PHYSIOLOGY OF BLOOD

FUNCTIONS OF BLOOD

HOMEOSTATIC FUNCTION

buffering

thermoregulation (transport of heat)

TRANSPORT OF SUBSTANCES

(blood gases, nutrients, metabolites, vitamins, electrolytes…)

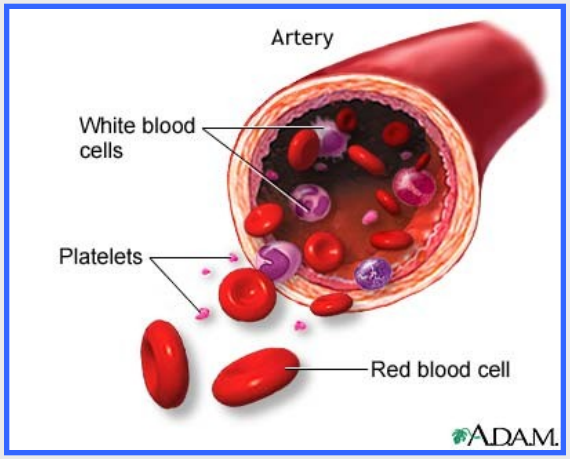
HUMORAL CONTROL OF ORGANISM (hormones)

DEFENCE OF ORGANISM (immune functions)

BLOOD CLOTTING

BASIC CHARACTERISTICS

- **Suspension** character
- 6 - 8% total body mass
  - 55% - **fluid** phase (plasma)
  - 45% - **formed** phase (blood cells and platelets)



- **Serum**: from plasma during blood clotting – after consumption of fibrinogen

BONE MARROW

Size (1600-3000 grams), activity.

Red bone marrow, **yellow** bone marrow.

Pluripotent stem cells.

Unipotent (determined) stem cells – differentiated cells.

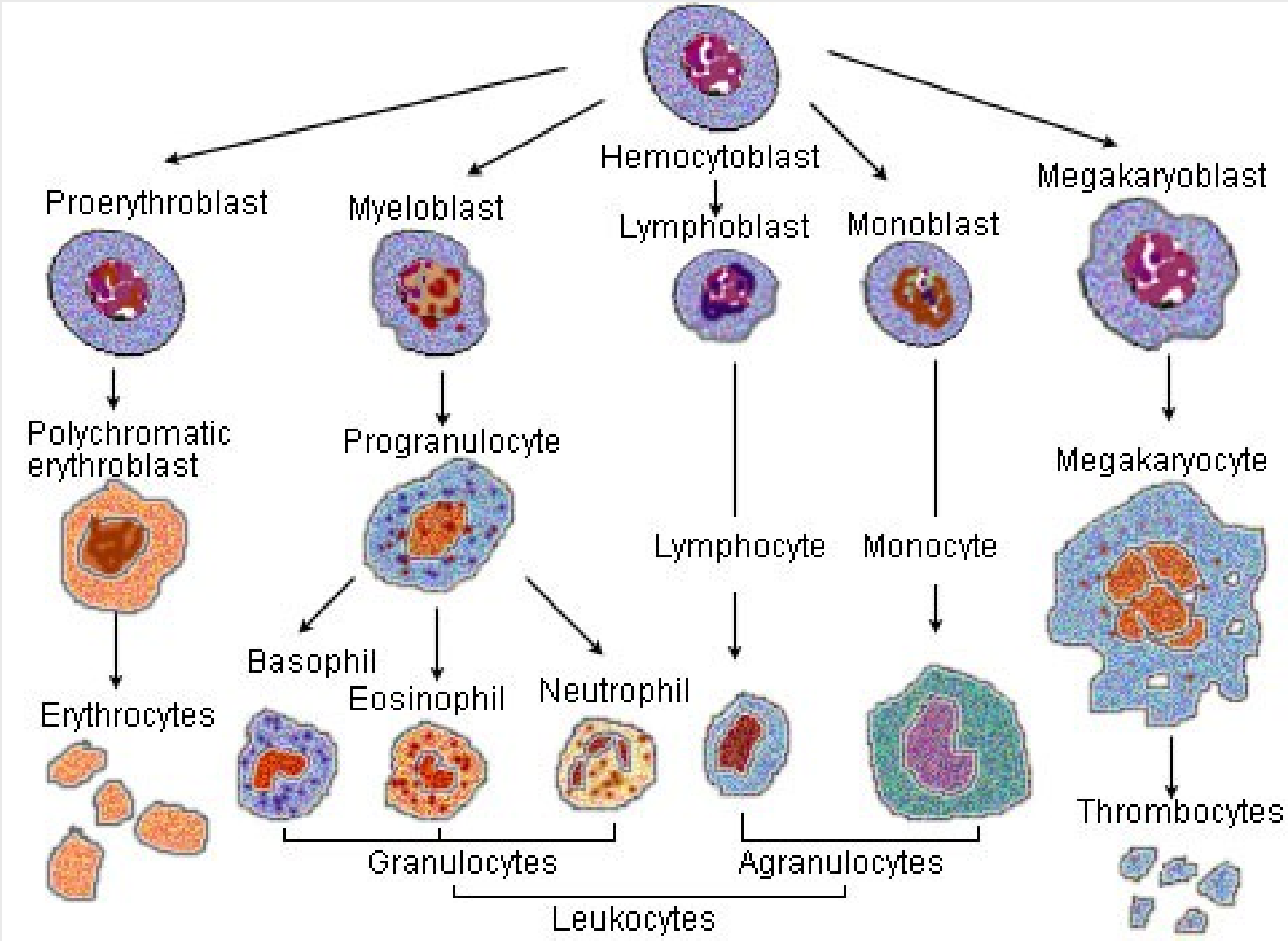
Medullar haematopoiesis – ADULTS.

Extra-medullar haematopoiesis

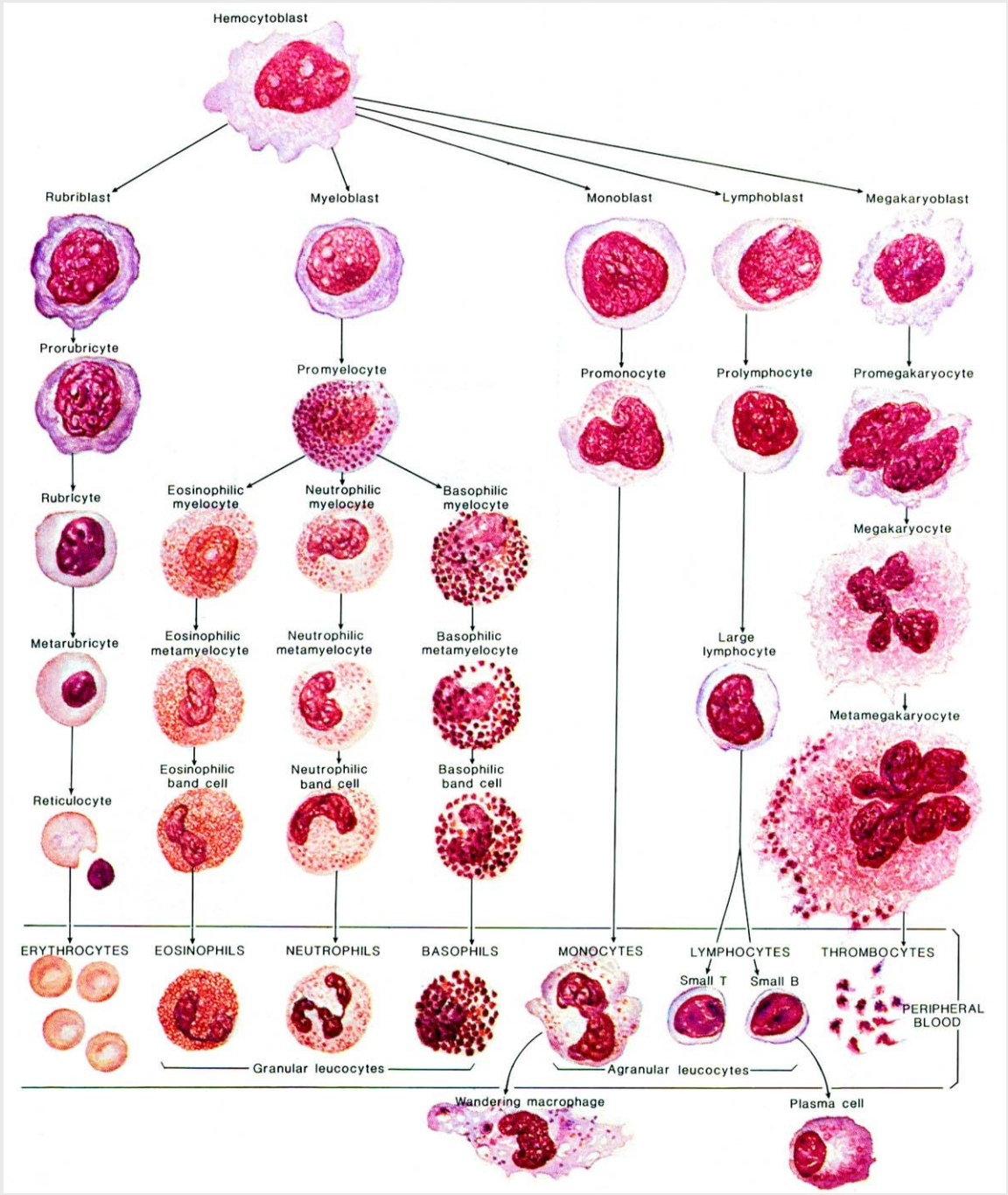
ERYTROPOESIS

Ontogenesis

3rd week – yolc sac
6th week – liver (formation in the yolk sac expires)
12th week – lien
20th week – bone marrow
32nd week – rearrangement from embryonic hemoglobin to HbF
newborn – only in bone marrow, rearrangement HbF to HbA
adult – sternum, vertebrae, ribs, clavicula, proximal epiphyses of some long bones

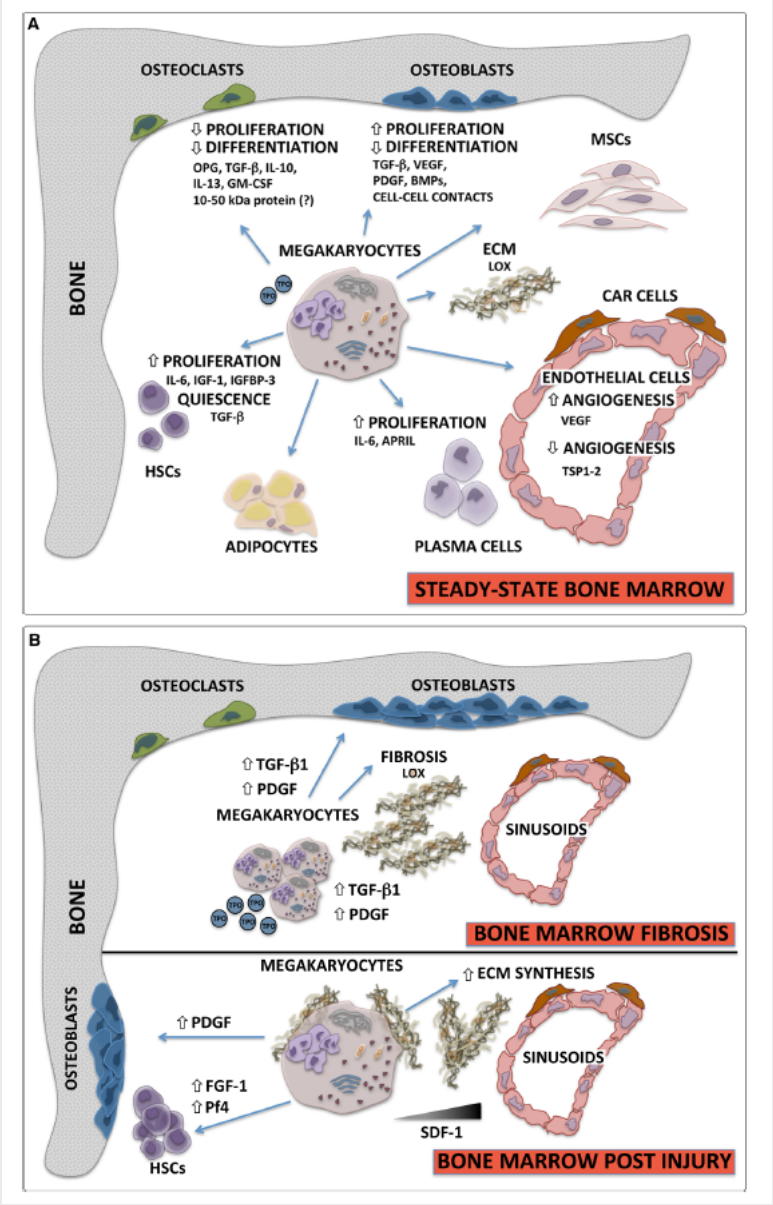


Source: Wikimedia Commons


Hemocytoblast
Rubriblast
Myeloblast
Monoblast
Lymphoblast
Megakaryoblast
Prorubricyte
Promyelocyte
Promonocyte
Prolymphocyte
Promegakaryocyte
Rubricyte
Eosinophilic myelocyte
Neutrophilic myelocyte
Basophilic myelocyte
Megakaryocyte
Metarubricyte
Eosinophilic metamyelocyte
Neutrophilic metamyelocyte
Basophilic metamyelocyte
Large lymphocyte
Metamegakaryocyte
Reticulocyte
Eosinophilic band cell
Neutrophilic band cell
Basophilic band cell
ERYTHROCYTES
EOSINOPHILS
NEUTROPHILS
BASOPHILS
MONOCYTES
LYMPHOCYTES
THROMBOCYTES
Small T
Small B
PERIPHERAL BLOOD
Granular leucocytes
Agranular leucocytes
Wandering macrophage
Plasma cell

Regulatory function of megakaryocytes (MKs)!

