The Chemistry of Hematology

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Objectives

- Explain how methylmalonic acid and homocysteine are related to B_{12} and folate deficiencies
- List the common laboratory findings in iron deficiency anemia
- Describe the chemical and molecular approaches to screening for Hereditary Hemochromatosis

Topics to be Covered

- Oxygen Concentration
  - Hemoglobin Species
- D-Dimer
  - Separate talk later in course
- Anemia
  - Hemolysis
  - Macrocytic Anemia
  - Microcytic Anemia

Analytes

- Hemoglobins
  - Oxyhemoglobin
  - Reduced (Deoxy) Hemoglobin
  - Methemoglobin
  - Carboxyhemoglobin
- D-Dimer
- LD (LD isoenzymes)
- Haptoglobin

- Vitamin B12 (cobalamin)
- Folic Acid
- Homocysteine
- Methylmalonic Acid
- Iron
- Transferrin/TIBC
- Ferritin

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O_2 Concentration in Blood

- not simply PaO_2
- not simply Hct (or, more precisely, Hgb)
- not simply O_2 saturation
- rather, a combination of all 3, "O_2 content"

$$= 0.003 * PO_2 + 1.4 * [Hgb] * [\%O_2sat]$$

(mm Hg; g/dL)

$$= 0.0225 * PO_2 + 1.4 * [Hgb] * [\%O_2sat]$$

(kPa; g/dL)

Different Scenarios Illustrating Oxygen Content Concepts

comments	PaO2	%O2Sat	Hgb	Hct	Dissolved Oxygen	Hgb-Bound O2	O2 Content
Normal	100	100	14	42	0.3	19.6	19.9
Low Hct	100	100	7	21	0.3	9.8	10.1
Low PaO2 (lung disease)	25	50	14	42	0.1	9.8	9.9
50% Methemoglobin	100	50	14	42	0.3	9.8	10.1
Very Low Hct No Transfusion	100	100	2	6	0.3	2.8	3.1
Hyperbaric Chamber	2200	100	2	6	6.6	2.8	9.4

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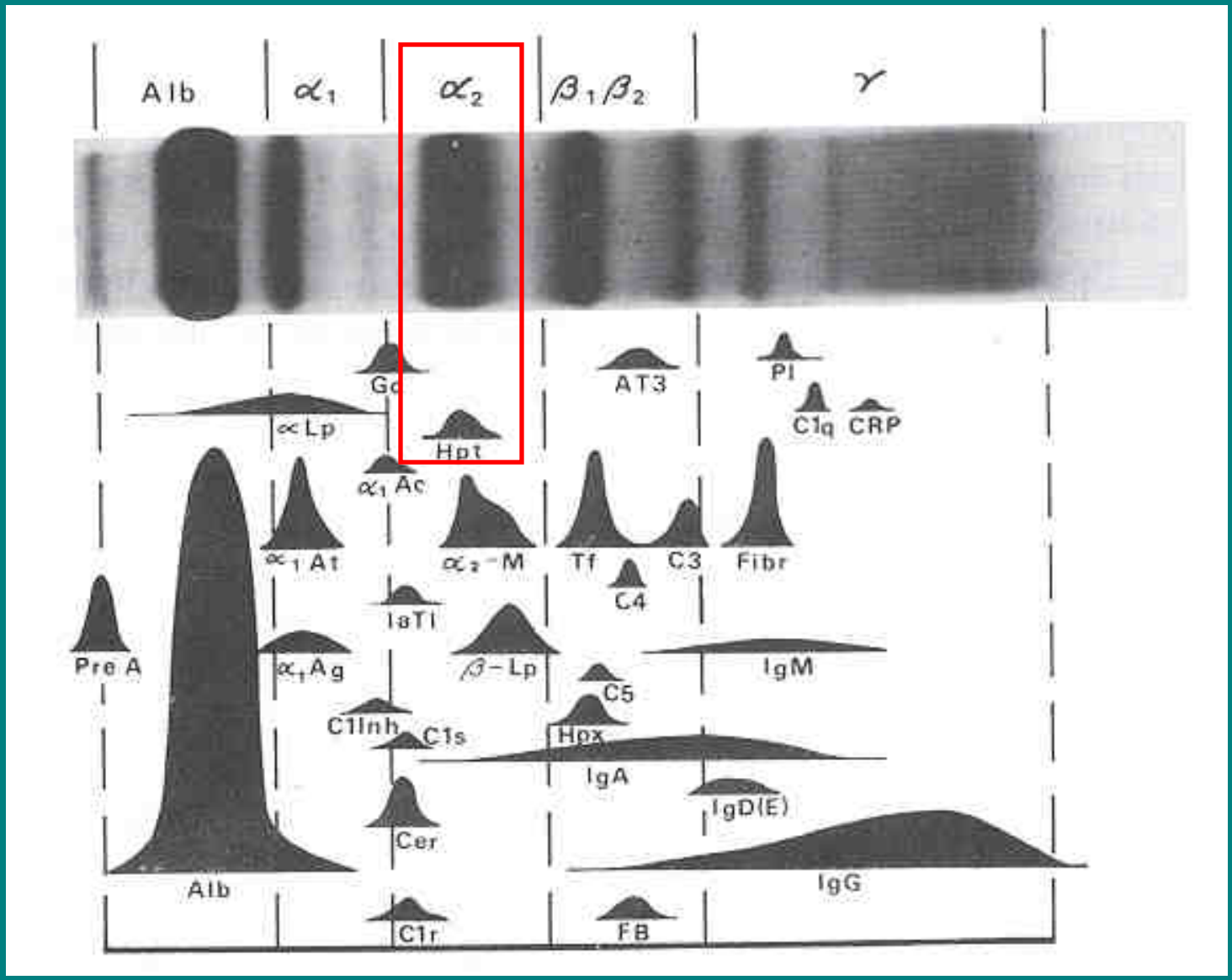
Hemolysis (any cause)

