Human red blood cells-1

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Abstract: Biochemists and cell biologists, who are interested in membranes, tend to regard the human red blood cell ambivalently. On one hand, red blood cells lack nuclei and the various intracellular organelles, yet that are highly specialized for a particular respiratory function. On the other hand, the human red blood cell presents an excellent model for membrane transport function.

Keywords: Human Erythrocyte, Electrochemical Potential, Donnan Equilibrium, Haemoglobin, Red Blood Cell Shape, Permean Ions, Osmotic Pressure, Band 3, Facilitated Transport, K^+/Cl^- Cotransport, $K^+(Na^+)/H^+$ Exchanger, Na^+/K^+-Atpase, Anion Transport

1. History

Red blood cells (also referred to as erythrocytes) are the most common blood cells that deliver oxygen to body tissues via a cardiovascular system. They take up oxygen in the alveoli and exchange it for carbon dioxide and the exchange of gases occurs by simple diffusion: higher pressure oxygen diffuses from the alveoli to a lower pressure of oxygen in the blood, whereas carbon dioxide diffuses in the opposite direction according to the concentration gradient [1]. We do this, of course, continuously to bring fresh air into the lungs and the alveoli and to exhale carbon dioxide through an active process of respiration that requires contraction of skeletal muscles [2].

Jan Swammerdam (1637-1680) was a Dutch naturalist and an expert microscopist. In 1658 Swammerdam documented small oval particles in frog's blood which were probably the first recorded observation of red blood cells [3,4,5]. Anton van Leeuwenhoek (1632-1723) was a Dutch scientist who provided in 1674 a more precise microscopic description of red blood cells, approximating their size "25,000 times smaller than a fine grain of sand" [6,7,8,9]. Karl Landsteiner (1868-1943) who identified agglutinins distinguished A, B and O blood groups [10,11]. He collaborated with Alexander Wiener (1907-1976) and they identified Rhesus factor in 1937, thus enabling physicians to transfuse blood without endangering the patient's life [11]. In 1902, Alfred von Decastello (1872-1960) and Adriano Sturli (1873-1964)   identified the fourth blood group AB [12]. We understand that while there are more than 200 minor blood groups today, they occur relatively rarely. The ABO system is still, 100 years later, the primary instrument for determining blood compatibility [13,14,15]. However, citrate anticoagulant was developed to prevent clotting and the use of blood transfusions [16] became routine therapy in medicine, just in time for World War I [17]. In 1959, Max Perutz (1914-2002) pioneered X-ray analysis of haemoglobin [18].

The future mode of investigation will be single cell based such as patch-clamp, flow cytometry or fluorescent lifetime cell imaging, because it enables to identify subpopulations of cells that can hardly be addressed by measurement of huge populations of cells such as tracer flux analysis [19, 20].

2. Hematopoiesis

The mature red blood cell is produced by a process called "normoblastic erythropoiesis" (Fig.1a). This occurs entirely in the red bone marrow that can be found in vertebrae, ribs, skull, sternum, scapula, and proximal ends of the limb bones [21]. Red marrow is also known as myeloid tissue. It is not generally found in other areas of the long limb bones which are instead filled with fatty yellow marrow. However, during extreme times, the marrow in these areas can switch to become red marrow. Red marrow is far more extensive during childhood [22]. Erythrocytes are formed in the stroma of the bone marrow [23,24]. The formation of erythroblast is controlled by a

glycoprotein hormone, erythropoietin (Fig.1b) which is produced in the kidneys from a plasma substrate (erythrogenin) in response to oxygen deficiency (alkalosis) [25]. The stimulatory effect of haemopoietin on the haemoglobin and RNA formation by bone marrow is inhibited by actinomycin D which is an antibiotic and a potent antitumor agent, in which DNA-dependent RNA synthesis is inhibited [26]. Patients become anaemic following bilateral nephrectomy

Adapted from: Ganong, W.F. (1977) *Review of Medical Physiology*, 8th edn, p.385, California: Lang Medical Publications.
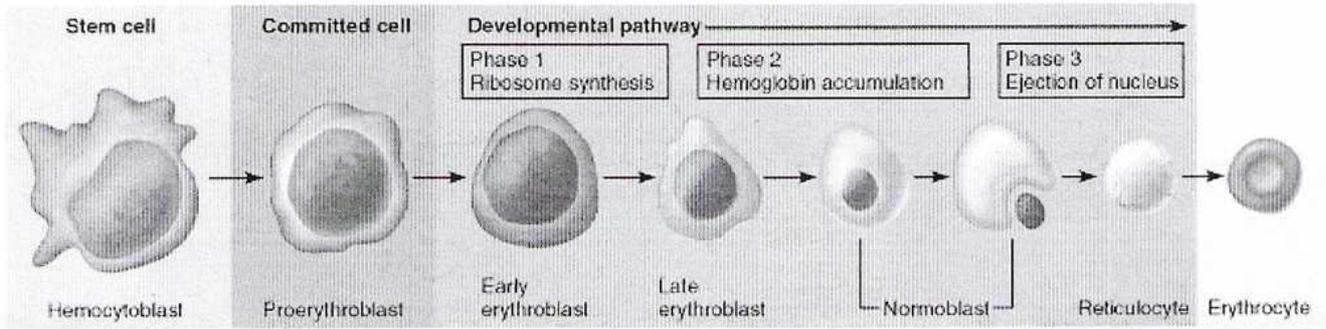

Fig.1a. *Normoblastic erythropoiesis*

Adapted from: Weissman, I.L, Anderson, D.J. and Gage F. (2001) " Stem and progenitor cells: origins, phenotypes, lineage commitments, and transdifferentiations", *Annu Rev Cell Dev Biol*., vol.17, pp. 387-403.
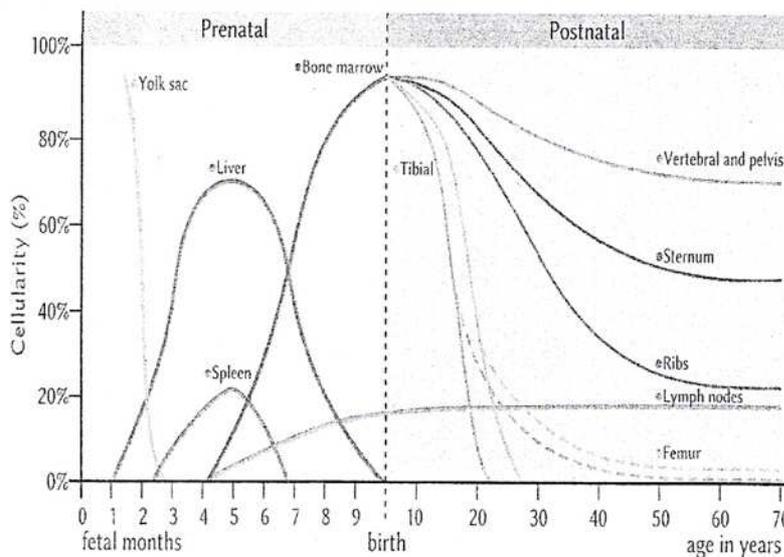
Fig.1b. *Haematopoiesis: Formation of blood cellular components in prenatal and postnatal periods.*
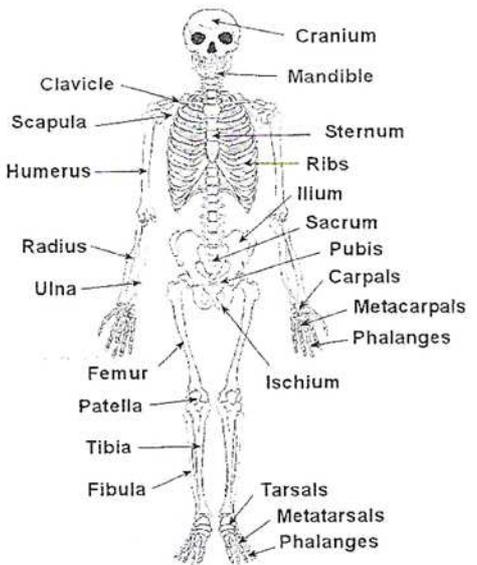
[23]. "Erythron" is a valuable constant defining the combined mass of mature and immature red blood cells. This constant emphasizes the functional unity of mature red blood cell and its precursors whether in the peripheral blood or bone marrow. The rate of red blood cell production is subject to feedback control which is related to tissue oxygen tension [27]. The normal value of oxygen pressure in the arterial blood is 60-80mmHg of oxygen saturation 90-95% [28]. Low oxygen tension in the blood causes a protein activator of haemopoiesis, haemopoietin and / or erythropoietin, to be released into the circulation from the kidneys. In turn, release of erythropoietin stimulates stem cells in the bone marrow to produce erythrocytes. Erythropoiesis is inhibited by a rise in the level of circulating red blood cells to subnormal values and stimulated by anaemia and by hypoxaemia [23]. The normal period for norrmoblastic erythropoiesis is 4 days [21,29]. The formation of red blood cell itself requires the usual nutrients and structural materials, in addition to iron, vitamin B_{12} and folic acid. The characteristics of normal, mature, and biconcave disk human red blood cells are shown in Table 1. Deviation of any of these average values may cause clinical disorders [28]. Red blood cells lose their nuclei before entering the blood circulation, but nucleated red blood cells, usually normoblasts, are found when erythropoiesis is vigorous where there is irritation of the bone marrow, as in leukaemia [23].

