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Rook's Textbook of Dermatology

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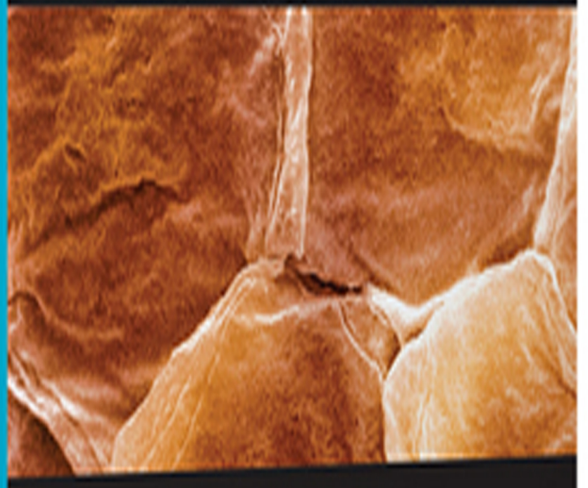

EDITED BY

Christopher Griffiths

Jonathan Barker

Tanya Bleiker

Robert Chalmers

Daniel Creamer

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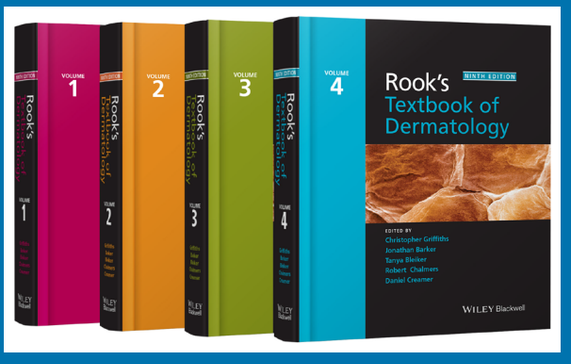

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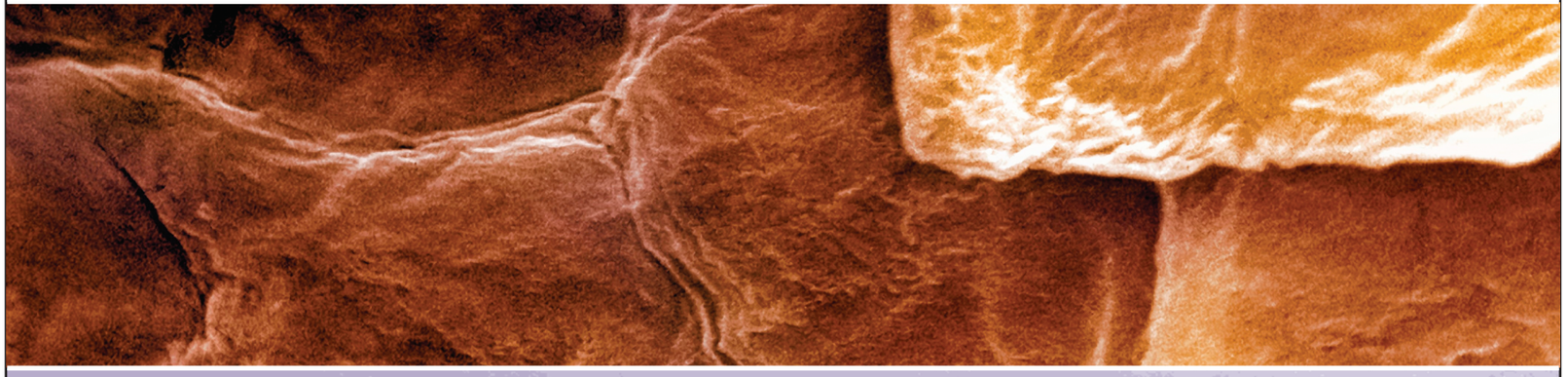

The Editors
From left to right: Robert Chalmers, Jonathan Barker, Christopher Griffiths, Tanya Bleiker, Daniel Creamer

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EDITED BY

Christopher E. M. Griffiths MD, FRCP, FMedSci
Professor of Dermatology
The Dermatology Centre, Salford Royal NHS Foundation Trust
The University of Manchester
Manchester Academic Health Science Centre
Manchester, UK

Jonathan Barker MD, FRCP, FRCPath
Professor of Medical Dermatology
St John's Institute of Dermatology
Division of Genetics and Molecular Medicine
Faculty of Life Sciences and Medicine
King's College London
London, UK

Tanya Bleiker FRCP
Consultant Dermatologist
Derby Teaching Hospitals NHS Foundation Trust
Derby, UK

Robert Chalmers FRCP
Honorary Consultant Dermatologist
The Dermatology Centre, Salford Royal NHS Foundation Trust
Manchester Royal Infirmary
Manchester, UK

Daniel Creamer MD, FRCP
Consultant Dermatologist
King's College Hospital
London, UK

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Associate Editors

Contributors

Christina Antoniou
MD
Professor of Dermatology, Department of Dermatology, University of Athens Medical School, Andreas Sygros Hospital, Athens, Greece
Chapter 132

Michael R. Ardern-Jones
BSc, MB BS, DPhil, FRCP
Dermatoimmunology, University of Southampton, Southampton General Hospital, Southampton, UK
Chapters 12, 41, 118

H. Ruth Ashbee
PhD
Principal Clinical Scientist, Mycology Reference Centre, Leeds Teaching Hospitals NHS Trust, Leeds, UK
Chapter 32

Matthias Augustin
MD
Director and Professor of Dermatology and Health Economics, Institute for Health Services Research in Dermatology and Nursing, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
Chapter 6

Robert Baran
University of Franche-Comté, Nail Disease Centre, Cannes, France
Chapter 95

Richard J. Barlow
MD, FRCP
Consultant Dermatologist, St John's Institute of Dermatology, Guy's and St Thomas' NHS Foundation Trust, London, UK
Chapter 23

Saqib J. Bashir
BSc(Hons), MB ChB, MD, FRCP
Consultant Dermatological Surgeon, King's College, Hospital, London, UK
Chapters 123, 125

Tanya N. Basu
MA, PhD, MRCP
Consultant Dermatologist, King's College Hospital, London, UK
Chapter 38

Jürgen C. Becker
MD, PhD
Head of Department, Translational Skin Cancer Research (TSCR), German Cancer Consortium (DKTK), University Clinic of Essen, Essen, Germany
Chapter 145

Emma C. Benton
MB ChB, MRCP
Consultant Dermatologist, St John's Institute of Dermatology, Guy's and St Thomas' NHS Foundation Trust, London, UK
Chapter 4

John Berth-Jones
FRCP
Consultant Dermatologist, University Hospital, Coventry, UK
Chapter 18

Anthony Bewley
BA(Hons), MB ChB, FRCP
Consultant Dermatologist, Department of Dermatology, Barts Health NHS Trust, London; and Senior Lecturer, Queen Mary College of Medicine, University of London, London, UK
Chapters 84, 86

Balbir S. Bhogal
BMS, BSc, MSc
Head, Immunodermatology Laboratory, St John's Institute of Dermatology, Guy's and St Thomas' NHS Foundation Trust, London, UK
Chapter 3

Michael Bigby
MD, FAAD
Associate Professor of Dermatology, Harvard Medical School and Beth Israel Deaconess Medical Center, Boston, MA, USA
Chapter 17

Laurence M. Boon
MD, PhD
Coordinator, Center for Vascular Anomalies, Cliniques Universitaires Saint-Luc; Professor, Division of Plastic Surgery, Cliniques Universitaires Saint-Luc; Professor of Human Genetics, Human Molecular Genetics, de Duve Institute, Université catholique de Louvain, Brussels, Belgium
Chapter 73

Elena Borzova
DMedSci
Associate Professor of Allergy, Russian Medical Academy of Postgraduate Education, Moscow, Russian Federation
Chapter 44

Johnny Bourke
MD, FRCPI
Consultant Dermatologist, South Infirmary-Victoria University Hospital, Cork; Clinical Senior Lecturer, University College, Cork, Ireland
Chapters 61, 64, 97

Stephen M. Breathnach
MA, MB BChir, MD, PhD, FRCP, DipDerm(USA)
Consultant Dermatologist, St John's Institute of Dermatology, Guy's and St Thomas' NHS Foundation Trust, London, UK
Chapter 37

Aparna Briggs
MA, MB BChir, MRCP, DipGUM, DFSRH, DipHIV
Specialist Registrar in Genitourinary Medicine, Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, UK
Chapter 30

Christine Bundy
PhD, CPsychol, AFBPS
Senior Lecturer in Behavioural Medicine and Honorary Consultant Health Psychologist, Institute of Inflammation and Repair, University of Manchester, Manchester, UK
Chapter 11

Christopher B. Bunker
MA, MD, FRCP
Consultant Dermatologist, University College London Hospitals and Chelsea & Westminster Hospitals, London, UK; Professor of Dermatology, University College and Imperial College, London, UK
Chapters 31, 111

A. David Burden
MD, FRCP
Consultant Dermatologist, Western Infirmary, Glasgow; Professor of Dermatology, University of Glasgow, Glasgow, UK
Chapter 35

Nigel Burrows
MD, FRCP
Consultant Dermatologist, Department of Dermatology, Addenbrooke's Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK
Chapter 72

Eduardo Calonje
MD, DipRCPath
Consultant Dermatopathologist, St John's Institute of Dermatology, Guy's and St Thomas' NHS Foundation Trust, London, UK
Chapters 3, 137, 138

Rino Cerio
BSc, MB BS, LRCP, MRCS, FRCP(Lond), FRCP(Edin), FRCPath, DipRCPath, ICPath
Consultant Dermatologist and Director of Dermatopathology, The Royal London Hospital, Bart's Health NHS Trust, London; Queen Mary's Medical and Dental School, University of London, London, UK
Chapter 47

Kelly B. Cha
MD, PhD
Assistant Professor of Dermatology, Department of Dermatology, University of Michigan Health System, Ann Arbor, MI, USA
Chapter 143

Amy Y.-Y. Chen
MD, FAAD
Assistant Professor of Dermatology, Department of Dermatology, University of Connecticut School of Medicine, Canton, CT, USA
Chapter 100

Ai-Lean Chew
MB ChB, MRCP
Consultant Dermatologist, Guy's and St Thomas' NHS Foundation Trust, London; King's College Hospital, London, UK
Chapters 123, 125

Fiona Child
MD, FRCP
Consultant Dermatologist, St John's Institute of Dermatology, Guy's and St Thomas' NHS Foundation Trust, London, UK
Chapters 135, 140

Olivier Chosidow
MD, PhD
Professor and Chairman, UPEC-Université Paris-Est Créteil Val de Marne, Department of Dermatology, Hôpital Henri Mondor, Créteil, France
Chapter 34

Anthony C. Chu
FRCP
Professor of Dermatology, Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, UK
Chapters 36, 87

Derek H. Chu
MD
Resident, Department of Dermatology, Children's Hospital of Philadelphia, Philadelphia, PA, USA
Chapter 63

Lis Cordingley
PhD, CPsychol
Senior Lecturer in Health Psychology, Institute of Inflammation and Repair, University of Manchester, Manchester, UK
Chapter 11

Ian H. Coulson
BSc, MB BS, FRCP
Consultant Dermatologist, Burnley General Hospital, East Lancashire NHS Trust IHC, Burnley, UK
Chapters 4, 94

Daniel Creamer
MD, FRCP
Consultant Dermatologist, Department of Dermatology, King's College Hospital, London, UK
Chapters 53, 119, 121

Robert Dawe
MB ChB, MD, FRCPE
Consultant Dermatologist and Honorary Reader, Photobiology Unit, University of Dundee, Ninewells Hospital and Medical School, Dundee, UK
Chapter 127

David A. R. de Berker
BA, MB BS, MRCP
Consultant Dermatologist, Bristol Dermatology Centre, Bristol Royal Infirmary, Bristol, UK
Chapters 89, 95

Pascal Delaunay
PharmD, PhD
Professor and Chairman, Department of Parasitology-Mycology, Centre Hospitalier Universitaire de Nice, Hôpital de l'Ardet, Nice, France; INSERM, Centre Méditerranéen de Médecine Moléculaire, Université de Nice-Sophia Antipolis, Nice, France
Chapter 34

Christopher P. Denton
PhD, FRCP
Professor of Experimental Rheumatology, Division of Medicine, University College London, London; Consultant Rheumatologist, Royal Free London NHS Foundation Trust, London, UK
Chapter 56

Nemesha Desai
MB BS(Hons), BSc(Hons), FRCP, PGCHE
Consultant, St John's Institute of Dermatology, Guy's and St Thomas' NHS Foundation Trust, London, UK
Chapter 92

Anne Dompmartin
MD, PhD
Coordinator, Consultation of Vascular Anomalies, Université de Caen Basse Normandie, Caen; Department of Dermatology, CHU Caen, Caen, France
Chapter 73

Christopher Downing
MD
Dermatologist, University of Texas Health Science Center, Houston, TX, USA
Chapters 33, 131

Reinhard Dummer
MD
Professor, Department of Dermatology, University Hospital Zurich, Zurich, Switzerland
Chapter 143

Alison B. Durham
MD
Assistant Professor of Dermatology, Department of Dermatology, University of Michigan Health System, Ann Arbor, MI, USA
Chapter 143

Jan Dutz
MD, FRCPC
Professor, Skin Care Centre, Vancouver General Hospital, Vancouver, BC, Canada
Chapter 51

E. Anne Eady
PhD
Principal Research Fellow, Department of Dermatology, Harrogate and District NHS Foundation Trust, Harrogate, UK
Chapter 90

David J. Eedy
MD, FRCP
Consultant Dermatologist, Department of Dermatology, Craigavon Area Hospital, Craigavon, UK
Chapter 85

Lennart Emtestam
MD, PhD
Professor, Department of Medicine Huddinge, Karolinska Institutet, Stockholm; Department of Dermatology, Karolinska University Hospital, Stockholm, Sweden
Chapter 147

Robyn Evans
MD, CCFP
Director, Wound Healing Clinic, Women's College Hospital; Lecturer, University of Toronto, Toronto, Ontario, Canada
Chapter 124

Khaled Ezzedine
MD, PhD
Doctor and Consultant, Service de Dermatologie et Dermatologie Pédiatrique, Hôpital St André, Bordeaux, France
Chapter 70

Paul Farrant
MB BS, BSc(Hons), FRCP
Consultant Dermatologist, Brighton and Sussex University Hospitals, Brighton, UK
Chapters 89, 107

Hiva Fassihi
MA, MD, MRCP
Consultant Dermatologist and Clinical Lead for the National XP Service, St John's Institute of Dermatology, Guy's and St Thomas' NHS Foundation Trust, London, UK
Chapter 78

Louise Fearfield
MA, DM, FRCP
Consultant Dermatologist, Chelsea & Westminster and The Royal Marsden Hospitals, London, UK
Chapter 120

Andrew Y. Finlay
CBE, FRCP(Lond, Glasg)
Professor of Dermatology, Department of Dermatology and Wound Healing, Cardiff University School of Medicine, Cardiff, UK
Chapter 16

Gary Fisher
PhD
Professor of Dermatology, University of Michigan Medical Center, Ann Arbor, MI, USA
Chapter 155

Carsten Flohr
FRCP, FRCPCH, MA, MPhil, DLSHTM, MSc, PhD
Reader and Consultant, St John's Institute of Dermatology, Guy's and St Thomas' NHS Foundation Trust, London, UK
Chapter 41

Paul D. Flynn
PhD, FRCP
Consultant Physician, Acute and Metabolic Medicine, Addenbrooke's Hospital, Cambridge; Associate Lecturer, Department of Medicine, University of Cambridge, Cambridge, UK
Chapter 62

John Frew
MB ChB, MRCP, FRCR
Consultant Clinical Oncologist, Northern Centre for Cancer Care, Freeman Hospital, Newcastle upon Tyne, UK
Chapter 24

Amit Garg
MD, FAAP
Associate Professor and Founding Chair, Department of Dermatology, Hofstra NSLIJ School of Medicine, Manhasset, NY, USA
Chapter 100

Caroline Gaudy-Marqueste
MD, PhD
Assistant Professor of Dermatology, Service de Dermatologie et Cancérologie Cutanée, Hôpital de la Timone, Marseille, France
Chapter 143

Andrew R. Gennery
MD, FRCPCH, MRCP
Reader in Paediatric Immunology and HSCT, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK
Chapter 82

Sam Gibbs
FRCP
Consultant Dermatologist, Great Western Hospital, Swindon, UK
Chapter 15

Mary T. Glover
MA, FRCP, FRCPCH
Consultant Dermatologist, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK
Chapter 117

Robert Gniadecki
MD, PhD
Consultant and Clinical Professor of Dermatology, Department of Dermatology, Bispebjerg Hospital and University of Copenhagen, Copenhagen, Denmark; and Division of Dermatology, University of Alberta, Alberta, Canada
Chapter 148

Chee-Leok Goh
MD, MB BS, MRCP(UK), MMed, FRCPE
Senior Consultant Dermatologist and Clinical Professor, National Skin Centre, National University of Singapore, Singapore
Chapter 159

Simone M. Goldinger
MD
Senior Physician, Department of Dermatology, University Hospital Zurich, Zurich, Switzerland
Chapter 143

Portia C. Goldsmith
MD, FRCP
Consultant Dermatologist, Barts Health and Homerton University Hospital, London, UK
Chapter 103

Mark Goodfield
MD, FRCP
Professor and Consultant Dermatologist, Department of Dermatology, Chapel Allerton Hospital, Leeds, UK
Chapters 51, 52, 54, 55

Kristiana Gordon
MB BS, MRCP, MD(Res)
Consultant in Dermatology and Lymphovascular Medicine, St George's Hospital, London, UK
Chapter 105

Patrick Gordon
FRCP, PhD, MB BS
Consultant Rheumatologist and Honorary Senior Lecturer, Department of Rheumatology, King's College Hospital, London, UK
Chapter 53

Michael Gossop
BA, PhD
Emeritus Professor, National Addiction Centre, King's College London, London, UK
Chapter 121

Clive E. H. Grattan
MA, MD, FRCP
Consultant Dermatologist, Norfolk and Norwich University Hospital, Norwich; and St John's Institute of Dermatology, Guy's and St Thomas' NHS Foundation Trust, London, UK
Chapters 42, 43, 44, 45, 46

Malcolm Greaves
MD, PhD, FRCP
Emeritus Professor of Dermatology and Honorary Consultant in Dermatology, Cutaneous Allergy Clinic, St John's Institute of Dermatology, Guy's and St Thomas' NHS Foundation Trust, London, UK
Chapter 83

Jean Jacques Grob
MD
Professor of Dermatology, Head of Dermatology and Skin Cancers, Service de Dermatologie et Cancérologie Cutanée, Hôpital de la Timone, Marseille, France
Chapter 143

Richard Groves
MB BS, FRCP
Head, Clinical Immunodermatology, St John's Institute of Dermatology, Guy's and St Thomas' NHS Foundation Trust, London, UK
Chapter 50

Girish Gupta
MB ChB, FRCP
Consultant Dermatologist and Skin Cancer Lead, Department of Dermatology, Monklands Hospital, Airdrie, UK
Chapter 142

Nadi K. Gupta
MB ChB, MRCP, DipGUM, DFFP, DipHIV
Honorary Senior Clinical Lecturer, University of Sheffield, Sheffield; Consultant Physician in Genitourinary Medicine, Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield; Rotherham Hospital NHS Foundation Trust, Rotherham, UK
Chapter 30

Richard H. Guy
MA, PhD
Professor of Pharmaceutical Sciences, Department of Pharmacy and Pharmacology, University of Bath, Bath, UK
Chapter 13

Jürg Hafner
MD
Professor, Dermatologist, Angiologist and Phlebologist (SIME/FMH) and Senior Staff, Department of Dermatology, University Hospital of Zurich, Zurich, Switzerland
Chapter 104

Philip J. Hampton
MB BS, BMedSci, PhD, FRCP
Consultant Dermatologist, Newcastle Hospitals NHS Trust, Newcastle Upon Tyne, UK
Chapter 49

Catherine A. Harwood
MA, PhD, FRCP
Professor of Dermatology and Consultant Dermatologist, Department of Dermatology, The Royal London Hospital, London; Centre for Cutaneous Research, Blizard Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, UK
Chapter 146

Roderick J. Hay
DM, FRCP, FRCPath, FMedSci
Professor of Cutaneous Infection, Dermatology Department, King's College Hospital, London, UK
Chapters 26, 32, 93

Elisabeth M. Higgins
MA, FRCP
Consultant Dermatologist, King's College Hospital, London, UK
Chapter 117

Colin A. Holden
BSc, MD, FRCP
Consultant Dermatologist, Department of Dermatology, Epsom & St Helier NHS Trust, St Helier Hospital, Carshalton, UK
Chapter 41

S. Walayat Hussain
BSc(Hons), MB ChB, MRCP(UK), FRACP, FACMS
Consultant Dermatological Surgeon, Leeds Centre for Dermatology, Chapel Allerton Hospital, Leeds Teaching Hospitals NHS Trust, Leeds, UK
Chapter 20

Sally Ibbotson
MB ChB, MD, FRCPE
Clinical Senior Lecturer in Photobiology and Honorary Consultant Dermatologist, Photobiology Unit, University of Dundee, Ninewells Hospital and Medical School, Dundee, UK
Chapters 21, 22, 127

John R. Ingram
MA, MSc, DM, MRCP(Derm), FAcadMEd
Senior Lecturer and Consultant Dermatologist, Department of Dermatology & Wound Healing, Cardiff University, Cardiff, UK
Chapter 39

Alan D. Irvine
MD, FRCP, FRCPI
Consultant Dermatologist, Our Lady's Children's Hospital, Crumlin; St. James's Hospital, Dublin; and Trinity College, Dublin, Ireland
Chapters 76, 77, 79, 80

Peter Itin
MD
Professor for Dermatology and Venerology, Head of Department of Dermatology, University Hospital Basle, Basle, Switzerland
Chapter 67

Natalia Jaimes
MD
Attending Physician, Dermatology Service, Universidad Pontificia Bolivariana and Aurora Skin Cancer Center, Medellín, Colombia
Chapter 144

Gregor B. E. Jemec
MD, DMSc
Professor and Clinical Lead, Department of Dermatology, Roskilde Hospital; Health Sciences Faculty, University of Copenhagen, Copenhagen, Denmark
Chapters 92, 93

Melinda V. Jen
MD
Assistant Professor, Department of Dermatology, Children's Hospital of Philadelphia, Philadelphia, PA, USA
Chapter 63

Marc G. Jeschke
MD, PhD, FACS, FCCM, FRCSC
Professor, Ross Tilley Burn Centre, Sunnybrook Health Sciences Centre, Toronto; Department of Surgery, Division of General Surgery, Plastic Surgery, Department of Immunology, University of Toronto, Toronto; Sunnybrook Research Institute, Toronto, Ontario, Canada
Chapter 126

Timothy M. Johnson
MD
Lewis and Lillian Becker Professor of Dermatology, Professor of Otolaryngology and Surgery (Division of Plastic Surgery), Department of Dermatology, University of Michigan Health System, Ann Arbor, MI, USA
Chapter 143

Charles G. Kelly
MB ChB, MSc, FRCP, FRCR, FBIR, DMRT
Consultant Clinical Oncologist and Lead for Radiotherapy, Northern Centre for Cancer Care, Freeman Hospital, Newcastle upon Tyne; Deputy Degree Program Director and Honorary Clinical Senior Lecturer, Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, UK
Chapter 24

Cameron Kennedy
MA, MB BChir, FRCP
Consultant Dermatologist, Bristol Royal Infirmary and Bristol Royal Hospital for Children, Bristol; Honorary Clinical Senior Lecturer, University of Bristol, Bristol, UK
Chapter 108

Alexandra B. Kimball
MD, MPH, FAAD
Department of Dermatology, Massachusetts General Hospital, Boston, MA, USA
Chapter 156

George R. Kinghorn
OBE, MD, FRCP
Honorary Professor and Consultant Physician in Genitourinary Medicine, Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, UK
Chapters 29, 30

Veronica A. Kinsler
MA, MB BChir, MRCPCH, PhD
Consultant Paediatric Dermatologist and Academic Lead Clinician, Paediatric Dermatology, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK
Chapter 75

Brian Kirby
MD, FRCPI
Consultant Dermatologist, St Vincent's University Hospital, Dublin; Associate Clinical Professor, University College, Dublin, Ireland
Chapter 35

Eubee Koo
BS
Department of Dermatology, Massachusetts General Hospital, Boston, MA, USA
Chapter 156

Magdalene Krensel
MSc
Project Manager in Health Economics, Institute for Health Services Research in Dermatology and Nursing, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
Chapter 6

Alison M. Layton
MB ChB, FRCP
Consultant Dermatologist, Department of Dermatology, Harrogate and District NHS Foundation Trust, Harrogate, UK
Chapter 90

John T. Lear
MD, FRCP
Consultant Dermatologist, Department of Dermatology, Manchester Royal infirmary, Manchester, UK
Chapters 133, 134, 141, 142

Laurence Le Cleach
MD
Consultant Dermatologist, Service de Dermatologie, Satellite Français du Cochrane Skin Group, Hôpital Henri Mondor, Créteil, France
Chapter 37

Haur Yueh Lee
MB BS, MRCP(UK), MMed(IntMed), FAMS(Derm)
Head of Department and Consultant Dermatologist, Department of Dermatology, Singapore General Hospital; Adjunct Assistant Professor, DUKE-NUS Graduate Medical School, Singapore
Chapters 118, 119

Jonathan N. Leonard
BSc, MD, FRCP
Consultant Dermatologist, Department of Dermatology, Imperial College Healthcare NHS Trust, London, UK
Chapter 109

Tabi A. Leslie
BSc(Hons), MB BS(Hons), FRCP(Lond)
Consultant Dermatologist, Royal Free Hospital, London, UK
Chapter 101

Nick J. Levell
MD, FRCP, MB ChB, MBA
Clinical Director of Dermatology, Norwich Medical School, Norfolk and Norwich University Hospital, Norwich; National Specialty Lead Dermatology, National Institute for Health Research, London; Clinical Vice-President, British Association of Dermatologists, London, UK
Chapters 1, 102

Fiona Lewis
MD, FRCP
Consultant Dermatologist, Wexham Park Hospital, Frimley Health; St John's Institute of Dermatology, Guy's and St Thomas' NHS Foundation Trust, London, UK
Chapter 112

Joyce Teng Ee Lim
MB BS, FRCPI, FAMS(Derm)
Senior Consultant Dermatologist, National Skin Centre, National University of Singapore, Singapore
Chapter 159

Dan Lipsker
MD, PhD
Professor, Faculté de Medicine, Université de Strasbourg, Strasbourg, France
Chapter 45

Diana N. J. Lockwood
BSc, MD, FRCP
Professor of Tropical Medicine, London School of Hygiene & Tropical Medicine, London; Consultant Leprologist, Hospital for Tropical Diseases, University College Hospital NHS Trust, London, UK
Chapter 28

Christopher R. Lovell
MB ChB, MD, FRCP
Consultant Dermatologist, Department of Dermatology, Royal United Hospital and Royal National Hospital for Rheumatic Diseases, Bath, UK
Chapters 45, 96, 154

Nicholas J. Lowe
MB ChB, MD, FRCP, FACP, FAmAcadDerm
Consultant Dermatologist, The Cranley Clinic, London, UK; Clinical Professor of Dermatology, UCLA School of Medicine, Los Angeles, USA
Chapter 158

Calum Lyon
FRCP
Dermatologist and Honorary Clinical Lecturer, York Hospital NHS Trust, York; Salford Royal Hospital NHS Trust, Salford, UK
Chapter 114

Vishal Madan
MD, FRCP
Consultant Dermatologist, Laser and Mohs Surgeon, Dermatology Centre, Salford Royal NHS Foundation Trust, Salford, UK
Chapters 23, 133, 134, 141, 142

Eleanor Mallon
MB BS, MD, FRCP
Consultant Dermatologist, Croydon University Hospital, Croydon; Honorary Senior Lecturer in Dermatology, St John's Institute of Dermatology, Guy's and St Thomas' NHS Foundation Trust, London, UK
Chapter 113

Juan Mañá
MD, PhD
Associate Professor of Medicine and Internal Medicine Clinical Chief, Department of Internal Medicine, Bellvitge University Hospital, Barcelona University, Barcelona, Spain
Chapter 98

Joaquim Marcoval
MD, PhD
Associate Professor and Consultant Dermatologist, Department of Dermatology, Bellvitge University Hospital, Barcelona University, Barcelona, Spain
Chapter 98

Ashfaq A. Marghoob
MD
Attending Physician, Dermatology Service, Memorial Sloan-Kettering Cancer Center, New York, NY, USA
Chapter 144

Alexander M. Marsland
BSc(Hons), MB ChB, FRCP
Consultant Dermatologist and Honorary Senior Lecturer, Salford Royal Foundation Trust and University of Manchester, Manchester, UK
Chapter 42

Marcus Maurer
MD
Professor of Dermatology and Allergology, Charité, Berlin, Germany
Chapter 43

Collette McCourt
MB, BCH
Clinical Fellow in Immunodermatology, Skin Care Centre, Vancouver General Hospital, Vancouver, BC, Canada
Chapter 51

John A. McGrath
MD, FRCP, FMedSci
Professor of Molecular Dermatology, St John's Institute of Dermatology, Division of Genetics and Molecular Medicine, Faculty of Life Sciences and Medicine, King's College London, London, UK
Chapters 2, 7, 71

Jane M. McGregor
MA, MB BChir, MRCP, MD
Senior Lecturer and Consultant Dermatologist, Department of Dermatology, The Royal London Hospital, London; Centre for Cutaneous Research, Blizard Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, UK
Chapter 146

Kevin McKenna
MD, FRCP
Consultant Dermatologist, Dermatology Department, Belfast Trust, Belfast, UK
Chapters 21, 22

Jemima E. Mellerio
BSc, MD, FRCP
Consultant Dermatologist and Honorary Senior Lecturer, St John's Institute of Dermatology, Guy's and St Thomas' NHS Foundation Trust, London, UK
Chapters 76, 77, 79, 80

Andrew G. Messenger
MB BS, MD, FRCP
Professor of Dermatology, Department of Dermatology, Royal Hallamshire Hospital, Sheffield, UK
Chapter 89

Dieter Metze
MD
Professor, Department of Dermatology, University Hospital Münster, Münster, Germany
Chapter 65

George W. M. Millington
PhD, FRCP
Consultant Dermatologist, Dermatology Department, Norfolk and Norwich University Hospital, Norwich, UK
Chapter 74

Sonja Molin
MD
Assistant Professor, Department of Dermatology and Allergology, Ludwig Maximilian University, Munich, Germany
Chapters 150, 151, 152, 153

Gentiane Monsel
MD
Professor and Chairman, UPEC-Université Paris-Est Créteil Val de Marne, Department of Dermatology, Hôpital Henri Mondor, Créteil, France
Chapter 34

Fanny Morice-Picard
MD, PhD
Doctor and Consultant, Service de Dermatologie et Dermatologie Pédiatrique, Hôpital St André, Bordeaux, France
Chapter 70

Andrew Morris
BM BCh, MA, PhD, FRCPCH
Consultant in Paediatric Metabolic Medicine, Manchester Centre for Genomic Medicine, Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK
Chapter 81

Rachael Morris-Jones
BSc, MB BS, MRCP, FRCP, PhD, PCME
Dermatology Consultant, Dermatology Department, King's College Hospital, London, UK
Chapters 26, 93

Peter S. Mortimer
MD, FRCP
Professor of Dermatological Medicine, St George's Hospital, London, UK
Chapter 105

Richard J. Motley
MA, MD, FRCP, FAcadMEd
Consultant in Dermatology and Cutaneous Surgery, Welsh Institute of Dermatology, University Hospital of Wales, Cardiff, UK
Chapter 20

Megan Mowbray
BSc(Hons), FRCP, MD
Consultant Dermatologist, Department of Dermatology, Queen Margaret Hospital, NHS Fife, Dunfermline, UK
Chapter 107

Chetan Mukhtyar
MB, MSc, MD, FRCP, FRCP(Edin)
Consultant Rheumatologist, Norwich Medical School, Norfolk and Norwich University Hospital, Norwich, UK
Chapter 102

Colin S. Munro
MD, FRCP(Glasg)
Honorary Consultant Dermatologist, Queen Elizabeth University Hospital, Glasgow, UK
Chapter 66

Rabindranath Nambi
MD, DD, DNB
Consultant Dermatologist, Royal Derby Hospitals Foundation NHS Trust, Derby, UK
Chapter 122

Janakan Natkunarajah
MRCP(Derm)
Consultant Dermatologist, Kingston Hospital, London, UK
Chapter 120

Tim Niehues
MD
Professor of Paediatrics, Centre for Child Health and Adolescence, HELIOS Klinikum, Krefeld; Academic Hospital, RWTH, Aachen; Immunodeficiency and Rheumatology Centre, Krefeld, Germany
Chapter 82

Síona Ní Raghallaigh
MRCPI, MD
Clinical Research Fellow in Dermatology, Charles Institute of Dermatology, University College Dublin, Dublin, Ireland
Chapter 106

Stephanie Ogden
MRCP, PhD
Consultant Dermatologist and Honorary Senior Lecturer, Salford Royal Hospital, Greater Manchester, UK
Chapter 4

Vinzenz Oji
MD
Private Lecturer, Department of Dermatology, University Hospital Münster, Münster, Germany
Chapter 65

Rasha Omer
MB BS, MRCP, DTM&H, DipGUM, DipHIV
Specialist Registrar in Genitourinary Medicine, Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, UK
Chapter 29

Anthony D. Ormerod
MB ChB, FRCP(Edin), MD, FRCP(Lond)
Emeritus Professor of Dermatology, University of Aberdeen, Foresterhill, Aberdeen, UK
Chapter 49

Catherine H. Orteu
MB BS, BSc, MD, FRCP
Consultant Dermatologist, Department of Dermatology, Royal Free London NHS Foundation Trust, London, UK
Chapters 56, 57

David Orton
BSc(Hons), MSc(Allergy), MB BS, FRCP
Consultant Dermatologist, Hillingdon Hospitals NHS Foundation Trust, Uxbridge; Honorary Consultant Dermatologist, Royal Free London NHS Foundation Trust, London, UK
Chapter 128

Edel A. O'Toole
MB, PhD, FRCP
Professor of Molecular Dermatology and Honorary Consultant Dermatologist, Centre for Cutaneous Research, Barts and the London School of Medicine and Dentistry and Barts Health NHS Trust, London, UK
Chapter 10

Carol Ott
MD, FRCPC
Physician, Wound Healing Clinic, Women's College Hospital, Toronto; Geriatrics and Wound Care Clinics, Baycrest Hospital, Toronto, Ontario, Canada
Chapter 124

David G. Paige
MB BS, MA, FRCP
Consultant Dermatologist, Department of Dermatology, Barts Health NHS Trust, London, UK
Chapter 116

Amy S. Paller
MS, MD
Walter J. Hamlin Professor and Chair of Dermatology, Professor of Pediatrics, Departments of Dermatology and Pediatrics, Northwestern University, Chicago, IL, USA
Chapter 69

Ralf Paus
MD, FRSB
Professor of Cutaneous Medicine and Director of Research, Centre for Dermatology Research, Institute of Inflammation and Repair, University of Manchester, Manchester, UK; Head, Laboratory of Hair Research and Regenerative Medicine, Department of Dermatology, University of Münster, Münster, Germany
Chapter 149

Vincent Piguet
MD, PhD, FRCP
Clinical Professor of Dermatology, Department of Dermatology, Cardiff University; Consultant Dermatologist, University Hospital of Wales, Cardiff, UK
Chapters 31, 37

Elena Pope
MD, MSc, FRCP(C)
Staff Dermatologist and Associate Professor, Hospital for Sick Children, Toronto, Ontario, Canada
Chapter 136

William M. Porter
BSc, MB BS, MRCP
Consultant Dermatologist, Gloucestershire Hospitals NHS Foundation Trust, Gloucester, UK
Chapter 111

Frank C. Powell
FRCPI
Professor and Consultant, Charles Institute of Dermatology, University College Dublin, Dublin, Ireland
Chapters 91, 106

Charlotte M. Proby
MA, FRCP
Professor of Dermatology and Consultant Dermatologist, Skin Tumour Laboratory, Division of Cancer Research, Medical Research Institute, Jacqui Wood Cancer Centre, Dundee, UK
Chapter 146

Deepti H. Radia
BSc(Hons), MRCPI, FRCPath, MSc(MedEd)
Haematology Consultant, St John's Institute of Dermatology; Department of Haematology, Guy's and St Thomas' NHS Foundation Trust, London, UK
Chapter 46

Madhuri Reddy
MD, MSc
Director, Wound Healing Program, Hebrew Senior Life Instructor, Department of Medicine, Harvard Medical School, Boston, MA, USA
Chapter 124

Luis Requena
MD, PhD
Chairman of Dermatology and Professor of Dermatology, Department of Dermatology, Fundación Jiménez Díaz, Madrid, Spain
Chapter 99

Nicole Revencu
MD, PhD
Consultant Clinical Geneticist, Center for Human Genetics and Center for Vascular Anomalies, Cliniques Universitaires Saint-Luc, Université catholique de Louvain, Brussels, Belgium
Chapter 73

Nick J. Reynolds
BSc, MB BS, MD, FRCP
Professor of Dermatology, Department of Dermatology, Royal Victoria Infirmary and Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK
Chapter 41

Bertrand Richert
MD, PhD
Clinical Professor, Dermatology Department, Brugmann – St Pierre and Children's University Hospitals, Université Libre de Bruxelles, Brussels, Belgium
Chapter 95

Franco Rongioletti
MD
Professor of Dermatology, Section of Dermatology, Department of Health Sciences (DISSAL), University of Genova, Genoa; Consultant in Dermatopathology, University of Genova, Genoa, Italy
Chapter 59

Adam Rubin
MD
Assistant Professor of Dermatology, Pediatrics, and Pathology and Laboratory Medicine, Hospital of the University of Pennsylvania, the Children's Hospital of Philadelphia and Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA
Chapter 69

Malcolm Rustin
BSc, MD, FRCP
Consultant Dermatologist, Royal Free London NHS Foundation Trust, London, UK
Chapter 47

Thomas Ruzicka
MD
Professor and Chairman, Department of Dermatology and Allergology, Ludwig Maximilian University, Munich, Germany
Chapters 150, 151, 152, 153

Berthold Rzany
MD, ScM
RZANY & HUND, Private Practice for Dermatology and Aesthetic Medicine, Berlin, Germany
Chapter 157

Dana L. Sachs
MD
Professor of Dermatology, University of Michigan Medical Center, Ann Arbor, MI, USA
Chapter 155

Nazanin Saedi
MD
Assistant Professor, Department of Dermatology and Cutaneous Biology, Thomas Jefferson University, Philadelphia, PA, USA
Chapter 160

Robert P. E. Sarkany
FRCP, MD
Consultant Dermatologist and Head of Photodermatology, St John's Institute of Dermatology, Guy's and St Thomas' NHS Foundation Trust, London, UK
Chapter 60

Karin Sartorius
MD, PhD
Consultant Dermatologist, Department of Clinical Sciences and Education, Karolinska Institutet, Stockholm; Department of Dermatology, Södersjukhuset, Stockholm, Sweden
Chapter 147

Valerie P. J. Saw
MB BS(Hons), FRANZCO, PhD
Consultant Ophthalmologist, Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology NIHR Biomedical Research Centre, London, UK
Chapter 109

Enno Schmidt
MD, PhD
Consultant, Department of Dermatology, Lübeck Institute of Experimental Dermatology, University of Lübeck, Lübeck, Germany
Chapter 50

David Schrama
PhD
Group Leader, General Dermatology Department, University Clinic of Würzburg, Würzburg, Germany
Chapter 145

Stephan Schreml
MD, PhD
Attending at the Department of Dermatology, University Medical Centre Regensburg, Regensburg, Germany
Chapter 58

Crispian Scully
CBE, MD, PhD, MDS, MRCS, BSc, FDSRCS, FDSRCPS, FFDRCSI, FDSRCSE, FRCPath, FMedSci, FHEA, FUCL, FSB, DSc, DChD, DMed(HC), Dr.h.c.
Co-Director, WHO Collaborating Centre for Oral Health-General Health; Council Member and Examiner, Royal College of Surgeons of Edinburgh; and Emeritus Professor, University College London, London, UK
Chapter 110

Neil J. Sebire
BSc, FRCPath, MD
Professor of Paediatric Pathology, Paediatric Pathology Department, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK
Chapter 75

Rodney D. Sinclair
MB BS, MD, FACD
Professor of Medicine (Dermatology), University of Melbourne, Melbourne; Director of Dermatology, Epworth Healthcare, Richmond, Victoria; Director, Sinclair Dermatology, Research and Clinical Trials Centre, Melbourne, Australia
Chapters 89, 107

Catherine H. Smith
MD, FRCP
Professor of Dermatology and Therapeutics and Consultant Dermatologist, Skin Therapy Research Unit, St John's Institute of Dermatology, Guy's and St Thomas' NHS Foundation Trust, London, UK
Chapters 14, 19

Reinhart Speeckaert
MD, PhD
Dermatologist, Department of Dermatology, Ghent University Hospital, Ghent, Belgium
Chapter 88

Eli Sprecher
MD, PhD
Professor and Chair, Department of Dermatology, Tel Aviv Sourasky Medical Center; and Department of Human Molecular Genetics and Biochemistry, Sackler Medical School, Tel Aviv University, Tel Aviv, Israel
Chapter 68

Sonja Ständer
MD
Dermatologist and Dermatopathologist, Department of Dermatology, University Hospital Münster, Münster, Germany
Chapter 83

Irene Stefanaki
MD
Dermatologist, Department of Dermatology, University of Athens Medical School, Andreas Sygros Hospital, Athens, Greece
Chapter 132

Martin Steinhoff
MD, PhD, MSc
Professorial Chair, Department of Dermatology; and Director, UCD Charles Institute of Dermatology, University College Dublin, Dublin, Ireland
Chapter 8

Jane C. Sterling
MB BChir, MA, FRCP, PhD
Senior Lecturer and Honorary Consultant Dermatologist, Department of Dermatology, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK
Chapter 25

Alexander Stratigos
MD
Professor of Dermatology, Department of Dermatology, University of Athens Medical School, Andreas Sygros Hospital, Athens, Greece
Chapter 132

Alain Taïeb
MD, PhD
Professor and Head of Department, Service de Dermatologie et Dermatologie Pédiatrique, Hôpital St André, Bordeaux, France
Chapter 70

Ruth E. Taylor
MRCPsych, PhD
Consultant Liaison Psychiatrist, Department of Liaison Psychiatry, Barts Health NHS Trust, London; and Senior Lecturer, Queen Mary College of Medicine, University of London, London, UK
Chapter 86

Fernanda Teixeira
MD, PhD
Consultant Dermatologist, Imperial College Healthcare Trust, London, UK
Chapter 87

Michael J. Tidman
MD, FRCP(Edin), FRCP(Lond)
Consultant Dermatologist, Department of Dermatology, Royal Infirmary of Edinburgh, Edinburgh, UK
Chapter 19

Thai Hoa Tran
MD, FRCP(C), FAAP
Research Fellow, Hospital for Sick Children, Toronto, Ontario, Canada
Chapter 136

Heiko Traupe
MD
Assistant Professor, Department of Dermatology, University Hospital Münster, Münster, Germany
Chapter 65

Kenneth Y. Tsai
MD, PhD, FAAD
Assistant Professor, Departments of Dermatology and Translational Molecular Pathology, University of Texas MD Anderson Cancer Center, Houston, TX, USA
Chapters 137, 139

Stephen Tyring
MD, PhD
Clinical Professor, University of Texas Health Science Center, Houston, TX, USA
Chapters 33, 131

Jouni Uitto
MD, PhD
Professor and Chair of Department of Dermatology and Cutaneous Biology, Thomas Jefferson University, Philadelphia; Director, Jefferson Institute of Molecular Medicine, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA, USA
Chapter 2

Hessel H. van der Zee
MD, PhD
Department of Dermatology, Erasmus Medical Center, Rotterdam, the Netherlands
Chapter 92

Nanja van Geel
MD, PhD
Dermatologist, Department of Dermatology, Ghent University Hospital, Ghent, Belgium
Chapter 88

Samantha Vaughan Jones
MD, FRCP
Consultant Dermatologist, Department of Dermatology, St Peter's Hospital, Ashford; and St Peter's Foundation Trust, Chertsey, UK
Chapter 115

Miikka Vikkula
MD, PhD
Coordinator, Center for Vascular Anomalies, Cliniques Universitaires Saint-Luc; Professor of Human Genetics, Human Molecular Genetics, de Duve Institute; Principal Investigator, Walloon Excellence in Lifesciences and Biotechnology (WELBIO), Université catholique de Louvain, Brussels, Belgium
Chapter 73

John J. Voorhees
MD, FRCP
Duncan and Ella Poth Distinguished Professor of Dermatology and Chair of Department of Dermatology, University of Michigan Medical Center, Ann Arbor, MI, USA
Chapter 155

Shyamal Wahie
MB BS, MD, FRCP
Consultant Dermatologist, University Hospital of North Durham, Durham, UK
Chapter 52

Sarah Wakelin
BSc, MB BS, FRCP
Consultant Dermatologist and Honorary Senior Lecturer, Imperial College Healthcare Trust, London, UK
Chapter 40

Stephen L. Walker
PhD, MRCP(UK), DTM&H
Clinical Lecturer, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK
Chapter 27

Sarah Walsh
MB BCh, BAO, BMedSci, MRCP
Consultant Dermatologist and Clinical Lead, Department of Dermatology, King's College Hospital, London, UK
Chapter 119

Timothy S. Wang
MD
Associate Professor, Department of Dermatology, and Director, Cutaneous Surgery Unit and Micrographic Surgery and Dermatologic Oncology (Mohs) Fellowship Program, Johns Hopkins Health System, Baltimore, MD, USA
Chapter 20

Molly Wanner
MD, MBA, FAAD
Department of Dermatology, Massachusetts General Hospital, Boston, MA, USA
Chapter 156

Sheila Weitzman
MB BCh, FCP(SA), FRCP(C)
Senior Staff Oncologist and Professor, Hospital for Sick Children, Toronto, Ontario, Canada
Chapter 136

Jonathan M. L. White
BSc, MRCP(UK)
Consultant Dermatologist, Department of Cutaneous Allergy, St John's Institute of Dermatology, Guy's and St Thomas' NHS Foundation Trust, London, UK
Chapters 129, 130

Sean J. Whittaker
MD, FRCP
Professor of Cutaneous Oncology, Division of Genetics and Molecular Medicine, King's College London, London; St John's Institute of Dermatology, Guy's and St Thomas' NHS Foundation Trust, London, UK
Chapters 135, 140

Mark Wilkinson
MD, FRCP
Consultant Dermatologist, Leeds Teaching Hospitals NHS Trust, Leeds, UK
Chapter 128

Hywel C. Williams
DSc, FRCP, FMedSci
Professor of Dermato-Epidemiology and Director, Centre of Evidence-Based Dermatology, Nottingham University Hospitals NHS Trust, Nottingham, UK
Chapters 5, 17

Niall J. E. Wilson
BSc(Hons), MB ChB, FRCP, FRCPI
Consultant Dermatologist, Royal Liverpool and Broadgreen University Hospitals, Liverpool, UK
Chapter 94

Albert C. Yan
MD, FAAP, FAAD
Section Chief, Associate Professor, Department of Dermatology, Children's Hospital of Philadelphia, Philadelphia, PA, USA
Chapter 63

Victoria M. Yates
MB ChB, FRCP, DTM&H
Honorary Consultant Dermatologist, Formerly at The Dermatology Centre, Salford Royal NHS Trust, Manchester, UK
Chapter 27

Antony R. Young
BSc, MSc, PhD
Professor of Experimental Photobiology, St John's Institute of Dermatology, Division of Genetics and Molecular Medicine, Faculty of Life Sciences and Medicine, King's College London, London, UK
Chapter 9

Christopher B. Zachary
FRCP
Professor and Chairman, Department of Dermatology, University of California, Irvine, Irvine, CA, USA
Chapter 160

Joanna M. Zakrzewska
MD, FDSRCS, FFDRCSI, FFPMRCA
Consultant and Lead for Facial Pain, Facial Pain Unit, University College London Hospitals NHS Foundation Trust, London, UK
Chapter 84

Mozheh Zamiri
MD, MRCP
Consultant Dermatologist, Queen Elizabeth University Hospital, Glasgow, UK
Chapter 66

Christos C. Zouboulis
Prof.Dr.med., Prof.h.c., Dr.h.c.
Director and Professor of Dermatology and Venereology, Departments of Dermatology, Venereology, Allergology and Immunology, Dessau Medical Center, Dessau, Germany
Chapters 48, 90

Axel zur Hausen
MD
Chair, Department of Pathology, Maastricht University Medical Center, Maastricht, the Netherlands
Chapter 145

Preface to the Ninth Edition

The ninth edition of *Rook's Textbook of Dermatology*, or 'Rook book' as it is known affectionately, marks a significant change from its traditional structure and format. The editorial team has changed: due to the retirements of Tony Burns and Stephen Breathnach and the untimely, early death of Neil Cox, only Chris Griffiths remains from the previous team. The current editors wish to pay tribute to these three, all of whom dedicated significant energy and knowledge to the success of previous editions. Four editors were deemed to be insufficient for a textbook of the complexity and size of Rook and thus a team of five editors supported by 12 associate editors was established for the ninth edition. The content has been reorganized into 14 sections with a total of 160 chapters, more than double the number in the previous edition although the overall size of the book is little changed. The new opening section, Foundations of Dermatology, provides a comprehensive introduction to the subject and there is an expanded section on Aesthetic Dermatology. The authorship has also enlarged with a mixture of authors from previous editions and newcomers, many of whom are from outside the UK and have thus added an important international dimension to the essential 'Britishness' of Rook.

The major change and the one which has catalysed the aforementioned restructuring is the requirement to bring the book into the twenty-first-century publishing world by designing it as much for online use as for a traditional print book. The hierarchical templating required for this has necessitated a complete rewrite and reformatting. The hard copy textbook mirrors the online version, the main difference being that only selected key references are printed in the former, the full reference list being available online. This has enabled us to increase the number of figures and images, all of which are downloadable as PowerPoint slides. We also listened to comments about the inconvenience of the index being printed in only one of the four volumes of the eighth edition and have ensured that it is available in each volume of the ninth.

We view our editorship of Rook as a privilege and are cognizant of our responsibilities as the current custodians of an institution of British dermatology. Thus, the changes we have wrought on the book have been undertaken with a sense of trepidation. Dermatology is at an important and exciting point in its evolution as a subject. The promise of translational research, whereby advances in the understanding of basic pathomechanisms of skin disease have resulted in higher quality patient care, is being realized, much as Arthur Rook, Darrell Wilkinson and John Ebling envisaged in their preface to the first edition of Rook in 1968. We have tried to encapsulate this approach in the ninth edition.

Our thanks go to the wonderful team of Jenny Seward, Catriona Cooper, Nick Morgan, Charlie Hamlyn, Oliver Walter and Martin Sugden at Wiley who have worked tirelessly to help us realize our vision for the new Rook, and to our outstanding project manager Lindsey Williams, and her indefatigable team of copy editors (Jane Andrew and Karen Stephenson), indexer (Jill Halliday) and artist (David Gardner).

Chris Griffiths
Jonathan Barker
Tanya Bleiker
Robert Chalmers
Daniel Creamer

Preface to the First Edition

No comprehensive reference book on dermatology has been published in the English language for ten years and none in England for over a quarter of a century. The recent literature of dermatology is rich in shorter texts and in specialist monographs but the English-speaking dermatologist has long felt the need for a substantial text for regular reference and as a guide to the immense monographic and periodical literature. The editors have therefore planned the present volume primarily for the dermatologist in practice or in training, but have also considered the requirements of the specialist in other fields of medicine and of the many research workers interested in the skin in relation to toxicology or cosmetic science.

An attempt has been made throughout the book to integrate our growing knowledge of the biology of skin and of fundamental pathological processes with practical clinical problems. Often the gap is still very wide but the trends of basic research at least indicate how it may eventually be bridged. In a clinical textbook the space devoted to the basic sciences must necessarily be restricted but a special effort has been made to ensure that the short accounts which open many chapters are easily understood by the physician whose interests and experience are exclusively clinical.

For the benefit of the student we have encouraged our contributors to make each chapter readable as an independent entity, and have accepted that this must involve the repetition of some material.

The classification employed is conventional and pragmatic. Until our knowledge of the mechanisms of disease is more profound no truly scientific classification is possible. In so many clinical syndromes multiple aetiological factors are implicated. To emphasize one at the expense of others is often misleading. Most diseases are to some extent influenced by genetic factors and a large proportion of common skin reactions are modified by the emotional state of the patient. Our knowledge is in no way advanced by classifying hundreds of diseases as genodermatoses and dozens as psychosomatic.

