R. B. Thompson

DISORDERS OF THE BLOOD

A Textbook of Clinical Haematology

Foreword by C. J. C. Britton

CHURCHILL LIVINGSTONE

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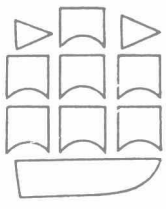

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Foreword

I am pleased to be asked to write a short foreword to this new *Disorders of the Blood*, a book, the revision of which has been for so long both a pleasure and a burden to me. I have read a considerable number of the chapters and, although the physiological, pathological and clinical intentions of the old book have been retained, this completely new book shows a wisdom and balance that will be appreciated by all haematologists.

It has always been my opinion that any modern scientific text should be completely rewritten by a new author after a few editions, so that a fresh outlook on the subject would be obtained. The first edition of *Disorders of the Blood* was published over 40 years ago so the onerous task of completely rewriting was long overdue. Dr Thompson is to be congratulated on having carried out this work with care and control and great success.

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C.J.C. BRITTON

Preface

This book is published by Churchill Livingstone as a successor to Whitby and Britton's *Disorders of the Blood*, the tenth edition of which appeared in 1969. As is indicated by the subtitle the book has been designed for use by haematologists with a predominantly clinical interest and by general physicians. With the present remarkable advances in factual knowledge no textbook can now be more than an introduction to its subject but each chapter contains an account of essential physiological, biochemical and pathological aspects. Three main sections deal with the red cells, the white cells and the platelets and the whole book is divided into forty-seven chapters each covering one specific subject. It is hoped that the value of the older work as a reference text has been continued, for as far as possible the source of every major statement is given and most of the numerous titled references cover the ten year period up to the middle of 1974. Technical methods have been excluded because the several admirable texts which are available are more suitable for use on the laboratory bench. Because of the expense the only colour illustration is the frontispiece but reference is made throughout the text to the relevant figures in the excellent *Atlas of Haematology* by G.A. MacDonald, T.C. Dodds and Bruce Cruickshank which is also published by Churchill Livingstone.

Acknowledgements

I particularly wish to acknowledge the kindness of Miss J.S. Emmerson, M.A., of the University Library, Newcastle upon Tyne, for allowing me the use of a carrel during the three years this book was in preparation. Thanks are also due to colleagues who have read much of the typescript. I am especially indebted to Dr Anne Collins, Dr Gordon Dale, Dr Mary Ellis, Dr Richard Evans, Dr Alan Horler, Dr Peter Jones and Dr William Walker. For his careful selection of material and for the preparation of photomicrographs illustrating the malignant lymphomas and the bone marrow histology I am greatly indebted to Dr Ian Lauder of the Department of Pathology, Royal Victoria Infirmary, Newcastle upon Tyne. The illustrations of the Philadelphia chromosome and of the 9q + chromosome have been reproduced here by courtesy of Dr Sylvia Lawler, Department of Cytogenetics and Immunology, The Institute of Cancer Research and The Royal Marsden Hospital, London. For the list of haemoglobinopathies included as an appendix to Chapter 5 I am greatly indebted to Professor Herman Lehmann of Cambridge. Many of the charts were drawn by Mr Harry Newman and for the preparation of the illustrations I am indebted to Miss Barbara James and Miss Dorothy Mustart of the Graphics Section, Department of Photography, University of Newcastle upon Tyne. Most of the radiographs are reproduced by courtesy of Dr C.K. Warrick, Department of Radiology, Royal Victoria Infirmary, Newcastle upon Tyne. Some illustrations have been reproduced from Whitby and Britton's *Disorders of the Blood* 10th Edition, Churchill Livingstone, and these are acknowledged in the legends as have the sources of a few other figures.

For the typing of the manuscript I am indebted to Miss Joan Ashton, Mrs Elizabeth Chisholm, Miss Maureen Edwards, Mrs Catherine Laidlow, Mrs D.E. Moore, Mrs Margaret Shepherd, Mrs Joan Templey, Mrs Hilary Turnbull and Miss Frances Upton.

This book is dedicated to Mr Harry Newman of the Clinical Laboratory, Royal Victoria Infirmary in appreciation of his meticulous standards and of 40 years of kindly interest and devotion to patients under the care of the late Dr C.C. Ungley and, more recently, of the author.

Contents

1. Haematopoiesis

This preliminary chapter begins with a description of blood formation in the fetus, its genesis in the yolk sac, its later occurrence in the liver, and its final colonization of the bone marrow. There follows a general description of the bone marrow in childhood and adult life, and its cellular content. Finally a brief survey is given of the concept of the stem cell. The three main activities of the marrow are the formation of erythrocytes (erythropoiesis), of granulocytes (granulopoiesis), and of platelets or thrombocytes (thrombopoiesis). Each of these have such distinctive features, specific control mechanisms and kinetics that they are separately discussed in later chapters as are the formation of lymphocytes and plasma cells.

