Systemic Pathology

SECOND EDITION

by THIRTY-EIGHT AUTHORS

VOLUME 2

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Blood and Bone Marrow
Lymphoreticular System
Thymus Gland

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* Please see also the Preface to Volume 1 of this edition.

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Note

Abbreviations.—The following abbreviations are used in this chapter:

DNA, deoxyribonucleic acid
G6PD, glucose-6-phosphate dehydrogenase
IgG, IgM, etc., immunoglobulin of class G, class M, etc.
MCH, mean red cell haemoglobin content
MCHC, mean red cell haemoglobin concentration
MCV, mean red cell volume.

Units of Measurement.—SI units (*Système international d'unités*) are used in this chapter. In accordance with the practice current at the time of writing (December 1975), the mass concentration of haemoglobin is still expressed in grams per decilitre (g/dl). All other concentrations are expressed in molecular units—moles (molecular weight in grams) or a submultiple (for example, millimoles)—per litre or (in the case of proteins) in grams or submultiples of grams per litre.

THE NORMAL BLOOD AND BONE MARROW

BLOOD VOLUME

The volume of the blood, and of its cell and fluid components, can be measured with much accuracy during life. The methods chiefly used depend on the degree of dilution of known amounts of some vital dye (such as Evans blue) or of isotope-labelled plasma proteins or erythrocytes after their injection into the circulation. In the average normal adult, the blood volume is about four litres in women and almost five litres in men (some 70 ml per kilogram of body weight); some variation is associated with differences of weight and height and in the amount of fatty tissue.

Much detailed work has been done on devising methods for determining total blood volume, in view of its importance in disease and in the circulatory failure that may follow severe trauma.[1]

Minor variations in blood volume result from changes in posture or in bodily activity, or through heavy sweating. A striking increase—even as much as 40 per cent—occurs during pregnancy, due mainly to a rise in plasma volume.[2] Considerable changes can also occur in disease.[3] Acute severe haemorrhage, extensive burns and prolonged dehydration through vomiting or diarrhoea may result in such a marked reduction in the volume of the blood that the systemic blood pressure falls dangerously. In cases of cardiac failure[4] and of polycythaemia[5] the blood volume may be substantially raised. These changes frequently affect the red cells and the plasma differentially. In cases of severe anaemia there is a relative loss of cells. In disorders accompanied by marked splenomegaly the plasma volume may be greatly increased:[5a] the resulting 'haemodilution anaemia' augments the severity of any anaemia that is associated with the condition responsible for the splenomegaly. The blood volume may rise by 1 to 2 per cent for each centimetre of enlargement of the spleen below the costal margin. The explanation of the increase in the volume of the plasma in association with splenomegaly is unknown; it does not appear to be accompanied by any significant abnormality of the plasma proteins.

THE CELLS OF NORMAL BLOOD

The composition of the blood depends largely on a dynamic equilibrium between its constituents (cells and plasma) and the tissues and tissue fluids. The blood can be regarded as a solution of colloids and crystalloids in which the cellular elements are

References to Other Chapters.: A list of the chapters in each of the five volumes is on page v at the front of this volume.

suspended in osmotic equilibrium. Many of its constituents—red cells, white cells, platelets and plasma proteins—are too large to escape freely through capillary walls, and can be regarded as belonging to the blood. In fact this statement is not strictly true—for example, the majority of the leucocytes in the body are in the tissues, although for our present purpose they may be considered as part of the blood. Other substances in the blood are merely in transit between tissue cells and sites of absorption or excretion. Some of these substances (such as iron and copper, trace metals, small amounts of haemoglobin, lipids and bile pigments) are temporarily bound to particular plasma proteins (see page 445);[6] others (including some of the hormones, sugars, fatty acids, amino acids and electrolytes) are more or less uniformly partitioned between the plasma and the tissue fluid.

All the constituents of the blood, including the water in which they are suspended or dissolved, are constantly undergoing replacement. There are wide differences in the rate at which their turnover occurs: information on this aspect of the various types of cells and plasma proteins that are specifically part of the blood is essential for an understanding of the pathology of the blood diseases. As all these elements are formed outside the circulation proper, most blood diseases are the reflection of some disturbance in the tissues of their origin—the bone marrow, the lymphoreticular tissues, the spleen and the liver.

The Red Cells

The red cells (see Table 8.1) are non-nucleate, discoid structures, composed of a lipoprotein envelope and a fluid interior that normally is saturated with haemoglobin. Their typical biconcave shape facilitates the rapid gaseous exchanges that occur in the capillaries of the lungs and of the tissues; at the same time, their ready distortability allows free movement through small vessels.

