

Frontespiece. Activated blood eosinophils.

1. Preface.

This book is designed to provide a comprehensive overview of eosinophils in man, and animals. It includes a review of over 2 000 selected scientific, and medical references in this area, published up to the end of 1987. The book covers the cell biology of eosinophils, and the clinical disorders in which eosinophils are important. The source database of 5 298 references, which was derived from 4 125 Medline entries, listed between January 1977, and January 1988, and an additional 1 173 references from other sources, is available as a supplement to the book.

Some of the significant advances in our knowledge of eosinophils during the past decade have come from the development of reproducible methods for purifying, and culturing them, the cloning of the eosinopoietic factors, the discovery of eosinophil activation, and the isolation, and characterization of many of the molecules synthesized, and secreted by eosinophils. Their role as effector cells in disease is now accepted, and they are now seen as specialized cells, with several unique constituents, which give them a distinctive range of effector functions in disease. The applications of this knowledge in human diseases is already showing promise, in our study, and treatment of several haematological, cardiac, respiratory, dermatological, parasitic, and other diseases. Current research is aimed at finding out what induces eosinophils to become activated, and degranulate, and the effects their secretion products have on cells, and tissues.

This book has been written as a guide to the literature on eosinophils, and the diseases where eosinophils may have a role. I hope that it will stimulate new approaches to this field, the questioning of dogmas about how eosinophils localize in vivo, and interact with other cells, parasites, and allergens, so that more can be learnt about how eosinophils contribute to the beneficial, or harmful effects of allergic reactions. I also hope that it will enable newcomers to the field to obtain an overview of eosinophils, and provide specialists at the bench, or in the clinic, with a ready source of information to help them in their work.

I owe my apprenticeship, what success I may have had in this field, to Professor Paul Beeson, and to my clinical, and scientific colleagues in Oxford, and London. The book could not have been written without the help of Dr. Po-Chun Tai, Peter Ison, Kathy Maguire, and the librarians in the Wellcome Library at the Royal Postgraduate Medical School, St. George's Hospital Medical School Library, and the library of the Royal Society of Medicine, London. I am also very grateful to Julia Maidment, and the staff at Oxford University Press, and many others who have contributed to the preparation of this volume.

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1. Chapter A 01. Introduction, and history.

Eosinophils are cells which are produced in the bone marrow, and migrate through the blood to the tissues, where they carry out a wide range of different functions. They are a well defined, and distinct cell type which are prominent in histological sections, as they stain strongly with eosin, which is used with haematoxylin, to define cytoplasmic structures, and nuclei in cells. They have been found in all vertebrates species which have so far been examined, and they are usually classified as polymorphonuclear leukocytes because the nucleus in the blood form of the cell has two, or three lobes, similar to neutrophils. Like neutrophils, they possess characteristic granules, which suggests that the two cell types have much in common. However, this apparent relationship is misleading, as eosinophils are derived from a different progenitor cell than neutrophils, and they should be considered as distinct from neutrophils as they are from macrophages.

The complex life history of eosinophils is only beginning to be unravelled with the availability of special techniques for studying them in vivo. They develop from primitive stem cells which are present in the bone marrow, and blood. These stem cells divide, and differentiate, acquiring the distinct granules which are the special feature of eosinophils. After a few hours maturing further in the bone marrow, they enter the blood stream, and are distributed throughout the body, where they first attach to vascular endothelium, and then migrate into perivascular sites. This is their normal place of residence in tissues, although in many diseases they migrate further into areas of inflammation. There are normally very few eosinophils in the blood (less than $0.6 \times 10^9/L$), and tissues, but in certain diseases they can be produced in enormous numbers. The highest reported blood eosinophil count in man is $326.4 \times 10^9/L$ in a Nigerian patient with eosinophilic leukaemia described in 1970²⁰⁸. Eosinophils are stimulated by one, or more of a range of signals from adjacent cells, including reticular cells, endothelial cells, lymphocytes, macrophages, and other cells, which induce them to differentiate further, and to become activated. Further stimulation with complexed IgG, IgE and/or C3b, and other products of inflammation, causes them to secrete their stored granule contents, and to synthesize, and secrete membrane-associated components, especially lipid mediators. These events take place over several hours, and can continue for as long as they remain alive, which may be for only a few hours in some settings, or many days, or weeks in others.

The principal function of eosinophils is to synthesize, and deposit their stored, and newly formed molecules, in areas of acute, and chronic inflammation. They are also able to ingest particles, such as bacteria, after they have become activated, although this function is more often carried out by neutrophils, and other phagocytic cells.

A comparison of eosinophils, and neutrophils in general terms is as follows: eosinophils are mainly perivascular (tissue) cells, whereas neutrophils defend intravascular sites; eosinophils respond to IgG, IgE and complement components, whereas neutrophils do not respond to IgE stimulation; eosinophils 'secrete' whereas neutrophils 'ingest'; eosinophils mostly take part in acute, and chronic hypersensitivity reactions, and are often found close to other intact cells, whereas neutrophils are most commonly present in areas of acute necrosis, where they are the main component of pus. Eosinophils are mainly associated with allergic, parasitic, and chronic inflammatory responses, whereas neutrophils are primarily found in acute bacterial infections, ischaemic lesions, and reactions to damaged cells, and tissues.

1.1. Discovery, and description of eosinophils.

At the beginning of the 19th century, compound microscopes were used to examine many different tissues, including blood samples. In 1846 Wharton-Jones, an anatomist at Charing Cross Hospital, London, U.K., was able to distinguish granulated cells from red cells in the blood of several species, including the frog, the elephant, and man. He added water, or acids to the preparations to make the cells swell, so that he could see the nuclei¹⁹²¹. With these simple microscopes, and without a staining method, it is unlikely that early microscopists could have distinguished eosinophils from other granulated cells. However eosinophils may have been seen in the blood of two patients with eosinophilic leukaemia in 1861, and 1876, and in bone marrow samples in 1862, and 1869, according to T.R. Brown (1898)²¹⁹.

It was not until the classical studies of Paul Ehrlich in 1879, when he was a young doctor in Leipzig, that the best known characteristic of eosinophils was discovered: its capacity to be stained with negatively charged chemicals, especially eosin. His paper on this subject 'Ueber die specifischen Granulationen des Blutes', was presented to the Berlin Physiological Society on 17 January 1879⁴⁹⁸. The history of Paul Ehrlich's discovery of the staining properties of eosinophils was described by the Hirschs in 1979, 100 years after their discovery⁸⁰¹. His technique was to make a blood smear, and to fix it by heating at 110° for 60 to 90 minutes. Then the smear was stained with a mixture of orange G, acid fuchsin, and methyl green (Ehrlich's triple stain). Tissue sections were stained with haematoxylin, and eosin.

Eosin is a brominated fluorescein derivative, and not an aniline dye. Its general use, with haema-

toxylin, as the standard stain for tissue sections, was one of the main reasons why eosinophils were so easily noticed in disease, as there are few cells, and structures which stain as prominently. We now know that eosin stains eosinophils because they possess granules which contain markedly cationic proteins which preferentially bind eosin, and other negatively charged stains.

In 1879 Ehrlich suggested that the bone marrow was the site of origin for eosinophils⁴⁹⁷. He published a number of papers in German, and later wrote a monograph in English⁴⁹⁹, which shows the high quality of his work, and the remarkable insight he had into the nature, and importance of eosinophils. For example in 1900, he suggested that 'eosinophils exert their functions in tissues'⁴⁹⁹. Initially, there was opposition from many physicians to his proposal that it was useful to stain blood cells. However, his methods soon became widely recognized as of great benefit in general medicine, and this led to the discovery of many eosinophil-disease associations. These findings dominated much of the literature on eosinophils during the subsequent thirty years.

The standard methods for studying eosinophils at the turn of the century was to stain either tissue sections, or blood smears. Differential blood counts were then carried out, and the results were given as a percentage of the total white cell count. Absolute counts were often provided as well, but many doctors thought that percentage data was more useful, than absolute counts. We now know that percentage data on its own, is of no clinical value.

In 1910, hypotonic solutions were introduced to lyse red cells in blood samples, so that eosin could be used to count the number of eosinophils directly⁴⁷⁵. From this grew the standard methods used today, in which an acetone/water/eosin solution, or a comparable mixture, is used to count eosinophils in suspension.

An important technical difficulty in this field is that eosinophils are predominantly tissue cells, and large numbers can be present in the tissues, when there are only a few in the blood. Blood counts reflect a complex relationship between cells leaving the marrow, marginating, and leaving the blood. There is no significant recirculation of eosinophils back from the tissues to blood. For this reason, blood eosinophil counts are of most value when there is a steady state. Single blood counts cannot be used either as a measure of the number which are being produced by the marrow, or the number being deposited in tissues.

The presence, and importance of eosinophils in different diseases soon began to be recognized. These included descriptions of them in asthma in 1889⁶⁹³, ankylostomiasis in 1891³⁷², skin diseases including prurigo, urticaria, psoriasis, and pemphigus in 1892^{266,1272}, malignant diseases in 1893¹⁴⁸⁵, and trichinosis in 1898²¹⁹.

1.2. The structure, cell biology, and effector functions of eosinophils.

Present day knowledge of eosinophils began with the research programmes set up in the early 1960s by James Hirsch at the Rockefeller Institute, New York, U.S.A., in which he, and G.T. Archer isolated horse blood eosinophils, and studied their granule enzymes, their capacity to phagocytose, and to degranulate in vitro⁵⁵. Cine films were made, and high quality photomicrographs were taken of this process. It was noted that cells often degranulated, releasing lysosomal enzymes, and large amounts of a peroxidase⁵⁶. This work was followed by high resolution electronmicrographs of the crystalloid structures inside human, and rat eosinophils, and changes within the cell undergoing phagocytosis. On his return to Australia, G.T. Archer began to look at the interactions between eosinophils, and parasites, and demonstrated that this induced eosinophil degranulation. He postulated that eosinophils might be involved in parasite killing, and possibly in other disease processes, such as mucus production in the bronchial epithelium in asthma.

During the early 1960s it was thought that one of the main roles for eosinophils was to ingest, and remove foreign proteins¹⁵³⁶. This concept was supported by experiments in rodents, which showed that, within five minutes of injecting immune complexes into the foot pad of rats, eosinophils appeared in the medulla of draining lymph nodes^{1085,1086}. Another hypothesis was that eosinophils processed antigens, and presented them to lymphocytes. Unfortunately these experiments did not throw light on the functions of human eosinophils, which seemed to have a much more limited

phagocytic capacity than rodent eosinophils.

It was also thought that one of the major functions of eosinophils was to inactivate histamine. In 1968, R.K. Archer published a review of what was then known about eosinophils, their biochemistry, numbers in tissues, methods for isolation, and their possible roles in antigen-antibody reactions⁶⁰.

The discovery that basophils, and mast cells, but not eosinophils, contain histamine, then led to the suggestion that eosinophils might inhibit the functions of mast cells, by degrading mast cell-derived mediators in tissues⁶⁸⁴. Experiments in Prof. K. Frank Austen's laboratory in Boston, U.S.A., using the latest techniques of protein chemistry began to define the constituents, and properties of eosinophils in acute hypersensitivity responses, especially their capacity to interact with, and inhibit mast cell products. Their initial findings supported the concept that eosinophils could inhibit several mediators from mast cells, especially histamine, the slow reacting substance of anaphylaxis (SRS-A), which had not then been characterized, platelet activating factor (PAF-acether), and heparin.

In the mid 1960s two research projects, one in Oxford, and the other in Cambridge, looked at eosinophils in animal models of disease. In Cambridge, Dr. A. Barry Kay, and his colleagues, started to work on the hypothesis that eosinophil localization in guinea pig tissues was due to the production of specific chemotactic factors. He continued this work in Prof. Austen's laboratory, and reported in 1971 the discovery of an 'eosinophil chemotactic factor of anaphylaxis' (ECF-A)⁹³³. Since then, with ECF-A as a model system, many chemotactic factors have been extracted in various degrees of purity from cells, tissues, and parasites, and shown to affect eosinophil directional movement in vitro.

In Oxford, Prof. Paul Beeson, and a small group, myself among them, studied the mechanisms of eosinophilia in rats. By 1968 Dr. Tony Basten, and Beeson had shown that T lymphocytes regulated eosinophil production in rats injected intravenously with *Trichinella spiralis* larvae. They concluded that the eosinophilia was an integral part of an immunological response, which was T cell dependent¹³⁶. Subsequent work has expanded, and built on this, and the T-lymphocyte dependency of the response to many molecules which stimulate, and affect eosinophil function has become an area of increasing importance.

In the early seventies, Dr. Sheldon Wolff, and colleagues at the National Institutes of Health, Bethesda, U.S.A., who were interested in studying vasculitic diseases, began to be referred patients with a marked eosinophilia. They were found to have distinct clinical features which set them apart from patients with other vasculitic disorders, such as polyarteritis nodosa. No cause for their eosinophilia was found, so they were classified under the general term of the 'idiopathic hypereosinophilic syndrome' (HES). Their findings in 14 patients with HES were published in 1975³²⁵. This group of patients increased in number over the subsequent decade, and it is now the largest group of patients with this disease.

In 1972 Dr. Inge Olsson, and colleagues in Lund, Sweden, who were interested in the biochemistry of granulocytes in patients with leukaemia, first isolated eosinophil cationic protein (ECP) from the blood cells of a patient with chronic myeloid leukaemia¹³²⁸. By the end of the 1970s it became apparent that ECP was one of the principal mediators of eosinophil-dependent effects on tissues, and cells.

In 1973 Dr. Gerry Gleich, and colleagues at the Mayo Clinic, Rochester, U.S.A., isolated the eosinophil major basic protein (MBP) from guinea pig eosinophils⁶⁶⁴. In 1976 his group showed that MBP was also present in human eosinophils⁶⁶⁵. The main thrust of this work, during the late 1970s, and early 1980s, was to determine the importance of MBP in immunobiology, including its capacity to injure parasites, and other cells.

In 1971 it first became possible to grow human eosinophils, as colonies in vitro^{312, 844}. The finding that they were distinct from neutrophil, and mixed neutrophil/macrophage colonies showed that they belonged to a different lineage. It soon became clear that eosinophils were also different from neutrophils in many other respects. Subsequent work on eosinophil production in vitro was slow,

due to the difficulties in preparing pure eosinopoietic factors. However by 1987, three of the principal eosinophil colony stimulating factors, IL-3, GM-CSF, and IL-5, had been cloned, and studied as recombinant molecules.

In the mid 1970s I became interested in looking after, and studying patients with a marked eosinophilia, and I began to collect together patients with HES, and eosinophilic leukaemia. As many of these patients had endomyocardial fibrosis, I then began to work on the association between eosinophils, and heart damage. With colleagues in the clinic and laboratory, we were able to show that eosinophils were responsible for causing this serious cardiomyopathy. At this time experiments also began to be done which have led to the concept that eosinophils are predominantly secretory cells. This was shown using a variety of immunological stimuli, and assays for each of the granule constituents, which had then become available.

Although several people in the late 1960s, and early 1970s had raised the possibility that eosinophils might be involved in parasite immunity, it was not until a paper was published by Butterworth, and colleagues in 1975 on eosinophil-dependent killing of the schistosomula of *Schistosoma mansoni*²⁵⁵, that major studies began in this area. This assay was then adapted to study how eosinophils might function. By 1977 it became clear that eosinophils were pro-inflammatory cells, and the concept of mast cell 'downregulation' by eosinophils had become less attractive. Although there was some evidence that enzymes in eosinophils could degrade basophil, and mast cell products in vitro, it was found that neutrophils contained larger amounts of histaminase than eosinophils, that eosinophil arylsulphatase could not break down SRS-A, and that eosinophils themselves produce large amounts of PAF-acether, and leukotriene C4.

1.3. The roles of eosinophils in disease.

Most of the hypotheses about the roles of eosinophils in disease have been based on particular properties which they were thought to possess at the time, which made them distinct from other cell types. At the turn of the century, it was thought that they had a protective role in removing debris, and detoxifying components of tissue reactions in allergic, and granulomatous diseases¹⁵⁸⁰. This view persisted, with minor variations, and was adapted in the 1960s, to include roles in histamine effects, and antigen presentation for antibody synthesis¹⁶⁶⁹.

In the mid 1970s most work on the roles of eosinophils in disease was based on the hypothesis that eosinophils 'down regulated' mast cell mediated responses⁸⁰. Other possible roles for eosinophils have also been proposed over the last one hundred years. These include the remodelling of tissues, for example by stimulating connective tissue catabolism in the uterus of animals undergoing oestrus¹³³, acceleration of repair mechanisms following trauma^{101, 135}, and the stimulation of mucus production in glandular epithelium⁵².

By the mid 1970s, the modern view of eosinophils, and their functions had come to the fore: that they are 'pro-inflammatory' cells involved in defence against large potentially harmful organisms, such as nematodes. They are also known to have a marked capacity to secrete their stored granule components, and newly formed lipid mediators, and products of oxygen metabolism, which affect adjacent cells, and tissues in inflammation. These effects may be reversible. For example, they may induce a permeability change in rat uterine blood vessels¹⁷⁶¹, and produce angioedema in man⁹¹⁹). Occasionally eosinophils can damage, and even kill adjacent cells.

My own approach to the issue of the functions of eosinophils, which has support of others¹²⁴, is to assess the roles of eosinophils in each setting in which they are found, in an unprejudiced way. There is no good reason for thinking that they have only one function. Just as it is no longer possible to provide a unifying concept of the 'role' of macrophages in disease, I feel that eosinophils also have no single function. They affect cells, and tissues in many different ways, according to the settings in which they find themselves, and the range of stimuli which act on them. For these reasons I suggest that no unifying hypothesis is possible, or even necessary, when one considers the remarkable variety of diseases in which eosinophils are present.

2. Chapter A 02. Eosinopoiesis.

The morphological features of human eosinophil differentiation in the marrow have been known for many years. This involves their development from the promyelocyte stage, through the myelocyte stage, to the metamyelocyte stage, when eosinophils can no longer divide, although they continue to mature. Light microscopy, and ultrastructural studies have shown the changes which occur in the crystalloid granules as the cells mature. The granule proteins are synthesized on the rough endoplasmic reticulum, and are transported to the Golgi apparatus, where they undergo several changes which have yet to be defined, but presumably involve glycosylation, and changes in tertiary structure. Then they are transported to the crystalloid granules, which gradually take on the characteristics found in peripheral blood eosinophils.

The microenvironment in which eosinophils develop in the marrow is important determining the rate, and the number of cell divisions that they undergo. Reticulum cells, which may have a 'nurse' function have been seen in electronmicrographs of rat bone marrow¹⁵⁴¹, but the way in which these cells might interact with eosinophil precursors is not known. Present day work on eosinopoiesis, and eosinopoietic factors is limited to studies on the effects of cloned stable molecules. The possible effects of factors with a short half-life, and direct stimulation by cell-cell contact remains to be explored.

Eosinopoiesis only occurs outside the human bone marrow in disease. However in rodents, eosinophil colonies containing dividing eosinophils are occasionally found in the spleen, and this is also the site in which rodent eosinophils mature after leaving the bone marrow, before they re-enter the blood.

2.1. Ontogeny of eosinophils.

In man, eosinophils, and basophils appear together in the early fetus, and increase in number in the blood during the first, and second trimester. This was documented in 1985, in blood samples from 99 normal fetuses which had undergone direct vision fetoscopy¹²¹¹. Unlike other species, myelopoiesis does not take place in the human liver, and it is confined to the marrow at birth.

The ontogeny of blood cells in cats has been studied in detail. Myelopoiesis occurs in the yolk sac longer than in other mammals, and in this site recognizable eosinophils appear after neutrophils, concomitantly with the first production of lymphocytes. The proportions of leukocytes which are eosinophils remain unchanged during embryogenesis. In cats, eosinophils are produced in the liver during the second half of embryogenesis, but this changes to the bone marrow. Some eosinopoiesis can be found in cat lymphoid tissues at birth¹⁷⁸². This sequence of events is also seen in the horse, where eosinopoiesis also occurs in the thymus for some time after birth.

2.2. The eosinophil lineage.

There are a number of reasons for believing that eosinophils, basophils, and occasionally erythrocytes, and lymphocytes, can develop from a common precursor cell. The relationship of these cells to neutrophils, monocytes, and megakaryocytes is more distant. Lineage pathways have been proposed for progenitor cell differentiation into mature blood cells. However, it is more likely that progenitor cells are susceptible to the differential effects of lineage-directing growth factors for much longer in their life history than lineage pathways suggest. This was shown in 1985 when the two progeny of single progenitor cells were able to develop into two different colonies, including eosinophil colonies¹⁰⁴⁴. Single progenitor cells from umbilical cord blood were also found to be able to develop into mixed colonies¹²⁶³.

2.2.1. Common origins with basophils.

Clinical evidence to link the differentiation of eosinophils, with basophils, and not neutrophils has come from several sources:

1. Defects in both eosinophils, and basophils have been seen^{899, 1793},
2. In patients with chronic myeloid leukaemia, a variable proportion of the immature, and more

mature leukaemic cells contain both eosinophilic, and basophilic granules^{1947, 1896}. In a study from Essen, West Germany published in 1986, leukaemic cells with both types of granule were detected in all 13 patients studied, when care was taken to look for them with special staining techniques¹²²⁷. Doubly granulated cells have also been seen in patients with myelofibrosis, and in cultured cord blood cells⁴³³.

3. A patient was described in 1983, who had cyclical depression of neutrophil, and monocyte production in the marrow, with normal eosinopoiesis¹⁶. Neutrophil promyelocytes disappeared from the marrow during these episodes.

4. A patient was described in 1980 with transformation of chronic myeloid leukaemia to a disease in which blood basophil counts rose to more than $100 \times 10^9/L$, with large of eosinophils in the tissues¹²⁸¹.

Biochemical studies on eosinophils, and basophils are equally convincing. Both cell types contain MBP, and the Charcot-Leyden crystal (CLC) protein, although eosinophils do not contain histamine. However MBP, and histamine are present together in 'eosinophil-type', or 'type II' colonies grown from human blood, which contain basophils⁴³². In 1984 it was shown that basophils, and eosinophils can develop in agar cultures from a common progenitor cell¹⁰⁴². In 1985 the plating efficiency, and the glucose 6 phosphate dehydrogenase isoenzyme content of type II colonies also showed that they were clonally derived⁴³³.

2.2.2. Common origins with some lymphocytes.

There is clinical evidence that lymphocytes, and eosinophils can develop from a common precursor cell. In 1977, a 41 year-old man in Copenhagen, Denmark, was reported with a T cell lymphoma, and hypereosinophilia with atypical eosinophil granules, which suggested that both cell types were involved in the malignant process¹¹⁵¹. In 1979, a two and a half year-old boy was seen in London, U.K., who had a lymphocytic leukaemia with increased numbers of eosinophils, and basophils¹³⁶⁰, and we have seen several patients who had lymphomatoid papulosis (a paraneoplastic disease affecting T cells), and the idiopathic hypereosinophilic syndrome (HES)¹⁹²⁶. I have also seen two patients with eosinophilic leukaemia, who had bony deposits of undifferentiated leukaemic cells which had several phenotypic features of lymphoid cells. Several patients with a biclonal leukaemia, involving lymphocytes, and eosinophils were described between 1984, and 1987^{154, 1923, 943, 995}.

2.3. Morphology of eosinophil differentiation.

There is little information on the nature of the cellular interactions that occur in the bone marrow at the sites of eosinophil differentiation, although it is probable that short range interactions are important³³². Weiss has shown that reticulum cells are often present in the bone marrow of rodents, adjacent to developing eosinophils¹⁵⁴¹. These may be sources of eosinopoietic factors.

A number of electronmicroscope (EM) studies have been carried out on the development of eosinophil granules in human bone marrow. EM studies in 1970 showed that condensation of the early granule contents transformed them into typical mature crystalloid granules¹⁵⁸⁴. This was confirmed in 1976²⁰⁴. In 1977, EMs of bone marrow from a patient with a marked eosinophilia led to the suggestion that granule formation begins in the endoplasmic reticulum. The granules appeared to become progressively more dense, possibly due to interaction with the Golgi vesicles, producing dense immature granules. Some granules were found within large vacuoles, and as the vacuole membranes were seen to fuse with the plasma membrane, it was suggested that some of the granule material was being secreted during their development⁴². The work of Zucker-Franklin on developing eosinophils in agar cultures suggested that there was continued transport of new granule material from the Golgi vesicles to the crystalloid granules during the promyelocyte stage²⁰⁰⁷.

In 1987, morphometric EM studies in Israel on normal rat bone marrow, and peritoneal eosinophils showed that the volumes of eosinophil granules in both sites were multiples of the smallest sized granules. This suggested that larger granules were formed by the fusion of smaller ones, and not by continuous growth in size of each granule⁵⁰⁸. This has not yet been studied in human eosinophil development.

There is ultrastructural evidence that eosinophil granules, and their protein contents, are secreted during eosinophil differentiation. Some newly synthesized lysosomal granule enzymes, which have been phosphorylated, but which fail to bind to mannose 6-phosphate receptors for transport, and packaging in granules, are known to be secreted⁹⁹⁹. However it is not known if the basic proteins in eosinophils are treated as lysosomal enzymes, or whether there is some other way in which they are packaged in the granules. Scott, and Horn first reported in 1970 that immature normal human bone marrow eosinophils sometimes contained tubular sinusoids filled with dense material, which often communicated with the extracellular space. Electron dense material, which resembled sinusoidal contents, was found on adjacent cell surfaces¹⁵⁸⁴.

This process could account for the reduced number of granules which can be seen in blood eosinophils from some patients with a marked eosinophilia, such as HES. In one patient with 'eosinophilic leukaemia', eosinophil granules were shown to be secreted at the promyelocyte stage of differentiation⁴².

This topic was studied further at the Mayo Clinic in 1984²⁴². Serial samples of blood were collected from bone marrow biopsy needles put in the posterior iliac crest of 15 normal individuals. Measurements of the concentrations of MBP, and CLC protein showed that initially high MBP levels decreased until they were the same as in the peripheral blood. On the other hand, although CLC protein levels also fell, they remained at over twice the serum level. Supernatants from their marrow cultures contained both MBP, and CLC in quantities which were related to the amount of eosinophil colony growth that had occurred. Electromicrographs of the colonies also suggested that there was some release of eosinophil granule constituents from immature eosinophils.

2.4. Kinetics of eosinophil production.

Normal adult human bone marrow contains 3 to 3.5 per cent eosinophils, of which 37 per cent are myelocytes, 26 per cent metamyelocytes, and 37 per cent granulocytes. Sixteen per cent of the myelocytes are undergoing DNA synthesis at any one time, and can be 'flash' labelled with radioactive nucleotides. The emergence time of eosinophils (the time from the end of the last cell division until they appear in the blood) in normal people is 2.5 days. In 1979 two studies were reported from Germany on the kinetics of eosinophil production, which was assessed using ³H-thymidine labelling. Flash labelling in three normal subjects showed a mean turnover of eosinophils of 0.22×10^9 cell/kg per day, and a mean post-mitotic eosinophil reserve capacity of the bone marrow of 0.14×10^9 cells/kg¹⁸⁷¹. The large reserve capacity could contribute to the rapid rise in blood eosinophil counts which occurs in some circumstances. Labelling by continuous infusions for three to 10 days in two patients with normal blood eosinophil counts, and six with chronic lymphocytic leukaemia showed that the mean eosinophil intramedullary maturation, and storage time was 103 hours¹⁷⁰⁷.

In 1971 I published details of the kinetics of eosinophil production in normal rats, and rats which were induced to produce an eosinophilia^{1676,1677}. The percentage of labelled eosinophil metaphases was assayed at intervals after a single injection of ³H thymidine into each animal. I noted that large sized eosinophils were cells which were about to undergo cell division, but that size alone did not define their stage of maturation. The cell cycle time for stimulated eosinophils was much shorter than that for unstimulated eosinophils. In rats with an eosinophilia, the number of cell divisions which took place before definable eosinophils entered the blood increased from three to five.

In inflammatory diseases with an eosinophilia, there is usually an increase in neutrophil as well as eosinophil counts in the blood, but there seems to be no competition for a precursor cell, i.e. eosinopoietic factors do not appear to divert cells from neutrophil synthesis. On the other hand, there is some evidence, from studies on neutropenic patients with an eosinophilia, that mature eosinophils may suppress the formation of neutrophils, by releasing prostaglandins¹⁷⁶⁴.

2.5. Assays for eosinopoietic factors.

A number of assay systems have been developed to study eosinophil development in vivo. These include assays with peritoneal chambers, and assays for the number of eosinophil colonies which develop in rodent spleens after intravenous injection of marrow cells. Alterations in eosinophil

labelling index have also been used to study the effects of eosinopoietic factors in vivo. None of these techniques have been very satisfactory, reflecting our ignorance of in vivo events in eosinopoiesis. All of the reproducible, and sensitive assays for human eosinopoiesis involve the culture of eosinophil progenitor cells in semi-solid, or liquid culture systems. The semi-solid agar technique was developed in the late 1960s, to assay marrow myeloid progenitor cells. Most of the in vitro colonies were derived from granulocyte-macrophage (GM) progenitor cells. The colony stimulating factor (CSF), which increased the number, and the size of these colonies is now known as GM-CSF.

Four types of colonies can be formed by progenitor cells (CFUc) in double layer agar cultures: eosinophil, granulocyte, macrophage, and mixed granulocyte-macrophage. In 1980, a comparison of the numbers of colonies which grew from 5×10^5 normal human bone marrow cells, and an equal numbers of lymphocyte-enriched blood cells, and cord blood cells, showed that there were a total of 89, 39, and 65 colonies respectively, of which 72 per cent, 48 per cent, and 23 per cent were eosinophil colonies⁹⁷².

2.5.1. Agar colony cultures.

The first descriptions of the in vitro growth of eosinophil colonies from human peripheral blood³¹², and bone marrow⁸⁴⁴ were published in 1971. Eosinophil colonies were separate from GM colonies, and grew more slowly. This was the first decisive evidence that eosinophils have a different origin in marrow from neutrophils. It was soon noted that eosinophil progenitor cells were present in surprisingly large numbers in human blood, and that they could be induced to form distinct colonies in agar cultures stimulated with certain types of conditioned media. Under optimal conditions, one third of the agar colonies which grow from human blood in conditioned medium were eosinophil colonies. These colonies were small, and they were usually distinguished by their special staining properties with dyes such as Biebrich scarlet, or Luxol fast blue^{879, 569}. A peroxidase-based method for distinguishing eosinophil colonies was described in 1985⁴⁷².

Eosinophil colonies were usually pure, although they occasionally contained small numbers of neutrophils, or macrophages¹⁰⁴³. Details of this technique for growing eosinophil colonies in agar are now well described, eg: ¹⁷⁵.

The EM appearances of eosinophils in soft agar colonies were reviewed in 1980, and it was noted that they did not form the characteristic granule crystalloid²⁰⁰⁷. It would be interesting to know if they would stain with anti-MBP antibodies.

The progenitor cells for eosinophil agar colonies have been shown to possess membrane HLA-DR antigens, and they did not have other membrane markers found on blood T, or B lymphocytes²⁴⁴. Bone marrow eosinophils are heavier than other cells. In a comparative study on the density of marrow cells in Percoll gradients, eosinophils had the highest density: 1.0904 g/ml¹³¹².

In patients with an eosinophilia the number of circulating eosinophil progenitor cells may increase. This was seen in a study of eosinophil progenitor cells in 12 Egyptians with schistosomiasis, and an eosinophilia, and eight healthy controls. The total number of colonies was doubled in the patients' blood samples, although there was no increase in the proportion which were eosinophilic⁹⁰³. The number of marrow-derived eosinophil progenitor cells may also increase. This was reported in a study on eosinophil colony development in bone marrow cultures, done in patients with the graft-versus-host disease¹²⁸⁰. However, as another study in 1984 on 17 patients with an eosinophilia, found no increase in eosinophil progenitor cells in the blood, or marrow⁹⁵², this topic remains unsettled.

Several studies have been carried out on the possible effects of drugs, and different disease processes on eosinophil development in agar cultures. It was found that eosinophil colony growth could be inhibited by cytotoxic drugs, and that steroids inhibited eosinophil progenitor cells directly, rather than through an effect on T lymphocyte-derived colony stimulating factors¹⁷⁷.

2.5.2. Bone marrow liquid cultures.

Liquid culture systems were introduced in 1976 for the study of bone marrow eosinophil development¹⁵³⁰. Initially they were used to study mouse marrow eosinophil development, and because they

only maintained dividing eosinophils for a few days, they were most useful for defining factors which had a rapid effect on eosinophil proliferation, such as eosinophil growth stimulating factor (eosinophil-GSF)¹¹⁸, and eosinophilopoietin (EPP)^{1138, 1140}.

Longer term liquid culture systems were then developed. These contained 0.9 per cent methylcellulose, and 30 per cent serum, and maintained marrow eosinophil growth for up to three weeks, providing that they were also stimulated with conditioned medium from lymphocytes, or certain cell lines, such as the EL-4 murine thymoma cell line¹⁸²⁰. In 1985, it was shown that eosinophils in mouse (but not human) cultures could be assessed conveniently by assaying eosinophil peroxidase¹⁷¹⁵.

In 1986 a two step culture system was developed which enabled eosinophil progenitor cells to grow from human bone marrow into large colonies containing up to 3 000 cells, after three weeks culture. In the first step, Percoll separated interface cells, which were nonadherent, were cultured for four days with EL-4 conditioned medium. Then the cells were separated on a Percoll gradient again, and cultured in 0.9 per cent methylcellulose with 30 per cent serum, and 10 per cent EL-4 conditioned medium²⁴⁷.

2.5.3. Assays in vivo.

The search for eosinophil specific growth factors which act in vivo has been a long one, and several groups have claimed success. In 1953, Samter showed that extracts from the lungs of guinea pigs, which had previously been injected intraperitoneally with antigen-antibody complexes, when injected into normal guinea pigs, would produce an eosinophilia, suggesting that the lungs could be a site of formation of eosinopoietic substances¹⁵⁴⁹. The main difficulty with this type of experiment has been to produce a reliable assay system.

In a series of papers, between 1977, and 1980, Mahmoud, and colleagues defined a low molecular weight pronase-sensitive substance called eosinophilopoietin (EPP), from mice, which increased the number of dividing marrow eosinophils in vivo, and raised the blood eosinophil counts of normal, and nu/nu mice^{1139, 1138, 1133}. Mouse bone marrow cells in liquid culture were also stimulated by EPP to develop into eosinophils, and similar effects were produced with products from antigenically stimulated spleen cells, with the maximal response occurring at two days¹¹⁹. Some activity was found in mice infected with *S. mansoni* or *T. spiralis*¹¹⁴⁰, but none was seen in sera from patients with HES¹¹³⁵.

2.6. Eosinopoietic factors.

Three different ways have been tried to find, and characterize the factors which might stimulate the production of bone marrow eosinophils. The first was based on the knowledge that T lymphocytes were required for animals to develop an eosinophilia. In these studies, eosinophil growth-promoting factors were purified from supernatants of stimulated, or cloned lymphocytes. The second approach, which has only been used in one centre, was to deplete circulating eosinophils with specific antibodies, and to examine the sera from these animals, to see if an eosinopoietin was produced, by analogy with the production of erythropoietin following acute anaemia. The third method, which has been outstandingly successful, was to screen purified, and recombinant colony stimulating factors (CSFs) for possible eosinopoietic activity.

The results of this work are now providing a comprehensive picture of how eosinophil production may be increased in disease. Two stages in eosinopoiesis can now be postulated: (1) an early event in which several CSFs, from adjacent cells in the marrow, stimulate eosinophil precursor cells to divide, and (2) a later process in which helper T lymphocytes, which are taking part in immunological responses, produce interleukin 5 (IL-5), which amplifies this proliferative response specifically. The relative roles of the different CSFs, and interleukins should soon become clearer, now that they have been isolated as recombinant proteins, although they may not have the full biological activity of the native molecule.

All of the purified factors which regulate eosinophil production are CSFs. Others, which remain to be defined have been described in cultures of tumour cells, in the complement system, and in parasites. No common basis for this wide range of stimuli has yet been defined, and it is not known

whether they act directly on eosinophil precursors, or via another cell type in the culture vessel. Little is known about the factors which induce the production of CSFs *in vivo*. Unstimulated cultured human vascular endothelial cells, which produced several molecules which could induced erythrocyte, and macrophage/granulocyte colony formation *in vitro*, did not stimulate eosinophil development⁷¹.

It has been known since 1976 that stimulated lymphocytes produce soluble factors, which increase the proliferation of eosinophils, in several assay systems. They were called (a) eosinophil-growth stimulating activity (GSF)¹⁵³⁰, (b) eosinophil stimulation promoter (ESP)^{342, 866}, and (c) eosinophilopoietin (EPP)^{118, 1138, 1140}.

It was later found that the eosinophil-GSF, and EPP were probably identical: they were both of low molecular weight, and had rapid effects on eosinophil proliferation¹¹⁹. This suggested that they were acting on a more differentiated eosinophil precursor than the CSFs¹²⁴, which take several days to produce a noticeable change in the number of eosinophils in culture.

2.6.1. Factors from T lymphocytes.

By 1970 it had been shown that T lymphocytes were involved in the regulation of eosinophil production, at least in rats^{136, 147}. In 1976, Ruscetti and colleagues found that primed mouse lymphocytes stimulated with *T. spiralis* extracts produced a supernatant, which increased the proportion of eosinophilic colonies in methycellulose cultures of femoral bone marrow cells, and a three to four fold increase in the number of eosinophils formed in liquid cultures. This response was antigen specific, as supernatants from BCG primed cells, which had been stimulated with the parasite antigens, were inactive¹⁵³⁰. In 1976 Miller, and McGarry also reported finding a soluble eosinopoietic factor released by mouse lymphocytes¹²¹². Subsequent work confirmed that antigen-stimulated human T cells produce an eosinopoietic activity, and aspergillus-sensitized human T lymphocytes produced an eosinopoietic activity⁵¹⁸. This stimulated the growth of eosinophils in both agar, and liquid cultures⁵¹⁹.

The types of lymphocytes involved in the induction of eosinopoiesis have been presumed to be helper T cells, but experiments in mice suggest that suppressor T cells may also be involved. It may not be limited to any one T cell subset, as human cortical thymocytes may have this property, see:⁹⁴⁵.

2.6.1.1. Interleukin 5, IL-5, EDF.

In 1985 Sanderson, and colleagues in London reported that a hybrid clone (NIMP-TH1) of a mouse thymoma cell line BW5147, and spleen cells from mice infected with *Mesocostoides corti*, produced an eosinophil-specific growth factor, after stimulation with phorbol myristate acetate (PMA)¹⁸⁷⁹. They also found that a high proportion of primary alloreactive T cell clones from mice with an eosinophilia produce this eosinopoietic factor^{1555, 1553}. This activity was called 'eosinophil differentiation factor' (EDF). In 1986 EDF was found to be able to stimulate human eosinophil differentiation¹¹⁰¹. The hybrid clone was grown in bulk, and used for further characterization of the molecule, including its capacity to act as a B cell growth factor¹⁵⁵². It was suggested that EDF should be called IL-4, but as the IL terminology is restricted to cloned molecules, this name was not generally accepted.

Mouse EDF had several properties which suggested that it could be a close analogue of the putative human eosinophil-specific CSF, as it was a selective stimulus for the clonal proliferation, and differentiation of human bone marrow eosinophils¹⁸²³.

In 1986, work by Honjo, and colleagues in Japan led to the cloning of a murine T cell factor which stimulated B cell growth: B cell growth factor II, which was given the internationally recognized name of IL-5⁹⁶⁶. It soon became clear that EDF, and IL-5 were identical molecules. In 1987, the Japanese workers cloned the human gene for IL-5⁸⁵. In 1987 a group in Australia, and the U.K.²⁶⁴ cloned, sequenced, and developed expression vectors for the gene for human IL-5. rHuIL-5 was found to be a unique molecule, specific for human eosinophil differentiation. Studies are now under way, to see how IL-5 affects human eosinophil differentiation, and whether IL-5 can be assayed in human samples, especially from patients with an eosinophilia. Preliminary studies have shown that

it may stimulate human eosinophil colony formation in agar cultures (Fukuda T, 1987, personal communication). Although recombinant IL-5 stimulated murine B cells to express IL-2 receptors¹¹⁰⁸, it did not appear to affect human B cells (Sanderson 1987, personal communication).

2.6.1.2. Interleukin 2, IL-2.

It has been found that injections of purified recombinant interleukin 2 (IL-2) into patients with malignant diseases, can give rise to a marked eosinophilia⁵⁸⁶. However, it is not known yet whether this is a direct, or indirect effect of IL-2 itself on eosinophil production, or a secondary effect of some contaminant, or even a hypersensitivity reaction to a component in the injected material. It was not prevented by injections of dexamethasone¹⁸⁵⁷. As IL-2 induced murine T cell clones to secrete GM-CSF (CSF alpha)⁹⁵⁰, the effects of IL-2 on eosinopoiesis may be indirect. This possibility is supported by a study on 11 patients with an eosinophilia, due to many different causes, in whom five had a raised blood helper/suppressor T cells ratio. In all eleven patients, culture of purified blood T lymphocytes with IL-2 increased the amount of eosinophil, and monocyte-macrophage CSF which was secreted into the medium during a five day culture. Surprisingly, this capacity persisted after the eosinophilia had disappeared in three of the patients⁵¹⁷.

2.6.2. Colony stimulating factors.

The properties of CSFs from cultures of placental, and other cells, have been studied for nearly two decades by Metcalf, Nicola, and their colleagues in Australia, and other centres¹¹⁹². A summary of the principal CSFs affecting eosinophil colony growth is shown in Figure A02-1. CSFs, with predominant effects on eosinophil colony growth in agar culture were described in several centres in the early 1970s. In 1974 Metcalf, and colleagues showed that supernatants, from pokeweed mitogen stimulated mouse spleen cells, contained a separate factor, which induced eosinophil development from mouse bone marrow. It was distinct from neutrophil, and macrophage CSFs¹¹⁹⁶.

Fig. 2-1: Factors affecting eosinophil differentiation.

Two of the well-defined CSFs, GM-CSF (previously known as CSF alpha), and multi-CSF (which is also called interleukin 3, IL-3), affect eosinophil proliferation especially¹⁸²⁴. The gene for murine GM-CSF was cloned in 1984⁷⁰⁴, and a cDNA expression product was produced from transfected monkey COS cells in 1986¹⁷⁸⁷, but it was not as potent as the native molecule. GM-CSF is a 127 amino acid protein with two internal disulphide groups.

In a review on eosinophil activating factors in 1984, Vadas emphasized the importance of differential regulation of eosinophils, and neutrophils with GM-CSF (CSF-alpha), and G-CSF (CSF-beta)¹⁸¹⁸. GM-CSF stimulated both eosinophil, and neutrophil mediated cytotoxicity, whereas G-CSF had a stimulatory effect only on neutrophils. It was reported in 1987 that recombinant G-CSF did not induce eosinophil colonies in vitro, from human marrow, or alter blood eosinophil counts in guinea pigs, although it gave them a neutrophil leukocytosis²⁰⁰⁵.

2.6.2.1. GM-CSF, CSF-alpha.

In 1986, two groups showed that rGM-CSF induced the development of eosinophil colonies from normal marrow. Metcalf, and colleagues in Australia used both rMuGM-CSF¹¹⁹⁵, and rHuGM-CSF¹¹⁹³. Tomonaga, and colleagues in California used rHuGM-CSF¹⁷⁸⁷. The work in California also showed that rHuGM-CSF induced eosinophil formation by HL-60 cells, although this has not been confirmed in other laboratories.

Earlier work from Australia had already shown that the activities of GM-CSF were identical to those of CSF-alpha, which had been previously purified from placental conditioned medium, and which increased the capacity of eosinophil to kill antibody-coated schistosomula²³².

The effects of GM-CSF are not always concentration dependent. Low concentrations of human placental-conditioned medium containing GM-CSF were most effective in stimulating eosinophil colony growth in agar cultures of human bone marrow, whereas stimulation was concentration-independent in suspension liquid cultures⁹⁵⁴.

Injections of large amounts of recombinant GM-CSF gives rise to a marked eosinophilia in man. This was first shown in a study on 16 patients with AIDS studied in Boston, and reported in 1987⁷¹⁸.

A bone marrow eosinophilia developed in eight patients. In one patient given 1×10^4 U/kg/day as a bolus on day 1, and by continuous infusion from days 3 to 17, the blood eosinophil count reached $15.5 \times 10^9/L$, at day 17, after which the injections were stopped, and the eosinophil counts then fell rapidly towards normal. These results are consistent with the hypothesis that GM-CSF is a natural stimulus for eosinopoiesis in disease.

2.6.2.2. Interleukin 3 (IL-3), multi-CSF (M-CSF).

In 1987 it was reported that cloned gibbon interleukin 3 stimulated the proliferation, and differentiation of human bone marrow cells to produce eosinophil colonies in semisolid agar¹¹⁰³.

Purified recombinant murine multi-CSF (IL-3) induced normal murine bone marrow cells to develop into colonies. At high concentrations (above 50-100 U/L), eosinophil colonies developed. Similar findings were made with native multi-CSF. When six mg was injected into the peritoneum of Balbc mice, more eosinophils were found at six days than in mice injected with serum-saline. This was dose-dependent. These results show that M-CSF affects a range of cell types. Although M-CSF has not been detected in serum, or organ extracts, it may be a stimulus for eosinophil production under some pathological conditions¹¹⁹⁴. Labelled murine M-CSF has been shown to bind to immature murine marrow eosinophils, but not to more mature eosinophils¹²⁷⁷. Further studies with rM-CSF in man are awaited with interest.

2.6.3. Factors in inflamed tissues.

In 1982, a GM-CSF-like activity was found in blister fluid of three out of five patients with bullous pemphigoid¹⁸⁴⁰. Nasal polyp epithelial cells from patients with allergic rhinitis were reported in 1987 to be able to produce eosinophil colony-stimulating factors⁴³¹.

2.6.4. Factors from tumour cells, and cell lines.

There have been a number of unsuccessful attempts to find eosinopoietic factors in the serum of patients with an eosinophilia^{1866, 117}, and in tumour cell extracts from patients with neoplasms, and hypereosinophilia. However in 1983, and 1984 eosinopoietic factor(s) were detected in the sera, and tumour extracts from patients with carcinoma of the lung, and a marked eosinophilia^{1646, 986}. Viruses with oncogenic potential can also stimulate the production of eosinopoietic factors. For example, supernatants from human lymphocytes infected with HTLV-1 retrovirus produce a number of lymphokines, including an eosinophil growth, and maturation activity¹⁵⁴³. In 1983, a factor from a malignant fibrous histiocytoma was shown to have eosinopoietic activity¹⁵⁹¹.

Several cell lines produce eosinophil growth promoting activities. In 1986 'pleuripoietin alpha' was described in the human bladder carcinoma cell line 5637⁶¹⁴. In 1987 it was shown that a T cell leukemia cell line (Mo) produced a conditioned medium containing an eosinophil growth stimulating activity. This was heat stable, and could be partly separated by DEAE chromatography from a basophil growth stimulating activity, when assayed by the methylcellulose technique with cord blood mononuclear cells¹⁷⁵⁴. The properties of several growth promoting factors from a number of other human cell lines were reviewed in 1987¹⁷⁵⁵.

2.6.5. Factors in serum from patients with an eosinophilia.

The presence of eosinopoietic factors in the serum of patients has not been reported very often, possibly because most eosinopoietic substances act as paracrine substances, eg. they act on adjacent cells only. In 1983 a weak CSF activity was detected in sera from a patient with a carcinoma of the lung-induced eosinophilia¹⁶⁴⁶. No comparable activity was found in sera from patients with acute lymphocytic leukaemia, and hypereosinophilia⁸⁰⁶. In 1986, it was reported from Berlin that serum, and a crude extract of a malignant fibrous histiocytoma, which had produced eosinophil counts up to $17.5 \times 10^9/L$ in a 63 year-old woman, increased the number of eosinophil colonies grown from normal bone marrow cultured by a plasma clot technique. As much activity was found in 10 per cent patient's serum as in a five per cent solution of the tumour extract¹⁵⁹¹.

A study from Sydney, Australia, in 1987 reported that sera from a group of patients with chronic myeloid leukaemia, two of whom were studied during blast cell transformation, contained an eosinopoietic activity, which increased the survival, and growth of bone marrow-derived 'persist-

ing' eosinophil colonies in semi-solid agar. These colonies had grown for 35 days, or longer. It was suggested that this might be IL-3, and that it could be involved in inducing the transformed cells to proliferate²¹⁷.

In 1986 it was reported that sera from IL-2 treated patients with AIDS who developed an eosinophilia, contained an 'eosinophil-producing activity' (EPA) in bone marrow liquid culture⁹⁵³. Similarly, sera from six other patients with HES, four with CML and eosinophilia, two with non-Hodgkin's lymphoma, and six with acute helminthic infections, and an eosinophilia, were found to be able to increase the number of eosinophils in 12 day-liquid cultures of human bone marrow from 0.1×10^5 eosinophils/250 ul, to 2.1×10^5 /250 ul. Four normal sera and sera from eight people without an eosinophilia had no effect. Serum was added at 5-40 vol per cent. Unfortunately, these experiments did not distinguish between differential survival of eosinophils in the cultures, and stimulation of eosinopoiesis. The activity was destroyed by heating at 65° for 20 minutes, and had an apparent molecular weight of 80-85 kDa on Sephadex G-100. Interestingly, active serum was ineffective in stimulating eosinophil colony growth in agar cultures⁹⁵⁴.

2.7. Drugs, and eosinophil production.

Injections of cyclophosphamide together with a parasitic, or other eosinopoietic stimulus, have been shown to be able to induce a marked eosinophilia in mice^{1816,1722}, and rats¹⁷⁷⁴. With this approach it was found in 1982 that genetic factors have a role in regulating eosinophil production in mice¹⁸¹⁷. There are no reports of a similar mechanism occurring in man.

A number of studies have found that steroids (hydrocortisone) inhibited the development of human eosinophil colonies in vitro. It was therefore surprising that experiments from Switzerland in 1987 showed that hydrocortisone can increase the capacity of human marrow, or blood cells to form eosinophil colonies in vitro, either when the drug is added to the cultures, or when the blood cells are obtained from people given hydrocortisone. It was suggested that the acute effects of steroids in causing a fall in eosinophil counts in vivo, are mainly a result of the redistribution of the cells in the blood¹¹⁵.

The addition of lithium, rubidium, and caesium salts to human bone marrow cultures increases the number of colonies, including eosinophils¹¹⁴, but there are no reports that patients taking lithium salts develop an eosinophilia.

2.8. Suppressor cells, and eosinophil production.

Suppressor T cells may be able to inhibit eosinopoiesis. This possibility was suggested when an excess of T suppressor cells were found in the blood of a patient with a thymoma, and a total absence of eosinophils¹²²⁰. No experimental work has been done yet to test this possibility, which could be of clinical importance in patients with a persistent eosinophilia, such as HES. However, in mice, suppressor T cells, which reduced the numbers of eosinophils in the peritoneal cavity, have been found in the spleens of mice injected with high doses of tetanus toxoid, or tetanus toxoid in complete Freund's adjuvant. These cells inhibited the development of a peritoneal eosinophilia in mice injected with tetanus toxoid with alum, following cyclophosphamide pretreatment¹⁷⁴⁶.

2.9. Eosinophil suppression of granulocytopoiesis.

As prostaglandins inhibit GM colony formation in agar cultures, it has been suggested that eosinophil-derived prostaglandins could act in this way. Experiments with mouse peritoneal eosinophils supported this idea: they inhibited the formation of mouse bone marrow colonies in agar, and this effect was prevented with indomethacin⁶²⁶. However, although there are a few clinical conditions in which eosinophils persist longer than neutrophils when granulocytopoiesis is inhibited, in patients with a marked eosinophilia, neutrophil numbers are almost invariably increased as well. There is also no evidence as yet that bone marrow eosinophils can secrete prostaglandins.

2.10. Genetic factors affecting human eosinophil production.

There are three lines of evidence which suggest that genetic factors influence the number of eosinophils that are produced in response to stimulation. These are studies in man, which have shown that certain chromosomal abnormalities are associated with a marked eosinophilia, and experiments in

inbred rodents, some of which can produce a marked eosinophilia to stimuli which only induce a small response in others. In addition there is a report that homozygous Sl/Sl and Wv/Wv mice had only 15 per cent of the number of blood eosinophils, as littermate controls. This was probably due to a defect in eosinophil production, as there were also less eosinophils in the bone marrow, spleen, and thymus¹⁹³¹.

2.10.1. Cytogenetic studies.

Cytogenetic studies on patients with malignant haemopoietic diseases involving eosinophils have shown G banding abnormalities in several chromosomes which may be involved in eosinophil differentiation. These include abnormalities with

- chromosome 5^{1980, 390}, where the gene for GM-CSF, IL-3, IL-5, and M-CSF are located.
- chromosome 7, which was abnormal in two patients with myeloblastic leukaemia, and a marked eosinophilia^{827, 1224}.
- chromosome 8, which is abnormal in the eosinophil cell line EoL¹⁵³⁷, and which was a trisomy in two patients with eosinophilic leukaemia^{1365, 1899}. An 8;21 translocation affecting eosinophils was seen in a girl with eosinophilic leukaemia in Japan, in 1983⁹⁰⁴.
- chromosome 12, which was abnormal in a patient with a malignant proliferation of eosinophils⁹⁴³.
- chromosome 16, where rearrangements, and other alterations have been seen in patients with M4-Eo acute leukaemia, with an eosinophilia. There is also an abnormality on the long arm of chromosome 16 in the HL-60 cell line, which has been induced to form eosinophil granules⁵⁶⁹.

There is no evidence that the c-fos proto-oncogene, which has been suggested to be important in the control of monocyte development, is involved in human eosinophil differentiation, as mRNA was not detected by in situ hybridization in blood eosinophils¹⁰⁰⁴.

2.10.2. Genetic studies in rodents.

In 1982, it was shown by Vadas, and colleagues that the eosinophil response in inbred mice was genetically controlled. Evidence that the major histocompatibility complex was involved soon followed¹⁵⁹³. High, and low responsiveness was shown to be determined by the bone marrow, as the development of an eosinophilia in radiation chimaeric mice was related to the origin of the bone marrow used to make the chimaeras¹⁷²⁰.

2.11. Eosinophil cell lines.

There are now several eosinophil cell lines, which have begun to be used to study the synthesis of eosinophil constituents^{567, 13}, and to prepare cDNA coding for eosinophil constituents. These have been derived from the HL-60 cell line, which was originally developed in the U.S.A., and the EoL cell line, which was derived from a patient with eosinophilic leukaemia in France.

The HL-60 cell line, which was developed from a patient with acute promyelocytic leukaemia in 1979⁶¹⁷, has a chromosome 17 inversion. HL-60 cells have several characteristics in common with eosinophils found in patients with acute myelomonocytic leukaemia with an eosinophilia (FAB class M4-eo). For example, in 1981 they were found to contain some luxol-fast-blue granules, a feature of eosinophils¹¹¹⁶. In 1983 it was shown that a small percentage of HL-60 cells in culture matured into typical eosinophils, but this was not influenced by additional human eosinophil CSFs¹¹⁹¹.

Fischkoff, and colleagues continued to develop eosinophil cell lines from cloned HL-60 cells. They showed that in soft agar cultures, a few HL-60 cells differentiated into eosinophils, some of which became mature cells, containing small granules. Although crystalloid were rarely seen, they contained eosinophil peroxidase (EPO), MBP, CLC protein, arylsulphatase, and acid phosphatase⁵⁶⁹.

In 1985 they reported that more HL-60 cell line were induced to synthesize eosinophil granules by culturing them at pH 7.4 with 0.5 mM butyric acid for seven days⁵⁶⁸. Neutrophil induction occurred with 176 mM dimethyl sulphoxide (DMSO). Enzyme assays, and electronmicroscopy showed that peroxidase was synthesized, and packaged in spherical granules, although the Golgi apparatus contained little peroxidase. Acid phosphatase, arylsulphatase, and lysophospholipase were synthesized in smaller amounts than in normal eosinophils⁵⁶⁷.

In 1986, it was reported that two stable eosinophil cell lines had been developed by serial subculture

of HL-60 colonies which contained many eosinophils. They retained lineage fidelity even after stimulation with GM-CSF¹⁷⁸⁶. It was suggested that these cell lines would be particularly useful for studying the production, and functions of human eosinophils, and for analysing leukaemic cell differentiation, and stem cell commitment.

In 1984, an eosinophilic leukemia cell line was established in Paris, France from a 33 year old man with a Phi-negative eosinophilic leukemia¹⁵³⁷. This was obtained from separated blood white cells grown in suspension culture, and in 0.3 per cent agarose. The cells began to proliferate after two months culture, and were subcultured at 10 weeks. Six clones were derived. Three clones, named 'EoL-1', 'EoL-2', and 'EoL-3' had eosinophilic features. Three other clones, 'Eo-B' had a B cell phenotype. No feeder cells were used. EoL-1 grew in single cell suspension. EoL-2, and EoL-3 formed cell clusters. Occasional cells contained eosinophil granules, but after induction with alkaline medium, or dimethylsulphoxide, 40 per cent contained granules. The EoL lines expressed Ia receptors, the myeloid antigens IF10, and MY9, and the IL-2.

2.12. Blood eosinophil kinetics.

Eosinophils leave the bone marrow, and enter the blood sequentially, and leave it randomly. This is easily shown by injecting labelled cells which appear in tissues sites (such as nasal secretions) immediately, and continue to do so during the next few hours. In the bloodstream, many eosinophils are believed to be marginated at any one time, in exchange equilibrium with an equal number in the circulation.

The kinetics of human blood eosinophils were measured accurately by two groups in Germany in 1976, and 1979 using ³H thymidine autoradiography. In the first study it was found that the eosinophil emergence time was 3.5 days. The blood half life was 13 hours, and the mean blood transit time 25 hours. The S phase was 13 hours, and myelocyte generation time about 34 hours: similar to neutrophils. There was some evidence for two pools of proliferating eosinophils. Similar results were also obtained for neutrophils kinetics which were measured at the same time¹³⁷¹. The second study, in 1979, showed that the mean blood eosinophil relative turnover rate was 4.0 per cent /hour, the mean blood transit time (or blood sojourn time) was 26 +/- 3 hours, and the blood half life was 18.0 +/- 2.1 hours¹⁷⁰⁷.

The eosinophil blood half-life is prolonged when there is an eosinophilia. This may be because the number of sites through which eosinophils can migrate into the tissues is limiting. The process of emigration from the bloodstream occurs in venules, and involves loose attachment to vascular endothelium, followed by migration between endothelial cells, and into the connective tissue around the blood vessels. (See page 185).

In many infectious diseases due to bacteria, or viruses the blood eosinophil count has been found to be low, or zero¹²⁴. Although the eosinopenia of infection may be due partly to the production of steroids during the stress produced by inflammation, it has been shown that eosinopenia can occur in infected mice, without an increase in blood corticosterone levels¹²¹. Steroids, other than large amounts of dexamethasone, had little effect on human eosinophil colony growth from blood, or marrow progenitor cells²⁴³.

The rapid decrease in blood eosinophil counts following infection, or stress involves both an increased uptake of eosinophils into tissues, as well as a decreased output of eosinophils from the marrow¹²². A host component, which was present in inflammatory reactions in the mouse, appeared to be responsible for these effects¹²³. Injections into rabbits of zymosan activated serum, partially purified C5a, and f-Met-Leu-Phe caused an immediate eosinopenia, suggesting that these could also mediate the acute eosinopenia of infection¹²⁵.

2.13. Kinetics of eosinophils in tissues.

It is not known how long eosinophils survive in tissues, although it has been assumed that they can remain there for several days at least. Human eosinophils can be induced to survive in culture for over 14 days with CSFs¹⁴⁸, or endothelial cell conditioned medium¹⁵²². It is probable that eosinophils can persist in tissues for equally long periods, as there are often more eosinophils in chronic inflam-

matory sites, than in the blood.

3. Chapter A 03. Methods. Eosinophil structure, constituents, and metabolism.

3.1. Eosinophil counts.

It is often necessary be able to count the number of eosinophils in the blood, and tissues. Unfortunately, there are no areas of the body where eosinophils are evenly distributed, although the blood comes closest to this. Even here, some eosinophils are marginated, and some in the circulating pool of cells. The best approach to this difficulty is to measure the number of eosinophils in several samples, and to use statistical methods to calculate their distribution. It is possible to measure the amounts of eosinophil specific constituents per unit mass of tissue, but most investigators count stained cells in suspension, or in sections. This is likely to underestimate the involvement of eosinophils in diseases, where degranulated eosinophils may not be stained as well as in normal tissues. Measurements of eosinophil numbers taken at a fixed time are also unable to provide any information about the flux, or kinetics of eosinophil transit into, and out of the tissues being studied.

Ottolenghi, and colleagues have assessed the extent of eosinophil involvement in rats by assaying tissue levels of lysophospholipase. This approach was based on their finding in 1967 of large amounts of phospholipase (lysolecithinase) in rat tissues using histochemical methods¹³⁴⁴. In 1970 this was related to their content of eosinophils¹³⁴², which led to the development of an enzyme-based assay for the involvement of eosinophils in inflammatory lesions produced by parasites in rats¹³⁴³. This type of measurement has not yet been used to study the eosinophil content of human tissues, or their contribution to inflammatory reactions, and it would need careful validation, before it could be used instead of cell counts.

Although the much criticized routine of using 'per cent eosinophils', as a crude measure of eosinophil counts, persists in many centres, fortunately most automated counting systems now provide printouts in which the total number of white blood cells is multiplied by the per cent eosinophils to provide clinically useful data. The term 'per cent eosinophils' should not be used unless there are specific reasons for knowing the proportions of eosinophils compared to other cells in a sample. It is no substitute for an accurate eosinophil count. It is a legacy from the early part of the century when it was thought (incorrectly) that the presence of one cell type in the blood could affect the numbers of another cell type.

3.1.1. Manual methods.

The most widely used, and one of the most accurate methods for measuring blood eosinophils counts in the internationally agreed SI notation (eosinophils $\times 10^9/L$), is to use an improved Neubauer counting chamber, into which is pipetted the blood, diluted 1 in 10 (or 1 in 20, if the blood eosinophil count is greater than $1 \times 10^9/L$) with a stock solution of one of the brominated derivatives of fluorescein which stain eosinophils strongly, such as eosin (tetrabromo-fluorescein), or phloxin (tetrabromo-tetrachloro-fluorescein). In the eosin method, which is widely used outside the U.S.A., the stock solution contains 1 per cent aqueous eosin Y (five volumes), acetone (five volumes), and distilled water (90 volumes) to lyse the red cells. This is an adaption of the counting method developed by Dunger in 1910⁴⁷⁵.

In the phloxin method, which was introduced in 1944 by Randolph in the U.S.A., where it is still often used, propylene glycol is added to 'clear' the red cells, which are not lysed. Methylene blue is used to stain the nuclei of the white cells, and phloxin to stain the eosinophils¹⁴⁷⁶. Randolph discussed the advantage of using propylene glycol to enable the eosinophil, and white blood cell counts to be done in the same counting chamber¹⁴⁷⁷. In both methods, an assessment is made of the volume of fluid in the counting chamber which contains 200 eosinophils, using both sides of the chamber if necessary, to ensure that the accuracy of the counts are consistent, and independent of the total eosinophil count. This is especially important when the count is less than $1 \times 10^9/L$.

Although total blood eosinophil counts can be calculated from differential blood counts done on blood smears, this is not recommended, as the accuracy is low⁵⁶⁴. This is because eosinophils are large cells, which tend to be pulled to the ends, and sides of smears. Also, as eosinophils comprise only a proportion of the 200 white cells counted, the accuracy of the count is only acceptable if they make up more than half of the total white blood cells examined.

A statistical analysis of the chamber method has shown its superiority to differential counts⁴⁵⁴. Although smears can give accurate counts in expert hands¹⁰³⁵, modern haematology laboratories now use automated counting methods, in which 2 000 white cells are assessed.

3.1.2. Automated methods.

The validity of automated differential counting systems for assessing the number of blood eosinophils have been shown by comparisons with manual counting methods in adults⁶⁵, and children³⁶⁶. Although most major laboratories find that automated counting systems are accurate^{99, 618}, they can produce errors in samples with high blood eosinophil counts⁹⁸. Besides their speed, and reproducibility, they have the added advantage that they may bring to light patient with eosinophil peroxidase deficiency¹⁷⁴⁸.

3.1.3. Normal blood eosinophil counts.

In a study of my own on 100 adults of both sexes in Oxford, U.K., blood eosinophil counts ranged from 0 to $0.55 \times 10^9/L$. I excluded people with allergic rhinitis, hayfever, and other mild allergic disorders whose counts were slightly higher than this. For practical reasons I consider blood eosinophil counts of between $0.55-1.5 \times 10^9/L$ as 'needing follow up', between $1.5-10 \times 10^9/L$, as 'needing investigation soon', and over $10 \times 10^9/L$, as 'needing urgent assessment'.

An analysis of a large number of blood eosinophil counts, which were done in the U.S.A. between 1971-75 for people aged 25-74 years, was published in 1982¹¹⁷⁴. However, these results were derived from differential counts, which is not very satisfactory for obtaining absolute values, as described above.

It was reported in 1987 that the blood eosinophil count in 740 medical students in Pittsburg, U.S.A., was $0.015-0.65 \times 10^9/L$ (95 per cent confidence limits)¹⁰⁰³.

In a group of normal children aged four to eight years, blood eosinophil counts were $0.206 \pm 0.270 \times 10^9/L$. Over 12 years of age they were lower: $0.096 \pm 0.140 \times 10^9/L$, and males were: 0.180 ± 0.160 , and females $0.145 \pm 0.120 \times 10^9/L$ ³⁷⁴.

There is a diurnal variation in blood eosinophil counts in normal people. The counts are inversely related to blood cortisol levels, eg. the counts are lowest in the morning, and highest at night. In 1981 the mean variation in blood eosinophil counts were assessed in 21 normal adults, using the Hemalog-D automated counter. The intra-subject within-day variation was 27 per cent, and the diurnal variation was over 40 per cent¹⁹³⁸. This showed the importance of doing serial blood counts at a fixed time of day.

In 1984, a comparative study of blood eosinophil counts in four ethnic groups living in Britain (Indian, black {African, and West Indian}, white {Northern European}, and oriental), showed no significant differences. This confirmed the generally accepted view that high eosinophil counts in tropical countries are related to parasitic infections, rather than genetic factors⁹⁷. A similar conclusion was reached in a study in Kenya, in 1979¹³⁰⁹.

It has been known for several years that exercise increases the blood eosinophil count³¹⁷, which can be exploited when normal blood eosinophils are needed for experimental studies.

3.2. Leukaphoresis.

Obtaining large numbers of human eosinophils from the blood is straightforward when the eosinophil count is high, but leukaphoresis has also been used successfully to obtain eosinophils from both normal, and patient's blood. In this technique dextran, methylcellulose, or hydroxyethyl starch⁶⁷¹ are used to sediment the red cells before the white cells are aspirated from the collection bag. This technique has provided up to 2×10^{11} eosinophils from an individual with a blood eosinophil count of $50 \times 10^9/L$ ⁶⁶¹.

In 1987, leukaphoresis using hydroxyethyl starch was used to separate blood eosinophils for biochemical, and ultrastructural studies from five patients with HES⁷⁸⁰. However in two patients, the presence of cytoplasmic Charcot-Leyden crystals in the purified eosinophils, which are never seen in freshly drawn blood, suggested that the procedure may have had a detrimental effect on the eosinophils.

3.3. Cell separation.

Many different methods for separating eosinophils from other cells have been described in the past, but none of them proved satisfactory until non-toxic density gradient methods were devised, which were able to exploit the greater density of eosinophils (1.088g/ml) compared to other leukocytes. These methods were reviewed in 1983⁶⁵⁵, and 1986¹⁵¹¹. Most methods have used blood cells, but they can also be used to separate eosinophils in exudates, and even in normal tissues, such as the lungs¹⁸³⁷.

Samples of blood are first anticoagulated. This can either be done with preservative-free heparin 5u/ml, if functional studies are to be done, or EDTA pH 7.2, 10 mM final concentration. It is best to remove the red blood cells before purifying eosinophils from other white cells. This can be done by sedimentation with 5 per cent dextran, although this usually leads to the loss of 30 per cent or more of the white blood cells within the sedimented red cells. A mixture of methylcellulose, and Hypaque in place of the dextran gives a higher yield in this first step⁸²⁵. We use this routinely. The removal of residual contaminating erythrocytes is best done with isotonic ammonium chloride, at pH 7.2, at 4°¹⁵¹². Other methods, for red cell lysis, such as rapid lysis with distilled water, damage eosinophils.

3.3.1. Differential binding to surfaces.

A method has been developed to separate human blood eosinophils from neutrophils on the basis of their different binding capacity to complexed IgG, using plastic dishes to which IgG was attached with carbodiimide¹⁷³⁹.

3.3.2. Density gradient separation.

The most commonly used density gradient solutions to separate eosinophil from blood, are metrizamide, and Percoll.

In 1980, Vadas, and colleagues developed what has become the standard method for purifying eosinophils from human blood, using metrizamide¹⁸²¹. The key steps were the use of DNAase to prevent cell clumping, and the use of slightly hypertonic discontinuous gradients. Normal blood eosinophils equilibrate in the 23-24 per cent bands, but in many diseases with an eosinophilia, lighter density eosinophils separate with neutrophils in the 22 per cent band, Figure A03-1.

Fig. 3-1: Eosinophil purification.

Percoll density gradients have been used to purify eosinophils since 1980⁶²⁸. A method for separating eosinophils from bone marrow, or blood samples using discontinuous Percoll gradients was described in 1984⁵⁰⁴. Using this method, it was found that, in gradients of 56 per cent, 64 per cent, 68 per cent, and 77 per cent Percoll, eosinophils were obtained in highest purity in the 77 per cent layer¹⁵³⁹.

A variety of other methods for using Percoll to isolate eosinophils have also been described. These include a method described from Rome, Italy in 1982⁴²⁰, a method described at the N.I.H. Bethesda, U.S.A., in which fMLP was used to activate neutrophils, which then became lighter, and easier to separate¹⁴⁹⁷. However, as eosinophils are also stimulated by fMLP^{1604, 1970}, this method is not recommended, a method described in 1987, from Amsterdam, Holland, in which eosinophils were separated over two successive Percoll gradients¹⁹⁶⁹.

In our hands, Percoll gradient separation of eosinophils has given less consistent results than metrizamide gradients, and there appears to be no method for removing the Percoll particles from the purified cells.

However, as the Percoll separation technique is carried out in isosmolar conditions, it can be used to measure the differences in density between eosinophils in normal blood (normal density, or normodense eosinophils), and patients' eosinophils, some of which may be of lighter density (light density, or hypodense cells)¹³⁸⁹.

The finding of light density eosinophils in patients' blood was first discussed in 1980 by Bass, and colleagues. They used gradients of metrizamide, and Hypaque, and found that in patients with an eosinophilia, eosinophils were often present in the lighter bands with the neutrophils, instead of in the denser layers where eosinophils from normal individuals always separated¹²⁷. This was also noted by other groups in 1982^{1944, 420}, and 1983¹⁴⁵².

Several years earlier, we had noted that many degranulated eosinophils in patients with HES had a similar density to neutrophils. The presence of contaminating neutrophils prevented us from comparing their functional properties with eosinophils of normal density. This problem persists, as there is no effective method for separating viable light density eosinophils from neutrophils. It is only when patients are found who have low blood neutrophil counts, that this type of study can be done. Percoll density gradients have been used to determine the density of light density eosinophils. The peak density of normal eosinophils is 1.088 g/ml, and light density eosinophils 1.076 g/ml⁶¹¹. Fractions containing light density eosinophils were seen to have a number of properties which suggested that they had been activated. Light density eosinophils were first called 'hypodense' cells in France. There are semantic, and scientific objections to the use of this hybrid Greek-cum-Latin word, in place of the better term 'light density'. 'Hypodense' also suggests, incorrectly, that there may be two populations of eosinophils: eosinophil density is a continuum.

There are many diseases in which light density eosinophils have been detected in the blood, including asthma⁶¹¹, allergic diseases, HES, and experimental hookworm infections¹⁹²⁵. Light density eosinophils have been found in samples taken from outside the blood stream, including bronchoalveolar eosinophils. This was first demonstrated in 1984 in bronchoalveolar lavage (BAL) fluid from three patients with pulmonary infiltrates¹⁴⁵³. This was confirmed in a study in 1987 in Kyoto, Japan, where it was found that 93 per cent of the eosinophils in BAL fluid from patients with PIE syndromes were of lower density than normal. This was a higher proportion than in other diseases with eosinophils in BAL fluid³¹⁶.

The presence of light density eosinophils in patients with an eosinophilia is still not explained. It is not known whether they result from accelerated eosinophil production giving rise to smaller, and lighter granules, or whether they are produced by an effect of some component of the blood stream, or vasculature on normal density eosinophils.

3.3.3. Elutriation centrifugation.

There have been no reports of the successful use of elutriation centrifugation to separate eosinophils, although several laboratories are assessing its potential value¹⁵¹¹.

3.4. Culture of mature eosinophils.

Although there has been anecdotal information for many years that eosinophils can survive in tissue culture for over 10 days, it is only recently that this has been studied formally, using conditioned medium for lymphocytes, and endothelial cells.

3.4.1. Lymphocyte products.

In 1985, during a study on the culture of HES blood eosinophils, we found that they would survive intact for three weeks in the presence of lymphocyte conditioned medium (Lai, P., Spry, C.J. 1985, unpublished). The light density, degranulated eosinophils appeared to survive as well as, if not better than the normal granulated cells, suggesting that degranulation was not inevitably associated with eosinophil death.

3.4.2. Endothelial cell products.

In 1987 it was shown that normal human blood eosinophils could be maintained for several weeks by co-culturing them with bovine aortic endothelial cells, or conditioned medium from endothelial cells¹⁵²². Under these conditions it was found that most of the surviving eosinophils were of light density.

3.4.3. Colony stimulating factors, CSFs.

It was first reported in 1986 that several CSFs can prolong the survival of human blood eosinophils in culture for up to nine hours. These factors were recombinant human GM-CSF¹¹⁰⁵, and partially purified preparations of murine GM-CSF, and murine IL-5, which increased the survival of blood

eosinophils in culture from a mean of 28 to 38 hours, but had no action on blood neutrophil survival¹⁸²³. Interestingly, recombinant human GM-CSF also enhanced the survival of neutrophils for six hours, but murine G-CSF, and its human analogue CSF beta only prolonged the survival of neutrophils, suggesting that there was cell lineage specificity for the actions of these CSFs on mature granulocytes¹⁴⁸.

Owen, and colleagues in Boston, U.S.A. were the first to show in 1987 that normal blood eosinophils can survive in culture for one, or two weeks in the presence of recombinant GM-CSF, providing it is replenished at each change of the culture medium. Sixty per cent of the inoculum survived. As GM-CSF can be produced by endothelial cells, which gave even better survival percentages in vitro, it was suggested that blood vessels might provide GM-CSF to enable perivascular eosinophils to survive for many weeks in vivo¹³⁴⁶.

3.5. Staining techniques.

There are many ways to stain eosinophils in sections, or when smeared, or centrifuged onto glass slides. As their granules are the most characteristic component of the cell, most staining methods detect these in fixed material. They can be fixed in air, absolute alcohol, methanol, or formalin, but care needs to be taken that their granule basic proteins are not extracted in weak acids, before they have been properly fixed.

3.5.1. Eosin, phloxin, and other fluorescein derivatives.

Because eosinophil granules contain basic proteins which are acidophilic, they can be stained with acidic dyes, such as eosin-Y, and phloxin. The component(s) in the granules which take up the acidic dyes have not been defined, but Ehrlich noted that the periphery of the granule was stained more than the centre⁴⁹⁹, and as EPO-deficient eosinophils stain normally with eosin¹⁴³⁷, so it is possible that ECP, and EDN/EPX are responsible.

The capacity of eosin Y, and the Romanowsky stains to stain eosinophil granules from man, pig, and horse is increased up to six times by pretreatment with 0.2 M EDTA, or 2 per cent sodium citrate for up to eight hours. This is believed to be due to chelation of metals which mask the reactive sites on the granule basic proteins for these stains. The absorption maximum of eosin is at 530 nm, and there is an additional component at 600-650 nm when Romanowsky stains are used. Both are increased by chelation treatment⁶⁹⁴.

In addition to eosin staining of eosinophils, other fluorescein derivatives, such as fluorescein-isothiocyanate (FITC) can also stain eosinophil granules, in immunocytochemical preparations, in man, and many other animals. This has often led to the incorrect impression that FITC-linked antibodies can stain eosinophil granules specifically⁵⁸¹, and can cause confusion when immunofluorescent studies are done on samples rich in eosinophils⁴⁴⁷.

The non-specific staining of eosinophils with fluorescein-labelled antibodies can be prevented by prior treatment with either (1) Lendrum's stain (chromotrope 2-R)⁸⁸², or (2) H₂O₂, and diaminobenzidine, which block the binding sites on the granules for the fluorescent conjugate^{1829,969}, or (3) p-benzoquinone⁵⁹⁹.

3.5.1.1. Eosinophil autofluorescence.

It has been known, at least since the mid 1960s, that alcohol-fixed eosinophils in bone marrow smears show intense autofluorescence when excited with green light, although some variation has been noted from patient to patient⁶⁰⁷. In 1987, this property was used to study the deposition of eosinophil granules in the bone marrow of patients with a variety of malignant diseases¹⁵⁴⁶.

In 1981, it was found that unfixed blood eosinophils could be separated from neutrophils in a fluorescent activated cell sorter, on the basis of their larger size, and greater autofluorescence¹⁸⁹⁵. An attempt was made to isolate the fluorescent component in eosinophils, but this was not successful. We have also examined this property of human eosinophils. We found that light density eosinophils in patients with an eosinophilia were of similar size, and fluorescence as neutrophils, so that they could not be distinguished by this technique. In 1982, blister fluids from six patients with bullous pemphigoid were found to increase human blood eosinophil autofluorescence¹⁸⁴⁰, but the signifi-

cance of this is not known.

3.5.2. Romanowsky stains.

The Romanowsky staining technique is the most commonly used method for detecting eosinophils in blood smears. Their morphology is best seen in cytocentrifuge preparations. The basis of this staining method was reviewed in 1987⁸¹⁶. In urine, Hansel's stain is said to be preferable to Wright's stain for detecting eosinophils¹²⁸⁴.

3.5.3. Chromotrope 2R.

As eosin (or phloxin) are not specific stains for eosinophils, Lendrum (1944) developed a method for staining fixed eosinophils using chromotrope 2R dissolved in phenol (carbol-chromotrope). Sections are stained first with haematoxylin, then with carbol chromotrope for 30 minutes. Other cells and tissues either do not stain red, or they develop a brown colour, which is easily distinguished from the bright red eosinophils¹⁰⁵⁶.

3.5.4. Biebrich scarlet.

Some acidophilic dyes can be made to stain eosinophils with great specificity, as they are only retained in eosinophils (and a few other cells with highly cationic constituents, such as spermatozoa, and Paneth cells), after washing in strong alkali solutions. Biebrich scarlet is one of the most useful stains to exploit this approach, as it provides a familiar red colour for eosinophils^{1671, 1564}. Adams' stain has been reported to be equally specific¹²²⁵.

3.5.5. Other stains.

A number of other acidophilic staining methods can be used to define eosinophils in tissue sections. These include basic orcein, ninhydrin, Luxol fast blue, aniline blue¹⁶², Congo red⁷²⁰, the triazo dye Chlorazol black E⁹¹⁵, p-dimethylaminobenzaldehyde-nitrite¹⁰⁴⁵, and most recently the xanthene dye C.I. acid red 52 (C.I. 45100) which stains eosinophil granules dark red⁹¹⁶. Mercurochrome has been suggested as a useful eosinophil stain on the grounds that it stains eosinophils, and is also fluorescent, and electron dense⁵⁵⁹. In electronmicroscopy, two per cent palladium chloride has been recommended to provide high quality contrasting images of eosinophil granules⁵⁶⁰. In 1987 it was reported that certain fluorescent-labeled lectins from *Griffonia simplicifolia*, and soybeans bound to human eosinophil granules, and could be used as specific markers for eosinophils in acetone-fixed cell smears, formalin-fixed, pepsin-treated tissue sections, and even eosinophils in suspension, after they had been made permeable with glycerol¹⁰⁴⁷.

3.6. Eosinophil structure.

The principal structures in human blood, and tissue eosinophils are shown schematically in Figure A03-2.

Fig. 3-2: The structure of eosinophils.

Eosinophils are usually oval in sections of tissues, although long pseudopodia can develop in culture, and these may be seen in samples of blood, and tissues⁷⁴⁹.

The classical study on the ultrastructure of human blood eosinophil granules was reported from the Rockefeller University, New York, U.S.A., by Palade, and colleagues in 1966¹²¹³. They produced fine pictures of the crystals in human eosinophils, and showed that they were more varied in shape than in those in rat eosinophils. A regular structure was seen within about one third of human blood eosinophil granule crystals. These gave interference patterns characteristic of cubic lattice crystals with a periodicity of 41 mu, and occasionally 28 mu. In addition, the granule matrices frequently contained small membrane-bounded vesicles, which have not been discussed in later work, although they are commonly seen in eosinophils which have degranulated.

In the late 1960s, questions were raised about the possible significance (in normal eosinophils) of dense crystalloids in a lighter staining matrix, as compared to the presence of light staining crystalloids, and a darker matrix. This was resolved by careful work in 1966, which showed that the crystalline core of the normal eosinophil granule is darker when stained with uranyl acetate, and lead citrate. However this can be reversed by adding precipitating substances such as phosphotungstic acid, or molybdic acid during the alcohol dehydration stage⁵³⁷.

In 1986, morphometric studies on electronmicrographs made from normal blood eosinophils from six people, has shown that eosinophils have a volume of 275 fl, and a diameter of 8 μ . The nucleus occupies one fifth of the cell volume, and the specific granules one fifth of the cytoplasm¹⁶⁵⁹. The same group reported similar findings in eosinophils from nine men, and nine women in 1987¹⁶⁶⁰. Electronmicrographs of human blood eosinophils show that they can often contain lipid droplets³⁴⁰.

3.7. Eosinophil constituents, and their properties.

Eosinophils are difficult cells to break down into their component granules, nuclei, and membrane components, by traditional mechanical methods. They are usually suspended in 0.34 M sucrose, and either repeatedly passed through an 18 gauge needle¹⁹¹⁸, or homogenized in a glass homogenizer. After 20, or 30 strokes, a small number of eosinophils are ruptured, and this is only slightly increased as more strokes are made. The use of a Waring blender increases the number of eosinophils that are ruptured, but this also breaks the nuclei. An efficient method for obtaining human blood eosinophil cytoplasts, containing their granules was reported in 1985¹⁹⁹¹. This technique uses saponin to rupture the plasma membrane, followed by Percoll gradient separation of the cytoplasts.

3.7.1. Cell nucleus.

In 1967, the number of nuclear lobes in blood eosinophils was measured in 14 normal individuals, 15 with asthma, 10 with ulcerative colitis, and six others. The 'lobe index' in normals was 2.05, and 2.3 in the others. Occasional blood eosinophils had three, or even four lobes, but there was no correlation between the lobe index, and the blood eosinophil count¹⁶⁶⁸. In 1987 it was shown that there were more nuclear segments in bronchoalveolar lavage fluid eosinophils in patients with pulmonary infiltrates, than in other diseases with a pulmonary eosinophilia, and eosinophils with four segments were only seen in these patients³¹⁶. This suggests that segmentation increases as eosinophils reach maturity in tissues. The causes, or the consequence for cell function of the increase in nuclear lobes is not known.

Although individual chromosomes can be seen in dividing eosinophils in the marrow of patients with an eosinophilia, there has been no formal attempt to study the nature of the nuclear constituents in human eosinophils. The main classes of histones have been seen in extracts of human blood eosinophils. These are highly basic proteins, and as some have molecular weights comparable with those of the basic proteins in eosinophil granules, they could be a cause of confusion, if their presence is not recognized in crude eosinophil extracts.

For over twenty years antinuclear antibodies have been noted in the serum of some patients which reacted with fixed human eosinophil nuclei, but not with neutrophil nuclei. Their significance, and the way in which they are induced are unknown. In a study which was reported in 1985¹⁵¹⁷, this unusual antibody specificity was found in low titres only (less than 1/250) in four (4 per cent) of 112 children with juvenile chronic polyarthritis, and 14 (27 per cent) of 38 adults with rheumatoid arthritis. Their blood eosinophil counts were normal. A related antibody which only bound to the nuclei of neutrophils, has been described in the serum of patients with rheumatoid arthritis, and chronic active hepatitis in Australia¹⁹²⁷.

3.7.2. Cytoplasmic constituents.

The cytoplast of adherent human blood eosinophils has been studied by stereo high-voltage electronmicroscopy in 1987 at Chapel Hill, U.S.A.¹⁴⁵⁵. This showed the presence of a highly organized cytoplast, containing an intricate network of strands: the microtrabecular lattice. This lattice can alter the shape of granules. In stimulated cells, there was reorganization of the cytoplast, so that the granules become more central, and a framework was formed for the granules. The cytoplasm of human eosinophils contains a wide variety of components. Only a few of these have been well characterized, and their biosynthesis, structure, and functions in eosinophil biology are largely unknown. A summary of the probable biosynthetic pathways for the crystalloid granule proteins, and their secretion from the cell, is shown in Figure A03-3.

Fig. 3-3: The synthesis of eosinophil granule proteins.

3.7.2.1. Lipid bodies.

Lipid bodies are often present in blood eosinophils from patients with an eosinophilia, including patients with the Spanish toxic oil syndrome⁶¹³, and in tissue eosinophils. In light microscopy they can be confused with endocytotic vesicles, and altered granules, but as they do not have a surrounding bilaminar membrane they are quite distinct in electronmicrographs. The lipid contents are removed by fixation, or washing in alcohol. They are also found in mast cells, and some macrophages. In 1985 lipid bodies were studied in blood eosinophils from two patients with an eosinophilia, by autoradiography, after incubation of the separated cells with ³H arachidonic acid. It was found that the labelled unsaturated fatty acid was esterified to form phospholipids, which were retained almost entirely within lipid bodies. This suggested that lipid bodies may be an important site for the metabolism of arachidonic acid in eosinophils¹⁹¹¹. It is also possible that lipid bodies are storage sites for plasma membrane lipids, which need to be internalized, and replaced in the plasma membrane as the cell alters its shape, and hence its surface area.

3.7.2.2. Ribonucleic acid.

The RNA content of human marrow eosinophils decreases as they mature, and none can be found in blood eosinophils using standard histochemical methods⁵⁹. Nucleic acid synthesis in human marrow eosinophils was studied in samples from seven patients, by incubating marrow aspirates with ³H-uridine. Autoradiographs showed that some of the radiolabel had become associated with the crystalloid granules, suggesting that RNA might be synthesized, (or may accumulate) on the surface of these granules¹⁹²⁹. ³H-thymidine-5-triphosphate has been found to attach to the cytoplasm of human eosinophils. Neutrophils were not labelled¹⁸⁷⁴. The significance of this is not known.

