

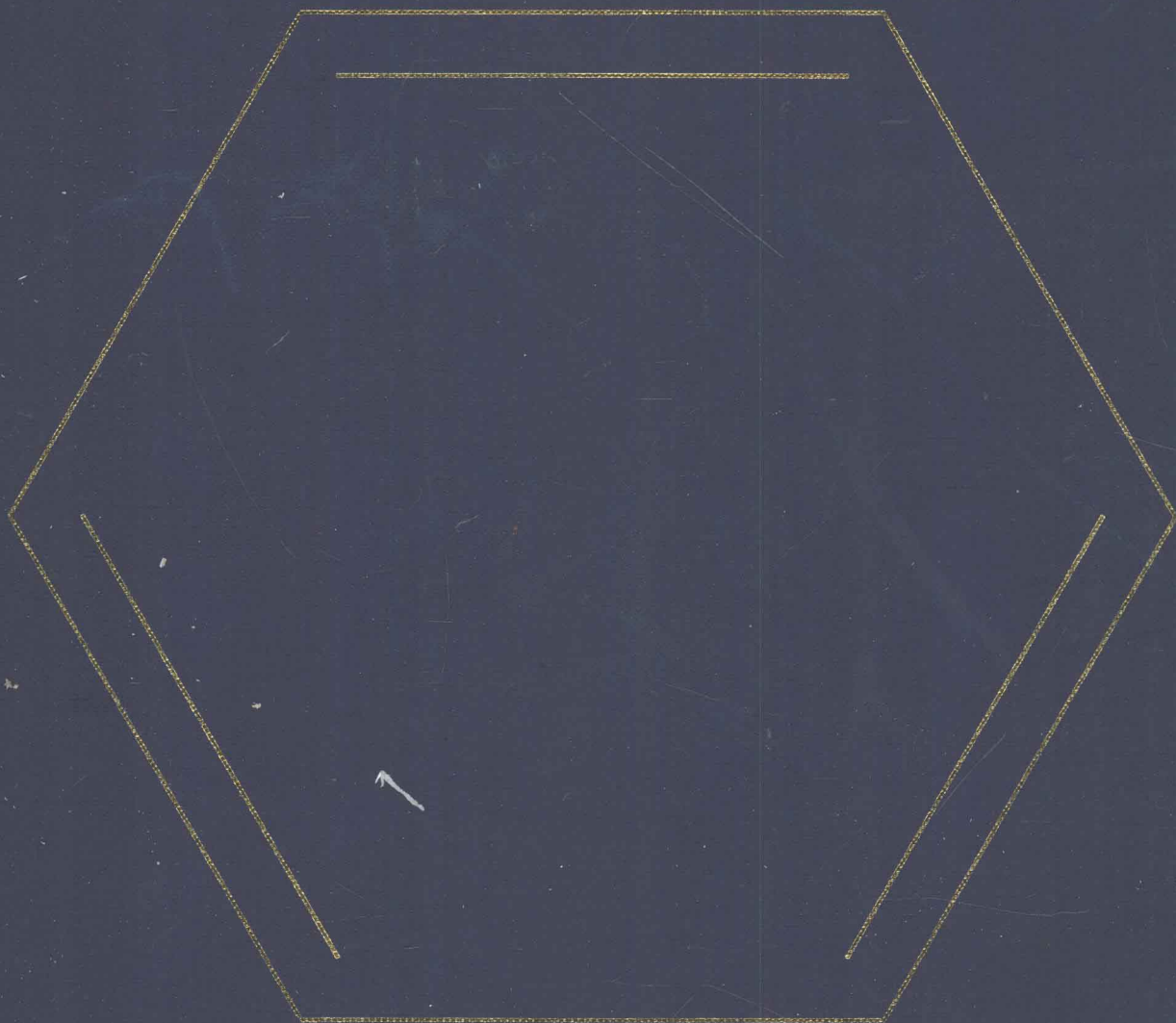
Histochemistry

VOLUME THREE

Edited by

PETER J. STOWARD

A. G. EVERSON PEARSE



Churchill Livingstone

Fourth Edition

Histochemistry

Theoretical and Applied

Edited by

Peter J. Stoward MA MSc DPhil FRSE

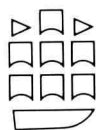
Professor of Histochemistry, Head of
Department of Anatomy and Physiology,
University of Dundee, UK

A. G. Everson Pearse MA MD FRCP FRCPath DCP

Emeritus Professor of Histochemistry,
University of London, UK

Volume 3 Enzyme histochemistry

FOURTH EDITION



CHURCHILL LIVINGSTONE

EDINBURGH LONDON MELBOURNE NEW YORK AND TOKYO 1991

CHURCHILL LIVINGSTONE

Medical Division of Longman Group UK Limited

Distributed in the United States of America by Churchill Livingstone Inc., 1560 Broadway, New York, N.Y. 10036, and by associated companies, branches and representatives throughout the world.

