CIRCULATION AND VITAMIN AND MINERALS

VITAMIN K

1. Discovery of Vitamin K

A. The process of discovery & extensive study of "anti-hemorrhagic" nutrient may represent the best illustration of the formula for a "success" in the field of nutrition!
B. Steps involved:

1) Observations:

a) Experimental diets & signs of "malnutrition" in the animal?
b) Accurate description of main features of pathological changes!

2) Testing of different classes of natural foods for prevention or cure of lesions.
3) Preparation of crude extracts from a "protective" food, and testing those extracts.
4) Further investigation of "curative" extracts/concentrates:

a) Separation of extracts into several constituents.
b) Determination of the value of fractions for prevention/cure of lesions by feeding experiments.
c) Identification of the species of molecule responsible for prevention/cure.

5) Determination of distribution of the dietary factor in natural or derived foods by feeding experiments.
6) Synthesis of the "factor!"

2. Introduction

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

1) Henrik Dam (1929, Denmark):

a) Fed chickens a low-fat diet to determine their ability to synthesize cholesterol.
b) Chickens were anemic & developed subcutaneous & intermuscular hemorrhages.
- Hemorrhagic signs were also reported by others using diets containing "ether-extracted" fish meal (McFarlene et al., 1931).

2) Investigations by others found that:

a) The curative factor was a "fat soluble," and hemorrhagic signs can be prevented by feeding "unextracted" fish meal.
b) "Known" fat-soluble vitamins at that time (A, D & E) or other physiologically active compounds were not effective in prevention/cure.

3) Dam (1935):

   a) Proposed that the anti-hemorrhagic factor to be classified as a new fat-soluble vitamin.
   b) He called it "vitamin K" (from the word "Koagulation," which is Danish word for "coagulation").
   (• Received the Novel prize for his discovery.)

4) In 1935, Almquist & Stockstad:

   a) Independently reported that ether extracts of alfalfa cured the hemorrhagic condition.
   b) Pointed out that the microbial action in fish meal & bran preparation could lead to a development of anti-hemorrhagic activity.

   • Actually, they discovered vitamins K1 and K2 in 1928, but the submission for publication was delayed & then the paper was rejected!

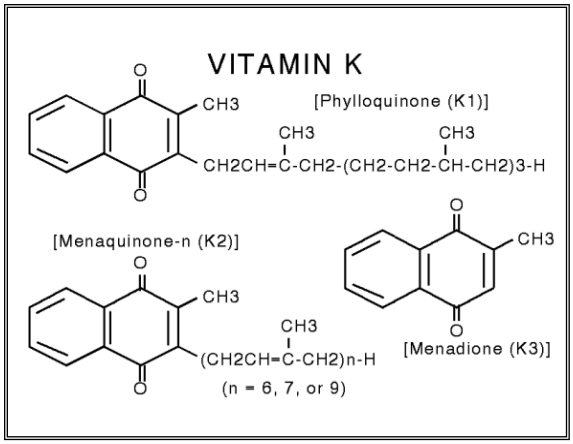
B. General:

1) The last fat-soluble vitamin to be discovered.
2) Unlike other fat-soluble vitamins, its function may be limited to a normal blood-clotting mechanism.

   • Vitamin K-dependent proteins have been discovered recently, thus, vitamin K may have other roles!

3) Previously referred to as the "coagulation vitamin," "anti-hemorrhagic vitamin" and "prothrombin factor."
4) Can be synthesized by intestinal microorganisms, but deficiency signs have been observed under field conditions.
5) Birds (and to a lesser degree, pigs) are susceptible to deficiency.

3. Structures, Properties, and Assay



A. Three forms of vitamin K: (Adapted & redrawn from Martin et al., 1983)

   1) Vitamin K - a generic term for a homologous group of vitamins consisting of 2-methyl-1,4-naphthoquinone derivatives, commonly called "menadione."
   2) K_1 = extracted from plants, K_2 = from materials that had undergone bacterial fermentation & K_3 = synthetic menadione.

B. Characteristics:

1) A viscous, golden yellow oil.
2) The vitamin in natural sources is fat-soluble & stable to heat, but labile to oxidation, alkali, strong acids, light and irradiation.
3) Some synthetic products are water soluble:

   a) Menadione sodium bisulfite (MSB).
   b) Menadione sodium bisulfite complex (MSBC):

      (1) With excess Na bisulfite, MSB crystallizes as a complex (additional mole of Na bisulfite).
      (2) Highly stable in diets & being used widely.

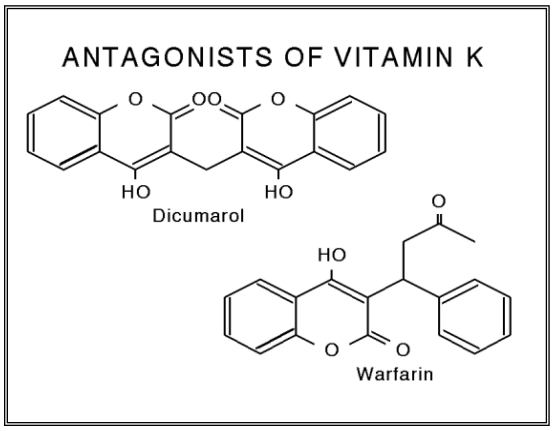
   c) Menadione pyridinol bisulfite (MPB) is formed by the addition of dimethyl-pyridinol.