3.7.3. Golgi apparatus.

The Golgi apparatus is prominent in blood eosinophils, distinguishing it from neutrophils. It has been known since 1968 that this is a major site for the processing of newly formed eosinophil granule components, when it was shown that 3(H)-lysine was incorporated into bone marrow eosinophils in vitro, and transported through the Golgi apparatus to the crystalloid granules⁵⁵². Microtubules probably link the Golgi apparatus to adjacent granules, although no direct connection has yet been shown in electronmicrographs.

3.7.4. Mitochondria.

Blood eosinophils contain more mitochondria than blood neutrophils. There has been no work on their properties, but there is no reason to think that they might be different from mitochondria in other cells.

3.7.5. Cytoplasmic granules.

Blood, and tissue eosinophils contain crystalloid granules which develop from immature granules, which first appear at the promyelocyte stage. A diagram of the localization of the main granule constituents is shown in Figure A03-4.

Fig. 3-4: Eosinophil granule proteins.

3.7.5.1. Crystalloid granules.

In 1975, eosinophils from the blood of five patients with HES at the N.I.H. Bethesda, U.S.A. were assayed for their enzyme content, and the results were compared with similar measurements on neutrophils. Eosinophil lysates had more total protein, and b-glycerophosphate than neutrophil lysates. Larger amounts of peroxidase, and b-glucuronidase activity were also found, which were present in extracts of isolated eosinophil granules, which had a density of 1.24g/ml. No lysozyme, or alkaline phosphatase was detected in eosinophils¹⁹¹⁸.

Eosinophils in the rat contain many of the enzymes described in human eosinophils, and these have been shown histochemically¹⁴⁰⁹. Ultrastructural studies have also shown the presence of alkaline phosphatase in rat eosinophils¹⁹³².

Rat crystalloid granules also contain peroxisomal enzymes^{1975, 1977}, which may be involved in the production of H₂O₂¹⁹⁷⁶. For this reason, rat eosinophil granules can be classified as having features of both lysosomal, and peroxisomal granules. It is not known if this is also true for human eosinophils.

3.7.5.2. 'Small' granules.

In electronmicrographs of blood, and tissue eosinophils, some small granules are visible. These were commented on by Parmley, and Spicer in 1974¹³⁶². The eosinophil small granule has a round, or elongated profile, 0.1-0.5 μm in diameter. They are moderately, but homogeneously stained in standard electronmicrographs, and contain uniform acid phosphatase activity, which is unmasked, and resists fixation. They stain strongly for arylsulphatase, although peroxidase activity is equivocal. Eosinophil promyelocytes, and myelocytes do not contain small granules. Blood eosinophils have 2-8 small granules per section. They are increased in number in tissue eosinophils, especially in Hodgkin's disease¹³⁶³. They have structural similarities with tertiary granules in rabbit heterophils, and some granules seen in mononuclear cells, and platelets. They were mainly found around the Golgi zone, and peripheral cytoplasm. It was suggested that they could fuse with pinocytotic vesicles, or supplement products in crystalloid granules.

Small granules have also been seen in rat uterine eosinophils¹⁷⁶¹, and as rat peritoneal eosinophils were found to take up gold particles within small granules, it was suggested that these could be small heterophagic organelles⁹⁹⁶.

I suspect that small granules in human eosinophils are the remains of crystalloid granules which have secreted most of their contents. My reasons for suggesting this are that (1) they contain similar components to the crystalloid granules, (2) the acid phosphatase in small granules is unmasked, as it is in granules without crystalloids, in contrast with crystalloid granules where acid phosphatase is mostly masked⁵²⁰ (3) they are most prominent in eosinophils which are in sites where they might be expected to be secreting their contents (4) the localization of arylsulphatase, which has been considered to be confined to the small granule, has only been assessed by histochemistry, and it may well be present as an inactive complex in the crystalloid matrix. Support for this would come from experiments showing that antibodies to arylsulphatase bound to both the crystalloid, and small granule components, but this has yet to be carried out.

3.7.5.3. 'Microgranules'.

In 1973, electronmicrographs of eosinophils from 20 different species were said to show the presence of 'microgranules', which were dumb-bell in shape¹⁵⁶⁶. However Zucker-Franklin in 1980, considered that these were small profiles of endoplasmic reticulum, and not specific granules, and that they were part of the cell's secretory machinery. This would explain their presence in dividing eosinophils (which are known to secrete their granule contents) and their peripheral location in the cell²⁰⁰⁷.

3.7.5.4. Comparisons with neutrophil granules.

There are several types of granules in human blood neutrophils: azurophil (primary) granules, which are lysosomal granules, specific (secondary) granules, which contain lactoferrin, and B12-binding protein, and the newly discovered tertiary granules, which are lighter than specific granules, and contain a gelatinase¹¹⁴⁷. It is probable that many lysosomal enzymes are common to both neutrophils, and eosinophils. This is supported by the finding that an antibody (Leu M1)¹⁸⁷⁸, recognized a common component in eosinophil, and neutrophil granules.

3.7.5.5. Comparisons with basophil granules.

Eosinophil crystalloid granules have several features in common with basophil granules. When eosinophil granules are first formed in the marrow they are basophilic, but they lose this property as they mature. MBP is present in both eosinophil crystalloids, and in the heparin-containing granules of human basophils. Normal basophils contained on average 140 ng MBP/ 10^6 cells, whereas purified eosinophils from normal donors, and patients with the hypereosinophilic syndrome had 4 979 and 824 ng MBP/ 10^6 cells, respectively⁷. MBP was not detected in mast cells¹⁰⁵³, although it may be synthesized by a mast cell line (Gleich 1987, personal communication).

Although guinea pig basophils can ingest horse eosinophil peroxidase (EPO) in vitro, which becomes associated with their granules⁴⁸⁶, and human basophil colonies can incorporate EPO in a similar way⁴⁸⁵, this is unlikely to provide a functionally significant quantity of EPO in basophils in vivo.

Basophils have also been shown to possess large amounts of CLC protein (lysophospholipase). In a recent study of this property, basophils were purified from the blood of normal people by flow microfluorometry with fluorescent anti-IgE antibodies. Charcot Leyden crystals formed after incubation for 24, or 48 hours. As there was staining of the basophil cell membrane with a polyclonal anti-CLC protein antibody, it was suggested that basophils, like eosinophils, have large amounts of this protein at their surface¹¹. The finding of CLC protein within basophils shows that the presence of this crystal type in tissues cannot be solely ascribed to eosinophils, but may represent basophil involvement in some instances. Mast cells do not contain CLC protein¹⁰⁵³.

3.7.5.6. Comparisons with mast cell granules.

Mast cells appear to be quite distinct from eosinophils. They do not contain MBP, or the CLC protein¹⁰⁵³, and eosinophils do not contain mast cell tryptase²⁸⁷.

3.7.6. Eosinophil major basic protein.

MBP has been detected, and characterised in eosinophils in a number of animals, including guinea pigs, rats, cattle, and horses, and man. It is believed to be an important mediator of eosinophil-dependent damage to parasites, and host tissues. The properties of MBP were reviewed by Gleich, and Adolphson in 1986⁶⁵⁶. There has been no work on the biosynthesis of MBP.

3.7.6.1. Purification, and structure.

MBP was first isolated from guinea-pig peritoneal eosinophils in 1973⁶⁶⁴, and subsequently from rats¹⁰⁶⁶. It was first purified from human eosinophils in 1976,⁶⁶⁵. The main method for purifying MBP involves its extraction in weak acids from isolated blood eosinophil granules, and partial purification on Sephadex G-50 columns followed by weak ion exchange chromatography⁶⁶⁵.

Early work on MBP centered on animal eosinophils. Guinea pig MBP was shown to have a molecular weight of about 11 kDa⁶⁶³. After electrophoresis in 8 per cent polyacrylamide gels it migrated as a single band. It contained 10 or 11 arginine residues, six half-cystine residues, and 0.8 per cent carbohydrate. When it was reduced, and carboxymethylated it was more stable in solution, and so easier to handle, and store. It had a pI of over 10.5. As MBP contains tryptophan it is not a histone-like protein.

The amino acid composition of MBP is different from ECP⁶⁶⁵. In 1987, the amino-terminal sequence of MBP from a patient with HES was reported to be unique: ?-?-Arg-Tyr-Leu-Leu-Va-Arg-Ser-Leu-Gln-Thr-Phe-Ser-Gln-Ala-?-Phe-Thr-?¹⁹⁰⁶. In 1987, sequence studies on purified, and mRNA translated human MBP showed it had 117 amino acids, with 8 half cysteines, with hydrophilic, and hydrophobic domains (Gleich 1987, personal communication). No work has yet been reported on possible differences between the storage, and secreted forms of MBP.

3.7.6.2. Assays for MBP.

Human MBP is homologous with guinea pig MBP, and antibodies to guinea pig MBP¹⁰⁶⁵ cross react with human MBP. Antibodies to human, and rat MBP do not cross-react. MBP can be assayed in serum, or other solutions with a radioimmunoassay¹⁸⁸⁸. MBP can be visualized in paraffin-embedded tissues, using a rabbit antibody after trypsin digestion⁵⁶².

The amount of MBP in blood eosinophils from 10 normal people was shown to be 8.62 +/- 1.45 ug/10⁶ eosinophils¹³⁸⁹, and it was thought to account for over half of the granule protein.

In 1978 MBP was shown to make up the crystalline core of guinea pig eosinophil granules, using peroxidase immunoelectronmicroscopy, and extraction from purified crystalloids¹⁰⁶⁴. In 1986, immunoelectronmicroscopy was used to localize MBP in human eosinophils. In studies with human blood eosinophils using gold-labelled rabbit antibody, MBP was shown to be present in the crystalloid granule cores of eosinophils from four normal individuals⁴⁹⁵. MBP in nasal polyps was found to be predominantly within eosinophil granule cores, and not within other eosinophil organelles, plasma cells, mast cells, lymphocytes, or neutrophils¹³⁹⁰.

In 10 normal subjects who were studied at the Mayo Clinic, Rochester Minnesota, U.S.A., the plasma MBP level was 0.307 +/- 0.038 ug/ml (mean +/- SD). In this group of normal people, and patients with an eosinophilia, plasma MBP levels were related directly to the blood eosinophil counts,

the per cent light density eosinophils, and the mean blood eosinophil MBP content¹³⁸⁹. It was shown previously that the serum MBP levels were related to blood eosinophil counts in a variety of diseases¹⁸⁸⁹, although in some skin diseases the serum MBP levels were higher than was expected from the counts. In some eosinophilic diseases there can be very high levels of MBP: up to 14 ug/ml (1.5×10^{-6} M) in serum; 30 ug/ml (3.2×10^{-6} M) in pleural fluid; and 93 ug/ml (1×10^{-5} M) in sputum⁶⁵⁶.

3.7.6.3. Functional properties of MBP.

The main effects of MBP are to damage, and/or kill parasites, and normal human cells, and tissues. It does not have any enzymatic activity. It became of particular interest to parasitologists when it was discovered in 1979 that MBP was highly toxic to the schistosomula of *S. mansoni*²⁵⁷. It is secreted from human blood eosinophils onto the surface of IgG antibody coated schistosomula, which are killed within a few hours. This was inhibited by heparin. Purified MBP induced a detachment, and ballooning of the tegumental membrane of schistosomula in vitro⁵.

In 1987 it was found that the reduced, and alkylated form of MBP was more effective than native MBP in killing newborn larvae of *T. spiralis*, which were killed rapidly⁷⁴⁷. However MBP can also have a protective effect by acting as a scavenger for HClO₂ ions. This reduced the toxicity of HClO₂ ions for newborn *T. spiralis* larvae²⁵⁹. MBP is also toxic for amastigotes, and epimastigotes of *Trypanosoma cruzi* in vitro¹⁸⁶⁴.

In 1980, a bovine equivalent of MBP was described. This was a 16 kDa protein, but poor in arginine. It was able to kill juvenile *Fasciola hepatica* in vitro⁴⁷³.

The initial studies with MBP in 1976 showed that it did not affect vascular permeability, or cause smooth muscle contraction⁶⁶⁵. However in 1984, it was reported that injections of MBP into the skin of normal subjects produced a wheal, and flare reaction⁶⁷².

MBP is also thought to be an important mediator of inflammation in the respiratory tract, especially in asthma. This was reviewed by Gleich, and colleagues in 1983⁶⁶². Evidence in favour of this suggestion came from the finding in 1979 that 10^{-7} to 10^{-5} M MBP killed tracheal epithelial cells in three to 23 hours⁶⁵⁸. MBP can kill some pneumocytes⁸³, and guinea-pig tracheal epithelium⁶⁰⁵. Although 10^{-5} M MBP was unable to kill cultured type II pneumocytes, EPO can do, so in the presence of H₂O₂, and a halide²⁴.

Purified human MBP reduced ciliary movement in rabbit tracheal explants, and directly inhibited isolated porcine tracheal ciliary axonemes⁷⁶⁸, suggesting that it could be responsible for mucus impaction, which is a prominent feature of severe asthma.

MBP is nearly as potent as poly-L-arginine in inducing a non-cytolytic release of histamine from human basophils. This is a slow response, taking place over 40 minutes, with half-maximal release at 17 minutes. Native MBP is more potent than reduced, and alkylated MBP¹²⁹⁴. There was day-to-day variation in the amounts of histamine released by MBP in vitro, from blood leukocytes (basophils) of patients with allergic disease, but there was no evidence that patients with allergic diseases released more histamine than normal¹⁹⁹⁷. Other work on MBP-induced effects on basophils were reported from Uppsala, Sweden in 1985¹⁶¹, and Chicago, U.S.A., in 1986¹⁷⁸⁵.

MBP caused the release of histamine from rat peritoneal mast cells within one minute¹²⁹⁴.

Guinea pig MBP, which was reduced, and alkylated to make it stable in solution, has been shown to be toxic for a number of cells in vitro. 10^{-7} to 10^{-5} M killed spleen cells, mononuclear cells, intestinal, skin, and tracheal cells in three to 23 hours. 5×10^{-5} M MBP destroyed endothelial cells in 24 h, and inhibited their growth at concentrations as low as 10^{-9} M⁶⁵⁸.

In 1974 it was shown that guinea pig MBP increased the clotting time⁶⁶³, but its site of action in the coagulation system has not been determined.

In 1986 an abstract of work on the properties of MBP on the human complement system stated that MBP can bind irreversibly to EAC4b3b, and inhibit the generation of the alternative pathway convertase. These activities may have been related to the strong charge on MBP, as reduced, and alkylated MBP was less effective than native MBP¹⁸⁹⁷.

3.7.7. Eosinophil cationic protein.

Eosinophil cationic protein is another of the principal basic proteins found within human eosinophils. It was first described in 1974 by Olsson, and Venge in Sweden¹³²⁹. It is not known whether eosinophils in other species contain ECP, or related ribonucleases.

ECP is synthesized as a larger molecular weight precursor which is processed to become the storage form of the protein¹³²⁵. Its biosynthesis was assayed by culturing bone marrow eosinophils from patients with an eosinophilia with ¹⁴C-leucine, followed by immunoprecipitation, PAGE electrophoresis, and fluorography. A 22 kDa ECP precursor protein was produced initially, which was processed into an 18-19 kDa molecule. This was inhibited by monensin¹³²⁷.

3.7.7.1. Purification.

ECP is usually prepared from isolated eosinophil granules which have been solubilized with weak acids. It is then chromatographed by molecular sieve, and ion exchange chromatography¹³³¹. Nowadays, this can be done easily using fast protein liquid chromatography¹³²⁷. ECP has also been purified from eosinophil granules by sequential chromatography on Sephadex G-50, and heparin-sepharose, where it was eluted in a broad peak at a higher salt concentration than EDN/EPX. This separated two forms in an early eluting (ECP-1), and a later eluting (ECP-2) fraction. In Uppsala, Sweden, Peterson and colleagues have developed a method for purifying ECP on a zinc-chelate Sepharose 6B column¹³⁹⁵.

3.7.7.2. Structure.

ECP exists as several species of molecular weights 18.5 to 22 kDa, but can dimerize. The smallest form is the most basic. These forms contain different amounts of carbohydrate. The isoelectric points of ECP are greater than pH 11, and it contains 11 per cent arginine, and 10 half-cysteines. It is a single polypeptide chain in SDS-PAGE gels, and contains 2.5 moles of zinc per mole of protein¹³³¹. When purified ECP was eluted isocratically from a high resolution cation exchange resin, it showed considerable heterogeneity. It was suggested that these could have different biological activities. There were five components probably differing in their hydrophobicity. They had molecular weights of 16.7-19.5 kDa, and virtually identical amino acid composition. The amino terminal sequence of the first 33 amino acids of ECP was determined in 1986¹³²⁷.

SDS-acrylamide electrophoresis of ECP-1 showed a major band at 18.3 kDa, and a minor band at 21.4 kDa. ECP-2 gave a doublet at 16kDa, and a band at 16.9kDa. In two-dimensional gels, ECP-1 migrated as a single spot with a heterogeneous tail in the acidic direction. ECP-2 migrated as a major spot, more acidic than ECP-1, with a heterogeneous tail in the basic direction. A minor spot of higher molecular weight showed comparable charge heterogeneity to the major band. Digestion of ECP-1 with Endo-F (which removes high-mannose, and complex oligosaccharides), reduced its size close to the two smaller forms of ECP-2. Endo-F digestion of ECP-2 removed the largest form. As Endo-F did not convert ECP-1 or ECP-2 to a single band, there may have been an O-linked oligosaccharide, or an amino acid difference at the C-terminal end of these two forms of ECP.

The N-terminal 59 amino acid residues of ECP-1, and ECP-2 were sequenced in 1986⁶⁶¹. These showed marked sequence homologies with human pancreatic ribonucleases, which was sequenced in 1984¹⁴⁹, and ribonuclease from several other species.

ECP has an affinity for zinc, which may account for the high levels of zinc in eosinophil granules. This enables the protein to be separated by zinc-affinity chromatography. If the association with zinc is linked with its ribonuclease activity, ECP can be classified as a metalloenzyme.

3.7.7.3. The secreted form of ECP.

Following solubilization, ECP undergoes further structural changes which have been demonstrated using monoclonal antibodies, one of which was shown to bind to the secreted form of the protein¹⁷⁴². This antibody (EG2) has shown the presence of the secreted form of ECP in areas of eosinophil degranulation in the skin, gut, heart, and spleen¹⁶⁹⁷. The secreted form of ECP has greater molecular weight heterogeneity than the storage form¹³⁹⁵.

3.7.7.4. Assays for ECP.

ECP in serum is usually assayed by a radioassay with either rabbit antibodies¹⁸⁵¹, or with monoclonal antibodies. The method which we use employs monoclonal antibodies EG1, and EG2 which can be purchased from SANBIO bv. Heinsbergenstraat 50. 5402 EG Uden. Postbus 540. 5400 AM Uden. The Netherlands. Phone: 04132 51115. Telex: 74827. In our technique, antibody EG1 first concentrates ECP onto the floor of the reaction microwell, and then labelled antibody EG2 is added, which binds to the fixed antigen, through a separate epitope. A commercial ECP assay kit, which uses rabbit antibody is being developed by Pharmacia, Uppsala, Sweden, for sale in 1988. A competitive binding radioimmunoassay with a monoclonal antibody has been used in some studies in the U.S.A.⁶⁶¹.

In 1987, a method was described for assaying ECP in urine, by an enzyme immunoassay with an antibody which was prepared against ECP from the urine of patients with interstitial cystitis. It was proposed as a useful assay for eosinophil involvement in this disease⁵⁹⁵. The cell content of ECP in normal human eosinophils is about 25 ug/10⁶ cells.

3.7.7.5. The localization of ECP in eosinophils.

In 1986, it was reported by three groups, using immunogold electronmicroscopy, that ECP was present in the granule matrix of human eosinophils: (1) This was shown with blood eosinophils from four normal people⁴⁹⁵. Small granules, which may correspond to those identified by Parmley and Spicer¹³⁶², were also positive. (2) Sections of nasal polyps showed that ECP was mainly in the granule matrix, with small amounts in the crystalloids¹³⁹⁰. (3) We localized the secreted form of ECP to the granule matrix of eosinophils using monoclonal antibody EG2¹¹¹⁵. ECP has only been found in eosinophils, unlike MBP. In contrast with the EM localization of ECP, subcellular fractionation of blood eosinophils has shown ECP in four bands of different density (Fattah, D. et al. 1985), unpublished observations). This was also noted by Peterson, and colleagues¹³⁹⁵, who also found that peroxidase was not present in three of these bands.

3.7.7.6. ECP in plasma, and serum.

Small amounts of ECP are found in normal serum. In 93 people, aged 18-64 years, serum ECP was 15 ug/L (2-116, 2SD)⁷³⁷. It binds tightly to the fast form of alpha 2 macroglobulin in vivo¹³⁹⁸, and this has been thought to inactivate its toxic properties, although this has not yet been tested. In 1977, serum ECP levels in normal people were found to correlate with their blood eosinophil counts¹⁸⁵¹.

3.7.7.7. Functional properties of ECP.

Like other strongly positive charged molecules, ECP binds to negatively charged surfaces, and this may localize its effects in vivo. Initial studies on ECP gave no clues about its roles in disease. It did not have bactericidal activity or esterolytic activity. It had no effect on histamine-induced, or guinea pig anaphylatoxin-induced contraction of ileal muscles¹³³¹, although it could augment the ingestion of immune complexes by phagocytic cells.

In 1986, it was found that ECP was a ribonuclease. Highly purified ECP completely degraded transfer RNA, and ribosomal RNA at concentrations down to 1.4 x 10⁻⁸M. No differences were found between the four ECP fractions which were separated by high resolution Mono-S cation resin exchange chromatography. However, the ECP ribonuclease activity was nonspecific, and less potent than the second ribonuclease in eosinophils, EDN/EPX⁷²⁹.

Both ECP, and EDN/EPX may belong to a ribonuclease family of proteins, which are derived from a common ancestral gene. The present day proteins of this type may include pancreatic ribonuclease, ECP, EDN/EPX, and angiogenin, which was cloned¹⁰¹⁹, and sequenced in 1985¹⁷¹⁷. Although these proteins have differing ribonuclease activity, they have considerable structural, and aminoacid homology which supports this possibility⁶⁵⁷. If ECP is found to have angiogenin-like properties this could account for the presence of many blood vessels in areas where eosinophil degranulation is prominent, and in diseases such as angiolymphoid hyperplasia with eosinophilia, where there is a close relationship between eosinophils, and abnormal vessel formation.

It is unlikely that secreted ECP (or EDN/EPX) could enter intact cells, or parasites, and destroy

their RNA, causing their death, as suggested in 1986⁷²⁹, although this may contribute to the final destruction of these targets. The toxicity of EDN/EPX, and ECP does not correlate with their ribonuclease activity, and pancreatic ribonuclease is not toxic for *T. spiralis* newborn larvae⁷⁴⁷.

ECP is a potent cytotoxic molecule for a range of parasites, especially the schistosomula of *S. mansoni*¹¹⁷⁸. In a study on the relative toxicity of purified ECP, and MBP for the schistosomula of *S. mansoni* in vitro, it was found that ECP was eight to ten times more toxic than MBP. However as eosinophils in some patients with an eosinophilia contain more MBP than ECP, it is possible that MBP may be more important under some circumstances⁵. In vitro, purified ECP caused the formation of blebs on the surface of schistosomula, followed by disruption of the surface, and extrusion of the parasite's contents^{1178,5}. ECP was able to paralyze the lung stage schistosomula of *Schistoma mansoni* in vitro, but did not cause structural damage, or parasite death¹¹⁷⁹.

In 1987, it was shown that ECP was as toxic as MBP in killing *T. spiralis* newborn larvae, although ECP killed more slowly⁷⁴⁷. ECP is also a powerful neurotoxin⁵⁹⁸. This was first discovered in 1982. 0.1-0.3 ug induced the Gordon phenomenon in guinea pigs, at concentrations 10-100 times less than those needed by the eosinophil neurotoxin, EDN/EPX, to produce a similar effect⁵⁹⁷. When 2-11 ug of purified ECP was injected into the cisterna magna of 17 rabbits, it produced paralysis in five animals. Minor symptoms were produced at doses below 2 ug. EDN/EPX had similar effects, but produced paralysis at doses less than 2 ug⁶⁶¹.

When ECP was instilled into the trachea of rabbits, it caused the epithelium to strip off, and inflammatory cells to accumulate in the lumen, with plugs of mucus, as seen in severe asthma (Dahl,R. et al. 1985, personal communication).

Although Zheutlin, and colleagues in the U.S.A., did not find in 1984 that partially purified ECP caused the release of histamine from blood mononuclear cells, containing basophils¹⁹⁹⁶, Bergstrand, and colleagues in Sweden reported in 1985 that ECP, at concentrations of 10 and 30 ug/ml caused direct histamine release from human basophils, and that ECP also inhibited anti-IgE-induced histamine release from basophils. These experiments, which have not yet been published in full, raise the possibility that eosinophils may either augment, or inhibit the effects of inflammatory stimuli in tissues giving rise to basophil secretion, and it might not be possible to predict how eosinophils would affect tissue responses in allergic diseases, such as the late-phase Ig E-mediated asthmatic responses¹⁶¹. Partially purified ECP caused the release of histamine from rat peritoneal mast cells¹⁹⁹⁶.

Two groups have shown that eosinophils can inhibit lymphocyte mediated responses in vitro. Experiments reported from Sweden using purified ECP, and EDN/EPX showed that at 10^{-10} to 10^{-7} M, both of these proteins inhibited ³H-thymidine incorporation into DNA, in cultures of human blood lymphocytes stimulated with PHA, or allogeneic lymphocytes. ECP, and EDN/EPX also appeared to increase the 'suppressor cell activity' generated by Con A¹³⁹⁶. In the U.S.A., extracts of human blood eosinophils, and secretion products from stimulated eosinophils were also found to inhibit PHA-induced lymphocyte proliferation in vitro, and a newly synthesized product from eosinophils appeared to mediate the effect¹⁴⁷¹. However, as immunological assessment of patients with HES have failed to show any consistent defects in blood T lymphocyte-mediated responses¹³⁶⁹, and there is no evidence that other patients with eosinophilic disorders have impaired T cell responsiveness, the relevance of these findings is not clear.

There has been no formal study on the possible interactions of ECP with tumour cells.

ECP affects the coagulation system in several ways. Initially, it shortens the coagulation time of normal plasma, but after 10 minutes, the clotting time becomes prolonged¹⁸⁴⁹. The recalcification time of plasma deficient in Factors V, VII, VIII, IX, X, and XI, is shortened by ECP, but the recalcification time of Factor XII-deficient plasma is prolonged, suggesting that ECP mainly interacts with Factor XII. For this reason, kallikrein activation, which is Factor XII dependent, was increased by ECP¹⁸⁴⁹. ECP also affects fibrinolysis. The hydrolysis of a plasmin-specific substrate by plasmin was increased by ECP, whereas ECP inhibited plasminogen activation by streptokinase, possibly

because ECP bound to streptokinase³⁸¹. Although plasminogen levels are normal in patients with an eosinophilia, marked defects in other coagulation factors have been found in patients with HES, showing that there is likely to be a clinically significant effect of eosinophils on coagulation *in vivo*. ECP inhibits the anticoagulant effect of commercially prepared heparin *in vitro*^{1850, 380}, and therefore has the potential to inhibit this component from mast cell.

There have been no reports of the possible interactions of ECP with the complement system, although this is likely to occur, in view of the strong basic charge on ECP.

3.7.8. The eosinophil-derived neurotoxin/eosinophil protein-X.

In 1933 M.H. Gordon reported that the injection into the brains of rabbits of extracts of eosinophil-rich lymph nodes from patients with Hodgkin's disease produced paralysis⁶⁹⁹. He thought that this was due to a virus in Hodgkin's tissue. A similar effect was found in 1939, following the injection of bone marrow extracts⁹⁶⁸. Then it became clear that eosinophils in these tissues were the cause of the brain damage¹⁸⁰². This reaction is now called the Gordon phenomenon.

The cause of the Gordon phenomenon began to be studied in the late 1970s in the U.S.A., where the name 'eosinophil derived neurotoxin' (EDN), was given to an activity in eosinophil extracts⁴⁷⁹, and in Sweden, where it was found to be due to a protein which had been called 'eosinophil protein-X' (EPX)¹³⁹⁷, because it was found in a column fraction which contained an unclassified protein (protein-X).

In 1982, work in Aarhus, Denmark showed that EPX¹³⁹⁷, was probably identical with EDN¹⁸⁴⁸. This review will use the term EDN/EPX for this protein, but clearly a better name is needed.

No work has been done on the biosynthesis of EDN/EPX, but it is likely to be similar to ECP, in view of their close structural homology, as described below.

3.7.8.1. Purification.

EDN/EPX was partially purified in 1979 by Durack, and colleagues in Seattle, U.S.A., by sequential sonication of blood eosinophils from three patients with HES, followed by ultracentrifugation, and fractionation on Sephadex G-50 at neutral pH. It was heat labile at 90°, but could be lyophilized⁴⁷⁹. Recent work on EDN/EPX was summarized in a report from the Mayo Clinic, U.S.A. in 1987⁶⁵⁷. It was purified from eosinophil granules by chromatography on Sephadex G-50, and heparin-sepharose, where it eluted at a lower salt concentration than ECP. In Sweden, EDN/EPX was purified by Bio Rex-70, and heparin-Sepharose chromatography¹³⁹⁵.

3.7.8.2. Structure.

The results were published in 1981 of a collaborative study by Durack with Ackerman, and colleagues at the Mayo Clinic, in which EDN/EPX was purified from extracts of whole eosinophils, and granules by Sephadex G-50 chromatography. This showed that EDN had an estimated molecular weights of 17.7-19.2 kDa⁴⁷⁸. In 1986, it was reported that EDN/EPX, which was purified from eosinophil granules by chromatography on Sephadex G-50, and heparin-sepharose, produced a major band at 17.4 kDa, and a minor band at 20.3 kDa. In two-dimensional gels, EDN/EPX migrated as two spots differing in charge, but not in apparent molecular weight. Two minor spots of slightly higher molecular weights were seen above each major spot. Endo-F, which removes high-mannose and complex oligosaccharides, affected the larger form of EDN/EPX only. Endo-H, which only cleaves high mannose linked oligosaccharides, had no effect, suggesting that EDN/EPX possessed a single complex oligosaccharide with some additions. The amino acid composition of EDN from two patients was similar, suggesting that the protein is not polymorphic⁶⁶¹. Similar findings, and confirmation of these results were reported by Peterson in 1987¹³⁹⁵. Like ECP, EDN/EPX undergoes a structural change when it is secreted, which can be detected with monoclonal antibodies. The nature of this alteration is not known.

3.7.8.3. Assays for EDN/EPX.

EDN/EPX can be assayed using rabbit antibodies, or mouse monoclonal antibodies to human EDN/EPX. It is not known whether eosinophils in other animals contain EDN/EPX. A monoclonal antibody which is specific for EDN/EPX has been described, which inhibited its ribonuclease activ-

ity, and could be used in a competitive binding radioimmunoassay⁶⁶¹.

The content of EDN/EPX in eosinophils has been estimated to be about 10ug/10⁶ eosinophils, which is about 40 per cent of the amount of ECP in eosinophils¹³⁹⁵. The serum levels of EDN/EPX in normal people, and patients with asthma were reported in 1977¹⁸⁵³.

3.7.8.4. Localization of EDN/EPX in eosinophils.

EDN/EPX has been presumed to be an eosinophil granule matrix protein since it was shown in 1982 that it could be extracted from isolated human eosinophil granules⁴⁷⁸, and that MBP accounted for all of the protein in crystalloids. Its localization in eosinophils within nasal polyps was shown in 1986, using colloidal gold particles with immunoelectronmicroscopy. EDN/EPX was localized to the granule matrix, with small amounts in the crystalloids¹³⁹⁰.

3.7.8.5. Functional properties of EDN/EPX.

In 1986 it was first reported by Gleich, and colleagues at the Mayo Clinic, that EDN/EPX had marked homology with ECP, and pancreatic ribonuclease⁶⁶¹. Later that year, in Lund, Sweden, it was found that purified EDN/EPX completely degraded tRNA at concentrations down to 5.7 x 10⁻¹⁰M⁷²⁹. Further work in the U.S.A. showed that ECP was 50 to 100 times less active as a ribonuclease than EDN/EPX. In addition, the ribonuclease activity associated with ECP was not significantly inhibited after exposure of ECP to polyclonal, or monoclonal antibodies to EDN/EPX^{1645, 657}.

There is some confusion as to whether EDN/EPX is toxic to schistosomula. One study showed that it had little effect⁵. Another study reported that EDN/EPX injured the schistosomula of *S. mansoni* in vitro, like ECP, and sub-lethal concentrations paralyzed the lung stage, causing structural damage to the sub-tegmental musculature¹¹⁷⁹. EDN/EPX was 10 times less effective than either MBP, or ECP in killing *T. spiralis* newborn larvae in vitro, and pancreatic ribonuclease was ineffective⁷⁴⁷.

It has been known since 1979 that intrathecal injections of partially purified EDN/EPX into rabbits caused stiffness in the fore-legs, and then the hind-legs. Ataxia, and weakness developed progressively, which prevented the animals, which appeared to have normal higher functions, from feeding, and drinking⁴⁷⁹. Histology showed lesions in the cerebellum, pons, and spinal cord, with loss of Purkinje cells of the cerebellum. The gray matter was normal, but the white matter in the spinal cord, cerebellum, and pons showed spongiform changes⁴⁷⁹.

In 1982, work in Aarhus, Denmark showed that EDN/EPX was highly toxic to the guinea pig brain, after intrathecal injection, and damaged all cells in proximity to the ventricular system. In these animals ECP was 100 times more toxic than EDN/EPX¹⁸⁴⁸. On the other hand in rabbits, ECP and EDN/EPX had equivalent effects, which suggests that there is some species differences in the effects of the human eosinophil neurotoxins.

In 1986 further work at the Mayo Clinic showed that as little as 0.15-7 ug EDN/EPX produced ataxia in rabbits after injection into the cisterna magna (five of eight rabbits). The onset of symptoms was three days at the higher doses, and seven days at the lower doses⁶⁶¹.

The mechanism for this toxic effect has not yet been defined, but presumably involved binding to, and interference with Schwann cell functions. No studies have yet been published on the effects of purified eosinophil granule proteins on isolated neuronal cell, or glial cell function, although the growth of brain cells in vitro may be inhibited (Gleich, G.J. 1987, personal communication). Measurements of cerebrospinal ECP levels have begun to show that ECP may also be toxic to the human brain in patients with a variety of brain disorders⁷⁴⁰.

Two groups have shown that partially purified EDN/EPX does not induce the release in vitro of histamine from blood mononuclear cells, containing basophils^{1996, 161}. In addition, partially purified EDN/EPX did not induce the release of histamine from rat peritoneal mast cells in vitro¹⁹⁹⁶.

It was reported in 1986 that EDN/EPX at 10⁻¹⁰, to 10⁻⁷ M, inhibited the uptake of ³H-thymidine by PHA-blasts, or MLR-blasts in a dose-dependent mechanism. This was irreversible, and it was not due to cytotoxic damage. It was suggested that the suppressive effect of EDN/EPX might involve suppressor cells, and it was proposed that EPX could have a regulatory role in immunological reactions¹³⁹⁶.

There are no reports on the possible effects of EDN/EPX on the respiratory system, or its localization in respiratory diseases, and it is not known whether EDN/EPX is toxic for the heart, or vasculature, or affect their function. No studies have been reported on the possible effects of EDN/EPX on the coagulation, or complement systems.

3.7.9. The relationship between ECP, and EDN/EPX.

In 1985 I suggested that ECP, and EDN/EPX could be products of a common gene family¹⁶⁸⁷, as they had similar physical, and antigenic properties. Direct evidence to support this was produced in 1986, when sequence homology between these two proteins was first established⁶⁶¹. Proof for this awaits sequencing of the genes for ECP, and EDN/EPX. However, they (1) have a similar amino acid content, (2) have a closely related N-terminal sequence, (3) possess at least one common antigenic epitope¹⁷⁴², (4) have ribonuclease activity, and (5) produce similar effects on brain cells, and lymphocytes¹³⁹⁶. For these reasons, it might be preferable to call ECP, and EDN/EPX by a common name, such as 'eosinophil ribonuclease', and to give each variant an internationally agreed enzyme classification term.

3.7.10. Eosinophil peroxidase.

In 1963, G.T. Archer, and colleagues first showed that EPO was distinct from myeloperoxidase, which had been studied in neutrophils. This conclusion was based on the finding that human, and horse EPO were not inhibited by 10^{-3} M cyanide, or azide, unlike myeloperoxidase⁶¹. Two years later it was shown that partially purified EPO from rat peritoneal eosinophils was a haemoprotein, with different reaction kinetics with guaiacol, and different spectral properties to myeloperoxidase⁵³. In guinea pigs the substrate requirements for EPO have also been shown to differ from neutrophil myeloperoxidase⁵²⁸.

3.7.10.1. Biosynthesis.

The sites of peroxidase transport from ribosomes to the storage granules of rat, and rabbit eosinophil myelocytes were studied in 1970 by a combination of cytochemistry, and electronmicroscopy, and shown to occur along the endoplasmic reticulum, and to involve the Golgi cisternae, from where the enzyme became localized in primary, and crystalloid granules. At a later stage in differentiation, the enzyme could only be detected in the granules. Acid phosphatase, and arylsulphatase followed the same pattern, but were not detected in the mature granules by this technique¹⁰⁰. In 1979, electronmicrographs of human bone marrow eosinophils also showed peroxidase to be present in the rough endoplasmic reticulum, and Golgi apparatus, and in large homogeneous granules (0.6 to 1.2 μ m) in early eosinophils. There was some structural evidence for secretion of peroxidase from these dividing eosinophils⁸³⁴.

Patients with a congenital absence of myeloperoxidase, have eosinophils containing normal amounts of EPO. This proves that the genes responsible for the production of eosinophil peroxidase, and myeloperoxidase are distinct.

The biosynthesis of human EPO in marrow samples from patients with an eosinophilia was studied by pulse-chase experiments with ¹⁴C-leucine in 1985. It was found that three large molecular weight proteins of 78, 72, and 25 kDa, were labelled, and the 53 kDa heavy chain of EPO was produced, by a pathway which was not inhibited by Monensin. As the antibody which was used in this study did not bind to the light chain of EPO, its biosynthesis from the labelled 25 kDa protein was only surmised¹³²⁶.

3.7.10.2. Purification.

In 1965 rat peritoneal EPO was purified by electrophoresis, and shown to be a haemoprotein with several differences from myeloperoxidase, including its reaction kinetics with guaiacol, and its spectral properties. These were a Soret maximum of 403 nm for oxidized EPO, and 437 for its reduced form⁵³. Guinea pig EPO was purified from bone marrow in 1972, and found to be a monomeric enzyme of 75 kDa, which could dimerize. It had a Soret maximum at 425 nm⁴⁴⁵. Horse EPO has been isolated from blood eosinophils⁸⁹⁵, and eosinophil granules⁸⁹⁴. They were very basic. Work on horse EPO, including its basic properties was reviewed in 1983⁹⁷⁶. Human EPO was isolated from

whole eosinophils in several studies reported between 1981, and 1984^{188, 1321, 1920}, and from eosinophil granules in 1985^{280, 1326}. In 1986, a method was developed, which used granule-rich eosinophil cytosomes¹¹⁸⁹, to give EPO of high purity.

3.7.10.3. Structure.

EPO is a highly basic two chain haemoprotein which loses much of its enzymatic activity when it is purified. The initial work on the enzymatic properties of EPO was done in rodents, and then horse EPO. Subsequent studies on human EPO have given a clear indication of its size, the nature of the haem component within the molecule, and its biophysical characteristics.

Human EPO has a molecular weight of between 71 kDa¹⁸⁸, and 77 kDa²⁸⁰. It consists of a heavy chain of 50-58 kDa, and a light chain of 14-15 kDa, and it has a Soret band at 412 nm¹⁸⁸.

Studies on the amino acid composition of EPO showed that it had a high content of arginine, leucine, and aspartic acid^{188, 280}, and an isoelectric point greater than 11²⁸⁰. Carbohydrate is associated only with the heavy chain, and although the light chain is homologous with the MPO light chain, they are distinct¹³²².

In 1987, the partial N-terminal amino acid sequence of the smaller 14.45 kDa unit of peroxidase was shown to have a unique sequence of: ?-?-Ser-Asp-Lys-Try-Arg-Thr-Ile-Thr-Gly-?-?-Asn-Asn-Glu-?-?-Pro-Leu-... The larger chain was 49.250 kDa¹⁹⁰⁶. So far neither component has been fully sequenced.

The haem prosthetic group in human EPO has been studied in detail^{188 1622}. This work showed that EPO is closely related to horseradish peroxidase, lactoperoxidase, and intestinal peroxidase, and unrelated to catalase.

No studies have been done to see whether EPO alters its antigenicity, or size when it is secreted from eosinophils.

3.7.10.4. Assays for EPO.

Semi-quantitative measurements of EPO concentrations have been made for many years by enzyme cytochemistry. Quantitative assays for EPO are either enzymatic, which have the disadvantage that EPO loses its activity relatively easily, or based on immunoassays with antibodies, which have the disadvantage that EPO can bind nonspecifically to surfaces, because of its basicity. Eosinophils contain 15 ug EPO /10⁶ eosinophils²⁸⁰.

EPO is commonly assayed by its ability to oxidize guaiacol, or p-phenylenediamine with H₂O₂. EPO is markedly inhibited by 3-amino-1,2,4-triazole, unlike myeloperoxidase, so that the two enzymes can be assayed when mixed together³⁶⁵. Conversely, EPO can be assayed when MPO has been inhibited¹⁹².

The histochemistry of human EPO has been studied extensively, see:⁷⁵⁰. EPO can be distinguished from myeloperoxidase because EPO is less easily inhibited by KCN in cell smears, and sections. However, as MPO in neutrophil promyelocytes is less easily inhibited than blood neutrophil MPO, this can cause some difficulties with marrow samples¹²²⁶.

A radioimmunoassay for EPO has been developed, with a specific rabbit antibody²⁸⁰. A monoclonal antibody to rat EPO has also been made, and used in immunocytochemical studies⁹⁴⁴.

A mouse monoclonal antibody has been produced, which binds to human EPO, and which has demonstrated the presence of large amounts of EPO in lymph nodes of patients with nodular sclerosing Hodgkin's disease¹⁵⁴⁵.

3.7.10.5. The localization EPO in eosinophils.

Human, and animal EPO is localized to the matrix of eosinophil crystalloid granules^{100, 520, 949, 1963}. Lesser amounts are found in the small granules. Quantitation of the peroxidase-reaction product in ultrathin sections of murine eosinophils, has been carried out by electronmicroscopy using a platinum technique⁴¹². EPO deficient human eosinophils have a thin matrix layer in electronmicrographs¹⁰⁶⁰.

3.7.10.6. Functional properties of EPO.

EPO has the property of catalyzing the formation of HClO⁻ ions, and H₂O₂. It has been mainly

studied for its capacity to act in the presence of hydrogen peroxide, and a halide as a cytotoxic effector system. This is a potent mechanism for killing some parasites, bacteria, tumour and cells in vitro. In addition to this effector role, it can diminish the effector roles of inflammatory cells, by breaking down LTC₄. It does not cause the Gordon phenomenon.

The principal role of EPO is to oxidize substances, by a catalytic action with H₂O₂, which is also generated by eosinophils^{975,977}. In the presence of iodide^{1412,1209}, bromide¹⁹⁰², or chloride⁸⁹⁰, this acts as a potent mechanism for killing a variety of organisms. EPO has a different pH optimum from MPO¹⁹¹⁹.

The EPO, H₂O₂, halide system is directly toxic to a number of parasites including schistosomula⁸⁹², *T. spiralis* newborn larvae¹³⁰, *Toxoplasma gondii*¹⁰⁸⁹, and *T. cruzi*¹²⁸³. EPO can also augment the capacity of neutrophils to kill schistosomula in vitro⁸⁸⁹. EPO is also able to kill newborn larvae of *T. spiralis* by generating HClO⁻ ions, although it is less efficient than MPO in generating hypochlorite ions²⁵⁹.

In 1965, G.T. Archer found that purified rat EPO caused mast cells to disrupt in vitro⁵⁷. This was confirmed in 1984, and studied by scanning electronmicroscopy³¹⁵. In 1980, a similar process was seen using human EPO⁷⁷⁸, with the additional finding that a complex of EPO with mast cell products was more effective than EPO alone. Unfortunately there are no studies on whether EPO can cause mast cell degranulation, or disruption in vivo. EPO has been found to attach to the surface of isolated rat mast cell granules, where it retained its enzymatic properties⁷⁸¹.

EPO appears to be able to 'arm' macrophages, so that they become more effective in killing staphylococci¹⁴⁷⁵, toxoplasma¹⁰⁸⁹, and trypanosoma¹²⁸³.

In a study reported in 1987 on the uptake of EPO by human neutrophils, it was found the the enzyme was internalized, and held in microvesicular bodies. This did not occur with MPO¹⁹⁹⁰. It is not known whether the uptake of EPO is a method for removing this potentially toxic molecule, or whether it increases the effector functions of inflammatory cells.

EPO can bind to neoplastic cell lines cells in vitro. This makes these cells susceptible to macrophage-mediated cytolysis, in the presence of H₂O₂ which is released from macrophages after contact with the coated cells¹²⁶⁶.

Under certain conditions, EPO, like other peroxidases, can inactivate the leukotrienes LTB₄, LTC₄, and LTD₄⁷⁸². Its action on LTC₄ is to produce biologically inactive isomers of LTB₄⁶⁷⁹, and other molecules¹⁷⁹⁹.

3.7.11. Other cytoplasmic granule constituents.

Many different lysosomal enzymes, and other components have been characterised in human blood eosinophils.

3.7.11.1. Phosphatases.

Large amounts of acid phosphatase have been extracted from human eosinophils, but in unstimulated eosinophils this enzymatic activity is masked, although the enzyme can be demonstrated with antibodies⁷⁷. In 1966, cytochemical electronmicroscopy of normal human eosinophils showed that acid phosphatase was not detectable in granules containing cores, but was found both in the specific granules which did not possess crystalloids, and in small granules^{520,639}.

In 1980, Bass, and colleagues noted that only eight per cent of granules in blood eosinophils from normal individuals stained for acid phosphatase, when assessed by cytochemical electronmicroscopy, whereas in patients with an eosinophilia, 49 per cent stained. The percentage stained could be increased by stimulating normal eosinophils with opsonized staphylococci, or the calcium ionophore A23187¹²⁷. They studied the activation of this enzyme in detail, and showed that it occurred rapidly¹²⁸. However it is not known whether activation is due to an alteration in the permeability of granule membranes, dissociation of the enzyme from a glycosaminoglycan backbone, or some other mechanism.

3.7.11.2. Proteases, collagenase, and elastase.

Although neutrophils contain a potent collagenase, and this enzyme had been described in rat¹³⁴,

and guinea pig eosinophils⁷⁹⁴, it was not until 1984 that human eosinophils were also shown to contain an enzymatic activity which degrades collagen⁴⁰⁹. In this study, extracts of human eosinophils were shown to degrade collagen types 1, and 3. Some experiments suggested that the collagenase was different from neutrophil collagenase which cleaves type I collagen preferentially. This paper also showed that guinea pig eosinophil granule extracts contained a 'masked' collagenase, which was only revealed after trypsin treatment. Unlike macrophages, and neutrophils, eosinophils were shown not to contain elastase, or other types of nonspecific neutral protease.

3.7.11.3. Arylsulphatase B.

Eosinophils possess more arylsulphatase activity than other leukocytes, and it is present in the granules in an inactive form¹⁰⁰, but the enzyme is active in small granules, most commonly in tissue eosinophils. In 1980, arylsulphatase B was purified from human eosinophils, and shown to have a molecular weight of about 60 kDa, and a pH optimum of 5.7, which is characteristic of type II arylsulphatase. It was suggested that it was involved in the catabolism of proteoglycans, and glycosaminoglycans¹⁹¹⁷. In 1983, further work showed that it probably had a four-subunit structure, with a minimum molecular weight of 70 kDa. Its variable activity could be accounted for by changes in the association of its subunits¹⁹⁰⁷.

Arylsulphatase B is inactivated by LTC₄ in vitro¹⁹¹⁰. Suggestions, which were made between 1974 and 1976, that eosinophil arylsulphatases could break down SRS-A^{1334, 1884, 932} are incorrect. This activity was due to the presence of contaminants in the arylsulphatase preparations¹³⁵⁹. In 1981 the content of arylsulphatase B in eosinophils was assayed, and shown to alter during the eosinophilia which occurred when bancroftian filariasis was treated with diethyl carbamazine¹⁹¹⁶.

3.7.11.4. Histaminase.

Histaminase (diamine oxidase), and histamine methyltransferase are the only constituents in granulocytes which degrade histamine in man. They have been shown to be secreted from human blood neutrophils after incubation with opsonized zymosan, or the calcium ionophore A23187¹⁹⁹⁴. Eosinophils possess an histaminase, but this is present in smaller amounts than in neutrophils¹⁹⁹⁵, although greater activity has been found in eosinophils from patients with an eosinophilia⁷⁸⁹.

Eosinophil histaminase secretion has been studied by Colten, and colleagues in Boston, U.S.A., who compared it with the secretion of histaminase from neutrophils⁷⁹⁰. A number of differences were found. Histaminase, and arylsulphatase were only secreted from purified blood eosinophils when they had phagocytosed C3b-, or cobra venom factor-coated particles, whereas attachment to C3b-particles was sufficient to stimulate neutrophils. Cytochalasin B inhibited the release of histaminase from eosinophils, but increased neutrophil secretion. Components of the alternative pathway of the complement system also appeared to be required to induce eosinophils to secrete, as it did not occur when serum was depleted of factor B.

3.7.11.5. Phospholipase B, and D.

It is not known whether human eosinophils contain phospholipase B, although this enzyme has been found in eosinophils in other species, including the rat, and horse. Phospholipase D was isolated from human eosinophil granules in 1976⁹¹⁷. In 1980 it was shown to be able to liberate 0.3 nmol choline/hour/5 x 10⁶ eosinophils, which is ten times more than neutrophils. It had a molecular weight of 60 kDa¹⁹¹⁷. This enzyme is also able to degrade PAF-aceter.

3.7.11.6. Catalase.

Human eosinophils contain catalase, which has been localized by immunoelectronmicroscopy⁸⁴².

3.7.11.7. Nonspecific esterases.

Eosinophils contain nonspecific esterase activity¹²³², which is associated with the membranes of eosinophil granules, mitochondria, rough endoplasmic reticulum, and perinuclear cisternae¹⁰⁷⁴.

3.7.11.8. Vitamin B12 binding proteins.

A vitamin B12 binding protein has been found in eosinophils¹¹⁵⁰. In 1984, measurements were made of serum unsaturated B12 binding proteins in nine patients with an eosinophilia, four of whom had HES. The serum B12 levels, and/or the unsaturated B12 binding capacity were markedly raised in

all of them²⁰⁰². In a further three patients with HES studied in India, normal levels of B12 binding proteins were found⁶⁴⁰.

3.7.11.9. Fibroblast growth factors.

Fibroblast growth factors have been described in supernatants, and cultures of many different cell types¹⁷⁷⁰. The eosinophil appears to be no exception. Take has unpublished work showing that sepharose-C3b stimulated human blood eosinophils released fibroblast growth-stimulatory factors of molecular weight 30-50 kDa. In 1987, Pincus, and colleagues in Boston, U.S.A., detected a similar activity in human, and guinea pig eosinophils¹⁴¹⁸. It was suggested that this might explain the occurrence of fibrosis in some lesions where eosinophils are found.

3.7.11.10. Glycosaminoglycans.

Glycosaminoglycans (acid mucopolysaccharides) have been demonstrated within the granules of immature, but not mature rat, and guinea pig eosinophils¹²⁵⁶.

3.7.11.11. Metals.

A number of heavy metals are present in eosinophils¹⁴⁰⁵. These include zinc, copper, manganese, magnesium, cobalt, and iron. They are probably bound through nitrogen ligands, especially the amine, and imidazole groups of basic amino acids. The precise localization of zinc in eosinophils remains to be established. It could be determined by electronprobe scanning electronmicroscopy. It is not an integral part of lysophospholipase¹⁹¹⁴, as was considered at one time. However in 1980 it was found with isolated ECP at 2.5 moles of zinc/mole protein¹⁸⁵⁰, so it is presumably a granule matrix constituent.

3.7.12. The Charcot-Leyden crystal protein.

An unexplained feature of human eosinophils is the presence of large amounts of lysophospholipase (lysolecithin acylhydrolase, EC 3.1.1.5, or Charcot-Leyden crystal protein, CLC protein) in eosinophils, and in sites where eosinophils have degranulated, where it forms into characteristic bipyramidal crystals: Charcot-Leyden crystals.

3.7.12.1. Biosynthesis.

In 1986, murine eosinophils were found to contain more lysophospholipase after culture with T cells, and macrophages with antigen, than when cultured alone, suggesting that cytokines could induce eosinophils to synthesize this protein¹⁹. The biosynthesis of CLC protein was studied in 1987, in human blood eosinophils, and HL-60 cells after culture for 20, and 4 hours respectively, using ³⁵S-methionine. The newly synthesized labelled protein was found to be post-translationally modified by covalent attachment to myristic acid¹³.

3.7.12.2. Purification, and structure of EPO.

A method for preparing large amounts of CLC protein was reported in 1980⁸. In the mid-1970s the CLC protein began to be studied at the Mayo Clinic, U.S.A., and several of its biochemical characteristics were defined: it was shown to be a single hydrophobic polypeptide chain of 117-119 amino acids, with 18-19 residues of glutamic acid, 12 of valine, six residues of arginine, and 1.2 per cent carbohydrate. Later it was shown to have one sulphhydryl group⁶⁶⁵. It could be solubilized at neutral pH after freeze drying, and had a pI of 5.7-5.1. and an apparent molecular weight of 13 kDa⁸. This molecular weight was subsequently revised to 17.4 kDa in 1984¹⁹⁰⁹. CLC protein gave rise to several bands on electrophoresis in polyacrylamide gels, and isoelectric focusing, but immunoprecipitation with antibodies, and electrophoresis in SDS-polyacrylamide gels showed only one band.

3.7.12.3. Charcot-Leyden crystals.

In vivo, lysophospholipase forms into characteristic bipyramidal crystals, with a hexagonal cross section in tissues, sputum, and faeces where many eosinophils have accumulated, and degranulated, and within eosinophils themselves. They can also be induced to form in vitro, using whole eosinophils, or purified lysophospholipase.

Robin first described these crystals in Paris, France in 1853, in the heart blood, and spleen of a patient with a leukaemia, being looked after by Dr. Charcot³⁰⁶. In 1872 Leyden noted them in the

sputum of a patient with asthma¹⁰⁷². Since then, Charcot-Leyden crystals have been seen in the tissues of patients with a wide range of other diseases¹⁴², including eosinophilic pneumonia⁹⁰⁶, pulmonary ascariasis, tropical eosinophilia, ulcerative colitis, HES⁴⁵², eosinophilic granuloma, histiocytosis X⁹⁰⁵, pemphigus vegetans¹⁰¹⁸, and pleural effusions of diverse causes¹²⁶⁸. Their presence is believed to show sites of extensive eosinophil inflammation.

Experimental work on crystals from eosinophils began in 1922, when eosinophils from a patient with hypereosinophilia were seen to form crystals after incubation at 37°. These were soluble in hot water, 2 per cent acetic acid, and sodium hydroxide¹¹⁷². Charcot-Leyden crystals were shown to consist of a small protein in 1956²²⁸, and 1962⁸¹³. In 1963 G.T. Archer, and Blackwood isolated blood eosinophils from a patient with $32.4 \times 10^9/L$ eosinophils. After the cells had been left for 1-2 days in a refrigerator, Charcot-Leyden crystals were found to be present. Basophils which were suspended in hypotonic solutions also formed Charcot-Leyden crystals within two minutes. The crystals appeared in the cytoplasm, and granules after the cells, and their nuclei had become swollen. They also found that Charcot-Leyden crystals could be formed from protein extracts of eosinophils, and that they were soluble in acid solutions⁵⁴. In 1971, it was noted that these crystals could form within the crystalloid granules, and electronmicrographs suggested that they formed by a rearrangement of the crystalloid constituents⁵⁰². This is probably untrue, as the crystalloid consists only of MBP.

CL crystals also form in vitro, when either eosinophils, or basophils have been left in hypotonic solutions, or after they have been treated with detergents. In tissues, although most crystals are probably derived from degranulated, or lysed eosinophils, it has also been shown that they can form in intact eosinophils. For example, an electronmicrograph which was made in 1980 of a bone marrow eosinophil promyelocyte showed a CL crystal within the cell²⁰⁰⁷.

The persistence of CL crystals in tissues is probably due to their resistance to proteolytic digestion, and they may continue to function as lysophospholipases in the crystalline form¹⁹¹⁷. They are soluble in alcohol, and so leave characteristic spaces in conventionally processed tissue sections.

3.7.12.4. Assays for CLC protein.

A radioimmunoassay for CLC protein, using a rabbit antibody, was described in 1980⁸, and 1983¹². Eosinophils contain more than two, and a half times the amount of lysophospholipase as mononuclear cells, and eight times as much as neutrophils¹⁹¹⁷. Initial work on the CLC protein in eosinophils suggested that it was only present in the plasma membrane, and not in granules, or cytoplasm¹⁹¹⁷. This seems to be unlikely, as CL crystals have been seen to form within granules in vivo, and in vitro, as described above. Normal human serum contains 37 ng/ml of CLC protein. High levels have been found in patients with raised blood eosinophil counts due to many different diseases (over 8 ug/ml in some patients). In these patients, the CLC protein levels were over twice as high as serum MBP levels⁸. Levels correlated (a) with peripheral blood eosinophil counts both in normals, and patients with an eosinophilia, and (b) with serum MBP levels. There was also a suggestion that CLC protein might exist in several forms in serum¹⁰.

3.7.12.5. Functional properties of CLC protein.

There is still no clear explanation for why eosinophils should contain so much lysophospholipase, or why it should be released from eosinophils to accumulate to form the characteristic crystals. Initial work on the CLC protein in 1976, showed that it did not alter vascular permeability, contract guinea pig ileum, or antagonize the effects of bradykinin, or histamine⁶⁶⁵, and it does not cause the Gordon phenomenon.

In 1980 Weller, and his colleagues in Boston, U.S.A., first showed that the CLC protein had lysophospholipase activity¹⁹¹⁴, and that purified lysophospholipase formed into CL crystals¹⁹¹⁴. Evidence to support the identity of the CLC protein, and lysophospholipase includes (a) that they both have this enzyme activity, (b) that they comigrate on SDS polyacrylamide gels, (c) that they have antigenic identity¹⁹⁰⁸, (d) that they have the same Michaelis constant, (e) that they have an identical aminoacid composition, with blocked terminal amino acids, and (f) that they are identical

with faecal CLC protein. CLC protein contains a single cysteine residue, and it may make up 10 per cent of the total proteins in eosinophils¹⁹⁰⁹.

It is possible that lysophospholipase protects eosinophils from the toxic effects of lysophospholipids¹⁹¹⁷, which have been shown to produce structural alterations in human eosinophils¹⁷⁴¹, or from toxic products released by parasites, or from metabolites generated in the plasma membrane itself, such as PAF-acether which would otherwise damage the cell.

It is also possible that CLC protein is involved in parasite killing. This has been suggested by Adewusi, and colleagues, who have been studying lysophospholipase activity in mice infected with *T. spiralis*. They showed that anti-thymocyte serum reduced the eosinophil response, and the tissue lysophospholipase activity in the tissues of infected mice. As the cuticle of *T. spiralis* contains phospholipids, it was suggested that lysophospholipase might have a hydrolytic action on the parasite cuticle^{21,20}, but this has not yet been tested directly.

3.8. Eosinophil metabolism in resting, and stimulated eosinophils.

Much of the work on human eosinophil metabolism has been carried out with blood eosinophils isolated from patients with a marked eosinophilia, especially patients with HES. As recent work has shown that the metabolism of eosinophils can vary considerably from the resting to the stimulated stage, it has proved difficult to make relative assessments of the amount of glucose, oxygen and other compounds utilized by eosinophils in normal, and diseased states. However, measurements made by several groups show that eosinophils have a remarkably high capacity to develop a respiratory burst, to generate oxygen radicals, to metabolize glucose anaerobically, and generate large amounts of leukotrienes, and other products of arachidonic acid metabolism. Much of the energy for this work is stored in ATP, which is the principal nucleotide in eosinophils⁴¹⁴.

3.8.1. Glucose metabolism.

Blood eosinophils metabolize glucose. When blood samples containing about $100 \times 10^9/L$ eosinophils were left without sodium fluoride, plasma glucose concentration fell from 140 to 5 mg/dL within three hours¹⁰⁵¹. When eosinophils are stimulated to produce a respiratory burst, there is a marked increase in glucose metabolism. This has been shown with radiolabelled glucose molecules, most of which are metabolized through the hexose-monophosphate-shunt.

In comparison with neutrophils, blood eosinophils in the resting state oxidize five times more ¹⁴C-glucose, incorporate five times more (125)iodide, and contain six times more NADPH oxidase. They have a comparable capacity to reduce nitroblue tetrazolium salts. Similar differences have been shown between blood eosinophils, and neutrophils when they are subjected to phagocytic stimuli. Eosinophils oxidized seven times more 1-¹⁴C glucose under these conditions. Nitroblue tetrazolium reduction, ¹²⁵I incorporation, and chemoluminescence were two times greater, whereas superoxide anion production was similar to neutrophils⁴²⁷.

In tissues, eosinophils may have an even greater capacity to oxidize glucose when they have been activated. This possibility was first suggested in 1977, when peritoneal eosinophils from a 14-year old boy with eosinophilic gastroenteritis were isolated, and shown to have a marked increase in hexose monophosphate shunt activity, H₂O₂ formation, superoxide anion generation, chemoluminescence, thyroid hormone degradation, iodination, and oestrogen binding, as a result of ingesting particles. This metabolic burst following endocytosis was greater than for neutrophils⁹⁷⁵.

In 1972 the capacity of human eosinophils to oxidize glucose was studied using blood eosinophils from six patients with an eosinophilia. In unstimulated eosinophils the oxidation of 1-¹⁴C glucose, 6-¹⁴C glucose, and ¹⁴C fumarate was greater in eosinophils than in a comparable number of neutrophils. When the cells were induced to phagocytose particles, the oxidation of both 1-¹⁴C glucose, and 6-¹⁴C glucose was also stimulated more in eosinophils than neutrophils¹²⁰⁷.

The carrier-facilitated transport of hexoses into blood eosinophils has been measured with ³H 2-deoxyglucose. It was three times higher in eosinophils from patients with an eosinophilia, and it could also be increased by stimulating the cells with zymosan activated serum¹²⁷, and other chemotactic factors¹³¹. The addition of arachidonic acid, or its lipoxygenase products 5-HETE, and 11-

HETE induced an increase in labelled deoxyglucose uptake into human eosinophils. Inhibitors of this pathway reduced the deoxyglucose uptake of blood eosinophils from patients with an eosinophilia. These results suggest that lipoxygenase pathway metabolism, and hexose transport are closely interrelated in eosinophils¹²⁶.

In 1980 measurements were made of the hexose monophosphate shunt activity using the metabolism of 1-¹⁴C glucose to ¹⁴CO₂ in normal blood eosinophils. Unstimulated eosinophils had the same activity as neutrophils, but whereas it was not significantly increased by stimulation of normal eosinophils, it increased seven to tenfold in eosinophils from patients with an eosinophilia¹²⁷.

3.8.2. Oxidative metabolism.

The capacity of eosinophils to respire is well known, and appropriately stimulated eosinophils produce a marked respiratory burst. This was first shown with eosinophils from the blood of patients with an eosinophilia.

3.8.2.1. The respiratory burst.

In 1983, a comparison study was reported on the oxygen uptake of blood eosinophils from five patients with malignant eosinophils, and six with a reactive eosinophilia³⁷³. This showed that malignant eosinophils had a greater respiratory burst in response to latex beads, or antibody, and complement-coated bacteria. No difference was found in their response to antibody coated bacteria, or immune complexes. In 1987, it was found that purified human blood eosinophils stimulated with PMA consumed twice as much oxygen as neutrophils from the same individuals. Zymosan-C3b gave a 70 per cent oxidative response. The bacterial-derived stimulus formyl methionyl-leucine-phenylalanine (fMLP), and the calcium ionophore A23187 induced only a small response¹⁹⁶⁹. However fMLP-stimulated eosinophils produced a marked respiratory burst after incubation with PMA, or opsonized zymosan, which was twice as great as neutrophils, and more prolonged¹⁴⁰⁰.

The capacity of eosinophil granule-free membrane-bound cytoplasm (eoplasts) to utilize oxygen was 20 per cent of the intact cell. However, after stimulation with A23187, they consumed more oxygen than the intact cell. This method for preparing granule-free cytoplasmic particles enables experiments to be carried out which compare the properties of granule constituents, and other cytoplasmic components¹⁹⁶⁹.

3.8.2.2. Chemoluminescence.

The remarkable capacity of stimulated blood eosinophils to produce chemiluminescence has been repeatedly confirmed. fMLP was twice as effective as LTB₄ in producing this effect¹³⁵⁵. In a study from Wisconsin, U.S.A., in 1985, eosinophils were found to be as effective as neutrophils in producing luminol-dependent chemoluminescence, and superoxide generation, after stimulation with opsonized zymosan, and twice as effective as neutrophils after stimulation with PMA¹⁶²¹. Blood eosinophils from patients with allergic rhinitis produced more luminol-dependent chemoluminescence in response to opsonized-zymosan, and PMA than normal, and the response was greatest when the patients' symptoms were worst¹⁶²⁰.

In 1984, work in Lille, France, showed that light density eosinophils from the blood, and tissues of patients with an eosinophilia had reduced PMA-induced chemoluminescence¹⁴⁵³. Other groups have found the opposite: that light density eosinophils are 'activated', and respond with a greater production of luminol-dependent chemoluminescence. For example the lighter density cells in patients with HES, and symptomatic asthma, have a greater capacity to develop chemoluminescence with PMA, and PAF stimulation, than intermediate, or normal density eosinophils from patients with asymptomatic asthma, or atopy (Chanez, P. et al 1987, personal communication).

3.8.2.3. Reactive oxygen species.

The respiratory burst in eosinophils is accompanied by the release of potentially toxic oxygen metabolites⁹⁴, including the superoxide anion O₂⁻¹⁰⁴¹, and H₂O₂¹⁴¹³. The amount of superoxide anion produced by eosinophils from two patients with an eosinophilia, three hours after stimulation with opsonized zymosan, was twice the amount produced by neutrophils from normal individuals¹⁷⁵⁷. Superoxide production was greater than normal in blood eosinophils from individuals with

experimental hookworm infection¹⁹²⁵, suggesting that superoxide formation increases in activated blood eosinophils. The respiratory burst is produced by an enzyme system which oxidizes NADPH, and reduces molecular oxygen to superoxide. The enzymes which do this are present in large amounts in eosinophils⁴²⁵. Cytochrome b-245, which is important in this process, has been shown to be present in the plasma membrane of human blood eosinophils¹⁵⁸⁶.

3.8.3. Unsaturated fatty acid metabolism.

Eosinophils can be distinguished from neutrophils by their marked capacity to produce large amounts of leukotriene C4 (LTC4), and D4 (LTD4), whereas neutrophils preferentially synthesize leukotriene B4 (LTB4). The importance of this is that LTC4 can cause bronchoconstriction, alterations in vascular permeability, and tone, and increased mucus secretion in man.

3.8.3.1. 15 lipoxygenase pathway products, HETEs.

The ability of human eosinophils to generate 15-HETE was first shown in 1982¹⁸⁰⁰, and in murine eosinophils in 1983¹⁸⁰¹. This occurred when the eosinophils were stimulated in cultures containing arachidonic acid, which diverts synthesis away from LTC4 synthesis. In a study reported in 1987 on arachidonic acid metabolism in collagenase-separated polyp eosinophils, the main metabolite was 15-HETE¹⁶⁴⁹. Although the functions of HETEs from eosinophils are unknown, it has been shown that 15-HETE can act as an internal regulator of 5-lipoxygenase activity, reducing the amount of LTC4 secreted after stimulation of human blood eosinophils²²⁰.

3.8.3.2. Leukotrienes.

Resting blood eosinophils do not synthesize significant quantities of leukotrienes. However when they are stimulated by the calcium ionophore A 23187, or opsonized zymosan, they make large amounts of LTC4. The capacity of stimulated horse eosinophils to produce leukotriene C4 was first reported in 1982⁸⁹³, and 1983¹⁹⁹⁸. Similar findings were made in 1983 using human eosinophils¹⁹¹⁵, where 38 ng LTC4/10⁶ eosinophils was produced after stimulation with A23187. The amounts of LTC4, and LTB4, and 15-HETEs produced by human eosinophils has been found to depend on the amount of arachidonic acid available to the cells to convert into these metabolites⁷⁷⁹. It has been suggested that 15(S)-HETE is the monohydroxy acid which leads to the preferential synthesis of 15-HETE²²⁴.

LTC4 secretion by human eosinophils was also reported from Holland in 1984¹⁸⁵⁵. In this study it was found that 87 per cent pure blood eosinophils secreted nearly 40 x 10⁶ LTC4 molecules / cell, after stimulation with 10 uM A23187. Only small amounts of LTB4 were made by stimulated blood eosinophils. On the other hand, neutrophils made large amounts of LTB4, and only small amounts of LTC4¹⁸⁵⁵.

In 1985 it was reported that light density eosinophils secreted more LTC4 than normal density eosinophils⁹⁰². In another study in 1987, it was found that this was also true when the cells were stimulated with zymosan-C3b, but not A23187⁹²¹.

It has been suggested that LTC4 from eosinophils may be an important bronchoconstricting agent in late phase asthmatic reactions where eosinophils are often found²²⁰. LTC4 has also been shown to induce mucus secretion from human lung cells in vitro¹¹⁵⁷. The stimulation of eosinophils to produce LTC4 has been shown to involve an initial transient intracellular accumulation of biologically active LTC4, extracellular release, and the apparent limitation of oxidative metabolism to the outside of the cell¹³⁴⁷. The properties of LTC4, and other products of arachidonic metabolism were reviewed in 1987 by Austen⁸¹. The main effect of LTC4 is to cause the contraction of vascular, and nonvascular smooth muscle. It also produces a wheal, and flare when injected intradermally in normal people.

A number of different stimuli have been shown to be able to induce human blood eosinophils to secrete LTC4. However there is no good comparative study of their efficacy, and there is no consensus on whether only a few stimuli¹⁸⁵⁵, or many stimuli have this effect. There is also no study showing that eosinophils make LTC4 in vivo. The range of stimuli which induce LTC4 secretion include:

- the calcium ionophore A23187, which has been one of the standard stimuli for producing this response, since it was first reported in 1983¹⁹¹⁵. In 1987 it was shown that another effect of A23187 was to induce eosinophils to make lipoxin A: 5(S), 6(R),15(S)-trihydroxy-7,9,13-trans-11-cis-eicosatetraenoic acid¹⁵⁹⁰.
- opsonized zymosan^{220,223}.
- fMLP,¹⁶⁰⁴.
- PAF-acetHer²²³.
- IgG complexes on the surface of beads^{1604,371}.
- eosinophil activating factors.

The capacity of normal (but not light density) eosinophils to secrete LTC₄ was increased when they were incubated with partially purified eosinophil activating factor (EAF)⁵⁷¹, and this effect was also shown when Ig-coated particles were used instead of complement-coated particles, or A23187⁵⁷⁰. After addition of the eosinophil cytotoxicity enhancing factor (ECECF), (a 1:3 dilution of LPS-stimulated blood mononuclear cell culture supernatants), to human blood eosinophils, additional LTC₄ was made when the calcium ionophore was added. However, this effect varied considerably between different individuals, and it never reached more than 140 ng/10⁶ eosinophils. Preincubation in the factor for 1-2.5 minutes was essential for this enhancement, and it was abolished by washing the cells. Although the responses were variable, and small, it was suggested that activation with this factor might be an important mechanism for affecting inflammatory responses⁴⁴¹. Tumour necrosis factor (TNF) had no effect on A23187-induced secretion of LTC₄¹⁶²⁷.

Leukotriene production by eosinophils was suppressed by E-prostaglandins (PGE), stimulators of adenylate cyclase, and phosphodiesterase inhibitors. The importance of this in relation to the treatment of asthma was discussed in 1987¹⁰¹¹.

3.8.3.3. Prostaglandins, and thromboxanes.

It was first shown in 1975 that human eosinophils could secrete prostaglandins E1 (PGE1), and E2 (PGE2)⁸²³. It was not until 1986, that prostaglandin synthesis by eosinophils was examined again. This was a study on the capacity of human peritoneal fluid eosinophils to secrete thromboxane B2 (TXB2), PGE2, and 6-keto-prostaglandin F1 (6-keto-PGF1). The basal release, and A23187 stimulated release of TXB2 was greater than PGE2 release. There was very little 6-keto-PGF1 release. When TXB2 release was inhibited, there was a corresponding increase in PGE2, and 6-keto-PGF1 secretion⁵⁸³. Stimulated guinea pig eosinophils, and neutrophils also released PGE2, but in quantities which were too small to inhibit mast cell degranulation¹²⁴².

3.8.3.4. Platelet activating factor.

Platelet-activating factor, which is also known as PAF, PAF-acether, AGEPC, and 1-O-alkyl-2-acetyl sn-glycero-3-phospho-choline, is an inflammatory mediator with a wide range of biological activities. It was first identified in 1972 in supernatants from basophils stimulated with antigen. Its main effects are to cause bronchoconstriction, platelet, and neutrophil activation, and inflammation. It is produced by many different cell types, including eosinophils, but on a cell basis, eosinophils are probably the most potent. Although normal density blood eosinophils release less than 5 pg PAF-acether, light density blood eosinophils produced 100 ng/10⁶ cells after stimulation with the calcium ionophore A23187^{1049, 898}. It is a puzzling feature that eosinophils are also strongly stimulated by added PAF-acether in vitro, but it is not known if eosinophil-derived PAF-acether can act as an autocrine molecule.

3.8.4. Protein synthesis.

Although there has been some interesting work on the synthesis of the individual granule proteins in bone marrow eosinophils, there is little information of the possible biosynthetic capacity of more mature eosinophils. This may be due to the dogma that blood eosinophils are 'end' cells, which simply deliver their preformed constituents to tissues. In 1978, it was found that³H-uridine, and ³H-leucine were taken up by mature bone marrow eosinophils after one hour culture, and the label localized on the surface of crystalloid-containing granules¹⁹³⁰.

In 1987, studies in Boston, U.S.A. showed that when blood eosinophils were incubated with ³⁵S-methionine, radioactivity was incorporated into a 43 kDa protein within 30 minutes, and this was maximal at four hours. Three other proteins of molecular weights 24, 46, and 60 kD were synthesized when the cells were stimulated with opsonized zymosan. Surprisingly, fMLP, A23187, and PMA did not induce new protein synthesis. It was suggested that changes in the functions of eosinophils may be affected by alterations in protein synthesis ¹⁴⁷². These findings suggest that measurement of the biosynthetic capacity of mature blood eosinophils could be an important area for future study, using the conditions which maximize the survival, and responsiveness of eosinophils in culture.

3.8.5. Proteoglycan synthesis.

The presence of carbohydrates in eosinophils has been demonstrated with fluorescent lectin markers in 1980. Sialic acid, galactose, and mannose were localized to the plasmalemma, cytoplasm, nuclear membrane, and granules, like basophil granules, although neutrophil granules did not stain ¹⁷¹³. Complex carbohydrates have also been identified in immature granules in human bone marrow eosinophils by cytochemistry, and autoradiography. Mature granules were not stained by these methods ¹³⁶⁴. Cultured blood eosinophils have been shown to incorporate labelled sulphate into complex glycosaminoglycans. They contained 70-81 per cent chondroitin 4-sulphate, 9-12 per cent chondroitin 6-sulphate, and 5-12 per cent dermatan sulphate ¹¹⁹⁸. Chondroitin 4-sulphate is also found in human neutrophils, and platelets.

4. Chapter A 04. The eosinophil cell membrane, and receptors.

The eosinophil cell membrane has a number of important features which set it aside from other cell membranes, although it has many characteristics in common with neutrophils, and other cells. The cell surface charge of normal human blood eosinophil is 2.45 +/- 0.03 umoles/sec/volt/cm, compared to 1.98 +/- 0.01 for neutrophils. This charge is lower in blood eosinophils from patients with an eosinophilia, and it can also be reduced by incubation in zymosan-activated serum ¹²⁷.

4.1. Scanning electronmicroscopy.

Scanning electronmicrographs (SEMs) have been published of blood eosinophils from patients with hypereosinophilia, but these show no features to distinguish them from blood neutrophils, and lymphocytes. Some cells appeared to be relatively smooth, some had small projections, and others had ridges characteristic of secretory cells ¹⁴²⁸. I have seen some blood eosinophils in SEMs, which appeared to bud off small amounts of their plasma membrane. Eosinophils also have the capacity to send out long cytoplasmic projections on glass surfaces. There may be crystalloid granules at the tips of these projections. The nature of the processes, and the factors which cause them to be produced have yet to be determined. Their formation must be energy-dependent, and require new membrane synthesis.

The SEM appearances have also been described of the surface of eosinophils on glass smears made from blood from normal cattle. The bulges made in the cell surface by underlying large granules enabled the cells to be recognized easily ¹⁴⁰³.

SEM studies of the surface of rat peritoneal eosinophils has shown two types of projection: finger-like microvilli, and flattened, and ridge-like projections ¹⁴⁰⁷.

4.2. Freeze-fracture studies on eosinophil membranes.

Freeze-fracture studies on human eosinophils have been done, both with eosinophils from patients with an eosinophilia, and in in vitro studies on eosinophils secreting onto the surface of the schistosomula of *S. mansoni*. Studies on blood eosinophils from three patients with a marked non-leukaemic eosinophilia showed that there were more intramembranous particles in the outer face of the plasma membrane, than in the inner face, and that the particles in the membranes of the crystalloid granules, were larger: 80-150A. Aggregates of particles were not seen ⁴⁶³.

A freeze-fracture study on peritoneal eosinophils from Am-1(2)/Tor rats found that there were few intramembranous particles in the plasma membrane compared to other similar cell types (706 and 549/um² in the P and E faces respectively), but there were many in the membrane of the specific granules (1720/um² in the P face)¹⁴⁰⁷. The significance of this is not known.

In 1987, freeze-fracture photographs were published of human blood eosinophils stimulated with the calcium ionophore A23187, and opsonized zymosan. This showed aggregates of intramembranous particles, and the formation of membrane pits⁷⁸⁰. Similar intramembranous particles had been seen in the freeze-etch studies on human eosinophil granules in 1975²³⁵.

4.3. Membrane proteins.

The plasma membrane of eosinophils contains many components, such as 5' nucleotidase, which are also found in the plasma membranes of other inflammatory cells. Studies on the membrane proteins which can be labelled with ¹²⁵I, or which bind antisera, show that they are most closely related to neutrophils in these respects, despite having a separate lineage. The proteins in the plasma membrane of murine peritoneal eosinophils were studied in 1980, using partially purified cells from BALB/c mice infected with *M. corti*. ¹²⁵I bound to an eosinophil membrane protein of molecular weight 79 kDa, and to four other proteins larger than 50 kDa. A 90 kDa component (a major constituent of mouse neutrophil plasma membranes), was not present²³³.

Membrane components are important in the interactions between eosinophils, and normal tissue components, and foreign molecules which induce them to spread out, and degranulate. A summary of the main immunological receptors on eosinophils is shown in Figure A04-1. The molecular basis for eosinophil attachment to particles, and surfaces is not known. The complement, and immunoglobulin receptors are clearly important, but other structures are also involved. This was suggested in experiments in which human eosinophils were found to attach to albumin-coated Sepharose beads, and microtitre plates, to a greater extent than neutrophils¹⁹⁴³.

Fig. 4-1: Eosinophil plasma membrane receptors.

4.3.1. Immunoglobulin, and complement receptors.

As eosinophils mature in the bone marrow, they lose Ia antigens⁹⁸⁸, and gain complement receptors (CR), and immunoglobulin receptors (FcR), which are most strongly expressed in stimulated, and activated eosinophils. Blood eosinophils from normal individuals bind immunoglobulins relatively poorly compared to neutrophils, and they generally express less CR than neutrophils, and monocytes. This may be partly the result of masking of receptors by sialic acid containing membrane glycoproteins, as neuraminidase treatment leads to the increased expression of Fc-receptor binding capacity by normal blood eosinophils. There is also evidence that eosinophil CR are recycled, (perhaps through coated pits), although this has not been formally demonstrated. We considered this possibility in 1976, after finding that CR were re-expressed within 30 minutes of digestion of these receptors from the surface of human blood eosinophils with proteolytic enzymes.

Normal blood eosinophils do not have IgM receptors¹³⁴⁰, although IgM binding to eosinophils can be induced by culturing them in vitro⁴¹⁸.

It was first claimed in 1976 that eosinophils can bind to sheep erythrocytes (E)¹⁶⁰⁹. In 1980, it was reported that a mean of 16 per cent of blood eosinophils from patients with parasitic diseases formed E rosettes, whereas they did not develop in normal individuals. It was suggested that this reflected an alteration in the membrane of patients' eosinophils⁴¹⁹. However, it is possible that the separation techniques, and culture conditions could have led to the release of cationic proteins which might bind erythrocytes to the eosinophil surface nonspecifically. Other groups, including ourselves, have not been able to detect the binding of sheep red cells to intact blood eosinophils¹³⁶⁷.

4.3.1.1. Receptors for IgG (Fc gamma R).

Much of the early work on eosinophil IgG binding capacity was done using rosetting techniques. Guinea pig eosinophils were first shown to bind IgG-coated erythrocytes in 1969⁷⁸⁷, and we demonstrated that human eosinophils had receptors for IgG in 1976¹⁷³⁸. They were found to bind complexed IgG almost as well as neutrophils, and eosinophils from the blood of patients with an eosinophilia

had an increased capacity to bind to complexed IgG, and form rosettes¹⁶⁹⁶. We also showed that complexed IgG1, and IgG3 myeloma proteins inhibited IgG rosettes more effectively than IgG2, or IgG4 complexes¹⁷³⁸. In 1978 Ottesen also found that eosinophils from the blood of seven patients with schistosomiasis, and four with filariasis had an increased binding capacity for mouse C3-, and rabbit IgG-coated sheep erythrocytes¹³⁴⁰.

Eosinophil Fc gamma R first appear on dividing eosinophils in human bone marrow at the promyelocyte stage, earlier than in the mouse. In one study marrow aspirates were studied from five patients using rabbit IgG-coated sheep erythrocytes. Twelve per cent of eosinophil myelocytes had detectable Fc gamma R, the same as blood eosinophils studied with this technique⁷⁸⁸.

Subsequent work has involved the use of monoclonal antibodies to define, and isolate the receptor proteins in the plasma membrane of phagocytic cells. At the Rockefeller University, monoclonal antibodies have been produced which distinguish between high affinity IgG receptors (Fc gamma Rhi, or Fc gamma RI), which are mainly found on immature granulocytes, and low affinity receptors (Fc gamma Rlo, or Fc gamma RII), which characterize more mature granulocytes. Using the monoclonal antibody (3G8) to Fc gamma Rlo, it was shown that there was a progressive loss of Fc gamma RI during myeloid differentiation, with reciprocal expression of Fc gamma RII. Mature eosinophils only expressed Fc gamma RII⁵⁷⁷. Further studies with antibodies 3G8, and CLB-FcR-1, have shown that human blood eosinophils have less Fc gamma RII than neutrophils¹⁹⁷¹.

In 1984, a report was published in which two molecules of 16, and 18 kDa were isolated from human blood eosinophils surface labelled with (125)iodide by the lactoperoxidase method¹⁷⁷⁵. These were thought to be components of Fc gamma R because they were eluted from immobilized immune complexes. However, it is possible that these molecules were secreted ECP, or EDN/EPX. In a careful study which was reported in 1984, and which set out to isolate Fc gamma R from human blood eosinophils, it was found that they were more sensitive to proteolysis than neutrophil Fc gamma R. The isolated eosinophil receptors had a molecular weight of 43 kDa, whereas neutrophils had receptors of 52, and 68 kDa. This work also gave some evidence that human IgG3 bound most effectively to eosinophil receptors¹⁰¹³, although studies by Walsh in 1986, using aggregates of myeloma IgG subclasses failed to show this¹⁸⁷³.

In 1986 it was shown that human blood eosinophils have a 40 kDa IgG Fc gamma R which is also found on neutrophils, monocytes, platelets, and K562 cells, and which was defined with monoclonal antibody IV3¹⁰⁹⁹. It was suggested that this receptor is the homologue of the murine macrophage FcRII, which is present on both neutrophils, and eosinophils in that species.

Although these experiments suggested that there are two Fc receptors on human blood eosinophils, it is possible that the different epitopes recognized by antibodies 3G8, and IV3 are on the same Fc receptor which has varying degrees of glycozylation. This is supported by our experiments which showed that neuraminidase treatment of normal blood eosinophils increased their capacity to bind to complexed IgG¹⁷⁴⁰.

Many different stimuli, especially endotoxins, have been shown to increase the proportion of blood eosinophils which bind IgG-coated particles or complexes. It is probable that this event is an integral part of the 'activation' step which is the final stage in eosinophil differentiation, and response to stimuli in tissues. Several factors increase the proportion of blood eosinophils which express Fc gamma R, including ECF-A^{271,78}, and beta-interferon (200 units/ml), which increased human blood eosinophil EA rosetting from 19 to 24 per cent after one hour incubation⁴²³.

The binding of eosinophils to IgG-coated surfaces can have several effects:

(a) If the particle is small, and if the eosinophils are activated, or come from the blood of patients with an eosinophilia, they will be ingested. This difference between normal, and patients' eosinophils was first shown in 1976¹⁶⁹⁶.

(b) If the surface on which the IgG is deposited is large, then the eosinophils will attach firmly, and generate LTC4, with a respiratory burst, and degranulate. This is the basis of the IgG-dependent cytotoxicity of eosinophils for antibody-coated parasites. Under these conditions eosinophils have

been shown to synthesize new membrane proteins¹⁷⁷⁶.

(c) If the IgG complex is in the blood, it will activate the complement system, and this will then bind to the eosinophil C3 receptors. This is probably the main way in which soluble IgG complexes induce eosinophil responses in vivo, as there is such a high concentration of IgG in tissue fluids, and plasma, that it is unlikely that soluble IgG complexes could bind to granulocyte Fc gamma R directly¹¹¹⁷. However, IgG immune complexes in vitro can interact with human eosinophils, and produce morphological changes in the granules, including the development of tubulo-vesicular structures, which have been proposed as a route for granule secretion¹³¹¹.

