Respiratory Proteins Or pigments:-

These are the substance which are/is found in blood & serve to carry the respiratory gases, particularly the oxygen. R.P. combined with O_2 by forming a temporary compound with O_2 at its higher conc. (i.e. lung etc) & release the O_2 where it is in less conc. (i.e. tissue)

R.P. are found to be different in different groups of animals. R.P. are circulating pigments which mediate the transfer of O_2 at the extracellular & intracellular levels. All pigments contains a metallic atom in their constitution. like iron in Hemoglobin.

Table: Different types of respiratory pigments

R.P.	Metallic atom	Site	Animal	Colour of oxygenated	Colour of deoxigenated
(1) Hemoglobin M.wt of Mammals 7×10^4 D.	Fe^{+2} $Fe:O_2$ 1:1 $Hb:O_2$ 1:4	R.B.Cs. Plasma	Mammal, Birds, Reptiles, Amphibians, Fishes. Annelids, Mollusca	orange red	purple red
(2) Myoglobin	Fe^{+2} $Fe:O_2$ 1:1 $Mc:O_2$ 1:1	muscles Plasma	Vertebrates & some invertebrates Annelids & Snails	"	"
(3) Haemoerythrin	Fe^{+2} $Fe:O_2$ 2:1	~~RBCs~~ or Plasma Coelomic Plasma	Some annelids, Sipunculids, Branchipods (Lingula, Crotida)	Brown	colourless
(4) Chloro cruorin erythrin	Fe^{+2}	Plasma	Annelids (Polychaeta) Serpula, Potamella	Pinkish	Green
(5) Haemocyanin	Cu^{+2} $Cu:O_2$ 1:2	Plasma	Molluscs (Gastropoda, Cephalopoda), Crustacean, Limulus, Scorpion)	Blue	colourless
(6) Vanadium ☆	Mg^{+2}		Protochordata		

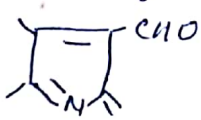
① Hemoerythrin:-

* It is concentrated in the coelomocytes that are circulate with the coelomic fluid.
* Binding ratio $Fe:O_2$ is 2:1.
* Rise in temp shift 0.0.C to right side, at the same time there is no influence of change in PH.
* CO can not effect it, so there is no poising effect of CO on He.
* Ex- Sipenculids, Polchati (Magelona), Priapulids (Priapulus & Halicryptus) Brachipods (Lingula, Glothidia)
* Its O_2 transport capacity is very low, Probably it is a storage pigment.

deoxyhemoerythrin (colourless) $Fe^{II} < H_2O\ H_2O\ H_2O\ H_2O > Fe^{II}$

Oxyhemoerythrin (Red) $Fe^{III} < O_2^-\ O_2^- < O\cdots H- > Fe^{III}$

② Chlorocruorin:-

* It occure in plasma.
* Its ~~is~~ ch. is a O_2 carrying pigment functioning normally at high O_2 tension
* It is interesting that within the same family of worms (Sabellidae, Serpulidae & amphertitidae) some species have ch. while other have Hb.
* In Serpula genus both (Hb & Ch.) are present, however their amount varies with age, Younger have more Hb.
* O_2 combining capacity is equal to Hb.
* 

Porphrin which differes from Heme in that one pyrrole ring a vinyl chain is replaced by -CHO
* It resembles Hb functionally else than it is much larger in size
* The O_2 equilibrium curve is sigmoid & like Hb shifts to right on increasing CO_2 conc. & acidity.
* O_2 carrying capacity is 9.1 ml O_2/100ml.
* It has a much higher affinity with CO than Hb & combine with CO at any conc. of O_2
* It function normally at high O_2 pressure.

Ex- 4 Family of Poly chaeta (Annelida) Sabellidae, Serpulidae, Chlorohaemidae, Amphertidae.