   • All these have roughly equal biological activity on the molar basis.

C. Assay:

1) "Classic" bioassay - uses whole-blood clotting time of the chick raised on a vitamin K deficient diet.
2) Can be analyzed by a variety of color reactions or by direct spectroscopy.
3) ↑ interest in the use of HPLC (sensitive, specific & accurate) in recent years.

D. Vitamin K antagonists:



   • "Dicumarol & Warfarin:" (Adapted & redrawn from McDowell, 1989)

1) Dicumarol [3,3'-methyl-bis-(4-hydroxycoumarin)] - produced by mold & combines with a proenzyme to prevent formation of the active enzyme.

   • Being used as a clinical agent for "anticoagulant" therapy (e.g., to prevent intravascular blood clots).

2) Warfarin [3-(α-acetonylbenzyl)-4-hydroxycoumarin] - a synthetic form of dicumarol (being used successfully to ↓ K-dependent clotting factors for a long time).

- Also, being widely used as a rodenticide, but > 10% of a random population of rats are now resistant to Warfarin, ∴ there is a need for developing more active compounds!

3) Many others vitamin K antagonists exist - e.g., Bromodifenacoum, 2-phenyl-1,3-indandione, chloro-K, tetrachloropyridinol, etc.

4. Metabolism

A. Biosynthesis:

1) Animals are not cable of synthesizing 2-methyl-1,4-naphthoquinone ring needed for the vitamin K activity, thus unable to synthesize this vitamin.
2) The biosynthetic pathway is active in plants and many microorganisms.
3) Although a vitamin K deficiency is relatively rare in humans because of microbial biosynthesis, it is fairly common among newborns.

B. Absorption:

1) Absorbed in association with dietary fats, and requires bile salts & pancreatic juice for the formation of "mixed micelle."
2) Absorbed from the proximal portion of the SI into the lymphatic system.
3) The rate & efficiency may differ among various forms of vitamin K:

a) Mode:

(1) Phylloquinone by the active transport.
(2) Menaquinone by the passive noncarrier-mediated process.
(3) Menadione is a water soluble, thus can be absorbed satisfactory from low-fat diets.

b) The efficiency ranges from 10 to 70%:

(1) Menadione may be completely absorbed, but a retention rate may be very poor - menadione must be converted to the biologically active form to have its effect!
(2) The opposite might be true for phylloquinone, i.e., ≈ 50% absorption rate, but a relatively higher retention rate.

C. Tissue deposition/excretion:

- In terms of alleviating deficiency signs of vitamin K, the biosynthesis and efficiency of absorption might be more important than the body storage.

1) "Phylloquinone" is concentrated and retained in the liver, but it does not have a long retention time in the liver - A half-life of ≈ 17 h in rats.

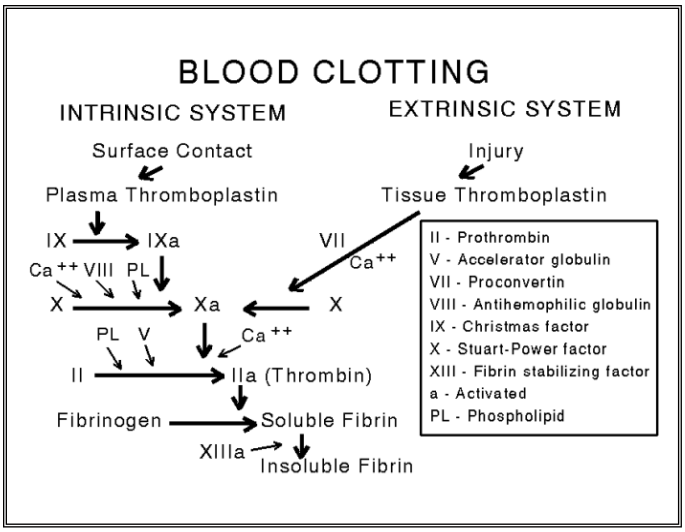
2) "Menadione" is widely distributed in all tissues, but it's rapidly metabolized & excreted. (Three different urinary conjugates, phosphate, sulfate & glucuronide, have been identified.)

   - Studies to elucidate/explore metabolic pathways for phylloquinone or menaquinone have not been conducted extensively!

3) Injected phylloquinone is mostly excreted in the feces - e.g., 20% in the urine & 40-50% in the feces via the bile in humans.

5. Functions

A. Vitamin K is involved in the post-ribosomal modification of several blood clotting factors, which are essential for the synthesis/activation of factor II (prothrombin), factor VII (proconvertin), factor IX (thromboplastin) & factor X (Stuart factor).



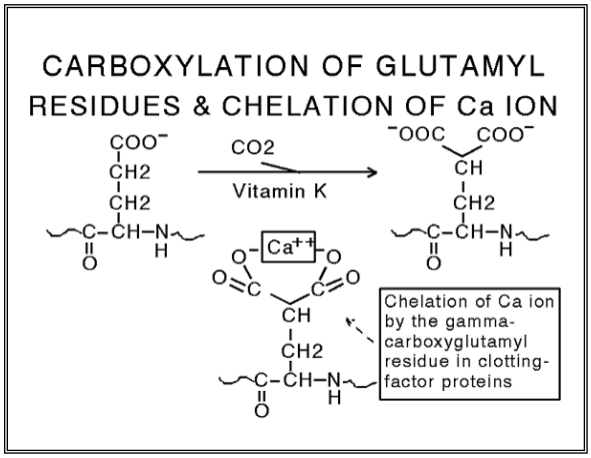
B. Blood clotting: (Adapted & redrawn from McDowell, 1989)

1) These proteins are synthesized in the liver as inactive precursors.
2) Other vitamin K-dependent factors:

   a) "Protein C" is not a procoagulant, but rather functions to inactivate the cofactor proteins & shut down the overall reaction.
   b) Also, protein S, protein Z, and protein M, but their function(s) - ???

