# CLINICAL HAEMATOLOGY IN MEDICAL PRACTICE

DE GRUCHY

SECOND EDITION

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## IN MEDICAL PRACTICE

BY

G. C. DE GRUCHY

M.D.(Melb.) F.R.A.C.P. M.R.C.P.(Lond.) M.C.P.A.

*Professor of Medicine in the University of Melbourne*
*St. Vincent's Hospital*

WITH A FOREWORD BY

J. V. DACIE

*Professor of Haematology in the*
*University of London*

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TO J.V.D.

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# FOREWORD

HAEMATOLOGY is a fascinating branch of medicine. The blood, however, is a complicated tissue and the many things that can go wrong with it are a constant challenge to the haematologist and provide him with innumerable problems for investigation. The last twenty years or so have witnessed an astonishing increase in knowledge, and so much so that even the vocabulary of haematology has been transformed. It seems hardly credible today that vitamin $B_{12}$, folic acid, Christmas disease, haemoglobin-S, the Rh blood groups and 6-mercaptopurine, to quote words which are now in daily use throughout the world, were unheard of at that time. To most people, unfortunately, all this new knowledge will not seem to have led to any obvious simplification in the classification, diagnosis or treatment of blood diseases. The reverse may even appear to have taken place, for as each new technique for investigation has been introduced the apparently inevitable consequence has been a further splitting up of disorders previously considered to be well circumscribed, even if ill-understood. The megaloblastic anaemias, congenital haemolytic anaemias, sickle-cell anaemia and the haemorrhagic disorders provide examples of this process.

To the physician, therefore, haematology must appear to be at least as complicated as any other branch of medicine. The standard textbooks on the subject increase in size (and in price) as the authors struggle desperately to include in each new edition at least something about each new development.

The above considerations have led me to believe for some years now that there was a real need for a new book on haematology which could bridge the gap between the inevitably short and incomplete descriptions of blood diseases found in textbooks of general medicine and the large comprehensive and heavily documented haematological reference books and monographs.

Dr Carl de Gruchy's new book will I think fulfil this need. It is written from both the clinical and laboratory standpoints by a physician of wide experience who himself is as equally at home in the laboratory as in the wards. In simple terms, unencumbered by hosts of references, Dr. de Gruchy covers the whole field of modern haematology. The text follows orthodox

lines; erythropoiesis and the anaemias are dealt with first, and leucopoiesis, the leukaemias, polycythaemia and allied disorders next. Later chapters include discussions of the tumours of lymphoid tissue, the haemorrhagic disorders and disorders of pigment metabolism. The emphasis is predominately clinical, but aetiology and pathogenesis have not been overlooked.

The reader will be struck by the clarity of the writing and the fine sense of proportion between the different sections. Helpful tables illustrating classification, aetiology and differential diagnosis are included as well as schemes for the investigation of particular types of blood disorder. Each chapter has its own bibliography in which a selection of the more important relevant papers and reviews are listed. Key references are given in bold type. All these factors will ensure that the book will be widely read and appreciated.

Postgraduate Medical School of London

June 1958. J.V.DACIE

# PREFACE TO SECOND EDITION

Since the first edition was written, significant developments have occurred in most fields of haematology. Thus in preparing the second edition the whole of the text has been reviewed and all chapters have been re-written, some extensively. Aspects which have been particularly revised include iron metabolism and deficiency, the sideroblastic and non-sideropenic hypochromic anaemias, vitamin $B_{12}$ and folic acid metabolism and deficiency, the treatment of aplastic anaemia, the drug-induced haemolytic anaemias with special reference to glucose-6-phosphate dehydrogenase deficiency, the non-spherocytic congenital haemolytic anaemias and pyruvate kinase deficiency, serum haptoglobins, the treatment of acute and chronic leukaemias, chromosomal abnormalities in leukaemia, erythropoietin, toxoplasmosis, the pathogenesis of polycythaemias and the pathological physiology of disorders of coagulation.

The number of chapters has been increased by two by dividing Chapters 3 and 14 of the first edition into two chapters each. Small print has been used in the description of some less common disorders and certain aspects of morphology and physiology.

