ESSENTIALS

ESSENTIAL ENDOCRINOLOGY AND DIABETES

RICHARD I.G. HOLT | NEIL A. HANLEY

SEVENTH EDITION

WILEY Blackwell

Essential Endocrinology and Diabetes

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Preface

Welcome to the latest edition of Essential Endocrinology & Diabetes. We are delighted that the previous edition was so well received around the world, selling over 5600 copies. The book also had the dubious honour of being the most frequently stolen book from the University of Southampton medical school library suggesting that the medical students, in Southampton at least, found it a useful accompaniment to their endocrinology and diabetes course. However, it is time for an update and a refresh that captures the latest research, clinical guidelines, investigational modalities and therapies. We hope you enjoy the new content.

The textbook aims to be valued by different groups of reader. Its core purpose is still to provide a foundation from understanding the science to clinical training in undergraduate medicine. In addition, the content here should also be invaluable for postgraduate clinicians training in the speciality of endocrinology and diabetes. Written 'exit' examinations have become increasingly common during the later stages of clinical speciality training and we hope our textbook provides complementary study material alongside larger reference textbooks and published clinical guidelines. From feedback, we realise that our book has been valued by biomedical undergraduate and masters students and by those pursuing clinical biochemistry. We hope the new content increases this reach across different audiences. Learning objectives, recap points, cross-referencing guides, boxes, and concluding key points help orientate the reader and emphasize the major topics.

Based on the success of previous editions, the book is still structured in much the same way. The first part is designed to create a knowledgeable reader, well prepared for the clinical sections or for more specific scientific study. To assist the many students coming from non-scientific backgrounds we limit assumptions on prior knowledge. Chapter 1 still covers the core principle of feedback regulation which underlies nearly all endocrine physiology and is vital for the correct interpretation of many clinical tests. Chapter 3 has advanced to encompass the latest research made possible from next-generation sequencing technology. The latter has already started to impact significantly on clinical investigation and diagnostics in endocrinology and diabetes. This is covered in Chapter 4, which also includes positron emission tomography (PET) imaging. It is important that aspiring clinicians, as well as scientists, appreciate these new approaches, their application and their challenges. The second part of the book still follows its organ or system-based approach. We have retained the more specific scientific knowledge at the start of these chapters to underpin understanding, diagnosing and managing the relevant clinical disorders. The third part on diabetes and obesity has seen the greatest change from the previous edition. Over the last 8 years, there have been significant advances in the treatment of both type 2 diabetes, such as an expansion of the indications for incretin-based therapies and the introduction of the SGLT2 inhibitors, and type 1 diabetes with the development of better insulins and the use of technology to support self-management. Clinical algorithms have also changed and these have been updated.

As previously, the book is founded on our collective clinical and research experience and has been a truly collaborative venture. The book is designed to read as a whole, however, inevitably one of us has taken a lead with each chapter according to our own particular expertise. When we wrote the 5th edition, NAH took the responsibility for Part 1 and Part 2, while RIGH was responsible for Part 3. Now on our third edition, we have each taken the opportunity to bring our experience to each of the chapters. Finally, we must thank a number of people. We are grateful for the skilled help of the team at Wiley. It is still important to recognise the excellent contribution of Charles Brook and Nicholas Marshall who authored the book up to and including the 4th edition. We are also very grateful to our scientific and clinical colleagues who have been the recipients of frequent questions and from whom we have sought valued opinions. The final thank you is to our families whose support and tolerance made this book possible. Particular thanks go to Tristan Holt, now a medical student, for his helpful comments in making the book as relevant as possible for our target audience.

The authors

Richard Holt is Professor in Diabetes and Endocrinology at the University of Southampton and Honorary Consultant in Diabetes at University Hospital Southampton NHS Foundation Trust. He is partly supported by the Southampton National Institute for Health Research Biomedical Research Centre. His research interests are broadly focused around clinical diabetes with particular interests in diabetes in pregnancy, cystic fibrosis diabetes and diabetes in young adults, and the relation between diabetes and mental illness. He also has a long-standing interest in growth hormone.

Neil Hanley is Professor of Medicine at the University of Manchester and Honorary Consultant in Endocrinology at the Manchester University NHS Foundation Trust. His research over three decades has focussed on human development. His wider roles now include all aspects of biomedical and clinical research including the application of data science, and interests in how to triangulate healthcare, academia and the commercial sector to drive improved outcomes including economic growth.

Both authors play a keen role in teaching and training at both undergraduate and postgraduate levels. RIGH is a Fellow of the Higher Education Academy. NAH established an Academy for early career researchers at the University of Manchester and has led aspects of reviewing and refreshing national training portfolios in health research.

Further reading

The following major international textbooks make an excellent source of secondary reading:
Melmed S, Polonsky KS, Reed Larsen P, Kronenberg HM, eds. *Williams Textbook of Endocrinology*, 14th edn. Saunders, 2019.
Holt RIG, Cockram C, Flyvbjerg A, Goldstein BJ. *Textbook of Diabetes*, 5th edn. Wiley-Blackwell, 2017.
In addition, the following textbooks cover topics, relevant to some chapters, in greater detail:
Delves PJ, Martin SJ, Burton DR, Roitt IM. *Roitt's Essential Immunology*, 13th edn. Wiley-Blackwell, 2017.
Johnson M. *Essential Reproduction*, 8th edn. Wiley-Blackwell, 2018.
Nelson DL, Cox MM. *Lehninger Principles of Biochemistry*, 7th edn. W.H. Freeman, 2017.

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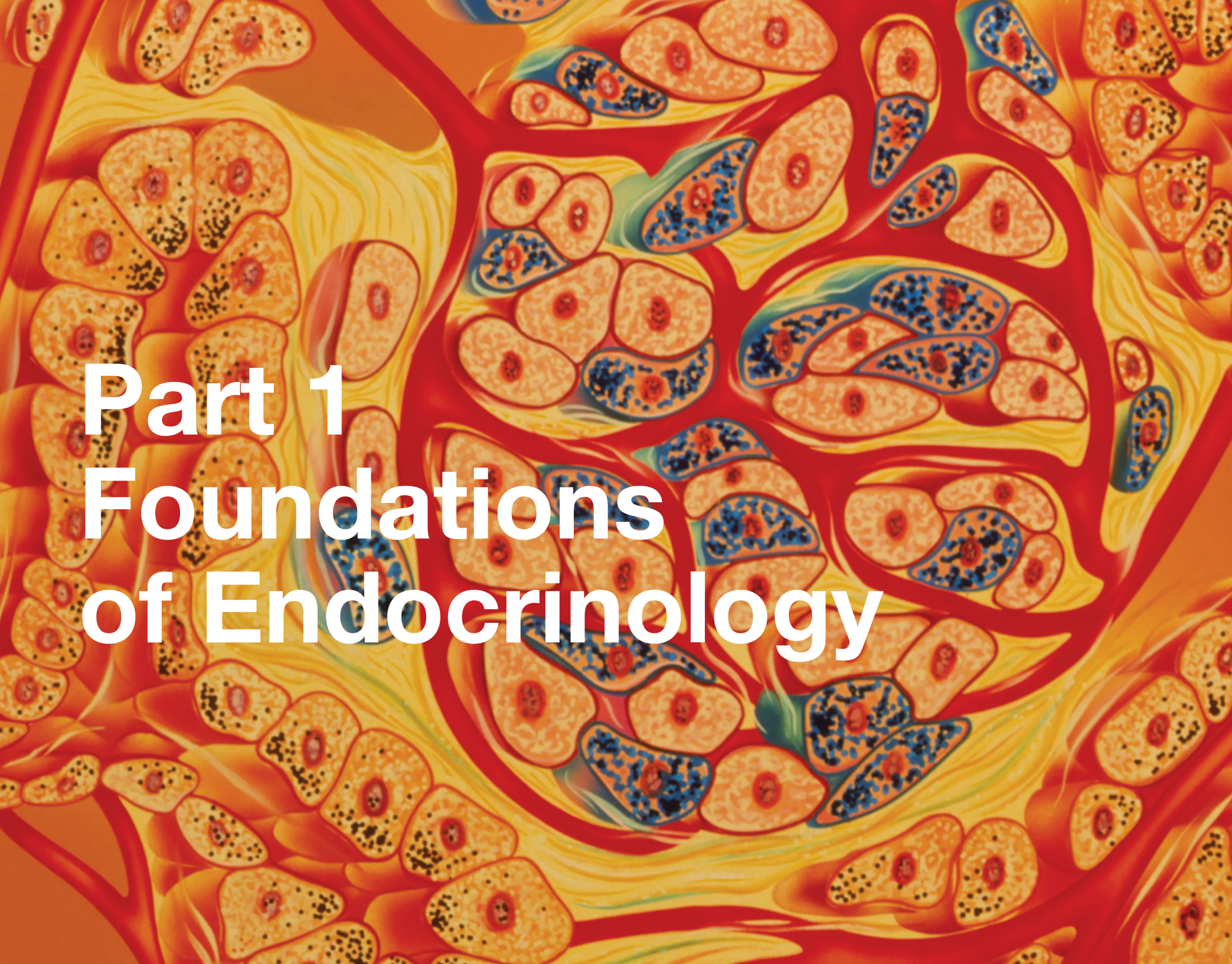
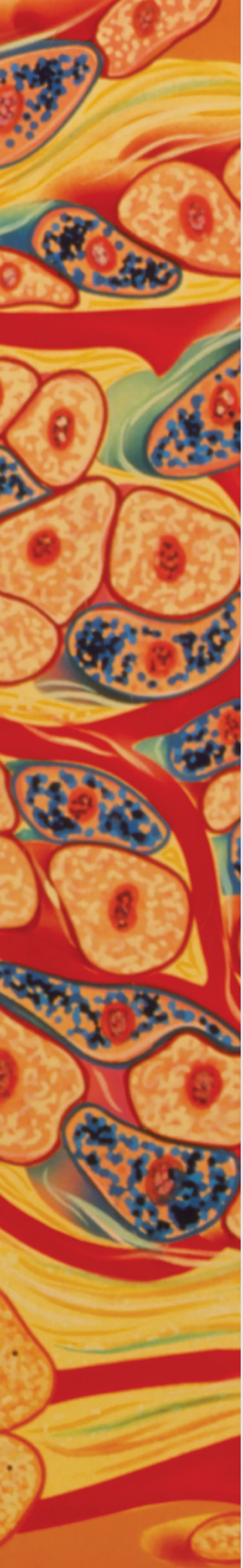
List of abbreviations