C. Activation of factors: (Adapted & redrawn from Martin et al., 1983)

1) Carboxylation of specific glutamyl residues by "vitamin K-dependent" carboxylase.
2) γ-Carboxyglutamyl (Gla) residues chelate Ca ions, which are essential for the blood clotting mechanism.



D. γ-Carboxyglutamyl residues:

1) Other vitamin K-dependent proteins have been shown to be present in the bone, kidney, lung, placenta, skin, spleen, etc.

2) No clear understanding of the function of those proteins, but may indicate that vitamin K has some other functions - e.g.:

   a) Osteocalcin (found in the embryonic chick bone & rat bone matrix at the beginning of mineralization) may be involved in the bone formation?
   b) Proteins found in the skin may be involved in the Ca metabolism in the skin?

6. Deficiency Signs

- Essentially, an impairment of blood coagulation!

1) Low prothrombin levels (factor II).
2) Increased blood clotting time.
3) Hemorrhaging - Subcutaneous & internal hemorrhages in severe cases.
4) Anemia.

7. Requirements & Sources

A. Most species:

1) Usually, met by feed/food sources & microbial synthesis in the hind gut, but the rate of biosynthesis & efficiency of absorption can influence the status!
2) Possible exceptions:

   a) The use of sulfonamides & other antibiotics, which can ↓ microbial synthesis of vitamin K. (Also, antibiotics can affect the synthesis of B-vitamins!)
   b) Molds in feeds can result in the production of antagonists such as "dicumarol."
   c) Limited access to feces - e.g., the use of slotted floors and cages.

3) As an insurance, diets are usually supplemented.

B. Requirements: (NRC & RDA)

Animal	mg/kg or µg/d
Poultry (NRC, 1984):	
Chickens & broilers	0.5
Turkeys	0.8-1.0
Swine (NRC, 1998):	
All classes	0.5
Horses (NRC, 1989)	No estimate
Cats, adults	0.1
Dogs, growing	1.0
Fish	0.5-1.0
Rats, all classes	0.05
Humans, µg/d (RDA, 1989):	
< 1 yr	5-10
1-10 yr	15-30
Males	45-80

Females	45-65
Cattle, sheep, horse & goats	Not established

C. Fish - Quantitative requirements have not been established:

1) Intestinal synthesis of vitamin K has not been fully evaluated in fish.
2) Channel catfish & trout - Need vitamin K for normal blood coagulation, but growth rate is not affected by dietary deletion of the vitamin.
3) 0.5 to 1 mg of menadione/kg may be sufficient for fingerling trout, and 10 mg/kg has been suggested for trout & salmon.
4) Fish meal & alfalfa are good source of vitamin K.

D. Possible reasons for ↑ needs for vitamin K in swine in recent years?

1) Increased use of grain-soy-based diets, i.e., less use of other ingredients, which may contain vitamin K.
2) The use of solvent-extracted oil-seed meals (less K vs. expeller).
3) Increased incidence of hemorrhagic gastric ulcers, which are associated with genetics, stress, processing of grains/feeds, etc.
4) Increased use of antibiotics.
5) Molds in feeds.
6) Reduced opportunity for coprophagy.
7) Increased productivity, which may increase the need for vitamin K (& other vitamins).

E. Sources:

1) Water-soluble menadione (K_3) salts - e.g., menadione Na bisulfite (MSB), menadione Na bisulfite complex (MSBC) & menadione dimethyl-pyrimidinol bisulfite (MPB).
2) Activity of these supplements - ≈ 50, 33, and 45% of menadione for MSB, MSBC & MPB, respectively.
3) Stability of MSBC & MPB in a vitamin-TM premix (% activity):

Item	1/2 mo	1 mo	3 mo	6 mo
MSBC	80	64	21	0
MPB	81	65	23	0

4) Vitamin K content of foods/feedstuffs: (McDowell, 1989)

Source	ppm (as fed)
Alfalfa hay, sun cured	19.4
Alfalfa dehy	14.2
Barley, corn, sorghum	0.2
Cabbage	4.0
Carrots	0.1
Eggs	0.2
Fish meal	2.2

Liver, cattle	1-2
Liver, swine	4-8
Meat, lean	1-2
Milk, cattle	0.02
Milk, human	0.2
Spinach	6.0
Tomato	4.0

8. Toxicity

A. "Natural" forms (phylloquinone & menaquinone) are generally nontoxic at very high dosage levels.
B. "Menadione" compounds can be toxic to humans, rabbits, dogs and mice in excessive amounts - may result in anemia, acute renal failure and death.
C. Upper safe levels - menadione can be ingested as much as 1,000 times the dietary requirement with no adverse effects.

