THE EOSINOPHIL LEUCOCYTES

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Research Haematologist at the Equine Research Station of the Animal Health Trust, Newmarket

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THE EOSINOPHIL LEUCOCYTES

To

Wm. C. M.

Foreword

This monograph may be looked upon as a new beginning—and it may well become a milestone—in our understanding of the function of the eosinophils, which can invade the tissues and which vary in number in different pathological conditions. Eosinophilia occurs in allergic states and parasitic manifestations, and the Charcot-Leyden crystals which may abound amongst the eosinophils in pronounced eosinophilia of the tissues, originate from the eosinophilic cytoplasmic granules, whereas eosinopenia which occurs in acute forms of illness is a feature of shock and a sign of an active adrenal cortex. But what is the function of the eosinophils, what is the mechanism by which eosinophilia is brought about or by which eosinopenia is produced following the injection of cortisone? So far the answers to these and other questions have been mainly based on speculation. In this monograph Dr Archer tries to answer many of these problems on the basis of new experimental findings which are the outcome of his investigations during the last eight years. The fact that he is working in an equine research station has not been a disadvantage—on the contrary—horse eosinophils have large and conspicuous granules which make them particularly suitable for staining as well as for certain other investigations. The isolation of eosinophils proved difficult until Dr Archer found that after centrifugation of whole blood the eosinophils were lying beneath the buffy coat layer and in addition that they were resistant to lysis in hypotonic solution. These findings formed the basis of his method for isolating viable eosinophils from normal blood. His method is an important step forward, and one that cannot be over-estimated as it opens up numerous new approaches to the study of these blood elements. One such approach, used by Dr Archer himself, was to inject suspensions or extracts of eosinophils into a tissue or animal and to study their effects. He found they antagonized the action of histamine and to some extent also of 5-hydroxytryptamine. Previously he had shown that

histamine, on the other hand, attracted the eosinophils. Here then are two new facts. Will they enable us to solve the riddle of the eosinophils? In this monograph an attempt is made.

Dr Archer himself shows he is aware that so far this is only the beginning, but his colleagues in various fields of medicine must be grateful to him for he was willing to stop at this stage for a moment in order to review the subject and to show us how his findings fit in with those of the past, yet giving them a different meaning, with the result that a new idea emerges about the possible function of these strange elements of the blood—the eosinophils.

W. FELDBERG

Preface

The astonishing rapidity with which eosinophil leucocytes may disappear from the blood and the equally surprising reappearance of them sometimes in large numbers, has a strange fascination. Tissues, too, may be subjected to invasion by large numbers of eosinophils, sometimes alone but sometimes accompanied by other leucocytes and the invaded areas may be many or few, small or large. The variations appear to be almost infinite. There have been numerous publications concerning eosinophils and many hypotheses have been presented to account for the known facts.

Eosinophils are conspicuous cells, especially when stained, but they are not numerous. In the blood they usually amount to no more than one cell in 100,000 of the others, while the tissue content may vary from zero to some thousands of eosinophils in a cubic millimeter.

This monograph is an attempt to present, as a connected entity, a series of experiments with eosinophils which have been made over a period of about eight years at Newmarket. For the most part, the horse has been used for these investigations, with limited use of material from man and some of the smaller laboratory animals. I have not made an attempt either fully to review the vast literature on eosinophils or to consider critically all the various theories that have been propounded about these cells, but I have tried to present a sufficient account of earlier work to indicate the complexity of the problem of the eosinophil in physiological and pathological conditions.

The book is divided into three parts. The first is intended as a review of the work on eosinophils, mainly prior to 1955, in such a way that the problem I have studied is presented.

The second part is a description of the experimental investigations that have been made in this laboratory. I have arranged this section substantially in chronological order, as the work was done, and I

have tried to show how the various facets of the puzzle were explored in turn. When it became necessary to prepare some of my earlier work for publication I was frequently struck with the desire to repeat or redesign certain experiments. However, this has not been done since the various questions that arise are, I believe, adequately covered by later work.

The third section is a general discussion of the experimental work from other laboratories and from my own. I have tried to present a hypothesis which will account for the strange behaviour of eosinophils, as concerns both their distribution and their functions. This hypothesis accounts, I think, for most of the known facts about eosinophils and allots to them a primary physiological function which is intimately concerned with histamine.

Finally, I am often asked why I have chosen to use the horse for experiments on eosinophils. There are several reasons for this, of which perhaps the most important is the large and most conspicuous cytoplasmic granules of equine eosinophils. The cells are easy to identify, whether fixed and stained, or alive, by these characteristic granules. Since the horse is a large animal, it can provide quite large volumes of blood or bone marrow fluid without any change in the physiological *status quo ante*. In addition, working at an Equine Research Institute implies that horses have been readily available to me, and they are tractable to handle. On the debit side, horses are expensive to purchase and to keep, and they are difficult subjects for certain major surgical interferences which might throw light upon physiological problems.

