

Pharmacokinetics of Botulinum Toxin

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Professor of Medicine

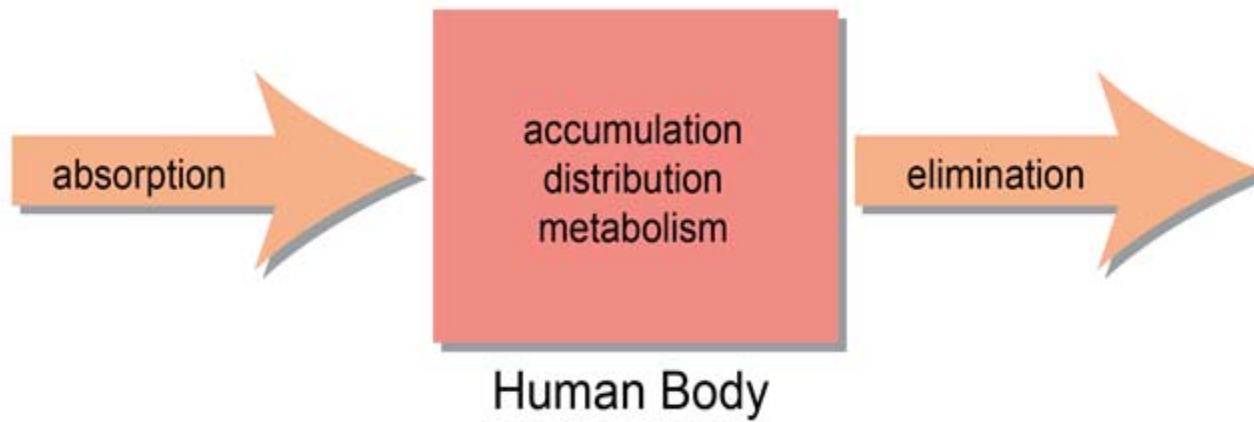
Primary Goals

1. To identify and characterize the mechanisms for absorption, distribution, metabolism and elimination of botulinum toxin.
2. To focus on the three serotypes (A,B, and E) that are most often implicated in human illness.

