BONE & VITAMINS/MINERALS

BONE IN GENERAL

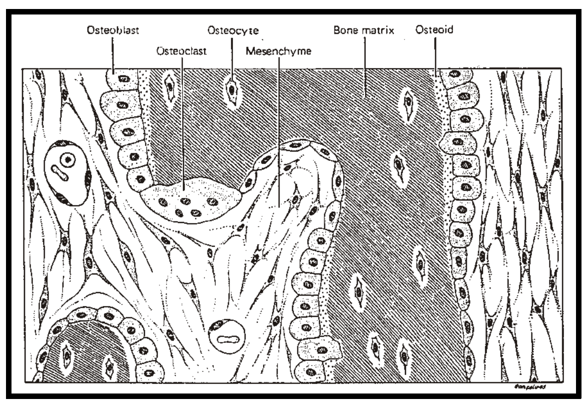
1. **Composition**

A. Composition of "normal" adult bones: (Maynard et al., 1979)

Item	%
Approximate composition:	
Water	45
Protein	25
Fat	10
Ash	25
Composition of ash (moisture- & fat-free basis):	
Calcium	36
Phosphorus	17
Magnesium	.8

B. The composition of bones is somewhat variable according to age, state of nutrition & species.
C. Bones also contain small amounts of Na, K, Cl & F, and traces of others.
D. The ratio of calcium & phosphorus in the bone is about 2:1.

2. **Bone Structure**



- Major constituents? - Bone cells, organic matrix & minerals.
- e.g., Junqueira & Carneiro (1983).

A. Bone cells:

1) Osteoblasts:

a) Found in active areas of bone formation.
b) Responsible for the synthesis of bone matrix.
c) Most cells eventually rise to osteocytes, while others remain as osteoblasts for a long period of time, and some return to the state of "osteoprogenitor cell" (i.e., slightly differentiated mesenchymal cell).

2) Osteocytes:

a) Mature bone cells found in the bone matrix.

b) Less active vs osteoblasts, and involved in maintenance of bony matrix.
c) During destruction of the matrix (e.g., in the process of remodeling), some osteocytes die, whereas others return to the state of osteoprogenitor cell.

3) Osteoclasts:

a) Multinuclear, giant cells found in sites of bone resorption.
b) A precise role of osteoclasts in bone resorption is not clear, but those cells are responsible for secretion of collagenase, acid & proteolytic enzymes.

B. Organic matrix:

1) Consists of ≈ 95% collagen, which is responsible for hardness & resistance of the bone.
2) Others (≈ 5%) - Amorphous (noncrystalline) ground substances:

a) Ground substances - Fill spaces, act as a lubricant & also serve as a barrier.
b) Contains glycosaminoglycans associated with proteins (mucopolysaccharides) - e.g., Chondroitin 4-sulfate, chondroitin 6-sulfate and keratin sulfate.

C. Inorganic matters:

1) Mostly calcium & phosphorus.
2) Deposited as tricalcium phosphate ($Ca_3(PO_4)_2$) - Amorphous & predominant in immature bones.
3) $Ca_3(PO_4)_2$ undergoes changes to form hydroxyapatite $\{3[Ca_3(PO_4)_2] \cdot Ca(OH)_2$ or $Ca_{10}(PO_4)_6(OH)_2\}$ - Crystalline & predominant in mature bones.

3. **Abnormal Bone Metabolism**

A. Rickets:

1) A disturbance of mineral metabolism in young animals.
2) Calcification of growing bones does not take place normally.

B. Osteomalacia - Similar conditions in mature animals.
C. Osteoporosis:

1) A failure of normal bone metabolism in adults.
2) Differs from osteomalacia, i.e., the mineral content is normal, but the absolute amount of bone is decreased.
☞ Common in humans after the age of 50, especially among ♀!

D. Osteopenia:

1) A general term used to describe bone pathology.
2) Simply means too little bone.

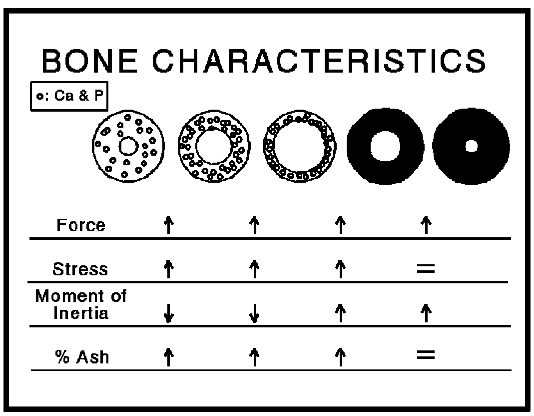
E. Osteosclerosis:

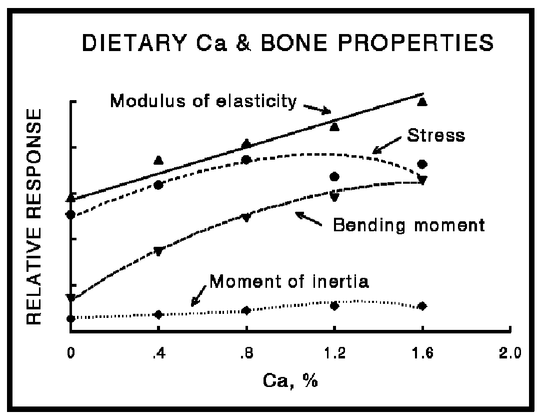
1) Presence of increased amounts of calcified bones.
2) Due to hypoparathyroidism, lead poisoning, etc.

4. **Assessing Bone Mineralization**

◦ *Reference: Crenshaw et al., 1981. Bone strength as a trait for assessing mineralization in swine: A critical review of techniques involved. J. Anim. Sci. 53:827.*

A. Cross section and characteristics of the bone - Figure on the left.
B. Dietary Ca & mechanical properties of the bone - Figure on the right.

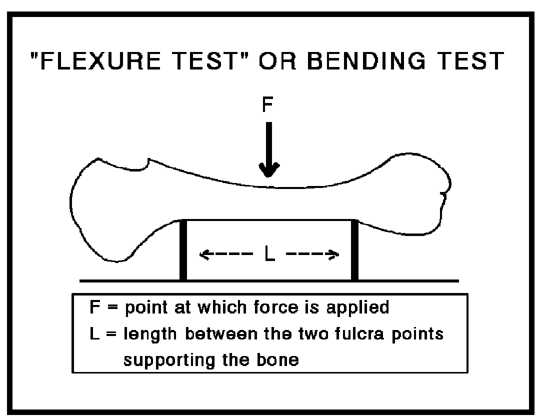




C. A “flexure test” (or bending test):

1) Considers the force, distance, inside & outside diameters, etc.
2) Based on measurements, can calculate:

Bending moment, stress (or strength), modulus of elasticity & other bone characteristics!



5. **Factors Affecting Bone Metabolism**

A. Parathyroid hormone (PTH):

1) Acts directly on bones to ↑ bone resorption, ∴ ↑ plasma Ca.
2) ↑ reabsorption of Ca in the distal tubule (↓ urinary Ca).
3) ↓ plasma phosphate by ↑ phosphate excretion in the urine.
4) Increase formation of 1,25-dihydroxycholecalciferol.

B. 1,25-$(OH)_2D_3$ (vitamin D):

1) Increase absorption of Ca and phosphate from the intestine.
2) Also, involved in mobilizing Ca & phosphate from the bone.

C. Calcitonin:

1) The exact role is unknown, and does not seem to be involved in homeostasis of Ca, P or others.
2) Hypercalcemia or hypermagnesemia stimulates secretion.
3) ↓ plasma Ca by ↓ bone resorption.
4) ↓ reabsorption of Ca, P & Mg.

☞ The 1° function might be to prevent hypercalcemia after ingestion of a meal?

D. Other hormones:

1) Glucocorticoids - Anti-anabolic effects.
2) Growth hormone - Anabolic (at the epiphyseal cartilage).
3) Estrogens & androgens - Anabolic (↑ Ca & P in the body, and involved in formation of spongy bones).
4) Thyroid hormones - Normal concentrations/activities are anabolic, whereas the excess may have negative effects.

E. Vitamin A:

1) Has a role in normal development of bones via a control over osteoblast & osteoclast activities.
2) Involved in the synthesis of mucopolysaccharides, which are components of cartilage & bones.

☞ The vitamin A deficiency can lead to disorganized bone growth & irritation of joints.

F. Vitamin C:

1) Important in collagen synthesis, thus involved in protein matrix formation.
2) Involved in hydroxylation of Pro & Lys, thus involved in stabilizing, e.g., collagen.

G Manganese (Mn):

1) Required for enzymes involved in the synthesis of chondroitin sulfates (component of mucopolysaccharides in bones & cartilage).
2) Involved in activation of alkaline phosphatase, which is involved in collagen synthesis & transfer of P-group to bone tissues.

H. Zinc (Zn):

1) A component of alkaline phosphatase.
2) A component of collagenase, which is involved in collagen synthesis (∴ in bone matrix formation & remodeling of the bone).

MINERALS IN GENERAL

1. **Historical Perspectives**

A. The importance of mineral salts (NaCl) has been known even in the ancient times.
B. The importance of minerals in bones & teeth has been recognized very early, even though Ca was not discovered until 1808.
C. More than 150 years ago, some scientists suggested a relationship between variations in supply of trace elements and human/animal health: (e.g.)

1) Fe and health - Fe as a constituent of blood (1832).
2) Goiter in man & deficiency of environmental iodine (1850's).
☞ But, these ideas were greeted with skepticism & controversy!

D. The study of mineral metabolism & feeding has only existed since 1920's or 1930's:

1) Advances in the science of chemistry & physiology enabled scientists to initiate mineral studies.
2) The use/development of synthetic diets was useful in identifying individual macro- & microelements.
3) Interrelationships observed between chemical composition of organisms and Earth's crust were useful in understanding the importance of minerals.

E. Refinements in research/analytical techniques & instrumentation, and general understandings of metabolism have taken great prominence since about 1950!

2. **Essentiality**

A. Criteria for essentiality - Stated & debated by many investigators, e.g., "*Considered to be an essential if its deficiency consistently results in an impairment of the function from optimal to sub-optimal!*"

B. Examples of some suggested criteria:

1) Present in all healthy tissues of all living things.
2) Concentration from one animal to the next is fairly constant.
3) Withdrawal from the body induces the same physiological and structural abnormalities regardless of species.
4) Its addition either reverses or prevents these abnormalities.
5) Abnormalities induced by deficiency are always accompanied by pertinent, specific biochemical changes.
6) Biochemical changes can be prevented or cured when the deficiency is eliminated.

C. Some minerals do not meet those criteria, but found in living tissues:

1) Occur more less constantly but in variable concentrations.
2) May reflect a contact with environment or contamination because distribution patterns are often similar to environmental levels!
3) Examples include aluminum (Al), antimony (Sb), cadmium (Cd), mercury (Hg), germanium (Ge), rubidium (Rb), silver (Ag), gold (Au), bismuth (Bi), titanium (Ti) & zirconium (Zr).

☞ An essential element shows a normal distribution, suggesting the existence of internal control mechanisms?

