Hematology

PHYSIOLOGY

☐ Sheet ☑ Slide ☐ Handout

Number: **1**

Subject: **Blood contents**

Doctor: **Salim Khrisha**

Date: **00/9/2016**

Price:

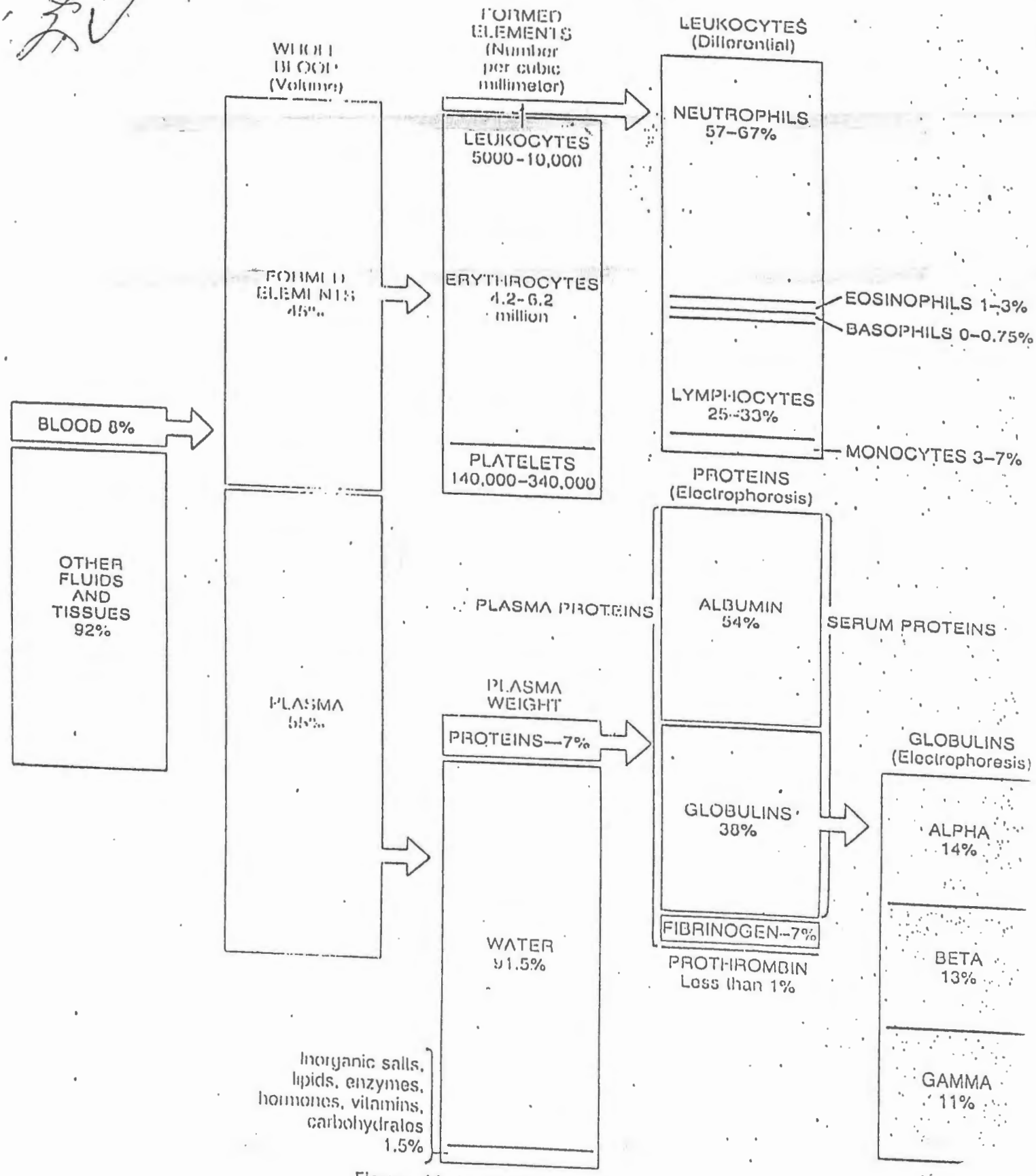

Figure 11–1. Composition of blood.

Table 13.2 The cellular elements of whole blood

Cell type	Site of production	Typical cell count (l^{-1})	Comments and function
Erythrocytes (red cells)	Bone marrow	5×10^{12} (men) 4.5×10^{12} (women)	Transport of O_2 and CO_2
Leukocytes (differential count)		7×10^9	
Granulocytes			
neutrophils	Bone marrow	5.0×10^9 (40–75%)	Phagocytes—engulf bacteria and other foreign particles
eosinophils	Bone marrow	100×10^6 (1–6%)	Congregate around sites of inflammation—have antihistamine properties; very short-lived in blood
basophils	Bone marrow	40×10^6 (<1%)	Circulating mast cells: produce histamine and heparin
Agranulocytes			
monocytes	Bone marrow	0.4×10^9 (2–10%)	Phagocytes, become macrophages when they migrate to the tissues
lymphocytes	Bone marrow, lymphoid tissue, thymus, spleen	1.5×10^9 (20–45%)	Production of antibodies
Platelets	Bone marrow	250×10^9	Aggregate at sites of injury and initiate hemostatis

Note that, while mean values are given, these are subject to considerable individual variation. The approximate percentage of individual types of leukocyte are given after the number per litre—this is called the differential white cell count.

Table 16.3 Constituents of Plasma

Constituent	Amount/Concentration*
pH	7.35 to 7.45
Water	*90% of plasma*
Electrolytes (inorganic)	*<1% of plasma*
Na^+	142 mEq/l (142 mmol/l)
K^+	4 mEq/l (4 mmol/l)
Ca^{2+}	5 mEq/l (2.5 mmol/l)
Mg^{2+}	3 mEq/l (1.5 mmol/l)
Cl^-	107 mEq/l (107 mmol/l)
HCO_3^-	27 mEq/l (27 mmol/l)
Phosphate (mostly HPO_4^{2-})	4 mEq/l (2 mmol/l)
SO_4^{2-}	1 mEq/l (0.5 mmol/l)
Gases	*about 1% of plasma*
CO_2	60 ml/100 ml plasma
O_2	0.2 ml/100 ml
N_2	0.9 ml/100 ml
Nutrients	*about 3% of plasma*
Glucose and other carbohydrates	100 mg/100 ml
Amino acids	40 mg/100 ml
Lipids	500 mg/100 ml
Cholesterol	150–250 mg/100 ml
Vitamins	traces
Trace elements	traces
Waste products	*about 1% of plasma*
Urea	<20 mg/100 ml
Creatinine	<1 mg/100 ml
Uric acid	5 mg/100 ml
Bilirubin	0.2–1.2 mg/100 ml
Proteins	*6% of plasma (2.5 mmol/l)*
Albumins	4.5 g/100 ml
Globulins	2.5 g/100 ml
Fibrinogen	0.3 g/100 ml
Hormones	*traces*

*Concentrations for some substances are expressed in both millimoles (mmol) and milliequivalents (mEq). One millimole is one-thousandth of a gram molecular weight of a substance. For substances that have a valence of 1, mEq and mmol are equal; for substances that have a valence of 2, 2 mEq equal 1 mmol.

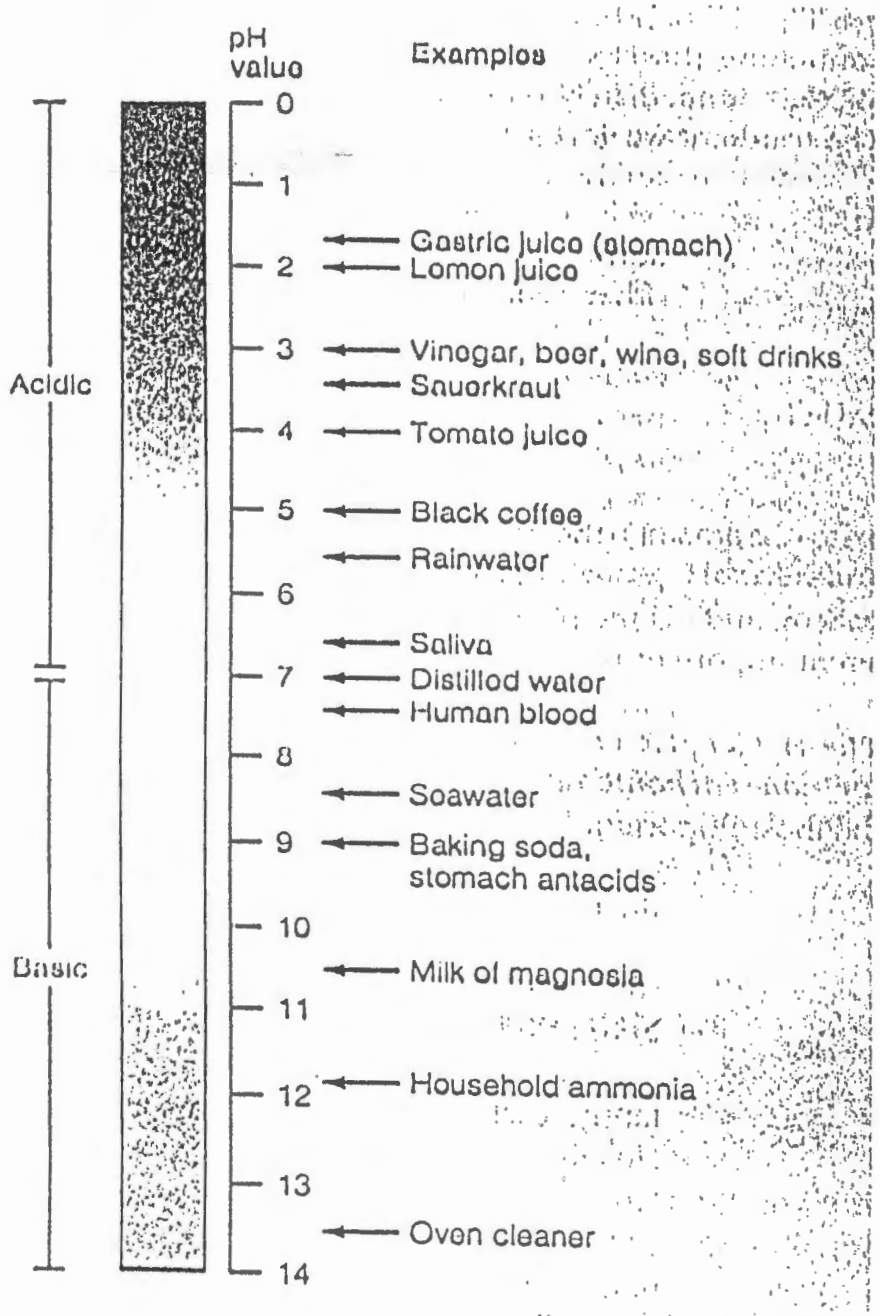

Figure 15-7 Comparison of pH Values of Common Solutions

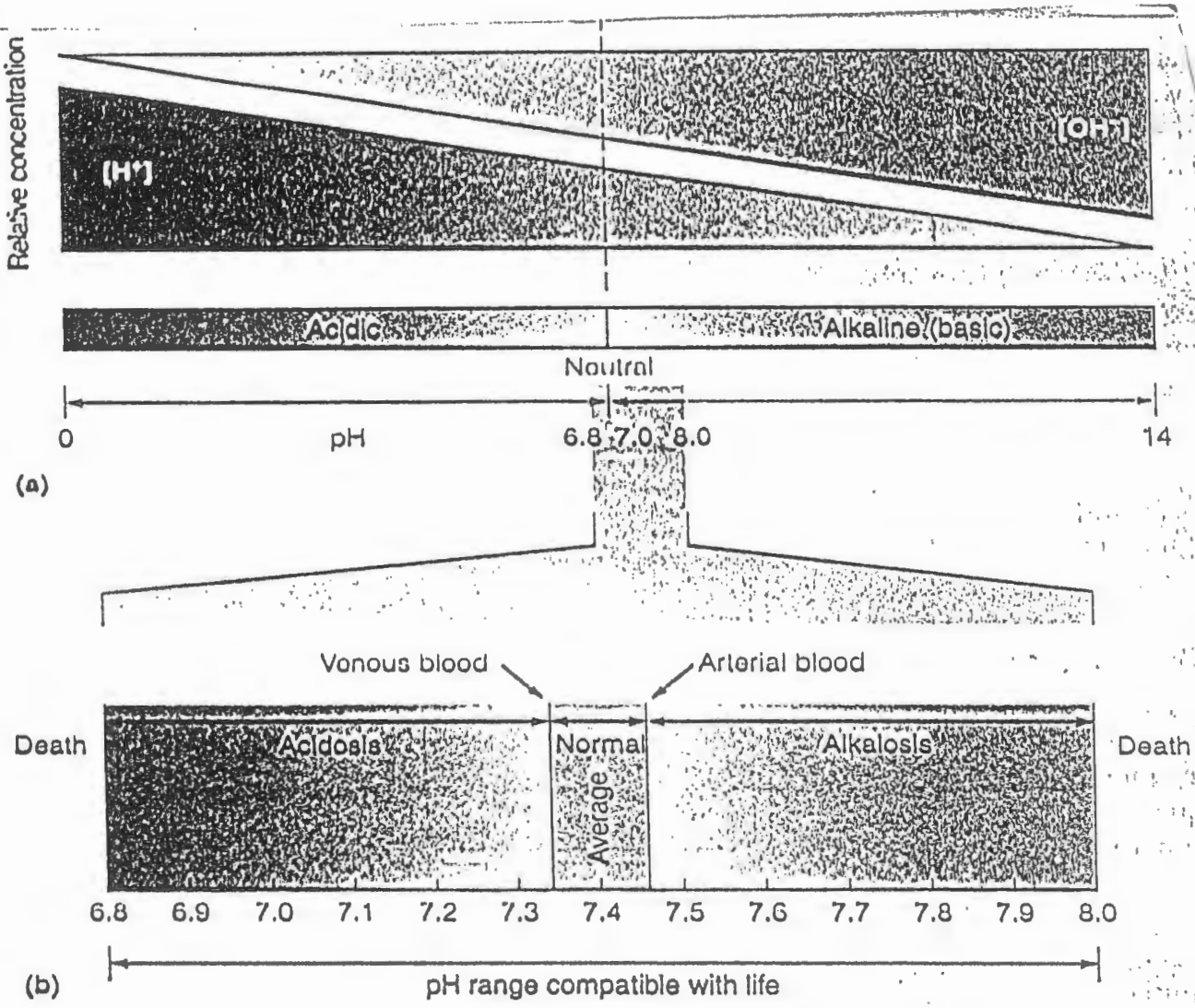

Figure 15-6 pH Considerations in Chemistry and Physiology
(a) Relationship of pH to the relative concentrations of H^+ and base (OH^-) under chemically neutral, acidic, and alkaline conditions. (b) Plasma pH range under normal, acidosis, and alkalosis conditions.

The principal role of the blood,

The blood is a vital vehicle of communication between the tissues of multi-cellular organisms. Its numerous functions include the following:

(1) delivery of nutrients from gut to tissues;

(2) gas exchange: the carriage of oxygen from the lungs to the tissues, and carbon dioxide from the tissues to the lungs.;

(3) transport of the waste products of metabolism from the sites of production to the sites of disposal;

(4) carriage of hormones from endocrine glands to specific target tissues; and

(5) protection against invading organisms—its immunological role.

Table 13.1 Principal constituents of the plasma

Constituent	Quantity	Units	Remarks
Water	94.5	g l^{-1}	
Bicarbonate	25	mmoles l^{-1}	Important for the carriage of CO_2 and for H^+ buffering
Chloride	105	mmoles l^{-1}	The principal extracellular anion
Inorganic phosphate	1.1	mmoles l^{-1}	
Calcium	2.5	mmoles l^{-1}	This is total calcium; ionized calcium is about 1.5 mmoles l^{-1}
Magnesium	0.8	mmoles l^{-1}	
Potassium	4	mmoles l^{-1}	
Sodium	144	mmoles l^{-1}	The principal extracellular cation
Hydrogen ions	40	nmoles l^{-1}	This corresponds to a pH value of c. 7.4
Glucose	4.5	mmoles l^{-1}	Major source of metabolic energy, particularly for the CNS
Cholesterol	2.0	g l^{-1}	
Fatty acids (total)	3.0	g l^{-1}	
Total protein	70–85	g l^{-1}	
Albumin	45	g l^{-1}	Principal protein of the plasma; binds hormones and fatty acids
α-Globulins	7	g l^{-1}	
β-Globulins	8.5	g l^{-1}	
γ-Globulins	10.6	g l^{-1}	Immunoglobulins (antibodies)
Fibrinogen	3	g l^{-1}	Blood clotting
Prothrombin	1	g l^{-1}	Blood clotting
Transferrin	2.4	g l^{-1}	Iron transport

Note that these values are approximate mean values and that even in health there is considerable individual variation.

The concentration of osmotically active particles is usually expressed in osmoles. One osmole (osm) equals the gram-molecular weight of a substance divided by the number of freely moving particles that each molecule liberates in solution. The milliosmole (mosm) is 1/1000 of 1 osm.

If a solute is a nonionizing compound such as glucose. the osmotic pressure is a function of the number of glucose molecules present. If the solute ionizes & forms an ideal solution, each ion is an osmotically active particle. For example, NaCl would dissociate into Na+ & Cl- ions. so that each mole in solution would supply 2 osm. One mole of Na_2SO_4 would dissociate into Na-, Na+ & SO_4^{2-} , supplying 3 osm.

The osmolarity is the number of osomoles per liter of solution - e.g. plasma whereas the osmolality is the number of osmoles per kilogram of solvent. Therefore , osmolality is affected by the volume of the various solutes in the solution & the temperature. While osmolality is not.

The freezing point of normal human plasma averages -0.54 °C, which corresponds to an osmolal concentration in plasma of 290 mosm/L. Solutions that have the same osmolality as plasma are said to be isotonic : those with greater osmolality are hypertonic ; & those with lesser osmolality are hypotonic.

The circulating blood volume is about 7–8 per cent of body weight, so that for a 70 kg man blood volume will be around 5 liters, but for a newborn baby weighing 3.2 kg (7 lb), blood volume will only be around 250 ml—an important point to remember when considering a blood transfusion on a small baby.

At any one time, assuming a blood volume of 5 liters, about 1 liter will be in the lungs, about 3 liters in the systemic venous circulation and the remaining liter in the heart, systemic arteries, arterioles, and capillaries (

Table 9.1 Approximate percentage distribution of the blood volume in an adult at rest

Veins	65%–75% (Capacitance)
Arteries	10%–15% (Resistance)
Capillaries	5%
Heart	5%
Lungs	10%

VARIATIONS UNDER DIFFERENT PHYSIOLOCAL CONDITIONS

1. **SEX**: for males the blood volume is 10% higher than in females. This is due to greater number of RBc.

2. **PREGNANCY**: B.V. rises due to increase in **both** cells & plasma. In pregnant women B.V. increases on the average by about 20 to 30%, in the last few weeks of pregnancy.

3. **MUSCULAR EXPERCISE**: It raises B.V. probably due to contraction of spleen.

4. **POSTURE**: In erect posture there is about 15% diminution of total plasma. It passes out into the tissue spaces.

5. **BLOOD PRESSURE**: Rise of B.P. lowers B.V. by pressing out more fluid into the tissue spaces.

6. **AL TITUDE**: At higher altitude the B.V. will rise. Due to hypoxia the number of RBc will increase.

7. **ADERNALINE INJECTION**: Raises B.V. probably by contraction of spleen.

The plasma proteins generally are synthesized by the liver, with the exception of the gamma globulins, which are produced by lymphocytes,.

Summary of the functions of plasma proteins

1 Transport functions (α- and β-globulins).

2 Defensive (immunoglobulins).

3 Reserve of body proteins

4 Osmotic function (albumin) through control of the exchange of fluid between blood and tissues

5 Viscosity of plasma is due mainly to fibrinogen and globulins.

6 Fibrinogen is the precursor of fibrin in the blood clot. Prothrombin is an α_2-globulin and most of the remaining clotting factors are β-globulins.

1. Non-nucleated circular biconcave disc shape.
2. They change shape remarkably as the cells pass through the blood vessels.

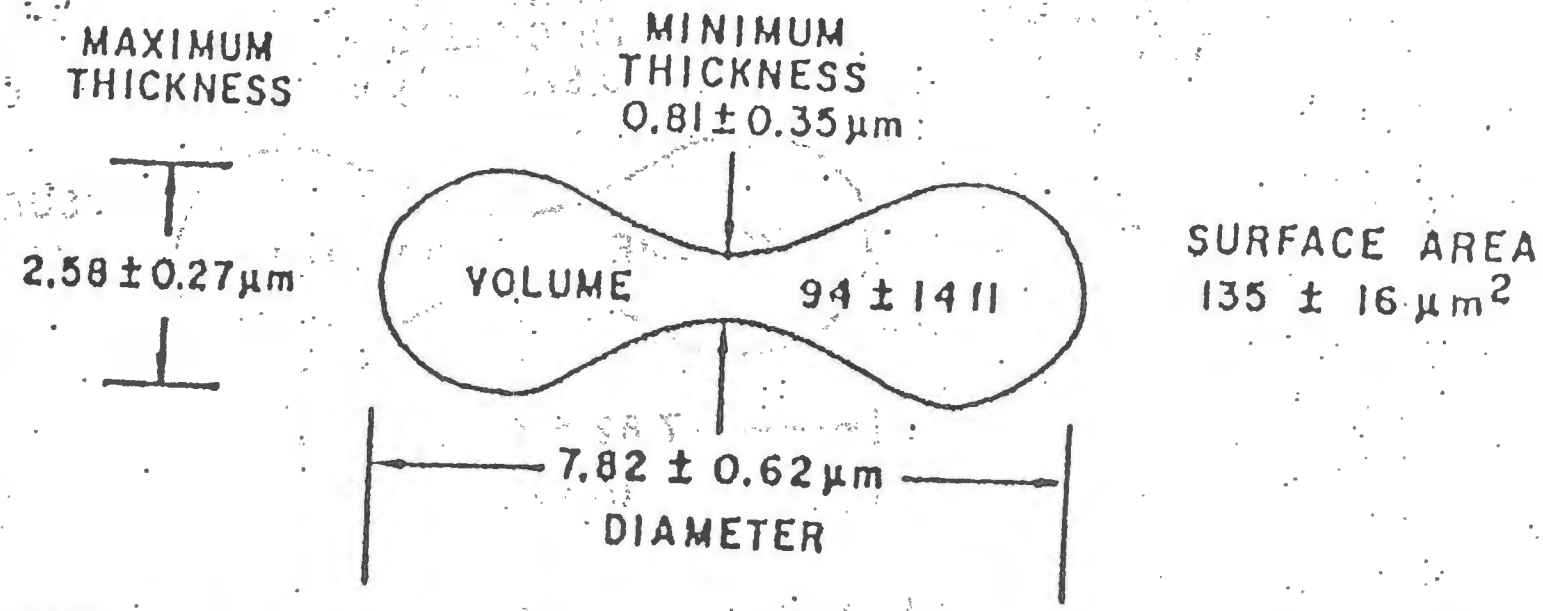

FIG. 4-3. Dimensions of a cross section of the erythrocyte in isotonic solution. Values are means ± one standard deviation.[20]

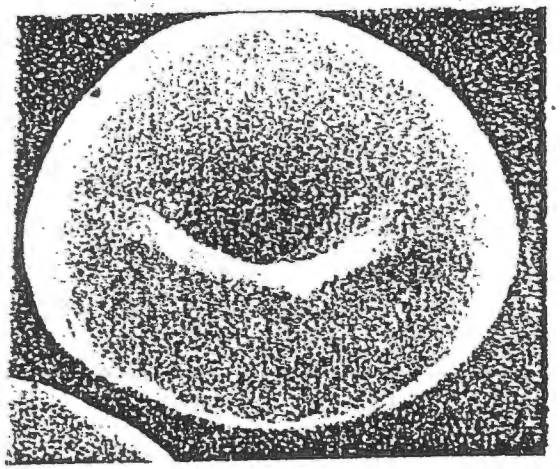

Table 3.3.
Approximate Concentrations and Daily Production Rates of Peripheral Blood Cells

Cell Type	Mean Concentration, per microliter	Daily Production Rate, per kg body weight
Erythrocytes		
Males	5.4×10^6 ($4.7–6.1 \times 10^6$)	3.0×10^9
Females	4.8×10^6 ($4.2–5.4 \times 10^6$)	
White blood cells		
Granulocytes	4500 (2600–7000)	1.6×10^9
Monocytes	300	1.7×10^8[a]
Eosinophils	150	Variable
Basophils	40	Unknown
Lymphocytes	2500 (1500–4000)	Unknown
Platelets	2.5×10^5 ($1.5–4.0 \times 10^5$)	2.8×10^9

Values in parentheses are ranges. [a]Based upon assuming production rate equals blood turnover rate and using an intravascular half-disappearance time of 8.4 h.

