



# Vitamins

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# Objectives



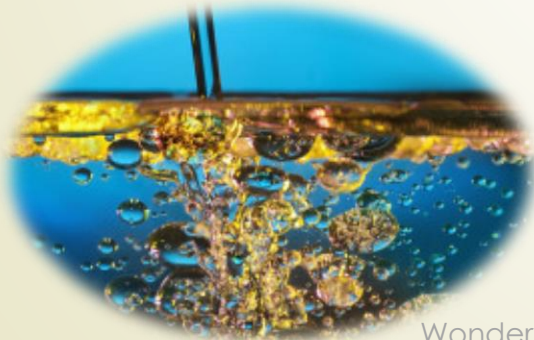
- ▶ Students will recall the common and chemical names for each vitamin
- ▶ Students will categorize the vitamins based on solubility
- ▶ Students will relate each vitamin to its purpose
- ▶ Students will correlate symptoms to a toxicity or deficiency of each vitamin
- ▶ Students will compare methods of patient preparation and chemical analysis
- ▶ Students will identify the purpose of testing each vitamin

# Vitamins are....

- Organic compounds required in small amounts
- Not significantly synthesized by body
  - Mostly obtained through diet
- Grouped by their function NOT structure
  - They belong to a diverse group of chemicals
- 2 Main categories
  - Fat soluble: stored until needed
  - Water soluble: Not stored, require regular consumption



npr.org



Wonderopolis.org

# Fat Soluble Vitamins

Common Name	Chemical Name
Vitamin A	Retinol
Vitamin D	Tocopherol
Vitamin E	Calciferol
Vitamin K	Napthoquinone Derivatives



# Water Soluble Vitamins

Common Name	Chemical Name
Vitamin B1	Thiamin
Vitamin B2	Riboflavin
Vitamin B3	Niacin
Vitamin B6	Pyridoxine
Vitamin B9	Folate
Vitamin B12	Cobalamin
Vitamin C	Ascorbic Acid

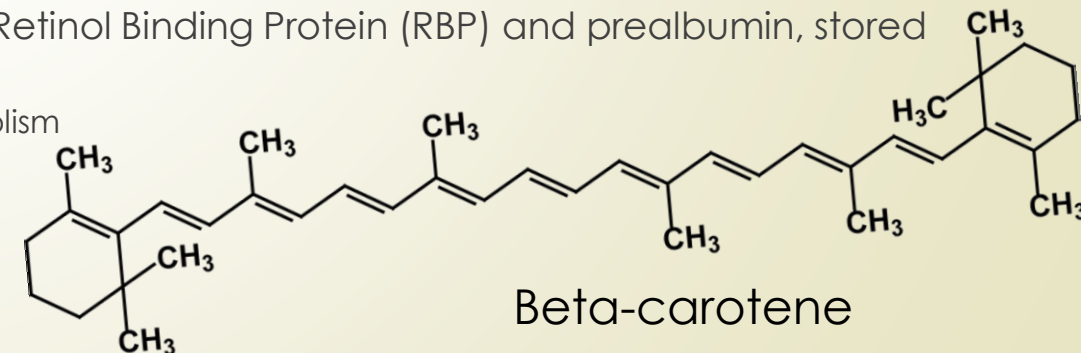




# Vitamin A



- Vitamin A
  - Several forms:
    - Alcohol- Retinol
    - Aldehyde- Retinal
    - Carboxylic Acid- Retinoic Acid
    - Fatty Acids- Retinyl esters
- Carotene (Vitamin A Precursor)
  - Looks like two Vit. A molecules hooked together
  - More stable than Vit. A
- Found in salmon, liver, green leafy vegetables, broccoli, carrots, squash, apricots, mangos, dairy, and fortified cereals.
- Absorbed as a lipid, transported attached to Retinol Binding Protein (RBP) and prealbumin, stored in the Liver.
  - RBP regulates Vitamin A absorption and metabolism



# Vitamin A Pathology



## Functions:

- Vision and maintenance of the surface lining of the eyes
- Growth, Reproduction
- Epithelial maintenance (Respiratory, urinary, and intestinal tracts)
- Immune Response

## Deficiency

- Nyctalopia (night blindness)
- Dry corneal epithelium (xerophthalmia)



## Dry skin, hair

- Hyperkeratosis (thickening of skin, bumps)

## Excess

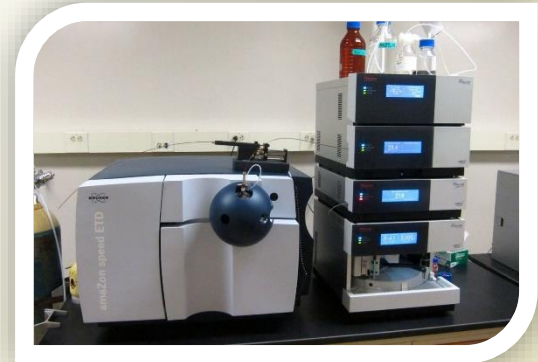
- Loss of hair, dry skin, joint pain
- Drowsiness, headaches
- Increased Cranial Pressure
- Lack of appetite, vomiting
- Death



