

# ERYTHROCYTES: KINETICS, DESTRUCTION AND ANEMIA

Erythrocyte has shape of biconcave disc (allows better surface area : volume ratio than a sphere) which appears as round with a central clear area (normally occupying 1/3 of the diameter) on blood smears. The function of the erythrocyte is to carry oxygen to the tissues at pressures sufficient to permit rapid diffusion of oxygen from the blood to the metabolizing cells. This is done by

- ✓ i) A carrier molecule (hemoglobin),
- ✓ ii) A vehicle (RBC) capable of getting the intact carrier molecule to cellular level, and
- ✓ iii) A metabolism geared to protect both the vehicle cell and the carrier molecule from damage.

Interference with hemoglobin synthesis, red cell production, or red cell metabolism causes diseases of the erythron.

Hemoglobin (Hb) is a complex molecule, the principal components of which are ① porphyrin - heme, and the ② globular protein - globin. Each molecule is formed of 4 heme and 4 globin (2  $\alpha$  and 2  $\beta$  globins) those are attached with each other. Since each heme contains an oxygen carrying iron molecule, each Hb molecule can carry 4 oxygen molecules to the tissues. Heme synthesis occurs enzymatically on the mitochondria and involves a series of condensation reactions that require pyridoxal phosphate and copper. Iron is added in the last step by the ferrochelatase enzyme. Several of the steps including the incorporation of iron are blocked by lead. Globin synthesis is under genetic control and balances heme production.

## 1) Erythropoiesis (Erythrokinetics)

It is the production of erythrocytes. At pre-natal stage it takes place in the liver whereas in post-natal stage bone marrow is the place. In mammals, erythropoiesis occurs extravascularly in bone marrow parenchyma. In avian species, erythropoiesis occurs within the vascular sinuses of the bone marrow (intravascular or intrasinusoidal development). Erythrocytes arise from multipotent stem cells called haematocytoblasts or granulocytes-erythrocytes-monocytes-megakaryocytes colony-



forming units (CFU-GEMM) or CD34+ cells. These cells have the capacity for self-renewal and differentiate into progenitor cells. Differentiation is controlled by growth-promoting stimuli produced by marrow stromal cells. Some early progenitor cells have the capability of differentiating into more than one cell line (e.g., CFU-GEMM has the potential to differentiate into granulocytes, erythrocytes, monocytes, or megakaryocytes). Other progenitor cells are unipotential (e.g., erythroid colony forming unit (CFU-E) can differentiate only into erythroid cells).

#### A) Regulation

The rate and degree of erythropoiesis in the marrow is related to levels of tissue oxygen tension. Erythropoiesis is regulated by erythropoietin (EPO) (which stimulates in times of demand and regulates normal production).

- a) The majority of EPO is produced by peritubular interstitial cells of the kidney in response to hypoxia, but the liver may account for 10-15% of EPO production by specific hepatocytes. Androgens increase EPO release. In contrast, estrogens and corticosteroids decrease EPO release, but their effect is probably not clinically significant.
- b) EPO produced from the kidneys in response to hypoxia, acts on bone marrow:
  - i) To increase the number of stem cells entering red cell production,
  - ii) To shorten red cell maturation time, and
  - iii) To cause early release of reticulocytes.
  - iv) Inhibition of apoptosis of newly formed progenitor cells and prorubricytes allowing them to differentiate into mature erythrocytes.
  - v) Stimulation of hemoglobin synthesis in already dividing erythroid cells. Switching of hemoglobin synthesis in sheep from one adult type to another (i.e., HbA to HbC).
- c) ~~Interleukin-3~~ (IL-3) and granulocyte/monocyte colony-stimulating factors (GM-CSF) and granulocyte-CSF (G-CSF):
  - i) IL-3 is produced by activated T lymphocytes; GM-CSF by activated T lymphocytes, macrophages, endothelial cells, and fibroblasts; and G-CSF by macrophages, monocytes, neutrophils, endothelial cells, and fibroblasts.
  - ii) In concert with EPO, these factors stimulate multiplication of a primitive erythroid progenitor cell, erythroid burst-forming units (BFU-E), and its differentiation into the CFU-E progenitor cell (Fig. 1).



