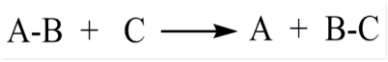
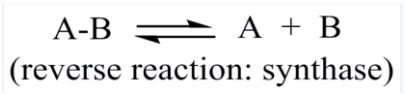
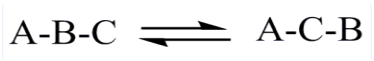
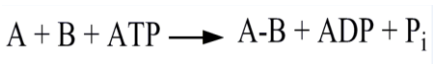
Enzymes

Enzymes are biological molecules (typically proteins) that significantly speed up the rate of virtually all of the chemical reactions that take place within cells. They are vital for life and serve a wide range of important functions in the body, such as aiding in digestion and metabolism.

Enzymes Classification

According to the type of reactions that the enzymes catalyze, enzymes are classified into seven categories, which are oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases, and translocases. Oxidoreductases, transferases and hydrolases are the most abundant forms of enzymes. Individual enzyme classes are further classified systematically based on the chemical name of the substrate and its reaction mechanism.

Enzyme class	Reaction type	Description
EC 1 Oxidoreductases		Catalyze redox reaction and can be categorized into oxidase and reductase.
EC 2 Transferases		Catalyze the transfer or exchange of certain groups among some substrates
EC 3 Hydrolases		Accelerate the hydrolysis of substrates
EC 4 Lyases		Promote the removal of a group from the substrate to leave a double bond reaction or catalyze its reverse reaction
EC 5 Isomerases		Facilitate the conversion of isoisomers, geometric isomers or optical isomers.
EC 6 Ligases		Catalyze the synthesis of two molecular substrates into one molecular compound with the release energy
EC 7 Translocases		Catalyze the movement of ions or molecules across membranes or their separation within membranes

Coenzyme:

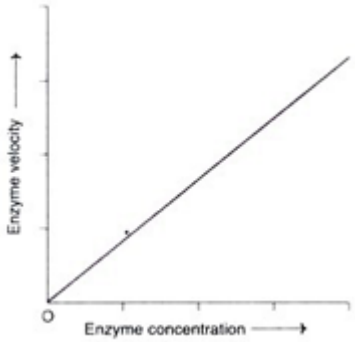
Coenzyme: A substance that enhances the action of an enzyme. (An enzyme is a protein that functions as a catalyst to mediate and speed a chemical reaction).

Coenzymes are small molecules. They cannot by themselves catalyze a reaction but they can help enzymes to do so. In technical terms, coenzymes are organic nonprotein molecules that bind with the protein molecule (apoenzyme) to form the active enzyme (holoenzyme).

A number of the water-soluble vitamins such as vitamins B1, B2 and B6 serve as coenzymes.

