BONE AND VITAMINS AND MINERALS

BONE IN GENERAL

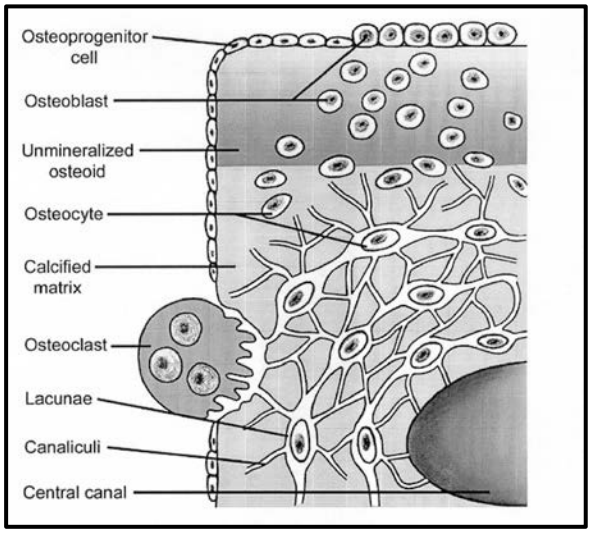
1. Composition

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	0.8

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.
- Bones cells – See, e.g., Junqueira & Carneiro (1983). Also, see the figure (unknown source).
- Structure of long bone – See the figure (unknown source).

A. Bone cells:

1) Osteoblasts:

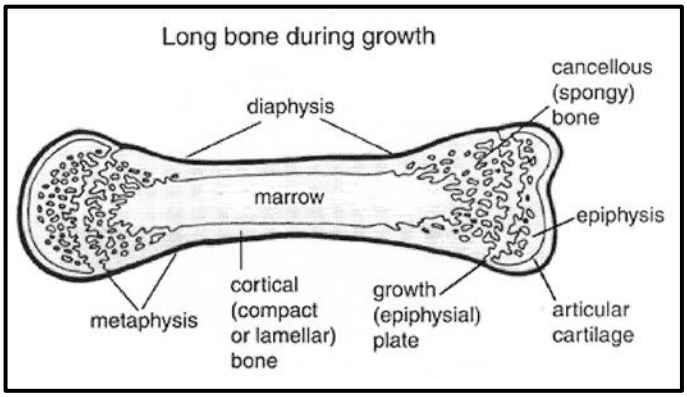
a) Found in active areas of bone formation.
b) Responsible for the synthesis of bone matrix.
c) Most cells eventually rise to osteocytes, while others remain as osteoblasts for a long period of time, and some return to the state of "osteoprogenitor cell" (i.e., slightly differentiated mesenchymal cell).

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: [See Heaney, 1993. Annu. Rev. Nutr. 13:287]

1) General:

a) Symptoms are usually not apparent until the disease is in the advanced stage.
b) Current estimates in the US - > 14,000,000 females may be affected by osteoporosis.
c) Males start losing bone density between 45 and 55 yr of age, and perhaps 10 yr earlier in females.
d) In the US, about 1/3 > 1 million fractures occur in females > 45 yr of age may be associated with osteoporosis.

2) A failure of normal bone metabolism in adults.
3) Differs from osteomalacia, i.e., the mineral content is normal, but the absolute amount of bone is decreased.
4) Common in the human > 50 yr, especially in females:

a) Adult females have less bone mass vs. males.
b) After menopause, estrogen would be reduced, which can increase sensitivity to PTH.
c) May be ameliorated by increasing Ca intake, increasing activity, taking a small dose of estrogen, etc.

(1) Ca intake among females? - Only 200 to 350 mg/d.
(2) Recommending 500 to 750 mg Ca/d with 375 units of vitamin D.
(3) Reduce activity – Can increases bone loss regardless of sex (. . . astronauts?!).
(4) Exercise – Can increase blood Ca & certain hormone levels.

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.

F. Others?

1) Osteochodrosis:

a) Skeletal disorder commonly observed in young, fast growing animals subjected to a high plane of nutrition.
b) Affects the shoulder, elbow, stifle, hock, and cervical vertebrae.
c) Might be related to insufficient collagen cross-linking because of a reduction in hydroxylation of Lys & Pro?

2) Tibial dyschondroplasia (TD):

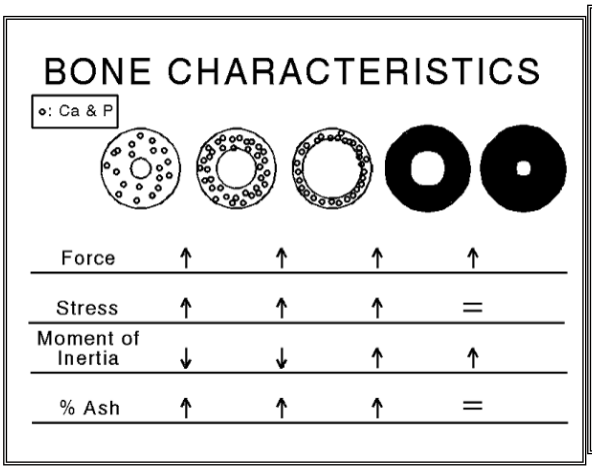
a) The poultry industry has developed this problem possibly because of breeding birds for a fast growth rate.
b) Perhaps, 30 to 50% of the male meat-type chickens and turkeys have TD, even though it only contributes to 5 to 25 per cent of the total lameness cases.
c) A tremendous weight burden placed on a juvenile skeletal system that was not meant or designed to accommodate the burden, i.e., the rate of muscle mass production is out of synchrony with skeletal development.

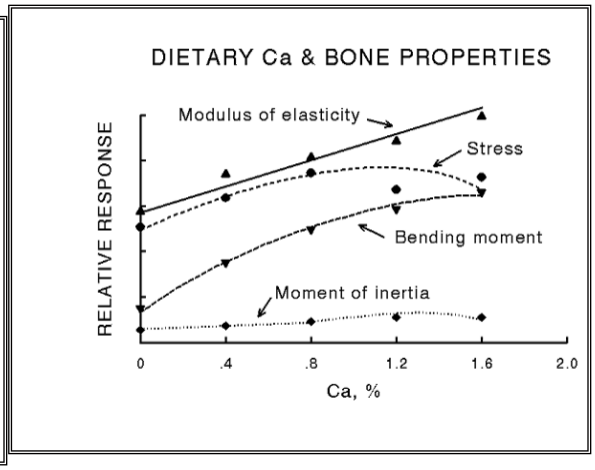
d) Abnormal development of skeletal system, skeletal deformities and ensuing leg weakness.
e) No known prevention/cure other than to restrict feeding?

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.*

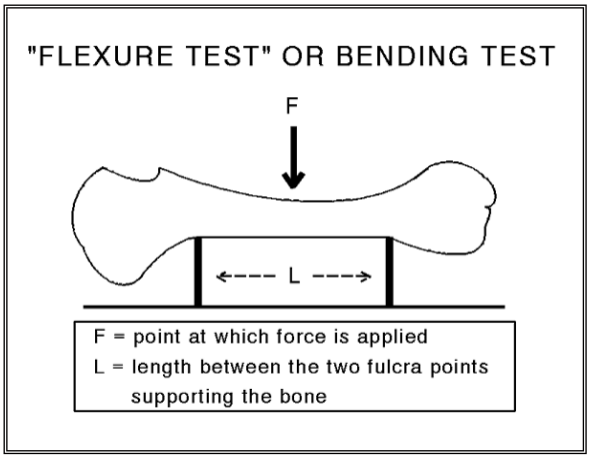
A. Cross section and characteristics of the bone – See the figure on the left.
B. Dietary Ca & mechanical properties of the bone – See the figure on the right.





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!"

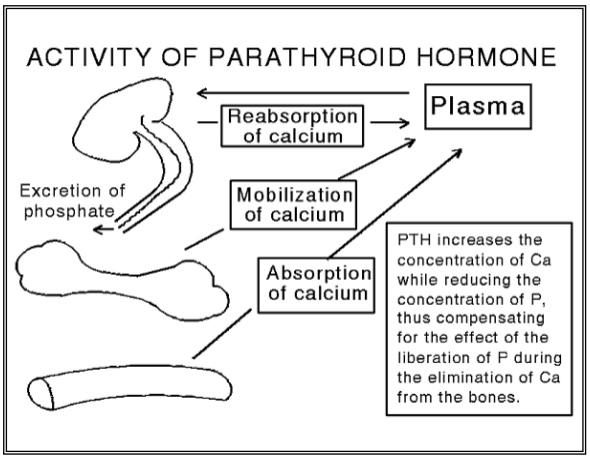


5. Factors Affecting Bone Metabolism

- See "Activity of PTH."
- See "Organization of bone cells

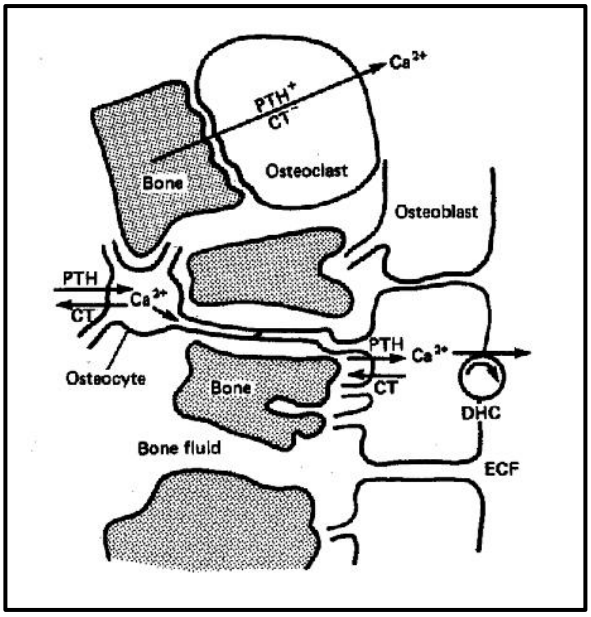
(unknown source)."

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) Estrogen & 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).

CALCIUM AND PHOSPHORUS

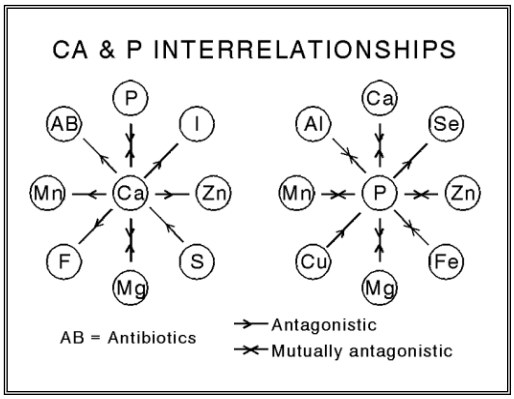
1. Introduction

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:

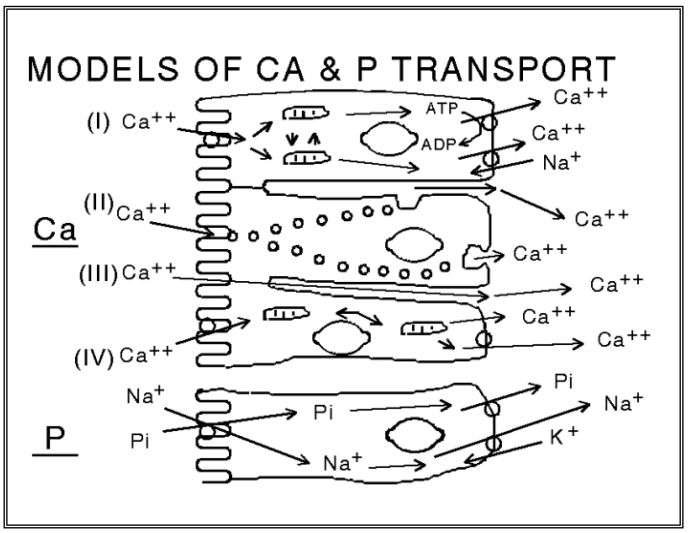
   a) Hardly any physiological function that does not involve P, directly or indirectly!
   b) Unlike Ca, a marginal deficiency of P can reduce rate & efficiency of growth.