- evidence of hemolysis
  - ↑K, ↑LD *(LD1)*, ↑AST
  - ↑Hemoglobin → ↓Haptoglobin
    - once haptoglobin depleted, plasma hemoglobin ↑s
      - by eye, one can detect as little as 30-50 mg/dL
      - automated indices more sensitive, precise, and accurate
  - ***↑folate, too***

- *NB*: in vitro hemolysis
  - will decrease, but not eliminate, haptoglobin


Alb
α1
α2
β1β2
γ
Gc
AT3
PI
C1q
CRP
α Lp
Hpt
α1 Ac
α1 At
α2-M
Tf
C3
Fibr
C4
IαTI
Pre A
α1Ag
β-Lp
IgM
C5
C1Inh
C1s
Hpx
IgA
IgD(E)
Cer
Alb
IgG
C1r
FB

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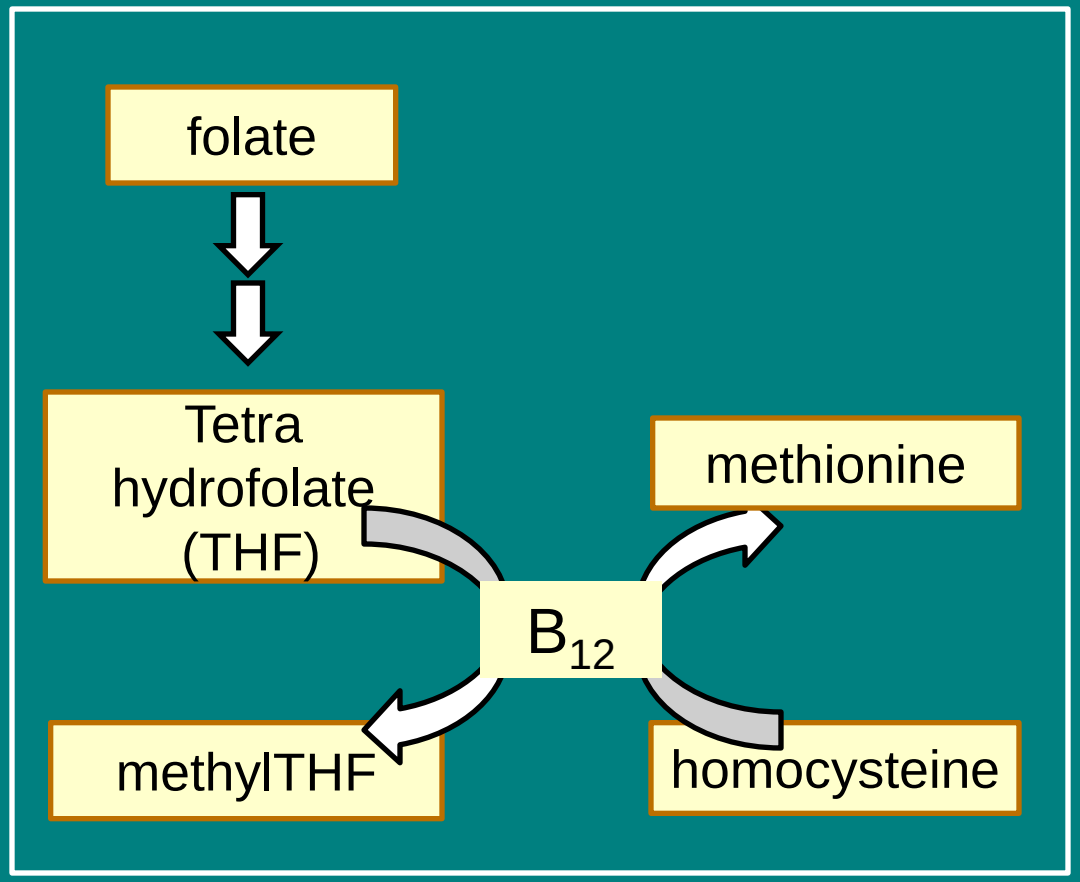
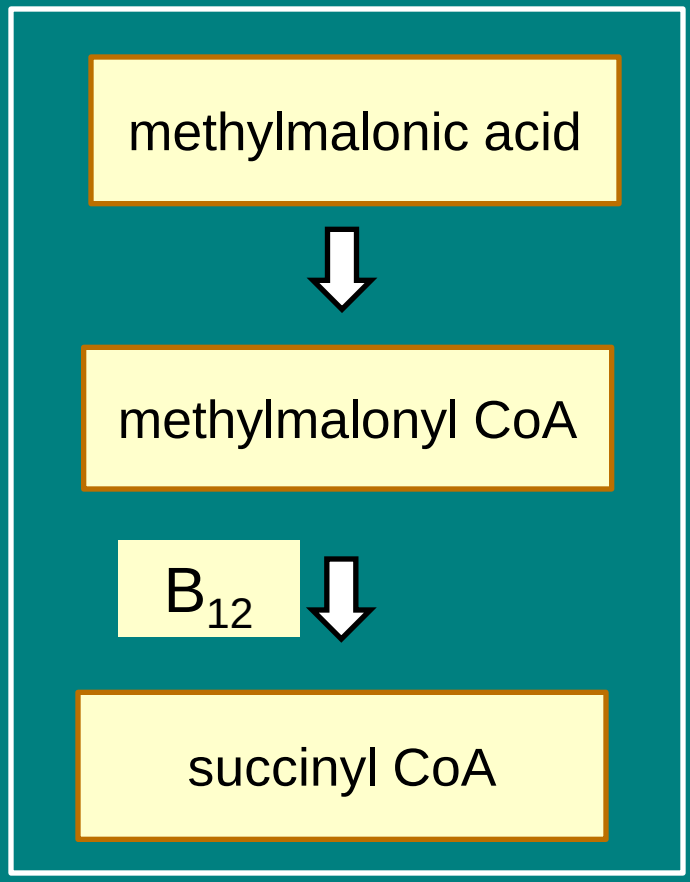
B12 & Folate

