THE MAST CELLS

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E. & S. LIVINGSTONE LTD.
EDINBURGH AND LONDON
1959

FOREWORD

DURING the past few years the so-called 'mast cells' have very rapidly acquired a new interest for the interpretation of phenomena and the solution of problems which have been much longer familiar to students of physiology and pathology. The initiative and the effective stimulus to the studies from which this new interest has arisen can be attributed to the original work carried out and published in a succession of papers by Dr. Riley, with Dr. G. B. West and his other colleagues. The publication of this Monograph by Dr. Riley, assembling and presenting for discussion all this new and highly suggestive evidence, will certainly be widely welcomed, not only by those who are themselves active in related fields of research, but also by the many others who may well have found it difficult to follow the rapid unfolding of knowledge about these cells, and especially its recent and rapid development.

As Dr. Riley duly records, we owe the first differential recognition of the mast cells to the late Paul Ehrlich, whose pioneer publication on the subject gave evidence, indeed, of the early ripening of his remarkable genius; for the paper which first described and named these cells, characterized by the packing of their cytoplasm with large, basophile granules, had been Ehrlich's graduation Thesis. I find myself less clearly convinced than Dr. Riley seems to be, with regard to the appropriateness of the name, 'Mastzellen' (=fodder cells, or cells concerned with fattening, or nutrition), as proposed by the young Ehrlich to indicate the function which he attributed to these cells, but for which, in fact, he had no evidence but their histological appearance, including the basophile staining of their granules by which he recognized them. A short name, however, is obviously needed for convenient reference. In its English translation, 'mast cells' has practically no such functional implication as the original German form had; and, in any case, a proper respect for priority and for Ehrlich's memory would make us retain the name which he proposed, and which has passed into such general use.

Dr. Riley's attention appears to have been first drawn to the functional possibilities of the mast cells, by the observation of their unusual abundance in the tissues adjacent to experimentally induced cancers of the skin in mice. Later he became acquainted with the results obtained by Jorpes and other Scandinavian workers, who had observed a significant correspondence between a special abundance of these cells in different tissues and the respective yields obtainable from them of the anticoagulant principle known as 'heparin'. This had been so named by its discoverer, W. H. Howell, on account of his original finding of it in extracts from the liver of the dog—an organ in which the mast cells were later found to be conspicuously abundant. It was apparently this association of the dog's liver with the production of heparin, which first led Dr. Riley, and those who became associated with him, to consider the possibility that the mast cells might also be the source of the histamine; since the anaphylactic shock and analogous reactions, in the form characteristic of the dog, were known already to be due, essentially, to the effects of histamine and heparin poured into the general circulation, as the result of a primary reaction in the liver. Dr. Riley and his co-workers were thus led to make a more general survey of the relation between the abundance, on the one hand, of mast cells in different organs and tissues of a number of species, including examples of mast-cell tumours, and the yields, on the other hand, of histamine obtainable by extraction from the same normal and pathological sources. I do not doubt that any careful student of their results will find that the evidence here presented shows a highly significant correlation between the mast-cell content of solid organs and their yield of histamine to artificial extraction; and such a student will surely be impressed, as I am, with the reasonableness of the deduction that the mast cells in such tissues, and, indeed, the characteristic, basophile, metachromatically staining granules which occupy so much of the cytoplasm of those cells, are by far the most probable source of that histamine.

It must be borne in mind, on the other hand, that this staining of the mast-cell granules does not, in itself, provide evidence for the presence in them of histamine. Histamine, on the contrary, being itself a base, has a special affinity for the acidic dyes, such as eosin and phloxin, with which it combines *in vitro* to form precipitates. Before the demonstration by Dr. Riley and his colleagues of the connexion between mast cells in a tissue and its yield of histamine, there had been highly suggestive evidence of a similar association between the yield of histamine from the blood of different animal species, including that of man, and its richness in white cells with an eosinophile granulation. In those of us who were familiar with these earlier observations, the news of the discovery that, in the tissues, the occurrence of histamine was associated with that of the basophile mast cells, produced, at first glance, a sense of paradox, or conflict of evidence. And it seems to me that we have the more reason for gratitude to Dr. Riley and his co-workers for having gone steadily forward, undeterred by any such prejudice, to collect and assemble the data, which have now so abundantly confirmed their first observations. For my own part, I am expecting that the new association which they have so convincingly established, between histamine and the heparin-containing mast-cells of the tissues, will be found to have an increasing importance, for the assignment to histamine, and to the other amines, such as serotonin, now coming into view as mast-cell constituents, of their various functional roles in a range of physiological and pathological reactions.

