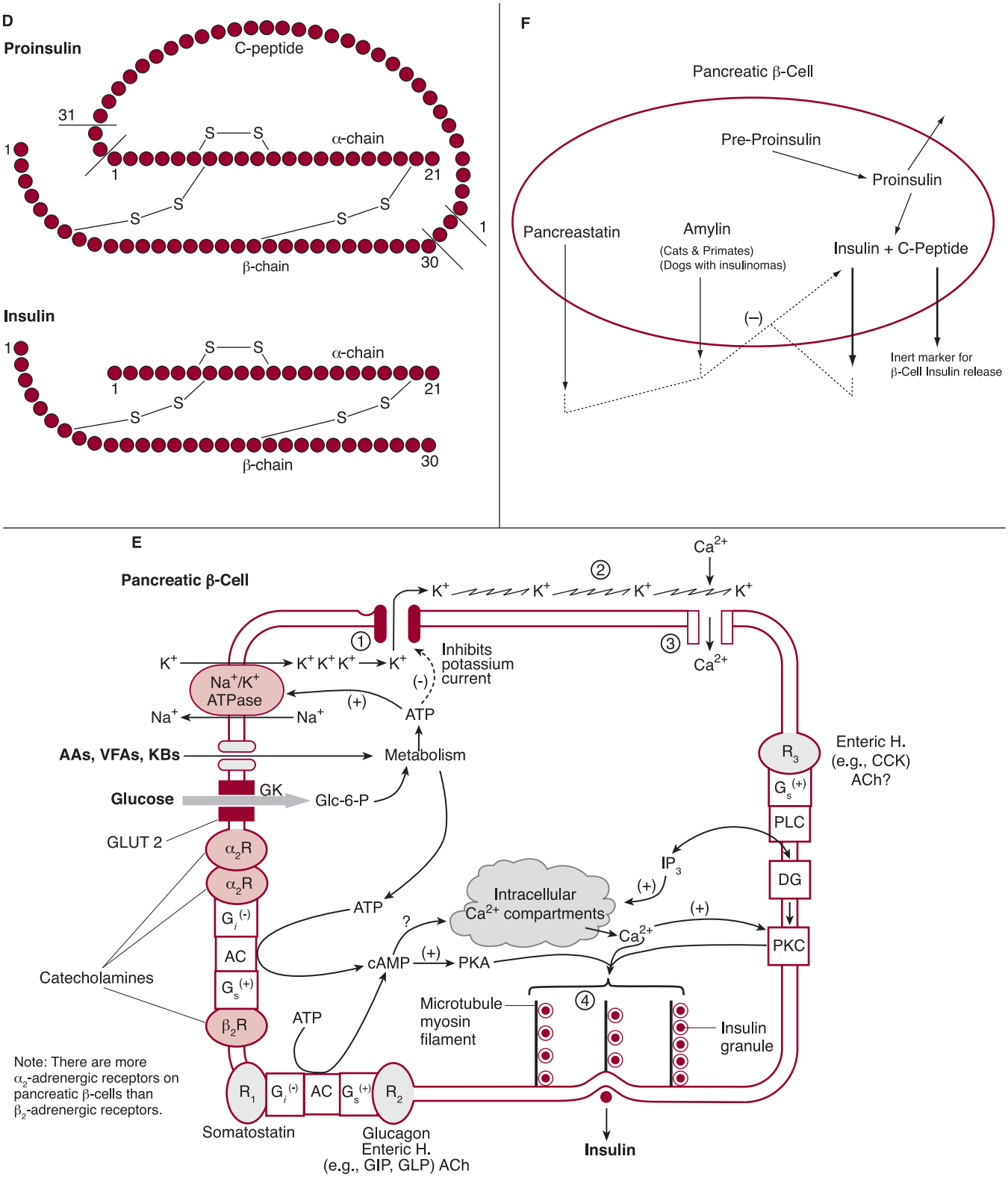
Pancreatic polypeptide contains 36 amino acid residues, and is closely related to two other polypeptides: **polypeptide YY,** which is found in the intestine and has been hypothesized to be a gastrointestinal hormone, and **neuropeptide Y,** which is found in the brain and in the ANS. Secretion of PP is largely under cholinergic and thus parasympathetic control. It is increased by digesta containing protein, and by starvation, exercise, and acute hypoglycemia, and decreased by somatostatin and IV glucose. Avian PP, unlike its mammalian counterpart, stimulates gastric acid secretion in chickens.

Insulin and Glucagon in Nonmammals

Insulin is a conserved protein, in that few amino acid substitutions have occurred across species lines. Insulin-secreting β-cells have been identified in the guts or digestive organs of many nonchordate invertebrate animals. As in mammals, insulin reduces blood sugar levels and promotes glycogen synthesis in gastropod mollusks. Furthermore, insulin-like molecules have been found in distantly related taxonomic groups including protozoans, bacteria, and fungi.

Glucagon also appears to be a well-conserved molecule, with the exception of some birds and teleosts. Chicken and turkey glucagon have substitutions at position 28 of the 29 polypeptide chain, and glucagon has 31 amino acids in salmon (yet 29 in other angler fish). Glucagon increases blood glucose in channel catfish, and glycogenolysis in isolated hepatocytes of goldfish. Compared to mammals, the avian pancreas contains about 5 to 10 times more glucagon per gram, emphasizing its probable importance to egg formation (Ch.72).

Ch41 Endocrine Pancreas: II
(Regulation of Insulin Release)



Source: Part D modified from Niewoehner CB. Endocrine pathophysiology. 1st ed. Madison, CT: Fence Creek, 1998:119.

Insulin Synthesis and Secretion

Insulin, like other peptide hormones, is synthesized as a larger molecule. This **proinsulin** includes a signal peptide that helps direct its folding and movement through the Golgi, but is removed before storage in secretory granules. The connecting peptide is cleaved from proinsulin in secretory granules, thus forming two separate molecules, **insulin** and **C-peptide**. This connecting peptide is reported to be metabolically inert, but is released in equimolar amounts with insulin, thus serving as a marker for insulin production by β cells.

Part D shows the structures of proinsulin, C-peptide, and insulin, with slashes representing cleavage sites, and the numbers representing the respective amino acid positions of cleavage and bridge sites.

Insulin is a protein consisting of two chains that are designated α and β, having 21 and 30 amino acids, respectively, and are connected by two disulfide bridges (see **Part D**). Differences in amino acid sequences between species are small; for example, cattle, sheep, horses, dogs, and whales differ only in positions 8, 9, and 10 of the α-chain. Consequently, biologic activities of insulin are not highly species specific. Porcine insulin differs from human insulin by one amino acid (Ala instead of Thr at the carboxy terminal of the β-chain), and bovine insulin differs by three amino acids (Ala instead of Thr at α-8 as well as the β-30 position, and Val instead of Ile at α-10). **Porcine** and **bovine insulin** have been used for many years to treat insulin-dependent humans. They are still used in dogs and cats, but insulin produced in bacteria via recombinant DNA technology is becoming more widely accepted in human medicine to avoid antibody formation.

Insulin-like growth factor-2 (**IGF-2**) immunoreactivity has been reported in association with pancreatic β-cells, whereas **IGF-1** immunoreactivity has been found in association with glucagon-secreting α-cells (Ch. 10). Both may influence islet cell function in a paracrine fashion.

Several nutritional, neural, paracrine, and endocrine variables govern **insulin** release from pancreatic β-cells (**Part E**). **Glucose** appears to be a primary secretagogue for insulin, particularly in omnivores, whereas several **essential amino acids** (AAs; particularly arginine, lysine, and leucine), short-chain **volatile fatty acids** (VFAs; acetate, propionate, and butyrate), and **ketone bodies** (KBs; particularly β-OH-butyrate) exert stimulatory influences as well. The relative importance of these secretagogues depends on the natural diet and nutritional status of the species in question.

Glucose enters pancreatic β-cells via **GLUT 2** transporters, which are insulin-independent (Ch. 42). Once inside β-cells, glucose is immediately phosphorylated to glucose 6-phosphate (Glc-6-P) by **glucokinase** (**GK**), an enzyme also present in hepatocytes, which has a low affinity (i.e., **high K_m of 180 mg%**) for glucose. It is thought that this enzyme (**GK**) functions as a **"glucose sensor"** in β-cells, allowing insulin release only at elevated plasma glucose concentrations. Intracellular metabolites of Glc-6-P, as well as those of the AAs, VFAs, and KBs entering pancreatic β-cells, increase the intracellular **ATP/ADP** ratio, thus making more ATP available to facilitate insulin release. Normally, K^+ efflux from β-cells (process **1** in **Part E**) polarizes the cell membrane and prevents **Ca^{2+}** entry by closing voltage-dependent Ca^{2+} channels (process **2**). However, when glucose and other secretagogues enter β-cells, **metabolic factors produced** (either intermediates of primary catabolic pathways or ATP itself) are thought to inhibit **K^+** efflux, thus depolarizing the cell and allowing **Ca^{2+}** to enter (process **3**). As intracellular **Ca^{2+}** levels rise, insulin-containing secretory vesicles attached to microtubules are expelled (process **4**).

In addition to nutrients, pancreatic β-cells are also strongly controlled by neurotransmitters, paracrine agents, extracellular K^+, and circulating hormones (**Parts C** and **E**). **Catecholamines** have a dual effect upon insulin secretion; they inhibit release via **α_2-adrenergic receptors** (**α_2Rs**), yet stimulate release via **β_2-adrenergic receptors** (**β_2Rs**; Ch. 33). The net effect is usually inhibition since α_2Rs outnumber β_2Rs on plasma membranes of pancreatic β-cells. However, if catecholamines are infused into an animal following administration of an α_2-adrenergic blocking drug, net inhibition is converted to stimulation.

Parasympathetic stimulation is facilitory to insulin release. **Acetylcholine,** acting through a muscarinic receptor (**M_4 subtype**; **R_2** in **Part E**) stimulates insulin release through activation of adenyl cyclase (AC) via G-stimulatory protein ($G_s^{(+)}$; although some investigators believe the M_4 glandular receptors may be working through inositol triphosphate (IP_3) and the Ca^{2+} messenger system (**R_3**)). Additionally, **glucagon,** acting in a paracrine fashion, and **gastric inhibitory polypeptide** (**GIP**), acting in an anticipatory fashion (see Ch. 50), are also thought to increase AC activity of β-cells. **Somatostatin,** secreted from pancreatic Δ-cells, acts through its receptor (**R_1**) to stimulate an inhibitory G-protein ($G_i^{(-)}$), which reduces AC activity and thus generation of cyclic-AMP (cAMP). Cyclic-AMP activates protein kinase A (**PKA**), which synergizes with **Ca^{2+}** to facilitate insulin release. Cyclic-AMP may also help to stimulate Ca^{2+} release from the endoplasmic reticulum. Certain enteric hormones like **CCK** (Ch. 49) act through their receptors (**R_3**) to activate phospholipase C (**PLC**), which in turn splits IP_3 off membrane-bound phospholipid leaving diacylglycerol (DG) behind. IP_3 induces Ca^{2+} release from intracellular compartments, while DG stimulates protein kinase C (**PKC**), another facilitator of insulin release (Ch. 5). Intestinal **GIP** (also called **glucose-dependent insulinotropic peptide**) is released into blood from duodenal K cells due to the presence of carbohydrates in the duodenal lumen of omnivores, and **CCK** is released from duodenal I cells due to the presence of fatty acids and amino acids in carnivores (Ch. 47).

Pancreatic β-cells are also known to synthesize and secrete **pancreastatin,** a 49-amino acid peptide, and **amylin,** a 37-amino acid peptide. These compounds are cosecreted with insulin, and may participate in autofeedback regulation since they **inhibit** insulin release (**Part F**).

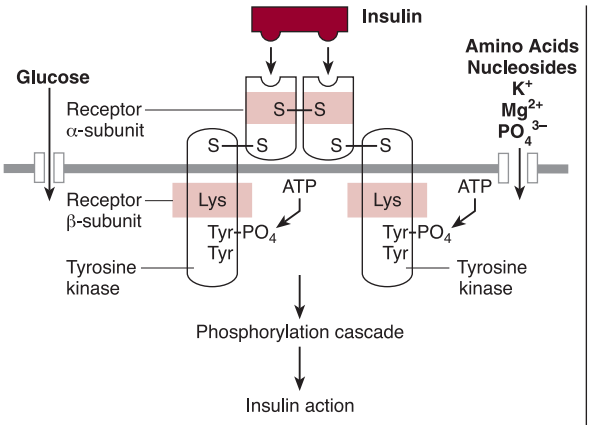
Amylin Secretion by β-Cells

Islet amylin (**or amyloid**) initially forms intracellularly in β-cells, then accumulates extracellularly after exocytosis or cell death. It is stored and co-secreted with insulin from secretory granules. Although amylin is reportedly secreted from β-cells of all species studied to date, only a few species reportedly develop amyloid deposits in association with diabetes mellitus–namely **humans**, other **primates**, and **cats**. Overproduction of amylin has been reported in dogs with insulinomas, but not in those with diabetes. Most diabetic dogs apparently experience islet cell destruction by the time of diagnosis; therefore, they are less likely to develop islet amyloidosis. Islet amyloid surrounds β-cells, isolating them from adjacent pancreatic tissue and blood capillaries, and is believed to act as a barrier to the diffusion of nutritive substances and glucose. Because amylin inhibits insulin secretion and stimulates muscle glycogenolysis, it may also play an important role in the control of insulin secretion and in the modulation of glucose homeostasis (**Part F**). Amylin is anorectic, shares a common amino acid sequence with calcitonin, and may be associated with diabetic hypertension.

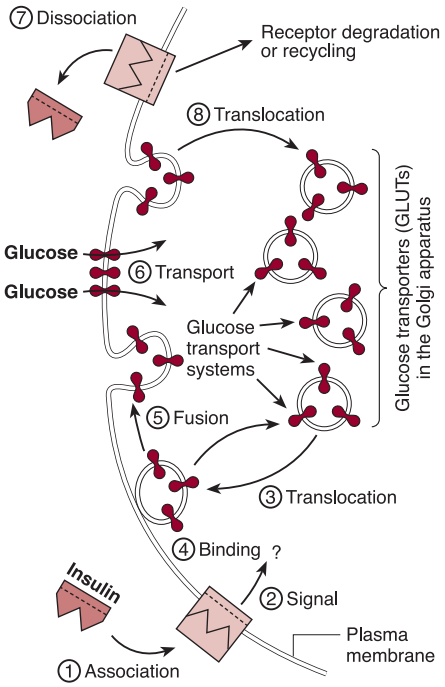
Islet amyloid polypeptide (**IAPP**), or amylin, is the primary constituent of amyloid isolated from pancreatic tissue of humans and cats with **noninsulin-dependent diabetes mellitus** (**NIDDM**). IAPP-derived amyloid fibrils can become cytotoxic, and are associated with β-cell apoptosis and insulin hyposecretion. The severity of islet amyloidosis determines in part whether diabetic cats have NIDDM, or the insulin-dependent form (**IDDM**; Ch. 44).

Ch42 Insulin
(Actions and Degradation)

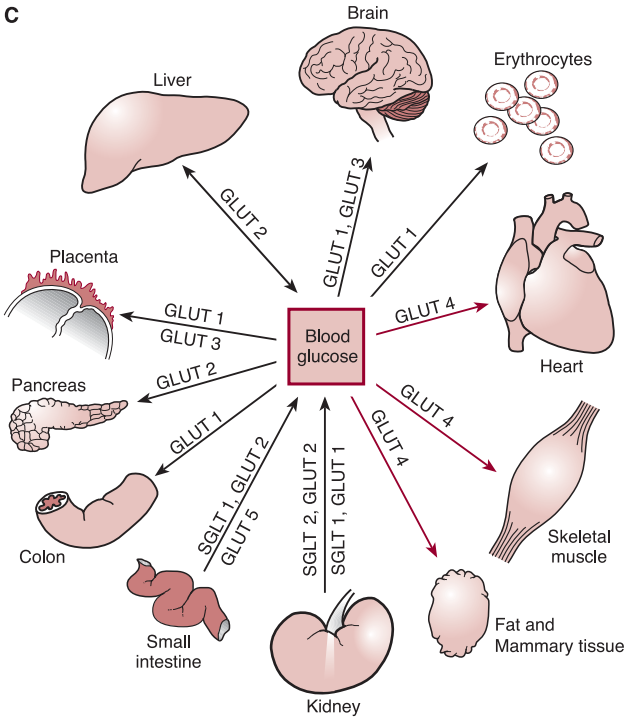
A Insulin Receptor



B GLUT 4 Transporter System



C



D Endocrine Regulation of Fuel Metabolism

Tissue	*Insulin*	*Glucagon*	*Catechol-amines*	*Cortisol*	*Growth hormone*
Liver					
Fuel storage					
Glycogenesis	↑	↓	↓	↑	—
Lipogenesis	↑	↓	—	—	—
Protein synthesis	↑	↓	↓	↓	↑
Fuel breakdown					
Glycogenolysis	↓	↑	↑	—	—
Gluconeogenesis	↓	↑	↑	↑	↑
β-Oxidation/ketogenesis	↓	↑	↑	↑	↑
Proteolysis	↓	—	—	—	—
Muscle					
Fuel storage					
Glucose uptake (glycogenesis)	↑	—	↓	↓	↓
Amino acid uptake (protein synthesis)	↑	—	—	↓	—
Fuel breakdown					
Glycogenolysis	↓	—	↑	—	—
Proteolysis	↓	—	—	↑	—
Adipose					
Fuel storage					
Lipoprotein lipolysis	↑	—	—	—	—
Glucose uptake	↑	—	—	—	—
Fatty acid esterification	↑	—	—	—	—
Fuel breakdown					
Lipolysis of stored triglyceride	↓	—	↑	↑	↑

Source: **Part A** modified from Niewoehner CB. Endocrine pathophysiology. 1st ed. Madison, CT: Fence Creek, 1998:121. **Part B** modified from Kamieli E, et al. Insulin-stimulated translocation of glucose transport systems in the isolated rat adipose cell. J Biol Chem 1981;256:4772.

Mechanism of Insulin Action

The **glycoprotein receptors for insulin** are found on the surface of target cells in **liver**, **muscle**, **fat** and **mammary tissue**; that is, on classic insulin sensitive tissues responsible for energy homeostasis. In addition, insulin can mediate anabolic effects in other nonclassic target tissues, such as the ovary, via cross-reactivity with **IGF-1** receptors. The **insulin receptor** is a tetrameric structure consisting of two α-subunits and two β-subunits held together by disulfide bonds. The α-subunits are extracellular in location and contain the insulin-binding site, which is rich in cysteine residues. The β-subunits span the plasma membrane and anchor the receptor to the cell, and they contain specific **tyrosine** residues that are **autophosphorylated** by the receptor when insulin binds to the α-subunit. This autophosphorylation turns the receptor into a **tyrosine kinase**, capable of phosphorylating other proteins, thereby mediating the effects of insulin on cellular metabolism (**Part A**). Numerous target cell enzymes are ultimately activated or inactivated via the **MAP K cascade** (Ch. 4), resulting in a shift in glucose metabolism, for example, toward glycogen and pyruvate formation. After the **insulin receptors** have been stimulated, they are ultimately taken into target cells by receptor-mediated endocytosis, where they enter lysosomes to be broken down or recycled, making their **t½** about **7 hrs**.

In insulin-sensitive tissues, insulin facilitates glucose entry into cells by increasing the number of specific **glucose transporters** (**GLUT 4s**) in the cell membrane. These glycoprotein transporters are recruited from the Golgi fraction to the plasma membrane, a process which is both temperature and energy dependent. **Exercise**, which is associated with low insulin levels, encourages **GLUT 4 recruitment** into plasma membranes of exercising muscle cells without the assistance of insulin. Seven different GLUTs have been identified, but only **GLUT 4** is **insulin-sensitive**. The 6 non-insulin-sensitive GLUTs appear to remain in their cell membranes.

The **GLUT 4 translocation process** is summarized in **Part B.** Insulin binds with its plasma membrane receptor (process **1** in **Part B**), which in turn brings about an intracellular signal (process **2**; mechanism unknown) that causes the translocation of **GLUT 4** (process **3**) from an inactive pool in the Golgi fraction. These transporters are moved and bound (process **4**) to active sites on the plasma membrane, where fusion (process **5**) to this membrane brings about **facilitated diffusion of glucose** into the cell (process **6**). Following removal of insulin from its binding sites on plasma membrane receptors (process **7**), the **GLUT 4** transporters are translocated back to the Golgi (process **8**).

The **hepatic cell** represents a notable exception to this scheme. Insulin does not promote facilitated diffusion of glucose into hepatocytes, but rather indirectly enhances net inward flux by converting intracellular glucose to glucose-6-phosphate through activation of **glucokinase**. Rapid phosphorylation keeps the free glucose concentration low in hepatocytes, thus allowing entry of the nonpolar glucose molecule by simple diffusion down its concentration gradient. Insulin also promotes entry of **amino acids** into cells, particularly in muscle, and enhances uptake of **K^+**, **Mg^{2+}**, **nucleosides**, and **inorganic phosphate**—actions independent of insulin's effects on glucose entry.

Two Na^+-dependent glucose transporter (**SGLT**) isoforms, and seven **GLUT** transporter isoforms have been identified in various tissues (**Part C**). The **SGLT 1** and **2** transporters are found in luminal (apical) membranes of proximal renal tubular epithelial cells, while both the **SGLT 1** and **GLUT 5** transporters are found in luminal (apical) membranes of mucosal cells in the small intestine. The **SGLT 1** transporter requires 2 Na^+/glucose molecule, while the **SGLT 2** transporter requires only 1, and both are **insulin-independent**. The **SGLT 1** and/or **2** transporters are responsible for secondary active transport (i.e., symport) of glucose (and galactose) out of the intestine and renal tubular filtrate, against their concentration gradients, and therefore are associated with aerobic processes. The **GLUT 5** transporter is associated with lesser amounts of anaerobic glucose and fructose (facilitated) diffusion into duodenal and jejunal mucosal cells. Three of the remaining 5 transporters are also **insulin-independent: GLUT 1** is responsible for glucose reabsorption across the basolateral membrane of proximal renal tubular epithelial cells (S-3 segment), and also glucose uptake into erythrocytes, the colon, and across the placenta and blood-brain-barrier, etc.; **GLUT 2** is found in pancreatic insulin-secreting β-cells. It also functions in transporting glucose out of intestinal and proximal renal tubular epithelial cells (S-2 segment), as well as into and out of the liver; **GLUT 3** is responsible for basal glucose uptake into neurons, the placenta and other organs; and **GLUT 7** (not shown) is a newly discovered intracellular Glc-6-P transporter into the endoplasmic reticulum. **GLUT 3** and **1** may also be involved with the transport of **dehydroascorbate** (**DHC**) out of neurons and into astrocytes (glial cells) of the brain.

Glycogen synthase in liver is activated by a specific **phosphatase** that removes key phosphate residues previously added by cAMP-dependent protein kinase A (**PKA**) in response to hormones such as glucagon and epinephrine. Some actions of insulin are apparently mediated through a phosphoinositol (IP_3) kinase, while others involve the MAP K cascade. These multiple downstream events in the action of insulin are referred to collectively as **postreceptor insulin actions.**

Lipoprotein lipase (**LPL**), anchored to the capillary endothelium by proteoglycan chains of heparan sulfate, has been found in the heart, adipose tissue, spleen, lungs, renal medulla, aorta, diaphragm, lactating mammary gland, and neonatal liver. This important enzyme, needed for the clearance of triglyceride from circulating chylomicrons (**CMs**) and very-low-density lipoprotein (**VLDL**), hydrolyzes the ester bonds of triglyceride, and its activity is greatly increased by **insulin**. Many of the macrovascular, atherosclerotic effects of **diabetes mellitus** are due to the lack of LPL stimulation by insulin.

Major Effects of Insulin on Liver, Muscle, and Fat Tissue

Insulin promotes fuel storage in the liver by stimulating glycogen synthesis and storage (**Part D**). It inhibits gluconeogenesis and glycogenolysis, thereby reducing hepatic export of glucose to the circulation, and promotes formation of precursors for fatty acid synthesis by stimulating glycolysis. Moreover, insulin stimulates hepatic lipogenesis, leading to increased synthesis and secretion of VLDL, which delivers triglyceride from the liver to fat tissue for storage. Insulin also inhibits hepatic fatty acid β-oxidation and the production of ketone bodies, which are water-soluble alternative fuels produced only in the liver that can be used by the brain and fetus when glucose supplies are low (e.g., starvation).

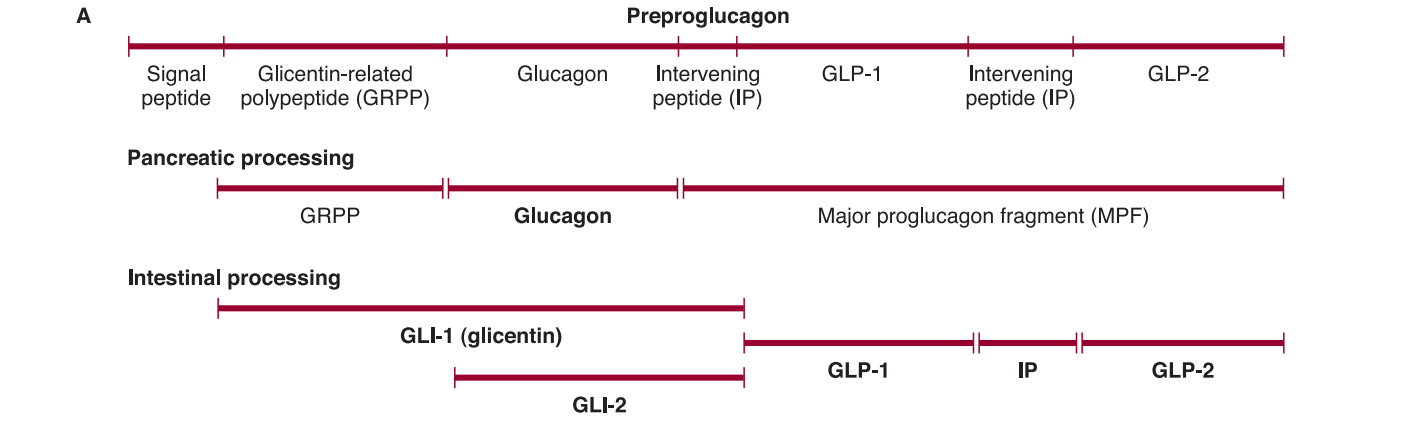
Although hepatic uptake of glucose is important, uptake by **muscle** generally accounts for the majority of insulin-stimulated glucose disposal (see **Part D**). However, insulin's effect on **K^+** entry into muscle cells is greater than its effect on glucose entry (Ch. 46). Insulin promotes muscle glucose storage by stimulating glycogen synthesis and inhibiting glycogen catabolism. It also promotes muscle protein synthesis.

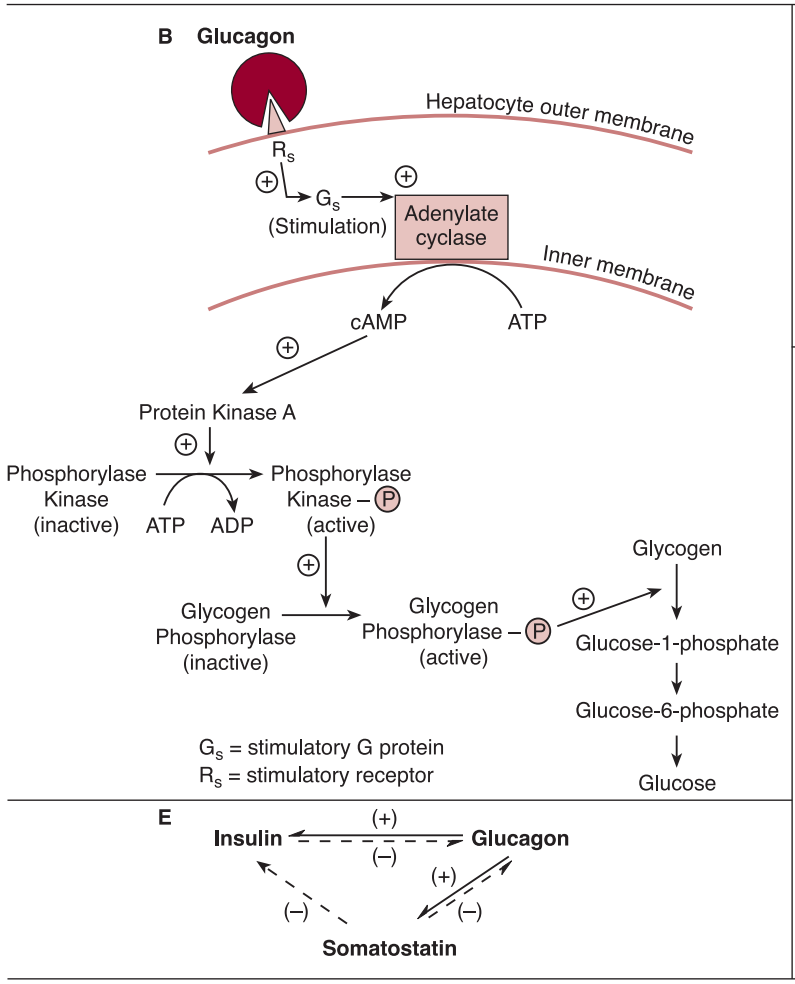
Insulin stimulates fat storage by stimulating **LPL** activity (see **Part D**). The FFAs cleaved from the triglyceride contained in circulating **CMs** and **VLDL** enter adipocytes, where they become available for triglyceride resynthesis. Insulin-stimulated **glucose uptake** by adipocytes via upregulation of the **GLUT 4** transporter increases intracellular levels of **glycerol-3-phosphate**, the activated triglyceride backbone needed for fat storage. Insulin also **inhibits lipolysis** by decreasing the activity of **hormone-sensitive lipase**, the enzyme in adipocytes that hydrolyzes fatty acids from stored triglyceride. Together, these effects of insulin result in increased fat storage.

Insulin Degradation

Insulin has a normal circulatory half-life of about **3-5 min**, and is catabolized by the liver, kidneys, and placenta. Normally about **50%** of insulin is degraded on its first pass through the liver. In contrast, both **C-peptide** and **proinsulin** are catabolized only by the kidneys, and therefore have half-lives three to four times longer. Insulin degradative activity (i.e., insulinase activity) involves an **insulin-specific protease** found in extrahepatic tissues, and a **glutathione-insulin transhydrogenase** present in the liver.

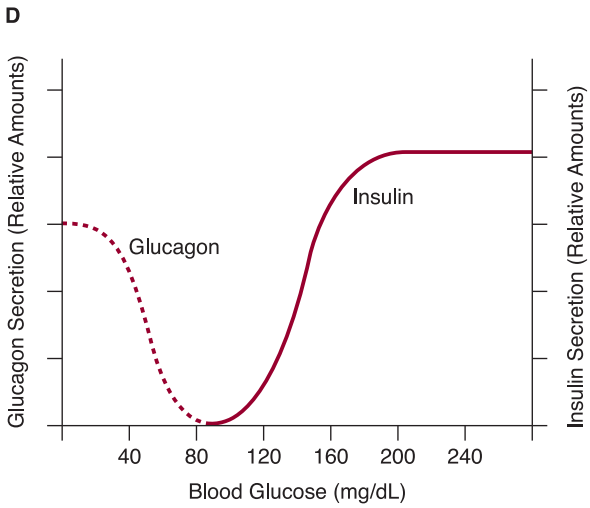
Ch43 Glucagon
(Biosynthesis and Metabolic Actions)





C

Diet	Hormone Levels
1. ↑Carbohydrate, ↓Protein	→ ↑Insulin, ↓Glucagon
2. ↓Carbohydrate, ↑Protein	→ ↑Insulin, ↑Glucagon
A. Glucagon → Liver (Fed/Starved)	→ Gluconeogenesis
B. Insulin → Extrahepatic tissues (Fed)	→ Anabolism



Source: **Part A** modified from McPhee SJ, et al. Pathophysiology of disease. 2nd ed. Appleton and Lange, 1997:428. **Part D** modified from Marliss EB et al. Normalization of glycemia in diabetics during meals with insulin and glucagon delivery by the artificial pancreas. Diabetes 1977;26:663. **Part E** modified from Ganong WF. Review of medical physiology. 18th ed. Stamford, CT: Appleton & Lange, 1997:323.

Glucagon Synthesis and Metabolism

Glucagon, a 29-amino-acid peptide, is derived from a much larger precursor protein, **preproglucagon.** This precursor molecule is proteolytically processed to form several biologically active peptides (**Part A**). Preproglucagon and the smaller proglucagon are synthesized in the pancreas, GI tract, and brain. However, only pancreatic **α-cells** have been shown to cleave **glucagon** from proglucagon. Other biologically active peptides derived from preproglucagon, such as glucagon-like peptides (**GLP-1** and **GLP-2**) and glucagon-like immunoreactivity peptides (**GLI-1** and **GLI-2**), are primarily synthesized by the GI tract in response to a meal. Of these, a metabolite of **GLP-1** is reported to be a more potent stimulator of **insulin release** than glucagon, and it may be an important enteric hormone associated with postprandial insulin release (Ch. 42, **Part D**). Glucagon-like peptide-1 decreases glucagon secretion from pancreatic α-cells, and **GLP-2** binds glucagon receptors, though with only 10% potency. The physiologic roles of these glucagon-related peptides are under investigation.

Glucogenic amino acids, β-adrenergics, cortisol, and exercise stimulate glucagon release, while glucose, insulin, somatostatin, secretin, FFAs and KB^-s inhibit it. In **birds** and **lizards**, circulating FFAs stimulate glucagon release, where this hormone may be the primary pancreatic regulator of CHO metabolism, with insulin playing only a minor role (Ch. 72).

Degradation of Glucagon

The circulatory half-life of glucagon is similar to that of insulin—about **three to six minutes**. Like insulin, glucagon is metabolized in the liver and kidney; however, the liver is thought to account for only **25%** of glucagon clearance (compared to **50%** for insulin—Ch. 42).

Mechanism of Action

Glucagon's major target organ is the liver, where it binds to cell surface receptors **(Part B).** Interaction of the receptor with a stimulatory G ($\mathbf{G_s}$) protein then activates adenylate cyclase. Cyclic-AMP (**cAMP**), generated by adenylate cyclase, activates protein kinase A (**PKA**), which then phosphorylates various enzymes (Ch. 4).

Effects of Glucagon on the Liver

Glucagon counters the effects of insulin on the liver by promoting output rather than input of glucose. This occurs through stimulation of both **glycogenolysis** and **gluconeogenesis**. Glucagon stimulates β-oxidation of fatty acids and thus promotes ketone body formation, providing alternative fuel for the brain when glucose supplies are low. Glucagon also stimulates hepatic uptake of amino acids, which are then used as gluconeogenic substrates (Ch. 42, **Part D**).

The plasma glucagon concentration reaches a peak on the third day of starvation, then decreases thereafter as FFAs and ketone bodies become major sources of energy.

Effects of Glucagon on Extrahepatic Tissue

Because glucagon lowers serum triglyceride and FFA concentrations while stimulating lipolysis, it may increase muscle uptake and catabolism of FFAs; however, it functions mainly at the level of the **liver**. Few effects of glucagon on extrahepatic tissues have been described in mammals.

The Insulin/Glucagon Ratio

The ratio of insulin to glucagon plays an important role in controlling the level of cAMP in liver cells and, thereby, the rate of glucose synthesis or biotransformation. In addition, in felines ingesting meals containing fat and protein (but little or no carbohydrate), and in ruminant animals in which little or no glucose is absorbed from the GI tract, insulin is still released at a rate greater than basal (**Part C**). Increasing concentrations of **amino acids** initiate this insulin release, while the lack of incoming glucose and the presence of glucogenic amino acids maintain glucagon release. Therefore, extrahepatic tissues receive the **"fed"** signal to take up circulating fuels (such as amino acids and fatty acids), yet the liver remains in the **fed/starvation** mode (i.e., the gluconeogenic mode) in order to maintain the blood glucose concentration. Thus, whether the liver is gluconeogenic or glycogenic-glycolytic is a function of the ratio of insulin to glucagon. In the absence of amino acid, if sufficient carbohydrate is absorbed from the GI tract to displace the need for hepatic glucose production, then the rise in glucose concentration is sufficient to increase β-cell insulin release and thus suppress α-cells from releasing glucagon. As a result, hepatic glucose production is suppressed (**Parts C** and **D**). In addition, this slight increase in the blood glucose concentration markedly synergizes β-cells to produce even more insulin as a response to increased amino acids, so that the insulin/glucagon ratio increases even more.

Insulin/glucagon molar ratios fluctuate markedly under different metabolic conditions. This ratio may be about **2.3/1** on a balanced diet, and with amino acid infusion the concentration of each will increase, but the ratio may not exceed **3/1**. Following 3 days of starvation,the ratio may fall to **0.5/1** or lower, but amino acid infusion can increase it again to ≈ **3/1**. Conversely, the ratio may be as high as **25/1** in animals receiving constant glucose infusion, and rise to **170/1** following protein ingestion during that infusion. This occurs because insulin secretion rises sharply, while the usual glucagon response to a protein meal is abolished. When energy is needed during starvation, the insulin/glucagon ratio is low, favoring glycogen breakdown and gluconeogenesis; when the need for energy mobilization is low, the ratio is increased, favoring deposition of glycogen, protein, and fat. **Insulin** is **glycogenic**, **antigluconeogenic**, **antilipolytic** and **antiketogenic** in its actions, favoring energy storage. **Glucagon**, which exhibits most of its actions in the liver, is **glycogenolytic**, **gluconeogenic**, and **ketogenic**.

Somatostatin

Somatostatin, a 14-amino-acid peptide, is also formed by the proteolytic cleavage of a preprohormone that is synthesized in the pancreas, GI tract, and brain (as well as other tissues). Unlike the situation for glucagon, however, all of these tissues apparently retain the ability to cleave the preprohormone and prohormone to form somatostatin.

Somatostatin was originally discovered in the hypothalamus as a factor responsible for the **inhibition of growth hormone (somatotropin) release**—hence its name. Only later was it appreciated that Δ cells of the pancreas also produce and secrete somatostatin. Unlike most prohormones, however, prosomatostatin, a 28-amino-acid peptide, is more potent than its derivative. **Octreotide,** a synthetic 8-amino-acid analogue of somatostatin used clinically, is also more potent than somatostatin. The half-life of somatostatin has been reported as less than **three minutes**.

Several of the same secretagogues that stimulate insulin release also stimulate somatostatin release. These include glucose, amino acids, certain enteric hormones, and glucagon (Chs. 40 and 41). Somatostatin acts as a paracrine agent to inhibit both insulin and glucagon release and, therefore, to modulate their output. Glucagon, however, stimulates insulin release in a paracrine fashion, while insulin inhibits glucagon release (**Part E**).

Summary

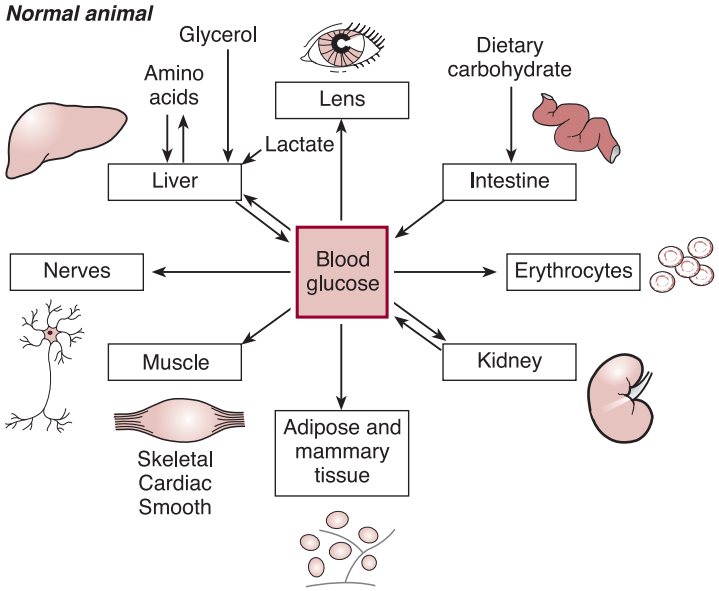
Insulin is anabolic, increasing the storage of glucose, fatty acids, and amino acids, while **glucagon is catabolic**. These two major pancreatic hormones are reciprocal in their overall action, and they are also reciprocally secreted in most circumstances. Insulin excess causes hypoglycemia, which leads to convulsions and coma, while insulin deficiency (either absolute or relative) causes diabetes mellitus, a complex and debilitating disease that can be fatal if left untreated. Glucagon deficiency causes hypoglycemia, and glucagon excess can worsen the hyperglycemia of diabetes.

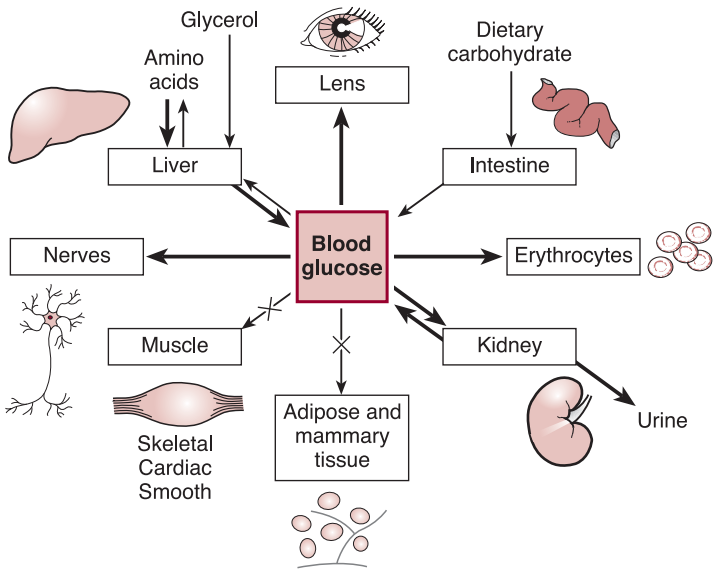
Several secretagogues that stimulate insulin release also stimulate **somatostatin** release. Somatostatin modulates insulin and glucagon release in a paracrine fashion, and it also reduces gastrin release from the stomach, and CCK release from the duodenum (Chs. 48 and 49). Patients with somatostatin-secreting tumors develop hyperglycemia and other symptoms of diabetes mellitus, and they may also develop dyspepsia and gallstones.

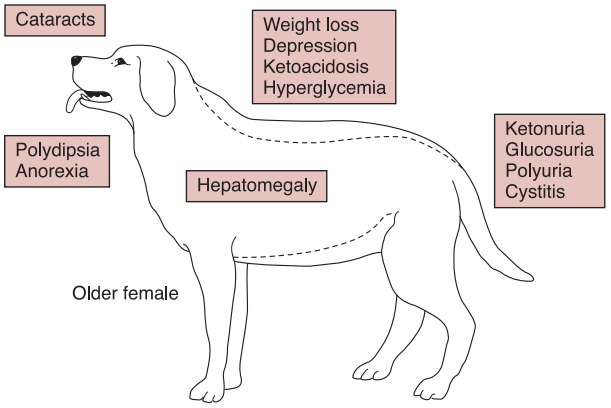
Other counter-regulatory hormones, such as **cortisol**, **epinephrine**, and **growth hormone**, play important roles as well in the regulation of carbohydrate, fat, and protein metabolism.

Ch44 Diabetes Mellitus
(The "Pissing Evil")

B







A

Heterogenic Causes of Diabetes Mellitus in Animals

Genetic predisposition
Pancreatic injury
- Trauma
- Neoplasia
- Infection
- Autoantibodies
- Inflammation
- Drugs

Hormone-induced β-cell exhaustion
- Growth hormone
- Thyroid hormones
- Cortisol
- Catecholamines
- Progestins

Target tissue insensitivity
- Decreased number of insulin receptors
- Defective insulin receptors
- Defect in postreceptor effects

Dyshormonogenesis of insulin

Tissues in Which Insulin Facilitates Glucose Uptake

Skeletal muscle
Cardiac muscle
Smooth muscle
Adipose
Mammary
(GLUT 4)
Liver (Activate GK)

Tissues in Which Insulin Does Not Facilitate Glucose Uptake

Nerves (except in part of the hypothalamus)
Kidney tubules and glomeruli
Intestinal mucosa
Erythrocytes (RBCs)
Lens
Blood–brain barrier
Pancreatic β-cell

The term **diabetes,** meaning **syphon** or **running through,** was used by the Greeks over 200 years ago to describe the striking urinary volume excreted by certain subjects. **Mellitus,** meaning **sweet,** distinguishes this urine from the large quantities of **insipid** urine produced by patients suffering from **ADH deficiency** (Ch. 13).

Sweet urine was first described in the 17th century, but in England the illness had long been called the **"pissing evil."** Because of marked weight loss despite a large food intake, the body's substance was believed to be dissolving and pouring out through the urinary tract (a view that is not far from the truth). In 1889, experimental diabetes mellitus (DM) was produced in dogs by surgical removal of the pancreas, and 32 years later **Banting** and **Best** discovered insulin.

Diabetes mellitus is characterized by a persistent hyperglycemia that most typically results from an absolute or relative deficiency of **insulin**. It is a commonly recognized endocrine disease in cats (with an approximate incidence of 1 in 800), and the second most common endocrine disorder in dogs (approximately 1 in 200). Diabetes mellitus is rare in birds, horses, and other domestic animals.

Canine DM most frequently occurs in small breeds (e.g., the dachshund and poodle), but all breeds are affected. German shepherds, cocker spaniels, collies, and boxers, however, appear to have a significantly decreased risk. The age of onset is usually 8 to 9 years, and affected intact and neutered female dogs outnumber males by two-to fourfold. The diabetogenic effect of **progesterone** or progesterone-induced hypersecretion of **growth hormone** during diestrus, has been cited as a potential cause of DM in sexually intact female dogs (Ch. 11).

In contrast, **feline DM** is apparently more common in males than females. Most affected cats are sexually altered (as is the usual feline hospital population). Domestic shorthairs are the most frequently affected breed, but again the incidence may not exceed that of the general hospital population. The usual age of onset is more than 9 years.

Experimental Diabetes and Other Domestic Animals

Early diabetic studies performed on pancreatectomized animals were confounded by the difficulty of removing the entire pancreas, and then dealing with the lack of both its exocrine and endocrine activities. It is possible to induce experimental diabetes in animals today with agents such as **alloxan** and **streptozotocin** that destroy pancreatic β-cells without loss of exocrine pancreatic activities and the complications of surgery.

Pancreatic islet β-cell tumors are common in **ferrets**, but DM is uncommon (Ch. 72). DM is recognized in **birds**, but the clinical condition is controversial due to unknown pathogenic variables, and the fact that normal blood glucose levels range from 150-300% above those of mammals. Most avian birds tolerate pancreatectomy well, except the owl, which is carnivorous and suffers severely from this surgical insult. Pancreatectomy in some birds (e.g., ducks) is followed by a lowering of the blood glucose concentration. This is undoubtedly associated with the fact that the avian pancreas contains about ten times as much extractable **glucagon** as the mammalian pancreas. Little work has been done on metabolic disturbances following pancreatectomy or naturally occurring DM in domestic animals (except in dogs and cats).

Excess glucagon must be considered a possible cause of DM. In humans, some diabetic patients have elevated levels of glucagon, which could come from pancreatic α-cells or an enteric source. The ensuing hyperglycemia may lead to β-cell exhaustion. In this regard, β-cells of most species are so susceptible to injected growth hormone (GH) that one wonders if overproduction of endogenous GH could also be a cause of DM.

Cattle that have recovered from viral foot-and-mouth disease have a high incidence of DM, and the incidence of DM in humans (2% to 10% of the American population) is thought to be higher than that in other domestic animal species.

Causes and Classifications of Diabetes

Although not enough research has been conducted to allow classification of all conditions that may contribute to the onset of DM in domestic animals, it is thought that the multiplicity of biochemical manifestations observed stems from five general heterogenic causes: **1**) genetic predisposition, **2**) pancreatic injury, **3**) hormone-induced β-cell exhaustion, **4**) target tissue insensitivity, and **5**) dyshormonogenesis of insulin (**Part A**).

Diabetes arises through two etiologically distinct routes. **Type I diabetes** in humans most commonly results from immunologically mediated destruction of pancreatic β cells, and usually requires replacement therapy with insulin. Type I diabetes is also referred to as **insulin-dependent diabetes mellitus** (**IDDM**), or **juvenile diabetes** in humans. **Type II** diabetes appears to result from a combination of alterations in insulin sensitivity and insulin secretion. It can be treated with dietary therapy or oral hypoglycemic agents, and infrequently requires exogenous insulin. Type II diabetes in humans is therefore referred to as **non-insulin-dependent diabetes mellitus (NIDDM)**, or **adult-onset DM.**

Dogs with DM (mostly middle-aged to old dogs) have been classified in two major groups:

1. **A minority of cases that are hyperinsulinemic** have elevated growth hormone levels and mild clinical signs (mainly ketotic).
2. **A majority of cases that are hypoinsulinemic.** This group has been further divided into two subgroups:
   a. Those that are **mildly ketotic.**
   b. Those that are **severely ketotic.**

Dogs in **group 1** are similar to type II human diabetics, while dogs in **group 2** are similar to type I human diabetics.

NIDDM occurs most frequently in **cats**, but no genetic predisposition has been demonstrated (as it has in humans). Obesity appears to be a significant risk factor. **Amyloidosis** of pancreatic islets is the most striking feline pancreatic lesion, which usually begins before the onset of diabetic symptoms (Ch. 41).

Glucose Intolerance in Diabetes

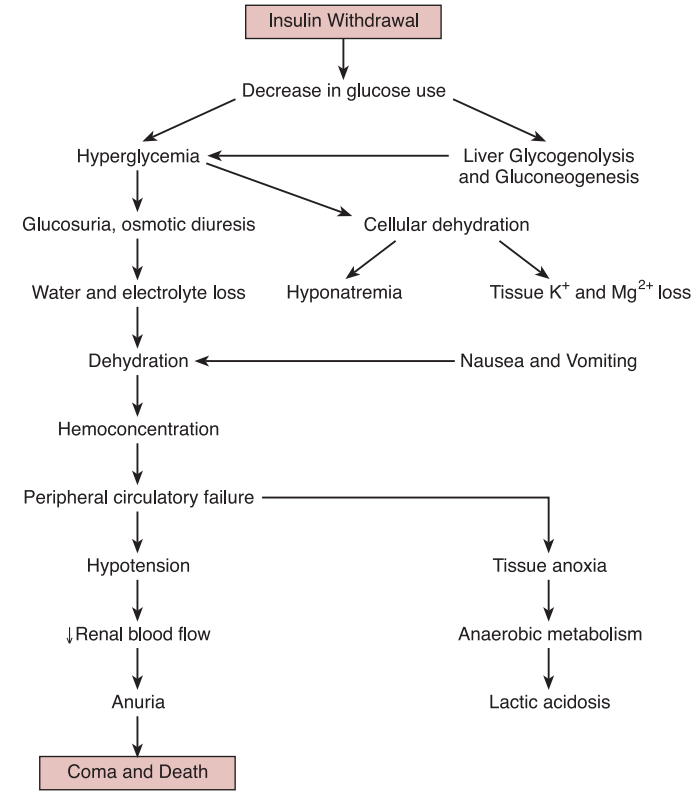
Glucose intolerance in diabetic animals is in part due to **reduced entry of glucose into insulin-sensitive tissues** (**Part B**). In the absence of insulin, the entry of glucose into skeletal, smooth, and cardiac muscle as well as the liver, adipose and mammary tissue is decreased. Intestinal absorption of glucose remains unaffected because mucosal cells are insulin insensitive, and proximal tubular reabsorption of glucose from the renal filtrate remains unaffected. However, as the renal threshold for reabsorption is reached (at a concentration of about 180 mg/dL), glucose begins to appear in urine. As the plasma glucose concentration rises, glucose also cannot be kept out of insulin-insensitive tissues. Therefore, intracellular glucose concentrations rise in glomerular renal tubular epithelial cells, erythrocytes, nerve cells (including those of the brain), and the lens. All are affected pathophysiologically.

The **liver** and **kidneys**, which are gluconeogenic organs, continue producing glucose from glycerol and incoming glucogenic amino acids under the effects of the **diabetogenic hormones** (**glucagon**, **epinephrine**, **growth hormone**, and **cortisol**), particularly in times of stress.

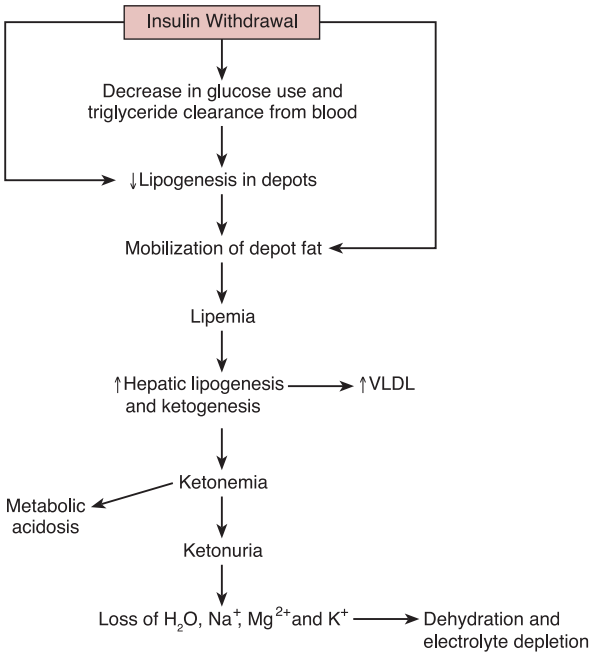
Perhaps the best way to appreciate what insulin means to the economy of the body is to consider in detail the **biochemical and physiologic manifestations of acute insulin withdrawal**. When insulin is withdrawn acutely from a severely diabetic patient, a remarkable sequence of intricately interconnected events begins and, if there is no intervention, the inevitable outcome will be **coma** and **death**. These events involve not merely carbohydrate metabolism, but fat and protein metabolism, and electrolyte and fluid balance as well. The repercussions of insulin withdrawal appear in the CNS, as well as in the respiratory, cardiovascular, renal, and gastrointestinal systems. Insulin withdrawal, the focus of the next two chapters, permits the unopposed action of the counter-regulatory diabetogenic hormones (which, unfortunately, elevate the plasma glucose concentration).

Ch45 Acute Insulin Withdrawal: I
(Carbohydrate and Lipid Metabolism)

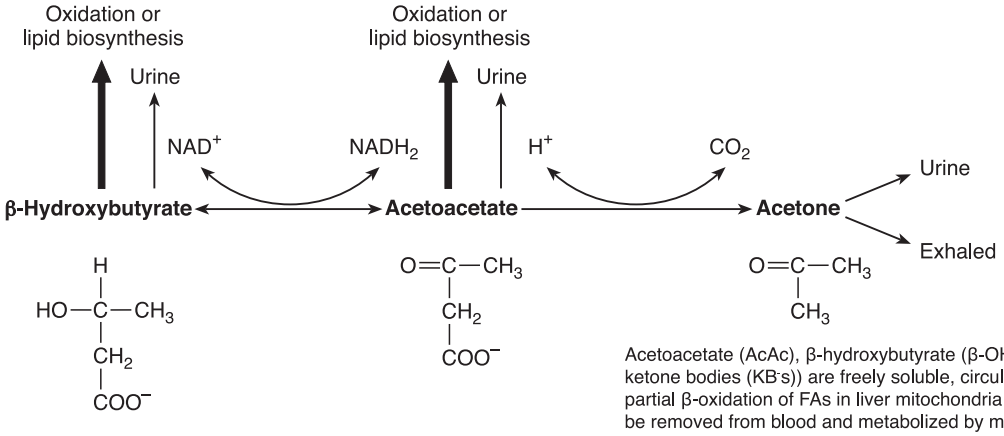
A Effects of acute insulin withdrawal on carbohydrate metabolism



B Effects of acute insulin withdrawal on lipid metabolism



C Hepatic ketone body formation and mitochondrial NAD^+, $NADH_2$, and H^+ concentrations



Mild ketonemia	**5**	**:**	**1**
Diabetic coma	**15**	**:**	**1**

Acetoacetate (AcAc), β-hydroxybutyrate (β-OH-butyrate) and acetone (the ketone bodies (KB^-s)) are freely soluble, circulating lipids that arise from the partial β-oxidation of FAs in liver mitochondria. AcAc and β-OH-butyrate can be removed from blood and metabolized by most aerobic tissues (e.g., muscle, brain, kidney, mammary gland, small intestine and fetal liver), but they cannot be oxidized by the adult liver from which they are produced. These small particles become important lipid fuel during starvation, and important substrates for both fetal and neonatal complex lipids biosynthesis. However, KB^-s are strong acids, and their accumulation in blood of the diabetic animal leads to a progressive academia, which can itself become debilitating, if not fatal.

Source: **Parts A** and **B** modified from Tepperman J, Tepperman HM. Metabolic and endocrine physiology. 5th ed. St. Louis: Mosby Year-Book, 1987:282–284.

The effects of **insulin deprivation** in the diabetic patient form a ghastly caricature of the normal adaptation to **starvation**. Many of the same types of changes occur, but with inappropriately violent consequences. Moreover, it is often difficult to understand interrelationships among the many biochemical events that are essentially occurring simultaneously. For this reason, an arbitrary division of this topic into three segments has been made in this and the following chapter, followed by a discussion of how all three processes are interrelated.

Carbohydrate Metabolism

The primary event that causes a disruption in normal carbohydrate metabolism is **relative insulin withdrawal** (**Part A**). In diabetic animals, this may not necessarily mean an absolute decrease in the amount of insulin the patient receives, but rather a sudden and unexpected increase in the insulin requirement. Diabetic ketoacidosis may be precipitated by an infection, physical trauma, or emotional distress, all of which tend to increase the need for insulin, or it may be initiated by the omission of insulin. Sometimes there is vomiting, which is then followed by inadequate food and water assimilation.

With insulin withdrawal, there is a decrease in glucose utilization by peripheral tissues, mainly muscle and adipose tissue. This contributes to a developing **hyperglycemia**, and liver glycogenolysis also contributes to this condition. Hepatic gluconeogenesis from glycerol, lactate, and glucogenic amino acids also adds to the increase in blood sugar.

The plasma **Na^+** concentration is generally low, owing to the hyperglycemic osmotic effect that draws intracellular water into extracellular spaces. The Na^+ concentration is thought to fall approximately 1.6 mmol/L for every 100 mg/dL increase in plasma glucose.

Total body stores of **K^+** are reduced by diuresis and vomiting. However, acidosis, insulinopenia, and cellular dehydration due to hyperglycemia cause a shift in both **K^+** and **Mg^{2+}** out of cells, thus maintaining normal or even elevated plasma concentrations of these electrolytes. With administration of insulin and correction of the acidosis, the plasma K^+ concentration falls as K^+ moves back into cells (along with Mg^{2+} and PO_4^{3-}). Without treatment the **plasma K^+ concentration can fall to dangerously low levels**, leading to potentially lethal cardiac arrhythmias. Therefore, K^+ supplementation is routinely given with insulin in the treatment of diabetic ketoacidosis.

When the plasma glucose concentration rises above the renal threshold for glucose (i.e., 180 mg/dL), **glucosuria** appears and an **osmotic diuresis** is instituted. This is the basis of the polyuria of diabetes, the first symptom of the disease to be recognized in antiquity. Loss of water in urine, especially combined with the fact that intake by ingestion has usually ceased, leads to dehydration and hemoconcentration. This, in turn, leads to peripheral circulatory failure because of the marked reduction in the effective circulating volume. One of the characteristic features of hypovolemic shock is hypotension followed by diminished renal blood flow, which may progress to the point of anuria. Generalized tissue anoxia, with a consequent shift to anaerobic metabolism, results in increasing concentrations of lactic acid in blood. Coma may appear sometime after the appearance of peripheral circulatory failure.

Lipid Metabolism

Oskar Minkowski, the man credited with describing the glucosuria associated with insulin withdrawal, is said to have **tasted** the urine of a pancreatectomized dog in 1889 because it attracted an inordinate number of flies. Had he **smelled** the urine instead, perhaps the emphasis on biochemical and physiologic disorders of insulin withdrawal initially would have been shifted toward disordered lipid metabolism instead of disordered carbohydrate metabolism.

It is well known today that the insulinopenia of diabetes, and the subsequent decrease in glucose use by adipose tissue, results in retention of triglyceride-containing lipoproteins in blood (mainly **chylomicrons** and **VLDLs**), as well as large-scale mobilization of depot fat (**Part B**). **Lipolysis** may result in secondary hypertriglyceridemia as free fatty acids (FFAs) are synthesized back into triglycerides and then packaged by the liver into VLDLs, which are then exocytosed into blood.

The liver is flooded with **long-chain FFAs**, many of which can be oxidized only as far as the acetyl-CoA stage. The 2-carbon fragments then generate **acetoacetic acid** and **β-hydroxybutyric acid,** the two primary **ketone bodies**, which appear in hepatic venous blood in increasing amounts (**Part C**). The adult liver cannot oxidize ketone bodies, and, other than rumen epithelial cells, the liver is the only tissue in the body known to produce them.

Acetone is formed by the spontaneous decarboxylation of acetoacetate, and is only detectable when the concentration of the latter is abnormally high (see **Part C**). Acetone is not further metabolized, but rather is excreted through the lungs and kidneys (where it accounts for the characteristic sweet or fruity smell on the breath and in the urine of severely diabetic patients).

The developing **ketonemia** has two prominent effects: **1**) it leads to a progressive **metabolic acidosis** (because ketone bodies are strong acids), which in turn initiates the characteristic deep Küssmaul breathing that is one of the cardinal signs of diabetic ketoacidosis; and **2**) as ketonemia exceeds the renal threshold for ketone body reabsorption, ketone bodies appear in urine. In the process of being excreted by the kidneys, ketone bodies, because they are anions, also pull Na^+, Mg^{2+}, and K^+ with them. This means in effect that the ionic "skeleton" of extracellular fluid (i.e., Na^+) is diminished and, therefore, can "support" progressively smaller volumes of fluid.

Also of pathophysiologic relevance is the fact that, in diabetic coma, **excessive hepatic β-oxidation of fatty acids** can cause the concentration ratio of **β-hydroxybutyrate to acetoacetate** to rise from about **5** to as high as **15** due to decreases both in the mitochondrial NAD^+/NADH concentration ratio, and in the intracellular pH (see **Part C**). Because a frequently used rapid test for ketonuria with **Clinistix** (Bayer Diagnostic) or similar material detects only acetoacetate (and acetone), it can sometimes result in serious underestimates of the extent of ketonuria.

Hyperglycemic Hyperosmolar Syndrome (HHS)

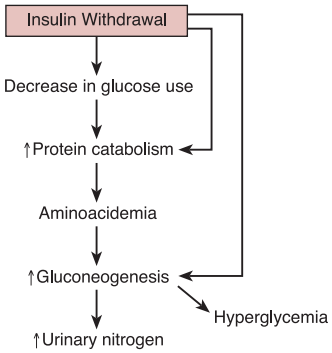
Diabetic animals with **HHS** usually exhibit extreme dehydration, renal and CNS dysfunction, and marked hyperglycemia. When this is accompanied without significant ketonemia, it is referred to as **hyperosmolar nonketotic syndrome** (**HNKS**). It is believed that peripheral insulin insensitivity, enhanced hepatic gluconeogenesis, and reduced renal glucose clearance contribute to HHS. If the diabetic liver remains partially responsive to insulin, free fatty acid oxidation and VLDL formation is minimized, ketone body formation is reduced, and HHS becomes HNKS. Coupled with a decreased portal insulin/glucagon ratio, diabetic renal glomerular disease can contribute significantly to hyperglycemia. Concurrent diseases such as pancreatitis, pyometria, hyperadrenocorticism, pyelonephritis, and/or congestive heart failure can exacerbate the effects of HHS. **Hypertonic dehydration** is a hallmark of this severe form of DM, which can cause severe neurologic symptoms. In HNKS, the glucosuria (in the absence of ketonuria) causes urinary H_2O loss in excess of electrolyte, causing an elevated plasma Na^+ concentration.

In contrast, **diabetic ketonuria** draws filtered cations (e.g., Na^+, K^+ and Mg^{2+}) into urine, which can promote a **hypotonic dehydration**. Severe ECF volume depletion follows since water and electrolyte loss into urine leads to dehydration and hemoconcentration, which in turn leads to peripheral circulatory failure (e.g., hypovolemic shock). As the GFR is reduced, anuria can ensue, and coma may appear sometime after the appearance of peripheral circulatory failure. Death then becomes inevitable in the untreated patient.

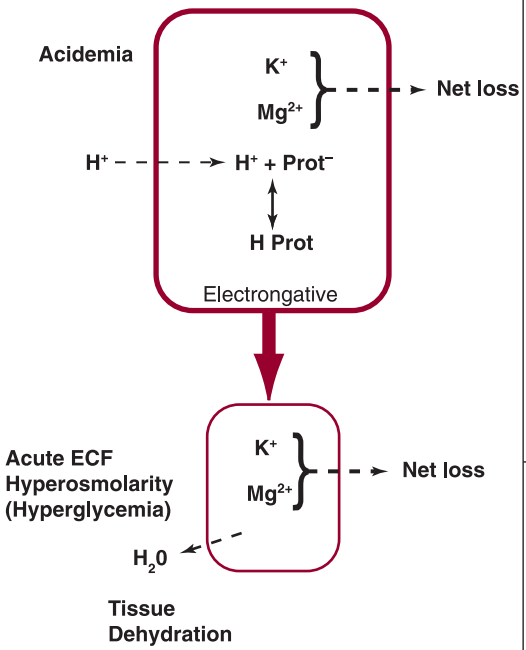
Ch46 Acute Insulin Withdrawal: II

(Protein Metabolism and Electrolyte Depletion)

D Effects of acute insulin withdrawal on protein metabolism

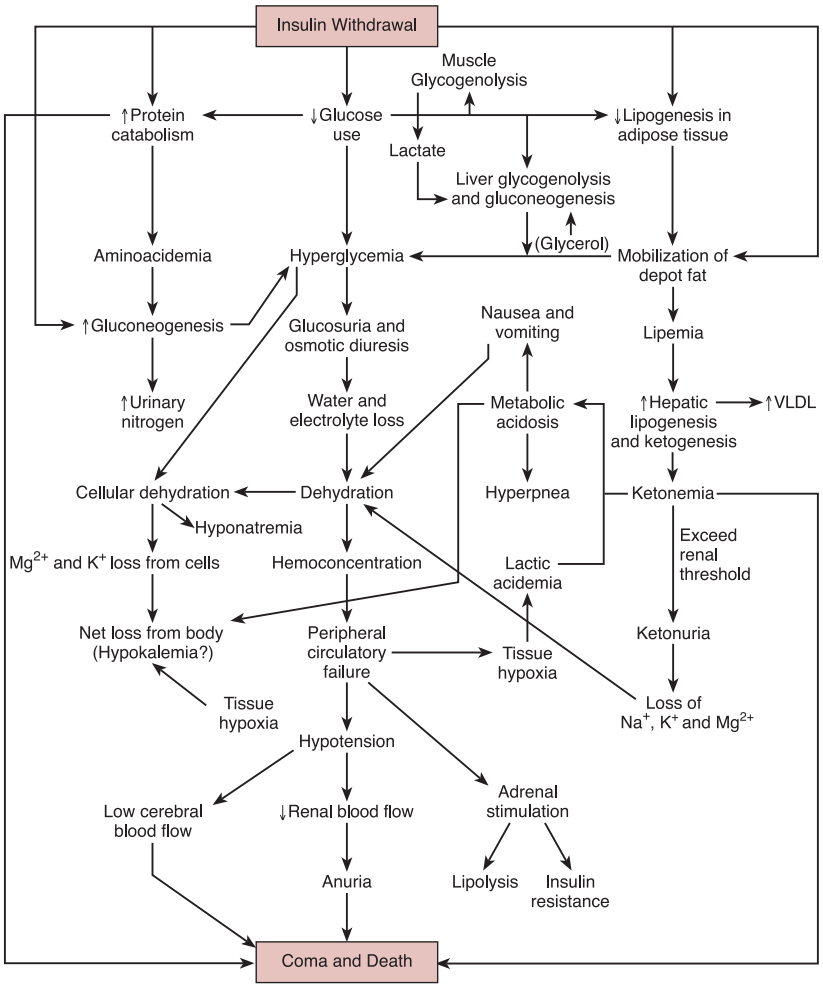


E Effects of acute insulin withdrawl on K^+ and Mg^{2+} balance

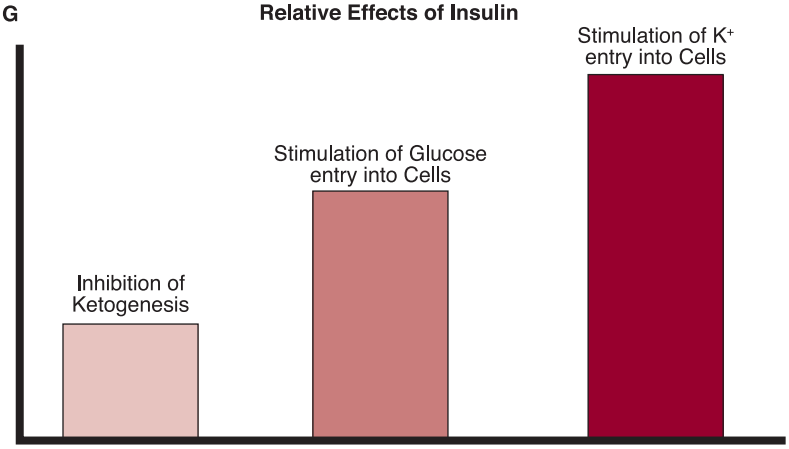


Acute hyperosmolarity of extracellular fluid due to hyperglycemia causes net loss of water from tissues, and as intracellular fluid volume is reduced major cations (K^+ & Mg^{2+}) become more concentrated, and diffuse out of cells. Protons from ketoacidemia are also buffered in tissue cells, and these cations continue to be lost in order to reestablish intracellular electronegativity.

F Combined effects of acute insulin withdrawal



G Relative Effects of Insulin



Source: Parts D and **F** modified from Tepperman J, Tepperman HM. Metabolic and endocrine physiology. 5th ed. St. Louis: Mosby Year-Book, 1987:282-284.

Protein Metabolism and Electrolyte Depletion

Withdrawal of insulin and impaired use of glucose cause a decrease in protein synthesis and, therefore, have the effect of promoting net **protein catabolism.** This effect is manifested largely in insulin-sensitive tissues (mainly muscle). Excess protein catabolism causes an **aminoacidemia**, which provides the liver with glucogenic amino acids that are converted to glucose (**Part D**). The ammonia left over goes into hepatic glutamine and urea production, with both the blood urea nitrogen (BUN) level and urinary nitrogen excretion increasing.

The **hyperglycemia** that ensues from excess hepatic glucose production (and decreased peripheral glucose utilization), causes the osmolarity of extracellular fluid (ECF) to increase, thus drawing water out of insulin-sensitive cells (**Part E**). This temporarily expands the ECF volume, thus creating an (apparent) **hyponatremia** (Ch. 45). The tissue dehydration which follows increases the concentration of intracellular molecules, and the two most prevalent intracellular cations, **K^+** and **Mg^{2+}**, diffuse down their concentration gradients into ECF. Additionally, acidemia also tends to promote cellular **K^+** and **Mg^{2+}** loss (whereas the opposite holds true for alkalemia). When protons enter cells they bind with intracellular buffers (e.g., inorganic phosphates and anionic protein ($Prot^-$)), thus momentarily decreasing electronegativity of the cell. In order to reestablish normal electronegativity, major inorganic cations are lost from the cell.

As glucose, ketone body anions and accompanying water are lost in urine, filtered electrolytes follow. Fluid and electrolyte replenishment will be required during the recovery phase, and when **insulin** is administered, particular attention must be paid to its abilities to not only drive **glucose** into insulin-sensitive tissues, but also **K^+**, **Mg^{2+}**, and **PO_4^{3-}** as well (Ch. 42).