## B) Different Steps of Erythropoiesis:

From the Rubriblast (Erythroblast) following cells take place:



**Prorubricytes (Pronormoblast)**

Cytoplasm is very little in amount and more basophilic



**Rubricytes (Basophilic Normoblast)**

These are small in size than pro-rubricytes, nuclear chromatin is more dense, more basophilic and appears in the form of coarse granules which often are clumped.

Sometimes the clumped granules arrange like the spikes of a wheel.

Cytoplasm is more basophilic.



**Rubricytes (Polychromatophilic normoblast)**



**Metarubricyte (Orthochromic normoblast)**

These cells have nucleus smaller with coarse clumped very basophilic granules but no nucleolus can be seen. These cells do not divide further.



**Reticulocyte**

Non-nucleated erythrocytes containing RNA.



**Mature Erythrocyte**

This is in the circulation.

a) Proliferation stage From hemocytoblast to metarubricyte

b) Hemoglobinization:

It takes place from prorubricyte to reticulocyte.

c) As is evident, the whole maturation process involves morphological changes.

As the cells mature:

i) Hemoglobin accumulates and becomes the principal protein (pink cytoplasm).



- ii) Mitochondria are lost and protein synthesis stops.
- iii) The nucleus becomes inactive and clumped (pyknotic) and is extruded from the cell.
- iv) Nuclear : Cytoplasm ratio decreases.
- v) Cytoplasm color changes from blue to gray to orange as hemoglobin accumulates and RNA is lost.
- vi) When the cell loses its nucleus it becomes the reticulocyte. These cells can appear in the peripheral blood and can be used as an index of effective marrow activity. The cytoplasm of the reticulocyte contains residual RNA that can be precipitated into a reticular network by certain special supravital stains. This residual RNA is lost after 1 to 2 days in the circulation.
- d) Reticulocytes and mature erythrocytes migrate into venous sinuses of the bone marrow and then into peripheral blood.
- e) It takes 120 hours from EPO stimulation of the erythrocyte stem cells to release a mature erythrocyte into circulation.
- f) Normally, four divisions produce 16 mature erythrocytes, if less, it is ineffective erythropoiesis. A current concept of <sup>my</sup>hematopoiesis is provided in Fig. 1. Specific growth factors can influence the development of cells at various stages in hematopoiesis.

### C) Nutritional Requirement

- a) Many vitamins, minerals, and proteins are necessary for normal RBC production.
- b) Clinically, folic acid, vitamin B<sub>12</sub> and iron are the most important. Deficiencies of these factors lead to characteristic anemia.

### 2) Erythrocyte Destruction

The age of erythrocyte varies from species to species (Table 1). Aged cells are destroyed which is also regulated. Two mechanisms exit for the removal of senescent red cells; both conserve the principal constituents of the cell for re-utilization. Approximately 1 % of normal aging red cells are removed by intravascular hemolysis (Fig. 2) which releases the free Hb, this is quickly converted to Hb dimers that form complexes with globulin, haptoglobin, which are transported to the liver. A portion of



the free Hb may also be converted to methemoglobin that is quickly broken down to heme and globin. The globin protein chains may be immediately recycled: heme is bound to a second serum carrier protein, hemopexin, and carried to the liver and spleen for processing by the fixed monocytes/macrophages system.

*2nd stage*  
*b shape + (purity)*  
Aged red cells are recognized by the macrophages of the liver, spleen and marrow (reticuloendothelial system) and are phagocytised/destroyed. There is a steady state between this destruction and production of new erythrocytes. As the cell ages, it loses its flexibility due to impaired adenosine triphosphate (ATP) production and becomes trapped in the spleen, unable to percolate through the spleen sinusoids. Following phagocytosis, Hb is converted to heme and globin and the latter is recycled. Iron is released from heme moiety and stored by the macrophages as ferritin or  hemosiderin or released into the circulation for transport back to the marrow. The remaining porphyrin is converted to free bilirubin, which is released by the macrophages into the systemic circulation where it complexes with albumin for transport to hepatocytes. In intravascular hemolytic anemia, the red cells have shortened life span and the same mechanisms occur at an increased rate.

