Veterinary Pathology

Radiation, Mycotoxins, Neurotoxic, Nutritional Toxicologic Pathology
Summarized by jwliao

Radiation Toxicologic Pathology

Introduction

1. Ionizing radiation and ultraviolet (UV) radiation are not only important as injurious agents in our environment, but also are used for therapeutic or cosmetic purposes

2. Nuclear weapons testing caused environmental contamination

Sources and occurrence

Ionizing radiation: Natural source
1. External exposure:
   ⇒ radiative materials in the earth and from cosmic radiation from space occurring naturally
   medical exposures: X-ray

2. Internal exposure:
   1) from naturally occurring radionuclides deposed in the body
   come from minerals in the earth crust and entrance to the body
   2) by inhalation and ingestion
   3) Radon (\(^{220}\)Rn) is most concerned to lung cancer
   4) Occupational exposure: underground miners, radium (\(^{224}\)Ra)
      induce bone sacomas
   5) Nuclear waste: radioiodine, \(^{144}\)Ce, \(^{90}\)Sr and \(\alpha\)-emmiting transuranic of nuclear fuels (plutonium)

Mechanisms of ionizing radiation
1. Interactions of ionizing radiation with biological materials
   1) direct ionization of biological molecules
2. Subcellular and cellular effects of ionization
   1) cell killing, to carcinogenesis to injury to DNA
   2) DNA lesions, DNA base alteration, DNA-DNA and DNA protein cross links, single and double strand break
   3) cell cycle delayes from G\(_1\) to S to G\(_2\) phase
   4) chromosome abberation
3. Cell and tissue radiosensitivity
   1) The frequency of cell division:
   2) the most sensitive populations are the undifferentiated and primitive cells, stem cells, hematopoietic, crypt epithelium, spermatogonia, and dermal basal cells
   3) the least sensitive populations are highly differentiated cells, neurons, skeletal muscles, PMN cells
Response to injury induced by ionizing radiation

Hematopoietic and lymphoid system

- Beagle Dogs, 400 days old
- Chronic Low Daily Dose, Whole-Body Gamma Irradiation (1.8-7.5 rads/day)
- Fractional or Duration-of-Life Exposures
- Serial Assessment of Hematopoietic Function with Time, Dose, Clinical Phase

Figure 5. Primary feature of a chronic radiation leukemogenesis model in the canine. Dogs were serially assessed for clinical phasing and hematopoietic function throughout the radiation exposure period. Reproduced from Seed (1987), with permission.

Respiratory system
Nature and action of UV

1. UV includes wavelengths of electromagnetic energy between 10 to 400 nm
2. UV (10-120 nm), far UV (120-200 nm), UV-C (200-280 nm), UV-B (280-320 nm), UV-A (320-400 nm)
Mechanisms of UV injury

1. Carbohydrates do not absorb light above 230 nm

2. Photosensitization:
   UV-A induced cellular damage is due to the formation of activated oxygen species, such as peroxides, superoxide anion, and hydroxyl radiation

3. Methionine, histidine, cysteine, and tryptophan and be photooxidized to cause protein denaturation

4. DNA photoadducts: xeroderma pigmentosum

5. UV-induced cell death (apoptosis), cell cycle arrest, mutation, and altered gene expression

Response to injury induced by UV

Skin

Carcinogenesis


Mycotoxins Toxicologic Pathology

Introduction
1. Mycotoxins have attracted worldwide attention since the 1970s, because of the economic loss, impacts on human health, decrease of animal production
2. Mycotoxin, myco=mold; toxin=poison; mycotoxicosis
3. Mycotoxins are secondary fungal metabolites (i.e., metabolites not essential to the normal growth and reproduction of the fungus)
4. Cause biochemical, physiologic, and/or pathologic changes in other microbes
5. predispose to mycotoxin production by fungi include: moisture (humidity), temperature, aeration and substrate type and availability
6. Corn at higher moisture, damage by machinery, fungi can initiate growth
7. T-2 toxin in USA, Canada, Soviet Union
8. Ochrtoxin in Denmark
9. Fumonasins, Deoxynivalenol (DON, vomitoxin), Aflatoxin, Zearalenone in Midwestern USA
10. Ergot alkaloids in USA, Australia, New Zealand
11. Mycotoxicosis may as acute, subacute, subchronic, or chronic
12. Mycotoxicosis may be carcinogenic, mutagenic, or teratogenic (Table III)
13 Post harvest control of mycotoxins is drying and storage regiments

Aflatoxins
1. Aflatoxins are secondary mold metabolites produced by some strains of *Aspergillus flavus* and *A. parasiticus*.
2. Aflatoxins can be produced in conditions of 85% relative humidity and temperatures over 25°C that persist for over 48 hours.
3. peanuts, corn, walnuts, pecans, almonds, cottonseed and grain sorghum are more susceptible to *A. flavus* contamination than soybeans, wheat, rye and oats.
4. Aflatoxins were first identified as turkey X disease in England in 1960, over 100,000 turkeys died in the outbreak

Toxicology
1. Chemically they are polycyclic furan compounds possessing intense blue or
green fluorescence under long wave UV light.