Factors affecting enzyme acivity

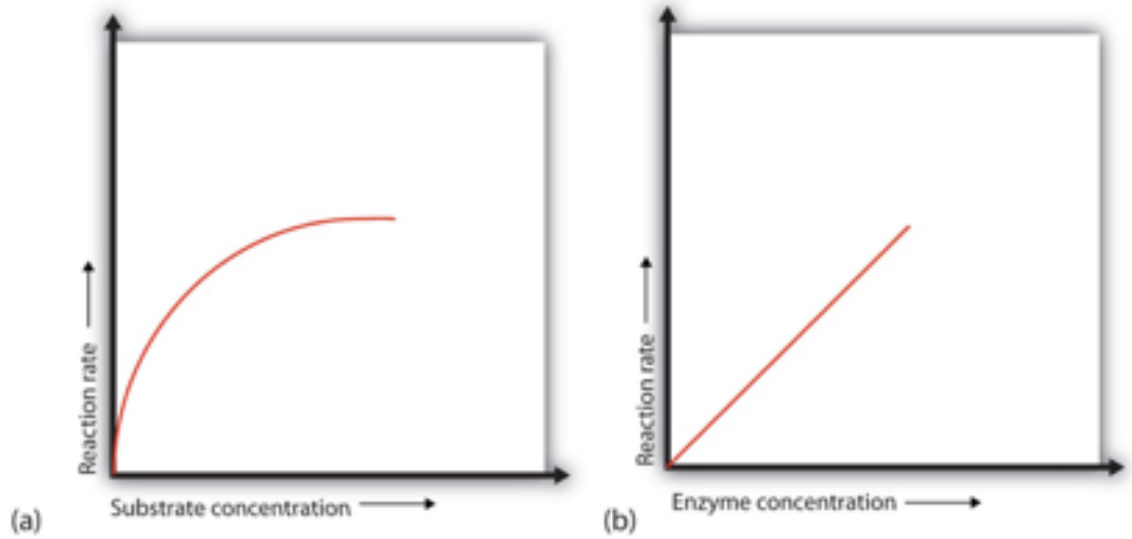
Factor 1: Concentration of Enzyme



Enzyme Concentration

- As the concentration of the enzyme is increased, the velocity of the reaction proportionately increases. This property is used for determining the activities of serum enzymes during the diagnosis of diseases.

Factor 2: Concentration of Substrate



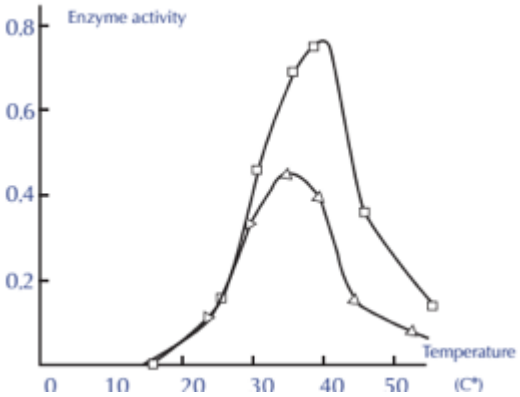
Substrate Enzyme Concentration

- In the presence of a given amount of enzyme, the rate of enzymatic reaction increases as the substrate concentration increases until a limiting rate is reached, after which further increase in the substrate concentration produces no significant change in the reaction rate. At this point, so much substrate is present that essentially all of the enzyme active sites have substrate bound to them.
- In other words, the enzyme molecules are saturated with substrate. The excess substrate molecules cannot react until the substrate already bound to the enzymes has reacted and been released (or been released without reacting).

Factor 3: Effect of Temperature

- The protein nature of the enzymes makes them extremely sensitive to thermal changes. Enzyme activity occurs within a narrow range of temperatures compared to ordinary chemical reactions. As you have seen, each enzyme has a certain temperature at which it is more active. This point is called the optimal temperature, which ranges between 37 to 40C°.
- The enzyme activity gradually lowers as the temperature rises more than the optimal temperature until it reaches a certain temperature at which the enzyme activity stops completely due to the change of its natural composition.

- On the other hand, if the temperature lowers below the optimal temperature, the enzyme activity lowers until the enzyme reaches a minimum temperature at which the enzyme activity is the least. The enzyme activity stops completely at 0C°, but if the temperature rises again, then the enzyme gets reactivated once more.



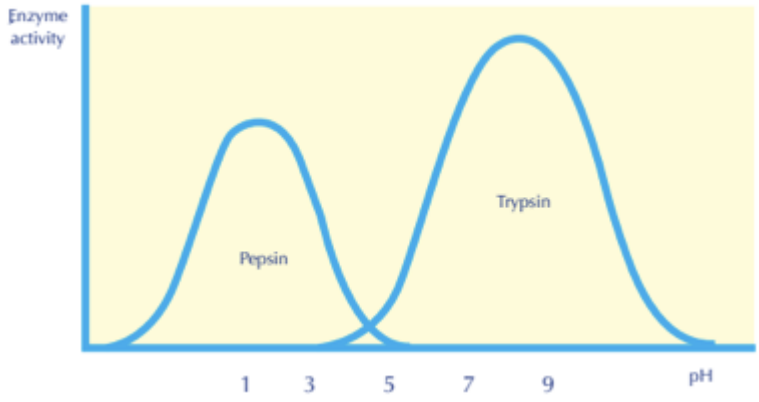
Enzyme activity and temperature

Factor 4: Effect of pH

- The potential of hydrogen (pH) is the best measurement for determining the concentration of hydrogen ion (H^+)in a solution. It also determines whether the liquid is acidic, basic or neutral. Generally, all liquids with a pH below 7 are called acids, whereas liquids with a pH above 7 are called bases or alkalines. Liquids with pH 7 are neutral and equal the acidity of pure water at 25 C°. You can determine pH of any solution using the pH indicators.

pH Indicators

- Enzymes are protein substances that contain acidic carboxylic groups ($COOH^-$) and basic amino groups (NH_2). So, the enzymes are affected by changing the pH value.
- Each enzyme has a pH value that it works at with maximum efficiency called the optimal pH. If the pH is lower or higher than the optimal pH, the enzyme activity decreases until it stops working. For example, pepsin works at a low pH, i.e, it is highly acidic, while trypsin works at a high pH, i.e, it is basic. Most enzymes work at neutral pH 7.4.