3. **Essential Elements**

A. Classification based on the content: [Georgievskii, 1982. In: Georgievskii et al. (Ed.)]

% BW	Element	Class
1-9	Ca	Macro
0.1-.9	P, K, Na, S, Cl	
0.01-.09	Mg	
0.001-.009	Fe, Zn, F, Sr, Mo, Cu	Micro
0.0001-.0009	Br, Si, Cs, I, Mn, Al, Pb	
0.00001-.00009	Cd, B, Rb	
0.000001-.000009	Se, Co, V, Cr, As, Ni, Li, Ba, Ti, Ag, Sn, Be, Ga, Ge, Hg, Sc, Zr, Bi, Sb, U, Th, Rh	Trace

B. Classification based on the function: [Georgievskii, 1982. In: Georgievskii et al. (Ed.)]

Essential	Essential?	Function (uncertain)	
Calcium	Fluorine	Lithium	Lead
Phosphorus	Silicon	Beryllium	Antimony
Potassium	Titanium	Boron	Caesium
Chlorine	Vanadium	Scandium	Barium
Sodium	Chromium	Aluminum	Mercury
Zinc	Nickel	Gallium	Tin
Molybdenum	Arsenic	Germanium	Bismuth
Selenium	Bromine	Rubidium	Radium
Sulfur	Strontium	Zirconium	Thorium
Magnesium	Cadmium	Silver	Uranium
Iron			
Copper			
Cobalt			
Manganese			
Iodine			

4. **Metabolism of Minerals**

A. General:

1) Present in feeds as organic compounds and mineral salts.
2) Once dissolved or acted by enzymes (e.g., phytin by phytase), then minerals become assimilable or utilizable.
3) Minerals in feeds & digestive juices:

   a) Present in chyme as ions.
   b) Interact & form various organic complexes.
   c) Under certain conditions, form salts of low solubility, which are practically inassimilable.

4) Minerals are excreted extensively into the GI tract (along the entire GI tracts):

   a) Ensures normal metabolism of minerals.
   b) Creates optimum conditions for absorption of minerals.

5) Exchange between the GI tract & blood:

   a) Very extensive for a number of elements.
   b) Quantitative estimation of secretion/absorption is very difficult!

B. Absorption and excretion:

1) The wall of the GI tract is permeable to minerals in both directions.
2) The site of absorption is generally known, but little is known about the mechanism(s):

a) Some minerals are absorbed by active transport (e.g., Ca & Fe).
b) Most by facilitated diffusion (e.g., Co, Cr, Cu, Mn & Zn).
c) Others by passive diffusion.
d) Few by more than one mechanism (e.g., Ca).

☞ With exception of Na & K, most minerals form salts & other compounds, thus relatively insoluble & not readily absorbed.

5. **Methods of Studying Mineral Metabolism**

◦ *Reference: Georgievskii, 1982. In: Georgievskii et al. (Ed.)*

A. Absorption & retention by the balance study:

1) Absorption = $Element_{feed}$ - $Element_{feces}$
2) Retention = $Element_{feed}$ - ($Element_{feces}$ + $Element_{urine}$)

☞ True absorption or retention cannot be determined by the balance method!

B. Absorption rates at various sections of the GI tract using inert substances (Cr, polyethylene glycol, etc.):

1) Sacrifice animals or use fistula.
2) % Absorption = 100 x [1 - (E:label) in feces or chyme/(E:label) in feed]

C. The use of radioactive indicators:

1) Possible to study: a) Exchange between digestive tract and blood, b) incorporation of minerals into tissues, c) magnitude of reserves in the body, d) transplacental passage, etc.
2) Interpretation of results is often very difficult.

D. Angiostomy of blood vessels and analysis of blood:

1) Use catheters in blood or lymph vessels.
2) Useful in studying dynamics of absorption, type of bonds formed with carriers, accumulation in the live/other organs, etc.

E. Perfusion of isolated rumen, intestine, etc. - Useful in studying ion transport mechanisms, role of various segments in absorption, interaction of elements, etc.
F. Obtain samples via fistula and incubate (*in vitro*) - Useful in studying the effect (or function) of minerals on microflora of the digestive tract, etc.

G. Slaughter or biopsy technique - Can determine the mineral content of important organs & tissues (liver, bones, skin, etc.).

6. **Determination of Requirements - Methods**

A. Factorial method:

1) Based on:

a) Deposition in the body (D) - Usually by chemical analysis of animals slaughtered at various stages of growth.
b) Endogenous losses (E) - By analysis of urine, and by radioisotope studies of losses via feces.
c) Deposition in fetus & reproductive tissues (F) - By chemical analysis during various stages of pregnancy.
d) Elimination via milk (M) - By estimating/determining milk yield/d and composition.
e) Actual assimilation of element from feed (Y) - Usually by radioisotope studies.

2) Formulas for estimating the daily requirement:

(D, E, F & M → mg or g/day & Y = % of daily intake)

a) Growing animals - (D + E)/Y * 100
b) Pregnant animals - (F + E)/Y * 100
c) Lactating animals - (M + E)/Y * 100

☞ Accuracy & reliability of the estimate depend on completeness and reliability of data used in calculation.

B. Balance tests:

1) The oldest and most popular method used until recently - Most often used for macroelements.
2) Complicated, laborious and require a high accuracy from collection to analysis.
3) The balance of minerals is affected by many factors (environment, physiological conditions, etc.).
4) Loss of minerals from skin?

☞ May have a limited-value in estimating the requirement.

C. Practical feeding trials:

1) Use various dietary levels, and estimations are made based on productivity, health, reproductive capacities, etc.
2) Advantages include simplicity, minimal use of equipment(s) and applicability under many different conditions.
3) Drawbacks include the need for more animals/standardized feedstuffs, time consuming, animal's access to other sources (e.g., soil).
4) Popular and fairly reliable method.

D. Analysis of organs, tissues & whole body:

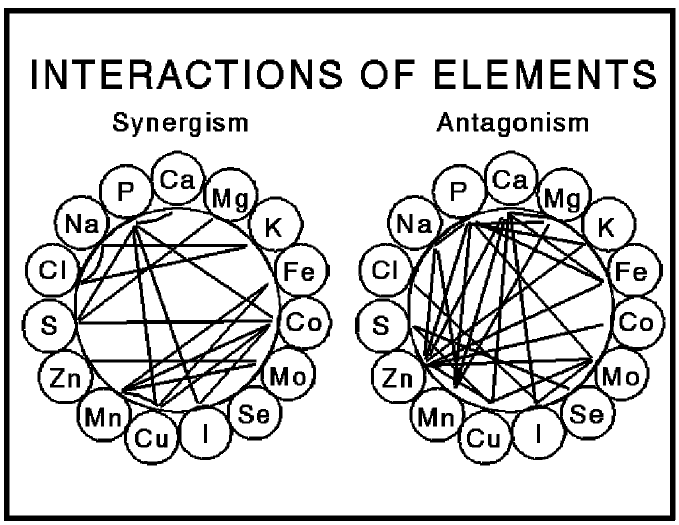
1) Based on the initial & final compositions.
2) Can determine actual deposition in organs and tissues, and it does not involve collection of urine or feces (& also less analytical work?).
3) Drawbacks include difficulty in determining mineral content of whole body of large animals, and also must be conducted over a long period of time.

7. **Interaction of Minerals**

A. Interactions in general:

1) May interact with each other, other nutrient and non-nutritive factors.
2) May be synergistic or antagonistic.
3) Interactions can take place: a) in the feed itself, b) in the digestive tract, and c) during tissue & cell metabolism.

B. Interactions among minerals:



1) Metabolic interactions of essential elements: [Adapted & redrawn from Georgievskii, 1982. In: Georgievskii et al. (Ed.)]
2) Synergism in the digestive tract - Mutually enhance their absorption?
    a) Direct interactions - e.g., Ca with P, Na with Cl, Zn with Mo.
    b) Through phosphorylation & activation of digestive enzymes - e.g., P, Zn & Co on their liberation from feed, and subsequent ↑ absorption of others.

c) Indirect interactions, i.e., stimulation of growth/activity of microflora - e.g., Stimulation of rumen microflora by Co, which in turn may have beneficial effects on others.

2) Synergism at the tissue and cell metabolism level:

a) Direct interaction in structural process - e.g., Ca & P in bone hydroxyapatite, Fe & Co in formation of Hb, etc.
b) Simultaneous participation in the active center of enzymes - e.g., Fe & Mo in xanthine & aldehyde oxidases, Cu & Fe in cytochrome oxidase, etc.
c) Activation of enzymes, i.e., ↑ synthetic processes that need other minerals - e.g., Mg on synthetases with subsequent participation of P, S & others.
d) Activation of endocrine systems - e.g., I & thyroid hormones → ↑ anabolic processes → ↑ retention of P & Mg in the body.

3) Antagonism in the GI tract - May be one- or two-sided:

a) P & Mg or Zn & Cu - Inhibit absorption of each other.
b) K inhibits absorption of Zn & Mn, but not the other way around.
c) Simple chemical reactions - e.g., Formation of Mg phosphate, Cu sulfate, Ca-P-Zn salt, etc.
d) Adsorption at the surface of colloidal particles - e.g., Fixation of Mn & Fe on particles of insoluble Mg or Al salts.
e) Antimetabolic effects of B, Pb, Tl & others - Interfere with breakdown of feed ingredients, liberation & absorption of ions.

4) Antagonism in the tissue metabolism:

a) Competition for an active center of enzyme - Mg^{2+} & Mn^{2+} in alkaline phosphatase, cholinesterase, etc.
b) Competition for a carrier - Fe^{2+} & Zn^{2+} for a bond with plasma transferrin.
c) Activation of enzymes with opposite functions - e.g., Cu and ascorbate oxidase ⇔ Zn/Mn and lactonases.
d) Antagonistic effect on a given enzyme - e.g., Activation of ATPase by Mg^{2+} and inhibition by Ca^{2+}.
e) Reduction of toxic effect - e.g., ↓ of Pb concentration in the body by addition of Cu, Zn & Mn.

C. Interaction with other substances:

1) Vitamin D - Affects absorption of Ca, P, Mg, Zn & others.
2) Fats - Affect absorption of Mg & Ca.

3) Protein - Affects the degree of utilization of P, Mg, Zn, Cu and others.
4) Chelates (AA, polypeptides, proteins, porphyrin derivatives & other heterocyclic compounds, organic acids, etc.) - Form complexes with minerals.
5) Others - Antibiotics, antioxidants, other vitamins, carbohydrates, etc.

8. **Fish & Minerals in General**

A. General:

1) One difficulty associated with fish research is that fish can absorb minerals from water via gills & skin.
2) 1° difference between fish & land animals is osmoregulation, and other aspects may be very similar.
3) Mineral requirements:

- ▹ Fish meal has been regarded as an adequate source of minerals in the past, but it is becoming apparent that some fish meal may cause deficiencies - e.g., White fish meal & widespread problems of cataracts in salmonids in the 70s ("Zn unavailability" in white fish meal).

a) Mineral requirements have been studied only sparsely, and there are still many questions on requirements/optimum physiological functions in most fish species.
b) Mineral mixtures developed for warm-blooded animals may not support maximum growth or prevent the deficiency, ∴ may not be appropriate to use those in estimating the requirement.
c) Some clinical signs & marginal nutritional deficiency signs can be due to mineral imbalances in the diet or in water.
d) Species differences exist in absorption & utilization - Especially between fresh water fish & marine fish.
e) The requirement & toxicity are influenced by acidity of water.