3. Absorption of Ca & P

- A good reference - "Littledike, E.T. and J. Goff. 1987. Interaction of calcium, phosphorus, magnesium and vitamin D that influence their status in domestic meat animals. J. Anim. Sci. 65:1727."
- See Models of Ca & P Transport [adapted and redrawn from Wasserman, 1981 (Fed. Proc. 40:68); also see Wasserman and Fuller, 1995 (Vitamin D and intestinal calcium transport: Facta, speculations and hypotheses. J. Nutr. 125:1971S-1979S].

A. General:

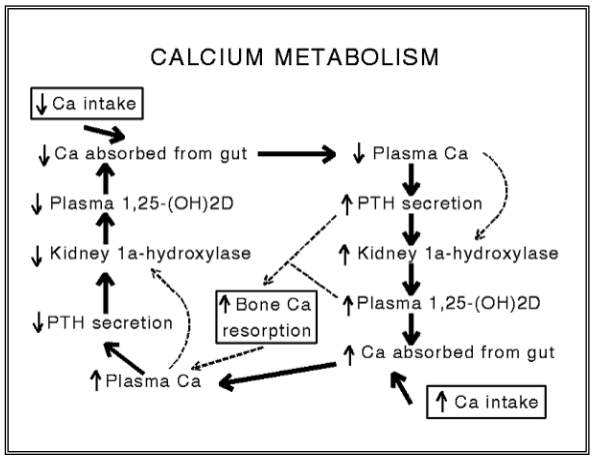
1) Mostly absorbed from the upper tract (duodenum).
2) Ca by a specific Ca-binding protein (also by a passive diffusion).
3) P, less clear, but probably similar to Ca.
4) Absorption rate for dietary Ca & P is ~ 30-50%.

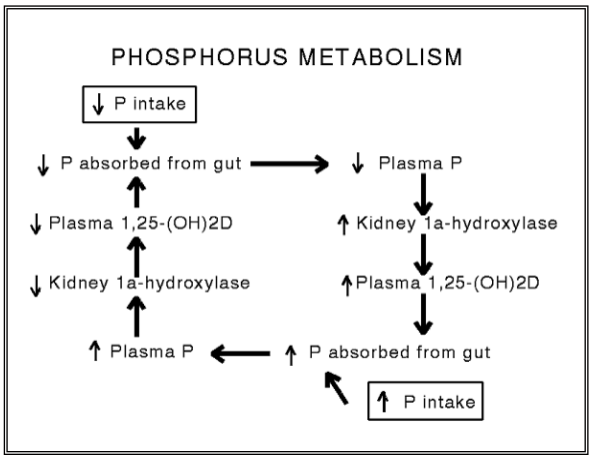


B. Factors affecting absorption:

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





A. Factors affecting homeostasis? – See the section on "Factors Affecting Bone Metabolism."
B. Ca and P metabolism - Redrawn from Horst, 1986. J. Dairy Sci. 69:604:
C. Homeostatic control of Ca?

1) Very strong and effective, but may take time to rectify a "low-Ca" situation.

a) Through PTH stimulation - May take approximately 48 h achieve homeostasis.
b) Through vitamin D stimulation – May take 15 to 24 h to achieve homeostatis.

2) A sudden increase in the Ca need, like onset of lactation? May end up with "hypocalcemia," such as "milk fever."

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. In general, can result in abnormal bone metabolism such as rickets, osteomalacia, etc.
B. Laying hens:

1) Eggs (with shell) contain 35.9 mg Ca and 2.0 mg P/g egg.
2) Deficiency can result in a poor quality shell & incubation quality.
3) Also, can increase mobilization of Ca, which can lead to thin & brittle bones, thus, fracture easily.

C. Sows:

1) Sow's milk contains 0.15 to 0.16% Ca and 0.12 to 0.13% P.
2) Deficient can lead to paralysis of hind limbs, which can be seen in socalled high-milking sows.

D. Depressed rate & efficiency of growth – Generally, because of a deficiency of P, not Ca! (Also, likely to reduce production of milk, eggs, etc.)

7. Fish & Calcium/Phosphorus

A. General:

1) Calcium - Readily derived from water & adequate amounts in most fish diets:

a) 0.5 to 1% of body weight (wet-basis).
b) 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.
(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 - ???
(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).

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:

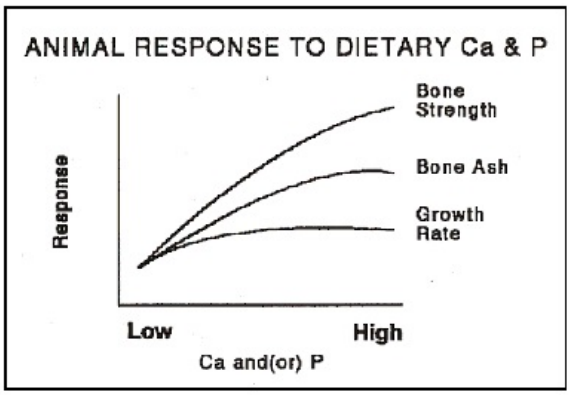
a) Carp - Signs include cranial deformity, ↓ growth, poor feed efficiency & low Ca & P content of vertebrae.
b) Signs for other fish species include anorexia, poor growth & feed efficiency, skeletal abnormalities, and poor bone mineralization.

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 the figure.

      (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, %:	0.65/0.50	0.65/0.50	0.98/0.75	0.98/0.75
Item	Gestation, g/d:	13.0/10.0	19.5/15.0	13.0/10.0	19.5/15.0
No. of ♀ started		23	22	24	22
Reproductive failure		4	3	7	4
Unable to stand		5	1	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

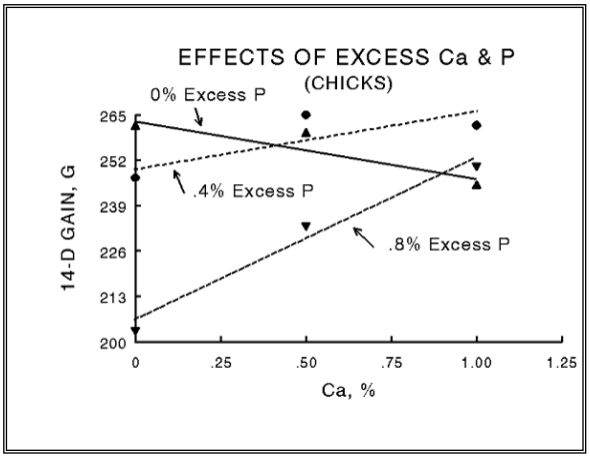
B. Ca and P ratio in pigs:

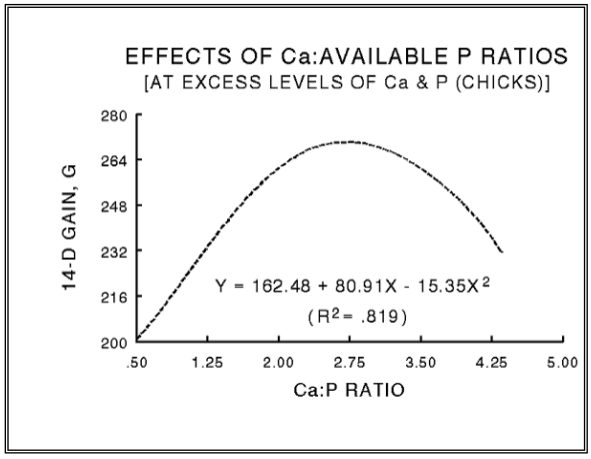
1) Important in establishing the requirement because of interactions.
2) Ideal ratio in feed = "1:1."
3) But, phosphorus is relatively expensive, thus acceptable ratios in swine range from 1.25 to 1.50:1:

   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

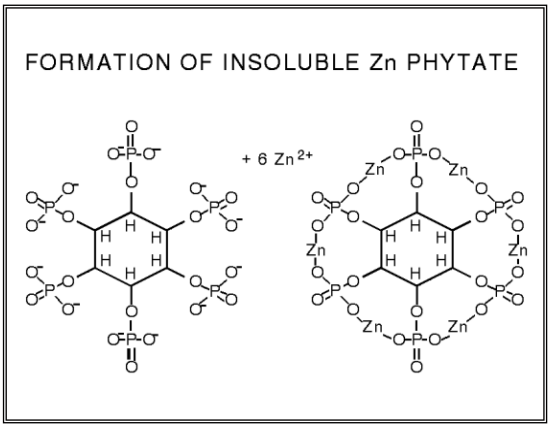
A. Calcium - Bioavailability is less critical because:

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 - e.g., for steers:

Item	Young	Old
Ca carbonate (standard)	100	100
Mono-Ca phosphate	125	137
Di-Ca phosphate	121	116
Bone meal	138	135
CaCl	117	128
Ground limestone	90	88
Alfalfa hay	*79*	*74*
Orchardgrass hay	*92*	*91*

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, and phytate P is really not available to nonruminant animals.



   a) Phytate can form complexes with Zn, Cu, Co, Mn, Fe, Ca, etc.
   b) Formation of insoluble zinc phytate - 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.

Example: Effect of rye bran (20%) on P utilization[a] in pigs (Pointillart, 1991. JAS 69:1109)

Item	Control	Rye bran
Performance:		
ADG (last mo.), kg*	0.56	0.63
G:F (last month)*	0.40	0.45
Balance:		
Absorption, % intake**	36	55
Retention, % intake**	36	50
Bone ash, % DM		
Epiphysis***	29.9	35.2
Diaphysis	58.8	60.1
Whole tibia**	36.7	41.3

[a]A diet with 20% rye bran contained 1,200 IU phytase/kg; **P* < .05; ***P* < .01; ****P* < .001.

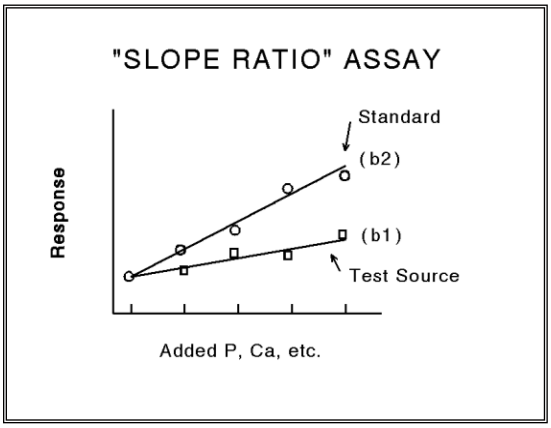
e) Ruminant species contain organisms capable of hydrolyzing phytate in the rumen.
f) Nonruminant species - Considerable variations in their ability to hydrolyze phytate.

(1) Only about 1/3 (15 to 50% for grains and 15 to 70% for grain byproducts.
(2) Even hydrolyzed, may not be absorbed because of the site of hydrolysis.

4) Determination of bioavailability:

a) Often "slope-ratio assay" is used - See the figure.
b) Bioavailability

$$= \frac{b\ (\text{Test source})}{b\ (\text{Standard source})} \times 100$$



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: (Also, see appropriate "Nutrition & Feeding" sections.)

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

1. Major Reasons for Including these Minerals in this Section?

A. Magnesium

1) Found mostly in the skeleton.
2) Involved in activation of alkaline phosphatase, etc.

B. Manganese

1) As a component of enzymes, involved in the synthesis of chondroitin sulfate.
2) Involved in activation of alkaline phosphatase, etc.

C. Zinc

1) Interacts with Ca.
2) Component of alkaline phosphatase & collagenase.

D. Fluorine - May inhibit excessive demineralization of bones.

2. Mg, Mn, and Zn:

A. There are extensive interactions among these elements.
B. These elements are a component of may enzyme systems.
C. These elements can activate many enzyme systems.

