# Chapter I

# Introduction

# GENETIC PRINCIPLES

Since hereditary principles are involved in many of the bl. disorders.

Every normal human somatic cell contains forty-Six chromosomes. Following fertilization the zygote receives two sets of twenty-three chromosomes, one set from each of the parents, twenty-two of these pairs are known as autosomes. The remaining pair is concerned with sex determination. The normal female has a sex chromosome constitution of xx while the normal male is xy the y chromosome of man appears to carry little specific genetic information other than the determinant for the male phenotype.

The gene may be considered as a small piece or section of chromosome concerned with a specific biochemical function It has been estimated that the total number of genes in a human being is about 42,000 with at least 2,000 of these on the x chromosome , The genes, like chromosomes exist in pairs If a pair of genes are identical the person is homozygous at this locus. If the two genes of a pair are different, the person is heterozygous for this gene pair Alternative forms of a gene which may occur at a single gene locus are called allelomorphs or alleles. Thus an individual with the same allele on each number of the chromosome pair is said to be homozygous for this gene locus. In contrast an individual with two different alleles at the corresponding gene locus of the chromosome pair is said to be heterozygous for that gene locus or a heterozygote. All

the traits or characteristics determined by a gene are probably mediated through the regulation or synthesis of proteins. A curious imbalance that appears to occur in nature is the possession of two x chromosomes by the female as compared with only one in the male, According to the Lyon hypothesis one of the two x chromosomes is in a tightly coiled or condensed state and is functionally inactive. At some point in embryogenesis one of the x chromosomes is turned off. Thus only one x chromosome is active in somatic cells of mammals regardless of the number actually present,. In females the inactive x chromosome becomes heteropyknotic and is termed the Barr body, a localized chromatin particle that is Feulgen positive and thus presumbly contains deoxyribonucleic acid or DNA, the number of Barr bodies is usually one less than the number of x chromosomes.

Most patients with Down's syndrome ( mongolism) have forty-seven chromosomes. The most common abnormality is a trisomy for chromosome 21, that is the occurrence of three chromosomes of a particular pair instead of two Nondisjunction of homologous chromosomes in the maternal germ cells is generally considered to be the basic mechanism by which the majority of affected individuals arise, Specifically, this is due to a failure of the two chromosomes of pair 21 to separate during gametogenesis in the mother, An abnormal ovum is produced and thus a child with trisomy 21. Mongolism can therefore result from either trisomy for chromosome 21

or translocation of chromosomes 13,15 and 22.

Bl. dyscrasias in relation to congenital anomalies:

According to current interpretation, single or coexisting anomalies represent deviations from orderly development induced by a known infection such as rubella or other as yet unidentified agents or a states acting upon the pregnant woman and the placenta if one accepts the principle that for all organs injury is most likely to occur during the stage of active differentiation, the vulnerable period of erythropoiesis would extend from the sixth to the twelfth week, when the liver, spleen and bone marrow are simultaneously involved in the proliferation of erythroblasts. Chronic thrombocytopenia present from birth may be related to suppression of platelet formation from the second month of fetal life when platelet production becomes evident. It is thus apparent that the type of deformity depends upon nonspecific noxious influences operating at " developmental moments " corresponding to periods when organs are in their most rapidly proliferating condition. The infrequent association of severe types of hematologic disorders with other congenital malformations suggests the relative resistance of hematopoietic tissues in comparison with the vulnerability of other systems during critical periods of development. Examples of bl. dyscrasias associated with congenital anomalies include congenital leukemia and mongolism, hypoplastic-aplastic anaemias with skeletal defects ( i-e Fanconi syndrome), sporadic congenital

spherocytosis associated with congenital hypoplastic thrombocytopenia and malformations and congenital factor V ( Labile factor ) deficiency with syndactylism.

## HAEMOPOIESIS

In human embryo bl. cells are first formed in area vasculosa of yolk sac, later they arise from body stalk and placental site, later still from liver, spleen and thymus finally from bone marrow so passing 3 stages : The mesoblastic phase till the 2nd month, the hepatic phase till the 5th month then the myeloid phase.

From the endothelial cells lining the bl. vessels of area vasculosa arise groups of central cells which become free and form in primitive bl. of circulation the free cells become haemoglobinised and become the primitive nucleated red cells ( magaloblasts ) first described by Ehrlich (1977).

In young embryo all haemoglobin ferrous cells are nucleated, but at 8th month all are non nucleated ( Whitby, 1963).

Medullary Haemopoiesis.

Bl. formation after birth is confined under normal conditions to the bone marrow. The bone marrow is either red or yellow, the red marrow is the bl. forming ( haemopoietic) marrow, it owes its colour to the haemoglobin in the developing and mature red cells and also to the bl. presented in the bl. vessels.

The yellow marrow is composed principally of fat, small number of capillaries and reticulum cells these cells are potentially haemopoietic and act as reserve that can be called when there is an increased demand for bl. formation ( Grucly, 1972) At birth the marrow in all the bones of the body appeared red, at the age of 7 years, the marrow of the shafts of long bones become less active and pale red with droplets of fat, at age of 10-14 years, yellow marrow appears at distal end of long bones and gradually extends proximally until the age of 20 years, the entire red marrow of long bones has been replaced by yellow marrow except upper end of femur and humerus, so in adults the red marrow is in bones of the skull, thorax, vertebrae and upper end of femur and humerus ( Whitby, 1963).

Structure of the bone marrow:

It is composed of : (1) Reticulum cells and reticulum fibres that form a network for other marrow elements. (2) The bl. vessels that arise from the nutrient vessels of the bone which break up into number of dilated thin walled sinusoids, they are lined by a single of endothelial cells that form part of the reticulo endothelial system. (3) Haemopoietic cells as the red marrow is the only normal site for formation of red cells granular leucocytes and platelets, the precursors of these cells lie free outside the bl. vessels in between reticulin fibres. (4) Fat cells that can be considered reticulum cells with stored fat ( Gruchy 1972).

