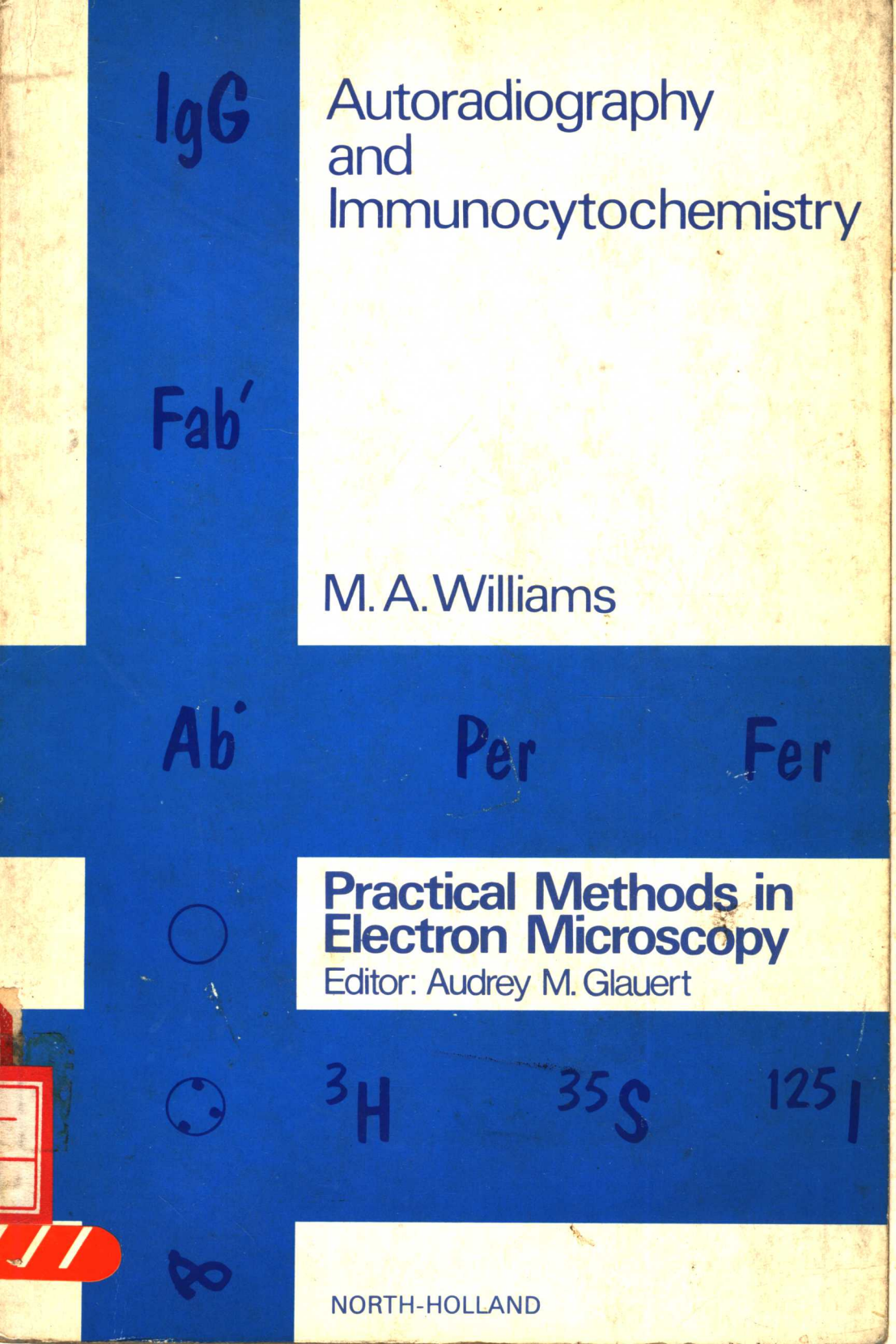

Autoradiography and Immunocytochemistry

M. A. Williams

Practical Methods in Electron Microscopy

Editor: Audrey M. Glauert

NORTH-HOLLAND

AUTORADIOGRAPHY AND IMMUNOCYTOCHEMISTRY

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Editor's preface

Electron microscopy is now a standard technique with wide applications in all branches of Science and Technology, and every year a large number of students and research workers start to use the electron microscope and require to be introduced to the instrument and to the techniques for the preparation of specimens. Many books are available describing the techniques of electron microscopy in general terms, but the authors of Practical Methods in Electron Microscopy consider that there is an urgent need for a comprehensive series of laboratory handbooks in which all the techniques of electron microscopy are described in sufficient detail to enable the isolated worker to carry them out successfully. The series of books will eventually cover the whole range of techniques for electron microscopy, including the instrument itself, methods of specimen preparation in biology and the materials sciences, and the analysis of electron micrographs. Only well-established techniques which have been used successfully outside their laboratory of origin will be included.

Great care has been taken in the selection of the authors since it is well known that it is not possible to describe a technique with sufficient practical detail for it to be followed accurately unless one is familiar with the technique oneself. This fact is only too obvious in certain 'one author' texts in which the information provided quickly ceases to be of any practical value once the author moves outside the field of his own experience.

Each book of the series will start from first principles, assuming no specialist knowledge, and will be complete in itself. Following the successful innovation, made by the same publishers in the parallel series Laboratory

Techniques in Biochemistry and Molecular Biology (edited by T. S. Work and E. Work), each book will be included, together with one or two others of the series, in a hardback edition suitable for libraries and will also be available in an inexpensive edition for individual use in the laboratory. Each book will be revised, independently of the others, at such times as the authors and editor consider necessary, thus keeping the series of books continuously up-to-date.

Strangeways Research Laboratory
Cambridge, England

AUDREY M. GLAUERT, SC. D.
General editor

Author's preface