H. H. DALE.

PREFACE

THIS booklet on the mast cells is in two parts. The first is a review of the literature, mainly up to 1950 when the work described in the second part began. The second part is essentially the thesis, 'Functions of the Tissue Mast Cells', which was accepted in 1958 by the University of St. Andrews for the degree of Doctor of Philosophy.

In preparing the manuscript for publication I have been reminded of the very great help which I have received from other workers at various stages, and which I wish freely to acknowledge.

Some of the early experiments on the histology and histochemistry of mast cells were carried out in Edinburgh in collaboration with Dr. J. M. Drennan of the Department of Pathology. Mr. (now Professor) D. M. Douglas and Dr. J. P. Graham of the Wilkie Research Department, Edinburgh, helped me in experiments on peptone shock in dogs. Later, in Dundee, when a pharmacological approach was required, Professor R. B. Hunter not only offered me the very considerable facilities of his department, but also introduced me to Dr. G. B. West, without whose cheerful enthusiasm and unlimited patience further progress would have been slow indeed. Much of the present work on histamine in mast cells has already been published in collaboration with Dr. West, and this forms the main body of the second part of the book: where it is appropriate, a reference to the original paper is given under the chapter heading. On the departure of Dr. West for London, the pharmacological investigations were continued by his successor, Dr. P. B. Marshall, and by Miss Rosemary Cass. Dr. West and I have acknowledged elsewhere the valuable contributions of Mr. K. W. Head of the Royal (Dick) School of Veterinary Studies, Edinburgh, who supplied us with much unusual veterinary material, and of Mr. S. W. Stroud of the Research Division, Boots Pure Drug Co. Ltd., Nottingham, for his assistance with the heparin assays. In some experiments the initial extraction of the heparin was carried out by Dr. D. M. Shepherd of the Department of Pharmacology, Queen's College, Dundee, to whom we also owe our thanks for his advice on problems connected with the chromatography of tissue extracts. An interesting off-shoot of the present study has been the work of Dr. J. D. B. MacDougall of the Department of Anatomy, who has succeeded in growing various types of mast cell in tissue culture and has himself published several papers on the subject. I record with special gratitude the constant flow of encouragement and advice of Professor A. C. Lendrum of the Department of Pathology: many of the paraffin sections were cut and stained by Mr. W. Slidders in Professor

Lendrum's department, and all the photomicrographs were taken there with the aid of his technician, Mr. J. W. Corkhill.

Finally, I wish to place on record the debt of gratitude which Dr. West and I owe to the constructive criticism and generous advice of Sir Henry Dale throughout the whole of this work. Sir Henry, himself a pupil of Ehrlich, was among the first to accept our evidence on the cytological location of histamine in mast cells and to suggest fruitful means for obtaining further information. For our part we were honoured in being invited in April 1955 to take part in the Symposium on Histamine organized by the Ciba Foundation to celebrate Sir Henry's eightieth birthday. Sir Henry now does me the even greater honour of writing a foreword to this account of a problem on which he himself has contributed so much.

JAMES F. RILEY.

Dundee, 1959.

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PART I

REVIEW OF THE LITERATURE

CHAPTER I

THE DISCOVERY OF THE MAST CELLS

ON the 17th January 1879 the Physiological Society of Berlin heard a remarkable paper by a remarkable young man. The speaker was Paul Ehrlich; his subject a granular cell of the loose connective tissues which he had discovered as a medical student some two years previously (Ehrlich, 1877) and for which he had proposed the name 'Mastzellen' (well-fed cells) since the cells are more numerous in connective tissue whose nutrition is enhanced (Ehrlich, 1878). Ehrlich pointed out that not only do the granules of mammalian mast cells display great avidity for basic dyes, but that they also tend to alter the shade of the dye ('metachromasia'). Later, with his own pupil, Westphal (1891), he stressed a second characteristic feature of the mast-cell granules in many species, their solubility in water. As Michels (1938, p. 262) remarks, 'Uncounted pages of useless and misleading research have been the result of the failure on the part of many investigators to heed the admonition originally given by Ehrlich and Westphal, that the mast granules are soluble in water and that to preserve them tissues must be fixed in 50 per cent alcohol and stained in alcoholic thionine'.

