



Human Serum Butyrylcholinesterase: The First Bioscavenger for Protection Against Organophosphate Chemical Warfare Agents

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Current medical regimen for nerve agent intoxication

- **Pretreatment** with carbamates, e.g., pyridostigmine, to protect AChE from irreversible inhibition by OPs
- **Post-exposure therapy** with:
 - Anticholinergic drugs, e.g. atropine to counteract the effects of excess acetylcholine
 - Oximes, e.g. 2-PAM to reactivate OP-inhibited AChE
 - Anticonvulsant drugs, e.g. diazepam to minimize convulsions and permanent brain damage

Goal

To improve the present medical regimen for nerve agent intoxication

To develop an antidote that can prevent any toxicity at low or high level of exposure of up to 5 LD₅₀ of soman

Requirements for an enzyme to be an effective bioscavenger

- Relatively high rate of reactivity with all OPs
- Long half-life of circulation *in vivo*
- Should be devoid of physiological or psychological side effects
- Repeated administrations should not cause adverse immune reactions
- Readily available in sufficient quantities

Production of Hu BChE From Cohn Fraction IV-4

Resuspension of Cohn Fraction IV-4 Paste in 25 mM Phosphate Buffer pH 8.0, containing 1 mM EDTA



Clarification of Cohn Fraction IV-4 Suspension by Continuous Flow Centrifugation



Purification of Hu BChE by Procainamide Affinity Chromatography



Further Purification of the Enzyme on a DEAE Anion Exchange Column



❖ 80 Kg starting material = 10 million units ~ 14 g Hu BChE

❖ **The final product:**

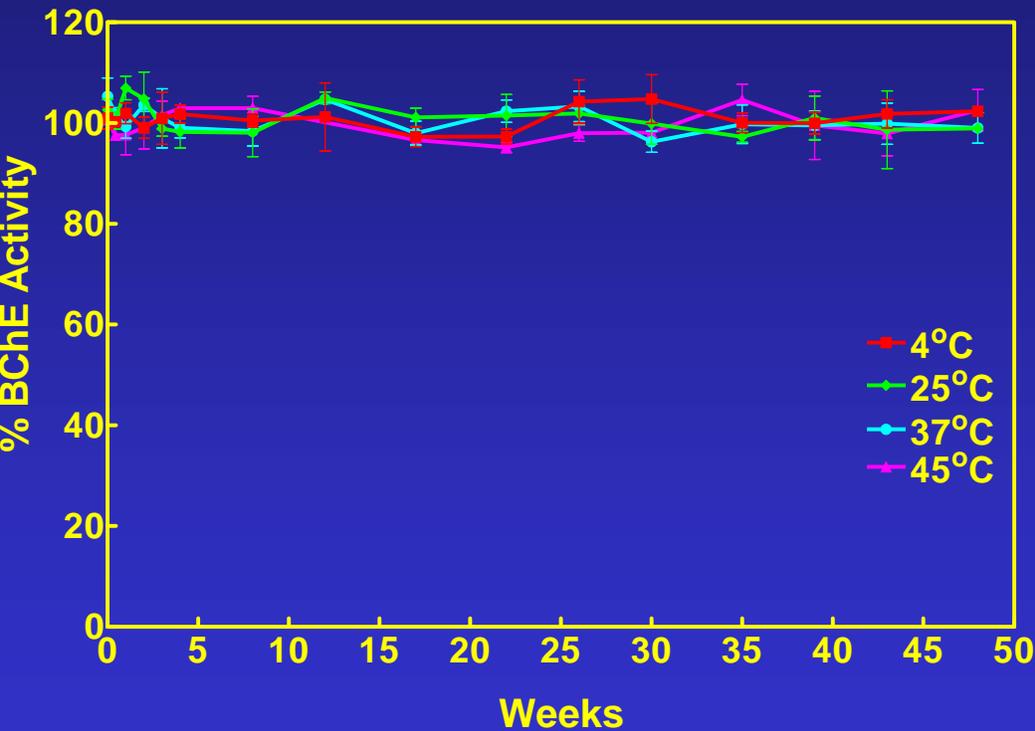
❖ ~ 6 g of purified BChE

❖ >98% pure; specific activity ~700 U/mg

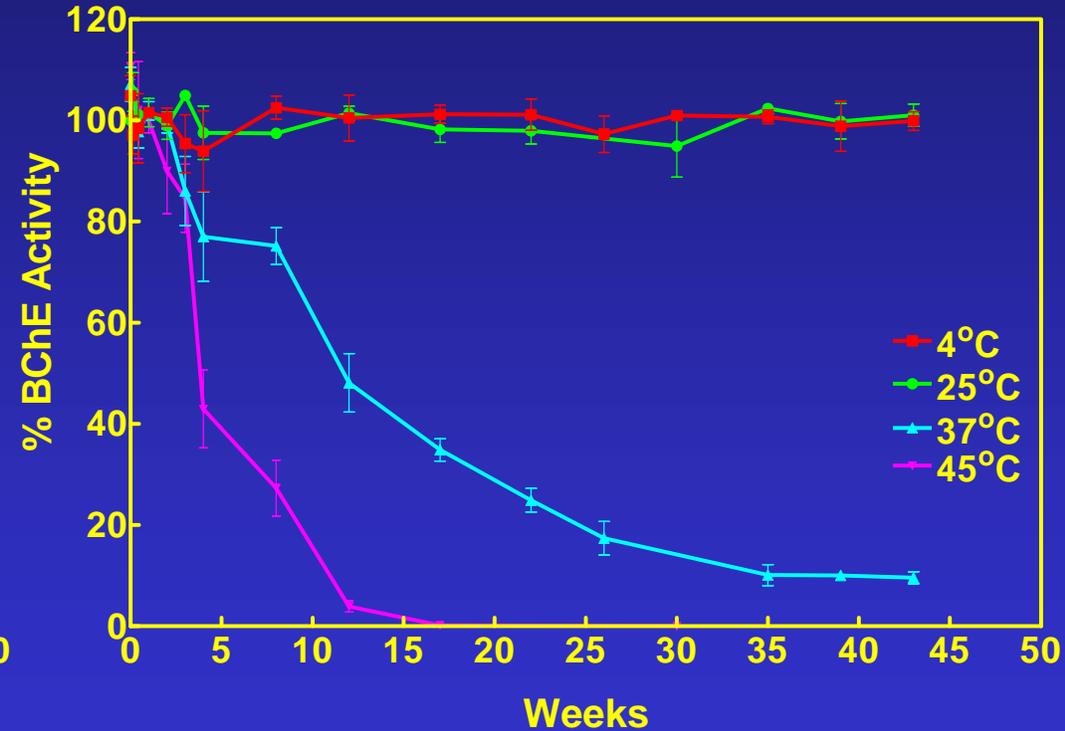
❖ single band on SDS-PAGE

Thermal Stability of Purified Hu BChE

Lyophilized



In 10% glycerol



Conclusions

- The use of Cohn fraction IV for the purification of large quantities of Hu BChE greatly improved the yields of purified enzyme without affecting:
 - Thermal stability
 - Bioavailability
 - Mean residence time
 - Safety
 - Scavenging efficacy
- This greatly enhances the utility of Hu BChE as a protective regimen for nerve agent toxicity in humans