

<b>COURSE CODE:</b>	VBB 204
<b>COURSE TITLE:</b>	Drugs & Nutritional Biochemistry
<b>NUMBER OF UNITS:</b>	2 Units
<b>COURSE DURATION:</b>	Two hours per week

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### COURSE DETAILS:

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<b>Office Location:</b>	<b>Glass house, COLVET</b>
<b>Other Lecturers:</b>	<b>Dr Babatunde Samuel Okediran B.Sc., DVM., M.Sc</b>

### COURSE CONTENT:

Biochemical basis of drug, chemical and toxin metabolism and elimination, drug metabolizing enzymes. Biochemistry of drugs of importance in vet. Medicine including antibiotics, cytotoxic drugs hypoglycemic drugs, steroidal drugs. General nutritional requirements of animals. Monogastric and ruminant biochemistry. Mechanism of nutrition-related diseases; nutrition and infection. Nutrient-drug interactions. Effect of nutrition on membrane functions and immune response. Water and the major ions. Fluid intake and output. Intracellular and extracellular fluids. Vomiting, diarrhea and dehydration and their correction. Calcium, phosphorus and trace element ions metabolism and dysfunctions. Water soluble and fat soluble vitamins: biochemical functions. Chemistry and biochemistry of vision.

## COURSE REQUIREMENTS:

This is a compulsory course for all students of Veterinary Medicine. In view of this, Veterinary students are expected to participate in all the course activities and have a minimum of 75% attendance to be able to write the final examination.

## READING LIST:

1. V.K Malhotra. Biochemistry for students. Tenth edition. Jaypee Brothers Medical Publishers (p) ltd.
2. J.Jerry Kaneko., John W. Harvey., Michael L. Bruss. Clinical Biochemistry of Domestic Animals. Sixth edition. Academic Press.
3. Harvey Lodish., David Baltimore., Arnold Berk., S. Lawrence Zipursky., Paul Matsudaira and James Darnell. Molecular Cell Biology. Third Edition. By Scientific American Books, Inc.
4. David A. Bender., Kathleen M. Botham., Daryl K. Granner., Frederick W. Keeley., Peter A. Mayers., Robert K. Murray., Victor W. Rodwell., P. Anthony Weil.(2006). Harpers Illustrated Biochemistry. 27<sup>th</sup> Edition. Published By Mc Graw Hill

## LECTURE NOTES

### WATER AND THE MAJOR IONS

#### Water

The living cell is made up of approximately 70% of water. It is an irregular tetrahedron molecule with oxygen at its center. It is essential for life and solubilizes and modifies the properties of biomolecules. H<sub>2</sub>O is its chemical formula.

#### PROPERTIES

- It is dipolar(It has unequally distributed electrical charge)
- The elements of this compound are joined by weak hydrogen bonds which accounts for its liquidity at room temperature

- It is highly viscous
- It has a high surface tension.
- good solvent: water dissolves more compounds than any other liquid
- high heat capacity
- high melting and boiling point

Sources; water as such by drinking, water in the feed supply and metabolic water obtained from the oxidation of carbohydrates, fat and protein in the body.

### FUNCTIONS

1. It's a vital constituent of cells and provides medium for chemical reactions
2. It provides fluidity to blood and other body fluids e.g. saliva, cerebrospinal fluid, gastric juices etc hence serves as a lubricant in the transport of feed to tissues
3. It acts as a medium of heat dissipation in the body.
4. An aid in excretion
5. A buffering agent to regulate pH (acidity or alkalinity) of body fluids 70% of the body is composed of water which is distributed in two major compartments in the body.
6. Water can act as both hydrogen donor and hydrogen acceptor i.e. acid and base, the basis of which it acts as a solvent to compounds which can also accept or donate protons themselves for H-binding with Water.

A number of factors affect the amount of water consumed by an animal including physiologic states, environmental temperature, and type of diet and so on. Dissolved within the body water are solutes composed chiefly of three categories of substances:

1. Organic compounds of large molecular e.g. proteins and these aid in distribution of water between the compartments of the by its effect on osmotic pressure.
2. Organic compounds of small molecular size e.g. glucose, urea etc exert little or no osmotic pressure but when in large quantities aid in retention of water.
3. Inorganic electrolytes or ions, these are found in large quantities within the fluids hence play a vital role in retention and distribution of body water.

## **MAJOR IONS: H<sup>+</sup>, NA<sup>+</sup>, K<sup>+</sup>, CL<sup>-</sup> AND HCO<sub>3</sub><sup>-</sup>**

Electrolytes are elements or compounds that dissociate in solution for example NaCl, KCl to give the constituent ions Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup>, ions that are completely surrounded by water molecules. Positive ions are called cations while negative ions are called anions. These elements are involved in the maintenance of homeostasis (water, osmotic and PH status).the law of electrical neutrality states that the total number of +ve ions always equals the total number of -ve ions.

### **Hydrogen ion (proton) - H<sup>+</sup>**

H<sup>+</sup> ions are present in all body compartments and the maintenance of appropriate concentrations is essential for normal cellular functions. It has the largest concentration amongst cations in the plasma. It has negligible osmotic activity

The gradient of H<sup>+</sup> concentration between inner and outer mitochondrial membranes acts as a driving force for oxidative phosphorylation. In addition H<sup>+</sup> concentration in a fluid medium determines the ionization of weak acids and hence their functions within the body. Also H<sup>+</sup>

Levels affect the surface charge and physical properties of proteins that make up the body. PH level is a measure of the H<sup>+</sup> concentration, hence H<sup>+</sup> concentration determines the PH of blood and determines optimum environment for body chemical reactions.

H<sup>+</sup> is excreted in urine as H<sub>2</sub>PO<sub>4</sub><sup>2-</sup>.

### **Sodium ion (Na<sup>+</sup>)**

It is the major cation of the ECF and helps to regulate the volume of the ECF. Total body sodium is about 4000mEq, 50% is found in bones, 40% in ECF and 10% in soft tissues.

Na<sup>+</sup> as well as other cations constitutes osmotically active particles. Sodium pump operates in all cells to keep the levels of Na<sup>+</sup> in the ECF always higher than that in the ICF, its activity is usually accompanied by opposite movement of K<sup>+</sup>. Normal plasma concentration of Na<sup>+</sup> is about 136-145mEq/l while in the ICF its 12mEq/l.

NaCl (common salt) is the major source of Na<sup>+</sup> to the body, although it is also widely distributed in food materials mainly of animal sources.

Na<sup>+</sup> is readily absorbed from the intestines by sodium pump located in the Basal and lateral plasma membrane enterocytes and renal cells and Na-pump actively transports Na into the ECF.

### **FUNCTIONS**

1. It maintains the crystalloid osmotic pressure of ECF, helping to retain water in the ECF.
2. It's involved in neuromuscular excitability/irritability.

3. It maintains viscosity by the sodium salt and along with  $K^+$  helps to maintain the degree of hydration of plasma proteins.
4. It plays an active role in resting membrane potential, by keeping the Na conc. Far in the ECF than in the ICF (known as the resting membrane potential) causing a polarization creating a potential difference of up -70 to -90mV across the membranes.
5. In the same vein, the sudden increased permeability of the membrane to Na causing a rapid influx of Na into the cell occurs in the generation of action potential.

99% of  $Na^+$  is filtered along in the glomerular filtrate and reabsorbed majorly in the proximal convoluted tubules. At the distal tubules, rennin (secreted from the juxtaglomerular cells) is produced due to decreased arterial pressure (decreased  $Na^+$ ) and this stimulates the secretion of Aldosterone that causes reabsorption of  $Na^+$ ,  $K^+$  and to a lesser extent  $H^+$  is lost in its place. Water subsequently moves in the direction of  $Na^+$ .

### **Potassium ion ( $K^+$ )**

It is the major cation of the ICF and helps to maintain the intracellular osmotic pressure.

Total body  $K^+$  is about 3500mEq/l.

This ion is easily obtained from many foods such as fruits and vegetables etc.

$K^+$  is easily absorbed into the blood.

#### **FUNCTIONS**

1. EC  $K^+$  is an important factor in skeletal and cardiac muscle contraction.
2. It is also involved in acid-base balance in the body
3. It is also actively involved in nerve impulse transmission and neuromuscular irritability.
4. Certain enzymes such as pyruvate kinase require  $K^+$  as cofactor.

Plasma levels are about 3.5-5mEq/l while IC levels reach up to 150mEq/l.