Adapted from: Bauer, J.D., Ackermann, P.G. and Toro, G. (1974) *Clinical Laboratory Methods,* 8th edn. St.Louis: The C.J.Mosby Company; Harris, J.W. and Kellermeyer, R.W.

(1974) The Red Cell-Production, Metabolism, Destruction: Normal and Abnormal. Cambridge, MA: Harvard University Press; Suresh, S. (2006)"Mechanical response of human red blood cells in health and disease: some structure-property-function relationships", *J.Mater.Res.*, vol.21 (8), pp.1871-1877; Diez-Silva, M., Dao, M., Han, J., Chwee-Teck Lim, C.T. and Suresh, S. (2010)"Shape and biomechanical characteristics of human red blood cells in health and disease*", Material Research Society Bulletin*, vol.35 (5), pp.382-389.

Table 1. Characteristics of human red blood cells

			Male	Female
Red blood cells count	10^{12} l^{-1}		5.4	4.8
Haematocrit	(%)		47.0	42.0
Haemoglobin	(g/100 ml)		16.0	14.0
Mean corpuscle volume	(μm^3)		87.0	87.0
Mean corpuscle surface area	(μm^2)		163.0	163.0
Mean corpuscle diameter	(μm)	= Mean diametere of 500 cells in smear	8.5	8.5
Iron　　0.0034 @g Hb	($x10^{-4}$g)		5.4	4.8
Anaemia　　: the concentration of haemoglobin in the blood cells below the normal level of a particular age and sex.				
Anisocytosis　: inequality of mean corpuscle volume.				
Elliptocytosis　: elliptical shape of erythrocytes.				
Hypochromia　: haemoglobin <25 x 10^{-9} g/corpuscle.				
Macrocytosis　: mean corpuscle volume >95 μm^3 .				
Microcystosis : mean corpuscle volume <80 μm^3 .				
Poikilocytosis　: irregularity in erythrocytes shape.				

Aging of the red blood cell revealed a decrease of cholesterol-to-phospholipids moral ratio , followed by a marked decrease in the activities of the membrane-bound enzymes, Na^+,K^+-ATPase [E.C.3.6.1.7] which is the marker of erythroid differentiation [30], is inactive in the intact membrane and is activated by disintegration of red blood cell membrane [31]. When the red blood cell membrane becomes defective, the red blood cell is removed from the blood by the phagocytes and broken down in the reticuloendothelial system [32]. Iron liberated in the breakdown of haemoglobin. Hb, is deposited as ferritin which a special iron-containing protein, if it is not utilized immediately [21]. The iron-free residual green bile pigment, biliverdin, is converted to yellow pigment, bilirubin, which is carried by the plasma to the liver for excretion as brown stercobilin. However, if the liver has undergone degeneration, and if breakdown of hemoglobin is excessive, or if the bile duct is obstructed, bilirubin may accumulate to high levels in the blood, causing jaundice, in which the skin acquires a yellowish tinge. Red blood cells are broken down by reticuloendothelial system and unconjugated bilirubin (0.2-1.4 mg/100ml) in the blood plasma [28] is carried by albumin to the liver. Red blood cells survive for the average period of 120±4 days [33].This involves 175 miles travel over the life span [34]. It follows that normally 2.5 x 10^6 red blood cells must be formed every second to replace those that are removed from the circulation [26].

Red blood cells, like other cells, shrink in solutions with an osmotic pressure greater than that of normal plasma which is 300 mosm/litre [35]. Also, red blood cells swell in a solution with a lower osmotic pressure and become spherical rather than discoid shape. Eventually, lysis occurs and red blood cells lose haemoglobin. Red blood cells which are spherocytic in normal plasma [36], are haemolyzed more readily than normal discoid cells in hypotonic sodium chloride solutions. This abnormal erythrocyte fragility is hereditary and the associated disease is termed congenital haemolytic icterus [37].

3. Function of the Human Red Blood Cells

Because of the native widely held opinion that erythrocyte biochemistry is a simple and uncomplicated, this cell type has been grossly underestimated as a dynamic functional unit. However, it has come into its own during the past twenty years through the appreciation of its complex ultrastructure and the dynamic mechanisms associated with its operation [15,38,39]. The developing red blood cell is organized for the biosynthesis of some 4 x 10^8 molecules of haemoglobin that ultimately make up 95% of its dry weight [29]. The haemoglobin of the adult erythrocyte affects the transfer of oxygen and carbon dioxide throughout the body (Fig.2).

If haemoglobin was not maintained within the corpuscle units, the resulting haemoglobin solution of 15 g 100 ml^{-1} would be too viscous for efficient circulation [40]. It has been demonstrated that haemoglobin in lysates, at an intracorpuscular concentration of 5 mM Hb, has a viscosity less than that of the erythrocyte suspended in the same diluting medium [41].

Haemoglobin (Hb) possesses a molecular weight of 64, 450 dalton. Hb is a tetramer molecule made up of four subunits, in which each contains a haeme moiety conjugated to a polypeptide. Haeme is an iron-containing porphyrin derivative. There are two pairs of polypeptides in each Hb molecule. In normal adult HBA_1 , the two types are $\alpha_2\beta_2$, in which α chain contain 141 amino acid residues and the β chain contain 146 amino acid residues. In foetal Hbf, the β polypeptide chains are replaced by γ chains of 146 amino acid residues each, but only 10 individual residues differ from those in β chains. When Hb binds to oxygen the distance between the iron (II) ions of the β_1 and

β_2 polypeptide chains is markedly reduced from 40 Å to 33 Å. The changes in contacts between α and β chains are of special interests. In the transition from oxy- to deoxyhaemoglobin , large structural changes take place at two of the four contact regions, namely $\alpha_1 \beta_2$ and the identical $\alpha_2 \beta_1$ contacts, but not at the others $\alpha_1 \beta_1$ and $\alpha_2 \beta_2$ contacts. The $\alpha_1 \beta_1$ pair rotates 15 degrees with respect to the other pair and atoms at the interface between these pairs

shift by as much as 6 Å. In fact, $\alpha_1 \beta_2$ contact   region acts as a switch between two alternative structures. The two forms of interface are stabilized by different sets of hydrogen bonds. This interface is closely connected to the haeme groups, and so the structural changes in it affect the haemes and vice versa. The quaternary structure of deoxyhaemoglobin is termed the T (taut or tense) form, whereas R (relaxed) form is termed for oxyhaemoglobin.
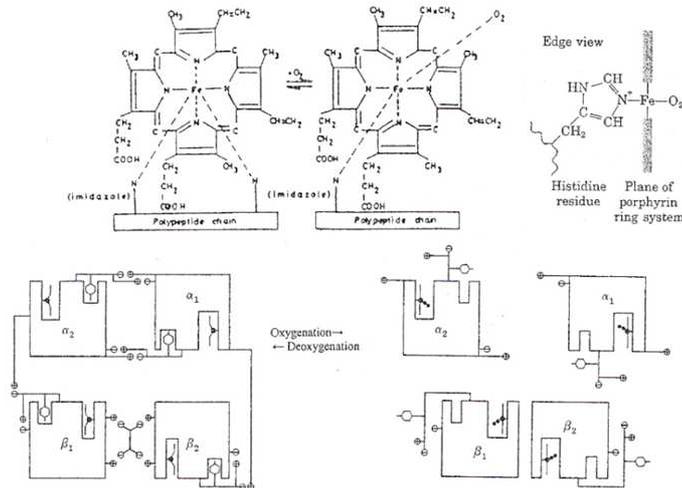

Fig.2. Chemistry of haemoglobin.

The schematic diagram shows the conformational change in the quaternary structure from deoxygenated Hb to fully oxygenated HB. Hb has all salt bridges intact; one mole of diphosphoglyverate is held between the two $\beta_1\beta_2$-chains; the four iron atoms are out of the plane of the porphyrins; the four tyrosine residues are present in the pockets, and the carboxyl-terminal residues of all four chains are anchored. In oxyhaemoglobin state, all of the salt bridges have been ruptured; the diphosphoglycerate has been released; the four iron atoms residue in the plane of the porphyrins; the four tyrosine residues have been released from the pockets, and the carboxyl-terminal residues of all four chains have almost complete freedom of rotation.

Adapted from: Perutz, M.F. (1970)" Stereochemistry of Cooperative Effects in Haemoglobin", *Nature*, vol.228, pp.726-739; Harris, J.W. and Kellermeyer, R.W. (1974) *The Red Cell- Production, Metabolism, Destruction: Normal and Abnormal.* Cambridge, MA; Harvard University Press; Perutz, M.F. (1979)"Regulation of oxygen affinity of hemoglobin: influence of structure of the globin on the heme iron", *Ann. Rev.Biochem.*, vol.48 (1), pp.327-386; Dickerson, R.E. and Geis, I. (1983) *Haemoglobin: Structure, Function, Evolution and Pathology.* San Francisco, CA: The Benjamin/Cummings Pub., Inc.; Manengo-Rowe, A.J. (2006)"Structure-function relations of human hemoglobins", *Proc. Bayl. Univ.Med.Cent.*, vol. 19(3), pp. 239-245.