# Vitamin A Testing



- ▶ Patient Preparation: Should fast for 12 hours.
  - ▶ With fat soluble vitamins, recent intake from diet will be measured during the absorption process.
- ▶ Sample Requirements:
  - ▶ Should be protected from light and analyzed soon after draw.
  - ▶ After centrifugation, samples should be frozen if testing not performed within 24 hours.
- ▶ Method Principle:
  - ▶ Solvent is used to extract lipids from the serum. HPLC used to measure Vitamin A or Vitamin A esters.
- ▶ Reference Range: 20 – 120  $\mu\text{g}/\text{dL}$





# Carotene Testing



- ▶ Patient Preparation: Should fast for 12 hours.
  - ▶ With fat soluble vitamins, recent intake from diet will be measured during the absorption process.
- ▶ Sample Requirements:
  - ▶ Should be protected from light and analyzed soon after draw.
  - ▶ After centrifugation, samples should be frozen if testing not performed within 24 hours.
- ▶ Method Principle:
  - ▶ Spectrophotometric carotene method used measure all Carotenoids. Serum proteins are denatured in ethanol, then carotene is extracted into petroleum ether. Absorbance of extract measured at 440-450 nm and concentration determined from a standard curve.
  - ▶ Specific, individual carotenes (such as  $\beta$ -carotene), can be measured by HPLC after extraction from serum using solvents.
- ▶ Reference Range: 50 – 250  $\mu\text{g/dL}$



# Why Test Vitamin A?

- ▶ Vitamin A is tested to:
  - ▶ Determine deficiencies and toxicities
  - ▶ Monitor patients on Vitamin A therapies
  - ▶ *As a measure of fat absorption*
- ▶  $\beta$ -Carotene is usually tested as a measure of fat absorption
- ▶ BOTH are absorbed in GI tract and are used to monitor patients with GI disorders
  - ▶ Cystic Fibrosis, Sprue, Pancreatic Insufficiency, IBD, Cholestasis, Small-Bowel bypass surgery, Small Bowel transplants



# Vitamin E



- ▶ Active form is  $\alpha$ -tocopherol
  - ▶ Found in vegetable oils, nuts (peanuts, hazelnuts, and almonds), sunflower seeds, green leafy vegetables, spinach, and broccoli.
- ▶ Lipid soluble, absorption tied to dietary fats
- ▶ Transported on lipoproteins, stored in adipose
- ▶ Functions as antioxidant
  - ▶ Unsaturated lipids need to be protected from oxidative damage
  - ▶ RBC membrane subject to damage from oxidative stress



# Vitamin E Pathology

- ▶ Toxicity:
  - ▶ Least toxic fat-soluble vitamins
  - ▶ Can cause malaise, easy fatigability
  - ▶ Most toxicity due to self-medication for poor reasons
- ▶ Deficiency:
  - ▶ Rare
  - ▶ Characterized by neurological problems, esp. nerve degeneration in hands and feet
  - ▶ Occurs in:
    - ▶ Premature, formula fed infants
    - ▶ Adults with fat malabsorption/on artificial diets
    - ▶ People with rare genetic abnormalities in the alpha-tocopherol transport protein

} Hemolytic anemia  
possible

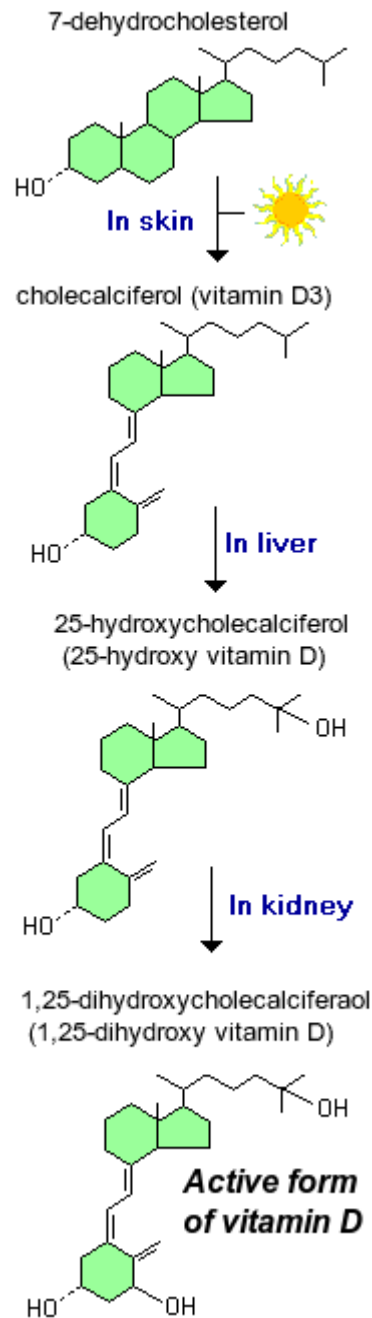




# Vitamin E Testing

- ▶ Measured similarly to Vitamin A:
  - ▶ Serum proteins are precipitated, lipid extraction is performed. Analysis performed by HPLC
- ▶ Reference Range: 0.5 – 2.0 mg/dL

