Megaloblastic Anaemia

- B12 and folate metabolism, absorption,functions,transport.
- Megaloblastic anaemia.
- Tests for B12 & folates.
- Pernicious anaemia.

Vit B12 & folate metabolism

They r present in normal diet.
Under physiologic conditions r absorbed from GIT in sufficient amounts to cover body needs.

	Vit B12	Folates
Source:	Mainly animal diet Vegetables r poor	Liver is very rich Vegetables r rich
Effect of cooking:	Little effect 10-30 % lost	Easily destroyed by heating. 70-100% lost
Daily requirements:	2 ug	100-200 ug
Daily intake:	5-30 ug	500-1000 ug
Site of absorption:	ileum	Duodenum & jejunum
Serum level:	160-925 ng/L Methyl cobalamine	3-15 ug/L Methyl tetrahydrofolate
Stores:	2-3 mg Sufficient for 2-4 ys	5-10 mg Sufficient for 4 ms

	Vit B12	Folates
Absorption mechanisms:	Mainly active mechanism Absorption of 90% of B12 Requires presence of intrinsic factor	Mechanism is unknown
Appears in blood:	After 6 hours	After 15-30 min
Red cell level:		20 times serum level
Transport in blood:	TCI,TCII binding	Weakly bound to albumin
Loss:		Urine, stool, skin
Intracellular form:	Methyl & deoxy adenosyl cobalamine	Reduced polyglutamate
Therapeutic form:	Hydroxy cobalamine	Folic acid

Folate metabolism

Source:

vegetable (spinach) liver

Daily intake:

500-1000 ug

Daily requirement:

100-200 ug

Body stores:

5-10 mg (depleted in 4 ms)

Loss:

urine, stool, skin

Absorption & transport

- Amount: only 100 ug is absorbed
- Site: duodenum & upper jejunum (unknown mechanism).
- Appears in blood: 15-30 min after ingestion (90% is absorbed).
- Mechanism:
- 50% of blood folates r absorbed after processing

Polyglutamates hydrolysis monoglutamates

Reduction 5 methyl tetrahydrofolate → Blood

Methylation

Monoglutamates reduction

methylation 5 methyl tetrahydrofolate (THF)

Folic acid methylation 5 methyl THF

Functions of folates:

1-Purine synthesis:

form C2, C8 of purine ring

2- Pyrimidine synthesis:

Meyhylation of deoxy uridine to thymidine (imp for DNA synthesis**) {Basis of deoxyuridine suppression test}.**

3- Amino acid interconversion:

serine → glycine

homocystine → methionine

foeiminoglutamic acid (FIGLU) → glutamic acid **{FIGLU test}.**

4- Carbon carrier:

folates act as a carrier of 1 carbon from 1 compound to another

e.g: CH3 methyl gp

CHO formyl gp

CH2 methylene gp

N.B:

- **B12 is important for folate entry in RBCs to perform its function. So B12 deficiency is always accompanied by folate deficiency**

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- **Folate deficiency is not accompained by B12 deficiency. But folate therapy leads to B12 deficiency (as folate uses B12 to enter RBCs).**

Biochemical functions of B12 & folates:

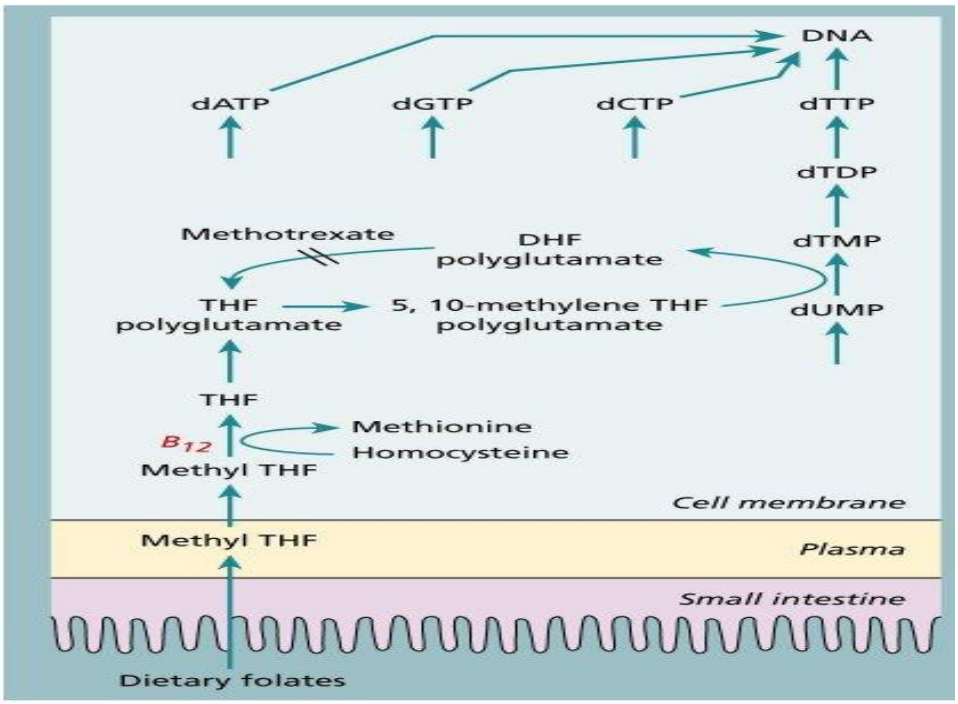


Figure 5.5 The biochemical basis of megaloblastic anaemia caused by vitamin B_{12} or folate deficiency. Folate is required in one of its coenzyme forms, 5,10-methylene tetrahydrofolate (THF) polyglutamate, in the synthesis of thymidine monophosphate from its precursor deoxyuridine monophosphate. Vitamin B_{12} is needed to convert methyl THF, which enters the cells from plasma, to THF, from which polyglutamate forms of folate are synthesized. Dietary folates are all converted to methyl THF (a monoglutamate) by the small intestine. A, adenine; C, cytosine; d, deoxyribose; DHF, dihydrofolate; DP, diphosphate; G, guanine; MP, monophosphate; T, thymine; TP, triphosphate; U, uracil.

Stages of folate deficiency:

1- ↓ serum folates (2 weeks)

2- ↑ FIGLU exceration (4 weeks)

3- ↓ RBCs folates (18 weeks)

4- Appearance of megaloblastic anaemia (20 weeks).

Causes of folate deficiency:

1-Deficient intake:

Infants, old age, alcoholics, psychiatric patients

2-Malabsorption:

coelic disease, gastrectomy, jejunal resection

Drugs: methotrexate, anticonvulsants, alcohol

Alcoholism: ↓ intake
↓ absorption
↓ folate dependent enzymes

3-Excess utilization: most important

i- ↑ demands:

in pregnancy, lactation & prematurity.

ii-Haematological disorders: (↑RBCs turnover)

N folates after doing its function, remain in RBCs to be reutilized.In haemolysis, it can't be completely reutilized as RBCs r lysed.

e.g: H.A, MF, CML

iii-Malignancy:

Leukemia, lymphoma, myeloma.

iv- Inflammatory conditions:

e.g: T.B, rheumatoid, bacterial endocarditis

Due to: ↓ intake

↓ absorption

↑ demands

fever → inhibition of folate enzymes

v- Long term dialysis:

Folates r loosely attached to proteins, so easily lost in dialysing fluid.

vi- Excess urinary loss:

in liver disease, CHF → release from damaged cells.

vii_ Antifolate drugs:

e.g: Methotrexate inhibit DHF reductase

anticonvulsants, alcohol.

4-Abnormal folate metabolism: Homocysteinuria

rare metabolic defect

Leads to ↓ conversion of homocysteine to methionine utilizing folate → folate deficiency.

5-Congenital abnormalities of folate metabolism:

↓ folate due to congenital ↓ in folate enzymes

e.g: cyclohydrase, methyl folate transferase, DHF reductase.