It is obligatorily lost during  $Na^+$  reabsorption from the tubules. It is also excreted in gastrointestinal tract, saliva, gastric juices, bile, pancreatic and intestinal juices.

### **Chloride ion ( $Cl^-$ )**

It is the major anion of the ECF and forms inorganic anion of greatest quantity in the body. It also forms part of the osmotically active particles in plasma.

$Cl^-$  is obtained from NaCl, and many other food substances. It is readily absorbed.

#### **FUNCTIONS**

1. It is involved in water distribution
2. Osmotic pressure maintenance and
3. Anion-Cation balance in ECF
4. It is important in the formation of gastric juices and hydrochloric acid.

Intake, output and metabolism of  $\text{Na}^+$  and  $\text{Cl}^-$  run in parallel; it is filtered in renal tubules and passively reabsorbed in the proximal tubules.  $\text{Cl}^-$  is also excreted in sweat.

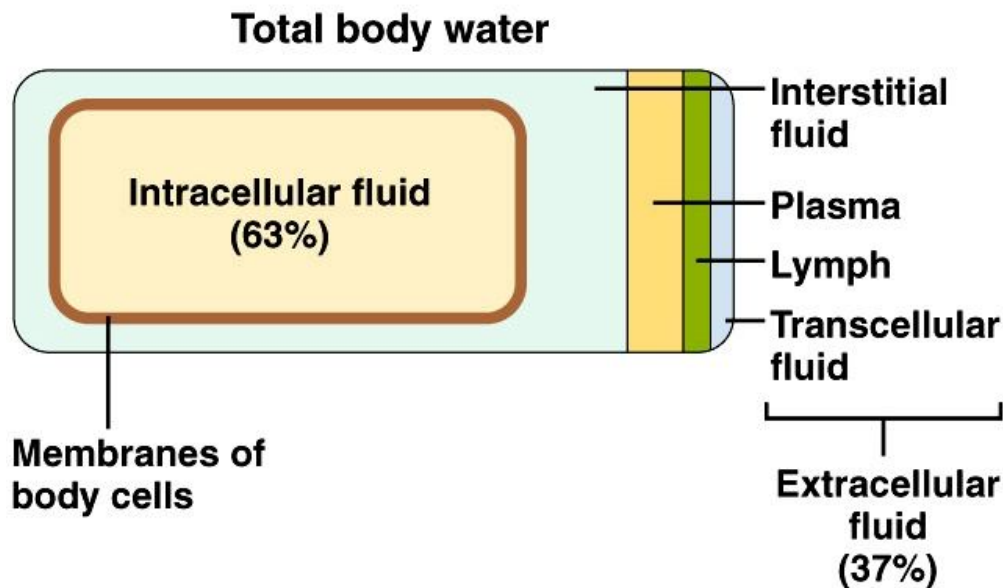
## FLUID AND ELECTROLYTE BALANCE

### TOTAL BODY WATER

Total body water (TBW) is divided into two major compartments- the intracellular fluid (ICF) and the extra cellular fluid (ECF).the ICF accounts for approximately half to two third, the volume of body water while the ECF accounts for the rest. The two body fluid compartments differ markedly in solute and electrolyte compositions but are in osmotic equilibrium and water is freely diffusible between them. Movement of fluids is due to hydrostatic pressure and osmotic pressure.

The ECF  $\text{Na}^+$  concentration largely determines the volume of the ECF, while that of the ICF is determined by  $\text{K}^+$  concentration. Since water is freely permeable through cell membrane is no major osmotic gradient between ECF and ICF.

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### ECF

This consists of all the fluids located outside the cell including fluid in plasma (5-8% of TBW), interstitial fluid (25%), lymph, transcellular fluid (fluid content of gastrointestinal, respiratory

tracts, intraocular fluids etc; 1-2%). All these fluid content  $\text{Na}^+$  as the predominant cation at values ranging between 130-150mEq/l, this determines the ECF volume.

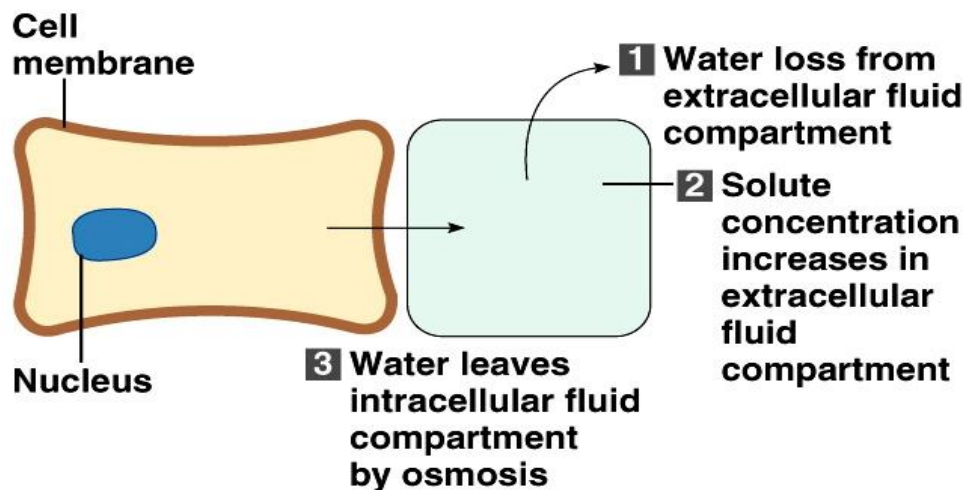
Deficient in  $\text{Na}^+$  results in decrease in ECF volume whereas excess results in water retention in the compartment and can result to a condition called Edema (accumulation of fluids within the interstitial spaces).  $\text{Cl}^-$  and  $\text{HCO}_3^-$  are the major anions found in this compartment. Fluid generally moves from plasma to interstitial space via hydrostatic pressure and from IF to plasma through the force of colloid osmotic pressure and from the IF to lymph to venous plasma.

Homeostasis is maintained in response to changes that occur in the ECF.

### ICF

This is all the fluid contained within the cells in the body.  $\text{K}^+$  provides the osmotic skeleton for the ICF just as  $\text{Na}^+$  does for the ECF. Changes in tonicity of the ECF are rapidly reflected in the ICF as also changes in tonicity as a result of movement of water out of the cells, thereby changing the ICF volume.

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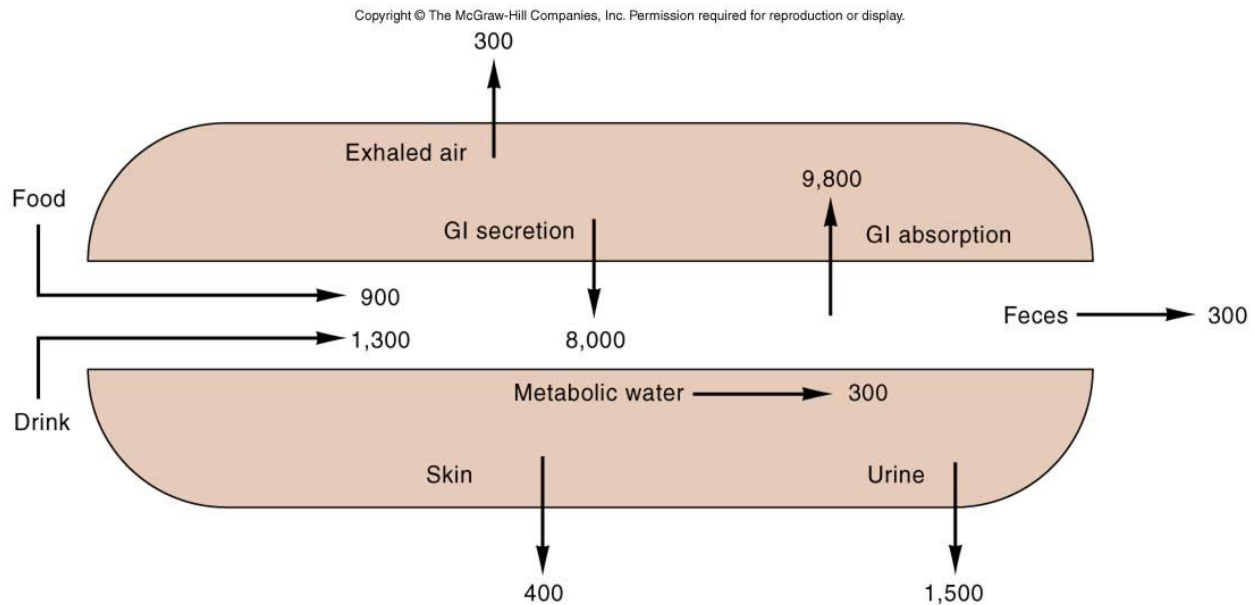


Hence when there is water retention in the ECF,  $\text{Na}^+$  concentration decreases, whereas the ICF volume increases. Other major ions found in this compartment are;  $\text{Mg}^{+2}$ ,  $\text{HPO}_4^{-2}$  and proteins<sup>-</sup>. Rapid fluid movement occurs between the ECF and ICF in response to changes in concentration of the ECF, and to ensure a balance, fluid or electrolyte gains must be equal to loss.

