

# ***TOXICOLOGY OF ANTICHOLINESTERASE COMPOUNDS (ANTICHOLINESTERASE PESTICIDES)***



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- **The pesticides** are any **substances or mixture** of substances intended **for preventing, destroying or mitigating any pest**.
  - The pesticides are usually more specifically classified on the basis of **their use** and **organism killed**:
    - **insecticides**
    - **herbicides**
    - **fungicides**
    - **acaricides**
    - **rodenticides**
    - **defoliants etc.**

# Classes Of Pesticides

## ➤ Insecticides (kill insects)

- Organochlorines
- Organophosphates
- Carbamates
- Synthetic Pyrethroids



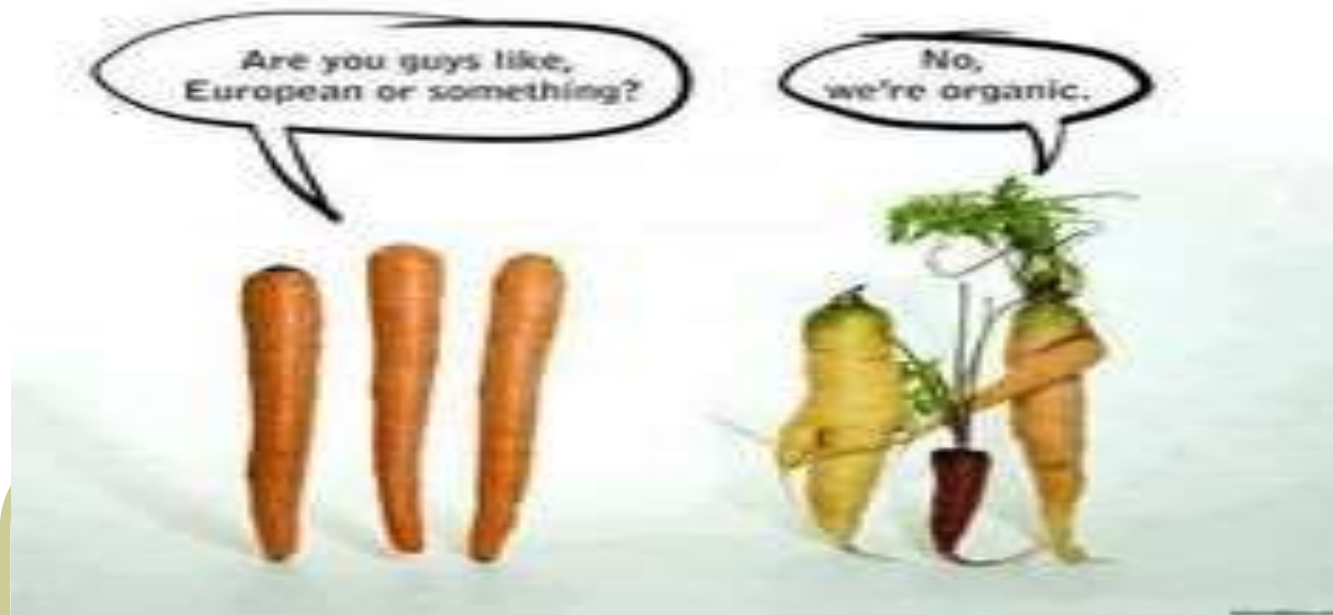
## ➤ Herbicides (kill plants)

## ➤ Rodenticides (kill rodents)

## ➤ Fungicides (kill fungus)

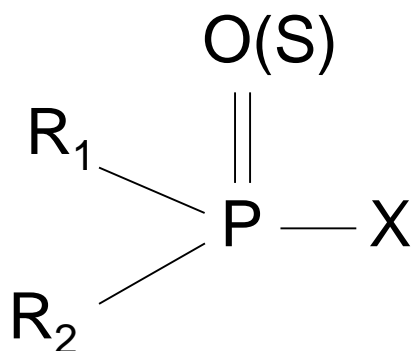
## ➤ Fumigants (kill whatever)





## **ANTICHOLINESTERASE PESTICIDES**

**Anticholinesterase pesticides** have a common mechanism of action, but arise from two distinctly different chemical classes - **the esters of phosphoric or phosphorothionic acid** and those of **carbamic acid**.



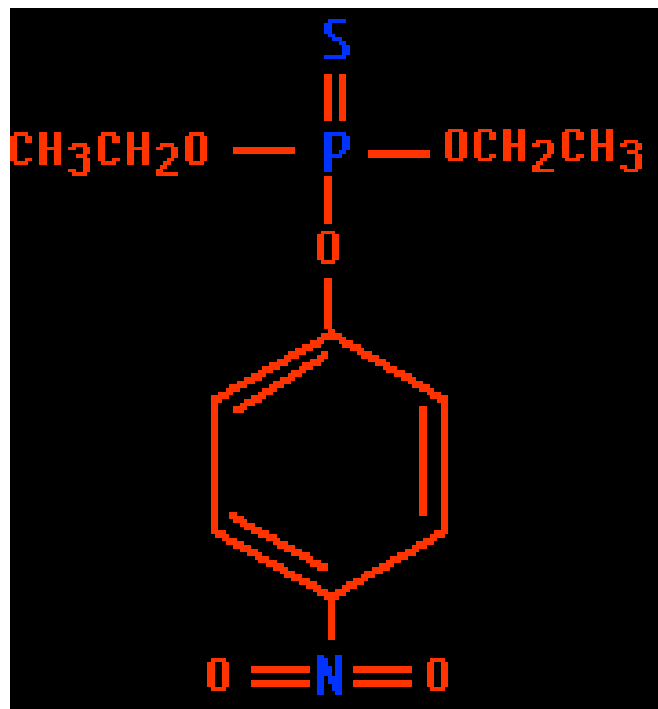
X represents the leaving group, R1 and R2 the side groups, usually alkoxy groups.

In very toxic agents the leaving group contains fluorine (F), which has high tendency to hydrolysis and thus extremely high AChE inhibition. In less toxic OPs the leaving group usually contains alkyl or aryl groups. The majority of novel OP pesticides possess the thion, P=S.

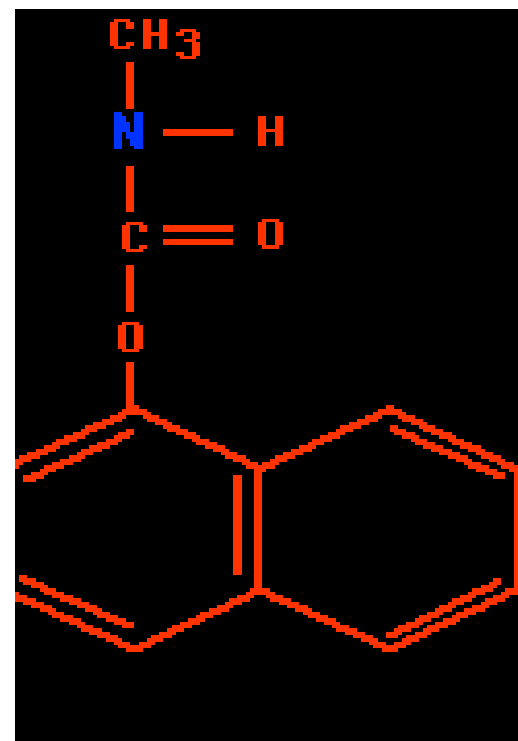
# Pesticides

## OPs and Carbamates

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**Parathion**



**Carbaryl**



# ***Toxicokinetics***

- **Absorption** can occur from all body surfaces and especially the **gastrointestinal tract, skin, lungs, and eyes**.
- **Distribution is rapid**, but organophosphorus and carbamate products **do not accumulate in the fat depots**.
- It is important to note that:
- **Condition that promote phase I or mixed function oxydase activity are likely to increase the toxicity of organophosphates** by converting them to the corresponding oxygen analog.
- **Hydrolysis of the ester linkage in organophosphates or carbamates markedly decreases toxicity**.



**Exposure to OP**

**ABSORPTION**

**DISTRIBUTION**

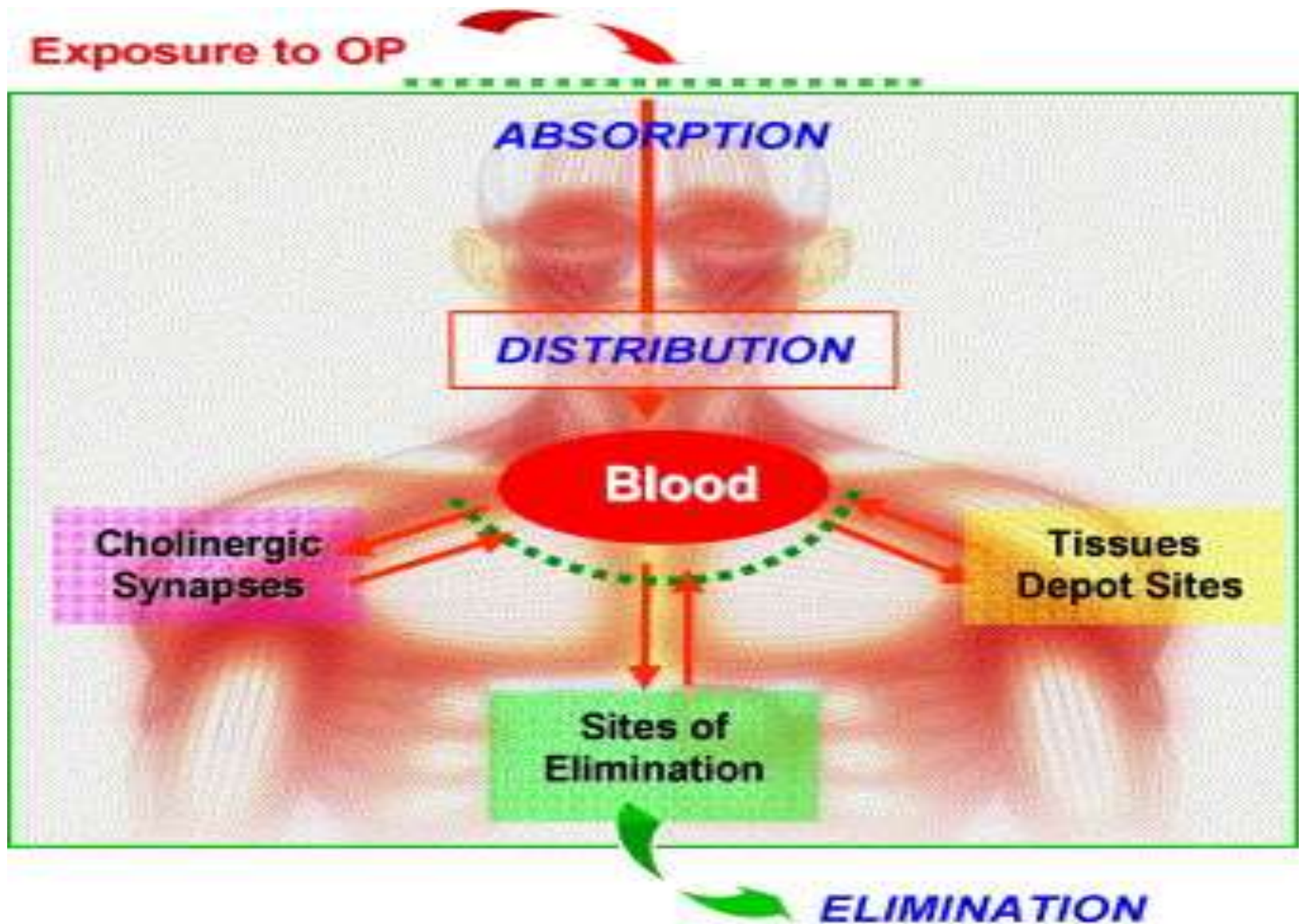
**Blood**

**Cholinergic  
Synapses**

**Tissues  
Depot Sites**

**Sites of  
Elimination**

**ELIMINATION**



## Mechanism of toxic action

Although the anticholinesterase type insecticides have a **common mode of action**, there are **significant differences between organophosphorus and carbamate esters**.

There are **two kinds of AchE** in the human body.

**True cholinesterase** is found primarily in the **nervous system** and **red blood cells**.

**Butyrylcholinesterase (BuChE)** or **pseudocholinesterase** is found **in the plasma and liver**. OP insecticides also inhibit its activity. BuChE is not subject to substrate inhibition, because BuChE is an enzyme present at the synaptic cleft that has a less specific activity than AchE.