The true prevalence of a disease may throw light on its aetiology but reported incidence figures are often unreliable and incorrectly interpreted. The scientific approach to the evaluation of racial and environmental factors has therefore been considered in some detail.

The effectiveness of any physician in practice must ultimately depend on his ability to make an accurate clinical diagnosis. Clinical descriptions are detailed and differential diagnosis is fully discussed. Histopathology is here considered mainly as an aid to diagnosis but references to fuller accounts are provided.

The approach to treatment is critical but practical. Many empirical measures are of proven value and should not be abandoned merely because their efficacy cannot yet be scientifically explained. However, many familiar remedies old and new have been omitted either because properly controlled clinical trials have shown them to be of no value or because they have been supplanted by more effective and safer preparations.

There are over nine hundred photographs but no attempt has been made to provide an illustration of every disease. To have done so would have increased the bulk and price of the book without increasing proportionately its practical value. The conditions selected for illustrations are those in which a photograph significantly enhances the verbal description. There are a few conditions we wished to illustrate, but of which we could not obtain unpublished photographs of satisfactory quality.

The lists of references have been selected to provide a guide to the literature. Important articles now of largely historical interest have usually been omitted, except where a knowledge of the history of a disease simplifies the understanding of present concepts and terminology. Books and articles provided with a substantial bibliography are marked with an asterisk.

Many of the chapters have been read and criticized by several members of the team and by other colleagues. Professor Wilson Jones, Dr R.S. Wells and Dr W.E. Parish have given valuable assistance with histopathological, genetic and immunological problems respectively. Many advisers, whose services are acknowledged in the following pages, have helped us with individual chapters. Any errors which have not been eliminated are, however, the responsibility of the editors and authors.

The editors hope that this book will prove of value to all those who are interested in the skin either as physicians or as research workers. They will welcome readers' criticisms and suggestions which may help them to make the second edition the book they hope to produce.

Arthur Rook, Darrell Wilkinson and John Ebling

PART 1

Foundations of Dermatology

CHAPTER 1

History of Dermatology

Nick J. Levell

Norwich Medical School, Norfolk and Norwich University Hospital, Norwich, UK

Introduction: when did dermatology history begin?

The history of dermatology is the history of mankind. Failure to care for the skin increases the risk of skin infections leading to morbidity and disability, and caring for the skin improves the chances of being perceived as being attractive to others thereby enhancing survival of an individual's genes. Primates demonstrate mutual grooming behaviour to reduce infestation and many species lick their wounds. Dermatology activity, removing parasites, applying grease to dry skin and cleaning and dressing wounds and burns, must have been an important role for the Shamans and Wise Women who were responsible for medical care in primitive hominid tribal groups.

Prior to the invention of writing, archaeologists provide evidence of disease and early medical activity. Findings, such as the radiological appearances of possible metastatic melanoma in 2400-year-old pre-Colombian Inca mummies, provide early evidence of the impact of skin disease [1].

The invention of writing coincided with early cities in Africa (Egypt *c*. 3100 BC), the Middle East (Mesopotamia *c*. 3000 BC), India (Indus valley *c*. 2500 BC), Europe (Crete *c*. 1800 BC) and China (Shang *c*. 1400 BC). Early texts were mainly for administrative or religious purposes, but medical writings soon appeared. Many of these related to skin disorders.

Ancient dermatology writings

The first medical texts

Medical writings between *c*. 3000 BC and 400 BC in most cultures had a theoretical basis founded on religious beliefs with pragmatic practical tips. Historians have often stated that there was an initial age of medicine dominated by magic, followed by the growth of rational medicine led by Hippocrates around 400 BC. However the reality was probably not so clear-cut. Experience makes it obvious that doctors who relied totally on magic and religion would not have cured many patients, so all systems of health care contained practical measures.

A pharmacopoea, written by an unknown Sumerian (Mesopotamia in the Middle East) in the third millienium BC may be the earliest medical writing [1]. It is on a clay tablet and describes a selection of external salves comprising cedar oil, wine and botanical, mineral and animal materials. Other preparations include clay mixed with honey, water and oil.

Medical writing moved from clay tablets, to papyrus to paper and now in the 21st century is moving back to tablets of a different type.

Ancient Egypt

Imhotep, the Chief Vizier to the Pharaoh Zoser (2700 BC), was renowned as a physician and was soon deified by the Egyptians (Figure 1.1). Egyptian medical writings date from a millennium

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Companion website: www.rooksdermatology.com

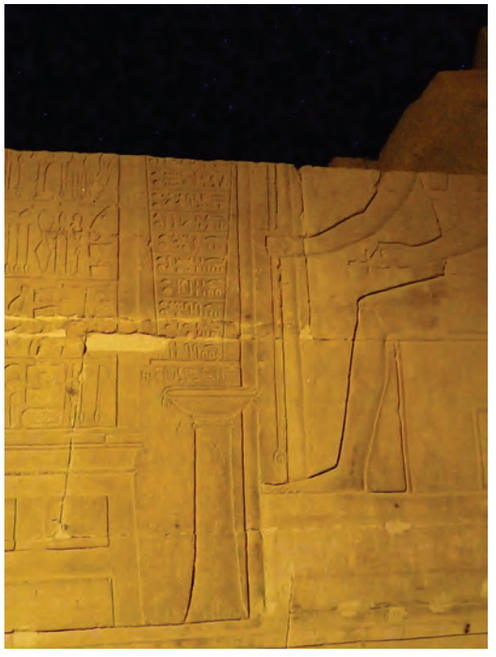


Figure 1.1 Imhotep, seated on the right with a surgical instrument.

later. The Edwin Smith Papyrus (*c*. 1600 BC) known as the 'Book of Wounds' mentions wound dressings using fresh meat and then honey, grease and lint. However, the most important medical text is the Ebers Papyrus (1550 BC) written on over 20 metres of papyrus [**2**]. This describes 700 magical formulae and 800 formulae to treat 15 diseases of the abdomen, 29 of the eyes and 18 of the skin. The text includes a baldness cure: a drink made from black ass testicles, or vulval and penis extracts from a black lizard. The Ebers Papyrus also describes an effective treatment for the Guinea worm: wrap the emerging end of the worm around a stick and slowly pull it out.

Mesopotamia

Over 1000 clay tablets that refer to medicine, of which 40 comprise a medical diagnostic handbook, and written c. 1000 BC, are attributed Esagil-kin-apli, a Babylonian physician. The writings on skin disease demonstrate the interplay of magic and empricisim [3]:

- Tablet 9, line 48: 'If his face is covered in white boils: Hand of the Sun God Ama, he will survive …'.
- Tablet 9, line 49: 'If his face is covered in black boils: Hand of the God I tar, he will die …'.
- Table 14, line 128: 'If his testicles are black he will die …' (possibly the first description of Fournier gangrene).

Ancient Greece

Asclepius is thought to have practiced about 1000 BC in Greece. The reality of his work is lost in stories around his deification. Greek legend confuses him with the Egyptian God/physician Imhotep and also describes him as a son of the God Apollo (the healer) and the Goddess Panacea. Ascelpius is said to have been executed by the gods for taking gold to raise the dead, a lesson for all modern clinicians tempted by greed. Temples to Asclepius included healing dogs to lick wounds. His followers in Greece persisted for centuries and included Hippocrates and Aristotle.

Ancient India

Early Brahmana (hereditary priests) guarded the Sanskrit religious teachings, the Veda (knowledge) from 1500 BC. Much of the medicine revolved around a magico-religious approach that paralleled that of Mesopotamia and Egypt. Vedic rites probably involved human sacrifice but writings included information on practical dermatology such as the use of cautery for haemostasis.

Growth of rational medicine

The Silk Road: the pathway of rational medicine

The trading routes known as the Silk Road linked China, India, the Middle East and eastern Europe *c*. 400 BC. At around this time, a growth of rational medicine appeared in Europe along with similar ideas in south India and China so it seems likely that a flow of medical ideas took place in parallel with the trade items. It can be argued whether knowledge flowed mainly from East to West or from West to East. The 'Diagnostic Handbook' from Mesopotamia, from 1000 BC, remained in print but between 600 and 400 BC changed radically in nature to show that disease was subject to the forces of nature and originated from the body rather than being of a divine nature.

China

Existing texts date back to at least 200 BC but some people contend that they originate over 2000 years before. Some of the original formulae are thought to still be in use today. Although there is little relationship to what western medicine considers to be an anatomical- or physiological-based system, the underlying concepts were not based on religion or spirits. Disease is seen to be based on a loss of harmony of the yin/yang system upsetting the qi (energy) and the meridians [1]. This is a generalist approach: skin disorders are considered to be an internal problem. Sections on skin disease exist in classic works from 652 BC. Urticaria or 'wind type concealed rash' was considered to be due to excess lesser yin causing fluid obstruction in the skin. The 'Yellow Emperor's Inner Canon' describes urticaria and eczema.

South Indian early Buddhism

The Pali scripts date back to around 400 BC and describe the work of the Buddha [**2**]. The *Girimananda Sutra* described dermatology nursing, psychodermatology, occlusion therapy for foot eczema and possible early descriptions of skin diseases including leprosy, boils/abscesses, scrofula, ringworm, scabies, pustular eruptions, plethora, fistula and sexually transmitted diseases.

The Holy Bible

The Book of Leviticus written *c*. 450 BC gave an account of how to diagnose 'leprosy' – although the descriptions of skin disease in this text could include many chronic cutaneous infections including tinea infection, impetigo and infected eczema. Practical tips on the management of contagious cutaneous disease include burning clothes and isolation of those afflicted.

Greeks: the rational age

Hippocrates (*c*. 400 BC) was known to his contemporaries as Hippocrates the Great – an accolade in the age of Plato and Socrates. He was an Asclepian physician and teacher on the island of Cos. Some

of his great ideas may have been written by his pupils who built up a body of medical knowledge at his school over later generations. Hippocrates' school moved away from the magical and religious approach to medicine and adopted a method based on logic and reason. His approach was, like the Chinese, to see disease in the context of the whole patient and to see people as physical entities subject to the same laws of nature as the world. He used diet and exercise as therapies and adopted an expectant approach, not rushing to intervene. His writings on leg ulcers are relevant now: 'In the case of an ulcer, it is not expedient to stand; more especially if the ulcer be situated in the leg; but neither, also, is it proper to sit or walk. But quiet and rest are particularly expedient …'.

The Roman Empire

Galen was born in Pergamon, Turkey in 120 AD and travelled to Egypt to learn about African and Indian medicine prior to settling in Rome. He studied anatomy through the dissection of animals (not humans), but then set Hippocratic ideas into an incorrect anatomical and physiological framework. This was based on four humours that might lead to fever if in excess: yellow bile, black bile, phlegm and blood. This led to an enthusiasm for blood-letting to restore balance in those with fever or if the physician wished to prevent fever.

Galen had a powerful intellect, an overbearing personality and a gift for self-publicity and was a prolific writer. Consequently, perhaps, this theoretical basis for medicine became entrenched in Europe and the Middle East. A period of relative intellectual stagnation regarding underlying disease processes persisted for over 1500 years. This may have been partly due to religious and cultural bans on human dissection until Renaissance times.

Over the next 500 years a series of Greek and Roman writers defined diseases within this flawed model of basic science. Therapeutic advances were made with various herbal and mineral remedies for skin disorders. Wood tars and coal tars were described for inflammatory skin disorders, presumably eczema and psoriasis [3]. The last of the series of Greco-Roman authors was Paul of Aegina (around 700 AD) who wrote a medical encyclopaedia in seven books of which book IV concerns skin disease [4]. This may be considered the earliest dermatology textbook.

Dermatology after the fall of Rome

Early Islamic medicine and dermatology

With the failure of the Roman Empire and the onset of the Dark Ages in Europe, the baton of medical knowledge in the West was passed back to the Middle East. Much would have been lost were it not for translations into Arabic by Christian and Islamic scholars at the Bayt al Hikma centre set up in 832 in Baghdad, the capital of the Islamic Empire. Hundreds of Greek, Latin and Sanskrit texts were translated, making Islamic culture the centre for learning. A series of medical compendia were produced, the first being the 'Paradise of Wisdom' (*Firdaws al-bikma*) by Ali ibn Rabban al-Tabari (c. 850 AD).

The great Persian physician, Muhammad ibn Zakariya al-Razi (865–925; known as 'Rhazes' in the West) studied in Rayy near Tehran, before settling in Baghdad. He wrote over 200 texts and initially challenged many of Galen's precepts – although ultimately describing himself as a Galen's disciple. He wrote *al-Jadari wa'l-basha* ('Smallpox and Measles') in which he was the first to distinguish between febrile exanthemas: 'The rash of measles usually appears at once, but the rash of smallpox spot after spot'. Al-Razi's work was renowned in the Arabic world and was translated to Latin, still being reprinted in the West in 1542, over 600 years after his death.

The Persian writers, al-Majusi (Haly Abbas: 10th century), Ibn Sina (Avicenna: 980–1037) and al-Zahrawi (Albucasis: 936–1013) all wrote influential medical texts.

Italy during the European Renaissance

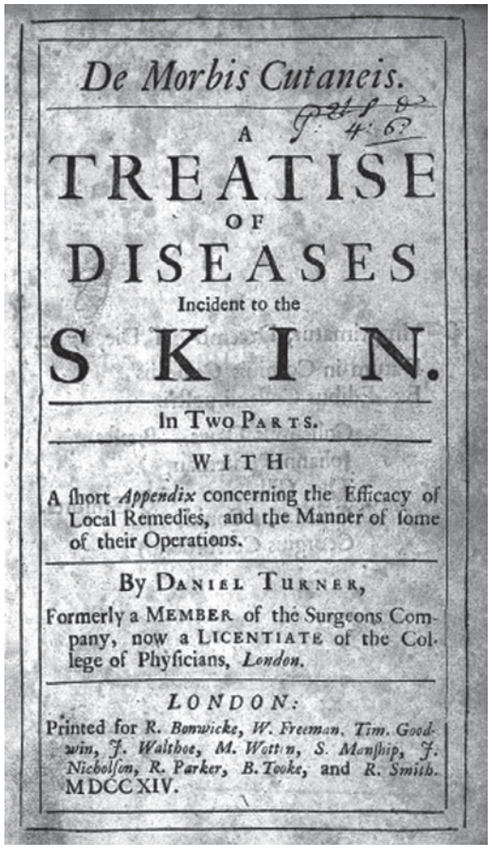
In the mid-16th century Europe was slowly struggling out of the religious superstition that characterized the Dark Ages and Middle Ages. A group of brilliant doctors in Padua, including Vesalius and Mercurialis (Geronimo Mercuriale), set up a system of learning and wrote medical texts that revitalized medicine in Europe. Mercurialis wrote *De Morbis Cutaneis* in 1572: this summarized work of earlier writers and had a focus on hair disorders, but still represents the first dermatology textbook in the West since the time of Paul of Aegina, 800 years before.

European Enlightenment

A series of dermatology textbooks written in the 18th century pulled dermatology through to the beginning of the modern age.

Daniel Turner (Figure 1.2) wrote the first English language dermatology textbook in 1712 [**1**] (Figure 1.3). This was a series of case reports and was popular, running to four editions over 20 years. Turner was an English surgeon, who aspired to be a physician, and he dedicated this book to the President of the London Royal

Figure 1.2 Daniel Turner. (From http://wellcomeimages.org/. Copyrighted work available under Creative Commons Attribution only licence CC BY 4.0 http://creativecommons.org/licenses/by/4.0/. Wellcome Library, London.)


De Morbis Cutaneis.

A TREATISE OF DISEASES Incident to the SKIN.

In Two Parts.

WITH

A short *Appendix* concerning the Efficacy of Local Remedies, and the Manner of some of their Operations.

By Daniel Turner,

Formerly a Member of the Surgeons Company, now a Licentiate of the College of Physicians, *London*.

LONDON:

Printed for R. Bonwicke, W. Freeman, Tim. Goodwin, J. Walthoe, M. Wotton, S. Manship, J. Nicholson, R. Parker, B. Tooke, and R. Smith. MDCCXIV.

Figure 1.3 The first English language dermatology textbook by Turner. (From http://wellcomeimages.org/. Copyrighted work available under Creative Commons Attribution only licence CC BY 4.0 http://creativecommons.org/licenses/by/4.0/. Wellcome Library, London.)

College of Physicians. He was awarded association to the Royal College as a 'Licentiate', but a medical degree from Oxford or Cambridge was required to be a full member. Turner then endowed a medical library at Yale University in America and was given the first medical degree awarded in America, but this distinction still failed to achieve his college membership.

Dermatology was linked with venereology in Europe, and Jean Astruc, physician to the Parisian Court, wrote a definitive text summarizing all knowledge on syphilis. He described the anatomy of the skin and linked cutaneous diseases to the sebaceous glands.

In Italy, Bernadino Ramazzini wrote a textbook on industrial disease in 1700, which classified occupational dermatoses ranging from varicose veins in priests to syphilis in midwives and wet nurses [2].

Classification was in the air: following Linnaeus, clinicians across Europe strove to classify cutaneous disease. Joseph Jacob Plenck, a Viennese-born professor in Buda, wrote a classification of skin disease in 1776 that divided skin disease into 14 categories [**3**]. This was a landmark for dermatology, being the first serious attempt to classify skin diseases. The following year, Antoine Charles Lorry in France wrote a text that considered the pathology, physiology and aetiology of skin diseases [4].

Growth of scientific dermatology

Willan and Bateman: definition of skin diseases

Prior to Robert Willan, terms were used loosely to describe skin diseases. Two doctors might use the same descriptive term to mean different appearances. Attempts to describe disease characteristics in classifications were ambiguous. Willan (Figure 1.4) defined precisely the terms used to describe skin disease. He wrote a classification based on these definitions, first published in Breslau from 1798 onwards [**1**] (Figure 1.5). He died before finishing his next work, but it was completed by his friend and student, Thomas Bateman in 1813. Bateman's *A Practical Synopsis of Cutaneous Disease* was translated into the main European languages and remained the standard textbook until the 1830s [**2**].

Willan produced images of skin diseases in his textbook (Figure 1.6), the first dermatology atlas, that was completed by Bateman in 1817 [3]. This atlas went through many editions and was still in print in 1877.

Willan and Bateman changed the way dermatology was practised, with followers all around Europe: Biett and Cazenave in France, Chiarugi in Florence, Alfaro in Spain and Klaatch and Schreiver in Germany.

L'Hôpital St Louis, Paris: the first skin hospital

The time of war in Napoleonic Europe not only produced a great step forwards in England, but also led to the encouragement of science in France. In 1801 the L'Hôpital St Louis became a dermatology hospital under the leadership of Jean-Louis Alibert (Figure 1.7). His flamboyant personality, prolific writing and elegant descriptions and atlases included the famous 'arbre des

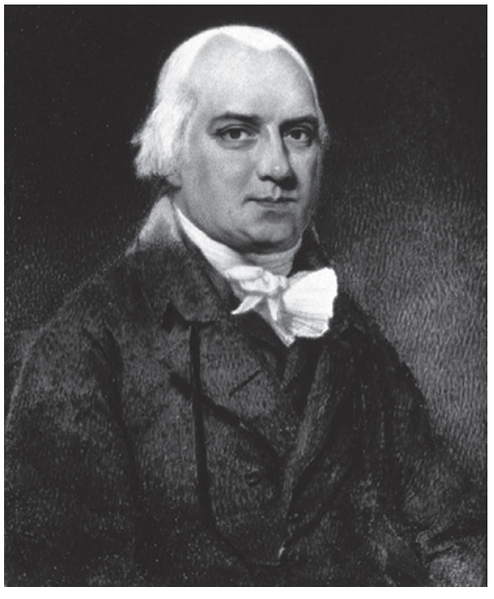


Figure 1.4 Robert Willan. (From http://wellcomeimages.org/. Copyrighted work available under Creative Commons Attribution only licence CC BY 4.0 http://creativecommons.org/licenses/by/4.0/. Wellcome Library, London.)

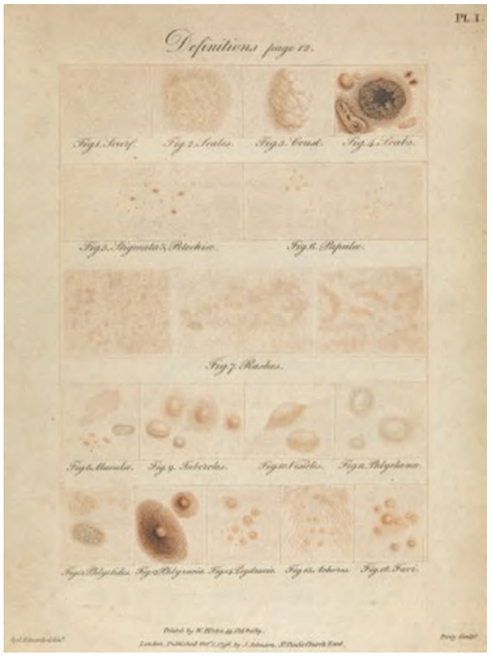


Figure 1.5 Willan's definitions. (From http://wellcomeimages.org/. Copyrighted work available under Creative Commons Attribution only licence CC BY 4.0 http://creativecommons.org/licenses/by/4.0/. Wellcome Library, London.)

dermatoses' classification (Figures 1.8 and 1.9). He was balanced by the scholarly Laurent Biett, his student, who studied under Bateman in 1816 and brought Willanism to France. Biett then further developed and refined this work with Cazenave, Rayer and Bazin, making France and L'Hôpital St Louis the leading centre for dermatology in the early 19th century.

Figure 1.6 Psoriasis gyrata in Willan's textbook. (From http://wellcomeimages.org/. Copyrighted work available under Creative Commons Attribution only licence CC BY 4.0 http://creativecommons.org/licenses/by/4.0/. Wellcome Library, London.)

Figure 1.7 Jean Louis Alibert. (From http://wellcomeimages.org/. Copyrighted work available under Creative Commons Attribution only licence CC BY 4.0 http://creativecommons.org/licenses/by/4.0/. Wellcome Library, London.)

Figure 1.8 Alibert's syphilide pustuleuse en grappe. (From http://wellcomeimages.org/. Copyrighted work available under Creative Commons Attribution only licence CC BY 4.0 http://creativecommons.org/licenses/by/4.0/. Wellcome Library, London.)

Figure 1.9 Alibert's arbre des dermatoses. (From http://wellcomeimages.org/. Copyrighted work available under Creative Commons Attribution only licence CC BY 4.0 http://creativecommons.org/licenses/by/4.0/. Wellcome Library, London.)

Figure 1.10 Ferdinand von Hebra. (From http://wellcomeimages.org/. Copyrighted work available under Creative Commons Attribution only licence CC BY 4.0 http://creativecommons.org/licenses/by/4.0/. Wellcome Library, London.)

Natural Sciences and the German-speaking Europeans

The integration between hospitals and universities was pioneered by German-speaking countries in central Europe in the second half of the 19th century. The new sciences of bacteriology and histopathology led to the understanding of underlying disease processes in dermatology. This finally ended Galen's ancient teachings and marked the origin of modern scientific dermatology.

In Vienna, Austria, the Allgemeines Krankenhaus became the world centre for medical teaching. From a group of brilliant clinicians, Ferdinand von Hebra (Figure 1.10) arose to introduce the new science of pathology into dermatology. He reclassified skin diseases using an anatomical and pathological framework, defined cutaneous fungal infections, and made great contributions to the definitions of many inflammatory disorders including eczema. Many great dermatologists studied under him and took his methods across the world; including Filip Pick to Prague and Schwimmer to Budapest [**4**].

Heinrich Köbner, and then Oscar Simon and Albert Neisser in Breslau (now Wrocław), developed the Allgemeines Krankenhaus as the centre for German and Polish dermatology. Köbner moved to Berlin in 1872 to set up the Berlin Dermatology Society. Neisser utilized his knowledge of histopathology, microbiology, immunology, X-rays and ultraviolet and by the age of 25 had discovered the gonococcus and worked with Hansen to stain the lepra bacillus. He became the pre-eminent figure in European dermatology with advances in many areas, including work with Wassermann in syphilis [**5**].

Britain in the late 19th century

Sir Erasmus Wilson (Figure 1.11) was the most famous Victorian dermatologist. A wealthy and charismatic surgeon, who founded a dermatology chair for himself at the Royal College of Surgeons, paid for Cleopatra's needle to be transported from Egypt to London, and founded and edited the first English dermatology journal in 1867, he was also responsible for abolishing flogging in the British Army. He co-founded the controversial St John's Hospital for dermatology in London, with John Laws Milton. Another co-founder, William Tilbury Fox, became the first British university hospital consultant, defined impetigo and wrote an early text describing skin disease in India.

Sir Jonathan Hutchinson was a polymath surgeon, ophthalmologist, neurologist and dermatologist who described signs in syphilis and a host of other dermatology conditions. He often renamed these, causing confusion when diseases had previously been named by others, for example the recurrent summer eruption of Hutchinson, previously described by Bazin as hydroa vacciniforme.

United States in the 19th century

Henry D. Bulkley studied under Cazenave and Biett in France, and then returned to New York City to set up the Broome Street Infirmary for Diseases of the Skin in 1837 with John Watson. He translated Cazenave into English and was the first President of the New York Dermatology Society in 1869 – the first dermatology society in the world. Noah Worcester was influenced by Willan and Bateman to write *Diseases of the Skin*, the first American dermatology textbook in 1845.

Figure 1.11 Erasmus Wilson. (From http://wellcomeimages.org/. Copyrighted work available under Creative Commons Attribution only licence CC BY 4.0 http://creativecommons.org/licenses/by/4.0/. Wellcome Library, London.)

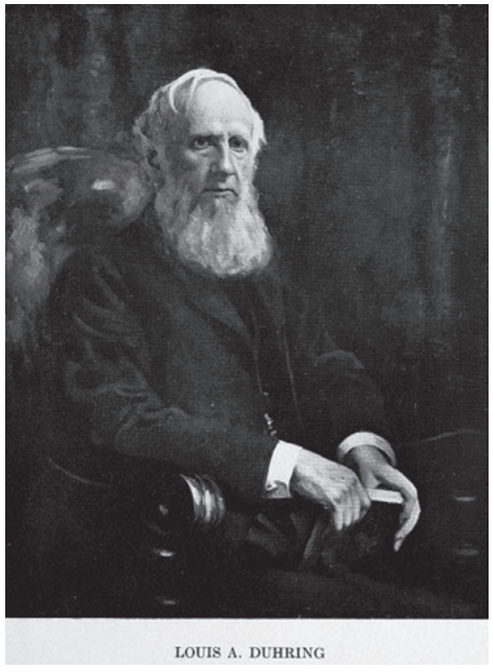


Figure 1.12 Loius Duhring. (From http://wellcomeimages.org/. Copyrighted work available under Creative Commons Attribution only licence CC BY 4.0 http://creativecommons.org/licenses/by/4.0/. Wellcome Library, London.)

Faneuil Weisse studied under Sir Erasmus Wilson in Britain, and then helped establish the New York Dermatology Society when working as lecturer on skin disease at the University of New York City from 1865. In 1871 James White, a student of von Hebra, was appointed to the new chair of dermatology at Harvard, after lecturing for 8 years at the medical school, then practising at Massachusetts General. White was a strong force in developing American dermatology and medical education at Harvard and was president of the Massachusetts Medical Society.

Louis Duhring (Figure 1.12), another student of von Hebra, occupied the first chair of dermatology at the University of Pennsylvania from 1875 for 35 years. He had also studied in Paris and London, influencing his vision of dermatology as a part of general medicine. Duhring's book, *A Practical Treatise on Diseases of the Skin*, and work on dermatitis herpetiformis, together with great wealth and benefactions to the specialty, helped establish dermatology in the United States.

Development of dermatology as a world specialty in the 20th century

At the beginning of the 20th century, dermatology was developing in most European and North American countries with fledgling dermatology societies and dermatology journals. The work often crossed national boundaries. From this point on we can consider the development of dermatology as a world specialty, considering the growth of subspecialty areas and science. The early 20th century saw the conquest of infections that had ravaged mankind for centuries and the development of effective treatments for inflammatory diseases. This, together with public health initiatives, led to great increases in longevity. The later 20th century saw skin surgery develop to deal with the skin cancers that arose in an ageing population, and the birth of molecular genetics that promises an even greater revolution in redefining diseases and predicting responses to treatment in a future era of personalized medicine.

Skin infections

Paul Ehrlich from Germany and Sarachiro Sata from Japan, working with Paul Uhlenhuth in Frankfurt, developed arsphenamine in 1910, Ehrlich's silver bullet, which offered effective chemotherapy for syphilis. This remained a mainstay of treatment until Alexander Fleming, in London, by serendipity discovered penicillin in 1928. This was produced commercially after its first synthesis by Florey, Heatley and Chain in Oxford and provided a cure for syphilis and many other cutaneous infections in the 1940s.

Leprosy has been a misdiagnosed, stigmatizing and confused condition since biblical times. It is likely that many cases who were excluded from society in all ages of mankind actually suffered from other skin diseases. Norwegian Gerhard Hansen together with Neisser in Breslau, described the bacterium between 1873 and 1880. Promin, a sulfone drug, was introduced in 1941; R. G. Cochrane then introduced dapsone in 1950 following research in Carville, Louisiana. Research in Malta resulted in multidrug

therapy in the 1970s, which was then adopted by the World Health Organization in 1981 and since has led to the reduction or eradication of the disease in many countries.

Inflammatory disease and immunomodulatory treatments

The discovery of corticosteroids revolutionized dermatology in the middle of the 20th century [1]. Further drugs that modified the immune system and that were introduced later in the 20th century, such as azathioprine, methotrexate and ciclosporin, enabled the control of serious inflammatory diseases that had scourged humankind. Management of these disorders changed from long-term admissions to hospital into management as outpatients with potentially toxic systemic agents. Increasing knowledge of the molecular basis for diseases has led to more targeted and personalized treatment as dermatology moves into the 21st century.

Dermatology surgery

The practice of dermatology changed in the last three decades of the 20th century in Europe and North America due to the great increase in skin cancer in white-skinned populations. This has been paralleled by the growth in dermatology surgery. Dermatologists have developed the technique of Mohs micrographic surgery and have adopted many plastic surgery procedures such as flap and graft repairs [2]. Many innovations in skin surgery have been introduced by dermatologists as the number of practitioners has grown. Recent advances in the understanding of the mechanisms underlying basal cell carcinoma and melanoma has heralded new drugs offering medical treatments for skin cancers.

The adoption of skin surgical techniques by dermatologists, consumer demand and financial rewards have led to the growth of cosmetic dermatology in affluent societies since the end of the 20th century. Hair dyes, tattooing and other cosmetic procedures have been found in mummies from ancient Egypt, however the growth and spending on cosmetic products to adorn and preserve the skin has paralleled economic growth. The spending on fillers, muscle relaxants, cosmetic surgery and hair transplantation has provided a change in direction for some dermatologists.

Contact dermatitis and allergy

Industrial skin disease has been present since antiquity but patch testing was developed by Jadassohn in Breslau in 1895 [**3**]. Bloch in Basle and Zurich, Bonnevie in Copenhagen and Sulzberger in New York developed the technique and led its introduction across the world. In 1967 Niels Hjorth founded the International Contact Dermatitis Research Group, a group of clinicians from Europe and the United States (including Darrell Wilkinson, an original editor of this book), which standardized patch testing technique across the world.

Phototherapy

Niels Finsen's academic studies from 1894–7 on the value of ultraviolet light as a treatment for tuberculosis put phototherapy on a scientific basis [**4**]. However, the use of topical and oral plant extracts combined with sunlight was used over 3000 years ago in India and Egypt. Broad-band UVB was used until the development of psoralen and UVA (PUVA) in 1974. The initial enthusiasm for this treatment was tempered by caution when the first patients who were on maintenance treatment began to develop skin cancers. Intermittent phototherapy was introduced and the use of PUVA further declined when narrow-band UVB equipment became available from 1990. Extracorporeal photophoresis for cutaneous T-cell lymphoma was introduced in 1987 and in the 1990s photodynamic therapy was developed.

Genital dermatology

In most of the world dermatology and genito-urinary medicine have developed as the same specialty, whereas in the USA and UK they have become separate specialties since the mid-20th century. In these countries, male and female genital dermatology has developed as a subspecialty with increasing awareness of the disability and distress caused by genital disorders.

Psychodermatology and social medicine

Medicine and dermatology has changed its focus diametrically in the latter half of the 20th century. It has moved from being a process applied to passive patients to correct disease as perceived by doctors, to an engagement with an active patient who is encouraged to express their needs and wishes, which the medical team aims to fulfil. With this change has come an awareness of the morbidity that all disease, and skin disease in particular, has due to depression and anxiety. There has been a change in how to measure success in medicine. Disease outcome measures focus on the experience of the individual who is being treated and the effect that the treatment has on the patient's ability to function in their normal activities.

The subspecialty of psychodermatology has developed in recent years, usually by practitioners working closely with psychiatrists and psychologists.

Paediatric dermatology and genetics

A greater understanding of molecular genetics is revolutionizing the relatively new subspecialty of paediatric dermatology. Diseases that were originally described by appearance, phenotype, are now being defined by their genetic abnormalities. This understanding is allowing a reclassification of dermatology based on genetic abnormality, which will progress onto a new understanding of more complex inflammatory diseases and cancers associated with multiple genetic abnormalities. This will result in splitting up diseases and enabling personalized treatment, maximizing benefit and reducing the risks from new drugs targeting the precise molecular abnormality.

Dermatology has never been at a more exciting time in its history. Read on.

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The full list of references can be found in the online version at www.rooksdermatology.com.

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CHAPTER 2

Structure and Function of the Skin

John A. McGrath[1] *and Jouni Uitto*[2]

[1]St John's Institute of Dermatology, Division of Genetics and Molecular Medicine, Faculty of Life Sciences and Medicine, King's College London, London, UK
[2]Department of Dermatology and Cutaneous Biology, Thomas Jefferson University, Philadelphia, PA, USA

Components of normal human skin

Skin is the largest organ in the body. In a 70 kg individual, the skin weighs over 5 kg and covers a surface area approaching 2 m^2. Human skin consists of a stratified, cellular epidermis and an underlying dermis of connective tissue, separated by a dermal–epidermal basement membrane (Figure 2.1). Beneath the dermis is a layer of subcutaneous fat, which is separated from the rest of the body by a vestigial layer of striated muscle.

The epidermis is mainly composed of keratinocytes and, for the living cell layers, is typically 0.05–0.1 mm in thickness. It is formed by the division of cells in the basal layer, which give rise to the spinous layer. This layer contains cells that move outwards and progressively differentiate, forming the granular layer and the stratum corneum. The cellular progression from the basal layer to the skin surface takes about 30 days but is accelerated in diseases such as psoriasis. The 'brick-like' shape of keratinocytes is provided by a cytoskeleton made of keratin intermediate filaments. As the epidermis differentiates, the keratinocytes become flattened. This process involves the filament aggregating protein, filaggrin, a protein component of keratohyalin granules. Indeed, keratin and filaggrin comprise 80–90% of the mass of the epidermis.

The outermost layer of the epidermis is the stratum corneum, where cells (now called corneocytes) have lost the nuclei and cytoplasmic organelles. The corneocyte has a highly insoluble, cornified envelope within the plasma membrane, formed by cross-linking of soluble protein precursors, including involucrin and loricrin, the latter contributing 70–85% to the mass of the cornified cell envelope. It also contains several lipids (fatty acids, sterols and ceramides) released from lamellar bodies within the upper, living epidermis. The stratum corneum can be divided into three distinct biochemical and functional zones – an outer absorber of solutes, a middle absorber of water for hydration, and an inner mechanical defence barrier.

Other cells in the epidermis are the melanocytes, Langerhans cells and Merkel cells. Melanocytes are dendritic cells that distribute packages of melanin pigment in melanosomes to the surrounding keratinocytes to give skin its colour. The number of melanocytes does not differ much between skin types. Rather it is the nature of the melanin and the size of the melanosomes that account for the different appearances. The Langerhans cells are also dendritic in nature, although these are of mesenchymal origin and originate from bone marrow. Langerhans cells are antigen-presenting cells and process antigens encountered by the skin to local lymph nodes and thus have a key role in adaptive immune responses in the skin. Merkel cells are probably derived from keratinocytes. They have a role as mechanosensory receptors in response to touch.

Human skin contains pilosebaceous follicles and sweat glands. The hair follicles comprise pockets of epithelium that are continuous with the superficial epidermis but which also envelop a small

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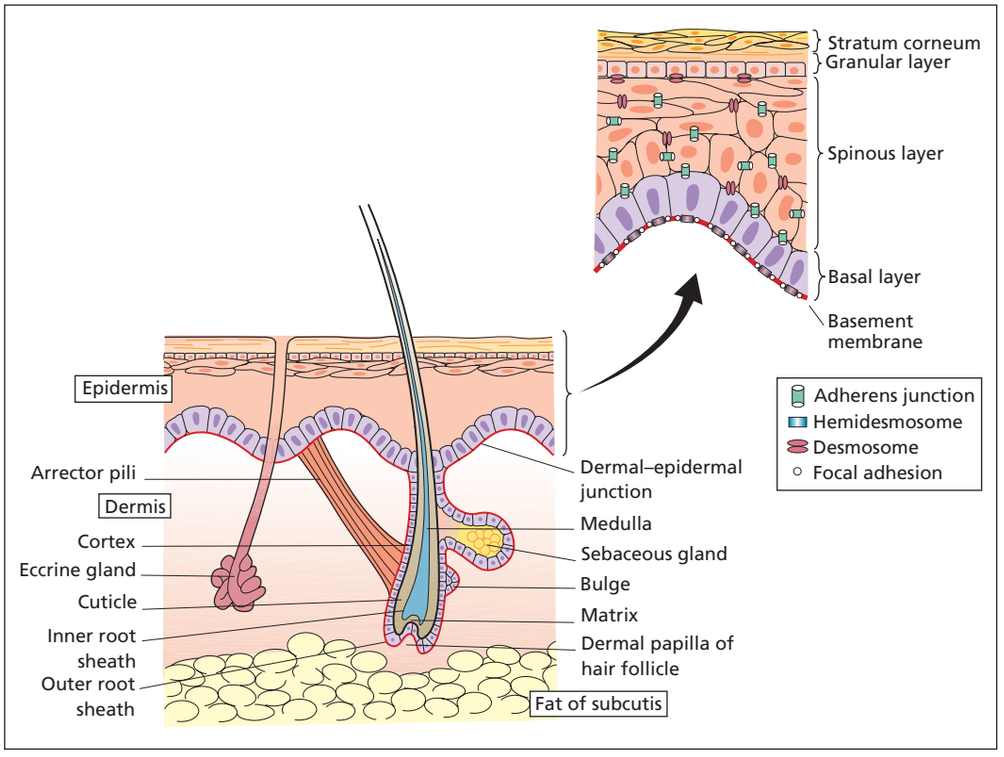


Figure 2.1 The skin and its appendages.

papilla of dermis at their base. A bundle of smooth muscle, the arrector pili, extends at an angle between the surface of the dermis and a point in the follicle wall. Above the insertion, there are holocrine sebaceous glands which open into the pilary canal. In some sites, such as the axillae, the follicles may be associated with apocrine glands. Also derived from the epidermis and opening directly to the skin surface are the eccrine sweat glands.

The epidermis is attached to the dermis via a complex network of proteins and glycoproteins that extend from inside basal keratinocytes into the superficial dermis. Besides adhesion, the dermal–epidermal junction components also contribute to cell migration (for example during wound healing) as well as epithelial–mesenchymal signalling events. Over 30 different macromolecules (collagens, laminins and integrins) interact within a basement membrane zone that is less than 200 µm across.

The dermis consists of a supporting matrix in which polysaccharides and proteins are enmeshed to a network that provides resilience to the skin and has a remarkable capacity for retaining water. The thickness of the dermis varies from less than 0.5 mm to more than 5 mm depending on the skin site. There are two principal types of protein fibre: collagen and elastic tissue.

Collagen is the major extracellular matrix protein, comprising 80–85% of the dry weight of the dermis. Twenty-nine different collagens have been identified in vertebrate tissue (depicted by Roman numerals in the order of their discovery, from I to XXIX), of which at least 12 are expressed in skin. The main interstitial dermal collagens are types I and III, whereas the principal basement membrane collagen (at the dermal–epidermal junction and around dermal blood vessels, nerves and appendages) is type IV collagen. Triple-helical collagen monomers polymerize into fibrils and fibres, which then become stabilized by the complex formation of both intra- and intermolecular cross-links. Collagen fibres are extremely resilient and provide skin with its tensile strength.

In sun protected adult skin, elastic fibres account for no more than 2–4% of the extracellular matrix in the dermis and consist of two components, elastin and elastin-associated microfibrils, which together give skin its elasticity and resilience. Elastic microfibrils are composed of several proteins, including fibrillin, which surround the elastin and which can extend throughout the dermis in a web-like configuration to the junction between the dermis and the epidermis. The dermis also contains a number of non-collagenous glycoproteins, including fibronectins, fibulins and integrins. These extracellular matrix components facilitate cell adhesion and cell motility.

Between the dermal collagen and elastic tissue is the ground substance made up of glycosaminoglycan/proteoglycan macromolecules. These contribute only 0.1–0.3% of the total dry weight of the dermis but provide a vital role by maintaining hydration, mostly due to the high water-binding capacity of hyaluronic acid. About 60% of the total weight of the dermis is water.

The dermis has a very rich blood supply, although no vessels pass through the dermal–epidermal junction. There is a superficial and a deep vascular plexus. The motor innervation of the skin is autonomic, and includes a cholinergic component to the eccrine sweat glands and adrenergic components to both the eccrine and apocrine glands, to the smooth muscle and the arterioles and to the arrector pili muscle. The sensory nerve endings are of several kinds; some are free, some terminate in hair follicles and others have expanded tips.

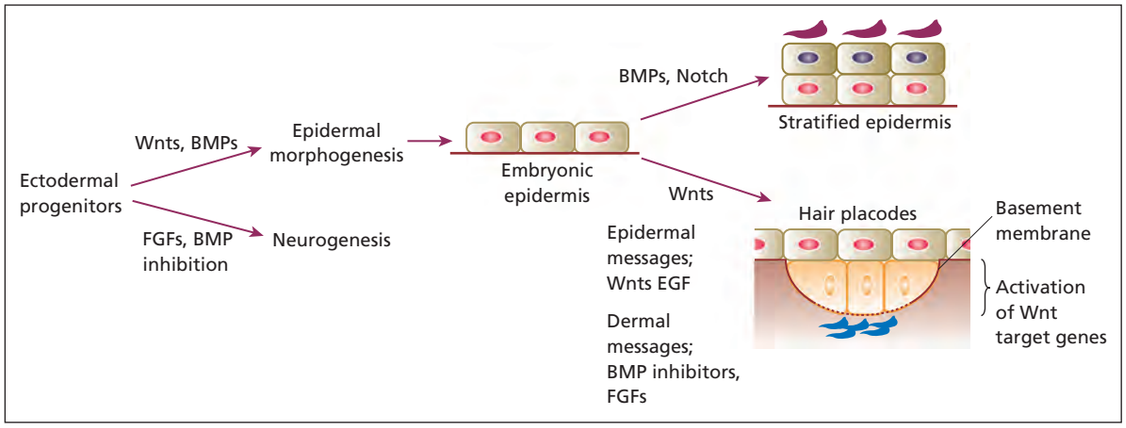


Figure 2.2 Embryonic development of the skin depends on specific signalling molecules. Relative stimulation or inhibition by these signalling molecules also determines whether embryonic epidermis progresses to a stratified epidermis or whether formation of skin appendages is induced. BMP, bone morphogenic protein; EGF, epidermal growth factor; FGF, fibroblast growth factor.

Skin development

The skin arises by the juxtaposition of two major embryological elements: the prospective epidermis that originates from a surface area of the early gastrula, and the prospective mesoderm that comes into contact with the inner surface of the epidermis during gastrulation. The mesoderm not only provides the dermis, but is essential for inducing differentiation of the epidermal structures, such as the hair follicle. The melanocytes are derived from the neural crest.

After gastrulation, there is a single layer of neuroectoderm on the embryo surface; this layer will go on to form the nervous system or the skin epithelium depending on the molecular signals it receives (Figure 2.2). Activation of Wnt signalling will block the ability of the ectoderm to respond to fibroblast growth factors (FGFs). Without FGFs the cells express bone morphogenic proteins (BMPs) and progress to an epidermal lineage. Conversely, lack of Wnt signalling promotes a neural fate [1]. One key transcription factor in skin development is p63, which contributes to epidermal lineage commitment, epidermal differentiation, cell adhesion and basement membrane formation [2]. The embryonic epidermis consists of a single layer of multipotent epithelial cells, which is covered by a special layer known as periderm that is unique to mammals (Figures 2.3 and 2.4). Periderm provides some protection to the newly forming skin as well as exchange of material with the amniotic fluid.

The embryonic dermis is at first very cellular, and at 6–14 weeks three types of cell are present: stellate cells, phagocytic

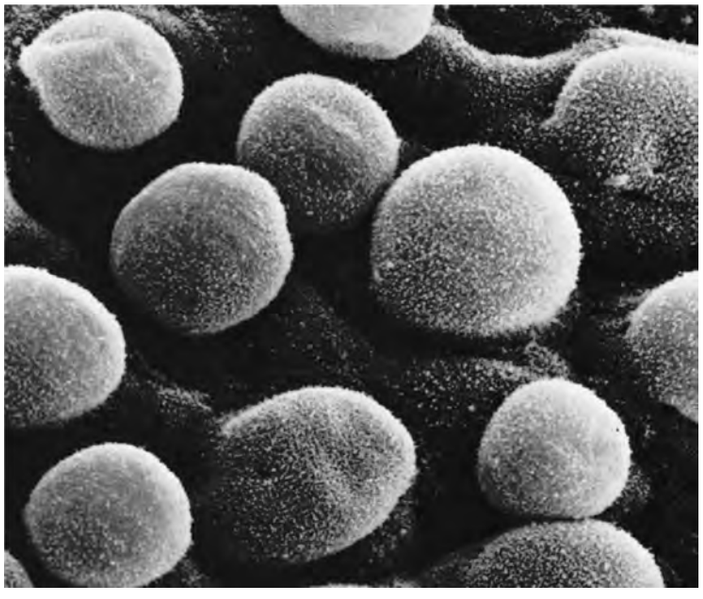


Figure 2.3 Scanning electron micrograph of an 85–110-day (estimated gestation age) human embryo. Single globular blebs project from the periderm cells. (Courtesy of Professor K. A. Holbrook.)

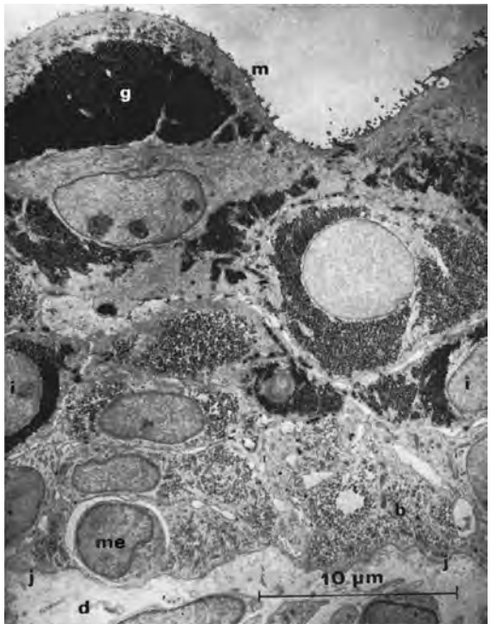


Figure 2.4 Electron micrograph of the full-thickness epidermis from the back of a 14-week human fetus. The periderm cells are full of glycogen (g) and have microvilli (m) at their amniotic border. Cells of the intermediate layer (i) also contain glycogen. Basal layer cells (b) have lost glycogen by this stage. Just above the dermal–epidermal junction (j) there is a melanocyte (me); the surrounding space indicates that it is a recent immigrant from the dermis (d). Osmium fixation and lead staining. (Courtesy of Professor A. S. Breathnach.)

macrophages and granule-secretory cells, either melanoblasts or mast cells. From weeks 14 to 21, fibroblasts are numerous and active, and perineural cells, pericytes, melanoblasts, Merkel cells and mast cells can be individually identified. Two distinct lineages of fibroblasts are present: one that gives rise to the upper dermis and hair follicle formation, and another that helps generate the deep dermis and subcutaneous fat [3].

The various structural components of the skin that can be recognized postnatally start to appear at different embryonic time points, for example hair follicles and nails (9 weeks), sweat glands (9 weeks for the palms and soles, 15 weeks for other sites) and sebaceous glands (15 weeks). Touch pads become recognizable on the fingers and toes by week 6 and reach their greatest development at week 15. After this, they flatten and become indistinct. It is these areas that determine the pattern of dermatoglyphics that take their place.

The mesoderm not only provides the dermis but is essential for inducing differentiation of the epidermal structures, such as the hair follicle in mammals [4]. The earliest development of the hair rudiments occurs at about 9 weeks in the regions of the eyebrow, upper lip and chin (Figure 2.5). Mesenchymal cells, derived from the dermomyotome, populate the skin and interact with the overlying epidermis to induce the formation of hair placodes [5]. Key components of the mesenchymal signals to produce hair follicles include FGFs and BMP-inhibitory factors such as Noggin; excessive BMP stimulation can reduce hair follicle density. The epidermal response to form the hair placode is generated by Wnt signals such as Wnt10b and sonic hedgehog (Shh), which also has a key role in the formation of the dermal papilla [6]. After it is formed, the dermal papilla sends further signals to transform the placode into a hair follicle. At the centre of the signalling cross-talk is the bipartite transcription factor composed of lymphoid enhancer-binding factor 1 (LEF1) and stabilized β-catenin, which is essential for hair follicle formation. Hair follicle development is also influenced by Smads, a group of signalling mediators and antagonists of the transforming growth factor β (TGF-β) superfamily. Smad-4 affects hair follicle differentiation by mediating BMP signalling; Smad-7 affects hair follicle development and differentiation by blocking TGF-β/Activin/BMP pathways [7]. Skin development is governed by complex, balanced waves of gene activation and silencing; cross-talk between small non-coding micro-RNAs and messenger RNAs is very important for the coordination of signal transduction and transcriptional activation [8].

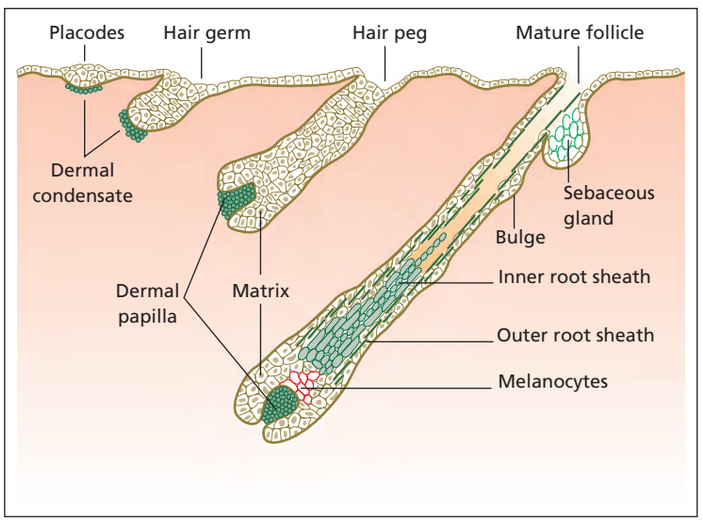


Figure 2.5 Embryonic stages of hair follicle morphogenesis.

Signalling responses differ between follicular and interfollicular epidermis: BMP signalling is active in the interfollicular epidermis and is both an epidermis-promoting signal as well as a follicle-inhibiting signal; epidermal growth factor receptor (EGFR) signalling may have a similar role in governing follicle density. As hair follicles mature to form inner and outer root sheaths, several signalling pathways are involved, including Wnt, Notch and BMP receptors. There are also marked changes in certain cell adhesion proteins, notably E-cadherin and P-cadherin. The hair follicles are arranged in patterns, usually in groups of three. It appears that the first follicles develop over the surface at fixed intervals of between 274 and 350 μm. As the skin grows, these first hair germs become separated, and new rudiments develop between them when a critical distance, dependent on the region of the body, has been reached. There is no large-scale destruction of follicles during postnatal development, only a decrease in actual density as the body surface increases; nor do any new follicles develop in adult skin. In interfollicular epidermis, the undersurface of the epidermis is smooth, but during the fourth month, at the same time as the hair follicle starts to develop, it becomes irregular.

Sebaceous glands first appear as hemispherical protuberances on the posterior surfaces of the hair pegs. The cells contain moderate amounts of glycogen, but soon the cells in the centre lose this, and become larger and accumulate droplets of lipid. The sebaceous glands become differentiated at 13–15 weeks, and are then large and functional. The sebum forms part of the vernix caseosa. At the end of fetal life, sebaceous glands are well developed and generally large. After birth, the size is rapidly reduced, and they enlarge to become functional again only after puberty. The molecular signals that induce sebaceous gland differentiation involve the c-Myc transcription factor as well as the adipogenic transcription factor peroxisome proliferator-activated receptor γ (PPAR-γ) [9].