5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptophan
αMSH	α-melanocyte-stimulating hormone
ACTH	adrenocorticotrophic hormone
ADH	vasopressin/antidiuretic hormone
AFP	α-fetoprotein
AGE	advanced glycation end-product
AGRP	Agouti-related protein
AI	angiotensin I
AII	angiotensin II
ALS	acid-labile subunit
AMH	anti-Müllerian hormone
AR	androgen receptor
APS-1	type 1 autoimmune polyglandular syndrome
APS-2	type 2 autoimmune polyglandular syndrome
CAH	congenital adrenal hyperplasia
CCK	cholecystokinin
cAMP	cyclic adenosine monophosphate
CBG	cortisol binding globulin
cGMP	guanosine monophosphate
CRE	cAMP response element
CREB	cAMP response element-binding protein
CNS	central nervous system
CRH	corticotrophin-releasing hormone
CSF	cerebrospinal fluid
CT	computed tomography
CVD	cardiovascular disease
DAG	diacylglycerol
DEXA	dual-energy X-ray absorptiometry
DHEA	dehydroepiandrosterone
DHT	5α-dihydrotestosterone
DI	diabetes insipidus
DSD	difference in sex development
EGF	epidermal growth factor
EPO	erythropoietin
ER	oestrogen receptor
FDG	fluoro-2-deoxyglucose
FFA	free fatty acid
FGF	fibroblast growth factor
FIA	fluoroimmunoassay
FISH	fluorescence *in situ* hybridization
FSH	follicle-stimulating hormone
fT_3	free tri-iodothyronine
fT_4	free thyroxine
GC	gas chromatography
GDM	gestational diabetes
GFR	glomerular filtration rate
GH	growth hormone (somatotrophin)
GHR	GH receptor
GHRH	growth hormone-releasing hormone
GI	glycaemic index
GIP	glucose-dependent insulinotropic peptide (gastric inhibitory peptide)
GLP1/2	glucagon-like peptide 1 or 2
GLUT	glucose transporter
GnRH	gonadotrophin-releasing hormone
GPCR	guanine-protein coupled receptor
GR	glucocorticoid receptor
Grb2	type 2 growth factor receptor-bound protein
hCG	human chorionic gonadotrophin
hMG	human menopausal gonadotrophin
HMG-CoA	hydroxymethylglutaryl coenzyme A
HNF	hepatocyte nuclear factor
HPLC	high-performance liquid chromatography
HRE	hormone response element
HRT	hormone replacement therapy
ICSI	intracytoplasmic sperm injection
IDDM	insulin-dependent diabetes mellitus
IFG	impaired fasting glycaemia
IFMA	immunofluorometric assay
IGF	insulin-like growth factor
IGFBP	IGF-binding protein
IGT	impaired glucose tolerance
IP	inositol phosphate
IPF	insulin promoter factor
IR	insulin receptor
IRMA	intraretinal microvascular abnormalities (Chapter 14)
IRMA	immunoradiometric assay (Chapter 4)
IRS	insulin receptor substrate
IVF	*in vitro* fertilization
JAK	Janus-associated kinase
LDL	low-density lipoprotein
LH	luteinizing hormone
MAO	monoamine oxidase
MAPK	mitogen-activated protein kinase
MEN	multiple endocrine neoplasia
mIBG	*meta*-iodobenzylguanidine
MIBI	sestamibi
MIS	Müllerian inhibiting substance
MODY	maturity-onset diabetes of the young
MR	mineralocorticoid receptor
MRI	magnetic resonance imaging
MS	mass spectrometry
MSH	melanocyte-stimulating hormone
NEFA	non-esterified fatty acid
NICTH	non-islet cell tumour hypoglycaemia
NIDDM	non-insulin-dependent diabetes mellitus
NPY	neuropeptide Y
NVD	new vessels at the disc
NVE	new vessels elsewhere
OGTT	oral glucose tolerance test

PCOS	polycystic ovarian syndrome
PCR	polymerase chain reaction
PCSK1	prohormone convertase 1/3
PDE	phosphodiesterase
PET	positron emission tomography
PGE2	prostaglandin E_2
PI	phosphatidylinositol
PIT1	pituitary-specific transcription factor 1
PKA	protein kinase A
PKC	protein kinase C
PLC	phospholipase C
PNMT	phenylethanolamine *N*-methyl transferase
POMC	pro-opiomelanocortin
PPAR	peroxisome proliferator-activated receptor
PPGL	phaeochromocytoma/paraganglioma
PRL	prolactin
PTH	parathyroid hormone
PTHrP	parathyroid hormone-related peptide
PTU	propylthiouracil
RANK	receptor activator of nuclear factor-kappa B
RER	rough endoplasmic reticulum
RIA	radioimmunoassay
rT_3	reverse tri-iodothyronine
RXR	retinoid X receptor
SERM	selective ER modulator
SDH	succinate dehydrogenase
SHBG	sex hormone-binding globulin
SIADH	syndrome of inappropriate antidiuretic hormone
SoS	son of sevenless protein
SRD5A1/2	5α-reductase type 1 or 2
SRE	serum response element
SS	somatostatin
StAR	steroid acute regulatory protein
STAT	signal transduction and activation of transcription protein
T1DM	type 1 diabetes
T2DM	type 2 diabetes
$t_{1/2}$	half-life
T_3	tri-iodothyronine
T_4	thyroxine
TGFβ	transforming growth factor β
TK	tyrosine kinase
TPO	thyroid peroxidase
TR	thyroid hormone receptor
TRE	thyroid hormone response element
TRH	thyrotrophin-releasing hormone
TSH	thyroid-stimulating hormone
UFC	urinary free cortisol
V	vasopressin/antidiuretic hormone (previously also known as arginine vasopressin)
VEGF	vascular endothelial growth factor
VIP	vasoactive intestinal peptide
WES	whole-exome sequencing
WGS	whole-genome sequencing



Part 1 Foundations of Endocrinology



CHAPTER 1

Overview of endocrinology

Key topics

Learning objectives

- To be capable of defining endocrinology
- To understand what endocrinology means as a basic science and a clinical specialty
- To appreciate the history of endocrinology
- To understand the classification of hormones into peptides, steroids and amino acid derivatives
- To understand the principle of how feedback mechanisms regulate hormone levels in the circulation

This chapter introduces endocrinology and diabetes including some of the basic principles that underpin the following chapters

Essential Endocrinology and Diabetes, Seventh Edition. Richard I.G. Holt and Neil A. Hanley.

Box 1.1 The endocrine and nervous systems are the two main communication systems in the body

- Monitor internal and external environments
- Allow appropriate adaptive changes
- Communicate via chemical messengers

} Ensure homeostasis

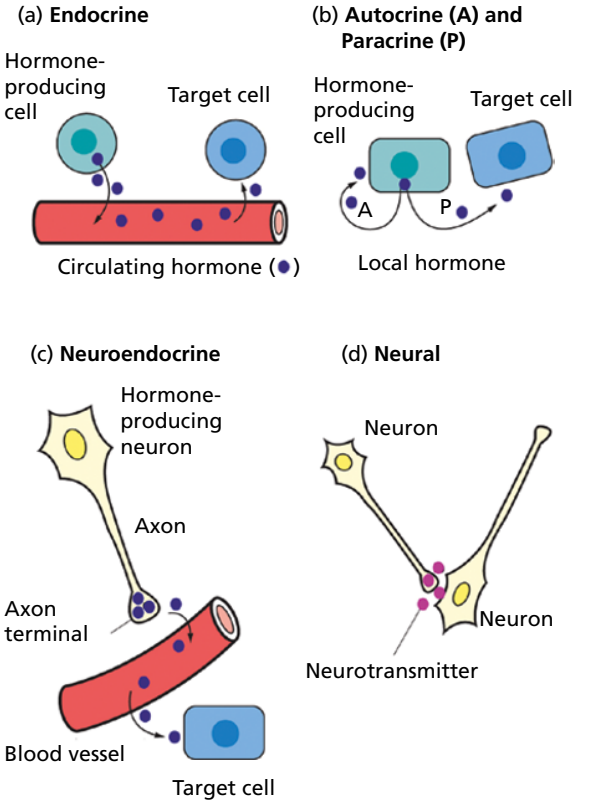


Figure 1.1 Chemical signalling in the endocrine and neural systems. (a) In endocrine communication, the producing cell secretes hormone into the blood vessel, where it is carried, potentially over large distances, to its target cell. (b) Sometimes hormones can act on the cell that produces them (autocrine, A) or nearby cells (paracrine, P) without the need for transport via the circulation. For instance, glucagon from α-cells and somatostatin from δ-cells can regulate insulin secretion by adjacent β-cells within the pancreatic islet. (c) In neuroendocrine communication, neurons can secrete hormones into the surrounding blood vessels to reach a more distant target. A good example is hypothalamic regulation of the anterior pituitary. (d) In pure neural communication, neurons activate other neurons via neurotransmitters released from axonic terminals into the synaptic space. Conceptually, neurotransmitters are similar to hormones and in some instances, such as for norepinephrine/noradrenaline, can actually be the same chemical.

All organisms need to be able to analyze and respond to their surroundings in order to provide a constant internal environment. Maintaining this internal constancy is called homeostasis. For an organism comprised of a single or a few cells homeostasis is relatively easy as no cell is more than a short diffusion distance from the outside world or its neighbours. This simplicity has been lost with the evolution of more complex, larger, multicellular organisms. Diffusion alone is inadequate in larger animal species where discrete functions localize to specific organs. In humans, there are ~10^{14} cells of more than 200 different types. With this compartmentalized function comes the need for effective communication to disseminate information throughout the whole organism – only a few cells face the outside world, yet all need to respond to it. Two communication systems facilitate this: the endocrine and nervous systems (Box 1.1).