MAGNESIUM

1. Introduction

A. General:

1) Early 1600s – English village of "Epsom" was a popular health spa!

- Why? - Water in that area contains "Mg sulfate," which has, among others, a laxative effect.

2) Used as an anesthetic in the early 20^{th} century, used as an euthanasia agent, and can be used for a relief of hyperactivity.

- Why? - For instance, overload can lead to: vasodilation, reduction of blood pressure, respiratory paralysis, cardiac arrest, depression of the central nervous system, etc.

B. About 70% of Mg is in the skeleton, and remaining Mg is found within cells of soft tissues.
C. Unlike Ca & P, Mg is not readily mobilized.
D. 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 – e.g.:

1) $Mg(OH)_2$ are held within the hydration shell of apatite crystal surface.
2) Can activate, e.g., a family of alkaline phosphatase, which may be involved in transfer P and synthesis of organic matrix of the bone.

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 – For decarboxylation, transketolase reactions, etc.
2) Activates enzymes involved in phosphorylation, carboxylation, and oxidation of pyruvate, etc. – Pyruvate kinase, creatine kinase, alkaline phosphatase, hexokinase, etc.
3) Activates polymerases (DNA & RNA), ribonuclease, and others involved in nucleic acid metabolism - e.g., Involved in ribosomal aggregation (binds mRNA to 70s ribosome?). Thus, involved in protein metabolism.

3. Deficiency

A. In general, can develop problems in growth, immunity, allegy, muscle contration, RBC survival, metabolism of collegen-rich tissues, Na & K metabolism, etc.
B. Poultry - Hyperirritability, convulsion, comatose, death (some times), reduction of egg production and hatchability, etc.
C. Swine - Hyperirritability, muscular twitching, reluctance to stand, loss of equilibrium, tetany, etc. & death.
D. 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 (Also, see appropriate "Nutrition & Feeding" sections.)

Animal	mg/kg or %
Poultry (NRC, 1994):	
Immature chickens	370-600
Laying hens	420-625
Broilers	600
Turkeys	500

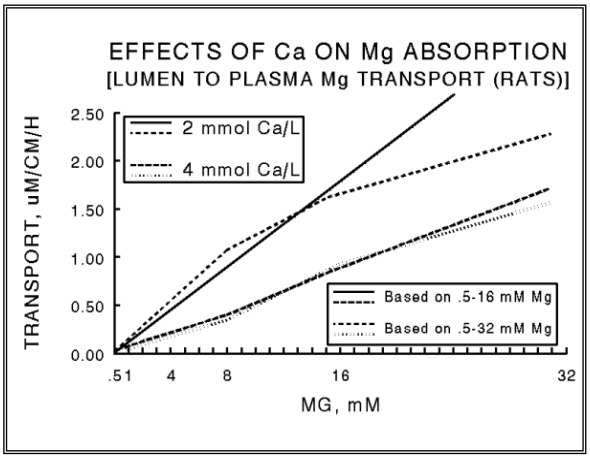
Swine (NRC, 1998):	
All classes	400
Horses (NRC, 1989; %)	0.07-0.12
Fish (NRC, 1993; %):	
Channel catfish	0.04
Rainbow trout	0.05
Pacific salmon	Not tested
Common carp	0.05
Tilapia	0.06

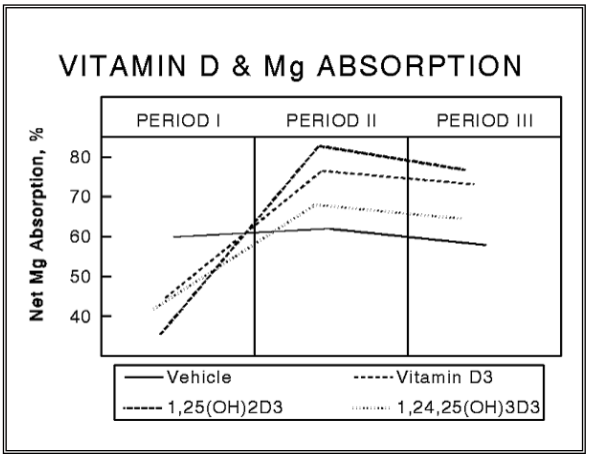
A. Swine - Not well established, and corn-soy diets usually contain enough Mg to meet the requirement.
B. Fish - Diets usually contain adequate levels of Mg (plus active uptake from the environment), thus probably no need for dietary supplementation.
C. Beneficial effects in humans? May prevent heart diseases, reduce blood pressure, may be effective in treating "housewife syndrome," etc.

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)

1) Calcium has a negative effect on Mg absorption and vice versa.
2) Vitamin D may affect Mg absorption, but have been some conflicting results.





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 – 480 to 560 μg/kg body weight, and 5 to 10 μg/dL. (Approximately, 55 to 57% in skeleton?)
2) Although there are, generally, no notable concentrations in any particular location, it is fairly high in bones. Also, substantial amounts can be found in liver, muscle, kidneys, gonadal tissues, and skin.
3) Also, fairly high in hair and feathers, and may be correlated with dietary needs!?
4) In tissues, concentrated more in mitochondria vs. cytoplasm or other organelles.
5) There seem to be little variations among species or with age.

C. Coordination chemistries of Mn^{2+} and Mg^{2+} are similar, thus:

1) May replace each other as a activator of various enzymes.
2) Nonspecific activator of many enzymes such as kinases, decarboxylases, etc.

2. Functions

A. Involved in bone formation, growth, reproduction, blood formation, etc.
B. 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. Reproductive problems (disturbances in estrus cycle, reduced egg production, etc.) are the first sign of deficiency, and bones can be shorter and thicker.

B. 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.)
C. Swine - Abnormal skeletal growth, increased fat deposition (Mn has a specific lipotropic effect), impaired reproductive performance and milk production, etc.
D. Fish - ↓ growth & skeletal abnormalities in rainbow trout, carp, and 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	0.88	204	36.5
$CaCO_3 + K_2HPO_4$	1,000	1.0	0.88	240	33.5
$CaCO_3 + KH_2PO_4$	1,000	1.0	0.88	261	37.4
KH_2PO_4	1,000	0	0.88	203	45.0
Dical	1,000	1.0	0.88	258	52.8
De-F rock PO_4	1,000	1.0	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.

a) Excess P – More antagonistic vs. Ca.
b) Other studies – High incidence of perosis with high P, but not with high Ca.

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. General:

A. Has been used by humans for utilitarian or ornamental purposes for almost 2,000 years - e.g., semitic bronzes contain as much as 23% Zn.
B. In 1934, a nutritional essentiality of Zn was demonstrated by Bertrand & Bhattacherjee in mice & Todd et al. in rats.
C. In 1940, Keilin & Mann isolated and purified a metalloenzyme (carbonic anhydrase which contains .33% Zn).
D In 1955, Tucker & Salmon discovered that "parakeratosis" in swine was due to inadequate dietary Zn.

- The first demonstration of the importance of Zn supplementation for food-producing animals.

E. In 1958, O'Dell et al. showed that "Zn deficiency" was responsible for poor growth & abnormal bone development in chicks receiving purified diets.

2. 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) Zinc is involved in the DNA synthesis, cell replication, differenciation, hypertrophy, etc.
2) Zinc can stabilize the structure of RNA, DNA, and ribosomes!?
3) Growth rate - Associated with nucleic acid biosynthesis, amino acid utilization or protein synthesis, etc.

4) Skin & wound healing - Skin is rich in Zn, and deficiency can lead to parakeratosis, scaling/cracking, loss of hair & dermatitis.
5) Immune response - Essential to the integrity of the immune system.
6) Water & cation balance - Early signs of deficiency in most species are dehydrated appearance, elevated hematocrit & diarrhea.
7) Others - Development of sex organs, reproductive functions, bone and blood formation, metabolism of nucleic acids, proteins & carbohydrates, etc.

    - Zinc can affect production, storage, and secretion of hormones – Testosterone, insulin, corticosterone, etc.

D. Other roles of Zn?

1) Its relationship with vitamin A:

    a) Zinc 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. Bones? – Shorter & thicker, bowing of the hind legs (calves), stiffness of joints, etc.
B. Poultry - Delayed growth, unnatural feather formation, shorter & thicker long bones of the legs & wings, lower egg production & hatchability, etc.
C. Swine - Parakeratosis, reduced rate & efficiency of growth, reduced testicular development in boars, small litters, small pigs, etc.

- Parakeratosis (affect other species, including humans):

    a) Hyperkeratinization of skin mostly because of the failure f complete nuclear degeneration of epithelian tissues.
    b) Mostly around eyes, mouth, scrotum, and lower part of the leg.
    c) Skin is rich in Zn - 2 to 3 % of total Zn in skin and hair.
    d) Severe Zn deficiency can result in scaling, cracking, loss of hair, dermatitis, etc.

    - Why Zn? – Zinc has antioxidative effects, I's related to vitamin A via retinol binding protein, and it's involved in DNA synthesis, replication, hypertrophy, etc.

D. 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

1) Supplements - Oxide, carbonate, sulfate & chloride salts are highly available sources, but sulfide salt is a poor source.
2) With the use of Cu as a growth promoter (e.g., 250 ppm), an adequate Zn (≥ 100 ppm) is must because of their interaction!

4. Zn, Ca, and 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, non-viable newborn, etc.
C. Maximum tolerable levels - 300 ppm for sheep, 500 ppm for cattle & 1,000 ppm for swine & poultry.

- But, a supplementation with, e.g., 3,000 ppm Zn has been shown to improve growth performance of weanling pigs (& others) in recent years.

FLUORINE

1. General

A. Identified as a constant constituent of bons and teeth as early as 1805.
B. 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).

C. No one ha demonstra6ed clearly that F is an essential nutrient for animals.
D. But, small amounts are beneficial in:

1) Development of a dental caries-resistance - 1-2 ppm in water may have beneficial effects.

a) Addition of 0.7 to 1 ppm is a common practice in many municipalities.
b) Also, topical application – effective, tooth paste – somewhat effective, and F-tablet – depending on the level.
c) Why? – Not sure, but may reduce enzyme(s) and(or) cariogenic bacteria, and reduce solubility!?

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 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.

- F – Cumulative poison, i.e., gradually accumulates in bones and teeth, and then in soft tissues.

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:

- Bones? – Rough/irregular surface, disorganized, and poorly mineralized.
- Effects? – Can binds Ca and induce secondary hyperparathyroidism, reduce DNA and protein synthesis, reduce activity of certain enzymes, induce dysfunction of kidneys, etc.

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 and Animals

A. According to a relatively 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 (e.g., mono- & dicalcium phosphate) - 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. History: [See Maynard et al. (1979), McDowell (1989) & others]

1) "Rickets" - well known since the antiquity!

- Soranus Ephesius (born 130 A.D.) provided a classic description of this disorder - "the backbone bending & legs twisted at the thighs!"

2) Since the middle ages, people suspected that it might be associated with "sunlight" because it was more common in smoky cities than in the country.
3) 1822 - Sniadecki suggested that rickets was caused by lack of exposure to the sunlight.

a) ≈ 70 yr later, Palm reached the same conclusion based on the epidemiological survey, and encouraged/recommended a systematic sunbathing as a means of prevention & cure.
b) But, scientists & physicians at the time were very skeptical!

4) Sir Mellanby (1921-22):

a) Successfully produced rickets in puppies by raising them indoors on a high-cereal, low-fat diet.

b) Addition of cod-liver oil or butterfat prevented rickets.
c) But, concluded incorrectly that vitamin A was responsible for prevention of rickets.

5) McCollum (1922): (Responsible for discovering vitamin A)

a) Found that antirachitic activity in cod-liver oil was distinct from antixerophthalmic activity because aeration & heating destroyed vitamin A, but antirachitic activity remained!
b) Named this new substance "vitamin D."

