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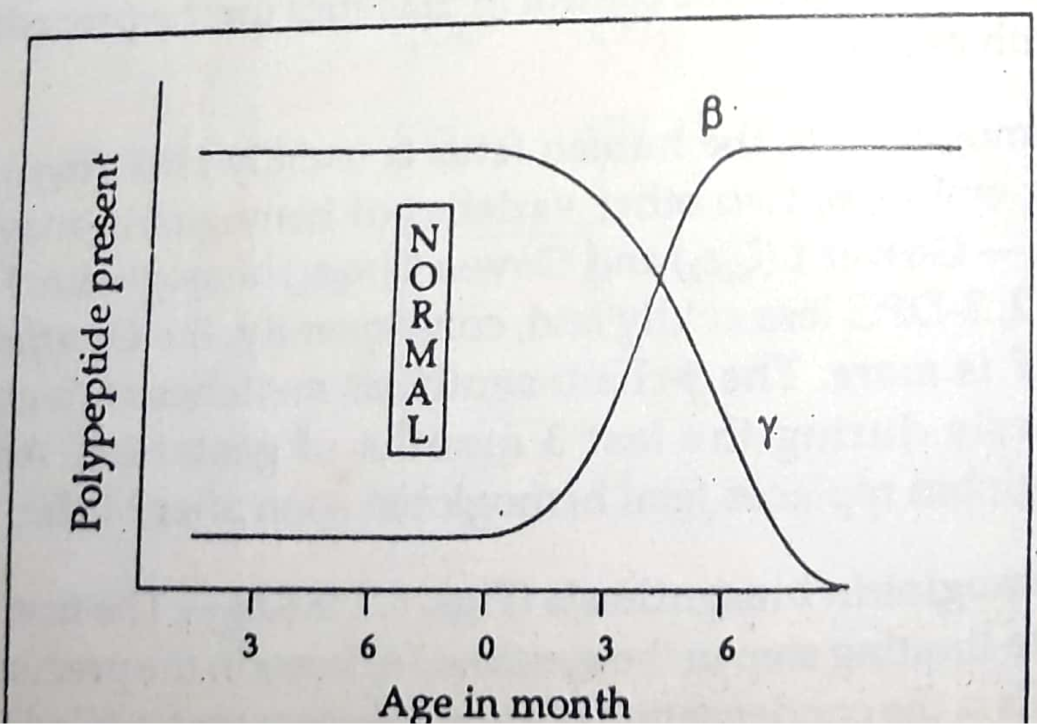
Hemoglobin abnormalities — Inherited disorders of hemoglobin in humans result either in defect in the structure of hemoglobin or in quantitative diminution in hemoglobin (globin chain, to be specific) production.

Qualitative defects in globin chains are referred to as **hemoglobinopathies. Sickle cell anemia** occurs due to the presence of an abnormal hemoglobin called **HbS**. In HbS, the glutamic residue at the 6th position of the β-globin chain is replaced by valine because of a genetic defect in the β-globin gene. HbS polymerizes at low O_2 concentrations producing long crystals of HbS and, thereby, deforming the RBCs to assume a sickle shape. Sickle cells are readily destroyed in spleen and they may occlude the capillaries. This results in hemolytic anemia and vasoocclusive signs and symptoms. Individuals heterozygous for the HbS gene have less severe disease (sickle cell trait). HbS confers resistance to falciparum malaria.

Quantitative decrease in globin chain production may also occur due to genetic defects giving rise to diseases referred to **as thalassemias**. In *α-thalassemia, production of α-globin chain is reduced* and *in β-thalassemia, production of β-globin chain is deficient*. When the deficiency is absolute, the thalassemia is referred to as major **(thalassemia major)**. In **thalassemia**



