The Biology & Physiology of Cholinesterase Running a Cholinesterase Program

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Objectives

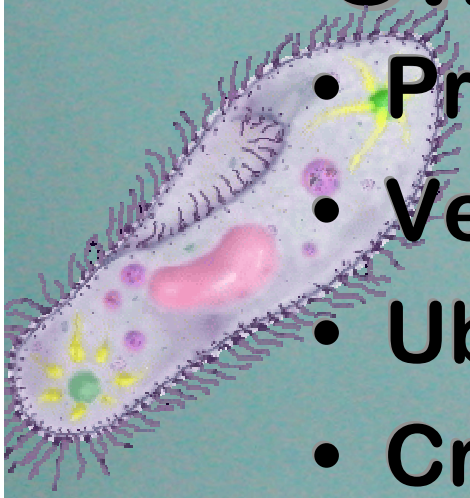
Review:

- Purpose of Monitoring
- Basic biology of cholinesterase and cholinesterase inhibiting pesticides, basic enzymology
- History and physical exam of the handler
- Appropriate testing methods and interpretation of monitoring results
- Responses to cholinesterase depression
- How to investigate for false positives
- Setting up a cholinesterase monitoring in the clinic/medical system
- Quality assurance evaluation

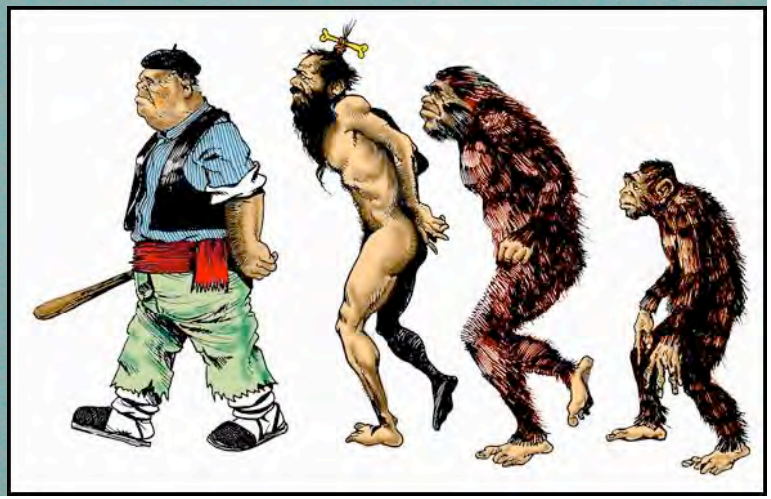
What ChE monitoring accomplishes ?

- Identifies hazardous conditions/practices
- Increases worker/employer hazard awareness
- Assists in medical return to work
- Avoids problems from chronic exposure
- Influences economic decisions:
  - Increases costs of production
  - May influence choice of pesticide

Biology of Cholinesterase



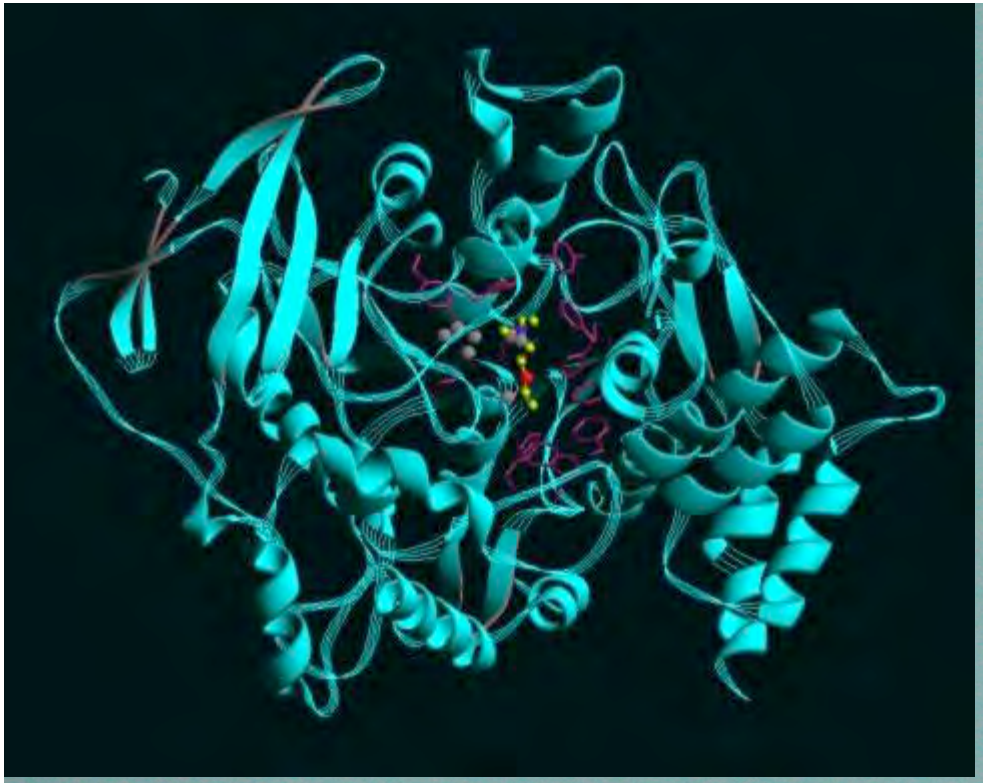
- Present from paramecia to sapiens
- Very Fast enzyme (perfect kinetics)
- Ubiquitous in the human body
- Critical for many nervous system functions



What is it?

