



PRINCIPLES OF
BIOLOGICAL
MICROTECHNIQUE

JOHN R. BAKER



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A Study of Fixation and Dyeing

by

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of Microscopical Science*

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DEDICATED
TO THE MEMORY OF
PAUL EHRLICH
GENIUS
IN CHEMOTHERAPY
IMMUNOLOGY
MICROTECHNIQUE

Preface

The principles of biological microtechnique may perhaps be reduced to one—the principle that when we make a microscopical preparation of any sort, we ought to try to understand what we are doing, for otherwise we shall examine an unknown object that has been treated in an unknown way. A scientific outlook has been introduced into certain branches of our subject, particularly histochemistry, but there are others in which rule of thumb rules indeed. One thinks at once of the most ordinary processes of the histological or cytological laboratory: of fixation, embedding, dyeing, and mounting. Here the empirical outlook is often manifest, and some workers are content to follow the recipe-book blindly, as though a scientific result could be obtained by unscientific means.

A very long book—and a very learned author—would be necessary if the attempt were made to illustrate the principles of microtechnique by a full consideration of all its branches. It seems best to concentrate on the most familiar processes, so that the principles may find most frequent application in practice. In fixation and in dyeing the tissues are *responsive*: they react to what we do to them. In embedding and mounting they are more passive, allowing us to surround them with what we will. I therefore choose fixation and dyeing as being even more interesting than the other familiar branches of microtechnique. I hope that a study of the principles that should guide us in these branches may engender an outlook towards microtechnique that will find application in a wider field. This outlook is at least as necessary when tissues are prepared for the electron microscope as in our more homely endeavours within the realm of light.

The book is addressed to research-workers, teachers, and students in the fields of pathology, histology, cytology, zoology, and botany. The primary intention has been to make it as useful and attractive as possible to the consecutive reader, but certain features have been introduced to help the casual inquirer. Thus there are numerous cross-references and rather a lot of repetitions. Three of the chapters (5, 6, and 9) can be used as though they were parts of a work of reference. A full index is provided.

This is in no sense a text-book. There is, I hope, nothing dogmatic in it. Its purpose is as much to show the gaps in knowledge as to knit together what is surely known. The possibilities for research in microtechnique seem endless, and every effort has been made to point out as many of them as possible.

The book contains a good deal of new material of three sorts. First, there are new contributions to the theory of fixation and dyeing. Secondly, there are many factual observations that have not been published previously. Thirdly, the Appendix contains full descriptions of new experiments illustrating the principles underlying the processes of fixation and dyeing. Most of these can be carried out in practical classes.

There is in the whole book no practical instruction on how to make a microscopical preparation. For this the reader should turn to one of the excellent guides to the subject, such as Langeron's *Précis de microscopie*,²⁸² or Pantin's *Notes on microscopical technique for zoologists*.³⁸³

Apart from my own little book on *Cytological technique*,³⁴ I know of only three that cover more or less the same field as the present work. These are Fischer's *Fixierung, Färbung und Bau des Protoplasmas* (1899),¹⁶⁹ Mann's *Physiological Histology* (1902),³²² and Zeiger's *Physikochemische Grundlagen der histologischen Methodik* (1938).⁵⁵³ There cannot be many equally important fields of science to which so few books have been devoted—and those few of such merit as these three.

In the historical parts of the book I have adopted the usual convention of giving as the date of a discovery the year in which it was first made known in print.