Enzyme PH activity

Factor 5: Effect of Activators

- Some of the enzymes require certain inorganic metallic cations, like Mg^{2+}, Mn^{2+}, Zn^{2+}, Ca^{2+}, Co^{2+}, Cu^{2+}, Na^{+}, K^{+} etc., for their optimum activity. Rarely, anions are also needed for enzyme activity, e.g. a chloride ion (CI^{-}) for amylase.

An enzyme inhibitor

An enzyme inhibitor is a molecule that disrupts the normal reaction pathway between an enzyme and a substrate

- Enzyme inhibitors can be either competitive or non-competitive depending on their mechanism of action

Types of Enzyme Inhibition

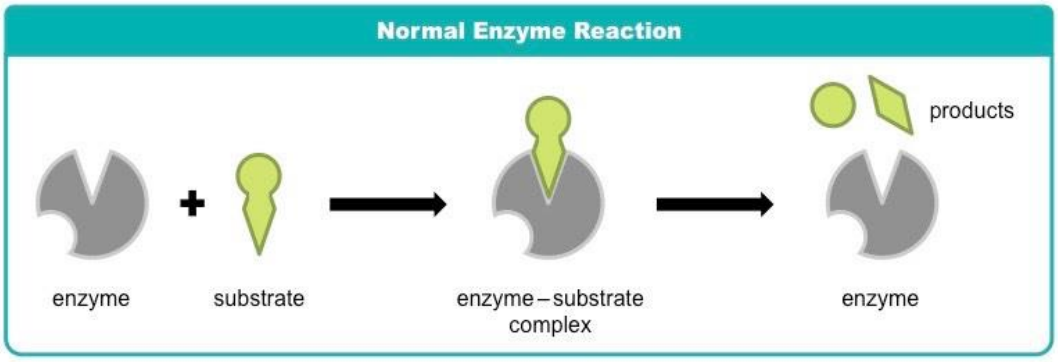
Enzyme inhibitors prevent the formation of an enzyme-substrate complex and hence prevent the formation of product

- Inhibition of enzymes may be either reversible or irreversible depending on the specific effect of the inhibitor being used

<u>Normal Enzyme Reaction</u>

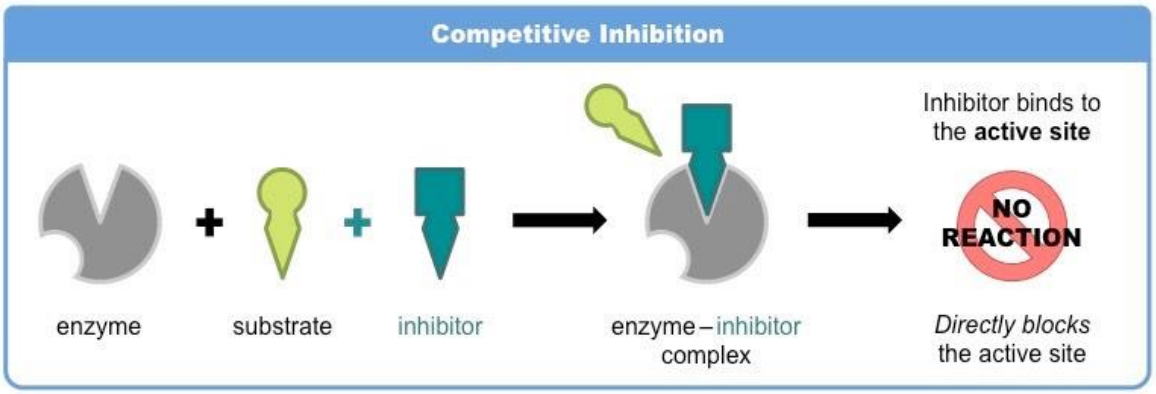
- In a normal reaction, a substrate binds to an enzyme (via the active site) to form an enzyme-substrate complex
- The shape and properties of the substrate and active site are complementary, resulting in enzyme-substrate specificity