## Fluid intake and output

Intake; fluid as water enters into the body by ingestion through drinking of water, as such, or from water in food. A small amount of water is also gotten from the metabolic breakdown of food products to carbon dioxide and water, via the oxidative phosphorylative pathway.

Output (or loss); is via kidneys (urine), sweat glands (insensible perspiration and sensible loss), & feces. Little amount of water is also lost through exhaled air.



## REGULATION OF TOTAL BODY WATER AND ELECTROLYTES.

Generally homeostasis of water and electrolytes is maintained by ion transport, water movement and kidney functions.

1. Thirst can be stimulated to increase water intake.
2. An hormone ADH (anti diuretic hormone) can be secreted by the which causes the reabsorbtion of water at the kidney tubules; depending on the state of the body water content there may be either an increased secretion (to facilitate retention of more water e.g. as in time of dehydration) or decreased secretion (to facilitate loss of more water from the body as in cases of overhydration). The effects of either an increase or decrease in ADH secretion are an increase or decrease in urine output.



3. Renin is also secreted and this causes increased reabsorption of  $\text{Na}^+$  and concurrently water by converting angiotensinogen to angiotensin I, which is subsequently converted to biologically active angiotensin II which exerts the effects. Renin is released in response to reduced renal perfusion produced by hypotension volume depletion or sympathetic activity.
4. Aldosterone produced in the adrenal cortex in response to changes in effective circulating fluid volume. It causes renal resorption of  $\text{Na}^+$  in exchange for  $\text{K}^+$  and  $\text{H}^+$  in response to  $\text{Na}$  depletion.
5. Atrial Natriuretic Factor (ANF); this hormone results in natriuresis and diuresis by the kidneys.

### **Functions of electrolytes**

Electrolytes are substances that exist as positive or negative charged particles in solution. To maintain electrical neutrality in biological fluids, there must be equal number of equivalents or milliequivalents of anions and cations in solution, therefore electrolytes in solution combine equivalents for equivalents i.e. positive and negative charges.

The osmotic properties of a solute in solution are related to the number of particles in solution and not on its weight or charge.

Concentrations of solutes in biological fluids are expressed as millimole (mmol/l), milliequivalents (mEq/l) or milliosmoles (mOsm/kg).

Electrolytes function to maintain the osmotic pressure and water balance between the compartment while individual electrolytes provide suitable environment for biological reactions and cell functions.

- $\text{Na}^+$  - major ion in ECF
  - Gain-loss imbalance is most common electrolyte problem
    1. Intake across digestive epithelium based on food content
    2. Loss in urine excretion & skin perspiration
  - Change in  $\text{Na}^+$  level causes water movement, maintaining ECF  $\text{Na}^+$  conc.

Ex. Salty meal increases  $\text{Na}^+$  level in digestive ECF, causing water input from digestive tract, increasing blood volume & pressure

- Homeostatic mech: ADH, Aldosterone, Natriuretic peptides
- $\text{K}^+$  - major ion in ICF

- Imbalance less common but more dangerous
  - Essential for nerve transmission
- $\text{Ca}^{+2}$  - in ECF & ICF
  - Absorbed by active transport, increased by PTH & calcitriol
  - Essential in muscle contraction, neurotransmitter release, clotting, bone formation
- $\text{Mg}^{+2}$  - mainly in ICF
  - Essential as enzyme cofactor, ATP use in contracting muscle, bone component
- $\text{PO}_4^{-3}$  - most important function in ICF
  - Essential for bone mineralization, ATP, phosphorylation
- $\text{Cl}^-$  - mainly in ECF, associated with Na

### **Dehydration and its correction**

Dehydration occurs when there is more loss than gain of water or fluids and results in an increase in the osmotic concentration of the ECF relative to the ICF causing water to shift from ICF to the ECF with a resultant more concentration and lower volumes of both the ECF and ICF. It is usually caused by pathological conditions such as vomiting and diarrhea, or low water intake or exercise in hot weather.

The goal in correcting dehydration is not just to replace lost fluid but also lost electrolytes as well in order to increase the volume of both the ECF and ICF.

### **Overhydration**

This occurs when there is more gain than loss of water from the body and results in the decrease in the osmotic concentration of the ECF and the shifting of water from the ECF into the ICF with resultant lower concentration in both compartments but higher volumes. It occurs in such conditions as excess intake of fluids, hypotonic solution infused, unable to eliminate urine, endocrine disorders etc

## **CALCIUM, PHOSPHORUS AND MAGNESIUM**

### **CALCIUM**

A total of approximately 1-1.5kg of body weight is made up of calcium where 99% is found in the bones and teeth and remaining 1% is in the ECF and other compartments.  $\text{Ca}^{2+}$  exists as carbonates or phosphates of calcium within the body, while in the plasma they exist in either the ionized form (which is the physiologically active form), bound to plasma proteins (mainly albumin) or complexes with organic acids, all these forms being in equilibrium with each other.

SOURCES; milk and other dairy products (cheese etc), egg yolk, bone meal, cabbage, nuts, figs etc

ABSORPTION; dietary  $\text{Ca}^{2+}$  is absorbed mainly from the duodenum and first half of the jejunum under the influence of a carrier protein CALBINDIN – a calcium dependent ATPase against an electrical and concentration gradient. Absorption is affected by certain factors including;

1. Vitamin D- presence of vitamin D in the gut promotes calcium absorption.
2. PH-acidity increases the absorption of calcium salts which are more soluble in these condition, while in alkaline medium decreases absorption by causes the formation of insoluble salts of calcium.
3. Composition of diet-a high protein diet (amino acids particularly Lysine and Arginine) and organic acids e.g. citric acid increases the solubility of  $\text{Ca}^{2+}$  salts, fatty acids on the other hand cause formation of insoluble  $\text{Ca}^{2+}$  salts thereby decreasing  $\text{Ca}^{2+}$  absorption.
4. Parathyroid hormone through its stimulation of  $1, \alpha$ -hydroxylase which increases the production of Calcitriol ( $1, 25\text{-(OH)}_2\text{-D}_3$ ), the active form of vitamin D, increases calcium absorption.
5. Calcitonin decreases the absorption of  $\text{Ca}^{2+}$
6. Glucocorticoids diminish intestinal transport of  $\text{Ca}^{2+}$  hence its absorption.
7. Phytic and oxalic acids- presence of phytates and oxalates especially in cereals (phytates) and vegetables (oxalates) cause the formation of insoluble calcium salts which are excreted in feces and decrease  $\text{Ca}^{2+}$  absorption.
8. Presence of other minerals such as phosphates, phosphorus, iron and magnesium decrease  $\text{Ca}^{2+}$  absorption.

#### FUNCTIONS

1.  $\text{Ca}^{2+}$  is involved in calcification or mineralization of bones and teeth.
2. Also involved in coagulation of blood as factor IV causing the chelating of prothrombin to form thrombin in the clotting cascade.
3. Plays a role in neuromuscular transmission of impulses particularly at the pre and post synaptic junctions.
4. It is actively involved in muscle contraction and relaxation that produces body movement.
5. It in addition regulates microfilament mediated processes such as degranulation, cell motility etc
6. It activates regulatory kinases with or sometimes without binding to the regulatory protein Calmodulin.

7. It is needed for the excitability of nerves.
8. It plays a role in permeability of gap junctions
9. It acts as a secondary and tertiary messenger in signal transduction and hormone action
10. It mediates secretion of hormones
11. It's involved in systolic myocardial contraction and general excitability of heart.
12. Affects (decreases) vascular permeability hence reduces allergic exudates.

#### REGULATION AND EXCRETION

Levels of calcium in the body and particularly in the blood are strictly regulated by vitamin D- Calcitriol, and hormones such as Parathyroid hormone and Calcitonin. Excess  $\text{Ca}^{2+}$  is normally excreted in urine with little amounts also excreted in stool.