**Acetylcholine (Ach)** is involved in **neurotransmission** at **motor, autonomic and central synapses**.

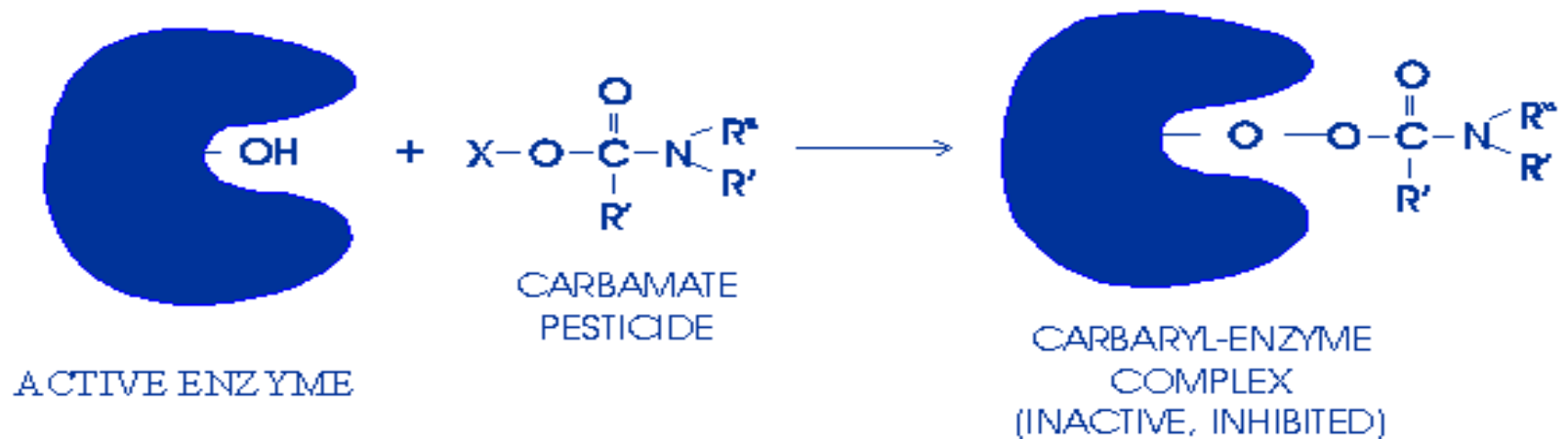
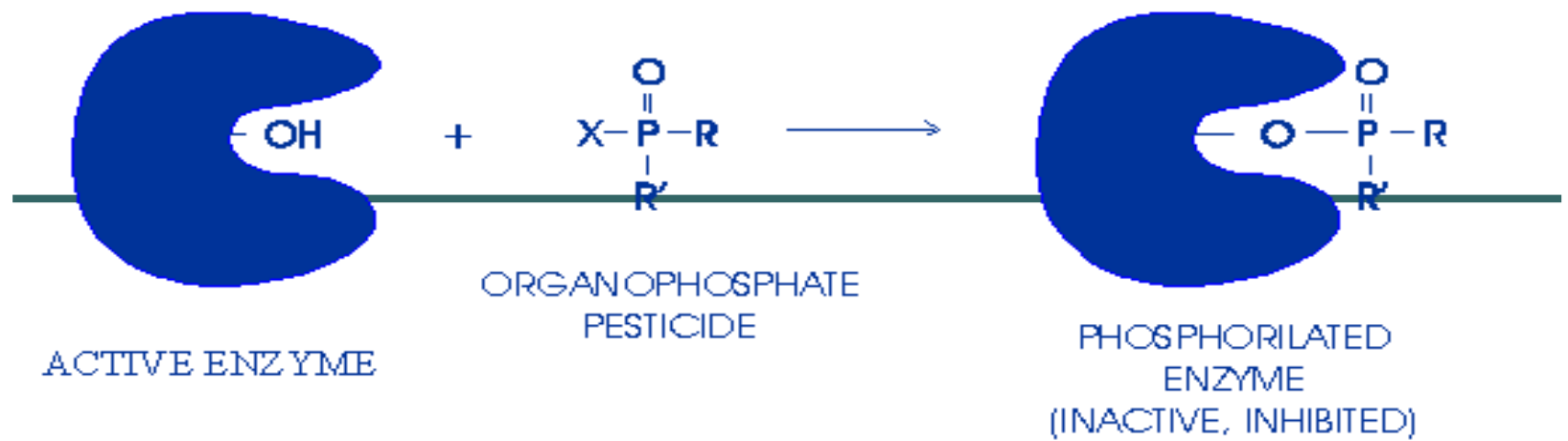
For **cholinergic neurotransmission** to occur properly, **acetylcholine** must be **inactivated rapidly** by the enzyme **acetylcholinesterase (AchE)**.

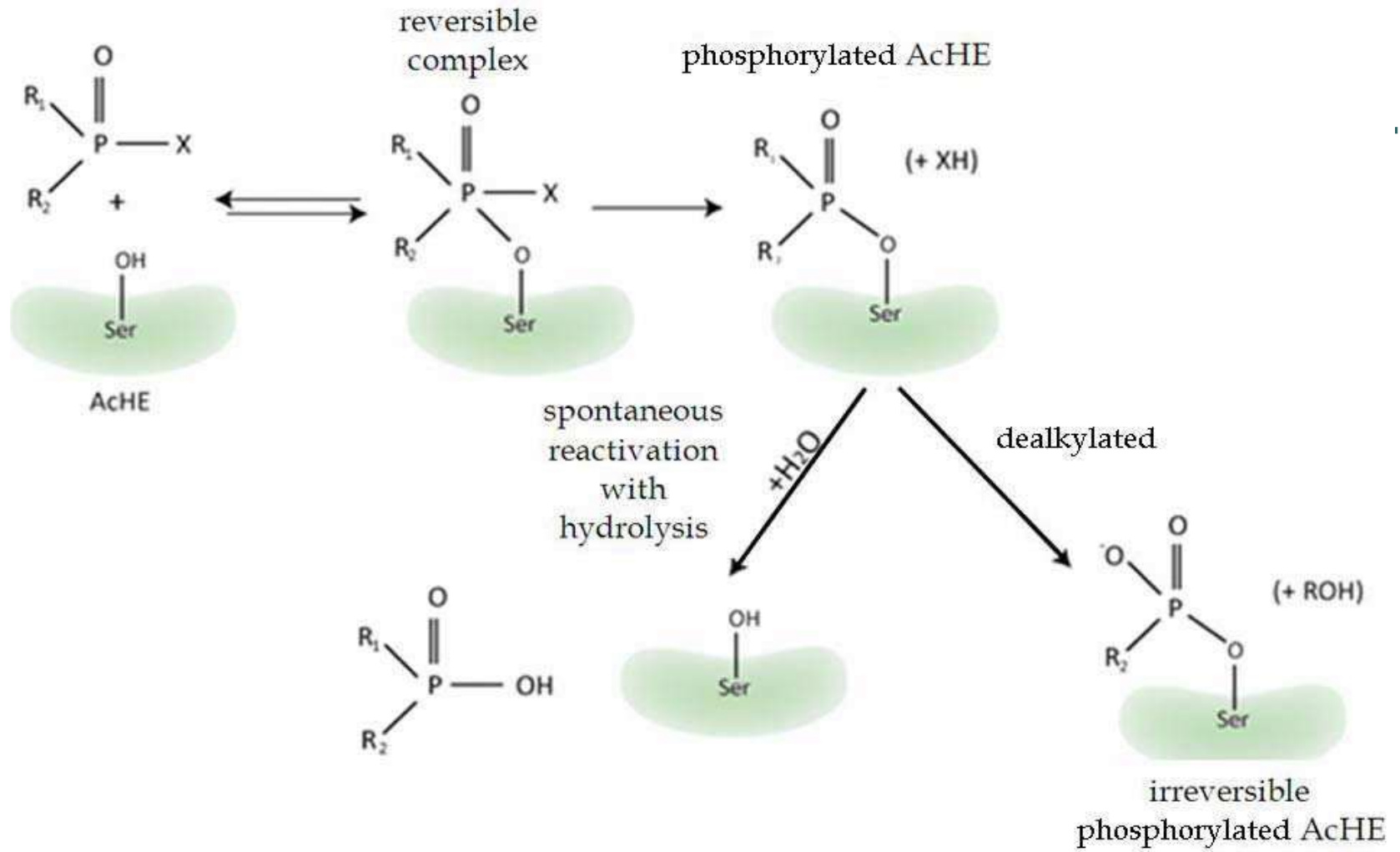
This removal **prevents repeated stimulation** of the receptors.

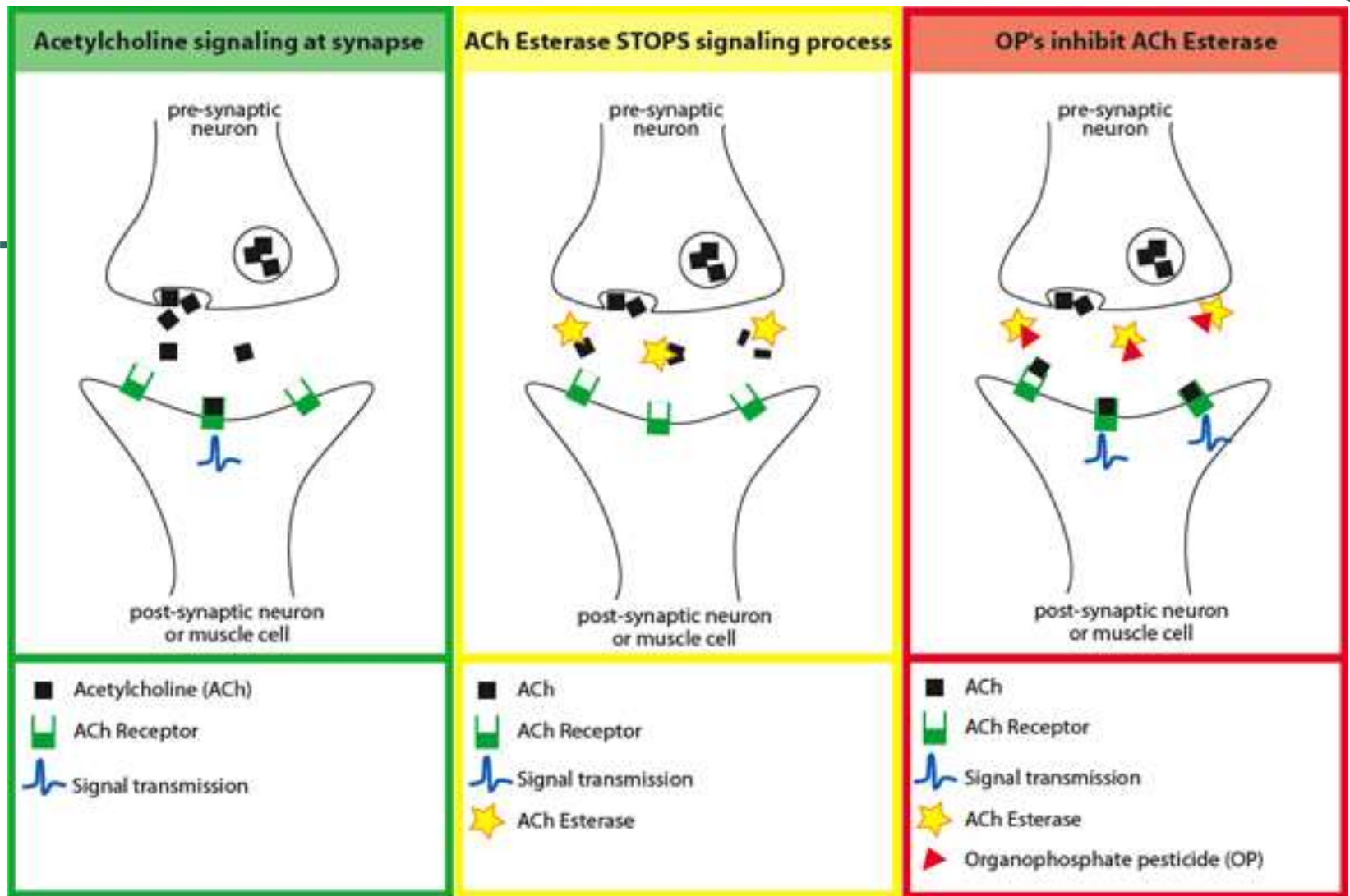
## Mechanism of toxic action


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- The organophosphorus pesticides **phosphorylate** and **inactivate** the enzyme.
- The reaction between the organophosphorus ester and **the active site in acetylcholinesterase protein (a serine hydroxyl group)** results in the formation of **a transient intermediate complex**.
- This complex **partially hydrolyses** leaving a **stable, phosphorylated and largely unreactive, inhibited enzyme**.
- Under normal circumstances the enzyme can be reactivated **very slow**.
- So, with many organophosphorus ester insecticides, an **irreversibly inhibited enzyme is formed**, and **the signs and symptoms** of intoxication are **prolonged** and **persistent**.