Development of red bl. cells:

Traditional theories:

(1) The monophyletic theory:- It was thought that all bl. cells are developed in the bone marrow from only one stem cell called haemocytoblast.

(2) The polyphyletic theory:- It was thought that bl. cells are developed from more than one stem cell. Red cells, granulocytes and megakaryocytes develop in the bone marrow from one stem cell called myeloblast, while lymphocytes and monocytes develop from other stem cells in the lymphatic tissues.

(3) The colony forming unite " CFU " as a stem cell by the recent electron microscopic studies.

It was established that all bl. cells are developed in the bone marrow from one stem cell called colony forming unit. CFU develop from the mesenchymal cells in the bl. islands of the yolk sac of the embryo. They are free bone marrow cells, they are small in diameter than the so called haemocytoblast and myeloblast. The CFU proliferate and differentiate in the bone marrow into:

a) CFU-E Responsible for the formation of the red cells.

b) CFU-G Responsible for the formation of the granular series and monocytes.

c) CFU Responsible for the formation of lymphocytes in bone marrow and thymus.

The CFU-E is responsible also for the formation of the megakaryocytes.

The mature red cells to reach the bl. stream pass through three processes :

(1) Multiplication, takes place by mitotic division, in normal marrow film mitosis can be seen in 1% of new cells but in hyperblastic ones it can be seen in 5% of new cells.

(2) Maturation, progressive development of cell characteristics both structural and functional.

(3) Release into the bl. stream this process is not clearly understood as the bl. vessels of the bone marrow form a closed system, so the red cells must cross the endothelial lining of the vessel wall either by diapedesis or more possibly by temporary rupture of the wall when the pressure of the accumulated cells become great.

Extra Medullary Haemopoiesis : ( Gruchy, 1972)

After birth, the spleen , liver and lymph nodes normally play no part in formation of red cells, However in certain circumstances these organs revert to their foetal role of haemopoiesis, as the reticulum cells retain their potential haemopoietic activity.

The term extramedullary haemopoiesis is applied to bl. formation in organs other than the marrow and organs showing such activity are said to be the site " myeloid

metaplasia". The usual cause of this activity is an increased demand for cells which can not be met by hyperplasia of the marrow alone, most common in infants and children following haemorrhage or haemolysis, may also occur in certain chronic severe anaemia e.g pernicious and haemolytic anaemias and also with bone marrow replacement as myelosclerosis and bone marrow secondary.

## ERYTHROPOIESIS

Comprises these biological processes of cell differentiation proliferation, biosynthetic activities and maturation which provide erythrocytes and hemoglobin in appropriate amount for the respiratory gas transport requirements of the organism ( Douglas 1974).

Colony Forming unit C F U.S

CFU-E.

Pronormoblast ( proerythroblast)

Basophilic normoblastic( basophilic erythroblast)

Polychromatic normoblast ( polyerythroblast)

Late normoblast ( normoblast)

Reticulocyte

Mature normal red cell.

Factors that determine erythropoiesis:

(1) Arterial $O_2$ content - tissue $O_2$ tension:

The major factor controlling the rate of red cell production is the $O_2$ content of the arterial bl. A decrease in $O_2$ content stimulates erythropoiesis while an increase depresses it. $O_2$ content of the bl. may be lowered either by a decrease in the amount of circulating Hb. or by an adequate oxygenation of Hb. e.g. a) haemorrage by causing a fall in circulating Hb. is followed by increase in the rate of R.b.cs production. b) chronic anoxia due to an inadequate oxygenation of bl. such as what occurs at high

altitudes and in certain cardiac and pulmonary disorders. The anoxia acts as stimulatory factor resulting in compensatory polycythemia.

There is also ample evidence of the depressant effect on erythropoiesis of increased $O_2$ tension of the arterial bl. Thus in normal subjects transfusion of bl. induced has been shown to cause depression of erythropoiesis. The degree of depression being related to the increase in red cell mass and thus to the increase in arterial $O_2$ content.

So it can be stated that the rate of erythropoiesis is controlled by tissue tension of $O_2$, this in turn depends on the relation between $O_2$ demand and $O_2$ supply, when $O_2$ demand exceeds $O_2$ supply the tissue tension of $O_2$ is reduced, and erythropoiesis is reduced.

In normal subjects the size of the total R.C. mass of the body is controlled by feed back mechanism between the erythropoietic tissue of the marrow and other tissue of the body.

The rate of red cell production determines the size of R.C. mass, the red cell mass determines the Hb. concentration.the Hb. concentration determines the degree of tissue oxygenation, the degree of tissue oxygenation determines the rate of red cell production ( Erslev, 1960).

(2) Erythropoietin:

( Haemopoietin = Erythrocyte stimulating factor)

The effect of $O_2$ tension on the rate of erythropoiesis is not a direct action of the bone marrow but rather it acts through a substance in the plasma produced in response to $O_2$ lack it is called erythropoietin. Erythropoietin is a low molecular weight glycoprotein its site of origin is equally doubtful, but there is significant evidences to suggest that the kidney is the main site of production or activation :

- Chronic renal diseases are usually associated with anaemia.
- Nephrectomised animals do not show an erythropoietic response when exposed to prolonged hypoxia.
- Renal extracts I.V. injected augment erythropoiesis ( Talaat, 1973).
- Erythropoietin can be extracted from both medulla and cortex it appears to act on the bone marrow primarily by stimulating the differentiation of primitive cells to pronormoblasts, it probably also affects the rate of multiplication and maturation of normoblasts it possibly also affects the rate of haemoglobin synthesis.
- Erythropoietin output is increased by renal hypoxia e.g at high altitudes, in cyanotic heart disease and in chronic lung disease plasma erythropoietin is high in all forms of anaemia except that of renal disease. A rare form of polycythaemia is associated with over

production of erythropoietin from renal tumours or cysts ( Kerr, 1975).