- control of bone marrow homeostasis
- mesenchyal stem cells (MSCs) = an important regulator of MKs function via production of cytokines and soluble factors
- role of MKs in modulating the replication and differentiation of osteoclasts and osteoblasts = regulation of bone formation and matrix reorganization
- MKs represent an important reservoir of bioactive hemopoietic and angiogenic factors
- MKs can directly regulate hemopoietic stem cells (HSCs) and next hemopoietic cells (mainly via IL-6)
- Mks participate in angiogenesis



Malara A, Abbonante V, Di Buduo CA, Tozzi L, Currao M, Balduini A: **The secret life of a megakaryocyte: emerging roles in bone marrow homeostasis control.** ***Cellular and Molecular Life Sciences 2015, 72(8):1517-1536.***

BLOOD CELLS

Cells	Cells /µl (average)	Normal range	Percent of total number of leukocytes
Leukocytes (total)	9000	3600 - 9600	White blood cell count
Granulocytes Neutrophiles	5400	3000 - 6000	42 – 75
Eozinophiles	275	150 - 300	1 - 4
Basophiles	35	0 - 100	0,4
Agranulocytes Lymphocytes	2750	1200 - 3400	20 - 50
Monocytes	540	110 - 590	1,7 – 9,3
Erythrocytes woman		$4{,}2 – 5{,}4 \cdot 10^6$	
men		$4{,}5 - 6{,}3 \cdot 10^6$	
Platelets	300 000	140000 – 440000	

RBC *(ERY)*

$3.5\text{-}5.5*10^{12}/l$

↑ POLYCYTAEMIA

↓ ERYTHROCYTOPENIA

HCT

haematocrite

0.38-0.49 l/l

HGB

concentration of hemoglobin

140-180 g/l

↑ POLYGLOBULIA

↓ ANAEMIA

MCV

mean corpuscular volume

80-95 fl

↑ MACKROCYTE

↓ MICROCYTE

MCH

mean corpuscular haemoglobin

27-32 pg – NORMOCHROMIA

↓/ ↑ HYPO/HYPERCHROMIA

MCHC

mean corpuscular haemoglobin concentration

320-360 g/l NORMOCHROMIA

↓/ ↑ HYPO/HYPERCHROMIA

RED BLOOD CELLS (ERYTHROCYTES)

		Men	Women
Hematocrit (Hct) (%)		47	42
Erythrocytes (RBC) (10^6/µl)		4,5 - 6,3 x10^6	4,2–5,4x10^6
Haemoglobin (Hb) (g/l)		140 - 180	120 - 160
Mean volume of ery (MCV) (fl)	= Hct x 10 / RBC (10^6/µl)	82 - 97	82 - 97
Mean content of Hb in ery (MCH) (pg)	= Hb x 10 / RBC (10^6/µl)	27 - 33	27 - 33
Mean concentration of Hb in ery (g/100ml)	= Hb x 100 / Hct	32 - 36	32 - 36
Mean diameter of ery (MCD) (µm)		7,5	7,5

Function of erythrocytes: blood gases transport

RED BLOOD CELL EXAMINATION

1. Red blood cell count

- normocytemia
- erytrocytopenia (oligocytemia)
- polyglobulia (polycytemia)

2. Concentration of haemoglobin

- anaemia

3. Hematocrit

SHAPE AND SIZE OF ERYTHROCYTES

Shape: biconcave disc
OPTIMAL RATIO OF SURFACE TO VOLUME!!!
By 30% larger surface in comparison with the cell of the same size but of round shape!!!
Anizocytosis – physiological, pathological. Price-Jones curve.

Size: 7,5 μm in diameter, 2 μm thickness – normocytes.
Microcytes (-osis): diameter below 6 μm, volume below 80 fl
Macrocytes (-osis), megalocytes: diameter above 8.2 μm, volume above 95 fl

Amount of haemoglobin in one red blood cell: hypochromia (below 27 pg Hb/ery), normochromia, hyperchromia

Deformation of red blood cells. Fahraeus-Lindqvist effect.

Gallagher PG: **Abnormalities of the Erythrocyte Membrane.** ***Pediatric Clinics of North America 2013, 60(6):1349-+.***

1. Transport proteins
2. Cell adhesion proteins
3. Structural proteins

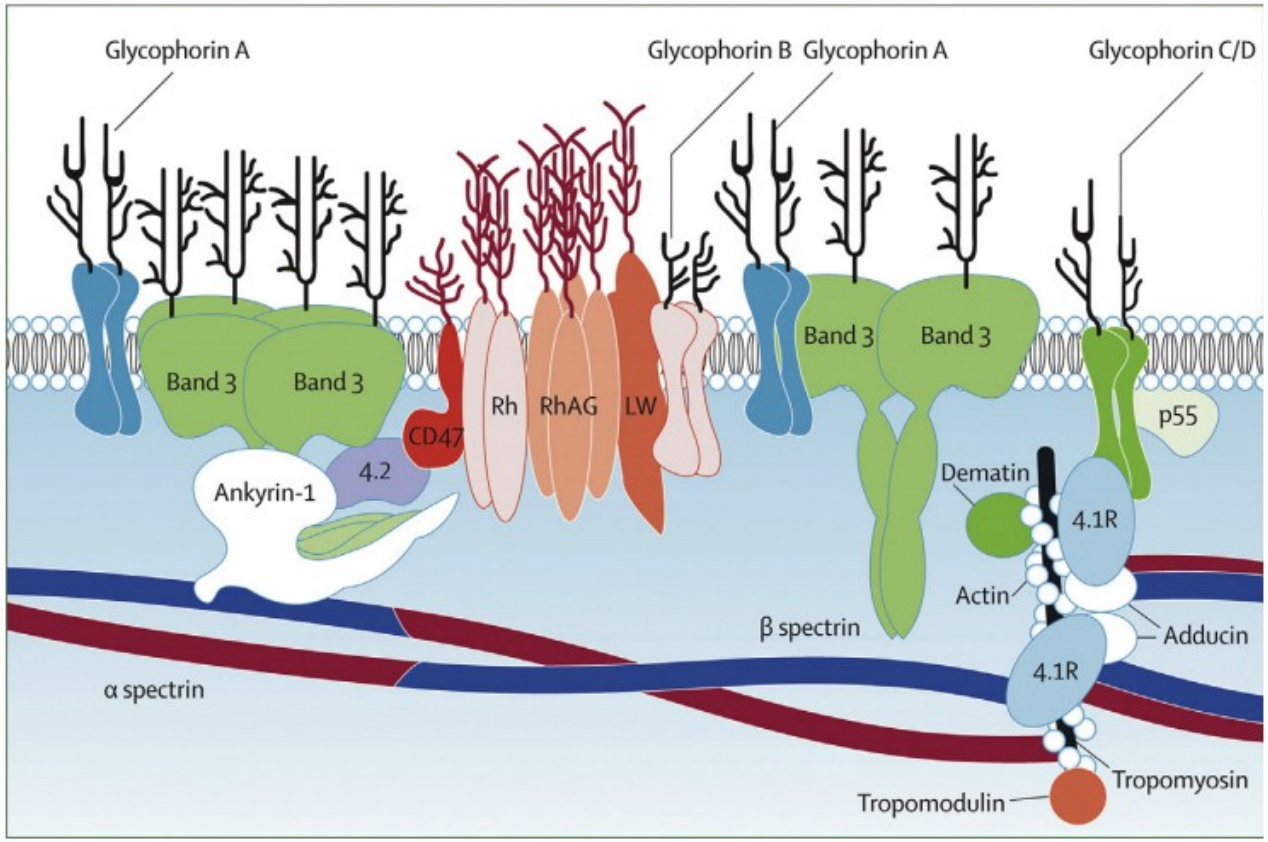


Fig. 1. The erythrocyte membrane. A model of the major proteins of the erythrocyte membrane is shown: α- and β-spectrin, ankyrin, band 3 (the anion exchanger), 4.1 (protein 4.1) and 4.2 (protein 4.2), actin and glycophorin. (*From* Perrotta S, Gallagher PG, Mohandas N. Hereditary spherocytosis. Lancet 2008;372:1412; with permission.)

- **Glycophorins A and B**
  - major sialoglycoproteins of the human erythrocyte membrane which bear the antigenic determinants for the MN and Ss blood groups (MNS blood group)
- **Spectrin**
  - the most prominent component (two isoforms α,β; a tetramer; a meshwork)
  - fixed to the membrane - ankyrin binding sites for several other proteins (glycophorin C, actin, band 4.1, adducin)
- **This organization keeps the erythrocyte shape.**

Transport proteins

- **Band 3 (Diego Blood group)**
  - mediating the exchange of chloride (Cl^-) for bicarbonate (HCO_3^-) across a plasma membrane
- **Aquaporin 1 = water channel (Colton Blood Group)**
- **GLUT1**
- **Jk antigen**
  - on a protein responsible for urea transport in the red blood cells and the kidney (aka human urea transporter 11- HUT11 or UT-B1)
- **Rh-associated glycoprotein (RHAG) (Rh Blood Group)**
  - an ammonia transporter protein
- **Na^+/K^+-ATPase**
- **Ca^{2+}-ATPase**
- **Na-K-Cl cotransporter**
- **Sodium-chloride symporter**
- **Chloride potassium symporter**
- **Potassium intermediate/small conductance calcium-activated channel (Gardos channel)**

Cell adhesion proteins

- **ICAM-4 (Landsteiner and Wiener Blood System)**
- **BCAM** = Basal cell adhesion molecule (**Lutheran blood group**)

Structural proteins

- Establish linkages with skeletal proteins
- Regulating cohesion
- Ankyrin-based macromolecular complex
- Protein 4.1R-based macromolecular complex
  - Protein 4.1 (Beatty's Protein)
  - Glycophorins C and D (**Gerbich Blood Group**)
  - XK (Kell blood group precursor) (**Kell Blood Group**)
  - RhD/RhCE (**Rh Blood Group**)
  - Duffy antigen/chemokine receptor (DARC)
  - Alpha-adducin
  - Dematin

Erythrocyte exceptions

They lack organelles

- no ATP production in oxidative phosphorylation
- no ability to replace damaged lipids and proteins (low metabolic activities, with no ability to synthesize new proteins or lipids)

Free radicals exposure

- haemoglobin autoxidation ($O_2^{\bullet -}$ release)
- a cell membrane rich in polyunsaturated fatty acids (susceptible to lipid peroxidation)
- deformation in tiny capillaries; catalytic ions leakage (cause of lipid peroxidation)

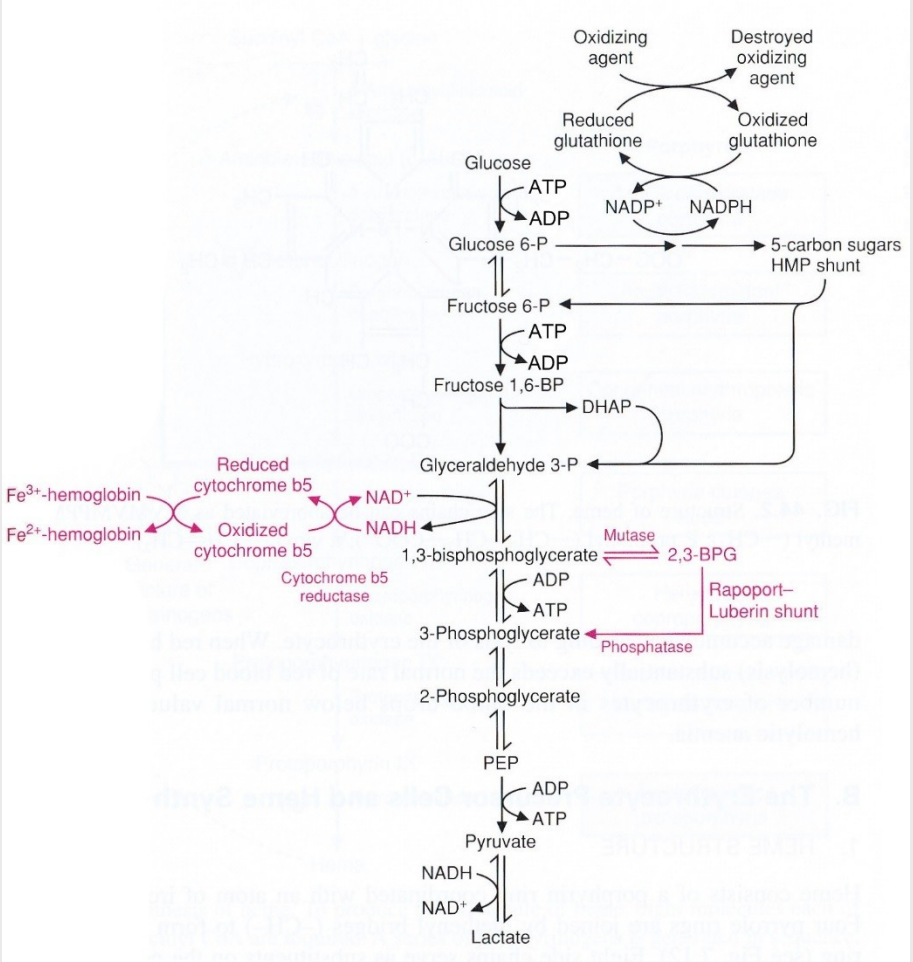
Erythrocyte metabolism

1. **Glucose as a source of energy** (GLUT1 transporter, insulin-independent)

2. **Glycolysis generates ATP and 2,3-bisphosphoglycerate** (the specific binding of 2,3-BPG to deoxyhemoglobin decreases the oxygen affinity of hemoglobin and facilites oxygen release in tissues)

3. **The pentose phosphate pathway produces NADPH**

4. **Glutathione synthesis - the antioxidant defence system**



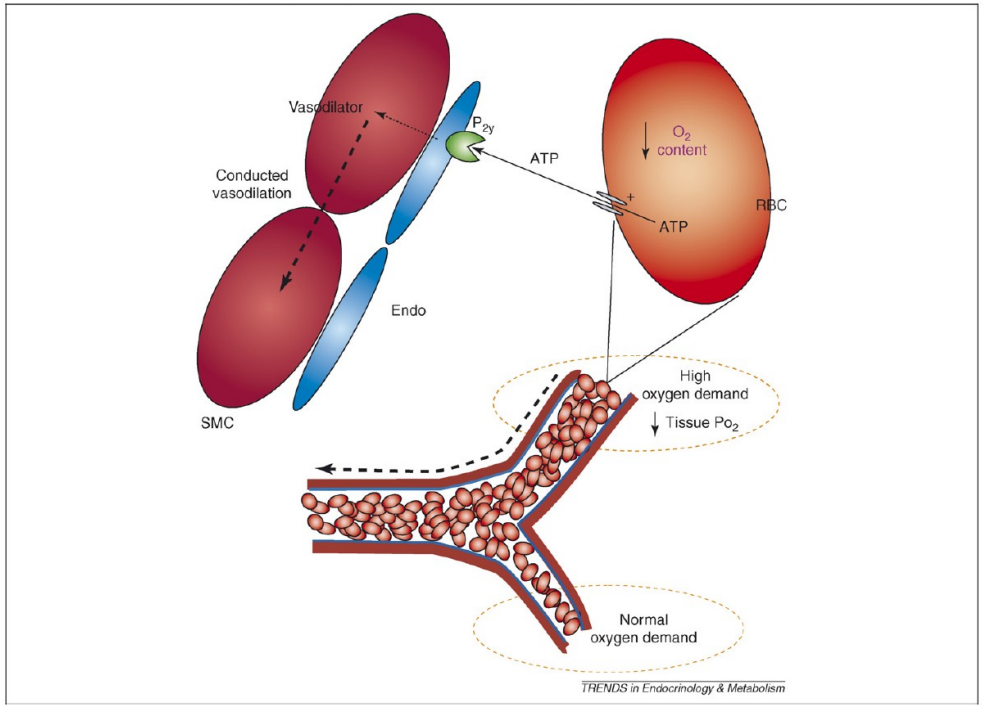


Figure 1. Cascade of events initiated by the entrance of erythrocytes into a tissue region (dashed oval) in which oxygen demand exceeds oxygen supply. [For clarity, a single erythrocyte (RBC) is enlarged along with the associated vascular cells to show the events that occur following the entrance of an erythrocyte into the region of tissue with high oxygen demand.] When oxygen supply does not meet oxygen demand, tissue oxygen tension (PO_2) decreases. This decrease in tissue PO_2 causes the hemoglobin oxygen content of the erythrocytes that perfuse the tissue region to decrease proportionally. This decrease in oxygen content initiates a series of events resulting in the release of ATP from the erythrocyte. The ATP then diffuses to the endothelium (Endo) where it binds to purinergic (P_{2y}) receptors resulting in the production of vasoactive mediators, either within the endothelium or the smooth muscle (SMC), which initiate vasodilation. This vasodilation is conducted (dashed arrow) in a retrograde fashion increasing flow and thus oxygen supply to the tissue region in need.