Calcitriol increases blood levels of calcium by increasing its absorption from the intestine while parathyroid hormone which is secreted by the parathyroid gland acts at three principal sites- the bones, kidneys and intestines also to increase blood levels of  $\text{Ca}^{2+}$  the hormone causes demineralization of bone leading to the release  $\text{Ca}^{2+}$  into blood, increased absorption of  $\text{Ca}^{2+}$  from the intestines and reabsorption of the filtered ions from the glomerular filtrate at the kidneys.

Calcitonin is a peptide hormone secreted by the thyroid gland, it decreases blood  $\text{Ca}^{2+}$  concentration by inhibiting the resorption of bone having an opposite effect to parathyroid hormone together with which it causes remodeling of bone to achieve proper bone growth and development. Secretion and activities of these two hormones that regulate calcium blood conc. is under feedback regulation depending on the levels of blood  $\text{Ca}^{2+}$  and this influences quantity excreted.

Other factors that may influence blood  $\text{Ca}^{2+}$  conc. includes levels of Phosphorus ion which decreases Ca (Ca and P ions have almost completely reciprocal relationships with respect to regulation and excretion from the body), pregnancy which places greater demands on blood Ca hence reduce total amount in blood, presence and absence of serum proteins, Ph of the blood with alkalosis decreasing Ca blood level through its facilitation of complexing of Ca with organic compounds in the blood.

#### PHOPHORUS

P levels in the body represent about 1kg of total body weight with 80% of this quantity found in the bones and teeth and about 10% in the muscles is found mainly intracellular, it occurs in either the organic (nucleic acids, phospholipids etc) or inorganic form.

SOURCES: milk, cereals, nuts, meat etc

Absorption; asorption is mainly from the jejunum and is influenced by Calcitriol which increases it. P in blood is mainly protein bound the skeleton is the major reservoir of P.

## FUNCTIONS.

1. It is involved in the formation of bones and teeth .
2. It is an energy source as high energy phosphate bonds in ATP and other high energy compounds (CTP,GTP and CP) that maintain muscle contractility, neurological functions, electrolyte transport etc
3. It is a constituent of cyclic adenine and guanine nucleotides,cGMP,cAMP .
4. Composition of nucleoside coenzymes e.g. NAD,NADP
5. Involved in DNA and RNA synthesis
6. Forms physiologically important phosphate esters such as Phospholipids, Phosphoproteins , Glucose-6-phosphate,Nucleic acids etc.
7. It also helps to maintain the critical intracellular concentration and provides substrate for bone mineralization.
8. It is also the source of the phosphate buffer system of the blood.
9. It helps in the activation of some enzymes by phosphorylation and is involved in the activities of several enzyme systems e.g. adenylate cyclase and 1,  $\alpha$ -25-hydroxy vitamin D-hydroxylase.

## REGULATION

Serum levels of P depends on levels from diet and on its excretion and reabsorption from the kidney tubules which is under the influence of parathyroid hormone and calcitonin.

## MAGNESIUM

This is the fourth most abundant cation in the body of animals and is second to potassium inside the cell. 60% of the body Mg is located in bones, 20% in skeletal muscles, 19% in other cells and 1% in ECF. It is an alkaline earth metal distinct from other transition elements in that it interacts with other chemical species with a stronger electrostatic bonding component and prefers oxygen to N atoms.

**SOURCES;** vegetables, cereals, nuts, beans, Bone Meal, dairy products etc.

20-30% of ingested Mg is absorbed from the small intestine, and this is influenced by malabsorption syndromes and other factors that affect passage of food. Other minerals such as

Ca and Phosphates also decrease Mg absorption, while presence of proteins, lactose and vitamin D increases Mg absorption.

## FUNCTIONS

1. It chelates important intracellular anionic ligands especially ATP. (Convert adenosine triphosphate (ATP) to adenosine pyrophosphoric acid (ADP), with the subsequent release of energy.)
2. It catalyses and activates more than 300 enzymes-being an essential cofactor for enzymes concerned with respiration, glycolysis and transmembrane transport of other cations e.g. Na and Ca. Mg affects enzyme activity by binding to the active sites of enzymes, ligand binding or induction of conformational changes during catalytic process as well as promotion of aggregation of multiple enzyme complexes
3. It helps to maintain low resting concentration of intracellular calcium by competing with Ca for binding sites on proteins (troponin molecule found at regular intervals along actin filaments) and membranes hence sequestering Ca into the sarcoplasmic reticulum. Magnesium acts to relax muscles after calcium stimulates contraction
4. It helps maintain normal muscle and nerve function.
5. Mg is known to play a crucial role in the maintenance of cell integrity such that deficiencies of Mg lead to development of cancer. Glutathione requires magnesium for its synthesis. Low magnesium is associated with dramatic increases in free radical generation without the cleaning and chelating work of glutathione (magnesium), cells begin to decay as cellular filth and heavy metals accumulate.
6. Magnesium has an effect on a variety of cell membranes through a process involving calcium channels and ion transport mechanisms. Magnesium is responsible for the maintenance of the trans-membrane gradients of sodium and potassium.

The major excretory pathway for Mg is through the kidneys, but 60-80% orally taken Mg is lost through feces while up to 0.75mEq/l is lost through sweat.

Deficiency of Mg manifests as impairment of neuromuscular functions such as hyperirritability, tetany, convulsions and electrocardiographic changes. In cattle an endemic disease called grass staggers or grass tetany characterized by restlessness and convulsions followed by death frequently occurs.\

## **TRACE ELEMENTS**

Trace elements occur in the human and animal body in milligrams per kilograms amount or less as against major elements which occur in gram per kg. Essential elements are elements required for life, whose deficient intake results in impairment of vital functions and only intake of physiologic amounts of the element can alleviate or prevent such a disturbance in function. Certain T.Es exists in the body whose exact role is not known such includes Arsenic, Mercury, and Cyanide etc

### **GENERAL CHARACTERISTICS OF TRACE ELEMENTS**

1. Amplification; a very small amount of the element is necessary for optimal performance in the whole organism, hence a lack of such elements even in small quantities can result in disturbances. Trace elements are constituents of or interact with enzymes or hormones and regulate the metabolism of large biochemical substrates.
2. Specificity; they are specific in their functions and are most times not replaceable by even similar compounds.
3. Homeostasis; There exists mechanisms that regulate to achieve optimal body distribution of these T.Es including their absorption, storage and excretion e.g. the rate of absorption of T.Es generally decreases with its increasing concentration in the intestinal lumen or associated tissues. Active transport mechanisms have been suggested for Fe, Zn, and Cu. Excretions of T.Es is mainly through feces.
4. There are interactions between two or more T.Es, such as an overabundance of one element interfering with the metabolic activities of another one present in normal or marginal concentrations.

## **SULPHUR**

Sulfur represents about 0.25 percent of our total body weight, similar to potassium. The body contains approximately 140 grams of sulfur-mainly in the proteins, although it is distributed in small amounts in all cells and tissues. Approximately half of the total of body sulphur is found in the Muscles, skin and bones, as well as concentrated amounts in hair and nails. A multivalent non-metal, it is an essential element for life as it is a building block of proteins, enzymes and vitamins. Sulfur is present in four amino acids: methionine (an essential amino acid) and in the non-essential cystine and cysteine, which can be made from methionine and taurine (used in production of bile acid for digestion).

**SOURCES;** “organic” sulphur is found in foods such as meat, fish, poultry, grains, legumes and vegetables such as Brussels sprouts, broccoli, onions and garlic.

## FUNCTIONS

1. It plays an important function in the formation of amino acids. It is a component of keratin, the main protein of hair and nails. Sulfur is also present in the fur and feathers of animals. It also makes up collagen and elastin, the main proteins found in skin and connective tissue. And is involved in repairing damaged skin and maintaining healthy detoxification of the skin.
1. Organic sulphur can add flexibility to cell walls and allows easier passage of fluids. This may aid in eliminating pain, softening tissues and help movement.
2. Sulphur is also a component of the B vitamin Biotin and therefore contributes to fat metabolism. Sulphur is essential for insulin and thiamine production and therefore plays a role in carbohydrate metabolism.
3. Sulphur is a major component of joint tissue where it functions in the formation of cartilage, tendons and ligaments
4. Plays a role in detoxification of heavy metals in conjunction with the transport of oxygen across the cell membrane.
5. It is important in cellular respiration, as it is needed in the oxidation-reduction reactions that help the cells utilize oxygen, which aids brain function and all cellular activities.