Eccrine glands start to develop on the palms and soles at about 3 months, but not over the rest of the body until the fifth month [10]. In embryos of 12 weeks, the rudiments of eccrine sweat glands are first identifiable as regularly spaced undulations of the developing epidermis. Cells that go on to form the eccrine sweat glands are oblong, palisading and lie closely together, but otherwise they do not differ from the rest of the developing basal epidermis. By 14–15 weeks, the tips of the eccrine sweat gland rudiments have penetrated deeply into the dermis, and have begun to form the coils. In the overlying epidermis, columns of cells that are destined to form the intraepidermal sweat ducts are recognizable. Each column is composed of two distinct cylindrical layers, comprising two inner cells that are elongated and curved so that they embrace the inner cylinder. The intraepidermal duct appears to form by the coalescence of groups of intracytoplasmic cavities formed within two adjacent inner cells. In the intradermal segment, the lumen forms by dissolution of the desmosomal attachment plaques between the cells that compose the inner core of the eccrine duct germ.

Nails begin to develop in the third month. Key signalling events in nail development involve the R-spondin family of transcription factors [11]. In fetuses at 16–18 weeks (crown to rump length 120–150 mm), keratinizing cells from both dorsal and ventral matrices can be distinguished. Melanocytes take their origin from the neural crest. This can be identified in early human embryos, but the elements arising from it soon lose themselves in the mesenchyme, and pigmented melanocytes cannot be identified, even in darker skin fetuses, before 4–6 months of gestation. However, dopa-positive melanocytes can be demonstrated earlier. Langerhans cells are derived from the monocyte–macrophage–histiocyte lineage and enter the epidermis at about 12 weeks. Merkel cells appear in the glabrous skin of the fingertips, lip, gingiva and nail bed, and in several other regions, around 16 weeks.

Although some cells of the dermis may migrate from the dermatome (ventrolateral part of the somite) and take part in the formation of the skin, most of the dermis is formed by mesenchymal cells that migrate from other mesodermal areas [12]. These mesenchymal cells give rise to the whole range of blood and connective tissue cells, including the fibroblasts and mast cells of the dermis and the fat cells of the subcutis. In the second month, the dermis and subcutis are not distinguishable from each other but distinct collagen fibres are evident in the dermis by the end of the third month. Later, the papillary and reticular layers become distinct and, at the fifth month, the connective tissue sheaths are formed around the hair follicles. Elastic fibres are first detectable at 22 weeks.

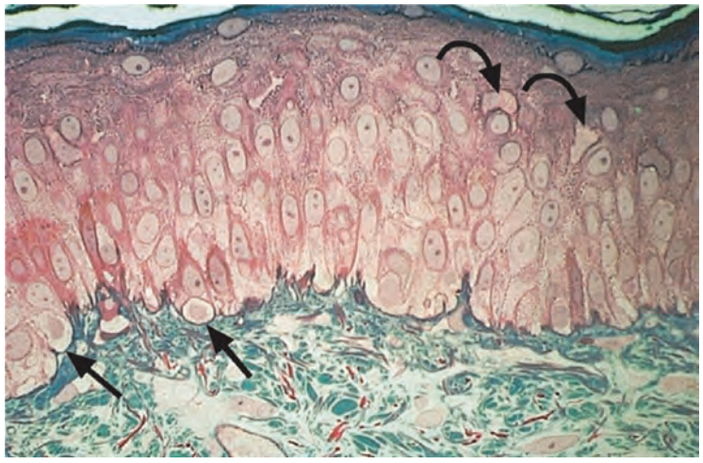


Figure 2.6 Photomicrograph of a 1 μm-thick plastic section of normal human skin. The tissue was fixed with half-strength Karnovsky medium and embedded in Epon. This technique allows the cellular components of the epidermis, including keratinocytes, melanocytes (straight arrows) and probable Langerhans cells (curved arrows) to be clearly resolved. Magnification 400× (basic fuchsin and methylene blue). (Courtesy of Professor R. A. J. Eady, St John's Institute of Dermatology, King's College London, UK.)

Epidermal and adnexal structures

The normal epidermis is a terminally differentiated, stratified, squamous epithelium. The major cell type, making up 95% of the total, is the *keratinocyte*, which moves progressively from attachment to the epidermal basement membrane towards the skin surface, forming several well-defined layers during its transit [1]. Thus, on simple morphological grounds, the epidermis can be divided into four distinct layers: *stratum basale* or *stratum germinativum*, *stratum spinosum*, *stratum granulosum* and *stratum corneum*. The term *Malpighian layer* includes both the basal and spinous cells. Other constitutive cells within the epidermis include melanocytes, Langerhans cells and Merkel cells (Figure 2.6).

The stratum basale is a continuous layer that is generally only one cell thick but it may be two to three cells thick in glabrous skin and in hyperproliferative epidermis. The basal cells are small and cuboidal (10–14 μm in diameter) and have large, dark-staining nuclei, and dense cytoplasm that contains many ribosomes and dense tonofilament bundles. Immediately above the basal cell layer, the epibasal keratinocytes enlarge to form the spinous/prickle cell layer or stratum spinosum (Figure 2.7).

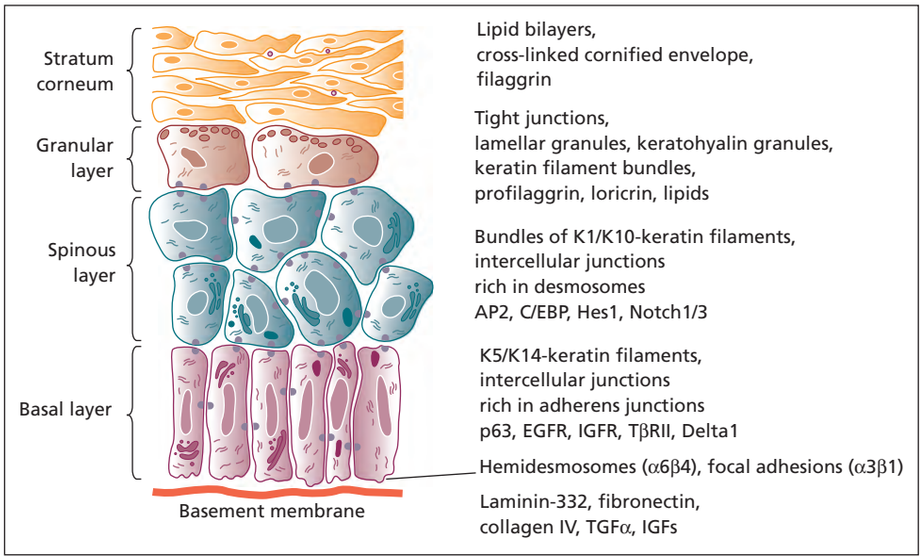


Figure 2.7 The process of epidermal differentiation is associated with the expression of different structures, macromolecules, transcription factors and other signalling molecules and their receptors in the different keratinocyte layers. EGFR, epidermal growth factor receptor; IGF, insulin-like growth factor; IGFR, IGF receptor; TGF, transforming growth factor.

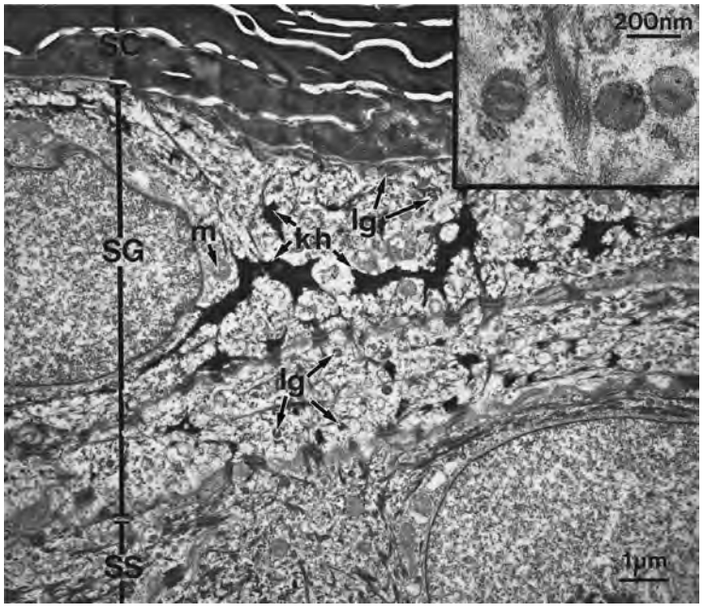


Figure 2.8 Electron micrograph showing details of the upper part of the epidermis including the stratum corneum (SC), stratum granulosum (SG) and most superficial cell layer of stratum spinosum (SS). Note the irregularly shaped keratohyalin granules (kh) and the small, round, lamellar granules (lg). The latter are present in both SS and SG and are smaller than mitochondria (m). The inset shows details of lamellar granules. (Courtesy of Professor R. A. J. Eady, St John's Institute of Dermatology, King's College London, UK.)

The stratum spinosum is succeeded by the stratum granulosum or granular layer, which contains intracellular granules of keratohyalin. At high magnification, the dense mass of keratohyalin granules from human epidermis has a particulate substructure, with particles of irregular shape, on average 2 nm in length, and occurring randomly in rows or lattices. The cytoplasm of cells of the upper, spinous layer and granular cell layer also contains smaller lamellated granules averaging 100–300 nm in size. These are known as lamellar granules or bodies, membrane-coating granules or Odland bodies. They are numerous within the uppermost cells of the spinous layer and migrate towards the periphery of the cells as they enter the granular cell layer (Figure 2.8). They discharge their lipid components into the intercellular space, playing important roles in barrier function and intercellular cohesion within the stratum corneum (Figure 2.9) [2].

The outermost layer of epidermis is the stratum corneum where cells, now known as corneocytes, have lost the nuclei and cytoplasmic organelles. The cells become flattened and the keratin filaments align into disulphide cross-linked macrofibres, under the influence of *filaggrin*, the protein component of the keratohyalin granule. Filaggrin is responsible for keratin filament aggregation, and is subsequently broken down into individual hygroscopic amino acids that form the basis of the natural moisturizing factor within corneocytes. The key role of filaggrin in skin biology has been demonstrated by the discovery of common loss-of-function mutations in the filaggrin gene as the cause of the genetic disorder

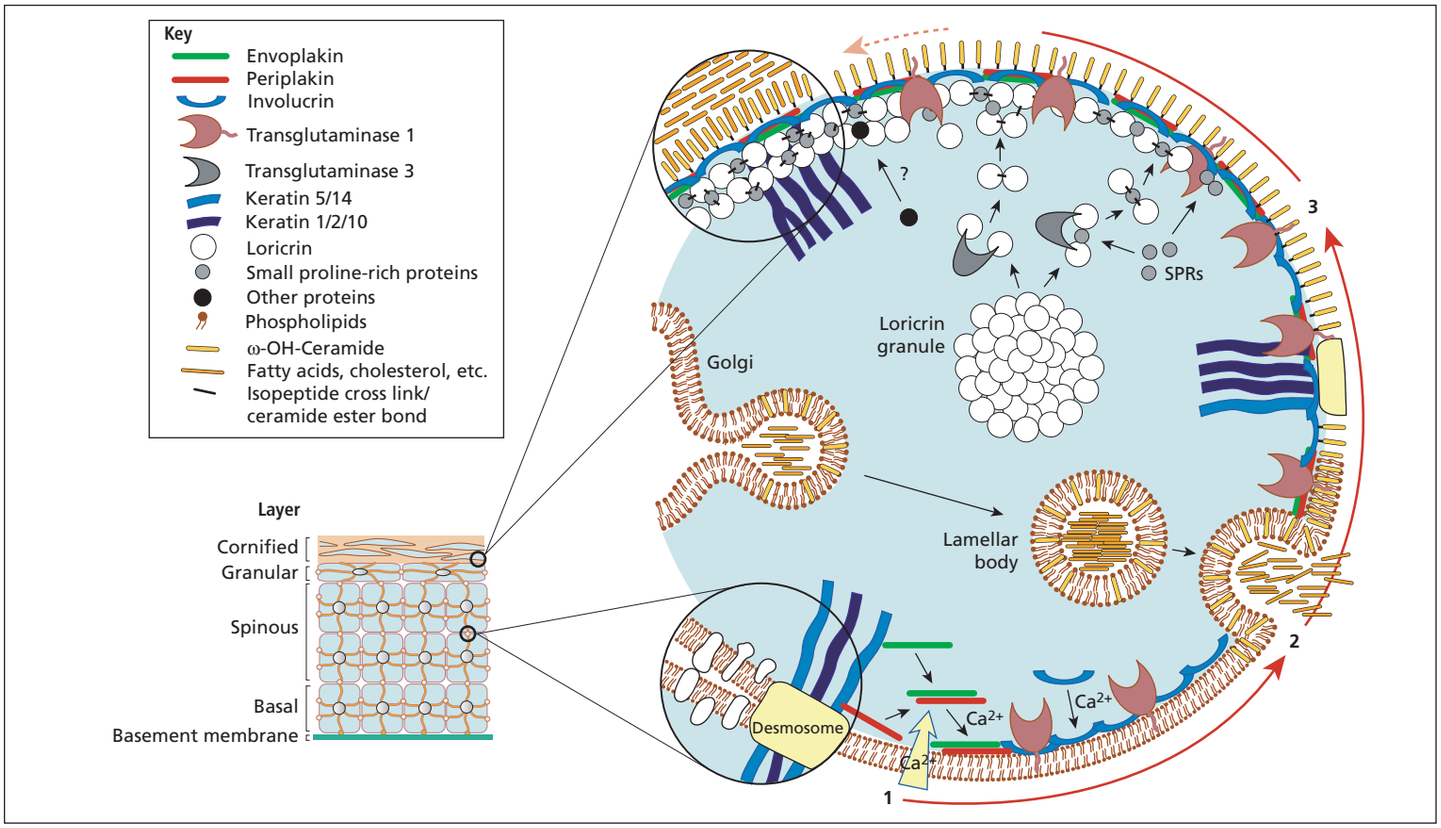


Figure 2.9 Assembly of the epidermal cornified cell envelope. In response to increasing intracellular calcium, an internal scaffold of desmosomal proteins is made along the plasma membrane. The contents of lamellar bodies (ceramides and other fatty acids, cholesterol and cholesterol esters) are released into the extracellular milieu to form a lipid membrane. The developing envelope is then added to and reinforced by the recruitment of various proteins, including loricrin, small proline-rich proteins (SPRs), other desmosomal remnants and attached keratin filaments. The resulting cornified cell envelope is durable and flexible and provides important mechanical and barrier functions.

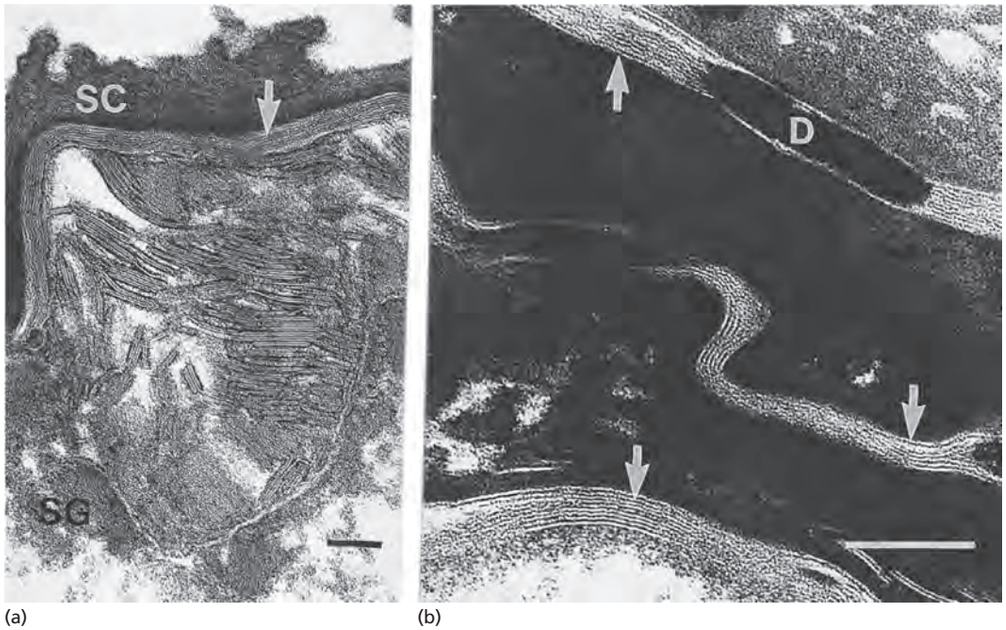


Figure 2.10 Electron micrograph showing the location of epidermal lipids by ruthenium oxide staining. (a) Extrusion of lamellar body lipids or sheets can be seen at the interface between the stratum granulosum (SG) and stratum corneum (SC). Scale bar 0.1 μm. (b) Sheets of lipid bilayers (arrowed) are present in the intercellular spaces of the SC. Some regions show a repetitive pattern of staining. D, desmosome. Scale bar 0.1 μm. (Courtesy of Dr M. Fartasch, Department of Dermatology, University of Erlangen, Germany.)

ichthyosis vulgaris and as a major risk factor for the development of atopic eczema, atopic asthma and systemic allergies [3]. The corneocyte has a highly insoluble, cornified envelope within the plasma membrane, formed by cross-linking of the soluble protein precursor, *involucrin*, following the action of a specific epidermal transglutaminase also synthesized in the high stratum spinosum (Figure 2.10). Many of the proteins involved in terminal differentiation are derived from a cluster of about 25 genes located within a *c*. 2 Mb region on the long arm of chromosome 1. Termed the epidermal differentiation complex (EDC), these coding elements are derived from at least three families of structurally, functionally and evolutionarily related genes. Together, the EDC proteins have roles in structural integrity, signal transduction and cell cycle progression and may be primarily or secondarily disrupted in several inflammatory or neoplastic disorders.

The process of desquamation involves degradation of the lamellated lipid in the intercellular spaces and loss of the residual intercellular desmosomal interconnections [**4**]. In palmoplantar skin there is an additional zone, also electron-lucent, the *stratum lucidum*, between the granulosum and corneum. These cells are still nucleated, and may be referred to as 'transitional' cells.

Keratinocytes

The filamentous cytoskeleton of all mammalian cells, including epidermal keratinocytes, comprises: actin-containing microfilaments approximately 7 nm in diameter; tubulin-containing microtubules 20–25 nm in diameter; and filaments of intermediate size, 7–10 nm in diameter, known as intermediate filaments. There are six types of intermediate filaments: keratins in epithelial cells; vimentin within mesenchymal cells; glial filament acidic protein (GFAP) in glial cells; neurofilaments in neurons; desmin in muscle cells; and peripherin in peripheral nerves. The nuclear matrix proteins, nuclear lamins A, B and C, are also intermediate filaments. The polypeptide building blocks of all intermediate filaments have a similar backbone structure of a classic α-helical region with heptad repeats, having four separate helical zones with interhelical linker sequences, and non-helical carboxy- and amino-terminals. There are 70 intermediate filament genes (including those encoding keratins, desmins and lamins), which are now known to be associated with at least 72 distinct human diseases, including skin blistering, muscular dystrophy, cardiomyopathy, premature ageing syndromes, neurodegenerative disorders and cataract [1,2].

The human genome possesses 54 functional keratin genes located in two compact gene clusters, as well as many non-functional pseudogenes scattered around the genome [3]. Keratin genes are very specific in their expression patterns. Each one of the many highly specialized epithelial tissues has its own profile of keratin gene expression. Hair and nails express modified keratins, containing large amounts of cysteine which forms numerous chemical cross-links to further strengthen the cytoskeleton. The genes encoding individual keratins fall into two gene families: type I (basic) and type II (acidic). Mapping the tissue distribution of keratins shows co-expression of particular acidic–basic pairs in a cell- and tissue-specific manner. Heterodimers are assembled into higher order protofibrils and protofilaments by an antiparallel stagger of some complexity.

Simple epithelia are characterized by the keratin pair K8/K18, and the stratified squamous epithelia by K5/K14 (Figure 2.11). In addition, stratified squamous epithelia express up to four other keratin pairs during epithelial differentiation. In skin, suprabasal keratins K1/K10 are characteristic of epidermal differentiation. In the stratum granulosum, release of filaggrin from the keratohyalin granules forms macrofibres. Retinoid levels, growth factors and hormones may regulate keratin gene expression. Mesenchymal

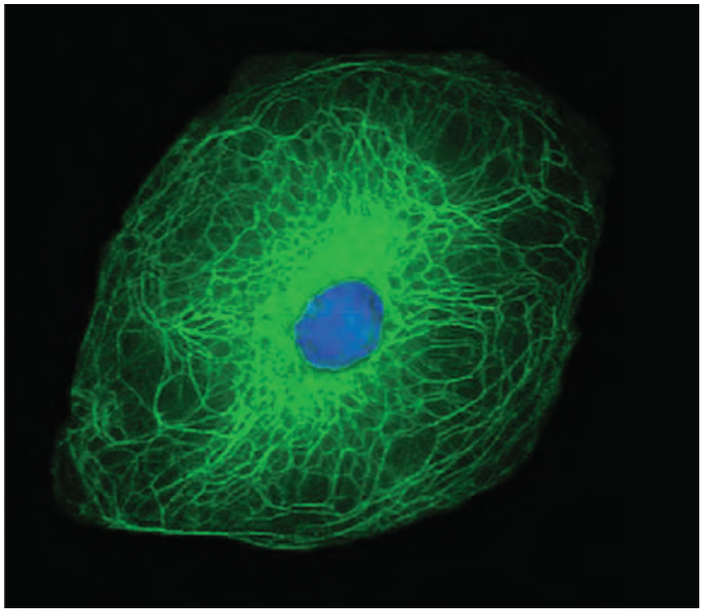


Figure 2.11 Structural organization of the keratin filament network within a keratinocyte. (Courtesy of Professor W. H. I. McLean, University of Dundee, UK.)

signals may also direct or permit intrinsic patterns of keratinocyte differentiation. K15 is expressed in basal keratinocytes of the hair follicle bulge region at the site of pluripotential stem cells. K9 and K2 expression is site restricted in skin: K9 to the palmoplantar epidermis and K2 to the superficial interfollicular epidermis. Apart from their structural properties, keratins may also have direct roles in cell signalling, the stress response and apoptosis [4]. In epidermal hyperproliferation, as in wound healing and psoriasis, the expression of suprabasal keratins K6/K16/K17 is rapidly induced.

Currently, 21 of the 54 known keratins (28 type I, and 26 type II) have been linked to monogenic genetic disorders, and some have been implicated in more complex traits, such as idiopathic liver disease or inflammatory bowel disease [5]. The first genetic disorder of keratin to be described was epidermolysis bullosa simplex, which involves mutations in the genes encoding K5 or K14. About half of the 54 keratin genes are expressed in the hair follicle (trichocyte 'hard' keratins), although only four of these have been linked to human genetic disorders (monilethrix, hair–nail ectodermal dysplasias, pseudofolliculitis barbae and woolly hair) [6].

Eccrine and apocrine glands

Human sweat glands are generally divided into two types: apocrine and eccrine [1]. The eccrine gland is the primary gland responsible for thermoregulatory sweating in humans. Eccrine sweat glands are distributed over nearly the entire body surface. Sweat glands become identifiable in the palms and soles in the 16th fetal week, and in the rest of the body from the 22nd week onwards. The number of sweat glands in humans varies greatly, ranging from 1.6 to 4.0 million. The structure of the eccrine sweat gland consists of a bulbous secretory coil leading to a duct. The secretory coil is located in the lower dermis, and the duct extends through the dermis and opens directly onto the skin surface. The active sweat glands are present most densely on the sole, forehead and palm, somewhat less on the back of the hand, still less on the lumbar region and the lateral and extensor surfaces of the extremities, and least on the trunk and the flexor and medial surfaces of the extremities. The uncoiled dimension of the secretory portion of the gland is approximately 30–50 μm in diameter and 2–5 mm in length. The size of the adult secretory coil ranges from 1 to 8×10^{-3} mm^3.

Human perspiration is classified into two types: insensible perspiration and active sweating. Insensible perspiration involves water loss from the respiratory passages and the skin. In the skin, the epidermis is supplied with water originating from blood in the skin microcirculation and interstitial spaces so that water can evaporate from its dry surface. Thus, the evaporation of water from the skin may depend on several environmental factors, such as ambient temperature and ambient humidity. Heat, mental stimuli and muscular exercise can all induce active sweating in human beings. Active sweating may be classified into two types: thermal and mental/emotional. Thermal sweating plays an important role in keeping the body's temperature constant and involves the whole body surface [2]. Mental or emotional sweating usually appears on the palms and soles. The physiological features of mental sweating differ considerably from those of thermal sweating. Mental sweating has a shorter latent period for its onset and immediately attains a certain rate of secretion that corresponds to the intensity of stimulation, remaining for the duration of the stimulation and subsiding quickly after it ends [3]. Eccrine glands contribute to both types of sweating.

The secretory nerve fibres innervated in human sweat glands are sympathetic, and seem to be cholinergic in character as sweating is produced by pilocarpine and stopped by atropine [4]. Vasoactive intestinal peptide (VIP) coexisting in the cholinergic nerve fibres may act as a candidate neurotransmitter to control the blood circulation of the sweat glands. The sudorific nervous system is also separated into parts for thermal and emotional sweating, each being controlled by its own regulatory centre in the brain that is associated with the sweat glands in its respective region of the skin. The exact neurological pathways responsible for sweating are not entirely understood.

Sympathetic nerve terminals cluster mainly around the secretory coil of the sweat gland, but a few projections extend to the sweat duct. Acetylcholine is the primary neurotransmitter released from cholinergic sudomotor nerves and binds to muscarinic receptors on the eccrine sweat gland, although sweating can also occur via exogenous administration of α- or β-adrenergic agonists. Released acetylcholine is rapidly hydrolysed by acetylcholinesterase, and this response may be one of a number of mechanisms by which the rate of sweating rate is regulated.

When acetylcholine binds to muscarinic receptors on the sweat gland, intracellular Ca^{2+} concentrations increase. This results in an increase in the permeability of K^+ and Cl^- channels, which initiates the release of an isotonic precursor fluid from the secretory cells [5]. This precursor fluid is similar to plasma but is devoid of proteins. As the fluid travels up the duct toward the surface of the skin, sodium and chloride are reabsorbed, resulting in sweat on

the surface being hypotonic relative to plasma. When the rate of sweat production increases, however, for example during exercise or heat stress, ion reabsorption mechanisms can be overwhelmed due to the large quantity of sweat secreted into the duct, resulting in higher ion losses. The sodium content in sweat on the skin's surface, therefore, is greatly influenced by sweat rate.

Apart from eccrine glands, the skin also contains apocrine sweat glands [6]. Eccrine glands do not show cytological changes during secretion, whereas apocrine glands are characterized by decapitation secretion, in which part of the cell is pinched off and released into the lumen. Apocrine glands are located only in genital, axillary and mammary areas, where they are always connected to a hair follicle. Apocrine glands have a low secretory output and hence no significant role in thermoregulation. They are composed of a coiled secretory portion and an excretory duct. The inner layer of the secretory portion contains a single columnar secretory cell type containing numerous, large, dense granules located at the apical aspect, which contribute to the lipid-rich secretion produced. The inner layer is also surrounded by a fenestrated layer of myoepithelial cells but the lumen is generally larger in diameter than that present in eccrine tissue. The apocrine excretory duct does not have any known reabsorptive function and consists of a double layer of cuboidal cells that merge distally with the epithelium of the hair follicle, resulting in emptying of the secretion into the hair follicle. The exact role of apocrine glands in humans is unknown. A third type of intermediate sweat glands, the apoeccrine glands, has also been described in axillary skin but their existence is not universally accepted.

Pilosebaceous unit

The pilosebaceous units develop from epidermal down-growths under the influence of specific mesenchymal cell condensations between the 10th and 14th week estimated gestational age. They have complex groups of specialized cell layers with distinctive pathways of differentiation. There are four classes of pilosebaceous unit: terminal on the scalp and beard; apopilosebaceous in the axilla and groin; vellus on the majority of skin; and sebaceous on the chest, back and face. The dermal papilla is located at the base of the hair follicle with a rich extracellular matrix. Around the papilla are germinative (matrix) cells that have a very high rate of division, and give rise to spindle-shaped central cortex cells of the hair fibre, and the single outer layer of flattened, overlapping cuticle cells. A central medulla is seen in some hairs, with regularly stacked, condensed cells interspersed with air spaces or low-density cores. The cortical cells are filled with keratin intermediate filaments orientated along the long axis of the cell, interspersed with a dense interfilamentous protein matrix.

Terminal differentiation of cortical cells is associated with the appearance of a contiguous, laminated, intercellular layer, which appears critical for filament integrity. The cuticular cells are morphologically distinct; these are flattened, outward-facing cells, with three layers inside the cuticle of condensed, flattened protein granules: endocuticle, exocuticle and 'a' layer [1]. Around the cuticle is the inner root sheath (IRS), which is composed of three distinct layers of cells that undergo keratinization: the IRS cuticle, the Huxley layer and the outermost Henle layer [2]. Differentiation in the IRS involves the development of trichohyalin granules, with 8–10 nm filaments orientated in the direction of hair growth. The IRS moves up the follicle, forming a support for the hair fibre, and degenerates above the sebaceous gland. The outermost layer is the outer root sheath (ORS), which is continuous with the epidermis and expresses epithelial keratins K5/K14, K1/K10 and K6/K16 in the upper ORS and K5/K14/K17 in the deeper ORS.

Normal growth of the hair fibre is 300–400 μm/day, generated by the high rate of proliferation of progenitor cells in the follicle bulb. Compartmentalization within the bulb gives rise to the different layers within the follicle, with the majority of bulb cells forming the IRS. There are three phases of cyclical hair growth: anagen, when growth occurs; catagen, a regressing phase; and telogen, a resting phase (Figure 2.12). The follicle re-enters anagen, and the old hair is replaced by a new one.

Immediately above the basal layer in the hair bulb, cells undergo a secondary pathway of 'trichocyte' or hair differentiation, and express a further complex group of keratins, the hard keratins [3]. Two families of hair keratins, types I and II, are present in mammals, which have distinctive amino- and carboxy-terminals with high levels of cysteine residues, and lack the extended glycine residues of epidermal keratins. The proteins differ from epithelial keratins in their positions on two-dimensional gels but they can be grouped into acidic and basic families; there are four major proteins in each of these families and several minor proteins, Ha 1–4 and Hb 1–4. Recent cloning of the hair keratin genes, which cluster on chromosomes 12 and 17, has shown a greater number of hair keratin genes, *HaKRT1–6* (including 3.1 and 3.2) and *HbKRT1–6*. Mutations in hair keratin genes have been found to be causative for autosomal dominant forms of the human disease monilethrix. In addition, keratin 17 null mice also demonstrate varying degrees of alopecia, depending on the age and strain of the mice.

Over the last two decades, several naturally occurring, inherited human disorders of hair have provided fascinating insight into hair development and growth. These include key signalling molecules such as ectodysplasin, as well as transcription factors, including hairless and the vitamin D receptor, structural hair keratins, desmosomal proteins, a G protein-coupled receptor, a serine protease and a copper transporter [4]. More common hair variants, such as curly hair, may be explained by dynamic changes during hair growth [5]. Curvature of curly hair is programmed from the very basal area of the follicle and the bending process is linked to a lack of axial symmetry in the lower part of the bulb, affecting the connective tissue sheath, ORS, IRS and hair shaft cuticle.

Nails

The main purpose of the nail apparatus is to provide protection to the digit tips, enhance sensory discrimination, help increase dexterity, facilitate scratching or grooming, and, in some individuals, to function as a cosmetic accessory [1]. The earliest signs of finer nail development occur at 8–9 weeks' gestation: there is an invagination of the primitive epidermis to form an uninterrupted groove delineating a flattened surface at the end of each digit, known as the nail field. A key transcription factor in nail

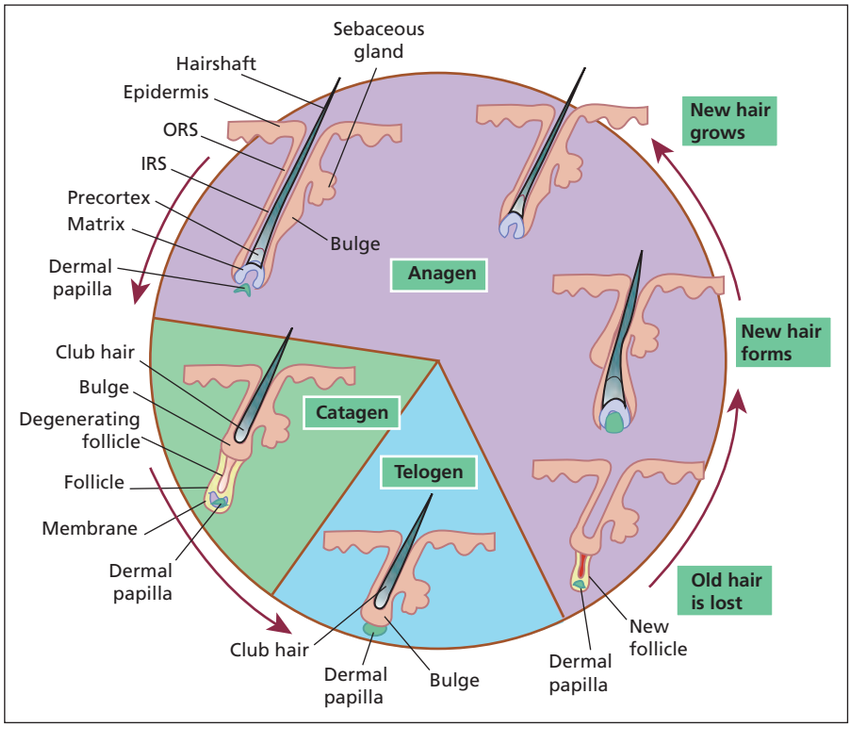


Figure 2.12 There are three components to the hair cycle: anagen (where new hair forms and grows), followed by catagen (regressing phase) and telogen (resting phase), and then loss of old hair. The hair cycle is associated with discrete changes in hair follicle anatomy, both in the shape of the follicle and in the subjacent dermal papilla. IRS, inner root sheath; ORS, outer root sheath.

initiation is R-spondin 4, mutations in which underlie congenital anonychia [2]. A group of cells from the proximal part of the nail fold then grows proximally into the digit, stopping approximately 1 mm from the phalanx and giving rise to the matrix primordium. This site will eventually contribute to the epithelium of the proximal nail fold as well as the distal and intermediate matrix epithelium. From the distal part of the nail fold, a visible mound of cells emerges on the dorsum of the distal tip of each digit, which is known as the distal ridge [3]. At 13 weeks' gestation, the proximal nail fold is formed and the first signs of nail plate growth are observed from the lunula. At this stage, the stratum corneum and the stratum granulosum start to materialize from the nail field epithelium, beginning distally and advancing toward the proximal nail fold. At 18 weeks' gestation, the granular layer recedes, and the nail bed epithelium takes on a postnatal appearance. Likewise, at 20 weeks' gestation, the process of cellular differentiation and maturation within the matrix is similar to that seen in adult nails. By 32 weeks' gestation, virtually all the components of the nail can be recognized. Toe nail development is similar to that of finger nails but the stages occur about 4 weeks later.

The nail unit is composed of the nail plate and four epithelial structures: the proximal nail fold, the matrix, the nail bed and the hyponychium (Figure 2.13) [4]. The nail plate is a rectangular, translucent and relatively inflexible structure, and contains calcium, phosphate, iron, zinc, manganese and copper, but it is mainly the sulphur within the nail matrix that is responsible for the nail plate's physical qualities. The nail plate arises from beneath the proximal nail fold and is bordered on both sides by the lateral nail folds. The proximal aspect may contain white semicircular areas called lunulae, which are the visible portions of the distal matrix [5]. The dorsal surface of the nail unit appears pink in colour because of the enhanced vasculature of the underlying nail bed. The proximal nail fold has a dorsal and a ventral epithelial surface. It is a continuation of the skin of each digit (the dorsal surface) that folds underneath itself, resting above the nail matrix (the ventral surface). The dorsal proximal nail fold is devoid of

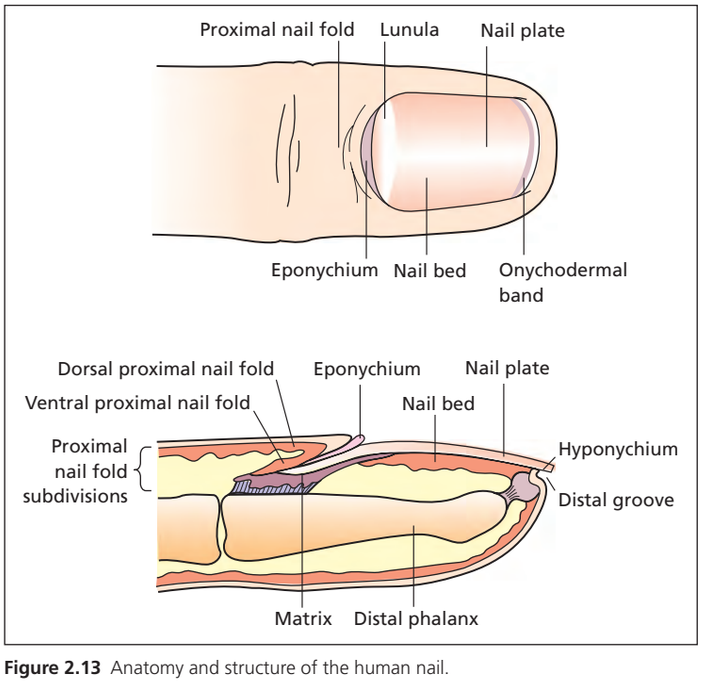


Figure 2.13 Anatomy and structure of the human nail.

hair follicles, sebaceous glands and dermatoglyphic markings and the ventral proximal nail fold also lacks rete ridges. At the junction between the dorsal and ventral surfaces is the eponychium (cuticle) which protects the matrix from damage. The lateral nail folds are extensions of the skin surface of the sides of the digits and join the nail bed medially.

The nail matrix forms the nail plate and is divided into three regions: the dorsal section of the matrix contributes to the most superficial layers of the nail plate, whereas the intermediate region of the matrix forms the deeper layers. The ventral subdivision is the most distal part of the nail matrix. The nail bed is the area underneath the nail plate (between the lunula and the hyponychium). It has a role in forming the deeper layers of the nail plate, as its thin epidermal layer represents the ventral portion of the nail matrix. The hyponychium is located underneath the free edge of the nail plate and denotes the transition of the nail bed to the normal epidermis of the fingers and toes. There is also part of the hyponychium, known as the onychodermal band, that reflects on to the ventral surface of the nail plate to protect the nail parenchyma from trauma.

The epithelium of the matrix is composed of at least two to three actively dividing, basal keratinocyte layers. These cuboidal cells have their vertical axes aligned in a diagonal manner, which allows the nail plate to develop in an upward and outward direction. As these cells differentiate and migrate they become flatter, losing their nuclei and becoming integrated into the developing nail plate as onychocytes, or nail plate cells. This process of cellular maturation is similar to stratum corneum formation within the epidermis but does not require keratohyalin. The matrix also contains melanocytes, which pigment the surrounding keratinocytes and manifest as longitudinal bands across the nail plate; this may be a common racial variant in darker skinned individuals. The nail bed is composed of a thin epidermal layer and a dermal layer, but there is no subcutaneous fat. As the epidermis is thin, the differentiation of keratinocytes to onychocytes occurs within one to two cell layers. The epidermis of the nail bed also contains parallel longitudinal ridges from the lunula to the hyponychium. These ridges interlock to provide strong binding between the nail bed and the nail plate. The dermal layer of the nail bed contains blood vessels to supply the nail unit, as well as lymphatics. Trauma to these vessels results in splinter haemorrhages.

Merkel cells

Merkel first gave the name *tastzellen* to certain cells that he found near the base of the rete peg in the snout skin of the mole. As there were intraepidermal neurites with expanded tips (Merkel discs) adjacent to them, he believed them to be transducers of physical stimuli. Merkel cells are post-mitotic cells scattered throughout the epidermis of vertebrates and constitute 0.2–5% of epidermal cells [1]. They are located amongst basal keratinocytes and are mainly found in hairy skin, tactile areas of glabrous skin, taste buds, anal canal, labial epithelium and eccrine sweat glands, all regions of high tactile acuity (Figure 2.14).

Sun-exposed skin may contain twice as many Merkel cells as non-sun-exposed skin. They form close connections with

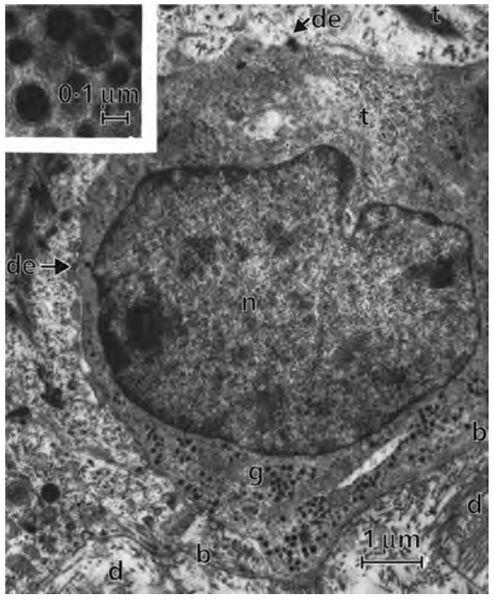


Figure 2.14 Merkel cell in human epidermis. The dermis (d) with collagen fibres is seen in the lower part of the picture; b, basement membrane; de, desmosomes making connections with adjacent basal keratinocyte; g, spherical granules (see inset); n, nucleus of Merkel cell; t, tonofilaments. (Courtesy of Professor A. S. Breathnach.)

sensory nerve endings and secrete or express a number of peptides. Human Merkel cells express immunoreactivity for various neuropeptides including *Met*-enkephalin and vasoactive intestinal polypeptide, in addition to neuron-specific enolase and synaptophysin-like and pancreastatin-like material. They also contain chromogranin A [2].

Merkel cells are easily identifiable on transmission electron microscopy. They are oval with a long axis of approximately 15 µm, orientated parallel to the basement membrane. They also have a large bilobed nucleus and clear cytoplasm, which reflects a relative scarcity of intracellular organelles. Merkel cells contain numerous neurosecretory granules, each 50–160 nm across; these are found opposing the junctions with the sensory nerve endings. The close contact between Merkel cells and nerve fibres represents a Merkel cell–neurite complex. Indeed, Merkel cells actively participate in touch reception, displaying fast, touch-evoked mechanotransduction currents, and provide evidence for a direct, functional, excitatory connection between epidermal cells and sensory neurons [3].

Human skin contains an extensive neural network that consists of cholinergic and adrenergic nerves and myelinated and unmyelinated sensory fibres. The skin also contains several transducers involved in the perception of touch, pressure and vibration, including Ruffini organs surrounding hair follicles, Meissner's corpuscles, Vater–Pacini corpuscles located in the deep layer of the dermis, and nerve endings which pass through the epidermal basement membrane. Some of these contain Merkel cells, which form the Merkel cell–neurite complex, while others are free nerve endings. The cell bodies for all these neurons reside in the dorsal root ganglion. The Merkel cell–neurite complexes are thought to serve as mechanoreceptors and to be responsible for the sensation of touch.

In glabrous skin, the density of Merkel cells is approximately 50 per mm^2. They are clustered near unmyelinated sensory nerve endings, where they group and form 'touch spots' at the bottom of rete ridges. These complexes are also known as hair discs, touch domes, touch corpuscles or Iggo discs. The complex is innervated by a single, slowly adapting type 1 nerve fibre. In hairy skin, Merkel cells also cluster in the rete ridges and in the outer root sheath of the hair follicle where the arrector pili muscles attach. The function of Merkel cells in hair follicles is unclear, although they may be involved in the induction of new anagen cycles.

There are two hypotheses for the origin of Merkel cells: one possibility is that they differentiate from epidermal keratinocyte-like cells and the other is that they arise from stem cells of neural crest origin that migrated during embryogenesis, in a similar fashion to melanocytes [4]. A unifying view, however, could be that there is very early migration of the Merkel cells from the neural crest and population of the epidermis during the sixth or seventh embryonic week in humans and that these cells subsequently only undergo further differentiation once in the epidermis.

Circulating autoantibodies against Merkel cells have been described in pemphigus and graft-versus-host disease. Merkel cells are absent in vitiligo lesions, in keeping with an autoimmune destruction or neural involvement. Merkel cell hyperplasia is a common histological finding and may accompany keratinocyte hyperproliferation as well as being frequently seen in adnexal tumours such as naevus sebaceus, trichoblastomas, trichoepitheliomas and nodular hidradenomas [5]. Merkel cell hyperplasia is associated with hyperplasia of nerve endings that occurs in neurofibromas, neurilemmomas, nodular prurigo or neurodermatitis. Merkel cell carcinoma is a highly aggressive neuroendocrine carcinoma, the incidence of which appears to be increasing; most cases are associated with the Merkel cell polyomavirus although the precise disease pathophysiology remains to be elucidated [6].

Innate immunity

The skin continuously encounters microbial pathogens, and to prevent infection, cells within the epidermis and dermis have evolved several innate strategies. One of the primary mechanisms used by the skin in the early stages of immune defence is the synthesis, expression and release of antimicrobial peptides [1]. There are more than 20 of them in the skin, including cathelicidins, β-defensins, substance P, RANTES, RNase 2,3,7 and S100A7 (Figure 2.15).

Many peptides have antimicrobial action against bacteria, viruses and fungi. The antimicrobial activity of most peptides occurs as a result of unique structural characteristics that enable them to disrupt the microbial membrane while leaving human cell membranes intact. Some may play a specific role against certain microbes in normal skin, whereas others act only when the skin is injured and the physical barrier disrupted [2]. Other peptides may play a larger role, signalling host responses through chemotactic, angiogenic, growth factor and immunosuppressive activity; these peptides are known as alarmins [3]. For example, some alarmins not only kill bacteria but also stimulate expression of syndecan-1 and -4 in dermal fibroblasts, which is critical to the process of wound healing. Alarmins may also stimulate elements of the host defence system, such as barrier repair and recruitment of inflammatory cells [3].

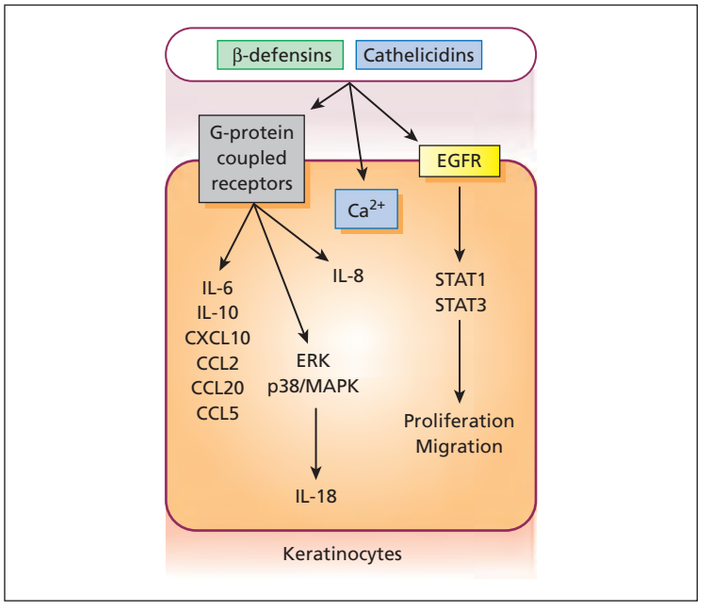


Figure 2.15 As part of the innate immune defence system, antimicrobial peptides can stimulate G-protein-coupled receptors to induce cytokine and chemokine release from keratinocytes as well as epidermal growth factor receptor (EGFR) signalling to influence cell proliferation and migration. CCL, chemokine (C–C motif) ligand; CXCL, C–X–C motif chemokine; IL, interleukin; MAPK, mitogen-activated protein kinase; STAT, signal transducer and activator of transcription.

The production by human skin of antimicrobial peptides such as defensins and cathelicidins occurs constitutively but also greatly increases after infection, inflammation or injury. Some skin diseases, including atopic eczema or rosacea, show altered expression of antimicrobial peptides, partially explaining the pathophysiology of these diseases [4]. In atopic eczema there is decreased expression of multiple antimicrobial peptides, which contributes to an increased susceptibility to infections, and in rosacea there are excessive and abnormally processed cathelicidin peptides, which can reproduce elements of the disease in mice. Certain antimicrobial peptides can influence host cell responses in specific ways. For example, the human cathelicidin peptide LL-37 can activate mitogen-activated protein kinase (MAPK) and extracellular signal-related kinase in epithelial cells, and blocking antibodies to LL-37 hinder wound repair in human skin equivalents. Defensins and cathelicidins have immunostimulatory and immunomodulatory capacities as catalysts for secondary host defence mechanisms. At nanomolar concentrations they are chemotactic for distinct subpopulations of leukocytes as well as some non-leukocytes. Human β-defensins (hBDs) 1–3 are chemotactic for memory T cells and immature dendritic cells. hBD2 attracts mast cells and activated neutrophils, whereas hBD3 and -4 are also chemotactic for monocytes/macrophages. Cathelicidins are chemotactic for neutrophils, monocytes/macrophages and CD4 T lymphocytes. Epidermal keratinocytes stimulated with either β-defensins or cathelicidins release an array of cytokines through the stimulation of G-protein-coupled receptors. In addition, antimicrobial peptides induce keratinocyte proliferation and migration, which involves EGFR signalling and STAT activation.

Skin microbiome

Present on the skin are thriving complex communities of bacteria, fungi and viruses, with approximately 1 million bacteria (involving hundreds of distinct species) inhabiting each square centimetre of skin [1]. The bacteria mostly comprise Actinobacteria, Firmicutes, Bacteroidetes and Proteobacteria, with numerous subspecies thereof. Actinobacteria represents the largest phylum and includes Propionibacteria and Corynebacteria; Firmicutes includes Clostridia and Bacilli, the latter including the class *Staphylococcus*. The precise composition of these organisms depends on sebaceous gland concentration, moisture content and temperature, as well as on host genetics and exogenous environmental factors, but may be very diverse (Figure 2.16). For example, a survey of the palm microbiome found 4742 distinct species in 51 healthy subjects, with an average of 158 species coexisting on a single palm [2]. It has also become clear that these organisms are not just commensals but play a much bigger role in immune modulation and epithelial health than previously expected. Understanding microbe–host interactions and discovering the factors that drive microbial colonization is likely to provide greater insight into the pathogenesis of skin diseases, such as the role of staphylococci in atopic eczema, and the development of new promicrobial and antimicrobial therapeutics [3].