Encapsulation of haemoglobin within the red blood cell membrane may also offer advantages to gas exchange within the capillary by producing a bolus flow instead of a laminar flow. With bolus flow there is a reduced stagnant

layer along the capillary wall [42].

Further advantages of packaging haemoglobin are as follows:

(1) the haemoglobin is removed from the general metabolic pool, preventing its rapid turnover (the half life of haemoglobin circulating free in the plasma is 200 min compared to 120 days for haemoglobin in the cytoplasm [43];

(2) the haemoglobin is kept in close proximity to enzyme systems that can effectively function to maintain it in a functional chemical state; and

(3) the osmotic pressure of the plasma (25 mmHg) is only 1/3rd that of the plasma plus haemoglobin (80 mmHg) [44].

3.1. Gas Exchange

Since the combination of the haeme iron with oxygen is stoicometric, the oxygen capacity of 1 g of haemoglobin is 1.38 ml, thus the oxygen capacity of 100 ml of normal blood, containing 15 g haemoglobin, is 20.7 ml [40]. With the average individual, in a basal state, about 250 ml of oxygen per minute is inspired, absorbed, transported and delivered to capillary tissues by erythrocytes. This quantity of oxygen is markedly increased by physical activity [45]. For a turnover of this magnitude, extremely rapid and nearly complete oxygenation of haemoglobin must occur in the lungs and comparably rapid deoxygenation must occur in the tissues. Some concept of the effectiveness of the actual reactions may be gained from the calculated average time in which a typical red blood cell spends in a pulmonary capillary, less than 1s at rest and 0.25 sec during

exercise [46]. The process of gas exchange is greatly aided by the total surface area of the circulating red blood cells, exceeding the body surface by 1500 times [29]. Furthermore, the discoid shape of red blood cell exposes 20-25% greater surface area than a sphere of equivalent volume [29].

The oxygen-binding curve for haemoglobin is sigmoid (Fig.3) in which the cooperativity exponent (Hill coefficient) of 2.8 [47] enables the haemoglobin to deliver twice as much oxygen than if the binding sites were independent.

The affinity of haemoglobin for oxygen is affected by pH, temperature and by 2, 3-diphosphoglycerate (2, 3-DPG) [48] (Fig.3 inset).
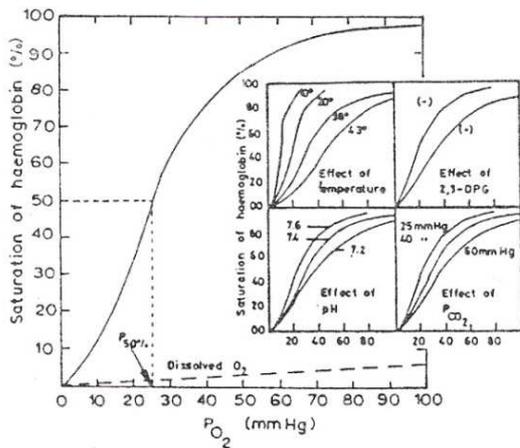

Fig.3. Haemoglobin-oxygen dissociation curve.

The Large curve applies to blood at 38°C and the normal arterial hydrogen-ion concentration (acidity). The inset curves illustrate the effects of altering temperature, 2, 3-DPG concentration, pH and P_{CO2} relationship between P_{O2} and haemoglobin saturation with oxygen.

Adapted from: Benesch, R. and Benesch, R.E. (1967)" The effect of organic phosphates from the human erythrocyte on the allosteric properties of hemoglobin ", *Biochem. Biophys. Res. Comm.*, vol.26 (2), pp.162-167.

It is found that haemoglobin in the human red blood cell has a lower affinity for oxygen than does pure haemoglobin in isotonic saline solution [26]. This difference is largely due to 2, 3-DPG

which binds with overwhelming preference for deoxy haemoglobin [49], effectively competes with oxygen for binding the haemoglobin (Eq. 1).

$$Hb_4(O_2)_4 + 2,3\text{-DPG} \overset{1.3 \times 10^5}{\underset{(pH7.4)}{\rightleftharpoons}} (Hb)_4 - 2,3\text{-DPG} + 4O_2 \quad (1)$$
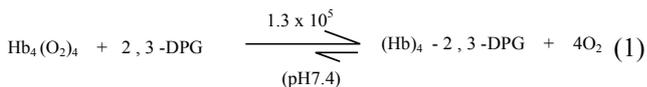
Thus an active catabolic tissue possessing a high concentration of 2, 3 –DPG would facilitate the dissociation of oxygen from haemoglobin [47] (Fig.4). However, people who live at 4,572 m above sea level where oxygen is in chronically short supply, have 20% higher concentration of erythrocyte 2 , 3 – DPG than do

people who live at sea level [50].

Adapted from: Kilmartin, J.V. (1976)"Interaction of haemoglobin with protons, CO_2, and 2, 3-diphopshoglycerate", *Brit. Med. Bull.*, vol.32, pp.209-222; Imaizumi, K, Imai, K. and Tyuma, I. (1982)"Linkage between carbon dioxide binding and four-step oxygen binding to hemoglobin", *J.Mol.Biol*, vol.159 (4), pp.703-719.
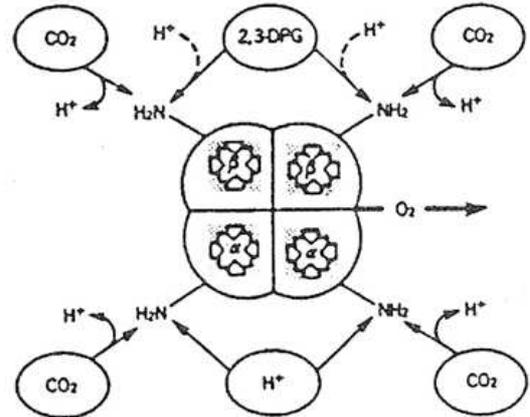

Fig.4. A diagrammatic representation of the interactions among the binding of O_2 , 2, 3 –DPG, CO_2 , and H^+ in haemoglobin. O_2 is released from haemoglobin when the proceed in the directions marked by arrows. On deoxygenation the direction of the processes is reversed.

Foetal haemoglobin ($\alpha_2\gamma_2$) which has a relatively poor affinity for 2, 3 –DPG, possesses a greater affinity for oxygen than that of adult haemoglobin ($\alpha_2\beta_2$) [49]. ATP which also constitutes a significant proportion of organic phosphates in the red blood cell, affects the oxygen equilibrium curve of haemoglobin in the same manner as 2, 3 –DPG [48]. Since the concentration of both 2, 3 –DPG and ATP is metabolically controlled, organic phosphate can act as a prevailing regulator for oxygen transport.

The CO ,carbon monoxide, combines with haemoglobin at the same site as does O_2 and in a similar way, but the affinity is 250 times higher than that of O_2 . Partial carbon monoxygenation in the circulating blood decreases the sigmoidicity of the oxygen equilibrium curve and that induces more serious effects than loss of a similar amount of haemoglobin due to anaemia [51].

The CO_2 produced in the tissue by diverse metabolic processes exerts a high partial pressure (P_{CO2}). Accordingly, CO_2 moves along the pressure gradients into the circulating plasma (Table 2) and then into the red blood cell. CO_2 is transported

Adapted from: Yoshikawa, H. and Rapoport, S.M. (1974) *Cellular and molecular Biology of Erythrocytes*. Tokyo: University of Tokyo Press; Kofstad, J. (1996) "Blood gases and hypothermia: some theoretical and practical considerations", *Scand. J. Clin. Lab. Invest. (Suppl)*, vol. 224, pp. 21–26; Middleton, P., Kelly, A.M., Brown, J. and Robertson, M. (2006) "Agreement between arterial and central venous values for pH, bicarbonate, base excess, and lactate", *Emerg. Med. J.*, vol.23 (8), pp. 622–624.

		Arterial blood	Venous blood
Oxygen			
P_{O_2}	(mmHg)	100.00	40.00
O_2 content	(vol %)	20.30	15.50
Dissolved O_2	(vol %)	0.30	0.12
Carbon dioxide			
P_{CO_2}	(mmHg)	40.00	46.50
Total CO_2	(vol %)	49.00	53.10
Dissolved CO_2	(vol %)	2.60	2.90
As H_2CO_3	(ml)	43.90	47.00
As carbamoni compound	(ml)	2.40	3.90
Plasma pH		7.40	7.38

by red blood cell in the following forms:

(i) A small portion (5%) remains physically dissolved in the cytoplasm.