The enigma that basophil degranulation can be induced with anti-IgG4 antibodies may have been resolved with the finding in 1986 that this is a two-step process in which anti-IgG4 first cross-links IgG4 in eosinophil Fc gamma R, resulting in the secretion of eosinophil cationic proteins, which then induce basophil degranulation. This may be a mechanism for amplifying eosinophil-dependent tissue injury in allergic diseases¹⁴¹.

4.3.1.2. Receptors for IgE (Fc epsilon R).

The first experiments to show that human eosinophils can interact with complexed IgE were carried out by Arbesman, and colleagues in 1974⁸⁴⁹, and 1975⁶⁰⁹. They showed that immune complexes of ragweed antigens, with IgE antibody in antigen excess, were taken up more by human eosinophils from nasal secretions than by neutrophils. IgE-antigen complexes in antigen, or antibody excess were ingested to a greater degree by eosinophils than neutrophils, and the reverse was true for IgG antigen complexes. Although IgE was not found on the surface of these patients' eosinophils, Hubscher detected them in other patients' eosinophils in 1975, using immunoelectronmicroscopy with ferritin-conjugated anti-IgE⁸²².

In 1981 Dr. Monique Capron, and colleagues in Lille, France first demonstrated that eosinophils had a low affinity receptor for IgE. In a series of studies they showed (1) that human blood eosinophils formed rosettes with IgE-coated sheep erythrocytes, (2) that they bound radiolabelled human IgE²⁷⁵, (3) rabbit antibodies to IgE, and (4) the monoclonal antibody BB10²⁷³, which recognizes the membrane signal site triggered by activation of cells by IgE²⁶⁹, and Capron M. (1987) personal communication. In patients with an eosinophilia, the proportion of eosinophils with IgE receptors was higher than in normal people, and the same was also found in rats infected with *S. mansoni*²⁷⁰. They showed that IgE was present on both circulating, and tissue eosinophils in a number of diseases²⁷⁶.

The Fc epsilon R was found to be of the low affinity type, and was named the Fc epsilon R2 in 1986²⁶⁷. In 1987 it was found that the CR3 receptor is closely associated with the IgE receptor on eosinophils: Monoclonal antibodies to the alpha-chain (but not the beta-chain) of the C3 receptor inhibited the binding, and cytotoxicity of human light density eosinophils for IgE coated schistosomula²⁷⁴.

Light density eosinophils from patients with an eosinophilia were more effective than normal density eosinophils in killing IgE-coated schistosomula. This killing was inhibited by preincubation of eosinophils with chemically-aggregated IgE, but not IgG, or anti-C3b receptor antibodies²⁷⁷. The IgE receptor may not be fully exposed on blood eosinophils, and other receptors may be also partly hidden in the plasma membrane. For example, IgG receptors are only partially exposed on normal blood eosinophils¹⁷⁴⁰. The presence of an IgE receptor on mouse eosinophils was demonstrated in 1987⁹⁹⁰.

In 1984, Dr. Monique Capron, and colleagues demonstrated the presence of IgE on the surface of blood eosinophils of 17 of 21 patients with a variety of eosinophilic disorders, including parasitic infections, HES, tumours, the Churg-Strauss syndrome, and asthma. In patients with IgE levels above 100 IU/ml, more eosinophils had surface IgE than when it was less than 100 IU/ml. Bronchoalveolar lavage eosinophils from five patients with respiratory disease, (three with eosinophilic pneumonia, one with polyarteritis nodosa, and one with a drug hypersensitivity) had a greater proportion of IgE occupied receptors than blood eosinophils²⁷⁶. In 1986 they found that

functional Fc epsilon R2 were restricted to light density (hypodense) eosinophils in patients' blood, although they could be detected by rosetting techniques on normal density eosinophils²⁷², and this was confirmed elsewhere¹⁸⁷³. It is possible that the functional receptor only appears as a result of eosinophil activation.

A possible role for IgE in eosinophil-parasite interactions has been considered at least since 1969, when G.T. Archer, and colleagues in Australia reported that rat peritoneal eosinophils degranulated within 10 minutes of culture with immune complex formed from extracts of *Amplictacum robertsi* or ascaris body fluids, and rat antibody. As the antibody was heat labile, they suggested that it might be IgE⁵⁸.

In 1984, Dr. Monique Capron, and her colleagues first showed that eosinophils could kill IgE coated parasites²⁷⁷, although an effect of the eosinophil CR3 receptor was also noted²⁶⁹. The killing of IgE-coated schistosomula took place in much the same way as IgG-dependent killing²⁷⁷. Rat eosinophils were also shown to have IgE receptors, and to be able to kill IgE-coated schistosomula²⁶⁸.

This was confirmed in studies from Japan in 1985, in which it was found that rat eosinophils bound to, and killed dinitrophenol (DNP)-haptenedated *S. japonicum* schistosomula when the parasites had been previously treated with a mouse IgE monoclonal anti-DNP antibody (B.53), but not when they had been pretreated with normal mouse serum. Eosinophils from the peritoneum of rats infected with *N. brasiliensis* were more adherent, and toxic than normal, suggesting that they had an increased capacity to bind to IgE^{991,992}.

In 1977 it was first shown that human IgE-antiIgE complexes could induce the release of EPO from human eosinophils in vitro¹⁷⁴⁷. The effects of crosslinking the Fc epsilon R on human eosinophil secretion began to be studied by Dr. Monique Capron, and her colleagues in 1985. They showed that the attachment of specific antigens to IgE antibodies in the Fc epsilon R2 receptor on human eosinophils induced the secretion of both EPO, and MBP²⁶⁹. In the first of these experiments blood eosinophils were isolated from eight patients with filariasis, and high serum IgE levels, and 10 patients with hypereosinophilia, and normal IgE levels, and the quantity of EPO secreted in response to stimulation with anti-IgE, or specific filarial antigens was measured. Light density patients' eosinophils, (but not normal density eosinophils) released large amounts of EPO, which was maximal one hour after incubation. As anti-IgG caused the release of equal amounts of EPO from normal, and light density eosinophils, it was suggested that IgG-mediated secretion was independent of the cell density, whereas IgE effects were limited to the light density cells⁹⁵⁷. This was supported by their experiments in 1984 showing that light density eosinophils had a greater capacity to kill schistosomula of *S. mansoni* through an IgE dependent mechanism²⁷⁷. It was also noted that IgE stimulation did not induce the release of ECP, and that IgG stimulation had the reverse effect. This surprising observation has been confirmed, and it provides the first clear evidence that there may be differential control of eosinophil granule protein secretion⁹⁵⁷.

4.3.1.3. Complement receptors (CR1, and CR3).

It was first shown in 1969 that guinea pig eosinophils could form rosettes with red cells coated with C3⁷⁸⁷. In 1976 we demonstrated the presence of complement receptors on human eosinophils using a similar technique¹⁷³⁸. We also showed that human blood eosinophils did not have C3d (CR2) receptors, and that complement receptor-dependent binding by blood eosinophils from patients with an eosinophilia was greater than blood eosinophils from normals¹⁶⁹⁶.

Human eosinophils possess C3b (CR1), and iC3b (CR3) receptors, which have been defined on other myelomonocytic cells. Binding to these receptors does not involve the cytoskeleton, unlike IgG Fc receptors, as attachment is not inhibited by cytochalasin B¹⁷⁴⁰. Monoclonal antibodies to these two receptors bind to eosinophils, although eosinophils have half the number found on neutrophils^{1971,566}. The CR1 receptor is a polymorphic single chain glycoprotein of about 250 kDa, coded for by a gene with four alleles. Several stimuli increase the number of CR1 receptors on the plasma membrane, possibly by transferring them from an intracellular site⁵⁵⁰, as our experiments suggested in 1980¹⁷⁴⁰.

The adherence of neutrophils to surfaces depends on at least three plasma membrane glycoproteins, which share a common beta-subunit of 95 kDa, but distinct alpha-subunits of 150-180 kDa. These are the iC3b (CR3) receptor, the LFA-1 glycoprotein, and the p150,95 glycoprotein. They are involved in cytoskeleton assembly following interactions with surfaces. Genetic defects in these proteins have been described, but it is not known if eosinophils were affected in these patients ¹¹⁴⁷. In 1977, Kay's group showed that the percentage of human eosinophils which formed rosettes with human C3b-coated sheep erythrocytes was increased from 30 to 60 per cent by treatment with histamine, or the two ECF-A tetrapeptides. No effect on IgG-binding, or on neutrophil C3-binding was seen. Optimal conditions were at 10⁻⁴M histamine, and following 80 minutes incubation ⁴⁵. It was suggested that this alteration could affect the functions of eosinophils. This effect of histamine was found to be H1-dependent ⁴⁶. In 1979, fMLP was also shown to increase eosinophil-C3b rosetting ⁹³⁰, and both effects could be prevented with disodium cromoglycate ⁹³⁴.

Eosinophil activating factors have the same effect: Mouse EDF increased the expression of the iC3b receptor in human eosinophils ¹⁸²³, and supernatants from cultured human monocytes (mono-sup), increased the number of iC3b, but not C3b receptors on eosinophils ¹⁹⁷². Several products of arachidonic metabolism increased the percentage of C3b receptors on blood eosinophils from four patients with an eosinophilia. These were LTB₄, 5-HETE, and 5-HPETE. LTC₄ had no effect ¹²⁵⁷.

The binding of particles to the surface of eosinophils by the complement receptors CR1, and CR3, is a potent method for inducing them to secrete their granule contents in vitro. This has been confirmed by many groups, who used zymosan- or sepharose-C3b. See for example ¹⁹⁹³. In 1977, a study using Sepharose particles, showed a requirement for the alternative complement pathway components to be present, in order to produce the most effective stimulus for the secretion of arylsulphatase, and b-glucuronidase ¹¹⁹⁷.

4.3.1.4. Receptors for the anaphylatoxins C3a, C5a.

In 1977 it was found that peritoneal eosinophils from a 14-year old boy with eosinophilic gastroenteritis responded to the chemotactic factor C5a ⁹⁷⁵, suggesting that they also possessed receptors for C5a. In 1979, ¹²⁵I labelled C3a, and C5a were shown to bind to the surface of human eosinophils using autoradiography ⁶⁷⁴. The C3a binding site was found to be of low affinity ⁶⁷⁸.

Stimulation of eosinophils by C5a may also lead to the secretion of their granule contents. This was first suggested in 1986, when isolated human blood eosinophils were incubated with C5a, prepared by adding zymosan to serum. There was 60 per cent lysis of ⁵¹Cr-labelled chicken red cells. As this was inhibited by catalase, sodium azide, and KCN, but not by superoxide dismutase, it was suggested that peroxidase, and H₂O₂, rather than superoxide anions were involved ⁴²².

4.3.1.5. Receptors for C1q.

Receptors for C1q were detected on normal human blood eosinophils in 1987, using radiolabelled C1q. There were twice as many on eosinophils, as on neutrophils. The binding of C1q took place through the collagen portion of the molecule. As C1q increased the cytotoxic capacity of eosinophils for schistosomula, in the presence of small amounts of IgG antibody, it was suggested that the C1 receptor could have a role in the effector functions of eosinophils ⁷⁴⁵.

4.3.2. Steroid receptors.

In 1981, radiolabelled glucocorticoids were used to demonstrate that eosinophils have glucocorticoid receptors, and that the number of receptors per cell were similar on eosinophils, and neutrophils ¹³⁹⁴.

There have been many publications from Chile by the Tchernitchins, and their colleagues on the interactions between oestrogens, and eosinophils in the uterus of a number of species, including rats, guinea pigs, and man. Their work showed that rodent eosinophils can be stimulated by oestrogens to take part in the process of oestrus, and implantation ¹⁷⁶³. In 1985, this group suggested that oestrogens could also induce rat eosinophils to degranulate in the blood, and uterus ¹⁷⁶². However, as experiments which were done in Seattle, U.S.A. in 1977 showed that radiolabeled oestradiol did not bind to resting normal human peritoneal eosinophils from a patient with eosinophilic gastroen-

teritis⁹⁷⁵, this work may not be applicable to man.

4.3.3. Receptors for chemotactic factors.

In 1976 it was suggested that the ECF-A tetrapeptides bound to a specific receptor on human eosinophils^{194,80}, but this remains to be confirmed.

4.3.4. Phospholipid-exchange protein.

A phospholipid exchange protein has been detected in human eosinophils. There were 199 units/5 x 10⁶ eosinophils, compared to 7.5 units in the same number of neutrophils. It may transfer phospholipids, or interact with phospholipids on other cells, or organisms¹⁹¹⁷.

4.4. Membrane carbohydrates.

Human blood eosinophils have a sialic acid-rich glycocalyx, which is reduced by incubation with neuraminidase¹⁷⁴⁰. The plasma membrane of peritoneal eosinophils from rats also has a glycocalyx rich in carbohydrates, including concanavalin A binding sites, and acidic carbohydrates, as shown by ultrastructural cytochemistry¹⁴⁰⁷, and by alterations in the electrophoretic migration of the cells after treatment with neuraminidase¹⁴⁰⁶. There are no reports yet on whether human blood eosinophils possess the glycoproteins found on other blood cells, which have been defined by galactose oxidase-tritiated borohydride labelling³⁷, and other methods. It is also not known whether human eosinophils can be agglutinated by wheat germ agglutinin, soybean lectin, or concanavalin A, as has been shown with guinea pig peritoneal eosinophils¹⁴¹⁴.

4.5. Eosinophil membrane antigens.

Eosinophils, like neutrophils, do not have blood group antigens on their surface, although they express HLA antigens. In the mid 1970s a number of groups claimed to have raised rabbit antibodies which only bound to the surface of eosinophils. However, later work with monoclonal antibodies has shown that there are few qualitative differences in the membrane antigens expressed on eosinophils, compared to neutrophils.

4.5.1. Polyclonal antibodies.

In the 1970s, Dr. Adel Mahmoud, and colleagues in Cleveland, U.S.A., raised rabbit antibodies to murine, and human eosinophils. Gleich, and colleagues at the Mayo Clinic, raised rabbit antibodies to guinea-pig eosinophils. These were used to 'ablate' eosinophils in vivo, with the hope that this would determine the function of eosinophils in parasitic, and allergic diseases. Although a number of papers were published in this area showing some changes in resistance to parasitic infections (reviewed in⁶⁵⁶), they were generally disappointing in their effects. The best results were seen with a combination of anti-basophil, and anti-eosinophil sera in tick immunity in guinea pigs²¹⁸. There has also been a suspicion that these rabbit antibodies to rodent eosinophils were not specific for eosinophils, although they caused a reduction in circulating, and tissue eosinophils in experimental animals. Doubts have also been raised about whether the antibody to human eosinophils was eosinophil-specific¹²⁴. No unique antigens have been detected on human eosinophils with mouse monoclonal antibodies.

4.5.1.1. Antibodies to mouse, and guinea pig eosinophils.

A rabbit anti-mouse eosinophil serum, which did not bind to mouse neutrophils, was described in 1973¹¹⁴¹. It was used to study the role of eosinophils in resistance to *S. mansoni*¹¹⁴², and *T. spiralis*⁷²².

In 1975, a rabbit antibody to guinea pig eosinophils was developed at the Mayo Clinic⁶⁶⁶. This was used to study the effects of depleting eosinophils in the blood, and intestines on *Trichostrongylus colubriformis* infections⁶⁶⁸, and immediate hypersensitivity reactions⁶⁷⁰. Neither anti-eosinophil serum, nor prednisolone reduced passive cutaneous, or systemic anaphylaxis, but histamine release was reduced. This antiserum also reduced histamine replenishment in guinea pig skin after passive cutaneous anaphylaxis⁸⁸⁷.

4.5.1.2. Antibodies to human eosinophils.

Several rabbit antibodies, which only bound to human eosinophils were reported in 1974, by Mahmoud, and colleagues in Cleveland, U.S.A. Sera from two rabbits immunized with blood eosinophils from

a patient with schistosomiasis contained high titres of agglutinating, and cytotoxic antibodies against human blood eosinophils. Absorption with red cells, and white cells of different types was done, to remove cross-reacting antibodies. Other antisera were produced against basophils, lymphocytes, and myeloblasts¹¹³⁶. Purified eosinophils from a patient with eosinophilia secondary to Hodgkin's disease, and a patient with an eosinophilia in chronic granulocytic leukaemia, were purified, and injected into rabbits. After absorption, these antibodies also appeared to be specific for eosinophils, causing agglutination, and cytotoxicity¹¹³⁷.

4.5.2. Monoclonal antibodies.

Monoclonal antibodies against eosinophil membrane constituents have been described by a number of groups. These fall into two broad categories: (a) antibodies which also bind to neutrophils, but not to other blood cells, lymphocytes, or platelets, and (b) antibodies which do not bind to neutrophils, but which also bind to platelets, and some other cells. It should also be noted that there are antibodies to neutrophil plasma membrane antigens, which do not bind to eosinophils³³¹. None of these antibodies are cell specific, e.g. only bind to one cell type. These antibodies are summarized in Figure A04-2.

Fig. 4-2: Monoclonal antibodies to eosinophil membrane antigens.

4.5.2.1. Antibodies which do not bind to neutrophils.

A monoclonal antibody (Eo-1), which binds eosinophils, but not neutrophils was described in 1986, by Saito, and colleagues, who raised it by immunizing a mouse with the eosinophil EoL cell line that they had developed. (See page 50). Eo-1 was shown to be a mouse IgG1 monoclonal antibody, which bound to the surface of 47 per cent of human blood eosinophils, dividing marrow eosinophils, 92 per cent of basophils, all platelets, and some lymphocytes, but not neutrophils. It recognized all cells of the pre-B cell lines NALM-1, and NALM-6. and (to a lesser extent) cells from other human haemopoietic cell lines. It bound to null ALL cells, and most pre-B cell leukaemic cells, and cells of a basophilic leukaemia. In two patients with chronic myeloid leukaemia, a higher proportion of blood, and marrow eosinophils bound the antibody than normal. The antigen on platelets was a single chain polypeptide of 23 kDa, which was distinct from the Fc epsilon receptor. A possible relationship was suggested between the antigen recognized by antibody Eo-1, and other 24 kDa antigens which are shared by platelets, and pre-B cells¹⁵³⁹. In unpublished result by Tai, she has found that all light, and normal density eosinophils from patients with HES bind this antibody, but there was greater uptake of labelled antibody by the HES light density cells.

A number of monoclonal antibodies have been described, which recognize antigens on the surface of human leucocytes, and which are classified by international agreement as belonging to the cluster of differentiation 9 (CD9). Dr. Jacqueline Breard has suggested that antibody Eo-1 may be of this type. We have tested 20 CD9 antibodies, and have confirmed this suggestion. Another closely related murine monoclonal antibody (TP82), which may also belong to the CD9 group, was described in Tokyo, Japan, in 1985. It binds to eosinophils, and the platelet-cALL associated antigen p24¹³⁰⁵. The presence of shared membrane antigens on pre-B cells, eosinophils, basophils, and platelets, is a feature which has been recognized (a) in relation to Fc epsilon binding, (b) as a feature of the CD9 group of antibodies, and (c) in relation to the capacity of IL-5 to stimulate eosinophil, pre-B cell, and platelet production. These associations, which may be accounted for by a common stem cell origin, may also point to a common set of membrane structures, which are involved in the regulation of their maturation, or their capacity to respond to haemopoietic stimulation.

4.5.2.2. Antibodies which also bind to neutrophils.

Dr. Po-Chun Tai has been raising antibodies to membrane antigens on human blood eosinophils, since 1981. It was hoped that some could be produced which would not bind to other haemopoietic cells. This assumption was based on the possibility that the earlier production of eosinophil-specific antibodies in rabbits, would be applicable to the mouse. She screened her hybridomas with normal blood cells, and cloned those which (a) had strong reactivity with eosinophils, and (b) which failed to bind to one, or more, of the other blood leukocytes. However, over 100 of the anti-eosinophil

antibodies which she developed also bound to all blood neutrophils, although they stained them less intensely than eosinophils. There was considerable variation in the percentages of blood eosinophils stained by her panel of antibodies. Alterations in the expression of these antigens were seen in patients with an eosinophilia.

She immunized mice with isolated blood eosinophils from patients with HES. Six antibodies (EoN1-7) were finally selected. These also bound to blood neutrophils, eosinophil myelocytes, K562, and HL-60 cells. They differed in the proportions of normal, and stimulated eosinophils which they stained. They bound to a higher proportion of intermediate, and light density cells than normal density eosinophils¹⁷³⁴. These antibodies demonstrated that there is a marked capacity for eosinophils to alter their membrane constituents in disease, and several of them recognized antigens preferentially expressed on eosinophils during activation, and degranulation.

There are other reports, published between 1981, and 1987, of monoclonal antibodies, which are not CD9 antibodies, or antibodies to the complement, or immunoglobulin receptors, which bind to antigens in eosinophil, and neutrophil plasma membranes. These include, antibody FMC10²⁰⁰³, antibody PM-81¹⁰⁷, which binds to a membrane glycolipid¹¹³², antibody Mo95^{584,1710}, antibodies to granulocyte functional antigens GFA-1, and GFA-2 which bound to both human eosinophils, and neutrophils, and stimulated the killing of schistosome of *S. mansoni*^{1104,1102}, a mouse monoclonal antibody which bound to both bovine eosinophils, and neutrophils¹⁵²⁰, antibody AHN-7¹⁶⁴⁰, antibody TG1, which also binds to monocytes⁸⁰⁷.

4.5.2.3. Antibodies which also bind to T lymphocytes.

In 1987, an abstract stated that 20 per cent of blood eosinophils from patients with an eosinophilia formed rosettes with a monoclonal antibody (MLR4), which also bound to mixed lymphocyte-stimulated, but not mitogen-stimulated T lymphocytes. This antigen was preferentially expressed on light density eosinophils, but was virtually absent from neutrophils⁴¹⁷. We have unpublished work showing that a number of monoclonal antibodies against CD1-CD9 antigens expressed on human T cells also bind to eosinophils.

4.6. Alterations in the eosinophil plasma membrane.

The human eosinophil plasma membrane undergoes striking changes during development, and following stimulation. This has been clearly shown in agar culture of mouse eosinophils, but it also occurs in cultured human eosinophils when the expression of Ia antigens is reduced, and they acquire additional C3b, and Fc binding capacity.

Following activation, eosinophils also alter their membrane structures, as defined by monoclonal antibodies. For example, mouse EDF increased the expression of antigens GFA-1, GFA-2, in human eosinophils¹⁸²³. Stimulation of eosinophils with endotoxin, and other stimuli, which cause secretion of the lysosomal granule constituents, also alters the membrane, as they digest sialic-acid rich components from membrane glycoproteins¹⁷⁴⁰.

4.7. Antigen presentation by eosinophils.

It has been suggested that eosinophils might be antigen-presenting cells. When antigens are injected into the footpad of rodents, they move rapidly to draining lymph nodes, and eosinophils are often found close these areas, initially in the cortex, and later in the medulla. However, there have been no formal attempts to show whether pure populations of eosinophils can present antigens to lymphocytes *in vitro*. This seems rather unlikely, in view of the occurrence of specialized antigen presenting cells in the immune system.

5. Chapter A 05. Endocytosis, secretion, and motility.

Eosinophils can ingest both small, and large particles, and respond to a variety of stimuli by secreting their stored, and newly formed constituents. This is under the control of a number of factors derived from adjacent cells in tissues, (Figure A05-1). Secretion in eosinophils appears to be finely regu-

lated. These products of secretion can have a protective function, but they can also damage tissues, as I proposed in 1978¹⁶⁸⁰.

Fig. 5-1: Eosinophil maturation, and secretion.

5.1. Endocytosis.

In vitro, normal blood eosinophils have only a quarter of the capacity of neutrophils to endocytose bacteria, and other small particles, although they may be as effective as neutrophils when they have been purified from patients with hypereosinophilia. This was confirmed in 1987, when it was found that tissue eosinophils, which were separated from colonic neoplasms by enzymatic dissociation, and countercurrent centrifugation, had a greater phagocytic capacity for *E. coli*, than neutrophils, or macrophages isolated at the same time. Sixty-seven per cent of mucosal eosinophils ingested a mean of 4.7 bacteria per cell¹⁴⁶.

However eosinophils, even when they have an impressive endocytic capacity, have a lower capability to kill staphylococci²²⁹, and *E. coli*⁴²⁶ than blood neutrophils. This was reviewed by Bass in 1982¹²⁴. The ultrastructural events during eosinophil phagocytosis of mycoplasma²⁰⁰⁹, and antibody-coated *Candida albicans*⁸⁴⁸ have been described. Complement-coated particles, such as opsonized-zymosan, and C3b coated Sepharose particles can also induce a phagocytic response. Surprisingly, the trypomastigotes of *T. cruzi* can be ingested by human eosinophils in serum-free medium, and killed⁹⁶².

Most of the factors which induce endocytosis by eosinophils, such as complexes containing C3b, are similar to those involved in neutrophil phagocytosis. There is some confusion about the capacity of eosinophils to ingest IgE-coated particles. In 1974, it was found that immune complexes containing IgE were taken up to a greater extent by eosinophils than complexes containing IgM, IgG or IgA⁸⁴⁹, although in 1978 it was reported that IgG-coated latex beads were phagocytosed by eosinophils more effectively than IgE-coated beads, and blood eosinophils from ragweed sensitive patients had a lower phagocytic capacity than normal eosinophils⁷²³.

In 1975, rat peritoneal eosinophils were shown to have a marked capacity to carry out microendocytose, as colloidal gold particles were taken up in large amounts⁹⁹⁶.

5.2. Secretion.

In 1879, Paul Ehrlich suggested that eosinophil granules were storage depots of materials that were secreted into the environment, as needed⁸⁰¹. Kanthack, and Hardy were the first to demonstrate this directly in 1895 in their studies on frog leukocytes⁹⁰⁷. They found that when anthrax bacilli were introduced into the dorsal lymph sac of the frog, eosinophils came in contact with the bacilli, but did not ingest them. While they were attached together, eosinophils began to discharge their granules, so that after a time, their cytoplasm became relatively homogeneous, lacking granules.

In the 1960s, work on horse⁵⁶, rat⁵⁸, and guinea pig eosinophils³⁶² suggested that an important property of eosinophils was to mobilize, and secrete their granule proteins into endocytic vacuoles, like macrophages, and neutrophils. However, this rarely happens with human eosinophils, which more commonly secrete their constituents to the outside of the cell. Eosinophils in tissues rarely contain endocytosed particles, even in areas where other phagocytic cells have ingested large amounts of material. Until the mid1970s, the only evidence for human eosinophil secretion came from electronmicrographs of the bone marrow, or tissues from patients with a variety of eosinophilic disorders. These showed that their crystalline granules become disrupted with an altered electron density, as described by Kelenyi, in 1968⁹⁴⁷. Within the last decade, the factors which induce eosinophil secretion, the nature of the products which are secreted, and their effects on cells, and tissues, have become subjects of great interest, as they seem to provide the key to understanding the roles of eosinophils in disease.

A wide range of stimuli can induce human blood eosinophil secretion in vitro, but few of them induce eosinophils to release more than about 30 per cent of their stored granule components. This may be because multiple stimuli are required for eosinophils to respond effectively. This possibility is supported by the finding that pairs of monoclonal antibodies to eosinophil membrane antigens

were more effective than individual antibodies in inducing secretion of ECP¹⁷³⁵. Neutrophils also have a low capacity to secrete their azurophil granule contents in response to stimulation *in vitro*¹¹⁴⁷. Activated eosinophils secrete more of their granule components than normal eosinophils, but no *in vitro* experiment has yet induced eosinophils to lose virtually all their granule proteins, as can be seen in blood eosinophils from some patients with HES.

Evidence is now being produced which supports the idea that there is control of eosinophil secretion of a far greater complexity than was originally thought. This control may extend to the selective release of individual components from the granules, with or without the generation of newly-formed products. In one study, complexed IgG induced the secretion of ECP, but not EPO, whereas complexed IgE induced the secretion of EPO, but not ECP⁹⁵⁷. Peterson, and colleagues have also found that a synthetic glyceride (IpOCOC9) induced eosinophils to release 60 per cent of the stored ECP, but only 10 per cent of EDN/EPX, and 3-5.5 per cent of EPO, after one hour incubation¹³⁹⁵. If differential secretion does occur *in vivo*, this might explain why eosinophil granule proteins can be found in several different types of inflammatory response: either chronic necrotic lesions, such as endomyocardial necrosis, or in acute inflammatory responses where necrosis does not occur, such as urticaria, where they may have reversible effects. See Figure A05-2.

Fig. 5-2: The effects of different stimuli on eosinophil secretion.

5.2.1. Secretory capacity *in vitro*.

The capacity of eosinophils to secrete their granule proteins in response to a variety of stimuli has been studied in many different laboratories. In 1981, the interaction of eosinophils with staphylococci was studied by interference contrast microscopy. The granules marginated, and were discharged¹⁶¹⁰. In the majority of studies on eosinophil secretion, the cells have been stimulated with either (a) soluble stimuli, such as PMA, complement activation products, immune complexes, and sepharose-C3b, (b) phagocytic stimuli, such as zymosan-C3b, or (c) large, non-phagocytosable surfaces such as sepharose-C3b, or agar layers on glass, coated with complexes⁶⁵⁰. Generally, about 15 per cent of each stored constituent is released by 40 minutes¹⁹⁴², although occasional reports have shown higher release. One reason for the low recovery of secreted proteins is that some of the secreted basic proteins may remain membrane-bound, or attached to adjacent surfaces. This may be an important mechanism for localizing, and limiting the effects of these eosinophil secretion products *in vivo*.

In 1987 a study was published on the capacity of purified normal blood eosinophils to secrete beta-glucuronidase, and arylsulfatase. Less than 9 per cent of the beta-glucuronidase, and less than 20 per cent of the arylsulfatase were released under optimal, non-lytic conditions¹⁹⁶⁹.

5.2.2. Mechanisms of secretion.

It is important to know how stored eosinophil granule components are solubilized, and exported to the outside of the cell. There are several possibilities. Granule-containing vacuoles may form, and burst open on the outside of the cell, but this is less common with human than rodent eosinophils. The absence, in most degranulating eosinophils, of tubules linking the granules to the outside of the cell, suggests that storage constituents are most commonly solubilized within the granule, transported through the cytoplasm as individual molecules, or via the endoplasmic reticulum, to be secreted at the cell surface. The energy required for this may be generated during the rapid respiratory burst that occurs when eosinophils are stimulated.

The routes taken by peroxidase from the storage granules to the outside of human eosinophil was studied by electronmicroscopy in nasal eosinophils, and blood eosinophils stimulated in culture, in 1984. The calcium ionophore A23187 was used to stimulate these eosinophils to degranulate. This produced granule lysis, and the secretion of eosinophil peroxidase, which was dose-dependent. It required the presence of calcium in the incubation medium, and it was inhibited by pretreatment with the calcium antagonist TMB-8. Electronmicrographs of the stimulated eosinophils suggested that peroxidase secretion occurred (a) through the dilated endoplasmic reticulum, and perinuclear space, and (b) by endoplasmic diffusion, and transudation through the cell membrane¹⁵⁴⁰. It was suggested

that these routes would enable the cell to protect itself from the toxic effects of peroxidase, and other cationic granule constituents which may be secreted in the same way¹³¹⁰.

The ways in which the crystal of MBP is solubilized, and the processes which enable molar concentrations of highly insoluble cationic proteins to be brought to the cell surface, are important topics for future research. It is likely to involve a configurational change in these molecules, perhaps brought about by limited proteolysis, or digestion, altering the 3-dimensional structure of these proteins, so that hydrophobic residues are internalized, and other sites are exposed which enable these proteins to be transported through the cytoplasm. If this is the case, then a search for 'transport' molecules might be worthwhile. (Could MBP itself act as a transport protein for the other basic proteins?) Secretory processes are also potential sites of action for drugs which could affect eosinophil functions. Steroids, which are the most effective inhibitors of eosinophil secretion, may act in this way, although this has yet to be determined.

There are at least three steps involved in secretion. In the first, cell stimulation leads to an alteration in the granules which begin to take up water, and form the characteristic vacuoles, which are refractile circular bodies seen in blood smears, but not in tissue sections. In the second step, the granule components are solubilized. Certain stimuli such as con A (Con A), induce the granules to become solubilized, but they are not secreted¹⁹⁴². However complement-coated particles, and complexed immunoglobulins, and other stimuli such as the calcium ionophore A23187 cause the third stage to occur: the export of the proteins from the cell.

In 1983, Henderson, and colleagues in Seattle, U.S.A. used A23187 to study the ultrastructural changes in secreting horse blood eosinophils. They showed that the granules moved to the cell periphery, and fused with each other. Electron-dense granule material was released into large intracellular vacuoles, which opened to the outside of the cell through surface pores, and peroxidase was released. These events appeared to depend on calcium-dependent activation of phospholipase A₂, and the generation of lipoxygenase products⁷⁷⁷.

In 1985, the same group reported that after incubation of human blood eosinophils with 10ug/ml calcium ionophore A23187, there was an alteration in the structure of their granules, within one minute. Many vesicles were seen in the matrix of some of the granules with loss of the electron dense crystalline core. Fusion of adjacent granules, and occasionally fusion of intact granules with the plasma membrane was noted, with probable secretion of whole granule contents to the outside. Large intracellular channels were found. These effects were most marked at 20 minutes, when many small granules were noted in the region of Golgi apparatus. Although the number of granules decreased, and the size distribution of the granules was lowered, it was suggested that exocytosis of mature granules could be accompanied by the formation of new cytoplasmic granules⁷⁷⁶. This work contrasts with a separate study reported in 1985 from the Mayo Clinic, which showed that A23187 only induced the release of MBP from blood eosinophils by a cytolytic process⁶¹⁰.

The time course of ECP solubilization from stimulated human blood eosinophils may take several hours. Kierszenbaum, and colleagues incubated purified normal human blood eosinophils with *T. cruzi* amastigotes, and the ultrastructural appearances of the phagocytosed parasites, and the changes in the granule correlated with the progressive increase in granule staining with monoclonal antibody EG2, which binds to the secreted form of ECP, and EDN/EPX. Although no antibody, or complement were present in the cultures, eosinophils ingested the amastigotes within 20 minutes, but the secreted form of ECP, and EDN/EPX only began to appear in the granules at four hours, and in the phagosomes at five hours⁹⁶². Experiments were not done with antibody or complement, which might have speeded up this process. Winqvist, and colleagues have shown that Sephadex-C3b induced normal human blood eosinophils to begin to release ECP at 10 minutes¹⁹⁴², whereas unpublished experiments of Dr. Po-Chun Tai have shown that IgG complexes only begin to stimulate release after two hours incubation. The reasons for this are unknown, but may reflect the need for eosinophils to increase their membrane receptor density, or metabolism before they can respond to stimulation by several types of stimuli.

Divalent cations are known to be involved in secretory events in most cells, and secretion does not occur in the presence of EDTA. Both calcium, and magnesium are involved in the secretion of ECP from human eosinophils¹⁹⁴⁵. Some secretagogues can alter the calcium content of eosinophils: fMLP (but not PMA) increased the eosinophil calcium content, as assessed with fura2¹⁴¹⁰.

Experiments are now being done to see whether protein kinase C, and/or phospholipase C are involved. This possibility has some support from experiments in which certain triacylglycerides caused blood eosinophils to secrete 30-50 per cent of their ECP: more than was induced with opsonized particles¹⁸⁴⁷. Preliminary experiments using supernatants from cultured monocytes, which contain eosinophil activating activities, have also suggest that these factors may induce the translocation of protein kinase C from the cytoplasm to the plasma membrane of eosinophils, priming the cell for subsequent stimulation¹⁹⁷².

5.2.3. Drugs affecting secretion.

Steroids are amongst the most potent inhibitors of eosinophil secretion in vitro. In the first report of this effect in 1984, blood eosinophils from normal people, and patients with an eosinophilia, were stimulated with complement coated Sephadex beads. This led to the secretion of 15 per cent of the ECP by 40 minutes, which was reduced by preincubating the eosinophils with 10^{-5} M hydrocortisone for 30 minutes¹⁹⁴².

During the last few years research has been carried out by Dr Harry Smith, and colleagues at Beecham's Group Research, Epsom, U.K. into the potential use of a wide variety of drugs to inhibit eosinophil secretion, eosinophil accumulation in tissues, and eosinophil antibody-dependent cytotoxicity against a number of target cells³⁵⁶. Most of this work has been done with rodent eosinophils. The principal findings were that many steroids, a few membrane-active drugs, (which included some drugs with local anaesthetic activity, adenosine receptor antagonists, and histamine beta blockers), and a number of anti-allergic compounds were effective in these different assays. They included betamethasone, hydrocortisone, dexamethasone, propranolol, chlorpromazine, ketotifen, isoprenaline¹⁶⁷⁰ chloradenosine, and dapsone. Sodium cromoglycate, nedocromil, and related drugs can also inhibit human eosinophil secretion in vitro.

Suramin may stabilize the granules of human eosinophils in patients with filariasis, reducing the proportion which are vacuolated, and degranulated¹²⁷⁵.

5.3. Motility.

Eosinophils are one of a limited number of cells which are produced in one part of the body, and which function in another. For this reason they possess mechanisms which enable them to respond to signals, which induce them to leave the bone marrow, travel through the blood, attach to vascular endothelium, and emigrate into inflammatory lesions. At the end of the last century, the classical studies of Metchnikoff on inflammation in lower marine organisms imprinted the idea of chemotaxis into the minds of those interested in the mechanisms leading to the accumulation of cells in tissues. Ehrlich himself used the term 'chemotaxis' to describe this process. However chemotaxis has not been shown to occur in vivo, in vertebrates, and granulocytes move randomly in inflamed tissues. There is no work to show that concentration gradients are formed in vivo, of the kinds that are set up in chemotactic assays in vitro. Inflammatory cells move out of blood vessels in the same direction as the flow of freshly filtered interstitial fluids, which would flush away any chemotactic molecules as they were formed in inflamed tissues.

Those who work on chemotaxis have rarely assessed the biological effects of their reagents in vivo, and when they have done so, they have been largely unsuccessful in inducing cell accumulation without first damaging tissues to stimulate the normal processes which lead to cell localization. Despite these criticisms, a large, self-fulfilling literature has been built up around the concept of chemotaxis, some of which has come from respected laboratories. This has given the field a respectability which it does not deserve. Few workers have attempted to distinguish between directed migration (chemotaxis), and alterations in the rate of movement (chemokinesis).

There is an extensive literature on the capacity of isolated human blood eosinophils to show direc-

tional motility *in vitro* in response to a variety of stimuli. The main impetus for this work has been to account for the tissue localization of eosinophils *in vivo*, although few experiments have been done to assess this directly. Skin window studies in the rabbit, which were carried out to study the localization of monocytes, and neutrophils, showed that directional movement after migration into injured tissues did not occur, and that accumulation of inflammatory cells was the result of the retention of cells in the lesion, and not directional migration towards it. It would be interesting to repeat these experiments with eosinophils, but there is no reason to suspect that they would be any different. The key events in the localization of blood-borne cells in tissues are margination, and attachment to endothelial cells. Marginated cells migrate between endothelial cells, and are retained in lesions, which they encounter during their random migration into the tissues. In any case, eosinophils probably do not travel very far in tissues, once they have crossed the vessel wall. Surprisingly, no work has been carried out to see whether chemotactic factors induce eosinophil margination, and attachment to endothelium, as this would seem to be the most effective way for them to induce eosinophils to localize in tissues.

The main reason why so many studies have been done on eosinophil chemotaxis is that they are easy to perform, provide statistically significant results, and provide some insight into the kinds of stimuli which can affect eosinophil properties, in this case directional migration. It is unfortunate that these studies have not been extended to see how far other properties of eosinophils, such as activation, secretion, and cytotoxic capacity, are affected.

5.3.1. Localization of eosinophils *in vivo*.

As the uptake of eosinophils into diseased tissues can be very marked, a number of groups have attempted to reproduce this effect by isolating components of the response, and injecting them into the skin. For example guinea pig IgG1 (which is the anaphylactic antibody in this species), induced eosinophil accumulation in skin injection sites, 12 to 24 hours after local passive anaphylaxis had been induced⁹²⁴. This type of experiment was done because it was hoped that there might be unique factors in these lesions which would cause eosinophils to accumulate. Differences in the number of eosinophils present in stimulated, and control tissue sites have been reported, with a wide range of substances, ranging from parasite extracts, to concanavalin A³⁷⁶, and individual components of the immune response.

Unfortunately much larger amounts of these substances have to be given than would be produced in disease, and the findings have often shown differences from the results of *in vitro* chemotactic experiments. For example: (1) PAF-acether is a potent chemotactic factor for eosinophils *in vitro*, but it does not induce eosinophil accumulation in normal human skin⁵¹, although there is a letter suggesting that it may do so in patients with atopy⁷⁸⁴; (2) 1 ug of LTB₄, which has a weaker capacity to induce eosinophil chemotaxis than PAF-acether¹⁶²⁶, does not cause significant eosinophil infiltration into the guinea pig eye, after topical application, whereas 0.1 ug LTE₄, and LTD₄, induced an eosinophil infiltrate in the conjunctival epithelium¹⁶⁶⁷.

However, as there are many technical difficulties in interpreting the results of injecting substances, other methods have been used, including topical application to the eye, the skin window technique⁵⁰⁰, and migration into peritoneal chambers, and other implanted fluid filled cell-permeable vessels. Unfortunately, these experiments have provided little insight into the ways in which eosinophils localize in tissues, and the relative 'potency' of putative localizing substances is still disputed.

As outlined above, I think that future work on the localization of eosinophil in tissues is more likely to be more fruitful if it became concerned with one, or more families of molecules on the surface of endothelial cells, and blood eosinophils, which are now known to be involved in the initial attachment of leukocytes to endothelium in lymph nodes, and areas of inflammation, and to be increased in response to inflammatory signals. A summary of this interesting potential area for future work is shown in Figure A05-3.

Fig. 5-3: Eosinophil localization in tissues.

In 1987, a glycoprotein antigen was described on the surface of murine leukocytes (including

eosinophils), which was recognized by monoclonal antibody MEL-14. This antibody blocked the attachment of neutrophils to vascular endothelium in mice, *in vitro*, and *in vivo*¹⁰⁶³, but it was not tested on eosinophil attachment. Clearly it is important to know whether these antigens are induced to appear on post capillary venules in areas of inflammation, as has been described in a large number of *in vitro* studies^{361, 1426, 165}.

Four patients with ragweed sensitivity were induced to form blisters on their arms, by applying ragweed extracts. The blister fluid was removed, and after it was shown not to contain ragweed antigens, it was injected into other skin sites. Eosinophils were found in skin biopsies from challenged sites as early as 30 minutes after injection. Ragweed antigen itself took two to four hours to induce a significant blistering¹⁷⁸⁴.

5.3.2. Eosinophil motility *in vitro*.

The most widely used method for assaying eosinophil motility *in vitro* has been to use the Boyden chamber, or its variants. Most work has measured chemotaxis, although chemokinesis is more likely to be relevant *in vivo*, (where inflammatory cell movement is random), as cells which migrate the furthest are most likely to reach areas of inflammation.

5.3.2.1. Eosinophil chemotactic factors.

Over one hundred and eighty papers (of very variable quality, and often with conflicting results), have been published on 'eosinophil chemotactic factors' since 1977. For this reason it is difficult to provide an overview of this work, although it seems that many of the substances secreted by eosinophils appear to be chemotactic for eosinophils themselves (LTC₄, PAF etc), and that a range of products from mast cells, parasites at different stages in their life cycles, T lymphocytes, and tumour cells, besides complement components, and many other stimuli, can induce eosinophil chemotaxis *in vitro*.

The difficulty in relating this work to *in vivo* events is borne out by the finding that eosinophils of different density have different chemotactic capacity, and that eosinophils can be 'deactivated' by prior incubation with many of these factors. This makes it difficult to know how to obtain 'normal' eosinophils for studies on eosinophil chemotaxis. It is also puzzling that stimuli which may have 'deactivated' eosinophils in the blood could then cause them to localize in tissues.

A large amount of research on eosinophils has been based on the hypothesis that stimulated mast cells release factors which attract eosinophils into tissues. In 1971 a search for this activity led to the description of eosinophil-chemotactic factor of anaphylaxis (ECF-A)⁹³³. Several molecules, of varying size are now known to be included under this general term. In 1975, two of these were shown to be the tetrapeptides Val-Gly-Ser-Glu, and Ala-Gly-Ser-Glu⁶⁸⁰. It was thought that these tetrapeptides, and an intermediate weight ECF might attract eosinophils to sites of mast cell degranulation, where eosinophils would be retained by a process of 'deactivation'⁸⁰. The properties of ECF-A were reviewed in 1977⁶⁸¹.

It then became clear that these tetrapeptides had no significant biological effects on eosinophils *in vivo*²²⁵. Surprisingly, no critique, has been published by the groups involved, or others. Later work to find other biologically active molecules which might have accounted for the earlier finding with 'ECF-A' has also been unsuccessful. It would be interesting to trace the origins, and development of this concept, which dominated the work of several laboratories working on eosinophils, in the 1970s. Its perceived importance at the time can be judged from the fact that so many papers were produced on the topic, that a separate heading of 'chemotactic factors, eosinophil' was made in 1980, in the Medline databases, and ECF-A found its way into the eosinophil section of most textbooks.

There is an equally difficult series of papers to review on the effects of histamine on human blood eosinophils. Initially, histamine was found to be chemotactic, and chemokinetic, and to interfere with the effects of ECF-A on eosinophil chemotaxis. Then it was suggested, on the basis of histamine-blocking experiments, that eosinophils had H₁, H₂, and a third type of receptor, each of which induced a different effect on eosinophil chemotaxis³²⁹. In 1982, further work using histamine, and specific inhibitors, was thought to show that eosinophils had H₁ receptors for eosinophil-mediated

cytotoxicity, and superoxide production¹⁴¹⁷, but not for chemotaxis, or chemokinesis. However in 1987 *in vivo* experiments showed that histamine was not involved in eosinophil localization, as injections of histamine into the normal skin, of man, and other primates, did not give rise to an increased number of eosinophils in the test sites⁵²⁹. Some aspects of this confusing area were reviewed in 1982⁸⁸.

There is often a close relationship between the development of a delayed hypersensitivity reaction, and the presence of eosinophil-rich lesions in tissues. For example, studies done in 1963 on guinea pigs which had developed delayed hypersensitivity reactions to injected proteins, showed a striking accumulation of eosinophils in the sites where they were reinjected with heat-killed tubercle bacilli. Eosinophil infiltration began two hours after reinjection, and was maximal at six to eight hours. At four to eight hours the eosinophil was the most prominent cell, even though blood eosinophil counts were still normal⁶⁶. Although *in vivo* studies could have been carried out, most subsequent work in this area centred on chemotaxis.

A T lymphocyte-derived chemotactic factor for eosinophils was first demonstrated in 1973, by Dr. Dan Colley. In this study lymphocytes from mice with schistosomiasis were found to produce a factor (eosinophil stimulation promotor, ESP), after stimulation with soluble schistosome egg antigens, which increased the migration of eosinophils from an agarose droplet³⁴². In 1976-7, Colley's group defined the conditions for producing it³⁴³, and assayed its size as 25-31 kDa¹⁰⁶⁷. A related factor from human lymphocytes was described by Parish in 1982¹³⁵⁸.

An unusual eosinophil chemotactic factor (ECF) was described in 1973, which was produced when a guinea pig lymphocytes-derived factor (ECFp), was incubated with immune complexes. It was thought to play some role in cellular immune processes¹⁷⁸⁹. In 1987 another 15-20 kDa lymphokine was detected in supernatants from primed mouse spleen cells stimulated with *S. japonicum* soluble egg antigen. This factor increased the eosinophil chemotactic response to *S. japonicum* egg-derived eosinophil chemotactic factor¹³⁴⁸.

A large number of eosinophil chemoattractants have been found in parasites which can infect man, and animals. Some of these have been purified, and partially characterized. Much of this work has been done in Japan, and has used guinea pig eosinophils. Unfortunately there are no comparative studies on these factors, or their properties. See for example, studies on a factor in extracts of *S. japonicum* eggs⁸¹².

PAF-acether 10^{-6} to 10^{-9} M is both chemokinetic, and chemotactic for human blood eosinophils of normal density, but affects neutrophils to the same extent. At these concentrations, LTB₄, histamine, and the alanyl, and valyl tetrapeptides had negligible effects on the movement of eosinophils^{1751, 1876}.

There are conflicting reports about the chemotactic activity of PAF on eosinophils of different density. In one study, PAF at 10^{-5} to 10^{-8} M caused directional movement, and chemokinesis of light density eosinophils more than normal density eosinophils, and lyso-PAF was ineffective¹⁸⁷⁷. In another study, the dense eosinophils were affected by concentrations of PAF 100 times less than those which stimulated light density eosinophils¹⁶²⁶.

LTB₄, 5-HETE, and 5-HPETE were equally chemotactic to blood eosinophils from four patients with an eosinophilia¹²⁵⁷. Normal, and light density eosinophils were both affected to the same extent by 30-100nM LTB₄¹⁶²⁶. These findings suggest that eosinophils had receptors for LTB₄, and analogues, although these have not yet been identified. However there is no evidence to suggest that LTB₄ induces eosinophil localization *in vivo*. Injections, or application of LTB₄ in the skin of volunteers did not induce a significant eosinophil accumulation in two studies reported in 1986^{173 1939}.

Many different eosinophil chemotactic activities have been reported to come from neoplasms, by workers in the U.S.A., U.K., and Japan. The first of these was a 500 Da chemotactic peptide for eosinophils which was obtained, in 1974, in Boston, U.S.A., from a large cell carcinoma of the lung containing many eosinophils, in a patient who also had a blood eosinophilia¹⁸⁸⁵. In 1978, the same group reported that serum from three patients with anaplastic carcinomas of the lung contained

eosinophil chemotactic activity⁶⁸³. A factor was found in Hodgkin's disease tissues in 1975⁹³¹. In Japan, a factor was obtained from a squamous cell carcinoma in 1979¹⁵⁶¹, and a malignant cervical carcinoma in 1981⁸⁴¹. In the U.S.A., in 1980, an acidic protein, was isolated from a histiocytic lymphoma in the brain of a patient with an eosinophilia of up to $8.0 \times 10^9/L$ ⁶⁸². Large squamous cell cervical carcinomas were found to contain an eosinophil chemotactic factor, in Fukuoka, Japan in 1981⁸⁴¹. In 1984 extracts of a large cell carcinoma of the lung in a Japanese patient were chemotactic for eosinophils⁹⁸⁶, and in 1986 a factor was derived from a malignant fibrous histiocytoma⁸⁵³. C5a shows strong chemotactic activity for human eosinophils. This factor can be generated by many different surfaces, and antigen-antibody reactions, via the classical, and alternative pathways. It has also been found that neutral proteases from human eosinophil granules can cleave C5 directly, to produce an eosinophil chemotactic activity¹²⁹⁹.

Chemotactic factors for eosinophils have been produced from alveolar macrophages stimulated with anti-IgE, or the corresponding allergen in patients with allergic asthma. This was described in 1984⁷⁰¹. In 1987, histamine-stimulated blood mononuclear cells were shown to produce a number of factors of 2.5 and 13-25 kDa, which were also chemotactic for eosinophils¹⁴⁵⁴.

In 1982, three eosinophil chemotactic activities were found in the serum of patients with idiopathic cold urticaria, in whom angioedema had been induced by immersion in ice water for three minutes¹⁸⁸³. An eosinophil chemotactic activity was also detected in the sera of patients with eosinophilic fasciitis¹⁸⁸⁶. In 1987 a heat-labile eosinophil chemotactic factor was described in the sera of patients with asthma, and the levels rose after antigen exposure¹⁴⁶⁸.

A wide variety of skin lesions have been found to contain chemotactic factors for eosinophils. A low molecular weight factor was found in the skin of patients with bullous pemphigoid, atopic eczema, drug reactions, and contact sensitivity⁴⁵⁰.

6. Chapter A 06. Eosinophil activation, cytotoxicity, and interactions.

The most important discoveries about eosinophils in the last decade have been (1) that they are effector cells in inflammatory reactions, able to kill certain parasites, and cells, and to secrete a range of biologically important molecules; (2) that their capacity to carry out these functions is only fully developed after they have been activated. Many of the earlier misconceptions about the roles of eosinophils in disease have stemmed from a failure to appreciate the importance of these activation mechanisms. The range of diseases in which eosinophils may be involved in tissue injury is also under investigation. Figure A06-1.

Fig. 6-1: The effector functions of eosinophils.

6.1. Activation.

A number of methods have been used to show whether eosinophils have been activated, or not. They include antibody-dependent killing of schistosomula, LTC₄ production, secretion of granule proteins, oxygen metabolism, etc. These assays used have reflected the interests of the laboratories concerned, and there has been no attempt to see whether one type of assay is more sensitive than another, and whether all of these activities run in parallel. It is probable that activation is a quantitative difference in effector function, rather than a qualitative difference in some aspect of the cell's ability to recognize, and interact with environmental signals. This means that no single assay would be able to tell whether a cell was activated or not, it would simply demonstrate the extent to which a population of cells had become activated.

The development of a monoclonal antibody (EG2) which only binds to the secreted form of ECP, and EDN/EPX has the particular advantage that it can be used to demonstrate individual activated cells.

There is no information about whether activation is reversible, or whether the various activating factors influence some, or all of the effector functions of eosinophils.

So far, no unique functions of activated eosinophils have been detected, which cannot be carried out by a larger number of non-activated eosinophils. If this proves to be generally true, then large numbers of nonactivated eosinophils should be able to carry out the functions of a smaller number of activated cells. It would be interesting to separate, and study populations of eosinophils from the blood, and tissues of patients, and to measure the amounts of each activating factor to which the cells had been exposed. This would show which of the many activating factors are important in vivo. This is of practical importance, as it could lead to the development of therapeutic methods for increasing, or decreasing the effector functions of eosinophils in inflammatory reactions. These drugs would be of great benefit in the treatment of diseases in which eosinophils have protective, or harmful effects.

6.1.1. In vivo studies.

In 1976, we showed that blood eosinophils from patients with an eosinophilia had several structural differences in their plasma membranes, and granules, and in their functions, compared to eosinophils in normal people. We concluded that: 'blood eosinophils in patients with an eosinophilia may be functionally mature, or altered in response to unknown stimuli while they are in the blood'¹⁶⁹⁶. This was one of the first pieces of evidence that eosinophils could be activated in disease.

In the same year, James, and Colley, in Nashville, U.S.A., were the first to show, in 1976, that eosinophils from infected animals had an increased cytotoxic capacity for parasites. They reported that peritoneal eosinophils from mice infected with *T. spiralis* were more effective than eosinophils from uninfected mice in killing *S. mansoni* eggs in vitro⁸⁶⁷. In 1978 this group showed that eosinophil-rich peritoneal exudate cells from *S. mansoni*-infected mice, were able to kill 20 per cent of *S. mansoni* eggs in vitro, which did not occur with eosinophils from uninfected mice. The culture of soluble products from isolated liver granulomas induced normal mouse eosinophils to become toxic to these eggs⁸⁶⁸.

Work in the rat, published in 1987, has confirmed that peritoneal eosinophils vary in their metabolic, and effector functions, depending on the types of stimuli which are used to induce them in vivo³⁵⁵. In 1980, an increased cytotoxic capacity for blood eosinophils in patients with an eosinophilia was first demonstrated by David, and colleagues. This study was done on blood samples from 30 patients in Brazil with a variety of disorders including infections with *S. mansoni*. Their eosinophil counts ranged between 2.2 and 3.2 x 10⁹/L. Blood eosinophils from patients with the highest blood eosinophil counts had the greatest capacity to kill schistosomula of *S. mansoni* in vitro after 40h of culture. The extent of eosinophil antibody-dependent adherence to the parasites was also increased at two hours³⁹⁶.

They showed that blood eosinophils from patients with the highest egg counts had more Fc gamma receptors, and were more effective in killing schistosomula in vitro, than eosinophils from patients with low egg counts, or normal individuals¹⁸²².

In 1980, experiments by Bass, and colleagues on the in vitro properties of eosinophils showed that there were alterations in the density, surface charge, metabolism, and other properties of blood eosinophils from patients with an eosinophilia, compared to normal blood eosinophils. They interpreted this as evidence that the cells had been 'stimulated or activated' in vivo¹²⁷.

Tissue eosinophils may have a much greater capacity to take part in inflammatory reactions, than was previously thought possible, because they are activated in tissues. This was suggested in a study published in 1987, using human eosinophils isolated from colonic neoplasms by enzymatic dissociation, and countercurrent centrifugation, which gave 1.8 x 10⁶ eosinophils/g mucosa. C3b receptors were found on 75 per cent of these cells, and Fc gamma receptors on 90 per cent. These eosinophils had a greater phagocytic capacity for *E. coli*, than neutrophils, or macrophages isolated at the same time¹⁴⁶.

6.1.2. Eosinophil activating factors.

Studies on eosinophil activation have followed similar lines to those taken during the investigation of macrophage activation, in which a small number of assay systems have been used to define the

relative potencies of a wide range of potential activating factor¹⁶²⁷. However, little is known about what activation is, how it comes about, and why all eosinophils are not normally activated in tissues. Most of the factors which cause eosinophil secretion are also activating factors, although there are some exceptions. For example lipopolysaccharides, and PMA do not activate eosinophils for antibody-dependent killing of schistosomula¹⁷⁷⁹, although they are potent inducers of eosinophil secretion *in vitro*.

6.1.2.1. Endogenous factors.

Most of the molecules which are released from stimulated monocytes, and lymphocytes, and which are loosely classified as cytokines, are able to augment several of the effector properties of human blood eosinophils. These factors were initially described in complex mixtures produced by stimulating T cells, or monocytes with different stimuli, or by culturing mononuclear cells without further stimulation. The availability of recombinant molecules, which have some of the properties of these impure preparations, is an important step forward. These have been used to show that eosinophils are receptive to most of the molecules which affect the properties of macrophages, and which stimulate lymphocytes to proliferate. Much of this area will become clearer, as these different activating factors are cloned, and as techniques are developed for studying their occurrence, and relative importance *in vivo*, as well as *in vitro*. A summary of the principal eosinophil activating factors is shown in Figure A06-2.

Fig. 6-2: Eosinophil activating factors.

A highly purified 41 kDa glycoprotein interleukin (HILDA) released from a human alloreactive T cell clone, was chemotactic for mouse eosinophils in a tissue air sac, and induced blood eosinophils from four patients with HES to produce chemoluminescence, similar to that developed by PMA. It did not stimulate the growth and differentiation of eosinophil colonies from human bone marrow¹²³⁹. Several features show that HILDA is distinct from EAF, and EDF, although it has a similar molecular weight to EAF.

In Boston, U.S.A. David, with Dessein, and Silberstein, and other colleagues have worked for several years on the capacity of cultured human blood monocytes to generate factors which enhance the capacity of eosinophils to take part in cytotoxic, and inflammatory reactions. These were initially called eosinophil cytotoxicity enhancing activity (ECEA). This was distinguished from T lymphocyte derived factors, and called monocyte eosinophil cytotoxicity enhancing factor (M-ECEF).

Mononuclear cells from patients with chronic schistosomiasis in Brazil produced only 25 per cent of the amount of ECEA as matched controls, 84 per cent of whom produced ECEA⁴⁴³. It was thought possible that this defect occurred because these patients had an altered immune responses to the parasite, which resulted in a failure of the mononuclear cells to produce ECEA⁴⁴².

A principal effect of ECEA was shown to be an increase in the amount of eosinophil electron dense material deposited onto the surface of schistosomula²⁹². Dessein, has summarized the work of the group which he subsequently set up in France, in a paper published in 1987⁵¹⁰.

In 1987 Silberstein, and colleagues described the production of an ECEF by a human cell line (U937) with monocyte features, which was derived from a patient with a histiocytic lymphoma. This was produced after stimulation in serum-free medium with PMA for two days, and LPS for a further two days. It had a subunit structure of about 9-10 kDa¹⁶²⁷, and migrated in gels with apparent molecular weights of about 17, and 32kDa, and isoelectric points 3.8-5.1. M-ECEF consisted of several components, including tumour necrosis factor (TNF) which accounts for part of its activity, and to which it may be related structurally¹⁶²⁷. It was concluded that M-ECEF is one of several cytokines which enhance the toxicity of human blood eosinophils to schistosomula *in vitro*¹⁶²⁸.

Butterworth, Thorne, and colleagues in Cambridge, U.K. have described an eosinophil activating factor (EAF), which is a 37 kD protein, pI 4.4. It was partially purified from supernatants prepared by culturing unstimulated blood mononuclear cells from moderately eosinophilic people for 1-25 hours. A smaller molecular weight activity was also produced, but this has yet to be characterized¹⁷⁷⁹. Mononuclear cell supernatants from individuals with an eosinophilia produced the most activity

In 1986, EAF was shown to be distinct from TNF, ECEF, and GM-CSF¹⁷⁷⁹. EAF enhanced the capacity of human blood eosinophils to kill antibody-coated schistosomula, and cell line cells. It increased several properties of eosinophils: the expression of some membrane antigens, the adherence of eosinophils to schistosomula, degranulation, and the production of hydrogen peroxide, superoxide, and LTC₄¹⁷⁸⁰.

A similar eosinophil activating activity to EAF has been described in Amsterdam, Holland. This was detected in supernatants from cultured human monocytes (mono-sup), and increased the oxidative metabolism of human eosinophils in response to a variety of stimuli, except A23187¹⁹⁷².

Mast cells, and basophils may produce factors which enhance the activities of eosinophils. This was suggested in a study in which stimulation of eosinophils with a F(ab')₂ rabbit anti-human IgE increases their cytotoxic capacity against schistosomula, if there were human basophils also present in the cultures¹²³⁴.

The discovery, in 1982⁴⁴⁴, that CSFs can also be activating factors, raises the question of whether newly formed eosinophils are activated within the bone marrow, but lose this capacity when they enter the blood, or whether an additional tissue factor(s) is required for this final step in differentiation. These possibilities await measurements of eosinophil activation in different locations, and the possible role of the blood components in inhibiting (and reversing?) the activation process.

In 1986 it was reported that biosynthetic (recombinant) human T lymphocyte GM-CSF enhanced eosinophil cytotoxicity toward schistosomula, and calcium ionophore A23187-induced generation of leukotriene C₄ (LTC₄). Augmentation of each eosinophil function by GM-CSF was time- and dose-dependent, which reached a plateau one hour after incubation, before stimulation with ionophore. There was an initial increase in intracellular levels of LTC₄¹⁶²⁹.

Colony stimulating factor alpha (CSF-alpha) derived from human placental conditioned medium, and which has identical activity to recombinant GM-CSF, was shown, in 1982, to alter human blood eosinophils so that they adhered to antibody-coated schistosomula more rapidly *in vitro*, by an irreversible binding mechanism. This led to an increase in their capacity to kill the parasites at 24 hours⁴⁴⁴.

In 1987 it was reported that, after culture for up to two weeks in small quantities of recombinant human GM-CSF, normal human blood eosinophils became lighter in density, and had increased capacity to secrete LTC₄, and to kill antibody coated schistosomula¹³⁴⁶. Both recombinant TNF, and GM-CSF increase eosinophil antibody-dependent killing of schistosomula. Recombinant TNF was more potent than GM-CSF. The time course of activation by the two factors was different: eosinophils were maximally stimulated by GM-CSF at ten hours, whereas this was 30 hours using TNF¹⁶³⁰. GM-CSF is produced by a number of cells, including endothelial cells, in response to stimulation with TNF¹²⁵³, which is mainly produced by macrophages.

In 1987, mouse EDF, (IL-5), was found to be an activating factor for human eosinophil antibody-dependent killing of some tumour cells, and phagocytosis of opsonized zymosan¹⁸²³.

In the presence of bovine endothelial cells, or their conditioned medium, cultured blood eosinophils developed an increased capacity to secrete LTC₄, after stimulation with the calcium ionophore, and to kill antibody-coated schistosomula. In endothelial cell cultures, eosinophils of normal density also became lighter. It was suggested that endothelial cells could activate blood eosinophils during their passage into tissues¹⁵²². The molecules from endothelial cells which activate eosinophils may be both GM-CSF, and G-CSF, as it has been found that human umbilical cord endothelial cells make both of these activating factors in response to stimulation with interleukin 1²¹³.

Substance P, which is released by sensory neurones in the lungs, and gut, and causes respiratory tract hyperreactivity, smooth muscle contraction, and urticaria, has been found to increase the expression of IgG, and IgE receptors on isolated human blood eosinophils, and to increase their capacity to lyse rabbit antibody-coated chicken red cells, labelled with ⁵¹Cr. This suggests that this undecapeptide may activate eosinophils, and contribute to their effector roles in disease⁴²¹.

PAF-acether, at concentrations of 10^{-7} to 10^{-9} M stimulated human blood eosinophils to kill complement-, and antibody-coated schistosomula more than normal. It was 100 times more potent than histamine¹²³⁶.

Tumour necrosis factor can activate eosinophils for antibody-dependent killing of schistosomula. It has a lesser effect than GM-CSF, and unlike the 'all or nothing' effect of GM-CSF, it induces an increased cytotoxic response over a 10^4 concentration difference. These differences were thought to show that there are several different ways in which eosinophils respond to cytokines¹⁶²⁷.

Interferon alpha did not activate human eosinophils in an antibody-dependent killing assay^{1779, 1627}, although it increased the secretion of granule enzymes from eosinophils in response to zymosan-C3b stimulation¹⁵³⁸. Injections of recombinant interferon alpha into patients with solid tumours decreased the percentage of bone marrow cells that were eosinophils from 4.5 to 2.2 per cent⁵²⁴. Interferon beta enhanced the capacity of eosinophils to lyse antibody-coated chicken red blood cells⁴²³.

IL-1 does not activate human eosinophils directly^{1779, 1627}, but it has some effects on superoxide production, and granule secretion in response to a number of stimuli¹⁴²⁰. IL-2 does not activate human blood eosinophils¹⁷⁷⁹. Cloned gibbon interleukin 3 (gIL-3) stimulated mature eosinophils to kill antibody-coated target cells, to produce superoxide anions, and to phagocytose opsonized yeast particles. It had more specificity as an activating factor than GM-CSF, as gIL-3 did not activate neutrophils¹¹⁰³.

6.1.2.2. Exogenous factors.

The possibility that schistosomula of *S. mansoni* produce activation factors for eosinophils was assessed in a study from Lille, France, in which secretion/excretion products from schistosomula were assessed for their capacity to induce rat, and human eosinophil activation. These effects were compared with the capacity of ECF-A to induce activation of eosinophils from rats, and man. The schistosome-released products (SRP) were of a larger molecular weight, and different heat lability from ECF-A, and caused eosinophils to develop a greater cytotoxic capacity, more Fc gamma receptor expression, and greater morphological changes after 4h incubation, than ECF-A. As SRP had less effect on human neutrophils, it was proposed this could partly explain why eosinophils were more toxic for schistosomula than neutrophils, and how parasites themselves could influence the way in which inflammation develops in tissues⁷⁸. It was later shown that rat eosinophil-dependent killing of schistosomula was enhanced by proteases released from schistosomes, and by collagenase. This suggested that the effect of SRP on rat, and human eosinophil Fc receptors, and antibody-dependent cytotoxicity involved eosinophil membrane structures which were changed by neutral proteases⁷⁹.

6.1.2.3. Effects of activating factors on eosinophils.

Incubation of human blood eosinophils with EAF induced them to produce more lamellopodia, and their granules moved rapidly in phase-contrast preparations¹⁷⁷⁸. A 25 kD product from histamine-stimulated blood mononuclear cells also induced morphological changes in cultured blood eosinophils¹⁴⁵⁴.

There is evidence that activation signals induce eosinophils to phosphorylate several proteins. This was seen when human blood eosinophils were cultured alone, or after stimulation with PMA, A23187, or opsonized-zymosan. There was also dephosphorylation of a 21 kDa protein. Alterations in phosphorylation were not seen with fMLP. As each stimulus affected a different range of proteins of varying size, it was suggested that phosphorylation is not only an early event following cell activation, but that the proteins which are affected depend on the nature of the signal¹⁴⁷³.

Cultures of partially purified mouse peritoneal eosinophils contained more lysophospholipase, after incubation with mononuclear cells stimulated with parasite antigens to which they were sensitized, than control cultures. This suggested that mononuclear cell products can increase the amount of this enzyme synthesized by eosinophils. These findings also explain why higher levels of lysophospholipase are found in the tissues of parasitized mice, than can be accounted for by the number of normal eosinophils²⁰.

6.2. Cytotoxicity.

It has been known for many years that eosinophils are less effective than neutrophils in killing bacteria. This is partly because less eosinophils ingest bacteria, and the number of bacteria ingested per cell is lower than for neutrophils. In addition it has been suggested that eosinophils have a less effective peroxidase-H₂O₂-halide killing system than neutrophils. The topic was reviewed by Bass in 1982¹²⁴. The possibility that eosinophils might kill parasites was not seriously considered until recently, although there were early observations which might have suggested this. For example in 1968 it was shown that the ultrastructure of tissue eosinophils was altered by a parasitic infection, and it was shown that the central area of the granule had been effected. It was proposed then that the release of the central electron-opaque material could play some part in the reaction to the infestation. This work, by Casley-Smith in Australia was done in rats infected with *Moniliformis dubius*²⁸⁵. He also compared this change in the crystalloid with changes in the matrix of eosinophil granule in patients with an eosinophilia, which had been described in 1966 by Zucker-Franklin, and her colleagues²⁰⁰⁹.

The capacity of eosinophils to kill the schistosomula of *S. mansoni* in vitro, which was discovered in 1975²⁵⁵, provided a major stimulus (and assay system) for the study of eosinophil-dependent cytotoxicity. Work in this area has led to the conclusion that eosinophils are major defensive cells in protection against certain helminthic, and other parasitic infections. Some work on eosinophil-dependent killing of tumour cells in vitro has also shown their potential for protection in neoplastic diseases. This work has also raised the possibility that eosinophils could damage normal cells, and tissues, and be responsible for several important clinical abnormalities, such as some forms of asthma, skin diseases, and endomyocardial fibrosis.

Most experiments in this field have been carried out using intact eosinophils, and the targets coated with a molecule which can bind to one of the main receptors on eosinophils. In these experiments, several hundred eosinophils were required to kill each parasite, and the mechanism of killing was a three-step process in which the parasite was first recognized, then attached to, and finally killed by secretion products from the attached cells. Similar types of experiments have been carried out with mammalian cells coated with a variety of molecules which eosinophils can recognize. For example in 1978 Sanderson, and colleagues, showed that August rat peritoneal eosinophils were able to cause the release of up to 30 per cent of the label from ⁵¹Cr-labelled chicken red blood cells coated with rat antibody, after incubation in a 2.5:1 cell:target ratio for four hours. (This was less than the amount of ⁵¹Cr released by neutrophils under the same conditions). The ways in which eosinophils attached, and partially ingested the labelled cells was different from neutrophil, as shown in a time-lapse film which they made¹⁵⁵⁴.

Mammalian cells, and tissues are probably killed in a different way. Normal cells, and tissues are not coated with immunoglobulin or complement in the same way as invasive parasites, and in this case, the effects take place without a receptor-mediated attachment phase. In this model of eosinophil-dependent tissue injury, eosinophil products would be generated by soluble stimuli. Release of products would attach to nearby structures, and damage them. Long range interactions of secreted eosinophil products with cells, and tissues could also occur. However, these are less likely in view of the basic properties of the eosinophil cationic proteins. Figure A06-3.

Fig. 6-3: Eosinophil-dependent cytotoxic mechanisms.

6.2.1. Parasite killing.

During the past decade, the view that eosinophils provide a first line of defence against infection by a number of nematodes, which induce the production of IgG, and IgE antibodies, has gained wide acceptance. This was reviewed in 1984²⁴⁹. Antibodies coat certain stages in the tissue life cycle of the parasite, so that eosinophil can attach, and degranulate onto their surface, and kill them. Details of how this interaction takes place with schistosomula in vitro were published in 1979²⁵⁶, and 1980¹⁸¹⁹, and have provided the model system for this type of interaction.

One of the main effects of the attachment process was to cause the release of highly toxic cationic

proteins onto the surface of the parasite. This has been studied by several groups using electronmicroscopy. Secreted EPO was found on the surface of the schistosomula¹¹⁷⁷, and *Angiostrongylus cantonensis* adults¹⁹⁸³, and MBP on schistosomula²⁵⁷, and *Onchocerca volvulus* microfilariae⁹⁵¹.

However, the capacity of many parasites to alter their antigenic characteristics within a short time of invasion of the host, means that cell-mediated immunity can only act on certain stages in the parasite life cycle. A temporal, and spatial association between an eosinophilia, and many parasites is often difficult to demonstrate in the early stages of an infection. An eosinophilia is usually more prominent in the later stages of the disease¹²⁴. Even so, eosinophils do appear to have a role in limiting the number of invasive parasites which migrate to their final tissue locations. At the least, eosinophils provide a 'hostile' tissue environment for helminths, which are so common in man's environment. Many nematodes may be able to resist or avoid eosinophil-dependent cytotoxicity, although there has been no formal study of the range of parasites which are susceptible to, or resistant to this cytotoxic response. For example, the infective larvae of *Toxocara canis* were not killed in vitro by human blood eosinophils, even after they have been activated in vitro. This was found to be due to the capacity of the larvae to shed their cuticle⁵⁴².

Eosinophil-dependent killing may also act in tandem with other forms of cytotoxicity in vivo, but experiments to assess this in vitro are obviously more difficult to carry out. For example, an effect of EPO on the killing of *T. cruzi* epimastigotes by macrophages has been shown.

Parasites are also susceptible to cytotoxic mechanisms when they lodge in sites where they are unable to develop further. For example migrating larvae of *T. spiralis* are always killed in the heart, where they commonly localize in heavy infections. Eosinophils are a prominent component of this response.

In the case of parasites which produce eggs in their definitive tissue locations, eosinophils again may play an important role in host defence. In some instances this is beneficial, but the marked granulomatous response that can occur around ova in the gut, liver, and bladder wall can also be detrimental, in the long term. The fibrotic reaction that occurs as a result of these granulomas may be partly due to products released during the tissue damage, or as a result of the release of fibrogenic molecules from inflammatory cells in these sites, including eosinophils. This possibility has still to be explored fully in the case of eosinophils, but the marked association of eosinophils with fibrotic reactions in many different tissues argues for their central role in this type of lesion.

6.2.1.1. Eosinophil killing of schistosomes.

Eosinophils may have a protective effect during penetration of the skin by schistosomula of *S. mansoni*. They may also kill migrating parasites in the lungs, and other sites, where the major depredation of migrating larvae is now known to take place. However cultured schistosomula have the ability to alter their tegument, so that they no longer become susceptible to eosinophil-dependent killing mechanisms^{1287,439}. This protective change may also occur in vivo.

IgG antibody enables eosinophils to attach to the surface of the parasite. This is present in the serum of some patients with schistosomiasis. It has also been produced by immunizing animals with the haemocyanin glycoprotein from the keyhole limpet, giving some protection against infection⁷²⁶. Complement mediated killing has also been reported using rat¹⁴⁷⁰, or human eosinophils⁴⁸.

Most of the assays for eosinophil-dependent killing of schistosomula have been done using skin-penetrated parasites, although mechanically disrupted schistosomula have been used by some groups. Details of the techniques have been given in a number of the papers discussed below. The signs of parasite death are a reduction in motility, loss of their normal structure, and penetration of dyes, such as methylene blue, into the worm surface. Although ⁵¹Cr release was used initially as an assay technique for eosinophil dependent killing²⁵⁴, this was replaced by these other methods. In a review of this assay system in 1985, it was found that each of the components of the assay could affect the result, including the source of the eosinophils, the antibody, and even the reaction vessel²⁵³.

The biochemical events during the killing of *S. mansoni* eggs by mouse eosinophils were reported by

James, and Colley in 1978. They showed that inhibitors of eosinophil glycolysis (iodoacetate, and 2 deoxy-D glucose), prevented eggs from being killed, and they also found that calcium, and magnesium ions were needed. Aerobic metabolism was essential for satisfactory eosinophil function in vitro, and there was also a requirement for microfilaments, and serine esterases⁸⁶⁹. Histological evidence, from liver biopsies of patients with schistosomiasis mansoni, supports the possibility that eosinophils can kill the miracidium in egg granulomas⁸²⁰.

Work with rodent cells continued in other laboratories in 1978, using rat eosinophils, and schistosomula. Electronmicroscopy of the interaction of rat peritoneal eosinophils with schistosomula showed that eosinophils migrated under the tegument, and prised it away from the body of the worm. Eosinophils released many of their granules, and had begun to degenerate in these areas. In contrast, eosinophils adherent to complement-coated Sepharose beads did not show any evidence of specific granule secretion, or vacuole formation, even after eight hours of culture¹¹⁸⁰. This is surprising, in view of previous studies in which complement-coated Sepharose has been shown to induce lysosomal enzyme release from eosinophils in man¹¹⁹⁷.

The interaction of eosinophils with the pentalaminar surface membrane of antibody-coated schistosomula is a close one, which was first studied in 1978 by phase-contrast, and electronmicroscopy. The cells adhered, flattened, and then after fusion of the eosinophil granule membrane with the cell plasma membrane, electron-dense granule material was spread closely over the surface of the parasites. This was followed by evidence of damage to the tegument within the first five hours of incubation. Eosinophils continued to attach to the exposed deeper muscle layers, and ingested fragments of the parasites⁶⁴⁹.

In 1980, the interaction was studied using freeze fracture techniques. This showed that eosinophils did not fuse their plasma membrane with the outer membrane of the schistosomula, unlike neutrophils, but discharged their granule contents directly onto the surface of the parasite²⁹⁰. When eosinophils were detached from the surface of antibody coated schistosomula, transmission EM, and freeze-fracture studies suggested that eosinophils adhered to the parasite surface by their discharged granule material²⁹¹. This makes it probable that the toxicity of eosinophils for this parasite is linked to their capacity to secrete their granules, and other components.

Although initial work in 1979 on eosinophil-dependent killing of schistosomula in vitro suggested that MBP was the most potent toxic component in eosinophils²⁵⁷, it is now known that on a molar basis, ECP is more toxic in vitro⁵. The capacity of ECP to kill schistosomula is greatly increased in the presence of oxygen metabolites. This was shown by incubating schistosomula with neutroplasts, or eoplasts, which contain membrane oxidative enzymes, but no granules. The importance of the respiratory burst was shown in experiments in which cytoplasts of granulocytes from patients with chronic granulomatous disease failed to synergize with ECP to kill schistosomula¹⁹⁷³. For further discussion on the effects of individual eosinophil proteins on parasites in vitro, see Chapter A 03.

6.2.1.2. Eosinophil killing of trichinella.

It was first shown in 1978, that *T. spiralis* newborn larvae could be killed by murine eosinophils in vitro^{942,939}. Human eosinophils, and neutrophils were also shown to be able to kill these larvae^{129,938}. Studies done in Paris, and reported in 1987, have shown that murine eosinophils are the main effector cells of in vitro antibody-dependent killing of larvae which are only two hours old, but that the parasite alters to become resistant by 20 hours⁶²⁰.

The mechanism of killing was initially ascribed to the generation by eosinophils of reactive oxygen species, and hydrogen peroxide¹²⁹. Then it was shown that MBP could kill newborn larvae¹⁸⁸⁷, but not muscle stage larvae²⁴⁶. A monoclonal antibody to newborn larvae, which could mediate eosinophil-dependent killing of the larvae in vitro, was able to reduce infectivity in vivo¹³³⁶.

It was not until 1987 that the toxicity of three of the basic proteins in eosinophils, native MBP, reduced and alkylated MBP, ECP, and EDN/EPX, was assayed in cultures of *T. spiralis* newborn larvae. MBP in its reduced, and alkylated form, was more toxic than the native molecule, and ECP was as toxic as MBP, but killed more slowly. EDN/EPX was about 10 times less active. Ribonucle-

ase was ineffective⁷⁴⁷. As this parasite has lysolethacin within its tegument, it has been suggested that eosinophil lysophospholipase could also damage this parasite²⁰, but this has not been tested.

6.2.1.3. Eosinophil killing of filaria.

Possible links between eosinophils, and the pathology of onchocerciasis have been studied for several years, and there is a growing body of evidence that although eosinophils may have a protective role in this disease, they may also be responsible for some of the tissue lesions which produce permanent injury in the skin, and eyes. IgE antibody may be involved, as sera from Sudanese patients with onchocerciasis was found to contain a heat-labile component which enabled blood eosinophils to adhere to third stage larvae of this parasite, during a three hour assay. Neutrophils did not adhere, and eosinophil binding to other filarial larvae was not seen. The most active sera came from patients with punctate keratitis¹⁹³⁴.

Eosinophils can kill the microfilariae of *Onchocerca volvulus*⁷¹⁴, and *Brugia malayi*¹⁶³² in vitro. Eosinophils in the lymph nodes of patients with onchocerciasis, who had been treated with diethylcarbamazine, had features to suggest that they were destroying the microfilariae¹⁴⁶⁵.

6.2.1.4. Eosinophil killing of trypanosomes.

In 1979 Thorne, and colleagues found that human eosinophils could phagocytose *Trypanosoma dionisii*, and kill the organism in endocytic vacuoles¹⁷⁷⁷. In 1981 Kierszenbaum, and colleagues also reported that trypomastigotes of *T. cruzi* were killed in vitro by reduced, and alkylated MBP⁹⁶⁰. Killing was prevented with greater than 312u/ml of heparin in cultures containing four eosinophils for each organism⁹⁶¹.

It has been shown that amastigotes of *T. cruzi* can be taken up, and damaged by human blood eosinophils. Intact granules, and MBP were also deposited onto extracellular organisms, and these may have been the cause of their death, as MBP was found to be directly toxic to epimastigotes¹⁸⁶³, and amastigotes¹⁸⁶⁴.

6.2.1.5. Eosinophil killing of other parasites.

There is experimental work suggesting that eosinophils may be able to kill malaria parasites. This has come from studies in infected rodents, and from in vitro experiments with eosinophil secretion products¹⁸⁹⁰.

Leishmania mexicana amazonensis promastigotes, and amastigotes were rapidly phagocytosed by rat peritoneal eosinophils, and destroyed both within endocytic vacuoles, and by secretion onto the surface of the parasites¹⁴⁰⁸. This reaction was complement mediated.

In 1983, it was shown that human blood eosinophils were able to kill *Giardia lamblia* in an antibody-dependent cytotoxicity assay, although this effect was less than with neutrophils¹⁶⁵³.

6.2.2. Mammalian cell killing.

There are a wide range of non-parasitic diseases in which eosinophils are prominent, and may have a toxic effect on cells, and tissues. Examples include atopic eczema, in which large amounts of eosinophil granule proteins are found in the dermis in areas where there are few eosinophils, in eosinophilic cystitis, in certain slowly-growing tumours where eosinophils are found, and around the tumour masses, in the gut in various inflammatory disorders such as Crohn's disease, and allergic gastroenteritis, and in granulomatous diseases where eosinophils deposit large amounts of their granule proteins.

Guinea pig MBP can damage a number of human cells in vitro, including spleen cells, mononuclear cells, intestinal, skin, and tracheal cells⁶⁵⁸. As the concentrations of MBP which had this effect could be reached in vivo, it has been suggested that this may be a cause of tissue injury in hypersensitivity diseases⁶⁵⁶.

6.2.2.1. Eosinophils, and respiratory tract injury.

The finding of large numbers of eosinophils in the bronchial epithelium of patients dying with severe asthma prompted experiments to see whether eosinophils could be responsible for some of the pathology of this disease. Experiments using guinea-pig tracheal rings in vitro showed that MBP cause desquamation, and damage to ciliated epithelial cells on the surface of the trachea, and re-

duced ciliary motility.

Eosinophil peroxidase which was purified from the blood of patients with HES killed cultured human A549 cells, (which have some of the characteristics of type II pneumocytes), and rat type II alveolar pneumocytes, in the presence of an H_2O_2 -generating system, and a halide. It was suggested that, as MBP at 10^{-5} mol/L did not cause lysis in this system, the EPO- H_2O_2 -halide system may be an important cause of lung injury in patients with asthma, and pulmonary eosinophilic syndromes²⁴.

Purified rat peritoneal eosinophils, either intact, or after disruption, were able to prevent the confluent growth in culture, of a human fetal lung fibroblast cell line, MRC-5. The inhibition was greater when the cells had been stimulated with PMA, and only then became as effective as neutrophil-dependent toxicity³⁵⁴.

6.2.2.2. Eosinophil killing of cell lines, and tumour cells.

There is a body of experimental work which show that eosinophils can kill tumour cells in vitro, raising the possibility that eosinophils may have a protective role against the growth of certain tumours. As mentioned above, during a two-hour incubation, EPO was toxic for cultured human lung A549 cells in the presence of an H_2O_2 -generating, and halide system²⁴. Eosinophils in bronchoalveolar lavage samples from patients with respiratory diseases, killed a cat lung diploid alveolar epithelial cell line (AK-D) spontaneously. Guinea pig eosinophils had the same effect and also killed a labelled human lung fibroblast cell line (HFL-1) spontaneously. Cultured fibroblasts, and mesothelial cells were killed, but less effectively. The mechanism of killing appeared to be through the production of oxygen radicals⁴⁰⁹.

6.2.2.3. Eosinophil killing of heart cells, and vascular constituents.

The association of a marked eosinophilia due to many different causes, with the appearance of endomyocardial fibrosis has led several investigators to raise the possibility that eosinophils might be able to damage the heart. Since 1976, we have been investigating the role of eosinophils in cardiovascular injury in patients with hypereosinophilia. We have shown that eosinophil secretion products are highly toxic for isolated rat heart cells in vitro. Toxicity was due to damage to the sarcolemma, with secondary effects on mitochondrial enzymes, which were inhibited stoichiometrically. The most sensitive enzymes were 2-oxoglutarate dehydrogenase, and pyruvate dehydrogenase¹⁷³⁶. Earlier studies using heart cell line cells, and intact human blood eosinophils failed to show significant toxicity¹³⁶⁷, but these experiments were done without prior activation of the eosinophils to enhance their potential cytotoxic capacities, and to cause them to secrete their contents. The intravenous injection of human eosinophil secretion products can also injure the right ventricular muscle of mice⁹⁷³. Isolated vascular endothelial cells can be killed by purified guinea pig MBP⁶⁵⁸.

6.2.3. Mechanisms of eosinophil-dependent killing.

Eosinophils can damage target cells, and multicellular organisms by both oxygen-dependent, and oxygen-independent mechanisms. Oxygen-independent killing mechanisms affect cultured bronchial cells⁸³, and schistosomula¹⁴¹⁵. Oxygen-dependent mechanisms are involved in schistosomula killing by eosinophils stimulated with activating factors¹⁴¹⁶. It is also interesting to note that eosinophils from patients with chronic granulomatous disease (CGD) kill schistosomula less well than normal eosinophils⁹⁴¹.