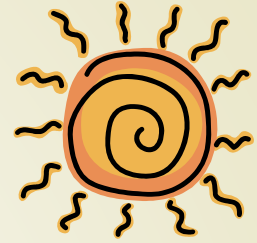




# Vitamin D

- Fat-soluble vitamin found in foods and made by body through UV exposure
  - Found in: salmon, tuna, dairy, egg (yolk), mushrooms, supplemented in cereals, juices and other beverages
- Different isoforms
  - D<sub>2</sub> Ergocalciferol from plants
  - D<sub>3</sub> Cholecalciferol from animals
- Cholesterol in skin changed to Vit D<sub>3</sub>
- Liver changes to 25-OH-D<sub>3</sub>
- Kidney activates to 1,25-[OH]<sub>2</sub>-D<sub>3</sub>
  - Active, signals absorption of calcium and phos in kidney and intestines

# Vitamin D Pathology

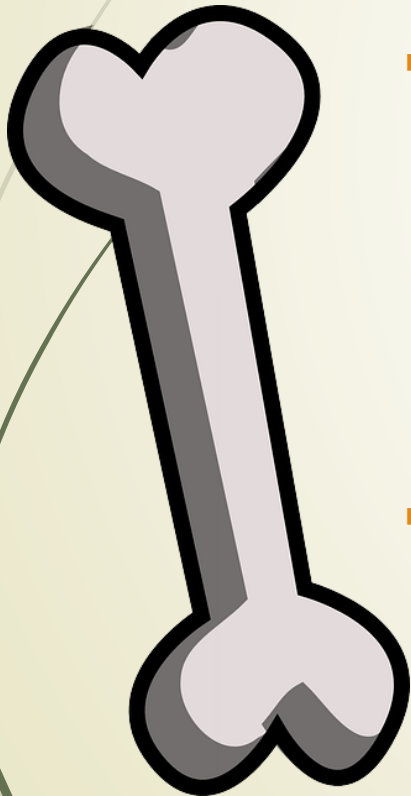


## ► Toxicity:

- nausea/vomiting, poor appetite, constipation, weakness, and weight loss
- Can raise blood levels of calcium:
  - Mental health status changes
  - Confusion
  - Heart rhythm abnormalities
  - deposition of calcium and phosphate in soft tissues like the kidneys
- Sun exposure and diet are unlikely to cause toxicity
  - Usually from high intake of supplements

## ► Deficiency:

- Causes rickets in children, and osteomalacia in adults.
  - Both are characterized by weakened, thin, brittle, and misshapen bones.
- Who?
  - Infants who are exclusively breast fed for extended periods.
  - Children with constant use of sunscreen and very limited access to sunlight.
    - Cereals and dairy products are fortified with Vitamin D.
  - Adults with fat malabsorption or on artificial diets.



# Vitamin D Testing

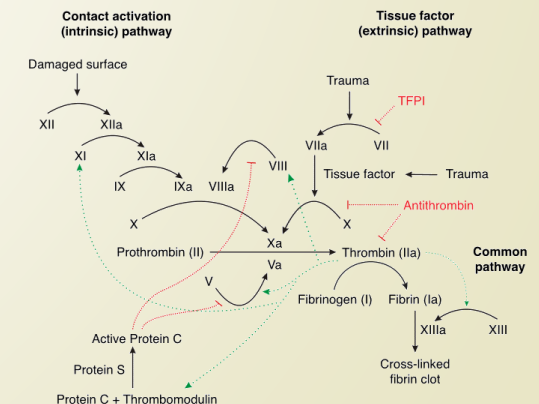
- ▶ The 25,OH Vitamin D is the most commonly measured form.
  - ▶ 25,OH Vitamin D by chemiluminescent immunoassay.
  - ▶ Both 25,OH and 1,25,OH Vitamin D can also be measured by LC/MS/MS methods.
- ▶ Reference Range:
  - ▶ Sufficiency: 31 – 80 ng/mL
  - ▶ Insufficiency: 15 – 30 ng/mL
  - ▶ Deficiency: <15 ng/mL
- ▶ Ryan's diatribe





# Vitamin K

- Fat soluble vitamin, from the German word **koagulation** due to its role in coagulation
- Obtained through diet in form of Phylloquinone ( $K_1$ )
  - synthesized by intestinal bacteria in form of Menaquinone ( $K_2$ )
- $K_1$  is in herbs, green leafy vegetables, asparagus, chili powder, curry, paprika, cayenne pepper, cabbage, cucumber, and prunes.
- A cofactor for a carboxylase that catalyzes glutamic acid residues on Vitamin K-dependent proteins, including:
  - Factors II (prothrombin), VII, IX, X, Proteins C, S, and Z



# Vitamin K Pathologies



- ▶ Toxicity:

- ▶ Rare, since it is not stored in the liver
- ▶ Menadione (synthetic Vitamin K) has double potency and can be toxic
  - ▶ Banned as a supplement in the U.S.
- ▶ Symptoms: Thrombosis, vomiting, kidney tubule degeneration
  - ▶ Also—Jaundice and hemolytic anemia in newborns

- ▶ Deficiency:

