Whitby and Britton

DISORDERS OF THE BLOOD

Diagnosis: Pathology: Treatment: Technique

NINTH EDITION

By

C. J. C. BRITTON

M.D. (New Zealand), D.P.H.

Physician and Hæmatologist to the Prince of Wales's Hospital, London: Pathologist to St. Andrew's Hospital, Dollis Hill, The Finchley Memorial Hospital and the Hendon District Hospital: Late Assistant Pathologist, Christchurch Hospital, New Zealand, and the Bland-Sutton Institute of Pathology, The Middlesex Hospital, London

With a chapter on

The Cytochemistry of Hæmopoiesis

By F. G. J. HAYHOE, M.A., M.D., M.R.C.P.

Lecturer in Medicine, University of Cambridge: Hon. Physician, United Cambridge Hospitals

and a chapter on

Blood Groups and Blood Transfusion

BY GEOFFREY H. TOVEY, M.D.

Director, South-Western Regional Blood Transfusion Service, Lecturer and Clinical Teacher in Hæmatology, University of Bristol

21 *Plates* (12 *in colour*) *and* 124 *Text-figures*

J. & A. CHURCHILL LTD.

104 Gloucester Place, London W.1.

1963

First Edition 1935
Second ,, ... 1937
Third ,, ... 1939
Fourth ,, ... 1942
,, ,, *Reprinted* . . 1944
Fifth ,, ... 1946
,, ,, *Reprinted* . . 1947
,, ,, *Spanish Translation* 1946
Sixth ,, ... 1950
Seventh ,, ... 1953
Eighth ,, ... 1957
Ninth ,, ... 1963

To
"D. of P."

Printed in Great Britain

DISORDERS OF THE BLOOD

PREFACE TO THE NINTH EDITION

THERE has been no diminution in the amount of research carried out in Hæmatology during the five years since the last edition, and this is indicated by the fact that more new references (over 1,500) have been included in this edition compared with any previous edition—and these constitute less than seven per cent of the papers examined. It is perhaps worth while to inform the reader of the methods used in choosing references. Key reference papers which have appeared since the last edition are of course included and many unnecessary references deleted. But references are made to many papers, particularly in the last few months before publication where they give an up-to-date bibliography, even if the merit of the papers themselves may be slight. In this way, the reader is able easily to look up the latest work on any subject.

The large amount of new information which has been added has necessitated considerable rewriting and deletion of much of the old text, and the pruning of some sections has been ruthless. By these means and by the use of a slightly larger page area together with the deletion, to my great regret, of the Index of Authors, the book has been increased by only some ten pages.

The most significant advances in Hæmatology, as would be expected, are chiefly at the biochemical level and the importance of disturbances in the enzyme systems of the hæmopoietic cells is rapidly being documented.

Chapters showing most alterations are those on the hæmolytic anæmias, the purpuric and hæmorrhagic diseases and the leukæmias. Some of the older methods have been retained in the technique chapter, as many laboratories in all parts of the world still have no spectrophotometer.

The author has been very fortunate to have had the assistance of Dr. Hayhoe, who has completely rewritten the chapter on "The Cytochemistry of Hæmopoiesis," and of Dr. Tovey, who has done the same for the chapter on "Blood Groups and Blood Transfusion." Both collaborators have given help and criticism for other parts of the book.

Again I am grateful to the many who have sent suggestions and criticisms of the last edition; they are always most welcome. I would like to express my sincere thanks to my Chief Technicians, R .W. Deacon and C. E. A. Tidd, to my secretary, Miss Hamilton and to my wife and son for their willing and constant co-operation in the considerable task of revising this book.

C. J. C. B.

HARLEY STREET
LONDON, W.1.