© Longman Group UK Limited 1991

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without either the prior written permission of the publishers (Churchill Livingstone, Robert Stevenson House, 1-3 Baxter's Place, Leith Walk, Edinburgh EH1 3AF), or a licence permitting restricted copying in the United Kingdom issued by the Copyright Licensing Agency Ltd, 33-34 Alfred Place, London, WC1E 7DP.

First edition 1953

Second edition 1960

Third edition 1968

Fourth edition 1980 (Vol. 1), 1985 (Vol. 2), 1991 (Vol. 3)

ISBN 0-443-02996-2

British Library Cataloguing in Publication Data

Stoward, Peter J.

Histochemistry: Vol 3. — 4th ed.

I. Title II. Pearse, A. G. Everson

574.19

Histochemistry
Volume 3

For Churchill Livingstone

Publisher: Tim Horne

Editorial: Jane Starling, Ruth Swan, Joanna Smith

Design: Design Resources Unit

Production: Lesley Small

Sales: Hilary Brown

Preface

This volume, child of many prayers (ours) and of many talents (others), completes for 'Histochemistry, Theoretical & Applied' a span of almost 40 years.

When the first volume of the work appeared in 1953, Enzyme Histochemistry was the rising star of a resurgent discipline, resurgent only after seven decades in a wilderness made for it by Johann Friedrich Miescher's 'Guild of Dyers', namely the histological fraternity.

For at least the next two decades it formed the advancing front of the whole discipline, and particularly for the field of Diagnostic Pathology. Here it promised, and made, considerable advances in the search for its 'Philosopher's Stone', distinction between a benign cell and its malignant counterpart.

By the early 1980s the relative importance of Enzyme Histochemistry had begun to decline, especially with respect to the younger disciplines of Immunocytochemistry and Hybridocytochemistry. Nevertheless it continues to form a significant and far from static branch of the whole subject.

For example, there have been enormous advances in quantification of enzyme activity *in situ*, and a far greater understanding of the fundamental mechanisms underlying many histochemical reactions. The size and complexity of the text provided in this volume is a direct response to the creation, since publication of the previous relevant volume in 1972, of a mass of new technology. This accompanies an ever widening range of histochemically demonstrable enzymes and enzyme groups.

We feel no need to apologise here for maintaining the principle enshrined in previous editions, of complete and up-to-date biochemical and histochemical coverage for each enzyme described.

We wish to acknowledge our great indebtedness to all those who have contributed to the final volume of the 4th Edition. We trust that they will feel that their time of waiting, whether patient or impatient, has been rewarded, and that this volume will stimulate and foster both the understanding and the application of the valuable techniques of Enzyme Histochemistry.

A.G.E.P., P.J.S.
Exeter and Dundee
1991

Contributors

Peter F. Altman BSc PhD DSc FIBiol DipRMS
Medical Publisher, Chapman and Hall Ltd;
formerly Lecturer in Cell Science, Institute of
Orthopaedics, Stanmore, Middlesex, UK

Sabine Angermüller DVM
Privatdozentin, Institute of Anatomy and
Cell Biology (II), University of
Heidelberg, Germany

Charles J. Arntzen BS MS PhD
Deputy Chancellor of Agriculture, Texas
A & M University, USA

Marcel Borgers PhD
Head, Life Sciences Department, Janssen
Pharmaceutica, Beerse, Belgium

R. G. Butcher BSc PhD (died 3 November 1986)
Senior Lecturer,
Cardiac Thoracic Institute,
University of London, Midhurst, UK

W. Deimann MD
Privatdozent, Institute of Anatomy and Cell
Biology (II), University of
Heidelberg, Germany

Dariusz Fahimi MD
Professor and Chairman, Institute of Anatomy
and Cell Biology (II), University of
Heidelberg, Germany

J. Anthony Firth MA PhD
Professor of Anatomy and Head of Department,
Imperial College of Science, Technology and
Medicine and St Mary's Hospital Medical
School, London, UK