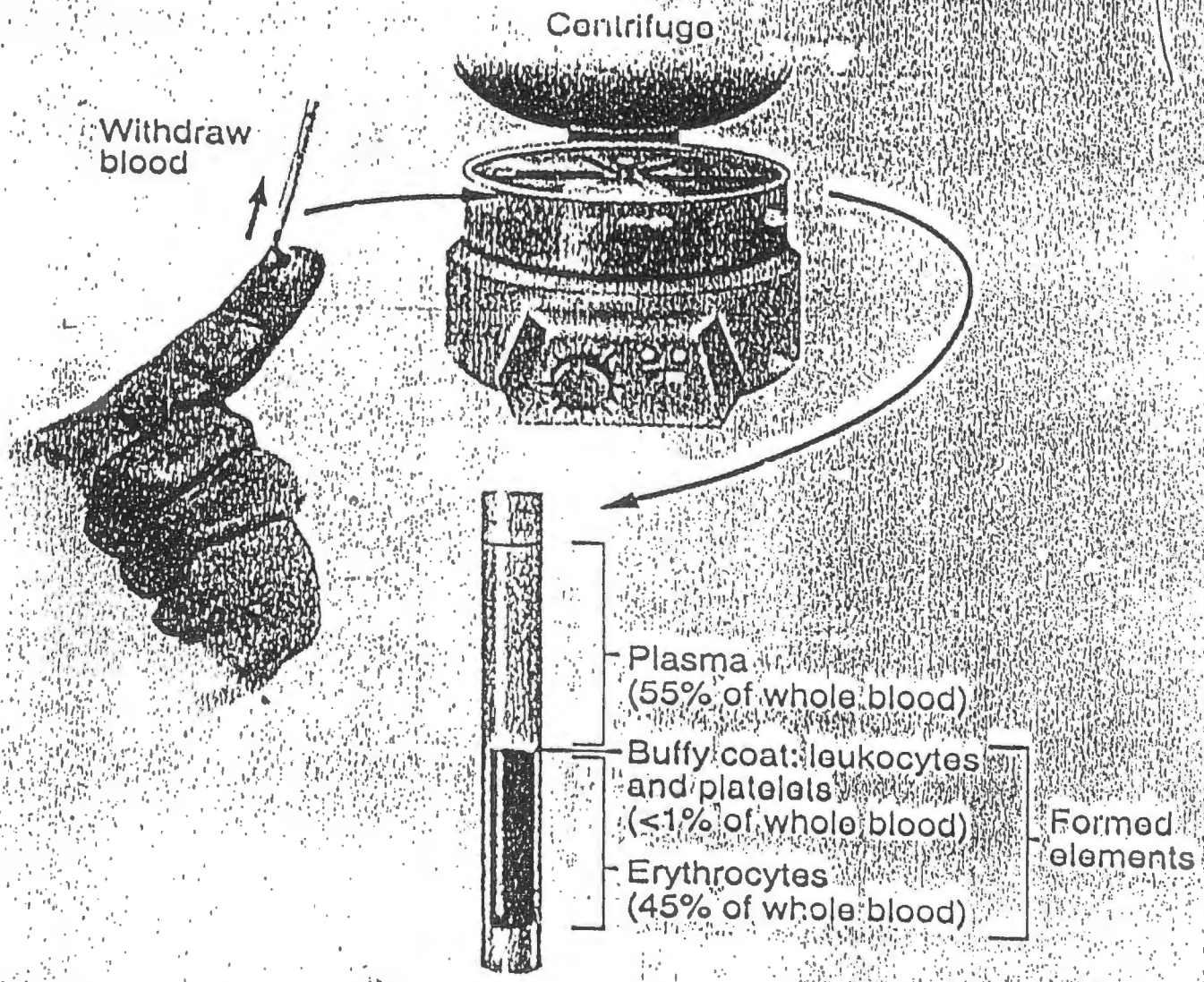

Figure 18.2 The Hematocrit. A small sample of blood is taken in a glass tube and spun in a centrifuge to separate the cells from the plasma. The percent volume of red cells (hematocrit) is then measured. In this example, the hematocrit is 45%.

HCT
or
Packed Cell Volume
PCV

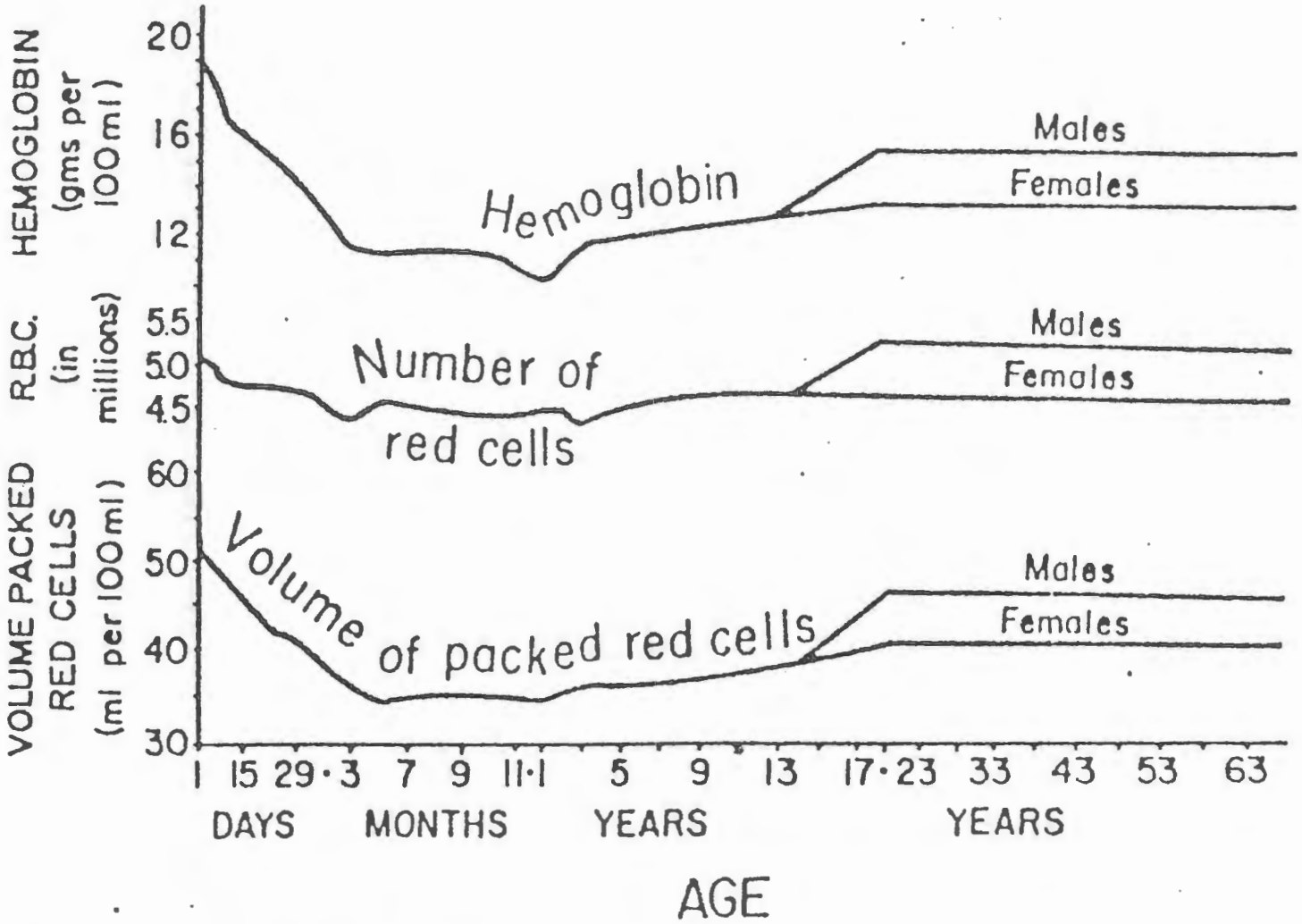

Figure 4–1. Relationship of age and sex to the hemoglobin content, red blood cell count, and hematocrit of the blood.

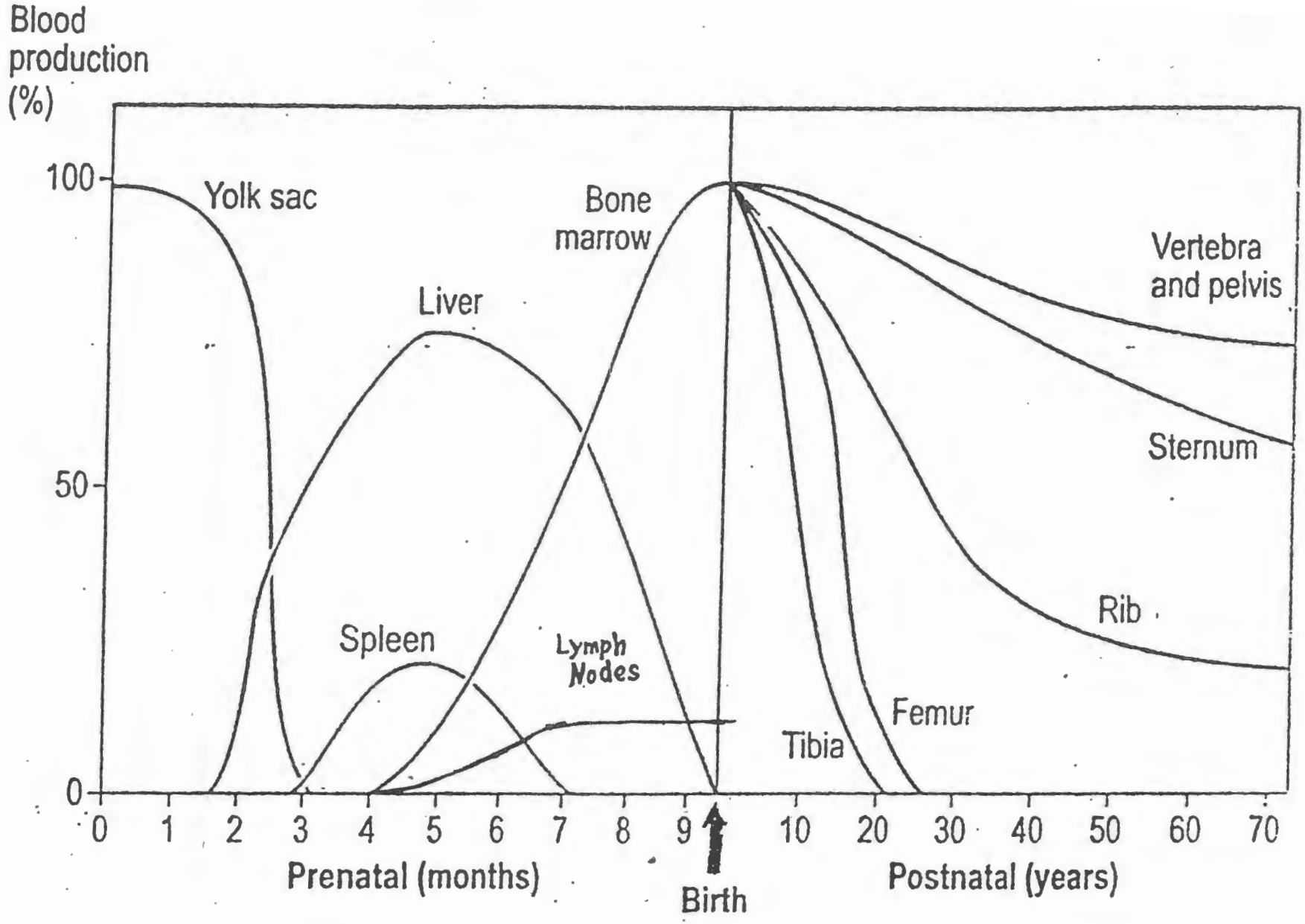

Fig. 1 Sites of blood production in the fetus and after birth. (Erythropoiesis)

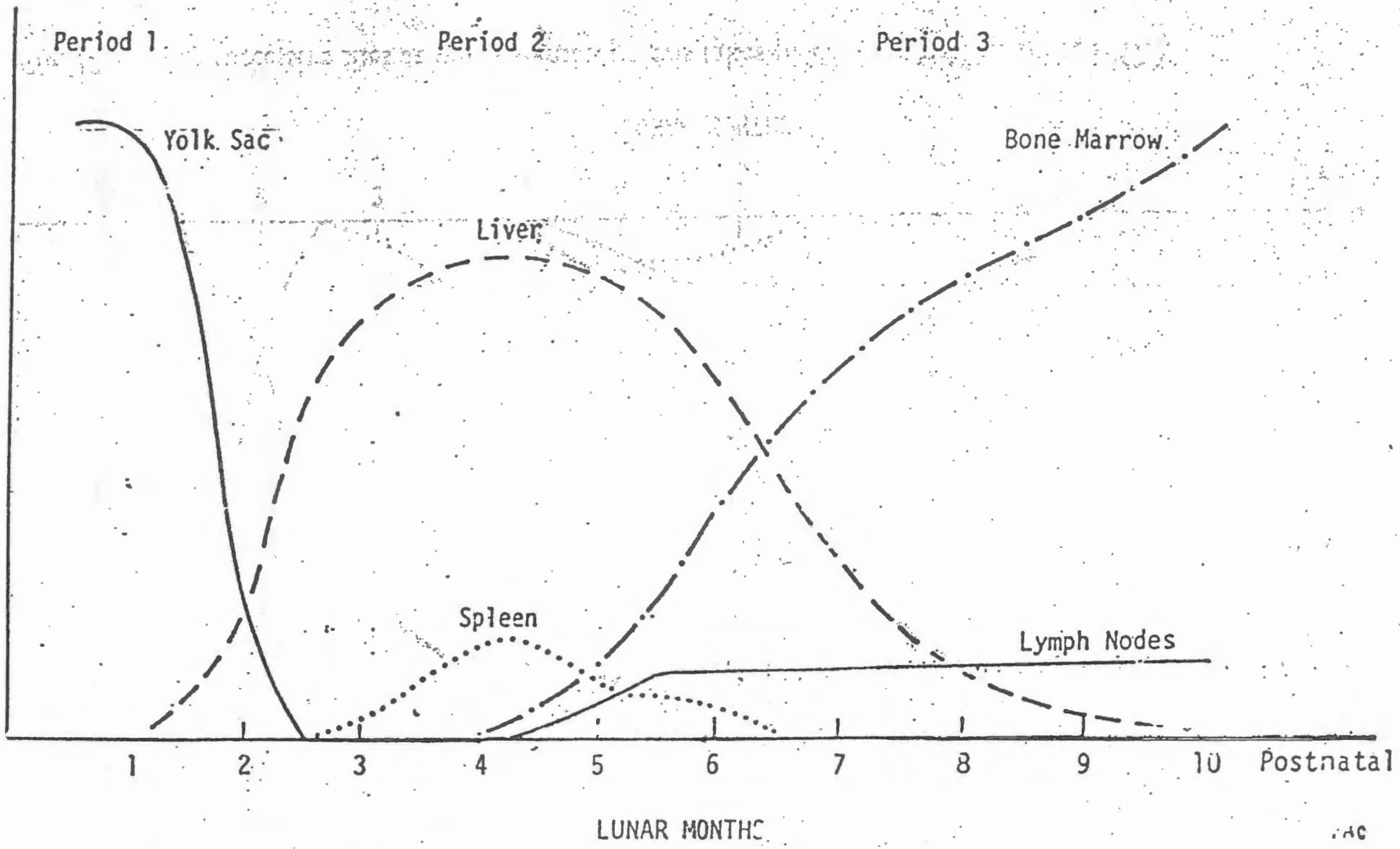

Fig. 17-3. The successive sites at which hematopoiesis takes place. (From Wintrobe, 1967.)

Foetus 0–2 months—yolk sac
2–7 months—liver, spleen

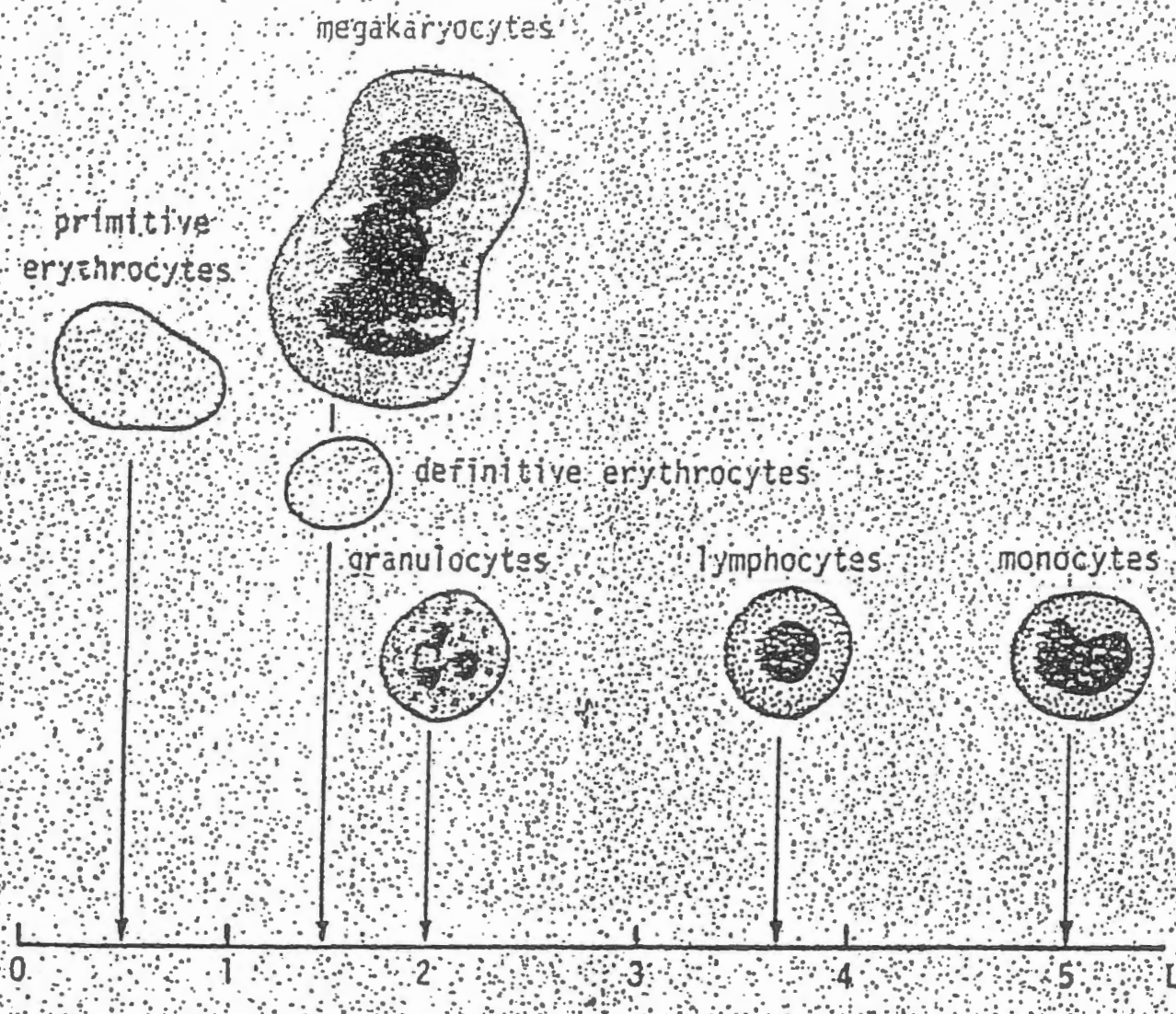

Fig. 17-6. The successive appearance of the different forms of blood cells.

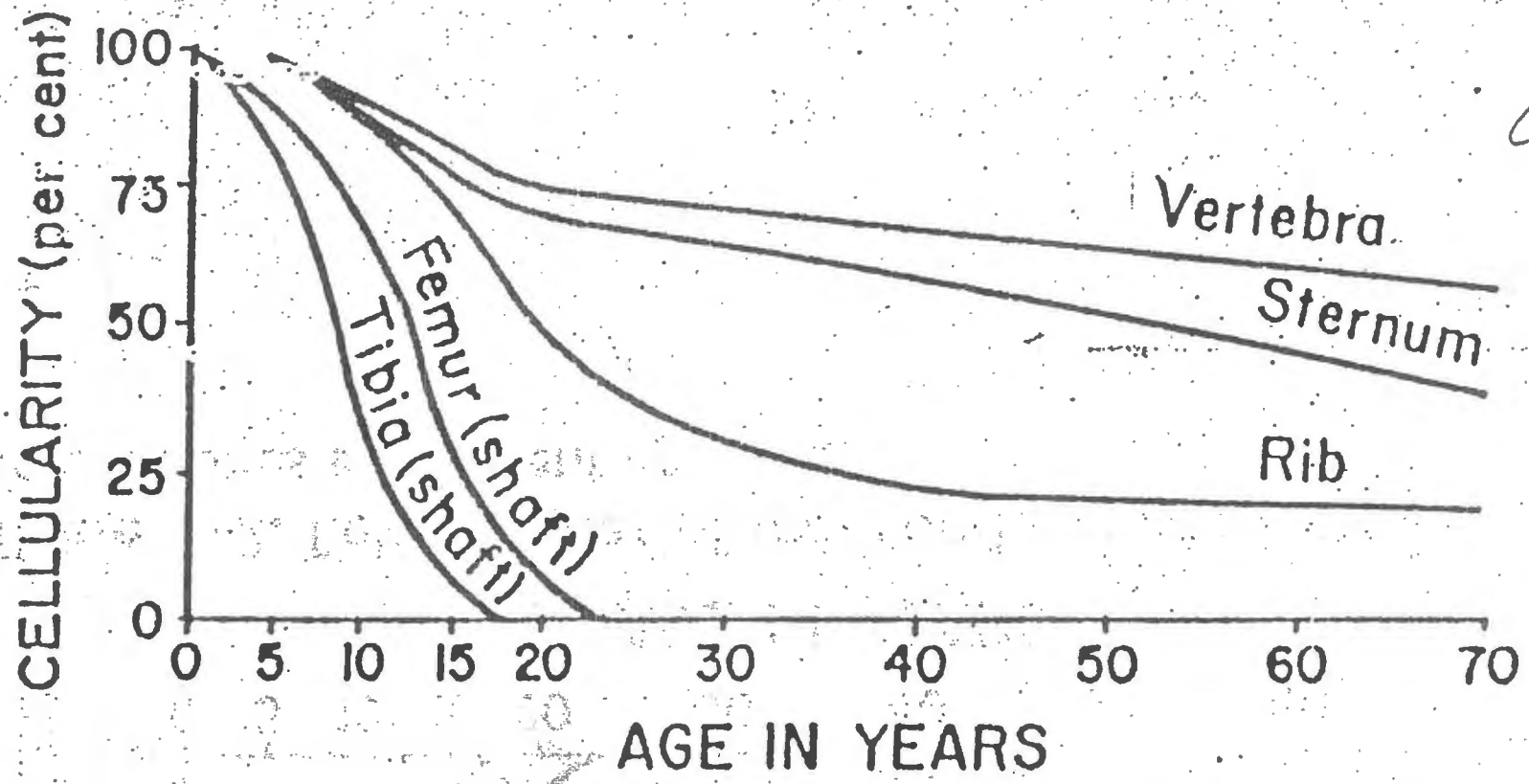

Figure 4–2. Relative rates of red blood cell production in the different bones at different ages.

