Disclosures: none

Objectives:

1. Recognize the clinical appearance of acute, subacute, and chronic mitochondrial failure.
2. Identify plant species known to cause mitochondrial disruption in humans.
3. Become familiar with the distribution of these toxic plants.
4. Become familiar with the toxins in these plants.
5. Become familiar with human outbreaks involving mitochondrial failure secondary to plant ingestion.
6. Understand risks, and identify at risk populations, for these exposures.
7. Explain potential treatment plans for patients who suffer from these exposures.
8. Think of preventive measures to limit further exposures.
OVERVIEW OF MITOCHONDRIAL FAILURE:

Concept: strength of mitochondrial toxin and duration of exposure contribute to differing clinical pictures:

1. Acute, short exposure to a strong toxin or a high dose, results in life-threatening mitochondrial dysfunction (acute mitochondrial failure)
2. Subacute, prolonged exposure (many months) to a weaker toxin or a lower dose, produces mitochondrial dysfunction (subacute mitochondrial failure)
3. Chronic, prolonged exposure (many years) to a still weaker toxin or a very low dose, produces mitochondrial dysfunction (chronic mitochondrial failure)

Acute mitochondrial failure:

Acute, severe dysfunction of the mitochondria frequently produces a rapidly progressive, often fatal, illness.

Acute mitochondrial failure is a metabolic failure that promptly produces acute neurological failure, followed by multiple system organ failure.

Clinically:

Metabolic Acidosis
Hypoglycemia
Seizures
Agitation
Encephalopathy

Multiple System Organ Failure (Respiratory Failure, Cardiac Failure, Hepatic Failure, Renal Failure)

Examples of plant toxins producing acute mitochondrial failure:

<table>
<thead>
<tr>
<th>Plant</th>
<th>Toxin</th>
<th>MOA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ackee</td>
<td>hypoglycin A and B</td>
<td>BOFA</td>
</tr>
<tr>
<td>Lychee*</td>
<td>MCPG</td>
<td>BOFA</td>
</tr>
<tr>
<td>Gifblaar and others (livestock primarily)</td>
<td>monofluoroacetate</td>
<td>KC</td>
</tr>
<tr>
<td>Tuba Plant and others</td>
<td>rotenone</td>
<td>ETC – I</td>
</tr>
<tr>
<td>Cocklebur. Blue-lime/Blue thistle, Ox-eye daisy</td>
<td>carboxytractyloside, atractyloside</td>
<td>ADP-ATP</td>
</tr>
<tr>
<td>Prunus spp. (apricot, peach, plum, cherries, etc)</td>
<td>cyanogenic glycosides: amygdalin</td>
<td>ETC – IV</td>
</tr>
</tbody>
</table>

*Can have some features of subacute mitochondrial poisoning.

Key: BOFA = inhibits beta-oxidation of fatty acids; KC = inhibits Krebs cycle; ETC – I = inhibits complex I of the electron transport chain; ETC – IV = inhibits complex IV of the electron transport chain; ADP-ATP = inhibits ADP-ATP
**Subacute and chronic mitochondrial failure:**

Prolonged exposure to a weaker toxin or a lower dose produces prolonged, low-grade mitochondrial dysfunction which produces slowly progressive neurodegeneration (however, the prolonged accumulation of toxin may culminate in a relatively abrupt onset of clinically evident neurological disease in subacute mitochondrial failure).

Subacute mitochondrial failure produces irreversible, often devastating, neurological disease:

- Upper motor neuron disease
- ALS-like (amyotrophic lateral sclerosis – like) disease (weakness, inability to walk with postural Instability and spastic paraparesis (LEs > UEs) or quadriparesis)

Chronic mitochondrial failure produces a chronic, often devastating, neurological disease:

- Atypical Parkinson’s disease
- Dementia

**Examples of toxins producing subacute and chronic mitochondrial failure:**

<table>
<thead>
<tr>
<th>Plant</th>
<th>Toxin</th>
<th>Subacute or Chronic Mitochondrial Failure</th>
<th>MOA (mechanism of action)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grass pea</td>
<td>BOAA or β-ODAP</td>
<td>Subacute</td>
<td>Neuroexcitation</td>
</tr>
<tr>
<td>Cycads with symbiotic cyanobacteria</td>
<td>BMAA</td>
<td>Subacute and Chronic</td>
<td>Neuroexcitation</td>
</tr>
<tr>
<td>Soursop</td>
<td>annonacin</td>
<td>Chronic</td>
<td>ETC – I</td>
</tr>
<tr>
<td>Cassava</td>
<td>cyanogenic glycosides (linamarin)</td>
<td>Subacute</td>
<td>ETC – IV</td>
</tr>
</tbody>
</table>

Key: ETC – I = inhibits complex I of the electron transport chain; ETC – IV = inhibits complex IV of the electron transport chain
BACKGROUND: UNDERSTANDING MITOCHONDRIAL FUNCTION:

Mitochondria = The Energy Bank:
1. energy (currency) exchange
2. store energy (currency)
3. transfer energy (currency) to distant sites

Mitochondrial Functions: The Energy Bank
1. Break up large molecules from food (energy source) into smaller molecules: fatty acids, amino acids, sugars. Continue to break these molecules into smaller, and smaller units. Example: long, branched fatty acids are broken down into smaller, 2-carbon fatty acids during beta-oxidation of fatty acids so that they can be utilized in the Krebs cycle. (Bank analogy: breaking a $100 bill down into five $20 bills so that it can be spent (utilized) more easily and at multiple sites)
2. Harvest and store the energy in the form of electrons, using electron carriers: NADH, FADH₂, generated via the Krebs cycle (Bank analogy: breaking $20 bills down into coins (electrons) that can be carried in pockets. Pockets = NAD+, FAD electron carriers. The coins in the pockets (NADH and FADH₂) are carried to the electron transport chain.)
3. Transport electrons down a chain of progressively favorable redox reactions, via the electron transport chain, to create a H+ gradient between 2 contained spaces, separated by the inner mitochondrial membrane. (Bank analogy: deposit coins in a machine to create an ion gradient; saving energy in the bank)
4. Use this H+ ion gradient to power an enzyme (ATP synthase) that will store energy in a high energy phosphate bond (ADP + Pi → ATP). (Bank analogy: making ATP is like melting the coins to make liquid gold. Liquid gold = ATP. It can be used in small aliquots and is accepted everywhere; perfect for travel to distant sites)
5. Transport ATP to distant sites that require energy for cell functions.
6. Bank analogy: Unlike a modern bank, there is no credit. You can only spend what you have.
7. Bank analogy: When the bank fails, everything around it fails (apoptosis).