Whereas gastrointestinal cells tend to secrete chemicals into ducts, the specialized cells that make up the glands and tissues of the endocrine system release their chemical messengers, called hormones, into the extracellular space, from where they enter the bloodstream. Historically, this blood-borne transit of hormones was what defined 'endocrinology'; however, the principle is identical for hormone action on a neighbouring cell (called a paracrine effect) or, indeed, the endocrine cell itself (an autocrine or intracrine effect) (Figure 1.1).

The nervous and endocrine systems interact. Endocrine glands can be under nervous control; the adrenal medulla is an excellent example (Chapter 6). Conversely, neural cells can themselves release hormones into the bloodstream. This is particularly relevant in the hypothalamus (Chapter 5). Indeed, this interplay between the body's two main communication systems has led to the composite specialty of 'neuroendocrinology' (Figure 1.1).

A brief history of endocrinology and diabetes

The term 'hormone', derived from the Greek word 'hormaein' meaning 'to arouse' or 'to excite', was first used in 1905 by Sir Ernest Starling in his Croonian Lecture to the Royal College of Physicians. However, endocrinology is built on foundations that are far older. Although Aristotle described the pituitary, gigantism, caused by excess growth hormone (GH) from the somatotrophs of the anterior pituitary, was referred to in the Old Testament. It was only two millennia or so later in the 19th century that the gland's anterior and posterior components were appreciated by Rathke, and Pierre Marie connected GH-secreting pituitary tumours to acromegaly and excess growth.

Diabetes was recognized by the ancient Egyptians. Areateus later described the disorder in the second century AD as 'a melting down of flesh and limbs into urine'. Consequently, diabetes comes from the Greek word meaning 'siphon'. The pancreas was only implicated relatively recently when Minkowski realized in 1889 that the organ's removal in dogs mimicked diabetes in humans.

The roots of reproductive endocrinology are equally long. The Bible refers to eunuchs and Hippocrates recognized that mumps could result in sterility. Oophorectomy in sows and camels was used to increase strength and growth in ancient Egypt. The dependence of endocrinology on technology is also long standing. It took the invention of the microscope in the 17th century for Leeuwenhoek to visualize spermatozoa and later, in the 19th century, for the mammalian ovum to be discovered in the Graafian follicle.

During the last 500 years, many endocrine organs and systems ('axes') have been identified and characterized. In 1564, Bartolomeo Eustacio noted the presence of the adrenal glands. Almost 300 years later (1855), Thomas Addison, one of the forefathers of clinical endocrinology, described the consequences of their inadequacy. Catecholamines (epinephrine/adrenaline and norepinephrine/noradrenaline) were identified at the turn of the 19th century, in parallel with Oliver and Schaffer's discovery that these adrenomedullary substances raised blood pressure. This followed shortly after the clinical features of myxoedema were linked to the thyroid gland, when, in 1891, physicians in Newcastle-upon-Tyne treated hypothyroidism with sheep thyroid extract. This was an important landmark, but long after the ancient Chinese recognized that seaweed, as a source of iodine, held valuable properties in treating swelling of the thyroid gland ('goitre').

These early aspects of clinical endocrinology and diabetes tended to rely on recognition and description. Since then our understanding has advanced through:

- Successful quantification of circulating hormones
- Molecular unravelling of complex hormone action
- Mechanistic identification of pathophysiology underlying endocrine dysfunction
- Molecular genetic diagnoses

Some of the landmarks from the last 100 years are shown in Box 1.2. Endocrinology and diabetes is notable for researchers who have been awarded the Nobel Prize for Medicine, Physiology or Chemistry for their landmark discoveries (Table 1.1).

Box 1.2 Some landmarks in endocrinology over the last century or so

1905	First use of the term 'hormone' by Starling in the Croonian Lecture at the Royal College of Physicians
1909	Cushing removed part of the pituitary and saw improvement in acromegaly
1914	Kendall isolated an iodine-containing substance from the thyroid
1921	Banting and Best extracted insulin from islet cells of dog pancreas and used it to lower blood glucose
Early 1930s	Pitt-Rivers and Harrington determined the structure of the thyroid hormone, thyroxine
1935–1940	Crystallization of testosterone
1935–1940	Identification of oestrogen and progesterone
1940s	Harris recognized the relationship between the hypothalamus and anterior pituitary in the 'portal-vessel chemotransmitter hypothesis'
1952	Gross and Pitt-Rivers identified tri-iodothyronine in human serum
1955	The Schally and Guillemin laboratories showed that extracts of hypothalamus stimulated adrenocorticotrophic hormone (ACTH) release
1950s	Adams and Purves identified thyroid stimulatory auto-antibodies
	Gonadectomy and transplantation experiments by Jost led to the discovery of the role for testosterone in rabbit sexual development
1955	Marcel Janbon and colleagues first recognized the hypoglycaemic effects of sulphonamide antibiotics during a typhoid epidemic in Marseilles in 1942. This led to the introduction of sulphonylureas into clinical practice
1955	Sanger reported the primary structure of insulin
1956	Doniach, Roitt and Campbell associated antithyroid antibodies with some forms of hypothyroidism – the first description of an autoimmune phenomenon
1957	Growth hormone was used to treat children with short stature
1966	First transplant of human pancreas to treat type 1 diabetes by Kelly, Lillehei, Goetz and Merkel at the University of Minnesota
1969	Hodgkin reported the three-dimensional crystallographic structure of insulin
1969–1971	Discovery of thyrotrophin-releasing hormone (TRH) and gonadotrophin-releasing hormone (GnRH) by Schally's and Guillemin's groups
1973	Discovery of somatostatin by the group of Guillemin
1981–1982	Discovery of corticotrophin-releasing hormone (CRH) and growth hormone-releasing hormone (GHRH) by Vale

1983	Cloning of gene encoding glucagon and two glucagon-like peptides, including GLP-1, by Bell and colleagues
1994	Identification of leptin by Friedman and colleagues
1994	First transplantation of pancreatic islets to treat type 1 diabetes by Pipeleers and colleagues in Belgium
1999	Discovery of ghrelin by Kangawa and colleagues
1999	Sequencing of the human genome – publication of the DNA code for chromosome 22
2000	Advanced islet transplantation using modified immunosuppression by Shapiro and colleagues to treat type 1 diabetes
2005	GLP-1 receptor agonists introduced into clinical practice
2010	SGLT-2 inhibitors entered clinical practice

Table 1.1 Nobel prizewinners in endocrinology and diabetes or those whose discoveries have profoundly affected the specialty

Year	Prizewinner(s)	For work on . . .
1909	Emil Theodor Kocher	Physiology, pathology and surgery of the thyroid gland
1923	Frederick Grant Banting and John James Richard Macleod	Discovery of insulin
1928	Adolf Otto Reinhold Windaus	Constitution of the sterols and their connection with the vitamins
1939	Adolf Friedrich and Johann Butenandt	Sex hormones
1943	George de Hevesy	Use of isotopes as tracers in the study of chemical processes
1946	James Batcheller Sumner, John Howard Northrop and Wendell Meredith Stanley	Discovery that enzymes can be crystallized and prepared in a pure form
1947	Carl Ferdinand Cori, Getty Theresa Cori (neé Radnitz) and Bernardo Alberto Houssay	Discovery of the course of the catalytic conversion of glycogen
1950	Edwin Calvin Kendall, Tadeus Reichstein and Philip Showalter Hench	Discoveries relating to the hormones of the adrenal cortex, their structure and biological effects
1955	Vincent du Vigneaud	Biochemically important sulphur compounds, especially for the first synthesis of a polypeptide hormone
1958	Frederick Sanger	Structures of proteins, especially that of insulin
1964	Konrad Bloch and Feodor Lynen	Discoveries concerning the mechanism and regulation of cholesterol and fatty acid metabolism
1964	Dorothy Hodgkin	X-ray crystallography, a method used to determine the three-dimensional structures of molecules, including insulin
1966	Charles Brenton Huggins	Discoveries concerning hormonal treatment of prostatic cancer
1969	Derek HR Barton and Odd Hassel	Development of the concept of conformation and its application in chemistry
1970	Bernard Katz, Ulf von Euler and Julius Axelrod	Discoveries concerning the humoral transmitters in the nerve terminals and the mechanism for their storage, release and inactivation
1971	Earl W Sutherland Jr	Discoveries concerning the mechanisms of the action of hormones
1977	Roger Guillemin, Andrew V Schally and Rosalyn Yalow	Discoveries concerning peptide hormones in the production in the brain and the development of radioimmunoassay from peptide hormones
1979	Allan M Cormack and Godfrey N Hounsfield	Development of computer-assisted tomography

Table 1.1 (*Continued*)

Year	Prizewinner(s)	For work on . . .
1982	Sune K Bergström, Bengt I Samuelson and John R Vane	Discoveries concerning prostaglandins and related biologically active substances
1985	Michael S Brown and Joseph L Goldstein	Discoveries concerning the regulation of cholesterol metabolism
1986	Stanley Cohen and Rita Levi-Montalcini	Discoveries of growth factors
1992	Edmond H Fischer and Edwin G Krebs	Discoveries concerning reversible protein phosphorylation as a biological regulatory mechanism
1994	Alfred G Gilman and Martin Rodbell	Discovery of G-proteins and the role of these proteins in signal transduction in cells
2003	Peter Agre and Roderick MacKinnon	Discovery of water channels, and the structural and mechanistic studies of ion channels
2003	Paul Lauterbur and Sir Peter Mansfield	Discoveries concerning magnetic resonance imaging
2010	Robert G Edwards	Development of *in vitro* fertilisation

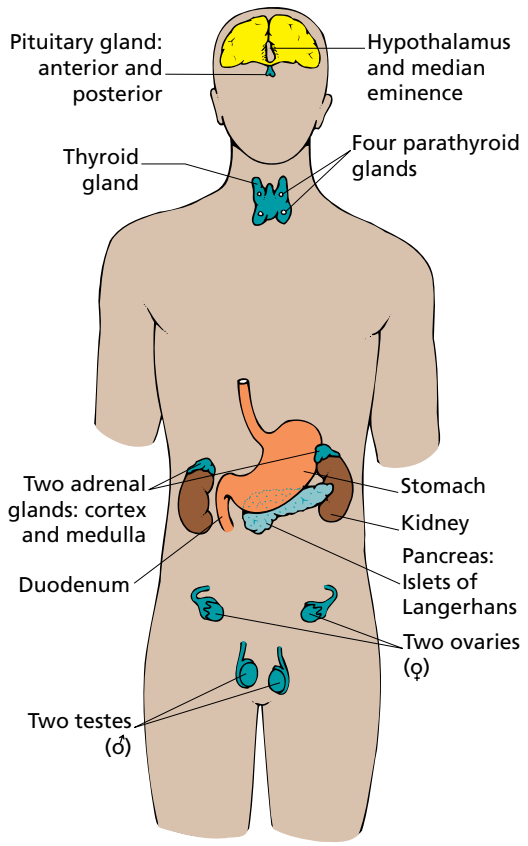


Figure 1.2 The sites of the principal endocrine glands. While the stomach, kidneys and duodenum are shown, a multitude of different hormones are secreted right the way along the gastrointestinal tract.