(ii) Another portion (25%) reacts directly with N-terminal amino acid residues of deoxy haemoglobin, forming carbamino-haemoglobin (Eq. 2).

$$CO_2 + R - NH_2 \rightleftharpoons R - NHCOOH \quad (2)$$
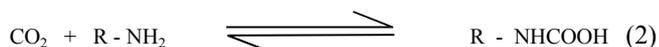
At P_{CO_2} values above 10 mmHg, the amount of caramino-haemoglobin formed is relatively constant and independent of the P_{CO_2} , because the tendency to form more, as the amount of available CO_2 increases, is offset by the formation of more protons (Eq.3).

$$H_2CO_{32} \rightleftharpoons HCO_3^- + H^+ \quad (3)$$

Where upon the terminal amino functions become protonated.

(iii) The major portion (70%) is rapidly converted to HCO_3^- .

3.2. Buffering Capacity

Various impermeable proteins in the erythrocyte cytoplasm act to buffer the intracellular medium against changes in pH incident to gas-exchange and Hamburger shift.

3.3. Haemoglobin

Haemoglobin itself (2.8×10^8 molecules / erythrocyte) is the most important buffer as various groups on haemoglobin molecules possess a powerful buffering function [21]. Much of the buffering is in the physiological range of pH (7.0-7.7), and is mainly achieved by imidazole groups of histidine residues (Eq. 4) which reach an effective concentration of 100mM [52]. Some of the imidazole groups on deoxy haemoglobin dissociate less readily than those on oxyhaemoglobin, this being particularly true for His-146β which forms a salt link with Glu-94β.

$$(4)$$
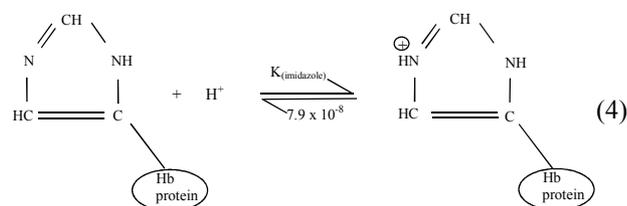
Indeed, it has been estimated at 40% of the Bohr effect is associated with protonation of this residue. However, the N-terminal amino function of the β-chain also makes an appreciable contribution at the Bohr effect (estimated to be 20%) [53]. Significantly, this region of haemoglobin forms the binding site of 2, 3 –DPG and Cl⁻ (Fig. 5), thus anions also influence the

**Fig. 5.** *2, 3 –DPG binding in deoxyhaemoglobin.*

Amino acid side chains around the 2, 3- DPG binding site. This top view of the central cavity in human deoxyhaemoglobin shows the positive charges lining the 2, 3- DPG site: two each of the amino terminus, His-2β, Lys-82β and His-143β. The 2, 3- DPG molecule with its five
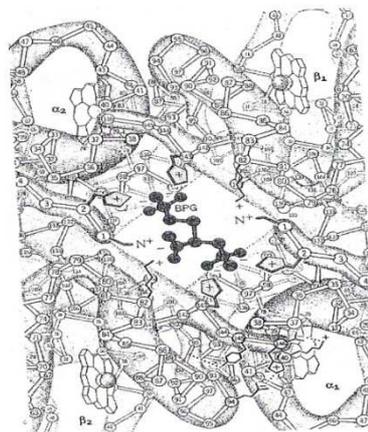
negative charges site in the middle of this ring of positive charges. The foetal γ chain loses two of the eight positive charges by substituting Ser for His at β143, decreasing the affinity for 2, 3- DPG. Also shown are the salt bridges and the hydrogen-bonding pattern around the carboxyl terminus

of the β chain (lower part of drawing), which emphasizes their contribution to the Bohr effect.

After: Arnone, A. (1972)"X-ray diffraction study of binding of 2, 3-diphosphoglycerate to human deoxyhaemoglobin", *Nature*, vol.237, pp.146-149; Di Dickerson, R.E. and Geis, I. (1983) *Haemoglobin: Structure, Function, Evolution and Pathology*. San Francisco, CA: The Benjamin/Cummings Pub., Inc.; Stryer, L. (1995) Biochemistry, 5th edn. New York: W.H.Freeman and Company.oxygen dissociation curve of haemoglobin by influencing the protonation states of the N-terminal amino function, His-2β and His-143β [50]. Therefore, deoxyhaemoglobin is a weaker acid and therefore, a better buffer than oxyhaemoglobin. As a result of an acid environment, the equilibrium curve of oxygen is shifted towards the deoxyhaemoglobin, facilitating the removal of medium H^+ ions (Eq.5). As the medium becomes less acidic, the

$$HbO_2 + H^+ \rightleftharpoons Hb^+ + O_2 \quad (5)$$
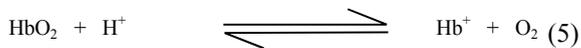
oxyhaemoglobin is favoured and H^+ ions are liberated [23]. The physiological implication of this behaviour is that generation of both lactic acid and H_2CO_3 cause acidification and thereby, encourage oxygen dissociation from haemoglobin. Furthermore, CO_2 which forms a carbamino derivative of the N-terminal function [50] (Eq. 6), inhibits the effect of 2, 3- DPG at pH $\geq$7. 3, but not at lower pH values [54].

$$Hb\text{-}NH + CO_2 \rightleftharpoons Hb\text{-}NHCOOH \rightleftharpoons Hb\text{-}NHCOO^- + H^+ \quad (6)$$

3.4. Carbonic Anhydrase [E.C.4.2.1.1]

Human red blood cell contains three isoenzymic forms of carbonic anhydrase (CA) A, B, and C which can be separated by electrophoresis. B is the most abundant and C exhibits three times the specific activity of B. Each has a molecular weight of about 30,000 daltons with one zinc atom per molecule [55,56]. CA is essentially absent from the plasma.

The majority of tissue CO_2 molecules having entered the red blood cell are rapidly converted to H_2CO_3, which then dissociates (Eq.7). Two Opposing phenomena then come into play: (1) increasing levels of H_2CO_3 would tend to lower the

$$CO_2 + H_2O \underset{5 \times 10^{-3}}{\overset{K_{eq}}{\rightleftharpoons}} H_2O \underset{1.6 \times 10^{-4}}{\overset{K_a}{\rightleftharpoons}} HCO_3^- + H^+ \quad (7)$$
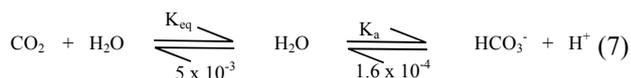
pH within the red blood cell (Fig.6), but (2) the transformation of oxygenated haemoglobin to deoxygenated haemoglobin involves a change of pK from 6.2 to 7.7, which leads to the removal of protons from the cytoplasm of the red blood cell.

The relative concentrations of bicarbonate and CO_2 at a pH of 7.4 and the effect of adding 14 mEq. Of strong acid per litre are shown. At first one would expect the result to

be a drop in pH to 6.06. However, 14 mEq. Of carbonic acid (as CO_2) is removed by the lungs and the pH would therefore be 7.10. Furthermore, breathing is stimulated at once (hyperventilation), and the carbonic acid concentration is reduced to o.80 mEq. Per litre to yield a final pH of 7.34, a condition of compensated metabolic acidosis.

After: Bauer,J.D., Ackermann,P.G. and Toro,G.(1974) Clinical Laboratory Methods, 8th edn., p.837. Sat Louis, MO:The C.J. Mosby Company.
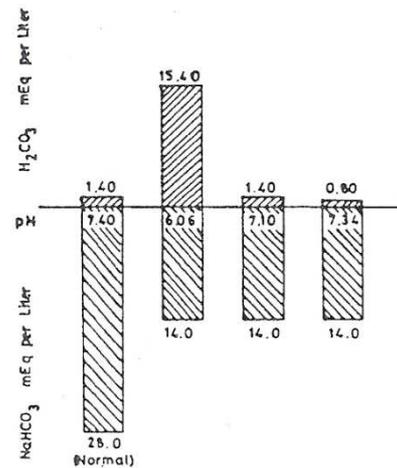
Fig. 6. *Comparison of bicarbonate as a chemical and physiological buffer of strong acid.*

Consequently, protons formed in the dissociation of H_2CO_3 are accepted by the imidazole nitrogen of the haemoglobin. The net result of these two events is to maintain the pH essentially unchanged, and K^+ ions within the red blood cell, previously neutralized by oxyhaemoglobin, are now neutralized by the newly formed HCO_3^- ions. Similarly, in the lungs, where there is a diminishing P_{CO2} and increasing P_{O2}, the haemoglobin becomes oxygenated. Thus plasma HCO_3^- moves into the red blood cell and combines with protons given up by dissociation of the newly formed oxygenated haemoglobin. The ratio of HCO_3^- to H_2CO_3 which is normally 28 mEq/L to 1.4 mEq (20:1) [28]. Also, since HCO_3^- is much more soluble in blood plasma than CO_2, the capacity of the blood to carry CO_2 from tissues to the lungs will therefore increase (Fig.7). The HCO_3^- re-permeates the erythrocyte and converted by carbonic anhydrase to CO_2 that is released in the lungs and eventually exhaled. The effectiveness of this shuttle is directly proportional with the rapid movement of HCO_3^- across the red blood cell membrane which has to be achieved in the 300-600ms period for the red blood cell traverses the pulmonary circulation [57].