Infants	Bone marrow (practically all bones)
Adults	Vertebrae, ribs, sternum, skull, sacrum and pelvis, proximal ends of femur

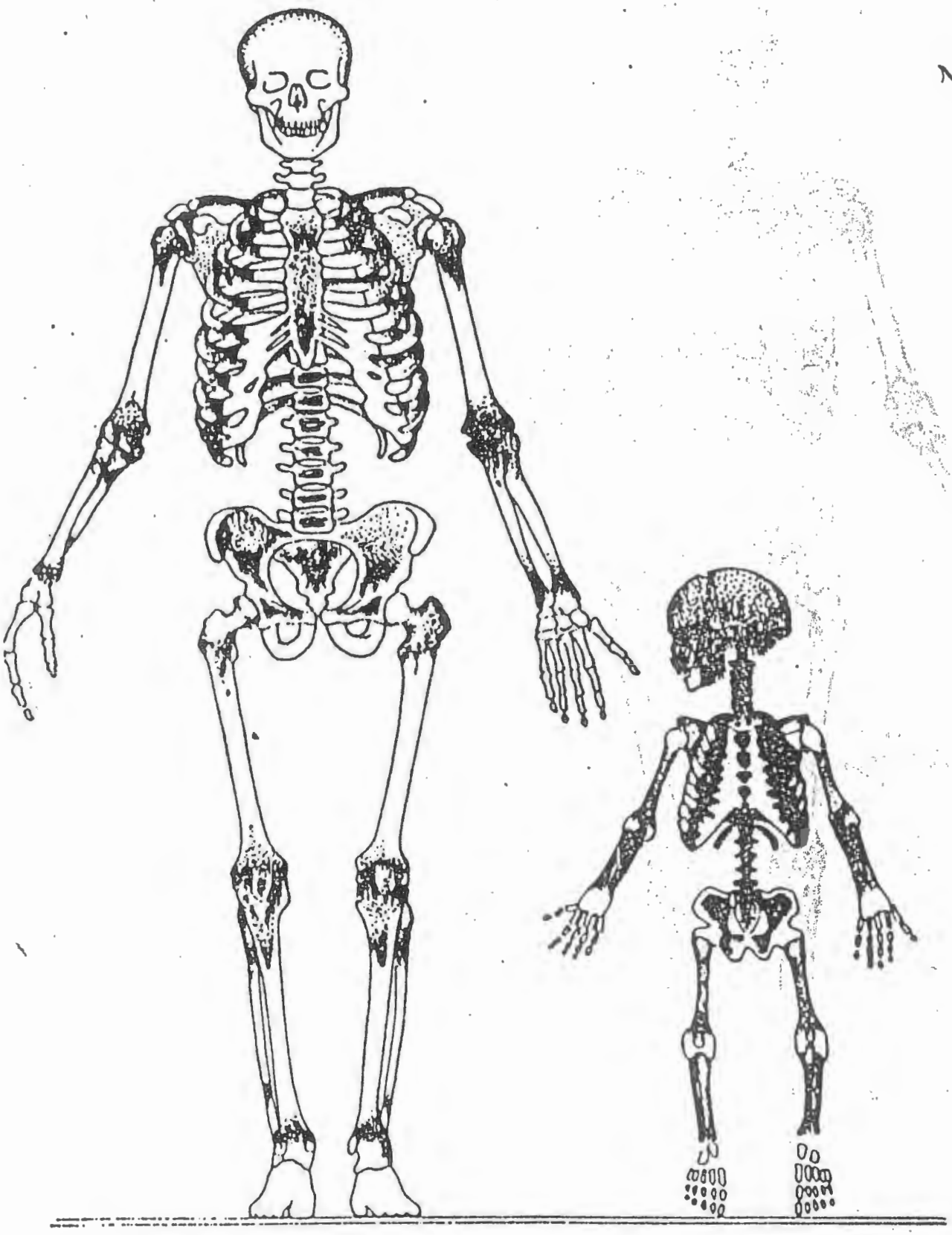

Fig. 8.4 Sites of active haematopoietic marrow (red marrow) in children and adults. There is a similar amount of red marrow (1000 to 1500 g) in each despite the differences in body weight. (*From* Bierman, H. R. (1961) In *Functions of the Blood*, ed. MacFarlane, R. G. & Robb-Smith, A. H. T., p. 357: Oxford: Blackwell.)

Hypoxia	Insufficient O_2 at the cellular level
Anemic hypoxia	Reduced O_2-carrying capacity of the blood
Circulatory hypoxia	Too little oxygenated blood delivered to the tissues; also known as stagnant hypoxia
Histotoxic hypoxia	Cells unable to utilize O_2 available to them
Hypoxic hypoxia	Low arterial blood P_{O_2} accompanied by inadequate hemoglobin saturation

Biogenesis of EP.

I. Kidney

↓ ← Hypoxia

REF (or pro. EP)

↓ ← plasma Globulin

EP

⟷

II. Kidney

↓ Hypoxia

REF (or EP activator)

↓

pro. EP (or plasma factor) → **EP**

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① EP. is glycoprotein ⟶ 70% protein, 30% $C_6H_{12}O_6$ ② M.W. 40^{10^3} – $70,^{10^3}$

③ Is found in plasma and urine.

④ stimulated by hypoxia and Inhibited by hyperoxia

⑤ Its half life 5 – 10 hours.

⑥ 90% of it released by kidneys and 10% from extra renal sources (e.g. like spleen and liver).

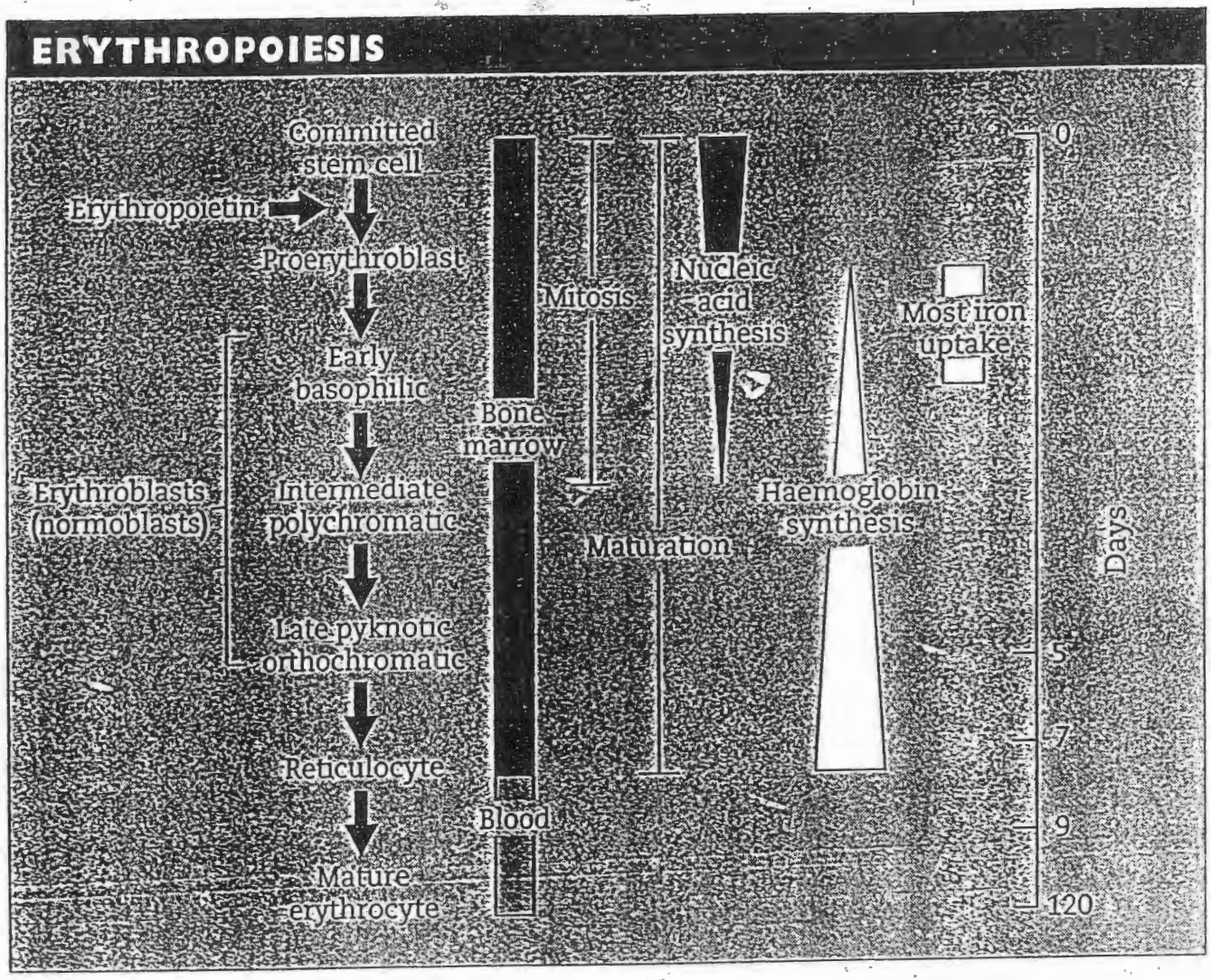


Fig. 12.3 Normal erythropoiesis.

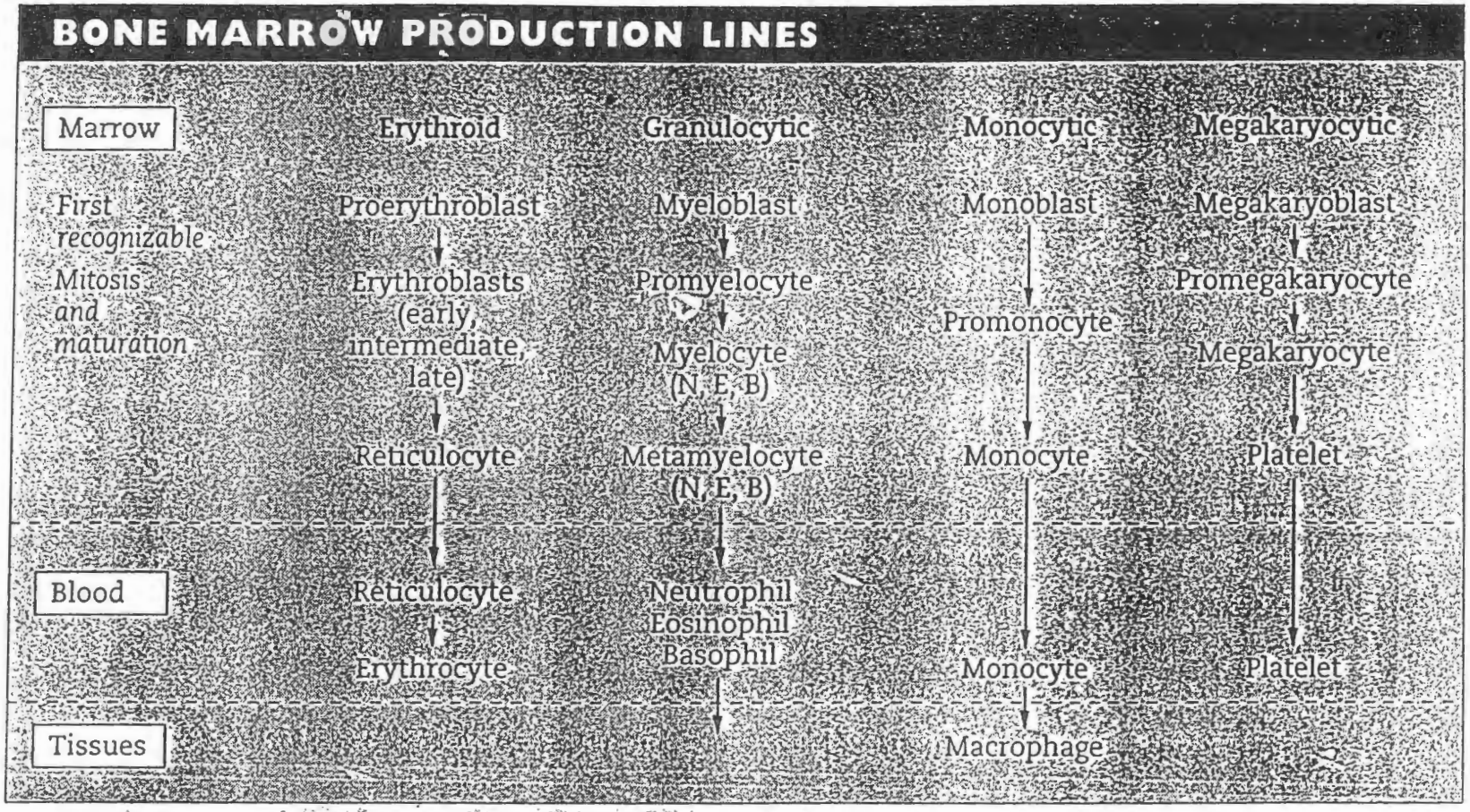


Fig. 12.2 Bone-marrow production lines. B, basophil; E, eosinophil; N, neutrophil.

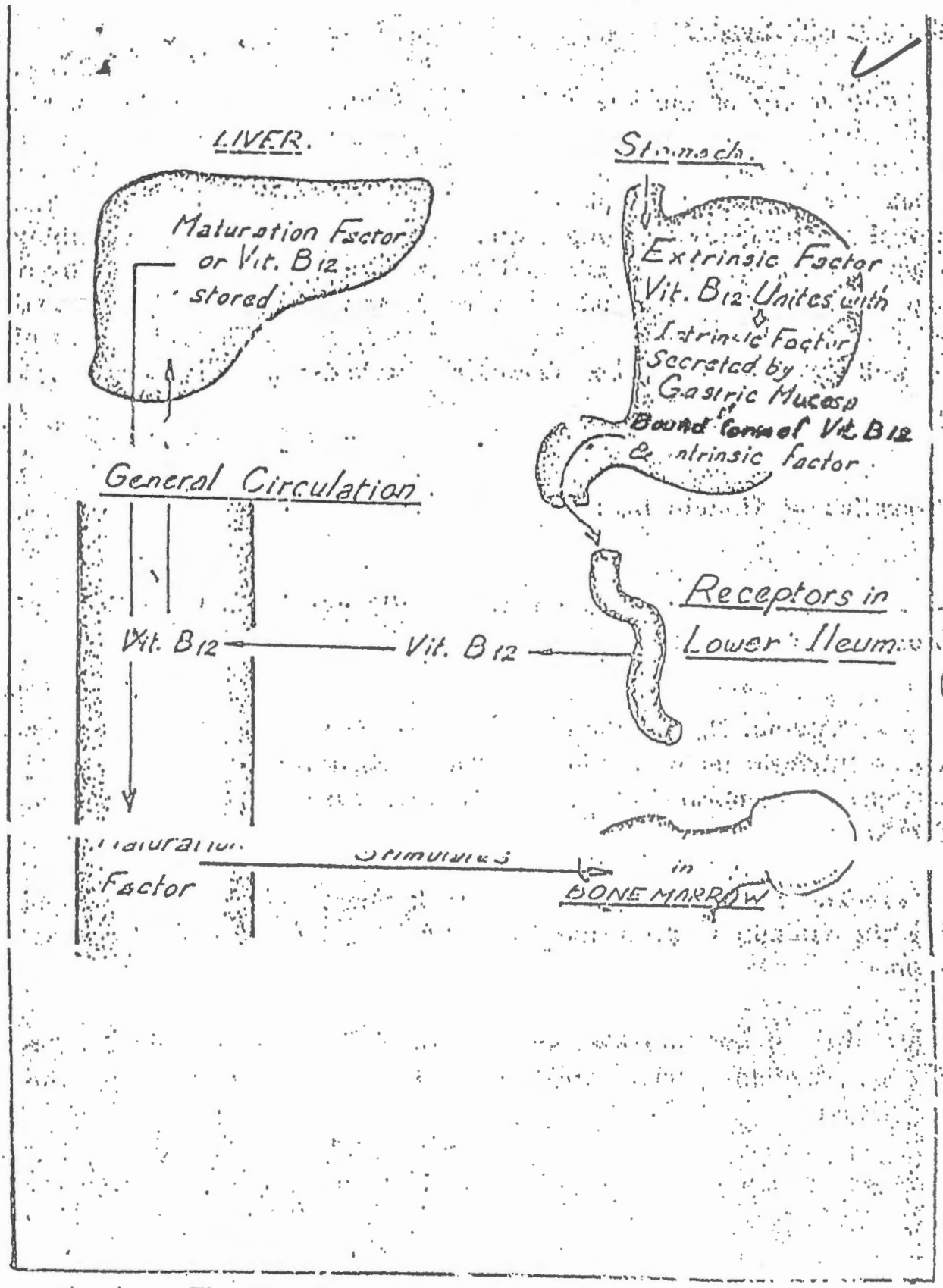


Fig. 11 : Absorption, storage and action of vit. B_{12}

Causes of vitamin B_{12} deficiency

1. VEGANISM
2. MALABSORPTION

a- gastric causes

Congenital lack of IF
Total or partial gastrectomy

b. intestinal causes

Chronic tropical sprue
Ileal resection

Causes of folate deficiency

1- Inadequate dielary inlake

2- Malabsorption

Coeliac disease, jejunal resection, tropical sprue

3- Increased requirement

Pregnancy, premature infants, chronic haemolytic anaemias

IRON REQUIREMENTS

The amount of iron required each day to compensate for losses from the body and growth varies with age and sex; it is highest in pregnancy and in adolescent and menstruating females (Table 2.3). These groups, therefore, are particularly likely to develop iron deficiency if there is additional iron loss or prolonged reduced intake.

Table 2.3 Estimated daily iron requirements. Units are mg/day.

	Urine, sweat, faeces	Menses	Pregnancy	Growth	Total
Adult male	0.5–1				0.5–1
Post-menopausal female					
Menstruating female*	0.5–1	0.5–1			1–2
Pregnant female*	0.5–1		1–2		1.5–3.0
Children (average)	0.5			0.6	1
Female (age 12–15)*	0.5–1	0.5–1		0.6	1–2.5

* These groups more likely to develop iron deficiency.

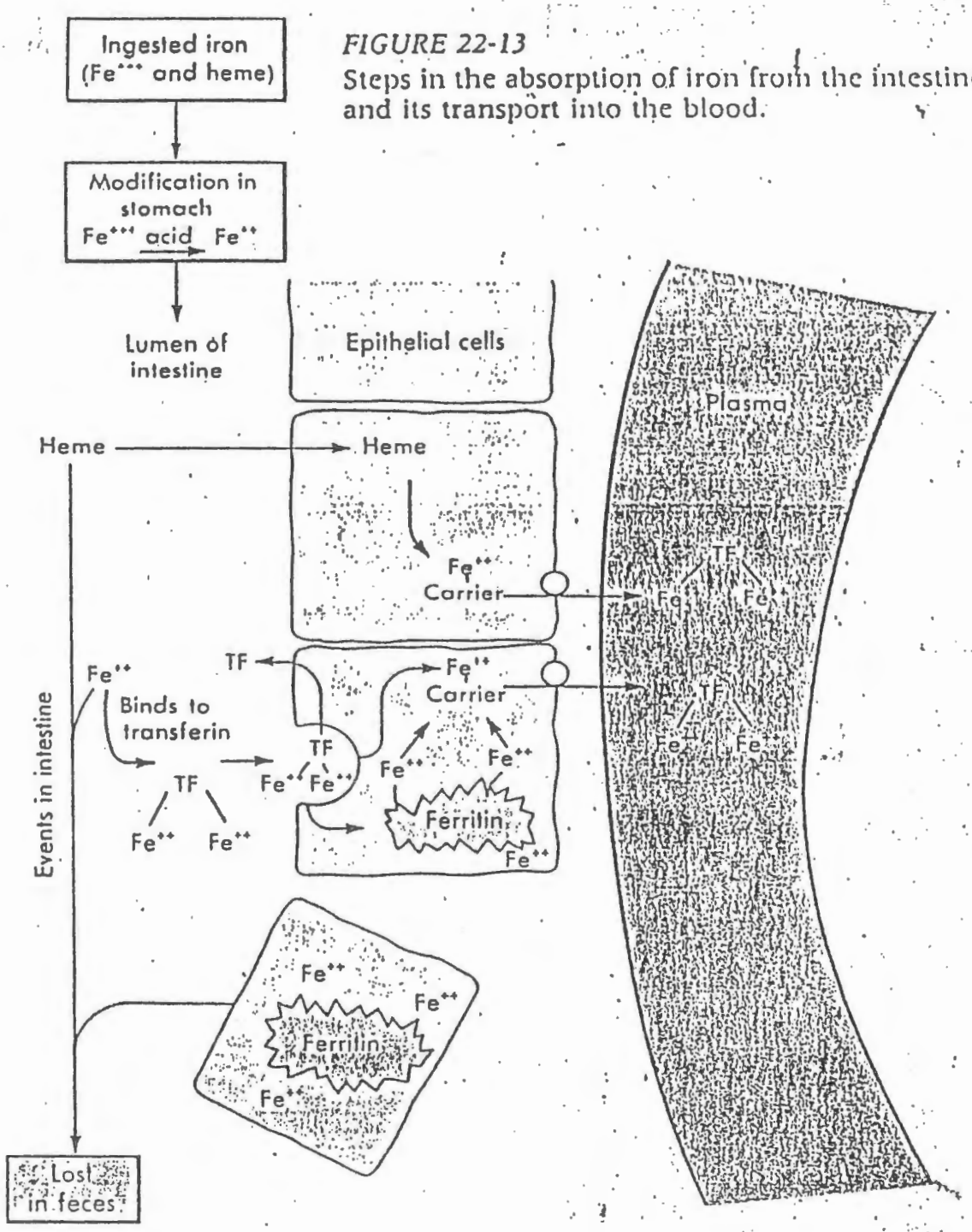


FIGURE 22-13
Steps in the absorption of iron from the intestine and its transport into the blood.

Table 2.2 Iron absorption.

Factors favouring	Factors reducing
1 Ferrous form	1 Ferric form
2 Inorganic iron	2 Organic iron
3 Acids—HCl, vitamin C	3 Alkalis—antacids, pancreatic secretions
4 Solubilising agents—e.g. sugars, amino acids	4 Precipitating agents—phytates, phosphates
5 Iron deficiency	5 Iron excess
6 Increased erythropoiesis	6 Decreased erythropoiesis
7 Pregnancy	7 Infection
8 Primary haemachromatosis	8 Tea
	9 Desferrioxamine

Table 2.1 The distribution of body iron.

	Amount of iron in average adult		
	Male (g)	Female (g)	% of total
Haemoglobin	2.4	1.7	65
Ferritin and haemosiderin	1.0 (0.3–1.5)	0.3 (0–1.0)	30
Myoglobin	0.15	0.12	3.5
Haem enzymes (e.g. cytochromes, catalase, peroxidases, flavoproteins)	0.02	0.015	0.5
Transferrin-bound iron	0.004	0.003	0.1

Table 2.4 Causes of iron deficiency.

1 BLOOD LOSS

Uterine.

Gastrointestinal. e.g. oesophageal varices, hiatus hernia, peptic ulcer, aspirin ingestion, partial gastrectomy, carcinoma of stomach or caecum, colon or rectum, hookworm, angiodysplasia, colitis, piles, diverticulosis, etc.

Rarely haematuria, haemoglobinuria, pulmonary haemosiderosis, self-inflicted blood loss.

2 INCREASED DEMANDS (see also Table 2.3)

Prematurity.

Growth.

Child-bearing.

3 MALABSORPTION

e.g. gastrectomy, coeliac disease.

4 POOR DIET

A contributory factor in many countries but rarely the sole cause.