- An enzyme with a sulfhydral active site
- Produced in tissues and blood
- Hydrolyses acetylcholine
- Present in the autonomic, central and peripheral nervous systems

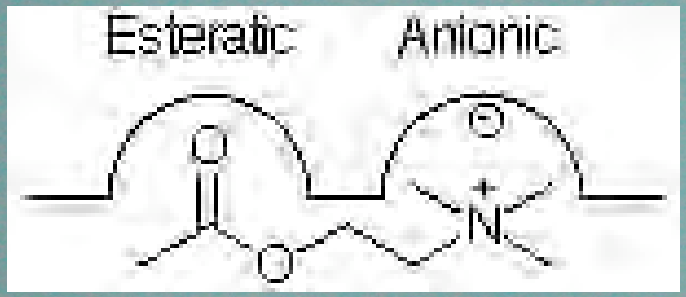
- Excellent web page on the enzyme http://www.weizmann.ac.il/Structural_Biology/Pages/Sussman/webpage2/kurt/che.html

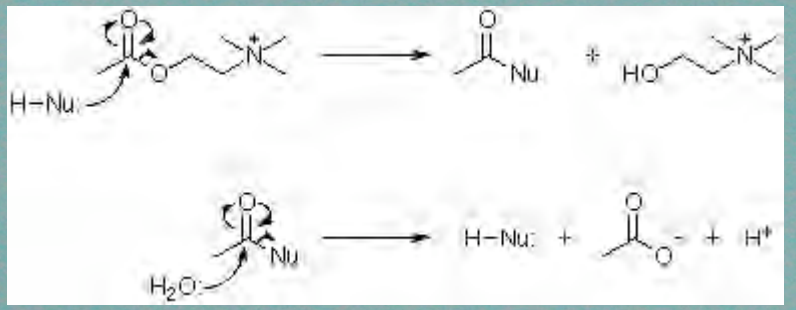


What it Does Chemically

Hydrolysis of Acetylcholine : A key neurotransmitter

- Thought to mediates a nucleophilic attack on carbonyl carbon acylating it and liberating choline and vinegar. Old model explains much but is not accurate.





Two Kinds of ChE in the Body

- Different enzymes with some behaviors in common
- Plasma Cholinesterase
  - Butyrylcholinesterase, pseudocholinesterase, PChE, or just cholinesterase and ChE
- RBC Cholinesterase
  - True cholinesterase, acetylcholinesterase, or AChE

Plasma ChE?

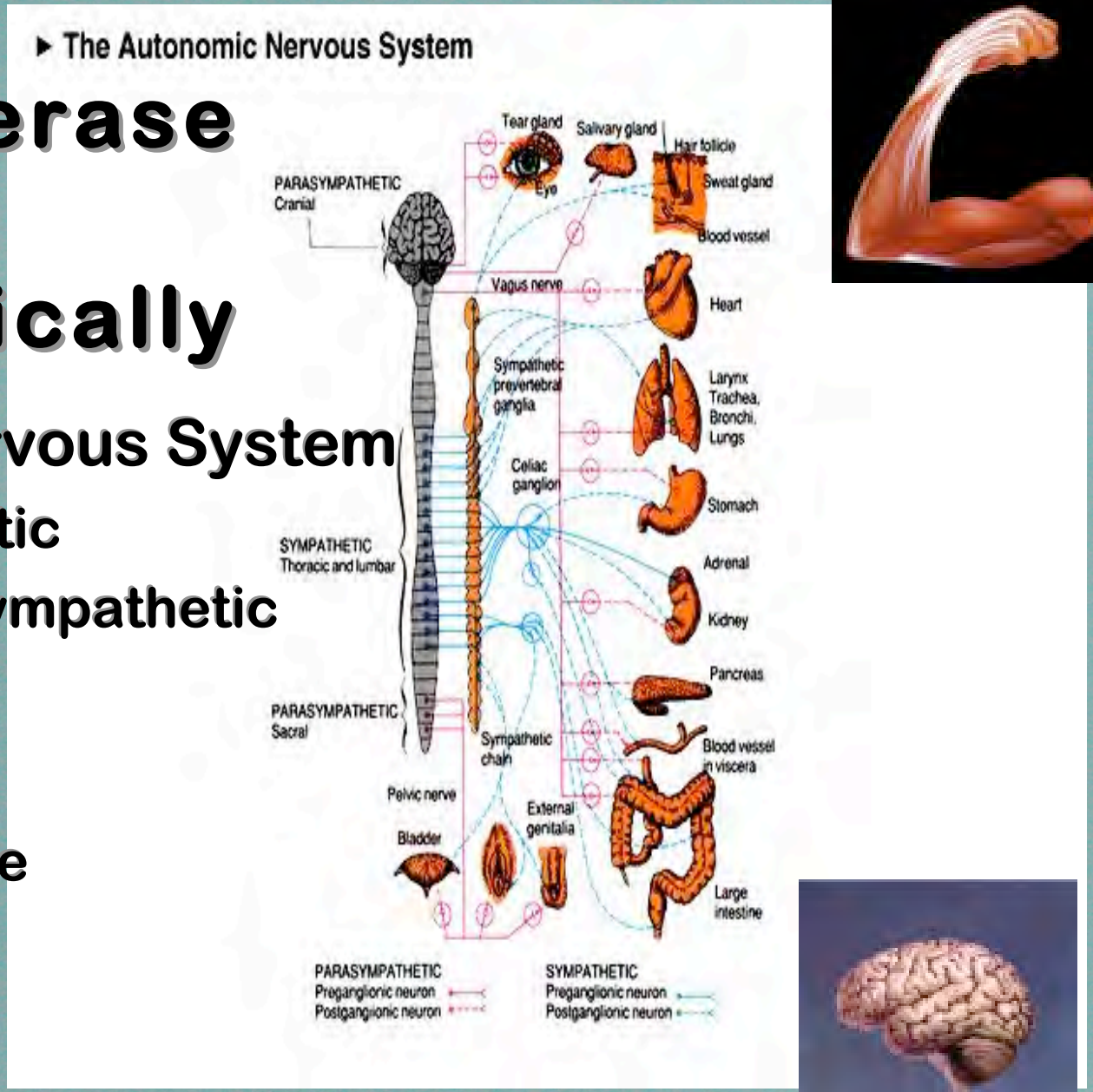
- Floats freely in plasma
- Made by liver
- Rapid recovery from depression
- Rapid replacement by new synthesis
- Liver disease may affect levels
- Sensitive to most ChE inhibitor pesticide exposures