Haematopoiesis in the embryo

This subject has been intensively studied for about one hundred years so that there is a considerable volume of literature. The earlier work was ably reviewed by Bloom in 1938 who commented on the number of contradictory statements and theories, the unwieldy terminology, and the unsatisfactory methods used for studying both cells and tissues. At that time, however, the essential facts were established and it was realized that the blood cells developed in fundamentally the same manner in all mammals. It was also realized that there were some differences between species in the relative importance of the various haematopoietic organs. These, as Bloom pointed out, are not of fundamental importance but they must be remembered when studies of the development of stem cell colonies are being compared; there are, for instance, important species differences in the haematopoietic activity of the spleen. Ingram (1971) has reiterated the close and obvious relationships between embryonic erythropoiesis in the chick, man, mouse and tadpole. The chick embryo has been most frequently used for *in vivo* studies and there are recent papers by Lemez (1964); Wilt (1967); Bank *et al*, (1970); Campbell *et al.* (1971) and by Weintraub *et al.* (1971). Much work has also been done utilizing short term cultures of the blastoderm by such investigators as Murray (1932); Settle (1954); O'Brien (1960); Hell (1964); Wilt (1967); Miura and Wilt (1969; 1970; 1971); Hagopian and Ingram (1971) and Reynolds and Ingram (1971). Embryonic haematopoiesis has been reviewed by Metcalf and Moore (1971).

Erythroid precursor cells first appear in the mesenchyme (Fig. 1.1) as was well known to earlier workers (Bloom, 1938). They are recognizable within 24 hours of incubation and it is presumed that they arise by a process of induction by endodermal cells (Miura and Wilt, 1969). Clusters of these early cells can be identified by both light and electron microscopy (Trelstad *et al.*, 1967; Rifkind *et al.*, (1969). Haemoglobin is already identifiable at 36 hours and by 48 to 60 hours a circulatory system is forming. A series of embryonic haemoglobins have been described in man; of these the latest type to appear is haemoglobin F (fetal haemoglobin) and this persists for the first 2 to 3 months of life though steadily declining as it is replaced by adult haemoglobin. The earliest embryonic haemoglobins are produced by a 'primitive' red cell series which have ceased dividing by the 5th day and only function briefly so that but few persist after the 13th day. This primitive system is replaced after the seventh day by a permanent erythroid series carrying adult types of haemoglobin (Wilt, 1967; Campbell *et al.*, 1971; Weintraub *et al.*, 1971). The nature of the cells giving rise to this later definitive red cell series is quite unknown. In the chick embryo it is separate from the circulation, occurs first in the yolk sac tissue and later in the bone marrow as this develops (Settle, 1954; Wilt, 1967; Hagopian and Ingram, 1971). In cell culture it is possible to demonstrate seven doublings of the cells and successive morphological changes as they mature from proerythroblasts to erythrocytes (Hagopian *et al.*, 1972).

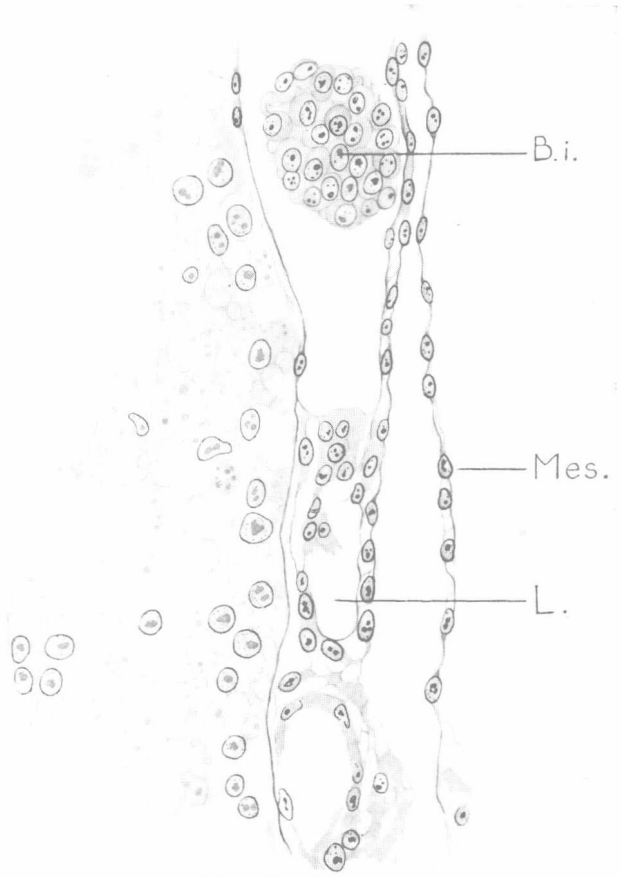

Fig. 1.1 Formation of blood cells from endothelium in the embryo chick. B.i. =blood islet. Mes. =mesoderm. L. =lumen of vessel (after Sabin).