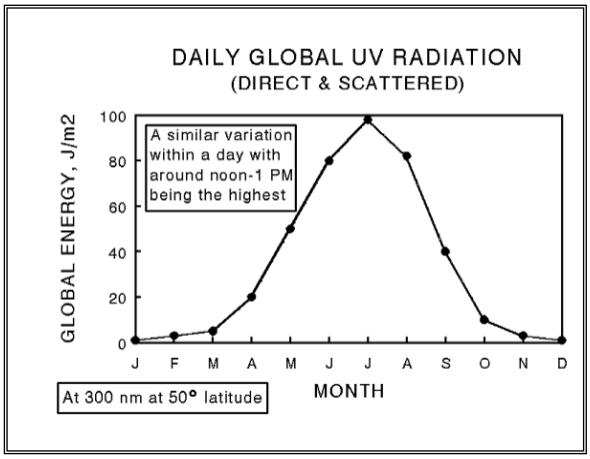
6) Goldblatt & Soames (1923), Steenbock & Black (1924) & Hess & Weinstock (1925):

a) Cured rickets by "irradiating" food before feeding to rats.
b) Irradiation of the skin produced a factor that was capable of preventing rickets.

B. General:

1) Referred to as a "sunshine vitamin:"

a) But, animals housed in the total confinement (swine & poultry) have little or no exposure to natural sunlight.
b) Even those on the outside lots may have problems during the winter.
c) "Ultraviolet radiation:" (Adapted & redrawn from Lawson, 1985)
d) "UV light" - varies considerably with the latitude, time of the year and time of the day!



2) Vitamin D:

a) A family of compounds that possess the antirachitic activity.
b) ≈ 10 provitamins have been identified, and there are variations in the antirachitic activity.
c) Two most prominent/important compounds:

(1) Cholecalciferol (vitamin D_3; in animals).
(2) Ergocalciferol (vitamin D_2; in plants).

d) The most potent vitamin D metabolite is 1,25-dihydroxy-D_3 (may be functioning as a hormone?).
e) Vitamin D activity - 1 IU (or USP) = 0.025 µg of vitamin D_3. [For poultry, use ICU (International Chick Unit) instead.]

3) General function of vitamin D - "To elevate plasma Ca & P for normal mineralization of the bones and other body functions."

C. Vitamin D and Ca & P:

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

2) Effects on the bone:

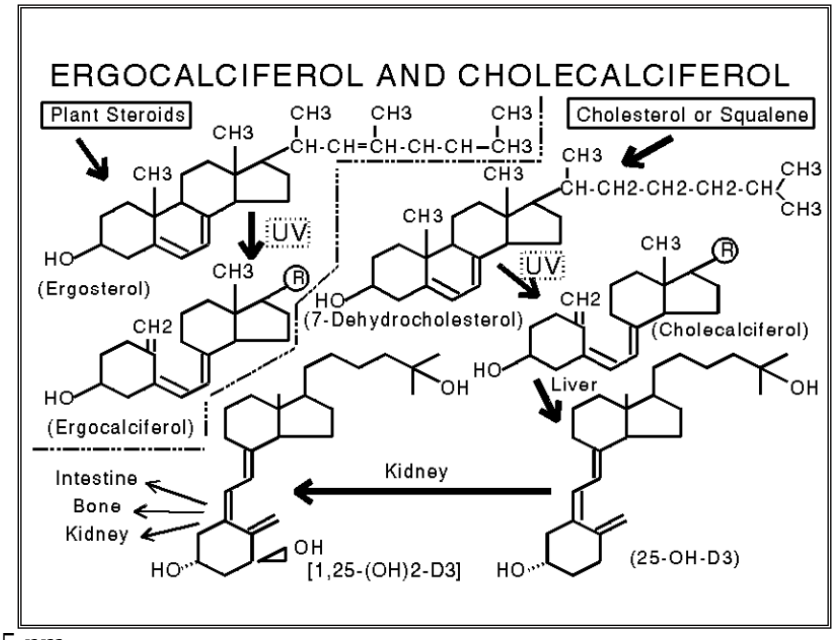
a) ↑ mobilization of Ca and P to the ECF, but little is known about the process.
b) 25-(OH)D (major metabolite in bones) & 24,25-$(OH)_2$D [found in a constant proportion with 25-(OH)D in bone] may have unique actions on the bone (e.g., promoting "normal" development of the bone?).

3) Effects on other organs/tissues - Poorly understood:

a) ↑ renal reabsorption of Ca & P - Less important for Ca since most of Ca is reabsorbed in absence of vitamin D, but quantitatively important for P.
b) May act on parathyroid glands to regulate PTH secretion.

2. Structure, Properties, and Assay

A. Structure: (Adapted & redrawn from McDowell, 1989)



B. Properties:

1) Occurs as colorless crystals.
2) Insoluble in water, but readily soluble in benzene, chloroform, ethanol, acetone & others.
3) Melting points = 84-85℃ (D3) & 121℃ (D2).
4) UV absorption (maximum) at 264-265 nm.

5) Can be destroyed by overexposure to the UV light & peroxidation.

C. Assay:

1) Chemical or physical means:

a) Generally, lack the sensitivity & somewhat difficult because of the existence of the No. of isomers.
b) Not all vitamin D compounds are biologically active.

2) Standard method for assay is a biological assay using rats or chicks:

a) Feed a rachitogenic diet until sever rickets develops (≈ 3-4 wk in rats & 21 d in chicks).
b) Then feed diets supplemented with a graded series of known amounts of standard vitamin D or unknown test substance.
c) After 7 d (rats) or 3 wk (chicks), sacrifice animals & examine bones.

3) Other biological methods include intestinal Ca absorption, bone Ca mobilization, growth rate, assay for CaBP, etc.

3. Metabolism

A. Absorption & transport:

1) Absorbed from the SI:

a) The most active site (faster rate of absorption) seems to be the duodenum.
b) But, the largest amount is absorbed at the ileum possibly because of retention time? (By passive diffusion.)

2) Only about 50% of dietary vitamin D is absorbed.
3) Absorbed in association with fats, i.e., requires the presence of bile salts (via chylomicrons):

a) Absorbed into the lymphatic system in the mammal.
b) Absorbed into the portal circulation in birds and fishes.

4) Transport:

a) Vitamin D & its metabolites are protein bound.
b) In most mammals & some other species, transported with vitamin D-binding protein (or transcalciferin).

- Vitamin D-binding protein has a high affinity for 25-(OH)D (a major circulating form of metabolites) compared to vitamin D or 1,25-(OH)2D3.

c) Other species - carried on albumin, lipoproteins and(or) α-globulin.
d) Avian sera contain two distinct proteins:

(1) Each has a different affinity for vitamin D & 25-(OH)D.
(2) Has a lower affinity for metabolites of D2 vs metabolites of D3.

◦ May account for the ability of birds to distinguish vitamin D2 & D3?

B. Conversion of vitamin D:

1) 25-(OH)D is a 1˚ circulating form of vitamin D:

a) Addition of a hydroxyl group to C-25 in the liver by the enzyme, vitamin D 25-hydroxylase.
b) Also in the kidney & intestine to some extent in the chick,.

2) 1,25-(OH)2D is the most active form of vitamin D:

a) Addition of a 2nd OH group to C-1 position in the kidney by the enzyme, 1-hydroxylase.
b) Transported to the intestine, bones, etc.
c) Activity of 1-hydroxylase is the 1˚ regulatory point in formation:

(1) An inverse relationship between 1,25-(OH)2D & 1-hydroxylase.
(2) The activity is influenced by Ca (& P?), PTH, vitamin D status, etc.

3) 24,25-(OH)2D - its exact function is unknown:

a) Addition of OH group to C-24 position in the kidney by the enzyme, 24-hydroxylase.
b) Involved in the suppression of PTH secretion & mineralization of bone?

- There are many other metabolites of vitamin D!

C. Catabolism & excretion:

1) The catabolic pathway(s) for 1,25-(OH)2D has not been well established!
2) Vitamin D & metabolites are excreted 1˚ in the feces in association with bile salts.

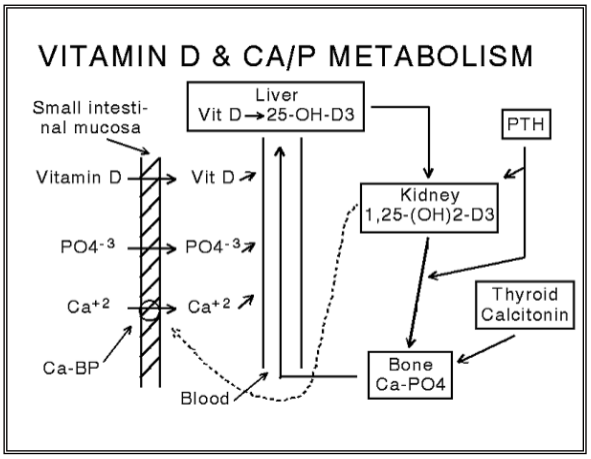
4. Functions

A. General functions?

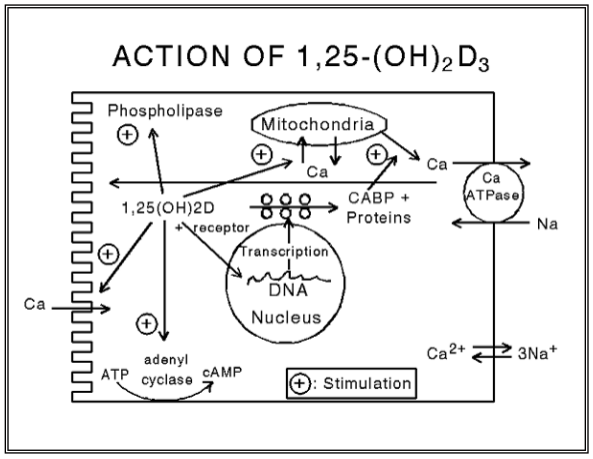
1) 1,25-$(OH)_2D_3$ stimulates the synthesis of Ca-binding proteins in the gut mucosa, which facilitate absorption of Ca, P & Mg.

2) Maintains the homeostasis of Ca/P along with PTH & calcitonin in the bone & kidneys.
3) Current view - general:

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

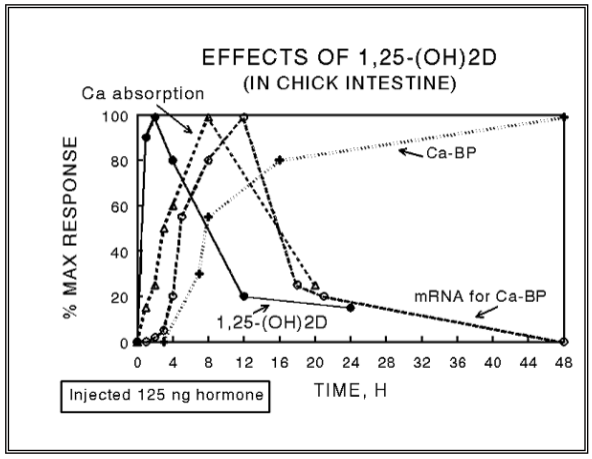


B. Vitamin D & Ca/P metabolism: (Adapted & redrawn from Peo, 1991. In: Miller et al., 1991)
C. Action of 1,25-(OH)2D3: (Adapted & redrawn from Lawson, 1985)



1) The mode of action of vitamin D seems to be "analogous" to the action of steroid hormones!
2) 1,25-(OH)2D3 is involved in transcription of genes for proteins at the intestine and bone.
3) Synthesis of calcium-binding protein and other proteins:

a) Two forms of vitamin D-dependent CaBP are known - MW of 27,000 & MW of 27,000 + smaller one with MW of 9,000.
b) Vitamin D stimulates the synthesis of Ca-binding proteins in the gut mucosa, which facilitates absorption of Ca, P & Mg.



