Megaloblastic Anemia
Folate and Cobalamin Deficiency

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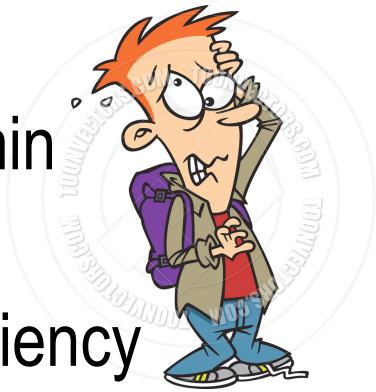
Bottom Line at Start (BLAST)

The Participant…

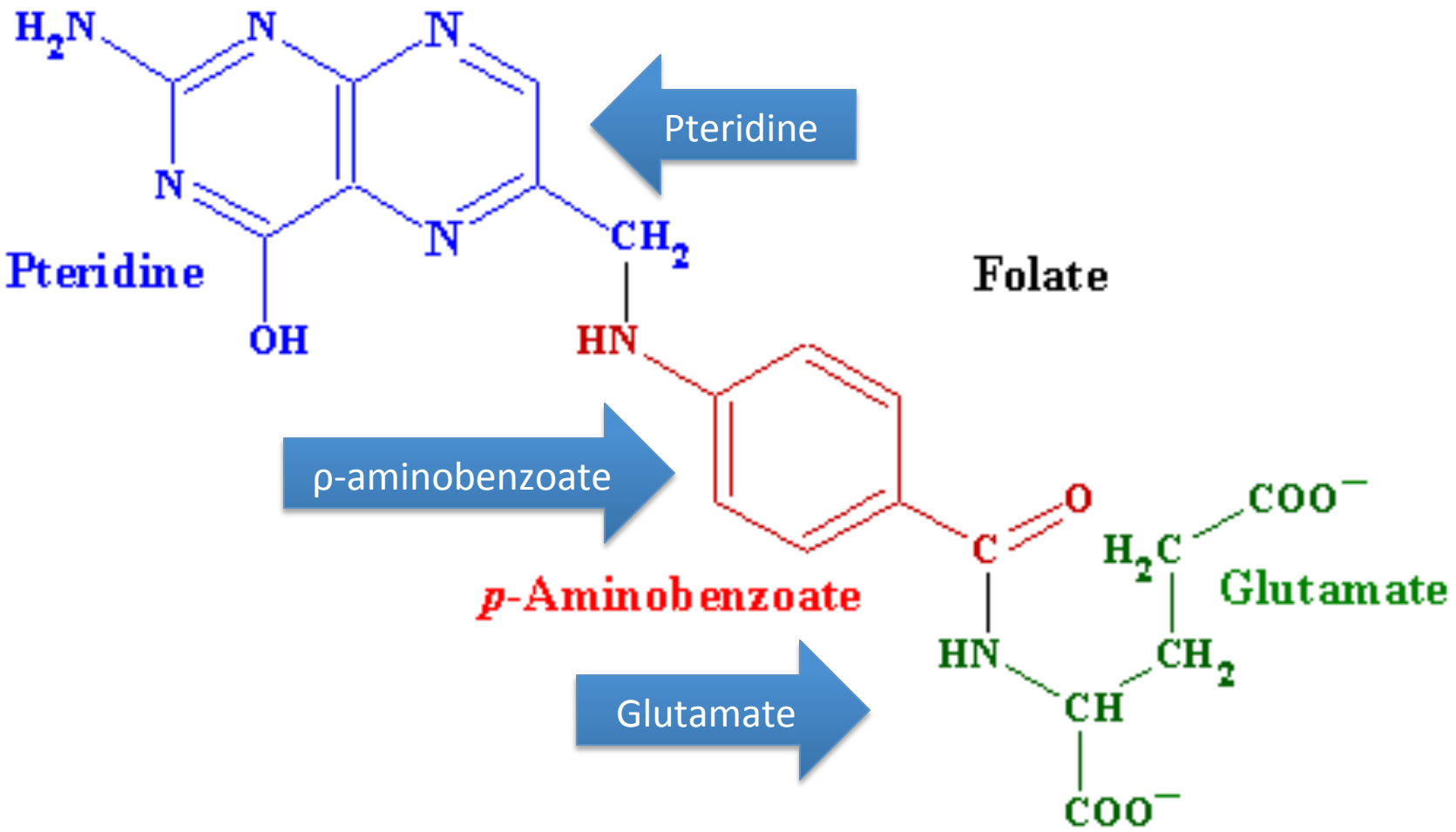


- Diagrams the biochemistry of folate and cobalamin (cyanocobalamin, vitamin B_{12})
- Relates the causes of folate and cobalamin deficiency
- Recognizes the clinical symptoms of megaloblastic anemia
- Recognizes the peripheral blood and bone marrow cell morphology in megaloblastic anemia
- Uses lab procedures to identify megaloblastic anemia and differentiate it from refractory and other macrocytic anemias
- Recounts the cause and effect of homocysteinemia

Folate Sources and Folic Acid

- Folate from leafy vegetables (foliage), fruits, organ meats, nuts, beans, orange juice, dairy products, grains, cereals
- Boiling reduces folate concentration
- Fortification with 140 µg folic acid/100g cereal grain in 1998
- Absorbed in jejunum, converted to 5-methyl tetrahydrofolate (THF)
- Minimum daily adult requirement
  - 400 µg synthetic folate equivalent (folic acid)
  - 600 µg folic acid during pregnancy, 500 µg during lactation
- Cleared to tissues, primarily liver
  - Absence of dietary folate leads to anemia in 3–6 months