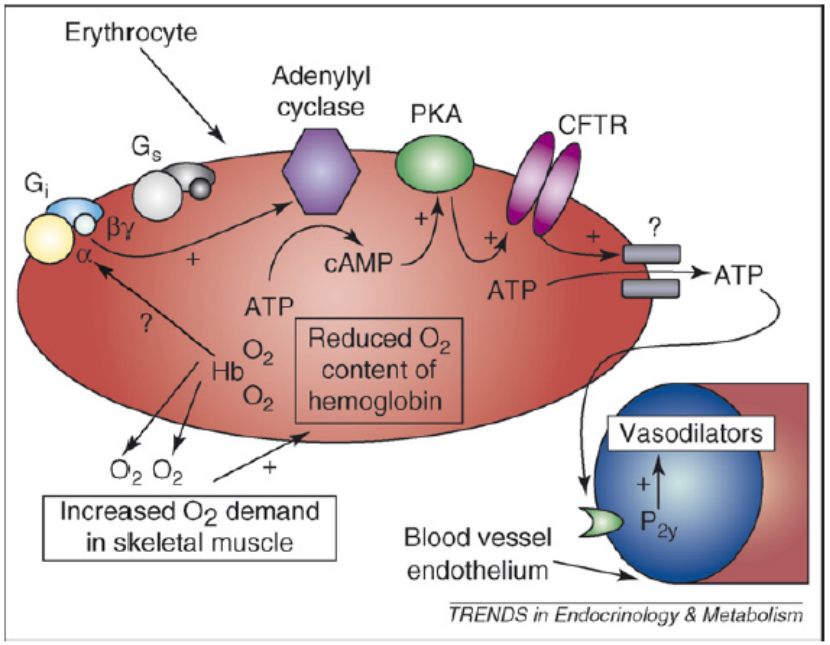


Figure 2. Proposed pathway for regulated ATP release from erythrocytes in response to passage of these cells through areas of increased oxygen demand in skeletal muscle. The increase in oxygen demand leads to oxygen release from hemoglobin within the erythrocyte. Consequently, hemoglobin oxygen content decreases resulting in activation of the heterotrimeric G protein, Gi, leading to ATP release. ATP released from the erythrocyte can bind to purinergic receptors (P_{2y}) on the vascular endothelium resulting in the release of vasodilators and, ultimately, an increase in blood flow (oxygen delivery). Abbreviations: Gi and Gs = heterotrimeric G proteins - i = inhibitory, s = stimulatory; ATP = adenosine 5′-triphosphate; cAMP = 3′5′-cyclic adenosine monophosphate; Hb = hemoglobin; PKA = protein kinase A; CFTR = cystic fibrosis transmembrane conductance regulator; ? = an as yet unidentified mechanism; P_{2y} = P_{2y} purinergic receptor; ± = stimulation.

Sprague RS, Stephenson AH, Ellsworth ML: **Red not dead: signaling in and from erythrocytes.** ***TRENDS in Endocrinology and Metabolism 2007, 18(9):350-355.***

MORPHOLOGICAL VARIATIONS OF ERYTHROCYTES

Poikilocytes – drop-like erythrocytes

Schizocytes – fragmented erythrocytes

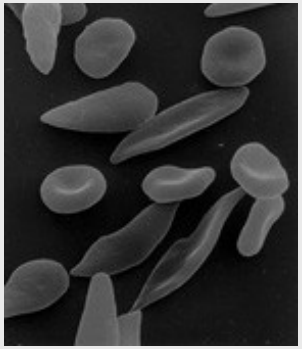
Spherocytes – volume normal, diameter smaller, thickness bigger

Eliptocytes – ecliptic shape

Leptocytes – thin, centrally concentrated haemoglobin

(thalasemia, after splenectomy)

Akantocytes – prickly prominences



FRAGILITY OF ERYTHROCYTES

Haemolysis – destruction of red blood cell membrane.

Types of haemolysis:
a) physical
b) chemical
c) osmotic
d) biological (toxic)
e) immunological

Spherocytosis
- disorders of protein net responsible for shape and elasticity of erythrocyte membrane – actin, ankyrin, spectrin.
Disorders of glucose-6-phosphate-dehydrogenase .

Erythrocytes life span: 120 days, role of lien (double circulation), splenectomy.
Reticulocytes.

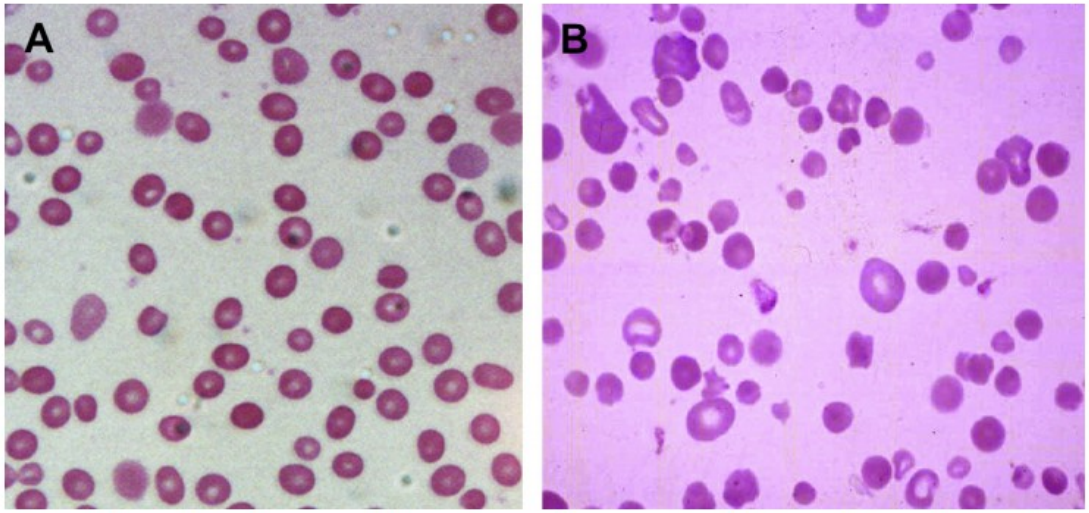


Fig. 2. Peripheral blood smears in hereditary spherocytosis. (*A*) Typical hereditary spherocytosis. Characteristic spherocytes lacking central pallor are seen. (*B*) Severe, recessively inherited spherocytosis. Numerous small, dense spherocytes and bizarre erythrocyte morphology with anisocytosis and poikilocytosis associated with severe hemolysis are seen.

Table 1
Classification of hereditary spherocytosis

	Carrier	Mild Spherocytosis	Moderate Spherocytosis	Severe Spherocytosis[a]
Hemoglobin (g/dL)	Normal	11–15	8–12	6–8
Reticulocytes (%)	≤3	3–6	≥6	≥10
Bilirubin (mg/dL)	0–1	1–2	≥2	≥2
Spectrin content (% of normal)	100	80–100	50–80	40–60
Peripheral smear	Normal	Mild spherocytosis	Spherocytosis	Spherocytosis
Osmotic fragility fresh blood	Normal	Normal or slightly increased	Distinctly increased	Distinctly increased
Incubated blood	Slightly increased	Distinctly increased	Distinctly increased	Distinctly increased

[a] Values in untransfused patients.

From Eber SW, Armbrust R, Schroter W. Variable clinical severity of hereditary spherocytosis: relation to erythrocytic spectrin concentration, osmotic fragility, and autohemolysis. J Pediatr 1990;117:409–16.

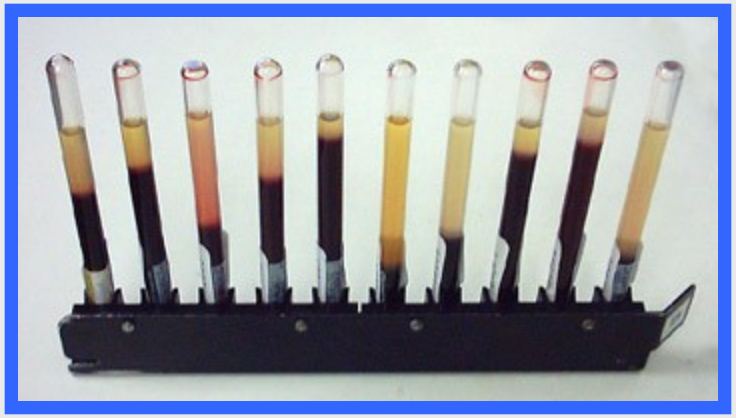
Gallagher PG: **Abnormalities of the Erythrocyte Membrane.** ***Pediatric Clinics of North America 2013, 60(6):1349-+.***

ERYTHROCYTE SEDIMENTATION

Sedimentation rate indirectly corresponds to suspension stability of blood.

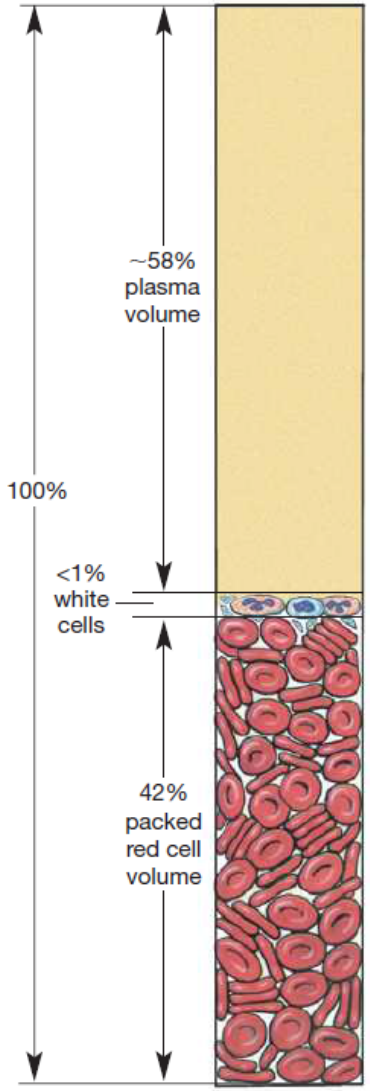
Method of Fahreus-Westergren (FW).

Physiological values: men – women
Units: mm to1 hr/2 hrs
Physiological causes of increased sedimentation.
Pathological causes of increased sedimentation.



THE BLOOD COUNT

This table lists the normal ranges of values.



	MALES	FEMALES
Hematocrit		
Hematocrit is the percentage of total blood volume that is occupied by packed (centrifuged) red blood cells.	40–54%	37–47%
Hemoglobin (g Hb/dL* whole blood)		
The hemoglobin value reflects the oxygen-carrying capacity of red blood cells. (*1 deciliter (dL) = 100 mL)	14–17	12–16
Red cell count (cells/μL)		
A machine counts erythrocytes as they stream through a beam of light.	$4.5–6.5 \times 10^3$	$3.9–5.6 \times 10^3$
Total white count (cells/μL)		
A total white cell count includes all types of leukocytes but does not distinguish between them.	$4–11 \times 10^3$	$4–11 \times 10^3$
Differential white cell count		
The differential white cell count presents estimates of the relative proportions of the five types of leukocytes in a thin blood smear stained with biological dyes.		
Neutrophils	50–70%	50–70%
Eosinophils	1–4%	1–4%
Basophils	<1%	<1%
Lymphocytes	20–40%	20–40%
Monocytes	2–8%	2–8%
Platelets (per μL)		
Platelet count is suggestive of the blood's ability to clot.	$150–450 \times 10^3$	$150–450 \times 10^3$

Fig. 16.3

Silverthorn, D. U. Human Physiology – an Integrated Approach. 6th. edition. Pearson Education, Inc. 2012.

Table 2. Factors causing false changes in Erythrocyte Sedimentation Rate

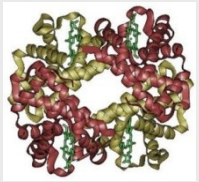
Factors causing false increases	Factors causing false decreases
Increased fibrinogen, globulin, cholesterol levels	Cachexia
High room temperature	Coagulation of the blood sample
Macrocytic anemia	Increase in bile salts
Menstruation	Increase in phospholipids
Pregnancy	Making the sedimentation sample wait more than two hours
Tilting or lying down of the ESR tube	Increase in adrenal steroids
Drugs: Dextrane, methyldopa, methysergide, penicillamine, procainamide, teophylline, trifluoperidole, vitamin A	Hypofibrinogenemia
	Hyperglycemia
	Hyperalbuminemia
	Leukocytosis
	Microcytic anemia
	Drugs: ACTH, cortisone, ethambutol, quinine, salicylates

(Adapted from A Textbook of Natural Medicine, Pizzorno and Murray, 1992)

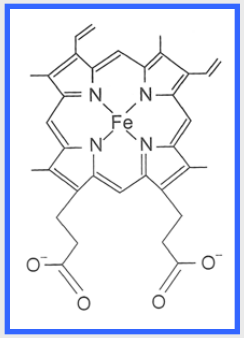
Table 3. Factors affecting Erythrocyte Sedimentation Rate (ESR)

Increased ESR	Decreased ESR
Acute Heavy Metal Poisoning	Congestive heart failure
Collagen Vascular Disease	Polycythemia
Carcinomas	Sickle Cell Anemia
Cell or tissue injury	
Gout arthritis	
Infections	
Inflammatory disorders	
Leukemia	
Myocardial infarction	
Nephritis	
Syphilis	

(Adapted from A Textbook of Natural Medicine, Pizzorno and Murray, 1992)



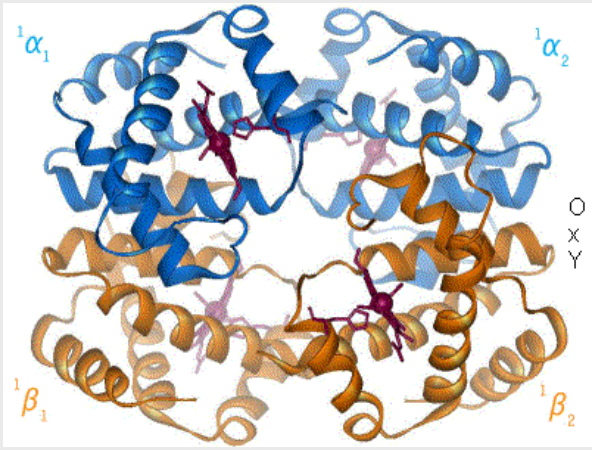
HAEMOGLOBIN



Red pigment transporting oxygen.
Protein, 64 450, 4 subunits.
Hem – derivative of porphyrine containing iron, conjugated with polypeptides (globin).