From what we know of Ehrlich's enthusiastic temperament (Marquardt, 1949) it is not difficult to picture him giving this, his first public dissertation. But the occasion was more than that. In discovering the mast cells Ehrlich had discovered a principle which was to guide him through his working life and inspire his later studies in chemotherapy. That the biological action of a drug is a direct consequence of its molecular architecture is now a pharmacological axiom so firmly established as to pass without comment: in Ehrlich's day when medical treatment was still empirical and symptomatic, the idea of 'specific affinity' between drug and protoplasm was a novel conception. It is said (Plesch, 1947) that the idea of specific affinities first germinated in Ehrlich's mind when he was working in the laboratory of his cousin, Karl Weigert, who had begun to use the new technique of staining histological sections. While examining one of Weigert's slides under the microscope Ehrlich began to speculate why one part of the cell should stain selectively with a particular dye leaving the rest of the cell unstained. It was not long before he was seeking the answer.

Success came early. Ehrlich obtained samples of the latest commercial dyes from German manufacturers who were then turning to such profitable account Perkin's original discovery in Manchester of the first synthetic aniline dye, mauve. On applying some of the new dyes to connective tissue Ehrlich discovered the mast cells. The strong affinity of the granules for basic dyes and the metachromatic staining reaction enabled Ehrlich to differentiate his Mastzellen from the heterogeneous collection of 'embryonal' or 'plasma' cells described by his teacher, Waldeyer (1875). Granular cells were first seen in the peritoneum of frogs by Von Recklinghausen (1863). Waldeyer, who described and depicted similar cells with refractile granules around small blood vessels, believed them to be precursors of the fat cells which form the outer layer of the perivascular sheath. Ehrlich showed that Waldeyer's granular cells do not contain fat by pretreating the tissues with fat solvents, and that dyes which stain fat leave the granules of mast cells unstained. According to Ehrlich the mast granules represent an albuminous reserve substance for the connective tissues and are the products of differentiation in healthy cells of the fixed mesenchyme.

Ehrlich then went on to study the staining reactions of the blood cells, laying the foundations of modern haematology on the basis of the specific affinities of the leucocytes for various dyes (Ehrlich, 1891; Ehrlich and Lazarus, 1898). Here again he encountered cells with basophilic, metachromatic granules, and thus came to recognize two types of mast cell; the first, derived from, and living in the connective tissues (tissue mast cell), the second—the counterpart of the neutrophil polymorph and eosinophil leucocyte—whose origin is in the bone marrow and whose habitat is the peripheral blood (blood mast cell, basophil or mast leucocyte). Not the least of Ehrlich's genius was his intuitive appraisal of the limitations of the experimental methods of his day. His later work on cancer is typical of this. So it was with the mast cells. He made no attempt to apply experimental methods to the problem of their function or to characterize chemically the granule substance. Having described the morphological features of blood and tissue mast cells, he left it to others to elucidate the role of these cells in the organism, concluding his brief introduction (Ehrlich, 1879) with the hope that one day the mast cells would be found to have an interesting function. It is the purpose of this monograph to trace the subsequent story and see to what extent Ehrlich's hopes have been fulfilled. It is an interesting story, and no less interesting in the certainty that the full account has yet to be written. Even now we are in the midst of one of the periodic waves of enthusiasm for the mast cells, and there may thus be workers in many fields to whom an interim account will be acceptable. This is built around the researches of my colleagues and myself in Scotland who have investigated the relationship of the mast cells to tissue histamine. At the same time we have endeavoured to retain something of the wider perspective which appealed so strongly to Ehrlich: that is perhaps the outstanding feature of his undergraduate Thesis which has only recently become available for study (Ehrlich, 1878).