- But, a precise role of CaBP in the transfer of Ca is unclear!

c) Effects of 1,25-$(OH)_2D$: (Adapted & redrawn from Lawson, 1985)

(1) Vitamin D may be inducing the synthesis of unknown proteins that are somehow involved in the transfer of Ca!?
(2) Vitamin D may alter membrane fluidity, ∴ ↑ Ca transport rate!?

- In the intestinal mucosa, also see alkaline phosphatase, Ca-stimulated ATPase, phytase enzyme & other activities, but their functions (if any?) have not been elucidated?

D. Effects on the bone:

1) ↑ mobilization/release of Ca and P to the ECF, but little is known about the biochemical process.
2) 25-(OH)D (a major metabolite in bone) & 24,25-(OH)2D [a constant proportion of 25-(OH)D in bone] may have unique actions on bones - e.g., promoting a normal development of bones?

E. Effects on other organs/tissues:

1) Poorly understood.
2) ↑ renal reabsorption of Ca & P:

a) Ca - less significant since most of Ca is reabsorbed in the absence of vitamin D.
b) P - quantitatively most important action of vitamin D on the kidney?

3) May act on the parathyroid gland to regulate PTH secretion.
4) Muscle weakness (associated with vitamin D deficiency) - vitamin D may have direct effect, i.e., "muscle weakness" may not be results of hypocalcemia or hyperparathyroidism.

5. Deficiency

A. Similar to the Ca-P deficiency in land animals.
B. Fish -Get relatively little UV light from the sun because of shallow depth of penetration of these rays in natural water, and dietary needs has been established for at least two species by feeding vitamin D deficient diets:

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

6. Vitamin D Requirements

A. Factors that influence requirements:

1) Sunlight.

2) The amount and ratio of dietary Ca and P.
3) Availability of Ca & P.
4) Species.
5) Physiological factors.

B. Requirements (1 IU = 0.025 μg of vitamin D_3): (NRC & RDA)

Animal	ICU/kg	IU/kg orothers
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
Beef(DM basis):		
All classes		275
Dairy (DM basis):		
Calf		600
Others		300
Sheep (per 100 kg BW):		
All classes		555
Humans (IU/d):		
≤ ½ yr		300
1-24 yr		400
> 25 yr		200
Pregnant/lactating		400

7. Sources and Supplementation

A. Sources: (Miller & Norman, 1984; McDowell, 1989)

Source	IU/100 g
Feedstuff (D2):	
Alfalfa, pasture	4.6
Alfalfa, sun cured	142
Corn	0
Corn silage	13
Molasses (sugar beet)	58
Red clover, fresh	4.7
Red clover, sun cured	192
Sorghum	2.6
Food (D3):	
Beef steak	13
Blue fin tuna liver oil	4,000,000

Cod liver oil	10,000
Eggs	100
Herring (canned)	330
Liver (raw):	
Beef	8-40
Pork	40
Milk (summer)	4
Milk (winter)	1

B. Supplementation:

1) Conditions/situations that may require dietary supplementation:

   a) When animals are kept indoors (swine, poultry, etc.).
   b) When animals are located in climates where sunlight may not be adequate.
   c) During the winter months? - housed outdoors only a part of the time, fewer sunny days, etc.

2) Natural feedstuffs are usually low or devoid of vitamin D, ∴ must be supplemented in many instances.
3) Vitamin D_3 may be 30 times more effective than D_2 in the poultry.
4) Recent data indicate that D_3 might be a preferred substrate (. . . perhaps, 2 to 3 times?) for pigs, ruminants & others.

   • But, D_2 is still an acceptable source for many species!

C. Commercial supplements:

1) Stabilized or protected by coating (e.g., gelatin, starch, sugars & antioxidant).
2) In a good storage condition & kept in the original, unopened container, can expect 90-100% retention of the vitamin activity up to 1 yr.
3) Stability can be ↓ by moisture, minerals, light, oxygen, heat, rancid fat & pelleting.
4) Vitamin D stability: [Coelho, 1991. Feed Management 42(10):24]

Condition	Retention, %
In vitamin-trace mineral 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

8. Toxicity

A. Pathogenesis of vitamin D toxicosis: (Adapted & redrawn from NRC, 1987)
B. Clinical signs of hypervitaminosis:

1) Cows - signs include anorexia, ↓ rumination, depression, premature ventricular systoles, bradycardia, etc.
2) Monkeys - signs include weight loss, anorexia, ↑ BUN, diarrhea, anemia, upper respiratory infections, etc.
3) Pigs - signs include anorexia, stiffness, lameness, arching of the back, polyuria, etc.

- "Postmortem examinations" - often see an extensive mineralization in cardiovascular systems & kidneys, and also in other areas such as the respiratory tract, salivary glands, GI tracts, etc.

C. Factors affecting toxicity:

1) Type & dose - D_3 may be more toxic than D_2.
2) A functional state of the kidney.
3) A route of administration.
4) Ca & P - the toxicity can be ↑ by high dietary Ca & P, etc.

D. Safe levels:

1) Effects of vitamin D on nursery-age pigs[a]: (Peo, 1991. In: Miller et al., 1992)

	Vitamin D_3, IU/kg						
Item	550	5,500	11,000	22,000	44,000	88,000	220,000
Gain, g/d	420	416	408	413	375	390	30
Feed, g/d	801	792	763	771	741	766	633
Feed:gain	1.91	1.92	1.89	1.89	1.97	1.94	10.36
Serum:							
Ca, mg/dL	11.7	11.2	11.8	11.6	12.0	12.0	16.7
P, mg/dL	9.8	10.3	10.1	9.9	9.8	9.8	7.8
AKP, IU/L	117.7	128.3	112.0	117.2	105.1	96.2	82.4

[a]"440,000 IU/kg" - five of eight pigs died during the first 7 d on the test.

2) 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. General

A. The role of vitamin A in the bone metabolism? - Involved in normal development of bone via a control of the activity of osteoblasts & osteoclasts!
B. Some review papers on vitamin A:

1) Blomhoff, R., M. H. Green, and K. R. Norum. 1992. Vitamin A: Physiological and biochemical processing. Annu. Rev. Nutr. 12:37-57.
2) Byers, T., and G. Perry. 1992. Dietary carotenes, vitamin C, and vitamin E as protective antioxidants in human cancers. Annu. Rev. Nutr. 12:139-159.
3) Hill, D. L., and C. J. Grubbs. 1992. Retinoids and cancer prevention. Annu. Rev. Nutr. 12:161-181.
4) Petrovich, M. 1992. Regulation of gene expression by vitamin A: The role of nuclear retinoic acid receptors. Annu. Rev. Nutr. 12:443-71.
5) Chew, B. P. 1993. Effects of supplemental β-carotene and vitamin A on reproduction in swine. J. Anim. Sci. 71:247-252.
6) Ross, A. C. 1993. Overview of retinoid metabolism. J. Nutr. 123:346-350.

2. Introduction

A. History: [See Maynard et al. (1979), McDowell (1989) & others]

1) "Night blindness" - probably the first nutritional deficiency disease to be recognized.

a) Ancient Egyptians recommended a topical application of juice squeezed from cooked liver to cure night blindness - "written records" since 1,500 B.C., but probably this practice started much earlier!
b) An early reference to vitamin A deficiency in livestock is the Bible (Jeremiah 14:6):

"*. . . and the asses did stand in high places, their eyes did fail, because there was no grass!"*

2) Early 20th century - "modern" science on vitamin A began:

a) Hopkins (1906-1912; UK) found a growth-stimulating principle in "alcoholic extract" of milk (not from "ash" portion).
b) Stepp (≈ same time; Germany) identified one of those minimal qualitative factors as a lipid.
c) McCollum & Davis (shortly thereafter; WI):

(1) Found that butter or egg yolk contained a lipid-soluble factor necessary for the growth of rats.

(2) Coined the term "fat soluble A" in 1915.

3) Colorless extracts of liver & colored plant lipids both showed biological activity:

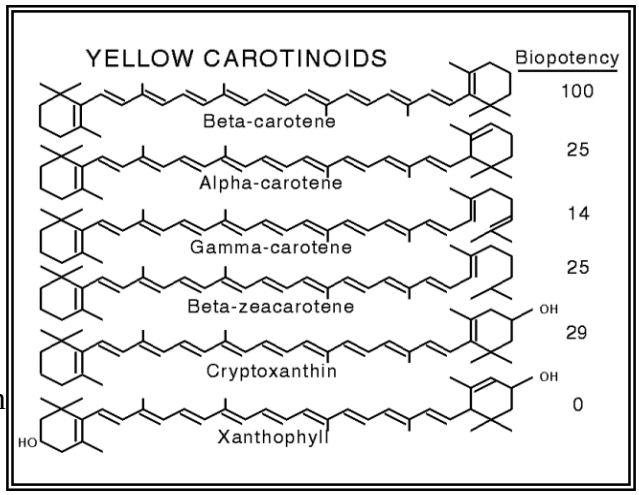
a) Steenbock (1919; WI) postulated "interconversion" of the two forms.
b) Moore (≈ a decade later; UK) demonstrated a conversion of the pigment, β-carotene, to vitamin A in the liver.

4) Karrer et al. (1930; Switzerland) determined the structure of both β-carotene & vitamin A, and synthesized some derivatives later.

B. General:

1) Necessary for a support of growth, health and life of higher animals.
2) From a practical standpoint, probably the most important vitamin because essentially all animals require dietary supplements!
3) The term "vitamin A" is used to describe all derivatives that contain the same ring (β-ionone) & show biological activity of "retinol."

- Excludes provitamin A, carotinoids, but they are included in a practice!



4) "Vitamin A" does not occur in plants as such:

a) But, occurs in plants as carotenoid pigments (provitamin A):

(1) Over 80 carotinoids exist with 15 having some degrees of activity, and according to some, over 400 carotenoids with about 50 having the biological activity.
(2) The most important one is "β-carotene" because of its vitamin A activity, and there are variations among species in their ability to convert β-carotene to vitamin A - e.g., may be only 25-30% in pigs!
(3) Yellow carotinoids: (Adapted & redrawn from McDowell, 1989)

(a) Found mainly in green leaves & to a lesser extent in corn.
(b) Among many carotenes, α-carotene, β-carotene, γ-carotene & cryptoxanthin (1˚ carotinoid of corn) are of particular importance because of their provitamin A activity.

5) The liver can store a large amount of vitamin A, which account for ≈ 90% of total body vitamin.

   - e.g., in one experiment, mature sows completed three normal pregnancies without vitamin A supplementation, and did not develop deficiency signs until their 4th pregnancy!

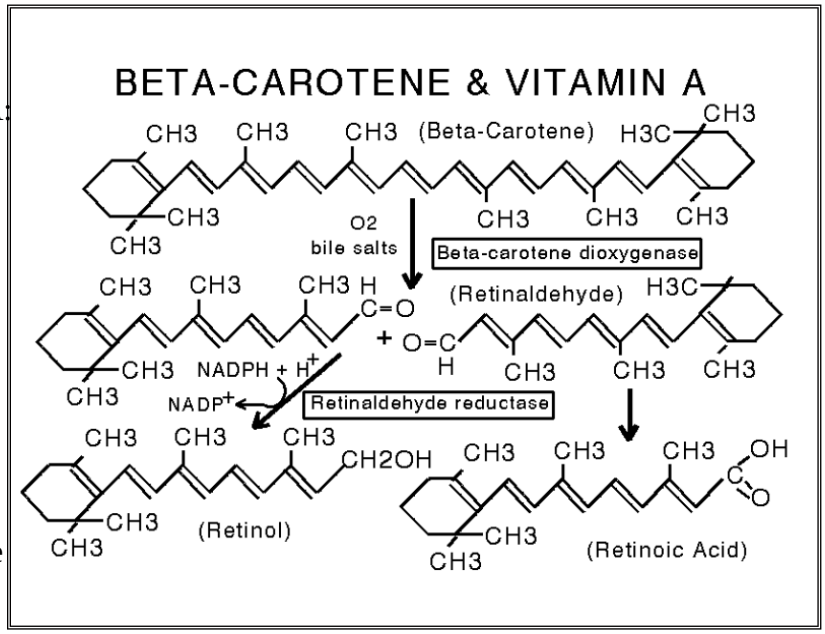
6) Vitamin A activity:

   a) 1 IU = .30 µg of vitamin A alcohol (retinol) or .55 µg of vitamin A palmitate.
   b) 1 IU of provitamin activity = .6 µg of β-carotene.
   c) May be expressed as a retinol equivalent (RE):

      (1) 1 RE = 1 µg of retinol, or 6 µg of β-carotene, or 12 µg of other provitamin A carotinoids.
      (2) 1 RE = 3.33 IU of retinol or 10 IU of β-carotene.