B. Difficulty in studying/establishing mineral requirements:

1) The exchange of ions from the environment across gills & skin complicates the quantitative determination.
2) Requirements for some trace elements are so small, it is difficult to:

a) Formulate a purified diet low in a certain mineral(s).
b) Maintain water sufficiently free of a test element.

3) Detection/measurement of some minerals:

a) Still difficult despite advances in the technique.
b) Samples & associated environment may affect measurements.
c) Reported "normal values" range widely from one Lab to the next.

4) The most commonly used measures to assess nutritional status, i.e., blood, muscle, liver & bones:

a) Intake levels & corresponding tissue levels - Changes in the function of organs/tissues are very slow until a clinical toxicity or deficiency develops.
b) Wide range of tissue levels are compatible with optimum growth & functions.
c) Thus, very difficult to establish the intermediate level/stage.

☞ Obviously, some of these difficulties are applicable to other species as well!

C. Distribution of elements:

1) Concentrations of minerals in the body are dependent on food sources, environment, species, stage of development, and physiological states.
2) Most species accumulate & retain minerals from environment, but their incorporation is highly selective.
3) Salmonid fish:

a) Can absorb Ca, Mg, Na, K, Fe, Zn & Cu from environment to satisfy their nutritional requirements.
b) Cl, phosphates & sulfates are more efficiently utilized from food sources vs from environment.

4) Fish eggs/embryos:

a) Eggs can absorb certain minerals from water - e.g., Absorption of Na, Fe & Zn by eggs (at hatching) has been demonstrated in rainbow trout.
b) Absorption of others (e.g., Mn, Se) by eggs after fertilization has been demonstrated, and absorption/accumulation in embryos may increase with development of gills.

D. Skeletal tissue metabolism:

1) Like most vertebrates, the skeleton represents a reservoir of Ca, P and other ions.
2) Fish bones:

a) Morphologically, consist of dermal bones of head, internal skeleton and scales.

b) Histologically, basically similar to that of higher vertebrates, but do not have any hematopoietic element within the bone.
c) Two types of fish bones:

   (1) Cellular - Confined to only a few groups of fish, e.g., Salmonidae, Cyprinadae & Clupeidae.
   (2) Acellular - Formed from osteoblast cells, which move away from the site of mineralization as bone deposition occurs (incapable of extensive modeling).

d) Scales:

   (1) Formed by replacement of dermal connective tissues during intramembranous ossification.
   (2) Consist of two layers:

      (a) A superficial mineralized bony tissues.
      (b) Hydrodentine layer, which is deposited over a deeper fibrous sheet described as the lamellar or fibrillar plate.

      ☞ But, there are many variations in fish scales & their structures.

e) Resorption of scales occurs during spawning migration of salmon & also during food deprivation.
f) The elemental concentration of skeletal tissues varies with age in both cellular & acellular bones.
g) The mineral phase of fish bone:

   (1) Poorly crystallized apatite.
   (2) Crystals in acellular bones are smaller & more strained vs cellular bones.
   (3) Main constituents of fish vertebrae are Ca, P, carbonate with small amounts of Mg, Na, Sr, Pb, citrate, F, hydroxide, & sulfate.

E. Environmentally induced toxic elements:

1) Fish & aquatic organisms can accumulate & retain trace elements drawn from their environment.
2) The solubility of trace elements in natural water is affected by pH, type & level of ligand & chelating agents, oxidation state, and "redox" environment of the system.
3) The soluble forms:

   a) Usually ions or un-ionized organometallic chelates or complexes.
   b) Absorbed via gills and body surfaces, and from ingestion of food or water.

4) Regulation of abnormal concentrations - variations among species:

   (a) Certain fish & crustaceans can excrete high proportions of excessive metal intake.
   (b) Fingerlings/newly hatched fish may be "poor" regulators of excess intakes.
   (c) Generally, gills, GI tract, feces & urine are involved in regulatory & excretory processes.

9. **Mineral Supplementation? (e.g., Pigs)**

- Mineral content of corn-soybean meal & mineral requirements:

Mineral	Corn	SBM	Corn-SBM	Requirement[a]
Calcium, %	0.02	0.28	0.07	0.60
Phosphorus, %	0.28	0.65	0.32	0.50
Available P, %	0.04	0.20	0.08	0.23
Sodium, %	0.01	0.01	0.01	0.10
Chlorine, %	0.05	0.05	0.05	0.08
Magnesium, %	0.11	0.14	0.14	0.04
Potassium, %	0.33	2.00	0.66	0.23
Sulfur, %	0.11	0.40	0.16	?
Copper, ppm	4	20	7	4
Iron, ppm	33	165	60	60
Manganese, ppm	6	28	10	2
Zinc, ppm	19	46	24	60
Iodine, ppm	0.03	0.16	0.06	0.14
Selenium, ppm	0.07	0.07	0.07	0.15

[a]Mineral requirements for 20-50 kg pigs (NRC, 1988).

CALCIUM AND PHOSPHORUS

1. **Introduction**

A. About 99% of total body Ca and 75% (80-85% in bones & teeth) of total body P are found in the skeleton - The ratio of Ca & P in the bone is 2.1:1.
B. The bone serves as:

   1) Structural framework of the body.
   2) A reservoir of Ca and P:

      a) The bone is in a dynamic state, i.e., a continuous exchange between the solid & liquid phases, and also between bone & body fluids.
      b) Ca & P are readily mobilized when needed, which is especially important for laying hens & lactating sows!

2. **Additional Functions**

A. Calcium:

- Serum Ca - ≈ 60% ionized, 35% bound to protein & 5% citrate, bicarbonate & phosphate complexes.

1) Involved in the development and maintenance of teeth.
2) Involved in a normal blood coagulation - Responsible for the conversion of prothrombin to thrombin, etc.
3) Involved in contraction of skeletal, cardiac & smooth muscles.
4) Involved in regulation of nervous system:

   a) Ionic permeability of the membrane.
   b) Generation of neuron stimulation.
   c) Stimulation of nerve extremities.

5) An activator or stabilizer of enzymes.

☞ (2) through (5) - Usually don't see deficiency symptoms because amounts needed to perform these functions are small, and also there are considerable reserves in bones.

B. Phosphorus:

1) P in other tissues:

   a) In soft tissues & body fluids:

      (1) Mostly organic - Phosphoproteins, nucleic acids, hexose phosphates, energy-rich phosphates (ATP, ADP, creatine-P, etc.), etc.
      (2) Inorganic - Ca-, Mg-, Na-, K- & ammonium-phosphate, etc.

   b) In plasma - 85% phosphate ions ($H_2PO_4^-$, HPO_4^{--}), 10% protein bound & 5% Ca & Mg complexes.

2) Other functions:

   a) Involved in development & maintenance of teeth.
   b) Involved in metabolism of energy, protein & lipid (glycolysis, citric acid cycle, protein synthesis, FA synthesis, etc.).
   c) Component of membranes (phospholipids).
   d) Component of coenzymes (NAD, FAD).
   e) Important buffer (acid-base) - A major intracellular buffer in regulation of urine pH, etc.

☞ The most widely involved mineral in various body functions:

a) Hardly any physiological function that does not involve P, directly or indirectly!
b) Unlike Ca, a marginal deficiency of P can reduce rate & efficiency of growth.

3. **Absorption of Ca & P**

A. General:

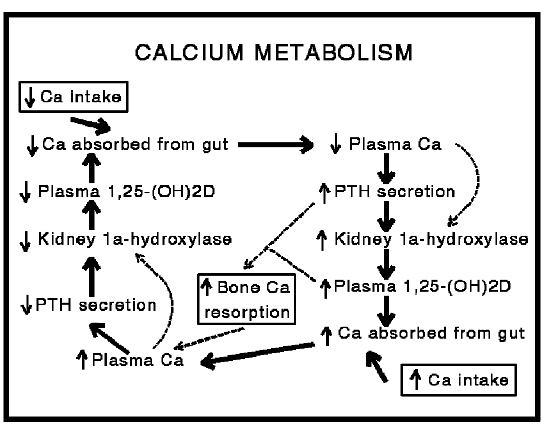
1) Mostly absorbed from the upper tract (duodenum).
2) Ca by a specific Ca-binding protein (also by a passive diffusion).
3) P, less clear, but probably similar to Ca.
4) Absorption rate for dietary Ca & P is ≈ 30-50%.

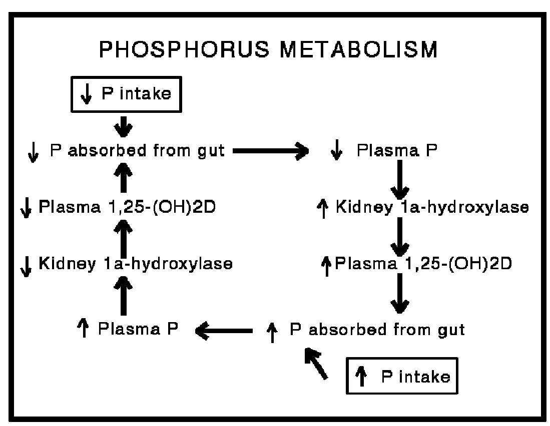
B. Factors affecting absorption:

1) Animal's needs.
2) Form of P - Inorganic > organic.
3) Ca:P ratio - Excess Ca can form insoluble tricalcium phosphate, ∴ ↓ absorption of P.
4) Vitamin D - Involved in the synthesis of Ca-binding protein.
5) Excess Fe, Al, Mg can form insoluble phosphates, ∴ ↓ P (& others) absorption rate.
6) Excess fats can ↓ Ca absorption by forming a Ca soap.
7) pH - A lower pH can ↑ absorption rate by ↑ solubility.
8) Presence of chelates ↓ absorption (e.g., oxalate & phytates).

4. **Ca & P Homeostasis**

◦ Adapted & redrawn from Horst, 1986. J. Dairy Sci. 69:604 (Cited by Litteledike & Goff, 1987. J. Anim. Sci. 65:1727).





5. **Excretion of Ca & P**

   A. Calcium:

      1) Almost 99% of Ca is reabsorbed by the kidneys, thus limited excretion via the urine.
      2) Undigested & endogenous Ca are mostly excreted in the feces.

   B. Phosphorus:

      1) Excreted in both the urine and feces - Via the kidneys & GI tracts equally in pigs, and mainly via the kidneys in poultry.
      2) Homeostasis by adjusting reabsorption from renal tubules.

6. **Deficiency of Ca & P**

   A. Abnormal bone metabolism (rickets, osteomalacia, etc.).
   B. Laying hens - A poor quality shell & incubation quality, and ↑ mobilization of Ca can lead to thin & brittle bones, thus easily fractured.
   C. Sows - Paralysis of hind limbs; often seen in high-milking sows.
   D. Depressed rate & efficiency of growth - Generally a deficiency of P, not Ca! (Also, likely to reduce production of milk, eggs, etc.)