Red Blood Cell Cholinesterase

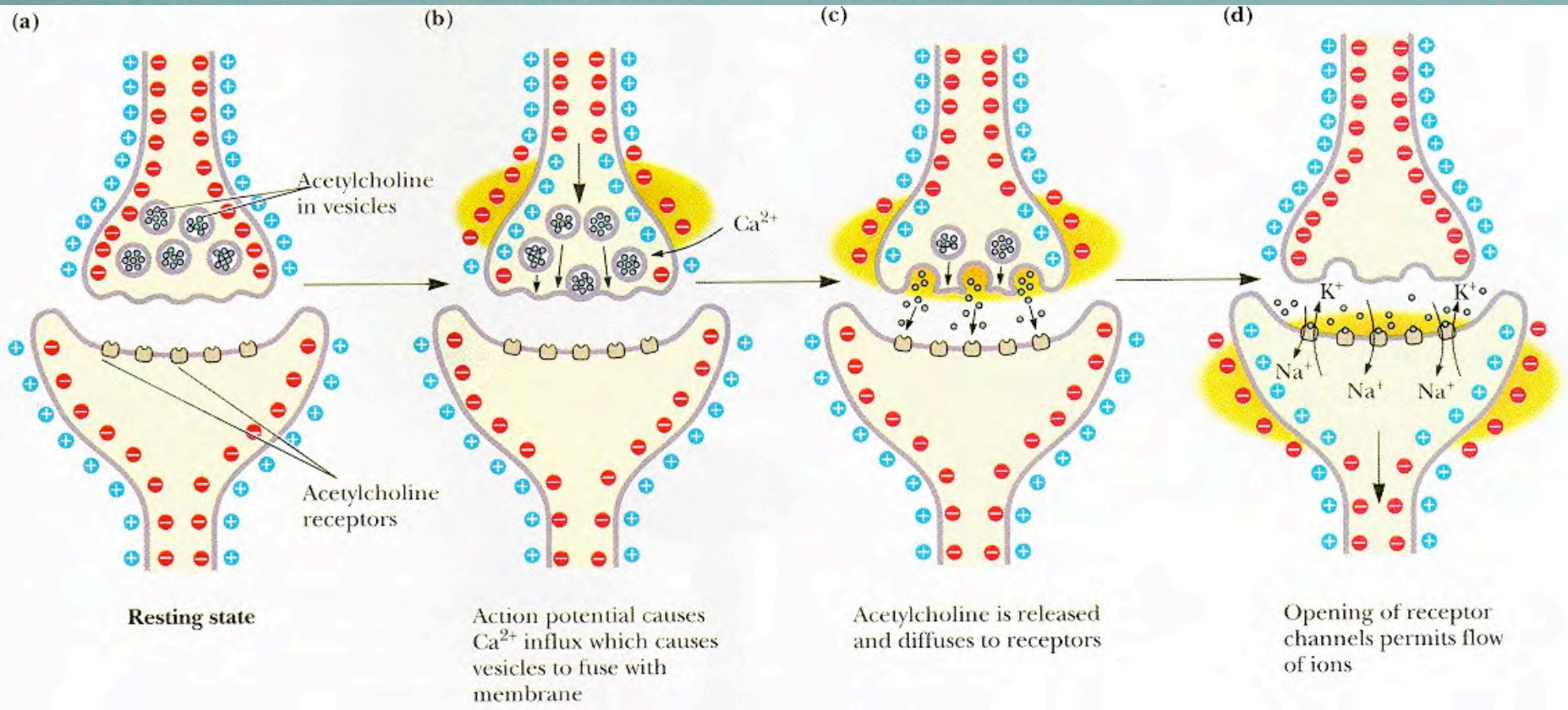
- Bound to red blood cells
- Made at the same time as the Rbc's
- Recovery from depression 0.8%/day
- Slower to depress, slower to recover
- Low RBC count may cause lower levels
- Identical to neuronal ChE