(3) Dietary factor:

In starvation the bl. volume decreases as a part of the general loss of body weight , erythropoiesis, however, continues and the necessary materials are obtained from the disintegrated erythrocytes as well as from the autolysed tissues.

The formation of Hb is given priority to plasma protein in fact plasma proteins are used to supply the amino acids necessary for the synthesis of haemoglobin.

(a) Protein and amino acids:

The protein globin which constitutes 96% of the Hb molecule, contains a number of essential amino acids thus it is obvious that an adequate dietary supply of good quality protein containing these amino acids is necessary for the formation of Hb. in sufficient amounts to maintain a normal concentration in the periperal blood. The food proteins are broken down by proteolytic enzymes the released amino acids are absorbed to enter the amino acid pool of the body to be available for synthesis of tissue, plasma proteins and Hb. Hb has a high priority for available protein and so in states of protein deficiency Hb formation takes the upper hand over tissue and plasma protein. So protein deficiency alone in man does not often act as a

limiting factor in HB synthesis and cause anaemia, very considerable depletion of body stores of protein must occur before interference in Hb production results.

Recent work suggests that adecrease of erythropoietin stimulation of the marrow is an important pathogenic factor in the anaemia in the protein depleted individual as it is a natural consequence of the reduction in $O_2$ requirements.

The fact that the protein deficiency alone is not the only etiological factor in the anaemia of many protein deficient subjects, is suggested by the fact that there may be a lack of correlation between severity of protein deficiency and degree of anaemia, and signs of folic acid and iron deficiency are present in some patients. Patients respond well to high protein diet but signs of iron deficiency may develop during recovery, as a subclinical iron deficiency often pre-exists and is unmasked by increased erythropoiesis so a supplement of iron is recommended as well as folic acid in the treatment of anaemia due to protein deficiency ( Adams et al 1967).

(b) Iron:

Iron is essential to human life because of its central role in the haeme molecule that permits $O_2$ and electron transport, every cell in the body contains some iron as myoglobin, cytochrome, cytochrome oxidase and peroxidase.

Total body iron content of the normal adult varies from 3-5 gm depending on sex and weight, it is distributed in several forms:

(1) Haemoglobin (1.5-3gm ).

(2) Tissue iron : storage tissue iron ( 0.6-1.5gm) and essential tissue iron ( 0.3 gm).

(3) Plasma ( Transport) iron.

Hb. iron is about 60-70% of total body iron so it is obvious that any major bl. loss will significantly lower the total iron content. The iron derived from breakdown of Hb released by destruction of old R.C is reutilised for Hb synthesis.

Tissue iron It may be subdivided into a) storage iron which when needed can be readily mobilised from body tissue for Hb synthesis. b) Essential which in general is not available for Hb synthesis as myoglobin ( iron in muscles) cytochrome, catalase and peroxidase ( iron in enzymes of cellular respiration) and iron present as constituent of the cells.

Storage iron increases in conditions of excessive iron storage as in transfusion haemosiderosis and haemochromatosis and is depleted in iron deficiency anaemia it occurs in 2 forms (1) ferritin (2) haemosiderin present in equal amounts in normal subjects 1/3 of storage iron in the bone marrow, 1/3 the liver and 1/3 in the spleen, muscles and tissues.

(1) Ferritin is colourless and is finally dispersed in tissues where it is not visible microscopically, when present in large quantity it may give a bluish tint to tissues stained by ferrocyanide method. It is composed of apoferritin ( large water soluble crystalline protein surrounding clusters of iron).

(2) Haemosiderin appears as golden yellow in unstained tissues and blue granules with K ferrocyanide, it contains more iron than ferritin and is not water soluble.

Plasma iron ( 3-5 mg of iron).

It is bound to specific protein that is known transferrin ( Siderophilin / each molecule of transferrin binds 2 atoms of ferric ions. Its main function is to transport iron, it carries iron from alimentary tract to the tissue stores, and from them to the marrow and from storage site to another one. Transferrin is present in the serum in a concentration which enables it to combine with 300,360 mg of iron / 100ml this value is known as total iron binding capacity.

(c) Vitamin $B_{12}$ and folic acid:

" The erythrocyte maturation factor are present in normal diet. Vit. $B_{12}$ plays a role in cell metabolism acting as coenzymes in the chemical reactions leading to synthesis of nucleic acid, it is essential for normal haemopoiesis and for maintainance of the integrity of

nervous system. vit. $B_{12}$ requirements are obtained from food mainly those of animal protein origin.

Vitamin $B_{12}$ in man is synthetised only in large bowel but it is not absorbed from that site but is excreted in faeces, thus man depends on dietary sources for his requirements. Absorption of Vit. $B_{12}$ occurs in the ileum, gastric juice of normal person contains a substance called intrinsic factor which acts on substance present in certain foods ( liver) called extrinsic factor ($B_{12}$) to give haemopoietic factor, this factor is absorbed from the ileum and stored mainly in the liver in recent discoveries, $B_{12}$(extrinsic) does not combine with intrinsic factor, but rather it in some way facilitates the absorption of Vit. $B_{12}$ through intestinal wall to blood stream. So the extrinsic factor and haemopoietic factor are identical and are in fact Vit. $B_{12}$ ( De Gruchy, 1972).

The intrinsic factor is secreted by the acid secreting parietal cells of gastric fundus, the $B_{12}$ absorption is an active process and it is calcium dependent following absorption into stream, most of $B_{12}$ is bound to protein ( alpha-globulin) and is carried to body tissues for storage or utilization.

Folic acid Plays an essential role in cellular metabolism and is essential for normal haemopoiesis. It is mainly absorbed from duodenum and jejunum and the body takes its requirements from naturally occuring folic acid

in food, it is mostly present in the form of polyglutamates to be available for tissue metabolism it must be splitted off to be free. Folic acid acts as catalyst in the chemical reactions leading to synthesis of nucleic acids ( desoxyribose n.a)and is also important in the metabolism of mucosal cells of the gut.