Traditionally, endocrinology has centred on specialized hormone-secreting organs (Figure 1.2), largely founded on the 'endocrine postulates' of Edward Doisy (Box 1.3). While the focus of this textbook remains on these organs, virtually all tissues make hormones of some description or, equally relevant, modulate the action of hormones from other sites. All of these different aspects are important for a complete appreciation of endocrinology and its significance.

Box 1.3 The 'Endocrine Postulates': Edward Doisy, St Louis University School of Medicine, USA, 1936

- The gland must secrete a substance (an 'internal secretion')
- Methods of detecting the secretion must be available
- The purified substance (the hormone) must be recoverable from gland extracts
- The hormone must be capable of isolation for its structure to be determined and for synthesis

To this could be added:

- The hormone must act on specific target cells via a receptor such that excess or deficiency causes a specific phenotype

The role of hormones

Endocrine (i.e. hormone-secreting) cells may exist as distinct glands or be located as single cells within other organs, such as the gastrointestinal tract (Table 1.2). The chapters in Part 2 are largely organized on this anatomical basis.

Hormones act by binding to specific receptors, either on the surface of or inside the target cell, to initiate a cascade of intracellular reactions, which frequently amplifies the original stimulus and generates a final response. These responses are altered in hormone deficiency or excess: for instance, GH deficiency leads

Table 1.2 The endocrine organs and their hormones*

Gland	Hormone	Molecular characteristics
Hypothalamus/ median eminence	Releasing and inhibiting hormones:	
	Thyrotrophin-releasing hormone (TRH)	Peptide
	Somatostatin (SS; inhibits GH)	Peptide
	Gonadotrophin-releasing hormone (GnRH)	Peptide
	Corticotrophin-releasing hormone (CRH)	Peptide
	Growth hormone-releasing hormone (GHRH)	Peptide
	Dopamine (inhibits prolactin)	Tyrosine derivative
Anterior pituitary	Thyrotrophin or thyroid-stimulating hormone (TSH)	Glycoprotein
	Luteinizing hormone (LH)	Glycoprotein
	Follicle-stimulating hormone (FSH)	Glycoprotein
	Growth hormone (GH) (also called somatotrophin)	Protein
	Prolactin (PRL)	Protein
	Adrenocorticotrophic hormone (ACTH)	Peptide
Posterior pituitary	Vasopressin [also called antidiuretic hormone (ADH)]	Peptide
	Oxytocin	Peptide
Thyroid	Thyroxine (T4) and tri-iodothyronine (T3)	Tyrosine derivatives
	Calcitonin	Peptide
Parathyroid	Parathyroid hormone (PTH)	Peptide
Adrenal cortex	Aldosterone	Steroid
	Cortisol	Steroid
	Androstenedione	Steroid
	Dehydroepiandrosterone (DHEA)	Steroid
Adrenal medulla	Epinephrine (also called adrenaline)	Tyrosine derivative
	Norepinephrine (also called noradrenaline)	Tyrosine derivative
Stomach†	Gastrin	Peptide
Pancreas (islets of Langerhans)	Insulin	Protein
	Glucagon	Protein
	Somatostatin (SS)	Protein
	Pancreatic polypeptide	Protein
	Ghrelin	Protein
Small and large intestine†	Secretin	Protein
	Glucagon-like peptide 1 (GLP-1)	Protein
Liver	Insulin-like growth factor I (IGF-I)	Protein
Ovary	Oestrogens	Steroid
	Progesterone	Steroid
Testis	Testosterone	Steroid

*The distinction between peptide and protein is somewhat arbitrary. Shorter than 50 amino acids is termed a peptide in this table.
†The list is far from exhaustive for the gastrointestinal tract (see Chapter 11).

to short stature in children, while excess causes over-growth (either gigantism or acromegaly; Chapter 5).

Hormone responses can be widespread or incredibly focussed according to how widely the hormone's receptor is distributed. For instance, thyroid hormone acts on many, if not all, of the more than 200 cell types in the body. The body's entire metabolic rate increases if it is present in excess and declines if there is a deficiency (Chapter 8). Similarly, insulin acts on most tissues; an importance underlined by its pivotal role in the survival and growth of many cell types in laboratory culture. In contrast, other hormones may act only on one tissue. For instance, thyroid-stimulating hormone (TSH), adrenocorticotrophic hormone (ACTH) and the gonadotrophins are secreted by the anterior pituitary and regulate specific cell-types in the thyroid gland, the adrenal cortex and the gonads, respectively (Table 1.2).

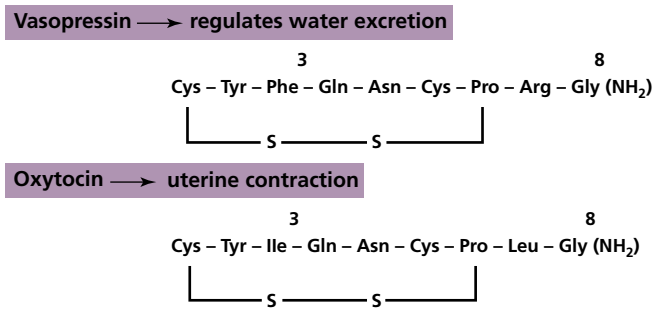


Figure 1.3 The structures of vasopressin and oxytocin are remarkably similar, yet the physiological effects of the two hormones differ profoundly.

Classification of hormones

There are three major groups of hormones, defined according to their biochemical synthesis (Box 1.4). Peptide or protein hormones are synthesized like any other cellular protein. The amino acid derivatives and steroid hormones originate from a cascade of intracellular enzymatic reactions.

Peptide hormones

The majority of hormones are peptides and range in size from tiny, only three amino acids [thyrotrophin-releasing hormone (TRH)], to small proteins of >200 amino acids, such as TSH or luteinizing hormone (LH). While some peptide hormones are secreted directly, a typical feature is their storage in granules, the release from which is commonly controlled by another hormone, as part of a cascade, or by innervation.

Some peptide hormones have complex tertiary structures or comprise more than one peptide chain encoded by multiple genes. Oxytocin and vasopressin, the two posterior pituitary hormones, have ring structures linked by disulphide bridges. Despite being remarkably similar in structure, they have markedly different physiological roles (Figure 1.3). Insulin consists of α- and β-chains linked by disulphide bonds. Like several hormones, it is synthesized as an inactive precursor that requires modification prior to release and activity. To some extent, this regulation protects the synthesizing cell from being overwhelmed by the action of its own hormone. The gonadotrophins, follicle-stimulating hormone (FSH) and LH, TSH and human chorionic gonadotrophin (hCG) also have two chains. However, these α- and β-subunits are synthesized separately from entirely different genes. The α-subunit is common to each of the hormones. It is the distinctive β-subunit of each that confers biological specificity.

Box 1.4 Major hormone groups

- Peptides and proteins
- Amino acid derivatives
- Steroids

Amino acid derivatives

These hormones are small water-soluble compounds. Melatonin is derived from tryptophan, whereas tyrosine derivatives include thyroid hormones, catecholamines, and dopamine, which regulates prolactin secretion in the anterior pituitary. The catecholamine hormones, epinephrine (also called adrenaline) and norepinephrine (noradrenaline) are secreted by the adrenal medulla as well as being sympathetic neurotransmitters, emphasizing the close relationship between the nervous and endocrine systems (Figure 1.2). Like peptide hormones, they are stored in granules prior to release.

Steroid hormones

Steroid hormones are lipid-soluble molecules derived from cholesterol. Cholesterol is present in all cells as a basic constituent of the cell membrane. However, in the steroid hormone synthesizing cells of the adrenal cortex, gonad and placenta, it is also extracted from the circulation and stored intracellularly as cholesterol esters. Steroid hormones are insoluble in water and circulate largely bound to plasma proteins.

Control systems regulating hormone production

The synthesis, release and circulating level of hormones are regulated by control systems, similar to those used in engineering. These mechanisms ensure that hormone signals can be limited in amplitude and duration. Central to the regulation of many different endocrine organs is the anterior pituitary gland, which, in turn, is controlled by several hormones and factors released from specialized hypothalamic neurones (Chapter 5). Thus, the body's major endocrine axes comprise the hypothalamus, anterior pituitary and an end organ, the adrenal cortex, thyroid,

testis or ovary. An understanding of these control mechanisms is crucial for appreciating endocrine physiology and its clinical investigation.

Simple control

An elementary control system is one in which the signal itself is limited, either in magnitude or duration, so as to trigger only a transient response. Certain neural impulses are of this type. Responsiveness of the target cell is set to discriminate between a positive signal, when a cell responds, and background 'noise', when a response is not triggered. An example is the pulsatile release of gonadotrophin-releasing hormone (GnRH) from the hypothalamus.