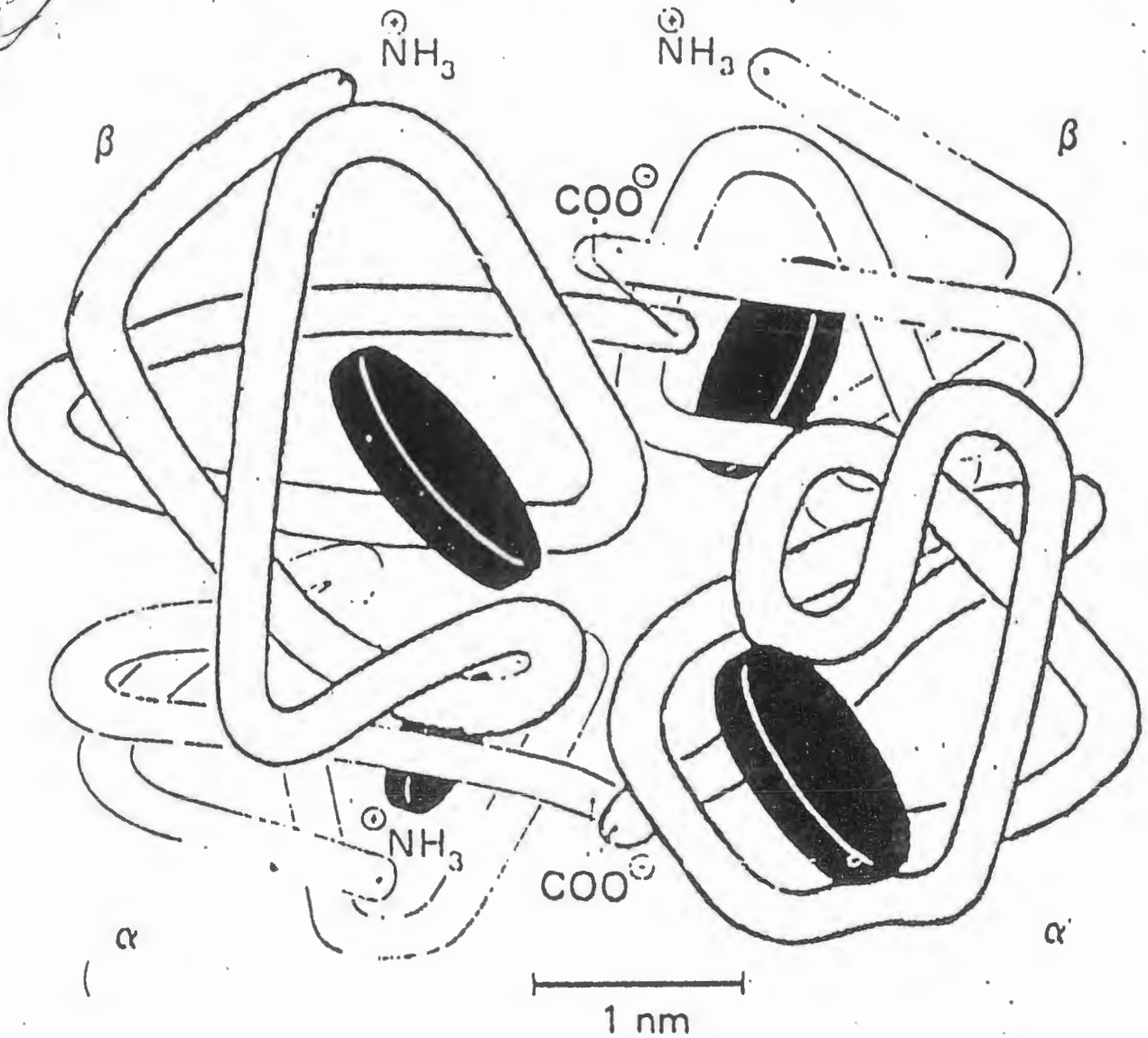


Figure 27–10. Diagrammatic representation of a molecule of hemoglobin A, showing the 4 subunits. There are two α and two β polypeptide chains, each containing a heme moiety. These moieties are represented by the disks. (Reproduced, with permission, from Harper HA et al: *Physiologische Chemie*. Springer-Verlag, 1975.)

*Each α = 141 a.a

*Each β = 146 a.a

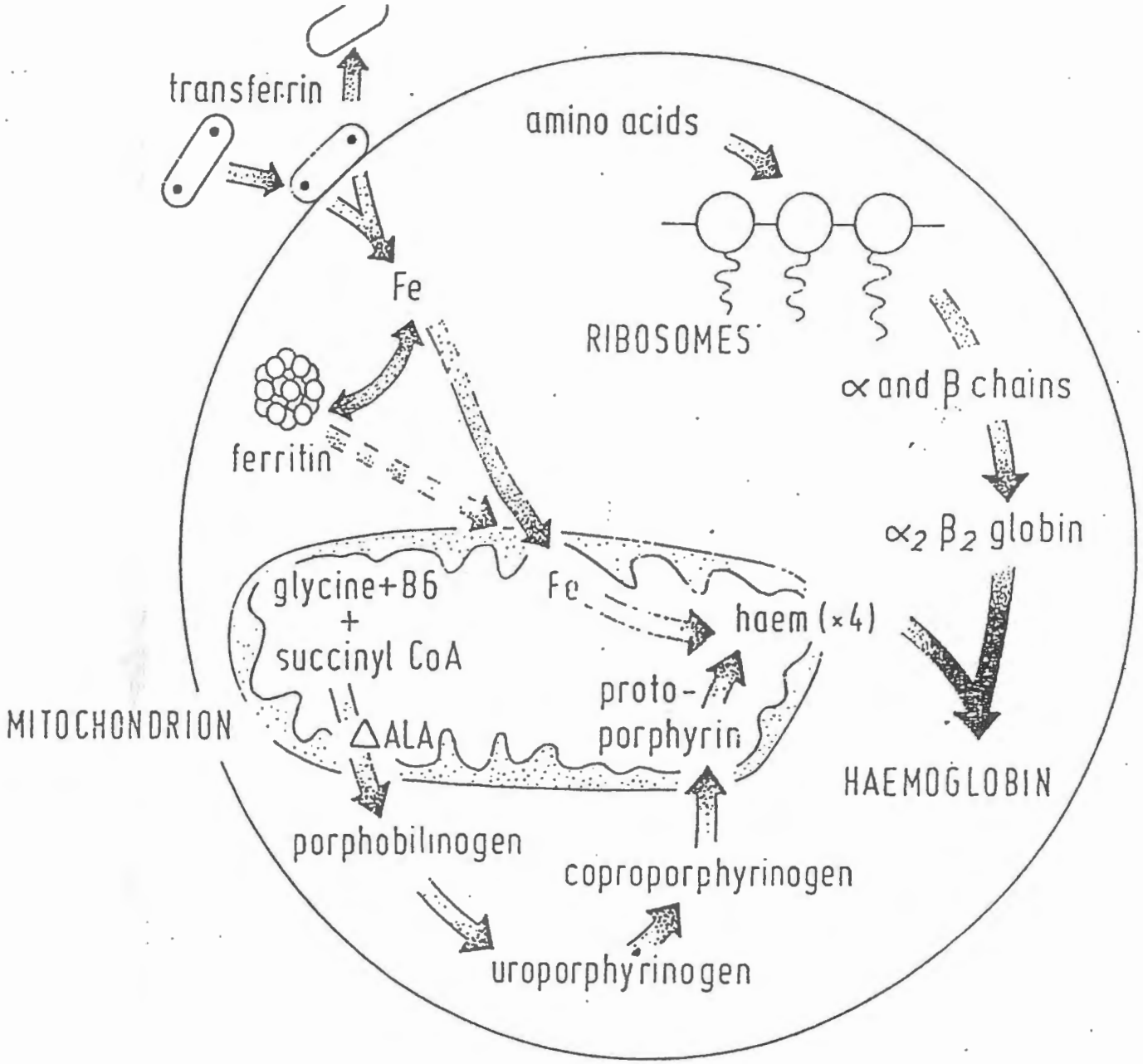


Fig. 1.7 Haemoglobin synthesis in the developing red cell. The mitochondria are the main site of protoporphyrin synthesis, iron is supplied from circulating transferrin and globin chains are synthesised on ribosomes. ΔALA = delta-amino laevolinic acid.

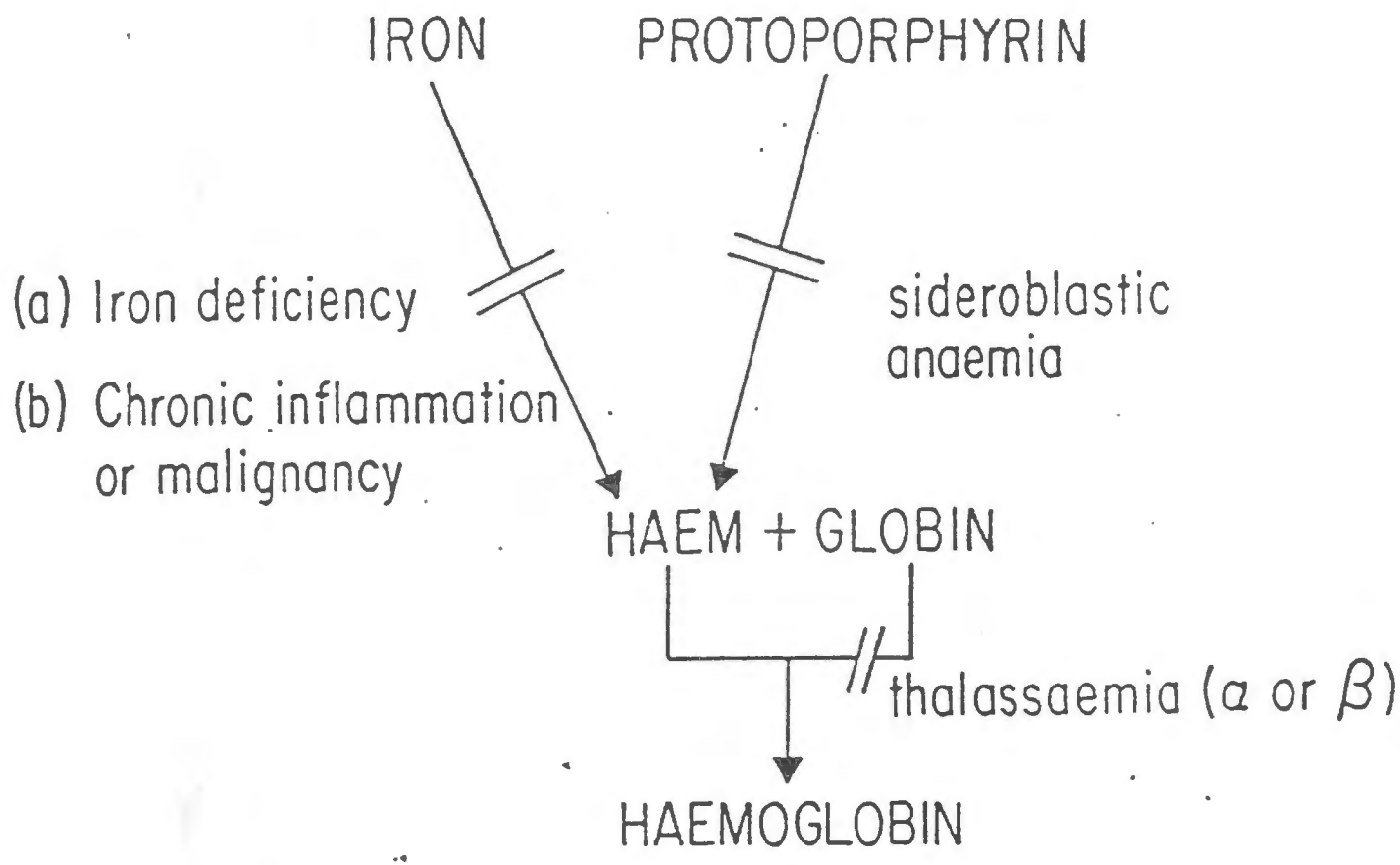


Fig. 2.1 The causes of a hypochromic microcytic anaemia. These include lack of iron (iron deficiency) or of iron release from macrophages to serum (anaemia of chronic inflammation or malignancy), failure of protoporphyrin synthesis (sideroblastic anaemia) or of globin synthesis (α or β-thalassaemia). Lead also inhibits haem and globin synthesis.

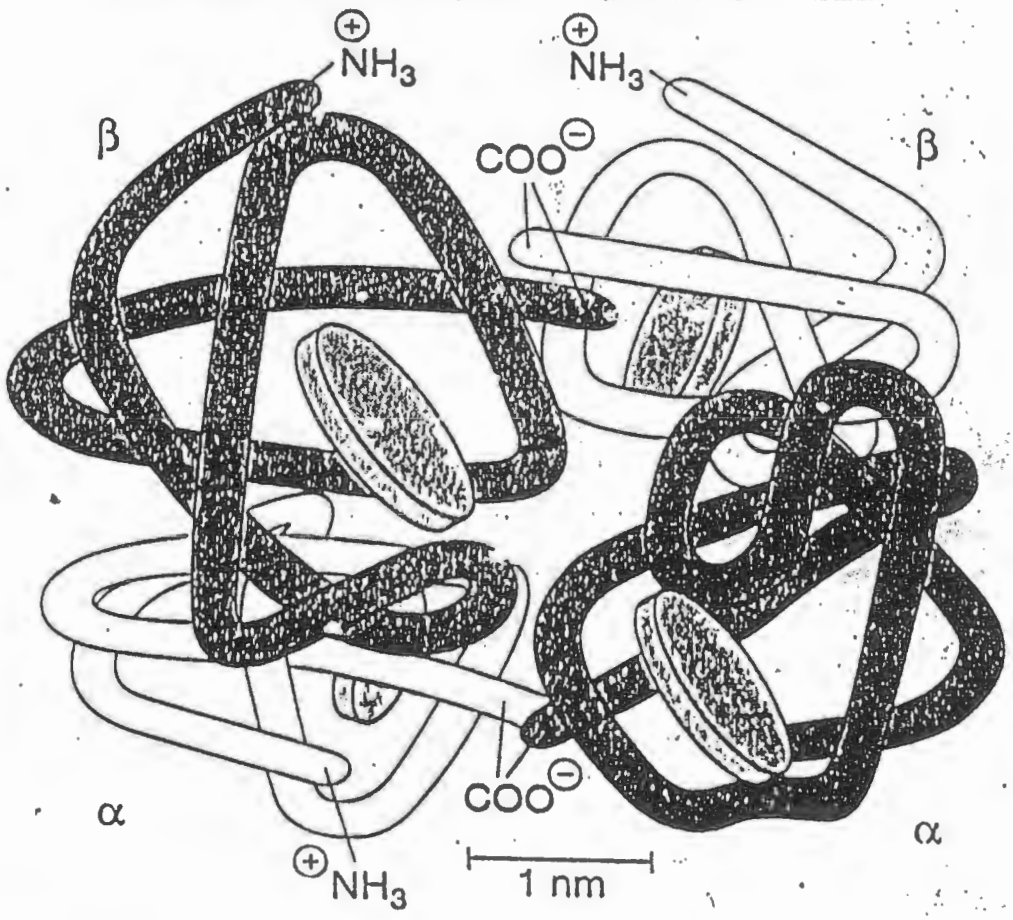


Figure 27–12. Diagrammatic representation of a molecule of hemoglobin A, showing the 4 subunits. There are 2 α and 2 β polypeptide chains, each containing a heme moiety. These moieties are represented by the disks. (Reproduced, with permission, from Harper HA et al: *Physiologische Chemie*. Springer-Verlag, 1975.)

Table 4-4. Normal Human Hemoglobins—Genetic Variants

Name	*Designation*	*Molecular Structure*	*Proportion in*	
			Adults	*Newborns*
Adult hemoglobin	A	$\alpha_2\beta_2$	97%	20%
Hemoglobin A_2	A_2	$\alpha_2\delta_2$	2.5%	0.5%
Fetal hemoglobin	F	$\alpha_2\gamma_2$	<1%	80%
Portland		$\zeta_2\gamma_2$	0	0
Gower I		$\zeta_2\epsilon_2$	0	0
Gower II		$\alpha_2\epsilon_2$	0	0

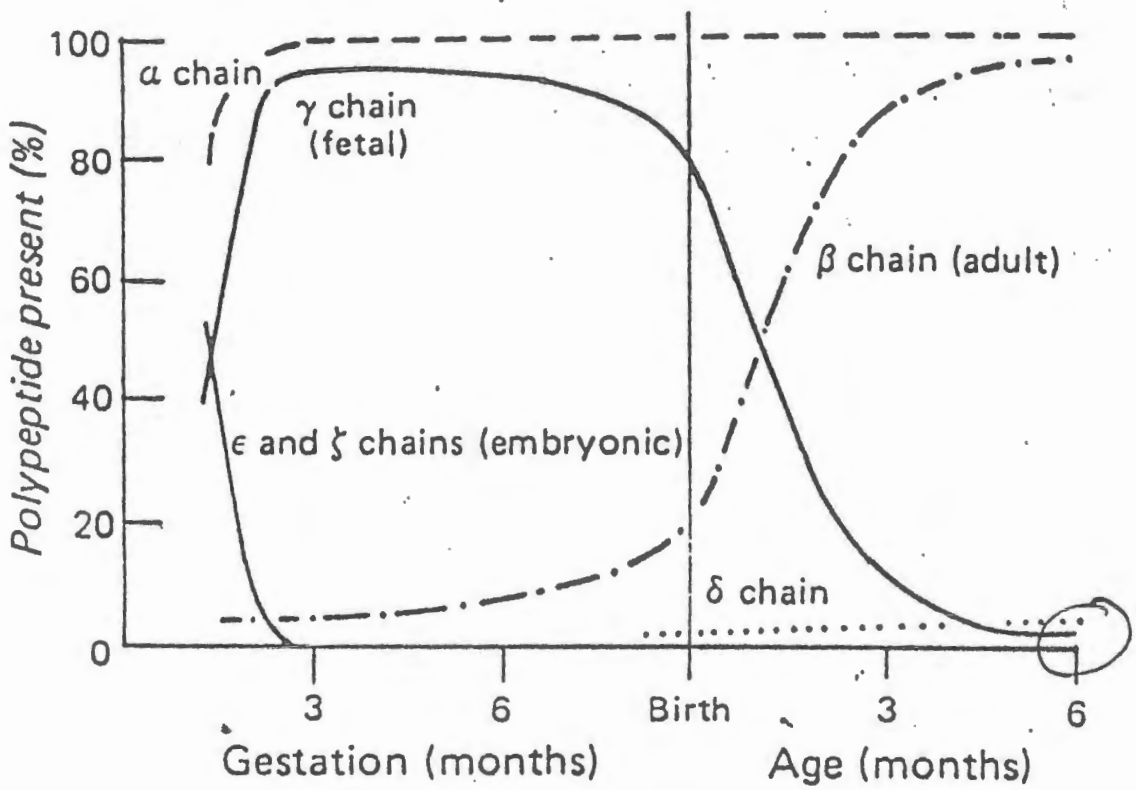


Figure 27–14. Development of human hemoglobin chains.

Figure 14.24 Plasma and whole blood that are brought into equilibrium with the same gas mixture have the same pO_2 and thus the same amount of dissolved oxygen molecules (shown with black dots). The oxygen content of whole blood, however, is much higher than that of plasma because of the binding of oxygen to hemoglobin.

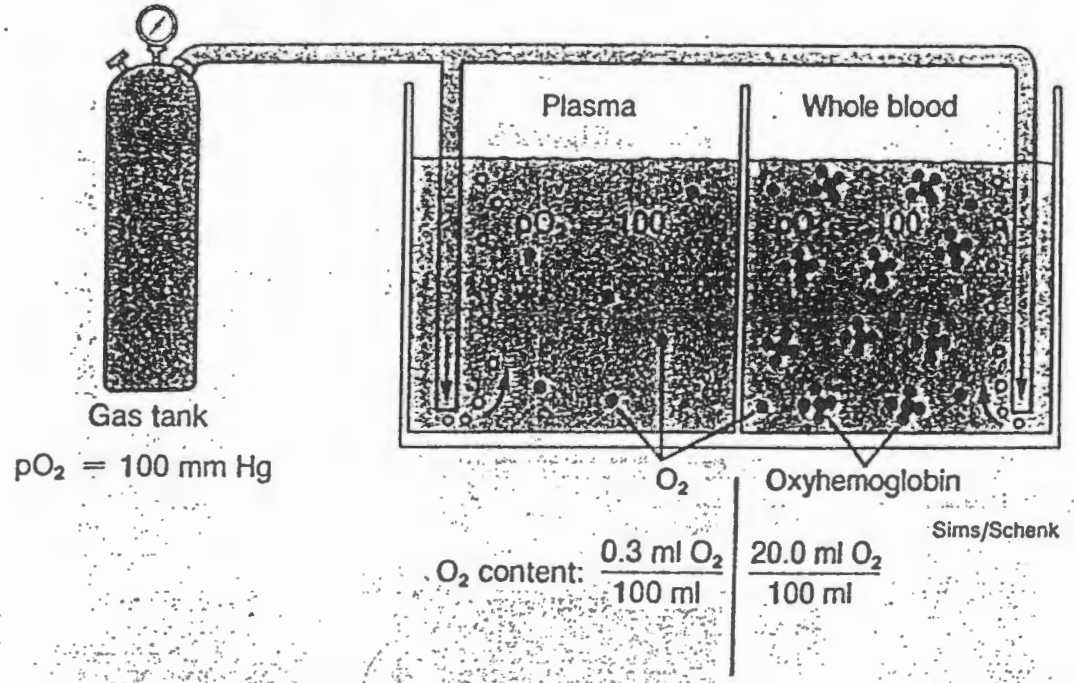


Table 35–1. Gas content of blood.

Gas	mL/dL of Blood Containing 15 g of Hemoglobin			
	Arterial Blood (P_{O_2} 95 mm Hg; P_{CO_2} 40 mm Hg; Hb 97% Saturated)		Venous Blood (P_{O_2} 40 mm Hg; P_{CO_2} 46 mm Hg; Hb 75% Saturated)	
	Dissolved	Combined	Dissolved	Combined
O_2	0.29	19.5	0.12	15.1
CO_2	2.62	46.4	2.98	49.7
N_2	0.98	0	0.98	0

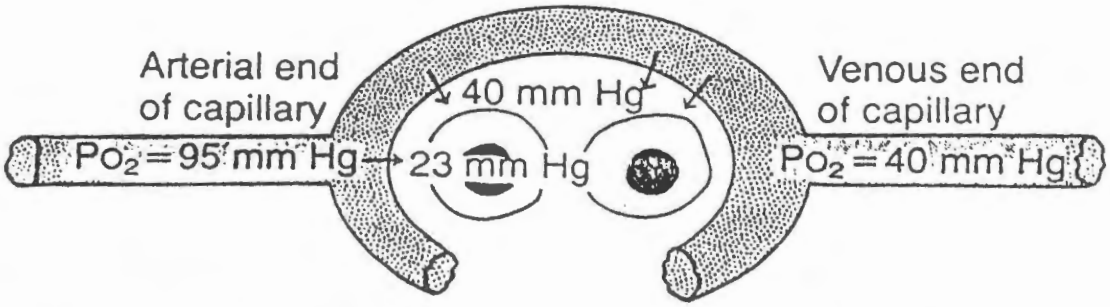


Figure 40–3. Diffusion of oxygen from a tissue capillary to the cells.

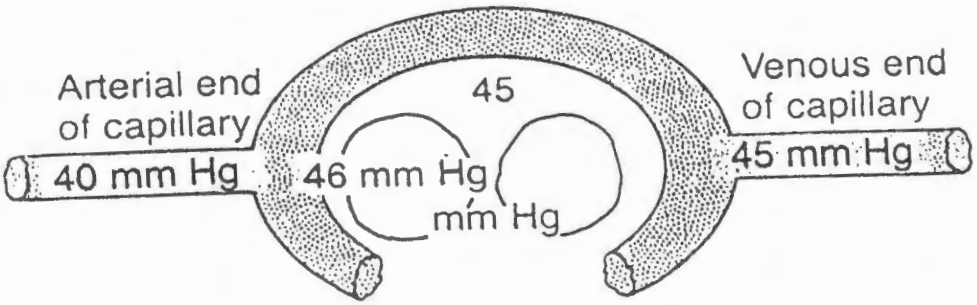


Figure 40–5. Uptake of carbon dioxide by the blood in the capillaries.