Langerhans cells

Dendritic cells of a form similar to melanocytes, but free from pigment and dopa negative, were first described by Langerhans, who demonstrated their existence in human epidermis by staining with gold chloride. More recently, the dynamic behaviour of epidermal Langerhans cells has been investigated by combining time-lapse, intravital, confocal imaging technology and I-Aβ-enhanced green

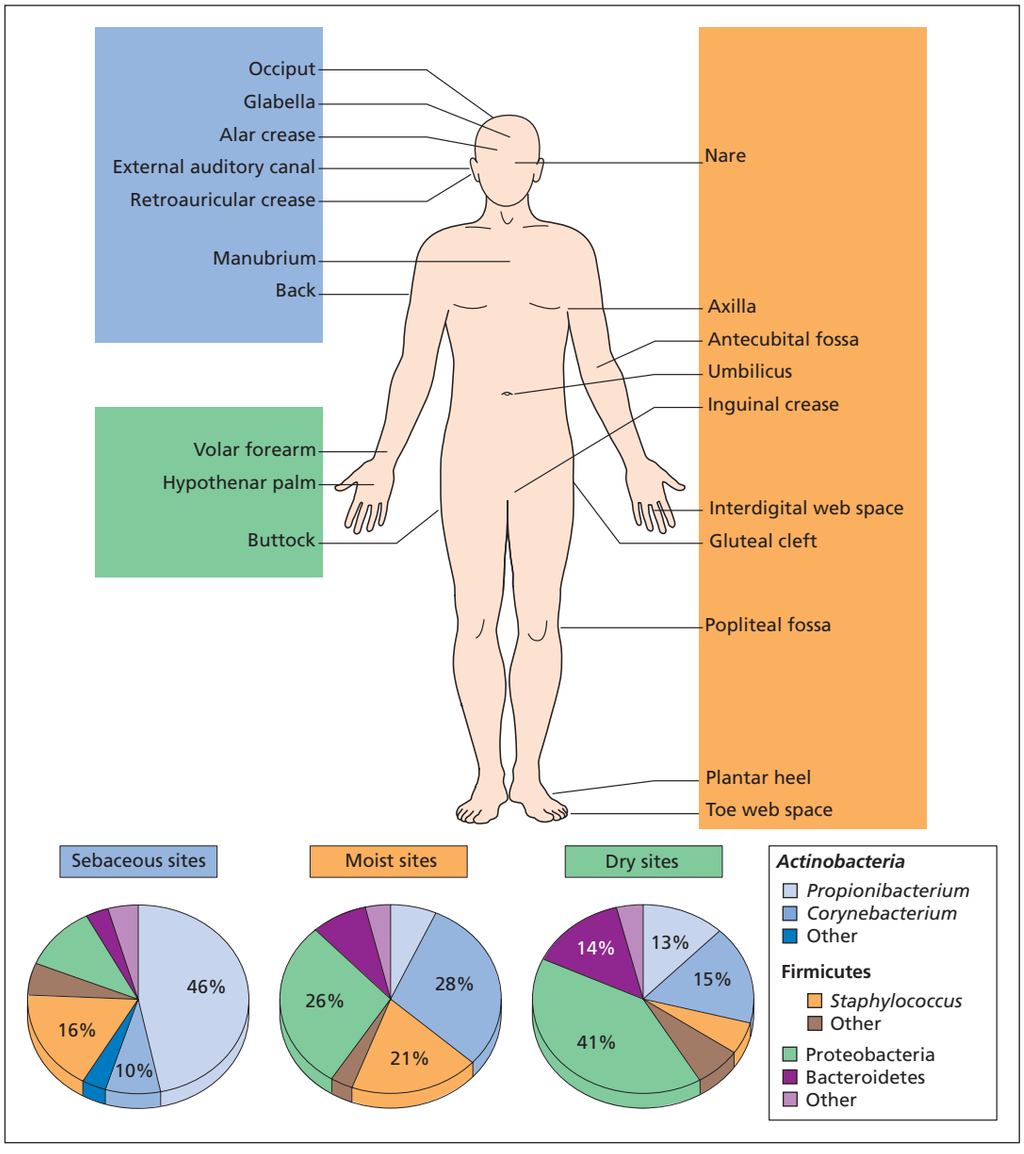


Figure 2.16 The skin microbiome contains numerous bacteria that are variably present in different body regions. (Adapted from Chen and Tsao 2013 [3].)

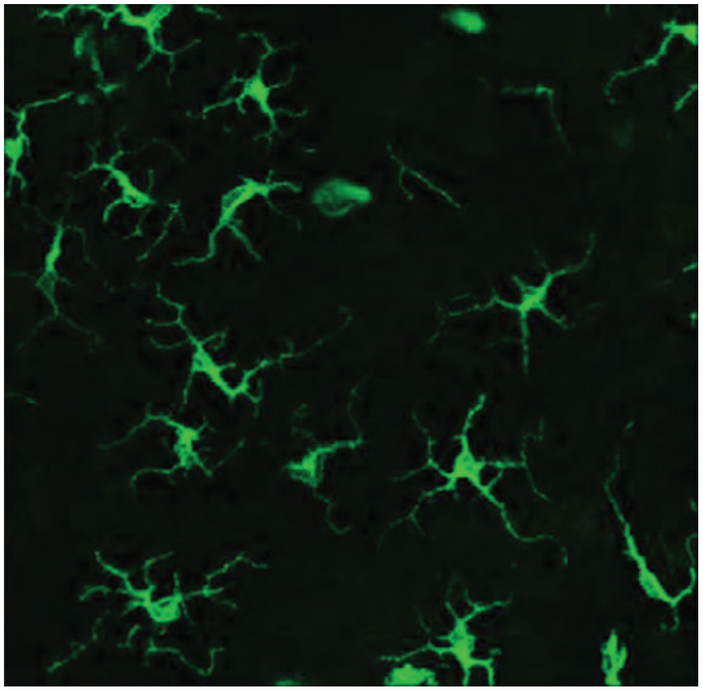


Figure 2.17 Dendritic appearance of epidermal Langerhans cells. Exposure to antigen provokes an increased movement of Langerhans cells as well as direct cell–cell contact between Langerhans cells. (Courtesy of Dr R. Mohr, University of Toledo, Ohio, USA.)

fluorescent protein (EGFP) knock-in mice in which Langerhans cells can be identified by EGFP-associated fluorescence. Without stimulation, some Langerhans cells exhibit a unique motion, which has been termed *dendrite surveillance extension and retraction cycling habitude* (dSEARCH), and which is characterized by rhythmic extension and retraction of dendritic processes between intercellular spaces. The topical application of an antigen such as dinitrofluorobenzene leads to greater dSEARCH motion and also triggers direct cell-to-cell contact formation between adjacent Langerhans cells. It appears that, *in vivo*, dSEARCH motion allows for a more efficient antigen sampling through scanning of a wide area. It is also evident that, under pathological stimulation, adjacent Langerhans cells may exchange antigens between cells (Figure 2.17) [1].

Langerhans cells, in combination with macrophages and dermal dendrocytes, represent the skin's mononuclear phagocyte system [2]. Langerhans cells are capable of phagocytosis, antigen processing, antigen presentation and interactions with lymphocytes (Figure 2.18). They can also release cytokines, such as interleukin-1, to promote lymphocyte chemotaxis and activation [3]. Langerhans cells are intraepidermal macrophages whose dendrites trap antigens among keratinocytes. The cells then leave the epidermis and migrate via lymphatics to a regional lymph node. In the paracortical region of lymph nodes, the Langerhans cell (or 'interdigitating reticulum cell' as it is then known) expresses protein on its surface to present to a T lymphocyte that can then undergo clonal proliferation. There may be some selectivity in whether certain antigens are presented to lymph nodes by Langerhans cells or by dermal dendrocytes. The timing of antigen presentation may also vary, with the possibility that sequential presentation of skin-acquired antigens may regulate cell-mediated immunity.

Langerhans cells may contribute to several skin pathologies including infections, inflammation and cancer, and they play a pivotal role in regulating the balance between immunity and peripheral tolerance [4]. Langerhans cells appear, however, to have characteristics distinct from other dendritic cells in that they are more likely to induce Th2 responses than the Th1 responses that are usually necessary for cellular immune responses against pathogens. It has also been shown that Langerhans cells are dispensable for contact hypersensitivity and that dermal dendrocytes can serve as antigen-presenting cells in the absence of Langerhans cells. Indeed, Langerhans cell-deficient mice appear to have enhanced contact hypersensitivity. With regard to a specific function, Langerhans cells, or a subset thereof, may have regulatory properties that counteract the proinflammatory

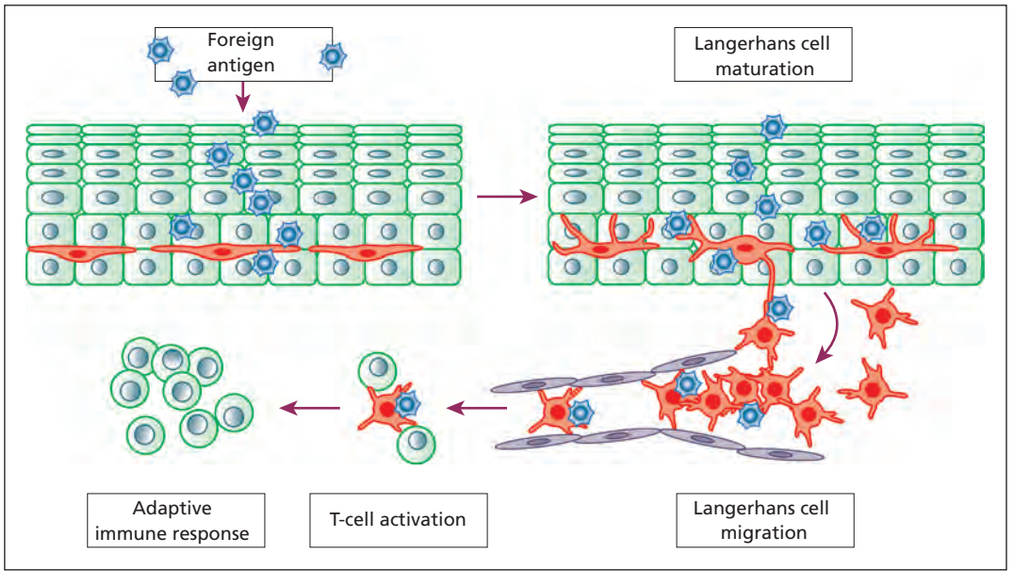


Figure 2.18 When exposed to foreign antigen, the activity of resting Langerhans cells increases and the cells mature. Antigen is then processed and transported to the lymph nodes. T cells are then activated and an immune response is triggered.

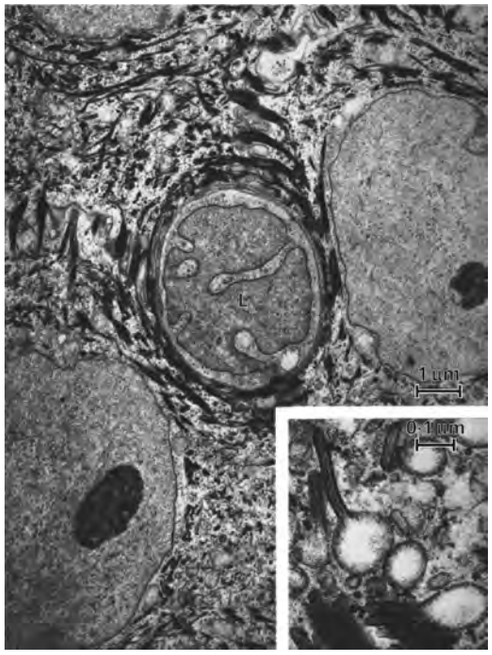


Figure 2.19 Langerhans cell (L) with its characteristically indented nucleus, situated between keratinocytes. The inset shows Langerhans cell granules with racquet-shaped profiles. (Courtesy of Professor A. S. Breathnach.)

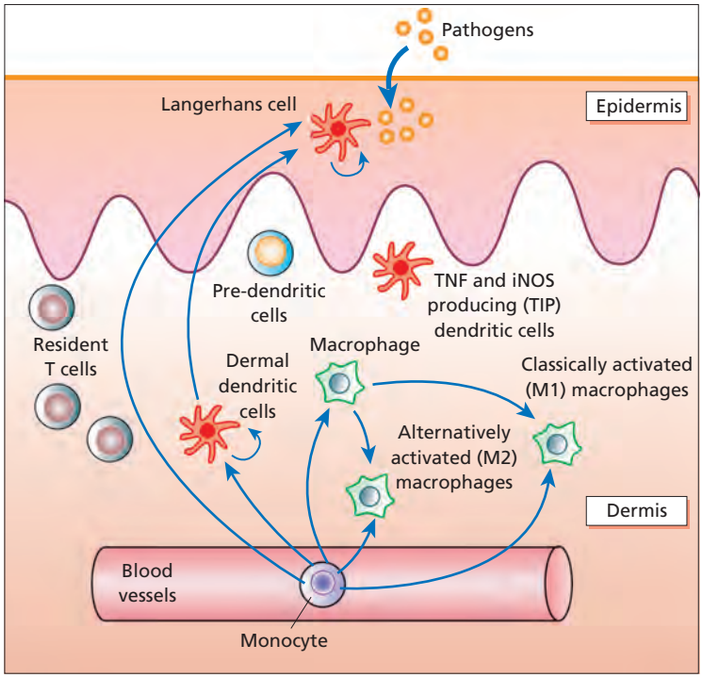


Figure 2.20 Immune surveillance in normal skin is carried out by an array of skin-based dendritic cells, macrophages and resident T cells. iNOS, inducible nitric oxide synthase; TNF, tumour necrosis factor.

activity of surrounding keratinocytes. It is plausible that under non-inflammatory steady state conditions, Langerhans cells carry skin-specific components to draining lymph nodes to prevent immunization and to induce peripheral tolerance against epidermal self-determinants [5]. It also appears that Langerhans cells may indeed consist of distinct subsets, since Langerhans cells that repopulate the skin after inflammation have been shown to derive from monocyte precursors.

Under the electron microscope, Langerhans cells share with melanocytes a lobulated nucleus, a relatively clear cytoplasm and well-developed endoplasmic reticulum, Golgi complex and lysosomes (Figure 2.19). They differ in lacking melanosomes or premelanosomes, and in possessing a characteristic granule that is either rod- or racquet-shaped. These 'Birbeck' granules have been shown to represent subdomains of the endosomal recycling compartment and form at sites where the protein Langerin accumulates. Using ultrastructural evidence of the presence of the characteristic granules, Langerhans cells have been identified in the outer root sheath of the human hair and the secretory duct of the sebaceous gland and in the epithelium of the crypts of the human tonsil. The discovery of similar granules in cells in the dermis in histiocytosis X resulted in the renaming of this condition as Langerhans cell histiocytosis.

Immune surveillance

Besides the antigen detection and processing role of epidermal Langerhans cells, cutaneous immune surveillance is also carried out in the dermis by an array of tissue-resident T cells, macrophages and dendritic cells (Figure 2.20) [**1**]. These immune sentinel and effector cells are able to provide rapid and efficient immunological backup to restore tissue homeostasis should the epidermis be breached. The dermis contains a very large number of resident T cells; remarkably, there are approximately 2×10^{10} skin-resident T cells, which is twice the total number of T cells in the circulating blood [2,3]. There are several distinct populations of dermal dendritic cells; some have potent antigen-presenting capacities, others have low antigen-presenting capacity but the potential to develop into CD1a+ and Langerin-positive Langerhans cells, while some are pro-inflammatory.

Another recent addition to the family of skin immune sentinels is type I interferon-producing plasmacytoid predendritic cells, which are rare in normal skin but which can accumulate in inflamed skin [4]. A further component of the dermal immune system is the dermal macrophage. This cellular diversity of dermal immune sentinels is reflected in some flexibility or plasticity in function. For example, immature dendritic cells, including dermal dendritic cells, can be phagocytic, which is a cellular function usually attributed to macrophages [5]. Alternatively, macrophages, which normally are phagocytic cells, can also be potent antigen-presenting cells for CD8+ T cells. This means that tissue-resident mononuclear sentinels of the dermis are likely to exist in a pluripotent state. Depending on microenvironmental factors and cues, they may acquire an antigen-presenting mode, a migratory mode or a tissue-resident phagocytic mode.

Mast cells

Mast cells were first described by Ehrlich in 1877, who distinguished them from other connective tissue cells by their ability to stain metachromatically with basic aniline dyes. Mast cells are larger than eosinophils and basophils. They occur in most tissues, but are particularly numerous in the skin, bronchus, nasal mucosa and gut. In the skin, mast cells are distributed close to blood vessels, nerves and appendages, and are most numerous in the subpapillary dermis, in the region of the superficial dermal vascular plexus. There are about 7000 mast cells per mm^3 in normal skin.

Dermal mast cells are ovoid or spindle-shaped, mononuclear or occasionally binuclear, and only rarely show signs of mitosis in normal skin. Their major distinguishing feature is the presence of numerous, round, cytoplasmic granules (Figures 2.21 and 2.22). Mast cells are heterogeneous and fall into two main types – connective tissue and mucosal – which can be differentiated by their morphology, tissue distribution, histochemical characteristics and responses to degranulating agents. Solubility of the granules in formaldehyde and the content of neutral proteinase, namely tryptase and chymase (chymotryptic proteinase), will vary according to the type of cell. For example, human foreskin mast cells contain both proteinases, whereas mast cells in intestinal mucosa and the lung contain mainly tryptase [1].

Human mast cells arise from CD34+ pluripotent stem cells in the bone marrow. They then circulate in the blood as precursors and home to tissues where they mature under the influence of stem cell factor (SCF) and local cytokines and other factors. Mast cell growth and differentiation are also influenced by several other cytokines, including interleukin 3 (IL-3), -4, -6, -9, -10 and nerve growth factor. Mast cells are long lived and may proliferate in association with IgE-dependent activation and in the presence of IL-4 [2].

Kit (CD117), expressed on haematopoietic stem cells and progenitor cells, is the tyrosine kinase transmembrane receptor for SCF that is involved in the differentiation of both myeloid and lymphoid lineages. While Kit is down-regulated on other bone marrow-derived cells during their differentiation, Kit remains highly expressed on mast cells and is critical for many mast cell functions such as survival, differentiation, chemotaxis and enhancement of signalling events during mast cell activation. The importance of Kit is shown by the finding of activating mutations in the *KIT* gene in patients with urticaria pigmentosa [3].

Upon activation of mast cells via cross-linking of the high affinity IgE receptor (FcεRI) or non-IgE-mediated activation through complement receptors or toll-like receptor (TLR) activation, mast cells can release histamine, serotonin and proteases as well as newly synthesized leukotrienes, prostaglandins, cytokines and chemokines. In addition to IgE-mediated activation, human mast cells exposed to interferon γ (IFN-γ) can be activated following IgG-mediated aggregation of FcγRI to release similar mediators. Additional IgE-independent mast cell triggers have been described, including SCF, complement (C3a and C5a), neuropeptides (substance P), adenosine, TLR and scavenger receptors.

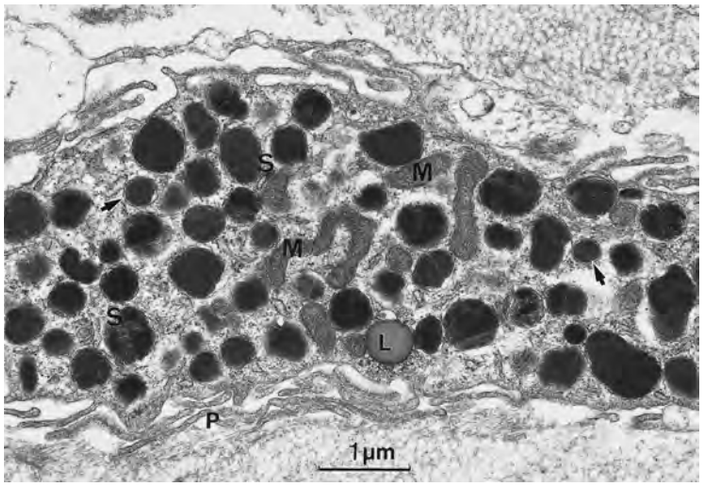
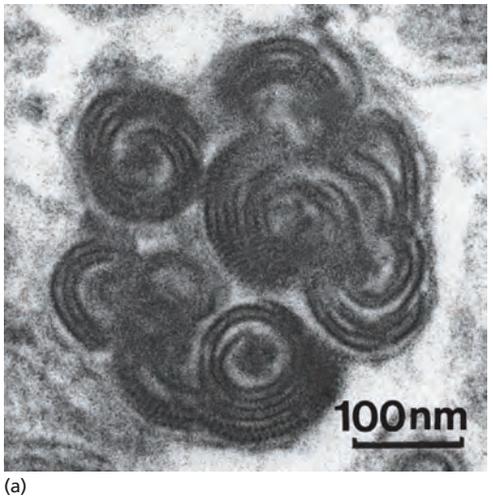
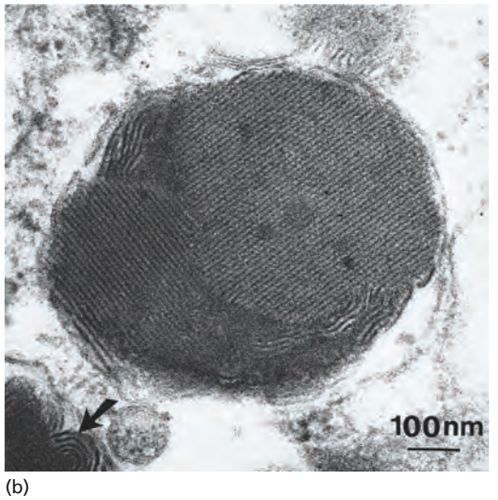


Figure 2.21 Part of a human skin mast cell showing characteristic granules, some with scroll-like profiles (S). Arrows indicate perigranular membrane; L, lipid droplet; M, mitochondria; P, peripheral processes. (Courtesy of Professor R. A. J. Eady, St John's Institute of Dermatology, King's College London, UK.)



(a)



(b)

Figure 2.22 High-magnification views of dermal mast cell granules. (a) Typical scroll-like configuration of lamellae, some of which show a cross-banding of regular periodicity. (b) The substructure of this granule is a highly organized lattice (arrow). (Courtesy of Professor R. A. J. Eady, St John's Institute of Dermatology, King's College London, UK.)

Mast cell products may both induce an immediate reaction and contribute to a late phase reaction. The immediate phase reaction occurs within minutes of FcεRI cross-linking and its consequences are referred to as an immediate hypersensitivity reaction. Late phase reactions peak 6–12 h following antigen challenge and are associated with cytokine and chemokines from eosinophils, neutrophils and basophils that have been secondarily recruited. Mast cell activation results in increased vascular permeability and smooth muscle contraction, as well as fibroblast deposition of collagen, induction of B cells to class switch to synthesize IgE, basophil histamine release, recruitment of neutrophils and eosinophils, and promotion of T cells to a T helper 2 (Th2) phenotype.

Mast cells play an important role in both adaptive and innate immunity, and contribute to the skin pathology seen in contact dermatitis, atopic eczema (AE), immunobullous disease, scleroderma and chronic graft-versus-host disease [4]. In AE, there is an increase in mast cell numbers in lesional skin. Mast cells reside in the papillary dermis and undergo migration through the basal lamina into the epidermis. Although overall levels of histamine are not increased in AE, tryptase and activation of proteinase-activated receptor-2 (PAR-2) may contribute to the pruritus seen in AE, as tryptase is reported to be increased up to fourfold in AE patients and PAR-2 expression is markedly enhanced on primary afferent nerve fibres in skin biopsies from patients with AE. Chymase may play a role in eliciting and maintaining chronic inflammation in AE by increasing spongiosis and compromising the skin barrier. Mast cell–nerve interactions may also play a role in promoting inflammation in AE [5]. There is an increased number of contacts between mast cells and nerves in both lesional and non-lesional skin, which may lead to inflammation mediated by neuropeptides such as substance P, calcitonin gene-related peptide, vasoactive intestinal peptide and nerve growth factor.

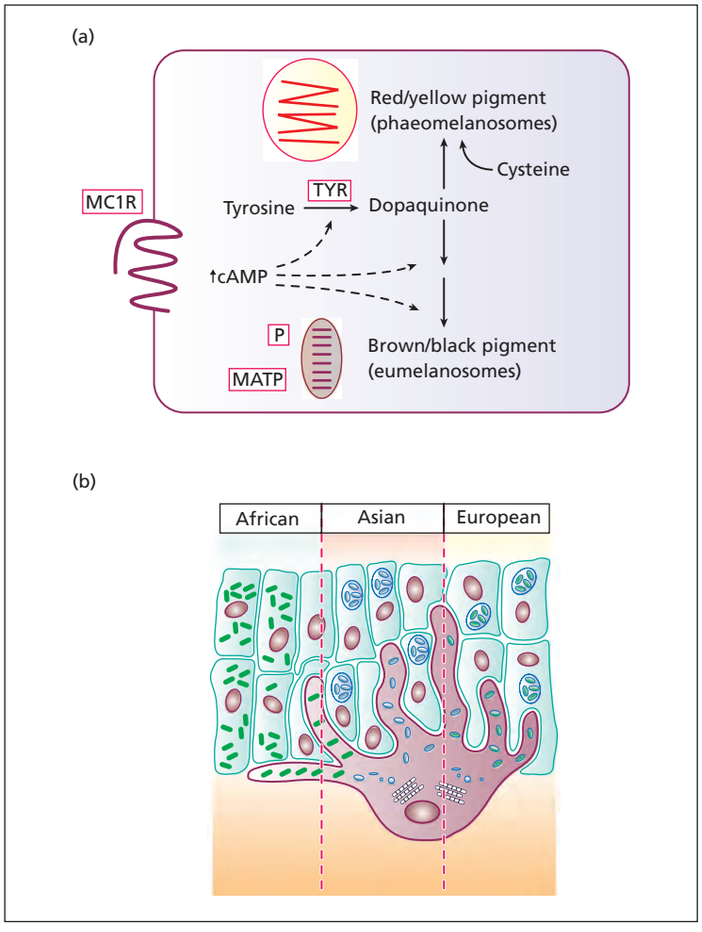


Figure 2.23 (a) Activation of the melanocortin 1 receptor (MC1R) promotes the synthesis of eumelanin at the expense of phaeomelanin. Oxidation of tyrosine by tyrosinase (TYR), however, is required for synthesis of both pigment types. Melanosomal membrane components, including the membrane-associated transport protein (MATP) and the pink-eyed dilution protein (P), play a role in determining the amount of pigment synthesis within melanosomes. (b) In African, Asian and European skin there is a gradient of melanosome size and number; in addition, melanosomes in African skin are more widely dispersed.

Melanocytes

Melanocytes are pigment-producing cells located in the skin, inner ear, choroid and iris of the eye. In the skin and hair, two forms of melanin pigment are produced: brown/black eumelanin and yellow/red phaeomelanin (Figure 2.23). The melanin is subsequently transferred in melanosomes to neighbouring keratinocytes in the epidermis and into the growing shaft in hair follicles. Variations in the types of melanin pigment produced and their distribution within the skin and hair contribute to the vast diversity in colour. A key protein involved in melanosome assembly is NCKX5, encoded by the gene *SLC24A5* [1]. Loss of expression of this gene in mice results in marked changes in skin colour with loss of pigment. Melanin production also provides skin protection by reducing damage from harmful ultraviolet radiation. In humans, alterations in melanocyte development and function can lead to various pigmentary disorders. These include disorders with reduced melanocytes in skin, such as piebaldism and Waardenburg syndrome, and disorders with defective pigment production or processing, such as albinism and Hermansky–Pudlak and Chediak–Higashi syndromes.

The melanin-producing melanocytes in adult skin and hair develop from embryonically derived melanocyte precursors called melanoblasts. During development, melanoblasts emerge from a subset of neural crest cells and migrate to the skin and developing hair follicles. In the hair follicle, melanocytes are divided into two distinct populations: differentiated melanocytes, located in the hair matrix region, and melanocyte stem cells, located at the lower permanent portion of the hair follicle. The life cycles of the follicular melanocytes and melanocyte stem cells are closely related to the cyclical nature of the hair follicle, and during anagen new melanocytes are generated from the pool of slow-proliferating melanocyte stem cells [2]. Differentiated melanocytes express *Sox10*, *Kit*, *Mitf*, *Pax3* and *Dct* but melanocyte stem cells only express *Pax3* and *Dct* [3]. Whether a subpopulation of melanocyte stem cells exists that may be relevant to the pathogenesis of melanoma is not yet known, although some of the melanocyte stem cells do express markers such as CD166, CD133 or Nestin, which are found in stem cells from other lineages and may be multipotent in culture, capable of differentiating into various cell types (e.g. adipocytes, chondrocytes).

Alterations in melanocyte signalling pathways also contribute to common hair abnormalities such as hair greying [**4**]. One particular pathway involves Notch signalling [5]. The Notch signalling pathway is an essential cell–cell interaction mechanism, which regulates processes such as cell proliferation, cell fate decisions, differentiation or stem cell maintenance. Notch signalling in melanocytes is essential for the maintenance of proper hair pigmentation, including regeneration of the melanocyte population during hair follicle cycling. Deletion of Notch1 and Notch2 or RBP-Jkappa in melanocyte lineages results in a gene dosage-dependent, precocious hair greying, due to the elimination of melanoblasts and melanocyte stem cells. Aberrant Notch signalling may also be relevant in the development or progression of melanoma.

Melanocytes possess melanocyte-specific receptors including melanocortin-1 (MC1R) and melatonin receptors [6]. The activation or the inhibition of melanocyte-specific receptors can augment normal melanocyte function, skin colour and photoprotection. Moreover, receptor polymorphisms are known to underlie red hair phenotypes. Receptor targeting may also be relevant to the treatment of melanoma. Notably, melanocytes also possess G-protein-coupled receptors, such as Frizzled5, and receptor tyrosine kinases, including c-Kit and hepatocyte growth factor receptor. These receptors activate two crucial cell signalling pathways, RAS/RAF/MEK/ERK and PI3K/AKT, integral to melanoma cell survival, and could serve as targets for future therapies of disseminated melanoma.

Desmosomes

Desmosomes are the major adhesion complex in the epidermis, anchoring keratin intermediate filaments to the cell membrane and bridging adjacent keratinocytes, and allowing cells to withstand trauma. Desmosomes are also found in the myocardium, meninges and cortex of lymph nodes. The desmosome has a characteristic ultrastructural appearance, in which the cell membrane of two adjacent cells forms a symmetrical junction with a central intercellular space of 30 nm containing a dense line (Figure 2.24). Plaques of electron-dense material run along the cytoplasm parallel to the junctional region, in which three ultrastructural bands can be distinguished: an electron-dense band next to the plasma membrane, a less dense band and then a fibrillar area [**1**].

The main components of desmosomes in the epidermis consist of the products of three gene superfamilies: the desmosomal cadherins, the armadillo family of nuclear and junctional proteins, and the plakins [2]. The transmembranous cadherins comprise mostly heterophilic associations of desmogleins and desmocollins. There are four main epidermis-specific desmogleins (Dsg1–4) and three desmocollins (Dsc1–3), all of which show differentiation-specific expression. For example, Dsg1 and Dsc1 are preferentially expressed in the superficial layers of the epidermis, whereas Dsg3 and Dsc3 show greater expression in basal keratinocytes. The intracellular parts of these glycoproteins are attached to the keratin filament network via desmoplakin, plakoglobin and other macromolecules, including the armadillo protein, plakophilin 1, an important stabilizer of keratinocyte adhesion in differentiated keratinocytes, as well as other site-specific plakin cell envelope proteins, such as envoplakin and periplakin [3]. The network of the major interactive desmosomal proteins is depicted in Figure 2.25.

Further clues to the biological function and *in vivo* contribution to keratinocyte adhesion of these desmosomal components have arisen from various mouse models and human diseases, both inherited and acquired [4,5], and desmosome proteins may also serve as autoantigens in several immunobullous blistering skin diseases [6].

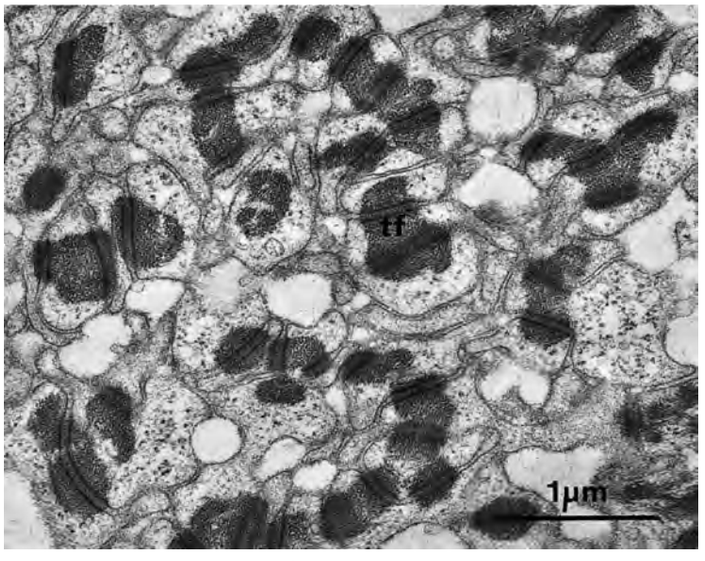


Figure 2.24 Electron micrograph of desmosomes in the spinous layer. These intercellular junctions are closely associated with tonofilaments (tf), many of which, in this view, are cross-sectioned. (Courtesy of Professor R. A. J. Eady, St John's Institute of Dermatology, King's College London, UK.)

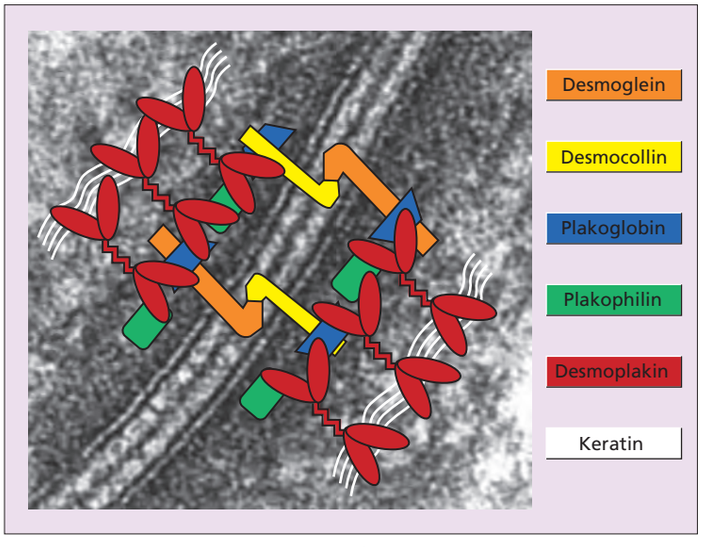


Figure 2.25 Macromolecular composition of desmosomes linking adjacent keratinocytes. Cells are connected via transmembranous cadherin glycoproteins (desmogleins and desmocollins). Attachment of these molecules to the keratin filament cytoskeleton occurs via a network of desmosomal plaque proteins (desmoplakin, plakoglobin and plakophilin). The background to this figure is a transmission electron micrograph of a desmosome to highlight how the molecules function as an adhesive complex.

Antibodies to multiple desmosomal proteins may develop in diseases such as paraneoplastic pemphigus, possibly through the phenomenon of epitope spreading [7]. Disruption of the extracellular domain of Dsg1 has also been demonstrated as the basis of staphylococcal scalded skin syndrome and bullous impetigo in which this desmosomal cadherin is cleaved by the bacterial toxin [8].

Adherens junctions

Adherens junctions are electron-dense, transmembrane structures that engage with the actin skeleton [1]. They can associate with tight junctions and desmosomes or exist separately from these junction complexes. Adherens junctions contribute to epithelial assembly, adhesion, barrier formation, cell motility and changes in cell shape. They are characterized by two opposing membranes separated by approximately 20 nm and are 0.2–0.5 μm in diameter. Adherens junctions may also spatially coordinate signalling molecules and polarity cues as well as serving as docking sites for vesicle release. They comprise two basic adhesive units: the nectin–afadin complex and the classic cadherin complex (Figure 2.26) [2,3].

There are several different nectins and cadherins and these may be variably incorporated into adherens junctions; the precise composition will impact on the adhesive specificity and other functions of the junction. The nectins form a structural link to the actin cytoskeleton via afadin (also known as AF-6) and may be important in the initial formation of adherens junctions. The cadherins form a complex with the catenins (α-, β-, and p120 catenin) and help mediate adhesion and signalling. Cell signalling via β-catenin can activate several Wnt pathways, which implicates adherens junctions in coordinating morphogenetic movements with cell fate determination. Adherens junctions are also associated with a variety of actin-binding molecules, suggesting multiple dynamic interactions with the cytoskeleton.

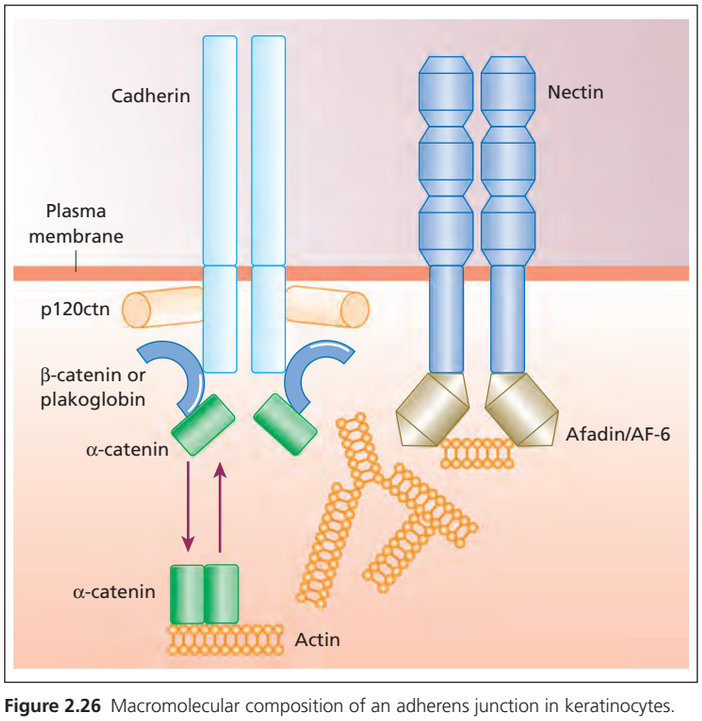


Figure 2.26 Macromolecular composition of an adherens junction in keratinocytes. There are two main components, nectin–afadin and the classic cadherin–catenin complex, which can both attach to the actin cytoskeleton.

The first human gene mutation reported in a component of adherens junctions was in plakoglobin, also a component of desmosomes, in individuals with Naxos disease [4]. However, mutations have subsequently been reported in the *CDH3* gene, which encodes P-cadherin; these mutations result in autosomal recessive hypotrichosis with juvenile macular dystrophy [5]. P-cadherin mutations are also found in a different disorder, ectodermal dysplasia–ectrodactyly–macular dystrophy (EEM) syndrome, in which there is hypotrichosis, macular degeneration, hypodontia and limb defects, including ectrodactyly, syndactyly and camptodactyly [6]. Mutations in nectin-1 and -4 have also been reported in a group of ectodermal dysplasia syndromes, sometimes refered to as nectinopathies [7].

Gap junctions

Gap junctions comprise clusters of intercellular channels, known as connexons, which directly form connections between the cytoplasm of adjacent keratinocytes (and other cells) [1]. Thirteen different human connexins have been described. Connexons originate following the assembly of six connexin subunits within the Golgi network that are then transported to the plasma membrane. The connexins are divided into three groups (α, β and γ) according to their gene structure, overall gene homology and specific sequence motifs [2]. At the plasma membrane, connexons associate with other connexons to form a gap junction (Figure 2.27).

Homotypic or heterotypic connexins (formed from one or more than one type of connexin, respectively) can be identified, and the formation and stability of gap junctions can be regulated by protein kinase C, Src kinase, calcium concentration, calmodulin, adenosine 3′,5′-cyclic monophosphate (cAMP) and local pH [3]. Apart from the connexins, vertebrates also contain another class of gap junction proteins, the pannexins, which are related to the innexins found in non-chordate animals [4]. The function of gap junctions is to permit sharing of low-molecular-mass metabolites (<1000 Da) and ion exchange between neighbouring cells, thus allowing intercellular coordination and uniformity to maintain tissue/organ homeostasis in multicellular organisms [3]. Gap junction communication is essential for cell synchronization, cell differentiation, cell growth and metabolic coordination of avascular organs, including epidermis.

Inherited abnormalities in genes encoding four different connexins (Cx26, -30, -30.3 and -31) have been detected in several forms of keratoderma and/or hearing loss but non-dermatological disorders can also arise from mutations in the higher-molecular-weight connexins (Cx32, -40, -43, -46 and -50).

Tight junctions

Tight junctions are the major regulators of permeability in simple epithelia, but they are also present in the skin, with a key role in

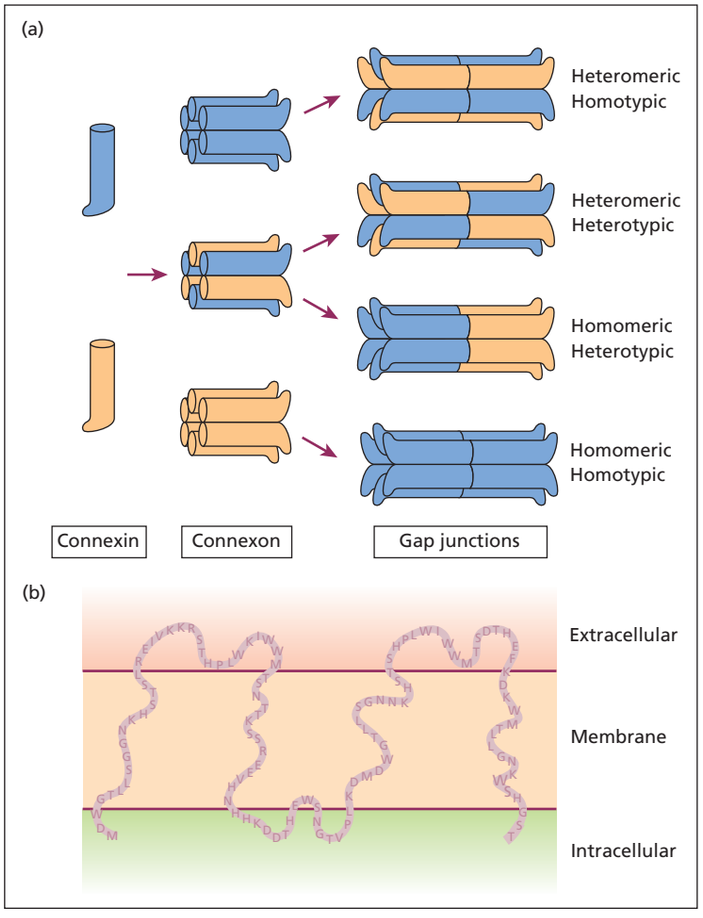


Figure 2.27 Formation and structure of gap junctions in human skin. (a) In the Golgi network six connexin subunits assemble to form a connexon. The connexon is then transported to the plasma membrane. Other connexons then co-aggregate to form homotypic or heterotypic gap junctions. (b) The gap junction protein is a transmembranous molecule with intracellular, transmembranous and extracellular domains (here illustrated for Cx26).

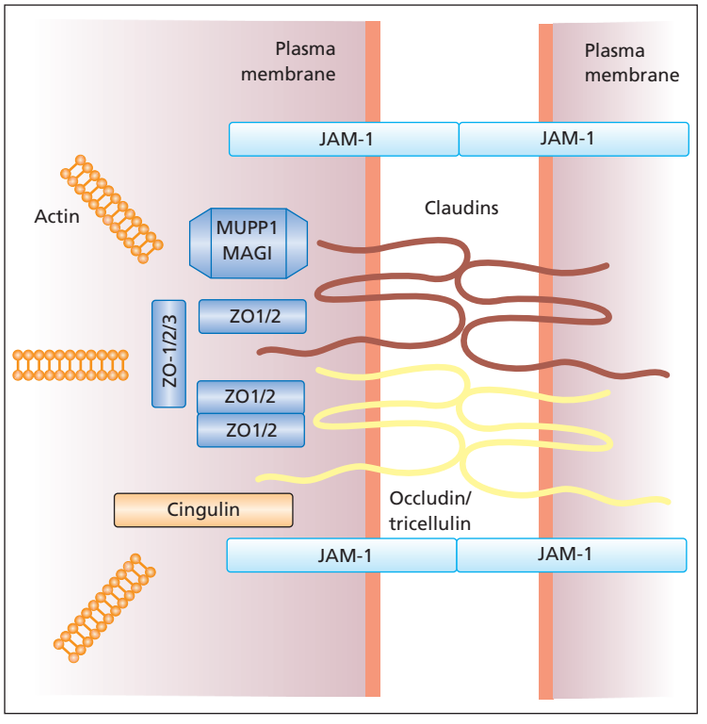


Figure 2.28 Structural composition of a tight junction in human skin. There are three transmembranous families of proteins, the junctional adhesion molecules (JAMs), the claudins and the occludins, of which the latter two bind to zonula occludens proteins (ZOs) and then directly to actin. MUPP1, multiple PDZ domain protein; MAGI, membrane-associated guanyl kinase inverted protein.

skin barrier integrity and maintaining cell polarity [1]. Tight junctions regulate the paracellular flux of water-soluble molecules between adjacent cells [2]. The principal structural proteins of tight junctions are the claudins, of which there are approximately 24 subtypes. The other component transmembranous proteins are the IgG-like family of junctional adhesion molecules (JAMs) and the occludin group of proteins. Seven claudins are expressed in human epidermis, although the main claudins are 1 and 4. These transmembranous proteins do not bind to one another, but the claudins and occludins can bind to the intracellular zonula occludens proteins ZO-1, ZO-2, ZO-3. These proteins can also interact with actin thus providing a direct link with the cytoskeleton [1,3]. The structural organization of a tight junction is shown in Figure 2.28.

Mutations in claudin 16 (also known as paracellin-1) result in familial hypomagnesaemia with hypercalciuria and nephrocalcinosis; mutations in claudin 14 underlie the autosomal recessive deafness disorder DFNB29 leading to cochlear hair cell degeneration; mutations in claudin 19 result in renal and ocular disease [4]. In addition, the gene encoding the ZO-2 protein may be mutated in some cases of familial hypercholanaemia. With respect to skin, mutations in claudin 1 have been demonstrated in one pedigree with clinical features of diffuse ichthyosis with large scales, hypotrichosis, scarring alopecia and sclerosing cholangitis [5]. Collectively, these human genetic disorders, all of which are autosomal recessive in nature, demonstrate the key role tight junctions play in the skin, kidney, ear, eye and liver.

Dermal–epidermal basement membrane

The interface between the lower part of the epidermis and the top layer of the dermis comprises the dermal–epidermal basement membrane zone (BMZ), which consists of a number of extracellular matrix macromolecules (Box 2.1; Figure 2.29) [**1**,2]. Many of these components are glycoproteins, and the BMZ can be recognized histologically by positive labelling with PAS stain. Ultrastructural examination of the BMZ by transmission electron microscopy shows the presence of two distinct layers with different optical densities (Figure 2.30).

The upper layer, the lamina lucida, is a less electron-dense region and directly abuts the plasma membranes of the basal keratinocytes. Below the lamina lucida is the lamina densa, an electron-dense region that at the lower part interacts with the mesenchymal matrix of the upper dermis. The major biochemical components of the BMZ are type IV collagen and a number of

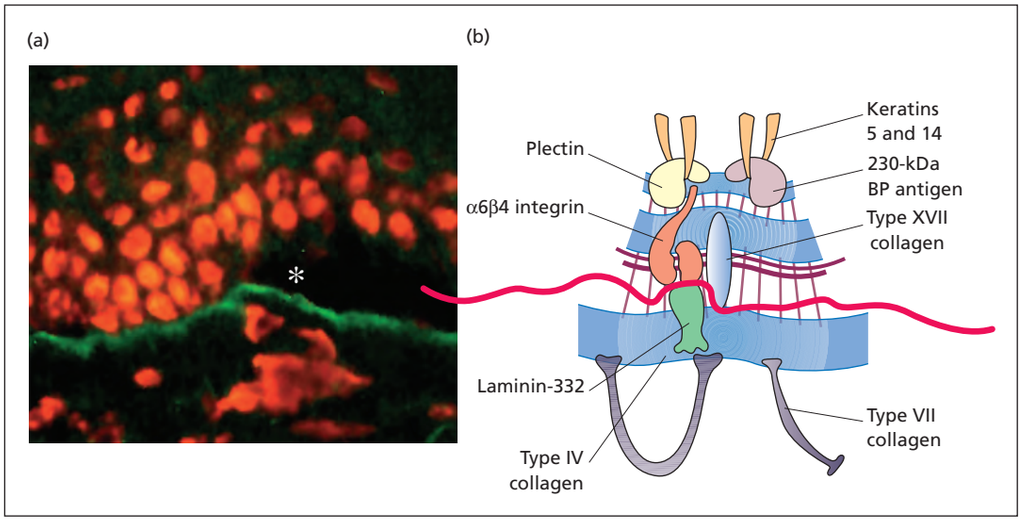


Figure 2.29 NaCl-induced separation between the epidermis and dermis and antigen mapping within the cutaneous basement membrane. (a) The dermal–epidermal basement membrane visualized by immunofluorescence staining with antibodies to type VII collagen. This maps to the base of the split because cleavage occurs through the lamina lucida, and type VII collagen is located below the lamina densa. (b) Molecular complexity at the dermal–epidermal junction and how various proteins map above or below the NaCl-induced split (red line and asterisk). BP, bullous pemphigoid.

non-collagenous glycoproteins, including laminin 322 [3]. Associated with the cutaneous BMZ are ultrastructurally recognizable attachment structures that form a contiguous network extending from the intracellular milieu of basal keratinocytes through the plasma membrane of basal cells, traversing the dermal–epidermal basement membrane and extending to the upper papillary dermis (Figure 2.29). The components of this network are the hemidesmosomes, anchoring filaments and anchoring fibrils. The biochemical components of the BMZ are synthesized by basal keratinocytes and dermal fibroblasts, which both contribute to the development and repair of the basement membrane [4].

The critical importance of this network structure in securing the adherence of the epidermis to the underlying dermis is reflected in the group of diseases, epidermolysis bullosa, in which components of the hemidesmosomes, anchoring filaments or anchoring fibrils are genetically altered or missing. As a result, fragility at the dermal–epidermal junction ensues, clinically manifesting as erosions and blisters following minor trauma (Figure 2.31) [5].

Box 2.1 Molecular components of the epidermal basement membrane zone

Intermediate filament (IF) components
- Keratin 5
- Keratin 14

Hemidesmosomal plaque components
- 230 kDa bullous pemphigoid antigen (BP230/BPAG1)
- Plectin

Transmembrane components
- α6β4 integrin
- Type XVII collagen (180 kDa bullous pemphigoid antigen/BPAG2)
- α3β1 integrin
- Type XIII collagen
- Syndecans 1 and 4

Lamina lucida/lamina densa components
- Laminin 332 (laminin 5)
- Laminin 311 (laminin 6)
- Laminin 511 (laminin 10)

Lamina densa components
- Type IV collagen
- Laminin 111 (laminin 1)
- Nidogen
- BM-40/SPARC
- Perlecan

Anchoring fibril components
- Type VII collagen
- GDA-J/F3 antigen

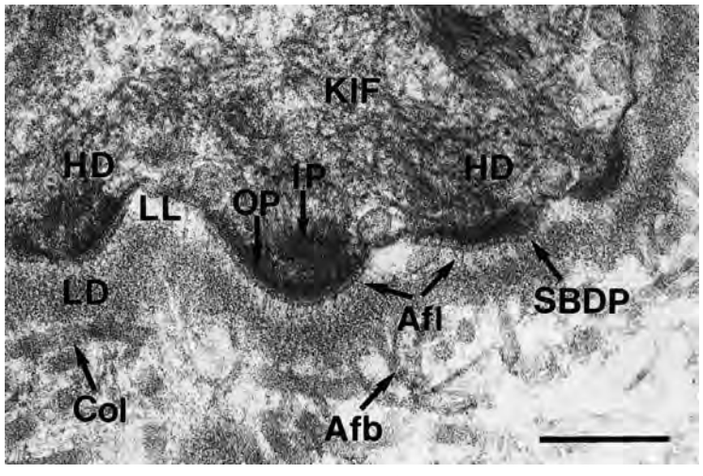


Figure 2.30 Transmission electron microscopy of the dermal–epidermal junction in human skin recognizing hemidesmosomes (HD), anchoring filaments (Afl) and anchoring fibrils (Afb). The HD consists of an intracellular inner plaque (IP) and outer plaque (OP) as well as a sub-basal dense plate (SBDP) in the upper lamina lucida (LL). The anchoring filaments traverse the LL, appearing as thread-like structures that concentrate under the HDs and merge with the lamina densa (LD). Anchoring fibrils extend from the lower part of the LD to the upper papillary dermis where they closely associate with interstitial collagen fibres (Col). Keratin intermediate filaments (KIFs) associate with intracellular components of the HDs. Scale bar 0.25 μm. (Courtesy of Professor R. A. J. Eady, St John's Institute of Dermatology, King's College London, UK.)

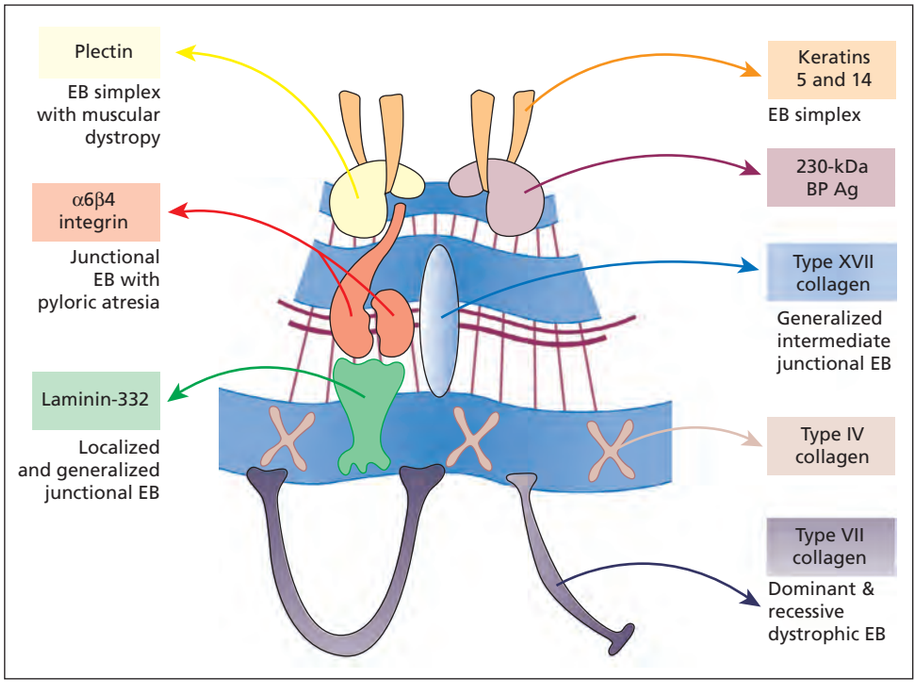


Figure 2.31 Gene/protein systems within the cutaneous basement membrane zone that can harbour mutations and result in blistering of the skin in different forms of epidermolysis bullosa (EB). BP, bullous pemphigoid.