In the human embryo haematopoiesis is also first evident in the yolk sac where numerous blood islands develop (Bloom and Bartelmez, 1940). This site is active for some 2 months though it rapidly declines in importance and by the 3rd month the liver is the predominant haematopoietic organ though some haematopoiesis is stated to occur in the spleen from the 3rd to the 6th months. In an 11 week fetus

examined by Rosenberg (1969) there was pronounced active erythropoiesis in the hepatic sinusoids but in spite of this nucleated red cells were rarely seen in the peripheral circulation. There was, however, sequestration of erythroblasts in the spleen though it showed no evidence of haematopoiesis. The bone marrow showed sparse myelopoiesis but no erythropoiesis. The predominance of erythropoiesis in the fetal liver was also noted by Thomas and Yoffey (1964) who considered that the erythroblast precursors arose in the hepatic trabeculae and differentiated from entodermal trabecular cells. The erythroid cells are morphologically very similar to those found in normal adult bone marrow (Ackerman *et al.*, 1961; Grasso *et al.*, 1962; Zamboni, 1965). Several observers have described erythroblastic islands of cells at all stages of maturation surrounding a central reticuloendothelial cell as illustrated by Rosenberg (1969) and as occurs in normal bone marrow (Bessis, 1972). Bone marrow activity usually commences about the 4th or 5th month when islands of mesenchymal cells which remain after the resorption of cartilage begin to differentiate, not only into erythroid cells as in the liver, but into haematopoietic cells of all types. The marrow activity becomes steadily more important until birth at which time hepatic erythropoiesis has virtually ceased.

Haematopoiesis in the adult—the bone marrow

Haematopoiesis begins to occur in the bone marrow at about the 16th to the 20th weeks of fetal life and by about the thirtieth week the marrow has achieved its full cellularity (Kalpaktsoglou, 1960). As the marrow haematopoietic activity increases that of the liver declines and ceases altogether at the time of birth (Langley, 1951; Morison, 1963). At birth there is a fall in the proportion of red cell precursors which persists for 4 to 6 weeks after which there is a steady but slow increase, the birth values not being regained until the end of the twelfth week. The marrow cellularity of children born at over 32 weeks' gestation varies after birth in the same way as does that of full-term infants. The changes in erythropoietic activity are no doubt largely due to changes in tissue oxygenation and in erythropoietin secretion. The cellularity of the haematopoietic marrow at birth in normal and in premature infants has been studied by Gairdner *et al.* (1952a, b) and by Kalpaksoglou and Emery (1965).

It has often been assumed that the bones are completely filled by red marrow at birth but the studies of Emery and Follett (1964) showed that the red marrow in the terminal digits may already have been replaced by inactive fatty marrow before haematopoiesis has ceased to occur in the liver; the marrow in the toes is almost completely fatty by the end of the first year of life. Little further recession of red marrow occurs during childhood but about the age of 7 years the marrow in the long bones becomes less active and by the age of 10 to 14 years areas of fatty marrow at the distal ends of the long bones have begun to extend proximally. At about the age of 18 years the normal adult distribution of marrow is established (Fig. 1.2). In the adult red marrow occurs only in the skull, axial skeleton, pelvic and shoulder girdle, sternum and ribs with a small extension into the proximal ends of the humeri and femora (Custer and Ahnfeldt, 1932; Custer, 1933; 1949; Jaffe, 1936). Later in life the proportion of fatty marrow increases so that in old age it occupies about half of the ribs and sternum.

Although the marrow is distributed throughout the skeleton it should be regarded as an organ. Estimates of its size are difficult and most of the figures quoted are derived from a paper by Mechanik (1926) but according to Hudson

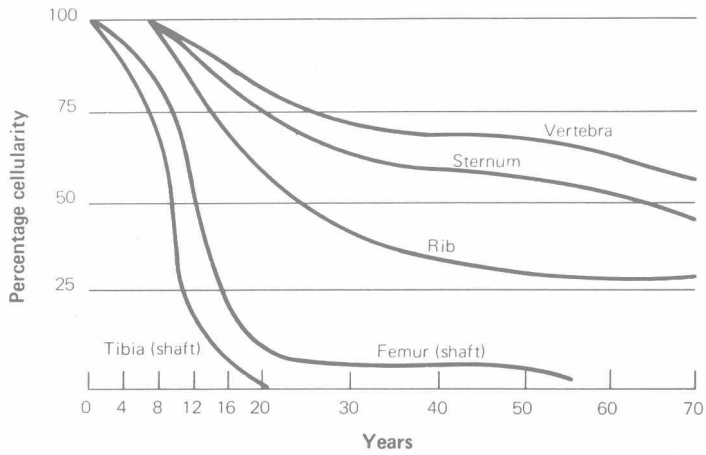

Fig. 1.2 Illustrating the distribution of active red marrow from birth until the age of 70 years. (Redrawn from Whitby and Britton, *Disorders of the Blood*, and derived from the data of Custer and Ahlfeldt, 1932).