Negative feedback

Negative feedback is the commonest form of regulation used by biological systems. For example, in enzymology, the product frequently inhibits its own catalyzed reaction. In endocrinology, a hormone may act on its target cell to elicit a response (often secretion of another hormone) that then inhibits production of the original hormone (Figure 1.4a). The same effect can come from a metabolic process. For instance, the pancreatic β-cell makes insulin in response to high surrounding glucose levels. The effect is to lower glucose, which, in turn, inhibits further insulin production. The hypothalamic–anterior pituitary–end organ axes are a slightly more complex extension of this model. The hypothalamic hormone [e.g. corticotrophin-releasing hormone (CRH)] stimulates release of the anterior pituitary hormone (e.g. ACTH), which, in turn, increases peripheral hormone production (e.g. cortisol). The peripheral hormone then feeds back via the circulation to inhibit further production of the anterior pituitary and hypothalamic hormones. Figure 1.4b illustrates the anterior pituitary and end-organ components of this model where hormone 1 could be ACTH and hormone 2 could be cortisol.

Positive feedback

Under certain more unusual circumstances, hormone feedback enhances, rather than inhibits, the initial response. This is called positive feedback (illustrated alongside the more usual negative feedback by the plus sign in Figure 1.4a). This is intrinsically unstable and always has built-in self-limiting features. Transiently, it can be beneficial. For instance, the action of oestrogen on the pituitary gland induces the ovulatory surge of LH and FSH, further stimulating oestrogen production in the developing follicle

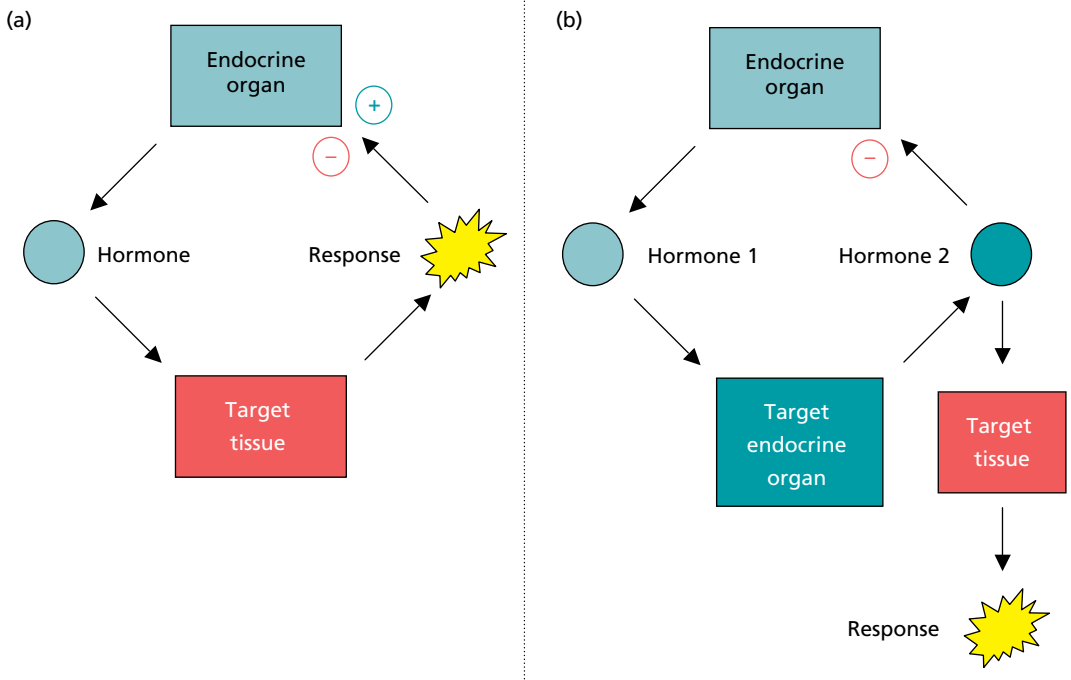


Figure 1.4 Control systems regulating hormone production and circulating levels. (a) The endocrine organ releases a hormone, which acts on the target tissue to stimulate a response. The response usually feeds back to inhibit (−) the endocrine organ and decrease further supply of the hormone. Occasionally, the feedback can act to enhance the hormone secretion (+, positive feedback). (b) In this slightly more complex scenario, the endocrine organ produces hormone 1, which acts on a second endocrine gland to release hormone 2. In turn, hormone 2 acts dually on the target tissue to induce the response and feeds back negatively onto the original endocrine organ to inhibit further release of hormone 1. This model is illustrative of the axes between the anterior pituitary and the peripheral end-organ targets.

Box 1.5 Endocrine cycles

Circadian = 24-h cycle
- Circa = about, dies = day

Ultradian < 24-h cycle
- E.g. GnRH release

Infradian > 24-h cycle
- E.g. menstrual cycle

(Chapter 7). During childbirth, stretch receptors and nerves from the distended vagina stimulate the posterior pituitary to release oxytocin. The rise in oxytocin causes more uterine contraction, further activating the stretch receptors. The role of oxytocin in the suckling–milk ejection reflex is similar. In each instance, the positive feedback amplifies a signal until there is a break in the circuit, either ovulation, birth of the baby or the cessation of suckling.

Inhibitory control

The secretion of some hormones is under inhibitory as well as stimulatory control. Somatostatin, a hypothalamic hormone, prevents the secretion of GH so that when somatostatin secretion is diminished, GH secretion is enhanced. Prolactin is similarly controlled by tonic inhibition from dopamine.

Endocrine rhythms

Superimposed on the regulatory systems described above, many of the body's activities show additional periodic or cyclical changes (Box 1.5). Control of these rhythms commonly arises from the nervous system via the hypothalamus. Some appear independent of the environment, whereas others are coordinated and 'entrained' by external cues (e.g. the 24-h light/dark cycle, which becomes temporarily disrupted in jetlag). Cortisol secretion is maximal between 0400h and 0800h as we awaken and minimal as we retire to bed. In contrast, GH and prolactin are secreted maximally ~1 h after falling asleep. Clinically, this knowledge is important as investigation must be referenced according to hour-by-hour and day-to-day variability. Otherwise, such laboratory tests may be invalid or, indeed, misleading.

Endocrine disorders

The chapters in Part 2 largely focus on organ-specific endocrinology and associated endocrine disorders. Diabetes and obesity in Part 3 has now become its own specialized branch of endocrinology. Nevertheless, it is possible to regard all endocrine abnormalities as *too much*, *too little* or *disordered* production of hormone. Some clinical features occur because of compensatory overproduction of hormones. For example, Addison disease is a deficiency of cortisol from the adrenal cortex (Chapter 6), which reduces negative feedback on ACTH production at the anterior pituitary. ACTH rises and stimulates melanocytes in the skin to increase pigmentation, especially in unusual locations, which is a striking sign of Addison disease.

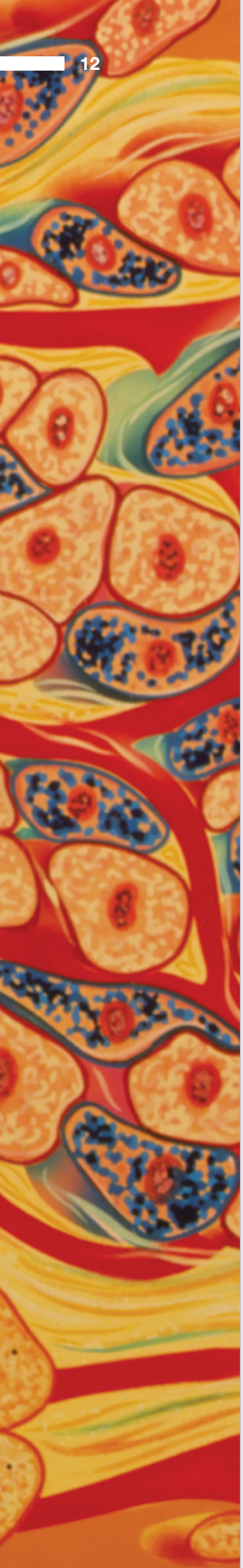
Imbalanced hormone production may occur when a particular enzyme is missing because of a genetic defect. For example, in congenital adrenal hyperplasia, lack of 21-hydroxylase prevents adequate cortisol synthesis (Chapter 6). Other pathways within adrenocortical cells remain intact, leading to excess production of sex steroids that masculinize aspects of the female body. Endocrine disorders may also arise from abnormalities in hormone receptors or downstream signalling pathways. The commonest example is type 2 diabetes, which is characterized, at least in part, by resistance to insulin action in target tissues (Chapter 13).

For endocrine organs under regulation by the hypothalamus and anterior pituitary, the associated disorders can also be categorized according to site. When disease is located in the end organ it is termed 'primary'. When the end organ is affected because of an upstream problem in the anterior pituitary (either under-activity or over-activity) it is termed secondary, while in tertiary disease, the pathology resides in the hypothalamus.

Tumours affect all organs and tissues. In endocrinology, these tumours are most commonly sporadic and benign but they may over-secrete hormones resulting in organ-specific syndromes. These are described in the appropriate chapters of Part 2. Endocrine tumourigenesis may also affect multiple endocrine organs as unusual clinical syndromes. These are described in Chapter 10.

Key points

- Endocrinology is the study of hormones, defined classically by their secretion into the bloodstream
- The endocrine and nervous systems are the body's two major communication systems
- A hormone is a chemical messenger that elicits specific effects by binding to a receptor on or inside target cells
- The three major types of hormones are peptides and the derivatives of amino acids or cholesterol
- Negative and, occasionally, positive feedback, and cyclical mechanisms operate to regulate hormone production, commonly as part of complex multiorgan systems or axes
- Clinical endocrine disorders usually reflect too much, too little or dysregulated hormone production



CHAPTER 2

Basic cell biology and hormone synthesis

Key topics

Learning objectives

- To appreciate the organization, structure and function of DNA
- To understand mitosis and meiosis
- To understand peptide or protein hormone production
- To understand how enzyme cascades generate steroid and amino acid-derived hormones

This chapter aims to introduce some of the basic principles that are needed to understand later chapters

Essential Endocrinology and Diabetes, Seventh Edition. Richard I.G. Holt and Neil A. Hanley.