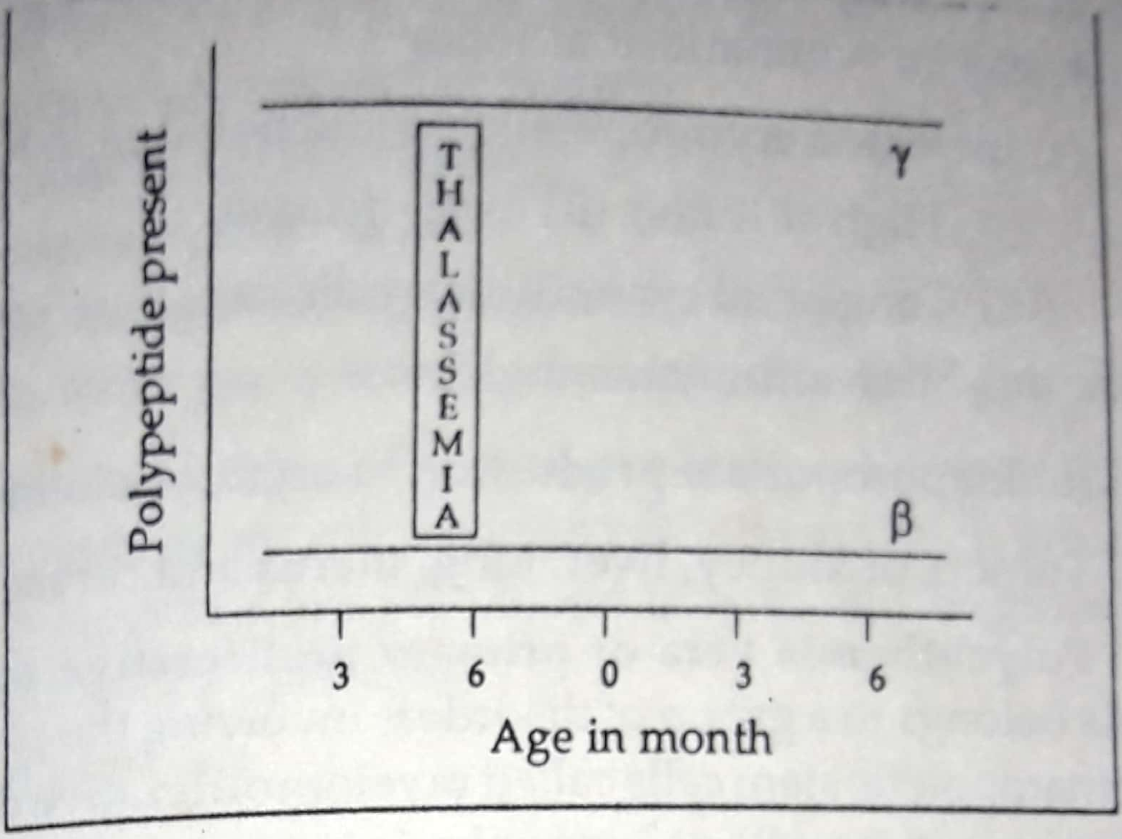


Fig. 6.12: Absence of β to γ switchover in case of β-thalassemia. Compare with Fig. 6.11.

minor, the deficiency is partial. **Thalassemia major** is found in individuals homozygous for the genetic defect and thalassemia minor in heterozygous individuals. In normal fetal development, dominant pattern of fetal hemoglobin (HbF, $\alpha_2 \gamma_2$) is gradually switched over to adult hemoglobin (HbA, $\alpha_2 \beta_2$) due to changing pattern of synthesis of β and γ chains (Fig. 6.11). This switch over takes place between 6 months of intra-uterine life to 6 months after birth. The intersection of the synthetic curve takes place at around $1\,^1/_2 - 3$ months postnatal (Fig. 6.11).

In case of thalassemia (say β thalassemia), due to moderate to severe deficiency of β chain synthesis, this switchover does not take place and, in affected infants, HbF concentration remains, high and HbA concentration remains low (Fig. 6.12).

In sickle cell anemia, an abnormal hemoglobin called *hemoglobin S (HbS)* is produced due to substitution of the glutamic acid residue at position 6 of β-globin chain by valine. At low oxygen concentration, HbS polymerizes to form long crystals that alter the shape of the RBCs to make them sickle-shaped. Sickle-shaped RBCs are easily ruptured in the capillaries and may block the capillaries. **In thalassemias, *production of globin chains is deficient*.** Quantitative deficiency of α-chain production leads to α-thalassemia while in β-thalassemia, β-chain production is deficient. *When the deficiency is complete*, the thalassemia is designated as **"major"**.