### 3) Erythrocyte Metabolism

Normal cell metabolism maintains both structural and functional integrity of the cell. Mature red cell has no nucleus or mitochondria. It depends on glycolysis for energy and cannot synthesize new proteins. Therefore, principal metabolic pathway of red cells is glycolysis and the main energy source in most species is glucose. Glucose enters the red cells by an insulin-independent mechanism and most of it is metabolized via the glycolytic scheme, producing ATP (there is a net gain of 2 ATP's in glycolysis) and reduced nicotinamide adenine dinucleotide (NADH). The energy of ATP is used to maintain red cell membrane pumps, which in turn are responsible for maintaining red cell shape and flexibility. The reducing potential of the NADH is utilized via the methemoglobin reductase pathway to maintain the iron in Hb in its reduced form.

The glucose not utilized in glycolysis is metabolized via second pathway, the hexose monophosphate shunt (HMP). Red cells depend on the HMP and glutathione to protect from oxidative denaturation. No energy is produced via the HMP; rather, like the methemoglobin reductase pathway, the principal effect of the HMP is reducing



potential, this time in the form of reduced nicotinamide adenine dinucleotide phosphate (NADPH). NADPH, in conjunction with glutathione reductase/peroxidase system, maintain the sulfhydryl groups of globin in their reduced state. The principal HMP enzyme is glucose-6 phosphate dehydrogenase (G6PD). G6PD deficient red cells are unable to protect themselves against oxidant drugs or stress (i.e., infection). Oxidation will cause Hb to precipitate within erythrocyte (Heinz bodies) and erythrocyte lysis within findings of intravascular hemolysis.

Numerous red cell disorders are the direct result of abnormal red cell metabolism: interference with glycolysis causes ATP deficiency, which leads to reduced red cell life span and hemolytic anemia. Excessive oxidant stress may overload the protective HMP shunt or methemoglobin reductase pathways, thereby causing Heinz bodies hemolysis or methemoglobin formation, respectively. Many drugs induce hemolytic anemias, such as phenothiazine hemolysis or methylene-blue-induced hemolysis are examples of this mechanism of disease.

#### 4) Anemia *etiology classification*

Anemia, a condition characterized physiologically by insufficient circulating hemoglobin (Hb). Clinically, there is pallor of the mucous membranes, weakness, lethargy, reduce exercise tolerance, loss of appetite, increased heart and respiration rates. There may be systolic murmur due to reduced blood viscosity. In anemia laboratory findings indicate:

- ✓i Decreased RBC numbers ✓✓
- ✓ii Decreased PCV ✓✓
- ✓iii Decreased Hb. Concentration ✓✓

