HISTOCHEMISTRY

THE WIDENING HORIZONS OF ITS APPLICATIONS IN THE BIOMEDICAL SCIENCES

Edited by
PETER J. STOWARD
and
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Histochemistry

The Widening Horizons of its Applications in the Biomedical Sciences

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To

A. G. Everson Pearse

with affection and grateful thanks
from his many friends for his
outstanding contributions
to the development and
application of histochemistry

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Abbreviations

ACTH	Adrenocorticotropic hormone
AO	Acridine orange
APUD cells	Amine precursor uptake and decarboxylation cells
m.ATPase	Myofibrillar adenosine triphosphatase
BUdR	5-bromodeoxyuridine
CGD	Chronic granulomatous disease
DAB	3,3′-dimethylaminobenzidine
EM	Electron microscope
LH	Luteinizing hormone
LHRF	Luteinizing hormone releasing factor
MPO	Myeloperoxidase
MSH	Melanocyte-stimulating hormone
OTAN	Osmium tetroxide-α-naphthylamine
PAMS	Periodic acid–methenamine–silver
PAS	Periodic acid–Schiff
PHA	Phytohaemagglutinin
PMA	Phorbol myristate acetate
PMN	Polymorphonuclear leucocyte
SIF cells	Small intensely fluorescent cells
TRF	Thyrotropin releasing factor

Foreword

It is a privilege to put on record an appreciation of the important revolution created in histopathology by Tony Pearse who was my inspiring colleague over many years at the Royal Postgraduate Medical School.

This whole-time academic centre was always on the look-out for leaders with original minds, often trained in more than one discipline relevant to the problems of medicine. Tony Pearse was such a man specially gifted in chemistry which he could apply to the problems of pathological histology. He contributed continuously and with new and original methods during his period at the School, enriching the whole atmosphere of the study of medicine by exciting new ideas and challenging observations. I remember well how he made new approaches to the identification of the various granules in endocrine organs, particularly in the pituitary as well as in the brain, contributing to new concepts of neuro-secretion. As many of these active substances were recognized and identified as polypeptides, this led him on to the establishment of certain methods for recognizing the possibility of these secretions by his amine precursor uptake and decarboxylation (APUD) system. This led on to the identification of many potential endocrine cells in the alimentary tract, some of which were previously unsuspected, and many have been discovered following the hints given by Pearse's method of their potential significance.

His mode of identification of enzymes in particular tissues, too, led to much progress in recognition of the sites of action of acetylcholine, adrenalin, and other active physiological substances.

When his colleague, Professor Ian MacIntyre, recognized the occurrence of calcitonin in the thyroid gland, the identification of the special cells derived from the neural crest concerned with its production was a splendid co-operative contribution by Pearse, and indeed the subsequent evolution of

knowledge concerning this important biological substance has been a rich field of collaboration between two departments.

In many of his publications, Pearse has been a valued collaborator with other investigators and he has never spared himself in trying to help others to understand their problems. He contributed quite unselfishly expert assistance to many colleagues at home and abroad in the identification of active biological substances concerned with both physiological and pathological phenomena in the human body. No one is better known internationally for his contribution to histochemistry than Tony Pearse, and his textbook is more widely quoted in research publications than any other comparable authoritative review.

In fact, his work is incomparable and has been recognized and acclaimed by prizes from research organizations and by honorary degrees from universities. As a former senior member of the staff of the Royal Postgraduate Medical School I have great pleasure in acknowledging our immense debt to Tony Pearse for his work which has so continuously contributed to the reputation of a unique medical research centre, the many pioneering efforts of which have done so much to sustain exemplary standards in British medicine over the last half-century.

J. McMICHAEL

Preface

According to data generated by the *Science Citation Index*, histochemistry is now the most cited discipline in the biomedical literature. Its techniques have insidiously invaded a multitude of fields from botany to routine histopathology. The old saying of Sir Roy Cameron that 'Morbid anatomy is not enough' has, at last, taken hold of the minds of most pathologists. Practitioners in other branches of science and medicine now also need histochemistry as the butter on their daily bread, but in the early days the potential of the new ideas was largely ignored.