According to the Henderson-Hasselbach equation, the general mathematical formulation for bicarbonate buffer may be expressed as follows:

$$pH = pK'_{H_2CO_3} + Log \frac{[HCO_3]^-}{[H_2CO_3]} \quad (8)$$

Where K' is the upper apparent dissociation constant of H_2CO_3 and it is equivalent to the constants K_{eq} , K_{a1} , 8 x 10^{-7}M (Eq. 7). But, the actual clinical measurement of CO_2 is expressed as:

$$[CO_2]_{Dissolved} = s \cdot P_{CO2} \quad (9)$$

Where s is the solubility coefficient of CO_2 gas, 0.301 $mM.mmHg^{-1}$. Thus, the buffer equation (9) may be written as follows:

$$pH = 6.1 + log \frac{[HCO_3^-]}{0.0301 \cdot P_{CO_2}} \quad (10)$$

Since electrochemical neutrality of both the intracellular fluid and the plasma is maintained by diffusion of Cl^- (chloride shift) into the red blood cell, the Cl^- content of the red blood cell in venous blood is appreciably greater than that in arterial blood. Also, the number of osmotically active anionic particles in the red blood cell increases as the hydrogen ions is buffered and HCO_3^- accumulates. Therefore, the red blood cell takes up H_2O, and the cell size increases. As a result, the haemotocrit (Hct) of venous blood is normally 3% greater that that of aerterial blood. In the lungs the Cl^- effluxes from the red blood cell and as a result they shrink.

Adapted from: Dailey, J. F. (2001) Blood, 2nd edn, pp. 87-88. Ipswich, MA; Medical Consulting Group; Nelson, D. L. and Cox, M.M. (2008) *Lehninger Principles of Biochemistry*, 5th edn. p.395.New York: W.H.Freeman and Company.
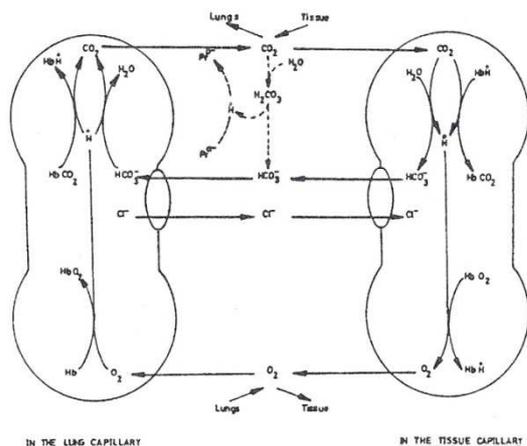
Fig.7. *Schematic diagram of chloride-bicarbonate exchange associated with O_2 and CO_2 transmembrane movement in the human red blood cell.*

3.5. Blood Clot Formation

In *Homo sapiens*, blood coagulation is only one of several important mechanisms for haemostasis [58,59]. The haemostatic mechanism is subject to the kind and number of vessels damaged and the location of injury. Haemorrhage occurs in bulk flow, the basic prerequisites for bleeding are: (1) loss of vessel continuity or marked increase in permeability so that cells are able to leak out; (2) pressure inside the vessel is greater than outside [21]. Accordingly, bleeding ceases if either blood loss is eliminated, or the damage portion of vessel is blocked and all haemostatic processes accomplish one of these two requirements. However, damage to small vessels (arterioles, capillaries, venules) is the most common source of bleeding in everyday life and haemostatic mechanisms are most effective in dealing with such injuries because blood clots [60,61,62,63]. In contrast, the bleeding from a severed artery of medium or large size is not usually controllable by the body and it requires radical aids such as application of pressure and ligatures. The bleeding from venous is less dangerous because of the vein's low hydrostatic pressure that can be induced by simple elevation of bleeding part [64].

The involvement of platelets in haemostatic events requires their adhesion to a surface. Although platelets have a propensity for adhering to many foreign or rough surfaces, they don't adhere to the normal endothelial cells lining the blood vessels. When a rupture occurs to a vessel, blood plasma, blood cells and platelets leak out into surrounding tissue spaces. The injury disrupts the endothelium and exposes the underlying connective tissue with its collagen fibers. This attachment somehow triggers the release from platelets' granules of potent chemical agent (integrin $\alpha_{IIb}\beta_3$); including thromboxane and adenosine diphosphate that causes the surface of adhered platelets to become extremely sticky, enabling new platelets adhere to the old ones. Mutations in either α or β of platelet integrin may cause bleeding disorder [65]. Also, the contact between platelets and collagen fibers cause some platelets to break open and leak out several agents, including serotonin and thromboplastin [66,67]. Serotonin is a small molecule that stimulates contraction of smooth-muscle cells of nearby arterioles to cut off blood flowing toward the injury site, and thromboplastin is an enzyme that stimulates blood clotting by initiating a remarkable cascade of plasma protein factors, each normally inactive until activated by the previous one in the sequence [68]. It acts on one of the globular proteins in blood plasma, prothrombin [E.C.3.4.21.6]. In the presence of sufficient level of Ca^{++} ions, thromboplastin catalyzes a massive conversion of prothrombin to thrombin, yet an other enzyme. It is an endolytic serine protease [E.C.3.4.21.5] that selectively cleaves the Arg-Gly bonds of fibrinogen which is a soluble ,large, rod shaped dimeric glycoprotein (M_{wt} $340x10^3$) produced by the lever and always present in the plasma of normal persons [69]. It forms fibrin and release fibrino peptides A and B [70]. Fibrin makes up the final cascade step forming fibrous strands that compose a clot and give the clot tensile strength. Platelets and red blood cells pouring from the ruptured blood vessel are trapped

within the three-dimensional fibrin matrix as clot forms [63].

Red blood cells have a physical and chemical effect on the interaction between platelets and blood vessel surfaces [71,72,73]. Several clinical observations have reinforced the active role of red blood cells in normal and disorders of haemostasis [74,75,76,77,78,79].

4. Metabolism of Human Red Blood Cell

The red blood cell (Fig.1a) possesses functioning glycolytic and phosphogluconate oxidative pathways, the tricarboxylic acid cycle, an intact cytochrome system and electron transfer mechanism [21]. Moreover, the reticulocyte can synthesize haemoglobin and diverse lipids, viz. cholesterol, phosphoglycerides and triacylglycerols and can achieve the *de novo* synthesis of purine nucleotides.

In contrast, the enucleated red blood cell which also lacks mitochondria, and many of these metabolic processes [80], but nevertheless, has the necessary means to sustain the red blood cell as a functional unit [81,82,83]. Thus red blood cell metabolism is dominated by anaerobic Embden-Meyerhof and pentose phosphate pathways [84,85].

2,3- DPG which plays a major role in the red blood cell physiology [86], is produced in the cytoplasm via a branch of glycolysis [21] (Fig.8).

(E_1) Di-P-glyceromutase [E.C.2.7.5.3]; (E_2) 3-P-glycerokinase [E.C.2.7.2.3]; (E_3) Di-P-glycerophosphatase [E.C.3.1.3.13];

(--) the broken lines indicate inhibitions.

After: Jacobasch, G., Minankami, S. and Rappport, S.M. (1974)"*Glycolysis of the erythrocyte* ", in *Cellular and Molecular Biology of Erythrocytes*, Yoshikawa, H. and Rapoport, S.M. (eds), pp.55-92.Tokyo: University of Tokyo Press; Lionetti, F.J. (1974)"*Pentose phosphate pathway in human erythrocytes*", in *Cellular and Molecular Biology of Erythrocytes*, Yoshikawa, H. and Rapoport, S.M. (eds.), pp.143-166. Tokyo: University of Tokyo Press.
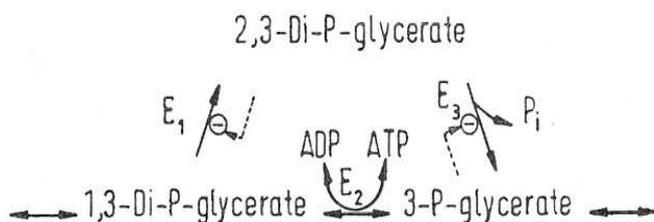

Fig.8. 2, 3-Diphosphoglycerate metabolic bypass.