Mitochondrial Functions: The Dump
The mitochondria also function as dumps for calcium and ROS.
1. Excessive calcium is taken into the mitochondria to protect the rest of the cell from excessive amounts of this second messenger.
   a. High (pathologic) loads of calcium can overwhelm the mitochondria.
   b. Calcium can leave the mitochondria via mitochondrial permeability transition pores. When these pores are in high conductance states (rather than low conductance states), irreversible mitochondrial swelling and leakage of molecules like cytochrome c occurs.
   c. This triggers apoptosis.
2. Toxic waste: reactive oxygen species (ROS) can accumulate in times of stress.
   a. ROS are made during cellular respiration
b. ROS may contribute to intracellular oxidative stress.
c. Mitochondria are the primary source of ROS.
d. Complex I and III of the electron transport chain are felt to be the main sites of ROS production.
e. Complex I is very sensitive to ROS induced damage.
f. Superoxide radical is the primary ROS (produced by single electron transfer to oxygen).
g. In complex I, several things increase superoxide production: low ATP production AND high NADH/NAD+ ratio in the matrix.

Bank and Dump Analogy: When the energy bank is not working, the dump gets overwhelmed with toxic waste (ROS), and the whole mitochondrion can fail. When mitochondria fail, the cell fails (apoptosis).

Some cells (such as dopaminergic neurons of the substantia nigra) are more susceptible to ROS than others. The neurons in the substantia nigra are more vulnerable to complex I dysfunction compared to neurons in other brain regions.

Further, ROS injure mtDNA and mtDNA codes for some subunits of complex I. With age, we all have some increase in mtDNA deletions and associated respiratory chain dysfunction. These mtDNA deletions are notably prevalent in the substantia nigra. Mitochondria are thought to play an important role in PD and aging.
CLASSIFYING MITOCHONDRIAL TOXINS BY SITE AND MECHANISM OF ACTION:

1. **Neuroexcitation (glutamate agonists):** these toxins that produce Ca^{++} influx into mitochondria and act as general toxins (Dump analogy: toxic waste is accumulating; the trash bins are overflowing)
   
   Examples:
   
   Grass pea (neurolathyrism from BOAA or β-ODAP)
   
   Cycads with symbiotic cyanobacteria relationship (ALS/PDC from BMAA)

2. **Beta-oxidation of Fatty Acids Inhibitors**
   
   Examples:
   
   Ackee (hypoglycin A and B)
   
   Lychee (α-methylenecyclopropylglycine)

3. **Krebs Cycle Inhibitors** (block one or more steps in Krebs cycle, thus, also interfere with complex II, which shares an enzyme with the Krebs cycle)
   
   Examples:
   
   Gifblaar (monofluoroacetate) – livestock toxicity
   
   Willow (methyl salicylate) – willow bark not as toxic as medication, ASA

4. **Electron Transport (Respiratory Chain) Inhibitors** (toxins that interfere with Complex I-IV)
   
   Examples:
   
   Tuba plant (rotenone; complex I)
   
   Soursop (annonacin; complex I)
   
   Grass pea (neurolathyrism from BOAA or β-ODAP; complex I, but likely secondary to ROS from neuroexcitation – see above)
   
   Cyanogenic plants (cyanide; cytochrome oxidase inhibitor; complex IV)

5. **ATP-ADP Translocase Inhibitors** (competitively bind ADP-ATP carrier sites)
   
   Examples:
   
   Cocklebur (carboxyatractyloside)
   
   Blue-lime or Blue Thistle (attractylloside and carboxyatractyloside)

6. **Uncouplers** (abolish linkage of electron transport chain and phosphorylation at the mitochondrial inner membrane)

   Example:
   
   Willow (salicylate) – willow bark not as toxic as medication, ASA
CASE PRESENTATIONS AND DISCUSSIONS OF SPECIFIC TOXINS:

CASES OF NEUROEXCITATION:

CASE 1 (Part of an outbreak)

CC: An Ethiopian teenage male farmer had irreversible spastic myelopathy, without sphincter or bulbar dysfunction.

HPI/PMH: 22 y.o. man presents with 10-year history of BLE spasticity, which had developed acutely and progressed over several days. He had no back pain or sensory symptoms. Initially, his weakness improved slightly over weeks, but he then developed bilateral lower limb spasms and rigidity.

SocH: He reported consuming guaya-kitta, guaya-shiro, and guaya-kollo for his meals. He was no longer able to work. Several people in his village developed similar symptoms around the same time.

Exam: Gait was spastic and scissored, with toe walking. He had sustained clonus and brisk DTRs, with positive Babinski sign. He had LE weakness. UEs normal. CNs intact.

Conclusion: Chronic neuroexcitation (excitotoxicity) from the glutamate agonists (BOAA or β-ODAP), producing neurolathyrism. Neurolathyrism occurs after eating grass pea, also called chickling pea, (Lathyrus sativus, L. cicero, Vicia sativa, V. ervilia).

Several recent epidemics have occurred in Ethiopia (they eat chapatti and (guaya-) kitta (bread from seeds), shiro (flour), kollo (roasted seeds). Other outbreaks have occurred in Bangladesh, India (they eat ghotu (porridge), and Nepal. Numerous historical outbreaks have been documented: in Hindu treatise, by Hippocrates, in Europe during the Middle Ages, in Francisco de Goya’s 1863 painting (Spain, and again in the Spanish Civil War; they eat almorta), and in Wapniarka Nazi labor camp (Ukraine).