Enumeration of Cells and Measurement of Haemoglobin Content.—Since quantitative determinations figure prominently in haematological diagnosis, it is necessary to define the normal limits of the numbers of the various types of blood cells. Formerly, a visual count of red cells and an estimation of the amount of haemoglobin in a sample of blood were regarded as the only practical, simple, quantitative investigations needed for the diagnosis of anaemia or polycythaemia. It has become realized, however, that the errors inseparable from the visual methods of red cell counting make these unreliable. Greater accuracy is obtained by the use of electronic counting instruments.

Separation of the red cells by centrifugation provides a valuable measure of the relative proportions of cells and plasma—the packed-cell volume (PCV) or haematocrit value.

Modern automated instruments measure the haemoglobin level, count the red cells and measure the mean red cell volume (MCV) automatically. They may measure or compute the packed cell volume (PCV) and calculate the mean cell haemoglobin (MCH) and the mean cell haemoglobin concentration (MCHC).[7] While the total red cell mass is the ultimate measure of whether an individual is normal or polycythaemic or anaemic, in practice it is the haemoglobin level and the related indices that are used in making the initial diagnosis of disorders affecting the red cells. The normal range of these parameters in men and in women is shown in Table 8.1.

Table 8.1. *The Normal Parameters of the Red Blood Cells in Adults (SI Units)**

	Men	Women
Number ($\times 10^{12}$/l)	5·4 ± 0·8	4·8 ± 0·6
Haemoglobin (g/dl)	16 ± 2	14 ± 2
Packed cell volume (%)	47 ± 5	42 ± 5
Mean cell volume (fl)	87 ± 5	
Mean cell haemoglobin (pg)	29 ± 2	
Mean cell haemoglobin concentration (g/dl)	34 ± 2	
Mean cell diameter (μm)	7·5 ± 0·3	

* Based on: Wintrobe, M. M., *Clinical Hematology*, 6th edn, page 86. London, 1967.

Blood Films.—In addition to the investigations noted above, study of the red cells requires examination of thin films of blood stained by one of the Romanowsky methods, such as those of Leishman, Wright, May and Grünwald, Jenner, and Giemsa. This study should include observation of the size, shape and staining of the cells and the presence of any inclusions (Fig. 8.9).

Red Cell Diameter.—It may be helpful to determine the distribution of variation in the diameter of the red cells, especially in certain macrocytic anaemias (see page 458). This was formerly done by the laborious method of Price Jones;[8] similar information can now be obtained by use of an automatic instrument that determines the distribution of the red cells according to their volume.[9]

Reticulocytes

When the red cells first enter the circulation they are not fully mature, and they appear a slaty blue in Romanowsky-stained films. By staining blood films, while the blood is still moist, with vital dyes, such as brilliant cresyl blue or Nile blue sulphate, the remains of a basiphile material (ribonucleic acid) within these immature cells can be precipitated as a conspicuous reticulum (see Fig. 8.9N, page 450). It is from this reticulum, which disappears in a day or two in the circulation, that the reticulocyte derives its name. A large number of reticulocytes in the blood indicates that erythropoiesis is currently proceeding at an unusually active level or that there is a gross marrow disturbance. A constant reticulocytosis indicates a shortening of the life span of the red cells (see page 472).

Red Cell Life Span

In mammals, the red blood 'cell' is not a cell in the usual sense of a nucleate unit, but rather an envelope filled almost entirely with a nearly saturated solution of haemoglobin. Under ordinary circumstances, the red cells remain in the circulation for about four months, at the end of which their remnants are ingested and destroyed by the reticulo-endothelial cells of the marrow, spleen, liver and other organs.[10] In some diseases of the blood, however, they may disappear from the circulation much more rapidly (see page 472).

The White Cells

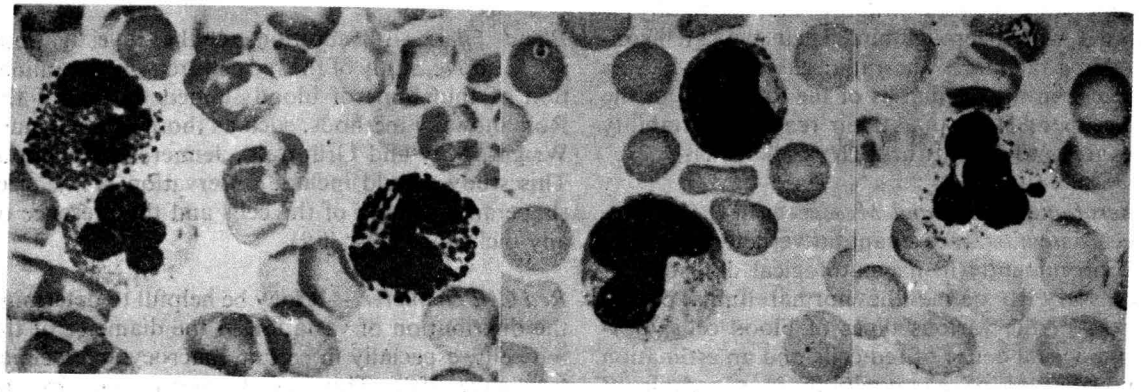
The white cells have a lower specific gravity than the red cells, and consequently they sediment less rapidly on centrifugation. In a haematocrit tube, therefore, they come to form, together with the platelets, a layer normally about a millimetre deep —the 'buffy coat'—between the plasma above and the packed red cells below. Although the depth of this layer varies with the number of white cells in the blood, the relationship is not very close. For the determination of the white cells in a specimen of blood, total and differential counts are required.