6.2.3.1. Cationic proteins.

Both eosinophils, and neutrophils can damage targets by secreting their stored cationic proteins. The toxic cationic proteins in neutrophils, which kill some bacteria, and fungi, are of two types: (1) small molecular weight cysteine-rich proteins containing only 29-30 amino acids, named defensins¹⁵⁸⁹. These can also kill tumour cells¹⁰⁷⁷, (2) larger molecules of 37, and 57 kDa¹⁵⁹⁴. Eosinophil cytotoxic cationic proteins are probably unrelated to the neutrophil antimicrobial proteins, but their capacity to damage bacteria has not been as well defined. All these proteins appear to damage their targets, through membrane interactions. In the case of the neutrophil defensins, this occurs within phagosomes, and less than 8 per cent is secreted after stimulation with PMA, or opsonized zymosan

The possibility that eosinophil basic proteins cause membrane damage by forming transmembrane lesions in target cells has been suggested on several occasions. Studies with purified human ECP, have shown that it can form ion channels in liposomes¹⁹⁸⁵, but these transmembrane pores were relatively voltage-insensitive, and non-ion-selective¹⁹⁸⁶.

It has also been suggested that MBP could disrupt cell membranes in a way similar to mellitin¹⁰⁰⁶, in which the basic N-terminal part of the molecule binds first, and the hydrophobic part then interacts with the cell membrane to damage it⁹⁹⁸.

6.2.3.2. Oxygen radicals.

Eosinophils may kill organisms by two oxygen-dependent mechanisms. One involves the generation of oxygen radicals (superoxide, and O_2^-), and the other the production of HIO_3 or $HBrO_3$. These two events are linked, as newly formed superoxide is rapidly converted to H_2O_2 , which is highly toxic in the presence of a peroxidase, and a halide. Superoxide, and H_2O_2 can diffuse easily, but they are detoxified rapidly by superoxide dismutase, catalase, and glutathione peroxidase. Other reactive oxygen metabolites probably travel only a short distance⁹⁴. The most toxic product of granulocyte oxygen metabolism is the hydroxyl radical¹¹⁴⁷.

6.2.3.3. Hydrogen peroxide, and EPO.

There is an extensive literature on the capacity of products of oxidative metabolism from neutrophils to damage cells, and tissues. This began with the classical study of Klebanoff who showed in 1967, that myeloperoxidase with H_2O_2 , and free iodine can iodinate, and kill bacteria⁹⁷⁴. It has also been known since 1971 that eosinophils generate large amounts of H_2O_2 ^{92,975}.

The toxicity of the EPO + H_2O_2 + halide system in eosinophils is different from that in neutrophils, as iodide¹²⁰⁹, and bromide¹⁹⁰², but not chloride, are important in bacteria killing by eosinophils. The product generated may be HIO_3 , and $HBrO_3$. However, when gelatin is absent from the reaction mixture, purified EPO can kill *E. coli* in the presence of H_2O_2 , and chloride⁸⁹⁰.

The range of organisms which are killed in this way in vitro is impressive. It includes schistosomula^{940,892}, toxoplasma¹⁰⁸⁹, trypanosoma¹²⁸³, leprosy bacilli⁹⁷⁸, and other bacteria⁸⁹⁰, viruses¹⁵⁰, mycobacteria, and fungi. However, it is not certain that eosinophils can mobilize this effector system very well. For example, phagocytosed *E. coli* are not destroyed by eosinophils as effectively as by neutrophils, although they appear to have the capacity to do so when purified peroxidase with H_2O_2 , and a halide are used in vitro⁸⁹⁰.

EPO can also kill mammalian cells in vitro. This has been shown with mast cells⁷⁷⁸, and some tumour cells⁸⁹¹.

6.2.4. Factors which increase eosinophil-dependent cytotoxicity.

A large number of factors have been described which increase the cytotoxic capacity of eosinophils for target parasites, and cells. These include the following endogenous factors:

- GM-CSF (CSF-alpha)¹⁷⁷⁹.
- mast cell-derived factors, including ECF-A, and histamine¹²³⁵.
- ECF-A^{47,271}.
- leukotriene B4¹²³⁷ (a minimal effect).
- fMLP⁹³⁴, which is normally considered as a bacterial product, but which can also be generated by mitochondria.
- factors from stimulated T lymphocytes including ESP⁸⁶⁸, and EDF.
- IL-3¹¹⁰³.
- monocyte products including EAF¹⁷⁷⁹, and M-ECEF.
- recombinant TNF¹⁷⁷⁹.
- Beta-interferon which increased⁵¹Cr release from antibody-coated chicken red blood cells from 24 to 30 per cent after 30 minutes, at an effector/target cell ratio of 25:1⁴²³.

6.3. Interactions of secreted products with normal cells, and tissues.

If the primary role of eosinophils is to release their granule components into areas of inflammation, it is clearly important to know what effects these secreted products have on other cells, and tissues.

With this in mind, several of the principal eosinophil constituents have been tested individually for their capacity to interact with other cells, and tissues, *in vitro* (see Chapter A 03). However, most of these studies have used (partially) purified eosinophil extracts, which may possess different properties from naturally secreted proteins. In the case of ECP, antigenic differences between storage, and secreted forms of the protein can be recognized with monoclonal antibodies. There may even be interactions between two, or more of the secreted eosinophil proteins, giving rise to molecules with either increased, or reduced capacity to interact with adjacent cells, and proteins. Although experiments with complex mixtures of secretion products from eosinophils are 'messy', they are more likely to provide relevant information in this area, especially when they are carried out in the presence of blood, and tissue components, which may modify their actions. Possible 'targets' for these effects of eosinophil secretion products are mast cells, basophils, lymphocytes, platelets, and other inflammatory cells, including other adjacent eosinophils.

In most of the *in vitro* studies in which individual eosinophil components have been tested on other cell types, their main effect has been to cause the target cell to release its own granule components, or to become 'deactivated', perhaps because important parts of the target cell membrane became covered with granule components. These effects would probably act at short range, as the eosinophil basic proteins would bind to surfaces, and cells immediately adjacent to them. Heparin is the only endogenous inhibitor of eosinophil granule protein functions so far described¹⁸⁵⁰, although others may exist, such as alpha 2-macroglobulin. They would also limit the range of action of these proteins. Eosinophil-derived reactive oxygen molecules could also have a very limited range of action. A summary of the possible actions of eosinophils on tissues is given in Figure A06-4.

Fig. 6-4: The interaction of eosinophils with other cells.

6.3.1. Interactions with blood vessels.

It is likely that eosinophils affect vascular permeability, causing oedema, since the formation of a transudate is a common feature of many eosinophilic disorders. There are no details of how this could occur, although it has been stated that MBP causes a wheal, and flare reaction when injected into human skin⁶⁷². More is known about how neutrophils affect vessel permeability to small molecules: most of their effector mechanisms appear to be involved, ranging from granule enzymes, and cationic proteins, to products of arachidonic acid metabolism, and products of oxygen metabolism

¹⁰⁷⁰.

1. Chapter B 01. Eosinophils in animals other than man.

Eosinophils in animals have been studied by many people since Ehrlich noted in 1879 that eosinophils were present in all the species he examined, including frogs, and birds⁸⁰¹. Research on eosinophils in different species is important for several reasons. It offers the opportunity to determine the properties, and roles of eosinophils *in vivo*, and to define the relationship between eosinophils, and other cell types, under controlled conditions. However there are species differences, which can cause difficulties if the findings in animals are to be related to man. For example rodent peritoneal eosinophils have a phagocytic capacity which is usually greater than human eosinophils. In some animals, including members of the cat family, hyracoids, rhinoceros, hyenas, okapi, and some birds, there is no eosinophil peroxidase, and eosinophils in hyenas do not stain with eosin¹⁸¹⁰. As eosinophil responses in animals are also often used in drug testing, as a measure of an allergic reaction, the pharmaceutical industry has built up an extensive literature on eosinophils in different drug regimes. At the same time, the normal values of blood eosinophil counts have been established. A database of eosinophil counts (and other baseline measurements) in the most commonly used laboratory animals has been set up by the American Cyanamid Company, New York. This was summarized in 1986¹⁹⁴⁹.

There is a great variety in the morphology of eosinophils in different vertebrates, both in the light

and electronmicroscope, and they can be difficult to distinguish from other blood cells by their capacity to bind eosin. When molecular probes become available for the unique constituents in eosinophils (ECP, EDN/EPX, and EPO), it will be of great interest to determine the phylogeny of eosinophils in animals.

1.1. Phylogeny of eosinophils.

All vertebrates appear to possess eosinophils. They have also been found in a number of cartilaginous fishes, and more primitive organisms. It is not known whether an equivalent cell they occurs in insects. There is no formal study on the phylogeny of eosinophils, although this is an interesting area, which could be helpful in defining the biological role of eosinophils in normal, and diseased organisms.

1.2. Eosinophils in amphibia, birds, and fishes.

Eosinophils are present in the frog, and triton, but their granules do not contain crystalloids^{948, 1190}. Eosinophil granules in chickens, and pigeons, do not have a crystalloid⁹⁴⁸, but Maxwell, in Roslyn, Scotland, has found crystalline cores in eosinophils from several other species of bird, including ducks^{1166, 1167}. In 1986, he described a method for inducing large numbers of eosinophils to accumulate in the peritoneal cavity of chickens, using bovine serum albumin¹¹⁶⁸, and he has studied the morphology, and enzyme content of avian eosinophils, including alterations in lipid bodies.

Eosinophils have been described in the blood, and tissues of a number of different fishes, including lower vertebrates such as the elasmobranchs. They have been in the nurse shark⁸³³, and torpedoes (*Torpedo marmorata* Risso, and *Torpedo ocellata* Rafinisque)⁷¹⁶. However, they are absent in hagfishes, which are Agnathan cyclostomes, and are direct descendants of the first fishes to emerge over four hundred million years ago. These are amongst the lowest living vertebrates, and have blood cells similar to neutrophils in higher organisms, but eosinophils, and basophils were not detected in one electronmicroscopy study, which suggests that eosinophils may have appeared at a later stage in evolution¹⁰⁸².

The majority of fishes have eosinophils without crystalloids in the specific granules. This was shown in a study reported in 1986 on eosinophils from the peritoneum of the striped bass, *Morone saxatilis*¹⁸⁴.

Eosinophil granules with crystalloids are present in the tench⁹⁴⁸, and a fresh water fish, the loach, *Misgurnus anguillicaudatus*⁸⁵⁰, in which eosinophils sometimes contain only one large granule, with separate small granules. One type of eosinophil granule in three teleosts (*Cyprinus carpio* L., *Tinca tinca* L., *Salmogairdneri* R.) has been shown to contain peroxidase, which varied in amount during development¹⁶⁹.

As the ultrastructure of eosinophil crystalloids in the loach was similar to man, it has been suggested that they might contain an analogous basic protein to human MBP⁸⁵⁰. The nurse shark, *Ginglymostoma cirratum*, also possesses eosinophils⁸³³.

There has been some recent work on the functional properties of fish eosinophils, which can be separated by differential adhesion, or density gradient separation¹¹⁴³. Eosinophils from the peritoneum of the striped bass, *Morone saxatilis* were found to have lamellipodia, and were phagocytic¹⁸⁴. Isolated eosinophils from the pronephros of the carp, *Cyprinus carpio* L, were also able to ingest bacteria, but unlike neutrophils in this species, the bacteria were not contained in endocytic vacuoles¹⁷⁶⁶.

1.3. Eosinophils in mammals other than man.

The majority of in vivo experimental studies on eosinophils have been carried out in inbred rodents, including mice, rats, and guinea pigs. Large animals, such as horses, cattle, and some primates have also been used when greater quantities of eosinophils were needed.

1.3.1. Buffalos, camels, cattle, goats, sheep, and swine (*Artiodactyla*).

In this group of animals, there are reports of blood eosinophil counts in calves⁵²³, and wild fallow deer in Australia⁵¹⁶. Blood eosinophil counts in the African buffalo (*Syncerus caffer caffer*), and in the red buffalo (*Syncerus caffer nanus*), were measured in 1985, and found to rise with age¹⁴³³.

Nematode infections in the yak, *Bos grunniens*, have been shown to produce an eosinophilia⁷⁷¹. Cattle have provided large numbers of eosinophils by a technique in which antigen primed cows had antigens injected into their nipples, which then gave an inflammatory exudate rich in eosinophils⁴⁷³. Bovine eosinophils can be purified by density gradient centrifugation²⁹⁸.

1.3.2. Cats, dogs, ferrets, mink, racoons (Carnivora).

The general features of eosinophils in dogs, and cats were reviewed in 1981²⁰⁰¹. Eosinophils in cats have some of the most elegant crystalloids in the animal kingdom^{1442, 2007}, although eosinophils in members of the cat family do not contain peroxidase^{1442, 1810}.

Eosinophils in dogs have been seen both in the blood, and in efferent lymph⁶²². They can be purified by density gradient centrifugation in Percoll²⁵⁸, and they can form rosettes with human erythrocytes, in the same way as T cells in this species¹⁹⁶⁷.

1.3.3. Rabbits (Lagomorpha).

Although the rabbit has proved an important experimental animal for the raising of antisera, it has proved less successful for the preparation, and study of the eosinophil, largely because heterophil granules in rabbits show some staining with eosin. Modern density gradient separation techniques have not been used to separate rabbit blood eosinophils, and this could be an interesting area for further work.

1.3.4. Kangaroos, opossums (Marsupials).

In a study on 112 opossums, although neutrophils were the commonest blood cell during the first few weeks of life, eosinophils increased rapidly so that they soon equalled, or exceeded the number of neutrophils. After this period, eosinophils remained a prominent, but smaller component of the blood³⁷⁵.

1.3.5. Horses (Perissodactyla).

In the 1960s horses were used as a major source of eosinophils, and their attractive large granules were thought to contain histamine. On the basis of this incorrect assumption, the role of eosinophils was thought to involve histamine-dependent processes. More recently, horses have been used in the preparation of large amounts of EPO. The ready availability of large numbers of eosinophils in individual horses still provides an important source for bulk separation of eosinophils, and their components.

In 1984, the blood eosinophil counts in 474 healthy horses at Newmarket, U.K., were assayed³². The counts were not normally distributed, but there was no difference in the blood eosinophil count in horses of different ages. The mean count was $0.2 \times 10^9/L$.

1.3.6. Primates.

The majority of studies with eosinophils in primates have been carried out on anthropoidea. Many reports on eosinophils in primates have been given for the macaque monkey, *Macaca mulatta*, and baboons (*Papio*). In 1982, eosinophil counts were reported for a colony of 104 captive golden lion tamarins (*Leontopithecus rosalia*) in the Smithsonian Institute, Washington D.C., U.S.A.²³⁶. In 1984, blood eosinophil counts in a captive group of 86 lesser mouse lemurs (*Microcebus murinus*) were reported as $0.54 \times 10^9/L$ ⁴³⁵.

1.3.7. Rodentia.

Rodents have proved to be the most valuable experimental animals for studies on eosinophils in vivo, and the availability of inbred strains of many of these species has been particularly important for providing reproducible animal models of eosinophilic disorders. The genetic basis of eosinophilia in some species of Rodentia has also begun to be explored.

1.3.7.1. Chinese hamsters, gerbils, hamsters (Cricetidae).

Eosinophil research has used these species only rarely, although there is a report in 1984 of the use of Chinese hamsters in the study of schistosomiasis³⁶⁴. The appearances of eosinophil in the bone marrow of the Mongolian gerbil were described in 1978¹⁸⁹⁴, and acid phosphatase in hamster eosinophils in 1980⁴⁹⁶.

1.3.7.2. Guinea pigs.

Large numbers of eosinophils can be induced to localize in the peritoneal exudate of guinea pigs after repeated lavage with saline, or saline containing a wide range of protein antigens^{1411,1081}. This technique provides 'tissue' eosinophils, which can also be obtained by bronchoalveolar lavage of sensitized animals. There is no published report on the preparation of blood eosinophils from guinea pigs.

1.3.7.3. Rats, and mice (Muridae).

Mice, and rats have been the most widely used experimental animals for studies on eosinophils, as they are available as inbred strains with reproducible, and defined characteristics. Many strains of mice have been used for studies on eosinophils, especially Balbc, C57Bl, and CBA mice. Mice which have been infected with *Mesocostoides corti* produce large numbers of eosinophils in the peritoneal cavity, and this provides an excellent source of eosinophils for experimental studies⁸⁸⁰. Eosinophils have also be obtained from mice with toxocariasis¹⁷²¹.

Many inbred strains of rats have been used for studies on eosinophils. Normal blood eosinophil counts in rats have been well defined¹⁹⁴⁸. A strain of August rat has been used extensively at the National Institute for Medical Research, London, as it has a spontaneously high level of eosinophils in its peritoneal cavity, which can be separated by density gradient centrifugation¹¹²⁴. Am-1(2)/Tor rats, which have been studied in Brazil, also have a spontaneously high number of these cells in peritoneal washings, and can be purified to over 90 per cent purity on metrizamide gradients¹⁴⁰⁷. The largest numbers of eosinophils are obtained from rats which have been induced to develop an eosinophilia. 2.5×10^8 eosinophils can be obtained from the peritoneum of a rat infected with *M. corti*, although the cells are in an activated state, with altered properties³⁵⁵. Rat, and mouse peritoneal eosinophils can be purified using discontinuous metrizamide gradients⁸⁷⁰.

1.4. Reptiles.

Eosinophils have been found in turtles, and lizards, but they do not contain a crystalloid internum⁹⁴⁸. A primitive reptile, the tuatara, *Sphenodon punctatus* (Gray), has eosinophils, but these do not have crystalloids⁴⁴⁶. The American alligator (*Alligator mississippiensis*) has blood eosinophil counts of about $0.6 \times 10^9/L$, which stain for alkaline phosphatase, and have some phagocytic capacity for bacteria in vitro¹¹⁶³.

1. Chapter C 01. Bacterial, fungal, and rickettsial infections.

1.1. Bacterial infections.

The majority of bacterial infections cause a rapid fall in blood eosinophil counts, and this has been used to help distinguish between allergic, and infectious causes of, for example, inflammatory lung disease. This decrease in blood eosinophil counts is not always associated with an absence of eosinophils in the inflammatory lesions of bacterial infections, suggesting that some of the fall in blood counts is due to the uptake of eosinophils into the lesions. However, bone marrow production of eosinophils is reduced during most bacterial infections. It is possible that eosinophils take part in bacterial infections, even though they may not be present in the blood. This possibility arises from the results of serial measurements which were made in 1978 of the serum concentrations of eosinophil granule-derived proteins in twelve patients with pneumonia, and twelve control subjects. Although patients with pneumonia had no circulating blood eosinophils, their serum ECP levels were raised on admission to hospital, and they only return to normal after thirty days. This supported the possibility that, although eosinophils are not found in the blood during an infection, they may continue to secrete their granule proteins in tissues¹⁸⁵². The occurrence of an eosinophilia with a bacterial infection is seen in certain immunodeficiencies in childhood, during drug hypersensitivity reactions, and occasionally as a response to the bacterial infection itself.

1.1.1. Actinomycetales infections.

An eosinophilia may occasionally occur in patients with pulmonary mycobacterial infections, includ-

ing *M. tuberculosis*⁵⁸⁰, atypical mycobacteria¹⁹⁵⁴, *C. pseudotuberculosis*⁹⁵⁶, and avian tuberculosis⁷⁵². More commonly, an eosinophilia in patients with tuberculosis is due to a drug reaction to antibiotics.

1.1.2. Enterobacteriaceae infections.

There are only a small number of reports of an eosinophilia occurring in patients with bacterial infections of the gut, including bacillary dysentery and salmonella infections, emphasizing the importance of looking for parasites in patients who have a predominantly diarrhoeal illness.

1.1.3. Staphylococcal, and streptococcal infections.

Staphylococcal infections are not normally associated with an eosinophilia, although there are a few reports of this unusual association, and although there were a number of earlier reports of an eosinophilia in patients with chronic streptococcal infections, and during the recovery phase from scarlet fever, there are few reports of this today. This may be related to the reduced incidence in developed countries of some types of streptococcal infection, such as rheumatic fever, and subacute bacterial endocarditis. However, a patient with a streptococcal sore throat, and a blood eosinophil count of $56.3 \times 10^9/L$ was reported from Athens, in 1980⁴⁹⁴.

1.1.4. Chlamydial infections.

A mild increase in blood eosinophil counts of $0.3 \times 10^9/L$, or more, is a feature of chlamydial pneumonia in infants. In 1982, 115 babies with this disease were reviewed in Berne, Switzerland¹⁵⁶³. The infection is acquired during birth, and becomes manifested during the first three months as a respiratory infection, with conjunctivitis, and serous otitis.

1.2. Mycoses.

Fungal infections are not normally associated with an eosinophilia, except when they induce a hypersensitivity reaction. The components of fungi which produce this type of response have not yet been defined, although this could be studied, as fungal antigens produce an eosinophilia after injection into the peritoneum of mice³⁰⁵. Occasionally, fungal infections in the skin can produce an eosinophilic pustular folliculitis¹⁰¹⁶.

1.2.1. Aspergillosis, and fungal lung diseases.

Aspergillosis is a well known cause of an eosinophilia, both as a primary response to the fungus, and as a hypersensitivity reaction to fungal antigen in the respiratory tract of patients with asthma. The eosinophil response is most marked when a hypersensitivity reaction develops to antigens released from persisting fungal mycelia. This can occur in the absence of tissue invasion by the fungus. In bronchopulmonary aspergillosis a hypersensitivity response develops to this organism, which produces an eosinophilia. A good review of this disease was published by R.G. Slavin in 1985¹⁶⁴⁴. It was first described in 1952 in the U.K., and in the U.S.A. in 1968. The American experience was reviewed in 1985¹⁶⁰. Other reports have come from Australia, where 42 patients were described in Perth in 1981. Eleven of these patients did not have asthma, although they had blood eosinophil counts between 0.7 and $4.5 \times 10^9/L$. As three patients had other fungi in their lungs, it was suggested that the term 'allergic bronchopulmonary fungal disease' was a better name for the disease⁶⁴⁸. Ten other patients were described in Concord, Australia, in 1984²⁰³.

There are also other patients with chronic asthma, and an eosinophilia, who have fungi in their sputum. In these cases, it is difficult to know whether the fungus, or the asthmatic process itself is responsible for the eosinophilia. The syndrome of allergic bronchopulmonary aspergillosis is characterized by episodes of fever, cough, purulent sputum, brown sputum plugs, and antibodies to aspergillus antigens. The basis of the disease is believed to be an immunological response to fungal antigens which give rises to immediate hypersensitivity reactions, immune complex damage, and delayed hypersensitivity lesions. It is important to diagnose, and treat this disease before irreversible changes have occurred, which can be progressive, without steroid treatment¹²⁰⁸, or even fatal⁷¹³. Occasionally other fungi, including *Curvularia lunata*⁷⁴⁴ can cause a similar syndrome.

Hypereosinophilic mucoid impaction is a disorder which is most commonly seen in allergic bronchopulmonary aspergillosis, but can occur in bronchocentric granulomatosis. It is characterized by

bronchial mucus plugs filled with degenerating eosinophils, and is distinct from the mucus plugs seen in asthma⁸⁷³. It usually occurs in adults, but there are several case reports in young children, including one studied at post mortem³¹⁸.

1.2.2. Coccidioidomycosis.

A peripheral blood eosinophilia occurs in as many as 88 per cent of patients with primary coccidioid infections⁴⁶⁷. Although eosinophils are not seen in the granulomas at sites of tissue invasion by this fungus, eosinophils are prominent in the tissue lesions when hypersensitivity reactions have developed to fungal antigens⁴⁹⁰. Two patients with a pulmonary eosinophilia due to coccidioid infections were reported from Stanford, U.S.A., in 1987¹⁰⁹⁶, which emphasized the difficulties in distinguishing this disease from cryptogenic pulmonary eosinophilia, and suggested that skin testing, and serological studies were important investigations to carry out in areas where *Coccidioides immitis* is endemic.

1.2.3. Histoplasmosis.

Histoplasmosis produces an eosinophilia in man. A clear account of this was given in a patient reported in 1979²³⁰. This was a man aged 21, who had a maximal eosinophil count of $1 \times 10^9/L$, 18 days after his illness started, with many vacuolated, and degranulated eosinophils

1.3. Rickettsial infections.

Although rickettsial infections are uncommon, they may be spreading in certain parts of western Europe, and the east coast of the United States, and an eosinophilia has been described in a number of these patients.

1.4. Yeast infections, cryptococcosis.

Cryptococcal infections of the brain can give rise to an eosinophilia in the cerebrospinal fluid¹²⁵⁰.

2. Chapter C 02. Viral diseases.

Viral diseases are not normally associated with an eosinophilia, although they can occur occasionally when the respiratory tract is involved. It is not known why virus infections do not give rise to an eosinophilia, especially as T lymphocyte-dependent responses are a normal component of the immune response to viruses.

Herpes viruses can occasionally give rise to an eosinophilia. There are also a few reports of viral hepatitis associated with an eosinophilia.

There are only a handful of reports of an eosinophilia occurring in patients with RNA virus infections. A 13 month-old boy with a silicon elastomere ventricular peritoneal shunt for porencephaly, developed a coxsackie B4 meningitis, and was found to have 45 eosinophils/ul in his cerebrospinal fluid. This disappeared when the infection resolved³¹³. The only previous reports of a viral infection associated with eosinophils in the cerebrospinal fluid were in patients with chronic lymphocytic choriomeningitis meningitis.

Two patients with a marked eosinophilia have been noted among the patients with AIDS looked after at St. George's Hospital Medical School, London (Lambert, 1987, personal communication). This may be related to the capacity of HTLV-I to induce T cells to produce an eosinophil growth, and maturation factor in vitro¹⁵⁴³. In Houston, U.S.A., bone marrow samples from eight men with AIDS showed 4.5 per cent eosinophils, compared to 2.7 per cent in controls⁸¹⁹. A patient with AIDS, and an eosinophilic temporal arteritis was described in 1986¹⁵⁷⁹.

3. Chapter C 03. Parasitic diseases.

Eosinophils are a striking feature of many parasitic diseases. As helminthic infections are so common, it is an essential part of the clinical assessment of patients with an eosinophilia, to look for

parasites in the gut. In countries where parasitic infections are endemic, many asymptomatic patients have eosinophil counts of over $1 \times 10^9/L$. The study of Indochinese refugees from the Vietnam wars, has provided a modern assessment of the parasite burden present in this part of South East Asia. Seventeen species of parasites were reported among 353 refugees examined in Japan in 1984¹⁹⁶⁴. Similar findings were reported in 128 refugees with an eosinophilia, who were studied in Bethesda, U.S.A., in 1987¹²⁹¹.

Helminthic infections are the commonest parasitic diseases which produce an eosinophilia, although ecto-parasitic diseases, and protozoan infections can also induce an eosinophilia. These diseases are largely confined to tropical, and sub-tropical developing countries, but parasitic diseases are also common in visitors to these areas. In a retrospective study of 119 patients with an eosinophilia after travel abroad from London, U.K., a parasite infection was diagnosed in 39 per cent, and the eosinophilia disappeared after appropriate chemotherapy. In 66 patients, in whom no parasitic infection was demonstrated, and who had mean blood eosinophil counts of $1.8 \times 10^9/L$, (which was not significantly different from the patients who had a demonstrable parasitic infection), the eosinophilia either resolved without therapy, or responded to empirical treatment with thiabendazole, or mebendazole⁷⁵⁹.

3.1. Helminthiasis.

These are the commonest cause of an eosinophilia throughout the world. It is unusual for patients with helminthiasis not to have an eosinophilia, but eosinophil counts can vary considerably during the course of the infection, with the highest counts occurring during the early invasive stage of the parasite life cycle, with waxing, and waning of the counts during the subsequent weeks, and months. Successful treatment of helminthic infection can produce a rapid rise in blood eosinophil counts, probably stimulated by the release of antigens from dead, and dying parasites. A summary of the main parasitic diseases to consider in people with an eosinophilia is shown in Figure C03-1.

Fig. 3-1: Parasitic diseases.

The question of why helminths induce an eosinophilia has intrigued students for many years. In Paul Beeson's group in Oxford, U.K. we showed that intact *T. spiralis* infective larvae had to be present within the vascular system for several hours, or days, in order to produce an eosinophilia. Larval extracts were ineffective, and larvae which were not injected into an artery, or vein were less effective⁷⁵⁹. This suggests that an endothelial cell product (possibly GM-CSF) may be at least partly responsible for the development of a marked eosinophilia to this parasite. This could also explain why parasites which survive, and migrate within blood vessel, are such potent inducers of an eosinophilia. Recirculating T lymphocytes were a key component in the development of the eosinophilia¹³⁶. This may be because T lymphocytes can produce IL-5¹¹⁰¹. Now that so much has been learnt about the nature of eosinopoietic molecules in rodents, and man, it would be interesting to reexamine these classical experiments, with these possibilities in mind.

3.1.1. Cestode infections.

Cestode infections give rise to an eosinophilia less commonly than nematode, or trematode infections. The reason for this could be due to the points discussed above. The principal organisms are *Taenia*, *Hymenolepis*, *Dipylidium*, *Diphyllobothrium*, and *Spirometra*²⁶³, and *Echinococcus*.

3.1.1.1. Echinococcosis.

An eosinophilia is found in about half of patients with hydatid disease, and it is most common in patients with hepatic, and/or pulmonary involvement.

3.1.1.2. Taeniasis, and cysticercosis.

Taenia infections are rarely associated with a marked eosinophilia, possibly because of the mild nature of the infection. In a study from Poznan, East Germany, in 1985, only 36 per cent of 296 patients with *T. saginata* infections had an eosinophilia. Eleven per cent had an eosinophil count greater than $1 \times 10^9/L$ ⁹⁸⁵. However, the eggs of *T. solium*, but not *T. saginata*, can develop to the larval stage in man, and produce cysticercosis, in which there is often lodgement of larvae in the brain. This usually presents with epilepsy, but it does not often give rise to an eosinophilia in the

cerebrospinal fluid, possibly because it is diagnosed late in the course of the disease¹⁸⁴⁶. In 1981, histological studies on a multi-lobulated mass of *Taenia* larvae in the brain of a 30-year old woman showed a large number of eosinophils adjacent to the parasite. Previous reports of this rare disease were reviewed, and it was noted that an eosinophilia is very variable, and may only follow surgical intervention, and the release of antigenic material from the parasites²⁸.

3.1.2. Nematode infections.

Nematode infections account for the majority of patients with an eosinophilia in tropical countries, especially in areas where filariasis is endemic. In many of these patients the infection is subclinical, and it is routine practice for patients in areas of endemic filarial, ascaris, and hookworm infections to take anti-parasite treatment once, or twice a year. In western countries, nematode infections are often asymptomatic, coming to light only when a blood count is carried out.

3.1.2.1. Angiostrongyliasis.

There are two main diseases caused by the *Angiostrongylus* spp. These are caused by *A. costaricensis*, and *A. cantonensis*. *A. costaricensis* infections are seen in the West Indies, and parts of South America, where they produce abdominal complications, such as pain, appendicitis, and bowel wall lesions requiring surgical resection¹¹⁰⁶. *A. cantonensis* infections are common in many parts of the Pacific basin, and South-East Asia, and are most noted for central nervous system involvement, meningitis with a marked eosinophil infiltrate into the cerebral spinal fluid, cranial nerve lesions, and a variety of focal signs.

Angiostrongylus cantonensis is the rat lung-worm. It is non-permissive in man, but migrates to the brain, where the young adult worm dies. During this time there is a marked eosinophilia in the brain, the meninges, cerebrospinal fluid, and in the blood, where eosinophil counts reach a peak, two to three weeks after infection.

Experiments have shown that mice, which are also a non-permissive host for *A. cantonensis*, have many features which make them a useful animal for studying the immunology of this infection¹⁷²³. The number of reports of eosinophilic meningitis are increasing in South East Asia, as it becomes more readily diagnosed, even when the parasite is not isolated. Details of 125 patients in southern Taiwan with eosinophilic meningitis, or meningoencephalitis were published in 1976. Most of these patients had a mild, or moderately severe illness, but three had permanent brain damage, and four died¹⁹⁷⁴.

The disease is established in Samoa. Three New Zealand children who had visited Western Samoa, and developed severe infections were reported in 1984¹⁴⁸³, and a particularly severe outbreak occurred in 16 Korean fishermen in American Samoa in 1982, when three became comatose, and one died⁹⁸⁰.

In Thailand, cases of this disease have occurred after eating uncooked *Pila* snails, which are a local delicacy. It produces eosinophilic meningitis, which is usually a relatively benign disease, with a mortality of less than 1 per cent. Most patients present with headache, with, or without signs of meningeal irritation. There may be blurred vision, with swelling of the optic disc. The cerebrospinal fluid looks like coconut juice because it contains 0.5 to 5 x 10⁹/L leukocytes, 70 per cent, or more of which are eosinophils¹⁸⁴⁶. Over a three year period up to 1968, 646 patients were seen with eosinophilic meningitis in one centre in Thailand¹⁴⁵⁹. The post mortem findings in three patients were reported in 1978¹⁶⁶³. These showed the tracks, and cavities left by migrating worms in the brain, and spinal cord.

3.1.2.2. Ascariasis.

Ascaris infections produce a higher blood eosinophil count in children than in adults, in whom infections are often asymptomatic, and associated with a mild or moderate eosinophilia, although a heavy infection can cause a serious illness.

Visceral larva migrans, which is due to the invasion of tissues with *ascaris* larvae gives rise to a moderately severe illness in childhood, associated with lung involvement, and systemic features linked to the migration of larvae into different tissues. It has a low mortality, and patients recover

over a few weeks, and can develop very high blood eosinophil counts. An ascarid of racoons, *Baylisascaris procyonis*, can produce visceral larva migrans, and a severe, or even fatal eosinophilic meningoencephalitis in children⁵⁹⁰.

Toxocara canis infection in man were identified as due to the infective, or second stage larvae of this nematode in 1952 by Beaver, and colleagues¹⁴³. About 5 per cent of children in New York, U.S.A., have antibodies to *T. canis*, and there is some evidence that seropositive children have lower scores than controls, in neuropsychological tests¹¹⁵⁶.

The eggs from female adult worms in the intestines of puppies, and pregnant bitches, are deposited in soil, where they are ingested by young children who play in the same areas as the dogs defaecate. The eggs then hatch into larvae in the stomach, and invade the tissues, where they become surrounded by a marked inflammatory reaction. Specific IgG, and IgE antibodies develop at this stage. Toxocariasis demonstrates a striking difference between childhood, and adult infections, in that an eosinophilia is most commonly seen in patients below the age of nine²⁰⁰⁰. In these children, who may have a marked eosinophilia¹⁶⁸⁶, the larvae are rapidly destroyed, although a few can persist in tissues where they usually cause little damage. In a controlled study on the use of thiabendazole in a group of Hispanic children with toxocariasis, reported in the U.S.A. in 1987, it had no effect on blood eosinophil counts, or antibody levels¹³².

In older children, and adults, migratory larvae can lodge in the eye, where they may cause a localized inflammatory reaction, with gross appearances similar to those of a retinoblastoma. The eye damage is possibly partly the result of a toxic effect of eosinophils on the retina, while they take part in the protective inflammatory reaction against the parasite¹⁵⁰⁸. *Toxocara* infections of the eye are best diagnosed by measuring serum antibody levels to secretory antigens from cultured *toxocara* larvae. If necessary, the aqueous humour can be aspirated to look for eosinophils¹⁶¹⁴.

Ocular toxocariasis can occasionally give rise to a marked eosinophilia. One example was in a 19 year-old man, who presented with a blood eosinophil count of $3.72 \times 10^9/L$. Interestingly, a friend who appeared to have contracted this disease at the same time, did not have an eosinophilia⁹⁷⁰. There are also occasional reports of a marked eosinophilia in older patients with toxocariasis. An example of this, in a 23 year-old woman, was published in 1987¹²⁴⁴.

Eosinophil-dependent killing mechanisms are probably less important than other components of the immune response in immunity to this parasite in man. This is surprising, as infected children can produce very high eosinophil counts. We found that although blood eosinophils were able to attach to antibody and/or complement coated infective larvae in vitro, and degranulate onto their surface, there was no evidence for parasite damage⁵⁴². However, the parasites are destroyed after several weeks residence in human tissues.

T. canis larvae can activate the alternative complement pathway directly, and fix C3 to their surface. Surface bound antibody can also cause the binding of guinea pig peritoneal eosinophils, which attach maximally in the presence of autologous IgG1, IgG2, and possibly IgE isotypes⁹¹.

3.1.2.3. Filariasis.

Filarial infections in man, and animals invariably induce a marked eosinophilia, which can produce some of the highest blood eosinophil counts found in any infectious disease. The high counts in *Loa loa* infections, especially in visitors to endemic areas, have been commented on many times. The reason for this marked host response is not known, but it may be related to the close spatial relationship that the adult parasite has with lymphocytes, and endothelial cells, in lymphatics, and lymph nodes. The continued production of microfilariae, some of which are destroyed in capillaries in the lung, and other tissues, probably accounts for the persistence of the marked eosinophilia in untreated patients.

Biopsy specimens from 64 patients with filariasis, acquired while American troops were stationed in the Far East, have provided detailed histological information about the principal features of filarial infection in man. In patients with lymphadenitis, lymph nodes often contained many eosinophils in distended sinuses, lymphoid cords, and the connective tissue around the hilum. However, eosinophils

were most prominent near adult worms, where they were the most conspicuous cell type. Eosinophils were also found in hydrocele fluid³⁴⁹.

Tropical (filarial) eosinophilia, which is often called tropical pulmonary eosinophilia (TPE), is a disease in which an immune response to microfilaria cause them to be sequestered in the lungs, where they are destroyed in multiple small inflammatory foci. These induce a marked blood eosinophilia, with absent microfilaria in the blood. The disease was reviewed by Neva, and Ottesen in 1978¹²⁷³, and by us in 1982¹⁶⁹⁵. Bronchoalveolar lavage has been done in TPE. This has shown large numbers of eosinophils in the lower airways, which return to normal after treatment¹⁴²².

As filaria of animal origin are often introduced by biting insects, into people living in areas of endemic filarial infection, it has been suggested that tropical eosinophilia may be the result of a marked host response due to these nonpatent infections. This was supported by experiments in Malaysia, which showed that sera from patients with tropical eosinophilia (but not elephantiasis) were able to induce blood cells to attach to the surface of the third stage larvae of *Brugia pahangi*, and *Breinlia booliati*, which do not give rise to patent infections in man¹⁶³³.

TPE was only recognized as being due to a filarial infection in the late 1950s, because of the difficulty in detecting microfilaria in the blood⁴⁵⁹. It is usually recognized by the asthmatic, and coughing attacks that it produces, and the rapid response to treatment with diethylcarbamazine (DEC). Chest radiographs can show a variety of abnormalities, including diffuse mottling, pleural effusions, cavitation, and even large areas of consolidation⁸⁶². There appears to be no relationship between the time of day when symptoms are worst, and blood eosinophil counts, which are usually highest at 2 am in these patients¹⁶³⁷.

Sometimes the respiratory symptoms are not prominent, and the marked eosinophilia is the main sign that the patient has this form of filarial infection. It is then often called tropical eosinophilia. This is commonest in male patients. It can occur in patients who have left filarial endemic regions several years previously. I have studied seven patients in London, and reported in 1981 the presence of prominent vacuoles in their blood eosinophils, an increase in their capacity to bind complexed IgG, and their response to treatment with DEC, including a fall in serum ECP levels¹⁶⁸².

Subsequent work has centered on the importance of IgE, and IgG subclasses, especially IgG4¹³³⁹ in the disease⁸³¹. In 1987, clinical details were published of a series of 50 patients with tropical pulmonary eosinophilia in the Army in Kerala, South India, where the disease is hyperendemic. The commonest symptom was a nocturnal cough, in 72 per cent⁶⁹⁸.

In a study from India on patients with TPE, as many as 63 per cent of the blood eosinophils were found to contain vacuoles. The greatest number of vacuolated cells was found in midday blood samples, and the lowest number at midnight¹⁵⁵⁷. The importance of this, is that it suggests that eosinophils are releasing their constituents into the circulation of these patients.

In 1981, serum MBP, and CLC protein concentrations were reported in 450 patients in a Pacific island where Bancroftian filariasis was endemic, to see whether the extent of eosinophil degranulation could be assessed before, and during treatment with DEC. Following treatment, it was found that MBP levels increased as the eosinophil counts rose, so that they were still high at three weeks. Serum CLC levels rose at 14 days, and returned to normal at 21 days. Pretreatment serum MBP, and CLC protein levels did not correlate with microfilaraemia, but serum MBP, and CLC levels correlated with eosinophil counts. These findings suggested that eosinophil constituents were released following treatment, but that their levels were not useful in assessing the worm burden⁶.

It has been suggested that localized, and dangerous systemic reactions produced by the treatment of filariasis with diethylcarbamazine (the Mazzotti reaction), may be mediated by the release of toxic granule proteins from eosinophils attached to dying microfilariae. This has been reviewed in 1987 by Ottesen¹³³⁸.

Dirofilarial infections are common in many parts of Africa, but rarely give rise to disease, or a marked eosinophilia. However, a number of patients have been found to respond to treatment with a fall in blood eosinophil counts, so it seems certain that Dirofilarial infections can cause a moderate

eosinophilia, especially in Europeans returning from tropical countries.

Loa loa infections, like many filarial infections, are mainly found in rural areas in tropical countries, where there are few public health facilities. The migrating worms gives rise to a marked tissue eosinophilia which seems unable to destroy the parasites, even though many eosinophils are found close to them. The main features in endemic regions are episodic angioedema, especially in the backs of the hands, the presence of migrating adult worms around the eyes, lymphadenopathy, microfilaraemia, and a moderate, or marked increase in blood eosinophil counts. The lesions in lymph nodes in tissues affected by Loa Loa have been shown to be distinct from those produced by other filarial infections¹³⁵³.

Loiasis is another cause of a marked eosinophilia in Caucasians returning from visits to Africa. There were two reports of this in 1986. In ten patients seen at the Hospital for Tropical Diseases, London, between 1978-84, the mean blood eosinophil count was $6.4 \times 10^9/L$, and it responded to treatment with DEC⁷⁵⁹. In 20 with loiasis who were reported from N.I.H., Bethesda, U.S.A., in 1986¹²⁹⁰, blood eosinophil counts were as high as $3 \times 10^9/L$, and most of them had developed a vigorous immunological response to the infection, so that only three had a microfilaraemia.

Onchocerciasis, which gives rise to a dermatitis, subcutaneous nodules, sclerosing lymphadenitis, and eye lesions, including punctate keratitis, is an important cause of a moderate, or high blood eosinophil count in many tropical countries. Much of the pathology of the disease is the result of the host's response to dead microfilariae³⁴⁸. Patients with large numbers of living microfilariae have the least tissue injury, although this can become severe when the microfilariae are killed by chemotherapy¹¹²⁵.

Eosinophils play an important role in these tissue lesions, as they are induced to degranulate, releasing several eosinophil granule components. Following treatment with DEC, damage occurs in lymph nodes¹⁴⁶⁶, and the skin⁹⁵¹, where dense deposits of MBP have been seen around dead, or dying microfilariae. Some MBP was also seen in areas where there were few eosinophils, suggesting that eosinophils could have effects in sites where few eosinophils are seen by conventional histological techniques.

Patients may develop a systemic reaction, the Mazzotti reaction, soon after treatment starts, and it has also been suggested that this may be due to the release of eosinophil granule components into the blood as a result of stimulation by parasite products released from dead, or dying organisms^{727, 831}.

3.1.2.4. Hookworm infections.

Hookworm infections which are common in both tropical, and subtropical regions are usually found associated with other parasitic infections of the gut. The eosinophilia may be related to the worm burden, and to the extent of damage to the duodenum. The blood eosinophil count is usually lower in patients with profound anaemia than in patients who have been treated, but counts can rise as high as $50 \times 10^9/L$ ³⁷².

Ancylostomiasis is readily diagnosed by examining the stools, and successful treatment results in the return of blood eosinophil counts to normal. Hookworm is one of the main causes of an eosinophilia in patients returning to Britain from tropical countries. In nine patients reported in 1986, the mean blood eosinophil count was $2.3 \times 10^9/L$ ⁷⁵⁹, but counts as high as $20 \times 10^9/L$ have been reported⁹⁸⁴. In an experimental study on two volunteers in 1986, in Western Australia, who were infected percutaneously with 1 200 larvae of *Ancylostoma ceylanicum*, an eosinophilia was first detected at four weeks, and eggs appeared in the faeces at five weeks²⁸³. Prior immunity is unlikely to affect the eosinophil counts, as there was no difference in the blood eosinophil counts in a dog model of ancylostomiasis, in animals which had been partially protected from a second infection, by the presence of an earlier infection²⁸².

The invasive stage of hookworm infections is associated with a systemic disorder in which there may be a marked eosinophilia, itching, and respiratory symptoms, similar to that found with visceral larva migrans due to *Ascaris* invasion. Cutaneous larva migrans is usually caused by *A. braziliense*, the

dog, and cat hookworm. This was reviewed in 1985²⁴⁰.

Necator americanus produces clinical features very similar to Ancylostomiasis. It responds equally well to treatment, once it has been diagnosed from stool microscopy. A course of mebendazole, 200 mg daily for two days, is often sufficient to treat this infection. Details of an experimental study on necatoriasis in volunteers was published in 1986¹⁹²⁵. The main effect of the infection was to produce a rise in blood eosinophils, with little effect on neutrophils. There is some preliminary work on the interaction of human blood eosinophils with the infective larvae of *N. americanus*⁴³⁶.

The course of an experimental infection of five American volunteers with a small number of *Necator americanus* third stage larvae was studied at N.I.H. Bethesda, and reported in 1987. After being infected in the skin with 50 larvae, they developed a marked eosinophilia, which was maximal at the seventh week, with mean counts of $3.5 \times 10^9/L$. At this time egg laying was occurring in the intestinal tract, and this was associated with abdominal symptoms. Only small changes in other immunological measurements, including T cell responses to larval antigens, were seen. Total, and specific serum immunoglobulins, including IgE, bronchoalveolar washings at 8-21 days, when larval migration was taking place, and biopsies of the skin sites of larval penetration at day five, showed no alterations from normal. A surprising feature of this primary infection was the massive eosinophil response, and the absence of significant changes in other measurements of the host response to this infection¹¹⁶⁵.

3.1.2.5. Capillaria infections.

Capillaria infections, which occur in carnivores such as cats, and dogs, can give rise to a marked eosinophilia in man, and this is one of the causes of a massive infiltration of eosinophils into the liver¹³⁴¹, and pulmonary eosinophilia²³.

3.1.2.6. Trichinosis.

Trichinosis is an underdiagnosed parasitic disease, largely because the persisting tissue form of the parasite can be asymptomatic following the initial disease process. Less than 200 cases are reported each year in the U.S.A. Although the chronic stage of the disease in mildly infected people is of little clinical importance, a systemic illness, with widespread painful lesions can occur, when a large number of larvae have been ingested. An outbreak of the latter type occurred outside Paris, France in 1979, affecting 125 people who had eaten contaminated horse meat¹⁹⁷, and 29 people in Alaska were infected in 1975, from eating contaminated walrus meat¹¹⁵². Occasionally, trichinosis can produce a hypersensitivity response, giving a necrotizing vasculitis, which can affect the brain¹⁵³⁵.

As the encapsulated worms can only encyst successfully in skeletal muscle, migratory larvae are destroyed when they settle in other tissues. Histology of the lesions in a fatal infection in a 46 year old woman showed extensive ventricular endocardial damage with superimposed thrombi. It was suggested that eosinophils may have been partly responsible for the endocardial damage⁴⁰, although dying parasites could also have injured adjacent heart cells. Interestingly, only 20 per cent of patients with fatal trichinosis have a blood eosinophilia, although it is one of the most useful investigations, with serum creatine phosphokinase, and *Trichinella* antibody measurements, in making the diagnosis in less severe infections¹⁶³⁴.

The introduction of compulsory methods for controlling trichinosis in animals for human consumption, has been successful in many parts of the world in reducing the spread of the disease to man. Individual outbreaks in temperate climates are usually linked to a failure to boil pig swill, or to cook infected meat adequately.

3.1.2.7. Trichuris infections.

Infections with *Trichuris trichiura*, which is limited to the colon, produce a moderate, or rarely a high blood eosinophil count. Results of blood eosinophil counts, and serum IgE levels were reported in 21 boys in an institution in Sao Paulo, Brazil, in 1982¹⁵¹⁵. The mean eosinophil count was 0.58 (range $0.14-1.58$) $\times 10^9/L$.

3.1.2.8. Strongyloidiasis.

Strongyloidiasis is another important parasitic infection to consider in patients in temperate, or

subtropical climates who have a marked eosinophilia, especially when stool examinations are normal. Failure to make the diagnosis can lead to an incorrect diagnosis of HES, and if the patient is then treated with steroids, the parasite may become disseminated²⁰¹⁹. It may be difficult to diagnose this parasitic infection on clinical criteria alone, in areas where other intestinal parasites are common. This was clearly shown in a study on 88 patients with strongyloidiasis in Brazil⁴¹⁵. As the infection can persist for over 20 years in patients who have left endemic areas, it is important to look for it in former servicemen, and others who have lived in endemic areas⁶³⁴.

Diagnosis is best carried out from duodenal aspirates, and biopsies, and this should always be done in patients who are to be treated with immunosuppressive drugs who might have contracted this infection. Strongyloidiasis in otherwise healthy people produces only a moderate eosinophilia: mean $1.0 \times 10^9/L$ in one group of six patients⁷⁵⁹. In another group of 152 patients, the mean percentage of eosinophils in the blood was 10 per cent, and serum IgE 2944 IU/ml¹¹⁸⁵. Occasionally, strongyloidiasis gives rise to appendicitis, as described in 1986 in a 47 year-old man in the U.S.A. Histology showed a marked eosinophilic inflammatory reaction, containing Charcot-Leyden crystals within epithelioid cell granulomas, and parasite remnants in the appendix¹⁵⁹⁵.

It has been suggested that eosinophils are involved in protection against disseminated strongyloides infections, as blood eosinophil counts were lower in four hyperinfected, immunosuppressed patients, than in five others who had severe infections without hyperinfection⁶³³.

3.1.2.9. Trichostrongyloidiasis.

Infections with this group of parasites is not very common, although it can give rise to a marked eosinophilia. Infections with *Trichuris trichuria* produce a moderate eosinophilia. In seven Caucasian patients seen in London, the mean blood eosinophil count was $1.4 \times 10^9/L$ ⁷⁵⁹.

3.1.2.10. Gnathostomiasis.

Gnathostoma spinigerum is a nematode, which infects dogs, and cats, and rarely man, to produce a severe, and even fatal disease. It was first described in the stomach of a tiger in the London zoo. Most human reports have come from Thailand, and other parts of South East Asia⁹²³. It was recognized in South America in 1984. The usual clinical presentation is with severe pain in the limbs, or trunk, due to irritation of nerve roots by the migratory larvae. This may progress to urinary retention, and paraplegia. The brain stem can be involved, and when this happens, the disease is usually fatal. Occasionally the presentation is with subarachnoid haemorrhage¹⁸⁴⁶. The parasite is acquired by eating poorly cooked intermediate hosts for the parasite, including eels, frogs, fishes, and snakes. The parasite migrates to many parts of the body, and produces two main clinical problems: recurrent skin swellings, and cerebral lesions, especially intracranial haemorrhage, and eosinophilic meningoencephalitis. It usually gives rise to a marked eosinophilia during the migratory phase, but the eosinophil count can be normal in some patients¹⁸⁹.

3.1.2.11. Enterobiasis.

Infections with *Enterobius vermicularis* (the pin worm, or thread worm) cause few clinical problems other than pruritus ani in children, unless they invade the tissues, where they can give rise to salpingitis, or acute appendicitis¹²²⁹, with an eosinophilia.

3.1.3. Trematode infections.

Of the several forms of trematode infections which give rise to an eosinophilia in man, two are of particular interest: fascioliasis, and schistosomiasis. These infections occur in temperate, and tropical countries respectively. Work on the role of eosinophils in the killing of the schistosome of *S. mansoni* has provided a great deal of insight into how eosinophils may function in parasitic infections. Although initial work suggested that they were important mediators of protection in both infections, this appears to be limited to certain stages in their life cycle, and they are only one of a number of effector mechanisms which partly controls these infections, and prevent reinfection.

3.1.3.1. Fascioliasis.

Fasciola hepatica is an important veterinary problem, and can occasionally occur in man where it is often misdiagnosed, leading to unnecessary investigations, and surgery on the biliary tree. An eosi-

nophilia, and alterations in liver function are the commonest findings. In 1984, a report was published from Puerto Rico of the value of a variety of diagnostic approaches, including radionuclide imaging, in making the diagnosis¹⁴⁹⁵.

3.1.3.2. Schistosomiasis.

The widespread occurrence of schistosomiasis mansoni, haematobium, and japonicum accounts for the common finding of schistosomal infections, both in endemic areas, and in people who have returned from these areas to temperate climates, when it is nearly always associated with an eosinophilia⁷⁵⁸. The eosinophil count may be remarkably high, especially after the parasite has first entered the skin (the Katayama syndrome)¹²³⁰, during diarrhoea produced by *S. mansoni*, when it can reach $18 \times 10^9/L$ ⁹⁸⁴, and when egg formation continues at a high rate.

The epidermal skin reaction induced by the cercariae of *S. mansoni* has been studied in Kenya in captured baboons with schistosomiasis. In animals which had been infected for eight months (but not less), many eosinophils surrounded schistosomula which were trapped, and killed in the epidermis. Eosinophil-rich abscesses were also seen in the deeper dermis, but here many schistosomula appeared to have escaped injury, and were not surrounded by eosinophils. This study suggests that in primates with several months infection, the epidermal reaction involving eosinophils may be an important part of the protective response to further infection with *S. mansoni*¹⁵⁸⁷.

As eosinophils can kill schistosomula in vitro, a search has been carried out to see whether this mechanism might be protective in patients with schistosomiasis mansoni. A group of 119 children were studied in Kenya, and some were found to have partial protection against reinfection following a course of treatment. The resistant children were two years older than susceptible children, but there was no difference in their blood eosinophil counts. Although differences were found in the capacity of blood mononuclear cells from different children to produce EAF, this did not correlate with their susceptibility to reinfection²⁵¹. There was no evidence that the possession of high-titre antibody to *S. mansoni* conferred resistance²⁵⁰. However, the possession of antibodies to egg antigens appeared to increase their susceptibility to reinfection, possibly by acting as 'blocking antibodies', which have been found to inhibit eosinophil-dependent killing of schistosomula in vitro. Three IgM monoclonal antibodies which bound to an egg antigen, and the surface of schistosomula, reduced the eosinophil killing in the presence of human infection serum⁴⁷⁶.

A research programme is also underway in the Gambia to study the immune response to *S. haematobium*. One interesting finding, which was not seen in East Africans with *S. mansoni* infections, was that blood eosinophil counts correlated with resistance to reinfection, although eosinophil induced antibody-, or complement-dependent killing of *S. haematobium* was barely detectable⁷³¹.

3.2. Protozoan infections.

Protozoan infections seldom give rise to an eosinophilia during an uncomplicated infection, although there are occasional case reports which show that this group of parasites can induce an eosinophilia. Amoebiasis due to *Entamoeba* is only rarely associated with an eosinophilia.

Amoebic meningitis due to *Naegleria* spp., can be associated with an eosinophil infiltrate in the heart, even though the organism is not found in this site. This was shown in a histopathological study on 15 patients with primary amoebic meningoencephalitis. Seven had a focal, or diffuse myocarditis, consisting mainly of neutrophils, although there were scattered eosinophils, plasma cells, and myocardial necrosis was seen in two¹¹⁵⁴.

Although *Giardia lamblia* can give rise to marked tissue invasion, and damage to the intestine, it is rarely associated with an eosinophilia.

Most leishmanial infections are not associated with an eosinophilia, although an eosinophilia may occasionally be seen in visceral leishmaniasis.

Patients with malaria who have an eosinophilia are usually found to have other parasitic infections as well, and the blood eosinophil count usually falls during the acute stage of the illness. The possibility that eosinophils might protect against these infections has some experimental support¹⁸⁹⁰.

Pneumocystis carinii pneumonia in infants can give rise to a marked eosinophilia. An example of this

was reported from Memphis, U.S.A., in 1987¹³⁸⁰. The patient was a boy with hypogammaglobulinaemia, who developed *P. carinii* pneumonia at aged five months. His blood eosinophil count was $14.2 \times 10^9/L$, and fell after treatment with gammaglobulin. Other previous patients with a T cell deficiency have been described with a similar syndrome.

Toxoplasmosis is rarely associated with an eosinophilia, although this has been described in a few patients, including a patient in South Africa who had a diffuse encephalopathy with a marked eosinophilia. Treatment with pyrimethamine, and sulphadiazine was effective in this patient, and there were negligible sequelae⁷¹¹.

Trypanosomiasis does not produce an eosinophilia in African patients, although there are some patients in South America who appear to have developed an eosinophilia.

3.3. Ectoparasitic infestations, and insect bites.

The human skin, and hair follicles are inhabited by mites, in at least 10 per cent of normal people, but these seldom produce an inflammatory response. However, there are several ectoparasites which can produce irritating skin lesions, including hair nits, and scabies. Other insects attack the skin to obtain blood, and tissue fluids. Where breaches of the skin are produced, eosinophils, and other inflammatory cells are often found in the lesions.

Many people have follicle mites in the skin of their face, or other parts of their body, and their prevalence increases with age. Men are commonly affected than women. Two species are involved: *Demodex folliculorum*, and *D. brevis*⁸⁴. Their importance in human disease has not been easy to define, and the nature of the eosinophilic material, which is often found adjacent to them in hair follicles, is not known.

Scabies usually produces an eosinophil-rich inflammatory response in the skin. In one study on 60 patients in Oslo, Norway, 10 had an eosinophilia, which related to the severity of the infestation⁵³⁶, and raised counts returned to normal after effective treatment had been given. The histological appearances of the skin lesions show a mixture of lymphocytes, histiocytes, with numerous eosinophils⁵⁵⁸.

4. Chapter C 04. Neoplasms.

An eosinophilia may precede, or be found at presentation in a wide variety of malignancies. This has been known since before the turn of this century. For example a report was published in 1893 of a 31-year old woman who had a tumour in the neck, associated with a blood eosinophil count of $57.6 \times 10^9/L$ ¹⁴⁸⁵.

Some tumours give rise to an increase in blood eosinophil counts, without a great increase in eosinophils within the tumour itself. Some patients have a normal blood eosinophil count with a heavy infiltrate of eosinophils into the tumour, and in some cases, both occur together. It is important to distinguish between blood, and tissue eosinophilia in discussions about tumour-associated increases in eosinophils, as evidence is accumulating that a tissue eosinophilia can be beneficial, whereas a blood eosinophilia may be a sign of tumour dissemination¹¹⁰⁹.

The commonest tumours which give rise to, or are associated with increased numbers of eosinophils in the blood, or tissues, are carcinomas of the lung, lymphoreticular malignancies, (especially Hodgkin's disease), and lymphocytic leukemia. Figure C04-1.

Fig. 4-1: Malignant diseases, and eosinophils.

Several groups have attempted to isolate eosinopoietic substances from tumours, and some success has been reported with large cell carcinomas of the lung. No work has been done to see whether these tumours induce T cells, endothelial cells, or other cells to generate eosinopoietic substances, such as IL-5, and GM-CSF.

4.1. Leukaemias.

An eosinophilia can occur in patients with leukaemia, (a) as a 'reactive' phenomenon, or (b) as an

integral part of the leukaemic process⁹⁶⁴. Few studies have distinguish between these two mechanisms, although it can be attempted by comparing clones of leukaemic, and eosinophil colonies using cytogenetic, and recombinant DNA techniques. However, this may be difficult in evolving leukaemias, and where there may be no detectable abnormality in the leukaemic clones.

4.1.1. Eosinophilic leukaemia.

There are now over 100 case reports of this unusual neoplasm, in which there is no doubt about the diagnosis. Twenty nine patients described as having eosinophilic leukaemia, were described by Chen, and Smith in 1960, and they reported their patient: a 36 year old man with a marked cough, and a blood eosinophil count of $43 \times 10^9/L$. He died one year later, in heart failure, with endomyocardial lesions, and thrombi³⁰⁸. Five patients with eosinophilic leukaemia, among a group of 20 patients with hypereosinophilia, were reviewed by Bentley in 1961¹⁵⁶. Since then there has been no comprehensive review of this disease.

The difficulties in distinguishing eosinophilic leukaemias from HES were best defined by Bousser in 1957¹⁹⁸, who carried out a multi-centre investigation, in which the clinical features, and prognosis of patients with eosinophilic leukemia, and what is now known as the HES, were clearly distinguished. A failure to recognize that most patients with a marked eosinophilia of unknown cause do not have a leukaemic process used to result in deaths from the incorrect prescriptions of cytotoxic drugs. Recognition of the non-malignant course of HES in most patients has been an important advances in this field.

Chronic eosinophilic leukemia occurs either with the classical Philadelphia chromosome, or with a number of other chromosomal abnormalities which are also seen in other forms of chronic myeloid leukemia. Here again, it has proved difficult to provide a single diagnostic group into which all these patients fall, and it may be impossible to distinguish patients with chronic eosinophilic leukaemia from patients with HES, on clinical grounds alone.

Eosinophilic leukaemia has been reported to occur following radiotherapy of Hodgkin's disease, and the treatment of multiple myeloma with melphalan. In the latter patient, there was 5q-, and monosomy 7¹¹³⁰, which is a characteristic of other treatment induced leukaemias.

4.1.1.1. Acute eosinophilic leukaemia.

Acute eosinophilic leukaemia can be distinguished most easily from the more aggressive forms of HES when there is a marked increase in the number of immature eosinophils in the blood, and/or marrow, with over 5 per cent blast forms in the marrow, infiltration of tissues with immature cells of predominantly eosinophilic type, and a clinical course similar to other defined types of acute leukaemia. The main complications are anaemia, thrombocytopenia with bleeding, and an increased susceptibility to infections, which usually results in the death of the patient within a few weeks, or months¹⁴⁹⁰. Occasionally, a marked eosinophilia may develop in patients who present with erythrocytosis. In one patient in whom this occurred, endocardial thrombi developed later in the illness¹⁷⁶⁹. An important feature of eosinophilic leukaemia, is the propensity for the disease to produce myeloblastomas in bones, such as the sternum⁸²¹, ribs,^{638,505} spine^{483,572}, head⁵⁰⁵, pelvic region¹⁹⁴¹, and tibia¹⁹⁶⁰. We are looking after a patient with widespread lytic lesions, which first appeared in his tibiae. An abdominal mass may have been produced by an eosinophilic chloroma, in a patient in Hong Kong⁶⁸⁷. Several other patients have been described with eosinophilic sarcomas, which had features to suggest that T lymphocytes were involved in the leukaemic process (see Chapter A02).

Localization of eosinophil leukaemic cells in the central nervous system, and in cranial nerves, is another important feature in some patients. Infiltrates of tumour cells into the Gasserian ganglion were described in one patient in 1951¹²⁸⁵, and into the cerebrospinal fluid in another in 1987⁹⁹⁵.

Chromosome studies, and agar colony cultures of bone marrow should be part of the assessment of these patients. As eosinophilic leukaemia occurs in childhood, and there are very few cases of HES below the age of 14, all children with a marked eosinophilia of unknown cause should have a particularly careful assessment for a leukaemic process, so that chemotherapy, and bone marrow transplantation can be considered as early as possible. It can occur in infants as young as five months, and

seven months of age.

Examples of eosinophilic leukaemia in childhood include:

- two infants, aged 5, and 7 months with eosinophilic leukaemia described in the U.K., in 1987³⁹⁰.
- a one-year old girl who died three months after presentation with widespread tissue infiltration with eosinophils, and thrombi⁴²⁴.
- a three-year old boy with an eosinophil count of $57 \times 10^9/L$, who was found to have acute eosinophilic leukaemia, and died 11 months later. Emphasis was placed on the isolation of *Mycoplasma orale* from his bone marrow, although the significance of this is unknown¹⁷⁴⁹.
- a five-year old boy who had a blood eosinophil count of $157 \times 10^9/L$ at presentation, and who died after seven months, with blast cell infiltrates in his liver and spleen¹⁵⁶.
- a nine year old girl with an enlarged liver, and spleen, and eosinophil counts of reached $77 \times 10^9/L$ ⁹⁰⁴.
- a 10 year old boy with lymphadenopathy, enlarged liver, and spleen, and endocardial fibrosis, whose eosinophil counts reached $95 \times 10^9/L$. He died after five months⁵³⁹.
- an 11-year old boy from Bangladesh who developed eosinophil counts of up to $120 \times 10^9/L$. The lymph nodes, liver and spleen increased in size, and despite treatment with steroids, and cytotoxic drugs he died in a blast cell crisis⁸⁵¹.
- an 11-year old boy, who lived for a year after he presented with 31×10^9 eosinophils /L blood, and developed endomyocardial fibrosis, with multiple thrombi. Many blast cells were seen in tissues at post mortem¹⁷⁷³.
- a 12-year old boy who had an eosinophil count of $51 \times 10^9/L$ at presentation, with structurally abnormal blood eosinophils. He developed endomyocardial fibrosis, pulmonary infarction, skin, and peripheral nerve lesions. When he died four months later, immature eosinophils, and blast forms were found in his liver, and lymphoid tissues¹⁹⁶².
- a 14 year-old Austrian boy with T-ALL, in whom a shift in tumour cell phenotype occurred, both in the blood, and cerebrospinal fluid. Eosinophil myeloblasts were first found in the bone marrow, and although the cerebrospinal fluid initially contained only lymphoblasts, four weeks after cytotoxic drug treatment was started, 35 per cent were found to be eosinophil myelocytes, increasing to 75 per cent within eight days⁹⁹⁵. This case report supports the suggestion that T lymphocytes, and eosinophils might have a common stem cell origin.

Examples of eosinophilic leukaemia in adults include:

- a 34 year old man with an eosinophil count of $7.9 \times 10^9/L$, up to 25 per cent blast cell in the blood, and 6 per cent in the bone marrow. There was enlargement of his liver, and spleen, a petechial rash, and cardiac involvement. He had a partial deletion of the long arm of chromosome 16 del(16)(q22) in 56 per cent of 68 metaphases. Treatment with hydroxyurea gave no improvement, and he died two months later from infections. Cyanide resistant peroxidase was demonstrated in the blast cells. but they also contain naphthol-AS-D-chloroacetate esterase, which is a feature of neutrophils. The proportion of blast cells in tissues was greater than expected from the number in the blood¹⁶⁸.
- a 46 year old Japanese man, in whom the blood eosinophil counts was $234 \times 10^9/L$ ⁸⁰⁰. Initially, only mature eosinophils were seen, and there was a partial response to steroids. However, two months after presentation, he died with blast cells in many tissues, and eosinophilic endomyocardial fibrosis in the acute necrotic stage, with large endocardial thrombi. Cytogenetics had shown 45 X0/46 XY.
- a 47 year-old woman, with a carcinoma of the breast, lymphocytic lymphoma, and a eosinophilic myeloproliferative disorder¹⁶⁵⁸.
- a 54 year-old man, with a three year history of alcohol intolerance, who then developed what appeared to be HES, with respiratory, lymphatic, liver, and endocardial disease, which became an acute eosinophilic leukaemia, with C group trisomy three months after presentation¹³⁵¹.
- a 60 year old Nigerian man who was described in 1970. He was anaemic, and had a total white cell count of $510 \times 10^9/L$, with $326.4 \times 10^9/L$ eosinophils, 9 per cent of which were blast cells. This

appears to be the highest blood eosinophil counts ever recorded. At post mortem there were renal, and splenic infarcts. His heart was enlarged, with endomyocardial disease, and the myocardium was infiltrated by leukaemic cells. This report also reviewed 19 previous patients who had a marked eosinophilia, and heart disease²⁰⁸.

- a 65 year old man, in whom the blood eosinophil counts rose, over a four year period, to $13 \times 10^9/L$. The serum B12 level increased, and the marrow contained some fibrosis. Finally, he developed a myeloblastoma involving the pleura⁶³⁸.

- a 67 year old man with a large cell carcinoma of the lung, cerebral meningioma, occult adenocarcinoma of the prostate, and follicular adenoma of the thyroid, who developed hypereosinophilia, which progressed to eosinophilic leukaemia, with karyotype 45, X, 15q22-⁶⁸⁵.

The cytochemical appearances of eosinophils from patients with eosinophilic leukemia have been reported in only a few patients since 1879, when Paul Ehrlich first described the staining characteristics of eosinophils in a patient with large numbers of marrow eosinophils, many of which were immature⁸⁰¹. In 1973, it was reported that leukaemic eosinophils stained for chloroacetate esterase, which is not present in normal eosinophils¹⁵⁶⁵. The use of cyanide-resistant peroxidase as an eosinophil marker was recommended in 1986, when maturation is arrested at an early stage. Four patients were studied in this way⁶¹². More details of histochemical reactions in eosinophils from two further patients with eosinophilic leukaemia were published in 1975¹⁹⁵⁶.

Four patients with eosinophilic leukaemia have been found to have increased serum levels of vitamin B12^{483, 572, 1222, 827}.

As early as 1965, the finding of an abnormal acrocentric chromosome in some metaphases from two patients with eosinophilic leukaemia, suggested that it was not just a variant of chronic myeloid leukaemia⁶⁸⁶. In 1975 an isochromosome 17 was found in a 50 year-old man with eosinophilic leukaemia. This patient had other features to suggest that he had a myeloid leukaemia, including raised serum B12 levels, a low alkaline phosphatase score, and increased numbers of platelets. Fifteen months after presentation, the eosinophil count had risen from 0.9 to $30 \times 10^9/L$ ¹²²². A second patient with isochromosome 17 was described in 1979¹⁰⁹⁷. In 1979 a review was made of published cytogenetic studies on 45 patients with a marked eosinophilia⁸²¹. Chromosomal abnormalities were found in 20, and an additional patient was reported: a man, aged 47, who had hypereosinophilia for five years, with malignant cells which had a hyperdiploid karyotype.

In the last 10 years, cytogenetic studies in patients with eosinophilic leukaemia have shown a variety of abnormalities, involving particularly chromosomes 5, 7, 8, 12, 16, and 22. In 1986, we reviewed 13 reports of chromosome banding findings in patients with eosinophilic leukaemia¹³⁶⁵, and in 1987 many of these findings were reviewed again, but emphasis was put on abnormalities seen in chromosome 12⁹⁴³. Sixteen earlier reports of G banding chromosome abnormalities in patients with eosinophilic leukaemia were listed, and the clinical, and cytogenetic findings were provided of four other patients with marked eosinophilia, who had translocations affecting chromosome 12p13.

Two infants, aged 5 and 7 months with eosinophilic leukaemia were described in the U.K., in 1987, who had translocations of chromosomes 1, and 5: t(1;5)(q23;q33), and (q23;q33)³⁹⁰. This translocation has not been reported previously in patients with haematological malignancies, although a t(5;14)(q31;32) has been reported in a patient with ALL, who had lymphoblasts in the marrow, and a blood eosinophil count of $120 \times 10^9/L$ ¹⁷⁸⁸, and a t(5;11)(p15;q13) in 1984¹⁹⁸⁰. The suggestion has been made that, as the GM-CSF gene is present on chromosome 5q31, and the c-fms oncogene is on 5q34, this region of chromosome 5 may be involved in the development of eosinophil malignancies³⁹⁰.

Patients with abnormal marrow eosinophils, and nonlymphoblastic leukaemia, particularly of the myelomonocytic subtype, have been found with abnormalities in chromosome 16, especially 16q22^{67, 1040, 1768}. Because of this finding, it was suggested that chromosome 16q22 may contain gene sequences that are important in eosinophil differentiation¹⁷⁶⁸.

In 1986, a 27 year-old woman with eosinophilic leukaemia was found to have the clonal chromo-

somal anomaly: t(10;11)(p14;q21)²¹⁴.

A 57 year old man with a short Y chromosome was reported in 1972⁵⁷², and a boy with monosomy 7 and a marked eosinophilia complicating a myeloproliferative disorder, in 1981⁸²⁷.

In 1986, we described a patient with eosinophilic leukaemia, and trisomy 8¹³⁶⁵, and reviewed a similar previous case¹⁸⁹⁹. Another patient with eosinophilic leukaemia, who had trisomy 8, and trisomy 7, was reported from Munich, West Germany in 1985¹¹⁶⁴. Two patients with t(8;21) have also been described^{904, 859}.

In 1986, three patients with acute myeloid leukaemia, and a marked marrow eosinophilia, were reported from New York, U.S.A.¹²⁶⁰. They were unique in having trisomy 22. One patient also had inversion of chromosome 16.

In 1984, a patient with acute myelomonocytic leukaemia, and a marked marrow eosinophilia was found with a 15;17 translocation¹²³¹.

Chemotherapy has not proved to be of any benefit in these patients, but the small number of patients involved has not enabled any clear studies to be carried out on the potential benefits of different forms of therapy. Bone marrow transplantation is of potential benefit to these patients, and a number of centres have considered this recently, but none has been carried out yet.

4.1.1.2. Chronic eosinophilic leukaemia.

In the last few years, there have been descriptions of patients with chronic myeloid leukemia, in whom the majority of cells are eosinophils.

It may be possible to distinguish this group of patients from others with acute myeloid leukemia, or a secondary eosinophilia, by carrying out agar colony cultures, which would show large numbers of eosinophil colonies in patients with chronic eosinophilic leukemia, but normal numbers of eosinophil colonies in other diseases. This was first shown to be useful in 1975, during a detailed study on an elderly woman with the clinical features of chronic myeloid leukaemia. She had a high blood eosinophil count, and was shown to have chromosome 10 tetraploidy. Large numbers of colonies developed when her bone marrow cells were cultured in agar⁶⁹⁰.

Over 90 per cent of patients with well-defined chronic myeloid leukaemia have the classical cytogenetic features of a Philadelphia chromosome (an abnormal chromosome 22)¹⁵²⁶, where there is a reciprocal translocation between chromosomes 9 and 22 t(9;22)(q34;q11), with the c-abl oncogene moving from chromosome 9, and fusing with the break cluster region (bcr) on chromosome 22, where an abl-bcr mRNA is transcribed.

Examples of patients with Phi positive chronic myeloid leukaemia, in whom there was a marked eosinophilia include two reported in 1965^{686, 725}, one seen in 1975³²⁴, and a man of 59, described in 1980, who had a double Philadelphia chromosome, with an eosinophilia of $21.2 \times 10^9/L$, and heart disease with widespread leukaemic deposits in his tissues¹⁷¹².

Rarely, chronic myeloid leukaemias are Phi-negative. In a few of these cases of Phi-negative CML, a similar genomic event occurs as in Phi-positive disease, without the visible chromosomal alteration, and this gives rise to a clinically identical disease. Work is now being done to see if the remaining cases of Phi-negative CML, without c-abl translocation, have a different form of CML, or a variant of the myelodysplastic syndrome, such as chronic myelomonocytic leukaemia, which has a worse prognosis than Phi-positive CML. Against this background of information about neutrophil-predominant CML, it is possible that some of the problems in differentiating HES, and eosinophil-predominant CML, would benefit from a similar search for abl-bcr juxtapositioning, and the abnormal fusion product mRNA, and protein. There are no reports, as yet, of studies of this kind in patients with a marked eosinophilia.

4.1.1.3. Eosinophilic leukaemia in animals.

Eosinophilic leukaemia has been described as a spontaneous disease in a pig⁹¹³, and in cats^{565, 1791}. In 1985, it was reported that eosinophilic leukaemia was induced in one cat by the injection of a recombinant feline retrovirus PR8. This virus also induced a marked eosinophilia in another cat, but did not induce a leukaemia. There was no evidence for the production of T cell tumours in either of

these animals, although they can occur in cats infected with this virus¹⁰⁶⁹. Spontaneous eosinophilic leukemia has been also described in a Syrian hamster¹⁴³⁰, and two horses¹²⁴³. Acute eosinophilic leukaemia has not been reported in dogs, although there may be a marked increase in blood eosinophils in dogs with chronic myeloid leukaemia⁷⁶⁴.

4.1.2. Myelocytic leukaemia.

Acute myeloid leukaemia (AML), and chronic myeloid leukemia (CML) are occasionally associated with a marked eosinophilia. A good example of a 'reactive' eosinophilia is given in a report from Fukushima, Japan in 1987, in which eosinophil colonies, which grew from the peripheral blood of a patient with AML, were cytogenetically normal, even though he had a blood eosinophil count of $13.4 \times 10^9/L$, and GM colonies contained chromosomal abnormalities⁹⁶⁴. In other patients, who probably have a reactive eosinophilia, the number of eosinophils in the bone marrow, and blood varies during the course of their illness, and in response to treatment.

4.1.2.1. Myeloblastic leukaemia, acute nonlymphocytic leukaemia.

In 1982, a distinctive type of leukaemia was described. These patients had acute nonlymphocytic leukaemia (ANLL) with an increased number of marrow eosinophils, which were morphologically, and cytochemically abnormal. The FAB classification for this group is M4Eo. It was soon found that they had a characteristic cytogenetic feature: rearrangements of chromosome 16, with break-points at 16p13, and 16q22. Many of these patients seemed to have a slightly better prognosis than others with classical ANLL, even though they had a high incidence of leptomeningeal disease, and occasional intracerebral myeloblastomas⁸¹⁰. It occurs in 5 per cent of patients with acute nonlymphocytic leukaemia, and was reviewed in 1987⁹⁸⁷.

In 1983, five patients were found with this disease, among a group of 61 patients with ANLL at the University of Minnesota Hospitals, U.S.A. They had partial deletion of the long arm of chromosome 16 del(16)(q22), and a marked increase in the percentage marrow eosinophils, which appeared to be part of the malignant clone, as they were structurally abnormal. Two patients who had blood eosinophil counts of $1.6 \times 10^9/L$, went into remission for two, and three years after treatment was started⁶⁷. A further 18 patients were reported by Le Beau, and colleagues in 1983¹⁰⁴⁰. In 1984, six patients were reported from Baltimore, U.S.A.⁸⁰⁸, and three from Boston, U.S.A.¹⁷⁵⁶.

In 1985, there were reports of this disease in seventeen patients in Paris, France¹⁵⁹, and 26 patients in Houston, U.S.A.,⁸¹⁰. Other reports were published that year from Tokyo, Japan¹³⁰⁷, Kiel, West Germany¹⁵⁷², Leuven, Belgium¹¹⁸⁶, and Barcelona, Spain¹⁹⁴⁷.

In 1986 six further cases were described in Bordeaux, France,³⁷⁸ who had an abnormal chromosome 16, which had a pericentric inversion inv(16)(p13;q22) in four patients. Two patients were in remission at 14 months, and four years, but three relapsed 7, 9, and 20 months after diagnosis, and one died, suggesting that some patients did not have as good a prognosis as in the initial reports. Thirty seven patients at the University of Chicago, U.S.A., were also described in 1986, and the previous reports were reviewed¹⁰³³. A nine-month old baby girl in Japan with this disease was reported in 1987¹³⁰⁴.

Difficulties in deciding whether the cytogenetic defect is an inversion, or partial deletion of chromosome 16 were discussed in another paper in 1986⁷⁶². There are also reports that FAB M2 acute myelocytic leukaemia, with t(8;21) can be associated with a similar syndrome⁸⁴⁵, and that M4-Eo leukaemia may develop in the absence of chromosome 16 abnormalities¹⁷⁰³.

In 1984, 30 patients with the 8; 21 translocation, and three with closely related variants were described. Auer rods were present in eosinophil precursors in two cases, and in one patient, the marrow contained an excess of eosinophils with abnormally staining 'basophilic' granules¹⁷³⁰. This suggested that the eosinophil cell line was involved in this malignant process. In 1987, there was a report of a 63 year old man with myeloblastic leukaemia, and an eosinophil count of $5.7 \times 10^9/L$, with a variety of cytogenetic abnormalities, including chromosome 7 defects¹²²⁴.

4.1.2.2. Chronic myelocytic leukaemia.

The blood, and marrow eosinophil count is commonly raised in patients with chronic myeloid leukemia

(CML). In one series of 50 patients with Phi-positive CML reported in 1977, 46 had a mean blood eosinophil count of $5 \times 10^9/L$, ranging from 0.3 to $30 \times 10^9/L$. There was an equally high incidence of eosinophil myelocytes in the blood of these patients, and some of the eosinophils were partly degranulated¹⁶⁷². There is a suggestion that young patients with CML have the highest marrow eosinophil counts, but that this is unrelated to survival times¹⁰²³.

It is probable that the eosinophils in CML are a component of the malignant process, as patients who are heterozygous for the glucose 6 phosphate dehydrogenase genes, have eosinophils, and eosinophil CFU of only one allotype. This has been shown with Phi positive⁹⁸⁹, and Phi negative patients⁵⁶¹. In patients with CML, eosinophils can show abnormal positive histochemical staining for chloroesterase activity, and have PAS, and Astra blue staining, with toluidine blue metachromasia¹⁰⁸⁴.

A unique case report of Phi-negative CML with an eosinophilia, and multiple eosinophil chloromas in the spleen, was described in 1984³⁰⁹.

4.1.3. Lymphocytic leukaemia.

A small number of patients with acute lymphocytic leukemia have been found to have a marked increase in eosinophil counts. This was first reported in two Australian patients in 1973. These were two girls aged 14, and 16 years with lymphoblastic leukaemia, and an eosinophilia of 109 and $3.3 \times 10^9/L$ respectively. They had a purpuric rash, and one had lung involvement¹⁶⁷⁴.

Since then a total of 26 patients with this disease have been described. The mean age at diagnosis was 12 years. The youngest was aged two years. Eighty one per cent were males. Median survival times were 18 months from diagnosis in children up to the age of ten, and 6 months in older individuals, but was not different from other patients with lymphocytic leukaemia. The eosinophilia preceded the leukaemia in half the patients, and in half recurrence, or an increase in eosinophil counts, preceded relapse of the disease. Cytogenetic studies have been reported on 14 patients. Three patients had 14q+ chromosomal abnormalities, with t(5,14),(q?,q32) in two, including a 19-year old man studied at the West Virginia University Medical Center, U.S.A, from where a detailed review of the 26 patients was also provided. The q32 area of chromosome 14 is a common site for chromosomal rearrangements in patients with leukaemias, and lymphomas, and this is also the site for the immunoglobulin heavy chain family of genes. It is not known whether the eosinophilia is 'reactive', or part of the malignant clone in these patients, but the unusual morphology of the eosinophils, and the absence of a detectable eosinopoietic activity in serum, and in normal marrow cocultured with the malignant lymphoblasts, is consistent with the eosinophils being a component of the neoplastic process⁸⁰⁶. Some of the leukaemic lymphocytes possess helper T cell markers, and some only markers of primitive lymphoid cells.

Nine of the cases which occurred in children under 13, and five adolescents, were reviewed in 1984, and an additional patient was reported. She was a 29 month-old girl in Chicago, who had acute lymphocytic leukaemia, with an eosinophilia of $106 \times 10^9/L$. Treatment was started with steroids, vincristine, and L-asparaginase. Methotrexate, and 6-mercaptopurine with cranial irradiation were also given. The eosinophilia disappeared six days from the onset of treatment, and she remained in remission for at least 10 months⁶²⁹. Nine of these 15 patients were boys, the same ratio as in HES. This disease can be compared with acute nonlymphocytic leukaemia where there is rearrangements of band q22 on chromosome 16. Although it is difficult to see a common genetic basis for the presence of abnormal eosinophils in these two diseases, these two chromosomal abnormalities should provide clues about the sites for the genes which regulate the synthesis of eosinophil granule constituents, and eosinophil differentiation.

Doubts about whether the eosinophilia is part of the malignant process are not resolved, but recent measurements of the nucleotide content of the eosinophils in a Dutch patient with lymphoblastic leukaemia showed abnormal levels in both the tumour cells, and the eosinophils⁴¹³. Reports on 15 patients with lymphoblastic leukemia associated with an eosinophilia were reviewed in 1984¹⁷⁹⁶.

In 1972 I showed that the injection of a rat T cell lymphoma into PVG/C rats produced a marked

eosinophilia. This was steroid resistant, and was unlikely to have been due to a graft-versus-host reaction, which induced a marked eosinopenia in the strain used. In this case it seemed probable that the circulating tumour cells were causing the retention of eosinophils in the blood, possibly by interfering with their normal emigration routes into the tissues¹⁶⁷⁸.

Example of hypereosinophilia in patients with lymphoblastic leukaemia include:

- a 2.5 year-old boy in London who developed a blood, and CSF eosinophilia ten months after his disease presented. The appearance of the eosinophils in both sites preceded the appearance of leukaemic cells during subsequent relapses¹³⁶⁰.

- a four-year-old boy with an eosinophil count of $53 \times 10^9/L$, with lung, liver and spleen involvement¹⁹⁶.

- a nine-year old boy with an eosinophilia of $2 \times 10^9/L$, with endomyocardial fibrosis, lymphadenopathy, and a macular rash¹⁸⁰.

- a 12-year-old boy with an eosinophilia of $10.5 \times 10^9/L$, with lung, and endomyocardial involvement, who died two months later¹²⁷⁰.

- a 13-year-old boy with an eosinophilia of $67 \times 10^9/L$, and heart and lung involvement¹³⁸⁷.

- a 17-year-old boy with an eosinophilia of $10 \times 10^9/L$, a purpuric rash, lymph node enlargement, and lung, heart, liver, and spleen involvement⁶³².

- a 27 year old man with a lymphoblastic lymphoma, which was preceded by typical features of HES for one year. The lymphocytes had the phenotype of cortical thymocytes⁹⁴⁵.

Seven male patients, aged between 4 and 41, were described in 1980 from a number of European centres. In several patients the eosinophilia preceded the appearance of the tumour, (or was present when the tumour was recognized), and the eosinophil counts usually fell during remissions. In five patients the blood eosinophils were hypogranular. Three of the tumours were of the C-ALL type, two were T-ALL, and one was a lymphoblastic lymphoma. One patient had several complications more commonly found in HES: eosinophilic endomyocardial disease, and intra-vascular thrombi²⁸⁹.

4.1.4. Other types of leukaemia.

Monocytic leukemia has only rarely been associated with an eosinophilia. There are reports of four patients with plasma cell neoplasms, and hypereosinophilia, which were reviewed in 1984⁵⁹⁴.

4.2. Lymphomas.

Malignant lymphomas, and tumours of Langerhans' cells (histiocytosis X) are the commonest types of malignancy which give rise to a moderate eosinophilia. However, these tumours seldom produce as marked an eosinophilia as can occur in patients with carcinomas. Hodgkin's disease tissue, and the granulomas of histiocytosis X can be densely infiltrated with eosinophils, even though the blood eosinophil count is only modestly raised. There have been no studies on eosinophil production in these disorders, which may be greater than appears from the blood eosinophil counts.