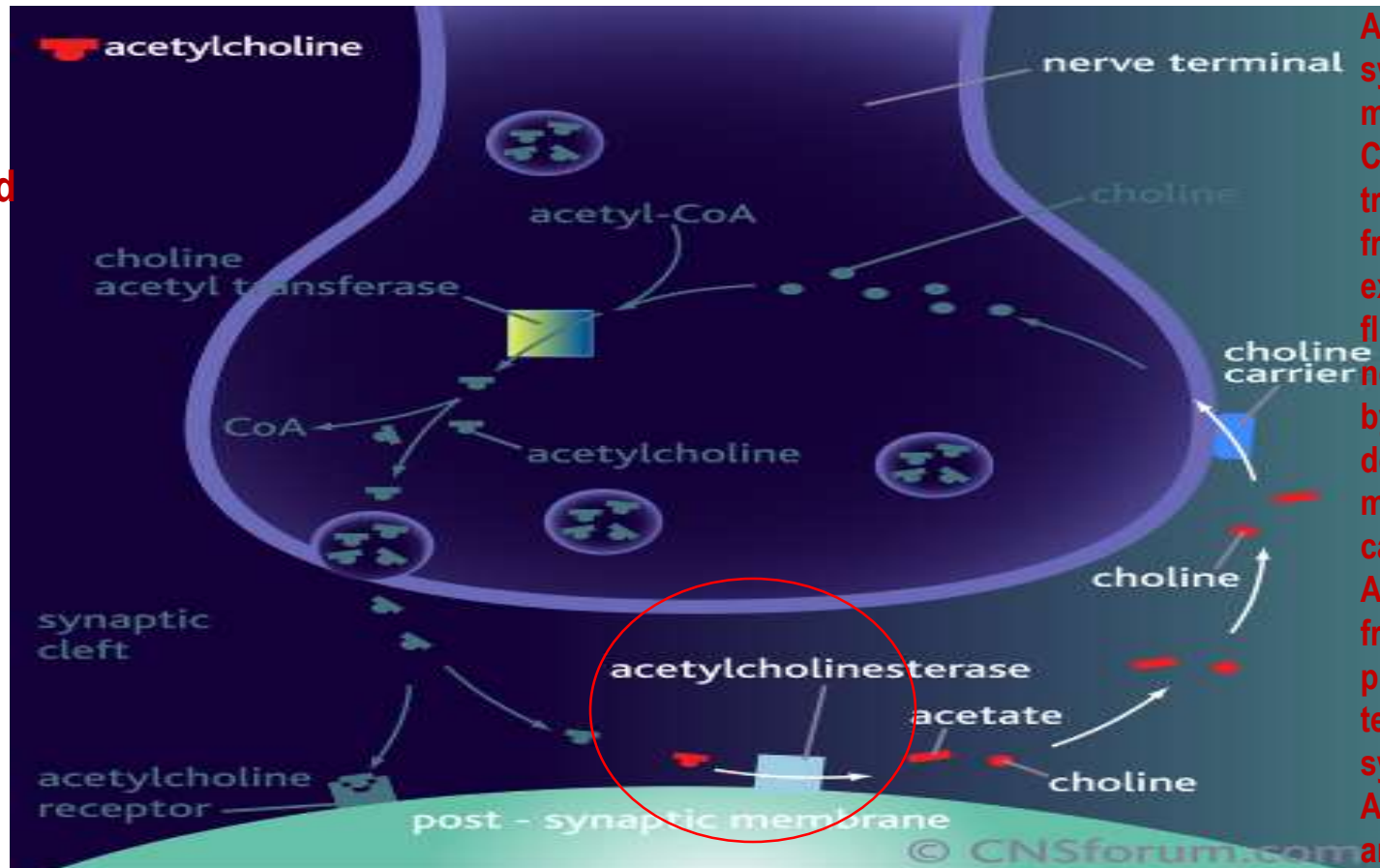
ACh  Choline  
Acetate

Free, unbound ACh,  
continual stimulation of  
electrical activity



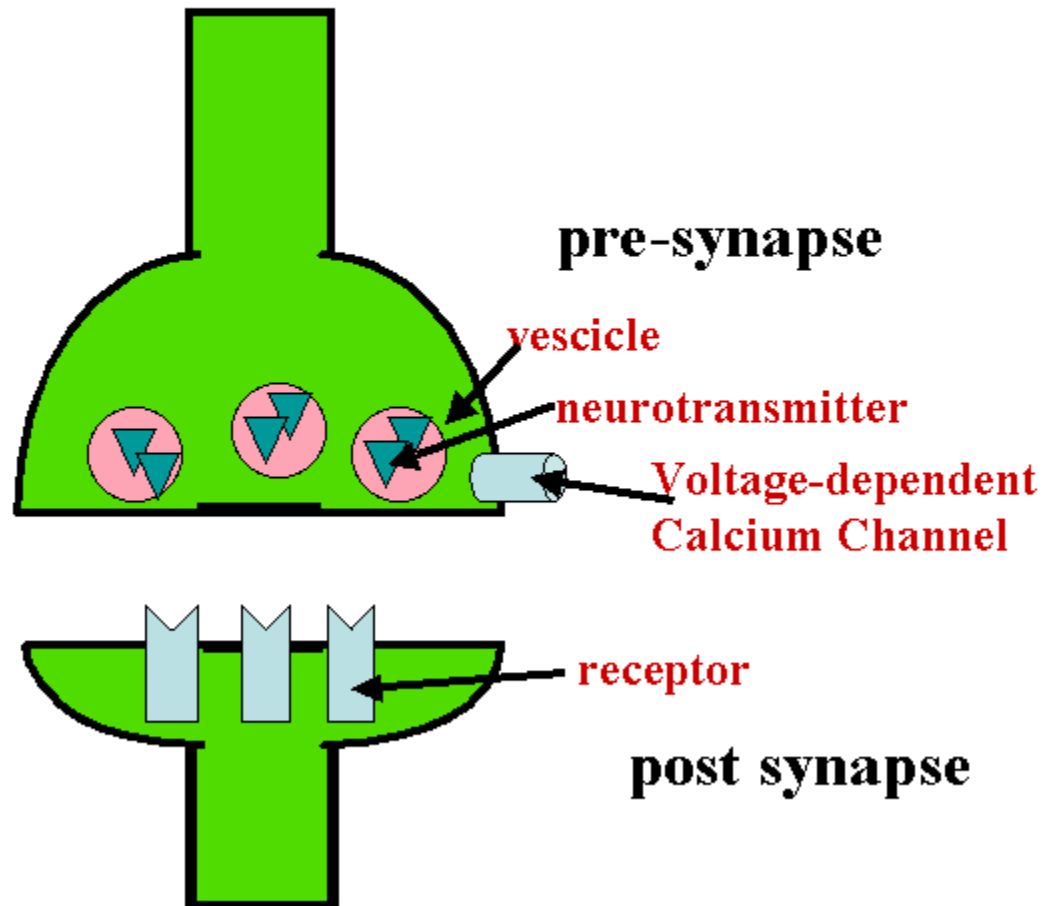
# Mechanism of OP's

Ach synthesized in the cytoplasm



Acetyl-CoA synthesized in mitochondria. Choline is transported from the extracellular fluid into the neuron terminal by Na dependent membrane carrier. After release from the presynaptic terminal into the synaptic cleft ACh may bind to and activate Ach R

# chemical transmission





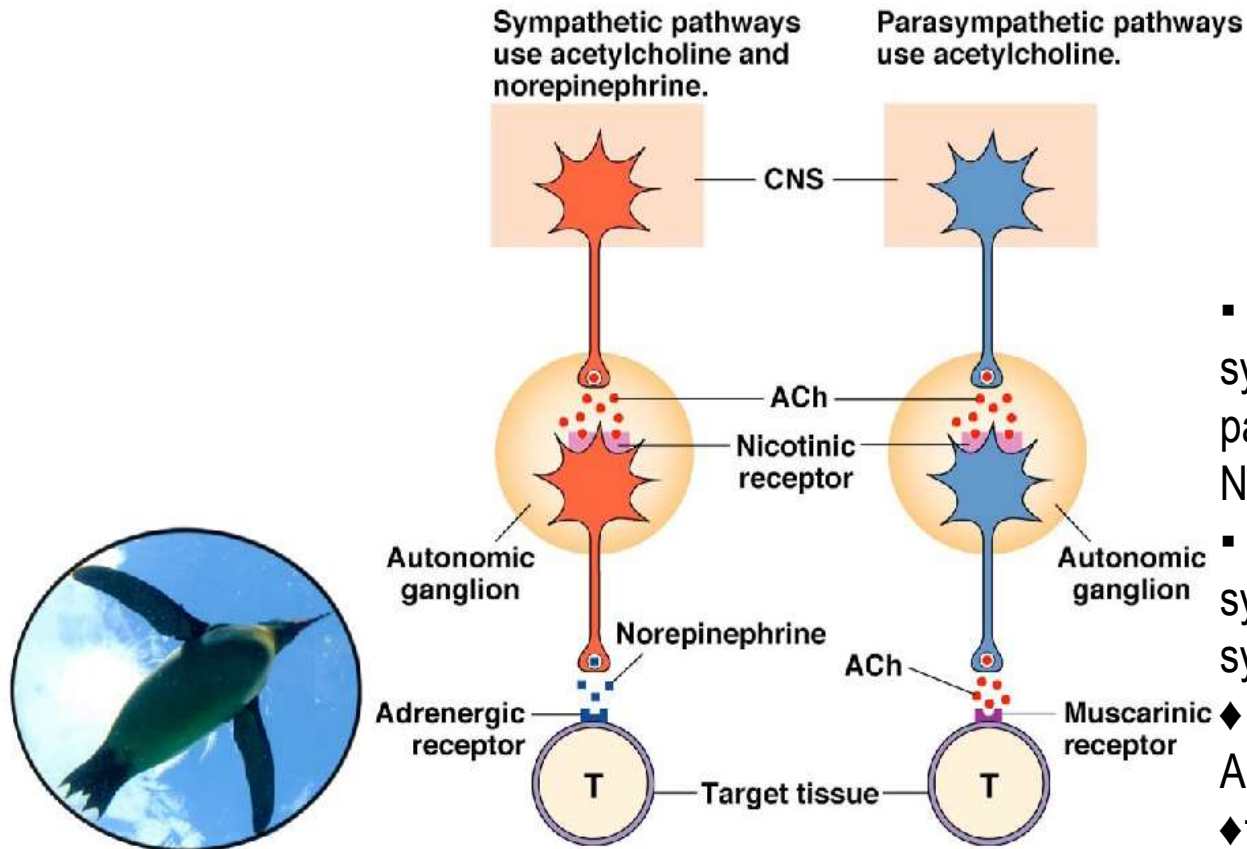
## Mechanism of toxic action

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- Without intervention, the toxicity will persist until sufficient quantities of **"new" acetylcholinesterase** are synthesized **in 20 to 30 day to destroy** efficiently the excess of acetylcholine.