2. at least 13 aflatoxins have been identified, blue (B1,B2), green (G1 and G2) are most common (Fig 1).

3. AF M1 and M2 are important metabolites of AFB1 and B2.

4. AFB1 is more toxic than AFG1 and M1

5. Toxicity:
   ⇒ LD50 aflatoxin B1=0.8 mg/kg
   ⇒ At 400-600 ppb in feed, severe clinical disease in feeder pigs
   ⇒ Adults: 450 ppb in feed of sows and boars had no effect on spermatogenesis, litter size, piglet quality or lactation.
   ⇒ Chronic toxicity, feeders 300-400 ppb in feed
   ⇒ Less than 223 ppb, no effect.
   ⇒ In corn, a maximum of 20 ppb aflatoxin is allowed in interstate shipments

5. AFs are potent mutagens carcinogens

![Chemical structures of representative aflatoxins.](image)

**Species susceptibility**
1. Pigs are more sensitive than sheep
2. Young animals are susceptible than adults
3. Ducklings and turkey poultries are sensitive than quail or chicks
4. Rats, GPs and rabbits are sensitive than mice
5. Trout are sensitive to hepatocarcinogenic effects

**Biodistribution, metabolism, and excretion**
1. In mammalians, cytochrom P450 (CYP) are responsible for phase I (CYP 1A2 and 3A4 ) in human
2. CYP-mediated AFB1 to AFB1 8,9-epoxide
3. AFB epoxides may react with phase II (GSH) conjugation and bind to macromolecules to form DNA adducts in N7 if guanine adducts
4. Differences among species and sexes are explained in Phase II metabolism
higher hepatocarcinogenicity related with higher GST activity in rats

Mechanism of action
1. AFs are genotoxic, potent hepatotoxins and hepatocarcinogens
2. May react with and modify the DNA template to interfere with RNA transcription or may specifically inhibit RNA polymerase which would also impair transcription
3. point mutation at the third base of codon 249 of the p53 tumor suppressor gene in human (Chinese and African) with liver tumor
4. Hepatitis B as a cofactor for aflatoxin-induced liver cancer

Clinical signs and pathology
1. Acute aflatoxin poisoning occurs less commonly than chronic
2. Decrease protein synthesis (hypproteinemia), decrease body weight gain, coagulopathy lead to extensive hemorrhage and anemia
3. target organ is the liver with fatty change, necrosis
   ⇒ Abnormally elevated AST, ALT, alkaline phosphatase…
   ⇒ Decreased albumin and A/G ratio
   ⇒ Increased prothrombin time (acute syndromes).
   ⇒ Centrilobular hepatic necrosis (GPs, dogs, pigs, cattle)
   ⇒ Periportal hepatic necrosis (rat, poultry, cat)
   ⇒ Vacuolization and anisocytosis, anisonucleosis
   ⇒ Bile duct cell proliferation and nodular regeneration (Fig 2)
   ⇒ Cirrhosis and hepatoma (< 1 ppm in diet)

![Image of liver tissue with liver damage](image)

Aflatoxin—Effects in Swine According to FDA

<table>
<thead>
<tr>
<th>Parts per Billion (ppb)</th>
<th>Clinical Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>Reduced growth, liver impairment, appetite loss</td>
</tr>
<tr>
<td>1000</td>
<td>Marked growth reduction; liver and kidney</td>
</tr>
</tbody>
</table>
Damage, petechial hemorrhages in muscles, viscera

2000 Severe hemorrhage, bloody diarrhea, death in 1-3 days

6. immunosuppression

**Diagnosis**
1. ELISA test: 20 ppb
2. Feeds containing concentrations less than 50 ppb of aflatoxins can generally be fed safely to all animals.
3. the actionable limit for the sale milk is 0.5 ppb AFB1

**Treatment**
- Change to aflatoxin-free ration
- Increase dietary protein
- Vitamin B₁₂ and Vitamin
- Selenium supplementation

**NovaSil (hydrate calcium aluminum silicates, HSCAS) and ammoniation of corn to reduce aflatoxin**
1. Corn turns brown
2. Can be used only for animal feed
3. Treated corn cannot be shipped across state lines
4. Ammonia may corrode steel, metal, copper and brass

### Ochratoxins

**A. Source**
1. Ochratoxins are produced by *Aspergillus ochraceous*, *Penicillium verrucosum*, and related species
2. Ochratoxins: A, B, C, D, and A is most common
3. Ochratoxin A contains a phenylalanine and attached to a dihydroisocoumarin group via an amide bond (fig 3)
4. Ochratoxin A is nephotoxin
5. the source of Ochratoxins is cereal grains, with moisture 19-22%, 24°C
6. the Northern European and Canada
B. Toxicology