R. Gossrau Dr med
Professor of Anatomy, Free University of
Berlin, Germany

Poul Erik Høyer MD
Professor of Medical Anatomy, University of
Copenhagen, Denmark

Margaret A. Johnson PhD
Research Lecturer in Neuromuscular Disease,
University of Newcastle-upon-Tyne, UK

Morris J. Karnovsky MB BCh DSc
Shattuck Professor of Pathological Anatomy,
Harvard University, Boston, USA

Brian D. Lake BSc PhD FRCPath
Professor of Histochemistry, Hospital for Sick
Children and Institute for Child Health, Great
Ormond Street, London, UK

P. R. Lewis MA DPhil ScD
Formerly Assistant Director of Research in
Physiology, University of Cambridge, UK

Z. Lojda MUDr DrSc
Vice-Rector and Professor of Pathology,
Charles University, Prague, Czechoslovakia

A. E. F. H. Meijer PhD
Professor of Histochemistry and Biochemistry,
Laboratory of Experimental Neurology,
Department of Neurology, Academic Medical
Centre, University of Amsterdam, The
Netherlands

C. Oliver BA MS PhD
Research Biologist, National
Institute of Dental Research,
Maryland, USA

John M. Robinson PhD
Associate Professor in Anatomy, Ohio State
University, USA

E. Seidler Dr sc nat
Privatdozent, Institute of Anatomy,
Humboldt University, Berlin, Germany

Peter J. Stoward MA MSc DPhil FRSE
Professor of Histochemistry, Head of
Department of Anatomy and Physiology,
University of Dundee, UK

Hewson Swift PhD
Professor of Molecular Genetics and Cell
Biology, University of Chicago, USA

Pieter van Duijn PhD
Emeritus Professor of Histology and
Cytochemistry, University of Leiden, The
Netherlands

C. J. F. van Noorden PhD
Senior Lecturer in Histology, University of
Amsterdam, The Netherlands

A. Verheyen PhD
Head of Laboratory, Department of
Cardiovascular Pharmacology,
Janssen Research Foundation,
Beerse, Belgium

Eugene L. Vigil PhD
Plant Physiologist, Climate Stress Laboratory,
Natural Resources Institute, United States
Department of Agriculture, Beltsville,
Maryland, USA

Frank Wohlrab Dr rer nat habil
Privatdozent, Institute of Pathological
Anatomy, University of Leipzig, Germany

Enzymes reviewed

The localisation and function of the following enzymes are described in this Volume.

<i>Enzyme</i>	<i>Contributors</i>	<i>Enzyme</i>	<i>Contributors</i>
Acetyl-CoA carboxylase	PJS	Aspartate aminotransferase	PRL
Acetylcholinesterase	CO, PRL	Aspartate carbamoyl transferase	PRL
β -N-Acetylgalactosaminidase	RG, ZL, PJS	ATPases	JAF
N-Acetyl- β -glucosaminidase	RG, ZL, PJS	Benzaldehyde dehydrogenase	PJS
β -N-Acetylhexosaminidase	RG, ZL, PJS	Benzylamine oxidase	PJS, PRL
Acetylserotonin methyltransferase	PRL, PJS	Betaine-aldehyde dehydrogenase	FW
Acid phosphatase	MB, AV, PJS	Calcdiol 1-monooxygenase	PJS
Acrosin	PJS	Carbamoyl-phosphate synthase	PJS
Acyl-CoA oxidase	PJS	Carbonic anhydrase	CO, PJS
Acylglycerol palmitoyltransferase	PRL	Carbonyl reductase	PJS
Adenylate cyclase	PJS	Carnitine acetyltransferase	PRL
Alcohol dehydrogenase	PJS, PRL	Catalase	WD, SA, HDF
Alcohol oxidase	PJS	Catechol methyltransferase	PJS
Aldehyde dehydrogenases	PJS, EW	Catechol oxidase	PJS
Aldehyde oxidase	PJS	Cathepsin B	ZL, RG, PJS
Aldehyde reductase	PJS	Cathepsin D	ZL, RG, PJS
Aldose reductase	PJS	Cathepsin G	ZL, RG, PJS
Alkaline phosphatase	MB, AV	Cathepsin H	ZL, RG, PJS
D-Amino-acid oxidase	PJS, JMR, MJK, PRL	Cathepsin L	ZL, RG, PJS
4-Aminobutyrate aminotransferase	PRL	Cellulase	RG, ZL, PJS
Aminopeptidase A	ZL, RG, PJS	Cellulose 1,4- β -cellobiosidase	RG, ZL, PJS
Amylases (α - and β -)	RG, ZL, PJS	Chitinase	RG, ZL, PJS
Apyrase	PJS	Cholesterol esterase	CO
α -L-Arabinofuranosidase	RG, ZL, PJS	Cholesterol oxidase	PJS, JMR, MJK
Arginine aminopeptidase	ZL, RG, PJS	Choline acetyltransferase	PRL, PJS
Aromatic L-amino-acid decarboxylase	PJS	Choline dehydrogenase	FW
Arylamine acetyltransferase	PRL	Cholinesterases	CO, PRL
Arylsulphatase	PJS	Chymase	ZL, RG, PJS
		Collagenases	ZL, RG, PJS
		Creatine kinase	PRL