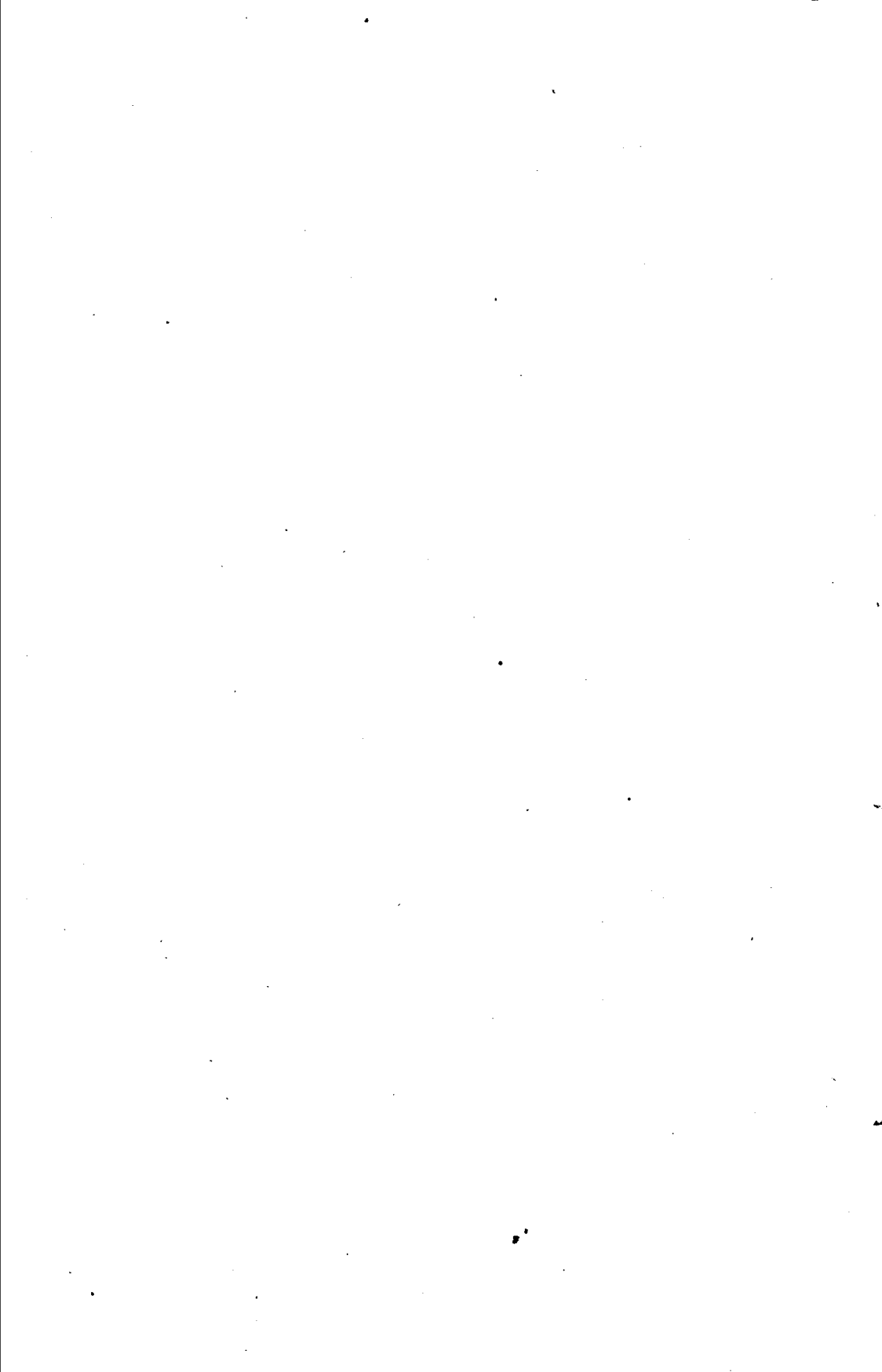
Acknowledgements. It was Mr Frank Sherlock, Head Technician of the Department of Zoology, Oxford, who first gave me instruction in microtechnique, and I have always owed a debt of gratitude to him. Dr H. M. Carleton was generous with good advice throughout our association of thirty-five years. Prof. A. C. Hardy, F.R.S., has encouraged cytological studies in his Department and I am thankful for all that he has done to help me. Dr M. Wolman generously sent me the proof of his article on fixation⁵⁴¹ before it was published. One learns best, perhaps, by continual association with lively young minds, and it has been my good fortune for many years to have a splendid succession of research-pupils from many lands, to whom I owe much. I have always been lucky in my assistants and must particularly mention Mrs B. M. Jordan-Luke,

whose skill in microtechnique has been of great benefit in my research. She has given much practical help in connexion with the experiments described in the Appendix. Mrs J. A. Spokes has helped me with this book more than anyone else, by acting as my secretary with uniform accuracy and good nature. Several of the illustrations have been copied photographically from old books and journals. Mr P. L. Small and Mr J. S. Haywood have taken a lot of trouble to produce as good copies as possible. Dr W. G. B. Casselman has most kindly made several readings of the oxidation-potentials of fixatives specially for this book.