Species susceptibility
1. Synergistic occur with AFB, citrinin, penicillic acid
2. potentially hazardous to horse, swine, duck, poultry, dog
3. cattle resistant due to ruminal microflora metabolize
4. teratogenicity

Biodistribution and metabolism
1. about 60% of orally administrated Ochratoxin A is absorbed from GI tract in rats
2. The T1/2 in pigs is 90hr and elimination from kidney and liver
3. Ochratoxin A accumulated in the kidney, liver, skeletal muscle and fat
4. Ochratoxin A is metabolized via cytochrom P450
5. The anionic transporter plays a major role in accumulation in kidney and absorption in the renal tubules

Mechanism of action
1. phenylalanine tRNA hydroxylase is inhibited by ochratoxins and reduced protein synthesis
2. inhibited mitochondrial respiration and cellular ATP
3. interfered cellular Ca\(^{2+}\) homeostasis
4. lipid peroxidation
5. induced DNA adducts and renal tumors in rats

Clinical signs and pathology
Laboratory animals:
1. Ochratoxin A is toxic and carcinogenic to rats (70-210 \(\mu\)g/kg) and mice (40 ppm) in diet for 2 yrs
2. Males are more sensitive than females
3. Kidney:
⇒ caused degeneration and regeneration in the tubules of the inner cortex and outer medulla in the C-M junction
⇒ tubular dilation, hyperplastic and multilayered, tubular atrophy
⇒ karyomegaly and cytomegaly of tubular epithelia (fig 4)
⇒ renal tubular adenoma and carcinoma
⇒ lower incidence of transitional cell papilloma in the urinary bladder

Swine:
1. swine is more sensitive to ochratoxin with chronic renal toxicity after fed with 0.2 to 4 ppm
2. Occur in northern European and first described in Denmark
3. kidney showed enlargement, gray-white and cyst formation

Human:
1. Balkan endemic nephropathy (BEN) in human
2. A high incidence of urinary tract cancers

Diagnosis, and treatment
1. Ochrtoxin $\alpha$ can be detected in kidney, liver, skeletal muscle, urine, feces
2. No specific treatment is available
3. Ammoniation

● Trichothecene mycotoxins

Source:
1. Trichothecene mycotoxins produced by *Fusarium* in grains with cool temperature ($15^{\circ}$C)
2. Deoxyivalenol (DON, vomitoxin), T-2 toxin, HT-2 toxin, diacetoxyscirpenol
3. T-2 toxin produced by *F. sporotrichiode*, and caused epidemics of alimentary toxic aleukia (ATA) in humans of Soviet
4. Mold corn toxicosis in North America
5. Bean hull poisoning in horse in Japan

**Toxicology**
Toxin: (fig 5)

**Biodistribution and metabolism**
1. Trichothecene mycotoxins do not accumulate in the body
2. metabolized by Phase I and II
3. Initial metabolites are often as toxic as the parent compounds

**Mechanism of action**
1. Inhibition of DNA synthesis
2. Induced lymphoid organs, the GI tract and others via apoptosis

**Biological effects and species susceptibility**
1. young animals are severity than adults
2. Vomiting is an acute reaction, as a result of central nervous stimulation
3. Cytotoxic to skin and oral activity (fig 9)
4. Immunosuppression
5. Lack mutagenic and no carcinogenic potential

**Clinical signs and pathology**
**T-2 toxin**
1. reduced feed intake, vomiting
2. hemorrhage and necrosis of GI tracts (fig 7)
3. lymphoid necrosis
4. Toxic to heart (fig 8)
4. LD50: 1.3 mg/kg iv

**Diagnosis and treatment**
1. ELISA with sensitivity at 25-250 ppb
2. activated charcoal

**Fumonisins**

**Source**
1. Fumonisins are produced by *Fusarium verticillioides* (*F. oniliforme*), *F. proliferatum*
2. associated with corn and corn-based foods
3. More than 15 homologues, FB1, 2, 3, are most common
4. Fungal growth including heat stress, insect damage, high humidity
5. Resistance to the GMO corn
6. high concentrations (1-100 ppm) have been found in China, Southern Africa
7. Fumonisin in mea, milk, eggs are negligible

**Toxicology**
1. Toxin structure (fig. 11)
Species susceptibility
1. Naturally occurs in cattle, horse and pig
2. Species-specific syndromes, equine leukoencephalomalacia (ELEM, porcine pulmonary edema (PPE) (table IV)
3. Male SD rats are extremely sensitive to the nephrotoxic effects (9 ppm for 90 days)
4. Tumor were induced in the diet > 50 ppm
5. No teratogenic

Biodistribution and metabolism
1. Poorly absorbed from the gut (10%)
2. Microflora can remove the tricarballylic acid groups
3. High concentrations in liver and kidney