The globin chains that cannot combine to form hemoglobin are precipitated within the RBCs forming inclusion bodies that damage the RBC membrane rendering them vulnerable to rupture during passage through the capillaries as well as to phagocytosis by macrophages. **Erythroblastosis fetalis** is a condition in which antibodies from Rh-negative mother (sensitized by Rh-positive RBCs from a previous pregnancy with a Rh-positive fetus) bind to Rh-positive RBCs of the fetus and lyse them leading to hemolytic anemia (Fig. 6.13b).

Significant jaundice may be produced due to overproduction of bilirubin and may lead to **kernicterus** (deposition of bilirubin in the CNS). A large number of blast cells of erythroid series are found in the fetal blood due to rapid production of RBCs. In **immunohemolytic anemia,** hemolysis occurs by immune mechanisms, eg, transfusion reactions, drug reactions etc. In malaria, a parasite called plasmodium grows inside RBCs and ultimately comes out by lysing the RBCs.

Reticulocyte count in peripheral blood (normally $<$ 1% of RBCs) is an useful laboratory test to distinguish between underproduction of RBCs from hemolysis. **A significant rise of reticulocytes in peripheral blood is, in most cases, suggestive of hemolysis.** Unconjugated hyperbilirubinemia is another useful guide to the presence of hemolysis.

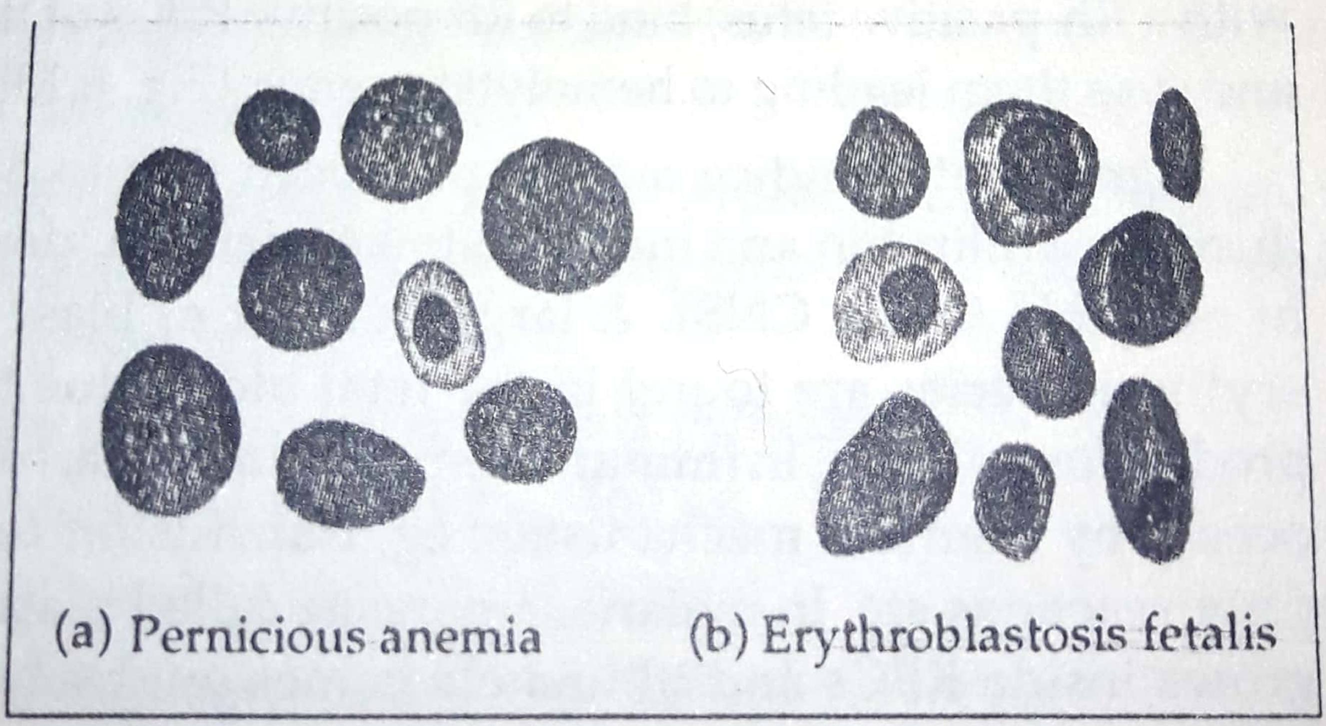


Fig. 6.13 : Peripheral blood picture in — (a)Pernicious anemia (one form of megaloblastic anemia); (b) Erythroblastosis fetalis