Such, then, are the intentions of this monograph. It is admittedly biased since I have concentrated on my own ideas and conclusions to the relative exclusion of others. I hope, however, that it will make some contribution to the better understanding of these fascinating cells which we call eosinophils.

Newmarket, 1963 R. K. ARCHER

Acknowledgments

No scientific monograph of this type would be written were it not for the work undertaken and published by workers all over the world in previous years. I have yet to see this indebtedness adequately expressed, but I am sure that every scientist will agree to its pertinence.

I have been fortunate to have as my chief Professor Wm. C. Miller, Director of the Equine Research Station of the Animal Health Trust. Without his constant encouragement and advice this whole project would have foundered many times. Professor Miller has made invaluable suggestions and has always taken a full and most gratifying interest throughout the whole of these studies. Furthermore, during the nine years that I have studied eosinophils, the Animal Health Trust has placed at my disposal all the animals, laboratory space and equipment which have been used. Indeed, without the contributions of many animal owners to the funds of the Trust, upon a purely voluntary basis, none of this project could have been undertaken.

The work described in Chapter 8 was done in conjunction with my colleague Dr D. Poynter, now with Messrs. Allen & Hanbury, Ware, and that in Chapter 11 with Dr D. Franks, now at the Cambridge University Department of Pathology. In Chapter 14 I have described experiments upon bronchospasm in guinea pigs which were performed with Dr W. Feldberg and Dr B. A. Kovacs of the National Institute for Medical Research, Mill Hill, London. Dr Feldberg has allowed me many greatly valued hours of his time in extensive discussions of my studies and of this manuscript, and his unique knowledge of histamine, first planted in me some twenty years ago when I was a pupil of his, has been of incalculable value to me.

In 1961 I had the good fortune to be joined by a chemist, Mr J. Broome. Mr Broome and I have been, and still are, engaged upon

chemical and other studies of eosinophils. Some of our results are reported in Chapters 5 and 15. Dr F. Wild of the Department of Medicine, University of Cambridge, has also helped me greatly by reading the manuscript and in many other ways, especially in the more chemical fields of my studies.

Throughout the period I have been fortunate indeed to have had first class technical assistants. Mrs Taylor was with me while almost the whole of the work described in this book was done and she has made most of the drawings in it. I have also had invaluable technical assistance from Miss Cowley and Miss Ritchie. Mrs Finch has somehow managed to decipher my almost illegible writing and has turned it into intelligible typescript, frequently, I fear, only to have it altered extensively again. Special centrifuge tubes have been made to my design by Wesley Coe (Cambridge) Ltd. Mr R. Flemans of the Cambridge University Medical School made the leucocyte mill, the skin biopsy punches and several other special pieces of equipment.

I am obliged to the Editors and Publishers of several scientific periodicals for permission to use some of the figures that have appeared in various papers that I have written. These are: *The British Journal of Haematology* (Figures 56, 57, 59 and 62); *The Journal of Pathology and Bacteriology* (Figures 35, 45, 46 and 47); *The Journal of Comparative Pathology and Therapeutics* (Figure 43); *Nature* (Figures 33 and 34); and the *British Journal of Pharmacology* (Figures 67 and 68).

PART I
A STATEMENT OF THE PROBLEM OF EOSINOPHILS

CHAPTER I

The Discovery and General Microanatomy of Eosinophil Leucocytes

Just about 100 years before I became interested in eosinophils, Wharton-Jones (1846) gave a description before the Royal Society of 'coarse granular cells' of the blood. Using a Ross compound microscope with a $\frac{1}{8}$ in. objective, he described and illustrated the leucocytes and erythrocytes. There is no mention of the use of any anticoagulant, yet the description and drawings are outstandingly clear (Fig. 1). The largest granules in any of the cells he examined were in the 'coarse granular cells' of the horse. Undoubtedly this description is of the cells now called 'eosinophil leucocytes' since only these have large and abundant cytoplasmic granules.

It was not until the classical work of Ehrlich, which appeared in 1879, that eosinophils were so named. The cytoplasmic granules of these leucocytes exhibit an intense affinity for eosin and this property has been used to identify and to enumerate the cells to the present day.

The origin and development of blood cells in the bone marrow was beautifully described by Sabin and her colleagues in 1925 (Cunningham, Sabin and Doan; Doan, Cunningham and Sabin; Sabin, Doan and Cunningham). Many papers have added details since this time and the marrow of many different species has been studied. Eosinophils can be identified in mammals, fish and reptiles. In birds, the position is less clear since the so-called heterophil is morphologically similar to mammalian eosinophils. It is generally assumed, however, that the 'true' avian eosinophil is distinguishable since the granules of the very much commoner heterophil are somewhat ovoid, while the eosinophil has spherical granules similar to those seen in

mammals. In all the mammalian species examined, eosinophils develop in the red bone marrow. Some of the stages of maturation in man and in the horse are illustrated in Figs. 2 to 5.