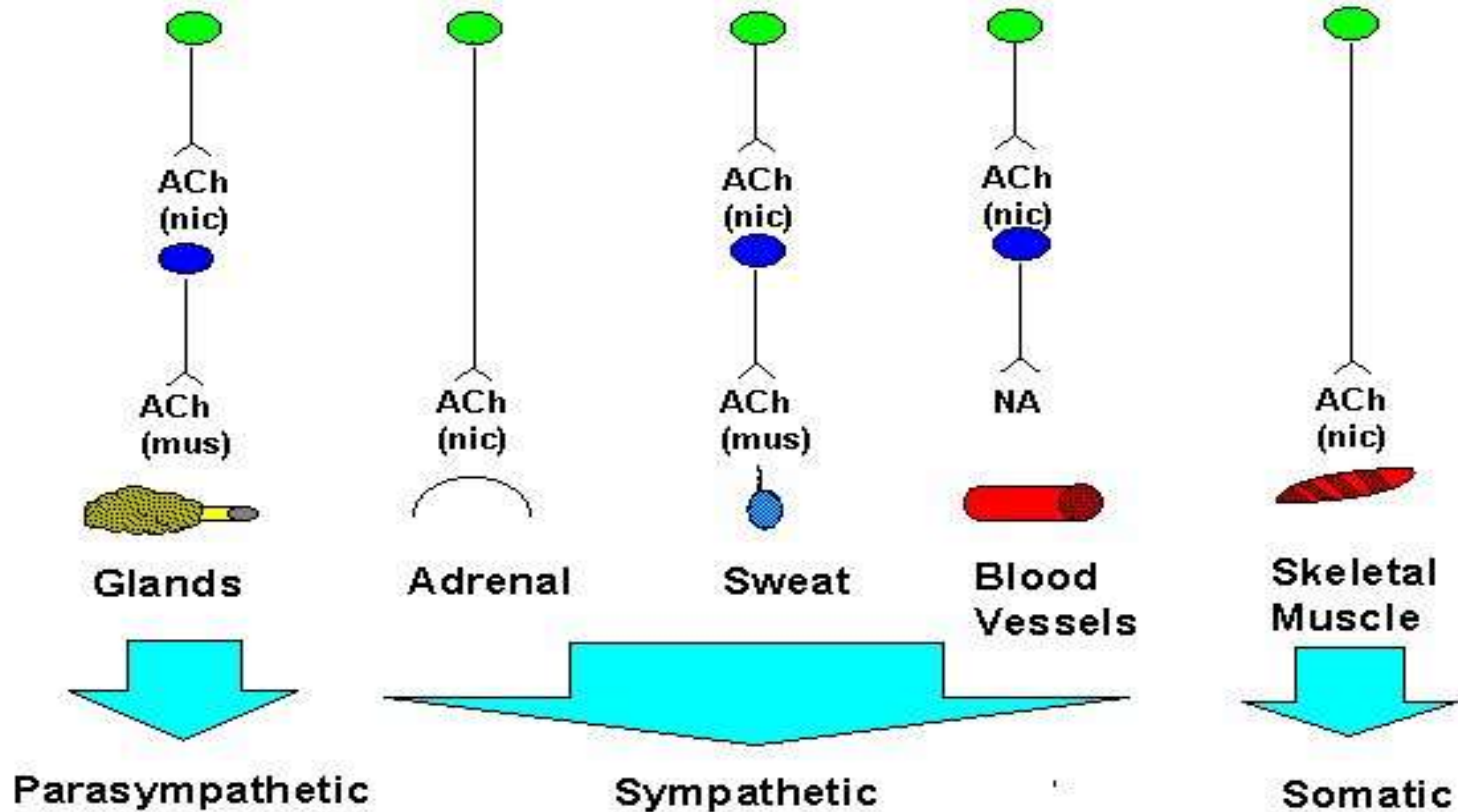
- In contrast, **carbamic acid** esters attaching to the **reactive site** of the **acetylcholinesterase** undergo **hydrolysis** very quickly and the enzyme activity **is recovered** soon.
- So, the **only distinctive difference** between the two anticholinesterase - type insecticides lies **in the rate** at which the **dephosphorylation or decarbamylation takes place**.
- The rate is exceedingly slow for organophosphorus esters and sufficiently rapid for the carbamic esters.
- The **adverse effect** of organophosphorus insecticides and **carbamate ester** insecticides are the result of **accumulation of acetylcholine** at the neuromuscular junctions, which causes muscular fasciculations and ultimately **paralysis**.
- The **excessive acetylcholine** also causes **excessive stimulation** of the **autonomic nervous system** and changes the **function of the lung, cardiovascular system, gastrointestinal tract**, etc.

# The Autonomic Nervous System



- at the ganglionic synapses in both the parasymp. and the symp. NS, the NT is ACh
- at the postganglionic synapses, the two systems are differ:
  - ◆ the parasymp. NT is ACh
  - ◆ the symp. NT is norepinephrine, but at sweat glands it is ACh

## CENTRAL NERVOUS SYSTEM



Acetylcholine  
(ACh)

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graph TD; A[Acetylcholine (ACh)] --> B[ACh receptors]; B --> C[Nicotinic receptors]; B --> D[Muscarinic receptors];
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ACh receptors

Nicotinic  
receptors

Muscarinic  
receptors

## Nicotinic receptors

N1 or Nm

Neuromuscular  
junction

N2 or Nn

Autonomic ganglia  
Central nervous system  
Adrenal medulla

## Muscarinic receptors

M1

Striatum, cortex,  
hippocampus

M2

Forebrain, thalamus, heart,  
pupil, spinal cord, exocrine

M3

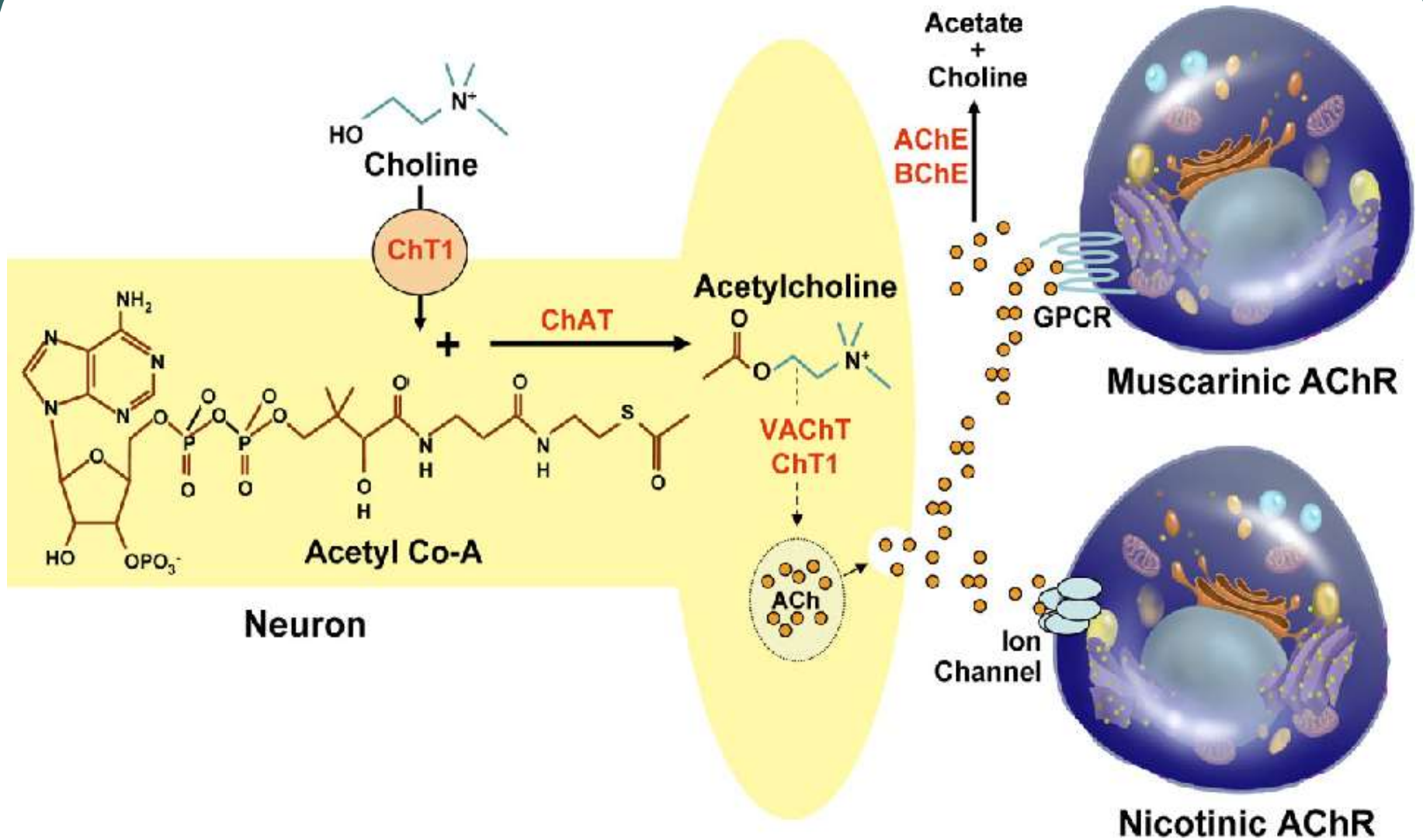
Brain, hypothalamus, pupils,  
exocrine, peripheral arteries

M4

Striatum, cortex,  
hippocampus, spinal cord

M5

Dopaminergic neurons, basal  
ganglia, brain vasculature





# ***Signs and Syptoms of poisoning***

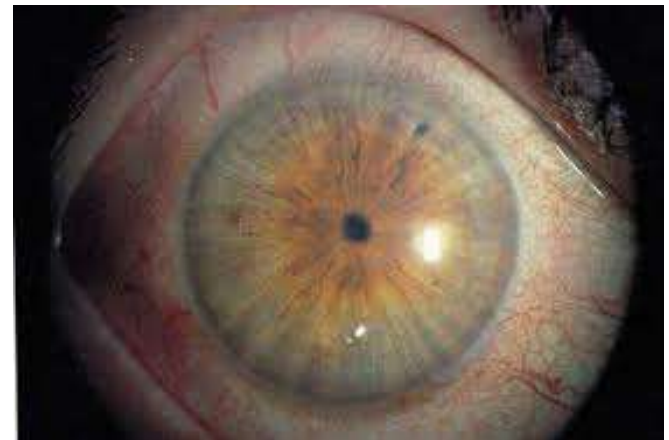
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## **A. Acute form of poisoning**

### **Muscarinic syndrome**

The **signs** of toxicity include those resulting from **stimulation** of the **muscarinic receptors** of the **parasympathic** autonomic nervous system:

- **increased secretions (hypersalivation, lacrimation)**
- **bronchoconstriction (dyspnea), bronchorrhea**
- **miosis**
- **gastrointestinal cramps**
- **diarrhea**
- **vomiting**
- **urination**
- **bradycardia, hypotension**