) Hemocyanin:-

* It is always found in plasma, never in R.B.Cs.
* Shows normal Bohr's effect like Hb.
* The Hcy of Limulus & Scorpion, Heterometrus shows a reverse Bohr effect i.e. one increase PH it shifts towards ~~left~~ Right (lower Acidity low CO2)
* The affinity of Hcy for O_2 is greatly influenced by ecological relationships. Thus in case of animals inhabiting deep waters the Hcy have high affinity for O_2 as the medium is O_2 poor. So it is extract Max^m O_2 for O_2 poor environment.
* Hcy also play some other role rather than respiratory function.
  Ex- Pure Hcy is able to act as pseudophenolase & Pseudocatalase. The enzymatic activities are due to the presence of Cu in protein molecule & it is inhibited by cyanide & copper reagent
* Binding ratio Cu : O_2 1 : 2.
* Ex- Most Molluscs & arthropodes.
* O_2 carring capacity 3.44/100ml but in Scorpion (Heterometrus) it is 1.82/100ml.
* Oxygenated Hcy shows a very broad absorption band (5600 A° to 5800°A) It is much stronger near UV region (3550 A°).
* ~~Heme~~ Cu is not readily detached from Hcy. but Heme can easily separated in Hb.
* The Hcy of molluscs have mol. wt about 3×10^6 to 7×10^6 & hence are among the largest protein molecule.

  In general those protein which occurs in cells have mol. wt below 10^6 while those which occurs in blood plasma may have mol. wt above 10^6.

* Hcy is also combine with CO but its affinity for CO is less, as compared to O_2. The CO not combine with Hcy permanently. Hcy ~~lose~~ separate from CO at high

Myoglobin:-

It is also a iron porphyrin compound. It is found in muscles of vertebrates & some invertebrates. It is also present in blood plasma of various annelids & planorbid snails. It's O_2 affinity is very high, so it can combine with O_2 at any low conc.

* Globin protein have 150 A.A. → Single chain
* Fe conten → 0.318%
* Seals & other diving mammals have high levels of myoglobin in their muscles. Myoglobin acts as an O_2 store, releasing O_2 only during periods when O_2 levels in the muscle decreased as they do during active.
* In some diseased states, the iron of Hb is oxidized & converted into ferric (Fe^{+3}). The condition is called Methemoglobinemia. and O_2 can not release itself from methemoglobin easily in tissue. Normally the enzyme Methemoglobin reductase keeps the iron in ferrous (Fe^{+2}) state.

Haemoglobin

* It is main R.P. pigment found in blood of vertebrates & invertebrates. It is found in R.B.Cs of vertebrates & in the plasma of annelids & molluscs.
* It combine with O_2 at high O_2 tension & release O_2 at low O_2 tension

$$Hb + O_2 \underset{\text{low } O_2 \text{ tension}}{\overset{\text{High } O_2 \text{ tension}}{\rightleftharpoons}} HbO_2$$