Basement membrane collagen

The major component of the dermal–epidermal basement membrane is type IV collagen, a heterogeneous group of macromolecules that are present in diverse combinations in various basement membranes (Figure 2.32) [1]. Like all collagens, each type IV collagen molecule consists of three polypeptide subunits, known as α-chains. Some collagen molecules are homopolymers, that is the three α-chains are genetically identical, as in the case of type II, III and VII collagens. Others are heteropolymers so that there are two or even three different kinds of polypeptides, as for example in type I and type VI collagens. For type IV collagen, there are six genetically distinct but structurally related α-chains, and the precise composition of the α-chains varies with the tissue location of the basement membranes. In the case of the cutaneous BMZ, the major type IV collagen consists of α1 and α2 chains, with the chain composition $[\alpha1(IV)]_2\alpha2(IV)$, although other type IV collagen subunit polypeptides are also present in lower quantities. The α3 chain of type IV collagen has been shown to be the antigen recognized by circulating autoantibodies characteristic of Goodpasture syndrome, while structural aberrations in the α5 chain of type IV collagen are associated with Alport syndrome [2,3]. Autoantibodies against α5 and α6 chain epitopes have also been reported in patients with glomerulonephritis and subepidermal blistering [4].

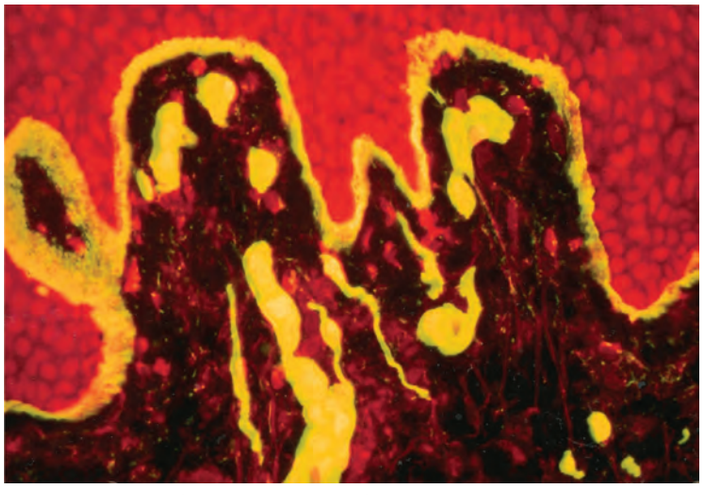
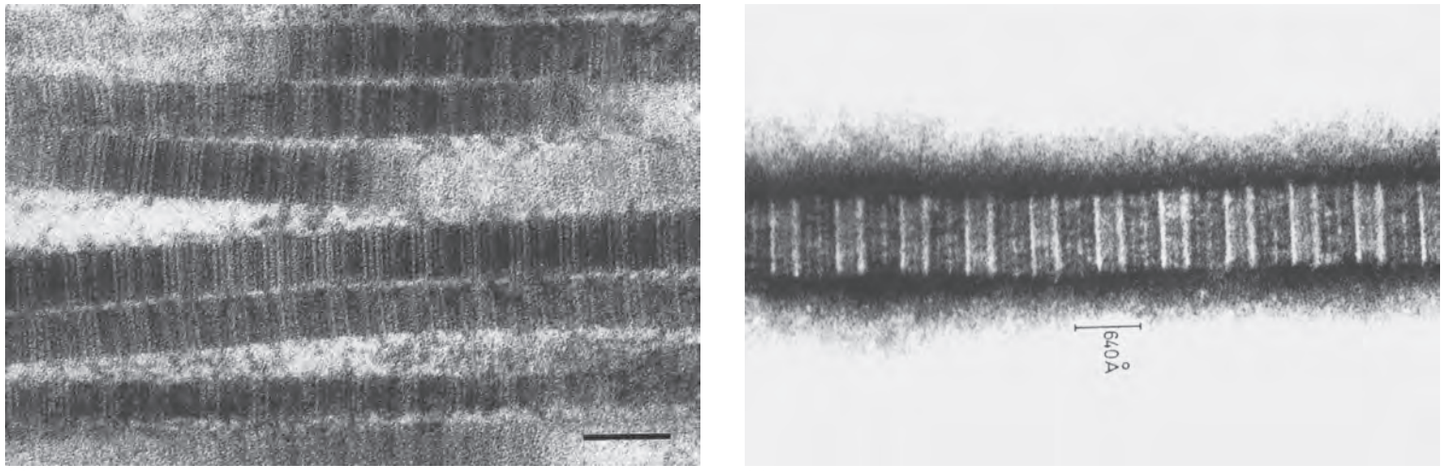


Figure 2.32 Immunofluorescence staining of the dermis and cutaneous basement membrane zone with an antibody for type IV collagen. Note positive staining at the dermal–epidermal basement membrane and around the dermal blood vessels. Original magnification 250×.

The characteristic fibre structure of interstitial collagens, as exemplified by type I collagen, results from the lateral aggregation of individual molecules in a quarter stagger array; this gives rise to a 64 nm cross-striation pattern when examined by transmission electron microscopy (Figure 2.33). In the case of type IV collagen, the non-collagenous globular domains both at the amino- and carboxyl-ends of the individual collagen molecules interact to form dimers and tetramers which then assemble into lattice-like structures and associate laterally in a complex hexagonal arrangement (Figure 2.34). This arrangement allows the basement membrane structure to be highly flexible and makes interactions with other collagenous and non-collagenous basement membrane components possible [1].



(a) (b)

Figure 2.33 Demonstration of periodicity in collagen fibres of 640 Å. (a) Collagen fibrils in the reticular dermis show a characteristic banding pattern after standard processing and staining with uranyl acetate and lead citrate for transmission electron microscopy. Scale bar 0.1 μm. (b) Transmission electron micrograph of a shadowed replica of unfixed, freeze-frozen and surface-sublimated rat-tail tendon collagen showing the step-like banding of the fibres. Original magnification 40 500×. (Courtesy of G. A. Meek.)

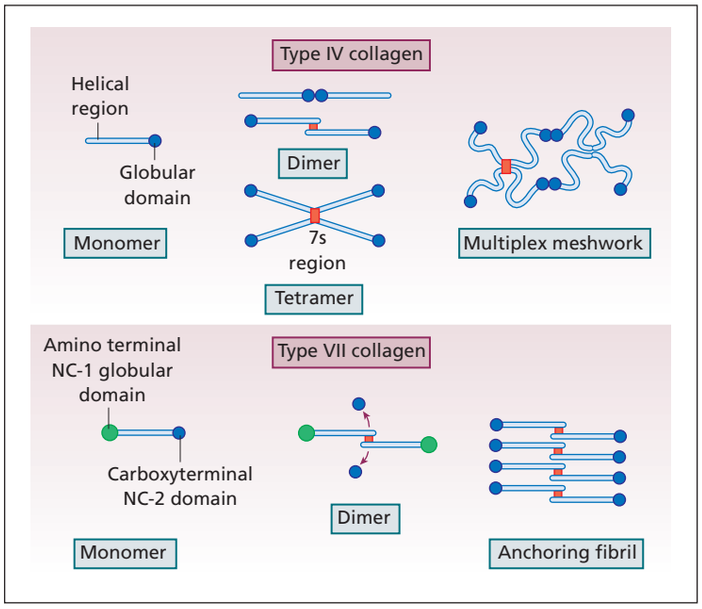


Figure 2.34 Assembly of type IV and type VII collagen molecules into supramolecular structures. The red boxes represent intermolecular disulphide bonds.

Table 2.1 Chain composition of the major laminins in the skin.

Type	Chain composition	Old designation	Distribution in basement membranes
111	α1β1γ1	1	Blood vessels, LD
332	α3β3γ2	5	LL/LD
311	α3β1γ1	6	LL/LD
511	α5β1γ1	10	LL/LD

LD, lamina densa; LL, lamina lucida.

Laminins

Other components that contribute to the cutaneous BMZ include members of the laminin family of multidomain proteins. As many as 16 different laminins have been identified thus far and at least four of them are physiologically present in the skin in significant quantities (Table 2.1) [1,2]. Each laminin molecule consists of three polypeptide subunits, α-, β- and γ-chains, which form a cruciform structure with three short arms and one long arm when visualized by rotary shadowing electron microscopy (Figure 2.35).

The short arms represent the N-terminal segments of the α-, β- and γ-chains. The long arm consists of an extended rod-like structure of the triple-stranded, coiled-coil domain of all three chains. This domain serves as the site of chain assembly of the three subunit polypeptides. The α-chain contains an additional C-terminal segment that has five globular segments located at the tip of the long arm, known as the G domain. The major laminin within the cutaneous basement membrane zone is laminin 332, previously known as laminin 5 (Figure 2.36). In addition, laminin 311 and laminin 511 are integral components of the cutaneous BMZ, while laminin 111 is also present in basement membranes of the blood vessels in human dermis [3].

The cell binding of laminins is mediated by integrins, a family of cellular receptors, each consisting of two subunit polypeptides (Figure 2.37). Integrins also mediate outside-in signal transduction elicited by laminins and regulate cell migration, proliferation, differentiation and adhesion [4]. The principal integrin in the cutaneous BMZ is the α6β4 integrin, which is critical for the adhesion of basal cells to the underlying BMZ.

The cruciform structure of laminins contains both globular and rod-like segments that have been individually implicated in various functions, including interactions with other extracellular matrix molecules, such as the hemidesmosomal components

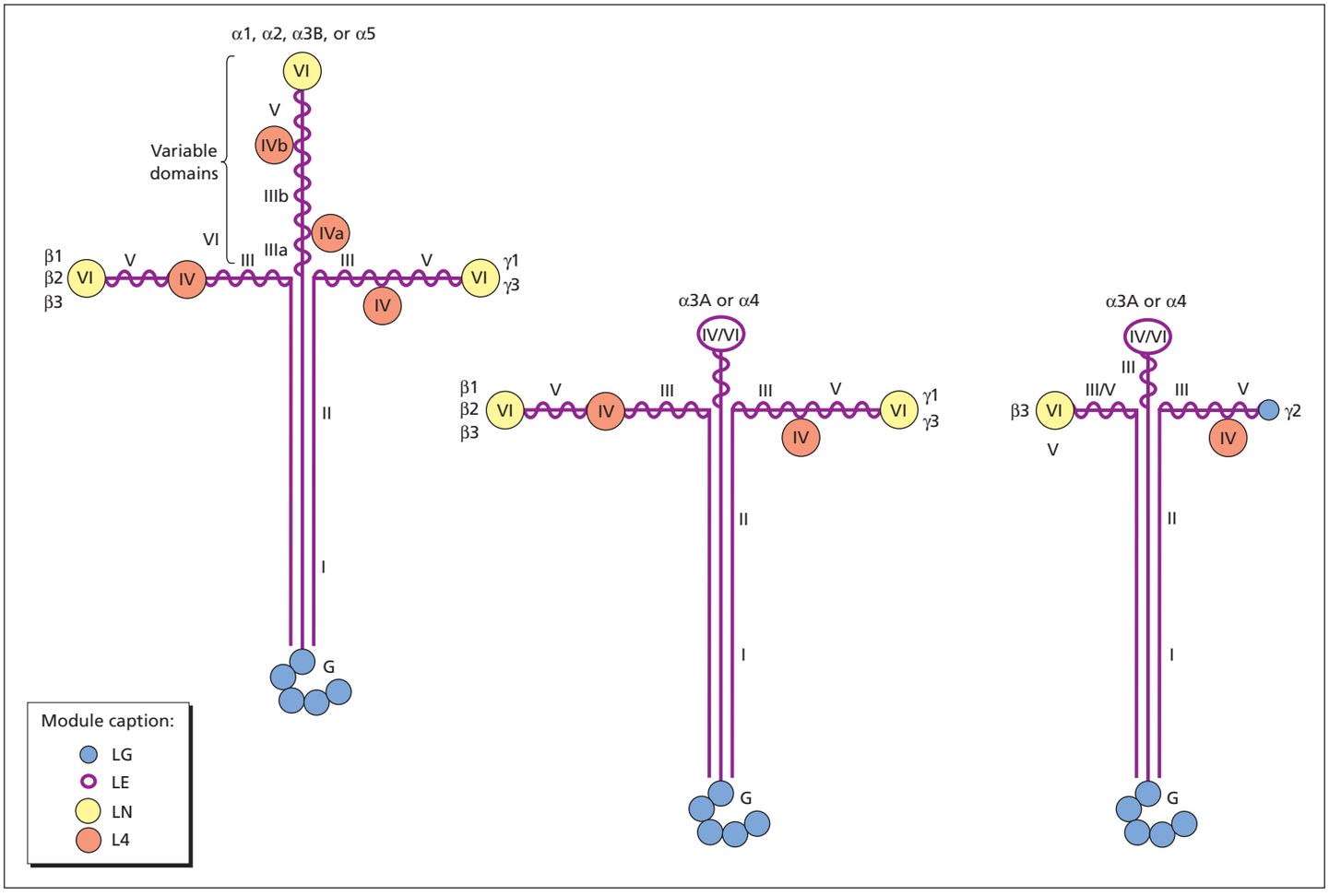


Figure 2.35 Different isoforms and domain organizations of laminin, each consisting of three distinct subunit polypeptides, α-, β- and γ-chains. The LE modules are formed by approximately 60 residues each and have homology to epidermal growth factor. The LN and L4 modules are folded into globular structures located between the LE modules (domain IV) or at the amino-terminus of each chain (domain VI). The coiled-coil central region, comprising all three polypeptides, is represented as vertical straight lines. Note the presence of a G domain consisting of five globular segments (LG) at the carboxy-terminus of the α-chains. (Adapted from Aumailley and Rouselle 1999 [8]. Reproduced with permission of Elsevier.)

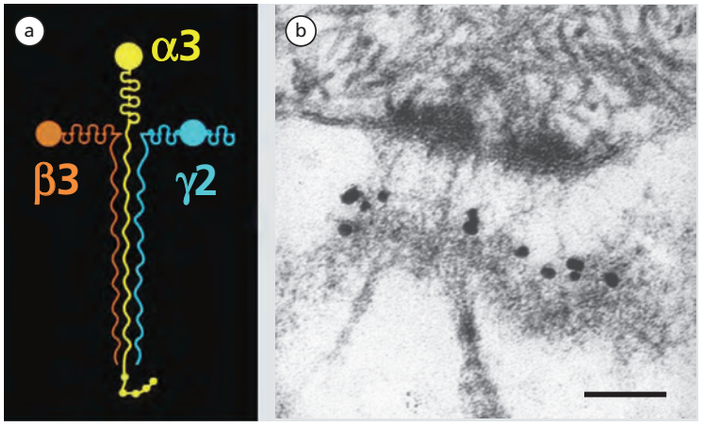
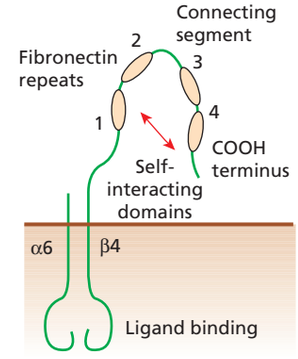


Figure 2.36 (a) Laminin 332 expressed in the cutaneous basement membrane zone. (b) Immunogold electron microscopy using an antibody to the γ2 chain of laminin 332 showing labelling at the lamina lucida–lamina densa interface below a hemidesmosome. Scale bar, 50 nm.

and type VII collagen, as well as in cell attachment and spreading, neurite outgrowth and cellular differentiation. Collectively, the laminins play vital roles in the development and maintenance of the supramolecular organization of the basement membrane [5]. The critical role of laminin 332 in providing integrity to the cutaneous BMZ is evident by observations that genetic mutations in any of the three polypeptide subunits – that is the *LAMA3*, *LAMB3* or *LAMC2* genes which encode the α3, β3, and γ2 chains, respectively – can result in junctional forms of epidermolysis bullosa (EB) with profound fragility of the skin. Furthermore, mutations in the *ITGA6* and *ITGB4* genes – encoding the α6 and β4 subunit polypeptides of integrin, respectively – cause a form of junctional EB frequently associated with pyloric atresia [6].

Other basement membrane zone components at the dermal–epidermal junction include a glycoprotein known as nidogen (previously called entactin) that interacts with type IV collagen



α6β4 integrin	Domain	Function
	β4 integrin	
	COOH terminus	Plectin binding
	COOH terminus	Proximal endodomain binding
	Connecting segment	Hemidesmosome assembly
	Connecting segment	Plectin binding
	Connecting segment	Tyrosine activation motif
	Connecting segment and 3rd FN repeat	Collagen XVII binding
	NH_2 half, endodomain	COOH terminus binding
	Exodomain	Extracellular ligand binding
	α6 integrin	
	Proximal exodomain	Collagen XVII binding
	Exodomain	Extracellular ligand binding

Figure 2.37 Molecular composition, domain organization and functions of the α6β4 integrin, the main keratinocyte integrin in hemidesmosomes. This integrin is important in hemidesmosome assembly and protein–protein interactions. FN, fibronectin.

either alone or as a laminin–nidogen complex (Figure 2.38) [7]. Nidogens are a family of highly conserved, sulphated glycoproteins within a spectrum of binding proteins. Although nidogens are an abundant component of various basement membranes, genetic analyses have shown that they are not required for the overall architecture of the basement membrane. Instead, nidogens appear to play a critical role in the development of basement membranes in tissues undergoing rapid growth or turnover.

Another class of integral basement membrane constituents is that of the heparan sulphate proteoglycans (HSPGs). These molecules consist of a core protein with different numbers of covalently associated heparan and sulphate chains which make these molecules highly negatively charged and hydrophilic. These proteoglycans are capable of interacting with a number of basement membrane components and they are likely to contribute to the overall architecture of the basement membrane. Specific cell surface HSPGs are found also on the surface of epithelial cells and possibly mediate cell–matrix interactions.

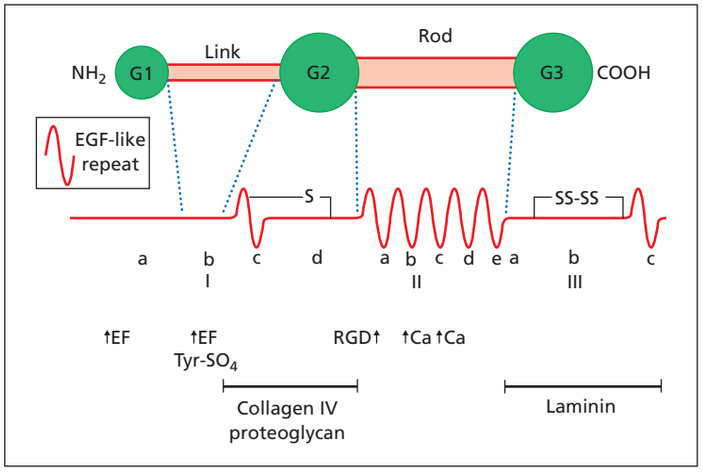


Figure 2.38 Model of nidogen, containing subdomains with predicted binding activities to type IV collagen, proteoglycans and laminin 1. EF and Ca refer to putative calcium-binding sites; RGD is a putative cell-binding sequence. EGF, epidermal growth factor. (Adapted from Niessen *et al.* 1994 [9]. Reproduced with permission of Elsevier.)

Hemidesmosomes

The dermal–epidermal junction of skin is characterized by the presence of specific structures that are critical for the functional integrity of the skin. These ultrastructurally recognizable components include hemidesmosomes, anchoring filaments and anchoring fibrils. The molecular composition and the specific domain organizations of the component macromolecules have been largely characterized (Figure 2.39).

The hemidesmosomes are seen ultrastructurally as electron-dense attachment complexes, which extend from the intracellular compartment of the basal keratinocytes to the lamina lucida in the upper portion of the dermal–epidermal basement membrane (Figure 2.40). The intracellular domains of hemidesmosomes within the basal keratinocytes attach to the keratin intermediate filament network, while in the extracellular space within the lamina lucida the hemidesmosomes are contiguous with anchoring filaments; this unit is termed the hemidesmosome–anchoring filament complex.

Early biochemical studies identified at least five major components of hemidesmosomes, originally designated as HD1 to HD5 with molecular masses of approximately 500, 230, 200, 180 and 120 kD, respectively. Various molecular and immunological approaches have subsequently identified HD2 and HD4 as the 230 and 180 kDa bullous pemphigoid antigens (BPAG1 and BPAG2), respectively [1]; HD3 and HD5 correspond to the β4 and α6 integrin subunit polypeptides, respectively; and HD1 corresponds to plectin, a large intracytoplasmic adhesion molecule. The intracellular hemidesmosomal plaque contains the 230 kDa bullous pemphigoid antigen, a non-collagenous protein of the plakin family that serves as an autoantigen in bullous pemphigoid. The 180 kDa bullous pemphigoid antigen, a transmembrane collagenous protein, also known as type XVII collagen, interacts with α6β4 integrin and extends from the intracellular compartment of basal cells to the extracellular space, thus stabilizing the association of basal keratinocytes to the underlying basement membrane.

Attesting to the critical importance of the hemidesmosomes in providing stability to the association of basal keratinocytes with the underlying BMZ, is the finding that mutations in the genes

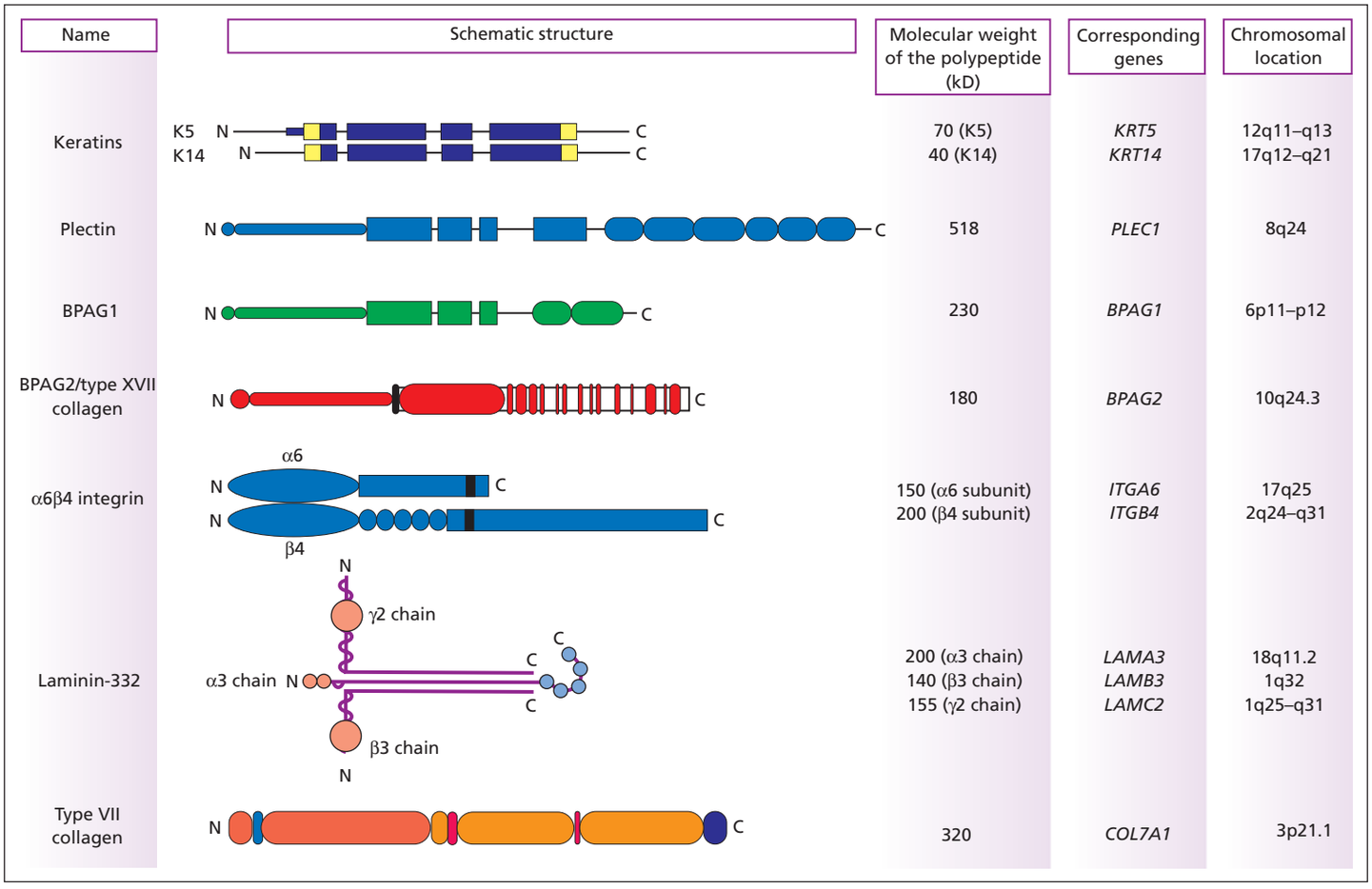


Figure 2.39 Structure and domain organization of the major protein components at the cutaneous basement membrane zone, with their molecular weights and chromosomal locations of the corresponding genes. BPAG, bullous pemphigoid antigen. (From Pulkkinen and Uitto 1998 [7]. Reproduced with permission of John Wiley & Sons.)

encoding hemidesmosomal proteins have been shown to result in different forms of EB (see Figure 2.31) [2,3]. The causative nature of the mutations in these hemidesmosomal genes has been verified by the development of targeted mutant ('knock-out') mouse models, which frequently recapitulate the clinical, genetic, histological and ultrastructural features encountered in patients with EB [4,5].

The hemidesmosomes are complexed with anchoring filaments, which are thread-like structures that tend to coalesce below the hemidesmosomal outer plaques (see Figure 2.36). Electron microscopy and immunohistochemical analyses have suggested that laminin 332 may be the major component of the anchoring filaments, although the presence of additional molecules has also been suggested. As indicated, laminin 332 is a disulphide-bonded complex of α3, β3 and γ2 chains that associate into a trimeric, cruciform structure. After the initial assembly, the α3 and γ2 chains are proteolytically processed, and many of the conformational epitopes are recognized by specific antibodies. Complete absence of any of the three subunit polypeptides of laminin 332, due to loss-of-function mutations in both alleles of the corresponding gene, results in severe generalized junctional EB [6]. Since laminin 332 binds to α6β4 integrin in the hemidesmosomes and to type VII collagen in the anchoring fibrils, the severity of skin fragility in this form of EB apparently reflects the loss of its ability to bridge the hemidesmosomes and the anchoring fibrils. This results in a separation of the epidermis from the dermis within the lamina lucida as a result of minor trauma to the skin.

Anchoring fibrils

Anchoring fibrils are ultrastructurally recognizable, U-shaped structures that extend from the lower part of the lamina densa to the upper reticular dermis (Figure 2.41). Type VII collagen is the major, if not the exclusive, component of anchoring fibrils [1]. Individual collagen molecules are approximately 450 nm long, consisting of a central, triple helical segment flanked by non-helical globular domains at each end of the triple helix: NC1 at the amino-terminus and NC2 at the carboxy-terminus of the molecule (see Figure 2.34). In addition, the triple helical segment of type VII collagen contains imperfections in the triple helix, including a central

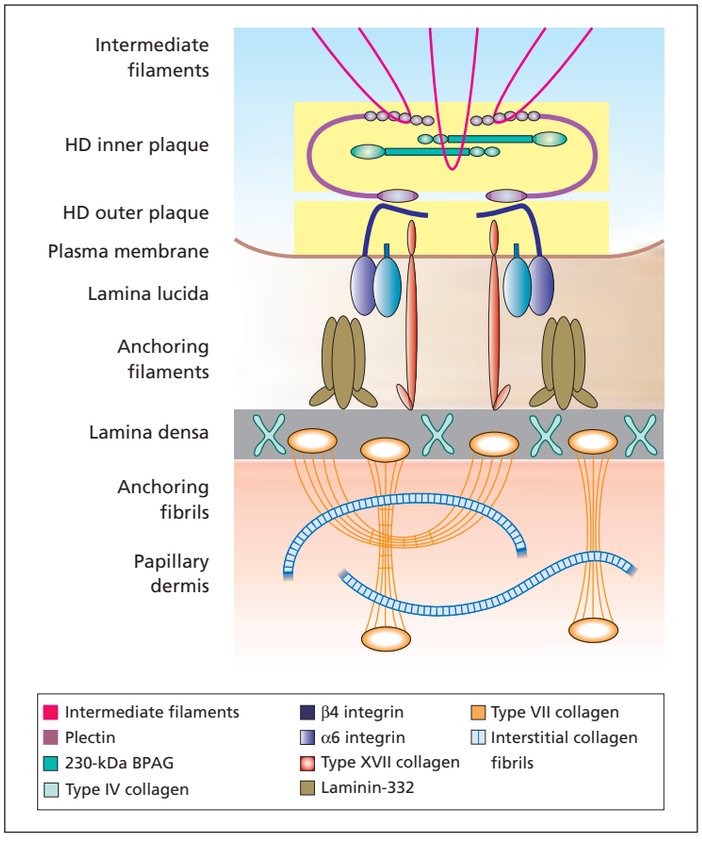


Figure 2.40 Molecular interactions of the major components of the cutaneous basement membrane zone. The individual components are identified in the colour key and their domain organizations are given in Figure 2.39. BPAG, bullous pemphigoid antigen; HD, hemidesmosome. (Adapted from Pulkkinen and Uitto 1998 [7]. Reproduced with permission of John Wiley & Sons.)

39 amino acid non-collagenous segment. These interruptions in the glycine-X-Y sequence are thought to provide flexibility to the type VII collagen molecules.

The gene encoding type VII collagen (*COL7A1*) is extremely complex, consisting of 118 exons on the short arm of human chromosome 3 [2]. Type VII collagen is synthesized by both dermal fibroblasts and epidermal keratinocytes, although the basal cell appears to be the major source of type VII collagen during the early prenatal development of skin. Upon secretion to the extracellular space, two type VII collagen molecules align into an antiparallel dimer with overlapping NC2 domains, and after partial proteolysis of the NC2 domain, the dimer is stabilized by intermolecular disulphide bonds. Subsequently, a large number of type VII molecules laterally aggregate to form anchoring fibrils in which the NC1 domains at both ends attach to the basement membrane. The U-shaped loops then entrap, and possibly interact with, large interstitial collagen fibres consisting of type I, III and V collagens [3].

The critical importance of the anchoring fibrils in securing the adhesion of the dermal–epidermal basement membrane to the underlying dermis as well as in wound healing is illustrated by the dystrophic forms of EB [4]. Specifically, a complete absence of type VII collagen results in severe, generalized, recessive dystrophic EB with fragility of the skin and mucous membranes, leading to mutilating scarring of the hands and feet. Missense mutations, particularly glycine substitution mutations, can result in somewhat milder, dominantly inherited dystrophic EB [5].

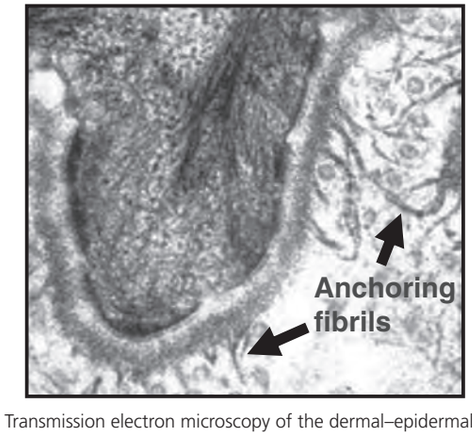


Figure 2.41 Transmission electron microscopy of the dermal–epidermal junction revealing wheatsheaf-shaped anchoring fibrils beneath the lamina densa. These fibrils help secure adhesion between the epidermal basement membrane and interstitial collagens within the dermis.

Extracellular matrix

The major component of human skin is the dermis, demarcated on the top by the lamina densa in the lower border of the dermal–epidermal basement membrane and at the bottom by the subcutis. In contrast to the epidermis, the dermis is largely acellular and consists primarily of the extracellular matrix of connective tissue, a complex meshwork of various macromolecules. There are four major classes of extracellular matrix components: (i) collagen fibres, which provide tensile strength to allow the skin to serve as a protective organ against external trauma; (ii) elastic structures, which provide elasticity and resilience to normal human skin; (iii) non-collagenous glycoproteins, such as fibrillins, fibulins and integrins, which often serve as organizers of the matrix and facilitate cell–matrix interactions; and (iv) proteoglycan/glycosaminoglycan macromolecules, which provide hydration to the skin. The maintenance of proper quantities and appropriate interactions between the extracellular matrix components is a prerequisite for the physiological homeostasis of the dermis.

The major extracellular matrix component in the dermis is collagen, which comprises a family of closely related yet genetically distinct proteins [1–4]. The major collagen fibres in the dermis provide tensile strength to the skin to serve as a protective organ against external trauma (Figure 2.42). A characteristic feature of all collagens is the triple helical conformation, which is predicated upon the primary amino acid sequence of the subunit polypeptides, α-chains, depicting a repeating glycine-X-Y sequence. The collagens also demonstrate non-collagenous flanking segments at the ends of the individual molecules. Currently, 29 distinct collagens have been identified in vertebrate tissues, and have been characterized to the extent that they are referred to by Roman numeral designation (I–XXIX) in the order of their discovery, many of them being present in the skin (Table 2.2).

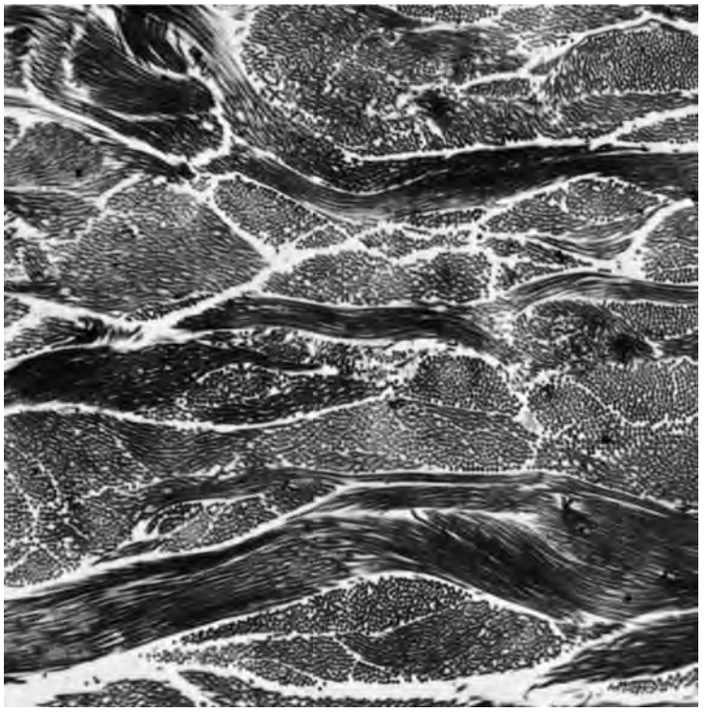


Figure 2.42 Transmission electron micrograph of a section of dermis from the human forearm showing bundles of collagen fibres, both in transverse and longitudinal sections. Original magnification 4900×. (Courtesy of Professor A. S. Breathnach.)

Table 2.2 Genetic heterogeneity of collagens.

Collagen type	Chain composition	Supramolecular assembly	Tissue distribution[a]
I	$[\alpha1(I)]_2\alpha2(I)$	Fibrillar	Dermis, bone, tendons
III	$[\alpha1(III)]_3$	Fibrillar	Fetal dermis, blood vessels, GI tract
IV	$[\alpha1(IV)]_2\alpha2(IV)$[b]	Basement membrane	Ubiquitous
V	$[\alpha1(V)]_2\alpha2(V)$[b]	Fibrillar	Ubiquitous
VI	$[\alpha1(VI)\alpha2(VI)\ \alpha3(VI)]$[b]	Microfibrils	Ubiquitous
VII	$[\alpha1(VII)]_3$	Anchoring fibrils	Epithelial basement membranes
VIII	$[\alpha1(VIII)]_3$	Network forming	Endothelia
XIII	$[\alpha1(XIII)]_3$	Transmembrane	Ubiquitous, including epidermis
XIV	$[\alpha1(XIV)]_3$	FACIT	Skin, cornea
XV	$[\alpha1(XV)]_3$	Basement membrane	Ubiquitous
XVII	$[\alpha1(XVII)]_3$	Transmembrane	Hemidesmosomes
XXIX	Unknown	Unknown	Epidermis

[a] Distribution in the skin and other major tissues is indicated; lesser amounts may be present in other tissues.

[b] Additional α-chains have been identified.

FACIT, fibril-associated collagens with interrupted triple helices; GI, gastro-intestinal.

All collagen molecules consist of three subunit polypeptides, which can either be identical as homotrimers or can consist of two or even three genetically different polypeptides in heterotrimeric molecules. Since the different subunits are all distinct gene products, there are over 40 different genes in the human genome that encode the different subunit polypeptides, the α-chains, of these distinct collagens [5].

Collagens

On the basis of their fibre architecture in tissues, the genetically distinct collagens can be divided into different classes [1,2]. Types I, II, III, V and IX align into large fibrils and are designated as fibril-forming collagens. Type IV is arranged in an interlacing network within the basement membranes, while type VI is a distinct microfibril-forming collagen, and type VII collagen forms anchoring fibrils. FACIT collagens (fibril-associated collagens with interrupted triple helices) include types IX, XII, XIV, XIX, XX and XXI. Many of the latter collagens associate with larger collagen fibres and serve as molecular bridges, stabilizing the organization of the extracellular matrices. The major collagens significantly contributing to skin physiology and pathology are presented in Table 2.2. Other collagen types, including types II, IX, X, XI, XIX, XX and XXI, are not discussed in detail because they are not present in the skin to a significant extent or their participation in maintaining skin physiology is unclear.

Type I collagen, the most abundant form, is the predominant collagen in human dermis, accounting for approximately 80% of the total collagen. Type I collagen contains two different kinds of α-chain with an $[\alpha(I)]_2\alpha2(I)$ stoichiometry. A collagen consisting of three identical α1(I) chains has also been identified (so-called α1(I) trimer), but it appears to be only a minor component of connective tissue in the skin. Type I collagen associates with type III collagen to form broad, extracellular fibres in the human dermis. Mutations in type I and III collagens, or in their processing enzymes, can result in connective tissue abnormalities in the different forms of Ehlers–Danlos syndrome, and mutations in the type I collagen gene are responsible for the fragility of bones in osteogenesis imperfecta.

Type III collagen accounts for about 10% of the total bulk of collagen found in adult human dermis. It was originally shown to predominate in human skin during embryonic development, but during the early postnatal period type I collagen synthesis accelerates, resulting in the ratio of type I to type III collagen in the adult human skin being approximately 8 : 1. Type III collagen is most prominent in vascular connective tissues, the gastro-intestinal tract and the uterus. It consists of three identical α-chains, α1(III), and mutations in the type III collagen gene can cause the vascular type of the Ehlers–Danlos syndrome [3].

Type IV collagen is a basement membrane collagen present within the dermal–epidermal junction as well as in the vascular basement membranes. The predominant form of type IV collagen in human skin is a heterotrimer of $[\alpha1[IV)]_2\alpha2(IV)$, although occasional homopolymers from these two chains may be assembled. The type IV collagen molecule is characterized by the presence of non-collagenous interruptions within the triple helical

domains, thus conferring flexibility to the molecule. In addition to α1 and α2 chains, four other polypeptides of type IV collagen have been identified [4]. Type IV collagen molecules containing these polypeptides are present primarily in the glomerular basement membranes, and their importance for renal physiology is attested by the fact that mutations in the gene encoding the α5(IV) polypeptide results in Alport syndrome. The α3(IV) chain harbours the epitopes recognized by antibodies in Goodpasture syndrome, and the α5 chain of type IV collagen has been shown to be a target of circulating autoantibodies in a novel autoimmune disease with subepidermal blisters and renal insufficiency.

Type V collagen consists of interrelated collagens containing four different types of α-chains. Type V collagen is present in most connective tissues, including dermis, where it represents less than 5% of the total collagen. Type V collagen is located on the surface of large collagen fibres in the dermis, and its function is to regulate the lateral growth of these fibres. Thus, in the absence of type V collagen, the collagen fibre diameter is variable and the contour of the individual fibres can appear irregular, some of them having 'flower-like' morphology in cross-section. The importance of type V collagen in contributing to connective tissue stability is attested by the fact that mutations in the type V collagen gene underlie most patients with classic, autosomal dominant forms of Ehlers–Danlos syndrome [3].

Type VI collagen, as originally discovered, consists of three distinct α-chains, α1(VI), α2(VI) and α3(VI), which fold into a relatively short, triple helical domain and contain large globular domains at both ends. More recently, three additional α-chains have been suggested to belong to the type VI family of collagens [5]. Type VI collagen is a relatively minor collagen in human dermis, where it assembles into thin microfibrils independent of the broad collagen fibres, which consist primarily of type I and type III collagens. The microfibrillar network has an anchoring function, stabilizing the assembly of collagen fibres as well as basement membranes. Mutations in the three type VI collagen genes can lead to different forms of muscular dystrophy with little effect on the physiology of skin [6].

Type VII collagen, the major if not the exclusive component of anchoring fibrils, consists only of one type of α1 chain, α1(VII). This polypeptide has a characteristic modular structure, with the central collagenous domain being flanked by amino-terminal (NC1) and carboxy-terminal (NC2) collagenous domains with homology to known protein sequences.

Type VII collagen molecules become organized into anchoring fibrils through the formation of antiparallel dimers linked through their carboxy-terminal ends (Figure 2.34). The large amino-terminal, non-collagenous NC1 domains interact with type IV collagen and laminin 332 at the dermal–epidermal basement membrane, forming U-shaped loops that entrap larger fibres in a manner that stabilizes the association of the lower part of the lamina densa to the upper papillary dermis (Figure 2.41). Consequently, altered expression or changes in the molecular interactions of type VII collagen with other basement membrane components can result in skin fragility as exemplified by the dystrophic forms of epidermolysis bullosa [7]. In addition to the heritable forms of EB, type VII collagen serves as an autoantigen in the autoimmune blistering skin disease EB acquisita, the majority of antigenic epitopes residing within the NC1 domain [8].

Type XVII collagen was initially identified as the 180 kDa bullous pemphigoid antigen (BPAG2) recognized by circulating autoantibodies in the sera of patients with bullous pemphigoid or herpes gestationis. Subsequent characterization of the protein and the corresponding gene has indicated that BPAG2 is, in fact, a collagenous molecule consisting of 15 collagenous domains with characteristic Gly-X-Y repeat sequences which form triple helices (Figure 2.43) [9]. Type XVII collagen is a transmembrane protein in type 2 topography, that is the amino-terminal segment of the molecule is intracellular while the carboxy-terminal ectodomain, containing the collagenous segments, is in the extracellular space. The importance of type XVII collagen to the stability of the dermal–epidermal junction is attested to by the fact that mutations in the corresponding gene (*COL17A1*) result in a generalized intermediate variant of junctional EB, originally designated as non-Herlitz or generalized atrophic benign EB, and circulating autoantibodies to type XVII collagen (BPAG2) are associated with bullous pemphigoid [10].

Type XXIX collagen is a putative epidermal collagen with a specific gene expression pattern; the highest level of expression is in the skin, lung and gastro-intestinal tract [11]. In the skin, expression is restricted to the epidermis with the highest level in suprabasal layers. This collagen was initially identified through genetic linkage of patients with AE to a locus on the short arm of chromosome 3q21. The locus contained a single gene encoding a collagenous segment flanked by multiple von Willebrand factor A-like domains, with a high degree of homology with the α3 chain of type VI collagen. Immunofluorescence staining of skin from patients with AE demonstrated a striking lack of collagen XXIX in the viable, outermost spinous and granular layers, suggesting a role in AE.

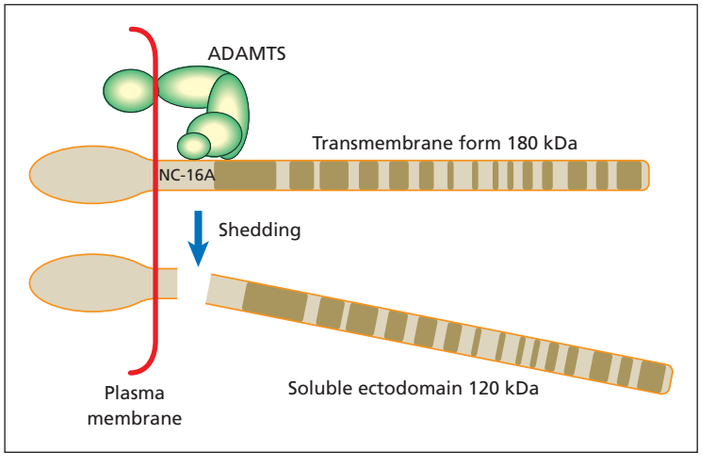


Figure 2.43 Type XVII collagen, a transmembrane protein in type 2 orientation. Note that the ectodomain traversing the lamina lucida contains 15 distinct triple helical collagenous segments (COL1–15). The non-collagenous segment between COL15 and the transmembrane domain, NC-16A, harbours the major epitopes recognized by autoantibodies in bullous pemphigoid. ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs, cleaves the protein at a sequence in the NC-16A, resulting in release of the ectodomain. (Adapted from Powell *et al.* 2005 [12].)

Collagen biosynthesis

The genetically distinct collagens demonstrate considerable tissue specificity, and, accordingly, they are synthesized by a number of different cell types, including dermal fibroblasts, epidermal keratinocytes, vascular endothelial cells and smooth muscle cells. The individual α-chains are initially synthesized as precursor molecules, pro-α-chains, with non-collagenous extensions at the ends of the collagenous domain (Figure 2.44).

While in the rough endoplasmic reticulum, three individual pro-α-chains assemble into a trimeric molecule through interactions of the non-collagenous sequences at the carboxy-terminal end. Upon completion of the prolyl hydroxylation reactions, the collagenous domains of the α-chains fold into a triple helical conformation, and the collagen molecules are then secreted through Golgi vesicles into the extracellular milieu. In the extracellular space, parts of the non-collagenous peptide extensions are cleaved by specific proteases, and the collagen molecules then assemble into their tissue-specific supramolecular organization. For example, the fibrillar collagens align into a characteristic quarter-stagger arrangement and form fibres, the growth occurring at the tip of the growing fibre. The coarse collagen fibres in the mid-dermis consist primarily of type I and III collagens, and type V collagen associates with them on the surface of the fibre so as to regulate the diameter of the growing fibre. Type VII collagen assembles into centrosymmetrical anchoring fibrils within the dermal–epidermal basement membrane zone, and type XVII collagen assumes a transmembrane type 2 orientation as a component of the hemidesmosomes. A characteristic feature of collagen is the presence of hydroxyproline and hydroxylysine residues, two amino acids that are post-translationally synthesized by hydroxylation of proline and lysine residues, respectively (Figure 2.45) [1].

These hydroxylation reactions are catalysed in the rough endoplasmic reticulum by prolyl and lysyl hydroxylases, respectively, enzymes that require ascorbic acid, molecular oxygen and ferrous iron as co-factors. The hydroxylation of prolyl residues is necessary for the stabilization of the triple helical conformation at physiological temperatures, and hydroxylysyl residues are required for the formation of stable covalent cross-links. Thus, for example, as a result of ascorbic acid deficiency in scurvy, the hydroxylation reactions are suboptimal, the newly synthesized collagen is poorly functional, and clinically scurvy manifests with connective tissue weakness. Similarly, low oxygen tension in chronic ulcers and

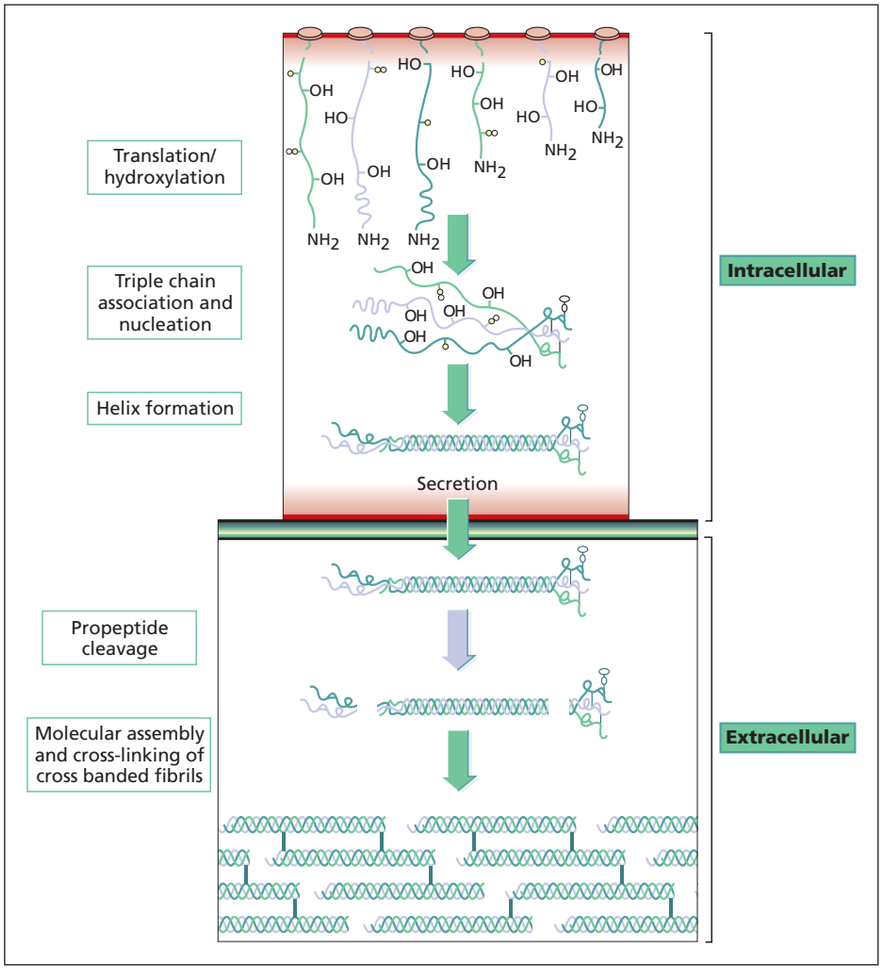


Figure 2.44 Steps in the intracellular biosynthesis of triple helical type I procollagen, its secretion into the extracellular space, and assembly and cross-linking of mature collagen fibres in the extracellular space.

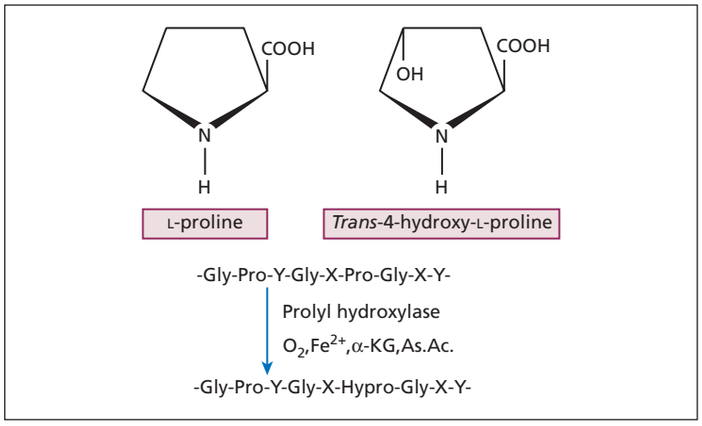


Figure 2.45 Enzymatic hydroxylation of prolyl residues in the Y-position of the repeating Gly-X-Y amino acid sequence to form hydroxyproline, an amino acid characteristic of collagen. Note that the reaction requires molecular oxygen, ferrous iron, α-ketoglutarate (α-KG) and ascorbic acid (As.Ac.) as co-factors.

wounds due to poor circulation may impair collagen production, resulting in poor healing.

Hydroxylation of the lysyl residues is followed by *O*-glycosylation, catalysed first by galactosyltransferase, which adds a galactosyl residue to the hydroxyl group of hydroxylysine, followed by a glucosyltransferase reaction to form glucosyl-galactosyl-hydroxylysine in *O*-glycosidic linkage. Additional glycosylation in *N*-glycosidic linkage will take place on the non-collagenous extensions at the end of the triple helical molecule, but the functional significance of these glycosylation reactions is currently unclear.