IRON

1. Introduction

A. General:

1) Has been used as medicinal agents for centuries - e.g., the ancient Greeks, Egyptians and Hindus prescribed Fe as a treatment for a general weakness, diarrhea, and constipation.
2) By the 17th century, the role of Fe in blood formation became apparent, i.e., as a constituent of Hb, Fe plays a central role in life processes.
3) Relatively high incidence of iron-deficiency anemia being observed in human populations (including in those affluent countries):

Country	% of cases with anemia[a]
Israel:	
Pregnant	47
Nonpregnant	29
Males	13.6
India:	
Pregnant	80.0
Nonpregnant	64.3
Mexico:	
Pregnant	26.6
Nonpregnant	11.7
Males	.9

[a]Based on Hb concentration, i.e., < 13 g for adult males, < 12 g for nonpregnant females & < 11 g/dl for pregnant females (NRC, 1979).

- Indicates the importance of considering dietary Fe as a "marginally adequate" or "borderline" nutrient for humans.

B. Distribution:

1) Occurs as a iron-porphyrin nucleus, heme:

a) Not only in Hb, but also in Mb, cytochromes, peroxidase, catalase & other enzymes - Hb & Mb account for ≈ 3/4 of body Fe.
b) A constituent of oxygen carriers and oxidation catalysts or enzymes.

2) Fe content in organs and tissues of mammals:

Tissue	mg/100 ml or 100 g
Whole blood	20-45
Spleen	20-40
Liver	10-20
Kidneys	4-6
Heart	4-8
Skeletal muscle	1.5-3.0
Brain	2.0-2.5
Bones	3.5-4.0

3) In the human body:

Item	g
Hemoglobin	3.00
Cytochromes, catalase, siderophilin	0.01
Ferritin & hemosiderin	0.70
Muscular Fe:	
Mb	0.15
Non-heme Fe	0.50

2. Absorption, Metabolism, and Excretion

A. Absorption:

1) "Fe complexes" are split by HCl & pepsin, and ferric Fe (Fe^{+++}) is reduced to ferrous Fe (Fe^{++}) by reducing agents.
2) Absorption occurs throughout the GI tract, but the duodenum & jejunum are major sites:

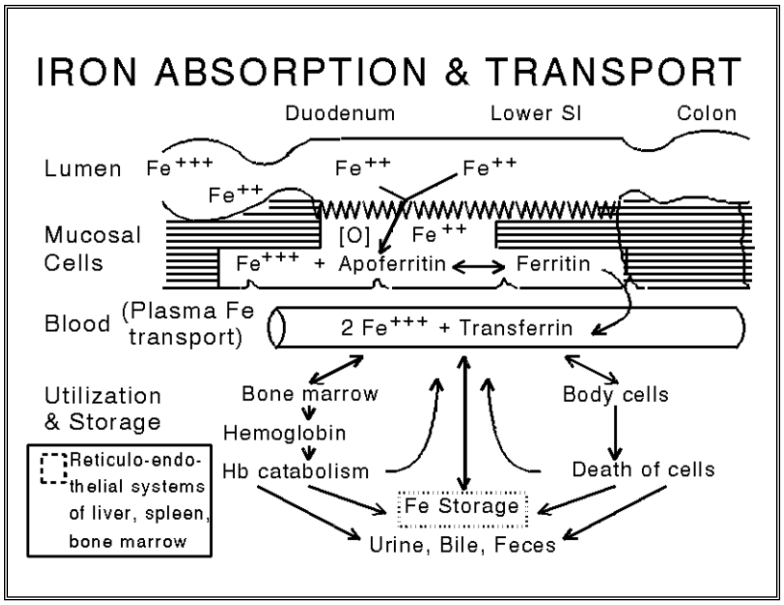
a) Absorbed in three stages – (1) uptake by the wall, (2) transit through the mucosal cell, and (3) passage through the serosal membrane into the blood.
b) Generally, Fe is poorly absorbed:

(1) ≈ 5-10% of intake might be common in the adult.
(2) The efficiency of absorption ↑ in the deficient state:

(a) ≈ 7-10% in a "normal" state, but ↑ to 80% in the Fe-deficient state in rats.
(b) ≈ 2-20% for normal subjects & 20-60% for anemic human subjects.

3) Absorption is facilitated by reducing substances or antioxidants in foods/feeds such as ascorbate, tocopherol, -SH groups of S-amino acids & glutathione.
4) Absorption is inhibited by organic acids (e.g., oxalate, citrate & phytate), chelating agents, phosphates, etc.
5) Fe competes with Cu, Mn, Pb & Cd for absorption sites, ∴ high levels of these minerals ↓ Fe absorption.

- Summary of Fe metabolism – See the figure (adapted & redrawn from McDowell (1992).



B. Transport/storage:

1) Transferrin:

a) Ferrous Fe entering blood is quickly oxidized to the ferric state.
b) Fe^{+++} form complexes with transferrin immediately, and transported through the body.
c) Transferrin links various cycles of the Fe metabolism, and thus regulating body Fe distribution.

2) Stored as:

a) Ferritin [nonheme protein (globulin) contains ≈ 20% Fe] & hemosiderin (contain little protein & as much as 35% Fe) are present throughout the body, but especially high in the liver.
b) Transferrin in serum.
c) Uteroferrin in placenta.
d) Lactoferrin in milk.

C. Excretion:

1) Very little Fe is lost from the body:

a) e.g., Fe released from Hb → liver → bile → reabsorption → reutilization.
b) Exceptions - menstruation, injury, etc.