In tissues such as nerve, retina, lens, kidney, intestinal mucosa, erythrocytes and small blood vessels, the uptake of glucose is **insulin-independent**. These tissues are most susceptible to chronic complications from too much available glucose. This carbohydrate is highly reactive with tissue proteins, forming **Amadori products** intracellularly, which in turn form **advanced glycosylation end products** (**AGEs**). These further cross-link matrix proteins, damage blood vessels, and interfere with leukocyte responses to infection. AGEs formed in the eyes, peripheral nerves, and basement membranes of glomeruli are particularly troublesome to diabetic patients. In plasma, **fructosamines** (glycosylated or glycated proteins) form similarly via nonenzymatic reactions driven solely by the hyperglycemia, and therefore become markers of the mean blood glucose concentration during their 1-3 week life-span. In erythrocytes, **glycosylated hemoglobin** (**HbA_{1c}**) forms as excess glucose becomes covalently linked to the globin chain. Although Hb glycosylation appears to exert only a minor effect on its functional properties, it is useful, like serum fructosamines, in monitoring the long-term blood glucose control of diabetic animals

Summary

The foregoing discussion of the biochemical and pathophysiologic manifestations of acute insulin withdrawal was used as an example to show how **carbohydrate**, **lipid**, and **protein** metabolism, as well as **water** and **electrolyte** balance, are interconnected. When all of these sequential events are united into a single diagram (**Part F**), the important points become obvious and intricate interrelationships are more readily appreciated.

Diabetic ketoacidosis develops when insulin levels are low. Early symptoms include weight loss, usually with increased appetite, thirst, and frequent urination (**Part G**). Initially plasma glucose levels rise, and once a concentration of about 180 mg/dL is reached, renal glucose reabsorptive mechanisms become saturated and glucose spills into urine, causing an **osmotic diuresis**. This stimulates thirst, which is compensated by taking in more fluid. As the condition worsens, excessive urinary loss of glucose and water leads to **dehydration** and stimulates release of catecholamines in order to maintain blood pressure and cardiac output (i.e., the early stages of hypovolemic shock). In the setting of low insulin levels, epinephrine secretion leads to lipolysis and release of **FFAs** from adipocytes. The **FFAs** are then taken up by the liver (which becomes increasingly engorged with fat), and in the presence of low insulin levels they are oxidized to **ketone bodies** in mitochondria. Although ketone bodies provide a critical source of energy for most tissues in the presence of intracellular glucopenia, their massive outpouring leads to their presence on the breath (acetone) and in the urine. Acetoacetate and β-hydroxybutyrate are strong acids and deplete the body's buffering systems, leading to **metabolic acidosis**. Excretion of these anions in urine results in a loss of balancing cations (namely Na^+, K^+ and Mg^{2+}). The obligatory loss of Na^+ and water worsens the dehydration, causing catecholamines to rise to even higher levels. **Growth hormone** and **cortisol** also increase in response to this **"metabolic stress,"** thus accelerating muscle and fat tissue breakdown, increasing hepatic glucose production, and further antagonizing the action of insulin. **Glucagon** is elevated due to the low insulin concentration. Thus begins a vicious cycle of tissue breakdown to release stored nutrients, poor uptake of those compounds, increased hepatic production of glucose and organic acids, and urinary loss of glucose, ketone bodies, water, and electrolytes. Eventually, acidosis leads to nausea and vomiting that preclude adequate oral intake of water; consequently, patients can become quite ill.

Fructose and **sorbitol** (a polyol also known as **glucitol**), are found in the lens and neurons, where they increase in concentration in hyperglycemic diabetic patients, and are involved in the pathogenesis of **diabetic cataract** and **neuropathies**. The sorbitol pathway from glucose (i.e., the **"Polyol Pathway"**), is responsible for fructose formation in certain insulin insensitive tissues. Glucose undergoes reduction by NADPH to sorbitol, catalyzed by **aldose reductase**, followed by oxidation to fructose in the presence of NAD^+ and **sorbitol dehydrogenase** (**SDH**). Although sorbitol and fructose can be metabolized to glycolytic intermediates, this process is slow. Additionally, sorbitol does not diffuse through cell membranes easily, and its accumulation causes **osmotic damage** by allowing ingress of H_2O with tissue swelling. Sorbitol also tends to reduce cellular Na^+-K^+ ATPase activity, making the osmotic damage worse.

Therapy is generally aimed at increasing the effective circulating volume and replacing lost electrolytes (IV fluids), suppressing the massive gluconeogenesis and ketogenesis, and facilitating nutrient uptake by muscle, adipose tissue, and the liver (insulin). Acidosis can be reversed by administration of **alkalinizing solutions**. Because a lack of insulin has triggered the adverse effects being evaluated, **insulin** can be given by vein if shock is profound and the likelihood of insulin being picked up from a subcutaneous depot is small. In spite of the fact that the plasma glucose concentration may be high, depletion of muscle and liver glycogen stores most likely has been so extensive that **carbohydrate** should also be given in order to help replenish those stores.

Particular attention is usually paid to the **net K^+** and **Mg^{2+} deficit** that has most likely developed. Infusion mixtures containing K^+ and Mg^{2+} are administered (with caution) in repairing the electrolyte disturbance. Efficacy of management is assessed by tests of plasma glucose, K^+, Mg^{2+} and nonprotein nitrogen (i.e., BUN). Electrocardiographic tracings may also be helpful for the purpose of guiding electrolyte administration.

Before this condition was as well understood biochemically and pathophysiologically as it is today, results of treatment were reported as disappointing, and mortality was high. The preceeding discussion emphasizes the importance of appropriate life-long insulin replacement therapy in the diabetic animal, as well as the catastrophic effects that might occur if insulin replacement therapy is withdrawn.

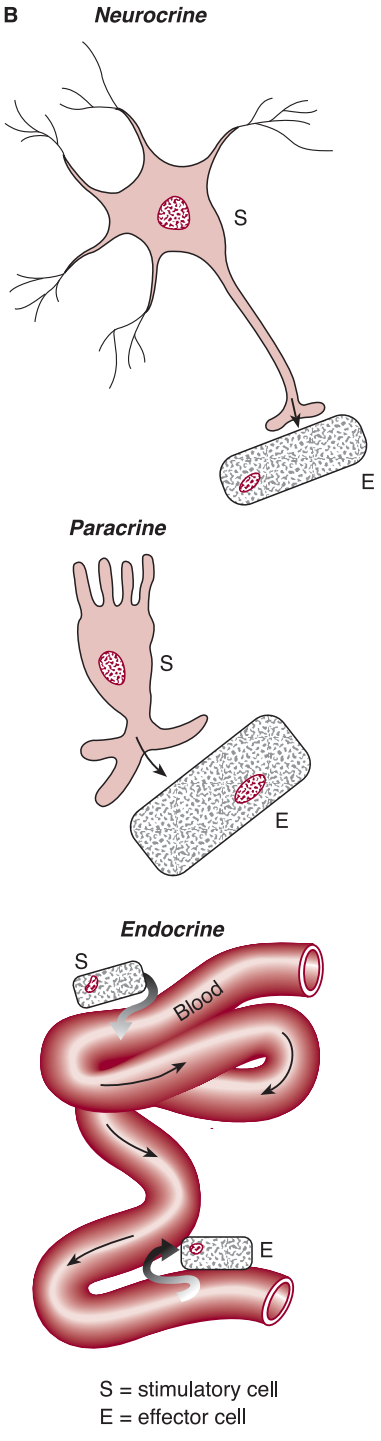
Ch47 Gastrointestinal Hormones: I
(Introduction and Historical Perspective)

A Distribution of Peptides Along the Digestive Tract

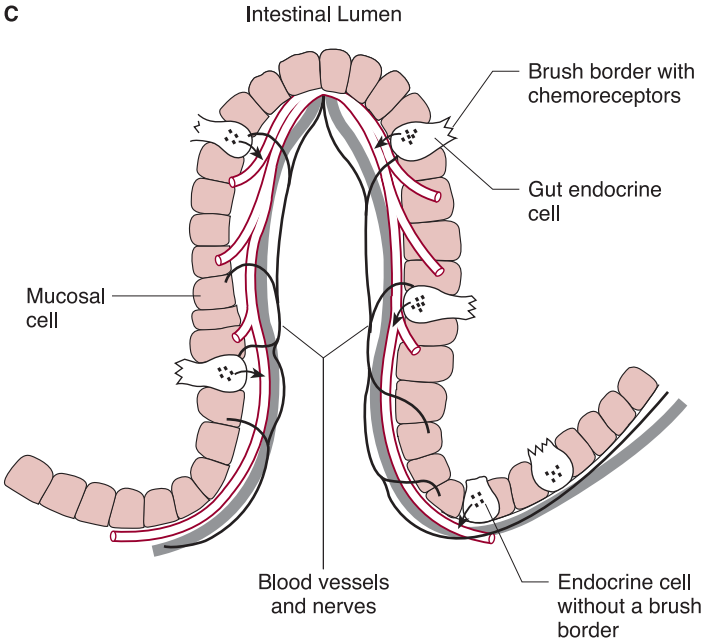
Hormone	*Stomach*	*Pancreas*	*Small intestine*	*Large intestine*
Gastrin family				
Gastrin (G cells)	++	+	+	+
CCK (I cells)	–	–	++	+
Secretin family				
Secretin (S cells)	–	–	++	–
Glucagon (α cells)	++	++	+	–
GLP (L cells)	+	–	++	++
GIP (K cells)	–	–	++	–
VIP (D_1 cells)	++	++	++	++
Others				
Insulin (β cells)	–	++	–	–
Somatostatin (Δ_1 cells)	++	++	+	+
GRP	++	–	+	+
Motilin (EC_2 and M cells)	–	–	++	–
Neurotensin (N cells)	–	–	++	–
Substance P (EC_1 cells)	++	–	++	++
Guanylin and Uroguanylin	–	–	+	++
Pancreatic polypeptide (Δ_2F cells)	–	++	–	–
Ghrelin	+	–	–	–
Obestatin	+	–	–	–

++ = present in large amounts; + = present; – = absent.

B



C



Source: Part C modified from Greenspan FS, Strewler GJ. Basic and clinical endocrinology. 5th ed. Stamford, CT: Appleton & Lange, 1997:577.

The gastrointestinal (GI) tract is the largest endocrine organ system of the body, and may secrete more hormones than all other organ systems combined. However, unlike other endocrine organs, which are more concentrated masses of endocrine tissue, endocrine cells of the GI tract are scattered throughout the pancreas and the mucosa of the stomach, small intestine, and colon. More than 15 types of hormone-secreting **enteroendocrine cells** have been identified. Many secrete only one hormone, and are identified by letters (G cells, I cells, S cells, α cells, etc. (**Part A**)), but some also manufacture serotonin or histamine, and are called **enterochromaffin** (or enterochromaffin-like) cells (Ch. 49). Cells that manufacture biogenic amines in addition to polypeptides are sometimes called **APUD cells**, or **neuroendocrine cells**, and are also found in the lungs, CNS and other organs. They are the cells that can form GI carcinoid tumors (**APUDomas**; Ch. 51).

Although **William Bayliss** and **Ernest Starling** are credited with initiating the "physiological era" of GI endocrinology with their discovery of **secretin** in 1902, the true "biochemical era" awaited the development of more modern laboratory techinques for the purification and analysis of extracts from the GI mucosa. Approximately 30 "new" GI peptides have been identified over the past 30 years in specialized enteroendocrine cells, and in GI neuroendocrine cells. Many of these are also found in the CNS, leading to the concept of a **brain-gut axis** (Ch. 51). The full physiological significance of many of these newly discovered GI peptides is unknown.

Although disease states attributable to disorders of gut endocrine cells are somewhat rare among domestic animals, certain neuroendocrine tumors are known to exist (Chs. 50, 51 and 72). **Insulinomas**, for example, are not uncommon among **North American ferrets**. With the exception of pancreatic insulin and diabetes mellitus, there are few **GI endocrine deficiency states**, and animal models of GI hormone dysfunction are largely unavailable for study. Naturally occurring and acquired dysfunctions of the thyroid, parathyroids, and adrenal glands are frequently observed among domestic species.

GI Regulatory Peptides

The principal stimulus for GI hormonal secretion is digesta in the lumen of the gut, yet hormone release is also affected by neural mechanisms and exposure to other hormones. Gastrointestinal hormones regulate digestive processes by influencing **secretion**, **motility**, and **blood flow** to the GI tract. Less evidence exists for direct hormonal control of **absorptive** processes.

Gastrointestinal peptides that regulate digestive processes may do so as **endocrine**, **paracrine**, or **neurocrine** agents (**Part B**). **Endocrines** are released into **blood**, which then allows them to reach all tissues (unless excluded from the brain by the blood–brain barrier); however, specific receptors that recognize and bind these peptides are present only on their target cells. **Paracrines** are released from endocrine cells and diffuse through **interstitial fluid** to their neighboring target cells. Their effects are limited by the short distances they diffuse. Nevertheless, these agents can affect large areas of the GI tract by virtue of the scattered and abundant distribution of cells containing them. A paracrine agent can also act on endocrine cells to influence their secretion. Some GI peptides are located in nerves and may act as neurocrines (or neurotransmitters). A **neurocrine** agent is released near its target cell and needs only to diffuse across a short **synaptic gap**. Neurocrines act to stimulate or inhibit release of other endocrine and/or paracrine agents.

Some peptides may possess more than one mode of delivery. **Somatostatin**, for example, is known to have endocrine actions (Chs. 10 and 50), is present in neurons, and also exercises paracrine actions in the pancreas (Ch. 43), as well as the gastric antrum and fundus (Ch. 48). Because of their various modes of delivery (**Part N,** Ch. 50), some investigators refer to these substances merely as **GI regulatory peptides** rather than hormones (a word first coined by **William Hardy** following the discovery of secretin by **Bayliss** and **Starling**).

Endocrine cells are scattered singly throughout the mucosa of the GI tract. Most have a broad base and narrow apex with a brush border that faces the intestinal lumen (**Part C**). These cells act as chemoreceptors, sensing luminal contents and then releasing their peptide hormones into adjoining blood vessels. Endocrine cells lacking a luminal brush border are presumably affected by neurocrine and paracrine agents.

When large doses of GI hormones are administered to animals, or when tumors of GI endocrine cells appear (Ch. 51), **certain GI peptides seem to mimic the actions of others through overlapping actions on each other's receptors**. On the basis of structural similarity (**Part A**), several of the hormones fall into one of two families: the **gastrin family** (with the only two members being gastrin and cholecystokinin, CCK); and the **secretin family** (with the members being secretin; glucagon; glicentin, GLP (also called glucagon-like immunoreactivity peptide); vasoactive intestinal polypeptide, VIP; and gastric inhibitory polypeptide, GIP).

Although some of the GI regulatory peptides clearly possess endocrine activities (e.g., gastrin, secretin, CCK, somatostatin, and GIP), investigators have had difficulty delineating precise digestive regulatory activities of other GI neurocrine and paracrine agents. Difficulties have also arisen in measuring the concentrations of these locally acting agents at their presumed sites of action, and in effectively replicating those concentrations in experimental animal models.

Cellular Mechanisms of Action

Gastrointestinal hormones are peptides, and therefore they bind to their respective receptors on plasma membranes of their target cells. These receptors are glycoproteins, whose production is subject to regulation, often by the peptide hormone itself. Carefully controlled studies involving gastrin, CCK, and somatostatin, reveal that their receptor concentrations are **down-regulated** by high circulating titers of the homologous hormone. This would imply that alterations in synthesis, function, internalization, and/or degradation of the receptor occur as a part of the down-regulation process.

Hormone-receptor affinity can be determined through measurement of binding in the presence of varying concentrations of the hormone. There is some evidence, particularly with CCK, that there may be two receptor subtypes; one with a high affinity and low capacity, and the other with a low affinity yet high capacity.

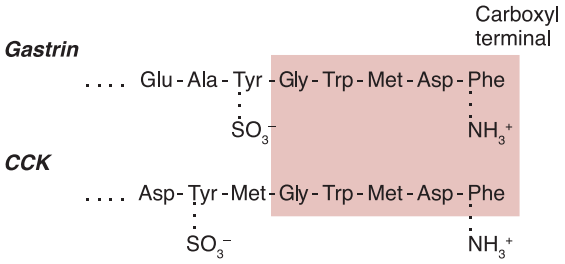
Gastrointestinal hormone binding to cell membrane receptors on target cells activates one of two major intracellular second-messenger systems discussed in previous chapters, the cyclic AMP (**cAMP**) messenger system, and/or the **Ca^{2+}** messenger system. It is clear that the **gastrin family** functions primarily through the release of intracellular Ca^{2+} from stores in the rough endoplasmic reticulum, whereas the **secretin family** activates adenyl cyclase, the enzyme responsible for catalyzing conversion of ATP to cAMP. Although these two second-messenger systems are distinct in their early stages (Chs. 4 and 5), they may nonetheless cause similar changes in later intracellular processes. For example, **histamine** acts through **H_2 receptors** to facilitate intracellular **cAMP** generation in gastric parietal cells (Ch. 49). Gastrin, acting through G protein-coupled receptors on the same cell type, and acetylcholine acting through the M_3 (muscarinic) receptor, facilitate release of intracellular **Ca^{2+}**, with both processes converging on parietal cell canalicular membranes to bring about active **H^+/K^+** exchange with net **HCl** release into the stomach lumen.

Brain-Gut Axis

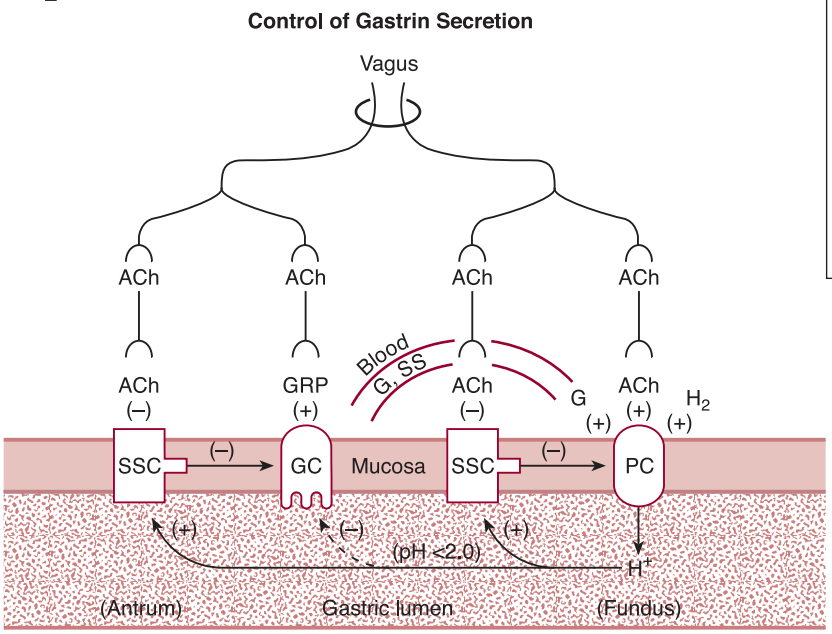
Most of the GI hormones are also found in the brain, where they are locally produced to function as neurotransmitters. This observation led investigators to postulate the existence of a brain-gut axis, where certain hormones from the gut could be traversing the blood-brain-barrier in support of processes such as feeding behavior and perhaps thermoregulation. Indeed, certain gut peptides have been associated with **hunger** (i.e., ghrelin and motilin), while obestatin (ghrelin-associated peptide), GLP, and CCK appear to induce **satiety**. Bombesin (gastrin-releasing peptide; GRP) has been linked to thermoregulation (Chs. 50 and 51).

Ch48 Gastrointestinal Hormones: II (Gastrins)

D



E



GC = gastrin-secreting cell
PC = parietal cell
H_2 = histamine
SSC = somatostatin-secreting cell

F

Control of Gastrin Release

Increase secretion

Primary
- Peptides and L-amino acids
- Volatile fatty acids (VFAs)
- Vagal activation (GRP)
- Stomach distention

Secondary
- Epinephrine
- Calcium
- Rise in abomasal pH
- Insulin-induced hypoglycemia

Decrease secretion

Primary
- Luminal gastric pH <2.0
- Secretin
- Somatostatin

Secondary
- Calcitonin
- GIP
- VIP
- Glucagon

G

Actions of Gastrin

Physiologic
- ↑Gastric HCl secretion
- ↑Intrinsic Factor (IF) secretion
- Trophic effect on gut
- ↑Gastric mixing
- ↑Pepsinogen secretion
- ↑Lower esophageal sphincter (LES) pressure
- ↓Ileocecal sphincter pressure
- ↑CT release

Pharmacologic
- ↑Exocrine pancreatic secretion
- ↑Insulin secretion
- ↑Biliary HCO_3^- secretion
- ↑Duodenal mucus and water secretion
- ↑Gallbladder contraction
- ↑Uterine contraction

Gastrins

Edkins discovered a potent gastric acid secretagogue in extracts of the gastric antral mucosa in 1905, and named it **"gastrin"**. By the early 1960s, gastrin had been isolated, sequenced and synthesized by **Gregory** and his co-workers.

Gastrin is primarily produced by **G cells** of the gastric antrum. Lesser amounts are produced throughout the small and large intestine. Gastrin is also found in the pancreas, pituitary gland, hypothalamus, medulla oblongata, vagus and sciatic nerves. Its functions in the pituitary gland, brain and peripheral nerves are unknown. Gastrin-secreting tumors (gastrinomas) occur in the pancreas, and have been reported in primates, dogs, and cats. **Gastrin excess** leads to **hyperchlorhydria** (HCl excess), and secondarily to gastroduodenal ulceration and maldigestion (since pancreatic digestive enzymes do not reach their optimal pH, and are further denatured by the excess luminal HCl). Maldigestion leads to malabsorption, then to steatorrhea. Ulcerations of the stomach and/or duodenum lead to melena (Ch. 51).

Gastrin and other gastrointestinal hormones demonstrate both **macro-** and **microheterogeneity.** Macroheterogeneity refers to the occurrence in tissues and in body fluids of peptide chains of various lengths; microheterogeneity refers to differences in molecular structure due to derivatization of single amino acid residues. **Preprogastrin** is biologically cleaved into fragments of various chain lengths. Three primary gastrin fragments contain **34**, **17**, and **14** amino acid residues, all with the same carboxyl terminal configuration. One form of derivatization is sulfation of the tyrosine (Tyr) positioned as the sixth amino acid residue from the carboxyl terminal (**Part D**). There are approximately equal amounts of nonsulfated and sulfated forms in blood and tissues, and they are equally active. Another derivatization is amidation of the carboxyl terminal phenylalanine (Phe).

There are differences in activity between the various gastrins, and proportions differ in various body fluids and tissues in which they are found. **Little gastrin** (**G 17**) appears to be the most abundant form, and the principal form stimulating gastric acid secretion. **Big gastrin** (**G 34**) and **mini-gastrin** (**G 14**) exhibit similar physiologic actions to little gastrin, primarily because the entire spectrum of activity known for gastrin is exhibited by the last four amino acids at the carboxyl terminus of all three forms. Little and mini-gastrin exhibit biologic half-lives of about **2-3 minutes**, whereas big gastrin has a half-life of about **15 minutes**. The gastrins are thought to be inactivated primarily by the small intestine and kidneys.

Gastrins obtained from the stomachs of pigs, cats, dogs, and sheep differ from each other and from primate gastrin by one or two amino acid substitutions in the nonspecific part of the molecule (i.e., residues more than six amino acids away from the carboxyl terminus).

Gastrin and CCK have five identical carboxyl terminal amino acids (**Part D**). For CCK, eight carboxyl terminal amino acids are necessary for full biologic activity, whereas for gastrin only four are necessary. The biologic activities of **gastrin** and **CCK** are similar in that both stimulate gastric acid and pancreatic enzyme secretion, and contract the gallbladder. However, their potency for eliciting these responses differs considerably. Gastrin is a strong stimulant of acid secretion, yet a weak stimulant of pancreatic enzyme secretion and gallbladder contraction. The relative potency of CCK for these actions is reversed. These differences are explained by the position of the tyrosine (Tyr) moiety located near the carboxyl end of the molecule. In gastrin, Tyr is the sixth amino acid from the carboxyl end, while in CCK it is the seventh (**Part D**). For CCK-like activity, the tyrosine must be sulfated.

Vagal efferents are directly facilitatory to gastric motility and secretion. Along with their effect on gastrin release, this accounts for the **cephalic phase** of gastric secretion, which is promoted by the sight, smell, taste, and chewing of food. Acid levels, sensed by gastrin-secreting as well as somatostatin-secreting cells, affect gastrin release, which partially controls acid secretion by parietal cells (**Part E**). Impulses reaching the antral gastric mucosa through vagal efferents stimulate gastrin release. The mediator for stimulation is thought to be **gastrin-releasing peptide** (**GRP;** also called **bombesin**), which is liberated from postganglionic parasympathetic neurons that directly innervate **G cells**. The primary mediator for inhibition is thought to be **somatostatin** (**SS**), which is liberated in a paracrine fashion in response to high concentrations of H^+ in the gastric lumen (i.e., pH <2.0—an example of negative feedback). Cholinergic input to SS-secreting cells apparently suppresses SS release, which leads to an increase in gastrin release (an example of stimulation by **disinhibition,** meaning the elimination of an inhibitory paracrine influence). Somatostatin-secreting cells in the acid-secreting fundic region of the stomach are closely coupled to parietal cells, and are thought to function similarly to those in the antral region. Thus, GRP, acting directly on gastrin-secreting cells, and acetylcholine (ACh), acting mainly to eliminate the inhibitory paracrine influence of SS on these cells, are two important neurotransmitters regulating gastrin release. Other stimuli for gastrin release include products of **protein digestion** in the stomach, particularly L-amino acids; solutions of Ca^{2+} salts, including milk; VFA solutions in the abomasum; a rise in abomasal ph; epinephrine; and ethanol (particularly in dogs, which are confirmed "teatotalers") (**Part F**). Carbohydrates, fats, and caffeine do not release gastrin, but decaffeinated coffee does (an effect attributed to the peptides in the brew). Insulin-induced hypoglycemia is also known to stimulate gastrin release. This pathway is presumably mediated by neurons of the enteric nervous system, and may involve both arms of the autonomic nervous system.

Gastrin has a **trophic** (or **growth**) effect on the mucosa of the stomach, small intestine, and colon (**Part G**), and it **increases gastric HCl** and **intrinsic factor** (**IF**) **secretion** from fundic parietal cells. It evokes gastric acid secretion directly or indirectly by facilitating **histamine** release from **enterochromaffin-like** (**ECL**) **cells** of the gastric fundus (Ch. 49). Prolonged **hypergastrinemia** is associated with **hyperchlorhydria**, **gastric mucosal hyperplasia**, **ECL cell hyperplasia** and, occasionally, **EC cell carcinoid tumors** (Ch. 51).

Other known physiologic actions of gastrin are to **enhance mixing actions** of the **stomach** (to increase acid emulsification and initiate digestion of ingesta), **increase lower esophageal** (**LES**) **sphincter pressure** (preventing gastroesophageal acid reflux), **decrease ileocecal sphincter pressure** (part of the urge to defecate following a meal), and **increase exocrine pepsinogen** (the first proteolytic enzyme of the digestive tract) **secretion**. This action on pepsinogen secretion is also shared by **CCK** and **secretin** (Chs. 49 and 50). In response to the presence of dietary Ca^{2+}, **gastrin releases calcitonin in an anticipatory fashion** (Chs. 16 and 17).

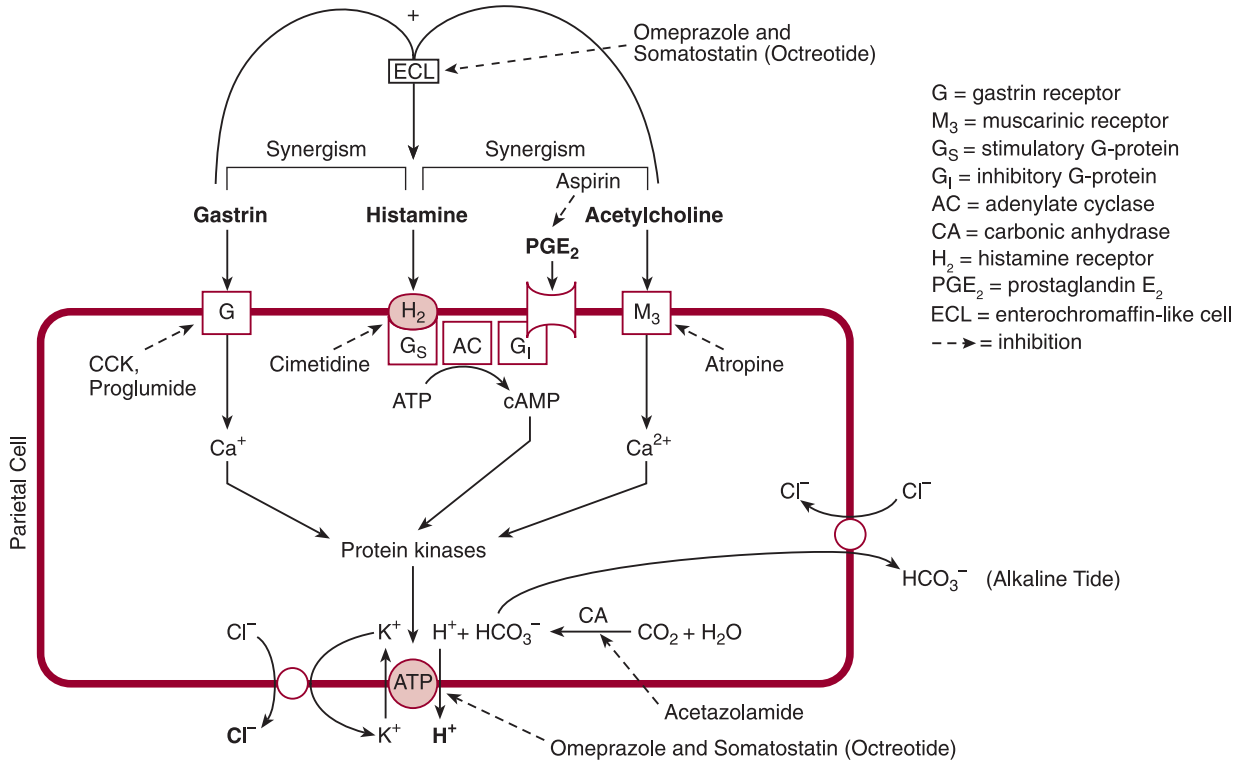
Gastrin's pharmacological actions (**Part G**), which are difficult to explain from a physiological perspective, are seen only when blood levels of gastrin are abnormally high (e.g., with **gastrinoma**). For example, why would high gastrin levels influence uterine contractility? This appears to indicate that gastrin receptors may be fairly widespread, and there is also a significant amount of heterogeneity among the different forms of gastrin. It may be that this peptide has an exciting evolutionary history, adapting its actions to the unique environmental, nutritional and physiologic needs of each species. Gastrin has been isolated from all vertebrates studied, two molluscan species and an insect (Chs. 2 and 72).

Pentagastrin

Stimulants are sometimes employed in diagnostic procedures to assess the ability of the stomach to secrete acid. **Pentagastrin** (**Peptavlon**), a synthetic pentapeptide derivative of gastrin, is commercially available, stable and water-soluble. Pentagastrin contains the active tetrapeptide sequence, and thus exhibits physiologic actions of the natural gastrins. It is usually administered subcutaneously with histamine and an H_1-receptor blocker to prevent side effects.

Ch49 Gastrointestinal Hormones: III
(Gastric HCl Secretion, and CCK)

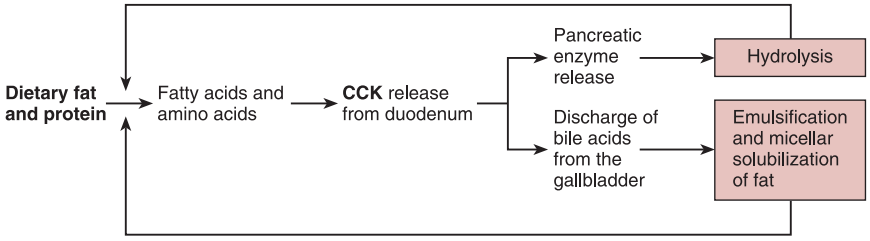
H **Control of Gastric HCl Secretion**



I

Stimulators of CCK Release	*CCK Actions*
Luminal fatty acids longer than 8 carbon atoms Protein digestion products Essential L-amino acids	**Primary** ↑Gallbladder contraction ↓Sphincter of Oddi pressure ↑Pancreatic enzyme secretion ↓Gastric emptying ↑Pepsinogen secretion ↓LES pressure (fatty meals)
Inhibitor of CCK Release	**Secondary** Trophic action on pancreas Synergistic with secretin (HCO_3^-) ↑Insulin, glucagon, and CT secretion ↑Satiety (present in brain)
Somatostatin	

J



Control of Gastric HCl Secretion

Histamine is synthesized, stored (in large amounts), and released locally from the fundic mucosa. It is not a true hormone. There is a continuous background concentration of histamine within gastric interstitial fluid, and its concentration increases when the gastric mucosa is injured. Histamine is found in **enterochromaffin-like** (**ECL**) **cells**, and both gastrin and acetylcholine (ACh) stimulate its release, while somatostatin and omeprazole act as inhibitors (**Part H**). Histamine plays a central role (along with gastrin and ACh) in the direct stimulation of gastric parietal cell HCl secretion. Synergism exists between gastrin and histamine, and between ACh and histamine in stimulating parietal cell acid secretion, but not necessarily between gastrin and ACh (since intracellular cAMP levels may not be elevated). **Cimetidine**, a drug that blocks histamine (H_2) receptors on parietal cells, blocks much of the effect of either elevated serum gastrin or abnormally high parasympathetic (vagal) discharge since cAMP levels are reduced. Less well understood roles for histamine include its ability to enhance gut motility (similar to prostaglandins), vasodilate arterioles of the digestive tract (an H_1 receptor action), enhance gastric pepsinogen release, and decrease pressure of the lower esophageal sphincter (LES).

The effects of **histamine** on gastric acid secretion are mediated through the **cAMP** messenger system. Histamine combines with **H_2** receptors on parietal cells, and through a stimulatory G-protein activates adenylate cyclase, thus catalyzing formation of cAMP. Through activation of a cAMP-dependent protein kinase (i.e., protein kinase A (PKA)), cAMP then performs the function of stimulus-secretion coupling through facilitation of **H^+/K^+ exchange**. H^+/K^+ ATPase molecules in apical membranes of parietal cells pump H^+ and K^+ against their respective concentration gradients. Potassium molecules are made available for this exchange by first diffusing out of parietal cells in electroneutral symport with **Cl^-**. Once H^+/K^+ exchange is complete, net **HCl** secretion results, and the overall electroneutrality of the parietal cell remains undisturbed. Histamine is further methylated within the gastric mucosa, and most methylated derivatives are inactive.

To make H^+ and Cl^- available, **CO_2** combines with **H_2O** inside parietal cells, and **H^+** and **HCO_3^-** are formed via the **carbonic anhydrase** reaction. **HCO_3^-** moves into blood in exchange for **Cl^-** (the most prevalent extracellular anion), and when gastric secretion is elevated following a meal, sufficient **HCl** may be secreted and **HCO_3^-** absorbed to slightly elevate systemic blood pH making the urine alkaline (i.e., the postprandial **"alkaline tide"**).

Acetylcholine and parasympathomimetic drugs such as carbachol also stimulate parietal cell acid secretion, but not through cAMP. These agents act through muscarinic receptors to increase inositol triphosphate (**IP_3**) formation from membrane-bound phospholipids, with IP_3 in turn serving as a second messenger to stimulate intracellular **Ca^{2+}** release (Ch. 5). Through further Ca^{2+} binding with calmodulin (**CaM**), specific CaM kinases can be activated leading to enzyme phosphorylation/dephosphorylation, and activation of **H^+/K^+ ATPase**. The Ca^{2+}-CaM avenue of H^+/K^+ ATPase activation is not entirely separate from that of cAMP, for in the absence of cAMP maximal HCl secretion cannot be achieved. Therefore, **ACh** as a stimulant must partially rely upon the simultaneous action of **histamine**.

Gastrin release from G cells in the antral mucosa stimulates acid secretion by combining with specific gastrin receptors on parietal cells. Like ACh, gastrin does not stimulate formation of cAMP, but works through mobilizing intracellular **Ca^{2+}** stores. Combination of gastrin with its receptors is competitively blocked by **CCK**, which shares gastrin's active C-terminal 5 amino acid sequence (Chs. 47 and 48, **Part D**). Because CCK is a much weaker stimulant of acid secretion than gastrin, CCK's net effect is to decrease gastrin-stimulated acid secretion by denying receptors to gastrin. **Histamine** also plays a central role in allowing for the maximal action of gastrin. Although histamine is a potent parietal cell secretagogue, gastrin is reported to be approximately 500 times more potent.

Acetazolamide inhibits acid secretion by inhibiting carbonic anhydrase, while caffeine, a phosphodiesterase inhibitor, enhances acid secretion (but not gastrin secretion) by increasing the half-life of cAMP. **Proglumide** blocks gastrin receptors, and **prostaglandins** inhibit acid secretion by inhibiting adenylate cyclase (through inhibitory G-protein) (see **Part H**). Nonsteroidal antiinflammatory drugs such as **aspirin** inhibit prostaglandin formation and, therefore, increase gastric HCl secretion. **Somatostatin** and **omeprazole**, a substituted benzimidazole, block H^+/K^+ ATPase, thereby reducing acid secretion.

Cholecystokinin

In 1928, **Ivy** and **Oldberg** observed that instillation of fat into the small intestine caused the gallbladder to contract. They postulated a hormonal mechanism and named their mediator **cholecystokinin** (**CCK**). In the early 1940s, **Harper** and **Raper** discovered a substance in extracts of the duodenal mucosa that stimulated secretion of enzyme-rich juice from the pancreas. They called the substance **pancreozymin** (**PZ**). In 1964, **Jorpes** and **co-workers** found that increasing the purification of a single extract from the small bowel proportionately increased its potency for gallbladder contraction and pancreatic enzyme secretion, indicating that the two actions were properties of a single hormone. For many years thereafter this hormone was appropriately called **CCK-PZ**; however, because **Ivy** and **Oldberg** discovered CCK first, their's is the name used today.

Like gastrin, **CCK** shows both macro- and microheterogeneity. It exists in six molecular forms (peptides of **58**, **39**, **33**, **12**, **8**, and **4** amino acid residues), with the smaller peptides likely representing progressive steps in the reductive creation of CCK 4. With the exception of CCK 4, all of these peptides have the same **8 amino acid sequence**, and the same 5 amino acid carboxylterminal as gastrin (Ch. 48, **Part D**). The carboxyl terminal of the CCKs is typically amidated, and the tyrosine moiety 7 amino acids away is typically sulfated. The circulatory half-life of CCK is about **5 minutes**.

In addition to its secretion by endocrine **I cells** of the upper small intestine, CCK is found in nerves of the distal ileum and colon, and also neurons of the brain. The CCK secreted by the upper small intestine is mostly CCK 8 and CCK 12, although CCK 58 is also present in the intestine and circulating blood of some species. Enteric and pancreatic nerves contain primarily CCK 4. In the cerebral cortex, CCK 58 and CCK 8 are found. These peptides are thought to be involved with satiety, and they have been reported to block enkephalin receptors like naloxone (Chs. 8 and 9).

Two **CCK receptors** (**A** and **B**) have been identified. CCK-A receptors are primarily located in the periphery, whereas both types are found in the brain. Both activate PLC, and thus produce IP_3 and DG as second messengers (Chs. 5 and 47).

The **physiologic actions of CCK** are listed in **Part I.** In general, CCK causes **exocrine pancreatic enzyme release** to hydrolyze ingested macromolecules, as well as **gallbladder contraction** to provide needed **bile acids** for fat emulsification and micellar solubilization of the products of lipid digestion (namely long-chain fatty acids and monoglycerides) (**Part J**). Hypertrophy of the pancreas is produced by intraperitoneal injections of CCK 8, and the number of acinar, ductal, and β-cells is increased.

CCK augments the action of secretin in producing secretion of an alkaline biliary and pancreatic juice, and also in stimulating gastric pepsinogen secretion. These two hormones also contract the pyloric sphincter, thus reducing gastric emptying as well as reflux of duodenal contents into the stomach.

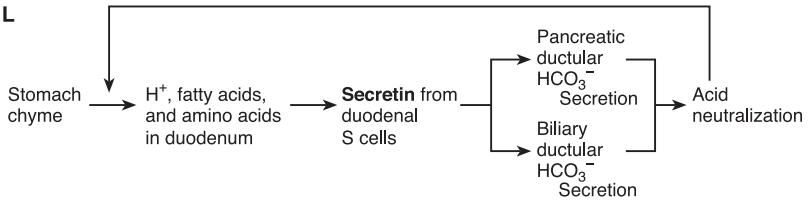
The secretion of CCK into blood is decreased by somatostatin, yet increased by contact of the intestinal mucosa with **products of fat and protein digestion**, namely fatty acids containing more than 8 carbon atoms, peptides, and essential L-amino acids. Since the bile and pancreatic juice that enter the duodenum in response to CCK further the digestion of fat and protein, additional stimulation of CCK release ensues. This positive feedback is terminated when the products of digestion are absorbed, or they move on to lower portions of the digestive tract.

Ch50 Gastrointestinal Hormones: IV
(Secretin and other GI Peptides)

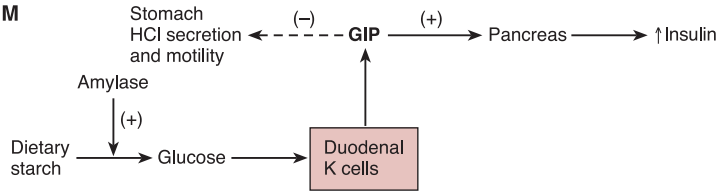
K ***Properties of Secretin***

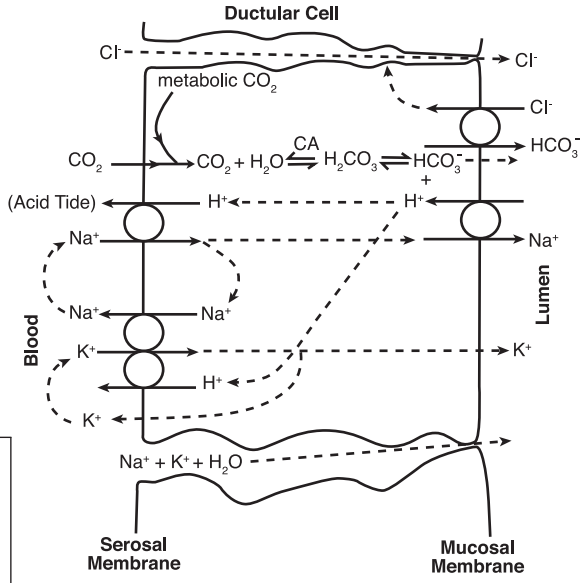
Stimulate secretin release	Secretin actions
Primary	**Primary**
pH <4.5 in duodenum	↑Pancreatic HCO_3^- secretion
Secondary	↑Biliary HCO_3^- secretion
Peptides in duodenum	**Secondary**
Fatty acids in duodenum	↓Gastric motility
Amino acids in duodenum	↓Intestinal motility
	↓HCl secretion
Inhibit secretin release	↑Pepsinogen secretion
Nicotine	↑Insulin secretion
	Opposes trophic action of gastrin
	Synergistic with CCK (enzymes)

L



M





Although the mechanisms of ion transport in the ductular epithelium are poorly understood, **secretin-stimulated $NaHCO_3$ secretion** from pancreatic and biliary ductular cells is dependent upon the carbonic anhydrase (CA) system. CO_2 is taken-up from plasma (as in gastric secretion), with H^+ and HCO_3^- being generated. In this instance HCO_3^- is either passively transported into the ductular lumen, or it enters via HCO_3^-/Cl^- antiport. Other ions and H_2O appear to enter via the paracellular space. H^+ is thought to enter blood via either Na^+/H^+ and/or K^+/H^+ antiport, thus promoting further HCO_3^- production. This **"acid tide"** in blood helps to neutralize the **"alkaline tide"** originating from the stomach. Na^+/H^+, K^+/H^+ and Na^+/K^+ antiporters are thought to be present in serosal membranes of pancreatic ductular cells, while Cl^-/HCO_3^- and Na^+/H^+ antiporters may well exist in mucosal membranes.

N ***Major Actions of the GI Hormones***

GI Hormone	Mode of Action	Major Action	Exist in both gut and brain?
Gastrin	E, (N)	Gastric acid and pepsinogen secretion	Yes
CCK	E, (N)	Gallbladder contraction, pancreatic enzyme secretion	Yes
Secretin	E	Pancreatic and biliary ductular HCO_3^- secretion	Yes
Glucagon	E, P	Enhances hepatic glycogenolysis and gluconeogenesis	Yes
GLP	(E), (N), (P)	Decrease gastric motility, promote satiety, increase insulin release	No
GIP	E	Enhances glucose-mediated insulin release, inhibits HCl secretion	No
VIP	N, (P)	Smooth muscle relaxation, electrolyte secretion	Yes
Insulin	E	Enhances lipogenesis and cell membrane permeability to glucose	Yes
Somatostatin	E, N, P	Numerous inhibitory effects	Yes
GRP	N, (P)	Stimulates release of gastrin	Yes
Motilin	(E)	Initiates interdigestive intestinal motility	Yes
Neurotensin	E, (N), (P)	Inhibits GI motility and blood flow	Yes
Substance P	N, (P)	Increases intestinal motility	Yes
Guanylin/Uroguanylin	E, P	Increases Cl^- secretion into intestinal lumen + Na^+ excretion into urine	Yes
Pancreatic polypeptide	(E), (P)	Inhibits pancreatic HCO_3^- and enzyme secretion	Yes
Ghrelin	(E)	Induce hunger, enhance gastric and intestinal motility	Yes
Obestatin	(E)	Promote satiety, decrease gastric and intestinal motility	Yes

E = endocrine; N = neurocrine; P = paracrine; ( ) = suggested, but not proven.

Source: Part N modified from Greenspan FS, Strewler GJ. Basic and clinical endocrinology. 5th ed. Stamford, CT: Appleton & Lange, 1997:576.

Secretin

Secretin (**"nature's antacid"**) holds the distinction in biology of being the first hormone discovered. In the afternoon of January 16, 1902, **Bayliss** and **Starling** found that when acid was placed in an intrinsically denervated loop of the upper small bowel of an anesthetized dog, the pancreas responded by secreting. "**This cannot be a nervous reflex!"**, **Starling** exclaimed. **"Then it must be a chemical reflex!"** He then demonstrated that a crude extract of the intestinal mucosa also stimulated pancreatic secretion when given intravenously. Although in retrospect the experiment was far from conclusive, **Starling's** exclamation announced **the birth of the science of endocrinology**; he noticed that a specific stimulus acting on a specific receptor organ released a specific messenger that traveled by blood to a distant, specific target organ, and elicited a specific response. In addition, the experiment by **Bayliss** and **Starling** demonstrated a negative feedback loop in which the stimulus evoked a response (HCO_3^- secretion) that ended in the elimination of the stimulus (H^+).

The amount of secretin normally released from the duodenum is proportional to the amount of acid entering, with secretion inhibited at a **pH** above **4.5**. Secretin has a circulatory half-life of about **four minutes**, and its chief physiologic action is to stimulate bicarbonate release from pancreatic and biliary ductular cells (**Parts K** and **L**). Because it is synergistic with **CCK**, secretin also enhances pancreatic enzyme secretion and gallbladder contraction in the presence of CCK. Secretin opposes the trophic action of gastrin on the GI mucosa. It decreases gastric and intestinal motility, and it noncompetitively inhibits gastrin-stimulated acid secretion in dogs (probably a pharmacologic action). Secretin and glucagon share a common 14-amino-acid sequence; therefore, in pharmacologic amounts they mimic each other's actions (Ch. 47, **Part A**).

Other GI Peptides

Glucagon is secreted by α-cells of pancreatic islets as well as α-cells of the GI mucosa. As discussed in Chs. 31 and 32, glucagon is thought to play a role in diabetic hyperglycemia. **Glucagon-like immunoreactivity peptide** (**GLP**) is found in L cells of the stomach, small and large intestines, and it exists in two forms (**GLP-1** and **GLP-2**). This GI peptide promotes satiety, decreases gastric motility, and increases insulin release in an anticipatory fashion (Chs. 40 and 47, **Part A**).

Gastric inhibitory polypeptide (**GIP**) is a polypeptide of 43 amino acid residues, and is produced by K cells of the small intestinal mucosa. Glucose and fat in the duodenum stimulate its release into the circulation. Gastric inhibitory polypeptide was so-named because it inhibits gastric secretion and motility; however, its more important role in metabolism may be its ability to stimulate insulin release (in an anticipatory fashion; **Part M**). Because of this important action, GIP is sometimes referred to as **glucose-dependent insulinotropic polypeptide**.

The gastric inhibitory effects of GIP appear only when blood levels of this hormone are quite high. However, the insulin-release promoting activity of this peptide occurs following oral doses of dextrose that produce normal blood glucose levels in omnivores. In carnivores, intestinal glucagon, GLP, and CCK may be more important anticipatory hormones for insulin release (Ch. 40, **Part C**).

Vasoactive intestinal polypeptide (**VIP**) contains 28 amino acid residues, and is found in nerves of the GI tract as well as D_1 cells. Tumors that secrete VIP, or VIPomas, are known to markedly stimulate intestinal electrolyte and water secretion, and hence cause diarrhea. Vasoactive intestinal polypeptide relaxes intestinal smooth muscle including sphincters, dilates peripheral blood vessels, and inhibits gastric HCl secretion. It is found in the brain as well as in several autonomic parasympathetic nerves.

VIP is a member of the secretin family, and thus possesses structural similarities with secretin, glucagon, GLP, and **GIP** (Ch. 47, **Part A**). This hormone potentiates the action of acetylcholine on salivary glands, and has a circulatory half-life of about **2 minutes**. Pre-pro-VIP contains both VIP and a closely related polypeptide (PHI-27) in animals. Physiologic actions of PHI-27, if any, have not been described.

Motilin is a 22 amino-acid peptide secreted by two specialized cell types within the duodenum. One is the EC_2 cell, and the other the M-cell (Chs. 47 and 48, **Part A**). Motilin contracts intestinal smooth muscle, and appears to be an important regulator of interdigestive intestinal motility (i.e., the housekeeper that helps to sweep leftover luminal contents down the gut between meals). The antibiotic erythromycin is known to stimulate motilin receptors, and thus may be of some value in treating patients with decreased intestinal motility. Motilin may also help to stimulate hunger, and to increase gastric emptying.

Neurotensin is a 13-amino acid polypeptide produced by neurons of the ileum, as well as endocrine N-cells. Its release is stimulated by fatty acids, and it reduces ileal blood flow and motility.

Substance P (**pain**) is a polypeptide containing 11 amino acid residues that is found in the intestine, various peripheral nerves, and several parts of the CNS. It is one of a family of six mammalian polypeptides called **tachykinins** that differ at the amino terminal end, but have in common a 4 amino acid carboxyl terminal sequence. Substance P is found in neurons and endocrine EC_1 cells of the GI tract, and it may enter the circulation. Unlike neurotensin, it increases small intestinal motility.

Gastrin-releasing peptide (**GRP**; also known as **bombesin**) contains 27 amino acid residues, and is present in parasympathetic nerve endings terminating on gastrin-secreting G cells of the gastric antrum (Ch. 48, **Part E**). Although endocrine actions of this peptide have not been described, it sometimes enters the circulation following efferent vagal discharge.

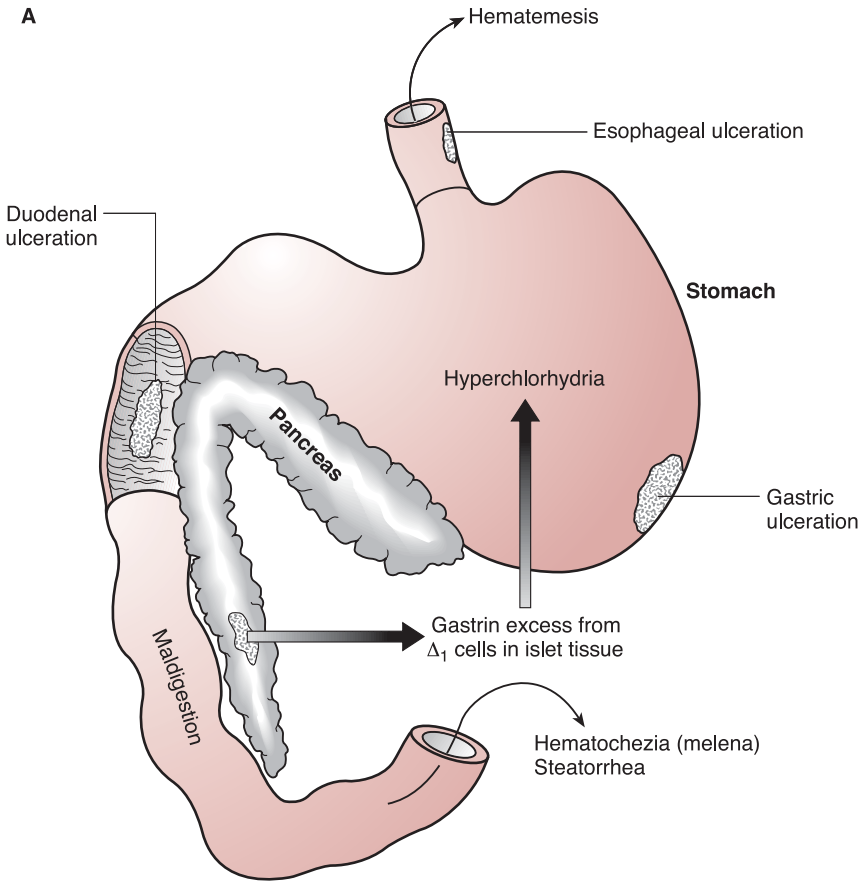
Guanylin/uroguanylin is a 15-amino acid polypeptide secreted by endocrine cells of the small and large intestines in response to **dietary NaCl**. It stimulates guanylyl cyclase in target cells, increasing intracellular cGMP concentrations, and appears to increase **Cl^- secretion** into the intestinal lumen and **Na^+ excretion** into urine (Ch. 31). Receptors are also found in the liver and female reproductive tract where this polypeptide may also be acting in an endocrine fashion to regulate electrolyte and fluid movement. Certain diarrhea-producing strains of ***E coli*** have enterotoxins similar in structure to guanylin, and activate intestinal guanylin receptors.

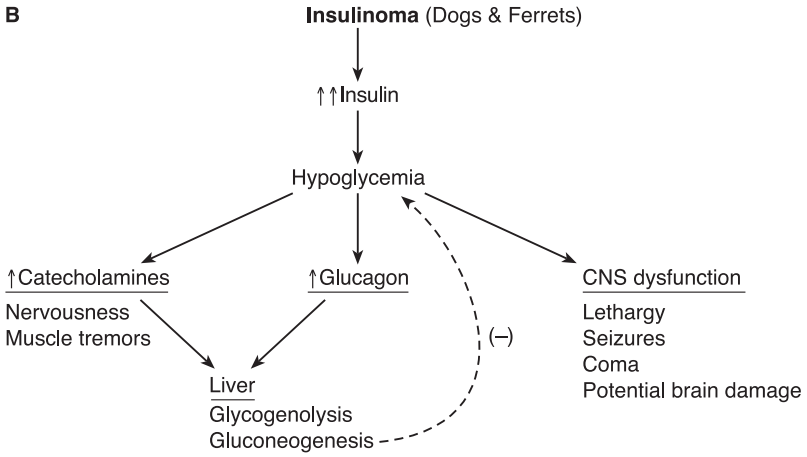
Pancreatic polypeptide is found in Δ_2-cells of the pancreas, and appears to reduce acinar enzyme and ductular HCO_3^- secretion (**Part N**). It contains 36 amino acid residues, and is closely related to peptide YY (also found in the intestine), and neuropeptide Y (found in the brain and ANS; Ch. 40). Secretion of pancreatic polypeptide is largely under parasympathetic control, but is also increased by protein digestion products, by starvation, exercise, and acute hypoglycemia. Somatostatin and hyperglycemia reduce its output.

Peptide YY (**PYY**) is secreted by the small intestine in response to dietary lipids. It inhibits gastric acid secretion and motility, and has **food intake-inhibiting activity** when infused into normal or obese subjects. The idea that food entering the GI tract triggers release from the mucosa of substances that act on the brain to produce satiety is attractive. The effects of **leptin**, a satiety polypeptide secreted by adipocytes, appear to be relatively prolonged compared to gut peptides that may provide shorter-term, meal-to-meal control. **Ghrelin** and its sibling, **obestatin**, are also gastric hormones that enhance and suppress feeding behavior, and both are derived from the same polypeptide precursor. In addition to promoting feeding behavior, ghrelin also enhances adenohypophyseal GH secretion (Ch. 10), gastric and intestinal motility, and gastric emptying. Obestatin (ghrelin-associated peptide), reduces gastric and upper intestinal tract motility. Other gut peptides that decrease food intake include **GRP**, **glucagon**, **somatostatin**, and **CCK**.

Adrenocorticotropic hormone (**ACTH**), **thyrotropin** (**TSH**), and **thyrotropin-releasing hormone** (**TRH**) have also been found in the digestive tract. Although this may not be a significant source of these hormones for the general circulation, their presence nonetheless implies physiological significance. There is some evidence indicating that locally produced TRH and TSH may be involved with regulation of intestinal secretory immunity.

Ch51 APUDomas
(Neuroendocrine Tumors of the Gut)





Source: **Part A** modified from Chastain CB, Ganjam VK. Clinical endocrinology of companion animals. 1st ed. Philadelphia, PA: Lea & Febiger, 1986:313.

Cytochemical and ultrastructural characteristics of **GI endocrine cells** were described by **Pearse** in 1966. The principal products of these cells were noted to be peptide hormones and biogenic amines (e.g., catecholamines and serotonin). A general characteristic observed was their ability to take up amine precursors and connect them to amines, and it was speculated that the uptake of 5-hydroxytryptamine (serotonin) was linked to the production of peptide hormones. Most of the cells possessing these characteristics are found in the **gut** or **CNS** (hypothalamus, pituitary axis, and pineal gland), but they are also present in the **thyroid** (in calcitonin-secreting cells), **parathyroids**, and **placenta**. **Pearse** referred to them as **amine precursor uptake and decarboxylation** (**APUD**) **cells**.

Although it was postulated that APUD cells originated from the primitive neural crest, whether all APUD cells actually stem from this crest is uncertain. Although studies using quail and chick embryos cast doubt on the claim that APUD cells of the GI tract originate from neural ectoderm, endocrine cells of the GI tract and pancreas do possess an enzyme specific to neural cells (neuronal-specific enolase, NSE), which supports the hypothesis of a common neural origin. It is clear that neuroendocrine cells of the gut are remarkably similar to cells within the hypothalamic–pituitary axis. Gut endocrine cells may also be "neuroendocrinologically" programmed, even though their origin is not clearly understood, thereby giving validity to the concept of the gut acting as a **"visceral brain."**

Within the gut's enteric nervous system is represented every one of the classes of neurotransmitter found in the brain. Indeed, **there are more nerve cells in the gut than in the entire remainder of the peripheral nervous system**, which demonstrates the importance of the neuroendocrine control mechanisms within.

The APUD cells that produce and release peptide messengers may indeed have a common embryologic origin. The concept is appealing because it posits the existence of a common ancestor for those cells in the gut and CNS with almost identical cytochemical characteristics (storage of peptides), and cellular actions (release of peptide messengers). It helps clarify why identical peptides are synthesized, stored, and released by gut epithelial cells, neurons of the GI tract, and nerve cells in the CNS. This **brain–gut axis** could be important in the regulation of feeding behavior. Furthermore, neurons of the CNS interact with those of the enteric nervous system to influence involuntary digestive processes. Many of these neurons are peptidergic, and this interaction occurs via both afferent and efferent pathways.

Neuroendocrine Tumors of the Gut

Neuroendocrine tumors of the gut may be found in the **pancreas** or **intestinal wall**. They are known by the general term **APUDomas**. Many GI endocrine tumors reportedly secrete more than one peptide, but they are generally named after the one responsible for the most demonstrable clinical manifestations. Biochemical confirmation is generally made by measurement of hormonal markers in blood and/or urine. Unfortunately, most APUDomas are small, and preopeative localization is often reported as inaccurate. However, tumor localization and excision is generally the desired treatment.

APUDomas that secrete peptides foreign to their cell of origin (e.g., an APUDoma from a G cell of the stomach that secretes large amounts of VIP) cause **paraendocrine syndromes,** whereas those that secrete excessive quantities of the peptide typical to their cell of origin cause **orthoendocrine syndromes.** The latter are reportedly more typical in veterinary medicine (e.g., gastrinoma, insulinoma, and pheochromocytoma; Ch. 34).

Gastrinoma (Zollinger-Ellison-Like Syndrome)

Gastrinomas have been reported in humans, **dogs**, and **cats**. In 1955, **Zollinger** and **Ellison** described the human gastrinoma syndrome, and 21 years later it was reported in the dog. Several other spontaneous canine and feline cases have been reported since that time, and the syndrome has also been produced experimentally in dogs.

Gastrinomas are usually carcinomas arising from gastrin-secreting Δ_1 cells of pancreatic islet tissue, which normally produce gastrin only during the **fetal** period. In adult animals, these cells normally produce somatostatin (Chs. 47 and 48), but they may revert back to the fetal state in gastrinoma. Most of the gastrin found in normal adult animals originates from G cells of the gastric antral mucosa, with a few gastrin-secreting cells found in the small and large intestines. Gastrinomas in dogs, cats, and humans can secrete other hormones as well (e.g., ACTH, insulin, glucagon, CCK, and pancreatic polypeptide). In about 30% of human cases, other endocrine tumors are reportedly present, usually in the parathyroids or pituitary. This condition is known as **multiple endocrine neoplasia** (**MEN**).

Hypergastrinemia causes parietal cell hyperplasia leading to hyperchlorhydria (HCl excess) and, secondarily, gastroduodenal ulceration (**Part A**). Disruption of intestinal digestive and absorptive functions also occurs, largely because excess acid in the duodenum denatures digestive enzymes. Signs and symptoms may include blood-stained vomit (hematemesis), steatorrhea, bloody stools (hematochezia or melena), abdominal pain, dehydration, regenerative anemia, depression, and weight loss. Esophageal reflux of excess acid can also lead to ulceration (**Part A**). As noted previously (Ch. 48), prolonged hypergastrinemia is also associated with enterochromaffin-like (ECL) cell hyperplasia and, occasionally, EC cell carcinoid tumors.

Duodenal acidification is the primary stimulus for secretin release, and secretin has been shown to cause gastrin release from pancreatic gastrinomas. Therefore, a vicious cycle may ensue (acid to secretin, to gastrin, to acid) that serves to perpetuate the hyperacidity of Zollinger-Ellison-like syndrome.

Insulinoma

This insulin-secreting tumor of pancreatic islets is the most commonly recognized APUDoma of **dogs** and domestic pet **ferrets**, but is seldom reported in other species. It is most often malignant in dogs, and will spread to duodenal, mesenteric, hepatic, and splenic lymphatics. Although local tumor recurrence is a common feature in pet ferrets in the U.S., metastasis to other organs is low. When it does occur,hepatic and splenic lymphatics appear to be affected (Ch. 72).

Results of a serum profile and urinalysis from animals with active insulinomas may be normal, except that plasma glucose, Mg^{2+}, PO_4^{3-} and K^+ concentrations can be low (Ch. 42). Severe hypoglycemia from an insulinoma leads to stimulation of the sympathoadrenal system, which in turn can cause muscle tremors and nervousness (**Part B**). Hypoglycemia-induced catecholamine and glucagon secretion result in stimulation of hepatic glycogenolysis and gluconeogenesis in an attempt to restore the blood glucose concentration.

Acute hypoglycemia seems to affect the CNS more than other organ systems, and if severe and prolonged can cause **irreversible brain damage**. Neuroglycopenic signs include lethargy, seizures and coma in dogs, and irritability, star-gazing, hind limb weakness, ataxia, nausea, excessive salivary flow (ptyalism), pawing at the mouth, and occasionally seizures in ferrets.

Some non-iselt, non-β-cell **hepatocellular tumors** may also be associated with hypoglycemia. Theoretically they could express insulin-like growth factors (**IGF-1** and/or **IGF-2**; Ch. 10).

Other APUDomas

Although **glucagonomas**, **VIPomas** (Verner-Morrison syndrome), **somatostatinomas**, and **pancreatic polypeptide-producing tumors** have been described in humans, they are rarely reported in domestic animals. **Serotonin-secreting carcinoid tumors** that arise from enterochromaffin cells of the GI tract have, however, been reported in older dogs and cats.

Reproductive Endocrinology

Introduction

The chapters which follow summarize important aspects of female and male reproductive endocrinology, as well as fetal, maternal and neonatal physiology. But before an understanding of the intricacies of reproductive endocrinology can be fully appreciated, we should first consider some of the external clues an animal may perceive that help to promote the actions needed to generate at least one replacement of itself.

Animal reproductive behavior, which includes the numerous and varied activities directed toward perpetuation of the species, has been carefully studied and documented. The dominant behavior form in mammals is sexual rather than asexual, although it would seemingly be easier for an organism simply to divide into two or more individuals. One explanation for the dominance of **sexual reproduction** relates to the fact that the environments in which mammals live can change in location and through time. Therefore, their evolutionary success is determined by how well they adapt to such changes. Sexual reproduction allows for the reshuffling of genes, resulting in offspring that possess a genetic makeup different from each of its parents, with this greater genetic variability hopefully allowing species to keep pace with their changing environments.

Several external and internal factors are known to initiate animal reproductive behavior, with the chief **external clues** being **visual, auditory** and **olfactory**. **Light**, usually in the form of increasing day length, appears to be a major visual environmental stimulus for many species (Chs. 60 and 61), especially those living away from the Equator. This seems logical given the fact that increasing day length usually signifies the onset of a more favorable period of time for the success of behavioral and physiologic activities related to perpetuation of the species. Superimposed on day length may be several internal factors, however, which if lacking may override the stimulatory effect of light, for mammals possess internal cycles of endocrine and cellular activity that must coincide with external forces before reproduction can occur (i.e., the estrous and menstrual cycles; Chs. 52-56). Most females with an estrous cycle are sexually receptive only during a brief period where ovulation occurs. **Visual** clues may also include appearance changes of either the male or female during the onset of reproductive activity. For example, the hindquarters of female baboons become bright red during this time, indicating that she is in estrus and sexually receptive. The so-called prenuptial molt of some male birds results in the attainment of the nuptial plumage, which differs from that possessed by the bird at other times of the year. Often associated with changes in appearance are changes in behavior, particularly the increase in aggressive behavior between males (which can sometimes be a prime feature in attracting females). In certain grouse, for example, females appear to be attracted to males that engage in the greatest amount of fighting. Some males are also territorial during the breeding season, thus reducing the amount of interference from other males, and perhaps making it easier for females to find them at the proper time. Females in season may also use behavioral stances that announce to potential male suitors that they are receptive and willing to engage in sexual activity.

Auditory clues, unlike visual clues, travel around and/or through most natural physical barriers. This undoubtedly accounts for their widespread use in indicating sexual receptiveness, especially among frogs, insects and birds. Like visual signals, a sound for advertising purposes can encode several pieces of information (e.g., the caller's species, its sex, and in some instances whether or not it has mated).

There is also considerable information passed between animals through **olfactory** means. Well known are the urine, feces and scent markings employed by mammals to "mark" their breeding grounds, and advertise their sexual state. Males of a number of mammalian species are capable of determining if a female will be sexually receptive simply by smelling her urine. A substance in the urine of male mice, on the other hand, induces and accelerates the estrous cycle of females. **Olfactory clues** (**ectohormones**; also called **pheromones**) are *"secreted or excreted chemical agents that trigger a **social response** in members of the same species."* For example, rabbit mothers are thought to release mammary pheromones that trigger immediate nursing behavior by their young, and dogs and cats deposit chemical markers in and around their territory which serve as indicators to other members of the species regarding the presence of the occupant in that territory. When the recently impregnated female mouse is exposed to the odor of a male other than the one with which she has mated, uterine implantation of the egg often fails; as a result, there is a rapid return to estrus. The odor of the strange male is thought to signify to the pregnant female an unfavorable situation in which to raise young, inasmuch as a number of male rodents attempt to attack offspring not their own. Pheromones are also occasionally used in the detection of estrus in sows. Boar pheromones, for example, can be sprayed into the sty, with sows exhibiting sexual arousal now known to be available for breeding purposes. Pheromones are thought to have evolved in most all animal phyla to signal **sex and dominance status**. These chemical signals are detected primarily by the **vomeronasal organ** (**VNO**), located at the base of the nasal septum, which is present in most amphibians, reptiles and non-primate mammals, but absent in birds. The VNO is present in fetal primates, but atrophied in adults. On a more cellular level, three distinct families of pheromonal receptors (V_1, V_2 and V_3) have been identified in the VNO, and all are G protein-coupled.

Mammalian fertilization normally involves contact during copulation (with artificial insemination (AI; most notably in the cattle industry) being the exception). However, many animals exhibit an aversion to bodily contact, which may be an anti-predator mechanism. Since females are usually in a semi-helpless situation during copulation, they can be particularly wary about this contact. In addition, males are particularly aggressive during the breeding period, which may further increase the uncertainty of both potential participants. These difficulties have been partially circumvented through the evolution of a collection of behaviors collectively known as **courtship** (e.g., *appearance, persistence, appeasement, persuasion,* and perhaps even *deception*). Courtship behavior has many advantages, including reduction of hostility between potential sex partners, especially in species where the male actively defends his territory. The term **display** has been used to describe the numerous social signals that not only convey information, but that in the course of evolution have become **ritualized**. Visual, auditory, olfactory, tactile and other patterns of communication by which organisms advertise their readiness to engage in reproductive activity provide a vast array of differing and unique examples of display.

One of the more unusual evolutionary reproductive behaviors, however, is exhibited by the **praying mantises**, predatory insects which target any species small enough to be successfully captured and devoured (e.g., small scorpions, lizards, frogs, birds, snakes, fish, and even rodents). Mantises are sometimes known to engage in **sexual cannibalism**, with the female biting off the male's head (as they do with regular prey) during copulation. This, apparently, makes the male's movements even more vigorous in the delivery of sperm (after his head has been removed). Although potential reasons for sexual cannibalism among mantises have been debated, it appears that feeding the female *ad libitum* before copulation (so she is not hungry), may reduce the cannibalism. Also, if left undisturbed the male may sometimes attempt to engage the female in a courtship dance, perhaps to alter her interests from feeding to non-lethal mating. Males also seem to approach hungry females with more caution, and sometimes remain mounted to the female's back following copulation for a longer period of time (indicating that he may be attempting to avoid being devoured, for the act of dismounting can also lead to cannibalism). Whatever the motivational force is for animal mating behavior, it appears to drive males to seek, fight for and sometimes die for the opportunity to pleasurably copulate with a receptive female.

During the breeding season, the typical male usually attempts to copulate and mate with as many females as available, whereas females, on the other hand, act more selectively in accepting several matings with a given male, while refusing to stand and copulate with other males. Although seasonality of the estrous cycle is well-described for many species, there is evidence as well that the frequency, quality, and fertility of ejaculates are improved during the breeding season (Ch 57). Another general aspect of animal reproductive behavior is that many animals do not normally form pairs. Exceptions occur, however, with certain animal species (e.g., carnivores and primates), in which parental care is divided between the male and female.

Between birth or hatching and the attainment of maturity, high mortality can occur. Therefore, some of the most elaborate evolutionary adaptations among animal species are seen during this period of time, including behaviors among both parents and offspring that serve to ensure maximal survival of the young to maturity. This primarily involves protecting them against environmental hazards, and providing them with adequate nutrition until they reach an age where they can reproduce in turn. Following the onset of puberty in most animals, an initial period of estrogen exposure followed by secondary exposure to progesterone is required to induce female behavioral receptivity (Ch. 59). However, the domestic cat (an induced ovulator) appears to be an exception since the queen develops receptive behavior in response to priming by estrogen alone, without the need for subsequent progesterone exposure.