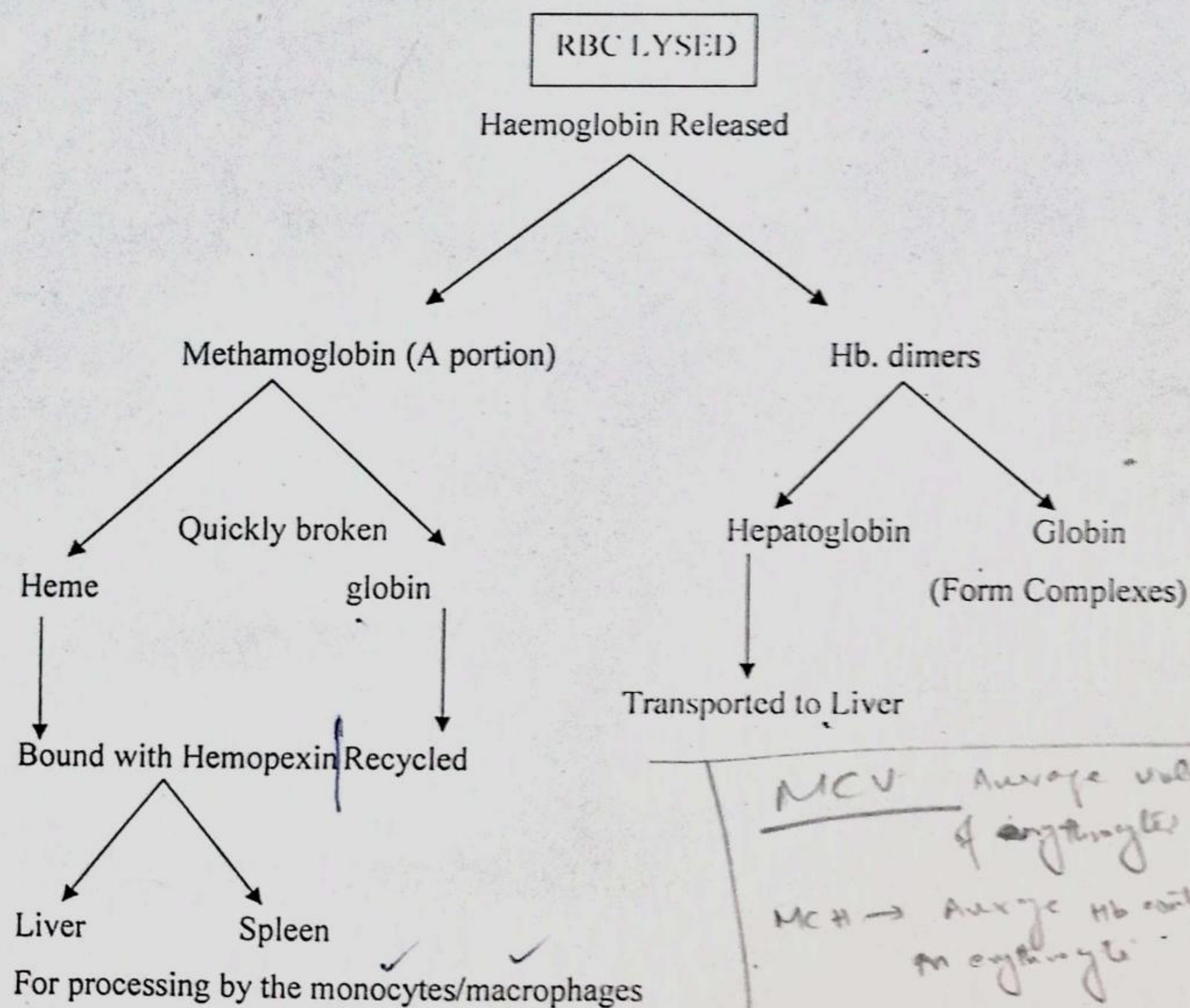
Anemia is not a disease but sign of underlying disease. Diagnostic approach will be based to know why there is

- ✓i Decreased production and/ or
- ✓ii Increased destruction or loss of RBCs are the factors that lead to anemia.



**Table 1: Life span of erythrocytes in various animal species**

Species	Mean Life span (days)
Birds	35-50
Buffalo	140-160
Cat	70
Cattle (adult)	160
Cattle Calf (3 months)	48-63
Dog	110-122
Goat	125
Horse	140-150
Man	120
Pigeon	17-25
Rabbit	68
Sheep	70-153



**Fig. 2: Fate of hemoglobin in intervascular hemolysis.**

MCV → Average volume of erythrocytes  
MCH → Average Hb content of erythrocytes  
MCHC → Av. Hb. conc. in erythrocytes



## 5) Classification of Anemia

Anemia is classified to provide a rational basis of treatment; either the anemia is unresponsive or responsive.