The unique feature of haemoglobin which is the main red

blood cell protein is the formation of a stable oxygen complex, in which the iron remains in the ferrous state. This special behaviour is due to the relatively low dielectric constant of the immediate vicinity of the haeme moiety which lies within a hydrophobic environment. However, oxygen may autoxidize the haeme iron from Fe^{2+} to Fe^{3+} rather than simply binding, thus yielding the unwanted methemoglobin (metHb) form of the molecule. Furthermore, the autoxidation of Hb to metHb results in the formation of superoxide anion (O^-_2.) which in turn can generate hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^.$) [29]. These are reactive species and potentially deleterious. However, the red blood cell has evolved an enzymic defense mechanism directed against superoxide (Eq.11 and Eq.12).

$$2CO^._2 + 2H^+ \xrightarrow{\text{Superoxide dismutase}} H_2O_2 + O_2 \quad (11)$$

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2 \quad (12)$$
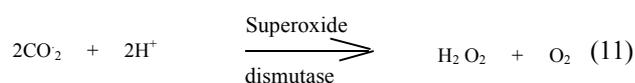
This is the dominant route of ferro Hb regeneration under normal conditions, 70 % of the oxidized Hb being renatured via this mechanism. However, ascorbate and GSH are also able to reduce ferrihaemoglobin and thus red blood cell catabolism is also used to maintain these two redox coenzymes in a reduced state. The red blood cell also possesses several mechanisms specifically designed to regenerate iron (II) haemoglobin. For instance, ferrihaemoglobin is reduced by both NADH and NADPH metHb reductases [29, 87] (Fig.9).

A congenital absence of the metHb reduction is one cause of hereditary methemoglobinemia, in which as much as 25-40 % of the total Hb is present as metHb [88].

(E_1) Glyceraldehyde-3-Pdehydrogenase [E.C.1.2.1.12]; (E_2) Lactate dehydrogenase [E.C.1.1.1.28]; (E_3) Glucose-6-P-dehydrogenase [E.C.1.1.1.49]; (E_4) 6-P-Gluconate dehydrogenase [E.C.1.1.1.44]; (E_5) Dehydroascorbic acid reductase (NADPH dependent); (E_6) Oxidized glutathione reductase (NADPH dependent); (E_7 and E_8) Methemoglobin reductases.

After: Harris Harris, J.W. and Kellermeyer, R.W. (1974) *The Red Cell-Production, Metabolism, Destruction: Normal and Abnormal.* Cambridge, MA: Harvard University Press; Jaffe, E.R. (1974)"*The formation and reduction of methemoglobin in human erythrocytes*" in *Cellular and Molecular Biology of Erythrocytes*, Yoshikawa, H. and Rapoport, S.M. (eds), pp.345-376. Tokyo: University of Tokyo Press.
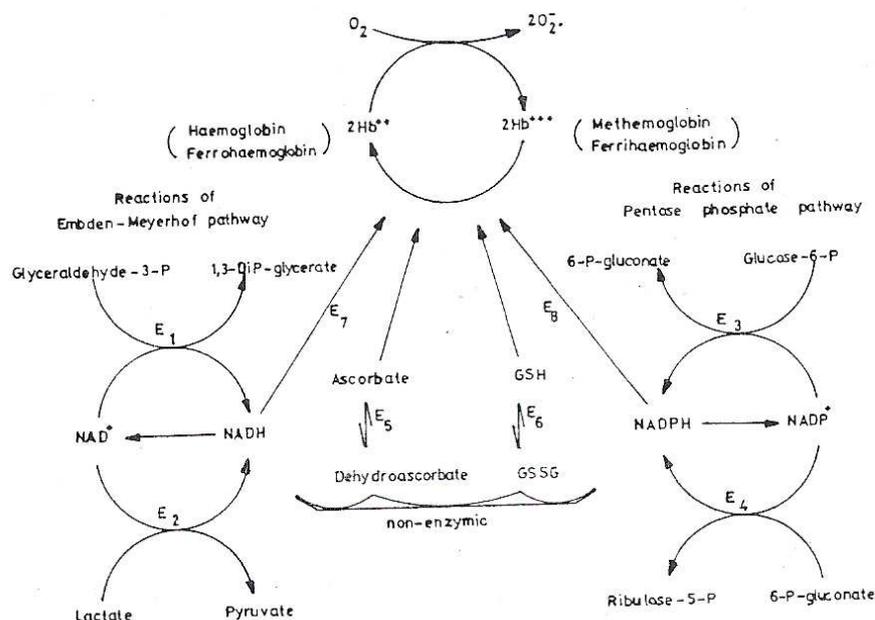
Fig.9. Mechanisms available in the red blood cell for effecting reduction of methemoglobin to haemoglobin.

5. The Human Red Blood Cell Membrane

The human red blood cell has proved to be a useful tool for many studies, as it has the particular advantage that the plasma membrane is the only membrane protein. Plasma membrane preparations (ghosts) can be produced from intact cells by simple hypotonic shock and then subsequent washings to remove the resealed haemoglobin [89,90,91,92]. Studies with this sytem have led to many advances in our present understanding of biological membranes [38,93,94].

5.1. Membrane Lipids

The chemical structures of various lipids that are present in red blood cell membrane [95, 96, 97, 98] is shown in Fig.10. Cholesterol (3.2 µmols/10^{10} cell), glycolipids (0.2 µmols/10^{10} cell) and phospholipids (4.01 µmols/10^{10} cell) (Van Deenen et al, 1963; Nelson, 1972) constitute the red blood cell membrane lipids. Thus, the ratio of cholesterol to phospholipids is 0.82. Phospholipids of red blood cell membrane [99] are distributed as follows:

Sphingomyelin (25.8 %), phosphatidylcholine (28.3 %), phosphatidylethanolamine (26.7 %), phosphatidylserine (12.7 %) and other phospholipids (3.9 %). The fatty acid composition and approximate molecular configurations of the most important lipids are shown in Table 3 and Fig.10.

Table 3. Fatty acid composition of the main individual phospholipids in the human red blood cell membrane.

Chain ength and unsaturation	Total phospholipids	Percentage Sphin-gomyelin	Of Total Phosphatidyl-choline	Fatty Phosphatidyl-ethanolamine	Acid Phosphatidyl-serine
16:0	20.1	41.3	34.7	15.5	4.4
18:0	17.0	9.1	13.8	14.1	39.7
18:1	13.3	5.2	21.1	17.2	9.8
18:2	8.6	3.7	21.4	5.6	2.6
20:0	<1 %	-	-	-	-
20:3	1.3	-	-	-	-
20:4	12.6	0.1	6.7	21.8	23.5
22:0	1.9	8.0	-	-	-
22:4	3.1	-	-	7.8	-
22:5	2.0	-	-	4.6	2.9
22:6	4.2	-	-	8.9	7.0
23:0	<1 %	-	-	-	-
24:0	4.7	15.0	-	-	4.1
24:1	4.8	15.5	-	-	3.7

Each class of lipid is shown with its hydrophobic area on the upper side.
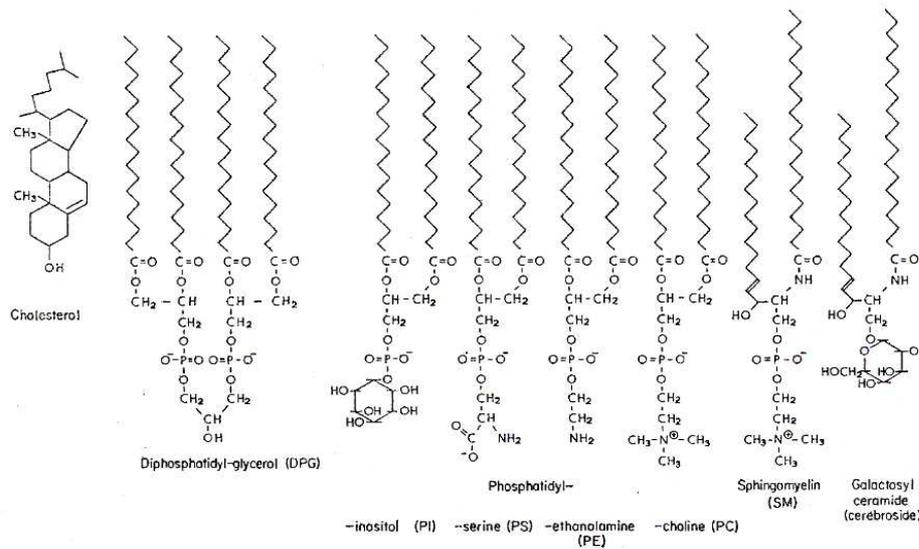
Fig.10. Chemical composition and approximate molecular configurations of the most membrane lipids.

After: Van Deenen, L.L.M., De Gier, J., Houtsmuller, U.M.T., Montfoort, A. and Mulder, E. (1963)*"Dietary Effects on the Lipid Composition of Biomembranes", in Biochemical Problems of Lipids*, Frazer, A.C. (ed.), pp. 404-414. Amsterdam: Elsevier; Nelson, G.J. (1972) *Blood Lipids and Lipoproteins: Quantitation Composition and Metabolism*, p.317. New York: Wiley-Interscience; Manku, M.S., Horrobin, D.F., Huang, Y.S. and Morse, N. (1983)" Fatty acids in plasma and red cell membranes in normal humans", *Lipids*, vol.18 (12), pp.906-908; Hsiao,L.L., Howard, R. J., Aikawa, M. and Taraschi,T.F.T.(1991)"Modification of host cell membrane lipid composition by the intra-erythrocytic human malaria parasite plasmodium falciparum", *Biochem.J.*, vol.27(1),pp.121-132.

