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BIOCHEMISTRY AND FUNCTION OF PHAGOCYTES

Edited by F. Rossi and P. Patriarca

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PREFACE

This volume collects the papers presented at the "Second European Conference on Phagocytic Leucocytes" which was held in Trieste, September 15-18, 1980. The Conference was the continuation of the thread of a discourse initiated with the "First European Conference on Phagocytic Leucocytes," which was held also in Trieste in 1976. In fact, in both Conferences the biochemical aspects of the basic functions of phagocytes--that is movement, metabolism, secretion, and bactericidal activity--were mainly emphasized. In the Second Conference, two additional subjects were also dealt with, and those are the tumoricidal activity of phagocytes and the clinical aspects of phagocyte functions. In connection with the latter subject a "Round Table on the Clinical Application of Leucocyte Function Tests" was held during the Conference, and the proceedings are reported in this book in the hope that they may be useful to those who are interested in the relationship between phagocytes and diseases. The number of papers which are being published concerning the phagocytic process is such that one wonders whether publication of a new book in this field is necessary. However, the philosophy which inspired the editing of this book differs from that underlying publication of original articles in scientific journals. We started from the common notion that the real progress of our knowledge is not necessarily proportional to the amount of published material in that specific field. Additionally, a true progress in knowledge requires a careful verification of the validity of the information that one receives, a realistic analysis of the reasons of conflicting results and of controversies, and a severe criticism of erroneous or false problems which generate unuseful and confusing papers. The two European Conferences on Phagocytic Leucocytes were organized to meet those requirements. The worldwide participation in both Conferences (77 scientists from 15 countries in the First Conference, and 106 scientists from 14 countries in the Second Conference) guaranteed a broad exchange of opinions. Consequently, this book does not represent a mere addition of new papers to an already vast literature, but defines the present status of the art and the future trends of research in the field of phagocyte functions. We hope that, owing to the value of the parti-

cipants and to the interest of their contributions, this volume can be useful to clarify problems and to stimulate new research projects.

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Gratitude is also expressed to the members of the organizing committee Drs. P.Patriarca, G.D.Rottini, G.Zabucchi, P.Dri, R.Cramer, M.R.Soranzo, A.Dobrina of the Institute of General Pathology of the University of Trieste, and Drs. F.Rossi, G.Berton, P.Bellavite, G. deNicola of the Institute of General Pathology of the University of Padua, Sede di Verona. The Conference could not have succeeded without the financial assistance of the Medical Faculty of the University of Trieste, the National Council of Research (CNR) of Italy, the Azienda Autonoma di Soggiorno e Turismo of Trieste, the Banca Cattolica del Veneto of Trieste, the Dr.Ing.G.Terzano & C. S.p.A. of Milano and the Beckman Analytical S.r.l. of Milano.

Finally, a particular mention is due to the precious collaboration of Mrs. Milvia Gioria throughout the Conference and during the preparation of these proceedings.

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THREE TYPES OF GRANULE FORMED IN GUINEA PIG AND RAT HETEROPHIL GRANULOCYTES

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INTRODUCTION

It is generally accepted that in heterophil (in man neutrophil) granulocytes, two types of granule are formed successively during the development of these cells in the bone marrow. The process of formation and maturation of these two types of granule was well documented by Bainton and Farquhar (1966) in their studies on the development of heterophil granulocytes in the bone marrow of the rabbit. From these studies it became clear that during the promyelocyte stage azurophil granules are formed, whereas during the myelocyte stage and shortly thereafter, specific granules are produced. Both types of granule originate from vesicles pinching off from Golgi cisternae and changing into mature granules by condensation of their contents. Mature azurophil granules are characterized by a homogeneous matrix of higher electron density than that of specific granules. In the mature heterophil granulocytes furthermore, azurophil granules are considerably outnumbered by specific granules.

More information about the morphological heterogeneity of the two types of granule was later obtained in cytochemical studies (Bainton and Farquhar, 1968), which showed that azurophil granules possess both peroxidase and acid phosphatase and can therefore be considered primary lysosomes, whereas the specific granules have alkaline phosphatase and no lysosomal enzymes.

Besides these two types of granule, mention has been made in the literature of ellipsoid and spindle-shaped granules, characterized by the presence in their matrix of periodic structures lying parallel to the long axis of the granule. The fine structure varied from regular crystalloid to irregular fibrillar. Such observations have been made mainly in rat heterophils and human neutrophils (for references, see: Brederoo and Daems, 1978; see also: Brederoo and