(d) Vitamines: ( Pyridoxin, Riboflavin and Vit.C):

Pyridoxal 5-phosphate is necessary for haemosynthesis so its deficiency results in a severe hypochromic anaemia, in man an abnormality of pyridoxin metabolism has been demonstrated in the sideroblastic anaemia. Riboflavin is necessary for normal erythropoiesis so its dificiency may produce normocytic normochromic anaemia with reticulocytopenia associated with marrow erythroid hypoplasia. Vit. C appears to be concerned with erythropoiesis it plays a role in folic acid metabolism, anaemia may occur usually with scurvy.

(e) Hormones:

Thyroxine stimulates the metabolic process of bone marrow, in myxoedema, anaemia occurs and responds most effectively with thyroxin.

Although anaemia has not been widely appreciated as a complication of primary hyperparathyroidism, 5.1% of the individuals with this disorder has a normocytic normochromic anaemia that could not be related to blood loss, a

deficiency state, or uraemia the anaemic group has more advanced bone disease and higher levels of serum calcium alkaline phosphatase and parathyroid hormone than the non anaemic group of patients showed variable degrees of myelofibrosis.

Removal of the abnormal of parathyroid glands lead to improvement or correction of the anaemia ( Micheal Boxer 1977).

Cation influcences on in vitro growth of erythroid stem cells ( CFU-E and BFU-E) ( Murphy 1983).

Recent developments have focused on long term bone marrow cultures and serum free media to establish clonal hematopoiesis in vitro. Prior investigation have suggested an important role for such ingredients as transferin, lipid, selenite and potassium provided by serum these factors when present can support clonal growth even in the absence of serum. Results reported here further define the importance of the cations $K^+$ and $Li^+$ in the regulation of in vitro erythropoiesis in the presence of both normal and dialyzed fetal calf serum and ouabain while $K^+$ proved essential for optimal erythroid colony formation. The presence of $Li^+$ in such cultures reduced optimal colony formation of erythroid stem cells this evidence suggests that in long term marrow cultures or serum free media $K^+$ is essential for successful maintenance of self renewal of erythroid stem cells and their differentiation and that the monitoring of $K^+$ levels may prove useful in such

cultures. Since in vitro erythropoiesis was reduced in the presence of $Li^+$ the mechanisms controlling differentiation of haematopoietic stem cell may be interpreted to be subject to cation influence.

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## HEMOGLOBIN

The unique metabolic property of erythrocyte precursors is the synthesis of the iron containing $O_2$ carrying protein hemoglobin. Human Hb has a molecular weight of 66,700 and is readily divided into two components:

(1) An iron binding prosthetic group, heme and

(2) A globin moiety.

- Heme constitutes about 4% of the weight of the Hb

  It consists of an atom of ferrous iron bound to a porphyrin ( tetrapyrrole) nucleus.

- Globin, Normal and abnormal hemoglobins vary in the globin moiety of hemoglobin.

Normal adult Hb (HbA) contains two pairs of polypeptide chains, designated $\alpha$ and B respectively and can be referred to as $\alpha_2 B_2$. In fetal Hb (HbF) the second pairs of chains differs from B and has been designated $\gamma$ . HB F therefore is $\alpha_2 Y_2$ . The Hb of normal human adults is heterogeneous and contains a minor constituent $A_2$ which contains two polypeptide chains different from B or Y and designated $\delta$ $HbA_2$ therefore is $\alpha_2 \delta_2$. The $\alpha$ chain contains 141 amino acid residues the B, Y and $\delta$ 146 each.

Hemoglobin Concentration:

From the 25th to 36th week of fetal life the Hb concentration appears to be relatively constant at about

of full term infants at birth are higher and vary more widely. Values from 11. 2 - 26.6 gm/ 100 ml have been reported but 95 per cent of Hb values fall between 13.7 and 20.2 g/100ml and it seems reasonable to take 13.5 g/ 100ml as the lower limit of the normal range . The mean Hb concentration of cord blood is 16.8 g/ 100 ml.

Prefetal hemoglobin:

Whereas in fetuses the main Hb is Hb F. in embryo three hemoglobins ( Gower I Gower 2 and Portland 1 ) Predominate.

Fetal hemoglobin:

Although both fetal and normal adult hemoglobins can be demonstrated to be heterogenous, the specific difference between the two lies in the sequence of animo acids in the second pair of peptide chains . The genetic deternimants for the synthesis of HbF are different from those of the hemoglobinopathies persistence of HbF throughout life occurs as a rare hereditary anomaly.

Hb F is also synthesized in patients with severe anaemia. both congenital and acquired that date from early life such as congenital spherocytosis sickle cell anaemia and particularly thalassaemia major. The postnatal formation of fetal Hb in these patients is compensatory rather than a genetically determined abnormality.

The time course of the transition from HbF to HbA production in the postnatal period is based on the time dependent pattern of total Hb and of HbF observed in an 8 months follow up of 25 premature newborns. The absolute amount of HbF decreases exponentially from birth to approximately 25 weeks ( from 15.56 ± 0.48 g/dl to 0.66 ± 0.08 g/dl) at a weekly rate of approximately 16% On the other hand the HbF shows a biphasic pattern: first it decreases slightly from birth to 6 weeks ( from 4.09 ± 0.32 gm/ dl to 2.63 ± 0.33 g/dl) afterwards it increases export exponentially at a weekly rate of completely 5% up to a plateau value until HbF is almost completely replaced by HbA.

A slowly moving Y chain variant was discovered in the cord bl. of a baby. The abnormality ( HF. Banaire-Ga) concerned the substitution of Gln residue in position 39 ($C_5$) of the A.Y chain by an arg residue resulting in an-Arg - Arg sequence at pesitions 39-40 the quantity of the AH chain variant was nearly 10% of the total HbF with 15% of the HbF having normal (A) Y chains and 75% of the HbF having (G) Y chains. High pressure liquid chromatographic and microsequencing methods greatly facilitated the structural analysis.