In 1947–1948, a young pathologist, A. G. E. Pearse, was settling down to his first job at the Hammersmith Hospital after service in the Royal Navy during the war when he became interested in the trichrome-PAS stain which was subsequently used for the full identification of the various cell types in the anterior pituitary. Professor J. H Dible, at the time the Head of the Department of Histopathology, was impressed by Pearse's special 'histochemical' gift and suggested that he write a monograph on the 'Histochemistry of Nucleic Acids'. Pearse immediately transformed this idea into the writing of a volume on 'The Whole of Histochemistry'—a decision typical of his all-embracing mind! This venture materialized in 1953 when the first edition of his masterpiece, *Histochemistry: Theoretical and Applied*, was published. A second edition appeared in 1960, a third in 1970 (extending to two volumes), and now the fourth edition is coming out in three instalments, the first of which appeared in 1980. The increase in size and number of volumes simply reflects the enormous expansion of the subject since he first wrote about it almost 30 years ago. Pearse's *Histochemistry* has been translated into Italian, Spanish, and Russian and on its account alone he is now the second most cited author in the biomedical literature.

For some time, we have been wondering how best to honour Tony Pearse's contribution to histochemistry. It has been largely through his efforts that this increasingly popular and multipurpose discipline, which satisfies all the rules of science, is now universally used. In the event, we decided to ask some of his distinguished friends to write an account of what they regarded as the most important recent advances made by histochemistry in their own particular fields, and to indicate what advances they saw in the future.

This book is the result. Human nature being what it is, each of our contributors has interpreted the common commission we gave them in a completely different way. We hope that this very diversity will help both the established histochemist and the novice to find a forceful indication of the applications and future of histochemistry.

The first histochemist was a botanist, Raspail. Today, 150 years later, we are delighted that two more distinguished botanists, Jack Heslop-Harrison and Bruce Knox, are the first in this book with their splendid essay setting out the development of histochemistry in its proper historical context.

One of Tony Pearse's major contributions to histochemistry in recent years has been the creation of the APUD concept and the discovery that the endocrine and neural systems are closely linked, not only by their cytochemical similarities and common products but also in their embryological origin. It gave us much pleasure, therefore, that Berta Scharrer, the doyenne of neuroendocrinology, agreed to bring this field up to date in her contribution. Of course, many advances in neuroendocrinology, and indeed the neurological sciences generally, have come about through the availability of histochemical techniques for visualising cholinergic and aminergic pathways. Olavi and Liisa Eränkö introduced and refined many of these techniques over twenty years ago, but they are still making new discoveries as may be seen in their chapter.

Nearly all biological investigations today employ immunohistochemical techniques of some kind. Nevertheless, as Earl Benditt and Allen Gown show in their chapter, the vistas of immunohistochemistry are broadening. Indeed, immunohistochemistry has established itself as a discipline in its own right. This is mostly due to the better understanding of its basic principles as well as to the rapid advances in chemistry and immunology which have provided not only better ways of fixing the substance to be demonstrated, but also synthetic and pure antigens and a wide range of monoclonal antibodies directed to smaller and well-characterized parts of the cell molecule to be visualized.

Perhaps the greatest use made of histochemistry to date is in pathology, something Tony Pearse always hoped would happen. It is not surprising, therefore, that most of the contributions in this book describe applications of histochemistry to various aspects of pathology. They range from Morris Karnovsky and John Robinson's exposition of how 'good' cytochemistry can help to unravel a fundamental pathological process (phagocytosis) to the masterly

surveys by Margaret Johnson and Sir John Walton, Bob Mowry, Ian Dawson, and Zdenek Lodja on the uses to which histochemistry can now be put in diagnostic pathology and medicine. In addition, histochemistry is now playing a considerable part, along with other experimental approaches, in furthering our understanding of the pathogenesis of several chronic diseases. This is amply illustrated in Colin Adam's chapter on the contributions histochemistry has made to the study of multiple sclerosis.