(1958) Mechanik's material was not normal and the average body weight of his subjects was only 55 kg. The methods used by Mechanik were, however, found to give comparable results to those used by Hudson (1958; 1965) and the original values were utilized by Woodard and Holodny (1960) and by Ellis (1961) in their studies. The distribution of the active red marrow by weight in the individual bones of a normal 40 year old man of 70 kg are given by Ellis (1961) who estimated the total to be some 1460 g so that the mass of the active marrow organ equals that of the liver. The distribution of the marrow spaces in the individual bones as a percentage of the total is given in some detail by Woodard and Holodney (1960). Their figures and those of Ellis are given by Miale (1972, p. 330). In the adult the total (red and yellow) marrow accounts for some 4.6 per cent of the body weight but in the fetus the figure is only about 1.4 per cent which, as he points out, would be expected on considering the structure of fetal bones. The skull of the fetus and newborn child contains proportionately much more marrow than does that of the adult and this is of some interest in view of the striking changes which occur in the skull bones in certain chronic congenital haemolytic syndromes (Figs. 6.1a, b).

The blood supply to the marrow is by the nutrient arteries to the bone. After entering the marrow cavity these arteries, which are muscular but rather thin-walled, branch widely into a fine network of capillaries which enter sinuses or sinusoids which run radially from the periphery towards a central lobular vein (Weiss, 1961; 1965). The sinus walls have a trilaminar structure consisting of a lining cell (termed endothelial by most authorities) a basement membrane, and an adventitial cell. At some vascular junctions a curious arrangement of endothelial cells has suggested the possibility of a 'throttle' mechanism (Burkhardt, 1971, pp. 28–29). Between the sinuses lie cords of haematopoietic cells, the blood cells, it is now agreed, being formed extravascularly. Mature cells enter the lumen of the sinuses through apertures in their walls (Pease, 1956; Weiss, 1961; 1965; 1970; Zamboni and Pease, 1961; De Bruyn *et al.*, 1966). What factors determine the passage of cells when, and only when, they reach maturity are unknown but there are interesting studies of the physicochemical changes occurring on the surface of maturing granulocytes which certainly appear relevant (p. 450). That abnormalities of the bone marrow microcirculation may be responsible for some aplastic states

has been suggested by Knospe and Crosby (1971) but there has been no confirmation of this. Large numbers of reticulo-endothelial cells occur in the marrow in relation to the sinuses and islands of erythropoiesis have been described in which a central reticuloendothelial cell is surrounded by erythroblasts and normoblasts (Bessis and Breton-Gorius, 1962; Bessis, 1972). The role of these cells is controversial, the last-named authors believe that they supply materials such as iron to the erythroblasts and Trubowitz and Masek (1968) have also attributed to them a transport role. Other evidence is against this, certainly in respect to iron metabolism as is discussed on page 146. The study of Trubowitz and Masek (1968) also demonstrated an intense alkaline adenosine tri-di- and mono-phosphatase activity in the capillary endothelial cells as well as about the individual marrow cells.

Even in red active marrow there occur variable numbers of fat cells and there is also an intercellular substance containing mucopolysaccharide (Carter *et al.*, 1961; Carter and Jackson, 1962). The usual techniques for the examination of marrow give but little idea of its three dimensional structure but such information may be obtainable with the scanning electron microscope. Preliminary studies have permitted adipose cells, marrow cells and blood vessels to be distinguished but specific cell types could not be identified (Trubowitz *et al.*, 1970).

It would be of clinical importance to determine the antatomical extent of marrow activity during life and although this has been done the methods have serious disadvantages and require the use of isotopes. Kramer and Wynne (1958) used ^{59}Fe while ^{198}Au was employed by Engstedt *et al.* (1958) and by Edwards *et al.* (1964); this latter material remains almost indefinitely in reticulo-endothelial cells and large amounts are taken up by the liver and spleen. Scanning methods for the detection of neoplastic bone deposits and bone infarction in sickle-cell anaemia have been much used and have proved very helpful (see page 77). Russell *et al.* (1966) have studied the distribution of active bone marrow throughout the skeleton and have compiled a series of tables giving the amounts at a large number of locations.

Bone marrow kinetics

Because erythropoiesis, granulopoiesis and thrombopoiesis proceed more or less independently and each is controlled by distinct specific factors it is not very useful to discuss bone marrow kinetics as a whole. One method of study which was discussed by Killman *et al.* (1963) is by the estimation of the time parameters of proliferation by measuring the mitotic indices of bone marrow cells. Mauer (1965) was able to show a general diurnal variation in the proliferative activity of human bone marrow. The number of mitotic figures was least at 6.00 a.m. and increased during the day to a maximum at 6.00 p.m. or midnight. The time of the least number of mitotic figures followed the period during which the smallest percentage of cells incorporated labelled thymidine. No consistent variation in synthesis could be found in erythroid precursors but the percentage of myeloid cells incorporating the label was lowest at midnight. A similar pattern of increasing mitotic indices in the marrow was noted in one case by Killman *et al.* (1962) and a similar relationship has been reported in mice in the diurnal variation of mitosis and the incorporation of tritiated thymidine into epidermal cells; this and several other instances of diurnal mitotic rhythms are quoted by Mauer (1965).