- When binding occurs, the active site undergoes a conformational change to optimally interact with the substrate (induced fit)
- This conformational change destabilises chemical bonds within the substrate, lowering the activation energy
- As a consequence of enzyme interaction, the substrate is converted into product at an accelerated rate



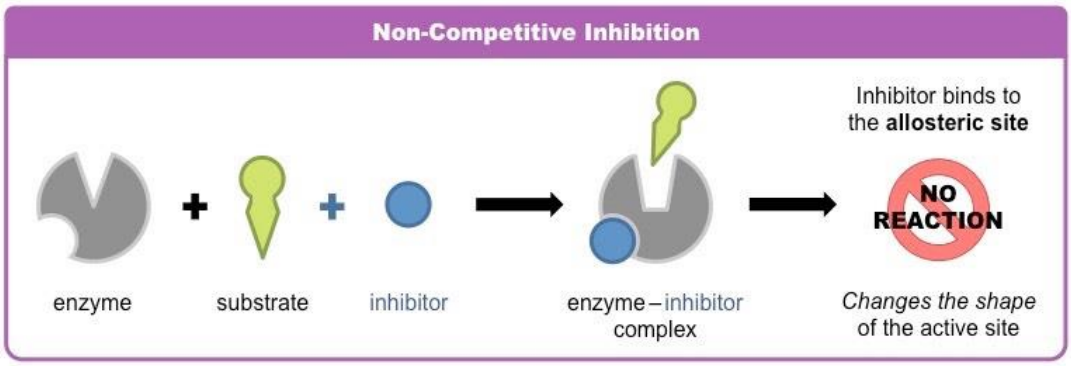
Competitive Inhibition

- Competitive inhibition involves a molecule, other than the substrate, binding to the enzyme's *active site*
- The molecule (inhibitor) is structurally and chemically similar to the substrate (hence able to bind to the active site)
- The competitive inhibitor blocks the active site and thus prevents substrate binding
- As the inhibitor is in competition with the substrate, its effects can be reduced by increasing substrate concentration



Noncompetitive Inhibition

- Non-competitive inhibition involves a molecule binding to a site other than the active site (an *allosteric site*)
- The binding of the inhibitor to the allosteric site causes a conformational change to the enzyme's active site
- As a result of this change, the active site and substrate no longer share specificity, meaning the substrate cannot bind
- As the inhibitor is **not** in direct competition with the substrate, increasing substrate levels cannot mitigate the inhibitor's effect



Examples of Enzyme Inhibition

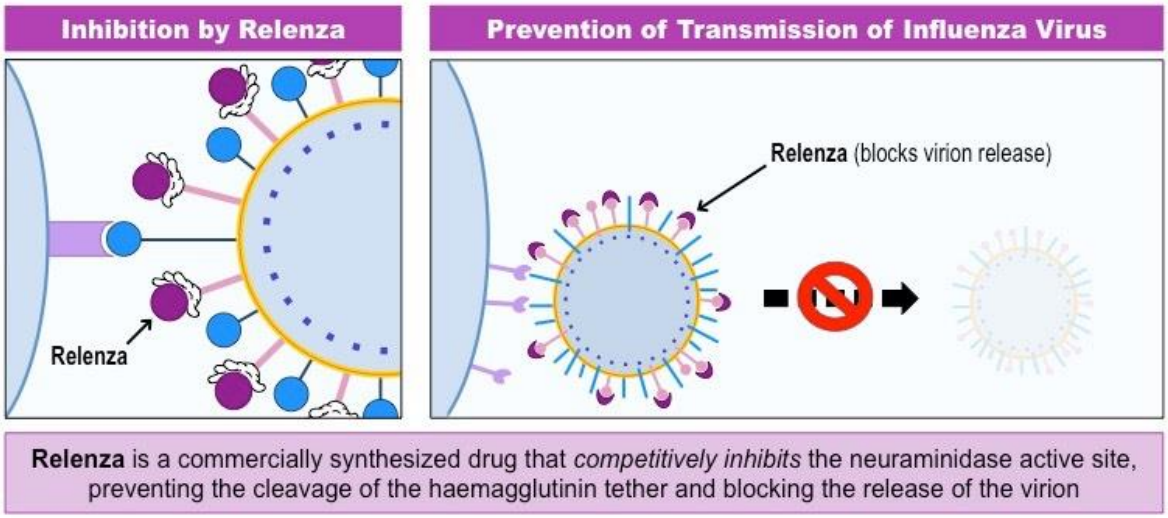
Enzyme inhibitors can serve a variety of purposes, including in medicine (to treat disease) and agriculture (as pesticides)