What Cholinesterase Does: Physiologically

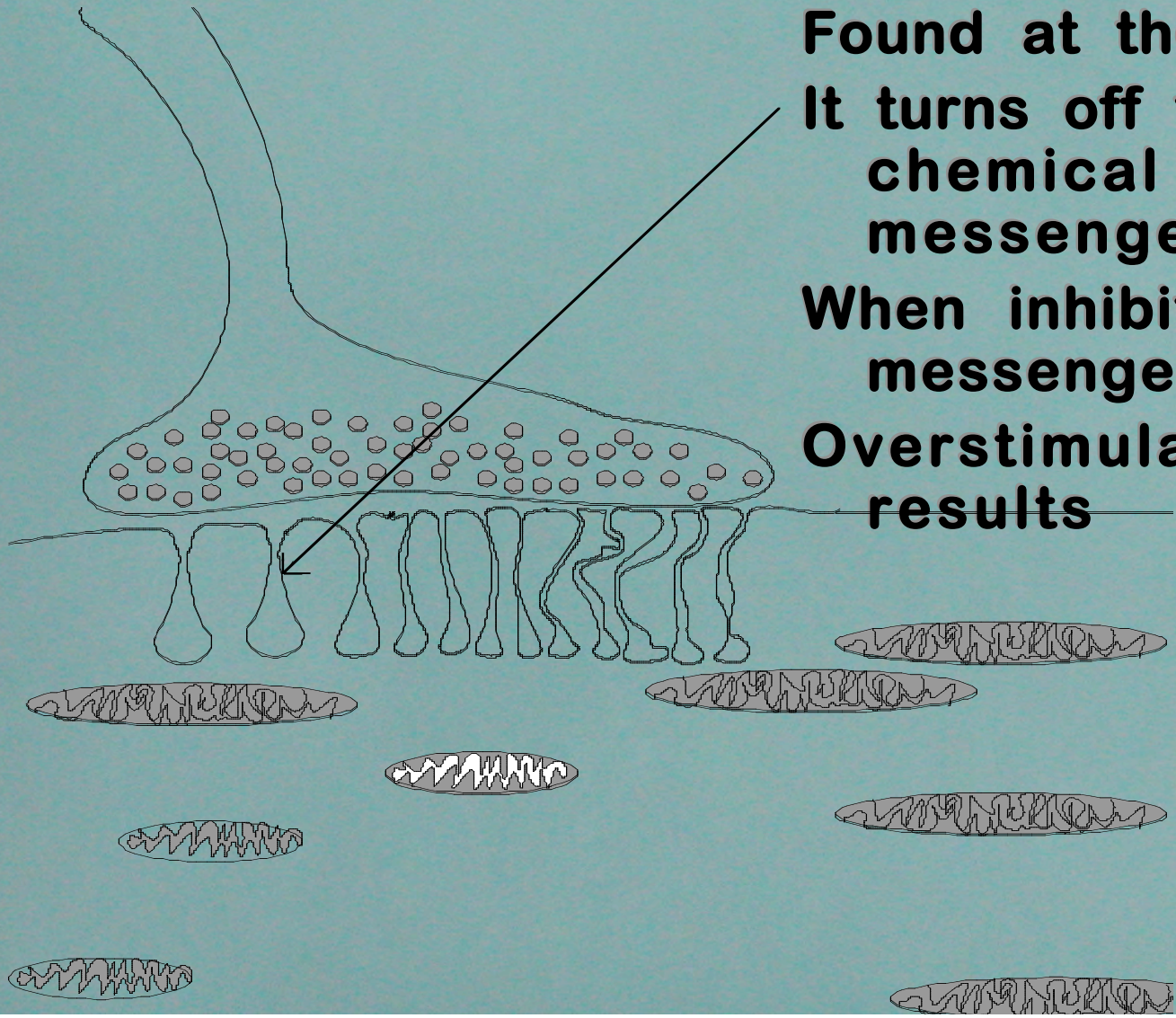
- Autonomic Nervous System
  - Parasympathetic
  - Presynaptic Sympathetic
- PNS
  - Skeletal muscle
- CNS
  - Memory & others



What It Does: Neurochemically First understand Acetylcholine



Cholinesterase



- Found at the synapse
- It turns off the chemical messenger
- When inhibited the messenger builds
- Overstimulation results

Why do we have this enzyme in the blood?

- A buffer for poisons
- Potatoes
  - Solanaceous alkaloids

- The Calabar Bean
  - Physiostigma venenosum

- Green Mamba Snake
  - Fascilin inhibits AChE

Cholinesterase Pharmacology

- Alzheimers Treatment with ChE inhibitors
  - Tacrine, Donepezil, Metrifonate, Galantamine
    - Metrifonate is converted to DDVP
- Myasthenia Gravis
  - Edrophonium,
  - Pyridostigmine bromide
- Glaucoma
- Prophylaxis for Nerve Gas Attacks

Pesticides That Inhibit Cholinesterase

- Organophosphates
  - Inhibit irreversibly
  - “aging of complex”
  - ChE must be replaced by the body
- Carbamates
  - Inhibit temporarily
  - No “aging”
  - Reversal is rapid and level related
  - ChE reactivates and is ready to go

Oral vs. Dermal LD_{50} of some OPs

Organophosphate	Oral mg/kg mg/kg	Dermal
Phorate	2	6
Azinphos-Methyl	13	220
Methamidaphos (rat)	32	94
Oxydemeton (rat)	75	250
Diazinon (rat)	108	900
Phosalone (rat)	130	1500
Chlorpyrifos (rat)	155	202
Malathion (rat)	1375	4444