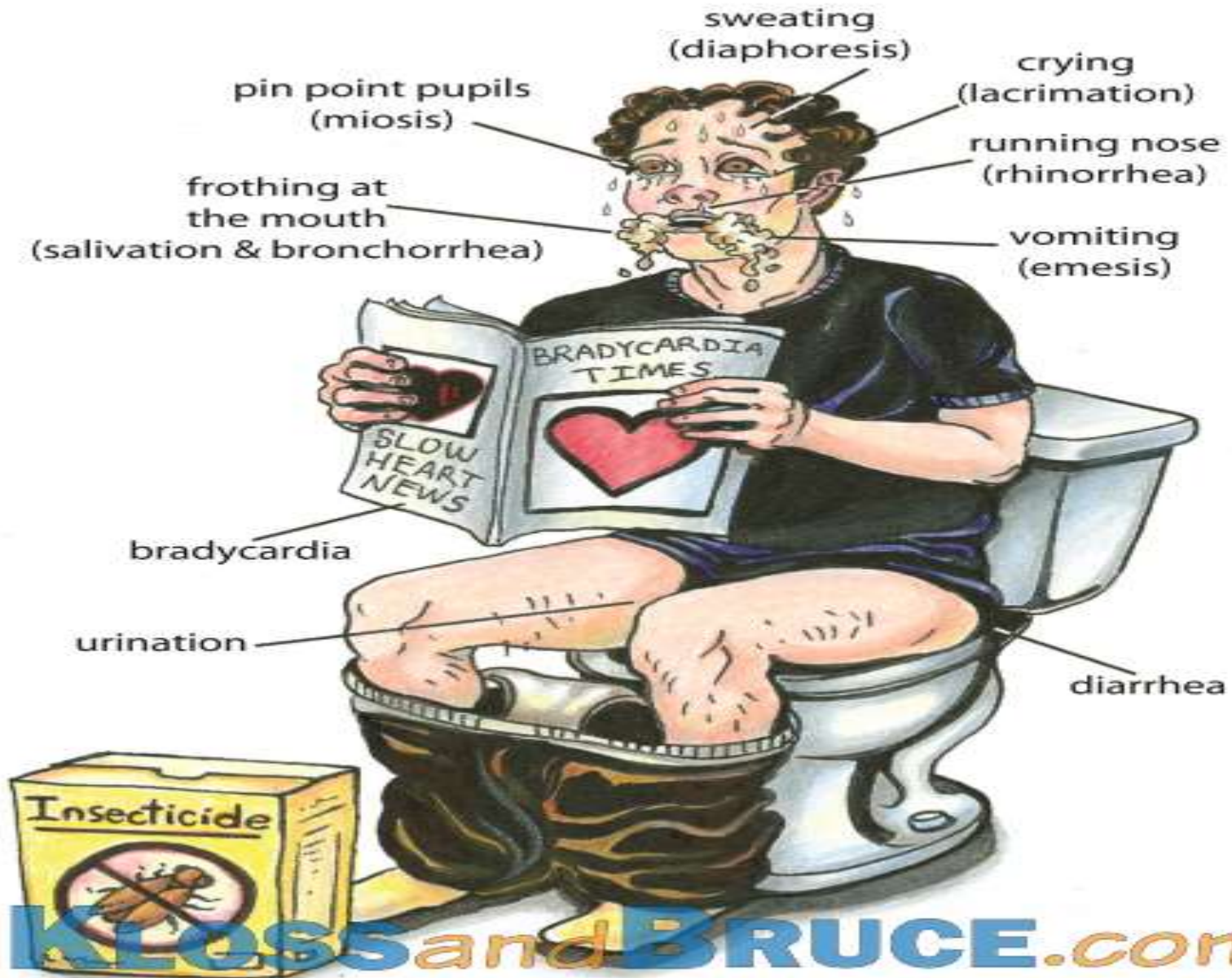
# Toxidromes – M syndrome

Various mnemonics have been used to describe the muscarinic signs of OP poisoning:

**S** Salivation  
**L** Lacrimation  
**U** Urine incontinence  
**D** Diarrhea  
**G** GIT cramps  
**E** Emesis

**D** Diarrhea  
**D** Diaphoresis  
**U** Urination  
**M** Miosis  
**B** Bronchospasm  
**B** Bronchorrhea  
**B** Bradycardia  
**E** Emesis  
**L** Lacrimation  
**S** Salivation

# Cholinergic Toxidrome



## ***Signs and Syptoms of poisonings***

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- The sings resulting from the **stimulation** and subsequent **blockade** of **nicotinic receptors**, including the **ganglia** of the **sympathetic** and **parasympathetic nervous** system as well as the **junction** between **nerves** and **muscles** form the ***nicotinic syndrome***:
  - tachicardia
  - hypertension
  - muscle fasciculations (myofibrilations)
  - tremor
  - muscle weakness
  - convulsions (tonic-clonic seizures)

# Toxidromes – N syndrome

**M** Muscle weakness and fasciculations

**A** Adrenal medulla activity ↑

**T** Tachycardia

**C** Cramping of skeletal muscle

**H** Hypertension

- 
- The signs caused by the effects on **CNS** form ***CNS syndrome***:
    - restlessness
    - emotional lability
    - ataxia
    - lethargy
    - mental confusion
    - loss of memory
    - coma

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## B) Delayed neurotoxicity

- In the **recent years** are **recognized additional and persistent signs of neurotoxicity** not previously associated with the organophosphorus pesticides.
- First and frequently associated with exposure to high concentration of these compounds are effects that may persist **for several months following** exposure and involve **neurobehavioral, cognitive and neuromuscular functions, lowered vitality, cephalgia, gastrointestinal and cardiovascular symptoms, premature decline in potency and libido, premature aging, amnestic or demential effects.**

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## C) Organophosphate-induced delayed Neurotoxicity (OPIDN)

This syndrome was known as "**ginger jake paralysis**" or "**jake led**".

**Symptoms:** initial flaccidity and muscle weakness in the arm and legs giving rise to:

- **a clumsy**
- **shuffling gait**
- **spasticity**
- **hyperreflexia and abnormal reflexes**
- **clonus**

They indicate the damage of the pyramidal tract.

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## D) Carbamate pesticides

- The signs and symptoms of acute intoxication by **carbamate insecticides** are **quite similar** to those described above for organophosphorus compounds, **differing** only in the **duration** and **intensity of the toxicity**.
- - The most apparent **reason** for the relatively short duration and the mild severity of signs of carbamate insecticides are that:
  - they are **reversible inhibitors** of nervous tissue acetylcholinesterase;
  - they are **rapidly biotransformed**;



# ***Diagnosis***

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- ◆ Diagnosis is based **on the history and clinical picture**.
- ◆ The examination of the **acetylcholinesterase activity** is very important for the diagnosis.
- ◆ A **20% to 50% reduction** in an individual baseline **cholinesterase level** indicates excessive exposure.

Mild form – **50-60%**, moderate form – **30-40%**, severe form – **10-20% - 0%**

- ◆ Signs and **symptoms may not appear in some individuals** until the cholinesterase level has **declined by 80%**.
- ◆ **Measuring the RBC cholinesterase level** is **more reliable** for the diagnosis of the organophosphorus poisonings, but in the health practice **the serum or plasma cholinesterase** is preferred to be defined.

**DD: *Amanita muscaria* (fly agaric, fly  
mushroom)**



# Treatment

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# Treatment

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The priorities in management are to:

- **Resuscitation**
- **Atropinization of symptomatic patients**
- **Decontamination**
- **Other Treatments - Oximes**



# ***Treatment***

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- **All cases of anticholinesterase poisoning** should be treated as **serious medical emergencies**.
- **Careful washing of the entire body** with tincture of **green soap** (contains alcohol, useful to remove **fat-soluble compounds**).
- **Remove all contaminated clothing**.
- The status of the patient **should be monitored** by repeated analysis of **the plasma (serum) cholinesterase** and the **erythrocyte cholinesterase**.
- The **life threatening signs** (respiratory depression, bronchospasm, bronchial secretion, pulmonary edema, muscular weakness) require unmediated **artificial respiration** and **suctioning via an endotracheal tube** to maintain a patient airway.
- **Arterial blood gases** and **cardiac function** should be monitored.

# GIT Decontamination

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- **Gastric lavage** and **activated charcoal** should be used if the patient presents within **4 hours after exposure**.
- **Ipecacuanha-induced emesis** should not be used in OP poisoning. Patients poisoned with organophosphorus can rapidly become unconscious, risking aspiration if ipecacuanha has been given.
- **Mechanically-induced emesis** with large quantities of water risks pushing fluid through the pylorus and into the small bowel, probably increasing the rate of absorption.

# GIT Decontamination

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- **Activated charcoal** binds organophosphorus in vitro. The absence of effect in patients might be due to rapid absorption of pesticides **into the blood**. No evidence suggests that patients with pesticide poisoning benefit from treatment with activated charcoal.
- **Magnesium sulphate** blocks ligand-gated calcium channels, resulting in **reduced acetylcholine release** from pre-synaptic terminals, thus improving function at neuromuscular junctions, and reduced CNS overstimulation (reduced mortality in people).

# Antidotes

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## 1. Cholinolitics

- **Atropine** is used to **counteract** the initial **muscarinic effects** of the **accumulating neurotransmitter acetylcholine**.
- **Small doses** of atropine (subcutaneously or intravenously) are indicated for mild signs and symptoms following **a brief, intensive exposure** (1 mg s.c. repeated after 30 min if necessary).
- Relatively **large, cumulative** doses of **atropine** up to **50 mg daily** may be necessary to **control severe muscarinic symptoms**.
- This drug can be administered every 15 minutes.



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<b>Mild form</b>	<b>1 mg s.c., repeated if necessary</b>
<b>Moderate form</b>	<b>2-4 mg i.v., every 15 min 1-2 mg → atropinization</b>
<b>Severe form</b>	<b>4-6 mg i.v., every 15 min 1-2 mg → atropinization</b>

# Antidotes

**Atropine** - belongs to a class of compounds known as alkaloids. It is derived from **Levohyoscyamine** – a component of plants such as **Belladonna, henbane, thorn apple, scopolia**.

Specific effects of atropine include: **the arrest of secretion** of sweat, mucus and saliva; inhibition of the **vagus nerve**, which result in **an increased heart rate, dilation of the pupils** and paralysis of accommodation of the lens of the eye and **relaxation of bronchial, intestinal and other smooth muscles**. Central effects include **excitement and delirium** followed by **depression and paralysis of the medulla oblongata**.

# ***Atropa belladonna***





# Henbane







# Torn apple



# *Treatment*

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## **Specific effects of atropine**

- The status of the patient must be **monitored continuously** by examine for **disappearance of secretions** (dry mouth and nose and sweating), **facial flushing** and **mydriasis, tachycardia**.

## **Anticholinergic Agents:**

**Atropine, Scopolamine, Benztropine etc.**



# What if you give too much Atropine ?



- Anticholinergic Syndrome:
  - Hot as a hare/hell/
  - Blind as a bat
  - Red as a beet
  - Dry as a bone
  - Mad as a hatter
- A sensitive indicator for ingestion, but poor predictor for toxicity.
- Full syndrome is rare





# Anticholinergic TOXIDROME

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- Mad as a hatter
  - ❖ confusion/hallucinations/seizures/coma
- Blind as a bat
  - ❖ mydriasis
- Dry as a bone
  - ❖ dry skin, urinary retention
- ❖ Red as a beet
- ❖ Flushed skin
- Hot as a pistol (hare)
  - ❖ hyperthermia

# ANTICHOLINERGIC SIDE EFFECTS



Hot as a hare

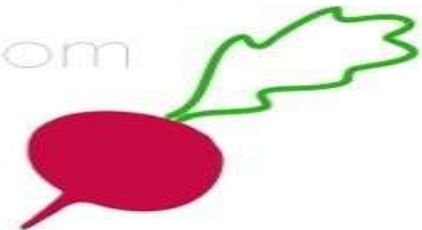


Dry as a bone

[sketchymedicine.com](http://sketchymedicine.com)



Blind as a bat



Red as a beet



Mad as a hatter

# Mad as a hatter



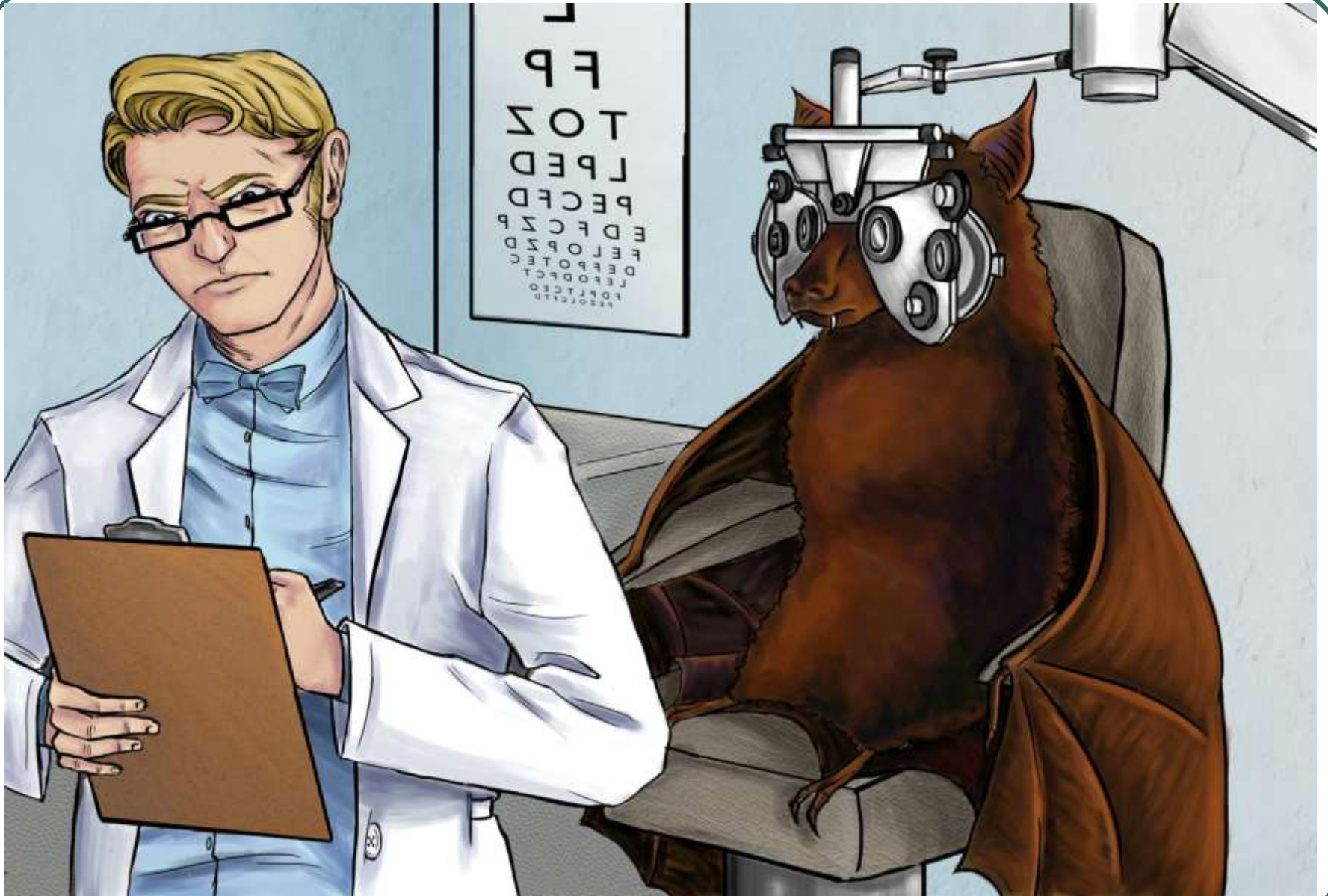
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## **Blind As A Bat**

Pupils will be very dilated!!  
Near vision will be blurry







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## Dry As A Bone

Blockade of cholinergic tone to salivary glands.

