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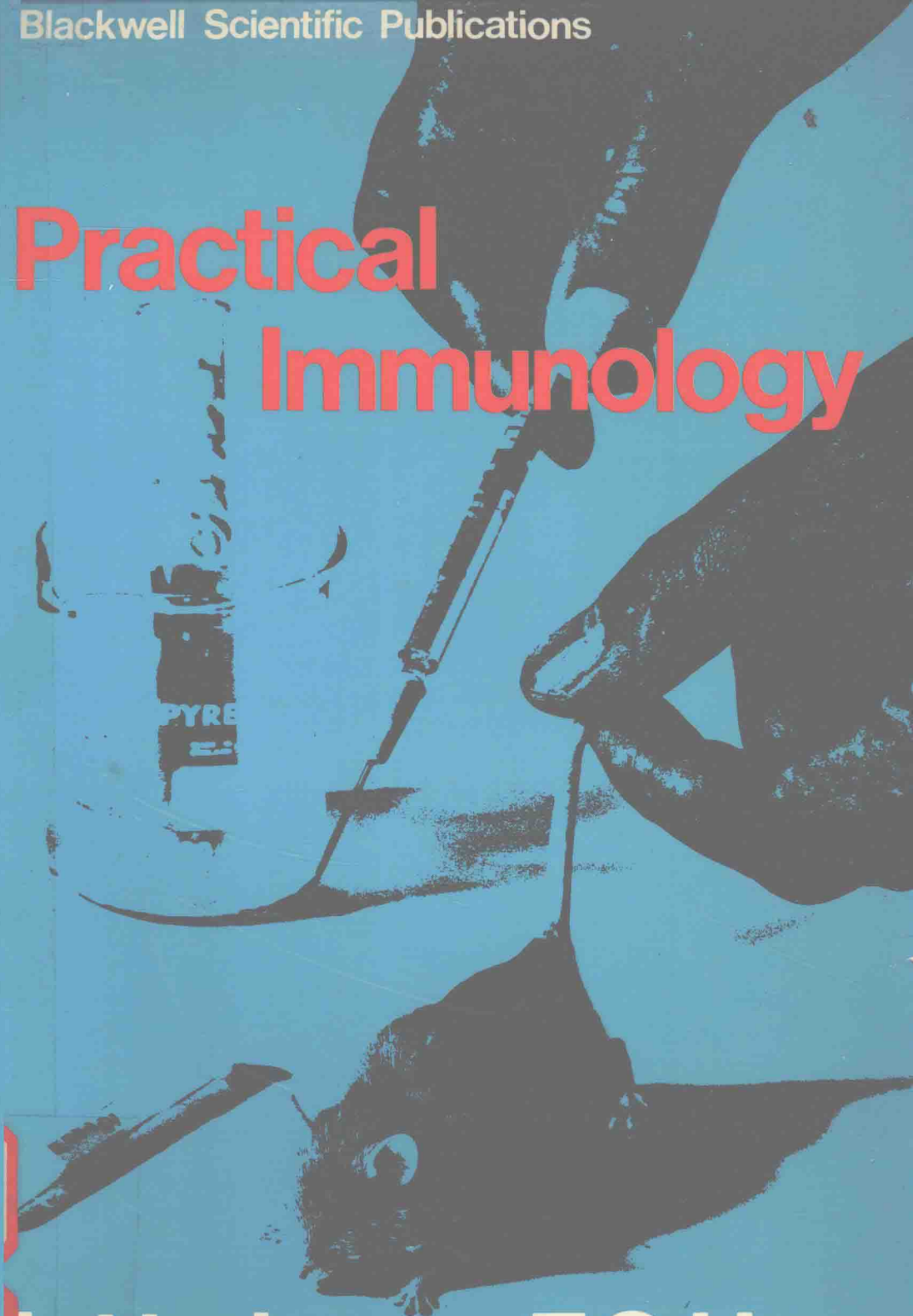
Practical Immunology



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Practical Immunology

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PRACTICAL IMMUNOLOGY

TO SARAH-LOUISE AND REBECCA

Foreword

Immunology might well claim to be the most popular and the most glamorous of the biological sciences today. I suspect that there has been a sharper increase in the number of research workers in immunology over the last two decades than in any other scientific discipline.

Applied immunology, plus the intangibles we lump together as the rising standard of living, has virtually rid the world of smallpox, yellow fever, diphtheria and poliomyelitis and helped in many other fields. Its prestige lingers on as the major tool of preventive medicine but, as one whose first immunological paper was published more than fifty years ago, I have seen a complete switch in the contemporary importance of immunology—but not a diminution.