I again wish to express my thanks to the many colleagues and friends who have assisted me in the preparation of this second edition. I am particularly grateful to Dr A.Baikie, Dr J.Owen, Dr H.Lehmann, Dr A.Waters, Dr J.Madigan, Dr M.Verso, Dr J.Niall, Dr G.Hale and Dr J.Carew who helped me with particular sections by making available their expert knowledge and advice. Dr A.Spiers and Dr A.Buenaventura greatly assisted by reading the proofs and making a number of helpful suggestions. Professor Dacie read a number of chapters and made valuable suggestions and criticisms; it is with pleasure that I again express my thanks for his help and encouragement. Dr R.Sawers kindly consented to rewrite the section on coagulation disorders which appears as Chapter 15; he has again produced an authorative and readable account of these disorders.

I wish to thank those authors who have given me permission to reproduce illustrations; detailed acknowledgments are given in the text. I also wish to

thank the Editors of the following Journals for permission to include illustrations: *Medical Journal of Australia* and *Australasian Annals of Medicine*. Mr A.Daniels not only took the new photographs but gave helpful advice on their composition. The new black and white figures were drawn by Miss G.Babarczy who also assisted greatly by her careful proofreading. It is with pleasure that I acknowledge the help and initiative of Miss M.Donohoe in the preparation of the manuscript and her skill in its typing. Finally I which to acknowledge the friendly and helpful collaboration of Mr Per Saugman and Mr J.Robson of Blackwell Scientific Publications.

*Melbourne* G.C.DEGRUCHY

Chapter I

# BLOOD FORMATION. BONE MARROW BIOPSY

Blood consists of three formed elements, namely the red cells (erythrocytes), white cells (leucocytes) and platelets (thrombocytes), suspended in a fluid medium, the plasma. These cells are continually being destroyed, either because of old age or as a result of their functional activities, and replaced by newly formed cells. In healthy subjects there is a finely adjusted balance between the rates of formation and destruction, and thus the number of each cell type remains remarkably constant, although there are minor daily physiological fluctuations.

In this chapter the site and control of blood formation will be discussed, together with the morphological appearances of the developing cells. Bone marrow biopsy, a method of investigation which gives much information about disorders affecting blood formation, will also be described.

## GENERAL ASPECTS OF BLOOD FORMATION

### Site of Blood Formation

In the *foetus* all the blood cells develop from cells having their origin in the mesenchyme—the embryonic connective tissue. During the first 2 months of foetal life blood formation takes place in the yolk sac. The liver then becomes the main site of haemopoiesis until about the seventh month, and the spleen makes a small contribution. Haemopoiesis commences in the bone marrow in the third month, and from the fifth month until term the marrow progressivley takes over from the liver, with the result that after the seventh month it is the major site of haemopoiesis, and that shortly after birth in the normal full-term infant it is the only site of formation of red cells, granulocytes and platelets. Although some lymphocytes and monocytes are formed in the liver and bone marrow, the main sites for their production are the spleen, lymph nodes and other lymphoid tissues. Occasional small erythropoietic foci are seen in the lymph nodes and thymus but their contribution to total erythropoiesis is not significant.

*After birth*, red cells, granular leucocytes and platelets are formed only in

the bone marrow. Lymphocytes are formed mainly in the lymph nodes and other collections of lymphoid tissues, but a small proportion are formed in the marrow. Monocytes appear to be formed mainly in the spleen and lymphoid tissue, but the bone marrow makes some contribution. The parent cell of all the blood cells, irrespective of their site of formation, is the undifferentiated stem cell or primitive reticulum cell of the reticulo-endothelial system.

*Extramedullary haemopoiesis (myeloid metaplasia).* After birth the spleen, liver and lymph nodes normally play no part in the formation of red cells, granulocytes or platelets. However, in certain circumstances these organs revert to their foetal role of haemopoiesis, as the reticulum cell retains its potential haemopoietic activity. The term extramedullary haemopoiesis is applied to blood formation in organs other than the marrow, and organs showing such haemopoiesis are said to be the site of 'myeloid metaplasia'.

The usual cause of extramedullary haemopoiesis is an increased demand for cells which cannot be met by marrow hyperplasia alone. It occurs most commonly in infants and young children, because the whole of their marrow cavity is occupied by red (haemopoietic) marrow and there is little or no room for expansion in response to increased demands, e.g. following haemorrhage or haemolysis. Extramedullary haemopoiesis may also occur in certain chronic severe anaemias in adults, e.g. pernicious anaemia and haemolytic anaemia, and in association with bone marrow replacement. It regularly occurs in myelosclerosis, and occasionally in secondary carcinoma of bone. Until recently it was thought that the extramedullary haemopoiesis occurring with myelosclerosis was a compensatory process to make up for the loss of normal blood forming marrow. However, it now seems probable that it represents another manifestation of the abnormal reticulum cell proliferation of this disorder (p. 457).