Folate is Pteroylmonoglutamate



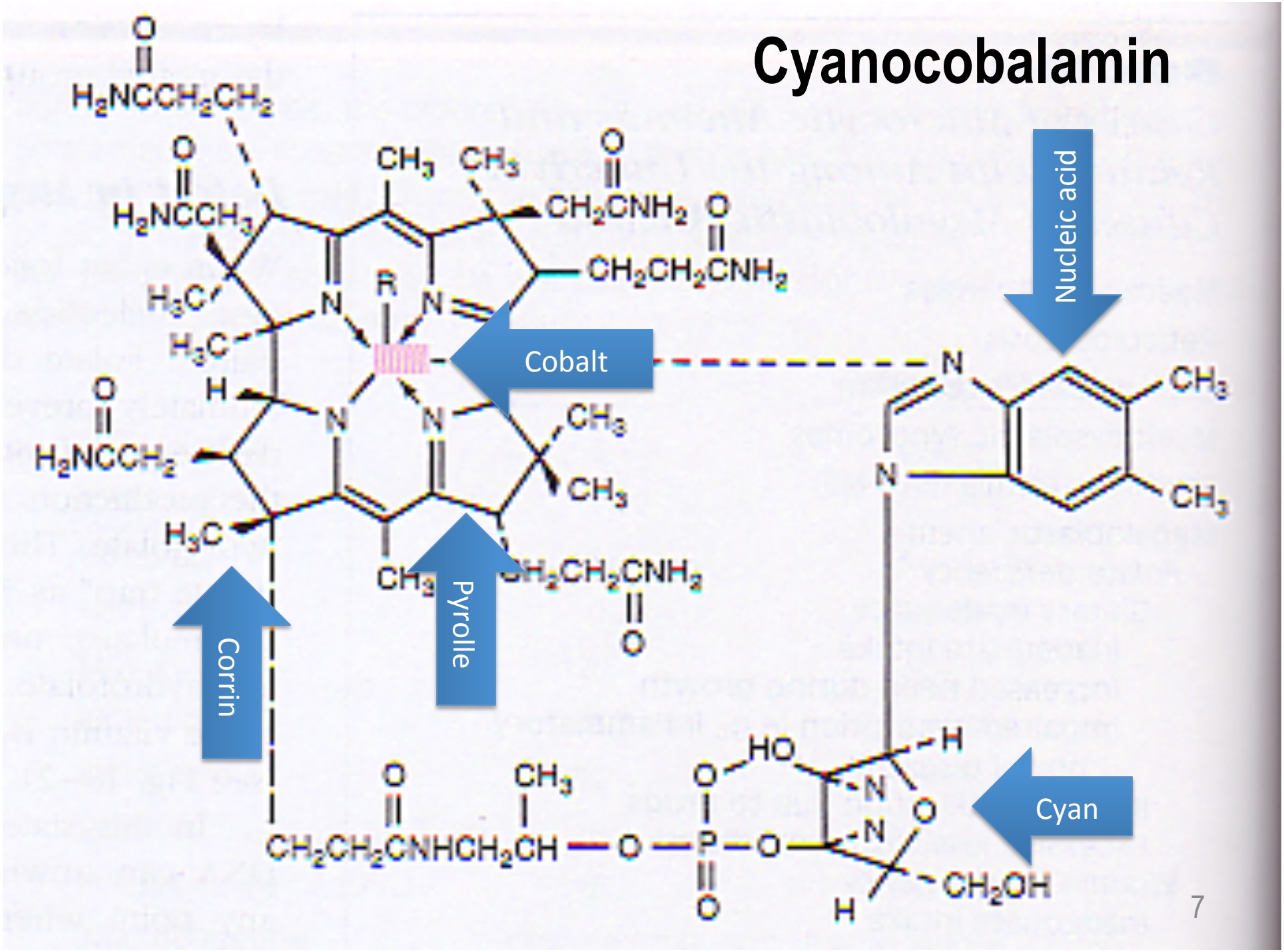
Folate Deficiency

- Decreased folic acid intake
  - Poor nutrition: alcoholism, poverty, premature infants
  - Impaired absorption: inflammatory bowel disease, tropical/non-tropical sprue, diverticulitis
  - Impaired folate utilization due to drugs; chemotherapy
- Increased folate requirements
  - Host competition: blind loop syndrome
  - Pregnancy, lactation
  - Chronic hemolytic anemia or chronic blood loss
  - Solid tumors, lymphoma, myeloproliferative neoplasms
  - Chronic renal dialysis

Dietary Cobalamin (Vitamin B_{12})

- Source: synthesized by bacteria
- Humans ingest from animal products, milk, cheese, eggs, cyanocobalamin supplements, not in fruits or vegetables
- MDR in adults is 5–7 µg/d, 70% absorbed
- Normal stores last 1000 days
- Structure
  - "Corrin" ring: four pyrolle groups surround cobalt
  - Connected by a nucleotide
  - β-group: cyano, hydroxyl (inactive); methyl, adenosyl (active)

Cyanocobalamin



Cobalamin Metabolism

- Cobalamin binds salivary haptocorrin (HC)
- Stomach pepsin digests HC–cobalamin in HCl environment
  - Pepsin absent in pancreatitis
- Free cobalamin rapidly binds parietal cell intrinsic factor (IF)
- IF-cobalamin pass safely to ileum and crosses the mucosal lining
- Free cobalamin binds transcobalamin (TC) for plasma transport
- In t ½ 6–9 minutes TC-cobalamin circulates to liver, BM, all rapidly dividing cells
- Adult body stores 2.5 mg, normal loss of 1µg/day

Cobalamin Deficiency

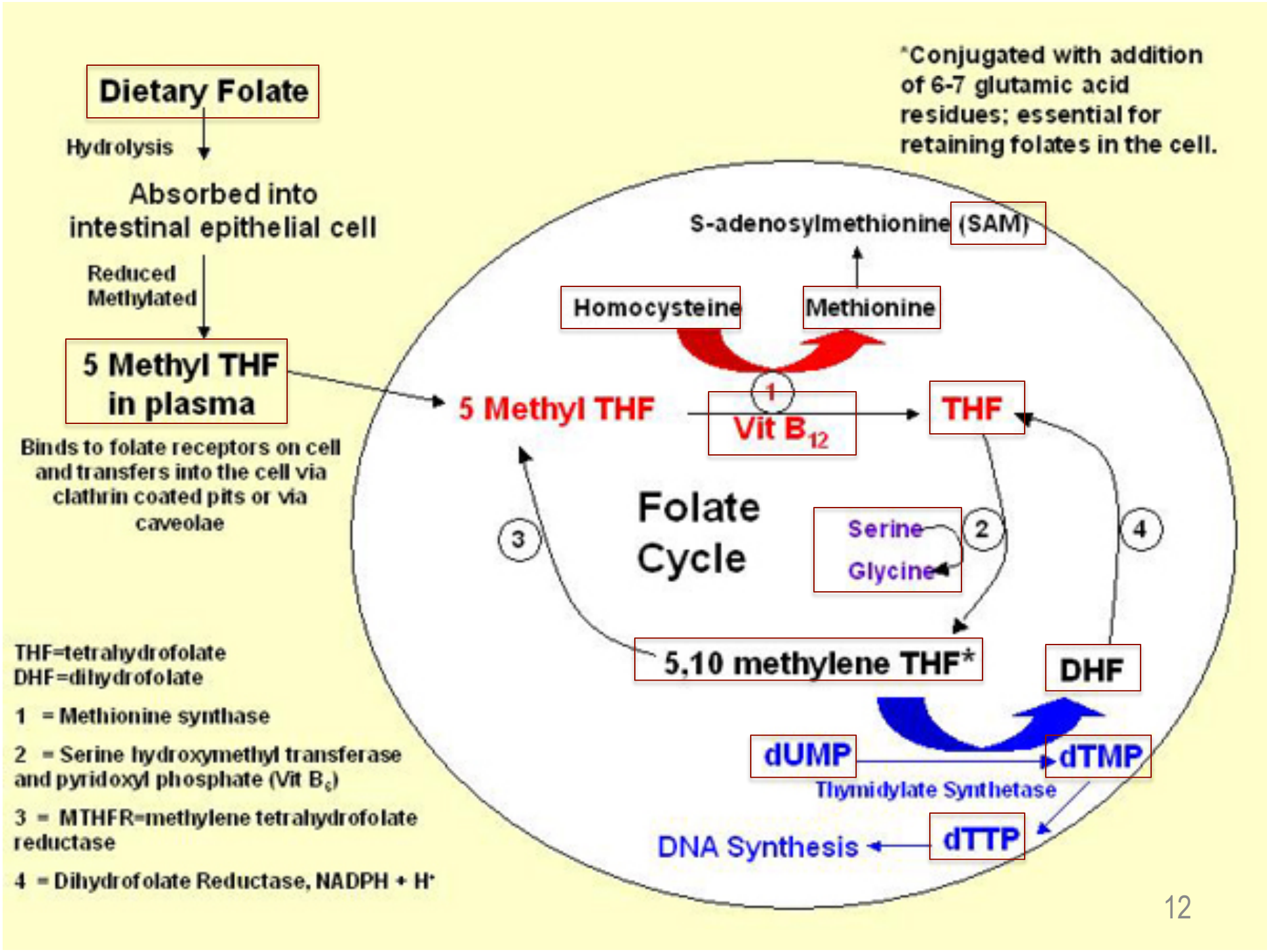
- Decreased intake
  - Strict vegetarianism only, and rare
  - Infants of mothers who are vegans or who have little or no intrinsic factor
- Impaired malabsorption
  - Ileal resection
  - IF deficiency in gastrectomy, bariatric surgery
  - Anti-parietal cell or anti-IF antibody: N. Europeans only
- Competition
  - Blind loop syndrome, diverticulitis, diphyllobothrium latum infestation

Auto-anti-parietal Cell or anti-IF Antibody

- Once called “pernicious anemia”
- Scandinavian, English, Irish parentage, females predominate
- Gastritis and parietal cell atrophy; achlorhydria
- Decreased IF; no cobalamin absorption
  - Autoimmune, genetic and environmental factors involved
  - Autoantibodies to IF present in half of patients
  - Associated with other autoimmune diseases
- Usually >50 YO; a rare congenital form in children
- Increased risk of gastric carcinoma

Non-immune Cobalamin Malabsorption

- Most common cause of deficiency >50 YO
- Decreased absorption: inability to digest cobalamin from food
  - Acid hydrolysis releases cobalamin from dietary proteins
  - Gastric HCl production diminishes with age
  - Excess use of proton pump inhibitors
- Chronic gastritis due to *Helicobacter pylori, alcohol abuse*
- Gastrectomy, ileal resection, Crohn disease, sprue, diverticulitis
- Differentiate from refractory anemia; myelodysplastic syndrome


