Vitamin B12 and Folate Metabolism

MBChB 3 Lecture series (2014)

Lecture outline

- Chemistry
- Functions & Role
- Sources, absorption, Storage
- Consequences of Lack
- Laboratory Studies

Lecture Objectives

At the end of this lecture you will be able to:

- State the dietary sources of Vit B12 and Folate
- Explain the metabolism & role of vit.B12,and folate
- Outline the laboratory studies for Vit B12 and folate status

Vit. B12

- A cobalt-containing compound produced by intestinal micro-organisms
- Consists of a group of compounds - cobalamins

- Higher plants do not concentrate vitamin B 12 from the soil therefore a poor source as compared with animal tissues

Forms of B12

- Active compounds:
  - Cyanocobalamin
  - Hydroxocobalamim
  - Nitrocobalamin

Sources:

- Animal sources only
  - ✓Liver
  - ✓Muscle Meats
  - ✓Fish
  - ✓Eggs
  - ✓Dairy products
  - ✓Vitamin B12 fortified foods (Cereals)
- Recommended daily allowance: 2 mcg/day

Role & function

- Transfer of methyl groups
- Intra molecular exchanges of H
- Synthesis of methionine from homocysteine

  (Methyl transferase,Nmethyl FH4 (folate),Vit B12)
- Synthesis of SuccinylCoA from MethylmalonylCoA

  (methylmalonylCoA mutase,5”adenoxyadenosyl)

Role

- Pyrimidine synthesis(thymidine acid)
- Conversion of RNA to DNA
- Vitamin B12 (and folate) regulate the formation of red blood cells and to help iron function better in the body.
- S-adenosylmethionine (SAMe), involved in immune function and mood regulation.

Normal B12 absorption pathway

- Acidic stomach environment breaks down food-bound B12
  - Insufficient acid decreases B12 absorption
- Intrinsic factor (IF) binds B12 in duodenum
  - IF produced in stomach parietal cells
- IF-B12 compound binds to specific receptor – cubilin which binds to another protein (amnionless) leading to absorption in distal ileum (endocytosis)

Transport

- Vit B12 absorbed into portal blood
- Binds Transcobalamin (TC) and delivered BM

Role

- Vitamins B12 and folate control blood levels of the AA homocysteine
- Homocysteine –methionine reaction is closely linked to metabolism of folate
- Elevated levels of homocysteine linked to heart disease, depression and Alzheimer's disease.

Folate

- **Folic acid** is also known as
  - Vit B9, folacin, folate, folic acid, folinic acid, pteroylglutamic acid, pteroylmonoglutamic acid, pteroylpolyglutamate, vitamin M.
- Folate occurs naturally as polyglutamates (conjugated)
- One of the water soluble B vitamins
- 90% of food folates are polyglutamates (hexaglutamates) in reduced form and methyl forms

Dietary sources

- Leafy green vegetables, liver, yeast.
- Destroyed by heat e.g. excessive cooking

Role and Function

- Folate derivatives are substrates in a number of single-carbon-transfer reactions, and also are involved in the synthesis of thymidine, a pyrimidine base essential for synthesis of DNA

Role and Function

- Folate coenzymes, Tetrahydrofolate , Dihydrofolate
- Reduction requires NADPH and enzyme

- Transfer of one C fragments between molecule in red cell.
- Steps in Purine synthesis. Transfer of carbons 2 and 8 to the purine ring.
- Degradation product of histidine metabolism

	VITAMIN B12	FOLATE
Rich sources	❖**Rich: Fish, dairy products, organ meats (particularly liver and kidney), eggs, beef, and pork.** ❖**Moderate: cereals, nuts ,cheese, milk** ❖**None in vegetables and fruits**	●**Rich: Leafy vegetables such as spinach, turnip greens, lettuces, dried beans and peas, fortified cereal products, sunflower seeds and certain other fruits and vegetables are rich sources of folate.** ●**Poor: Liver ,bakers yeast, breakfast cereals**
Daily requirements	**Newborns – 6/12: 0.4 mcg** **Infants : 0.5 mcg** **Children 1 - 13: 0.9- 1.8 mcg** **Adolescents: 2.4 mcg** **Adult: 2.4 mcg** **Pregnant females: 2.6 mcg** **Breastfeeding females: 2.8 mcg**	**Adults : 100 – 200 (max 400 µg)** **Pregnant females: 600 µg** **Daily diet: 100 – 500 mcg**