CONTENTS

PLATES

CHAPTER 1

THE ORIGIN, DEVELOPMENT, FUNCTIONS AND FATE OF THE CELLS OF THE BLOOD

BLOOD FORMATION IN THE EMBRYO AND FŒTUS

STUDIES made on the yolk sac of the three-day-old chick show that in embryonic life blood cells first appear in the area vasculosa (Fig. 1). In the human embryo also, blood cells are first formed in the area vasculosa of the yolk sac; later they arise from the body stalk and placental site, later still from the liver, spleen and thymus, and finally from the bone marrow. There are in fact three successive phases of blood formation in the embryo and fœtus. These are known as the *mesoblastic phase*, occupying the first two months of embryonic life; the *hepatic phase*, which includes also splenic and thymic blood production and which is most active from the second to the fifth month, and the *myeloid phase*, which begins during the fifth month. Blood vessels are formed from blood-islets in

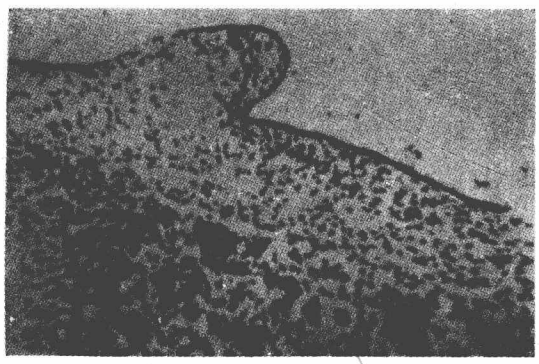


FIG. 1. Yolk sac showing primitive erythroblastic tissue

the area vasculosa by a hollowing-out of the syncytial mesoderm; proliferation of the endothelial cells lining these tube-like vessels gives rise to groups of central cells which become free and form the primitive blood cells of the circulation (Fig. 2). The free cells become hæmoglobinized and are the primitive nucleated red cells or *megaloblasts*, first described by Ehrlich. The hæmoglobin appears to be formed in the cells themselves from precursor substances of porphyrin type. The megaloblasts multiply in the fœtal blood stream to form smaller nucleated red cells containing more hæmoglobin. In the young embryo all hæmoglobiniferous cells are nucleated; at eight months nearly all are non-nucleated.

Thompson (1951) has calculated the rate and time of loss of nuclei from a study of 71 human embryos of various ages. He found the percentages of non-nucleated cells to be as follows: at six weeks, 0·1; at seven weeks, 0·5; at eight weeks, 9·0; and by the

end of nine weeks, 69; after eleven weeks the percentage remained steady at 98. These findings may be of value in estimating the age of fragmented or mutilated embryos.

Gilmour (1941), from an extensive study of hæmopoiesis carried out with more than fifty embryos and fœtuses, describes two types of megaloblast, the primitive and the definitive. Primitive megaloblasts are found in embryos less than 10 mm. and are considered to be relics of premammalian days. Definitive megaloblasts, which are derived from hæmocytoblasts (p. 12), are found in older embryos; they mature in the

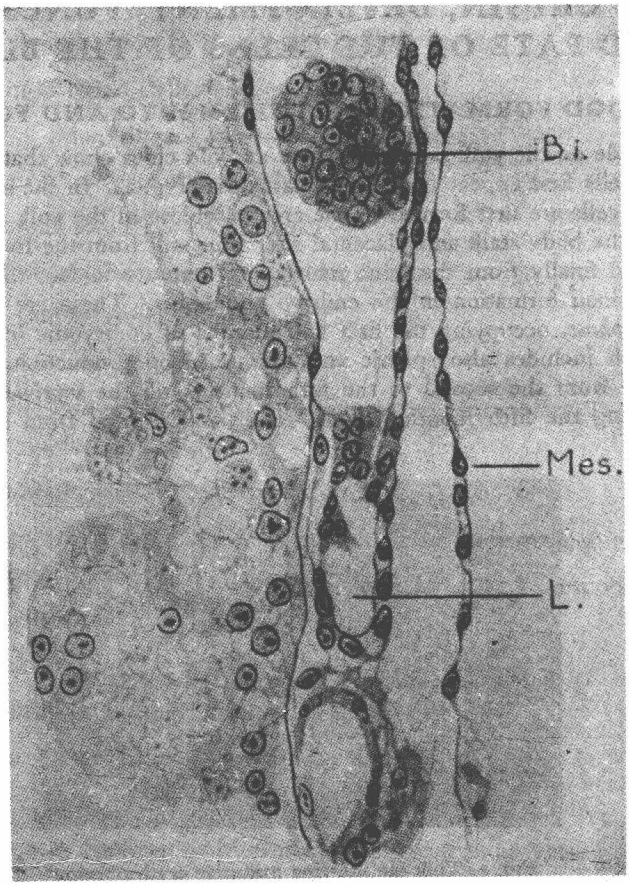


FIG. 2. Formation of blood cells from endothelium in the embryo chick. B.i.=blood islet. Mes.=mesoderm. L.=lumen of vessel. (After Sabin.)