Chapter II

THE MAST CELL IN EVOLUTION

DURING the sixty years which followed Ehrlich's discovery, research on the mast cell was almost entirely histological. Controversies arose but their resolution for the most part merely emphasized the soundness of Ehrlich's original work.

Thus Ranvier (1890) described his 'clasmatocytes'—cells which cast off ('clasmatose') fragments of protoplasm; but it is clear from Ranvier's own descriptions that many of the cells which he originally recognized as clasmatocytes are tissue mast cells. In the newt and salamander especially the mast cells are exceptionally large and irregular in shape and are easily damaged during preparation of the tissues. Moreover, Ranvier used a watery fixative (1 per cent osmic acid), a watery stain (violet 5B) and a watery mountant (glycerine) whereby the granule substance rapidly diffuses from the cytoplasm. Using the same stain and a more adequate fixative, Raudnitz (1883) had already successfully demonstrated mast cells in man. The situation was finally clarified by Jolly (1900) who clearly distinguished mast cells from clasmatocytes in mammals and showed that the huge arborizing cells in amphibia are the tissue mast cells of these species.

The use of a watery technique likewise led Pappenheim and his school to assert that mast myelocytes are lacking in the bone marrow of the rabbit and in the marrow of patients with myeloid leukaemia, thereby implying that the numerous blood mast cells in both are derived from precursors in the tissues. This misconception persisted until Maximow (1913) and Ringoen (1919) conclusively re-established Ehrlich's views and again stressed the extreme water-solubility of the mast granules in certain species (Kanthack and Hardy, 1894). Not all mast granules are so readily soluble; the mast granules of the rat are surprisingly resistant to water (Paff and Mergenthaler, 1955). It will be shown later that once the limitations of a watery technique for mast cells are appreciated the method can be used to yield information concerning the physiological function of the cells.

In only one respect did Ehrlich's early work fail to find complete acceptance later. It will be recalled that Ehrlich's classification and nomenclature of the granular leucocytes rested on the 'specific affinities' of the granules for various dyes. As the scope of the search for mast cells widened, it became increasingly apparent that in many lower organisms not only are the mast granules frequently non-metachromatic, but that, at least during certain phases of their life history, they may even be acidophilic. The significant homology for the mast cell is rather the connective tissue habitat of the cell and the size and number of its granules than the immediate staining qualities of the granules themselves: 'coarse granulocyte' is perhaps the more appropriate term for such cells. With these considerations in mind we may first review in general terms the evolutionary development of the mast cell in a series of organisms of increasing complexity, and later examine in somewhat greater detail the distribution of tissue mast cells in vertebrates.

INVERTEBRATES

It has been suggested that the metachromatic bodies in certain bacteria and the volutin granules in yeasts (Guilliermond and Marvas, 1908; Wermel and Sassuchin, 1927) correspond to mast cells in higher organisms; but in view of the doubtful histo-chemical significance of metachromasia no great importance can be attached to these claims (Tulasne and Vendrely, 1947; Duguid *et al*, 1954). Nevertheless, in such lowly multicellular organisms as sponges (Cotte, 1904) and simple coelenterates (Kollmann, 1908) well-defined basophilic granular cells are already present in the connective tissues from which, in some species, they escape as free cells into the primitive body cavity. Both Cotte (p. 547) and Kollmann (p. 213) are of the opinion that these cells correspond to the mast cells in higher forms. Such 'amoebocytes with spherules' are common in echinoderms, especially in the walls of the ambulacratory pockets of the star fish and sea urchin (Kollmann, 1908; Kindred, 1926) from which a yellowish substance can be extracted from the granules by transferring the organisms to tap water (Pequegnat, 1948). According to Kindred (1926) the 'coarse granulocyte' in echinoderms is first recognizable as a connective tissue cell with clear cytoplasm, and thus corresponds to the 'hyaline cell' of Kollmann (1908). Under conditions of good feeding the cell develops bulky refractile spherules which stain with basic dyes. The spherules then progressively become yellow and shrunken; finally often red and acidophilic. Thus the intermediate coarse granulocyte in invertebrates, the cell with colourless, basophilic spherules, bears the most obvious resemblance to the mast cell of higher animals. Kollmann found these cells in the connective tissues of various invertebrate forms which he examined. 'Les cellules spheruleuses . . . restent habituellement cantonnées dans le tissu conjonctif. On peut vraisemblement les comparer aux Mastzellen des Vertébrés' (Kollmann, p. 200). Thus it will be observed from the standpoint of comparative morphology that mast cells are regularly demonstrable *before* the demand for oxygen and nutriment has led to the evolution of a definitive blood-vascular system. Whatever may be the function of the mast cells in these simple organisms it can hardly be concerned with the blood.