5.2. Membrane Proteins

Red blood cell phospholipid bilayers provide the cell with both hydrophilic and hydrophobic environments. A hydrophilic surface of a protein will therefore, preferentially interact with the polar membrane surface, whereas a hydrophobic protein will position itself within the environment provided by the acyl chains. The major membrane proteins and glycoproteins present in the human red blood cell are presented in Table 3. Several major proteins are resolved by SDS-PAGE into well –defined bands [100,101,102,103] together with 15-20 faint bands that taken together constitute ~ 1% of total membrane proteins [104,105,106]. These proteins consist of structural polypeptides, transport polypeptides and enzymes (Table 4).

Table 4. Some properties of human red blood cell membrane proteins

Peptide Fraction	Mol. Weight (x 10³)	Copies per cell (x 10⁻⁵)	Ref.	Protein Content[1] (% of total)	Function	Association with
Band 1 (spectrin)	240	2.2	212, 219	15%	Membrane skeleton	Band 3 (to Band 1) Ankyrin (to Band 2) Band 4.1
Band 2 (spectrin)	220	2.2	117			
Band 2.1 (ankyrin) Band 2.2 Band 2.3	220 183 165	1.1	16 206 206	15% 15%	Connects membrane Skeleton with intrinsic domain	Band 2 Band 3
Band 3	95	12.0	21, 31, 212	25%	Anion transporter Skeleton attachment site Protein kinase C Phosphorylation site.	Band 1, Ankyrin Band 4.2, Band 6 Glycophorin A
Band 4.1 a Band 4.1 b	80 78	2.3	117, 157 83	4% 5%	Connects membrane Skeleton with Intrinsic domain. Spectrin-actin interaction	Spectrin Glycophorin C
Band 4.2 (paladin)	72	2.3	117,		ATP binding site	Band 3

Peptide Fraction	Mol. Weight (x 10³)	Copies per cell (x 10⁻⁵)	Ref.	Protein Content[1] (% of total)	Function	Association with
			155, 157, 213			Ankyrin
Band 4.5 / 1 2 3 4 5 6	59 52	1.3 1.4 0.7 1.0 1.3 1.3	49, 116		Glucose transporter monosaccharide, L-lactate and nucleoside transfer system	
Band 4.9	48	1.0	25		Membrane skeleton	Band 2
Band 5 (actin)	43	5.1	117		Membrane skeleton	Spectrin Band 4.1
Band 6	35	4.1	117, 213	6%	Glyceraldehyde-3-P-dehydrogenase	Band 3 Spectrin -actin
Band 7.1 7.2 (stomatin) 7.3	30 31 26		131, 211	3%	Cytoskeletal anchor Na⁺, K⁺ flux control Glucose transporter	
Band 8	23		165, 186		Ca⁺⁺ binding	
Glycophorin A Glycophorin B Glycophorin C Glycophorin D	39 28 38 32	2.0 2.5 0.35 0.2	95, 165, 172, 232 72 72 187		major sialoglycoproteins for antigenic determinants. Skeleton attachment site	Band 3 Band 4.1

Spectrin (bands 1 and 2), ankyrin (bands 2.1, 2.2 and 2.3) and actin (band 5) form a polymeric network on the cytoplasmic surface of the membrane, cytoskeleton [106,107,108]   (Fig.11) which presumably endows the membrane with enhanced mechanical strength.
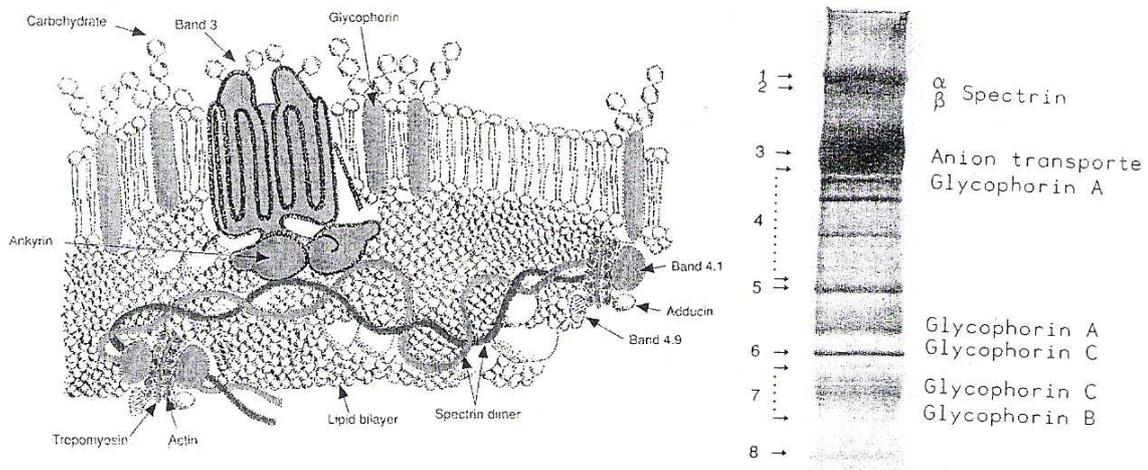
Fig.11. A schematic diagram of the proposed binding of red blood cell membrane skeleton to the plasma membrane.

The fatty acyl chains in the interior of the membrane form a fluid, hydrophobic region. Integral proteins held by hydrophobic interactions with their nonpolar amino acid side chains. The gycoproteins penetrate into or through the hydrophobic zone with all their carbohydrates exposed to the extracellular medium. The other polypeptides are, in general, less firmly anchored at the cytoplasm side, where they participate in specific interprotein association. Spectrin is linked to the anion channel protein by ankyrin, and to glycophorin by protein 4.1 that binds an actin filament. The Inset is red blood cell membrane major proteins separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and silver stained.

Adapted from: Singer, S.J. and Nicolson, G. L. (1972)"The fluid mosaic model of the structure of cell membranes", *Science*, vol. 175, pp. 720-731; Steck,T.L.(1974)"The organization of proteins in the human red blood cell membrane", *J.Cell Bio.*,vol.62,pp.1-19; Branton, D. (1983)"Erythrocyte membrane protein associations and erythrocyte shape", *The Harvey Lectures,* vol.77, pp.23-42; Lux, S.E. and Shohet, S.B. (1984)"The erythrocyte membrane skeleton: I-biochemistry, *Hospital*

Practice, vol.19, pp.76-84; Korsgren, C, Lawler, J., Lambert, S., Speicher, D. and Cohen, C.M. (1990)"Complete amino acid sequence and homologies of human erythrocyte membrane protein Band 4.2", *Proc.Natl.Acad. Sci.USA*, vol.87 (2), pp.613-617.

All of the major polypeptides of erythrocyte are available for modification or proteolytic digestion in leaky ghosts [35, 90, 109, 110, 111]. Thus none are located totally within the hydrophobic core of the bilayer [80].

In intact cells, glycophorin and Band 3 are the only proteins labeled with periodate-Schiff's stain [100,107,112,113]. Glycoprotein is characterized by a high content of sialic acid whereas Band 3 contains a small amount [114]. In unsealed membranes, only specific regions of these two proteins become available for labeling, indicating that they span the bilayer [105,115,116]. Indeed, these integral membrane proteins behave as amphiphiles [117], in that they possess both hydrophilic and hydrophobic regions. The so-called peripheral proteins may be displaced from the membrane by treatment with high ionic strength solutions, increased pH or by chelating agents such as ethylene-diamineteraacetic acid (edetic acid). The amino acid sequence of glycoprotein [118,119] is known and a schematic diagram of its structure is shown in Fig.11.

The second major integral protein is Band 3 [100,112] which has a molecular weight in the region 89,000-110,000 [100,106,120]. The broadness of Band 3 is attributed to the heterogeneity [106] of a mono- or dioligosacharaide chains [121,122], but not due to any structural variation in the polypeptide chain [112]. The Carbohydrate content of Band 3 is estimated at 4-9% that consists of focose, mannose, galactose, N-acetylglucosamine, and sialic acid [106,121]. Jenkins and Tanner (1977) and Fujinaga et al. (1999) have argued the amino acid composition of Band 3 and it consists of charged residues (lysine, histidine,arginine,aspartic acid , glutamic acid), intermediate residues (threonine, serine, proline, glycine, alanine, half-cystine) and hydrophobic residues (valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, tryptophan). It is unusual in that it possesses a high percentage of non-polar residues (~41 %) [123]. This finding is consistent with the solubility characteristics of Band 3 which can not be extracted by solutions of high or low ionic strength, but is dissolved in the presence of detergents [100,103,106,124]. Band 3 contains at least six –SH groups in which five groups are exposed to the cytoplasm and a 'cryptic SH' is in the bilayer [125,126,127].Surprisingly, only one disulphide bond is formed per Band 3 dimer, whereas all five –SH groups on the cytoplasm surface can participate in cross-linking [128]. The major part (90%) of band 3 is homogeneous with respect to solubility [106], proteolysis [102,110] and undergoes dimmer cross-linking with the presence of orthophenanthroline [101]. This structure consists of a cytoplasmic domain and a membrane-bound domain. Some sections of Band 3 protein have been sequenced [116,129,130] is known and a schematic diagram of its structure is shown in Fig. 11. The structure of Band 3 protein in red blood cell membranes has been determined by the use of proteolytic enzymes, impermeant covalent chemical probes, iodination of intact cells by lactoperoxidase, and interaction of concavalin A coupled to ferritin in intact cells [117,129,131].