Decreased salivation, dry mouth, intense thirst and difficulty swallowing



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## **Red As A Beet**

Describes a marked flushing on the face and chest.  
This is not an effect of cholinergic blockade.



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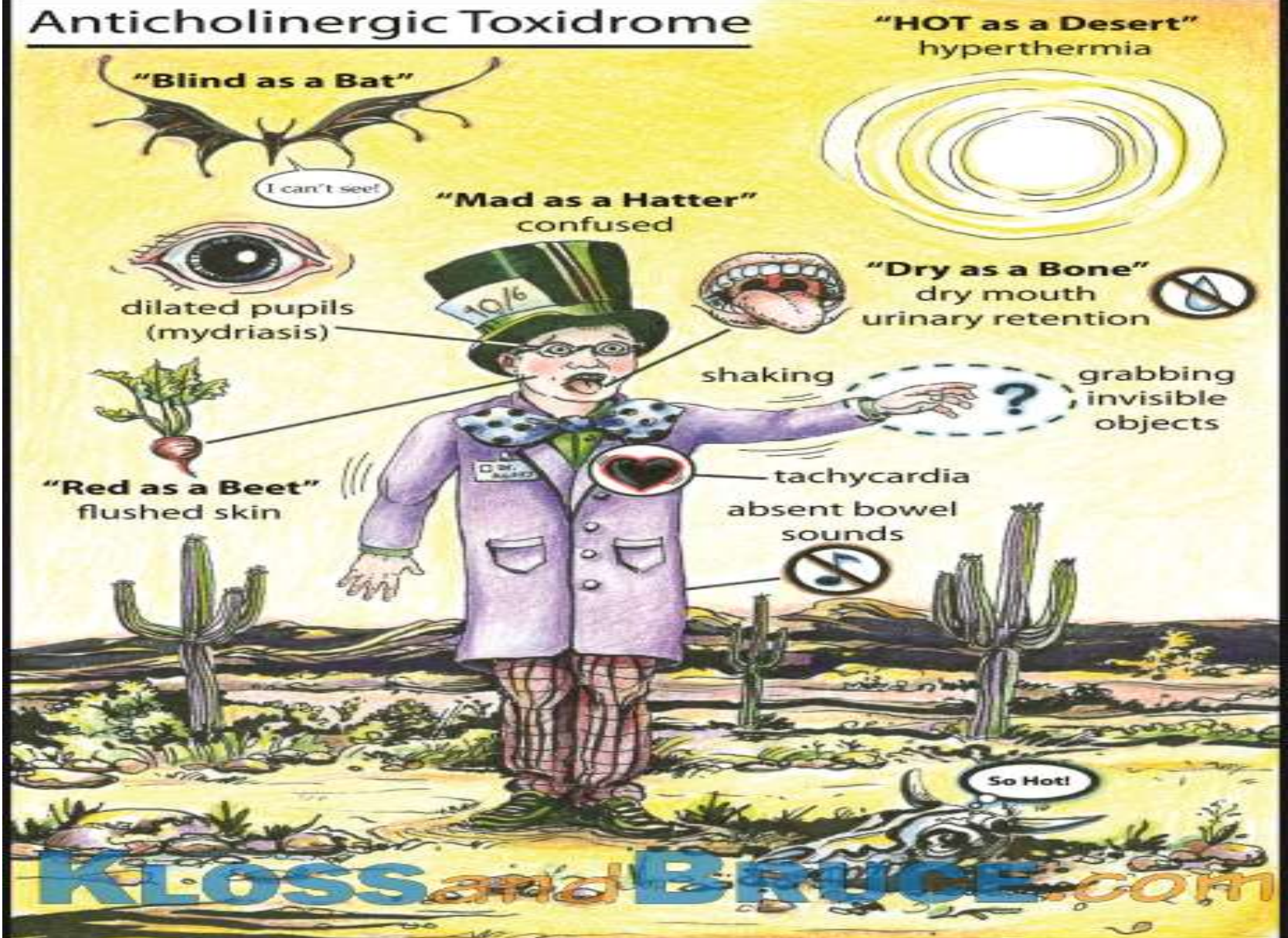
## **Hot As A Pistol**

Refers to an elevated body temperature. This is a result of blockade of sweat glands.





# Anticholinergic Toxidrome



# Treatment

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- **2. Oximes**

The oximes (**pralidoxime or 2-PAM, toxogonin, obidoxime**), administered **intravenously** reactivate the inhibited nervous tissue **AchE**.

The use of these agents are **not necessary** for **cases of mild Intoxication** and they should be used for **moderate to severe** poisonings.

Treatment by **slow intravenous infusion** of doses of **1 g Pralidoxime** should be initiated **as soon as possible**.

The clinical treatment of **carbamate toxicity** is similar to that for organophosphorus insecticides **intoxication with the exception** that the **use at oxymes** is **contraindicated**.

## ***Treatment***

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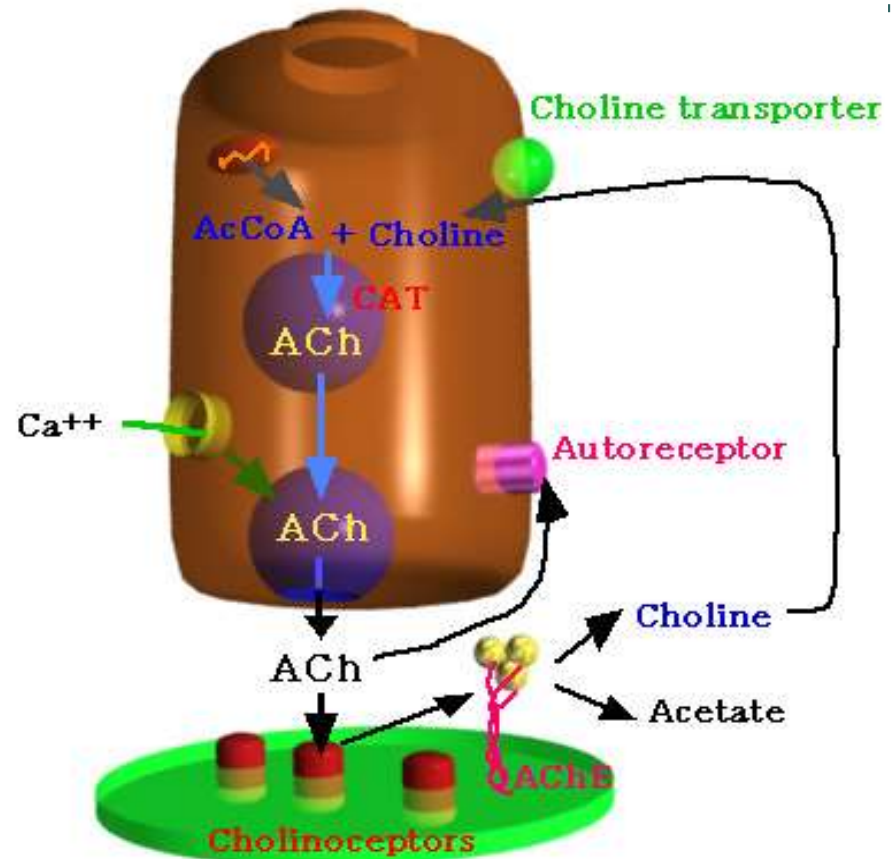
- **Diazepam**

This drug **counteracts** some aspects of the **CNS and neuromuscular signs** that are not affected by atropine. **Doses of 10 mg** (s.c. or i.v) are appropriate and may be repeated.

- **Other centrally acting drugs** that may **depress respiration** is **not recommended** in the absence of artificial respiration.

# Alternate sites for antidotes

- Protect AChE
- Supply AChE
- Reduce ACh
- Protect ACh Receptor
- Reduce OP Load
- Multiple Mechanisms



## New aspects of treatment

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- The **alpha 2-adrenergic receptor agonist clonidine** also reduces acetylcholine synthesis and release from presynaptic terminals. Animal studies show benefit of clonidine treatment, especially in combination with atropine, but effects in human beings are unknown.
- **Sodium bicarbonate** is sometimes used for treatment of organophosphorus poisoning. Increases in blood pH (up to 7.45–7.55) have been reported to improve outcome.



# Enzymes

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**Butyrylcholinesterase** scavenges organophosphorus in plasma, reducing the amount available to inhibit acetylcholinesterase in synapses. It has been cloned and military research now aims to inject soldiers with the enzyme before exposure to organophosphorus nerve gases. Such a prophylactic approach is not practical for self-poisoning with organophosphorus because we cannot predict when a person is going to ingest the pesticide. Some researchers have reported the use of **butyrylcholinesterase in fresh frozen plasma** to treat poisoned patients.

# Enzymes

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- A better approach than use of butylcholinesterase might be to give **recombinant bacterial phosphotriesterases, or hydrolases**. These proteins break down organophosphorus pesticides **enzymatically**.



# **TOXICOLOGY OF CHEMICAL WARFARE AGENTS**



# **Classification of chemical warfare agents, so-called "medical classification"**

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## **I. Agents liable to be met in warfare**

Nerve agents (**G and V gases**, **sarin**, **tabun**, **soman**)

Lung-damaging (**phosgene and chlorine**) agents

Vesicant agents (**sulphur mustard**, **lewisite**, etc.)

Psychotomimetic agents (**LSD**, **BZ**, etc.)

Heterogenous (other) agents

a) Cyanide

b) Arsine

## **II. Agents liable to be met in riot control/or war**

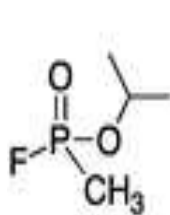
1. 1. Sensory irritants (**CS**, **CN**, **CR**, etc.)