Adult hemoglobin (Hb A)

In this type the 2 polypeptides are called the $\alpha$ chains each of which contains 141 amino acid residues. Not all the Hb in the blood of normal adults is (HbA). about 2-5% of the Hb is ( $HbA_2$) in which B chains are replaced by $\delta$ chains. The $\delta$ chains also contain 146 amino acid residues but 10 individual residues differ from those in the B chain. Normal adult Hb ( HbA) is rapidly denatured by an alkali like potassium hydroxide so that it can not combine with $O_2$ any longer. Determination of HbA (IC) provides an integrated index of glycemic control in diabetic patients during the previous 6 to 8 weeks However interference in HbA (IC) assays from a labile glucosylated Hb fraction the concentration of which is strongly correlated to the actual blood glucose level has been described. It has been reported that the labile Hb compound is Co-determined in all chromate graphic assays and that this interference can be eliminated by a saline incubation of erythrocytes or dialysis of haemolysate before quantitation.

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## RED CELL METABOLISM

The metabolism of the red cell is more limited than that of most body cells. Since there is little ability to metabolise fatty acids, amino acids and no mitochondrial apparatus for oxidative metabolism.Energy is generated almost through break down of glucose.

Red cell metabolism may be subdivided into the anaerobic ( Embden Meyerhof) pathway and three ancillary pathways which act in different ways to maintain the function of hemoglobin.

The Embden Meyerhof Pathway:

Is responsible for about 90% of the red cell glucose utilization and provides essential energy for membrane maintenance. In the breakdown of a molecule of glucose to lactate, 2 moles of ATP are consumed during the hexose portion of the pathway and 3-4 moles are generated at the triose level. It is this net gain in ATP which provide high energy phosphate for maintenance of the disc shape and flexibility of the red cell, for maintenance of membrane lipids and for energizing the metabolic pumps controlling sodium and potassium flux the essential role of ATP in the red cell is demonstrated in at least 2 conditions a) early cell death ( hemolytic anaemia) which occurs when ATP is deficient due to inherited defects in glycolysis b) the loss of viability accompaning ATP depletion of bl.

Stored in Vitro ( Dern et al. 1967).

This pathway also maintains the pyridin nucleotides in a reduced state to permit their function in oxidative reductive haemostasis within the cell.

The oxidative pathway : or hexose monophosphate is an ancillary energy system which couples oxidative metabolism with pyridine nucleotide ( TPN and NADP) and glutathione reduction. The activity of this pathway increases with increased oxidation of glutathione, when the pathway is functionally deficient the amount of reduced glutathione becomes insufficient to neutralise oxidants causing globin denaturation and precipitation as aggregates ( Heinz bodies) within the cell these masses attach themselves to the cell membrane and are ultimately removed by R.E. cells within the spleen along that portion of the red cell membrane to which they are attached, if the process inflicts sufficient membrane damage, the cell is destroyed a severe enzyme defect in the oxidative pathway may in itself cause a chronic haemolytic anaemia.

The methemoglobin reductase pathway:

Is another important component of red cell metabolism. Just there is a mechanism for protecting hemoglobin against oxidative denaturation, so there is need to prevent the oxidation of heme iron Methemoglobin which results from the conversion of the bivalent iron of heme to the trivalent form can no longer combine reversibly with oxygen

and oxygen transport function is lost maintenance of heme iron in a functional state ( $Fe^{++}$) requires the reduction action of the pyridine nucleotide ( DPN and NAD) and the enzyme Methemoglobin reductase.

In the absence of this system about 2% of circulation hemoglobin is oxidised daily until 20-40% methemoglobin is present within the cell.

Luebering - Rapaport pathway:

By passes the direct formation of 3PG from 1,3 DPG and permits the accumulation of 2,3 DPG the apparent reason for the large amount of this compound found in the red cell lies in its profound effect on the affinity of hemoglobin for $O_2$ so the red cell DPG is essential for maintaning the basal $Po_2$ at a level suitable for $O_2$ transport ( Oski - Gottlieb 1971).

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## RED CELL BREAKDOWN

The normal red cell has a definite life span in circulation of 120 ± 20 days . As the cell becomes older, certain glycolytic enzymes decrease in activity membrane is lost, the mean hemoglobin concentration increased and cell pliability decreased, when these changes have reached a critical point, the red cell is no longer able to traverse the microvasculature and is phagocytosed by the reticuloendothelial ( R.E.) tissues. While all R.E cells participate in the destruction of old red cells, those in the spleen are so situated anatomically to be the most sensitive detectors of a red cell abnormality. Blood enters the reticular mesh-work of the splenic red cell pulp through terminal arterial branches, blood flow is slow and volume of plasma is reduced exposing the red cell to the phagocytic action of R.E. cells ( weiss 1972).

Intact red cells return to circulation via the venous sinusoids , where the pliability is tested by the small sinusoidal orifices, there, abnormal particles are removed along with some cell membrane the quality control which the spleen exerts on the circulating red cell mass is evident from the increase in circulating abnormal forms after splenectomy including nuclear remnants ( Howell Jolly bodies) denaturated Hb inclusions ( Heinz bodies) Siderocytes and misshaper or fragmented cells ( Roberts, et al, 1974).

Haemoglobin breakdown:

When the senile red cells undergo phagocytosis their Hb is released and then broken down inside the phagocytes. The protein globin is split off from the haem and the haem is then broken into its constituents iron and porphyrin. The globin and its amino acids are reutilized. The iron passes into the blood stream where it combines with the iron binding protein and is carried either to the marrow for reutilization in Hb synthesis or to the body iron stores.