4.2.1. Hodgkin's disease.

Eosinophils are a common feature in tissues affected by Hodgkin's disease, and the number of tissue eosinophils reflects the Rye subtypes of the disease⁶⁰⁸. It is also a well-known cause of a moderate increase in blood eosinophil counts. A study on the possible significance of an increase in bone marrow eosinophils in Hodgkin's disease, was reported from London, U.K., in 1987¹¹²³. No clinical differences were seen in the 28 patients with increased numbers of marrow eosinophils, compared with 108 who did not. However the same group showed that, among 193 of 1260 (15 per cent) patients with Hodgkin's disease who had an increase in blood eosinophils at presentation, there was a survival advantage in 95 with disease of mixed cellularity, and grade I nodular sclerosis, whether their disease was localized, or widespread¹⁸⁴³. This compares with a small series of ten patients with the fibrotic type of Hodgkin's disease in Hungary, in whom over half of the cells in the tumour deposits were eosinophils, who only had a short survival¹⁷⁹⁰.

Although Hodgkin's disease rarely induces a marked increase in blood eosinophils, two patients with hypereosinophilia were described in Australia, in 1966¹⁷⁵⁸. One was a 43 year-old man whose blood eosinophil counts rose to $48 \times 10^9/L$. The other was aged 34 year, and had abdominal Hodg-

kin's disease, and emboli to the lungs. The tumour was found to be infiltrated with eosinophils, and his blood eosinophil counts reaching $108 \times 10^9/L$. A marked eosinophilia with Hodgkin's disease can also occur when the lungs are involved²⁰². The highest blood eosinophil count yet seen in a patient with Hodgkin's disease is $160 \times 10^9/L$, which was reported in 1939¹¹⁴⁴.

It is not known if eosinophils affect the tumour cells, or the surrounding tissues. They may play a more extensive role than appears from the number of intact eosinophils in affected tissues, as large amounts of EPO were detected by immunocytochemistry in the lymph nodes of four patients with nodular sclerosing disease, even though there were relatively few intact eosinophils¹⁵⁴⁵. EPO was also found in the affected tissues of other patients with non-Hodgkin's lymphomas¹⁵⁴⁷. Eosinophil MBP has also been found within affected lymph nodes of 13 of 18 patients with each of the principal histological types of Hodgkin's disease²⁴⁵.

4.2.2. Non-Hodgkin's lymphomas.

Seven patients with non-Hodgkin's lymphomas, and a marked increase in blood eosinophils were reviewed in 1984¹⁴²⁹. Lymphosarcomas rarely cause an eosinophilia. One example of this was seen in a four year-old boy who presented with a lymphosarcoma which involved the lungs, lymphatic tissues, and cerebrospinal fluid. He had a blood eosinophil count of $25 \times 10^9/L$, and some of these cells had morphological abnormalities⁹⁶.

In 1987, two patients were described who had pruritic skin lesions with a lymphocytic vasculitis, and hypereosinophilia. Several years after presentation they developed peripheral T lymphocyte malignancies, and died rapidly¹³³⁷. These patients have some features in common with three patients with lymphomatoid papulosis whom we have seen in London, U.K.¹⁹²⁶.

Many non-Hodgkin's lymphomas contain eosinophils in the stroma of the malignant tissues, and this can be extensive in some. A review was published in 1987 of 28 cases of gastrointestinal tract T cell lymphomas which contained massive numbers of eosinophils¹⁶⁰⁸. Two were in the stomach, and 26 in the small intestine, and eight were associated with coeliac disease. These tumours had several distinctive features on low, and high power microscopy.

4.2.3. Angioimmunoblastic lymphadenopathy.

This disease is probably a prelymphomatous state of immunoblastic sarcoma. It was reviewed in 1985¹⁷¹⁸. Although eosinophils, with deposits of eosinophilic material are a characteristic feature of the lesions, only a third of affected people have an increase in blood eosinophils. Occasionally there may be a particularly high count, as seen in a man, aged 70, in Angers, France. His blood eosinophil count at presentation was $10 \times 10^9/L$, and reached $45 \times 10^9/L$, despite treatment with cytotoxic drugs⁶²³. The presence of an eosinophilic infiltrate in the affected tissues was a poor prognostic feature, according to a report in 1978 on eight patients in Southampton, U.K.⁸⁸⁶, and 30 in Lyon, France, who were followed prospectively for 42 months⁶³.

4.2.4. Sezary's syndrome, and mycosis fungoides.

There are very few case reports of the cutaneous T cell lymphomas (Sezary's syndrome, and mycosis fungoides) being associated with an eosinophilia¹³⁵⁴, or an infiltrate of eosinophils into the tumours. This suggested that these tumours are seldom of the type which can produce eosinopoietic molecules.

4.2.5. Lymphomatoid granulomatosis.

This premalignant, or frankly malignant condition can be associated with a peripheral blood eosinophilia.

4.3. Reticuloendotheliosis.

The histiogenesis of the tumour cells involved in various clinical forms of reticuloendotheliosis was not known with certainty until it was recognized in 1983, that the malignant cell in most (if not all) of the malignancies classed as histiocytosis X belong to the Langerhans' cell lineage. They usually contain the characteristic Lx-bodies⁸³⁸, and are able to be stained with anti-Ia antibodies, and the CD6 monoclonal antibodies^{1577, 593, 765}.

As these granuloma cells can secrete IL-1⁶⁴, which is a potent stimulus for vascular endothelial cell

production of G-CSF, and GM-CSF²¹³, it is probable that the eosinophilic response in these diseases is the result of an indirect effect of granuloma cell products on endothelial cells, which cause the localization of eosinophils in the lesions, eosinophil activation, and occasionally increased eosinophil production in the marrow.

4.3.1. Histiocytosis-X.

Histiocytosis-X is a general term used to cover the three diseases commonly referred to as Hand-Schuller-Christian disease, Letterer-Siwe disease, and eosinophilic granuloma. The relationships between the various clinical disorders in which granulomas affect the bone were reviewed in 1977²⁰¹². Eosinophilic granuloma, which comprises 60-80 per cent of the total cases of histiocytosis-X, is the least aggressive form of this disease. It may be (1) unifocal, unisystem, (2) multifocal, unisystem, or (3) multifocal, multisystem. Other classifications based on morphology of the lesions, or clinical criteria, have not proved useful.

4.3.1.1. Eosinophilic granuloma.

Eosinophilic granulomas, which were first described in 1940, are a well known cause of lytic lesions in the bones of children, and young adults. They occur slightly more commonly in males, and are usually solitary, in the skull, femur, or vertebral bodies⁶¹⁹ of children, and the ribs, and other sites in adults. They can occur in soft tissues, including the thymus¹⁶²⁵. There are innumerable case reports of this condition, including some recent reports. In a typical example, published in 1987, a 37 year-old woman was reported, who had a headache for three weeks. She was found to have a solitary eosinophilic granuloma in the temporo-parietal region, which was cured by curettage^{391, 1384}. The computed tomographic findings in vertebral lesions have been described in 1985¹⁴⁴⁹.

It is now known that Langerhans' cells are the predominant cell type in these lesions²⁰¹³, although many eosinophils can be found in them, and more rarely in the blood. Peanut agglutinin, which binds to Langerhans' cells, also binds to the granuloma cells, and this can be used to distinguish this disease from other granulomatous lesions⁷³². The bony lesions may extend into adjacent soft tissues, and occasionally they originate in other sites. Computed tomographic scans are helpful for determining the extent of individual lesions. Although vertebral granulomas show increased density due to vertebral collapse, the lesions in other bones usually show decreased X-ray density, and can be highly characteristic¹⁰⁰². Biopsy with curettage is usually curative, but occasionally low doses of radiotherapy are given with success¹⁹²². All patients should be followed up for many years, as new lesions can develop as long as 12 years later¹⁴⁸⁰.

As there are so many case reports of this condition this review will only describe some of particular interest:

- an eosinophilic granuloma which was close to the foramen magnum, and produced torticollis in a five year-old girl. It responded to a course of low dose radiotherapy¹¹⁰. There is another report of eosinophilic granuloma affecting the L4 vertebral body in a four year old girl³⁰⁷.
- eosinophilic granulomas affecting lymph nodes were described in two men aged 34, and 25 in 1977. Both had lymphadenopathy, with areas of necrosis, and many eosinophils in the lymphoid tissues. It is possible that this is a variant of the more common diseases in which eosinophilic granulomas develop in skeletal tissues, and it may also progress to a malignant histiocytosis in some patients¹⁴⁸².
- a 48-year old black patient in Nashville, U.S.A., with eosinophilic granuloma was described in 1987 who had painful deep draining sinuses, and nodules in both axillae, inguinal regions, and perianal areas. The diagnosis was made by lymph node biopsy. His disease responded to electron beam therapy. It was not stated whether the patient also had an eosinophilia⁴⁷⁴.
- an 11 year old boy with eosinophilic granulomas, which affected many sites, and which was resistant to chemotherapy, was treated successfully, by bone marrow transplantation in Sweden in 1987¹⁴⁹³.

4.3.1.2. Hand Schuller Christian disease.

This syndrome, consisting of some, or all of the classical triad of exophthalmos, diabetes insipidus,

and focal bony lesions, occasionally shows a marked eosinophil infiltration into the tumour. Many different forms of treatment have been used, including etoposide in 1986⁸⁰².

4.3.1.3. Letterer-Siwe disease.

Letterer-Siwe disease is seen in infants, who present with lymphadenopathy, hepatosplenomegaly, and a bleeding diathesis, with bone, and skin lesions, which are usually fatal.

4.3.1.4. Familial reticuloendotheliosis with eosinophilia, Omenn's syndrome.

Among the 200 or more patients who have been found to have familial histiocytosis¹⁶⁷⁵, there is a group of infants with skin lesions, hepatomegaly, variable splenomegaly, and lymphadenopathy, fever, and infections, and a marked eosinophilia, now known as Omenn's syndrome. This is a rare autosomal recessive disease, first described in 1965, of which there are now about 20 reported cases. During the first, or second month of life, these children begin to develop erythrodermia, chronic diarrhoea, severe infections, lymphadenopathy, hepatosplenomegaly, failure to thrive, and leukocytosis with a marked eosinophilia. These babies have hypogammaglobulinaemia, and low B cell counts, with increased levels of serum IgE, and reduced T-cell functions, associated with an absence of ecto 5'-nucleotidase in fresh, but not cultured lymphocytes⁶³¹. There is a deficiency of IL-2 and interferon-gamma production in vitro. Four patients, two of whom received bone marrow transplants, were reported in 1987 from Rome, Italy. Their blood eosinophil counts range between 0.4 and $11.1 \times 10^9/L$ ²³⁹.

It has been suggested that Omenn's syndrome is Letterer-Siwe disease in an immunodeficient individual. Lymph node biopsies show diffuse proliferation of histiocytes with eosinophil infiltration. The histiocytes are usually of the Langerhans'-type with abundant cytoplasm, an eccentric round-to-oval nucleus with a nuclear groove, and no nucleolus¹³⁹.

4.4. Neoplasms of vascular tissue.

Each of the main types of vascular neoplasm has been associated (in small numbers of patients) with an eosinophilia, involving both an increase in blood, and tissue eosinophils. Examples of this include:

- a patient with a histiocytoid hemangioma of the heart with an eosinophilia, who was reported from Taiwan in 1985. The eosinophilia disappeared after surgical removal of the tumour¹⁰¹⁷. The tumour cells appeared metabolically active, suggesting that they might be secreting an eosinopoietic factor.

- eight patients with epitheloid haemangiomas who were described in Fukuoka, Japan, in 1987¹⁸¹¹. Although the lesions had some clinical similarities with Kimura's disease, they were smaller, and not in the preauricular region. They also contained irregularly hypertrophied vascular structures, and not lymphoid follicles, containing Langerhans' cells. For these reasons they were considered to be a distinct disorder.

4.4.1. Angiolymphoid hyperplasia with eosinophilia, (Wells' syndrome, eosinophilic granuloma of soft tissue, Kimura's disease).

This tumour, which is often called Kimura's disease after the first author of a paper describing it in Japan in 1948⁸⁶⁵, is an uncommon, and benign tumour affecting endothelial cells, lymphocytes, and eosinophils, in the head, and neck region. A similar disease was described in London by Wells in 1969, which he called angiolymphoid hyperplasia with eosinophilia. Some earlier reports in the English literature were reviewed in 1982, and stress was given to the abnormalities in endothelial cells in this disease¹³⁸. Not all patients have an increased number of eosinophils in the blood, although they are always found in the tissue lesions.

Some authors distinguish Kimura's disease from Wells' syndrome¹⁰¹⁵, as there are differences in sex incidence, and histological appearances of the lesions. These have been compared in a paper from Japan in 1986⁸³⁷, when it was suggested that the morphological differences could be due to the long interval, (a mean of nine years) between onset, and histology in the Japanese patients. There were 171 cases by 1978⁷⁸⁶.

In Japan, 94 per cent are men, the mean age of onset is 32 years, and the lesions tend to be in deep tissues. In Europe, nearly all the patients are women, and the lesions are more superficial. In both

regions, they are usually cured by simple excision. There were case reports of the disease from Japan in 1986⁸³⁷, Hong Kong in 1984¹⁰¹⁵, London, U.K. in 1987¹⁹⁰⁴, and Memphis, U.S.A. in 1981⁶²⁵, which also provided good pictures of the gross appearances, and histology of the lesions.

Histology shows varying degrees of infiltration with lymphocytes, histiocytes, mast cells, and eosinophils. There can be marked germinal centre formation in the Japanese patients. Fibrosis is common in Japan, but rare in non-Japanese patients. The histopathological features in 116 cases referred to the Armed Forces Institute of Pathology, Washington, U.S.A., were reviewed in 1985¹³²³. Oriental, blacks, and patients from the Middle East tend to have larger lesions (about 3 cm diameter), than other groups (about 1 cm diameter). An eosinophilia occurs in 17 per cent of Caucasians, and 62 per cent of Orientals. Its behaviour is benign, and this is confirmed in the histology. There is a tendency for recurrences, but spontaneous regression usually occurs. Treatment can include removal, and irradiation.

The clinical, and histopathological features of this benign disease in 12 patients studied at the Pittsburg School of Medicine were published in 1980, with a review of the world literature then available, and the importance of differentiating it from an angiosarcoma was emphasized¹¹¹. Two further patients with this disease were reported in detail in 1981, a 49 year old woman, and a 24 year old man, and the histological appearances were provided¹⁰⁶. Details of the histological appearances in Japanese patients were reported in 1986⁸³⁷. A case report in a 12 year-old boy, which responded to intralesional injections of triamcinolone, was reported in 1984¹²⁷¹.

A characteristic feature of the lesions is the presence of 'flame figures', which contain eosinophil granules¹⁷⁰⁹. Immunofluorescent studies using rabbit antibodies to MBP on skin biopsies from four patients with Wells' disease, were published in 1983, and showed MBP in the infiltrating eosinophils, and in the 'flame figures'. The localization of such large quantities of MBP in these lesions suggested that eosinophils may be involved in the pathogenesis of this disorder¹³⁹¹. A histopathological study from Stockholm, Sweden, in 1986, showed that the flame figures consisted of eosinophil granules, and not collagen bundles²⁰¹, as suggested previously. It is also important to note that eosinophil-rich skin lesions with 'flame figures', have been seen in skin lesions from patients with a number of different diseases, besides Wells' syndrome. These include other skin diseases¹⁹⁵¹, vascular malformations¹²⁸², and renal diseases, including membranous glomerulonephritis, which disappeared after successful treatment of the skin lesion¹⁶⁶⁵.

In a critical review which was published in 1982, of the relationship between Kimura's disease, and angiolymphoid hyperplasia with eosinophilia, Rosai, in Minneapolis, U.S.A., argued that they were different diseases, and that the lesions were not always limited to the skin. He suggested that the lesions were due to a 'histiocytoid change' in endothelial cells, which then secreted substances that caused inflammatory cells to localize in the abnormal areas¹⁵¹⁴. Eosinophil chemotactic substances from the lesions of Kimura's disease, and the infiltrating T cells, have been described⁷⁹⁹. I suspect that the abnormal endothelial cells themselves are responsible for inducing the inflammatory changes, now that it is known that endothelial cells can secrete GM-CSF, and increase the survival, and toxicity of eosinophils in vitro¹⁵²². These abnormal cells may release several other potent molecules into the blood. For example large amounts of renin were secreted by the lesions in one patient, who had hypertension, which disappeared when the skin lesions were removed.⁵⁵⁷

In Toulouse, France, a patient has been described who had angiolymphoid hyperplasia with eosinophilia, and a carcinoma of the lung. He was a 58 year old man with these skin lesions affecting his neck, ears, and scalp. Cytotoxic drugs were effective in eliminating the skin lesions, and in temporarily reducing the size of the tumour⁴⁷⁷.

4.4.2. Neoplasms of lymphatic vessels.

A number of tumours in which the malignant cells appear to be endothelial cells of lymphatic origin have been found to have a marked eosinophilia in the blood or tissues. We have a record of this in an African patient with Kaposi's sarcoma (which is now known to be of lymphatic origin), and it can occur with lymphangiomas, and lymphosarcomas.

4.5. Neoplasms of solid tissues.

Solid tumours, especially those involving body surfaces, may contain many eosinophils, and/or may give rise to a marked increase in blood eosinophils. The same types of tumour produce both types of eosinophil response, but a peripheral blood eosinophilia is more common in patients with carcinomas of the kidney, adrenal, thyroid, liver, gallbladder, pancreas, and breast, and in patients with peritoneal mesothelioma, or liposarcoma¹¹¹³. Recent work has suggested that this could be of clinical importance, as patients with tumour-associated tissue eosinophilia, without an increase in blood eosinophils, may have a better prognosis than those without eosinophils in the tumour stroma. Conversely, an increased blood eosinophil count is often linked to the presence of metastases, and a poor prognosis¹¹¹³.

It has been known since before the turn of the century that eosinophils can be a prominent component of solid neoplasms, and there are a large number of case reports describing this feature. One of the most widely quoted studies was carried out at the Mount Sinai Hospital, New York, U.S.A. in 1946. Eighteen patients were described, and 15 further case reports were assessed. Most of the tumours were of epithelial, or connective tissue origin. It was concluded that an eosinophilia usually indicated that the tumour had disseminated, and that there was a poor prognosis. 90 per cent had metastases, and these were suspected in a further 7 per cent⁸⁴³. By 1966 at least 29 patients had been reported with a marked eosinophilia, in association with solid tumours¹³⁷, since the first case report in 1893. This number had increased to 64, by 1977, when they were reviewed in a paper from Zurich, Switzerland¹⁶²⁴. Although there was some evidence at that time that an eosinophilia might be a poor prognostic sign in patients with solid tumours, this view has had to be modified in the last few years, as it may be a good sign in certain neoplasms.

The eosinophil response may involve the tumour itself, the draining lymph nodes, or the blood. In lymph nodes their presence may simply reflect the presence of tumour cells in areas where eosinophils are commonly found. This was supported by the finding that eosinophils were more often seen in the draining lymph nodes of adenocarcinomas of the large bowel, than carcinomas of the breast, and the number of eosinophils in these nodes was not altered by the presence of lymphatic metastases⁸¹⁴. The incidence of an eosinophilia in patients with a solid tumour has been assessed in several studies. It was 4.8 per cent in a group of 252 patients with solid neoplasms, who were reported from Israel in 1986¹⁶¹⁷, 3 per cent of patients with cervical carcinoma in Malawi¹¹¹⁴, and 2 per cent in an unselected series in London¹¹¹³.

4.5.1. Bone neoplasms.

The majority of eosinophil-rich lytic lesions in bones are eosinophilic granulomas, but there are also a number of primary bone neoplasms in which a marked eosinophilia has been noted.

4.5.2. Breast neoplasms.

An increase in blood eosinophil counts is extremely rare in patients with carcinomas of the breast. In 1983, a study on 419 patients with carcinoma of the breast suggested that when the blood eosinophil count was less than $0.055 \times 10^9/L$, that there was a greater risk of recurrence¹³⁴⁹. This was not confirmed in a separate study on 83 patients reported in 1984¹⁵²⁴.

In 1987, experiments were reported, which showed that tumour-specific serum factors were involved in causing eosinophil infiltration into murine mammary tumours. Mice were implanted with the mammary carcinoma MC2, and after three weeks, although blood eosinophil counts only rose from 0.1 to $0.5 \times 10^9/L$, eosinophils began to infiltrate the tumour, with maximal numbers at five weeks. Unrelated tumours in the same animals were not infiltrated¹⁸¹⁵. The nature of the factor(s) remains to be determined.

4.5.3. Cardiac neoplasms.

A six month-old baby boy was reported in 1965, who had an eosinophil of $53.6 \times 10^9/L$, and an unresectable fibroma containing healthy-looking myocardial fibres in the right ventricle, giving rise to tricuspid stenosis⁷⁷⁰. A 59 year-old man with a malignant rhabdomyosarcoma infiltrating the right ventricular cavity, was described in 1983, who developed an eosinophil count of $51 \times 10^9/L$.

The tumour cells were of a large size, and contained NADH-positive striations¹⁷²⁴. Another adult, with an histiocytoid haemangioma in the heart, which produced an eosinophilia, which declined after the tumour was removed, was described in 1985¹⁰¹⁷.

4.5.4. Digestive system neoplasms.

Neoplasms affecting the gastrointestinal system may give rise to a marked eosinophilia. In some patients the blood eosinophilia precedes the clinical recognition of the tumour, and in others, the tumour is found to be heavily infiltrated, without producing a marked blood eosinophilia. There is preliminary evidence that a marked infiltrate into some gastrointestinal tumours may indicate a better survival than normal¹⁹⁸¹.

4.5.4.1. Oesophageal neoplasms.

There are only a handful of reports of oesophageal neoplasms with an eosinophilia, or eosinophil infiltrates into the tumour. In 1980 there was a report of a patient who had a marked eosinophilia due to a leiomyoma of the oesophagus, which caused superior vena cava obstruction⁷⁹⁵.

4.5.4.2. Stomach neoplasms.

Adenocarcinomas of the stomach are the commonest tumours of the digestive system associated with an increased blood eosinophil count¹⁷⁹⁷. These tumours may also be infiltrated with eosinophils. The possible clinical importance of this was studied in Fukuoka, Japan, in 1986⁸⁵⁶. A stromal eosinophilia was seen in 157 of 649 (24 per cent) resected tumours, and survival studies showed that in 59 with a marked infiltrate, 29 (57 per cent) were still alive at five years, compared with 61 of 158 (39 per cent) patients with lesser numbers of eosinophils in the resected material.

4.5.4.3. Small intestinal neoplasms.

Neoplasms of the small intestine are rarely associated with an eosinophilia. We have reported an unusual case of a patient with chronic strongyloidiasis, who developed a small intestinal lymphoma with an eosinophilia³³⁷, and we raised the possibility that chronic parasitic infections could induce a malignant change at the site of parasite localization.

4.5.4.4. Caecal, colonic, and rectal neoplasms.

It has been known for many years that there may be a marked infiltrate of eosinophils in gastrointestinal carcinomas¹⁹⁸¹. In 1983, an analysis, of 67 colorectal carcinomas in Cleveland, U.S.A., showed that, although there was great variation in the numbers of eosinophils in sections of the tumours, varying from 0 to 233/mm², there was a positive correlation between the numbers of eosinophils, and the survival time¹⁴⁴⁸. In patients with a good prognosis, these eosinophils were more concentrated in the centre, than the edges of the resected specimens¹⁴⁴⁷.

In 1987, a semi-quantitative assessment of the cells infiltrating 22 invasive adenocarcinomas of the large intestine in Kiel, West Germany has also showed that, although mononuclear cells were the most frequent infiltrating cells, there were sometimes large numbers of eosinophils in the tumour stroma. A moderate, or marked infiltrate was seen in nine of the tumours. The follow up period was too short to tell whether this might correlate with prognosis⁸¹⁵.

Eosinophils have been isolated from colonic tumours by enzymatic dissociation, and countercurrent centrifugation, which gave 1.8 x 10⁶ eosinophils/g mucosa¹⁴⁶. This should enable studies to be done on their possible cytotoxic capacity against these neoplasm.

4.5.4.5. Peritoneal, and retroperitoneal neoplasms.

There are occasional reports of patients with slowly growing lesions in the peritoneal cavity who have a marked eosinophilia¹⁰⁵¹. We have noted an unusual progressive fibrotic reaction in the abdomen which had many features of a malignant condition, recurring after repeated surgery. See also page ***.

4.5.4.6. Liver, and biliary tract neoplasms.

Neoplasms in these sites have given rise to a marked eosinophilia on rare occasions. In 1986, a patient was reported with a sclerosing bile-duct carcinoma, and hypereosinophilia¹²¹⁸.

4.5.4.7. Pancreatic neoplasms.

Pancreatic neoplasms are rarely associated with an eosinophilia, except in the case of tumours which

produce large amounts of lipases, which can also give rise to an unusual skin disorder resembling erythema nodosum¹⁵⁰³. There is a single report in 1981 of a malignant islet cell tumour, which produced a persistent eosinophilia, and non-bacterial thrombotic endocarditis¹²⁹².

4.5.4.8. Mouth neoplasms.

Tumours of the lip, and tongue can be associated with a marked eosinophilia. A large mandibular cyst, associated with an eosinophilia, was reported from India in 1985⁷³⁰. Eosinophilic granulomas have also been seen in the soft tissues of the mouth⁶⁷⁷.

4.5.5. Endocrine neoplasms.

Tumours of the thyroid^{1215,1831}, and pituitary can occasionally produce a marked eosinophilia.

4.5.6. Nervous system neoplasms.

There are only a few reports of tumours of the brain, or spinal cord producing an eosinophilia. In 1980, a cerebral histiocytic lymphoma was described which produced an eosinophilia, and in 1981, a glioblastoma was reported which produced eosinophilic meningitis⁴²⁸.

In 1972 it was reported that a 62 year old woman with a glioblastoma had blood eosinophil counts of up to $28.5 \times 10^9/L$ ³⁵³. The tumour was localized, and not infiltrated with eosinophils. She only lived for two months, but during that time she developed cardiac failure, and other features of eosinophilic heart disease. This case report is important (a) for showing that a localized malignant tumour can induce hypereosinophilia, and (b) that a localized tumour with an eosinophilia can induce the development of a distant lesion: eosinophilic endomyocardial disease.

4.5.7. Neoplasms in the ear, nose, and throat regions.

Primary tumours in this region seldom produce an increase in blood eosinophil counts, although tumours of the nasopharynx¹¹¹³, and maxillary sinus¹⁵⁶⁰ are frequently infiltrated with eosinophils. The presence of eosinophils in the stroma of head, and neck tumours appears to be a good prognostic indicator, according to a study of 82 tumours, which were reported from Chapel Hill, U.S.A., in 1987⁶⁹¹.

Twenty six per cent of nasopharyngeal carcinomas in a series of 422, reported from Kuala Lumpur, Malaysia in 1987, contained a stromal eosinophilia¹⁰⁹⁸. Eosinophil accumulation was also seen in lymphatic metastases, but the clinical significance of these findings remains to be determined.

4.5.8. Melanomas, and squamous cell carcinomas of the skin

The commonest primary neoplasm in the skin, are vascular tumours, and melanomas. In one series, which was reported in 1977, of 132 patients with malignant melanomas in Houston, U.S.A., 22 per cent had blood eosinophil counts greater than $0.3 \times 10^9/L$. This group responded slightly better to chemotherapy, in the short term, than the others, although their survival was not affected¹¹⁷⁵. In 1977, a 58 year-old man was reported to have developed an eosinophil count of $66.5 \times 10^9/L$, four years after he presented with a malignant melanoma. The high count persisted for the last two months of his life¹⁶²⁴.

In a study from Bari, Italy, in 1986 it was found that 30 advanced, and metastatic squamous cell carcinomas contained more inflammatory cells, including eosinophils, than 22 basal cell carcinomas³³⁰. Studies have been reported on the assessment of eosinophils in squamous cell carcinomas of the skin in several sites¹¹⁰⁹, and the value of a tissue eosinophilia in distinguishing keratoacanthomas from squamous cell carcinomas¹¹¹².

4.5.9. Respiratory tract neoplasms.

Respiratory tract neoplasms are amongst the commonest causes of tumour-associated eosinophilia. Eosinophil-rich tumours are almost always large cell bronchial carcinomas. Eosinophils have not been reported in association with oat cell carcinomas, which more commonly produce paracrine effects. In a study, which was reported in 1987, from New York, U.S.A., on the occurrence of unexplained high blood counts in 105 patients with large cell carcinomas of the lung, there were three with hypereosinophilia, and it was suggested that these tumours were producing haemopoietic growth factors⁷⁰. Three patients were reported from London, U.K., in 1981¹¹⁵⁵, and five from Leeds, U.K. in 1986⁹⁸³. A possible role for eosinophils in determining the survival of patients with

carcinomas of the lung has come from a study in 1979, showing that an eosinophil infiltrate improved the survival time⁹⁹⁴.

Malignant diseases involving the lung may produce both a peripheral blood, and tissue eosinophilia. The commonest are respiratory metastases, but bronchial tumours themselves can cause both local accumulations, and occasionally very high peripheral blood eosinophil counts. Bronchial adenomas have also been described with mucoid impaction, and local eosinophil accumulation. Histological studies of lung tumours show that diffuse eosinophil infiltration occurs in as many as 50 per cent¹²⁴⁹. In some cases eosinophilia is marked, and eosinophilic substances have been extracted from some of these tumours⁶⁸³. A marked eosinophilia can also occur both in Hodgkin's disease, and non-Hodgkin's lymphomas with lung involvement²⁰².

4.5.10. Urogenital neoplasms.

Amongst the many different forms of tumour which affect the urogenital tract, carcinomas of the cervix, bladder, and kidney are most commonly associated with an eosinophilia.

4.5.10.1. Female genital tract neoplasms.

Carcinomas of the cervix may produce a peripheral blood eosinophilia, but biopsies of the cervical tumour more commonly show a marked eosinophil infiltration into, and around the tumour cells. Adenocarcinomas of the uterine body may occasionally be infiltrated with large numbers of eosinophils. In 1937, a 47 year-old woman was described who had a carcinoma of the cervix with blood eosinophil counts of $10-22 \times 10^9/L$. Earlier work on the presence of eosinophils in tumours was reviewed, and it was suggested that they may have stimulated the bone marrow to produce excessive numbers of eosinophils¹⁵⁴².

In a study on 460 cases on cervical carcinoma from Malawi, which was reported in 1981¹¹¹⁴, 13 (3 per cent) had a marked infiltrate of eosinophils into the tumour. They were all large cell non-keratinizing squamous carcinomas. There was no tumour necrosis or ulceration. In 37 per cent, there was a slight infiltrate of eosinophils. In London, U.K., 2 per cent of cervical squamous cell carcinomas had an equally marked eosinophil infiltrate¹¹¹³. Six patients were reported from Ann Arbor, U.S.A., in 1982¹⁹³. In 1985, eight patients with tumour stromal eosinophilia were reported from Jacksonville, U.S.A.¹⁹⁸⁷.

There is a suggestion that the presence of eosinophils in cervical neoplasms, is linked to an improved prognosis^{909,1374}, and that a peripheral blood eosinophilia is a poor prognostic feature. However, in five patients with cervical neoplasms containing many eosinophils, and raised blood eosinophil counts, who were treated surgically in Taipei, Taiwan, there was a fall in blood eosinophil counts in four patients, and these patients had no recurrences. In the one patient with metastases, blood eosinophil counts remained high, and she died two years later¹⁹⁶⁶.

4.5.10.2. Male genital tract neoplasms.

Tumours of the male genital tract have only rarely been reported as producing an eosinophilia.

4.5.10.3. Neoplasms of the kidneys, ureters, and bladder.

Renal tumours may occasionally produce a marked eosinophilia¹³⁷. Some bladder tumours have a marked infiltrate of eosinophils. A study from South Africa in 1978, found a marked eosinophil infiltrate in 16 of 151 tumours (11 per cent). Biopsies of adjacent normal tissue also contained an eosinophil infiltrate¹⁷⁸³. In 1984, in a study of 1 305 transitional-cell, and squamous-cell carcinomas of the bladder, 36 (2.8 per cent) had a marked eosinophilic infiltrate within the tumour-cell islands, and adjacent stroma. They were most numerous where the tumour was invading the muscularis¹¹¹¹. Further work on this series showed that they did not give rise to an increase number of eosinophils in the urine¹¹¹⁰.

4.5.11. Eosinophilia in animals with solid neoplasms.

There are a number of reports of an eosinophilia in dogs with tumours. One example was of a 2-year old dog with a palatal fibrosarcoma. The eosinophilia was maximal at diagnosis ($11.6 \times 10^9/l$). It fell after radiotherapy, then increased as the tumour metastasized³⁶³. Details of a dog with a mammary carcinoma, and a marked eosinophilia, were published in 1986¹¹⁰⁷.

5. Chapter C 05. Musculo-skeletal diseases.

Rheumatologists seldom see patients with an eosinophilia, but when they do, the eosinophilia is often strikingly high. The commonest musculo-skeletal diseases which produce an eosinophilia are the fasciitis syndromes, and rheumatoid arthritis. Patients with musculo-skeletal diseases can also develop an eosinophilia due to drug reactions. Occasionally, large numbers of eosinophils are found in the joint fluid of patients with rheumatoid arthritis. The demonstration of raised ECP levels in joint fluid has raised the possibility that eosinophils could be involved in destructive processes within joints.

5.1. Bone, and joint diseases.

Primary bone diseases of developmental origin are seldom associated with an eosinophilia, although this has been reported in patients with fibrous dysplasia of bone.

An eosinophilia is an unusual feature of joint diseases, including rheumatoid arthritis in which it has been most commonly described. Occasionally an eosinophilia occurs as an isolated finding in patients with joint disease, and its significance is difficult to define. One example of this was seen in a woman with subchondral osteolysis of the femoral condyles⁸⁶⁰.

5.1.1. Rheumatoid arthritis.

A slight increase in blood eosinophil counts is common in patients with rheumatoid arthritis, but some can have a marked eosinophilia. This can persist for months or years, and in some patients can be life-long, raising the possibility that they have both rheumatoid arthritis, and HES. As joint involvement is uncommon in HES, it seems unlikely that the joint disorder is secondary to an underlying eosinophilic process. In these rare cases it is more probable that the two diseases occur together, or that the arthritis has triggered the development of a persistent eosinophilia. There are three settings in which an eosinophilia occurs in patients with rheumatoid arthritis: in association with a vasculitis, in patients with associated respiratory disease, and in patients taking drugs known to produce an eosinophilia, which is discussed under drug hypersensitivity reactions, page ***. Occasionally patients with juvenile, or adult rheumatoid arthritis have been found to have serum anti-nuclear antibodies which bind to eosinophil, but not neutrophil nuclei¹⁵¹⁷. None of these patients had an eosinophilia.

Examples of rheumatoid arthritis, with a marked eosinophilia were published in the early 1970s^{1731, 1937}. Five patients were described in 1971, with the classical features of rheumatoid disease, and eosinophil counts of 0.7, 1.5, 2.6, 4.7, and 24 x 10⁹/L, with raised serum immunoglobulin levels, and relatively low serum complement levels¹³⁵⁶. Another patient with rheumatoid arthritis, and an extensive eosinophilic vasculitis was reported in 1979, from Sweden⁷⁹⁸. In 1980, a 58 year old man was reported with an eosinophil infiltrate into the soft tissues around his joints, and the synovium, which showed fibrinoid degeneration. Many degranulating eosinophils were also found in his skin nodules²¹¹. Two patients, who presented with active rheumatoid arthritis with nailfold vasculitis, and hypereosinophilia were reported from Birmingham, U.K., in 1985. One, a woman aged 50, had an eosinophil count of 2.8 x 10⁹/L at presentation, which persisted. The second patient had an eosinophil count of 1.5 x 10⁹/L at presentation, but the eosinophilia disappeared after successful treatment with penicillamine⁴¹¹. The features of rheumatoid arthritis with vasculitis, including observations on eosinophils in this disease were reviewed in 1985¹⁵⁷³.

Some patients with rheumatoid arthritis, and an eosinophilia, also have respiratory diseases, raising the possibility that the lungs, rather than the joints, are responsible for the eosinophilia. This is most likely in patients with a high serum IgE level, as reported in 1978, in two patients, a man aged 57, and a woman aged 54, who had asthma, pulmonary infiltrates, high serum IgE levels, and cardiovascular involvement²³⁷. Other patients with respiratory diseases, and rheumatoid arthritis, who developed a marked eosinophilia have been described in 1966¹⁴³², 1981³⁵⁷, and 1982³⁶⁸.

In 1985, serum ECP levels were assayed in 42 patients with rheumatoid arthritis in Uppsala, Sweden⁷³⁷. They had normal blood eosinophil counts, but the mean serum ECP level was 81 ug/L compared to 15 ug/L in normal people. Serum ECP levels fell after treatment with steroids, and the highest levels were seen in patients with early, and active disease. Synovial fluid did not contain eosinophils, although there was a mean ECP concentration of 523 ug/L, which did not correlate with serum ECP levels.

5.1.2. Synovitis, and joint fluid.

The finding of more than a small number of eosinophils in synovial fluid, and in synovial biopsies is rare. Among the known causes are metastatic adenocarcinomas, parasitic diseases, including ascariasis¹⁷⁹⁴, and guinea-worm infections⁵⁰¹, radiation⁷⁶⁶, rheumatoid arthritis¹⁹³⁷, HES²¹¹, and arthrography⁷⁶⁷. In one patient there was an associated hookworm infection, although no parasite was found in the synovial biopsy¹⁷⁴. Occasionally, an eosinophilic synovitis can occur without any obvious disease associations¹¹²⁰.

A small number of patients have been described who had a strong allergic history⁹⁸¹. In these patients, the joint involvement was mild, and although it recurred, it seldom lasted for more than a few weeks or months. The eosinophil count in their synovial fluid did not relate to their blood eosinophil counts. In some them, an eosinophilic synovitis was identified by the presence of Charcot-Leyden crystals in the joint fluid¹¹⁸⁸. Thirteen patients were seen in Sherbrooke, Canada, in 1986, and details of seven of these were reported²¹⁵. They were aged 18-51 years, and had developed an acute, painless monoarthritis, following mild injury. The swelling resolved rapidly in most patients. They had a strong personal, and family history of allergic diseases, and they all had dermatographism. The synovial fluid contained $5.7-19 \times 10^9$ white blood cells/L, and $2.4-9.7 \times 10^9$ eosinophils/L, although the blood eosinophil counts were normal. Some intracellular, and extracellular Charcot-Leyden crystals were seen, and some of the eosinophils were partially degranulated. Other reports of patients with an increased number of eosinophils in synovial fluid are:

- a woman, discussed in 1980, who presented with a swelling of her elbow, a year after it had been injured. The fluid contained 83 per cent eosinophils, and resolved without therapy¹⁴²⁷.

- an Iraqi child aged 10 with a history of atopy, and an eosinophilic monoarthritis, described in 1983²⁶.

- a 15 year-old boy, described in 1987, with a three month history of recurrent swelling of his knee joints, and a history of occasional asthma attacks for several years. The fluid was aspirated, and found to contain 2.2×10^9 /L eosinophils. He had a blood eosinophil count of 0.8×10^9 /L. The synovitis resolved without treatment⁵⁴⁸.

- a patient, who was described in France in 1987, who had delayed pressure urticaria, and joint effusions containing 9.5×10^9 eosinophils /L¹²¹⁷.

Raised levels of ECP have been found in joint fluid from patients with a variety of inflammatory arthritides, and these correlated with the amounts of lactoferrin present⁷³⁵. It is not known what effects, if any, eosinophil products may have within inflamed joints.

5.1.3. Ankylosing spondylitis.

Although patients with ankylosing spondylitis do not have an eosinophilia, or histological signs of eosinophil accumulation in their affected joints, serum ECP levels have been found to be three times higher than normal in a group of 48 patients in Uppsala, Sweden. Treatment with sulphasalazine led to an improvement in nearly all the patients, with falls in serum ECP levels, possibly due to a direct, or indirect action of this drug on ECP secretion⁵⁵⁴.

5.2. Muscle diseases.

Chronic inflammatory diseases affecting skeletal muscle seldom produce an eosinophilia. It does not occur in giant cell arteritis, or polymyalgia rheumatica. An acute inflammatory myopathy, with an eosinophil-rich infiltrate, is most commonly seen in patients with parasitic diseases such as trichinosis, but it can also occur in patients with actinomycosis⁷⁶, dermatomyositis, and eosinophilic myositis.

5.2.1. Eosinophilic myositis, and polymyositis.

There are several reports of an eosinophilic myositis, or polymyositis of unknown cause in man, with either widespread lesions in skeletal muscle, or a lump in one muscle group¹⁹⁷⁹, which improve on treatment with steroids. Examples of this were reported in:

- 1977, when three patients were described who had a systemic illness involving the heart, Raynaud's syndrome, splinter haemorrhages, a rash, and anaemia, and eosinophilic polymyositis. Two of the patients had previous asthma, and hay fever¹⁰³⁹.
- 1979, in a 43-year old man with swelling of the leg muscles, and a marked eosinophilic infiltrate into the muscle, but normal blood eosinophil counts¹⁷⁰⁴.
- 1980, when two patients were described: The first was a 47 year old man with asthma, who had non-pitting oedema of the legs, and an eosinophilia of $1.96 \times 10^9/L$. This responded to treatment with indomethacin. The second was a 30 year old woman had tender leg muscles, but no eosinophilia, whose symptoms responded to treatment with steroids¹⁵⁹².
- 1981, in a 14 year old boy, with a mass in the left side of the neck²⁵.
- 1983, in three men, who had a peripheral blood eosinophilia, and a myositis showing focal necrosis, and degeneration with an inflammatory infiltrate containing histiocytes, and eosinophils¹⁶⁴¹.
- 1986, in a 40 year-old man who presented in Saudi Arabia with a swollen left calf muscle. He had pronounced cramps, and weakness, but the muscles were not tender. The blood eosinophil count was $1.1 \times 10^9/L$, and the serum creatine phosphokinase level was raised to 872 IU/L. Biopsy showed 'focal degenerative changes with collection of eosinophils'⁵³⁸.
- 1986, in a 40-year-old man who had eosinophilic myositis, with episodic weight gain of up to 11 per cent of his normal weight, hyperimmunoglobulin-E, and blood eosinophil counts as high as $41 \times 10^9/L$ ¹⁷³². His illness lasted for nine years, finally responding to alternate day steroid treatment. Some features of his illness, especially the weight gain, and marked eosinophilia, are similar the those seen in patients with episodic angioedema (See Chapter C17). Like them, he had none of the complications which are commonly seen in patients with HES.

Eosinophilic myositis is also a well recognized disease in dogs, sheep, and cattle, where it can present with muscle rupture, possibly as a result of weakening of the tissues by eosinophil granule components¹⁷². Bovine eosinophilic myositis affects up to 5 per cent of cattle in some parts of the U.S.A. It is usually of unknown cause, although in about 7 per cent of cases, it is due to infection with *Sarcocystis* spp.. This organism is a common incidental finding in skeletal, and cardiac muscle in adult cattle, and sheep, where it rarely gives rise to eosinophil infiltrates, or muscle necrosis. The histological, and parasitological findings in 53 bovine carcasses, and seven ovine carcasses with eosinophilic myositis in Colorado, U.S.A. were reported in 1986⁸⁷⁵. *Sarcocystis* is spread by cats, and dogs, which eat contaminated meat, and excrete the sporocysts, which infect the livestock. It is possible that some cases of an unexplained eosinophilia in man may be due to infection with this parasite, although this has not yet been described.

5.3. Fasciitis syndromes.

Chronic inflammation in the vascular subcutaneous tissues, which is characteristic of eosinophilic fasciitis, and the Spanish toxic allergic syndrome, can produce a very marked increase in blood eosinophil counts. The common denominator in these disorders may be an inflammatory response affecting certain blood vessels, and in both cases there is a dense deposit of fibrous tissue in the affected areas. Eosinophilic fasciitis was first defined by Shulman in 1974¹⁶¹⁹, so it is sometimes called Shulman's syndrome.

5.3.1. Eosinophilic fasciitis.

There are now over 100 reported cases of this striking, but usually benign disease in adults, and (less commonly) in children. The main features of the disease, which is a variant of systemic sclerosis, are pain, swelling, and tenderness of the distal part of the forearms, and legs, and diffuse induration of the skin, and subcutis. There is limitation of movement of the hands, and feet. In several patients, the skin of the abdominal wall, and face was involved. It can also produce the carpal tunnel syn-

drome⁸⁸⁸.

Most series of patients with eosinophilic fasciitis have been published in the U.S.A.¹⁰⁹⁴, but a woman with the disease was also reported from Zambia. She had an eosinophil count of $2.4 \times 10^9/L$, and her symptoms improved after treatment with chloroquine, but not with aspirin, or prednisolone³⁴. It has been reported in a child as young as 2 years 10 months¹⁹³³.

In 1975, a report of six patients showed that blood eosinophil counts varied from 0.4 to $2.7 \times 10^9/L$, and they were above $1.2 \times 10^9/L$ in four patients. Serum IgG levels were elevated in most of the patients. There was inflammation, and thickening of the fascia between the subcutis, and muscle, and the inflammatory lesions contained lymphocytes, plasma cells, and histiocytes, with some fibrin, but few eosinophils were found in the tissues¹⁵¹⁰. Initial descriptions of this condition in 1975 did not describe a blood eosinophilia^{1619, 112}, and in 1978 only 13 of 67 patients in a large series had a transient eosinophilia⁵⁷⁶.

It is distinguished from systemic sclerosis by the absence of Raynaud's phenomenon, and autoantibodies, by its responsiveness to steroid treatment, and from linear scleroderma, which can produce a moderate eosinophilia in half of patients⁵³². Nailfold capillary defects are also less common in eosinophilic fasciitis, than in systemic sclerosis¹⁵²⁷.

Eosinophilic fasciitis can be asymptomatic⁸², but later studies have also noted an overlap of eosinophilic fasciitis with systemic sclerosis⁶⁹², especially as involvement of the oesophagus, liver, lymphatic tissues, lungs, and heart has been noticed in an increasing number of patients.

The pathogenesis of eosinophilic fasciitis is not clear. In the acute stage, eosinophils are usually present in the affected tissues, but there are no reports yet of whether eosinophil granule constituents are secreted in the areas of injury, or whether they might cause some of the dermal injury, including oedema, and fibrosis. The disease may have several causes. It has appeared in association with a number of diseases, including thyroid diseases, and haematological disorders, including malignant diseases^{465, 858, 466}, but their relationship remains unknown. In 1987 a 34 year-old man was given an HLA identical bone marrow transplant, in an attempt to treat his eosinophilic fasciitis, and myelodysplasia, but he died from an infection too soon for it to be clear whether the transplant would have been of long term benefit¹⁷⁵⁰. In one patient, a graft-versus-host reaction appeared to have induced the development of eosinophilic fasciitis¹⁸³².

5.3.2. The Spanish toxic oil syndrome.

From 1 May to 26 December 1981, 20 000 people in Spain were affected by a disease, which produced a scleroderma-like syndrome, known as the 'toxic oil syndrome'. There were 277 deaths¹⁴⁹². A marked eosinophilia was a characteristic feature of this extraordinary disease. In a study of 32 patients, blood eosinophil counts rose in 28 during the first month to a mean of $3.9 \times 10^9/L$ (range $0.6-11.1 \times 10^9/L$)³⁵. Many of these eosinophils contained lipid bodies⁶¹³. The diagnostic features were exposure to contaminated oil, the development of an eosinophilia, and/or chest X-ray abnormalities, and a variety of musculo-skeletal lesions.

This syndrome was probably the result of an allergic response to, or a direct toxic effect of one, or more chemicals in cooking oils. Unfortunately, although considerable efforts have been made to discover the exact cause of the disease, and its sequelae have been described in detail⁷⁰⁹, the syndrome has not been reproduced in experimental animal models, so that its cause, and the possible underlying nature of the inflammatory process have not been defined.

As several features of the disease such as thrombi, vascular leakage, and peripheral nerve injury, are also seen in patients with other causes for hypereosinophilia, it has been suggested that some of the complications of the disease, including the peripheral nerve lesions¹⁴⁹¹, could have been caused by the release of granule proteins from eosinophils. Thrombi, which a major problem in patients with the idiopathic hypereosinophilic syndrome, caused the deaths of 46 of 258 (18 per cent) affected people, in one series, and it was suggested that the eosinophilia, which was present in 88 per cent of the patients for over six months, could have been an important factor in producing the thrombi, as the highest blood eosinophil counts occurred at the time when thrombi were most prevalent: in the

second, and third months of the disease²⁸⁸.

5.4. Sjogren's syndrome.

Sjogren's syndrome can produce a moderate increase in blood eosinophil counts. A case report of a 24 year old man who had this disease, with $3.8 \times 10^9/L$ eosinophils, was described in 1984⁵⁴¹. Eosinophils were also present in the skin, liver, and bone marrow.

6. Chapter C 06. Digestive system diseases.

A wide variety of diseases of the gastrointestinal tract are associated with eosinophil infiltrates. These include parasitic infections, inflammatory bowel diseases, connective tissue diseases⁴³⁷, drug hypersensitivity reactions¹¹⁶¹, and eosinophilic gastroenteritis²⁹⁴, but only in a proportion of these patients is there an increase in blood eosinophil counts.

The presence of many eosinophils in the lamina propria of the small, and large intestines of patients with intestinal parasitic diseases has suggested that this is one of the major sites in which eosinophils act as effector cells in immunity to parasites, and other potential disease-forming components of the bowel lumen. Although the intestine has been proposed as a possible site for the elimination of eosinophils in patients with a blood eosinophilia, intestinal biopsies in man have not shown an increased number of eosinophils in patients with an eosinophilia, but without intestinal disease⁹¹⁸. In rats aboard the Cosmos 1129 satellite, eosinophils were seen to accumulate in the lamina propria of the intestines, possibly as a result of the stress involved⁷²⁴. It is not known if a similar event occurs in human astronauts, or people in other stressful situations.

The finding, which we reported in 1985, of large numbers of activated eosinophils in intestinal biopsies from patients with allergic gastroenteritis, with deposits of ECP, and EDN/EPX, has suggested that eosinophils could be involved in the toxic reactions which cause damage to the bowel wall in this disease⁹⁵⁵. The peritoneal cavity can also contain many eosinophils in patients with the serous form of eosinophilic gastroenteritis, and they also appear to be activated.

6.1. Oesophageal diseases.

Eosinophils have been described in the oesophagus in a number of disorders, including eosinophilic gastroenteritis^{585, 551, 1048}, herpes simplex infection of the oesophagus, reflux oesophagitis, and achalasia¹⁰²⁶, where it has been suggested that they damage the myoenteric plexus (Dahl, 1987, personal communication).

There has been an interest since the early 1980s in the possibility that the presence of oesophageal intraepithelial eosinophils may be a useful histological feature for diagnosing reflux oesophagitis. Their occurrence in 10 patients with reflux oesophagitis were reviewed in 1985, when it was linked to the presence of oesophageal strictures in three¹⁰⁴⁸. This was supported by two studies in adults in 1984²¹⁶, and children with the disease in 1985¹⁶¹⁸. However, in 1987, a report from Yale, U.S.A. showed no differences in intraepithelial eosinophils in 73 adults with reflux oesophagitis, compared with 12 normal volunteers¹⁷⁹⁸.

6.2. Diseases of the stomach, and peptic ulcers.

Eosinophils can be prominent in biopsy samples from patients with gastritis. Eosinophils are not a component of duodenal ulcers, or most forms of duodenitis.

6.3. Eosinophilic gastroenteritis.

Eosinophilic gastroenteritis has been recognized since 1937⁹⁰¹ to be an uncommon disease which can affect several parts of the intestines, producing oedema of the bowel wall, and a dense infiltrate of eosinophils. The gastric antrum¹⁸³⁸, and proximal small intestine, are most commonly involved, but the oesophagus, and large bowel can also be affected. The blood eosinophil count is raised in only 20 per cent of patients. Males have the disease twice as commonly as females, and it usually presents in adults in their third decade, although 15 children with the disease had been described by 1977⁸⁰³. Further cases in children were reported in 1983³⁴⁵.

It has been divided into two forms: a polypoid form, (which has sometimes been confusingly called inflammatory fibroid polyps, or eosinophilic granuloma of the gastrointestinal tract - see below), and a diffuse type²¹². When the disease is localized it is sometimes difficult to distinguish from a tumour. A review of the literature up to 1967 showed 33 were of the polypoid type, and 104 had diffuse disease¹⁵⁴⁴. When the diffuse form of the disease affects the mucosa, it produces malabsorption, protein loss, and anaemia. When the muscle layer is affected it can cause intestinal, and biliary tract obstruction¹⁵²⁸, and when the serosa is infiltrated with eosinophils, there is peritoneal involvement with ascites, which may contain many eosinophils. Figure A06-1. It is unlikely to be caused by a hypersensitivity reaction, as the serum IgE level is raised in only 20 per cent patients, and proven food hypersensitivity reactions involving the gut in adults only rarely produce an eosinophilia¹⁵⁸⁵.
Fig. 6-1: Eosinophilic gastroenteritis.

The disease can remit spontaneously, but relapses may also take place¹⁶⁸⁵. It was first suggested in 1979, on the basis of the histological appearances of the small intestine of a 19 year old man with eosinophilic gastroenteritis, that eosinophil products may injure the gut in eosinophilic gastroenteritis²⁹⁴, but this has yet to be shown directly. It is usually treated with 20-40mg prednisolone for one, to two weeks, but a tissue diagnosis is desirable before this is begun, as other disorders can produce a similar clinical picture¹⁸³⁸. Surgery is only necessary if perforation, or continued bleeding occur. The oesophagus can be involved in eosinophilic gastroenteritis. Seven cases had been reported up to 1985⁵⁵¹, including a report in a 3 month-old infant boy in 1978⁵⁸⁵. They may have upper oesophageal strictures, and/or polypoid lesions in the oesophagus.

The colon can occasionally be involved in eosinophilic gastroenteritis. Twenty patients were reported up to 1985¹²⁶⁷, and a further case report was published in 1986¹¹³¹.

The ultrasound appearances of antral thickening in a patient with eosinophilic gastroenteritis has been reported in 1987, in a 15 year old boy in Karachi, Pakistan⁹³⁶. The radiological appearances were discussed in 1981¹¹⁵⁸.

The pathology of eosinophilic gastroenteritis has been well described in a review of 120 patients in the U.K. made in 1978, which included nine new patients⁸⁸³. Immunohistochemical studies on a resected stomach from a 50 year-old patient with eosinophilic gastroenteritis, in 1985, showed many IgE stained mast cells in the areas rich in eosinophils, and it was suggested that mast cell degranulation may have contributed to the development of the lesions¹³⁵⁰.

A 14 year old girl with eosinophilic gastroenteritis who was described in 1985, was found to have large numbers of activated eosinophils in the lamina propria in duodenal biopsies, and their presence correlated with the degree of histological abnormality. She had $1.2-3.1 \times 10^9/L$ blood eosinophils, which were activated. Unlike her brother, who also had eosinophilic gastroenteritis, but no activated eosinophils, she had malabsorption. This suggests that degranulation of activated eosinophils in the gut could have caused her mucosal defects⁹⁵⁵.

6.3.1. Eosinophilic ascites.

Eosinophilic ascites is a feature of eosinophilic gastroenteritis, when the serosal layer of the small intestine is involved. Adjacent lymph nodes are often enlarged, and the spleen may be increased in size. By 1981, there had been 15 case reports of this condition⁷⁵⁷.

- A man aged 36, was described in 1967 who developed ascites with a blood eosinophil count of $2.1 \times 10^9/L$. There were 15 pints of ascites containing up to 98 per cent eosinophils. A pleural fluid collection contained $1.7 \times 10^9/L$ eosinophils. The small bowel was thickened, and there was delayed transit time. He responded well to treatment with ACTH¹⁵⁴⁴.

- In 1970 two adults, a woman aged 25, and a man aged 57 were reported with these features, and eosinophilic ascites⁹⁷⁹.

- In 1979, a patient was described with widespread eosinophilic gastroenteritis involving the intestines from the gastric antrum, to the sigmoid colon, with eosinophilic ascites¹⁰⁶².

- In 1979, a further example was given, and earlier reports were reviewed¹¹⁸⁴.

- In 1985 a boy aged nine years was described with eosinophilic ascites¹⁷⁹⁵.

An eosinophilic ascites has also been described in patients undergoing peritoneal dialysis (see Chapter C12), and a small number of other diseases, including granulomatous diseases. Two men aged 34, and 25 with widespread eosinophilic granulomas affecting lymph nodes, contained areas of necrosis, and many infiltrating eosinophils were reported in 1977¹⁴⁸². A patient with HES, and endomyocardial disease had ascites which contained 86-90 per cent eosinophils, $3.2 - 10.4 \times 10^9/L$ ¹⁵.

There is one report of spontaneous *E. coli* peritonitis, with large numbers of eosinophils in the peritoneal fluid¹⁵²⁵. This association is difficult to explain.

6.4. Inflammatory fibroid polyps.

Granulomas, which are occasionally rich in eosinophils, can be found in the gastric antrum, and occasionally in the wall of the small intestine. The pathogenesis of these diffuse, or polypoid benign lesions is not clear. They have been referred to as eosinophilic granulomas of the gastrointestinal tract, but are probably best called inflammatory fibroid polyps⁸⁸⁴. A review of the pathology of 64 polyps was published in 1984¹⁶¹⁵. It is unusual for these patients to have an increase in blood eosinophils, even though the lesions can contain many eosinophils. Remnants of the herring parasite *Eustoma rotundum* have been seen in a few patients¹⁰¹². The typical clinical features of this disease, which affected the ileum of a 55 year-old man, and produced an intersusception, was described in 1987³⁸⁷. It was treated successfully by resection.

6.5. Chronic eosinophilic pseudotumours in the abdominal cavity.

There are a number of reports of a puzzling disease, or group of diseases, which produces progressing nonmalignant, but chronic inflammatory lesions in the abdomen, with infiltrating eosinophils, and a blood eosinophilia. Examples include:

- a patient who was reported from Houston, U.S.A. in 1980¹⁸³. There were extensive necrotic eosinophil-rich lesion in the upper part of the stomach. These spread to the colon, diaphragm, and lung, and did not respond to steroid treatment.
- a patient in Bilbao, Spain, who developed a retroperitoneal 'inflammatory fibrous histiocytoma' with an eosinophilia¹⁸⁶².
- a woman aged 23 years, from the Antilles, who had a marked enterocolitis, and eosinophilia, which did not improve with steroid treatment, reported in 1983¹¹⁴⁵. A colectomy was done, but the disease recurred in her rectum, producing tumour-like granulomas infiltrated with eosinophils, which caused her death seven years after presentation.
- a 17 year-old Laotian woman who had a progressive disease with necrotic granulomas in lymphoid tissues, which induced a marked eosinophil infiltration, and blood eosinophilia, with raised serum IgE³³⁸. The lesions regressed on steroid treatment, then recurred, and produced abdominal venous thrombi, and small bowel necrosis, which were fatal.
- I have also seen sections of the abdominal masses which developed in a young woman in the U.K. with an undiagnosed disease of this type. Her illness progressed slowly over several years, to produce large abdominal sclerosing lesions containing eosinophils, which finally produced a cuirasse of chronic inflammatory tissue around the lower abdominal contents.

6.6. Enteritis, and enterocolitis.

6.6.1. Coeliac disease.

Patients with coeliac disease do not have an increase in the number of blood eosinophils, although there is an increased number of eosinophils in their jejunal biopsies. In 1984, a report on 47 patients in Budapest, Hungary found that the number of eosinophils in the lamina propria was raised, and inversely related to villous height, and the number of mast cells. The relative numbers of mast cells, and eosinophils returned towards normal after gluten withdrawal¹⁰⁰⁰.

6.6.2. Cow's milk, and soy protein allergy.

Cow's milk, and soy protein allergy are an important cause of intestinal disease in young children. This may take the form of rectal bleeding, or chronic diarrhoea, with the small intestine, or rectum affected, providing the two main forms of the disease: allergic gastroenteritis, and allergic proctitis. Some patients have a similar disease, called chronic nonspecific diarrhoea. In eight boys aged

between one, and five years, with chronic nonspecific diarrhoea, with normal IgE levels, blood eosinophil counts were normal, but the number of eosinophils in duodenal biopsies was increased to $155/\text{mm}^2$ ²⁹⁷, from a mean of $62/\text{mm}^2$ in seven normal subjects.

6.6.2.1. Allergic gastroenteritis.

In 1986, details of 38 children, aged less than two years, with allergic gastroenteritis, were analyzed in Boston, U.S.A.⁶⁸⁹. They had vomiting, pain, some rectal bleeding, and weight loss, an allergic history, anaemia, and increased serum IgE. The blood eosinophil count was raised in most patients, up to $7.2 \times 10^9/\text{L}$, and they were greater than $1 \times 10^9/\text{L}$ in 21 patients. Mucosal abnormalities were present in biopsies from the gastric antrum, in the small intestine in 79 per cent, the oesophagus in 60 per cent, and the body of the stomach in 52 per cent. The inflammatory lesions, which contained scattered eosinophils, were usually diffuse, and marked in the antrum, and oesophagus, but generally focal, and mild in the small intestine, and stomach. Most of these children had multiple relapses, despite dietary elimination of possible allergens, but many recovered only after steroids were given. In seven children in the U.K. with diarrhoea due to cow's milk allergy, who had high serum IgE levels, and mean blood eosinophil counts of $0.87 \times 10^9/\text{L}$, there was a marked increase in the number of eosinophils in duodenal biopsies from a mean of $62/\text{mm}^2$ in controls, to $368/\text{mm}^2$ ²⁹⁷. In a study from Lund, Sweden in 1982, serial serum ECP measurements were made after oral challenge of eighteen patients with cows milk intolerance¹⁹⁴⁶. No consistent patterns emerged. In seven, serum ECP levels fell, whereas in five others it rose before falling again.

6.6.2.2. Allergic proctitis.

Allergy to milk, and soy proteins in babies, can give rise to a severe eosinophilic inflammatory reaction in the colon, and rectum^{321, 874}. Fifteen infants with this disorder were studied in Boston, U.S.A., in 1986⁶⁸⁹. Their disease began at less than six months of age, and all were under two years old when they presented with rectal bleeding alone, or in combination with diarrhoea. Eight had increased blood eosinophil counts, reaching $1.9 \times 10^9/\text{L}$ in one. In most cases there was a diffuse increase of eosinophils in the rectal lamina propria, together with a focal infiltration of the epithelium by eosinophils. They all recovered when the dietary antigens, to which they had become sensitized, were stopped.

6.7. Crohn's disease.

There is a small body of evidence that eosinophils are an important feature of the lesions of Crohn's disease. For example in a study on 114 patients with Crohn's disease treated by 'curative' resection of the terminal ileum, and part of the colon, there was a recurrence rate of 72 per cent within the first year, and ileal biopsies at this stage showed a marked inflammatory cell infiltrate in the lamina propria, containing numerous eosinophils, with fusion, and blunting of the villi¹⁵³⁴. These abnormal histological features were not always associated with symptoms.

In 1980, ultrastructural studies on surgical specimens from 13 patients with Crohn's disease showed that some eosinophils had become partially degranulated, with loss of many of the granule cores^{484, 488}. When considered in relation to the degranulation of mast cells, and damage to vascular endothelium, autonomic nerves, and muscle cells in these sites, it was suggested that eosinophil constituents could be responsible, in part, for these changes.

6.8. Colonic diseases.

Eosinophils may be a prominent component of the lesions in inflammatory bowel diseases.

6.8.1. Ulcerative colitis.

Little is known about the properties, or possible roles of eosinophils in ulcerative colitis, although they became more prominent in the blood, and/or rectal mucosa as the disease progresses, and becomes chronic. Genetic factors may be involved, as Indian patients in one series had a blood eosinophilia at presentation¹⁵⁵, whereas there was no increase in the number of tissue eosinophils in another series of Caucasian patients with a first severe attack of ulcerative colitis¹⁹³⁵. Rectal biopsies have usually shown increased numbers of eosinophils just prior to¹¹⁰⁰, or during relapses.

In 1966, during a trial of dietary treatment of ulcerative colitis, measurements were made of the

number of blood, and colonic eosinophil numbers. There was little relationship between them, although in some patients, blood counts rose during relapses, when the tissue eosinophil counts were highest¹⁹⁵⁵. A report in 1987 failed to find a direct relationship between the number of eosinophils in rectal biopsies, and the severity of ulcerative colitis, or an unfavourable response to treatment¹⁵⁵⁸. Eosinophilic enterocolitis has been described in dogs, cats, and in a captive orangutan, which had diarrhoea with malabsorption, steatorrhoea, and a blood eosinophilia of up to $7.2 \times 10^9/L$. The lamina propria of the small intestine, and colon contained many eosinophils, and there was stunting of villi. The diseases responded to treatment with oral prednisolone³²⁶. A dog with marked thickening of the colon, and mucosal ulcers with a marked eosinophilic infiltrate, has been described in Holland. The animal did not have an increase in marrow, or blood eosinophils, but did have thrombi on the heart valves, with emboli in several organs¹⁸³⁶.

6.9. Rectal diseases.

Increased numbers of eosinophils in rectal biopsies are seen in a number of different disorders. These include parasitic infections, inflammatory bowel disease⁷⁷³, and allergic proctitis due to dietary allergens in children, where the eosinophil infiltrate is largely confined to the crypts⁶⁸⁹.

6.10. Liver, and biliary tract diseases.

Many different types of eosinophil-rich granulomas can produce an eosinophil infiltrate in the liver. Other major causes are parasitic infections, drug hypersensitivity reactions, and involvement in malignant diseases. There are two reports of chronic active hepatitis with an eosinophilia^{1357, 761}. Both patients improved with steroid treatment. Acute liver rejection with a marked eosinophil infiltrate following liver transplantation, has also been noted in one patient¹⁶⁵⁶. Patients with HES often show an infiltrate of eosinophils into the liver.

Sometimes, large numbers of eosinophils are found in gallbladders removed for cholecystitis. In one series of 625 resected gallbladders, reported in 1972, 16 contained many eosinophils⁵⁹². The infiltrate consisted almost entirely of eosinophils in three patients, all of whom were young women. The eosinophils were mainly subserosal, and in the muscle coats of the gallbladder. There are other case reports of patients with an eosinophilic infiltrate into the gallbladder¹⁰⁵⁰, including patients with eosinophilic infiltrates into the cystic duct, and lymph nodes²⁴¹, and a patient with hepatic echinococcosis¹⁵³¹. An eosinophilia has also been noted in a patient with primary sclerosing cholangitis¹²⁶⁹.

6.11. Pancreatic diseases.

An acute pancreatitis can occasionally cause a marked eosinophilia¹²⁵², and there are reports of three patients with an unusual combination of partial lipodystrophy, pancreatitis, and eosinophilia^{1654, 1367}. Although alcohol-related pancreatitis is rarely found associated with an eosinophilia, in 1985, a report from Toronto, Canada, suggested that the pain of chronic pancreatitis related to alcohol, could be due to a toxic effects of eosinophils on nerves in pancreatic perineural areas⁹⁴⁶. This was based on the finding of a correlation between the number of eosinophils in these sites, and pain severity, in resected pancreatic tissues from 50 patients with chronic pancreatitis.

7. Chapter C 07. Diseases of the mouth.

Eosinophils are not prominent in most of the diseases that affect the mouth, and teeth, except for eosinophilic granuloma, which can affect the jaws, and adjacent teeth. Eosinophils are probably not an important part of the protective mechanism of the mouth against infectious agents. The reason for this is not known, but it may be related to the different types of epithelial barrier which are found in each part of the gastrointestinal tract: eosinophils are commonest in sites where there is columnar epithelium.

There is an unusual type of chronic (traumatic) inflammatory ulcer of the oral cavity, which is often rich in eosinophils. Eighteen cases had been reported by 1981. The features have been the subject of reviews in 1983⁵¹¹, 1984¹⁶³⁹, and 1986¹⁹⁹². It is a rare but benign disease of the oral cavity, and

usually affects the tongue, and occurs in all age groups. It has been described under a number of different names, and is unrelated to histiocytosis X. It can last for several weeks, but may be treated conservatively with steroids. In 1987, a patient with this disease was reported to have multiple linear ulcers, and fissures on the tongue, in association with Waldenstrom's macroglobulinaemia²⁰¹⁰.

8. Chapter C 08. Respiratory tract diseases.

The respiratory tract, like the gastrointestinal tract, is one of the major sites for eosinophil localization in tissues, in a wide variety of diseases. As in the gut, it has been suggested that this is because eosinophils are involved in the protective mechanisms of columnar epithelium. The capacity of eosinophils to secrete toxic basic proteins, which can kill a variety of potential pathogens, and damage the airways, and their ability to make molecules which affect the functions of smooth muscle, and goblet cells could also be important in these sites. The eosinophil EPO-H₂O₂-halide system can kill type II pneumocytes in vitro, and could produce tissue injury in patients with asthma, and pulmonary eosinophilia²⁴.

The special relationship between eosinophils, and mast cells in the lung has been a subject of research for many years, as discussed in Chapter A04. A consensus view is now emerging that eosinophils are involved in inducing tissue mast cell degranulation. The earlier view, that they were involved in inhibiting mast cell mediated products, now seems rather unlikely.

The principal disorders in which eosinophils are involved in respiratory tract diseases are allergic, and hypersensitivity disorders, but the presence of particularly large numbers of eosinophils in intrinsic asthma is an important fact which cannot be explained on the basis of an exogenous allergic stimulus, producing eosinophil localization within the lung. It suggests that the factors involved in localizing eosinophils in the lung, are produced locally within the lung itself.

Eosinophils are only rarely found in normal lung tissue, and their presence in moderate, or larger amounts is always associated with a disease process, either in the main bronchi, or the peripheral lung tissues. It is unusual to find eosinophils in sputum, except when there is damage, and loss of the respiratory epithelium. Eosinophils may be involved in stripping epithelial cells from bronchi, and there is experimental evidence to support this view.

Measurements of the amounts of eosinophil granule components in bronchial secretions in different diseases has now begun, but it is not yet clear whether this is useful in distinguishing between different lung diseases, or following the effects of treatment.

There are many disorders affecting the respiratory tract in which eosinophils are prominent, and a number of classifications have been proposed. In 1952, it was recommended that a distinction should be made between patients with, and without asthma³⁶⁹. In 1981, Schatz, and colleagues suggested that classifications should be based on the aetiology of the respiratory disease¹⁵⁶⁷, and I used this approach in my review of this topic in 1983¹⁶⁸⁴. It is also useful to subdivide these diseases on an anatomical basis, into those which predominantly affect the bronchi, lung, and pleura.

8.1. Sputum eosinophils.

Sputum is readily available from most patients with respiratory tract disease. It is best to look for eosinophils in unstained sample, viewed under a cover slip on a glass slide. If necessary, the sputum can be smeared, and stained with one of the Romanowsky stains. In patients with a history, and other signs of asthma, the presence of large numbers of eosinophils, without many other inflammatory cells, favours this diagnosis, aspirin-sensitive asthma, eosinophilic pneumonia, or bronchopulmonary aspergillosis. The presence of few eosinophils, and many neutrophils favours a chest infection, with, or without one of the other causes of chest disease. Other components of the sputum which are worth looking for are Curschmann's spirals, Charcot-Leyden crystals, corpora amylacea, ciliated epithelial cells, and macrophages, including lipid laden macrophages.

8.2. Bronchoalveolar lavage.

The introduction of bronchoalveolar lavage (BAL) in the early 1970s, has led to a greater knowledge of the nature of the cells, and components which are secreted into the bronchial lumen, and bronchoalveolar spaces in different disorders. Comparisons are usually made between the components in bronchial washings, and in segmental washings. The largest yields of alveolar eosinophils have been from patients with eosinophilic pneumonia^{429, 715}, but they are also prominent in BAL fluid from patients with allergic bronchopulmonary aspergillosis, asthma, and asbestosis¹⁵⁰⁶.

Eosinophils were not found in BAL fluid from 18 non-smoking normal subjects in 1987⁵²⁵. Smokers have both a mild increase in blood eosinophil counts⁹²², and increased numbers of eosinophils in BAL fluid.

In 1984, a study at the N.I.H., Bethesda, U.S.A. showed that in patients with interstitial lung diseases, often more than 5 per cent of BAL cells obtained by BAL were eosinophils⁴⁰⁹. The highest proportions of eosinophils were seen in patients with idiopathic pulmonary fibrosis (44 per cent of patients had greater than 5 per cent eosinophils), and they were also seen in fluid from some patients with sarcoidosis, hypersensitivity pneumonitis, collagen-vascular diseases, histiocytosis-X, and chronic eosinophilic pneumonia.

The use of BAL to study the cellular, and protein content of the bronchi, in patients with asthma has been carried out in France, the U.S.A., and in other countries since about 1980. It was found to be a safe procedure in patients with mild symptomatic asthma, both before⁶⁷⁶, and after bronchial challenge¹²⁰³.

8.3. Bronchial diseases.

Asthma, and hay-fever are the commonest cause of a raised blood eosinophil count in western countries. The suggestion that eosinophils might be involved in the pathogenesis of several diseases affecting the bronchi, and interstitial tissues of the lung has been a major stimulus for research into the association between eosinophils, and respiratory diseases.

8.3.1. Asthma.

The presence of eosinophils in the blood, and sputa of patients with asthma has been known since 1889⁶⁹³. Later, histological studies showed that there were often many eosinophils in the bronchial submucosa of patients who died from asthmatic attacks, although this was not always the case^{1276, 667}. The functions of eosinophils in these lesions were unknown, but their presence in the blood, and sputum was taken to indicate that an allergic reaction was taking place in the lungs. This was useful in distinguishing patients with exacerbations of asthma, from those who had acute respiratory infections, in whom blood eosinophil counts usually fell.

Later, as knowledge of the possible pathogenetic roles of mast cells in asthma developed, experiments were done with blood eosinophils, to see whether they were able to detoxify products which were secreted from mast cells. The outcome of this work was interpreted in some centres as showing a beneficial role for eosinophils in asthma.

It is only in the last decade that knowledge of the toxicity of eosinophils themselves for human tissues, has led to different experiments being done to see whether eosinophils could induce some of the pathophysiological features of asthma. Many of these ideas developed from studies at the Mayo Clinic which were reviewed in 1985⁶⁶⁰, and 1986⁶⁰⁴. Today, it seems probable that eosinophils may produce acute, and chronic effects on the lungs in asthma, through their capacity (1) to secrete leukotriene C₄, which can cause bronchial smooth muscle contraction, (2) to secrete PAF-aceter which produces bronchial hypersensitivity, and vascular permeability¹³⁵², (3) to secrete toxic granule basic proteins, including MBP, and EPO, which can induce mucosal cell desquamation, and injury to ciliated epithelial cells, which help to reduce bronchial tone⁵⁷⁴, and remove mucus, and injured epithelial cells, (4) to release reactive oxygen products, which can cause direct membrane damage to respiratory cells, and (5) to stimulate mucus production from goblet cells. Figure C08-1.

Fig. 8-1: Eosinophils, and asthma.

Current clinical research is now designed to test these hypotheses. Experiments are beginning to show that in spontaneous, or bronchial challenge-induced asthma there are alterations in blood,

sputum, and bronchoalveolar eosinophil structure, metabolism, activation, and functions. Eosinophils from patients with asthma have many of the properties required to mediate toxic-effector functions against normal tissues, although the overall importance of these findings in spontaneous attacks of asthma is still not known.

Antigen challenge of patients with mild asthma is beginning to show how these alterations in the properties of eosinophils come about. At the same time, it has become clear that eosinophils are probably more important in producing some of the features of the late asthmatic response after antigen challenge, than the acute changes which are linked more closely to the presence in the lungs of other inflammatory cells, such as mast cells, neutrophils, and macrophages. The efficacy of steroids in preventing late (nocturnal) asthmatic reactions, following antigen challenge, and their variable effects in the acute response, confirms that the pathogenesis of the two events is different. As steroids are effective inhibitors of eosinophil degranulation *in vitro*, this finding supports an important role for eosinophils in late stage reactions. There are also animal models of late phase asthma, which have shown that granulocyte depletion, at the time of antigen challenge, prevents the late response, and the increased bronchial reactivity¹⁰³².

The possibility that eosinophils may cause some of the serious features of asthma has led several pharmaceutical companies to search for drugs which may inhibit eosinophil activation, and degranulation. In most of these studies, human, or animal eosinophils have been stimulated *in vitro*, with, and without test drugs. There are also several more ambitious projects in experimental animals, to study the systemic effects of drugs on eosinophil reactions in the lungs, peritoneum, and other sites (see below). The fruits of these experiments may well extend to other diseases where eosinophils are also thought to have deleterious effects.

8.3.1.1. The histopathology of asthma.

The pathology of fatal asthma has shown large numbers of eosinophils associated with areas of the desquamation, bronchial plugging, and basement membrane thickening, which are the characteristic features of the late stage. Eosinophils are usually found around bronchial vessels, in the parenchyma of the lung, and under the submucosa. The most marked histopathological feature in light, and electronmicrographs of bronchial biopsies from patients with asthma is damage to ciliated, and other epithelial cells. This finding was reported in 1985, in a study of 24 endobronchial biopsy samples from different levels of the respiratory tract, in eight patients with asthma, who were studied by Laitinen, and colleagues in Finland¹⁰²¹. Eosinophils were not seen in any of these samples.

The presence of MBP in histological sections of the lungs of patients with asthma was shown in a study, which was reported in 1982, using fluorescein-labelled rabbit antibodies to MBP. After digestion of lung sections, the MBP was visualized in tissue eosinophils, and along the lining of bronchioles close to areas of damage to bronchial epithelium, and mucus plugs. It was suggested that MBP was secreted into the lungs, and that it could have been responsible for the mucosal damage⁵⁶³.

8.3.1.2. Blood eosinophils in asthma.

Although an eosinophilia has been thought to be a hallmark of intrinsic asthma¹⁵⁶⁸, this is not always the case. For example, among 15 patients with extrinsic, and 12 with intrinsic asthma not on steroids, who were studied in Holland, mean blood eosinophil counts were only 0.29 and $0.24 \times 10^9/L$ respectively²²¹. This shows that an increase in blood eosinophil counts is not a prerequisite for this disease, especially in its milder forms. But in patients with an eosinophilia, there may be a link between blood eosinophil counts, and bronchial responsiveness, as measured by the histamine PC₂₀ method¹⁷⁶⁰.

It has been taught for many years, that alterations in the number of eosinophils in the blood, and/or sputum can be useful for predicting steroid responsiveness, and monitoring steroid treatment. However, in a study in 1983 on acute exacerbations in eleven patients with chronic asthma, three had normal blood eosinophil counts. Measurements of the total number of eosinophils present in the sputum per day were more useful in predicting the response to steroids, and monitoring its effects⁹⁵. The number of light density eosinophils in the blood may be a more useful measure of their involve-

ment in asthma. This possibility arises from a study, which was reported from the Mayo Clinic in 1985, showing that the proportions of light density eosinophils in the blood of normal people, patients with asthma, and patients with HES were 10 per cent, 35 per cent and 95 per cent respectively⁶¹¹. There was a correlation between the log eosinophil count, and the per cent light density eosinophils among 22 patients studied. Further studies on the relationship of the number of light density eosinophils to disease activity, and responses to treatment are awaited with interest.

There is a possibility that blood eosinophils from patients with extrinsic, and intrinsic asthma are more activated than in normal people. This stems from the finding, in a study which was reported in 1986¹⁷⁵², that when blood eosinophils from 26 patients with extrinsic asthma, and 27 with intrinsic asthma were incubated with the calcium ionophore, they released significantly more LTC₄ than normal subjects: 23.5 ± 14.8, 24.6 ± 20.6, and 8.3 ± 7.7 ng/10⁶ cells, respectively. However this was not seen in a previous study in 1984¹⁶⁰³, on 32 other patients.

Little is known about the properties of eosinophils in asthma, although it was shown that the content of arylsulphatase in the light density eosinophils of patients with asthma was normal⁹²¹.

8.3.1.3. Serum eosinophil protein levels in asthma.

In 1977, work in Scandinavia showed that resting serum ECP levels were lower in patients with asthma, than normals, or patients with an eosinophilia due to other causes, although EDN/EPX levels were invariably raised¹⁸⁵³. This was not due to a defect in eosinophils, as the ECP content of blood eosinophils in patients with asthma was normal³⁸². There was also no difference in the diurnal variations in blood eosinophil counts, or serum ECP levels in patients with asthma, compared with controls³⁸⁴. In twelve patients with asthma who were given an inhalation challenge, the serum ECP level rose at one hour, while blood eosinophil counts fell at three hours, only rising to higher levels at 24 hours. There was no relationship between the peak serum ECP levels, and the severity of late reactions. As the EDN/EPX levels did not follow this pattern, it was suggested that serum ECP was being cleared at a different rate from EPX³⁸³.

8.3.1.4. Sputum eosinophils in asthma.

Large numbers of eosinophils in sputum are a feature of several diseases, especially eosinophilic pneumonia, and aspirin-induced asthma. They are also increased in other forms of asthma, and bronchial allergic responses. They are easily seen in phasecontrast preparations of fresh sputum, but it is also possible to carry out accurate differential counts on stained smears¹⁸⁵⁸. However they are best assessed, and quantified in timed samples. In 24 hour collections of sputum from a group of 11 patients with asthma, the mean number of cells was 8 x 10⁷, and 26 per cent were eosinophils⁹⁵.

8.3.1.5. Sputum eosinophil protein levels in asthma.

In a study on 15 patients admitted to hospital with asthma, the MBP content of the sputum was 0.3 to 92 ug/ml, geometric mean 7.1 ug/ml. Raised levels in sputa from a consecutive series of 100 patients with a variety of chest diseases, showed that high sputum MBP levels were related to the presence of asthma, and not other diseases. In a later study, by the same group, on 116 patients with a variety of respiratory diseases, including 10 patients in hospital with asthma, raised sputum MBP levels were again correlated with the presence of asthma⁴⁶⁰. As 10 ug MBP/ml was previously found to be capable of causing damage to human bronchial epithelium in tissue culture¹²⁴⁷, it was suggested that the MBP in these patients was causing direct mucosal injury⁶⁰⁶.

The amount of CLC protein in sputum was raised in patients with asthma, but it was also high in other respiratory diseases, suggesting that this measurement does not have as high a discriminant value as sputum MBP assays⁴⁶⁰. LTC₄ was not detected in the sputum of 17 patients with asthma, although it was detected in sputum from patients with cystic fibrosis¹²⁹⁵.

8.3.1.6. Bronchoalveolar lavage in asthma.

By 1987, over 12 studies had been reported on the use of BAL to assess the number of inflammatory cells in the lower airways of patients with asthma. They all showed a similar picture: that in most patients eosinophils were increased in BAL fluid, but that they were only a small proportion of the recovered cells: less than 1 per cent^{448, 579, 971}, except in patients with aspirin-sensitive asthma⁶⁷⁶,

when larger numbers were found.

Data on 171 patients who were studied in Montpellier, France, were summarized in 1987. There were often no excess eosinophils in the lavage fluid in asymptomatic patients, and the mean number of eosinophils in 50 ml fluid in 93 symptomatic patients was only 7×10^5 . No excess eosinophils were found in patients taking steroids⁶⁷⁵. In another study, reported in 1987, it was found that only 0.6 +1.0 per cent of the cells in BAL fluid from 14 patients with mild asthma were eosinophils⁵²⁵. Even though they are present in small numbers, BAL eosinophils are metabolically active, and can develop chemoluminescence *in vitro*³³³, and synthesize LTC₄, and 15-HETE¹⁰³⁷.

In 1987, two studies were reported on MBP concentrations in BAL fluid from patients with mild asthma. In London, U.K., the amount of MBP correlated with the number of eosinophils in BAL fluid (Wardlaw A. 1987, personal communication), whereas in Ontario, Canada, MBP levels were not elevated⁹⁷¹.

8.3.1.7. Bronchial challenge in asthma.

As a number of environmental antigens have been identified as causes of asthma, especially the house dust mite faecal particles, it has become possible to study the events which follow bronchial challenge. The response in the lungs has followed several patterns. In some patients there is an acute increase in bronchial resistance, which disappears after about four hours. In others there is a 'late phase' reaction, occurring between four, and 12 hours after challenge. Some patients have both reactions. These two types of response are mediated by different mechanisms. Eosinophils may take part in both events, but they are believed to be especially important in the late phase reactions, by secreting LTC₄, and toxic granule basic proteins²²⁰.

There is no consistent alteration in blood eosinophil counts during the late phase reaction following antigen challenge of patients with asthma. This reaction is not usually associated with a leukocytosis, or fever, although patient may feel hot, and sweat excessively. About half of the patients develop an eosinophilia in a study in 1986¹³⁸⁶. In a study from Scandinavia in 1979, the blood eosinophil count fell slightly at three hours, and then increased at 24 hours³⁸³. However in a report from London, U.K., in 1985, eleven patients with late phase reactions to inhaled allergens had raised blood eosinophil counts at 24 hours, whereas this did not occur in six with single reactions, or after methacholine challenge. The initial blood eosinophil counts were related to the methacholine reactivity in the 11 patients⁴⁸¹.

In 15 patients with asthma, who were studied at the Mayo Clinic, and reported in 1985, plasma MBP levels correlated with the number of blood eosinophils⁶¹¹. An eosinophil chemotactic activity was reported to be present in the serum, and to correspond to the times of bronchospasm, in 10 patients with seasonal asthma, who developed biphasic, or late phase responses after bronchial challenge¹²⁰⁴.

In 1985, de Monchy, and colleagues in Holland reported on the results of bronchoalveolar lavage, which was done six hours after antigen challenge, in six patients with asthma due to house dust antigens. The number of eosinophils was greater than in control subjects, or patients who only had early reactions⁴¹⁶. Twelve atopic patients were studied in Iowa City, in 1986¹²⁰⁵. Lavage eosinophils, which were increased in number at four, and 24 hours, were also found to have lost their central cores. Taken together, these studies supported the view that eosinophils are particularly involved in the late asthmatic reactions to inhaled antigens. This was confirmed in a study from Chile which was published in 1987, in which bronchoalveolar cell numbers were assessed in the late phase asthmatic reactions induced by antigen challenge in six patients⁴⁴⁹.