2) Fe is excreted in the feces & urine, but also a continual loss via sweat, hair & nails.
3) Most of Fe present in the feces is unabsorbed dietary Fe.

D. In fish?:

1) Relatively little information on absorption/metabolism of Fe, but probably similar to other species.
2) Absorption of Fe takes place across the gill, but the intestinal mucosa is the major site.
3) Dissolved Fe (e.g., ferrous sulfate) in water may serve as a source in certain warm-water fish, but may precipitate out as ferric hydroxide.

3. Biological Functions and Supplementation in General

A. Specific function (Georgievskii, 1982):

Compounds (1° source)	Function
Heme compounds:	
Hb (erythrocytes)	Oxygen transport
Mb (skeletal muscles)	Oxygen transport
Cytochrome oxidase (heart muscle)	Electron transfer
Cytochrome C (heart muscle)	Electron transfer
Catalase (horse erythrocytes)	Peroxide decomposition
Peroxidase (horse radish)	Peroxide decomposition
Nonheme compounds:	
Succinate dehydrogenase (heart)	Electron transfer
Reduced NAD dehydrogenase (heart)	Electron transfer
Xanthine oxidase (milk)	Electron transfer
. . . and many others.	

B. Supplementation in general?

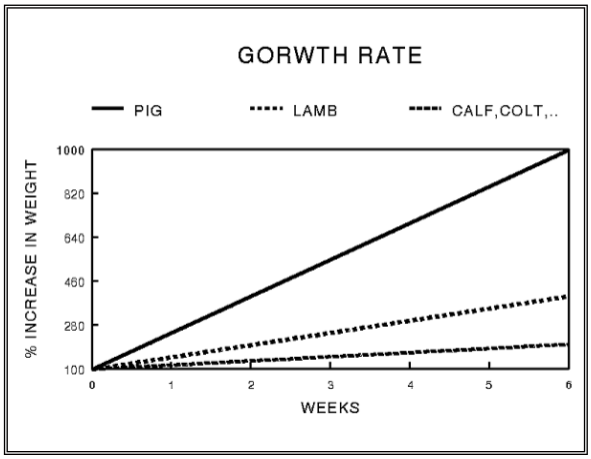
1) Natural feed ingredients usually supply enough Fe to meet the postweaning requirements of pigs and poultry.
2) The Fe-deficiency is unlikely to occur under practical conditions in fish.
3) "Borderline nutrient" for humans, and Fe-deficient anemia is common.
4) Very important for newborn or suckling piglets.

4. Iron Deficiency Anemia in Nursing Pigs

A. Reasons?

1) Low body storage in newborn pigs - Born with only about 40-50 mg of Fe, and with the daily requirement of 7-16 mg/day, deplete rapidly!

2) Low Fe content in sow's milk - About 2 ppm in colostrum & 1 ppm in milk, thus piglets receive only about 1 mg/day from milk!
3) Rapid growth rate vs. other species - See the figure.
4) No access to iron sources (e.g., soil) in the confinement.



B. Fe deficiency:

1) Characterized by pale mucous membranes (around eyes, ears, nose & mouth).
2) Slow growth, rough hair coat, wrinkled skin, and may be listless.
3) Labored breathing or “thumps” (spasmodic jerking of the diaphragm muscles), and sudden death from anoxia.
4) More susceptible to infectious diseases.

C. Hb or hematocrit levels - The best index of the Fe status in pigs:

Item	Normal	Anemic
Hb, g/100 mL	12	5
Hematocrit, % RBC	35	17

D. Prevention of baby pig anemia:

1) Treatment of sows with oral or injectable Fe during gestation:

- Injection of 22 mg Fe-dextran/kg BW (divided among 5 injections at d 40, 45, 50, 55 & 60 of gestation): (Ducsay et al., 1984. J. Anim. Sci. 59:1303)

Item	Control	Treated
Fetal liver:		
Fe, mg/g DM	1.3	1.2
Total Fe, mg	3.7	3.5
Placenta:		
Uteroferrin, mg/g	1.6	2.4
Uteroferrin, mg	287	427
Allantoic:		
Fe, μg/mL	5	7
Fe, mg	204	1,327

  - No appreciable effect on body stores of newborn pigs!

2) Treatment of sows with Fe during lactation:

- Hemoglobin levels in young pigs (g/100 mL): (Univ. of Kentucky data)

Treatment	Birth	Wk 1	Wk 3
None	9.2	7.7	5.9
Pigs injected	9.0	9.3	9.1
Sows fed 700 ppm Fe	9.6	8.4	8.9

  - No effect on the Fe content of milk!

3) Thus, limited placental & mammary transfer of Fe in pigs, and it is a common practice to inject pigs with 100-150 mg of Fe as Fe-dextran or Fe-dextrin at 1-3 days of age.

5. Iron Requirements/Supplementation

A. Requirements:

Animal	mg/kg
Poultry (NRC, 1994):	
Immature chickens	56-80
Laying hens	38-56
Broilers	80
Turkeys	50-80
Swine (NRC, 1998):	
3-120 kg	40-100
Sows/boars	80
Horses (NRC, 1989; DM)	40-50
Fish (NRC, 1993):	
Channel catfish	30
Rainbow trout	60
Pacific salmon	Not tested
Common carp	150
Tilapia	Not tested
Beef cattle	50
Dairy cattle:	
Calf starter & milk replacer	100
All other classes	50
Humans (mg/d; RDA, 1989):	
Children, < 10 yr	6-10
Males, > 11 yr	10-12
Females, 11- 50 yr	15
Females, 50+ yr	10
Pregnant	30
Lactating	15

B. Bioavailability:

1) Ferrous sulfate ($FeSO_4$) - Highly available.
2) Ferrous carbonate ($FeCO_3$) - Variable.
3) Ferrous oxide (FeO) - Poorly available.