- ▶ Malabsorption, prolonged antibiotic use, ingestion of rat poison
- ▶ Symptoms: Hemorrhagic disorders, easy bruising, bleeding in various areas, (internally, gum)



# Vitamin K Testing

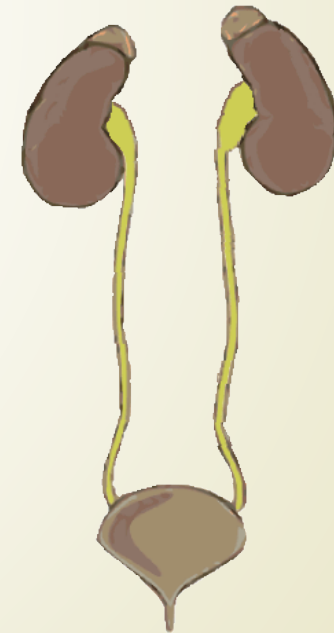
- ▶ Not routinely performed (or even rarely, it's super-rare)
- ▶ Prothrombin Time (PT) and International Normalized Ratio (INR)
  - ▶ Vitamin K deficiency and therapeutic anticoagulation
    - ▶ Usually elevated in both cases
- ▶ Vitamin K levels can be measured by HPLC.
  - ▶ First, Vitamin K is extracted into a solvent,
  - ▶ Separated by preparatory HPLC
  - ▶ Then measured by analytical HPLC with electrochemical or fluorometric detection
- ▶ Reference Range:
  - ▶ Prothrombin Time: 9.7-13 secs
  - ▶ INR: 0.9-1.3
  - ▶ Vitamin K: 0.1-2.2 ng/mL



# Vitamin B1



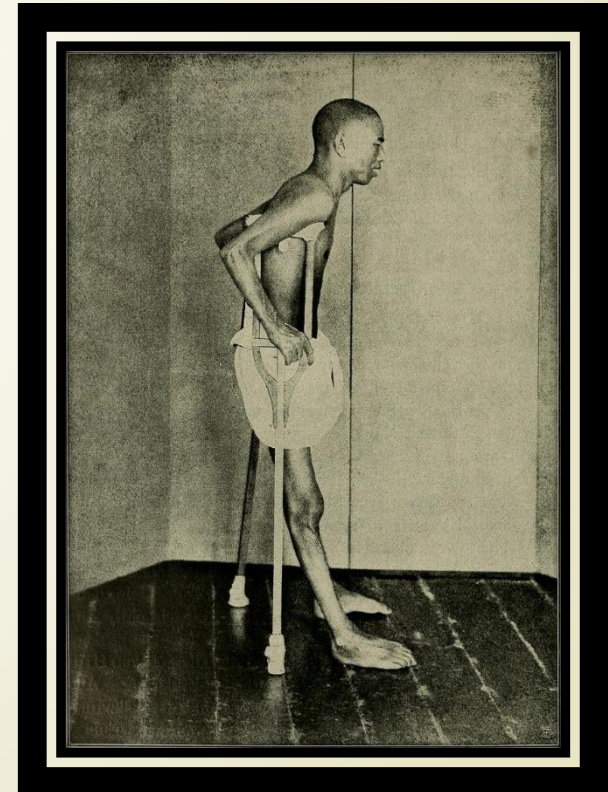
- ▶ Thiamin- A coenzyme in the metabolism of fats, carbs, and alcohols
  - ▶ Small amounts in most plant, animal tissue
    - ▶ trout, pork, nuts, sunflower seeds, peas, squash, asparagus, soy beans, and navy beans
  - ▶ Supplemented into flour and cereals
- ▶ Absorbed in small intestine
  - ▶ Able to freely circulate
    - ▶ Some albumin
  - ▶ Phosphorylated to active thiamine pyrophosphate
- ▶ Excess may be excreted by the kidney





# Vitamin B1 Pathology

- ▶ Toxicity
  - ▶ Not documented
  - ▶ IV Thiamine can cause itching, tingling, pain, anaphylaxis
- ▶ Deficiency
  - ▶ Beriberi
    - ▶ Dry: peripheral neuropathy
    - ▶ Wet: short breath, tachycardia, lower leg edema
  - ▶ Chronic alcoholism
    - ▶ Wernicke-Korsakoff syndrome
      - ▶ Memory loss, confabulation
  - ▶ Mild deficiency common (tea and toasters)
    - ▶ Cognitive impairments



# Vitamin B1 Testing

- ▶ Measure transketolase activity
  - ▶ Thiamine pyrophosphate is catalyst
  - ▶ Hemolysate
- ▶ Direct Measurement
  - ▶ Plasma/red cells/whole blood
    - ▶ Plasma is influenced by recent eating
  - ▶ HPLC



# Vitamin B2

- ▶ Riboflavin- water soluble, not metabolically active
  - ▶ Absorbed in intestines and converted
    - ▶ Riboflavin-5-phosphate (FAD)
    - ▶ Flavin mononucleotide (FMN)
  - ▶ Excess secreted by kidney
  - ▶ Used to obtain energy, build tissue
- ▶ Found in mushrooms, venison, beef, liver, spinach, milk, soybeans, nuts, legumes, eggs



