Detoxification of Xenobiotics

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Detoxification

Biphasic process carried out by a suite of microsomal enzymes.

Mixed Function Oxidases (MFOs) are located on the endoplasmic reticulum.

- convert lipophilic compounds to more water-soluble forms
- water-soluble compounds more easily excreted

The process of detoxification involves two steps with the two phases placing different demands on nutrient stores in the body.

Phase 1 Detoxification

Substrates undergo oxidation, reduction or hydrolysis to yield more polar derivatives.

Biological activity of a compound is reduced, increased, or undergoes no change.

- may also activate biologically inert compounds.

Oxidation reactions are catalyzed by mixed function oxygenase (MFO) system.

- at least four separate enzymes are involved
- most important is cytochrome P-450

Inhibition of Phase I biotransformation:

- chemicals such as piperonyl butoxide (used to increase pyrethrin toxicity in insects).
- need to assess the potential toxicity of, not only the active pesticide, but any component chemicals in the mix.

Phase 2 Detoxification

Conjugation of a molecule containing a functional group such as:

- hydroxyl, thiol (glutathione) or amino group.
- obtained from Phase I with an endogenous moiety such as:
  - glucuronic acid, sulphate, glycine or cysteine.

Compounds with OH, COOH, NH_2 or SH group

- can enter Phase II without going through Phase I.

Conjugate inactive and less lipid soluble than its precursor

- excreted in the urine or in the bile.

Conjugation

Costly exercise as leads to a heavy drain on both energy and nutrients involved in conjugation.

Animals don't maintain complete sets of microsomal enzymes on the off chance that they will encounter a particular toxin.

- enzymes occur at low concentrations
- energy is required to:
  - synthesise and maintain enzyme systems
  - for specific nutrients used as conjugates and
  - for water in which to excrete the end products,

The conjugating moiety is lost with excretion

- compounds include:
  - glucose, glucuronic acid, glutathione, glycine, ornithine, glutamine or a sulphate ion.

If conjugates are limited

- foreign compound that normally conjugates with one type of group may conjugate with a different group.

Conjugates for carnivores

- ratios of sulphate to glucuronide conjugates suggest that carnivorous or insectivorous animals rely more on sulphuric acid for conjugation

Conjugates for herbivores

- herbivores and folivores show low levels of sulphate utilisation.
- sulphate groups are in limited supply from diets based on plant material
- herbivores show a greater reliance on glucuronide for conjugation.
- can lead to a drain on the reserves of glucuronic acid which is a major constituent of connective tissue.

Alterations to Detoxification

Ingestion of toxic foods is limited by:

- rate of detoxification
- availability of specific materials for conjugation.

Capacity to detoxify is under genetic control but MFO efficiency depends on:

a) **animal's size**

b) **age**

- older animals have larger store of nutrients to draw on for detoxification
- age differences in enzymes for detoxification
- GI mucosa and BBB less well developed in younger animals
- Active transport systems less effective in neonates
- Changes in body composition affect distribution and storage of toxicants
  - Newborns have higher body water and lower fat
  - Older animals lose structural proteins, increased stores of collagen and fat

c) **sex**

- males have more rapid microsomal enzyme function than females
- due to high levels of androgens
- progesterone during breeding can further inhibit detoxification in females

d) **sexual state**

- increase in organs size and protein content during pregnancy
- liver, adrenals, ovaries and uterus
- additional protein may be microsomal protein, which enhances biotransformation.

Health Status that Influence Detoxification

Liver disease

- may reduce the activity of MFOs
- regeneration of liver tissue after hepatic injury may actually increase biotransformation.
- reduction in the hepatic synthesis of protective binding macromolecules such as glutathione, ligandin and metallothionein can increase the effects of toxicants.

Feathers Quality

- tripeptide glutathione (glu-cys-glu) may provide a reserve of cysteine
- supports keratin synthesis during overnight period of fasting
- results in short-term deficit in the availability of sulphur amino acids
- greater demands for glutathione in detoxification mechanisms may be evident in poor feather quality.

Bile

- retention prevents excretion
- allows some toxicants to accumulate in the blood.

Kidney

- filtration and reabsorption by the kidney may be altered by disease
- may affect the excretion or secretion of xenobiotics.

Nutrient Deficiencies

- Ca, Cu, Fe, Mg, Zn
- Vitamins: B complex, C and E
- Proteins
- Limit chemicals necessary for synthesis of enzymes or conjugating agents

Fasting

- energy deficits reduce blood glucose
- decrease activity of xenobiotic metabolising enzymes

Detoxification and Impact on Vitamin C

Glucuronate is a precursor in the biosynthesis of ascorbate in most animals

- deficiency can lead to a reduced ability to detoxify foreign chemicals.
- not known whether the production of large amounts of glucuronic acid for conjugation has an effect on ascorbate synthesis.

Stress may also modify (increase) the rate of ascorbate metabolism

- changes in environmental attributes (structural or nutritional) may impact on ascorbate metabolism
- may influence a species' ability to detoxify xenobiotics in its diet.