Embryonic haemoglobin: Gower I a Gower II ($\tau 2\varepsilon 2$, $\alpha 2\varepsilon 2$), Portland
Fetal haemoglobin: Hb F, $\beta 2\gamma 2$, weaker binding of 2,3 DPG
Adult haemoglobin: Hb A, $\alpha 2\beta 2$ (141/146)

Forms of haemoglobin:
oxyhaemoglobin - O_2
carbaminohaemoglobin – CO_2
methaemoglobin – Fe^{3+} in hem
carboxyhaemoglobin – CO



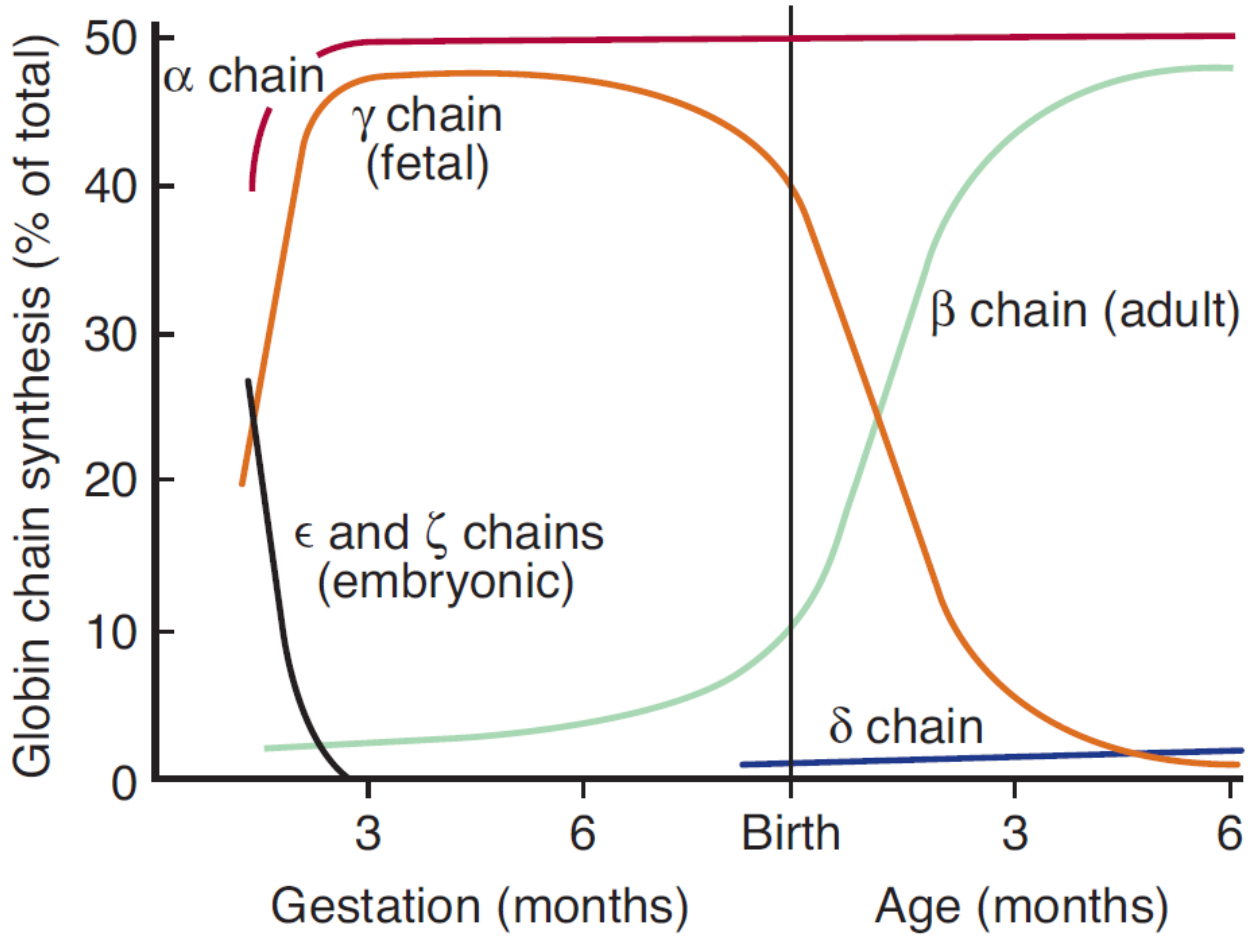
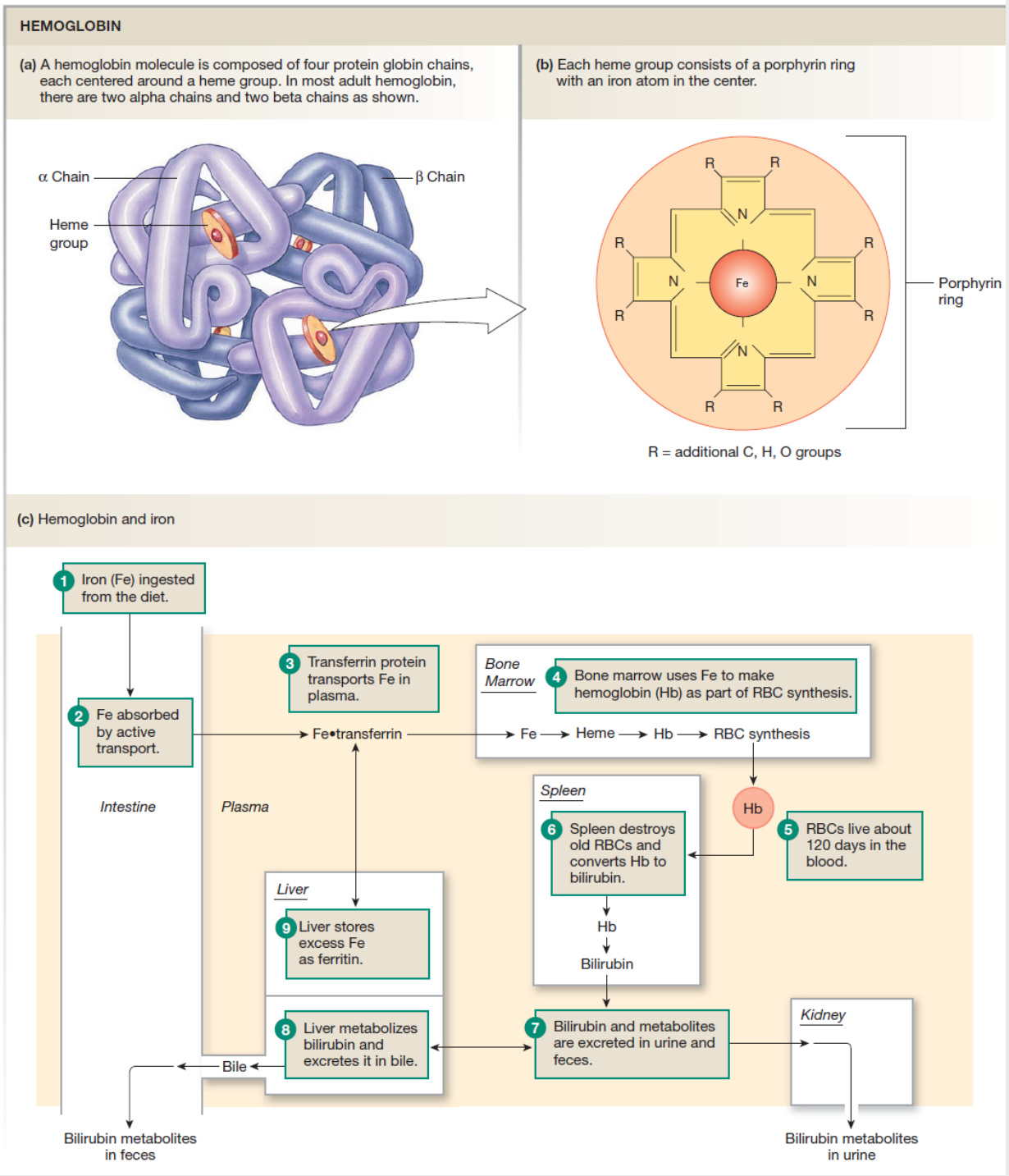


FIGURE 32–8 **Development of human hemoglobin chains.**



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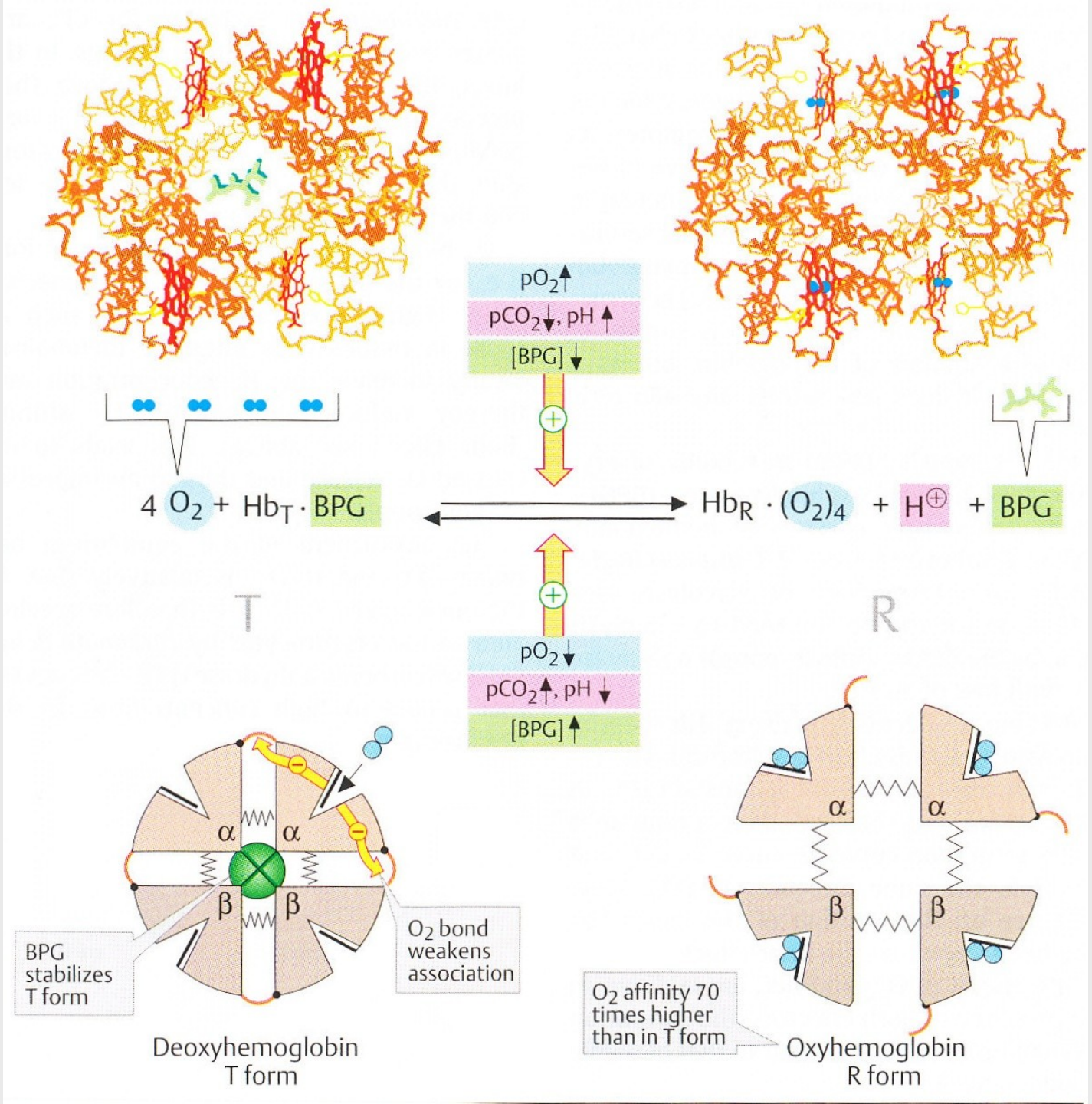

pO2↑
pCO2↓, pH↑
[BPG]↓
4 O2 + HbT · BPG
HbR · (O2)4 + H⊕ + BPG
T
R
pO2↓
pCO2↑, pH↓
[BPG]↑
α
α
β
β
BPG stabilizes T form
O2 bond weakens association
Deoxyhemoglobin T form
O2 affinity 70 times higher than in T form
Oxyhemoglobin R form

Abnormalities of haemoglobin production

- haemoglobinopathy (abnormal structure of chains)
- thalasemia (lower production of normal chains)
- Sickle cell anaemia (Hb J)

Synthesis and destruction of haemoglobin

Hem: glycin a succinyl-CoA
Globin: AMK
Hem - globin: biliverdin, bilirubin (lumirubin – photo-therapy), bil

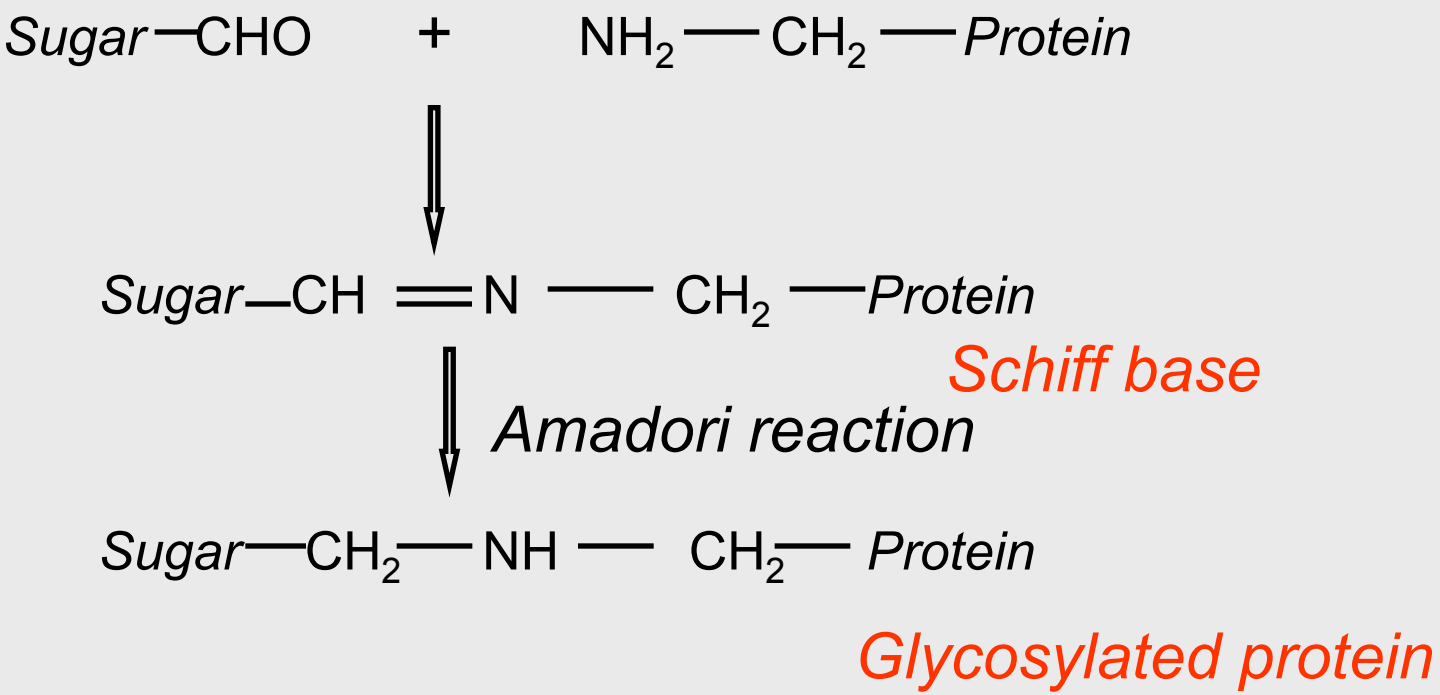
TABLE 32–3 **Partial amino acid composition of normal human β chain, and some hemoglobins with abnormal β chains.[a]**

	Positions on Polypeptide Chain of Hemoglobin						
Hemoglobin	**1 2 3**	**6 7**	**26**	**63**	**67**	**121**	**146**
A (normal)	Val-His-Leu	Glu-Glu	Glu	His	Val	Glu	His
S (sickle cell)		Val					
C		Lys					
$G_{San\ Jose}$		Gly					
E			Lys				
$M_{Saskatoon}$				Tyr			
$M_{Milwaukee}$					Glu		
O_{Arabia}						Lys	

[a]Other hemoglobins have abnormal α chains. Abnormal hemoglobins that are very similar electrophoretically but differ slightly in composition are indicated by the same letter and a subscript indicating the geographic location where they were first discovered; hence, $M_{Saskatoon}$ and $M_{Milwaukee}$.