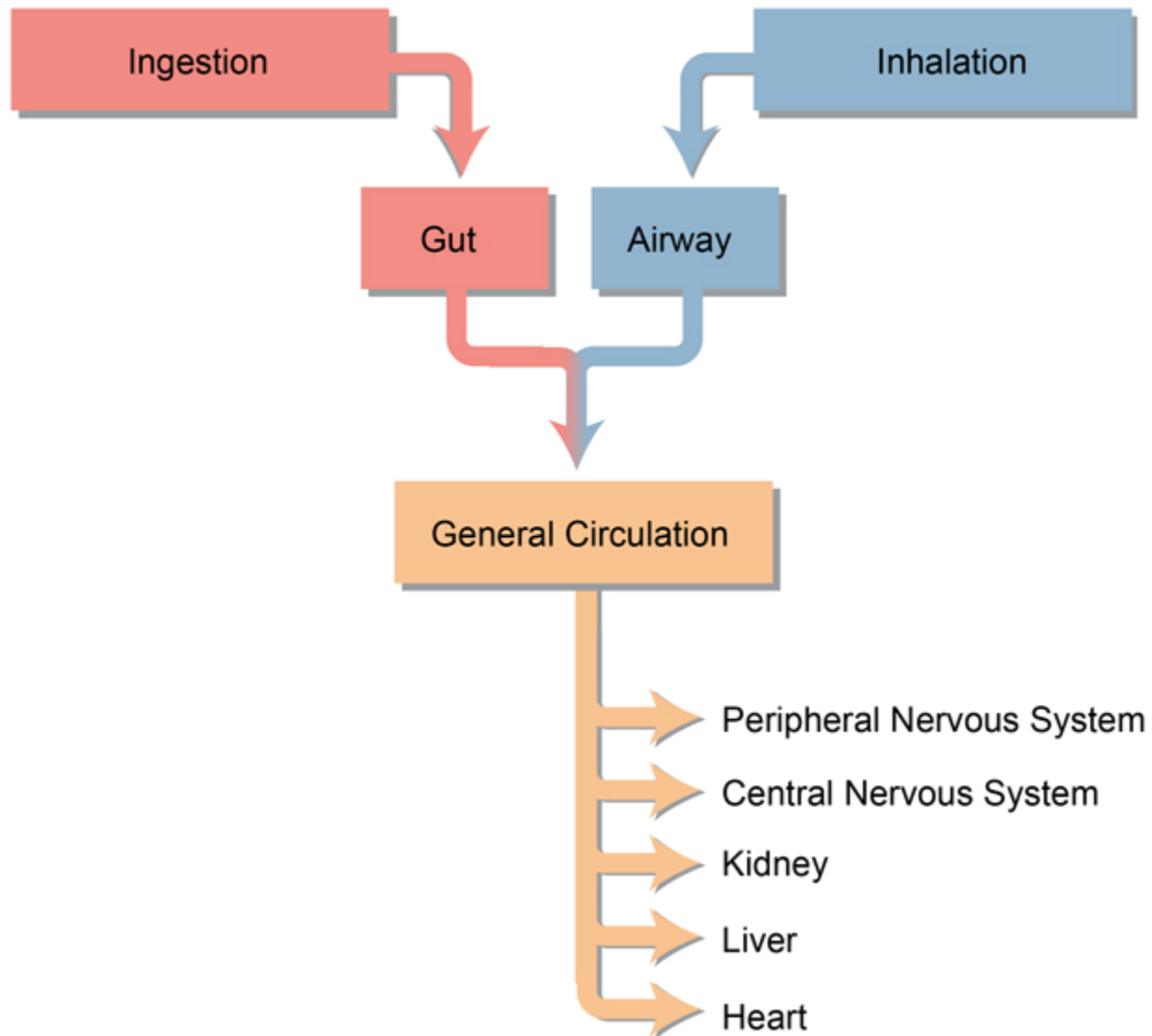
Secondary Goals

1. To develop *in vitro* and *in vivo* assays for characterizing the mechanisms of action of agents that antagonize botulinum toxin.