2. 2. Vomiting agents

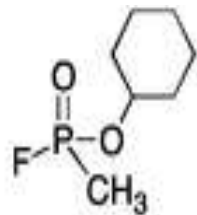
# **ROUTES OF ENTRY**

- ☐ Ingestion
- ☐ Eyes
- ☐ Respiratory Tract
- ☐ Injection
- ☐ Skin

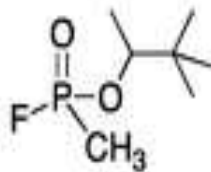




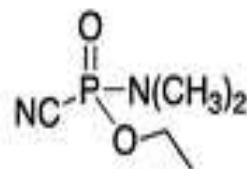
sarin



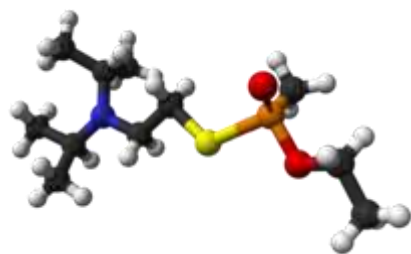
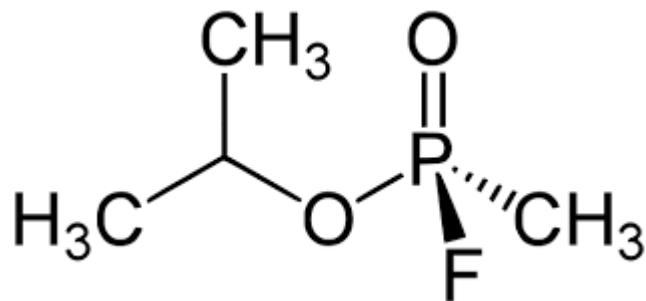
cyclosarin



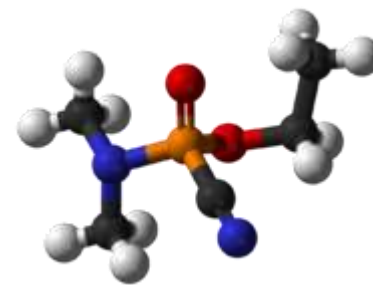
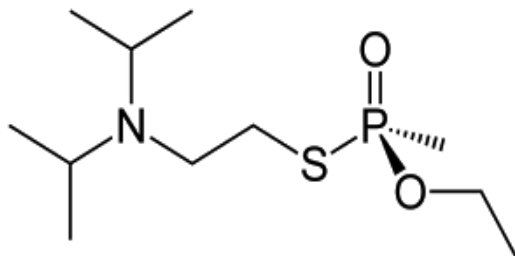
soman