7. **Fish & Calcium/Phosphorus**

   A. General:

      1) Calcium:

         a) Readily derived from water & occurs in adequate amounts in most diets consumed by fish.
         b) Content - 0.5 to 1% of body weight (wet Weight basis).
         c) 99% of Ca in bones & scales with 20-40% of total Ca in scales.

      2) Phosphorus - 85-90% of P in bones and scales.

   B. Functions and metabolism of Ca & P:

      1) Calcium:

         a) One of the most abundant cations in the fish body.
         b) In addition to skeletal tissues, widely distributed in soft tissues.
         c) Other functions are similar to other species.

d) Unlike terrestrial animals, the bone is not the 1° site of Ca regulation:

(1) Gas exchange across gills provides continuous access to an unlimited Ca reservoir.
(2) Regulation of Ca influx & efflux by gills, fins & oral epithelia:

(a) All structures are important in marine fish, with gills being the most important site in both marine & freshwater fish.
(b) Gills are probably more efficient in freshwater fish.
(c) The gut is not a major site of Ca absorption in marine fish, which drink water continuously.

e) Others:

(1) Endocrine control of Ca metabolism is not fully understood.
(2) Some minerals (e.g., Mg, Sr, Zn & Cu) may ↓ Ca absorption.
(3) Calcitonin inhibits Ca influx across salmon gills.
(4) Prolactin stimulates Ca uptake by tilapia.
(5) Vitamin D has no effect on the Ca homeostasis???

f) Absorbed Ca:

(1) Deposited in the bone & skin.
(2) The rate of uptake, deposition pattern & retention by skeletal tissues? Similar for both freshwater & marine water fish, and similar for all species regardless of bone types, i.e., cellular or acellular types.
(3) Ca exchange - Three time higher in scales vs bones, and scales are the site of labile Ca storage. Cellular bones must also play an important role in the Ca turnover in smooth skin fish (eels & catfish).

g) Excretion - 1° by gills & kidneys (feces also contain endogenous Ca secreted in the gut).

2) Phosphorus:

a) General:

(1) In addition to being 1° constituent of structural components of skeletal tissues, located in every cell of the body.
(2) Other functions are similar to other species.

b) Metabolism:

(1) Has not been studied extensively compared to Ca.
(2) Feed is the main source because the water content of phosphate is low in both freshwater & seawater.
(3) Absorbed P accumulates mainly in soft tissues (heart, liver, kidney & blood), and limited extent in skeletal tissues.
(4) Regulation of P is considered to be more critical vs Ca, but mechanism(s) has not been elucidated.
(5) Excretion:

(a) ≈ 90% of P is lost via kidneys from the body in marine fish.
(b) Freshwater fish produce more urine, thus more loss via urine - The difference can be demonstrated by comparing freshwater eel & seawater-adapted eel.

C. Deficiency:

1) Calcium:

a) Not detected in carp & catfish, and Atlantic salmon.
b) Rainbow trout, eel, red sea bream & tilapias require a low level for optimum growth.
c) Deficiency may ↓ growth (& feed efficiency) & ash content (under the conditions of Ca-free diet & Ca-free water).

2) Phosphorus:

a) Carp - Ssigns include cranial deformity, ↓ growth, poor feed efficiency & low Ca & P content of vertebrae.
b) Signs for other fish species include anorexia, poor growth & feed efficiency, skeletal abnormalities, and poor bone mineralization.

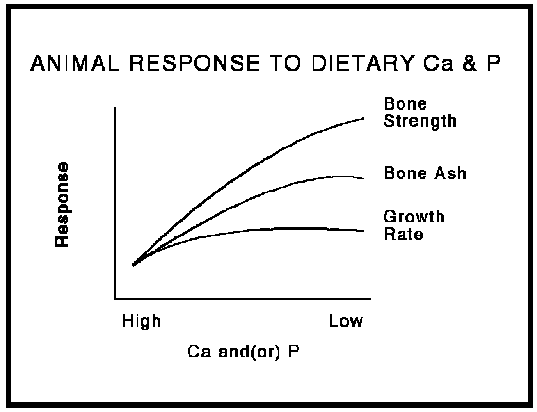
8. **Establishing Ca & P Requirements**

A. Methods used:

1) Growth trial.
2) Balance trial (Ca & P retention) - Difficult to interpret the results because of endogenous Ca & P.
3) Blood Ca & P - May not be useful because of the homeostatic mechanism (especially, Ca).
4) Blood enzymes - e.g., Alkaline phosphatase (↑ or higher with when deficient, and concentrations stabilize when the requirement is met).

5) Characteristics of the bone:

a) For the general response patterns of animals to dietary Ca & P, see Figure (Erratum: High & Low in the X-axis should be switched!).



(1) For the maximum bone strength and ash, animals require higher levels of Ca & P than those required for maximum growth.

(2) Example? Pigs need at least 0.1% higher Ca to maximize bone strength!

b) Effect of Ca & P on growing animals - e.g., Grower-finisher pigs (Cera & Mahan, 1988. J. Anim. Sci. 66:1598)[a]:

	Ca/P level (%) during the finisher phase		
Item	0.45/0.32	0.52/0.40	0.65/0.50
Gain, kg/d	0.70	0.73	0.73
Serum mineral, mg/dL:			
Ca	11.41	10.70	10.89
P	7.65	8.87	9.20
Mg	1.88	1.78	1.80
Bone ash, %	57.92	60.00	60.79
Bone bending moment:			
Humerus	435	511	543
Femur	553	686	738

[a]Ca/P levels during the grower phase were 0.52/0.40, 0.65/0.50 & 0.80% Ca/0.60% P.

c) Effects of Ca & P on breeding animals - e.g., Sows (parities 1 to 2. Nimmo et al., 1981. J. Anim. Sci. 52:1330):

	Ca/P level			
Growing, %: Gestation, g/d:	0.65/0.50 13.0/10.0	0.65/0.50 19.5/15.0	0.98/0.75 13.0/10.0	0.98/0.75 19.5/15.0
No. of ♀ started	23	22	24	22
Reproductive failure	4	3	7	4
Unable to stand	1	5	0	0
Weaned	14	18	17	18
Lost after weaning	2	0	0	1
Total remaining	12	18	17	17
Bone (metatarsal):				
Weight, g	33.4	33.4	35.5	334.7
Length, cm	9.18	9.30	9.46	9.42
Strength, kg/cm^2	437	498	528	549

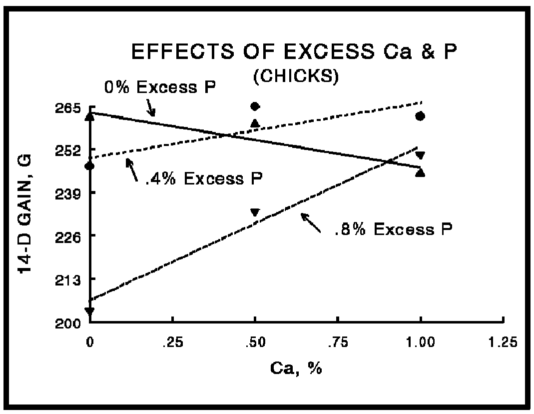
B. Ca and P ratio in pigs:

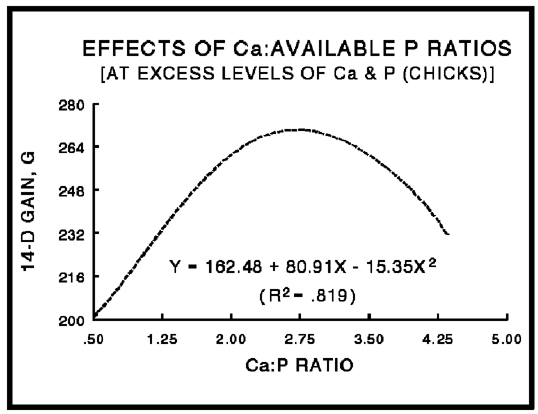
1) Important in establishing the requirement because of interactions.
2) Ideal ratio in feed = "1:1."
3) But, phosphorus is relatively expensive, thus acceptable ratios in swine range from 1.25 to 1.50:1:

    a) The most commonly used ratio is 1.3:1.
    b) Unacceptable ratio is ≥ 2:1, especially when P level is marginal or the diet is high in phytate P.
    c) If P is above the requirement, pigs can tolerate a relatively high Ca:P.

C. Ca:P ratio in poultry:

1) Effect of excess Ca & P on performance of chicks - Figure on the left (Adapted & redrawn from Wedekind & Baker, 1990. Poult. Sci. 69:1156).
2) Ca:available P ratios on performance of chicks -Figure on the right (Adapted & redrawn from Wedekind & Baker, 1990. Poult. Sci. 69:1156).





☞ For poultry, generally Ca to available P ratio of 2 to 1 is recommended!

9. **Availability of Ca & P**

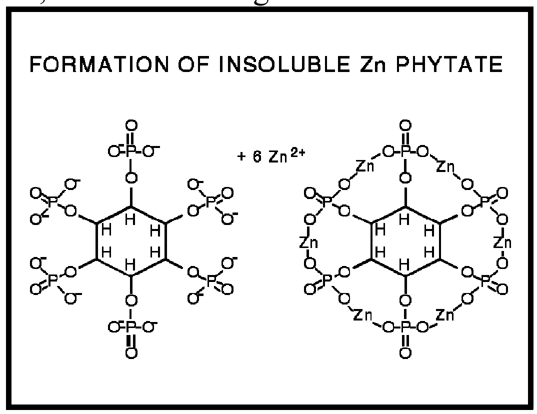
A. Calcium - Bioavailability is less critical because:

1) Ca in most feedstuffs is very low - e.g., Corn = 0.03%. (An exception is alfalfa, which contains ≈ 1.40% Ca.)
2) Most of Ca sources/supplements (calcium carbonate, calcium sulfate, oyster shell, marble dust, etc.) are 100% or close to 100% available.

B. Phosphorus:

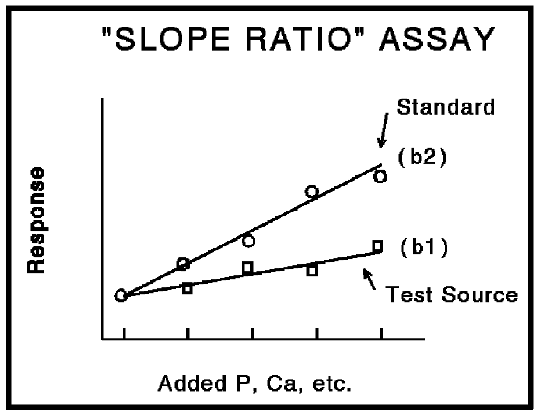
1) The content in feedstuffs is variable, thus the amount of dietary inorganic P needed to meet the requirement.
2) Also, considerable variations in bioavailability of P in plant feedstuffs.
3) About ⅔ of total P in plant feedstuffs is phytate, which is a storage form of P in seeds.

    a) Phytate can form complexes with Zn, Cu, Co, Mn, Fe, Ca, etc.
    b) Formation of insoluble zinc phytate - Adapted & redrawn from Georgievskii et al., 1982. In: Georgievskii et al.
    c) Can be utilized after hydrolysis by phytase.
    d) Phytase is present in some feeds (relatively high in wheat, barley & rye), and also produced by some microbes.
    e) Ruminant species contain organisms capable of hydrolyzing phytate in the rumen.
    f) Nonruminant species - Considerable variations in their ability to hydrolyze phytate.