# Vitamin B2 Pathology

- ▶ Toxicity

- ▶ No known cases
- ▶ Large amounts cause bright yellow urine

- ▶ Deficiency

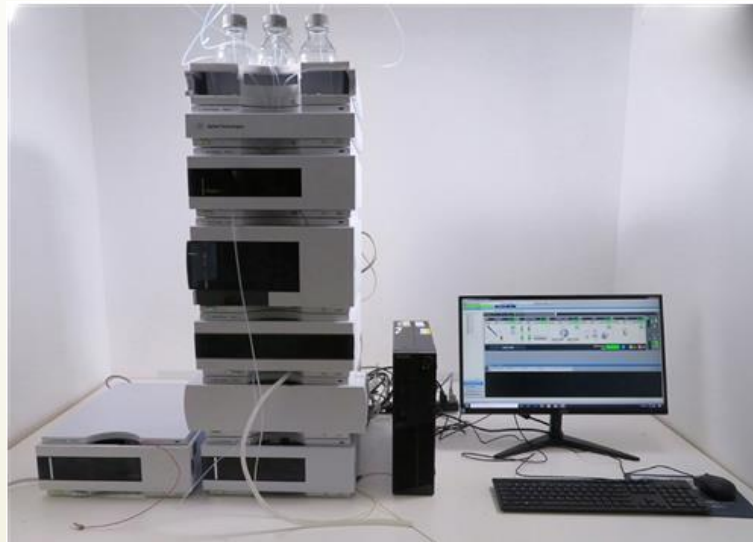
- ▶ Poor diet leading to hypothyroidism, adrenal insufficiency
- ▶ Alcohol interferes with digestion and absorption
- ▶ Symptoms include, lesion on skin & GI tract, bloodshot itchy burning sensitive eyes, inflamed and burning mouth and tongue, cracks in lips and corner of mouth, dull/oily hair, split nails





# Vitamin B2 Testing

- Specimen Consideration
  - Light sensitive, must be protected
- Testing
  - Riboflavin, FAD, FMN all measured with HPLC, fluorescent detection



# Vitamin B3

- ▶ Niacin
  - ▶ CAN be synthesized by the body from tryptophan
    - ▶ More easily obtained through diet
  - ▶ Stored by liver until needed, excreted through kidney
- ▶ Used for energy
  - ▶ Regulates lipid levels
  - ▶ Promotes HCl creation in stomach



# Vitamin B3 Pathology

- ▶ Toxicity
  - ▶ Can increase blood glucose
  - ▶ Liver damage (storage area)
  - ▶ Peptic ulcers
  - ▶ Skin rashes
- ▶ Deficiency
  - ▶ Pellagra
    - ▶ 3 Ds-dermatitis, dementia, diarrhea



# Vitamin B3 Testing

- Specimen Considerations
  - Light sensitive, protect from light
- HPLC performed on plasma
- Reference Range:
  - Niacin: 0.50 – 8.91  $\mu\text{g/mL}$





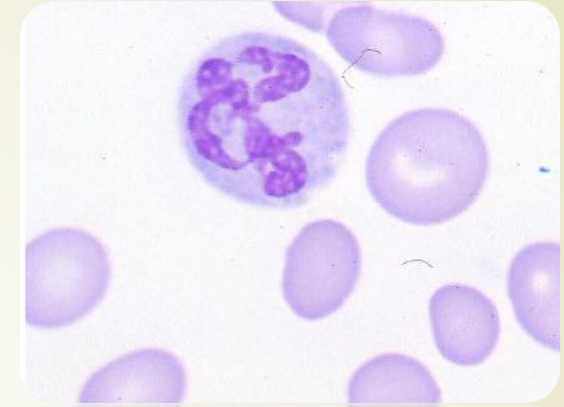
# Vitamin B9



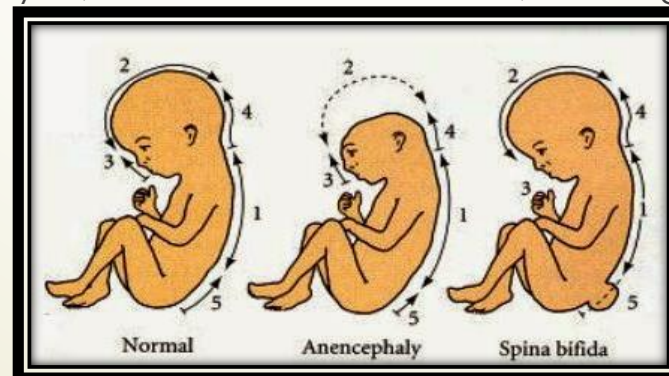
- ▶ Folate or Folic Acid
  - ▶ Folic acid is changed to folate by body
  - ▶ Found in leafy greens, legumes, oranges, cauliflower, lentils, asparagus, liver, yeast, salmon, liver, avocados, and milk
- ▶ Absorbed in small intestine
  - ▶ Modified by enzymes
  - ▶ Circulates bound to protein
  - ▶ Excess excreted by kidney
- ▶ Regulates hematopoiesis along with B12
  - ▶ Vital for DNA synthesis and cell division
  - ▶ Cardiovascular healthy by lowering homocysteine
  - ▶ Fetal development