<i>Enzyme</i>	<i>Contributors</i>	<i>Enzyme</i>	<i>Contributors</i>
Cystathionine γ -lyase	PJS	Glucosylceramidase	RG, ZL, PJS
Cysteine lyase	PJS	β -Glucuronidase	RG, ZL, PJS
Cytochrome c oxidase	JMR, MJK	Glucuronosyltransferase	PJS
Cytochrome P450	PJS	Glutamate decarboxylase	PJS
Cytochrome P450-dependent oxygenase	PJS	Glutamate dehydrogenases	FW, PJS
Cytosol aminopeptidase	ZL, RG, PJS	γ -Glutamyltransferase	ZL, RG, PJS
α -Dextrin	RG, ZL, PJS	Glutathione peroxidase	PJS
endo-1,6- α -glucosidase		Glutathione reductase	PJS
Diamine oxidase	PJS	Glutathione transferase	PJS
Dihydroorotate dehydrogenase	PJS	Glyceraldehyde-3-phosphate dehydrogenase	AEFHM, PJS
Dihydrofolate reductase	PJS, FW	Glycerol-3-phosphate acyltransferase	
Dihydrolipoamide dehydrogenase	PJS	Glycerol-3-phosphate dehydrogenase	AEFHM, PJS
Dipeptidase	ZL, RG, PJS	Glycerol-3-phosphate dehydrogenase (NAD ⁺)	FW, PJS
Dipeptidyl peptidase I	ZL, RG, PJS	Glycogen synthase	PRL
Dipeptidyl peptidase II	ZL, RG, PJS	Glycolate dehydrogenase	PJS
Dipeptidyl peptidase IV	ZL, RG, PJS	Glycolate oxidase	PJS
Dipeptidylcarboxypeptidase I	ZL, RG, PJS	Glycosylceraminidase	RG, ZL, PJS
Dopamine β -monooxygenase	PJS	Guanylate cyclase	PJS
Endo-1,3- β -glucanase	RG, ZL, PJS	L-Gulonolactone oxidase	PJS
Enolase	PJS	Hetero- β -galactosidase	RG, ZL, PJS
Enoyl-CoA hydratase	PJS	Hexokinase	PRL
Enteropeptidase	ZL, RG, PJS	Histidine decarboxylase	PJS
Eosinophil peroxidase	PJS	Hyaluronidase	RG, ZL, PJS
Ferredoxin-NADP ⁺ reductase	PJS	L-2-Hydroxy-acid oxidase	JMR, MJK
β -Fructofuranosidase	RG, ZL, PJS	3-Hydroxyacyl-CoA dehydrogenase	PJS
Fructose-bisphosphate aldolase	AEFHM, PJS	3-Hydroxyanthranilate 3,4-dioxygenase	
Fructose-bisphosphatase	MB, AV, PJS,	3-Hydroxybutyrate dehydrogenase	FW
β -D-Fucosidase	RG, ZL, PJS	9-Hydroxyprostaglandin dehydrogenase	FW, PJS
α -L-Fucosidase	RG, ZL, PJS	3 α -Hydroxychololate dehydrogenase	PEH, PJS
Galactose oxidase	PJS, JMR, MJK	15-Hydroxyprostaglandin dehydrogenase (NAD ⁺)	FW, PJS
Galactosidases (α and β -)	RG, ZL, PJS	3 α -Hydroxysteroid dehydrogenase	PEH
Galactosylceramide sulphotransferase	PJS	11 β -Hydroxysteroid dehydrogenase	PEH
Galactosyltransferase	PJS, PRL	3 β -Hydroxy- Δ^5 -steroid dehydrogenase	PEH
Glucan 1,4- α -glucosidase	RG, ZL, PJS	L-Iditol dehydrogenase	FW, PJS
1,4- α -Glucan branching enzyme	PRL	L-Iduronidase	RG, ZL, PJS
Glucokinase	PRL		
Glucose dehydrogenase	PJS		
Glucose oxidase	PJS, JMR, MJK		
Glucose-6-phosphatase	MB, AV		
Glucose-6-phosphate dehydrogenase	PJS		
Glucose-6-phosphate isomerase	PJS		
Glucosidases (α -and β -)	RG, ZL, PJS		