2. To determine the “Window of Opportunity” within which medical countermeasures can be administered to block toxin action.

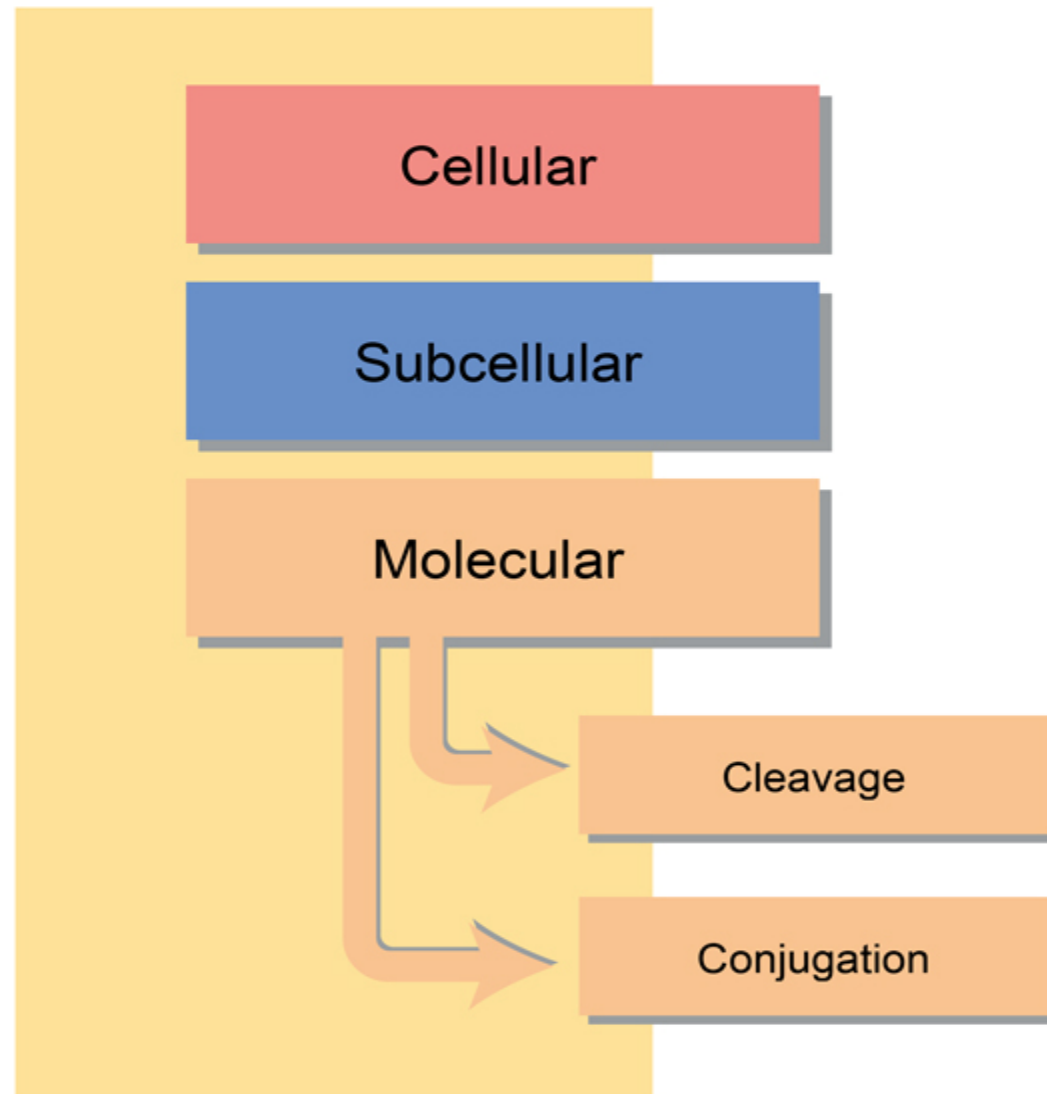
Pharmacokinetics (Dynamic Analysis of Compartments)