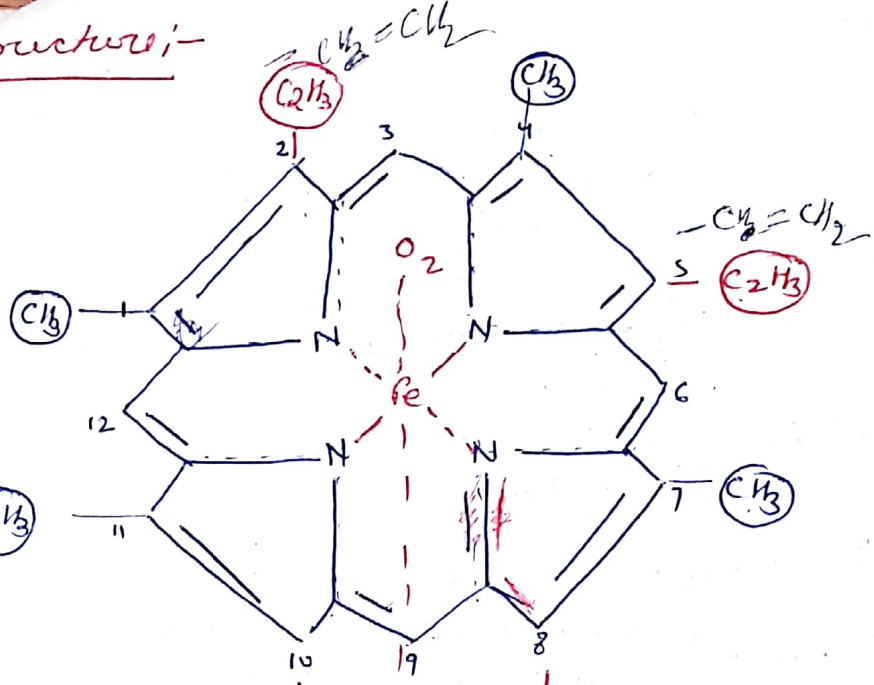
This is oxygenation & not oxidation because it is a non-enzymatic** reaction & totally
** depends upon physical factor of O_2 tension, partial pressure of CO_2, pH etc.

* Function of Hb in invertebrates —

Hb functions in invertebrates are interesting. In some it transfer O_2 at atm. pressure while in some at low pressure. In some invertebrate in hypoxia condition Hb store O_2.

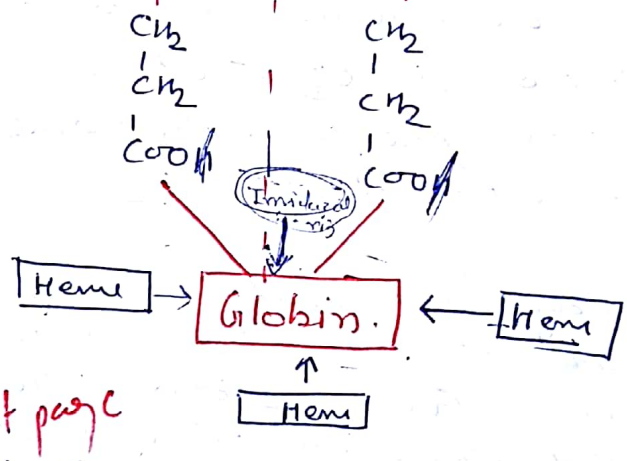
* The haemo is an iron containing compound belonging to the class compounds called Protoporphyrins. Globin belongs to the class protein called globulins. Thus Hb is a Conjugated protein.
* Formula of adult Hb ⟶ $\alpha_2\beta_2$

Structure:-



1, 4, 7, 11 → CH_3

2, 5 → Venyl (C_2H_3 or $CH=CH_2$)



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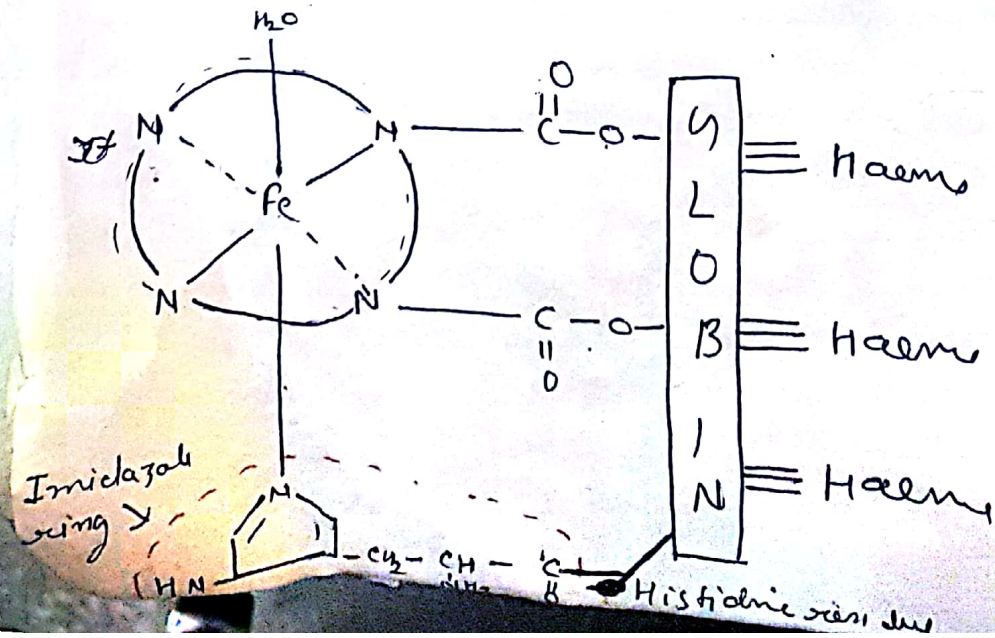
Acetic acid + Glycin = Porphyrin