When there are increased demands for erythrocytes or granulocytes the volume of red marrow expands at the expense of the yellow. In a case of haemolytic anaemia red marrow may expand well down the shafts of the long bones and those fat spaces normally present in red marrow will be encroached upon. Such changes were well seen in patients dying of pernicious anaemia in relapse and in chronic haemolytic states such as thalassaemia and sickle-cell disease there may be a remarkable expansion of the marrow cavities (p. 107). In these conditions, and very rarely in congenital spherocytosis, there may occur islands of erythropoietic activity outside the normal adult sites (p. 107 and p. 316). Such extramedullary haematopoiesis is also a feature of myeloid metaplasia (p. 722).

The assessment of marrow activity

This cannot be usefully discussed in general terms. Clearly if all the formed elements of the peripheral blood are normal and are present in normal numbers it is reasonable to assume that the marrow activity is normal. Even so the results of other investigations and of bone marrow aspiration may show the presence of some pathological process unsuspected from the blood count. If one or all the cellular elements of the blood are depressed there may be either depressed or enhanced marrow activity. Again, the presence of a hypercellular apparently hyperactive marrow may be associated with findings suggestive of aplastic anaemia. Some idea of the degree of erythropoietic activity may be gained from the reticulocyte count but as discussed on page 11 this too may be misleading. A reasonable assessment of clinical value can usually be made if a complete peripheral blood picture and an adequate marrow biopsy report is available but an accurate and detailed knowledge of marrow activity can only be obtained by specialized investigations and kinetic studies which are outlined in the appropriate chapters.

An examination of the bone marrow is an essential investigation in many disorders of the blood, nevertheless many common conditions can be correctly diagnosed and treated without it, the procedure is not without discomfort and accidents, very occasionally fatal, have occurred. As with any investigation the reasons for its performance must be clear in the physician's mind, a marrow biopsy is not an infallible way of reaching a diagnosis but should be done as one of a logically planned series of investigations much as a move in chess is designed eventually to reach mate. The methods of biopsy are not discussed here for they are well described in books dealing with technical methods (Wintrobe, 1967, pp. 29–36; Dacie and Lewis, 1968, pp. 114–134; Miale, 1972, pp. 318–328). For most purposes aspiration biopsy taken from either the sternum or the iliac crest will provide very satisfactory material. If cellular detail is required smears should be stained by the usual Romanowsky methods although special techniques will be required for the demonstration, for instance, of siderotic granules, or for the determination of cell types in acute leukaemia. The cellular content may be better assessed by the concentration of aspirated material and the preparation of sections and the presence of tumour cells is often more readily detected in this way. If the actual marrow cellularity and architecture is of importance, as in aplastic anaemia or in myelofibrosis, either needle, drill, or open biopsy is necessary: a marrow cannot be pronounced acellular simply because of a 'dry tap' or the aspiration of poorly cellular material. Smears of material obtained post mortem are often so severely autolyzed that they are of little value. When obtained 3 hours after death reasonable smears may be obtained but even then the cells may be severely autolyzed (Miale, 1972, p. 328). Preparations may, however, be obtained as long as 23 hours post mortem if bovine albumin is added to the aspirate before smears are made (Rickert and Vidone, 1968).

The normal marrow

The appearance of a section of normal marrow is shown in Figure 1.3. This shows a spicule of normal lamellar bone with a few osteoblasts and osteoclasts. A small blood vessel is also visible. There is some variation in the proportion of fat cells to marrow cells but the figure is representative of that normally found (McDonald *et al.*, 1970, pp. 49–50; Burkhardt, 1971, pp. 46–47). The normal ranges for differential counts on aspirated bone marrow are presented in Table 1.1 which is taken from Dacie and Lewis (1968, p. 130) and is compiled from data obtained from Hynes (1939); Plum (1941); Osgood and Seaman (1944); Israels (1948); Fadem and Yalow (1951) and Leitner *et al*, (1949).

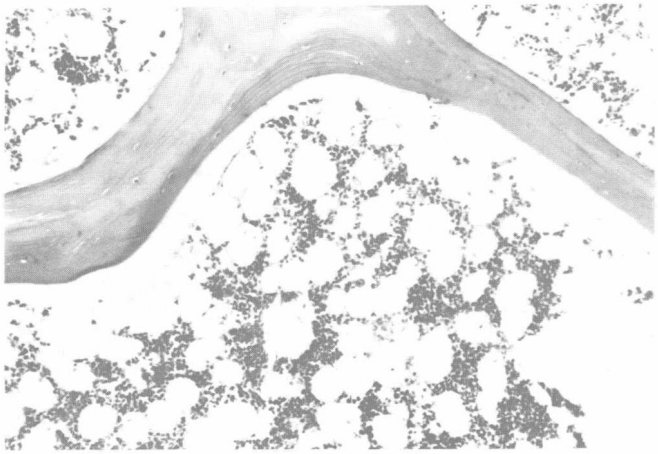

Fig. 1.3 Section of normal bone marrow. Bony trabeculae support the marrow-containing compartments which consist of islands of haematopoietic cells separated by fat. When haematopoiesis is enhanced the proportion of haematopoietic tissue increases at the expense of the fat. At first this is limited to the sites normally containing red active marrow but later there may be an extension into the shafts of the long bones which, in the adult, contain fat only. (Decalcified and stained with H & E. ×96. Courtesy of Dr. Ian Lauder).