## ABSORPTION

It is generally believed that the sulphate ion is poorly absorbed. Sulfur is absorbed from the small intestine primarily as the four sulfur-containing amino acids or from sulfates in water or fruits and vegetables. Sulfur is stored in all body cells, especially the skin, hair, and nails. Excess amounts are eliminated through the urine or in the feces.

## IODINE

The body contains about 25 mg of iodine. A small percentage of this is in the muscles, 20% is in the thyroid, and the rest is in the skin and bones.

Iodine is well absorbed from the stomach into the blood. About 30 percent goes to the thyroid gland, depending on the need. Iodine is eliminated rapidly. Most of the remaining 70 percent is filtered by the kidneys into the urine.

Iodine's main role in animal body is as constituents of the thyroid hormones, *thyroxine* (T4) and *triiodothyronine* (T3). These are made from addition condensation products of the amino acid tyrosine, and are stored prior to release in an iodine-containing protein called thyroglobulin. T4 and T3 contain four and three atoms of iodine per molecule, respectively. The thyroid gland actively absorbs iodide from the blood to make and release these hormones into the blood, actions which are regulated by a second hormone TSH from the pituitary. Thyroid hormones are phylogenetically very old molecules which are synthesized by most multicellular organisms, and which even have some effect on unicellular organisms. The thyroid hormones, thyroxine and triiodothyronine, are also needed for normal growth and development, protein synthesis, and energy metabolism

Sources; Iodized salt -- table salt with iodine added -- is the main food source of iodine. Seafood is naturally rich in iodine. Cod, sea bass, haddock, and perch are good sources. Kelp is the most



common vegetable seafood that is a rich source of iodine. Dairy products also contain iodine. Other good sources are plants grown in iodine-rich soil.

## MANGENESE

Mn is usually bound to proteins in the body either in the =2 or +3 valency states, it is associated with formation of connective tissue and bony tissue, growth reproduction as well as lipid and carbohydrate metabolism.

Sources; include cereals, vegetables, fruits nuts, liver, kidney etc

## FUNCTIONS

1. It functions as a constituent of metalloenzyme and as an enzyme activator
2. It binds directly to substrates e.g. ATP or protein causing conformational changes that lead to enzyme activation. Some of the enzymes activated are non specific hence this can mask Mn deficiency, however Mn activation of glycosyltransferase, phosphoenol pyruvate carboxykinase and glutamine synthetase are specific.
3. Arginase, pyruvate carboxylase and Mn superoxide dismutase are examples of enzymes composed of Mn, while hydrolases, kinases and decarboxylases are examples of enzymes that can also be activated by Mn.

Mn within the cells are concentrated in the mitochondria and the body stores are located within the skeleton, absorption of Mn is poor especially in increased intake of the same. Ca and P also decrease its absorption. Absorbed Mn is bound to plasma proteins in blood and then transported to the liver from where its excess are removed via the kidney, as well as in bile and pancreatic secretions.

## COPPER

Copper is distributed widely in the body and occurs in liver, muscle and bone. Cu is an important trace element associated with some metalloproteins. It is present both in the +1 and +2 valency states within the body and is involved mainly in oxidation-reduction reactions. It is an important constituent of many compounds and enzymes including Ceruloplasmin, Cytochrome C Oxidase, Super oxide Dismutase, Tyrosinase (necessary for pigmentation of skin via the production of melanin) etc. most of these copper containing enzymes bind to and react directly with molecular oxygen.

It in addition plays a significant role in iron metabolism- Cu deficiency impairs Fe absorption and is accompanied by anemia.

Sources; meat, legumes, nuts and cereals etc.

## FUNCTIONS

1. In addition to its enzymatic roles, copper is used for biological electron transport. Blue copper proteins participate in electron transport and include Azurin and Plastocyanin.

Duodenum is the site of maximum absorption of Cu; it may also be absorbed in the stomach. Within the intestinal mucosa Cu reacts with metallothionein (a sulphurhydryl group rich protein that binds Cu) which can be competed with by other metal ions. The amount of ingested Cu that is eventually absorbed depends on sex (females have been shown to absorb more), chemical form of the ingested compound, other dietary constituents like trace elements, and the amount ingested.

Cu is stored in the liver after being transported as Cu- albumin or Cu –histidine complexes from blood as metallothionein like Cuproproteins, ceruloplasmin, from where it is transported to other cells to be used in Cu containing enzymes. It is excreted in feces after being secreted from bile into the intestine.

Deficiency of Cu results in weight loss, bone disorders, anemia and myocardial atrophy.

## ZINC

Zn is the second most abundant trace element in the body with about 1.4-2.3g of it occurring in a matured animal body.

Prostrate, semen, liver, retina, bone and muscle tissues are rich in Zn. It is found in the +2 valency state and is an essential component of many metalloenzyme involved in all aspects of metabolism.

Sources; meat, fish, dairy products are good sources of available Zn.

## FUNCTIONS

- 1.) It is an integral part of nearly 300 enzymes, contributing to their structural stability, in different species of life e.g. superoxide dismutase, carbonic anhydrase, alkaline phosphatase, RNA and DNA polymerases, thymidine kinase, carboxypeptidase, alcohol dehydrogenase etc
- 2.) It also plays a role in protein synthesis and is involved in gene expression e.g. Zn finger proteins.
- 3.) It stabilizes the structures of proteins and nucleic acids
- 4.) Zn has been shown to be an important element in wound healing as it is necessary factor in the biosynthesis and integrity of connective tissue

- 5.) It is involved in normal fetal development and influences pregnancy outcome
- 6.) It is also involved in insulin secretion
- 7.) Biosynthesis of mononucleotides
- 8.) Vitamin A metabolism (stimulate the release of vitamin A into the blood).

20-30% of ingested Zn is absorbed mostly in the duodenum and early p

Art of jejunum by active energy dependent transport. This absorption is varied and depends on certain factors such as presence of dietary Ca, P, Fe, and Cu (which decrease Zn absorption); however diets rich in protein have the opposite effect.

Zn is transported in the blood bound to albumin (mainly)  $\alpha$ -macroglobulin, transferin and free amino acids. It is mainly excreted in feces smaller amounts are excreted in urine and sweat.

## **MOLYBDENUM**

It is a component of about 3 metalloenzymes in animals and man including Xanthine oxidase, Aldehyde oxidase and Sulfite oxidase.

Sources; cereals and dry legumes

### **FUNCTIONS**

- 1.) Xanthine oxidase is involved in degradation of purines to uric acid.
- 2.) Aldehyde oxidase is involved in oxidation of aldehydes
- 3.) Sulfite oxidase is involved in final oxidation of sulphur containing amino acids.

Presence of Mo helps in the utilization of Cu while excessive amounts may result in Cu deficiency.

It is absorbed mainly in the stomach and small intestine, and stored in the liver small amounts are retained in the kidney and a skeleton.

Excess are excreted from kidneys and some from bile.

## **CHROMIUM**

Cr is a transitional element that occurs mainly in the +3 and +6 valency state in biological systems. It is widely distributed throughout the body.

Sources; meat, whole grain products and yeast.

## **FUNCTIONS**

It helps in the control of glucose, protein and lipid metabolism i.e. it is a potentiator of insulin action.

It is absorbed poorly from the upper small intestine and is bound to  $\beta$ -globulin fraction of serum proteins (transferin). It is excreted via the kidneys and it is found mainly in the liver-mitochondria, microsomes and cytosol.

Deficiency results in impaired glucose tolerance secondary to parenteral nutrition.

## **SELENIUM**

It is a constituent of glutathione peroxidase and iodothyronine deiodinases and thioredoxin reductase (selenoproteins). It is present in tissues as selenocysteine and selenomethionine which serves as a store for Se which is released in cases of dietary insufficiencies.

The biologically active form of Se is selenocysteine and it is present in the selenoproteins.

Sources; plant (grown on Se rich soils) and animal tissues.

## **FUNCTIONS**

It helps to defend the body against oxidative stress and is also involved in the synthesis and metabolism of thyroid hormones.

GSH (reduced glutathione) catalyses the breakdown of  $H_2O_2$ , phospholipid hydroperoxides etc, it is present in RBC. Thioredoxin reductase is thought to have an immunological function and plays a role in reproduction.

Se is well absorbed in the GIT and may not be regulated homeostasis of Se is achieved by regulation of its excretion via the urine, however high intake of Se leads to exhalation of volatile forms.

## **COBALT**

Co is an integral part of the B vitamin B12- cyanocobalamin which is required for the maturation of RBCs.

Sources; animal tissues.