Temperature and Detoxification

Body temperature

- decreases may decrease the activity of microsomal enzymes
- diurnal variations in cytochrome P-450 and reduced glutathione alters detoxification

Environmental temperature

- low temperature may enhance biotransformation of xenobiotics
- possibly due to the increase in metabolic activity to maintain body temperature
- high environmental temperatures increase susceptibility to poisons that alter metabolism for thermal regulation
- oxidative uncouplers increase body temperature, and their toxic effect is increased in hot weather.

Actions of Pesticides

Cellular damage is the basis for most toxicologic injury, and the toxic response to any specific chemical is the result of dysfunction of relatively few basic biologic processes. Normal processes can be suppressed or stopped completely, or they can be enhanced beyond normal physiologic limits and, in turn, affect other systems dependent on their controlled functions. Cellular responses of chemical toxicants occur through both structural and metabolic mechanisms in the cell. A single response can be elicited from an individual pesticide or a number of actions can be attributed. These can include:

1. Altered membrane integrity:

- interference of fluid and electrolyte movement
- effects nervous system, with changes to the permeability of nerve cell membranes to ions.
- DDT and pyrethrins

    - alter sodium and potassium currents
    - change threshold for action potentials of the membrane
    - influence generation and transmission of action potentials by affecting sodium or potassium flux.
- DDT and methoxychlor (diphenyl aliphatics)
    - in inhibit both the "turning off" of the inward sodium current and the "turning on" of the outward potassium current
    - decrease the action potential threshold and stimulating nervous activity.

Alteration of cell volume regulation:

- results from direct membrane damage or from a loss of metabolic energy.
- alterations in energy metabolism can reduce energy availability required to drive active transport systems, synthesis of macromolecules and maintenance of osmotic balance
- interference with body metabolism or synthesis causes loss of products used for energy, structure components or growth
- oxidative uncouplers such as dinitrophenol and chlorophenol fungicides and arsenates increase oxygen utilisation, but energy is dissipated as heat rather than stored in high-energy phosphate bonds increasing body temperature
- inhibition of oxidative phosphorylation by trisubstituted tin fungicides results in limited oxygen uptake with lower ATP formation. The effects of fatigue and weakness are similar to the effects of oxidative uncouplers but there is no fever.

Abnormal accumulation of lipids and pigments:

- results from metabolic defects.

Alteration of protein synthesis:

- results from disturbance of nucleic acid control or denaturing of enzymes and structural proteins.
- enzymes can be inactivated or denatured by toxicants.
- toxicants can also interfere with enzyme action through competitive inhibition e.g. oxalic acid and fluoroacetate (rodenticides), which inhibit succinic anhydrogenase and aconitase respectively in the TCA cycle
- organophosphate and carbamate insecticides inhibit cholinesterase
- systemic toxins can damage cells with high metabolic activity and cellular replication, including renal tubules, hepatocytes, bone marrow, and intestinal epithelium
- toxicants affect key enzymes or metabolic intermediates in these cells
- inhibition of enzymes essential to balanced synaptic function changes the characteristics of synaptic transmission (acetylcholinesterase inhibition by organophosphate insecticides).

Disturbance of growth regulation:

- DNA damage:

- not properly repaired
- exceeds homeostatic control
- can result in hyperplasia or carcinogenesis.

Free Radicals

- can result in the damage of cellular macromolecules
- = compounds with an unpaired electron, a result of an enzyme-catalyzed addition of electrons to a carbon bond, with subsequent cleavage.

Superoxides (active oxygen)

- formed when some compounds are oxidised by MFOs to free radicals
- electrons transferring to oxygen (paraquat herbicides)
- reacts with PUFA, initiating auto-catalytic chain reaction, leading to lipid-free radicals and then lipid peroxidation

Peroxidation

- damages organelle and cellular membranes
- reduces structural integrity and control of selective absorption and active transport
- loss of calcium sequestration in the organelles, leading to a marked increase in cytosolic calcium.
- loss of calcium homeostasis leads to activation of phospholipases, autodigestion of membranes, loss of membrane function, swelling of cells, and eventual irreversible cell injury progressing to necrosis.

Time Period for Expression of Toxicity

The toxicity of certain pesticides may be underestimated

- accumulative affects
- delayed toxicity
- may not manifest for years
- can occur long after exposure stops
- confusing interpretation of signs and symptoms that can be accredited to pesticide toxicology

Synergistic effects

- when the combined effects of two or more chemicals are greater than the sum of the individual effects
- may result if one chemical affects the
  - solubility,
  - binding,
  - metabolism or
  - excretion of the other.

So, while studies on the affects of individual pesticides may not indicate any detrimental actions, a combination of two or several pesticides may enhance toxicological actions.

Potentiation

- when one chemical enhances the toxicity of another,
- toxicity of the potentiator may be minor or nonexistent