The porphyrin is not reutilised but is broken down through series of intermediate compounds to bilirubin. The bilirubin passes into the plasma to form loose complex with albumin that is taken by the liver to be separated from albumin and is conjugated with glucoronic acid to form bilirubin glucoronide ( conjugated bilirubin) that is excreted by the liver into the bile ducts so there are 2 types of bilirubin, the prehepatio ( non conjugated) bilirubin it is impermeable to the glomeruli and does not appear in the urine, and the posthepatic ( conjugated) bilirubin that is permeable to glomeruli and appears in the urine, the conjugated bilirubin passes via bile ducts to the intestine where it is reduced to stercobilinogen by the bacterial flora of the colon, part of stercobilinogen is absorbed to the liver through portal circulation this is called the enterohepatic circulation of the bile pigments. A small quantity of the stercobilinogen passes to the systemic blood stream to be

excreted by the kidney as urobilinogen. In the air both stercobilinogen and urobilinogen are oxidised to stercobilin and urobilin ( De Gruchy 1972).

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## RED CELL KINETICS

- Splenic erythrocyte pooling:

Red cells accumulate in the spleen in asplenic erythrocyte pool which increases significantly with increasing splenomegaly and which may constitute up to 50 per cent of the total red cell volume. Red cells in the splenic erythrocyte pool are in dynamic equilibrium with circulating red cells red cells being passively accumulated in the spleen so that the erythrocyte content of the spleen depends on the red cell concentration in the circulating blood.

- Splenic hyperhaemolysis:

Red cell sequestration ( destruction) in the spleen increases with increasing splenomegaly, and in cases of myelofibrosis with predominant splenomegaly ( splenic volume > 1000ml) it averages more than twice the normal red cell production.

Expansion at the total plasma volume

The increment increases with increasing splenomegaly After splenectomy the plasma volume usually returns to normal within a period of about 30 to 60 days At the same time the venous Hb concentration rises due almost exclusively to a reduction in the plasma volume.

An important result of red cell kinetic clinical studies is that red cell destruction in the enlarged spleen in myelofibrosis usually exceeds the splenic extramedullary red cell production. From a red cell kinetic point of view then, splenectomy is indicated in M.F.Predominated by splenomegaly and anaemia, as this operation is followed by a decreasing tendency to anaemia and a reduced requirement for transfusions. On the basis of the results of red cell kinetic studies and of clinical characteristics, patients with splenomegaly may roughly be divided into two main categories:

(1) Patients characterised by a near normal total erythropoietic capacity ( in iron kinetic studies only moderately reduced recovery percentage) with moderate anaemia without any requirement of transfusions and due mainly to expansion of plasma volume ( haemodilution anaemia). After splenectomy the venous Hb concentration rises parallel to the reduction in plasma volume within 30 to 60 days. Then follows a slower rise which may be attributed chiefly to an increase in total red cell volume when splenic hyperhaemolysis ceases.

(2) Patients characterised by a distinct reduction of the total erythropoietic capacity ( maximum recovery percentage $< 20$) with severe anaemia, often with a marked requirement for transfusions, splenectomy is followed by a short-lasting increase in venous Hb

concentration parallel to a reduction in plasma volume. Therafter, the venous Hb falls slowly, due to a slow reduction of total red cell volume caused by a still insufficient erythropoiesis.

In this category the requirement for transfusions is reduced after splenectomy.

In concluding this chapter it should be emphasised that in patients with myelofibrosis, anaemia and splenomegaly splenectomy is generally indicated from a red cell kinetic point of view.

Irradiation of the spleen is usually insufficient, time consuming and to the patient, a stressful treatment However, the operative risk has to be considered individually, According to the literature, splenectomy on patients with MF and splenomegaly carries a relatively high operative mortality ( strumia, strumia and Bassert 1966) quite high in the case of very large spleens. ( Christensen 1970) therefore early splenectomy is advisable in cases of increasing splenomegaly and anaemia. The most common operative and postoperative complication are haemorrhage from the splenic bed and infections, usually in pulmonary focus in the most serious cases leading to septicaemia and circulatory failure, the incidence of these complications may be reduced by centralising and systematizing the splenectomies. In principle, splenectomy in haematological patients is a difficult operation which should be performed only by specialised surgeons.

## PHYSIOLOGY OF RED BLOOD CELLS

The red blood corpuscle is a non nucleated cell shaped as flat biconcave disc. It is flexible and can be readily distorted e.g in passage through small capillaries but it quickly resumes its normal shape.

It may be regarded as a frame work of protein known as stromatin in which is contained a heavily concentrated solution of haemoglobin and at its outer surface is the red cell membrane.

The red cell membrane:

It is thought to be consisted of bimolecular leaflet of phospholipids covered externally and internally by a layer of protein, the phospholipids are the predominant lipids in the red cell membrane but unestrerified cholesterols are also present in large portion, small amounts of glycolipids, glycerides, phosphatidic acid, polyglycerol phosphatides and free fatty acids are also found .

The quantity and nature of red cell membrane lipids affect the physical characteristics of the membrane both passive cation permeability and mechanical flexibility of the red cell have been shown to be influenced by changes in the lipid composition of the membrane ( Cooper et al, 1975).

The mature human erythrocyte does not synthesize fatty acids donovo. However, during its four months life the membrane lipid is continuously remodelled and new lipid is incorporated from the plasma by both active and passive mechanism. The red cell membrane contains approximately 50% protein. Many investigators believed that the protein matrix of the red cell consisted of a single structural protein which was insoluble in aqueous media.

Functionally the membrane is semipermeable it is permeable to water and to a less extent to anions it is impermeable to Hb and selectively permeable to cations. An important component of the red cell membrane are the bl. group antigens. They are mucopolysaccharides in nature and are situated on the external surface. The surface of the cell carries a negative charge, following haemolysis and rupture of the membrane and escape of the hemoglobin, the remaining stromal frame work retains the shape of the original cell.

Chemical Composition:

Analysis of the red cell shown that it is composed of 60% water and 40% solid dry substances the dry substances are 90% Hb and 10% stroma, there is no turnover of Hb in the mature red cell, the Hb present when the mature cell is formed lasting throughout the life of the cell. However some constituents as phospholipid and cholesterol, appear to be continuously exchanged between red cell and plasma.

Haemoglobin: is a conjugated protein consisting of a red pigment haem and colourless protein globin, four molecules of haem being attached to each molecule of globin.