## FUNCTIONS

As a cofactor for some enzymes e.g. glycyl-glycine dipeptidase, involved in bone marrow development for RBC maturation and in the formation of cobamide enzyme (adenosyl co-enzyme).

## FLOURINE

It is obtained mainly from drinking water other sources include tea, salmon, sardine and mackerel. It is present in calcified tissues like bones and teeth.

It is absorbed from intestine and excreted mainly in the urine.

## FUNCTIONS

It helps in tooth development-i.e. normal maintenance and hardening of enamel and prevention of dental caries. It also helps in the normal bone development as catalytic amounts of F is required for the conversion of phosphates and Ca hence helps prevent osteoporosis (softening of the bones).

Toxic levels of F in the body result in fluorosis characterized by mitochondrial damage, certain enzyme inhibition and negative effects on protein, steroid and collagen synthesis.

## NICKEL

This occurs in trace amounts in humans and animal tissues. Dietary intake is poorly absorbed and it is excreted mainly in feces.

Ni plays an important role in some enzyme activities e.g. Arginase, carboxylase, trypsin and acetyl CoA synthetase.

It is also required for growth and reproduction.

## VITAMINS

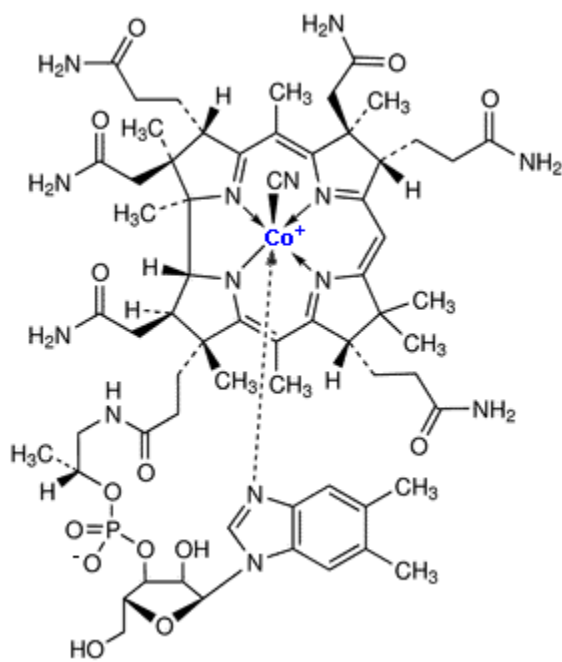
### **WATER SOLUBLE VITAMINS: B AND C, BIOMEDICAL IMPORTANCE IN MAN AND ANIMALS.**

#### VITAMIN B12;- CYANOCOBALAMINE

It is a hemopoietic vitamin required for maturation of RBCs, and also involved in nucleic acid metabolism, methyl transfer, and myelin synthesis and repair. It serves as a carrier of one carbon

group in metabolic reactions. The compound is composed of physiologically active substances classified as cobalamines or corronoids which is made up of tetrapyrrole rings surrounding a central cobalt atom and nucleotide side chains. It has a molecular weight of about 1355.

Sources include clams, oysters, turkey, chicken, beef, and pork. Absorption is under the influence of an intrinsic factor, which takes place in the terminal ileum, it is then released from the factor and transported into blood. it is stored in liver and released to meet plasma needs. Excess vit. B12 is excreted by the kidneys.

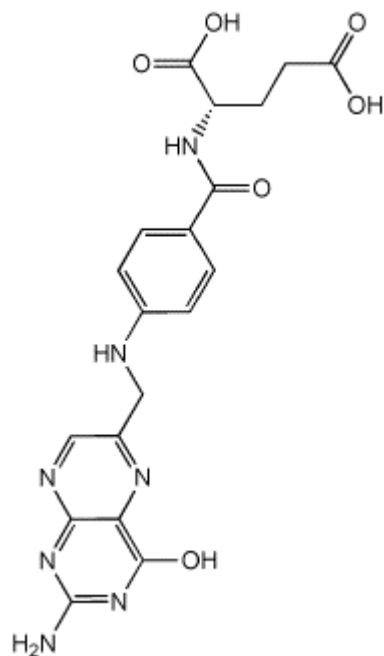


### **FOLIC ACID**

This consists of one molecule of P-aminobenzoic acid and glutamate to which a base, pteridine, has been attached sometimes (rarely) referred to as vitamin B-9, it is also involved in maturation of red blood cells and the synthesis of purines and pyrimidines which are required for development of the fetal nervous system.

Sources include dried peas, dried beans, yeast, and leafy green vegetables such as spinach, endive, lettuce, and mustard greens.

It is absorbed in the duodenum and upper jejunum.



FOLIC ACID

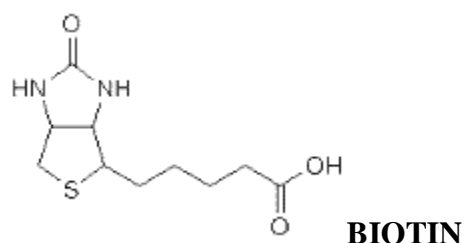
## BIOTIN

This is an imidazole derivative, chemical name – Cis-tetrahydro-2-oxothienol [3,4-d]-imidazole-4- valeic acid.

Liver, egg yolks, green vegetables, and whole grains are rich sources of biotin. It acts as a coenzyme for carboxylation reactions essential to fat and carbohydrate metabolism e.g. pyruvate carboxylase, acetyl co A carboxylase, serving as a carrier on the enzyme.

Avidin found in egg white binds biotin and makes it unavailable for absorption into the body.

Biocytin a form of biotin is readily absorbed and in the plasma is hydrolyzed to biotin and taken up by tissues for use attached to an apoenzyme. By products of biotin in form of biotin sulfoxides and bisnorbiotin (trace amounts) is excreted along with free vitamins.



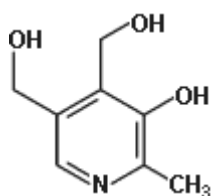
### **VITAMIN B6 (PYRIDOXINE)**

Derivatives of pyridines and their phosphates including pyridoxine, pyridoxal and pyridoxamine.

Sources include brewer's yeast, liver, mackerel, avocado, bananas, meat, vegetables and eggs. Vitamin B6 is readily absorbed by intestinal mucosa cells that contain cytoplasmic pyridoxal kinase that catalyse the phosphorylation of the vitamin form, which is then absorbed to other cells by diffusion (the active form of the vit is the phosphate form especially pyridoxamine-5-phosphate).

B6 serves as a coenzyme in the catalysis of transamination, decarboxylation and threonine aldolase reactions. Vitamin B6 is important in the biosynthesis of heme and nucleic acid, as well as in lipid, carbohydrate, and amino acid metabolism. As a coenzyme in the breakdown of glycogen-phosphorylase and in condensation of L-serine with palmitoyl Co A to form sphingomyelins.

The main by product of vit B6 metabolism is excreted in urine as 4-pyridoxic acid formed by oxidation of the aldehyde and aldehyde dehydrogenation.



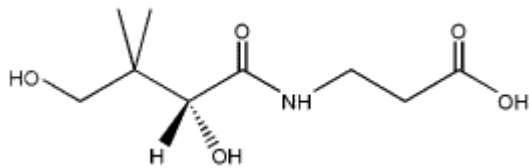
### **PYRIDOXINE(VITAMIN B6)**



## PANTOTHENIC ACID

This is a combination of pantoic acid and  $\beta$ -alanine. Also called vit B5. It is richly distributed in foods- whole grain cereals, legumes, eggs, and meat. It is critical in the metabolism and synthesis of carbohydrates, proteins, and fats.

It is readily absorbed in the intestine and goes into the cell for the formation of coenzymes. It is absorbed as pantetheine and pantothenate into circulation and it is within the cells that coenzyme forms are synthesized. The  $\beta$ -mecarptoethylamine derivative is excreted in urine and smaller fractions excreted in milk and colostrums.



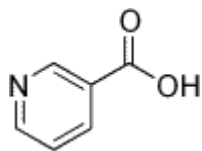
## PANTOTHENIC ACID

## NIACIN

This comprises nicotinic acid and nicotinamide. It can be synthesized from tryptophan in man but cats lack the metabolizing enzymes. Derivatives include nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), which are coenzymes in oxidation-reduction reactions vital in cell metabolism.

Sources include most plants and animal products, mushrooms and fish are good sources of niacin.

Both the acid and amide forms are readily absorbed in the GIT into circulation from where it diffuses into the cerebrospinal fluid. They are converted to the coenzyme forms in the liver, kidney blood and brain cells. Metabolites of this vitamin are excreted in urine.