Accepted values for the numbers of the various types of white cells are given in Table 8.2. The criteria used for their differentiation in Romanowsky-stained films are indicated in Table 8.3.

Table 8.2. *The Normal Total and Differential White Cell Count per Litre (SI Units)*

	Average count ($\times 10^9$/l)	Normal range ($\times 10^9$/l)
Total cell count	7·0	4·0 to 11·0
Neutrophils	4·5 (65%)	2·5 to 7·5
Lymphocytes	1·8 (25%)	1·5 to 4·0
Monocytes	0·55 (8%)	0·2 to 1·0
Eosinophils	0·15 (2%)	0·05 to 0·5
Basiphils	'occasional'	0 to 0·1

Young neutrophile granulocytes can be identified by the incomplete segmentation of their nucleus. In the earliest stage the nucleus is reniform, but segmentation into two, three, four or more lobes soon follows. Attempts have been made to ascertain the rate of formation of granulocytes by determining the relative numbers of cells showing the various degrees of lobation. In the case of the neutrophils, the presence of many bilobate cells—the so-called



A B C

Fig. 8.1. Normal leucocytes in blood films. *Jenner–Giemsa stain.* ×1250.
A. Left to right: an eosinophil, a neutrophil and a basiphil.
B. Above, a lymphocyte; below, a monocyte.
C. A neutrophil from a female, showing a sex 'drumstick', which is believed to be formed from the inactive X chromosome.

Table 8.3. *Appearance of White Blood Cells in Romanowsky-stained Films* (Fig. 8.1)

	Size (μm)	Nucleus	Chromatin	Cytoplasm	Granules
Neutrophils	12–14	2–5 lobes	Heavy clumps	Abundant; yellow	Fine; purple
Eosinophils	12–17	2–3 lobes	Heavy clumps	Overlaid with granules	Coarse; orange-red
Basiphils	10–12	Indented	Heavy clumps	Overlaid with granules	Coarse; purple-blue
Small lymphocytes	8–11	Unlobed	Heavy clumps	Clear; blue	Clumps of 5–6 red granules
Large lymphocytes	11–18	Unlobed	Heavy clumps	Clear; blue	Clumps of 5–6 red granules
Monocytes	14–25	Overlapping	Fine lacework	Abundant; cloudy; blue	Fine red stippling

'shift to the left'—implies active leucopoiesis, while an unusually large proportion of cells with four or five lobes—the 'shift to the right'—indicates the persistence of senescent cells in the circulation. Determinations of the frequency of these differing lobate forms are known as Arneth counts; they may have some value in estimating the responsiveness of the marrow in some forms of infection. No criteria have yet been recognized by which to estimate the age of the other types of white cells in man; in animals, this can be done by isotopic labelling methods.

Measurement of the life span of the leucocytes in the blood[11] is complicated by the fact that the great majority of these cells are in the tissues, where they cannot be examined readily. By labelling the mature granulocytes with diisopropylfluorophosphonate containing radioactive phosphorus (^{32}P) it has been found that their life span in the blood is about one day. However, about four days may be spent in the bone marrow and rather longer in the tissue compartment, the total life span being therefore, perhaps, 10 days. In contrast, the lymphocytes, which recirculate through the lymph nodes and thoracic duct, appear to consist of two populations, one of which survives for many years (see page 519).

Neutrophils from donors with the Pelger–Huët neutrophil nuclear anomaly (Fig. 8.25C, page 476) are easily recognized in the circulating blood of recipients: they survive at most only six hours in the circulation of normal recipients.[12]