Dietary Folate
Hydrolysis
Absorbed into intestinal epithelial cell
Reduced Methylated
5 Methyl THF in plasma
Binds to folate receptors on cell and transfers into the cell via clathrin coated pits or via caveolae
*Conjugated with addition of 6-7 glutamic acid residues; essential for retaining folates in the cell.
S-adenosylmethionine (SAM)
Homocysteine
Methionine
5 Methyl THF
1
Vit B12
THF
Folate Cycle
3
Serine
Glycine
2
4
5,10 methylene THF*
DHF
dUMP
dTMP
Thymidylate Synthetase
DNA Synthesis
dTTP
THF=tetrahydrofolate
DHF=dihydrofolate
1 = Methionine synthase
2 = Serine hydroxymethyl transferase and pyridoxyl phosphate (Vit B6)
3 = MTHFR=methylene tetrahydrofolate reductase
4 = Dihydrofolate Reductase, NADPH + H+

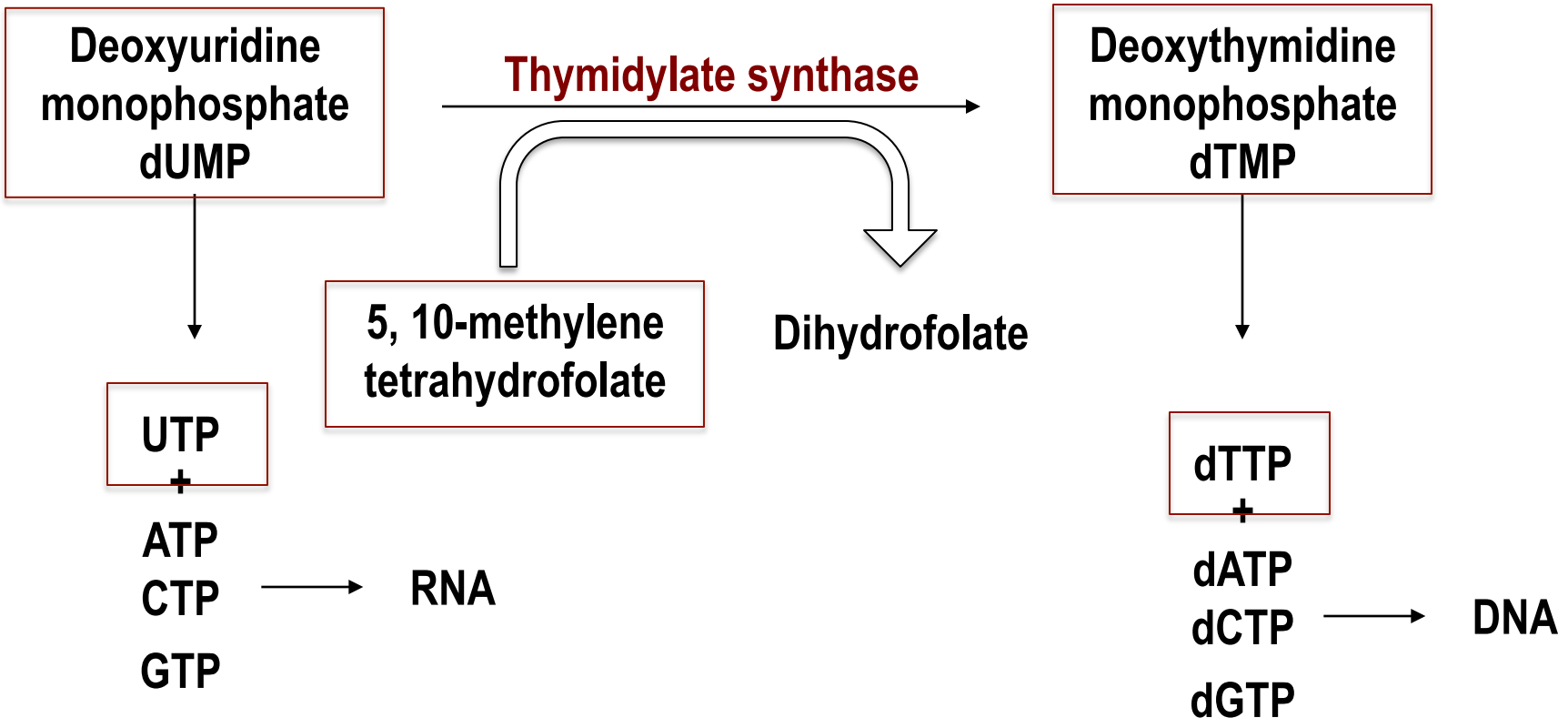
Folate Cycle: Enzymatic Steps

1. 5-methyl THF is demethylated to form THF by cobalamin catalyzed by methionine synthase
2. THF is methylated from serine by serine hydroxymethyl transferase, requiring pyridoxyl phosphate (vitamin B_6)
3. 5, 10 methylene THF demethylated to 5 methyl THF by methylene tetrahydrofolate reductase (MTHFR)
4. DHF becomes condensed to THF by dihydrofolate reductase in the presence of coenzyme $NADPH^+ H^+$

Folate and Cobalamin: Two Important Steps

- Cobalamin demethylates 5-methylene THF
  - Coupled to homocysteine-methionine
  - Methionine converts to S-adenosyl methionine (SAM) needed for normal neurologic function
- 5,10 methylene tetrahydrofolate and thymidylate synthetase
  - Convert deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP)
  - dTMP converted to dTTP used in DNA synthesis

Uridine to Thymidine



The "Folate Trap"

- Folate enters cell as 5-methyl THF
- Demethylated by cobalamin to form tetrahydrofolate
- THF is re-methylated by 1–4 glutamic acid molecules to 5, 10 methylene tetrahydrofolate, which cannot diffuse from the cell
- With cobalamin deficiency, folate remains "trapped" as 5-methyl THF, leaks out of the cell
  - This is sometimes called the "folate trap" since the folate is trapped in the inactive 5-methyl form and escapes the cell
- So, cobalamin deficiency allows folate to escape the cell

Effect of Folate or Cobalamin Deficiency

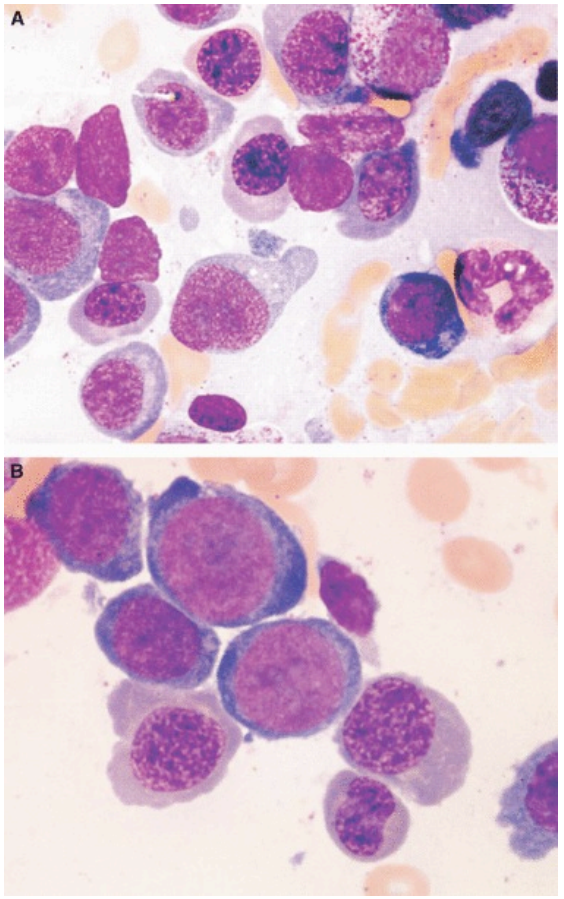
- Uridine nucleotide production is normal
  - Normal RNA production
- Thymidine nucleotide production is impaired
  - Inadequate carbon transfer, poor dUMP methylation
  - Deoxyuridine misincorporated in DNA causing double-stranded breaks
  - Interphase and S-phase arrested at several points

Biochemical Defect Consequences

1. Uridine replaces thymidine in DNA
2. DNA uracil glycosylase excises dUTP
3. DNA strand breakage, nuclear fragmentation
4. Ineffective hematopoiesis, increased production
5. Delay in cell cycle increases cell size
6. RBC survival 25–35 days, rapid turnover
7. Affects survival of all rapidly dividing cells; myeloblastic, megakaryocytic, normoblastic, and intestinal epithelium