BLOOD VOLUME

Definition. The term **blood volume** means the total amount of blood in circulation, as well as in the blood stores.

Normal blood volume. It can be expressed in two ways: (*a*) **In relation to body weight**—78 to 97 ml (average 90 ml) per Kg of body weight; or about 1/11th (9%) of the total body weight. That of plasma is 50 ml per Kg body wt. or 1/20th (5%) of the total body weight. (*b*) **In relation to body surface**—2·5 to 4 litres (average 3·3) per sq. metre of body surface. A man, weighing 70 Kg, has about 5 litres of blood in his circulation.

Variations under different physiological conditions. (1) **Age**—In infants the blood volume is greater in proportion to the body weight but is lesser in proportion to the body surface. [The body surface of infants is

* **Defibrinated blood.** When a sample of blood is constantly stirred or shaken with fine twigs or pieces of wire or glass beads, the blood clots and the fibrin gradually collects upon the surface of the foreign bodies. In this way all the fibrinogen becomes converted into fibrin in a short time and is quickly removed from the blood sample. This blood will not clot any further and remains fluid. It is composed of serum in which red and white cells remain suspended. Defibrinated blood is frequently used in physiological experiments, instead of using any anticoagulant.

proportionally larger than their body weight.] This larger volume is due to greater number of corpuscles as well as larger amount of plasma. (2) **Sex**—In males the blood volume is 7·5% higher (per sq. metre of body surface) than in females. This is due to greater number of red cells in the males. The plasma volume is same in both. (3) **Body weight** and **surface area** (*vide* above). (4) **Pregnancy**—Blood volume rises due to increase of both cells and plasma, but the rise in plasma volume is much greater than the rise in cell volume. It falls after delivery. (5) **Muscular exercise**—Raises blood volume probably due to contraction of spleen. (6) **Posture**—In erect posture there is about 15% diminution of total plasma. It passes out into the tissue spaces. (7) **Blood pressure**—Rise of blood pressure lowers blood volume by pressing out more fluid into the tissue spaces. Lowered blood pressure draws in more fluid from the tissue spaces and raises the blood volume. (8) **Altitude**—At higher altitude the blood volume rises, due to anoxia produced the number of red cells increase in such condition. (9) **Anoxia** due to any other cause will raise blood volume. (10) **Adrenaline injection**—Raises blood volume probably by splenic contraction.

Methods of determination of Blood Volume. (A) **Direct Method.** (1) (Welcker)—An animal is bled to death and the blood is collected. Then its blood vessels are washed out by pumping saline solution into the vessels. The saline washings are added to the already collected blood. The colour of this mixture is matched against the sample of normal blood of the same animal. From these data the volume may be calculated. In dogs the total blood volume is 7·7% of body weight. (2) (Bischoff)—The above method was applied upon decapitated criminals. It is obvious that the direct methods have got no place in clinical medicine, for which one of the following indirect methods are used. (B) **Indirect Method**—In this method a known amount of a particular substance is introduced into the blood stream. After some time a sample of blood is drawn out and the concentration of the injected substance is determined in it. From this, the degree of dilution is calculated. Total blood volume can be found out from these data. The substance to be used must have certain special qualities. It must not be toxic, must not alter blood volume, must not easily pass into the tissue spaces or be excreted, and must not be taken up by the phagocytic cells of the blood. Also it must not change its colour and chemical composition while in the body. Usually, two classes of substances are used for this purpose. (1) Dye-stuff like Congo red, Evans' blue, etc. (2) Radioactive substances.