Ch52 Estrous Cycles (Types and Phases)

A

Spontaneous (Silent) Ovulators	*Induced (Reflex) Ovulators*
Seasonal	**Seasonal**
Horse	Mink
Sheep	Cat
Goat	Ferret
Donkey	Skunk
Dog	Wild rabbit
Nonseasonal (continuous breeders)	**Nonseasonal**
Cow	Lab rabbit
Buffalo	Llama
Pig	
Human	
Mouse	
Monkey	
Rat	
Guinea pig	
Chinchilla	
Hamster	

Nonseasonal Polyestrous

Cow
Pig

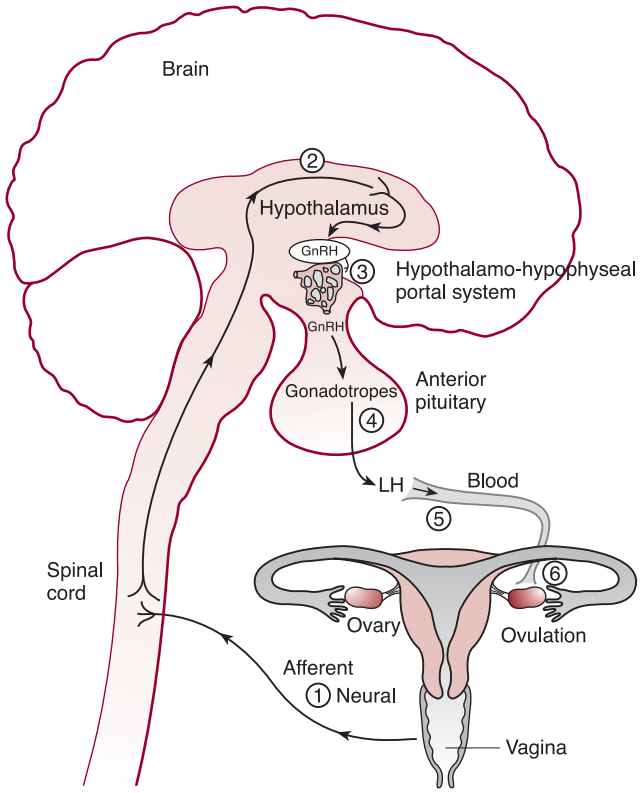
Seasonal Polyestrous

Horse
Sheep
Goat

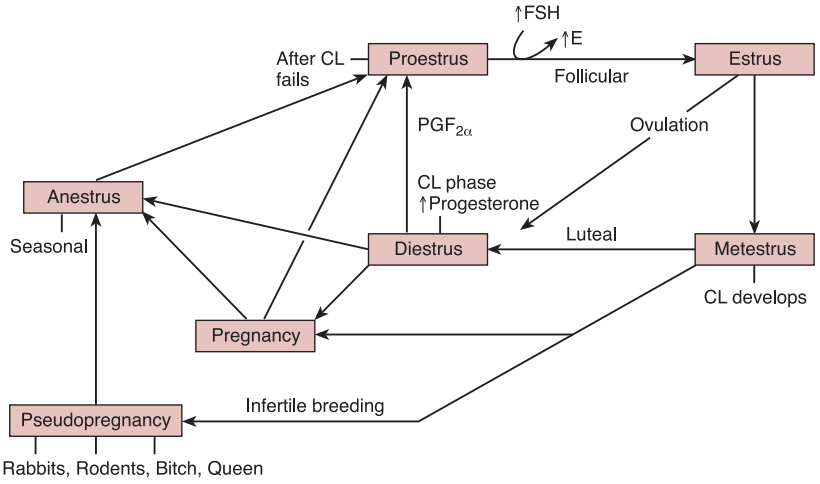
Monestrous

Dog

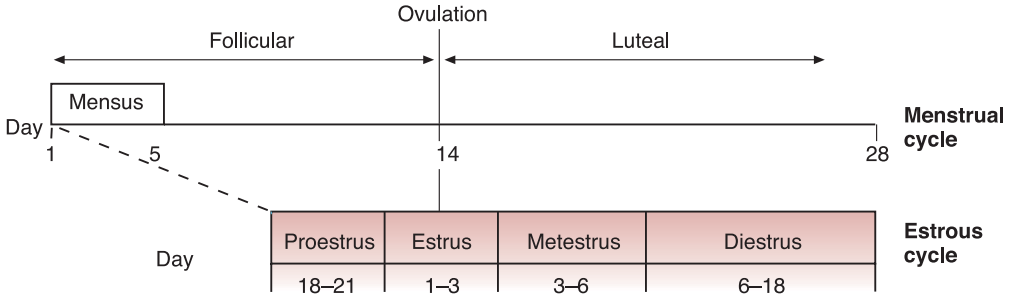
B



C



D Timing of Human Menstrual Cycle Versus Porcine Estrous Cycle



Reproductive patterns of animals vary within and between species (**Appendix Table II**). The following chapters will summarize and contrast basic ingredients of the estrous cycles of domesticated species, the menstrual cycle of primates, and then discuss the basic physiology of male reproduction. This will be followed by chapters dealing with the pineal gland, pregnancy and parturition, lactation, fetal and neonatal physiology.

Types of Estrous Cycles

Two types of estrous cycles are commonly observed in domestic animals (**Part A**). One type is exemplified in nonseasonal breeders, such as the cow and sow, and in the bitch, mare, and ewe during the breeding season. The infertile cycle of these species culminates in **spontaneous** (**silent**) **ovulation** of mature follicles; CLs automatically form, become functional, and exist for a defined period of time. Species such as the rat and mouse are also included in this type of estrous cycle in which ovulation is spontaneous, but CLs that form in these species are dysfunctional unless mating occurs. The estrous cycles are short (5 days) when rats are not mated, and longer (12 days) if cervical stimulation occurs. Some of these animals are **polyestrous** (exhibit more than one estrous cycle in sequence), while others are **monestrous** (come into heat once, then exhibit an anestrus period).

In the second type of estrous cycle, maturation and ovulation of follicles fail unless the male copulates with the female. The rabbit, cat, and mink are examples of this type, commonly referred to as **reflex** or **induced ovulators.** Successive groups of follicles mature and degenerate rhythmically in these animals during the breeding season, and at any time there are a number of follicles capable of being ovulated if copulation occurs. The cat and mink could therefore be considered seasonally **pseudopolyestrous**, because if mating does not occur in these animals, follicles regress and subsequent periods of follicular growth and estrus recur several times during the breeding season. Copulation in these species is thought to stimulate afferent neural pathways to the hypothalamus (processes **1** and **2** in **Part B**), causing release of **GnRH** (process **3**), which stimulates the adenohypophysis (process **4**) to release **LH** into blood (process **5**). Luteinizing hormone in turn, promotes the **ovulatory** process (process **6**).

Phases of the Estrous Cycle

The **estrous cycles** of domestic animals are generally divided into four phases (plus alternates) (**Part C**). These are called **estrus, metestrus, diestrus,** and **proestrus.** The duration of each varies with species, but in the sow are 2 to 3 days, 3 days, 11 to 13 days, and 3 days, respectively.

Estrus is the period of sexual receptivity (i.e., **heat**), during which **ovulation** occurs in many species and CLs begin to form. The beginning is usually a gradual phenomenon, its detection is difficult, and its onset is dependent upon such factors as behavior of the male and female. Duration is based upon the period of receptivity of the male, which varies from 14 to 18 hours in the cow, to 7 to 10 days in the mare and bitch. Age, breed, and environmental factors such as temperature may affect duration. Generally, high environmental temperatures have a tendency to shorten the duration of estrus. At the end of estrus circulating estrogen and LH decline. In cows, ovulation occurs 12 to 16 hours following estrus (Ch. 54) .

Metestrus is the immediate postovulatory phase, in which CLs develop before producing significant amounts of **progesterone**. In some species CLs produce progesterone quickly, and in others the follicular wall initiates progesterone production before ovulation. Therefore, in these species metestrus is not recognized, and animals proceed from estrus directly into diestrus. Examples are the mare, sow, ewe, goat, and bitch that ovulate before the end of estrus, and reflex ovulators like the queen. In these and similar species, metestrus is partially or totally included within the phase of estrus. In the cow and sow, metestrus lasts two to three days from ovulation until significant quantities of progesterone are produced.

Diestrus is the period during which the influence of luteal progesterone on accessory sex structures predominates. Together, metestrus and/or diestrus are referred to as the **phase of the CL**. Generally, diestrus is identified as the first day the female refuses to mate with the male, an effect thought to be due to high circulating levels of progesterone (Ch. 53). This negative effect of progesterone on sexual behavior may not be exhibited in dogs.

Pregnancy may occur during metestrus or diestrus as a result of a fertile mating. Gestation length varies with species, from 31 days in the Western chipmunk to 660 days in the African elephant.

Proestrus is the period after the CL fails (usually due to **$PGF_{2\alpha}$**), when progesterone levels drop, FSH release stimulates follicular growth, and rising estrogen levels lead to estrus. Proestrus and early estrus are referred to as the **follicular phase** (before ovulation). Proestrus is short (two to three days) in domestic animals compared to the follicular phase of menstruating primates (14 days), due largely to the fact that regeneration of the endometrial stratum functionale is unnecessary in domestic animals, because it does not fully degenerate when the CL fails (Ch. 56).

Pseudopregnancy, or **false pregnancy**, is an exaggerated diestrual response of the bitch and queen. It may be related to the extreme sensitivity of the canine endometrium and mammary glands to progesterone, in synergy with prolactin. The CL of the nonpregnant bitch remains functional for an extended period of time after ovulation. In **overt pseudopregnancy** (which may sometimes last as long as pregnancy), mammary glands develop, the uterus enlarges, the abdomen may relax, the pelvis and external genitalia may change as they would during pregnancy, and the bitch may develop a whelping nest. In fact, some pseudopregnant bitches have been reported to adopt and effectively nurse puppies from other bitches. Queens, rodents, and rabbits that are induced to ovulate by mechanical stimulation of the vagina, exogenous hormones, or matings with sterile males may also become pseudopregnant. Pseudopregnancy lasts from 30 to 70 days in the queen, yet it is not associated with the profound organic and behavioral changes seen in the bitch, and seldom leads to lactation and nesting behavior. However, pseudopregnant queens undergo vaginal, uterine, and oviductal changes induced by progesterone secreted by CLs.

Anestrus is a stage of **sexual quiescence** characterized by the lack of estrus behavior. It is a normal stage of reproductive function in prepubertal and aged animals, and in pregnant animals of all species. In fact, pregnancy is the most common cause of anestrus in polyestrous species. After puberty, anestrus in nonpregnant animals is normal for monoestrous species such as dogs, for seasonally polyestrous species during the nonbreeding season, and for lactating females of most species. Anestrus may also occur due to pathological conditions such as infections, nutritional or endocrine imbalances, or to diseases of the ovaries and uterus.

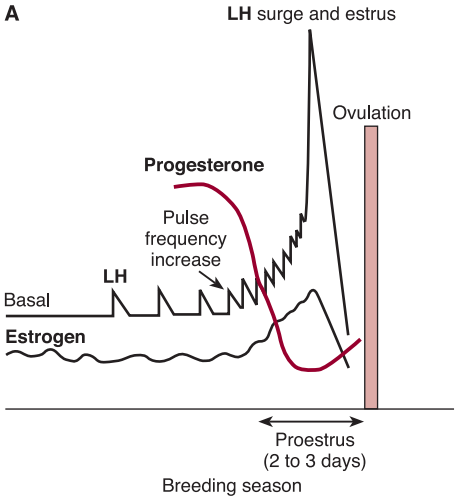
Studies indicate that anestrus can be shortened in bitches through dopamine–agonist (bromocriptine) administration (Ch. 56). Low-dose treatment is associated with a rise in basal plasma FSH levels (without a concomitant increase in LH), which leads to folliculogenesis.

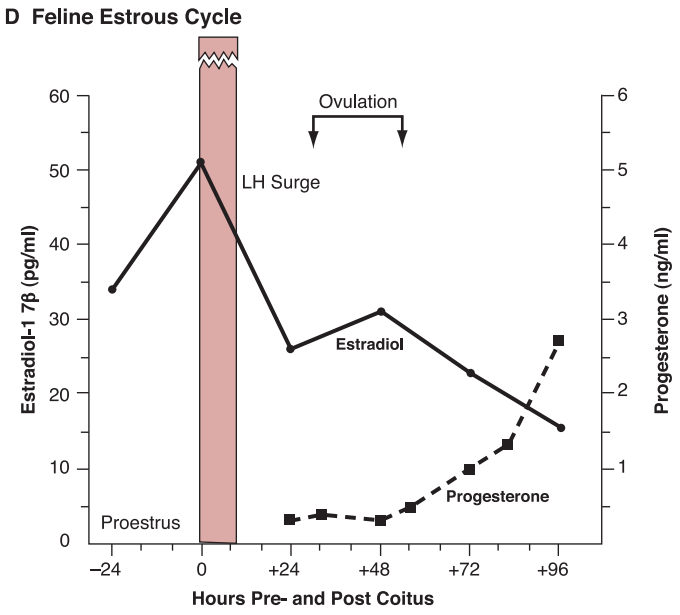
Timing of the Estrous vs. Menstrual Cycles

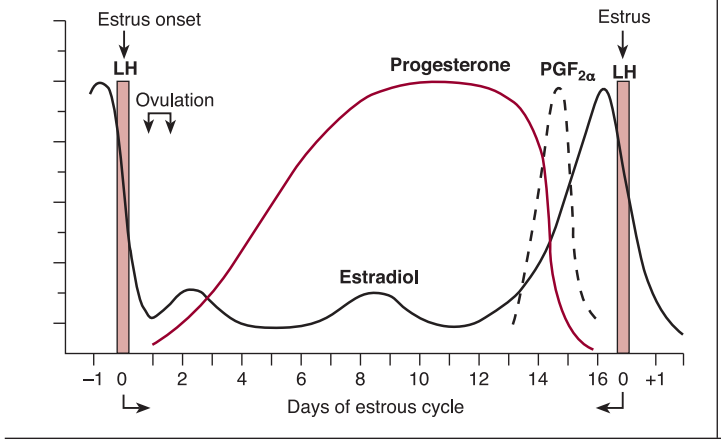
The primary difference between menstruating and nonmenstruating species is the **anatomy of the endometrial arterial blood supply** (i.e., menstruating species have coiling arteries supplying the endometrium). Note that in nonmentruating species, the endometrium does not degenerate following the decline of the CL (to the same extent as in menstruating species). The preovulatory (or follicular) phase of the estrous cycle (**proestrus**) is generally short (2 to 3 days), because there is no need to fully regenerate the endometrium (**Part D**). Timing of the mentrual and estrous cycles also differs. **Day 1** of the **estrous cycle** is the first day of **"heat"** (i.e., **estrus**, the most conspicuous *behavioral* event), while **day 1** of the **mentrual cycle** is the first day of **mentstruation** (i.e., the most obvious *physical* event).

Ch53 Ovine, Canine and Feline Estrous Cycles

(Endocrine Parameters)



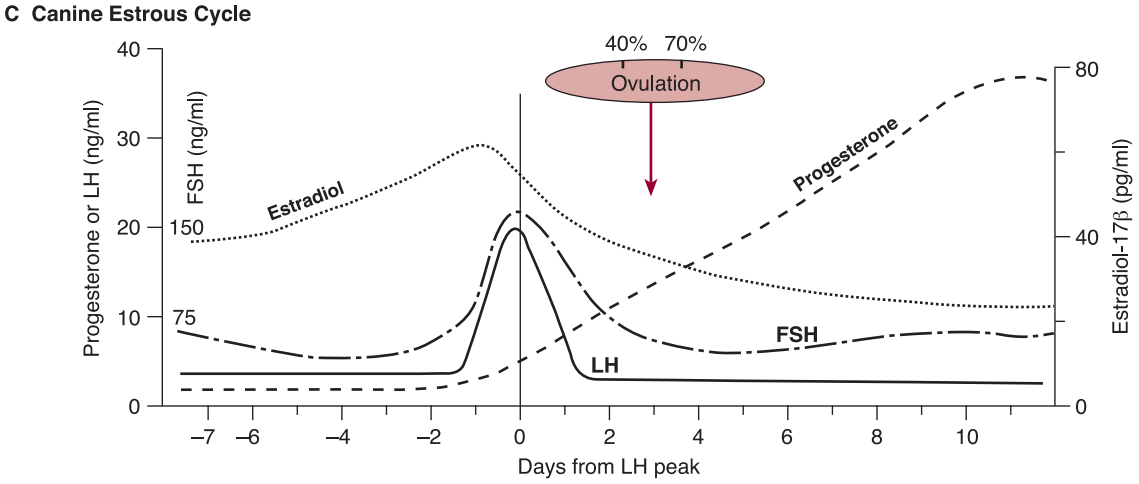




Canine Interestrus Intervals

Basset Hound	5.8 months
Beagle	7.4
Boston Terrier	8.1
Boxer	8.0
Chihuahua	7.2
Cocker Spaniel	6.0
Dachshund	7.0
German Shepherd	5.0
Pekingese	7.7
Scottish Terrier	6.5
Toy Poodle	8.0

From Pineda MR, Dooley MP [eds]: McDonald's veterinary endocrinology & reproduction. 5th ed, Ames, IA, Iowa State Press, 2003.



Sources: Part B modified from Caldwell BV, Tillson SA, Brock WA, Speroff L: The effects of exogenous progesterone and estradiol on prostaglandin $F_{2\alpha}$ levels in ovariectomized ewes. Prostaglandins 1972;1:217. **Part C** modified from Pineda MR, Dooley MP [eds]: McDonald's veterinary endocrinology & reproduction. 5th ed, Ames, IA: Iowa State Press, 2003;477. **Part D** modified from Banks DH, Stabenfeldt G. Biol. Reprod. 1982; 26:603.

Two hormones from the adenohypophysis that most affect the gonads of mammals are the gonadotropins **luteinizing hormone** (**LH**), sometimes called **interstitial cell-stimulating hormone** (**ICSH**) in males, and **follicle-stimulating hormone** (**FSH**). **Prolactin** exerts luteotropic effects in certain species, including the mouse, rat, and ferret.

Mammalian ovaries are dependent on **FSH** for follicular growth and maturation, and on **LH** for estrogen synthesis, ovulation, and initial growth of the **corpus luteum** (**CL**). Both gonadotropins are continuously synthesized and stored in the pituitary, from which they are released during the estrous cycle. The pattern of release takes three forms: **basal**, **pulses**, and **surges** (**Part A**). **Basal** release refers to low and relatively constant concentrations of gonadotropins in blood; **pulses** are sharp, increased concentrations above basal levels; and a **surge** is a large increase in concentration significantly above basal levels, lasting for more than one hour (Ch. 56). The **LH surge** is associated with estrus (i.e., mating) behavior, with ovulation generally occurring a few hours later.

Ovine Estrous Cycle

Examination of the **ovine estrous cycle** (**Part B**) shows the following:

1. The **CL** is the main source of **progesterone**. Blood levels of progesterone are low during estrus, then increase rapidly from day 2 following estrus. High levels of progesterone from the CL inhibit gonadotropin release.
2. If embryos are not present in the uterus, progesterone levels decline rapidly due to **prostaglandin ($PGF_{2\alpha}$)-induced CL regression** about two weeks following estrus.
3. As progesterone declines, **estrogen** increases due to FSH-stimulated follicular growth and maturation.
4. **Estrogen** peaks on day 16, followed by an **LH surge** 12 hours later. Behavioral **estrus** begins.
5. **Ovulation** occurs 10 hours after the end of estrus.
6. The **CL develops** and progesterone rises.

Although hormonal patterns during the estrous cycle may differ somewhat between animal species, those occurring in the **ewe** are not atypical.

During the nonbreeding season, the ovaries of the ewe undergo some follicular development, but ovulation does not occur, and the ewe does not express behavioral estrus. However, as the breeding season approaches, gonadotropic hormones stimulate ovarian follicles to mature, secrete estrogen, and ovulate (**Part A**). Estrogen secreted by maturing follicles also stimulates changes in the oviducts, uterus, and vagina that help prepare them for copulation and pregnancy.

Although spermatozoa are thought to survive within the reproductive tract of the ewe for about 48 hours, the fertilizable life of the ovulated ewe oocyte is considered to be only about 10-12 hours. However, ovulation in ewes usually occurs at the end of estrus, and matings are most likely to occur during estrus.

Like sheep, domestic **goats** are also seasonally polyestrous, and their breeding activity is influenced by photoperiod (Chs. 60 and 61). Although there are several similarities observed in hormonal patterns of the estrous cycles in sheep and goats, there are also distinct differences. Additionally, there are genetic, anatomic, and physiologic differences between reproductive processes in these animals. Unfortunately, space limitation precludes a discussion of those differences.

Canine Estrous Cycle

Periods of the four primary phases of the canine estrus cycle, **proestrus** (mean length = 9 days, range = 2-15), **estrus** (mean = 10 days, range = 3-12), **diestrus** (mean = 65 days, range = 55-90), and **anestrus** (mean = 120 days, range = 40-270) are highly variable. Examination of endocrine changes in the few days before and after the LH peak are shown in **Part C**.

1. As **preovulatory** blood levels of **estrogen** increase, the bitch develops a few external signs associated with estrogenic stimulation, such as edema of the vulva, bloody discharge, and increased receptivity to the male.
2. Near the **end of proestrus**, increasing blood levels of LH stimulate follicular luteinization, as in the ewe, which results in increasing blood levels of progesterone.
3. About 40% of bitches spontaneously **ovulate** within two days, and 70% within three days following the onset of estrus.
4. The bitch, unlike farm animals, continues to accept the male for mating several days after ovulation.
5. **Estrogen waves** are not seen in the bitch during the luteal phase as are seen in the ewe.
6. **Corpora lutea** continue to survive and secrete **progesterone** for 50 to 70 days from ovulation in the bitch, whether she is pregnant or not. Luteolytic factors during nonpregnancy, such as **$PGF_{2\alpha}$** in the ewe, may be lacking in the bitch, or she may be highly resistant. Unlike the ewe, corpora lutea regression in the bitch appears to be due to **aging**.

Each **oocyte** is released from the follicle of the bitch before completion of meiosis. However, completion is thought to occur during oviductal transport, with oocytes remaining viable for several days following ovulation. Pregnancy and conception rates are not reported to be different when bitches are mated only once, either on the 1st or 7th day following the onset of estrus. Canine spermatozoa are also thought to retain their viability for over 1 week within the genital tract of the bitch.

Feline Estrous Cycle

Cats are seasonally polyestrus, and induced (i.e., reflex) ovulators (Ch. 52). Therefore, hormonal characteristics of the feline estrous cycle are influenced greatly by mating (**Part D**). The onset of puberty in the queen is, on average, about 10 months.

Nonmated queens generally display a series of nonovulatory estruses, each lasting about one week. Each nonmated estrus is followed by an interestrous period of nonsexual receptivity, lasting about 10 days. Values reported for the length of nonmated estruses and interestrous intervals varies, most likely because it is difficult to assess accurately the onset and end of estrus solely through behavioral signs in the absence of a tom. Nonmated queens, in colonies maintained under conditions of controlled light and temperature, have been reported to display an average of 13 estruses per year, with a range of 4 to 25. The average elapsed time from the onset of one nonovulatory estrus to the next is reportedly 17 days.

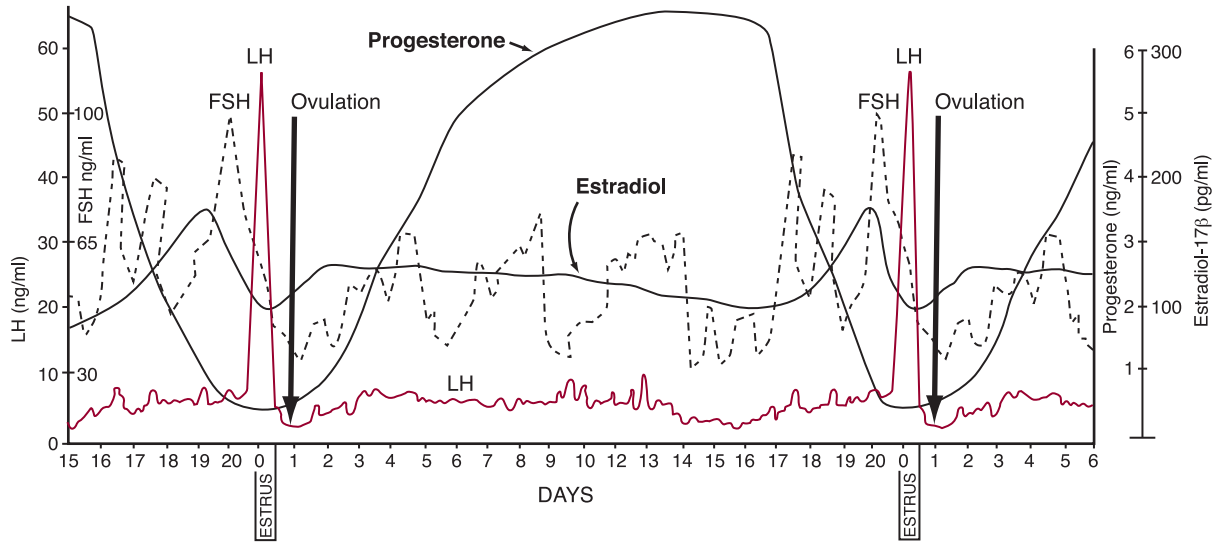
The period of proestrus in the **queen** is known to be a rather short one to three days, with follicular growth occurring rapidly. Due to this short period of follicular growth, this phase of the estrous cycle can be difficult to detect. Like in the ewe and bitch, however, it is associated with a rise in circulating estrogen (17β-estradiol).

During the first five days of estrus, as developing follicles reach a mature stage, concentrations of circulating estrogen decline. During this period of time, however, developing follicles retain their capacity to respond to an ovulatory surge of LH. Should mating occur, copulation will stimulate afferent neural pathways to the hypothalamus, causing release of gonadotropin releasing hormone (GnRH), which in turn stimulates LH release into blood from the anterior pituitary (Ch. 52). This hormone will in turn promote the ovulatory process, with potential fertilization. **Ovulation** normally occurs 24-50 hours following mating. The number of follicles that succeed in ovulating and the number of oocytes released are dependent upon the number of matings. This may be related to the day of estrus, since ovulation is more likely to occur after the second day of estrus.

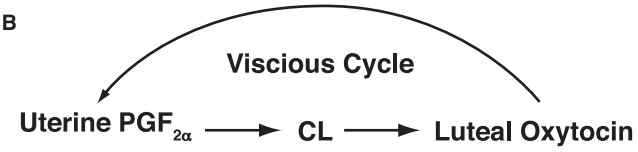
Those follicles in the queen that have reached the mature stage during a given estrus, but have not succeeded in ovulating, will undergo atresia (i.e., become atretic) as blood levels of estrogen continue to decline. This process will be repeated several times during the breeding season if mating and fertilization do not occur. New waves of follicles will develop at each succeeding proestrus, and continue developing during each estrus. In general, blood levels of **progesterone** have been found to remain at basal levels during these nonovulatory estruses.

Ch 54 Bovine, Equine and Rodent Estrous Cycles
(Endocrine Parameters)

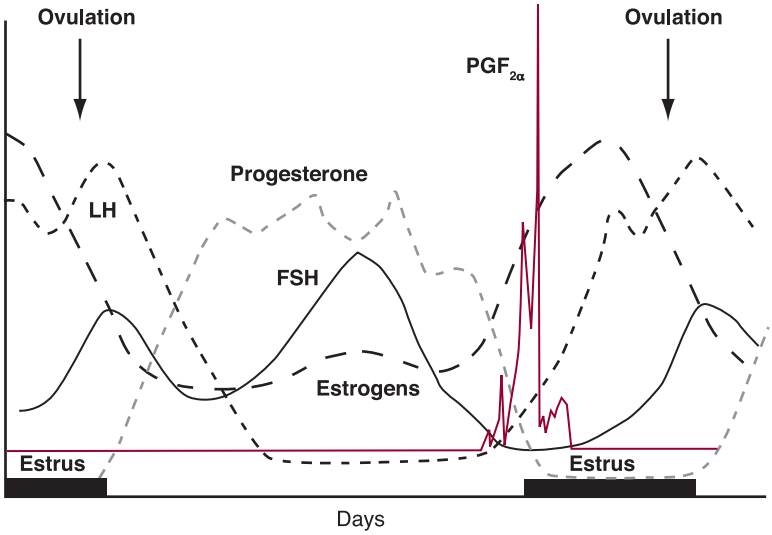
A **Bovine Estrous Cycle**



B

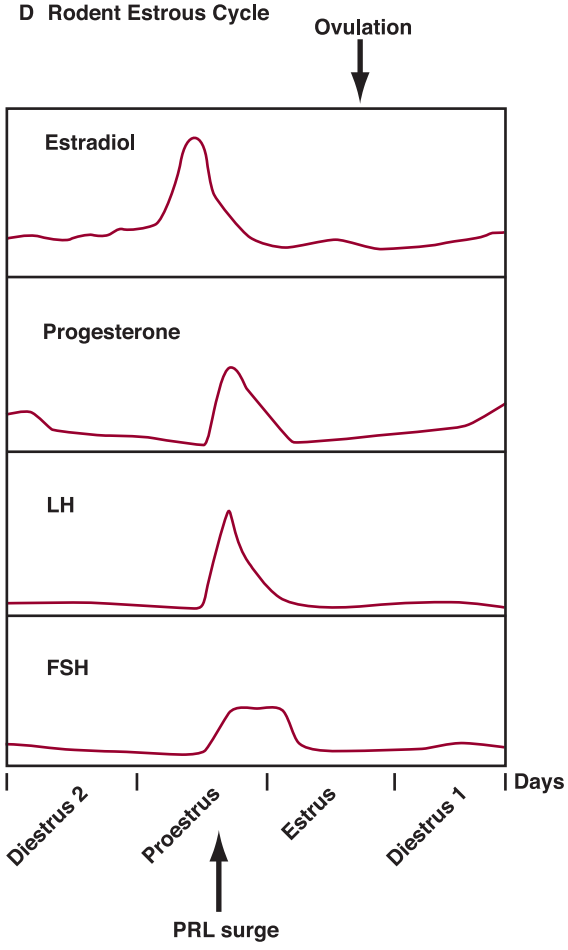


C **Equine Estrous Cycle**



During the mare's winter anestrus, melatonin secretion from the pineal gland suppresses hypothalamic GnRH output, which further suppresses pituitary gonadotropin release (Ch. 60), therefore this aspect of the hypothalamic-pituitary axis is thought to be inactive. However, the suppressive effects of melatonin due to long periods of winter darkness can be overcome by exposure to artificial lighting regimens.

D **Rodent Estrous Cycle**



Sources: Part A modified from Pineda JH, Dooley MP. McDonald's veterinary endocrinology and reproduction. 5th ed. Ames, IA: Iowa State Press, 2003:396, and Schams D, et. al., Acta Endocrinol. 86:180, 1977, Theriogenology 10:453, 1978. **Part C** modified from Eilts BE, Jones E, and Huey E. **Part D** modified from Butcher RL, Collins WE, Fugo NW: Endocrinology 94:1704, 1974.

Onset of Puberty

The onset of puberty in animals is governed by maturity of the hypothalamic-adenohypophyseal axis, nutrition, photoperiod, and body weight. GnRH and gonadotropin secretion are generally kept in check before puberty because the hypothalamus is highly sensitive to negative feedback inhibition exerted by low levels of estrogen in females, and testosterone in males. Hypothalamic maturation at puberty results in reduced sensitivity, thus resulting in GnRH release, which triggers gonadotropin secretion (in pulsatile form), which then promotes ovulation and spermatogenesis.

Bovine Estrous Cycle

Endocrine parameters during the four primary phases of the **21 day** bovine estrus cycle, **proestrus** (day 19 to onset of estrus), **estrus** (day 0), **metestrus** (days 1 to 4), and **diestrus** (days 5 to 18), show cyclic changes (**Part A**), similar to those of other ruminants, with control exerted by the hypothalamic-pituitary axis in concert with cyclic ovarian structures and the uterus. Cows exhibit a short period of sexual receptivity (estrus), about **18 hours**, with ovulation occurring less than a day later. The duration of estrus in heifers is shorter, about **15 hours**.

Follicular growth and regression occurs in distinct waves throughout the bovine estrus cycle in response to **FSH** secretion. About 2 to 3 waves occur, each consisting of a group of developing follicles, where one becomes dominant. In the presence of elevated **progesterone**, the dominant follicle increases in size, and eventually ovulates. Cohort follicles, which eventually undergo atresia, produce **estradiol**, which synergizes with progesterone to suppress **GnRH** and **LH** release. Absence of an implanted embryo allows pulsatile release of endometrial prostaglandin $F_{2\alpha}$ (**$PGF_{2\alpha}$**; not shown in **Part A**), which enters the utero-ovarian vein, passing into the ovarian artery and subsequently reaching the corpus luteum (**CL**). This eicosanoid stimulates **luteal oxytocin** release into blood, which then stimulates further secretion of **$PGF_{2\alpha}$** from the endometrium (**Part B**). This "vicious cycle" continues until luteal oxytocin is depleted, signifying CL regression and cessation of progesterone production. With removal of progesterone suppression and a rise in circulating estradiol, the hypothalamus releases GnRH, the LH pulse frequency increases, behavioral estrus ensues, and ovulation occurs 27 to 30 hours later. If pregnancy occurs, this luteolytic cascade is blocked, and **progesterone** levels continue to rise (thus "prolonging gestation"). Cows require a functional CL for the first 200 to 220 days of gestation.

Equine Estrous Cycle

Mares are seasonally polyestrous (spring-fall), with estrus cycle onset influenced by **photoperiod** (Chs. 60 and 61), and interestrus intervals during the breeding season generally last 19-26 days. Although equine, like many other domestic animals, express each of the 4 primary phases of the estrus cycle during the breeding season (i.e., **proestrus**, **estrus**, **metestrus** and **diestrus**), only 2 phases are routinely recognized (**estrus**, days 16-21, and **diestrus**, days 2-15). This species is unique in that proestrus occurs during estrus, and metestrus during diestrus.

Subsequent to the initial secretion of **$PGF_{2\alpha}$** from the unimplanted endometrium at days 14-16 (**Part C**), and **luteal oxytocin** (not shown), the CL rapidly regresses so that $PGF_{2\alpha}$ concentrations in blood are at baseline in approximately 24-48 hours. This occurs in the absence of a counter-current anatomical system (i.e., utero-ovarian vein to ovarian artery) demonstrated in cows, sows and ewes. With the sharp decline in progesterone and rise in estrogens (mainly estradiol-17β and estrone), mares exhibit **estrus behavior** quickly. During estrus, regression of the CL from the previous cycle has been completed and follicles grow rapidly under LH and FSH stimulation. Initial recruitment of ovarian follicles begins 12-14 days prior to ovulation, when progesterone levels are high. Further maturation of the dominant follicle begins 6-7 days before ovulation. Because the mare ovulates 24-48 hours before the end of estrus, metestrus (the postovulatory period during which the CL forms and begins secreting progesterone) is contained within the last 2 days of estrus. The mare is unusual in that **LH** is still rising when **ovulation** occurs, and elevated **progesterone** levels cause her to reject the stallion.

Stallions also undergo seasonal reproductive changes, however they are not as evident as in the mare. Maximal ejaculate volumes, spermatozoal concentrations, LH and FSH levels occur during the breeding season, and are minimal during winter months. The stallion does not undergo seasonal reproductive quiescence like most mares.

Return of Estrous Cycles During Pregnancy and After Parturition

Estrus behavior is occasionally observed in **pregnant** cows, sows, ewes, mares, and queens, usually without ovulation. Parturition is generally followed by a period of **lactational anestrus** (except in cows and mares). Estrous cycles reappear about a week after offspring are weaned. It is usual for sows to exhibit an **anovulatory estrus** within 2 days postpartum, and mares experience a fertile estrus, termed **"foal heat,"** 7-14 days postpartum. Mares are frequently bred during foal heat, despite results indicating a reduced fertilization and high abortion rate. Cows present with a fertile ovulation about 21 days postpartum, but it is usually not preceded by overt estrus signs. It is termed **"silent heat,"** and is infrequently detected. Ewes of most sheep breeds reproduce only during short day periods. After lambing, they experience long periods of daylight which suppresses LH secretion, producing **seasonal anestrus**. Photoperiod manipulation or administration of gonadotropins or melatonin can sometimes overcome this dilemma (Chs. 58 and 59).

Rodent Estrous Cycle

The short estrous cycle of the rat makes it an ideal investigative model, however, changes in endocrine parameters during the cycle are somewhat atypical compared to other mammals. Mean duration is 4-5 days, however some animals present with longer regular or irregular cycles. Like other mammals, the rat exhibits periods of **proestrus**, **estrus**, **metestrus**, and **diestrus**, but the period of **metestrus** overlaps into **diestrus**, which is then commonly subdivided into **diestrus 1** (**metestrus**), and **diestrus 2** (**Part D**). Sexual maturity is reached by 1-2 months (**Appendix, Table II**).

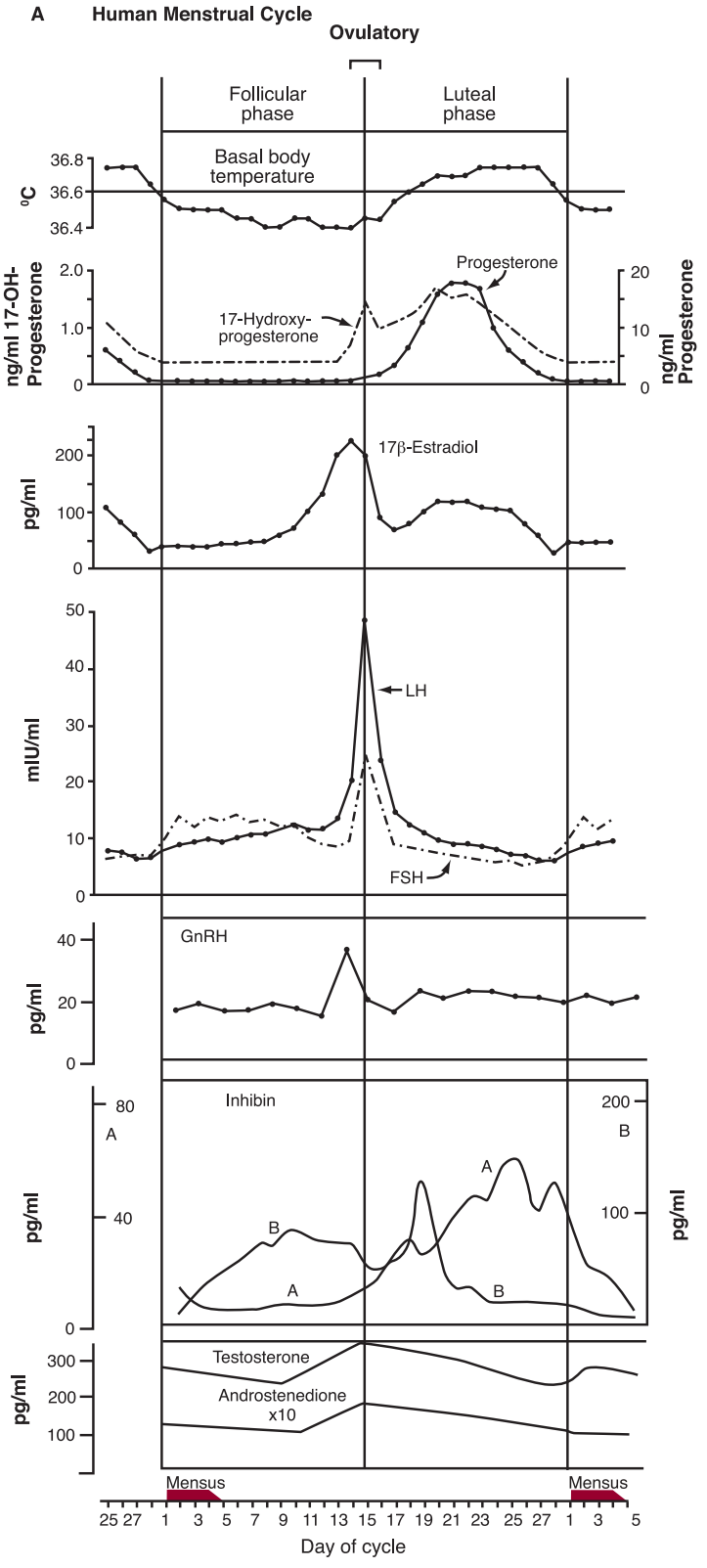
During the estrous cycle, **LH**, **FSH**, **estradiol**, **progesterone**, and **prolactin** (**PRL**) blood concentrations peak during **proestrus**, then progesterone concentrations increase again, but to lower levels during diestrus 1 and 2. The progesterone rise during proestrus, along with estradiol, promotes **GnRH** (also called **LHRH** in rodents) release. Estradiol induces the PRL surge, but the mechanism is not entirely clear. Although seldom reported, this rise in PRL during the follicular phase of the estrous cycle may be common to several species. Mating during estrus causes twice-daily PRL surges that last for 8-10 days, and act to rescue corpora lutea from the previous estrous cycle, establishing progesterone secretion necessary to maintain pregnancy. **Ovulation** occurs during the last one-third of estrus.

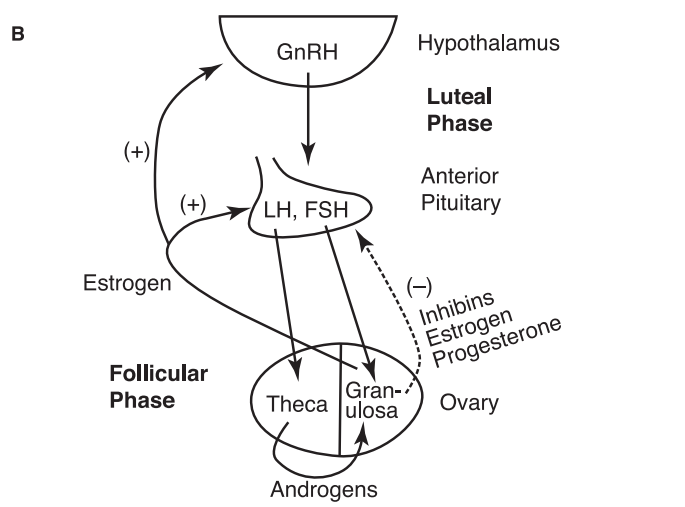
The estrus cycle of the **mouse** is similar, but varies in length (4-6 days), and is less well defined. Ovulation occurs during the first one-third of estrus. Sterile copulations in rodents can induce a state of **pseudopregnancy** (Ch. 52), which can extend the period of diestrus 1 (metestrus) by 10-13 days.

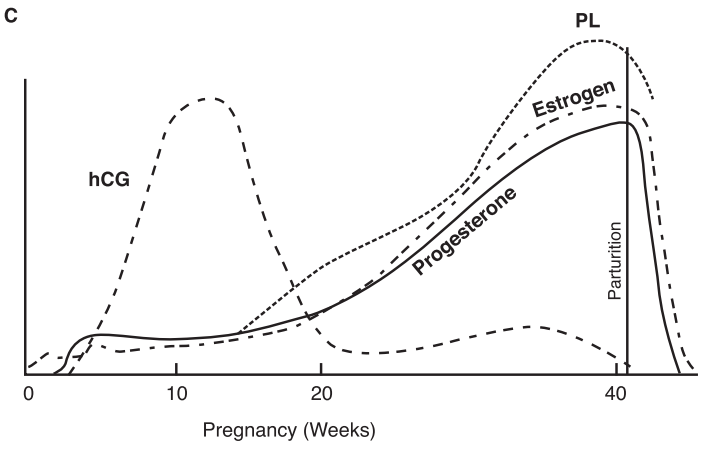
Seasonal changes in photoperiod, which represent a natural and reliable proximate cue of environmental change, promote alterations in the reproductive activity of many rodent species. Although this allows them to conserve energy during harsh winter conditions, winter breeding is frequently observed, which is thought to reflect differential responsiveness to photoperiodic changes among rodent populations.

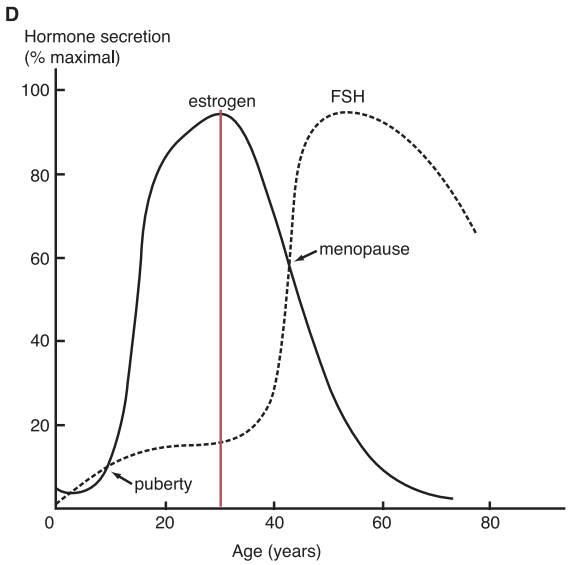
Ch55 Primate Menstrual Cycle

(Endocrine Parameters)









Sources: Part A modified from Ganong WF: Review of medical physiology, 11th ed, Los Altos, CA, Lange Med. Pub., 1983; Midgley AR: Human reproduction. Hafez ESE, Evans TN [eds], Harper & Row, 1973; and Berne RM, Levy MN: Physiology, 4th ed, St Louis, MO: Mosby, 1998. **Part B** modified from Norris DO: Vertebrate endocrinology. 3rd ed, San Diego, CA: Academic Press, 1997. **Part C** modified from Strand FL: Physiology, a regulatory systems approach, New York, NY, Macmillan Pub. Co., 1965.

The primate menstrual cycle is routinely subdivided into 4 phases; **1**) the **follicular** or **proliferative** phase, **2**) the **ovulatory** phase, **3**) the luteal or secretory phase, and 4) menses (**Part A**). The follicular phase begins with the onset of menstrual bleeding (menses), and averages 15 days (range = 9-23 days). Overt menstruation (where there is blood flow from the uterine endometrium through the vagina) occurs in humans and their close evolutionary relatives (Ch. 56). The ovulatory phase lasts 1-3 days, culminating in ovulation near the time of the gonadotropin (LH & FSH) surge. The luteal phase has a more consistent length of about 14 days, ending with menstruation. Duration averages 28.5 days, but can vary from 21-35 days, depending on the length of the follicular phase.

A series of cyclic changes in **gonadal steroid** and **protein hormone** production characterize adult ovarian function. This profile results from cyclic alterations in pituitary gonadotropin release coupled with paracrine and autocrine effects on the ovarian follicle. Changes in gonadotropin secretion reflect changes in pituitary sensitivity to **GnRH**, and changes in pulsatility of the **hypothalamic GnRH generator** (Ch. 53). The pattern of gonadotropin release is also regulated by negative and positive feedback from gonadal steroids, gonadal inhibins and activins (Ch. 57).

A few days before the onset of **menses**, plasma gonadotropin concentrations are at their lowest levels. **FSH** begins to rise, followed by a slow elevation in plasma **LH**. **Estrogen** (17β-estradiol and estrone) levels increase gradually, stimulated by the modest rise in FSH during the first half of the **follicular phase**. During the second half, FSH levels fall modestly, whereas LH levels continue their slow ascent, with the LH:FSH ratio increasing to about 2. Concurrently **estrogen** production increases sharply (in the absence of progesterone), providing positive feedback to the **GnRH pulse generator**. **Androstenedione** and **testosterone** levels also rise modestly in parallel with estrogen, indicating extensive aromatization of these androgens in ovarian granulosa cells to estrogen (Ch. 57). **17-OH-Progesterone** (a weak progestational steroid) concentrations in plasma also increase, and will continue to be secreted by the CL during the luteal phase. If pregnancy ensues, 17-OH-progesterone becomes a good indicator of CL function (since progesterone biosynthesis will eventually become placental).

The succeeding **ovulatory phase** is characterized by a sharp surge in plasma gonadotropin concentrations, taking an average of **14 hours** to occur. Plasma estrogen levels fall from their peak at the same time LH and FSH are on their ovulatory upswing. Plasma androgen concentrations decline, but a significant elevation in **progesterone** is initiated. Typically, a single follicle in one of the ovaries will reach maturity in a given cycle, with ovulation often occurring in the alternate ovary during the following cycle. Following ovulation, **luteal output** of progesterone is significant, and a second rise in 17β-estradiol occurs. Progesterone is thermoginic, and tends to shunt blood toward the internal organs causing a slight rise in the **basal body temperature** for about 1-2 days following ovulation.

Inhibin levels fluctuate systematically throughout the menstrual cycle, and there are two inhibins (and two activins) in extracts of the testes and in antral fluid from ovarian follicles (Ch. 57). **Inhibin B** rises during the follicular phase in parallel with FSH, displaying a periovulatory phase peak, then becoming low during the luteal phase. **Inhibin A** levels are low during the follicular phase, but increase markedly in parallel with progesterone during the luteal phase. Inhibin B is secreted from granulosa cells of the dominant follicle, whereas inhibin A arises predominantly from the CL. Inhibin B plus estradiol feedback negatively on the piutitary to reduce FSH secretion during the latter part of the follicular phase, whereas inhibin A plus estradiol and progesterone feedback negatively on the pituitary to suppress gonadotropin output throughout the luteal phase (**Part B**).

Regression of the CL (**luteolysis**) starting 3-4 days before menses is a key event in the menstrual cycle, for without it menses will not occur. **$PGF_{2\alpha}$** appears to be a physiologic luteolysin in primates, like in domestic animals, but this eicosanoid is only active when endothelial cells producing **endothelian-1** (**ED-1**, a vasoconstrictor) are present. In most domestic animals, **oxytocin** secreted by the CL appears to exert a local luteolytic effect by promoting endometrial release of $PGF_{2\alpha}$ (Chs. 53 & 54). Once luteolysis begins, **estrogen** and **progesterone levels fall** and gonadotropin secretion increases. A new crop of follicles develops, then a single dominant follicle matures. Another cycle has been initiated.

Midcycle Control

The midcycle gonadotropin surge is short (12-24 hrs.), and is one of the few examples of normal physiologic positive feedback. In order for this surge to occur, **estrogen** must be maintained at a concentration of about 200 pg/ml for sufficient duration (≈ 36-48 hrs.). The presence of elevated progesterone with estrogen during this period of time (e.g., the traditional birth control pill), inhibits this surge. Estrogen exerts its positive feedback effects on the pituitary as well as the hypothalamus. This concept was developed from experiments in monkeys whose medial basal hypothalamus, including the GnRH-producing neurons, was destroyed by lesioning, resulting in a marked decrease in plasma LH. Administration of exogenous GnRH at a fixed frequency restored LH release. When estrogen was given at an optimal concentration for an appropriate period of time, an LH surge was generated. Estrogen appears to increase the number of GnRH receptors on pituitary gonadotropes, thus increasing responsiveness to GnRH. It also increases activity of the GnRH pulse generator (Ch. 56), serving to fine-tune this mechanism. A small but distinct rise in **progesterone** also occurs before the gonadotropin surge, which appears to be important in augmenting it.

Pregnancy

Should fertilization occur following ovulation, trophoblastic cells of the blastocyst secrete **human chorionic gonadotropin** (**hCG**; **Part C**) prior to implantation, an LH-like protein that maintains luteal output of gonadal steroids (Ch. 62). **hCG** output and luteal function are maintained for several weeks, until the fetal adrenal-placental unit produces sufficient progesterone and estrogens to maintain further pregnancy. During this entire period , pituitary gonadotropin release is inhibited.

The stimulus for birth is related to a marked increase in the **estrogen:progesterone ratio** (Ch. 69). High estrogen levels nullify the anesthetic properties of progesterone on uterine smooth muscle, and allow **oxytocin** and **$PGF_{2\alpha}$** to initiate uterine contractions and the onset of labor. This action of oxytocin is a consequence of increased uterine receptors (stimulated by estrogen) rather than an immediate elevation in circulating blood levels. Premature birth is often correlated with enlarged **fetal adrenals**, whereas delayed births are associated with fetuses that have underdeveloped adrenals (Ch. 63).

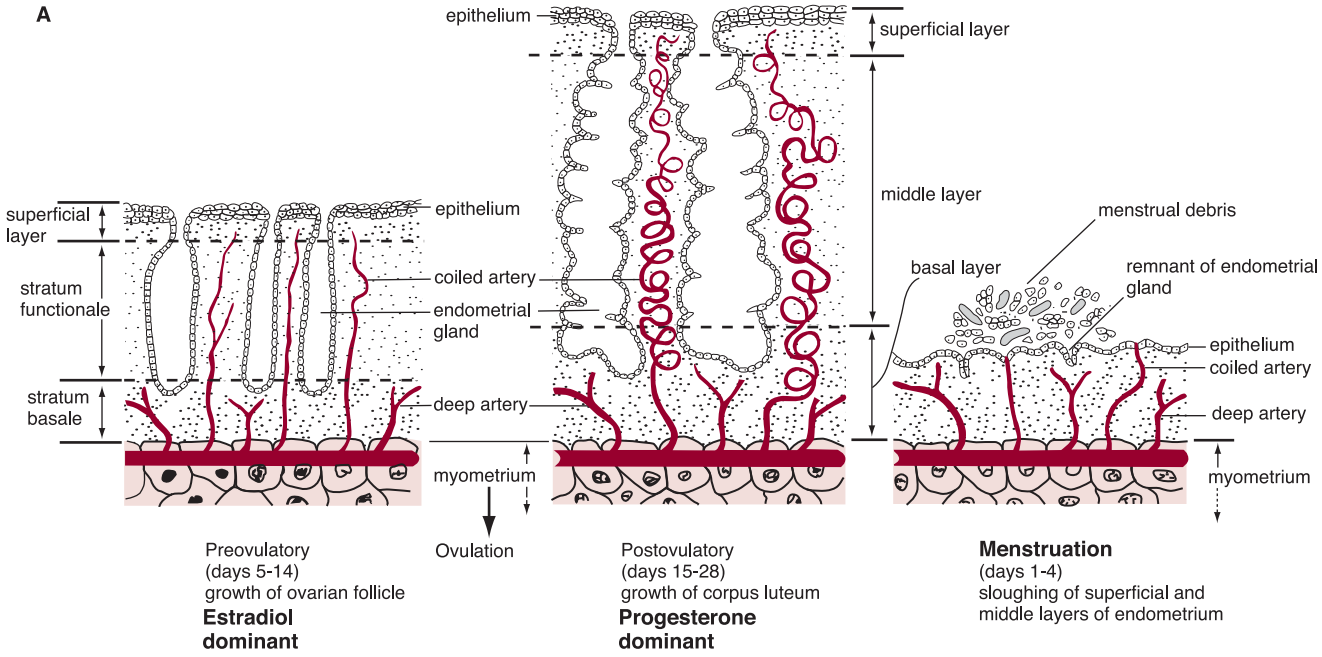
Menopause

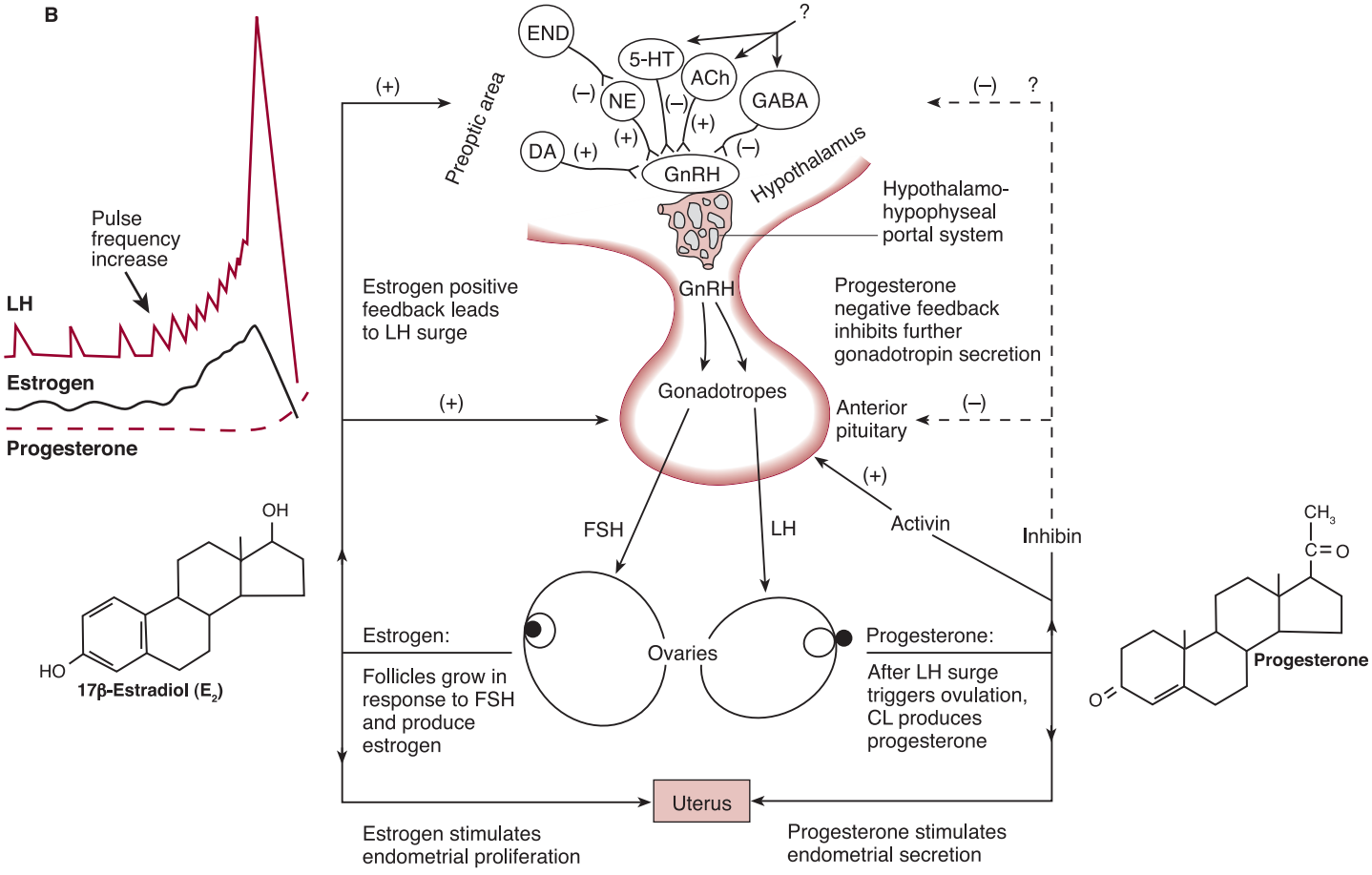
Life after reproductive age is common among humans. Whereas men may produce viable sperm most of their lives, the ovaries become refractory to gonadotropins, usually during the mid- to late 40s, the transitional stage of **menopause**. Few animals live beyond their peak of reproductive activity owing to predation, disease or other environmentally related phenomena, thus menopause is rare.

The menstrual cycles of menopausal women become irregular and eventually they cease to ovulate and menstruate. This is accompanied by a marked depression in circulating gonadal steroids, and elevation in pituitary gonadotropins (**Part D**). The transition is usually gradual , and may be accompanied by vaginal atrophy, hot flashes, reduced libido, and accelerated bone resorption leading to osteoporosis (Ch. 20). Studies demonstrate that cardiovascular disorders increase exponentially in postmenopausal women, with death rates due to cardiac disease being several times greater than for uterine and breast cancer combined.

Ch56 Menstruation and Regulation of Gonadotropin Release

(Control of the GnRH Pulse Generator)





Menstrual cycles occur in humans, old world monkeys (e.g., Rhesus, macaques, mandrills, chimpanzees and baboons), gibbons, the slender loris, marmosets, zebras, the elephant shrew, and 3 species of bats. Sloughing of the uterine lining results in a vaginal discharge of uterine epithelial cells and trapped blood (**overt menstruation**). Some mammals are known to exhibit **covert menstruation**, during which sloughed tissues of the uterine endometrium are reabsorbed, but there is little, if any uterine discharge. Although other animals may shed minor amounts of blood from the uterus during the estrous cycle, **domestic animals do not generally menstruate**. Cows may shed some blood from the uterus during metestrus (about 48 hours after the onset of heat), due largely to intensive endometrial stimulation by estrogen during proestrus and estrus. A similar condition occurs in the bitch, but usually blood is shed earlier in proestrus. Again, the cause is thought to be overstimulation of the endometrium by estrogen from growing follicles. There may also be some shedding of the endometrial epithelial lining in domestic animals at the end of the estrous cycle. These patches of epithelium, sometimes referred to as **casts,** are shed because of a failing gonadal hormone supply that is unable to maintain the highly developed endometrium. In other words, it is the reaction of a steroid-deficient endometrium when pregnancy does not occur. However, the physiologic shedding of blood or epithelial casts from the uterus of domestic animals is relatively unimportant, and should not be confused with menstruation.

Menstruation

The most accepted theory regarding the cause of menstruation is that when the corpus luteum fails, because pregnancy has not occurred, there is an abrupt drop in estrogen and progesterone (sometimes referred to as **estrogen-progesterone withdrawal**). This withdrawal reduces hormone support to the endometrium, thus allowing rather sudden shrinking. Spiral arteries soon begin to kink upon themselves, thereby shutting off blood supply to the endometrium. Although the precise mechanism for vascular necrosis is uncertain, many believe it to be due in part to spasm of blood vessels caused by locally produced prostaglandins (e.g., **$PGF_{2\alpha}$**). There have been large quantities of prostaglandins found in the secretory endometrium and in menstrual blood, and infusion of prostaglandins produces endometrial necrosis and bleeding. One credible theory holds that in necrotic endometrial cells, lysosomal membranes break down, causing activation of enzymes (e.g., phospholipase A_2) that foster release of unsaturated fatty acids (e.g.. arachidonic acid) from the two position of membrane-bound phospholipids. These unsaturated fatty acids then become substrates for the formation of prostaglandins, which in turn produce vasospasm, vascular necrosis, and menstrual flow. This concept seems plausible, for without a blood supply endometrial tissues necrose, the epithelium is sloughed off, and several layers of endometrial tissue are lost by hemorrhage.

Menstrual blood is mostly **arterial**, with only about 25% normally being of venous origin. Blood lost does not usually coagulate well because of the presence of relatively large amounts of **fibrinolysin** from endometrial tissue. Fibrinolysin is known to lyse clots, thus keeping menstrual blood clot-free unless flow is excessive. Large numbers of **leukocytes** are released during this physiologic process, along with necrotic material and blood (which contains iron). It is probable that some substance liberated through endometrial necrosis causes this outflow of leukocytes. As a result of this menstrual leukorrhea, the uterus is considered to be resistant to infection during menstruation, even through the endometrial surface is denuded. Obviously this is of protective value.

Blood and tissue debris are normally voided for about 1-4 days, and at the end of this stage endometrial regeneration begins. The outer and middle layers that are shed during menstruation, the **superficial layer** and **stratum functionale**, are supplied by long, coiled arteries, whereas the basal layer that is not shed, the **stratum basale**, is supplied by short, deep arteries (**Part A**). Under the influence of **preovulatory estrogen** during the follicular, proliferative phase, endometrial glands increase in number, epithelial cells proliferate, and coiled arteries grow linearly. As a result of this growth endometrial thickness greatly increases. **Progesterone** becomes the dominant sex steroid during the **postovulatory**, luteal secretory period, where a tremendous increase in the growth and coiling of superficial glands and arteries occurs, thus increasing their surface area. In preparation for an implanting blastocyst, the stroma becomes loose and edematous. Endometrial glands also become active, producing large quantities of **mucin**, **glycogen** and **other nutrients**. Mucin helps the blastocyst adhere to the endometrium, and glycogen and other nutrients (i.e., **"uterine milk"**) are needed for embryonic nourishment until the placenta has developed. Progesterone also slows contractions of the myometrium, so that embryos are not expelled. These progesterone effects, however, are largely dependent on the prior priming actions of estrogen.

Regulation of Gonadotropin Release

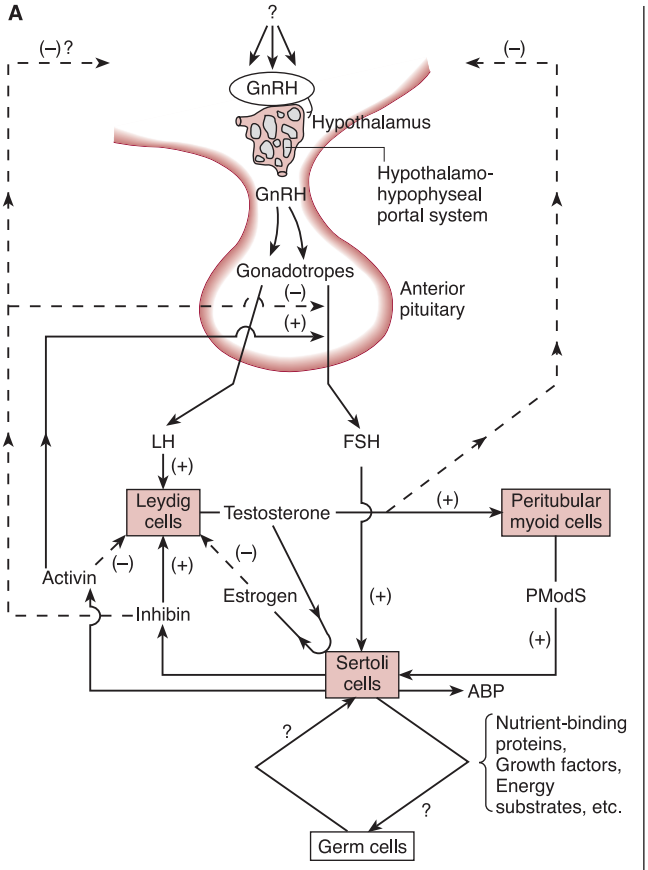
Hypothalamic control of gonadotropin release is exerted by **GnRH** (also called **LHRH**), which is secreted into portal hypophyseal blood (**Part B**). GnRH stimulates pituitary release of **FSH** as well as **LH**, and it is uncertain whether there is an additional separate FSH-releasing hormone (**FRH**).

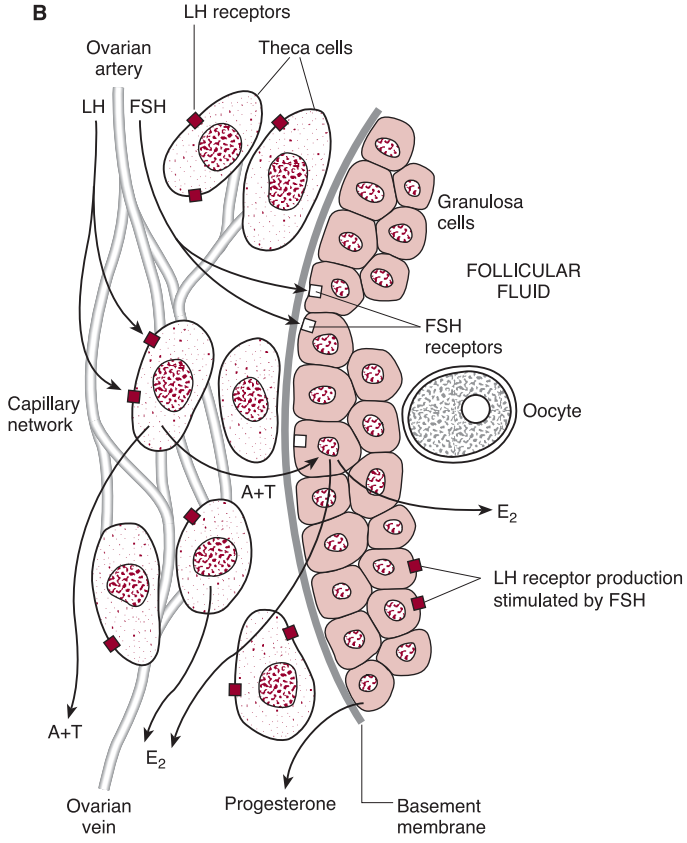
GnRH is normally secreted in a **pulsatile** fashion, which is essential for normal LH and FSH release. If large amounts are administered by constant infusion, GnRH receptors in the adenohypophysis downregulate, and LH secretion ceases. However, if GnRH is administered in small amounts episodically at the rate of approximately 1 pulse/hr, LH and FSH release is stimulated. It is clear that fluctuations in both frequency and amplitude of GnRH bursts are important in generating other hormonal changes responsible for the menstrual and estrous cycles (Ch. 53). Frequency is **increased** and amplitude decreased by high levels of **estrogen** in the absence of progesterone (preovulatory, follicular phase), yet frequency **decreases** with high levels of **progesterone** (luteal phase) or testosterone. Frequency increases late in the follicular stage, culminating in the **LH surge** and ovulation. Duration of the gonadotropin surge is relatively short (usually 12 to 24 hours). During the luteal phase, frequency decreases due in large part to high levels of progesterone and **inhibin A**. When progesterone secretion decreases at the end of the cycle, frequency once again increases (due to lack of negative feedback). **Activin** modulates the effects of inhibin by promoting FSH release (Ch. 57).

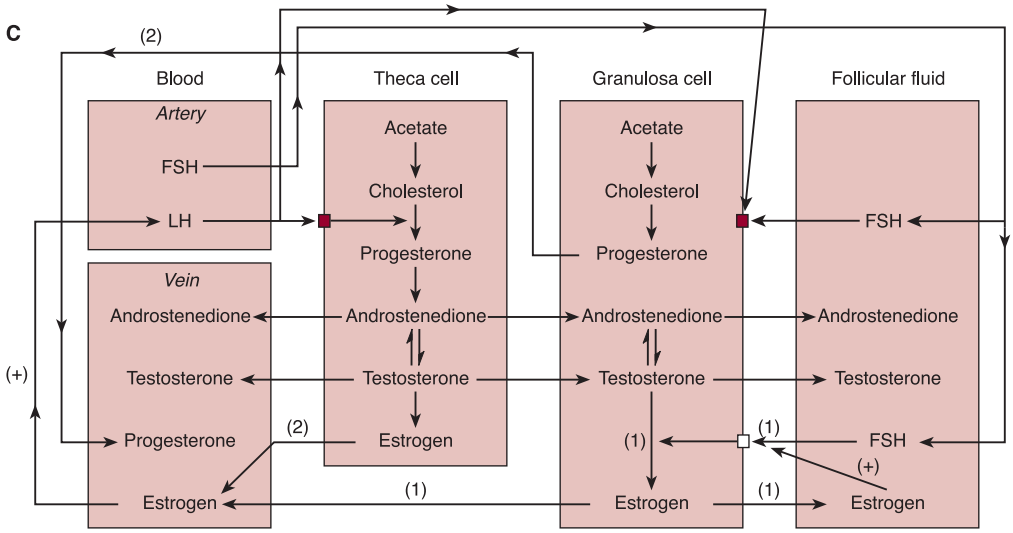
Although the precise location and nature of the **"GnRH pulse generator"** in the preoptic area of hypothalamus are still undefined, it is generally recognized that catecholamines and acetylcholine (ACh) increase GnRH pulse frequencies, while serotonin (5-HT), γ-aminobutyric acid (GABA), and opioid peptides (END) reduce them (Ch. 7). Other agents such as neurotensin, galanin, neuropeptide Y, and angiotensin II are thought to play important roles in the disinhibition-excitation control of the hypothalamic pulse generator, and pituitary gonadotropin release.

At least three observations support the concept that the **ovaries control the basic rhythm of the hypothalamic pulse generator: 1)** no cyclic release of gonadotropins was observed in animals whose ovaries never functioned, or in those whose ovaries had been removed; **2)** the ovulatory gonadotropin surge does not occur until the dominant follicle (or follicles) has/have reached the appropriate stage of development, whatever number of days that may take; and **3)** administration of estrogen in a format that resembles the normal pre-ovulatory estrogen surge, induces an LH surge. Such observations indicate that the GnRH pulse generator in the CNS is required to initiate and sustain follicular development. However, it is the pattern of ovarian secretion, most critically from dominant follicles, that apparently conditions this pulse generator.