Compartments of Interest (Influx)



Compartments of Interest (Metabolic Transformation)



Liver

Compartments of Interest (Metabolism and/or Elimination)



The diagram shows a large yellow rectangle representing a 'Nerve Cell'. Inside this cell, three smaller, overlapping rectangles represent different compartments of interest. From top to bottom, they are: a red rectangle labeled 'Export', a blue rectangle labeled 'Redistribution', and an orange rectangle labeled 'Cleavage- Lysosome' and 'Cleavage- Proteasome'. The orange rectangle is wider than the others and overlaps with the blue one. The entire diagram is flanked by two vertical blue bars on the left and right sides.

Export

Redistribution

Cleavage- Lysosome
Cleavage- Proteasome

Nerve Cell

Compartments of Interest (Routes of Elimination)

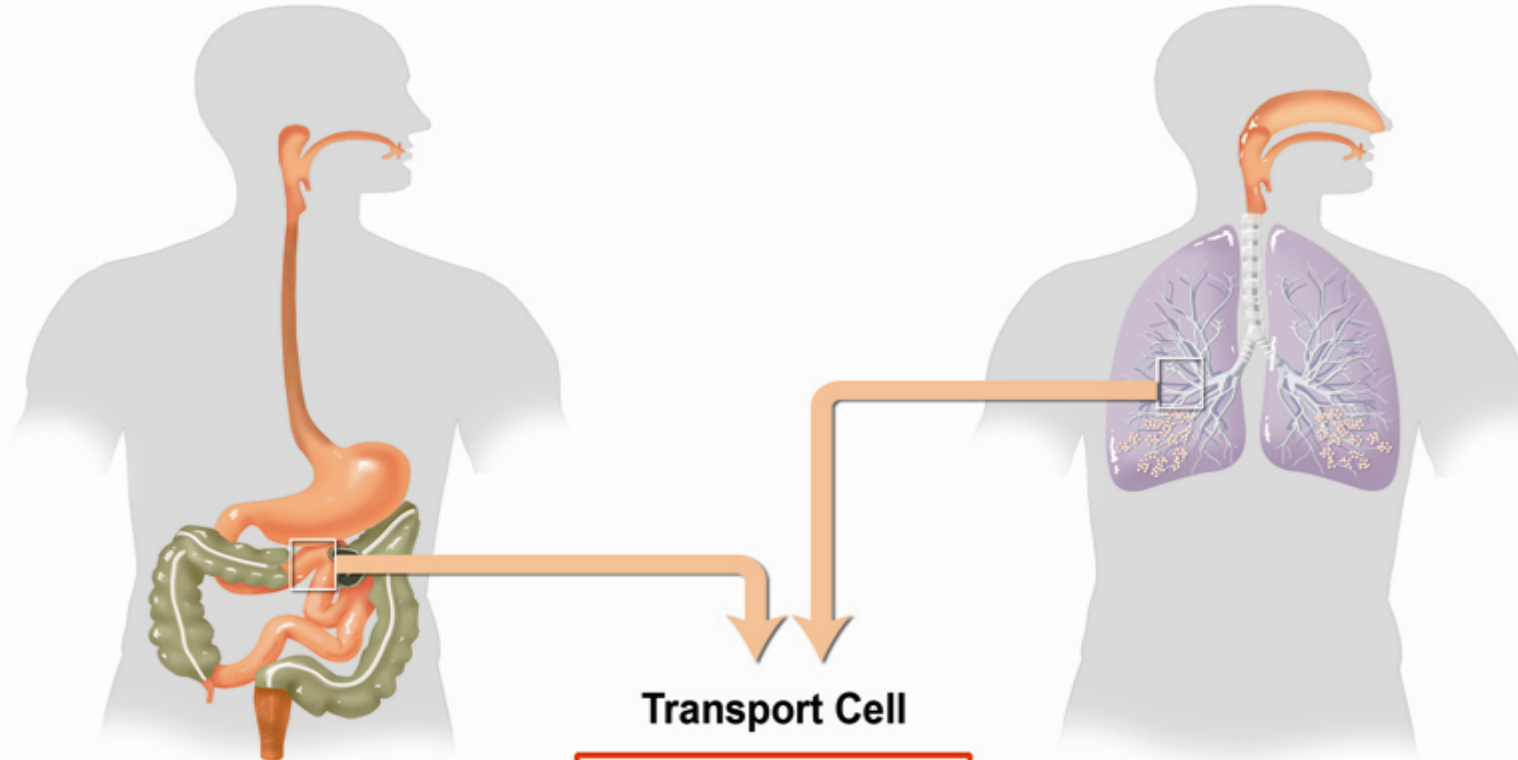
Lower Bowel

Kidney and Bladder

Airway

Skin

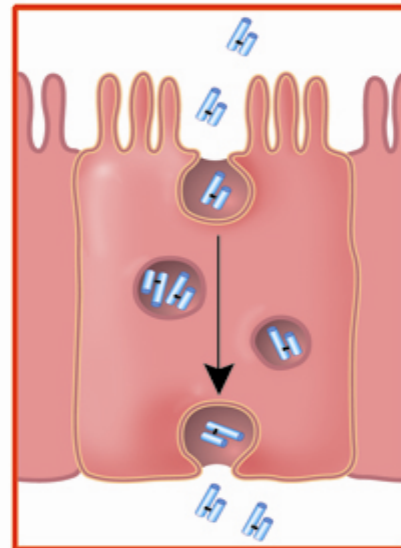
Absorption of Botulinum Toxin



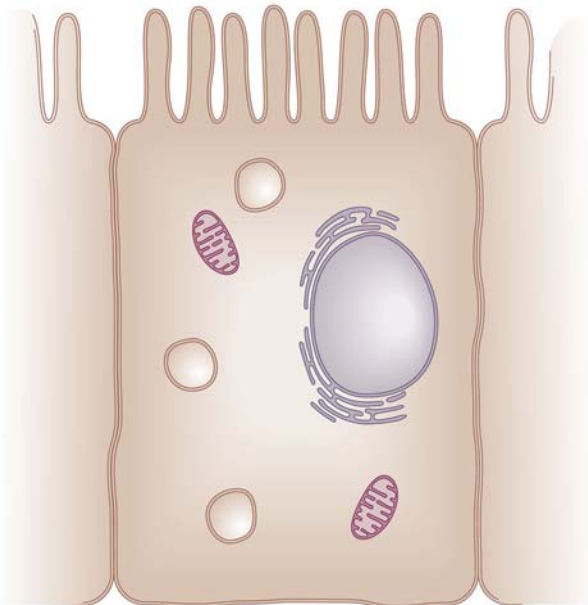
Foodborne Botulism
(Primary Intoxication or
Primary Infection)