Found esp. in Japan

Vit B12 metabolism

Source: animal

Daily intake: 5-30 ug

Daily requirement: 2 ug

Stores: 2-3 mg (suff. For 2-4 ys)

Forms:

A- Natural: Methyl cobalamine (in blood)

Deoxy adenosyl cobalamine (in liver & other tissues)

B- Pharmacological: Cyano cobalamine

Hydroxy cobalamine

Absorption:

Passive:

- inefficient (less than 1%)
- in jejunum & ileum

Active:

- more important
- need intrinsic factor
- in ileum

Intrinsic Factor

It is a glycoprotein.

MW 45,000-60,000.

It is synthesised in fundus & body of stomach by microsomes or endoplasmic reticulum of gastric parietal cells.

When forms complex e' vit B12, it resists digestion by enzymes (contrary to IF alone).

Mechanism : Active mechanism of vit B12 absorption:

Stomach :

Dietry B12 pass to stomach

↓

released from protein complexes (by enzymes in stomach)

↓

binds to R- binder protein in stomach

Duodenum: ↓

reaches the duodenum & neutralized

↓

leaves R-binder protein (digested by pancreatic secretion) & B12 attaches to IF (2 IF mol bound to 2 vit B12 mol)

Ileum: ↓

pass to ileal receptors & attach to brush border of ileal mucosa, binds to IF ileal R

↓

Receptor mediated endocytosis of B12 & release IF

↓

Blood:

B12 appear in blood after 6 hs & attach to TCII (probably synthesized in ileum)

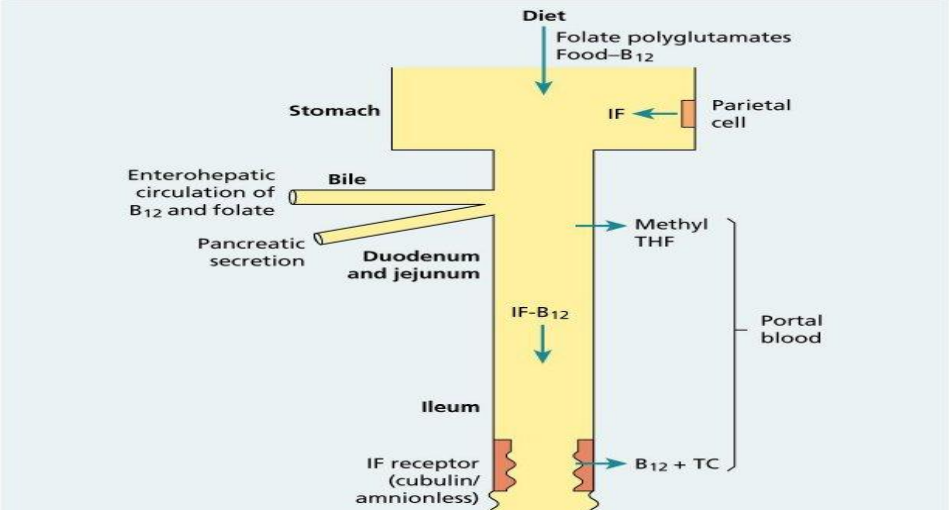


Figure 5.2 The absorption of dietary vitamin B_{12} after combination with intrinsic factor (IF), through the ileum. Folate absorption occurs through the duodenum and jejunum after conversion of all dietary forms to methyl-tetrahydrofolate (methyl THF). TC, transcobalamin.

Regulation of absorption:

-Ileum has limitd capacity to absorb vit B12 due to limited no. of receptors.

-Also receptors become refractory to absorption for 6 hours after absorption.