5.3. Asymmetry of the Membrane

The chemical composition of human red blood cell membrane is as follows:

Protein, 49%; lipid, 43%; and carbohydrate, 8% [108, 132].
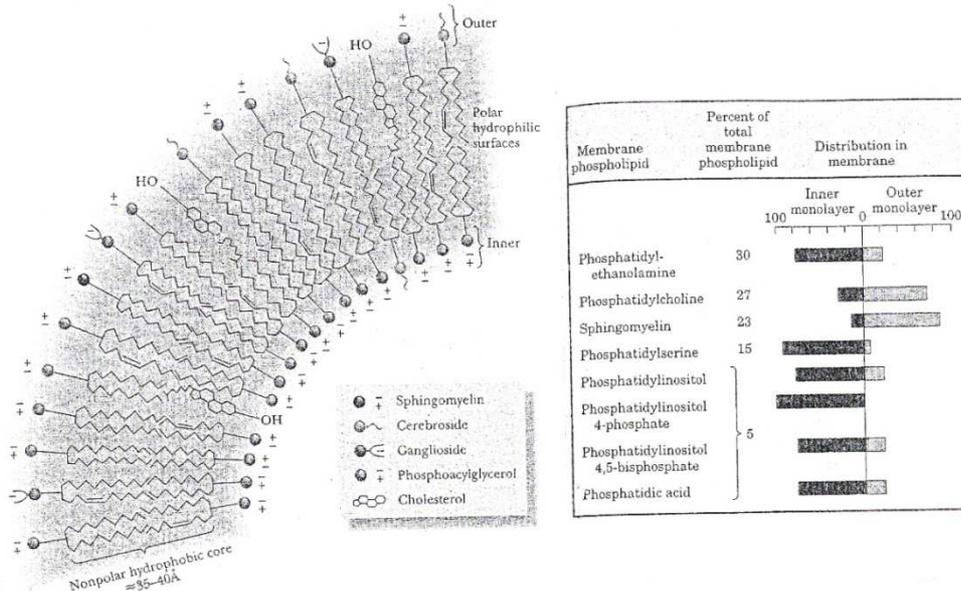
Fig. 12. Lipid bilayer asymmetry.

All of the membrane carbohydrates are located on the outer surface and the cytoskeleton proteins are on the inner surface (Fig. 12). Both the well characterized intrinsic proteins, glycoprotein and Band 3, are asymmetrically orientated in the membrane. Phospholipids are also asymmetrically distributed and this asymmetry is apparently related to the net charge of the head group [133]. The anionic aminophosphatides being almost totally located on the cytoplasmic face. This ratio of zwitterionic phospholipids species (Sph +PC + PE) to strongly anionic phospholipid species (PS + PI) is maintained in the two halves of the bilayer throughout the life time of the red blood cell [99,134]. This implies almost total absence of transbilayer movement (flip-flop) in normal red blood cells.

The positions of the outer and inner layers differ as well as the concentration of ohospholipid species are higher in the inner layer whereas the bulky molecules are higher in the outer layer. Inset shows the effect of double bonds on the conformations of the hydrocarbon tails of fatty acids that have kinks in their tail.

Adapted from: Steck, T.L. and Kant, J.A. (1974) *"Preparation of Impermeable Ghosts and Inside-out Vesicles from Human Erythrocyte Membranes "*, *in Methods in Enzymology*, Fleischer, S. and Packer, S. (eds.), vol. 31, pp.172-180 ;Van Deenen, L.L.M., De Gier, J., Houtsmuller, U.M.T., Montfoort, A. and Mulder, E. (1963*)"Dietary Effects on the Lipid Composition of Biomembranes", in Biochemical Problems of Lipids*, Frazer, A.C. (ed.), pp. 404-414. Amsterdam: Elsevier; Bretscher, M.S. (1985)"The molecules of the cell membrane", *Sci. Am.*, vol.253 (4), pp.100-108; Campbell, M.K. and Farrell, S.O. (2006) *Biochemistry,* 5th edn., p.193. Belmont, CA: Thompson Learning, Inc.

However, in the membrane of homozygous reversible sickle cells the phosphatidylcholine molecules experience increased 'flip-flop' rates [135], and this may well be associated with their decreased life span. Normally, the outer surface phospholipids do not trigger blood coagulation due to the absence of phosphatidylserine [136].

Associated with head group asymmetry is an asymmetry of the ratio of unsaturated to saturated fatty acyl side chains (Van Deenen et al., 1974). Also, the concentration of cholesterol in the outer leaflet of the bilayer is greater than that in the inside leaflet [137]. This asymmetric distribution of lipid side chains may well be essential for the controlled function of many trans membrane proteins. Furthermore, the anionic cytoplasmic phospholipids may be required for a specific conformational requirement of cytoplasmic membrane enzymes [138].

The perturblance of red blood cell membrane lipid asymmetry can induce morphological changes in which externalization of phosphatidylserine can ultimately lead to rapid thrombin generation and efficient blood clotting (139,141). Exposed phosphatidyserine provides a site for the assembly of coagulant enzymes, suggesting the development of thrombus formation and cardiovascular diseases.

5.4. Fluidity of the Membrane

The biomembrane phospholipid chains show (i) flexing and twisting of the methylene (HCH) groups; (ii) oscillations and rotational disorder of the methylene groups; (iii) mobility of the head groups; and (iv) lipid self-diffusion. The temperature dependent nature of this process lead to the concept of membrane fluidity, [141]. The hydrocarbon chains in the bilayer core are not as disordered as they are in a pure liquid hydrocarbon, and the ΔH values associated with the gel to liquid-crystal transition are lower than the ΔH values for melting of pure hydrocarbons [142]. Thus the core of the bilayer is liquid-like in character. Although membranes consisting of single phospholipid types possess sharp transition temperature, the red blood cell membrane, by virtue of possessing a wide range of phospholipids with hydrocarbon side chains of varying unsaturation (Fig.12), lacks a sharp transition temperature [143,144]. This broad temperature range is probably enhanced by the presence of cholesterol [144,145]. Cholesterol which is inserted rather deeply into the bilayer and in an orientation perpendicular to the bilayer plane [132,146] has a condensing effect on the bilayer fatty acyl chains [147]. At 37°C, the lipid environment of red blood cell membranes are highly fluid, there being appreciable translational motions of individual phospholipid molecules.

As a result, fluid mosaic models of the red blood cell membrane have been proposed [105,133] (Fig.11) which emphasis is placed on the highly mobile character of the individual membrane components in a fluid matrix [143]. Thus, the mobility which is permitted for both phospholipids and intercalated proteins, are as follows:

(i)   Intra-chain motion of fatty acyl chains attached to the glycerol backbone of the phospholipid molecule. The fatty acyl chains are continually being distorted in the fluid state of the bilayer by rotation about C-C bonds, yielding "kinks" in the chain [148]. These are extremely short lived states lasting for only ~ 10^{-9}s [149].

(ii) Fast lateral diffusion within the plane of the bilayer is constrained to the two dimensions of the horizontal plane of the bilayer (Fig.12). The membrane proteins can induce a significant restriction on phospholipids lateral mobility [150,151]. Band 3 has been represented to exhibit lateral diffusion coefficient of 4×10^{-11}cm^{2}s^{-1} at 37°C [152].

(iii) Both phospholipids and Band 3 possess a fast rotational diffusion about an axis which lies perpendicular to the plane of the bilayer (Fig.12). Although the diffusion relaxation for Band 3 is 1000 s^{-1} [153], Golan and Veatch (1980) (1979) have reported that the Band 3 is not immobolised as might be expected if it was anchored to spectrin. Moreover, no change in the rotation was observed when spectrin and actin were removed from the

membrane [155]. Thus, it is more likely that integral proteins are entrapped within the interstices of the spectrin-actin network [106], and no physical linkage is involved.

(iv) 'Flip-flop' process for membrane proteins does not occur due to the obvious thermodynamic difficulties that would have to be overcome in translocating bulky hydrophilic carbohydrates through the hydrophobic core of the bilayer [155,156]. The movement of phospholipid molecules from one leaflet of the bilayer to the other on a 'one-for-one' exchange basis is extremely unlikely [151]. A very slow diffusion could be specifically catalyzed by flippases, floppases or scramblases [157,158].

Spectrin in cooperation with other cytoskeletal proteins is probably essential for mechanical stabilization of the lipid bilayer [143,154]. Also, caveolins and flotillins are an integral membrane protein that has been linked to inner leaflet of the membrane that is forced to curve inward, forming caveolae [159,160,161]. Integrins are also transmembrane proteins of the plasma membrane that facilitates cells attaching to each other and ferries messages between extracellular and intercellular matrices [162,163,164].

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