Inhalation Botulism
(Primary Intoxication)

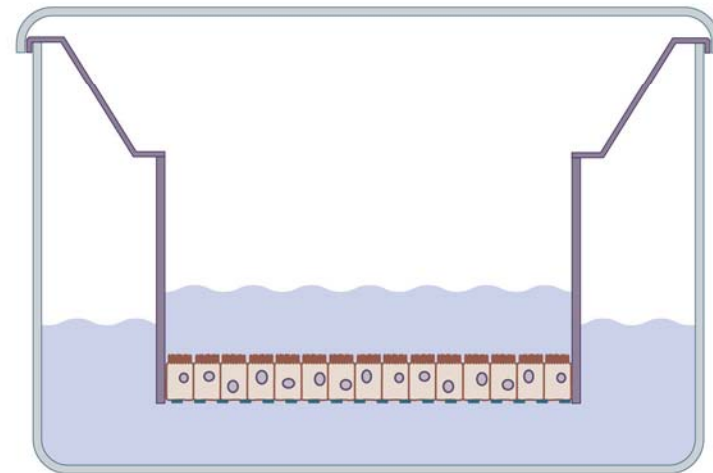
Transport Cell



Apical (Mucosal)



Basal (Serosal)

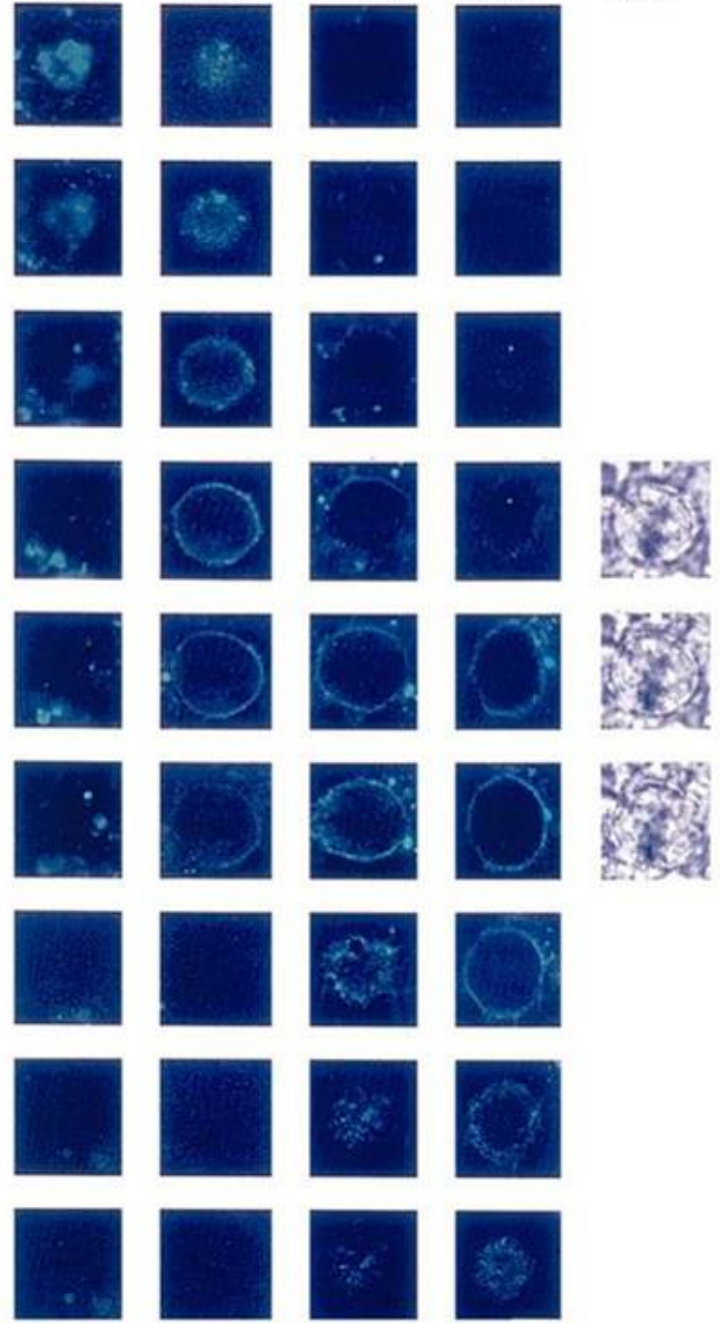


Transwell Apparatus

Characterization of ^{125}I -BoNT/A Transcytosis in Polarized Epithelial Cell Cultures

Cell Line	Conditions	fmol / hr / cm ²	% Control	<i>p</i>
T -84	37°C (A→B)	11.29 ± 0.30	100	—————
	37°C (B→A)	8.98 ± 0.20	80	<i>p</i> < 0.001
	18°C (A→B)	2.26 ± 0.46	20	<i>p</i> < 0.001
Caco-2	37°C (A→B)	8.46 ± 0.49	75	<i>p</i> < 0.001
MDCK	37°C (A→B)	0.32 ± 0.07	2.8	<i>p</i> < 0.001