### A) Anemia Due to Bone Marrow Hypo-functions (Un-responsive)

#### a) Decreased Erythrocyte Production.

##### i) Nutritional Anemia

- a. Vitamin B<sub>12</sub> and folic acid are required for DNA synthesis. In deficient cases  $\rightarrow \downarrow$  in erythroid mitosis thus  $\rightarrow \downarrow$  RBC numbers. Macrocytic and normochromic anemia will be observed which will be characterized by ineffective erythropoiesis or abnormal cells that are not released in to the circulation.
- b. Folic acid deficiency anemia is mild in animals and limited to animals with fast growing tumors.
- c. Anemia due to carbohydrate deficiency; may be due to starvation - mild.
- d. Protein deficiency causes severe anemia in animals and this will also lead to reduction in blood volume. Prior to increase erythropoiesis plasma proteins be replaced due to general debility and reduced activity. Recovery is very slow.
- e. In chronic diseases,  $\downarrow$  RBC production is the primary cause of anemia, though some hemolysis is also present. e.g.,
  - (1) Toxemia in abscess or uremia,
  - (2) Lack of iron utilization,
  - (3) Diffuse liver disease: There is inadequate detoxification of normal metabolites.

##### ii) Injury to Marrow Stem Cells

Damage to marrow stem cells is usually related to drug or toxic exposure.

Diagnosis of this category of anemia is through bone marrow examination.

e.g.,

- a. Chloramphenicol and hydrocarbons  $\rightarrow$  aplastic anemia.
- b. Irradiation
- c. Bracken fern poisoning: in cattle causes hard marrow aplasia.



d. Virus of feline panleukopenia causes aplasia and pancytopenia; deaths due to agranulocytosis, dehydration and thrombocytopenia; therefore, in hypoplastic and aplastic anemias shortage of RBC is not as critical as lack of platelets or neutrophils is. In total aplasia, death of individual occurs in 10-14 days due to hemorrhages and sepsis.

iii) Marrow Replacement by Abnormal Cells (Myelophthisis)

- a. Myelofibrosis: Marrow cavity is filled by connective tissue that is very rare in animals.
- b. Lymphoid tumors are common.
- c. Diagnosis: Appearance of rubricytes in peripheral blood in the absence of polychromasis, especially if anemia is mild or absent is indicative of such type of anemia. Then be alert that cells may be displaced by tumorous growth in bone marrow; then examination of bone marrow is required to look for tumor cells in peripheral blood and bone marrow.

b) Anemia Due to Decreased Hb. Production

i) Deficient Heme Synthesis

Microcytic hypochromic anemia is observed which may be due to deficiency of

- a. Iron
- b. Copper: Copper is required to recharge the ferroxidase enzyme; deficiency of copper blocks iron utilization resulting in iron deficiency. If there is absolute iron deficiency then there will be no hemosiderin in bone marrow.
- c. Vitamin E is required for heme synthesis.
- d. Vitamin B<sub>6</sub> or pyridoxin deficiency also reduces heme synthesis.

ii) Deficient Globin Synthesis

Synthesis of  $\alpha$  and  $\beta$  globin is under genetic control. This deficiency is not reported in animals.

B) Anemia due to loss of abnormal erythrocytes (responsive)

- a) Erythrocytes with deficient enzymes
  - i) Glucose 6-phosphate dehydrogenase (G6PD) deficiency



- a. This enzyme is a part of an intra-erythrocytic metabolic chain that protects Hb. from oxidative denaturation. In deficient cells oxidative agents cause globulin to precipitate → Heinz bodies formation → rapid lysis.
- b. Old cells are more susceptible to oxidative denaturation because due to lowered G6PD; similar changes are brought by the phenothiazine, phenylhydrazine and primaquine drugs. In such cases no treatment required because young erythrocytes are spared from such reactions. However, blood transfusion be considered if PCV drops below 15%.
- c. Bone marrow is usually functioning; spontaneous recovery will be rapid.
- d. Diagnosis of G6PD deficiency or Heinz bodies anemia:
  - (1) Heparinized test and control blood = 0.1 ml separately.
  - (2) Acetylphenylhydrazine = 100 mg/ml
  - (3) Buffer saline = 2 ml
  - (4) Incubation = 2 hours at 37 °C
  - (5) The RBCs are stained with new methylene blue and examined under microscope for Heinz body formation.

#### ii) Pyruvate kinase deficiency

This deficiency is reported in dogs not in other animals. Example is congenital anemia with marked polychromatic RBC response.