The results of a similar study in Padua, Italy, in patients challenged with toluene diisocyanate which was causing occupational asthma, showed similar increases in the number of bronchoalveolar eosinophils in six late responders, but not in six early responders, or six normal subjects. As there was also an increase in neutrophils, it was suggested that late asthmatic reactions, and the associated increase in airway responsiveness, could be induced by both cell types during airway inflammation⁵²⁷. In a study on 44 patients in 1987, with asthma due to plicatic acid from red cedars, BAL with bronchial biopsies, after bronchial challenge showed many eosinophils in lavage fluid, but little neu-

trophil infiltrate¹⁰²². Although eosinophils were seen infiltrating the bronchial epithelium, and sub-mucosa, no relationship was seen between the number of eosinophils, and sloughing of bronchial epithelial cells, albumin leakage, or airflow obstruction. It was suggested that macrophages were responsible for the sloughing. There was a small increase in blood eosinophil counts at 24 hours. A useful assessment of the effects of bronchial challenge on the human bronchial mucosa, and BAL cells was reported from Iowa City, in 1987¹²⁰⁶. The local effects on the lower bronchi were assessed in 11 asthmatic patients with a history of late reactions. There were six controls. An immediate pallor was followed by reactive hyperaemia, oedema, and bronchial narrowing. The challenge site, and a control site were relavaged at 48 or 96 hours. Eosinophils were a significant component of the BAL fluid at both times, whereas neutrophil counts rose initially, then returned to normal at 96 hours. Eosinophils were also found to be degranulated at both times, and macrophages had ingested intact eosinophil granules.

In a study reported from Holland in 1985, in which BAL was done six hours after antigen challenge, in six patients with late phase reactions due to house dust antigens, the ECP/albumin ratio in the lavage fluid was greater than in control subjects, or in patients who only had early reactions⁴¹⁶.

8.3.1.8. Asthma, and parasitic infections.

There has been an interest for many years in the occurrence of asthma in populations having many parasitic infections. The importance of this is that eosinophils induced by one disease could affect the other¹⁸⁰³, either by altering the severity of the asthma¹⁸, or by influencing the capacity of the individual to resist parasitic diseases. Although these possibilities have not been investigated adequately, it seems likely that any effects are probably minor⁷²¹.

This was supported by a study from Papua New Guinea in 1985, where it was found that the incidence of hookworm infections in asthmatic, and control patients was equal, 75 per cent⁴⁶⁴. However a study in this region in 1980 had shown that patients with asthma had significantly less parasite eggs in their stools than parasitized patients without asthma¹⁶¹².

8.3.1.9. Intrinsic asthma.

In patients with intrinsic asthma, who are often of a older age group than those with extrinsic asthma, blood eosinophil counts are often higher. This has not been explained, and it is not known whether this is linked in any way to morbidity, and mortality in this patient group.

8.3.1.10. Aspirin-induced asthma.

The syndrome of asthma, drug intolerance, and nasal polyps (AIA) has been known for many years². It is caused by a number of drugs, including aspirin, and other non-steroidal, antipyretic analgesic drugs, and some food colourants. The clinical details of this triad have been described in a number of papers from the West, and recently from Osaka, Japan. There were eighteen patients, six males, and 12 females. The mean age of onset for rhinitis was 25, and for asthma 32 years. Thirteen had nasal polyps, eight had urticaria, and 15 sinusitis. Fourteen of 16 patients had a blood eosinophilia, but no relationship with skin test results, or IgE levels was found¹³⁰¹. It has been suggested that this syndrome results from an effect of these cyclo-oxygenase pathway inhibitors causing an over production of SRS-A, and altered prostaglandin synthesis. This would cause bronchoconstriction.

8.3.2. The Churg-Strauss syndrome

The classic post mortem study of patients with asthma, who developed a disease with granulomas containing necrotizing vasculitic lesion with many eosinophils affecting many different tissues, and high blood eosinophil counts, was carried out by Churg, and Strauss in 1951³²³. This disease, which is named after them, may present with a granulomatous pulmonary eosinophilia, and there may be considerable difficulty in diagnosing it, if tissue is not available for biopsy. It was previously known under a variety of different names, which are sometimes used today, and I suspect that many of the earlier descriptions of patients with eosinophilia, respiratory tract disease, and involvement of the pleura, pericardium, peritoneum, kidneys, gut etc. (many of whom were called polyarteritis nodosa) would be classified today as having the Churg-Strauss syndrome. A possible example of this is 'polyserositis', which was described in eight patients in 1941. Six of the patients in this report

developed pleural effusions; three had ascites containing many eosinophils, and two developed constrictive pericarditis, which was confirmed at post mortem⁷⁵⁴. Occasionally a disease similar to the Churg-Strauss syndrome can occur without the presence of a vasculitis. Two patients of this kind were reported in 1986¹⁸³³.

The aetiology of the syndrome is unknown, but it may be exacerbated by an excessive eosinophil inflammatory response to allergens, as was reported in six patients who developed systemic vasculitis with an eosinophilia after allergic hyposensitization therapy. In these patients, the eosinophilia continued after the treatment had been stopped¹⁴⁰².

8.3.2.1. Clinical features of the Churg-Strauss syndrome.

Characteristic clinical features of the disease are a family history of asthma, or a recent presentation with asthma⁷²⁸, and a marked peripheral blood eosinophilia. In a group of 43 patients reported from Paris, France in 1987, the average blood eosinophil count was $8.2 \pm 6.2 \times 10^9/L$ ⁷²⁸. Granulomas can occur in many different tissues including the pericardium, heart, gastrointestinal tract, skin, and peripheral nerves where they produce a mononeuritis multiplex. The eosinophil count can fluctuate, and may occasionally be normal¹⁰³⁰. A patient with the Churg-Strauss syndrome has been described with an eosinophil-rich vasculitis affecting the sural nerve¹³⁰³. In previous patients, nerve damage has not been reported with eosinophil infiltrates, although this probably occurs frequently. Asthma is one of the principal features of the disease, and this was emphasized in a paper describing 10 patients in Lille, France, in 1984. In three, BAL fluid contained many eosinophils¹²⁷⁴.

8.3.2.2. Cardiac complications of the Churg-Strauss syndrome.

The cardiac complications of the Churg-Strauss syndrome are particularly dangerous, as they can give rise to arrhythmias, and sudden death. This probably accounts for the high mortality of the disease. Long term effects of the disease are also suspected: Among three patients with this disease, who were seen at the Brompton Hospital, London, two had pericardial involvement, and one progressed to a dilated cardiomyopathy⁴¹⁰. There has been a special interest in this disease at the Hammersmith Hospital, London. Our work there showed deposition of the activated form of ECP and EDN/EPX in two patients at post mortem. These deposits, and activated eosinophils, were found within the heart of a patient who died with an arrhythmia, and in the spleen, and other tissues of both patients. The close apposition of the eosinophil granule proteins to damaged cardiac myocytes suggested that they could have caused the myocyte necrosis¹⁷³⁷.

8.3.2.3. Treatment of the Churg-Strauss syndrome.

Immediate treatment with large doses of corticosteroid treatment can be life-saving³²⁰, but even with steroids, immunosuppressive drugs, and plasma exchange, the mortality rate is 16 per cent⁷²⁸. The use of alternate day steroids to treat the Churg Strauss syndrome is not recommended during the active phase of the disease. For example, in an account of a patient with widespread mononeuritis multiplex, 100 mg prednisolone on alternate days failed to control the disease⁶⁴³. Pulse methylprednisolone has been effective in one a patient whose disease was resistant to prednisolone¹¹²².

8.3.3. Respiratory hypersensitivity.

Inhaled antigens can give rise to many different types of respiratory tract damage. There may be reversible bronchial obstruction, or extensive damage, giving rise to fibrous tissue formation. Eosinophils are probably involved in both types of response, and are prominent in the peripheral airways of patients with fibrosing alveolitis.

Many organic, and chemical dusts can give rise to hypersensitivity pneumonitis, which was first recognized in maple bark strippers, and in farmers working with mouldy hay. In 1986, an association was found with exposure to the neoprene rubber thermoinjection process, which produced an eosinophilia in 10 of 18 people¹⁷⁷¹. There may be a genetic component in susceptibility to these diseases, which are due to both antibody, and cell-mediated reactions to the inhaled antigens in the lungs³³.

8.3.3.1. Extrinsic allergic alveolitis.

In some patients with moderate, or severe respiratory tract involvement, often with X-ray changes,

and defects in respiratory gas exchange, rather than obstruction, eosinophils can be a prominent feature of their disease.

8.3.3.2. Hayfever.

Hayfever is the commonest cause of a moderately raised blood eosinophil count in western countries. This causes difficulty in defining the 'normal' blood eosinophil count, as patients with mild disease may not report that they have it. A longitudinal study on 16 children with hayfever, and 16 with hayfever, and asthma was reported in 1986 from Aarhus, Denmark⁷⁸⁵. The main findings were that the blood eosinophil counts increased in both groups during the pollen season, and it was greatest in those with asthma. Asthma sufferers also had the greatest alteration in exercise-induced bronchoconstriction, suggesting that the two may have been related.

In patients with chronic hayfever, and allergic rhinitis, who develop polyps, eosinophils are often a prominent component of the nasal washings, and the mucosa of the polyps. It is possible to obtain them in nasal washings, and their properties may then be studied.

8.3.4. Acute, and chronic bronchitis.

Bronchitis is seldom associated with an eosinophilia. Although eosinophils are found in the sputum of patients with chronic bronchitis, their numbers are much less than is seen in asthma¹⁶⁵⁵. The presence of large numbers of eosinophils in the sputum of these patients suggests that the epithelium may have been colonized with fungi, especially *aspergillus* spp..

8.4. Lung diseases.

There are a wide range of parenchymal lung diseases in which eosinophils are an important component. They range from granulomatous, and vasculitic disorders, to the large group of patients classified as having pulmonary eosinophilia. Their causes are usually unknown, and they present a challenge for diagnosis, and treatment in many instances. In patients with a parasitic, fungal, or bacterial hypersensitivity reaction, the pulmonary eosinophilia probably develops as a reaction to antigens released from these organisms. However, there is little evidence for an allergic cause of the lung disease in patients with cryptogenic pulmonary eosinophilia.

In many of these patients, chest radiographs may show pulmonary infiltrates, which can be characteristic of the disorder (eosinophilic pneumonia), and more marked than was suspected from the clinical features. Investigations often prove to be difficult to interpret. There is also no consensus view about the diagnostic groupings, and pathological descriptions of these lung disorders, which makes the topic difficult to review.

8.4.1. Pulmonary eosinophilia.

Pulmonary eosinophilic syndromes are characterized by the presence of an interstitial inflammatory reaction in the lung, containing large numbers of eosinophils. The blood eosinophil count is often raised as well.

Some classifications of pulmonary eosinophilia have as their basis, the occurrence of asthma in some patients^{369, 1804}. The principal difficulty with this approach is that asthma can occur (albeit rarely in some diseases) in every clinical syndrome associated with pulmonary eosinophilia. I have argued¹⁶⁸⁴ that it is better to separate pulmonary eosinophilic syndromes of extrinsic, or known cause (secondary pulmonary eosinophilic syndromes), from diseases of unknown pathogenesis (cryptogenic pulmonary eosinophilic syndromes). The second group consists of three main subtypes: (a) acute, and (b) chronic disorders, which may respond well to steroids and are usually easy to manage, and (c) more severe syndromes, which are chronic with multi-system involvement, and are usually difficult to treat, with multi-system involvement. The more serious disorders includes the chronic eosinophilic pneumonias, and some vasculitis, and granulomatous diseases of the lung, such as Wegener's granulomatosis.

8.4.1.1. Loeffler's syndrome.

The classical description of patients with a fleeting infiltrate, and moderate eosinophilia was made in 1932, by Loeffler in Switzerland¹⁰⁹. Two of the five patients who had recurrent infiltrates developed blood eosinophil counts of only 7 per cent and 9 per cent. Symptoms were mild in all of the patients.

Loeffler's syndrome is now seldom seen, or described in publications, and is probably due to ascaris, and other parasitic infections in childhood.

8.4.1.2. P.I.E. syndromes.

Patients with eosinophilic inflammatory reactions in the lung, similar to eosinophilic pneumonia, but of less severity have been designated as having the pulmonary infiltrates with eosinophilia (PIE) syndrome. They showed similar clinical features to those described below.

8.4.1.3. Chronic eosinophilic pneumonia.

Chronic eosinophilic pneumonia, which became widely known following a pathological study in 1969²⁸¹, is the most severe form of cryptogenic pulmonary eosinophilia. It requires hospital treatment, and careful follow-up. The principal difficulties are in distinguishing it from infective lesions, emboli, and malignant diseases involving the lung.

About 50 per cent of these patients have preceding adult-onset asthma, but most are not atopic. Patients usually present with fever, night sweats, weight loss, cough and dyspnoea, which becomes progressively worse over several days, or weeks.

Twenty seven patients with cryptogenic pulmonary eosinophilia were described in 1973, among a group of 43 patients with pulmonary eosinophilia of different causes, who were seen during a two year period at the Brompton Hospital, London. Interestingly, two of the patients later developed polyarteritis nodosa¹¹⁷⁰. In 1976 a detailed description was published of a further 15 patients who were seen in this hospital. Ten were women (mean age 35 years), and five men (mean age 42 years). Eight had had previous attacks of asthma. The mean blood eosinophil counts were $4.3 \times 10^9/L$, range $0.8-12.8 \times 10^9/L$. The principal clinical features were anaemia, weight loss, and fever, in addition to their respiratory symptoms. Four had splenomegaly, and three hepatomegaly. One also had hilar node enlargement. In each case, there was a dramatic resolution after treatment with steroids. Some patients had minor elevations in serum IgE. The pathology of the lung lesions was described in lung biopsies from three of the patients¹⁸⁰⁵.

In 1977 four women with this disease were reported from Salt Lake City, U.S.A., and their features were correlated to give a picture of this disease as seen in all 27 patients whose details had been published up to this time⁴¹. They were nearly all women, with breathlessness, weight loss, fever, an eosinophilia, and peripheral alveolar infiltrate in chest radiographs.

Details of a further eight patients with chronic eosinophilic pneumonia were described in 1978. Four were allergic to penicillin¹³⁸².

Although the pathogenesis of cryptogenic pulmonary eosinophilia, presenting as eosinophilic pneumonia, is unknown, in one series about half the patients had intrinsic asthma which began within five years of diagnosis¹⁵⁶². Other series have shown a lower incidence of preceeding asthma¹³⁸². However relatives often have a history of atopy in relatives, drug hypersensitivity, or allergic rhinitis. A strong genetic element in the diseases was suggested in a report from the U.K. of cryptogenic pulmonary eosinophilia in identical twin sisters, aged 25, and 29 years¹¹³. Both responded well to treatment with steroids.

Six patients with chronic eosinophilic pneumonia were reported from Chapel Hill, U.S.A. in 1986⁸⁵⁴. Two had atypical features with diffuse abnormalities on chest radiographs. They developed the adult respiratory distress syndrome, which responded slowly to treatment with steroids.

About two-thirds of the patients have raised blood eosinophil counts which can be over $5 \times 10^9/L$ ²⁸¹, and are generally higher in this group of patients than in those with pulmonary eosinophilia of known cause¹¹⁷⁰. However in 13 per cent of histologically confirmed cases, a raised blood eosinophil count was intermittent, or absent⁵⁹¹.

Pulmonary function tests can show airway obstruction in addition to restricted volumes, and reduced diffusing capacity¹²⁴⁵.

Chest radiographs show areas of patchy consolidation, which are usually peripheral, with dense and ill-defined areas that lack lobar, or segmented distribution. They may be unilateral, or bilateral, and the latter give the appearance of a 'photographic negative' of pulmonary oedema²⁸¹. In 1977, six-

teen patients in a large series of 24 with pulmonary eosinophilia had these characteristic 'ground glass' abnormalities, which related to areas where positive lung biopsies were obtained. Details on 81 other patients with pulmonary eosinophilia were assessed in this comprehensive paper⁶¹⁵. Opacities may recur in the same site on several occasions.

These radiological features, in association with an eosinophilia (or occasionally without) are sufficiently distinct for a diagnosis of chronic eosinophilic pneumonia to be made without a lung biopsy. However, in some patients with an atypical illness, bronchoscopy, with BAL (looking for large numbers of eosinophils), and/or trans-bronchial lung biopsy may be required to establish the diagnosis.

BAL has been used to diagnose⁷¹⁵, and to follow the effectiveness of steroid treatment of eosinophilic pneumonia⁴²⁹. In 1987, large amounts of prostaglandin E2 were found with many eosinophils in BAL fluid from two patients with eosinophilic pneumonia, which fell after steroid treatment¹³⁰². In a study on four patients with eosinophilic pneumonia seen in Israel, blood eosinophil counts were $0.78-1.8 \times 10^9/L$, and BAL showed that eosinophils made up 23-76 per cent of the cells recovered in the fluid⁷¹⁵.

In BAL of three patients with eosinophilic pneumonia, one with polyarteritis nodosa, and one with a drug hypersensitivity pneumonitis, who were studied in Lille, France, in 1986, light density, and degranulated eosinophils were noted to have an increased capacity to generate chemoluminescence after stimulation with alveolar macrophage supernatants. They also possessed IgE on their surface¹⁴⁵¹.

By 1981, there had been over 60 published case reports of the histological appearances of chronic eosinophilic pneumonia⁵⁹¹. The focal areas of infiltration contain eosinophils, mononuclear cells, and occasional plasma cells. Granulomas with giant cells, and Charcot-Leyden crystals also occurred¹⁰⁷⁸. Bronchiolitis obliterans, and vasculitis were sometimes noted, and eosinophils in the lesions were frequently degranulated. Deposits of IgG, and IgA have been seen by immunofluorescence.

Electronmicroscopy, and tissue immunofluorescence studies for MBP showed extensive eosinophil degranulation in one patient¹⁴⁶³, with deposits of MBP, and free granules within the pulmonary microvasculature, in the lesions of another patient with chronic eosinophilic pneumonia, reported from Galveston, U.S.A., in 1987⁶⁹⁵. MBP was found in the lung, and pleural fluid of a patient studied at the Mayo Clinic in 1986⁷¹². Intracytoplasmic inclusions in other patients have been interpreted as Birbeck granules, but recent work has shown that these were probably phagolysosomes¹⁴⁶³. As there was also exfoliation of alveolar lining cells, with clustering of eosinophil granules against a denuded basement membrane, it was suggested that these eosinophils may have contributed to the damage which occurred in the respiratory parenchyma.

Over 80 per cent of the 31 reported patients responded to steroid treatment. This resulted in a rapid resolution of clinical and radiological features. Oblique, or vertical lines may persist in chest radiographs, and cavitation can develop in rare instances. This disease is a chronic one, and lesions can recur in previously affected parts of the lung after steroid treatment has been stopped. For this reason a long follow-up period is important. As spontaneous remissions occur in less than 10 per cent, almost all patients require some form of treatment. Corticosteroids usually produce such a dramatic response, that this can be used to confirm the diagnosis. As little as 10 mg prednisolone on alternate days may be effective.

Treatment is usually continued for several weeks after clinical, and radiological resolution is complete. Unfortunately 30 per cent of patients, in whom steroids have been stopped, relapse, and require further treatment. Some patients may require long-term corticosteroid therapy. One group of patients had to be treated for a mean of four years to control relapses, and some patients needed treatment for eight years¹³⁸². There appears to be no place for inhaled steroid derivatives in treating chronic eosinophilic pneumonia. Although clinical, and radiological appearances may resolve rapidly, lung function tests appear to be slower to improve, and these are useful to monitor relapses.

Blood eosinophil counts, red cell sedimentation rates, and chest radiographs can also be helpful to detect relapses at an early stage.

8.4.2. Pulmonary fibrosis.

Studies on BAL cells in patients with cryptogenic pulmonary fibrosis usually show an increased number of eosinophils¹⁹⁶⁸, although this is not so in all series¹⁶¹⁶.

However, when large numbers of eosinophils are present, patients have more severe disease, and a worse prognosis. This was first shown at the Brompton Hospital, London, U.K., where serial BAL was also shown to help in management¹⁸⁰⁶. The initial observation was confirmed in 1987, in a group of 27 patients who were studied in Iowa, U.S.A.¹³⁹⁹, and in Denver, U.S.A.¹⁸⁹². Increased numbers of eosinophils have also been seen in BAL fluid from patients with the CREST syndrome, and interstitial fibrosis¹³⁸⁸.

The reasons why an eosinophilia in BAL may have a poor prognostic value in pulmonary fibrosis, compared with other disease such as eosinophilic pneumonia, and histiocytosis X, where the eosinophil infiltrate is usually greater, may be related to the different sites in which eosinophils localize in the lungs in these diseases¹⁰⁷⁶, and the capacity of eosinophils to induce a fibrotic reaction, under certain circumstances.

A guinea pig model of interstitial lung disease has been described, in which guinea pigs were given aerosols of polymyxin B for four weeks. They developed an eosinophilic alveolitis, but no fibrosis. In this model, it was found that alveolar eosinophils, but not blood eosinophils were able to kill foetal lung fibroblasts spontaneously¹⁷²⁶.

8.4.3. Wegener's granulomatosis.

Wegener's granulomatosis is occasionally associated with an eosinophilia, although the necrotic lesions in the lungs, and kidneys seldom contain eosinophils. A wide range of pulmonary eosinophilic syndromes can be associated with vasculitic, and granulomatous reactions in the lungs. Although apparently distinct syndromes have been defined, the illness in individual patients often does not correspond with them. This difficulty is seen in occasional patients who present with chronic eosinophilic pneumonia, but who then develop a systemic vasculitis. For this reason these disorders are best described as a spectrum of diseases⁵⁴⁵. A general description, and a modern attempt to provide a rational classification of these diseases was put forward by A. Churg in 1983³²². Earlier work stressed the importance of pulmonary involvement in polyarteritis nodosa. For example, in the widely quoted study of Rose, and Spencer in 1957¹⁵¹⁶, of 111 patients with polyarteritis, 14 had lung involvement, 10 of whom also had a peripheral blood eosinophilia. However, these patients had many features which suggest today that they had Wegener's granulomatosis, in which the characteristic histological features would be necrotizing vasculitis, and eosinophil accumulation. At present, polyarteritis involving the lungs is rarely diagnosed, probably because of the distinctions which are now being made between the various types of vasculitis. Granulomatous disorders which produce pulmonary eosinophilic syndromes are much more common^{1844, 828}.

The proportion of patients with Wegener's granulomatosis who develop an eosinophilia varies from 60 per cent in some series, to none in others⁵⁴⁶. It has been suggested that a preceding history of pulmonary infection is an important feature which distinguishes this type of granulomatous disease from the Churg-Strauss syndrome. Lesions are usually better defined than in other diseases with pulmonary eosinophilia, and cavitation can occur leaving areas of scarring.

Extrapulmonary lesions are an important feature, and involve the upper respiratory tract, kidneys, eyes, brain, skin, and other sites. In these patients the pulmonary lesions can recur at an early stage during relapses, and chest radiographs are useful to monitor the progress of the disease. Cyclophosphamide appears to be of particular benefit in Wegener's granulomatosis, and patients are usually also treated with high doses of corticosteroids initially.

8.4.4. Lymphomatoid granulomatosis.

Lymphomatoid granulomatosis is a somewhat similar disease to Wegener's granulomatosis, but it is much less common than other granulomatous diseases of the lung with eosinophilia¹⁰⁷⁹. This dis-

ease, which is often fatal, is characterized by lesions containing a mixture of inflammatory cells infiltrating blood vessels, and tissues, without necrotizing vasculitis. Cavitating lung nodules appear, and lesions also occur in the skin, and kidneys. The upper respiratory tract is seldom affected. Patients may show leukopenia, unlike other forms of granulomatous, and vasculitis disorders affecting the lung. Early treatment with cyclophosphamides, and corticosteroids may reduce the high mortality.

8.4.5. Eosinophilic granuloma of the lung.

Another rare cause of eosinophilia, and pulmonary disease is eosinophilic granuloma of the lung. This was first described in 1951, and over 200 patients have been reported since then. It was discussed in 1986²⁰¹⁷. It occurs in 20 per cent of patients who have the histiocytosis X group of diseases.

Patients usually present with bilateral reticulonodular lesions, which may be found in chest radiographs carried out to investigate nonspecific respiratory symptoms. Pneumothorax is an important complication, and 10 per cent of the patients have hemoptysis: six of 74 patients in one series¹⁰⁶⁸. In these patients granulomas, with eosinophil infiltrates, may also develop at sites outside the lung, including the skeletal system, brain, and other systems. Patients with localized disease respond well to treatment with corticosteroids, and cytotoxic drugs, but diffuse disease can be rapidly fatal.

The lesions are small nodules, which centre on small airways, and develop into fibrotic areas³⁴¹. Electronmicroscopic studies on the abnormal histiocytic cells in lung washings, and lung biopsies show characteristic cytoplasmic rod-shaped bodies (X-bodies)¹⁰¹⁴. When these are found in lung washings, and used in conjunction with the staining of Langerhans'-type cells with CD6 monoclonal antibodies, it is possible to make a firm diagnosis.

Experimental work on the induction of granulomas in the lungs of rabbits, has suggested that prostaglandin E2, and other cyclooxygenase products, may be important in the development of eosinophil-rich granulomas after the inhalation of toxic substances⁷⁴⁶.

8.4.6. Obstructive lung diseases, and pulmonary emphysema.

The chronic effects of obstructive lung disease, which give rise to pulmonary emphysema, are rarely associated with an increased number of eosinophils in the blood, and tissues.

8.4.7. Sarcoidosis.

Sarcoidosis is characterized by macrophage, and lymphocyte infiltrates into tissues, and eosinophils are not a prominent part of the disease pathology.

8.4.8. Adult respiratory distress syndrome.

Studies in Uppsala, Sweden have implicated eosinophils in the adult respiratory distress syndrome (ARDS). This conclusion was based on the finding of high ECP levels in serum⁷³⁶, and BAL fluid, which correlated with the severity of the lung disease. The study was carried out on 12 patients with ARDS, 15 patients undergoing major surgery, and 17 having minor surgery¹²²⁸. BAL was done just after anaesthetic induction. Patients having major surgery also had BAL one hour after surgery was completed. Patients with major surgery had higher levels of BAL fluid ECP after surgery, increasing from 25 to 50 ug/L, but blood ECP levels did not increase. Eight patients with severe ARDS had mean lavage fluid ECP levels of 200 ug/L. The highest level was 272 ug/L in a patient who subsequently died. In four less severely affected patients, lavage fluid contained 75 ug/L. Serum ECP levels were only slightly elevated in most ARDS patients, but the highest serum level was 107 ug/L, in a fatal case. As very few eosinophils were found in the blood, it was suggested that eosinophils were trapped in the lungs, where they degranulated, and may have caused tissue injury. Neutrophil lactoferrin, and myeloperoxidase were also increased in the lavage fluid⁷³⁹, suggesting that neutrophils were affected in much the same way as eosinophils.

8.5. Pleural diseases.

Several of the parenchymal diseases of the lung, which are associated with an eosinophilia, can also affect the pleura. Reactive eosinophilic pleuritis⁷³, is a disease of unknown cause, which can give rise to a spontaneous pneumothorax. The pleural biopsy shows sheets of histiocytes, mixed with

eosinophils in the pleural tissues.

8.5.1. Pleural effusions.

Eosinophils have been noted in pleural effusions since 1894. Particular note is taken of the presence of eosinophils in pleural fluid when they comprise 10 per cent, or more of the cells in the effusion. Five to eight per cent of pleural effusions are of this type. Eosinophilic pleural effusions have been seen in a wide variety of diseases, especially following introduction of air into the chest, trauma³⁸⁹, infections, including tuberculosis¹²¹⁰, and more rarely in patients with a malignancy, benign asbestos lesions, pulmonary infarction, sarcoidosis⁴⁸⁰, and rheumatological diseases, including systemic sclerosis⁴⁵⁶. In a series of 30 patients, reported in 1979, trauma was involved in 11, and an allergy in eight, but no cause was seen in 11¹⁸⁵⁴. This report commented on the large numbers of lymphocytes in these effusions, and the benign course of the effusions in most patients. In 1974, a study on 78 patients with an eosinophilic pleural effusion showed that 48 had tuberculosis, and six a tumour. No definite diagnosis was made in 16, even after pleural biopsies had been taken⁹⁹³. In 1981, a study from Finland found that tuberculosis was no longer an important cause of an eosinophilic pleural effusion¹⁴⁰¹. Occasionally, an eosinophilic pleural effusion develops in patients with viral respiratory diseases⁸³⁶. An historical review of 343 published patients was done in 1985, and showed no clear association with any single disease, although the presence of an eosinophilic effusion reduced the likelihood of a malignant disease, or pulmonary tuberculosis, compared to other benign diseases¹⁷. A negative correlation between an eosinophilic pleural effusion, and malignant disease was also found in a study of 334 patients in Sweden in 1985¹¹⁵⁹, and in 36 patients studied in Israel in 1985¹⁹⁵⁹. In about 10 per cent of eosinophilic pleural effusions no cause can be detected, although some of these may be due to asbestos, as half of known asbestos-associated pleural effusions are eosinophilic. These idiopathic cases usually resolve spontaneously, and empirical treatment for other diseases, such as tuberculosis is probably unwarranted in most instances.

9. Chapter C 09. Diseases of the ear, nose, and throat.

9.1. Diseases of the ear.

The external ear may be involved in a number of diseases which affect other parts of the skin, including Kimura's disease, and angiolymphoid hyperplasia with eosinophilia. Eosinophils have been seen in the exudate from the ear in a proportion of patients with chronically discharging ears, (17 per cent in one series of 84 patients¹³⁰⁶), but the reasons for this are not known.

In otitis media, eosinophils are occasionally found in cytological preparations, but they are seldom present in any large numbers, except in patients with aspergillus infections.

9.2. Diseases of the nose.

Eosinophils are not found normally in the nose, although large numbers can be found in patients with allergic nasal diseases. Early reports described eosinophils in the nose of normal children under six months of age, but this was not confirmed in a groups of 22 children aged two days to 12 months, who were studied in San Francisco, U.S.A. in 1985³³⁵. Although patients may occasionally have focal lesions in the nose rich in eosinophils due to such rare disorders as nasal tumours, and the Churg-Strauss syndrome, the majority of patients with nasal symptoms, and eosinophil accumulation in the nose, or in the blood, have allergic rhinitis, with or without nasal polyps. An important non-allergic form of rhinitis which does not appear to have an allergic cause (NARES) can also present with many eosinophils in nasal washings. Occasionally, patients with allergic bronchopulmonary aspergillosis can also present with allergic rhinitis⁷⁰⁷.

9.2.1. Rhinitis.

Rhinitis is usually divided into allergic, and non-allergic types. Allergic rhinitis may be perennial, or seasonal. A general review was published in 1984¹³⁸¹. The nasal smear contained eosinophils in 71 per cent of one group of 770 patients with allergic rhinitis studied in Helsinki, Finland in 1984¹⁷¹.

Nasal eosinophilia is more common in those with perennial allergic rhinitis, than in seasonal allergic rhinitis. Nasal eosinophil levels are related to blood eosinophil counts, but as there may be a significant difference in the numbers of eosinophils recovered from each nostril, the association is not close.

Non-allergic rhinitis has been described both in adults, and children¹⁵²⁹. It has many different causes, including drug reactions, aspirin sensitivity, vasomotor rhinitis, and NARES. NARES is associated with more than 5 per cent eosinophils in nasal smears. (Occasionally a 25 per cent lower limit is used to make this diagnosis). In a study from Providence, U.S.A., in 1985 on 78 patients with non-allergic rhinitis who had negative skin tests, 61 per cent had vasomotor rhinitis, 33 per cent NARES, and 6 per cent sinusitis. Among the 25 patients with NARES, three had mean blood eosinophil counts of $0.95 \times 10^9/L$. These are sometimes classified separately as BENAR (Blood Eosinophilia Non-Allergic Rhinitis) syndrome. There is some evidence that patients with non-allergic rhinitis, and an increased number of eosinophils in nasal secretions respond best to medication¹²⁴⁸.

9.2.2. Nasal polyps.

Nasal polyps, in patients with chronic rhinitis, are often rich in eosinophils, and nasal secretions from these patients usually contains large number of these cells⁶⁰⁰. The proportion of polyps which contain eosinophils varies from 26 per cent in Japan, to 94 per cent in several parts of Europe^{811,231}. The incidence of atopy in patients with eosinophilic polyps is one in four, and about half have asthma. Polyps in children with cystic fibrosis contain few eosinophils¹⁶⁶⁶.

A recent study on nasal polyps from 77 patients in Tokyo, Japan, showed that 43 (56 per cent) contained eosinophil infiltrates. Thirty per cent were atopic. IgG-immune complexes were found in five of the six polyps studied¹²⁹⁸, and IgE has also been detected in nasal polyps⁶⁰⁰. Polyps also occur in patients with aspirin sensitivity, who may have a marked blood eosinophilia. The incidence of nasal polyps in patients with aspirin-induced asthma ranges from 60-100 per cent¹³⁰¹. In a recent study on 22 nasal polyps, it was found eosinophils were responsible for the formation of large amounts of 15-HETE in this site, but their role(s) within, and on the surface of polyps remains unknown¹⁶⁴⁹.

9.2.3. Paranasal sinus diseases.

Aspergillus infections of the sinuses have been noted to be able to cause a marked eosinophil-rich infiltrate in this site⁹²⁰. In 1987, the clinical features of allergic fungal sinusitis due to *Curvularia lunata* were described¹¹²⁷.

9.3. Pharyngeal, and laryngeal diseases.

The pharynx, and larynx may contain many eosinophils in rare individuals with tumours, granulomatous diseases, or rheumatoid arthritis. In 1985, three patients were described in Norwich, U.K., with an eosinophil-rich fibrotic reaction in the submucosa of the upper respiratory tract, and sub-epiglottic region of the larynx¹⁴⁹⁶. Their illnesses may have been related to primary eosinophilic granuloma of the larynx, which was described in three patients in 1981⁶⁰³.

10. Chapter C 10. Diseases of the nervous system.

Eosinophils are not normally found within the nervous system, and their presence there is always evidence for a disease process. Much of the recent interest in the relationship between eosinophils, and the nervous system has centred on the capacity of ECP and EDN/EPX to cause demyelination in experimental animals. However, there is no direct evidence that ECP, or EDN/EPX injure the brain in man, and high levels of ECP in the cerebrospinal fluid do not seem to cause demyelination. However, it is possible that one or more of the eosinophil effector mechanisms may accentuate lesions in the meninges, and within the brain in inflammatory disorder where they are prominent, and further work in this area will be of interest. The peripheral nervous system is commonly affected in patients with hypereosinophilia, but it is not known whether eosinophil constituents affect nerve

conduction.

10.1. Central nervous system diseases.

Few primary diseases of the central nervous system result in eosinophil accumulation within the brain, and it is very seldom that they give rise to a peripheral blood eosinophilia. The common forms of cerebrovascular diseases do not give rise to eosinophil infiltrates.

10.1.1. Diseases affecting meninges, and eosinophils in the cerebrospinal fluid.

The finding of eosinophils in the cerebrospinal fluid (CSF) is uncommon¹⁰⁰⁷, except in countries where eosinophilic meningitis due to *Angiostrongylus cantonensis* is prevalent, and gives rise to the highest CSF eosinophil counts. This was reviewed by Kuberski in Honolulu in 1981¹⁰⁰⁸. A number of other parasitic diseases produce an increase in CSF eosinophils¹⁶⁷, including echinococcosis, cysticercosis, and toxoplasmosis. It can also occur in other infectious diseases, including (rarely) tuberculous meningitis, coxackie B meningitis³¹³, chronic lymphocytic choriomeningitis, and Rocky Mountain spotted fever³⁶⁷. The other main causes are neoplastic diseases, including lymphocytic leukaemia^{967, 1360, 995}, Hodgkin's disease^{1716, 295, 1375, 809, 260}, myeloproliferative disorders⁸⁶¹, pneumococcal meningitis³⁰², following myelography, and surgical operations on the brain⁸⁷², and the presence of shunts in children treated for hydrocephalus¹⁸⁰⁸. Other causes are meningeal carcinomatosis³⁵², cerebral eosinophilic granulomas¹⁶³⁸, and drug hypersensitivity reactions, such as gentamycin hypersensitivity, which induced a blood, and CSF eosinophilia in a patient with a ventricular shunt¹²¹⁶, and ibuprofen, which produced an aseptic meningitis¹⁴⁶². There is only one report of a minor increase in CSF eosinophils in a patient with HES¹⁹⁰⁰. The meninges can be infiltrated with eosinophils in some patients with central nervous system involvement in eosinophilic leukaemia.

In one series of patients with meningitis, measurements of ECP in the cerebrospinal fluid showed that patients with the highest levels had the slowest recovery⁷⁴⁰.

In 1985, ultrastructural studies were carried out on subdural hematomas from five patients in whom eosinophils were seen in the outer membranes of the haematomas. They appeared to be degranulated, and free granules with altered matrix were found outside the cells. It was suggested that eosinophils might release constituents which could contribute to the increase in fluidity of chronic subdural haematomas causing further leakage of blood into the capsule of the hematoma¹⁹⁶⁵.

10.2. Peripheral nerve diseases.

There are no specific disorders in which the peripheral nerves, or cranial nerves are involved by eosinophils, although cranial nerve lesions, and peripheral damage is common in patients with hypereosinophilia. The pathology of these lesions has not been investigated, but it is possible that the damage is vascular in origin, probably due to occlusion of central, and perineural blood vessels. In 1986 four patients were described from Stanford, U.S.A., who had multiple peripheral nerve lesions in association with an eosinophilia. They also had chronic sinusitis, and asthma, and responded to steroids, with relapses. Biopsies failed to show any vasculitic lesions, although these could have been localized, and difficult to detect. Whether these patients fall into the HES clinical spectrum remains to be seen. It was postulated that eosinophil granule components might have caused the nerve lesions⁴⁶¹.

10.3. Schizophrenia.

Although there is no clinical evidence to suggest that eosinophils are involved in schizophrenia, a study from Uppsala, Sweden, in 1982, showed raised serum levels of ECP in several patients⁷⁴³. As these correlated with raised serum lactoferrin levels, it was suggested that these changes might accompany an alteration in inflammatory reactions which have been noted previously in schizophrenia.

11. Chapter C 11. Eye diseases.

The principal eye diseases associated with eosinophils, are parasitic, and allergic diseases.

11.1. Conjunctival diseases.

The presence of eosinophils in tarsal conjunctival scrapings is a feature of a number of hypersensitivity disorder such as vernal conjunctivitis, seasonal allergic (hay fever) conjunctivitis, atopic conjunctivitis, and other non-specific allergic diseases. In some patients with these diseases it may be difficult to demonstrate the eosinophils in tear fluid, or scrapings, although they are present in conjunctival biopsies¹. Occasionally only eosinophil granules may be seen⁶⁰². Other causes of an eosinophil-rich conjunctivitis are filarial infections, and the Churg-Strauss syndrome¹⁶¹³.

11.1.1. Vernal conjunctivitis.

In vernal conjunctivitis, which often starts in childhood, and is associated with itching, and mucus secretion, eosinophils can be a prominent cell type in the affected tarsal conjunctiva. In ten patients with vernal conjunctivitis, biopsies showed the presence of many eosinophils in the epithelium, and the substantia propria. As similar features were seen in 38 per cent of the biopsies of the tarsal conjunctiva from 15 patients who wore hard or soft contact lenses, and who had developed a similar clinical picture of giant papillary conjunctivitis, it was suggested that the two diseases were related^{31, 30}. Both MBP, and CLC protein were detected in significant amounts in the tears from five of seven patients with vernal conjunctivitis¹⁸⁰⁹.

11.1.2. Seasonal, and perennial allergic (hay fever) conjunctivitis.

Allergic conjunctivitis is an IgE-mediated acute allergic reaction in the conjunctiva, due to air-borne allergens, especially pollens. It gives rise to relatively mild symptoms, but these can last for several months, and a large number of people are affected each year. It responds well to treatment with antihistamine, and 2 per cent disodium cromoglycate. The conjunctival fluid usually contains eosinophils, and raised levels of IgE. In one study on 25 patients with seasonal allergic conjunctivitis, and 14 with perennial allergic conjunctivitis, eosinophils were found in carefully prepared samples of tear fluid in 25 per cent and 43 per cent respectively. Seventy eight per cent of the patients with perennial allergic conjunctivitis had higher levels of IgE antibodies in their tears than in serum, suggesting that house dust mite antigens were responsible for their disease³⁹².

The rabbit, and guinea pig conjunctival sacs have been used for many years as sites in which to test for immediate hypersensitivity reactions to drugs, and chemicals. It has been suggested that this assay detects the presence of mast cells sensitization with IgE. It was also proposed that antigen caused them to release histamine, which attracted blood eosinophils into the conjunctival epithelium¹⁹⁵³.

11.2. Corneal diseases.

The significance of finding eosinophils in the human cornea has been reviewed in 1986¹⁰⁸⁰. They have been seen in failed corneal transplants, and smaller numbers were seen in a wide variety of other corneal diseases.

11.3. Retinal diseases.

One of the best known causes of an eosinophilic lesion in the retina are *Toxocara canis* infections, which can produce a diffuse unilateral subacute neuroretinitis¹³³³, in addition to focal lesions, which may mimic a retinoblastomas.

11.4. Diseases of the orbit.

There are several reports of eosinophilic granulomas affecting the orbital frontal bones, and the lateral orbital wall⁵⁵³.

12. Chapter C 12. Urological diseases.

Eosinophils are prominent in the urological system in several parasitic diseases, in a variety of granulomatous, and allergic diseases, and in patients undergoing renal, or peritoneal dialysis, Figure C12-1. The presence of eosinophils in the urine can be of diagnostic value in a number of diseases.

Fig. 12-1: Eosinophils in urological diseases.

12.1. Kidney, and ureteral diseases.

Drug-induced interstitial nephritis, and acute tubular necrosis can both produce an eosinophil-rich infiltrate into the kidneys, and eosinophiluria. In a study reported in 1987, on 81 patients, 28 of whom had a hypersensitivity reaction to non-steroidal inflammatory drugs, or antibiotics, both types of renal lesion were detected by renal biopsy¹⁰²⁵. Eosinophil-rich allergic reactions to drugs can also produce glomerular lesions⁴⁹. See page 564.

12.2. Renal dialysis, and eosinophils.

Patients with renal failure often have a small increase in blood eosinophil counts, and their serum ECP levels are usually raised⁷³⁸. In a study from Denmark, in 1987, 13 per cent of 99 patients about to start maintenance hemodialysis had mean counts of 0.8, range 0.4-2.7 x 10⁹/L, and many of them were male⁶⁰¹. Renal haemodialysis can produce a rapid fall in blood eosinophil counts. This was well documented in 1980, in eight patient whose blood eosinophil counts fell to 80 per cent of the initial levels. They gradually returned towards normal by three hours¹²⁵. Haemodialysis also decreased the density of white blood cells, possibly as a result of the formation of C5a by the dialysis membrane¹⁰⁷¹.

It has been known for over 20 years that treatment of end-stage renal failure by either peritoneal dialysis, or haemodialysis, can induce a marked increase in the number of eosinophils in the peritoneal fluid, and occasionally in the blood.

As eosinophils are rarely seen in the peritoneal fluid from patients with bacterial peritonitis, a search for eosinophils in cloudy peritoneal fluid can be a useful, and rapid way to distinguish these two types of peritonitis¹⁶⁷³.

Peritoneal fluid from patients having ambulatory dialysis can also be a useful source of eosinophils, which have traversed into tissues, for structural, and functional studies. In one report from Washington D.C., U.S.A. in 1986, 0.3 to 288 X 10⁸ eosinophils were obtained from each bag⁵⁸³.

12.2.1. Peritoneal dialysis, and eosinophils.

There are many reports of an eosinophilia in patients undergoing maintenance peritoneal dialysis. In 1981 three patient were reported from Newcastle-upon-Tyne, U.K.⁶⁸⁸, and ten were described in the U.S.A.⁸²⁶. Eight more were reported in 1982¹⁶⁷³. Twenty-nine patients with this complication were reviewed in 1985, and previous cases were reviewed³⁰⁴. The typical clinical features of these patients were that two weeks, to four months after implantation of the peritoneal catheter, they developed a cloudy peritoneal fluid, without having fever, abdominal pain, nausea, vomiting, or rebound tenderness, which are characteristic of a peritoneal infection. They did not have a skin rash, or itching. The fluid contained as many as 1.9 x 10⁹ white blood cells /L, and up to 95 per cent were eosinophils. Some patients also developed a blood eosinophilia. In most patients the fluid became clear again after several days, or weeks, without the use of antibiotics. Quantitative counts of peritoneal fluid eosinophils, and blood eosinophils, and the time course of disappearance of peritoneal eosinophilia was assessed in 61 patients in Pittsburgh, U.S.A., in 1984¹⁴²³, and it was found that the counts fell from initial high levels, and continued to fall at six months.

The cause(s) of this eosinophilic response has been the subject of considerable interest in the last few years. One possibility was that they had become sensitized to impurities released from the tubing, bags, or fluids used for the dialysis. Ethylene oxide was considered to be a possible sensitizing agent⁷⁰⁸, as it can be eluted from dialysis tubing¹³⁷⁸. Plasticisers in dialysis bags were also considered¹²⁶⁵. An explanation of this kind was supported by a study in 1986 which showed that the frequency of tubing changes was linked to the incidence of eosinophilia⁹⁰.

The possibility that eosinophils in the peritoneal fluid appear as a result of the entry of air into the peritoneal cavity was tested in two patients on continuous ambulatory peritoneal dialysis (CAPD) in 1985. The number of eosinophils increased from a mean of 1.5 to 50 in one patient, and from 0 to 60 x 10⁹/L in the other, during the week following air injection, showing that this could be a factor in the appearance of eosinophils in peritoneal fluid³⁹⁴. In 1987 the same group reported that a peritoneal fluid eosinophilia was produced in four of five patients on CAPD by the injection of 100-

500 ml air into the peritoneum. With the larger amounts of air, the eosinophilia persisted for five weeks³⁹⁵.

12.2.2. Haemodialysis, and eosinophils.

Nephrologists have known for many years that patients with end stage renal failure on haemodialysis can develop an eosinophilia. As the highest counts were usually seen in patients who had been on dialysis the longest, and as a raised serum IgE level was more common in haemodialysis patients with an eosinophilia than those without^{1288,1875}, it was natural to suppose that an allergic reaction to a component in the dialysis fluid was responsible. However alterations in the rinsing procedure did not affect the development of the reactions in a study reported in 1984¹⁸⁷⁵.

Among a group of 240 patients undergoing haemodialysis in Lisbon, Portugal, 14 per cent had blood eosinophil counts greater than $0.4 \times 10^9/L$. The mean eosinophil count was 0.613, and the range $0.410-1.780 \times 10^9/L$. Thirty one per cent of the HBsAg positive patients who were on coil dialysis had an eosinophilia. There was no difference in the incidence of eosinophilia between patients on coil, and on capillary dialysis machines, and there was also no relationship to their parathyroid status¹⁴³⁶.

In studies from Uppsala, Sweden, reported in 1982⁷⁴², and 1984³⁸⁸, it was found that blood eosinophil, and neutrophil counts fell during the first ten minutes of haemodialysis, although they subsequently returned to normal levels. During the first two hours of dialysis, the serum ECP level also rose. It was then seen that, when fresh blood containing eosinophils was passed through a dialyser, the ECP level rose steadily, so that at two hours it was 76 times higher than at the start, and the eosinophil count had fallen to 67 per cent of the initial level. This effect on eosinophils was most marked when cuprophane membranes were used. It was suggested that during hemodialysis, the contact of eosinophils with the dialysis membrane caused them to release their constituents into the blood. These would not attach to the membrane, as experiments showed that only 10 per cent of added ECP bound to dialysis membranes. This possibility was supported by a study from Italy in 1985, which suggested that MBP was released from eosinophils, and that this neutralized some of the heparin anticoagulation which their patients were receiving¹⁵⁵⁶. Dialysis membranes can cause complement activation, with aggregation, and stimulation of neutrophils degranulation in the lungs. Whether eosinophils could also be affected by complement activation products in this way is not known.

Microangiopathic changes in the skin of patients undergoing haemodialysis were reported in 1987, from a group of 63 patients with end stage renal failure in Japan. There was marked basement thickening of epidermal capillaries, and it was suggested that this was due to inflammatory reactions induced by uraemic toxins. As some of these patients has an eosinophilia, it was thought possible that eosinophils could have been involved in the production of these vascular changes⁸³⁵.

12.3. Eosinophilic cystitis.

Eosinophil cystitis, which was originally defined in 1960 as 'eosinophilic granuloma of the bladder', is a rare disease. Only 39 cases were reported by 1982¹⁰⁸⁷, although by 1987 there were a total of 50 case reports. It is more common in men than women, and there may be a history of allergy, or previous trauma to the bladder. These patients had episodes of haematuria, frequency of urination with dysuria, and suprapubic pain. At cystoscopy there were raised erythematous mucosal lesions which often looked like polyps, or even tumours. In the acute stages of the disease, the mucosa, submucosa, and muscularis of the bladder wall was found to be infiltrated with many eosinophils, and mast cells, with muscle necrosis, and surrounding oedema.

Later, chronic inflammation lead to fibrosis, especially of the superficial layer, and this required urinary diversion, and cystectomy in some patients¹⁶²³. About one third had an eosinophilia. One patient with eosinophilic cystitis also had Glanzmann's thrombasthenia¹⁹⁵.

Light microscopy, and EM studies on biopsies from the bladder of a 63-year old woman with eosinophilic cystitis showed giant cells ingesting eosinophils, and free granules⁴⁴. An assay for ECP in urine was developed in 1986, and it was recommended as a useful technique for identifying patients

with eosinophilic cystitis⁵⁹⁵, but this was not confirmed in a study the following year¹¹²¹. Eosinophilic cystitis can cause obstruction of the ureteral orifices with eosinophil-rich inflammatory tissue¹²¹⁹. This may necessitate surgical resection, and reimplantation of the ureters, and/or repeated hydrostatic dilatation¹³⁰⁸. Rarely, the ureters only are effected by a similar process⁷⁷⁵, or an eosinophilic granuloma¹⁸¹⁴.

An 'allergen provocation test' with tomatoes in a 35-year old woman with eosinophilic cystitis showed an eosinophil-rich infiltrate into the bladder, and it was suggested that this, or similar allergens could be the cause of this disease in some patients¹⁵⁵¹.

Eosinophilic cystitis can also occur in children, when it appears to have a more benign course than in adults¹⁷²⁷.

12.4. Eosinophils in the urine.

Eosinophils can occur in the urinary sediment, with other inflammatory cells, in a wide variety of diseases, as reviewed in 1987³⁵⁹. In a study from Chicago, U.S.A., in 1985, eosinophils were found in the urine of 65 of 470 patients whose urine was specifically examined. Infections in the upper, and lower urinary tract was responsible in 29 (45 per cent), and nine patients had acute interstitial nephritis³⁶⁰. About 60 per cent of patients with interstitial nephritis have eosinophiluria^{321, 1728}, but the incidence can be even higher. Eosinophiluria was found in ten of 11 patients with drug induced acute interstitial nephritis, reported in 1986¹²⁸⁴. Other diseases where they have been seen include rapidly progressive glomerulonephritis, acute prostatitis, and occasionally acute cystitis, and postinfectious glomerulonephritis. A patient with systemic atheroembolism, renal damage, and eosinophiluria was described in 1986²⁰¹⁸.

12.5. Prostatic diseases.

Eosinophils are prominent in the prostate gland in several types of granulomatous prostatitis, including (1) allergic eosinophilic prostatitis, in which eosinophil-rich granulomas are found in patients with asthma, or other allergic diseases, (2) non-specific granulomatous prostatitis, and (3) post-transurethral resection prostatitis. Seven patients at the Johns Hopkins Hospital, Baltimore, U.S.A. with eosinophil-rich granulomatous prostatitis, were described in 1984⁵²². Three had nonspecific granulomas, and four had post-transurethral resection granulomas. Two other cases of non-allergic eosinophilic prostatitis were described in 1984^{1978, 1481}.

12.6. Renal allograft rejection.

In renal transplant patients, an increase in blood eosinophil counts is invariably associated with an acute rejection episode 1-4 days later, but it is not related to the extent of the cellular infiltrate into the graft¹⁰³⁶. In one patient, the eosinophilia returned to normal four days after the removal of a rejecting graft¹⁵⁹⁶. Eosinophils are usually present within rejecting renal allografts, where they may play a role in the rejection process itself, by causing further tissue injury. This possibility was discussed in 1986 following an analysis of acute rejection episodes in 112 patients with renal allografts, which showed that an increased percentage of eosinophils in the blood, and in renal biopsies was linked to a poor outcome from rejection episodes¹⁹⁰¹.

13. Chapter C 13. Female genital tract diseases, and pregnancy.

It has been known for many years that eosinophils are prominent in the endometrium of rodents undergoing oestrus. Experimental studies have looked for oestrogen receptors on rodent eosinophils, and an effect of eosinophils on the endometrium. This work has been based on the hypothesis that eosinophils may release a number of molecules, including leukotrienes, during oestrus which could affect the permeability of uterine blood vessels. However, in man, eosinophils do not appear to have oestrogen receptors, and only small numbers are found in the endometrium. The presence of large numbers of eosinophils in the human genital tract is always abnormal. The principal disorder of the genital tract in which eosinophils are prominent is carcinoma of the cervix as described in Chapter

C04. There have been no studies using monoclonal antibodies to look for secreted eosinophil products in the uterus in different diseases. There may be a role for MBP in pregnancy, although there is no evidence that eosinophils are involved in gestation.

13.1. The uterus, and uterine diseases in man.

The number of eosinophils in the human uterus varies during the menstrual cycle, so that they are least during the luteal phase, when implantation may occur²⁸⁶. They are mainly localized, with larger numbers of neutrophils, in the endocervix, and lower uterine segments. It has been suggested that peroxidases, which are produced by granulocytes in these sites, may have a protective role against bacterial infections¹⁴⁴⁵. A marked cervical eosinophil-rich infiltrate, of unknown cause, was reported in 1977 in a baby who died at birth, with dysplastic kidneys, and hypoplastic lungs³⁷⁷.

13.1.1. The uterus in animals.

There is an extensive literature, much of it from Tchernitchin's group in Chile, on the occurrence of eosinophils in the rodent uterus. This was reviewed in 1986⁶⁵⁶. Their main conclusions were that oestrogens induced eosinophils to localize, and degranulate in the wall of the uterus, where they contributed to the large amounts of uterine peroxidase which is present in late oestrus. It is not known whether these eosinophils induce a vascular permeability increase that occurs at this time, or whether they have a role in protecting the rodent uterus from infection.

13.2. Pregnancy.

Although there is either no change in blood eosinophil counts during pregnancy, or a slight fall¹⁴²⁴, plasma, and serum MBP levels (but not ECP, EDN/EPX or CLC protein levels) have been found to increase to about 7.5 ug/ml, at 20 weeks, when it levels off, only returning to previous levels at term¹¹²⁸. MBP has been detected in the placenta, in anchoring villi, placental X-cells, and giant cells¹⁸⁸², and this may be the source of the serum MBP in pregnancy. Pregnancy-associated MBP appears to be identical with eosinophil-derived MBP¹¹²⁹. As there was a relationship between increased plasma MBP levels late in the third trimester, and the onset of spontaneous labour, it was suggested that plasma MBP levels could be useful in predicting when this was about to occur¹⁸⁸¹. An increase in plasma MBP has also been noted in the pregnant gorilla, cynomolgus monkey, and rhesus monkey, and MBP was also found in primate placental X cells¹⁸⁸⁰.

14. Chapter C 14. Cardiovascular diseases.

Eosinophils have an important relationship with a number of heart diseases. Recent research has suggested that eosinophils may cause endomyocardial fibrosis, both in patients with a marked, and persistent eosinophilia, and in tropical endomyocardial fibrosis. There are also a range of inflammatory heart muscle disease in which eosinophils are often found, including cardiac rejection, drug reactions affecting the heart, and the Churg-Strauss syndrome. Cardiac involvement in systemic diseases producing an eosinophilia is usually a serious prognostic sign, and many of these patients develop arrhythmias, or heart failure. It is therefore important to examine the heart carefully in all patients who have an eosinophilia. Any abnormalities which are detected at the bedside, or following electrocardiograms (ECGs), and chest radiographs should be followed up with further studies, providing treatment is not delayed, as many of these disorders can progress rapidly.

14.1. Diseases of the heart, and pericardium.

14.1.1. Pericardial diseases, and pericardial effusion.

There is an unusual eosinophilic disease of unknown cause, which gives rise to constrictive pericarditis. In a study from Hawaii, U.S.A., reported in 1984, three patients were described with constrictive pericarditis, in whom eosinophils were found in the resected tissue. In one of them there was a blood eosinophil count of $2.2 \times 10^9/L$. They were treated successfully by pericardectomy¹⁸⁶⁷. I have also seen two women, aged 45, and 65 years with this disease, and both needed pericardectomies. Granulomas were not found in the pericardium, and there was no evidence for a systemic illness to

account for their pericardial lesions, and eosinophilia, although one had a history of asthma. Details of the first patient were published in 1978³⁵⁸. The second patient had a similar history, although her eosinophilia persisted, despite continuous treatment with steroids for over 10 years.

There are very few reports of the discovery of large numbers of eosinophils in pericardial fluid, but they have been described in patients with asthma, malignant lymphomas, Hodgkin's disease, and drug reactions. A 28 year-old woman was described in 1964, who had pulmonary eosinophilia, and pericarditis, in whom the pericardial fluid contained $11.3 \times 10^9/L$ white cells, 96 per cent of which were eosinophils⁷³³. Four previous case reports of eosinophils in pericardial fluid were discussed in a case report in 1983, of a woman with asthma, and blood eosinophil counts of $6.2 \times 10^9/L$, who developed a pericardial effusion, containing 540 ml, and 80 per cent eosinophils⁸⁸⁵.

14.1.2. Cardiac allograft rejection.

Rejection of the transplanted human heart is usually associated with an increased inflammatory infiltrate. In a study on 18 transplants, reported in 1987 from the Johns Hopkins Hospital, Baltimore, U.S.A., more eosinophils were seen in biopsies showing myocyte necrosis, than in pre-rejection biopsies, but they were not useful as a predictor of rejection episodes⁷⁹².

14.1.3. Coronary heart disease.

As coronary artery occlusion induce blood neutrophils to localize in areas of ischaemia, and necrosis, with some extension of the areas of myocardial injury¹⁶⁹⁰, it is important to consider whether eosinophils can also be localized in the areas of myocardial ischaemia. This is of potential importance as eosinophils can produce large amounts of reactive oxygen species, and secrete their cardiotoxic proteins.

Raised levels of serum ECP were reported in 1979, in a group of Swedish patients admitted to hospital with a myocardial infarction, even though none of them had an eosinophilia. This report suggested that eosinophils might be involved in the inflammatory response to myocardial injury, although they are seldom seen in histological sections of myocardium. This study also showed that treatment of normal individuals with 0.5 mg methylprednisolone did not alter serum ECP levels, although it caused blood eosinophil counts to fall, suggesting that the rise in serum ECP following myocardial infarction was not a steroid-induced effect⁷⁴¹. Eosinophils may also affect the long-term outcome of a myocardial infarction, as a study from Nashville, U.S.A., in 1985 found that cardiac rupture following myocardial infarction seems to be related to the presence of an increased number of eosinophils in the area of myocardial damage⁷⁴.

14.1.4. Myocardial diseases.

Eosinophil infiltrates can also be found in the heart in a wide variety of inflammatory disorders, which we have reviewed in 1985¹⁷⁰⁰.

They include eosinophilic, and tropical endomyocardial fibrosis, which are reviewed in the next Chapter, drug hypersensitivity reactions¹⁷⁰ (see page 563), cardiac transplantation, and a poorly defined disorder called necrotizing eosinophilic myocarditis⁷⁹³. Focal areas of vasculitis, and granulomatous reactions, as seen in the Churg-Strauss syndrome, can also give rise to large numbers of eosinophils in areas of myocardial injury. Parasitic diseases, such as trichinosis, can also induce areas of myocardial injury rich in eosinophils⁴⁰. Congestive cardiomyopathy is the end result of a wide range of different types of myocardial injury. We raised the possibility that some patients may have a disease process involving cardiotoxic eosinophil constituents has been raised in one small study in which we found that serum ECP levels were higher than normal¹⁶⁹⁸.

14.2. Vascular diseases.

Eosinophils are often found with mast cells in the adventitia of veins in the lung, intestine, and other sites, but the presence of eosinophils in the vessel wall itself is uncommon, except in vasculitic disorders.

The occurrence of thromboemboli complications in patients with a persistent eosinophilia, especially HES, is an important clinical observation, suggesting that eosinophils could produce effects on the endothelium, or platelets. Although there is limited work in this area, it has been shown that

ECP can (a) produce changes in the intrinsic coagulation system, (b) reduce thromboxane production by isolated endothelial cells, and (c) occasionally stimulate platelet aggregation³⁹⁸. The relative importance of these processes in thromboembolic complications of hypereosinophilia are not known, and it is also not clear whether therapy to prevent platelet aggregation is beneficial in patients with a persistent hypereosinophilia.

14.2.1. Atherosclerosis.

Eosinophils are not a component of atherosclerotic lesions. However patients who develop the 'multiple cholesterol emboli syndrome', with visceral infarction, due to the release of cholesterol-rich plaques, for example during arterial catheterization, may have a marked eosinophilia, with thrombocytopenia, and hypocomplementaemia. Biopsies may be needed to distinguish this syndrome from vasculitic diseases¹⁹⁸⁴. An eosinophilia occurs in 80 per cent of reported patients with atheroembolic renal disease, according to a literature review made in 1987. The eosinophilia is often mild, and may last only a few days⁹¹⁴.

14.3. Vasculitis.

A large number of diseases have a vasculitic element which can contain many eosinophils. A useful review of these diseases was published in 1985¹⁵⁰⁴, and the difficulties in providing distinct categories of arteritis were discussed in 1986³⁰³.

Polyarteritis nodosa is the best known example of a systemic vasculitis, without granulomatous inflammation, and many textbooks on general medicine state that it is an important cause of a marked eosinophilia. This stemmed from early reports¹⁴⁶⁴, and difficulties at that time in distinguishing polyarteritis nodosa from the Churg-Strauss syndrome which does give rise to a marked eosinophilia. However both diseases are closely related and in the classification of patients with vasculitis put forward by Dr. Anthony Fauci in 1983⁵⁴³, they were combined under the general term 'polyarteritis nodosa group of systemic necrotizing vasculitis'.

Although hypereosinophilia is not a characteristic of classical polyarteritis nodosa⁵⁴⁵, these patients often have a mild, or a transient eosinophilia^{1581, 1246}. A moderate increase in blood eosinophil counts is also common in patients with microscopic polyarteritis with crescentic, and necrotizing glomerulonephritis. This was discussed in a study on polyarteritis nodosa, and necrotizing glomerulonephritis, from Florida, U.S.A. in 1987³⁷⁰.

Serum antibodies, which stain the cytoplasm of circulating neutrophils, are a recently discovered finding in patients with necrotizing vasculitis. These were found in two patients who also had an eosinophilia. One was a 17-year old boy with the Churg-Strauss syndrome, and cardiac involvement, and blood eosinophil counts of $18 \times 10^9/L$, and the second was a 55-year old man with pulmonary eosinophilia, and a blood eosinophil count of $4 \times 10^9/L$ ¹⁸⁹¹.

Wegener's granulomatosis which was first described in the 1930s, is a systemic disease which gives rise to necrotizing granulomas in the upper, and lower respiratory tract, glomerular lesions, and injury to the skin, joints, and peripheral nerves. It mainly affects the respiratory tract, and is discussed in Chapter C08.

Temporal arteritis can be found in young adults, and there is a recent case report of this disease in a young adult with an eosinophilia of 28-48 per cent of $9.9-13.9 \times 10^9/L$ total white blood cells¹⁸⁷.

Spontaneous dissection of the left anterior descending coronary artery, which was reviewed in 1982¹⁵⁰⁵, has been described in 54 people who developed chest pain, or died suddenly. A striking feature is an eosinophil-rich inflammatory reaction in the adventitia, with dissection in the outer third of the vessel. The coronary sinus can be involved in a similar disease process as described in a 44 year-old woman in Sydney, Australia in 1980²⁰⁶.

14. Chapter C 14 a. Endomyocardial fibrosis.

14.1. History.

Eosinophilic endomyocardial fibrosis (E-EMF) was probably first seen in 1893 in a 31-year old woman who had a tumour in the neck, with a blood eosinophil count of $57.6 \times 10^9/L$. At post mortem the right ventricular endocardium was found to be covered with a greyish-white mural thrombus¹⁴⁸⁵. In 1936 Professor W. Loeffler published detailed clinical descriptions of two patients in Basle, Switzerland, who had E-EMF, although at that time it was called endocarditis parietalis fibroplastica, or Loeffler's endomyocardial fibrosis. His first patient was a woman of 45 who died one year after presentation, with severe right, and left sided eosinophilic endomyocardial disease. The second was a 37-year old man who died after 21 months, with similar complications. In one patient the heart disease was associated with a severe illness whereas the second patient presented with heart failure without a systemic illness. Another difference was that the eosinophilia increased in one patient whereas it disappeared shortly before death in the other¹⁰⁹². An English translation of Loeffler's seminal paper was published in 1948¹⁰⁵⁹, and the disease was reviewed in a German paper published that year¹⁵⁸.

A second form of this disease, called tropical endomyocardial fibrosis (T-EMF), as it is mainly confined to tropical regions, has been known since 1946¹⁴⁵. For several years these diseases were considered to be distinct, although the possibility that there was a disease spectrum ranging from 'tropical' endomyocardial fibrosis to 'Loeffler's' endomyocardial fibrosis on the other, was suggested by Davies, and Ball, as early as 1955⁴⁰⁶. Histological studies in the 1970s demonstrated that they were pathologically indistinguishable, despite the different clinical settings in which they were found. In the first of these studies, in 1970, detailed post mortems were carried out on three men aged 25, 26 and 35 who died with HES. The findings supported the possibility that tropical endomyocardial fibrosis might be a later stage, or an inactive form of E-EMF¹⁵⁰⁰.

In the second, a comparative pathological, and clinical review of 90 patients with E-EMF was carried out in 1973. The eosinophilia was of unknown cause in 50 per cent, reactive in 25 per cent (polyarteritis, asthma, drug sensitivity, Hodgkin's disease, and carcinomas), and due to eosinophilic leukaemia (or HES) in 25 per cent. Among 30 hearts examined, 10 were in the acute necrotic stage, with an illness lasting a mean of 5.5 weeks, eight were in the later thrombotic stage, with an illness of 10 months, and 12 were in the fibrotic stage, with an illness lasting 24.5 months. Figure C14a-1. Samples from 16 patients in the thrombotic, and fibrotic stages of E-EMF were compared with 32 specimens from patients with T-EMF, and no significant pathological differences were found. It was concluded that T-EMF, and E-EMF had a similar pathogenesis, such as a toxic effect of eosinophils on the myocardium²⁰⁹.

Fig. 14-1: Eosinophilic endomyocardial fibrosis.

By 1972 the frequent occurrence (20 per cent or more) of E-EMF in patients with 'eosinophilic leukaemia', (two rare disorders occurring together with high frequency), led to the suggestion that there might be an intimate cause-effect relationship between the two disorders, similar to that of the cardio-pulmonary lesions in the metastatic carcinoid syndrome:

'forceful cardiac contractions might cause excessive destruction of eosinophils, and a continued high concentration of their chemicals, and hydrolytic enzymes within the heart. This in turn may cause endocardial inflammation, and injury. In prolonged eosinophilia, regardless of etiology, this sort of change is likely to occur more frequently, and to be more severe. It would appear that the endocardial lesion is the result of the eosinophilia ...'¹⁹⁶².

It is now thought likely that eosinophils are important in the development of the endocardial lesions in both forms of the disease. This association has been most clearly demonstrated in E-EMF, and there is strong circumstantial evidence that a similar, but more slowly progressive disease occurs in the tropical form of the disease.

However the praedilection of the endocardium for this type of damage has not been explained, but it does raise the possibility that regional differences in disease susceptibility within the heart may account for a number of other focal, or diffuse cardiac diseases of unknown cause. It also shows how an inflammatory response can give rise to distant effects on other otherwise normal tissues,

especially when the inflammatory process is prolonged.

Twenty six patients with E-EMF were described during the period 1936-57, and reviewed by Weiss Carmine. Several patients had arteritic lesions, and thrombo-emboli were also noted to be an important feature of the disease¹⁹⁰³. Another review was published in 1955⁸⁰⁴.

A second major review of E-EMF was carried out in 1963 with details of 14 further patients, including three more of their own, by Brink, and Weber (1963). This paper suggested that high blood neutrophil counts might be associated with endomyocardial fibrosis, although this has not been substantiated subsequently²⁰⁷.

During the past decade, the development of effective invasive techniques for studying the heart have enabled clinicians to diagnose both diseases at an earlier stage than was previously possible. The advent of 2-D echocardiography, endocardial biopsy, and surgical treatment, has also enabled clinical, and experimental studies to be carried out on the nature, and pathogenesis of EMF.

We reviewed the clinical features, and pathophysiology of E-EMF in 1983¹³¹⁸, 1985¹³¹⁹, and 1987^{1691, 1689}.

By 1980, over one hundred patients with E-EMF had been described¹⁸⁹³. Because an underlying eosinophilic disorder, which was often HES, often dominated the clinical picture, the heart disease was usually described in less detail. However, the general awareness that cardiac complications were the main presentation in at least half of the patients with HES, and that over 80 per cent would finally develop heart disease¹⁶⁸³, stimulated detailed cardiological studies on these patients.

Two series of patients with HES, who have a high incidence of E-EMF, have been studied over more than a decade in the U.S.A.¹³⁶⁶, and by us in the U.K.¹⁶⁹⁴.

Although EMF, with or without an eosinophilia, was initially only known in Uganda⁴⁰⁴, and Nigeria, there have been detailed reports from a number of other countries in the last few years including South India, in 1983^{402, 310} and 1986¹⁰⁴, Ivory Coast in 1985¹⁶³ Venezuela in 1983,¹⁴⁵⁷ Brazil in 1984²⁸⁴, Japan in 1985¹⁷⁴³, Thailand in 1984¹⁷¹⁹, Spain in 1982¹⁸²⁸, the Middle East in 1985⁵⁴⁹, and France in 1983⁴⁷¹. In french speaking regions, no attempt was made to distinguish the tropical forms of the disease from the eosinophilic form. In other countries, they are usually described separately.

In 1979, an analysis of the cardiological features of E-EMF was reported from the N.I.H., Bethesda, U.S.A.¹³⁶⁶. This paper reviewed 65 previous case reports, in which 57 per cent had histological features of E-EMF, combined with descriptions of a further 26 patients. M-mode echocardiograms were thought to show an increased left ventricular wall thickness and raised left ventricle mass in 18 of the 22 patients (82 per cent). It was suggested that this was an early feature of cardiac involvement in HES, and might be helpful in following the progression of the disease. In seven of eight patients, who did not show a good response to treatment with prednisolone, with or without hydroxyurea, there appeared to be a progression of echocardiographic abnormalities, whereas in eight of 10 patients adequately treated, this did not occur. Although this was one of the first detailed descriptions of the disease, using a combination of echocardiography, and ventricular angiocardiograms, cardiac biopsies were not done in any of the 26 patients. Three of the 26 patients died: one following busulphan treatment, one with a possible arrhythmia, and one with the Budd Chiari syndrome. Although only one patient had emboli, because of the likely presence of thrombi in the left ventricle of some patients, it was recommended that anticoagulation should be carried out. Several patients had angiographic evidence for coronary artery disease, but this was not a clinical problem in any of these patients.

The cardiological features of E-EMF seen in outpatients range from apparent normality, in patients at the first stage, to gross heart failure in patients in the late fibrotic stage of the disease. In 1974, a review of the cardiovascular complications which occurred in the N.I.H. patients with HES, and eosinophilic leukaemia, showed that they were the same, and that the endocardial lesions were indistinguishable from those seen in T-EMF¹⁴⁹⁹. A further report from the same centre was made in 1983⁷⁵⁵.

The awareness of the high incidence of eosinophilic heart disease in patients with a persistent eosinophilia has made many clinicians more alert to the earlier signs of cardiac involvement: asymptomatic murmurs, micro-embolic disease, especially splinter haemorrhages, minor strokes, and non-specific ECG abnormalities²⁶². Today, eosinophilic heart disease can be recognized when it gives rise to a number of different abnormalities, besides a restrictive cardiomyopathy. In the most recent series of 14 patients, which was published in 1985 by Take, and colleagues in Japan¹⁷⁴³, five patients had acute carditis, three had electrical disturbances, three had ventricular dilatation, and three had a restrictive cardiomyopathy.

14.2. Eosinophilic endomyocardial fibrosis.

The clinical features of the underlying eosinophilic disorders may mask or affect the clinical presentation of the heart disease, which is usually diagnosed at a late stage in the development of the cardiac pathology, although methods are now available for recognizing this at an early stage.

14.2.1. Eosinophilic diseases with endomyocardial fibrosis.

By definition, E-EMF always occurs in patients with an eosinophilia.

It can occur in patients with any of the principal causes of a chronic eosinophilia. For example a 52 year old man was described in 1959, who had tuberculosis, treated with isoniazid, and PAS. He developed an eosinophil count of $14 \times 10^9/L$, and died later with E-EMF. Other early work, describing patients with infections, an eosinophilia, and endomyocardial disease were also reviewed in this report. These included patients with syphilis, malaria, tonsillitis, amoebiasis, dengue fever, and allergic reactions to parasites, including *Loa loa*¹⁴⁸⁶. In Western countries the commonest disease association is HES, but it also includes parasitic diseases, chronic drug reactions, and tumour-induced eosinophilia.

E-EMF complicating HES can occur in infancy, and childhood. Examples include:

- a girl aged five years, who died within a month of presenting with HES. At post mortem, there were thrombi in the left ventricle, and right atrium, and emboli in a pulmonary artery, but there was no endomyocardial fibrosis, probably because the illness was of such short duration¹⁴⁷⁹.

- a child aged seven years, who developed HES. She died after a 10 month illness, and was found to have bilateral E-EMF, with peripheral emboli originating from the left ventricle⁹⁸².

- a 9-year old boy died after a 10 month illness due to HES, and showed the features of E-EMF at post mortem¹³²⁴.

- a boy aged 12, who was found to have an eosinophil count of $170 \times 10^9/L$. Three years after the onset of his illness, he died with congestive cardiac failure, due to bilateral E-EMF¹⁰⁷⁵.

E-EMF is an important complication of eosinophilic leukaemia. A review in 1961 showed that, among five of nine patients with acute eosinophilic leukaemia with blast cells, five had cardiovascular abnormalities. Seven of the 11 patients, who only had mature blood eosinophils (which might today be called HES), also had heart involvement. This is a total of 12 cases of heart disease in 20 patients, (60 per cent)¹⁵⁶. In 1978 E-EMF was described in a patient with Phi positive chronic myeloid leukaemia, and a hypoplastic right ventricle¹⁶⁵¹.

Although it is unusual for patients in Western countries with E-EMF to have a parasitic infection, in 1972 a 40 year old woman was described who died with E-EMF, and an eosinophilia of '60-70 per cent', and who was found to have a 10 metre-long *Taenia saginata* worm in her intestines⁵⁰. Patients with E-EMF, and fascioliasis have also been reported from France in 1975³⁸⁶, and 1978¹⁴³⁴.

Patients with a drug-induced hypersensitivity disease may develop E-EMF. In 1946 a 41 year old man was described, who had been treated with neoarsphenamine for syphilis, and developed an eosinophilia of $2.5 \times 10^9/L$, with heart failure, which was found to be due to E-EMF⁴⁹². In 1959, E-EMF was described as a complication of an eosinophilia due streptomycin hypersensitivity⁶²⁴.

Vasculitic diseases with an eosinophilia are also known to be able to lead to the development of E-EMF, including the Churg-Strauss syndrome¹⁰²⁹.

E-EMF has been seen in patients with lymphocytic leukaemia-associated hypereosinophilia^{180, 629}.

An unusual clinical story of a woman with chronic eosinophilic pneumonia, who developed

hypereosinophilia, and E-EMF, was given in a clinico-pathological conference in 1980. When she was 31, she developed pulmonary infiltrates with an eosinophil count of $6.4 \times 10^9/L$. This improved on treatment with prednisolone, 13 mg per day. At age 35, after the steroids had been stopped, and the eosinophilia had recurred, she developed chest pain, and went into severe heart failure with an enlarged heart, and a pericardial effusion. A right ventricular biopsy showed E-EMF in the acute necrotic stage. Treatment with steroids, warfarin, and digoxin, resulted in an improvement. The discussion by Dr Shelly Wolff, centered on the differential diagnosis of this unusual combination of clinical problems. It illustrated the wide range of eosinophilic disorders in which E-EMF can occur

2014.

14.2.2. Clinical features.

There are now over a hundred case reports of patients with E-EMF.

As the majority of patients with E-EMF have HES, the most extensive studies have been carried out in this group of patients, and most is known about the clinical features of the disease in this setting. About half the patients presented with late stage disease, and half the developed it during the subsequent several years. The age, and sex incidence of the patients reflect that of HES: a mean age of 37 years.

In the late stage of the disease it has many features in common with other forms of restrictive cardiomyopathy, and it can be confused with pericardial constriction. This difficulty in distinguishing constrictive pericarditis, and E-EMF was well illustrated by a case report from the Johns Hopkins Hospital in 1952. The correct diagnosis was only made when surgery was carried out to remove what was thought to be a constricting pericardium¹¹⁷⁶. Loeffler himself described the ways in which E-EMF is now known to affect cardiac function: 'endocardial thickening prevents normal diastolic relaxation of the ventricles, and thrombotic masses decrease the capacity of the ventricles'¹⁰⁹³. Unlike T-EMF, ascites is uncommon, and oedema of the face and upper extremities is not often seen, even when the patients are in gross heart failure, with persistently high right atrial pressures, and pulmonary oedema.

The main features at the bedside are signs of chronic heart failure, with wasting, and oedema, and heart murmurs which are most marked in patients with mitral incompetence.

E-EMF is invariably associated with a systemic disorder giving rise to the eosinophilia. This ranges from a malignant process, which may remain hidden for several months, or years, and HES, to rare cases of eosinophilic leukemia. In some patients with acute eosinophilic leukemia their blood disease may progress so rapidly that they do not have time to develop cardiac disease before they die.

14.2.3. Investigations of E-EMF.

The investigations of E-EMF can be considered under two headings: outpatient investigations, and inpatient invasive studies designed to determine the extent of the lesions, and the potential value of surgery.

The cardiac silhouette is generally normal in patients with the early, or later stages of E-EMF, until heart failure supervenes. In occasional patients calcification can be seen in lateral chest radiographs, although this is much more common in T-EMF. Marked calcification was reported in one patient¹⁰⁵⁷. When pulmonary oedema occurs, this has no special features. Pleural effusions, and pericardial effusions are sometimes seen.

14.2.3.1. Electrocardiography in E-EMF.

Electrocardiograms (ECGs) are normal until the later thrombotic, and fibrotic stage of the disease have developed, when there are repolarization changes, characteristic of endocardial lesions. Following the development of heart failure, a number of other abnormalities in the ECG can develop, but none of these are diagnostic.

Holter monitoring has generally not been of great help, except in defining the types of arrhythmias that often develop sporadically in patients who have the late stage of the disease, and who may be improved by anti-arrhythmic therapy. However, as arrhythmias usually respond to successful cardiac surgery (valve replacement with, or without endocardectomy), arrhythmias seem to be more related

to heart failure than to involvement of conducting system, or other sites by the fibrotic process³³⁶. Conduction defects have been reported on several occasions⁹¹⁰. Arrhythmias were a problem in a 48 year old man with E-EMF, who had conduction defects, a wandering atrial pacemakers, and intermittent 2:1 dissociation, which required treatment with a pacemaker¹⁶⁵². Another example was in a 79 year old man who developed complete heart block¹⁴⁶⁷. This study also reviewed 65 other cases of E-EMF, and found that 19 (29 per cent) had conduction disturbances. An His bundle electrogram has been carried out on a patient with E-EMF. Right atrial pacing showed increased P1-A interval from 25 msec to 100 msec, followed by Mobitz type 1 block. This suggested that there was a conduction delay in the atrium, and AV node, possibly due to fibrous tissue. Details of the catheterization, and angiocardiology findings in this patient were also published⁷⁰⁵. It may be very difficult to place a transvenous pacing electrode into the right ventricle, due to the presence of thrombus, and fibrosis in the cavity,¹⁵⁸³, and an open procedure may be preferable.

Ventricular fibrillation has been documented in a few patients.

14.2.3.2. Echocardiography in E-EMF.

Echocardiography is the most useful technique for defining the structural changes within the heart, and the abnormalities in the ventricular wall which are characteristic of endomyocardial fibrosis.

M mode echocardiograms are most useful in defining the mitral valve defect that occurs in the late stage of the disease. M-mode echocardiography findings were described in 1977 in ten patients with HES studied at N.I.H., Bethesda, U.S.A. Symmetrical thickening of the left ventricle of greater than 11 mm, with increased left ventricular mass (greater than 275 g) was reported in all the patients. These observations have not been confirmed in other series of patients with E-EMF, and they remain puzzling¹⁹¹.

2-D echocardiography, which can be combined with colour coded enhancement of the images, has provided clear evidence during life of the sites of involvement of the heart in endocardial fibrosis, and the abnormalities in the septal wall, and the presence of thrombi within the heart. It has become the technique of choice in first-line investigations of patients suspected of having endomyocardial disease^{182, 1509}. Unfortunately, only patients in the late stage of the disease show abnormalities which can be considered characteristic of this disorder. These include enhanced echos from the endocardium in either ventricle, septum, and the base of the posterior papillary muscle.

In 1980 the findings were published of 2-D echocardiography in 21 patients at N.I.H.¹³⁶⁶. In nine patients who had clinical evidence of mitral regurgitation, the 2-D echo features were correlated with the gross features seen at operation, or at post mortem. In six patients who had peripheral emboli, the presence of thrombus, or thickening of the posterobasal wall of the left ventricle was detected, and it was suggested that this was the site of origin of these emboli⁷⁰³. Three patients with HES were described from Philadelphia, U.S.A., in 1983, with cardiac dilatation on echocardiography⁴³⁴. Mural thrombi have also been clearly shown by echocardiography¹⁰⁰⁹, and computed tomography¹³⁷⁷. In 1987, pulsed Doppler echocardiography showed the marked contribution of right atrial systole to pulmonary flow, in a woman with HES, and a large right ventricular thrombus¹⁴⁴⁶.

A detailed report on echocardiographic studies on E-EMF was reported from Hungary in 1982¹⁰⁵⁸, and this led to the correct diagnosis in six of eight patients⁶⁹.

In London, M-mode, and two-dimensional echocardiography were done on nine patients with E-EMF, among our series of patients with HES looked after at Hammersmith Hospital. The value of this series, which was also published in 1982, was that all the patients had undergone full cardiologic investigations, and the extent and staging of their heart disease was known. Amplitude processed 2-D echocardiography appeared to be more sensitive than conventional 2-D echocardiography in detecting areas of increased relative echointensity. M-mode echocardiography only showed non-specific abnormalities, but was particularly useful in assessing the functional defects in myocardial function, and mitral valve disease. It was also clear from this study that echocardiography was not as sensitive as endocardial biopsy in detecting the earlier stages of the disease. This limitation may be important if echocardiography is to be used to monitor the effects of treatment on the progression

of endocardial fibrosis, as it may not be able to detect structurally small, but functionally significant deteriorations in cardiac performance³⁹⁷.

14.2.3.3. Cardiac catheterization in E-EMF.

Before the introduction of echocardiography, E-EMF was often mistaken for constrictive pericarditis, and cardiac catheterization, with pressure studies, was then the only technique for providing a definitive diagnosis of the disease prior to operation. One of the first patients in whom this was done, was reported in 1956³²⁷.

Intracardiac pressure studies are used today to help in the assessment of pulmonary hypertension, the work of each side of the heart, and to assist in functional studies on the possible effectiveness of steroids, and other drugs in preventing the progression of the disease to the later thrombotic, and fibrotic stages. Angiocardiography, which is usually carried out at the same time, is also helpful in defining the extent of the lesions, and the effects of these lesions on ventricular function. Angiocardiography is particularly good at demonstrating intracavity thrombi.

We published the results of detailed cardiovascular assessments of 11 patients with E-EMF who were studied at Hammersmith Hospital, between 1975, and 1981⁴⁰¹.

The characteristic lesions of endocardial fibrosis have been described in many patients with E-EMF. Good examples of this are given for two patients reported from St. Thomas' Hospital, London in 1976¹⁵², and a 45 year old man, described in the U.S.A. in 1977. The latter patient died 14 months after the onset of heart failure due to biventricular E-EMF, complicating HES. He underwent a variety of cardiac investigations, including catheterization, with pressure studies, and cardiac biopsy, and although his blood eosinophil counts were reduced with hydroxyurea, and vincristine, his disease appeared to progress⁷³⁴. An earlier report in 1974 of right sided catheterization showed almost complete obliteration of the ventricle¹⁷⁹.

Pressure studies during left ventricular catheterization of a woman in 1987 with an eosinophilia of $6.068 \times 10^9/L$ showed a 90 mm Hg subaortic gradient, while the catheter was pulled back from the apex to the aortic root. This was interpreted as evidence for an obstructive cardiomyopathy, but was more likely due to thrombus which was also demonstrated in the cavity¹²¹⁴.

Cardiac biopsy of E-EMF has been carried out since 1972. Histological studies on these biopsies, and post mortem material have defined the three stages of the disease. It has become one of the principal methods for diagnosing it in its early stages. This technique has the theoretical advantage that it samples the endocardium, which is the principal site of the lesion being studied¹⁴⁵⁶. Occasionally, the endocardium is so thickened by fibrous tissue that it may not be possible to obtain a biopsy. As the fibrotic areas are surrounded by softened areas of acute inflammation, the myocardium may also be perforated. There are also several examples of pericardial haematoma which have resulted from cardiac biopsy.

The use of endocardial biopsy to help in the diagnosis of endomyocardial fibrosis, has been used in London, U.K., since 1976, when the findings in two patients were reported¹⁵², and in the U.S.A. since 1980^{616, 1278}. The experience at N.I.H. were reviewed in 1982⁵⁴⁴.

Right ventricular biopsy is most commonly carried out, because the lesions of E-EMF are always bilateral. If the tropical form of the disease is suspected, it may be also necessary to carry out left ventricular biopsy, because the lesions can occasionally be restricted to the left side of the heart.

In the earlier stages of the disease, biopsy of the septal wall can usually provide diagnostic material. Generally four to seven biopsies are taken. In the late stage of the disease fibrotic lesions may not be easily sampled, and the catheter may move to unaffected endocardium from which normal samples may be obtained. This means that careful placement of the biopptome is essential.

14.2.3.4. Histopathology of E-EMF.

The histological appearances of E-EMF show involvement of the endocardium, underlying areas of the myocardium, small vessels, and interstitial tissues in the granulation tissue layer of the heart. These lesions are confined to the inflow tract, and part of the outflow tract of the heart, where a rolled edge is often seen in post mortem samples. Occasionally, thrombi can be found attached to

the mitral, or tricuspid valve leaflets, and less commonly to the walls of the atria.

Results of histological studies of E-EMF, using both light, and electronmicroscopy, were reported from the U.K. in 1981⁸⁷, and 1982¹³¹⁴, Denmark in 1977⁸⁶, and Japan in 1985^{1588, 1264}.