4) Ferric oxide (Fe_2O_3) - Totally unavailable.

- Others such as ferrous ammonia sulfate, ferrous chloride, ferrous fumarate, ferrous gluconate, ferric chloride, ferric citrate, ferric choline citrate & ferric sulfate are available, but not commonly used!

6. Fe Toxicity

A. Excess Fe (pigs):

1) 600 mg Fe/kg BW - Develop toxic signs within 3 h (incoordination, shivering, heavy breathing, convulsion, diarrhea, etc.).
2) Injection of > 200 mg Fe/day - May increase bacterial growth, thus become susceptible to infections & diarrhea.

B. Toxicity in general:

1) Chronic - Reduced feed intake, growth rate and feed efficiency.
2) Acute - Anorexia, diarrhea, hypothermia, shock, metabolic acidosis, vascular congestion of various organs & death.

C. Maximum tolerable levels - 500 ppm for sheep, 1,000 ppm for cattle & poultry, and 3,000 ppm for swine.

COPPER

1. Introduction

A. General:

1) Copper played significant roles in the civilization since the Stone Age - e.g., from crude hammered artifacts (dating back to ≈ 6,000 B.C.) to today's electrical uses.
2) Can form more than 200 minerals, but Cu is mainly found as sulphide ores.
3) The importance of Cu in animals was provided by Hart et al. (1928. J. Biol. Chem. 77:797), who worked with anemic, milk-fed rats:

a) Anemia was not corrected by a Fe-supplementation alone or by a liver-extract alone.
b) Feeding both resulted in a marked elevation in the Hb level and packed cell volume.
c) A bluish tinge of ashed-liver preparation was a clue to its Cu content, and prompted simultaneous Cu & Fe supplementation.

4) Subsequently, the role of Cu demonstrated in:

a) Enzootic ataxia (swayback) of lambs.
b) Bovine falling disease.

c) Aortic rupture in rabbits, swine, guinea pigs & turkeys.
d) Wool and hair depigmentation.
e) Bone disorders.

- All these disorders were preventable with a Cu supplementation!

B. Distribution: (Georgievskii, 1982. In: Georgievskii et al., 1982)

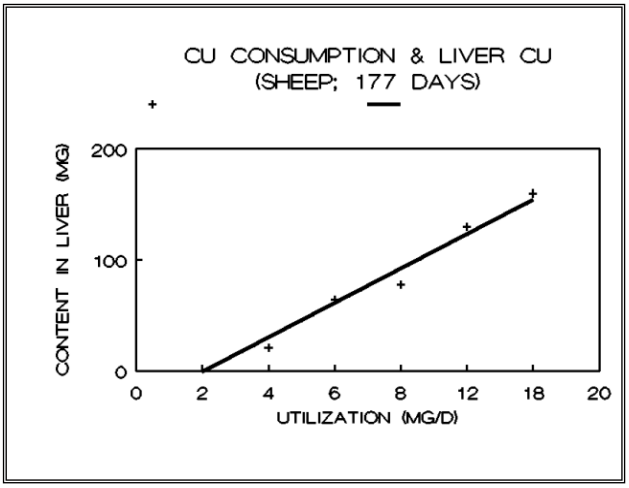
1) Cu contents in tissues & organs of farm animals:

Tissue	µg/100 g (wet tissue)
Whole blood	80-120
Liver	800-10,000
Spleen	120-1,200
Kidneys	200-400
Heart	300-400
Skeletal muscle	200-300
Brain	50-530
Bones	370-4,000

2) Variations in the content:

a) Skeletal muscle, heart, endocrine glands and kidneys - relatively independent of diets.
b) Liver, spleen, brain and bones - strongly dependent on diets (especially true for the liver!).

◦ Example (mammals) - 800 to 10,000 µg/100 g (wet wt) in livers & 370 to 4,000 µg/100 g (wet wt) in bones.



c) Ruminants seem to have much higher concentrations of Cu in the liver vs nonruminants - a reflection of greater requirements for Cu?

(1) e.g., normal concentrations are 100-400 ppm (DM basis) in ruminants vs. 10-50 ppm in nonruminants.
(2) Can reach 1,000-3,000 ppm, and 1,000 ppm may be toxic.

2. Metabolism

A. Absorption:

1) For Cu to be absorbed, it must be in the cupric state (Cu++)!

2) Sites:

a) "Adults" - Cu is absorbed in all segments, but duodenum > jejunum > ileum. (May be some absorption at the stomach?)
b) "Suckling rats" - mostly at the ileum.
c) "Sheep" - a considerable net absorption at the LI.

3) Absorption rate:

a) Poorly absorbed in most species (generally, < 5-10% of dietary Cu).
b) Young animals may absorb 15-30% of intake - e.g., in one study with lambs, an apparent availability estimate at 2 wk before weaning was 47%, but it was 8-10% after weaning.
c) Higher in deficient-animals, and may be higher in pregnant animals (latter stages of pregnancy).
d) Factors interfere with absorption include Ca, Cd, Zn, Fe, S, Mo, etc.