Immunology today is a science in its own right. The enthusiasm of younger workers like the authors of this book, is primarily directed toward understanding; medical applications of the new knowledge will be wholeheartedly welcomed but they are not central. For me, and to some extent all of us in immunology, the excitement is in the lead that our subject is giving toward a real understanding of the form and strategy of living process. Thanks to the *recognizability* of the significant molecules, antibody, antigen and the like, we have been able to apply the new techniques of molecular biology to the elucidation of one of the essential bodily functions. We are leading the field, for nowhere else have genetics, biochemistry and every other basic science that can help, been so effectively applied to living function. It is the first step toward a sophisticated understanding of what we are and how we became so.

This book is basically an introduction to the techniques and ideas on which immunology is based but to one who grew up with the older, predominantly medical approach, the new version can be sensed everywhere in the authors' approach.

I wish them every success.

F.M. Burnet

Basel, Switzerland

Acknowledgements

This book was started while we were carrying out research for our doctorates in the department of Professor Ivan M. Roitt. He both encouraged and, indeed, stimulated us to become interested in the teaching of immunology. Our grateful thanks are also due to Dr Giorgio Torrigiani for initiating us into the world of immunochemistry.

We wish to thank our colleagues Siraik Zakarian, Harold von Boehmer, Andrew Kelus, Hansruedi Kiefer, Clive Loveday, Jan Obel, Marcus Nabholz, Richard Pink and Jonathan Sprent both for their helpful discussions and in many cases for allowing us to use their unpublished material. We are particularly grateful to Sir Macfarlane Burnet for writing the foreword to this book.

Without the valuable assistance of Lynn Nineham and Anthony Finch it would have been impossible for us to gather all the information and data required for this book. In addition, we wish to acknowledge the tenacity with which Penny Hamilton-Jones converted our pages of hieroglyphs into typewritten sheets.

We have written this book for the use of those individuals, from the undergraduate to post-doctoral level, with a sound theoretical knowledge of immunology, who wish to extend their knowledge by experimentation.

We have been made painfully aware of the interdependence of the branches of immunology in writing this book. It proved extremely difficult to find a point at which to begin. The approach finally adopted was one from the view of cellular biology rather than the classical immunological approach which starts with antibody, a mediator produced half-way through the immune response, and leads to the logical gymnastics of proceeding backwards, to the cellular basis of this response, and forwards, to the secondary mechanisms initiated by antibody–antigen interaction. We believe that the development of the book from the basic 'lymphocyte unit' to the complete immune system avoids the fast approaching dichotomy between cellular immunology and immunochemistry.

Leslie Hudson
Frank C. Hay

November 1975

Introduction and Additional Reading

Reading to accompany this book

BURNET F.M. (1969) *Self and Not Self.* Cambridge University Press.
ROITT I.M. (1974) *Essential Immunology*, 2nd ed. Blackwell Scientific Publications, Oxford.
STEWARD M.W. (1976) *Immunochemistry*, 2nd ed. Chapman and Hall, London.

For the advanced student

BURNET F.M. (1969) *Cellular Immunology* (hardback including *Self and Not Self*). Cambridge University Press.
GREAVES M.F., OWEN J.J. & RAFF M.C. (1973) T and B lymphocytes. *Excerpta Medica.*
HUMPHREY J.H. & WHITE R.G. (1970) *Immunology for Students of Medicine*, 3rd ed. Blackwell Scientific Publications, Oxford.
KABAT E.A. (1968) *Structural Concepts in Immunology and Immunochemistry.* Holt, Rinehart & Winston Inc, New York.
WEIR D.M. (editor) (1974) *Handbook of Experimental Immunology*, 2nd ed. Blackwell Scientific Publications, Oxford.

Many of the techniques described in this book involve radioactive isotopes; it is therefore essential that individuals be familiar with some manual dealing with the 'Code of Practice against Radiation Hazards'.