Diagnosis of neurolathyrism - Requirements for toxicity:

- Diet of Grass Pea:
  - 300-400 g/day for 2-4 months, with little else to eat
  - Usually malnourished
  - Often, occurs in growing or active, young men
  - Often acute or semi-acute onset, even though toxin has been accumulating for months

About the toxin (BOAA or β-ODAP):

BOAA (Beta-N-oxalylamino-L-alanine) or beta-ODAP (beta-N-oxalyl-alpha, beta-diaminopropionic acid) is a non-protein amino acid that acts as an AMPA glutamate receptor agonist and indirect metabotropic (mGluR1) receptor agonist to produce
excitotoxicity. BOAA is transported into the cell by an Xc\textsuperscript{-} antiporter. BOAA blocks glutamate/cysteine transporter of astrocytes, which contributes to increased glutamate at synapse and increased neuroexcitation. It also limits uptake of cysteine and glutathione production. Reduced glutathione limits the ability to detoxify ROS. Further, BOAA inhibits complex 1 activity in the electron transport chain (secondary to ROS formed during neuroexcitation with resultant excitotoxicity).

**Mechanisms of Action:**

Mitochondrial respiration is essential in preventing calcium overloads.

1. Activation of glutamate receptors can result in increased intracellular calcium with leads to alteration in mitochondrial membrane potential and opening of the mitochondrial permeability transition pore (the beginning of excitotoxicity).
2. Calcium is loaded into the mitochondria to protect the cell, but eventually the mitochondria are overwhelmed and ROS levels increase.
3. Neuroexcitation also decreases formation of glutathione, limiting the neurons ability to manage oxidative stress.
4. Early mitochondrial damage plays a key role in glutamate neurotoxicity (mitochondrial damage results in apoptosis of neurons).
5. Neurons containing calcium-binding proteins are relatively resistant to excitotoxic injury.
6. Motor neurons lack some important calcium-binding proteins (the oculomotor tract is an exception).

Some areas of the CNS are more sensitive, such as the motor cortex and lumbar spinal cord. An upper motor neuron disease is evident after exposure to BOAA. There are alterations of cortical motor regions of brain and loss of upper motor neurons of cortex of brain.

Other diseases can show similar patterns of excitotoxicity. Damage from other illnesses (such as ischemia, hypoglycemia, and ALS), also results in increased Ca++ release and excitotoxicity. Excitotoxicity from these illnesses are also associated with excessive accumulation of calcium in the cell and mitochondria, resulting in ROS that inhibit mitochondrial complex I (due to oxidation of critical thiol groups). Oxidation of protein thiol groups secondary to ROS occurs after calcium overwhelms the mitochondria.

*Key point:* glutamate excitotoxicity, and the associated calcium and ROS overload, is a major factor in many neurodegenerative disorders.

**Treatment:**

Treatment of neurolathyrism has included tolperisone (expensive); surgical resection of thigh adductor muscles; botulinum toxin IM injection; physical therapy; increase methionine and cysteine in diet with cereals rich in sulphur-containing amino acids (such as: wheat, maize, Ethiopian teff).

**Prevention:**
Prevention has included autoclaving the seed with lime, which removes the toxin; however, bacterial or fungal fermentation and moist heat (soaking/steaming/boiling) also reduce the toxicity (more practical). Famine relief would be helpful.

Experimental prevention of toxicity:

1. Pre-treatment with thiol delivery agents (e.g., alpha-lipoic acid) abolishes L-BOAA-mediated complex I dysfunction (experimentally in lab).

ALS-PDC of Guam:

Another cause of neuroexcitation from a glutamate agonist is endemic ALS-Parkinsonism-Dementia complex of Guam, which presents as both a subacute or chronic mitochondrial failure. This outbreak has resolved, but it provides insight into plant-induced mitochondrial failure. Interestingly, although the mechanisms of action are very similar to those seen in neurolathyrism, the disease presents differently, with a spectrum of subacute mitochondrial failure (ALS-like picture when onset is in adolescents) to chronic mitochondrial failure (dementia is seen with senescence onset). This illustrates the fact that the strength of the toxin and the duration of the exposure can produce different neurological diseases. In the case of ALS-PDC of Guam, the toxin accumulates over many years. (Trojsi F, 2013 and Banack SA, 2006 and Murch SJ, 2004)

Spectrum of disease:

![Spectrum of disease diagram](image)

History: In the 1950s an endemic foci of ALS with a frequency of 100X that in the rest of the world was noted (200 cases/100,000 people/year) in Guam. In Guam, at that time, up to a third of adult deaths were due to ALS-PDC. In historical review, the first reports of this syndrome were actually recorded in the late Spanish Period (1700-1800); however, it was not until 1944 when a Navy neurologist, Dr. Zimmerman, noted the high incidence of ALS in Guam that it was spotlighted in modern medicine.

The indigenous people (Chamorro) of Guam call ALS “lytico” and the Parkinson-dementia complex “bodig.” They had already divided the disease into separate entities, which now appear to be subacute mitochondrial failure (lytico or ALS) and chronic mitochondrial failure (bodig or PDC).
An age-dependent phenotype was noted: adolescent onset associated with ALS, middle-life onset associated with Parkinsonism, and senescence onset associated with dementia. This may reflect a strength (or dose) of toxin (see diagram above).

The toxin associated with ALS-PDC of Guam, BMAA (beta-methylamino-L-alanine), is a non-protein amino acid that acts as an AMPA and KA glutamate receptor agonist (also a NMDA and mGluR1 and 5 glutamate agonist).

Epidemiology: The natives of Guam use a cycad flour made from *Cycas micronesica* (cycads, which are small palms, are now critically endangered due to the introduction of the exotic pest the Cycad Scale (*Aulocapsis yasumatsui*)). In time, it was recognized that a symbiotic relationship between the cycads and a cyanobacteria living in its roots existed. The cyanobacteria, which produce BMAA, are not felt to be responsible for the disease. Consumption of animals (flying foxes or fruit bats (*Pteropus tokudae*)) that consumed cycads was felt to result in increased toxicity due to biomagnification of BMAA. The prevalence of disease has declined significantly, with the extinction of the flying foxes (due to over-hunting) and the loss of cycads (due to pest-infestation). The diet has changed and the disease has disappeared.