<i>Enzyme</i>	<i>Contributors</i>	<i>Enzyme</i>	<i>Contributors</i>
IMP dehydrogenase	PJS	Phosphoenolpyruvate carboxykinase	PJS
Indoleamine 2,3-dioxygenase	PJS	6-Phosphofructokinase	PRL
Inorganic pyrophosphatase	MB, AV	Phosphoglucomutase	PRL, PJS
Isocitrate dehydrogenases	AEFHM	Phosphogluconate dehydrogenase	ES, CJFVN, PJS
Ketohexokinase	PJS	Phosphorylase	PRL
Lactase	RG, ZL, PJS	Plasmin	ZL, RG, PJS
Lactate dehydrogenase	AEFHM, PRL, PJS	Plasminogen activator	ZL, RG, PJS
Leucocyte elastase	ZL, RG, PJS	Platelet peroxidase	PJS
Lysophospholipase	CO	Polyphenol oxidases	PJS, JMR, MJK
Lysozyme	RG, ZL, PJS	Prolyl hydroxylase	PJS
Lysyl oxidase	PJS	Prostacyclin synthase	PJS
Malate dehydrogenases	AEFHM, PJS	Prostaglandin synthase	PJS
Malate synthase	PJS	Protochlorophyllide reductase	PJS
Mannosidase	RG, ZL, PJS	Protein-disulphide reductase	PJS
Membrane metallo-endorpeptidase	ZL, RG, PJS	Purine-nucleoside phosphorylase	PRL
Microsomal aminopeptidase	ZL, RG, PJS	Pyruvate dehydrogenase complex	PJS
Monoamine oxidase	PJS, JMR, MJK, PRL	Pyruvate kinase	PRL, PJS
NAD(P)H oxidases	PJS, JMR, MJK	Renin	ZL, RG, PJS
NAD ⁺ kinase	PJS	Retinol dehydrogenase	PJS
NADH dehydrogenases	PJS	Ribose-5-phosphate isomerase	PJS
NADH-ferrihemoprotein reductase	PJS	Ribulose biphosphate carboxylase	PJS
NADP(H) phosphohydrolases	MB, AV, PJS	Secondary alcohol dehydrogenase	PJS
NADPH dehydrogenases	PJS	Serine-pyruvate aminotransferase	PJS
Nicotinamide-nucleotide adenyltransferase	PRL	Sialidase	RG, ZL, PJS
Nicotinate-nucleotide pyrophosphorylase	PJS	Submandibular protease A	ZL, RG, PJS
Nitrate reductase	PJS	Succinate dehydrogenases	PJS
Non-specific esterases	CO, PJS, PRL	Succinate-semialdehyde dehydrogenase	PJS
Nucleoside-diphosphatase	PJS, MB, AV	Sucrose- α -glucosidase	RG, ZL, PJS
Nucleoside-triphosphatase	MB, AV	Sulphinoalanine decarboxylase	PJS
3'-Nucleotidase	MB, AV	Superoxide dismutase	PJS
5'-Nucleotidase	MB, AV	Terminal deoxynucleotidyl transferase	PJS
Oligo-1,6-glucosidase	RG, ZL, PJS	Tetrametaphosphatase	PJS
Ornithine aminotransferase	PJS	Thiamine monophosphatase	MB, AV
Ornithine carbamoyltransferase	PRL	Thiamine pyrophosphatase	MB, AV, PJS
Ornithine decarboxylase	PJS	Thioglucosidase	RG, ZL, PJS
Peroxidases	WD, SA, HDF	Thioredoxin reductase	PJS
Phenolases	PJS	Thiosulphate sulphurtransferase	PJS, PRL
Phenylethanolamine N-methyltransferase	PJS	Tissue kallikrein	ZL, RG, PJS
Phosphoenolpyruvate carboxylase	PJS		