Collagen biology

The regulation of collagen gene expression has to be tightly controlled in order to maintain normal amounts and ratios of genetically distinct collagens under physiological conditions. At the same time, regulatory mechanisms have to be responsive to the needs of rapid collagen synthesis in repair processes, such as wound healing. On the other hand, uncontrolled collagen synthesis can lead to excessive accumulation of collagen in fibrotic diseases, as exemplified by systemic sclerosis, keloids and hypertrophic scars [1,2].

An important control mechanism is at the level of collagen mRNA formation through regulation of the transcriptional activity of the corresponding genes. In general, there is a good correlation between the rate of collagen biosynthesis and the corresponding procollagen mRNA levels, as demonstrated in several *in vitro* models, including cultured fibroblasts. The transcriptional regulation of collagen gene expression involves a number of both *cis*-acting elements and *trans*-acting factors. The *cis*-acting elements, representing nucleotide sequences within the regulatory regions of the gene that serve as binding sites for *trans*-acting regulatory proteins, have been identified in most collagen gene regulatory regions. Such factors can either up-regulate or suppress the transcriptional promoter activity. An example of such *trans*-acting factors are the retinoic acid nuclear receptors (RAR and RXR) that form a complex with the ligand (a retinoid), which then binds to the retinoic acid-responsive elements (RARE) in the target gene. Retinoids, such as all-*trans*-retinoic acid, have been shown to modulate collagen gene expression both *in vitro* and *in vivo*, and quiescent non-proliferating cells can be stimulated by retinoic acid to activate type I collagen synthesis. These observations may have relevance to the elevated rate of collagen synthesis observed in photodamaged dermis treated by the topical application of retinoids [2]. All-*trans*-retinoic acid has also been demonstrated to increase the density of anchoring fibrils along the cutaneous BMZ in adult human skin, suggesting that retinoids are capable of up-regulating type VII collagen gene expression.

Collagen gene expression can also be modulated by a number of cytokines and growth factors, and one of the most powerful modulators of connective tissue gene expression is TGF-β [1,3]. In general, TGF-β is pro-fibrotic and it has been shown to up-regulate the expression of a number of extracellular matrix protein genes, including those encoding collagen types I, III, IV, VI and VII. Elevated levels of TGF-β have also been demonstrated in various fibrotic lesions, including the skin in systemic sclerosis and keloids. The up-regulatory activity of TGF-β can be counteracted by other cytokines, including tumour necrosis factor α and interferon γ, which antagonize the TGF-β action [1]. These cytokines have been tested for their efficacy for the treatment of keloids and other fibrotic diseases, with variable results.

A number of hormones clearly regulate collagen gene expression, as certain endocrine disorders dramatically change the amount of collagen found in connective tissues, including the skin. Glucocorticosteroids also affect collagen biosynthesis; inhibition is much more pronounced with fluorinated steroids compared with hydrocortisone. The glucocorticosteroid inhibition of collagen biosynthesis occurs in lower concentrations at the transcriptional level through inhibition of promoter activity. In higher concentrations and with more potent glucocorticosteroids, inhibition of prolyl hydroxylase activity also is evident, leading to deficient hydroxylation of collagen polypeptides and subsequently to reduced amounts of newly synthesized collagen. These mechanisms would explain the connective tissue side effects, such as dermal atrophy, associated with intralesional or prolonged topical application of fluorinated glucocorticosteroids.

Collagen cross-linking

The alignment of collagen molecules into their specific supramolecular organization occurs spontaneously, but these fibre structures do not attain the necessary tensile strength until the molecules have been covalently linked together by specific intra- and intermolecular cross-links [1]. The commonest forms of cross-link in type I collagen are derived from lysine and hydroxylysine residues, and in some collagens there are also cysteine-derived disulphide bonds. The first step in the cross-linking process is enzymatic synthesis of aldehyde residues from lysyl and hydroxylysyl residues by removal of the ε-amino group of these amino acids (Figure 2.46). This oxidative deamination reaction is catalysed by the lysyl oxidases, a group of enzymes that require copper as a co-factor. These enzymes act primarily upon native collagen fibrils and poorly,

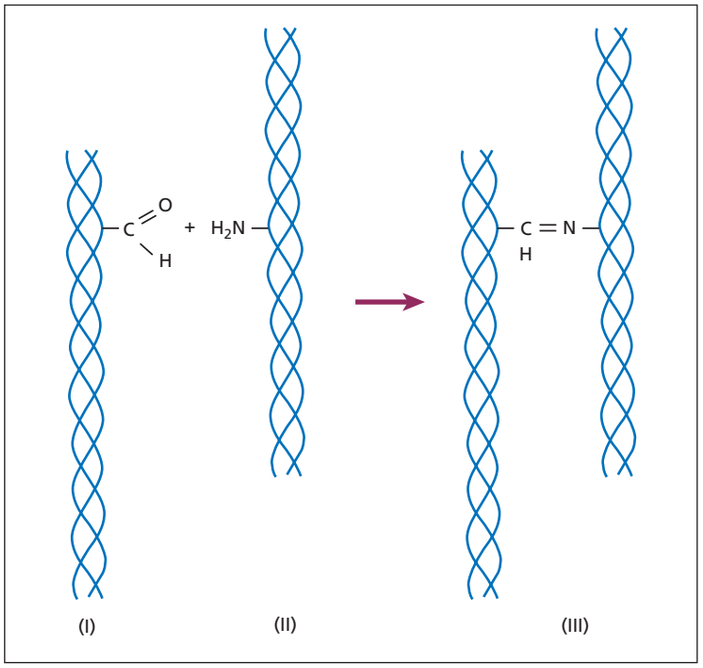


Figure 2.46 Formation of intermolecular cross-links between individual collagen molecules. The cross-linking is initiated by the conversion of lysine or a hydroxylysine residue that contains an ε-amino group to a corresponding aldehyde (I). The aldehyde then reacts with an unmodified ε-amino group in an adjacent collagen molecule (II) to form a Schiff base-type covalent cross-link (III).

if at all, on denatured collagen (gelatin) or isolated α-chains. Similar deamination reaction catalysed by lysyl oxidase occurs also in elastin.

In addition to the classic human lysyl oxidase, four additional lysyl oxidase-like (LOXL1–4) genes/proteins have been identified. These lysyl oxidase-like enzymes have been postulated to play a role in a number of disease processes. For example, *LOXL1* gene sequence variants confer susceptibility to exfoliation glaucoma and elastin was found to be the major component of the ocular lesions in this disease [2,3]. The expression of LOXL2 is increased in a number of cancers, and LOXL2 has been shown to be a marker for poor prognosis with decreased overall and disease-free survival in squamous cell carcinomas [4]. LOXL3 and -4 demonstrate structural features similar to lysyl oxidase and have wide tissue distribution [5,6]. Alterations in lysyl oxidase activities have also been described in a number of experimental systems involving age-related changes in the cardiovascular system [7].

Collagen degradation

Collagen fibres, once fully matured by the cross-linking processes, are relatively stable and can exist in tissues under normal physiological conditions for long periods. However, there is continuous, yet slow, degradation and turnover of collagen in normal situations, as attested by continuous urinary excretion of hydroxyproline as a marker of collagen degradation. In addition, in certain physiological situations, as exemplified by reabsorption of the postpartum uterus, and in pathological conditions, such as tissue invasion and tumour metastases, degradation of connective tissues and particularly collagen is accelerated. There are a number of enzymes that comprise the family of matrix metalloproteinases (MMPs), enzymes capable of degrading the extracellular matrix components. These proteinase families include the collagenases, gelatinases, stromelysins, matrilysins and membrane-type MMPs (Figure 2.47) [1,2].

Native collagen is resistant to non-specific proteolytic degradation in physiological situations due to the fact that the triple helical conformation is not readily degradable by general proteases. However, collagenases have the ability to degrade collagen triple helix at physiological pH and temperature. The vertebrate collagenase was initially isolated from tadpole tails which, when cultured upon reconstituted type I native collagen substrate, exercise proteolytic activity. Similar techniques were subsequently employed to demonstrate the presence of collagenase in human skin. Interstitial collagenase (MMP1) was initially shown to be synthesized as a proenzyme by cultured fibroblasts, and later, different cell types, including epidermal keratinocytes, were shown to express a similar or identical enzyme. The ability of interstitial collagenase to digest the type I collagen triple helix is based on its ability to specifically cleave the α1(I) chain at a particular glycine–isoleucine peptide bond, or the α2(I) chain at a glycine–leucine peptide bond. This initial cleavage results in two degradation products, three-quarters and one-quarter of the size of the original collagen molecule. These shortened triple helical fragments have a lower helix-to-coil transition temperature (T_m) than the full-length molecule. Subsequently, at temperatures below 37°C the triple helix unravels, rendering the individual polypeptides susceptible to general proteolytic

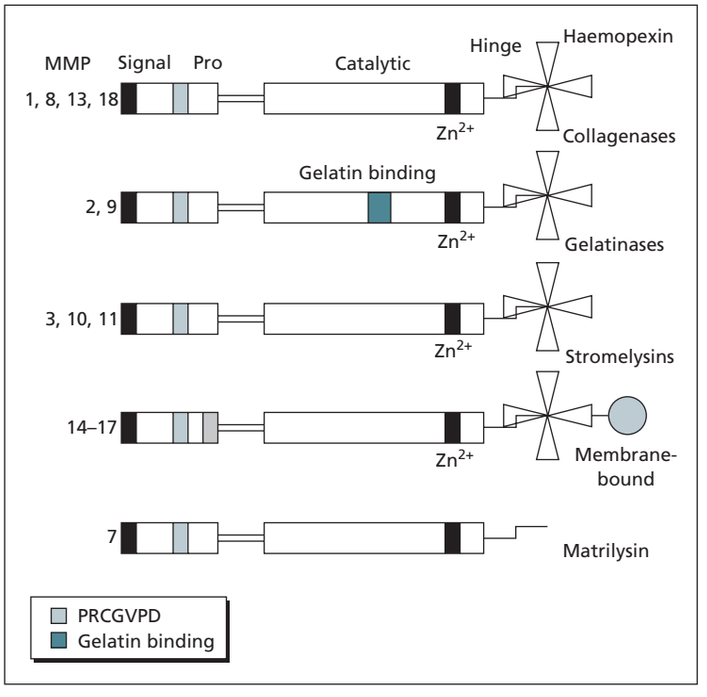


Figure 2.47 Structural organization of various matrix metalloproteinases (MMPs), divided into different subclasses. The signal, propeptide, active catalytic hinge and haemopexin regions are indicated. Note that MMP7 lacks the haemopexin region, while MMP14–17 harbour membrane-binding sequences at the carboxy-terminal end.

degradation. It should be noted that type I collagen has several additional glycine–isoleucine and glycine–leucine sequences, but these are not susceptible to collagenase degradation in this collagen when in the native triple helical conformation. A similar enzyme (MMP8) has been identified in human neutrophils with comparable degrading characteristics. The neutrophil collagenase is stored in neutrophil granules and released upon stimulation. MMP1 and MMP8 can, in addition to type I collagen, degrade a number of other collagens, including types III and VII.

Another group of extracellular proteolytic enzymes is that of the gelatinases, which are able to degrade denatured collagen (gelatin) but can also cleave certain native collagens, such as types IV, V and VII, with certain interruptions or imperfections in their collagenous triple helices, thus allowing the proteolytic cleavage at these sites. Basement membrane collagen IV can also be degraded by MMP3 (stromelysin-1) and MMP10 (stromelysin-2).

In general, MMPs are synthesized and secreted as inactive proenzymes, which become activated proteolytically by removal of the propeptide. The MMPs are zinc metalloenzymes and require calcium for their activity. Consequently, the enzymes can be inhibited by chelators of divalent cations, and, pharmacologically, tetracyclines have been suggested to inhibit MMP proteolytic activity due to their ability to bind calcium. The MMPs also have specific, small-molecular-weight peptide inhibitors, so-called tissue inhibitors of metalloproteinases (TIMPs). These proteins complex stoichiometrically with MMPs to prevent the degradative events.

In normal human skin, a number of MMPs are synthesized and secreted by fibroblasts and keratinocytes. The expression of these enzymes is activated in various pathological situations, including the invasion and metastasis of cutaneous malignancies, as well as during dermal wound healing and epidermal regeneration [2–4]. Finally, proteolytic enzymes play a pathophysiological role in tissue separation in a number of blistering diseases, such as bullous pemphigoid, dermatitis herpetiformis and epidermolysis bullosa acquisita [5].

Another metalloproteinase family has been designated as ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs). The prototype, *ADAMTS-1* gene can be induced by interleukin 1 *in vitro* or by lipopolysaccharide injection in mice, and, thus, this metalloproteinase was initially associated with inflammatory processes. Subsequently, a number of ADAMTS genes have been identified with similar domain organizations, consisting of a signal sequence, a propeptide, a metalloproteinase domain, a disintegrin-like domain, a cysteine-rich region and a variable number of thrombospondin type 1 molecules (Figure 2.48). These molecules are zinc-dependent proteases with a high level of expression in fetal tissues. ADAMTS proteases also have high levels of expression in tumour cells and tissues, including melanoma and colon carcinoma. Of particular interest in the context of collagen processing is ADAMTS-2, which serves as a procollagen I/II amino-propeptide processing enzyme.

Regulation of extracellular matrix turnover and collagen degradation during postpartum involution of the uterus has been attributed to relaxin, a hormone initially implicated in pregnancy-related conditions. More recently, it has become clear that a number of tissues, including skin, can serve as targets of relaxin. These tissues contain a relaxin family peptide receptor 1 (RFPR1) that mediates the relaxin effects on connective tissue metabolism, contributing to the maintenance of tissue homeostasis. The critical role of relaxin and its receptor has been illustrated by targeted mutant mice in which the absence of relaxin leads to collagen accumulation in a number of tissues, similar to systemic sclerosis [6,7].

Elastic fibres

An integral component of the dermal connective tissue is the elastic fibre network, which provides resilience and elasticity to the skin [1,2]. Elastic fibres are a relatively minor component in normal sun-protected adult skin, being less than 2–4% of the total dry weight of

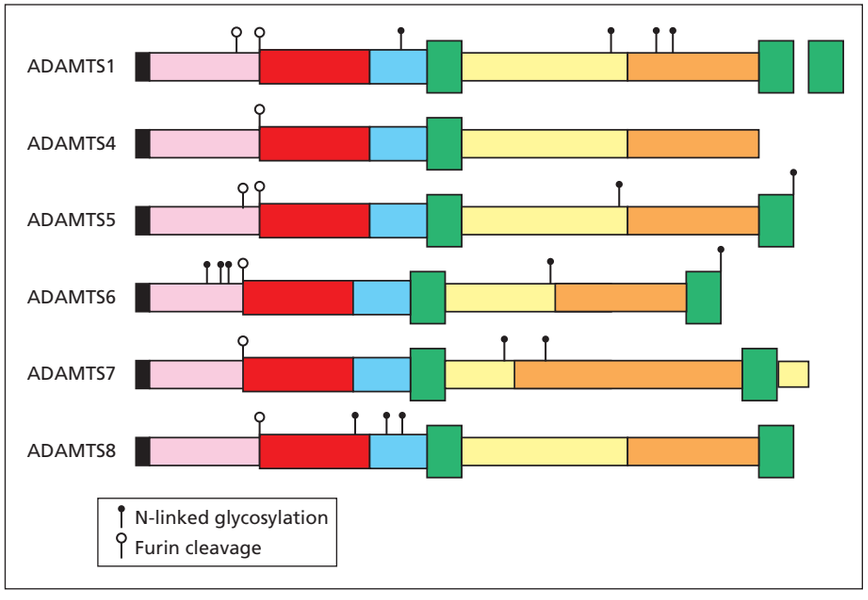


Figure 2.48 Main organization of various ADAMTS family metalloproteinases. The catalytic domain is shown in red, while other domains include the thrombospondin type I repeat sequences (green), disintegrin-like domain (blue) and cysteine domain (yellow). The signal peptidase is shown in black, the propeptide sequence is coloured pink and the spacer domain is in orange. ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs.

the dermis. The elastic fibre system in the reticular dermis consists of horizontally orientated fibres that interconnect to provide a network structure. Extending from these horizontal fibres is a network of vertical extensions of relatively fine fibrils, which consist either of bundles of microfibrils (oxytalan fibres) or of small amounts of cross-linked elastin (elaunin fibres) (Figure 2.49).

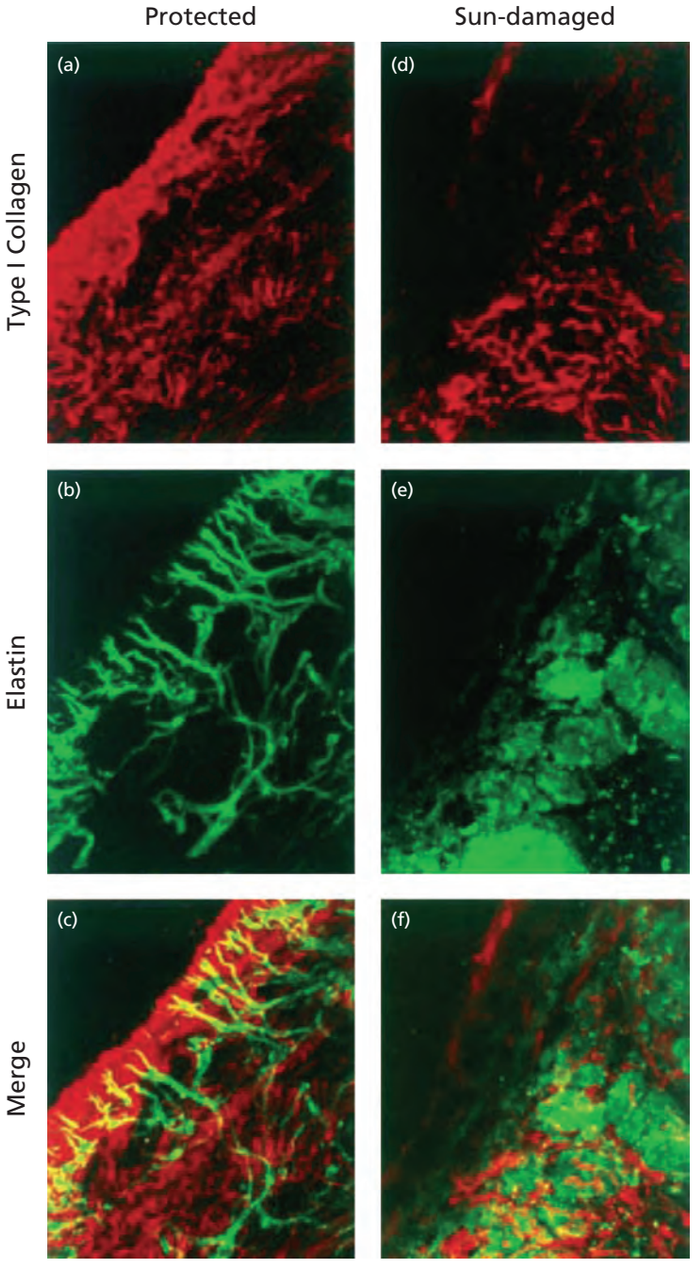


Figure 2.49 Immunofluorescence staining of type I collagen (a,d) and the elastic fibre network (b,e) in the dermis of human skin visualized by confocal laser scanning microscopy. Merging of the images (c,f) reveals that the elastic fibres assume a horizontal orientation in the mid-dermis while vertical extensions (oxytalan and elaunin fibres) reach the upper dermis, terminating just below the dermal–epidermal junction. Note that in sun-damaged skin there is a dramatic decrease and disorganization of both collagen and elastic fibres in comparison with sun-protected skin. (Adapted from Uitto and Bernstein 1998 [3].)

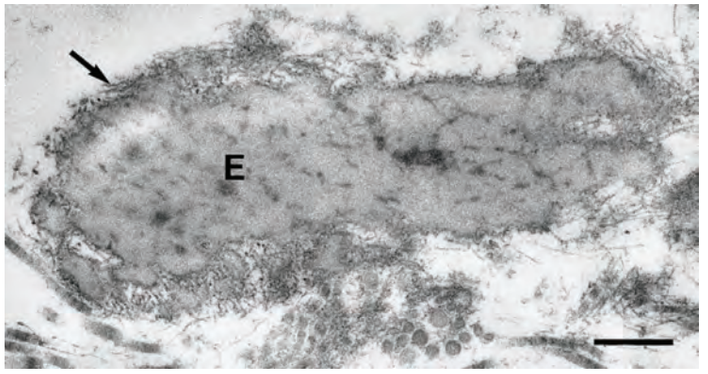


Figure 2.50 Transmission electron microscopy of an elastic fibre in the reticular dermis. The central electron-pale core consists of elastin (E), while the electron-dense areas represent the elastin-associated microfibrillar proteins which are particularly evident at the periphery of the fibre (arrow). Scale bar 0.5 μm. (Courtesy of Professor R. A. J. Eady, St John's Institute of Dermatology, King's College London, UK.)

Elastic fibres have two principal components: (i) elastin, a well-characterized connective tissue protein that forms the core of the mature fibres; and (ii) the elastin-associated microfibrils which consist of a family of proteins, some of them less defined. Examination of mature elastic fibres by transmission electron microscopy reveals an electron-lucent core that consists of elastin (Figure 2.50). Surrounding the elastin core are distinct microfibrillar structures, which appear electron dense under routine electron microscopy staining. While elastin is the major component and the microfibrils are less conspicuous in fully mature elastic fibres, the relative proportion of these two components varies during the embryonic development of elastic fibres and/or connective tissue repair. The first elements of elastic fibres that form consist of bundles of microfibrils, which can be visualized by electron microscopy during the first trimester of gestation. These microfibrils form a scaffold, allowing alignment of the elastin molecules in parallel array so as to guide the growth of fibres with relatively uniform diameters. During the second trimester of fetal development, the elastic fibres remain immature, but with increasing fetal age maturation of the fibres occurs and the elastin component becomes more prominent. In fully developed elastic fibres, well over 90% of the total content is elastin with relatively few microfibrillar components, mostly confined to the outer surface of the fibres.

Elastin

Elastin is initially synthesized as a precursor polypeptide, 'tropoelastin', which consists of approximately 700 amino acids with a molecular mass of approximately 70 kDa [1]. The amino acid composition of tropoelastin is similar to collagen, in that about one-third of the total amino residues consist of glycine. However, glycine is not evenly distributed in elastin in every third position as it is in a typical collagenous sequence. Instead, the tropoelastin primary sequence shows domains rich in glycine, valine and proline, alternating with lysine- and alanine-rich sequences. A characteristic sequence motif in the latter setting is the presence of two lysine residues separated by two or three alanine residues (Figure 2.51).

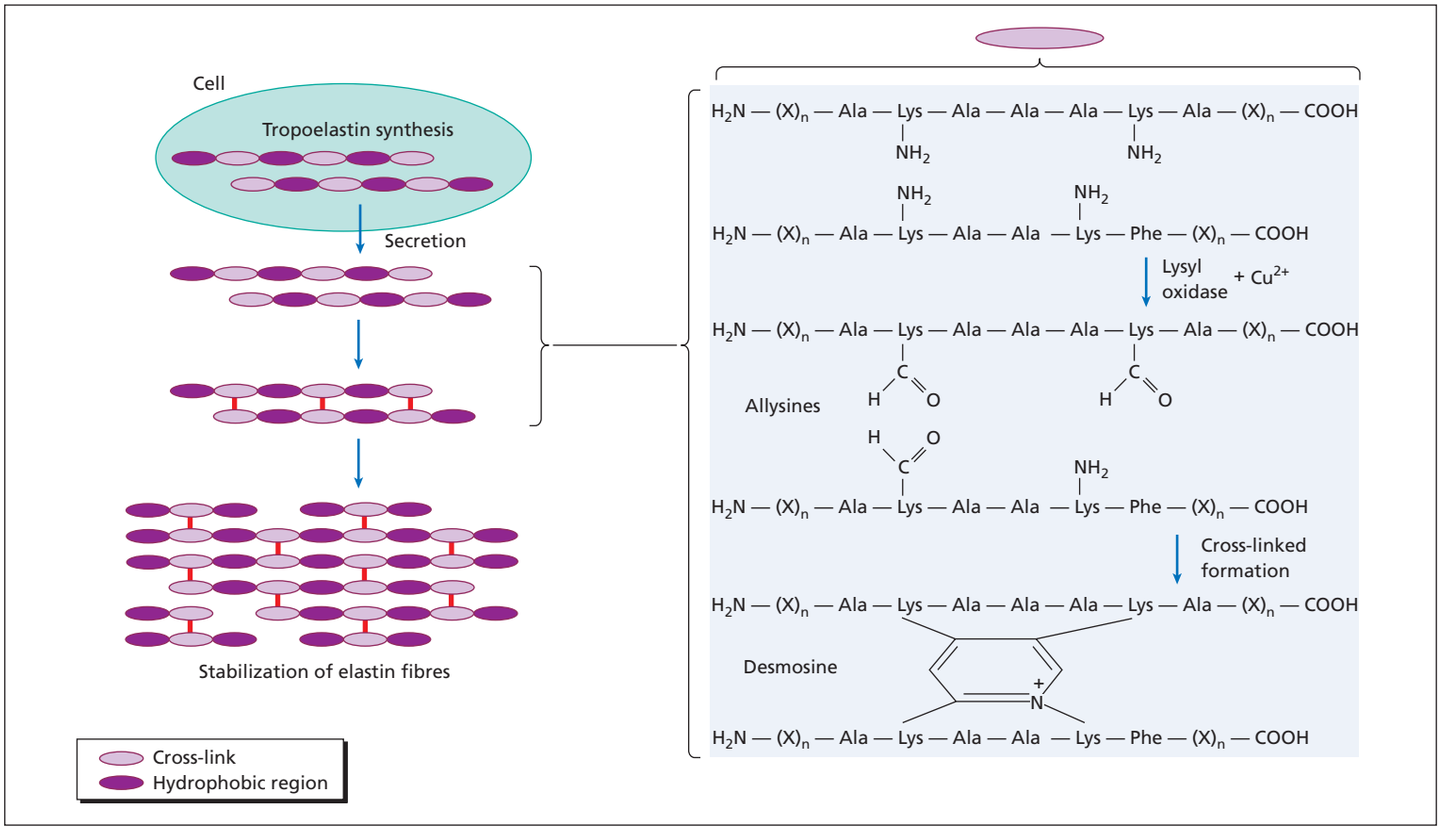


Figure 2.51 Assembly and cross-linking of elastic fibres. Newly synthesized elastin precursor polypeptides, tropoelastins, with alternating hydrophobic and cross-link regions are secreted into the extracellular milieu. Lysine residues in characteristic lys-ala-ala-lys or lys-ala-ala-ala-lys sequences in the cross-link region undergo oxidative deamination of the ε-amino groups catalysed by lysyl oxidase, an enzyme requiring copper as a co-factor. Three resulting allysine residues fuse with an unmodified lysine residue to form desmosines, elastin-specific cross-link molecules. (Adapted from Mahoney *et al.* 2009 [7].)

The lysine residues in tropoelastin are critical for the formation of covalent cross-links, desmosine and its isomer, isodesmosine, which are unique to elastin. The first step in the formation of these elastin-specific cross-links is oxidative deamination of three lysine residues to form corresponding aldehydes, so-called allysines. Three of these resulting aldehydes, together with a fourth lysine residue containing the intact unmodified ε-amino group, spontaneously fuse to form a stable desmosine compound which covalently links two of the tropoelastin polypeptides. The addition of desmosines to other parts of the molecule progressively converts tropoelastin molecules into an insoluble fibrous structure which can be stretched, but upon release the fibres recoil, providing resilience and elasticity to the skin (Figure 2.52). The content of desmosine in various elastin preparations is fairly constant, with approximately 1.5 residues per 1000 amino acids, and desmosine or isodesmosine can therefore provide a quantitative measure of the insoluble elastin content in tissues.

The human elastin gene spans approximately 45 kb of genomic DNA on chromosome 7 and consists of 34 exons corresponding to 3.5 kb of human elastin mRNA sequences. Examination of the gene structure reveals that the alternating cross-link domains, characterized by the presence of lysyl residues separated by two or three alanines, and the hydrophobic domains, are encoded by individual exons. The elastin mRNAs are synthesized in a number of cell types present in elastin-rich tissues, as for example, the vascular smooth muscle cells in arterial connective tissues. In the skin, the primary cell type responsible for elastin production appears to be the fibroblast which, under tissue culture conditions, expresses the elastin gene. Keratinocytes have also been suggested to express

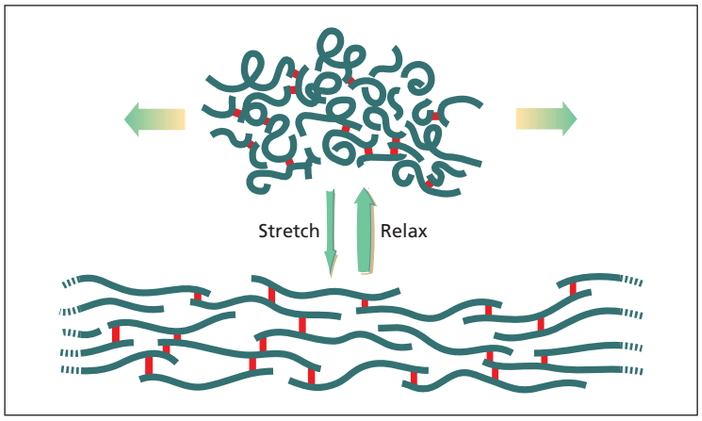


Figure 2.52 Elastic fibres cross-linked by desmosines (red). In the relaxed state, the fibres assume coiled-coil conformations. When the fibres are stretched and then released, they return to a relaxed state, the contraction providing elasticity and resilience to the skin.

the elastin gene, but the level of expression is very low in comparison with dermal fibroblasts and the potential significance of elastin in the epidermis remains unclear.

Primary mutations in the elastin gene have been demonstrated in cutis laxa, a group of diseases that manifest with loss or fragmentation of elastic fibres [2,3]. It should be noted, however, that this group of heritable diseases is highly heterogeneous, and mutations in the fibulin-4 and fibulin-5 genes have also been observed. Williams syndrome is a contiguous gene deletion syndrome that also involves the elastin gene, with clinical manifestations predominantly in the cardiovascular system [4]. Finally, cutis laxa can develop as a post-inflammatory condition, probably mediated by proteolytic enzymes released from the inflammatory cells [5].

An interesting observation during the processing of elastin mRNA precursor molecules is that they undergo extensive alternative splicing, leading to the formation of elastin molecules of varying primary sequences. In fact, at least six exons in the human elastin gene have been reported to be subject to alternative splicing, and this mechanism can provide significant variation in the primary sequence composition of elastin polypeptides, leading to different types of elastic fibres in different tissues. However, the physiological significance of the alternative splicing has not been established.

The oxidative deamination of lysyl residues to corresponding aldehydes is catalysed by a group of enzymes, lysyl oxidases, which require copper for their activity. Thus, copper deficiency can lead to reduced lysyl oxidase activity and the synthesis of elastic fibres that are not stabilized by sufficient amounts of desmosines. In such a situation, the individual tropoelastin polypeptides remain soluble and susceptible to non-specific proteolysis, and the elastin-rich tissues are fragile. Clinical manifestations of copper deficiency can vary depending on the level of copper and its circulating transport protein, caeruloplasmin, as manifested by Menkes syndrome and the occipital horn syndrome, two allelic conditions due to mutations in the copper transporter protein gene, *ATP7A* [6]. Copper deficiency can also occur in patients undergoing long-term treatment with high doses of D-penicillamine, a copper chelating agent, which can result in abnormalities in the elastic structures in the skin and other tissues.

The metabolic turnover of elastin is slow, but a portion of elastin in the body is continuously degraded, as reflected by the continuous presence of desmosines in the urine. Thus there may be an ongoing turnover and repair of elastic fibres in normal tissues. In addition, there are a number of pathological conditions in which degradation of elastin is the histopathological hallmark, such as in some forms of cutis laxa and cutaneous ageing. Elastic fibres are degraded by elastases, a group of elastolytic enzymes in different tissues and with different cleavage specificities. The classic elastases, such as those originally isolated from the pancreas, are serine-proteases, and their activity can be inhibited by serum factors such as α_1-antitrypsin and α_2-macroglobulin. In addition to these classic serine-elastases, there are a number of metalloenzymes that are capable of degrading elastic structures, particularly the microfibrillar components. These metalloelastases are present in the skin and originate from fibroblasts and monocyte–macrophages.

Elastin-associated microfibrils

Elastin-associated microfibrils consist of tubular structures of approximately 10–12 nm in diameter. Both ultrastructural evidence and biochemical analyses have confirmed that the microfibrils differ from elastin, and they may also be found in a number of tissues as individual microfibrillar structures without direct association with elastin. It is now known that elastin-associated microfibrils consist of a number of proteins, which can be divided into several different categories based on their molecular characteristics. Many of them form gene families with closely related structure and function, but clearly different from other groups in their structural features.

One of the microfibrillar protein families is that of the fibrillins, which are a critical part of the microfibrillar structure [1]. Two distinct, yet closely homologous, human genes encode fibrillin 1 (FBN1) and fibrillin 2 (FBN2), proteins characterized by multiple repeats of sequence motifs previously observed in the epidermal growth factor (EGF) precursor molecule, with each motif having six conserved cysteine residues. Electron microscopy has established that monomeric fibrillin molecules synthesized by fibroblasts show an extended flexible molecule, which is approximately 148 nm long and 2.2 nm wide. Multiple fibrillin molecules can then align in a parallel, head-to-tail fashion to form microfibrils associated with elastin in tissues, such as skin and the arterial connective tissues. It should be noted that fibrillin is also a major component of microfibrils in tissues such as the ocular ciliary zonule and the periodontal ligament, without microscopic or immunoreactive evidence of elastin. The importance of fibrillin 1 is illustrated by the fact that the mutations in the corresponding gene (*FBN1*) underlie Marfan syndrome, manifesting with skeletal abnormalities, aortic dilatations, subluxation of the lens and cutaneous laxity [2]. Fibrillin 2 (*FBN2*) mutations cause congenital contractural arachnodactyly with some similarities, but also differences, to Marfan syndrome.

The latent TGF-β binding family of proteins (LTBP) has some structural similarities with the fibrillins, including repeating EGF-like domains [3]. TGF-β, a pro-fibrotic cytokine, is secreted as a latent complex bound to LTBP. There are at least four distinct proteins in the family, with a molecular weight ranging from 125 to 310 kD. One of the putative functions of LTBP is to facilitate the secretion of TGF-β or binding of the inactive complex to the cell surface where activation takes place. However, LTBPs have also been found as free proteins associated with components of the extracellular matrix. LTBP1, a prototype of this subfamily of elastin-associated microfibrillar proteins, is clearly a component of the elastic fibres in human skin, and its levels are altered in solar elastosis.

Another family of the elastin-associated microfibrillar proteins consists of fibulins, extracellular matrix glycoproteins with characteristic calcium binding EGF-like domains. Five distinct fibulins have been characterized (fibulins 1–5; FBLN-1–5), and at least four of them have been located within the elastic fibres in different tissues [4]. In addition, FBLN-5 has been shown to bind both muscle cells and elastin, thus apparently facilitating cell–matrix interactions. The importance of FBLN-4 and FBLN-5 in skin physiology is attested by the demonstration of mutations in the corresponding

genes in patients with cutis laxa, manifesting with loose and sagging skin and loss of recoil [5].

In addition to fibrillins, LTBPs and fibulins, a number of other proteins have been shown to be associated with elastic fibres in the microfibrillar network. Several of these proteins belong to the families of microfibril-associated glycoproteins (MAGPs) or microfibril-associated proteins (MFAPs), highly acidic, relatively small molecules, some of which have been characterized in detail. Finally, interface proteins, so-called emilins, as well as lysyl oxidases critical for the cross-linking and stabilization of elastic fibre structures, have been shown to be associated with elastic fibres [6].

Proteoglycan/glycosaminoglycans

Proteoglycans form a number of subfamilies defined by a core protein to which polymers of unbranched disaccharide units, glycosaminoglycans (GAGs), are linked by an O-linkage to serine residues (Figure 2.53). There are a number of distinct core proteins, the number of attached disaccharides varies, and the molecular mass of GAGs is highly variable. Commensurate with variability in structure, different proteoglycans are of different functional importance as critical components of cell membranes and the extracellular matrix of the skin during development, homeostasis and disease [1].

GAGs are highly charged polyanionic molecules that attach to the core protein. The characteristic feature of GAGs is their primary structure, consisting of alternating pairs of different monosaccharides, glucose or galactose, joined in 1–3 or 1–4 linkage (Figure 2.54). After the initial synthesis of GAGs, the polymers undergo complex post-assembly modifications catalysed by specific enzymes. Sulphatases catalyse replacement of *N*-acetyl by *N*-sulphate and epimerases convert D-glucuronic to L-iduronic acid. The linear GAG chains, consisting of linked disaccharide units, are highly variable in size, ranging from just a few to several thousands. Consequently, the molecular mass of naturally occurring GAGs can range from 5×10^3 to 5×10^7 Da.

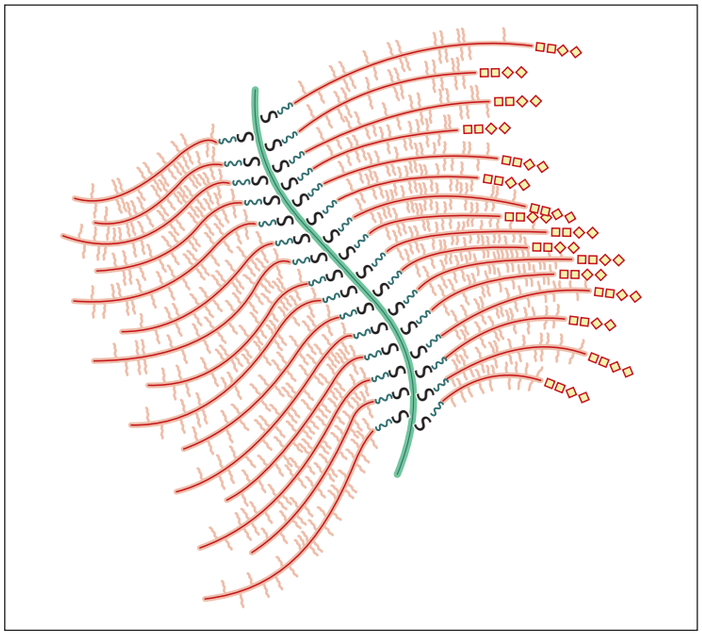


Figure 2.53 Prototypic proteoglycan in which the central core (green) is hyaluronic and the link proteins are represented by S-shapes, joining the protein side chains and carbohydrate polymers. (Adapted from Stryer 1995 [6].)

The degree of post-assembly modifications is highly variable, and the control of this reaction depends on the specific characteristics of the GAGs, the associated core protein as well as the cell type and tissue environment (Figure 2.55). The simplest GAG, hyaluronic acid, is not sulphated, while other GAGs show sulphation of varying degrees. Within the individual GAG chains, there are regions that show either a low or high degree of sulphation, a feature that may facilitate interactions of proteoglycans and GAGs with their numerous binding partners.

The core proteins of proteoglycans have been increasingly characterized through cloning and sequencing of the corresponding genes. These genes are expressed in a number of different types of cells, including dermal fibroblasts which are the principal cell type for proteoglycan synthesis in the dermis. Newly synthesized core protein polypeptides are transferred to Golgi vesicles where the attachment of GAG chains occurs. The final product, consisting of a core protein with attached GAG chains, allows classification of the proteoglycans. It should be noted that hyaluronic acid is a GAG produced without synthesis of a core protein; instead, this macromolecule is synthesized by a complex of enzymes at the plasma membrane, with subsequent extrusion into the extracellular space.

Known core proteins with their predominant tissue distribution and associated GAG components are listed in Table 2.3. The core proteins can be intracellular, reside on the cell surface or be part of the extracellular matrix. For example, serglycin shows an intracellular core protein present in the secretory granules of haematopoietic cells, such as mast cells and eosinophils, associated with either heparan sulphate or chondroitin sulphate GAGs. This proteoglycan is found in the skin in areas infiltrated by mast cells or eosinophils, and, on subsequent release, serglycin is a major form of highly sulphated heparan sulphate GAG in the skin.

There are a number of cell surface proteoglycans that function at the interface between the plasma membranes and the extracellular matrix. The mode of attachment to the cell surface is variable. For example, the glypican family of proteoglycans is attached to the cell surface by a phospholipid anchor, while the syndecans have membrane-spanning core proteins. Syndecans and glypicans are present in a number of cells and tissues, including abundant expression in the skin. Syndecan expression varies during the development and maturation of tissues, and, for example, syndecan-1 is particularly abundant in keratinocytes. The nature of the attached GAG chains, however, changes as keratinocytes differentiate. Syndecans-1 and -4 are also induced in the dermis and granulation tissue, and it has been shown that deletion of syndecan-4 from mice greatly decreases the rate of wound repair. Furthermore, there are alterations in syndecan-1 expression as a result of malignant transformation.

The extracellular matrix contains a number of different proteoglycans as an integral component of the connective tissue meshwork. In the dermis, fibroblasts produce large proteoglycans, as exemplified by versican, consisting of a core protein with attachment sites for 12–15 GAG side chains (Figure 2.56). The GAGs in

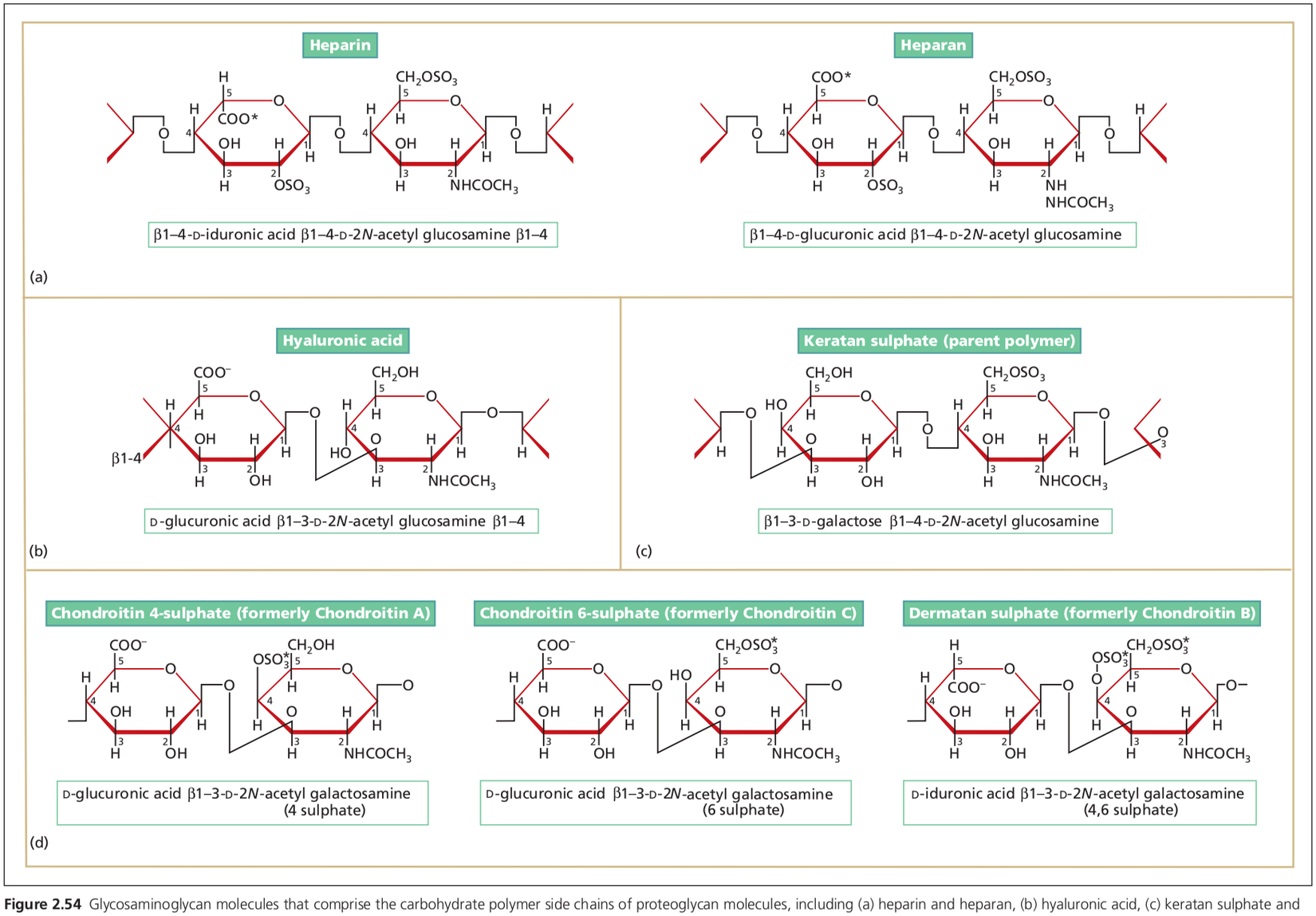


Figure 2.54 Glycosaminoglycan molecules that comprise the carbohydrate polymer side chains of proteoglycan molecules, including (a) heparin and heparan, (b) hyaluronic acid, (c) keratan sulphate and (d) various chrondroitin sulphates. Note the variants that include *O*-sulphation at the 6 position of both glucosamine and galactose.

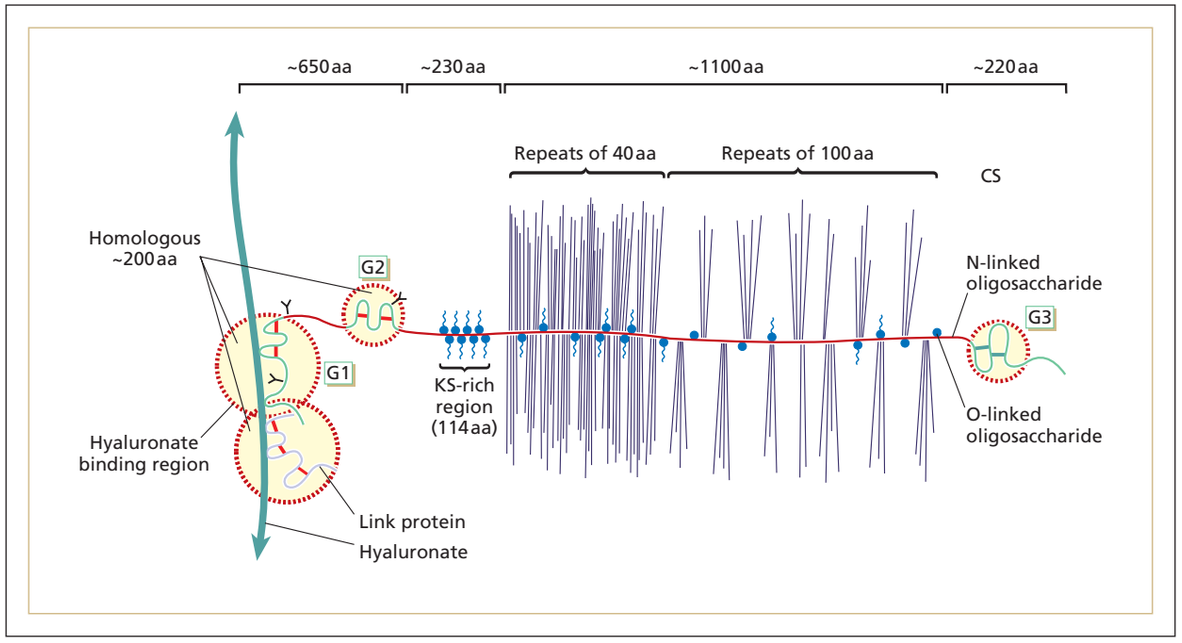


Figure 2.55 Core protein aggrecan is joined by link proteins to hyluronate, with keratan sulphate (KS, blue) and chondroitin sulphate (CS, purple) side chains. aa, amino acids. (Adapted from Heinegard and Oldberg 1993 [7].)

Table 2.3 Molecular characteristics and tissue distribution of selected proteoglycans (PG).

PG	Protein (kDa)	Glycosaminoglycan(s)	Gene location	Tissue distribution
Decorin	36	CS/DS	12q21–23	Connective tissue
Biglycan	38	CS/DS	Xq28	Cell surface
Fibromodulin	42	KS	1q32	Collagen matrix
Lumican	38	KS	12q21–22	Cornea, bowel, cartilage, muscle
Epiglycan	36	CS/DS		Epiphyseal cartilage
Versican	260–370	CS/DS (10–30)	5q13	Skin, blood vessel, cartilage, brain
Aggrecan	220	CS (100)	15q26	Cartilage, blood vessel, brain
Perlecan	400–470	HS/CS	1p36	Cartilage, bone, marrow
Agrin	210	HS (3–6)	1q32	Cell membranes, kidneys, neuromuscular
Neurican	136	CS (3–7)		Brain
Brevican	100	CS (1–3)		Brain
Testican	44	HS/CS	21	Testis

CS, chondroitin sulphate; DS, dermatan sulphate; HS, heparan sulphate; KS, keratan sulphate.

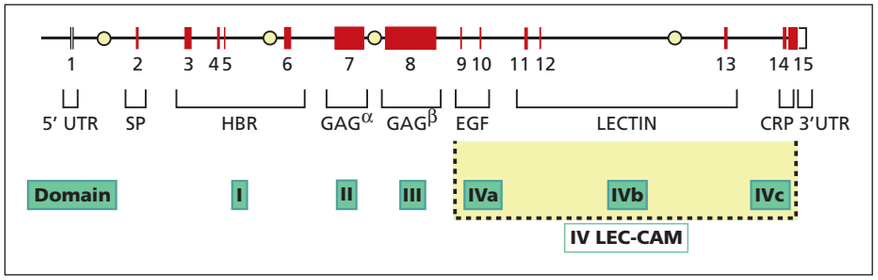


Figure 2.56 Human versican gene: intron–exon organization (top) and deduced functional domains of the encoded protein. CRP, complement regulatory protein; EGF, epidermal growth factor; GAG, glycosaminoglycan; HBR, hyaluronan-binding region; SP, signal peptide; UTR, untranslated region. (Adapted from Dours-Zimmerman and Zimmerman 1994 [8].)

versican are primarily chondroitin sulphate or dermatan sulphate, but versican can also bind hyaluronic acid, resulting in the formation of large aggregates. In the skin, versican has been identified in the dermis and epidermis as a product of fibroblasts and keratinocytes, respectively.

Extracellular matrix contains a number of small proteoglycans, exemplified by the family of leucine-rich repeat motifs. The prototype of this family is decorin, abundantly present in the skin. The decorin core protein is relatively small in size and has a single dermatan sulphate side chain covalently bound to a serine residue at the amino acid position 4 of the core protein. This proteoglycan was designated 'decorin' due to the observation that it associates with collagen and 'decorates' the fibres *in vivo*. This binding is attributed to the availability of decorin core protein to bind type I collagen, but the single GAG chain of decorin also binds to tenascin X, another extracellular component with affinity for collagen fibrils. Consequently, these interactions contribute to the connective tissue organization and architecture with functional consequences for normal skin physiology [2].

Proteoglycan–GAG complexes have a multitude of functions. For example, the proteoglycans containing heparan sulphate and dermatan sulphate have the ability to bind extracellular matrix components, including various collagens. In addition, these proteoglycans bind several growth factors, cytokines, cell adhesion molecules and growth factor-binding proteins and they can serve as antiproteases. In addition to binding to a number of extracellular molecules, proteoglycans also play a role in the adhesion of cells to the extracellular matrix. For example, syndecan-4, which is selectively enriched in dermal fibroblasts, facilitates the adherence of the cells in conjunction with other extracellular matrix-binding molecules, such as the integrins. Furthermore, the formation of focal adhesions requires heparan sulphate and subsequent activation of protein kinase C by a domain in the syndecan-4 core protein cytoplasmic tail [3].

Proteoglycans also interact with other extracellular matrix molecules besides collagen. In addition to decorin, which is known to associate primarily with type I collagen, chondroitin sulphate and dermatan sulphate bind fibronectin and laminin. The largest extracellular GAG, hyaluronic acid, plays an important role in providing physicochemical properties to the skin, mediated at least in part by its hydrophilicity and viscosity in dilute solutions. Most notably, hyaluronic acid has an expansive water-binding capacity, providing hydration to normal skin. The expression of hyaluronan is developmentally regulated in the skin, and the gene required for its synthesis, hyaluronan synthase, has been characterized. During wound healing, the physicochemical properties of hyaluronan may serve to expand the matrix and thus aid cell movement. The relatively high content of hyaluronan may also explain the finding that wounds in fetal skin heal without scarring. Other properties attributed to large proteoglycan complexes, such as those formed with versican or basement membrane proteoglycans, include their ability to serve as ionic filters, to regulate salt and water balance and to provide an elastic cushion [4].