3. Structures & Properties

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



1) Retinol (alcohol) - probably serving as a sterol hormone?
2) Retinal (aldehyde) - a precursor of the visual pigment, "rhodopsin."
3) Retinoic acid - can maintain normal growth & general state of health, but:

   a) Not a visual pigment precursor.
   b) Can't support normal functions of the reproductive system.
   c) But, the recent data indicate that it might be involved in the production of steroid hormones, so ...?

4) Isomers of vitamin A:

   a) Vitamin A2 (3,4-dehydroretinol):

      (1) Isolated from freshwater fish.
      (2) Contains an additional double bond in the β-ionone ring.

(3) Liver oils of marine fish usually average less than 10% vitamin A2 of the total vitamin A content, and the relative biological activity is 40 to 50% that of vitamin A or A1.

b) More common isomeric forms of vitamin A are all-trans, 13-cis, 11-cis, 9-cis, 11,13-di-cis & 9,13-di-cis.

(1) Considerable variations in the biological activity among these isomers.
(2) All-trans form is the most active form, and usually found in mammalian tissues.

B. Properties:

1) A nearly colorless, fat-soluble, long-chained, unsaturated alcohol with five double bonds.
2) Made up of isoprene units with alternate double bonds, starting with one in the β-ionone ring.
3) Moisture, heat, light others cause structural changes & changes in the activity.

C. Assay methods:

1) Physicochemical - color reactions with antimony trichloride (Carr-Price reaction), gas chromatography, thin-layer chromatography, spectrophotometric procedures & HPLC method.
2) Bioassay:

a) Integrates all aspects of the vitamin, i.e., the value of provitamins, a mixture of provitamins and(or) vitamin, and the metabolism.
b) e.g., growth response tests with rats or chicks, liver storage assays in rats and chicks, quantitative evaluations of cell changes in the vaginal smear technique using rats, and organ & cell culture systems.

4. Metabolism

A. Digestion:

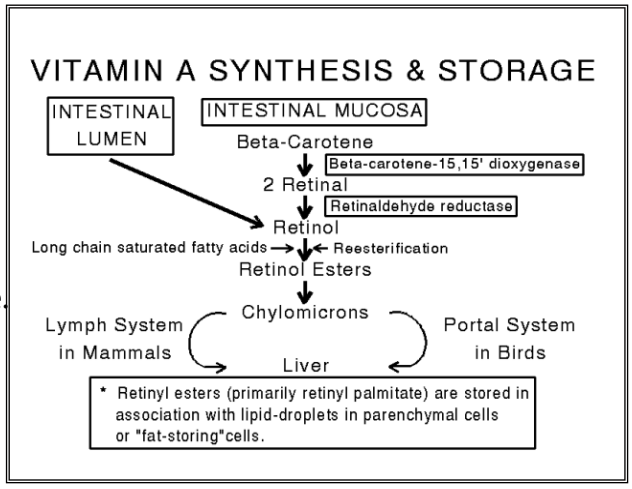
1) Free carotenoids and retinyl esters tend to congregate in fatty globules in the stomach, and then enter the duodenum.
2) "Mixed" micelles diffuse into a glycoprotein layer surrounding microvilli, and make a contact with the cell membrane.
3) Rapidly absorbed into mucosal cells (mainly at the upper half of the intestine).
4) Factors that influence carotene & vitamin A digestibility include forage type (hay, silage, etc.), harvest season, species of the plant & dry matter content.

B. Absorption/transport:

1) A conversion of β-carotene to vitamin A takes place mostly in the intestinal mucosa by two enzymes:

   a) β-carotene dioxygenase catalyzes "cleavage" to yield two molecules of retinaldehyde.

      (1) Many biological tests have shown consistently that pure vitamin has twice the potency of β-carotene on a weight-to-weight basis, indicating that only one molecule of vitamin A is formed from one molecule of β-carotene.
      (2) This enzyme is not present in the cat or mink, thus, those species cannot utilize carotene.



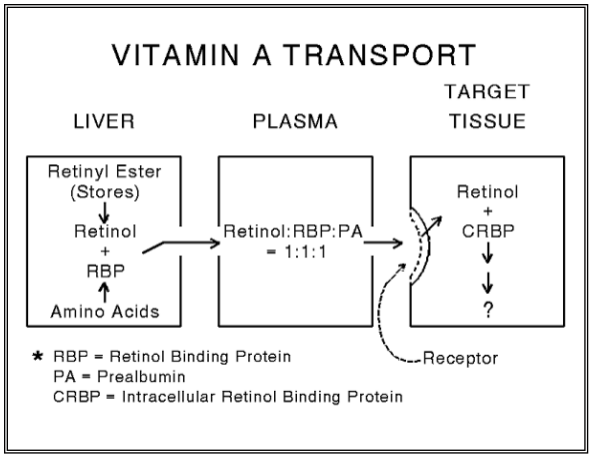
   b) Retinaldehyde reductase reduces retinaldehyde to retinol.

2) Humans, cattle, horses & carp can absorb significant amounts of carotene - Can store in the liver and fatty tissues, thus, having yellow body & milk fat.

   - Variations in the carotene absorption - e.g., Holstein (a more efficient converter of carotene to vitamin A) vs. Guernsey and Jersey.

3) Factors affecting absorption/transport:

   a) Cis/trans forms - trans-forms are absorbed more efficiently.
   b) Dietary lipids enhance absorption by serving as a vehicle & also by stimulating the flow of bile.
   c) Dietary protein - protein deficiency ↓ vitamin absorption from the intestine - serving as surface-active agents?



4) ≈ 75% of retinol is esterified in the mucosa with fatty acids (primarily palmitate) and incorporated into chylomicra, and then transported into the liver:

   a) Via the portal blood system in the bird.
   b) Via the lymph system in mammals.
   c) Two vitamin A binding proteins:

(1) Cellular retinol-binding protein (CRBP).
(2) Cellular retinoic acid-binding protein (CRABP).

- e.g., in rats, CRBP are found in all organs/tissues except heart, skeletal muscle & serum, and CRABP are detected only in eye, brain, testes, ovary and uterus.

5) Under normal conditions:

a) Over 90% of the vitamin is stored in the liver & the remainder in the kidneys, lungs, adrenal and others.
b) The liver can store enough to protect the animal from a long period of dietary scarcity.

C. Excretion:

1) ≈ 20% is unabsorbed & excreted in feces within 1-2 d.
2) ≈ 20 to 50% of absorbed A is conjugated or oxidized, and excreted in the feces or urine within ≈ 1 wk.
3) Stored vitamin A - metabolized or degraded, and conjugated/oxidized forms are eventually excreted:

a) Derivatives with the intact carbon chain are excreted in the feces?
b) Acidic chain-shortened products are excreted in the urine?
c) The ratio of excretion in the feces & urine is ≈ 2:1.

5. Deficiency/Functions

- Necessary for the support of growth, health and life of higher animals.
- The metabolic functions of vitamin A are still not completely understood!

A. Deficiency signs:

1) General - loss of appetite, retardation of growth, etc.
2) Nervous system - incoordination, nerve degeneration, etc.
3) Reproduction - a cessation of spermatogenesis, abnormal estrus cycle, fetal malformation & resorption, etc.
4) Eyes - night blindness, xerophthalmia, blindness, etc.
5) Bone - defective modeling, restriction of brain cavity & spinal cord, etc.
6) Others - drying & keratinization, cysts on endocrine & other glands, lactation disorders, increased infections, etc.

- At least four different, physiologically distinct deficiency problems exist: bones, vision, reproduction, and epithelial tissues.

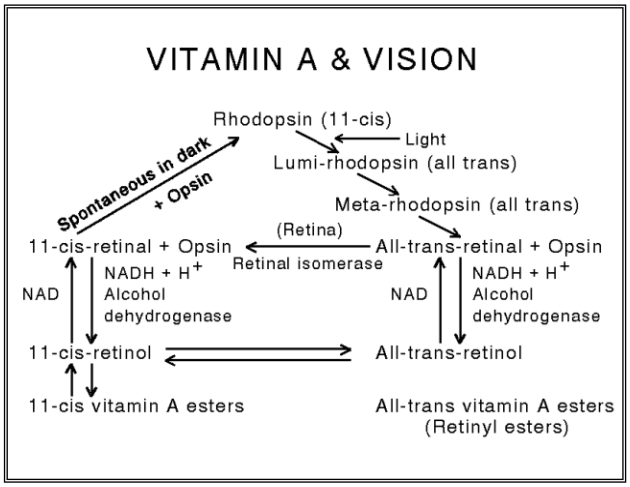
B. Abnormal bone growth:

1) Deficiency can result in a disorganized bone growth & irritation of joints.
2) Vitamin A affects the activity of osteoblasts, which are found in the site of active bone formation, and osteoclasts, which are found in the bone resorption site.
3) Involved in the synthesis of mucopolysaccharides (ground substances), which are important constituents of the cartilage & bone.
4) Changes/abnormality in the bone may be responsible for muscle incoordination & other nervous system related symptoms seen in vitamin A-deficient animals.

C. Impairment in vision:

1) Impaired vision is due to a failure of rhodopsin formation:

   a) 11-cis-retinal (aldehyde form of vitamin A) combines with opsin to form rhodopsin (visual purple).
   b) Need vitamin A for resynthesis of rhodopsin - lose some in the reaction process in the retina & must be replaced by vitamin A from the blood.



2) Deficiency:

   a) If blood level is too low, can result in a "functional" night blindness (slow, dark adaptation) first.
   b) A continuous deficiency can result in the total blindness.
   c) Xerophthalmia:

      (1) Advanced stage of the deficiency seen in all species.
      (2) Characterized by dry condition of cornea & conjunctiva, cloudiness & ulceration.

3) Vitamin A & vision: (Adapted & redrawn from McDowell, 1989)

D. Abnormal epithelial cells:

1) Epithelial cells/mucous membranes (e.g., respiratory tracts, GI tracts, urogenital tracts, eyes, etc.) can form a "protective lining" against environmental factors.
2) Vitamin A may be involved in the differentiation of cells?

   a) "Retinol-CRBP" binds to nuclear receptor sites & retinol binds to chromatin, which may alter the expression of genetic information?
   b) May be acting as cell surface receptors or as regulators of cellular differentiation?

3) Vitamin A & synthesis of cell surface glycoproteins - "retinyl phosphate" may be involved in the transfer of sugars to specific glycoproteins.

- Mucopolysaccharides can be found in all tissues, but mostly in mucus-secreting epithelia & bones/cartilage.

4) Stabilization of membranes - vitamin A can penetrate lipoprotein membranes, ∴ acting as a cross-linkage agent between lipid & protein in the membrane?

- The vitamin deficiency can result in damages to mucus-secreting cells of epithelium:

(1) Keratinization of epithelial tissues.
(2) Interferes with normal absorption, secretion/elimination, etc.
(3) Consequences would be ↑ infections, diarrhea, kidney & bladder stones and others adverse conditions.

E. Reproductive problems:

- e.g., a failure of spermatogenesis, resorption of fetus, abortion, retained placenta, reduced hatchability, etc.