The substance of this volume spent its early foetal life as a chapter intended for inclusion in a multi-author work. However, its growth easily outstripped my early imaginings and it grew first to two chapters, then to three, and then, apparently, into a book in its own right. Finally, that book has on delivery proved to be a pair of twins. I am extremely grateful to the editor Audrey Glauert and to the staff of North-Holland Publishing Co. for their forbearance during the years of gestation.

Writing this book has been possible because it has been my great fortune for the past twelve years to work at number 3, Clarkehouse Road, the annexe to the Department of Human Biology and Anatomy at the University of Sheffield. The friendship, support and skills of all those persons (students, technicians, office staff, researchers and academics) who work or who have worked at Clarkehouse Road are of immeasurable value to me. The creation of such a vital environment owes much to the head of Department, Professor Robert Barer.

I have gained considerably from meeting Andrew Rogers and teaching with him and some others a series of autoradiography courses under the auspices of the Anatomical Society of Great Britain and Ireland. The expertise of the teachers on these courses has been made available to me freely, and very many of the participants have willingly permitted insight into their research problems. However, in so far as opinions are offered in this book, they represent my own.

The aim of this series of books is to provide detailed laboratory guides from which workers, even geographically isolated ones, can carry through

electron microscope techniques with success. The texts, each of which covers a relatively narrow area, are *not* intended as reviews of all the available literature and techniques. Only well-established methods have been included, the criterion being that to merit consideration they have to have been used successfully in at least two independent laboratories. Doubtless the application of this rule has caused some excellent methods to be excluded. However, many less than excellent ones have been avoided and new methods of excellence will doubtless find their way, in any case, into later editions.

Da mi basia mille, deinde centum,
take, cut; take, cut: always something
dein mille altera, dein secunda centum,
ever-so-slightly imperfect sets us trying again;
deinde usque altera mille, deinde centum,
sustained by the silence of many held breaths;
vivamus, mea Lesbia, atque amemus,
and if at times another voice whispers
soles occidere et redire possunt,
in your ear or mine, we are professionals,
nobis cum semel occidit brevis lux,
stretch out our hands again and again, to touch;
nox est perpetua una dormienda.
I think we shall perfect it in the grave.