## THE DEVELOPMENT OF THE BLOOD CELLS

### Origin of the Blood Cells

All blood cells are derived from the undifferentiated primitive reticulum cell of the reticulo-endothelial system. Ferrata has called the potentially haemopoietic reticulum cell the haemohistioblast. In the marrow this cell gives rise to the haemocytoblast, the primitive cell which has the power to differentiate and develop along the red cell series, granulocyte series, or as a megakaryocyte. Thus, the haemocytoblast may develop into a pronormoblast, myeloblast or a megakaryoblast according to the nature of the stimulus it receives (Fig. 1.1). The cells of the lymphocytic series and the monocytic series also develop from the primitive reticulum cell but most are formed outside the marrow. The primitive cells of each family (the 'blast' cells), are

similar in appearance and cannot always be differentiated from each other by their morphological appearance alone.

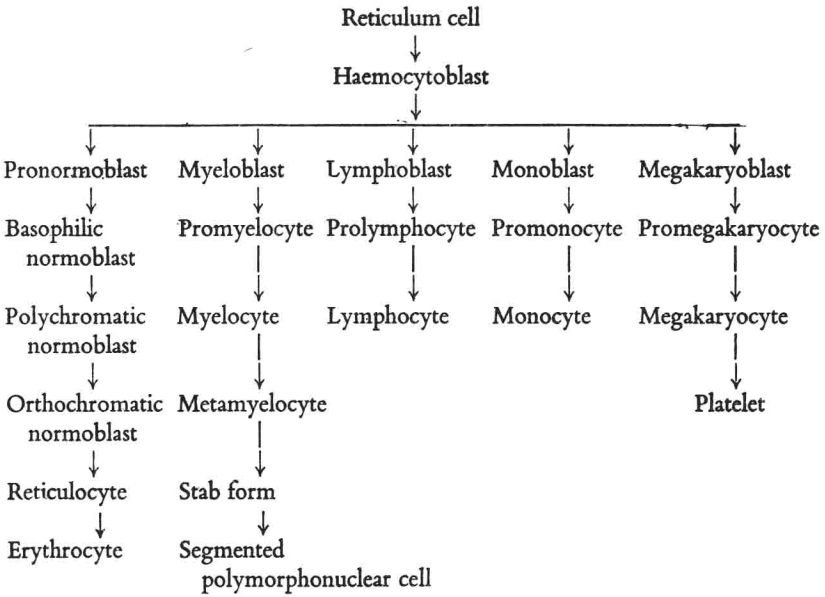


FIG. 1.1. Diagrammatic representation of blood cell development

The reticulum cell and haemocytoblast each constitute about 1 per cent of the cells seen in a Romanowsky stained bone marrow film. The *reticulum cell* varies in size from 20 to 30 μ. Its cytoplasm is abundant, palely basophilic, and may contain a few azurophil granules. As the cytoplasm is delicate it is usually of irregular outline and commonly it ruptures. The nucleus is large, round or oval, with a pale staining, finely reticular chromatin pattern; it contains one or more round or oval nucleoli. The haemocytoblast is a large cell of about 20 μ in diameter. The cytoplasm is less abundant than in the reticulum cell, moderately basophilic, non-granular, and often somewhat irregular in outline. The nucleus is round, averages about 15 μ in diameter, and stains palely to give a finely stranded and stippled appearance; it contains one or more nucleoli.

## Steps in Blood Formation

Formation and delivery to the blood stream of mature blood cells of all three series from the primitive haemopoietic reticulum cell of the marrow involve three processes:

1. Multiplication of the developing cells.
2. Maturation.
3. Release of mature cells from the marrow into the blood stream.

*Multiplication* of cells takes place by mitotic division. In a normal marrow film mitosis can be seen in about 1 per cent of the marrow cells, while in hyperplastic marrows the proportion of mitoses may increase up to 5 per cent. In the normal marrow, mitoses are more numerous in normoblasts than in developing granulocytes, suggesting that normoblasts divide more frequently.

*Maturation* may be defined as the progressive development of cell characteristics, both structural and functional. The changes which occur with each series of cells are described in detail below.