This chapter introduces five major themes: chromosomes and DNA, the synthesis of the three different categories of hormone (peptide and proteins, and hormones derived from either amino acids or cholesterol), and hormone transport in the circulation. How hormones exert their actions is covered in Chapter 3.

The human genome is made up of deoxyribonucleic acid (DNA), assembled into 46 chromosomes in the nucleus (Box 2.1). The DNA contains genes, which are the templates for synthesizing proteins. There are approximately 30,000 human genes that encode proteins. There are many thousands of other genes which are never translated into protein and play important regulatory roles, mostly within the nucleus. Each gene serves as the template for generating many copies of messenger ribonucleic acid (mRNA) by a process called transcription or gene expression. This transcription into mRNA is the means by which the information contained within a single gene becomes amplified and turned into many replica proteins. Specific proteins define the particular phenotype of a cell-type (e.g. a thyroid cell that synthesizes thyroid hormone rather than the complement of proteins that, for instance, might lead to a beating cardiomyocyte). More commonplace proteins carry out basic functions such as the metabolic processes common to all cells. Proteins on the cell surface act as receptors that initiate intracellular signalling, which in turn is reliant on proteins that function as enzymes. Eventually, this signalling cascade reaches the nucleus and the proteins within it, called transcription factors. These latter proteins bind or release themselves from areas of DNA around genes to determine whether a gene is expressed (i.e. mRNA is transcribed) or silenced.

Box 2.1 The structure of DNA

- A molecule of deoxyribose (a five-carbon sugar) is linked covalently to one of two types of nitrogenous bases:
  - Purine – adenine (A) or guanine (G)
  - Pyrimidine – thymine (T) or cytosine (C)
  - The base plus the sugar is termed a 'nucleoside', e.g. adenosine
- The addition of a phosphate group to a nucleoside creates a nucleotide. E.g. adenosine mono-, di- or tri-phosphate (according to how many phosphate groups have been added)
- Phosphodiester bonds polymerize the nucleotides into a single strand of DNA
- Two strands, running in opposite directions, 5 prime (5′; upstream) to 3′ (downstream) assemble as a double helix:
  - Hydrogen bonds form between the strands, between the base pairs A–T and G–C
- ~3 billion base pairs comprise the human genome

Chromosomes, mitosis and meiosis

Genomic DNA is wrapped around proteins called histones and packaged into chromosomes. The DNA–histone complex is referred to as chromatin. There are 22 pairs of 'autosomes' and two sex chromosomes; two Xs in females, one X and a Y in males. This paired composition ('diploid') makes females '46,XX' and males '46,XY'. 46 refers to the total number of chromosomes. Distinct chromosomes are only apparent when they are lined up in preparation for cell division. Cell division occurs by two processes, either 'mitosis' or 'meiosis' (Figure 2.1). Mitosis generates two identical daughter cells, each with a full complement of 46 chromosomes, and occurs ~10^{17} times during life in humans. In contrast, meiosis creates gametes (i.e. spermatozoan or ovum), each with 23 chromosomes so that full diploid status is reconstituted at fertilization.

Several chromosomal abnormalities can result in endocrine disorders. During meiosis, if a chromosome fails to separate properly from its partner or if migration is delayed, a gamete might result that lacks a chromosome or has too many. Turner syndrome (45,XO) occurs when one sex chromosome is missing while in Klinefelter syndrome (47,XXY) there is an extra one. Similarly, breakages and rejoining across or within chromosomes produce unusual 'derivative' chromosomes or ones with duplicated or deleted regions (see Figure 4.4). These events can disrupt gene function, e.g. deletion causing congenital loss of a hormone. Duplication can be equally significant. For instance, on the X chromosome, duplication of a region that includes the *dosage-sensitive sex reversal, adrenal hypoplasia critical region gene 1* (*DAX1, also called NR0B1*) overrides normal male development in the 46,XY embryonic gonad to result in an ovarian pathway.

Synthesizing a peptide or protein hormone

Gene transcription and its regulation

The stretches of DNA within genes that are pieced together as mRNA are called exons. Where there is more than one exon (and there may be very many), the intervening lengths of DNA are called introns (Figure 2.2). Introns may be very large. Upstream of the first exon is the 5-prime (5′) flanking region of the gene, which contains the promoter. The promoter is responsible for binding transcription factors at very precise short DNA sequences ('elements') leading to the recruitment of RNA polymerase II, and the onset of transcription. RNA polymerase is the enzyme that 'reads' the DNA code. Commonly, the signal that recruits RNA polymerase to the DNA occurs at a 'TATA' box, a short run of adenosines and thymidines, ~30 base pairs upstream of exon 1 (Figure 2.2) or an area rich in G and C residues. Alternatively, other types of transcription factor can bind sites in and around the promoter leading to a shut-down of transcription ('repression').

Superimposed on this, gene expression often depends on more cell- or tissue-specific transcription factors binding more distantly to specific stretches of DNA ('binding motifs') within 'enhancers' (short stretches of DNA which serve to upregulate

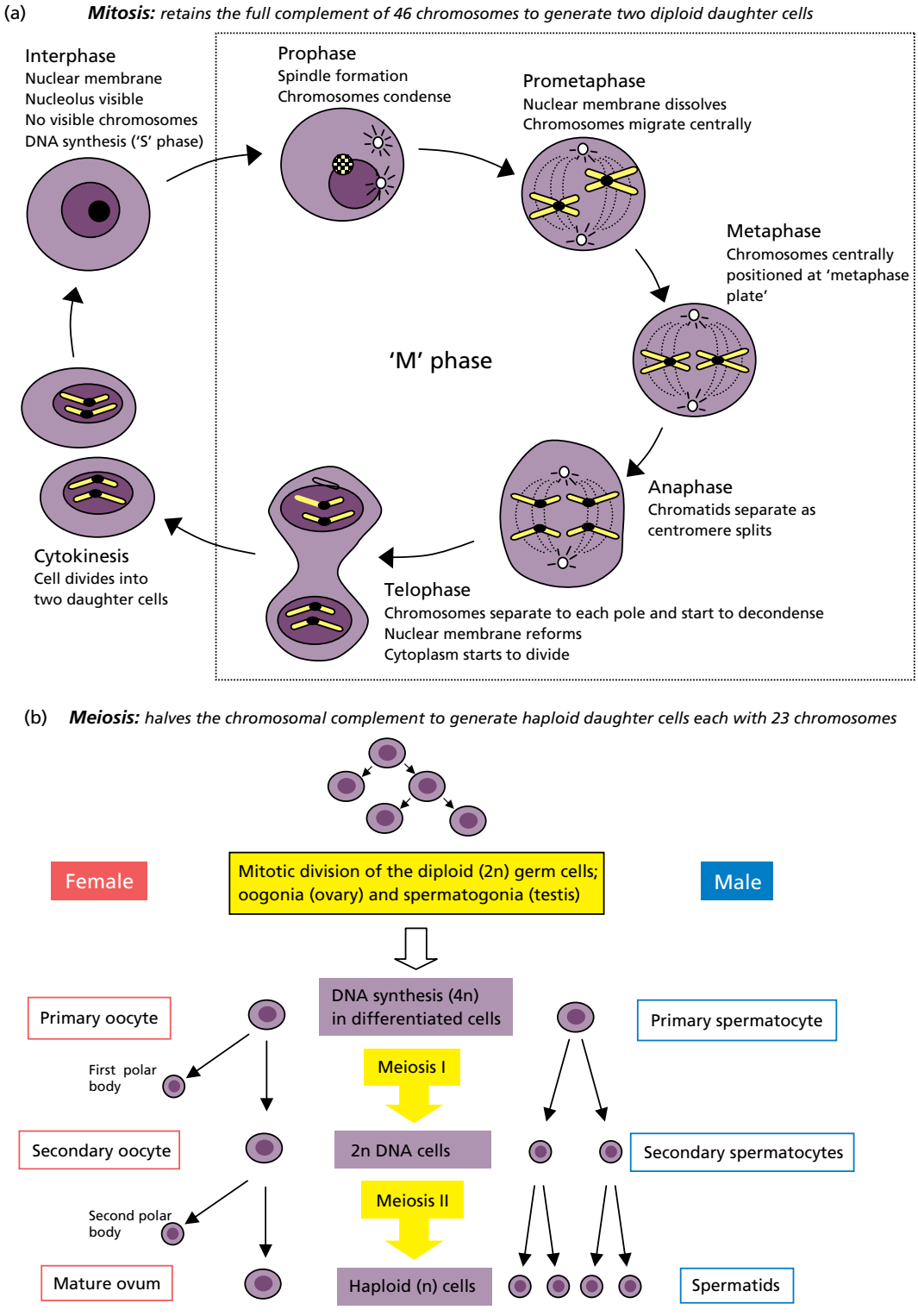


Figure 2.1 Cell division. Prior to mitosis and meiosis, the cell undergoes a period of DNA synthesis ('S' phase) so that the normal diploid status of DNA (2n) temporarily becomes 4n. (a) The stages of mitosis result in each daughter cell containing diploid 2n quantities of DNA. (b) Meiosis is split into two stages, each of which comprises prophase, prometaphase, metaphase, anaphase and telophase. During prophase of meiosis I, the maternally and paternally derived chromosomes align to allow crossing over ('recombination'), a critical aspect of genetic diversity ensuring that each of the final haploid cells is genetically different from the parent cell. The two sister chromatids do not separate, so that the secondary oocyte and spermatocytes each contain 2n quantities of DNA. During the second stage of meiosis, separation of the chromatids results in haploid cells (n). In males, meiosis results in four spermatids. In females, only one ovum is produced from a primary oocyte, with smaller polar bodies extruded at both stages of meiosis.