Ch57 Spermatogenesis and Ovulation
(Gonadotropin Control)







Male Gonadotropins and Spermatogenesis

The release of **gonadotropin-releasing hormone** (**GnRH**), and subsequently of **gonadotropins**, is thought to proceed in a more continuous fashion in males, compared with the typical cyclic pattern described for females. The **preoptic area** of the **hypothalamus**, which contains neurons responsible for regulating the ovulatory surge of gonadotropins in females (Ch. 56, **Part B**), does not appear to be as functional in males. However, some seasonal variation in male gonadotropin release does occur, indicating that sensory input to the hypothalamus from higher centers in the CNS is involved in male GnRH output. In general, the frequency, quality, and fertility of ejaculates are reportedly improved during the breeding season.

As in females, **GnRH** stimulates **luteinizing hormone** (**LH**) release from the anterior pituitary (**Part A**). Luteinizing hormone binds to specific membrane receptors on **Leydig cells** (**interstitial cells**) of the testes, which leads to generation of cAMP and other messengers that ultimately cause secretion of androgens (i.e., androstenedione and testosterone). Elevation of androgens, in turn, inhibits further LH secretion. This inhibitory effect on the hypothalamus is mediated principally by estrogen, which is formed locally in hypothalamic glial cells from testosterone.

After stimulation by **GnRH**, the gonadotropes also secrete **FSH** into the circulation. This glycoprotein hormone binds to specific receptors on **Sertoli cells** of the testes, stimulating production of **androgen-binding protein** (**ABP**). FSH is necessary for the initiation of spermatogenesis. However, full maturation of spermatozoa also appears to require **testosterone**. Indeed, the major action of FSH on **spermatogenesis** may be stimulation of ABP production, which allows a high intratubular concentration of testosterone to be maintained. In addition to ABP, there is substantial evidence that other compounds are synthesized and secreted by the gonads. For example, testicular Sertoli cells (like ovarian granulosa cells) secrete peptide and protein products that act in endocrine, paracrine, and even autocrine fashion to modulate the processes of gametogenesis. **Inhibin** and **activin** are members of the same superfamily of growth-regulating factors as discussed in Ch. 4.The inhibins (A and B) are glycoproteins that circulate in plasma and inhibit GnRH-stimulated FSH release by the pituitary. It is not known whether they also exert a significant negative feedback at a hypothalamic locus. Activin, another gonadal glycoprotein, has the opposite action, stimulating FSH release. At the gonadal level, inhibin increases whereas activin decreases testosterone secretion; thus, FSH can influence Leydig cell function indirectly by modulating production of inhibin and activin. Other paracrine interactions may also be important in maintaining a proper testicular environment for spermatogenesis. Testosterone from Leydig cells further stimulates differentiation and proliferation of **peritubular myoid cells.** The latter secrete a protein known as **PModS** that stimulates Sertoli cell function. Each of these pathways may vary in functional activity and significance at different points in the cycle of **spermatogenesis**. **Part A** is an overall diagram of pituitary and testicular control of Leydig cell, peritubular myoid cell, and Sertoli cell secretion, as well as spermatogenesis.

Not depicted in **Part A** are other testicular-derived factors that also appear to play important roles in spermatogenesis. **Follistatin** is another FSH-suppressing protein that is produced by the gonads. It may act by binding activin. **Insulin-like growth factor 1** is also synthesized by this cell line and appears to modulate cell growth and hormonal responses within the gonads. Leydig cells also synthesize and secrete **proopiomelanocortin products** and **oxytocin**. A peptide functionally resembling GnRH (but structurally dissimilar to it) is also produced by Sertoli cells. **Intragonadal (gonadocrinin)** has been hypothesized to function in modulating the effects of LH on interstitial cell testosterone secretion. However, a specific role for locally produced GnRH has not yet been established. In addition, a variety of **trace metal-binding proteins**, **steroid-binding proteins**, **proteases**, **prostaglandins**, **lymphokines**, and **extracellular matrix molecules** such as **laminin**, **collagen types I** and **IV**, and **proteoglycans** are also produced. These compounds are thought to exert local actions in the nurturance and development of germ cells, as well as in the exodus of these cells from their gonadal enclave.

Female Gonadotropins and Ovulation

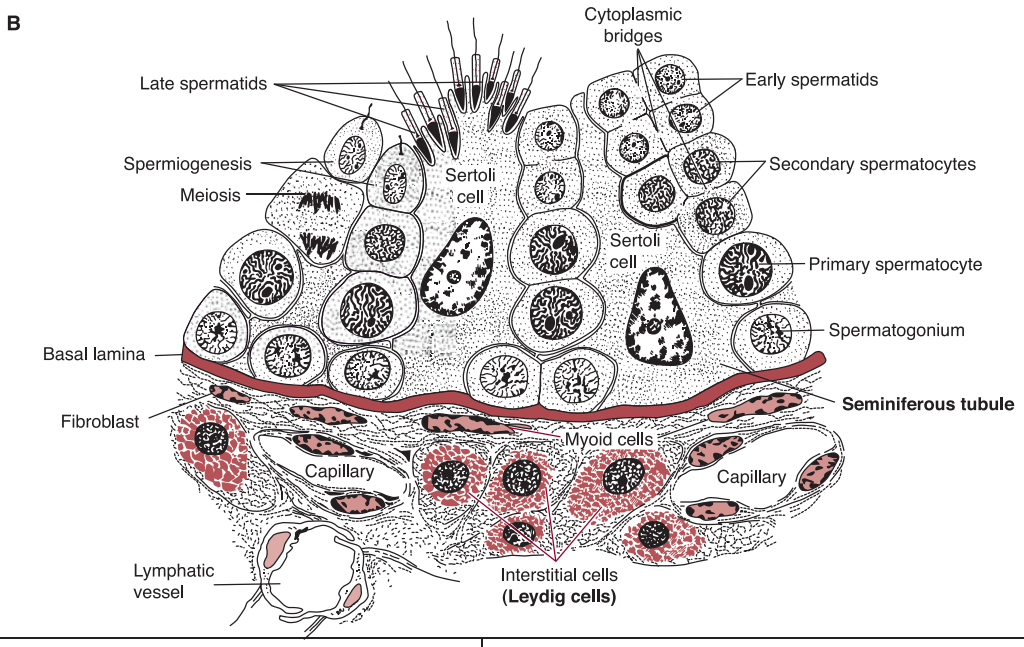
During the early follicular phase of the estrous cycle, ovarian **theca interna cells** (similar to testicular Leydig cells) produce **androgens** [androstenedione (A) + testosterone (T)], and some **estrogen** (estradiol, E_2) in response to **LH** (**Parts A** and **B**). During this time, **granulosa cells** (similar to testicular Sertoli cells) proliferate, and they aromatize the androgens to estrogen under the effect of **FSH** (**Parts B** and **C**). The estrogen so produced also synergizes with FSH to promote replication of granulosa cells (positive feedback; **Part C,** process [**1**]), and the **FSH surge** induces enough **LH receptors** on granulosa cells for **luteinization** to occur. During the midfollicular phase, theca cells continue producing androgens, and granulosa cells estrogen. Also during this time, granulosa cells initiate production of **inhibin**, which inhibits further FSH secretion (Ch. 56, **Part B**). During the late follicular phase, extensive aromatization of androgens to estrogen occurs in granulosa cells (**Part C,** process [**1**]), causing a positive feedback demand on the hypothalamic-pituitary axis, which results in an **LH surge**. During the very late follicular phase (**just before ovulation**), granulosa cells begin secreting **progesterone** (**Part C,** process [**2**]), and theca cells enhance their production of estrogen. During the luteal phase (**following ovulation**), granulosa cells of the CL produce mainly **progesterone**, with theca interna cells continuing their production of **estrogen** (albeit at decreased levels; see **Part C,** process [**2**]).

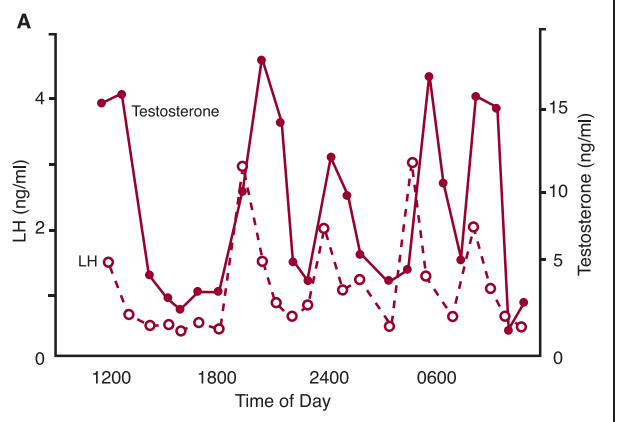
As in testicular Sertoli cells, FSH also stimulates production of a variety of nonsteroidal compounds by granulosa cells that likely have paracrine effects. As previously mentioned, **inhibin A**, whose secretion parallels that of progesterone, inhibits FSH secretion, which is thought to keep competitor follicles (with fewer FSH receptors) from developing. Inhibin also increases androgen secretion from theca cells. **Activin** (also produced by granulosa cells) modulates the effects of inhibin by increasing pituitary FSH secretion and decreasing androgen secretion by theca cells. However, because there is a greater production of inhibin than activin around the time of ovulation, the supply of precursor androgens from theca cells is increased.

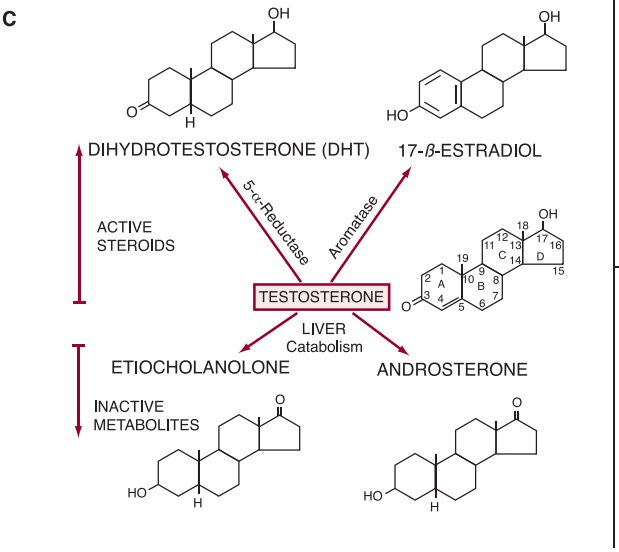
Other paracrine agents from granulosa cells include **transferrin** and **ceruloplasmin** (which pick up iron and copper, respectively, from their plasma-binding analogues and transfer these vital elements to the oocyte). Various **granulosa growth factors** (such as **IGF-1**) modulate growth and steroid hormone secretion by neighboring endocrine cells, and conceivably by the oocyte itself. For example, IGF-1 is thought to potentiate FSH action on granulosa cell differentiation and progesterone synthesis, and also to potentiate LH stimulation of androgen production by theca cells. Other agents thought to play a role in **ovulation** (because of their strong presence in follicular fluid) include **$PGF_{2\alpha}$**, **oxytocin**, **proteolytic enzymes**, **plasminogen activator**, **renin**, and **angiotensin**, as well as some **GnRH-like peptides**. Indeed, **ovulatory processes** appear to utilize the same biochemical factors as **spermatogenesis**.

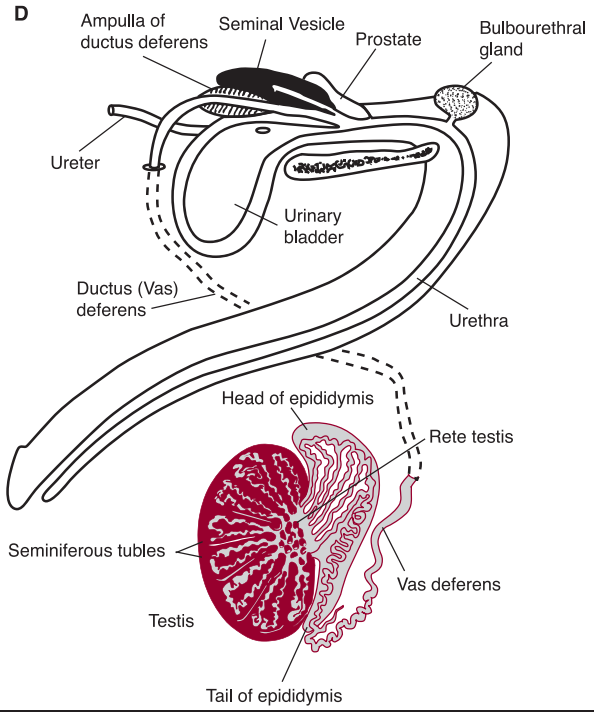
The participation of follicular **myoid cells** (also called **theca externa cells**) and/or sympathetic neurons in the ovulatory process is incompletely understood. Studies indicate that myoid element contraction in the follicular wall may contribute to mechanical rupture and hemorrhage, yet myoid elements may also participate in the postovulatory tissue repair process. Other contributing factors to ovulation include the **LH surge**, activated **collagenase**, and locally produced **estrogen** and **prostaglandins**.

Ch58 Male Reproductive System
(The Testes and Accessory Sex Glands)









E

Species	Seminal vesicles	Prostate	Bulbourethral
Canine	No	Yes	No
Feline	No	Yes	Yes
Ovine	Yes	Yes	Yes
Porcine	Yes	Yes	Yes
Bovine	Yes	Yes	Yes
Equine	Yes	Yes	Yes
Llamas & alpacas	No	Yes	Yes

Sources: Part A modified from Norris DO: Vertebrate endocrinology. 3rd ed, San Diego, CA: Academic Press, 1997. **Part B** modified from Ganong WF: Review of medical physiology. 22nd ed, New York, NY: Lange/McGraw-Hill, 2005; and Junqueira LC, Carneiro J: Basic histology: text & atlas, 10th ed, New York, NY: McGraw-Hill, 2003.

Pulsatility of Male GnRH, LH and Testosterone Secretion

Although GnRH release is thought to proceed in a more continuous fashion in males than females, a pulsatile pattern is observed. The pattern of **LH** and **testosterone** secretion in the bull is shown in **Part A**. Pulsatile release of **GnRH** (not shown) would precede each **LH peak**, which precedes each **testosterone peak**. Although daily secretory patterns for gonadotropins show considerable variation among species, hourly fluctuations in GnRH, LH and testosterone secretion are not uncommon. In animals that exhibit distinct seasonal breeding, spermatogenesis may be restricted to a few months or less.

The Testes and Accessory Sex Glands

The **testes** are paired, encapsulated, ovoid organs with a mass of about **1%** of the body weight in ungulates, but as much as **8%** in gerbils. They reside in the abdominal cavity during development, but migrate in some species to a subcutaneous evagination of the peritoneum, the **scrotum**. Descent, a process assisted by testicular Leydig cell **IGF-3**, occurs during fetal life in large animals, but after birth in others (such as rats and dogs). Cryptorchidism (incomplete descent) is sometimes observed in horses and dogs. The testes of some species are in the scrotum during sexual activity and ascend during sexual quiescence. In birds and a few mammals, they remain permanently intra-abdominal.

The testes contain 2 major components, which are structurally separate and serve different functions. The **Leydig**, or **interstitial cells**, are a major endocrine component (**Part B**). Their primary secretory product, **testosterone**, is responsible either directly or indirectly for embryonic differentiation of the external and internal genitalia (Ch. 62), male secondary sexual development at puberty, and maintenance of libido and potency in the adult. Two active steroids produced in target cells from testosterone are **dihydrotestosterone** (**DHT**), and/or estrogen (**17-β-estradiol**), and the inactive **17-ketosteroid metabolites** produced by the liver are **androsterone** and **etiocholanolone** (**Part C**). These are further **glucuronidated** and/or **sulfated**, then secreted into bile or returned to the circulation where they are filtered by the kidneys and excreted in urine.

Androgens and estrogens exist in blood in either the free (unbound) state or bound to serum proteins. Although about 38% of testosterone is bound to albumin and other plasma proteins, the major binding protein is **sex hormone binding protein** (**SHBP**), also called **sex steroid binding globulin** (**SSBG**). This protein is similar to **androgen-binding protein** (**ABP**), synthesized and secreted by Sertoli cells (Ch. 57), however SHBP is synthesized in the liver. While 60% of circulating estrogens are bound to albumin, SHBP binds estrogens about 5-times less avidly than it binds testosterone or DHT, and SHBP has little affinity for progesterone and cortisol. Progesterone and cortisol bind with nearly equal affinity to **transcortin** (also called **cortisol binding protein** (**CBP**)), which has little avidity for estrogen, and even less for testosterone and DHT.

The **seminiferous tubules** comprise 80-90% of testicular mass, and are responsible for spermatozoa production during male reproductive life, puberty to death. Both of these testicular components, the interstitial cells and seminiferous tubules, are interrelated, and require an intact hypothalamic-pituitary axis for initiation and maintenance of function. In addition, several accessory sex glands are required for the functional maturation and transport of spermatozoa (**Part D**). Disorders of the testes, hypothalamus, pituitary or accessory sex structures may result in abnormalities of androgen or gamete production, infertility, or a combination of these problems.

Androgen-producing **Leydig cells**, PModS-producing **myoid cells**, **blood**, **lymphatic vessels**, **nerves** and **fibroblasts** are all interspersed between seminiferous tubules (**Part A**). **Testicular lymph production** is high in the boar, intermediate in the ram and ferret, and low in the rat (which has large lymphatic vessels). In mammalian species, lymph returns a peptide-rich fluid (e.g., **inhibins** and **activins**) to the circulation. Spermatic arteries to the testes are tortuous, and blood in them runs parallel but in the opposite direction to blood in the pampiniform plexus of spermatic veins. This arrangement permits countercurrent exchange of heat and steroid hormones. Tight junctions between adjacent **Sertoli cells** and **peritubular myoid cells** near the basal lamina form a **blood-testis barrier** that prevents many large molecules from passing from interstitial tissue into the seminiferous tubules. Steroids penetrate this barrier with ease, as do some protein hormones (Ch. 57). Tubular fluid at this location is quite different from plasma; it contains little protein and glucose, but is rich in androgens, estrogens, K^+, inositol, glutamic and aspartic acids. The blood-testes-barrier protects germ cells from blood-borne noxious agents, prevents antigenic products of germ cell division and maturation from entering the circulation, thus generating an autoimmune response, and helps establish an osmotic gradient that facilitates fluid movement into the tubular lumen.

The seminiferous tubules empty into a highly convoluted anastomotic network of ducts called the **rete testis** (**Part D**). Its walls possess **estrogen receptors** (**ER-α**), and it is where fluid is reabsorbed and spermatozoa concentrated. Spermatozoa are transported through efferent ductules into a single duct, the **epididymis**, by testicular fluid pressure, ciliary motion and contraction of the efferent ductules. Cells lining the epididymis possess **oxytocin receptors**. During transit through this structure spermatozoa undergo morphologic and functional changes essential to confer upon the gametes the capacity for fertilizing an ovum. The epididymis also serves as a reservoir for sperm, and studies show that an acidic environment is attained here through **Na^+/H^+** exchange and **HCO_3^-** reabsorption (not unlike similar transport processes present in proximal tubular epithelial cells of the kidneys). Spermatozoa entering the epididymis lack forward motion, so a glycoprotein known as **forward motility protein** is thought to be secreted here, giving sperm "direction." Spermatozoa pass through the tail of the epididymis into the **vas deferens** (or **ductus deferens**), which is more than a simple conduit from the epididymis to the urethra, since it possesses a complex epithelium capable of both absorptive and secretory function, and has **endorphin** and **oxytocin receptors**.

The **Ampulla**, which is present in the bull, rabbit, ram, stallion and other animals, is a spindle-shaped thickening of the terminal portion of the ductus deferens, and the dorsal wall of the urethra in the hamster, mouse and rat. It's secretions include Na^+, Ca^{2+}, Mg^{2+}, citric acid and ergothionine.

The **seminal vesicles** (**vesicular glands**), absent in dogs and cats, are paired, bag-shaped glands in guinea pigs, rats, primates and stallions. In bulls, boars and rams they consist of multiple lobes containing a system of ramified secretory ducts. Seminal vesicle secretions, where present, contribute significantly to the volume of semen, and contain fructose (needed for sperm nutrition), amino acids, phosphorylcholine, fibrinogen, inositol, ergothionine, flavins and prostaglands (**Note**: scientists may have misnamed the prostaglandins because they thought their presence in semen was due to prostatic secretion).

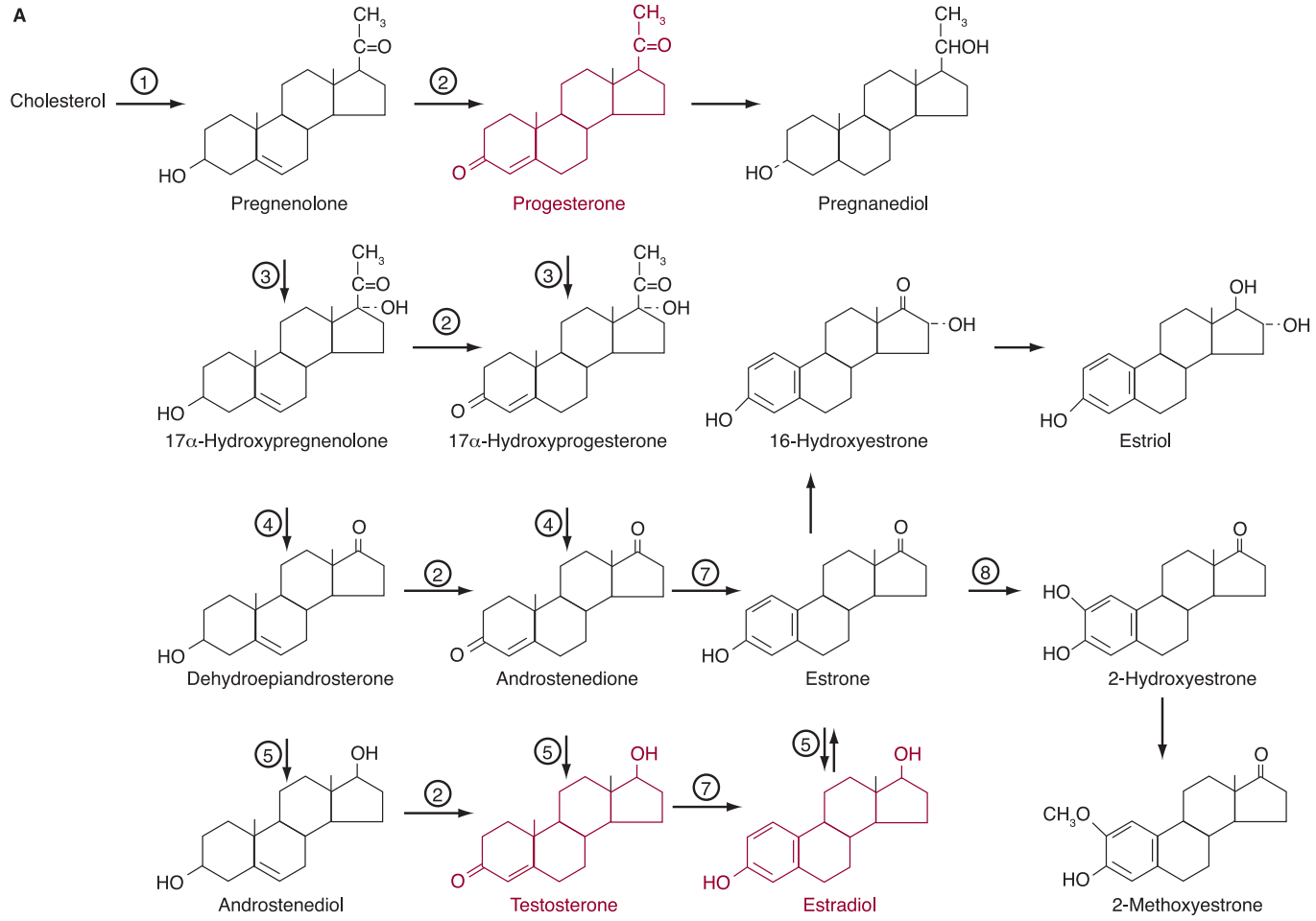
The **prostate** is a tubuloalveolar gland that may be diffuse if it is confined around the urethra, or discrete if it forms a definite body outside the urethral muscle. In rams it is of the diffuse type. Both types exist in boars and bulls. It is the only accessory sex gland in dogs, and is known to secrete buffers that help to neutralize acidic vaginal secretions and increase sperm motility. Prostatic secretions also contain cholesterol, citrate, Ca^{2+}, Zn^{2+}, clotting enzyme, profibrinolysin, acid phosphatase and a serine endopeptidase known as **prostate-specific antigen** (**PSA**). PSA hydrolyzes the sperm motility inhibitor semenogelin in semen, and although it is thought to have several substrates in plasma, its precise function in blood is unclear. Besides man, the dog is the only animal known to develop **benign prostatic hyperplasia** (**BPH**), a common condition in old, intact dogs. Dogs don't usually develop urethral obstruction from BPH like humans. Prostate cancer is, unfortunately, another prostatic abnormality in some castrated dogs, and **PSA** is a routine scan for **BPH** and **prostate cancer**.

Bulbourethral (**Cowper's**) **glands** are absent in dogs, but are present in many other animals (**Part E**). They secrete HCO_3^- and sialomucin, the latter causes the gelation reaction of boar semen. **Urethral glands** also produce a mucus secretion during coitus.

Ch59 Testosterone, Estrogen and Progesterone

(Biosynthesis, Metabolism and Functions)

A



Enzymes

1. 20,22-Desmolase
2. 3β-Hydroxysteroid dehydrogenase, and Δ^5, Δ^4-Isomerase
3. 17α-Hydroxylase
4. 17,20-Desmolase (or 17,20-Lyase)
5. 17-Ketoreductase
6. 5α-Reductase
7. Aromatase
8. 2-Hydroxylase

B Relative contributions (approximate percentages) to circulating levels of sex steroids in males

	Testicular secretion	*Adrenal secretion*	*Peripheral conversion of precursors*
Testosterone	95	< 1	< 5
DHT	20	< 1	80
Estradiol	20	< 1	80
Estrone	2	< 1	98
DHEA Sulfate	< 10	90	....

Testosterone

Testosterone, the principal hormone of the testes, is a **C_{19} steroid** synthesized by **Leydig cells** from cholesterol. A common enzymatic pattern underlies the capability of all steroid hormone-secreting cells, with biochemical differentiation (between cells) involving deletion or de-emphasis of certain enzymes (Ch. 21). In Leydig cells the 11- and 21 β-hydroxylases in the adrenal cortex are absent, but **17 α-hydroxylase** is present (**Part A**). **Pregnenolone** is hydroxylated in the 17 position, then subjected to side chain cleavage to form 17-ketosteroids. These are converted to testosterone. Testosterone is also formed via **progesterone** and **17-hydroxyprogesterone** in domestic animals, a pathway less prominent in primates. Dihydrotestosterone (DHT) and estrogens are also formed by the testes, but circulating levels derive largely from peripheral conversion of precursors (**Part B**).

Male sex steroids are **androgenic** and **anabolic**, with **androgenic activity** stimulating growth and functional expression of accessory sex organs (Ch. 63), and development of special sex characteristics. Deletion of oxygen at C_3 was initially found to remove some of the androgenic activity, which became important in development of the synthetic anabolic steroids. Anabolic activity promotes myotropic activity (e.g., nitrogen, Ca^{2+} and PO_4^{3-} retention), and development of general sex characteristics.

Males are typically larger, with enhanced muscular development related to copulatory postural requirements and aggressive social mannerisms coincident with mating behavior. Testosterone, following conversion to estrogen, causes the epiphyses to fuse to the long bones, and halts linear body growth. Male sex steroids promote hair pigmentation and growth in mammals, and feather pigmentation and growth in birds. Hair fibers are coarser than those of females, and hair coat is usually darker. Testosterone also stimulates erythropoiesis, increases the BMR, and maintains, with FSH, spermatogenesis.

Estrogen

The naturally occurring estrogens (of most mammals) are **estradiol** (**17-β**) and **estrone**, which have been isolated from the ovaries, adrenals, placenta and testes. **Estrogen**, like testosterone, stimulates development and maintenance of reproductive tissues through stimulation of protein synthesis. In females, the vaginal epithelium proliferates and differentiates, growing to adult size; the cervix secretes an alkaline mucus; the uterine endometrium proliferates, glands hypertrophy and elongate, and antibody levels increase, thus protecting against infection; mammary ducts proliferate, and the uterine myometrium develops an intrinsic rhythmic motility during proestrus and estrus. Estrogen simulates progesterone and oxytocin receptor synthesis, as well as endometrial cyclooxygenase synthesis. In most domestic species, estrogen, in association with declining progesterone levels, induces behavioral estrus (Chs. 52-55). The bitch requires declining plasma estrogen levels and increased progesterone for full expression of standing behavioral estrus (Ch. 53).

Estrogen exhibits osteoblastic activity through decreasing PTH sensitivity and promoting calcitonin release. It favors ossification of the epiphyses, which retards postpubertal growth of the long bones. Estrogens are used for the treatment of urinary incontinence, because they upregulate activity of **α_1-adrenergic receptors** on the bladder's internal sphincter. Estrogens tend to impair erythropoiesis, and when given in excess can lead to aplastic anemia. Estrogen is protein anabolic, but less so than testosterone. It stimulates hepatic biosynthesis of a number of proteins, including the thyroid (TBG) and steroid binding globulins (e.g., CBG, SSBG), angiotensinogen, albumin, fibrinogen and prothrombin. Estrogen increases **hepatic LDL receptor synthesis**, which lowers plasma cholesterol (an action shared with **T_4** and **insulin**). Estrogen, unlike progesterone, produces peripheral vasodilation and increases heat dissipation by increasing local nitric oxide (NO) production.

There are two types of nuclear **estrogen receptors**, **ER-α**, and **ER-β**, with some tissues containing one type over the other. ER-α is found primarily in the uterus, rete testis, kidneys, liver and heart. ER-β is found in the ovaries, prostate, lungs, GI tract, hemopoietic system and CNS.

Phytoestrogens, which are nonsteroidal dietary substances with estrogenic activity, and plant **proestrogens** which can convert to estrogen during ruminal sumbiotic digestion, are often consumed by ruminants and other herbivores. These substances, which are cleared readily by the primate liver following beef consumption, can disrupt the estrous cycle and cause infertility in herbivores. Chronic ingestion causes cell differentiation resembling a pubertal pattern in the cervix, increased uterine weight, increased protein synthesis by endometrial cells, and changes in enzyme activity.

Sulfated and **glucuronidated derivatives** of **estrogenic compounds** are normally excreted in bile and urine. An additional pathway of estrogen metabolism involves **2-hydroxylation**, producing the **catechol estrogens** (**Part A**). These compounds resemble catecholamine neurotransmitters in their hydroxylated benzene rings (Ch. 32). Because **2-hydroxylase** activity is present in the hypothalamus, the catechol estrogens produced seem to modulate **estradiol effects on GnRH release**. Although the catechol estrogens bind to ER-α and ER-β, they do not activate those receptors, but act as blocking agents, becoming **antiestrogenic**.

Ethinyl estradiol is a synthetic estrogen that, unlike natural estrogens, has a low first-pass hepatic clearance, and is relatively active when taken orally. **Diethylstilbestrol** (**DES**) and a number of related compounds are estrogenic, probably because they are converted to a steroid-like ring structure in the body. Because of its protein anabolic effect, DES was used to "fatten" beef cattle, but is no longer recommended because of public health implications.

Progesterone

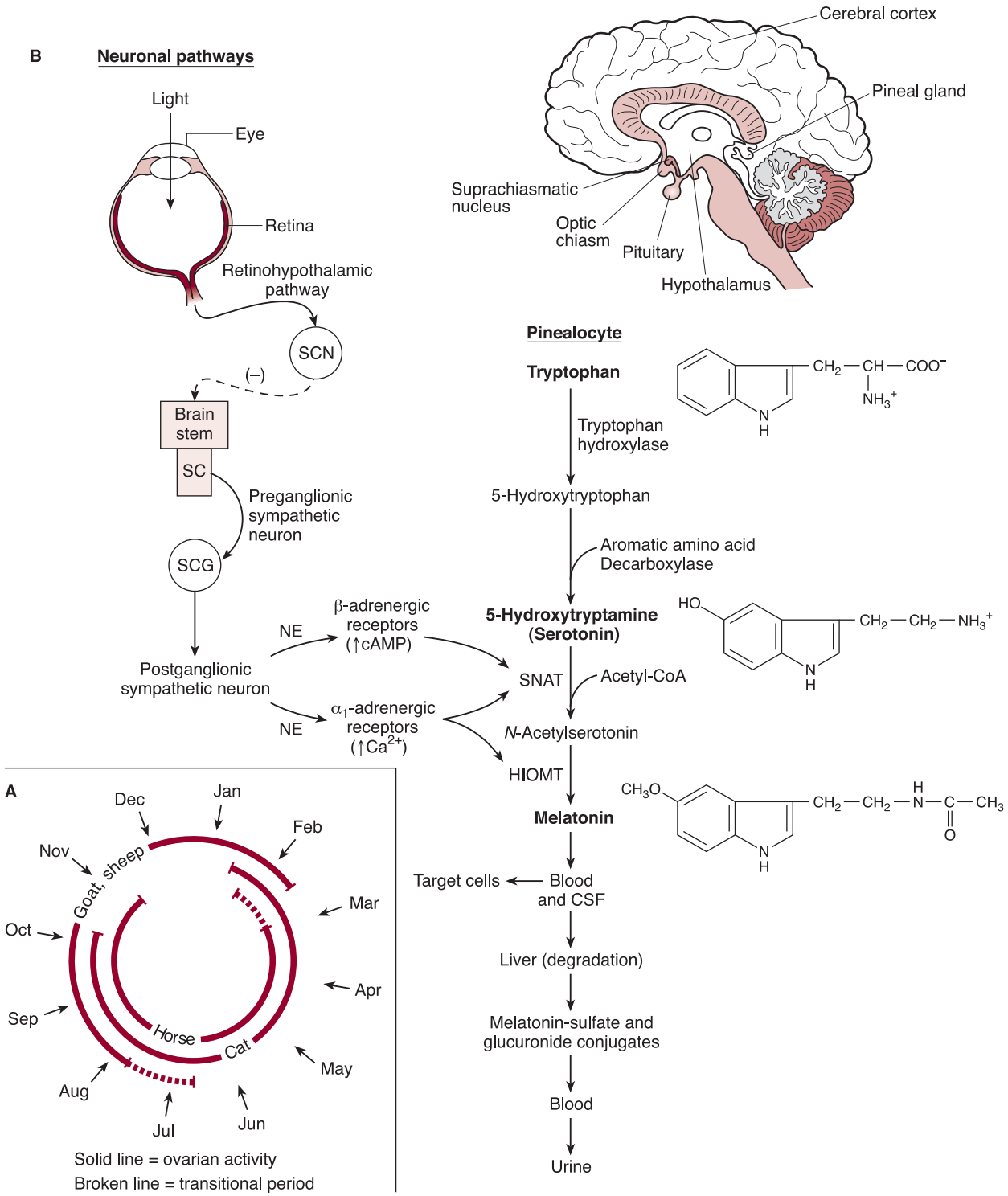
Progesterone, meaning to **"promote gestation,"** is the pro-hormone to all adrenal steroids (Ch. 21), and the gonadal sex steroids (**Part A**). Blood levels are established by the corpus luteum (CL) of the nonpregnant animal, and both the CL and placenta of the dam. Since progesterone has a short circulatory half-life (≈ **25 min**), abortion will follow if it is secreted in insufficient amounts, or if progesterone receptors are blocked. **Epostane** is a drug that inhibits **3β-hydroxysteroid dehydrogenase**, and **mifepristone** (**RU 486**) is a progesterone analog that binds to progesterone receptors (**PR_A** and **PR_B**), but does not release **heat shock protein** (**HSP**; Ch. 6). RU 486 effectively blocks PR_A and PR_B. Both are effective abortifacients that can control mismating in bitches. There are species differences in the amino acid compositions of PR_A and PR_B, and some animals fail to respond to RU 486 (e.g., cats, hamsters and chickens).

Progesterone is normally **45%** bound to plasma **transcortin** (**CBG**), **50%** to **albumin**, and **1-2%** is **free**. Cellular effects are seen after target cells are subjected to a period of "estrogen priming." Progesterone quiets the myometrium, partially by increasing the **β-** to **α-adrenergic receptor ratio**, and induces uterine milk secretion by endometrial glands. Uterine glands and blood vessels increase in depth, branching and tortuosity under progesterone stimulation (Ch. 56). High levels of progesterone inhibit the hypothalamic GnRH pulse generator, and correlate well with rejection of the stallion by mares. Aside from favoring gestation in all species through inhibiting uterine smooth muscle contraction, progesterone may also be **nature's immunosuppressant** against rejection of the fetus (since the fetus contains paternal antigens that would otherwise be incompatible with those of the dam). This may be likened to the immunosuppressant activity of glucocorticoids.

Progesterone exhibits antiestrogenic effects on the uterus, **decreasing** its excitability and sensitivity to **oxytocin**, and **reducing cyclooxygenase activity**. It decreases the number of estrogen receptors in the endometrium, and increases the rate of converstion of 17 β-estradiol to less active estrogens. In mammary glands, progesterone stimulates development of lobules and alveoli (rather than ducts), and supports secretory activity. It is thermogenic and responsible for the rise in basal body temperature at the time of ovulation (Ch. 55). It stimulates respiration, thus lowering the Pco_2. It competes with aldosterone for distal renal tubular receptors, and since it is less potent than aldosterone, natriuresis occurs at low levels, but increased Na^+ retention and blood volume expansion occur during pregnancy. The only hepatic protein known to increase during progesterone stimulation is fibrinogen.

Ch60 Pineal Gland: I

(Photoperiodic Regulation of Reproductive Events)



Source: **Part A** modified from Stabenfeldt GH, Edqvist L-E: Female reproductive processes. In Swenson MJ, Reece WO [eds]: Dukes' physiology of domestic animals. 11th ed. Ithaca, NY: Cornell University Press, 1993:691.

The **pineal gland** is found in all vertebrate groups, with the exception of crocodilians (Ch. 72). Several years following the discovery that pineal extracts caused the skin of frogs to lighten by causing a concentration of melanin in melanosomes around the nucleus of melanophores (Ch. 8), in 1958 investigators succeeded in isolating and characterizing the active ingredient, **N-acetyl-5-methoxyserotonin** (**melatonin**). Since that time other biologically active indolamines and related compounds have been isolated from pineal tissue.

Historically the pineal gland has been viewed as being **"a third eye,"** as **"the seat of the soul,"** and at one time as a **"sphincter to control the flow of thought."** Today, however, it is viewed as being a **neuroendocrine transducer organ** in mammals. This classification did not come easily, as early workers wanted to define pineal endocrine action along the lines of other endocrine gland functions known at the time. All were thought to be entirely dependent upon substances in the bloodstream both for their own control and for their effects on the remainder of the body. Endocrine glands secreted hormones into blood, and were themselves regulated by other hormones, which were delivered to them by the circulation. Secretory activity of a gland was thought to be maintained within narrow limits by simple homeostatic mechanisms: as the level of a particular hormone in blood rose, the gland invariably responded by decreasing its secretion of that hormone; when the level of the hormone fell, the gland increased its secretion. Studies conducted within the past 20 years indicate that the pineal gland is an intricate and sensitive **"biologic clock,"** converting cyclic **nervous activity** generated by **light in the environment** into endocrine secretions.

Since circadian (circannual, circalunar or circatidal) environmental cycles were found to influence biological rhythms in both plants and animals, the annual "biologic clock" became evident. These cycles have been shown to influence modalities such as body weight, breeding behavior, testicular size, prolactin secretion, udder development, hibernation, and the molting of birds, partially because changes in photoperiod affect environmental temperature, and thus the quantity and quality of feed. Evolution of this biologic timing device has clearly provided selective advantages for animals as they cope with substantive annual fluctuations in their environments, which are particularly important to seasonal breeders so they can initiate estrous cycles at the appropriate time to ensure survival of their offspring.

It should not be surprising, therefore, to find that the major physiologic actions of the mammalian pineal gland relate to **reproduction**, and are most pronounced in species that breed only during the spring or fall. Many years ago investigators discovered that the frequency of estrus was increased in rats kept under conditions of constant light. Several years later it was discovered that pinealectomy also increased the frequency of estrus in rats maintained under normal photoperiods, and a surge of investigations were launched into possible roles of photoperiod, the pineal gland, and melatonin in controlling mammalian sexual maturity and reproductive cycles. The study of the pineal gland and its hormones has succeeded in significantly advancing our understanding of the **"biologic clocks"** that help to synchronize various important physiologic events.

Photoperiodic Regulation of Reproductive Events

Photoperiodism is known to influence the reproductive cycles of a number of seasonal breeders, including cats, goats, horses, and sheep, resulting in periods of continuous (cyclic) ovarian activity followed by periods of anestrus (**Part A**). However, the response to photoperiod changes is different among species. For example, **horses** and **cats** appear to be positively affected by increasing day-length, whereas **goats** and **sheep** are positively affected by decreasing day-length. The primary translators of photoperiod appear to be the **suprachiasmatic nucleus** (**SCN**; reported to control a number of circadian rhythms in mammals), and the **pineal gland**, which produces **melatonin** in response to darkness (**Part B**). Although melatonin is antigonadal in some species, it apparently is not in sheep and goats, in which melatonin levels rise during the short-day-length breeding season. Administration of melatonin to sheep during the spring results in early onset of ovarian activity.

The **cat** is reported to be highly sensitive to changes in photoperiod, with a day-length increase of as little as 15 minutes in January perceived (presumably through the pineal gland) and translated by the hypothalamus into gonadotropin output, and thus ovarian activity. Also, the suppressive effects of melatonin due to long periods of darkness are reportedly overcome by exposure to artificial lighting regimens in cats and horses. The customary time for placing **mares**, for example, under lights is December 1 (Northern Hemisphere), with cyclic ovarian activity anticipated early in February.

In mammals that experience reproductive suppressive effects from melatonin (e.g., cats and horses), data indicate that **melatonin** released from the pineal gland acts through either blood or cerebrospinal fluid (CSF) on the hypothalamus to **lower GnRH output**, thus lowering LH output from the pituitary. As indicated in **Part B**, light inhibits sympathetic input to the pineal, resulting in decreased melatonin synthesis, followed by increased levels of GnRH and LH leading to estrus. Phototic signals that strike the retina pass via the retinohypothalamic tract to the **SCN** of the hypothalamus, and then to the brain stem. From there, sympathetic preganglionic pathways leave thoracic segments of the **spinal cord (SC)**, and terminate on the **superior cervical ganglion (SCG)**. The postganglionic sympathetic fibers of the SCG travel along the tentorium cerebelli, and enter the pineal gland via the conarian nerve. The SCN, operating through the brain stem and SC, **reduces** activity of sympathetic fibers to the SCG and pineal gland in the presence of light. In pinealocyte synthesis of melatonin, **tryptophan** is hydroxylated in position 5 to hydroxytryptophan by the enzyme tryptophan 5-hydroxylase. 5-Hydroxytryptophan is next decarboxylated to **5-hydroxytryptamine** (**serotonin**) by L-aromatic amino acid decarboxylase. The concentration of serotonin undergoes circadian variation: during daylight hours it increases, while at night it decreases due to increased activity of **serotonin N-acetyltransferase** (**SNAT**), and the dark-adapted enzyme **hydroxyindole-O-methyltransferase** (**HIOMT**). Both β- and α_1-adrenergic receptor agonists seem to increase activity of SNAT in rats, whereas melatonin synthesis in sheep may be controlled more by α_1-adrenergic receptor stimulation of HIOMT. β-Adrenergic receptor agonists work through cAMP, whereas α_1-receptor agonists work primarily through the Ca^{2+}-CaM second messenger system.

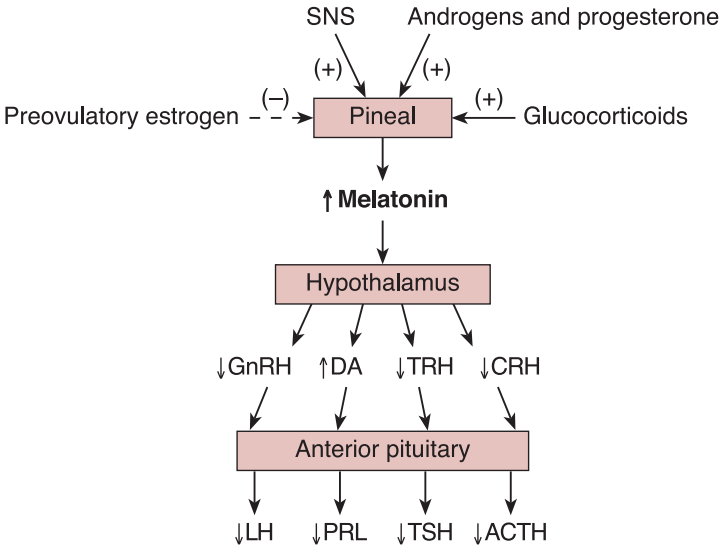
The morphology of the pineal gland reportedly differs between pregnant and nonpregnant sows, implying that functional modifications occur in this gland to meet physiologic requirements during pregnancy. Evidence from human studies indicates that the pineal gland may help control the onset of puberty. Circulating melatonin decreases by 75% between the ages of 7 and 12 years, when LH levels are observed to rise (Ch. 60, **Part D**). The pineal gland of the rat also synthesizes **arginine vasotocin** (**AVT**). If AVT is administered to neonatal mice during the period when the brain is undergoing sexual differentiation, increased growth of reproductive organs occurs. In contrast, if AVT is administered after the brain has undergone sexual differentiation, growth of accessory organs (and in some cases the gonads themselves) is reduced. The presence of other hypothalamic peptides, including **oxytocin**, **TRH**, and **somatostatin**, has also been demonstrated in human pineals.

Pineal Tumors

Although pineal tumors are uncommon, they sometimes occur in young males. Some have been reported to impair release of antigonadotropic melatonin, thus allowing sexual maturation to occur prematurely. In contrast, other pineal tumors have been related to delayed puberty. These tumors probably secrete more antigonadotropic factor (melatonin) than does a normal gland.

Ch61 Pineal Gland: II
(Nonreproductive Actions of Melatonin)

C



D

Melatonin Targets in Mammals	*Actions*
Photoperiodic regulation of reproduction	Melatonin, released from the pineal gland according to light/dark cycles, acts on the hypothalamus to reduce GnRH secretion. Major effects of the pineal gland are seen in seasonal breeders. Preovulatory estrogen decreases melatonin output, helping to promote a GnRH and thus LH surge.
Puberty	Circulating melatonin decreases by 75% between the ages of 7 and 12 years in humans.
Stress response	Both acute and chronic stress affect pineal function through the sympathetic nervous system and glucocorticoids, respectively (See **Part C**)
Hibernation	Melatonin has a pronounced effect on sleep–awake and arousal cycles.
Immune response	Melatonin enhances and 5-HT impairs immune function.
Free radicals	Melatonin reduces and 5-HT enhances free radical formation.
Hair	Melatonin inhibits hair growth in rodents, but may stimulate hair growth in other animals.
Melanophores (melanocytes)	Melatonin implants in weasels (*Mustela erminea*), cause them to grow white winter coats in the spring instead of brown coats.
Adrenal cortex	A pineal substance (e.g., adrenoglomerulotropin) may directly stimulate aldosterone release, and melatonin inhibits cortisol release (probably by reducing hypophyseal CRH release; see **Part C**). Excessive aldosterone release leads to hypertension.
Parathyroids	Pinealectomy causes parathyroid hypertrophy in rats.
Thyroid	Melatonin reduces thyroid function, probably by inhibiting hypophyseal TRH release (see **Part C**). Thyroid function is involved with thermoregulation and hibernation.
Lactation	Melatonin enhances hypothalamic DA release, thereby reducing adenohypophyseal PRL release (see **Part C**).
Cardiovascular system	Vasopressor activity has been reported for pineal extracts (probably AVT).

Other Factors Affecting the Pineal Gland

In addition to the actions of light on the pineal gland, hypophysectomy, stress, gonadal and adrenal steroids influence pineal function. **Androgens** (e.g., testosterone and dihydrotestosterone) and **progesterone inhibit monoamine oxidase (MAO)** activity in the pineal, which in turn allows for **increased melatonin synthesis** (see **Part C**). Monoamine oxidase is a catecholamine-degrading (i.e., norepinephrine-degrading) enzyme (Ch. 32). Increased melatonin can reduce LH release, which decreases testosterone synthesis in testicular Leydig cells. In contrast to androgen effects in males, the preovulatory **estrogen** surge in females reportedly increases MAO activity in the pineal and, therefore, decreases melatonin secretion (thus enhancing GnRH release and the LH surge).

Acute stress stimulates pineal melatonin synthesis, presumably through enhanced sympathetic stimulation. **Chronic stress** (e.g., starvation) is associated with elevated **glucocorticoids**, which reduce pineal MAO activity, and allow for increased melatonin production (see **Part C**). Thus, **stress**, working through either or both pathways, can **depress reproductive function via the pineal gland**.

Nonreproductive Actions of Melatonin

In addition to the inhibition of GnRH and therefore LH secretion, **melatonin** may influence the secretion of other adenohypophyseal hormones (**Part C**). **Thyroid function** in some mammals is affected by photoperiod changes. This influence appears to be exerted through the control of melatonin release (**Part D**). Melatonin treatment reduces thyroid function, presumably by limiting hypothalamic release of **TRH**. This would seem to be advantageous to hibernating animals who have a need to reduce their basal metabolic rate during periods of hibernation. Melatonin is also directly involved in sleep-wake and arousal cycles, which also aids hibernating animals.

Long photoperiods are correlated with increased **prolactin** (**PRL**) secretion (and lactation) in ruminant ungulates (sheep, cattle, goats). Melatonin treatment decreases adenohypophyseal PRL release in both sheep and goats, presumably through stimulating hypothalamic **dopamine (DA)** release. Exogenous **glucocorticoids** stimulate melatonin release, which in turn may be involved in reducing **ACTH** release as part of its negative feedback loop. A pineal substance known as **adrenoglomerulotropin** is also thought to stimulate **aldosterone** release, which in turn can cause hypertension if secreted in excess (**Part D**). The vasopressor activity reported for pineal extracts could also be due to arginine vasotocin (**AVT**).

Biologic aging may also involve the pineal gland. One popular theory of aging involves the formation and accumulation of **free radicals,** compounds that can interact with and damage certain proteins, carbohydrates, phospholipids, and nucleic acids. One of the most dangerous free radicals is produced during the breakdown of hydrogen peroxide. In a number of disorders (including Parkinson's disease, atherosclerosis, muscular dystrophy, multiple sclerosis, and rheumatoid arthritis), free radicals are responsible for cell damage. In certain *in vitro* systems, **melatonin** has been found to **reduce free radical formation**, whereas **serotonin** (**5-HT**), the precursor to melatonin, **increases free radical formation**. One theory of aging holds that as mammals age, the **SCN**, which sends important regulatory messages to the pineal, becomes dysfunctional, and the pineal reduces its production of melatonin while elevating that of 5-HT. Another influence of the pineal on aging may be related to effects of melatonin on the immune response. **Melatonin** appears to **enhance immune function**, and has been claimed to increase immune surveillance and decrease the risk of cancer. In contrast, **5-HT** may **impair immune function**. As decreased immune function in general is associated with aging, melatonin may have a dual retarding effect on the aging process (by reducing free radicals and enhancing immune surveillance) (see **Part D**).

Biodegradation of Melatonin

Circulating melatonin is highly lipid-soluble, and therefore is taken up by virtually all tissues, including brain. It is rapidly metabolized by hydroxylation in the liver, followed by conjugation with either sulfate or glucuronic acid. Once returned to blood, these conjugates are filtered by the kidneys and excreted in urine.

Extrapineal Sources of Melatonin

The **harderian gland,** described by Harder in 1694 in the red deer, is located directly behind and around the eye of all vertebrates that possess nictitating membranes (reptiles, birds, and many mammals). In humans this gland is rudimentary. Melatonin has been demonstrated in the rat harderian gland, and continuous illumination causes enlargement of this gland and an increase in **hydroxyindole-*O*-methyltransferase** (**HIOMT**) activity. Harderian HIOMT apparently differs from that found in the pineal, where continuous illumination decreases pineal weight and HIOMT activity. The full significance of these observations remains to be determined.

The **retina** of the eye appears to be another viable source of melatonin. Although a pineal is absent in alligators, melatonin, presumably of retinal origin, is present in the blood of these animals (Ch. 72).

Evolution of Melatonin Functions

Both retinal and pineal melatonin exhibit nighttime (scotophasic) peaks of synthesis. This compound may have initially been a local hormone, regulating the distribution of melanosomes in the retina. During the day, melanosomes are dispersed in retinal pigment cells, thus protecting photoreceptors from intense light. At night, elevated melatonin levels cause concentration of melanosomes, thus allowing dim light to stimulate photoreceptors maximally. This sensitivity of melanosomes to melatonin is retained in skin melanophores of modern fishes and amphibians. The diurnal rhythm in melatonin production appears to have been coopted as the blood-borne signal entraining a number of other internal events during the evolution of vertebrates (Ch 72).

Birds

The **avian pineal** is innervated by sympathetic fibers as reported for mammals. It also appears to be biochemically similar to the mammalian counterpart. However, unlike most mammals and other vertebrates, the role of the pineal in the reproductive biology of some birds may be **progonadal** (Ch. 72). Avian pinealectomy inhibits androgen synthesis, whereas administration of melatonin stimulates androgen synthesis, presumably by stimulating gonadotropin release from the adenohypophysis. Pinealectomy of quail has also been reported to delay ovarian development, an observation that supports a progonadal role. Since some birds are less susceptible to the effects of melatonin treatment (e.g., white-throated sparrows and border canaries), marked species differences may occur.

Neural control of melatonin secretion in birds also differs from that in mammals, for increased sympathetic activity from the SCG decreases avian melatonin release. This inhibitory action of catecholamines appears to involve a G-inhibitory (G_i) protein in pinealocytes, with subsequent inhibition of cAMP formation, SNAT activity, and melatonin synthesis and release.

The avian hypothalamus may be the site of melatonin action, and thus explain effects of this hormone on **gonadal function**, **thermoregulation**, and **locomotor activity**. Pinealectomy abolishes endogenous body temperature rhythms and free-running locomotor activity rhythms in house sparrows, *Passer domesticus* (Ch. 72).

Ch62 Placental Hormones
(Fertilization, the CL and Placenta)

A

Animal Species Known to Produce Chorionic Gonadotrophin (CG)

Humans (hCG)

Sheep (oCG)

Horses (eCG)

Nonhuman primates (e.g., Rhesus CG, RhCG)

B

Species Known to Produce Placental Lactogen (PL)

Rats	Cattle
Sheep	Goats
Primates	Mice
Voles	Guinea pigs
Chinchillas	Hamsters

C

Major Physiologic Actions of Placental Lactogen (PL) During Pregnancy

GH-like effects:

Decreased use of glucose and amino acids by the mother and increased transport of these compounds across the placenta for fetal growth and development.

Increased use of free fatty acids by the mother.

Decreased maternal responsiveness to insulin.

Increased maternal erythropoietin production and therefore red blood cell mass.

PRL-like effects:

Stimulate growth and development of mammary tissue (along with estrogen and progesterone).

Assist in maintaining the CL (of some animals).

CNS stimulation of maternal behavior.

D

Placental Hormones

Sex steroids (e.g., progesterone, estrogen)

Vitamin D

Chorionic gonadotropin

Chorionic thyrotropin

Chorionic corticotropin

Growth hormone variant

Relaxin

Placental lactogen

Gonadotropin-releasing hormone

Prolactin

Insulin-like growth factors I and II

Ovulation occurs either during or immediately after estrus, and estrus leads to mating. Sperm deposited in the vagina through copulation are transported mainly by **peristalsis** through the uterus and ascend into the oviduct, through which recently ovulated ova are descending. **Fertilization** typically occurs in the upper part of the oviduct, and cleavage begins soon after fertilization, giving rise to a small, multicellular **blastocyst**. The blastocyst is comprised of an **inner cell mass** that will develop into an **embryo,** and an outer **trophoblast** that will become the **chorion.** The trophoblast enables the blastocyst to erode the highly vascularized, secretory uterine endometrium (i.e., receive **"uterine milk"**), and settle in (i.e., **implant**) for development. Gestation may last as long as 22 months in elephants (eutherians), or be as short as 12 days in the opossum (a marsupial).

In contrast to the mammalian norm, species such as **bats**, **skunks**, and **mink** evolved a mechanism of **delayed implantation**, whereby development of the blastocyst is arrested, and the unimplanted blastocyst remains in the oviduct or uterus for an extended period of time. Delayed implantation appears to be an adaptation allowing copulation and fertilization to occur at a time that is advantageous to the parents, while ensuring that the young are born at a time more favorable to their survival. The biochemical basis for delayed implantation is unknown. A similar phenomenon occurs in marsupials, and its continuation is related to the presence of young that suckle at the teat. This is not, however, the eutherian mechanism.

Several animal species such as the dog, rat, mouse, hamster, rabbit, pig, and goat have corpora lutea (CLs) that produce progesterone throughout pregnancy. Other animals such as guinea pigs, sheep, cattle, horses, and primates maintain their CLs only during the early phase of pregnancy, and allow the placenta to be the primary source of progesterone thereafter. The question remains: How do these animals **"know"** they are pregnant during the preplacentation phase, so that they can prolong CL progesterone production, and thus prevent premature regression of the endometrium?

Rescue of the Corpus Luteum (CL)

The signal for prolongation of CL function in some species is the synthesis of LH-like **chorionic gonadotropin** (**CG**) by trophoblastic cells (**syncytiotrophoblasts**) (**Part A**). The trophoblast will eventually become the fetal component of the placenta, which will continue to secrete CG throughout pregnancy. In order for trophoblastic tissue to produce CG, it must have intimate contact with the interstitium of the endometrium. This contact occurs by **interstitial implantation** in primates, wherein the trophoblast penetrates the endometrium approximately 7 days following fertilization. Secretion of CG begins 24 to 48 hours after implantation, with immediate enhancement of luteal progesterone production (Ch. 55). In horses and pigs, relatively minor trophoblastic invasion of the endometrium occurs, and in ruminants there is minor invasion of endometrial caruncles. **Eccentric implantation** of the dog and cat is, however, less invasive.

Equine CG (**eCG**), formerly called **pregnant mare's serum gonadotropin** (**PMSG**), is similar in structure to **LH** in the mare, and **FSH** in other species. An **ovine CG** (**oCG**) may also exist, and other species may secrete proteins that function similarly. Equine CG enhances progesterone production by the primary CL of pregnancy, and aids in the formation of additional (secondary) CLs through the luteinization, or ovulation, or preformed follicles. Whether eCG is essential for pregnancy maintenance is unknown, because the primary CL seems to be adequate in this regard. Unlike **human CG** (**hCG**), eCG is not detectable in urine. However, it rises in plasma at 36 to 40 days following fertilization, peaks at 60 days, then declines at 120 to 150 days.

A slow, pulsatile release (1 pulse/2-3 hrs) of **LH** seems to maintain the CL of some animals, as does **pituitary prolactin** (**PRL**). PRL, or possibly **placental lactogen** (**PL**), may maintain the CL of the ewe during the first trimester. Rodents who maintain their CL throughout pregnancy seem dependent on pituitary PRL, and **PL-1**. The mating stimulus induces twice-daily PRL surges that continue for 8-10 days, and rescue CL from the previous estrous cycle (Ch. 54). PL-1 (from the placenta) continues to assist in maintaining CL steroidogenesis, but at parturition **PL-II** induces further pituitary PRL release, which induces **luteolysis**.

Other Placental Hormones

During the last third of pregnancy in several animal species, another pituitary-like hormone, **PL** (also called **chorionic somatomammotropin** (**CS**), or **chorionic growth hormone-prolactin** (**CGP**)), is secreted by the placenta (**Part B** and Ch. 55). Secretion occurs in direct proportion to placental size and maturity, and 2 PLs occur in rodents (PL-I & PL-II). One is secreted early in pregancny (PL-I), the other later. They are found in high concentrations in the maternal circulation; however, little reaches the fetus. There are some mammals, including rabbits, dogs, and pigs, that apparently do not express a PL, and it is thought that in these species **pituitary PRL** secretion is elevated throughout the latter half of gestation in lieu of PL.

PL shares activity and a common 161-amino acid sequence with **GH** (which possesses 191 amino acids), and **PRL**. Additionally, antibodies to PL cross-react with both GH and PRL. Major roles for PL appear to involve effects on metabolism (GH-like), stimulation of the mammary glands to initiate milk synthesis (PRL-like), maintenance of the CL (in some species), and CNS stimulation of maternal behavior (PRL-like) (**Part C**). PL secretion may be influenced by placental blood glucose levels.

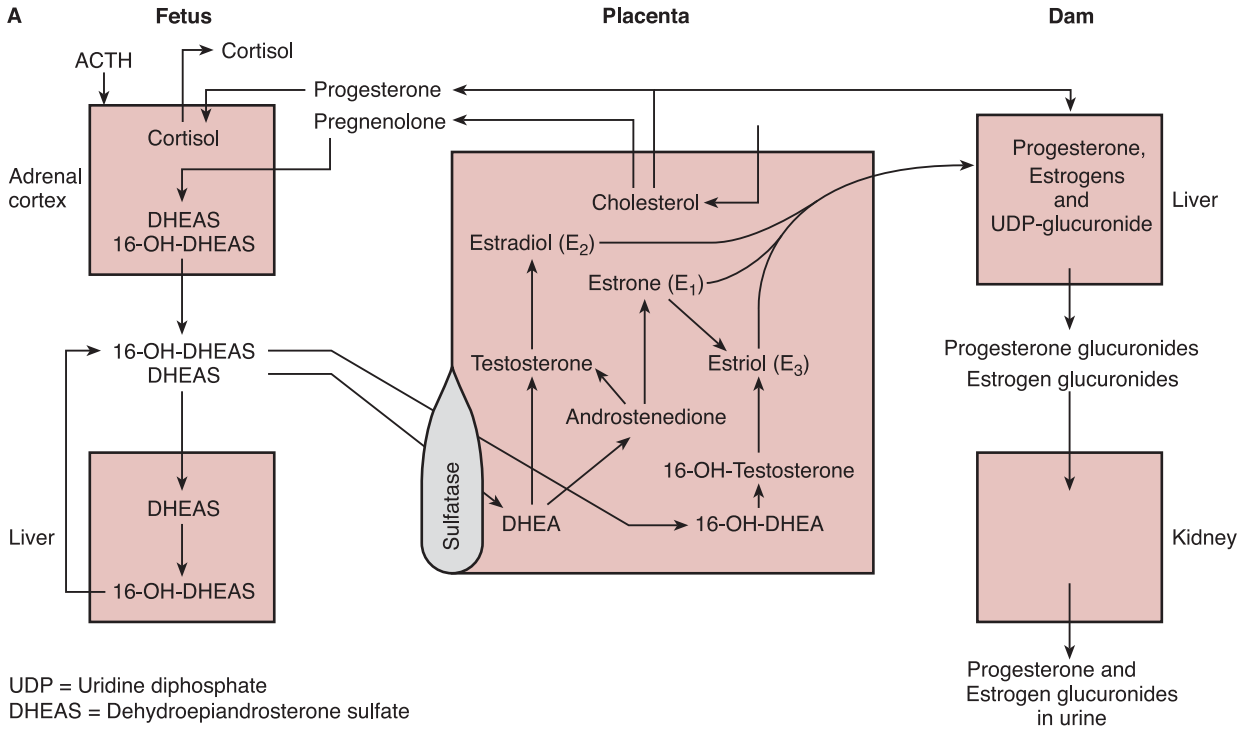
The **primate placenta** also secretes small amounts of **PRL** identical in structure to pituitary PRL. Placental PRL accumulates in amniotic fluid during pregnancy, where it is thought to help regulate both volume and ionic composition. Four additional hypothalamic/adenohypophyseal-like hormones have been identified in placental tissue; **GnRH**, **chorionic thyrotropin**, **chorionic corticotropin**, and **GH variant** (**Part D**). Although placental GnRH appears to increase CG production, functions of chorionic thyrotropin and chorionic corticotropin are not well established. Some speculate that these hormones may help to replace their adenohypophyseal counterparts (i.e., TSH and ACTH), which are inhibited throughout pregnancy. The placental GH variant and the **placental somatomedins** (**IGF-1 and IGF-2**) are secreted into both maternal and fetal circulations, where they are thought to help modulate energy metabolism and fetal growth.

Relaxin is an **insulin-like peptide** best associated with pregnancy. It relaxes the pubic symphysis and other pelvic joints of animals, and also softens and dilates the uterine cervix during pregnancy. In the cow and pig, the CL is the primary source of relaxin. In both species, prepartum release of $PGF_{2\alpha}$ causes luteolysis with a concomitant decline in progesterone production and release of preformed relaxin. In cats, dogs, and horses, the primary source of relaxin is apparently the placenta. In these species, significant relaxin production begins during the 1st trimester, with values sustained through parturition. Relaxin may play a role in **enhancing insulin sensitivity** during the 1st trimester, and also in maintaining pregnancy (in synergism with progesterone).

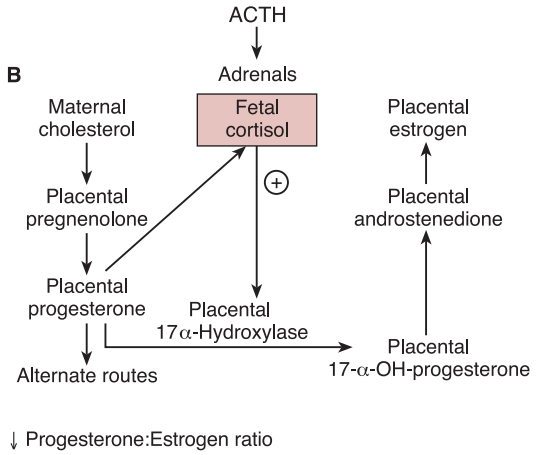
In pregnant primates, relaxin is found in both the CL (granulosa cells), and placenta. In non-pregnant primates it is found in the CL and endometrium during the secretory, but not the proliferative phase of the menstrual cycle. This hormone may be mammotropic in primates, and it may enhance myometrial glycogen content and decrease contractility (like progesterone). It is found in semen, and appears to be produced by the prostate. Relaxin has also been shown to induce expression of vascular endothelial growth factor in the endometrium, and hence may be responsible for the formation of new blood vessels essential for embryonic growth and development. In addititon, relaxin has been shown to dilate blood vessels, and it is produced in the primate heart to (perhaps) redress the cataclysmic consequences of congestive heart failure.

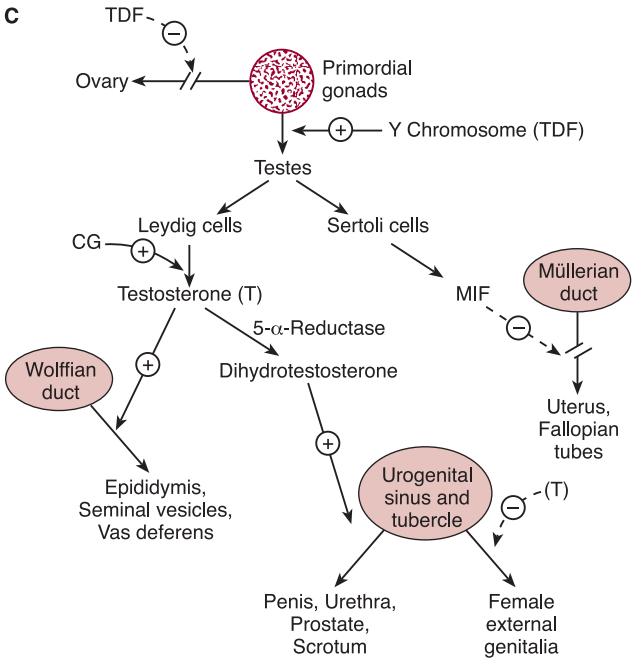
Ch63 Fetal Endocrine System: I

(The Fetoplacental Unit)



The placenta of the ewe, and perhaps other farm animals, possesses **17-α-hydroxylase**, that is stimulated by fetal cortisol. Through fetal adrenal development, the progesterone:estrogen ratio is reduced, thus initiating biochemical processes that lead to parturition.





Physiologic function of most organ systems begins in the embryonic or early fetal period. Although inaccessibility makes the mammalian fetus difficult to study, investigators believe that the endocrine system is one of the first organ systems to develop.

The fertilized ovum generally implants within the endometrium soon after reaching the uterine cavity. Following progressive cell division and growth, the **embryonic stage** begins. The **fetal stage** follows sometime after the outline of most organ systems has been established, and the placenta has developed sufficiently to provide needed nutrients. The **preplacentation period** is generally referred to as the **"period of the embryo,"** with the **postplacentarion period** being the **"period of the fetus."** Most intrauterine deaths are thought to occur during the preplacentation period.

Fetal Substrate Utilization

Studies using fetal sheep demonstrate that the fetus utilizes **amino acids**, **volatile fatty acids** (**VFAs**), **glucose**, and **lactate** as major metabolic substrates, with amino acids supplying 40% to 50% of required calories. Lactate is normally present in fetal plasma in concentrations higher than in the dam, and is derived from both fetal and placental glucose oxidation. Roughly half of the glucose, VFAs, lactate, and amino acids are used to provide energy. Under normal circumstances, free fatty acids (bound to albumin) seem to be less utilized by the fetus, which may be due to a reduced capacity for transport across the placenta. However, their maternal hepatic products, **ketone bodies,** are water-soluble and, therefore, cross the placenta with ease. These are used by the fetus as energy substrates and building blocks for needed macromolecules.

Hormones that Cross the Placenta

Few maternal protein or peptide hormones effectively traverse the placenta (with the exception of TRH, ADH, and lesser amounts of PL and PRL). Although maternal thyroid hormones also have difficulty crossing the placenta, because they are tightly bound to plasma proteins, **catecholamines** readily cross. **Steroid hormones** (and their precursors), particularly cortisol, progesterone, pregnenolone, dehydroepiandrosterone sulfate (DHEAS), and 16-OH-DHEAS, also cross the placenta to participate in the fetoplacental synthesis of estrogen. Estrogen enters both fetal and maternal circulations, but is tightly bound to a plasma protein in the fetal circulation (**α-fetoprotein**), which keeps this steroid from exerting undo influence on fetal development.

The Fetoplacental Unit

The biosynthesis of estrogens during pregnancy is considerably more complicated than the biosynthesis of progesterone. **Estrogens** (e.g., **estrone** (**E_1**), **estradiol** (**E_2**), **estriol** (**E_3**), **equilin**, and **equilenin**) are synthesized in varying amounts throughout pregnancy in different mammalian species; however, the presence of estrogen conjugates in the urine of the pregnant animal is generally a reflection of the **fetal state**, because the complete synthesis of estrogen requires certain steps in the fetal adrenal glands, fetal liver, and/or fetal gonads. This interaction has been best described in primates (**Part A**).

Although the placenta possesses enzymes for the synthesis of **cholesterol** from acetate, it instead takes cholesterol from maternal blood for progesterone and pregnenolone synthesis. Because many mammalian placentas lack the **17-α-hydroxylase** needed for further metabolism to estrogen (Ch. 22), **progesterone** and **pregnenolone** are released into umbilical venous blood and carried to the fetal adrenal cortex, where progesterone is used to form **glucocorticoids** (**e.g., cortisol**), and pregnenolone is used to form **DHEAS** and **16-OH-DHEAS**. Some 16-hydroxylation of DHEAS also occurs in the fetal liver. Then DHEAS and 16-OH-DHEAS are transported back to the placenta, where active sulfatases split the ester linkages, with the resulting **DHEA** converted to **E_1** and **E_2**, and **16-OH-DHEA** converted to **E_3**. While E_3 is the major maternal estrogen of primates, E_1 and E_2 are the major maternal estrogens of most domestic animals.