N-Methyl-Carbamates

Pesticide	Oral mg/kg	Dermal mg/kg
Aldicarb	0.5	3
Carbaryl	5 -13	>1000
Propoxur	100	1000 -2400
Oxamyl	5.4	3000
Carbofuran	5-13	>1000
Methomyl	17-24	>5000

Toxicity of ChE Inhibitors

Mild cases:

tiredness, weakness, dizziness, nausea and blurred vision

Moderate cases:

headache, sweating, tearing, drooling, vomiting, tunnel vision, and twitching

Severe cases:

abdominal cramps, urinating, diarrhea, muscular tremors, staggering gait, pinpoint pupils, hypotension (abnormally low blood pressure), slow heartbeat, breathing difficulty, and possibly death

Extoxnet http://ace.ace.orst.edu/info/extoxnet/

Why is ChE Testing Useful?

- ChE reflects the toxicant on its target
- Integrates exposure over time
- The test is widely available
- A blood sample all that is needed
- BUT!
  - Baseline is needed
  - Good lab methods needed
  - Interpretation and timing important
  - Sample handling important

When Do Testing?

Class I and II Carbamates & Organophosphates

DANGER or WARNING

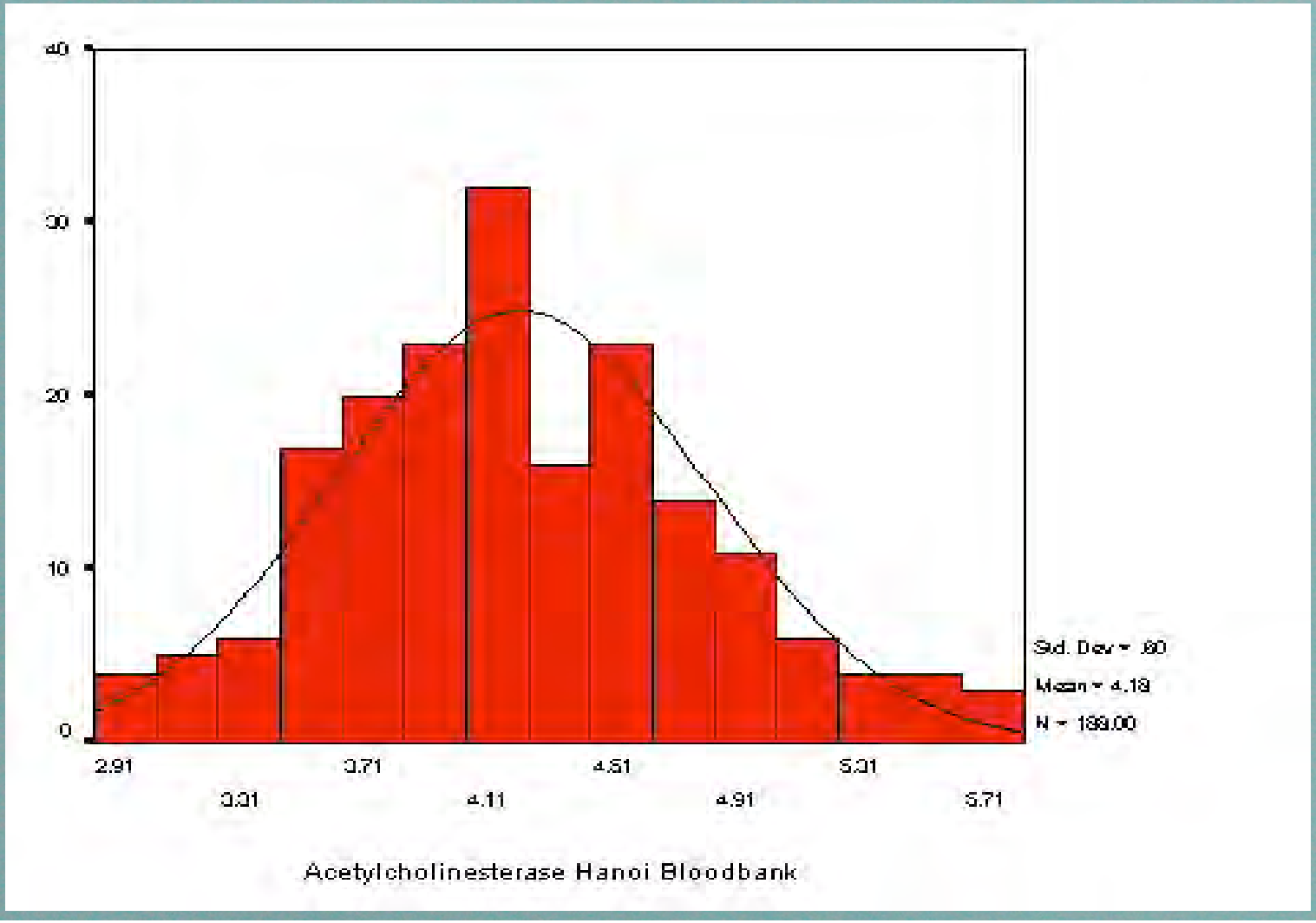
LD 50 of < 50 mg oral or 100 dermal

LD 50 of >50 <500 oral or <1000 dermal

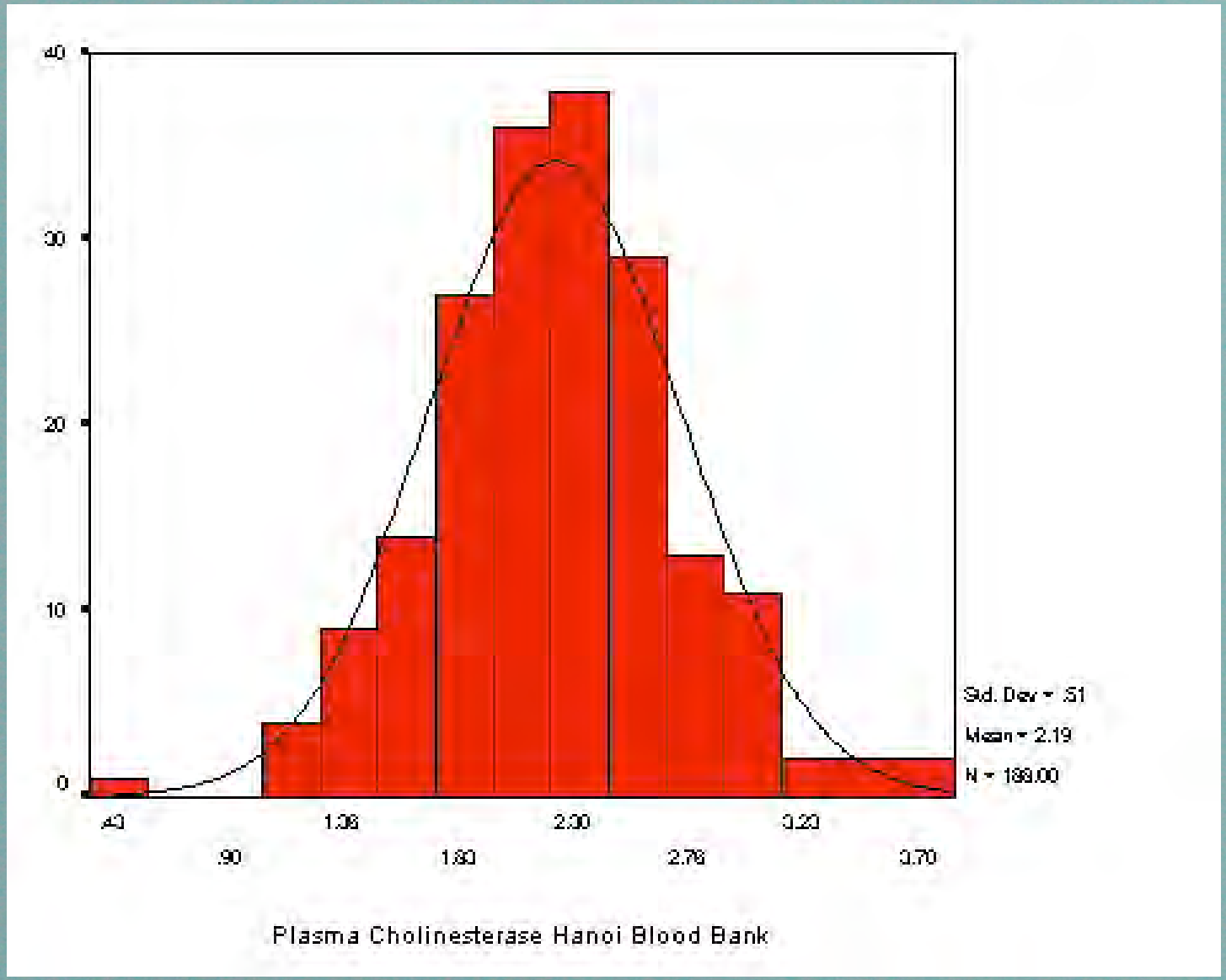
Threshold: 50 hrs in 30 days

How to Interpret Cholinesterase Monitoring Data: Why Baselines?