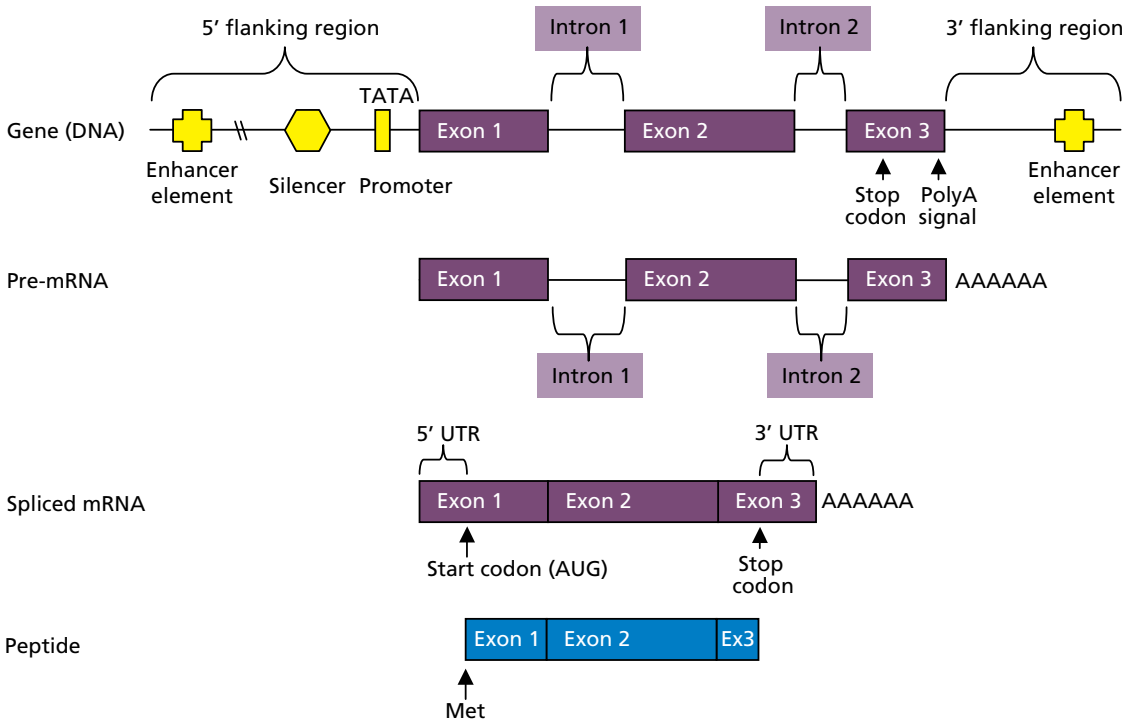


Figure 2.2 Schematic representation of a gene, transcription and translation. In this example, the gene comprises three exons with enhancer elements in the 5′ and 3′ flanking regions and a silencer element upstream of the promoter. UTR, untranslated region; Met, methionine (encoded by the start codon).

gene expression) or 'silencers' (which suppress transcription of the gene). For instance, the transcription factor, steroidogenic factor-1 (SF-1) binds most preferentially to motifs comprising CCAAGGTCA nucleotides to turn on many genes specific to the adrenal cortex and gonad; when SF-1 is absent, both organs fail to form. Enhancers may be many thousands of base pairs (kilobases) up or downstream from the gene they regulate. It is now realised that a significant part of their function, once bound by specific transcription factors, is to create three-dimensional looping so that the enhancers and promoters come together allowing stable recruitment of RNA polymerase. Silencers are most commonly located in the immediate 5' flanking region of the gene.

There is another layer of complexity governing how genes are expressed. Epigenetics is the study of how gene expression is regulated by mechanisms beyond the precise DNA sequence. Methylation of DNA around genes tends to silence expression. Modification to histones, such as acetylation or methylation, alters the chromatin structure to make enhancers and promoters either accessible or inaccessible to transcription factors. Acetylation tends to open up the chromatin structure, facilitating gene expression, whereas methylation tends to close it down and silence transcription. Genomic imprinting is an epigenetic phenomenon involving DNA methylation and modifications to histones such that gene expression varies according to which parent the particular chromosome came from.

RNA contains ribose sugar moieties rather than deoxyribose. RNA polymerase attaches ribonucleotides together to generate a single strand of mRNA that correlates to the DNA code of the gene, except that in place of thymidine, a very similar nucleoside, uridine, is incorporated. The initial mRNA strand (pre-mRNA) is processed so that intronic gene regions are removed and only the exonic sequences are 'spliced' together. Not all exonic regions encode protein; stretches at either end constitute the 5′ and 3′ untranslated regions (UTRs) (Figure 2.2). Within the 3′ UTR, mRNA transcription is terminated by a specific motif, the polyadenylation signal, ~20 base pairs upstream of where the mRNA gains a stretch of adenosine residues. This polyA tail provides stability as the mRNA is moved from the nucleus to the cytoplasm for translation into protein.

Translation into protein

mRNA is transported to the ribosomes, where protein synthesis occurs by 'translation' (Figure 2.3a). The ribosomes are attached to the outside of the endoplasmic reticulum (ER), leading to the description of 'rough ER'. The ribosome is an RNA–protein complex that reads the mRNA sequence in groups of three nucleotides, called a codon. Each codon represents an amino acid. The start codon is the sequence of A–U–G nucleotides (corresponding to ATG in the genomic DNA) and specifies the amino acid methionine (Figure 2.2). From this point onwards, translation continues until a 'stop' codon is encountered (UAA, UGA or UAG).

By understanding these normal events of gene transcription and protein translation, it becomes possible to appreciate how mutations (sequence errors) in the genomic DNA lead to a miscoded, and consequently malfunctional, protein (Box 2.2).

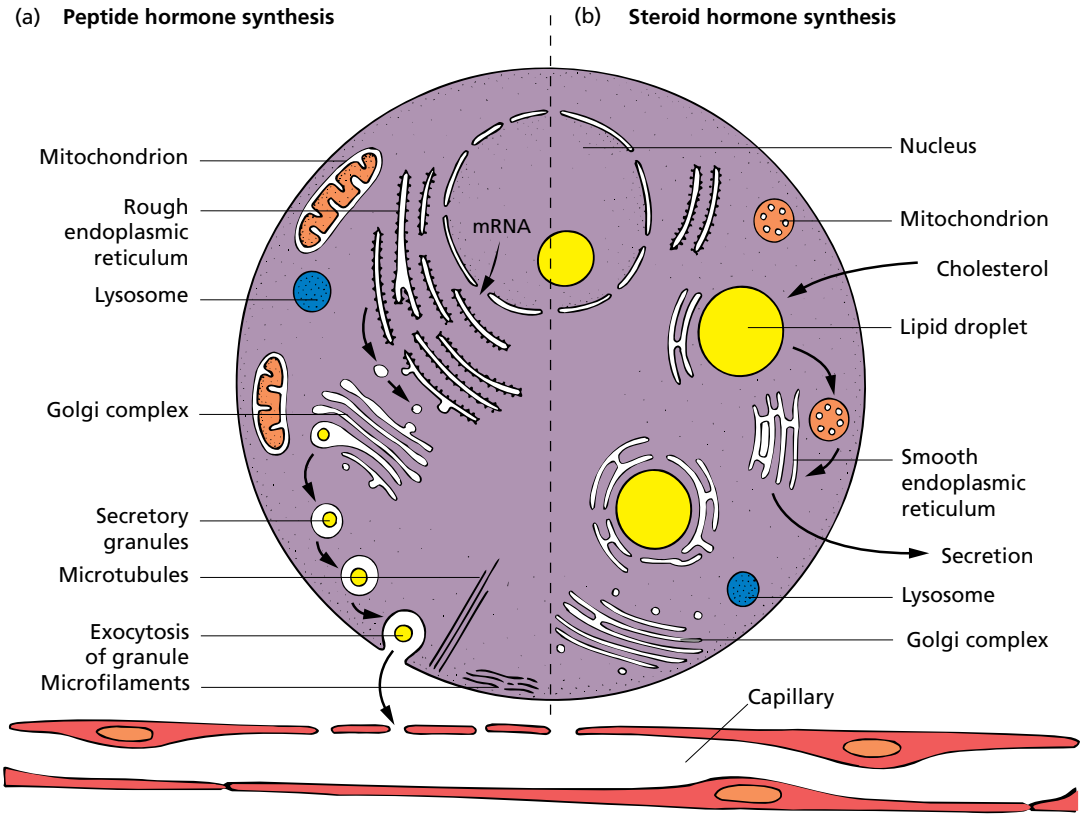


Figure 2.3 (a) Peptide hormone-synthesizing and (b) steroid hormone-synthesizing cells. In (b), cholesterol enters the cell via the low-density lipoprotein receptor which is internalized.

Box 2.2 Genetic, genomic and epigenetic abnormalities that can result in endocrinopathy

Abnormalities in DNA (genetic)

- Base substitution – swapping different nucleotides
- Insertion or deletion – alters frame if exonic and not a multiple of three

Chromosomal abnormalities (genomic)

- Numerical – loss of an X chromosome as in Turner syndrome (45,XO)
- Structural:
  - Inversion – region of a chromosome is turned upside down
  - Translocation – regions swapped between chromosomes
  - Duplication – region of a chromosome is present twice
  - Deletion – region of a chromosome is excised and lost

Imprinting abnormalities (epigenetic)

- Methylation – altered methylation changing local gene expression, such as Beckwith–Wiedemann syndrome with neonatal hypoglycaemia or transient neonatal diabetes mellitus associated with over-expression of the gene called PLAGL1
- Structural chromosomal abnormalities (above) can also cause imprinting errors

An entire gene may be missing ('deleted') or duplicated. An erroneous base pair in an enhancer or the promoter region may impair binding of a critical transcription factor and lessen a gene's expression. A similar error in a coding exon might translate a different amino acid or even miscode a premature stop codon. Small deletions or insertions of one or two base pairs throw the whole triplet code out of frame. A mutation at the boundary between an intron and an exon can corrupt splicing so that the intron is included in the mature mRNA. All of these genetic events can affect endocrinology either as developmental disorders when that the fault is likely to be present in all or many of the body's cells, or as acquired change later in life, potentially predisposing to the formation of an endocrine tumour (Chapter 10).