Mechanisms of action
1. Potent inhibitors of ceramide synthesis from sphinganine
2. induced apoptosis, cytokines, TNF-α
3. Apoptosis in liver, kidney
4. Cardiocontraction to cause pulmonary edema in swine
5. equine leukoencephalomalacia may be affected secondary
6. No evidence of genotoxicity in Ames test
7. Vitamin C can protect in hepatotoxicity

Clinical sins and pathology
Horse
1. equine leukoencephalomalacia outbreak in 1901-1902
2. morality is more than 50%
3. Neurotoxic and hepatoxic forms
4. Neurotoxic:
⇒ anorexia and glossopharygeal paralysis, circling, and the onset time as 7 days
⇒ Liquefactive necrosis of the white matter in the cerebrum (fig 12)
⇒ Gitter cells, edema and hemorrhage
⇒ perivascular eosinophilic infiltration
5. Hepatoxic forms
⇒ hepatic apoptosis and necrosis (fig 13)
6. Cardial toxic:
⇒ cardiac contraction (Dr. Pang)

**Swine:**
1. porcine pulmonary edema (PPE)
   ⇒ Pulmonary edema in the interstitial and perivascular edema (fig 14)
2. Liver injuries similar to horse

![Liver injury image](image)

**Laboratory animals**
1. hepatopathy and nephropathy
2. Liver:
   ⇒ hepatic apoptosis, vacuolation and mitotic figures, cytomegaly
   ⇒ neoplastic lesions
3. Kidney:
   ⇒ major targets, apoptosis (fig 15)
   ⇒ Regenerative tubules
   ⇒ Renal tubular adenoma and carcinomas (fig 16)

**Diagnosis and treatment**
1. HPLC, ELISA
2. No effective therapy
3. Diet should less than 10 ppm FB1
I. Introduction

1. The adult neuron does not divide, replacement of lost cells is not possible
2. A barrier to passage of many blood-borne substances, unless are nonpolar or are actively transported
3. Even a small damage can result in marked effects on function
4. Neurons are dependent on glucose as a constant source of energy

II. Structure and function

A. Structure of the central nervous system (fig 1)

B. Cell types of the CNS

Four types of cells: neurons, neuroglia (astrocytes, oligodendrocytes, ependymal cells), microglia and mesenchymal cells with capillaries
III. Mechanism of toxicity

1. toxicants may direct or indirect changing the cell environment
   1) OP, malathion, combine with receptors of postsynapses
   2) carbon disulfide combine with neurofilaments
   3) vinca alkaloids combine with microtubules

2. Chemicals uncouple oxidative synthesis of energy, ATP
   1) cyanogens (plant), dinitrocreosol (herbicide) cause energy loss, and increase body temperature

1. Hypoxia and ischemia
   1) acute or chronic
   2) neurons are more sensitive than glial cells
   3) Large neurons, Purkinje cells, are more sensitive than small neurons, such as granule cells in the some brain region
   4) the degree of vascular:
      a. large white matter are less vascularized than gray matter
   5) hypoxia may result from ischemia, cardiovascular toxicity
      a. cyanide intoxication: metahemoglobin, inhibit cytochrome oxidase, ↓ oxygen, hypoxia
      b. ischemia: hypo tissue perfusion, lactic acid accumulation in the neurons
      c. lower cytosolic pH: activated acid phosphatase in lysosome, then necrosis

a. Diffuse neuropathies
1. Methylmercury
   1) a waste product of paper, electric apparatus, fungicide
   2) was first recognized as an environmental health hazard in the Minamata Bay and Niigata of Japan in 1950
   3) methylmercury bioaccumulation in fish and enters the food chain,
   4) both inorganic and organic mercury cause neurotoxicity
   5) damage granule cells of layer IV in the visual cortex, cerebellum, sensory neurons of the dorsal root ganglia

   a. neuronal degeneration and necrosis with axonal dystrophy and demyelination (fig 10, 11)
   b. toxic damage to vascular epithelial and BBB
c. edema, hemorrhage, perivascular lymphocytic infiltration, d. repair and scar formation is provided by astrocytes and perivascular mesenchyma cells in survivors

6) pathogenic mechanisms (Chang 1986)
   a. BBB dysfunction leading to irregular neural metabolism
   b. changes in RNA and protein synthesis
   c. enzymatic system disruption
   d. denaturation of cellular proteins and breakdown of biological membranes

3. Methanol
   1) as a solvent in industry, adulterant of alcoholic beverage, major automotive fuel
   2) acute toxicity in human is immediately depression, headache, nausea, vomiting, impaired vision, difficult breathing, coma and death
   3) pathology:
      a. retinal and photoreceptor cell necrosis, and permanent loss of vision
      b. neuronal degeneration and necrosis of the basal ganglian putamen in the brain
      c. high conc. (5000-10000 ppm) in monkey cause lesions in putamen, but not in the eye
      d. no eye or brain lesions have been produced in rodents with any dose
      e. 500-1000 times in human
   4) pathological mechanisms:
      a. methanol is metabolized to formaldehyde, then formate, then CO₂ and H₂O in all mammalians
      b. formate is considered the toxic metabolite
      c. the difference in susceptibility depend on the formate metabolism
a) non primates are very efficient in formate metabolism