Normal Range of cholinesterase activity

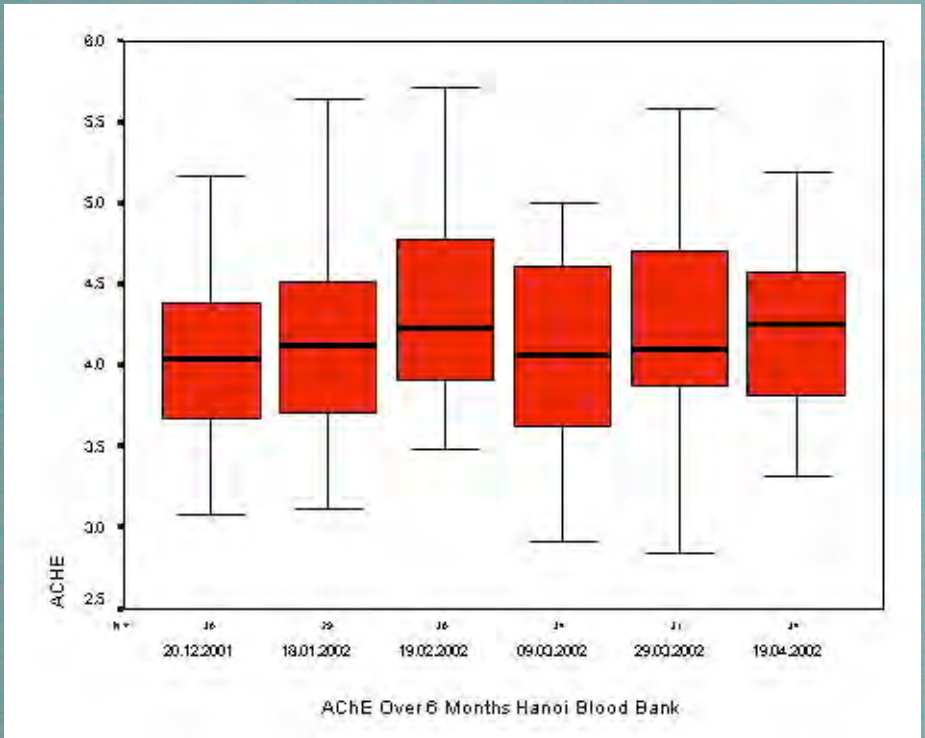


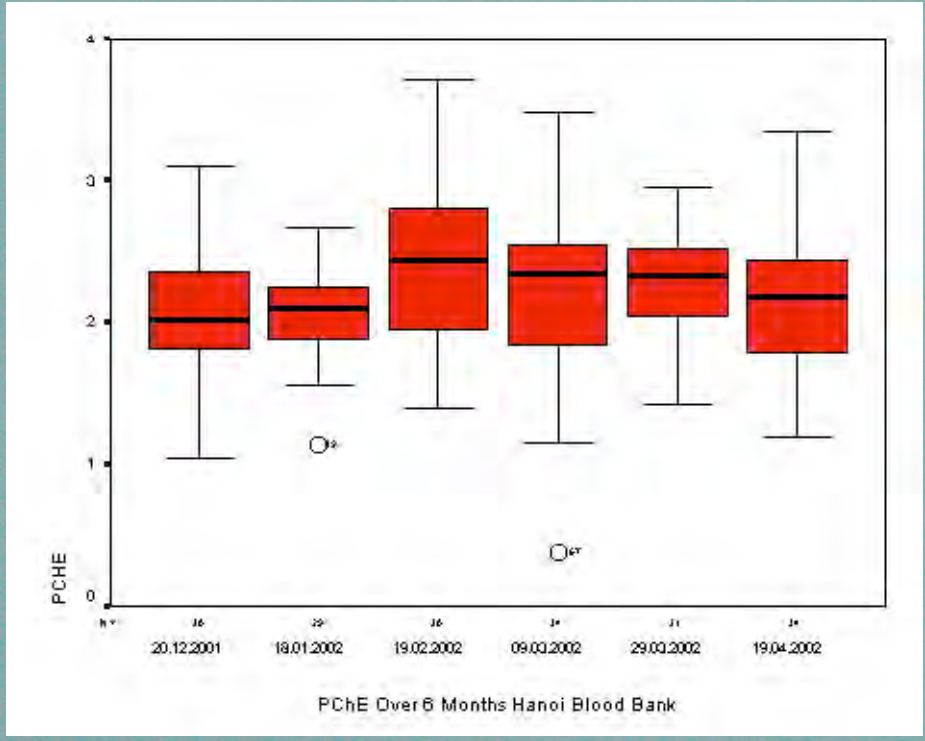
Plasma Normal Population



Variation, Month to Month

- Relatively Stable in the Population





Carbamates Alone

Is it worth testing?