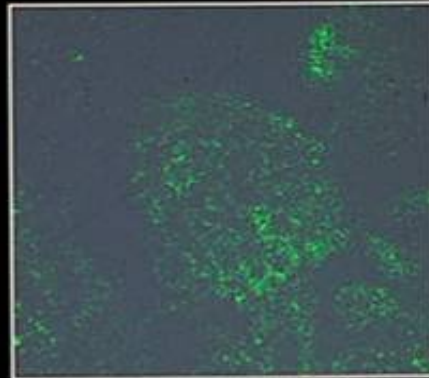
Top 0 min. 5 min. 10 min. 20 min. Phase



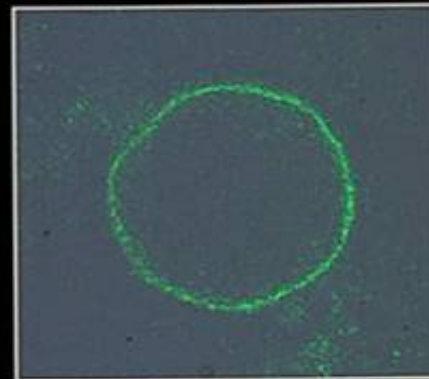
Bottom

Visualization of Transcytosis

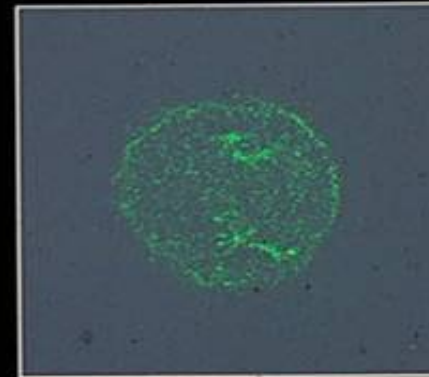