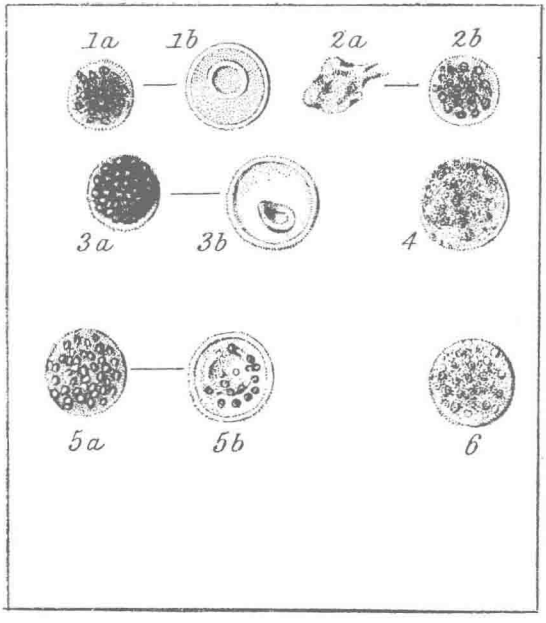

FIG. 1. *Drawings by Wharton Jones (1846) of blood leucocytes*

Cell 1a is probably a human eosinophil, cell 3a an equine eosinophil and cell 5a an elephant eosinophil. The original legend is as follows:

'Legend 1a—the granule cell of human blood in its coarsely granular stage distended by water.
" 1b—the same, after being acted upon by acetic acid.
" 2a—the granule cell of human blood in its finely granular stage in a state of collapse.
" 2b—the same, distended by water.
" 3a—the granule cell of the horse in its coarsely granular stage.
" 3b—the same, after being acted upon by acetic acid.
" 4 —the granule cell of the horse in its finely granular stage.
" 5a—the granule cell of the elephant in its coarsely granular stage.
" 5b—the same, after being acted upon by acetic acid.
" 6 —the granule cell of the elephant in its finely granular stage'.

In bone marrow, the earliest identifiable form of eosinophil leucocyte is a late myeloblast or early promyelocyte. At this early stage of development the differentiation into the eosinophilic form of this

Contents

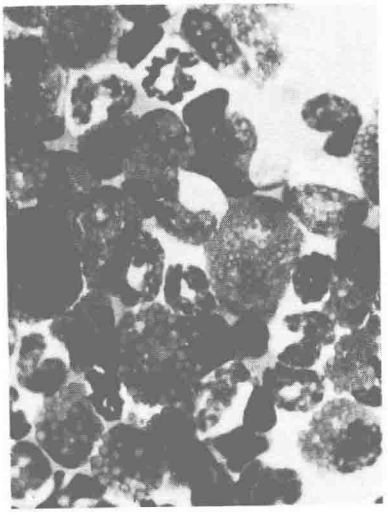

FIG. 2. *Equine bone marrow film, Leishman stain, × 231*

Three eosinophil myelocytes are near the centre; note the small but very abundant cytoplasmic granules. There are also various mature or staff eosinophils with fully developed granules.

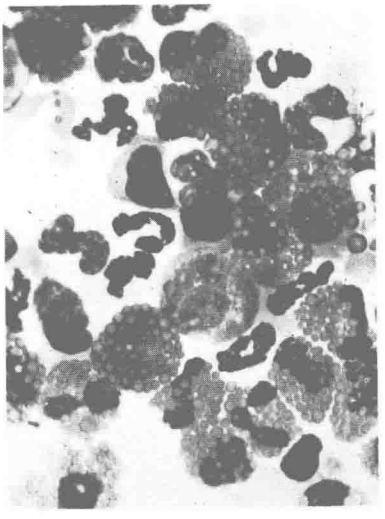

FIG. 3. *Equine bone marrow film, Leishman stain, × 231*

An eosinophil promyelocyte is at the centre; note the small and scarce granules. A metamyelocyte at the right centre shows granules larger than those of the myelocytes of Fig. 2.

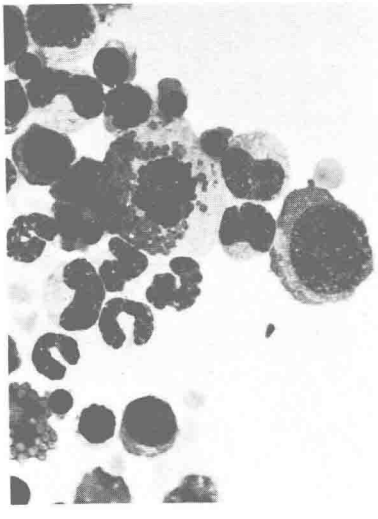

FIG. 4. *Equine bone marrow film, Leishman stain, × 231.*

An eosinophil myelocyte in mitosis.

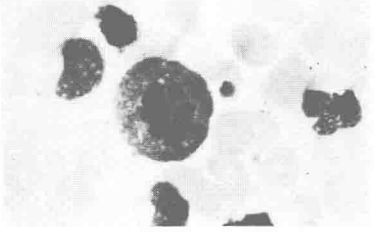

FIG. 5. *Human bone marrow film, Leishman stain, × 231.*

An eosinophil in mitosis.

facing p. 2