D.M. Thomas, *Computer 70: Dreams and Love Poems*

Acknowledgements

I am particularly grateful to the following persons and holders of copyright who have kindly given me permission to reproduce material. Micrographs were supplied by Dr. J. D. Jamieson (Fig. 3.8), Dr. S. Avrameas (Figs. 3.13 and 3.14), previously unpublished, Dr. J. A. Chapman (Figs. 3.9 to 3.12), courtesy of the Royal Microscopical Society, and Dr. Councilman Morgan (Fig. 3.6), courtesy of the *Journal of Virology*. The lines of poetry come from *Computer 70: Dreams and Love Poems*, which appears in *Logan Stone* by D. M. Thomas. It is published by Cape Goliard Press (distributed in the U.S.A. by Grossman Publishers Inc.). I am indebted to D. M. Thomas and Cape Goliard Press for permission to publish them.

Sheffield, September 1976 MIKE WILLIAMS

Contents

Chapter 1

Introduction

The contributions of electron microscopy to cellular biology during the last twenty five years have been prodigious. Few general texts in Anatomy, Physiology, Biochemistry, Botany, Zoology or Microbiology are prepared without recourse to illustrative electron micrographs. Text books in many specialized fields such as Immunology and Pathology rely heavily on pictures of microanatomy. Research and review papers in these fields utilizing electron microscopy are legion.

The very great majority of these books and papers simply draw upon morphological findings made with thin-sectioning techniques (Glauert 1974; Reid 1974) or by the viewing of stained particulates, although in very recent times observations made by the freeze-fracture approach have become common. For the most part this great volume of experimental work has sought pictorial results upon which to base textural commentaries on biological structure. However, the last 10–15 years has seen the grafting of new ideas on to this older stock and the emergence of techniques which seek not merely anatomical detail but chemical and/or numerical information concerning particular microanatomical structures.

Techniques for the localization of particular enzymatic activities via the deposition of electron-dense precipitates are described by Lewis and Knight (1977) and instrumental methods for placing such methods on a quantitative basis are being developed. Staining methods for various classes of macromolecule such as glycosaminoglycans have also become available (Lewis and Knight 1977).

However, certain very particular technical approaches based principally on standard thin-sectioning have come into prominence during the last 5–10 years. These include localization of processes by means of radioactive

chemicals and localizations based on specific antibody binding. Both of these approaches have in common the potential for providing numerical data by means of counts or measurements upon micrographs, such determinations generally being made by the human eye rather than by any specialized ancilliary equipment. Both areas of technology require, for the full realization of their potential, the application of objective sampling techniques and statistical methods to the finished preparations. Both combine well with a stereological approach.

This book sets out in detail the preparation of autoradiographs, methods of preparing immunological staining reagents and methods of the use of such reagents. Detailed accounts of methods for the collection, collation and interpretation of data derived from these approaches and their connection with stereological methods are given in a companion book (Williams 1977). The aim here has been to describe methods in enough detail to allow the student and solitary worker to exploit them successfully. The text has been centred upon well-established techniques. Where a choice is available the simpler alternatives are concentrated upon, although the more difficult and exotic methods are generally considered in outline.

1.1 The demand for autoradiography and immunocytochemistry

The demand for these methods derives partly from the growing area of common ground lying between the disciplines of anatomy, biochemistry and physiology in both animal and plant fields and partly from the expected evolution of microanatomy into a quantitative subject.