<i>Enzyme</i>	<i>Contributors</i>	<i>Enzyme</i>	<i>Contributors</i>
Tissue-endopeptidase	ZL, RG, PJS	Tryptophan 2,3-dioxygenase	PJS
degrading		Tyrosine 3-monooxygenase	PJS
collagenase-synthetic		UDPglucose dehydrogenase	PJS
substrate		Urate oxidase	PJS, JMR,
Transglutaminase	PRL		MJK, PRL
Triglyceride lipase	CO	Xanthine dehydrogenase	ES
Trimetaphosphatase	MB, AV, PJS	Xanthine oxidase	PJS, JMR,
Tryptase	ZL, RG, PJS		MJK

Contents

- Preface v
Contributors vii
Enzymes reviewed ix
Contents xiii
21. Principles of oxidoreductase histochemistry 1
P. J. Stoward, F. P. Altman and E. Seidler
22. Dehydrogenases 27
P. J. Stoward, A. E. F. H. Meijer, E. Seidler and F. Wohlrab
23. Dehydrogenases involved in steroid and prostaglandin metabolism 73
P. E. Hoyer, F. Wohlrab and P. J. Stoward
24. Oxidases 95
J. M. Robinson, M. J. Karnovsky, P. J. Stoward and P. R. Lewis
25. Reductases and oxygenases 123
P. J. Stoward
26. Peroxidases 135
W. Deimann, S. Angermüller, P. J. Stoward and H. D. Fahimi
27. Transferases 161
P. R. Lewis and P. J. Stoward
28. Phosphatases 187
M. Borgers, J. A. Firth, P. J. Stoward and A. Verheyen
29. Esterases 219
C. Oliver, P. R. Lewis and P. J. Stoward
30. Glycosidases 241
R. Gossrau, Z. Lojda and P. J. Stoward
31. Proteases 281
Z. Lojda, R. Gossrau and P. J. Stoward
32. Lyases, isomerases and ligases 337
P. J. Stoward
33. Quantitative enzyme histochemistry 355
C. J. F. van Noorden and R. G. Butcher
34. Model systems. Principles and practice of the use of matrix immobilized enzymes for the study of the fundamental aspects of cytochemical enzyme methods 433
P. van Duijn
35. Applications of enzyme histochemistry in pathological diagnosis 473
B. D. Lake
36. Applications of enzyme histochemistry in muscle pathology 489
M. A. Johnson
37. Cytochemistry of plant haem oxidases 515
E. L. Vigil, H. Swift and C. J. Arntzen
- Appendices**
22. Histochemical methods for dehydrogenases 537
P. J. Stoward and C. J. F van Noorden
24. Histochemical methods for oxidases 559
C. J. F. van Noorden and P. J. Stoward
26. Histochemical methods for peroxidases 573
W. Deimann, S. Angermüller, P. J. Stoward and H. D. Fahimi

27. Histochemical methods for transferases 577
P. R. Lewis
28. Histochemical methods for phosphatases 591
M. Borgers, P. R. Lewis and P. J. Stoward
29. Histochemical methods for esterases 607
C. Oliver, P. R. Lewis and P. J. Stoward
30. Histochemical methods for glycosidases 619
R. Gossrau and P. J. Stoward
31. Histochemical methods for proteases 639
Z. Lojda, R. Gossrau and P. J. Stoward
32. Histochemical methods for lyases, isomerases and ligases 653
P. J. Stoward
34. Preparation of model systems 661
P. van Duijn
37. Histochemical methods for plant haem oxidases 663

Indices

- Enzyme synonyms 667
- Subject index 677
- Author index 693

21. Principles of oxidoreductase histochemistry

P. J. Stoward F. P. Altman E. Seidler

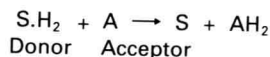
The commonest method of localizing oxidoreductases histochemically is based on one simple principle — the reduction, by the electrons released in the reaction catalysed by the enzyme, of a colourless, soluble tetrazolium salt to an intensely-coloured, highly insoluble derivative called a formazan. This is an oversimplification of what actually occurs. In 1972, when the previous edition was published, it was not widely appreciated how serious this oversimplification was. Several techniques recommended at that time have since been shown to be grossly inadequate and to give false results. For example, it was not realised that soluble enzymes diffused very rapidly out of sections into aqueous incubation media; consequently, the amount and distribution of the final reaction product (i.e. staining) subsequently seen in sections bore little relation to the original activity of the enzyme *in situ*. Unfortunately, many workers have continued to use these techniques despite the wealth of evidence of their shortcomings published in the two decades since 1970.