Recurrent publications, for the advanced student and specialist

Advances in Immunology. Academic Press, London and New York
Contemporary Topics in Immunobiology. Plenum Press, New York
Contemporary Topics in Molecular Immunology. Plenum Press, New York
Essays in Fundamental Immunology. Blackwell Scientific Publications, Oxford
Modern Trends in Immunology. Butterworths, London
Perspectives in Immunology. Academic Press, London and New York
Transplantation Reviews. Munksgaard, Copenhagen

Major Journals for Immunologists

Cellular Immunology

Clinical and Experimental Immunology
European Journal of Immunology
Immunochemistry
Immunological Communications
Immunology
Infection and Immunity
International Archives of Allergy and Applied Immunology
Journal of Experimental Medicine
Journal of Immunogenetics
Journal of Immunology
Journal of Immunological Methods
Lancet
Nature
Proceedings of the National Academy of Science
Scandinavian Journal of Immunology
Science
Transplantation

The growth of knowledge within the field of immunology has been so immense within the last five years that we would recommend that those individuals who wish to keep up to date with the current literature should consult either:

Current Titles in Immunology, Transplantation and Allergy. Sciences, Engineering, Medical and Business Data Ltd, Oxford; or
Current Contents (*Life Sciences*). Institute for Scientific Information, Philadelphia, USA.

Contents

Appendixes

1 Initial preparations

1.1 INTRODUCTION TO PRACTICAL IMMUNOLOGY

It would be very expensive to purchase immunological reagents in the quantities required for some of the procedures described in the book. With time, patience and experience it is possible to prepare all the reagents you will require. We have designed the book such that reagents used at later stages will already have been prepared in the earlier chapters. Accordingly this introductory section is not included in the logical sequence of the book. A full explanation of all the techniques and their applications is given in later chapters.

1.2 ANTIGENS

Many are not expensive and so may be purchased commercially.

1.2.1 ERYTHROCYTES

Sheep erythrocytes (SRBC) are used widely in immunology both as antigens and indicator cells. They should be purchased in Alsever's solution (See Appendix II), and have a shelf life of 3–4 weeks at 4°.

Horse RBC are sold commercially as oxalated whole blood (See Appendix II). Their shelf life at 4° is only 2 weeks.

1.2.2 SOLUBLE ANTIGENS (ammonium sulphate precipitation)

Fowl gamma globulin (FγG). This is a very powerful antigen, (a strong immunogen) especially in mice, and is usually prepared as an ammonium sulphate precipitate of whole chicken serum. It is not strictly γ globulin but is serum depleted mainly of albumin.

1.2.2a **Preparation of FγG**

Materials and equipment

Solid ammonium sulphate
Dilute ammonia solution
Chicken (adult, preferably cockerel)
UV spectrophotometer

Method

1 Dissolve 1000 g ammonium sulphate in 1000 ml distilled water at 50°, allow to stand overnight at room temperature and adjust the pH to 7·2 with dilute ammonia solution or sulphuric acid.
2 Bleed chicken by cardiac puncture (Section 2.5.1) and separate the serum from the clotted whole blood.
3 Dilute serum 1:2 with saline and add saturated ammonium sulphate solution (prepared in 1) to a final concentration of 45% (v/v.).
4 Stir at room temperature for 30 min.
5 Spin off precipitate (1000 *g* for 15 min at 4°).
6 Wash precipitate with 45% saturated ammonium sulphate and re-centrifuge.
7 Redissolve the precipitate in the same volume of PBS as the original serum.
8 Centrifuge to remove any insoluble material.
9 Re-precipitate the γ-globulin using a final concentration of 40% saturated ammonium sulphate.
10 Spin off the precipitate and wash with 40% saturated ammonium sulphate.
11 After centrifuging the washed precipitate, re-dissolve in a minimum volume of PBS.
12 Dialyse the FγG against 5 litres of PBS at 4° overnight. Centrifuge off any precipitate.
13 Prepare a 1:20 dilution of the FγG and determine the absorbance at 280 nm using a UV spectrophotometer.

Calculation of protein content. At 280 nm, an OD of 1·0 (1 cm cuvette) is equivalent to an FγG concentration of 0·74 mg ml^{-1}.

Example: if OD at 1:20 = 0·95,
FγG concentration = $0{\cdot}95 \times 0{\cdot}74 \times 20$
= 14·1 mg ml^{-1}.

Technical notes

1 Calculation of volume of saturated solution required to achieve a required concentration of ammonium sulphate:

volume saturated solution (ml) to be added per 100 ml volume, $S_i =$

$$\frac{100\,(S_f - S_i)}{1 - S_f},$$

where S_f = final saturation (fraction, not per cent),
S_i = initial saturation (fraction not per cent).

2 To minimize excessive volumes of solution when working in bulk, add solid ammonium sulphate according to the nomogram on the front inside cover.
3 18% sodium sulphate may be used to precipitate a crude γ-globulin fraction of serum. Unlike ammonium sulphate however, its