Megaloblastic Maturation

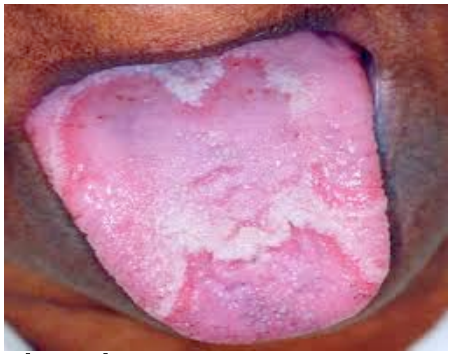
- DNA production inhibited
  - Mitosis delayed
  - Nuclear development delayed
- RNA production not inhibited
  - Near-normal cytoplasmic development
  - Near-normal protein production
- Consequence: panmyelosis
  - Nuclear-cytoplasmic asynchrony
  - Abundant cytoplasm
  - Retarded chromatin condensation
  - Chromatin abnormally dispersed



Ineffective Erythropoiesis

- Intense marrow erythropoietic activity
- Marrow normoblast destruction
- Increased marrow phagocytic activity
- Anemia with low red cell count
- Elevated LD and nucleic acids
- Elevated total and indirect bilirubin
- Also occurs in iron deficiency anemia & thalassemia

Clinical Effects of Cobalamin and Folate Deficiency



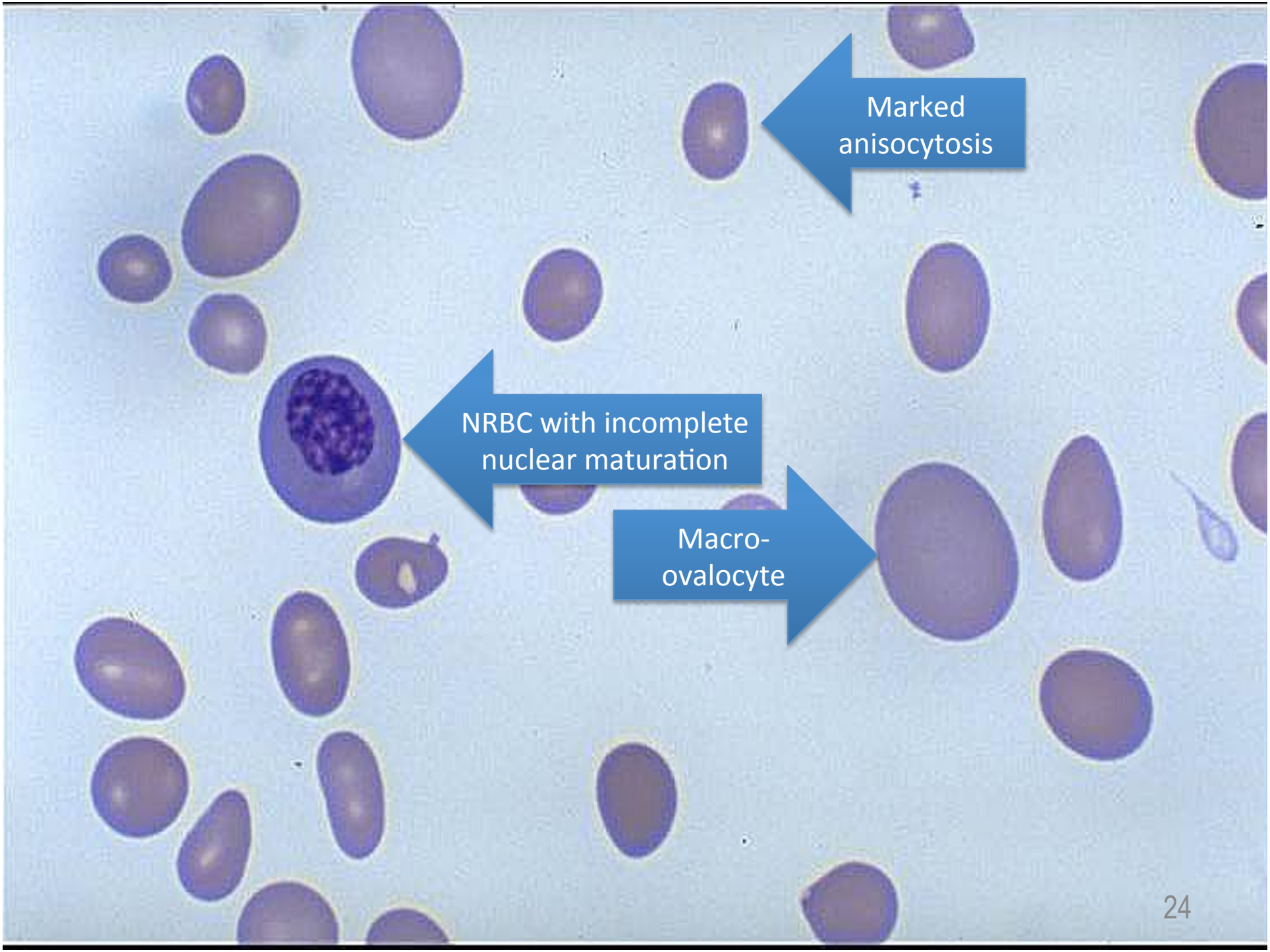
- Anemia: fatigue, weakness, shortness of breath, jaundice
- Glossitis: loss of tongue epithelium
- Stomatitis: loss of mouth epithelium
  - Mostly in cobalamin deficiency, causes nausea, constipation
- Demyelinization of spinal cord: cobalamin deficiency alone
  - Memory loss, numbness, tingling, loss of balance, irritability, depression, cognitive deficits, convulsions, psychosis
- Hyperhomocysteinemia: folate or cobalamin deficiency
  - Also pyridoxyl phosphate deficiency
  - Venous thrombosis, cardiovascular disease
  - Neural tube closure defect: spina bifida

Blood Findings in Megaloblastic Anemia

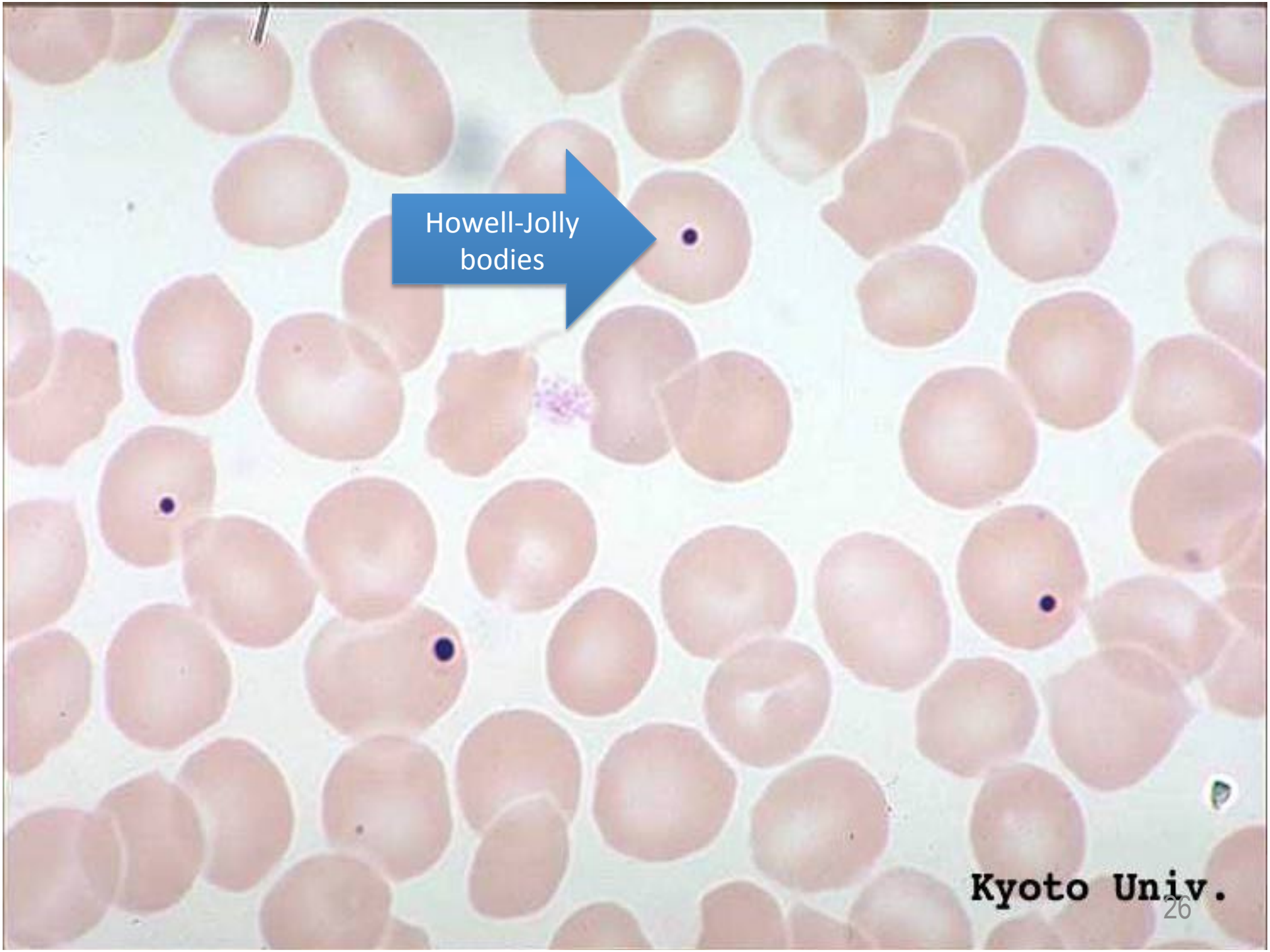
- HGB <12.0 g/dL and HCT <36 %
- MCV 100–150 fL, RDW >15.5, MCHC 32–36%
  - MCV rises long before HGB and HCT fall
- Oval macrocytes, Howell-Jolly bodies, basophilic stippling, Cabot rings, dacryocytes, schistocytes
- Absolute reticulocyte count (ARC) <84 × 10^9/L
- Neutropenia with ≥5% hypersegmented PMNs presenting with 5 segments (macropolycytes)
  - Only one macropolycyte with 6 segments supports the diagnosis
  - Hydroxyurea therapy and steroids also cause this
- Thrombocytopenia with functional platelet impairment