1) The role of vitamin A is not well known:

a) Possibly, the result of a failure to maintain normal epithelium?
b) "Direct" effects - e.g., involved in cell differentiation & proliferation & ranscription of specific genes?
c) "Indirect" effects - e.g., involvement in the production of steroid hormones & roles in the immune function?

2) β-Carotene:

a) Believed for a long time that it was only a precursor of vitamin A.
b) But, may have roles in the reproduction that are independent of vitamin A?

- A more intense estrus & ↑ conception rate in ruminants, ↑ litter size (by ↓ embryonic mortality), heavier birth & weaning weights in swine have been reported!

c) Also, may be effective as an inhibitor of lung cancer, stomach cancer, prostate cancer & others in humans. (Bosco, 1989)

- Beneficial effects might be related to its antioxidative effects against lipid peroxidation (β-carotene is a very powerful antioxidant!).

F. 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.

6. Vitamin A Requirements

A. 1 IU = 0.30 μg of retinol or 0.55 μg of vitamin A palmitate.
B. Requirements: (NRC & RDA)

Animal	IU/kg or others
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
Cat (gestation)	6,000
Dog (growing)	3,336
Human (RDA):	
Infants (up to 1 yr)	375 µg/d
Children (1-10 yr)	400-700 µg/d
Male	1,000 µg/d
Female	800 µg/d
Lactating	1,200-1,300 µg/d
Dairy:	
Growing	2,200
Lactating cows & bulls	3,200
Calf milk replacer	3,800
Sheep:	
Replacement ewes (60 kg)	1,567
Pregnancy (70 kg)	3,306
Lactation (70 kg)	2,380
Replacement rams (80-100 kg)	1,976
Beef cattle:	
Feedlot cattle	2,200
Pregnant heifers & cows	2,800
Lactating cows & breeding bulls	3,900

C. Factors that can influence requirements:

1) Genetics - species, breed, strain.
2) Carryover effects of stored vitamin (especially, in the liver).
3) The efficiency of converting carotene to vitamin A:

"Conversion of β-carotene to vitamin A:" (McDowell, 1989)

Animal	1 mg β-carotene to IU of vitamin A
Standard	1,667
Cattle	400
Sheep	400-450
Swine	500
Horse	333-555
Poultry	1,667
Dog	833
Rat	1,667
Human	556
Cat & mink	0

4) Variations in the vitamin activity due to the type, level & isomerization of precursors.
5) Stability of the vitamin due to oxidation, length of storage, temperatures, pelleting, etc.
6) Health status of animals due to diseases and(or) parasites.
7) Dietary factors such as levels of fat, protein, minerals, antioxidants, etc.

7. Source and Supplementation

A. Sources - "Retinol & β-carotene content:" (McDowell, 1989)

Source	IU/g (Vitamin A) or mg/kg (β-carotene)
Vitamin A source:	
Whale liver oil	400,000
Swordfish liver oil	250,000
Tuna liver oil	150,000
Butter	35
Cheese	14
Eggs	10
Milk	1.5
Carotene source:	
Fresh legumes & grasses	33-88
Dehydrated alfalfa meal (no field curing; bright green color)	242-297
Dehydrated alfalfa (after storage)	120-176
Legume hay (minimum sun exposure)	77-88
Non-legume hay (good green color)	20-31
Corn & sorghum silage (wet basis)	4-22
Grains, protein concentrates, etc.	0.02-0.44

B. Factors that can affect vitamin A stability:

1) Pure vitamin A - probably the least stable vitamin among those commonly used for supplementation.
2) Commercial dry products - protected by esterification (usually with palmitate) & coating (e.g., gelatin, starch, sugars and antioxidants).
3) Stability can be ↓ by moisture, light, oxygen, high temperatures & humidity, long storage, pelleting, extrusion, presence of trace minerals & rancid fat, etc.

a) The stability of vitamin A in feeds is somewhat unpredictable because many factors are involved!
b) Because of this uncertainty, diets are usually fortified with several-fold higher levels to compensate for a possible loss during the storage.

8. Hypervitaminosis

A. General:

1) "Massive" doses - reactions include a general malaise, anorexia, nausea, hyperirritability, peeling of the skin, muscular weakness, twitching, convulsions, paralysis and death.
2) "Chronic" toxicity:

a) Generally, caused by intakes of 1,000 times requirements for a prolonged period, but may be observed at intakes of 10 times of the requirements.
b) Signs include skeletal malformation, spontaneous fractures & internal hemorrhage, loss of appetite, slow growth, loss of weight, skin thickening & increased blood clotting time.

3) The storage capacity of the liver offers some protection, but can be overwhelmed, or can result in malfunctions in the metabolism:

a) Not uncommon to see 500-1,000 IU/g liver in most species, but 13,000-18,000 IU/g is common in fish livers & 20,000 IU/g has been observed in human livers (NRC, 1987).
b) Blood vitamin A:

1) Contains between 20 & 100 µg vitamin A/100 ml.
2) Upon depletion of liver stores, can be down to 5 to 20 µg/100 ml.
3) Persistence of > 100 µg/100 ml may indicate a vitamin A "toxicity."

B. Upper safe levels: (NRC, 1987; McDowell, 1989)

1) Presumed upper safe levels:

a) 4-10 times the requirement for nonruminant species.
b) 30 times the requirement for ruminant species.
c) Adverse effects are generally produced by feeding over 100 times the requirement!

2) 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. General

A. Vitamin C in bone metabolism?

- "Involved in the biosynthesis of collagen, which ia a principal component of the organic substances of bones and teeth and many others!"

B Some references on vitamin C:

- Wegger, I., F. J. Tagwerker & J. Moustgaard (Ed.). 1984. Proc. Ascorbic Acid in Domestic Animals. The Royal Danish Agric. Soc., Copenhagen.
- Wenk, C., R. Fenster, and L. Völker (Ed.). 1992. Proc. 2nd Symp. Ascorbic Acid in Domestic Animals. Institut für Nutztierwissenschaften & F. Hoffmann-La Roche Ltd., Switzerland.

2. Introduction

A. General: [See Mcdowell (1989) & others]

1) "Scurvy" is a disorder associated with inadequate vitamin C intake:

a) Scurvy has been known since the ancient times. Written records - as early as 1,550 B.C.!
b) Potentially fatal disease, and signs include:

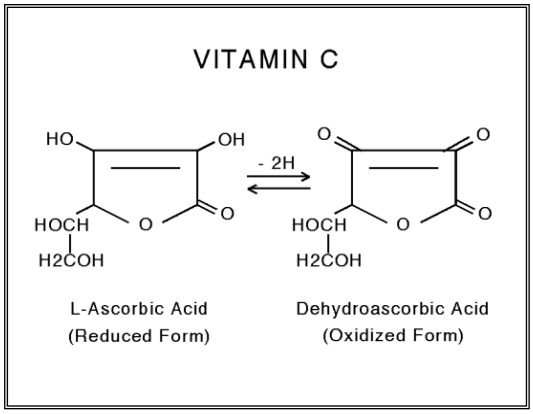
(1) Anemia, weakening of collagenous structures (bone, cartilage, teeth, connective tissues).
(2) Swollen, bleeding gums with loss of teeth.
(3) Hemorrhages in various tissues.

(4) Delayed healing of wounds.
(5) Fatigue & lethargy.
(6) Degeneration of muscles & skin lesions ..., etc.

2) Most species can synthesize vitamin C in adequate amounts, except primates, guinea pigs, fishes, fruit-eating bats, insects, some birds & certain fish.
3) Suggested beneficial effects of megadoses (2.3 g to 9-10 g/day) of vitamin C for humans include: (Pauling, 1971. Vitamin C and the Common Cold. Freeman, San Francisco; Pauling, 1974. Am. J. Psychiatr. 131:1251; Jaffe, 1984. In: Machlin, 1984)

a) Prevention & reduction of severity of a common cold.
b) Prevention of cancer & prolong life of cancer patients.
c) Lower serum cholesterol & severity of atherosclerosis.
d) Increase wound repair & normal healing processes.
e) Increase immune response for prevention and treatment of infections.
f) Control of schizophrenia.
g) Inactivation of disease viruses.

3. Structure, Properties & Assay



A. Structure (McDowell, 1989):
B. Vitamin C can occur in two forms, a reduced (majority exists in this from) & oxidized, and both are biologically active.

- Activity - 1 IU = .05 mg of L-ascorbic acid (equivalent to ≈ 1 ml of lemon juice).

C. Stability:

1) Very susceptible to destruction via oxidation (accelerated by heat & light).
2) More stable in the acid medium vs alkaline medium.
3) A cooking loss of the vitamin is considerable.

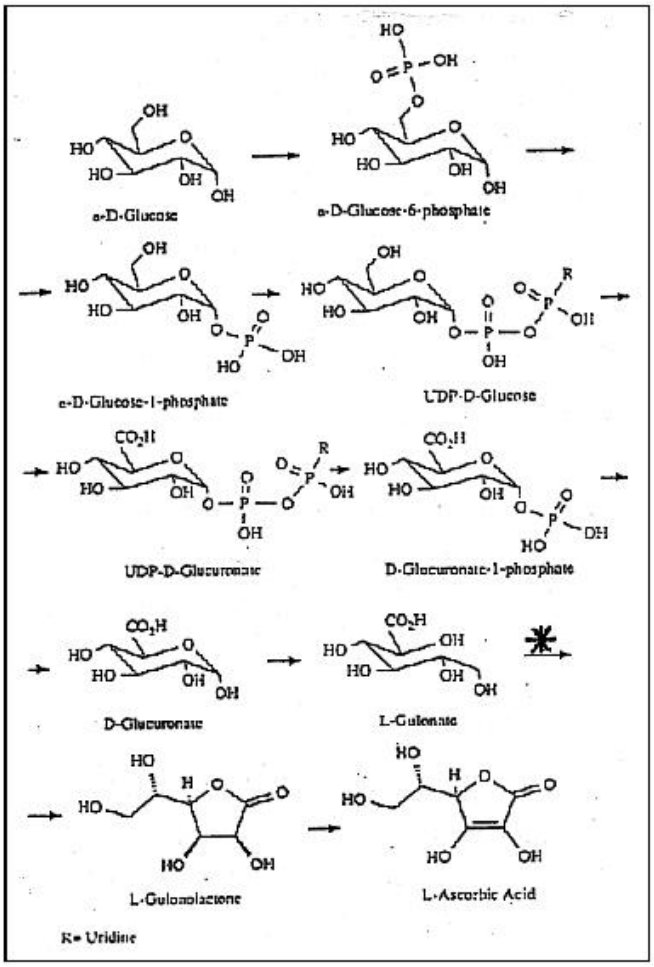
D. A reversible "reduction-oxidation" reaction - probably the most important property & probably the basis for known physiological activities of this vitamin.

E. Analytical procedures - chemical & physical methods are precise, faster & less expensive vs biological methods.

- e.g., Vanderslice, J. T., and D. J. Higgs. 1993. Qualitative determination of ascorbic acid, dehydroascorbic, isoascorbic, and dehydroascorbic acids by HPLC in foods and other matrices. J. Nutr. Biochem. 4(4):184.

4. Metabolism

A. Biosynthesis:



1) Produced by the glucuronic acid pathway in the liver of mammals & in the kidneys of other vertebrates:

   - D-glucose → D-glucuronic acid → L-gulonic acid → L-gulono-γ-lactone → L-ascorbate.

2) Some species, again, primates, guinea pigs, fishes, fruit-eating bats, insects, some birds & certain fish, etc.:

   a) Cannot synthesize the vitamin because they lack "L-gulono-γ-lactone oxidase," which is responsible for conversion of L-gulono-γ-lactone to 2 -oxo-L-gulono-γ-lactone.
   b) "2-oxo-L-gulono-γ- lactone" is transformed spontaneously into the vitamin.