Absorption	• **Cobalamin + carrier protein - pancreatic proteases in the proximal small intestine - transferred to the IF then absorbed together in the ileum**	**Folate occurs naturally attached to multiple glutamic acid molecules which must be removed by hydrolysis prior to absorption by Vit B12 dependent enzyme to form pteroylmonoglutamate Absorption is by by passive diffusion at the deudenum and jejunum**
Absorption requirement	**Intrinsic factor**	**No requirement**
Serum levels	**160 – 925 ng/l**	**3 – 6 µg /ml**
Half Life	**Approximately 6 days (400 days in the liver)**	**3 - 4 months**
Cook/process	**20 – 50%**	**50 - 90%**

Functions	**- General metabolism** **- Coenzyme in nucleic acid synthesis** **- Maintain integrity of CNS** **- Required in folate metabolism**	**- Role in cellular metabolism** **-Catalysts in nucleic synthesis** **-Folate co-enzymes are required for transfer of one carbon units from one compound to another**
Transport	**Once the IF/B-12 complex is recognized by specialized ileal receptors, it is transported into the portal circulation. The vitamin is then transferred to transcobalamin II (TC-II/B-12), which serves as the plasma transporter of the vitamin.** **Also have TCI and TCIII** **TCII carries 70-90% of circulating Vit B12 as methylcobalamin (store)**	**Weakly bound to many serum proteins** as **methyltetrahydrofolate**
Stores	**2000 – 5000 µg** **80% in liver**	**Liver and RBC: 5 – 20 mg; mainly in liver as polyglutamate**
Normal loss	**Bile, desquamation, enterohepatic circulation**	**Sweat, saliva, urine and faeces**

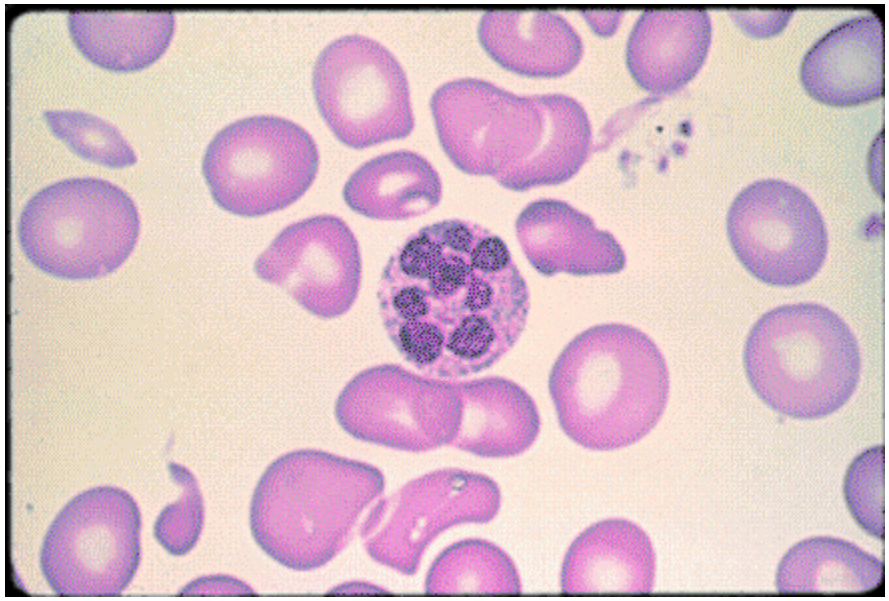
LAB STUDIES

1. TBC & PBF(macro ovalocytes,hypersegmented neutrophils)
2. Reticulocyte count
3. ASSAYS

- Serum vit B12 (ref 160 – 925 ng/l)
- Serum folate levels (3 – 15 ug/l)
- Red cell folate (160 – 640 ug/l)
- Serum homocysteine (Increase in urine in B12 def. sensitive marker for B12 depletion)
- Figlu Assays in Urine –Increase in Folate def.

4. BMA - megaloblasts

PBF



6. Investigation for cause.

   Malabsorption- Schilling test(give physio dose of labelled B12)

   Assay feacal or urinary excretion. <5% of test dose in deficiency

   Normal >10%

7. IF Assays

8. Ab demonstration

   - Parietal cells

   - IF Factor

Older tests prev done

Microbiological assays (L.gracilis,L.leischmanii, E. coli, L. casei)

(b) Radiolsotope Dilution Assays

4. Deoxyuridine suppresion test (DU) – specific and sensitive test.

(suppression less in B12 & folate deficiency)

5. Serum Methylmalonic acid & urinary methylmalonic acid excretion

Urine – colorimetric measurement,Serum – gas chromatography and mass spectophotometry,,High liquid chromatography.