Megaloblastic Anemia with Additional Pathology

- Ineffective erythropoiesis may lead to iron deficiency
  - MCV may return to normal
- Megaloblastic anemia plus blood loss, anemia of chronic inflammation, kidney disease, thalassemia
  - MCV not elevated
- Macropolycytes are key when MCV is ambiguous
- Or go to bone marrow aspirate smear

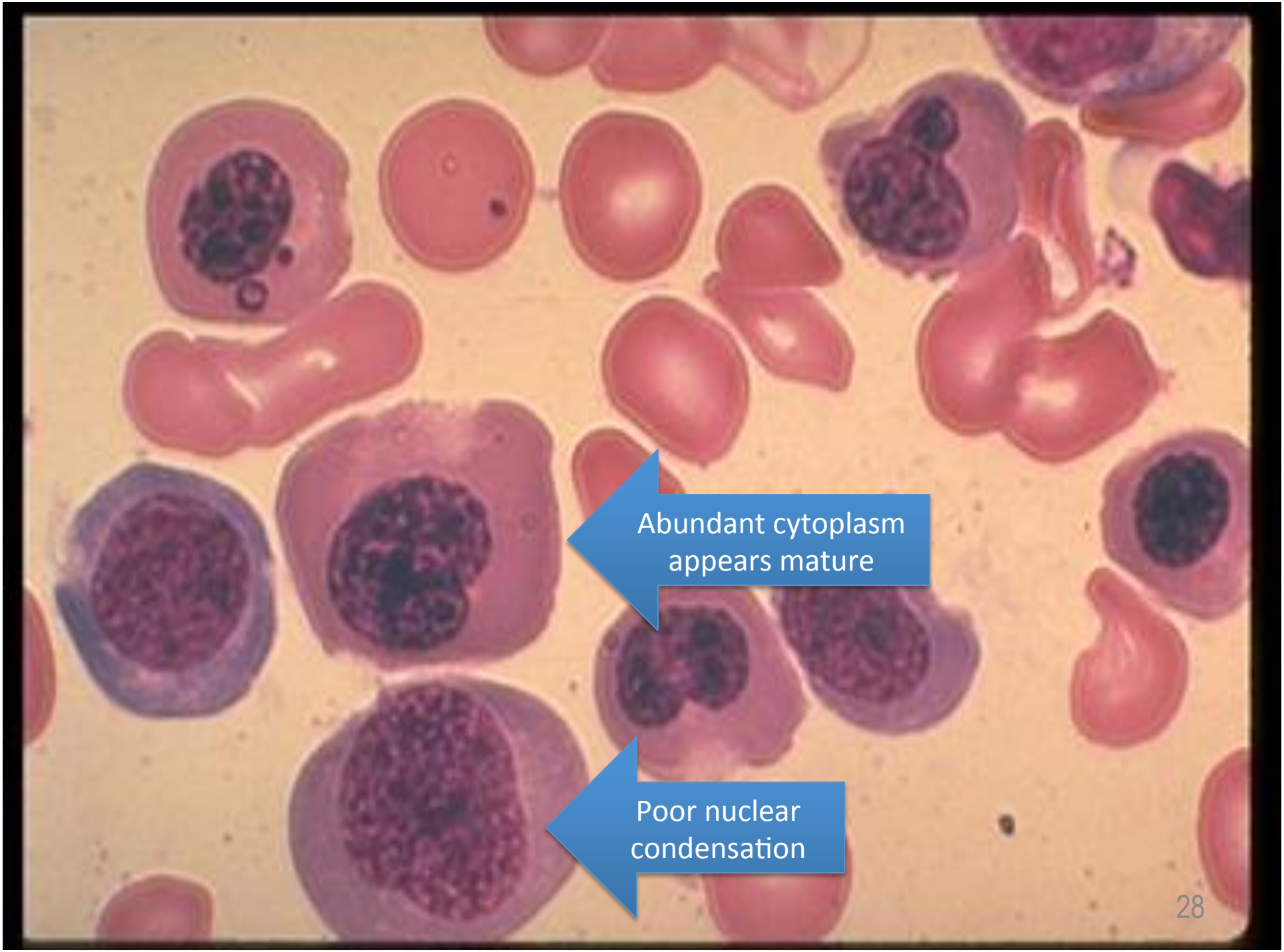

Marked anisocytosis
NRBC with incomplete nuclear maturation
Macro-ovalocyte

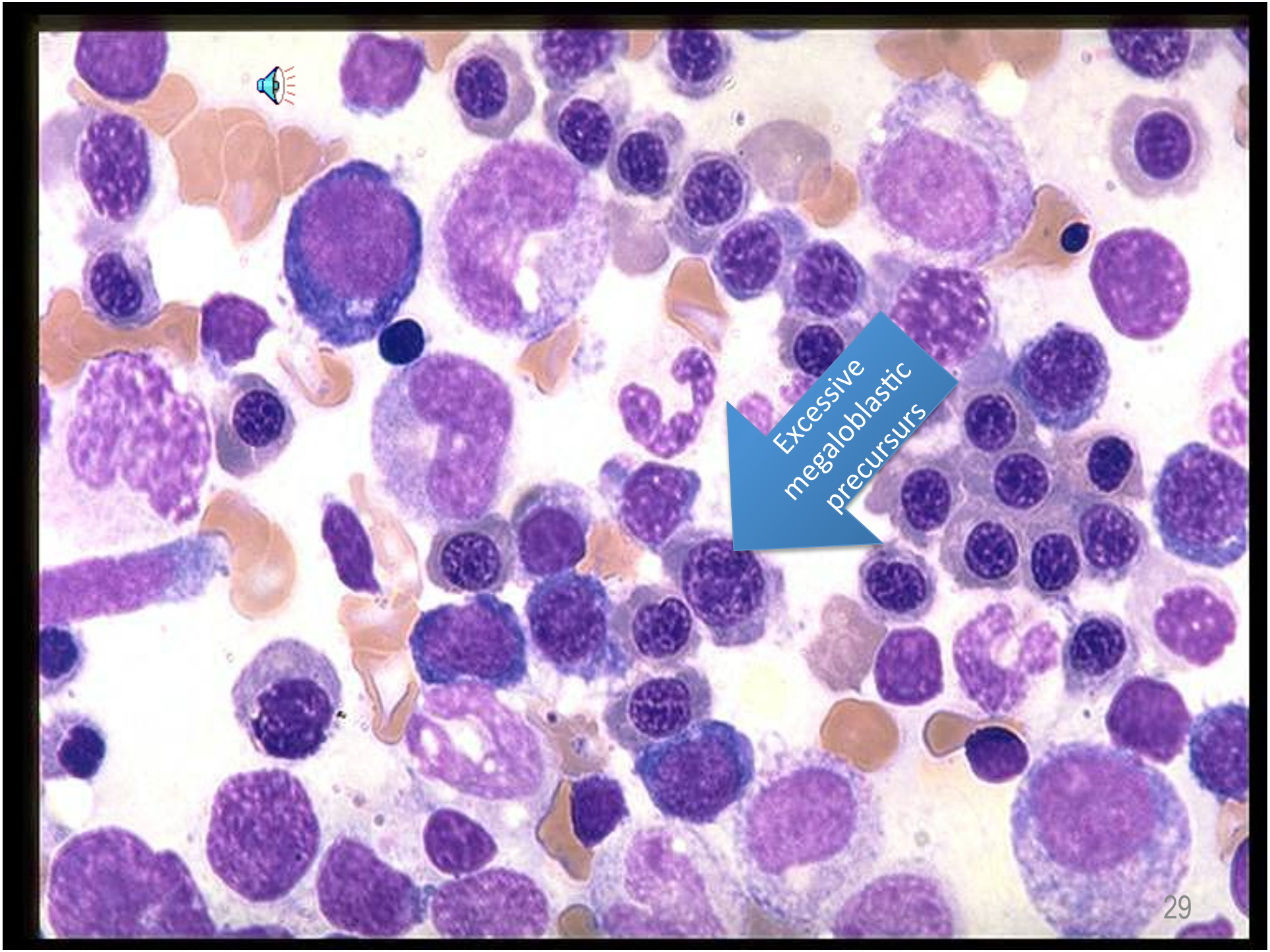

Macropolycyte

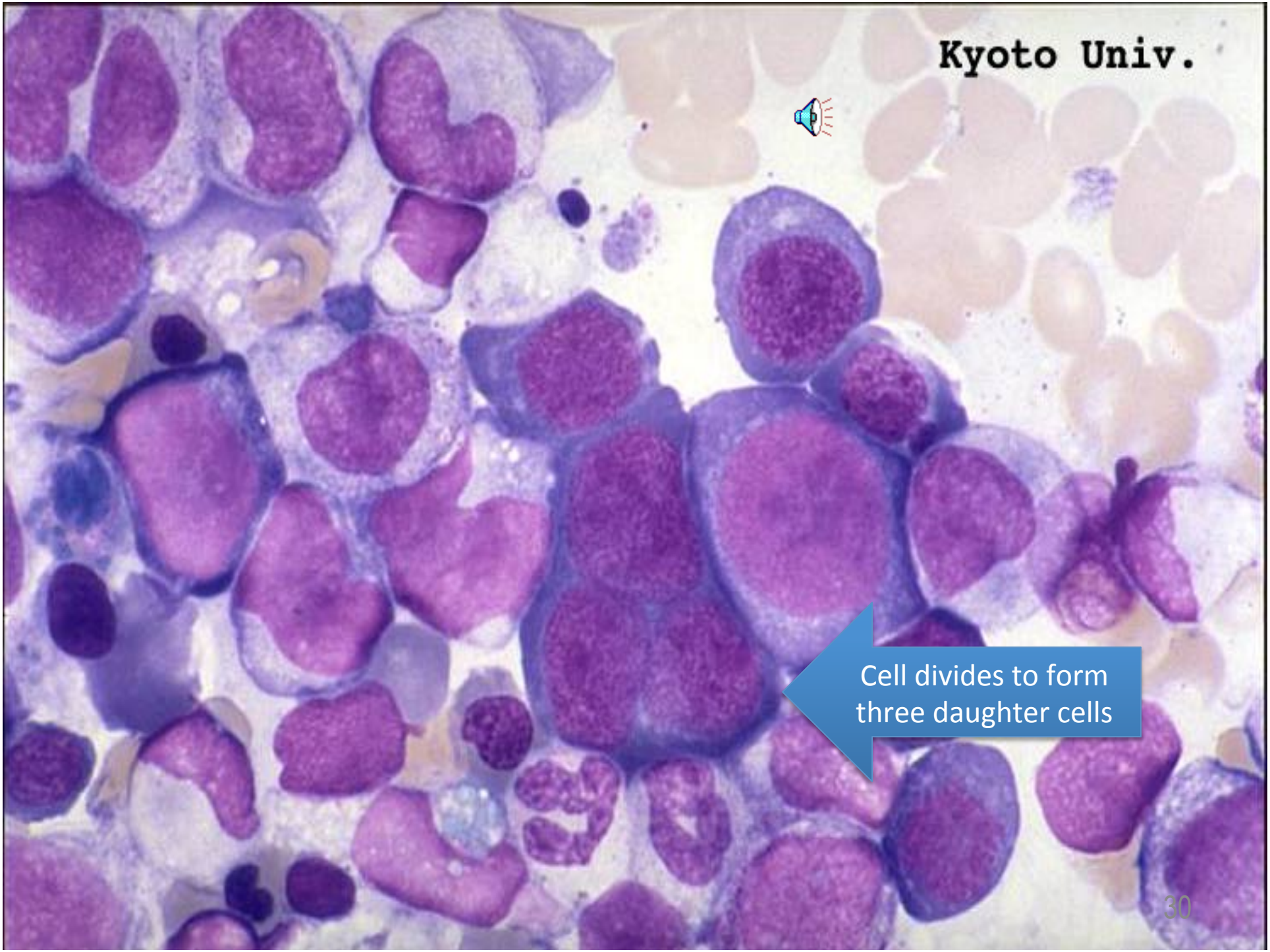

Howell-Jolly bodies
Kyoto Univ.

Megaloblastic Maturation: Bone Marrow

- Bone marrow hypercellular
- M:E ratio 1:1 due to increased erythropoiesis