# Vitamin B9 Pathology

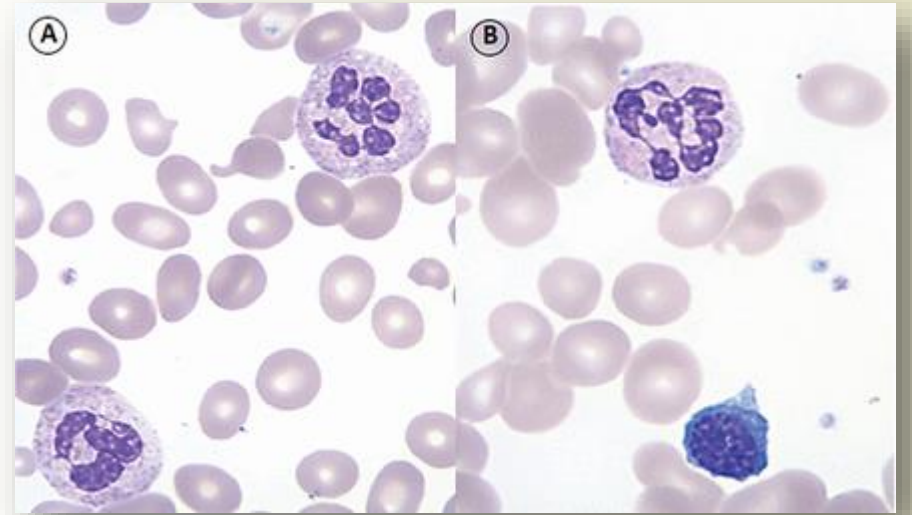


- Toxicity
  - Rare, as it is excreted in urine
  - Extreme OD can cause digestive problems, insomnia, skin reaction, and seizure
- Deficiency
  - Pregnant women: needed to prevent neural tube defects
    - Spina bifida, anencephaly, encephalocele, hydranencephaly, or cleft palate
  - Megaloblastic anemia
    - Macrocytic, normochromic anemia, indistinguishable from B12 deficiency



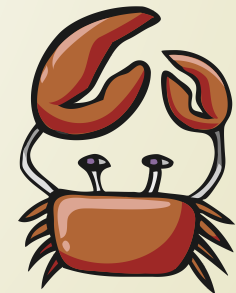
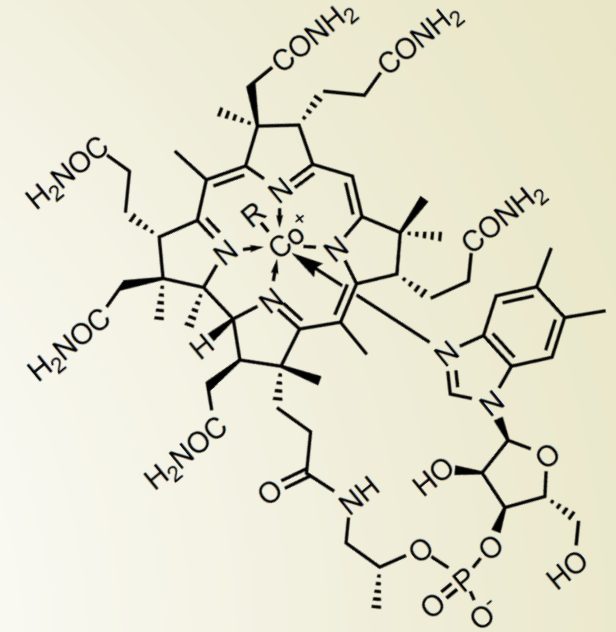
# Vitamin B9 Testing

- ▶ Immunoassay on serum
- ▶ Macrocytic anemia appearance
  - ▶ Low RBC
  - ▶ Low HGB
  - ▶ Hypersegmented neutrophils
  - ▶ High LDH (esp. LDH 2)