Clinical aspects - Glycosylated haemoglobin (HbA_1)

- formed by hemoglobin's exposure to high plasma levels of glucose
- non-enzymatic glycolysation (glycation)- sugar bonding to a protein
- normal level HbA_1- 5%; a buildup of HbA_1- increased glucose concentration
- the HbA_1 level is proportional to average blood glucose concentration over previous weeks; in individuals with poorly controlled diabetes, increases in the quantities of these glycated hemoglobins are noted (patients monitoring)



ERYTHROPOETIN

Glycoprotein, 39 000, α2-globulin.
Recombinant erythropoetin.
Small amount in plasma, urine, lymph, foetal blood.
Inactivation: liver
Origin: kidneys (85-90%) – endothelial cells of peri-tubular capillaries in kidney core, liver (10-15%)
Stimulation of release: tissue hypoxia of any origin, alkalosis, cobalt salts, androgens, catecholamines (β-receptors)
Effects:
Erythropoetin responsive cell – differentiation into erythroid line: increase of synthesis of nucleic acids, increase of iron absorption in erythroid cells, stimulation of cells release from bone marrow into circulation

Acclimation – adaptation to high altitude

Osteoblasts – next cellular source of erythropoietin

HIF signaling in cells of the osteoblastic lineage regulate EPO expression in bone under physiologic and pathophysiologic conditions.

In addition to regulating erythropoiesis, EPO has also been implicated in the regulation of bone formation and repair.

Source	Model	Phenotype	Reference
Osteoblast (OSX-VHL)	Remodeling (mouse)	Increased trabecular bone volume associated with increased angiogenesis and erythropoiesis.	Rankin et al.
EPO (4500; 6,000 U/Kg)	Remodeling (mouse)	Increased bone volume in neonatal and adult mice associated with increased osteoblasts and erythropoiesis.	Shiozawa et al.
EPO (300 U/Kg)	Remodeling (mouse)	Modest decrease in bone volume.	Singbrant et al.
EPO (5000 U/Kg)	Repair (mouse)	Increased torsinal stiffness, callus density, and mineralized bone.	Holstein et al.
EPO (40 ng)	Repair (mouse)	Increased cartilaginous callus formation and bone healing associated with increased angiogenesis.	Wan et al.
EPO (1000 U)	Repair (mouse)	Increased BMP-2 induced bone regeneration in a cranial defect model associated with enhanced angiogenesis.	Sun et al.
EPO (500 IU)	Repair (mouse)	Increased bone volume in an bridging calvarial defect model.	Nair et al.
EPO (500 IE/Kg)	Repair (mouse)	Increased bone volume and repair in an femoral segmental defect model associated with increased angiogenesis.	Holstein et al.
EPO (500 U/Kg)	Repair (mouse)	Increased callus formation in a closed femoral fracture model.	Garcia et al.
EPO (250 IU/Kg)	Repair (rabbit)	Increased bone fusion in a posterolateral spinal fusion model associated with enhanced angiogenesis.	Rolfing et al. (2011)
EPO (900 IU)	Repair (porcine)	Modest increase in bone formation in a calvarial defect model.	Rolfing et al. (2013)
EPO (900 IU)	Repair (porcine)	Increase in bone formation when combined with bone marrow concentrate in a osteochondral defect model.	Betsch et al.

EPO erythropoietin

Wu C, Giaccia AJ, Rankin EB: **Osteoblasts: a Novel Source of Erythropoietin.** ***Current Osteoporosis Reports 2014, 12(4):428-432.***

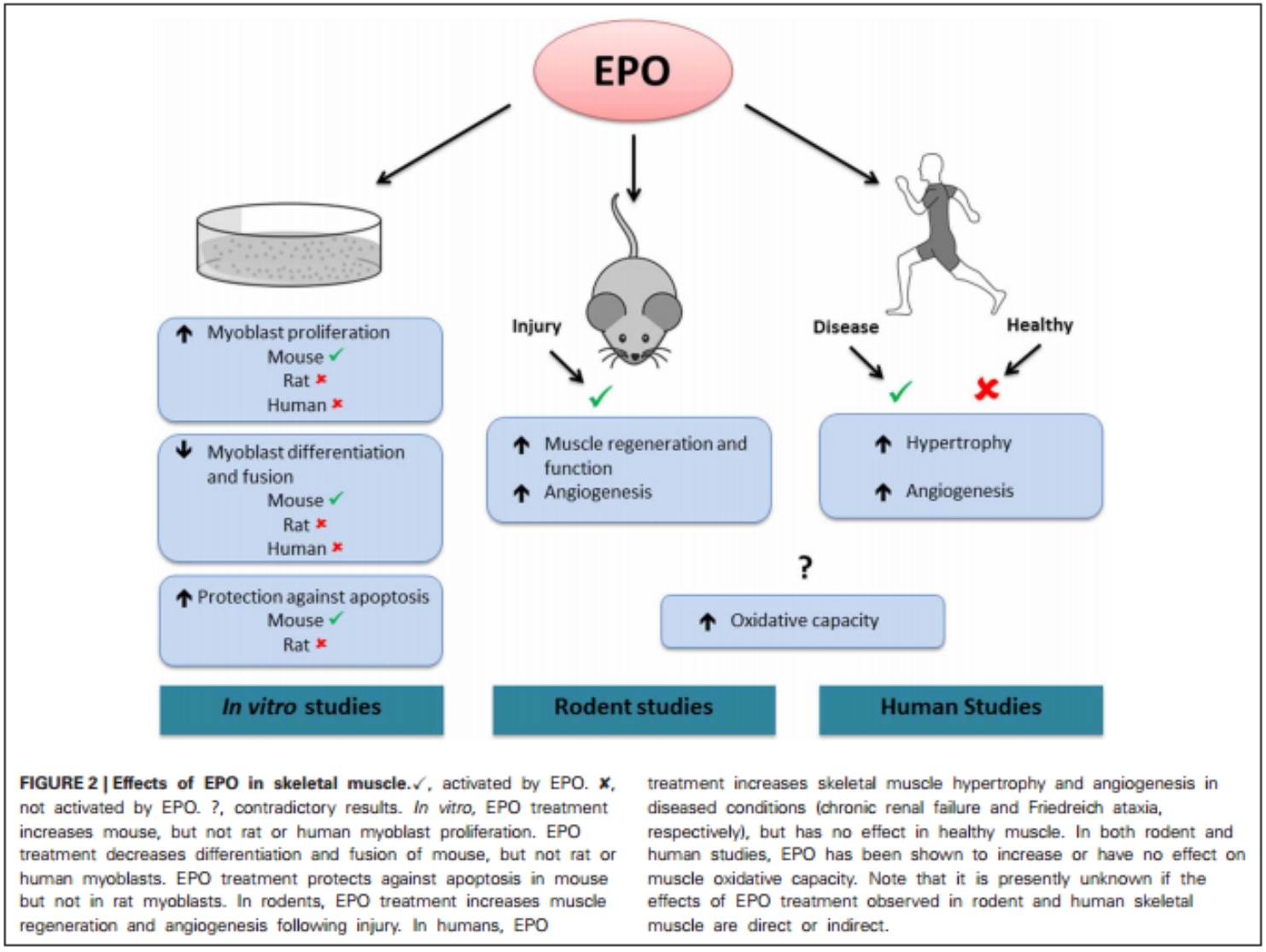


FIGURE 2 | Effects of EPO in skeletal muscle. ✓, activated by EPO. ✗, not activated by EPO. ?, contradictory results. *In vitro*, EPO treatment increases mouse, but not rat or human myoblast proliferation. EPO treatment decreases differentiation and fusion of mouse, but not rat or human myoblasts. EPO treatment protects against apoptosis in mouse but not in rat myoblasts. In rodents, EPO treatment increases muscle regeneration and angiogenesis following injury. In humans, EPO treatment increases skeletal muscle hypertrophy and angiogenesis in diseased conditions (chronic renal failure and Friedreich ataxia, respectively), but has no effect in healthy muscle. In both rodent and human studies, EPO has been shown to increase or have no effect on muscle oxidative capacity. Note that it is presently unknown if the effects of EPO treatment observed in rodent and human skeletal muscle are direct or indirect.

Lamon S, Russell AP: **The role and regulation of erythropoietin (EPO) and its receptor in skeletal muscle: how much do we really know?** ***Frontiers in Physiology 2013, 4.***

EPO and brain

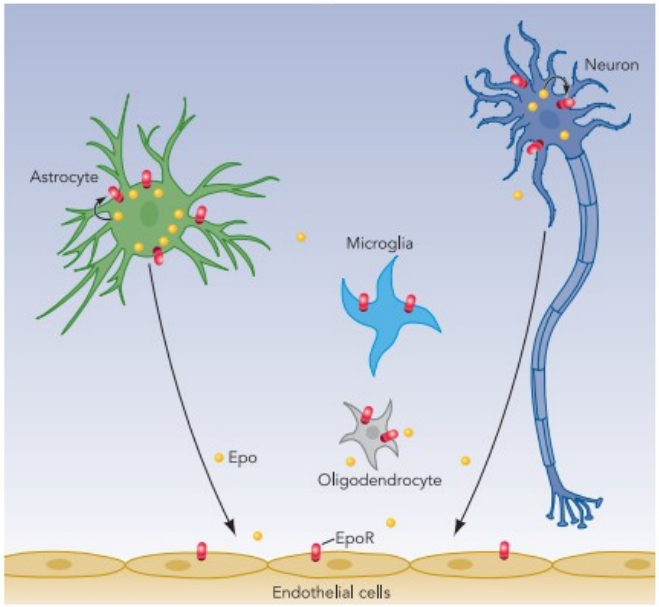


FIGURE 1. **Expression pattern of Epo/EpoR in the brain**
Whereas Epo expression is restricted to astrocytes and neurons, EpoR is expressed on the surface of endothelial cells, microglia, astrocytes, oligodendrocytes, and neurons. Epo is thought to act in an autocrine as well as paracrine manner.

Table 1. Functions of Epo

Function	Description	Refs.
Neuroprotection	Infusion of soluble EpoR into the brain of gerbils, subjected to a mild form of ischemia, caused neuronal death in the hippocampus.	95
Neurotrophic factor	Regeneration of septal cholinergic neurons in adult rats, which had undergone fimbria-fornix transections.	107
	Promotion of the survival and differentiation of dopaminergic precursor neurons in vitro.	107
Neurogenesis	Hypoxia-induced Epo production acts directly on neuronal stem cells in the forebrain.	99
	Indirectly by inducing BDNF expression.	113
Anti-inflammation	Reduced production of inflammatory mediators leading to:	112
	Cerebral ischemia: smaller infarcts.	
	Multiple sclerosis: protection.	
	Optic neuritis: improved survival of retinal ganglion cells.	2, 96
Angiogenesis	Mitogenic action on:	
	Human umbilical vein.	4
	Adrenal capillary endothelial cells.	4
	Brain capillary endothelial cells.	121
	Angiogenic action on:	
	Rat aortic rings.	19
	Mouse endometrium.	123
	Chick embryo chorioallantonic membrane.	90
Vascular permeability	In vitro: BBB protection against VEGF-induced increase in vascular permeability	75

BDNF, brain-derived neurotrophic factor; BBB, blood-brain barrier; VEGF, vascular endothelial growth factor.

Rabie T, Marti HH: **Brain Protection by Erythropoietin: A Manifold Task.** ***Physiology 2008, 23(5):263-274.***

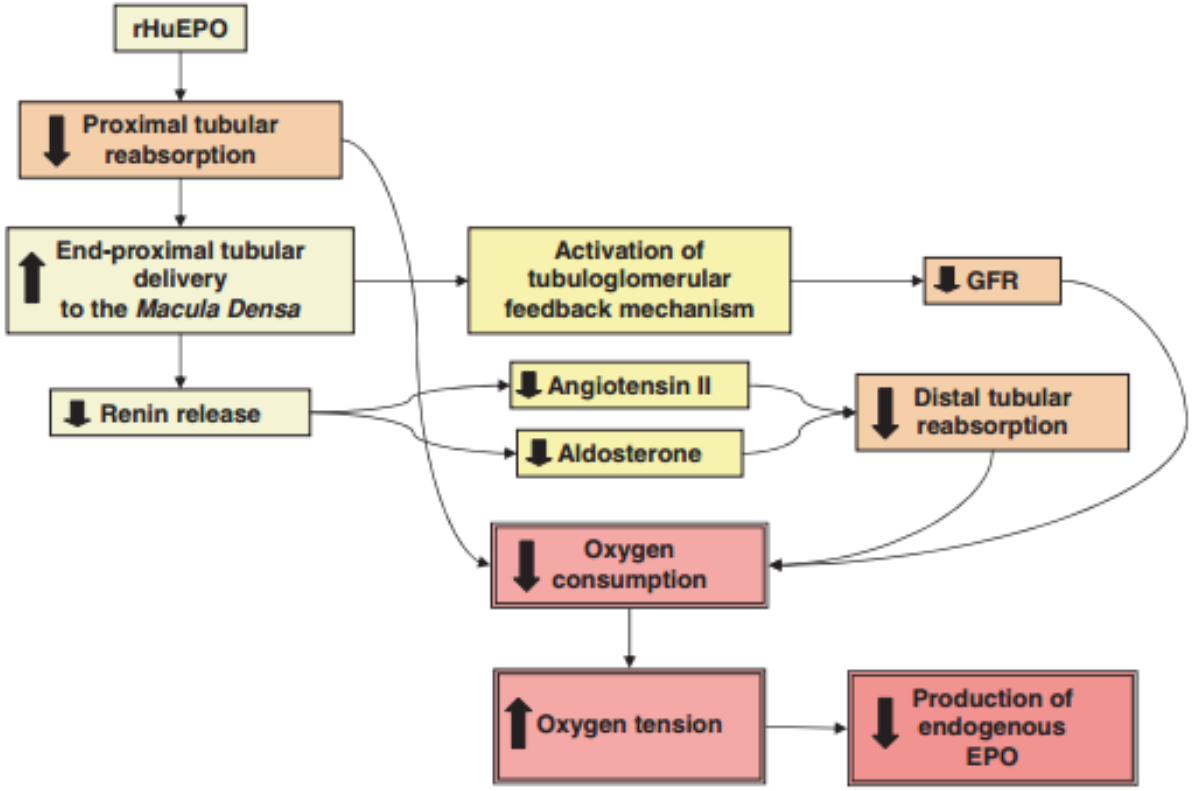


Figure 1. How high levels of circulating recombinant EPO may result in suppression of endogenous EPO synthesis secondary to a decrease in intrarenal oxygen consymption, by intrinsic renal effects
(1) EPO decreases reabsorption of sodium and fluid in the proximal tubule, thereby directly reducing the major oxygen-consuming process in the kidney; (2) increase in end-proximal tubular delivery to the macula densa decreases renin release and subsequent angiotensin II- and aldosterone-dependent reabsorption in more distal nephron segments; (3) decreased proximal tubular reabsorption activates the tubuloglomerular feedback mechanism producing a fall in GFR and reduction of the filtered load; (4) the resulting increase in renal oxygen partial pressure in the environment of interstitial fibroblast-like cells down-regulates the hypoxia-inducible factor-2-dependent production of endogenous EPO.