Iron metabolism and iron deficiency

MBChB 2 Lecture series

Dr J. N. Githang'a

Lecture Outline

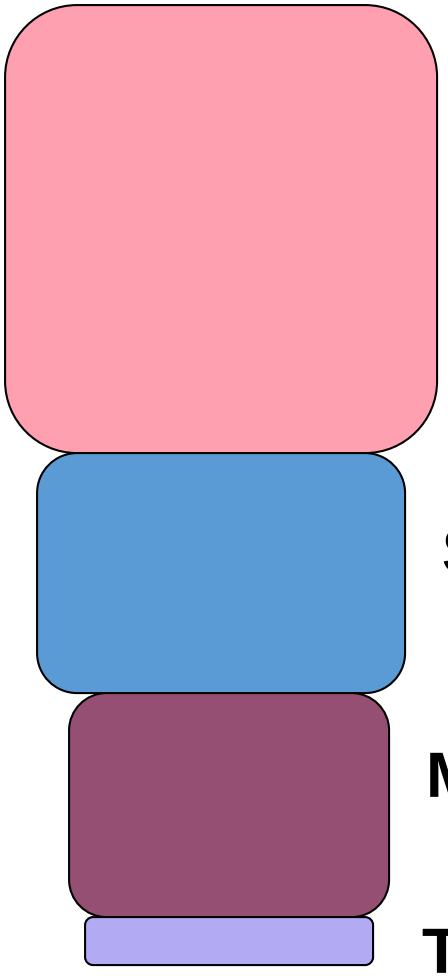
- Iron metabolism
  - Body iron compartments
  - Requirements, sources and absorption
  - Internal iron cycle, plasma iron transport
  - Iron storage
- Iron deficiency
  - Aetiology
  - Clinical features
  - Laboratory features

Iron metabolism

- Iron vital for living organisms, essential for multiple metabolic processs:
  - Oxygen transport
  - DNA synthesis
  - Electron transport
- In tissues usually in-cooperated into various proteins: heme, iron flavoproteins, heterogonous groups
- Nearly ½ of enzymes & co-factors of krebs cycle contain iron or need its prescence

Body Iron compartments

Total body iron approx 4g (3.5 – 4.5) in adult male, less in female



Hb 67% (2.5 – 3g)

Stores – 30% (0.8 – 1g)

Myoglobin - 3% (0.1g)

Tissue iron - 6 - 8mg

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Requirements

- 1-2 mg/day
- Higher requirements in
  - Growth periods
  - Pregnancy
  - Lactation
  - Females of reproductive ages

Sources of iron

- Heme iron:
  - Meats, poultry, fish
  - 10 – 15% of Fe in diet

- Non haem iron:
  - Cereals, vegetables, fruits, roots
  - 70% of dietary Fe
  - Mostly ferric form

Dietary iron:

- Variable, 10-30mg in “well balanced”
- 5 – 10 % absorbed (0.6 – 1 mg)
- Heme iron better absorbed and it enhances absorption of non-haem iron
- Reducing agents in HCL in gastric juice
- Dietary substances that enhance or inhibit dietary iron absorption

- Loss:
- I mg/day
- Desquamation epithelial cells (GIT, GU, skin,etc)

Iron balance:

Intake = loss

- Balance achieved via control of absorption
- Iron content in mucosal cells affects absorption
- Absorption increased in iron deficient states; reduced in overload states