Estrogens finally produced by the placenta eventually travel to the maternal liver, along with maternal progesterone, where they are conjugated to glucuronides, returned to the circulation, filtered by the kidney, and excreted in urine. The measurement of **urinary estrogen** levels is thus an indirect indicator of fetal function. Depending on species, maternal estrogens (or estrogen precursors) may also be synthesized in the corpus luteum, ovarian follicles, adrenals, fetal gonads, or a combination of these tissues (in addition to the placenta).

The fetal adrenal cortex is well developed after the first trimester of gestation due to stimulation by fetal ACTH. It is proportionally larger than the adult gland, and begins to involute some following parturition. As the fetus continues to mature, the fetal adrenals increase production of cortisol and estrogen precursors (from placental progesterone and pregnenolone, respectively), thus decreasing the **progesterone-to-estrogen ratio** (a biochemical cue for parturition). As this ratio declines, endometrial prostaglandin synthesis is increased, the inhibitory effects of progesterone on myometrial contraction are lessened, and the stimulatory effects of estrogen (and prostaglandin) on myometrial contraction are thus increased (Ch. 68).

Not all species lack placental 17-α-hydroxylase. The placenta of the ewe (**Part B**), and ostensibly other farm animals, possesses 17-α-hydroxylase, and can complete the synthesis of estrogen from progesterone. Increased diversion of progesterone into fetal cortisol synthesis, however, serves a similar purpose in ewes as it does in other animals, because cortisol in turn stimulates activity of placental 17-α-hydroxylase. This, in turn, lowers the progesterone-to-estrogen ratio at the time of parturition (**Part B**).

The equine fetoplacental unit produces two unique estrogens termed **equilin** and **equilenin**, in addition to the more typical estrogens (E_1 and E_2). Equilin and equilenin appear after about the eighth week of gestation, and are thought to be produced in the placenta by aromatization of precursors arriving from fetal gonads. It appears that equine and rodent fetal gonads, not fetal adrenals, are the key fetal endocrine organs involved in the cooperative synthesis of estrogens. Equine fetal gonads enlarge to a size greater than those of the mare during the latter part of gestation.

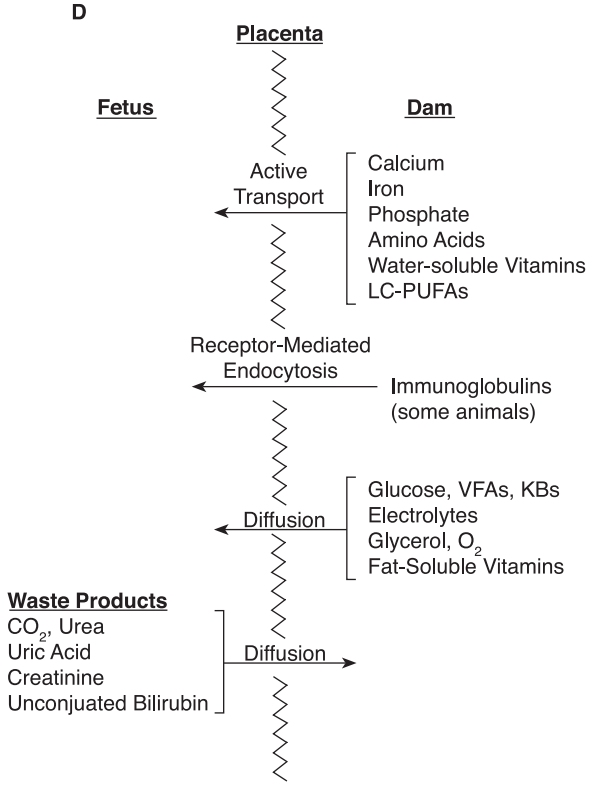
Male Sexual Differentiation

Fetal testicular development begins during the preplacentation period. **Testicular differentiation factor** (**TDF**) on the Y chromosome directs differentiation of Sertoli cells, which are the sites of **Müllerian duct-inhibiting factor** (**MIF**) production (**Part C**). Production of MIF keeps the Müllerian duct from developing into a uterus and fallopian tubes. Embryonic androgen production begins in developing **Leydig cells** due to the presence of **placental CG** (or a similar protein). Fetal testosterone is needed for **Wolffian duct** development into an epididymis, vas deferens, and seminal vesicles, and also to inhibit the urogenital sinus and tubercle from developing into female external genitalia. Another important fetal testicular product is the reduced testosterone metabolite, **dihydrotestosterone** (**DHT**), which is required for proper male differentiation of the **urogenital sinus** and **tubercle** into a prostate, penis, urethra, and scrotum (Ch. 58). In contrast to the male fetus, ovarian steroid production is not considered essential for female phenotypic development.

An example of intersexuality in cattle is the **freemartin heifer**, produced when a (sterile) genetic female is modified in the male direction by masculinizing factors from a male co-twin. Placental fusion with vascular anastomosis is reported to occur in about **90%** of bovine twins. **Testicular evocators** (i.e., TDF, MIF, testosterone, and DHT) from the male twin enter the vascular system of the female prior to development of the ovary and Müllerian system. Therefore, all degrees of masculinization are noted, and the gonads resemble testes to some degree. Similar intersexes have been reported less frequently in sheep, pigs, and goats.

Ch64 Fetal Endocrine System: II
(Glandular Development)

D



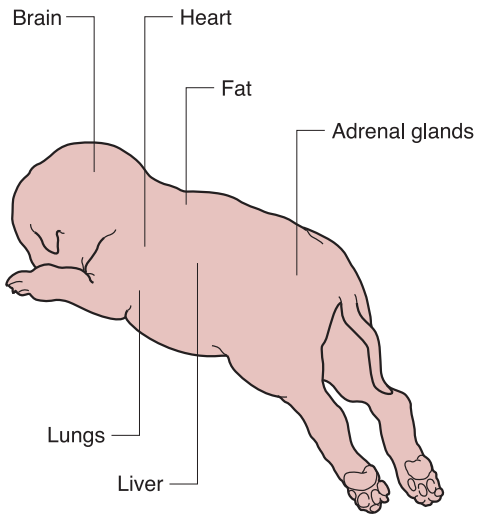
E

Fetal Hormones	
Chorionic gonadotropins	Glucagon
Testosterone	ADH and oxytocin
Dihydrotestosterone	Thyroxine
Müllerian-inhibiting factor	PTH_{rp}
TRH and TSH	Calcitonin
GnRH	Catecholamines
Somatostatin	Cortisol
GH	Aldosterone
PRL and PL	DHEAS and 16-OH-DHEAS
ACTH	IGF-2
Insulin	

F

Fetal/Neonatal Stress Response (Catecholamines, ACTH, Cortisol)

1. **Improves breathing**
   - Increases lung surfactant
   - Increases lung-liquid absorption
   - Improves lung compliance
   - Dilates bronchioles
2. **Protects heart and brain**
   - Increases blood flow to vital organs
3. **Mobilizes fuel**
   - Breaks down normal fat into fatty acids
   - Breaks down glycogen (in liver) to glucose
   - Stimulates new production of glucose by liver



Although a number of placental transport proteins help to regulate movement of substrates across the placenta, only **calcium**, **iron**, **phosphate, water-soluble vitamins, amino acids** and some (essential) **long-chain polyunsaturated fatty acids (LC-PUFAs;** e.g., omega-3 fatty acids) are thought to be transported **actively** from **maternal to fetal blood.** In contrast, **no active transport** mechanisms for the movement of substances **from the fetal to maternal circulation** have been identified (**Part D**). In primates and smaller animals, **immunoglobulins** may be transferred from the dam to fetus, but in farm animals neonatal immunoglobulins are normally gained from colostrum.

Although the fetus is exposed to placental and some maternal hormones, it also produces hormones (discussed below) thought to be instrumental in influencing fetal growth and development, as well as potentially directing the transport of nutrients across the placenta (**Part E**).

Fetal Adenohypophysis

Characteristic **anterior pituitary** cell types are discernible during the first trimester, with all adenohypophyseal hormones being extractable at that time. Similarly, hypothalamic **TRH**, **GnRH**, and **somatostatin** are also present at this time, although the circulatory connection between the hypothalamus and pituitary develops somewhat later. The role of the fetal pituitary in organogenesis, however, appears to be negligible during the first trimester. As discussed in Ch. 62, development of the gonads during the first trimester appears to be directed more by chorionic gonadotropins than by fetal pituitary gonadotropins.

During the second trimester there is increased secretion of all anterior pituitary hormones, which coincides with maturation of the hypothalamo-hypophyseal portal vascular system. There is a rise in **GH** and **TSH** production, with an increase in **thyroidal iodide uptake**. **Gonadotropin** release also increases, with the female achieving higher **FSH** levels than the male. Although fetal pituitary gonadotropins do not apparently direct early gonadal development, they are thought to be important for continued development of differentiated gonads and external genitalia in the second and third trimesters. Fetal **ACTH** rises and helps direct maturation of the adrenal cortex (as previously discussed). Fetal **PRL** levels also rise in the second trimester, and may exert some GH-like effects. Fetal and placental **GH**, **PL**, **PRL**, and **insulin** all stimulate hepatic production of **somatomedins** (e.g., **IGF-1** and **IGF-2**, with **IGF-2** likely being the most important growth factor in fetal life).

The third trimester is the period when most fetal development occurs, and all fetal pituitary hormones except **PRL** have been found to rise during this time.

Fetal Neurohypophysis

During the second trimester, **ADH** and **oxytocin** are demonstrable in fetal neurohypophyseal tissue. During parturition, the umbilical artery oxytocin level is reportedly higher than that in the umbilical vein. Therefore, the fetal posterior pituitary may be contributing to the onset and/or maintenance of labor (Ch. 68).

Fetal Thyroid

The fetal thyroid gland has been reported to develop in the absence of detectable TSH, and it is capable of concentrating iodide early in fetal life. However, during the second trimester, **TRH**, **TSH**, and free **thyroxine** (**T_4**) all begin to rise. **Triiodothyronine** (**T_3**) and **reverse T_3** (**rT_3**) only become detectable during the third trimester. Because little placental transfer of thyroid hormones occurs, most thyroid hormones found in fetal blood are thought to have originated in the fetus. A relative **hyperthyroid** state exists in the fetus during the last half of gestation in order to assure proper growth and development, particularly **neural** development, and prepare the fetus for thermoregulatory adjustments needed in extrauterine life. **Fetal hypothyroidism** results in **cretinism** (and irreversible mental retardation; Ch. 38).

Fetal Parathyroids

Although fetal parathyroids are capable of synthesizing **PTH** by the end of the first trimester, only low levels have been reported in umbilical blood. However, active transport of **Ca^{2+}** from maternal to fetal blood is probably stimulated by another protein hormone from the fetal parathyroids known as **PTH-related peptide** (**PTH_{rp}**) (Ch. 16). This hormone is a natural product of several additional tissue types including the amnion, squamous cell tumors (in which it was originally discovered), the uterus, avian shell gland, and lactating mammary gland (Ch.72). It relaxes uterine smooth muscle and is present in milk, where it may aid in the transport of Ca^{2+} across the neonatal intestinal mucosa. Although PTH_{rp} has a similar structure to PTH, a completely different segment of the molecule uniquely stimulates placental calcium transport, thus allowing the fetus to maintain a 30% to 40% increased ionized calcium concentration gradient over the dam. This **fetal hypercalcemia** also stimulates fetal **calcitonin** release, which promotes bone formation (i.e., accretion). Fetal **vitamin D**, a fat-soluble vitamin, reflects maternal levels, yet does not appear to be highly significant in fetal calcium metabolism.

Fetal Pancreas

Although fetal pancreatic islets are functional in the first trimester, **insulin** and **glucagon** secretion are relatively low. Neither appears to be critical for substrate metabolism, as glucose and amino acids are generally in plentiful supply from the mother. **Fetal pancreatic α** and **β cells** are reported to respond to their usual stimulators and suppressors in blunted fashion until birth, when responsiveness rapidly increases. Fetal insulin contributes to anabolism and to deposition of adipose tissue, and fetal glucagon may help to establish and maintain hepatic gluconeogenesis in ruminant animals, a metabolic process that is established early in fetal life. It should be noted, however, that if the dam is **diabetic** and therefore chronically hyperglycemic, the fetus could become the same. Fetal pancreatic β cells can become exhausted under sustained hyperglycemia, with the fetus born early, diabetic and unusually large.

Fetal Adrenal Glands

As previously discussed, the fetal adrenal cortex is identifiable early in the first trimester, with sex steroid biosynthesis occurring in the inner fetal zone (i.e., the **zona reticularis**). It differs anatomically and functionally from the adult gland in that the cortex increases to a mass that is considerably larger than its relative postnatal size. In most mammals, **ACTH** is thought to be somewhat less important for early fetal life, although later it is more important in stimulating **glucocorticoid** production. **Catecholamine** production in the inner adrenal medulla is critical during parturition and early postnatal development. During parturition, the newborn mounts an immediate **"stress response,"** as shown by high circulating **ACTH**, **cortisol**, and **epinephrine** levels in umbilical cord blood (**Part F**). If endogenous ACTH and cortisol cannot be secreted at this time, death will ensue unless replacement therapy is provided (Chs. 21, 69 and 70).

Fetal Gonads

The testes are detectable structures early, increasing in size, activity, and developmental importance throughout gestation. The ovaries become recognizable during the first trimester but less is known about the importance of fetal ovarian function. In **horses** and **rodents** fetal ovaries are reportedly participating, like the testes and adrenal glands, in fetoplacental estrogen biosynthesis (Ch. 63), and are thought to add significantly to biochemical aspects of the fetoplacental unit.

Ch65 Maternal Endocrine System
(Functional Alterations during Pregnancy)

A

Gland	*Hormone*	*Pattern of change during pregnancy*
Adenohypophysis	GH	Unchanged
	LH, FSH	Low or basal levels
	ACTH	Unchanged until parturition
	TSH	Unchanged
	PRL	Rises to term in some mammals (e.g., primates)
Fetoplacental sex steroids	Estrone (E_1) and 17-β-estradiol (E_2)	Rise to term in varying amounts in most domestic animal species
	Estriol (E_3)	Rises to term in primates
	Equilin and equilenin	Rise to 240 days in mares, then decline slightly to term
	Testosterone	Rises to term, but free concentration falls
	DHEA	Declines to term
Placenta and CL	Progesterone	Variable in domestic animals, but rises to term in primates
	17-Hydroxyprogesterone	Levels in plasma correlate well with CL activity
Adrenal cortex	Glucocorticoids	Rise to term
	Mineralocorticoids	Rise to term
Thyroid	Total T_4 and T_3	Increase during first trimester, then plateau
	Free T_4 and T_3	Unchanged
Parathyroids	PTH	Rises to term
Pancreas	Insulin	Low in early pregnancy, but increases later
	Glucagon	Responsive to usual stimuli

Source: **Part A** modified from Greenspan FS, Strewler GJ. Basic and clinical endocrinology. 5th ed. Stamford, CT: Appleton & Lange, 1997:550–551.

The **fetus**, like a successful parasite, manipulates the **dam** for its own gain, but normally avoids imposing exessive stress that would jeopardise this **"host,"** and thus itself. Several endocrine function tests in the dam are significantly altered during pregnancy. Some changes are due to increased production of plasma-binding proteins by the liver, others to decreased circulating levels of albumin. Additionally, some are mediated by altered clearance rates owing to increased glomerular filtration, decreased heptic excretion of metabolites, or metabolic clearance of steriod and protein hormones by the placenta. Although maternal endocrine adaptations to pregnancy (summarized in **Part A** and below) undoubtedly occur in all mammalian species, those in primates have been best detailed.

Maternal Anterior Pituitary

Maternal adenohypophyseal hormones generally have little influence on pregnancy after implantation. In some mammalian species, the pituitary gland enlarges somewhat during pregnancy, primarily due to hyperplasia of lactotrophs in response to high plasma estrogens. **Prolactin** (**PRL**), the product of lactotrophs, is the only maternal adenohypophyseal hormone to significantly increase during pregnancy in some species, and established pregnancy can continue following hypophysectomy. In cases of pituitary hyperfunction, the fetus is generally unaffected unless hyperglycemia results.

Serum PRL levels vary throughout pregnancy in rodents. **Placental lactogens** (**PL-I** and **PL-II**) are structurally homologous to PRL, and

may assume the role of PRL during gestation in some animals. There is evidence indicating that placental lactogens and PRL are capable of crossing the blood-brain-barrier to promote expression of **maternal behavior** toward foster young, and that PRL may act as an endogenous **anxiolytic** agent (Ch. 68).

Maternal Estrogens

As previously discussed, estrogen production by the placenta of most mammals depends on circulating precursors derived from the fetus. The estrogen **17β-estradiol** (**E_2**) rises in varying amounts throughout gestation in the bitch, mare, cow, ewe, rat, sow, rabbit, goat, and primate (among others). Higher levels of **estrone** (**E_1**) are also noted in most domestic animal species. Large elevations in maternal **estriol** (**E_3**) (e.g., by 1000-fold in humans) appear to be unique to primates. In the mare, rises in **equilin** and **equilenin** appear to parallel that of **E_1**. Immediately prior to parturition, estrogen levels generally rise while those for progesterone fall (a likely biochemical cue for the onset of parturition (Ch. 69)).

Maternal Androgens

In addition to the placenta, the adrenal gland and ovaries also slightly increase the production of androgens during pregnancy. The most important determinant of the androgen plasma concentration apparently is whether or not they bind to hepatic-derived **sex hormone–binding globulin** (**SHBG**). **Testosterone**, which binds avidly, may increase into the normal male concentration range by the end of the first trimester. **Dehydroepiandrosterone sulfate** (**DHEAS**) does not bind to SHBG; therefore, plasma concentrations of this androgen reportedly decrease during pregnancy. Placental desulfation of DHEAS (Ch. 63), and subsequent conversion of DHEA to E_1 and E_2, also appear to be important factors in its increased metabolic clearance.

Maternal Progestins

Progesterone synthesis relies on maternal cholesterol or the cholesterol precursor, acetate. Fetal death has no immediate influence on maternal plasma progesterone levels, thus indicating that the fetus is a negligible source of progesterone substrates. Maternal plasma progesterone concentrations rise in varying (species-dependent) amounts throughout pregnancy, then fall immediately before the onset of labor. Progesterone (meaning to **prolong gestation**) is necessary for the establishment and maintenance of pregnancy. Insufficient CL production of progesterone may contribute to implantation failure, and luteal phase deficiency is also implicated in some cases of infertility. Progesterone is indispensable to a relatively quiescent state of the myometrium, and it is also active as an immuno-suppressive agent in some systems, inhibiting T-cell–mediated tissue rejection. Thus, high local concentrations of progesterone, a steroid with only a **20 min.** circulating half-life, may contribute to immunologic tolerance by the uterus of invading embryonic trophoblast tissue. **17-Hydroxyprogesterone** is considerably less active than progesterone, and because it originates from the CL, urinary levels indicate CL, not placental function (with the exception of animals that possess placental 17-α-hydroxylase).

Maternal Adrenal Cortex

Plasma **cortisol** concentrations increase significantly by the third trimester in primates, yet mares may not exhibit similar increases. Most of the increase in primates is due to an increase in circulating **cortisol-binding globulin** (**CBG** or **transcortin,** which binds **progesterone** with equal affinity). Increased **estrogen** levels account for the increase in hepatic CBG synthesis, which in turn accounts for decreased catabolism of cortisol by the liver. The result is an increase in plasma cortisol half-life, while production of cortisol by the adrenal zona fasciculata is reduced. The net effect of these changes is an increase in plasma free cortisol that can be significant by late pregnancy, and probably contributes to some of the insulin resistance experienced by the dam (though PL also causes insulin resistance). High progesterone levels may act as a glucocorticoid antagonist (because they compete for similar receptors), thus preventing excessive glucocorticoid effects.

Blood levels of **aldosterone** are markedly elevated during pregnancy, probably due to increased production by the adrenal **zona glomerulosa** (not to increased plasma protein binding or decreased clearance). Peak serum aldosterone levels are reached by mid-pregnancy and maintained until parturition. **Renin substrate** (**angiotensinogen**) is increased by the influence of estrogen on hepatic synthesis, and both renin and angiotensin II levels are increased. In spite of these dramatic changes, normal pregnant animals show few signs of hyperaldosteronism. There is no tendency toward hypokalemia or hypernatremia, and blood pressure at mid-pregnancy, when changes in the renin–angiotensin system are maximal, tends to be lower than in the nonpregnant state.

Maternal Thyroid

The thyroid becomes palpably enlarged during the first trimester, with ^{131}I uptake by the gland increased. These changes are thought to be due in part to the increased renal clearance of iodide, which causes relative iodide deficiency. While **total serum thyroxine** (**T_4**) is elevated as a result of increased **thyroid-binding globulin** (**TBG**) synthesis by the liver, **free T_4** and **triiodothyronine** (**T_3**) are normal. Although estrogen is known to stimulate **thyroid-stimulating hormone** (**TSH**) release, TSH is not elevated in pregnancy (perhaps due to negative feedback by T_4). Rhesus monkey chorionic gonadotropin (RhCG), which is similar in structure to TSH, is known to increase T_4 production, however.

Maternal Parathyroids

The Ca^{2+} demand imposed by fetal skeletal development is significantly increased by the third trimester. This is met by hyperplasia of the maternal parathyroids and elevated serum **PTH**. Total serum Ca^{2+} declines to a nadir late in the third trimester, largely due to the hypoalbuminemia of pregnancy. Serum ionized Ca^{2+}, however, is usually maintained at normal concentrations.

Maternal Pancreas

The size of pancreatic islets increases, and insulin-secreting β cells undergo hyperplasia. Basal levels of **insulin** are low or unchanged in early pregnancy, but increase during the second trimester. Thereafter, pregnancy is **diabetogenic**, with resistance to peripheral metabolic effects of insulin apparent (perhaps due to glucocorticoids and PL). Increased insulin results from increased secretion rather than decreased metabolic clearance. The effects of pregnancy on the pancreas can be mimicked by appropriate treatment with estrogen, progesterone, PL, and glucocorticoids. Insulin is not transported across the placenta. Pancreatic production of glucagon remains responsive to usual stimuli, and is suppressed by glucose loading.

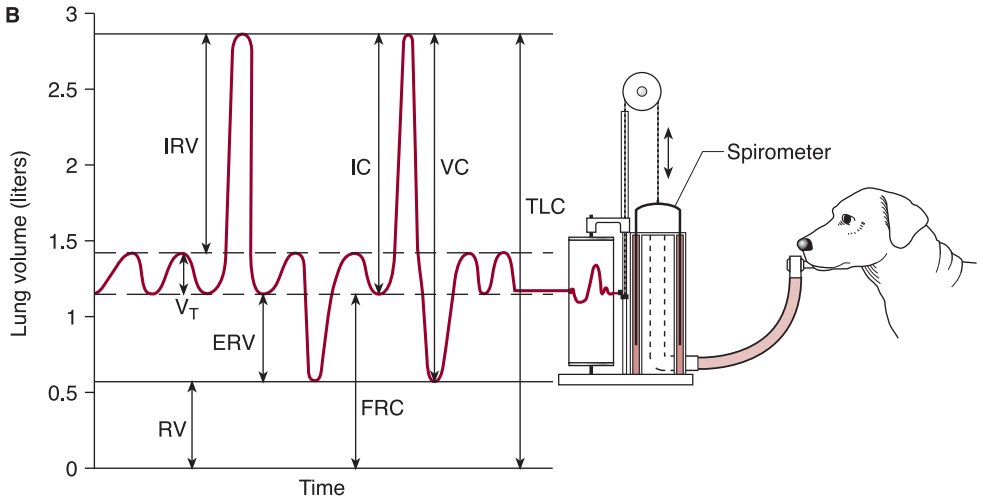
Excess carbohydrate during the first trimester is converted to fat, and fat is readily mobilized during the second half of pregnancy, particularly during periods of decreased caloric intake. Amino acid metabolism may also be altered during pregnancy at the expense of maternal needs. The normal effect of pregnancy, then, is to elevate maternal glucose levels modestly, thus providing glucose for fetal needs while maternal energy requirements are met increasingly by the peripheral metabolism of fatty acids. These changes in energy metabolism are beneficial to the fetus and generally innocuous to the mother (with an adequate diet). Even modest starvation, however, can cause ketosis.

Ch66 Maternal Organ Systems (Patterns of Change)

A

System and parameter	Pattern of change
Cardiovascular	
Heart rate	Gradually increases
Blood pressure	Gradually decreases until the end of pregnancy, then returns to prepregnancy values about the time of parturition
Stroke volume	Gradually increases
Cardiac output	Gradually increases
Peripheral venous distention	Progressively increases to term
Peripheral vascular resistance	Progressively decreases to term
Hematopoietic	
Blood volume	Progressively increases
Hematocrit	Decreases slightly
Fibrinogen	Increases
Electrolytes	Concentration unchanged (isotonicity)
Gastrointestinal	
Sphincter tone	Decreases
Gastric emptying	Decreases
Intestinal motility	Decreases
Respiratory	
Respiratory rate	Unchanged
Tidal volume	Increases
Expiratory reserve volume	Gradually decreases
Vital capacity	Unchanged
Respiratory minute volume	Increases
Renal	
Blood flow	Increases, then returns to normal before parturition
Glomerular filtration rate	Increases early, then plateaus
Urine formation	Increases slightly

B



RV = residual volume (the amount of air remaining in the lungs after the most forceful expiration; V_T = tidal volume (the amount of air breathed in or out during one normal respiratory cycle); IRV = inspiratory reserve volume (the amount of air that may still be inspired after inhalation of the V_T); ERV = expiratory reserve volume (the amount of air that may still be expired after exhalation of the V_T); TLC = total lung capacity (the sum of all lung volumes); IC = inspiratory capacity (V_T plus IRV); FRC = functional residual capacity (the amount of air remaining in the lungs after a normal expiration); Respiratory minute volume (mL/min) = V_T (mL/breath) × respiratory rate (breaths/min).

Source: Part A modified from Greenspan FS, Strewler GJ. Basic and clinical endocrinology. 5th ed. Stamford, CT: Appleton & Lange, 1997:555–556.

Hormone production by the fetoplacental unit during pregnancy, as well as by the dam herself, results in physiologic adaptations of virtually every maternal organ system. These alterations are summarized in **Part A** and below.

Maternal Cardiovascular System

The first two trimesters of pregnancy are generally associated with **low blood pressure** (**BP**). In fact, a normal BP during this time may be associated with hypertension. Increased placental blood flow causes arteriovenous shunting of blood, which in turn causes peripheral vasodilation. Other contributors to low BP are increased endothelial synthesis of vascular relaxation factors in the placenta, such as prostaglandins (PGE_2 and PGI_2), and nitric oxide (NO). The **increased cardiac output** during pregnancy is a result of cardiac afterload reduction (peripheral vasodilation). Because progesterone increases the ratio of β-adrenergic to α-adrenergic receptors in myometrial smooth muscle, it may similarly affect vascular smooth muscle, thereby lowering BP.

The rapid rise in arterial BP preceding parturition (**preeclampsia** or toxemia of pregnancy sometimes seen in bitches, mares, and primates) may be associated with loss of protein in urine due to an elevated glomerular filtration rate (GFR), or more probably to a rise in vasoconstricting placental thromboxanes (from circulating platelets). This rise in BP has also been associated with salt and water retention, weight gain, arterial compression by the fetus, and edema. The **Ca^{2+}** and **Mg^{2+}** drain by the fetus is also known to increase neuromuscular irritability, and it tends to elevate BP in the last trimester, when the majority of fetal growth occurs (Ch. 19).

Maternal Hematopoietic System

Maternal blood volume (**BV**) shortly before term is above normal. The cause is mainly hormonal, as both aldosterone and estrogens increase renal fluid retention. Although there is an increase in red blood cell (RBC) mass (as PL increases erythropoietin production), **hematocrit** (**Hct**) decreases slightly because the degree of renal fluid retention is greater than that of erythropoiesis. Maternal estrogens also stimulate hepatic **fibrinogen** production. The electrolyte concentration of extracellular fluid (ECF) remains **isotonic** (i.e., unchanged) due to compensatory renal fluid retention.

Maternal GI Tract

Progesterone decreases not only uterine smooth muscle contraction, but also GI motility. This less desirable secondary effect can delay gastric emptying, cause generalized constipation, and decrease tension of the lower esophageal sphincter (LES). This latter effect of progesterone can result in gastroesophageal reflux.

Maternal Pulmonary System

Because the total oxygen requirement of the dam (and fetus) increases throughout pregnancy, and a commensurate amount of carbon dioxide is produced, **tidal volume** and **respiratory minute volume** (**Part B**) steadily rise. **Progesterone** increases sensitivity of the medullary respiratory center to carbon dioxide, compounding the above effect and also effectively reducing alveolar (and arterial) Pco_2 significantly below that of nonpregnant animals. Because **tidal volume** increases and **residual volume** remains unchanged, **expiratory reserve volume** decreases. Physical compression of the fetus against the diaphragm can also have a tendency to limit total excursion of the diaphragm.

Maternal Renal System

Urine formation is slightly increased due to an increased load of excretory products from the fetus. Because electrolyte and water reabsorption is increased, the ECF volume is expanded. The increased BV leads to a progressive increase in the GFR until the second trimester, when it levels off throughout the remainder of pregnancy. Renal blood flow increases similarly, but for unexplained reasons returns toward normal during the final stage of pregnancy (while the GFR remains elevated).

Maternal-Parental Transition

Mares, **cows**, and **ewes** stand shortly after delivery, and start licking the newborn, usually at the head. Nearly all cows and about one-third of ewes eat the **birth membranes** during the cleaning process. These membranes are thought to facilitate, but not be essential to initiation of parental care. A short period of contact (less than 30 min) is usually needed to establish a bond that enables the mother to discriminate her own offspring from others. This attachment appears to be learned, not imprinted, for during this initial period animals that have given birth can approach other young animals, and sometimes become attached to them.

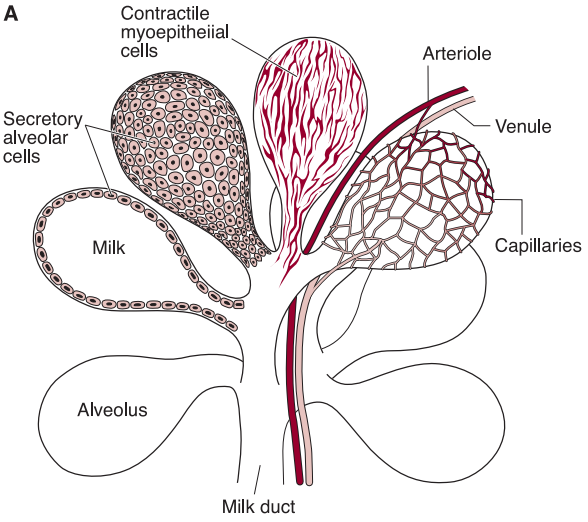
The mother **dog** or **cat** spends most of the day with its young during the first week following parturition. One month after whelping, some bitches begin regurgitating their food, which is quickly consumed by the puppies. **Rabbits** seem to visit their young only once a day, and allow suckling for only a few minutes.

Summary

Maternal adaptations to pregnancy occur in most all organs of the body. The pituitary gland enlarges somewhat during pregnancy, but, with the exception of PRL, the output of most adenohypophyseal hormones remains unchanged. Maternal blood levels of estrogens, progestins, and androgens generally rise in varying amounts among all domestic animal species. Plasma glucocorticoid concentrations generally increase in the third trimester, with most of the increase accounted for by an increase in hepatic-derived CBG. Plasma aldosterone levels also rise, but the dam generally shows few signs of hyperaldosteronism. The thyroid is palpably enlarged early in pregnancy, with total T_4 and T_3 levels elevated in the circulation. Maternal parathyroids are also enlarged, with increased PTH output helping to meet the increased Ca^{2+} demands of the fetus. The size of pancreatic islet tissue increases, and insulin-secreting β-cells undergo hyperplasia. There is an increased blood volume throughout pregnancy, and thus an increase in cardiac output. However, since progesterone stimulates β-adrenergic receptor synthesis, and since the placenta is a low-resistance, highly vascularized organ, the blood pressure usually remains low throughout pregnancy, returning to pre-pregnancy levels at the time of parturition. The Hct generally decreases during pregnancy because the increase in ECF volume is usually greater than that of total erythrocytic volume. However, the electrolyte concentration of the ECF remains isotonic due to compensatory renal fluid retention. Since progesterone decreases smooth muscle contraction, there is general constipation. Progesterone also reduces LES tension, thereby making gastroesophageal reflux more likely. Since the dam becomes the only avenue for fetal CO_2 excretion, there is an increase in maternal respiratory tidal volume and minute volume. Progesterone increases sensitivity of the medullary respiratory center to CO_2, thus assisting with these respiratory changes which can potentially lower the arterial Pco_2. Urine formation is slightly increased during pregnancy, due mainly to an increased GFR and load of excretory products from the fetus.

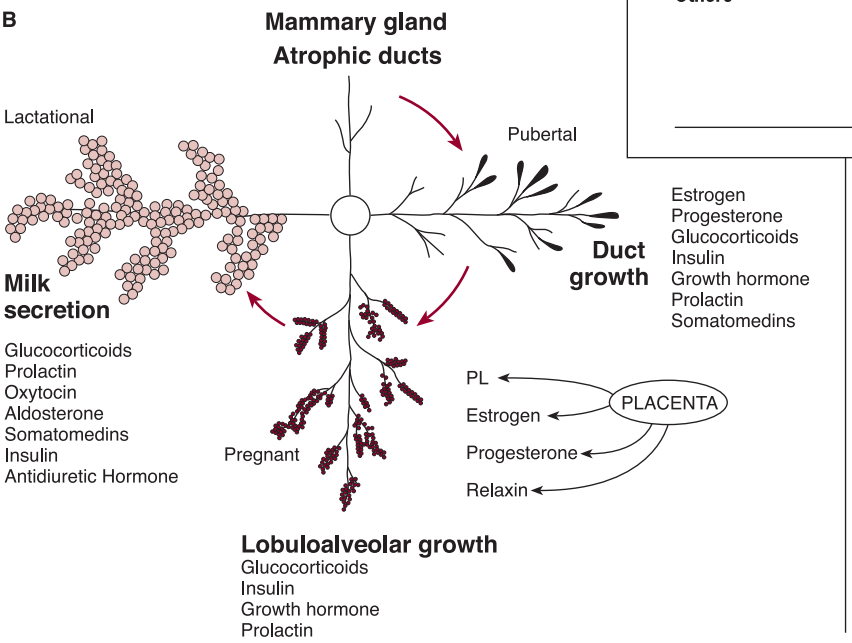
Most maternal organs, including the reproductive tract, normally recover rapidly from the effects of pregnancy and parturition. The uterus progressively decreases in size (uterine involution), with a reversal of myometrial hypertrophy. Individual myometrial cells usually decrease in size rather than in number. The CL, cervix and vagina also recover rapidly. The mammary glands, on the other hand, which have been endocrinologically prepared for lactation throughout pregnancy, now become available to perform their important physiologic task of providing nutriment for the neonate (Chs. 67-71).

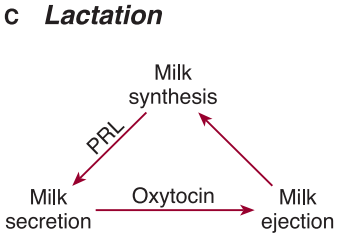
Ch67 Mammary Glands
(Mammogenesis and Lactation)



D

Chemical Mediators Identified in Milk	
Anterior pituitary hormones	PRL GH TSH FSH LH ACTH
Neurohormones	TRH GnRH GHIH GHRH Oxytocin
Steroid hormones	Estrogen Progesterone Testosterone Glucocorticoids Mineralocorticoids Vitamin D
Gastrointestinal peptides	VIP CCK Gastrin Gastric inhibitory peptide
Growth factors (Somatomedins)	IGF-I Nerve growth factor Epidermal growth factor Platelet-derived growth factor
Others	Prostaglandins (PGE, $PGF_{2\alpha}$) cAMP Relaxin Thyroid hormones (T_4, T_3, rT_3) Calcitonin PTH_{rp}





Source: **Parts A** and **B** modified from Cowie AT: Lactation. In Austin CR, Short RV [eds]: Hormonal control of reproduction. Reproduction in mammals, Vol 3, 2nd ed. Cambridge: Cambridge University Press, 1984:195–231.

All mammals are uniquely dependent upon milk for nutrition during the neonatal period. Mammary glands have the capacity to synthesize a variety of essential nutritional compounds, including milk fat, proteins, and lactose, and to secure others from the blood stream.

The number and conformation of mammary glands, as well as the organization of ductular systems, varies somewhat between mammalian species. Pigs, for example, possess up to 18 separate mammary glands, each with 2 lobular sections that open to the exterior through independent canals. Primates, like pigs, have no gland sinus, and within the nipple there is a series of ducts that drain independent lobular systems. A similar arrangement can be found in cats, dogs, and rabbits. The udder of the cow has 4 mammary glands with independent ductular systems, so that milk formed in one gland cannot pass directly into adjacent glands. Posterior mammary glands of ruminants are generally larger, and hence produce more milk than anterior glands.

Mammary glands are essentially skin structures, and therefore they adopt the blood supply and lymphatic drainage of the skin in that region. As ruminant animals enter their first lactation, there is a significant increase in regional blood flow, placing severe stress upon regional veins. These veins distend and valvular incompetence sometimes develops. Mammary veins in lactating dairy cows are in effect varicose veins, reflecting the enormous strain imposed by lactation in a species manipulated by humans, to produce milk greatly in excess of that required to fulfill the needs of its young. Lymph flow in the udders of lactating cows and sheep has been shown to be similar to the rate of milk secretion. Extensive edema of the udder sometimes seen in ruminant animals, can be attributed to the increased hydrostatic pressure within capillaries of the udder due largely to back pressure from veins, and an inability of distended lymphatic vessels to remove adequately the large amounts of interstitial fluid formed.

Growth and development of the mammary glands (**mammogenesis**), milk synthesis and secretion (**lactogenesis**), and milk ejection (**letdown**) are regulated by hormones. Lactogenesis is primarily controlled by **prolactin** (**PRL**), whereas milk ejection is stimulated by **oxytocin**.

Mammogenesis

The internal structure of a functional mammary gland is highly vascularized, and it is organized into clusters of minute, sac-like structures known as **alveoli,** the glandular epithelium responsible for **lactogenesis** (**Part A**). Alveoli are continuous with **ducts** and their enlargements for storing milk. In addition, alveoli are surrounded by modified epithelial cells (**myoepithelial cells**) that contain muscle-like myofilaments. These cells can contract to cause ejection of milk from alveoli into the ductular system and out of the gland in the region of the nipple.

Androgens suppress mammary gland growth, and are presumably responsible for their arrested development in the male fetus. **Postnatal mammary development** in females is conditioned by the degree of development achieved during each successive estrous cycle, and involves hormones from the pituitary (**ACTH**, **GH**, and **PRL**), ovaries (**estrogen** and **progesterone**), pancreas (**insulin**), liver (**somatomedins**), and adrenal cortex (**glucocorticoids**). **Estrogen** is primarily responsible for **ductular** development, while **progesterone** stimulates **lobuloalveolar** development. In this regard, the induction of lactation by steroid hormone treatment is sometimes desirable in animals with a productive lactational history, but poor reproductive performance. The use of a combined estrogen and progesterone treatment over a relatively short period of time (1 to 2 weeks) in dairy cows has induced ductular and lobuloalveolar development sufficiently to result in milk production. However, in all animals so far studied, the amount of milk produced is usually less than normal. Estrogen and progesterone are also ineffective in stimulating mammogenesis and subsequent lactation in the absence of normal anterior pituitary output of ACTH, GH and PRL.

During pregnancy, **PL**, **relaxin**, **estrogen**, and **progesterone** from the placenta continue stimulating expansion of alveoli, as do **glucocorticoids**, **insulin**, **GH**, and **PRL**. **During lactation**, continued milk secretion requires optimal physiologic levels of **glucocorticoids**, **PRL**, **oxytocin**, **ADH**, **aldosterone**, **insulin**, and **somatomedins**. **Part B** depicts mammary gland growth through the **pubertal**, **pregnant**, and **lactational** stages, and the hormones stimulating each.

Lactation

Lactation is generally separated into two separate phases. The first phase involves **milk synthesis** and **secretion** (lactogenesis), and the second phase involves **milk ejection** (**Part C**). If milk ejection is voluntarily halted, lactation ceases, milk is eventually reabsorbed, and functional alveoli are replaced by adipose tissue.

Although **lactogenesis** requires several hormones, it is primarily controlled by **PRL**, **PL**, **growth factors** (somatomedins), **insulin**, and **corticosteroids**. Lactogenesis involves alveolar cell synthesis of milk fat, protein, and lactose, and numerous vitamins and minerals are also secreted into the ductular lumen. Many hormones have been identified in milk, and their potential physiologic benefits to the neonate are being investigated (**Part D**).

Milk ejection is caused by a simple reflex mechanism controlled by **oxytocin** from the posterior pituitary (pars nervosa). Mechanical stimulation of the nipple (suckling) evokes release of oxytocin via a spinohypothalamic neuronal pathway (Ch. 68). Oxytocin then stimulates contraction of myoepithelial cells that cause milk to be ejected from alveoli. Suckling young strip milk from the gland by expressing it between the tongue and hard palate. **Prolactin** is also released when milk is ejected, stimulating further milk synthesis.

The first milk ejected from the mammary gland following pregnancy is known as **colostrum**, and has more protein and less carbohydrate than later milk. Colostrum contains **antibodies** (**immunoglobulins**) and other substances that protect the neonate against infection while its own immune system develops.

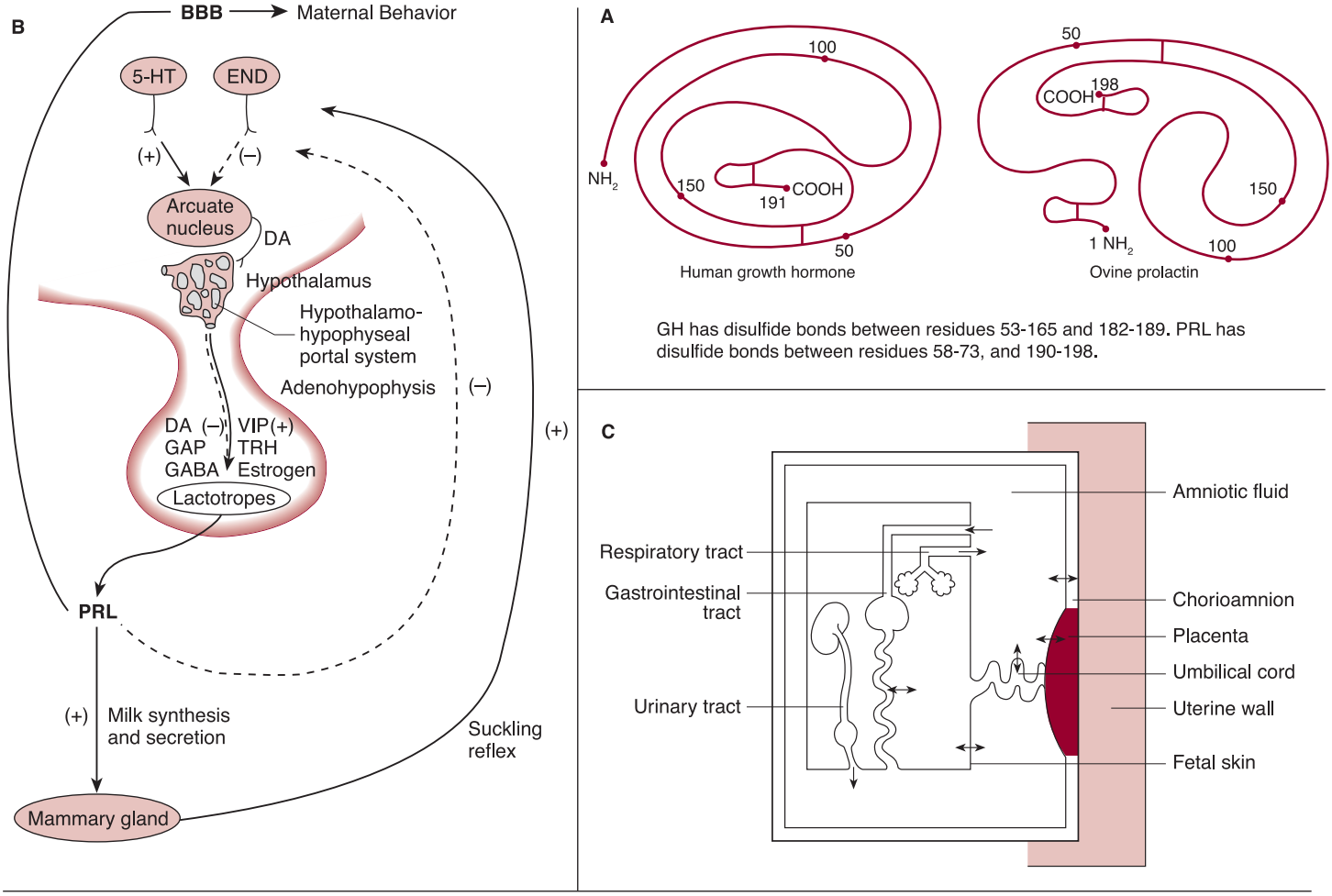
The immunoglobulins are produced in the mammary glands by **plasma cells**, which gain access to the secretory system through migration from adjacent tissue sites. Neonates generally have a limited amount of time (24 to 36 hours following birth) with which to assimilate these proteins through the gut wall. Additional antimicrobial factors found in colostrum are **lactoferrin**, which binds iron and is bacteriostatic, and **lysozyme**, which attacks the walls of bacteria. The immunoglobulins as well as these latter protective agents found in colostrum are also natural secretory products of the salivary glands.

Colostrum is a good source of **vitamin A**, which plays a central role in both photopic (day) and scotopic (night) vision, in bone remodeling, and in the maintenance and differentiation of epithelial tissues. The time required for changeover from colostral to post-colostral milk varies with species. In general, colostral milk tends to be more viscous, and is usually withheld from commercial milk production for a few days until its consistency (i.e., aesthetic appeal) improves.

Lactation is **metabolically expensive**. The lactating udder of the goat utilizes 60-85% of the **glucose** (largely from hepatic gluconeogenesis), 14-44% of the **acetate**, and a significant proportion of available **amino acids**. In lactating dairy cows producing 80 lbs. of milk/day, the blood glucose pool turns over every **5 min**, with mammary gland uptake accounting for **60-85%** of total glucose turnover. **Electrolytes** and **water** accompany the **lactose**, **proteins** and **triglycerides** secreted into milk. From 3-6% of body water can be passed into milk each day during lactation, and a 40 kg ewe producing 1 L of milk daily secretes 20-40 mEq of Na^+, 30 mEq of K^+, and 60 mEq of Ca^{2+}. These losses require homeostatic adjustments by various endocrine organs and the kidneys, where compensatory reabsorption of electrolytes and water takes place. The **pH** of milk is about **6.8**, and the kidneys usually adjust plasma pH by secreting a slightly alkaline urine.

Ch68 Prolactin

(Secretory Control and Physiologic Actions)



GH has disulfide bonds between residues 53-165 and 182-189. PRL has disulfide bonds between residues 58-73, and 190-198.

Source: Part B modified from Wallenburg HCS: The amniotic fluid. I. water and electrolyte homeostasis. J Perinat Med. 5:193, 1977, and **Part A** modified from Murray R, Rodwell V, et. al.: Harper's illustrated biochemistry. 28th ed, New York, NY: McGraw-Hill Medical, 2009.

Structural Similarity

Prolactin (**PRL**), **GH** and **PL** show considerable structural similarity (**Part A**), and therefore may promote similar physiologic actions (Chs. 10 and 62).

Control of Prolactin Secretion

Prolactin concentrations begin to increase progressively in the plasma of pregnant bitches, for example, about 35 days prior to parturition, peaking 1 to 2 days before whelping. They then decrease for about 36 hours before increasing to new peaks in response to each suckling experience.

Prolactin is the only pituitary hormone for which there is evidence of at least two releasing hormones, as well as two or three release-inhibiting hormones (**Part B**). Primary control over PRL release is **inhibitory** in mammals, with the pituitary releasing PRL when freed either surgically or chemically from hypothalamic control. This is in marked contrast to GH control, where both the presence of GHRH and the absence of somatostatin are necessary to elicit secretion.

Although there is some debate, most investigators believe that the physiologic mammalian **PRL release–inhibiting hormone** (**PRIH**) is the catecholamine neurotransmitter **dopamine** (**DA**), which is released into the hypothalamo-hypophyseal portal system from hypothalamic neurons in the arcuate nucleus. There is also some support for a peptide PRIH, namely the 56-amino-acid C-terminal fragment of the GnRH prohormone known as **GnRH-associated peptide** (**GAP**). It remains to be demonstrated, however, that GAP is a physiologic regulator. Large amounts of **γ-amino butyric acid** (**GABA**) have also been shown to block PRL release by direct action on lactotropes. However, GABA also stimulates PRL release through actions on hypothalamic neurons. Because PRL itself increases GABA synthesis and release into the hypothalamo-hypophyseal portal system, GABA could be acting as part of PRL's major negative feedback loop. **Serotonin** (**5-HT**) also reduces PRL output by stimulating DA release from the arcuate nucleus.

The physiologic PRL-releasing hormone (PRH) was at first thought to be **thyrotropin-releasing hormone** (**TRH**). However, the neuropeptide known as **vasoactive intestinal polypeptide** (**VIP**) stimulates PRL release during suckling when TRH is ineffective.

Endogenous opiate peptides (**END**) are also known to block the activity of DA-secreting neurons that normally prevent PRL release. **Estrogen** (in some mammals) tends to increase PRL secretion during the latter stages of pregnancy by increasing sensitivity of the lactotropes to hypothalamic PRHs. This effect may involve an estrogen-induced reduction in the sensitivity of lactotropes to DA by altering receptor levels and/or intracellular second messenger systems. Estrogen is also concentrated by certain GABA-secreting neurons that decrease their activity, thus increasing PRL release. Evidence suggests that **ADH** and **oxytocin** act as PRL-releasing factors during suckling (Chs. 7 and 67).

Prolactin, TRH and Galactorrhea

Secretion of lactescent (milky) fluid from the breasts of either males or non-lactating females is called **galactorrhea.** It is generally caused by **excessive PRL secretion**, and frequently occurs in **primary hypothyroidism**, characterized by decreased circulating levels of thyroid hormones, lack of their negative feedback, and therefore elevated circulating levels of **TRH** (and TSH). Prolactin release associated with primary hypothyroidism is thought to be evoked by high TRH levels (Ch. 38).

Prolactin, the "Mother Love Hormone"

Evidence suggests that systemic **PRL** and/or **PL** can gain access to CSF via a saturable, carrier-mediated process in the choroid plexus, from which these chemical messengers can diffuse to numerous brain sites. PRL of brain origin has also been reported. PRL receptors in the CNS have been identified, and their presence strongly resembles estrogen receptor (ER-α) distribution. PRL receptor expression appears to be directly influenced by estrogen, and progesterone. Much of this work was on gonadectomized, virgin rats treated with a steroid regimen that mimicked pregnancy. The hormone-treated, behaviorally inexperienced virgin rats were reported to elevate their systemic PRL levels, then retrieve, group and crouch over their test young. When PRL secretion was suppressed, the onset of **maternal behavior** was delayed.

PRL is recognized as an endogenous **anxiolytic agent**, and, like ACTH, is secreted into blood in response to a wide variety of stressors. Cortisol, secreted in response to ACTH, apparently feeds back negatively on PRL release. Chronic PRL treatment reportedly blocks stress-induced increases in **corticotropin-releasing hormone** (**CRH**; Ch. 22), and reduces neuronal activation in response to stress. Lactation is characterized by hyperprolactinemia, and is a stress hyporesponsive state. These physiologic adaptations apparently protect the infant and/or fetus from excessive exposure to high cortisol levels.

PRL release reportedly increases following orgasm in males and females. This prolonged postcoital elevation may provide a feedback system, reducing sexual drive during the refractory stage following intercourse.

PRL may also stimulate **food intake** and lactation during pregnancy, and promote leptin resistance. A reciprocal relationship seems to exist between **PRL** and **oxytocin** (**Oxy**). PRL stimulates Oxy release, and Oxy stimulates PRL release (Ch. 69). This relationship appears to be optimal during lactation, where both hormones are required.

Hyperprolactinemia

Circulating PRL concentrations in males are generally only slightly lower than those in nonlactating females. By itself, PRL apparently has little effect on the male reproductive apparatus. However, PRL receptors are present on the plasma membranes of testicular **Leydig cells**, where PRL is thought to potentiate the effect of interstitial cell–stimulating hormone (ICSH) on the steroidogenesis of testosterone. Prolactin may also increase the molecular population of androgen receptors on cells of the prostate and seminal vesicles.

Hyperprolactinemia is a common endocrine disorder of the hypothalamic-pituitary axis, and can be a major cause of reproductive dysfunction in both males and females. It is associated with a decrease in pituitary **ICSH** (**LH**) and **FSH** release, and **testicular atrophy** in males. High circulating titers of PRL may directly inhibit testicular function, **inhibition of GnRH release** seems to be the mechanism for reduced testosterone secretion, a hypogonadal state, decreased sperm production and infertility. Males may develop gynecomastia and galactorrhea. Females with hyperprolactinemia may develop infertility, galactorrhea, amenorrhea, reduced libido and habitual abortion.

Prolactin in Nonmammalian Vertebrates

Prolactin is also present in teleosts, amphibians, reptiles, and birds. In amphibians, PRL accelerates larval growth and blocks metamorphosis. It appears to be osmoregulatory in fishes and amphibians, where it is also, like PTH, a hypercalcemic factor. North American migratory birds exposed to a long day cycle release PRL, which induces premigratory fattening. As in mammals, TRH and VIP stimulate PRL release in birds. PRL stimulates brooding behavior (egg incubation) in chickens, and they stop laying eggs. When DA agonists are administered, egg-laying resumes (Ch. 72).

Prolactin and Amniotic Fluid Formation

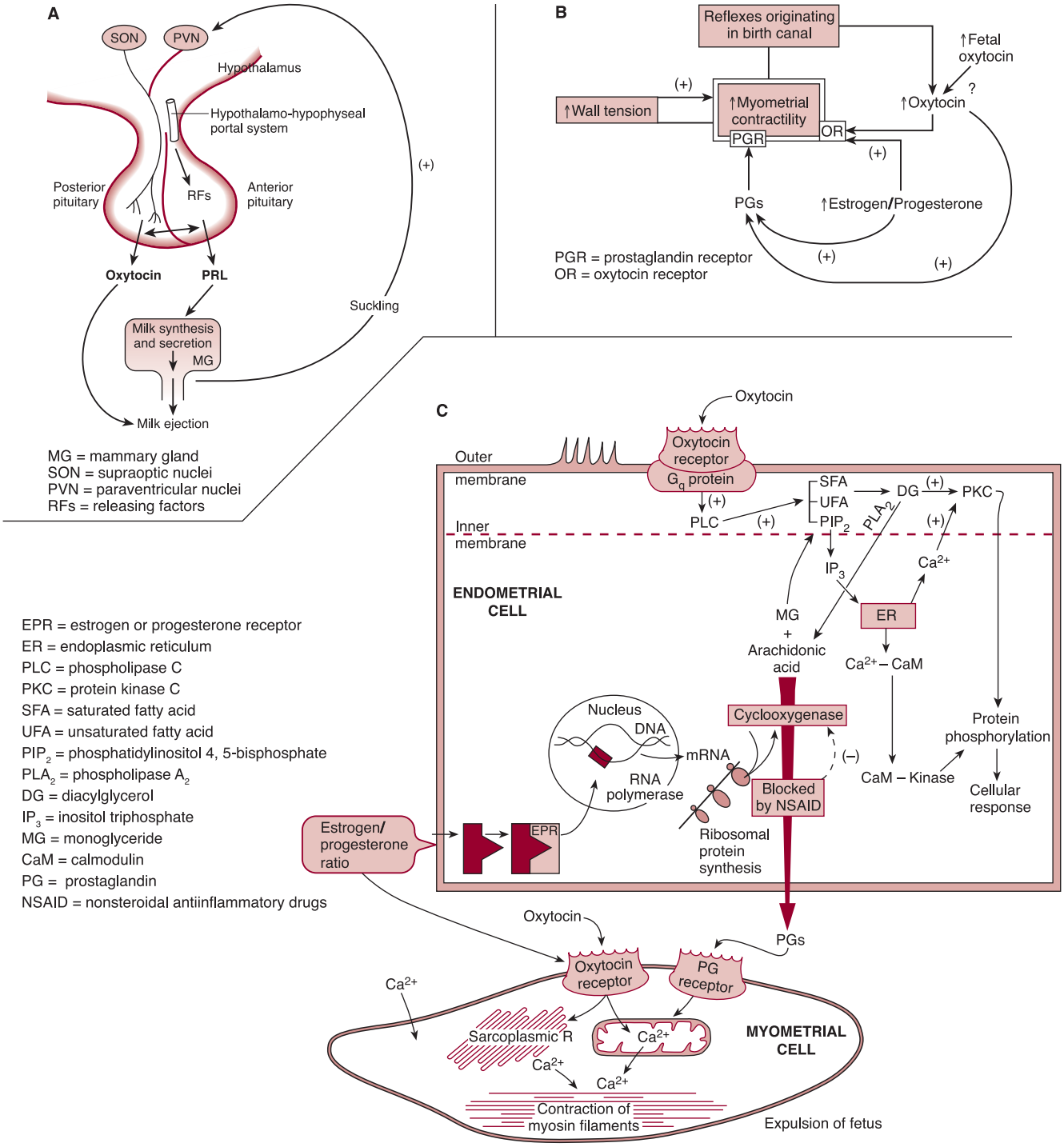
Amniotic fluid formation may be under hormonal control. It has been shown that PRL, which is present in amniotic fluid, can increase the permeability of the chorioamnion to water. Injection of PRL into the amniotic sac of rhesus monkeys in the third trimester of pregnancy significantly reduces the volume of amniotic fluid for 24 hours. Thus, prolactin's **osmoregulatory role** in amniotic fluid formation may be similar to its role in fish and amphibians (Ch. 72).

Part C depicts the pathways involved in amniotic fluid formation. The placenta, umbilical cord, and chorioamnion exchange materials continuously; fetal skin exchanges materials up to the time of keratinization; and the kidneys, respiratory tract, and gastrointestinal tract exchange materials phasically. Liquid is naturally secreted by the lungs (and thus into amniotic fluid), and moves through the lungs as if it were air. This phenomenon appears to be important for alveolar development during gestation, but must reverse itself at the time of birth. For at least 50 years it has been known that fetuses drink amniotic fluid, with the amount per day increasing in late pregnancy. By the time of parturition, the fetus may swallow approximately half of the amniotic fluid (in approximately 6 episodes) in a 24 hour period. Urine movement into amniotic fluid (micturition) is also high during the third trimester, and the urine is significantly hypotonic.

Amniotic fluid has several important physiologic functions: It physically protects the fetus from jolts associated with maternal activity; it assists in dilating the cervix and lubricating the birth canal at the time of parturition; it helps to keep the fetal environment at a relatively consistent temperature; it gives relative freedom of movement to the floating fetus without hindrance from, or damage to, the amnion; it prevents adhesion of fetal skin with the amniotic membrane; it allows for exchange of fluids and solutes in the respiratory tract; and swallowed amniotic fluid, once it is absorbed by the digestive tract, supplies lipids, essential amino acids and growth-promoting vitamins (e.g., pantothenic and folic acids) to extra- and intracellular fluid sites.

In certain animal species (e.g., pigs, horses and cattle), an **allantoic fluid** cavity (in addition to the amniotic fluid cavity) is prominent. The allantoic cavity is continuous with the cranial extremity of the urinary bladder by way of a urachus, which passes through the umbilical cord. Allantoic fluid originates from fetal urine and from secretory activity of the allantoic membrane. As gestation progresses, the composition of allantoic fluid steadily increases in electrolytes, total nitrogen and volume.

Ch69 Oxytocin
(Physiologic Actions)



Oxytocin (**Oxy**) is a nonapeptide (9 amino acids) synthesized in nuclei of the hypothalamus, and stored in the posterior pituitary (see Ch. 12). Lesser amounts are also synthesized and secreted by the corpus luteum (CL). In concert with **estrogen** and **prostaglandin** (**$PGF_{2\alpha}$**), CL-derived oxytocin may be involved with dissolution of the CL after an estrous cycle in which pregnancy did not occur (Ch. 54).

Plasma levels of oxytocin are normally low in males and nongravid females. In pregnant females, however, concentrations increase substantially during parturition, and following suckling during lactation. The two primary functions of pituitary-derived oxytocin appear to be: **1**) contraction of myoepithelial cells that surround alveoli and ducts of mammary glands to eject milk; and **2**) facilitation of uterine myometrial contraction during (and after) labor.

Oxytocin and Lactation

Exposure of mammary glands to **estrogen** during pregnancy causes development of myoepithelial cells that line ducts and alveoli, as well as development of **oxytocin receptors** on these cells so that they can respond to oxytocin following the suckling stimulus. As discussed in Ch. 67, PRL stimulates milk synthesis and secretion, while oxytocin causes contraction of myoepithelial cells for milk ejection (**Part A**). Oxytocin release is brought about through a neuroendocrine reflex, whereby suckling of the neonate sends neural impulses from the mammary gland to the hypothalamus, which then directs release of oxytocin from the posterior pituitary into the general circulation. Oxytocin may also have a stimulatory effect on **PRL** release (Chs. 7 and 68); however, a distinct hormone-releasing role for this peptide has not been clearly established.

Lactation is generally considered to be a vegetative experience. Fright and stress are known to **inhibit** milk ejection through mobilization of **catecholamines** into the general circulation. Catecholamines, in turn, are thought to act at three levels to **block milk ejection**: **1**) centrally by blocking oxytocin release; **2**) peripherally by vasoconstricting arterioles, thus reducing the amount of oxytocin available to myoepithelial cells of mammary glands; and **3**) directly as an antagonist to oxytocin on myoepithelial cells.

Oxytocin release may also be conditioned by a variety of visual, olfactory, and auditory stimuli (such as crying of the young or rattling of the milk bucket).

Oxytocin and Copulation

Genital stimulation involved in coitus releases oxytocin, which produces rhythmic contractions in the female reproductive tract as well as contraction of the vas deferens and epididymis in the male during ejaculation. The sensation of orgasm, which involves rhythmic contractions of reproductive smooth muscle in both males and females, may be induced by oxytocin. Whether the specialized contractions of the uterus and oviducts that propel sperm involve oxytocin has not been proven; however, it is possible that increased contraction of smooth muscle of the epididymis and vas deferens by oxytocin propels sperm toward the urethra.

Oxytocin and Parturition

The precise role of oxytocin in labor is enigmatic. Unquestionably, this peptide hormone causes **myometrial contraction** (providing the uterus is under estrogen dominance). Furthermore, its secretion can be reflexively increased by stretching the lower genital tract (**Part B**). However, maternal plasma oxytocin levels show no consistent rise immediately prior to or during early labor, although they do increase during the later stages. Fetal production is thought to contribute to this latter rise, and it has been suggested that protracted labor seen in mothers carrying anencephalic fetuses (those lacking a brain) may be a consequence of the lack of fetal stimulus.

On the other hand, uterine oxytocin receptors do increase throughout gestation, and dramatically so at term (under the stimulatory effects of estrogen). While labor can proceed without oxytocin, its most important roles may be to: **1**) work synergistically with estrogen in promoting endometrial prostaglandin synthesis, and **2**) work synergistically with those prostaglandins in promoting maximal uterine contractions after delivery of the fetus, thereby minimizing blood loss.

A relatively low **estrogen-to-progesterone ratio** is important in maintaining uterine quiescence throughout pregnancy. An increase in this ratio precedes labor (due to increased fetal adrenal cortical activity and therefore estrogen precursor synthesis from pregnenolone), and is most likely a parturition-initiating event in most domestic animal species, because **estrogen stimulates and progesterone inhibits endometrial prostaglandin and myometrial oxytocin receptor synthesis** (**Part B** and **Part C**). Evidence also indicates that a placental progesterone-binding protein exists, whose concentration may be increased by estrogen near term, causing an effective removal of free progesterone from the myometrium, thereby decreasing uterine quiescence. It has also been reported that unconjugated estrogen levels rise at term, thus increasing the amount of active estrogen available to enter target cells.

Estrogens and progesterone, respectively, act through receptor-mediated processes to stimulate and suppress the synthesis of mRNA essential for production of **cyclooxygenase** in the endometrium (**Part C**). The local decrease in the effectiveness of progesterone associated with continued action of estrogen also increases **myometrial oxytocin receptor** synthesis as well as **prostaglandin** (**PG**) levels in uterine fluid. In addition, PG production is increased by oxytocin, which also acts via receptor binding to the endometrium. Prostaglandins and oxytocin then act directly on uterine musculature to simulate contractions. Activation of contractile processes requires an increase in myometrial Ca^{2+}, which is achieved by decreased binding of Ca^{2+} to subcellular membranes and by influx into the cell.

When oxytocin interacts with its plasma membrane receptors on endometrial cells, activation of membrane-bound phospholipase C (PLC) through G_q protein catalyzes hydrolysis of the phosphatidylinositol 4,5-bisphosphate (PIP_2) moiety off membrane-bound phospholipid to produce diacylglycerol (DG) and inositol triphosphate (IP_3). Both DG and IP_3 act as intracellular messengers, DG (with Ca^{2+}) as a membrane-associated activator of protein kinase C (PKC), and IP_3 as a water-soluble inducer of Ca^{2+} release from mitochondria and the endoplasmic reticulum (ER), thereby causing a transient rise in the Ca^{2+}-CaM concentration of the cytosol. These two effects initiate a cellular response (Ch. 5). The DG is hydrolyzed further by phospholipase A_2 (PLA_2) to form arachidonic acid, a precursor to the prostaglandins, plus a monoglyceride (MG, with a saturated fatty acid (SFA) attached to the A_1 position). The MG is recycled into the membrane during the formation of another membrane-bound phospholipid. Cyclooxygenase inhibitors, such as **nonsteroidal antiinflammatory drugs** (**NSAIDs**) that reduce endometrial cell prostaglandin formation, can potentially abolish premature labor; however, they also **close the ductus arteriosus**, leading to fetal pulmonary hypertension.

Other Effects of Oxytocin

Oxytocin normally reduces blood pressure in birds, but severe water retention can occur in mammals receiving high doses of this hormone to promote uterine contractions during labor. This occurs because oxytocin shares structural similarity with ADH (Ch. 12), and thus can stimulate its receptors.

Intranasal administration of oxytocin has been shown to promote prosocial behavior. Receptors for this nonapeptide appear to be distributed in various brain regions associated with pair bonding, maternal care, sexual behavior, and the ability to form normal social attachments. It has thus been called, by some, the **"mother trust hormone."**

Ch70 Neonatal Physiology: I
(Thermal, Nutritional, and Cardiovascular Adaptations)

A ***Neonatal Thermal Adaptations***

Increase BMR
- Thyroid hormones
- Sympathetic activation
- Muscle movement

Brown fat
- Uncouple oxidation from phosphorylation

B ***Neonatal Glycogen***

Modest reserves

Mobilization requires a low insulin:glucagon ratio

C ***Neonatal Fat***

Reserves vary with species

Milk has a high fat content

Neonates frequently become ketonemic

D ***Fetal Circulation***

Low O_2 tension

Acidemia

Different from the adult due to the DV, FO and DA

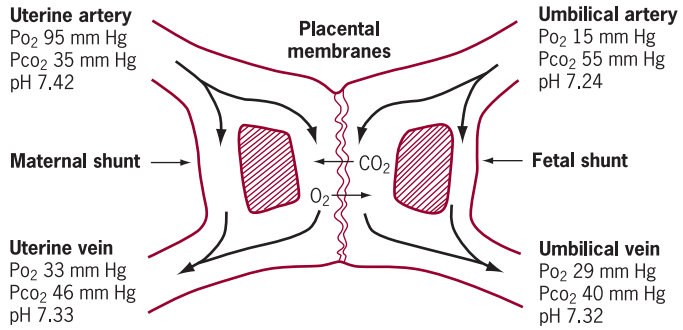
Reduced pulmonary circulation

F ***Neonatal CV Adjustments***

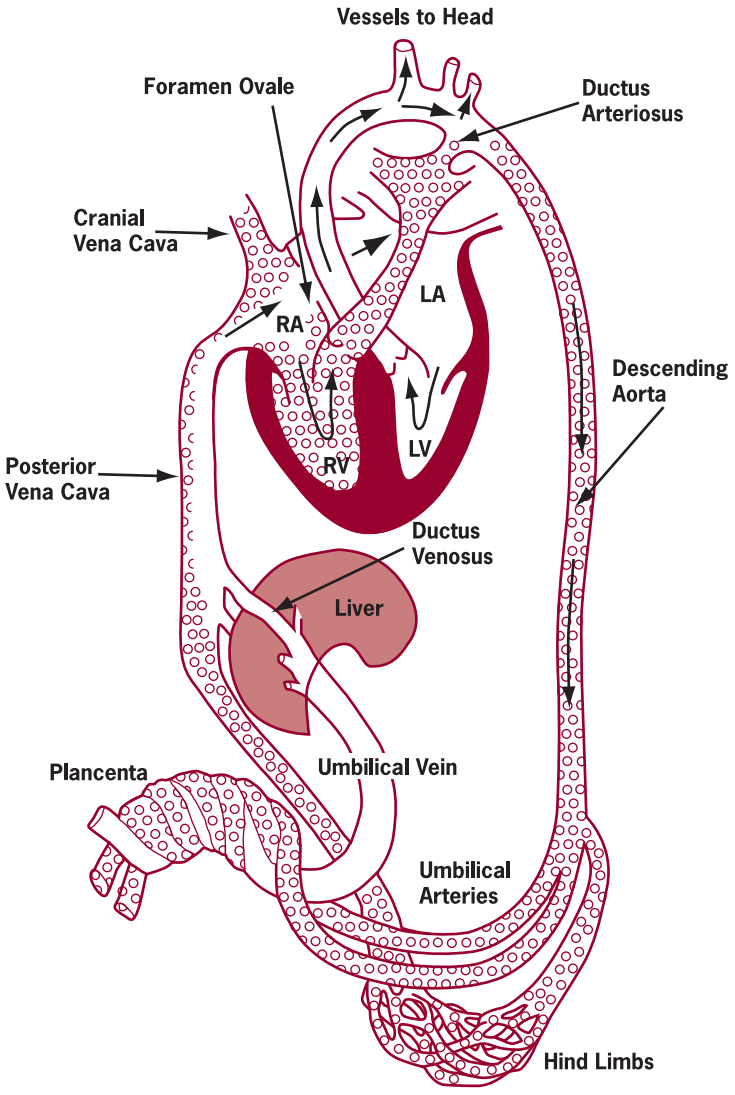
Glucocorticoids and a rise in blood O_2 tention normally close the DA following birth

Relative to the adult animal:
- Reduced BV
- Increased CO
- Low BP

E



G



Between conception and the onset of suckling the organism is faced with significant environmental changes, and alterations in its physiology. The newborn is launched into a world of stimuli for which it seems unprepared, but to which it must quickly adapt for survival. Studies of neonates in natural surroundings indicate that many of the signs we perceive as weakness are marks of strength, if strength means the ability to survive.

Thermal Adaptation of the Neonate

Neonates usually adjust to lower, more variable temperatures. The **basal metabolic rate** (**BMR**) after birth rises to a level about 3-times greater than the fetal rate (**Part A**). The **body temperature** (T_B) may fall sharply following birth, but quickly recovers. The fall and recovery times vary with species, and environmental conditions. In foals and calves it is transient; in lambs recovery takes a few hours, but in pigs it may take a day. These animals are relatively mature at birth, as are primate and guinea pig infants. Newborn mice and rats are relatively immature, and it takes longer for their thermoregulatory mechanisms to develop. The kitten, puppy and rabbit are intermediate between these groups.