# Vitamin B12

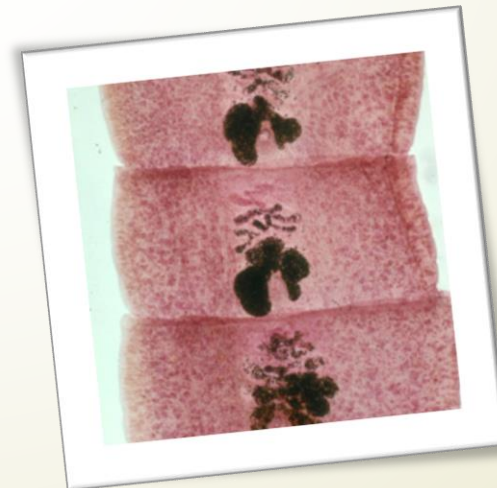
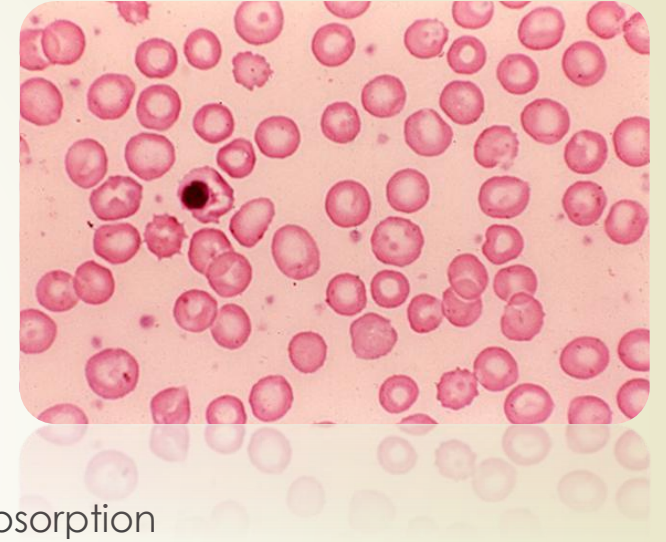
- ▶ Cobalamin
  - ▶ Not 1 compound, but group of related
    - ▶ Cobalt atom, nucleotide side chains, tetrapyrrole rings
- ▶ Found in clams, liver, many fish, shellfish, beef, eggs, and dairy
- ▶ Complex absorption
  - ▶ Absorbed in small intestine
    - ▶ Intrinsic factor
  - ▶ Stored in liver
    - ▶ Excess eliminated by kidney





# Vitamin B12 Pathology

- ▶ Toxicity
  - ▶ None reported
- ▶ Deficiency
  - ▶ Megaloblastic anemia
    - ▶ Restrictive diets, gastric bypass, IBD, congenital malabsorption
    - ▶ Pernicious anemia
    - ▶ *Diphyllobothrium latum*



# Vitamin B12 Testing

- Directly by immunoassay
  - Pretreatment to release from transport proteins
- Indirectly
  - Homocysteine- increased in b12 def.
  - Methylmalonic acid- increased in b12 def.



# Vitamin C

- ▶ Ascorbic acid
  - ▶ Water soluble
    - ▶ Absorbed in small intestines
    - ▶ Stored mainly in adrenal glands
    - ▶ Excretes by kidney
  - ▶ Found in citrus, strawberries, broccoli, tomatoes, potatoes, and cantaloupe
  - ▶ Most plants, animals can synthesize
    - ▶ Many primates cannot
    - ▶ Parallels inability to break down uric acid
      - ▶ Also functions as reducing agent



# Vitamin C Pathology

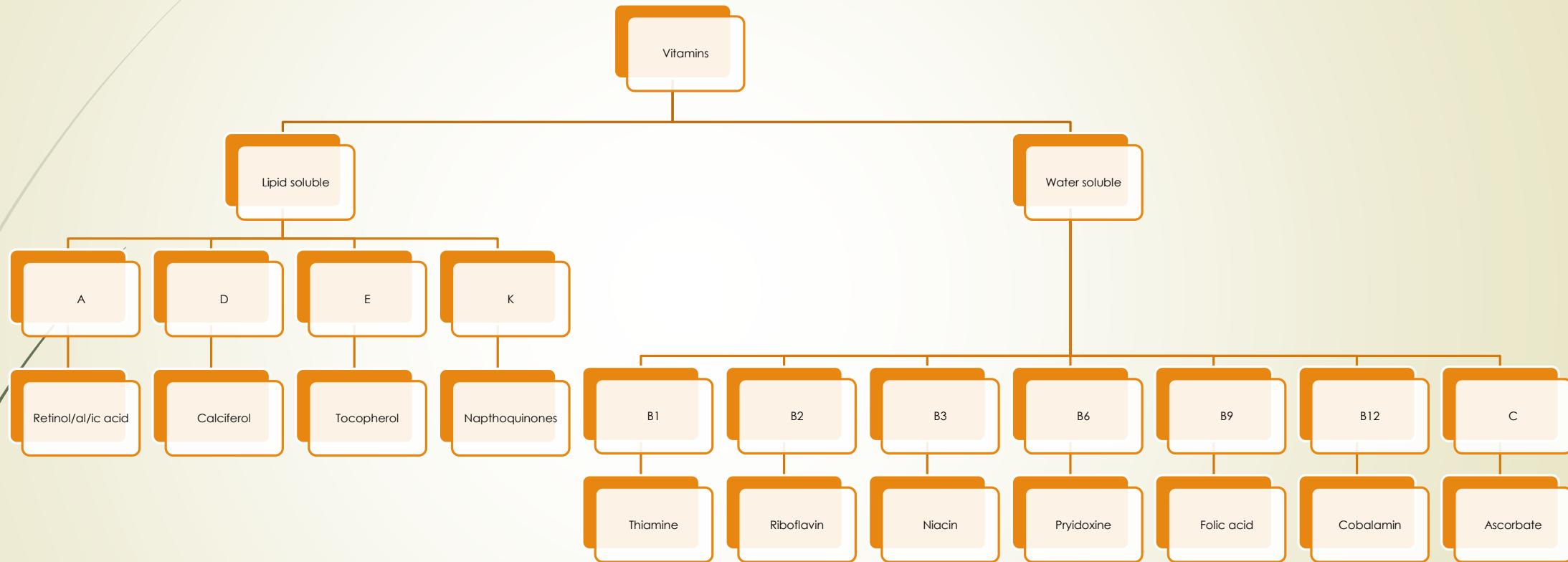
- ▶ Acts as reducing agent and in formation of collagen
  - ▶ Connective tissue
- ▶ Reduces iron and allows for absorption
- ▶ Toxicity
  - ▶ Rare, causes diarrhea, kidney stone, hemolysis, interfere with absorption of other nutrients
- ▶ Deficiency
  - ▶ Scurvy
    - ▶ Swollen & bleeding gums, tooth loss, poor wound healing, pain and weakness in lower extremities