Lundby C, Olsen NV: **Effects of recombinant human erythropoietin in normal humans.** ***Journal of Physiology-London 2011, 589(6):1265-1271.***

ERYTHROPOESIS

Substances affecting erythropoesis

Need of copper

Ceruloplasmin – binding protein (α2-globulin) with ferroxidase activity. Oxidation of Fe^{2+} to Fe^{3+} is necessary for binding of iron to transferrin.

Need of cobalt

Part of vitamin B_{12} molecule.

Vitamin B12 (cyancobalamin)

Produced by bacteria in GIT.

Source: liver, kidneys, meet, milk products…

Resorption: necessity of s.c. **intrinsic factor** secreted by parietal cells of gastric fundus and body. Bound to transcobalamins in blood.

Stored in liver, pancreas, kidneys, brain, myocardium.

Function: synthesis of nucleic acids, co-factor in conversion of ribonucleotids to deoxyribonucleotids, production of metabolic active forms of folic acid

NECESSARY FOR NORMAL DIVISION AND MATURATION OF RED BLOOD CELL LINE ELEMENTS.

Symptoms of anaemia after years only!!!

Pernicious anaemia.

Folic acid (pteroylglutamic)

Produced by higher plants and micro-organisms.

Source: green vegetables, yeast, liver, kidneys…

Function: part of co-enzymes during synthesis of DNA, participation in cell division and differentiation

Deficiency: deficient nutrition, treatment with cytostatics (methotrexate)

Symptoms of anaemia already after couple of months!!!

Macrocyte hyperchromic anaemia.

Other vitamins

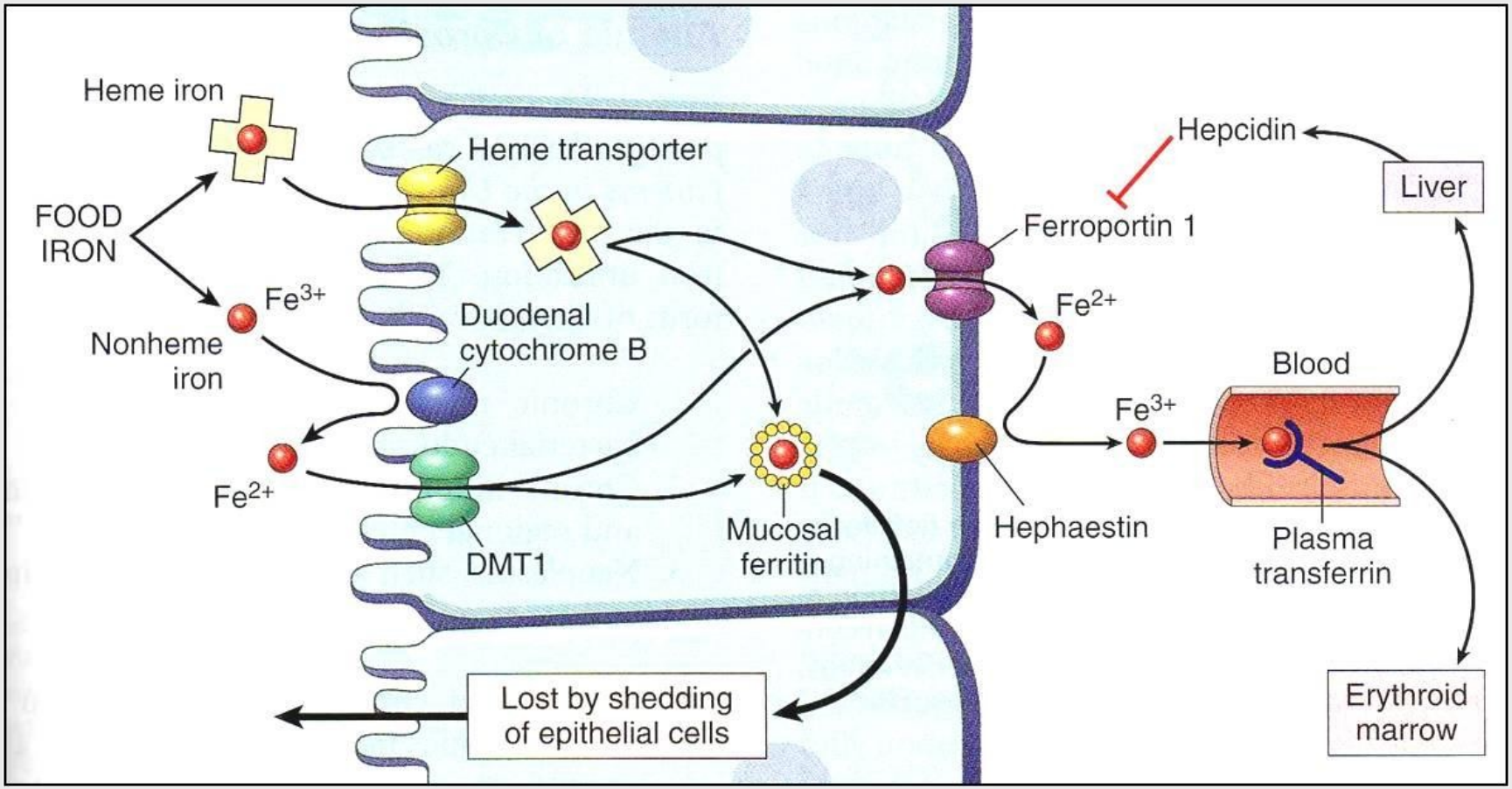
Vitamin B6 (pyridoxine) – metabolism of amino acids, synthesis of hem

Vitamin B2 (riboflavin) – part of flavoprotein enzymes – reductases of erythrocytes (normal function and survival of erythrocytes). Normocyte anaemia with lower reticulocytes count.

Vitamin C (ascorbic acid) – non-specific function in erythropoesis.

Hormonal influences

Androgens, estrogens, hormones of thyroid gland, glucocorticoids, growth hormone.


Heme iron
FOOD IRON
Nonheme iron
Fe3+
Fe2+
Heme transporter
Duodenal cytochrome B
DMT1
Mucosal ferritin
Lost by shedding of epithelial cells
Hepcidin
Liver
Ferroportin 1
Fe2+
Hephaestin
Fe3+
Blood
Plasma transferrin
Erythroid marrow

ANAEMIA

Disorder, in which basic and characteristic feature is **lower amount of haemoglobin.** Usually also haematocrit and red blood cell count in 1 litre of blood are below physiological value.

CLASSIFICATION OF ANEMIAS

MORPHOLOGICAL CLASSIFICATION

Evaluation of erythrocyte volume and concentration of haemoglobin in erythrocytes

1. Normocyte anaemia
2. Microcyte a.
3. Macrocyte

1. Normochromic anaemia
2. Hypochromic a.

PATHOPHYSIOLOGICAL CLASSIFICATION

Anaemias caused by inefficient blood production
Sideropenic anaemias – lack of iron
Megaloblastic a. – lack of vitamin B_{12} or folic acid
Anaemias caused by suppression of blood production
Anaemias in chronic diseases and symptomatic anaemias
Thalasemia

Anaemias caused by increased losses
Haemolytic a.– caused by increased destruction of erythrocytes
Chronic posthaemorhagic anemia

Acute posthaemorhagic anaemia

ANTIGENS AND ANTIBODIES OF RED BLOOD CELLS

1) History of blood transfusions.

2) ***Posttransfusion reactions***: aglutination, haemolysis (immediate or delayed), life-threatening complications (jaundice, damage of kidneys, anuria, death – in case of full blood or RBCs administration, in case of plasma – dilution of aglutinins!!!
Autoimmune diseases. Paternity tests, event. transplantology.

3) ***Antigens of blood cells*:**
a) 30 antigen systems (ABO, Rh, MNSs, Lutheran, Kell, Kidd, Lewis, Diego, P, Duffy…)
b) hundreds of other – „weak" – antigens (important for paternity testing, organ transplantations)

4) ***Aglutinogen*:** antigen of plasmatic membrane of cells
- complex oligosaccharide
- erytrocytes, salivary glands, pancreas, liver, kidney, lungs, testes
- saliva, sperm, amnionic fluid, milk, urine

5) ***Aglutinin:*** antibody against aglutinogen, γ-globulin (IgM –AB0 system, IgG – Rh system), produced in the same way as other antibodies
- **after births almost zero concentration in blood**
- production of aglutinins begins 2-8 months after birth: **stimulation by antigens similar to aglutinogens – in food, in GIT bacteria**
- maximal concentration of antibodies is reached in 8-10 years, decreases gradually with age

Blood group systems

ISBT №[1]	System name	System symbol	Epitope or carrier, notes	Chromosome
001	ABO	ABO	Carbohydrate (N-Acetylgalactosamine, galactose). A, B and H antigens mainly elicit IgM antibody reactions, although anti-H is very rare, see the Hh antigen system (Bombay phenotype, ISBT #18).	9q34.2
002	MNS	MNS	GPA / GPB (glycophorins A and B). Main antigens M, N, S, s.	4q31.21
003	P	P	Glycolipid. Three antigens: P_1, P, and P^k	22q13.2
004	Rh	RH	Protein. C, c, D, E, e antigens (there is no "d" antigen; lowercase "d" indicates the absence of D).	1p36.11
005	Lutheran	LU	Protein (member of the immunoglobulin superfamily). Set of 21 antigens.	19q13.32
006	Kell	KEL	Glycoprotein. K_1 can cause hemolytic disease of the newborn (anti-Kell), which can be severe.	7q34
007	Lewis	LE	Carbohydrate (fucose residue). Main antigens Le^a and Le^b — associated with tissue ABH antigen secretion.	19p13.3
008	Duffy	FY	Protein (chemokine receptor). Main antigens Fy^a and Fy^b. Individuals lacking Duffy antigens altogether are immune to malaria caused by *Plasmodium vivax* and *Plasmodium knowlesi*.	1q23.2
009	Kidd	JK	Protein (urea transporter). Main antigens Jk^a and Jk^b.	18q12.3
010	Diego	DI	Glycoprotein (band 3, AE 1, or anion exchange). Positive blood is found only among East Asians and Native Americans.	17q21.31
011	Yt	YT	Protein (AChE, acetylcholinesterase).	7q22.1
012	XG	XG	Glycoprotein.	Xp22.33
013	Scianna	SC	Glycoprotein.	1p34.2
014	Dombrock	DO	Glycoprotein (fixed to cell membrane by GPI, or glycosyl-phosphatidyl-inositol).	12p12.3
015	Colton	CO	Aquaporin 1. Main antigens Co(a) and Co(b).	7p14.3
016	Landsteiner-Wiener	LW	Protein (member of the immunoglobulin superfamily).	19p13.2
017	Chido	CH	C4A C4B (complement fractions).	6p21.3
018	Hh	H	Carbohydrate (fucose residue).	19q13.33
019	XK	XK	Glycoprotein.	Xp21.1
020	Gerbich	GE	GPC / GPD (Glycophorins C and D).	2q14.3
021	Cromer	CROM	Glycoprotein (DAF or CD55, regulates complement fractions C3 and C5, attached to the membrane by GPI).	1q32.2
022	Knops	KN	Glycoprotein (CR1 or CD35, immune complex receptor).	1q32.2
023	Indian	IN	Glycoprotein (CD44 adhesion function?).	11p13
024	Ok	OK	Glycoprotein (CD147).	19p13.3
025	Raph	RAPH	Transmembrane glycoprotein.	11p15.5
026	JMH	JMH	Protein (fixed to cell membrane by GPI). Also known as Semaphorin 7A or CD108.	15q24.1
027	Ii	I	Branched (I) / unbranched (i) polysaccharide.	6p24.2
028	Globoside	GLOB	Glycolipid. Antigen P.	3q26.1
029	GIL	GIL	Aquaporin 3.	9p13.3
030	Rh-associated glycoprotein	RHAg	Rh-associated glycoprotein.	6p21-qter
031	Forssman	FORS	Globoside alpha-1,3-N-acetylgalactosaminyltransferase 1 (GBGT1)	9q34.13
032	Langereis[4]	LAN	ABCB6. Porphyrin transporter	2q36
033	Junior[4]	JR	ABCG2. Multi-drug transporter protein	4q22
034	Vel	Vel	Human red cell antigens	1p36.32
035	CD59	CD59		11p13

A-B-O SYSTEM

Genotype	Blood group	Aglutinogen	Aglutinin
00	0	(H)	anti-A a anti-B
0A or AA	A	A	anti-B
0B or BB	B	B	anti-A
AB	AB	A and B	-

Described by Landsteiner in 1901, 1930 – awarded by Nobel Price.
Janský -1906.

Frequency of blood groups in ABO system:

O	47% (38%)
A	41% (42%)
B	9% (14%)
AB	3% (6,5%)

Subgroups in A a B blood groups.
A_1 (1 million copies of antigen on 1 ery), A_2 (250 thousands copies).

Heredity: both A and B is inherited dominantly, according to Mendel s law.

Rh SYSTEM

Monkey *Maccacus rhesus*.
40th of the 20th century, Wiener a Landsteiner.
Frequency: 85% - Rh^+, 15% - Rh^-.

Antigens D, C, E, d, c, e. Present only on erythrocytes.

D – the „strongest“ antigen: Rh – positive, Rh – negative (produces anti-D aglutinin after contact with D-erythrocytes).

Aglutinins production: only after the contact with D-erythrocytes (transfusion, foetal erythroblastosis).
High concentration of anti-D antibodies lasts for many years!!!

HAEMOLYTIC JAUNDICE OF NEWBORNS

Rh-negative mother x Rh-positive foetus.

First pregnancy – immunisation of mother during delivery (or interruption or miscarriage!!!).