4) Determination of bioavailability:

    ▸ Often "slope-ratio assay" is used - See Figure (Erratum: Y-axis should be moved to the right where slopes for the standard and test source intersect!).



5) Bioavailability of some feedstuffs: (NRC, 1988)

Feedstuff	Avg., %	Range, %
Alfalfa meal	100	
Barley	31	
Bone meal, steamed	82	
Corn	15	9-29
Corn, high moisture	49	42-58
Cottonseed meal	21	0-42
Defluorinated rock phosphate	87	83-90
Dicalcium phosphate	100	
Fish meal	100	
Meat & bone meal	93	
Oats	30	23-36
Peanut meal	12	
Rice bran	25	
Sorghum	22	19-25
Sorghum, high moisture	43	42-43
Soybean meal	38	36-39
Soybean meal, dehulled	25	18-35
Wheat	50	40-56

Wheat bran	35	
Wheat middlings	45	34-55

6) Supplemental P sources - Most commonly used sources such as monosodium phosphate, mono- and dicalcium phosphates are 95-100% available.
7) Phosphorus sources for fish:

   a) Phytate P (≈ 67% of grain P) has similar effects on fish, i.e., poor availability. (Also, phytic acid can form insoluble salt with Ca in the digestive tract.)
   b) Fish meal - 40 to 75% available in fish having "stomach."
   c) Inorganic P (e.g., Na or mono-Ca P) sources are highly available, but the availability of tri-Ca P is low compared to mono- or di-Ca P.

10. **Ca & P Requirements (%)**

A. Requirements:

Animal	Ca	P	Available P
Poultry (NRC, 1994):			
Immature chickens	0.80-2.00		0.30-0.40
Laying hens	2.71-4.06		0.21-0.31
Broilers	0.80-1.00		0.30-0.45
Turkeys, growing	0.55-1.20		0.28-0.60
Turkeys, laying	2.25		0.35
Swine (NRC, 1998):			
3-120 kg	0.45-0.90	0.40-0.70	0.15-0.55
Sows	0.75	0.60	0.35
Boars	0.75	0.60	0.35
Horses (1989)	0.21-0.62	0.15-0.34	
Fish (NRC, 1993):			
Channel catfish	?	0.45	
Trout, salmon & carp	?	0.60	
Tilapia	?	0.50	

B. Factors that influence the requirement & supplementation:

1) The variability of nutrient contents in ingredients.
2) Nutrient availability.
3) Animal performance potential, and the variability in animal response.
4) Energy content of feed.
5) Stress from diseases, overcrowding, poor ventilation, etc.
6) Interactions among ingredients & among nutrients.
7) Adequacy of vitamin D and(or) liver/kidney integrity.

11. **Toxicity**

A. Neither Ca nor P is generally considered toxic - Under normal conditions, Ca & P are absorbed according to the needs, and excess Ca & P are promptly excreted (homeostatic mechanisms).
B. Excess of either one can cause bone disorders, and reduced feed intake, weight gain and efficiency.
C. Excess Ca may cause deficiency of other essential elements such as P, Mg, Fe, I, Zn and Mn, and adverse effects are generally due to interactions!
D. Excess P may cause mild diarrhea, and also interacts with others (e.g., Ca & Mg).
E Maximum tolerance levels: (McDowell, 1992)

Animal	Ca, %	P, %
Cattle, horses & rabbits	2	1
Sheep	2	0.6
Swine	1	1.5
Poultry	1.2	1
Laying hens	4	0.8

OTHER MINERALS IN BONE PHYSIOLOGY

(*Major reasons for including these minerals in this section?)*

1. **Magnesium**

A. Found mostly in the skeleton.
B. Involved in activation of alkaline phosphatase, etc.

2. **Manganese**

A. As a component of enzymes, involved in the synthesis of chondroitin sulfate.
B. Involved in activation of alkaline phosphatase, etc.

3. **Zinc**

A. Interacts with Ca.
B. Component of alkaline phosphatase & collagenase.

4. **Fluorine** - May inhibit excessive demineralization of bones.

MAGNESIUM

1. **General**

   A. About 70% of Mg is in the skeleton, and remaining Mg is found within cells of soft tissues.
   B. Unlike Ca & P, Mg is not readily mobilized.
   C. Remaining 30% in soft tissues, and it is a main intracellular cation along with K.

2. **Functions**

   A. Constituent of bones & teeth & important for maintaining the integrity of bones & teeth - $Mg(OH)_2$ are held within the hydration shell of apatite crystal surface.
   B. Serving as a cofactor or activator of a number of enzyme systems:

      1) An active component of enzymes with thiamin pyrophosphate (TPP) as a cofactor.
      2) Activates enzymes involved in phosphorylation, carboxylation & oxidation of pyruvate, etc. and also polymerases (DNA & RNA), ribonuclease & others involved in nucleic acid metabolism.

3. **Deficiency**

   A. Poultry - Hyperirritability, convulsion, comatose, death (some times), reduction of egg production and hatchability, etc.
   B. Swine - Hyperirritability, muscular twitching, reluctance to stand, loss of equilibrium, tetany, etc. & death.
   C. Fish:

      1) Carp, catfish, eel & rainbow trout - Aanorexia, ↓ growth, sluggishness & high mortality.
      2) Carp - Convulsions & cataracts.
      3) Rainbow trout - Calcinosis of kidney, vertebrae deformity & degeneration of muscle fibers & epithelial cells of pyloric cecum & gill filaments.

4. **Mg Requirements**

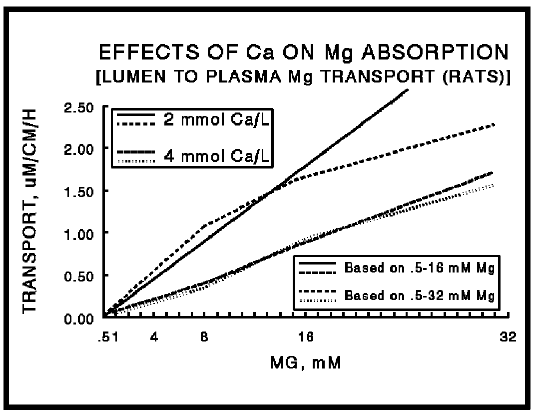
Animal	mg/kg or %
Poultry (NRC, 1994):	
Immature chickens	370-600
Laying hens	420-625
Broilers	600
Turkeys	500
Swine (NRC, 1998):	
All classes	400
Horses (NRC, 1989; %)	0.07-0.12

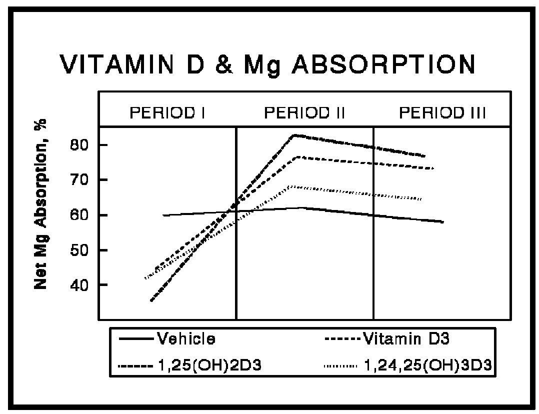
Fish (NRC, 1993; %):	
Channel catfish	0.04
Rainbow trout	0.05
Pacific salmon	Not tested
Common carp	0.05
Tilapia	0.06

A. Swine - Not well established, and corn-soy diets usually contain enough Mg to meet the requirement.
B. Fish - Diets usually contain adequate levels of Mg (plus active uptake from the environment), thus probably no need for dietary supplementation.

5. **Mg and Ca/Vitamin D**

- Effects of Ca & vitamin D on Mg absorption: (Adapted & redrawn from Hardwick et al., 1991. J. Nutr. 121:13)





6. **Toxicosis**

A. A toxicosis due to ingestion of natural feedstuffs has not been reported, and does not likely to occur unless making a mistake in supplementation process, or water is very high in Mg (≈ 1% or higher).
B. Some toxicity signs include lethargy, disturbance in locomotion, diarrhea, lowered feed intake & performance, and death. (Certain concentrations of Ca & P may protect animals from "Mg toxicosis.")
C. Maximum tolerable levels (NRC, 1980) - 0.50% for cattle & sheep, and 0.30% for poultry & swine.

MANGANESE

1. **General**

   A. Involved in normal growth, reproduction and skeletal development.
   B. Distributed widely throughout tissues & fluids:

      1) Present in a small amount.
      2) Although there are, generally, no notable concentrations in any particular location, it is fairly high in bones. (Also significant amounts can be found in liver, muscle, kidneys, gonadal tissues & skin.)
      3) In tissues, concentrated more in mitochondria vs cytoplasm or other organelles.
      4) There seem to be little variations among species or with age.

   C. Coordination chemistries of Mn^{2+} and Mg^{2+} are similar, thus may replace each other as a activator of various enzymes.

2. **Functions**

   A. Bone tissue:

      1) Mn is required for enzymes involved in the synthesis of chondroitin sulfate (component of mucopolysaccharides in bone matrix and cartilage).
      2) Activate alkaline phosphatase, which is involved in formation of collagen & transferring phosphate to the organic base of bone tissues.
      3) Activate many enzymes (or may be acting as a cofactor?) involved in carbohydrate, lipid and protein metabolism:

         a) Examples include arginase, hexokinase, pyruvate carboxylase, and isocitrate dehydrogenase.
         b) Some enzymes with specific needs for Mn include arginase, pyruvate carboxylase & superoxide dismutase.

3. **Deficiency**

   A. Poultry - Impaired growth & development, perosis or slipped tendon, lower egg production & shell strength, poor hatchability, etc. (Perosis is also associated with choline & biotin, and this condition is aggravated by excess Ca & P.)
   B. Swine - Abnormal skeletal growth, increased fat deposition (Mn has a specific lipotropic effect), impaired reproductive performance and milk production, etc.
   C. Fish - ↓ growth & skeletal abnormalities in rainbow trout, carp & tilapia.

4. **Mn Requirements**

Animal	mg/kg
Poultry (NRC, 1994):	
Immature chickens	28-60
Laying hens	17-25
Broilers	60
Turkey	60
Swine (NRC, 1998):	
Growing swine	2-4
Sows & boars	20
Horses (NRC,1989; DM)	40
Fish (NRC, 1993):	
Channel catfish	2.4
Rainbow trout	13
Pacific salmon	Required, but not determined
Common carp	13
Tilapia	Required, but not determined

5. **Mn and Ca/P**

- Manganese utilization in chicks fed various sources of Ca & P in excess[a]: (Wedekind and Baker, 1990. Poult. Sci. 69:977)

	Amount added				
Source	Mn, mg/kg	Ca, %	P, %	Gain, g	MN availability, %
None	0	0	0	269	
None	500	0	0	258	
None	1,000	0	0	255	97.3
$CaCO_3$	1,000	1.0	0	238	87.5
$CaCO_3$ + K_3PO_4	1,000	1.0	.88	204	36.5
$CaCO_3$ + K_2HPO_4	1,000	1.0	.88	240	33.5
$CaCO_3$ + KH_2PO_4	1,000	1.0	.88	261	37.4
KH_2PO_4	1,000	0	.88	203	45.0
Dical	1,000	1.0	.88	258	52.8
De-F rock PO_4	1,000	1.0	.56	279	55.9

[a]The basal diet contained 1.1% Ca, 0.51% available P & 37 mg Mn/kg; Estimated the availability based on total tibia Mn & supplemental Mn intake.