The Platelets

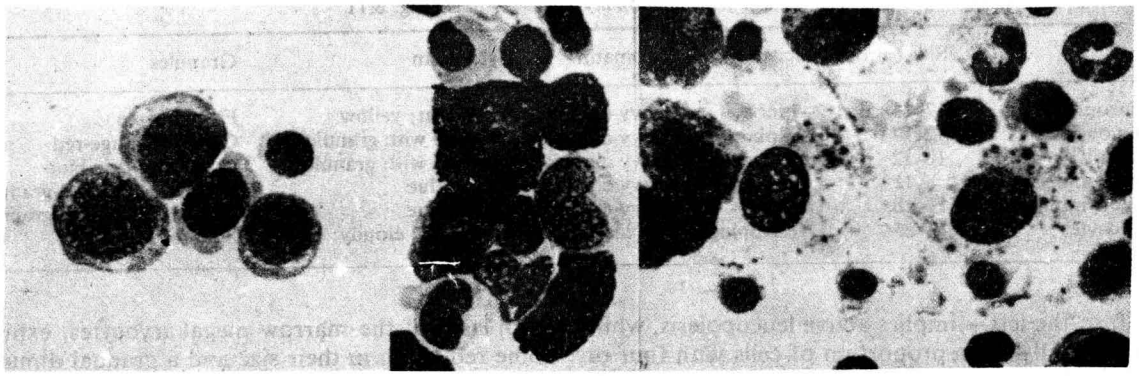
In the stained films that are used for differential leucocyte counts, platelets are seen lying either singly or in small clumps, and a rough estimate of their number can thus be made. Accurate platelet counts are obtained by using electronic counting equipment. The normal number is from $200 \times 10^9/l$ to $400 \times 10^9/l$.

The platelets, which are merely fragments of the cytoplasm of the marrow megakaryocytes, exhibit some reduction in their size and a gradual diminution of their stickiness as they age in the circulation. Their life span, measured by labelling with radioactive isotopes, is about nine days.

THE BONE MARROW

Since most of the so-called blood diseases are in reality diseases of the bone marrow, a general knowledge of haemopoiesis is essential for an understanding of their pathogenesis. Moreover, while these diseases were formerly diagnosed merely by an examination of the peripheral blood, nowadays much use is made of cytological studies of samples of marrow obtained by needle puncture of the sternum or of the crest of the iliac bone.

In the healthy newborn infant, haemopoietic marrow is present in the cavities of all the bones of the body. With time, the extent of haemopoietic marrow recedes; in the adult it is confined mainly to the bones of the skull and the vertebrae, ribs and pelvic bones, with small remnants in the head of the humerus and of the femur. In health, even the haemopoietic portions of the marrow are composed mainly of adipose cells, bands of haemopoietic cells lying between them (Fig. 8.20A, page 461). It is estimated that the medullary cavity of the bones of an adult contains in all about 2 kg of marrow, the greater part of which consists of adipose tissue. When haemopoiesis is stimulated, as may happen in widely differing types of disease, the haemopoietic tissue becomes progressively more abundant. In the severer forms of haemolytic anaemia the adipose tissue is almost wholly replaced by erythropoietic cells, and the rate of production of red cells is increased five-fold or more. At the other extreme, in depression of marrow activity, as in aplastic anaemia, the haemopoietic elements may be so diminished that the medullary cavities are wholly filled by adipose cells (Fig. 8.20B); sometimes, in such cases, the marrow may be replaced by fibrous tissue (Fig. 8.22).



A B C

Fig. 8.2. Normal marrow. *Film preparations: Jenner–Giemsa stain.* × 1000.

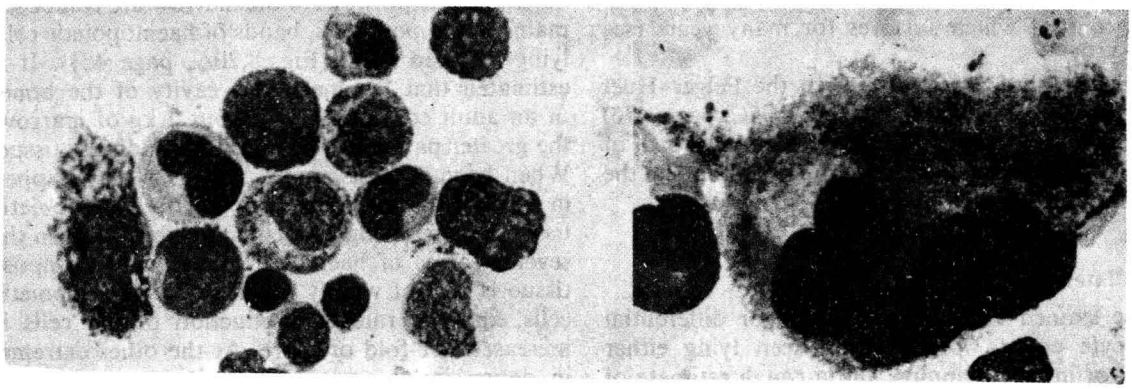
A. Erythropoiesis. A group of normoblasts—in order of decreasing nuclear size and increasing nuclear density the cells are early, intermediate and late normoblasts.

B. A mast cell, above the centre, on the left. The nucleus appears pale in comparison with the heavily stained cytoplasmic granules.