Very little good haematology can be done these days without recourse to histochemical techniques at some stage or other. We were, therefore, very pleased that Daniel Catovsky and his colleagues and Jake Hanker and Beverly Giammara agreed to write on the employment of histochemistry in investigations of leukaemias.

In the past, specialists in disciplines other than pathology and haematology have not always exploited the histochemical approach as much as they might have done. However, in his chapter, Ernst Wachsmuth suggests how much pharmacology and toxicology, for example, has gained, and could gain further, from the selective application of histochemical techniques for investigating those problems which are not easily studied by other means.

Until comparatively recently, histochemistry has generally been practised and regarded as a qualitative discipline. This is reflected in this book too. However, this situation is changing rapidly, thanks to the recent development and commercial availability of many kinds of sophisticated instruments for rapidly analysing cells and tissues on the basis of the products formed in them as the result of a histochemical reaction. One particularly exciting development is the possibility of sorting cells with fluorescence-activated flow cytometers, as described by two leaders in the field, Myron Melamed and Zbigniew Darzynkiewicz, in their chapter. But there are other developments in the pipeline, and in the last chapter of this book, one of us has tried to indicate how quantitative enzyme histochemistry and the generation of instruments currently coming into service will make a powerful combination in the years ahead, if properly used, for widening the horizons of many areas of the biological sciences.

We should like to thank our Publishers, Dr. S. D. Thornton and his colleagues at Wiley, for their help in putting this book together, and Sir John McMichael for his Foreward and his characteristically gracious praises of Tony Pearse's gifts. But we wish especially to thank our contributors for generously agreeing to have all royalties paid into a tax-exempt trust fund for establishing a Pearse Prize and Lectureship. We hope, therefore, that this book will sell widely. The Prize will be in the gift of the Histochemistry and Cytochemistry Section of the Royal Microscopical Society, which Tony Pearse served as President from 1972–4, and will be awarded periodically to a scientist who has made a major contribution either to the development

of histochemical techniques and instrumentation or to the application of histochemical methods in elucidating important biological problems or phenomena.

Histochemists, your time has come: your horizons are wider than ever.

PETER J. STOWARD
JULIA M. POLAK

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J. HESLOP-HARRISON and R. B. KNOX

Plant Histochemistry: Retrospect and Prospect

In the introductory paragraphs of Volume 1 of *Histochemistry: Theoretical and Applied* (3rd edn., 1968), Everson Pearse attributed the beginnings of histochemistry as a science to the years 1830–55, and observed that in its origins it was primarily botanical. '. . . For some decades', he noted, 'the whole practice of histochemistry in its true sense was in the hands of botanists.' The main work in the early years was indeed that of the French botanist François-Vincent Raspail, whose *Essai de Chemie Microscopique Appliquée à la Physiologie* appeared in 1830. Raspail's earlier work had been mainly morphological, and included splendid papers on the reproductive structures of the Gramineae; later his attention passed to the chemistry of the tissues he was investigating. Pearse, following Baker's earlier discussion in his *Cytological Technique* of 1945, attributes the early—although perhaps not the first—use of the starch–iodine reaction in microscopy to Raspail, and also gives him the credit for the introduction of methods for protein and cell-wall carbohydrates.

Botanical histochemistry thus got off to an impressive start. Its early development took place over the period of the 1820s and 1830s when biological microscopy in general was expanding apace, and when Dutrochet, Brown, and others were laying the foundations of the cell theory, shortly to be given a fuller expression by the botanist Schleiden and the zoologist Schwann. The omens for continued development were therefore good; but the botanical impetus in histochemistry was progressively lost as the nineteenth century progressed. In retrospect, it is easy enough to identify some of the reasons. Plant cells are bounded by walls, and the study of plant anatomy is in large