B. Transport/excretion:

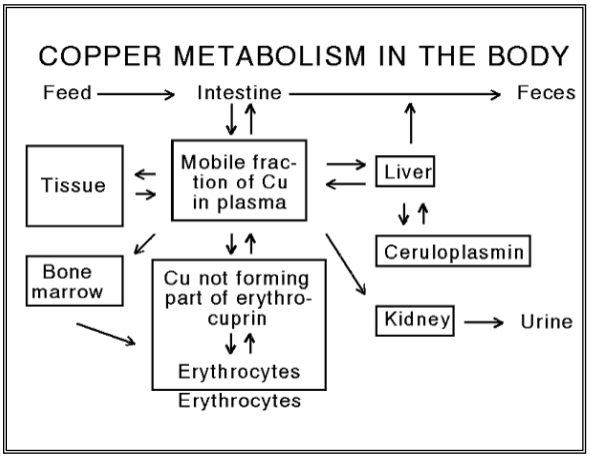
1) "Absorbed" Cu is loosely bound to serum albumin & some amino acids (His, Thr & Glu), and transported throughout the body.
2) CU is taken up quickly by the liver (& other tissues):

a) The liver is a major storage site, and also it is a site of ceruloplasmin synthesis.
b) "Ceruloplasmin" - a carrier of Cu:

(1) Contains ≈ 0.2-0.4% Cu (6 Cu atoms/molecule).
(2) The major circulating form of Cu (≈ 90% of plasma Cu).

3) Excretion:

a) A high proportion of ingested Cu appears in the feces (1° unabsorbed Cu).
b) Active excretion via bile, and to a lesser extent with other digestive juices.
c) Some excretion in the urine & milk.
d) Also, small amounts in the sweat & menstrual flow.



C. Summary of Cu metabolism: (Adapted & redrawn from Georgievskii, 1982. In: Georgievskii et al., 1982)

3. Deficiency/Functions

- Several enzymes are known to contain Cu, and are very sensitive to Cu depletion - e.g., Ceruloplasmin, lysyl oxidase, cytochrome C oxidase, superoxide dismutase, tyrosinase, uricase, dopamine beta hydroxylase, amine oxidases, ceramide galactosyl transferase, prostaglandin reductase, etc.

A. Anemia:

1) Cu deficiency can result in a poor iron mobilization, abnormal hemopoiesis, etc., ∴ Cu is essential for the blood formation.
2) Cu enhances the transport of Fe, and also catalyzes incorporation of Fe into Hb:

   - "Ceruloplasmin" (ferrioxidase) oxidizes Fe from ferrous to ferric state, which in turn can be bound to transferrin.

3) Assists a maturation of erythrocytes.

B. Abnormal bone development:

1) A Cu deficiency can result in a low osteoblastic activity, which in turn results in "bowing" of legs, spontaneous fractures, etc.
2) Cu is involved in the synthesis of collagen (& elastin) via serving as a component of lysyl oxidase:

   a) Lys to allysine → desmosine & isodesmosine → cross-linking of collagen in the bone.
   b) Also, "cross-linkages" can provide the structural rigidity & elasticity to connective tissues.

C. Cardiac and vascular disorders:

   - e.g., aortic rupture in turkeys & "falling disease" in cattle (heart failure, atrophy of myocardium, etc.).

1) Lysyl oxidase is involved in the synthesis of elastin (cross-linking).
2) Ceruloplasmin has an antioxidative activity, ~ may inhibit peroxidation?
3) Cu is a component of superoxide dismutase (convert superoxide to hydrogen peroxide + oxygen), thus, may protect the circulatory system!

D. Abnormal pigmentation & keratinization of hair & wool:

1) Pigmentation:

   a) A loss or lack of pigmentation due to ↓ activity of tyrosinase, thus lack of melanin.
   b) Tyrosinase is involved in the conversion of Tyr to dopa (dihydroxy-Phe), and then dopa is converted to melanin.

2) Impaired keratinization:

    a) Characteristics of wool (e.g., crimp) depend on disulfide groups for cross-linkages.
    b) Cu is required for the formation or incorporation of disulfide groups in the process of keratin synthesis.

E. Others:

1) Disorders in the central nervous system [e.g., swayback (enzootic ataxia) of lambs] - probably the results of ↓ cytochrome oxidase activity.
2) Reproduction failure (e.g., fetal death & resorption) - probably related to red blood cell and connective tissue formations during the early embryonic development.
3) Impaired immune response (e.g., formation of T & B cells, neutrophils & macrophages) - probably via superoxide dismutase (Cu-, Zn- & Mn-dependent enzyme), and Cu's role in the microbial system of phagocytes.

4. Some Cu Metabolism Disorders

A. Menke's syndrome:

1) X-linked, autosomal recessive disease that affects male infants.
2) Arises from malabsorption of Cu (∴ Cu accumulation in the gut mucosa), and most of the small amount of Cu absorbed accumulates in the kidney.
3) Severe vascular, skeletal and CNS abnormalities presumably due to low activity of Cu-dependent enzymes ..... death before age of 12 mo!