6. **Toxicosis**

A. Although some metabolic alterations may occur (e.g., effects on metabolism of Cu, Ca, P & Fe), generally, 1,000 ppm or less has no adverse health effects on most species.

☞ One of the least toxic trace elements for poultry & mammals!?

B. With above 2,000 ppm, may see growth retardation, anemia, gastrointestinal lesions & neurological signs.

C. Maximum tolerable levels - 1,000 ppm for sheep & cattle, 2,000 ppm for poultry, and 400 ppm for swine.

ZINC

1. **Functions**

A. Component of many metallo-enzymes - e.g., Alkaline phosphatase, collagenase (bone collagen), dehydrogenases (alcohol, malic, lactic, etc.), carbonic anhydrase, aldolase, RNA & DNA polymerases, thymidine kinase, carboxypeptidase, etc.
B. Activates many enzymes - e.g., Glycylglycine dipeptidase, arginase, dipeptidases, tripeptidase, His deaminase, enolase, oxalacetate dehydrogenase, lecithinase, etc.
C. Has a wide range of functions/effects:

1) Growth rate - Associated with nucleic acid biosynthesis, amino acid utilization or protein synthesis, etc.
2) Skin & wound healing - Skin is rich in Zn, and deficiency can lead to parakeratosis, scaling/cracking, loss of hair & dermatitis.
3) Immune response - Essential to the integrity of the immune system.
4) Water & cation balance - Early signs of deficiency in most species are dehydrated appearance, elevated hematocrit & diarrhea.
5) Others - Development of sex organs, reproductive functions, bone and blood formation, metabolism of nucleic acids, proteins & carbohydrates, etc.

D. Other roles of Zn?

1) Its relationship with vitamin A:

a) Zn deficiency reduces retinol-binding protein (Mobarhan et al., 1992. Int. J. Vit. Nutr. Res. 62:148), which influences mobilization of vitamin A.
b) Thus, Zn may aid in maintaining normal concentration of vitamin A in plasma, which in turn maintains normal functions of epithelial tissues.

2) Protection of membranes - Zn has antioxidative effect in protecting sulfhydryl group in the membrane.
3) Prostaglandin metabolism - Affects metabolites of PG.
4) Lipid metabolism - Zn deficiency ↓ incorporation of glucose into FA.
5) Microbial growth - Microorganisms need Zn for growth.
6) Behavior & learning ability - Severe maternal Zn deficiency has severe consequences in learning abilities & emotional responsiveness.

2. **Deficiency**

A. Poultry - Delayed growth, unnatural feather formation, shorter & thicker long bones of the legs & wings, lower egg production & hatchability, etc.

B. Swine - Parakeratosis (hyperkeratinization of skin), reduced rate & efficiency of growth, reduced testicular development in boars, small litters, small pigs, etc.

C. Fish:

1) A widespread occurrence of cataracts in rainbow trouts (1973-1974) was traced back to Zn unavailability in white fish meal.
2) Other signs may include ↓ growth, high mortality, erosion of fins & skin, short body dwarfism in rainbow trout, and ↓ growth, feed intake, bone Zn & Ca in catfish.

3. **Zn Requirements**

Animal	mg/kg
Poultry (NRC, 1994):	
Immature chickens	33-40
Laying hens	29-44
Broilers	40
Turkeys	40-70
Swine (NRC, 1998):	
Growing pigs	50-100
Sows & boars	50
Horses (NRC, 1989; DM)	40
Fish (NRC, 1993):	
Channel catfish	20
Rainbow trout	30
Pacific salmon	Required, but not determined
Common carp	30
Tilapia	20

•. Supplements - Oxide, carbonate, sulfate & chloride salts are highly available sources, but sulfide salt is a poor source.

4. **Zn, Ca & Phytate**

A. Phytate binds Zn and reduces its availability.

B. High dietary Ca also reduces absorption of Zn, thus Zn requirement is directly proportional to dietary Ca.

C. Phytate, Ca and Zn (growing rats): (Unknown source)

Phytate, mg/kg	Ca mg/kg	Zn mg/kg	Molar ratio[a]	4-wk Gain, g
10,000	16,000	2	197.8	8.8
10,000	16,000	15	26.3	12.5
4,000	16,000	15	10.5	15.3
4,000	16,000	70	2.3	37.5
4,000	16,000	125	1.3	48.5

[a]Molar ratio = (Phytate)(Ca)/Zn

1) Should not exceed 2.0 (maximum) for optimum performance.
2) In typical corn-soy diets (.75% Ca & 75 ppm Zn), the ratio is ≈ 1.80.

5. **Toxicosis**

A. Generally no adverse physiological effects at < 600 ppm.
B. Supplemental Zn at > 1,000 ppm caused some adverse effects in most studies - signs include GI tract distress, ↓ feed intake & weight gain, anemia, reduced utilization of Ca & bone ash, ↓ tissue concentrations of Fe, Cu & Mn, damage to pancreas, nonviable newborn, etc.
C. Maximum tolerable levels - 300 ppm for sheep, 500 ppm for cattle & 1,000 ppm for swine & poultry.

FLUORINE

1. **General**

A. Generally classified as a toxic element, and excessive accumulation in bones & teeth can result in:

1) Bones - Thick & soft bones, reduced breaking strength (low ash content), etc.
2) Teeth - Chalky and brittle teeth, and an enamel becomes pitted & stained (yellow to black) & may chip off (mottling).

B. Small amounts are beneficial in:

1) Development of a dental caries-resistance - 1-2 ppm in water may have beneficial effects. (Addition of 0.7-1 ppm is a common practice in many municipalities.)
2) Inhibit an excessive demineralization of bones (especially in aged).

C. Concentrations in bones & teeth:

1) Normal (dry, fat-free basis) - 300-600 ppm in bones & 200-540 ppm in molar teeth.
2) After high F intake, can expect 3,000 to 4,500 ppm in bones.

a) Generally, F toxicosis occurs when the F content in bones exceeds 5,500-7,000 ppm.
b) "Saturation" point is ≈ 15,000 to 20,000 ppm.

D. Classification of dental fluorosis: (NRC, 1974)

Score	Description
0	**Normal** - smooth, translucent, glossy white enamel; tooth has a normal shape.
1	**Questionable effect** - slight deviations; may have enamel flecks, but is not mottled.

2	**Slight effect** - slight mottling of enamel; best observed as horizontal striations with transmitted light; may be slightly stained but no increase in normal rate of wear.
3	**Moderate effect** - definite mottling; large areas of chalky enamel or generalized mottling of entire tooth; tooth may have slightly increased rate of wear and may be stained.
4	**Marked effect** - definite mottling, hypoplasia, and hypocalcification; may have pitting of enamel; with use, tooth will have increased rate of wear & may be stained.
5	**Severe effect** - definite mottling, hypoplasia, and hypocalcification; with use, tooth will have excessive rate of wear, and may have eroded or pitted enamel. (Tooth may be stained or discolored.)

E. Other symptoms associated with F toxicosis:

1) Low levels - Anorexia & reduced performance (secondary to dental & skeletal damages?), unthriftiness, dry hair, thick, nonpliable skin, etc.
2) High levels - High-F content of blood & urine, restlessness, stiffness, excessive salivation, nausea, vomiting, urinary & fecal incontinence, clonic convulsions, necrosis of GI mucosa, weakness, severe depression & cardiac failure.
3) Difficult to predict possible/potential problems because:

a) No histologic or functional changes in blood or soft tissues that can be used to assess the status.
b) The severity of the problem is influenced by the form, duration of ingestion, general nutritional status, species, age, other dietary components, etc.

2. **F & Animals**

A. According to a recent pig research:

1) ≈ > 132 ppm may resulted in reduced feed intake & weight gain.
2) ≈ > 7 ppm - Seems to have detrimental effects on the integrity of bones.

B. The fluoride content in some phosphate compounds:

1) Defluorinated (mono- & dicalcium, mono- & diammonium phosphate, etc.) - 0.12 to 0.18% F.
2) Soft rock phosphate - 1.2% F.
3) Ground rock phosphate - 3.7% F.

C. Use of phosphates in the diet:

1) Raw rock phosphate (3% F) at 1.5% of the diet provides 450 ppm F.
2) Defluorinated phosphate (0.18% F) at 1.5% of the diet provides 27 ppm F.

D. Maximum tolerable levels:

Young & mature cattle	40 to 50 ppm
Finishing cattle	100 ppm
Horses & rabbits	40 ppm
Breeding sheep	60 ppm
Finishing sheep	150 ppm
Swine & turkey	150 ppm
Chickens	200 ppm

VITAMIN D

1. Introduction

A. Often referred to as a sunshine vitamin:

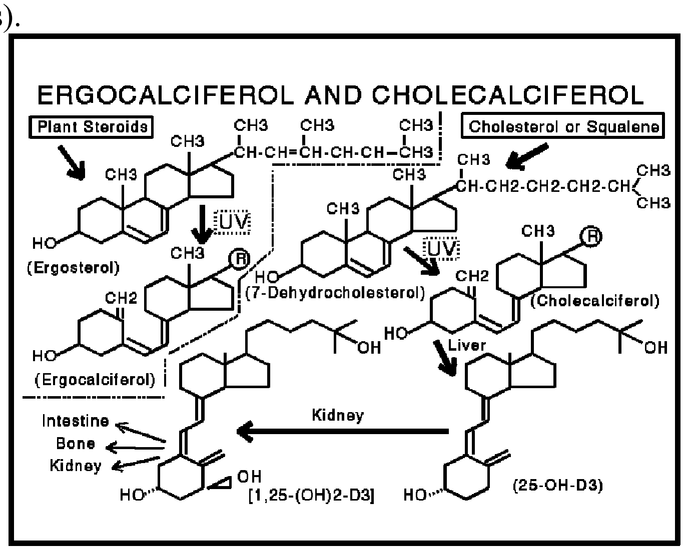
1) In the total confinement facility (swine & poultry), little or no exposure to the natural sunlight, ∴ must be supplemented.
2) Even those on pasture/outside lots, may develop deficiency symptoms during winter months.

B. A general function of the vitamin is to elevate plasma Ca & P for normal mineralization of bones & also for other body functions.

C. Two major natural sources or provitamins,

1) Cholecalciferol (D_3 - animal sources).
2) Ergocalciferol (D_2 - plant sources).

D. The most potent vitamin D metabolite is 1,25-dihydroxy-D_3. (Based on the mode of action, "1,25-dihydroxy-D_3" may be considered as a hormone?)



2. Structure & Metabolism

A. Ergo- & cholecalciferol: Adapted & redrawn from McDowell, 1989.

B. Vitamin D and Ca & P:

- Interrelationships? - See, e.g., Peo (1991) in Miller et al.