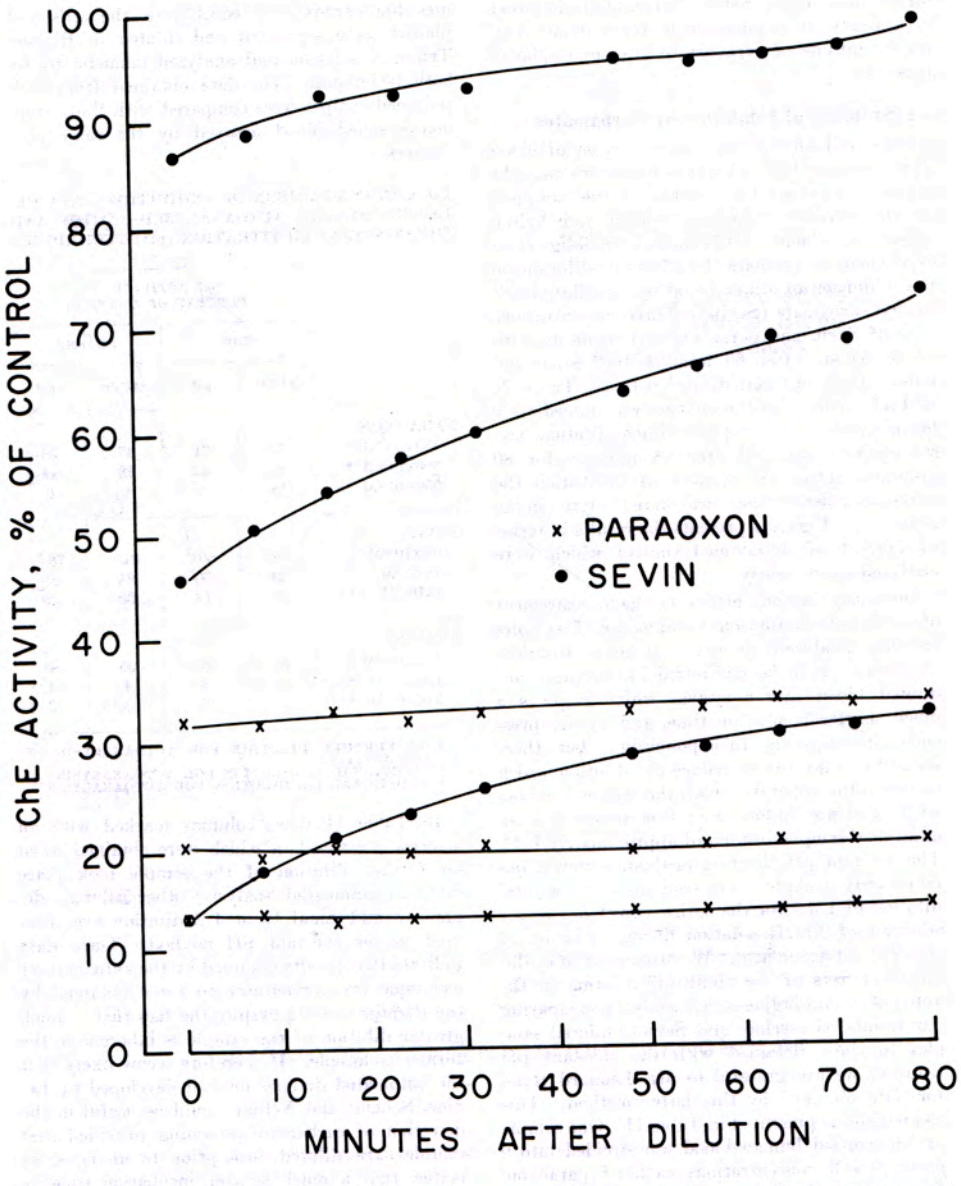


FIGURE 4. Reversibility of inhibition by Sevin after dilution of whole blood. Concentrations of Sevin (in undiluted sample) from top: 1, 10 and 100 x 10-6M. Concentrations of paraoxon: 3, 4 and 5 x 10-7M.

History and Physical of Handler

Presence of ChE inhibitor based symptoms

Experience with pesticides
- Attitude toward inhibitors
- Medications
- Previous medical history

Probable contraindications
- Asthma/COPD
- G.I. Ulcer

Treatment with ChE inhibitor
- myasthenia gravis
- Alzheimers
- glaucoma

Other possible problems
- Anemia
- degenerative diseases of the central nervous system
- chronic colitis
- psychosis

Baselines

- Obtain before exposure
  - 30 days since last handling
- Maintain records for future comparison
- If its abnormally low
  - Recheck, average or discard
- More tests are better than less
  - What does regression to the mean mean?

How Often to Test?

- Retest with the same laboratory, same methods
- Retesting every 30 days
  - When to do follow-up?
    - Rules state within 3 days of reaching threshold
  - Why are you testing?
    - To prevent future exposure
    - To evaluate work exposure
  - Decrease frequency with experience

How to Interpret Results

Large difference between upper and lower range of normal

20% depression- Significant

30% AChE- Removal*

50% AChE- Poisoning

40% PChE- Removal*

60% PChE- Poisoning

*California, WHO and ACGIH recommendations on removal thresholds

What Response to Depressed Results

- Act promptly
  - You're already late
- Evaluate for false positives
- Assure removal if meets threshold
- Be sure the workplace is evaluated
- Communicate with worker and with employer
  - The teachable moment

False Positives

Plasma Cholinesterase

- Drugs: therapeutic and recreational
  - BCPs, metaclopramide, cocaine?
- Liver Disease-alcoholism
- Congenital Deficiency (3%)
- Pregnancy
- Nephrotic syndrome
- Carbon disulfide, organic mercury

RBC Cholinesterase

- Drugs and Reticulocytosis

False Negatives

- Hard to find, Hard to know
- Lack of depression when depression is truly present
  - Laboratory phenomena
  - Low baseline
  - Sample confusion

Medical Removal

- What else can they do?
- Thinning? Probably not in sprayed orchards*
- Know the operation
- General work

*Engel and Keifer 1998, Keifer, Miller, Fenske 1995 Schnieder et al 1991)

Return to Work

Return to regular duty

- When both PChE and AChE get to 80%

File a Claim?

- If worker is sick, yes

Quality Assurance Policing Your System

- Records and Response
  - Dry run your response
  - Dry run you communication options
  - Check out removal options
- Test the quality of your ChE laboratory
  - Blinded split samples to laboratory
  - How far off should they be?
    - If they approach thresholds, you're in trouble

Responsibilities of Medical Supervisor

- Know the rules of Monitoring (WA state)
  - Obligations regarding confidentiality
- Know something of the pesticide practices
  - Which pesticides, application frequency, PPE
- Know your population
  - Language, culture, beliefs
- Know how to respond to a depression
  - Check PPE & pesticides, removal options
- Know the non-pesticide related causes of depression

Responsibilities of Medical Supervisor

- Assure quality performance and worker protection
  - False positives
  - False negatives
  - Laboratory accuracy
  - Response to depressions
    - Prompt
    - Appropriate
  - Advise employer
  - Counsel worker

Abnormal Baselines Plasma ChE

- Congenital cholinesterase deficiency
  - 3% of Anglos, 1% of Blacks carry the gene
  - May influence susceptibility to ChE inhibitors
  - Will have low baseline values for PChE
  - Will have normal RBC ChE values