Autoradiography is rooted firmly in biochemistry and is used to localize the binding of particular chemicals and to study the sites of ingestion or incorporation of various selected chemicals and to further study their subsequent fate, be it digestion, translocation or secretion. Absolute quantitation is possible in certain circumstances. Kinetic data can be derived in translocation and secretion systems. Immunological staining has a less secure factual basis than autoradiography, but nevertheless can contribute highly specific data on the localization of documented antigens or occasionally of particular chemical residues. Certain dynamic experiments are possible and in some cases staining can be quantitated and its pattern studied.

1.2 The most advantageous use of the techniques

Both techniques are highly refined and technically demanding and if used unwisely can result in a great expenditure of effort to produce results of minimal value. It is essential to have the true role of these sophisticated methods clearly in mind before committing oneself to an extensive project.

It is generally true that the fruitfulness of an advanced technique in a given situation is proportional to the amount of already established knowledge. The more you know about the system the more likely it is that a highly refined technique will be useful. Almost always it is prudent to be fully acquainted with the material at the light microscopical (LM) level before electron microscopical (EM) methods are applied. Thus EM autoradiography is best applied in a situation wherein LM autoradiography has been already applied. Immunological staining is best used at the EM level when serological experiments are complete and when a study using fluorescent or peroxidase-labelled antibody at the LM level has indicated that an EM study would be advantageous. In short, few projects are appropriately *commenced* at the EM level, although very many are usefully extended or concluded using these sophisticated methods.

In both autoradiography and immunocytochemistry the markers of the cytochemical events can be enumerated and hence these approaches are potentially open to quantitation. The valid utilization of a quantitative approach depends, however, upon a careful consideration of the theoretical and practical bases of the experiments and thence upon the judicious choice of an experimental design. Such preparations are necessary if the full potential of autoradiographic and immunocytochemical methods is to be realized, although very many useful but simple applications of these methods can be found. A companion book to this one (Williams 1977) gives a detailed account of the interpretive side of autoradiography and sets out also at some length, details of stereological methods, which form alternatively an excellent foundation for, or a fine correlative approach for, research employing autoradiography and/or immunocytochemistry.

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Chapter 2

Immunocytochemistry at the EM level: preparation of labelled antibodies

If an animal is injected repeatedly with a preparation of a foreign macromolecule, after a time its serum is found to contain specific 'antibodies'. These antibodies are proteins which will combine specifically with the chemical that induced them. A good basic account of the antigen–antibody reaction is given by Clausen (1969). Those antibodies most frequently encountered are molecules of sedimentation coefficient about 7 S, termed Immunoglobulin G (IgG), and molecules of about 19 S, termed IgM. (See Fig. 2.1 for generalized structure of IgG molecules.) These immunoglobulins occur in the electrophoretic fractions of serum protein which are termed gamma and beta globulins.

Many species of plant and some species of invertebrate animal contain proteins with the specific ability to interact with other proteins and often

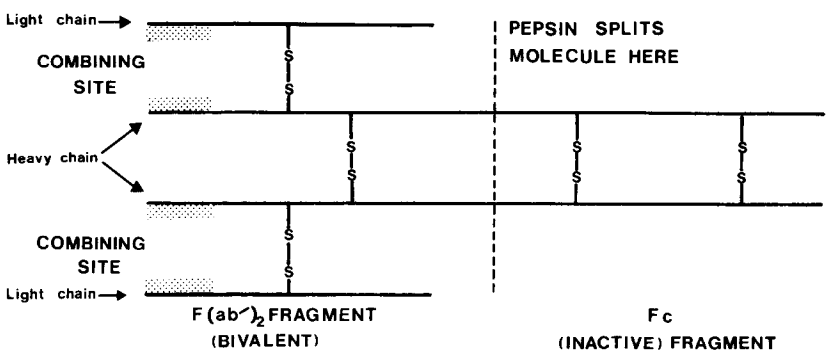

Fig. 2.1. General structure of immunoglobulin G. Note the presence of two heavy and two light chains. The molecules are bivalent, the combining sites being shown at the left side. The other (inactive) end of the molecule is also known as the F_c portion.