C. Two macrophages, one on each side of the centre of the field. The abundant, ill-defined, pale cytoplasm contains ingested granules; the nucleus is oval and its chromatin has a net-like pattern.

The haemopoietic cells comprise two main series, the red cell precursors (Fig. 8.2A) and the precursors of the granular leucocytes (Fig. 8.3A). In normal marrow, the precursors of the granulocytes predominate over the precursors of the red cells in a ratio of about four to one. The marrow also contains megakaryocytes (Fig. 8.3B), fat cells and other supporting tissue cells, macrophages (Fig. 8.2C), plasma cells and mast cells (Fig. 8.2B). The haemopoietic cells are conventionally regarded as arising from a common ancestor, sometimes called the haemohistioblast, which supposedly is also the ancestral cell of lymphocytes and monocytes (see below). After birth, these primitive cells ('colony-forming units') are very scanty, both in the haemopoietic tissues of the marrow and in the lymphoreticular tissues elsewhere.

Division of a haemohistioblast gives rise to two daughter cells: one of these may retain the attributes of the parent cell while the other is able to differentiate toward any of the definitive cell series. The first of the specific cells in each series is the 'blast' cell, characterized by the relatively large size and uniform chromatin content of the nucleus, which



A B

Fig. 8.3. Normal marrow. *Film preparations: Jenner–Giemsa stain.* × 1000.

A. Leucopoiesis. Clockwise from the left, clustered round an early myelocyte in the centre of the field, a myeloblast, a late myelocyte, a promyelocyte, a myelocyte and a metamyelocyte.

B. A megakaryocyte, with a group of platelets to the left.

contains one or more nucleoli, and by the absence of granules in the cytoplasm (Fig. 8.3A). The blast cell that is the precursor of the red cell series, the proerythroblast, is recognizable from the blast cells of the other series by the granular appearance of its chromatin, the poor definition of its nucleoli and the greater opaqueness of its cytoplasm. The earliest blast cell of the granulocyte series, the myeloblast, can be distinguished morphologically from the megakaryoblast, and by cytochemical methods (especially the periodic-acid/Schiff reaction) from the earliest precursors of the non-granular leucocytes—lymphoblasts (Fig. 8.4C) and monoblasts.[13] Lymphoblasts and monoblasts are commoner in the lymphoreticular tissues elsewhere in the body than in the bone marrow. In practice, the main guide to the identification of the nature of any particular blast cell is the character of the mature cells that are associated with it and that presumably are its derivatives.

Recently, doubt has been raised about the occurrence of the elusive haemohistioblasts. When leucocytes are cultured in the presence of phytohaemagglutinin (a substance extracted from beans), lymphocytes, although apparently adult cells, revert to a more primitive type and undergo mitosis.[14] This may not happen in the case of other cell types, but the fact that it occurs at all suggests that the principle of differentiation is not absolute and that it is, perhaps, not necessary to postulate the presence of ancestral cells to account for expansion of the lymphoreticular and haemopoietic tissues (see page 522).[15]

Erythropoiesis

The *proerythroblast* divides by mitosis, and this initiates a series of developmental steps, leading by further mitotic divisions and progressive maturation to the orthochromatic red cells found in the peripheral blood. These stages are defined according to the state of the nucleus and the degree to which haemoglobin synthesis has taken place in the cytoplasm (Fig. 8.2A). The proerythroblast gives rise to the *early normoblast*, which differs from the former in being rather smaller and in having no nucleoli. The next stage, the *intermediate normoblast*, has a smaller and more compact nucleus; a more important distinguishing feature is the commencing haemoglobinization of the cytoplasm. The final nucleate stage is the almost completely haemoglobinized *late normoblast*. This cell eventually extrudes its pyknotic nucleus, and passes into the blood stream as a *polychromatic red cell* (*reticulocyte* —see page 432). It matures into the *orthochromatic red cell* and loses its mitochondria after being in the circulation for a day or two. Occasionally, particularly after splenectomy, some of the red cells in the circulation still contain coarse basiphile nuclear fragments: these are known as Howell–Jolly bodies (Fig. 8.9K, page 450).

During the nucleate stages of development a series of enzyme-controlled reactions is needed both to build up deoxyribonucleic acid (DNA) in the chromatin, preparatory to cell division, and to effect the synthesis of haemoglobin. In addition to its constituent pyrimidines (thymine and cytosine), purines (adenine and guanine) and phosphates, the

A B C D

Fig. 8.4. Leucocytes—special staining and other techniques.

A and B. Demonstration of alkaline phosphatase in neutrophils (grades 4 and 1 respectively). × 1000.