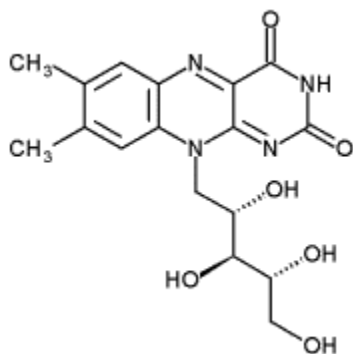
NIACIN

## RIBOFLAVIN

Riboflavin – 7,8 dimethyl[1'-D-ribityl] isoaloxazine; a heterocyclic isoalloxazine ring attached to a sugar alcohol, ribitol. The coenzyme forms are flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). It is involved in carbohydrate metabolism as an essential coenzyme in many oxidation-reduction reactions.

Sources are yeast, liver, kidney, heart and vegetables. It is synthesized in plants and microorganisms. Coenzyme forms of the vitamin release riboflavin during digestion in the intestine upon acidification in the stomach, it is absorbed in the proximal small intestine aided by bile salts and then absorbed into cells the flavins are converted to the coenzymes.

Excess riboflavin is excreted in urine and to a lesser extent in feces.



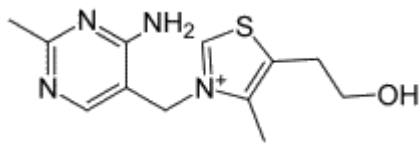
RIBOFLAVIN

## **THIAMIN(B1)**

Thiamin is 3-[4-amino-2-methyl-pyrimidyl-5- methyl]-4-methyl-5-[β-hydroxyethyl] thiazole. The active coenzyme form is thiamin pyrophosphate (TPP) or the diphosphate. It is widely available in the diet. Small amounts are present in animal and plant tissues but are more abundant in unrefined cereals grains, liver ,heart, kidney and pork.

Thiamin is involved in carbohydrate, fat, amino acid, glucose, and alcohol metabolism, coenzyme in transketolase reactions etc.

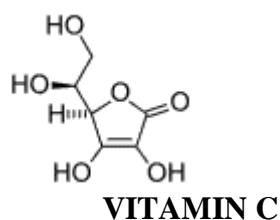
It is readily absorbed by the small intestine by active transport process and phosphorylated to TPP in the jejuna mucosa, then to portal blood. The diphosphate or triphosphate forms maybe stored in minute quantities in skeletal muscles, liver, heart and nervous tissue. Excess and metabolites of thiamin are excreted in urine.



## **THIAMIN (VIT.B1)**

## **VITAMIN C**

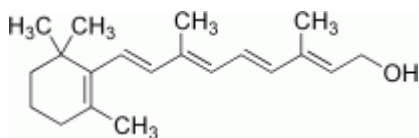
Vitamin C (ascorbic acid) plays a role in collagen, carnation, hormone, and amino acid formation. It is essential for wound healing and facilitates recovery from burns. Vitamin C is also an antioxidant, supports immune function, and facilitates the absorption of iron. Higher amounts can cause stomach upset and diarrhea. Vitamin C is found in fresh fruits and vegetables. Citrus fruits like oranges and lemons are good sources of vitamin C.



## **FAT SOLUBLE VITAMINS A,D,E,K THEIR BIOCHEMICAL FUNCTIONS IN MAN AND ANIMALS**

### **VITAMIN A**

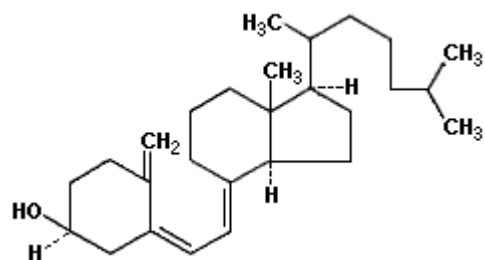
Vitamin A (retinol) is required for the formation of rhodopsin, a photoreceptor pigment in the retina. Vitamin A helps maintain epithelial tissues. Normally, the liver stores 90% of the body's Vitamin A. To use Vitamin A, the body releases it into the circulation bound to prealbumin (transthyretin) and retinol-binding protein.  $\beta$ -carotene and other provitamin carotenoids, contained in green leafy and yellow vegetables and deep- or bright-colored fruits, are converted to Vitamin A. Carotenoids are absorbed better from vegetables when they are cooked or homogenized and served with some fats or oils. Deficiency impairs immunity and causes skin rashes and typical ocular effects such as dry eyes and night blindness.



### **VITAMIN D**

Vitamin D has two main forms: D<sub>2</sub> (ergocalciferol) and D<sub>3</sub> (cholecalciferol). Vitamin D<sub>3</sub> is synthesized in skin by exposure to sunlight (ultraviolet radiation) and obtained in the diet chiefly in fish liver oils and egg yolks. Vitamin D is a prohormone with several active metabolites that act as hormones. Vitamin D<sub>3</sub> is metabolized by the liver to 25(OH)D, which is then converted by the kidneys to 1,25(OH)<sub>2</sub>D (1,25-dihydroxycholecalciferol, calcitriol, or active vitamin D hormone). 25(OH)D, the major circulating form, has some metabolic activity, but 1,25(OH)<sub>2</sub>D is

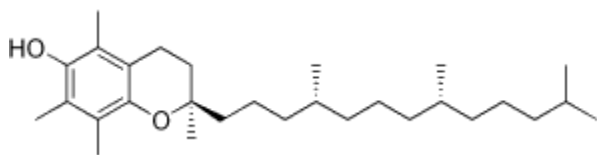
the most metabolically active. Inadequate exposure to sunlight may cause vitamin D deficiency. Deficiency impairs bone mineralization and may contribute to osteoporosis.



CHOLECALCIFEROL  
(VITAMIN D<sub>3</sub>)

## VITAMIN E

Vitamin E is a group of compounds (including tocopherols and tocotrienols) that have similar biologic activities. The most biologically active is  $\alpha$ -tocopherol, but  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherols also have important biologic activity. These compounds act as antioxidants, which prevent lipid peroxidation of polyunsaturated fatty acids in cellular membranes. Plasma tocopherol levels vary with the total plasma lipid levels. Vitamin E deficiency causes degeneration of the axons of neurons (nerve cells) resulting in neurologic deficits, and fragility of red blood cells which is generally diagnosed as hemolytic anemia. Vitamin E is found in spinach, watercress, mustard greens, and many green leafy vegetables. Good sources of Vitamin E are oily plant seeds such as peanuts and sunflower kernels.

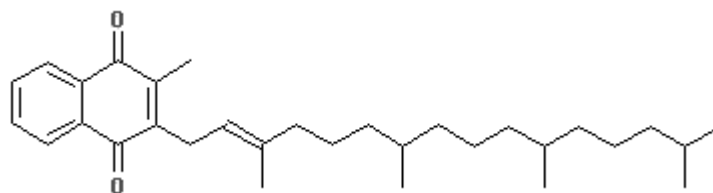


ALPHA-TOCOPHEROL (VITAMIN E)

## VITAMIN K

Vitamin K<sub>1</sub> (phylloquinone) is dietary vitamin K. Dietary fat enhances its absorption. Vitamin K<sub>2</sub> refers to a group of compounds (menaquinones) synthesized by bacteria in the intestinal tract; the amount synthesized does not satisfy the vitamin K requirement. Vitamin K controls the

formation of coagulation factors II (prothrombin), VII, IX, and X in the liver. Vitamin K is widely distributed in green vegetables such as kale, spinach, and mustard greens. The bacteria of the normal gut also synthesize menaquinones.



VITAMIN K<sub>1</sub> (PHYLLOQUINONE)

## DETOXIFICATION OF XENOBIOTICS

A variety of toxic substances or potentially toxic substances may enter human body. They are food additives, poisons, toxins, certain drugs, chemicals, environmental pollutants, pesticides and other foreign substances. They are called as Xenobiotics (Xenos (Greek) - Strange). When they are ingested either accidentally or some other way they may be absorbed from the gastrointestinal tract and gain access to the organs and tissues of the body. In the body xenobiotics undergo changes. These changes reduce the toxicity of xenobiotics. The conversion of highly toxic xenobiotics to less toxic substances is called detoxification or detoxication or biotransformation.