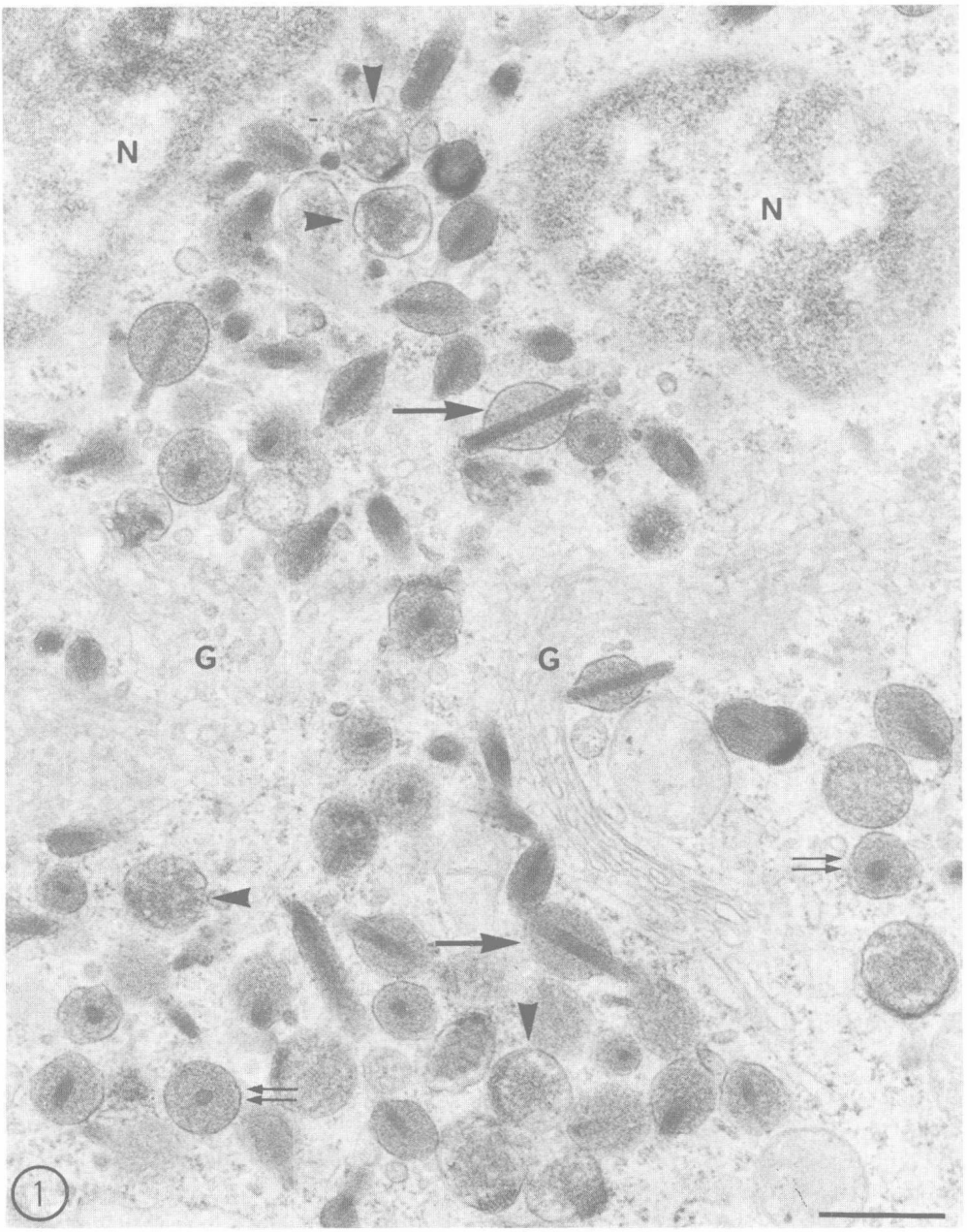

Fig. 1. Golgi area (G) of an early promyelocyte from a rat, showing numerous nucleated granules. Arrowheads point to vacuoles with a flocculent content. Almost-mature granules can be seen in longitudinal section (arrows) and cross-section (double arrows). N = nucleus. Bar: 0.5 µm.

van der Meulen, 1980). After incubation in a medium for the demonstration of the enzyme peroxidase, granules with an internal fine structure react in the same way as azurophil granules (Daems, 1968; Bainton et al., 1971; Breton-Gorius and Reyes, 1976; Brederoo and Daems, 1977).

On the basis of these observations it has been stated that the formation of two types of granule is not restricted to the rabbit, but is a general phenomenon in mammalian species. Furthermore, because both the azurophil and the fine structure-containing granules are peroxidase-positive, the latter have considered to be types or forms of the azurophil granule. In addition, study of the granules with fine structure has been hampered by the recommendation that the peroxidase reaction be used as a means to distinguish between azurophil and specific granules (Farquhar and Bainton, 1972).

This paper presents observations showing that these conclusions are not correct, at least as far as the guinea pig and the rat are concerned.

GUINEA PIG AND RAT HETEROPHILS

Bone marrow from femurs of male and female albino guinea pigs and rats was studied after fixation in osmium tetroxide alone as well as after fixation first in glutaraldehyde and then in osmium tetroxide. For details of the preparation for electron microscopy and the terminology used, the reader is referred to Brederoo and Daems (1978). Because the development of the heterophil of the guinea pig does not differ essentially from that of the rat heterophil, no distinction will be made here between the two species except where specially mentioned.

The steps in the process of development from promyelocyte via myelocyte, metamyelocyte, and band cell, to the mature heterophil of the guinea pig and rat are morphologically the same as those described for other mammals, including man. During maturation the cells become smaller and the nucleus changes in shape from slightly indented in the promyelocyte, via more and pronounced indentation in the myelocyte and metamyelocyte, to a multilobulated nucleus in the mature cell. After the myelocyte stage, the condensation of the nuclear chromatin increases and the amount of rough endoplasmic reticulum and Golgi cisternae decreases considerably. Throughout the development the total number of granules increases, and the mature cell shows a huge number distributed over the cytoplasm. Early stages of development, i.e., the promyelocyte and the myelocyte, are easily recognized due to the formation and maturation of typical granules. Formation of the specific granules starts in the myelocyte, as is the case in other mammals. However, in contrast to the other species, the guinea pig and rat were found to show two separate steps in the development of the promyelocyte, each characterized by the initiation and maturation of a typical granule. The production of granules containing fine structure, here called