1) Intestine:

   a) Two forms of vitamin D-dependent CaBP are known (MW of 27,000 & smaller one, MW of 9,000).
   b) But, a precise role of CaBP on transfer of Ca is unclear!

      (1) Vitamin D may be inducing synthesis of unknown protein(s) that involves in transfer of Ca!?
      (2) Vitamin D may alter membrane fluidity, thus ↑ Ca transport rate!?

2) Effects on the bone:

   a) ↑ mobilization of Ca and P to the ECF, but little is known about the biochemical process.
   b) 25-(OH)D (major metabolite in bones) & 24,25-$(OH)_2D$ [found in a constant proportion with 25-(OH)D in bone] may have unique actions on the bone (e.g., promoting "normal" development of the bone?).

3) Effects on other organs/tissues:

   a) Poorly understood.
   b) ↑ renal reabsorption of Ca & P:

      (1) Ca - Less important since most of Ca is reabsorbed in absence of vitamin D.
      (2) P - Quantitatively most important action of vitamin D at the kidney.

   c) May act on parathyroid glands to regulate PTH secretion.

3. **Functions**

A. 1,25-$(OH)_2D_3$ stimulates the synthesis of Ca-binding proteins in the gut mucosa, which facilitate absorption of Ca, P & Mg.
B. Maintains the homeostasis of Ca/P along with PTH & calcitonin in the bone & kidneys.
C. Current view:

   1) Conversion of D_3 to 25-$(OH)D_3$ in the liver.
   2) Conversion to 1,25-$(OH)_2D_3$ in the kidneys.
   3) 1,25-$(OH)_2D_3$ - Transported to the intestine & bone.
   4) 1,25-$(OH)_2D_3$ - Unmask a specific DNA which is transcribed into the mRNA.
   5) Synthesis of a protein (or proteins), which appears at the brush border as an ATP-requiring transport system. (Ca-binding protein & others.)
   6) Thus, enhancing absorption of Ca & P (also, Mg).

4. **Deficiency**

A. Similar to the Ca-P deficiency in land animals.
B. Fish:

1) Fish get relatively little UV light from the sun because of shallow depth of penetration of these rays in natural water.
2) Dietary needs has been established for at least two species by feeding vitamin D deficient diets:

a) Channel catfish - ↓ weight gain & ↓ body ash, Ca & P (fed for < 16 wk).
b) Rainbow trout - ↓ weight gain, tetany in white muscle & structural changes in muscle fibers.

4. **Vitamin D Requirements** (1 IU = 0.025 µg of vitamin D_3)

Animal	ICU/kg	IU/kg
Poultry (NRC, 1994):		
Immature chickens	190-300	
Laying hens	250-375	
Broilers	200	
Turkeys	1,100	
Swine (NRC, 1998):		
3-120 kg		150-220
Sows & boars		200
Horses (NRC, 1989; For those not exposed to sunlight, DM)		300-800
Fish (NRC, 1993):		
Channel catfish	500	
Rainbow trout	2400	
Others	Not tested	

A. Vitamin D_3 may be 30 times more effective than D_2 in poultry - Eliminates D_2 rapidly via bile.
B. Similarly, D_2 is poorly utilized or not at all by fish - e.g., D_3 was utilized ≈ 3 times more efficiently than D_2 by rainbow trout.
C. Recent data indicate that pigs, ruminants & others also utilize D_3 more efficiently.
D. Natural feeds are generally low or devoid of vitamin D, thus needs to be supplemented.
E. Factors influencing requirements? - Sunlight, amount and ratio of dietary Ca and P, availability of Ca & P, species, physiological factors, and others.

5. **Supplementation/Supplements**

A. Commercial supplements are stabilized or protected by coating (e.g., gelatin, starch, sugars & antioxidants).
B. In the original unopened container with good storage conditions, 90-100% retention of the vitamin activity can be expected up to one year.

C. Stability can be ↓ by moisture, minerals, light, oxygen, heat, rancid fat & pelleting.
D. Vitamin D stability: [Coelho, 1991. Feed Management 42(10):24]

Condition	Retention, %
In vit-TM premix (½ → 6 mo)	96 → 65
Pelleting - 140°F/3 min → 220°F/3 min	97 → 89
Extrusion - 230°F/3 min → 330°F/3 min	95 → 83
In feed (Avg. of the industry - ½ → 6 mo	93 → 55

6. **Toxicity**

A. Signs in swine include anorexia, stiffness, lameness, arching of the back, polyuria, etc.
B. Postmortem examination reveals extensive mineralization in cardiovascular systems, kidneys, respiratory tracts, salivary glands, GI tracts, etc.
C. Safe upper dietary levels of vitamin D_3 (IU/kg): (NRC, 1987)

		Exposure time	
Species	Requirement	< 60 d	> 60 d
Birds:			
Chicken	200	40,000	2,800
Turkey	900	90,000	3,500
Swine	220	33,000	2,200
Fish:			
Catfish	1,000	20,000	
Trout	1,800	1,000,000	
Horse	400	2,200	
Sheep	275	25,000	2,200
Cattle (cow)	300	25,000	2,200

VITAMIN A

1. **Introduction**

A. From a practical standpoint, may be considered as the most important vitamin because all animals require supplementation.
B. Necessary for support of growth, health and life of higher animals.
C. Vitamin A itself does not occur in plants as such:

1) Occurs in plants as carotenoid pigments (provitamin A).
2) Identified over 80 carotinoids with ≈ 15 having vitamin A activity.

- According to some, there are over 600 carotenoids in nature with < 10% having provitamin A activity!

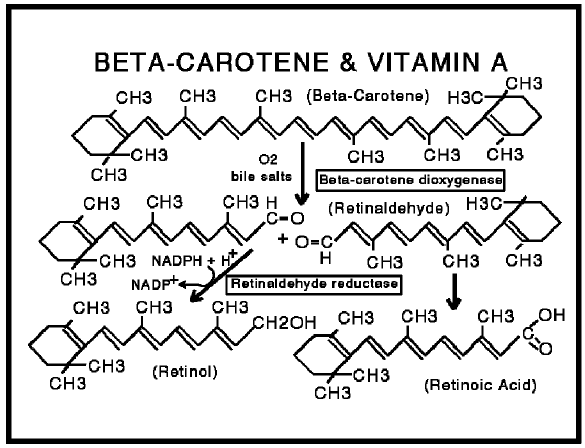
3) The most important one is β-carotene:

a) Variations among species in their ability to convert carotene to vitamin A.
b) In pigs, it might be only 25-30% [much higher in other nonruminant species such as poultry & rats, and also fish (especially channel catfish)].

D. Animals can store large amounts of vitamin A in the liver - e.g., mature sows completed three normal pregnancies without vitamin A supplement in one study (showed signs of deficiency in the fourth).

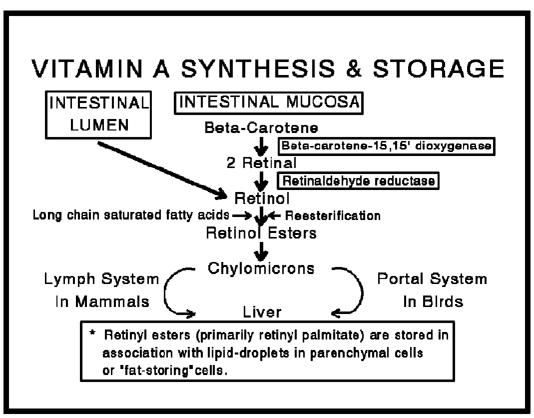
2. **Structures**

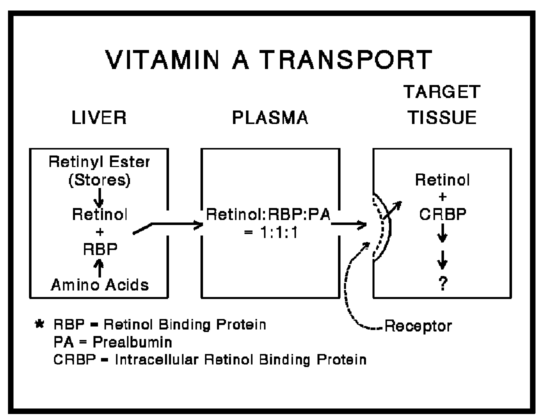
- β-Carotene and vitamin A: (Adapted & redrawn from Martin et al., 1983)



A. Retinol (alcohol) - Probably serving as a sterol hormone?
B. Retinal (aldehyde) - Precursor of a visual pigment, rhodopsin.
C. Retinoic acid - Supports normal growth & differentiation, but not a visual pigment precursor & can't support normal function of the reproductive system in male or females.

3. **Biosynthesis, Transport & Storage**





A. Over 90% of vitamin A is stored in the liver with remainder in the kidneys, lungs, adrenals & others.
B. Liver can store enough to protect animals for a long period of dietary scarcity.
C. Vitamins E & D might be using the same transport mechanism, thus excess of one of these (especially, vitamin A) may cause deficiency of others.

4. **Deficiency/Functions**

◦ Metabolic functions of vitamin A have not been completely elucidated!

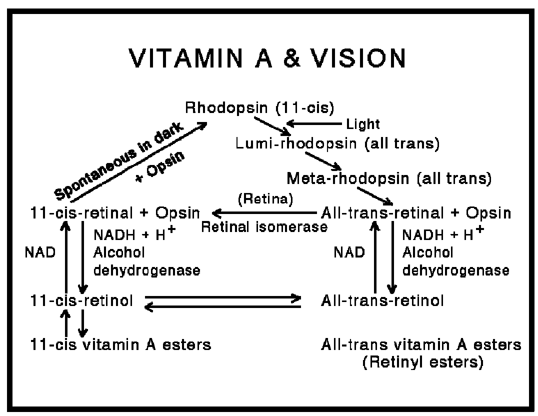
A. Defects in bone growth (disorganized bone growth & irritation of joints):

1) Vitamin A affects the activity of osteoblasts and osteoclasts.
2) Involved in mucopolysaccharide synthesis.
3) Changes in bones may be responsible for muscle incoordination & other nervous symptoms associated with vitamin A-deficient animals?

B. Abnormal epithelial tissues:

1) Mucus membranes protect respiratory, GI and uro-genital tracts.
2) Vitamin A deficiency can cause damages to mucus-secreting cells of epithelium (keratinization), which can lead to ↑ incidence of infections, diarrhea, kidney & bladder stones, etc.
3) Vitamin A is stabilizing membranes? - May be acting as a cross-linkage agent between lipid & protein in the membranes!
4) Involved in a normal cell differentiation?
5) Involved in the synthesis of mucopolysaccharides.

C. Impaired vision - The role of vitamin A in vision: (Adapted & redrawn from McDowell, 1989)



◦ Impaired vision is due to a failure of rhodopsin formation, which can lead to night blindness and also total blindness!

D. Reproduction problems:

1) Vitamin A deficiency can lead to a failure of spermatogenesis, resorption of fetus, abortion, retained placenta, reduced hatchability, etc.
2) Possible reasons?

   a) Probably the results of failure to maintain normal epithelium?
   b) Direct effects - Effects of vitamin A on cell differentiation & proliferation, transcription of specific genes, etc.?
   c) Indirect effects - Production of steroid hormones, enhancing the immune status, etc.?