Quantitative changes in the deposition of tissue proteoglycans have been encountered in a number of pathological processes. These include elevated hyaluronic acid synthesis in keloids and other fibrotic processes, as well as in pretibial myxoedema. In other skin conditions, including lichen myxoedematosus, systemic scleroderma and pseudoxanthoma elasticum, the lesional areas of skin have been reported to display abnormal amounts of proteoglycans. In most of these cases, the changes in proteoglycan/GAG content are secondary to an unrelated primary event. Finally, during innate cutaneous ageing in sun-protected areas of skin, the content of hyaluronic acid diminishes, possibly explaining the reduced turgor in aged skin [5].

Fibroblasts

The principal cell type responsible for the synthesis of connective tissue in the dermis is the fibroblast, which is of mesenchymal origin. The term fibroblast refers to a fully differentiated, biosynthetically active cell, while the term fibrocyte refers to an inactive cell. Biosynthetically active fibroblasts, as detected in developing or regenerating tissues, have an abundant cytoplasm, well-developed rough endoplasmic reticulum and prominent ribosomes attached to the membrane surfaces – features characteristic of cells engaged in active synthesis and secretion of extracellular matrix macromolecules (Figure 2.57). As indicated in the case of collagen, the newly synthesized polypeptides are first assembled in the cisternae of the rough endoplasmic reticulum, and the precursor polypeptides subsequently undergo extensive post-translational modifications. The polypeptides are then transferred to the Golgi vesicles and secreted to the extracellular milieu.

Human skin fibroblasts are the principal cell synthesizing collagen in the dermis. While the source of elastic fibres in the skin is less clear, fibroblasts clearly have the capacity to synthesize elastic tissues *in vitro*, and they probably are the primary source of elastin within the dermis as well. Finally, fibroblasts are the primary, if not the exclusive, cellular source of proteoglycan/glycosaminoglycan macromolecules. There is, however, considerable heterogeneity within fibroblast populations, and it has been demonstrated that

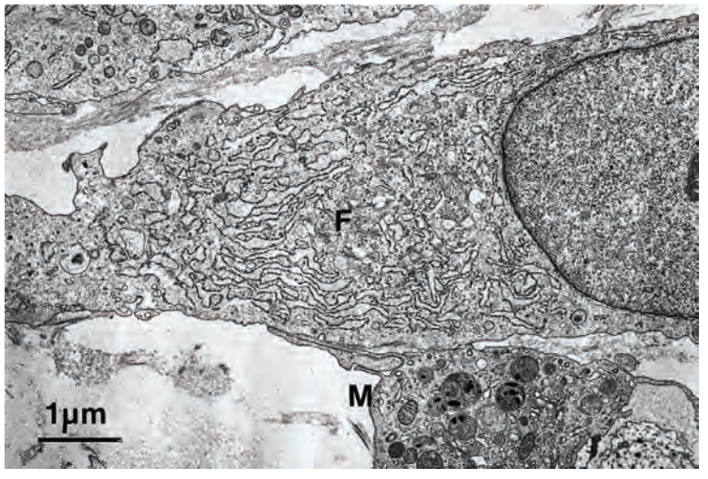


Figure 2.57 Transmission electron microscopy of an activated dermal fibroblast (F) in a healing wound. Note the prominent rough endoplasmic reticulum in the cytoplasm of this cell. There is an adjacent macrophage (M) with characteristic phagolysosomes, some of which contain ingested melanosomes. (Courtesy of Professor R. A. J. Eady, St John's Institute of Dermatology, King's College London, UK.)

the ratio of type I : III collagen synthesis or fibronectin expression in any given fibroblast population can be variable. For example, fibroblasts isolated from the papillary versus reticular dermis of skin have a higher rate of synthesis of type III collagen, and there can be as many as 30-fold differences in the level of fibronectin expression within individual cells [1].

The multiple functions of the stroma of vertebrate animals is dependent on the architecture of the extracellular matrix, which contains mesenchymal cells and provides a structural scaffold for blood and lymphatic vessels and nerves. Reciprocal interactions between the mesenchymal and epithelial cells are known to play a critical role in the development and morphogenesis of tissues, such as skin. More recently, the specific gene expression patterns in cultured fibroblasts derived from fetal and adult human skin at different anatomical sites have been explored [2,3]. Fibroblasts from different sites were shown to display distinct and characteristic transcriptional patterns, and groups of differentially expressed genes include some involved in extracellular matrix synthesis, lipid metabolism and cell signalling pathways that control proliferation, cell migration and fate determination. Large differences in the gene expression programmes were also related to anterior–posterior, proximal–distal and dermal versus non-dermal anatomical divisions.

Remarkably, adult fibroblasts maintain key features of *HOX* gene expression patterns established during embryogenesis, suggesting that the *HOX* genes direct topographical differentiation and retain a detailed positional memory in fibroblasts. In that sense, fibroblasts from different parts of the skin should be considered distinct, differentiated cell types. Collectively, these findings suggest that site-specific variations in fibroblast gene expression programmes are systematically related to their positional identities relative to the major anatomical axes [2,3].

While fibroblasts demonstrate certain variability in their gene expression profile, they are considered fully differentiated cells with relatively little plasticity. Recent, remarkable observations suggest, however, that fibroblasts can be induced to become pluripotent stem cells, essentially indistinguishable from embryonic stem cells [4,5]. Specifically, the transduction of cultured fibroblasts with four transcription factors, *Oct4*, *Sox2*, *Klf4* and *cMyc*, generated pluripotent stem cells, and similar protocols have been developed without the participation of the *Myc* retrovirus [6]. Furthermore, the transduction of human skin cancer cell lines with mir-302, a member of the micro-RNA family, which is highly expressed in slow-growing human embryonic cells and which quickly decreases after differentiation and proliferation, rapidly converted the cancer cells into a pluripotent state with the expression of key embryonic cell markers [7]. Collectively, development of these technologies holds promise for the reprogramming of fibroblasts in a manner that allows the development of patient- and disease-specific pluripotent stem cells for the treatment of diseases without a significant risk of immune rejection.

Micro-RNAs (miRNAs) are short, non-coding RNAs involved in the post-transcriptional regulation of gene expression, and over 500 miRNAs have been identified so far in humans [8]. Their biological importance was initially demonstrated for a role in cancer and, subsequently, they have been suggested to play a role in a number of clinical conditions. Certain miRNAs have also been suggested to contribute to skin development, for example by invoking differentiation through suppression of 'stemness' of the stem cells [9,10]. Recent results have also suggested that miRNA deregulation may be involved in the pathogenesis of psoriasis by contributing to the dysfunction of the cross-talk between resident and infiltrating cells [11].

Blood vessels and lymphatics

The arteries entering the skin form a deep plexus, the 'fascial' network, from which individual vessels rise to the border between the subcutaneous adipose tissue and the dermis to form a 'cutaneous' vessel network. These vessels then branch out towards various cutaneous appendages and provide ascending arterioles to generate a subpapillary plexus, which forms capillary loops entering the papillary dermis between the rete ridges. From these capillaries the blood is drained by venules which form intermediate plexuses. Thus, the cutaneous vasculature is rather elaborate and limited to the dermis, while the epidermis has no blood vessels (Figure 2.58) [1–3].

In addition to providing nutrients and oxygen to the skin, the vasculature plays a major role in regulating the body temperature. This is accomplished by controlling the blood flow through the capillaries in the upper dermis so that opening blood vessels allows dissipation of excess heat while constriction of blood vessels slows the blood flow to the skin and conserves the core energy. The amount of blood flowing through the superficial layers of the dermis can also be controlled by arterial–venous anastomoses, which act as shunts to short-circuit the flow.

The innermost component of the blood vessels is the endothelium, consisting of adjoining endothelial cells that surround the lumen. Arterioles are characterized by a subendothelial layer of elastic tissue (Figure 2.59), while venules generally do not have elastic tissue in their walls (Figure 2.60). The endothelium of

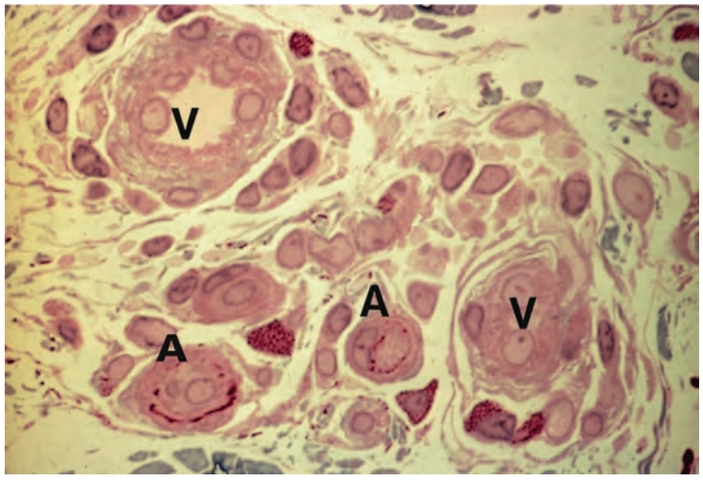


Figure 2.58 Histology of microvessels in the reticular dermis. Arterioles (A) can be distinguished from venules (V) by the presence of elastic lamina, which stains red. Surrounding mast cells can be distinguished by their prominent red/blue cytoplasmic granules. Original magnification 400× (basic fuchsin and methylene blue stain). (Courtesy of Professor R. A. J. Eady, St John's Institute of Dermatology, King's College London, UK.)

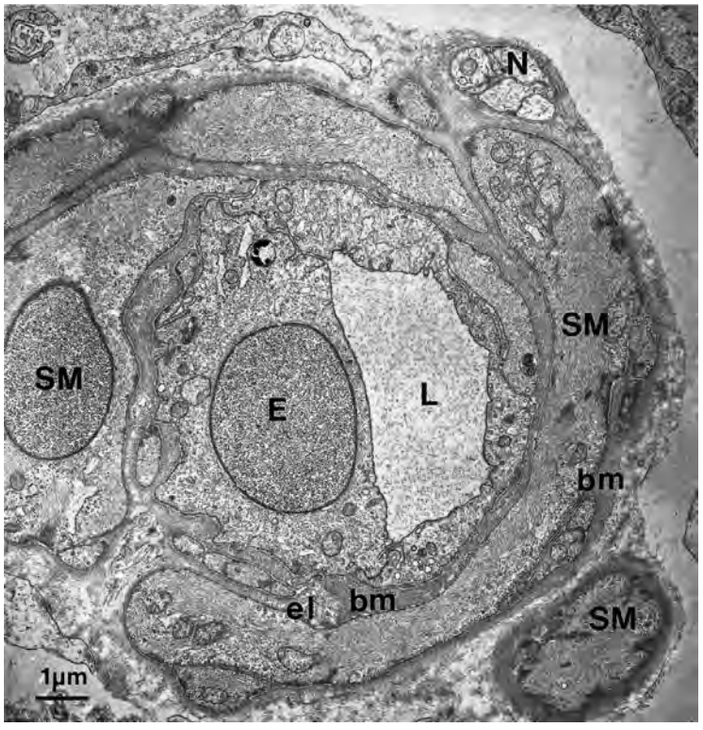


Figure 2.59 Transmission electron microscopy of a cross-section through a small arteriole in the skin. Note the relatively smooth surface of the endothelial cell (E) surrounding the lumen (L) and the presence of smooth muscle (SM) with an associated nerve (N). There is a small amount of elastic tissue (el) adjacent to the endothelial basement membrane (bm). (Courtesy of Professor R. A. J. Eady, St John's Institute of Dermatology, King's College London, UK.)

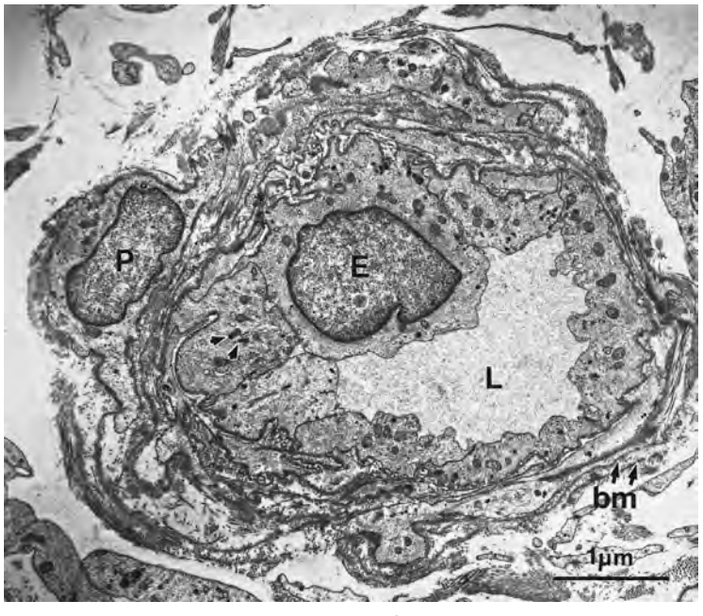


Figure 2.60 Transmission electron microscopy of a transverse section through a venule in the skin. The surface of the endothelial cells (E) in the lumen (L) is more convoluted than in its arteriolar counterpart (see Figure 2.59). The endothelial cells are surrounded by pericytes (P), and not smooth muscle cells, and the basement membrane (bm) contains dense strands (small arrows). The arrowheads indicate Weibel–Palade bodies. (Courtesy of Professor R. A. J. Eady, St John's Institute of Dermatology, King's College London, UK.)

capillaries and small arterioles and venules is surrounded by pericytes, which appear to share certain characteristics with both endothelial and smooth muscle cells. Capillaries contain a single, discontinuous layer of pericytes, whereas venules may include more than one pericyte layer in their periendothelial investment. Smooth muscle cells are found chiefly in the walls of ascending arterioles but also within the arterioles of the superficial and deep plexus and in collecting venules. Smooth muscle cells and pericytes are surrounded by a basement membrane, which also encompasses the outer surface of endothelial cells. Veil cells are long, thin cells with an attenuated cytoplasm, and they more closely resemble fibroblasts than pericytes. They do not have a basement membrane investment and are located outside the vessel wall [4].

At the ultrastructural level, endothelial cells possess many of the common cellular organelles, including rough and smooth endoplasmic reticula, mitochondria and lysosomes; micropinocytotic vesicles are also evident. Intermediate filaments containing vimentin are present and have been reported to be more abundant on the venous than on the arterial site. Dense bodies associated with actin-like filaments of 5–6 nm diameter are found in the endothelial cells of the larger arterioles, and they may have a role in endothelial contraction.

Weibel–Palade bodies are endothelium-specific inclusions that occur more frequently in the venous side of the microvasculature (Figure 2.61). They are not found in dermal lymphatics but have been reported in larger lymph vessels. Weibel–Palade bodies contain factor XIII-related antigen, von Willebrand factor and GMP-140, a protein that was first described in platelet α-granules.

A major feature distinguishing arterial from venous microvessels is the ultrastructural appearance of the basement membrane. Venules and venous capillaries have a multilaminated basement membrane, whereas arterioles possess a more homogeneous matrix, lacking the electron-dense strands. Vascular basement membrane contains laminin 111, type IV collagen, fibronectin and heparan sulphate proteoglycans. It does not contain, however,

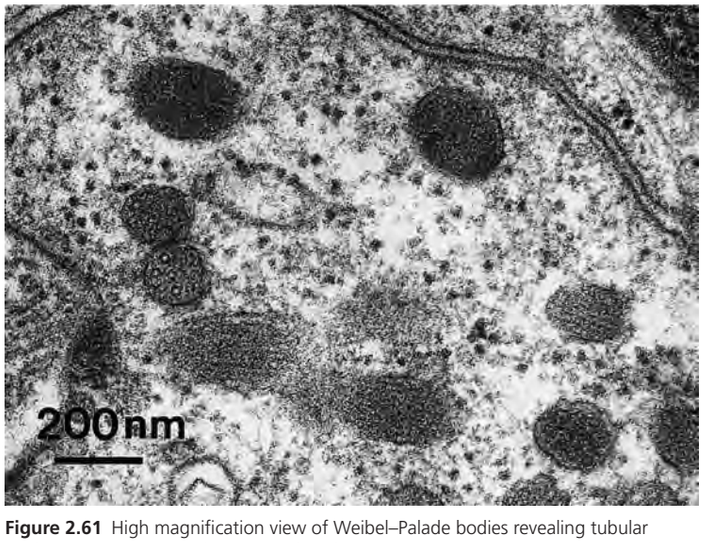


Figure 2.61 High magnification view of Weibel–Palade bodies revealing tubular profiles in cross-section. (Courtesy of Professor R. A. J. Eady, St John's Institute of Dermatology, King's College London, UK.)

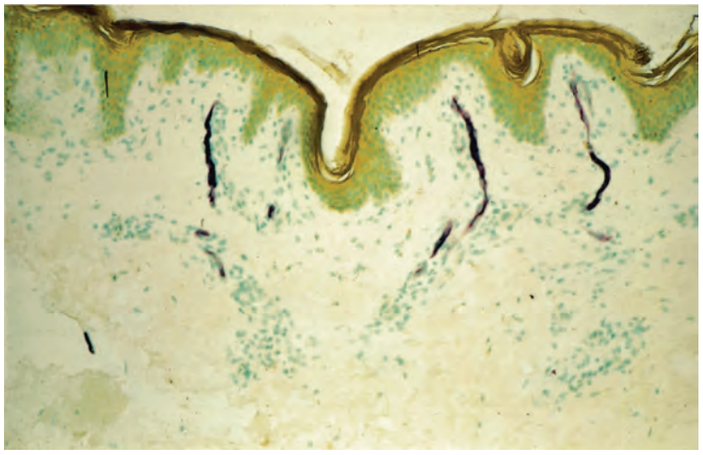


Figure 2.62 Histochemical detection of alkaline phosphatase activity indicating the presence of arterial microvessels in the superficial dermis. Original magnification 160×. (Courtesy of Professor R. A. J. Eady, St John's Institute of Dermatology, King's College London, UK.)

bullous pemphigoid antigens, type VII collagen or laminin 332, components of the epidermal basement membrane zone.

A number of endothelium-specific antigens have been recognized, and they may have a special value in studies of cutaneous pathology. Endothelial cells are the major source of angiotensin-converting enzyme as well as various cytokines and adhesion molecules. The microvasculature is also a rich source of enzymes that may be involved in cellular processes, such as endocytosis and vesicular transport. Acid phosphatase has been localized to lysosome-like structures in the endothelium, and alkaline phosphatase reactivity has been used extensively to map the distribution and arborization of the arterial network in the upper dermis (Figure 2.62).

The lymphatic network in the skin serves to transport particulate and liquid materials, such as proteins, from the extravascular compartment of the dermis. Interconnecting lymphatic spaces arise from terminal bulbs in the papillary layer and ultimately form the system that drains into the lymph nodes. The vessels have a broad lumen surrounded by a single endothelial layer, which is discontinuous in the terminal components and rests on an often discontinuous basal lamina. These processes are critical for the normal function of skin, as altered function and development of lymphatics can lead to diseases, including primary and secondary lymphoedemas [5].

Subcutaneous fat

Fat is a major component of the human body and approximately 80% of fat is in the subcutis; the rest surrounds internal organs. In non-obese males, 10–12% of body weight is fat, while in females the figure is 15–20%. Fat comprises white and brown adipose tissue. Brown fat is more common in infants and children and is characterized by different mitochondrial properties and increased heat production [1]. The function of fat is to provide insulation, mechanical cushioning and an energy store. In addition, fat may have an endocrine function, communicating with the hypothalamus via secreted molecules such as leptin to alter energy turnover in the body and to regulate appetite [2]. Adipocytes also have important signalling roles in osteogenesis and angiogenesis, and additional physical functions such as phagocytosis. Multipotent stem cells have been identified in human fat, which are capable of developing into adipocytes, osteoblasts, myoblasts and chondroblasts. Molecular biological insight into genes, proteins, hormones and other molecules that influence fat deposition and distribution are gradually being realized, both from research on rare inherited disorders (such as the lipodystrophies or obesity syndromes) as well as population studies on more common forms of obesity [3,**4**].

Physiological functions of skin

A key role of skin is to provide a mechanical barrier against the external environment [**1**]. The cornified cell envelope and the stratum corneum restrict water loss from the skin, while keratinocyte-derived endogenous antibiotics (defensins and cathelicidins) provide an innate immune defence against bacteria, viruses and fungi [2]. The epidermis also contains a network of about 2×10^9 Langerhans cells, which serve as sentinel cells whose prime function is to survey the epidermal environment and to initiate an immune response against microbial threats, although they may also contribute to immune tolerance in the skin. Melanin, which is mostly found in basal keratinocytes, also provides some protection against DNA damage from ultraviolet radiation.

An important function of skin is thermoregulation. Vasodilatation or vasoconstriction of the blood vessels in the deep or superficial plexuses helps regulate heat loss. Eccrine sweat glands are found at all skin sites and are present in densities of 100–600/cm^2; they play a role in heat control and produce approximately 1 litre of sweat per hour during moderate exercise [3]. Secretions from apocrine sweat glands contribute to body odour (pheromones). Skin lubrication and waterproofing is provided by sebum secreted from sebaceous glands.

Subcutaneous fat has important roles in cushioning trauma as well as providing insulation and a calorie reserve. In non-obese subjects, about 80% of the body's total fat is found in subcutaneous tissue. Fat also has an endocrine function, releasing the hormone leptin, which acts on the hypothalamus to regulate hunger and energy metabolism. Other functions of fat cells include tissue remodelling and phagocytosis [4].

Nails provide protection to the ends of the fingers and toes as well as being important in pinching and prising objects. Hair may have important social and psychological value, reflecting the notion that the appearances of human skin and its associated structures have a major impact on interpersonal relationships and personal well-being. Skin also has a key function in synthesizing various metabolic products, such as vitamin D.

There are two main kinds of human skin: glabrous skin (non-hairy skin) and hair-bearing skin. Glabrous skin is found on the palms and soles and has a grooved surface with alternating ridges and sulci giving rise to the dermatoglyphics (fingerprints). Glabrous skin has a compact stratum corneum which may be up to 10 times thicker compared with other body sites such as the flexures,

where the epidermis is at its thinnest. Glabrous skin also contains encapsulated sense organs within the dermis, as well as a lack of hair follicles and sebaceous glands. In contrast, hair-bearing skin has both hair follicles and sebaceous glands but lacks encapsulated sense organs [5]. Hair follicle size, structure and density can vary between different body sites; the scalp has large hair follicles that may extend into the subcutaneous fat whereas the forehead has only small, vellus hair-producing follicles although sebaceous glands are large. The number of hair follicles remains unchanged until middle life but there is a changing balance between vellus and terminal hairs throughout life. In certain hair-bearing sites, such as the axilla, there are apocrine glands in addition to the eccrine sweat glands. Sebaceous glands are actively functioning in the newborn, and from puberty onwards, and the relative activity modifies the composition of the skin surface lipids. The structure of the dermal–epidermal junction also shows regional variations in the number of hemidesmosomal-anchoring filament complexes that exist (more in the leg than the arm). In the dermis, the arrangement and size of elastic fibres varies from very large fibres in perianal skin to almost no fibres in the scrotum. Marked variation in the cutaneous blood supply is found between areas of distensible skin such as the eyelid and more rigid areas such as the fingertips. There are also regional differences in biomechanical properties which can affect percutaneous absorption of creams and ointments.

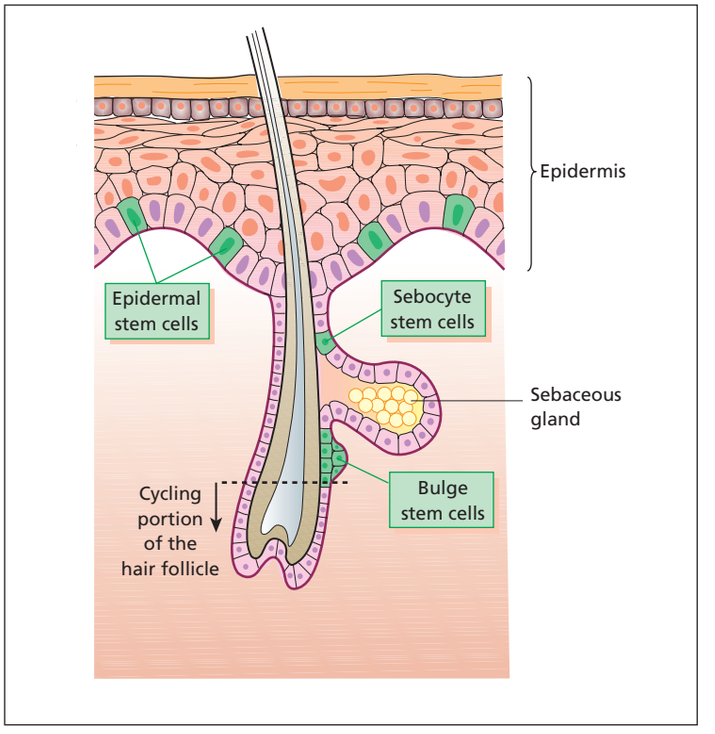


Figure 2.63 Epithelial stem cells are found within the interfollicular epidermis, the base of sebaceous glands and in the bulge area of hair follicles.

Skin homeostasis

To maintain skin homeostasis, regenerate skin appendages and repair itself after injury, the skin contains stem cells that reside in the bulge area of hair follicles, the basal layer of interfollicular epidermis and the base of sebaceous glands (Figure 2.63) [1]. These stem cells generate a proliferative progeny that can undergo differentiation. The molecular signals involved in regulating epidermal stem cell proliferation and differentiation are illustrated in Figure 2.64.

Stem cells are able to self-renew as well as give rise to differentiating cells [2]. In the epidermis, some basal cells can periodically withdraw from the cell cycle and commit to terminal differentiation. It is still not clear, however, what proportion of cells in the basal layer can function as a stem cell. One long-established theory divides basal keratinocytes into epidermal proliferation units, which comprise one self-renewing stem cell and about 10 tightly packed, transient, amplifying cells (each capable of dividing several times and then exiting the basal layer to undergo terminal differentiation) [3]. This unit gives rise to a column of larger and flatter cells that culminates in a single hexagonal surface. Stem cells within epidermal proliferation units are associated with a profile of particular chemical, molecular and biological characteristics. For example, stem cells retain labelling with injected ^{3}H-thymidine or 5-bromo-2-deoxyuridine after repeated cell division. In culture, actively growing clones present after serial passaging are considered to indicate an origin from stem cells. Potential markers of interfollicular epidermal stem cells are α6 and β1 integrin as well as p63, whereas sebocyte stem cells express Blimp1. Markers of hair follicle bulge stem cells include CD34, NFATc1, vitamin D receptor, TCF3, Sox9 and Lhx2, although considerably more markers exist in this and other parts of the hair follicle, highlighting the protean nature of the stem cell population in being able to respond to the requirements of tissue homeostasis, injury or growth spurts [4].

In the epidermal proliferation unit concept of stem cell behaviour, the division of basal cells has been viewed as a symmetrical process in which equal daughter cells are generated; the basal

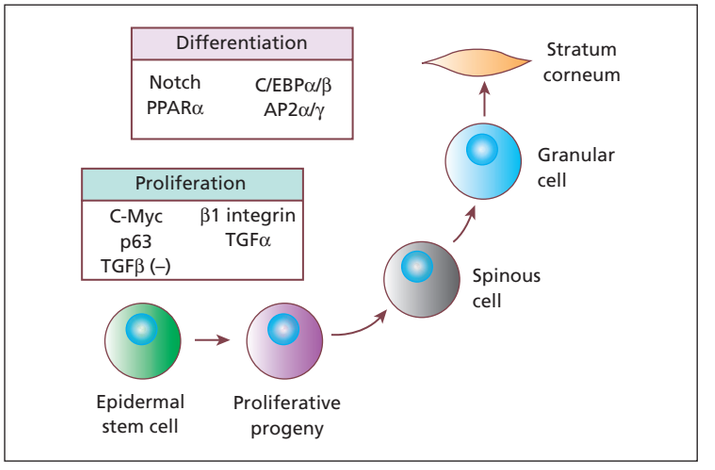


Figure 2.64 Epidermal stem cell proliferation is regulated positively by β1 integrin and transforming growth factor α (TGF-α) and negatively (–) by TGF-β signalling. The transcription factors c-Myc and p63 also promote epidermal proliferation. Notch signalling and the transcription factors peroxisome proliferator-activated receptor α (PPARα), AP2α/γ and C/EBPα/β control the differentiation of epidermal cells.

cells progressively reduce their adhesiveness to the underlying epidermal basement membrane, delaminate and commit to terminal differentiation (Figure 2.65). However, data also suggest that basal cells can also undergo asymmetrical cell division, shifting their spindle orientation from lateral to perpendicular [5]. Asymmetrical cell divisions provide a natural means of maintaining a proliferative daughter cell that retains the cell markers associated with stem cells, while the other daughter cell has reduced markers such as β1 integrin, increased expression of Notch signals, and is committed to terminal differentiation. Asymmetrical cell divisions, therefore, can bypass the need for transient amplifying cells.

The structural and biological composition of the dermal–epidermal junction also influences the proliferative properties of basal keratinocytes. Laminin 332 promotes anchorage as a ligand via α6β4 integrins in hemidesmosomes and signalling/migration via its association with α3β1 in focal adhesions. Signalling via α3β1 integrin stimulates the MAPK pathway, turnover of focal adhesions and epidermal migration. The basement membrane is also a reservoir for growth factors that can promote epidermal proliferation (e.g. TGF-α, EGFs, insulin growth factors) or restrict it (e.g. TGF-β). EGFR signalling also enhances proliferation and migration in the epidermis, possibly by phosphorylating β4 integrin and promoting hemidesmosome disassembly. Thus, the control of basal keratinocyte stem cell activity in maintaining homeostasis and responding to injury is through the regulation of at least two opposing tyrosine kinase pathways and two integrin structures.

A key transcription factor in regulating the self-renewal and long-term proliferative capacity of the stem cell is p63, a member of the p53 family of proto-oncogenes [6]. However, the precise role of p63 is not clear; it may have a direct effect on stem cell renewal, or lineage commitment, and/or an effect on switching from proliferation to terminal differentiation [6]. Notch signalling also appears to have an important gatekeeper function in the transition from basal to suprabasal cells; there is basal expression of Notch ligands such as Delta1 and suprabasal expression of Notch receptors and Notch downstream targets such as Hes1.

Stem cells in hair follicles are located in the lowest permanent part of the follicle, within the outer root sheath. These cells cycle more slowly than other cells and have the capacity to migrate (e.g. to the base of the hair follicle in follicular regeneration), as well as to differentiate into diverse lineages (e.g. outer root sheath, inner root sheath, hair shaft, sebocytes, interfollicular epidermis). Despite this multipotency, however, the follicle stem cells only function in pilosebaceous unit homeostasis and do not contribute to interfollicular epidermis unless the skin is wounded [7].

Hair follicles undergo cycles of degeneration and regeneration throughout life. During the growth phase (anagen), which requires activation of hair follicle stem cells, matrix cells proliferate rapidly but then undergo sudden apoptosis (catagen). The hair bulb and root shrivel to form an epithelial strand which forces the dermal papilla to rest at the base of the non-cycling part of the hair follicle [8]. The hairs then enter a resting phase (telogen). At a molecular level, inhibition of BMP signalling and activation of Wnt signalling converge to regulate stem cell activation. From microarray studies, *Sox9*, *Tcf3* and *Lhx2* appear to be markers of follicular stem cells whether they are quiescent or proliferative.

Apart from stem cells in the hair follicles and interfollicular epidermis, other cells in the dermis and subcutis may have stem cell properties. These include cells that have been termed skin-derived precursors, which can differentiate into both neural and mesodermal progeny [9]. In addition, a subset of dermal fibroblasts can have adipogenic, osteogenic, chondrogenic, neurogenic and hepatogenic differentiation potential [10]. Moreover, dermal fibroblasts can be reprogrammed into cells bearing an embryonic stem cell (pluripotent) phenotype by the insertion of just four key transcription factors, *Oct4*, *Sox2*, *Klf4* and *Myc* [11].

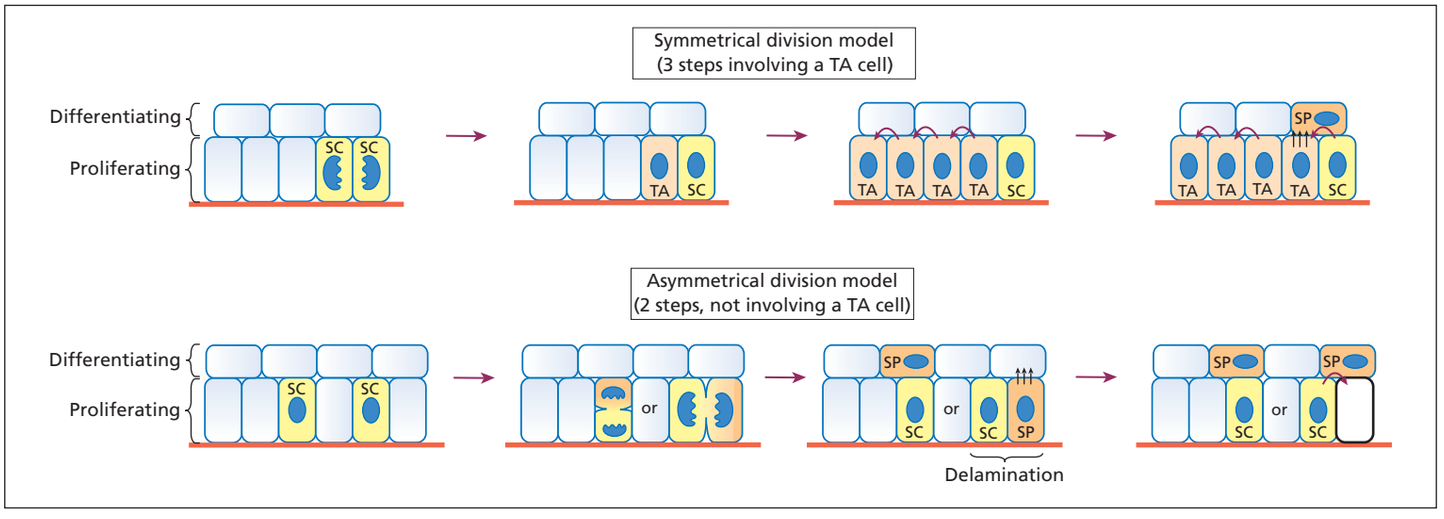


Figure 2.65 Possible mechanisms for the proliferative potential of stem cells (SC) in the basal keratinocyte layer. In the symmetrical division model, two stem cells are produced. Some of these cells in contact with the epidermal basement membrane are transient-amplifying cells (TA). These cells are capable of dividing four to five times before leaving the basal layer (delamination, black arrows) to become a spinous layer cell (SP) and entering terminal differentiation. In the asymmetrical division model, there is preferential partitioning of proliferation-associated factors into the stem cell daughter cell and, conversely, preferential partitioning of differentiation-inducing components into the daughter cell that is destined to become an SP. Depending on the orientation of the cell spindle, the daughter cell destined for differentiation can either become an SP directly or delaminate from the basal layer to enter terminal differentiation. *In vivo*, both mechanisms may exist.

Skin ageing

Skin ageing represents an inevitable physiological consequence of getting older but the impact on personal health and well-being can be significant. Considerable efforts have therefore been made to understand the biology and pathophysiology of skin ageing to try to identify new targets that might offer therapeutic intervention and prevention.

For many years, attempts have been made to define and characterize the physiological and pathological changes that occur in skin ageing [1], which is often subdivided into intrinsic (chronological) skin ageing and photoageing (sun-exposed sites). However, it is clear that this distinction is somewhat arbitrary, with light microscopy typically showing overlapping features of loss of collagen fibres, elastic fibre disruption, irregular pigmentation, a reduced number of thin hair follicles with grey hairs and a reduced number of inflammatory cells in the dermis, as well as shared age-associated physical changes such as reduced epidermal water-retaining capacity and reduced skin surface acidification (Figure 2.66) [2,3]. The often lax appearance to aged skin, however, does not reflect changes in skin water content. Indeed, the amount of water may not alter in intrinsically aged skin and may even increase in photoaged skin [4]. In skin ageing, there is an increase in the number of mast cells, mononuclear cells and neutrophils. In photoageing, the fibroblasts show a stellate phenotype and, ultrastructurally, a highly activated endoplasmic reticulum, reflecting increased biosynthetic activity [5].

Age often decreases sensory perception and increases the threshold for pain [6]. Aged skin displays a progressive disorganization or loss of some sense organs; for example, the density of Meissner corpuscles in terminal digits skin falls from over 30/mm^2 in young adults to approximately 12/mm^2 by the age of 70 years [7]. Langerhans cells become considerably reduced in number in elderly people, even in sun-protected areas [8]. There is a reduction in the number of epidermal Langerhans cells with age, coupled with a reduced ability to migrate from the epidermis in response to tumour necrosis factor α [9], although exogenous IL-1β can improve the impaired Langerhans cell migration in aged skin [10].

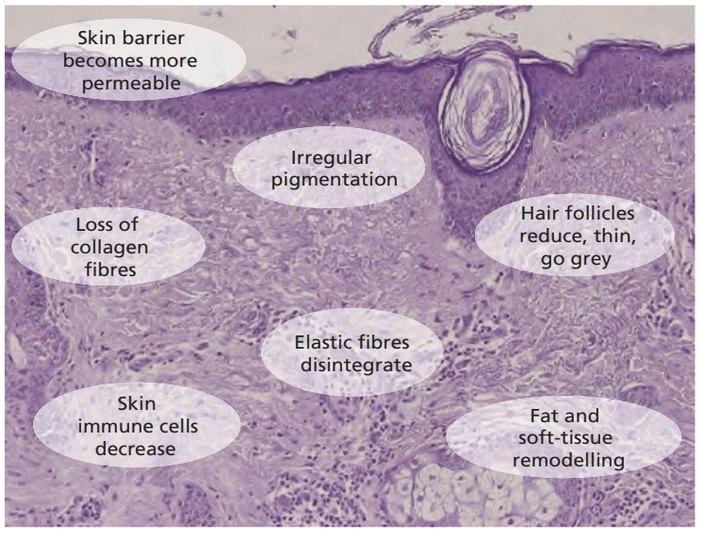


Figure 2.66 Structural and functional changes associated with skin ageing.

Peripheral immune function is altered in the elderly. The responses of both T and B cells to specific mitogens change in elderly skin, despite the fact that the absolute numbers of T and B cells do not alter significantly [11]. Nevertheless, a decreased intensity in delayed hypersensitivity reactions [11,12], an increased risk of photocarcinogenesis, and a greater susceptibility to chronic skin infections are all consequences of the ageing of the (skin) immune system [11,12].

Additional contributors to ageing may include endocrine factors, nutritional or calorie intake [13], mechanical stress/tissue tension or inflammation [14] and electromagnetic radiation [15].

Understanding why and precisely how these changes in the skin occur is a major challenge and one that is pivotal in trying to develop new and effective therapeutic agents that can delay or prevent the ageing skin phenotype. One approach in trying to address this has involved the comparison of young and old skin, using various chemical, physical, biological and molecular techniques, and over the last 60 years several theories have been proposed to explain ageing (in general and in the skin). In the 1950s, oxidative damage, with an accumulation of free oxygen radicals capable of damaging cell proteins, lipids and nucleic acids, was considered to be an aetiological factor [16]. Further cellular changes that promoted ageing were suggested to include the accumulation of nuclear DNA damage, misfolded proteins and increased frequency of mitochondrial DNA deletions. These are not mutually exclusive contributors and indeed chronic inflammation, both immune and non-immune mediated, has been identified as another factor that can promote ageing, in a manner linked to oxidative damage [17].

Apart from cellular damage, other factors may include telomere shortening, as ageing cells fail to express sufficient telomerase to maintain the telomere ends that prevent replicative senescence [18]. Overall, it is plausible that different types of damage occur at different rates in a population of individuals, with a variable impact (timing and extent of ageing) as thresholds for toxicity are reached. Indeed, studies on fibroblast cell senescence in culture have identified the contribution of cell stress, the accumulation of intracellular damage and the secretion of factors that can affect the behaviour of other cells in their vicinity [19]. Thus the surrounding microenvironment that bathes cells may have a direct impact on cellular ageing, tissue integrity and the ageing phenotype.

One other investigative approach in skin ageing has been to focus on differences in the severity and time course of skin ageing between different individuals. This has been done predominantly using genomics platforms facilitated by improvements in the annotation of the human genome as well as new technical advances for functional studies. The concept has been that key events in ageing might be gleaned from the analysis of cohorts of individuals who demonstrate traits such as shorter or longer than average lifespans, as well as those with phenotypically accelerated forms of ageing such as the progeria syndromes [20]. Such studies do not relate exclusively to skin ageing but, as an expression of the ageing process, skin provides a useful model to observe the downstream functional consequences of specific variations in the genome that might be implicated in ageing.

With regard to longevity, gene association studies using whole genome screening and targeted approaches have revealed certain

genes or genetic variants that may be associated with an increased lifespan [21]. For example, polymorphisms in the p16/p15 locus (INK4a/INK4b, CDKN2a/b) have been identified in several age-associated disease studies, potentially with relevance to stem cell function [22]. In addition, other studies have identified several genes associated with the insulin or insulin-like growth factor 1 signalling pathways [23]. The development of suitable animal models to study these pathways in more detail is likely to generate further insight into longevity and their relevance (or not) to the biology of tissue ageing, including skin health and either resistance or susceptibility to skin ageing.

From a reductionist point of view, rare genetic diseases, in which the pathology associated with normal ageing seems to accumulate at an accelerated rate, also have the potential to improve our understanding of the pathophysiology of normal skin ageing. These conditions include diseases such as the Werner syndrome, Hutchinson–Gilford progeria syndrome, Cockayne syndrome and trichothiodystrophy [8]. Collectively, molecular characterization of these diseases has identified particular abnormalities in enhanced DNA damage, defective DNA repair, genomic instability, susceptibility to genotoxic stress and impaired epigenetic homeostasis – processes which, when studied in specific individual disease models, demonstrate some overlap with ageing-related events [24]. For example, fibroblasts from individuals with Hutchinson–Gilford progeria syndrome show similar changes to normally aged fibroblasts with regard to increased reactive oxygen species accumulation, increased basal DNA damage, reduced proliferation and early senescence [25]. It is plausible, although not yet proven, that more common coding or non-coding variants in the genes that cause these rare monogenic syndromes (i.e. less disruptive changes than the disease-associated mutations) may confer some increased or decreased susceptibility to ageing via similar processes that occur on a grander scale in the rare genetic diseases.

With regard to photoageing, studies on the pathological changes following UV exposure have provided other insights into skin ageing mechanisms. For example, it is known that UV radiation can induce the synthesis of various destructive enzymes, specifically the MMPs [26]. Notably, there is increased expression of MMP-1, MMP-3 and MMP-9 [27]. The MMP-1 enzyme can cleave the major interstitial collagens type I and type III and these fragments can be further degraded by MMP-3 and MMP-9. It has also been shown that different individuals can have variable activity in the MMP-1 enzyme and that some of this variability may be due to functional polymorphisms within the promoter of the MMP-1 gene [28]. Of note, at the –1607 position, there is a common polymorphic variant that either gives a single G nucleotide or a GG sequence. This GG sequence creates a binding site for ETS (E26 transformation-specific) transcription factors that leads to increased activity of MMP-1 and a greater breakdown in dermal collagen.

It is possible that this single nucleotide polymorphism in MMP-1 (or others with similar functionality) might provide some insight into differential rates of skin ageing: those with the GG genotype might be prone to accelerated or more marked visible changes of skin ageing. For now, however, this possibility is merely speculative. Nevertheless, the GG genotype in the MMP-1 promoter has been shown to have a marked effect on contrasting skin phenotypes, for example, in the severity of scarring in the inherited blistering skin disorder, recessive dystrophic epidermolysis bullosa [28]. MMP-1 activity has also been shown to be greater in the skin of smokers (sun-exposed and sun-protected), a finding that may partially explain why many smokers display greater signs of skin ageing compared with age-matched non-smokers [29,30]. Nevertheless, MMP-1 is just one of c. 20 000 genes and it is clear that the analysis of single genes in isolation is not the optimal approach in trying to dissect out what contributes to a skin ageing phenotype in the general population.

An alternative approach to the study of ageing is to examine comparative gene expression, taking a more global assessment of different patterns of gene activation or repression. Transcriptome analysis has been performed in lymphocyte RNA derived from young and old subjects. This revealed that intrinsic ageing was associated with the modification of RNA processing genes and disruption of alternative splicing [31], but thus far only limited data on skin ageing have been published. Moreover, by comparing global gene expression in young skin versus old skin or UV-exposed skin versus UV-protected skin, there is the possibility of generating hypothesis-free data that also provide a distinct insight into both intrinsic ageing and photoageing.

Indeed, the analysis of gene expression in intrinsically and photoaged skin has revealed new insights into skin ageing processes – with some common changes in transcript levels and others that differ [32]. Notably, both types of ageing are associated with the reduced expression of genes involved in lipid biosynthesis and epidermal differentiation and display an increased expression of certain genes associated with inflammation and wound healing; changes in extracellular matrix are variable. Looking at processes rather than individual genes highlights pathways that are connected to proteases, matrix proteins and inflammation.

With regard to particular changes noted on transcriptome analysis, all aged skin shows alterations in lipid synthesis with significant reductions in many enzymes that are necessary for the synthesis of cholesterol, fatty acids and sphingolipids, consistent with a reduced capacity for skin barrier maintenance and repair. Aged skin also shows a decreased expression of several genes expressed in the epidermis, or hair follicles, a finding that indicates that skin ageing is not exclusively a dermal problem. Notably, a decreased expression of epidermal differentiation genes indicates that skin ageing affects epidermal integrity, keratinocyte structure, tight junctions, keratinocyte adhesion and stem cells. Although lipid metabolism and keratinocyte-associated gene expression changes may be common to intrinsic ageing and photoageing, the changes in epidermal differentiation-related genes tend to be greater in intrinsic ageing.

With regard to the extracellular matrix gene expression patterns, there is typically a markedly increased expression of elastic fibre components in photoaged skin, with reduced expression of some dermal collagens during skin ageing (particularly in intrinsically aged skin), although a paradoxical increase in some collagens can occur in both photoageing and intrinsic ageing. Aside from different changes in extracellular matrix patterns of gene expression, some similarities occur for markers of stress in aged skin that are indicative of oxidative stress. Of note, all aged skin shows a decreased capacity to detoxify hydrogen peroxide and other free radicals along with reduced antioxidant defences.

Overall, it is evident that intrinsic ageing and photoageing clearly affect many of the same biological processes. Themes common to both types of skin ageing include abnormalities in lipid biosynthesis, epidermal differentiation and oxidative stress. Contrasting differences between photoaged and intrinsically aged skin mainly involve extracellular matrix composition. Transcriptomic analysis also provides clues to biomarkers of aged skin, particularly involving abnormalities of proteases, matrix proteins and inflammation as well as structural protein pathology in both the epidermis and dermis. The challenge will be to see whether these types of data will generate new targets for intervention and a rationale for developing new agents that delay or prevent some of the pathophysiological changes noted at both an individual gene/protein level and as well as in the analysis of integrated or interactive biological pathways. An -omics approach to dissecting the pathophysiology of skin ageing is insightful at a basic science level, and at least provides a platform for further anti-ageing research and therapies, particularly to counter the visible effects of skin ageing.

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The full list of references can be found in the online version at www.rooksdermatology.com.

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CHAPTER 3

Histopathology of the Skin: General Principles

Eduardo Calonje with a contribution from Balbir S. Bhogal (Immunofluorescence techniques)

St John's Institute of Dermatology, Guy's and St Thomas' NHS Foundation Trust, London, UK

Introduction

As well as clinical examination, microscopic examination of skin tissue is probably the single most important diagnostic ancillary technique used by dermatologists in the management of patients with skin disorders. The correlation of clinical appearances with the dermatopathological findings is not only of direct benefit to individual patients, but has also led to the recognition of many new skin disorders and increased our understanding of the mechanisms of skin disease. The science and art of dermatopathology had its beginnings in early 19th century Europe with the writings of pioneers such as Simon, von Baerensprung and Gans. It is interesting that these individuals were dermatologists, and this tradition of dermatologists writing about histopathological aspects of skin disease was carried on by researchers such as P. Unna, F. Pinkus, A. Civatte, J. Darier, H. Montgomery, H. Pinkus and W. Lever, and in the last half of the 20th century by R. K. Winkelmann, E. Wilson-Jones and A. B. Ackermann. During the last 20 years, the definition of numerous new disease entities and great advances in histopathological and related techniques have led to a wealth of publications on the histopathology of skin disease. Many major reference texts are now available [**1–3**,4].

Close cooperation between the clinician and the diagnostic dermatopathologist is not only desirable, but also essential. The spectrum of skin disease, including rare genetic disorders, infectious diseases, neoplasms and a wide range of inflammatory disorders, is huge, and although in many conditions the histological features are relatively distinctive of a particular skin disorder, in others the changes may be fairly non-specific. Only by close liaison between the disciplines of clinical dermatology and histopathology can the usefulness and limitations of skin biopsy examination be appreciated. The clinician who reviews the histology of his or her own biopsies appreciates the problems of interpretation of an inadequate biopsy, a biopsy from an inappropriate or unrepresentative lesion, and the effects of artefact caused by undue trauma at the time of biopsy. The pathologist in turn can learn, for instance, that epidermal spongiosis, exocytosis of lymphocytes and mild dermal inflammatory changes are the hallmark of a spongiotic process and that a wide variety of clinical entities may show a similar histological picture. Using the term 'chronic non-specific dermatitis' to denote this picture is unhelpful and misleading. What the pathologist should do is to closely correlate the clinical picture with the histological findings and try to reach a more specific diagnosis. Even if this is not possible, the clinical often finds it very useful if a number of differential diagnoses are ruled out.

Dermatopathology as a medical specialty is a rapidly expanding discipline, as witnessed by the plethora of recent publications, the development of many national societies for dermatopathology and the many international meetings currently taking place. In the UK for more than two decades, the Diploma in Dermatopathology of the Royal College of Pathologists has assessed proficiency in dermatopathology for both pathologists and dermatologists. In Europe, a similar exam has been established by the European Union of Medical Specialists/Sections of Dermatovenereology and of Pathology [5]. This initiative has been received enthusiastically by physicians in both specialties not only throughout Europe but also worldwide. The exam has been so successful that it also takes place in Latin America under the auspices of the Ibero-Latin American Society of Dermatopathology.

Rook's Textbook of Dermatology, Ninth Edition. Edited by Christopher Griffiths, Jonathan Barker, Tanya Bleiker, Robert Chalmers and Daniel Creamer.

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Biopsy of the skin

A thorough understanding of both the indications for skin biopsy and the various biopsy techniques and their limitations is essential if the histopathologist is to provide the maximum useful information from the study of biopsy sections [6–8]. In addition to light microscopic examination of paraffin-embedded tissue, material obtained from skin biopsy may be used for a variety of investigative procedures. These include ultrastructural examination (only very seldom used nowadays), immunofluorescence studies, antigen mapping in congenital bullous disorders, immunohistochemistry, microbiological studies, tissue culture and molecular biological methods such as *in situ* hybridization (including FISH: fluorescent *in situ* hybridization) and polymerase chain reaction (PCR), mainly for immunoglobulin and T-cell-receptor gene rearrangement studies. These various investigative techniques often require specific specimens and transport conditions. The individual undertaking skin biopsy should have a clear idea, before carrying out the procedure, of the studies that are to be performed on the specimen obtained. It should be borne in mind that division of the specimen into many small portions for various techniques such as culture, direct immunofluorescence studies and light microscopy may lead to specimens being too small, too unrepresentative or too traumatized to provide useful results. It is often better to perform two biopsies or more to guarantee enough tissue to maximize the amount of information from the samples obtained.

Of the various indications for skin biopsy (Box 3.1), a diagnostic skin biopsy is frequently used to confirm a clinical diagnosis or to aid in the establishment of a diagnosis where a clinical diagnosis is not apparent. An excisional biopsy is the method of choice in treatment of skin lesions, particularly malignant neoplasms. It is less often used to remove lesions for cosmetic reasons.

Box 3.1 Indications for skin biopsy.