1. **Dye method.** For clinical purposes the blood volume can be determined by dye method. The dye Evans' blue (T-1824) which is non-toxic and escapes slowly from the blood vessels, is used mostly in present times. 10 ml of venous blood from the subject is taken in a heparinised tube. This serves as the control sample. 5 ml of a 5% solution of Evans' blue in distilled water is then injected intravenously. 10 minutes after beginning of the injection another 10 ml sample is withdrawn from the vein of the opposite side into another heparinised tube. The Haematocrit of both samples are determined. The optical density of the dye stained plasma is estimated. 0·01 ml of the dye is then diluted to 5 ml (dilution 1 : 500) with control plasma and its optical density is determined.

Plasma volume (ml) =

$$= \text{dye solution injected (ml)} \times \text{dilution of standard} \times \frac{\text{density of standard}}{\text{density of unknown}}.$$

$$\textit{Blood volume (ml)} = \frac{\text{plasma volume}}{1-(0{\cdot}96 \times \text{Haematocrit})} \quad \text{(0·96 is the correction factor).}$$

Red cell volume (ml) = blood volume − plasma volume.

2. **Radioactive methods.** (*a*) *Radio-iodine Plasma Albumin Method*. To a sample of plasma, iodine-containing radioactive isotope ^{131}I or ^{132}I are added, and allowed to incubate for some time. This plasma is slowly injected intravenously. The degree of dilution of its radioactivity is determined, which is a measure of plasma volume. The advantage of this method is that the albumin, cannot permeate through the capillary endothelium and is not affected by lipaemia and haemolysis. The plasma and blood volume are measured in a similar way as the dye dilution method. The plasma volume determined by this method is about 2·5 litres in women and 3 litres in men.

simultaneously.

Regulation of Blood Volume. Although it is customary to talk about a constant blood volume yet it has been already shown that total blood volume does not remain constant and varies widely under different physiological conditions. This variation is mostly due to alteration of the cells and not due to that of plasma. The plasma volume remains fairly constant under normal conditions. The problem of regulation of blood volume is intimately linked up with that of water balance. The maintenance of *blood volume depends upon a balance between water intake and water loss and also upon the adjustment of fluid interchange between plasma and tissue spaces through the capillary walls.* A number of factors are involved: (1) **Physical factors.** Blood pressure, osmotic pressure, diffusion, the state of permeability of the capillaries, etc., are the important factors concerned in the regulation of the blood volume. (2) The **tissue spaces,** due to their enormous capacity, act as a ready reservoir. Any increase in the blood volume will lead to passage of more fluid from the plasma to the tissue spaces. While any decrease will draw in more fluid from the tissue spaces and maintain the blood volume. (3) **Vitamins.** Some vitamins, specially C, by controlling the permeability of the capillaries—take part in the process. (4) **Endocrines.** A number of endocrine factors are also involved here. (*a*) The **antidiuretic factor** of the posterior pituitary controls excretion of water through the kidneys. When blood is diluted the secretion of the factor is inhibited and thus more water is lost. When blood becomes concentrated, reverse changes occur. (*b*) **Parathyroids,** by their effect on calcium metabolism, control the permeability of the blood vessels and thereby the rate of interchange between blood and tissues. (*c*) **Adrenal cortex** is believed to exert important influence upon salt balance, kidney function and excretion of water. (5) **Thirst.** Another important mechanism for replenishing the reduced blood volume is the phenomenon of thirst. When the water content of the body becomes low, 'thirst' is felt. The subject takes water and thus the blood volume is kept up. [Effects of saline injection, fluid loss and such others have been discussed elsewhere.]

Causes of decrease of Blood Volume. Blood volume is reduced in the following conditions:

1. Loss of whole blood, e.g., haemorrhage.
2. Reduction in number of R.B.C., e.g., anaemia.
3. Loss of plasma alone.
4. Loss of blood water or anhydraemia.
5. Acute exposure to cold causes moderate loss.
6. Posture—Blood volume is low in the erect position than in the recumbent state.

Causes of increase of Blood Volume. Blood volume is increased due to

1. High temperature.
2. Muscular exercise.
3. Emotional excitement.
4. Pregnancy.
5. Congestive heart failure.
6. Administration of mineralocorticoids (deoxycorticosterone and aldosterone).