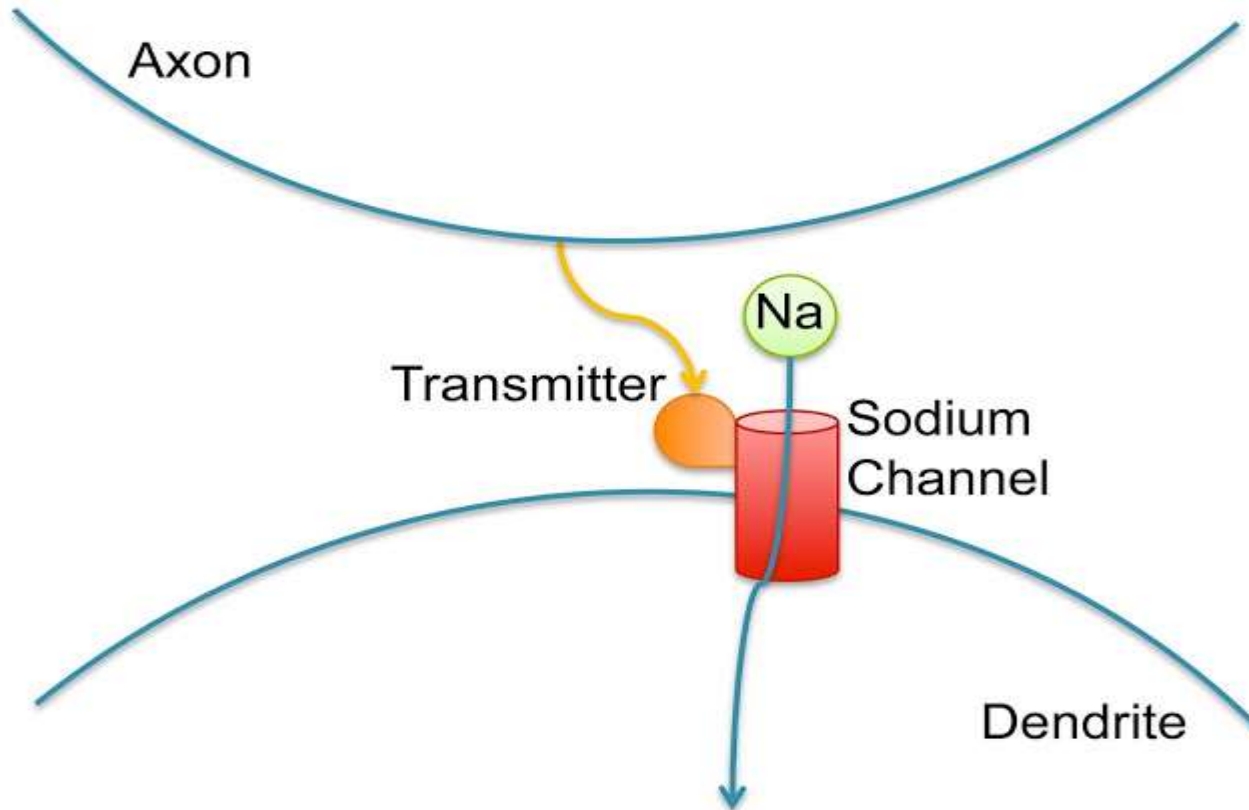
tabun

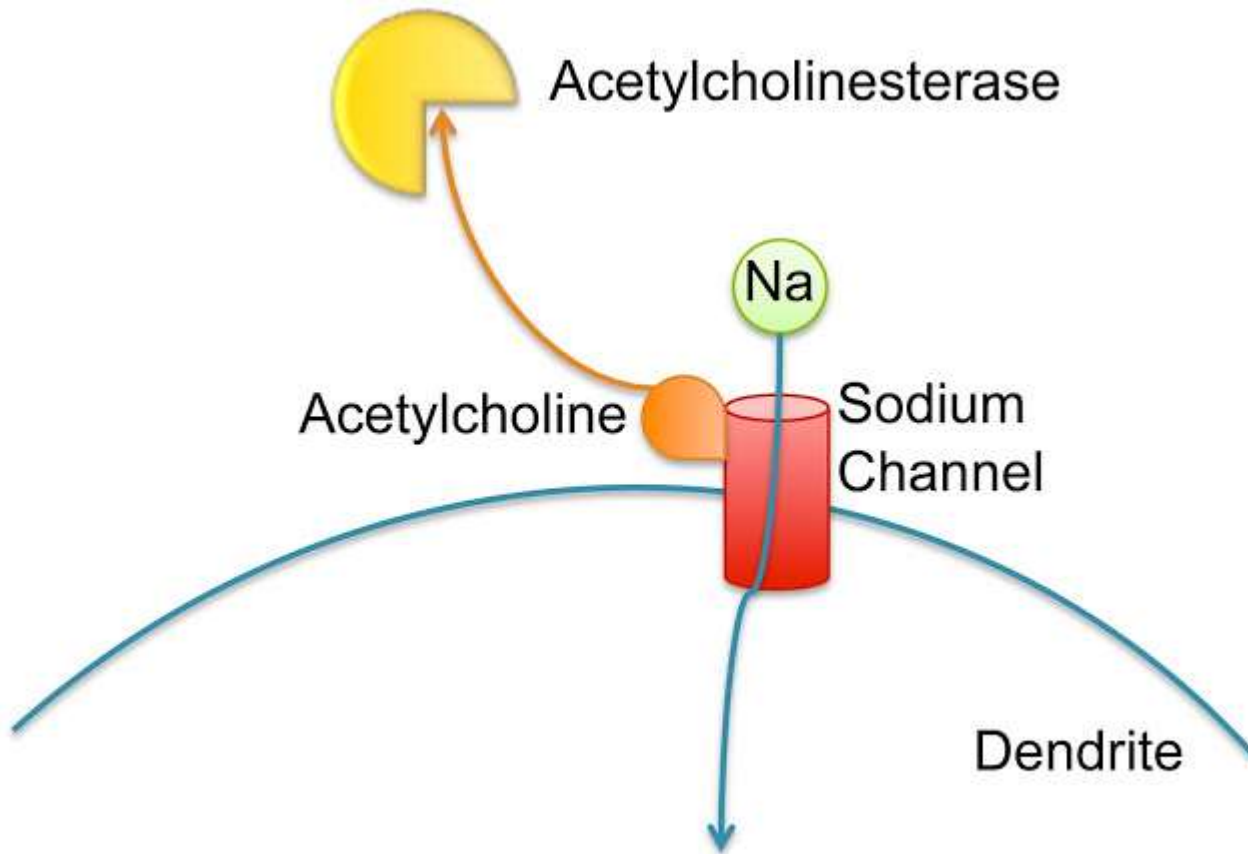


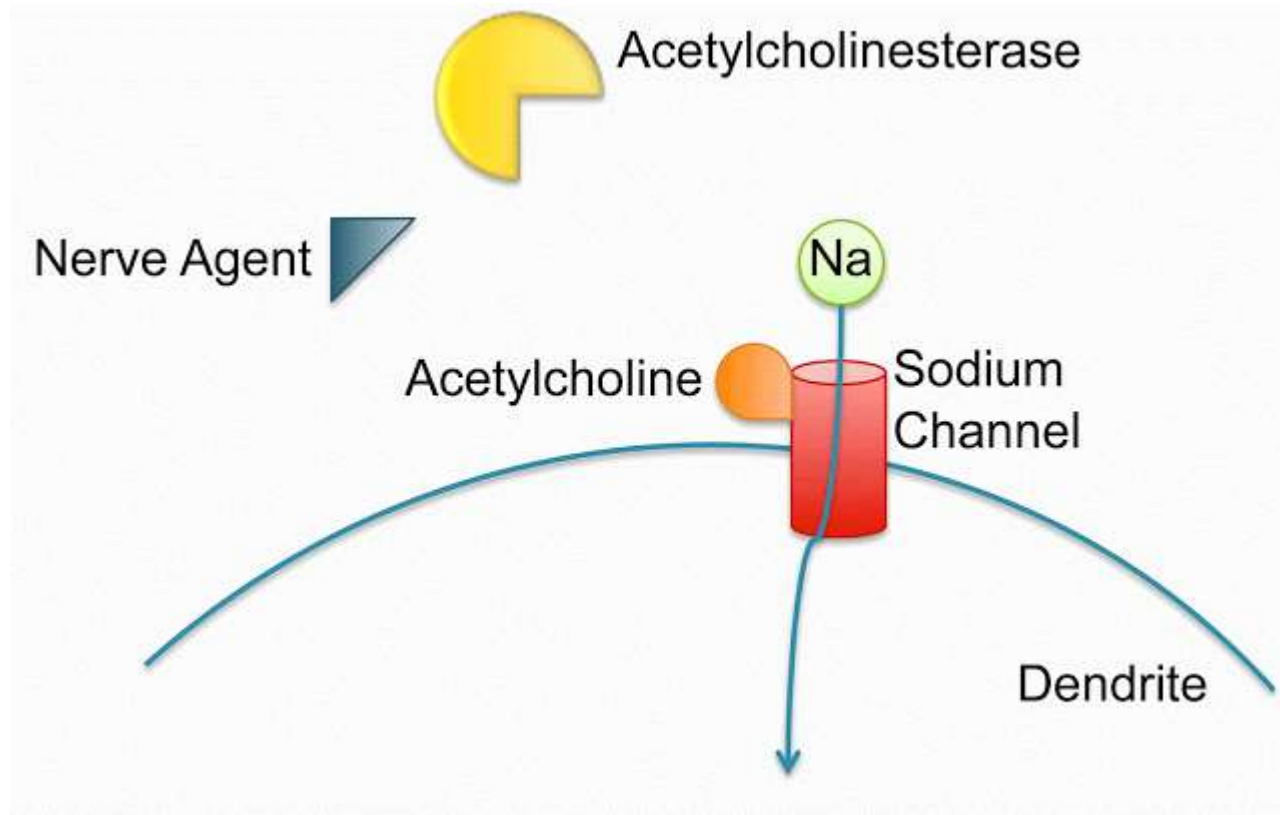
Vx gases

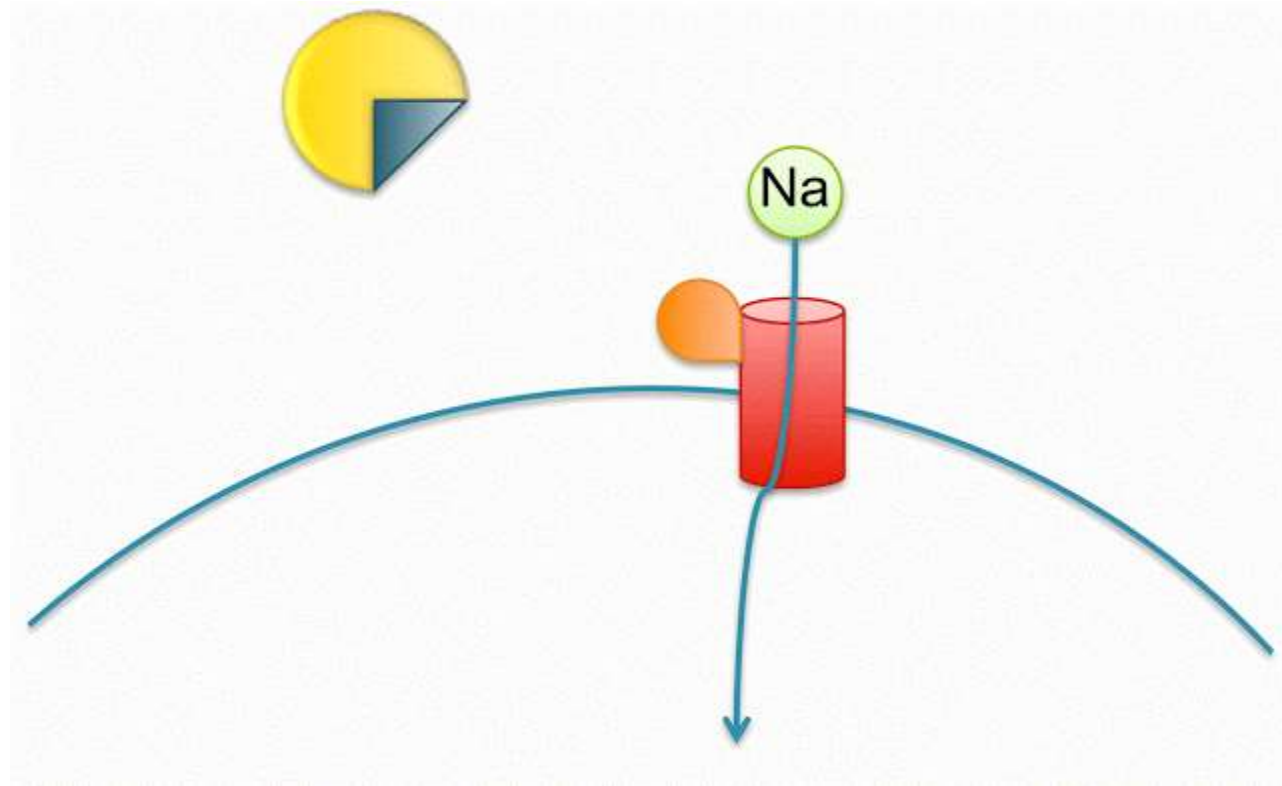


tabun











## Sarin

- 100mg can kill a person in a few minutes
- 500x toxic as cyanide

## V<sub>x</sub> Nerve Gases

Deadliest nerve agent

Fraction of a drop absorbed through the skin is deadly 100 x more deadly than sarin gas when absorbed, 2 x if inhaled.





# **VESICANT COMPOUNDS**

## **(Blister agents)**



- **Vesicant compounds** were introduced as **chemical warfare agents** on 12 July 1917 (I World War) when **German forces** used sulphur mustard **at the river Ypres, in Belge**.
- The most important agents of this group are **sulphur mustards** and **nitrogen mustards**
  - Mustards got their name from their pungent **mustard - garlic odor**. **Sulphur mustard** vapours pass quickly **through clothing**. It is **lipid soluble and is absorbed across the skin**.
  - The majority of inhaled sulphur mustards is absorbed in the **upper airways**.

Mustard gas (sulphur mustard)



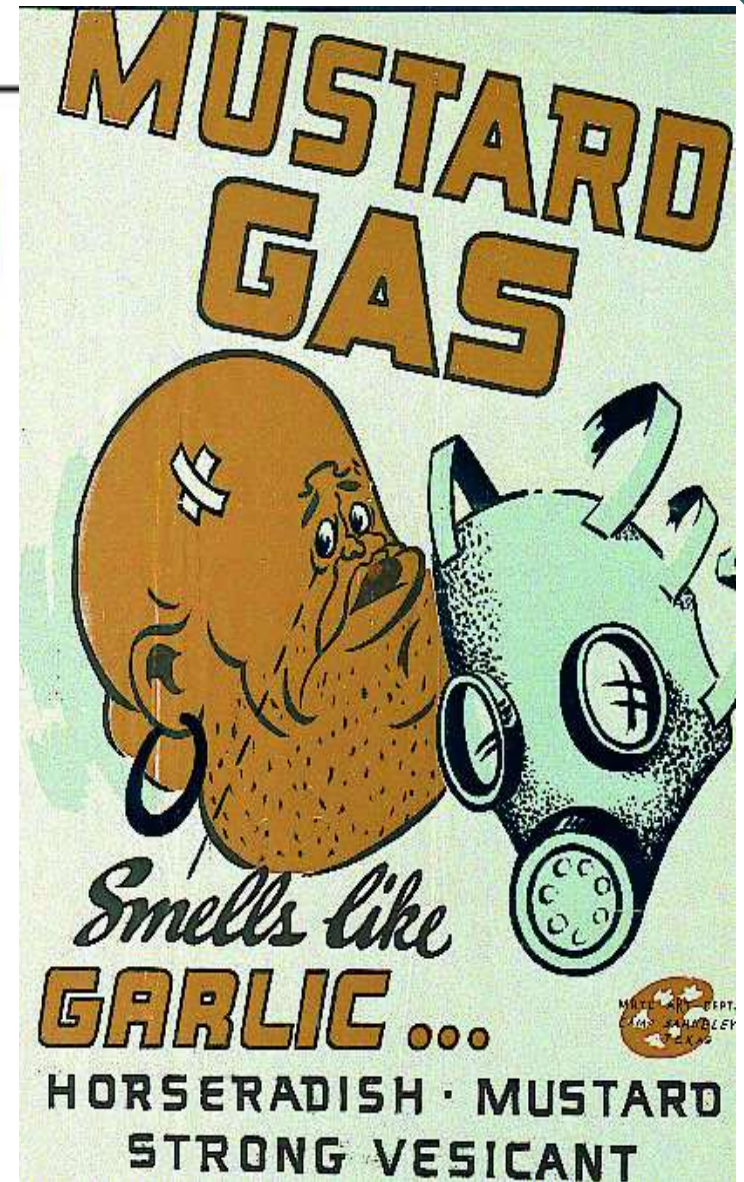
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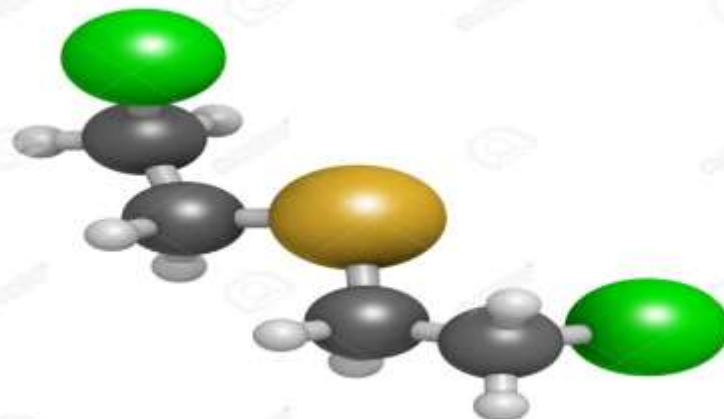
Nitrogen Mustard



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## Mechanism of action



- Mustard gases (**lperite**) are powerful **alkylating agents** and react with **amino, thiol, carboxyl, hydroxyl**, and primary **phosphate groups**.
- As a result of its alkylating and electrophilic properties, mustard gas is able to change the structure of **nucleic acids, cellular membranes, and proteins**.

# Clinical presentation

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- Mustard gas (either vapor or liquid) causes damage to the **skin, eyes, respiratory system, and gastrointestinal tract.**



# Eyes

## Clinical presentation

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- **After 20 - 60 min** of the contact with the poison is observed: **nausea, retching and eye smarting**. It is followed by a **latent period** of up **an hour**.
- **Later (2 - 6 h.):**
  - **inflammation of the eyes**
  - **intense eye pain**
  - **lacrimations**
  - **blepharospasm**
  - **photophobia**
  - **rhinorrhea**



## Skin

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- **Blisters** develop between **6 - 24 hours**. They **are not painful**, but pain may be produced on moving. Blisters heal **slowly** and healing areas of skin are **sensitive**. Reddening of exposed skin.
- **Darkening of the skin**, due to an increase of **melanin** in the skin and areas of brown or black **hyperpigmentation** are produced.

## Respiratory system

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- After a delay of 24 hours, **inhalation of the gas** produces initially **hoarseness**, which may progress to **loss of voice**.
- A **cough** (worse at night) appears early and later becomes productive. **Fever, dispnea, and moist rhonchi and rales may develop**.
- **Bronchopneumonia** frequently intervenes develops.
- Symptoms may **persist for 1 or more years**.

## **Gastrointestinal tract**

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- **Ingestion of food or water contaminated by liquid mustard produces:**
- **nausea**
- **vomiting**
- **pain bleeding diarrhea**
- **dehydration**
- **prostration**



# Treatment - the main treatment is to relieve symptoms

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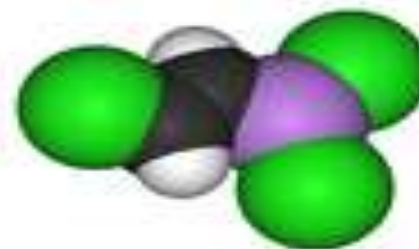
**Management of eye lesions** - early decontamination of liquid splashes in the eye is essential. It is necessary also:

- **daily saline irrigations;**
- **chloramphenicol eye drops** to prevent infection;
- **mydriatics** (e.g. hyoscine drops) to prevent iridolenticular adhesion;
- **dark glasses;**

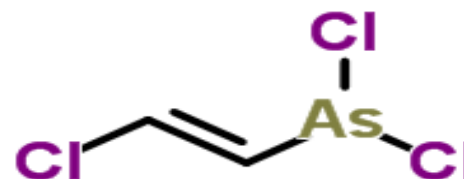
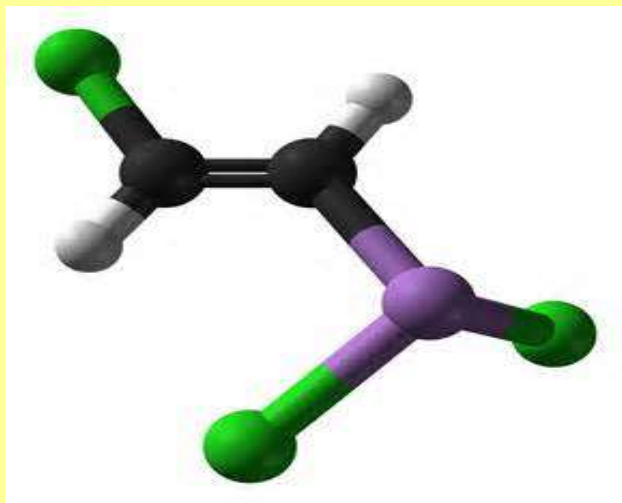
**Management of lesions of respiratory tract includes:**

- **antibiotic cover** is recommended if the respiratory effects are more than very mild;
- **mucolitics**, including acetyl cysteine;
- **intensive care** in case of very severe damage of respiratory tract;

## Lewisite



- Lewisite, developed as a chemical warfare agent in 1918 by Lee Lewis, has never been used in war. Despite this it has acquired a reputation as an agent of likely great effectiveness and lethality.





**The toxicology of the Lewisite is very similar to this one of the sulphur mustard. However, certain differences exist:**

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- Exposure to the eyes to Lewisite vapor is immediately painful and the damage produced is more severe than that produced by mustard gas vapor.
- Skin blisters produced by Lewisite appear more quickly post-exposure than those produced by mustard gas.
- The inflammatory response associated with Lewisite lesions is more severe than that associated with mustard lesions.
- Healing of Lewisite - induced skin lesions is more rapid than of those occurring as a result of exposure to sulphur mustard.

# Treatment

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- A specific, antidotal therapy is available for treatment of Lewisite poisoning.
- **Dimercaprol (BAL)** binds the arsenical groups of Lewisite and produces a harmless complex. BAL competes avidly with binding sites in the body for arsenic and removes arsenic from them. BAL is used also in the treatment of poisoning by a range of heavy metal compounds.