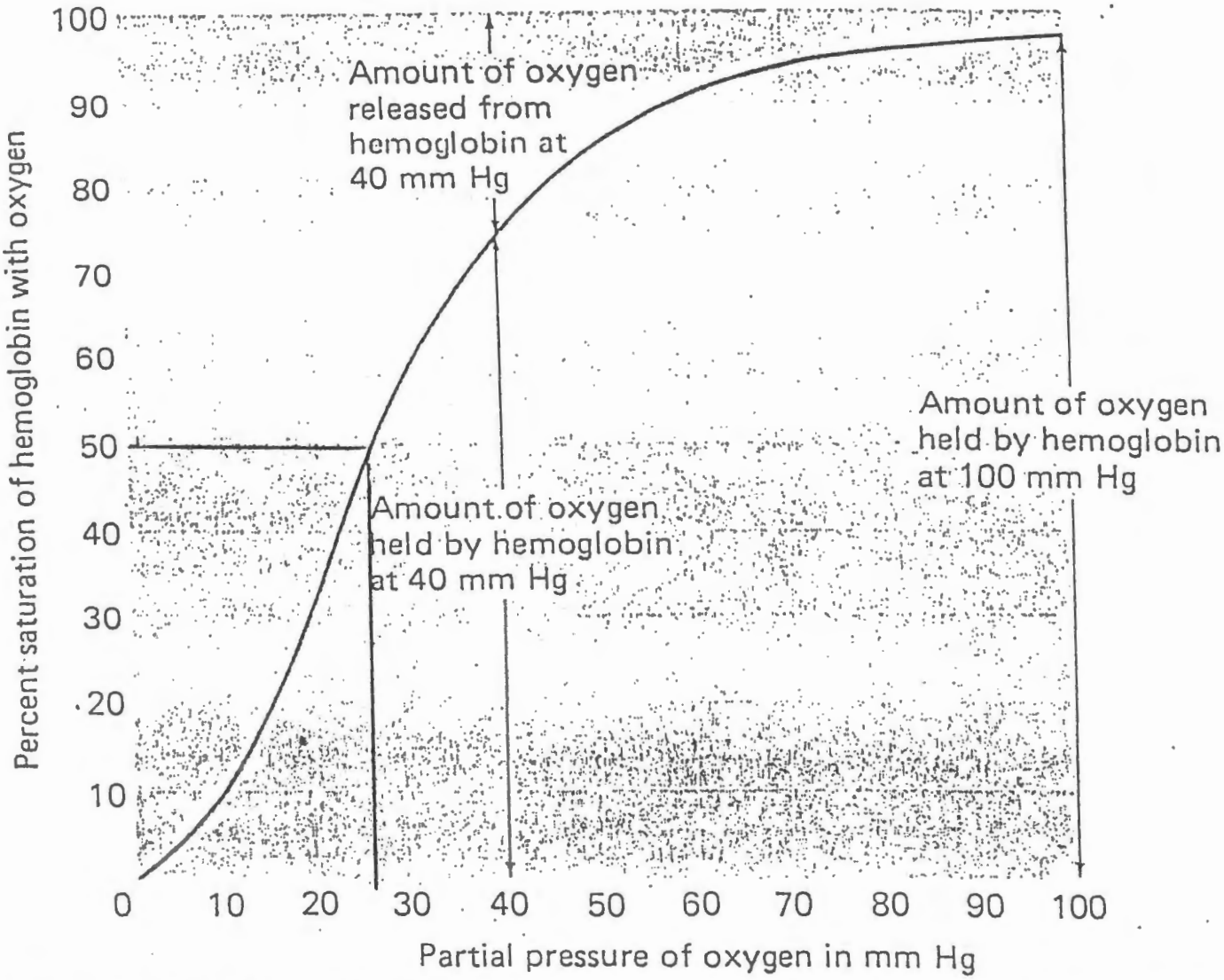


Figure 17.15 Hemoglobin Dissociation Curve for Oxygen in an Adult Human. The curve shows the extent to which hemoglobin picks up or releases oxygen as the oxygen pressure in the blood changes. When blood passes through the lungs, where the partial pressure of oxygen is about 100 mm Hg, the hemoglobin becomes about 97 percent saturated with oxygen. But when blood passes through distant tissues, where the partial pressure of oxygen is ordinarily about 40 mm Hg, the hemoglobin releases about 25 percent of its oxygen. (Adapted from J. W. Severinghaus, *J. Appl. Physiol.* 21 [1966]:1111.)

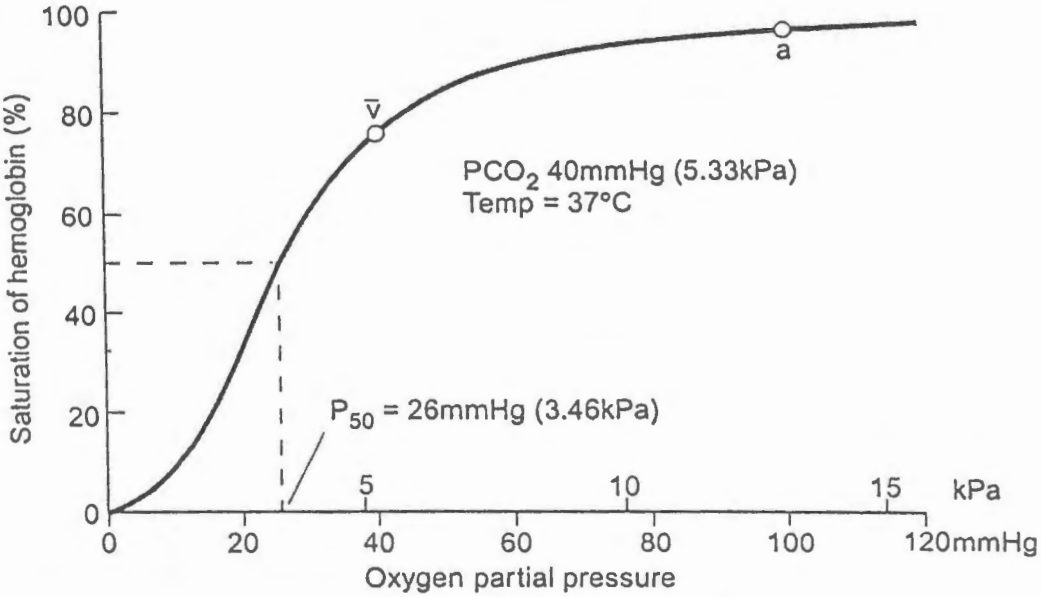
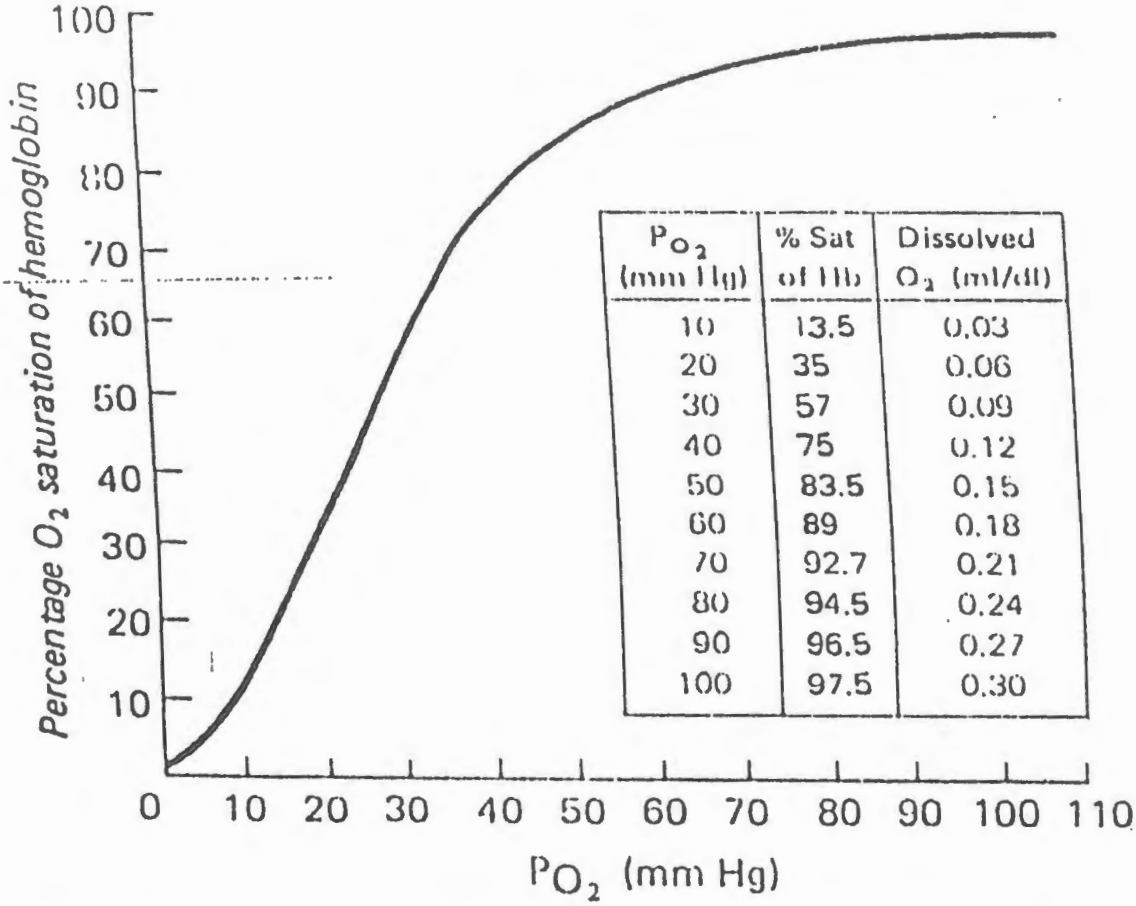


Fig. 13.6 The oxyhemoglobin dissociation curve for a PCO_2 of 5.33 kPa (40 mmHg) at 37 °C. Under these conditions, the P_{50} value is 3.46 kPa (26 mmHg). a, the PO_2 in arterial blood (97 per cent saturated); $\bar{v}$, the PO_2 for mixed venous blood (5.33 kPa or 40 mmHg) at which value the hemoglobin is still 75 per cent saturated. Note that as the PO_2 falls below 8 kPa (60 mmHg) the curve becomes progressively steeper.



P_{O_2} (mm Hg)	% Sat of Hb	Dissolved O_2 (ml/dl)
10	13.5	0.03
20	35	0.06
30	57	0.09
40	75	0.12
50	83.5	0.15
60	89	0.18
70	92.7	0.21
80	94.5	0.24
90	96.5	0.27
100	97.5	0.30

Figure 35–2. Oxygen-hemoglobin dissociation curve. pH 7.40, temperature 38°C. (Redrawn and reproduced, with permission, from Comroe JH Jr et al: *The Lung: Clinical Physiology and Pulmonary Function Tests*, 2nd ed. Year Book, 1962.)

$$Hb_4 + O_2 \rightleftarrows Hb_4O_2$$
$$Hb_4O_2 + O_2 \rightleftarrows Hb_4O_4$$
$$Hb_4O_4 + O_2 \rightleftarrows Hb_4O_6$$
$$Hb_4O_6 + O_2 \rightleftarrows Hb_4O_8$$

Combination of the first heme in the Hb molecule with O_2 increases the affinity of the second heme for O_2, and oxygenation of the second increases the affinity of the third, etc, so that the affinity of Hb for the fourth O_2 molecule is many times that for the first.

[illegible] Factors Affecting the Position of the Oxyhemoglobin Dissociation Curve

Factor	*Source*
Decreased pH	Results from increased production of CO_2 and lactic acid in active tissues
Increased P_{CO_2}	Increased in venous blood from tissues with high rate of oxidative metabolism; acts by formation of carbaminohemoglobin
Increased temperature	Higher in fever and in active tissues, especially skeletal muscle
Increased 2,3 DPG	Produced by red blood cells; increased in anemia and high-altitude adaptation

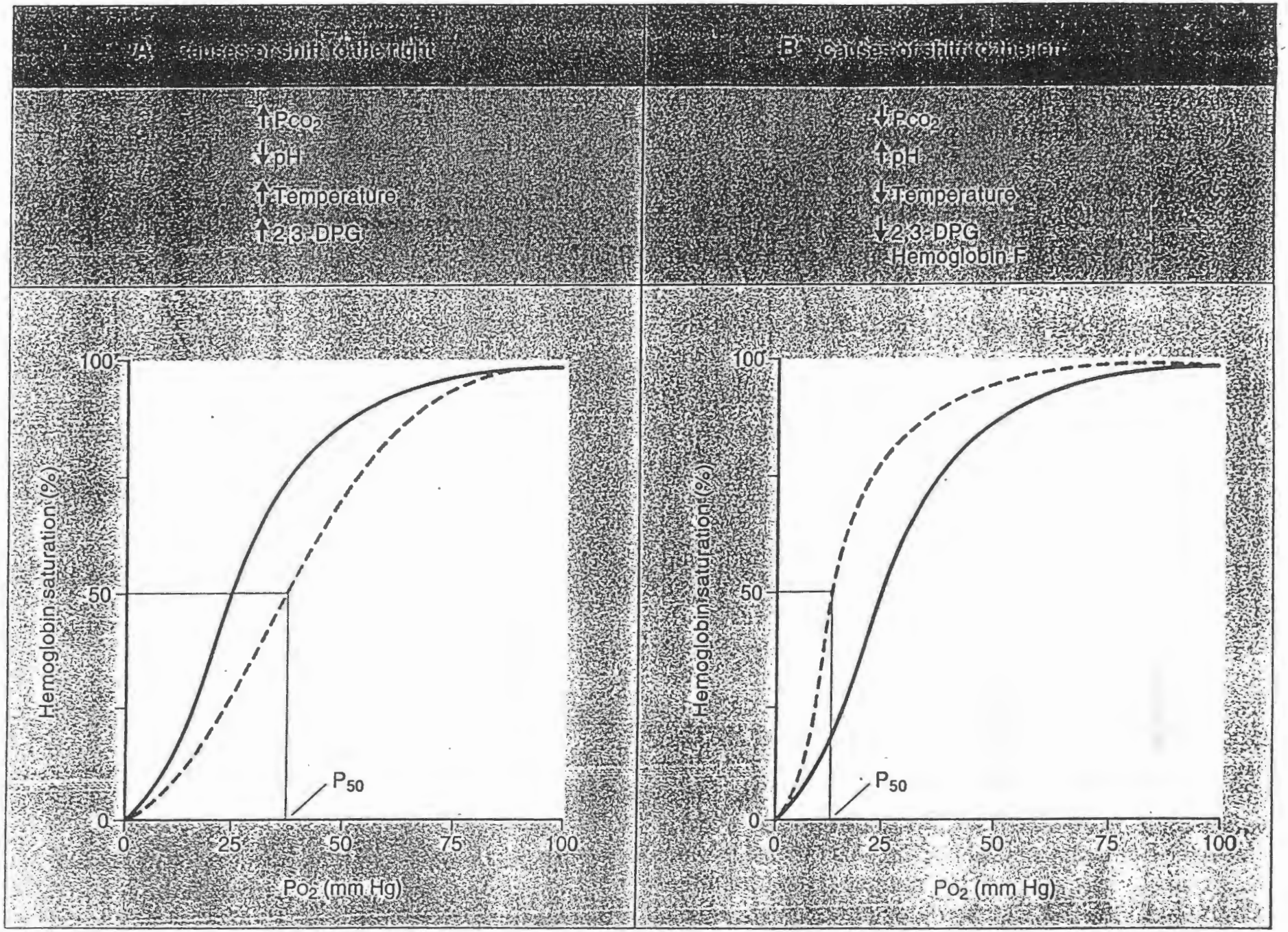
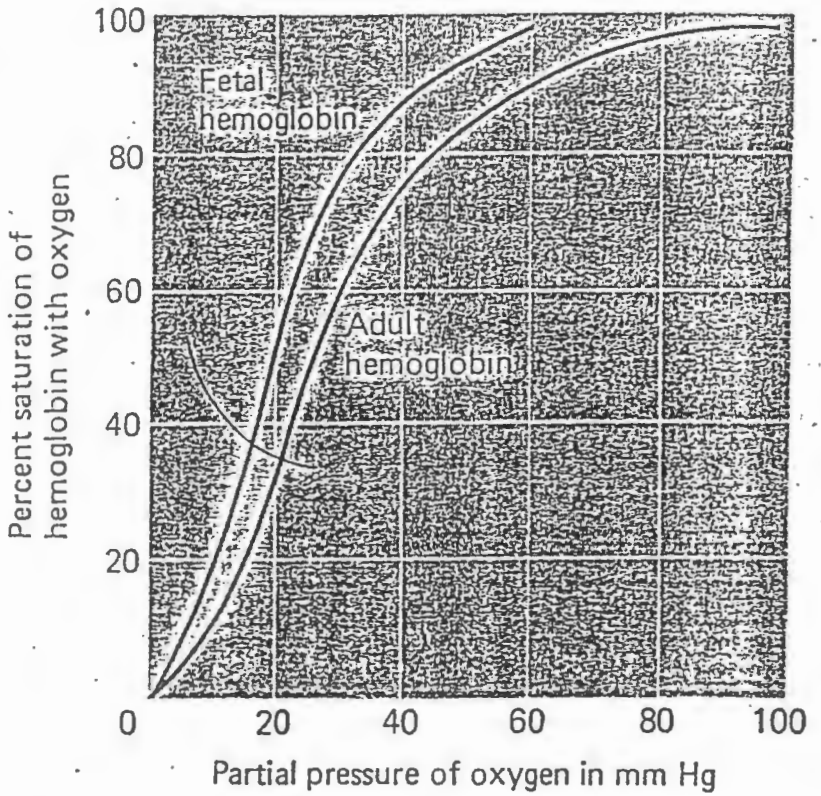


FIGURE 5–20. Shifts of the O_2-hemoglobin dissociation curve. A, Shifts to the right are associated with increased P_{50} and decreased affinity. **B,** Shifts to the left are associated with decreased P_{50} and increased affinity.



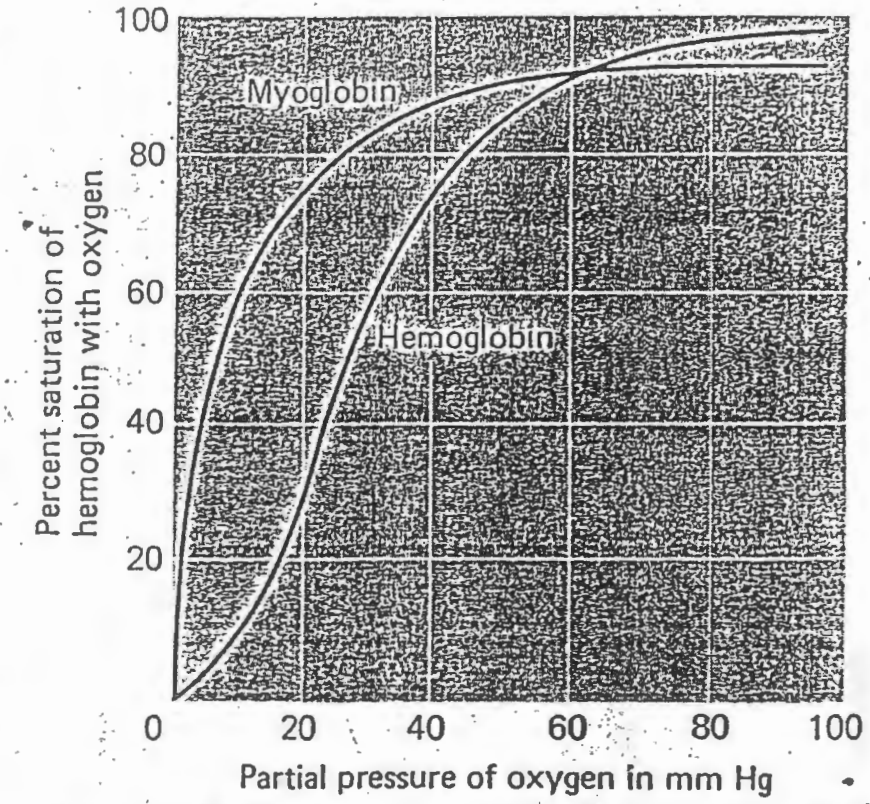


Figure 17.19 Differences in Dissociation of Oxygen from Fetal Hemoglobin, Adult Hemoglobin, and **Myoglobin.** The dissociation curve for fetal hemoglobin is to the left of that for maternal hemoglobin, indicating that fetal hemoglobin has a higher affinity for oxygen. Thus, when the mother's blood enters the placenta, it transfers oxygen to the fetus's blood. The dissociation curve for myoglobin (muscle hemoglobin) is far to the left of that for adult hemoglobin and has a hyperbolic shape. Thus, hemoglobin transfers oxygen readily to myoglobin. The myoglobin stores this oxygen until the oxygen pressure drops, as in exercise. Then the myoglobin releases its oxygen for use in cellular respiration. (Modified and reproduced with permission from J. H. Comroe, Jr., *Physiology of Respiration*, 2d ed., p. 185. Copyright © 1974 by Year Book Medical Publishers, Inc., Chicago.)

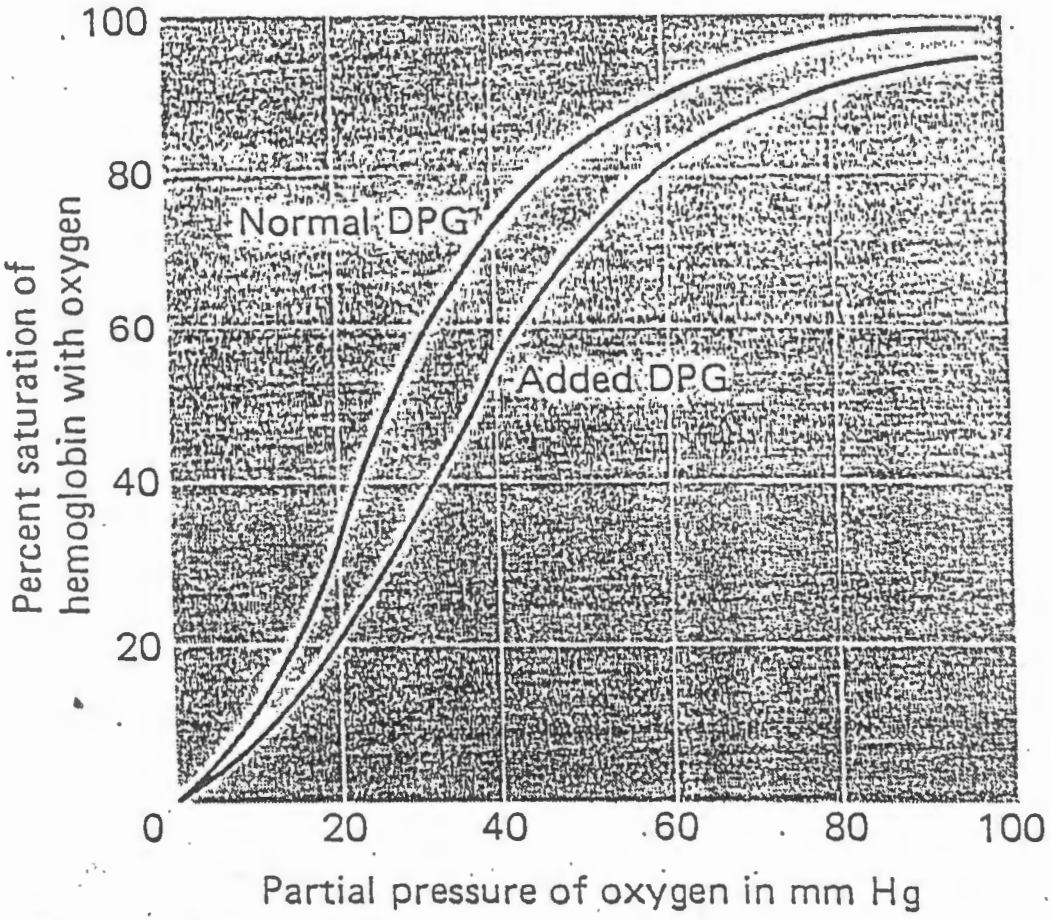


Figure 17.17 Effect of 2,3-Diphosphoglycerate (DPG) on Oxygen Dissociation from Hemoglobin. The formation of extra DPG in red blood cells, as occurs at high altitudes, shifts the dissociation curve to the right. In other words, DPG promotes the release of oxygen from hemoglobin. (Modified and reproduced with permission from J. H. Comroe, Jr., *Physiology of Respiration*, 2d ed., p. 185. Copyright © 1974 by Year Book Medical Publishers, Inc., Chicago.)