The common blood cell of many insects appears to be concerned functionally with the connective tissues (Lazarenko, 1925). In certain species the basophilic cytoplasmic bodies stain strongly with the periodic acid-Schiff method for a neutral mucopolysaccharide (Ohuye, 1952; Ohuye and Horikawa, 1956) 'which forms or contributes to the connective tissues and basement membranes' (Wigglesworth, 1956).

With the evolution of an 'open' type of circulation, as in arthropods, more typical mast cells become demonstrable around the walls of the smaller arteries whose contents empty direct into the tissue spaces. Over a century ago Häckel (1857) described an adventitial cuff, a 'Zellgewebe', surrounding the smaller blood vessels of the crayfish. By injecting a suitable vital stain into the pericardial sac or primitive heart of the crayfish, myriads of cells filled with metachromatic granules can be demonstrated in Häckel's perivascular sheath. Only rarely does such a tissue basophil escape into the circulation (Hardy, 1892).

VERTEBRATES

However, it is at the next evolutionary level that tissue mast cells appear in an abundance which makes it difficult to escape the conclusion that their number and situation foreshadow something of their function in higher organisms. The tissues of many Ichthyopsida (fishes and amphibians) are teeming with mast cells in contrast to the virtual absence of mast cells in the blood. So intimately is the distribution of the mast cells bound up with the evolution of the blood vascular system that it is worth while pausing for a moment to review briefly the development of the blood-forming tissue itself and the progressive specialization of its component parts (Jordan, 1926; Jordan and Speidel, 1930).

Evolution of the blood-vascular system

In the lancelet amphioxus (branchiostoma) the few free mesenchymal cells in the blood are derived from undifferentiated precursors scattered throughout the body. With the advent of the primitive craniate fishes, the jaw-less cyclostomes, there begins a process of segregation and specialization of blood-forming tissue which may be traced progressively forwards to the mammals. From being distributed diffusely throughout the wall of the intestine, as in the hagfish, the haemopoietic tissue becomes aggregated in the so-called spiral valve of the larval lamprey and forms a compact nodule in the wall of the stomach of the African lung fish. In the cartilaginous fishes the active tissue is now attached to the stomach by a mesentery and may thus more properly be termed a spleen. At the amphibian level, especially in the tailless anurans, the spleen is concerned almost exclusively with the production of erythrocytes and thrombocytes, granulopoiesis still being carried on at former sites of blood formation throughout the body. With the evolution of the bony fishes the marrow begins to usurp some of the functions of the spleen and becomes the predominant tissue for haemopoiesis. According to Jordan and Speidel (1930, p. 375) the mechanical advantage conferred on the organism by the development of tubular bones also led indirectly to ideal conditions for blood formation. 'The diaphyseal content of hollow bones is fundamentally related to the nutritive requirements of the bones. Thus, in a sense, the practical postnatal restriction of hemocytopoiesis to bone marrow in higher forms, especially mammals, was accidental. Arising apparently as an incidental aspect of the mechanism providing for the most favourable nutritive conditions for hollow bones, it becomes appropriated as offering more favourable conditions for blood formation. The spleen, accordingly, becomes of less importance at the higher evolutionary levels after the appearance of long bones. Its original multiple function becomes apportioned among lymph nodes, perhaps thymus, and bone marrow.' It is, of course, well known that extensive destruction of red bone marrow even in mammals can be followed by the resumption of extra-medullary blood formation in the original haemopoietic centres of the body, and that after splenectomy the marrow may perform the reciprocal function of producing lymphocytes and monocytes. In view of the multipotent capacity of the marrow for blood formation, it is not surprising that along with the neutrophil and eosinophil leucocytes the marrow also produces variable numbers of blood mast cells. In general there is an inverse numerical relationship between the numbers of blood and tissue mast cells in a particular species (Maximow, 1910): however, with few exceptions the number of blood mast cells is small compared with the number of tissue mast cells which continue to be produced at sites of former haemopoietic activity.