*Release into the blood stream.* The developing blood cells lie free outside the blood vessels of the marrow. The exact mechanism by which the mature cells are expelled into the circulation is not clearly understood. The vessels of the marrow form a closed system with no openings communicating with the extravascular spaces as is the case in the spleen. Hence the newly formed cells have to cross the vessel walls before being expelled into the circulation. In the case of granulocytes, which are motile cells, the passage into the blood vessels is easy to understand, but the red cells must cross the endothelial lining of the vessels either by diapedesis or more possibly by temporary rupture of the wall when the pressure of the accumulated cells in the extravascular spaces becomes sufficiently great. The factors controlling the release are not fully understood. The spleen probably plays some role; this is suggested by the fact that immediately following splenectomy there is a sharp rise in leucocytes and platelets in the peripheral blood (p. 508).

## ERYTHROPOIESIS

Two terms are used to describe the developing nucleated red cells, namely erythroblast and normoblast; however, authors vary in their definition of these terms. In this book the term erythroblast is used as a generic term to describe all nucleated red cells, and the term normoblast to describe erythroblasts showing the features of normoblastic (normal) erythropoiesis. In normal marrow the pronormoblast is the first cell recognizable as definitely belonging to the erythroid series; from it the red cell develops through a succession of maturing erythroblasts, namely, basophilic, polychromatic and orthochromatic normoblasts. Developing red cells are also sometimes described as early, intermediate and late normoblasts; these cells roughly correspond to basophilic, polychromatic and orthochromatic normoblasts

respectively, but are classified on nuclear rather than cytoplasmic characteristics.

The process of normoblastic maturation is characterized by the following progressive changes:

(*a*) diminution in cell size.

(*b*) ripening of the cytoplasm. In Romanowsky-stained preparations, this is accompanied by a change in colour from deep blue to pink, due to the progressive formation of acidophil staining haemoglobin and the simultaneous lessening of the ribose nucleic acid, which is responsible for the basophilia of the cytoplasm.

(*c*) ripening of the nucleus. This is manifest by loss of nucleoli, decrease in total size and size relative to the cytoplasm, progressive clumping and condensation of the chromatin and deepening in colour. Thus the large reddish purple open network nucleus of the pronormoblast is converted to the small deeply staining blue-black structureless nucleus of the orthochromatic normoblast.

The time for maturation from the pronormoblast to the mature red cell is estimated as about 7 days.

The characteristics of the developing normoblasts, as seen in Romanowsky stained films, are described below; however, it must be realized that the process of maturation is continuous and progressive and that transitional forms between the various types can be seen. In bone marrow films and sections, normoblasts are usually found in groups.

Mitotic division occurs up to the stage of the polychromatic normoblast, and mitosis is most active at this stage. The orthochromatic normoblast is not considered to be capable of mitotic division.

The *pronormoblast* is a round cell with a diameter ranging from 12 to 20 μ. It has a relatively large nucleus which occupies most of the cell, surrounded by a small amount of cytoplasm. The cytoplasm is of clear deep blue colour, often staining slightly unevenly, and showing a pale perinuclear halo; the blue colour is somewhat deeper than that of the blast cells of the white cell series, e.g. the myeloblast. Small rounded or pointed processes are commonly present at the periphery of the cell. The nucleus is round, and consists of a network of fairly uniformly distributed chromatin strands, giving a finely reticular appearance; it is reddish purple in colour and contains several nucleoli.

The *basophilic normoblast* varies in diameter from 10 to 16 μ, and still has a relatively large nucleus. The cytoplasm in general is similar to that of the pronormoblast but may be even more basophilic, and is usually regular in outline. The chromatin strands of the nucleus are thicker and more deeply staining, giving a coarser appearance, the nucleoli have disappeared.

The *polychromatic normoblast* varies in diameter from 8 to 14 μ, the nucleus

occupying a relatively smaller part of the cell. The cytoplasm is beginning to acquire haemoglobin and thus is no longer a purely blue colour, but takes on an acidophilic tint, which becomes progressively more marked as the cell matures. The chromatin of the nucleus is arranged in coarse, deeply staining clumps.

The *orthochromatic normoblast* varies from 8 to 10 μ in diameter. Typically the cytoplasm is described as acidophilic. However, in well-stained films the cytoplasm still shows a faint polychromatic tint, and for this reason some authors prefer the term *pyknotic normoblast*. The nucleus is small and initially may still have a structure with very coarse clumped chromatin, but ultimately it becomes pyknotic, and appears as a deeply staining, blue-black, homogeneous structureless mass. The nucleus is often eccentric and is sometimes lobulated. The nucleus is then lost; the mechanism is not certain but it is probably extruded.