4 Porphyrin + iron = Heme

4 Heme + 1 Globin = ~~Protein~~ (Haemoglobin)
(Protein)

Haem ≡ Globin ≡ Heme
Haem ≡ Heme
Haem ≡ Haem



* It consist a non protein part haem & protein part globin.

* The haem part is made up by the combination of porphyrin (4) & iron. 4 Haem combines with 1 globin to form Hb.

* The molecule of globin is made up of two symmetrical halves, each half contain two different polypeptide chains an α-chain (141 A.A) & a β-chain (146 A.A.). (Total 4 chain 2α, 2β in a globin).

* The chemical str of Hb resemble with cytochrome oxidase (cytochrome (3)), cytochrome oxidase is extensively present in all animal cells.

Foetal Hb:- In human (& other) foetal Hb differs from adult Hb in the str of β chains.

Hb foetus — α γ
Hb adult — α β

Genes for β & γ are of same chr but differ only at 6-7 place in a.a. residue.

* Later on the foetal Hb completly replaced by adult Hb.

* The change from foetal to adult Hb is genetically determined not environmentally. It is possible that hormonal factors initiate the change. There are however quantitative changes in Hb content in response to environmental influence Ex- men who move from low to high altitudes develop an increased Hb content in the blood after a period of exposure to the lower O_2 pressure at altitude.

Chr of Hb:

* Mamalian Hb is "4 unit Hb molecule" (2 unit in polychaetes). Each haem molecule combine with a mol of O_2 so adult Hb mol. can carry 4 O_2 mol.

* The oxygenation of each haem unit is independent if the units are separate, but when first O_2 combine with Hb it accelerates the combination of other 3 O_2 to haem this is called haem-haem effect.

★ CO combines ;
CO & Hb

★ The affinity of Hb is about 200 times a great for CO as for O_2. As a result, CO will displace O_2 & saturate Hb, even at very low partial pressure of CO, causing a marked reduction in O_2 transport to tissues.

Hb saturated with CO is called ~~carboxy~~ carboxyhemoglobin. The effect of such saturation on oxidative metabolism is similar to that of O_2 deprivation; which is why the CO produced by cars, coal or wood stoves is so extremely toxic

★ Deoxygenated (Reduced) Hb has +ve magnetic movement (i.e paramagnetic). Combination with O_2 or CO results in loss of magnetic movement (i.e. it become diamagnetic) which indicate an absence of unpaired electron.

Deoxygenated Hb ⟶ Paramagnetic
Oxygenated Hb ⟶ Diamagnetic.

★ It is concluded that on oxygenation there is a partial transfer of an electron from Fe^{+2} to O_2; the iron become Fe^{+3} & the O_2 become a super oxide.

$Hb(Heme\,d_2^6) + O_2 \rightleftharpoons Hb(heme\,d_2^5).O\dot{O}$

Then Fe^{+2} goes to formal Fe^{+3} & O_2 to $O\dot{O}^-$.