Chronic BMAA exposure leads to misincorporation of BMAA into brain proteins, which in turn produces protein misfolding, aggregates, and cell death. BMAA accumulates as a neurotoxic reservoir in the brain and is subsequently released during protein metabolism. This results in a slow, sustained neurotoxic insult. BMAA may require the presence of bicarbonate to become toxic. Like BOAA, with neurolathyrism, BMAA increases intracellular Ca++ while depleting glutathione and increasing ROS.

Unique exam finding (eyes): linear retinal pigmentary epitheliopathy

Autopsy (brain): elevated brain BMAA levels, neurofibrillary tangles

Aside: Though previously seen in the Mariana Islands (Guam and Rota), later there were been a similar outbreak in Japan (northern Kii peninsula), where a very similar disease pattern was noted. Also, an increased incidence of ALS-PD was noted in the Auyu and Jakai people of West New Guinea. The etiologies of these epidemics are unclear. The Kii outbreak and the West New Guinea outbreak are gradually resolving. (Gajdusek DC, 1982)

Caution: BMAA levels are elevated in patients with ALS and PD from other countries (including US) with no known cycad exposure. However, cyanobacteria are ubiquitous and over 90% of cyanobacterial species produce BMAA. There are also free living *Nostoc* species of cyanobacteria in fresh and salt water, which contributes to BMAA bioaccumulation in fish, mussels and oysters. Some believe that cyanobacteria exposure may contribute to neurodegenerative diseases. (Trojsi F, 2013)

In summary, neurodegeneration is induced by activation of glutamate receptors with exposure to both BOAA and BMAA.
Summary of neurolathyrism and ALS-PDC: Subacute and chronic exposure to mitochondrial toxins can produce mitochondrial failure that results in permanent, devastating neurological disease. The clinical appearance of this disease may be delayed for months (neurolathyrism, a form of subacute mitochondrial failure) or years (ALS-PDC; a spectrum of subacute to chronic mitochondrial failure).
CASES OF INHIBITION OF BETA-OXIDATION OF FATTY ACIDS:

CASE 2 – An Outbreak

In India (Muzaffarpur district of Bihar), in June of 2013, an outbreak of acute neurologic illness in 133 young children (most 5 yrs and younger) occurred.

The findings included:

- Afebrile
- Hypoglycemia
- Early morning seizures followed by AMS
- Non-inflammatory encephalopathy
- Cerebral edema
- Upper motor neuron findings (hypertonia; Babinski’s sign)
- Victims tended to be chronically undernourished
- High fatality rate (44%)

Cause of outbreak determined by epidemiologic study: lychee fruit (*Litchi sinensis* or *Litchi chinensis*)

- Occurred during lychee harvesting season
- Methylenecyclopropylglycine (MCPG) found in lychee seeds was previously known to produce hypoglycemia

Similar outbreaks in Vietnam:

- Since 1990s, unexplained outbreaks of acute encephalitis
- Specific location: Bac Giang Province (highest lychee production in Vietnam)
- Seasonal (92% in May-July, the litchi season)
- Young (88% < 15 yo)
- Fever, coma, seizures during the night, high fatality rate
- Conclusion: may be from lychee fruits
- Other causes excluded: no viral cause by CSF evaluation

Other areas with risk of lychee outbreaks (lychee production):

- Taiwan*
- Thailand*
Lychee (*Litchi chinensis*) toxin:

1. Lychee fruit contains methylenecyclopropylglycine (MCPG) which produces hypoglycemia and encephalopathy in undernourished children.
2. MCPG forms compounds with carnitine and coenzyme A (as does hypoglycin).
3. MCPG is found in high concentrations in the seed and semi-ripe pulp (similar to ackee fruits).

MCPG (Rat Studies):

1. MCPG inhibits beta-oxidation of fatty acid.
2. Inhibition of beta-oxidation decreases gluconeogenesis by decreasing NADH supplies.
3. MCPG suppresses ketogenesis, due to inactivation of acetoacetyl-CoA thiolase (note: with hypoglycin, there is marked hyperketonemia).

When the toxin MCPG is given to starved rats, the following are seen (Melde K, 1989):

1. Hypoglycemia
2. Elevated lactate
3. Elevated non-esterified fatty acids
4. Decreased activity of 2-methyl-(branched-chain) acyl-CoA dehydrogenase (similar to hypoglycin)
5. Activity of enoyl-CoA hydratase (crotonase) is inhibited by the toxic metabolite of MCPG (methylenecyclopropylformyl-CoA)
6. The inhibition spectrum of MCPG is different from hypoglycin A, with MCPG causing accumulation of medium-chain acyl-CoA thioesters.
It was noted by CDC, who consulted medical toxicologists, and others that this was very similar to the encephalopathy, hypoglycemia and seizures caused by Ackee (*Blighia sapida*). Both ackee and lychee fruit trees are members of the Sapindaceae (soapberry) family, as is longan (*Dimocarpus longan*).

Ackee outbreaks:

Fatal encephalopathy in West Africa:

- children 2 - 6 years
- vomiting, hypotonia, convulsions, coma
- all died within 48 hours of onset of vomiting
- necropsy: liver steatosis, severe hypoglycemia
- urine: dicarboxylic acids

100 cases of ackee poisoning in Haiti:

- similar findings; many deaths
- carnitine derivatives (octanoylcarnitine and hexanoylcarnitine) found in urine

Brief review of ackee poisoning:

Ackee (*Blighia sapida*) poisoning is generally from the consumption of unripe fruit. Unripe fruit, with a closed yellow aril, is toxic, while ripe fruit, with an open red aril, is nontoxic. Ackee seeds contain hypoglycin B and are always toxic while the unripe ackee fruit contains hypoglycin-A (L(R,S)-2-amino-3-methylene-cyclopropylpropionic acid) which is converted to methylenecyclopropylacetyl-coenzyme A. Both are hypoglycemic agents, but the latter is a suicide inhibitor of beta-oxidation of fatty acids.

Hypoglycin and MCPG have very similar chemical structures and both have toxic metabolites, with similar structures.