- An example of a use for a competitive inhibitor is in the treatment of influenza via the neuraminidase inhibitor, Relenza™
- An example of a use for a non-competitive inhibitor is in the use of cyanide as a poison (prevents aerobic respiration)

Relenza (Competitive Inhibitor)

- Relenza is a synthetic drug designed by Australian scientists to treat individuals infected with the influenza virus
- Virions are released from infected cells when the viral enzyme neuraminidase cleaves a docking protein (haemagglutinin)

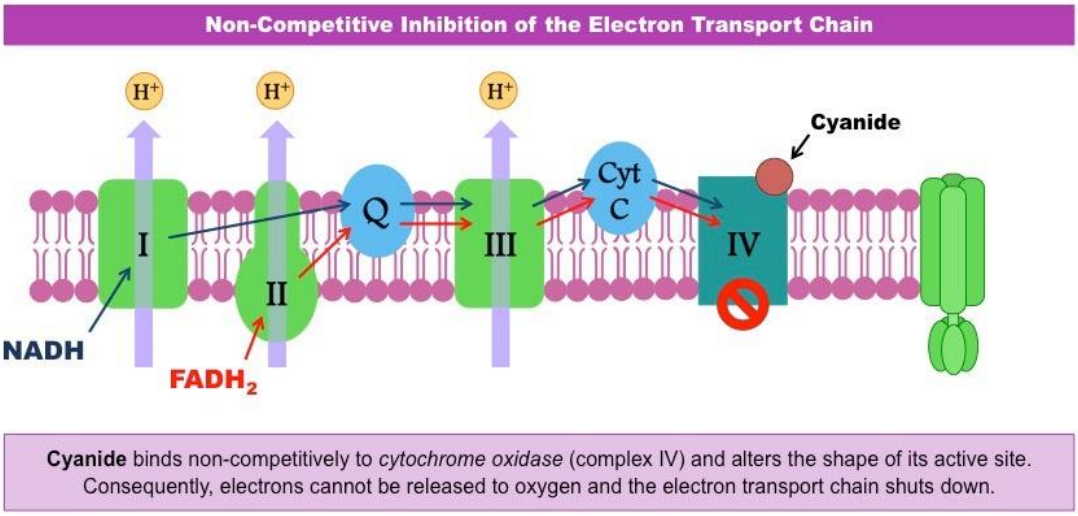
- Relenza competitively binds to the neuraminidase active site and prevents the cleavage of the docking protein
- Consequently, virions are not released from infected cells, preventing the spread of the influenza virus



Host Status: ○ Normal Infection ○ Treatment with Relenza

<u>Cyanide (Noncompetitive Inhibitor)</u>

- Cyanide is a poison which prevents ATP production via aerobic respiration, leading to eventual death
- It binds to an allosteric site on cytochrome oxidase – a carrier molecule that forms part of the electron transport chain
- By changing the shape of the active site, cytochrome oxidase can no longer pass electrons to the final acceptor (oxygen)
- Consequently, the electron transport chain cannot continue to function and ATP is not produced via aerobic respiration



Electron Transport Chain: Normal Function Treatment with Cyanide

Liver function tests

Liver function tests measure certain proteins, enzymes, and substances, including:

- Albumin, a protein that the liver makes and globulin.
- Total protein (TP)
- Enzymes that are found in the liver, including alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transpeptidase (GGT)
- Bilirubin, a yellow substance that is part of bile. It is formed when your red blood cells break down. Too much bilirubin in the blood can cause jaundice. There is also a urine test for bilirubin.
- Prothrombin time, which measures how long it takes for your blood to clot. Prothrombin is made by the liver.

Kidney function tests

- Kidney function tests are common lab tests used to evaluate how well the kidneys are working. Such tests include:
- BUN (Blood urea nitrogen)
- Creatinine - blood
- Creatinine clearance
- Creatinine - urine
- Uric acid
- Sodium
- Potassium
- Chloride.

Vitamins

Vitamins are organic compounds that are essential in very small amounts for supporting normal physiologic function. We need vitamins in our diet, because our bodies can't synthesize them quickly enough to meet our daily needs.