A. Apical



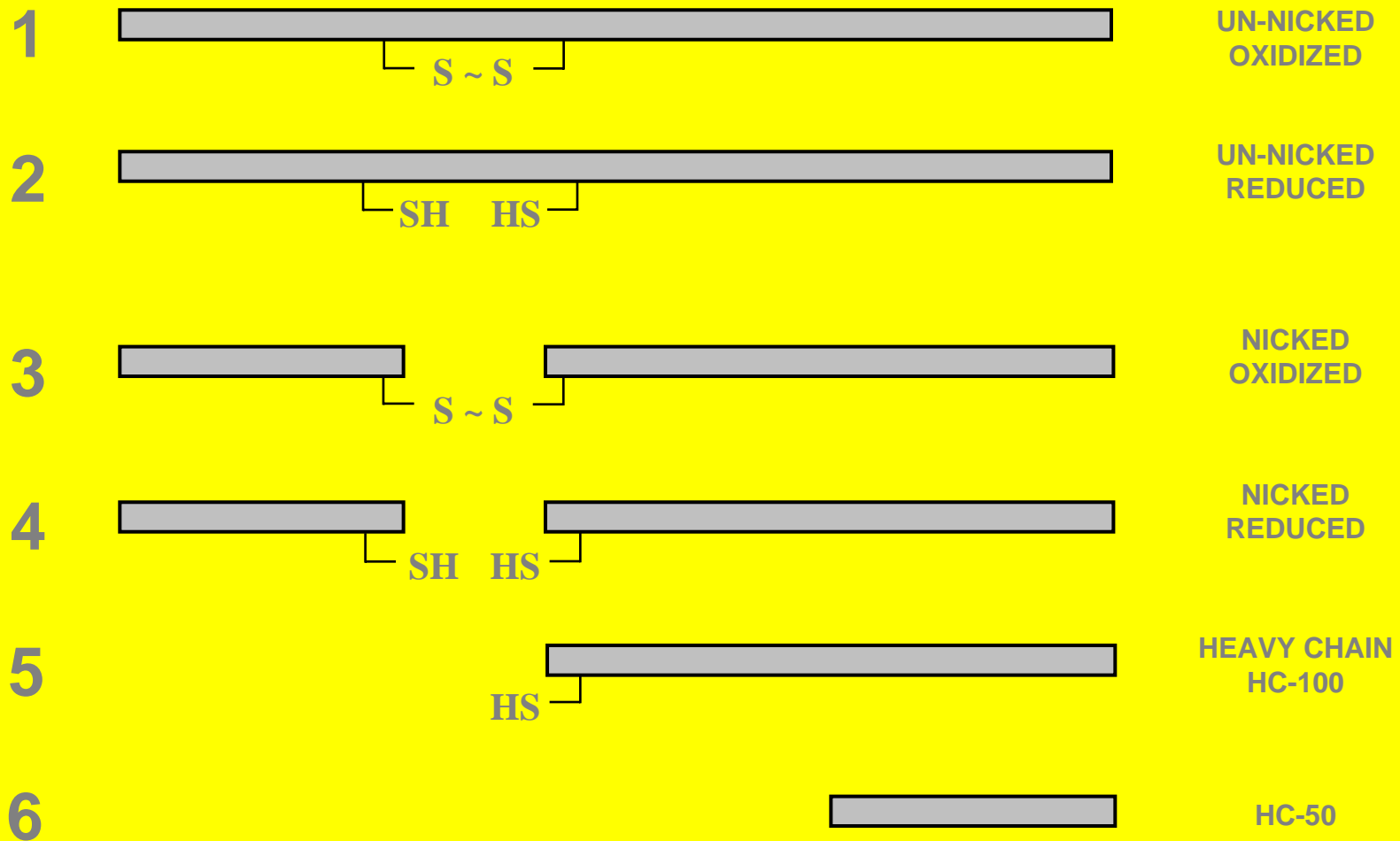
B. Medial



C. Basal



Structure-Activity Relationships



Distribution and Availability

In vitro

Blood: 100%