Haem: is composed of protoporphyrin and ferrous iron ( Porphyrin iron Complexes), the basic structure unit of all porphyrins is the porphin nucleus which is made up of pyrole rings connected by methene linkages (-ch.) to form large ring, the protoporphyrin is one of the 3 main naturally occuring porphyrins ( protoporphyrin, coproporphyrin and uroporphyrin).

The protoporphyrin contains 3 methyl groups 2 vinyl and 2 propionic groups, this compound is widely distributed in the body, in addition to forming the pigment moiety of haemoglobin and myoglobin, it also forms that of the important respiratory substances cytochromes and catalases.

Protoporphyrin is synthesized in the body through series of intermediated from relatively simple substances glycin and acetate. Free protoporphyrin is present in red cells ( normal values ranging from 20-40 mg/100ml) (Gruchy 1972).

Globin: is formed from amino acids and contains all the essential, and a number of non essential amino acids the molecular weight of Hb is 64, 458., globin making up about 96% and haem 4% of the molecule.

Normal human Hb exists in two main forms mainly adult and foetal Hb which differ slightly in the structure of their Hb moeity, however the haem is identical in all types and they all act as efficient oxygen carrying pigments. The Hb molecule is composed of equal parts in each half there are 2 different polypeptide chains. In normal adult haemoglobin the peptide chains are known as alpha and Beta chains, each alpha chain consists of 141 amino acids and each B-chain of 146 amino acids. Normally occuring haemoglobin types are 3 in number (1) Adult haemoglobin (A) composed of 2 alpha and 2 Beta chains ($\alpha_2$ $B_2$ ) in adults practically all haemoglobin is of this type.

(2) Foetal haemoglobin (F) composed of 2 alpha and 2 gamma chains ( $\alpha_2$, $\gamma_2$) this is the predominant type in foetus at birth red cells contain from 10-30% adult Hb and 70-90% foetal Hb, after birth this proportion progressively decreased until the age of 6 months foetal haemoglobin has usually disappeared.

(3) a minor adult haemoglobin ($A_2$) composed of 2 alpha and 2 delta chains, it is present normally in small quantities.

<u>Abnormal haemoglobin types:</u> in addition to the 3 normally occuring types, a large number of abnormal types have been described these includes haemoglobins C,d,E,G,H,I,J,K,L,M, N,P, and S ( sickle cell). ( Lehmann H. Huntsman 1966).

The various Hb types differ in their physiochemical properties, there are 2 main methods used in routine clinical laboratory work for their identification:
I. Electrophoresis and II. determination of the rate of denaturation in an alkaline medium.
Electrophoresis: the two most commonly used techniques are paper and cellulose acetate electrophoresis in addition agar gel electrophoresis is of value in differentiation between haemoglobin E,O and D from Hb-S, so it is of help in distinguishing between sickle cell anaemia and sickle cell thalassaemia. Alkali denaturation for detection of Hb-F that is resistant to denaturation by alkali.

The stroma of the red cell consists mainly of protein (40 - 60%) and of lipid ( 10-20%) the lipids are chiefly phospholipids but cholesterol both free and ester forms and neutral fat are present.

Potassium is the chief electrolyte of the cell, its content being about 6 times that of sodium.

Glucose and the intermediate compounds in the breakdown of glucose to lactic acid are present and the organic phosphate is largely in the form of 2,3 diphosphoglycerate.

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## FUNCTION OF RED CELLS

The primary role of the red cell is to transport oxygen a function made possible by the Hb within the erythrocytes so the fundamental object of an Hb determination is therefore to estimate oxygen combining capacity of the blood the results of this capacity are expressed as grams of Hb/100 ml of blood.

In man the bl. contains Hb in 2 forms oxy Hb and reduced Hb, both of these compounds are fully capable of carrying oxygen to the tissues and can be considered as active Hb, but other compounds derived from these compounds may be also found in the blood but are inactive as they are incapable of carrying $O_2$ for example methaemoglobin and carboxy Hb, they are potential oxygen carries as they can retransformed into oxy-Hb in the capable of reconversion.

The amount of $O_2$ delivered by fully oxygenated blood is dependent not only on the Hb content and rate of blood flow, but also on the affinity of the Hb for $O_2$ at existing $O_2$ tensions, the effectiveness of Hb in $O_2$ transport is the result of its variable $O_2$ affinity, properly depende it on the tetrameric. Structure of Hb molecule, as $O_2$ is bound successively to each of the 4 iron atoms, streochemical changes occur in the Hb molecule that cause an increased affinity of the remaining iron atoms for $O_2$,

pure Hb has too high an affinity for $O_2$ to release appreciable amounts at normal tissue $O_2$ tensions.

Within the red cell, the $O_2$ affinity of the Hb is modified by several mechanisms : Hydrogen ions stabilise the deoxy state of Hb so lessening the $O_2$ affinity carbon dioxide binds to Hb and decreases the affinity to $O_2$ both these reactions facilitate the release of $O_2$ in the tissue capillaries.

Certain phosphate compounds within the red cell significantly reduce the $O_2$ affinity of Hb, of these is 2,3 diphosphoglcerate ( D.P.G.) that occurs in high concentration and is most important in regulation of $O_2$ dissociation, the deoxyconformation of Hb facilitates D.P.G. binding to the beta chains, stabilising this conformation and lessening $O_2$ affinity.

Hb. F binds less D.P.G. than Hb.A, therefore $O_2$ affinity of red cell containing Hb-F is higher than that of normal adult red cell even though the $O_2$ dissociation curves of both Hb F and Hb-A are similar Each red cell produces its own D.P.G. as an intermediate in glycolytic metabolism, the concentration of D.P.G. within the red cell is markedly influenced by the environment of the erythrocyte, anaemia or anoxia causes the concentration of D.P.G. within normal red cell to rise, intrinsic disorders also may change the D.P.G. concentration as in erythrocyte pyruvate kinase deficiency the D.P.G. is usually elevated

when the concentration of D.P.G rises $O_2$ affinity is lessened. So that more $O_2$ is released in the tissues without a change in the tissue $O_2$ tension. In persons acclimatized to low $O_2$ tension ( high altitude) the D.P.G. concentration is high assuming that relatively high amount of $O_2$ are released at tissue tension even though the arterial blood is not fully saturated.