4. Carbon monoxide
   1) CO is high comparative (over 200 times) binding to hemoglobin (Hb) with oxygen
   2) caboxyhemoglobin (COHb) 50-60%: coma, 70-80%: cardiorespiratory failure and death
   3) pathology:
      a. few hours: hypoxic-ischemia, congestion in the meningeal vessels and petechial hemorrhage, particularly in the subcorticol white matter
      b. days and weeks: edema, as ischemic neuronal necrosis (the laminar necrosis of layers III, V, VI), hippocampus(CA1 and CA3-5) and globus pallidus (75% cases), cerebellar cortex, substantia nigra
      c. chronic: extensive phagocytosis, gliovascular proliferation, multifocal necrosis in the cerebral white matter
      d. myelinopathy in delayed CO toxicity
   4) pathological mechanisms:
      a. the binding of CO to hemoglobin
      b. decrease of $O_2$
      c. similar to hypoxic-ischemia

5. Cyanide
   1) in electroplating, rodenticide and fumigant, cyanide-containing fruit seeds (apricot, peach, wild cherry, bitter almond) and cassava plants
   2) acute toxicity resulting in death within few minutes
   3) petechia and congestion are the main lesions
   4) survivors for 24-36 hours reveal necrosis and softening of the globus pallidus
   5) pathological mechanisms:
      a. anorexia due to inhibition of cytochrome oxidase, transport chain the uses $O_2$ derived from oxyhemoglobin
I. Introduction

1. Dietary components traditionally are divided into macronutrients and micronutrients (table 1)

2. Macronutrients:
   1) provide energy: lipids, carbohydrates, proteins, fibers
   2) vitamins:
   3) minerals: Ca, K, Cu,
   4) trace elements: F,

3. Recommended dietary allowance (RDA)
   1) optimal growth
   2) The Food and Nutrition Board

4. The availability of vitamins and mineral supplements on the market permits opportunity for excess intake and overdose
   1) niacin used to depress elevated plasma cholesterol levels
      ⇒ flushing and pruritis can be allayed by the use of aspirin
   2) competitively inhibit intestinal uptake:
      a. molybdenum excess produces Cu deficiency
      b. Mn excess produces Fe deficiency anemia
      c. Zn overdose, Cu and Fe deficiencies

5. Carcinogens (antioxidants: BHT, BHA) are regulated carefully
   ⇒ risk for cancer: $10^{-6}$ per lifetime

6. Non-carcinogenicity:
   1) no observable adverse effect level (NOAEL)
   2) lowest observable adverse effect level (LOAEL)
   3) acceptable daily intake (ADI) = NOALE/uncertain factors (10x10) or/and magnificent factor (1-10)
      ⇒ Se (55µg/day for females; 70µg/day for males, RDA)
      ⇒ 10 µg/L in drinking water (EPA)
Dietary contaminants
1. The problem of pesticides and other contaminants in the diet is a complex and controversial for human and domestic animals
2. An acceptable daily intake (ADI) for human is based on the no observed adverse effect level (NOAEL) in rodents tests
3. ADI = NOAEL/ species difference (10) x individual viability (10) x Magnified factor (target organ, 10) (mg/kg/day)

II. Macronutrients

- Proteins
  
  Deficiency:
  1. Protein deficiency usually is combined with carbohydrate/fat (energy) deficiency
  2. Kwashiorkor leads not only to wasting of muscle but also to atrophy of internal organs
     ⇒ 1) plasma protein decrease
     2) serous atrophy, anemia
     3) abnormal bone
     4) endocrine atrophy
     5) skin lesions
  3. Malnutrition
  4. Protein restriction as a major means of preventing cancer

Excess
  1. Protein excess seldom is of pathological relevance in most species
  2. Increase protein intake increase the renal rate of uremia
3. Increase of chronic progressive nephropathy

- **Fat**
  1. Fat is important to the certain fatty acid for the absorption of certain essential nutrients, such as the fat soluble vitamins

**Deficiency:**
1. Fat deficiencies of linoleic, linolenic and arachidonic acid can be induced under experiment, naturally is rare.
2. Fatty acid deficiency may occurs if biliary or pancreatic dysfunction
3. Showed skin abnormalities:
   ⇒ dermatitis, dehydration, failure of wound to heal

**Excess:**
1. Enhance carcinogenesis
   ⇒ polyunsaturated fatty acid, increase of mammary gland, skin, lung, colon, liver, pancreas tumors in animal models

2. Cardiovascular diseases
- **Fiber**
  ⇒ Dietary fiber is the sum of polysaccharides and lignin that are not digested by the endogenous secretions
  ⇒ Cellulose, hemcelluloses, pectin, and lignin…