- Nuclear-cytoplasm asynchrony
- Giant metamyelocytes and BANDs
- Diffuse nuclear condensation; "cut salami" appearance

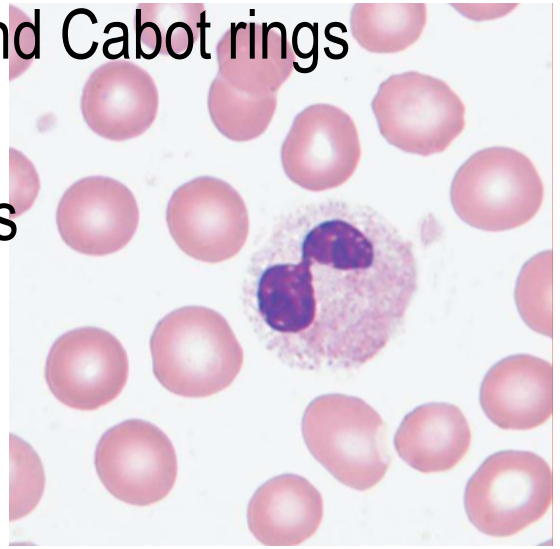

Abundant cytoplasm appears mature
Poor nuclear condensation


Excessive megaloblastic precursurs


Kyoto Univ.
Cell divides to form three daughter cells

Other Macrocytosis Causes

- Hemolytic anemias or chronic blood loss, elevated reticulocyte count = polychromatophilia, MCV 100–110 fL
- Liver disease and alcoholism membrane lipid imbalance
- Myelodysplastic syndrome: refractory anemia
  - Oval macrocytosis with H-J bodies and Cabot rings
  - Hypogranular pelgeroid PMNs
  - Thrombocytopenia with giant platelets
- Antiviral, immunosuppressive and cytotoxic drugs