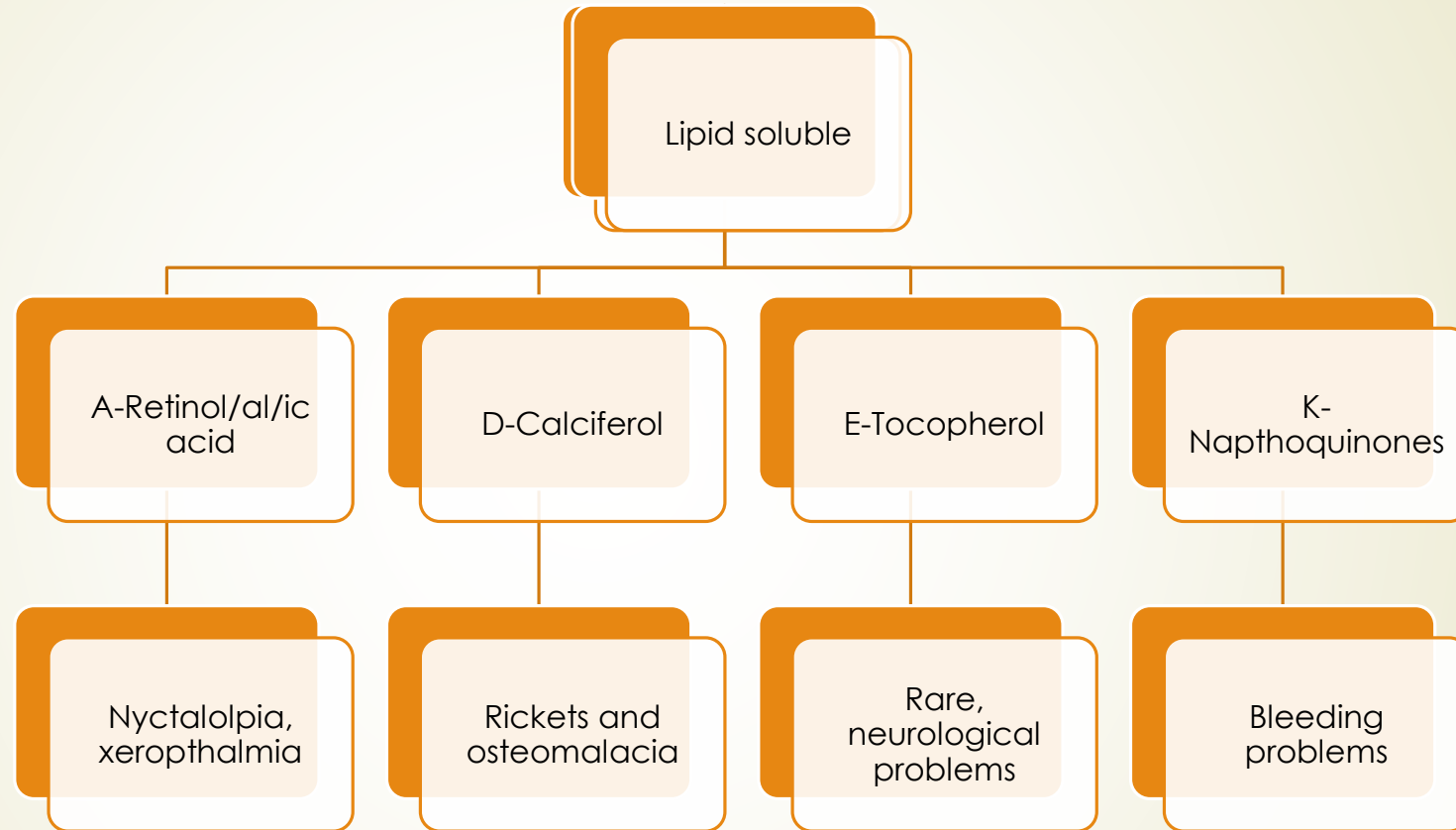
Infantile Scurvy. Ellen S. Five years old. The gums are swollen or beefy and hanging in funnel-like masses. There are also blood tumors on the forehead. (From the pathological laboratory of the Great Ormond Street Hospital, London. Courtesy of Sir Thomas Barlow.)  
\*I am indebted to Dr. Richard Armstrong, of the Great Ormond Street Hospital, London, for valuable assistance in procuring Plates XIV and XV.



# Recap Solubility and Names



# Recap-Deficiency



# Recap-Deficiency