**Deficiency:**
1. Decrease of fermentation
2. Decrease of short chain fatty acids
3. Decrease of cellular differentiation in the colonic gut

**Excess**
1. Reduce gut transit time, increase of harmful substance exposure
2. Bound mineral elements,
   ⇒ Mg$^{+2}$ ↓ (hypomagnesemia)
   ⇒ Ca$^{+2}$ ↓ (hypocalcemia, osteoporosis)
3. Decrease or delay mono-saccharide
   ⇒ hyperglycemia, IDDM↑
4. Bowel obstruction
Micronutrients

Vitamin A

Deficiency:
1. Daily intake is less than 70% of RDA (5000 IU for human) for long period
2. Liver damage (e.g., secondary to alcoholism, > 90% vit. store loss in liver)
3. Squamous metaplasia of pseudostratified columnar epithelium
   ⇒ Night blindness is followed by keratinization of conjunctiva
   epithelium and lacrimal glands, leading to dry eye and atrophy retina
   and loss of vision
   ⇒ metaplasia of glandular ducts, in pancreas and salivary gland (Fig 1)
4. Hyperkeratosis raised plaques by overproduction of basal cells in the
   epidermis on the skin
5. Impaired functions of lymphocytes and macrophages
6. imbalance of osteoclast and osteoblast lead to excessive cancellous bone

Excess
1. Acute toxicity:
   ⇒ by eating marine fish livers, neonates are more sensitive
   ⇒ signs of vomiting, diarrhea, redness of skin
   ⇒ human, headache, hyperirritability
2. Chronic toxicity
   ⇒ more common, improperly mixed diets, bovine liver
   ⇒ redifferentiated of simple types of epithelium
   ⇒ pruritis, cracking of the skin, lips, gums
   ⇒ enlargement of the liver
   ⇒ teratogenic by induced apoptosis, in face, ears, eyes, digits.

Figure 1: Vitamin A deficiency. Photomicrograph of salivary gland from a lamb
illuminating squamous metaplasia of the salivary ducts (*) and surrounding inflamma-
tory response. Keratin and cellular debris fill the ducts. Hematoxylin and eosin. Bar:
25 μm.
and brain
⇒ osteoporosis, bone fraction
⇒ decrease of iron, zinc deficiency

- The B vitamins

Thiamine (vit B₁)

Deficiency:
1. as a cofactor of Kreb’s cycle
2. beriberi (peripheral vasodilation)
3. non specific demyelination, Wernicke-Korsakoff synrom, in alcoholism

Excess:
1. RDA: 1-1.5 mg
2. headache, convulsion, paralysis, cardiac arrhythmias

Riboflavin (vit. B₂)

1. Riboflavin is an essential component of flavin mononucleotide and flavin adenine dinucleotide (FAD)
2. Cofactors in oxidation reactions, including Kreb’s cycle
3. ruminants receives from microflora

Deficiency:
1. inadequate intake of food, alcoholics, elders, debilitated
2. conjunctivitis, opacity, dermatitis, alopecia, scaling, normocytic hypochromic anemia, neuropathy (chicks)

Excess
1. riboflavin is eliminated very rapidly into the urine
2. difficult to achieve high levels

- **Vit. C**
  - Deficiency:
    1. Water soluble, do not accumulate in tissue
    2. hydroxylation of praline in collagen
    3. poor fruit or leaf green vegetable
    4. deficiency only in GPs, bats, primates (scurvy)
      1) gingivitis with hemorrhage
      2) loosening of teeth
      3) abnormal ossification (fig. 5)
      4) poor wound healing

- **Excess:**
  1. do not appear acute toxicity
  2. 1 g/kg for weeks or months showed diarrhea, nausea,
  3. increase excretion of oxalate uroliths
  4. increase vit C, Fe uptake
Vit. D.

Deficiency:
1. the sunshine vitamin
2. free factors:
   1) insufficient exposure to sunlight
   2) chronic renal disease
   3) inadequate dietary intake
3. Rickets
   1) enlargement of metaphyseal regions
   2) delayed osteoclasification
   3) osteomalacia

Excess
1. Toxicity:
   1) hypervitaminosis
   2) imbalance Ca and P: hypercalcemia and hyperphosphatemia
   3) calcification: renal, arteries, joint capsule, pulmonary (fig 2, 3)
   4) increase osteoclastic activity, mineralization
2. rodenticides: cholecalciferols (vit D₃)
3. Secondary hyper-parathyroidism
- **Vitamin E:**
  - **Deficiency:**
    1. $\alpha$-tocopherol, as antiaging vitamin protect cancer
    2. antioxidant in cell membrane
    3. relative increase dietary polyunsaturated fatty acids
    4. Keshan disease (necrotizing cardiomyopathy in human),
    5. White muscle disease (nutritional myopathy) (fig 4)
  6. **Pathology:**
    1) muscle cells showed swelling of mitochondria
    2) dissociation of myofibrils
    3) fragmentation of sarclemma reticulum
    4) oncotic necrosis
    5) steatites
  7. **Encephalomalacia in chicken, cats, dogs, and monkey**
  8. **Testicular atrophy and apermatogenesis in rats, GPs**