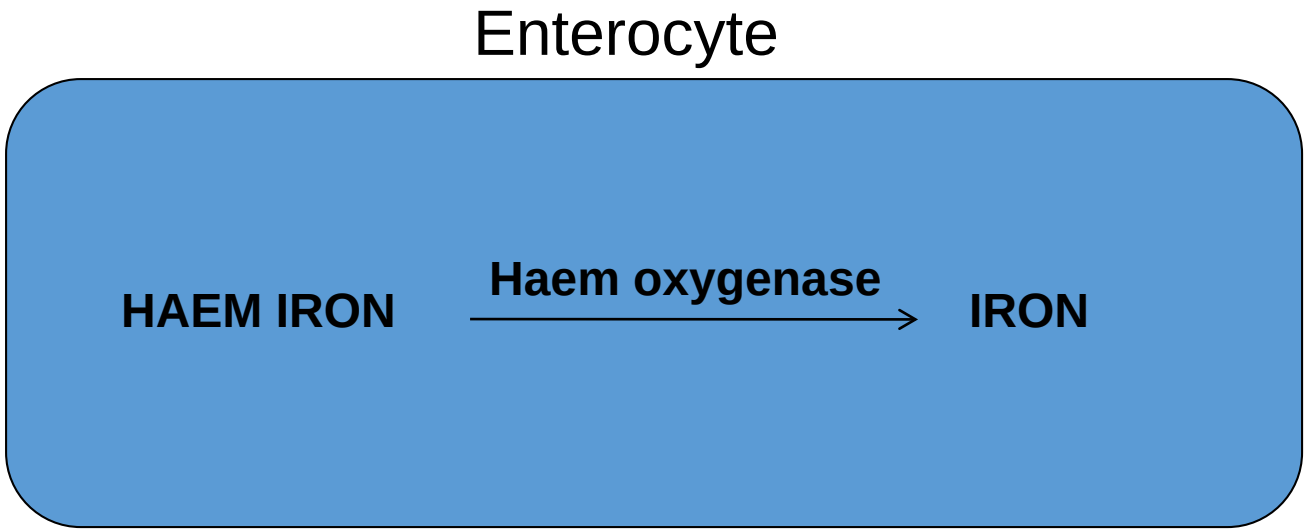
Iron Absorption

- Absorption takes place in the duodenum and upper jejenum
- Iron uptake in the proximal bowel is through 3 independent pathways for haem, ferrous (2+) and ferric (3+) forms

Iron Absorption cont.

1. Haem Pathway:

Absorbed into enterocyte by independent pathway as intact metalloprotein



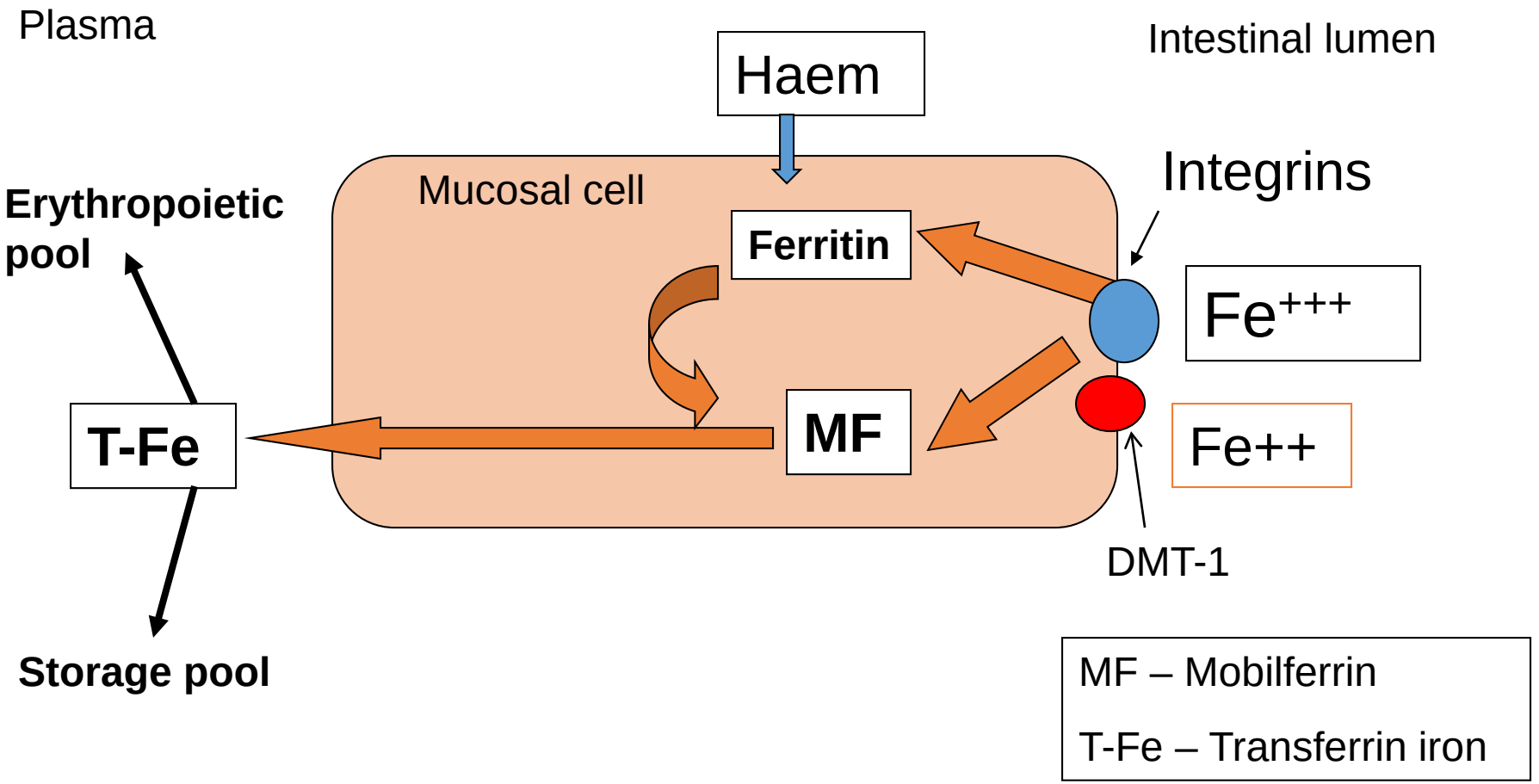
Iron Absorption cont.