Table 1.1 Normal ranges for differential counts on aspirated bone-marrow

	%
Reticulum cells	0.1–2
Haemocytoblasts	0.1–1
Myeloblasts	0.1–3.5
Promyelocytes	0.5–5
Myelocytes	
neutrophil	5–20
eosinophil	0.1–3
basophil	0–0.5
Metamyelocytes	
young forms, stab forms	10–30
Polymorphonuclears	
neutrophil	7–25
eosinophil	0.2–3
basophil	0–0.5
Lymphocytes	5–20
Monocytes	0–0.2
Megakaryocytes	0.1–0.5
Plasma cells	0.1–3.5
Pronormoblasts	0.5–5
Normoblasts	
polychromatic	2–20
orthochromatic	2–10
Myeloid: erythroid ratio	2.5–15:1

Stem cells

With the exception of most lymphocytes the formed elements of the blood—erythrocytes, granulocytes and platelets—have a finite life-span, that of granulocytes being measured in days and that of erythrocytes being about four months. Remarkably constant numbers and proportions of these cells are maintained in the circulating blood and when there is an increased demand, as for erythrocytes after haemorrhage or for neutrophil granulocytes during a bacterial infection the appropriate cellular increase promptly occurs. There clearly must be sensitive mechanisms to maintain the normal state and to evoke any necessary response. In addition there must be a supply of cells capable of rapid division which can replace those lost from the circulation. In the case of granulocytes a considerable marrow pool of mature cells exists which can meet immediate demands but there is no such pool of erythrocytes and increased requirements are met by the increased activity of nucleated precursors capable of division, a process which takes several days. The immediate progenitors of erythrocytes, granulocytes and platelets are the erythroblasts and normoblasts, myeloblasts and myelocytes, and the megakaryocytes which are readily recognized morphologically. Maintaining the populations of these cells however, are compartments of more primitive cells about the morphological identity of which there is no certainty. The first of these compartments consists of pluripotential stem cells, these give rise to cells which are committed to produce cells of one or other cell line; they include the erythropoietin-sensitive cells which form the erythroid series. This present concept reconciles the earlier arguments between those holding the monophyletic and the polyphyletic theories of haematopoiesis: arguments pursued with almost theological fervour. The monophyletic theory of Maximow (1909) held that all types of differentiated cell could arise from one pluripotential stem cell variously named the 'haemocytoblast' or the 'lymphoid wandering cell'. The polyphyletic school held that there were two, three, or more separate and distinct types of stem cell each capable of giving rise to one cell line only. Unfortunately there is still no general agreement about the nature of these stem cells and different groups of workers often use the same names with different shades of meaning. A stem cell is, however, generally taken to mean a primitive haematopoietic cell capable of extensive self-replication and endowed with a multiple differentiating capacity (Metcalf and Moore, 1971). These authors define the next cell compartment as consisting of cells with little or no self-replicating capacity but sensitive to specific regulatory factors and capable of differentiating into one cell line only. These are named 'precursor cells' by Ebbe and Stohlman (1965) by Morse and Stohlman (1966) and by Bennett and Cudcowicz (1968) or 'early differentiated cells' (McCulloch, 1968) while the name 'progenitor cells' was used by Moore and Metcalf (1970). These cells in turn give rise to the morphologically identifiable cells which populate the bone marrow (Figure 2.1 and *see* McDonald *et al.*, 1970 maturation chart in end board).

Since the pluripotential haematopoietic stem cells are not morphologically identifiable their existence, activities and numbers can only be assessed indirectly by observing their progeny. Knowledge of their nature and capabilities has largely come from observations of the repopulation of haematopoietic tissues rendered aplastic by radiation or by

various cytotoxic drugs. The earliest experiments of this type were those of Woenkhaus (1930). He gave a usually lethal dose of radiation to one of a pair of parabiotic rats and found that both survived; this result, it is now appreciated, was due to a repopulation of the irradiated animal by pluripotential stem cells from the other. In later experiments an animal was exposed to sublethal radiation with the exception of, say, the spleen (Jacobson *et al.*, 1949) or part of the bone marrow (Kaplan and Brown, 1952) and the repopulation of the irradiated area was studied. Jacobson *et al.* (1949; 1952) concluded that there was a humoral 'protecting factor' in the shielded spleen but cellular transfer is the mechanism now known to be involved. In further experiments cells from the bone marrow or from the peripheral blood were injected into irradiated animals. These procedures not only permitted the animal to survive but brought about the repopulation of the blood forming tissues and organs. Many workers have carried out such studies on the transfusion of large numbers of leucocytes (Merwin, 1959; Smith *et al*, 1960; Smith and Congdon, 1961; Goodman and Hodgson, 1962; Malinin *et al.*, 1965; Lord, 1967).