This chapter is, therefore, devoted to the principles on which the modern histochemistry of oxidoreductases is based. It is particularly concerned with the special factors that should be taken into account in order to obtain a reliable, accurate and specific localization. The most important concerns are; the reaction mechanisms underlying oxido-reductions, the characteristics of tetrazolium salts, intermediate electron acceptors and cofactors; and the suppression of the leakage of enzymes from unfixed cells and tissues during incubation. The account that follows is based partly on Chapter 20 of the last edition, but to a greater extent on texts by Altman (1972,

1976a), Wohlrab et al, (1979), Van Noorden (1984) and Stoward (1991).

OXIDOREDUCTASE-CATALYSED REACTIONS

Oxidoreductases (EC class 1) catalyse reactions between *two* substrates in which the principle substrate (S.H₂), called the donor, is oxidized by the other, the acceptor (A). Such reactions are generally written in the form



The donor is not always dehydrogenated as shown in this equation, but when it is, the catalysing enzyme is known as a dehydrogenase. However, two electrons are always removed from the donor. The electrons are transferred to, and thus accepted by, either the enzyme itself or separate molecules such as coenzymes or special proteins within the cell. The vast majority of dehydrogenases fall into two groups, *flavin-containing dehydrogenases* and *coenzyme-linked (or pyridine-linked) dehydrogenases*.

The flavin-containing dehydrogenases contain electron acceptors bound to the enzyme molecule itself. These acceptors are known as prosthetic groups, and are either a flavin adenine nucleotide (FAD) or a flavin mononucleotide. Succinate dehydrogenase belongs to this group.

The second group of dehydrogenases have no integrally-bound acceptors, and function only in the presence of separate electron acceptors or carriers known as coenzymes, usually NAD⁺ or NADP⁺.

Apart from these two groups, there is a small group of oxidoreductases which require other

acceptors, of which the most important are hydrogen peroxide (the peroxidases) and molecular oxygen (the oxidases).

The initial products of any biochemical reaction catalysed by a dehydrogenase are either a reduced flavin (for example, FADH_2 in the case of succinate dehydrogenase) or a reduced coenzyme (for example, NADH in the case of NAD^+ -dependent lactate dehydrogenase) plus, in both instances, the appropriate oxidized substrate. The reduced acceptors normally must be immediately re-oxidized to allow the enzymes concerned to go on functioning. Cells and tissues have a number of pathways to achieve this re-oxidation, the most well-defined ones being the respiratory chain in mitochondria and the cytochrome P_{450} system. They are discussed in detail later in connection with their relationships to the reduction of tetrazolium salts.

Thus, oxidation and reduction reactions catalysed by oxidoreductases do not necessarily involve oxygen and hydrogen. The fundamental event is the exchange of electrons; the passage or transfer of hydrogen or oxygen atoms is incidental. Nonetheless, it is often more convenient to visualize oxido-reduction reactions as 'hydrogen transport' rather than as 'electron transfer'.

PRINCIPLES UNDERLYING THE HISTOCHEMICAL LOCALIZATION OF DEHYDROGENASES

In most techniques, cell smears or sections are incubated in a suitably buffered medium containing:

1. the substrate of the enzyme to be localized
2. any necessary cofactor (NAD^+ or NADP^+)
3. an intermediate electron acceptor
4. a tetrazolium salt or some other final electron acceptor.

In addition, if the dehydrogenase is soluble, an inert tissue stabilizer, such as suitable grade of polyvinyl alcohol (PVA), should be dissolved in the medium to prevent the leakage of the enzyme from the section into the medium during incubation.