- Excision of epidermal or dermal neoplasm, whether benign or malignant. Clear margins are required in a malignant neoplasm
- An incisional biopsy (shave or punch biopsy) for confirmation of diagnosis of a lesion too big for removal, which will be treated by alternative methods, e.g. more complex surgery (Mohs), radiotherapy or cryotherapy. Most useful for basal cell carcinoma or *in situ* squamous cell carcinoma (Bowen disease); avoid in melanocytic lesions, particularly lesions suspected of melanoma
- An incisional biopsy of a hard-to-categorize skin eruption. Most will be inflammatory and a punch biopsy is often performed; sometimes cutaneous T-cell lymphoma is suspected and more than one biopsy may be necessary
- Fresh-tissue incisional skin biopsies for immunopathological study, especially immunofluorescence, in suspected autoimmune dermatoses, e.g. blistering disorders (perilesional skin) or lupus erythematosus (lesional skin)
- Simultaneous processing of contiguous incisional biopsies for pathology and for culture of fresh, unfixed tissue when infection is suspected. The tissue can be cultured for various organisms including mycobacteria and deep fungi or examined for protozoa or filarial worms

The type of biopsy, the selection of the site to be biopsied and the type of lesion to be biopsied, where there is a widespread eruption, are of utmost importance. Ideally, the lesion biopsied should be an early and untreated lesion and representative of the skin disorder as a whole. Avoiding secondary changes is crucial to obtain adequate information. Excoriated lesions and areas where secondary changes may obscure the primary features should be avoided. If lesions are present at all stages of evolution, such as may be seen in pityriasis lichenoides, it may be appropriate to biopsy more than one lesion. Multiple biopsies are also often helpful in conditions such as early cutaneous T-cell lymphoma, where definite histopathological diagnosis is often difficult. Normal skin should be included with a diagnostic biopsy wherever possible, and the inclusion of perilesional skin is essential when submitting biopsies for direct immunofluorescence studies. In certain conditions, such as connective tissue naevi, the changes may be subtle and comparison with normal neighbouring skin may be very helpful. When this is done the clinician should mark the area of normal skin with a stitch to facilitate comparison. It is equally important to ensure that the biopsy is deep enough. It is frustrating for the pathologist and clinician alike to receive a specimen with a request form suggesting a diagnosis of panniculitis where sections from the biopsy show only a portion of epidermis and superficial dermis. The general tendency in cases suspected of panniculitis is to perform a punch biopsy. However, not infrequently, the information obtained from this type of biopsy in the setting of panniculitis is minimal and, ideally, a large, deep incisional biopsy should be performed to obtain maximum yield. If lesions are widespread and there is a choice of biopsy sites, it is sensible to avoid areas liable to heal badly, such as areas over bony prominences and the lower limbs, and to avoid cosmetically important areas. Secondary changes in some areas of the body, such as changes of venous stasis in biopsies from the lower legs of older people, may be confusing to the inexperienced diagnostic dermatopathologist, and such sites are also best avoided.

Prior to skin biopsy, written informed consent is normally obtained from the patient, and in all but the smallest biopsies, local anaesthetic – usually 1% or 2% lidocaine (lignocaine) with or without epinephrine (adrenaline) – is injected around the biopsy site. Superficial blebs resulting from injecting local anaesthetic into the skin itself should be avoided. Injection of too much local anaesthetic into one area of the skin can cause a prominent distortion artefact in sections that are prepared from biopsy tissue. The effect of epinephrine on dermal blood vessels and mast cells has probably been overemphasized in the past. However, when biopsying conditions such as urticaria pigmentosa, it may be prudent either to avoid the biopsy site itself and inject the anaesthetic in a circle around it or to use an anaesthetic not containing epinephrine. Epinephrine and other vasoconstrictors should not be used in biopsies taken from the fingers or toes, as occasional intense vasospasm can result in tissue necrosis. Topical anaesthetic gels are available as an alternative to injections for removal of superficial skin lesions. These topical anaesthetics are particularly helpful with biopsies performed in children. The main topical anaesthetic used is a combination of lignocaine and prilocaine (EMLA: eutectic mixture of local anaesthetics). Although this topical preparation is extremely useful, it is important to know that it may cause

Table 3.1 Relative contraindications to skin biopsy.

Patient group	Comments
Infants	Although local anaesthetic creams/gels make this easier
Upper trunk	In order to avoid keloid formation
Lower legs in elderly patients	Poor healing may occur
Cardiac patients	Valvular disease means there is a potential risk of subacute bacterial endocarditis
Patients on aspirin/ anticoagulants	Can produce extensive bleeding
Melanoma (incisional) biopsy	Primary excision is preferable to allow proper evaluation and an accurate diagnosis In large lesions on acral sites and face, incisional biopsies are sometimes performed to confirm the diagnosis and to plan definitive treatment

secondary histological changes that may alter the interpretation of the biopsy. This is due to an irritant contact dermatitis and seems to be associated with the time of exposure to the medication and is more common in samples taken from patients with inflammatory dermatoses [9,10]. The histological changes consist of vacuolar change of keratinocytes in the upper layers of the epidermis, clefting at the dermal–epidermal junction with pallor of the superficial layers of the epidermis, lysis of keratinocytes and granular basophilic material, papillary dermal oedema and congestion of small superficial blood vessels.

There are circumstances when skin biopsy is best avoided unless absolutely essential (Table 3.1).

Techniques of skin biopsy (Table 3.2)

Elliptical surgical biopsy [8]

This is one of the most commonly used diagnostic skin biopsy techniques. The equipment required includes scalpel, fine-toothed forceps, needle holder, scissors and eyeless needle with suture (Figure 3.1). The use of a skin hook greatly facilitates manipulation of the biopsy specimen and avoids undue trauma. A reasonable size for an elliptical biopsy is about 5 mm, but smaller specimens may be adequate where indicated for cosmetic reasons. Small lesions may be totally excised, but a biopsy of a larger lesion should be at right angles through the margin to include adjacent normal skin. The long axis of the wound should, where possible, follow the natural crease lines of the skin. For suspicious melanocytic lesions, a simple excision with narrow margins is performed and this is followed by a wider excision on confirmation of the diagnosis. A full discussion of surgical techniques and suture materials is beyond the scope of this chapter, but there are several excellent reviews [7,8] and issues relating to dermatological surgery are discussed in Chapter 20.

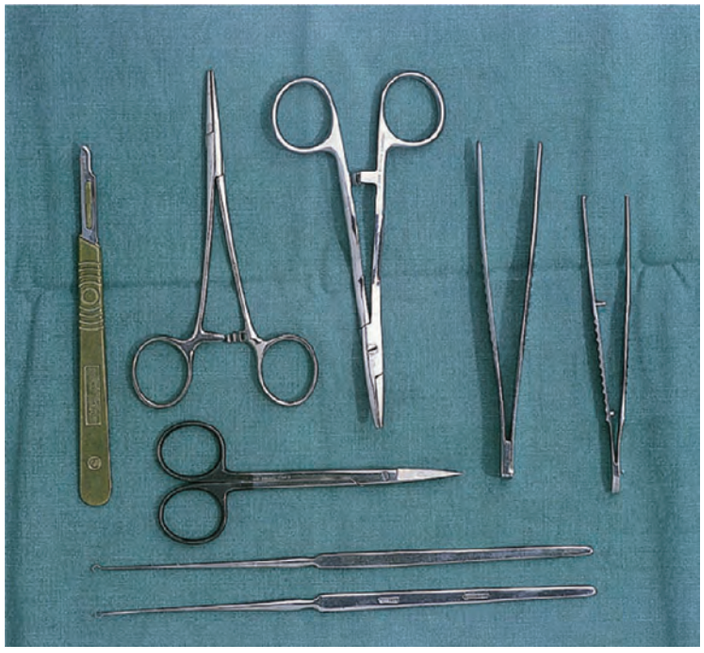


Figure 3.1 Instruments for skin biopsy, including scalpel, scissors, needle holder and skin hooks.

Table 3.2 Skin biopsy techniques [6–8].

Technique	Application
Excision	For removal of a single lesion; an elliptical or fusiform-shaped area of skin is removed
Incision biopsy ('wedge')	Similar to excision, but narrower; to include fat in suspected panniculitis; some normal perilesional skin is included for comparison
Punch biopsy (3–6 mm)	Useful if tissue available is limited, but accurate sampling is essential; a rapid procedure, sometimes useful in children
Curettage	For hyperkeratotic lesions, e.g. seborrhoeic keratoses, viral warts, basal cell carcinomas; usually accompanied by cautery
Shave biopsy	For facial protuberant lesions, e.g. benign intradermal naevi
Snip	For skin tags; skin snips also for onchocerciasis

Punch biopsy [11]

The biopsy punch is a metal cylinder of variable diameter with a sharp cutting edge at one end, usually attached to a plastic handle (Figure 3.2). The punch is pushed into the skin with a downward twisting movement, and then removed. The tissue specimen is lifted and separated from the underlying tissue, and removed from the biopsy punch. The wound may be left to heal without suturing, the base of the wound being cauterized by electrocautery or some other haemostatic agent. Many operators, however, prefer to insert one or two sutures to secure haemostasis.

Punch biopsies are convenient and quick to use, but it is preferable to use at least a 3 mm punch to obtain a satisfactory specimen. Biopsy punches blunt easily, and therefore disposable punches are recommended. Another problem with small punch biopsies is that the specimen is often difficult to orientate, and most pathologists greatly prefer elliptical excision biopsies for diagnostic interpretation. In general, a punch biopsy should not be used for the diagnosis of cutaneous tumours, and this is particularly true for melanocytic lesions. Nevertheless, punch biopsies do have a place

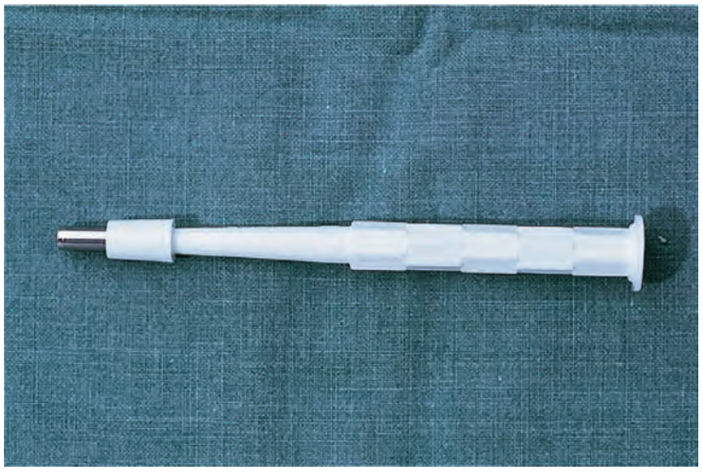


Figure 3.2 Disposable punch for cutaneous biopsy.

in busy out-patient departments and office practices, particularly for the diagnosis and management of small cutaneous lesions.

Curettage [12,13]

The technique of curettage with a sharp-edged Volkmann spoon or disposable curette followed by cautery is often used for the treatment of small benign and malignant skin lesions, such as viral warts, solar and seborrhoeic keratoses, and basal cell carcinomas. The resulting specimen is fragmented, and it is often impossible to comment on adequacy of removal. A combination of curettage and shave excision has been proposed to overcome this problem. Curettage alone is more useful as a therapeutic procedure than as a technique for providing ideal specimens for histopathological diagnosis. It must be emphasized that all specimens should be submitted for histopathological examination. Haemostasis may be achieved by electrocautery, alginate dressings or using aluminium chloride hexahydrate solution on a cotton bud rolled over the wound. It is important to mention a further haemostatic agent, Monsel's solution (ferric subsulphate) that is no longer widely used but may cause problems in the histological interpretation of re-excision specimens. The pigment coats the collagen bundles and induces a histiocytic reaction with numerous siderophages [14]. This may make the interpretation of biopsies very difficult, particularly in the setting of melanocytic lesions.

Shave biopsy

Certain superficial, benign papular or nodular lesions may be treated by shaving off the lesion flush with the surface of the surrounding skin. Many sorts of superficial skin lesion may be treated in this way, in particular melanocytic naevi. Although often a useful cosmetic result may be obtained, this technique has several drawbacks. The whole lesion is rarely removed, and recurrence of the lesion is sometimes a problem. In the case of melanocytic naevi, it is known that lesions that recur following partial excision may often demonstrate atypical histopathological features, sometimes leading to an erroneous diagnosis of a malignant lesion [15]. As with the techniques of curettage and punch biopsy, the wounds resulting from the procedure of shave biopsy normally require some form of cauterization or use of aluminium chloride hexahydrate to achieve haemostasis.

Other biopsy techniques

Various other ways of obtaining portions of skin tissue for diagnostic purposes have been described, including needle biopsy, similar to that used for tissue diagnosis of liver disease and lymph node pathology (see section on cytodiagnosis and Tzank smears). The results from such a technique are generally unsatisfactory for skin lesions, and in no way compare with those achieved using more conventional surgical procedures. Techniques for slit-skin smears for leprosy and skin snips for onchocerchiasis are discussed in Chapters 28 and 33, respectively.

Information to be provided with the specimen

For the clinician to obtain the optimum help from the pathologist, it is essential that full clinical details be provided. A fully completed histopathology request form should include the following details for each specimen. The patient should be identified by name, sex, age and usually a hospital reference number, or some other identification record number. It is useful to know the patient's racial group, as prominent epidermal basal layer melanin pigmentation may be pathological in some situations but represents normal skin for other individuals. A brief clinical history of the duration of the skin condition should be provided, together with details of any treatment including topical and systemic therapy. The site of each biopsy taken should be clearly identified on the request form, and accompanied by specimens in separate, individually labelled containers. Unfortunately, it is not uncommon to receive several biopsies from what are thought to be trivial lesions from the same patient in one container. Sometimes it turns out that one of these lesions is histologically malignant and the others are benign, and in these circumstances it may be impossible to determine the site of the malignant tumour.

Details of previous biopsies, and where possible the histopathology report reference numbers, should always be included on a request form, and finally a suggested clinical diagnosis or list of differential diagnoses is helpful. Where possible, abbreviations should be avoided. It may be that many pathologists will recognize the letters PLC as standing for pityriasis lichenoides chronica, but perhaps there would be fewer who would immediately recognize the letters TMEP as representing a form of urticaria pigmentosa.

Finally, but most importantly, the dermatologist or surgeon sending the biopsy material to the pathologist should give some clear indication on the request form from whom the biopsy is being sent, or to whom the report should be forwarded. If some of the simple advice above were more often heeded by clinicians performing biopsies, it would make the life of a dermatopathologist very much easier.

Care of the specimen

Care is required throughout the biopsy procedure to avoid trauma to the specimen with forceps or any of the other instruments used during biopsy. The use of skin hooks in manipulating the specimen during biopsy is helpful in this respect [16] and, in order to avoid trauma artefact, the division of small specimens into multiple smaller portions of tissue for different diagnostic purposes should

Table 3.3 Michel's transport medium for fresh cutaneous tissue.

Component of medium	Reagents
Ammonium sulphate	55 g in 100 mL buffer
Buffer	1 mol/L potassium citrate (pH 7), 2.5 mL
	0.1 mol/L magnesium sulphate, 5 mL
	0.1 mol/L ethyl maleimide, 5 mL
	Distilled water, 87.5 mL
	Mix 1 : 2 with 1 mol/L potassium hydroxide to pH 7.0

be avoided. If a specimen is needed for four separate studies – such as paraffin embedding, direct immunofluorescence studies, electron microscopy and microbiological culture – it is often better to take two separate specimens and divide each of these into two portions than to attempt to divide one specimen into quarters. Biopsies taken for ultrastructural studies should be small (of the order of 1–2 mm cubes) to allow for adequate fixation. Once the biopsy specimen has been taken, it should be placed epidermal side uppermost on a small portion of filter paper, to prevent curling, and transferred promptly to the appropriate transport medium.

For routine diagnostic microscopy of paraffin-embedded material, 10% neutral buffered formalin is still the most widely used fixative and is satisfactory for most purposes [17]. Many other fixatives [17] and transport media such as Michel's medium (Table 3.3) [18] are available, and these are indicated for either the study of specific diseases or tissue components, or for the application of specific diagnostic techniques such as immunofluorescence [19]. The use of Michel's medium allows the preservation of antigens for immunofluorescence studies for up to 6 months [20]. By using Michel's medium, specimens may be sent safely without the need for immediate freezing. This medium is also useful for fixation of samples used in immunoelectron microscopy studies [21] and also seems to preserve the antigens relevant for the investigation of genetic blistering skin diseases [22]. The specimen has to be washed thoroughly on receipt before freezing it to avoid artefacts. Sodium chloride 0.9% has been proposed as an alternative transport medium to Michel's medium [23]. However, preservation of antigens only lasts for 24 h and frequent artefacts, including hydropic degeneration of keratinocytes and splitting of the dermal–epidermal junction, are seen. Furthermore, this method is not suitable for samples obtained for immunoelectron microscopy or antigen mapping. Table 3.4 gives some details of the more important fixatives and transport media.

Laboratory methods

Specimen preparation

Frozen sections are not used routinely in dermatopathology, except for Mohs micrographic surgery (described in Chapter 20) and immunofluorescence. Most antibodies used in diagnostic dermatopathology work adequately in samples fixed in formalin, and except in the context of research, frozen sections are therefore not used on a regular basis for immunohistochemical studies. Frozen sections are used for the diagnosis of autoimmune blistering disorders and also for the diagnosis of blistering genetic skin diseases (antigen mapping, see Chapter 50). Although it has been advocated that autoimmune blistering disorders may be diagnosed with immunohistochemistry performed on samples fixed in formalin, the results are often of inferior quality and interpretation is difficult, leading to false negative and false positive results. This method is therefore not recommended.

Careful identification and preparation of tissue specimens prior to processing is most important. The first requirement is that the

Table 3.4 Fixatives and transport media for skin biopsy specimens.

Investigative technique	Fixative/transport medium	Comments
Most routine diagnostic studies on paraffin-processed material	10% neutral buffered formalin solution (pH 7)	Most useful fixative for general use
	10% aqueous formalin (unbuffered)	H&E staining generally better than with buffered preparation Formalin pigment a nuisance
	Carnoy's solution (pH 2.8)	Good preservation of nuclear chromatin Haemolyses red blood cells; use small specimens
Where rapid fixation required	Place specimen in the oven in alcohol	Microwave fixation
When good preservation of mucopolysaccharides is required	Formal saline solution (pH 3.8)	Nuclei badly distorted Not satisfactory for routine purposes
Demonstration of leprosy bacilli	FMA fixative or Zenker's fluid	Good results for acid-fast bacilli with Wade–Fite stain Mercury deposits must be removed
Transmission electron microscopy and some electron immunocytochemistry	Karnofsky's solution or 2.5% buffered glutaraldehyde solution Post fixation with osmium tetroxide	Small specimens required, normally no more than 1 mm cube
Immunofluorescence and immunoenzyme techniques	Various; periodate lysine paraformaldehyde fixation and cold processing useful for some endothelial cell markers	Various fixatives suitable depending on technique and antigen/substance to be identified Frozen tissue may be required
Transport of specimens for immunofluorescence studies	Michel's medium	Specimen must be thoroughly washed in phosphate-buffered saline before immersion in Michel's medium
Microbiological studies, studies on fat tissue and some immunohistological investigations	Fresh or frozen tissue	Specimens for microbiological examination should be placed in sterile containers and transported to the laboratory as soon as possible

biopsy specimen be placed immediately into a fixative solution. Various routine fixatives are employed; most contain 10% formalin. Because a stock solution of formalin consists of 40% formaldehyde, 10% formalin is really 4% formaldehyde. A minimum of 12 h fixation is recommended for most specimens, but small specimens may only need 6 h, and larger specimens a longer period of fixation, to produce optimal results. After fixation, the biopsy tissue is removed from its container, double-checking that the specimen corresponds with details given on the request form, and the pathologist produces a macroscopic description together with details of 'the cut up'.

Special care should be taken where multiple fragments of tissue are present, for example in specimens obtained by curettage. It may be necessary to pass the contents of the biopsy container through a filter paper to ensure that all relevant portions of tissue are processed and examined. After orientating the specimen, a description should be made of the size and shape of the specimen, and a note made of whether subcutaneous or other tissue is included. A careful description of surface changes is then made, with particular reference to changes of colour and surface texture, such as erosion or ulceration. Any obvious clinical lesion present on the gross specimen should be described. In some surgical specimens, an identification label, such as a skin suture, is left in place in the biopsy specimen by the surgeon to enable precise orientation of the specimen. This is particularly important when dealing with neoplastic lesions, where clearance of tumour in the margins of the biopsy specimen needs to be assessed.

Prior to sectioning a gross specimen, especially excised tumours, it is good practice to paint the margins of the biopsy specimen with coloured dyes that are resistant to tissue processing. Various commercial preparations are available, and one or several colours may be used. If the dye is visible on the final tissue section examined by the pathologist, this implies that no tissue has been lost in processing, and that the edge of the section corresponds to the genuine margin of the biopsy. After painting the biopsy margins, the gross specimen is then sectioned prior to the preparation of histological blocks. There are various techniques for cutting up a standard elliptical skin biopsy. The ideal method depends to some extent on the nature of the suspected diagnosis. A specimen from an excision biopsy of a benign or malignant skin tumour is better handled by taking transverse blocks through the specimen, so that the narrowest excision margins may be examined. For malignant lesions, particularly for suspected melanoma, ideally transverse blocks should be taken at 2 mm intervals throughout the whole length of the lesion (Figure 3.3a) [1]. An elliptical biopsy taken, for instance, from the margin of a patch of an inflammatory dermatosis, is best sectioned longitudinally, so that both normal and abnormal skin can be visualized (Figure 10.3b). With larger specimens, examination of a selection of transverse blocks made from various portions of the tumour may be adequate for diagnostic purposes. Any biopsy tissue not processed should be labelled, and returned to the fixative in the container and stored. Laboratories have different policies regarding the retention of fixed tissue specimens. Ideally, all specimens should be retained, but limitations of space often mean that most routine specimens can be discarded after a period of a few weeks after the histopathological report has been approved and signed.

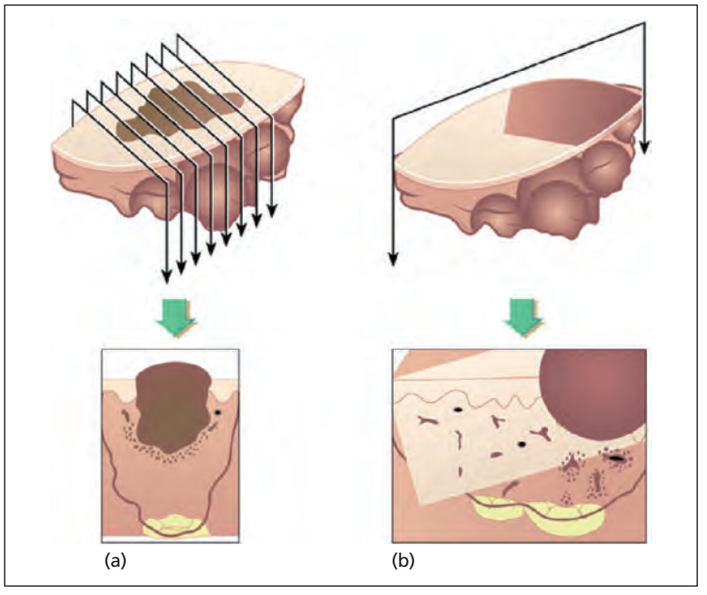
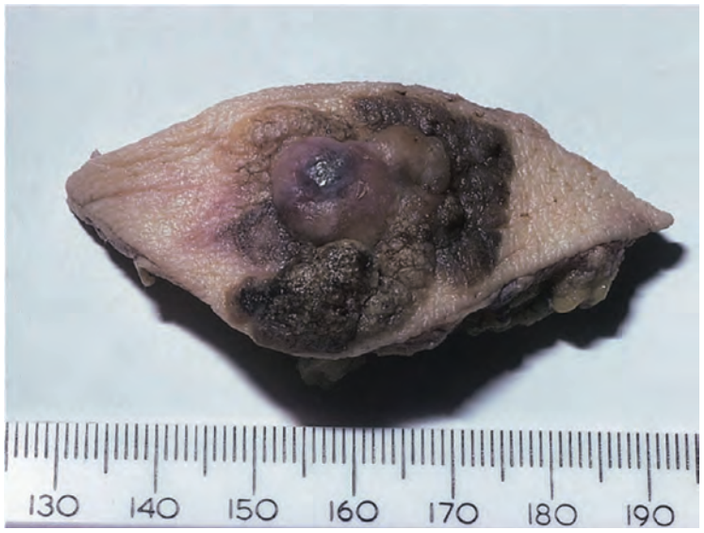


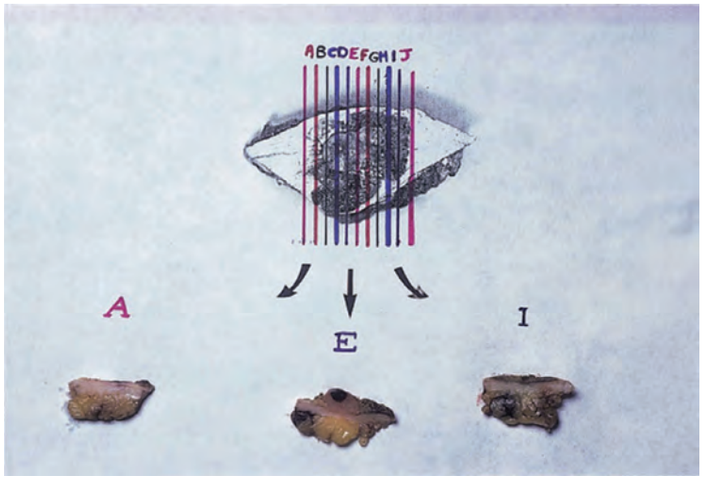
Figure 3.3 Blocking of elliptical skin biopsy specimens. (a) Neoplastic lesions. Multiple transverse blocks through the whole lesion allow for histopathological examination of the tumour at all levels, and assessment of the narrowest excision margins. (b) Incisional biopsy of inflammatory lesion. Longitudinal blocking is recommended; this allows optimal visualization of the affected and adjacent normal skin.

In addition to a written description of the gross specimen, and how it has been prepared for blocking, a rough diagram of the specimen is very useful, particularly with larger specimens. This enables clear identification of the portion of the specimen from which various blocks have been taken. An alternative approach for larger specimens, especially when dealing with neoplastic lesions, is either to photograph the specimen or to wrap the specimen in clear cling-wrap film and take a photocopy of the surface. This produces an image the same size as the gross specimen, and details of the blocks taken can be recorded directly on the photocopy image (Figure 3.4).

Very small specimens may be blocked in their entirety after trimming. Other blocking techniques may be used for specific purposes. An example is the blocking of transverse sections of cylindrical punch biopsies (at least 4 mm in diameter) of scalp disorders [2–5]. This technique is particularly useful in the assessment of various forms of inflammatory alopecia, and facilitates the quantitative morphometric analysis of pilosebaceous follicles and the hair itself. The technique also provides a useful method of studying the morphological details of the normal transverse anatomy of follicular structure, including the various phases of the normal hair cycle. Ideally, when investigating a condition presenting with alopecia, two biopsies should be provided: one for vertical sectioning and one for horizontal sectioning. It has been shown that the interpretation of both horizontal and vertical sections in scalp biopsies is better than each method on its own to obtain a high diagnostic yield [4]. If immunofluorescence studies are required, the specimen for vertical sectioning can be divided into two portions.



(a)



(b)

Figure 3.4 Photocopy procedure for recording the preparation of blocks. (a) The appearance of a macroscopic specimen of melanoma. (b) Transverse blocks are taken from the specimen, and their exact position is recorded on a photocopy made from the surface of the gross specimen.

Routine tissue processing

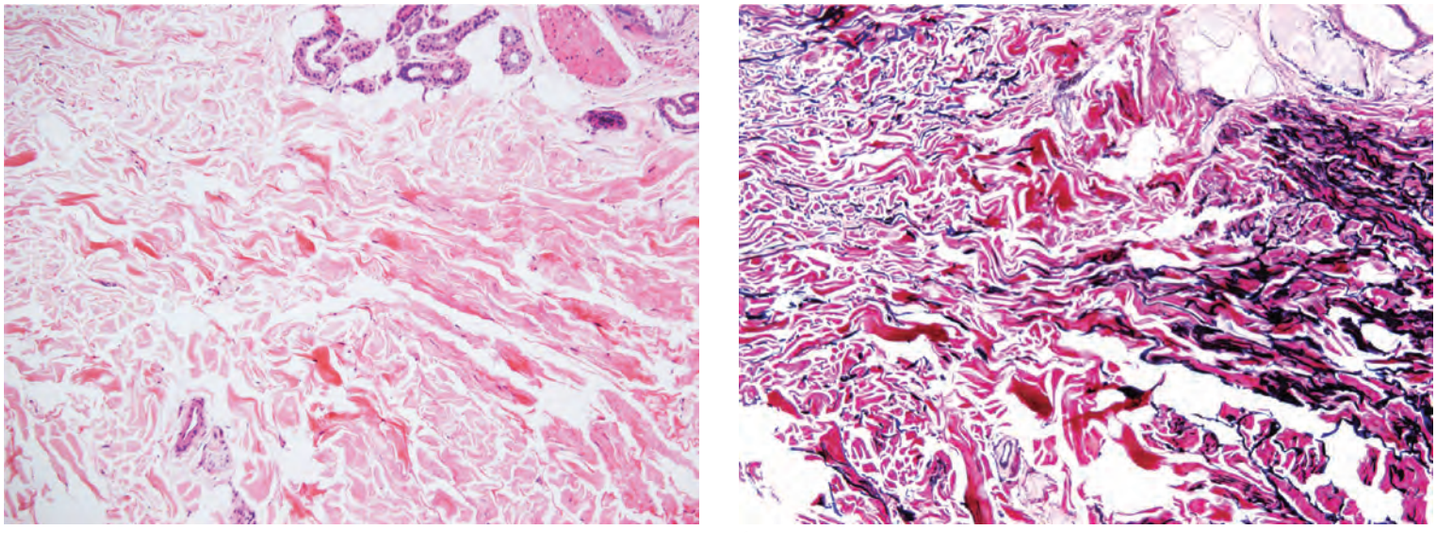
Although the skin biopsy specimen may be examined with various techniques, at least a portion of most skin biopsies is routinely processed for light microscopic evaluation of sections from paraffin-embedded tissue. In most modern histopathology departments, the tissue processing is carried out by the use of automated machines. Although it is maintained that superior results may be possible by manual means, this often involves the changing of processing fluids at inconvenient times, and is a time-consuming and labour-intensive procedure. Two main types of automated tissue-processing machine are in use: the traditional carousel type and the enclosed pumped fluid type. Both types of machine have the facility for multiple separate stages in processing. Whether tissue processing is carried out by machine or by hand, after completion of fixation the same basic steps of dehydration, clearing and embedding are involved. The process of dehydration removes aqueous fixative and any tissue water. Clearing refers to the use of a substance such as xylene, which is totally miscible with both the dehydrating agent that precedes it, and the embedding agent that follows it.

For embedding, paraffin wax at 56°C remains by far the most popular material. It is cheap, large numbers of tissue blocks may be processed in comparatively short times, and later sectioning and staining are straightforward. The use of vacuum impregnation in modern and automated tissue-processing machines considerably reduces the overall processing time. At the end of the embedding procedure, paraffin blocks are cut and stained and are then ready for microscopic examination. The dermatopathologist should be aware of relatively common potential artefacts that can cause confusion and misinterpretation (see Box 3.3) [6]. When urgent preparation of tissue specimens is required, various processing steps can be shortened. The use of very thin portions of tissue, increasing the temperature of the fixative, microwave-fixation techniques [7,8] and shortening the time used for clearing can all facilitate rapid processing. Alternatively, cryostat sections can be examined.

Routine staining techniques, including histochemistry

By far the most widely used stain for sections from skin biopsies used in histopathology laboratories is haematoxylin and eosin (H&E). This staining technique gives good definition of many cellular and tissue structures in the skin, and is sufficient for the diagnosis of most skin diseases. This stain, however, does not clearly demonstrate certain tissue components, such as elastic fibres (Figure 3.5), and does not allow differentiation between melanin, haemosiderin and other skin pigments. Special stains are required for these purposes, and also for confirming the nature of abnormal dermal deposits, such as calcium, mucin and amyloid. Another important indication for special staining techniques is the demonstration of microorganisms. Full details of the techniques and applications of special stains used in diagnostic dermatopathology are given in standard reference texts [**9**,**10**,11]. Examples of commonly used staining techniques that are useful in the diagnosis of specific conditions are given below and in Table 3.5.

The periodic acid–Schiff (PAS) stain demonstrates the presence of carbohydrates, particularly some polysaccharides such as glycogen, and mucoproteins containing neutral mucopolysaccharides. These substances are stained reddish purple by the PAS reaction. Because the cell walls of fungi and yeasts contain neutral polysaccharides, they also stain positively with the PAS reaction (Figure 3.6). This is a cheap and reliable diagnostic method and it has been advocated that a PAS stain should be routinely used in all biopsies of inflammatory dermatoses [**12**]. The reasoning for this proposal is based on the fact that the clinical and histological appearances of tinea may be fairly non-specific. The technique is also useful in demonstrating blood vessel walls, basement membrane (Figure 3.7), fibrin deposition and the presence of glycogen deposits, for instance in certain sweat gland and follicular tumours, such as clear cell hidradenoma, trichilemmoma and some epithelial lesions of uncertain histogenesis, such as the clear cell acanthoma of Degos. The identification of glycogen can be further confirmed by removal by enzyme digestion with diastase in 1% aqueous solution at 37°C for 30 min. Positive



(a) (b)

Figure 3.5 (a) Connective tissue naevus. The section stained with H&E only shows focal condensation of the collagen. (b) An elastic van Gieson stain shows marked focal increase in the number of elastic fibres.

Table 3.5 Some tinctorial stains used in dermatology.

Special stains	Tissue constituent	Appearance
Periodic acid–Schiff (PAS)	Glycogen	Magenta red (diastase sensitive)
	Mucopolysaccharides	Red (fungal wall red)
Van Gieson	Collagen	Red
	Muscle, nerve	Yellow
Congo red	Amyloid	Red with green birefringence
Acid orcein–Giemsa	Elastic fibre	Dark brown
	Collagen	Pink
	Melanin	Black
	Haemosiderin	Green/yellow
	Amyloid	Light blue
	Mast cell granules	Purple
Masson's trichrome	Collagen	Green
	Muscle + fibrin	Red
Aldehyde fuchsin	Elastic fibres	Purple
Gomori's	Reticulin	Black
Alcian blue (pH 4.5, 0.5)	Acid mucopolysaccharides	Blue
Toluidine blue	Acid mucopolysaccharides	Metachromatic purple including mast cells
Perls' Prussian blue	Iron (haemosiderin)	Blue
Masson's Fontana	Melanin	Black
Von Kossa	Calcium salts	Brown/black
Grocott's	Fungus wall	Black
Methenamine silver	Bacteria	
	Gram positive	Blue/violet
	Gram negative	Red/pink
Ziehl–Neelsen/Wade–Fite	Acid-fast bacilli	Red

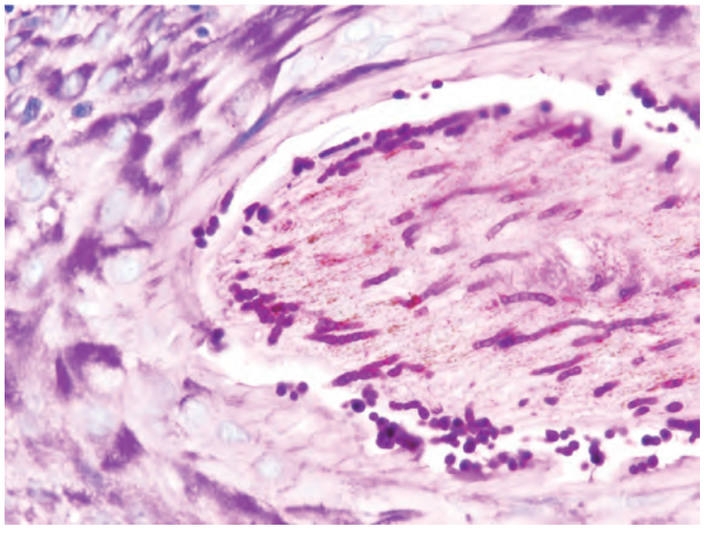


Figure 3.6 Periodic acid–Schiff stain showing numerous hyphae within the hair shaft in an endothrix infection.

staining after the use of diastase indicates the presence of neutral mucopolysaccharides.

The Alcian blue reaction produces a blue coloration in the presence of acid mucopolysaccharides. In addition to demonstrating the presence of mucin in cutaneous mucinoses, the technique is also of value in some cases of extramammary Paget disease, and occasionally in the demonstration of goblet cells in metastatic carcinoma of the gut. There are small amounts of acid mucopolysaccharide present in the ground substance of normal dermis, and the

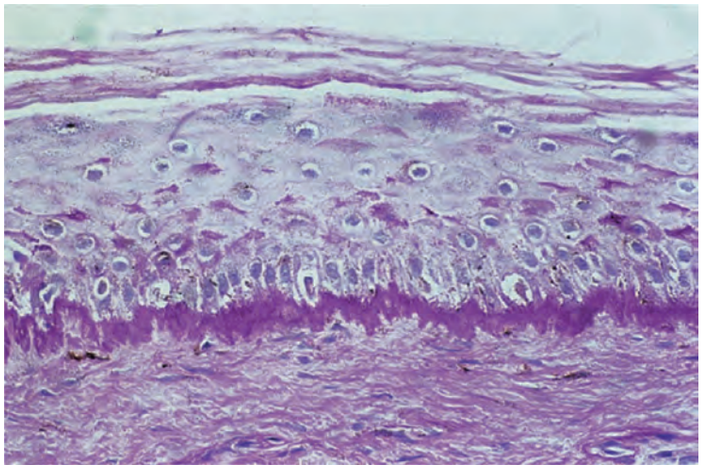


Figure 3.7 Periodic acid–Schiff stain showing thickening of the basement membrane zone in cutaneous lupus erythematosus.

Alcian blue reaction is very pH dependent. Care should therefore be exercised in the interpretation of a positive result.

Acid orcein and Giemsa stain was popularized by Pinkus and Mehregan who recommend its use as a second routine stain [13]. It is a valuable technique that demonstrates, in addition to structures normally visible with H&E stain, the presence of mast cells, eosinophils, metachromatic substances and elastic fibres. The routine use of the orcein–Giemsa stain often avoids the recutting of blocks for other special stains. Orcein itself is a constituent in other staining methods, particularly those used to demonstrate elastic fibres.

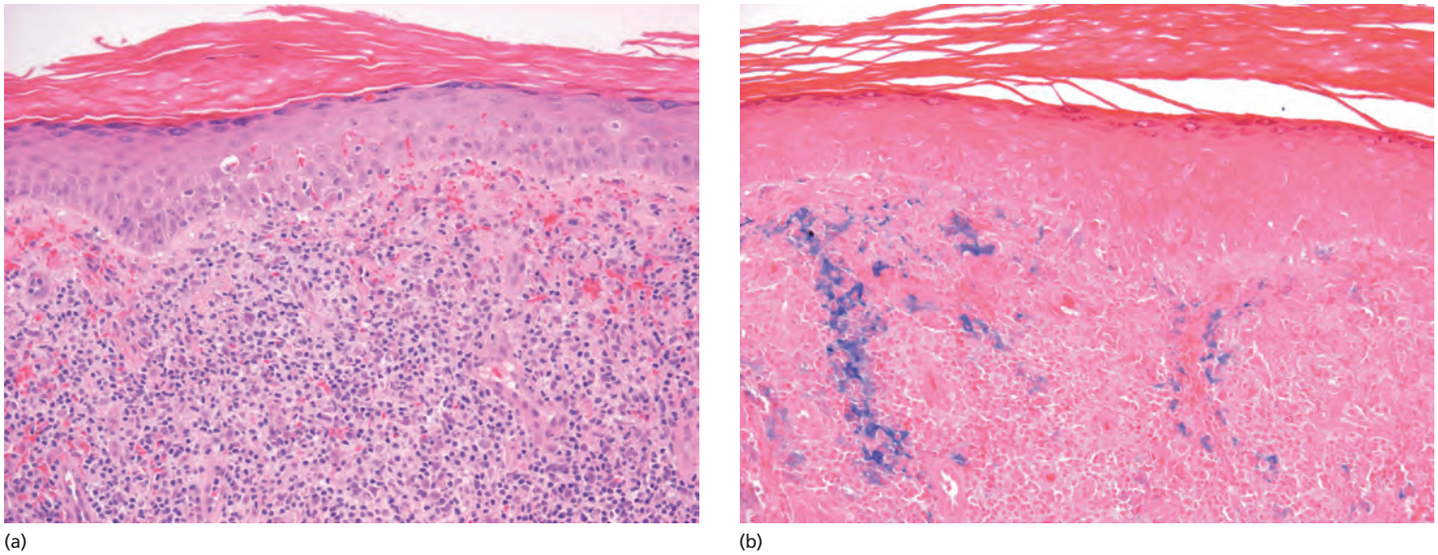
Special staining techniques are often essential to differentiate between epidermal and dermal deposits of melanin, haemosiderin and other substances. The Masson ammoniacal silver nitrate technique produces a densely black reaction product with melanin. Melanin deposits appear greenish black with the acid orcein–Giemsa stain. Iron, which in the context of skin biopsy material normally means haemosiderin pigment, is demonstrated by the Perls Prussian blue reaction, which yields a deep blue reaction product in the presence of ferric and ferrous iron (Figure 3.8).

Trichrome stains can demonstrate various elements of connective tissue. Common examples are the van Gieson stain, in which collagen appears red and muscle and nerves yellow, and the Masson trichrome stain, in which collagen is green and muscle red. The latter stain is seldom used nowadays.

Reticulin fibres are demonstrated by the Gomori silver impregnation technique. This technique is particularly useful in the study of certain cutaneous tumours, including vascular tumours and melanoma. The application of this method to melanocytic lesions may reveal foci of expansile growth undetectable in normal H&E-stained preparations.

Certain types of cell present within the skin may be difficult to recognize on conventional H&E-stained material. Mast cells are best demonstrated either by the use of a metachromatic staining technique, such as toluidine blue, or with one of the few enzyme histochemical methods that may be carried out on paraffin-embedded tissue, such as the chloroacetate esterase reaction. The use of this technique applied to formalin-fixed paraffin sections was described by Leder [14], and mast cells and myeloid white cells are easily identified by their bright pinkish red staining (Figure 3.9). Other histiocytic and dendritic cells that are difficult to visualize on routinely stained material are best visualized with immunohistochemical methods.

Cutaneous deposits of various naturally occurring and foreign substances often require special staining techniques for their demonstration. The von Kossa method produces a black coloration in the presence of calcium salts, and amyloid deposits may be demonstrated using crystal violet or Congo red. With this latter technique, amyloid deposits stain pinkish red, and under polariscopic examination there is a green birefringence. Another technique



(a) (b)

Figure 3.8 (a) A prominent lymphocytic infiltrate which is focally lichenoid and is associated with extravasation of red blood cells in a case of lichen aureus (H&E). (b) Perls' stain highlighting numerous haemosiderin-containing macrophages, a useful technique to confirm the diagnosis of lichen aureus.

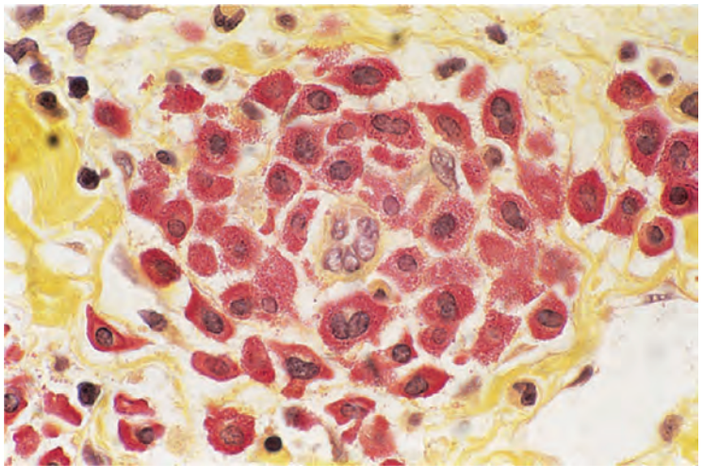


Figure 3.9 Chloroacetate esterase stain. Mast cells appear red with this technique.

useful for the demonstration of amyloid deposits is the thioflavine T method, yielding green fluorescence on examination with a fluorescence microscope. Unfortunately, several of the staining techniques recommended for the demonstration of amyloid are technically unreliable, and none is absolutely specific for amyloidosis. Some cotton dyes other than Congo red, such as pagoda red, have been claimed to be as sensitive as Congo red while being more specific [15]. It is recommended that more than one special stain be used if amyloid is suspected. Occasionally, even with the use of several special stains, amyloid cannot be demonstrated and electron microscopy is necessary for this purpose.

A wide range of techniques is employed for the demonstration of microorganisms in the skin. Reference has already been made to the usefulness of the PAS stain in the demonstration of yeasts and fungi. The silver staining technique of Grocott is also very helpful in the identification of fungal hyphae and yeast bodies. These structures stain black on a pale green background. Other silver techniques, including the Warthin–Starry technique, are useful in demonstrating spirochaetes, *Bartonella henselae* (the organism that causes bacillary angiomatosis) and *Borrelia* in tissue sections. *Bartonella* may also be demonstrated by the use of a Giemsa stain. The traditional Gram stain demonstrates coccal and bacillary organisms in the skin. Several techniques are available for the demonstration of acid-fast bacilli. The Ziehl–Neelsen method is widely used for the demonstration of mycobacteria, particularly *Mycobacterium tuberculosis.* It is based on the capacity of the lipid-rich cell wall of mycobacteria to take up strong phenol dye solutions. This dye is retained after differentiation in acid or alcohol. Mycobacteria stain magenta and the background is blue. Auramine–rhodamine stain is also a sensitive method for demonstrating mycobacteria in tissue sections. Its main drawback is that it requires the use of a fluorescence microscope for interpretation [16]. The Ziehl–Neelsen method should not be used for the demonstration of leprosy bacilli. The use of this technique often leads to a false negative result. The reason for this is that the leprosy bacilli are less acid- and alcohol-fast. The Wade–Fite stain, or a modification of this technique, is more appropriate for identification of leprosy bacilli and atypical mycobacteria because it uses minimal treatment with alcohol and acid. In fact, in cases with very few organisms, if Ziehl–Neelsen is used instead of Wade–Fite, the diagnosis may be missed.

Even with the above techniques, sensitivity remains very low and the demonstration of small numbers of microorganisms in the skin may be very difficult, and may require the examination of many sections. Thankfully, since the last decade of the last century, increasing numbers of newer tests for the detection of microorganisms in paraffin-embedded tissue have become available, simplifying the task of pathologists and dermatopathologists. These techniques include *in situ* hybridization, PCR and immunohistochemistry and are used for the detection of a wide variety of microorganisms, including bacteria, fungi, mycobacteria, viruses, rickettsia and leishmania [17–19,**20**,21–29,**30**]. PCR is a very valuable tool for confirming the diagnosis in these infections, but very stringent conditions are required to avoid false positive results, especially in the setting of mycobacterial infections. PCR is particularly useful in cases with suspicious histology in which special stains and cultures have been negative. It is important to highlight that mycobacterial DNA has been found in cutaneous lesions of nodular vasculitis and papulonecrotic tuberculid [31,32]. While this confirms the relationship between these two entities and tuberculosis, it does not necessarily mean that the finding may be interpreted as evidence of tuberculous infection in the affected sites. A polyclonal antibody for BCG is very useful as a screening technique for the presence of fungi and bacteria in tissues and this is based on the presence of wide cross-reactivity of this antibody between different species [33–35]. The antibody is, however, not useful in detecting viruses, leishmania or spirochaetes and it is unfortunately no longer commercially available. A monoclonal antibody to *Treponema pallidum* is very useful and much more sensitive than the silver technique to demonstrate organisms in tissue sections [36]. *Borrelia burgdorferi* can be identified in tissue sections by an immunohistochemical method using focus floating microscopy [37]. *In situ* hybridization for the Epstein–Barr encoding region (EBER) has been established as the method of choice for the detection of Epstein–Barr virus in paraffin embedded tissue [38]. Immunohistochemistry and *in situ* hybridization (on paraffin-embedded sections) may be used for the detection of human papillomavirus [39].

Immunopathology

There are some situations where even after careful biopsy of an appropriate lesion, expert tissue processing and sectioning, and the use of several special staining techniques, a specific histological diagnosis is still not possible. The use of immunological methods permits the identification of antigens, antibodies and various other cell and tissue components, and has greatly facilitated our ability to achieve a specific diagnosis. Hybridoma monoclonal antibody technology paved the way for the development of numerous antibodies to cell and tissue structures (Box 3.2).

Immunofluorescence methods

Immunofluorescence technique was pioneered by Coons and Kaplan [**1**]. It has been widely used both in research and clinical diagnostics. Applications include the evaluation of cells in

Box 3.2 Immunocytochemistry panels of cell markers

1 Undifferentiated malignancy panel
- MNF 116 (keratin)
- AE1/AE3 (keratin)
- CAM 5.2 (low-molecular-weight keratin)
- Epithelial membrane antigen (EMA)
- Leukocyte common antigen (LCA)
- S-100
- CD31

2 Spindle cell panel
As in 1 above plus:
- Desmin
- H-caldesmon
- Calponin
- Smooth muscle actin
- Q bend 10 (CD34)

3 Melanocyte panel
- S-100
- HMB45
- Mart-1 (Melan-A)
- MIFT-1
- Tyrosinase
- Melanoma cocktail
- SOX10

4 Small blue cell panel
- Chromogranin
- Synaptophysin
- NSE (neuron-specific enolase)
- Cam 5.2
- EMA
- CEA
- S-100
- Cytokeratin 20
- CD99
- FLI-1
- Desmin
- Myogenin
- Muscle-specific actin
- Smooth muscle actin

5 Lymphoma panel
- LCA (CD45)
- CD20, CD79a (pan B-cell markers)
- CD2, CD3, CD5, CD7, CD43 (pan T-cell markers)
- CD4 (T-helper/inducer lymphocytes)
- CD8 (T-cytotoxic/suppressor lymphocytes)
- UCHL (CD45Ro) (pan T-cell marker)
- Ber-H2 (CD30)
- κ light chain λ light chain (now also done by *in situ* hybridization)
- ALK-1
- Bcl-2
- CD10 (marker of follicle-centre cells)
- Bcl-6 (marker of follicle-centre cells)
- Ki-67 (proliferation marker)
- CD56 (natural killer cell marker)
- TIA-1 (marker of cytotoxic granules)
- Granzyme (marker of cytotoxic granules)
- Perforin (marker of cytotoxic granules)
- MUM 1
- FPOX-1
- PD1
- ICOS
- CXCL13

6 Leukaemia panel
- LCA
- Lysozyme
- Myeloperoxidase
- HLA-DR
- MT1 (CD43)
- KP1
- PGM1
- CD163
- TDT
- CD34
- CD4
- CD56

7 Langerhans cell panel
- S-100
- CD1a
- Langerin (CD207)

8 Vascular panel
- ERG
- CD31
- Q bend 10 (CD34)
- FLI-1
- LYVE-1
- Podoplanin (D2–40)

9 Mast cell markers
- CD117
- Tryptase
- Microphthalmia transcription factor

10 Immunofluorescence labelling
- IgG, IgM, IgA, C3, fibrin (much better results with frozen sections)

suspension, cultured cells, tissue, beads and microarrays for the detection of specific protein(s). In this technique, antibodies are chemically conjugated to fluorescent dyes such as fluorescein isothiocanate (FITC) or tetramethyl rhodamine isothiocyanate (TRITC). These labelled antibodies bind (directly or indirectly) to the antigen(s) of interest in cells or tissue sections, which can be visualized by fluorescence or confocal microscopy and quantified by flow cytometer, array scanner or automated imaging instrument.

In clinical immunodermatology there are two basic types of immunofluorescence staining methods: (i) direct immunofluorescence staining in which the primary antibody is labelled with fluorescence dye (Figure 3.10a); and (ii) indirect immunofluorescence staining in which a secondary antibody labelled with fluorochrome is used to recognize a primary antibody (Figure 3.10b). These techniques are simple to perform, they are both reliable and reproducible and have allowed major advances in the diagnosis of autoimmune diseases.

Accuracy in the diagnosis of bullous diseases is always important as some of these disorders can be life threatening. Although bullous diseases can have definitive clinical and histological features, a positive diagnosis is confirmed by immunofluorescence techniques. These techniques are now regarded as the 'gold standard' in the investigation and management of bullous diseases. In other conditions such as connective tissue diseases and vasculitis, they are not diagnostic but can be helpful.

Photobleaching or fading is a technical limitation of immunofluorescence. Prolonged or repeated examination reduces the intensity of emission, which also diminishes if preparations are exposed to sunlight. This fading can be reduced by the addition of *p*-phenylenediamine to the buffered glycerol mounting fluid [**2**].

Findings of immunofluorescence techniques have enabled dermatologists to classify blistering diseases and to develop new techniques such as immunoblotting, immunoelectron microscopy and enzyme-linked immunosorbent assay (ELISA). Investigating autoimmune diseases by these techniques continues and is likely to lead to further advances in our understanding of their pathogenesis.

Using these techniques, the antigen recognized by autoantibodies in autoimmune blistering diseases have been identified and characterized. Their respective genes have been cloned and used to produce recombinant proteins, which can be used to study the functional aspect of these molecules. The collective information obtained from the findings of these techniques is extremely valuable to practicing dermatologists.