Electronmicroscopy of eosinophilic heart disease has shown the presence of degranulated cells within the heart.

14.2.4. Treatment of E-EMF.

The treatment of E-EMF depends on the stage at which it is diagnosed. In the early acute necrotic stage, the aim is to prevent progression of the disease. In the later thrombotic phase, the goal is to prevent emboli, and further disease progression. In the final stage it becomes necessary to treat heart failure, and to consider the use of surgery.

As yet there is no medical treatment which will prevent the progression of E-EMF, although we have preliminary evidence that prednisolone 5-15/d slowed the progression of the disease. The insensitivity of echocardiography in following the progression of the disease, except in the later stages, is clearly a difficulty here, and cardiac biopsy has not been carried out routinely in treated, and untreated patients, to determine which form of therapy might be most effective.

The recognition that endocardial thrombi were clinically important¹⁷⁵³, and that thromboembolic complications were the most common cause of death in patients with E-EMF¹⁶⁹⁴, has led to the general use of anticoagulation in this disorder. However, there is no convincing evidence that this has resulted in a reduction in vascular occlusive episodes. Indeed, we have looked after several patients who developed large emboli after anticoagulation was begun.

Heparin may not as effective as warfarin in controlling the formation of clots in patients with HES. This was seen in a patient who had mitral, and tricuspid valve replacement with Bjork-Shiley prostheses for E-EMF, and was then treated with subcutaneous heparin eight hourly, instead of warfarin. One month later he was admitted to hospital with a thrombosed mitral valve, and peripheral emboli. The mitral valve was replaced with a porcine graft. When the coagulation response to heparin was assessed, he was found to need over twice the expected amount of heparin, and it was recommended that the activated clotting time should be carefully assessed in these patients, in view of their increased thrombotic tendency⁷⁵¹.

Anti-platelet drugs, including aspirin, and dipyridamole have also been used, but again, their efficacy in this condition has not been proven. Anticoagulation should to be continued for life after cardiac surgery for E-EMF¹⁵³³.

Medical treatment which improves the underlying eosinophilic disorder in patients with HES, in turn may prevent the progression of heart disease in these patients, but no long-term reports have yet been provided of their use, of different forms of medical treatment, such as chronic steroid treatment, cytotoxic drugs, or other medication. Steroids may benefit patients by inhibiting eosinophil granule secretion, and as a result, the amount of granule toxin present within the heart. This was shown to have occurred in a 51 year-old woman with the acute necrotic stage of E-EMF, complicating asthma, and a radiculopathy, who was treated with prednisolone 60 mg/day for several days, followed by tapering lower doses. Cardiac biopsies were done before this, and two months later, when the acute changes had resolved⁹⁶³.

14.2.4.1. Surgery.

At one time the apparently bad prognosis of the underlying eosinophilic disorder discouraged surgeons from attempting a surgical approach to E-EMF. However, the outlook of the underlying eosinophilic disorder has improved so much in the last decade, that surgery has become a worthwhile form of treatment in patients with severe endomyocardial fibrosis.

There is no consensus view about the stage at which surgery should be carried out, whether the mitral valve should be preserved¹⁹⁵⁰, the type of valve that should be used, and the possible danger of an increased thrombotic tendency affecting the new valve. Prior to 1980, early work in Switzerland, and the U.S.A. had led some surgeons to suspect that valve replacement would not benefit these patients due to the high incidence of thromboembolic complications. Indeed, occasional pa-

tients have been described who developed prosthetic valve endocarditis. Three of the patients in the series at N.I.H., Bethesda, U.S.A. who developed heart failure complicating HES had atrioventricular valve replacements. One patient had recurrent thrombotic involvement of the valves despite anticoagulation, and after these had been replaced with pig allografts, the patient died with *Staphylococcus aureus* endocarditis. The other two patients, one of whom also had removal of a left ventricular thrombus, did much better post-operatively, although there was little improvement in pulmonary hypertension. This report emphasized the serious feature of the hypercoagulable state in this disease, the importance of carrying out valve replacement early, before irreversible changes have occurred in the lungs, and other organs as a result of the heart failure, and the possible value of steroids⁷⁵⁶. This danger was seen in a 45-year old Finnish woman with asthma, and an eosinophilia of $9.4 \times 10^9/L$ who developed intracardiac thrombi. Removal of the thrombus, and replacement of the mitral valve with a Bjork-Shiley tilting disk valve, only prolonged the life of the patient for 14 months, as she died after the prosthesis had become blocked with thrombus, despite treatment with anticoagulants⁸³⁹. A patient with HES in India, was reported to have died from a pulmonary embolus from the right ventricle, in 1985¹⁴⁶⁹. Removal of a large intracavity thrombus can be successfully carried out, as described in 1982, in a patient with an underlying malignant eosinophilic disorder⁵⁸⁹. Each centre has its own approach as to how surgery should be carried out. The principal points to emphasize are that the restricting fibrotic tissue can be removed successfully, and that it does not recur at the sites of surgery. The lesions may be more difficult to see at operation than in angiograms, and the extent of fibrous tissue removal should be decided pre-operatively. A plain of cleavage can be found in some patients, but in others a ragged surface will be left, but this does not cause any special postoperative problems. The valves should be replaced with the best known material on which thrombi will not form, in view of this high risk in these patients.

When mitral valve incompetence suddenly occurs in a patient with left ventricular disease, the effects are much more serious than when a similar process occurs on the right side of the heart, and surgery then becomes urgent. Surgery for E-EMF was first carried out in 1972 in London, and this patient still remains well in 1987.

By 1985, there had been reports of the use of surgery in 41 patients with E-EMF.

Four of these patients were operated on in Budapest, Hungary between 1979, and 1984. Two required thrombectomy, two had bilateral valve replacements, and two two died. Although an eosinophilia persisted in the two survivors, there was no evidence for recurrence of the endocardial disease, and the patients were much improved^{69, 68}.

In 1980, we published details of the first two patients at Hammersmith Hospital with severe E-EMF who underwent open heart surgery. One patient, with predominant right ventricular disease, was treated by right ventricular endocardectomy with tricuspid, and mitral xenograft valve replacement. In the second patient, it was only necessary to replace the mitral valve. Both patients showed marked clinical improvement post-operatively, which was documented by cardiac catheterization, and angiography. Our review of the results of surgery on 22 other patients with endocardial fibrosis, some of whom had an eosinophilia, showed equally encouraging results overall. In none of these patients was there any evidence for recurrence of the endocardial lesions, or progression of the heart damage for periods of up to seven years³⁹⁹.

Three patients with HES, and E-EMF were reported from St. Thomas' Hospital, London in 1976. One of them, a 47 year old man, underwent mitral annuloplasty, and coronary bypass surgery, and remains well 13 years later¹⁵².

Probably the first patient to have surgery for E-EMF in the U.S.A. was a 16 year-old boy with HES, and heart failure, who had a Hancock mitral valve replacement on 1 Apr 1976. However his disease, including the heart failure, progressed, and he died 19 months later¹⁸²⁶. Mitral valve surgery was also successful in a 23 year-old black woman with blood eosinophil counts reaching $8.75 \times 10^9/L$, who had E-EMF causing mitral regurgitation. This responded well to mitral valve surgery¹⁵. A further account of the successful use of surgery for endomyocardial fibrosis was reported from

Huston in 1981⁷⁰⁶, but there has been no comprehensive survey of the North American experience of surgery for EMF.

In 1985 it was proposed that it might be easiest to destroy the thickened endocardium by laser photoablation, using the focused beam of a carbon dioxide laser, or argon laser light delivered through a 200-microns optical fiber⁸⁵², but this has not been described to date.

14.2.5. The pathogenesis of E-EMF.

The first suggestions that E-EMF, and T-EMF might have a common pathogenesis was made in the Ivory Coast in 1956⁶³⁶, and in Nigeria in 1970, where it was proposed that 'the eosinophil leucocytes in susceptible persons, by some unknown mechanism, caused endo- and myocardial damage'²⁰⁸. Subsequent papers have emphasized this possibility, describing the way in which eosinophils might be damaged as they hit the wall of the ventricle, where they could release toxic substances, or binding to thrombotic material on the endocardium, and damaging underlying contractile tissues.

We first became interested in these possibilities in 1975, and our subsequent experimental studies have confirmed that eosinophils have a marked propensity to damage myocardial cells through a unique series of interactions with cardiac cells.

Why the lesions are localized to the endocardium in this disease is still unknown. The two main possibilities are that the thrombus layer on the endocardium concentrates the cells, and the toxic proteins, so that the underlying tissue receives the largest amounts of toxic granule constituents. The second possibility is that endocardial cells have a metabolic, or structural difference from other parts of the heart, and other tissues, which makes them susceptible to eosinophil-mediated damage. The peculiar blood supply to the cells in this region indicates that they might differ from other parts of the heart in their response to inflammatory cells, but this has yet to be documented.

14.2.5.1. Human studies.

In 1976, we reported that a distinctive feature of these patients, which was linked to the development of E-EMF, was the presence of vacuoles, and a decreased number of specific granules in many blood eosinophils. This was documented in four patients, who had more than 1×10^9 eosinophils / L, which were vacuolated, and contained reduced numbers of crystalloid granules. A higher proportion than normal of these patients' eosinophils Fc gamma R for rabbit IgG-coated erythrocytes, and could phagocytose erythrocytes coated with rabbit IgG, or human C3b, suggesting that a large proportion of the blood eosinophils were activated, or stimulated in these patients¹⁶⁹⁶.

As eosinophils had been shown to be able to damage some antibody-coated parasites in vitro, a study was done at N.I.H. in 1978 to see whether eosinophils could kill antibody-coated heart cells in vitro. Blood eosinophils were isolated from 10 patients with HES, and their cytotoxic capacity was assessed against antibody-coated human, or chicken red blood cells, Chang cells, and Girardi human heart cells during an 18 hour assay. As only 15 per cent of the Chang, or Girardi heart cells had been killed, and only 33 per cent, and 43 per cent of human, and chicken red blood cells respectively were lysed, it was concluded that eosinophils were unlikely to damage the heart in this way¹³⁶⁷. A direct effect of eosinophils, or their products on the heart seemed more likely, as seen in post mortems showing eosinophils microabscess in the heart¹³⁸⁵.

At that time our principal reason for believing that E-EMF was caused by eosinophils, was that it was described in any of the many diseases which produce an eosinophilia. The only common pathogenic feature of these different diseases was the presence of a marked increase in blood eosinophils^{1317, 2015, 1692, 1688}.

There are few examples of E-EMF developing in patients with eosinophilic leukaemia, because they usually die from leukaemic complications soon after diagnosis. However, a patient has been described who had a marked blood eosinophilia with blast cells in the circulation, and who developed endomyocardial fibrosis¹⁰⁹⁵.

There are several case reports of carcinoma of the lung-induced E-EMF, in which the onset of the heart disease could be correlated with the eosinophilia. We reviewed these reports in 1985, and added details of a patient of our own in 1985¹⁷⁰¹.

One of the first papers to describe this association was reported from London in 1975. The patient was a 51 year-old man with a large cell carcinoma of the lung, who was studied over a two year period before he died in heart failure, and was found to have biventricular E-EMF at post mortem. He also had alcohol-induced flushing attacks, violent headaches, and eosinophil counts which rose as the tumour mass increased, and fell after excision of the main tumour mass, and subsequent radiotherapy. It was suggested that E-EMF was the result of an allergic response to the tumour, involving eosinophils¹¹⁷.

In 1978, a patient was studied in detail in Boston, U.S.A.,⁸⁷¹. In this case report, the association between degranulated eosinophils in the blood, and the development of heart disease was particularly well documented, supporting the view that eosinophil granule products might be involved in the development of the heart disease.

Another report of a similar disease process were published in the U.S.A. in 1982¹⁶⁴⁷, and 1983¹⁹⁶¹. One of the most striking demonstrations of the interaction between eosinophils, and the heart, is in an ultrastructural study from Japan, where a degranulating eosinophils were seen adjacent to a cardiac myocyte, in a cardiac biopsy from a patient with eosinophilic endomyocardial disease¹²⁶⁴. Figure C14a-2. A monoclonal antibody which only binds to activated eosinophils¹⁷⁴² can be used to look for the presence of these cells in the blood, but no formal study has yet been carried out to see whether the number of activated cells in the circulation can be used to define, or monitor patients most likely to develop E-EMF.

Fig. 14-2: Degranulating eosinophil in a cardiac biopsy.

There is a growing body of evidence that eosinophils may stimulate the coagulation system, not only by effects on the clotting sequence^{1148,381,1849}, but also by affecting endothelial cells, and platelets¹⁰⁴⁹. Patients with a marked eosinophilia, and E-EMF, appear to be forming thrombi continually, as judged by high serum levels of platelet factor 4, beta thromboglobulin, and high fibrinogen (Davies et al. 1986, unpublished). The possible importance of this mechanism in the induction of E-EMF has been outlined in an echocardiographic study in which thrombi were considered to precede and/or augment the fibrotic process, leading to atrioventricular valve dysfunction. It was suggested that scar formation in the ventricles in patients with E-EMF was the result of the organisation, and incorporation of thrombus into the ventricular cavity wall⁷⁰³, although this has not been seen in other studies.

In 1987, we reported the results of an immunopathological study on cardiac tissues taken at necropsy, or at cardiac biopsy, from 18 patients with E-EMF, to look for the presence of the toxic eosinophil granule proteins within the heart. Serial sections were stained for MBP by indirect immunofluorescence, and for ECP, EDN/EPX, and activated eosinophils with alkaline-phosphatase-linked monoclonal antibodies. Activated eosinophils, and secreted eosinophil granule proteins were mainly detected within the necrotic, and later stage thrombotic lesions, in areas of acute tissue damage in the endocardium, and in the walls of small blood vessels. These findings suggested that eosinophil granule proteins could be causing the cardiac muscle damage, and vascular injury which leads to the development of endomyocardial fibrosis¹⁷³³.

Several disease which give rise to a persistent eosinophilia are not commonly associated with eosinophilic heart disease. These include bronchial asthma, cryptogenic eosinophilic pneumonia⁴¹, hayfever, and other allergic disorders. This implies that there may be protective factor(s) in the blood of these individuals, or that the stimuli which cause eosinophil degranulation in these diseases are different, and only affect eosinophils which are distant from the heart.

14.2.5.2. Experimental studies.

Direct evidence that eosinophil granule proteins were involved in the killing of heart cells has come from our studies on isolated rat heart cells which were killed in a dose dependent manner by secretion products from human blood eosinophils¹⁷³⁶. These produced an alteration in the plasma membrane of isolated myocytes, which stimulated increased oxygen uptake to provide additional ATP. A second mechanism was shown to involve a direct toxic effect of eosinophil granule proteins on

cardiac mitochondrial enzymes. 2-oxoglutarate dehydrogenase was inhibited in a stoichiometric fashion, and pyruvate dehydrogenase was also inhibited. These were sufficiently potent, and irreversible effects for it to be clear that heart cells would die if these proteins could traverse the plasma membrane, and reach mitochondrial constituents.

There is recent confirmation for the effects of eosinophil cationic proteins on cell membranes, as they can induce pores to form in the surface of lipid bilayers¹⁹⁸⁶.

Two of the most toxic cationic proteins in eosinophil granules, ECP, and EDN/EPX, both have ribonuclease activity, and are probably evolved from a common gene⁶⁶¹. Whether ribonucleases have a predilection for the endocardium has not yet been studied, but this could explain the unusual distribution of cardiac necrosis, and fibrosis in this condition. Other possibilities which have been considered are the special blood supply to this part of the heart, or physical effects of the blood stream within the cavity of the heart affecting the inflow tract, and apex of the ventricles.

Future work on endocardial fibrosis will be concentrating on the nature of the eosinophilic disorders themselves which give rise to this disorder; the nature of the stimuli which induce eosinophil secretion within the heart, and the factors within the endocardium which localize the lesions to these unusual anatomical sites. It is hoped that when more is known about these, effective forms of therapy, and prevention of E-EMF may become available, and it may be possible to distinguish patients in the tropics at risk of developing T-EMF.

14.3. Tropical endomyocardial fibrosis.

Tropical endomyocardial fibrosis (T-EMF), was first described in 1946 in African troops serving in the Middle East by Bedford, and Konstam¹⁴⁵. They summarized their finding of 40 African troops, mainly from West Africa who had unexplained heart failure. Some of the patients had an eosinophilia, and many had parasitic infections. Post mortem examination of 17 showed 'an obvious and extensive subendocardial fibrosis, with fibrous areas resembling shallow infarcts in the ventricles, adherent to which was organized antemortem clots'. The disease was rediscovered by J.N.P. Davies in 1948 in Uganda. It occurs in regions five degrees north, and south of the Equator in Africa, India, and South America. Sporadic cases occur in other parts of the world. Most patients who present in the U.K. have lived in the tropics for several years before the disease presents²¹⁰. In parts of Nigeria 21 per cent of acquired heart disease is due to this disease⁴³, and it is equally common in parts of South India. It has many features in common with eosinophilic endomyocardial fibrosis, and the diseases are pathologically indistinguishable. However, the different clinical backgrounds of the patients with each type of heart disease was the reason why the two disorders were treated separately for many years.

It now seems likely that the pathogenesis of both disorders is linked to a marked blood eosinophilia, and eosinophil granule deposition within the endocardium in the early stages of the heart disease. In the case of the tropical form of the disease, evidence to support this has been difficult to obtain. Few patients are seen in the early stages of the disease, and so no cardiac biopsies have been done at a time when the lesions are developing. The tropical form of the disease may progress slowly, so that the heart has time to hypertrophy, and most patients only present when they develop heart failure. In 1982, a workshop on endomyocardial fibrosis was held in London, with contributions from Africa, India, and South America, and Europe. The clinical features of the various forms of the disease in each of these parts of the world was presented, with work on pathology, pathogenesis and treatment

¹³²⁰

14.3.1. Clinical features of T-EMF.

T-EMF occurs in a younger age group than the eosinophilic form of the disease. Children are affected as well as teenagers. Occasionally the disease is seen in older people. It occurs equally in males, and females¹⁵⁹⁷. In some patients the disease is unilateral, affecting the right ventricle only in 15 per cent of cases, and the left ventricle only in five per cent of cases.

These patients usually come from poor, malnourished communities, who presumably have a high parasite worm burden, although no comparison has been done of this in relation to unaffected peo-

ple living in the same region. A marked association with filarial infections has been noted in Nigeria⁸⁵⁵, but this is unlikely to be an important cause of the disease in patients with EMF in South America. On the other hand in Kerala, South India, where filariasis is hyperendemic, this may be the major parasite inducing an eosinophilia, hypergammaglobulinaemia, and subsequent endocardial damage¹⁸⁶¹.

The classical clinical appearances of patients in the late stage of T-EMF include an oedematous face, and eyelids, ascites, relatively few heart murmurs, but a markedly enlarged heart, and no ankle oedema⁴⁰⁵. The patients looks malnourished, and chronically ill, but the heart failure appears to be remarkably well compensated for in many cases. Goodwin has noted that marked right ventricular disease with almost complete obliteration of the ventricular cavity in T-EMF, is seldom seen in E-EMF⁶⁹⁷.

Thrombotic, and embolic lesions are uncommon, and retinal abnormalities, which are common in HES, have not been reported in T-EMF.

T-EMF is seldom associated with gross heart murmurs even in the late stage of the disease, in contrast with E-EMF, where a murmur is almost invariably present. Amongst a group of five patients in Ibadan, Nigeria, with clubbing of the fingers, and cyanosis, the cardiac murmurs most often noted were an opening snap, and a mid-diastolic murmur at the apex⁵³⁵. The classic features of a restrictive cardiomyopathy are present in most patients in the late state of the disease. Occasionally patients may have a markedly dilated left and/or right ventricle, particularly in the tropics, and in rare cases, it may be difficult to distinguish endocardial fibrosis from constrictive pericarditis.

The differential diagnosis includes Ebstein's anomaly²⁹. Atrial fibrillation, myocardial calcification, and pericardial effusions are more common in endomyocardial fibrosis, than in Ebstein's anomaly¹⁰². A comparison of the ECG findings in 18 patients with Ebstein's anomaly, and 20 with right ventricular T-EMF was published from South India in 1982⁸⁶³. Other important differential diagnoses are rheumatic heart disease, cardiac tumours, ventricular thrombi, and congenital heart diseases presenting in later life.

14.3.2. Investigations in T-EMF.

Now that surgery has become available for T-EMF, investigations need to be as thorough as in the eosinophilic form of the disease. In tropical countries chest radiographs, and echocardiograms are most useful, but cardiac catheterization is usually carried out prior to surgery. Investigations for associated disorders such as parasitic infections are obviously important in patients in the tropics, and these should be treated before surgery is done.

The cardiac silhouette is usually markedly increased, with or without pulmonary infiltrates, and occasionally marked calcification can be seen¹²⁵⁸. ECGs show axis deviation, repolarization changes, and rhythm abnormalities in proportion to the degree of heart failure.

14.3.2.1. Echocardiography in T-EMF.

Echocardiography is the most useful single investigation of T-EMF, and several groups have now been reported their findings.

M-mode echos provide measurements of the degree of mitral valve involvement, and the abnormalities in the septal wall. These are helpful prior to cardiac surgery, but cannot be used for diagnostic purposes, as they may be found in other cardiac disorders. There have been several echocardiographic studies on EMF²⁶⁵. M-mode echocardiographic findings have been well described in one group of 21 patients⁶³⁵.

2-D echos provide a clearer picture of the left and right ventricles, and the extent of endocardial fibrosis. In our studies in Kerala, South India, we found that it was helpful to use colour coded 2-D echocardiography to define the sites of fibrosis¹⁸⁶⁰. Abnormalities in the movement of the ventricle can help to distinguish ischaemic, and dilated ventricular muscle diseases from T-EMF, in which ventricular contractility is often well maintained, and infundibular contractility is enhanced. This was seen in a 2-D echocardiography study in Venezuela, which also demonstrated the value of this technique in distinguishing T-EMF from Chagas' heart disease which is common in that region.

Preservation of the left ventricular apical systolic inward motion in T-EMF contrasted with other diseases which caused apical abnormalities¹⁴.

14.3.2.2. Cardiac catheterization in T-EMF.

Cardiac catheterization is rarely carried out in T-EMF, except in patients who are to undergo surgery. The procedure is not without danger, especially in patients with severe, and long-standing heart disease with rhythm abnormalities. It is rarely carried out for diagnostic purposes, although this may occasionally be necessary when 2-D echocardiograms are difficult to interpret.

In South India, the results of cardiac catheterisation of patients with T-EMF were described in 1983³¹⁰. The appearances of minimal involvement of the right ventricle in patients with predominant left ventricle disease have been reported¹⁵⁵⁹. Selective coronary angiography was done in 24 patients with T-EMF in the study carried out in the Sree Chitra Tirunal Institute for Medical Sciences and Technology, Kerala, South India. Eight had right ventricular disease, two left ventricular disease, and 14 biventricular disease. Abnormalities included a vascular blush, localized ventricular filling from the region of the blush, and tortuosity of the coronary arteries, lending support to the suggestion that mural thrombi could be important in the development of the endomyocardial lesions¹⁰³. Post mortem coronary angiograms have been reported from Japan⁸⁴⁰. In four cases studied in China in 1983, massive enlargement of the right atrium was noted¹⁰⁸⁸.

The outline of the left, and right ventricles is clearly shown to be abnormal in classical angiocardiographic studies.

Cardiac biopsies have been carried out on a small number of patients with EMF, since it was first reported in 1971¹⁶⁶². The procedure can be especially difficult in patients in gross heart failure, and because heavy deposits of fibrous tissue can resist the jaws of the bioprobe.

14.3.2.3. Histopathology of T-EMF.

The gross appearances of T-EMF have been well described, and reviewed: The thickened endocardium may end in a thick-rolled edge in the outflow tract, and granulation tissue often extends from the thickened endocardium into the underlying myocardium¹³¹³. The post mortem, and histological appearances of T-EMF are indistinguishable from those seen in the late fibrotic stage of the eosinophilic form of the disease¹⁵⁰¹.

The post mortem histological features of T-EMF have been reviewed in studies from Uganda¹⁵⁹⁸, and South India⁹¹². The immunohistochemical features in some patients in South India have also been published⁹¹¹.

Only a few ultrastructural studies on T-EMF have been published. The appearances in two patients who were studied in France were reported in 1977. One showed the classical appearances of pure fibrosis, which was acellular, and associated with degenerative changes in the heart muscle cell; the other consisted of young fibrous tissue with numerous fibroblasts secreting a copious fibrillary substance which may have been the precursor of collagen fibres⁷¹⁹. In 1984, the ultrastructural features of the lesions were described in seven patients in South India. The eosinophils showed alterations in their granule morphology, which were similar to those seen in patients with E-EMF¹⁰⁰⁵.

14.3.2.4. Extra-cardiac lesions in T-EMF.

Although there is little clinical evidence for involvement of skeletal muscle in the process which leads to T-EMF, in 17 of 20 consecutive, angiographically proven cases of EMF in South India, there was evidence for myopathic changes in electromyograms of skeletal muscle, which was more pronounced than in a control group of patients with heart failure due to other causes. However light-microscopic study of the quadriceps muscles in eight of these patients showed no abnormality⁷².

Renal involvement, although well known in HES, has only recently been noted in T-EMF. Glomerular lesions included capillary wall thickening, basement membrane duplication, mesangial expansion, and interposition, intraluminal fibrin, and dense subendothelial deposits. These changes were thought to result from the deposition, and organisation of immune complexes³⁹³.

Feminization, which is not seen in patients with E-EMF in temperate climates, has been described in

patients in Nigeria with T-EMF. This was related to low serum testosterone levels.¹⁸⁵ Interestingly, the same group also found raised serum immunoreactive prolyl hydroxylase protein levels, galactosyl-hydroxylsyl glucosyl transferase activity, and type III procollagen aminopropeptide levels in the serum of some of these patients¹⁸⁶.

14.3.3. Treatment of T-EMF.

At present the treatment of T-EMF is largely limited to the late fibrotic stage, when surgery is the only effective treatment for patients who may have been in heart failure for several months, or years. Preparation for surgery is clearly important, and bedrest combined with carefully regulated diuretic treatment is usually required. A problem with diuretics is that the cardiac output depends to some extent on a high filling pressure, so treatment should be given carefully, and over several weeks, in order to prevent a sudden fall in cardiac output.

14.3.3.1. Surgery.

By 1985, there had been reports of the use of surgery in 94 with T-EMF⁶⁹, and many more have been carried out successfully since then. Surgery for endomyocardial fibrosis is now carried out in each of the major areas of the world in which this disease occurs, and the success rate has been much higher than might have been expected a decade ago. This is largely because the underlying myocardium remains intact in the majority of patients, and the relief of incompetent valves, and removal of restricting fibrotic tissue can return the cardiac output to near normal.

The main issues to consider before surgery is carried out, are whether the procedure should involve valve repair, or replacement, endocardectomy, and/or thrombus removal. A small number of patients with dilated hearts, and poorly functioning left ventricular muscle, may not benefit from any of these procedures¹⁷⁹², emphasizing the importance of considering surgery before the cardiac disease has led to irreversible myocardial injury. Occasionally, it may be possible to remove localized areas of fibrosis from the left ventricle, leaving the mitral valve intact¹²⁵⁹.

In 1971, Dubost's group in Paris began to use open heart surgery to treat endomyocardial fibrosis. His patients came from the Gabon, a French colony with a high incidence of filariasis, especially *Loa loa*. The importance of this pioneering work was not immediately recognized outside France, although it is now clear that Dubost's, and Carpentier's group have operated on more patients with this disease than any other outside the tropics^{1450, 637}. The techniques, and results of cardiac surgical treatment in five patients with T-EMF treated in Paris, were reviewed by Dubost, and his colleagues in 1976, and 1977. The operations included endocardectomy on the left, and/or right side, and valve replacements, with Starr, or Bjork prostheses, or heterografts. Survivals ranged from two to 56 months, or more^{469, 470}.

Between 1975-85 four women with T-EMF in Budapest, Hungary, had endocardectomy, and two had thrombectomy successfully performed, among a group of 11 patients with endomyocardial fibrosis. One had bilateral valve replacement. The outcome was successful in all four patients⁶⁹.

The indications for surgery for T-EMF in French speaking areas in the tropics have been defined¹⁶³, and the extensive work in Abidjan, Ivory Coast have been reported. Results in 55 operated patients were given in 1985¹¹⁹⁹. Twenty patients were reported in an earlier paper from the same centre¹²⁰⁰. The value of mitral valve repair in four patients was also described by this group¹²⁰¹. Difficulties in diagnosing localized left ventricular disease affecting the mitral valve has also illustrated in a report of six patients in the same centre¹²⁰².

The indications, and results of cardiac surgery were reported from South India in 1983: from Kerala¹⁸²⁷, and Tamil Nadu³¹¹. In Kerala 17 of 25 patients with T-EMF were followed up for a mean of over 13 months after endocardectomy, and atrioventricular valve replacement. Although there was no change in right ventricular shape after endocardectomy, left ventricular shape was restored to near normal. This study, like earlier reports, stressed that the benefits were mainly a result of the correction of mitral regurgitant lesions, and an improvement in ventricular compliance¹⁰⁴. Successful surgical correction of T-EMF has been carried out in India, in a child only 12 years old⁸⁷⁸.

In Recife, Brazil, thirty patients with T-EMF were submitted to endocardial decortication, and atrio-

ventricular valve replacement between 1977, and 1981, and the benefits of cardiac surgery were considerable¹²³⁸.

As T-EMF is rarely seen in the U.S.A., there have been no large series of surgically treated patients, although I know of one who had an unsuccessful cardiac transplant in 1983. Similarly, in other parts of the world, only occasional reports have been published. One of these was from Tehran, Iran, in 1979, of successful decortication of the right ventricle, mitral valve repair, and tricuspid valve replacement¹⁶⁰⁶.

14.3.4. Pathogenesis of T-EMF.

Many different pathogenetic mechanisms have been proposed for T-EMF, but there is still no generally accepted explanation for why it is largely limited to regions close to the equator, and to the poorer people living in these areas. As it may occasionally develop in western visitors to these places^{210, 719}, it is likely that environmental factor(s) are involved. There is little evidence for a familial susceptibility, although the disease has been seen in more than one member of a family in Kerala (Vijayarhagavan 1985, personal communication).

The unusual geographical distribution of T-EMF, especially its high incidence in Rwandan immigrants to Uganda, compared to the local population, led to the suggestion that genetic factors might be important, in addition to environmental factors, such as diseases causing an eosinophilia. However, as a comprehensive epidemiological study on the prevalence and natural history of T-EMF is not available, it has not been possible to determine the relative importance of genetic, and other factors⁸³².

In Nigeria, where T-EMF is a disease of the rain-forest belt, several prospective studies are under way to look at these questions⁵³³. The finding of raised IgM levels, with normal IgG, and IgA levels, in some patients in Nigeria, strengthens the case that an unusual response to an infectious disease may underly this disease^{279, 864}.

There has been circumstantial evidence for many years that an inflammatory reaction might be the initiating event in the development of T-EMF. In 1965 Parry, and Abrahams noted in Nigeria that in some patients who developed T-EMF, a constitutional illness could precede clinical cardiac disease. In patients who were judged to be at the earlier stage of the disease, there were often inflammatory cells, and necrosis in the endocardium¹³⁷⁰. Shaper in Uganda in 1972 detected antibodies to heart muscle antigens in some patients, but there was a closer association of this disorder with malaria antibody levels¹⁵⁹⁹. He suggested that some unknown mechanism induced both the heart damage, and the eosinophilia which is often associated with the disease¹⁵⁹⁷.

14.3.4.1. Eosinophils, and T-EMF.

The suggestion that eosinophils could be responsible for the endocardial lesions in T-EMF stems from the clear evidence that they are involved in causing the eosinophilic form of the disease. There are also reports in western countries of patients who had a marked eosinophilia, which disappeared several years before they were shown to have endomyocardial fibrosis¹⁸⁶⁸. Unfortunately, T-EMF is seldom diagnosed at an early stage, and there are few reports of accurate eosinophil counts in this disease. However 12 of the first 24 patients with T-EMF who were reported by Davies in 1948 had an eosinophilia⁴⁰³. In a detailed study reported in 1967 on 16 patients with T-EMF in Uganda, although absolute eosinophil counts were not reported, six had eosinophil differential counts of up to 64 per cent³⁵⁰. In 1977, mean blood eosinophil counts in 15 patients with T-EMF in Uganda were $1.13 \times 10^9/L$, compared with $0.72 \times 10^9/L$ in a control group, but the range of counts was similar, and the differences were not significant¹³⁷⁶.

Amongst a group of 77 patients who were studied in 1967 in Nigeria, 33 (43 per cent) had eosinophil counts greater than $1.0 \times 10^9/L$ ⁸⁵⁵. Several surveys are continuing in West Africa, and India, where there is some evidence that patients with an eosinophilia may later develop T-EMF. In Venezuela, a small prospective study was carried out on 14 patients with an eosinophilia for a mean of 14.5 months, but echocardiography, and clinical studies showed no abnormality¹⁴. It may be more important to assess the numbers of degranulated eosinophils, rather than total eosinophil counts, as

two patients with T-EMF in the Venezuelan study were noted to have degranulated blood eosinophils¹⁴. In a study on 10 patients with T-EMF in Nigeria, serum ECP levels were not statistically different from 15 controls. This may have been because they were in the late stage of the disease¹⁸¹³.

An alternative view is still held, which stems from early work in Uganda, showing connective tissue swelling in the endocardium, and inner myocardium at post mortem³⁵¹. This view proposes that the cardiac injury is primarily the result of an immediate hypersensitivity reaction, in which eosinophils only play a secondary role³⁹. These issues were discussed by Falashe in 1985⁵³⁴.

There has been a strong possibility for many years that some patients with filariasis may go on to develop EMF. Amongst a group of 77 patients who were studied in 1967 in Nigeria, 70 (91 per cent) had Loa Loa. This study was one of the first to suggest that there might be an association between filariasis, an eosinophilia, and the development of T-EMF in this area⁸⁵⁵. There is a suggestion from India that obstruction of heart lymphatics in lymphatic filariasis could produce T-EMF¹⁴⁷⁸, but there is no supporting evidence for this.

The possibility that filariasis, and eosinophilia may lead to the development of T-EMF was examined in a prospective study on 1,956 patients aged less than 20 in northern Nigeria, which was reported in 1981. Forty-four patients (2.2 per cent) had eosinophil counts greater than $3 \times 10^9/L$. Twenty-two patients had filariasis (loaiasis), with mean eosinophil counts of $1.48 \times 10^9/L$, and all responded to treatment with diethylcarbamazine. Thirteen patients had heart disease at presentation, and eight were found to have T-EMF, even though their eosinophil counts had returned to normal with treatment. Angiocardiograms were done in six patients. One patient died, and was shown to have T-EMF at post mortem. This study adds further evidence to the possibility that a chronic eosinophilia due to filarial infections can lead (over several months, or years) to the development of endomyocardial fibrosis. This sequence of events was documented in a six-year old boy in this series³⁸. Full details of his illness were published later: He was admitted to hospital with acute filariasis, and despite effective treatment, and disappearance of the eosinophilia, he died 2 years later with predominant right-sided T-EMF³⁹.

By 1981, 25 Europeans resident in the tropics had been reported to have developed T-EMF. Nineteen had a marked eosinophilia, and 15 had microfilaria in their blood. Disappearance of the eosinophilia at the late stage of the heart disease has also been noted in some of these patients³⁹.

14.4. Animal models of endomyocardial fibrosis.

There is some hope that an animal model of EMF could be produced. For example, in male Sprague-Dawley rats, endomyocardial fibrosis, which mostly affected the left ventricle, was observed one to 14 weeks after a course of treatment with the carcinogen N-nitrosomorpholine¹¹⁶⁹. Endomyocardial lesions were also seen in mice which were given a lethal infection of *Plasmodium berghei*⁵⁰³.

14.5. Comparisons of E-EMF, and T-EMF.

In 1983 Olsen in London, U.K. reviewed the concept which unites T-EMF, and E-EMF¹³¹⁶. He felt that the evidence linking these apparently separate diseases together, with a common pathogenesis (eosinophil-mediated cardiac injury), means that endomyocardial fibrosis should now be classified as a specific heart muscle disease, rather than a cardiomyopathy, which by definition is of unknown aetiology¹³¹⁵.

In the same year, we reported the results of a comparative study of T-EMF, and E-EMF in patients with HES, in London, to try to explain the different clinical features of the two disorders. Forty-seven patients with T-EMF were studied in South India, and eight in Brazil. Their clinical features were compared with eleven patients with E-EMF studied in London. Figure C14a-3. The patients in Britain were older than the patients in the tropics, and the male predominance in the U.K. was not found in the other patient group. In Britain, half of the patients presented in the acute necrotic stage of the disease, whereas patients in the tropics only presented in the late stage. This was linked to the association of endomyocardial fibrosis in London with HES, which has a mean age incidence of 37, whereas in the tropics, the patients were from poor malnourished communities, with heavy parasite loads, especially filariasis. Another important difference was that isolated right, or left ventricular

disease was found in a quarter of the patients in the tropics, whereas this did not occur in patients in London. In order to account for these differences, it was suggested that the rate at which the heart disease developed in these different regions was determined by the underlying disorder producing the eosinophilia. In temperate climates, the systemic effects of toxic eosinophil granule proteins was thought most likely to produce bilateral rapidly progressive disease, whereas in the tropics, the eosinophilic disorder might be more chronic, and the effects could be limited to one or other side of the heart, depending on where eosinophil degranulation was induced to occur ⁴⁰².

Fig. 14-3: A comparison of eosinophilic, and tropical endomyocardial fibrosis.

15. Chapter C 15. Diseases of the blood.

Although any disease which affects the bone marrow may affect eosinophil production, this is seldom commented on, and primary disorders which principally, or exclusively affect eosinophils are relatively rare.

15.1. Inherited diseases affecting eosinophils.

A few inherited blood disorders such as Omenn's syndrome (see p 306), and other immunodeficiency diseases, can be associated with a marked increase in blood eosinophil counts. Other inherited disorders can produce defects in eosinophils. For example they occur in eosinophils from patients with gangliosidase deficiencies. This was shown in 1985 in blood, and bone marrow samples from four patients with GM1 gangliosidosis type I. The cells contained poorly stained, and unevenly spaced granules, many of which were larger than normal, and had abnormal ultrastructural appearance ⁶⁴⁷.

Several of the major immunodeficiency diseases which are noted mainly for their effect on neutrophils also affect eosinophils. These include chronic granulomatous disease, the inherited Pelger-Huet anomaly, and the Chediak-Higashi syndrome.

15.1.1. Eosinophil peroxidase deficiency.

The only genetic disorder confined to eosinophils is peroxidase (EPO) deficiency, which has been noted sporadically. Genetic defects in neutrophil myeloperoxidase are common. The gene for this tetrameric protein was cloned in 1987 ⁸⁸¹, and it was found that the deficiency was due to the inheritance of abnormal myeloperoxidase gene(s) which did not undergo correct post-translational processing. There is also a case report of a patient with deficiency of the neutrophil cationic proteins defensin, and gelatinase ¹¹⁴⁷, but genetic abnormalities in the eosinophil basic proteins, ECP, EDN/EPX, and MBP, has not yet been noted.

EPO deficiency occurs either as a complete, or partial deficiency. Interestingly, members of the cat family, and some other animals have blood eosinophils which do not stain for peroxidase ¹⁸¹⁰. See all Chapter B-01.

In 1968, two children aged 3, and 7 were found by Presentey to have blood eosinophils which had hypersegmented nuclei, decreased numbers of granules, and complete absence of staining for EPO, and phospholipids. Myeloperoxidase was unaffected ¹⁴³⁷. This appeared to be a new inherited enzyme deficiency ¹⁴³⁹, which was due to an autosomal recessive defect ¹⁴⁴⁰.

He then made a detailed assessment of the staining characteristics of EPO-deficient eosinophils from two families, which showed that as Sudan black staining phospholipids were also reduced, phospholipids were probably associated with EPO in eosinophil granules ¹⁴³⁸. By 1976, he had extended his studies to more families, and was able to show shown that this anomaly was not associated with clinical problems ⁸⁹⁷.

In a six year study of 63,465 Jews resident close to Rehovet, and in Galilee, Israel, he reported in 1982 that there were 74 with complete absence of EPO, and phospholipid deficiency in blood eosinophil granules. These people were mainly Yemenite Jews. It was shown to be an autosomal recessive trait, and in 14 individuals, more than one member of a family was affected. Partial loss of

peroxidase staining was seen in 14 people. None of the EPO deficient people had a related clinical disorder, and they were able to produce an eosinophilia normally. It was suggested that this deficiency could be used as a useful genetic marker in affected populations¹⁴⁴⁴.

Although complete EPO deficiency is very rare in Caucasians, twins with this defect were reported from Lille, France, in 1987¹⁰⁶⁰. As yet no functional studies have been reported using EPO-deficient eosinophils. It is not known, for example, if they can kill parasites *in vitro*, or degranulate normally, and whether they have a normal complement of the other basic proteins. Ultrastructural studies have showed enlargement of the cores of the granules, and thinning of the matrix region, where EPO, and phospholipid are usually found^{1441, 1060}.

Partial EPO deficiency is seen less commonly, but the use of automated flow cytochemistry, which uses peroxidase staining, has brought some case to light. In 1980, partial deficiency of myeloperoxidase, with complete absence of EPO, was detected using an automated Hemalog D machine. The patient was a 48-year old man with intellectual deterioration due to ceroid lipofuscinosis: Kuf's disease. Electronmicroscopy studies showed many eosinophil granules did not stain for peroxidase. It was thought that the peroxidase deficiency did not explain the neurological disorder, as other patients with complete EPO deficiency have not had cerebral diseases²⁰⁰. However, partial deficiency of EPO in a 4-year old Turkish boy with mental retardation was also noted in Holland using a Technicon H-6010 automated flow-cytochemical analyzer. The residual peroxidase activity was almost completely cyanide resistant⁸⁰⁵, like the peroxidase in normal blood eosinophils¹²²⁶.

15.1.2. Other inherited blood diseases affecting eosinophils.

The Chediak Higashi syndrome affects many species, besides man. The eosinophils are structurally abnormal with giant cytoplasmic granules. These probably arise from the fusion of pre-existing granules⁵³¹.

In the X-linked form of chronic granulomatous disease, there is an abnormality in a gene which codes for a transmembrane protein, which anchors cytochrome b558 to the plasma membrane. The nature of the defects in the other genetic forms of the disease have not yet been defined¹¹⁴⁷. In 1986, a report on the eight patients seen at N.I.H., Bethesda, showed that they all had a minor increase in blood eosinophil counts. Eosinophils in four patients had a small capacity to form formazan from nitrobluetetrazolium salts, and it was suggested that this could produce a false positive result in this test for the disease¹⁴⁹⁸.

An autosomal dominant trait, in which eosinophils, and basophils contained unusual cytoplasmic crystalline inclusions was described in 1978. This did not appear to have caused any clinical problems¹⁷⁹³. In 1981, a familial trait in eosinophil, which contained more than five cores per granule, was also reported¹³⁶¹.

The Pelger-Huet anomaly can occur as an inherited, or an acquired abnormality in eosinophils. An example of the inherited form was described in 1987 in a three year-old boy, who had blood eosinophil granules which were reduced in number, and contained crystalloids of many different shapes¹³³².

The acquired form of Pelger-Huet may be more common, as it occurs in patients with myeloproliferative diseases¹¹⁵³, and it can be limited to the eosinophil series. The fourth example of this defect in eosinophils from a patient with myelofibrosis was described in Italy in 1980⁵⁸⁸.

15.2. Fanconi's anaemia.

Abnormalities of eosinophil development have been seen in occasional patients with Fanconi's anaemia. Abnormal crystalloids were seen within eosinophil granules in one patient. In another patient there were nuclear pockets, and eosinophil granules contained globular material, with some autophocytic vacuoles in the cytoplasm¹²⁰.

15.3. Cyclical haematopoiesis, and eosinophils.

Several patients have been described with cyclical alterations in the number of eosinophils in the bone marrow, and blood^{16, 513}. This appears to be part of a disturbance of haematopoiesis which affects some, or all of the blood cells, and produces maturation arrest, for a period, at the myelocyte,

or myeloblast stages of differentiation. The most significant alteration is in blood neutrophil counts, which fall periodically. About 20 per cent of these patients have a moderate eosinophilia¹⁰²⁸.

15.4. Acquired platelet dysfunction with eosinophilia.

The syndrome of acquired platelet dysfunction with eosinophilia, which is mainly seen in children in tropical countries, has been known for over a decade¹²²³. Although its pathogenesis is unknown, it has been linked to the presence of intestinal nematode infections¹⁰¹⁰. Most publications on this syndrome have come from Singapore, Thailand¹⁷²⁹, Singapore, and India. I have seen several patients with the syndrome in Kerala, South India. There is also a report of a patient in the U.S.A.¹⁴⁰, and another in the U.K.: This was a girl aged 8.5 years, who returned from a visit to Malaysia with multiple painless bruises. Her blood eosinophil count was $1.66 \times 10^9/L$. Her bleeding time was 20 minutes, and her platelets did not aggregate with collagen. Immune complexes were also found in her serum. It was suggested that the disease could be either due to a defect in the platelets, making them unable to release ADP after stimulation with collagen, or that there was a 'storage pool' effect of immune complexes on blood platelets¹²⁵⁵. Seven adults aged 19-21 years were reported from Singapore in 1984¹⁷⁶⁷. The defect can be partially corrected by platelet transfusions⁷⁶⁹.

15. Chapter C 15 a. The idiopathic hypereosinophilic syndrome, HES. I. Clinical features.

The idiopathic hypereosinophilic syndrome (HES) is a term used to cover a variety of eosinophilic diseases, in which the cause is unknown, but which share a number of unusual features. Figure C15a-1. The term was introduced by Hardy, and Anderson in 1968⁷⁵³, and then used it to cover both malignant, and non-malignant diseases affecting the eosinophil series. The first patient with HES was probably reported in the the U.S.A. in 1912¹⁷¹¹. The first case report of HES in the U.K. (from Durham) was published in 1922. This was a man who had an eosinophil count of $23.8 \times 10^9/L$. It was noted that the eosinophils had more lobes than normal, and that the cytoplasm contained vacuoles, and smaller numbers of granules than normal. These cells were able to form crystals (possibly Charcot-Leyden crystals), after they had been incubated at 37°. The crystals were soluble in hot water, 2 per cent acetic acid, and sodium hydroxide. It was thought that this patient had an eosinophilic leukaemia but, in retrospect, it is possible that he had HES¹¹⁷².

Fig. 15-1: The principal complications of HES.

In 1938 a detailed clinical description was made of a 35 year old man who died with HES, complicated by heart disease, and cerebral thrombo-embolic disease. Eighteen earlier reports were reviewed: Fourteen of the patients were male, and five female. They were aged between six and 54. Eleven had died, and six had post mortems. Four had heart failure, two cerebral haemorrhages, and five had thrombi in the heart¹⁴⁵⁸. This was not a complete review, as it did not include the patients already described by Loeffler in 1936 in German.

By 1969 there were at least 48 reported cases of 'eosinophilic leukaemia', and details of five were reported by Benvenisti, and Ultmann¹⁵⁷. In 1971 three patients with HES were reported from St. Thomas' Hospital, London, two of whom died from endomyocardial fibrosis¹⁶⁰⁷.

In 1975, the syndrome was redefined by Wolff, and his colleagues at the N.I.H., Bethesda, U.S.A.³²⁵. They argued that a clear distinction of eosinophilic leukaemia was now possible, and they restricted HES to patients with a non-malignant, but persistent eosinophilia of more than $1.5 \times 10^9/L$, with evidence for organ damage, such as endomyocardial fibrosis, skin lesions, and respiratory tract involvement. This was reviewed in 1980⁴³⁸.

The diagnosis is made by the exclusion of other distinct diseases, such as parasitic infections, which can give rise to a similar clinical picture. Even after a diagnosis of HES has been made, it is important to bear in mind that any of these diseases, such as tumours, parasitic diseases, and hypersensitivity disorders may only become apparent later. The main reason for classifying patients as having HES is that they share a number of serious complications, many of which are probably caused by

toxic effects of eosinophils on normal tissues. Although HES appears to occur world-wide, and there are over 100 reports of patients with the disease, only two centres have described their clinical experience with more than 10 patients with HES. Most haematologists in large hospitals have had experienced of treating these patients, but they are also seen in other clinical departments, especially departments of cardiology, dermatology, and neurology, because of the wide range of organs affected in this group of diseases.

The problems involved in distinguishing eosinophilic leukaemia from nonmalignant (leukaemoid) diseases with a marked eosinophilia, were carefully studied by Bousser in 1957¹⁹⁸. As these diseases were rare, questionnaires were sent to well known haematology centres in France, and abroad, to obtain their experience of diagnosing, and treating patients with high blood eosinophil counts of unknown cause. As a result, it was clear that there were two distinct diseases. In one group of patients, death was usually the result of heart failure. These patients also had pulmonary infiltrates, and central nervous system disease. This illness was clearly different from eosinophilic leukaemia which affected a smaller number of patients. As there seemed to be no overlap between the two diseases, he proposed that the main treatment for patients who did not have the malignant form of the disease should be steroids, and anticoagulants. He stressed the importance of continually searching for possible causes of the eosinophilia, (allergic, and parasitic diseases for example), as specific treatment would be curative. A small number of patients with HES also develop a malignant bone marrow disorder, several years after they presented with HES¹³⁴⁵.

In 1969, a group was set up at the National Institutes of Health (N.I.H.), Bethesda, U.S.A. by Dr Shelly Wolff, to study patients with unusual vasculitic diseases. As a result of this interest, patients began to be referred who had hypereosinophilia, which they defined as a blood eosinophil count greater than $1.5 \times 10^9/L$. Many of them did not have a vasculitis, which was surprising, as polyarteritis nodosa was considered at that time to be one of the main causes of a marked eosinophilia. However, they all had a number of serious clinical complications, which fell into a single syndrome, which they called 'the hypereosinophilic syndrome'. This was the first time that a significant number of these patients had been studied. Their results have formed the basis for all the subsequent work which has been carried out on this syndrome³²⁵.

They showed that the blood eosinophil counts were almost always persistently greater than $1.5 \times 10^9/L$, and (by definition) no cause for the eosinophilia could be found. In addition, a majority of the patients had evidence for tissue damage. Their first report described the clinical and haematological features of 14 patients, all of whom were male, and a comprehensive review of the English language literature brought to light 57 other patients with the same syndrome, 91 per cent of whom were also male. As the mean survival time of the earlier patients was only nine months, and as many deaths appeared to have been due to myelotoxic side-effects of cytotoxic drug therapy, treatment was more expectant in their own patients. In patients with evidence of active tissue injury, steroids were given first, followed by busulphan, chlorambucil, and 6-mercaptopurine in patients who remained ill. No attempt was made to bring eosinophil counts into the normal range: treatments was based on the extent, and severity of ongoing tissue damage. This approach resulted in a marked improvement in survival. The N.I.H. series of 50 patients was reviewed in 1982⁵⁴⁴.

It is likely that HES embraces a number of different diseases. Although most of the patients initially studied at N.I.H. did not have a malignant disease, (many have been followed up for more than 15 years), several had circulating myeloblasts, and raised serum B12 levels, with low folate levels, basophilia, and low alkaline phosphatase scores, suggesting that their illnesses was related to chronic myeloid leukaemia³²⁵.

15.1. Disease processes underlying HES.

It is still not known whether HES is an intrinsic bone marrow defect in which eosinophils, or their precursors themselves are at fault, or whether a factor, or regulatory mechanism affecting eosinophil production is abnormal. In 1982, it was reported that a 26 year-old woman with HES, and pulmonary lesions, E-EMF, and a blood eosinophil count of $54 \times 10^9/L$ had given birth to a healthy

boy with a blood eosinophil count of $55 \times 10^9/L$ ²⁷⁸. At one month it was still $61 \times 10^9/L$, but it then began to fall, so that it was $13 \times 10^9/L$ at four months, and in the normal range at eight months. It was suggested that the maternal eosinophilia had induced the eosinophilic response in the baby, but no special studies were done on this possibility. If this reaction were due to an eosinopoietic factor from the mother, its effect must have persisted for a remarkably long time. The activity of injected CSFs on eosinopoiesis are lost within a three days.

No genetic studies have been reported on whether eosinophils in patients with HES are derived from a single clone, and no patient with HES has yet receive a bone marrow transplant, which might help to clarify this question. There are also no reliable animal models of the disease.

A number of patients with HES have a preceding parasitic, or allergic illness in which an eosinophilia probably occurred. In these patients, it is tempting to suggest that this was the initial stimulus which led to the persistent eosinophilia. Two patients with eosinophilic leukaemia, who were studied in South Africa, also had schistosomiasis. This finding suggested to these authors, that chronic stimulation of eosinophil proliferation might have been followed by a structural change in an adjacent part of chromosome 12, where eosinophil proliferation may be regulated, possibly involving the c-Ki ras 2 gene, which is present at 12p12.1⁹⁴³. It is hoped that studies will develop along these lines into the genetic basis of eosinophil proliferation in normal, and malignant diseases.

15.2. Variants of HES.

It is possible to describe four clinical variants of HES which show some overlap.

- Some patients with HES have asthmatic symptoms, or a hypersensitivity, or allergic disorders. They may have raised serum IgE levels, suggesting that the disease has an allergic basis. The symptoms, and signs of disease in these patients usually responds within a few days to treatment with steroids, and their blood eosinophil counts can return to normal while they are taking steroids.

- A second group of patients with HES have a disease which gives rise to few symptoms or signs, despite the presence of a persistently raised blood eosinophil count. Treatment is not needed, and the disease may be of little inconvenience to the patient.

- In a third group of patients, who form the majority of patients with HES, there are one, or more, less serious complications of the disease, which requires treatment. This may range from coughing attacks, and skin lesions, to endomyocardial fibrosis. Treatment is again symptomatic, and in periods of relative quiescence of symptoms, the patients often feel well. Blood eosinophil counts are consistently raised, on, or off treatment. They have a life-long illness, and their lesions can progress.

- Patients in the fourth group present with the more serious complications of the disease, such as heart failure, and vascular occlusions, including strokes, or diffuse central nervous system involvement. These patients often have the highest blood eosinophil counts, and their blood eosinophils are often hypogranular. Eosinophil production in these individuals appears to be poorly regulated, compared to other patients with HES. For this reason cytotoxic drug treatment, in addition to steroids, may be required to suppress their bone marrow, and to help patients to recover from acute episodes of their illness, which generally recur at intervals of several weeks, or months.

15.3. Animal models of HES.

It would be of great value if an animal models of HES could be developed. This may well be possible, now that methods have been devised for producing a marked eosinophilia in rodents by treating them with cyclophosphamide, followed by an allergic, or parasitic eosinopoietic stimulus. We came across this possibility in 1980, when six LOU strain rats were infected with *T. spiralis* two larvae/g, two days after a heat wave. They developed eosinophil counts of up to $32 \times 10^9/L$. There was thrombus within both ventricles, extensive eosinophil infiltration into lymphoid tissues, and lungs, and one rat had cirrhosis¹⁶⁹⁹. It would be worth seeing whether recombinant GM-CSF, or IL-5 could be used to develop a model of HES in rodents.

A spontaneous hypereosinophilic disorder has been well-defined in cats. Six were described in 1981⁷⁸³, and in 1985, three cats which had the disease for four months, 1.5 years, and 3.5 years, were found at necropsy, to have many mature eosinophils in lymphatic tissues, the liver, and subendocardium,

but without endomyocardial fibrosis¹¹⁷³. Two dogs with a similar disease were described in 1983⁷⁰².
15.4. Patients affected.

HES is a sporadic disease which does not appear to have a strong genetic element in its pathogenesis, as there are no reports of the disease occurring in more than one member of a family. The remarkable sex incidence of HES, (and a number of other eosinophilic disorders), has yet to be explained. Nine males with the disease are reported for each female affected.

The mean age of onset of HES is 37 years, and few cases have been reported in childhood, or old age. The youngest child with the disease was aged 5.5 years at presentation³²⁵. A review of previously published cases of hypereosinophilia of unknown cause in children was published in 1987. There were only 18, which emphasizes the rarity of HES under the age of 15. Five of the children died with a leukaemic process, and the others probably had HES²⁷.

Examples of HES in children under 13 years of age include:

- A five year old girl described in 1973, who died 25 days after she presented with abdominal pain, vomiting, and fever. Her blood eosinophil counts rose from 78 to 126 x 10⁹/L. She also had cardiac, central nervous system, renal, liver, and spleen involvement¹⁴⁷⁹.
- A seven-year old boy reported in 1950, who presented with an eosinophil count of 10 x 10⁹/L, and had skin, heart, lymphatic, and joint involvement. Although the marrow was said to contain 53 per cent blast forms, he was alive 12 years later after treatment with only adrenocorticotrophic hormone (ACTH), making it probable that he had HES, not eosinophilic leukaemia as originally proposed⁴⁵⁸.
- A seven-year old boy described in 1942, who had an eosinophil count of 79 x 10⁹/L, with cardiac, respiratory, joint, and thrombotic complications. He died 11 weeks after presentation with coronary artery thromboses⁵¹².
- An eight-year old boy described in 1956, who presented with respiratory, cardiac, and abdominal symptoms. His liver, and spleen were enlarged, and his blood eosinophil count was 79 x 10⁹/L. Treatment with ACTH reduced the size of these organs, and the eosinophil count was lowered to 7.7 x 10⁹/L. However, the eosinophil count rose again, and he died six months later, after the marrow eosinophils had become increasingly immature. There was thrombus, and fibrosis in the heart, and eosinophilic infiltrates in tissues. Vascular changes were also noted in the spleen, and kidneys⁵¹⁴.
- A 10 year old boy discussed in 1956, who developed an illness which affected his skin, cornea, heart, kidneys, joints, and muscles. His liver, and spleen were enlarged. The eosinophil count rose to 10.5 x 10⁹/L. He also had a marked increase in serum immunoglobulins. Surprisingly, treatment with ACTH increased the eosinophil count, although the immunoglobulin levels fell. This disease appears to be different in many ways from classical HES⁵¹⁴.
- A six-year old girl described in 1984, who had an eosinophil count of 4.8 x 10⁹/L, with heart, skin, kidney, lung, and liver disease, while taking anti-tuberculous drugs. She died three, and a half years later. The possibility of rifampicin-induced hypersensitivity was a possibility in the aetiology of her eosinophilia, especially as she had circulating immune complexes, and a glomerulonephritis, but the illness was diagnosed as HES⁵⁸².
- A nine year old boy described in 1985, with blood eosinophil count of 64 x 10⁹/L, and heart, central nervous system, skin, respiratory, and lymphatic involvement. He died six months later with endomyocardial fibrosis⁷⁹¹.
- A seven year old girl described in 1958, who had an eosinophil count of 18 x 10⁹/L, with heart, central nervous system, skin, respiratory, and lymphatic involvement. She died after 10 months, with severe heart lesions, and peripheral emboli⁹⁸².
- A nine-year old boy described in 1985, who had a blood eosinophil count of 64 x 10⁹/L, with heart, central nervous system, skin, respiratory, and lymphatic involvement. He died six months later with endomyocardial fibrosis⁷⁹¹.
- Two Indian children aged three, and five years, described in 1983, who had abdominal pain, and a cough respectively. Although their blood eosinophil counts were between 4 and 9 x 10⁹/L, no

clinical complications developed during follow-up periods of over 18 months¹⁶⁰².

- a preterm boy described in 1981, who died aged four days with a perforation of the terminal ileum, which was infiltrated with eosinophils, as were his kidneys, lymph nodes, bone marrow, portal tracts of liver, gall bladder, and bile duct.¹²⁵⁴

15.4.1. Demography.

All races appear to be affected, although no accurate statistics are available. Besides the patients described above, there are also reports of HES from Scotland¹⁵¹, Eire¹⁴⁶¹, France^{1024, 509, 526}, Italy³³⁴, and Austria¹⁵⁷¹. Most of the patients described in the U.S.A., and England were white, although several were blacks^{530, 325, 2016, 1900}. Although HES was only described in temperate climates until recently, and it may be very difficult to distinguish it from chronic parasitic diseases, there are now several reports of HES from India^{1841, 1602, 1469}.

15.5. Presentation.

HES may present in a number of different ways. Sometimes the raised blood eosinophil count is found accidentally. In the N.I.H. series this occurred in 12 per cent of patients⁵⁴⁴. However in others, a serious illness leads to urgent hospital admission, and the diagnosis soon becomes clear. Most patients fall between these two extremes, and present with thrombotic complications, coughing, weight loss, or general malaise. The frequency of symptoms, and signs in the N.I.H. series⁵⁴⁴ was tiredness in 26 per cent, cough in 24 per cent, breathlessness in 16 per cent, muscle pains or angioedema in 14 per cent, a rash, or fever in 12 per cent, and retinal lesions in 10 per cent.

15.6. Nonspecific symptoms.

Sweating is a common feature of HES, and some patients also have a low grade fever. Although eosinophils were thought at one time to be able to produce endogenous leukocyte pyrogen¹²⁰⁷, which is now known as IL-1, this now seems to be unlikely, although no formal study has been done on this, using eosinophils from patients with HES, and a fever.

Twelve per cent of the N.I.H. series of patients presented with fever. It is seldom due to an infection, and the large amount of sweating in some patients is out of proportion to the rise in body temperature.

Few patients have weight loss, unless the disease is active, and they have lost their appetite. They seldom develop a catabolic state, as seen in some patients with malignant diseases.

Some patients with a marked eosinophilia have been found to have alcohol-related symptoms, such as flushing attacks, redness of the face, weakness, nausea, and diarrhoea. I have one patient with HES, who had these symptoms, with a lymphoma-induced hypereosinophilia. It has also been reported in a patient with eosinophilic leukaemia, in 1984¹³⁵¹, and in a patients with bronchial carcinoma-induced hypereosinophilia¹¹⁷.

15.7. Cardiovascular complications.

Cardiovascular complications are amongst the most serious features of HES. The principal ones are eosinophilic endomyocardial fibrosis (see Chapter C14a), and thromboemboli. Arterial calcification has also been noted in three patients with HES^{1286, 646}.

15.8. Thromboembolic complications.

Eosinophils are able to affect the coagulation system by actions on endothelial cells, platelets, and clotting factors. IL-1 has been found to be a potent inducer of tissue factor-like procoagulant activity, and this maybe an important event in intravascular coagulation produced by immunological, and inflammatory diseases¹⁶⁴. For this reason it is not surprising that small vessel, and large vessel thrombotic occlusions are an important feature of HES, and may well cause the death of patients if they are not recognized, and treated. Extensive cardiac ventricular intracavity thrombi, and emboli can develop in some patients with HES.

A striking example of this was described in 1977 in a 30 year old Japanese man who presented with thrombophlebitis, and an eosinophil count of $109.7 \times 10^9/L$. He was treated with prednisolone, and busulphan until his death three years later, when he had also developed eosinophilic heart disease. At post mortem the heart was found to contain a large mural thrombus below the mitral valve,

overlying fibrous endocardium. Thromboemboli were also seen in other organs: the spleen, kidneys, liver, and brain ⁸⁴⁶. A Swedish patient with similar cerebral, renal, and pulmonary infarcts, and thrombotic endocarditis, was reported in 1979 ⁸⁴⁷.

The principal small vessels which are known to be involved, include small vessels in the nail beds of the fingers, where they produce 'splinter haemorrhages' ¹⁴⁶⁰, and retinal vessels, which were occluded in as many as 80 per cent of patients in one series.

Large vessel occlusions probably occur mainly as a result of emboli leaving the wall of the left ventricle. These have been noted most commonly in femoral arteries, and the lower aorta, but can also occlude coronary arteries, or give rise to minor strokes, and major thrombi in the central nervous system.

Other forms of arterial disease can also affect the large vessels of patients with HES. Two patients with arterial calcification were described in 1971 ¹²⁸⁶. There is also a report of the development of thromboangiitis obliterans requiring amputation of the legs in a 47 year-old man with HES ⁵⁵⁶. This patient also had an occluded temporal artery, with eosinophils infiltrating the vessel wall. It was suggested that these vascular lesions had occurred as a result of eosinophil-dependent damage to the vessel wall, but the patient was also a smoker.

15.9. Respiratory tract complications.

One of the main presenting features of HES is an intractable nocturnal cough which wakes the patient, and forces him to cough repeatedly for several minutes. A small amount of sputum may be produced. These coughing attacks can occur during the day, and may be very disabling. Chest radiographs are normal before, and during these attacks. In one patient, I was able to show that large numbers of blood eosinophils were being sequestered in the lungs during an attack.

Pulmonary infiltrates are often seen in patients with HES. These have to be distinguished from heart failure, embolic lesions, infective episodes, and tumour deposits, which can also give rise to an eosinophilia ⁵²¹. When lung infiltrates in patients with HES are biopsied, they usually show an interstitial infiltrate of mononuclear cells, and eosinophils ⁴⁹³. In a report in 1984 on a patient with progressive infiltrates, the eosinophils were found surrounding, and infiltrating small pulmonary arterioles ⁷⁹⁷.

In 1982, details were given of a patient with HES, and endomyocardial fibrosis with heart failure, who had respiratory failure, due to respiratory infiltrates. His lung lesions cleared within two weeks of treatment with steroids ⁹³.

15.10. Digestive system complications.

Many patients with HES complain of indigestion, and discomfort in the abdomen, but it is uncommon for them to have diarrhoea, gastrointestinal bleeding, or other serious problems affecting the gut. When they do occur, a careful search should be made for the site of the abnormality, and the nature of the organ involvement can vary considerably. Detailed inpatient investigation may be required. Unusual complications include small bowel obstruction ¹⁶⁵², and jaundice due to pancreatic involvement ⁵²⁶.

There are now several patients with HES, who have developed ulcers in mucous membranes. Two patients with oro-genital ulcers were reported from the Mayo Clinic in 1982 ¹⁰⁵⁴. This preceded other more serious complications of HES, such as heart disease. We are also looking after a patient with HES, and lichen planus, which is producing severe pain in the mouth, and pharynx, and has been refractory to a wide variety of drugs.

Diarrhoea occurs in about 20 per cent of patients with HES, and the bowel wall may be infiltrated with eosinophils ^{325, 1694}. In one of our patients who had repeated attacks of gastrointestinal bleeding, this was eventually shown to be due to a vascular malformation in the ileum. In another patient, persistent diarrhoea was the presenting feature for an eosinophilic disorder which was subsequently shown to be eosinophilic leukemia.

The liver can be enlarged in patients with HES, and biopsies often show the presence of many periportal eosinophils. One patient with the Budd-Chiari syndrome complicating HES was reported

from Creteil, France, in 1985⁵⁰⁹.

15.11. Skin complications.

A wide variety of skin lesions have been noted in patients with HES. These were reviewed in 1979, when the findings in eight patients at N.I.H. were described⁹³⁷. They were usually mild, with erythematous pruritic papules, and nodules, or urticaria, and angioedema. There was a variable response to treatment with steroids. It was reported in 1984 that an intensely pruritic erythematous rash in a patient with HES, responded to oral sodium cromoglycate 200 mg, four times daily. Topical steroids had no effect¹⁹⁵².

Many patients have itching of the legs, and scratch marks are common. Some patients show marked dermatographism, but this does not appear to correlate with any features of the illness, and a few patients may have urticaria within two minutes of applying pressure to the skin for 30 seconds, a condition not seen in other type of pressure urticaria⁹⁰⁸.

Eczematous skin lesions can occur in HES, but they have no diagnostic features.

Papules, and macules are common in patients with HES⁹³⁷. An unusual skin disorder was seen in a man of 24 with HES who had pruritic papules involving many parts of his skin. He also had an enlarged liver, and an eosinophil count of $2.7 \times 10^9/L$. Treatment with prednisolone gave some benefit, but dapsone was more effective. On the basis of the results in this patient, and a review of three others, it was suggested that dapsone might be helpful in other patients with similar skin complications¹²⁷⁹. The term 'hypereosinophilic dermatitis' was used to describe this skin abnormality.

In other patients, localized abnormalities occur in the skin, which may ulcerate. We have looked after one patient with severe ulcerating lesions, which appeared several weeks before she died. They were associated with thrombi in dermal arterioles¹⁶⁹⁴. Similar lesions were described in a patient with HES in Puerto Rico in 1982¹⁵⁵⁰. We have also noted three patients with HES, who were later found to have lymphomatoid papulosis¹⁹²⁶. In these patients we suggested that the T cell abnormality which gave rise to the skin disorder, could also induced the eosinophilia.

15.12. Nervous system involvement.

The central nervous system is often involved in patients with HES, and individual peripheral nerves can also be affected. Many patients have psychological problems, although no formal studies of this have been carried out. Several of the major neurological complications of HES were defined in a detailed study in the U.K., in 1971⁶²⁷. Four patients were described who had optic ataxia, and paralysis of visual fixation. Post mortem studies in one patient showed brain infarcts, with subintimal fibrous tissue in the left posterior cerebral artery. These were also present in a 59 year old man, who had cerebral infarcts, and emboli, but without arteritis. These central nervous system complications only seem to occur in the most seriously affected patients, who seldom live for more than one or two years after they have developed. It is not known whether they are the result of microemboli, or local thrombosis in cerebral vessels, or a more subtle effect of the disease on the brain tissues.

The ways in which HES causes neurological problems is still not known, although a number of post mortems have been carried out on these patients. There have been no reports of demyelination, or loss of Purkinje cells, which occurs when ECP, or EDN/EPX are injected into the cerebrospinal fluid of experimental animals. As high levels of these proteins have been detected in the cerebrospinal fluid in a variety of eosinophilic disorders, it is probable that these effects of the eosinophil basic proteins in animals are unimportant in human neurological complications of HES. This area needs further work, and its importance may only come to light when experimental studies have been done using isolated nerve cells, and purified eosinophil constituents.

15.12.1. Central nervous system complications.

Patients with HES may have diffuse central nervous system disease, or focal lesions, or combinations of these. The commonest abnormality is an alteration in their capacity to think rationally. This has been difficult to define, but I have noticed it especially during periods when my patients have had progressive disease. Neurological disease affecting the N.I.H. group of patients with HES was

assessed in 52 patients, and reported in 1985. Thirty-four (65 per cent) had neurological abnormalities, including encephalopathy, peripheral neuropathy, and focal lesions. Seven patients had more than one of these abnormalities

In the N.I.H. series, five patients were described who had a distinctive encephalopathy, characterized by changes in behaviour, confusion, loss of memory, and ataxia. They had upper motor neuron signs, with increased muscle tone, deep tendon reflexes, and a positive Babinski sign. Two had epileptic fits¹²³³. The nature of the alteration in brain function is not known. In our series of patients, four of whom had post mortems, no lesions were found in the brain, and this was also the case in a post mortem of another patient with HES, and confusion, reported from Texas, U.S.A. in 1985¹⁹⁰⁰.

Major complications of HES are repeated minor, or major strokes which can cause the patient's death. These are usually multifocal, and can involve any of the major centres of the brain. They probably involve occlusions of vessels by emboli from the left ventricle¹³⁸³, and possibly by local thrombus formation. At the N.I.H., six patients had focal central nervous system disease, which was transient. Five others with these features also had heart disease, suggesting that they may have had thromboemboli, and two had recurrent attacks¹²³³.

15.12.2. Eye diseases.

Although only one patient with retinal haemorrhage was reported amongst a group of 52 patients with HES at the N.I.H.¹²³³, we have found retinal abnormalities in many of our patients with HES, Figure C15a-2. This was documented in 12 of our patients in whom lesions were visible, either by direct examination, or by fluorescent retinal angiography²⁹⁶. Defects were the result of vascular occlusions affecting the retinal, and choroidal vessels, possibly by emboli, such as probably occurs in patients with HES, and splinter haemorrhages. One patient developed showers of splinter haemorrhages, and had coincidental retinal lesions. Other case reports of patient with HES, and retinal vascular lesions were reported from Louvain, Belgium, in 1980¹⁰⁷³, and from Creteil, France in 1982⁵⁴⁰.

Fig. 15-2: Retinal vascular occlusion in a patient with HES.

15.12.3. Peripheral nerve diseases.

Several types of peripheral nervous system disease may develop in patients with HES^{151,717}. These include mononeuritis multiplex, symmetrical sensorimotor neuropathy, multifocal neuropathy, and radiculopathy. In some patients it may be difficult to distinguish nerve involvement in HES, from other causes of a neuropathy, and an eosinophilia, especially vasculitic, and granulomatous disease. This is well shown in a study on three patients with a peripheral neuropathy reported from Palo Alto, U.S.A., in 1983⁴⁶², and in these patients the presence of allergies, asthma, and granulomas, mitigated against a diagnosis of HES.

Mononeuritis multiplex is common in patients with HES, involving the peritoneal nerve, and other long nerve supplying the legs, and arms. They may cause considerable pain, and discomfort, but tend to resolve over several weeks, sometimes leaving no residual abnormality. An example of this was in a 16 year old man in Kuwait, who also had respiratory, and cardiac involvement, which were eventually fatal⁵⁰⁷. Another patient was described in 1985¹²³³.

A sensorimotor neuropathy was described in 1982⁵⁴⁴, and 23 patients among 52 with HES at N.I.H. had various forms of sensorimotor loss. Three other had a radiculopathy¹²³³. Seven of these patients, three of whom had clinical evidence of peripheral nerve injury, underwent nerve conduction studies, electromyograms, and sural nerve biopsies. These showed axonal neuropathy, axonal loss, and some demyelination. It was suggested that these changes could have been produced by EDN/EPX¹⁹²⁸. A patient with HES, and a purely motor neuropathy was described in 1986. This patient also had bilateral facial palsies, and focal central nervous system lesions¹¹⁶².

Multifocal neuropathy in HES has been described in 1975³²⁵, 1983⁴⁶², and 1985⁷¹⁷.

15.13. Musculoskeletal complications.

Many patients with HES have aches, and pains, which are often poorly localized. Muscle biopsies

have shown no specific abnormalities. Bony deposits of primitive eosinophils are a feature of eosinophilic leukaemia, but it has only been reported in one patient with HES, who also had hypercalcaemia⁶⁴⁶.

Joint involvement is also unusual, but there are a number of patients with a disease similar to rheumatoid arthritis who have a marked, and persistent eosinophilia for many years. We are looking after a woman with this association, who has had HES, and rheumatoid arthritis for 24 years. A 58 year old man with an eosinophilia of $4.5 \times 10^9/L$, was described in 1967 who developed swollen, tender, and stiff joints. His skin became doughy, and erythematous. The erythrocyte sedimentation rate was 68 mm/h. Although he was treated with steroids, his eosinophil counts increased, splenic infarcts occurred, and he died in heart failure. However, as blast cells eventually appeared in his blood, and these were also found infiltrating into the heart, he may have had eosinophilic leukaemia¹⁴⁰⁴.

Skeletal muscle wasting associated with eosinophil infiltrates may develop in a few patients with the hypereosinophilic syndrome¹⁶⁵², although muscle biopsies usually show no specific abnormalities.

15.14. Renal complications, and hypertension.

About 10 per cent of patients with HES have renal complications, but less than this have hypertension. One of the first was reported in 1965¹²⁹³. There are also some reports of renal interstitial eosinophil infiltrates, and vascular damage to renal arteries, shown by intimal proliferation, and medial injury¹³⁵¹. These vascular changes have also been seen in other sites³⁰⁸.

Renal impairment with glomerulonephritis has been described in a small number of patients with HES, and eosinophilic endomyocardial fibrosis¹⁶⁶¹. In two cases, which were reported in 1969, the renal damage may have been due to associated infections in the heart. A post mortem on a 25 year old man who died 1.75 years after onset of his illness, showed thickened glomerular basement membrane, with fibrinoid necrosis of arterioles, but he was also found to have vegetations on the aortic and mitral valves. A second man, aged 35, with eosinophilic heart disease complicated by bacterial endocarditis, and myocardial abscesses, had renal disease during life. Similar glomerular lesions were found at post mortem¹⁵⁰².

Several patients with HES have had hypertension, and impaired renal function. This has been shown to be associated with arteriolar thickening in renal biopsies, and it has been suggested that eosinophils might be involved in damaging blood vessels in these areas. The hypertension responds to therapy, but the renal impairment does not appear to improve. No patient with HES has progressed to end-stage renal failure.

15.15. Breast involvement.

A single report of breast involvement was reported in 1985¹⁷⁷². This was a 56 year old woman with HES who developed swollen, painful, red breasts, in which the ducts were heavily infiltrated with eosinophils, and contained areas of necrosis, but no fibrosis. The skin was indurated, and the breasts were stony hard. This lesion resolved following treatment with steroids.

15. Chapter C 15 b. The idiopathic hypereosinophilic syndrome, HES. II. Investigations.

The clinical investigation of HES involves an assessment of the nature, and mechanisms of the complications which are usually the presenting features of the illness, and a careful search for the underlying causes which may only become apparent several months, or years, after the patient becomes ill. As this disease is usually life-long, it is suggested that patients be admitted to hospital for several days for a careful assessment of each of the major organ system which might be involved at the time, or subsequently, and in order for a careful long-term plan of management to be organized. It is also recommended that these patients be reviewed at one of the major centres where there is a special interest in the disease, although their subsequent care can be carried out in the outpatient departments of most district hospitals. As many organ systems can be involved, patients may need

to be seen by different specialists for this initial workup.

This chapter will outline the special investigations which have proved to be useful in discovering the nature of the underlying abnormalities in the eosinophil series in this disorder. Details of cardiologic studies are given on page 439.

15.1. Blood eosinophils in HES.

An unusually high blood eosinophil count in patients with HES is the first investigation which points to this diagnosis. Figure C15b-1. Nearly every patient with HES has an eosinophilia throughout his life, with counts remaining remarkably constant in the majority of patients. In a few patients with the aggressive forms of the disease, the counts may rise to higher levels, but these can return towards their previous levels after treatment, and they may then persist at this level for several years, before relapses occur. The majority of patients with HES have eosinophil counts between 2 and 20 x 10⁹/L. The highest count I have seen recorded in HES is 220 x 10⁹/L¹⁰⁰⁹. There is no direct relationship between the eosinophil count, and the severity of the clinical complications. Some of the highest blood eosinophil counts have been found in patients with the more benign forms of the disease. Serial blood eosinophil counts have only been published in a relatively small number of patients, but the management of these patients is greatly helped by graphing their blood counts on log paper, over a prolonged period. This is important if minor fluctuations in the counts are not to be overinterpreted as signs of alterations in the underlying disease process.

Fig. 15-1: Blood sample from a patient with HES.

A wide range of special studies have been carried out on blood eosinophils from patients with HES, and many of the papers published on the properties of eosinophils are describing HES eosinophils. Comparisons of their properties with eosinophils from other disorders are still limited, but it is likely that differences will be found. Some of these may be linked to the increase rate of production of eosinophils in HES, but others may be due to the underlying abnormality in the eosinophil series, which probably occurs in some patients with HES.

15.1.1. Eosinophil density.

In a study reported from the Mayo Clinic in 1987, blood eosinophils from 10 normal people had a mean peak density in Percoll of 1.088 g/ml, and only 10 +/- 7 per cent (mean +/- SD) had a density less than 1.082, which was defined as the cut off point to distinguish normal density from light density eosinophils. In four patients with HES, the mean peak density was much lower: 1.075, and 97 per cent of the eosinophils were of light density. The density of blood eosinophils correlated with their MBP¹³⁸⁹. Current research in several centres is looking at how normal eosinophils could become lighter in density. The first successful in vitro technique, reported in 1987¹⁵²², involved the use of prolonged culture with endothelial cells, or their products.

15.1.2. Morphology of blood eosinophils in HES.

It has been known for many years that blood eosinophils in patients with a marked eosinophilia may be structurally abnormal, and these were often thought to be leukaemic. For example in 1919, a patient with 'eosinophilic leukaemia' in New York, U.S.A., was seen to have many blood eosinophils with fewer eosinophilic granules than normal. This important observation was recorded in a painting¹⁶⁰⁰, Figure 15b-2. Similar findings were reported in 1960³⁰⁸.

Fig. 15-2: Degranulated blood eosinophils.

15.1.2.1. Light microscopy of HES blood eosinophils.

In blood smears, eosinophils from patients with HES show a range of abnormalities, which include hypersegmentation of the nucleus, cytoplasmic vacuoles, a decrease in the number of specific granules, and granules of smaller size than normal. Measurements of the proportions of cells in the blood which show these abnormalities may be of clinical value in distinguishing HES from other causes of an eosinophilia. The presence of many degranulated eosinophils in a blood smear, can be striking. When there are more than 1 x 10⁹/L, it is likely that the patient has HES, and eosinophilic endomyocardial fibrosis.

Hypersegmented blood eosinophils with decreased numbers of granules, and vacuoles are not con-

ined to patients with HES. For example eosinophil peroxidase deficiency can occur with these abnormalities in blood eosinophils¹⁴³⁷, and I have seen them in a patient with severe tropical (filarial) eosinophilia.

Histochemical, and immunocytochemical studies on HES blood eosinophils have shown several qualitative differences from normal eosinophils, but they have not been studied sufficiently to know whether these can be used in diagnosis. The alterations were well illustrated in a detailed study in 1964 on the morphological, and histochemical characteristics of blood, and bone marrow eosinophils from a 38 year-old man with ' eosinophilic leukaemia', which would be diagnosed today as HES. Abnormalities in the neutrophil series were also seen³.

15.1.2.2. Ultrastructural studies on HES blood eosinophils.

Blood eosinophil ultrastructure in patients with HES shows several features consistent with the possibility that they are activated, and secreting their granule contents.

Scanning electronmicroscopic studies on human blood eosinophils from patients with HES, have shown no surface features to distinguish them from eosinophils, or neutrophils in normal individuals. As yet, no studies have been done to see if activated eosinophils develop ridges, and projections which are found in other cells which are stimulated.

The first detailed morphometric study on HES blood eosinophils was carried out in 1986, on peripheral blood eosinophils from a woman aged 41 with HES, and a blood eosinophil count of $202 \times 10^9/L$. The principal findings were that the eosinophils were larger in size than normal, with a 34 per cent increase in volume, and 19 per cent had three or more nuclear lobes. Mitochondria were more numerous, and 50 per cent larger than normal. The volume of the specific granules was 35 per cent smaller than normal, and there were slightly less of them than normal. These findings suggested that in this patient blood eosinophils were metabolically more active than normal, and that the granules were undergoing dissolution, or secretion¹⁶⁵⁹.

A detailed ultrastructural study was carried out in Seattle, U.S.A., in 1987, on five patients with HES. The mean diameter of the crystalloid granules was reduced to 82 per cent of normal, and there were only 27 per cent of the normal number of granules. Lipid bodies were prominent in the cytoplasm, and in two patients, some eosinophils were found to contain Charcot-Leyden crystals⁷⁸⁰.

There are two reports on ultrastructural appearances of blood eosinophils in patients seen at the Mayo Clinic. In the first, published in 1976, there were unusual electron-dense inclusions in the cells¹⁶⁶¹. The appearances of the cells in three other patients were reported in 1987. Over 90 per cent of the eosinophils were of light density: less than 1.082. The total number of granules were the same as in normal people, although the granules were of smaller mean area than normal: $0.14 \mu^2$, compared to $0.26 \mu^2$. The eosinophils were of the same size, with the same number of lipid bodies, and equal proportions of eosinophils with reversed staining were seen¹³⁸⁹. The reasons for this are not known, but it was suggested in 1975 that the hypogranular eosinophils could be less mature than normal⁴⁵⁵.

Freeze-fracture studies of HES blood eosinophils have shown the presence of membrane structures similar to those seen in blood neutrophils⁷⁸⁰.

15.1.3. HES blood eosinophil cell membrane components.

The plasma membrane of blood eosinophils from patients with HES has been studied using a variety of different techniques. In 1976 we showed that HES blood eosinophils had more Fc gamma R, and complement receptors than normal¹⁷³⁸. In one patient with HES it was found that the cell surface charge was low, similar to that of stimulated normal blood eosinophils¹²⁷.

We have raised monoclonal antibodies to HES blood eosinophil membrane constituents¹⁷³⁴. These recognized a number of structures which were also present on neutrophils, but which were occasionally found in greater amounts on the surface of HES blood eosinophils, than on normal eosinophils. The nature of the membrane antigens which they bound to, has still to be defined, but they appear to be components of the membrane which are involved in activating the cells to secrete.

15.1.4. Constituents in HES blood eosinophils.

Many of the studies which have described the purification of eosinophil constituents have used blood eosinophils from patients with HES, as they are the easiest to obtain. However, in some patients with HES, there is less ECP, and MBP in blood eosinophils than normal. In the past, this led to difficulties in measuring the absolute quantities of the different basic proteins in human eosinophils. The reasons why there are less of these proteins in these patients' cells is not known, but could be due to either reduced synthesis in the marrow, or to preferential secretion from the blood cells. A study in 1983 on blood eosinophils from six patients with HES showed that they contained 6.3-63.5 more MBP than ECP. This may have been related to the amount of degranulation that has occurred, as this ratio was smallest when the cells contained the highest concentration of ECP⁹.

Blood eosinophils from patients with HES have a lower, and a more variable content of MBP than normal⁷. The MBP content of blood eosinophils, with a density less than 1.082, in four patients with HES was 3,670 ± 368 ng/10⁶ cells, compared to 8,619 ± 1,445 ng/10⁶ cells in normal subjects¹³⁸⁹. The content of ECP in blood eosinophils from patients with HES is usually less than normal¹⁹⁴⁴. There are no reports on the eosinophil content of EDN/EPX, or EPO in HES blood eosinophils. Cytochemical electronmicroscopy has shown that acid phosphatase was unmasked in eosinophil granules from one patient with HES¹²⁷. Blood eosinophils from patients with HES have over twice the capacity to secrete LTC₄ in response to stimulation with A23187, as eosinophils from normal blood: 69 ng compared with 38 ng/10⁶ eosinophils¹⁹¹⁵. There is a case report describing PAF-acether production by blood eosinophils from a 32 year-old woman in Brazil, who had HES, with cerebral, and retinal vascular occlusions, and endomyocardial fibrosis. These produced 4 x 10⁻¹⁰ M PAF-aceter per 1 x 10⁶ eosinophils, and it was suggested that this could have contributed to the development of her thrombotic lesions²⁹⁹. HES eosinophils produce large amounts of PAF in response to the calcium ionophore A23187⁸⁹⁸.

15.1.5. Metabolism of HES blood eosinophils.

Many metabolic studies on human eosinophils have been carried out using blood eosinophils from patients with HES. An example of this was the study done by Mickenberg, and colleagues in 1971. They isolated blood eosinophils from a 45 year-old man with HES, eosinophilic endomyocardial fibrosis, and multiple embolic episodes. The cells' capacity to kill bacteria, and metabolize glucose after a phagocytic stimulus was compared with blood eosinophils from other patients with an eosinophilia. After incubation of the patient's eosinophils with bacteria, more of the organisms were killed at two hours, than when control eosinophils were used. There was also a higher oxidative capacity for 1-¹⁴C-glucose in the patient's eosinophils compared to controls, either resting, or after phagocytosing particles in vitro¹²⁰⁷.