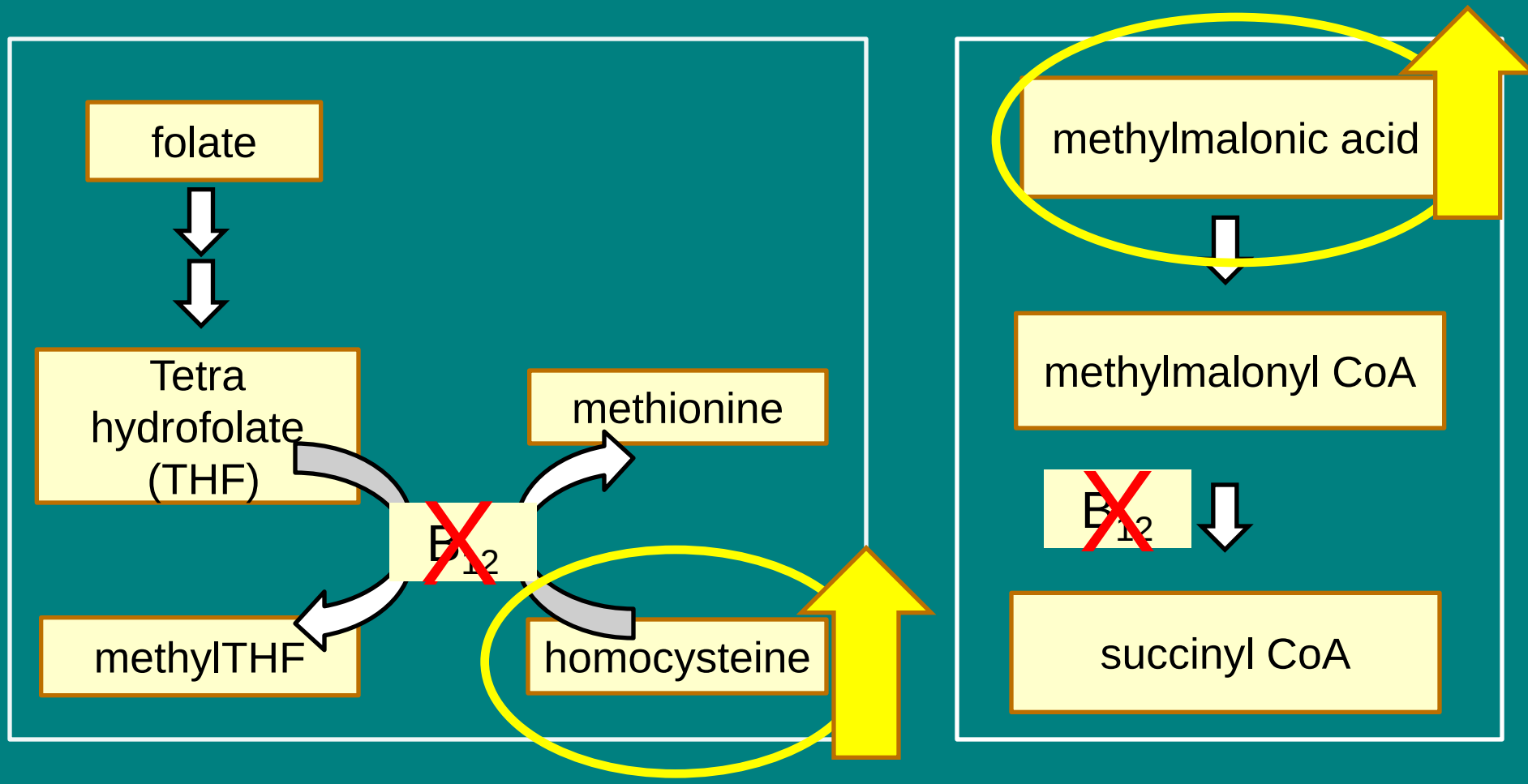
- linked in a key metabolic pathway
  - methyl transfers
  - along with homocysteine (!)
- historically, used to establish cause of megaloblastic anemias
- radioassays (RA) [not radioimmunoassays (RIA)]
  - use binding proteins not antibodies
    - e.g., for B12, use Intrinsic Factor
  - by RA, can be measured simultaneously: $^{125}I + {}^{57}Co$
    - isotopes have different gamma radiation energies and are distinguishable