normal way to form erythrocytes. The definitive megaloblast is smaller than the primitive whilst the nucleus is not only smaller but also darker staining. The megaloblasts found in pernicious anæmia are derived from the definitive megaloblasts. In the fœtus the liver shows an overwhelmingly erythropoietic pattern of hæmopoiesis (Thomas *et al.*, 1960).

As to leucocytes: In the fœtus precursors of white cells arise extra-vascularly from the mesoderm and migrate into the primitive blood stream. Until about the middle of fœtal life comparatively few white cells are produced; at this time the bone marrow is developed and begins to function as a blood-forming organ. Soon after birth the marrow is normally the only place where red cells and granular

leucocytes are formed, though the liver of both premature and full-term infants may contain active intralobular blood islands at birth and sometimes up to the fifteenth day of life (Gilmour, 1941). After great blood loss or in severe anæmias, and especially in infants, the liver, the spleen and the pelvis of the kidney may again assume blood-forming activity. Osgood (1955) reviews the quantitative and qualitative growth of the hæmopoietic tissue in the fœtus and growing child.

BLOOD FORMATION IN THE ADULT

General Principles. In the adult the mode of origin of the blood cells has been the source of so much speculation that a comprehensive description of the many suggested theories would be valueless. Extensive reviews have been published by Doan, Cunningham and Sabin (1925), Naegeli (1931), Doan (1932), Maximow (1932) and others. Descriptions of the process of blood development as well as the nomenclature are greatly confused and difficult to correlate, partly because the studies have been made in different animals and partly because of differences in technique.

The appearances of cells are remarkably different according to whether they are examined in fixed tissue sections, wet preparations, dried smears or tissue culture. Nomenclature still needs to be settled by international agreement. All hæmatologists are agreed that the mother tissue for the formed elements of the blood is the reticulo-endothelial system of fixed cells; in this system reticulum and endothelial cells are for histological purposes indistinguishable. The fixed cells of the reticulo-endothelial system, by division and maturation, give rise to the first recognizable free blood cells with primitive nuclei and basophilic cytoplasm, which divide and mature further to produce the well-differentiated cells of the circulation. The main points of controversy are the site of origin, whether intravascular or extravascular, and the phase at which differentiation of the first free cells becomes sufficiently fixed to allow of nothing but the formation of specific type cells.

The *monophyletic* theory of blood formation states that the first recognizable cell is a *hæmocytoblast*, which is totipotential and capable of giving rise to red cells, granular leucocytes, lymphocytes or monocytes, according to the requirements of the body; the stimulus for the formation of any specific blood cell falls upon the totipotential cell.

Alternative names for the hæmocytoblast (Maximow) are *lymphoidocyte* (Pappenheim) and *hæmohistioblast* (Ferrata), whilst other authorities (Maximow, Jordan, Bloom), regard the *large lymphocyte* as the mother of all blood cells.

In its complete form the *polyphyletic* theory states that the first recognizable blood cells are already differentiated precursors of all the cells of the circulation. These are a series of "blast cells" (myeloblasts, lymphoblasts, monoblasts, erythroblasts) so differentiated that they are unable to form any cell other than the specific mature cells.

The stimulus for differentiation is applied to the primitive reticuloendothelial cell.

Partial polyphyletism recognizes two (dualistic theory) or three (trialistic theory) "blast cells."

Thus Naegeli made a sharp distinction between the basophilic cells of myeloid and lymphatic tissue and regarded all blood cells as derived from either myeloblasts or lymphoblasts.

The basic theories are shown in Table I, and some of the evidence is discussed on pp. 7, 8.