Meanwhile, in course of evolution, another specialized type of tissue has come into being. This is the lymphatic system whose origins can also be traced to the widespread primitive haemopoietic tissue of lower animals. Portions of the multipotent mesenchyme become aggregated into foci specializing in the production of lymphocytes; capsulated lymph nodes make their first appearance in water birds (Further, 1913). Even so, the spleen in birds continues to play a significant role in haemopoiesis, its removal being followed by increased haemopoietic activity elsewhere, as in cold-blooded animals: 'A striking example is presented by the differentiation of the mast cells along the vessels of the omentum. . . . An analogous development of mast cells is seen under the epithelium of the intestine' (Dantschakoff, 1916, p. 485). As the importance of the marrow for haemopoiesis increases still further, the spleen eventually produces little else but lymphocytes and monocytes. Yet there remain, even in the mammals, scattered traces of the original blood-forming tissue which continues to produce the granular cells of the connective tissues. These are the tissue mast cells. It will be helpful to keep this genealogical background in mind when the apparently capricious distribution of mast cells in higher oıganisms is under consideration.

Detailed distribution of mast cells in vertebrates

LOWER VERTEBRATES. In lower vertebrates, production of coarse granulocytes is for the most part restricted to certain well-defined sites, notably gut, mesentery, subcutaneous and intermuscular tissue, liver, spleen, kidney, gonads, perivascular tissues generally, lungs (when present) and to a lesser extent pancreas and even skull (Drzewina, 1905; Michels, 1923).

Alimentary tract. In the alimentary tract of Ichthyopsida (fishes and reptiles) the 'lymphoid tissue' (Drzewina, 1905) forms an almost continuous sheath between mucous membrane and muscle, a region which corresponds to the location of the Peyr's patches and isolated lymphoid nodules in man. Drzewina failed to find granulocytes in the alimentary lymphatic tissue of carp though they have been described there by Al-Hussaini (1948). In the carp coarse granulocytes are particularly abundant in the perivascular tissues and serous membranes, especially mesentery (Michels, 1938). Greene (1912) found in the gut of the King salmon a layer of coarse granular cells so dense that he refers to it as the 'stratum granulosum'. However, Arvy (1955*a*) points out that apparently similar cells in the trout more closely resemble the granular cells of the exocrine pancreas; they are decidcdly more acidophilic than basophilic in their staining properties. Numerous coarse granulocytes were seen in the gut of other salmonoids by Bolton (1933) who has no hesitation in calling them 'mast cells'. Migratory activity and concomitant change in staining properties of the coarse granulocytes in teleosts were clearly recognized by Al-Hussaini (1949). Duthie (1939) shows coarsely granular basophils migrating towards mucosal and serous surfaces: as the surface is reached the now acidophilic granules become orientated in a manner which suggests (p. 40) 'a discharge of the granules on to the surface of the epithelium'. It will be seen later that there is evidence for limited migratory activity of perivascular mast cells towards mucosal and epithelial surfaces in higher vertebrates (Riley, 1953*a*; Arvy, 1956*a*; McGovern, 1956). Typical mast cells have been described in the gut of various Batrachians (anurans and urodeles) by Arvy (1955*b*).

Heart and blood vessels. As long ago as 1857 Leydig described a richly cellular sheath surrounding the mesenteric vessels in several teleostean (bony) fishes. The regular occurrence of haemopoietic tissue in this situation is well seen in certain 'ganoids' (*e.g.* sturgeon). Drzewina (1905) describes such a tissue clothing the heart of the sturgeon and extending along its blood vessels; Ohuye and Ochi (1954) observed a similar genesis of basophilic granulocytes in a urodele, the Japanese newt. According to Drzewina the formative tissue