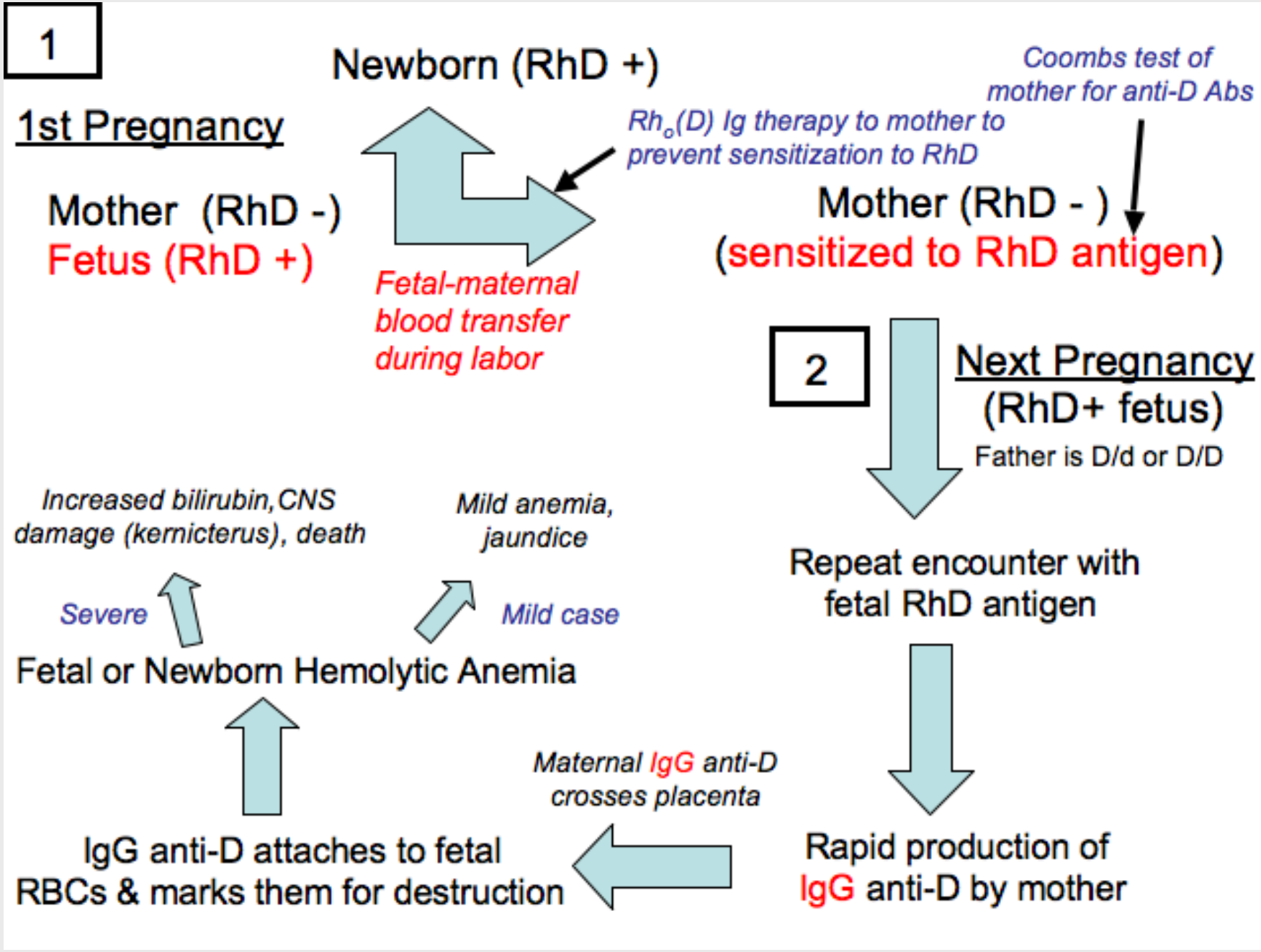
Next pregnancy – anti-D aglutinins (IgG) cross foetoplacental barrier.

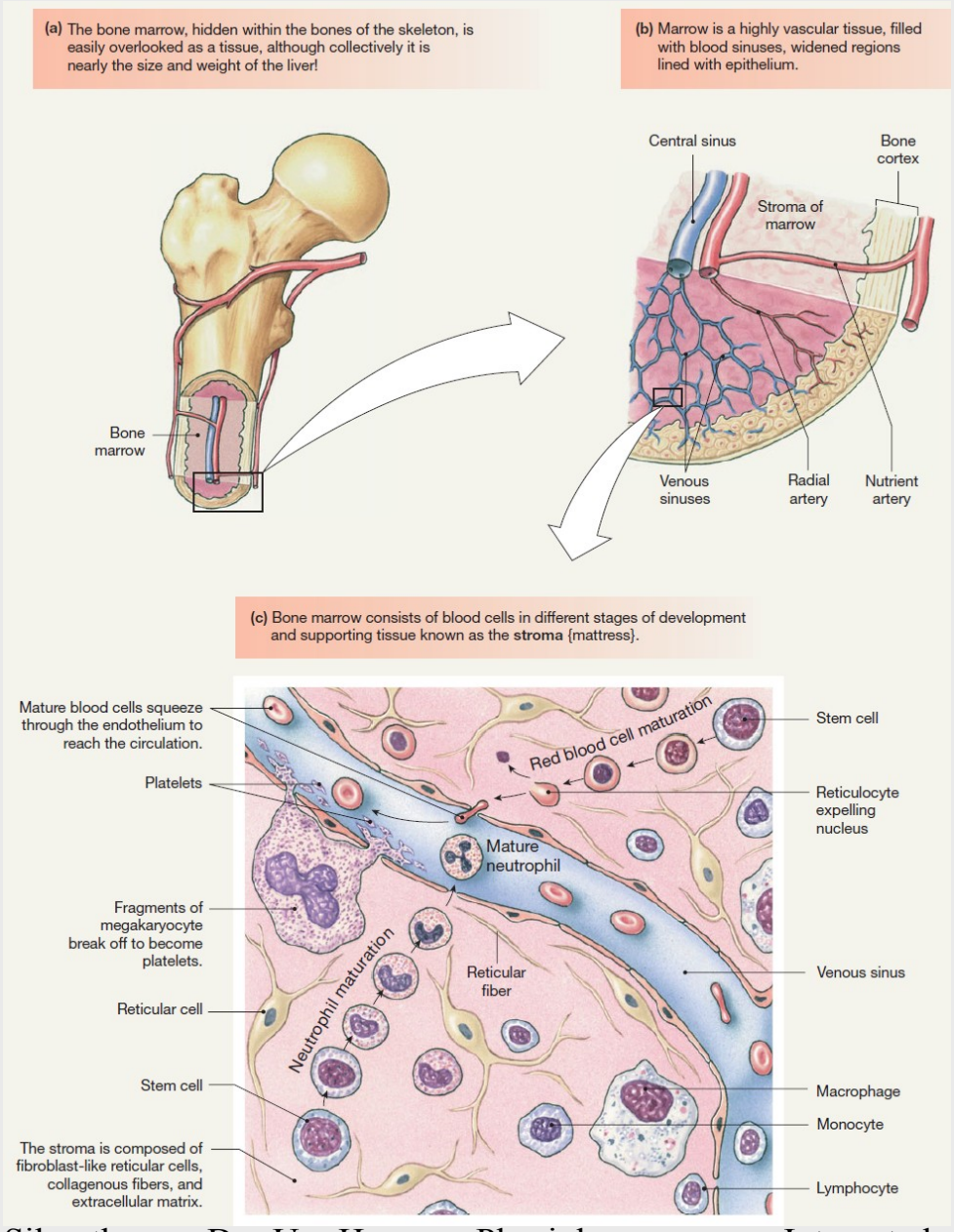
Foetus damage: approx. in 17% of next pregnancies

Haemolysis of foetal erythrocytes – haemolyti disease of newborn (erythroblastosis fetalis):
•anaemia
•jaundice
•oedemas – event. hydrops fetalis
•CNS damage (icterus) –bile acids enter CNS (no haematoencephalic barrier!)
•deaths of foetus in utero

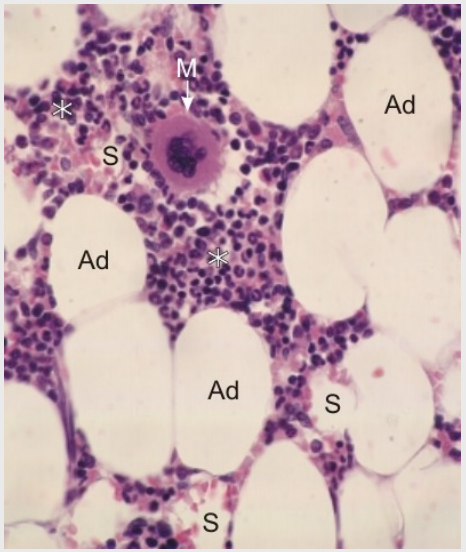
***Prevention of foetal damage*:**
1) administration of small doses of anti-D antibodies to mother during pregnancy
2) administration of one dose of anti-D antibodies during postpartum period

Success of therapy: up 90%.

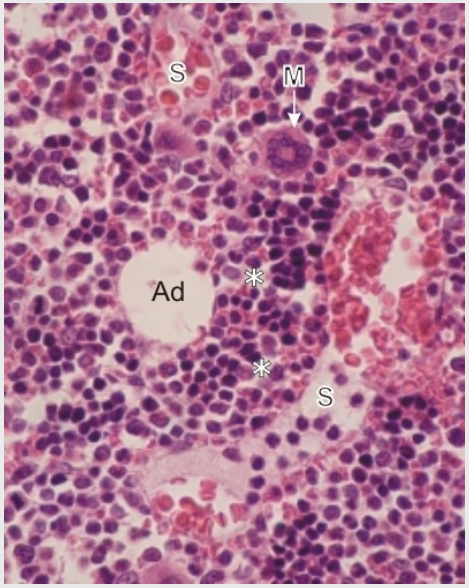

1
Newborn (RhD +)
1st Pregnancy
Mother (RhD -)
Fetus (RhD +)
Fetal-maternal blood transfer during labor
Rh$_o$(D) Ig therapy to mother to prevent sensitization to RhD
Coombs test of mother for anti-D Abs
Mother (RhD -)
(sensitized to RhD antigen)
2
Next Pregnancy
(RhD+ fetus)
Father is D/d or D/D
Repeat encounter with fetal RhD antigen
Rapid production of IgG anti-D by mother
Maternal IgG anti-D crosses placenta
IgG anti-D attaches to fetal RBCs & marks them for destruction
Fetal or Newborn Hemolytic Anemia
Severe
Increased bilirubin, CNS damage (kernicterus), death
Mild case
Mild anemia, jaundice



Silverthorn, D. U. Human Physiology – an Integrated Approach. 6th. edition. Pearson Education, Inc. 2012.



Section of yellow bone marrow. This bone marrow is yellow in its fresh state because of the abundance of yellowish adipocytes present. The hemopoietic (*) tissue is comparatively less abundant than in red bone marrow. The adipocytes, or fat cells, (Ad) appear as large circular clear spaces in this field. A megakaryocyte (M) and venous sinuses (S) are also labelled. Source: http://audilab.bmed.mcgill.ca/HA/html/blood_7_E.html



This bone marrow is referred to as red marrow because it contains few adipocytes, or fat cells, among an abundance of hemopoietic cells. It is difficult to identify the individual precursors of red and white blood cells because they are closely packed and condensed during the fixation of the tissue (*).

The following elements are identified: a megakaryocyte (M), which is a very large polyploid cell responsible for the production of blood platelets one adipocyte (Ad) two blood sinuses (S).

The walls of these vessels are the sites where newly formed erythrocytes and leukocytes pass from the connective tissue into the blood circulation.

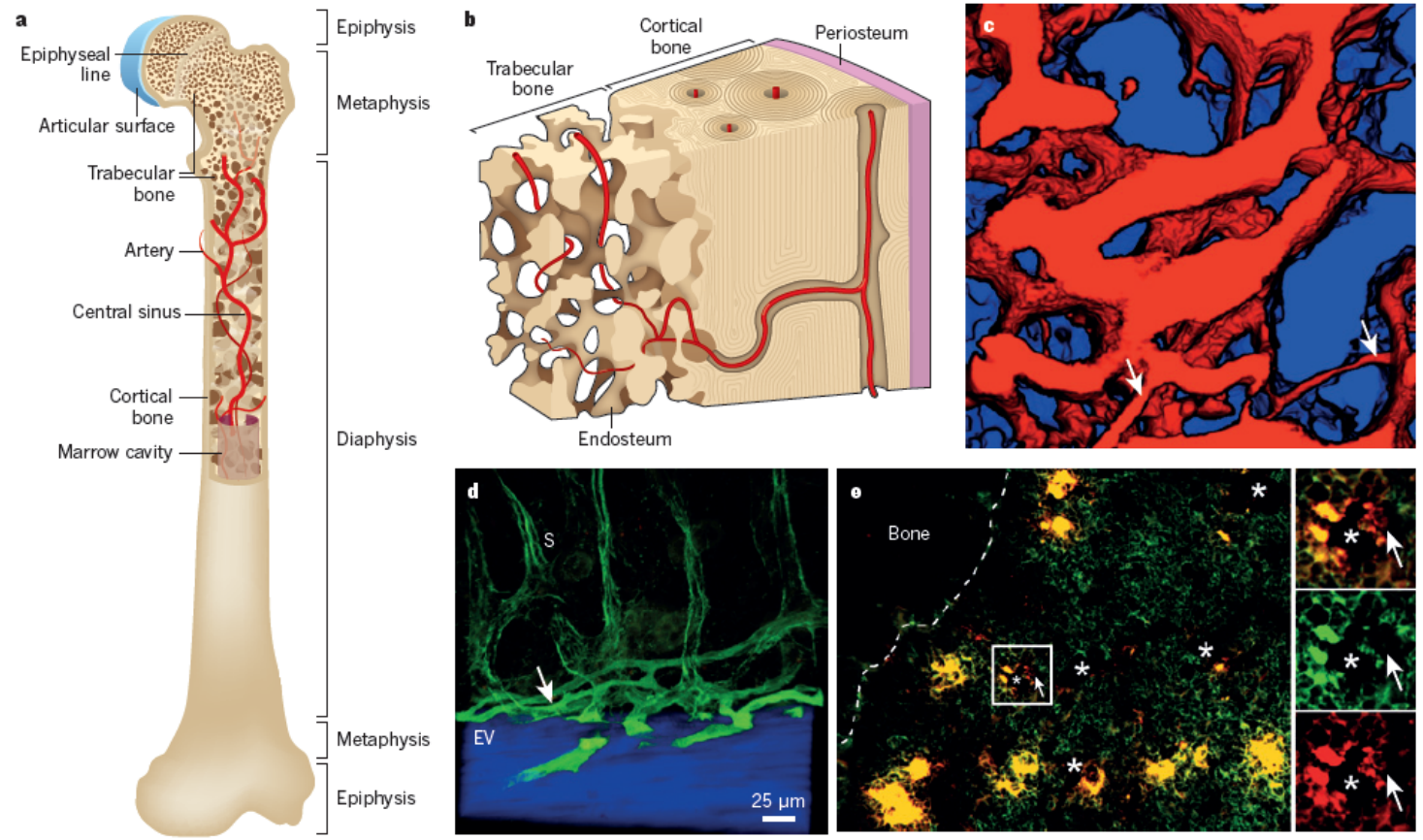


Figure 1 | Bone marrow anatomy. Haematopoietic stem cells (HSCs) reside mainly within bone marrow during adulthood. Bone marrow is a complex organ, containing many different haematopoietic and non-haematopoietic cell types, that is surrounded by a shell of vascularized and innervated bone. **a**, Minute projections of bone (trabeculae) are found throughout the metaphysis such that many cells in this region are close to the bone surface. **b**, The interface of bone and bone marrow is known as the endosteum, which is covered by bone-lining cells that include bone-forming osteoblasts and bone-resorbing osteoclasts. Arteries carry oxygen, nutrients and growth factors into the bone marrow, before feeding into sinusoids, which coalesce as a central sinus to form the venous circulation. Sinusoids are specialized venules that form a reticular network of fenestrated vessels that allow cells to pass in and out of circulation. There is a particularly rich supply of arterioles, as well as sinusoids, near the endosteum. **c**, Three-dimensional reconstructed photomicrograph from the bone marrow towards the endosteal surface (blue) from 50 µm below the surface, revealing the rich network of vessels (red) (image courtesy of C. Lin, J. Spencer and J. Wu). Smaller arteriolar vessels (white arrows) become larger sinusoidal vessels. The field of view is 350 µm × 350 µm. **d**, A cross-sectional view of blood vessels that run along the endosteal surface (EV) and that transition (white arrow) into sinusoids (S) that then course towards the central sinus (adapted with permission from ref. 31). **e**, The bone marrow is cellularly complex with $CD150^{+}CD48^{-}CD41^{-}lineage^{-}$ HSCs (arrow) residing in close contact not only with vascular and perivascular cells (*, sinusoid lumens) but also megakaryocytes (large yellow cells) and other haematopoietic cells (image adapted with permission from ref. 125). In the enlargement on the right, CD150 is shown in red and CD48, CD41 and lineage are shown in green.