Nomenclature. The nomenclature of the cells involved in normal blood development has been confused by the incorporation of terms which were originally

TABLE I

Theories of Blood Formation (Modified from Wiseman, 1934)

PHASE			
Undifferentiated	Reticulo-endothelial cells		
Partial Differentiation	Hæmocytoblast or Lymphoidocyte	Two Blast Cells: Myeloblast / Lymphoblast	Three Blast Cells: Myeloblast / Lymphoblast / Erythroblast
Complete Differentiation	All Blood Cells	Red cells, Granulocytes, Monocytes and Clasmatocytes / Lymphocytes, Monocytes and Clasmatocytes	Granulocytes, Monocytes and Clasmatocytes / Lymphocytes / Red cells
Hypothesis	Unitarian	Dualistic	Trialistic
School	Monophyletic	Polyphyletic	Polyphyletic

NOTE; Four "blast" cells may be postulated if a separate monoblast precursor for the monocyte is accepted.

used to describe cells found only in embryonic or pathological states. Hence, particularly in older literature, it may not be clear whether an author regards the cell he is describing as identical with the embryonic or pathological types or whether he regards embryonic and pathological blood formation as essentially the same process as the normal. Hæmocytoblast, lymphoidocyte and hæmohistioblast may be considered as synonymous terms. But the word *megaloblast* is often loosely used in hæmatology in senses never intended by its originator, Ehrlich. Ehrlich used the term to describe the large hæmoglobinized but nevertheless primitive nucleated red cell, characteristic of the embryo, and found, in the adult, invariably but not exclusively in the pathological state of pernicious anæmia. The cell is not found in normal marrow and takes no part in normal adult erythropoiesis; it signifies an abnormal line of development.

Such eminent authorities as Doan *et al.* (1925) unfortunately applied Ehrlich's term "megaloblast" to the earliest differentiated red cell found in small numbers in normal adult bone marrow. Had they used Ferrata's word, *proerythroblast*, much confusion would have been saved. Comparison between Doan's supravitally stained "megaloblasts" and Romanowsky-stained proerythroblasts is not possible, but it is probable that the two cells are identical.

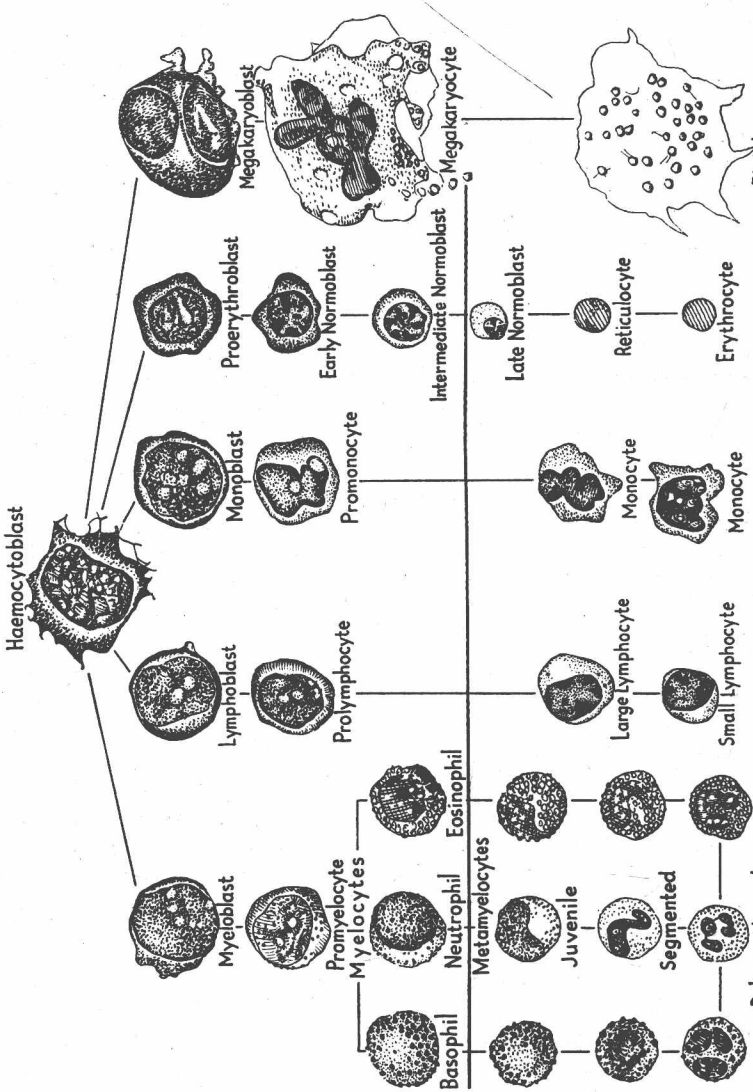
Confusion also arises as to the meaning of the word *erythroblast*. Ehrlich used this as a comprehensive term to include all nucleated red cells, normal or pathological; he regarded normal blood formation as essentially normoblastic and so described a series of normoblasts intermediate between the primitive cell and the mature erythrocyte. Doan *et al.* (1925), on the other hand, applied the word erythroblast to specific cells, to the earlier maturing form derived from the primitive cell, and they restricted the term normoblast to the final nucleated phase which is incapable of division and in which the nuclear material is condensed and pyknotic. There is much to be said for so differentiating a cell capable of division from one which is incapable; many hæmatologists therefore restrict the term normoblast to the last-named cell.