C. Periodic-acid/Schiff reaction in a leukaemic lymphoblast. × 1000.

D. Lupus erythematosus: the neutrophil at the centre of the field has engulfed a large spheroidal mass of pale, degenerate nuclear material ('LE cell'). *Incubated leucocyte preparation; Jenner–Giemsa stain.* × 700.

formation of DNA requires vitamin B_{12},* ascorbic acid (vitamin C) and folinic acid (the active derivative of folic acid). Lack of these essential substances hinders the enzymatic synthesis of the nucleoproteins of chromatin and consequently interferes with the further multiplication of the cells. As a result, while maturation and haemoglobinization can advance in the cytoplasm, nuclear development is delayed or prevented. Under these conditions, the cells grow abnormally large, and have a delicate expanded nucleus: they are then known as *megaloblasts* (Fig. 8.5C). A defect in the action of one of the enzymes would impair development of the nucleus: this may account for some of the cases of megaloblastic anaemia that are refractory to treatment.

Formation of Haemoglobin

The formation of haemoglobin takes place within the cytoplasm of the developing cells. The haemoglobin molecule is built in two parts: a large protein mass, the globin, and the porphyrin ring structures of the four haem molecules. The globin acts as a vehicle for the four haems, which each contain an atom of iron in a union suited to rapid uptake and liberation of oxygen. The many amino acids that form the four long polypeptide chains of the globin are linked in a specific order in accordance with the hereditary pattern of the DNA (see page 468). Enzymes control each step in the synthesis of the haem, from the union of relatively simple materials, glycine and succinate, through the intermediary stages of delta-aminolaevulic acid and porphobilinogen, to the final chelation of the iron atoms into the porphyrin ring by chelatase.[16]

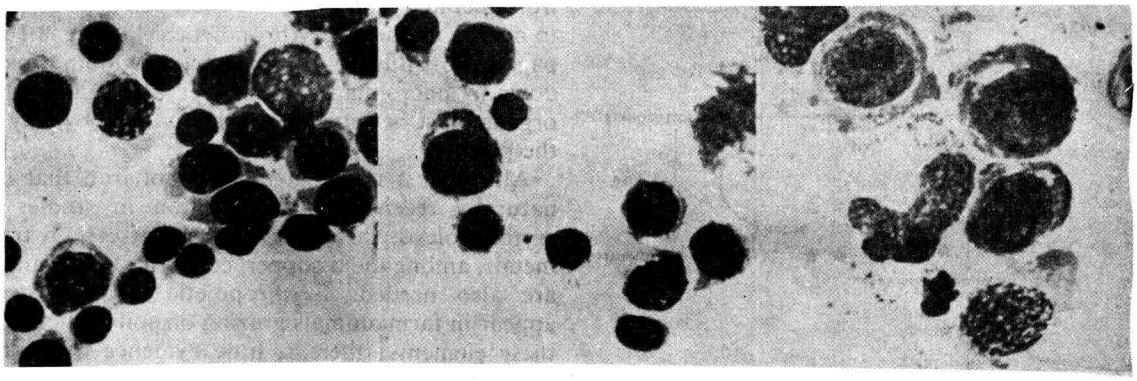
The synthesis of haemoglobin may be impeded at any stage, either through some congenital or acquired enzymatic defect, or through a lack of an essential substance, particularly iron. Deficiency of iron generally follows prolonged loss of blood but may be due to malabsorption. Non-utilization of iron can be due to an enzyme defect in the red cell precursors or to interference with the transport of iron from the reticuloendothelial cells to the erythropoietic tissues.[17] Whatever the cause of diminished haemoglobin synthesis, it results in poor development of the cytoplasm. The abnormal red cell precursors that are found in these circumstances are known as *micronormoblasts*, and are characterized by having only a narrow and ragged rim of cytoplasm and a rather over-developed, pyknotic nucleus (Fig. 8.5B). The red cells that develop from these micronormoblasts are small (MCV below 80 fl) and deficient in haemoglobin (MCH below 27 pg) (see page 452 and Figs 8.9C and 8.11A).

When the stroma of the red cells is also defective the cells may vary in size (anisocytosis) or shape (poikilocytosis) (Fig. 8.9C, page 450).