B_{12} Metabolic Pathways



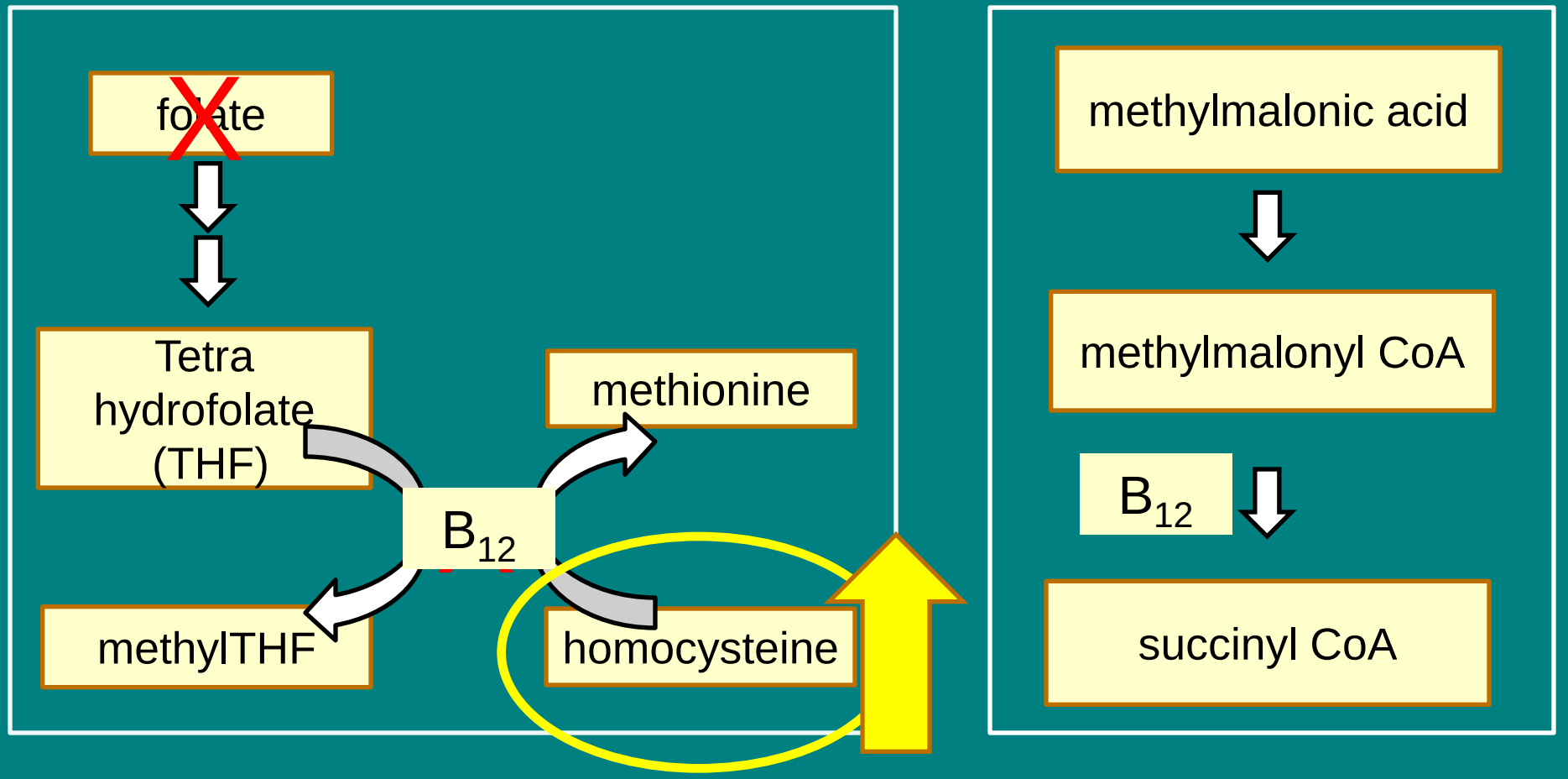


B_{12} Metabolic Pathways



B_{12} deficiency: homocysteine **and** methylmalonic acid increase

B_{12} Metabolic Pathways



B_{12} deficiency: homocysteine **and** methylmalonic acid increase
folate deficiency: **only** homocysteine increases

Fast Forward to 2005

- radioisotopes are no longer in wide use
- ordering panels (B_{12} + folate) are frowned upon
- most orders are not in patients with anemia
    - B_{12} ordered most often in peripheral neuropathy
- clinical scenarios are different
  even with megaloblastic anemia, one test probably suffices
    - diet/time course
        - vegans get B_{12} deficiency (years)
        - without green, leafy vegetables, folate deficiency (months)
        - with new US fortification, difficult to get folate deficiency

Jurassic Lab
(James D. Faix, MD)

- B_{12}, even when measured well, has limitations
  - low values may not mean deficiency
  - normal values may be functionally low
- transcobalamins: binding proteins
  - TCII-bound B_{12} is the only physiologically active form
  - but the majority of B_{12} (70-80%) bound to TCI and TCIII
- methylmalonic acid
  - not easily run
  - increases with renal insufficiency
  - (but not with folate deficiency)

A Few Words about Pernicious Anemia

- lack of intrinsic factor (IF), causing B_{12} deficiency
- increased stomach pH (less acidic) ➔ high gastrin
  - second most common cause of hypergastrinemia
  - most common: antacids
    (not Zollinger-Ellison syndrome (gastrinoma)!)
- review Schilling's Test (if only for historical reasons)
  - why is it OK to give radioisotope orally?
  - note 2 phases