Treatment of lychee fruit poisoning should be similar to treatment of ackee fruit poisoning: supportive care with close monitoring and replacement of glucose, benzodiazepines and barbiturates for seizures (after dextrose), and administration of L-carnitine. Experimentally, riboflavin, clofibrate, glycine, and methylene blue have also been tried for ackee fruit poisoning; however, it is unknown if these would be clinically helpful.

**Summary of lychee and ackee fruit toxicity:** Acute mitochondrial failure results in acute metabolic and neurological failure followed by multiple organ system failure. Mortality is high.
CASES OF INHIBITION OF KREBS CYCLE:

Paucity of Cases:

There are no human cases of significant toxicity from monofluoroacetate or methyl salicylate toxicity following plant exposure found by PubMed search. There are many cases of monofluoroacetate toxicity and death in livestock animals, following toxic plant exposure. Presumably, humans would suffer significant toxicity in they consumed sufficient quantities of plants containing monofluoroacetate.

Monofluoroacetate containing plants:

Over 40 plants, worldwide, that contain monofluoroacetate:

- **Africa**: *Dichapetalum cymosum* (Gifblaar)
- **Sierra Leone**: *Chailletia toxicaria*
- **Brazil**: *Mascagnia* spp. (*M. rigida*), *Palicourea* spp. (*P. aenofusca*) and *Arrabidaea* spp.

Mechanism of action: Fluoroacetate and fluorocitrate are preferentially taken up by glial cells, leading to inhibition of the glial Krebs cycle.

Fluorocitrate, which is formed after exposure to monofluoroacetate, inhibits the enzyme aconitase of the Krebs cycle, thereby inhibiting the conversion of citrate to isocitrate.

Clinically, human exposure to fluoroacetate produces:

- Seizures
- Encephalopathy
- Muscle spasms and facial twitching
- Metabolic acidosis
- Hyperglycemia followed by hypoglycemia
- Cardiac dysrhythmias and hypotension
- Multiple organ failure (respiratory, hepatic, renal)
- Death with rapid onset of rigor mortis

Treatment: glycerol monoacetate or ethanol or NAC (experimental) and correction of acid/base and electrolyte disturbances.
CASES OF INHIBITION OF ELECTRON TRANSPORT CHAIN:

CASE 3 (Outbreak; case reported by Caparros-Lefebvre D, et al, 2002)

An African-Caribbean man from Marie-Galante (Guadeloupe archipelago) developed mental and physical slowing at age 53. He was apathetic. His gait and movements slow. He drank teas of Annonaceae leaves as an aphrodisiac.

Later he developed a frontal lobe syndrome with urinary incontinence, sexual disinhibition, visual hallucinations and delusions. On neurological evaluation, he was felt to have frontal dementia with euphoria, echolalia, impaired verbal fluency, disorientation, memory loss, frontal lobe type bladder incontinence. Postural instability with falls, Parkinsonism with prominent nuchal rigidity, vertical supranuclear palsy involving up-gaze and down-gaze ensued. Eventually, he developed severe dysarthria and moderate dysphagia.

The outbreak:

A high incidence of atypical Parkinson syndrome in the French West Indies (Guadeloupe) has been noted (current).

- Postural instability with early falls (symmetry of bradykinesia, rigidity)
- Frontal lobe dysfunction
- Pseudo-bulbar palsy
- L-dopa treatment resistance

Some have progressive supranuclear palsy (PSP, which is generally characterized by movement disorders, speech and swallowing difficulties, visual changes, and mood and behavioral changes)

3 Autopsies:

- 3/3 PSP
- 3/3 Early postural instability (history)
- 3/3 gaze palsy (history)
- 3/3 Parkinsonian symptoms
- 3/3 frontolimbic dementia and corticobulbar signs (after Parkinsonian sx)

Found: accumulation of tau proteins, especially in midbrain

Unlike typical Parkinsonism and progressive supranuclear palsy, the tremor is jerky (cortical myoclonus) and hallucinations are seen.

**Proposed causes:** herbal teas and soursop fruits (by epidemiologic studies and finding of toxins in soursop fruits: acetogenins)
Guadeloupean Parkinsonism is felt to be secondary to soursop (Annona muricata of the Annonaceae family) fruit exposure. Sometimes this plant is used to make teas.

**Toxin:** annonacin – an inhibitor of complex I in electron transport

Annonacin is 1000X more toxic than MPP+ (1-methyl-4-phenylpyridinium) to cultured mesencephalic neurons.

**Mechanism of action:** In cultures of rat striatal neurons treated for 48 hrs with annonacin, there is a concentration-dependent decrease in ATP, a redistribution of tau from the axon to the cell body, and cell death. Annonacin induced the retrograde transport of mitochondria, some of which had tau attached to their outer membrane. This shares characteristics with some other neurodegenerative diseases (tauopathies, such as PSP, frontotemporal dementia with Parkinsonism, the Parkinsonism-dementia complex of Guam).

ATP depletion can lead to somatic accumulation of tau. Agents that reduce mitochondrial membrane potential or inhibit ATP production increase the retrograde transport of mitochondria, clustering in the perinuclear region. Tau plays a role in the retrograde transport of damaged mitochondria.

Rodent studies suggest a genetic susceptibility to annonacin-induced tauopathy. (Yamada ES, 2014)

**Clinical findings:**

Clinically, this appears similar to ALS-PDC in Guam (PDC component; similarities include: L-dopa unresponsiveness, akinetic rigid syndrome with axial predominance, postural instability and frontal type dementia).

MRI of Guadeloupean Parkinsonism patients: changes in temporal and occipital lobes, limbic areas and cerebellum. A flat superior profile of the midbrain and midbrain atrophy are seen vs the typical hummingbird sign (concave superior profile of midbrain) in traditional progressive supranuclear palsy.

Of note, this is also seen in New Caledonia (South Pacific), where annonaceous plant products are also consumed.