Many neonates respond to lower temperatures through increased muscular movement rather than shivering. Extra heat can also be generated through the unique properties of **brown fat**, which is abundant in newborns. Brown fat tissue is well vascularized, and has a high content of mitochondria and cytochromes, but low activity of ATP synthetase. Oxidation of both glucose and fatty acids is emphasized.

Catecholamines and **thyroid hormones** are important in increasing lipolysis in both white and brown adipose tissue. Under the influence of these hormones, oxidation and phosphorylation are **uncoupled** in mitochondria of brown fat tissue, producing much heat, and little free energy is trapped as ATP. The proton gradient, normally present across the inner mitochondrial membrane of coupled mitochondria, is dissipated in brown adipose tissue by **thermogenin**, a unique uncoupling protein.

Neonatal Glycogen

Carbohydrate is an important source of energy for many newborns. Although neonatal ruminant and carnivore liver **glycogen** reserves are quite low, those in other species are higher, but fall rapidly following birth. In most species reserves usually reach 10% or less of their initial values within 2 to 3 hrs (**Part B**), even when the newborn is suckled. In a few days liver glycogen reserves rise toward adult levels. Both liver and skeletal muscle glycogen reserves are important energy stores that can be used during the postnatal thermoregulatory struggle. The overall nutritional state of the dam during pregnancy influences glycogen content of newborn animals, and affects their ability to maintain body temperatures following birth. Glycogen mobilization following birth requires a low **insulin:glucagon ratio** (Ch. 71).

Neonatal Fat Metabolism

There is considerable species variation in the proportion of fat in the bodies of newborn animals, and their immediate dependence on fat metabolism (**Part C**). The piglet retains about **1%** body fat, and the primate infant about **12%**. Lack of fat in piglets emphasizes the immediate dependence upon glycogen metabolism. Piglets subsequently lay down fat at a high rate.

The milk of most mammals is high in fat relative to carbohydrate, and during suckling neonates are faced with the problem of conserving a limited supply of glucose for cells that depend exclusively on this substrate, while providing sufficient alternative substrates for the energy requirement of developing tissues. The neonatal liver commonly converts some incoming **fatty acids** to **ketone bodies** (**acetoacetate and β-OH-butyrate**), which are water-soluble compounds that can cross the **blood-brain-barrier** (**BBB**). During the early postnatal period, ketone bodies are preferred over glucose as substrates for the synthesis of phospholipids and sphingolipids for brain growth and myelination. During the first weeks of postnatal development, when the accumulation of complex lipids accelerates, the proportion of ketone bodies incorporated into those lipids increases. In the lungs, acetoacetate serves better than glucose as a precursor for the synthesis of **surfactant** in the neonate.

Cardiovascular (CV) Adjustments of the Neonate

The fetal and neonatal circulations of sheep and goats have been studied. Data from fetal lambs indicate that the placenta and fetal membranes receive ≈**57%** of the cardiac output, lungs <**10%**, foreparts of the body ≈**15%**, and hindparts ≈**18%**. The fetus exists in an **acidemic**, relatively **low O_2 tension environment**, with hypoxemia due largely to maternal and fetal arteriovenous shunts at the placental level (**Parts D** and **E**). The placenta consumes about **10-30% of O_2** supplied via the uterine artery, and the Po_2 of blood in the umbilical vein is roughly **29 mmHg**. However, since **fetal hemoglobin** (**HbF**) has a greater O_2 binding affinity than adult Hb (HbA), at equal O_2 tensions HbF carries more O_2 than HbA (Ch. 30). The fetal heart reportedly operates at lower O_2 tensions more effectively than the adult heart due to more stored glycogen/gm muscle tissue.

To prevent hepatocytes from consuming the vital O_2 reserve, most unbilical venous blood drains through a low resistance **ductus venosus** (**DV**) in the **liver** on its way to the posterior vena cava (**Parts F** and **G**). As oxygenated blood in the posterior vena cava reaches the right atrium, a **crista dividens** directs most of it through an open **foramen ovale** (**FO**) into the left atrium, where it enters the left ventricle. As deoxygenated blood in the cranial vena cava flows into the right atrium, it mixes somewhat with blood from the posterior vena cava, and moves into the right ventricle. When the ventricles contract, oxygenated blood (Po_2 25 mmHg) from the left side of the heart enters the aorta and blood vessels to the head. Less oxygenated blood (Po_2 19 mmHg) in the right side of the heart enters pulmonary arteries. Since the lungs are collapsed, pulmonary blood vessels are not pulled open by surrounding alveolar septa, and fetal hypoxemia maintains pulmonary blood vessels in a vasoconstricted condition. Less than 10% of the cardiac output passes through fetal pulmonary capillary beds. The high vascular resistance in the pulmonary circulation forces blood leaving the right ventricle through an open **ductus arteriosus** (**DA**) in the pulmonary artery, where it enters the aorta (downstream from the brachycephalic vessels).

The **FO** and **DA** do not immediately close following birth. As umbilical blood flow is arrested through vasoconstriction, the loss of low-resistance placental blood flow increases systemic vascular resistance, which increases pressure in the aorta and left side of the heart. This change in pressure gradient from the right to left side of the heart temporarily reverses blood flow through the DA and FO. Flow reversal in the FO forces a flap valve to close, which over time adheres to the atrial wall, thus permanently closing this channel. Flow reversal in the DA can sometimes be heard as a chest murmur for several hours following birth. Closure of the DA is associated with the **rise in blood O_2 tension** which follows the onset of breathing, as well as high circulating **glucocorticoid** levels. Localized **prostaglandin** release throughout the fetal period **keeps the DA open**.

Blood Volume (BV), Cardiac Output (CO), and Arterial Blood Pressure in the Neonate

The **BV** of the newborn animal immediately following birth is low compared to the adult on a per kg basis; but, if the infant is left attached to the placenta for a few minutes after birth, or if the umbilical cord is stripped to force blood out of its vessels into the infant, additional blood enters the neonatal circulation. Then, during the ensuing few hours, fluid is lost into tissue spaces, which increases hematocrit and returns the BV toward normal. This "extra" BV runs the risk of causing mild pulmonary edema with some respiratory distress. The **CO** of newborns is normally about twice, in relation to body weight, that of adults. **Arterial blood pressure** is normally low; but, it increases over time, with the adult pressure attained at puberty.

Ch71 Neonatal Physiology: II
(Organ System Development)

H Neonatal Respiratory Function

High respiratory rate
Compensation for metabolic acidosis

First inspirations are powerful

I Neonatal Kidney Function

High urine output, low osmolarity

Contributes to the relative acidemia and dehydration of infants

Neonate possesses a relatively large ECF volume

J Classification of Newborn Mammals

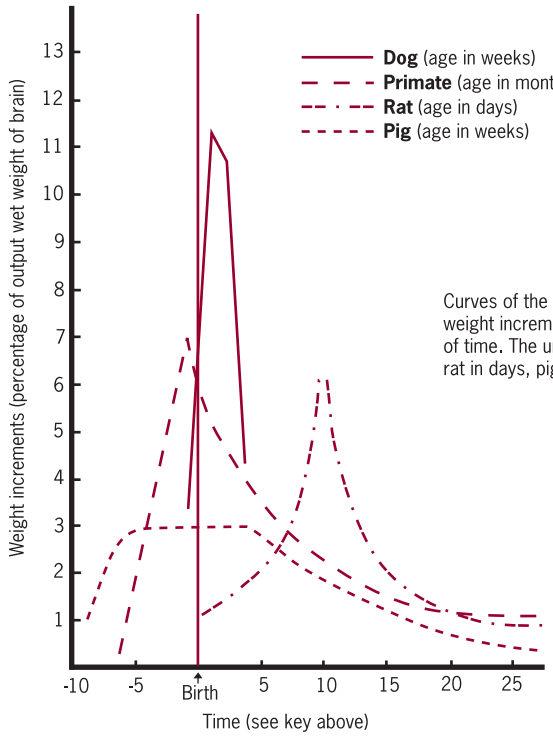
Herd animals (e.g., bovine)

Nest animals (e.g., pig and rat)

Mother-clinging animals (e.g., primates)

Animals with pouch protection (e.g., marsupials)

K



Curves of the rate of brain growth in different species expressed as weight increments (percentage of adult weight of brain) per unit period of time. The unit of time on the time scale is different for each species: rat in days, pig and dog in weeks, and primate in months.

L Neonatal Liver Function

Conjugate bilirubin poorly

Synthesize plasma proteins poorly

Exhibit sub-optimal gluconeogenic activity

M Neonatal Digestive Activity

Colostral protein (immunglobulins) absorbed early in some species

Pancreatic amylase and lipase secretion may be low

Intestinal starch and fat digestion may be low

Source: Part K modified from Davison AN, Dobbing J: Br Med Bull 1966; 22:40.

Respiratory System of the Neonate

The normal **rate of respiration** in the newborn is about 2-times as great as the adult in relation to body weight, partially in compensation for a **metabolic acidosis** (**Part H**). During birth, the walls of alveoli are collapsed by the surface tension of the fluid that fills them. More than 25 mmHg of negative pressure is required to pull viscous fluid down the airways for absorption, to oppose the effect of this surface tension, and to open alveoli for the first time. The first inspirations of newborns are usually powerful, and capable of creating as much as 60 mmHg negative pressure in the intrapleural space. Stimuli for these inspirations include **hypoxia** and **hypercarbia**, which result from loss of placental gas exchange, environmental cooling, and a generalized increase in sensory input.

Neonatal Kidney Function

Newborns tend to retain more water and salt than adults, and the **extracellular fluid** (**ECF**) **volume** is relatively large (**Part I**). Coupled with this is an apparent **immaturity of kidney function** which, relative to the size of the newborn, is characterized by a low level of tubular function, and an inability to concentrate the urine as well as the adult. These observations have led some to conclude that the newborn kidney is **"inefficient."** Such a view is unjustified when the level of renal function is considered in relation to the whole organism. For example, as long as the ECF volume is kept within appropriate limits, which are wide in newborns, the kidney is functionally adequate. The newborn uses so much of its protein intake for growth, incorporating amino acids into new tissue protein, that it can deal with large intakes of protein. Growth has a stabilizing effect which leaves only a small proportion of the nitrogen intake to be excreted by the kidneys.

The **rate of fluid intake and excretion** in suckling infants is about 7-times as great in relation to weight as in adults. Even a slight alteration in fluid balance can cause rapidly developing abnormalities. Second, the **rate of metabolism** in infants is about 2-times as great as in adults, which yields 2-times more acid. Therefore, there is a tendency toward **acidosis** in the infant. Third, functional development of the kidneys is not complete at birth, and therefore neonates cannot concentrate urine as effectively as adults. Considering the relative immaturity of the kidneys, the marked fluid turnover in infants and rapid formation of acid, it is understandable that two of the more important physiologic problems of infancy are **acidosis** and **dehydration**.

Neonatal Nervous Systems

The level of development and operation of the **central** and **autonomic nervous systems** in the postnatal period determines the level of response to environmental stimuli of which the newborn is capable. Newborn rats, without vision or locomotor ability, must exist largely as a sessile attachment to the mother, and for the first few days the kitten is not much more capable of independent action. Guinea pigs are born in a relatively advanced state of development, and newborn pigs and lambs have locomotor ability, open eyes, and the ability to shiver.

Investigators have pointed out that the patterns of activity of newborn mammals are appropriate to the environment in which they are born. They divide mammals into four classes (**Part J**). Of these, **herd animals** must be born in a state of **relative maturity** if they are to follow the herd successfully, whereas such an advanced stage of development may not be demanded of other animals.

The central nervous system is generally well developed in most neonatal mammals. As can be seen from **Part K**, the rate of **brain growth** accelerates in the third trimester, and then generally declines following birth in primates and pigs, but accelerates shortly after birth in dogs and rats.

Neonatal Liver Function

During the first few days of life, liver function may be somewhat deficient (**Part L**). **Bilirubin** is poorly conjugated with glucuronic acid, and biliary bilirubin excretion may not keep pace with bilirubin formation. Consequently, an unconjugated hyperbilirubinemia may develop. The liver of the newborn is also deficient in forming **plasma proteins**, and the plasma protein concentration falls to about 1 gm% less than that of normal adults. Occasionally, the protein concentration falls so low that the infant develops **hypoproteinemic edema**. Protein factors produced by the liver that are needed for blood coagulation (e.g., fibrinogen and prothrombin) may also be deficient. The gluconeogenic function of the liver is generally well developed in ruminant neonates, but may be deficient in non-ruminant animals. Some newborn animals may become **hypoglycemic**, and depend on stored fats for energy. Upon commencement of suckling, **ketonemia** frequently develops, which can compound the problem of **neonatal acidosis** (previously discussed).

Neonatal Endocrine System

Ordinarily the endocrine system of neonates is highly developed at birth, and rarely exhibits any immediate endocrine abnormalities. About the only exception to this generalization is **erythropoietin** (**EPO**) production. There is an early postnatal shift from the liver to the kidney as a major source of EPO in newborns (Ch. 30). This change usually occurs gradually over a period of weeks, and neonates may undergo a period of relative **anemia** during this time.

It is important for all mammalian neonates to maintain a low **insulin/glucagon ratio** during the immediate postnatal period, when infants are abruptly cut off from maternal fuel supplies. A low insulin/glucagon ratio allows them to mobilize their own glycogen and fat reserves until they can efficiently assimilate exogenous fuels through suckling. When this ratio is excessive, as may occur in infants born to diabetic mothers, **hypoglycemia** will develop.

Digestion, Absorption and Metabolism of Ingesta in the Neonate

During fetal existence the gut is not entirely inactive. Substances injected into amniotic fluid have been recovered from the fetal circulation, urine and tissues, which is evidence that the fetus is capable of digestion and absorption. Gastrointestinal movements occur *in utero*, and during the latter part of fetal life the gut is capable of the same order of motor activity as occurs at birth. Peristaltic movements have been demonstrated in animal fetuses, and toward the end of gestation the ingestion of material and its movement along the gut become more pronounced. Hypoxia stimulates gastrointestinal activity, and may lead to excretion of **meconium** from the fetal gut *in utero*. Meconium is material derived from desquamated mucosal cells and other organic matter, including bilirubin.

Absorption of intact protein by the neonatal intestine (for a **short** period of time; ≈36-48 hrs) is one means of antibody transfer for passive immunity. Production of antibodies in response to antigenic stimuli is low at birth, and the neonate would be overcome by invasive micro-organisms if it were not for maternal **immunoglobulins** (γ-globulins; IgA, IgD, IgE, IgG, IgM). In some species these are partially or wholly obtained after birth, through **colostrum** (e.g., horses), and in others they are obtained before birth by placental transfer.

The ability of newborns to digest, absorb and metabolize milk is not altogether different from that of the adult, with the exception of those listed in **Part M.** Secretion of **pancreatic enzymes** in newborns may be somewhat deficient, particularly **amylase**, leading to sub-optimal starch digestion and absorption. **Fat digestion** and absorption are also partially deficient, such that milk fat frequently appears in feces. The neonate is quite capable of digesting about 90% of ingested protein, and using the amino acids derived there from for its own protein synthesis.

Ch72 Comparative Aspects of Endocrinology

Nonmammals

- **Aquatic**
  - **Piscenes (fish)**
    - **Agnatha (Cyclostomes; jawless, round-mouthed fishes) (e.g., lampreys & hagfishes)**
    - **Chondrichthyes (jawed (Gnathostomes); cartilaginous fishes) (e.g., sharks, skates & rays)**
    - **Osteichthyes (Teleosts; jawed, higher bony fishes)**
- **Terrestrial**
  - **Herptiles**
    - **Amphibians**
    - **Reptiles**
  - **Birds**

Mammals

Hypothalamic-Pituitary Axis

Mammalian tropic hormones from the anterior pituitary (Ch. 7) have molecular counterparts among nonmammalian (or submammalian) vertebrates, and several nonmammalian functions have been experimentally stimulated by these mammalian agents. Early in vertebrate evolution 3 cell types appear to have differentiated to elaborate **glycoproteins** (**LH**, **FSH**, and **TSH**), **large polypeptides** (i.e., proteins; **GH** and **PRL**), and the **POMC-related peptides** (**ACTH**, **MSH**, and **endorphins**). These cell types attained functional significance and gave rise via amino acid substitutions, modified cleavage of prohormones, or both, to the additional hormones that characterize each group. Nonmammals produce the same tropic hormones as mammals, with considerable homology among them. Nonetheless, there is evidence of certain functional alterations elicited by these molecules related to minor structural and receptor modifications.

Gonadotropins (LH and FSH)

Several variants of the decapeptide **GnRH** are known, and purified GnRH preparations are effective in eliciting **LH** and **FSH** release from teleosts, amphibians, reptiles, and birds. Although some species exhibit more than one form of GnRH (e.g., chicken GnRH-I and GnRH-II), usually only one is distributed appropriately to release gonadotropins, while the other ostensibly functions as a neurotransmitter or neuromodulator. Hypothalamic control of anterior pituitary function in teleosts is slightly different than in tetrapods. There is no distinct portal system, and no true median eminence. Control of tropic hormone release is neuroglandular by direct peptidergic or aminergic innervation of the pituitary rather than neurovascular as found in several primitive fishes and in tetrapods (Ch. 7). Unlike mammals, DA appears to inhibit release of gonadotropins from the pituitary of teleosts.

It is speculated that a **gonadotropin-inhibiting hormone** (**GnIH**) of hypothalamic origin does not exist in vertebrates, but a hypothalamic dodecapeptide was discovered in **Japanese quail** and confirmed in several other types of birds. Accordingly, GnIH receptors are expressed in the anterior pituitary and several brain regions of birds, including the hypothalamus. GnIH has not been extensively studied, but it is thought that **melatonin** plays a key role in stimulating its release, playing a part in photoperiodic regulation of avian reproductive events.

It appears that the **most primitive glycoprotein hormone** is **LH-like**, and the LH gene later duplicated and became modified to produce a **TSH-like** molecule. **FSH** presumably diverged later from **TSH**. Bullfrog LH stimulates reptilian and avian thyroids, emphasizing structural proximity and functionality of the LH and TSH molecules.

FSH appears to be the most effective gonadotropin in **scaled reptiles** (**squamates**), although there are some conflicting reports, and GH appears to be gonadotrophic in fish. Since both FSH-like and LH-like gonadotropins are found in other reptiles, the squamate condition may be secondarily derived, and not represent a primitive condition.

Birds conform to the typical mammalian pattern of LH and FSH sensitivity, although avian species are rather insensitive to mammalian gonadotropin preparations. Testicular interstitial (Leydig) cells are stimulated by avian LH, and FSH stimulates Sertoli cells of the seminiferous tubules (Ch. 57). In birds, both LH and FSH appear to be important hormones for ovarian folliculogenesis and steroidogenesis, whereas ovulation results from a positive feedback mechanism promoted by LH and progesterone. Unlike mammals, LH and FSH are released in an asynchronous fashion, indicating that a separate FSH-stimulating factor may exist in birds (which has not been discovered). It is speculated that an FSH-stimulating factor may

also exist in mammals. Via negative feedback, both estradiol and inhibin play roles in controlling avian FSH secretion, like in mammals, and when PRL levels are elevated, gonadotropin levels appear to be reduced. Although norepinephrine (NE) is known to stimulate LH release in birds, DA, as in teleosts, inhibits it. Removing eggs from an incubating female bird will increase LH secretion, secondary to an increase in GnRH release.

Thyrotropin (TSH)

Although mammalian **TRH** (a Glu-His-Pro tripeptide; Ch. 36) is present in the brain of all nonmammalian vertebrates studied, it apparently has little effect on **TSH** release in fishes, amphibians, reptiles, and birds where responsiveness to mammalian **CRH** (and possibly **GnRH** and **GHRH**) appears to be greater than **TRH**. Pituitary thyrotropin activity in birds appears to be controlled **indirectly** by TRH. The avian hypothalamus secretes TRH during stress, and periods of decreased circulating thyroid hormones. Rather than stimulating TSH release, **TRH** appears to enhance pituitary **GH** release. GH then activates the **deiodinase** that converts T_4 to T_3 in target cells (Ch. 39), and since selenium is an essential component of the deiodinases, **selenium deficiency** affects growth in birds. Avian thyroid hormones and GH appear to be directly related since thyroid hormones feedback negatively on GH release. TSH is thyrotrophic, like in mammals, with no influence on peripheral T_4 deiodination.

Bullfrog **LH** exhibits **thyrotropic** activity **in fishes**, **reptiles**, and **birds**, and highly purified mammalian **LH** also has intrinsic **TSH activity** in teleosts. **This supports the concept that the modern TSH gene developed from the LH gene.**

Growth Hormone (GH)

GH release is altered predictably by mammalian hypothalamic neuropeptides (Ch. 10), and treatment of teleosts, amphibians, reptiles, and birds with mammalian **GHRH** reportedly promotes GH release. **Somatostatin** (**GHIH**) inhibits GH release, at least in teleosts, reptiles, and birds. Several other neuropeptides cause GH release, including **TRH** (in amphibians, reptiles, and birds), **GnRH** and neuropeptide Y (in teleosts). Reports of the actions of catecholamines on GH release fail to show any consistent pattern, with responses varying greatly among vertebrates. Responses to TRH and GHRH in birds can be blocked by **IGF** treatment, indicating that a negative feedback effect of IGFs may act on avian GH-secreting cells as in mammals. A synergistic relationship between thyroid hormones and GH can be assumed in nonmammals similar to that reported for mammals (Ch. 10). Androgens produce positive effects on fish growth, as in mammals, indicating a protein anabolic action for these steroids that is probably independent of GH. GH secretion correlates directly with induction of IGF-1 mRNA in livers of growing **coho salmon**, with similar relationships reported for other fish.

Mammalian GHs are effective in most nonmammals, but comparative studies of GH effects have been hampered by the **structural similarities of GH and PRL** (Ch. 68). For example, PRL exhibits clear growth-promoting actions in larval amphibians, and studies have failed to clearly separate effects of amphibian GH from amphibian PRL. A teleostean hormone named **somatolactin** (**SL**), which is chemically similar to GH and PRL, has been isolated from the pars intermedia, and appears to be involved with Ca^{2+} balance. Plasma SL peaks at spawning in Pacific salmon, and appears to stimulate ovarian and testicular steroidogenesis. Mammalian GH, like PRL, stimulates growth in juvenile **snapping turtles**, and in **lizards** GH stimulates appetite, as reported for PRL, but also induces a marked growth of the digestive tract. PRL may share this latter activity.

Bird GHs are more similar to their reptilian than their mammalian counterparts, and appear to influence post-hatching somatic growth through hepatic-derived IGF-1. GH stimulates lipolysis and influences the adrenal cortex to release corticosterone. The major stimulus for GH release in birds is **TRH**, with somatostatin (GHIH) playing an inhibitory role.

Prolactin (PRL)

The structure of PRL has changed throughout evolution, as evidenced by failure of **piscine** PRLs to work in avian and mammalian bioassays, although mammalian PRLs appear to have retained piscine activity. Piscine PRLs are sometimes referred to as **paralactins**. New PRL target tissues evolved (e.g., the crop sac of birds and the mammary glands of mammals), that possess receptors specific for "newer" portions of the PRL molecule that do not exist in piscines.

As in mammals (Ch. 68), major control over nonmammalian PRL release appears to be inhibitory. **DA inhibits PRL release** in teleosts and amphibians, as do **VIP** and **GAP**. The inhibitory action of VIP is unexpected in light of its stimulatory role in birds and mammals (Chs. 7 and 68). **Peptide histidine isoleucine** (**PHI**) is a VIP-like peptide that inhibits PRL release in teleosts, but stimulates TSH release in amphibians. Stimulation of PRL release by TRH occurs in nonmammals as reported for mammals, and major control of **PRL release in birds** appears to be **stimulatory** (unlike in mammals), under the direct control of **TRH** and **VIP**.

PRL exhibits so many different actions in vertebrates that it is difficult to describe them all. Investigators have suggested a variety of alternative, and perhaps more appropriate names for this hormone (e.g., **versitilin**, **ubiquitin**, **panaceanin**, and **miscellanin**). Most nonmammalian PRL actions can be grouped into 6 general categories: **1**) *growth phenomena*, **2**) *reproduction,* **3**) *H_2O and electrolyte balance,* **4**) *integumentary actions,* **5**) *behavioral actions* and **6**) *synergistic actions with other hormones.*

PRL increases Na^+ uptake across the gills of **teleosts**, with resultant alterations in plasma Na^+ concentrations. In most **amphibians**, PRL induces the **"water drive"** characterized by migration of juvenile terrestrial forms back to water for breeding, and specifically in **newts** and **salamanders**, integumentary actions of PRL have been described. Return to land after breeding (i.e., the **"land drive"**) by **newts**, **salamanders**, **frogs** and **toads** appears to be controlled by **thyroid hormones**. In **reptiles**, PRL has been reported to stimulate appetite and growth, and a possible effect on H_2O balance.

Estrogen synergizes with PRL in **birds** to create a **brood patch**, a ventral portion of the body which becomes defeathered and highly vascularized. This patch facilitates transfer of heat from parent to incubating eggs. PRL appears to reduce circulating avian gonadotropin levels, which increase again after brooding. PRL stimulates production of **"crop milk,"** and increases the number of mucosal cells in the crop sac gland of pigeons and doves (a biologic assay for PRL). During the final stages of incubation, the crop increases in weight in direct proportion to the plasma PRL concentration. PRL also promotes fat deposition and induction of migratory restlessness in birds. This behavior, or **zugunruhe** (German), appears immediately prior to the actual migration. Since PRL receptors are present in the brain, its affects on behavior appear to be well justified (Ch. 68).

PRL may be associated with **hypercalcemia** in birds, amphibians and mammals, for studies have related intravenous PRL administration to elevated serum Ca^{2+} levels and urinary Ca^{2+} excretion.

The Proopiomelanocortin Group (ACTH, MSH, and Endorphins)

As in mammals, the **ACTH**, **MSH**, and **endorphin-like peptides** of nonmammals are all derived from the same precursor, **POMC** (Ch. 8). Although the endorphin-like peptides are present in the pituitaries of all jawed vertebrates studied, they have not been detected in agnathans. Physiological roles for **β-endorphin** in nonmammals are

not well documented, but it may play a role in the **modulation of GnRH release**, as in mammals (Ch. 56).

Ovine CRH appears to be an effective releaser of **ACTH** in teleosts, amphibians, and birds, but studies on the natural hypothalamic-hypophysiotropic factors of nonmammals are not well documented. Most investigators feel that there must be considerable homology between mammalian and nonmammalian CRH, although it has yet to be isolated. As in mammals (Ch. 12), **AVT** and **AVP** can stimulate **ACTH release** in amphibians and birds, but solid justifications for these actions are lacking.

Secretion of **ACTH** in **teleosts** has been correlated with enhancement of cortisol secretion from the **interrenal gland** (homologous to the adrenal cortex of mammals), and **3β-hydroxysteroid dehydrogenase** activity is enhanced in the bullfrog interrenal gland following ACTH treatment (Ch. 21). Blood levels of corticosterone are reduced through hypophysectomy in **lizards**, **turtles**, and **birds**, whose interrenals all respond to ACTH. PRL, GH, serotonin (5-HT), and PTH also tend to stimulate the interrenal glands of **birds**. Although ACTH stimulates mainly cortisol release in mammals (Ch. 22), it stimulates **corticosterone**, **aldosterone**, and **11-deoxycorticosterone** release in birds. Corticosterone becomes the major circulating avian glucocorticoid. As in mammals, actions of ACTH are cAMP-mediated.

Many vertebrates demonstrate **pigmentary changes** correlated with environmental factors or particular life events. Rapid responses are generally under **neural control**, while slower responses are under **endocrine** (**or neuroendocrine**) **control**. As discussed in Chapter 9, one of the most important chromatophores of lower vertebrates is the **dermal melanophore**, which contains melanin granules concentrated in special organelles called **melanosomes**. When melanosomes are concentrated around the nucleus of the melanophore, the skin appears lighter than when they are dispersed throughout the cytoplasm. Coloration patterns of some vertebrates may be determined by the distribution of different chromatophore types, and by the relationship of dermal melanophores to other chromatophores. For example, dark spots on **frog skin** are due to concentrations of melanocytes and extracellular deposition of melanin, whereas adjacent regions of the skin that may vary from light green to black are occupied exclusively by dermal melanophores and other chromatophores. The major target for **MSH** in nonmammals is the **dermal melanophore**. In a few cases, other chromatophores may be affected. Although the mechanism of melanosome migration is incompletely understood, it appears that MSH is acting through cAMP.

Although it is not clear what mechanism predominates in controlling release of MSH in **piscines**, the **pars intermedia** exhibits innervation, with peptidergic fibers controlling MSH synthesis, and aminergic fibers controlling its release. MSH responses in **sharks** are slow, taking up to 100 hrs. to achieve maximal adaptations to changing backgrounds. In many **teleosts**, MSH does not appear to play a major role in physiologic color change, since dramatic pigmentary responses are under control of direct aminergic innervation of melanophores and other chromatophores. A **melanin-concentrating hormone** (**MCH**; 17 amino acids), has been isolated from teleost pituitaries, and it appears to **block both MSH** and **ACTH release**.

Catecholamines appear to control MSH release in **amphibians**, where evidence exists for the presence of both **α-** and **β-adrenergic receptors** in the **pars intermedia**. These receptors are also present on **amphibian iridophores**, another special chromatophore, where aggregation of reflecting platelets is involved in color change. The iridophores are also affected by MSH.

Although innervation of the **reptilian pars intermedia** does not appear to exist, catecholamine innervation of pigment cells reportedly occurs in some reptiles. Therefore, pigmentary control of dermal melanophores may be neural, endocrine, or both. Color changes in some **chameleons** (e.g., *Chamelo pumilis*) are under direct neural control, with no sensitivity to MSH, while in others (e.g., *Chameleo jacksoni*), the opposite is true. **Horned lizards** appear to exhibit both neural and hormonal regulation of melanophores.

The observation that **feather pigments** (including melanin) are under gonadal, thyroidal, and gonadotropic hormone control seems to be related to **loss of the pars intermedia and perhaps MSH in birds**. The only reported physiologic action for MSH in birds relates to development. Embryonic implants of chicken pituitaries cause formation of black feathers, where normally only white feathers would develop. This effect can be mimicked by treatment with either MSH or ACTH.

Pars Nervosa and Epiphysial Complex

Vasopressin and Oxytocin

Structures of nonapeptides from the pars nervosa (i.e., neurohypophysis – **oxytocin** (**Oxy**), **arginine vasopressin** (**AVP**; also called **antidiuretic hormone** (**ADH**)), and **arginine vasotocin** (**AVT**)), are presented in Chapter 12. **Lysine vasopressin** (**LVP**) is a variant of **AVP** occurring in pigs, hippopotamuses, one mouse and several marsupials, and **mesotocin**, a nonapeptide of birds, varies from AVP by having Ile in position 3, and Leu in position 8. These basic molecules (AVP, AVT, LVP, and mesotocin) tend to exhibit good pressor and antidiuretic properties among vertebrates, yet weak oxytocin-like properties (i.e., uterotonic, milk-ejecting, and depressor-like; Ch. 69) when injected into mammals. Mesotocin exhibits good depressor activity in birds, and reduces circulating aldosterone levels. It appears that **AVT** is the most primitive form of nonapeptide from which the other vertebrate nonapeptides evolved, and that **Oxy** is restricted to mammals (Ch. 69).

Parapineal and Pineal Organs, and Melatonin

Almost all vertebrates possess 1 or 2 epithalamic structures that constitute the **epiphysial complex**. The components are the **pineal gland** (or **organ**), and a more anterior projection, the **parapineal organ** (**parietal eye**). These structures apparently arose in primitive fishes as a pair of diverticula, and both organs have been retained only in **cyclostomes** and **lizards**. The pineal gland is found in all vertebrate groups, with the exception of **crocodilians** (Chs. 60 and 61).

The **parapineal** is thought to inhibit melanotropin release in cyclostomes, and the **pineal** may be involved with suppression of thyroid activity. In **teleosts**, a reduced parapineal has been described in only a few, but the pineal is prominent. Pigmentation, responses to light, and thyroid changes are influenced by the pineal of teleosts. Melanophore changes have been reported in several teleost species, including rainbow trout, following injection of melatonin and/or epinephrine, but circulatory melatonin levels exhibit no correlations to adaption by rainbow trout to different backgrounds. Attempts to relate pineal function with thyroid function are equivocal in **amphibians**, but reproduction appears to be under an inhibitory pineal influence. In **reptiles**, melatonin has been localized in the blood, pineal, and retinas of snakes, lizards, and turtles. Definitive effects of the epiphysial complex on reproduction are reported in lizards, and the **parietal eye** appears to be the transducer through which photoperiod influences reproduction.

Avian pineals are similar to their mammalian counterpart. The brain and especially the hypothalamus in birds may be the primary site of melatonin action, and may explain effects of melatonin on gonadal function, thermoregulation, and locomotor activity (Ch. 61).

The **harderian gland** (Ch. 61) is found directly behind and around the eye of all vertebrates possessing nictitating membranes (i.e., the third eyelid found in most reptiles, birds and mammals, but not

primates). It is a lipid-secreting lacrimal tear gland draining into the nasolacrimal duct that secretes **protoporphyrin IV**, a porphyrin intermediate in heme biosynthesis. This **reddish pigment**, most often recognized in Norway rats, undergoes fluctuations correlated with stress and lighting conditions, staining red or pink under UV light. In addition to the retinas, parietal eyes and pineal gland, the harderian gland appears to be another source of melatonin.

An hypothesis, by **William Gern** and **David Norris**, for the **original function of melatonin and the evolution of other functions**, is based on the presence of melatonin-synthesizing systems in **retinas**, **parietal eyes**, **harderian glands**, and **pineals**, and on observations that pineals of more primitive vertebrates are photoreceptive. Evidence indicates that retinal, pineal, parapineal, and harderian melatonin exhibit night-time (scotophasic) peaks of synthesis (Ch. 60). Melatonin was initially a local hormone, regulating melanosome distribution in the retina. During the day, melanosomes are dispersed in retinal pigment cells of these "early" species, protecting photoreceptors from intense light. Elevated melatonin at night causes melanosome concentration, allowing dim light to maximally stimulate rod photoreceptors. Melanosome sensitivity to melatonin is retained in the skin of both modern fishes and amphibians, and similar mechanisms presumably operate in the photoreceptive outer portion of the pineal, and in the parietal eye (parapineal). Increases in melatonin synthesis during scotophase, whatever the source, causes a greater proportion of melatonin to appear in blood. Consequently, scotophasic elevations are reliable internal cues for obtaining information about seasonal photoperiods. According to their hypothesis, diurnal melatonin rhythms have been coopted as blood-borne signals entraining a number of other internal events during the evolution of vertebrates (Part D, Ch. 61).

Thyroid

The thyroid gland, which stores its secretions extracellularly (Ch. 36), is one of the most highly vascularized endocrine glands, and may be the **oldest phylogenetically**. Regardless of gross morphological differences, follicular structure and function are reportedly mammalian-like in all gnathostomes with respect to iodide metabolism, hormone biosynthesis, production and storage of thyroglobulin, hormone release, and responsiveness to TSH. Biochemical differences are quantitative, not qualitative, and thyroid hormones present in all vertebrates are also found in some invertebrates. There is considerable diversity in the actions of thyroid hormones among vertebrates. Investigators report that thyroid function evolved hand-in-hand with endocrine control of **reproductive function**, and that thyroid function is associated primitively with gonadal maturation. In **higher bony fishes** peak thyroid activity appears to coincide with spawning behavior.

Pituitary **thyrotropes** and **TSH** are apparently **absent in agnathans**, and these cells may have evolved later from **gonadotropes**. Exogenous thyroid hormones and gonadal steroids have inhibitory actions on thyrotropes, and on gonadotropes in **teleosts**. Similar actions are reported in **amphibians**, **reptiles**, and **birds**. The observation that mammalian **LH** stimulates thyroid function in fishes is interpreted as supportive evidence of this hypothesis. Effects of thyroid hormones on growth, metabolism, development, and the integument are interpreted as later evolutionary events.

A general **antagonism** appears to exist between **PRL** and the **thyroid axis** of **nonmammalian vertebrates**. Studies also demonstrate a **goitrogenic** action of **PRL** directly on the thyroids of **teleosts**, **amphibians**, **lizards**, and **birds**, but the **peripheral antagonism** between **T_4** and **PRL** in certain tissues of **larval amphibians** is best known (Ch. 68). **TRH** causes **PRL release** from the pituitaries of **bullfrogs**, **turtles**, **birds**, and **mammals**, but not from those of **newts**. **TRH**, which is abundant in the amphibian brain, may be a **PRL-releasing agent**, especially since attempts to demonstrate a role for this tripeptide in the activation of the amphibian thyroid axis have failed. **TRH also stimulates ACTH release from the pars intermedia of horses with Cushing's-like Syndrome** (**pars intermedia dysfunction**), but under normal circumstances does not appear to be a physiologic stimulator.

Thyroid hormones induce **metamorphosis** in amphibians, and aid in the transformation from aquatic larval to terrestrial or semiterrestrial forms. It appears that metamorphosis not only involves the thyroid hormones, but also PRL, CRH, ACTH and the corticosteroids. **CRH may be the endogenous hypothalamic stimulator of TSH (and ACTH) release in amphibians**, as TRH appears to be the PRL releaser. Goitrogens or PRL appear to accelerate larval growth, but block metamorphosis.

Molting or shedding of the skin appears to be under thyroid hormone control in **reptiles** and **some amphibians**, yet in others corticosteroids seem to be involved.

Wild and domestic **birds** appear to exhibit thyroid structural and functional characteristics similar to mammals, except that the **calcitonin-secreting clear (C) or parafollicular cells of mammals are absent in the avian thyroid**. Calcitonin is secreted from separate **ultimobranchial glands** of nonmammalian vertebrates, and it is believed that ultimobranchial cells became incorporated into the thyroid glands of mammals, just as some parathyroids have done (see below). Avian thyroid hormones affect reproduction, growth, metabolism, temperature regulation, molting, and migratory activities. Albumin and **transthyretin** are plasma binding proteins, and there is evidence that transthyretin plays a role in assisting with the transport of T_4 through the choroid plexus. TRH appears to have an indirect stimulatory affect on avian thyroid hormone activity (through GH; see above), and somatostatin appears to have an inhibitory affect. **T_4** may contribute to seasonal testicular regression in birds by **reducing testicular LH sensitivity**.

Although thyroid abnormalities are described in nonmammalian vertebrates, they are not common.

Parathyroids

Comparative aspects of **Ca^{2+}** and **PO_4^{3-} homeostasis** are complicated by the **absence of parathyroid glands in fishes**, and the presence of the **corpuscles of Stannius** and **ultimobranchial glands** imbedded in the kidneys. Ca^{2+} metabolism in fishes appears to be regulated by one or more **hypercalcemic factors** from the pituitary (e.g., **PTHrp**, **PRL** or **hypercalcin**), and a **hypocalcemic factor** (**CT** or **hypocalcin**) from the corpuscles of Stannius and/or the ultimobranchials. **Definitive parathyroid glands and PTH first appear fully differentiated in amphibians**. Scales are important Ca^{2+} reservoirs in **teleosts**, with pituitary factors being important regulators of Ca^{2+} homeostasis in **fresh water species**, and the corpuscles of Stannius in **sea water species**. Mammalian or fish **CT** can decrease the free ionized Ca^{2+} concentration of blood, with the major target organ being the **gills**. Salmon CT is a more potent hypocalcemic factor in mammals than mammalian CT. This greater potency is a result of its relative resistance to clearance from blood.

Parathyroid glands are distinct in **amphibians** and **reptiles**. The effects of PTH and parathyroidectomy are similar to those observed in **birds** and **mammals**, but occur with lessened intensity. Ca^{2+} is a mineral that needs to be consumed in a reptile's diet (perhaps as part of environmental water), and for many ingestion of whole prey seems to have led to an evolution whereby vitamin D is completely (or nearly completely) collected. For many commonly kept **lizards** that are diurnal and insectivorous or herbivorous, UV light is needed to activate the cholecalciferol biosynthetic pathway (Ch. 17). **Metabolic bone diseases** (**MBDs**) are common in captive **reptiles** and

amphibians, and provide classic examples of **nutritional secondary hyperparathyroidism** in older animals and **renal secondary hyperparathyroidism** (Ch. 18).

Estrogens increase liver production of **vitellogenic (yolk) phosphoproteins** which bind Ca^{2+} when secreted into blood. Consequently, there is an elevation in total plasma Ca^{2+} and PO_4^{3-} associated with vitellogenesis that appears to be a mechanism whereby increased Ca^{2+} becomes available for **egg shell production** (**birds and reptiles**), and/or for incorporation into eggs. A similar mechanism operates in mammals, whereby maternal dietary Ca^{2+} is directed during development or lactation to the offspring by actions of **PTH**. Protection of the maternal skeleton is afforded through the actions of **estrogen-stimulated CT release** (Ch. 17). Estrogens also stimulate **$1,25(OH)_2D$ production** in birds and mammals. Conversion of provitamin D to cholecalciferol (D_3) is stimulated by UV light, especially on featherless areas of avian skin. Birds kept indoors must be supplied with dietary vitamin D_3, since vitamin D_2 is considered to be an ineffective supplement (Ch. 17). **Avian hypercalcemia** is most often associated with increased estrogen secretion and production of Ca^{2+}-binding proteins, whereas **hypocalcemia** is most often dietary (low Ca^{2+}, PO_4^{3-}, or vitamin D_3). Excessive dietary Mg^{2+} can also reduce intestinal Ca^{2+} absorption.

Expression of the **PTH_{rp}** gene (Ch. 16) in the isthmus and **shell gland** of the adult avian oviduct is related to entrance of the egg into the oviduct, and to calcification of the shell. **PTH_{rp}** also relaxes vascular smooth muscle and increases blood flow to the shell gland during calcification. Relaxation of oviductal smooth muscle by **PTH_{rp}** allows entrance and passage of the egg through the oviduct.

Rabbits appear to be an exception to the mammalian norm of **Ca^{2+} homeostasis**, since total serum Ca^{2+} values are usually about **30-50% higher than those of other mammals**. The amount of Ca^{2+} absorbed from the intestinal tract appears to be more dependent on the Ca^{2+} gradient than on the serum $1,25(OH)_2D$ concentration. In contrast to most mammals, a rabbit's teeth are constantly erupting, and the life-long demand for Ca^{2+} is interpreted by some as the reason for higher serum Ca^{2+} levels. Rabbits, through constant dental wear, continually release Ca^{2+} from their teeth, swallow it and reabsorb it from their intestines. This high intestinal absorption and serum Ca^{2+} level impose a **high filtered Ca^{2+} load** on the kidneys. It is not uncommon to see calcium carbonate crystals in alkaline rabbit urine. When metabolic demands for Ca^{2+} are elevated in rabbits (e.g., growth, pregnancy, lactation or metabolic disorders), serum Ca^{2+} levels are reduced and the urine is clear.

Adrenals

Although **zonation** of mammalian adrenocortical areas is evident (Ch. 21), there is less evidence of these distinct anatomical/functional relationships in nonmammals. However, general sequences for corticosteroidogenesis appear to be similar in all vertebrates with respect to precursor-product relationships. Many of the same enzymes are involved. Among **nonmammalian tetrapods**, the nature of corticosteroid secretion and the secretory pattern are similar to that described for cells of the mammalian zona glomerulosa. Daily rhythms and seasonal variations in corticosteroid secretion occur in nonmammals and mammals (Chs. 3 and 22). Peak seasonal adrenocortical activity is roughly correlated with periods of reproductive activity, although cause-effect relationships are difficult to firmly establish. Some studies indicate that "stress" may be the critical factor, and that stressors associated with reproduction may be only one component involved (albeit a major one). Nonmammalian adrenal responses to stressors such as surgery, forced exercise and handling are similar to those described for mammals.

Corticosteroids have been isolated from agnathans, and are believed to be of adrenal origin. No evidence exists for either **renin** or a **JG apparatus** in **cyclostomes**, as this specialization may not have evolved until the onset of the bony fishes. There is evidence of **ANP** in **agnathans**, indicating **a mammalian-like role for the natriuretic peptides before the renin-angiotensin system developed**.

Shark interrenals (adrenals) synthesize **corticosterone** and **11-deoxycorticosterone**, but apparently **no aldosterone** and **cortisol**. Although somewhat controversial, it appears that components of the renin-angiotensin system may exist in sharks.

Teleost adrenal tissue responds to **ACTH** by secreting **cortisol** under stress, and **CRH** stimulates **ACTH** release. **Angiotensin II** has been shown to stimulate **cortisol** secretion in teleosts, which is **their principal salt-retaining corticosteroid**.

Amphibians have both **adrenocortical cells** and **chromaffin cells** in their interrenal glands, with the major corticosteroids synthesized in most adult cortical cells being **aldosterone** and **corticosterone**. **Larval amphibians** and those that are **permanently aquatic** exhibit a preponderance of **cortisol over corticosterone**. **Ovarian 11-deoxycorticosterone production** has been reported, which may be an important adrenal steroid source in mature females. **AVT** indirectly stimulates secretion of **corticosterone** and **aldosterone** in amphibians by promoting **ACTH** release. ADH, in mammals, plays a similar role (Ch. 12). **Reptilian** interrenals exhibit structural and functional similarities to mammalian adrenals. Like in amphibians, **aldosterone** and **corticosterone** appear to be the primary steroids secreted. Although no macula densa has been described in fishes, amphibians and reptiles, **angiotensin II** appears to stimulate corticosterone release from the reptilian interrenals.

The adrenal glands of **birds** are organized in the same manner as those of **turtles**, **crocodilians**, and most **snakes**. The relative quantities of chromaffin with respect to adrenocortical cells can vary. Their major corticosteroids are **corticosterone** and **aldosterone** (like in amphibians and reptiles). Bird adrenals are also partially responsive to **PRL**, **hGH**, **5-HT**, and **PTH**.

Although all tetrapods have JG cells, the **macula densa** is present only in **birds** and **mammals**. Angiotensin II stimulates corticosteroid release throughout the vertebrates, which produce mineralocorticoid-like effects.

Although it is tempting to speculate that the mammalian fetal pattern of high **norepineprhine** (**NE**) production with increasing **epinephrine** (**Epi**) following birth is an example of ***ontogeny recapitulating phylogeny***, (a concept supported by the need to methylate NE to form Epi), studies are less than convincing. **NE** does predominate in the adrenals of **nonmammals**, while tetrapods appear to exhibit more reliance on **Epi** than NE (although this is variable). **The natriuretic peptides** are present in all vertebrate groups, implying that they perform a primitive functional role. That role in fishes appears to be **stimulation of corticosteroid secretion**, which is the opposite of its role in amphibians, most birds, and mammals. Little information is available regarding the role of natriuretic peptides in reptiles.

Adrenal gland disease appears to be common in **American ferrets**, with about **70% of pet ferrets in the U.S. being affected**. This disease is caused by adrenocortical adenoma (16%), hyperplasia (56%), or adenocarcinoma (26%), causing release of excess sex hormones, particularly estradiol. Plasma cortisol concentrations are apparently unaffected. A theory regarding the high incidence of this disease in ferrets relates to oophorohysterectomy and neutering at 4-6 weeks of age. It has been proposed that early sterilization can result in adrenal neoplasia or adrenocortical nodular hyperplasia arising from undifferentiated gonadal cells incorporated into the adrenal capsule during embryologic development. The hypothalamus of the sterilized ferret will continue to secrete GnRH (loss of negative feedback), which releases gonadotropins (LH and FSH) from the

pituitary. If ferrets incorporate gonadal cells into their adrenal glands, like sterilized mice, the gonadotropins will stimulate the zona reticularis, resulting in increased sex hormone secretion.

Pancreas

Insulin and glucagon are well-conserved among vertebrate organisms, furthermore, **insulin-like molecules** are found in distantly related taxonomic groups including **protozoans**, **bacteria**, and **fungi**, and immunoreactive **glucagon** has been localized in the digestive glands of **crabs**, two gastropod **mollusks**, and extracts of **invertebrate chordates**. Insulin and glucagon evolution apparently involve a long and interesting process, most of which remains to be elucidated.

Epinephrine from chromaffin cells of the interrenal system and pancreatic **glucagon** are **hyperglycemic** in **teleosts** and **amphibians**, while **insulin** is **hypoglycemic**. **Lizards are particularly insensitive to insulin**, as large quantities are required to invoke hypoglycemic responses.

Avian hyperglycemia, initially observed following pancreatectomy, is believed to result from overlooking the **islet-rich splenic lobe** (rich in glucagon-secreting α-cells) during pancreatectomy. **Glucagon is thought to play a major role in avian blood glucose regulation**. The avian pancreas contains about 5-10 times more extractable glucagon per gram of tissue than the mammalian pancreas. The normal blood glucose level of most birds ranges from 150-300% above mammals. How birds can survive this sustained "hyperglycemia" is unknown. It appears that **insulin release** in birds is stimulated **less by glucose**, and more by **glucagon**, **amino acids** and **CCK** (Chs. 40 and 43). **Glucagon release** is supported by **fatty acids** and **CCK**, while **glucose has an inhibitory affect**. These release mechanisms support the contention that glucagon is the dominant hormone in avian carbohydrate metabolism.

Somatostatin in the avian pancreas is greater in concentration than it is in the mammalian pancreas, and appears to help regulate the **insulin:glucagon ratio**. This ratio is normally about half that of mammals, which helps to maintain a slightly catabolic state, ensuring a plentiful fuel supply to meet the high BMR for conditions such as egg laying, starvation, and sustained migratory flight. The occurance of classical **diabetes mellitus** in birds is controversial, since knowledge of the pathophysiologic mechanisms leading to this condition is less complete than it is in mammals, and a relative hyperglycemia appears to be the avian norm (Ch. 40).

Although **hyperglycemia** is a relatively uncommon endocrinopathy of **domestic ferrets**, **hypoglycemia** from **pancreatic islet β-cell tumors** is a common finding among those in America. Domestic pet ferrets in the U.S. are supplied by a small number of breeders, thus limiting their genetic diversity, leading to the hypothesis that their **insulinomas** have a genetic component. Another theory focuses on diet. Their commercial diet, rather than a whole prey diet, is apparently high in carbohydrate, which may be contributory since ferrets are carnivores. Some believe that their commercial diet, in combination with domesticated husbandry conditions, may contribute to a propensity for insulinomas. Pancreatic β-cell tumors secrete indiscriminately, somewhat like pheochromocytomas (Ch. 34), and they are not responsive to inhibitory stimuli such as hypoglycemia and hyperinsulinemia. A rapidly increasing blood glucose, even in the presence of hypoglycemia, can apparently stimulate excessive insulin release from these tumors, causing a profound **rebound hypoglycemia**. Although local tumor recurrence is a common feature in ferrets, metastasis to other organs appears to be low. This is in contrast to the insulinomas of **dogs**, which are usually malignant, and have a high rate of metastasis at the time of diagnosis (Ch. 51).

Gastrointestinal Peptides

Investigation into regulation of nonmammalian physiology by GI peptides has been hampered by a lack of detailed information regarding their digestive physiology. There are several differences in digestive processes among nonmammals. Many fishes lack stomachs, and may have other specialized structures (e.g., pyloric caeca) that imply major differences in control mechanisms. Also, digestion in **fishes**, **amphibians**, and **reptiles** may require days to accomplish what **birds** and **mammals** manage to do in a few hours. Thus, comparative studies of GI hormones have been largely limited to demonstrations of the **presence of these peptides** in nonmammalian vertebrates. It is apparent that they occurred early in vertebrate evolution (class Agnatha), and some counterparts are in evidence among **invertebrates**. Several studies indicate that GI peptides may be involved with **temperature regulation** in nonmammals, particularly since several species are known to undergo nocturnal hypothermia as a means of conserving energy during food deprivation.

Gastrin has been extracted from the GI tract of two **molluscan** species, and neuroendocrine cells of an **insect**, supporting a possible neural origin for this peptide. The **atlantic hagfish** (an early Agnathan), exhibits open-type endocrine cells extending from the basal portion of the intestinal epithelium to border on the gut lumen. These cells do not apparently possess APUD characteristics, and they do not resemble zymogen cells either, indicating a probable separate origin for gut endocrine and enzyme-secreting cells. The intestinal epithelium of larval and adult **lampreys** does contain APUD-type cells. Immunoreactive **CCK**, **gastrin**, **glucagon-like peptides**, **somatostatin** (**SS**), **substance P**, **GIP**, and **VIP** (GI peptides present in mammals (Chs. 47-50)), have been reported in the **hagfish** intestine. Gall bladder strips prepared from a pacific hagfish, however, did not respond *in vitro* with contractions to **porcine CCK**, although **ACh** did cause contractions. Secretion of pancreatic **lipase** in this same species was stimulated by **porcine CCK**. **Secretin-like** and **CCK-like** activities are present in extracts from the **lamprey** intestine. Both activities in these extracts were assayed by monitoring pancreatic secretions of an **anesthetized cat**.

Most of the GI peptides have been found among **teleosts**, **amphibians**, and **reptiles**, indicating a clear pattern in their evolutionary development. Knowledge of the GI physiology of **domesticated birds** is clearly more extensive than for any other nonmammalian group, because of abundant research in the poultry sciences. Less information is available on **wild avian species**. **Gastrin stimulates HCl secretion in birds, and CCK promotes exocrine pancreatic enzyme release**. Extracts prepared from chicken intestines appear to be strong stimulants of pancreatic secretion in turkeys, but weak stimulants in cats and rats, indicating amino acid substitutions have most likely developed in the polypeptide chains of mammalian GI Peptides.

Reproduction

Reproduction among nonmammals involves a precise integration of environmental factors (photoperiod, temperature, availability of nesting sites, etc.), physiological factors (nutritional state, general endocrine state with respect to thyroid hormones, adrenocortical functions, etc.), and specific endocrine secretions (LH, FSH, androgens, estrogens, progestogens, PRL, etc.), not necessarily different from similar factors detailed in mammals. Space limitations and the scope of this text do not leave room for an in-depth discussion of nonmammalian reproductive physiology.

Reproductive patterns in all vertebrates are finely tuned to environmental conditions in order to maximize survival. As these conditions change, the nervous system, specifically the hypothalamus, alters

gonadotropin and PRL release, hormones that have been identified in all **tetrapods**. **Fishes** possess a PRL-like hormone, but some have only one LH-like gonadotropin that exhibits both LH and FSH activity in other groups (see above).

As in mammals, ovulation in **birds** (e.g., chickens) occurs after a peak in the LH blood concentration, which occurs 4-8 hrs prior to ovulation. Following fertilization, the egg remains in the reproductive tract of the mother hen for about 24 hrs, thereafter, the embryo, and later the chick, develops inside the eggshell. Before the shell is formed the hen must supply the egg with all nutrients required for fetal development. Except for sounds exchanged across the eggshell during the final days prior to hatching, the chick develops without any influence from the parents, other than heat transfer during brooding. During this period, only O_2, CO_2 and H_2O vapor diffuse across the wall of the eggshell.

Follicular atresia is common among females of all species. It appears to be a mechanism for effectively reducing biotic potential, placing reliance on production of a smaller number of offspring possessing (theoretically) enhanced survival potential. Corpora lutea form in many vertebrates, primarily from granulosa cells of ruptured follicles, and synthesize **progesterone** that is related to gestation and/or behavior in many viviparous species. Autocrine and paracrine control of gonadal function is common to all vertebrates.

Courtship and breeding behavior appears to be regulated primarily by gonadal steroids. Estrogens produce dramatic effects on **vitellogenesis** in the nonmammalian liver, which causes a consequent (temporary) disturbance in Ca^{2+} homeostasis. The basic **oviparous** (egg laying) mode of reproduction among nonmammalian vertebrates has become modified with respect to the development of **ovoviviparity** in some reptiles (i.e., rattlesnakes), and **viviparity** (birth to live young) in mammals.

Appendix Table I

Blood Chemistry Values for Domestic Animals

Constituent	Units	Dog	Cat	Horse	Cow	Pig	Sheep
AG (plasma)							
$(Na^+) - (Cl^-+HCO_3^-)$	mEq/L	10-16	12-19	9	11-25	20-21	14-18
Ammonia (NH_3)	µmol/L	0-40	0-40	0-40	–	–	–
ALP (SAP)	IU/L	12-127	10-79	109-352	29-99	26-362	68-387
ALT (SGPT)	IU/L	14-86	25-145	4-12	17-37	32-84	60-84
AST (SGOT)	IU/L	9-54	5-42	189-385	48-100	9-113	98-278
Amylase	IU/L	409-1250	496-1940	9-34	12-107	–	–
Bicarbonate	mEq/L	18-24	17-21	20-28	17-29	18-27	20-25
Bile acids – fast	µmol/L	<5	<2	<15	–	–	–
Postprandial	µmol/L	<15	<15	–	–	–	–
Bilirubin (total)	mg/dl	0.10-0.30	0.10-0.30	0.3-3.10	0.04-0.74	0-0.6	0.1-0.39
Direct	mg/dl	0.06-0.12	0.05-0.07	0.0-0.50	0-0.3	0-0.3	0-0.12
Indirect	mg/dl	0.04-0.18	0.05-0.23	0.2-3.00	0.04-0.44	0-0.3	0.1-0.27
BUN	mg/dl	8-30	15-33	11-27	10-26	8-24	18-31
Calcium	mg/dl	9.4-11.8	8.8-11.7	11.0-13.9	7.9-10.0	8-12	10.4-13
Chloride	mEq/L	106-116	110-125	99-105	94-104	100-105	98-115
Cholesterol	mg/dl	82-355	38-186	77-258	87-254	36-54	50-140
Cholinesterase	IU/L	1347-2269	1000-2000	–	–	–	–
CO_2 (content)	mEq/L	14-28	13-22	24-31	24-32	18-26	21-28
Pco_2	mmHg	38	36	42	40	–	41
Cortisol (basal)	µg/dl	1.0-6.8	0.3-2.6	–	–	–	–
CPK (CK)	IU/L	22-422	59-527	58-524	44-228	24-225	81-129
Creatinine	mg/dl	0.6-2.0	0.9-2.1	1.0-1.9	0.7-1.1	1.0-2.7	1.2-1.9
Fibrinogen	g/L	1-4	1-3	1-5	2-7	1-5	1-5
Folate	µg/L	7.5-17.5	13.4-38	–	–	–	–
GGT	IU/L	2-10	0-5	5-24	20-48	–	–
Glucose	mg/dl	67-135	70-120	60-128	37-71	65-95	50-80
Hemoglobin	g/L	130-190	90-150	110-170	80-150	100-180	80-160
Hct (PCV)	%	37-54	30-47	32-47	24-46	33-50	24-49
Iron	µg/dl	84-233	65-233	74-209	57-162	91-199	166-222
LDH	IU/L	10-36	16-69	41-104	178-365	96-150	60-111
Lipase	IU/L	13-200	0-83	–	–	–	–
Magnesium	mEq/L	1.8-2.6	2.0-2.7	1.8-2.6	1.4-2.3	2.7-3.7	2.2-2.8
Osmolarity	mosm/L	291-315	292-356	282-302	–	–	–
pH	pH units	7.31-7.42	7.24-7.40	7.32-7.44	7.31-7.53	–	7.32-7.54
Phosphorus (Pi)	mg/dl	2.6-7.2	3.0-6.3	1.9-6.0	4.6-9.0	5.3-9.6	5.0-7.3
Potassium	mEq/L	3.7-5.4	3.4-5.2	2.7-4.8	4.0-5.3	4.9-7.0	4.0-6.0
Protein (total)	g/dl	5.5-7.8	6.0-8.4	5.6-7.0	5.9-7.7	7.0-8.9	6.0-7.9
Albumin	g/dl	2.8-4.0	2.2-4.0	2.4-4.0	2.7-4.3	1.9-3.3	2.4-3.9
Globulin	g/dl	2.3-4.2	2.5-5.8	2.5-4.9	2.5-4.1	5.3-6.4	3.5-5.7
SDH	IU/L	2.9-8.2	3.9-7.7	1.9-5.8	4.3-15.3	1-6	6-28
SID	mEq/L	34	33-36	29-37	40-42	39-47	38-39
Sodium	mEq/L	140-150	146-158	128-142	136-144	139-152	136-154
T_3	ng/dl	85-250	85-250	–	–	–	–
T_4	µg/dl	1.2-3.0	1.2-3.0	–	–	–	–
T_4 (free)	ng/dl	0.7-3.0	–	–	–	–	–
TG	mg/dl	29-40	25-191	9-52	0-14	–	–

Continued

Appendix Table I Continued

Constituent	Units	Dog	Cat	Horse	Cow	Pig	Sheep
Other Miscellaneous Variables							
Body Temperature	°F	99.5-102.5	100-102.5	99-100.5	100-102.5	100.5-104	102-104
Respiration	Per min	10-30	20-30	8-16	10-30	10-20	10-20
Pulse	Per min	60-120	110-130	28-40	40-80	60-80	70-80
Urine SG		1.025	1.030	1.040	1.032	1.012	1.030
Urine pH		Acidic	Acidic	Alkaline	Alkaline	Neutral	Alkaline

Data from various sources, including the **Tufts Small** and **Large Animal Hospitals**, and the **Veterinary Laboratory Medicine (Interpretation and Diagnosis)** text by **Meyer DJ**, and **Harvey JW**. It should be noted, however, that most all of the standard ranges presented in this table will vary to some degree between and within diagnostic laboratories.

Appendix Table II

Reproductive Patterns of Domestic Animals

Species	Puberty (months)	Estrous Cycle Length (days)	Estrus Duration (days)	Ovulation Time in Relation to Estrus	Gestation Length (days)	Litter Size (Average)
Buffalo (Am. Bison)	24	21	2	—	270	1
Cat	7-12	8-30 (non-mated)	4-20 (with and without male)	24-48 hr. postcoitus	63	4-5
Cattle	6-18	21	0.75	12-16 hrs. after	280	1
Chinchilla	7-10	24	2	—	111	1-4
Dog	6-12	—	3-12	First 1/3	58-63	1-8
Donkey	12	21-28	2-7	Last 1/3	365	1
Ferret	8-12	—	Prolonged	30 hrs. postcoitus	42	7-14
Goat	4-8	19-24	1-3	30-36 hrs. following onset	150	1-3
Guinea Pig	2	16.5	0.5	Last 1/2	67	0-4
Hamster	2	4	1	Last 1/2	15-18	5-9
Horse	12-24	19-26	5-7	Last 1/3	330	1
Llama	5-24	—	2-90 (with and without male)	48 hrs. postcoitus	350	1-2
Mouse (lab)	1-2	4-6	1	First 1/3	29	10-12
Pig	5-10	21	2-3	Last 1/2	114	4-14
Rabbit	5-10	—	Somewhat continuous	10 hrs. postcoitus	31	2-10
Rat (lab)	1-2	4-5	1	Last 1/3	21	7-9
Sheep	6-12	16.5	1.5	Last 1/2	150	1-3

Data from various sources

Appendix Table III

Basic Physiological Units

The relationship of concentration to mass and volume can be used in a number of physiological situations (e.g., fluid and electrolyte balance, xenobiotic administration, and renal function):

	Concentration	= Mass/Volume (or Amount/Volume)
Units:	Amount	= gm, mg, moles, osmoles, milliosmoles, etc.
	Volume	= liter (L), ml, 100 ml (deciliter, dl or dL), etc.
	Concentration	= gm/L, mg/ml, milliosmoles/L, etc.
		% = gm% = gm/100 ml = gm/dl
		mg% = mg/100 ml = mg/dl

Knowing these relationships and two of the three values above, the third can be calculated.

Rearranging:

Volume = Amount/Concentration

This relationship taken over time yields:

Flow = Volume/Time = (Amount/Time)/Concentration

Units (example): ml/min = (mg/min)/(mg/ml)

Solutions

Molar (M)	= One gram-molecular wt. made up to 1L in solvent
Millimolar (mM)	= M/1000
Molal (m)	= One gram-molecular weight dissoved in 1000 gm solvent
mOsmolar	= mOsm/L
1 mmole NaCl	= 2 mOsm
1 mmole $CaCl_2$	= 3 mOsm

Milligrams/deciliter (mg/dl or mg%) can be converted to milliequivalents/L (mEq/L) as follows:

mEq/L = (mg/dl x 10 x valence)/mg atomic mass

Example: If the plasma Na^+ concentration = 346 mg/dl, then the plasma contains 3460 mg Na^+/L.
The equivalent mass of Na^+ is 23, and the valence is 1; therefore:

mEq/L = (346 x 10 x 1) / 23 = 150

Units for plasma electrolytes are sometimes given as millimoles/L (mmol/L or mM), and for Na^+ would be the same as mEq/L.

IU = International Unit (i.e., a unit of biological material (e.g., enzyme, hormone, vitamin, etc.), established by the **International Conference for the Unification of Formulas**).

Prefixes representing powers of ten

Powers of ten	Prefix	Symbol
10^{12}	tera-	T
10^{9}	giga-	G
10^{6}	mega-	M
10^{3}	kilo-	k
10^{2}	hekto-	h
10	deka-	da
10^{-1}	deci-	d
10^{-2}	centi-	c
10^{-3}	milli-	m
10^{-6}	micro-	μ
10^{-9}	nano-	n
10^{-12}	pico-	p
10^{-15}	femto-	f
10^{-18}	atto-	a

Learning Objectives and Questions

Chapter 1

Objectives

- Identify major endocrine glands of the body, and the hormones they secrete.
- Define the word "hormone."
- Summarize basic factors that contribute most to endocrine action.
- Distinguish different hormones of the body based upon structure.
- Define the term "homeostasis," and contrast it to "steady state."

Questions

1. Hormones affect diverse metabolic functions by:

a. Increasing or decreasing the rates of specific reactions inside target cells.
b. Contributing energy to various broad anabolic and catabolic processes.
c. Serving as substrates for the reactions they catalyze.
d. Simply functioning as both extra- and intracellular enzymes.
e. All of the above

2. All of the following are considered to be endocrine organs, <u>except</u> the:

a. Kidneys.
b. Pineal gland.
c. Lungs.
d. Gastrointestinal tract.
e. Parathyroids.

3. Thyroid hormone action is dependent upon:

a. Concurrent effects of antagonistic or synergistic hormones.
b. The rate of synthesis and/or secretion of T_4 from the thyroid gland.
c. The plasma concentration of liver-derived transport proteins.
d. Conversion of T_4 to a more active form within target cells.
e. All of the above.

4. The CNS is a major component of the endocrine system, and neurons can produce and secrete hormones into blood:

a. True
b. False

Chapter 2

Objectives

- Distinguish differences between positive and negative feedback control of chemical regulators, and discuss why negative feedback is generally advantageous to survival.
- Define "target cell."
- Explain why catecholamine receptors are normally found "on" rather than "in" target cells.
- Discuss why nonmammals and invertebrates are more dependent than mammals on their endocrine systems.
- Identify where the neural and endocrine systems of mammals have anatomically and functionally converged.
- Differentiate an endocrine from an exocrine gland.
- Know how hormones are degraded in the body.
- Recognize how endocrine disrupters could function.

Questions

5. Which chemical regulator below (if any), would have difficulty passing through most cell membranes of the body?

a. Eicosanoid (e.g., prostaglandin)
b. Biogenic amine (e.g., epinephrine)
c. Steroid (e.g., aldosterone)
d. Thyroid hormone (e.g., thyroxine)
e. None of the above, for they all exhibit high lipophilicity.

6. Select the <u>true</u> statement(s) below:

a. A given hormone might affect several different cell types.
b. Over 50 different hormones are known to exist in mammals.
c. More than one hormone can affect a given cell type.
d. Hormones exist in bacteria, worms, insects, and plants.
e. All of the above

7. Which one of the following best exemplifies an autocrine secretion?

a. A pheromone secreted through an exocrine duct to the body surface
b. Estrogen secreted from the ovary into the vascular system
c. A chemical messenger acting on neighboring target cells without entering the circulation
d. Thromboxane secreted by a platelet to then act on that platelet to promote its aggregation to other platelets
e. A neurotransmitter released into blood to affect target cells at distant locations

8. Select the <u>false</u> statement below:

a. Cats eliminate glucocorticoid metabolites primarily in urine, whereas dogs eliminate them primarily in bile.
b. Some metabolites of hormones are biologically active.
c. Serum proteases are known to degrade certain hormones.
d. Hormones are sometimes eliminated from the body in their active forms.
e. Invertebrates possess primitive nervous systems, and thus must depend upon paracrine and autocrine metabolic control.

9. Select the <u>true</u> statement below:

a. Prostaglandins are polypeptide hormones secreted in an autocrine fashion.
b. The pars nervosa is primarily controlled by release and release-inhibiting hormones from the hypothalamus.
c. There is considerable structural conservation of chemical messengers across species lines (from invertebrates to vertebrates).
d. The anterior pituitary controls release of PTH from the parathyroids, and glucagon from the pancreas.
e. The only known endocrine function for prolactin (PRL) is to stimulate milk production and secretion in mammals.

Chapter 3

Objectives

- Define "clearance," and recognize how the units differ from "excretion."
- Differentiate "plasma clearance" from "systemic clearance."
- Explain why the V_d for estrogen would be expected to be larger than that for ACTH.
- Understand the nature of the relationships that exist between plasma t½, CL_p, and V_d of a hormone.
- Explain how hormone immunoassays are performed, and discuss their limitations.
- Recognize under what conditions an average plasma hormone concentration (over time) might be a useful index of the hormone production rate (PR).

Questions

10. Given the following:

Plasma concentration of a hormone = 15 mg%
Plasma t½ = 10 min
Body excretion rate (at steady state) = 0.94 mg/min

The plasma clearance is __, and the distribution volume __.

a. 20.63 ml/min; 3 liters
b. 6.27 ml/min; 90 ml
c. 1.67 mg/min; 15 ml
d. 13.65 ml/min; 49 ml
e. Not possible to calculate from the data given.

11. The plasma half-life (t½) of a steroid hormone:

a. Is normally 0.693 min, and if this increases, disease is usually evident.
b. Usually increases when the CLp increases.
c. Is a function of both the distribution volume and plasma clearance.
d. Usually decreases when the Vd increases.
e. Is independent of plasma protein binding.

12. Immunoassays may not always distinguish sufficiently between:

a. Two sex steroids secreted by the same gland.
b. Peptide hormones and their prohormones.
c. Two adrenal steroids.
d. A hormone and its metabolic products.
e. All of the above

13. The rate at which a hormone is produced is equal to:

a. $CL_p \times P_c$
b. ER - $CL_{systemic}$
c. $V_d + CL_p$ - ER
d. $(P_c - Cl_p) \times t½$
e. None of the above

14. Why is it hazardous to conclude too much from the hormone concentration of a single plasma sample?

a. Because many hormones are secreted in episodic bursts, therefore a single plasma sample may not be reflective of the average plasma concentration.
b. Because most diagnostic laboratories are not experienced with plasma hormone analysis.
c. Because most hormones are degraded rapidly in plasma, thus if the plasma was not assayed soon following sample collection, the concentration might be seriously underestimated.
d. Because the blood hormone concentration can only be accurately determined in vivo since concentrations are normally so small.
e. All of the above

Chapter 4

Objectives

- Recognize how the physiologic state of an animal might alter the number of hormone receptors per target cell.
- Explain how receptor specificity, number, distribution and/or affinity might affect the functional expression to and/or plasma concentration of a hormone.
- Discuss how cAMP is formed and degraded in target cells, and also distinguish its intracellular actions.
- Identify hormones known to activate the MAP K cascade, and recognize the intracellular consequences.
- Summarize how "cross-communication" between hormone second messenger pathways is accomplished.
- Explain how the JAK-STAT messenger pathway operates in response to either GH or PRL stimulation.

Questions

15. Hormone receptor concentration and/or affinity may be affected by:

a. Ionic balance.
b. The plasma concentration of heterologous hormones.
c. Antibodies against the receptor.
d. The plasma concentration of the homologous hormone.
e. All of the above

16. Which of the following statements regarding GTP-binding proteins is/are <u>true</u>?

a. They are associated primarily with intracellular membranes of the endoplasmic reticulum.
b. They play roles in the cAMP second messenger system only.
c. They exist in both stimulatory and inhibitory forms.
d. They bind glycoprotein hormones in plasma, thus keeping them in the circulation.
e. They are largely activated by protein kinase A (PKA).