E. Deficiency signs in fish:

1) Salmonids - ↓ growth rate, light skin color, pathological conditions of the eye characterized by exophthalmos, hemorrhagic eyes, eye lens displacement, thinning of cornea, degradation of the retina, etc.
2) Channel catfish - Exophthalmos, edema & kidney hemorrhage.
3) Common carp - Light skin color, fin & skin hemorrhages, exophthalmos & deformed gill opercula.

F. β-Carotene:

1) May have a role in reproduction that is independent of vitamin A - A higher intensity of estrus, ↑ conception rate, ↓ embryonic mortality, heavier birth & weaning weights have been observed with β-carotene supplementation.
2) Also, it has been suggested that it is an inhibitor of some type of cancers in humans?

☞ β-carotene might be serving as an antioxidant against lipid peroxidation, thus having beneficial effects on reproductive performance?

5. **Vitamin A Requirements** (1 IU = 0.30 μg of retinol or 0.55 μg of vitamin A palmitate)

Animal	IU/kg
Poultry (NRC, 1994):	
Immature chickens	1,420-1,500
Laying hens	2,500-3,750
Broilers	1,500
Turkeys	5,000
Swine (NRC, 1998):	
Growing pigs	1,300-2,200
Sows	2,000-4,000
Boars	4,000
Horses (NRC, 1989)	2,000-3,000
Fish (NRC, 1993):	
Channel catfish	1000-2000
Rainbow trout & pacific salmon	2500
Common carp	4000
Tilapia	Not tested

6. Sources/Supplementation

A. Rich sources - Ffish oils (e.g., swordfish liver oil contains 250,000 IU/g).
B. Factors detrimental to stability include long storage, high temperature & humidity, pelleting, extrusion, presence of trace minerals, etc.

☞ Vitamin A might be a least stable vitamin among the vitamins commonly supplemented!

C. Vitamin A palmitate (synthetic):

1) Esterified with palmitate to increase stability.
2) Also encapsulated in gelatin beadlets for further protection.
3) But, still very unstable, thus usually fortified with several-fold levels to compensate for potential losses during the storage.

7. Hypervitaminosis

A. General:

1) Common reactions to massive doses of vitamins include general malaise, anorexia, nausea, hyperirritability, peeling skin, muscular weakness, twitching, convulsions, paralysis and death.
2) Chronic toxicity (generally with intake of 1,000 times requirements for a prolonged period, but may be observed with 10 times) - Skeletal malformation, spontaneous fractures, internal hemorrhage, loss of appetite, slow growth, loss of weight, skin thickening, increased blood clotting time, etc.
3) Vitamin A toxicity:

a) Signs in fish include enlargement of liver and spleen, abnormal growth, skin lesions, epithelial keratinization, hyperplasia of cartilage in the head, abnormal bone formation, etc.
b) Because of the storage capacity of the liver (which offers protection to some degree), probably require feeding an unusually high level for a long period to induce the vitamin toxicity.

B. Upper safe levels: (NRC, 1987)

1) Presumed upper safe levels - 4-10 times the requirement for nonruminant species, and ≈ 30 times for ruminant species.
2) Usually, can expect toxicity signs by feeding over 100 times the requirement.
3) Some examples: (chronic intake)

Animal	Requirement, IU/kg diet	Safe level, IU/kg diet
Chicken, growing	1,500	15,000
Chicken, laying	4,000	40,000
Swine, growing	2,000	20,000
Swine, breeding	4,000	40,000
Cattle, feedlot	2,200	66,000
Goats	1,500	45,000

VITAMIN C

1. Introduction

A. Scurvy is a disorder associated with inadequate vitamin C intake:

1) Scurvy has been known since the ancient times (as early as 1550 B.C.?!).
2) Potentially fatal - Signs include anemia, weakening of collagenous structures (bone, cartilage, teeth, connective tissues), swollen, bleeding gums with loss of teeth, hemorrhages in various tissues, delayed healing of wounds, fatigue & lethargy, rheumatic pains in the legs, degeneration of muscles & skin lesions.

B. Vitamin C can be synthesized by almost all species, except primates & few others (guinea pigs, many fishes, etc.):

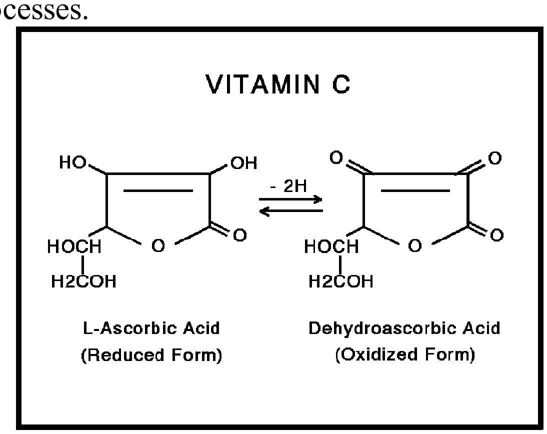
1) Biosynthesis by the glucuronic acid pathway in the liver of mammals & kidney of other vertebrates - D-glucose → D-glucuronic acid → L-gulonic acid → L-gulono-γ-lactone → L-ascorbate.
2) Humans, primates, guinea pigs, fruit bats, some birds and certain fish & insects lack "L-gulono-γ-lactone oxidase," which is responsible for conversion of L-gulono-γ-lactone to 2-oxo-L-gulono-γ-lactone (which is transformed spontaneously into the vitamin).

C. Suggested beneficial effects of megadoses (2.3 g to 9-10 g/day) for humans include: [Pauling, 1971. Vitamin C and the Common Cold; Pauling, 1974. Am. J. Psychiatr. 131:1251; Jaffe, 1984. In: Machlin (Ed.) Handbook of Vitamins]

1) Prevention & reduction of severity of a common cold.
2) Prevention of cancer & prolonged life of cancer patients.
3) Lower serum cholesterol & severity of atherosclerosis.
4) Increase wound repair & normal healing processes.
5) Increase immune response for prevention and treatment of infections.
6) Control of schizophrenia.
7) Inactivation of disease viruses.

2. **Structure** (Adapted & redrawn from McDowell, 1989)

- 1 IU = 0.05 mg of L-ascorbic acid, which is equivalent to ≈ 1 ml of lemon juice.



3. **Functions**

   - The exact role in the living system is not clearly known, i.e., to date, a coenzyme form has not been reported.
   - Functions are probably related to its reversible oxidation and reduction characteristics.

   A. Collagen biosynthesis:

      1) The most clearly established role of vitamin C.
      2) Involved in hydroxylation of Pro and Lys:

         a) OH-Pro - Gives a greater stability (H-bonding).
         b) OH-Lys - Involved in the formation of cross-links.

      3) Also, may protect hydroxylase enzymes!

   B. Other functions?

      1) Involved in the electron transfer (NADH & cytochrome b_5).
      2) Involved in metabolic oxidation of excess Tyr.
      3) Enhances absorption of minerals, and also mobilization (e.g., Fe).
      4) Involved in the synthesis of carnitine, and also norepinephrine.
      5) Has a stimulating effect on phagocytic activity of leukocytes.
      6) Is a natural inhibitor of nitrosamines (carcinogens).
      7) Is a powerful antioxidant, and spares vitamin E & enhance immune responses.
      8) Involved in activation, inhibition or ↑ activity of enzymes.
      . . . , etc.

4. **Deficiency**

   A. Ascorbate-synthesizing animals:

      1) Under practical situations, not likely to see vitamin C deficiency.
      2) Under certain conditions (stress, infectious diseases, etc.), may develop deficiency symptoms (scurvy-like symptoms).

   B. Fish:

      1) Highly sensitive to dietary deficiency, especially young fish.
      2) Curvature of spinal column is a prominent, early sign of deficiency in finfishes.
      3) Rainbow & brook trout, coho salmon, tilapia, channel catfish & young carp - Scoliosis & lordosis (lateral & vertical curvature of spinal column, respectively).

4) Channel catfish - Signs include deformed spinal columns, external & internal hemorrhages, erosion of fins, depigmented vertical bands around midsection, distorted gill filament cartilage & ↓ rate of wound healing.
5) Black death in shrimp - A condition characterized by melanized hemocytic lesions distributed throughout the collagenous tissues.
6) The deficiency also ↓ resistance to bacterial diseases.

5. **Vitamin C Supplementation (e.g., Swine)**

A. Has resulted in very inconsistent response:

1) Improved performance of pigs in some experiments, but no response in others (vitamin C used ranged from 150 to 5,000 ppm in those studies).
2) Reported a rapid cessation of naval bleeding & rapid growth of pigs by vitamin C supplementation of the sow diet (1 g/day), but observed no response in subsequent experiments.

B. Probably, a routine vitamin fortification of swine diets is not necessary.

C. But, in some situations, supplementation might be beneficial - e.g., Deficient in other nutrients (vitamin E, Se, protein, Fe, etc.), highly productive animals, transporting a long distance or handling animals extensively, adjusting to a new environment, ambient temperatures (too high or too low), diseases & parasite infestations, etc.

6. **Vitamin C & Fish**

A. Dietary requirement: (NRC, 1993)

Channel catfish	25-50 mg/kg
Rainbow trout, pacific salmon & tilapia	50 mg/kg
Common carp	Required, but not determined

B. Supplementation:

1) Commercial ingredients - Almost devoid of vitamin C, thus must be supplemented.
2) Vitamin C is highly sensitive to oxidative destruction during processing & storage:

a) 25% destruction during steam pelleting & 50% loss during extrusion process.
b) ½ life of L-ascorbic acid in feed is ≈ 2.5 mo under warm weather conditions.
c) Phosphate & sulfate conjugates are much more stable vs L-ascorbic acid.

7. **Vitamin C for Humans?**

A. Three levels of daily intake:

1) "5 to 10 mg" can prevent scurvy.
2) "100 to 250 mg" can achieve a saturation in the blood.
3) "1000 to 10,000 mg:"

    a) May produce favorable mega-vitamin effects.
    b) Pauling suggests 2.3 g/day for optimum health, and 9-10 g in presence of some ailments.

        ☞ Personally, he'd taken 10 g/day based on the fact that many species produce 10 g of ascorbic acid/day/70 kg BW.

B. Annual vitamin C consumption in the US:

1) ≈ 1 kg/year, i.e., 2,740 mg/person/day.
2) But, mostly used by the food industry for enrichment of canned vegetables, fruits and beer (adding color, flavor, etc.).

C. Requirements: (RDA, 1989)

Item	mg/d
Children	35-50
Adult	60
Pregnant	70
Lactating	90-95

D. Sources: (McDowell, 1989)

Food	mg/100 g (as fed)
Cauliflower, raw	50-90
Peppers, raw	100
Spinach	10-60
Apple, unpeeled	10-30
Grapefruit	35-45
Lemons	80
Limes	250
Oranges	40-60

D. Toxicity - Generally safe, but a consumption of > 4-10 g/d for a prolonged period may results in kidney stones, iron overload or hemochromatosis & other disorders in some people.