Metabolism of the Mature Red Blood Cell

In comparison with its nucleate precursors, the fully formed red blood cell shows little metabolic activity and its aerobic respiration is negligible.[18] Nevertheless, it has to expend energy to preserve its integrity, to keep its water-repellent lipoprotein surface intact, to retain its biconcave shape, to hold the haemoglobin in the active ferrous state, and to maintain a higher concentration of potassium and a lower concentration of sodium within its envelope than is present in the plasma. Most of the energy that is needed for these purposes is derived from anaerobic glycolysis. During the serial enzymatic degradation of diphosphoglycerate to pyruvate in that part of the main Embden–Meyerhof pathway that includes the activity of the enzyme pyruvate kinase, adenosine diphosphate (ADP) is phosphorylated to adenosine triphosphate (ATP) to provide a considerable amount of potential energy (see Fig. 8.6). A smaller amount is produced in the pentose phosphate shunt and at the same time nicotinamide adenine dinucleotide phosphate (NADP) is reduced ($NADPH_2$) by the activity of the enzymes glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. This maintains glutathione in the reduced state and in turn prevents the degradation of the haemoglobin in the living red cells. In the region between the glyceraldehyde-3-phosphate and the diphosphoglycerate not only is some energy produced to help maintain haemoglobin in its reduced form, but there is also a small side shunt involving the synthesis of the important substance 2,3-diphosphoglycerate. This substance controls the release of oxygen from haemoglobin: its increased production helps to compensate for relative hypoxia at high altitudes and for anaemia. When stored blood is transfused its capacity for oxygen release is defective during the first 24 hours because its content of 2,3-diphosphoglycerate diminishes progressively during storage and time is needed for it to form again.

* For some years vitamin B_{12} was believed to be cyanocobalamin; cyanide was later found to have become incorporated accidentally in the cobalamin molecule during the course of the purification of the vitamin (E. Lester-Smith, *Vitamin B_{12}*, 3rd edn, page 26; London, 1965).



A B C

Fig. 8.5. Abnormal erythropoiesis. *Film preparations of marrow: Jenner–Giemsa stain.* × 1000.

A. Over-active normoblastic erythropoiesis in haemolytic anaemia.

B. Micronormoblastic erythropoiesis in iron-deficiency anaemia.

C. Megaloblastic erythropoiesis in pernicious anaemia (Addisonian anaemia). A giant metamyelocyte is also seen, at the centre of the field, surrounded by the megaloblasts.

Once the developing red cell has lost its nucleus, production of further enzyme molecules probably ceases. Recent investigations of these enzyme systems have provided much information regarding disorders of red cell metabolism (see page 468).

The constant replacement of senile red cells through erythropoiesis involves the continuous synthesis of haemoglobin. Studies on the metabolism of iron have been of particular importance in throwing light on the problems of haemoglobin formation: in recent years, use of the radioactive isotope, ^{59}Fe, has much advanced knowledge of the absorption, transportation, utilization and excretion of iron, both in health and in disease.

The amount of iron normally present in the body is from 4 to 5 g. Of this, about 2·5 g are combined organically in haemoglobin (1 g of haemoglobin contains 3·39 mg of iron). From 1 to 1·5 g of iron is stored in the reticuloendothelial cells; a small quantity (about 200 mg) is present in the myoglobin of muscle, and in respiratory enzymes such as the peroxidases, and a trace (about 3 mg) is bound to a plasma globulin, transferrin (siderophilin). Despite its small quantity, the iron present in the transferrin fraction of the plasma proteins represents a vital link in the chain of iron metabolism.[19]

Although the quantity of iron in the plasma is so small, its turnover is rapid. Every day, a number of red cells equivalent to the normal red cell content of over 40 ml of blood is replaced, and this means that about 20 mg of iron have to be made available for the synthesis of the appropriate amount of haemoglobin. Some 18 mg of this comes from the recycling of haemoglobin iron released during the degradation of effete red cells in the reticuloendothelial system. Only 1 to 2 mg of iron are lost from the body daily, mainly in enzymes of cells desquamated from the skin and from the mucosa of the gastrointestinal and urinary tracts: to maintain a balance, this amount of iron must be absorbed daily from the alimentary canal. Menstruation accounts for the equivalent of a regular loss of 1 mg of iron daily throughout reproductive life. While this additional loss is ordinarily made good by a corresponding increase in iron absorption, a comparatively slight excess of blood loss during menstruation or a slight deficiency in the amount of iron absorbed may suffice to upset the balance. Even in the poorer diets in a country such as Britain there are probably about 15 mg of iron in the food consumed daily. In iron-deficiency anaemia iron administered therapeutically may raise the amount absorbed to over 20 mg a day.

Before it can be absorbed, much of the iron in food must be liberated from its combination with organic substances; this is effected by digestion in the stomach. Conversion of the iron into the ferrous form precedes its absorption through the mucosa of the duodenum and the upper part of the jejunum. Some foods contain large amounts of phosphates and phytic acid, which impair iron absorption. Achlorhydria does not itself block the absorption of therapeutically-administered iron, or even of iron in the food,[20] but indirectly it probably hinders the increased degree of absorption from the food that would be needed to prevent develop-