17. Endocrine signals from multiple extracellular stimuli on target cells can be integrated into specific patterns of altered cellular response through:

a. The cAMP 2nd messenger system.
b. The JAK-STAT pathway, which relays these multiple signals to responsive nuclei.
c. The MAP K cascade.
d. Cross-communication between the four primary intracellular 2nd messenger pathways.
e. None of the above, for multiple endocrine signals cannot be integrated.

18. The effects of stimulatory kinases inside cells are usually offset by:

a. Isomerases
b. Phosphorylases
c. Mutases
d. Lyases
e. Phosphatases

19. Which one of the following hormones normally functions through the MAP K cascade?

a. EPO
b. ADH
c. ACTH
d. Epinephrine
e. Insulin

Chapter 5

Objectives

- Describe differences and similarities between the cAMP and Ca^{2+}/DG messenger systems.
- Provide examples of physiologic effects due to Ca^{2+} signaling.
- Distinguish differing actions of PKC, PLA_2, and PLC.
- Recognize how Ca^{2+} can be involved with both intracellular and intercellular communication.
- Explain how both DG and IP_3 can act as 2nd messengers.
- Understand the function of calmodulin in the Ca^{2+} messenger system.
- Identify multiple sources of Ca^{2+} for diffusion into the cytoplasm of a cell, and recognize how the cytoplasmic Ca^{2+} concentration can be either beneficial or detrimental to cell survival.
- Explain why responses of the Ca^{2+}/DG pathway are usually faster than those of the cAMP pathway.
- Discuss how the Ca^{2+}/DG messenger system is related to the cAMP, MAP K and JAK-STAT messenger systems.

Questions

20. Intracellular Ca^{2+} and membrane-bound DG promote:

a. PLA_2 activation.
b. PKC activation.
c. PKA activation.
d. PLC activation.
e. All of the above

21. Where are catecholamine receptors normally located?

a. On plasma membranes of target cells.
b. In the cytoplasm of target cells.
c. At the same sites as thyroid and steroid hormone receptors.
d. In nuclei of target cells.
e. On mitochondrial membranes of target cells.

22. Which one of the following is a water-soluble inducer of Ca^{2+} release from mitochondria and the ER?

a. Calmodulin
b. DG
c. PLC
d. IP_3
e. PKC

23. Phosphorylation of the EGF receptor by PKC:

a. Is a normal mechanism for activating the MAP K cascade.
b. Opens voltage-gated Ca^{2+} channels in the plasma membrane.
c. Decreases its affinity for EGF.
d. Normally follows norepinephrine binding to an α_1-adrenergic receptor.
e. Initiates the arachidonic acid cascade.

Chapter 6

Objectives

- Distinguish intracellular locations of sex steroid receptors vs. mineralocorticoid receptors.
- Explain how HSP and HREs function in processes of steroid hormone signaling.
- Understand how and why certain steroid, catecholamine and/or polypeptide hormones work in harmony at various target cell locations.
- Examine similarities and differences in the mechanisms of thyroid and steroid hormone action.
- Recognize how cellular deiodinases function to maintain differences in thyroid hormone ratios (Ch. 39).
- Understand how plasma protein binding of steroid and thyroid hormones can influence their overall metabolic effectiveness.

Questions

24. The predominant nuclear receptor for thyroid hormones is specific for:

a. T_4
b. rT_3
c. T_3
d. T_2
e. TSH

25. Heat shock protein is:

a. Normally bound to a cytoplasmic steroid receptor when that receptor is not bound with a steroidal ligand.
b. Normally found in the nucleus of thyroid hormone target cells, where it helps to maintain receptors in their proper configuration for hormone binding.
c. A plasma protein made by the liver that binds steroid hormones, thus keeping them in the circulation.
d. Known as the hormone response element in nuclei of steroid hormone target cells.
e. Rapidly denatured when the body temperature gets one degree above normal.

26. What percentage of all genes expressed by responsive cells are regulated by steroid and thyroid hormones?

a. 1%
b. 15%
c. 33%
d. 66%
e. 99%

27. Which of the following is an example of heterologous up-regulation of receptors?

a. Desensitization of target cells to the presence of insulin.
b. Glucocorticoid-mediated up-regulation of its own receptors.
c. Translocation of the steroid receptor containing HSP to the nucleus, thus masking the receptor DNA-binding domain.
d. Estrogen priming of progesterone receptors.
e. None of the above

Chapter 7

Objectives

- Recognize why hypophysectomy is nonfatal.
- Compare the types of hormones secreted by the pituitary to those secreted by the adrenal glands.
- Distinguish animals possessing a pars intermedia from those that don't, and know which hormones are secreted by this structure.
- Contrast hypothalamic-neurohypophyseal communication to hypothalamic-adenohypophyseal communication.
- Recognize the developmental correlation between the pars intermedia and Rathke's pouch.
- Identify the circumventricular organs, and indicate how they differ from other areas of the CNS.
- Describe the difference between a hypophyseal hormone, and a hypophysiotropic hormone.
- Identify the release and release-inhibiting hormones that control anterior pituitary hormone release.
- Explain what is meant by neuromodulation of hypothalamic release and release-inhibiting hormone secretion.

Questions

28. Which adenohypophyseal hormone stimulates hepatic IGF-1 release?

a. FSH
b. ACTH
c. PRL
d. α-MSH
e. GH

29. Which one of the following is known to inhibit PRL, ACTH, TSH and α-MSH release from the adenohypophysis?

a. Acetylcholine
b. Dopamine
c. GnRH
d. GHIH
e. Norepinephrine

30. What is the major adenohypophyseal endocrine-secreting cell type in mammals?

a. Melanotroph
b. Lactotroph
c. Gonadotroph
d. Corticotroph
e. Somatotroph

31. Which of the following statements regarding the anterior pituitary is/are <u>true</u>?

a. It secretes tropic hormones that promote insulin release from the pancreas, and PTH release from the parathyroids.
b. It comprises about 80% of total pituitary weight in most species.
c. It secretes primarily release and release-inhibiting hormones into the systemic circulation.
d. It stores neuropeptides, like the posterior pituitary.
e. All of the above are true

32. Select the <u>false</u> statement below:

a. Oxytocin is stored in and secreted from the neurohypophysis.
b. Circumventricular organs appear to be independent of the blood brain barrier evident in other sections of the CNS.
c. ACTH is produced by the equine and canine pars intermedia.
d. Opioid peptides have no influence on anterior pituitary hormone secretion.
e. The gonadotropins and TSH are glycoproteins.

Chapter 8

Objectives

- Compare structural characteristics of morphine and met-enkephalin, two compounds that appear to stimulate common receptors.
- Recognize the importance of POMC being synthesized by both the anterior and intermediate lobes of the pituitary, and identify other regions of the body where it is produced.
- Identify commonalities and differences between the endorphins, dynorphins, and enkephalins.
- Differentiate "nonpain" from "antipain."
- Outline the effects of the opiate-like peptides on gonadotropin, GH and PRL release.
- Explain why animals with PDH (Ch. 25), might also exhibit skin darkening.
- Distinguish differences between "opiates," and "opioids."

Questions

33. Met- and leuenkephalin are:

a. Steroids that exert morphine-like effects.
b. Produced in the pituitary gland.
c. Derived from proenkephalin.
d. Derived from β-endorphin.
e. Not found in the CNS.

34. From which of the following is α-MSH derived?

a. β-Endorphin
b. CLIP
c. β-MSH
d. Pro-γ-MSH
e. ACTH

35. Structural similarity exists between:

a. ACTH and β-endorphin.
b. αMSH and β-MSH.
c. Codeine and naloxone.
d. POMC and heroin.
e. Morphine and β-endorphin.

36. Opiate peptides are known to reduce hypothalamic release of which one of the following?

a. GnRH
b. GHRH
c. PRL
d. Glucagon
e. PTH

Chapter 9

Objectives

- Explain why MSH undergoes both release- and a release-inhibiting control from the hypothalamus.
- Identify and discuss differences between melanocytes, meanophores and melanosomes, and between melanocytes and keratinocytes.
- Define the terms melanoderma, leukoderma, poliosis, melanotrichia and leukotrichia.
- Recognize differences between MSH and melatonin in terms of structure and source, and in terms of effects on skin coloring.
- Understand functions of MSH unrelated to skin darkening.
- Explain why some white-coated animals (e.g., cats) exhibit sensorineural deafness.
- What is the source of β-LPH, and what (potentially) are its functions.

Questions

37. Skin darkening often occurs in patients with Addison's-like disease (Ch. 29), because:

a. Of pituitary hyperplasia.
b. Excessive amounts of α-MSH are promoting melanoderma.
c. Of lack of corticosteroid negative feedback on hypothalamic CRH and pituitary ACTH release.
d. Of β-LPH hypersecretion from the pituitary.
e. None of the above

38. In response to α-MSH, skin darkening occurs in mammals because:

a. Melanosomes transfer melanin to dendritic processes, which then translocate it into keratinocytes.
b. Melanosomes disperse their melanin throughout the cytoplasm of dermal melanophores.
c. Keratinocytes, which are unstable cells, quickly die, releasing their melanin into interstitial fluid near the skin.
d. Dermal melanophores concentrate their melanin in melanosomes.
e. Keratinocytes take up the MSH, thus causing melanoderma.

39. Sensorineural deafness may occur in albino cats because:

a. Cells of the ciliary process fail to properly respond to α-MSH.
b. Of cochlear melanocyte deficiencies.
c. The choroid plexus fails to maintain proper Na^+/K^+ ATPase activity.
d. The stria vascularis fails to secrete melanin into perilymph.
e. None of the above

40. The major target of α-MSH in lower vertebrates is the:

a. Pars intermedia.
b. Keratinocyte.
c. Neurohypophysis.
d. Adrenal cortex.
e. Dermal chormatophore.

Chapter 10

Objectives

- Explain how and why GH exhibits both anabolic and catabolic properties.
- Distinguish the indirect growth-promoting actions of GH (that occur in concert with IGF-1), from the direct anti-insulin actions.
- Understand why adenohypophyseal GH secretion is under the control of GHRH, GHIH, IGF-1 and ghrelin (Ch. 50).
- Know why a protein meal and starvation are associated with increased GH release, whereas a carbohydrate meal reduces it.
- Recognize the numerous factors influencing somatotropin release.
- Describe the physiological relationship between megestrol acetate and GH.
- Identify the different second messenger pathways activated in response to IGF-1 and GH (Ch. 4).
- Understand why GH, in the absence of IGF-1, reduces hexokinase activity in muscle and adipose tissue.
- Explain why sustained elevations in GH might lead to diabetes mellitus.

Questions

41. GH secretion normally increases during deep sleep because:

a. It is needed, like glucagon, to exert its anabolic actions.
b. It is needed, like cortisol, to exert its catabolic actions.
c. Hypothalamic somatostatin secretion increases.
d. It is needed to inhibit adipocyte lipolysis.
e. Of adenohypophyseal IGF-1 stimulation.

42. Prenatal growth and development is largely promoted by:

a. Estrogen.
b. α-MSH, ACTH and the opioid peptides.
c. Somatostatin and cortisol.
d. IGF-2, thyroxine and GH.
e. None of the above

43. All of the following stimulate hepatic somatomedin C secretion, <u>except</u>:

a. Growth hormone.
b. Insulin.
c. Glucocorticoids.
d. A protein meal.

44. Which of the following stimulates GH secretion in cats?

a. Cortisol
b. β-Adrenergics
c. Arginine
d. Glucose
e. IGF-1

45. Synthetic progestins such as megestrol acetate stimulate GH secretion in:

a. Cats.
b. Primates.
c. Dogs.
d. All of the above

46. The intracellular second messenger system best associated with somatotropin, is the:

a. JAK-STAT messenger system.
b. MAP K messenger system.
c. cAMP messenger system.
d. Ca^{2+}/DG messenger system.
e. None of the above, for somatotropin does not work through a second messenger system.

Chapter 11

Objectives

- Recognize the numerous causes of short stature among domestic animals, and the most common cause of hyposomatotropism in prepubertal dogs.
- Explain why serum IGF-1 levels are usually reduced in pituitary dwarfism.
- Identify and discuss the causes of acquired hyposomatotropism in domestic animals.
- Differentiate acromegaly from gigantism.
- Explain the signs and symptoms of hypersomatotropism, and understand how this condition might lead to diabetes mellitus.
- Understand why progestins have been used to treat dwarfism in dogs.
- Summarize the effects of bST administration on bovine lactational physiology.

Questions

47. Pituitary dwarfism in the German shepherd dog may be associated with normal adenohypophyseal secretion of:

a. TSH.
b. GH.
c. The gonadotropins.
d. ACTH.
e. PRL.

48. Select the <u>false</u> statement below:

a. Somatotropin is diabetogenic (like glucagon, cortisol and epinephrine).
b. The only endocrinopathy associated with short stature in dogs is hyposomatotropism.
c. Acromegaly can develop in dogs following prolonged administration of progestins.
d. Cats with acromegaly may exhibit PU/PD.
e. GH is secreted into milk.

49. Pituitary dwarfism in prepubertal dogs is most often associated with:

a. Insensitivity of target cells to IGF-1.
b. Failure of somatocrinin release.
c. A cystic Rathke's pouch.
d. Defective GH receptors.
e. Excessive hypothalamic GHIH release.

50. Which one of the following is best associated with hypersomatotropism?

a. Testicular atrophy.
b. Hypoglycemia.
c. Delayed epiphyseal closure.
d. Megaesophagus.
e. Prognatism.

Chapter 12

Objectives

- Differentiate potential causes of hyposthenuric polyuria from those promoting isosthenuric polyuria.
- Discuss differences between neuronal polypeptide and catecholamine biosynthesis.
- Distinguish control of adenohypophyseal activity from that of neurohypophyseal activity.
- Recognize the mechanisms by which blood volume, pressure and osmolarity independently influence ADH secretion.
- Explain how ADH promotes renal water retention.
- Summarize the primary and secondary control mechanisms for ADH release.
- Know why all three ADH receptor types are considered "metabotropic."
- Understand why ADH is also referred to as vasopressin.
- Explain why symptoms of SIADH are similar to those of water intoxication.

Questions

51. Which one of the following is a symptom of diabetes mellitus, but not diabetes insipidus?

a. Glucosuria
b. Hyposthenuric polyuria
c. Hypernatremia
d. Polydipsia
e. Dehydration

52. Which of the following ADH receptors is tied to adenyl cyclase activation?

a. V_{1A}
b. V_{1B}
c. V_2
d. V_3
e. None of the above

53. Select the <u>false</u> statement below:

a. The arginine in position 8 of ADH is critical to its diuretic action, but not its pressor activity.
b. An increase in plasma osmolarity of only 1% causes an increase in posterior pituitary ADH release.
c. Angiotensin II stimulates ADH release.
d. The baroreceptor system involved in ADH release is less sensitive than the osmoreceptor system.
e. Desamino-8-D-arginine vasopressin has potent antidiuretic properties.

54. Select the <u>true</u> statement below:

a. The pressor actions of ADH include vasoconstriction of renal blood vessels.
b. Hypervolemia stimulates ADH release.
c. Lactation inhibits ADH release.
d. ADH stimulation of target cell receptors on collecting ducts causes aquaporins to be inserted into apical membranes of those cells.
e. Cortisol stimulates ADH release.

55. The equation for "free water clearance:"

$C_{H2O} = V - (U_{osm}\ V/P_{osm}) = ml/min$

Where:
V = Urine flow (ml/min)
U_{osm} = Urine osmolarity (μM)
P_{osm} = Plasma osmolarity (μM)

a. C_{H2O} would be negative when the urine is hypotonic.
b. Isosthenuric urine would yield a positive free water clearance.

c. Diuretic administration would be expected to yield a negative free water clearance.
d. Land mammals frequently vacillate between a $+C_{H2O}$ and a $-C_{H2O}$, depending upon how much water they consume.
e. Vasopressin has no direct influence on C_{H2O}.

56. The SIADH secretion would be expected to produce:

a. A positive free water clearance.
b. Diabetes insipidus.
c. Hyposthenuric urine.
d. Hypertonic dehydration.
e. None of the above

Chapter 13

Objectives

- Identify multiple factors associated with PU/PD in animals.
- Discuss differences in the causes of CDI and NDI, and outline possible treatments.
- Explain how an erythropoietin-producing tumor could lead to symptoms of DI.
- Differentiate between primary and acquired NDI.
- Show how hypercalcemia can lead to secondary NDI.
- Know why both hyper- and hypoadrenocorticism can lead to symptoms of NDI.
- Explain how over-administration of DDAVP could lead to diuresis.
- Know the relationships between liver disease and PU/PD, and between hypokalemia and PU/PD.

Questions

57. ADH is known to stimulate release of which pituitary hormone?

a. Oxytocin
b. PRL
c. FSH
d. TSH
e. ACTH

58. Hepatic insufficiency can lead to NDI because:

a. Of reduced hepatic ADH inactivation.
b. The BUN concentration may decrease, thus decreasing the renal medullary concentration gradient.
c. Hypocalcemia ensues.
d. Liver disease causes aldosterone deficiency, which in turn causes renal medullary solute washout.
e. Of secondary damage to the neurohypophyseal ADH release system.

59. Select the <u>true</u> statement below:

a. Hypokalemia, like hypercalcemia, is thought to cause collecting ducts of the kidney to become more responsive to ADH.
b. Glucocorticoids are known to stimulate ADH release and decrease the glomerular filtrate rate (GFR).
c. PU/PD is a common early symptom of hypercalcemia, and it is also seen in animals with hyperthyroidism.
d. The urine of patients with CDI is usually isosthenuric.
e. Animals with DI usually develop hypotonic dehydration within a short period of time if water is withheld.

60. Patients with polycythemia may develop PU/PD because:

a. Of impaired microcirculation and diminished ADH release.
b. They generally develop pyelonephritis, which leads to secondary NDI.
c. Of the ensuing hypokalemia.
d. They secrete high amounts of DDAVP from bone marrow.
e. Excessive erythrocytic ADH degradation.

Chapter 14

Objectives

- Outline the primary functions of Ca^{2+} in the body, and recognize how Ca^{2+} is distributed.
- Describe the 3-step intestinal mechanism by which Ca^{2+} is absorbed.
- Show how changes in acid/base balance affect the free ionized plasma Ca^{2+} concentration.
- Identify the calcium complexes normally present in the glomerular filtrate.
- Recognize unique features of Ca^{2+} metabolism in rabbits.
- Identify the proton acceptors in bone that result in Ca^{2+} loss during acidemic conditions.
- Recognize why the kidneys are important organs in the control of Ca^{2+} homeostasis.

Questions

61. Which one of the following is <u>least involved</u> in regulating plasma Ca^{2+}, Mg^{2+}, and PO_4^{3-} levels?

a. Kidney
b. Parathyroid gland
c. Bone
d. Hypothalamic-pituitary axis
e. GI tract

62. The free cytoplasmic Ca^{2+} concentration is normally about how many times lower than the plasma concentration?

a. 2
b. 10
c. 100
d. 1,000
e. 10,000

63. Approximately what percentage of plasma Ca^{2+} is normally protein-bound?

a. 10%
b. 20%
c. 40%
d. 60%
e. 80%

64. Which one of the following attributes is best associated with Ca^{2+}?

a. Activates protein kinase A (PKA)
b. Intracellular 2nd messenger
c. Urinary buffer
d. Second most plentiful cytoplasmic cation
e. Normally chelated cytoplasmic ATP

65. What percentage of Ca^{2+} traversing the canine intestinal lumen is normally absorbed each day?

a. 10%
b. 20%
c. 40%
d. 60%
e. 80%

66. Intestinal Ca^{2+} absorption is reduced by:

a. Acidic intestinal contents.
b. The active form of vitamin D.
c. Parathormone.
d. Glucocorticoids.
e. Antidiuretic hormone.

Chapter 15

Objectives

- Describe how intestinal absorption and renal PO_4^{3-} reabsorption differs from that of Mg^{2+}.
- Identify the primary intra- and extracellular locations and physiologic actions of Mg^{2+} and PO_4^{3-}.
- Explain why the phosphate buffer system is quantitatively significant in the renal tubular filtrate, but not in plasma.
- Understand why loop diuretic overuse may lead to hypomagnesemia.
- Summarize the effects of insulin on Mg^{2+} and PO_4^{3-} homeostasis.
- Know why $\mathbf{k = [Ca^{2+}]\ [PO_4^{3-}]}$ in plasma.
- Explain why both PTH and calcitonin are phosphaturic.
- Know why Mg^{2+} is wasted by the kidneys in acidosis.

Questions

67. Which one of the following normally has the <u>lowest</u> renal reabsorption efficiency?

a. Tyrosine
b. PO_4^{3-}
c. Glucose
d. Ca^{2+}
e. Mg^{2+}

68. Which one of the following typically has the highest absorption efficiency in the GI tract, and is not bound in plasma to protein?

a. Ca^{2+}
b. PTH
c. PO_4^{3-}
d. Mg^{2+}
e. Insulin

69. Renal diseases affecting the proximal tubules could have profound influences on the plasma concentration of which of the following?

a. Ca^{2+}
b. Glucose
c. PO_4^{3-}
d. Tyrosine
e. All of the above

70. Phosphate is found in erythrocytes in association with:

a. Membrane-bound phospholipids.
b. 2.3-BPG.
c. Carbohydrate metabolism intermediates.
d. ATP.
e. All of the above

71. The greatest amount of renal Mg^{2+} reabsorption occurs in:

a. The collecting ducts.
b. The distal tubule.
c. The ascending thick limb of the LOH.
d. The descending limb of the LOH.
e. The proximal tubule.

Chapter 16

Objectives

- Explain why cats suffering from hyperthyroidism sometimes experience severe hypocalcemia following thyroidectomy, and why vitamin D supplementation can help.
- Know why extreme fluctuations in the serum Mg^{2+} concentration reduce PTH release, and how stress increases it.
- Understand why PTH secretion increases in renal secondary hyperparathyroidism.
- Show how PTH can exert both anabolic and catabolic actions on bone.
- Outline the actions of PTH on bone and on the kidneys.
- Discuss the physiological benefits of PTH-stimulated bicarbonaturia and hyperchloremia.
- Explain how PTH and $1,25(OH)_2D$ promote Ca^{2+} release from bone.
- Identify and explain similarities and differences between sources and functions of PTH and PTH_{rp}.
- Recognize the extrathyroidal sources of CT.

Questions

72. PTH, either directly or indirectly, stimulates all of the following, <u>except</u>:

a. Bone resorption.
b. Adenyl cyclase activity in its target cells.
c. Renal Ca^{2+} reabsorption.
d. Renal PO_4^{3-} reabsorption.
e. Renal vitamin D activation.

73. Which of the following is/are <u>true</u>?

a. Osteoblasts possess $1,25(OH)_2D$ receptors.
b. Osteoclasts possess CT receptors.
c. Osteoblasts are capable of producing cytokines that activate osteoclasts.
d. Osteoblasts possess PTH receptors.
e. All of the above

74. Which one of the following exerts the greatest positive influence on PTH release?

a. Stress
b. A low serum ionized PO_4^{3-} concentration.
c. A low serum ionized Ca^{2+} concentration.
d. A low serum ionized Mg^{2+} concentration.
e. Estrogen

75. Select the <u>true</u> statement(s) below regarding the kidneys and Ca^{2+}/PO_4^{3-} homeostasis:

a. PTH promotes Ca^{2+} reabsorption and urinary PO_4^{3-} excretion.
b. Loop diuretics promote Ca^{2+} retention.
c. PTH promotes HCO_3^- retention and urinary Cl^- excretion.
d. Thiazide diuretics enhance urinary Ca^{2+} excretion.
e. All of the above

76. The circulating t½ of PTH in mammals is about:

a. 2 min.
b. 20 min.
c. 1 hr.
d. 2 hrs.
e. 20 hrs.

77. Pseudohyperparathyroidism is due to elevated serum levels of:

a. Indian hedgehog protein.
b. Ca^{2+}.
c. $1,25(OH)_2D$.
d. PO_4^{3-}.
e. PTH_{rp}.

78. Which portion(s) of the functional nephron has/have no PTH receptors?

a. Proximal tubule
b. Loop of Henle
c. Distal tubule
d. Collecting ducts
e. All of the above

Chapter 17

Objectives

- Identify the physiological relationships that exist between CT and gastrin, and between CT, estrogen and PTH.
- Explain why CT is considered to be antiosteoclastic.
- Understand differences between the sources of D_2 and D_3 in animals.
- Outline the endocrine regulation of intestinal Ca^{2+} absorption, renal Ca^{2+} reabsorption, and bone resorption.
- Recognize at least 7 factors that influence renal 1α-hydroxylase activity, and explain why $1,25(OH)_2D$ inhibits renal 24-hydroxylase.
- Explain why $1,25(OH)_2D$ is used to treat patients with PTH deficiency.
- Understand why 25(OH)DHT might be a good treatment for vitamin D deficient patients with kidney disease.
- Identify the roles played by estrogen, GH, PRL, PL, IFG-1 and cortisol in Ca^{2+} homeostasis.

Questions

79. Select the <u>true</u> statement below regarding calcitonin (CT):

a. It has a greater influence on overall Ca^{2+} homeostasis than does PTH.
b. It inhibits intestinal Ca^{2+} absorption.
c. It's release is inhibited by gastrin.
d. It may protect the skeleton of the dam from excess Ca^{2+} loss during pregnancy.
e. It is synthesized and secreted by the parathyroid glands.

80. Select the <u>true</u> statement below regarding calcitriol:

a. Formation from D_3 requires normally functioning hepatocytes and kidneys.
b. It inhibits bone resorption.
c. Receptors for the seco-steroid are found in osteoclasts.
d. It inhibits the action of PTH in the kidney.
e. The dehydroxylated form of the compound is known as calcitonin.

81. Which hormone below stimulates CT secretion, inhibits the action of PTH on bone, and helps to activate vitamin D in the kidney?

a. Estrogen
b. Growth Hormone
c. Cortisol
d. Insulin
e. Prolactin

82. Which one of the following vitamin D derivatives is physiologically active without being hydroxylated by the kidneys?

a. 7-Dehydrocholesterol
b. Ergosterol
c. D_3
d. Ergocalciferol
e. DHT

83. The "anticipatory" hormone for CT release appears to be:

a. Cortisol.
b. PTH.
c. Gastrin.
d. Estrogen.
e. $1,25(OH)_2D$.

84. A more potent <u>hypocalcemic factor</u> in mammals than endogenous CT appears to be:

a. PTH.
b. $1,25(OH)_2D$.
c. Estrogen.
d. Growth Hormone.
e. Salmon CT.

Chapter 18

Objectives

- Identify the most probable cause of PU/PD in primary hyperparathyroidism.
- Understand why the plasma Cl^-:PO_4^{3-} and Ca^{2+}:PO_4^{3-} ratios increase in primary hyperparathyroidism.
- Know the primary causes and symptoms of hyper- and hypocalcemia.
- Discuss why severe hypomagnesemia can lead to hypocalcemia.
- Explain why a decrease in the number of functional nephrons can lead to elevated PTH secretion.
- Establish a correlation between the signs and symptoms of small bowel disease, and hyperparathyroidism.
- Identify probable consequences of feeding animals all-meat diets with low Ca^{2+}:PO_4^{3-} ratios.
- Understand how loop diuretic overuse can lead to hypocalcemia.

Questions

85. Plasma ionizied Ca^{2+} levels are typically elevated in:

a. Ethylene glycol toxicity.
b. Nutritional secondary hyperparathyroidism.
c. Oxalate toxicity.
d. Primary hyperparathyroidism.
e. Hypomagnesemia.

86. Pathophysiologic effects associated with hypocalcemia include all of the following, <u>except</u>:

a. Coagulopathies.
b. Prolonged Q-T intervals of the ECG.
c. Bronchospasm.
d. Milk fever.
e. Nephrocalcinosis.

87. Malabsorption secondary hyperparathyroidism is best associated with:

a. Hypocalciuria.
b. Hypophosphaturia.
c. Decreased fecal Ca^{2+} excretion.
d. Hypercalcemia.
e. Nephrocalcinosis.

88. What is the second most reported cause of hypercalcemia in dogs?

a. Renal secondary hyperparathyroidism.
b. Primary hyperparathyroidism.
c. Malabsorption secondary hyperparathyroidism.
d. Vitamin D toxicosis.
e. Pseudohyperparathyroidism.

89. Renal secondary hyperparathyroidism is best associated with:

a. Decreased bone formation.
b. Reduced renal 1α-hydroxylase activity.
c. Metabolic alkalosis.
d. Hypercalcemia.
e. An increased plasma Ca^{2+}:PO_4^{3-} ratio.

90. Pathophysiologic effects associated with hypercalcemia include all of the following, <u>except</u>:

a. Fatigue.
b. Bone dissolution.
c. Increased neuromuscular excitability.
d. Constipation.
e. Metabolic acidosis.

Chapter 19

Objectives

- Explain the pathophysiologic effects associated with hyper- and hyponatremia, hyper- and hypokalemia, hyper- and hypophosphatemia, and hyper- and hypomagnesemia.
- Understand and explain the neuromuscular irritability (NI) equation.
- Know why a modest hyperkalemia is depolarizing, and why a severe hyperkalemia can lead to cardioplegia.
- Explain effects of hyper- and hypokalemia on the SA node action potential.
- Recognize how and why hyperphosphatemia can lead to symptoms of hypocalcemia.
- Discuss how and why excessive loop diuretic therapy could lead to hypomagnesemia and kaliuresis.
- Outline and explain the functional neuromuscular correlations between Na^+ and Ca^{2+}, and between Mg^{2+} and Ca^{2+}.
- Understand why alterations in the ECF Na^+ concentration exert profound influences on blood pressure and volume.

Questions

91. Select the <u>true</u> statement(s) below regarding hyperphosphatemia:

a. It is not as common in domestic animals as hypophosphatemia.
b. It can promote aciduria.
c. It can precipitate a hypocalcemia.
d. It is associated with rickets and osteomalacia.
e. All of the above

92. A decrease in the ECF K^+ concentration:

a. Is associated with increased neuromuscular irritability.
b. Will decrease the diffusion gradient for K^+ between intra- and extracellular fluid sites.
c. Will cause the equilibrium potential for K^+ (i.e., EK^+) to decrease.
d. Is, generally, hyperpolarizing.
e. All of the above

93. Select the <u>true</u> statement(s) below:

a. NaCl engorgement is generally associated with ECF volume expansion and hypertension.
b. The plasma K^+, Ca^{2+} and Mg^{2+} concentrations are usually inversely correlated with pH.
c. Slope of the phase 4 SA node prepotential is increased in hypokalemia.
d. Depolarizing effects of hyperkalemia close Na^+ inactivation gates, thus slowing development of ventricular action potentials.
e. All of the above

94. Select the <u>false</u> statement(s) below:

a. The Ca^{2+} current (I_{Ca}) through both T and L channels of SA node pacemaker cells is increased in hyperkalemia.
b. A decrease in the ECF Na^+ concentration will promote aldosterone release, particularly when associated with ECF volume expansion.
c. Mg^{2+} excess causes tachycardia by stimulating the SA node and cardiac conducting system.
d. Hypermagnesemia increases neuromuscular excitability, and usually leads to kaliuresis.
e. All of the above

95. Diabetes insipidus is best associated with:

a. Hypophosphatemia and hemolysis.
b. Increased neuromuscular excitability and cardiac toxicity.
c. Grass tetany in ruminant animals.
d. Hypernatremia and ECF volume contraction.
e. Hyperkalemia and tachycardia.

Chapter 20

Objectives

- Explain why a short period of hyperexcitability usually precedes collapse in animals with milk fever.
- Understand why an elevated Ca^{2+} prepartal diet may predispose the dairy cow to milk fever.
- Provide an explanation as to why acidifying salts such as NH_4Cl might prevent milk fever when added (in appropriate amounts) to the prepartal diet.
- Compare parturient hypocalcemia in dairy cows to that in dogs and cats.
- Identify various causes of osteoporosis in animals, and explain the positive feedback nature of this condition.
- Understand the roles of estrogen in Ca^{2+} homeostasis.
- Discuss why milk fever is both a hypocalcemic and a hypophosphatemic condition.

Questions

96. The acute hypophosphatemia seen with milk fever in dairy cows is potentially associated with:

a. Hyperexcitability.
b. Dilutional acidosis.
c. Cellular dehydration.
d. Hemoglobinuria.
e. Cardiac toxicity.

97. Dairy cows fed a low rather than a high Ca^{2+} prepartal diet:

a. Generally decrease their efficiency of intestinal Ca^{2+} absorption.
b. Are more capable of mobilizing Ca^{2+} from bone at the time of parturition.
c. Are more likely to develop parturient hypocalcemia.
d. Are more likely to develop osteoporosis once lactation has begun.
e. Are more likely to develop parathyroid atrophy.

98. Parturient hypocalcemia in bitches and queens:

a. Is similar to pre-eclampsia in women.
b. Can be prevented through dietary Ca^{2+} supplementation during pregnancy.
c. Develops similarly to parturient hypocalcemia in dairy cows.
d. Usually develops within minutes following parturition.
e. Is not a serious abnormality, and therefore does not require electrolyte therapy.

99. Osteoporosis:

a. That develops in hibernating animals is usually reversed following arousal from hibernation.
b. Is best associated with parathyroid atrophy.
c. Can be effectively treated with glucocorticoids.
d. Is, unfortunately, a result of weight bearing exercise and over-exposure to UV light.
e. Would not be a problem if we (and our animals) lived on the moon.

Chapter 21

Objectives

- Describe the structural/functional zonation of the mammalian adrenal gland, and discuss primary differences between the cortex and the medulla.
- Explain why the adrenal medulla can be considered a specialized ganglion of the sympathetic nervous system with neuro-secretory characteristics.
- Identify trophic agents for the zona glomerulosa and the zona fasciculata, and explain why the zona reticularis is considered to be the cortical graveyard.
- Recognize why the zona glomerulosa and the zona reticularis cannot synthesize cortisol.
- Understand effects of cortisol on epinephrine biosynthesis.
- Predict the consequences of an adrenal 21β-hydroxylase deficiency.
- Recognize how adrenal steroids are metabolized and eliminated from the body.
- Describe how plasma protein binding can affect the concentrations of steroid hormones in blood.
- Know what 17-ketosteroids are, and from where they are derived.

Questions

100. The parent steroid from which all corticosteroid hormones can be produced is:

a. Estrogen
b. Cholesterol
c. Acetate
d. Aldosterone
e. Pregnenolone

101. When the inner two zones of the adrenal cortex are removed, they regenerate from:

a. Glomerulosa cells.
b. Chromaffin cells of the adrenal medulla.
c. Renal tubular epithelial cells.
d. Reticularis cells.
e. None of the above, for the adrenal cortex does not possess regenerative capacity.

102. Which of the following statements regarding corticosteroid tetrahydroglucuronides is/are <u>true</u>?

a. They normally appear in urine.
b. They normally appear in feces.
c. They are produced by the liver.
d. They are water-soluble.
e. All of the above

103. Enzymes of which area of the adrenal gland are most affected by ACTH?

a. Zona glomerulosa
b. Zona fasciculata
c. Zona reticularis
d. Zona arcuata
e. Zona medulla

104. Aldosterone can be synthesized from:

a. Acetate.
b. Progesterone.
c. Cholesterol.
d. Corticosterone.
e. All of the above

105. The zona glomerulosa cannot synthesize cortisol because it lacks which enzyme?

a. 17,20 Lyase
b. 3β-Hydroxysteroid dehydrogenase
c. 17α-Hydroxylase
d. Adrenal sulfokinase
e. 21β-Hydroxylase

Chapter 22

Objectives

- Explain why cortisol, which binds to aldosterone receptors, does not normally activate those receptors.
- Understand why 9α-fluorocortisol is used to treat patients with Addison's-like disease.
- Recognize the primary corticosteroids of birds, amphibians, reptiles and mammals (Ch. 72).
- Differentiate between the selectivities of HSD-1 and HSD-2.
- Describe and explain the molecular mechanism for ACTH-stimulated cortisol secretion.
- Identify factors regulating CRH and ACTH output, and discuss the actions of these peptides.
- Recognize the circadian rhythmicity of cortisol release, and suggest reasons why this exists (Ch 23).
- Know the natural feedback modulators of ACTH release.

Questions

106. Select the false statement below:

a. Corticosterone has more mineralocorticoid activity than cortisol.
b. Prednisone possesses primary glucocorticoid activity.
c. 9α-Fluorocortisol administration would stimulate renal Na^+ retention and K^+ secretion more than it would hepatic gluconeogenesis.
d. Dexamethasone possesses primary glucocorticoid activity.
e. Plasma concentrations of aldosterone in dogs are normally greater than those of cortisol.

107. All of the following are produced in the adrenal cortex, except:

a. Androstenedione.
b. Corticosterone.
c. 17-OH-Progesterone.
d. Cortisone.
e. Pregnenolone.

108. Select the true statement below:

a. Cortisol secretion, like that of GH, decreases during sleep.
b. Steroid-secreting endocrine cells typically store their hormones, then secrete them upon demand.
c. Cortisol circulates in plasma primarily in the free, unbound form.
d. NADPH is required for steroid biosynthesis.
e. Transcortin is synthesized in the adrenal glands, and its production is increased by estrogen.

109. ACTH appears to be operating through:

a. The Ca^{2+}/DG messenger system.
b. The MAP K messenger system.
c. The cAMP messenger system.
d. The JAK-STAT messenger system.
e. Binding to cytoplasmic steroid hormone receptors.

110. ACTH:

a. Exerts a permissive effect on adrenal aldosterone release.
b. Is bound in plasma to transcortin.
c. Promotes GH release from the anterior pituitary.
d. Is a glycoprotein containing 236 amino acid residues.
e. Possesses mineralocorticoid activity.

Chapter 23

Objectives

- Explain why glucocorticoids are considered to be "anti-insulin," and "anti-calcium."
- Understand relationships between hormone structure and plasma clearance.
- Name three other diabetogenic hormones (besides cortisol).
- Know why cortisol, thyroxine, and progesterone are sometimes considered to be synergistic with epinephrine.
- Understand why cortisol is considered to be hyperlipidemic.
- Recognize the effects of glucocorticoids on adenohypophyseal TRH release.
- Explain the mechanism for glucocorticoid-stimulated glycogen deposition.
- Know why glucocorticoids are sometimes used to treat patients with vitamin D toxicity.
- Discuss the mechanisms for glucocorticoid-stimulated hypertension and ketosis.

Questions

111. Cortisol is cleared more rapidly from the circulation than:

a. Thyroxine.
b. Aldosterone.
c. Insulin.
d. Testosterone.
e. All of the above

112. Which one of the following would not be expected to result from sustained glucocorticoid excess in dogs?

a. Muscle wasting
b. Hyperthyroidism
c. Excessive bone loss
d. Lactescent serum
e. Vacuolar hepatopathy (excessive glycogen deposition)

113. Actions of cortisol include:

a. Promoting collagen synthesis.
b. Enhancing the anti-osteoclastic actions of calcitonin on bone.
c. Stimulation of pancreatic insulin secretion.
d. Fat redistribution.
e. Suppression of hepatic ketogenesis.

114. Approximately what percentage of plasma cortisol is normally protein-bound?

a. < 1
b. 25
c. 50
d. 75
e. > 90

Chapter 24

Objectives

- Know why glucocorticoid excess causes capillary fragility and promotes intracutaneous hemorrhages.
- Understand how cortisol assists in maintaining normal blood pressure and volume.
- Explain why patients receiving prednisone therapy sometimes develop PU/PD.
- Recognize the nature of the gastric mucosal barrier, and explain gastrointestinal symptoms of glucocorticoid excess.
- Predict why glucocorticoid excess would retard the growth of young animals.
- Know why glucocorticoids are sometimes used to treat lymphomas and lymphocyte leukemia.
- Examine the physiological benefits and liabilites of glucocorticoid therapy.

Questions

115. Which one of the following would <u>not</u> be expected to result from sustained glucocorticoid excess?

a. Peptic ulcer formation
b. PU/PD
c. Neutropenia
d. Reduced eicosanoid biosynthesis
e. Hyperglycemia

116. Select the <u>false</u> statement below:

a. When inflammatory reactions become intense, and spread to uninjured tissues, glucocorticoids help to prevent their destruction.
b. Glucocorticoids used therapeutically may retard wound healing.
c. Glucocorticoids suppress appetite and pulmonary surfactant biosynthesis, but enhance memory.
d. Although glucocorticoids enhance hepatic and renal gluconeogenesis, they have a tendency to suppress antibody formation.
e. Glucocorticoids may exacerbate the symptoms of diabetes mellitus.

117. Glucocorticoids aid in maintenance of blood pressure and volume by:

a. Decreasing permeability of the vascular endothelium.
b. Permitting normal responsiveness of arterioles to the constrictive actions of angiotensin II and norepinephrine.
c. Decreasing production of vasodilator prostaglandins from the vascular endothelium.
d. Helping to sustain myocardial performance.
e. All of the above

118. Which of the following statements regarding cortisol is/are <u>true</u>?

a. Antagonizes the actions of insulin
b. Anabolic in fat and muscle tissue
c. Increases sensitivity of the pituitary to TRH
d. Decreases hormone-sensitive lipase biosynthesis
e. All of the above

119. Physiologic/pharmacologic actions of cortisol include:

a. Decreasing erythrocyte production.
b. Opposing the actions of vitamin D in the intestine.
c. Stimulating phospholipase A_2 (PLA_2) and cyclooxygenase (COX) activity.
d. Increasing renal Ca^{2+} and PO_4^{3-} reabsorption.
e. All of the above

Chapter 25

Objectives

- Describe the causes and effects of PDH, primary hyperadrenocorticism, and iatrogenic Cushing's-like syndrome.
- Identify and discuss reasons for the signs and symptoms of glucocorticoid excess.
- Differentiate between Cushing's-like disease, and Cushing's-like syndrome.
- Explain why "iatrogenic hyperadrenocorticism" would be better termed "iatrogenic secondary hypoadrenocorticism."
- Understand why Lysodren can be used to treat animals with adrenal hyperplasia.
- Discuss why glucocorticoid excess causes a steroid hepatopathy in dogs.
- Explain why animals with Cushing's-like syndrome become hyposthenuric.
- Recognize why long-term glucocorticoid therapy could lead to secondary hypothyroidism, testicular atrophy and anestrus, short stature among young animals, and when discontinued, Addison's-like disease.

Questions

120. The plasma profile in Cushing's-like syndrome would typically include:

a. Decreased liver enzymes.
b. Hypocholesterolemia.
c. Hyperglycemia.
d. Decreased VLDL.
e. Azotemia.

121. Cutaneous hyperpigmentation would be expected with:

a. Primary hyperadrenocorticism.
b. Pituitary-dependent hyperadrenocorticism.
c. Exposure to excessive amounts of exogenous glucocorticoids.
d. Thyrotoxicosis.
e. All of the above

122. All of the following are signs of Cushing's-like syndrome, <u>except</u>:

a. Hyperactivity.
b. Muscle wasting.
c. Hepatomegaly.
d. Cutaneous infections.
e. Bone mineral loss (osteoporosis).

123. Which of the following could potentially cause Cushing's-like syndrome?

a. Excessive hypothalamic CRH release
b. Primary hyperadrenocorticism
c. ACTH-secreting tumors
d. Chronic excesses of exogenously administered glucocorticoids
e. All of the above

124. The CBC in Cushing's-like syndrome would be expected to include all of the following, except:

a. Lymphopenia.
b. Neutrophilia.
c. Mild erythrocytosis.
d. Eosinophilia.
e. Mild leukocytosis.

Chapter 26

Objectives

- Identify factors governing adrenal aldosterone release, and discuss the physiologic actions of this steroid.
- Explain why cortisol binds to but does not activate mineralocorticoid receptors.
- Contrast intracellular second messenger systems used by angiotensin II and ACTH.
- Recognize why ANP is anti-aldosterone.
- Know what percentage of renal Na^+ reabsorption is normally affected by aldosterone.
- Understand the mechanisms for aldosterone-stimulated renal Na^+ reabsorption, K^+ and H^+ secretion. Show how the transepithelial and transmembrane potential differences are affected.
- Discuss how hypothyroidism could lead to hyperkalemia and hypotension.

Questions

125. Aldosterone affects Na^+/K^+-ATPase activity in:

a. Muscle and liver tissue.
b. Mucosal cells of the large intestine.
c. Salivary ducts.
d. Tubular epithelial cells in distal nephrons of the kidney.
e. All of the above

126. Factors associated with increased adrenal aldosterone release include all of the following, except:

a. Decreased GFR.
b. Hypokalemia.
c. Decreased effective circulating volume (ECV).
d. Anxiety.
e. Increased circulating levels of angiotensin II.

127. Effects of aldosterone on renal target cells are enhanced by the concurrent presence of:

a. Insulin.
b. PTH.
c. GH.
d. T_4.
e. Epinephrine.

128. Aldosterone fails to exert any influence on Na^+ reabsorption in the kidney for 10-30 min following injection into the renal artery. This latent period represents the time needed to:

a. Remove Na^+ from the cytoplasm of distal renal tubular epithelial cells.
b. Actively transport Na^+ from the filtrate into distal renal tubular target cells.
c. Increase protein synthesis within it's target cells.
d. Transport aldosterone from the renal artery to target cells in the distal nephron.
e. None of the above

129. Chronic high levels of aldosterone will result in:

a. Hyponatremia.
b. Metabolic alkalosis.
c. Hyperkalemia.
d. Hypotension.
e. Azotemia.

Chapter 27

Objectives

- Discuss functional roles for renal "renin," and gastric/abomasal "rennin." Learn (and remember) how to properly pronounce both words.
- Explain why daily fluctuations in the plasma $[K^+]$ affect aldosterone release more than fluctuations in the plasma $[Na^+]$.
- Identify the hormone most affected by small changes in the plasma $[Na^+]$.
- Understand the factors that most affect urinary Na^+ excretion.
- Know how the JG apparatus functions, and identify factors that increase or decrease renin release.
- Understand why it is more efficient to have specialized cells in the distal nephron sensing the [NaCl] of the tubular filtrate (i.e., the macula densa), than specialized cells in afferent arterioles (e.g., JG cells) sensing the [NaCl] of plasma.
- Identify and discuss the physiologic actions of angiotensin II & III.
- Give the source and control of renin substrate production.
- Recognize the primary location of and importance of ACE to the renin-angiotensin system.

Questions

130. Which of the following statements regarding angiotensinogen is true?

a. Production decreased in hyperthyroidism.
b. A steroid produced by the liver.
c. Synthesis and secretion reduced by glucocorticoid administration.
d. A protein produced by the kidneys.
e. A substrate acted upon (enzymatically) by renin.

131. Hemorrhage causes an increase in renin release through all of the following mechanisms, except:

a. It leads to a fall in intrarenal afferent arteriolar pressure, which directly promotes renin release into blood.
b. It causes a decrease in the GFR, which gives more time for proximal tubular NaCl reabsorption. Thus, the distal tubular filtrate will contain less NaCl, causing the macula densa to signal JG cells of the afferent arteriole to release renin into blood.
c. It leads to an increase in sympathetic nervous system (SNS)

activity (i.e., hypovolemic shock), which in turn causes renin release since JG cells are directly innervated by the SNS.

d. It results in a reduction in circulating ANP, which in turn removes an inhibitor influence on renin release.

e. It causes the plasma osmolarity to decrease, which is sensed directly by osmoreceptors on JG cells of afferent arterioles. This, in turn, causes renin release.

132. Select the <u>true</u> statement below:

a. Angiotensin II is a vasoconstrictor, and angiotensin III a vasodilator.

b. Rennin splits the end off a liver-derived plasma protein called angiotensinogen.

c. Angiotensin converting enzyme (ACE) splits a peptide bond that converts angiotensinogen to angiotensin I.

d. The AT_1 receptors (for angiotensin II) are tied to the Ca^{2+}/DG intracellular 2nd messenger system.

e. Angiotensin II is known to promote thirst, and inhibit neurohypophyseal ADH release.

133. In addition to aldosterone, renal Na^+ excretion is affected by:

a. Changes in renal tubular Na^+ reabsorption independent of aldosterone.

b. Factors influencing the GFR.

c. The presence or absence of osmotic diuretics.

d. Natriuretic peptides.

e. All of the above

Chapter 28

Objectives

- Know how the JG apparatus operates, and recognize the numerous factors regulating renin release.
- Recognize the endocrine control of hepatic angiotensinogen production.
- Distinguish 3 cardiovascular variables best associated with HT.
- Understand the difference between "essential" and ""secondary" HT.
- Contrast differences between treatments for high- and low-renin HT.
- Summarize the negative feedback control of renin release.
- Recognize why ACE inhibitors and drugs such as Losartan, Enalkiren and the NSAIDs would have minimal effects in normalizing blood pressure and volume in patients with low-renin HT.
- Know why it is important for the JG body of the afferent arteriole to act as a baroreceptor.

Questions

134. Select the <u>false</u> statement below:

a. Use of ACE inhibitors may lead to hyperkalemia.

b. Angiotensin II vasoconstricts arterioles and stimulates catecholamine release from noradrenergic nerve fibers.

c. Although hypokalemia may result from sustained vomiting, the renin-angiotensin system will nonetheless be activated due to the hypovolemia that ensues.

d. Angiotensin II reduces ADH release from the neurohypophysis and aldosterone release from the adrenal glands.

e. JG cells possess β_1-adrenergic receptors.

135. Select the <u>true</u> statement(s) below regarding hypertension (HT):

a. Cushing's-like syndrome could lead to HT because of excessive hepatic angiotensinogen release.

b. β-Blocking agents such as propranolol can be effectively used to treat low-renin HT.

c. Vasoconstricting drugs that induce HT would include those that bind to angiotensin II receptors, preventing them from illiciting physiologic responses.

d. High-renin HT is usually the result of inadequate renin degradation.

e. All of the above

136. Which of the following would be an effective treatment for low-renin HT?

a. Nonsteroidal antiinflammatory drugs

b. Aldosterone antagonists

c. ACE inhibitors

d. Angiotensin receptor antagonists

e. Any of the above

137. Which one of the following <u>decreases</u> renin release?

a. Insulin

b. ACTH

c. PGI_2

d. Aldosterone

e. Isoproterenol

Chapter 29

Objectives

- Determine the causes of Addison's-like disease in animals, and distinguish between primary and secondary hypoadrenocorticism.
- Distinguish between idiopathic and iatrogenic causes of Addison's-like disease in animals.
- Understand reasons for each of the signs and symptoms of Addison's-like disease.
- Recognize the important connection between prolonged glucocorticoid therapy, and secondary hypoadrenocorticism.
- Explain the connection between hypercalcemia and hypoadrenocorticism.
- Know why Addisonian-like patients sometimes develop bloody diarrhea.
- Understand why plasma ACTH levels are increased in Addisonian-like patients.
- Provide reasoning for the glucocorticoid deficiency occurring first in Addisonian-like patients, then the mineralocorticoid deficiency occurring second.

Questions

138. What percentage of the adrenal cortex is generally destroyed before clinical signs and symptoms of Addison's-like disease become obvious?

a. 1%

b. 15%

c. 30%

d. 60%

e. 90%

139. Hypercalcemia may develop in Addison's-like disease because of:

a. Decreased urinary Ca^{2+} excretion.
b. Decreased intestinal Ca^{2+} absorption.
c. Bone dissolution.
d. High circulating titers of vitamin D.
e. All of the above

140. Addison's-like disease is usually associated with:

a. Alkalemia.
b. A low plasma Na^+/K^+ ratio.
c. Low circulating titers of ACTH.
d. Constipation.
e. Hyperglycemia.

141. What is the most common cause of Addison's-like disease in animals?

a. Iatrogenic primary hypoadrenocorticism
b. Trauma
c. Iatrogenic secondary hypoadrenocorticism
d. Pituitary insufficiency
e. Adrenal neoplasia

142. All of the following are signs and symptoms of Addison's-like disease, <u>except</u>:

a. Hypotension.
b. Decreased urine specific gravity.
c. Vomiting and bloody diarrhea.
d. Prerenal azotemia.
e. Erythrocytosis and neutrophilia.

Chapter 30

Objectives

- Recognize why HbF might be considered a "physiologic luxury."
- Identify sites of fetal vs. adult Hb and EPO production.
- Know the numerous factors controlling EPO release.
- Explain why it takes 2-3 days for bone marrow to respond to the presence of EPO.
- Discuss the mechanism of estrogen-induced aplastic anemia.
- Know the average turnover rate of erythrocytes in mammals.
- Recognize potential uses of rHuEPO-α in veterinary medicine.
- Understand the correlation between respiratory alkalosis and EPO release.

Questions

143. Which of the following statements regarding fetal hemoglobin is/are <u>true</u>?

a. It is produced in the fetal liver.
b. It may be more of a physiologic luxury than a necessity in mammals.
c. Production is stimulated by fetal EPO, which is produced in the liver.
d. It may not bind 2,3-DPG with as high an affinity as does HbA.
e. All of the above

144. All of the following stimulate erythropoietin (EPO) secretion, <u>except</u>:

a. Cardiopulmonary disease.
b. Hypertension.
c. Decreased blood hemoglobin concentration.
d. Testosterone.
e. High altitude.

145. Select the <u>true</u> statement below regarding EPO:

a. It is produced by the adult kidney.
b. It is produced by the fetal liver.
c. It is a protein.
d. It has been produced in culture.
e. All of the above

146. Select the <u>false</u> statement below:

a. Recombinant human EPO is active in dogs.
b. Hyperestrogenism can cause anemia.
c. The major factor controlling EPO release is the Po_2 of blood perfusing the kidneys.
d. Small amounts of EPO are produced by the uterus and oviducts.
e. Catecholamines inhibit EPO release.

147. Erythropoietin:

a. Inhibits erythropoiesis in a negative feedback fashion.
b. Has a circulating half-life of about 5 minutes.
c. Is produced by interstitial cells in peritubular capillaries of the kidneys.
d. Secretion is inhibited by placental lactogen (PL).
e. Is produced by Sertoli cell tumors.

Chapter 31

Objectives

- Explain how and why atria participate in blood volume and pressure regulation.
- Describe the affects of natriuretic peptides on blood vessels, the hypothalamus, and the adrenal cortices.
- Contrast opposing effects of the natriuretic peptides and the renin-angiotensin-aldosterone system.
- Summarize the actions of ANP in reducing cardiac output.
- Know how urodilatin functions, and explain why it does not circulate in blood.
- Recognize how increased circulating levels of ANP and/or BNP could reflect different cardiac abnormalities.
- Identify the source of CNP, as well as its physiologic properties.
- Name the GI peptide that induces natriuresis.
- Recognize the role for ANP-like peptides in fishes.

Questions

148. ANP works though which second messenger system?

a. cGMP
b. cAMP
c. Ca^{2+}/DG
d. MAP K
e. JAK-STAT

149. Select the false statement below:

a. Natriuretic peptides have been isolated from mammalian brain tissue.
b. ANP inhibits NaCl reabsorption in collecting ducts of the kidney.
c. Natriuretic peptides have yet to be found in nonmammalian vertebrates.
d. Myocytes of mammalian atria appear to possess fluid volume receptors.
e. BNP blood levels may increase in ventricular hypertrophy.

150. Which one of the following is considered to be the most potent natriuretic and diuretic hormone?

a. ADH
b. Cortisol
c. ANP
d. Urodilatin
e. Aldosterone

151. ANP effectively antagonizes the action of:

a. ADH
b. Aldosterone
c. Angiotensin II
d. Renin
e. All of the above

152. ANP and angiotensin II share which one of the following functions?

a. Stimulate aldosterone release
b. Increase thirst
c. Constrict efferent arterioles
d. Stimulate ADH release
e. Constrict peripheral arterioles

153. Natriuresis is promoted by:

a. ANP
b. BNP
c. Guanylin
d. Urodilatin
e. All of the above

Chapter 32

Objectives

- Explain why the adrenal medulla is considered to be a neuroendocrine structure.
- Know why the adrenal medullae are not considered "essential" for life, but the adrenal corticies are.
- Identify the source of most circulating metenkephalin.
- Describe the pathway of catecholamine biosynthesis, and understand how it is controlled.
- Explain the symbiotic nature of cortisol and epinephrine.
- Discuss the commonalities and differences between catecholamine and other biogenic amine (e.g., histamine and 5-HT) degradation.
- Identify at least 8 degradative products (including their conjugates) of the catecholamines that appear in urine.
- Know the difference between homovanillic acid and vanillylmandelic acid.

Questions

154. Which one of the following enhances the activity of PNMT, thus promoting adrenal medullary conversion of NE to Epi?

a. Insulin
b. Glucocorticoids
c. Propranolol
d. Metanephrine
e. Dopamine

155. Pheochromocytes:

a. Contain and secrete opioid peptides.
b. Secrete NE and/or Epi directly into blood when the SNS is activated.
c. Produce adrenomedullin.
d. Remove tyrosine from interstitial fluid, and convert it to catecholamines.
e. All of the above

156. Select the true statement below regarding catecholamine biosynthesis:

a. Conversion of Dopa to DA is the rate-limiting step in NE biosynthesis.
b. Serine serves (directly) as a methyl donor in the conversion of NE to Epi.
c. Epinephrine is produced largely through hydroxylation, decarboxylation, and methylation of tyrosine.
d. Like cholinergic neurons, NE-secreting neurons lack a re-uptake mechanism, and therefore must continually synthesize NE upon demand.
e. NE biosynthesis is inversely correlated with the cAMP concentration in pheochromocytes.

157. Select the false statement below regarding catecholamine degradation:

a. COMT uses SAM as a methyl donor.
b. MAO-A is found largely in neural tissue.
c. VMA is generally the most plentiful catecholamine metabolite found in urine.
d. While NE and Epi use COMT and MAO as degradative enzymes, DA uses phenylalanine hydroxylase.
e. Homovanillic acid is an inactive degradative product of DA found in urine.

Chapter 33

Objectives

- Identify the primary effects of sympathetic activation, and associate each effect with an adrenergic receptor sub-type.
- Explain how Epi and angiotensin II augment the sympathetic response to hypovolemia.
- Recognize how NE brings about arteriolar smooth muscle contraction, and Epi smooth muscle relaxation.
- Know the post-receptor molecular events that occur when β_1-adrenergic receptors on heart muscle and the SA node are stimulated.
- Contrast post-receptor molecular events that occur with α_1-, α_2-, and β-adrenergic receptors are stimulated.
- Explain how NE feeds-back negatively on the neuron that produces it.
- Summarize how Epi brings about the "fight or flight" response.
- Understand the effects of catecholamines on insulin and glucagon secretion.
- Describe the effects of catecholamines on the renin-angiotensin system, and upon EPO release.

Questions

158. Stimulation of α_2-adrenergic receptors on presynaptic membranes of NE-secreting neurons:

a. Increases adenyl cyclase activity.
b. Is blocked by clonidine.
c. Mobilizes intracellular Ca^{2+}.
d. Decreases NE release.
e. None of the above

159. Which one of the following is <u>not</u> a part of the "fight or flight" response?

a. Mobilization of liver glycogen
b. Constriction of gastrointestinal sphincters
c. Miosis of the eye
d. Bronchiolar smooth muscle relaxation
e. Reduction of pancreatic insulin output

160. Which one of the following adrenergic receptor subtypes responds to NE and Epi equally?

a. α_1
b. α_2
c. β_1
d. β_2
e. β_3

161. Smooth muscle cells relax under β_2-adrenergic receptor stimulation, because:

a. cAMP levels rise.
b. MLCK activity rises.
c. MLC phosphatase activity decreases.
d. The cytosolic $[Ca^{2+}]$ rises.
e. All of the above

162. Stimulation of β_1-adrenergic receptors in the heart causes:

a. cAMP levels to rise.
b. Phosphorylation of Ca^{2+} L-channel proteins in ventricular myocytes.
c. Increased Ca^{2+} conductance through T and L channels in the SA and AV nodes.
d. Increased inotropy and chronotropy.
e. All of the above

Chapter 34

Objectives

- Explain why patients with pheochromocytomas develop hyperglycemia.
- Draw symptomatic correlations between pheochromocytoma, hyperthyroidism, and secondary hypertension.
- Recognize the episodic nature of catecholamine release from a pheochromocytoma.
- Compare the physiologic actions of NE to those of Epi and DA (see previous Chs.).
- Indicate how biochemical confirmation of catecholamine excess is made.
- Explain how the clonidine suppression test works, and why this drug can be used as an anti-hypertensive agent.

Questions

163. Select the <u>true</u> statement(s) below regarding mammalian chromaffin cells (pheochromocytes):

a. Those associated with sympathetic ganglia normally regress in adulthood.
b. They may be involved in paraganglioma formation.
c. They are largely localized within adult adrenal medullae.
d. They arise from neuroectoderm.
e. All of the above

164. Select the <u>true</u> statement below regarding pheochromocytomas:

a. They are usually innervated.
b. They usually cause a decrease in the basal metabolic rate (BMR).
c. They typically promote hyperinsulinemia.
d. They may suppress pituitary ADH release, thus causing PU/PD.
e. They generally decrease their activity under general anesthesia.

165. All of the following may be associated with pheochromocytoma, <u>except</u>:

a. Ketonuria.
b. Hyperglycemia.
c. Decreased red blood cell (RBC) mass.
d. Mydriasis.
e. Increased urinary VMA excretion.

166. Select the <u>true</u> statement below regarding clonidine:

a. It will increase catecholamine release from a pheochromocytoma.
b. It can be used (effectively) to treat hypertension.
c. It is a β_2-adrenergic receptor agonist.
d. It is an α_2-adrenergic receptor antagonist.
e. None of the above

167. Select the <u>false</u> statement below:

a. Epi, NE, and DA can all be found circulating in blood.
b. Epi seems to be more involved with triglyceride and blood glucose homeostasis, whereas NE appears to be more involved with counteracting hypotension.
c. Pheochromocytes possess adrenergic, but not cholinergic receptors.
d. Many signs and symptoms of pheochromocytoma are similar to those of hyperthyroidism.
e. α_1-Adrenergic receptor blocking agents can be used (effectively) to treat hypertension.

Chapter 35

Objectives

- Explain why responses of the SNS and adrenal medullae to stress are not necessarily parallel, and give an example.
- Know why hypophysectomized or adrenalectomized animals have difficulty dealing with traumatic stress.
- Recognize the neuroendocrine components at play during phase 1 of the response to trauma, and understand how they defend against volume depletion.
- During phase 2 of the response to trauma, show how cortisol synergizes with thyroxine and the catecholamines to maintain survival.

- Outline the independent actions of cortisol and aldosterone in restoring blood pressure and volume following hemorrhage.
- Show how the "capillary fluid shift" operates in restoring blood volume following hemorrhage.
- Recognize how the endocrine complement of phase 3 differs from that of phase 2, and indicate how it is anabolic.
- Understand how and why circulating levels of Epi increase following birth.

Questions

168. Which one of the following is usually at a low level in the circulation during phase 3 of the response to trauma?

a. Somatotropin
b. Thyroxine
c. EPO
d. Glucagon
e. IGF-2

169. Select the <u>false</u> statement below regarding the phasic (physiologic) response to trauma:

a. It appears to be independent of the nutritional state.
b. A negative N balance is characteristic of the early phase.
c. The sequence of compensatory events that normally transpires cannot take place in the absence of the hypophysis.
d. Phase 3 is generally catabolic.
e. Phase 1 typically involves SNS activation.

170. All of the following are typically elevated in the circulation during phase 1 of the response to trauma, <u>except</u>:

a. Insulin.
b. Angiotensin II.
c. Vasopressin.
d. Epinephrine.
e. ACTH.

171. During the catabolic phase of the response to trauma, adipose tissue lipolysis is driven by:

a. Cortisol.
b. Epinephrine.
c. Thyroxine.
d. Low circulating insulin levels.
e. All of the above

172. Which one of the following hormones is usually at a low level in the circulation during phase 2 of the response to trauma?

a. Glucagon
b. Epinephrine
c. Insulin
d. Thyroxine
e. Cortisol

173. During physiologic starvation, which one of the following ratios would be expected to be <u>increased</u>?

a. Epi:NE
b. T_3:rT_3
c. Insulin:Glucagon
d. NE:T_4
e. Insulin:Cortisol

Chapter 36

Objectives

- Describe the functions of follicular and parafollicular cells of the mammalian thyroid gland.
- Recognize how negative feedback of the hypothalamic-pituitary-thyroid axis functions.
- Explain differing roles for E_2 and cortisol in adjusting sensitivity of the adenohypophysis to TRH.
- Know how the thyroid gland responds to the presence of TSH.
- Describe how T_4, T_3 and rT_3 are synthesized (Ch. 6).
- Explain why T_4 is considered to be the prehormone to T_3.
- Identify the monovalent anions that compete with I^- for uptake into the thyroid gland, and discuss their clinical relevance.
- Suggest a physiologic reason for the stimulation of hepatic TBG production by E_2.
- Know how thyroid hormones are synthesized, secreted, metabolized and excreted by the body.
- Compare the mechanism of thyroid hormone target tissue action to that of the steroid hormones (see Ch. 6).
- Discuss the control of TRH and TSH release, and predict how their aberrant production/secretion can lead to hypo- or hyperthyroidism.

Questions

174. In which of the following species are thyroid hormones primarily bound in plasma to albumin (rather than thyroid-binding globulin)?

a. Primates
b. Equine
c. Canine
d. Bovine
e. Porcine

175. Select the <u>false</u> statement below:

a. TRH has a longer amino acid chain length than TSH.
b. Estrogen enhances sensitivity of the anterior pituitary to TRH.
c. Cortisol decreases sensitivity of the anterior pituitary to TRH.
d. TSH enhances cytoplasmic HMS activity in follicular cells of thyroid tissue.
e. Photoperiod affects TRH release from the hypothalamus.

176. Select the <u>true</u> statement below:

a. Thyroglobulin and thyroid hormone-binding globulin (TBG) are produced by the liver, and circulate in plasma.
b. A Na^+/I^- antiporter transports Na^+ out and I^- into thyroid follicular cells.
c. The thyroid gland typically synthesizes more T_3 than T_4.
d. TSH promotes I^- uptake into thyroid follicular cells as well as into acinar cells of salivary glands.
e. During the process of thyroid hormone biosynthesis, two DIT molecules condense to form T_4 (+ Ala).

177. Cathepsins:

a. Are intracellular proteolytic enzymes found in the thyroid gland.
b. Bind T_4 and T_3 in plasma, thus helping to maintain circulating levels of these important hormones.
c. Are deiodinases present in thyroid hormone target cells throughout the body.
d. Simulate TSH release from the anterior pituitary.
e. Are intracellular thyroid hormone binding proteins.

178. Select the false statement below regarding T_4 and T_3:

a. Circulating levels are about 20:1, respectively, in dogs.
b. The thyroid secretory ratio is about 5:1, respectively.
c. T_4 is more tightly protein-bound in plasma than T_3.
d. Most T_4 and T_3 that appears in the glomerular filtrate is reabsorbed by the proximal nephron.
e. Thyroid hormone conjugates are normally excreted in bile, but not urine.

Chapter 37

Objectives

- Explain what is meant by a "step up" and a "step down" in T_4 deiodination.
- Understand how and why epinephrine and thyroxine are synergistic in releasing free fatty acids from adipocytes.
- Understand the metabolic effects of thyroid hormone stimulation.
- Identify tissues where O_2 consumption is not increased in the presence of T_4.
- Explain what affects throxine has on the β:α-adrenergic receptor ratio, and because of this how overall physiologic activity might change in hyper- or hypothyroidism.
- Recognize how thyroid hormones help to "provide more glucose and fatty acids" during periods of food deprivation.
- Understand how and why the total serum thyroid hormone concentration changes during pregnancy.
- Know how T_4 affects erythrocytic 2,3-DPG activity.
- Recognize the synergistic nature of T_4 and aldosterone.

Questions

179. In muscle tissue, nuclear activation occurs via binding of which thyroid hormone to specific high-affinity receptor sites?

a. T_4
b. T_3
c. rT_3
d. T_2
e. T_1

180. In which of the following do thyroid hormones increase O_2 consumption?

a. Smooth muscle
b. Neurons of the brain
c. Ovaries
d. Spleen
e. Testes

181. Which of the following processes do thyroid hormones support?

a. Na^+ excretion into urine
b. Erythropoiesis
c. Lipid deposition in adipocytes
d. Membrane permeability to glucose in muscle tissue
e. All of the above

182. All of the following hormones help to increase clearance of cholesterol from the circulation, except:

a. Estrogen.
b. Insulin.
c. Parathormone.
d. Thyroxine.

183. It appears that pituitary thyrotropes may have evolved from pituitary:

a. Somatotropes.
b. Lactotropes.
c. Corticotropes.
d. Gonadotropes.
e. Melanotropes.

Chapter 38

Objectives

- Identify common causes of hypothyroidism.
- Differentiate between 4 types of goiter.
- Recognize common signs and symptoms of hypothyroidism, and compare to those of hyperthyroidism (Ch. 39).
- Explain why patients with hypothyroidism develop hypotension, hypercholesterolemia, and anemia.
- Differentiate between physiologic and pathophysiologic hypothyroidism.
- Explain why some patients with primary hypothyroidism develop galactorrhea.
- Know why juvenile-onset hypothyroidism results in stunted growth.

Questions

184. A hypothyroid animal in which TSH blood levels do not increase following administration of TRH most likely has:

a. Acquired primary hypothyroidism.
b. Secondary hypothyroidism.
c. Hypothyroidism due to an overdosage of antithyroid drugs.
d. Tertiary hypothyroidism.
e. None of the above

185. Signs and symptoms of canine hypothyroidism include all of the following, except:

a. Obesity.
b. Atherosclerosis.
c. Coarse hair coat.
d. Diarrhea.
e. Anemia.

186. What is the most common cause of hypothyroidism in cats?

a. Naturally occurring primary hypothyroidism ($\downarrow T_4$)
b. Naturally occurring secondary hypothyroidism ($\downarrow$TSH leading to $\downarrow T_4$)
c. Naturally occurring tertiary hypothyroidism ($\downarrow$TRH leading to $\downarrow$TSH, leading to $\downarrow T_4$)
d. Iatrogenic hypothyroidism
e. Panhypopituitarism

187. A cretinous puppy differs from a pituitary dwarf by exhibiting:

a. Stunted growth.
b. Hyperthermia.
c. Subnormal mentality.
d. Ventricular hypertrophy.
e. Alopecia.

188. Galactorrhea may occur in sexually intact bitches with acquired primary hypothyroidism, because of:

a. High TSH levels that stimulate milk production.
b. High TRH levels that stimulate PRL release.
c. High TSH levels that stimulate oxytocin release.
d. An elevated estrogen/progesterone ratio.
e. None of the above

189. Somatotropin secretion may be depressed in hypothyroidism because:

a. Hypothyroidism reduces secretion of all hormones.
b. The hypothalamus ceases producing pituitary release and release-inhibiting factors.
c. T_4 normally stimulates hypothalamic GHRH output.
d. Of pituitary atrophy.
e. T_4 stimulates hypothalamic GHIH output.

Chapter 39

Objectives

- Explain how the type I, II, and III deiodinases operate, and identify the tissues in which they are found.
- Indicate the primary cause of feline hyperthyroidism.
- Know the primary signs and symptoms of hyperthyroidism.
- Recognize why the diastolic blood pressure may be low in hyperthyroidism.
- Compare the hyperthyroid CBC to that in Cushing's-like syndrome.
- Understand why signs and symptoms of steatosis may appear in hyperthyroidism, and why hypocholesterolemia develops.
- Discuss the reasons for ventroflexion of the head in hyperthyroidism.
- Recognize how the thiocarbamide group of the goitrogens affects functions of the thyroid gland.

Questions

190. An animal with hyperthyroidism would be expected to exhibit all of the following changes in it's combined blood count, except:

a. Erythrocytopenia.
b. Leukocytosis.
c. Eosinopenia.
d. Lymphopenia.