Very important experiments were reported by Till and McCulloch in 1961. They showed that mouse bone marrow contained cells which, when transfused into irradiated recipients, could colonize the spleen giving rise to nodules of haematopoietic tissue. Initially these colonies consisted of undifferentiated cells together with a few others belonging to a single cell line. Eventually erythroid, granulocytic and megakaryocytic cell lines emerged so that the original colony forming cells were pluripotent. There is good evidence that these colonies are clonal—that is that they are derived from a single stem cell or colony-forming unit (CFU). This clonal origin was demonstrated by Becker *et al.* (1963) in the case of spleen colonies derived from transfused bone marrow cells. They exposed mice to radiation, transfused them with normal marrow cells, and then gave two small doses of radiation to induce chromosomal abnormalities. The few colonies which did contain marker chromosomes consisted of 95 to 99 per cent of cells containing the chromosomal abnormality so that they must have been derived from one progenitor. Similar evidence has been produced by Wu *et al.* (1967) and by Fowler *et al.* (1969). Even though a clonal colony may apparently consist of only one cell type it may still contain pluripotential cells capable of acting as colony-forming units. For instance a pure erythroid colony, when transferred to another host, may give rise to secondary colonies containing various specific cell types (Lewis and Trobaugh, 1964; Curry *et al.*, 1967). An important factor governing the differentiation of such stem cells is the precise microenvironment in which they grow (Curry and Trentin, 1967). The mouse spleen is evidently more immediately favourable to erythroid maturation and the marrow to granulocytic (Wolf and Trentin, 1968). The conditions under which the transfused cells are harvested and grown were shown to be important by Grigorin *et al.* (1971) for when leucocytes from severely bled rabbits were introduced into diffusion chambers in the peritoneal cavity of the donor animals erythroid cells only were produced. The pretreatment of the host by cyclophosphamide makes the environment more favourable to colonization but the reason is unknown (Gregory *et al.*, 1971). Although the colony-forming cells are not themselves affected their differentiation into erythroid cells is influenced by erythropoietin and a suppression of erythropoietin inhibits erythroid differentiation (Bruce and McCulloch, 1964; Curry *et al.*, 1967).

It is possible to culture stem cells on semi-solid media, a method pioneered by Pluznik and Sachs (1965) and by Bradley and Metcalf (1966) and many recent studies have made use of it. There are, however, certain differences in the colonies obtained in this way. Only granulocytic colonies can be grown and kinetic studies would indicate that there are differences in the colony-forming units—indeed some prefer to use the term agar colony units (ACU) to distinguish them (Lajtha *et al.*, 1969; Lajtha, 1972; Testa and Lajtha, 1973). If a stimulating factor is present colonies may be grown on agar from cells obtained from the spleen, the bone marrow, and even the peripheral blood (Senn *et al.*, 1967; Pike and Robinson, 1970; Chervenick and Boggs, 1971). The number of normal peripheral blood cells capable of initiating such colonies is small but many more colonies appear when the cells are obtained from patients suffering from infectious mononucleosis and other common viral illnesses (Kurnick and Robinson, 1971). The number of colony forming (or agar colony) units is in direct proportion to the number of atypical mononuclear cells in the circulation of the donor patients. Various techniques have been used to concentrate the colony forming units and to determine their morphological characteristics (Cudkowicz *et al.*, 1964; Bennett and Cudkowicz, 1966; Turner *et al.*, 1967; Brunette *et al.*, 1968). Tyler and Everett (1966) studied the types of cell which crossed from the shielded to the non-shielded marrow in pairs of parabiotic animals. They concluded that a monocytoid cell was responsible for generating both erythroid and granulocytic cell lines. They did not think that the monocyte was the cell type involved—indeed all the evidence indicates that this cell transforms into the tissue macrophage and in culture it may slowly evolve into a fibroblast capable of laying down collagen (Petrakis *et al.*, 1961). There is, however, evidence that bone marrow cells of the monocyte-macrophage line are capable of elaborating a colony stimulating factor in liquid cultures (Golde and Cline, 1972; 1973; 1974; Golde *et al.*, 1972). The evidence would suggest that the stem cell is likely to be a variety of transformed lymphocyte resembling the cells evoked by a variety of antigenic and mitogenic stimuli and discussed on page 491. Studies by Dicke *et al.* (1973) suggest that the mouse haematopoietic stem cell and the primate bone marrow colony-forming cell may be identical. Confirmation of this would, however, require the careful examination of both types of cell by the same techniques. Many of the authorities quoted give general reviews of stem cell problems but valuable recent reviews are those by Metcalf and Moore (1971) by Schooley and Lin (1972) and those contained in Ciba Foundation Symposium 13 (1973).

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2. Erythropoiesis and its Regulation: the Red Cell

In this chapter the process of erythropoiesis is described from the earliest recognizable precursor cell to the mature erythrocyte. The various factors which influence erythropoiesis are also discussed and this is followed by a description of the red cell and of the normal values for the red cell count, haemoglobin, and packed red cell volume. The red cell indices are then discussed together with the morphological red cell abnormalities met with in disease. A brief discussion of blood volume is also given and the chapter ends with an account of the erythrocyte sedimentation rate.