#### b) Hemolytic anemia due to acquired defects of RBCs.

##### i) Immune mediated anemia

- a. RBC may become the target of those antibodies that coat their surface and cause them to be removed from circulation by reticuloendothelial system (RES).
- b. The adsorption of virus to RBC (e.g., equine infectious anemia) may cause hemolytic crisis because the attachment of antiviral antibody to RBC-bound virus often takes place. These cells are destroyed then by the RES, known as "innocent bystander reaction".
- c. Other examples could be of adsorption of drugs to RBC and isoimmune hemolytic anemia. This type of anemia occurs in foals, kittens, puppies, and calves from vaccinated dams. The RBC of



newborn are agglutinated or hemolysed by specific isoantibodies produced in their dams.

ii) Hemolytic anemia due to intravascular fragmentation

a Diseases that cause vasculitis activate.

- |                        |   |   |
|------------------------|---|---|
| (1) Platelets, and     | } | → disseminated intravascular coagulation (DIC). |
| (2) Clotting mechanism |   |   |

b. Blood is moving with fast speed in blood vessels, when ever there is damage to arterioles (vasculitis) → fragmentation of RBC: as they impinge on intraluminal strands of fibrin. Damaged cells are removed by RES.

c. The above pathogenesis is referred to as microangiopathic hemolytic anemia (MHA). It is characterized clinically by anemia with marked poikilocytosis and reticulocytosis that occurs in young calves.

(1) Usually occurs with hemolytic uremic syndrome in which there is

- Anemia ✓
- Uremia ✓
- Poikilocytosis ✓
- Hemoglobinuria and ✓
- Collapse ✓

(2) Other disease conditions in which MHA is observed are:

- Malignant catarrhal fever ✓
- African swine fever ✓
- Anemia with marked poikilocytosis may occur in some parasitic diseases such as strongyloides, canine heart worm disease ✓
- MHA may occur in hemangiosarcomas ✓

c) Anemia due to loss of normal RBC

i) Hemolysis of normal RBC (responsive)

It is seen in splenomegaly and hypersplenism, various examples are:

a. In condition that leads to enlargement of spleen → Increased erythrophagocytic activity.



- b. Splenomegaly may occur as a result of hyperplasia due to
- (1) Chronic infection ✓
  - (2) Autoimmune disease ✓
  - (3) Splenic neoplasia ✓
- c. Diseases of liver resulting in cirrhosis and portal hypertension → congestive splenomegaly. ✓
- d. Stretching of the splenic reticulum in the enlarged spleen is a stimulus to both phagocytosis and fiberplasia.
- e. Blood is pooled in enlarged spleen, environment of
- (1) ↓ Glucose and cholesterol
  - (2) ↑ pH
- } → Premature aging which causes them to become spherocytes and removed from circulation.
- f. Splenomegaly then for hemodynamic reasons alone → hypersplenism.

(1) Hypersplenism is defined as a spleen enlarged due to any cause with marrow hyperplasia and a decrease in one or more of the cellular elements of the blood.

(2) Hypersplenism is seen in thrombocytopenic purpura, hemolytic anemia or neutropenia or any combination of these. Splenectomy is the remedy.

## ii) Hemolysis due to RBC parasitism

Erythrocyte parasitism → Infected RBC, parasite may be removed by the spleen from RBC and cell → circulation or RBC along with the parasite entirely removed by RES. Intravascular hemolysis of RBC occurs with hemoglobinemia.

a. Hemoglobinuria is seen in babesiosis and theileriosis in cattle, hemobartonellosis in cats, anaplasmosis in sheep and cattle, eperythrozoonosis and plasmodium in mammals and birds. Other examples of such parasitism are leishmania, ehrlichiosis



b. In general, the bone marrow is hyperplastic in anemias due to parasitism. There is marked peripheral blood macrocytes and reticulocytes.

c. Hemoglobinuria with iron and protein loss → recovery slow.

iii) Lysis of RBC due to bacterial (hemolysins), plant, chemicals or physical agents

a. Bacterial (hemolysins)

(1) Bartonella bacilliformis causes a generally fatal hemolytic anemia in man, dogs and rodents.