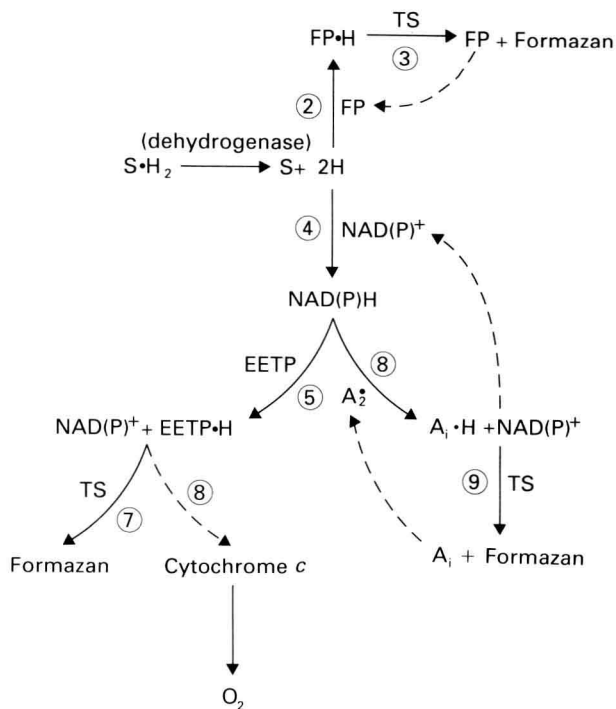
The dehydrogenase being localized oxidises the substrate ($\text{S}\cdot\text{H}_2$) in the medium to a reduced

intermediate reaction product (S), releasing electrons and hydrogen which pass either to the flavin component (FP) of the enzyme molecule or, if a coenzyme-linked dehydrogenase is being demonstrated, to exogenous oxidised coenzymes (NAD^+ or NADP^+) in the substrate medium. These are shown in Fig. 21.1 as reactions 2 and 4.

When a flavin-linked dehydrogenase, such as succinate dehydrogenase, is being localized, the reduced flavoprotein (FADH_2 or FADH) usually has a sufficiently high redox potential to reduce the tetrazolium salt in the medium to form an insoluble formazan, which then becomes deposited on the subcellular site of the enzyme.

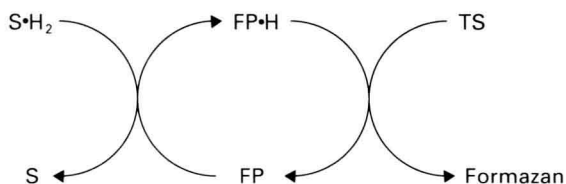
In contrast, the reaction paths followed during the localization of a coenzyme-linked dehydrogenase are more complex. The reduced coenzymes (NADH or NADPH) formed in reaction 4 (Fig. 21.1) are, unless steps are taken to prevent it, almost immediately oxidized by the *endogenous electron-transfer protein* (EETP) systems present in most cells. The reducing equivalents thus formed are either passed onto endogenous acceptors, such as cytochrome *c* (reaction 7), or onto exogenous acceptors such as molecular oxygen (reaction 8), or they reduce the tetrazolium salt in the substrate medium. If reaction 5 occurs to a significant extent, the insoluble formazan arising from the reduction of the tetrazolium salt is deposited on the subcellular site of the endogenous electron-transfer system, and not on the site of the enzyme. Thus, the resulting localization is 'false'.

In order to achieve a specific localization of a coenzyme-linked dehydrogenase, the endogenous electron-transfer systems of the tissue under study must be bypassed. This is achieved by adding an intermediate electron acceptor (A_i), such as phenazine methosulphate, to the substrate medium. The acceptor is reduced by the reducing equivalents (i.e. the electrons and protons released by the enzyme) to form a reduced intermediate which, in turn, immediately reduces the tetrazolium salt in the substrate medium. This results, in theory at least, in the deposition of an insoluble formazan on the subcellular site of the initial substrate-specific dehydrogenase (reactions 8 and 9 in Fig. 21.1). In this situation, the



Alternatively the above reaction sequences may be written in the form normally drawn in biochemistry textbooks for coupled oxidation-reduction reactions as follows

For flavin-containing enzymes:



For pyridine-linked dehydrogenases:

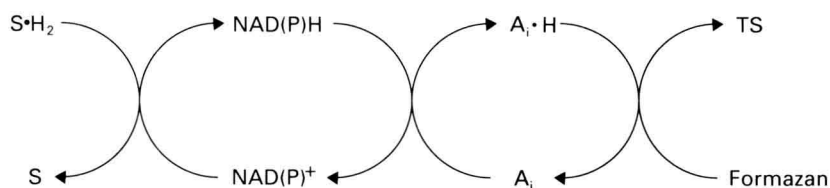


Fig. 21.1 Reaction sequences involved in the localization of coenzyme-linked and flavoprotein-linked dehydrogenases.