Non Haem Pathways:

2. Ferric iron (3+) absorbed into the enterocyte using beta3-integrin and mobiliferrin
3. Ferrous iron (2+) uses the divalent metal transporter-1 (DMT-1) to enter the enterocyte

- HCL in gastric juice facilitates the conversion of ferric iron to ferrous iron

Absorption:


Plasma
Haem
Intestinal lumen
Integrins
Mucosal cell
Erythropoietic pool
Ferritin
Fe+++
T-Fe
MF
Fe++
DMT-1
Storage pool
MF – Mobilferrin
T-Fe – Transferrin iron

Factors affecting Fe absorption

Enhancers:

Ascorbic acid

Heme

Organic acids

Amino acids

Simple sugars

Cysteine

Inhibitors:

Phytates

Phosphates

Polyphenols

Tanin

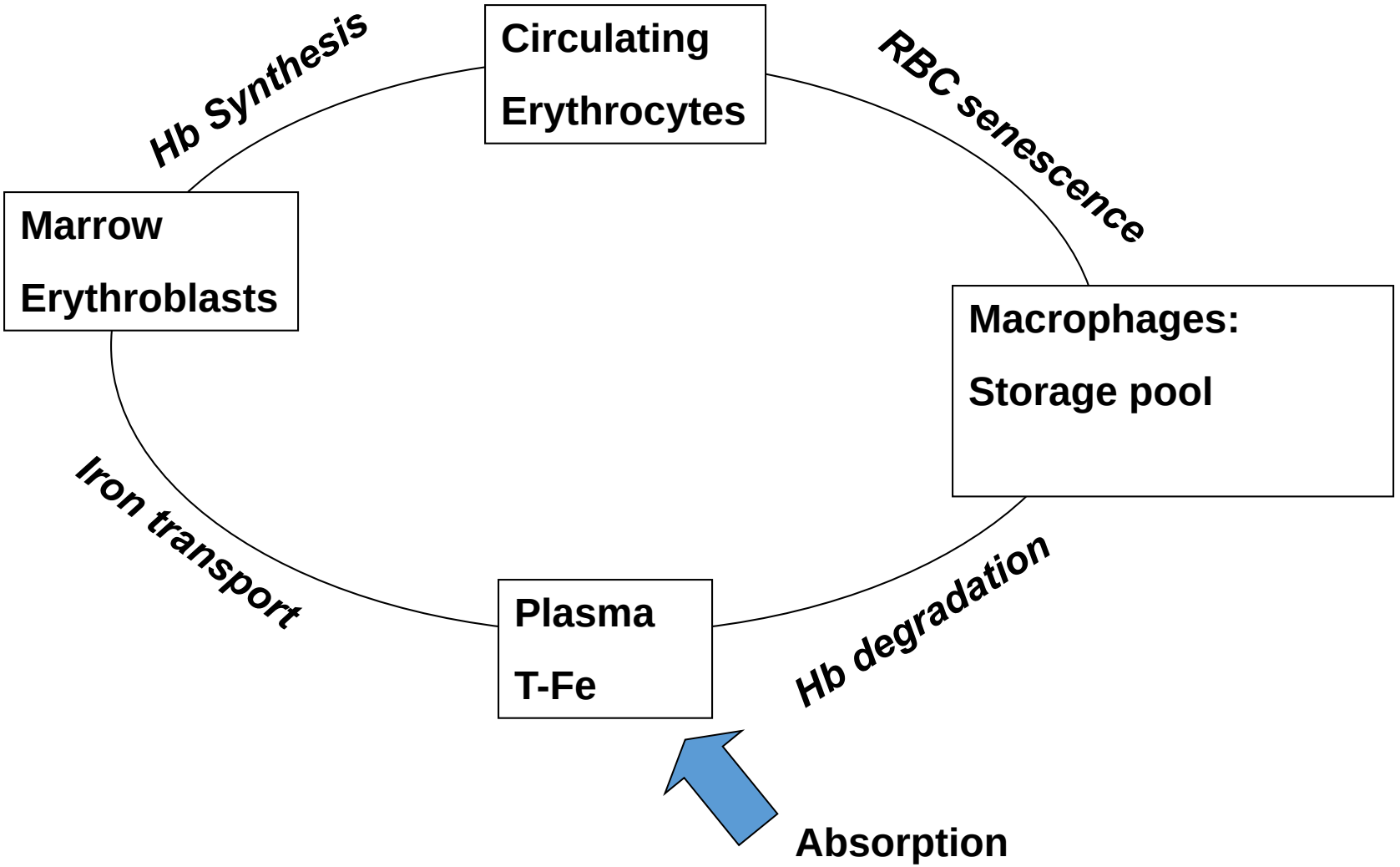
Calcium

Zinc

Soil clay

Cadmium

Internal Iron cycle



Plasma iron transport

Transferrin:

- MWt 80,000 Daltons
- Glycoprotein, synthesized in liver
- Many genetic variants
- When not compounded to iron – apotransferrin
- Normally 33% saturated with iron
- Concentration varies in physiologic and pathologic conditions

Iron storage

Ferritin & haemosiderin

Ferritin:

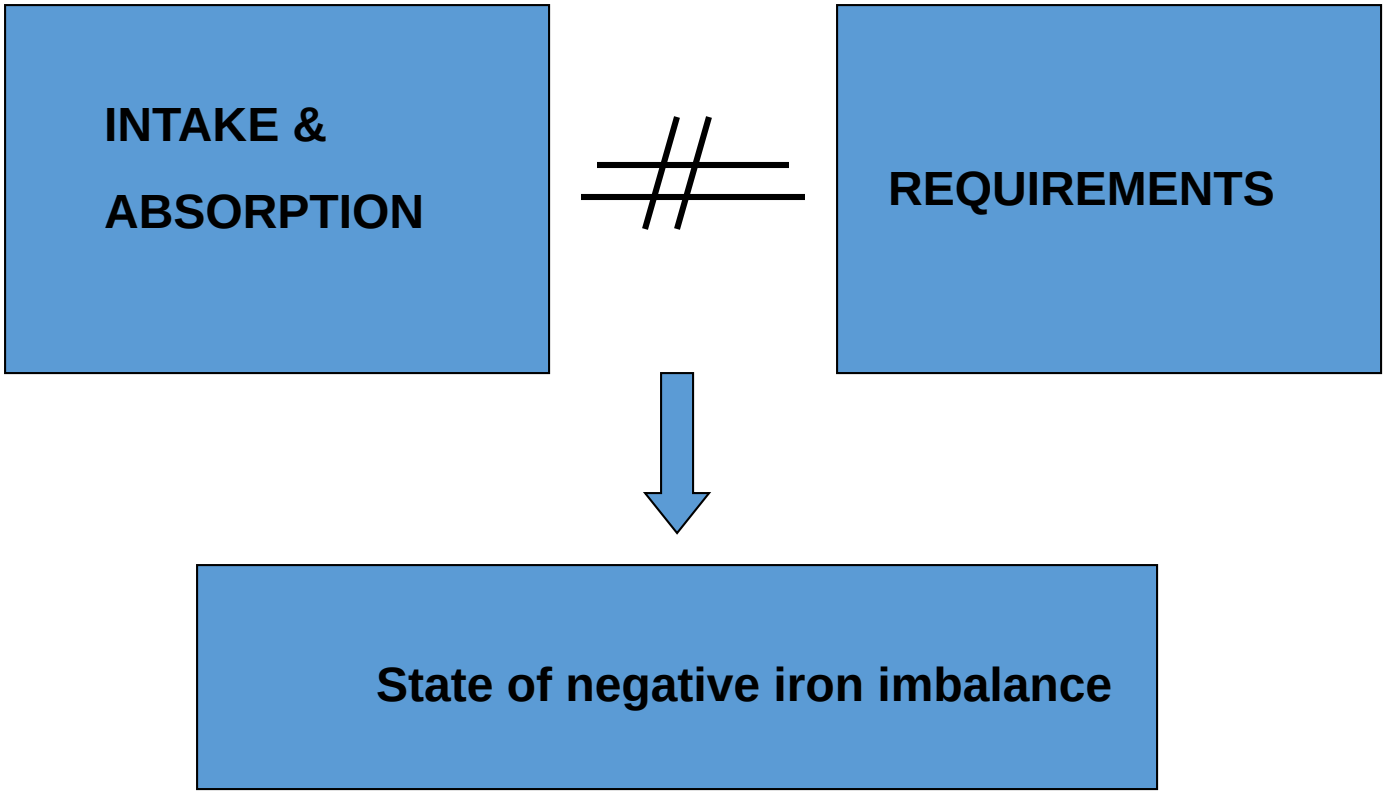
- Water soluable – ferric hydroxide and apoferritin
- Found in all body cells and tissue fuids
- Plasma content closely correlates with body stores