(2) Staphylococcus and Streptococcus toxins are other examples.

(3) Leptospirosis ✓

(4) Bacillary hemoglobinuria ✓

(5) Anthrax ✓

b. Plant hemolysins

(1) Most of the plants (rape, turnips and onions) that cause hemolytic anemia do to depletion of RBC enzyme, G6PD. Deficiency of this enzyme → Heinz-body production; such RBC are removed by RES.

(2) Direct lysis of RBC membrane can be caused by saponin or ricin.

(3) Hemolysins are the toxic principles in most spider and snake venom.

c. Chemical hemolysins:

(1) Wide range of chemicals produce hemolysis → aplastic anemia; examples are hydrocarbons chemicals such as benzene, toluene, phenacetin, etc.

(2) Heavy metals such as lead, silver inhibit Hb. synthesis. Arsenicals → hemolysis.

(3) Phenylhydrazine and other oxidant compounds such as phenothiazine, vitamin K<sub>1</sub> → Heinz-body production → Hemolysis.

d. Physical agents

(1) Burns: Intravascular hemolysis if more than 20% skin is affected.



(2) Excessive intake of cold water especially in calves may cause hemolysis likely of osmotic origin with anemia, dyspnea and hemoglobinuria

iv) Loss of normal RBCs

a. Hemorrhages: May be external or internal.

(1) Wounds, uterine prolapse, lacerations, surgical trauma due to dehorning or castration.

(2) Acute hemorrhages → hypovolemia and hypotension with normal PCV. In such cases blood transfusion is recommended.

(3) Chronic hemorrhages → decreased PCV; Bone marrow unresponsive due to iron loss; therefore iron and hematonics should be given

(4) Internal hemorrhages could be acute or chronic as a results of surgical wounds, tumors or abscesses or may be due to enteric or urinary tract ulcerations.

b. Parasitism: may internal or external.

(1) Blood sucking insects such as ticks, lice, flies, fleas, mosquitoes cause more irritation than blood loss.

(2) There are numerous internal parasites that suck blood e.g., hookworms, coccidia, liver flukes, stomach worms, whipworms etc.

## 6) Clinical Evaluation or Diagnostic Approach

### A) History

#### a) Time of onset of clinical signs

##### i) Abrupt Onset: suggests:

##### a. Acute hemorrhages may be due to

##### (1) Trauma

- Accidental or surgical
- Internal trauma
- External trauma
- Loss through uterus

(2) Diseases by which large vessel ruptures, e.g., rupture of friable neoplasm e.g. splenic hemangiosarcoma.



(3) Coagulative defect.

b. Hemolysis

(1) Bacterial infections:

- Leptospirosis ✓
- *Clostridium hemolyticum*
- Bovine bacillary hemoglobinuria
- Staphylococcus toxins
- Streptococcus toxins
- Anthrax

(2) Viral Infections: Equine infectious anemia

(3) Parasitic infestations:

- Haemobartonella, ✓
- Plasmodium ✓
- Anaplasma ✓
- Microflaria ✓
- Anaflaria

(4) Antibodies mediated destruction of erythrocyte

- Hemolytic disease of newborn ✓
- Vaccines ✓
- Autoimmune hemolytic anemia ✓

(5) Chemical Agents: Phenothiazine, methylene blue, copper, lead

(6) Snake venom

(7) Poisonous Plants: Wild onion, broom, oak shoots.

(8) Mechanical causes: Burns

(9) Physical agents: Heat, UV lights.

ii) Gradual Onset: Suggests:

✓ a. Chronic hemorrhages: May be due to

(1) Gastrointestinal lesions: ulcers; enteritis

(2) Bleeding from neoplasms ✓

(3) Parasitism: Internal or external ✓

(4) Urogenital tract bleeding ✓

✓ b. Bone marrow depression:

(1) Kidney disease - end stage kidneys

(2) Splenic disease - removal of spleen



(3) Liver disease – commonly seen.

b) Evidence of blood loss:

May be due to hematuria or blood loss through vomits e.g. hepatitis C or with feces as seen in coccidiosis.