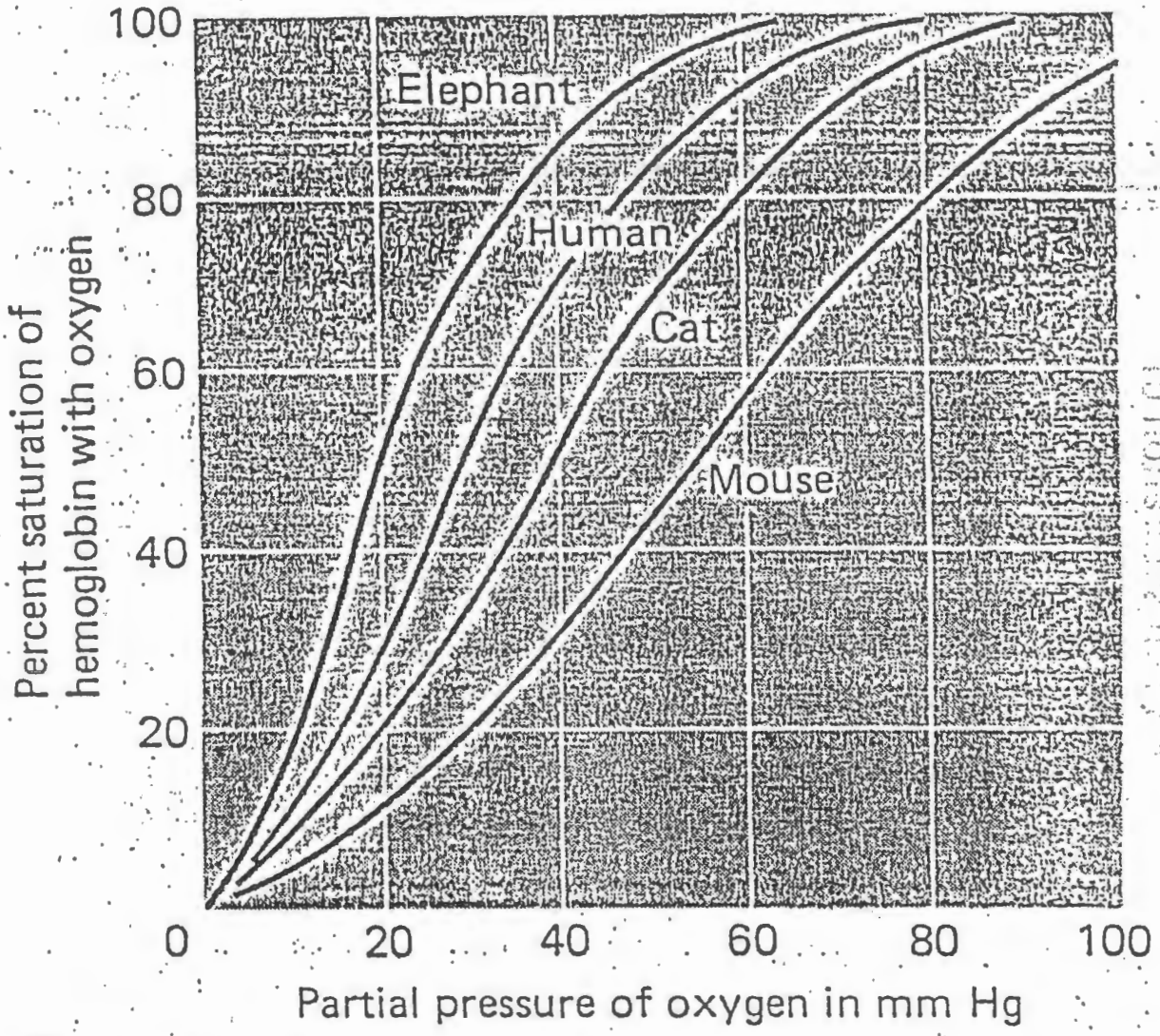
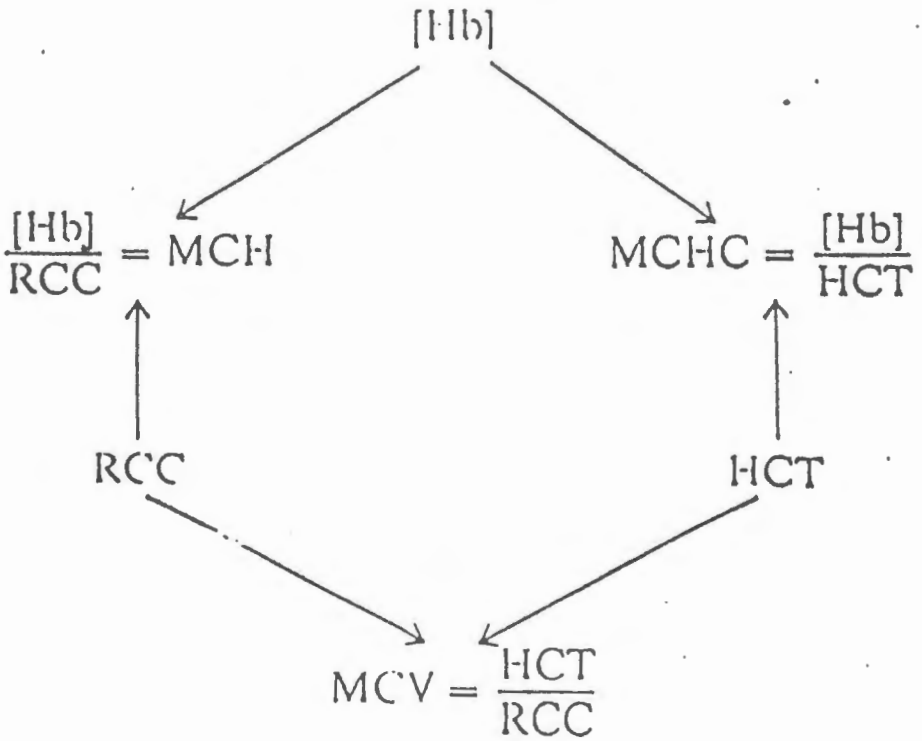


Figure 17.18 Effects of Mammalian Size on Oxygen Dissociation from Hemoglobin. Small mammals release oxygen more readily from hemoglobin than do large mammals. This difference is probably related to the greater need for oxygen in small mammals to support a greater heat production per unit of body weight. (Reprinted from K. Schmidt-Nielsen, *Federation Proceedings* 29 [1970]:1529.)

Relationships between the erythrocyte parameters. For the diagnostic evaluation of erythrocyte function it is usually necessary to measure three quantities: the *red cell count* RCC (μl^{-1}), the *hemoglobin concentration of the blood* [Hb] (g/l), and the *hematocrit* HCT. From these, three other characteristic parameters can be derived: the *mean corpuscular hemoglobin* MCH, the *mean corpuscular hemoglobin concentration* MCHC, and the *mean corpuscular volume* MCV. The relationships underlying these calculations are reflected directly in the definitions of the parameters and are summarized in the following diagram:



Given, for example, that RCC = $5 \cdot 10^6 \mu l$, [Hb] = 150 g/l and HCT = 0.45, the other parameters are as follows: MCH = 30 pg, MCHC = 333 g/l, and MCV = $0.09 \cdot 10^{-6} \mu l$ = 90 fl (femtoliters) = 90 μm^3 (the conversion among units is given on pp. 796f.).

---

* Values for Central Europe; for North America (according to Wintrobe) MCH = 29 pg

Mean Corpuscular Hemoglobin (MCH)

The MCH indicates the average weight of hemoglobin in the red blood cell.

$$\text{MCH} = \frac{\text{Weight of hemoglobin in 1 } \mu\text{l of blood}}{\text{Number of red blood cells in 1 } \mu\text{l of blood}}$$

If:

$1 \text{ g} = 10^{12} \text{ pg}$

$1 \text{ ml} = 10^{3} \mu\text{l}$

Then:

Weight (in pg) of hemoglobin in 1 μl of blood

$$= \frac{\text{Hemoglobin} \times 10^{12} \text{ pg}}{100 \times 10^{3} \mu\text{l}}$$

$$= \text{Hemoglobin} \times 10^{7} \text{ pg}/\mu\text{l}$$

If:

Hemoglobin = 15.0 g/dl
Red blood cell count = 5,000,000/μl

Then:

$$\text{MCH} = \frac{15 \times 10^{7} \text{ pg}/\mu\text{l}}{5 \times 10^{6} \mu\text{l}}$$

$$= \frac{15 \times 10 \text{ pg}}{5}$$

$$= 30 \text{ pg}$$

$$\text{MCH} = \frac{\text{Hemoglobin} \times 10}{\text{Red blood cell count in millions}} \text{ pg}$$

Normal value for the MCH: 27–31 pg

DISCUSSION

The MCH indicates the amount of hemoglobin in the red blood cell and should always correlate with the MCV and MCHC. An MCH lower than 27 pg is found in microcytic anemia and also with normocytic, hypochromic red blood cells. An elevated MCH occurs in macrocytic anemias and in some cases of spherocytosis in which hyperchromia may be present.

Mean Corpuscular Hemoglobin Concentration (MCHC)

The MCHC is an expression of the average concentration of hemoglobin in the red blood cells. It gives the ratio of the weight of hemoglobin to the volume of the red blood cell.

$$\text{MCHC} = \frac{\text{Hemoglobin in g/dl}}{\text{Hematocrit/dl}} \times 100 \text{ (to convert to \%)}$$

If:

Hemoglobin = 15.0 g/dl
Hematocrit = 45%

Then:

$$\text{MCHC} = \frac{15.0 \text{ g/dl} \times 100}{45 \text{ volumes/dl}} \% = 33\%$$

Therefore, the formula:

$$\text{MCHC} = \frac{\text{Hemoglobin} \times 100}{\text{Hematocrit}} \%$$

Normal value for the MCHC: 32–36%

DISCUSSION

The MCHC indicates whether the red blood cells are normochromic, hypochromic, or hyperchromic. An MCHC below 32% indicates hypochromia, an MCHC above 36% indicates hyperchromia, and red blood cells with a normal MCHC are termed normochromic.

Mean Corpuscular Volume (MCV)

The MCV indicates the average volume of the red blood cells.

$$\text{MCV} = \frac{\text{Volume of red blood cells in femtoliters (fl)/}\mu\text{l of blood}}{\text{Red blood cells/}\mu\text{l of blood}}$$

If:

Hematocrit = 45% (or 0.45)

Red blood cell count = 5,000,000/μl (or $5.0 \times 10^6/\mu l$)

$1\ \mu l = 10^9$ fl

Then:

$$\text{MCV} = \frac{0.45 \times 10^9\ \text{fl}/\mu l}{5.0 \times 10^6/\mu l}$$

$$= \frac{45 \times 10\ \text{fl}}{5}$$

$$= 90\ \text{fl}$$

Therefore, the formula:

$$\text{MCV} = \frac{\text{Hematocrit} \times 10}{\text{Red blood cell count in millions}}\ \text{fl}$$

Normal value for the MCV: 80–97 fl

DISCUSSION

The MCV indicates whether the red blood cells appear normocytic, microcytic, or macrocytic. If the MCV is less than 80 fl, the red blood cells are microcytic. If the MCV is greater than 97 fl, the red blood cells are macrocytic. If the MCV is within the normal range, the red blood cells are normocytic.

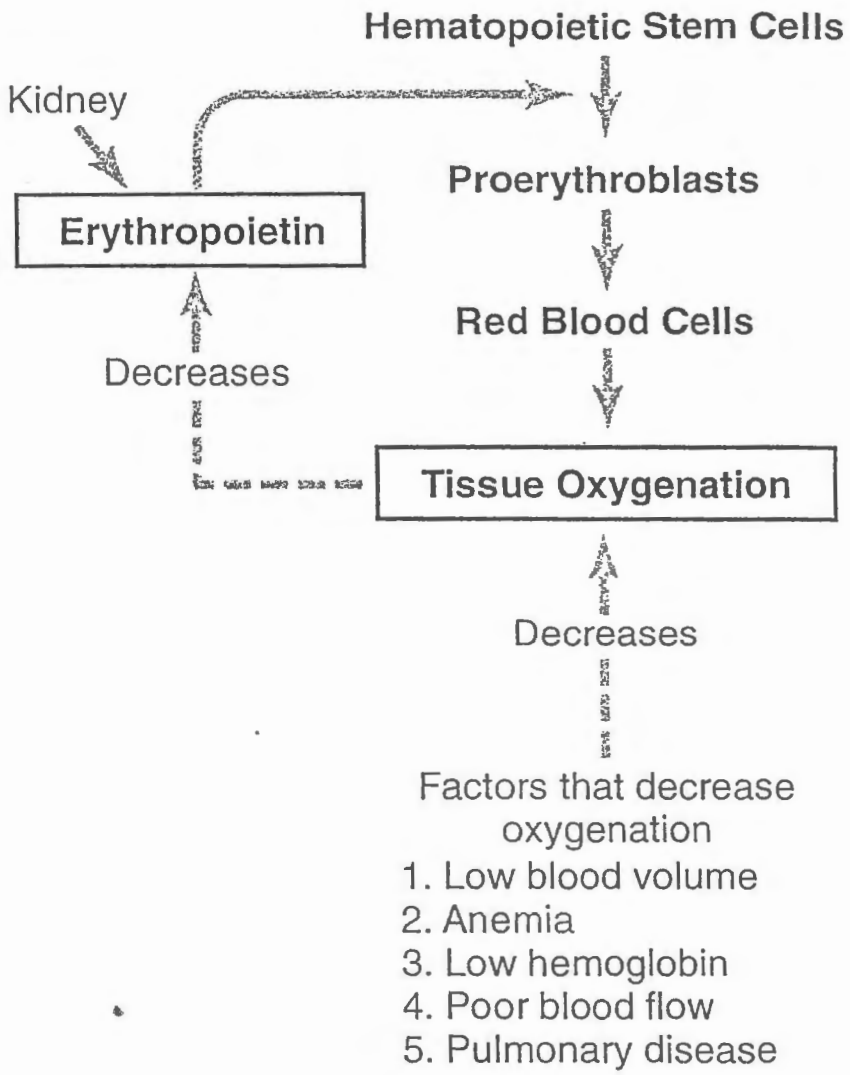


Figure 32–4

Function of the erythropoietin mechanism to increase production of red blood cells when tissue oxygenation decreases.

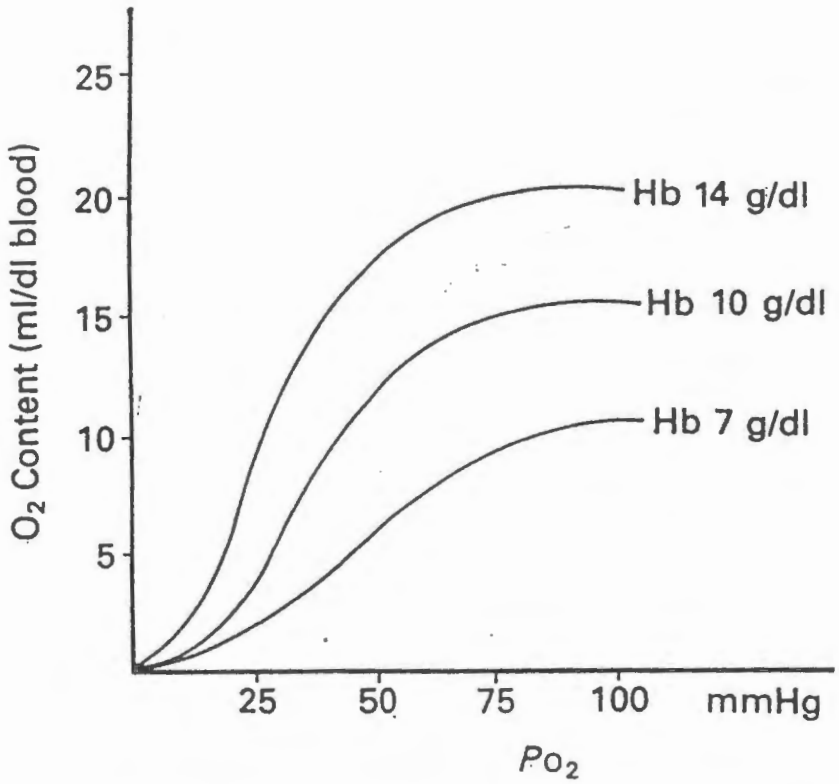
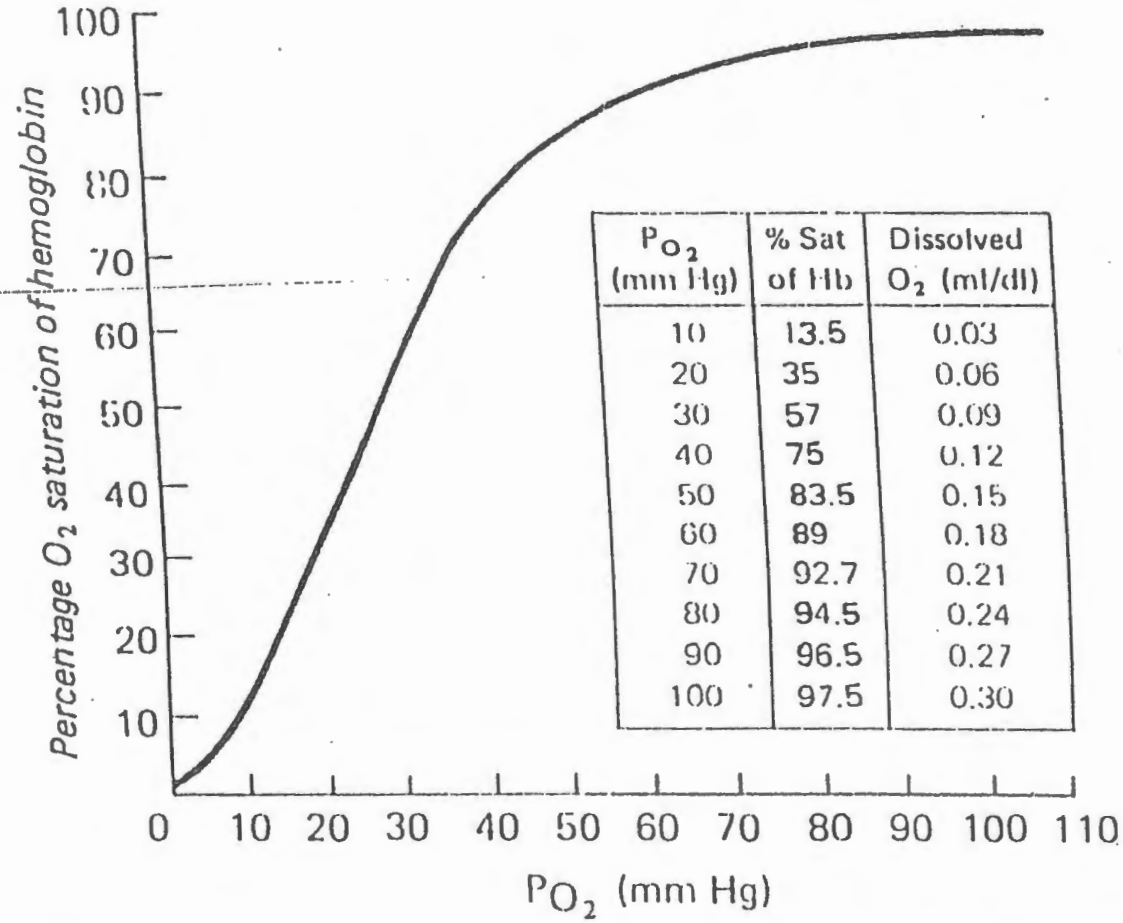


Fig. 6.12 Effect of anaemia on oxygen content of the blood at different Po_2 values.

It is evident that the **quantity** of oxygen carried in a volume of blood is dependent on the Po_2 as well as the haemoglobin concentration. The percentage saturation of haemoglobin with oxygen is dependent on Po_2 and totally independent of **haemoglobin concentration**. If oxygen content (instead of percentage saturation of haemoglobin with oxygen) is plotted against Po_2, the level of the curve will be dependent on the haemoglobin concentration of the sample of blood (Fig. 6.12). But when plotting **percentage saturation** against Po_2, as is usually done, the curve will always be the same, whatever the haemoglobin concentration is, if other factors remain the same.



P_{O_2} (mm Hg)	% Sat of Hb	Dissolved O_2 (ml/dl)
10	13.5	0.03
20	35	0.06
30	57	0.09
40	75	0.12
50	83.5	0.15
60	89	0.18
70	92.7	0.21
80	94.5	0.24
90	96.5	0.27
100	97.5	0.30

Figure 35-2. Oxygen-hemoglobin dissociation curve. pH 7.40, temperature 38°C. (Redrawn and reproduced, with permission, from Comroe JH Jr et al: *The Lung: Clinical Physiology and Pulmonary Function Tests*, 2nd ed. Year Book, 1962.)

$$Hb_4 + O_2 \rightleftarrows Hb_4O_2$$
$$Hb_4O_2 + O_2 \rightleftarrows Hb_4O_4$$
$$Hb_4O_4 + O_2 \rightleftarrows Hb_4O_6$$
$$Hb_4O_6 + O_2 \rightleftarrows Hb_4O_8$$

Combination of the first heme in the Hb molecule with O_2 increases the affinity of the second heme for O_2, and oxygenation of the second increases the affinity of the third, etc, so that the affinity of Hb for the fourth O_2 molecule is many times that for the first.

Table 27–5. Characteristics of human red cells.[1]

		Maie	Female
Hematocrit (Hct)(%)		47	42
Red blood cells (RBC) ($10^6/\mu L$)		5.4	4.8
Hemoglobin (Hb) (g/dL)		16	14
Mean corpuscular volume (MCV) (fL)	$= \frac{Hct \times 10}{RBC\ (10^6/\mu L)}$	87	87
Mean corpuscular hemoglobin (MCH) (pg)	$= \frac{Hb \times 10}{RBC\ (10^6/\mu L)}$	29	29
Mean corpuscular hemoglobin concentration (MCHC) (g/dL)	$= \frac{Hb \times 100}{Hct}$	34	34
Mean cell diameter (MCD) (μm)	= Mean diameter of 500 cells in smear	7.5	7.5

[1]Cells with MCVs > 95 fL are called macrocytes; cells with MCVs < 80 fL are called microcytes; cells with MCHs < 25 g/dL are called hypochromic.

*The red blood cell indices are used as an aid in differentiating anemias. When these indices are combined with an examination of the red blood cells on the stained smear, a clear picture of red blood cell morphology may be obtained.

with Corresponding Red Blood Cell Morphology

1. $$MCV = \frac{41 \times 10}{4.5} = 91 \text{ fl}$$

$$MCH = \frac{14.0 \times 10}{4.5} = 31 \text{ pg}$$

$$MCHC = \frac{14.0 \times 100}{41} = 34\%$$

The red blood cells are normocytic and normochromic.

2. $$MCV = \frac{30 \times 10}{4.5} = 67 \text{ fl}$$

$$MCH = \frac{9.8 \times 10}{4.5} = 22 \text{ pg}$$

$$MCHC = \frac{9.8 \times 100}{30} = 33\%$$

The red blood cells are microcytic and normochromic.

3. $$MCV = \frac{30 \times 10}{4.5} = 67 \text{ fl}$$

$$MCH = \frac{9.0 \times 10}{4.5} = 20 \text{ pg}$$

$$MCHC = \frac{9.0 \times 100}{30} = 30\%$$

The red blood cells are microcytic and hypochromic.

4. $$MCV = \frac{45 \times 10}{4.0} = 113 \text{ fl}$$

$$MCH = \frac{15.0 \times 10}{4.0} = 38 \text{ pg}$$

$$MCHC = \frac{15.0 \times 100}{45} = 33\%$$

The red blood cells are macrocytic and normochromic.