Haemosiderrin:

- Mainly in macrophage system (marrow, kupffer cells, spleen)
- Water insoluable
- May pathologically accumulate in tissue
- Formed from aggregates of ferritin
- Perl's stain (Prussian blue reaction) stains iron stores

Iron Deficiency Anaemia

Commonest type of anaemia worldwide



Aetiological factors for IDA

- Usually more than one implicated

1. Nutritional deficiency
2. Defective absorption

Aetiological factors for IDA cont….

3. Increased physiological requirements:

- Growth
- Menstruation
- Pregnancy
- Lactation
- Frequent blood donation

4. Pathological causes of ↑ iron loss:

- Blood loss (haemorrhage) - Chronic
  - Acute

a) Overt loss - e.g. epistaxis, haemorrhoids, haematemesis.

b) Occult loss - Peptic ulcer, helminths, chronic NSAID ingestion, cancer, etc.

CAUSES OF GI BLOOD LOSS

- OESOPHAGUS - Varices, hiatus hernia
- STOMACH - ulcer, carcinoma, gastritis, varices
- S.I.- Ulcer, Helmenthiasis, enteritis, polyps, vascular abn, volvulus, intussusception, etc

Causes of GI blood loss cont…..

- COLON- ulcerative colitis, amoebiasis, schistosomiasis, cancer, vascular abn.
- RECTUM- haemorrhoids, ulceration, cancer
- BILIARY TRACT- Trauma, cholelithiasis, neoplasm e.t.c.

CAUSES OF GENITO-URINARY BLOOD LOSS

- Menstrual bleeding (menorrhagia etc)
- Uterine fibroids
- Neoplasm’s
- Renal stones
- Inflammatory renal disease
- Parasites (urinary schistosomiasis- bilharzia)