On the other hand, it is logical to use the term *normoblast* consistently for cells involved in normoblastic erythropoiesis and to differentiate three general classes, early, intermediate and late normoblast, the last being the equivalent of the normoblast of Doan *et al.*

Throughout this book the nomenclature follows the Ehrlich tradition. The specific terms proerythroblast and normoblast (early, intermediate and late) are used for the cells involved in normal blood production and simple hyperplasia: the term erythroblast is used comprehensively to include all nucleated red cells, normal or pathological: whilst the word megaloblast is restricted to the series of cells characteristic of pernicious anæmia.

A committee formed for the Clarification of the Nomenclature of Cells and Diseases of the Blood and Blood-forming Organs published their first two reports in *Blood*, 1949, **4**, 89. The most drastic reforms naturally concerned the cells involved in red cell development and proposed the substitution of the following terms for the nomenclature used in this book (stated in brackets): rubriblast (hæmocytoblast); prorubricyte (proerythroblast); rubricyte (early and intermediate normoblast); metarubricyte (late normoblast). When megaloblastic development occurs the descriptive names were to be qualified by the phrase "pernicious anæmia type." This nomenclature has not been accepted by hæmatologists and has not come into general use.

Intravascular and Extravascular Blood Formation. Many studies have been made to determine whether blood cells are formed within blood vessels or



All cells below the horizontal line may be found normally in the blood stream with the exception of the late normoblast. All cells above the horizontal line and the late normoblast may be found in normal bone marrow.

without. Much depends upon the species of animal as well as maturity, whether embryo, fœtus or adult.

Gilmour (1941) found that in human embryos over 48 mm. long, erythropoiesis was undoubtedly extravascular except for the sinuses of the liver and for cells which multiplied within the circulation. Using pigeons, Jordan and Johnson (1935) considered that the hæmocytoblast was able to migrate into marrow sinusoids, and there develop into erythroblasts. On the other hand, Doan, Cunningham and Sabin (1925), using supravital methods in rabbits and pigeons, suggested that red cells had an intravascular origin from endothelial cells lining the intersinusoidal capillaries of the bone marrow. Leucocytes, in contrast, appeared to originate from extravascular reticulum cells. Migration to within the blood vessels occurred when the leucocytes had developed sufficiently to become actively motile. In fresh preparations of marrow a group of granulocytes was seen to move *en masse* against the wall of a capillary until the wall bent inwards. When stretching reached a certain point a leucocyte close to the wall flowed in between two endothelial cells and the remainder followed in rapid succession (Sabin, 1928).

Most authorities consider that both red cells and leucocytes have an extravascular origin in the human subject.

Experimental Evidence for the Various Theories. The monophyletic theory of blood formation is supported by the work of Gilmour (1941) in the human subject and by Jordan and Johnson (1935) who used pigeons. Turnbull (1936) described a hæmocytoblast, a bone marrow cell recognizable in fixed tissue sections which appeared to be less differentiated than any cell which could be recognized as a definite red cell precursor. Turnbull suggested that these hæmocytoblasts were derived from the cells of the supporting reticulum, that they were the undifferentiated precursors of granular leucocytes, erythrocytes and megakaryocytes in the bone marrow and of lymphocytes in other parts of the body.