B. Metabolism:

1) Absorption:

   a) The mechanism is very similar to monosaccharides.
   b) Vitamin C-dependent animals use the Na-dependent, active transport system?
   c) Usually, efficiently absorbed by animals, but a limited intestinal absorption with excess intakes.
   d) Absorbed mostly in a duodenal portion in guinea pigs, but the highest absorption rate at the ileum in rats.

2) Distribution:

   a) Widely distributed throughout tissues/organs.
   b) Highest in the pituitary & adrenal glands, and fairly high in the liver, spleen, brain & pancreas in experimental animals.
   c) Tends to localize around "healing-wounds."

3) Excretion:

a) A half-life is inversely related to intake, and it is usually 13-40 d in humans & 3 d in guinea pigs.
b) Generally, excreted in the urine, sweat (probably low) and feces (also low & ≈ 6-10 mg/d in humans).
c) The urine contains numerous metabolites of vitamin C (e.g., dehydroascorbic acid, diketogulonic acid, ascorbate-2-sulfate, oxalate, methyl ascorbate & 2-ketoascorbitol).
d) In guinea pigs, rabbit & rats, CO2 is the 1° excretory mechanism.
e) In primates, mostly excreted in the urine. (Normally, primates do not utilize the CO2 catabolic pathway.)
f) In humans, urinary oxalate is the major metabolite (30-50 mg/d) when consuming 60-100 mg/d, but the vitamin is excreted in the urine & feces (mostly un-metabolized vitamin) with higher intakes (e.g., up to 10 g/d).

5. Functions

- The exact role in the living system is not clearly known & the coenzyme form has not been identified/reported.
- All functions of vitamin C are probably related to its reversible oxidation and reduction characteristics.

A. Collagen biosynthesis:

1) General

a) The most clearly established role of vitamin C.
b) A failure of wounds to heal & changes in the gum and bone may be directly related to reduction of insoluble collagen fibers.

2) Vitamin C is involved in hydroxylation of Pro and Lys:

a) OH-Pro - provides a greater stability to extracellular protein matrix by H-bonding (stabilization of the alpha helix).
b) OH-Lys - involved in glycosylation and participate in intra- & intermolecular cross-links.

- Thus, both OH-Pro & OH-Lys are integral constituents in the formation and stabilization of collagen fibrils and resulting collagen fibers.

c) Probably, protecting hydroxylase enzymes (lysyl & prolyl hydroxylases) and(or) acting as a specific physiological reducing agent in the enzyme system.
d) Or, stimulating the collagen biosynthesis in a manner unrelated to hydroxylation (Schwartz, 1984. In: Wegger et al., 1984).

B. Other functions:

1) Involved in the electron transfer in cells - NADH & cytochrome systems.
2) Involved in the biosynthesis of carnitine, dopamine, corticosteroids, and others via its effect on other hydroxylases.
3) Metabolic oxidation of amino acids - e.g., oxidation of excess Tyr.
4) Metabolism of ions - enhances the absorption/mobilization of minerals via its reducing and chelating properties. Example? - Reducing ferric Fe at acidic pH in the stomach & forming complexes with Fe ions, which stay in the solution at alkaline conditions.
5) Metabolism of B-vitamins - e.g., folic acid transformation, storage, etc.
6) Stimulation of the phagocytic activity of leukocytes, formation of antibodies, etc.
7) May alleviate their toxic effects of metal ions, e.g., Cd, Cr, Ni, Pb, and V.
8) Natural inhibitor of nitrosamines (carcinogens) - a direct reaction with nitrate.
9) Others include:

a) Metabolism of vitamin D (Weiser et al., 1992. In: Wenk et al., 1992) & Ca (Orban et al., 1993. Poult. Sci. 72:691).
b) Synthesis of catecholamine (Lewin, 1976. Vitamin C: Its Molecular Biology and Medical Potential. Academic Press, London) & a role of vitamin C in stress (Dvořak, 1984 & Thaxton and Pardue, 1984. In: Wegger et al., 1984).
c) Synthesis of steroid and polypeptide hormones (Hornig et al., 1984 & Thaxton and Pardue, 1984. In Wegger et al., 1984; Padh, 1990. Biochem. Cell Biol. 68:1166) and(or) semen quality (ARC, 1981; McDowell, 1989).
d) Immune functions (Cummins and Brunner, 1992 & Bendich, 1992. In: Wenk et al., 1992; Li et al., 1993. Aquaculture 117:303).

6. Deficiency

A. Deficiency:

1) Not likely to develop deficiency signs in ascorbate-synthesizing animals under practical situation.
2) The requirement for domestic animals have not been established by the NRC or others.
3) But, may develop deficiency symptoms (scurvy-like symptoms) under certain conditions because of "disturbances" in the biosynthesis:

a) Dietary deficiencies - energy, protein, vitamin E, Se, Fe, etc.
b) High performance/production - e.g., growth rate, milk production, etc.
c) Transportation, handling, stressful practices, new environment, etc.
d) High ambient temperatures or cold trauma.
e) Diseases, parasites, etc.

B. Deficiency in fish:

1) Highly sensitive to dietary deficiency, especially young fish.

2) Deficiency can reduce resistance to bacterial diseases.
2) Curvature of spinal column is a prominent, early sign of deficiency in finfishes.
3) Signs in some species:

   a) Rainbow & brook trout, coho salmon, tilapia, channel catfish & young carp - Scoliosis & lordosis (lateral & vertical curvature of spinal column, respectively).
   b) Channel catfish - Deformed spinal columns, external & internal hemorrhages, erosion of fins, depigmented vertical bands around midsection, distorted gill filament cartilage & ↓ rate of wound healing.
   c) Black death in shrimp - A condition characterized by melanized hemocytic lesions distributed throughout the collagenous tissues.

7. Vitamin C Supplementation

A. General - Has resulted in very inconsistent response (e.g., in swine):

1) Improved performance of pigs in some experiments and a rapid cessation of naval bleeding in one study by vitamin C supplementation of the sow diet (1 g/day), but observed no response in subsequent studies.
2) Probably, a routine vitamin fortification of swine diets is not necessary.
3) Beneficial in some situations? - e.g., Deficient in vitamin E, Se, protein, Fe, etc., highly productive, transporting a long distance & handling extensively, adjusting to a new environment, ambient temperatures, diseases, parasite infestations, etc.

B. Vitamin C & stressed-chicks: (Thaxton & Pardue, 1984. In: Wegger et al., 1984)

- Effects of heat stress & vitamin C:

Item	Vitamin C, ppm: 0	1,000
Mortality, %		
No heat	2.4	2.4
Heat	22.0	7.3
Plasma corticosteroids, ng/mL		
No heat	3.2	4.9
Heat	18.1	8.8

  ◦ Vitamin C ameliorated the immunosuppressive effects of both exogenous cortisol & high environmental temperatures.

C. Vitamin C for swine:

1) Effect of ascorbic acid on weanling pigs: (Yen & Pond, 1981. JAS 53:1292)

	Vitamin C, ppm					
Item	0	330	660	990	Stat	r
Plasma C, mg/dL	1.19	1.35	1.60	1.57	Ln	
Gain, g/d	238	279	281	278	Ln	.33*

Plasma Fe, µg/dL	150	126	122	136	Qd	-0.36*
Hb, g/dL	10.7	11.0	10.3	10.7	Cu	0.09
Hematocrit, %	33.8	34.9	33.4	33.9	NS	0.10

* Correlation coefficient with vitamin C, $P < 0$ .05.

2) Effect of ascorbic acid & carbadox (Carb) on weanling pigs (28-d study): (Yen & Pond, 1983. JAS 56:621)

	Diet					
Item	Basal	C	Carb	C + Carb	Stat	r
Plasma C, mg/dL	1.67	1.90	1.94	2.24	Both	
Gain, g/d	344	339	422	435	Carb	0.54**
Plasma Fe, µg/dL	150	152	171	185	Carb	-0.01
Ceruloplasmin, unit, mL	86	79	77	78	NS	0.00
Plasma Cu, µg/dL	192	186	203	195	NS	0.19

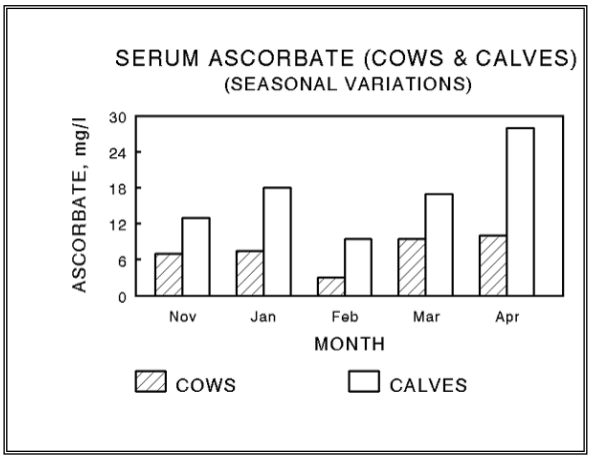
** Correlation coefficient with vitamin C, $P < 0.01$.

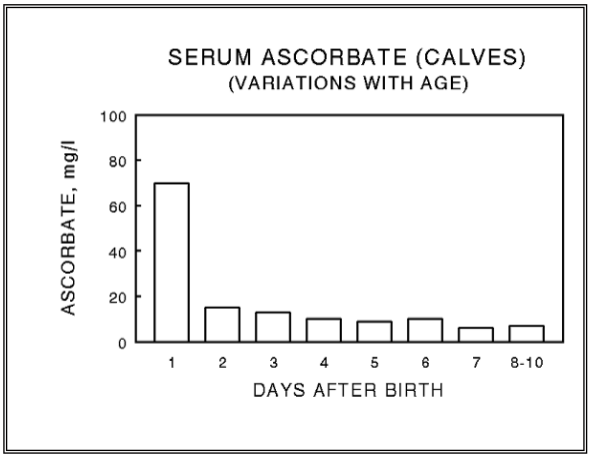
- The response to the vitamin has been very inconsistent, and a routine vitamin fortification of swine diets is probably not necessary!?

D. Vitamin C & ruminant species:

1) Vitamin C reserves in calves decline rapidly after birth, and may need supplementation until the synthetic rate becomes adequate!
2) A "recommendation" - a daily oral supplementation of 2.5 g vitamin C + parental application of 500 mg vitamin C in two doses immediately after moving animals to new facilities (McDowell, 1989).

Serum vitamin C: (Itze, 1984. In: Wegger et al., 1984)





8. 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.

9. 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 suggested 2.3 g/day for optimum health, and 9-10 g in presence of some ailments.

        • Personally, he took 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)
Vegetables:	
Asparagus, canned	15
Brussels sprout	90
Red cabbage	55
Cauliflower, raw	50-90
Celery, raw	7
Corn	12
Peppers, raw	100
Spinach	10-60
Fruits:	
Apple, unpeeled	10-30
Bananas	6-12
Grapefruit	35-45
Lemons	80
Limes	250
Oranges	40-60
Animal products:	
Fish	5-30
Kidney, lamb	9
Liver, calf	13
Milk, cow	1-7
Milk, human	3-6

10. Toxicity (McDowell, 1989; NRC, 1987)

A. General:

1) In general, a high intake of vitamin C is not toxic to humans - The toxicity is unlikely in humans because of a limited ability for intestinal absorption, and also an efficient renal elimination mechanism.

2) Nevertheless, a number of toxicity symptoms or signs in humans and laboratory animals have been reported:

- e.g., oxaluria, uricosuria, hypoglycemia, excessive absorption of Fe, diarrhea, allergic response, destruction of vitamin B_{12}, etc. (But, also there are a number of contradictory reports as well!)

3) Little information on any toxicity signs in domestic animals.

B. Upper safety levels?

1) Poultry - no adverse effect with up to 3,300 mg/kg (based on studies with > 60 d).
2) Swine & fish - as much as 10 g/kg had no adverse effect. (But, most studies with swine were very short, i.e., only 1 to 5 wk.)
3) Cats & dogs - 0.5 g/d for cats & 3 g/d for dogs had no adverse effects.