CAUSES OF BLOOD LOSS FROM RESPIRATORY TRACT

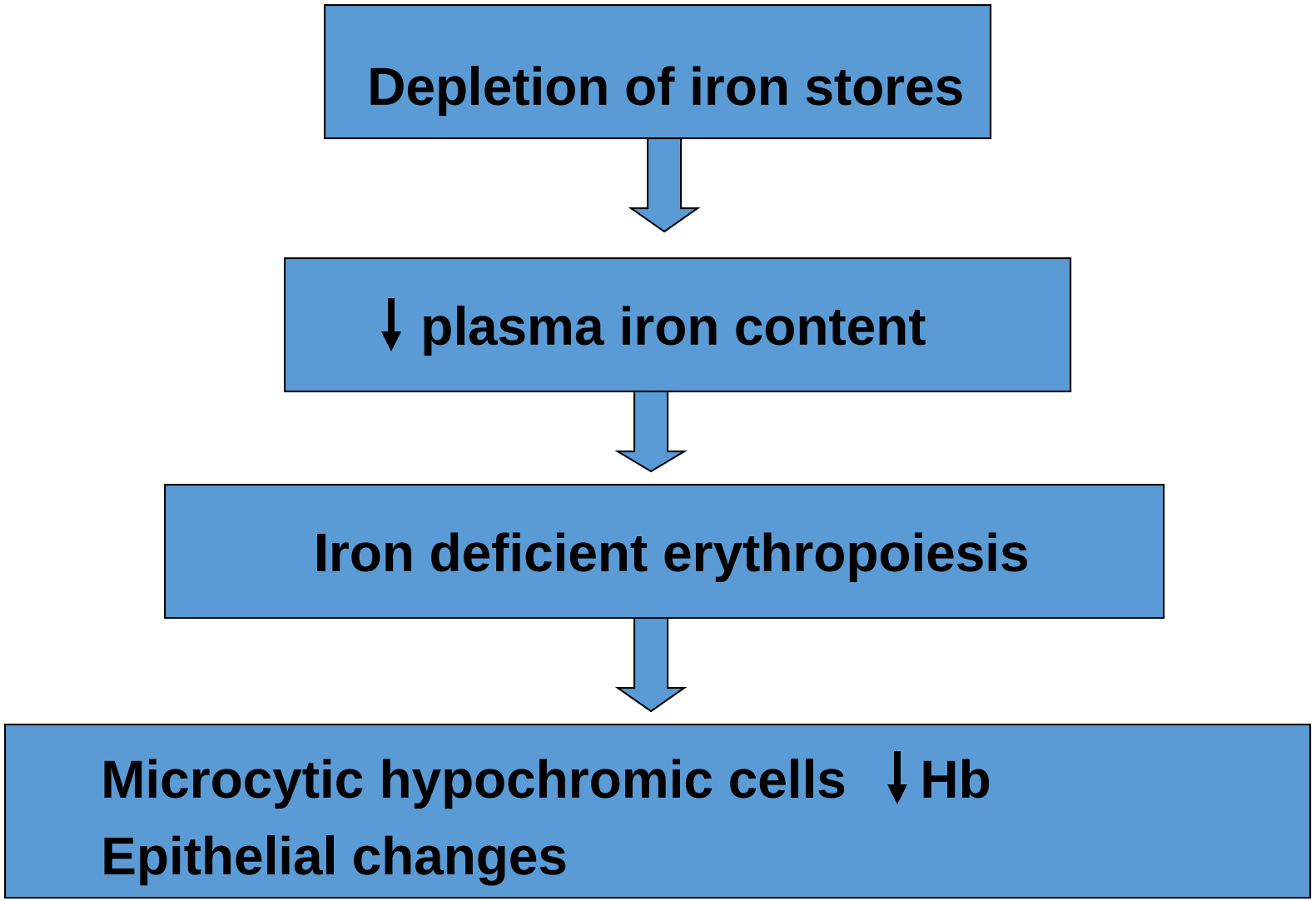
- Recurrent haemoptysis
  - Chronic infection e.g. P.T.B., Lung cancer, Valvular heart disease etc

INTRAVASCULAR HAEMOLYSIS, HAEMOGLOBINURIA

WIDESPREAD BLEEDING DISORDERS

- With chronic bleeding E.g. chronic thrombocytopenia

STAGES OF DEVELOPMENT OF IRON DEF.



Clinical Features of Iron Deficiency

- Anaemia:

  Fatigue, tiredness, dyspnoea, exercise intolerance, palpitation, headache, visual disturbances, CCF. Pallor

- Effect of chronic iron deficiency:

  Koilonychia, atrophic glossitis, angular stomatitis

- Features of underlying/causative disorder

LABORATORY FEATURES OF IDA.

1. Full blood counts:
   - Hb ↓
   - HCT/PCV ↓
   - MCV ↓ (<76 fl)
   - MCH ↓ (<26 pg)
   - MCHC ↓

- WBC – Usually N
- PLTs – N or ↑

2. PBF - Microcytic hypochromic cells
Anisocytosis, Poikilocytosis
Target cells

Microcytic hypochroic anaemia:

Low HB, Red cells with MCV and MCH less than normal

3. Biochemical Features

- Serum Fe reduced
- Serum ferritin reduced
- Transferrin saturation reduced < 33%
- TIBC increased
- Others:
  - Red cell porphyrin increased
  - Transferrin receptors increased

4. B.M.A (not necessary in uncomplicated IDA)
   - Absent stores

5. Investigate appropriately for the cause of iron deficiency (guided by Hx/PE)

E.g. Stools exam - O/C, occult blood

Thank you!

Questions?