c) Performance Status: Weakness; fatigue after exercise.

d) Progressive Weight Loss: May be due to renal disease or leukemia.

e) Exposure to drugs or toxic chemicals in environment e.g. lead

### B) Laboratory Diagnosis

Laboratory diagnosis of anemia is based on the following tests:

a) RBC counts

b) Hb. concentration

c) PCV

d) Erythrocyte indices including MCV, MCHC, MCH

e) Blood smear examination for immature and abnormal erythrocytes or leukocytes

f) Total plasma proteins

g) Reticulocyte counts

h) TLC and DLC

i) Platelets counts

j) Bone Marrow examination

For comparison purpose average red blood cell values in healthy individuals in different species of animals are presented in Table 2.

**Table 2: Haemogram in different species of healthy animals.**

Animal	RBC ( $\times 10^{12}/l$ )	Hb. Conc. (g/dl)	PCV (%)	MCV (fl)	MCHC (g/dl)
Buffalo	5.5 – 8.8	8-17	24-46	35-60	30-36
Cat	5.0-10.0	8-15.4	24-46	39-55	30-36
Cattle	5.0-10.0	8-15	25-45	35-60	28-36
Dog	4.95-8.5	12-18	35-57	60-77	32-36
Horse	6.0-12.0	10-18	27-48	34-58	31-39
Sheep/Goat	8.0-18.0	8-12	22-38	18-34	30-40



### C) Morphological classification of anemia

The characterization of anemia is aided by the RBC indices i.e., mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH).

MCV	MCHC Normal	MCHC Decreased
MCV Normal	Normocytic Normochromic	Normocytic Hypochromic
MCV Increased	Macrocytic Normochromic	Macrocytic Hypochromic
MCV Decreased	Microcytic Normochromic	Microcytic Hypochromic

### D) Erythrocyte Indices

#### a) Mean corpuscular volume (MCV):

It expresses the average volume of the individual erythrocyte.

##### i) Calculation:

$$\text{MCV} = \frac{\text{PCV} \times 10}{\text{RBC counts (x } 10^{12}/\text{l)}} = \text{Femtoliter (fl)} \checkmark$$

##### ii) Interpretations:

#### a. MCV Normal-Normocytic ✓

- (1) Acute Haemorrhages ✓
- (2) Hemolysis ✓
- (3) Lack of blood formation ✓

#### b. MCV Increased - Macrocytic

- (1) Increased activity of the bone marrow ✓ in some conditions usually associated with normocytic anemia.
- (2) Some deficiencies of hematopoietic factors ✓

#### c. MCV Decreased - Microcytic

- (1) Iron deficiency ✓
- (2) Copper deficiency ✓
- (3) Some deficiencies of hematopoietic factors ✓

#### b) Mean corpuscular hemoglobin concentration (MCHC):

It is the concentration of hemoglobin in the average erythrocyte or the ratio of weight of hemoglobin to the volume in which it is contained.



i) Calculation:

$$\text{MCHC} = \frac{\text{Hb. Conc. (g/dl)} \times 100}{\text{PCV (\%)}} = \underline{\text{g/dL}}$$

ii) Interpretations:

a. MCHC Normal -Normochromic

In many types of anemia an increase or decrease in the average size of the cell is accompanied by a corresponding increase or decrease in the average hemoglobin contents, so that the MCHC remains within the normal range.

b. MCHC Decreased -Hypochromic

In true hypochromic anemia the reduction in hemoglobin is relatively greater than average decrease in erythrocyte volume.

c. MCHC Increased

There is no condition in which the MCHC is increased above the normal range, since the erythrocytes cannot be over saturated with hemoglobin.

c) Mean corpuscular hemoglobin (MCH):

It is the amount of hemoglobin by weight in the average erythrocyte.

i) Calculation:

$$\text{MCH} = \frac{\text{Hb. Conc. (g/dl)} \times 10}{\text{RBC counts (x } 10^{12}/\text{l)}} = \underline{\text{picogram (pg)}}$$

ii) Interpretations:

a. It is not as accurate as MCHC. ✓