Schilling's Test

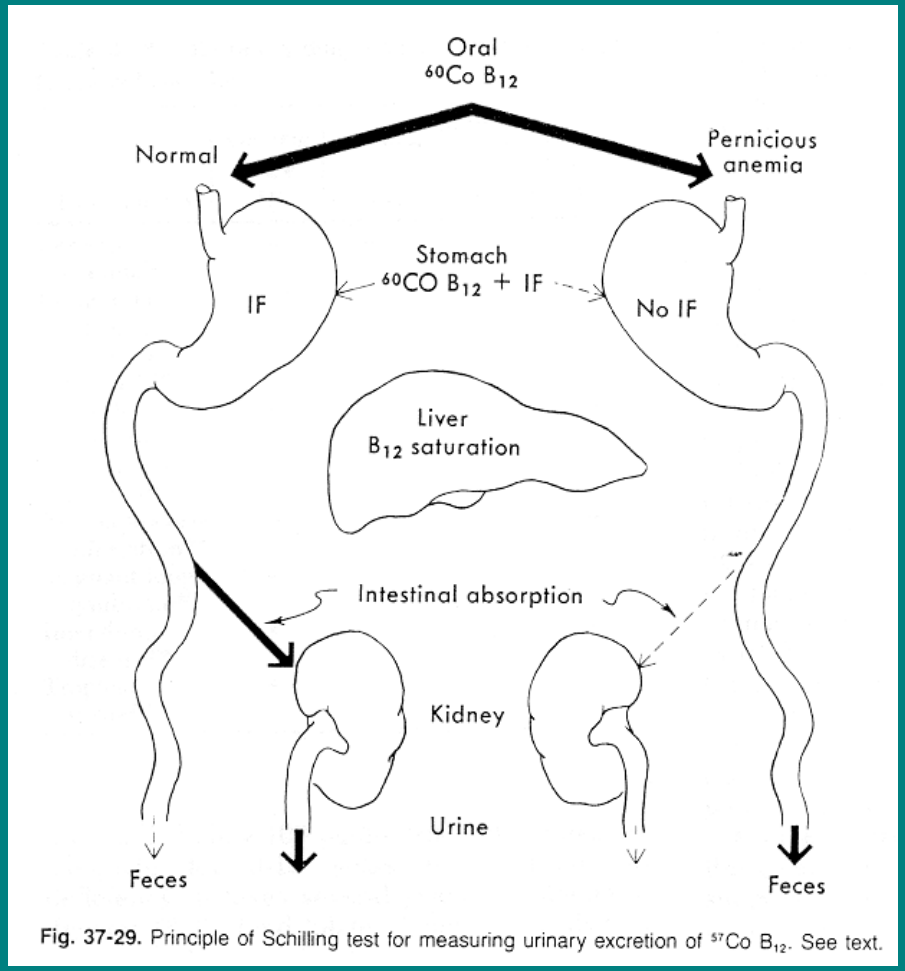


Fig. 37-29. Principle of Schilling test for measuring urinary excretion of ^{57}Co B_{12}. See text.

- **Phase 1**
  - give "cold" B_{12} by injection
  - give labeled B_{12} orally
  - measure urinary radioactivity
- **If low, →Phase 2**
  - give "cold" B_{12} by injection
  - give labeled B_{12} orally **+ IF**
  - measure urinary radioactivity
    - if it corrects, PA
    - if not, malabsorption

Folate

- may belong in Jurassic Lab, too
    - with recent increase in US dietary supplementation,
        - folate deficiency is a rare phenomenon
        - it's been months since we've seen a low one at BIDMC *(for 2006, 7900 folates ordered, 2 values <2.0 ng/mL)*
    - on the other hand, a normal value can be reassuring
- as noted earlier,
    - RA (not RIA)
    - homocysteine high, but MMA normal (vs. B_{12} deficiency, where both are elevated)

Serum vs RBC folate

- RBCs are rich in folate
  - theoretically, RBC folate should be more sensitive
  - empirically, it adds little to diagnostic accuracy
  - of course, I would argue that folates are rarely needed
  - logistically, adds a step
  - if you measure folate at all, use serum folate

- NB: hemolyzed samples . . .
  - “serum” folate will be falsely elevated
  - in slide for effects of hemolysis, I’ve added “folate”

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Stages in Iron Deficiency Anemia

1. bone marrow iron stores get depleted

2. ferritin decreases
   - specific but not sensitive
     - a low value is helpful
     - a normal value does not rule out iron deficiency
       - » acute phase reactant