Erythropoietic cells

Stem cells and precursor cells have been discussed in Chapter 1 and the following description is of the recognizable nucleated red cells which are present in normal bone marrow. The terminology used for the various stages of maturation is essentially that in general use and the sequence is illustrated in Figure 2.1. The appearance of the cells is well shown in Figures 1 and 2 of the Atlas of Haematology published by McDonald *et al.* (1970).

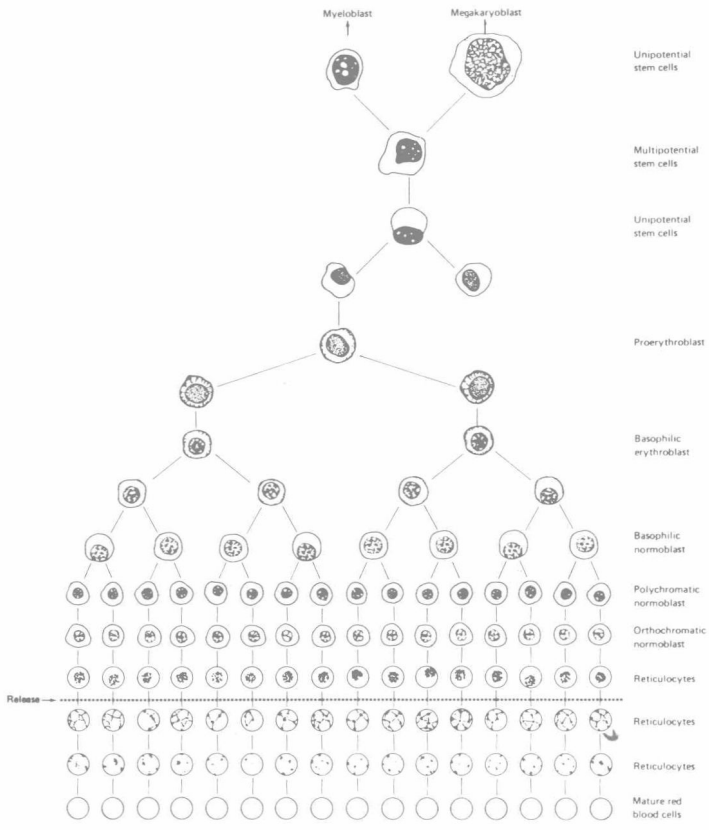

Fig. 2.1 Illustrating the origin of the three main cell lines from a multipotential stem cell and the processes of differentiation, proliferation and maturation of the red cell series (after Erslev and Silver, 1967).

Basophil proerythroblast (pronormoblast)

This cell is the most immature cell of the series. It is between 15 and 20 μm in diameter. The round or slightly oval nucleus occupies about 80 per cent of the cell. It is more or less centrally placed and has a uniformly fine reticular appearance. There are usually pale blue nucleoli present, the number varying from 1 to 5 but they are not clearly demarcated from the rest of the nucleus. The cytoplasm is densely basophilic and has a characteristic appearance, often staining rather unevenly and there is usually a paler perinuclear halo. With a little experience the much more deeply blue rather more abundant cytoplasm together with the appearance of the nuclei and nucleoli at once distinguish the proerythroblast from the myeloblast, a cell of much more feminine appearance.

Basophilic normoblast

This cell shows evidence of maturation in that the nucleoli have disappeared and the nuclear structure is much coarser. The size is a little smaller, 10 to 15 μm, and the cytoplasm occupies a greater volume of the cell because of nuclear shrinkage; by definition it is still markedly basophilic.

Intermediate (polychromatic) normoblast

It is at this stage that the presence of haemoglobin becomes obvious. It first appears near the nucleus and then spreads throughout the cytoplasm giving either a patchy or a uniform polychromatophilia due to a mixture of the earlier basophilia with the pink-staining acidophilic haemoglobin. The total diameter of the cell is a little smaller still, 8 to 12 μm, and the nucleus has shrunk even further and become more dense.

Late orthochromatic (pyknotic) normoblast

Here the haemoglobinization is complete or almost so. Strictly *ortho*chromatic normoblasts are rarely seen in normal marrow for the cytoplasm of many still stains a blue-grey colour resembling that of reticulocytes. Their diameter is 7 to 10 μm—little greater than that of a mature red cell and similar to that of many reticulocytes. The nucleus is small, dense, darkly-staining and almost structureless. It is usually eccentric and sometimes assumes an odd lobulated appearance before it is lost. Because of these changes many authorities prefer the name pyknotic normoblast for these cells.

Ultrastructure

As the nucleated red cell precursors mature there is a progressive clumping of the nuclear chromatin until at the orthochromatic stage it appears almost uniformly dense and structureless. The progressive disappearance of the nucleoli seen by light microscopy is confirmed. In the earlier cells the perinuclear clear zone is seen to contain the rather poorly-developed Golgi apparatus and a few mitochondria; both of these structures decrease even further as the cells mature. The centrosome is relatively small, contains two