5. $$MCV = \frac{41 \times 10}{4.5} = 91 \text{ fl}$$

$$MCH = \frac{11.8 \times 10}{4.5} = 26 \text{ pg}$$

$$MCHC = \frac{11.8 \times 100}{41} = 29\%$$

The red blood cells are normocytic and hypochromic.

1. Iron deficiency is estimated to affect about 30% of the world population.
2. Iron deficiency Anemia is still the most important deficiency related to malnutrition.
3. Iron deficiency anemia (IDA) and thalassemia trait (TT) are the most common forms of microcytic anemia.
4. Some discrimination indices calculated from red blood cell indices are defined and used for rapid discrimination between TT and IDA.
5. Iron-deficiency anemia (IDA) is a common clinical problem throughout the world and an enormous public health risk in developing and even in industrialized countries.
6. Traditionally, several methods other than serum ferritin were used to assess IDA.

Functions of Red Blood Corpuscles :

(1) ... The main function of the red blood corpuscles is to carry oxygen to & take up carbon dioxide from the tissues.

<u>The biconcave shape of the cells is best suited for this function as it provides maximum surface for diffusion of gases for the volume of the corpuscles.</u>
(fig. 7).

(2) ... Hemoglobin in the red blood corpuscles is an important buffer & helps to keep the pH of blood constant.

(3) ... The red corpuscles keep hemoglobin inside them & prevent its loss in the urine.

(4) ... If the hemoglobin was free in the plasma, this would lead to :

(■) ... The viscosity of blood will be increased → increase the work done by the heart to pump the blood in blood vessels.

*** <u>Thus the red blood corpuscles by keeping hemoglobin inside them decrease the work performed by the heart.</u>**

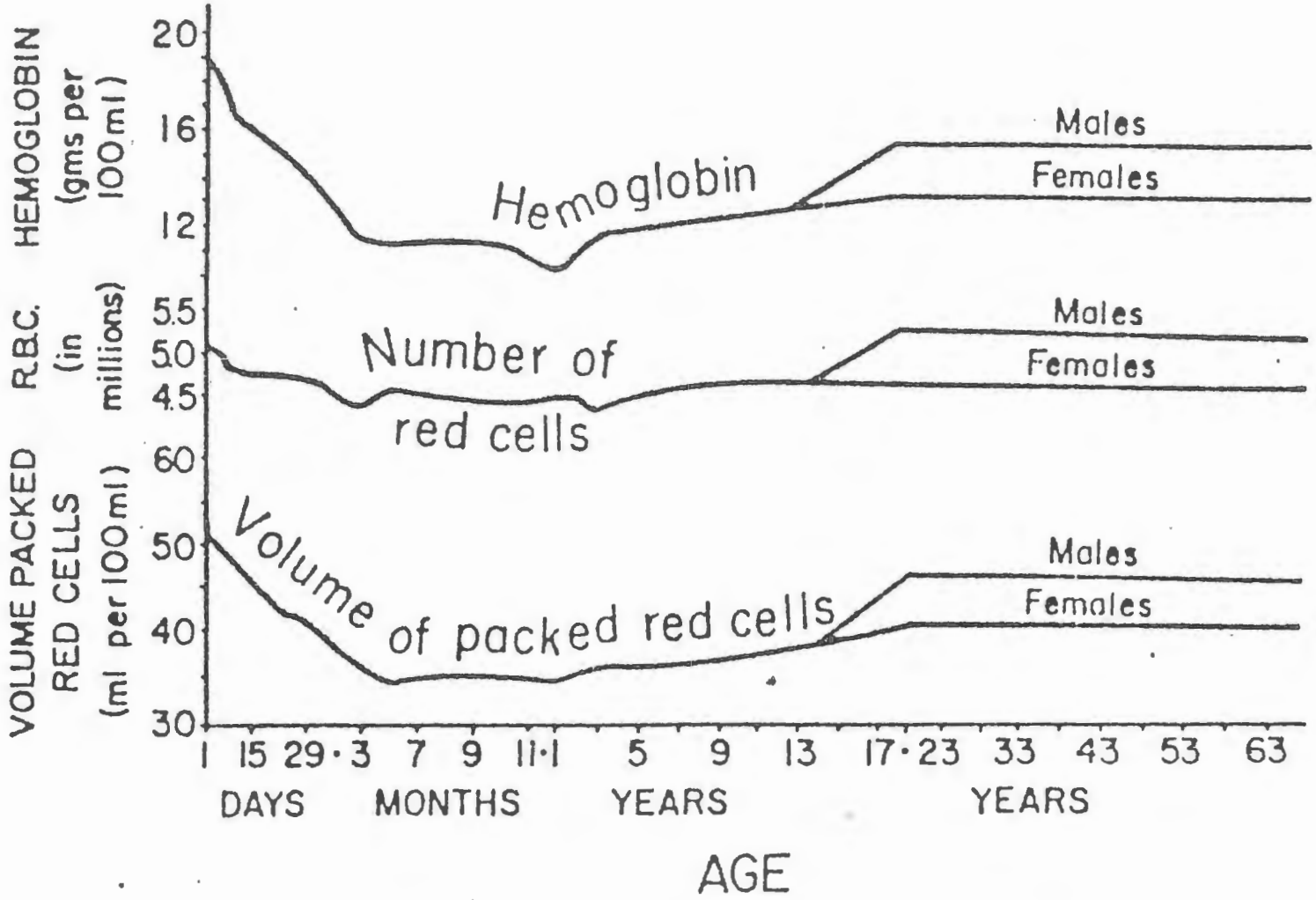


Figure 4–1. Relationship of age and sex to the hemoglobin content, red blood cell count, and hematocrit of the blood.

Table 27–1. Normal values for the cellular elements in human blood.

Cell	Cells/μL (average)	Approximate Normal Range	Percentage of Total White Cells
Total WBC	9000	4000–11,000	...
Granulocytes			
Neutrophils	5400	3000–6000	50–70 = 60%
Eosinophils	275	150–300	1–4 = 04%
Basophils	35	0–100	0.4 = 01%
Lymphocytes	2750	1500–4000	20–40 = 30%
Monocytes	540	300–600	2–8 = 05%

Table 19.3 Summary of Formed Elements in Blood

Name and Appearance	Number	Characteristics*	Functions
Red blood cells (RBCs) or erythrocytes 	4.8 million/μL in females; 5.4 million/μL in males.	7–8 μm diameter, biconcave discs, without a nucleus; live for about 120 days.	Hemoglobin within RBCs transports most of the oxygen and part of the carbon dioxide in the blood.
White blood cells (WBCs) or leukocytes	5000–10,000/μL.	Most live for a few hours to a few days.†	Combat pathogens and other foreign substances that enter the body.
Granular leukocytes			
Neutrophils 	60–70% of all WBCs.	10–12 μm diameter; nucleus has 2–5 lobes connected by thin strands of chromatin; cytoplasm has very fine, pale lilac granules.	Phagocytosis. Destruction of bacteria with lysozyme, defensins, and strong oxidants, such as superoxide anion, hydrogen peroxide, and hypochlorite anion.
Eosinophils 	2–4% of all WBCs.	10–12 μm diameter; nucleus has 2 or 3 lobes; large, red-orange granules fill the cytoplasm.	Combat the effects of histamine in allergic reactions, phagocytize antigen–antibody complexes, and destroy certain parasitic worms.
Basophils 	0.5–1% of all WBCs.	8–10 μm diameter; nucleus has 2 lobes; large cytoplasmic granules appear deep blue-purple.	Liberate heparin, histamine, and serotonin in allergic reactions that intensify the overall inflammatory response.
Agranular leukocytes			
Lymphocytes (T cells, B cells, and natural killer cells) 	20–25% of all WBCs.	Small lymphocytes are 6–9 μm in diameter; large lymphocytes are 10–14 μm in diameter; nucleus is round or slightly indented; cytoplasm forms a rim around the nucleus that looks sky blue; the larger the cell, the more cytoplasm is visible.	Mediate immune responses, including antigen–antibody reactions. B cells develop into plasma cells, which secrete antibodies. T cells attack invading viruses, cancer cells, and transplanted tissue cells. Natural killer cells attack a wide variety of infectious microbes and certain spontaneously arising tumor cells.
Monocytes 	3–8% of all WBCs.	12–20 μm diameter; nucleus is kidney shaped or horseshoe shaped; cytoplasm is blue-gray and has foamy appearance.	Phagocytosis (after transforming into fixed or wandering macrophages).
Platelets (thrombocytes) 	150,000–400,000/μL.	2–4 μm diameter cell fragments that live for 5–9 days; contain many vesicles but no nucleus.	Form platelet plug in hemostasis; release chemicals that promote vascular spasm and blood clotting.

Table 12.7 Types of white cell in Romanowsky-stained blood films.

WHITE-CELL TYPES

Cell	Diameter (μm)	Nucleus	Cytoplasm	% of total (adults)
Neutrophil	12–15	2–5 lobes	Pink, granular; fine purple granules	40–75
Lymphocyte	6–8 (small) 12–16 (large)	Round; heavy chromatin	Thin rim, pale blue; occasional granule	20–45
Monocyte	12–20	Large, irregular; fine chromatin	Bulky, pale blue-grey	2–10
Eosinophil	12–15	Two lobes	Many large, oval, orange-red granules	1–6
Basophil	12–15	Large; irregular lobes	Few dark-blue granules; often overlie nucleus	<1

Table 1.3 Ninety five per cent confidence limits for the concentrations of various types of circulating blood cell in adult Caucasians and their life-span in the blood.

Cell type	Normal range (95% confidence limits)	Life-span in blood
Red cells	Males 4.4–5.8 × 10^{12}/litre Females 4.1–5.2 × 10^{12}/litre	110–120 days
White cells (leucocytes)	4.0–11.0 × 10^{9}/litre	
Neutrophil granulocytes	1.5–7.5 × 10^{9}/litre	$t_{1/2}$ approx. 7 hours
Eosinophil granulocytes	0.02–0.60 × 10^{9}/litre	$t_{1/2}$ approx. 6 hours
Basophil granulocytes	0.01–0.15 × 10^{9}/litre	
Monocytes	0.2–0.8 × 10^{9}/litre	$t_{1/2}$ approx. 70 hours
Lymphocytes	1.2–3.5 × 10^{9}/litre	
Platelets	160–450 × 10^{9}/litre	9–12 days

The bone marrow is actually one of the largest or-
ans in the body, approaching the size and weight of
e liver. It is also one of the most active. Normally,
5% of the cells in the marrow belong to the white
lood cell-producing myeloid series and only 25%
re maturing red cells, even though there are over
00 times as many red cells in the circulation as
here are white cells. This difference in the marrow
eflects the fact that the average life span of white
ells is short, whereas that of red cells is long.

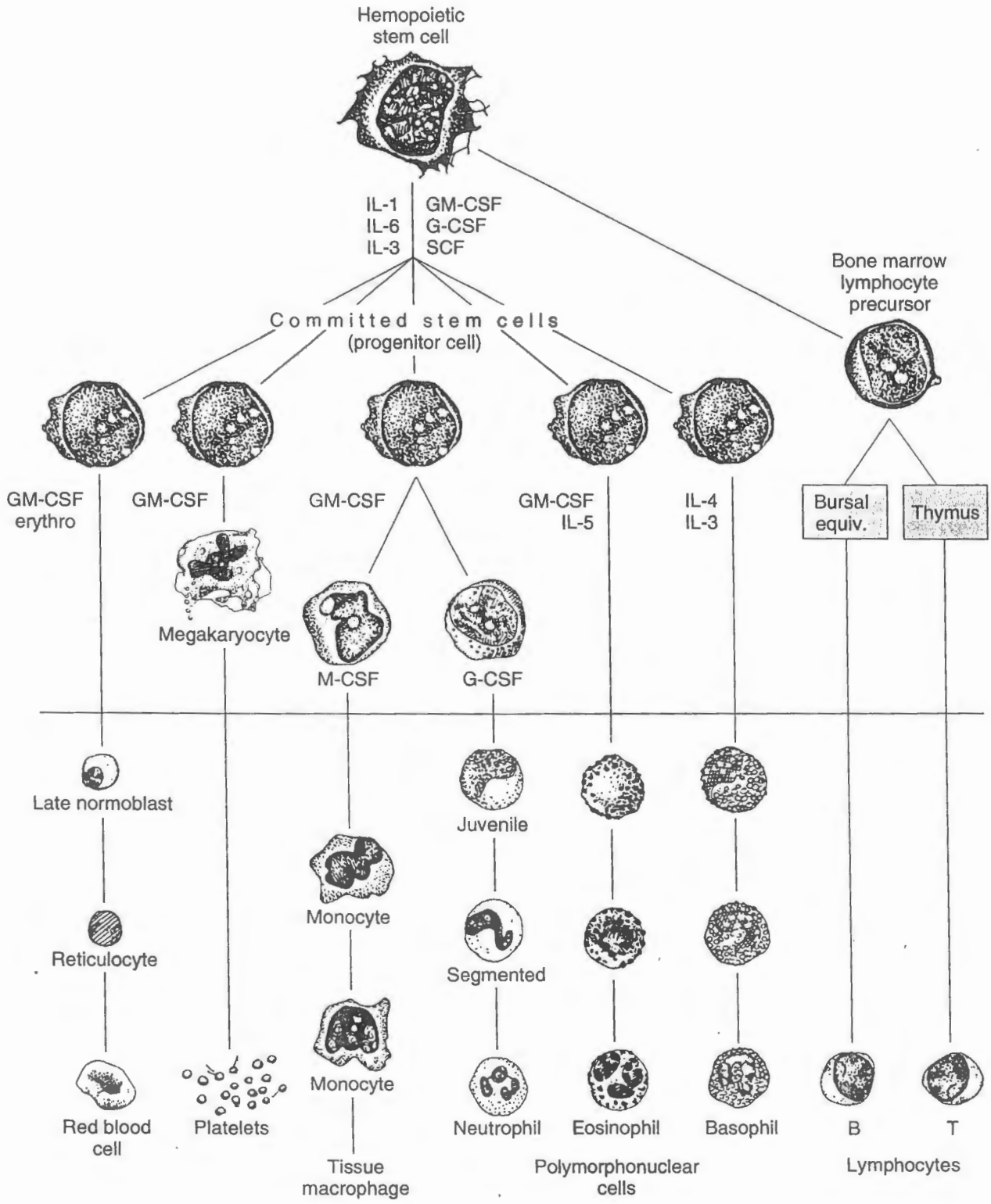


Figure 27–2 Development of various formed elements of the blood from bone marrow cells. Cells below the horizontal line are found in normal peripheral blood. The principal sites of action of erythropoietin (erythro) and the various colony-stimulating factors (CSF) that stimulate the differentiation of the components are indicated. G, granulocyte; M, macrophage; IL, interleukin; see Tables 27–2 and 27–3.

Table 27–2. Principal cytokines.

Cytokine	Cell Lines Stimulated	Cytokine Source
IL-1	Erythrocyte Granulocyte Megakaryocyte Monocyte	Multiple cell types
IL-3	Erythrocyte Granulocyte Megakaryocyte Monocyte	T lymphocytes
IL-4	Basophil	T lymphocytes
IL-5	Eosinophil	T lymphocytes
IL-6	Erythrocyte Granulocyte Megakaryocyte Monocyte	Endothelial cells Fibroblasts Macrophages
IL-11	Erythrocyte Granulocyte Megakaryocyte	Fibroblasts Osteoblasts
Erythropoietin	Erythrocyte	Kidney Kupffer cells of liver
SCF	Erythrocyte Granulocyte Megakaryocyte Monocyte	Multiple cell types
G-CSF	Granulocyte	Endothelial cells Fibroblasts Monocytes
GM-CSF	Erythrocyte Granulocyte Megakaryocyte	Endothelial cells Fibroblasts Monocytes T lymphocytes
M-CSF	Monocyte	Endothelial cells Fibroblasts Monocytes
Thrombopoietin	Megakaryocyte	Liver, kidney

Key: IL = interleukin; CSF = colony stimulating factor; G = granulocyte; M = macrophage; SCF = stem cell factor

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actin in the neutrophils does not polymerize normally, and the neutrophils move slowly. In another, there is a congenital deficiency of leukocyte integrins. In a more serious disease (chronic granulomatous disease), there is a failure to generate O_2^- in both the neutrophils and monocytes and consequent inability to kill many phagocytosed bacteria. In severe congenital glucose 6-phosphate dehydrogenase deficiency, there are multiple infections because of failure to generate the NADPH necessary for O_2^- production. In congenital myeloperoxidase deficiency, microbial killing power is reduced because hypohalite ions are not formed.

Lymphocytes

Lymphocytes are key elements in the production of immunity (see below). After birth, some lymphocytes are formed in the bone marrow. However, most are formed in the lymph nodes (Figure 27–4), thymus, and spleen from precursor cells that originally came from the bone marrow and were processed in the thymus or bursal equivalent (see below). Lymphocytes enter the bloodstream for the most part via the lymphatics. At any given time, only about 2% of the body lymphocytes are in the peripheral blood. Most of the rest are in the lymphoid organs. It has been calculated that in humans, 3.5×10^{10} lymphocytes per day enter the circulation via the thoracic duct alone; however, this count includes cells that reenter the lymphatics and thus traverse the thoracic duct more than once. The effects of adrenocortical hormones on the lymphoid organs, the circulating lymphocytes, and the granulocytes are discussed in Chapter 20.

IMMUNITY

Overview

Insects and other invertebrates have **innate immunity.** The key to this system is receptors that bind sequences of sugars, fats, or amino acids in common bacteria and activate various defense mechanisms. The receptors are coded in the germ line, and their fundamental structure is not modified by exposure to antigen. The activated

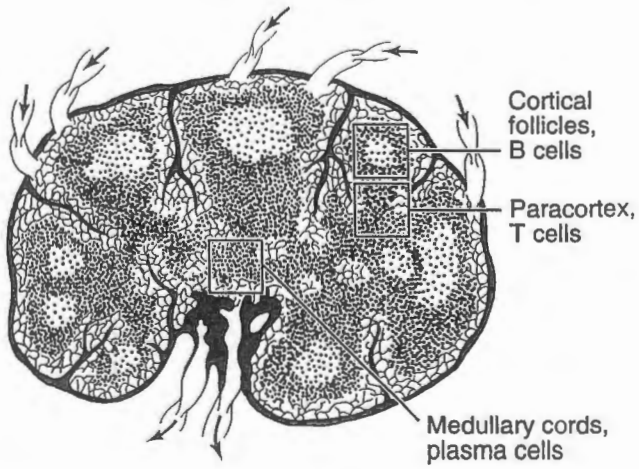


Figure 27–4 Anatomy of a normal lymph node. (After Chandrasoma. Reproduced, with permission, from McPhee SJ, Lingappa VR, Ganong WF [editors]: *Pathophysiology of Disease,* 4th ed. McGraw-Hill, 2003.)

Interleukins IL-1 and IL-6 followed by IL-3 act in sequence to convert pluripotential uncommitted stem cells to committed progenitor cel

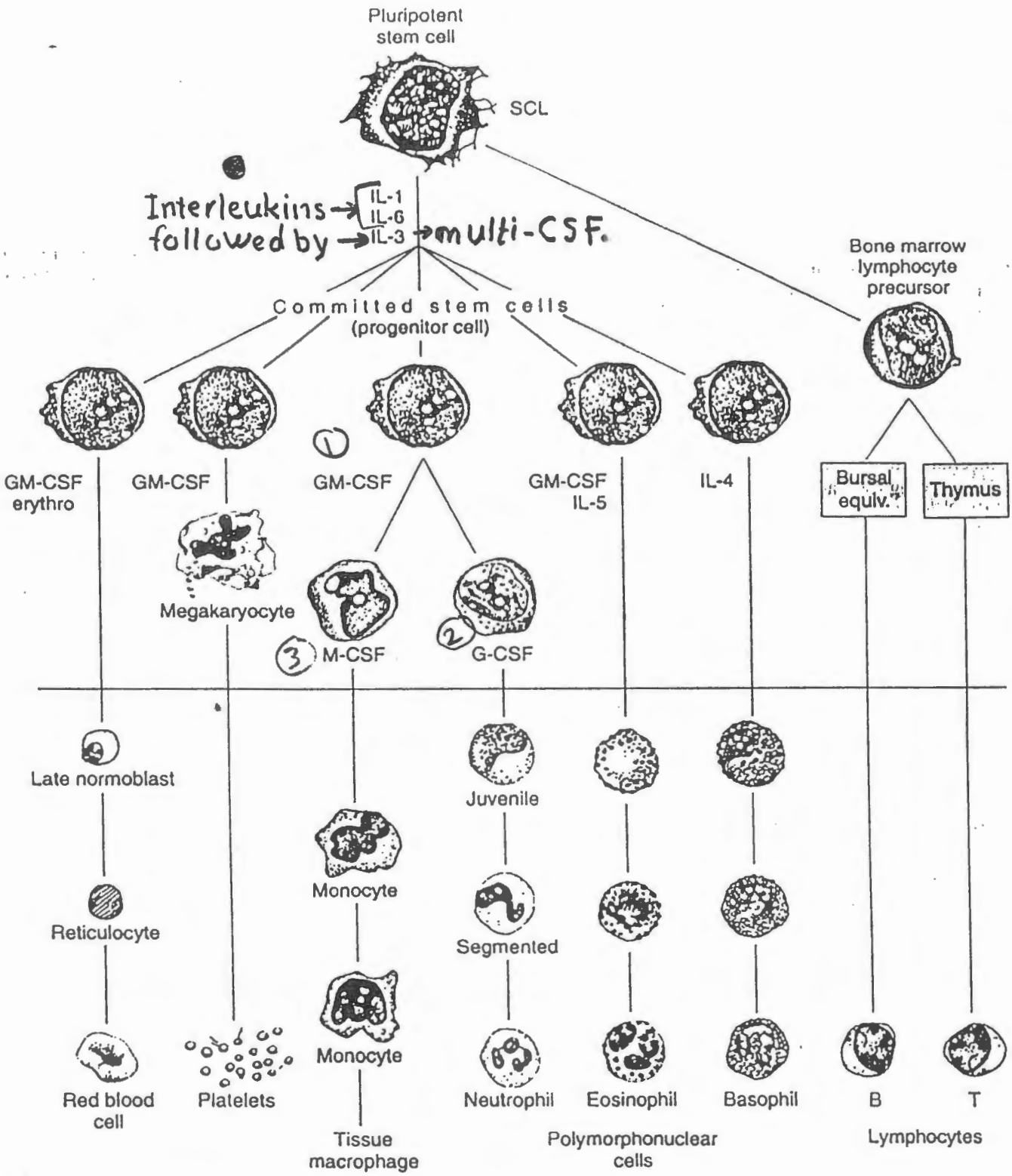


Figure 27–2. Development of various formed elements of the blood from bone marrow cells. Cells below the horizontal line are found in normal peripheral blood. The principal sites of action of erythropoietin (erythro) and the various colony-stimulating factors (CSF) that stimulate the differentiation of the components are indicated. G, granulocyte; M, macrophage; IL, interleukin; see Tables 27–2 and 27–4.

The factors stimulating the production of committed stem cells include 1 + 2 + 3 ↑. These factors are called colony-stim factors (CSFs), because they cause appropriate single stem cells to proliferate forming colonies in culture medium. Each of the CSFs a predominant action, but all the CSFs and interleukins also have other overlapping actions. Also activate and sustain

Table 27–2. Factors regulating hematopoiesis.

Name	Cellular Sources	Cell Type Produced in Increased Numbers
SCL	?	Pluripotential cells
Erythropoietin	Kidney cells, Kupffer cells	Red blood cells
G-CSF	Monocytes, fibroblasts, endothelial cells	Neutrophils
M-CSF	Monocytes, fibroblasts, endothelial cells	Monocytes
GM-CSF	T cells, monocytes, fibroblasts, endothelial cells	Neutrophils, monocytes, eosinophils, megakaryocytes, red blood cells
IL-1	Macrophages, fibroblasts, endothelial cells	Neutrophils, monocytes, eosinophils, basophils, megakaryocytes, red blood cells
IL-3	T cells	
IL-4	T cells	Basophils
IL-5	T cells	Eosinophils
IL-6	Macrophages, fibroblasts, endothelial cells	Neutrophils, monocytes, eosinophils, basophils, megakaryocytes, red blood cells

Functions of the Leucocytes:-

All leucocytes possess, to some degree, four basic properties that relate to their functions in the body.