Similar studies were done in 1981, on nine patients with HES, with blood eosinophil counts between 2.1 and 7.2 x 10⁹/L, by Bass, and colleagues. They showed that the patients' blood eosinophils had a higher uptake of deoxyglucose than normal, both resting, and after stimulation with zymosan activated serum, than normal blood eosinophils¹²⁷. The amount of superoxide produced by purified eosinophils from a patient with HES was shown to be related to the degree to which the cells were stimulated¹⁴¹⁹.

15.1.6. Motility, and chemotaxis of HES blood eosinophils.

Histamine diphosphate induced purified blood eosinophils from four patients with HES, and one other patient, to migrate up a concentration gradient, but random motility was not increased. At high concentrations of histamine, migration was inhibited³²⁸. In a paper published from Lille, France in 1987, it was found that light density blood eosinophil from four patients with HES migrated less well towards LTB₄ than normal density eosinophils from the same patients. The sera from five patients with HES was found to be as effective as LTB₄ in inducing eosinophils from other patients without HES, to migrate into polycarbonate filters, eg. the sera contained chemotactic activity. This activity was found in 600 kDa, and 240 kDa fractions. Preincubation of normal density HES eosinophils with this active sera reduced their capacity to migrate towards LTC₄, and fMLP. From these results, it was suggested that the sera of these patients with HES contained two

(deactivating) components which might inhibit the capacity of their blood eosinophils to leave the circulation, and that this could explain their high blood eosinophil counts⁷⁰⁰. Unfortunately some data was missing from this report, and it did not address the question of why HES blood eosinophils should be able to migrate in vitro, if they had already been deactivated in vivo.

15.1.7. Activated blood eosinophils in HES.

The monoclonal antibody EG2, which distinguishes activated from nonactivated blood eosinophils¹⁷⁴², has shown that a variable proportion of eosinophils in the blood of patients with HES are reactivated. So far, no study has been carried out to see whether this can be clinically useful in determining the degree of activation, or severity of complications in these patients.

15.1.8. Secretion from HES blood eosinophils.

The capacity of HES blood eosinophils to secrete their granule contents in response to stimuli, such as complement-coated particles, is very variable when different patients are studied. The reason for this is not clear. However, in any one patient, the secretory response to a set stimulus is reproducible, and this has enabled studies to be carried out on the effects of drugs on eosinophil secretion. In 1987, blood eosinophils from five patients with HES were isolated, to study the amounts of EPO which were secreted following stimulation with opsonized zymosan, and the calcium ionophore A23187. Under optimal conditions, 23 per cent of the EPO was secreted following stimulation with A23187, which was seen to induce vesiculation of granule contents, and fusion of adjacent granules in electronmicrographs. Opsonized zymosan also induced vesiculation, with granule fusion, and secretion into the endocytic vacuoles, but only 13 per cent of the EPO was secreted⁷⁸⁰.

15.1.9. Cytotoxic capacity of HES blood eosinophils.

The capacity of HES blood eosinophils to kill a variety of targets has been assessed: They have been shown to be able to kill a number of antibody, and complement-coated parasites, and nucleated cells¹³⁶⁷.

15.1.10. The half-life of injected blood eosinophils.

Isolated blood eosinophils have been labelled with ⁵¹Cr or (111)In, and reinjected into patients with HES. This has shown that they have the unusual capacity to marginate in the circulation for 1-2 hours, and then to re-emerge with a subsequent prolonged half-life compared to blood neutrophils, or eosinophils in patients with parasitic diseases³⁸⁵, and I have similar unpublished data on five other patients injected with (111)In-labelled blood eosinophils.

15.2. Serum, and plasma constituents in HES.

Plasma MBP levels in four patients with HES were 2.928 +/- 1.567 ug/ml compared to 0.307 +/- 0.038 ug/ml in 10 normal subjects¹³⁸⁹. In one patient with HES, large amounts of ECP were found complexed to alpha 2 macroglobulin¹³⁹⁸.

Several patients with HES have been noted to have high serum B12 levels^{910, 575, 1461, 1024}. In the large study from the N.I.H., Bethesda, U.S.A., in 1981, it was the commonest haematological abnormality in 32 patients with HES⁵⁷³. In 1984, serum cobalamin (vitamin B12), and unsaturated B12 binding capacity were measured in 16 patients with HES²⁰⁰². They were markedly elevated, and this was principally due to an increase to R binders (transcobalamins I and III). The cell source of this additional B12-binding capacity is not known. A proportion may have come from neutrophils, which normally contain over four times as much unsaturated B12 binding proteins as normal eosinophils. In a study in 1984 on partially purified eosinophils from a patient with HES, transcobalamin I was detected at 10 ng / 10⁸ eosinophils: half the amount found in normal neutrophils²².

Although Strath, and Sanderson in 1986 detected an eosinopoietic activity in the sera of mice infected with *Moscocestoides corti*¹⁷¹⁴, they have had no success in developing a reliable assay system using human sera. However in 1987, the serum from six patients with hypereosinophilia, who were studied in Hamburg, West Germany, induced additional production of eosinophils in 12 day liquid cultures of normal bone marrow⁹⁵⁴.

15.3. Bone marrow features of HES.

In patients with HES, up to 80 per cent of the dividing cells in the bone marrow may be of the

eosinophil lineage. There are no morphological features distinguishing these dividing eosinophils from eosinophils in normal bone marrow, but additional studies with these cells have shown that there are important abnormalities in up to ten per cent of these patients. The quantity of reticulin, and fibrous tissue in the bone marrow is often increased. In the majority of patients the red cell series is well-preserved. There is a report of one patient with polycythaemia and HES¹⁸⁴². Platelet production is normal, or increased, although the blood platelet count is usually low, due to platelet consumption. The neutrophil production is usually not affected, although some patients have unusually basophilic granules in a proportion of their neutrophils. A normal neutrophilia can develop in patients with HES, who developed a bacterial infection¹²⁴.

Between 1968 and 1979, 32 patients with HES, who presented at N.I.H., had bone marrow, and other haematological studies done. A retrospective analysis of their clinical, and haematological features, enabled each one to be given a numerical score, which was proposed as a simple method for deciding on the severity of the disease, and the necessity for different forms of treatment⁵⁷³.

There are a small number of patients with HES, who have a marked increase in fibrous tissue in the marrow. Examples of this include:

- a patient reported as having 'eosinophilic leukaemia' in 1969¹⁵⁷.
- a 57 year old man with refractory heart failure, and an eosinophil count of $12.4 \times 10^9/L$ in 1972⁵⁷².
- a 65 year old man with blood eosinophil counts of up to $13 \times 10^9/L$ in 1972⁶³⁸.
- three patients reported from Copenhagen, Denmark, in 1986. It was suggested that eosinophils could be producing a factor, such as platelet-derived growth factor, which could induce the fibrotic reaction¹⁰⁴⁶.
- four patients were reviewed in 1974 in a report which also described a 16 year old black patient who died in a blast cell crisis, and was found to have endocardial disease⁷¹⁰.

15.3.1. Bone marrow culture in HES.

In 1981, we measured the number of eosinophil colonies which could be grown from the bone marrow, and blood of four patients with HES. The total number of colonies which grew from the marrows of two of the patients was not different from normal, but the proportion which were eosinophilic was increased. No eosinopoietic effect of the patient's lymphocyte conditioned medium was found⁷⁷⁴. In 1982, 13 patients with H.E.S. at N.I.H., were assessed for the number of CFU-Eo which grew from bone marrow, and blood. In seven, normal numbers were found. In five, increased numbers developed, and in three of these, bone marrow cells produced excess CSF. One patient produced a smaller number of colonies than normal. None of them showed the in vitro growth characteristics of leukaemic cells¹⁷⁶. This study demonstrated that there may be heterogeneity in HES, depending on whether there is a defect in eosinophil progenitor cells, or in the cells which produce CSFs.

15.3.2. Cytogenetic studies in HES.

A wide range of chromosomal abnormalities have been described in patients with a persistent eosinophilia. Some of these patients have an eosinophilic leukemia, either of the acute or chronic form. Whether any of these patients should be included under the general term of HES is questionable. My own feeling is that, once a cause has been found for hypereosinophilia, the illness should be placed in a separate category from HES. The cytogenetic defects in eosinophilic leukemia are described in Chapter C 04.

15.4. Immunological investigations in HES.

The commonest immunological abnormality in patients with HES is a raised immunoglobulin level in serum. This is nearly always polyclonal, mainly affecting IgM, IgG, and/or IgE. Defects in lymphocytes have been less consistent.

The clinical significance of a polyclonal increase in serum immunoglobulins in patients with HES is not known, as it does not appear to be related to any particular clinical complication. Occasionally, as reported in 1976, in a 56 year old London man, a paraprotein can be present¹⁵².

Although many tests of T lymphocyte numbers, and functions have been carried out in patients with

HES, no consistent abnormality has been detected¹³⁶⁹.

It was reported in 1983 that blood mononuclear cells from a 54 year old man with HES, who had eosinophil heart disease, (but no thromboembolic episodes) produced three, or four times more procoagulant activity than normal monocytes on stimulation with endotoxin in vitro. As this abnormality returned to normal during treatment, it was suggested that this might be a cause of thromboembolic complications which are common in patients with HES¹³⁷³.

15.5. Coagulation studies in HES.

We have carried out coagulation studies in nine patients with HES, and compared them with normal subjects. There were marked elevations in the levels of B-thromboglobulin, platelet factor 4, fibrinogen, factor VIII, C:Ag, and R:Ag. In one patient, eosinophil supernatants produced platelet aggregation in vitro. As many of these patients had a low platelet count with increased numbers of bone marrow megakaryocytes, it was probable that there was continuing spontaneous coagulation even when there was no clinical evidence of thrombotic lesions³⁹⁸. It would be interesting to study patients with an eosinophilia of other causes, to see whether eosinophils induce these coagulation abnormalities. Abnormalities in fibrinolysis, and increased procoagulant activity secretion from monocytes in patients with HES would also be worth studying.

15. Chapter C 15 c. The hypereosinophilic syndrome, HES. III. Course of the disease, and treatment.

The management of HES has to take into account the life-long nature of the disease, its propensity to relapse, and remit, the prevention, and treatment of complications as they occur, and the avoidance of harmful side effects of powerful drug therapy which may be employed.

A number of charts of the course of the disease in individuals with HES have been published see for example some of patients' charts¹⁶⁹⁴. Studies on the survival rate of patients with the disease have shown a progressive improvement since the early sixties, when therapy was based on current treatments for leukaemia. The realization that a leukaemic disease process was not present in the majority of patients with HES has altered this approach, and now very few patients die from complications of their treatment. A breakdown of patients into the groups described in Chapter C15a can be helpful. Patients (1) with allergic features are given steroids intermittently in relation to symptoms, (2) with disease of mild severity are treated with low doses of steroids, and anti-platelet drugs, (3) with moderately severe disease are treated with moderate doses of steroids, intermittent courses of hydroxyurea, and anticoagulants, and (4) with severe progressive disease are treated with high doses of steroids, continuous hydroxyurea, or another cytotoxic drug, with intermittent injections of vincristine, and anticoagulants. Treatment can enable most patients to lead normal lives, and hospital admissions can be kept to a minimum. Many patients are surviving for more than 10 years after diagnosis. Two patients, who were reported in 1971, survived for 11 and 17 years after the onset of their illness¹⁴⁸⁷. On the other hand, other patients, especially those who develop the thromboembolic complications involving the central nervous system may die within a few days, or months. All patients are at risk from developing endomyocardial disease, and major vascular occlusions.

15.1. Treatment of HES.

Radiotherapy was one of the first treatments to be given to patients with HES. This was reported in 1912 in two patients. One had an eosinophil count of $7 \times 10^9/L$, and was in heart failure. There was a transient increase in the eosinophil count immediately following each treatment, but no long term reduction was seen⁷⁵. A second patient with a persistent eosinophilia of $4.5 \times 10^9/L$ was given an unspecified amount of irradiation. The blood eosinophil count increased to 8.1, and then fell to $3.2 \times 10^9/L$ the following day¹⁰³⁴. Despite these intriguing findings, no formal study of the effects of irradiation on patients' eosinophil counts has been published subsequently.

Splenectomy was first used by Giffin in 1919, to treat a patient with a persistently high blood eosi-

nophil count at the Mayo clinic. The patient was a 31 year-old man, who had eosinophil counts of up to $16 \times 10^9/L$. He died four years later from obliterative endocarditis, pericarditis, and cirrhosis⁶⁴². In 1922 splenectomy in a patient with HES led to an increase in blood eosinophil counts¹¹⁷². In 1971 splenic irradiation was used in a patient with HES, without obvious benefit¹²⁸⁶.

The use of modern drugs to treat HES began with corticotropin, and then steroids in the late 1950s. Corticotropin (ACTH) was first used in 1953 to treat a 61 year old woman with hypereosinophilia, and this producing a fall in her blood eosinophil counts¹⁵²³. The development of anti-neoplastic drugs in the 1960s was soon followed by the use of these compounds to treat HES, but the outcome was generally bad, as patients died from infections due to agranulocytosis. Bone marrow transplantation has not been attempted in this condition, although it is possible that it could cure some patients in whom the disease is confined to the marrow. The difficulty has been to decide whether the disease would recur in the transplanted marrow.

15.1.1. Steroids in HES.

In 1978, a review of the value of drug treatment in 24 patients seen at the N.I.H., showed that some responded to steroids, and others to cytotoxic drugs. Among these 24 patients, 15 were given steroids. Five improved, five showed little change, and five did not improve. The five unresponsive patients were also given hydroxyurea, and four responded favourably. Six patients did not need to be treated with steroids or cytotoxic drugs. Ninety six per cent of the patients survived for over three years, and 77 per cent for over 10 years¹³⁶⁸. This series was extended, so that 32 patients had been seen by 1980, when their treatment was reviewed again¹⁵⁷⁵. Six had no specific therapy. Twenty six were given steroids, nine of whom responded. Seventeen were treated with cytotoxic drugs, of whom nine responded, but four had progressive disease despite these treatments. Additional details were published in 1981^{573, 1574}.

Most patients with HES receive steroids at some time during the course of their illness, to treat the less serious complications, such as coughing attacks, malaise with sweating, and uncomfortable skin lesions. Doses of between 7.5 mg, and 20 mg/day are usually given, and the lowest dose which is effective is maintained for several weeks. Alternate day therapy is used in some centres, but it has not been particularly useful in my hands. Few patients have suffered from the serious complications of steroid therapy, although some have developed mild diabetes, hypertension, or a cushingoid appearance. One patient was described in 1982 who developed disseminated tuberculosis⁵⁴⁰.

It was suggested in 1978 that steroids may be particularly effective in treating patients with HES who have a history of asthma, and pulmonary infiltrates. This was put forward as a result of the successful treatment of two patients, a man aged 57, and a woman aged 54, who also had asthma with pulmonary infiltrates, high serum IgE levels, cardiovascular involvement, and rheumatoid arthritis. One of these patients had a mononeuropathy, and the other purpura²³⁷. In the N.I.H. series of 32 patients, 14 of 17 with splenomegaly at presentation did not respond to steroids, but three of nine who did not have splenomegaly responded well¹⁵⁷⁴. The reasons for this are not known.

Steroids may improve patients' symptoms by their capacity to inhibit eosinophil production, and the release of mature eosinophils from the marrow. This effect has been studied in rats. Injection of 5mg cortisol into the peritoneum of normal rats, 66 hours after injection of ³H thymidine, reduced blood eosinophil counts, but the percentage of labelled blood eosinophils was not lowered, suggesting that eosinopoiesis was not altered by single doses of steroids. However, when several doses were given over a three day period inhibited the emergence of labelled eosinophils from the marrow was inhibited³⁶.

Steroids also reduce the secretion of eosinophil granule components, which may cause some of the complications of HES.

Oral steroids usually produce a fall in blood eosinophil counts during the first few hours, or days after starting therapy, but they then usually return to their pretreatment levels. This can be a useful way of distinguishing steroid-sensitive allergic diseases from HES. However, as mentioned above, in asthma-associated HES, steroid treatment can return the patient's symptoms, and blood counts to

normal within a few days²⁷.

There have been no formal studies on the possible benefits of high dose pulse therapy with steroids on rapidly progressive disease, although these have been used occasionally.

Troleandomycin 250 mg/day, which has steroid sparing effect in asthma¹⁸⁶⁹, was used successfully in a 26 year-old woman with HES, after she failed to respond to steroids, and hydroxyurea⁴⁹³.

15.1.2. Anti-neoplastic drugs in HES.

Many different anti-neoplastic drugs have been given to patients with HES, but the ones which are most commonly used today, are those which are effective in chronic myeloid leukaemia. The point at which to change from steroids alone, to steroids with cytotoxic drugs should be judged on the clinical state of the patient, and not on a wish to produce a 'remission', or to lower high blood eosinophil counts in the absence of signs of continuing tissue damage. Treatment should also be based on the principle that HES is usually a non-malignant, and life-long disease, so that the long-term acceptability of each treatment, and its side effects are of great importance.

Doses of hydroxyurea between 1 and 2 grams per day appear to be effective in patients with more severe forms of the illness, and blood eosinophil counts may return to near normal with a combination of steroids, and hydroxyurea⁹³.

Vincristine has been particularly effective in patients with hypereosinophilia (a) who do not respond to steroids, and hydroxyurea, or (b) who have serious complications which would benefit from a reduction in eosinophil production, or (c) who have thrombocytopenia. Vincristine is also effective in patients with eosinophilic leukemia, and its variants.

One useful regime in adults with the most active forms of the disease is to give 1.5 grams of hydroxyurea, and 10-15 mg prednisolone per day, with 1.5 mg of vincristine intravenously at two week intervals. One of our patients has remained well, and at work on this treatment for five years, marrying, and having two healthy children. There are several case reports of its use in HES^{1839, 334}. Intravenous cytarabine, and oral 6-thioguanine, was given successfully to a patient with HES in 1982⁴⁸⁹.

15.1.3. Anticoagulation, and anti-platelet drugs.

As many patients with HES have thrombotic, and embolic complications, and as endomyocardial lesions are commonly coated with thrombus, many patients are treated with oral anticoagulants. However there is no proof that these prevent thromboembolic disease in these patients, and several patients in our care have had major embolic episodes after effective anticoagulation has been introduced. Most patients are treated with warfarin, and the dosage is carefully regulated with blood tests every two weeks. As this treatment is likely to be continued for the rest of their lives, care should be taken in deciding which patients might benefit.

The anti-platelet drugs, aspirin and dipyridamole, are also often prescribed for these patients, on the basis that platelet aggregation could be an important feature, and in view of the low risk of side effects from these drugs, they are commonly given. Again, there is no controlled study to show that they are effective.

15.1.4. Leucaphoresis, and plasma exchange in HES.

Leucaphoresis in patients with extremely high blood eosinophil counts is effective in lowering the viscosity of the blood, but there is no evidence at present that this is of therapeutic benefit in patients with an eosinophilia¹⁴²¹, despite a report from Boston, U.S.A. in 1974 that it could have helped a patient with a drug-induced hypereosinophilic state. This was 25 year-old woman who had a hypersensitivity reaction to sulphisalazole. She developed myositis, myocarditis, and a vasculitis, with an eosinophilia of $10 \times 10^9/L$. On two separate days, 22 and 9.6×10^{10} cells were removed, and she improved during the subsequent two weeks⁵⁰⁶. However, the fall in blood eosinophil counts was probably mainly due to stopping the sulphonamide. A therapeutic leucaphoresis was also done in 1979 in a 18 year old New Zealand boy, with HES, and retinal disease¹⁷⁸.

Surprisingly, plasma exchange produces a larger, and more consistent fall in blood eosinophil counts than leucaphoresis. This may be because some plasma components are involved in retaining eosinophils

in the circulation. Davies, and I reported in 1982, a comparison of the effects of plasma exchange, and leukapheresis on blood eosinophil counts in four patients with HES⁴⁰⁰. In three patients there was a larger percentage fall in eosinophil counts following plasma exchange (90 per cent, 83 per cent, and 35 per cent) than following leukapheresis (38 per cent, 27 per cent and 35 per cent). Serial plasma exchanges continued to be as effective on three consecutive days. These findings suggested that there might be a plasma factor retaining eosinophils within the circulation, and that removing it allowed eosinophils to become marginated. This might not always be beneficial, and it is not recommended that plasma exchange should be used as a treatment for the hypereosinophilic syndrome, until more is known about its effects.

15.1.5. Bone marrow transplantation in HES.

If HES is due to a defect in dividing eosinophil precursors in the bone marrow, then marrow ablation followed by marrow transplantation could be curative. However as HES may have a number of different causes, and there is no way of distinguishing between them at present, there has been a natural reluctance to consider this therapeutic option, except in patients who appear to have a clonal (malignant) eosinophilic disease.

16. Chapter C 16. Diseases in neonates, and infants.

In normal infants less than 28 days old, the eosinophil count is less than $0.70 \times 10^9/L$,⁵¹⁵, although occasionally counts of $1-2 \times 10^9/L$ have been found in otherwise normal babies¹⁸⁹⁸. In a study on cord blood samples, which were collected from 242 babies of five different ethnic groups living in London, U.K., there was no difference in eosinophil counts between the groups. This suggested that raised blood eosinophil counts, which were previously reported in Indian, and African infants, may have environmental causes³⁰¹.

The discovery of an eosinophilia in neonates, and in infants is not often described, although it may be quite common: 22 per cent in one series¹⁰³⁸. Cord blood contains more eosinophils, and more progenitor cells than adult peripheral blood, and is a good source of GM-CSF¹⁹⁸⁹, so neonates appear to have a well developed capacity to produce high blood eosinophil counts. In one series of 21 neonates receiving surgical treatment in Milwaukee, U.S.A., in 1979, 14 (67 per cent), had blood eosinophil counts greater than $740/10^9/L$ ⁹⁵⁹.

16.1. Premature infants.

A number of preterm, but otherwise normal infants have been reported to develop a marked eosinophilia soon after birth. The reasons for this are not known. Twenty of 38 premature infants, who were described in a report from the University of Arizona, U.S.A., in 1979, develop an eosinophilia greater than $1.0 \times 10^9/L$, which began 19 days after birth, and lasted for two weeks. It was suggested that the eosinophilia was linked to the development of an anabolic state⁶⁴¹. In 1982, a prospective study of 45 premature infants found that preterm babies had the highest blood eosinophil counts, especially when they had been subjected to invasive procedures¹⁶⁶. A second prospective study in 1983 in Johannesburg, South Africa, on six premature babies, who developed mean blood eosinophil counts of $2.7 \times 10^9/L$ three weeks after birth, and four comparable babies who did not have an eosinophilia, suggested that the eosinophilia was part of a biphasic granulopoietic response in infants¹⁵²¹.

16.2. Familial, and genetic diseases.

Relatively few newborn children with familial, or genetic diseases have been described with an eosinophilia. A syndrome of 'hereditary' or 'familial eosinophilia' has been previously noted, in which several members of a family, including children, were found to have a raised blood eosinophil count¹¹⁵⁵. As it has not been reported for the past 20 years, it may not exist today.

In a study on eight people with Down's syndrome in Australia, the morphology of bone marrow, and blood eosinophils was normal, except for an increase in the percentage of marrow eosinophils with

three, or more nuclear lobes. As this is the reverse of the findings in neutrophils, it is likely that the mechanisms which regulate nuclear lobation in neutrophils, and eosinophils are different⁶².

Intrauterine blood transfusions, with exchange transfusions, can produce a hypersensitivity reaction, with a rash, eosinophilia, thrombocytopenia, and lymphopenia. This was described in 21 of 35 (60 per cent) neonates in San Francisco, U.S.A., in 1982³¹⁹. It did not appear to be a graft-versus-host reaction, as it did not occur when intrauterine transfusions alone were used, and its cause remains to be determined.

16.3. Eosinophilic diseases in infants.

Malignant diseases are an important causes of a marked eosinophilia in infants. The malignant process may involve the eosinophil series, as in eosinophilic leukaemia, or act indirectly. Examples include:

- eosinophilic leukaemia in a one-year old girl who died three months after presentation with widespread tissue infiltration with eosinophils, and thrombi⁴²⁴. I have details of a similar case in a neonate in London.

- two infants with lymphoblastic leukaemia, and a marked eosinophilia^{1936,629}.

Occasionally a marked eosinophilia of unknown cause can occur in infants. One example was of an 18 month-old child who had an eosinophilia reaching $47.6 \times 10^9/L$. A lymphocyte-derived factor may have been involved, as conditioned medium from the child's blood cells induced marrow cells to make more eosinophil colonies in agar than normal¹⁰⁸. In 1987 it was reported that a boy aged 5.5 months had developed a marked eosinophilia, skin lesions, a hemiparesis, and hepatosplenomegaly. His blood eosinophil counts rose to $115 \times 10^9/L$. He was treated with steroids, hydroxyurea, and the eosinophilia disappeared 15 months after presentation¹⁹⁵⁸. Clearly, this illness had much in common with HES in older children, and adults, but I do believe that HES should not be diagnosed unless the eosinophilia persist.

Pertussoid eosinophilic pneumonia is a diseases of unknown cause, which produces a severe bilateral eosinophil-rich infiltrative inflammatory response in the lungs of infants. Twenty three cases were reviewed in 1977¹²⁹⁶. High immunoglobulin levels suggest that it might be an infectious disease, but no pathogen has yet been isolated from the lungs in this disease. It appears to have a good prognosis.

17. Chapter C 17. Skin diseases.

The normal skin contains very few eosinophils, but they are found in large numbers infiltrating from venules into lesions as different as atopic eczema, bullous pemphigoid, lymphomatoid papulosis, drug reactions, and insect bites. In these diseases, many eosinophils in the skin show ultrastructural evidence for secretion of their granule contents²⁰⁰⁶, and the amount of deposited eosinophil granule material can be extensive. This leaves little doubt that eosinophils are an integral part of the pathogenic processes in these lesions. However, the ways in which this occurs, and the different clinical manifestations of these disorders, suggest that there is no single disease mechanism which eosinophil serve in the skin. These may include alterations in permeability leading to oedema¹⁰⁵⁵, the stimulation of fibrous tissue deposition, the formation of bulli, itching, and chronic inflammatory injury. Figure C17-1. .IE

Eosinophils and skin diseases.

Research on the relationship between eosinophils, and skin diseases is just beginning. It is only in the last few years, that a close a relationship of eosinophils to scleroderma, Well's syndrome, recurrent angioedema, and filarial dermatitis has begun to be described, and explained. This is obviously an important area for research on the properties of eosinophils in disease, in view of the accessibility of the skin for experimental studies, and the large background of research on the pathogenesis of skin disorders. There is little related work on animals, although the characterization of ectoparasite-

induced eosinophil responses in guinea pigs, and other animals has been studied in Yale University, U.S.A. since the late 1970s.

Several diseases give rise to a marked eosinophilic infiltrate into the fatty tissues of the skin, producing the histological appearances of 'eosinophilic panniculitis'. This term is not particularly helpful when considering the possible roles of eosinophils in skin diseases, as there is no suggestion that eosinophils interact with fat cells. A description of 18 patients seen at the Mayo Clinic, U.S.A., with this histological appearance was made in 1986. The diseases encompassed by this term included erythema nodosum, immune vasculitis, eosinophilic dermatitis, Well's syndrome, injection granulomas, and lymphomas affecting the skin¹⁹⁴⁰.

17.1. Dermatitis.

Eosinophil-rich inflammatory diseases of the skin associated with itching occur in several well-defined skin disorders. The numbers of eosinophils in the lesions varies considerably from patient to patient, and there is no unifying concept about how eosinophils are involved in these disorders, and some unusual skin disorders of this type remain to be classified. For example, a 66 year old man has been described with papules, pruritus, and urticarial lesions on his thigh, and trunk, with an eosinophilia of up to $1.6 \times 10^9/L$, and raised serum IgE, which responded to a short course of treatment with ketotifen. Differences with Kimura's, and Well's syndrome were outlined¹⁵⁷⁰.

17.1.1. Atopic dermatitis.

Eosinophils are clearly involved in atopic dermatitis, as large amounts of secreted MBP were seen in the skin of 18 patients studied at the Mayo Clinic. The distribution of the MBP followed a fibrillar pattern, similar to that seen in the lichenified lesions of untreated onchocerciasis. As few intact eosinophils were seen in the skin, the extent of eosinophil involvement in atopic dermatitis has probably been underestimated in the past. It was suggested that MBP deposition occurred as a consequence of an IgE-mediated late phase reaction, and that they contributed to the tissue damage present¹⁰⁵².

As eosinophils are involved in the development of late phase asthmatic responses, and skin test reaction in patients with atopic dermatitis are often delayed, an experimental study was carried out to examine the possible role of eosinophils in atopic dermatitis. Fifteen patients with atopic dermatitis, 10 atopic patients with asthma, and rhinitis without atopic dermatitis, and 10 non-atopic subjects were studied to see whether epicutaneous patch tests with aqueous allergen preparations, and intracutaneous tests would give different inflammatory reactions in skin than normals. Eosinophils first appeared in the dermis at two to six hours, and in the epidermis at 24 hours. Dermal eosinophils were activated (stained with antibody EG2), whereas epidermal eosinophils were non-activated, and often lay adjacent to Langerhans' cells. It was suggested that eosinophils might influence the late responses in the skin, possibly by affecting the capacity of Langerhans' cells to present antigens in the skin of atopic patients²²².

17.1.2. Urticaria.

There are many different forms of urticaria, which are often distinguished by their mode of induction, and by the type of cellular reaction which develops. Chronic urticaria in children, which is often associated with an eosinophilia, is usually of unknown cause⁷⁶⁰. Intact eosinophils are only found in urticaria in which there is an acute inflammatory response, such as delayed pressure urticaria³⁷⁶. Typical of the types of eosinophil-rich urticarial skin lesions, which are difficult to define, is a report of a 68 year-old man with widespread itching papular eruptions, following an episode of facial oedema. He had raised blood eosinophil counts, and IgE, and skin biopsies showed a diffuse perivascular infiltrate of eosinophils. It responded to treatment with steroids¹⁸³⁴.

In 14 patients with urticarial vasculitis, we noted the presence of activated eosinophils in biopsies from seven with dense perivascular infiltrates¹⁵³², and secreted ECP was seen in the dermis of 12¹⁷⁴². Deposits of MBP had also been seen in the skin of patients with chronic urticaria in 1983¹³⁹², and in the skin lesions of 10 patients with pressure urticaria. The extent, and intensity of anti-MBP staining was not related to the number of intact eosinophils in the tissues. Deposits were seen in both

spontaneous, and induced lesions. It was suggested that eosinophil mediators, particularly MBP, were involved in the development of oedema in the skin¹³⁹³.

Recently, MBP, ECP, and EDN/EPX have been shown to produce a dose-related wheal-and-flare reaction after intradermal injection into human skin, and MBP has also been found in the late phase of the immediate wheal-and-flare reaction (Leiferman, Haugen, and Gleich 1987, personal communication).

Itching papular eruptions preceded by angioedema have been described in a 68 year old man who developed a blood eosinophil count of up to $9 \times 10^9/L$. Several forms of treatment were ineffective, until he was given PUVA therapy. After a three month course, his symptoms, and eosinophilia disappeared, and no abnormalities were found during the next two years¹⁸³⁵.

17.2. Episodic angioedema with eosinophilia.

The syndrome of episodic angioedema associated with an eosinophilia was described at the Mayo Clinic in 1984. Four patients were reported, who had recurrent attacks of angioedema with weight gain of up to 18 per cent, urticaria, fever, and blood eosinophil counts of up to $95 \times 10^9/L$. They were aged four, seven, 16, and 28 years. Three were male, and one female. Steroid treatment appeared to help the patients over each episode. MBP was found in skin biopsies, and high serum MBP levels were measured. Blood eosinophils had ultrastructural features of degranulation. Only one patient had raised serum IgE levels. It was suggested that eosinophils induced the oedema, and weight gain, through an effect of their granule proteins, and LTC₄ on blood vessels, and adjacent mast cells. Although these patients had features of HES, it was suggested that they had a distinct disorder, as follow-up for five to 37 years has shown no signs of visceral organ involvement^{672, 673}.

A related disease, in which recurrent oedema was restricted to the face, was described in 1985 in two patients, who had an eosinophilia. One had a high level of serum MBP. In both patients, skin biopsy samples showed a nonspecific mononuclear cell infiltrate, with deposits of extracellular MBP¹⁶⁶⁴.

Other patients with episodic angioedema, with eosinophilia have been described. They include:

- a two and a half year old girl in Boston who had monthly attacks of angioneurotic oedema, in which she gained up to 20 per cent of her total body weight, with malaise, and fever, preceded two days earlier by pruritic papules. She had pulmonary infiltrates on one occasion, which resolved 10 days later. The blood eosinophil count was $17.2 \times 10^9/L$ in an attack, and $6.8 \times 10^9/L$ on recovery. A slight increase in serum IgE (154 IU), and IgM (2.1 g/L) were found. MBP was found in a skin biopsy, and a few scattered eosinophils were also seen there. Thirty two per cent of her CD4 positive blood lymphocytes expressed HLA-DR antigens. Steroid treatment resolved the oedema in four days, and eosinophil counts were transiently reduced, but they returned to high levels, reaching $58 \times 10^9/L$ at their highest⁹¹⁹.

- the only child reported from the N.I.H. series of patients with HES in 1975, who was a ten year-old boy with had attacks of fever, angioneurotic oedema, malaise, and an eosinophilia which responded to steroid treatment³²⁵.

- a 64 year-old man, reported from Creteil, France, in 1987, who had eight years of cyclical fever, itching, diffuse muscle pains, and angioedema lasting 5-7 days, which recurred with regular 28 day cycles. Blood eosinophil counts rose to $30 \times 10^9/L$ during these episodes from baseline levels of $1.5-2 \times 10^9/L$. No MBP deposition was seen in biopsies of stomach, skin, or muscle. As there was no evidence for cardiac injury, or other serious complications of HES, it was suggested that the diseases was the result of a cyclical stimulation of eosinophil progenitor cell proliferation²⁶¹.

- a 65 year old man described in 1985 in the U.K., who presented with episodic facial angioedema, and associated eosinophilia up to $14 \times 10^9/L$. Marrow cultures grew an increased number of eosinophil colonies than normal, and T suppressor cell counts in his blood were increased⁴⁰⁷.

- a 12 year old boy described in 1986 in Australia, who had episodic facial, and peripheral angioedema for seven years, and an associated eosinophilia up to $48 \times 10^9/L$. No excess eosinophil colonies grew from his bone marrow, and no excess CSF production was detected⁷⁹⁶.

In one of the patients with episodic angioedema, and an eosinophilia of $13 \times 10^9/L$ who was studied at the Mayo Clinic, 90 per cent of the blood eosinophils were of density less than 1.082, and they contained only 37 per cent of the MBP found in normal eosinophils: 3,197 compared to 8,619 ng/ 10^6 eosinophils. In this patient plasma MBP levels were 7.8 times more than in normal plasma: 2,408 compared to 307 ng/ml¹³⁸⁹.

These descriptions appear to be distinct from earlier reports of patients with eosinophilia with a vasculitis, angioedema, and hypocomplementaemia. A six-year old girl with the latter disease was reported from Beirut, Lebanon, in 1977⁶³⁰. Biopsies of her skin, and muscle showed many eosinophils in the walls of blood vessels, which also contained deposits of immunoglobulins, and C3. Her illness responded to steroid treatment.

In 25 patients with cancers treated with recombinant IL-2 - activated autologous peripheral blood cells, 16 gained more than 10 per cent of their initial weight, and 20 had pulmonary oedema. Although no mention was made in the publication of this study¹⁵¹⁸, or in other reports of patients treated with IL-2, in a personal communication from one of the group doing this work, it was stated that a marked eosinophilia of up to $20 \times 10^9/L$ can develop, and 24 of their patients had greater than 5 per cent circulating eosinophils.

It has been suggested that treatment of patients with IL-2, which causes an eosinophilia, fluid accumulation, and respiratory difficulties, is due to stimulation of eosinophil proliferation, and eosinophil secretion by T cell factors which have been produced in response to the IL-2. This possibility was based on the finding that eosinophils secrete LTC₄, and PAF, and can damage endothelial cells in vitro. It was also suggested that the anti-tumour effect of IL-2 might be mediated via eosinophils, and that steroids, which inhibit eosinophil functions, could prevent the oedema caused by IL-2 treatment¹⁰⁰¹.

Another syndrome, which may have a common pathogenesis with episodic angioedema with eosinophilia, comprises oedema, hypergammaglobulinaemia M, eosinophilia, and tiredness. Several patients have been described¹¹⁶⁰, and although the disease persists, they seem to have an excellent prognosis.

17.3. Skin eruptions in pregnancy, and in infancy.

Eosinophils are one of the main cell types found in the nonspecific pleomorphic skin lesions which are sometimes seen in pregnancy. They occur with histiocytes, and lymphocytes in the dermis, and epidermis¹²⁴¹, but their pathogenesis is unknown.

Skin diseases in infants which can give rise to a marked eosinophil-rich exudate include erythema toxicum neonatorum, and acropustulosis, which was described in a baby in 1982¹¹¹⁹.

17.4. Scleroderma syndromes.

There are several subgroups of the diseases which fall into the broad classification of the scleroderma syndromes. Localized forms of skin thickening can occur, which may be extended on the arms, and legs. In some patients there is more generalized disease, systemic sclerosis. An eosinophilia can occur in any of these, but a marked blood eosinophilia is most commonly seen in eosinophilic fasciitis. Eosinophils are usually not a prominent part of the lesions themselves, and eosinophilic fasciitis might be better called fasciitis with an eosinophilia. Eosinophilic fasciitis is reviewed in Chapter C 05.

17.5. Bullous skin diseases.

Several different bullous diseases of the skin can be associated with the presence of eosinophils both in the blood, and in the skin lesions. The most striking example of this is bullous pemphigoid. In 1968, the term 'eosinophilic spongiosis' was introduced for the histological appearances of many eosinophils in the skin, with patchy areas of swelling of the connective tissue. It was thought at first that these features were confined to patients with pemphigus, but it has been shown subsequently, that this can also occur in bullous pemphigoid, bullous impetigo, pruriginous erythematous exanthema, and melanoerythroderma. In one 68 year-old woman with the latter diagnosis, who was reported in 1986, her blood eosinophil count was about $5 \times 10^9/L$, the serum IgE level was raised,

and she had eosinophilic spongiosis, with deposits of IgG in the epithelium⁴⁸².

17.5.1. Bullous pemphigoid.

It has been known for nearly 100 years that this disease can occur with large numbers of eosinophils in the inflammatory lesions, and blood²³⁸. It has been suggested that the lesions are due to binding of antibody to the basement membrane, where complement is activated. This induces eosinophils to release toxic molecules directly onto the basement membrane zone, to produce a loss of dermo-epidermal adherence, which causes blister formation¹⁵⁴⁸. In 1982 it was shown that eosinophils in the lesions had ultrastructural features to suggest that they could be degranulating, and damaging the basement membrane⁴⁸⁷. Eosinophil granule proteins have been found in the bullous fluid of some of these patients, and eosinophil peroxidase has been found along the basement membrane⁴⁶⁸. Eosinophil activating factors, which might be involved in triggering eosinophil degranulation, have also been extracted from blister fluid¹⁸⁴⁰.

Treatment is difficult. In 1987 a 48-year old man with bullous pemphigoid lesions containing many eosinophils responded to treatment with tetracycline. It was suggested that drugs which can inhibit the binding of tRNA to mRNA, and so block protein synthesis, could be effective in this disease by inhibiting the toxic properties of granulocytes, including eosinophils¹⁷⁸¹.

17.5.2. Dermatitis herpetiformis, and linear IgA disease.

Eosinophils were prominent in the skin of six of 26 patients (23 per cent) with dermatitis herpetiformis reported from London, U.K., in 1983¹⁸¹. In linear IgA disease there is a continuous, and homogeneous band of IgA along the basement membrane of the skin, and the formation of subepidermal bullae. It is closely linked to dermatitis herpetiformis, although it does not occur in patients with intestinal diseases. Eosinophils can be prominent in, or below the bullae. They were seen in this site in seven of 30 patients studied in London, U.K., who were reported in 1983¹⁸¹.

17.6. Incontinentia pigmenti.

This rare inherited disease, is named after the characteristic areas of increased skin pigmentation, which occur in whorls, stripes, and other angular shapes. These are present at birth, or first appears in the newborn period, and in 60 per cent of children they are associated with neurectodermal defects. The lesions begin with an inflammatory (vesiculobullous) phase, which may be extensive. The patients are usually afebrile, and they may have a marked increase in eosinophils in the lesions, and blood. The inflammatory phase may evolve through a verrucous phase, to the pigmented stage, which fades with age. In Sendai, Japan, an eosinophil chemotactic factor was defined, with LTB₄, in the eosinophil-rich lesions¹⁷⁴⁵.

17.7. Eosinophilic pustular folliculitis.

Eosinophilic pustular folliculitis (Ofuji's disease) was first described in 1970 in three young Japanese patients who had a marked eosinophil infiltrate into areas of pruritic follicular papulopustular eruptions, and a high blood eosinophil count¹²⁹⁷. Since then other patients have been reported from Japan¹⁷⁴⁴, North America in 1985¹¹¹⁸ the U.S.A. in 1986^{865, 453, 346}, including three patients with AIDS¹⁶⁵⁷, Italy in 1985¹²⁸⁹, and France in 1986¹⁸⁵⁹.

This is a rare disease, in which there are recurrent, sterile, papulopustules, and plaques which increase in size. They are commonest in hair-bearing areas, although the palms, and soles can also be involved. Microscopy shows subcorneal, and intrafollicular abscesses containing many eosinophils, or neutrophils, with spongiosis of the outer root sheath, and a dense infiltrate of eosinophils around dermal vessels. Occasionally, tinea infections can mimic this disease¹⁰¹⁶. It also occurs in dogs¹⁵⁸², and in both species it is a chronic disease, with a variable response to steroids. Dapsone was effective in one patient¹⁷⁰⁵.

17.8. Psoriasis.

Eosinophils can be a prominent component of the skin lesion in some patients with psoriasis¹⁷⁰⁶, and there may be an increase in blood eosinophil counts, especially in patients with psoriatic arthropathy.

17.9. Erythema nodosum.

Occasionally, erythema nodosum can be associated with an eosinophilic panniculitis, and a marked

eosinophilia¹⁹⁴⁰.

17.10. Lymphomatoid papulosis.

There are a small number of patients with lymphomatoid papulosis who have large numbers of eosinophils in their skin lesions¹²⁴⁰, in addition to atypical lymphocytes. Occasionally the lesions may contain more histiocytes than lymphocytes, and it has been suggested that these may be a separate form of the disease¹¹⁸¹. In 1988 we reported three patients who had lymphomatoid papulosis, and the hypereosinophilic syndrome¹⁹²⁶. As the dermal lymphocytes had some of the phenotypic features of helper cells, we suggested that the eosinophilia could have been due to the secretion of an eosinopoietic cytokine, such as IL-5, from these atypical T cells.

17.11. Erythema annulare.

Increased numbers of eosinophils have been found in the skin lesions of patients with granuloma annulare. They were present in 18 of 45 biopsies (40 per cent) in a study reported in 1985¹⁶³¹. They are also found in other cutaneous granulomas, such as necrobiosis lipoidica, and reactions to insect bites.

18. Chapter C 18. Immunological diseases.

Diseases of the immune system are often associated with an eosinophilia, especially the hypersensitivity group of disorders, and immunodeficiency diseases. This is not surprising in view of the importance of T cell regulation of eosinophil production, and the capacity of lymphokines to affect the properties of eosinophils.

18.1. Autoimmune diseases.

Although eosinophil infiltration into affected tissues has been observed in experimental animals injected with autologous endocrine tissues, a blood eosinophilia with eosinophils in the affected organ seldom occurs in autoimmune diseases. However Addison's disease can rarely present with an eosinophilia. I reported my studies on one patient in 1976¹⁶⁷⁹. There is also some clinical, and experimental evidence to suggest that eosinophils could be involved in the development of certain types of diabetes mellitus. In 1978, an eosinophil infiltrate was found in the pancreas of a premature baby, born to a diabetic mother¹¹⁶. An eosinophil infiltration into the islets of Langerhans has been noted in guinea pigs, in which diabetes was induced by immunization with pancreatic tissues. In 1987 it was noted that BB rats, which develop diabetes spontaneously, developed an eosinophilia at the onset of diabetes¹⁰²⁰.

18.2. Drug hypersensitivity reactions.

The normal immune response to allergens often includes eosinophils, and it is likely that they play an important role in protecting lymphoid tissues against potentially toxic, or harmful products which enter the body through afferent lymphatics. This mechanism may occasionally lead to a detrimental host response such as occurs during drug hypersensitivity reaction.

Eosinophils can be involved both in immediate hypersensitivity, and in chronic or delayed hypersensitivity responses. In each case, the blood eosinophil count is usually raised as well as there being large numbers of eosinophils in the lesions themselves. In hypersensitivity diseases, blood eosinophil counts are seldom as high as can be seen in patients with HES, although this can occur in drug hypersensitivity reaction.

Drug reactions are an important cause of an eosinophilia¹⁶⁸¹. The finding of a raised blood eosinophil count in patients taking drugs, can be useful as an early warning of potentially dangerous hypersensitivity reactions, which could lead to permanent tissue injury, or even death. The eosinophilia forms part of an immediate hypersensitivity reactions, or delayed cell-mediated response to the drug, or its breakdown products. Most of the diagnostic tests which are used to decide whether a drug is responsible for an adverse reaction are based on these two possible mechanisms.

It is not known why these reactions occur in some patients, except that they can be dose-related, and

it is clear why eosinophils are involved in some hypersensitivity reactions, and not others. No work has been done on how eosinophils could be involved in drug reactions, either as effector cells of the immune response, or as protective cells against the damaging effects of drugs, and their metabolites. Most reactions end when the drug treatment is stopped, but steroid treatment can be valuable, accelerating recovery from cell-mediated reactions. Very rarely, a drug-induced eosinophilia may progress to a chronic persistent eosinophilia.

In the majority of patients it is not difficult to recognize this complication of drug treatment. Problems mainly arise when the drug history is incomplete, or when several drugs are being used to treat a disease which may itself give rise to an eosinophilia. Then special investigations are worthwhile to determine the aetiology of the eosinophilia, especially when drug treatment ought to be continued. There does not appear to be any common chemical, or pharmacological feature to these agents to account for the development of the eosinophilia. In treated patients the incidence of eosinophilia is probably less than 0.1 per cent except for a few treatments, such as sodium aurothiomalate therapy, which can produce an eosinophilia in 20-50 per cent of treated patients⁴⁹¹. The prevalence of drug-induced eosinophilia is, however, relatively common in hospitals, largely because drugs are so widely prescribed there. Figure C18-1.

Fig. 18-1: Drug-induced hypersensitivity reactions with an eosinophilia.

18.2.1. Clinical features of drug hypersensitivity diseases.

Allergic, and hypersensitivity reactions to drugs can occur without any alterations in blood eosinophil counts, and acute anaphylactic reactions are usually not accompanied by an eosinophilia. There is also work to suggest that a drug-induced eosinophilia may not be related to the occurrence of toxic side effects in patients taking D-penicillamine¹⁶⁴⁸. Drug-induced hypersensitivity reactions with an eosinophilia usually only develop after several weeks or months of treatment, but it may recur rapidly, when the drug is taken again. This suggests that an immune response has developed. Sometimes it can be difficult to recognize that an eosinophilia is drug-related, especially when it is overshadowed by other complications. Failure to do so may be responsible for the development of progressive lesions which may be fatal. Useful clinical features which suggest that an eosinophilia is due to drug treatment are fever, skin rashes, lymphadenopathy, splenomegaly, pulmonary infiltrates, and (more rarely) hepatic, renal or cardio-toxicity. However, in a review of drug fever, which was published in 1987, fever was infrequently associated with an eosinophilia¹¹²⁶.

The eosinophilia that accompanies drug reactions ranges from just above normal, to very high levels, but the majority of affected patients have counts between 1 and $10 \times 10^9/L$. The eosinophilia may take several weeks to return to normal after the drug has been stopped.

Fever with an eosinophilia may be the only clinical manifestation of a drug reaction, and this is well described for hypersensitivity to the penicillin, barbiturate, and hydantoin groups of compounds. In 1987, details were given of a 15 year-old boy who developed a hypersensitivity reaction to phenytoin, with blood eosinophil count of $2.1 \times 10^9/L$. A review of 17 similar patients aged two to 21 years with hypersensitivity reactions to phenytoin showed that an eosinophilia occurred in 76 per cent¹⁴³⁵.

18.2.1.1. Reactions affecting the skin.

Skin reactions with an eosinophilia are common in patients taking antibiotics, antifungal agents, and allopurinol^{234, 1027}. Fatal angioedema due to bleomycin was reported in 1984⁹⁵⁸. The varied skin lesions which have been described under the general term of eosinophilic panniculitis, are often due to a drug reaction, although this can be difficult to prove¹⁹⁴⁰. Carbamazepine-induced erythema multiforme with an eosinophilia has been described in a patient in 1984¹¹⁸⁷.

18.2.1.2. Reactions affecting the lung, and heart.

Pulmonary hypersensitivity reactions can develop with an eosinophilia in patients taking nitrofurantoin¹¹³¹, sulphonamides^{1807, 293, 1825}, penicillin, mephenesin, and azathioprine¹⁶⁰¹, methotrexate¹⁹²⁴, bleomycin¹⁹⁸⁸, chlorpropamide¹⁵³, tolbutamide, carbamazepine¹⁷⁰⁸. Occasionally the reaction can progress to fibrosis¹⁵¹⁹, and naproxen⁵⁷⁸.

Several drugs which are used to treat pulmonary tuberculosis can cause damage to the lungs with an eosinophilia. These include para-aminosalicylic acid¹⁶³⁵, isoniazid, rifampicin, and capreomycin sulphate (16 of 38 patients in one series)¹³³⁵.

Eosinophilic myocarditis is an important complication of some types of drug-induced hypersensitivity reactions. Twenty four patients with this reaction were reviewed in New York, U.S.A., in 1982⁵⁵⁵. Twenty had died suddenly, and it was recommended that a careful search for this dangerous reaction should be made in patients with a drug-induced eosinophilia. Pericardial lesions can also develop. Sodium cromoglycate produced an allergic response in a 46 year-old woman who presented with pericardial symptoms, and signs, and a blood eosinophil count of $12.5 \times 10^9/L$, which resolving when this drug was stopped¹⁶⁴².

18.2.1.3. Reactions affecting the kidneys.

Both glomerular, and tubular injury can occur during eosinophilic hypersensitivity reactions to drugs⁴⁹. Glomerular lesions may develop during treatment with penicillins, amphotericin B, sulphonamides, and phenylbutazone¹⁰⁶¹.

Interstitial nephritis, in which there is an eosinophil infiltrate into areas of renal damage, can complicate treatment with a wide variety of drugs. It was reviewed in 1980¹⁰⁸³. Offending drugs include penicillins, including methicillin, and ampicillin (4 of 7 patients in one series)¹⁰⁵, cimetidine, non-steroidal antiinflammatory drugs, and allopurinol^{1183, 748}.

It has been suggested that the interstitial lesions are due to the production of antitubular basement membrane antibodies¹⁹⁰. Although a peripheral blood eosinophilia is not always found in drug-induced interstitial nephritis, when it occurs, it can be marked, and persist for weeks after stopping treatment. Phenytoin can cause marked eosinophil infiltration into the kidneys^{1611, 1171}, as can cephalosporin¹⁸⁵⁶, sulphasalazine³¹⁴.

Rarely, drugs can cause an eosinophilic reaction in the bladder, as described in a patient taking an anti-allergy drug in Japan¹²⁶¹.

18.2.1.4. Reactions affecting the liver.

Many drugs are able to produce a granulomatous hepatitis, in which eosinophils are a prominent feature. In a series from Charleston, U.S.A., published in 1981, they accounted for 29 per cent of granulomas in liver biopsies¹¹⁸². Drug reactions only rarely produce extensive hepatic injury with eosinophil infiltration, and a peripheral blood eosinophilia. Phenothiazines are probably the commonest type of drug causing liver injury, and eosinophilia, with jaundice of an obstructive type. A patient with carbamazepine-induced acute cholangitis and hypereosinophilia was described in 1987¹⁰³¹. Other drugs which have been reported to induce liver injury include tolbutamide, chlorpropamide,¹⁹⁹⁹ and allopurinol¹⁶³⁶. Eosinophilic hepatitis has also been seen in patients with juvenile rheumatoid arthritis treated with aspirin: six in one series¹⁴⁸⁹, and acute rheumatic fever treated with high doses of aspirin: 10 of 11 in a series from South Africa⁶⁴⁵.

18.2.2. Drugs which induce hypersensitivity reactions.

Many different types of drug can induce an eosinophilia¹⁹⁵⁷. Especially important causes of an eosinophilia are antibiotics, and fungicides, drugs acting on the central nervous system such as phenytoin⁷⁶³, anticoagulants such as phenindione, and chlorophenindione, and with them adverse reactions usually becomes apparent one to three months after treatment has started¹⁰⁹⁰, anti-inflammatory and anti-neoplastic drugs, including dacarbazine¹³⁷², and oral hypoglycaemic agents. Even small inorganic substances such as potassium iodide have been shown to produce eosinophilic hypersensitivity reactions as high as $63 \times 10^9/L$ ⁸⁵⁷. Radiographic contrast media, which may release small quantities of free iodine, can also produce an eosinophilia, and mild urticaria (21 of 101 patients undergoing intravenous urography in a series in Boston, U.S.A.,¹⁸⁶⁵), but this is rarely of clinical importance¹⁴²⁵.

18.2.3. Susceptibility to drug hypersensitivity reactions.

Although as many as 10 per cent of patients in hospital have adverse reactions to drugs, and approximately 11 per cent of these are hypersensitivity reactions⁸³⁰, and skin rashes may develop in 5-7 per

cent of patients receiving ampicillin and amoxicillin¹⁴³¹, the prevalence of hypersensitivity reactions with an eosinophilia is probably less than 1 per cent. For example, although penicillin, and cephalosporin, and their related derivatives are highly immunogenic in man, and are well known for their capacity to produce an eosinophilia^{109, 430}, allergic reactions develop in only about 1 per cent of treated patients⁸¹⁷.

Renal failure is an important predisposing factor in the development of drug-hypersensitivity reactions. The reasons for this are not known, but may involve slow elimination of immunogenic metabolites. The incidence, and severity of reactions to allopurinol is also increased in acute and chronic renal failure³⁰⁰. However, in many patients with renal failure who develop an eosinophilia, it may be difficult to incriminate the drug responsible for the reaction. We have seen eight patients with renal failure who developed an eosinophilia of unknown cause, and who were receiving several different drugs. In each case resolution of the eosinophilia occurred as renal function improved, suggesting that the response was in some way associated with the excretion of a sensitizing agent. In one of these patients tartrazine was suspected to be the cause of the eosinophilia, and the eosinophilia closely followed renal function²⁰⁵. Tartrazine may be a common cause of an eosinophilia in patients with renal failure. It is a yellow dye which can also give a red, or green colour, and it is widely used in foodstuffs, and medications. The incidence of hypersensitivity reactions to drugs is also increased in patients with liver failure¹⁹⁹⁹, probably for the same reasons. However, eosinophilia does not seem to develop as frequently as in renal failure.

The main genetic factor known to predispose to drug-induced eosinophilia is slow acetylator status, which is important in patients taking drugs which are altered by this pathway, such as the sulphonamides. For this reason, some reactions are dose related, and slow elimination through the liver or kidneys then becomes more important. Patients with atopy, or a family history of allergic diseases do not appear to have a higher risk of developing drug hypersensitivity reactions, although there is the possibility that they may be more severe when they do occur. A previous history of drug reactions or allergic diseases is often held to increase the likelihood of hypersensitivity reactions developing to other drugs⁸²⁹, but this has not been substantiated in the case of penicillin sensitivity⁸¹⁷. Patients with asthma also rarely develop hypersensitivity reactions to drugs. But when it occurs in them it may be particularly severe. It should be noted that there is a rare syndrome of asthma, nasal polyps and eosinophilia which is regularly aggravated by aspirin ingestion⁶⁴⁴.

18.2.4. Mechanisms of drug hypersensitivity reactions.

18.2.4.1. Introduction.

Drugs produce hypersensitivity reactions by binding to proteins, forming larger immunogenic hapten-protein conjugates. This usually leads to the production of antibodies which can form immune complexes with the drug. Large amounts of circulating complexes can injure blood vessels, producing the clinical features of serum sickness (type III reactions). IgE antibodies may be produced. These are present on the surface of mast cells, and combine with the drug to give acute type I reactions including urticaria, angio-oedema, and anaphylactic reactions. However, the most important mechanism in drug-induced eosinophilic hypersensitivity reactions is T lymphocyte dependent: the cell-mediated (type IV) reaction. Histologically, the main features of drug-induced lesions are a mononuclear cell, and eosinophil-rich inflammatory reaction in all three layers of the walls of small arteries, arterioles, and venules, without the presence of a necrotizing vasculitis, or fibrinoid necrosis¹²⁵¹.

18.2.4.2. IgG-mediated reactions.

Many drugs give rise to small amounts of IgG antibodies but generally these have no clinical significance. Eosinophilic hypersensitivity reactions to nitrofurantoin often occur in patients with higher IgG antibodies to the drug than in control patients, but as some patients with the highest IgG antibody levels do not have reactions, it is likely that other factors are also involved. In the majority of patients who develop allergic reactions to the penicillin group of drugs there is no correlation with IgG or IgM antibody levels²⁰⁰⁴. However, in a few patients, immune complexes can develop, caus-

ing serum sickness, accompanied by an eosinophilia, and complement activation. This has been described in a patient given cephalothin ¹⁹⁹.

18.2.4.3. IgE-mediated, and mast cell-mediated reactions.

IgE-mediated (anaphylactic) responses to drugs have often been incriminated in the development of drug-induced eosinophilia. Penicillin allergy is the best known. Here bivalent penicilloyl-haptens cross-link IgE antibodies on the surface of mast cells, and induce the secretion of mast cell mediators. In other drug reactions the involvement of IgE has been conjectural, and largely based on increased total IgE levels in serum, such as in patients with allergic reactions, and eosinophilia after treatment of rheumatoid arthritis with gold salts ⁴⁰⁸. Among 50 patients who received sodium aurothiomalate, 20 developed an eosinophilia. Of these, 35 per cent had raised IgE levels, whereas only 11 per cent among the other patients showed increased levels. Unfortunately, specific IgE antibodies to gold salts or their metabolic products have yet to be defined, and the possible importance of an eosinophilia as a useful indicator of an adverse reaction to gold salts was not confirmed in two other studies ^{876, 935}.

As mast cells degranulate during acute allergic reactions to drugs, a number of diagnostic tests for drug hypersensitivity have been developed using basophil degranulation *in vitro*. These are technically difficult, as basophils can degranulate non-specifically, so they are now seldom done, except in specialized laboratories. Another test which has now been largely abandoned, is serial measurements of blood cell accumulation in skin abrasions, to which glass coverslips or chambers containing the drug have been applied ('skin windows'). It is not clear what type of response this measures, and it is difficult to interpret.

18.2.4.4. Cell mediated (Type IV) reactions.

It is probable that most drugs produce an eosinophilia by stimulating T lymphocyte responses *in vivo*. This is studied by incubating patient's blood lymphocytes with the suspect drug, and measuring the degree of lymphocyte proliferation by the uptake of ³H-thymidine. This mitogenic response has been widely used in attempts to find the drug responsible for hypersensitivity reactions, and there are a number of reports where a positive response has been detected using lymphocytes from patients with a drug-induced eosinophilia ¹⁴⁸⁴. Positive results with this type of test have also been reported in patients with hypersensitivity reactions, and an eosinophilia, which developed during treatment with antituberculous drugs, and nitrofurantoin ⁸⁹. Five of seven patients with an eosinophilia due to carbamazepine had lymphocytes which showed a positive mitogenic response. In the other two patients, a positive response was seen only in samples taken several months later, possibly due to a depression of lymphocyte responsiveness by the illness itself. These patients also produced positive patch tests for the drug, suggesting that delayed (type IV) hypersensitivity had developed ⁸¹⁸.

The value of the mitogenic response in diagnosis has, however, been questioned. For example, in a group of patients with asthma who developed hypersensitivity reactions to sodium cromoglycate (cromolyn sodium) there was no difference in lymphocyte responsiveness to the drug compared with other unaffected asthmatic patients. However, by measuring lymphocyte-derived migration inhibitory factor, which is secreted during delayed hypersensitivity reactions, it was possible to discriminate between the two groups ¹⁶⁰⁵. Other drugs, in which mitogenic responses have been correlated with hypersensitivity reactions, and an eosinophilia, include phenytoin ¹⁶¹¹, and allopurinol ¹¹⁴⁶. Evidence that lymphocytes are involved directly in these responses also comes from studies on circulating lymphocytes, which may show a number of morphological abnormalities, for example in patients with phenytoin hypersensitivity ¹⁷⁰².

These findings fit in well with experimental work in animals on the role of T-lymphocytes in the induction of eosinopoiesis. Basten, and Beeson ¹³⁶, showed that an eosinophilia did not occur in rats injected with *T. spiralis* larvae unless they possessed normal T-lymphocyte populations. A similar conclusion was reached in regard to basophilopoiesis in recent studies in rats infected with *T. spiralis*. Congenitally T-lymphocyte-deficient rats did not show either basophilia, or eosinophilia when

infected¹³⁰⁰. However, it is not known whether the same lymphocyte subpopulation induces both an eosinophilia, and delayed (type IV) reactions, or whether one response influences the development of the other.

In further experimental studies, the importance of inflammatory lesions in the induction of eosinophilia was clearly demonstrated¹⁸⁷². In the absence of an inflammatory reaction, stimuli which would normally produce an eosinophilia appeared to be ineffective. How far this was due to a non-specific effect of the inflammatory reaction on the immune response, or a direct effect of these reactions on eosinopoiesis was not clear. However, it may be useful to consider whether a drug-induced hypersensitivity reaction has an underlying inflammatory reaction if there is an associated eosinophilia.

18.2.5. The roles of eosinophils in drug reactions.

Although tissue eosinophils are a prominent component of tissue lesions in patients with hypersensitivity reactions, it is not known what part they play there, and they are not involved in acute anaphylactic reactions⁶⁶⁹. It is possible that they are involved in the production of chronic tissue injury in drug hypersensitivity reactions, but as they are rarely associated with endomyocardial fibrosis, their effects are probably localized.

Blood eosinophils in these patients may contain many vacuoles³⁴⁷, and degranulated eosinophils have been seen in the blood,¹⁴⁴³ and in drug-induced tissue lesions. Tissue eosinophils containing vacuoles have been seen in 'skin window' preparations in a group of 45 allergic patients. The maximum number of vacuolated eosinophils (42 per cent) was seen four hours after the drug was applied to the skin⁴⁵¹. However, it is not clear whether eosinophils contribute in any way to the clinical, or pathological consequences of these reactions.

18.3. Graft-versus-host disease.

A mild eosinophilia is common in chronic graft-versus-host (GvH) disease, but occasionally the blood eosinophil count can be strikingly high. Blood eosinophil counts did not correlate with increases in IgE levels in 14 patients with GvH disease¹⁴⁹⁴. In one patient reported in 1986, an eosinophilia due to GvH disease after a bone marrow transplant was also accompanied by polymyositis¹⁸¹².

18.4. Immunological deficiency syndromes.

Immunological deficiency syndromes in childhood, and later life, and several acquired immunodeficiency syndromes can all be associated with a marked increase in eosinophil counts. At first sight this seems surprising in view of the role of T cells in eosinophil production. However, in these disorders it is likely that there is an imbalance in the regulation of the production of lymphokines, which leads to an over production of lymphokines with eosinopoietic activity from other cells, such as endothelial cells.

Children with severe defects in cell mediated, and humoral immunity, such as the severe combined immune deficiency syndrome may have a marked eosinophilia⁸⁹⁶. See also Omenn's syndrome, Chapter C04.

A number of children with hypogammaglobulinaemia, and *Pneumocystis carinii* pneumonia have been found to have an eosinophilia. In a five month-old child with this syndrome, and a blood eosinophil count of $14.2 \times 10^9/L$, who was reported in 1987, the eosinophilia disappeared after he was treated with intravenous gammaglobulin¹³⁸⁰.

18.5. Absence of eosinophils.

Occasionally, blood films may have less than three eosinophils per 200 white blood cells, although eosinophils are present in the bone marrow. As I have found that germ-free rats, and mice have very few blood eosinophils, this may be a reflection of the absence of stimuli, which might otherwise increase the eosinophil count, rather than an abnormality in these individuals. In hospital practice, low eosinophil counts are also sometimes seen, but it is very rare for this to occur in serial blood samples. This was studied in 1987 in 24 300 patients admitted to hospital in Pittsburg, U.S.A. Only 24 had blood eosinophil counts of less than $0.010 \times 10^9/L$, and in each case this was linked to current

steroid treatment, or the presence of a serious underlying disease¹⁰⁰³.

There are a small number of patients with a variety of immunodeficiency syndromes, which are often associated with a thymoma, or asthma, who have no eosinophils in their marrow, blood, or peripheral tissues, as judged by staining with eosin, and other similar stains. During the 1950s, and 1960s, there were several single case reports of this association^{696, 1474, 587, 1870}. The syndrome was reviewed in 1968 by Good, and colleagues, who had made several of the earlier descriptions of these patients⁸⁷⁷. In 1977 five patients with this syndrome were discussed in a short review²⁰¹¹, and an additional patient was mentioned in a review in 1977¹⁴⁷. Other reports of patients with no eosinophils are:

- a 52 year-old man in Uppsala in 1977, who had a wide range of recurrent, and persistent infections, including chest infections, tuberculosis, multiple warts, recurrent gastroenteritis, a persistent salmonella infection, and scabies. He also had a haemolytic anaemia, selective IgA deficiency, asthma, and an absence of eosinophils, and basophils in his marrow, and blood. A small number of mast cells were found in tissues. The plasma was found to induce white cells from normal individuals to degranulate, suggesting that newly formed cells were unable to reach maturity in this patient⁸⁹⁹.

- a woman in the U.S.A. in 1981, with episodic urticaria, and rhinitis who had no circulating eosinophils, or serum MBP⁵⁹⁶. She had 0.4 per cent basophils, and normal immunoglobulins. In 1984 it was reported that she had an eosinophil-specific serum inhibitor of CFU-Eo growth¹²⁶².

- a 59 year-old woman in Brussels, Belgium in 1982, who had no eosinophils in her blood, or marrow for at least eight years after she had recovered from an episode of drug-induced agranulocytosis¹⁷⁶⁵. The lack of eosinophils was thought to be due to a defect in the marrow environment for eosinopoiesis, as she had normal numbers of CFU-Eo, and could make Eo-CSA, and she did not have an antibody preventing the growth of immature eosinophils.

- a man aged 55 years reported in London, U.K., in 1983, who had a spindle cell thymoma, with hypogammaglobulinaemia. Two years after he had a thymectomy, blood, and marrow eosinophils disappeared, and eosinophils were noted to be absent in skin allergen patch test sites. As there was a marked increase in his blood T lymphocyte suppressor activity, it was possible that suppressor cells were inhibiting T lymphocytes which would normally stimulate eosinophil production¹²²¹. This report also suggested that an alternative mechanism for his lack of eosinophils. It was possible that the defective T cells might be the ones which would normally supply the 'helper' functions for eosinopoiesis¹²²⁰.

- a 52 year old woman described in 1984 with asthma, diabetes, and seasonal rhinitis, with a history of drug allergy, who had a mild leucocytosis, but no blood eosinophils. Her serum IgG inhibited CFU-Eo growth specifically, and it was suggested that the eosinopenia was due to an autoimmune process¹²⁶².

- a second patient with reported from Uppsala in 1988, who had no blood or marrow eosinophils or basophils, although there was a low level of serum ECP level⁹⁰⁰. The cause of the eosinopenia in this patient was not determined.

18.6. Hypergammaglobulinemia E, and infection syndrome.

The syndrome of recurrent pyogenic infections, and high serum IgE levels is occasionally associated with a marked eosinophilia. Two boys with recurrent infections, very high serum IgE levels, and poor secondary antibody responses, and reduced delayed hypersensitivity reactions, with a marked eosinophilia were described in 1972²²⁷. In 1975 serum IgE levels of over 200,00 U/ml, and increased numbers of IgE-staining B cells, were noted in a patient who had blood eosinophil counts of 4 to 18 x10⁹/L¹³⁷⁹.

In 1976 eleven other patients with this syndrome were reported from Minneapolis, U.S.A. They all had recurrent staphylococcal abscesses involving the skin, and the respiratory tract, chronic eczematous dermatitis, and high levels of serum IgE. In ten patients the peripheral blood eosinophil count was 10 per cent or more of the total white blood cell count³⁷⁹. A patient was reported from Denmark, in 1980¹⁴⁴, and 16 patients, who were seen at N.I.H., Bethesda, U.S.A., were reviewed in 1983⁴⁵⁷. Six had blood eosinophil counts above 1 x 10⁹/L, and eosinophils were also present in

abscess fluid, and sputum.

A. Appendix A. Research Groups working on eosinophils, and eosinophilic diseases, 1988.

There are many scientific, and clinical centres with an interest in the study of eosinophils, and eosinophilic diseases. The following list includes the major centres which have published several english language papers on eosinophils, in the period immediately preceeding 1988.

A.1. Australia.

- Division of Human Immunology, Institute of Medical and Veterinary Science, Box 14 Rundle Mall Post Office, ADELAIDE, South Australia 5000. Dr. Mathew Vadas. Genetic factors and eosinophil production. Colony stimulating factors, and eosinopoiesis. Eosinophil activation.

- The Walter and Eliza Hall Institute of Medical Research, Post Office, Royal Melbourne Hospital, MELBOURNE, Victoria 3050, Australia. Dr. Donald Metcalf. Eosinophil CSFs.

A.2. Chile.

- Laboratory of Experimental Endocrinology, University of Chile Medical School, Casilla 21104, Correro 21, SANTIAGO, Chile. Dr. Andrei Tchernitchin. The relationship between oestrogens, and eosinophils in the uterus of rodents.

A.3. France.

- Centre d'Immunologie et Biologie Parasitaire, Institut Pasteur, 1 Rue du Professeur Calmette, 59019 LILLE, Cedex, France. Dr. Monique Capron. Eosinophil-dependent killing of parasites. IgE receptors on human eosinophils. The properties of light density, and activated eosinophils.

A.4. Italy.

- Clinica Malattia Infettiva, Universita La Sapienza, ROME, Italy. Dr. C. DeSimone. Eosinophil activation, and cytotoxicity.

A.5. Japan.

- Clinical Research Centre for Rheumato-Allergology, National Sagamihara Hospital, KANAGAWA, Japan. Dr. H. Saito. Eosinophil malignancies. Eosinophil cell lines.

- Department of Pathology, Kumamoto University Medical School, Japan. Dr. M. Hirashima. Eosinophil chemotactic factors.

- Department of Medicine and Clinical Immunology, Dokkyo University School of Medicine, MIBU, Tochigi-Ken 321-02, Japan. Dr. Sohei Makino. Eosinophils in asthma. Eosinophil heterogeneity.

A.6. Sweden.

- Research Dept. 2.E. Blocket Floor EB-14, University of Lund, Lund Hospital, S-221 85 LUND, Sweden. Dr. Inge Olsson. Purification, and characterization of ECP. Biosynthesis of eosinophil granule proteins.

- University Hospital, Department of Clinical Chemistry, S-751 85 UPPSALA, Sweden. Dr. Per Venge. Separation, and properties of ECP. Characterization of EDN/EPX. Eosinophils and coagulation. Eosinophils, and lymphocyte proliferation. The involvement of eosinophils in asthma, and other diseases.

A.7. U.K.

- Immunology Division, Cambridge University Department of Pathology, Tennis Court Road, CAMBRIDGE CB2 1QP, U.K. Dr. Anthony Butterworth. Eosinophil-parasite interactions, especially eosinophil killing of schistosomula. Eosinophil activating factors.

- Beecham's Group Research, Biosciences Research Centre, Great Burgh Yew Tree Bottom Road, EPSOM, Surrey KT18 5XQ, U.K. Dr. Harry Smith. Effects of drugs on rodent eosinophils.

- Department of Allergy and Clinical Immunology, The Cardiothoracic Institute Brompton Hospital, Fulham Road, LONDON SW3 6HP, U.K. Dr. Barry Kay. Eosinophil chemotactic factors. The properties of eosinophils in the blood, and BAL of patients with asthma. Eosinophil leukotriene production.

- National Institute for Medical Research, Mill Hill, LONDON NW7 1AA, U.K. Dr. Colin Sanderson. Liquid culture of murine, and human eosinophils. T cell clones making eosinopoietic factors. Murine, and human eosinophil differentiation factor (IL-5).
 - Department of Immunology, St. George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, U.K. Dr. Christopher Spry. The clinical features of HES, and other diseases with an eosinophilia. Preparation of monoclonal antibodies to eosinophil granule, and secreted components, and eosinophil membrane antigens. Mechanisms of eosinophil secretion. The pathogenesis of eosinophilic endomyocardial fibrosis, and tropical endomyocardial fibrosis.
 - Agricultural Research Council, Poultry Research Centre, Roslin, MIDLOTHIAN EH25 9PS, U.K. Dr. M.H. Maxwell. Avian eosinophils.
- A.8. U.S.A.
- Building 31, National Institutes of Allergy and Infectious Diseases, NIH, BETHESDA, Maryland, MD 20205, U.S.A. Dr. Tony Fauci. The idiopathic hypereosinophilic syndrome. The properties of eosinophils in patients with hypereosinophilia.
 - Laboratory of Parasitic Diseases, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, BETHESDA, Maryland 20205, U.S.A. Dr. Eric Ottesen. Eosinophil interactions with filaria, and other parasites.
 - Harvard-Thorndyke Laboratory, Department of Medicine, Beth Israel Hospital, 330 Brookline Avenue, BOSTON, Massachusetts MA 02215, U.S.A. Dr. Peter Weller, and Dr. Stephen Ackerman. Characterization of the components in human eosinophil granules, and cell membrane. The biochemistry of the Charcot-Leyden crystal protein, lysophospholipase. Eosinophil-complement interactions. Lipid metabolism in eosinophils.
 - The Seeley G Mudd Building, Room 604, 250 Longwood Avenue, BOSTON, Massachusetts 02115, U.S.A. Dr. K. Frank Austen, and Dr. John David. Eosinophil chemotactic factors. Eosinophil-mast cell interactions. The constituents in eosinophils. The production of leukotriene C₄, and other lipid mediators. Extended culture of human eosinophils. Eosinophil activating factors. Properties of activated eosinophils.
 - Division of Allergy, New England Medical Centre Hospital, Tufts University School of Medicine, BOSTON, Massachusetts 02142, U.S.A. Dr. Stephanie Pincus. Guinea pig, and human eosinophils biochemistry, and responses to parasitic infections.
 - Department of Microbiology, Vanderbilt University School of Medicine, NASHVILLE, Tennessee, U.S.A. Dr. Dan Colley. Murine eosinophils. Eosinophil-parasite interactions. Eosinophil activation. T cell involvement in eosinophil functions in vivo.
 - Research Laboratory for Allergic Diseases, Mayo Clinic and Foundation, ROCHESTER, Minnesota 55905, U.S.A. Dr. Gerry Gleich. The isolation, and biochemistry of eosinophil granule components, especially MBP. Study of the roles of eosinophils in diseases. Clinical disorders in which eosinophils may have a pathogenetic role, especially asthma, and allergic diseases. MBP in pregnancy.
 - School of Medicine, University of California Medical Centre, SAN FRANCISCO, California, 94143, U.S.A. Dr. Ed Goetzel. Eosinophil-derived lipid mediators of inflammatory reactions. Eosinophil chemotactic factors.
 - University of Washington. SEATTLE, Washington 98195, U.S.A. Dr. Seymour Klebanoff. Eosinophil peroxidase. The interaction of EPO with mast cells, and parasites. Eosinophil ultrastructure, and secretion.

B. Appendix B. The scientific literature on eosinophils.

Over 400 papers on eosinophils, and eosinophilic diseases are published each year. Only one third of the literature on eosinophils is in journals which are readily available in most medical schools; one

third is in less commonly taken journals, and a third is in literature which is difficult to retrieve. The principal journals in which papers on eosinophils have appeared during the last decade, and which are indexed in Medline in decreasing order of frequency of publication are: the Journal of Immunology, Blood, the Journal of Allergy and Clinical Immunology, the New England Journal of Medicine, the American Journal of Tropical Medicine and Hygiene, Annals of Internal Medicine, the Lancet, Annals of Allergy, Clinical and Experimental Immunology, Immunology, Internal Archives of Allergy and Applied Immunology, the American Journal of Medicine, Archives of Dermatology, and the Journal of Experimental Medicine.

The National Library of Medicine Medline MEZZ database has indexed 8 759 articles, 6 283 in English, on eosinophils, and eosinophilic diseases under the Medical Subject Headings 'eosinophils', and 'eosinophilia', since it began in 1966. 3 825 of these were published from 1977, up to the time that this book was completed on 15 January 1988. 1977 was chosen as the starting date for this review, as this was the year in which Beeson, and Bass published their comprehensive book on 'The Eosinophil'¹⁴⁷. A free text search of the MEZZ database for the same period led to the selection of a further 300 references, providing a core material of 4 125 references, many of which also had abstracts. These were downloaded, and read into a Cardbox-Plus reference database on my own microcomputer. Over 1 000 photocopies were obtained of selected articles, which could not be read in my Medical School libraries, or at the library of the Royal Society of Medicine, London. As the work of extracting information from these references, and preparing the book proceeded, 1 173 earlier references, and related material was added to the database, so that my database finally contained 5 298 references on eosinophils, and eosinophilic diseases. Over one third of these references are discussed in this book. The order of the Chapters on eosinophils in disease, and individual diseases broadly follows the listings in the Medline Medical Subjects Headings Tree Structure, to make it easier to search for subsequent references from the Medline databases.

As this book was designed to be a source, and synthesis of information on human eosinophils, and diseases affecting eosinophils in man, animal studies were only included where they related to human eosinophils, except for one Chapter which centres on eosinophils in normal animals.

B.1. Books, and reports of meetings.

Few books have been written on eosinophils. The most comprehensive on 'The Eosinophil', was published in 1977 by Paul Beeson, and David Bass¹⁴⁷. It describes eosinophil production, their structure, biochemistry, and occurrence in a wide range of clinical disorders, and reviewed 1 485 references in a scholarly, and critical fashion. This book was the first to bring together clinical information about diseases in which eosinophils were either common, less common, or rare. It also gave a detailed, and balanced account of experimental studies on eosinophils in animals, and man. It remains an indispensable source of information on the literature dealing with eosinophils in disease. In 1979, on the centenary of the discovery of the eosinophil by Paul Ehrlich, a meeting was organized at Brook Lodge, Michigan, U.S.A. The proceedings were published, with chapters from different groups in several parts of the world. It is one of the most useful reviews of the properties of eosinophils, and their constituents¹¹³⁴.

The proceedings of a small meeting at the Royal Society of Tropical Medicine and Hygiene in London in 1980, was published as a supplement to Transactions²²⁶.

In 1981, the 'First International Workshop on the Eosinophil' was held in Fukuoka, Japan, and the presentations were published in 1983. This is the most recent book on the properties of eosinophils¹⁹⁸². This meeting was one of the first occasions in which eosinophil activation was discussed in detail by several groups.

B.2. Review articles in books, and journals.

There are many articles relating to eosinophils which are useful for obtaining an overview of various aspects of eosinophil cell biology, and their occurrence in disease. It is difficult to learn about these, as multi-author books are not indexed in reference databases, and many reviews are provided in the introduction, and discussion sections of more detailed papers. In this section, articles are listed

which provide a detailed assessment of the literature, and provide useful sources for further reading.

B.2.1. Published in 1914-1976.

1914: Schwarz E. Die Lehre von der allgemeinen und ortlichen 'Eosinophilie'. 2 758 references¹⁵⁸⁰.

1975: Chusid M J, Dale D C, West B C, Wolff S M. The hypereosinophilic syndrome: analysis of fourteen cases with review of the literature. 94 references³²⁵.

B.2.2. Published in 1977.

Butterworth A E. The eosinophil and its role in immunity to helminth infection. 239 references²⁴⁸.

Gleich G J. The eosinophil: structure and biochemical composition. 30 references⁶⁵¹.

Gleich G J. The eosinophil: new aspects of structure and function. 26 references⁶⁵².

Kay A B. Eosinophil leucocytes: recruitment, localization and function in immediate-type hypersensitivity. 30 references⁹²⁵.

B.2.3. Published in 1978.

Konig W. (Structure and function of the eosinophil leucocytes'. 56 references⁹⁹⁷.

Spry C J. Eosinophils as effector cells in disease. 51 references¹⁶⁸⁰.

Zucker-Franklin D. Eosinophil function related to cutaneous disorders. 45 references²⁰⁰⁶.

B.2.4. Published in 1979.

Schiffmann E, Gallin J I. Biochemistry of phagocyte chemotaxis. 300 references¹⁵⁶⁹.

Weller P F, Goetzl E J. The regulatory and effector roles of eosinophils. 210 references¹⁹¹².

Olsson I, Venge P. The role of the eosinophil granulocyte in the inflammatory reaction. 81 references¹³³⁰.

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Legends to Figures:

Frontispiece: Two blood eosinophils, one of which is activated, and stained with a monoclonal antibody (EG2), which only binds to the secreted form of the eosinophil granule proteins, ECP, and EDN/EPX. From¹⁶⁸⁷, with permission.

Figure A02-1. Factors affecting eosinophil differentiation. The earlier cells are probably stimulated by multi-CSF, and possibly by IL-2. GM-CSF induces further differentiation, and in the final step, Eo-CSF induces the dividing cells to become eosinophils.

Figure A03-1. The separation of human blood eosinophils by isopycnic density gradient centrifugation on discontinuous metrizamide. Eosinophils are found between the denser bands. Blood eosinophils from patients with an eosinophilia can be separated into light density, and normal density eosinophils, with this method.

Figure A03-2. The principal structures seen in electronmicrographs of mature human eosinophils. This schematic cell has not yet been stimulated to secrete its granule contents, which remain packaged in the granules. The presence of the Charcot-Leyden crystal, and the lipid body are mainly seen in stimulated, or tissue eosinophils.

Figure A03-3. The synthesis of eosinophil granule constituents. The proteins undergo a complex series of alterations, from the time that they are synthesized, to when they are secreted from the cell. At present most is known about the storage proteins, as these can be extracted easily.

Figure A03-4. The localization of different eosinophil granule constituents within the granule. The crystalloid is comprised of MBP, and the remaining components are present in the surrounding matrix.

Figure A04-1. The different types of immunological receptors found on human blood, and tissue eosinophils. The spatial distribution of the receptors, and their relationship with each other remain to be determined.

Figure A04-2. Monoclonal antibodies to human eosinophil membrane antigens. Antibodies which bind to eosinophils, and neutrophils, can be distinguished from antibodies which bind to eosinophils, T cells, platelets, and pre-B cells.

Figure A05-1. Development of eosinophils, leading to their activation, and degranulation. At each stage, eosinophils secrete some of their constituents. In the bone marrow, this occurs after they are synthesized, and possibly in response to CSFs. When non-dividing eosinophils are stimulated with activating factors, they secrete some granule constituents, and become metabolically active, so that immunological signals can induce them to degranulate, and to synthesize, and release newly-formed mediators.

Figure A05-2. Differential secretion by eosinophils. There is evidence that the 'strength', or the type of immunological stimuli applied to eosinophils, affect the type of molecules secreted. Inflam-

matory signals appear to cause the secretion of each of the main effector molecules, whereas IgE stimulation may only cause the secretion of some of these.

Figure A05-3. Stages in the emigration of eosinophils from post-capillary venules into inflammatory lesions. Each of the stages 'a' to 'e' may be regulated separately. The first steps are probably the most important in determining the extent of eosinophils localization in tissues.

Figure A06-1. The range of effector functions of eosinophils: an overview of the major areas in which eosinophils are believed to be involved in inflammation.

Figure A06-2. Eosinophil activating factors. These are derived from adjacent cells in tissues. Several cell types produce each of these activating factors. The way in which they interact, and the importance of each one in different diseases has still to be determined.

Figure A06-3. Eosinophil-dependent cytotoxic mechanisms. These occur either at short range, with parasites, and at surfaces, to which they have become attached, or at a distance, through the secretion of their granule proteins, which localize in sites of injury.

Figure A06-4. The effects of eosinophils, and their products on other cells. In some cases, the interaction occurs in both directions.

Figure C03-1. The main types of parasitic diseases in man, and the tissues they affect.

Figure C04-1. The principal tumours which are associated with an increase number of eosinophils in the blood, or in the tumour, or in both.

Figure C06-1. Eosinophilic gastroenteritis. Involvement of each of the layers of the intestine produces different syndromes. Submucosal lesions can be polyploid, or diffuse. Adapted from ¹⁶⁸⁵, with permission.

Figure C08-1. Eosinophils, and asthma. Eosinophils are associated with each of the major structural abnormalities seen in the bronchi of patients with asthma.

Figure C12-1. Eosinophils in urological diseases.

Figure C14a-1. Three stages in the development of eosinophilic endomyocardial fibrosis. Modified from ¹⁶⁹³, with permission.

Figure C14a-2. Electronmicrograph of an eosinophil adjacent to a cardiac myocyte. The eosinophil granules have undergone changes in electron density, and electron-dense material appears to be discharged from one of the granules close to the surface of the heart cell. This was obtained from a left ventricular cardiac biopsy in a 68 year old Japanese man with HES. Eosinophils in the heart muscle were also activated. From ¹²⁶⁴, with permission.

Figure C14a-3. A comparison of EMF in temperate, and tropical regions. Although differences are seen in the clinical settings in which these diseases occur, the pathogenesis of the heart damage is probably identical. Adapted from ⁴⁰², with permission.

Figure C15a-1. The principal complications of the idiopathic hypereosinophilic syndrome, HES.

Figure C15a-2. Photograph of the retina of a patient with HES, and thromboembolic occlusion of a major retinal artery, producing a quadrant hemianopia. Photographed by Mr. James Govan, and reproduced with permission.

Figure C15b-1. A blood sample obtained from a patient with HES on presentation, containing 180×10^9 eosinophils/L blood. He responded rapidly to treatment with steroids, and remains well six years later on prednisolone 5 mg/d.

Figure C15b-2. Degranulated eosinophils in the blood of a patient with an eosinophilia, and eosinophilic heart disease. This painting was produced in 1919 by L.G. Shapiro ¹⁶⁰⁰. Identical appearances are seen in blood samples from patients with HES today.

Figure C17-1. Eosinophilic diseases involving the skin, and the effects they may produce there.

Figure C18-1. Drug-induced hypersensitivity reactions, and the major target organs affected by eosinophils, and other inflammatory reactions in these patients.