The work of Doan, Cunningham and Sabin (1925) supports the polyphyletic theory.

They used rabbits and pigeons and a supravital technique. They employed an ingenious method for simplifying marrow tissue so that the early stages of leucocyte and red cell production could be studied. In pigeons this was accomplished by alternate starving and feeding; the former causes red marrow to become yellow and inactive, the latter brings about the opposite change; thus the early phases of erythropoiesis could be observed. In rabbits a diminution in white cell elements was produced by injecting typhoid bacilli, thus allowing observations on a relatively pure process of erythropoiesis. From these extensive studies it was concluded that red cells have an intravascular origin from endothelial cells passing through various phases of maturation named "megaloblast" (see Nomenclature, p. 4), erythroblast, normoblast, reticulocyte and erythrocyte. It was also concluded that leucocytes have an extravascular origin from a *primitive white cell*, itself derived from reticulum; later work by Sabin *et al.* (1936) showed that this primitive white cell appeared not to be a lymphocyte as has sometimes been suggested.

According to this work, therefore, both leucocytes and red cells are derived entirely from the reticulo-endothelial system, though the first division of the cells of this system commits the cell produced either to leucocyte or red cell formation, whilst specific leucocyte production is determined by differentiation of the primitive white cell. Other observations suggested that for the maintenance of the numbers of blood cells at normal level in health, division of the undifferentiated reticulum and endothelial cells was unnecessary; the normal level was thought to be maintained by the already differentiated precursor cells though these, even in the adult, have been originally derived from the reticuloendothelial system. Megakaryocytes

probably have an extravascular origin from reticulum cells; the nucleus divides without division of the cytoplasm.

Peabody (1926) obtained some confirmation of these views by examining serial sections through the marrow of a patient who had died of typhus fever. In typhus fever the yellow marrow becomes hyperplastic with very little increase in the white cell elements. Serial sections passing from frank red marrow to completely inactive yellow marrow provided human material confirming many of the views of the Sabin school. The work of Israëls (1940) also gives some support to the polyphyletic theory. In cultures of bone marrow from different types of leukæmia he observed pure development of the cells without any forms intermediate between the different series.

The polyphyletic theory is illustrated in Plate II, p. 7; it explains most of the phenomena of blood disorders in a satisfactory and practical manner for clinical work.

THE ANATOMY AND PHYSIOLOGY OF THE BONE MARROW

Active marrow in the adult is estimated at from 3·5–6 per cent of bodyweight (1,500–3,500 grams); it roughly equals the weight of the liver. The volume of the marrow is large; it is estimated at 70 ml. at birth and about 4,000 ml. in the adult. In the adult only about half the marrow is in an active state, but it has an enormous functional capacity as well as considerable room for expansion and is estimated to be capable under stress of producing seven or eight times the normal physiological turnover (p. 35). The parent cells of the marrow can produce many times their own volume of mature cells in a relatively short time, and in so doing they metabolize oxygen freely. On account of the diversity of the function and of the nature of the marrow cells the factors which govern production and release into the circulation are likewise multiple and variable according to the type of the cell and the nature of the demand, whether physiological or pathological (pp. 19, 17).

Macroscopic Appearances. The bone marrow of the adult is either red in colour and actively hæmopoietic, or yellow, fatty and inactive as far as blood formation is concerned.

Custer (1932) and Jaffé (1936) give good descriptions of the red marrow in regard to the amount and the situations where it is normally found at different ages. At *birth* and for the first three or four years of life all the bones in the body contain red hæmopoietic marrow. About the *age of seven* the marrow in the shafts of the long bones becomes less active. At this age the marrow is pale red and the cut surface is greasy from definite droplets of fat. At the *age of ten to fourteen* a patch of yellow fatty marrow appears at the distal end of the shafts of the long bones and this gradually extends proximally, becoming complete distally, until at the *age of twenty* the entire red marrow in the long bones has been replaced by yellow marrow, with the exception of the upper end of the femur and humerus. The change from red to yellow occurs in the carpus before the bones of the forearm and leg, and, again, in the forearm and leg before the humerus and femur. The change from red to yellow thus occurs in distal sites before proximal ones, and this is true also for any individual bone. The change is well shown in Fig. 3. The fibula, radius and ulna follow a similar course to the tibia, whilst the humerus resembles the femur, and the os innominatum the vertebræ.