The remarkably ability of patients with chronic severe anaemia to tolerate extremely low concentration of Hb in the blood is largely attributable to the high values of D.P.G. in the erythrocytes, permitting release of greatly increased amounts of $O_2$ per cell at normal tissue $O_2$ tensions ( Haris & Kellermeyer, 1970).

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## RED CELL VALUES IN INFANCY AND CHILDHOOD

### The first two weeks

During the first few hours after birth the Hb. concentration of the infants blood rises by about 15-20% over that of the cord blood. This increase is mainly due to the transfer of blood from the placenta to the infant before the umbilical cord is clamped. At delivery the placenta contains about 125 ml of bl. or 33% of the total blood volume. If blood is allowed to drain from the placenta into the infant as completely as possible before the cord is clamped, the infant may receive a transfusion is complete within three minutes of delivery and no further transfer of blood takes place if clamping the cord is delayed longer. Within the first four hours of postnatal life fluid is lost from the blood and the plasma volume may contract by 24% the loss of plasma produces haemoconcentration which is shown as an increase in Hb. concentration and haematocite though the total red cell volume is unaltered. At four hours of age the mean Hb concentration of capillary blood is between 19-21 gm/ 100ml with haematocrite of 60-66%.

After this initial increase the Hb concentration begins to fall but it remains high during the first week of life and does not fall below that of the cord blood until sometimes between the first and third week of life. The values in both arterial and capillary blood differ

significantly from that in venous blood during the first week of life.

These for arterial blood are usually 0.5 gm/ 100ml higher than those for venous blood whilest values for capillary blood. Obtained by heal prick, may be up to 6gm, higher . A mean of 20.3gm / 100ml for capillary blood Hb during the 1st hour of life compaired with 16.7 gm/ 100ml.

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Table I

Normal haematological values during the first twelve weeks of life

Hb = Haemoglobin concentration

R.C. = red cell count ( electrononic counting)

PCV= Packed cell volume

MCV = mean cell volume

MCHC = mean corpuscular haemoglobin concentration

From Matoth, Y. et al. (1971) . Acta paediatrica stockholm 60,317-23 .

| Age. | Number of cases | Hb gm/100ml ± SD | Hb gm/100ml ± SD | PCV% ± SD | MCV H.± SD | MCHC% ± SD |
|---|---|---|---|---|---|---|
| days | | | | | | |
| 1 | 19 | 19.0 ± 2.2 | 5.14 ± 0.7 | 61 ± 7.4 | 119 ± 9.4 | 31.6 ± 1.9 |
| 2 | 19 | 19.0 ± 1.9 | 5.15 ± 0.5 | 60 ± 6.4 | 115 ± 7.0 | 31.6 ± 1.4 |
| 3 | 19 | 18.7 ± 3.4 | 5.11 ± 0.7 | 62 ± 9.3 | 116 ± 5.3 | 31.1 ± 2.0 |
| 4 | 10 | 18.6 ± 2.1 | 5.00 ± 0.6 | 57 ± 8.1 | 114 ± 7.5 | 32.6 ± 1.5 |
| 5 | 12 | 17.6 ± 1.1 | 4.97 ± 0.4 | 57 ± 7.3 | 114 ± 8.9 | 30.5 ± 2.2 |
| 6 | 15 | 17.4 ± 2.2 | 5.00 ± 0.7 | 54 ± 7.2 | 113 ± 10.0 | 32.2 ± 1.6 |
| 7 | 12 | 17.9 ± 2.5 | 4.86 ± 0.6 | 56 ± 9.4 | 118 ± 11.2 | 32.0 ± 1.6 |
| weeks | | | | | | |
| 1-2 | 32 | 17.3 ± 2.3 | 4.80 ± 0.8 | 59 ± 8.3 | 112 ± 15.0 | 32.1 ± 2.9 |
| 2-3 | 11 | 15.6 ± 2.6 | 4.20 ± 0.6 | 46 ± 7.3 | 111 ± 8.2 | 33.9 ± 1.9 |
| 3-4 | 17 | 14.2 ± 2.1 | 4.00 ± 0.6 | 43 ± 5.7 | 105 ± 7.5 | 33.5 ± 1.6 |
| 4-5 | 15 | 12.7 ± 1.6 | 3.60 ± 0.4 | 36 ± 4.8 | 101 ± 8.1 | 34.9 ± 1.6 |
| 5-6 | 10 | 11.9 ± 1.5 | 3.55 ± 0.2 | 36 ± 6.2 | 102 ± 10.2 | 34.1 ± 2.9 |
| 6-7 | 10 | 12.0 ± 1.5 | 3.40 ± 0.4 | 36 ± 4.8 | 105 ± 12.0 | 33.8 ± 2.3 |
| 7-8 | 17 | 11.1 ± 1.1 | 3.40 ± 0.4 | 33 ± 3.7 | 100 ± 13.0 | 33.7 ± 2.6 |
| 8-9 | 13 | 10.7 ± 0.9 | 3.40 ± 0.5 | 31 ± 2.5 | 93 ± 12.0 | 34.1 ± 2.2 |
| 9-10 | 12 | 11.2 ± 0.9 | 3.70 ± 0.3 | 32 ± 2.7 | 91 ± 9.3 | 34.3 ± 2.9 |
| 10-11 | 11 | 11.4 ± 0.9 | 3.70 ± 0.4 | 34 ± 2.1 | 91 ± 7.7 | 33.2 ± 2.4 |
| 11-12 | 13 | 11.3 ± 0.9 | 3.70 ± 0.3 | 33 ± 3.3 | 88 ± 7.9 | 34.8 ± 2.2 |