Vitamins have three characteristics:

- They are natural components of foods; usually present in very small amounts.
- They are essential for normal physiologic function (eg. growth, reproduction, etc).
- When absent from the diet, they will cause a specific deficiency.

Vitamins are generally categorized into the following types

1. Water soluble
2. Fat soluble

Water-soluble vitamins

Vitamin B1 (Thiamine)

- **Deficiency:** Symptoms include burning feet, weakness in extremities, rapid heart rate, swelling, anorexia, nausea, fatigue, and gastrointestinal problems.
- **Toxicity:** None known.

- **Sources:** Sunflower seeds, asparagus, lettuce, mushrooms, black beans, navy beans, lentils, spinach, peas, pinto beans, lima beans, eggplant, Brussels sprouts, tomatoes, tuna, whole wheat, soybeans.

Vitamin B2 (Riboflavin)

- **Deficiency:** Symptoms include cracks, fissures and sores at corner of mouth and lips, dermatitis, conjunctivitis, photophobia, glossitis of tongue, anxiety, loss of appetite, and fatigue.
- **Toxicity:** Excess riboflavin may increase the risk of DNA strand breaks in the presence of chromium. High-dose riboflavin therapy will intensify urine color to a bright yellow (flavinuria) – but this is harmless.
- **Sources:** Almonds, soybeans/tempeh, mushrooms, spinach, whole wheat, yogurt, mackerel, eggs, liver

Vitamin B3 (Niacin)

- **Deficiency:** Symptoms include dermatitis, diarrhea, dementia, and stomatitis.
- **Toxicity:** Niacin from foods is not known to cause adverse effects. Supplemental nicotinic acid may cause flushing of skin, itching, impaired glucose tolerance and gastrointestinal upset. Intake of 750 mg per day for less than 3 months can cause liver cell damage. High dose nicotinamide can cause nausea and liver toxicity.
- **Sources:** Mushrooms, asparagus, peanuts, brown rice, corn, green leafy vegetables, sweet potato, potato, lentil, barley, carrots, almonds, celery, turnips, peaches, chicken meat, tuna, salmon

Vitamin B5 (Pantothenic acid)

- **Deficiency:** Very unlikely. Only in severe malnutrition may one notice tingling of feet.
- **Toxicity:** Nausea, heartburn and diarrhea may be noticed with high dose supplements.
- **Sources:** Broccoli, lentils, split peas, avocado, whole wheat, mushrooms, sweet potato, sunflower seeds, cauliflower, green leafy vegetables, eggs, squash, strawberries, liver

Vitamin B6 (Pyridoxine)

- **Deficiency:** Symptoms include chelosis, glossitis, stomatitis, dermatitis (all similar to vitamin B2 deficiency), nervous system disorders, sleeplessness, confusion, nervousness, depression, irritability, interference with nerves that supply muscles and difficulties in movement of these muscles, and anemia. Prenatal deprivation results in mental retardation and blood disorders for the newborn.
- **Toxicity:** High doses of supplemental vitamin B6 may result in painful neurological symptoms.
- **Sources:** Whole wheat, brown rice, green leafy vegetables, sunflower seeds, potato, garbanzo beans, banana, trout, spinach, tomatoes, avocado, walnuts, peanut butter, tuna, salmon, lima beans, bell peppers, chicken meat

Vitamin B7 (Biotin)

- **Deficiency:** Very rare in humans. Keep in mind that consuming raw egg whites over a long period of time can cause biotin deficiency. Egg whites contain the protein avidin, which binds to biotin and prevents its absorption.
- **Toxicity:** Not known to be toxic.
- **Sources:** Green leafy vegetables, most nuts, whole grain breads, avocado, raspberries, cauliflower, carrots, papaya, banana, salmon, eggs

Vitamin B9 (Folic acid)

Folate is the naturally occurring form found in foods. Folic acid is the synthetic form used in commercially available supplements and fortified foods. Inadequate folate status is associated with neural tube defects and some cancers.

- **Deficiency:** One may notice anemia (macrocytic/megaloblastic), sprue, Leukopenia, thrombocytopenia, weakness, weight loss, cracking and redness of tongue and mouth, and diarrhea. In pregnancy there is a risk of low birth weight and preterm delivery.
- **Toxicity:** None from food. Keep in mind that vitamin B12 and folic acid deficiency can both result in megaloblastic anemia. Large doses of folic acid given to an individual with an undiagnosed vitamin B12 deficiency could correct megaloblastic anemia without correcting the underlying vitamin B12 deficiency.