I take the opportunity of mentioning the benefit I have derived during the last eleven years from my association with Dr C. F. A. Pantin, F.R.S., in joint editorship of the *Quarterly Journal of Microscopical Science*.

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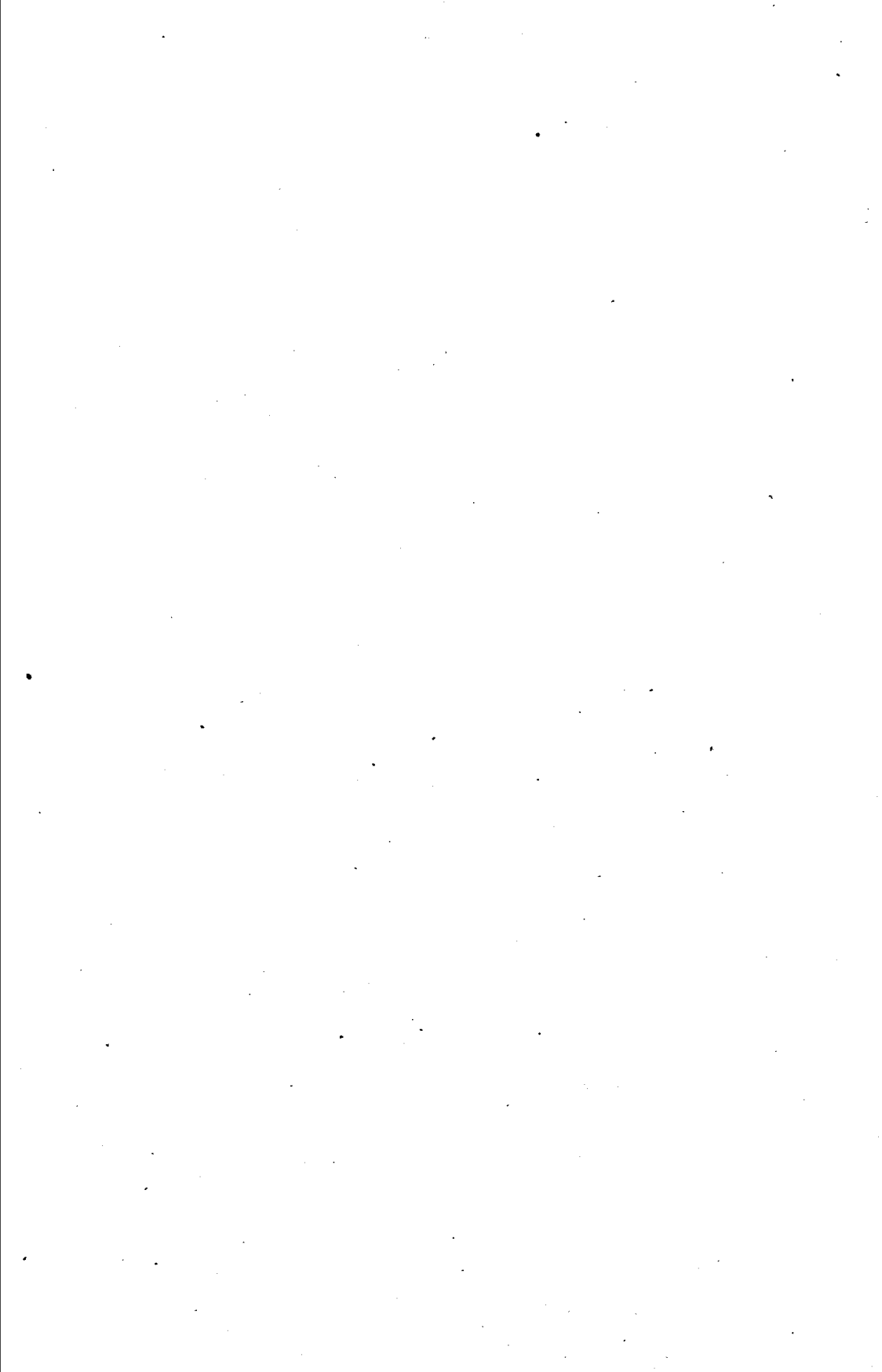
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PART ONE
FIXATION