### MEDICAL IMPORTANCE

1. Detoxification protects body and its organs from deleterious effects of toxins.
2. Detoxification removes most of drugs consumed from the body. Because of this drugs must be taken frequently during recovery from illness or disease.
3. Occasionally detoxification may generate toxic substance from relatively non-toxic substance.
4. Many anticancer agents work by inducing enzymes of detoxification.
5. Polymorphisms of enzymes of detoxification is associated susceptibility to diseases like myocardial infarction, cancer, inflammatory disease, alcoholic cirrhosis etc.

Generally detoxification converts less soluble toxic substance to more polar water soluble and hence the compound is easily excreted in urine. Some detoxified compounds may be excreted in feces through the bile. Liver is the organ involved in detoxification reactions. Detoxification of xenobiotics occur mainly in two stages (phases). In the first phase (stage) xenobiotics undergo three types of chemical reactions. They are oxidation, reduction (hydroxylation) and hydrolysis. The second phase involves conjugation of xenobiotics with variety of substances. Occasionally the detoxified products are sometimes more toxic than the original substance. Biotoxification is the word used to indicate such process.

**(a) Oxidation.** Indole and Skatole are produced from tryptophan by the action of microbes. They are responsible for the disagreeable odour of the feces. They undergo oxidation.

Skatole → Skatoxyl, indole → Indoxyl

Benzene → Phenol, Benzaldehyde → Benzoic acid

Chloral → Trichloro acetic acid, Toluene → Benzoic acid

Ethylalcohol may be oxidized completely to CO<sub>2</sub> and water. Similarly methanol may be oxidized to formaldehyde and formate.

Methanol → Formaldehyde → Formate

(b) **Reduction.** It is less common and less important than oxidation.

Picric acid → Picramic acid

Chloral hydrate (Sedative) → Trichloro ethyl alcohol

(c) **Hydroxylation.** Detoxification of number of drugs and steroids occur by hydroxylation.

These reactions are catalyzed by cytochrome P450 dependent monooxygenases.

Phenobarbitol → Hydroxy phenobarbitol

Meprobamate (Tranquilizer) → Hydroxy meprobamate

Felbamate is structurally related to meprobamate. It is used in the treatment of epilepsy.

It is eliminated by hydroxylation.

Felbamate → Hydroxyfelbamate

### **Cytochrome P450 (CYP) Enzymes**

They are most important phase-I enzymes. They are involved in the detoxification and bio activation of xenobiotics present in food, organic solvents, tobacco smoke, drugs, pesticides, environmental pollutants and alcoholic drinks. They are products of CYP super family of genes. Over 100 mammalian CYP genes and their products are studied extensively. Some members of CYP super family with their function are given below :

#### **CYP Form Function**

CYP1A1 Inducible member of CYP super family helps in detoxification of carcinogens, toxins.

CYP1A2 Catalyzes activation of carcinogenic aryl amines and aflatoxin B.

CYP3A4 Involved in biotransformation of many drugs.

CYP2E1 Involved in oxidation of volatile environmental chemicals and anesthetics.

#### **Medical Importance**

1. CYP enzymes are involved in biotransformation of several endogenous compounds and activation of certain carcinogens. Certain compounds of dietary origin inhibit activities of these enzymes thus acting as selective inhibitors of carcinogens or toxicity of chemicals.
2. Polymorphisms in the genes coding for CYP enzymes is associated with susceptibility to different diseases including alcohol related diseases like alcoholic cirrhosis and alcoholic pancreatitis.

(d) **Hydrolysis.** Many drugs are detoxified by hydrolysis.

Aspirin (Acetyl salicylic acid) → Salicylic acid + Acetic acid

Atropine (Psychoactive) → Tropic acid + Tropicine

**II. Conjugation.** Conjugation means the chemical combination of one compound with another compound. Many toxic substances are detoxified after combining with compounds like glucuronic acid, glutathione, sulfate, cysteine, acetate, glycine and glutamine.

(a) **Conjugation reaction using glucuronic acid.** Glucuronic acid participates in detoxification reactions as its UDP derivative.

Phenol is detoxified by conjugation with glucuronic acid as UDP-Glucuronic acid. The enzyme is UDP Glucuronyl transferase

Phenol → Phenyl glucuronide

Paracetamol → Conjugated product. The conjugating agent is UDP-Glucuronic acid

Benzoic acid → Glucuronide monobenzoate

Antibiotic chloramphenicol undergo conjugation with glucuronate.

Chloramphenicol → Complex with glucuronate

Lamotrigine an antiepileptic drug is conjugated with glucuronic acid and excreted in urine.

Lamotrigine → Conjugated product

Diclofenac sodium an analgesic and antipyretic is eliminated from the body by conjugation with glucuronic acid.

Diclofenac sodium → Conjugated product

Morphine, menthol, camphor, chloralhydrate, salicylic acid, PABA are excreted in conjugation with glucuronic acid.

(b) **Conjugation with glutathione.** Aliphatic or aromatic halogen substituted hydrocarbons are conjugated with glutathione. The conjugation is catalyzed by an inducible enzyme glutathione-S-transferase.

Dichloronitrobenzene is a halogen substituted aromatic hydrocarbon undergo conjugation with glutathione. The conjugated product is further acted upon by other enzymes to produce mercapturic acids which are excreted in urine.

Dichloronitrobenzene → Conjugated product → Mercapturic acid → Urine

### **Glutathione transferases (GST)**

Glutathione-S-transferases are major enzymes of detoxification. They are involved in bioactivation

and detoxification of xenobiotics present in food, tobacco smoke, alcoholic drinks, pesticides, drugs, environmental pollutants, antitumor agents etc. They catalyze binding of large variety of electrophiles to sulfhydryl group of glutathione. Three types of mammalian glutathione-S-transferases

are identified. They are cytosolic, mitochondrial and microsomal GST.

### **Medical importance**

1. Glutathione-S-transferases are involved in removal of chemical carcinogens. Since reactive ultimate carcinogenic form of chemical carcinogens are electrophiles GST is considered as important detoxification mechanism of carcinogen.

2. GST are involved in activation of unsaturated aldehydes, quinones, epoxides and



hydroperoxides formed during oxidative stress.

3. Mammalian cytosolic GST exhibits polymorphism which increases susceptibility to carcinogenesis and inflammatory diseases.

4. Polymorphism of human microsomal GST is associated with increased risk of myocardial infarction and stroke.

(c) **Conjugation reactions using sulfate.** Paracetamol, phenol, cresol, indoxyl and skatoxyl are compounds conjugated with sulfate. PAPS or active sulfate donates sulfate group.

Paracetamol → Etheral sulphate. The enzyme for the reaction is PAPS Transferase

PAPS is 3'-phosphoadenosine-5'-phosphosulphate

Skatoxyl/ Indoxyl → Etheral sulphate. The enzyme is PAPS Transferase

Pain killer diclofenac sodium is conjugated with sulfate and excreted as etheral sulphate.

(d) **Conjugation reactions using cyteine.** Naphthalene, anthracene, bromobenzene, chlorobenzene, iodobenzene and benzyl chloride are converted to mercapturic acids by conjugation with cysteine and acetylation.

(e) **Conjugation reactions using acetate.** Sulfa drugs are detoxified by acetylation.

Zonisamide an epilepsy drug is acetylated and excreted in urine.

Isonicotinic acid hydrazide used in treatment of tuberculosis undergo acetylation.

(f) **Conjugation reactions using glycine.** An example of conjugation with glycine is the detoxification of benzoic acid.

(g) **Conjugation with glutamine.** Phenyl acetate is conjugated with glutamine.

**Detoxification of cyanide :** Cyanide is converted to thiocyanate. The reaction is catalyzed by Rhodanase.

**Methylation.** Some compounds are detoxified by methylation. S-adenosyl methionine serve as methyl donor.

BAL (British anti Lewisite) is methylated and excreted. BAL removes toxic metals such as arsenic, mercury and cadmium from body.

BAL is used as antidote for arsenic poisoning.

### **Biomethylation**

Arsenic ingested is detoxified by methylation and excreted in urine. Biomethylation reduces toxicity of arsenic and facilitates its elimination from the body. Initially inorganic arsenic is methylated to monomethylarsenic acid and finally to dimethyl arsenic acid.

### **Anti carcinogens and enzymes of detoxification**

1. Several anticarcinogens exert their effect by inducing phase-I and phase-II enzymes. Most important phase-I enzymes are CYP enzymes.

2. Phase-II enzyme induction is common feature of many chemoprotectants of cancer. Induction of phase-II enzymes before or during exposure to carcinogen decreases or inhibits carcinogenesis.

3. Glucuronyl transferases and GST of phase-II enzymes are induced by some anti-carcinogens.

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