Lab Assays Assist in Differential Diagnosis

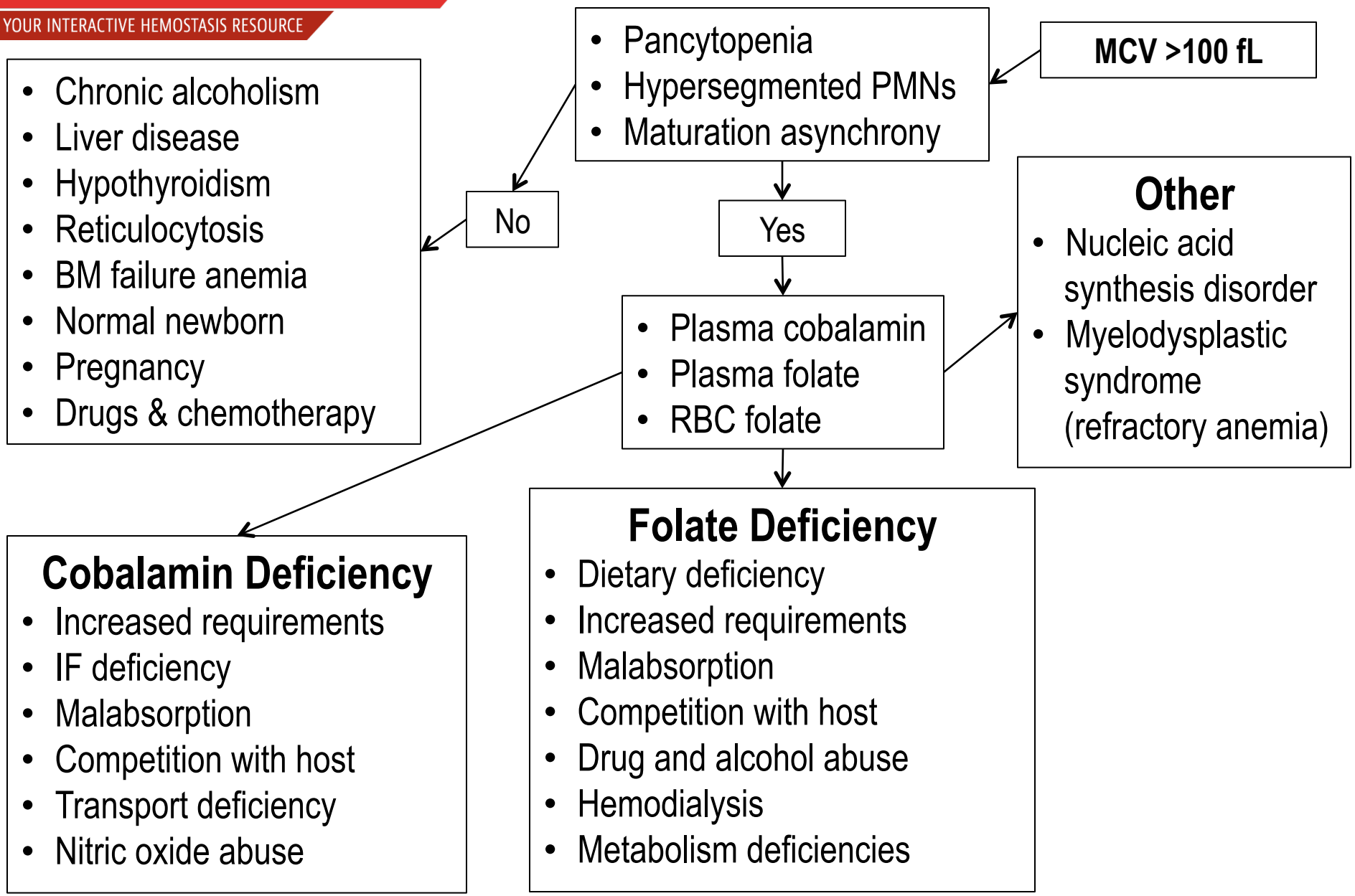
Assay	Purpose	RI
Serum cobalamin	Cobalamin deficiency	<200 ng/L
Serum folate	Folate deficiency	<2.5 ug/L
RBC folate (whole blood)	Follow-up serum folate deficiency	<160 ug/L
Serum homocysteine	Early folate deficiency	>18 nM/mL
Methylmalonic acid	Early cobalamin deficiency	>280 nM/L
Schilling test (obsolete)	^{57}Co-labeled oral cyanocobalamin. If low excretion, follow-up with second stage using cyanocobalamin and IF	>8% excretion In 24 h urine

Lab Assays Assist in Differential Diagnosis

- Chemiluminescent immunoassay of folate and cobalamin
- Serum folate: reflects folic acid ingestion over past few days
  - Hemolysis causes false elevation
- RBC folate: folate incorporated during erythropoiesis
  - Reflects folic acid ingestion over months
  - Reticulocytosis causes false elevation
- Cobalamin deficiency “folate trap;” folate escapes RBCs
  - Falsely raises serum folate
  - Falsely reduces RBC folate

Anti-IF; Anti-Parietal Cell Antibodies

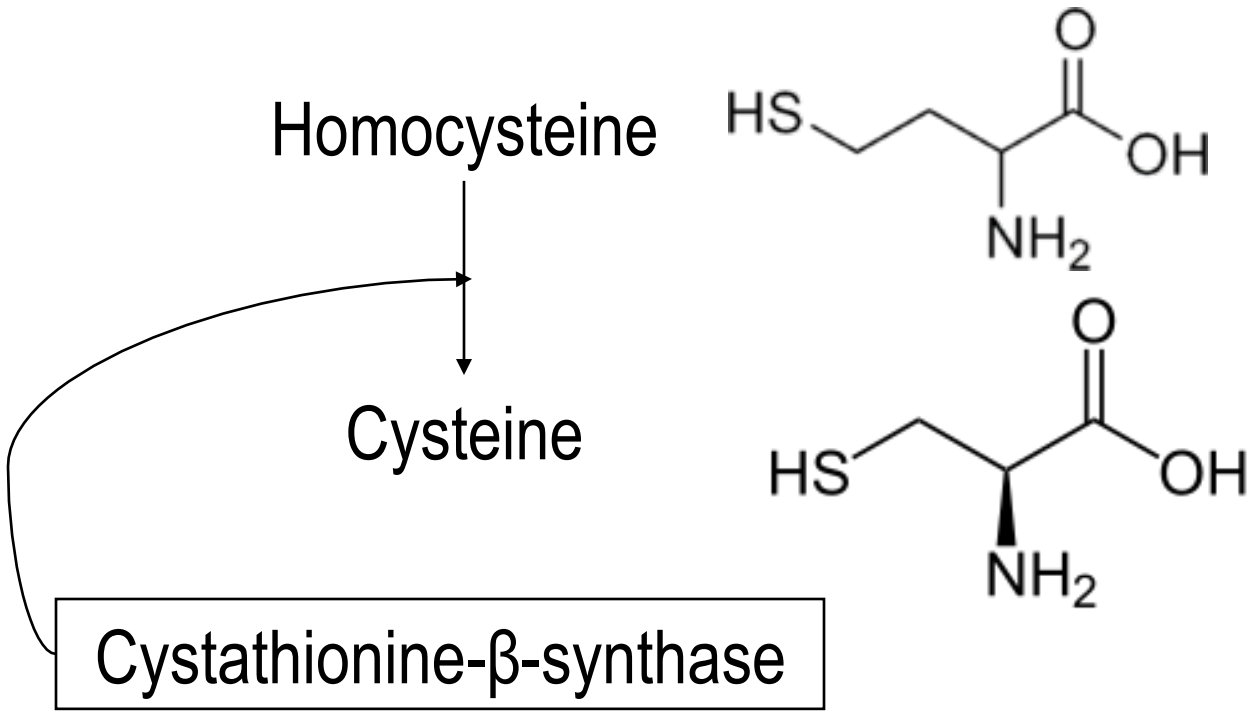
- Auto-anti-IF in 56% of cobalamin deficiency cases
- Auto-anti-gastric parietal cells in 85–90% of cases
  - Detected in other conditions including normals
- Gastric acid titration (obsolete)
- Schilling test results (obsolete)
  - Decreased absorption of oral radioactive cyanocobalamin
  - Corrected by ingestion of IF administered with a second dose of oral cyanocobalamin
  - Similar results seen after gastrectomy