191. The dehydration that may accompany hyperthyrodism may be due to:

a. Vomiting.
b. An increase in the GFR.
c. Diarrhea.
d. A decrease in the concentrating abilities of the kidneys.
e. All of the above

192. Which one of the following would be expected in the serum profile of a hyperthyroid cat?

a. ↓ Na^+
b. ↓ PO_4^{3-}
c. ↑ Unconjugated bilirubin
d. ↑ K^+
e. ↓ ALT

193. Cardiac abnormalities in hyperthyroidism may include:

a. Tachycardia.
b. Ventricular hypertrophy.
c. Dilatation.
d. Enhanced β_1-adrenergic receptor synthesis.
e. All of the above

194. Which one of the following would not be expected in the serum profile of a hyperthyroid cat?

a. ↑ LDH
b. ↓ Cholesterol
c. ↓ Glucose
d. ↑ Free fatty acids
e. ↑ BUN

Chapter 40

Objectives

- Explain how the autonomic nervous system controls pancreatic output of insulin and glucagon.
- Identify insulin's counter-regulatory hormones, and discuss how they help in maintaining adequate CNS function.
- Identify specific roles for insulin and glucagon in hepatic function.
- Identify "anticipatory" hormones promoting insulin release.
- Recognize where insulin and glucagon receptors are located in the body.
- Show how the different islet cells are organized in the pancreas, and how they are perfused.
- Identify primary physiologic regulators of pancreatic insulin, glucagon and somatostatin release.

Questions

195. Select the false statement below:

a. Pancreatic endocrine tissue is more highly vascularized than pancreatic exocrine tissue.
b. Insulin inhibits glucagon release.
c. Pancreatic islet tissue is abundantly innervated by autonomic nerve fibers.
d. Glucagon inhibits insulin release.
e. Biologic activities of insulin are not highly species specific.

196. Select the true statement(s) below:

a. Like neurons, pancreatic islet tissue has little regenerative capacity.
b. A 20% loss of pancreatic islet tissue will precipitate hypoglycemic shock.
c. Glucagon-secreting -cells normally comprise about 80% of pancreatic islet tissue.
d. Insulin-secretion β-cells are normally located in the islet cell periphery.
e. All of the above are true.

197. All of the following are diabetogenic, except:

a. Insulin.
b. Cortisol.
c. Epinephrine.
d. Glucagon.
e. Growth hormone.

198. The ANS affects pancreatic endocrine secretion in which way?

a. PNS activation: ↑ Insulin, ↓ Glucagon
b. SNS activation: ↑ Insulin, ↑ Glucagon
c. PNS activation: ↓ Insulin, ↓ Glucagon
d. SNS activation: ↓ Insulin, ↑ Glucagon
e. PNS activation: ↓ Insulin, ↑ Glucagon

199. Which of the following stimulate insulin release?

a. K^+
b. Glucose and amino acids
c. Volatile fatty acids
d. Mg^{2+} and PO_4^{3-}
e. All of the above

200. Select the <u>true</u> statement(s) below:

a. The avian pancreas contains more glucagon (per gm) than the mammalian pancreas.
b. Somatostatin is normally found in pancreatic islets, and it inhibits both insulin and glucagon secretion.
c. Most glucagon receptors in the body are found on hepatocytes.
d. Secretion of pancreatic polypeptide is largely under parasympathetic control.
e. All of the above

Chapter 41

Objectives

- Describe how insulin is synthesized, and identify secretory components of pre-proinsulin.
- Recognize other secretory products of pancreatic β-cells, and summarize the negative feedback control of insulin release.
- Identify the nutritional, neural, paracrine, and endocrine variables that govern insulin release (Ch. 40).
- Know the K_m for glucokinase, and explain why this enzyme is considered to be the pancreatic β-cell glucose sensor.
- Recognize how K^+, Ca^{2+}, cAMP , PKA and IP_3 are involved in insulin release.
- Understand why SNS stimulation normally reduces insulin secretion, and predict how muscarinic, α_2 and β_2-adrenergic drugs might affect insulin release.
- Discuss the significance of glucose-dependent insulinotropic peptide.
- Summarize the importance of islet amyloidosis to the health of diabetic cats.

Questions

201. Select the <u>true</u> statement(s) below:

a. Islet amylin (or amyloid) is stored in pancreatic β-cells.
b. Islet amyloid deposits are sometimes associated with feline IDDM.
c. Islet amylin may surround pancreatic β-cells, isolating them from adjacent endocrine cells and blood capillaries.
d. Islet amylin shares a common amino acid sequence with thyrocalcitonin.
e. All of the above are true.

202. Orally ingested glucose ultimately induces greater amounts of insulin release than similar quantities of glucose infused intravenously:

a. This would be an abnormal situation, seen perhaps in a diabetic, hyperglycemic animal.
b. This is most likely due to the augmenting effect of enteric stimulatory hormones.
c. This may happen in a goat, but I would not expect it to happen in a dog.
d. This is clearly due to the stimulatory effect of gastric somatostatin on pancreatic glucagon release, which in turn stimulates pancreatic insulin release.
e. Impossible!

203. Which one of the following statements most accurately describes the effects of secretagogues on insulin release?

a. The glucose sensor in insulin-secreting cells appears to be tyrosine kinase.
b. Glucose entry into pancreatic β-cells causes an increase in ATP, which inhibits K^+ efflux, thus depolarizing the cell and allowing Ca^{2+} to enter.
c. Somatostatin and glucagon stimulate insulin release through the cAMP second messenger system.
d. Glucose entry into pancreatic β-cells causes them to hyperpolarize, thus increasing the stimulus for insulin release.
e. α-Adrenergic agents depolarize pancreatic β-cells, thus promoting Ca^{2+} entry and insulin release.

204. C-peptide:

a. Is an inert marker for β-cell insulin release that is produced from insulin.
b. Possesses an α and β-chain held together by disulfide bonds.
c. Circulates in blood with insulin, but possesses a longer half-life.
d. And pancreastatin are produced by pancreatic α-cells, and inhibit insulin release.
e. Possesses glucagon-like activity.

205. Select the <u>false</u> statement below:

a. IGF-1 and IGF-2 immunoreactivities have been reported in association with pancreatic β-cells.
b. Porcine and bovine insulin can be used effectively to treat diabetic dogs and cats.
c. β-OH-Butyrate, a ketone body, stimulates insulin release.
d. Pancreatic β-cells possess muscarinic receptors.
e. Although IAPP-derived amyloid fibrils are known to block insulin release, they have not been found to be cytotoxic.

Chapter 42

Objectives

- Identify tissue types where insulin receptors are located.
- Explain the GLUT 4 translocation process, and know the approximate t½ of the insulin receptor.
- Understand how insulin affects metabolic activities of hepatocytes.
- Recognize locations where the SGLTs and GLUTs are found.
- Discuss the role played by insulin in lipid metabolism, and LPL activation.
- Describe the actions of insulin on plasma K^+, Mg^{2+}, PO_4^{3-}, amino acid and nucleoside clearance.

- Explain how insulin enhances triglyceride formation, clearance and deposition.
- Know the circulating half-life of insulin, and explain how (and where) it is degraded.
- Provide an explanation for the amelioration of ketoacidemia following insulin administration.

Questions

206. Which of the following statements regarding the plasma membrane-bound insulin receptor is/are true?

a. It acts as a tyrosine kinase following autophosphorylation.
b. It can be endocytosed and degraded intracellularly.
c. It has α and β-subunits held together by disulfide bonds.
d. It can be downregulated.
e. All of the above are true.

207. Activity of which one of the following enzymes is increased by insulin?

a. Hepatic glucose-6-phosphatase
b. Hexokinase in muscle cells
c. Lipoprotein lipase on the capillary endothelium
d. Phosphoenolpyruvate (PEP) carboxykinase in hepatocytes
e. Hormone-sensitive lipase in adipocytes

208. All of the following hormones exert positive influences on hepatic ketone body production, except:

a. Glucagon.
b. Insulin.
c. Growth hormone.
d. Cortisol
e. Epinephrine.

209. Which one of the following is not stimulated by insulin?

a. K^+ uptake into muscle cells
b. Hepatic lipogenesis and glycogenesis
c. Muscle amino acid uptake
d. Hepatic proteolysis
e. Lipoprotein lipolysis

210. Approximately what percentage insulin secreted by the pancreas is normally destroyed as it passes through the liver?

a. 10%
b. 25%
c. 50%
d. 75%
e. 99%

Chapter 43

Objectives

- Identify normal stimuli for glucagon release, as well as compounds that inhibit its release.
- Indicate the source of GLP-1, and also its effects on insulin and glucagon secretion.
- Understand how the intracellular second messenger system stimulated by glucagon operates, and how it counters the effects of insulin.
- Identify the primary target organ for glucagon, and show how this polypeptide affects pancreatic insulin and somatostatin secretion.
- Discuss the significance of the insulin/glucagon ratio, particularly to ruminants and carnivores.
- Contrast glucagon's effects on carbohydrate and lipid homeostasis to those of insulin.
- Explain why patients with somatostatin-secreting tumors develop hyperglycemia and dyspepsia.
- Show how the role of glucagon in carbohydrate metabolism is similar to those of GH, cortisol and epinephrine.

Questions

211. GLI-1:

a. Contains a 29-amino acid sequence similar to that of pancreatic glucagon.
b. Is also known as proglucagon.
c. Is a steroid with proteolytic properties.
d. Is the signal peptide of preproglucagon.
e. Is cleaved from preproglucagon in the pancreas.

212. The vast majority of glucagon receptors are found:

a. In renal tubular epithelial cells.
b. On mucosal cells of the digestive tract.
c. In the pancreas.
d. On hepatocytes.
e. Next to insulin receptors in muscle and fat cells.

213. Somatostatin inhibits the release of:

a. Growth hormone.
b. Insulin.
c. Gastrin.
d. Glucagon.
e. All of the above

214. Circulating half-lives of insulin and glucagon are about:

a. 5 min.
b. 30 min.
c. 1 hr.
d. 3 hrs.
e. 5 hrs.

215. Select the false statement below:

a. Glucagon reaches a peak in the circulation about 3 days following the onset of starvation in dogs.
b. A low insulin/glucagon ratio favors hepatic cAMP formation.
c. Glucagon inhibits insulin release in a paracrine fashion.
d. Insulin and glucagon levels rise in the circulation following a high-protein, low-carbohydrate meal.
e. Glucagon governs carbohydrate metabolism in birds more than insulin.

Chapter 44

Objectives

- Explain why the liver, usually a rather considerate organ, continues producing glucose in the hyperglycemic diabetic animal.
- Describe different types of endocrinopathies that can result in persistent hyperglycemia.
- Identify the 5 most common causes of DM in domestic animals.
- Explain why obesity is a risk factor for DM.

- Describe similarities and differences between human and canine DM.
- Identify the most common signs and symptoms of DM.

Questions

216. Select the false statement below:

a. Most dogs with DM are similar to type I human diabetics.
b. In the severely hyperglycemic diabetic patient, the kidney no longer reabsorbs glucose, and thus it appears in urine.
c. Progestins can exert a diabetogenic effect in sexually intact female dogs.
d. The uptake of dietary carbohydrate from the intestine remains unaffected in diabetic animals.
e. Pancreatic islet β-cell tumors are common in American ferrets, but they seldom develop DM.

217. In the hyperglycemic diabetic patient, glucose uptake is high in all of the following tissues, except:

a. Nerves.
b. Erythrocytes.
c. Hepatocytes.
d. Proximal renal tubular epithelial cells.
e. The lens.

218. Insulin facilitates glucose uptake into:

a. Mammary tissue.
b. Intestinal mucosal cells.
c. Proximal renal tubular epithelial cells.
d. Lymphocytes.
e. All of the above

219. Select the true statement below:

a. Tissues most affected pathophysiologically in the hyperglycemia of DM are insulin-sensitive tissues.
b. Tissues most affected pathophysiologically in the hyperglycemia of DM are insulin-insensitive tissues.

220. The liver continues producing glucose in the hyperglycemic diabetic animal because:

a. It is occasionally a "stupid" organ, playing into this "pissing evil" concept.
b. It is responding more to the counter-regulatory diabetogenic hormones than it is to insulin (which may be absent).
c. It cannot directly sense the hyperglycemia, and thus does not recognize it.
d. It is doing what it is being "told" to do (endocrinologically).
e. All of the above

Chapter 45

Objectives

- Explain how insulin deprivation affects carbohydrate and lipid metabolism.
- Predict how the plasma Na^+, K^+ and Mg^{2+} concentrations might change as an animal develops diabetic hyperglycemia and ketonemia.
- Know why K^+ is usually administered with insulin when treating the uncontrolled diabetic animal.
- Recognize how HHS develops in diabetic animals.
- Understand why some animals with HHS are non-ketotic.
- Explain how and why severe dehydration and hypotension develops in the uncontrolled hyperglycemic diabetic animal.
- Know how the β-OH-butyrate: acetoacetate ratio is determined in diabetic ketoacidosis.

Questions

221. The ketoacidosis associated with acute insulin withdrawal:

a. Is associated with a reduction in cellular K^+ stores.
b. May lead to excessive urinary Na^+, K^+ and H_2O loss.
c. May lead to compensatory increases in the respiratory minute volume.
d. May lead to hypotension.
e. All of the above

222. Which one of the following is generally increased in acute insulin withdrawal?

a. Release of glucose into the circulation from skeletal muscle glycogen stores
b. Cerebral blood flow
c. Nucleoside uptake by muscle tissue
d. Hepatic glucose production
e. Adipocyte triglyceride deposition

223. Select the true statement below regarding ketone bodies and DM:

a. Hormone sensitive lipase catalyzes the hepatic conversion of acetoacetate to acetone.
b. In diabetic coma, there is usually more β-hydroxybutyrate than acetoacetate in blood.
c. Ketonemia leads to a progressive metabolic acidosis, with compensatory hypoventilation.
d. Excessive hepatic β-oxidation of fatty acids increases the hepatic mitochondrial NAD^+/NADH concentration ratio.
e. The characteristic sweet or fruity smell on the breath and in the urine of severely diabetic patients is due to the presence of acetoacetate.

224. Hyperosmolar Nonketotic Syndrome (HNKS):

a. May develop in diabetic patients when the liver remains partially responsive to insulin, and hepatic FFA β-oxidation is minimized.
b. Is exemplified in diabetic patients by a hyperglycemia, metabolic acidosis and a hyponatremia.
c. Is a form of hyperglycemic hyperosmolar syndrome (HHS) where the liver stops making ketone bodies and glucose.
d. Is exemplified in diabetic patients as a severe form of hypotonic dehydration.
e. All of the above

225. As the plasma glucose and ketone body concentrations begin to rise in diabetes:

a. The first dehydration that occurs is usually a tissue dehydration.
b. Extracellular K^+ and Mg^{2+} concentrations usually decline.
c. The kidneys stop reabsorbing them, and move these compounds into urine.
d. Cells begin to take-on water, and hypotension develops.
e. Hypercholesterolemia develops as ketone bodies are converted to cholesterol in circulating low-density lipoprotein (LDL).

Chapter 46

Objectives

- Describe the effects of insulin withdrawal on overall protein metabolism.
- Explain why a diabetic hyperglycemic animal may be either hypo- or hypernatremic.
- Know why K^+ and Mg^{2+}-containing alkalinizing solutions are used in diabetic therapy.
- Discuss the mechanisms driving hepatic gluconeogenesis in diabetic hyperglycemic animals.
- Recognize why the liver has trouble simultaneously synthesizing glucose and completely oxidizing acetyl-CoA through the TCA cycle.
- Understand why lipolysis is frequently exaggerated in the hyperglycemic, ketotic diabetic animal.
- Explain why cortisol is considered to be "anti-insulin."
- Show how insulin withdrawal affects carbohydrate, protein and lipid metabolism, and discuss how those affects might lead to coma and death if there is no rationale intervention.

Questions

226. Therapy for diabetic ketoacidosis should be aimed at:

a. Replacing lost electrolytes, particularly K^+.
b. Increasing the effective circulating volume (ECV).
c. Suppressing hepatic gluconeogenesis and ketogenesis.
d. Facilitating nutrient uptake by muscle cells, hepatocytes, and adipocytes.
e. All of the above

227. Urinary nitrogen excretion increases in diabetic hyperglycemic ketoacidosis because of:

a. Mobilization of depot fat.
b. Tissue hypoxia.
c. Increased protein catabolism.
d. Hypernatremia.
e. All of the above

228. The hyperglycemic, ketoacidemic diabetic animal will frequently exhibit:

a. A reduction in tissue K^+ and Mg^{2+} stores.
b. Hypoventilation in an attempt to reverse the initial left-shift in the bicarbonate buffer equation due to metabolic acidosis.
c. Increased lipogenesis in adipocytes.
d. All of the above
e. None of the above

229. In hypoglycemic shock, the hypothalamus appropriately responds by:

a. Activating the SNS, which in turn elevates circulating catecholamine levels and promotes pancreatic glucagon release.
b. Increasing CRH release, which stimulates adenohypophyseal ACTH secretion.
c. Activating the SNS, which in turn reduces endogenous insulin secretion.
d. Increasing GHRH output, which stimulates somatotropin release from the adenohypophysis.
e. All of the above

230. Select the <u>true</u> statement below:

a. Insulin, like cortisol and aldosterone, is not stored in secretory cells, but is synthesized and secreted upon demand.
b. β-Adrenergics and insulin both have a tendency to decrease the amount of K^+ entering muscle cells.
c. Arginine is thought to decrease feline pancreatic insulin release.
d. In DM, glucose forms fructosamines via nonenzymatic reactions driven solely by the elevated plasma glucose concentration.
e. Insulin activates adenyl cyclase in the plasma membrane of its target cells.

Chapter 47

Objectives

- Distinguish differences between neuroendocrine cells of the gut and enterochromaffin-like cells.
- Discuss what is meant by the gut being a visceral brain.
- Give examples of the endocrine, neurocrine and paracrine actions of somatostatin.
- Explain why some of the gut peptides are referred to as regulatory peptides rather than hormones.
- Know how and why gut endocrine cells can sense luminal contents.
- Recognize why certain GI regulatory peptides are segregated into families.
- Identify the second messenger systems activated by the GI regulatory peptides.
- Know which hormone was first discovered, and who discovered it.

Questions

231. Vasoactive intestinal polypeptide (VIP) is structurally similar to:

a. Secretin.
b. CCK.
c. Somatostatin.
d. Gastrin.
e. All of the above

232. Cells of the GI tract, lungs and CNS that manufacture biogenic amines in addition to polypeptides are known as:

a. Enterochromaffin cells.
b. Kupffer cells.
c APUD cells.
d. JG cells.
e. Glial cells.

233. Which one of the following digestive processes is <u>least</u> affected (directly) by GI hormones?

a. Secretion
b. Motility
c. Absorption
d. Blood flow
e. Excretion

234. Gut endocrine cells with a brush border facing the intestinal lumen may act as:

a. Mechanoreceptors.
b. Baroreceptors.
c. Osmoreceptors.
d. Chemoreceptors.
e. Thermoreceptors.

235. The secretin family of peptides works primarily through which second messenger system?

a. Map K
b. Jak-Stat
c. cAMP
d. cGMP
e. Ca^{2+}/DG

Chapter 48

Objectives

- Identify the pharmacologic actions of gastrin, and explain each of the physiologic actions.
- Describe the effects of hypergastrinemia (Ch. 51).
- Understand the differences between gastrin micro- and macroheterogeneity.
- Explain why CCK and gastrin exhibit different potencies in stimulating gastric acid, pancreatic enzyme secretion and gallbaldder contraction.
- Discuss differences between the cephalic and gastric phases of HCl secretion.
- Describe the roles played by ACh, somatostatin, luminal pH and GRP in gastrin release.
- Identify various primary and secondary factors controlling gastrin release.
- Explain why gastrin and calcitonin secretions are related (Chs. 16 and 17).

Questions

236. All of the following are stimuli for gastrin release, <u>except</u>:

a. Vagal activation.
b. Luminal carbohydrates and fats.
c. Stomach distention.
d. Products of protein digestion.
e. Ca^{2+} salt solutions.

237. Which one of the following does gastrin <u>decrease</u>?

a. Calcitonin release
b. Ileocecal sphincter pressure
c. Gastric mixing
d. Lower esophageal sphincter (LES) pressure
e. Pepsinogen secretion

238. In which of the following are gastrin-containing cells located?

a. Vagus nerve
b. Medulla oblongata
c. Gastric antrum
d. Large intestine
e. All of the above

239. In pharmacologic amounts, gastrin appears to increase all of the following, <u>except</u>:

a. Duodenal mucus secretion.
b. Gallbladder contraction.
c. Uterine contraction.
d. Gastric emptying.
e. Exocrine pancreatic secretion.

240. Gastrin:

a. Excess can cause gastric mucosal hyperplasia.
b. Stimulates histamine release from ECL cells in the fundic portion of the stomach.
c. Exhibits a circulating half-life of 15 min. or less.
d. Exists in plasma in both sulfated and unsulfated forms.
e. All of the above

Chapter 49

Objectives

- Explain how gastrin, ACh and histamine interact in bringing about gastric HCl secretion.
- Know why ACh and gastrin may not be synergistic in stimulating gastric HCl secretion.
- Describe how and why the gastric alkaline, and the pancreatic acid tides are created.
- Explain the mechanisms by which omeprazole, somatostatin, acetazolamide, cimetidine, atropine and proglumide reduce gastric HCl release.
- Understand why oral NSAID administration may cause hyperchlorhydria.
- Show how CCK exhibits both macro- and microheterogeneity.
- Recognize why CCK and gastrin exhibit overlapping actions, and why CCK might reduce gastric HCl release.
- Know how CCK release is controlled, and understand the primary and secondary actions of this hormone.

Questions

241. Gastrin:

a. Like ACh, acts to simulate HCl secretion through the cAMP messenger system.
b. Receptors on parietal cells can be competitively blocked by CCK.
c. Along with ACh, can inhibit histamine-stimulated acid release from gastric parietal cells.
d. Exerts its effects on gastric HCl secretion in a paracrine fashion.
e. Inhibits pepsinogen secretion.

242. PGE_2 affects the gastric parietal cell by:

a. Interacting with a membrane-bound G-protein to reduce adenyl cyclase activity.
b. Reducing H^+/K^+ ATPase activity.
c. Competitively blocking H_2 receptors.
d. Acting as a parasympathomimetic agent.
e. Inhibiting cAMP-dependent phosphodiesterase activity.

243. CCK:

a. And gastrin are confined to neurons.
b. Is thought to play a part in satiety.
c. Release from the duodenum is caused by fat and protein digestion products in the lumen.

d. Is synergistic with secretin, and causes gallbladder contraction as well as exocrine digestive enzyme release from the pancreas.
e. All of the above

244. Select the false statement below regarding gastric ECL cells:

a. ACh stimulates release of histamine from these cells.
b. Hypergastrinemia would be expected to produce ECL cell hyperplasia.
c. Gastrin stimulates release of histamine from these cells.
d. Prostaglandins inhibit histamine release from these cells.
e. These cells are not considered to be neuroendocrine cells of the gut.

245. The gastric alkaline tide:

a. Is normally offset by a quantitatively equal pancreatic and biliary acid tide.
b. Normally occurs before the acid tide.
c. Is dependent upon the activity of parietal carbonic anhydrase.
d. It an indirect reflection of how much HCl is being produced by the stomach.
e. All of the above

Chapter 50

Objectives

- Explain why secretin is sometimes referred to as "nature's antacid," and why it is considered to be a part of the glucagon family.
- Review factors controlling secretin release, and identify its primary and secondary actions.
- Identify 17 GI peptides, as well as their major physiologic actions.
- Draw a correlation between the actions of uroguanylin and the natriuretic peptides (Ch. 31).
- Identify various GI peptides that theoretically help to regulate feeding behavior.
- Note which hormone was the first discovered.
- Explain why GIP has two names.
- Draw mechanistic correlations between secretin-stimulated $NaHCO_3$ secretion, and gastrin-stimulated HCl secretion.

Questions

246. Secretin:

a. Is a member of the structural CCK/gastrin family.
b. Is primarily a neurocrine agent.
c. Stimulates release of gastrin and CCK.
d. Release from the duodenum (into blood) is inhibited by a luminal pH above 4.5.
e. Is absent from brain tissue.

247. Which one of the following is known as the "interdigestive housekeeper," and why?

a. Gastrin, because in relaxes the ileocecal sphincter, thus prompting the urge to defecate following a meal.
b. GRP, for grab, recycle and process.
c. Motilin, because it sweeps leftover luminal contents down the gut between meals.
d. Uroguanylin, because it cleans and waters the small intestinal mucosa between meals, and stimulates urination.
e. Substance P, for the "pain" of pick-up and polish.

248. Which of the following promote insulin release in an anticipatory fashion?

a. GLP and GIP.
b. CCK and Gastrin.
c. Neurotensin and GRP.
d. Pancreatic polypeptide and ACTH.
e. Glucagon and VIP.

249. Which GI hormone stimulates lipogenesis?

a. Neurotensin
b. Insulin
c. GLP
d. GRP
e. CCK

250. Which one of the following is considered to be the most "inhibitory" GI peptide?

a. Glucagon
b. Somatostatin
c. Secretin
d. VIP
e. GIP

Chapter 51

Objectives

- Explain why identical peptides are synthesized, stored and released by gut epithelial cells, neurons of the GI tract, and nerve cells in the CNS.
- Predict the symptoms of a patient with a VIPoma.
- Differentiate between paraendocrine and orthoendocrine syndromes.
- Identify the gastrin source in patients with Zollinger-Ellison-Like Syndrome.
- Understand why patients with hypergastrinemia may have steatorrhea, ECL cell hyperplasia, and regenerative anemia.
- Explain the signs and symptoms of insulinoma, and discuss potential reasons for irreversible brain damage.

Questions

251. Insulinomas:

a. Are most always benign.
b. Occur more often in cats than in dogs.
c. Seem to affect kidney function more than neural function.
d. May cause hypophosphatemia and hypokalemia.
e. May cause hyperchlorhydria.

252. Hypergastrinemia:

a. Can be caused by hypersecreting Δ_1 cells of pancreatic islets.
b. Can lead to gastroduodenal ulceration.
c. May be perpetuated by duodenal secretin release in Zollinger-Ellison-like syndrome.
d. May lead to hematochezia.
e. All of the above

253. Orthoendocrine syndromes:

a. Are caused by APUDomas that secrete peptides foreign to their cell of origin.
b. Are more common in veterinary medicine than paraendocrine syndromes.
c. Are caused by non-endocrine-secreting tumors of the brain.
d. Include such conditions as Cushing's-like syndrome.
e. Are caused by steroid-secreting tumors that most often affect bone.

254. Select the false statement below:

a. APUD cells of the gut appear to be similar to several neuroendocrine cells of the brain.
b. Domestic pet ferrets in the U.S. are known to develop insulinomas.
c. Most gastrin circulating in the blood of normal adult animals originates from G cells in the gastric antral mucosa.
d. Animals with insulinomas would be expected to be hyperkalemic.
e. GI endocrine cells were first described by Pearse in 1966.

Chapter 52

Objectives

- Identify animal species where spontaneous ovulation occurs, and also those where reflex ovulation occurs.
- Differentiate the seasonal from the nonseasonal breeders.
- Know why some animals are classified as being pseudopolyestrous.
- Explain the physiological manifestations of estrus, metestrus, diestrus, proestrus and anestrus.
- Discuss divisions of the estrous in relation to corpus luteum development, activity, and demise.
- Recognize why proestrus is rather long in menstruating species, yet short in non-menstruating species.
- Know the causes of pseudopregnancy in rabbits, rodents, bitches and queens.
- Understand neural and endocrine involvements in reflex ovulators.

Questions

255. In which one of the following species is reflex ovulation most likely?

a. Primate
b. Chinchilla
c. Buffalo
d. Rabbit
e. Mouse

256. The period of the estrous cycle in which the influence of luteal progesterone predominates is called:

a. Estrus.
b. Proestrus.
c. Diestrus.
d. Anestrus.
e. None of the above

257. Canine pseudopregnancy may be characterized by:

a. Uterine enlargement.
b. The mammary glands developing lactational capabilities.
c. The bitch developing a whelping nest.
d. The bitch exhibiting an exaggerated diestrual response.
e. All of the above

258. Which one of the following is a polyestrous, nonseasonal breeder?

a. Mare
b. Sow
c. Ewe
d. Bitch
e. Queen

259. In which of the following settings might anestrus occur?

a. During pregnancy in polyestrous species
b. Infectious disease
c. In polyestrous species during the nonbreeding season
d. During lactation
e. All of the above

260. Select the false statement below:

a. The first day of "heat" is also the first day of the estrous cycle.
b. Ovulation occurs during estrus in all domestic animal species.
c. Proestrus is normally shorter than diestrus in the porcine estrous cycle.
d. Metestrus is not recognized in some domestic animal species.
e. LH promotes the ovulatory process in induced ovulators.

Chapter 53

Objectives

- Outline the effects of the gonadotropins on reproductive physiology.
- Distinguish between gonadotropin pulse frequencies and surges.
- Show how LH, estrogen and progesterone blood levels differ from each other throughout the estrous cycle.
- Discuss how the estrous cycle of the ewe differs from that of the queen and bitch.
- Show how interestrous intervals differ among different canine breeds.
- Explain why hormonal characteristics of the feline estrous cycle are influenced greatly by mating.
- Understand what controls CL regression in the ewe, queen and bitch.

Questions

261. Regression of the canine corpus luteum (CL) appears to be due to:

a. $PGF_{2\alpha}$.
b. LH.
c. Aging.
d. Progesterone.
e. Estradiol.

262. Mating behavior, ovulation and initial growth of the CL are dependent upon the presence of:

a. GH.
b. LH.
c. PRL.
d. $PGF_{2\alpha}$.
e. All of the above

263. Blood levels of progesterone in nonpregnant animals are derived largely from the:

a. Adrenal glands.
b. Adenohypophysis.
c. Mammary glands.
d. Corpus luteum.
e. Uterine endometrium.

264. The highest levels of estrogen during the estrous cycle are found:

a. During proestrus.
b. Immediately following ovulation.
c. During the luteal period.
d. During diestrus.
e. None of the above

265. Which one of the following is characteristic of the ovine estrous cycle?

a. Blood estradiol levels are normally higher during diestrus than during proestrus.
b. Ovulation normally occurs during estrus.
c. $PGF_{2\alpha}$ causes CL regression.
d. It occurs throughout the year (i.e., sheep are non-seasonal breeders).
e. The uterine endometrium is the main source of progesterone during the post-ovulatory period.

266. The bitch:

a. Unlike farm animals, may continue to accept the male for mating several days after ovulation.
b. Exhibits estrogen "waves," like the ewe, during the luteal phase of the estrous cycle.
c. Exhibits a luteal phase to her estrous cycle that is approximately the same length as proestrus.
d. Normally ovulates during estrus.
e. All of the above

267. The queen:

a. Is a spontaneous ovulator.
b. Comes into puberty at about 2 months of age.
c. Exhibits a rise in blood levels of progesterone during nonovulatory estruses.
d. Exhibits an LH surge and the beginning of estrus when estradiol levels peak at the end of proestrus.
e. All of the above

Chapter 54

Objectives

- Know the duration of bovine estrus, and when ovulation occurs in relation to estrus.
- Describe the luteolytic cascade that develops between endometrial $PGF_{2\alpha}$ and luteal oxytocin in the absence of an implanted embryo.
- Indicate how and why FSH, LH, estradiol and progesterone blood concentrations change throughout the bovine estrous cycle.
- Explain why only 2 (rather than 4) phases of the equine estrous cycle are routinely recognized during the breeding season.
- Compare LH blood levels in the mare to those of the cow in relation to estrus and ovulation.
- Explain what is meant by "foal heat" in mares, and "silent heat" in cows.
- Compare blood levels of progesterone in relation to estrus in rodents and the mare.
- Identify the role of the PRL surge during the rodent estrous cycle.

Questions

268. Sexual receptivity (i.e., estrus) in the cow normally lasts about:

a. 8 minutes.
b. 18 minutes.
c. 8 hours.
d. 18 hours.
e. 8 days.

269. During the bovine estrous cycle, $PGF_{2\alpha}$:

a. Promotes luteal oxytocin release.
b. Is released from the uterine endometrium in the absence of an implanted embryo, and causes demise of the CL.
c. Gains access to the ovarian artery through the utero-ovarian vein.
d. Production in the uterine endometrium is stimulated by oxytocin.
e. All of the above

270. Gestation is normally prolonged by:

a. Progesterone.
b. Estrogen.
c. Prolactin
d. LH.
e. Prostaglandins.

271. Which hormone in the mare is best associated with rejection of the stallion?

a. Estrogen
b. LH
c. Progesterone
d. FSH
e. Prolactin

272. The fertile estrus of the cow that occurs about 21 days postpartum is referred to as:

a. Foal heat.
b. Anovulatory estrus.
c. Silent heat.
d. Seasonal anestrus.
e. None of the above

273. Diestrus 1 in rodents is:

a. Proestrus.
b. Estrus.
c. Metestrus.
d. Anestrus.
e. Pseudoestrus.

274. All of the following hormones peak in the blood of rodents during proestrus, <u>except</u>:

a. Estradiol.
b. LH & FSH.
c. Progesterone.
d. Oxytocin.
e. Prolactin.

275. Which hormone below is thought to maintain luteal progesterone production in rodents following copulation?

a. Estradiol.
b. Prolactin.
c. LH.
d. GnRH.
e. Oxytocin.

Chapter 55

Objectives

- Compare the 4 phases of the primate menstrual cycle to proestrus, estrus, metestrus and diestrus in terms of timing, as well as estradiol, gonadotropin and progesterone blood levels.
- Show how plasma concentrations of GnRH, LH, FSH, 17β-estradiol, progesterone, 17-OH-progesterone, androstenedione, testosterone, inhibin A and inhibin B change throughout the human menstrual cycle.
- Explain why the basal body temperature rises (slightly) during the luteal phase of the menstrual cycle.
- Recognize the sources and functions of inhibin A and inhibin B.
- Contrast the control of luteolysis in the primate to that in the cow.
- Identify the source and function of hCG.
- Explain why menopause is recognized in women, but not in domestic animals.

Questions

276. Which one of the following hormones generally increases in the circulation following the onset of menopause?

a. Estrogen
b. Progesterone
c. Inhibin A
d. FSH
e. None of the above

277. Which phase of the menstrual cycle has the most consistent length?

a. Follicular phase
b. Luteal phase
c. Menses
d. Proliferative phase
e. Proestrus

278. Androstenedione and testosterone levels rise modestly during the proliferative phase of the menstrual cycle because of:

a. Aromatization of these androgens by ovarian granulosa cells.
b. Gonadotropin stimulation of adrenal androgen secretion.
c. Inhibin B stimulation of ovarian granulosa cell secretion.
d. $PGF_{2\alpha}$ stimulation of luteal secretion.
e. None of the above

279. The core body temperature rises slightly following ovulation because of the thermogenic activity of:

a. LH.
b. Estrogen.
c. Progesterone.
d. FSH.
e. GnRH.

280. Gonadotropin output is normally suppressed throughout the luteal phase of the menstrual cycle due to the presence of:

a. Inhibin A, 17β-Estradiol, and Progesterone.
b. Inhibin B, 17-OH-Progesterone, and $PGF_{2\alpha}$.
c. Inhibin A, Testosterone, and 17β-Estradiol.
d. Inhibin B, Progesterone, and Testosterone.
e. Inhibin B, 17β-Estradiol, and Progesterone.

281. Luteolysis in primates appears to be due to the combined action of:

a. Oxytocin and Prolactin.
b. ED-1 and Oxytocin.
c. $PGF_{2\alpha}$ and ED-1.
d. Prolactin and $PGF_{2\alpha}$.
e. Oxytocin and $PGF_{2\alpha}$.

282. Human chorionic gonadotropin is similar in structure and function to:

a. GnRH.
b. LH.
c. FSH.
d. Prolactin.
e. Inhibin.

Chapter 56

Objectives

- Recognize the difference between "overt" and "covert" menstruation.
- Identify the physiologic cause of menstruation.
- Explain why menstrual blood is normally clot-free.
- Discuss the meaning of menstrual leukorrhea.
- Identify the stimulus for "uterine milk" secretion.
- Compare uterine arterial and glandular development during the estrogen dominant preovulatory period to that occurring during the postovulatory period.
- Identify factors controlling the hypothalamic GnRH pulse frequency.
- Explain why it is believed that the ovaries control the basic rhythm of the hypothalamic GnRH pulse generator.

Questions

283. Which area of the endometrium is usually conserved during menstruation?

a. Stratum basale
b. Superficial layer
c. Stratum functionale
d. Epithelium
e. None of the above

284. Blood shed from the uterus of the cow during metestrus is secondary to:

a. Overt menstruation.
b. Estrogen and progesterone withdrawal.
c. Intensive endometrial stimulation by estrogen during proestrus and estrus.
d. $PGF_{2\alpha}$ and ED-1 stimulation.
e. None of the above

285. The uterus is resistant to infection during menstruation because of:

a. Fibrinolysin.
b. $PGF_{2\alpha}$.
c. Estrogen-progesterone withdrawal.
d. Menstrual leukorrhea.
e. None of the above.

286. If large amounts of GnRH are administered by constant infusion:

a. LH secretion ceases because of down-regulation of adenohypophyseal GnRH receptors.
b. Multiple ovulations occur.
c. LH and FSH secretion substantially increase.
d. Menstruation is initiated.
e. None of the above

287. GnRH release is inhibited by:

a. Dopamine
b. Preovulatory estrogen
c. Norepinephrine
d. Acetylcholine
e. None of the above

288. Which of the following increase(s) the discharge frequency of the GnRH pulse generator?

a. LH
b. Estrogen in the absence of progesterone
c. Estrogen and progesterone
d. Progesterone in the absence of estrogen
e. Ovulation

Chapter 57

Objectives

- Identify the roles played by LH and FSH in spermatogenesis and ovulation.
- Outline interactions between the hypothalamus, pituitary, Leydig cells, peritubular Myoid cells, and Sertoli cells in the process of spermatogenesis.
- Explain similarities and differences between ABP and sex hormone binding protein (SHBP) (Ch. 58).
- Explain the autocrine, paracrine and endocrine roles of the inhibins and activins.
- Identify testicular-derived and ovarian-derived factors that play important roles in spermatogenesis and ovulation.
- Show how ovarian theca interna cells function similarly to testicular Leydig cells, and how ovarian granulosa cells function similarly to testicular Sertoli cells.
- Identify the sources of pre- and postovulatory estrogen, as well as androstenedione, testosterone and progesterone.

Questions

289. Inhibin A:

a. Secretion parallels that of progesterone in females.
b. Is produced by ovarian granulosa cells, and testicular Sertoli cells.
c. Is a glycoprotein.
d. Inhibits FSH secretion in both males and females.
e. All of the above

290. Testicular testosterone secretion is stimulated by:

a. FSH
b. Activin
c. Inhibin
d. Estrogen
e. PmodS

291. Sertoli cells:

a. Are biochemically similar to ovarian granulosa cells.
b. Produce ABP under PmodS and FSH stimulation.
c. Are capable of aromatizing testosterone to estrogen.
d. Produce inhibin, which decreases FSH output from the adenohypophysis.
e. All of the above

292. Testicular androgen-binding protein (ABP):

a. Originates from Sertoli cells.
b. Release is stimulated by LH.
c. Is needed in order to bind activin for spermatogenesis.
d. Stimulates testosterone synthesis in Leydig cells.
e. All of the above

293. Which of the following occurs during the preovulatory, proliferative phase of the estrous cycle?

a. Ovarian theca cells primarily produce estrogen.
b. Ovarian granulosa cells are stimulated by LH to produce androgens.
c. Estrogen synergizes with FSH to promote replication of ovarian granulosa cells.
d. Estrogen is aromatized in ovarian theca cells to produce androgens.
e. All of the above

294. Theca externa cells are also known as:

a. Leydig cells.
b. Granulosa cells.
c. Interstitial cells.
d. Follicular myoid cells.
e. Oocytes.

Chapter 58

Objectives

- Discuss differences between patterns of cyclic and pulsatile gonadotropin secretion.
- Identify the inactive, conjugated 17-ketosteroid metabolites of testosterone that are produced by the liver.
- Describe the affinity of SHBP for testosterone, estrogen, progesterone, and cortisol.
- Distinguish anatomical and functional differences between the seminiferous tubules and interstitial tissues of the testes.

- Know what is typically contained in testicular lymph.
- Understand what the blood-testis barrier is composed of.
- Recognize what the function of the rete testis is.
- Know which domestic animal species possess seminal vesicles, a bulbourethral and/or prostate gland.
- Define and explain BPH, and site its connection to PSA.

Questions

295. Which of the following can be produced from testosterone?

a. Estrogen
b. DHT
c. Etiocholanolone
d. Androsterone
e. All of the above

296. The major plasma protein to which testosterone is bound is:

a. ABP.
b. CBP.
c. SSBG.
d. Albumin
e. TBG.

297. 80-90% of testicular mass is:

a. Lymphatic tissue.
b. Rete testes.
c. Interstitial tissue.
d. Seminiferous tubules.
e. Leydig cells.

298. Forward motility protein is produced by the:

a. Sertoli cells.
b. Vas deferens.
c. Epididymis.
d. Seminal vesicles.
e. Prostate.

299. Benign prostatic hyperplasia is recognized in:

a. Horses.
b. Goats.
c. Cats.
d. Sheep.
e. Dogs.

Chapter 59

Objectives

- Outline the biosynthetic pathways for progesterone, testosterone, and estradiol.
- Differentiate between sex steroid anabolic and adrogenic activity.
- Describe and compare the primary physiologic actions of testosterone, estrogen, and progesterone.
- Explain differences between estrogen and progesterone on oxytocin receptor synthesis and endometrial cyclooxygenase activity.
- Know why estrogens have been used for the treatment of incontinence.
- Compare the effects of estrogen and progesterone on plasma protein biosynthesis in the liver.
- Know the differences and affects of phytoestrogens and proestrogens on ruminant animals.
- Explain why the catechol estrogens are antiestrogenic.
- Understand how epostane and mifepristone exert their antiprogestational activities.
- Contrast the plasma protein binding of estrogen to progesterone.
- Recognize the contrasting physiologic activities of estrogen and progesterone.

Questions

300. Aplastic anemia could be due to excessive administration of:

a. Testosterone.
b. Estrogen.
c. Progesterone.
d. Erythropoietin.
e. Pregnenolone.

301. Which one of the following enzymes, blocked by Epostane, helps to convert pregnenolone to progesterone?

a. 20,22-Desmolase
b. 3β-Hydroxysteroid Dehydrogenase
c. 17α-Hydroxylase
d. Aromatase
e. 17-Ketoreductase

302. Dihydrotestosterone (DHT):

a. Can be formed from estradiol through the activity of 5α-reductase.
b. Circulating in blood primarily derives from testicular secretion.
c. Is formed in the adrenal glands through the action of 17-ketoreductase on dehydroepiandrosterone.
d. Circulating in blood primarily derives from peripheral conversion of its precursor (testosterone).
e. Can be converted to estradiol through the action of 5α-reductase.

303. Catechol estrogens:

a. Resemble catecholamine neurotransmitters.
b. Can be produced from estrogen in the hypothalamus.
c. Are produced through the action of 2-hydroxylase on either estrone or estradiol.
d. Block estrogen receptors in the CNS.
e. All of the above

304. Progesterone is normally 45% bound in plasma to:

a. The same protein that binds most of the estrogen.
b. Albumin.
c. The same protein that binds cortisol.
d. Sex steroid binding globulin.
e. Prothrombin.

305. Progesterone:

a. Inhibits the hypothalamic pulse generator in mares during the luteal phase of the estrous cycle.
b. Usually requires a period of "estrogen priming" before target cells fully respond to its presence.
c. Exhibits antiestrogenic effects on the uterus.

d. Stimulates development of lobules and alveoli in mammary glands.
e. All of the above

Chapter 60

Objectives

- Explain why the pineal gland of mammals is considered to be a neuroendocrine transducer organ.
- Identify the primary translators of photoperiod in mammals.
- Contrast the effects of melatonin on reproductive activity in sheep and horses.
- Describe the roles of light and the sympathetic nervous system on SNAT and HIOMT activity.
- Discuss the role of AVT in the pineal gland.
- Show the biosynthetic pathway for melatonin, and identify the rate-limiting enzymes.
- Explain how light striking the retina can ultimately affect pineal gland activity.

Questions

306. The suprachiasmatic nucleus (SCN) of the hypothalamus:

a. Reduces activity of sympathetic fibers to the pineal gland in the presence of light.
b. Increases activity of sympathetic fibers to the pineal gland in the presence of light.
c. Synthesizes melatonin.
d. Reduces activity of sympathetic fibers to the pineal gland in the absence of light.
e. None of the above

307. Which one of the following amino acids is used in the biosynthesis of melatonin?

a. Alanine
b. Tyrosine
c. Serine
d. Tryptophan
e. Methionine

308. Which one of the following (dark-adapted) enzymes converts N-acetylserotonin to melatonin in the pineal gland?

a. Tryptophan hydroxylase
b. Aromatic amino acid decarboxylase
c. Serotonin-N-acetyltransferase
d. Hydroxyindole-O-methyltransferase
e. None of the above

309. The pineal gland is found in all vertebrate groups, <u>except</u>:

a. Birds.
b. Frogs.
c. Carnivores.
d. Ruminants.
e. Crocodilians.

Chapter 61

Objectives

- Identify the way in which MAO activity influences pineal function.
- Describe the effects of "stress" on pineal activity, and thus reproductive function.
- Explain how thyroid function is affected by changes in photoperiod.
- Know why long photoperiods are correlated with increased PRL secretion in ruminant ungulates.
- Understand hypothetical relationships between pineal gland function and biologic aging.
- Discuss how melatonin is metabolized and eliminated from the body.
- Identify extrapineal sources and functions of melatonin (Ch. 72).
- Contrast the effects of catecholamines on avian and mammalian melatonin release.

Questions

310. Melatonin:

a. Stimulates brown hair growth in mice.
b. Excess is a cause of parathyroid hypertrophy.
c. Is synthesized only in the pineal gland of reptiles and birds.
d. Secretion is enhanced in response to stress.
e. Is antigonadal in all mammalian species studied to date.

311. Pinealocyte melatonin secretion would by highest during?

a. The preovulatory estrogen surge
b. Extended periods of daylight in long-day breeders.
c. The breeding season in sheep and goats (short-day breeders).
d. Periods when HIOMT activity is low.
e. Periods when MAO activity is elevated.

312. Which of the following statements is/are correct regarding theoretical relationships between melatonin and aging?

a. Melatonin appears to reduce free radical formation.
b. Blood melatonin levels decrease with age.
c. Melatonin appears to enhance immune surveillance.
d. Activity of the SCN is age-related, thus influencing pineal output of melatonin.
e. All of the above

313. Melatonin is thought to stimulate hypothalamic release of which one of the following in cats?

a. DA
b. GHRH
c. GnRH
d. TRH
e. CRH

314. Pineal function is affected by:

a. Stress.
b. Light.
c. Hypophysectomy.
d. Steroid hormones.
e. All of the above

315. Melatonin appears to influence all of the following in birds, except:

a. Gonadal function.
b. Lactation.
c. Thermoregulation.
d. Locomotor activity.
e. Adrenal steroidogenesis.

Chapter 62

Objectives

- Explain how animals "know" they are pregnant during the preplacentation phase.
- Identify the hormones that prolong CL function.
- Know the functions of PL-1 and PL-11 in rodents.
- Explain why PL, GH and PRL exert similar physiologic actions.
- Identify the sources and actions of relaxin in primates and domestic animals species.
- Recognize which hormones can be produced by the placenta.
- Identify species that produce PL, as well as those that produce CG.
- Summarize the physiologic actions of PL during pregnancy.

Questions

316. Which pituitary hormone is thought to function in place of placental lactogen in pregnant dogs, rabbits and pigs?

a. GH
b. ACTH
c. LH
d. PRL
e. FSH

317. Relaxin:

a. Has GnRH-like properties.
b. Is found in females, but not males.
c. Has vasoconstrictive properties.
d. Is an insulin-like polypeptide.
e. All of the above

318. Placental lactogen (PL):

a. Shares a common amino acid sequence with PRL and GH.
b. Is normally found in high concentrations in the fetal circulation.
c. Stimulates pituitary gonadotropin release during pregnancy.
d. Increases maternal responsiveness to insulin.
e. Suppresses maternal erythropoiesis.

319. The placenta:

a. Produces protein, but not steroid hormones.
b. Produces and secretes PL in direct proportion to its size and metabolic activity.
c. Is the sole source of sex steroid biosynthesis during pregnancy.
d. Produces steroid, but not protein hormones.
e. Is known to produce GnRH, which appears to reduce chorionic gonadotropin output.

320. Primate trophoblastic cells secrete which one of the following to maintain CL function?

a. FSH
b. GnRH
c. CG
d. IGF-1
e. PMSG

321. The CL of rodents is maintained throughout pregnancy by:

a. PL-II and GH.
b. PRL and PL-II.
c. GH and PL-I.
d. GH and PRL.
e. PL-I and PRL.

Chapter 63

Objectives

- Explain how the fetoplacental unit participates in estrogen biosynthesis.
- Recognize which substrates cross the placenta for use in fetal development and energy production.
- Identify which maternal hormones are capable of crossing the placenta.
- Discuss species differences in placental 17α-hydroxylase presence/activity, and recognize how the placental progesterone: estrogen ratio is controlled in all domestic animal species.
- Understand why maternal urinary estrogen excretion can be an index of fetal well-being.
- Recognize the testicular evocators that participate in male sexual differentiation.
- Note how and why intersexuality sometimes occurs in cattle.

Questions

322. Testicular differentiation factor (TDF):

a. Is a protein also referred to as Müllerian duct-inhibiting factor.
b. Directs differentiation of Sertoli cells.
c. Is carried on the X-chromosome.
d. Causes the Wolffian duct to develop into a penis, urethra, prostate and scrotum.
e. All of the above

323. Which one of the following maternal hormones is least likely to cross the placenta?

a. Estrogen
b. Epinephrine
c. Cortisol
d. Growth hormone
e. Aldosterone

324. The fetus utilizes which of the following fuels as major metabolic substrates?

a. Free fatty acids, vitamins and amino acids
b. Proteins, triglycerides and phospholipids
c. Amino acids, lactate and glucose
d. Ketone bodies, mineral and lipoproteins
e. Steroids, complex carbohydrates and apolipoproteins

325. The canine placenta synthesizes:

a. 17-Hydroxypregnenolone.
b. DHEA from progesterone.
c. Equilenin.
d. Progesterone.
e. All of the above

326. The absence of estrogen conjugates in maternal urine indicates:

a. Normal pregnancy.
b. Liver dysfunction.
c. Placental dysfunction.
d. Renal shutdown.
e. Fetal death.

327. 17α-Hydroxylase activity in the placenta of the ewe is stimulated by:

a. Maternal estrogen.
b. Placental progesterone.
c. Fetal aldosterone.
d. Placental lactogen.
e. Fetal cortisol.

328. Estrogen is normally tightly-bound in the fetal circulation by:

a. Cortisol binding globulin (CBG).
b. α-Fetoprotein.
c. Albumin.
d. Androgen binding protein.
e. TBG.

329. Estrogen precursors in animals used to finalize estrogen biosynthesis in the placenta are thought to derive from:

a. The maternal pituitary.
b. The maternal liver.
c. The fetal adrenal glands and/or gonads.
d. The fetal liver.
e. The maternal adrenal gland and/or ovaries.

Chapter 64

Objectives

- Recognize which compounds are actively transported across the placenta (from the maternal to fetal circulation), which cross by facilitated diffusion, and which compounds move from the fetal to maternal circulation by simple diffusion.
- List the physiologically relevant hormones that are known to exist in the fetal circulation.
- Understand the basic elements of the fetal/neonatal stress response, and why it is essential for survival.
- Recognize the period (first, second or third trimester) of accentuated fetal development.
- Understand why placental size and development correlates directly with fetal development.
- Know why the last half of gestation is associated with a fetal hyperthyroid state.
- Summarize the importance of PTH_{rp} to the fetus.
- Explain why a hyperglycemic, diabetic dam might give birth to diabetic offspring.
- Explain why the fetal adrenal gland differs anatomically and functionally from the adult gland.

Questions

330. Select the <u>true</u> statement below:

a. Diabetic mothers generally give birth to unusually small fetuses.
b. Fetal hypercalcemia stimulates PTH_{rp} release.
c. Fetal catecholamine release from the adrenal medulla is critical during parturition.
d. Relative fetal hypothyroidism is a normal state during the third trimester.
e. Dihydrotestosterone (DHT) is a metabolic breakdown product of testosterone, and has little, if any, biologic activity.

331. Which of the following is/are thought to be actively transported across the placenta (from maternal to fetal blood)?

a. Ca^{2+}
b. Fe^{3+}
c. PO_4^{3-}
d. Water-soluble vitamins
e. All of the above

332. All of the following stimulate hepatic somatomedin release, <u>except</u>:

a. ACTH.
b. GH.
c. PL.
d. PRL.
e. Insulin.

333. Select the <u>false</u> statement below:

a. Because little placental transfer of thyroid hormones occurs, most T_4 found in fetal blood is thought to have originated in the fetus.
b. Characteristic anterior pituitary cell types are discernible in the fetus during the first trimester.
c. The fetal gonads of horses and rodents participate in fetoplacental estrogen biosynthesis.
d. Maternal estrogen helps direct maturation of the fetal adrenal cortex.
e. During the second trimester, ADH is demonstrable in fetal neurohypophyseal tissue.

334. Which one of the following normally has a diffusion gradient from fetal to maternal blood?

a. Creatinine
b. O_2
c. Glycerol
d. Glucose
e. Acetoacetate (a ketone body)

Chapter 65

Objectives

- Discuss the involvement of the maternal anterior pituitary in maintaining pregnancy.
- Explain why maternal estrogen levels usually rise and progesterone levels fall immediately before the onset of parturition.
- Know why serum testosterone levels rise during pregnancy, and DHEA levels decline.

- Understand why fetal death has little influence on maternal progesterone levels, and why fetal retention may occur.
- Recognize why maternal plasma aldosterone and angiotensinogen concentrations increase during pregnancy, yet few signs of hyperaldosteronism appear.
- Explain why maternal cortisol concentrations increase in blood during pregnancy, yet adrenal cortisol production decreases.
- Know why the maternal thyroid gland may be palpably enlarged during pregnancy.
- Explain why the first two trimesters of pregnancy are generally hyper-insulinemic, yet the third trimester is normally a diabetogenic experience for the dam.

Questions

335. Elevated plasma cortisol levels in the dam are largely a result of:

a. Enhanced adrenal biosynthesis.
b. Decreased hepatorenal clearance.
c. Enhanced renin substrate availability.
d. Enhanced pituitary ACTH output.
e. Increased circulating transcortin levels.

336. Secretion of which maternal anterior pituitary hormone may be significantly increased during the third trimester of pregnancy?

a. LH
b. GH
c. PRL
d. ACTH
e. TSH

337. Which one of the following is normally decreased during pregnancy (compared to the nonpregnant state)?

a. Serum progesterone levels
b. Serum aldosterone levels
c. Total serum thyroxine levels
d. Arterial blood pressure
e. Serum GH levels

338. During pregnancy, urinary 17-OH-progesterone levels are a check on:

a. Placental function.
b. CL activity.
c. Fetal well-being.
d. Maternal adrenal activity.
e. Maternal ovarian function.

339. Which one of the following stimulates maternal hepatic synthesis of steroid-binding globulins, fibrinogen, thyroid binding globulin, and angiotensinogen?

a. Estrogen
b. Progesterone
c. Thyroxine
d. Prolactin
e. Insulin

Chapter 66

Objectives

- Explain why the blood pressure is normally low during pregnancy, yet cardiac output increases.
- Know potential causes of preeclampsia.
- Recognize why RBC mass normally increases during pregnancy, yet the Hct. may decrease.
- Understand why gastroesophageal reflux and constipation may occur during pregnancy.
- Explain why pulmonary tidal volume and respiratory minute volume steadily rise during pregnancy, yet vital capacity remains unchanged.
- Indicate why ECF volume and the GFR increase during pregnancy.
- Explain why serum electrolyte concentrations normally remain unaffected throughout pregnancy.

Questions

340. Which of the following normally decrease(s) during pregnancy?

a. RBC mass
b. Plasma osmolarity
c. Hematocrit
d. Blood volume
e. All of the above

341. The respiratory tidal volume (V_T), which is thought to rise throughout pregnancy, is:

a. Equal to the difference between FRC and ERV.
b. Driven by relatively lower Pco_2 and higher Po_2 levels throughout pregnancy.
c. Equal to the difference between IRV and FRC.
d. Equal to the sum of RV, ERV and IRV.
e. None of the above

342. Which one of the following parameters remains largely unchanged throughout pregnancy?

a. Urine formation
b. Na^+ concentration of plasma
c. Renal blood flow
d. Plasma fibrinogen levels
e. Heart rate

343. Which of the following physiologic factors serve(s) to lower arterial blood pressure during pregnancy?

a. Low placental vascular resistance
b. Progesterone-stimulated β-adrenergic receptor biosynthesis
c. Placental arteriovenous shunts
d. Increased placental endothelial synthesis of vascular relaxation factors
e. All of the above

344. Which of the following normally increases during pregnancy?

a. Respiratory residual volume
b. Gastric emptying
c. Expiratory reserve volume
d. GI motility
e. Respiratory minute volume

Chapter 67

Objectives

- Describe the various anatomical arrangements of mammary glands between species.
- Indicate how blood and lymph flow in the mammary gland compares to other areas of the body.
- Discuss endocrine control of lobuloalveolar and ductular development during pubertal, pregnant and lactational stages of life.
- Discuss endocrine control of lactogenesis and milk ejection.
- Identify the hormones and growth factors present in milk.
- Show how colostrum differs from milk secreted during the later stages of lactation.
- Identify those species to which colostrum is most important for survival.
- Explain why lactation is "metabolically expensive," and show how the kidneys assist in maintaining physiologic stability during lactation.
- Identify structural and functional similarities between ADH and oxytocin.

Questions

345. Select the false statement below:

a. During pubertal development, progesterone is primarily responsible for mammary gland ductular growth, while estrogen stimulates lobuloalveolar growth.
b. Valvular incompetence sometimes develops in mammary gland veins during lactation.
c. The cow udder exhibits 4 mammary glands with independent lobular systems.
d. Adrenal steroidogenesis is essential for the maintenance of lactation.
e. Secretory alveolar cells of mammary glands possess insulin receptors.

346. Which of the following is/are normally identifiable in breast milk?

a. PRL
b. GH
c. IGF-1
d. PTH_{rp}
e. All of the above

347. Select the true statement below:

a. Prolactin stimulates milk ejection.
b. Mammary glands are essentially modified lymphatic structures.
c. The pH of milk is normally about 8.6.
d. Androgens suppress mammary gland growth.
e. Mammary glands secure virtually all of their secretory fats, proteins and carbohydrates from blood.

348. Immunoglobulins:

a. In colostrum are produced by mammary gland plasma cells.
b. Cross the canine placenta (from dam to fetus) with ease, but not the equine placenta.
c. Cross the neonatal GI tract intact (for a short period of time following birth).
d. Protect the neonate against infection while its own immune system develops.
e. All of the above

Chapter 68

Objectives

- Identify and discuss the control of PRL release.
- Explain why bitches with primary hypothyroidism might develop galactorrhea.
- Outline the relationship between PRL and maternal behavior.
- Recognize the relationships between PRL, CRH, ACTH, and cortisol, and explain why lactation is considered to be a stress hyporesponsive state.
- Understand the role PRL plays in the immediate postcoital state.
- Describe the reciprocal relationships that exist between PRL and Oxy, and between ADH and PRL.
- Discuss the effects of hyperprolactinemia on male and female reproductive function.
- Identify the effects of PRL in birds.
- Identify the osmoregulatory role of PRL in amniotic fluid formation.
- Know the primary functions of amniotic fluid.

Questions

349. Which of the following stimulate PRL release from the anterior pituitary?

a. GnRH
b. DA
c. 5-HT
d. TRH
e. Cortisol

350. Select the false statement below:

a. PRL has been shown to enhance permeability of the chorioamnion to H_2O.
b. Primary control over PRL release is inhibitory in mammals.
c. ADH and oxytocin act as PRL-releasing factors during suckling.
d. PRL treatment has been found to induce stress.
e. PRL crosses the blood-CSF barrier via a carrier-mediated, saturable process.

351. Hyperprolactinemia:

a. Causes reproductive dysfunction in females.
b. Decreases GnRH, and thus pituitary gonadotropin release.
c. Causes reproductive dysfunction in males.
d. Can produce galactorrhea in males.
e. All of the above

352. PRL:

a. Potentiates the effect of LH on the steroidogenesis of testosterone in males.
b. Is present in amphibians, where it accelerates larval growth and blocks metamorphosis.
c. Is osmoregulatory in fishes.
d. Stimulates brooding behavior in chickens.
e. All of the above

353. Which of the following are appropriately matched:

a. PRL : Reduce food intake
b. 5-HT : Stimulate PRL release
c. PRL : Promote maternal behavior
d. Estrogen : Inhibit PRL release
e. All of the above

Chapter 69

Objectives

- Identify the actions of Oxy in dissolution of the CL, in postpartum uterine contractility, and in lactation.
- Name the steroid hormone that aids in the synthesis of Oxy receptors.
- Explain why Oxy is considered to be the "trust" hormone, and PRL is considered to be the "mother love" hormone.
- Give the 3 levels at which catecholamines inhibit milk ejection.
- Outline proposed actions of Oxy in the male and female reproductive tracts.
- Explain why administered Oxy does not induce labor at mid-gestation.
- Discuss the roles of Oxy, estrogen and progesterone in endometrial PG production.
- Explain why NSAID administration to a pregnant animal may induce fetal pulmonary hypertension.

Questions

354. Select the <u>true</u> statement below:

a. Oxytocin (Oxy) is synthesized in the hypothalamus and stored in the adenohypophysis.
b. Oxy activates phospholipase C in its garget cells.
c. Oxy synthesis and release is inhibited by NSAIDs.
d. DAG gives rise to IP_3 in Oxy target cells.
e. IP_3 is a membrane-associated activator of PKC.

355. All of the following statements regarding Oxy are true, <u>except</u>:

a. Its release from the neurohypophysis is generally inhibited by release-inhibiting factors (i.e., peptides) from the hypothalamus.
b. It is not a protein.
c. It may, in high enough concentrations, cause water retention.
d. It works synergistically with PGs in promoting uterine myometrial contractions.
e. It is generally released from the neurohypophysis through a neuroendocrine reflex.

356. Oxytocin:

a. Helps promote endometrial PG production during parturition.
b. May play a role in minimizing maternal blood loss following parturition.
c. Uterine receptor synthesis is facilitated by estrogen.
d. Is, like ADH, a nonapeptide.
e. All of the above

357. Select the <u>false</u> statement below:

a. Stress may inhibit oxytocin-induced milk ejection.
b. Genital stimulation involved with coitus releases Oxy in both males and females.
c. Catecholamines are known to block Oxy release.
d. Receptors for Oxy are found in nuclei of its target cells.
e. Oxy causes milk ejection by facilitating contraction of myoepithelial cells surrounding alveoli of mammary tissue.

Chapter 70

Objectives

- Describe the physiological adjustments that take place immediately following birth that allow neonates to maintain their own body temperature.
- Discuss how and where ketone bodies are metabolized in neonates.
- Explain why fetal blood is normally more acidemic than maternal blood.
- Distinguish differences between the circulation of fetal and adult blood.
- Recognize factors that keep the DA open in the fetal state, and close it following birth.
- Compare blood volume, cardiac output and blood pressure in neonates to those in adult animals.
- Know why neonatal ketonemia may be considered normal.
- Discuss effects of the stress hormones (e.g., ACTH, cortisol, catecholamines and T_4) on neonatal physiology.
- Explain why the Po_2 of umbilical venous blood is low.

Questions

358. Compared to the fetal state, which one of the following neonatal physiological parameters usually <u>decreases</u> soon after birth?

a. Cardiac output
b. Basal metabolic rate
c. Body temperature
d. Aortic blood pressure
e. Circulating cortisol levels

359. The pH and Po_2 of blood leaving the placenta in the umbilical vein are normally about ___ and ___ , respectively:

a. 7.7; 95 mmHg
b. 7.6; 78 mmHg
c. 7.5; 63 mmHg
d. 7.4; 45 mmHg
e. 7.3; 29 mmHg

360. Fetal blood normally passes from the right to left atrium through the:

a. Ductus venosus.
b. Aorta.
c. Foramen ovale.
d. Coronary vasculature.
e. Ductus arteriosus.

361. Which one of the following tends to keep the ductus arteriosus open?

a. Glucocorticoids (cortisol)
b. Prostaglandins (PGE_2)
c. The high Po_2 of aortic blood
d. Epinephrine (Epi)
e. Arginine vasotocin (AVT)

362. All of the following would favor hepatic glucose output into neonatal blood following birth, <u>except</u>:

a. PNS activation.
b. A low insulin:glucagon ratio.
c. Release of ACTH from the anterior pituitary.
d. High circulating catecholamine levels.
e. Thyroid hormone mobilization.

363. Approximately what percentage of the fetal cardiac output perfuses the placenta and accompanying fetal membranes:

a. 7
b. 37
c. 57
d. 77
e. 97

Chapter 71

Objectives

- Know the causes of neonatal acidosis.
- Understand why infants are frequently dehydrated.
- Explain why infants sometimes develop hypoproteinemic edema, and unconjugated hyperbilirubinemia.
- Recognize why some infants may become anemic.
- Explain how antibody transfer occurs from dam to fetus in various animal species.
- Recognize why neonatal kidney, liver and intestinal digestive activity are considered to be "immature."

Questions

364. The first inspirations of the newborn are partially facilitated by all of the following, <u>except</u>:

a. Hypoxemia.
b. Hypocarbia.
c. Environmental cooling.
d. Generalized increase in sensory input.
e. Loss of placental gas exchange.

365. Compared to the adult animal, which one of the following is normally <u>less</u> in the neonate?

a. Urinary nitrogen excretion (per kg body wt.)
b. Degree of acidemia
c. Respiratory rate
d. Extracellular fluid volume (per kg body wt.)
e. Plasma bilirubin concentration

366. The two most common physiologic problems encountered in newborn infants are:

a. Hypoglycemia and respiratory alkalosis.
b. Hypochloremia and hyperkalemia.
c. Hyperbilirubinemia and uremia.
d. Acidosis and dehydration.
e. Proteinuria and anemia.

367. Which of the following physiologic systems appears to most "mature" at birth?

a. Hematopoietic system.
b. Hepatobiliary system.
c. Endocrine system.
d. Exocrine pancreas.
e. Renal system.

368. Under normal circumstances, which one of the following would be expected to have a higher concentration in neonatal than in adult blood?

a. Glucose
b. HCO_3^-
c. Albumin
d. Erythropoietin
e. Acetoacetate

Answers

1. **A**
2. **C**
3. **E**
4. **A**
5. **B**
6. **E**
7. **D**
8. **A**
9. **C**
10. **B**
11. **C**
12. **E**
13. **A**
14. **A**
15. **E**
16. **C**
17. **D**
18. **E**
19. **E**
20. **B**
21. **A**
22. **D**
23. **C**
24. **C**
25. **A**
26. **A**
27. **D**
28. **E**
29. **B**
30. **E**
31. **B**
32. **D**
33. **C**
34. **E**
35. **C**
36. **A**
37. **C**
38. **A**
39. **B**
40. **E**
41. **B**
42. **D**
43. **D**
44. **C**
45. **C**
46. **A**
47. **D**
48. **B**
49. **C**
50. **E**
51. **A**
52. **C**
53. **A**
54. **D**
55. **D**
56. **E**
57. **E**
58. **B**
59. **C**
60. **A**
61. **D**
62. **E**
63. **C**
64. **B**
65. **C**
66. **D**
67. **B**
68. **C**
69. **E**
70. **E**
71. **C**
72. **D**
73. **E**
74. **C**
75. **A**
76. **B**
77. **E**
78. **D**
79. **D**
80. **A**
81. **A**
82. **E**
83. **C**
84. **E**
85. **D**
86. **E**
87. **A**
88. **E**
89. **B**
90. **C**
91. **C**
92. **D**
93. **E**
94. **E**
95. **D**
96. **D**
97. **B**
98. **C**
99. **A**
100. **B**
101. **A**
102. **E**
103. **B**
104. **E**
105. **C**
106. **E**
107. **D**
108. **D**
109. **C**
110. **A**
111. **A**
112. **B**
113. **D**
114. **E**
115. **C**
116. **C**
117. **E**
118. **A**
119. **B**
120. **C**
121. **B**
122. **A**
123. **E**
124. **D**
125. **E**
126. **B**
127. **D**
128. **C**
129. **B**
130. **E**
131. **E**
132. **D**
133. **E**
134. **D**
135. **A**
136. **B**
137. **D**
138. **E**
139. **A**
140. **B**
141. **C**
142. **E**
143. **E**
144. **B**
145. **E**
146. **E**
147. **C**
148. **A**
149. **C**
150. **D**
151. **E**
152. **C**
153. **E**
154. **B**
155. **E**
156. **C**
157. **D**
158. **D**
159. **C**
160. **C**
161. **A**
162. **E**
163. **E**
164. **D**
165. **C**
166. **B**
167. **C**
168. **D**
169. **D**
170. **A**
171. **E**
172. **C**
173. **A**
174. **C**
175. **A**
176. **E**
177. **A**
178. **E**
179. **B**
180. **A**
181. **B**
182. **C**
183. **D**
184. **B**
185. **D**
186. **D**
187. **C**
188. **B**
189. **C**
190. **A**
191. **E**
192. **C**
193. **E**
194. **C**
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196. **A**
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203. **B**
204. **C**
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206. **E**
207. **C**
208. **B**
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211. **A**
212. **D**
213. **E**
214. **A**
215. **C**
216. **B**
217. **C**
218. **A**
219. **B**
220. **E**
221. **E**
222. **D**
223. **B**
224. **A**
225. **A**
226. **E**
227. **C**
228. **A**
229. **E**
230. **D**
231. **A**
232. **C**
233. **C**
234. **D**
235. **C**
236. **B**
237. **B**
238. **E**
239. **D**
240. **E**
241. **B**
242. **A**
243. **E**
244. **D**
245. **E**
246. **D**
247. **C**
248. **A**
249. **B**
250. **B**
251. **D**
252. **E**
253. **B**
254. **D**
255. **D**
256. **C**
257. **E**
258. **B**
259. **E**
260. **B**
261. **C**
262. **B**
263. **D**
264. **A**
265. **C**
266. **A**
267. **D**
268. **D**
269. **E**
270. **A**
271. **C**
272. **C**
273. **C**
274. **D**
275. **B**
276. **D**
277. **B**
278. **A**
279. **C**
280. **A**
281. **C**
282. **B**
283. **A**
284. **C**
285. **D**
286. **A**
287. **E**
288. **B**
289. **E**
290. **C**
291. **E**
292. **A**
293. **C**
294. **A**
295. **E**
296. **C**
297. **D**
298. **C**
299. **E**
300. **B**
301. **B**
302. **D**
303. **E**
304. **C**
305. **E**
306. **A**
307. **D**
308. **D**
309. **E**
310. **D**
311. **C**
312. **E**
313. **A**
314. **E**
315. **B**
316. **D**
317. **D**
318. **A**
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320. **C**
321. **E**
322. **B**
323. **D**
324. **C**
325. **D**
326. **E**
327. **E**
328. **B**
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330. **C**
331. **E**
332. **A**
333. **D**
334. **A**
335. **E**
336. **C**
337. **D**
338. **B**
339. **A**
340. **C**
341. **C**
342. **B**
343. **E**
344. **E**
345. **A**
346. **E**
347. **D**
348. **E**
349. **D**
350. **D**
351. **E**
352. **E**
353. **C**
354. **B**
355. **A**
356. **E**
357. **D**
358. **C**
359. **E**
360. **C**
361. **B**
362. **A**
363. **C**
364. **B**
365. **A**
366. **D**
367. **C**
368. **E**

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