They are able to pass through the walls of capillaries, to enter the tissue spaces in accordance with the local needs. This process is known as *diapedesis*. Once within the tissue spaces, the leukocytes (particularly the polymorphonucleocytes) have the ability to move through the tissues by an ameboid motion at speeds of up to 40 μm min^{-1}. Furthermore, they seem to be attracted by certain chemical substances released by bacteria or by inflamed tissues (*chemotaxis*).

Phagocytosis: The ability to engulf and digest or kill bacteria and products of cell death.

One of the remarkable features of neutrophils is their fine capacity to distinguish foreign cells like bacteria from homologous body cells and aged or damaged cells from fresh ones. This is due to the presence in plasma of certain substances (**opsonins**), such as **γ-globulins** (especially immunoglobulin G (IgG)) and **complement C4**, which coat bacteria and ageing cells, thereby making them 'palatable' to neutrophils. To opsonize means to prepare for eating. An opsonin is an agent in plasma which acts on foreign particles to increase their palatability to phagocytes.

Table 1.4 Main functions of blood cells.

Type of cell	Main functions
Red blood cells (erythrocytes)	Transport O_2 from lungs to tissues; transport CO_2 from tissues to lungs
Granulocytes	
Neutrophil	Chemotaxis, phagocytosis, killing of phagocytosed bacteria
Eosinophil	All neutrophil functions listed above, effector cells for antibody-dependent damage to metazoal parasites, regulate immediate type hypersensitivity reactions (inactivate histamine and slow-reacting substance of anaphylaxis released by basophils and mast cells)
Basophil	Mediate immediate-type hypersensitivity (IgE-coated basophils react with specific antigen and release histamine and slow reacting substance of anaphylaxis), modulate inflammatory responses by releasing heparin and proteases
Monocytes	Chemotaxis, phagocytosis, killing of some microorganisms, become macrophages
Platelets	Adhere to subendothelial connective tissue, participate in blood clotting (see p. 162)
Lymphocytes	Involved in immune responses

Table 1.2 Morphology of normal white cells in Romanowsky-stained smears of peripheral blood.

Cell type	Cell size (μm)	Colour	Cytoplasm: Ratio of cytoplasmic volume to nuclear volume	Cytoplasm: Granules	Nucleus
Neutrophil granulocytes	9–15	Slightly pink	High	Numerous, very fine, faint purple	Usually 2–5 segments
Eosinophil granulocytes	12–17	Pale blue	High	Many, large and rounded, reddish-orange	Usually two segments
Basophil granulocytes	10–14		High	Several, large and rounded, dark purplish-black	Usually two segments, granules overlie nucleus
Monocytes	15–30	Pale greyish-blue, cytoplasmic vacuoles may be seen	Moderately high or high	Variable number, fine, purplish-red	Various shapes (rounded, C- or U-shaped, lobulated), skein-like or lacy chromatin
Lymphocytes	7–12 (small lymphocytes); 12–16 (large lymphocytes)	Pale blue	Low or very low	Few, fine, purplish-red	Rounded with large clumps of condensed chromatin

Types of Leukemia.

Leukemias are divided into two general types:

1- lymphocytic leukemias.
2. myelocytic leukemias.

* The leukemia cells are bizarre & undifferentiated & not identical with any of the normal white blood cells.

* Usually the more undifferentiated the cells the more acute is the leukemia.

* But with some of the more undifferentiated cells, the process can be quite chronic, sometimes developing slowly over a period of 10-20 years.

* Leukemic cells, especially the very undifferentiated cells, are usually nonfunctional.

Effects of Leukemia on the Body:

1. The first effect of leukemia is metastatic growth of leukemic cells in abnormal areas of the body.

2. The leukemic cells of the bone marrow invade the surrounding bone.

3. Almost all leukemias spread to the spleen, the lymph nodes, the liver & vascular regions.

4. In each of these areas the rapidly growing cells invade the surrounding tissues, utilizing the metabolic elements of these tissues & consequently ~~casing~~ causing tissue destruction.

5. Very common effects in leukemia are the development of infections, severe anemia & bleeding tendency caused by thrombocytopenia (lack of platelets).

6. The most important effect of leukeima on the body is the excessive use of metabolic substrates by the growing cancerous cells.

7. Tremendous demands are made on the body for foodstuffs, especially the amino acids & vitamins. Consequently, the energy of the patient is greatly depleted, rapid deterioration of the normal protein tissues of the body.

8. Obviously, after metabolic starvation has continues long enough, this alone is sufficient to cause death.

Leucocytes

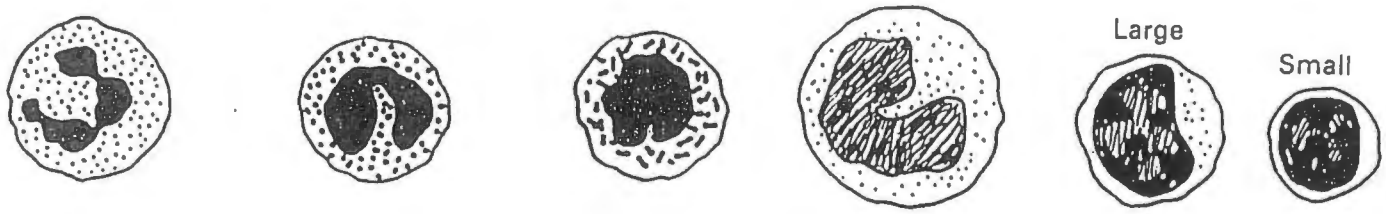
Classification

The blood leucocytes (white blood cells) are a heterogeneous population of nucleated cells lacking haemoglobin. There are five distinct morphological types classified into two groups on the basis of the presence or absence of granules in their cytoplasm:

1 **Granulocytes** (with cytoplasmic granules): these are the neutrophils, eosinophils and basophils.

2 **Agranulocytes** (without cytoplasmic granules): these are the monocytes and lymphoctyes.

Figure 2.5 gives the dimensions and morphological characteristics of the leucocytes.



Neutrophil 40–60%	Eosinophil 0.5–1%	Basophil 0.5–1%	Monocyte 6–10%	Lymphocyte 20–40%
Morphological features:				
Diameter 10–16μm	Diameter 12–18μm	Diameter 10–14μm	Diameter 15–20μm	Diameter 5–8μm (small lymphocyte) 9–15μg (medium and large lymphocyte)
Nucleus: Lobulated (2–5 lobes) connected by chromatin strands. Nucleus is made of dense masses which take the purple stain	Made usually of 2 lobes – stains less deeply than neutrophil	Rarely segmented and its margins are usually obscured by the overlying cytoplasmic granules	Usually kidney shaped. Nuclear chromatin made of interwoven threads which are without compact blocks. Stains faintly	Rounded or slightly indented. Nuclear chromatin is clumped in the form of dark masses
Cytoplasm: Contains fine granules which stain purplish	Contains the characteristic large spherical bright red granules	Contains large coarse rounded or oval dark deeply staining granules which overlie the nucleus	Grey-blue giving a ground glass appearance with fine reddish azurphilic granules	Large lymphocyte has abundant cytoplasm which usually takes pale blue stain and may contain fine reddish granules Small lymphocyte has a very scanty cytoplasm forming a small rim around the nucleus

Fig. 2.5 The shapes, dimensions and special morphological features of the various types of leucocytes.

3

Formation of leucocytes (leucopoiesis)

Sites of formation

1 **Granulocytes**: bone marrow.

2 **Lymphocytes**: bone marrow, thymus, lymph nodes and other collections of lymphoid tissues, e.g. wall of the intestine.

3 **Monocytes**: bone marrow.

Formation of granulocytes (granulopoiesis)

The life history of the granulocytes begins in the bone marrow, where there is progressive division and maturation from the earliest cell, the stem cell, successively through the cell types myeloblast, promyelocyte, myelocyte, metamyelocyte, band neutrophil and segmented neutrophil. The myeloblasts, promyelocytes and myelocytes are capable of mitotic division and cell replication; hence, these are collectively called the **proliferating granulocyte pool**. From the metamyelocyte stage onwards, no cell division occurs and therefore the metamyelocytes, band neutrophils and segmented neutrophils are together referred to as the **maturation pool**. Maturation takes the form of biochemical and morphological changes in both the nucleus and the cytoplasm. The nucleus becomes condensed and broken up into lobes. In addition, fine neutrophilic granules appear in the cytoplasm. The maturation pool is sometimes called the **marrow granulocyte reserve**, as it is believed to be the main source of extra neutrophils which enter the bloodstream in acute infections and other pathological states. The mature neutrophils, once released into the bloodstream, stay there for about 7–10 hours before they migrate to the tissues, where they function as mobile phagocytes.

Haemopoietic growth factors

The formation of all blood cells is sustained throughout life by a group of glycoprotein growth factors, the *haemopoietic growth factors* (collectively called *colony-stimulating factors*, **CSF**). The first to be discovered was erythropoietin. Others which control the production of white blood cells include: multipotential CSF, granulocyte–macrophage CSF and granulocyte CSF. Recently, these growth factors have found important clinical uses by stimulating the bone marrow activity in disease conditions such as bone marrow failure, haematological malignancies and infectious diseases.

Neutrophils in the bloodstream

Mature neutrophils leave the bone marrow to enter the blood. Some of them join the blood circulation—the so-called **circulating granulocyte pool**. These are the

cells available for blood sampling and counting. Others are deposited along the walls of the small vessels (**marginal granulocyte pool**), where they are in a state of rapid and continuous exchange with the circulating cells, and from this site they can be mobilized by exercise or by an adrenaline (epinephrine) injection. The entry of these cells into the circulating pool accounts for the increased white cell count (leucocytosis) that accompanies exercise and other stressful situations.

Functions of leucocytes

The general function of leucocytes is defence against infection. However, the different types of leucocytes contribute to a different extent towards this general function.

Functions of neutrophils

The neutrophils are also called **polymorphonuclear leucocytes** because the nucleus is formed of two to five lobes. This cell is the most important cell in the cellular defences of the body against infection. To achieve this goal, neutrophils execute several integrated functions: (i) the neutrophils must reach the site of infection (**chemotaxis**); (ii) they must ingest the foreign organism (**phagocytosis**); and (iii) they must kill or inhibit the multiplication of the microorganism (**microbial killing**).

Chemotaxis

The neutrophils are actively motile cells; they can move more rapidly than any other cell in the body. Their movement is directed towards bacteria in a purposeful manner, being attracted to bacteria or the site of infection or inflammation by a variety of chemotactic substances, e.g. products of certain bacteria, damaged leucocytes or other tissue components. The property of directed movement of the neutrophils is named **chemotaxis**. It accounts for the accumulation of neutrophils at sites where they are needed, e.g. infected wounds. Impaired chemotaxis can lead to increased susceptibility to infectious diseases, especially in children.

When neutrophils approach the infected site, they lie along the walls of the closest capillaries—a process called **margination**. Then individual neutrophils squeeze themselves between endothelial cells and gradually move out from the capillary—a process called **diapedesis**. Since neutrophils are motile cells, they move towards the bacteria.

Phagocytosis

Phagocytosis is the process whereby a cell eats particulate matter (Fig. 2.6).

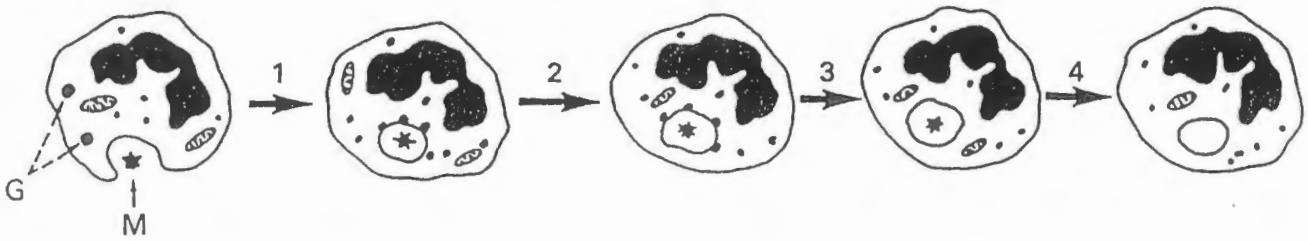


Fig. 2.6 Schematic diagram of the process of phagocytosis by a neutrophil. An ops onized microbe (M) after being recognized by th· neutrophil is contained in an invagination of the neutrophil membrane. T hereafter, the particle is enclosed in a phagocytic vaaıole. Soı of the neutrophil granules (G) stick to the wall of the vacuole and th en release their bactericidal substances, which induce kiling and ultimate digestion of the microbe.

One of the remarkable features of neutrophils is their fine capacity to distinguish foreign cells like bacteria from homologous body cells and aged or damaged cells from fresh ones. This is due to the presence in plasma of certain substances (**opsonins**), such as **γ-globulins** (especially immunoglobulin G (IgG)) and **complement C4**, which coat bacteria and ageing cells, thereby making them 'palatable' to neutrophils. To opsonize means to prepare for eating. An opsonin is an agent in plasma which acts on foreign particles to increase their palatability to phagocytes.

Recognition is followed by close adhesion between the outer membrane of the neutrophil and the bacterium. This is followed by invasion of the neutrophil membrane and complete encirclement of the bacterium by pseudopodia. The pseudopodia fuse to enclose the bacterium in a phagocytic vacuole.

9) *Microbial killing*

Following the ingestion (phagocytosis) of the bacterium, the following sequence of events take place:

1 The fusion of the neutrophilic granules with the phagocytic vacuole.

2 Discharge of antimicrobial agents from the granules into the vacuole. These agents include lysozymes, myeloperoxidase and lactoferrin, which are capable of destroying a wide range of bacteria.

3 Killing and digestion of the ingested organism.

Functions of eosinophils (acidophils)

The eosinophils are characterized by the presence of coarse, bright red granules in their cytoplasm. These granules contain an arginine-rich basic protein which attracts red acidic dyes like eosin. The eosinophil nucleus is often seen as two large lobes. Eosinophil functions are not very different from those of the neutrophil.

Chemotaxis

Unlike neutrophils, eosinophils are attracted more towards

areas of chronic inflammation rather than acute inflammation. **Chemotactic substances** for eosinophils include histamine, antigen–antibody complexes, 5-hydroxytryptamine (5-HT), bradykinin and a specific 'eosinophil chemotactic factor'.

Eosinophils tend to accumulate at the sites of histamine release, as is seen in allergic diseases of the skin or lungs.

Phagocytosis

Eosinophils are capable of ingesting a variety of particles, ranging from bacteria and destroyed cells to antigen–antibody complexes. Phagocytosis involves the same sequence of events as already described for neutrophils. However, antimicrobial activity is considerably less than that of the neutrophil. Eosinophils also release **major basic protein** (MBP) which is highly toxic to larvae of parasites.

(1)

Eosinophils and inflammation

In inflamed tissues, eosinophils have been shown to be capable of antagonizing and inactivating histamine and other chemical mediators of inflammation, such as 5-HT and bradykinin. Through this function, the eosinophil helps to limit and circumscribe the inflammatory process.

The eosinophilic response to an inflammatory stimulus is characterized by accumulation of eosinophils in the inflamed tissues, with a simultaneous increase both in the production of eosinophils by the bone marrow and in the number of circulating eosinophils (eosinophilia), on their way from the bone marrow to the inflammatory sites. Chronic eosinophilia occurs in response to complex antigens, as in helminthic (worm) infestations (e.g. hook-worm, ascaris and bilharzia) and in response to allografts (e.g. skin grafts).

The accumulation of eosinophils in inflammatory sites may be inhibited by high doses of corticosteroids, which also depress the chemotactic attraction of eosinophils.

Eosinophilia

From the foregoing account we may deduce that eosinophilia occurs in pathological states as a result of an antigen —antibody reaction.

Common causes of eosinophilia include:

1 Parasitic disease, e.g. worm infestations of the gut.

2 Allergic conditions:

(a) Bronchial asthma.

(b) Allergic rhinitis (hay fever).

(c) Drug reactions, e.g. penicillin sensitivity.

3 Tropical eosinophilia, which represents a reaction to the filaria parasite.

4 Dermatological diseases.

Functions of basophils

The distinguishing morphological feature of the basophil is the large blue-black granules which appear to fill the cytoplasm, overlie the nucleus and tend to obscure nuclear configuration. Basophil granules contain abundant acid mucopolysaccharide, which accounts for their strong affinity for basic dyes such as methylene blue. Heparin is one of the important acid mucopolysaccharide constituents; other constituents include histamine, 5-HT and ribonucleic acid (RNA). The basophil is the carrier of **histamine** in the blood and, due to its being rich in both heparin and histamine, it bears a strong resemblance to **tissue mast cells.** The function of basophils is not known with certainty but they may have a role related to their content of the physiologically active substances, such as heparin, histamine and 5-HT. Mast cells and basophils have surface Igt receptors which bind IgE coated antigens, degranulate and release histamine leading to allergic reactions e.g. urticaria.

Monocytes (blood macrophages)

This macrophage has its origin in the bone marrow monoblast and promonocyte. The mature monocyte reaches the bloodstream, where it stays for a variable period of time, ranging from a few hours to 6 days. Then it leaves the circulation for the tissues, where it undergoes transformation to the larger and more effective phagocyte—**tissue macrophage (histiocyte)**.

Functions

The macrophage contributes directly to the body defence systems by phagocytosis and killing of invading bacteria and, indirectly, by interacting and cooperating with lymphoid cells in both the afferent (or recognition of foreign material) and efferent (effector) limbs of the **immune response.** In the afferent limb, macrophages process the antigen and present it to lymphocytes.

Macrophages phagocytose damaged or altered host cells and microscopic debris, which justifies the descriptive name '**tissue scavengers**'.

Lymphocytes

Much of our knowledge about the cellular elements of the blood has been based on the concept that cells may be recognized and classified by morphological criteria. This concept, however, does not hold in relation to recognizing and classifying cells of the lymphoid series. The blood lymphocytes constitute a family of cells of different origins, migration patterns, sizes, staining characteristics, ultrastructure, lifespan and function.

Formation (lymphopoiesis)

Lymphocytes originate from the primitive unipotent stem cell (lymphoid-committed precursor) in the **thymus, lymphoid tissues** and **bone marrow** and then proceed along a known maturation line via the 'lymphocyte production pathway', which includes the following cellular stages:

1 *Lymphoblasts*. Normally these are only seen in lymphopoietic organs and almost never observed in peripheral blood.

2 *Intermediate (transitional) forms* (large blast cells).

3 *Small and large lymphocytes* (blood lymphocytes).

These stages are not unidirectional. The process can, under certain circumstances, go in the reverse direction and small lymphocytes can grow into large lymphocytes and lymphoblasts. Such blastic transformation can be demonstrated *in vitro* by growing small lymphocytes in a suitable culture medium containing a non-specific mitogen, such as *phytohaemagglutinin (PHA)*, or a specific antigen, e.g. *tuberculin*.

Lymphocytes in the bloodstream

Lymphocytes enter the peripheral blood either directly, by passing through the walls of blood-vessels in the various lymphopoietic organs, or indirectly, by entering the lymph stream and eventually reaching the bloodstream through the thoracic duct and other lymph ducts in the neck.

Classification

When seen under an ordinary light microscope, blood lymphocytes can be divided into **small** (5–8 μm diameter) and **large lymphocytes** (8–15 μm diameter). The majority of blood lymphocytes are of the small type.

Functions

Lymphocytes are the central cells in immunity. On the

basis of this function, lymphocytes are divided into two types:

1 *Thymus-dependent lymphocytes (T cells)* are so called because they originate in the thymus or bone marrow and migrate to the thymus where they mature and are reprogrammed to recognize foreign antigens. They have a lifespan of 100–300 days or even more (hence the name long-lived lymphocytes). This long lifespan is closely related to their property of constant movement from blood to tissues to lymph to blood again (*recirculation of lymphocytes*).

T lymphocytes are the principal mediators of **cellular immune responses**, such as rejection of tissue graft, e.g. kidney transplant, and delayed hypersensitivity reactions. They also play a minor role in the synthesis of immunoglobulins (antibodies).

2 *Thymus-independent lymphocytes (B cells)*. In humans, the B cells develop in the bone marrow, the germinal centres of lymph nodes and the red pulp of the spleen. Their lifespan is 2–7 days (hence the name short-lived lymphocytes). They have been called B cells because they are known as bursa cells. When the B cells are properly stimulated by an antigen, they develop successively into large lymphocytes and, lastly, plasma cells. The plasma cells are lymphoid cells which are capable of producing **antibodies**. Thus, the B lymphocytes are the principal mediators of the **humoral immune response**.

Total leucocyte count

Although it is usually quoted in textbooks that the total leucocyte count is 4000 to 10 000 cells per cubic millimetre of blood, it should be emphasized that this range applies more to Europeans than to residents of hot tropical countries. It is not uncommon to find a total leucocyte count among healthy students and blood donors in these geographical locations of between 2000 and 4000 cells/mm^3. Because there is a relatively low count of neutrophils, this is called *neutropenia*.

Differential white cell counts

The normal proportions of white blood cells are as follows:

Neutrophils	60–70%
Lymphocytes	20–30%
Monocytes	2–8%
Eosinophils	2–4%
Basophils	0–2%

Leucocytosis and leucopenia

An increase in the total leucocyte count above the normal

is called leucocytosis. This may occur in health (physiological leucocytosis) or disease.

Physiological leucocytosis may occur under several conditions:

1 Diurnal variation: leucocyte counts are lowest in the morning and increase to a maximum in the afternoon.

2 After a protein meal.

3 Following physical exercise.

4 Stimulation by stress or an injection of adrenaline (epinephrine).

Disease states which commonly cause leucocytosis are bacterial infections (pyogenic infections), e.g. tonsillitis, infected wounds or inflamed appendix. In these conditions, measurement of the total leucocyte count is essential for diagnosing the existence of the infection. The differential white cell count is also useful. In general, acute bacterial infections cause an increase in the neutrophil count, while chronic and viral infections are associated with an increased lymphocyte count.

Leucopenia is a decrease in the total leucocyte count below the normal. It is often seen in conditions of malnutrition and is also an important feature of typhoid fever. Some drugs may depress the bone marrow and therefore result in leucopenia and, in particular, a decrease in the granulocyte count (agranulocytosis). Leucopenia can also be caused by a deficiency of vitamin B_{12} or folic acid.

Table 19.2 Significance of High and Low White Blood Cell Counts

WBC Type	High Count May Indicate	Low Count May Indicate
Neutrophils	Bacterial infection, burns, stress, inflammation.	Radiation exposure, drug toxicity, vitamin B_{12} deficiency, and systemic lupus erythematosus (SLE).
Lymphocytes	Viral infections, some leukemias.	Prolonged illness, immunosuppression, and treatment with cortisol.
Monocytes	Viral or fungal infections, tuberculosis, some leukemias, other chronic diseases.	Bone marrow suppression, treatment with cortisol.
Eosinophils	Allergic reactions, parasitic infections, autoimmune diseases.	Drug toxicity, stress.
Basophils	Allergic reactions, leukemias, cancers, hypothyroidism.	Pregnancy, ovulation, stress, and hyperthyroidism.

Effects of Leukemia on the Body:

1. The first effect of leukemia is metastatic growth of leukemic cells in abnormal areas of the body.

2. The leukemic cells of the bone marrow invade the surrounding bone.

3. Almost all leukemias spread to the spleen, the lymph nodes, the liver & vascular regions.

4. In each of these areas the rapidly growing cells invade the surrounding tissues, utilizing the metabolic elements of these tissues & consequently casing tissue destruction.

5. Very common effects in leukemia are the development of infections, severe anemia & bleeding tendency caused by thrombocytopenia (lack of platelets).

6. The most important effect of leukeima on the body is the excessive use of metabolic substrates by the growing cancerous cells.

7. Tremendous demands are made on the body for foodstuffs, especially the amino acids & vitamins. Consequently, the energy of the patient is greatly depleted, rapid deterioration of the normal protein tissues of the body.

8. Obviously, after metabolic starvation has continues long enough, this alone is sufficient to cause death.