**Caution:** North American Pawpaw (False Banana) (Asimina triloba, Annonaceae family) also contains acetogenins. Like other herbal medicines or Traditional Chinese Medicines that interfere with mitochondrial function, whole fruit Pawpaw and isolated acetogenins have been advocated as chemotherapeutic agents. (see table of proposed chemotherapeutic agents that are plant mitochondrial toxins)
Differential Diagnosis: Sporadic and Familial (Plain Ol') Parkinson’s Disease (PD)
(Haelterman NA, 2014 and Subramaniam SR, 2013)

Parkinson’s disease, a movement disorder associated with progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta, is generally considered a sporadic disease, but several genetic mutations have been linked to the disease. Certain gene mutations appear to be risk factors for the disease, but environmental factors likely contribute to the disease. General mitochondrial abnormalities linked with PD include mitochondrial electron transport chain impairment. Post-mortem brain tissue from patients with PD shows reduced activity of complex I in the substantia nigra and frontal cortex. Further, alteration in mitochondrial morphology and dynamics, and alterations in calcium homeostasis have been seen in sporadic and familial PD. This helps explain why toxins that affect mitochondrial function (e.g., cause excitotoxicity or effect the electron transport chain) and can present similarly to sporadic and familial PD.

Even sporadic and familial PD are felt to be secondary to genetic susceptibility plus environmental factors. The known mitochondrial toxins contained in plants that are discussed here may represent stronger than typical environmental factors, resulting in higher incidences of PD than weaker (and yet unidentified) environmental factors typically produce.

Interestingly, substantia nigra pars compacta dopaminergic neurons normally use calcium channels for pacemaking, with continual influx of calcium into the cytoplasm. This is unusual for a neuron. Given the sensitivity of neurons to calcium and the danger of calcium producing excitotoxicity, it is likely than this calcium influx contributes to the substantia nigra’s increased susceptibility to mitochondrial insult.

A complex set of mitochondrial defects may cause Parkinson’s disease, including dysfunction of: electron transport chain, mitochondrial protein homeostasis, mitophagy, and fusion and fission of mitochondria. Oxidative damage to complex I of the electron transport chain is felt to contribute to PD.

In PD, alterations in numerous cell processes have been recognized, including alterations in: oxidative stress, excitotoxicity, the ubiquitin-proteasome system and mitochondrial dysfunction.

Haelterman’s Theory: Subtle mitochondrial defects in combination with other (environmental) insults trigger the onset and progression of familial and idiopathic PD.

Further, mitochondrial dysfunction likely underlies many other motor neuron disorders (ALS, Friedreich’s ataxia, Huntington’s disease, Alzheimer’s disease), as well. (Pan-Montojo F, 2014 and Trojsi F, 2013)
CASE 4 (reported by Fu PK, 2012)

A 54 y.o woman presents to a hospital in China. In the ED she c/o acute onset of diffuse abdominal pain, watery diarrhea and lethargy after eating 40 pieces of yam bean seeds \textit{(Pachyrhizus erosus or Pachyrhizus tuberosus)} for breakfast 4 hours earlier. She became dyspneic and drowsy. Exam: HR 34 bpm, BP – not measurable (both HR and BP responded to atropine). GCS = 4, resulting in intubation. AG = 31, BE = -11.5. Head CT normal at 24 hours. Negative cyanide testing.

After 2 weeks, patient remained altered.

Head MRI (3 weeks after exposure): diffuse irregular high signal intensity on T2W1 and FLAIR, without contrast enhancement over the periventricular white matter. In conjunction with her neurobehavioral deficits, a diagnosis of leukoencephalopathy from toxin-induced brain insult was made.

Yam bean seeds \textit{(Pachyrhizus erosus or Pachyrhizus tuberosus)} were carried to Asia and the Pacific islands from Mexico and Central America. Compounds extracted from the yam bean seeds contain rotenone and rotenoid compounds. Rotenone is more typically associated with the tuba plant \textit{(Derris elliptica)}.

Modern day cases are more likely to occur from the ingestion of the isolated toxin, rotenone. Two examples include:

A 47 y.o. married Indian woman (in India), with DM on metformin, was found to be coughing and vomiting after a domestic dispute. Later, she had difficulty breathing and admitted to ingesting rotenone. Upon hospital arrival, GCS was 3, BP 104/64 and pH 7.09, with respiratory distress. She received hemodialysis and had correction of the metabolic acidosis and some clinical improvement, but had multiple system organ dysfunction, including centrilobular hepatic necrosis, and required vasopressors. The patient ultimately died.

A young child ingested rotenone and suffered cardiopulmonary failure and death, with autopsy revealing hemorrhages in gastric mucosa, lungs, heart, thymus and hypoxic brain damage.

**Summary of signs and symptoms of acute exposure (acute mitochondrial failure):**
N/V, incoordination, seizures, encephalopathy, metabolic acidosis, respiratory distress, bradycardia, dysrhythmias.

**In the past:**

Derris was likely used by primitive peoples as a fish poison, since prehistoric times. The derris root is still placed in corals to kill fish in some small fishing communities.

Derris has also been used as a suicidal agent in the Philippines and Netherland East Indies, where the victim usually drinks a juice from the tuba plant \textit{(Derris elliptica)} or eats the root. In the 1930s, derris root (known as a “bun”; a “tuba” or an “akar-tuba”)}
was the most common method of suicide in New Ireland, a mandated territory of New Guinea (East Indies). Prior to death, the suicidal victims had weak pulses and mydriasis (in tribal communities), and the post-mortem examinations revealed acute congestive heart failure.

**Amount required for toxicity:** The cortex nibbled from a piece of fresh root <1/4 inch and 3 inches long can kill an adult.

**The toxin and its mechanism of action:** Rotenone inhibits mitochondrial cellular respiration. It is an inhibitor of NADH dehydrogenase (Complex 1), binding to the acceptor end of the enzyme, producing leakage of electrons that combine with oxygen to form superoxide. The resultant oxidative stress is damaging. Rotenone may induce apoptosis.

Interestingly, rotenone exposure is suspected to increase the risk for PD in humans. (Tanner CM, 2011) This illustrates that the strength or dose of a toxin determines the type of mitochondrial failure seen. Acutely, high doses produce encephalopathy and are often fatal, while prolonged low doses produce chronic mitochondrial failure, including PD.