Post-translational modification of peptides

Some polypeptides can function as hormones after little more than removal of the starting methionine, e.g. thyrotrophin-releasing hormone (TRH), which comprises only three amino acids. Larger peptides can fold into three-dimensional structures, which may contain helical or pleated domains. These shapes provide stability and affect how one protein interacts with another (e.g. how a hormone might bind to its receptor).

For hormones that require secretion out of the cell, additional modifications are important (Figure 2.4). The precursor

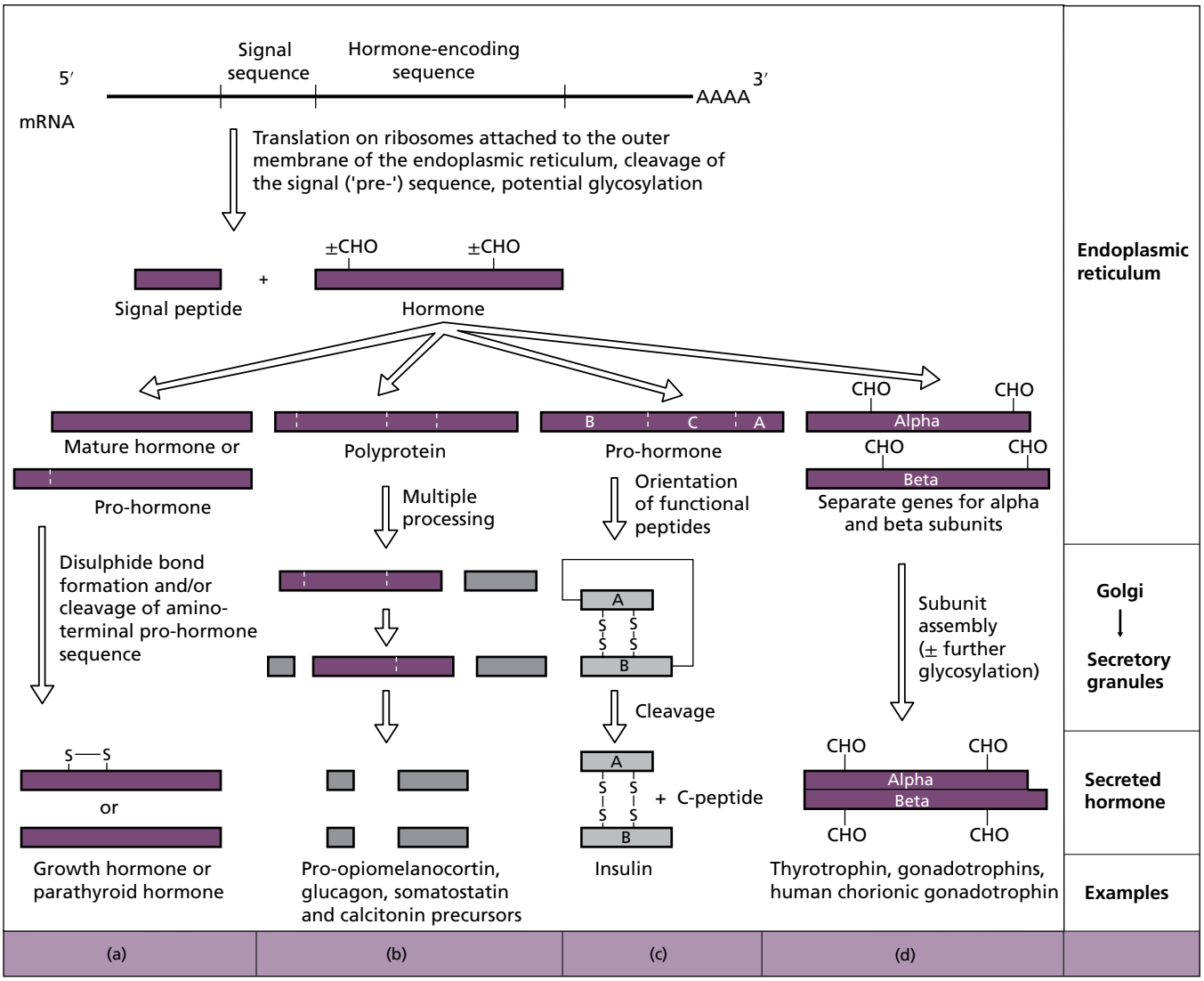


Figure 2.4 Potential post-translational modifications of peptide hormones. Four types are shown. (a) Simple changes such as removal of the amino-terminal 'pro-' extension prior to secretion (e.g. parathyroid hormone) or the addition of intra-chain disulphide bonds (e.g. growth hormone). (b) Multiple processing of a 'polyprotein' into a number of different peptide hormones (e.g. pro-opiomelanocortin can give rise to adrenocorticotrophic hormone plus melanocyte-stimulating hormone and β-endorphin). (c) Synthesis of insulin requires folding of the peptide and the formation of disulphide bonds. The active molecule is created by hydrolytic removal of a connecting (C)-peptide so that proinsulin gives rise to insulin plus C-peptide in equimolar proportion. (d) Synthesis of larger protein hormones (e.g. thyroid-stimulating hormone, luteinizing hormone, follicle-stimulating hormone and human chorionic gonadotrophin) from two separate peptides that complex together. The four hormones share the same α-subunit with a hormone-specific β-subunit.

peptide, called a pre-prohormone, carries a lipophilic signal peptide at the amino terminus. This sequence is recognized by channel proteins so that the immature peptide can cross the ER membrane. Once inside the ER, the signal peptide is excised in preparation for other post-translational changes (Figure 2.4a–d).

Disulphide bridges are formed in certain proteins (e.g. growth hormone or insulin; Figure 2.4a and c). Certain carbohydrates may be added to form glycoproteins (Figure 2.4d). Some prohormones (e.g. pro-opiomelanocortin and pro-glucagon) are processed into several final products, whereas others are assembled as a combination of distinct peptide chains, each synthesized from different genes, e.g. thyroid-stimulating hormone (TSH) or luteinizing hormone (LH).

The complete protein is then packaged into membrane-bound vesicles, which may contain specific 'endopeptidases'. These enzymes are responsible for final hormone activation by cleaving off the 'pro-' portion, as occurs with the release of insulin and its by-product, C-peptide (Figure 2.4c). Such post-translational modifications are essential stages in hormone synthesis (Box 2.3).

Box 2.3 Role of post-translational modifications

Post-translational modifications mean:

- Great diversity of hormone action can be generated from a more limited range of protein-coding genes
- The synthesizing cell is protected from being overwhelmed by its own hormone action

Storage and secretion of peptide hormones

Newly synthesized peptide hormone is stored within the cell in small vesicles or secretory granules. Movement of these vesicles to a position near the cell membrane is influenced by two types of filamentous structure: microtubules and microfilaments (Figure 2.3a). Consequently, the secretion of stored hormone tends to be rapid but only occurs after appropriate stimulation of the cell. Whether this is hormonal, neuronal or nutritional, it usually involves a change in cell membrane permeability to calcium ions. These divalent metal ions are required for interaction between the vesicle and plasma membrane, and for the activation of enzymes, microfilaments and microtubules. The secretory process is called 'exocytosis' (Figure 2.3a). The membrane of the storage granule fuses with the cell membrane at the same time as vesicular endopeptidases are activated. The active hormone is expelled into the extracellular space from where it enters the bloodstream. The vesicle membrane is then recycled within the cell.

Synthesizing a hormone derived from amino acids or cholesterol

In addition to peptides or proteins, hormones can also be synthesized by sequential enzymatic modification of either the amino acids tyrosine and tryptophan, or cholesterol.

Enzyme action and cascades

Enzymes can be divided into classes according to the reactions they catalyze (Table 2.1). In endocrinology, they frequently operate in cascades where the product of one reaction serves as the substrate for the next. The most simplistic representation of an enzymatic reaction is a physical interaction between the substrate and the enzyme at the latter's 'active site'. This proximity catalyzes a molecular modification of the substrate into the product. The product has less affinity for the active site and is released. Other macromolecules can also bind to the enzyme outside the active site and function as co-factors, adding more complex regulation to the biochemical reaction.

Patients can present with many endocrine syndromes because of loss of enzyme function. For instance, gene mutation might lead to substitution of an amino acid at a key position of an enzyme's active site. The three-dimensional structure might be affected so significantly that the substrate is no longer converted to product. In the enzyme cascade that synthesizes cortisol, such mutations can cause various forms of congenital adrenal hyperplasia (CAH) (Chapter 6). Understanding the biochemical

Table 2.1 Definition and classification of enzymes

Definition		
An enzyme is a biological macromolecule – most frequently a protein – that catalyzes a biochemical reaction		
Catalysis increases the rate of reaction, e.g. the disappearance of substrate and generation of product		
Enzyme action is critical for the synthesis of hormones derived from amino acids and cholesterol		
Classification		
Enzyme	**Catalytic function**	**Example (and relevance)**
Hydrolases	Cleavage of a bond by the addition of water	Cytochrome P450 11A1/cholesterol side-chain cleavage (CYP11A1; an early step in steroid hormone biosynthesis)
Lyases	Removal of a group to form a double bond or addition of a group to a double bond	Cytochrome P450 17α-hydroxylase/17–20 lyase (CYP17A1; step in the synthesis of steroid hormones other than aldosterone)
Isomerases	Intramolecular rearrangements	3β-Hydroxysteroid dehydrogenase/δ-4,5-isomerase isoforms (HSD3B; a step in the synthesis of many major steroid hormones)
Oxidoreductase	Oxidation and reduction	11β-Hydroxysteroid dehydrogenase isoforms (HSD11B; inter-conversion of cortisol and cortisone)
Ligases or synthases	Join two molecules together	Thyroid peroxidase (TPO; a step in the synthesis of thyroid hormone)
Transferases	Transfer of a molecular group from substrate to product	Phenolethanolamine *N*-methyl transferase (PNMT; conversion of norepinephrine to epinephrine)