★ 15 gm Hb / 100 ml blood < ♂ Human → 14-17 gm/100 ml
♀ → 12-16 gm/100 ml

★ The iron content of mammalian Hb is 0.376%
& heme content is 4% (Myoglobin → Fe - 0.318%

★ ~~The~~ chemi a

* The Hb of amphibians & reptiles tend to be slightly larger than that of mammal, bird & fishes.

* Tan mol. wt of Hb → 64,500 D (man 67,000 D)
  Myoglobin → 17,450 D
  D = Dalton

Amino acid str of chains —

* Genetic analysis shows that the α & β genes are on different chromosome, but β & λ are on the same chromosome.
* The β chain is more subject to mutation than α-chain
* Tertiary structure of α & β chains is similar in oxyhemoglobin & deoxyhemoglobin, but quaternary st. may be different.
* In human α & β differ in respect to 85 a.a. out of 141 a.a. sequences. β & λ chains differ only by 7-8 a.a.
  The λ chain (Hb foetal) has higher glutamic acid & methionine content & lower valine & proline content than β-chain (adult Hb)

Hb estimation —

* Hb is commonly estimated by converting it into acid hematin by adding N/10 HCl & diluting with water (Sahlis method) & matching with the standard. In clinical laboratories, Cyanmethemoglobin is popular method. In this Hb is converted into cyanmethemoglobin & colour developed is compared against a standard in a suitable colorimeter

* The affinity with O_2 :-

* Hot or acid (low pH) or high CO_2 decrease the O_2-affinity with Hb

* The working muscles are hot as well as their pH is low; in these conditions the affinity for O_2 is low & the O_2 is released vigorously

* Another compound 2,3 DPG (2,3 diphosphoglyceraldehyde), which accumulates during metabolism, also causes loss of affinity. This results in rapid & easy release of O_2 in exercising muscles.

* Synthesis of Hb :-

* The Hb is synthesized by the cells of erythroid series in the red bone marrow. Hb first seen at the stage of intermediate normoblast

* The essential steps —

Succinyl CoA (4'C') + glycine (2'C') = α amino β keto adipic acid (6'C')

α amino β - keto dipic acid ⟶ ALA + CO_2 (5C)

ALA + ALA ⟶ Porphobilinogen (a pyrrole)

(ALA = δ amino levulinic acid)

* 4 molecules of Porphybilinogen combine after some further change to form Protoporphyrin

* Protoporphyrin now incorporates an iron atom to become haem

* Four haem molecules are now joined with one globin molecule to form one molecule of haemoglobin

Factors affecting Hb synthesis

① Globin a.a, Glycine, Succinyl CoA → Raw material are available from metabolic intermediate.
Iron → has to be supplied in the food

② Cu & Co, B_{12}, Pyridoxine (B_6) → necessary for Hb synthesis, because their absence leading to failure of Hb synthesis.

IRON metabolism →

* milk → Fe deficient → "milk injury" → in those persons who depends upon milk.

* food iron < Animal source, Vegetable "

* Iron on absorption basis < Haem iron → Not depend upon phytic acid
Non-haem iron → absorption facilated by phytic acid (plant source) & phosphate

* Fe not generally absorbed in stomach, but HCl facilate the Fe absorption.

* Fe^{+3} (most food iron) (ferric) —vit'c' reduce in gastric→ Fe^{+2} (absorption occur)

* 10-20 mg Fe consumt per day (24 H) by average individual.

* A vegitarian consumed enough iron but most of the Fe may not be absorbed.

* Fe absorbed from the upper part of small intestine including duodenum.

* The Fe absorbed according to the needs of the body & regulation of this done by intestinal mucosal epethilum (Mucosal block)
The person who has Fe deficiency will absorb greater quantity of Fe than a person who has no Fe-deficiency.