**Experimental treatment:** The loss of dopaminergic neurons after rotenone exposure was significantly attenuated by methylene blue (an agent that is an alternative electron carrier that by-passes complex I/III blockade). (Wen Y, 2011)

**Future uses:** Rotenone has been investigated experimentally used to treat neuroblastomas. Will patients be at increased risk for PD?
CASE 5 (Case Series)

At a bamboo shoot pickling factory in Thailand:

Patient 1 dropped 20 kg fresh bamboo shoots into shoot pickling well and jumped in to retrieve the bag. He immediately lost consciousness in the well.

Patient 2 jumped in the well to rescue Patient 1, but immediately lost consciousness.

Patients 3, 4 and 5 jumped in the well to rescue Patients 1 and 2. All lost consciousness.

Patient 6 jumped into the well and immediately lost consciousness.

Patients 7 and 8 jumped into the well and immediately lost consciousness.

Other workers wore cloth masks and tied themselves with ropes and went down to lift the 8 patients out of the well.

Rescue workers arrived 30 minutes later.

Diagnosis: acute mitochondrial failure

CASE 6

A 41 yo woman ingested apricot kernels purchased from a health food store and became weak and dyspneic within 20 minutes. She was comatose and hypothermic upon presentation.

Diagnosis: acute mitochondrial failure

CASE 7 (Epidemic; reported by Howlett WP, 1990)

At the Christian Medical Center, Moshi, Tanzania:

An epidemic of spastic paraparesis was noted.

Uniform clinical findings were found in 39 cases, with symmetric, spastic paraparesis.

[aged 4-46 y.o. (most were 10-19 y.o.); 30 males; 9 females]

Abrupt onset of difficulty walking, with progressive paraparesis over 2-3 days
Typically, awoke with symptoms or symptoms developed after a long walk
Complained of generalized weakness (inability to rise from supine to sitting)
No fever or symptoms of infection
Lumbar pain radiating down legs and leg numbness
Some with difficulty speaking (hoarseness; muteness; often transient)
Some with visual changes (associated with pale discs)
Hyperreflexia and ankle clonus
Extensor planter responses
Increased tone with spasm in flexion of LEs
Eventual contractures
Eventual thoracolumbar kyphoscoliosis
UE relatively spared, but some had weakness
Sensation remained intact

10 month follow up: unchanged, with scissor gait, flexion at hips and knees, valgus ankle deformity, plantar flexion of feet with abduction of forefoot and toes. The damage was permanent, but not progressive.

Diagnosis: subacute mitochondrial failure.

**Diet (exposure) history:** stiff porridge made from flour called "kigoma," “ugali” and “ndiaraye.” The flour is made from processed cassava roots.

Due to drought, and failure of other crops, diet at the onset of illness consisted almost exclusively of bitter cassava roots. The drought increased the cyanogenic glycosides in the cassava roots and the processing was cut short due to the food shortage.

This epidemic was similar to epidemics of paraparesis in Mozambique and Zaire, given the name, Konzo. Low sulphur diets may contribute to toxicity, due to sulphur being used to detoxify cyanide (rhodanese in mitochondria transfers a sulfur atom from thiosulfate to cyanide to produce thiocyanate, which is much less toxic than cyanide).

Konzo is now known to be an upper motor neuron disease entity caused by toxic effects from insufficiently processed cassava.

The toxins: cyanogenic glycosides, yield hydrocyanic acid on hydrolysis via linase, in the case of linamarin induced cyanide toxicity.

<table>
<thead>
<tr>
<th>Cyanogenic Glycoside</th>
<th>Plant (example)</th>
<th>Clinical Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amygdalin</td>
<td>Almonds (<em>Prunus amygdalus</em>)</td>
<td></td>
</tr>
<tr>
<td>Prunasin</td>
<td>Stone fruits (<em>Prunus</em> spp.)</td>
<td>Acute cyanide toxicity</td>
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<tr>
<td>---------------</td>
<td>------------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Linamarin</td>
<td>Cassava (<em>Manihot esculenta</em>)</td>
<td>Konzo</td>
</tr>
<tr>
<td></td>
<td>Lima beans</td>
<td></td>
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<tr>
<td>Lotaustralain</td>
<td>Cassava (<em>Manihot esculenta</em>)</td>
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<tr>
<td></td>
<td>Lima beans</td>
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<tr>
<td>Dhurrin</td>
<td>Sorghum (<em>Sorghum album</em>)</td>
<td></td>
</tr>
<tr>
<td>Taxiphyllin</td>
<td>Bamboo shoots (<em>Bambusa vulgaris</em>)</td>
<td>Acute cyanide toxicity</td>
</tr>
</tbody>
</table>

**Mechanisms of action:**

Cyanide binds cytochrome oxidase in the mitochondrial inner membrane. Cytochrome oxidase is the terminal enzyme (complex IV) in the electron transport chain and transfers electrons onto oxygen to produce water (cytochrome oxidase accepts electrons from cytochrome c and passes them on to oxygen). Cyanide thereby inhibits electron transport, which prevents downstream oxidative phosphorylation and oxygen consumption. This results in a metabolic acidosis.

**Clinical presentation (acute, severe toxicity):**

Organs most sensitive to energy deprivation (brain and heart) are most affected in acute poisoning, as is demonstrated by plant-induced illnesses that produce acute mitochondrial failure. Clinically, acute cyanide toxicity reveals: rapid onset of CNS (coma, seizures) and cardiac dysfunction (tachycardia or bradycardia and dysrhythmias), with concomitant metabolic acidosis. Dyspnea and respiratory failure, as well as other organ failure, may be seen. Survivors may have permanent neurologic sequelae, including necrosis of the basal ganglia.

**Treatment:** supportive and cyanide antidote kits
CASES OF INHIBITION OF ATP-ADP TRANSLOCATION:

CASE 8 (Outbreak)

Bangladesh, November 2007:

Flooding ruined rice crops and prices rose. Poultry died. Government prohibited villagers to collect and sell stones.

A woman and her child presented unconscious to the hospital.