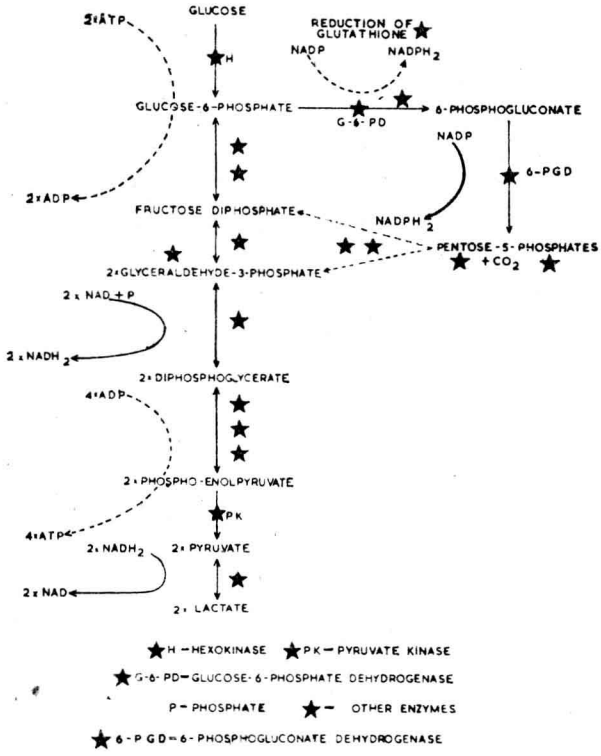


Fig. 8.6. The Embden–Meyerhof pathway, with pentose phosphate shunt. In addition to the reactions indicated in the diagram there is a lesser, but important, shunt at the first of the three steps in the conversion of diphosphoglycerate to phosphoenolpyruvate. In the direct pathway, 3-phosphoglycerate is formed by means of phosphoglyceric acid kinase while adenosine diphosphate (ADP) is converted to high energy adenosine triphosphate (ATP). In contrast, in the side pathway 2,3-diphosphoglycerate is formed as an intermediate product and energy is lost. These pathways are believed to alternate, when needed, to maintain the appropriate level of ADP ATP conversion.

ment of hypochromic anaemia when there is excessive loss of iron from the body.

When iron is absorbed by the epithelial cells of the intestinal mucosa, it is converted into the ferric form and bound to an intracellular protein, apoferritin, to form ferritin. The subsequent passage of the iron across the cell membrane to the iron-binding plasma globulin, transferrin, requires conversion from the ferric through the ferrous and back to the ferric form. Although the turnover of transferrin-bound iron is rapid, this transportation mechanism, operating from the intestine and from sites of haemoglobin degradation, may not suffice to supply all the 20 mg of the element required for the daily production of haemoglobin. It is possible that the macrophages in the marrow may transfer iron liberated from ingested effete red cells directly to nearby maturing erythropoietic cells (Fig. 8.15A, page 455).[21] But the iron from haemoglobin broken down in macrophages in the liver, spleen and other organs must be returned to the marrow by way of the plasma.

Although it is the metabolism of iron that has naturally received most attention in studies of erythropoiesis, there is evidence that certain trace metals, among them copper, cobalt and manganese, are also needed. Erythropoietic disorders may appear in farm animals grazing on soils deficient in these elements; there is little evidence that man, with his very mixed diets, is ever similarly affected.

Certain vitamins are intimately concerned in erythropoiesis; of great importance is the cobalamin, vitamin B_{12}. This has a complex tetrapyrrole molecule with a linking cobalt atom. The principal form used in therapy is hydroxocobalamin. In health nearly 0·5 μg of vitamin B_{12} is bound to protein (transcobalamins I and II) in the plasma for transport to the tissues, where it enters the cells, including the red cell precursors, and participates in nucleic acid synthesis. Ample quantities of this vitamin are present in the average diet, but a mucoprotein secreted by the mucosa of the fundus of the stomach—the intrinsic factor of Castle—is needed for its absorption from the small intestine. This factor may be missing as a result of atrophy of the mucosa (Fig. 8.17, page 457) or after extensive resection of the stomach. Alternatively, absorption of vitamin B_{12} may be impaired by disease of the lower part of the ileum, or by a change in the bacterial flora of the small intestine, such as may be brought about by the presence of a blind loop, diverticulum or stricture. The normal daily requirement of the vitamin is about 1 to 2 μg, and as about 3 mg are ordinarily stored in the body, even complete lack of intake of the vitamin would have little effect on haemopoiesis for several years. Usually, evidence of abnormal haemopoiesis is the first manifestation of a deficiency of vitamin B_{12}, although, as with iron deficiency, other types of cell may suffer before those of the marrow. Soreness and ulceration of the tongue, and even subacute combined degeneration of the spinal cord, may precede the development of anaemia.

Folic acid, which is a pteroylglutamic acid, is now known to be of fundamental importance in many metabolic processes, among them those concerned in erythropoiesis.[22] The daily requirement of this substance is much greater than that of vitamin B_{12}, and is measured in milligrams. In the body, folic acid is converted into folinic acid, which is required