★ Recycling of Fe:- After destruction of the erthrocytes the Hb is liberated and the Fe is released from the Hb. This Fe is not thrown out of the body but utilised again by the body for Hb synthesis.

★ Iron requirment →

♂ → 1 mg/day, ♀ → more

★ ~~1 mg/10~~

★ Women of reproductive age (15-50 y) require more Fe, because they either carry fetus, menstruation or lactate.

- During M.C. some blood is lost
- During pregnancy, although there is no M.C., Fe has to be supplied for development of Hb the fetus; further total RBC mass also increases in pregnancy.
- Lactating mothers lose Fe through their breast milk, amount of which is around 0.5 mg/day.

★ Blood loss by any mean (disease etc.) increase the Fe requirment.

Transport & distribution of Iron —

Absorbed in upper part of intestine → Plasma → Fe combine with 'transferrin' in plasma (only 20-35% → transferrin is saturated by Fe, Rest 70% is unsaturated) so only 30% of the 'total Fe binding capacity' of blood is utilized)

↓

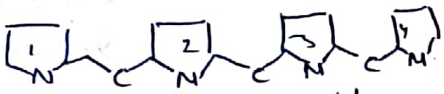
- Most Fe reach to Bone marrow → for Hb synthesis
- Rest Fe reach to other tissue for synthesis of other compounds such as — Myoglobin, oxidase, Peroxidase, cytochrome oxidase, cytochrome etc

* After @120 days (lifespan) the R.B.C. dies & release Hb (which has been phagocytozed by the phagocyte of reticulo endothelial system.
* From Hb the Fe is released, this Fe is retained in body as "Ferritin" → Bone marrow → Hb / other tissue → ...

* Body of human → 4 gm Fe → 70% → Hb
  20% → As / ferritin
  10% → myoglobin, peroxidase, cytochrome oxidase, cytochrome etc.

fate of Hb

Old R.B.Cs rupture (also in Bone marrow & liver) mostly in spleen → Hb phagocytes by RES. → within phagocyte of RES the the 4 pyrrol ring open



(Bile pigment) Tetrapyrrol ring.

↓

Fe & globin separate from this tetrapyrrol ring ↓,

Tetrapyrrol change into Biliverdin

↓ oxidised

Bilirubin

↓

All the above change occure within phagocyte of RES.

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conjugated bilirubin discharged into biliary canaliculi & mixed with Bile → & colour the bile

↑

Small amount of bilirubin conjugate with S to form bilirubin sulphate

Albumin is removed & bilirubin conjugate with ~~gluco~~ glucuronic acid (derivative of glucose) to form bilirubin glucuronides (water soluble) (conjugated Bilirubin)

↑

free bilirubin enters in liver

↑

Formed bilirubin - albumin complex (or "free bilirubin")

↑

combine with albumin in plasma

Bilirubin comes out from phagocyte in plasma

Bile with conjugated bilirubin enters to duodenum

↓

come in contact with intestinal bacteria

↓

Bilirubin glucuronide $\xrightarrow{\text{Bacterial enzyme hydrolized}}$ free bilirubin

↓

free bilirubin $\xrightarrow{\text{Reduced}}$ Urinobilogens Sterobilinogens

↓

- Both Urinobilogens & Sterobilinogens are absorbed by blood & excreted by Urine

⊕

- Some part which is not absorbed in blood pass with Stool & give it golden yellow colour.

Stool yellow $\xrightarrow[\text{Oxidating pigment}]{\text{Exposed to sun}}$ Black colour

★ Conjugation of bilirubin in liver catalyzed by glucoronyl transferase. Unless bilirubin conjugate with glucoronic acid it can not be excreted via bile.