In an *adult* the red marrow is contained in the bones of the skull and thorax (ribs, sternum, scapulæ and clavicles), the vertebræ and the os innominatum; there is also a little in the upper end of the femur and humerus (Plate I, Frontispiece).

The bone marrow of an adult has therefore ample room for expansion, there being an immense reserve of space which can fill with active marrow in times of

need (p. 35). In an infant, on the other hand, no such reserve space exists, because all the bones are filled with active marrow, leaving no room for expansion (Plate I, Frontispiece). It is for this reason that under conditions of anæmic stress an infant

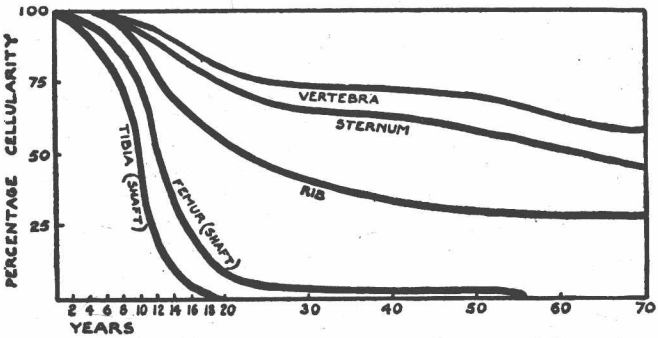


FIG. 3. To show the amount of cellular red marrow in different bones at different ages. (From the data of Custer and Ahlfeldt, 1932.)

develops accessory hæmopoietic centres in the liver, spleen, kidney and sometimes even in the muscular tissue (p. 468).

As age advances there is a gradual diminution in the amount of red marrow and by the age of seventy more than half the ribs and about half the sternum are filled with fatty marrow. At all times, even in the most active red marrow, fatty tissue can be seen in histological sections.

Benda, Orinstein and Depitre (1940), by an injection method, showed that the marrow blood supply depends on the distribution of red marrow. In the femur of the normal adult the nutrient artery breaks up into smaller vessels in the shaft of the bone, and these branches pass as well-defined vessels into any red marrow that may be present. Here the vessels divide into a fine network so that the whole of the red marrow appears like a mass of injected material. The stagnation of blood in such a suddenly increased tissue bed explains why metastases, cancerous or infective, tend to settle in such a site. Lymphatics are plentiful in the periosteum, but they have never been demonstrated in the bone marrow.

Microscopic Appearances. The yellow marrow is constructed simply of round fat cells with a few strands of fine connective tissue. Nevertheless, even obvious fatty marrow contains small groups of normoblasts and myelocytes. Bleedings stimulate erythropoiesis, whilst transfusions diminish it. By observing the effect of bleedings and transfusions it has been suggested that the microscopical structure of the bone marrow is as follows: The nutrient artery breaks up into smaller and smaller branches until finally there is a network of inter-communicating, thin-walled, but comparatively large vessels, lined with endothelium, which are known as *sinusoids*. Joining these sinusoids one to another, and opening into them by a conical aperture are the *intersinusoidal capillaries*, the walls of which are in apposition, so that only a potential cavity exists. This fine structure of the intersinusoidal capillaries is not evident in ordinary sections, but can be demonstrated in experimental animals. Some believe that the intersinusoidal capillaries are an artefact, whilst others maintain that the conical apertures are, in fact, closed. Those who support the intravascular theory of origin of red cells believe that the site of active erythropoiesis is the intersinusoidal capillary. A description of the living microscopy of the blood vessels is given by Brånemark (1959).

Red bone marrow is extremely cellular. Sections show concentrations or islands of cells consisting of leucoblastic and erythroblastic elements in various stages of