• Pancytopenia
• Hypersegmented PMNs
• Maturation asynchrony
MCV >100 fL
No
Yes
• Chronic alcoholism
• Liver disease
• Hypothyroidism
• Reticulocytosis
• BM failure anemia
• Normal newborn
• Pregnancy
• Drugs & chemotherapy
• Plasma cobalamin
• Plasma folate
• RBC folate
Other
• Nucleic acid synthesis disorder
• Myelodysplastic syndrome (refractory anemia)
Cobalamin Deficiency
• Increased requirements
• IF deficiency
• Malabsorption
• Competition with host
• Transport deficiency
• Nitric oxide abuse
Folate Deficiency
• Dietary deficiency
• Increased requirements
• Malabsorption
• Competition with host
• Drug and alcohol abuse
• Hemodialysis
• Metabolism deficiencies

Megaloblastic Anemia Therapy

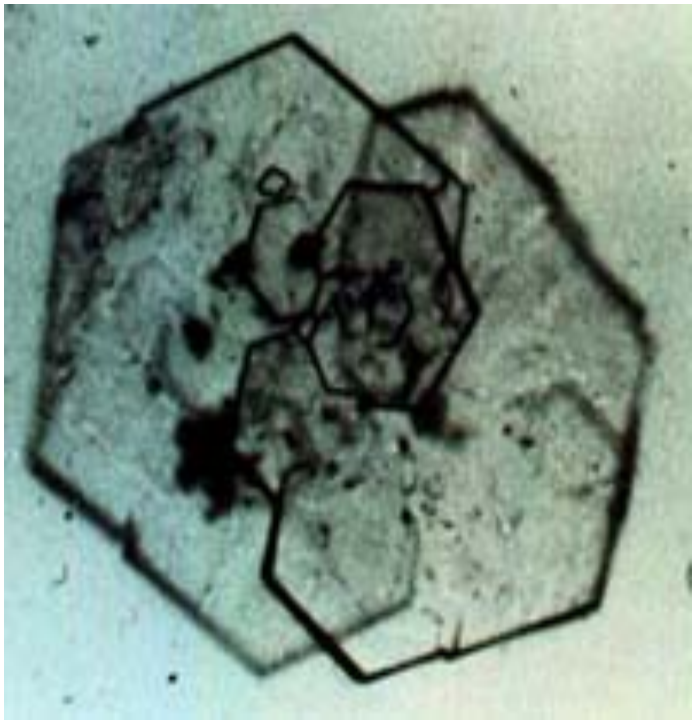
- Therapeutic trial: oral folic acid or subcutaneous cyanocobalamin
- Watch for response
  - Reticulocytes rise in 2–3 days, peak at 7 days
  - Hypersegmented PMNs disappear in 12–14 days
  - HCT begins to rise in 5–7 days and is normal in 4–8 weeks
  - MMA (cobalamin deficiency only) and homocysteine (cobalamin or folate deficiency) decline within a few days
- Folic acid may partially correct the anemia of a cobalamin deficiency, but will not correct the neuropathies, nerve damage and neuropsychiatric complications; must identify a cobalamin deficiency before permanent neurologic damage occurs

Homocysteine Transsulfuration

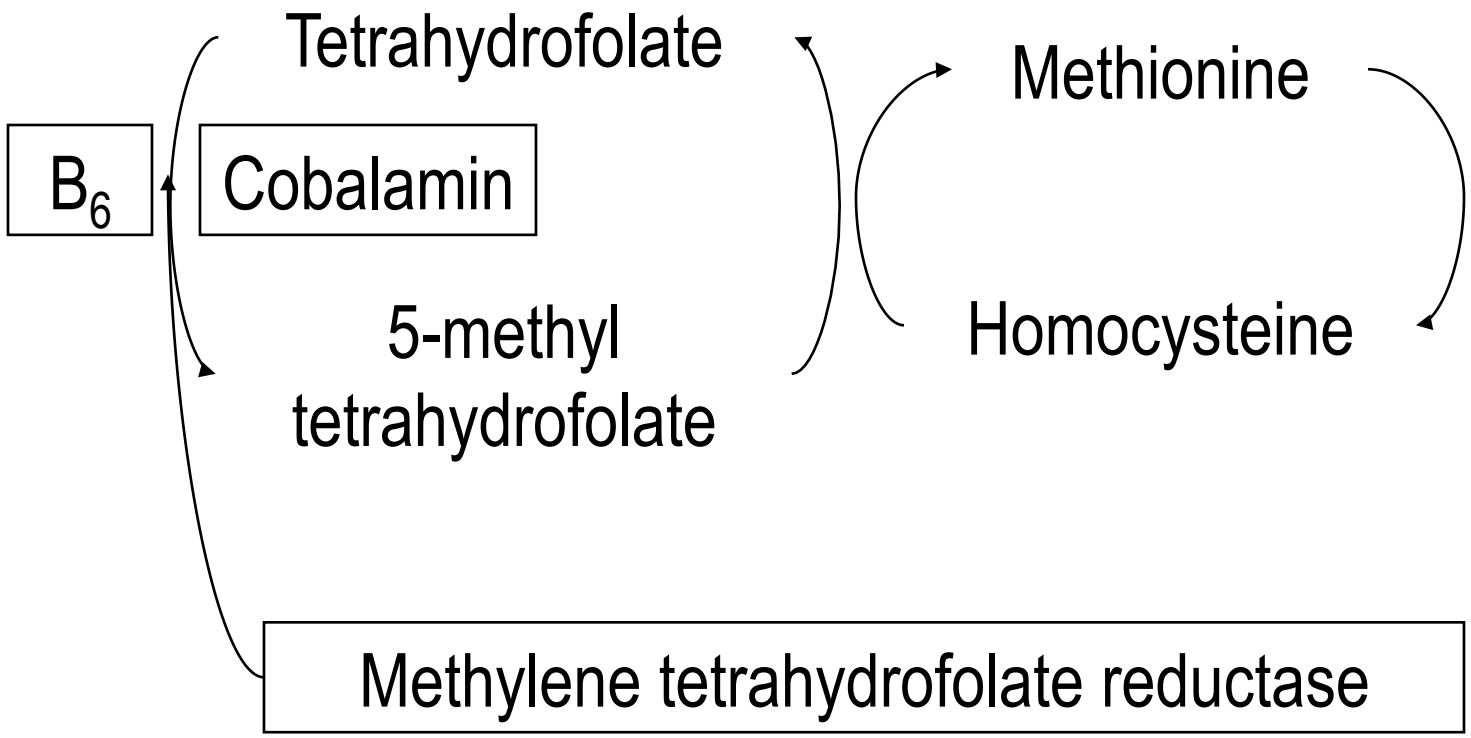


Cystathionine-β Synthase Deletion

- Autosomal recessive
- Homozygous deletion in 1 in 200,000 live births
  - Homocysteine 40x normal = 400 μM/L
  - Homocystinuria with cystine crystals
  - Severe premature atherosclerosis and arterial and venous thrombosis
- Heterozygous in 1 in 300 live births
  - Plasma total homocysteine 20–40 μM/L
  - No homocystinuria



Homocysteine Transmethylation



MTHFR Polymorphisms

- Heterozygous C677T substitution
  - 50% of unselected individuals
  - Total homocysteine may be unaffected
  - Enzyme is mildy thermolabile, becomes inactivated at 42°C
- Homozygous C677T
  - 11% of population
  - Total homocysteine > 20 mM/L
- Cosegregated A1298C
  - 33% of unselected population
  - Increases odds of homocysteinemia when present with C677T

Dietary Deficiencies and Homocysteinemia

- Dietary deficiency in chronic alcohol, smoking, excessive coffee
  - Pyridoxyl phosphate
  - Cobalamin
  - Folate (mitigated by grain enrichment)
- Disorders that cause cobalamin, folate, or pyridoxyl phosphare deficiency
  - Renal disease, organ transplantation, hypothyroidism, hypertension, cancer

Homocysteinemia and Odds of Venous Thromboembolism

- Homocysteine >18 nM/mL
  - –Men <50: 2.5×
  - –Women <50: 7.0×
  - –>50 YO: 5.5×

Reducing Homocysteinemia

- Folic acid: 0.65 mg/d min
  - Supplement with cyanocobalamin to avoid masking cobalamin deficiency with neuropathy
- Pyridoxyl phosphate: 250 mg/d minimum
- Cyanocobalamin: 0.2–0.4 mg/d minimum

Bottom Line at the End (BLEAT)

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