3. serum Fe ↓s, serum TIBC ↑s, Fe/TIBC ↓s (<15%)

4. hemoglobin ↓s

5. MCV ↓s
   - may not happen at all (!)
   - other causes of hypochromia (e.g.,thalassemia)

Tests for Iron Deficiency (page 1 of 3)

- Serum Fe
  - measures Total Fe
  - based on Beer's Law (A = abc)
    - add dye specific for Fe
    - then measure absorbance (proportional to [Fe])
  - note diurnal variation
    - high in am (like cortisol)

Tests for Iron Deficiency (page 2 of 3)

- TIBC
  - add excess Fe
  - separate free Fe from bound Fe (manual)
  - measure Total Fe (bound) as above
- Transferrin (Trf)
  - immunoassay
    - homogeneous (turbidimetry, using antibodies to Trf)
    - more easily automated
  - calculate TIBC (to be used in Fe/TIBC calculation)
    - based on empirical correlation/regression

Tests for Iron Deficiency (page 3 of 3)

- Ferritin
  - standard heterogeneous immunoassay
  - still, fully automated
  - as noted earlier, it's an acute phase reactant (APR)
    - if it's low, it's helpful
    - if it's normal, it's not

Bottom Line

- For Fe deficiency, order
  - Ferritin
    - if low, you're set
    - but a normal value doesn't rule it out (APR)
  - Serum Fe
    - should be low
  - TIBC (or Transferrin to get TIBC)
    - should be high
  - ➔ Fe/TIBC should be low (<15%)

Mixed Reviews on Tests So Far

The Good

-
-
- haptoglobin is a good test
-
-
-
-
- Fe/TIBC is a good test

The Not So Good

- O_2 concentration interplay of 3 factors
- pulse oximetry can be (dangerously) misleading
-
- B12 & folate are questionable tests
- homocysteine & MMA may not add much
- be careful with ferritin

Hereditary Hemochromatosis (HH)

- a common disease
  - incidence = 1:200 Caucasians!
- effective therapy available = phlebotomy
  - especially if instituted early (<40 years old)
- genetics
  - HFE gene, short arm chromosome 6
  - 2 mutations identified: C282Y, H63D
    - >90% of HH patients are C282Y/C282Y
    - another 3-5% C282Y/H63D
    - among C282Y homozygotes
      - » 100% have increased Fe/TIBC
      - » **but** only 58% have iron overload

HH Screening

- controversy: chemical vs molecular
  - Fe/TIBC
    - costs $2,700 per case
  - C282Y
    - costs $110,000 per case
    - because of false positives/incomplete penetrance
- Fe/TIBC screening is more cost-effective than most laboratory tests currently done

HH Screening Protocol

- Step 1: fasting Fe/TIBC
  - >50% for women, >60% for men
  - 92% sensitivity, 93% specificity, PPV=86%
  - overnight fast eliminates 80% of false positives
  - lowering thresholds ↑s sensitivity but ↓s PPV
- Step 2: molecular
- some advocate adding ferritin to Step 1

References on HH Screening

- Schmitt B, Golub RM, Green R. Screening primary care patients for hereditary hemochromatosis with transferrin saturation and serum ferritin level. **Ann Intern Med 2005;143:522-36**

- Whitlock EP, Garlitz BA, Harris EL, Beil TL, Smith PR. Screening for hereditary hemochromatosis. (genetic testing) **Ann Intern Med 2006;145:209-23**.

Back to the Future

- “winning” tests from this lecture
  - haptoglobin
  - Fe
  - TIBC (calculated from transferrin)

- not only for evaluating (some forms of) anemia
- but also for screening for hemochromatosis (despite the previous references)

Self-Assessment Question 1

Which of the following tests should rarely, if ever, be ordered in the US?

A) Serum B12

B) Serum Haptoglobin

C) Serum Iron

D) Serum Folate

Self-Assessment Question 2

Which of the following does ***not*** suggest iron deficiency?

A) A low ferritin concentration
B) A low iron concentration
C) A high transferrin concentration
D) A high methylmalonic acid

Self-Assessment Question 3

Which of the following is ***not*** associated with hemolysis?

A) Low serum folate concentration
B) High serum LD activity
C) Low serum haptoglobin concentration
D) High serum K concentration

Answers

1 (D) Serum Folate

2 (D) A high methylmalonic acid

3 (A) Low serum folate concentration