Transport of vit B12

2 main transport proteins r found in plasma

	Transcobalamin I	Transcobalamin II
Synthesis:	By granulocytes	By liver, ileum & macrophages
Structure: **MW:**	Glycoprotein 56,000-58,000	Glycoprotein 38,000
Binding to B12	Tightly (not available for tissues), so it is a store for B12	Loosely (gives it easily to tissues)
Carries:	Most of B12 (2/3 of it) 450 ng/L	Small amount (only 20-60 ng/L)
Clearance:	slowly	rapidly
Deficiency: (Congenital def.)	Doesn't cause megaloblastic an. but ↑serum B12	causes megaloblastic an. N B12 in serum
Increased:	In any disease ↑ granulocytes e.g: MPD (CML,PCV) infections	In liver diseases Autoimmune diseases Gaucher
If increased:	↓ serum B12	No elvation in serum B12

Functions of B12:

- Methyl malonyl Co A $\xrightarrow{B12}$ succinyl Co A
- Acts as a carrier of methyl group from MTHF in:
  Homocystiene ⟶ methionine
  deoxy uridine ⟶ thymidine
- B12 is important for folate entry to RBCs, so B12 deficiency is always accompanied by folate deficiency.

Causes of vit B12 deficiency:

1- Inadequate intake:

adults: in some religions & vegetarians

infant : of severly deficient mothers (develop megaloblastic an. 3-6 ms after birth).

2- Malabsorption:

a- Gastric causes:

pernicious anaemia

gastrectomy

B- Intestinal causes:

- Stagnant loop syndrome
- Ileal resection
- Tropical spure
- Fish tape worm
- Selective malabsorption of B12: **(Imersland – Grasbeck syndrome):**

AR

absent ileal Receptors

proteinuria or aminoaciduria inspite of N kidney functions

- Malabsorption causing minimal deficiency of B12:
  Crohn's disease
  Coeilic disease
  Simple atrophic gastritis
  Drugs e.g: neomycin
  Severe chronic pancreatitis
  Altered ph of ileum (ph below 6 ileal uptake of IF-B12 complex) e.g: **Zolinger Ellison Syndrome** (congenital acidic ph of intestine), ttt e' KCL.

3-Abnormal metabolism of vit B12:

A- Congenital:

i- Transcobalamin II deficiency or abnormalities:

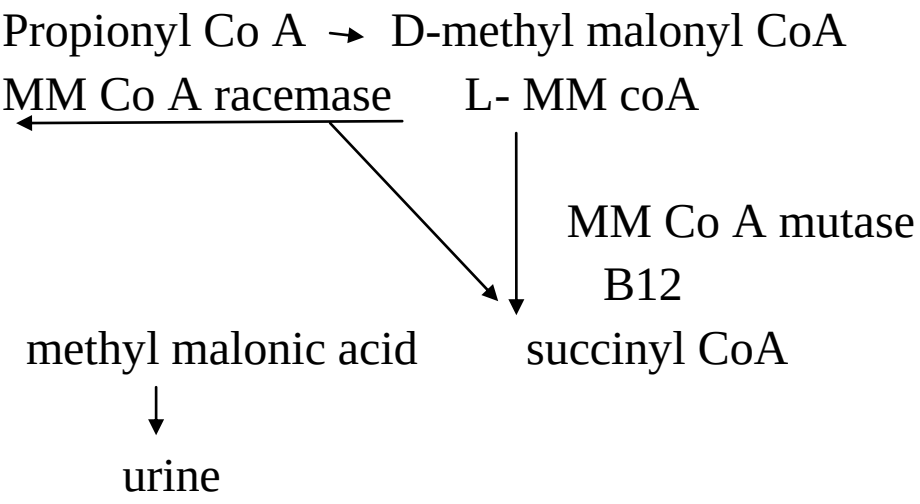
Infants develop megaloblastic an. In few weeks after birth (because of failure of B12 to enter BM & other cells from plasma).

ttt e' large doses of B12 → enter BM by passive diffusion (x tranport protein).

N.B: serum B12 is N as most B12 in plasma+ TCI (functionally dead).

{Megaloblastic anaemia e' Normal serum B12}

2- Congenital methyl malonic aciduria:



B- Acquired:

i- Nitrous oxide inhalation:

in anaesthesia, ICU

Active Co b I inactive Co b II

ii- Cyanide inactivation: of B12