- **Sources:** Green leafy vegetables, asparagus, broccoli, Brussels sprouts, citrus fruits, black eyed peas, spinach, great northern beans, whole grains, baked beans, green peas, avocado, peanuts, lettuce, tomato juice, banana, papaya, organ meats

Vitamin B12 (Cobalamin)

Vitamin B12 must combine with intrinsic factor before it's absorbed into the bloodstream. We can store a year's worth of this vitamin – but it should still be consumed regularly. B12 is a product of bacterial fermentation, which is why it's not present in higher order plant foods.

- **Deficiency:** Symptoms include pernicious anemia, neurological problems and sprue.
- **Toxicity:** None known from supplements or food. Only a small amount is absorbed via the oral route, thus the potential for toxicity is low.
- **Sources:** Fortified cereals, liver, trout, salmon, tuna, haddock, egg

Vitamin C (Ascorbic acid)

- **Deficiency:** Symptoms include bruising, gum infections, lethargy, dental cavities, tissue swelling, dry hair and skin, bleeding gums, dry eyes, hair loss, joint paint, pitting edema, anemia, delayed wound healing, and bone fragility. Long-term deficiency results in scurvy.
- **Toxicity:** Possible problems with very large vitamin C doses including kidney stones, rebound scurvy, increased oxidative stress, excess iron absorption, vitamin B12 deficiency, and erosion of dental enamel. Up to 10 grams/day is safe based on most data. 2 grams or more per day can cause diarrhea.
- **Sources:** Guava, bell pepper, kiwi, orange, grapefruit, strawberries, Brussels sprouts, cantaloupe, papaya, broccoli, sweet potato, pineapple, cauliflower, kale, lemon juice, parsley.

Fat soluble vitamins

Vitamin A (Retinoids)

Carotenoids that can be converted by the body into retinol are referred to as provitamin A carotenoids.

- **Deficiency:** One may notice difficulty seeing in dim light and rough/dry skin.

- **Toxicity:** Hypervitaminosis A is caused by consuming excessive amounts of preformed vitamin A, not the plant carotenoids. Preformed vitamin A is rapidly absorbed and slowly cleared from the body. Nausea, headache, fatigue, loss of appetite, dizziness, and dry skin can result. Excess intake while pregnant can cause birth defects.
- **Sources:** Carrots, sweet potato, pumpkin, green leafy vegetables, squash, cantaloupe, bell pepper, Chinese cabbage, beef, eggs, peaches

Vitamin D (Calciferol, 1,25-dihydroxy vitamin D)

Cholecalciferol = vitamin D3 = animal version; ergocalciferol = vitamin D2 = plant version

- **Deficiency:** In children a vitamin D deficiency can result in rickets, deformed bones, retarded growth, and soft teeth. In adults a vitamin D deficiency can result in osteomalacia, softened bones, spontaneous fractures, and tooth decay. Those at risk for deficiency include infants, elderly, dark skinned individuals, those with minimal sun exposure, fat malabsorption syndromes, inflammatory bowel diseases, kidney failure, and seizure disorders.
- **Toxicity:** Hypervitaminosis D is not a result of sun exposure but from chronic supplementation. Excessive supplement use will elevate blood calcium levels and cause loss of appetite, nausea, vomiting, excessive thirst, excessive urination, itching, muscle weakness, joint pain and disorientation. Calcification of soft tissues can also occur.
- **Sources:** Sunlight, fortified foods, mushrooms, salmon, mackerel, sardines, tuna, eggs

Vitamin E (tocopherol)

- **Deficiency:** Only noticed in those with severe malnutrition. However, suboptimal intake of vitamin E is relatively common.
- **Toxicity:** Minimal side effects have been noted in adults taking supplements in doses less than 2000 mg/day. There is a potential for impaired blood clotting. Infants are more vulnerable.
- **Sources:** Green leafy vegetables, almonds, sunflower seeds, olives, blueberries, most nuts, most seeds, tomatoes, avocado

Vitamin K

- **Deficiency:** Tendency to bleed or hemorrhage and anemia.

- **Toxicity:** May interfere with glutathione. No known toxicity with high doses.
- **Sources:** Broccoli, green leafy vegetables, parsley, watercress, asparagus, Brussels sprouts, green beans, green peas, carrots.