Morrison SJ, Scadden DT: **The bone marrow niche for haematopoietic stem cells.** ***Nature 2014, 505(7483):327-334.***

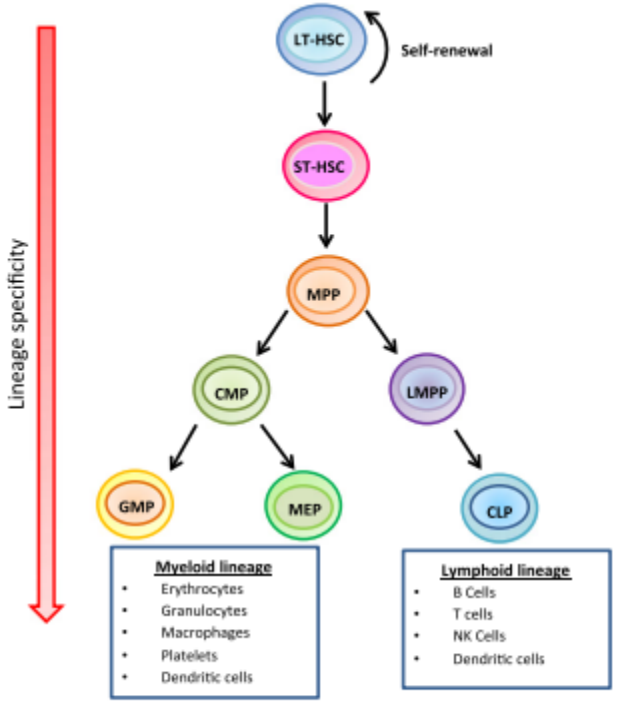


Fig 1. Hierarchical organization of the haematopoietic system. The long-term haematopoietic stem cell (LT-HSC) resides at the apex of the hierarchical haematopoietic system and can undergo self-renewal or sequential multilineage differentiation to produce all the specialized blood cells in the body. The LT-HSC first differentiates into the short-term haematopoietic stem cell (ST-HSC), which yields one of two types of multipotent progenitors (MPP): the common myeloid progenitor (CMP) or the lymphoid multipotent progenitor (LMPP). Downstream, these progenitor cells gradually become more restricted in their potential to differentiate into cells of other lineages. Eventually, the committed progenitors granulocyte–macrophage progenitor (GMP), megakaryocyte–erythrocyte progenitor (MEP) and common lymphoid progenitor (CLP), can only give rise to one lineage and produce mature blood cells.

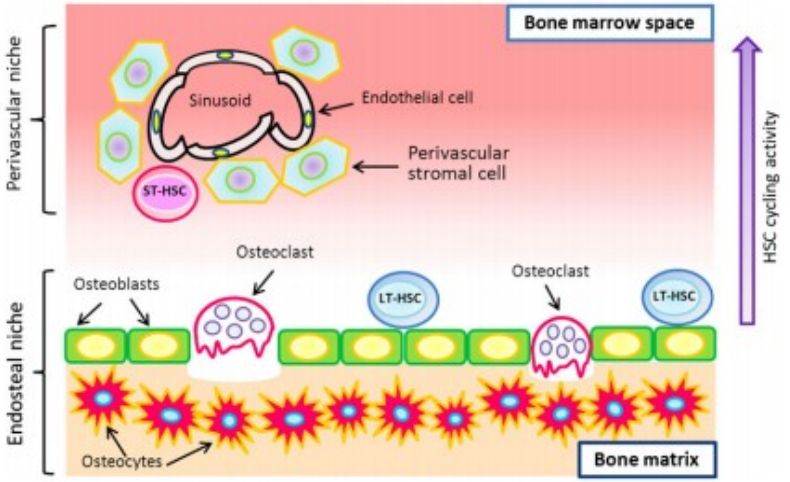


Fig 2. Haematopoietic stem cell niches in the bone marrow. Haematopoietic stem cells (HSCs) reside in specialized supportive microenvironments or niches within the bone marrow. Quiescent or slow-cycling long-term haematopoietic stem cells (LT-HSCs) localize close to the bone and bone marrow interface, a site known as the endosteal or osteoblastic niche. In contrast, fast-cycling short-term-HSCs (ST-HSCs) may be found in close proximity to sinusoidal endothelial cells and perivascular cells. This site, which is known as the perivascular niche, supports the proliferation and differentiation of HSCs.

Ho MSH, Medcalf RL, Livesey SA, Traianedes K: **The dynamics of adult haematopoiesis in the bone and bone marrow environment.** ***Br J Haematol 2015, 170(4):472-486.***

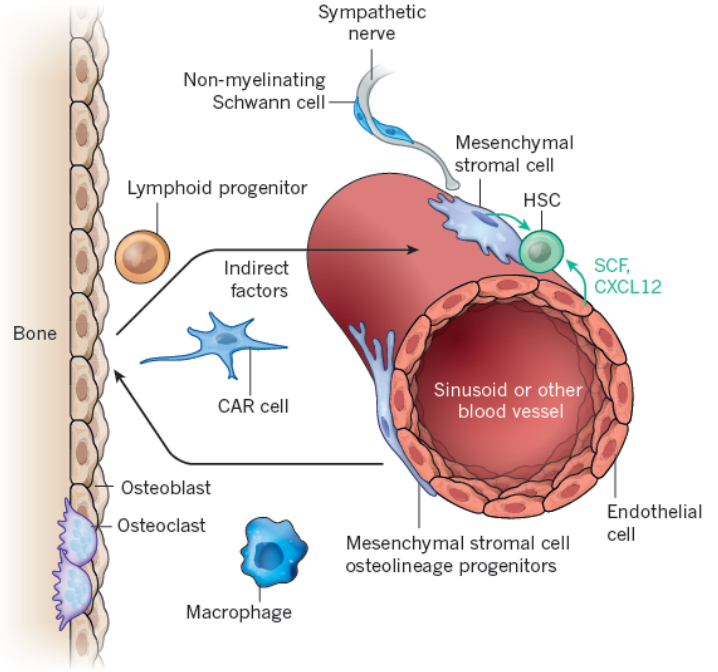


Figure 3 | Haematopoietic stem cells (HSCs) and restricted haematopoietic progenitors occupy distinct niches in the bone marrow. HSCs are found mainly adjacent to sinusoids throughout the bone marrow[27,30,31,33], where endothelial cells and mesenchymal stromal cells promote HSC maintenance by producing SCF[64], CXCL12 (refs 17, 33, 62) and probably other factors. Similar cells may also promote HSC maintenance around other types of blood vessels, such as arterioles. The mesenchymal stromal cells can be identified based on their expression of *Lepr*-Cre[64], *Prx1*-Cre[62], *Cxcl12*-GFP[33] or *Nes*-GFP transgenes[63] in mice and similar cells are likely to be identified by CD146 expression in humans[54]. Perivascular stromal cells, which probably include Cxcl12-abundant reticular (CAR) cells[33], are fated to form bone *in vivo*, express Mx-1-Cre and overlap with CD45/Ter119$^-$PDGFRα$^+$Sca-1$^+$ stromal cells that are highly enriched for mesenchymal stromal cells in culture[66]. It is likely that other cells also contribute to this niche, these probably include cells near bone surfaces in trabecular-rich areas. Other cell types that regulate HSC niches include sympathetic nerves[91,92], non-myelinating Schwann cells (which are also Nes$^+$)[96], macrophages[95] and osteoclasts[97]. The extracellular matrix[120,121] and calcium[56] also regulate HSCs. Osteoblasts do not directly promote HSC maintenance but do promote the maintenance and perhaps the differentiation of certain lymphoid progenitors by secreting CXCL12 and probably other factors[13,17,39,40]. Early lymphoid restricted progenitors thus reside in an endosteal niche that is spatially and cellularly distinct from HSCs.

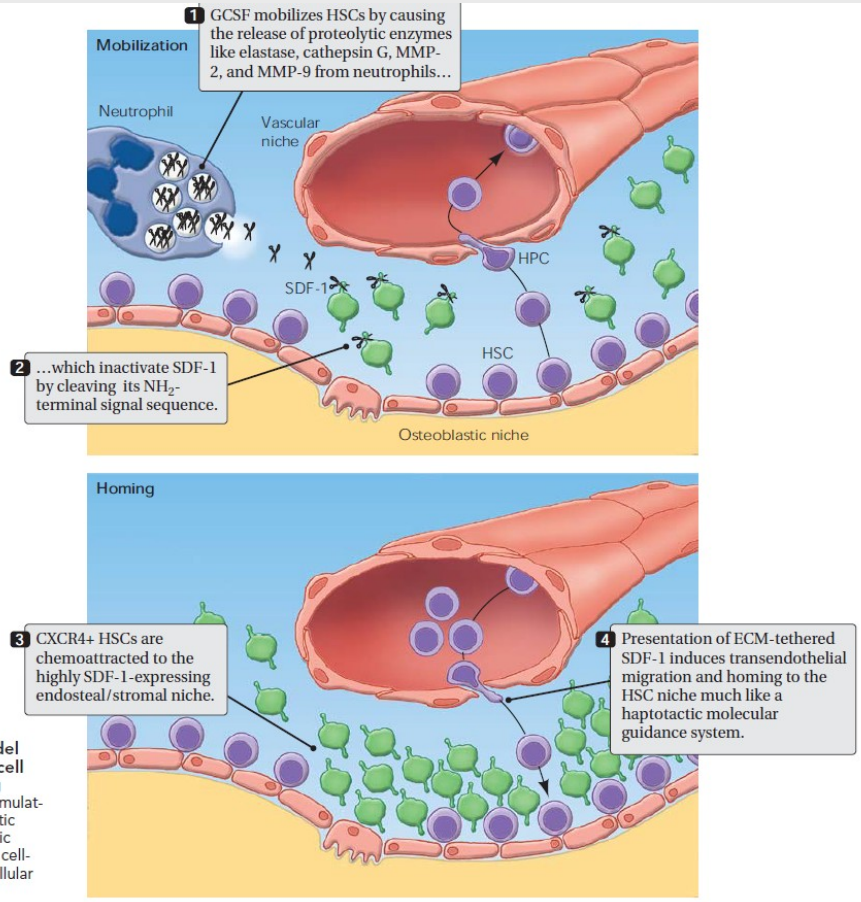


FIGURE 3. **Simplistic model for hematopoietic stem cell mobilization and homing**
GCSF, granulocyte colony-stimulating factor; HSC, hematopoietic stem cell; HPC, hematopoietic progenitor cell; SDF, stromal cell-derived factor; ECM, extracellular matrix.

Kopp HG, Avecilla ST, Hooper AT, Rafii S: **The bone marrow vascular niche: Home of HSC differentiation and mobilization.** ***Physiology 2005, 20:349-356.***

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bone marrow contains endothelial cell precursors

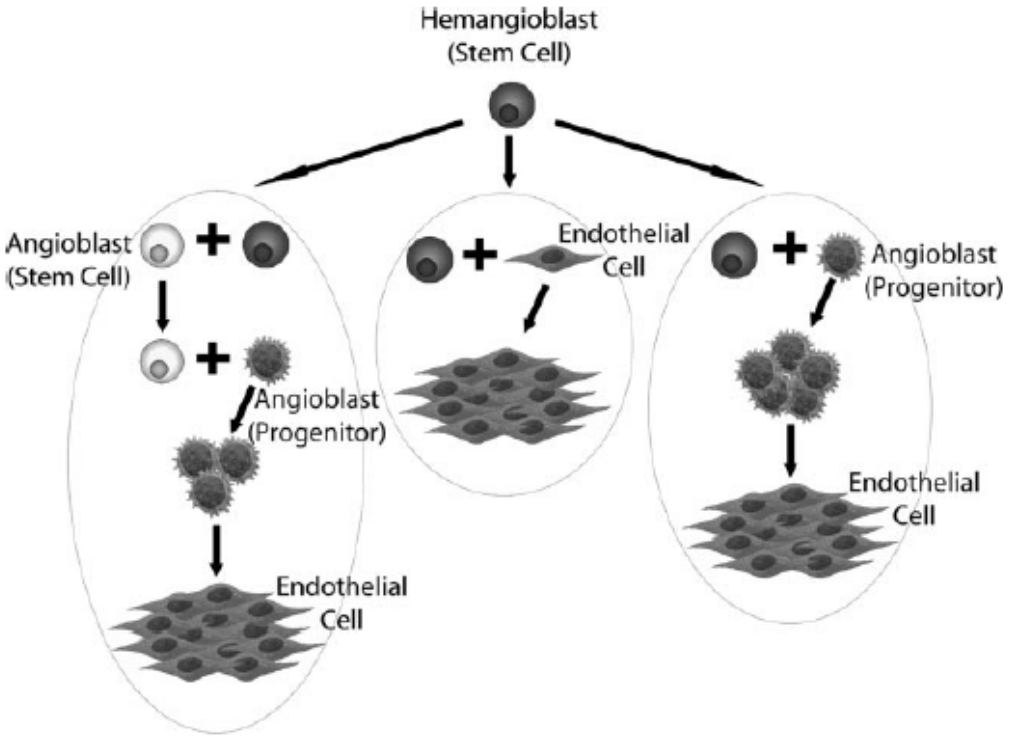

Fig. 1. Some possible differentiation pathways for endothelial cells (ECs) derived from hemangioblasts of bone marrow origin. Data to date have not clearly defined the pathway(s) through which hemangioblasts differentiate into ECs or even whether the process begins before or only after they leave the bone marrow. However, "final" differentiation into bone marrow-derived ECs (bmdECs) does not occur until cells have left the bone marrow and probably not until they enter the vessel wall. Whereas the progenitors may proliferate in the blood or bone marrow, it is likely that the bmdECs proliferate only in the vessel wall since their numbers are low in the circulation. As shown in the left-hand pathway, hemangioblasts may first produce a differentiated progeny, an angioblast, which is itself a stem cell. The angioblast stem cell might then produce progenitors (e.g., myeloid progenitors), which could differentiate into one or more fully differentiated progeny, including ECs. Alternatively, as suggested by the central pathway, hemangioblasts could undergo asymmetric cell division, producing a stem cell and an EC as daughters. Yet another possibility is that hemangioblasts produce angioblast progenitors (e.g., myeloid progenitors), which in turn differentiate into ECs and possibly other cell types. These pathways are not mutually exclusive, and this diagram does not include all possible mechanisms through which bone marrow-derived hemangioblasts may ultimately produce ECs.

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