![Image](image.png)

**Excess:**
  1. relative nontoxic
  2. human:
    1) headache, double vision
    2) depression of prothrombin time
    3) impaired vit K utilization

- **Vit K**
  - **Deficiency:**
    1. as a cofactor, II, VII, IX, X
    2. antibiotic therapy
    3. Sweet clover hay, coumarin, hemorrhage
Excess:
1. almost impassible naturally
2. treated synthetic vit K3 (menadion), renal tubular necrosis
3. Vit. K rich vegetable (cruciferous vegetables)
4. high dietary Ca, interfered with vit K

Minerals
Major minerals

• Calcium

Homeostasis
1. Ca is most abundant divalent cation in the body, 1-2% of body weight, 99% in the bone
2. Maintenance of extracellular (10^{-3} M) and intracellular (10^{-7} M)
3. Only 30% of ingested Ca is absorbed
4. Decrease in serum Ca triggers the PTH/calcitriol-dependent upregulation of absorption from the gut and deceased urinary excretion

Deficiency:
1. Low Ca and the chronic hyperparathyroidism cause **osteodystrophy in adult and rickets in growing young** (fig 6)
2. Diets high in protein or fiber can cause chronic Ca loss
3. Acute Ca loss can occur in a chelator, EDTA
4. Osteomalacia is most pronounced in vertebrae, mandible, the proximal and distal ends of the long bones

Excess:
1. Above 1% Ca causes food intake decrease, fecal weight increase and retarded
2. high Ca cause renal tubular calcification in C-M junction and progressing in the inner cortex
3. high Ca leads to P and Mg deficiency

• Phosphorous

Homeostasis and deficiency:
1. Most P (70-80%) excreted from kidney via urine and feces
2. P deficiency is seen most commonly in ruminants
3. hypophosphatemia results in osteomalacia
Excess:
1. RDA for P is 800 mg/day, no adverse when P is raised to 2000 mg/day
2. P : Ca ratio is above 1:2, secondary hyperparathyroidism results in osteomalacia and fibrous osteodystrophy in mandible
3. high intake of P in sodas is popular in America today that is concerned as osteoporosis of old age
4. acutely rises Ca produces calcification in heart and kidney

Synthetic-diet-induced nephrocalcinosis
1. Commercialized diet, AIN-76
2. intratubular mineral deposits of calcium and phosphate salt in the pars recta
3. Change Ca: P ratio, increase Ca, Mg deceased or prevents mineral precipitation
4. alternating water and pH
5. associated with estrogen

<table>
<thead>
<tr>
<th>Observation</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein concentration (%)</td>
<td>NIH-07</td>
<td>NTP-2000</td>
</tr>
<tr>
<td>Feed consumption (g/rat)</td>
<td>16.0</td>
<td>16.9</td>
</tr>
<tr>
<td>Protein consumption (g/rat)</td>
<td>3.68</td>
<td>2.38</td>
</tr>
<tr>
<td>Water consumption (g/rat)</td>
<td>20.0</td>
<td>17.0</td>
</tr>
<tr>
<td>Nephropathy grade (1 to 4)</td>
<td>2.6</td>
<td>1.8</td>
</tr>
<tr>
<td>Maximum body weight (g)</td>
<td>515</td>
<td>514</td>
</tr>
<tr>
<td>Survival at 110 weeks (%)</td>
<td>496</td>
<td>485</td>
</tr>
<tr>
<td>Inhalation studies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noninhalation studies</td>
<td>25</td>
<td>51</td>
</tr>
<tr>
<td>Ca:P molar ratio</td>
<td>1.0</td>
<td>1.28</td>
</tr>
<tr>
<td>Nephrocalcinosis (%)</td>
<td>0</td>
<td>96</td>
</tr>
</tbody>
</table>

*During a 13-week study (12).
*From 2-year studies (grade 1 = minimal, 2 = mild, 3 = moderate, 4 = marked).
*During the course of the 2-year studies. Average of 5 to 6 control groups with 50 rats/group.
*At 110 weeks of age or at the end of 2-year studies. Average of 5 to 6 control groups with 50 rats/group.
*After 13 weeks on study or 19 weeks of age (12).