Antecedent vomiting and restlessness prior to LOC had been noted.

They died within hours (another one of her 2 remaining children died within hours)

In the following days, 18 patients presented with similar symptoms

Ultimately, 76 patients were identified (many died before seeking care):

- Vomiting (100%)
- Fever (61%)
- Elevated LFTs
- AMS (59%; 38% unconscious)
- Fatality (25%) – children accounted for the majority of deaths, with a mortality closer to 35%
- Consumed “ghagra shak” a few hours prior to illness onset

Ghagra shak = Common Cocklebur = Xanthium strumarium

While the population had previously consumed small amounts of this plant, new dependence on X. strumarium resulted in increased toxicity due to an increased dose.

Toxin: Cocklebur (Xanthium strumarium and X. spinosum) have a worldwide distribution. The seeds taste like sunflower seeds, but contain the toxin carboxyatractyloside which inhibits translocation of ADP and ATP across mitochondrial membranes.

Clinical presentation: Abdominal pain, nausea, vomiting, diaphoresis, respiratory depression is seen. Also, as is characteristic of acute mitochondrial failure, encephalopathy, seizures and metabolic acidosis are seen. The seizures can be difficult to treat. Multiple organ system failure may ensue (hepatic, renal). A consumptive coagulopathy has also been described.

EKG - ST segment abnormalities seen

Autopsy: centrilobular hepatic necrosis, renal proximal tubular necrosis, microvascular hemorrhage of CNS, leukocytic infiltrates of muscle, pancreas, lungs and myocardium
CASE 8b

A 7 y.o. boy drank an extract of the root of *Atractylis gummifera* (bird-lime, blue thistle, glue thistle) as a traditional medicine.

He was admitted to the hospital 2 days after ingestion:

- Epigastric pain
- Vomiting
- General anxiety
- Coma

Labs: liver failure, acute renal failure

He died 8 days after admission

Autopsy: panlobular hepatic necrosis (differential dx: Reye syndrome)

Toxicity has occurred after topical application, as is illustrated by this case:

A 30-month old boy was admitted with coma.

Hepatic cellular injury, cholestasis, decreased prothrombin level, increased Cr were noted.

History revealed repeated occlusive cutaneous application of *A. gummifera*

He survived, with residual renal insufficiency.

**Plant locations:** *Atractylis gummifera*, is found in North Africa (Morocco, Tunisia, Algeria), Asia Minor and southern Europe (Spain, Portugal, Italy, Greece, France)

**Exposures:** accidental with high fatality in children, chewing the sweet gum from the latex, traditional medicine use, misidentification (mistaken for artichoke (*Scolymus hispanicus* L)).

**Toxins:** atracyloside and carboxyatractyloside (diterpenoids)

These toxins inhibit ATP-ADP translocase, a transporter protein that enables ATP and ADP to transverse the inner mitochondrial membrane, so that ATP may leave the mitochondrial matrix and journey to the cytoplasm and so that ADP may return to the matrix from the cytoplasm and be utilized by ATP synthase to make more ATP. This is so important to the function of the cell that more than 10% of the protein in the inner mitochondrial membrane consists of ATP-ADP translocase.

**Clinical presentation:** Coma, seizures, hypoglycemia (may be transiently hyperglycemic), and multiple system organ failure (hepatorenal failure, which may also be secondary to P450 cytochrome inhibition). Hypotension and dyspnea may be seen.
**Prognosis:** High fatality rate, even if unintentionally consumed; represents the leading cause of death by plant poisoning in Morocco. Most fatalities are in children. Hepatitis and encephalopathy are strongly associated with mortality.

**Treatment:** Supportive; there is research on Fab antibody fragments; liver transplantation for fulminant hepatic failure

Of note, Ox-eye daisy (*Callilepis laureola*), found in South Africa, where it is sometimes used as an herbal medicine. It produces coma and hypoglycemia, with hepatorenal failure, and is often fatal. It may appear similar to Reye’s syndrome. (Watson AR, 1979)

Like *A. gummifera*, *C. laureola* contains atractyloside and carboxyatractyloside. The clinical toxicity appears similar.
THE NEW FRONTIER - USE OF PLANT-DERIVED MITOCHONDRIAL TOXINS TO TREAT CANCERS:

Mitochondria play an important role in cell death; this is achieved by changing outer and inner mitochondrial membrane permeabilities, leading to release of cytochrome c and activation of caspases (cysteine-dependent aspartate-directed proteases). This results in a cascade that induces apoptosis (programmed cell death).

Mitochondrial-induced apoptosis:

1. Intrinsic (mitochondrial) pathway
   Induce cell death by activation of caspases (cysteine proteases), with a resultant cascade of cell death.
   
   Once you start the cascade, there’s no turning back.

   Release of proteins from the intermembrane space of mitochondria
GENERAL TREATMENTS FOR PLANT TOXINS THAT INDUCE MITOCHONDRIAL DYSFUNCTION:

Antioxidants, such as N-acetylcysteine and glutathione, reverse berberine-induced apoptosis in cell line research. Perhaps, NAC would be beneficial after exposure to plants that produce mitochondrial injury or to treat toxicity from mitochondrial toxins used as chemotherapeutic agents.

Methylene blue, an alternative electron carrier that bypasses complex I and III, may be useful for inhibitors of the electron transport chain.

Carnitine may be useful for inhibitors of beta-oxidation of fatty acids.

Glucose supplementation and correction of acid/base disturbances (with hemodialysis, if needed) should be considered for acute mitochondrial failure, regardless of the site of inhibition.
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**List of Abbreviations Used:**

ALS/PDC = amyotrophic lateral sclerosis-parkinsonism dementia (of Guam)

BMAA = beta-methylamino-L-alanine

BOAA = beta-N-oxalylamino-L-alanine

β-ODAP = beta-N-oxalyl-alpha, beta-diaminopropionic acid

ECT = electron transport chain

MPP+ = 1-methyl-4-phenylpyridinium, the metabolite of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), which together inhibit complex I, III, and IV

mtDNA = mitochondrial DNA

PD = Parkinson’s disease

ROS = reactive oxygen species