Jundice is due to accumulation of "free bile" → caused by drugs steroids Virus or bacteria. → because they harm the glucouronyl transferase

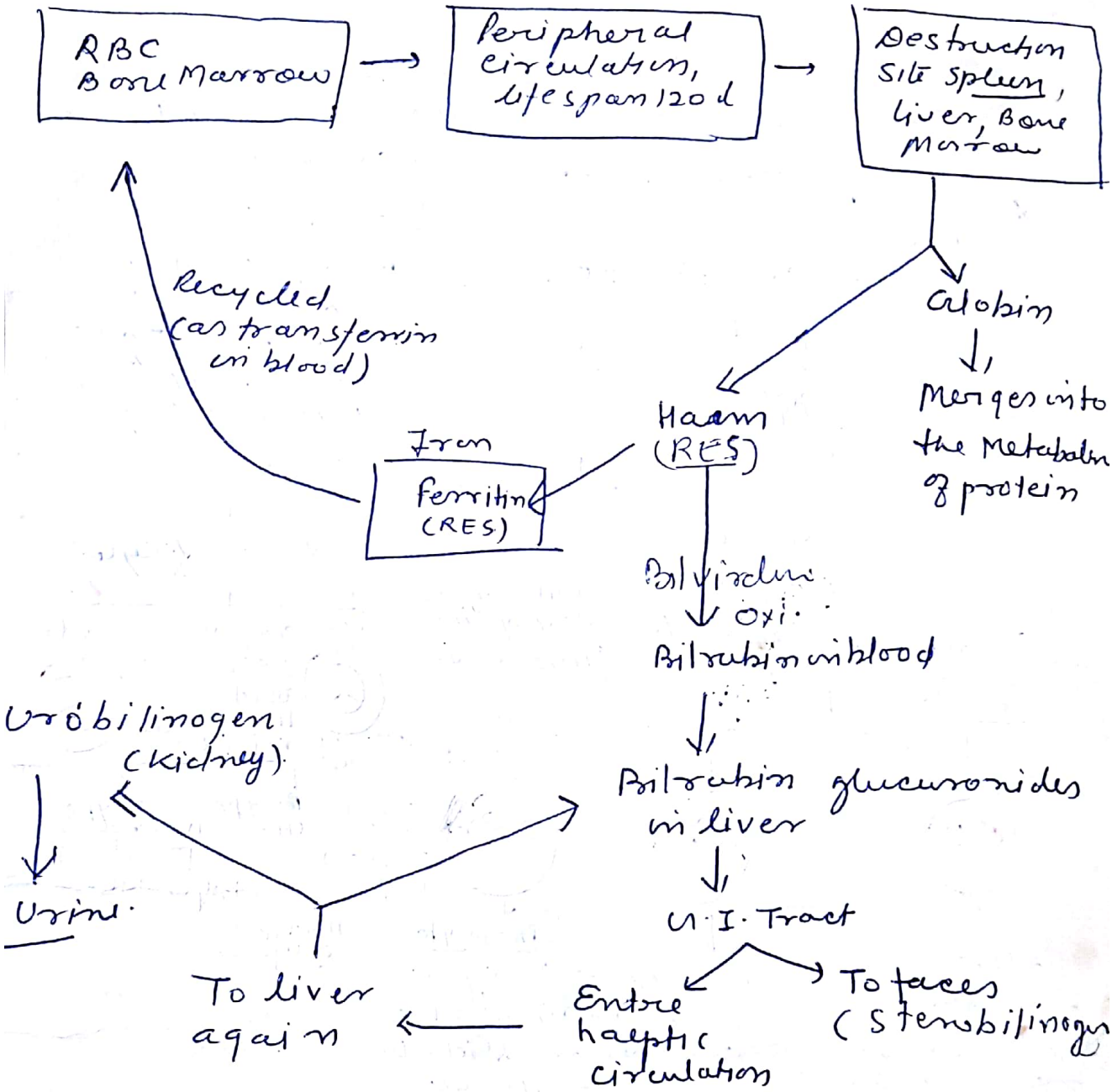

Fig:- The RBC story.