Osteodystrophia fibrosa in delivery goats
Magnesium

Deficiency:
1. Whole body Mg is found 60-65% in bone, 30% in muscle and 1% in the extracellular fluid
2. Mg is regulated in the renal proximal tubule and the ascending arm of the loop of Henle
3. The RDA is 4.5 mg/day and about 30% is absorbed
4. Chronic hypomagnesemia is associated with abnormal neuromuscular function, as tremor, tetany, and myocardial damage and arterial hypertension
5. Winter tetany in ruminants
6. Hypomagnesemia is associated to cancer mortality

Excess:
1. high Mg causes vasodilation, hypotension
2. hypermagnesemia is thought to interfere with the release of ACh, produces a curare-like action in neuromuscular junction and CNS depression
3. 0.3% Mg shows no toxicity in sheep, 0.8% causes diarrhea
4. i.v. 40 mg/kg MgSO4 to cattle and horse produces disruption, respiratory paralysis, and cardiac arrest

Sodium/Chloride/Potassium

Deficiency:
1. Plants foods do not provide sufficient Na, Cl
2. Salt must be added to animal feeds
3. Extracellular fluid is Na, intracellular is K
4. Homeostasis is lost in renal disease
5. Salt restriction is found to lower pressure in hypertensive
6. Blood pressure increases with age is concerned from lowering NaCl intake in Western

**Excess:**
1. In sheep, 13 g NaCl/L decreased reproduction
2. 30 g NaCl/L with no adverse, 6-8% NaCl in the diet causes staggering, weakness, paralysis
   \[ \Rightarrow \text{cerebral edema, eosinophilic infiltration in Vrichow-Robins spaces and necrosis of neurons in the cerebral cortex in swine} \]
3. Hyperkalemia (6% K) in the grazing pastures causes tachycardia

(King, et al., 1976)

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**Trace minerals**

- **Copper**
  1. Electron transfer reactions
  2. 1-2 ug/g in human tissue
  3. Liver and kidney have highest concentration
  4. Plasma Cu is 1.5-3 mg/day for human, 4-5 ppm for swine
  5. Cu is absorbed from duodenum

**Deficiency**

1. Low Cu soil
2. Swayback disease, bovine falling disease, aortic rupture in swine
3. Neurologic lesion is bilateral symmetrical demyelination and loss of white matter in spinal cord and cerebral cortex.
4. Absence of normal Cu/Zn superoxide dismutase to lipid are oxidized
5. RBC fragility, microcytic, hypochromic anemia

**Excess**
1. sheep are most sensitive, hemolytic crisis, hemoglobin nephrosis (fig 8)
2. Cu accumulated in liver lysosome, rupture, necrosis, and cirrhosis
3. Wilson’s disease in human

![Figure 8. Copper toxicity. Hemoglobin nephrosis in the kidney of a sheep with necrotic hemoglobin. Due to excessive copper ingestion, tubules containing hemoglobin pigment (*) are dilated, and the epithelium is atrophied or sloughed. Hematoxylin and eosin. Bar: 50 μm.](image)

- Selenium (Se)

**Deficiency:**
1. antioxidant
2. White muscle in young lamb, hepatosis dietetica in swine, reproductive failure in rats, pancreatic dystrophy, exudative diathesis in chicks
3. reversible by feeding Se

**Excess:**
1. lameness. Malformation of hoofs, loss of hair, emaciation
2. hemorrhagic enteritis, myocardial hemorrhage
3. fatty change, necrosis, hemorrhage in livers, kidneys, pancreas
4. necrosis in ventral horn areas of pigs, horses (fig 9)

![Figure 9. Selenium toxicity. Poliomyelomalacia in the gray matter (GM) of the ventral horn in the spinal cord of a pig, characterized by degeneration and loss of nerve cell bodies. White matter (WM) is unaffected. Hematoxylin and eosin. Bar:](image)
Iodine Deficiency:
1. $I_2$ is absorbed from stomach and colon and is taken up in the thyroid
2. $I_2$ is stored and incorporated into the hormone thyroxine for the growth and regulated metabolic rate and temp.
3. $I^2$ deficiency is associated with decrease of fertility, embryonic and postnatal development
4. $I_2$ deficiency in adult is hyperplasia of thyroid, thyroid follicles
5. Goiter is due to overproduction of colloid
6. Cassava (樹薯) diet contains cyanogenic glycosides and can produce overt $I_2$ deficiency

Excess:
1. Thyrotoxicosis cause weight loss, cardial diseases
2. 5 ppm in horse as a maximum tolerance
3. Fatty deposits and necrosis in liver, kidney, and GI tracts
4. Retinal changes in dogs, rabbits, GPs

Goiter in newborn piglets
Iron Deficiency:
1. Iron deficiency is the most common nutritional deficiency in human and produces microcytic anemia
2. Iron is absorbed by the GI tract
3. Men store 30-40% Fe; Women store 10-15% due to loss of blood during menses, pregnancy, and lactation
4. Neonates depend on milk for nutrition
5. Young pigs raised in confinement with no soil and must be supplemented via injection Fe

Excess:
1. 500 ppm in calves, poultry, sheep cause adverse effects
2. 3000 ppm has no adverse in Swine
3. Hemolytic anemia in premature infants low in Vit. E
4. Hemorrhagic necrosis of GI tract, with bloody vomitus and black stools in acute intoxication
5. Hereditary hemochromatosis (a genetic) cause Fe accumulation and producing hepatic cirrhosis, DM, and heart failure