B. Wilson's disease:

1) X-linked, autosomal recessive defect.
2) Accumulation of excessive Cu, first in the liver & then in the brain, kidneys, eyes, erythrocytes & other tissues → pathological changes!
3) Ceruloplasmin is absent in plasma of about 25% of patients, and reduced levels in another 70% of patients.
4) Symptoms:

    a) Never appear before 6 yr of age.
    b) Nonspecific signs, ∴ often mistaken for others (hepatitis, cirrhosis, etc.).
    c) Signs include injuries to the liver & scarring of tissues (not fatal), tremors, clumsiness, rigidity, slurring of speech, drooling, difficulty in gait/coordination, seizures, behavioral changes & psychosis.

5) "Treatment" - D-penicillamine can metabolize Cu from tissues & promotes its excretion via the urine, thus effective. (Also, Zn supplementation may be effective.)

5. Requirements/Toxicity

A. Requirements:

Animal	mg/kg or others
Poultry (NRC, 1984):	
Immature chickens	4-5
Laying hens	?
Broilers	8
Turkeys	6-8
Swine (NRC, 1998):	
Growing	3-6
Adults	5
Horses (NRC, 1989; DM)	10
Fish (NRC, 1993):	
Channel catfish	5
Rainbow trout & common carp	3
Others	Not tested or determined
Dairy cattle	10
Beef cattle	8
Humans	Not established

B. Toxicity:

1) Signs include loss of appetite, ↑ thirst, apathy, ↑ breathing rate, intensified heart beat, jaundice, hemolysis, necrosis of liver & death.
2) Maximum tolerable levels:

Sheep	25 ppm
Cattle	100 ppm
Chickens & turkeys	300 ppm
Rats	1,000 ppm
Swine	250 ppm
Trout	100 ppb
Lobsters, crayfish	15 ppb

6. The Use of Copper as a Growth Stimulant

A. A high dietary level of Cu (100-250 ppm) has antimicrobial activity, and acts like an antibiotic.
B. Widely used as a growth promotant for pigs in the U.K. and Europe.
C. Dietary copper supplementation?

1) Effect of Cu on performance of starter pigs (a summary of seven 7 28-d studies with 4-wk old pigs): (Cromwell, Univ. of Kentucky)

Itm	Cu, ppm: 0	125	250	500
ADG, g	245	291	305	236
G:F .49	.52	.54	.45	

Liver Cu, ppm	23	24	191	349

2) The most effective supplementation seems to be 250 ppm Cu.

D. Dietary copper and age of pigs:

1) A summary of 12 starter and 18 grower/finisher studies: (Cromwell. Pers. Comm.)

Item	0	250	% ↑
Starter:			
ADG, g	227	286	26
G:F	.49	.54	9
Grower:			
ADG, g	659	714	6.9
G:F	.36	.37	3.6
Finisher:			
ADG, g	714	736	3.1
G:F	.31	.32	2.5
Liver Cu, ppm (DM)	22	244	

2) Dietary Cu supplementation is the most effective during the starter phase.

E. Sources:

1) Copper oxide & sulfide - Totally unavailable.
2) Copper sulfate - Available and most commonly used. (☞ 2 lb of $CuSO_4 \cdot 5H_2O$/ton of diet provide 250 ppm Cu.)
3) Copper carbonate/chloride, sequestered Cu & chelated Cu Met - Effective, but costly.

F. Relationship between Cu & Fe:

1) When using 250 ppm Cu, may have to provide ~ 50% more dietary Fe (≥ 150 ppm?).
2) Supplemental Cu and Fe on performance & hematology of weanling pigs: (Dove & Haydon, 1991. J. Anim. Sci. 69:2013)

	5 ppm Cu			250 ppm Cu		
Item + Fe, ppm[a]:	100	200	300	100	200	300
Hematocrit, %[b]	37.2	39.9	40.5	39.9	39.3	41.4
Hb, g/dL[b]	10.6	11.2	10.8	11.2	11.3	11.9
Plasma Fe, μg/dL[b]	245	248	233	218	233	241
Gain, g/d[c]	320	360	330	410	400	430
Feed:gain[c]	1.62	1.54	1.65	1.55	1.59	1.59

[a]Basal diet contained 169 ppm Fe & added 50, 100, 150, 200, 250 & 300 ppm; [b]Cu x Fe interaction, $P = 0.05$ to 0.06; [c]Effect of Cu, $P < 0.01$.

G. Mode of action?

1) Not well defined, but probably similar to the action of antibiotics - Unclear, but some suggestions?

   a) Metabolic effects - Direct influences on metabolic processes such as ↓ FA oxidation, ↑ protein synthesis, etc.
   b) Nutritional effects (cannot separate completely from the metabolic effect):

      (1) May be ↓ undesirable microbes & ↑ desirable microbes, i.e., ↑ the population of microbes that synthesize vitamins & amino acids.
      (2) Inducing changes in the GI tract - e.g., Thinner wall of the gut, thus may ↑ absorption rate & ↓ energy expenditures.

   c) Disease controlling effects.

2) Effect of Cu is “additive” to antimicrobial agents.

H. Some concerns regarding the use of high levels of copper:

1) Toxicity in pigs - The optimum level for growth promoting effect & toxicity level are similar!
2) Adverse effects on humans - e.g., Consumption of high-Cu liver.
3) Rapid deterioration of galvanized metals (buildings & equipment).
4) Reduced microbial decomposition of wastes in lagoons.
5) Can be an environmental pollutant.