The *reticulocyte* is a flat, disc-shaped, non-nucleated cell, of slightly larger volume and diameter than the mature erythrocyte. In Romanowsky stained films it shows a diffuse pale basophilia, while with a supravital stain such as brilliant cresyl blue the basophil material appears in the form of a reticulum. The haemoglobin content is approximately the same as that of the mature cell, but because of its larger size the haemoglobin concentration is slightly lower. The reticulocyte loses its basophil material and becomes a mature red cell; the average life span of the reticulocyte is probably about 1 to 2 days.

The *erythrocyte* is described in Chapter II.

## LEUCOPOIESIS

### The Granulocytic (Myeloid) Series

The mature granular leucocyte (granulocyte) is a cell with a polymorphous nucleus, and a cytoplasm containing granules which with Romanowsky stains appear either neutrophilic, eosinophilic or basophilic. Because of their polymorphous nucleus these cells are often referred to as polymorphonuclear or polymorph leucocytes. The first recognizable cell of the granulocytic series is the myeloblast, from which the mature granulocytes develop through a series of cells, namely the promyelocyte, myelocyte, metamyelocyte and stab form. At the myelocyte stage the cell develops the specific granules which determine the nature of the mature cell, that is whether it is a neutrophil, eosinophil, or basophil.

#### THE POLYMORPHONUCLEAR NEUTROPHIL

Maturation of the neutrophilic granulocyte is characterized by:

(*a*) the development of specific granules in the cytoplasm.

(*b*) loss of basophilia of the cytoplasm.

(*c*) progressive ripening of the nucleus which ultimately becomes segmented.

(*d*) the development of motility and ability to act as a phagocyte.

Mitotic division occurs up to the stage of the myelocyte, in which it is most active. The metamyelocyte is not considered to be capable of mitotic division.

The *myeloblast* varies in diameter from 15 to 20 μ. It has a large round or oval nucleus which occupies about four-fifths of the cell. The cytoplasm is non-granular, moderately deep blue, may stain somewhat unevenly being lighter next to the nucleus than at the periphery and may show pointed or rounded tags at the margin. The nucleus is round or oval, and stains reddish purple with the chromatin arranged in fine strands or granules giving a fairly evenly staining reticular appearance. Nucleoli are present, varying from 1 to 5 or 6 (usually 2 or 3); they are of medium size and are sharply defined, being surrounded by a well-marked border of chromatin. The cell is peroxidase negative.

The *promyelocyte* in general resembles the myeloblast except for the fact that the cytoplasm contains a number of granules which stain purplish red—azurophil granules, and may be slightly more abundant than in the myeloblast. The granules of the promyelocyte and all cells later in the series give a positive reaction with the peroxidase stain. The nucleus still contains nucleoli but they are less well defined than in the myeloblast and the chromatin strands are somewhat coarser and less evenly stained.

The *myelocyte* differs from the promyelocyte in two major respects: (1) the cytoplasmic granules have assumed their specific neutrophilic character, and (2) the nucleus has no nucleoli. Early myelocytes are commonly larger than myeloblasts and may be up to 25 μ in diameter. The amount of cytoplasm relative to the nucleus is increased; in the earlier stages it stains light bluish, but it progressively acquires a pinkish tint and the mature myelocyte has a predominantly or completely pale pink cytoplasm. The nucleus is round or oval, and the chromatin strands are thicker and stain more deeply than in the promyelocyte.

The *metamyelocyte* has a smaller, slightly indented nucleus with coarser chromatin. The cytoplasm is pink and contains fine purplish (neutrophil) granules.

The *band or stab form* is slightly smaller than the metamyelocyte. It has a deeply indented U-shaped nucleus, which may be twisted and rather irregular in contour, composed of fairly coarsely clumped chromatin. The cytoplasm is pink and contains fine evenly distributed granules which stain purplish.

The *segmented neutrophil* is commonly about 12 to 14 μ in diameter. The nucleus is lobulated, having two to five lobes which are connected by thin strands of chromatin; the lobes vary in shape and size and may overlap so that the joining strands are not visible. The chromatin is arranged in large, deeply staining purple clumps. The cytoplasm is pink and contains numerous fine evenly distributed granules which stain purplish. A morphological difference between the neutrophil leucocytes of males and females has been demonstrated recently. Some neutrophils of the female have nuclear appendages shaped like a drum stick, which do not occur in neutrophils of the male. The drum stick has a well-defined head and is attached to one lobe of the nucleus by a thin strand of chromatin. In a film of normal female blood it is usually possible to identify at least six typical drum sticks in 500 neutrophils.