Serum: 88%

In vivo

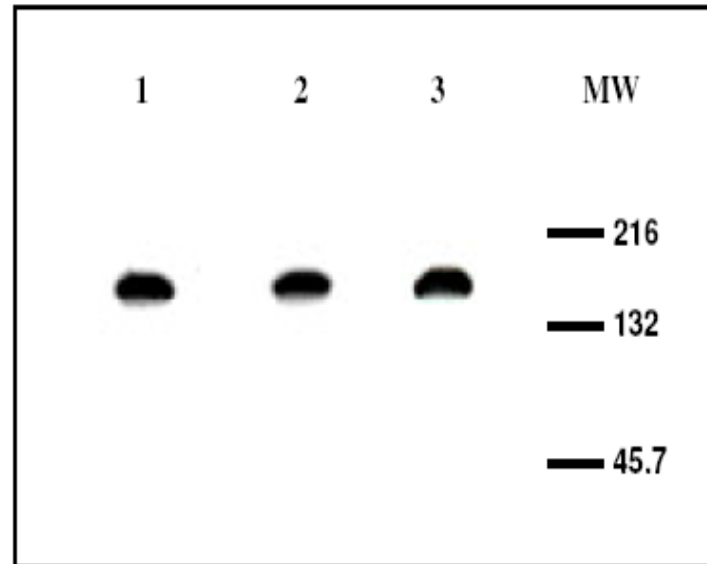
Blood: 100%

Serum: 85%

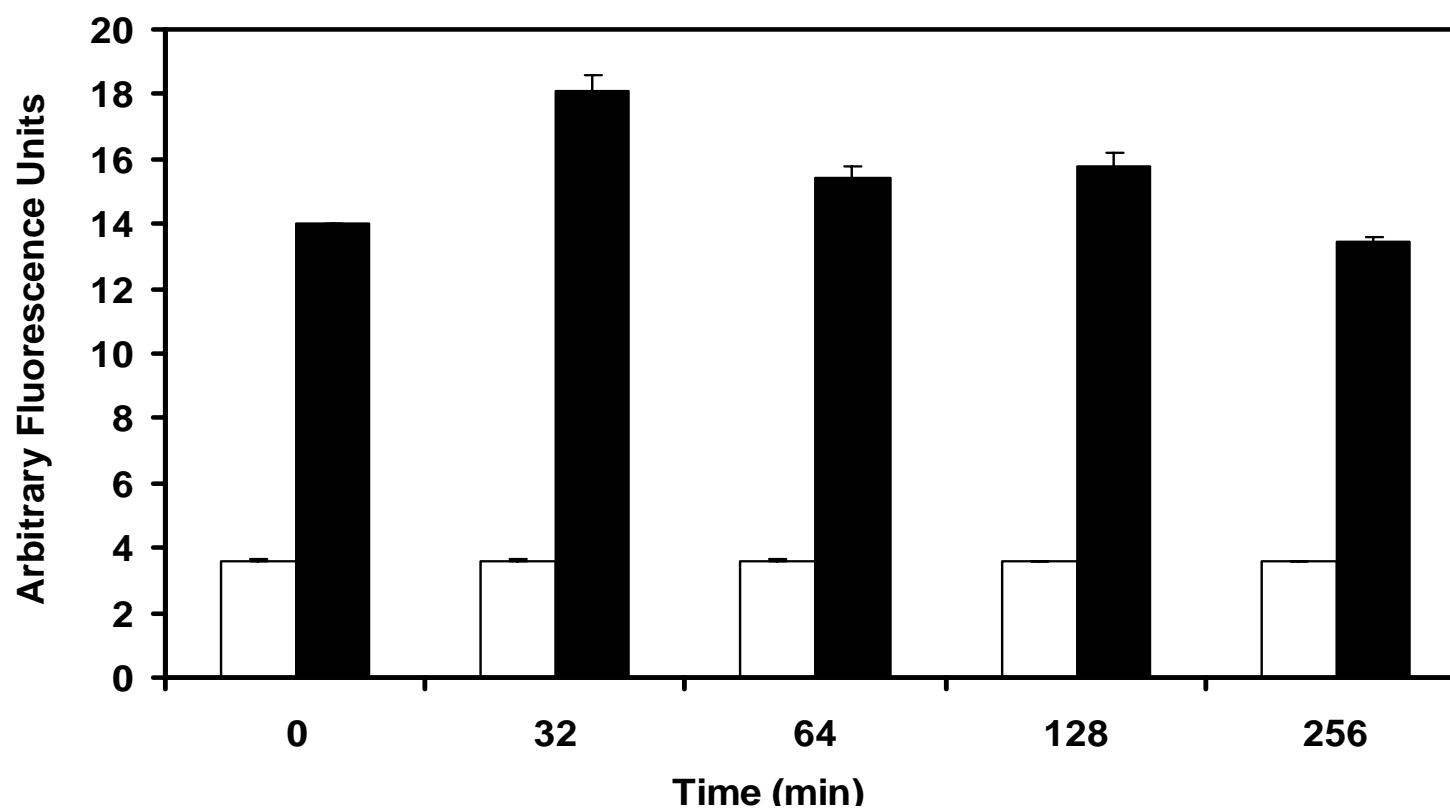
Serum albumin binding:

ca. 27% bound and 73% free

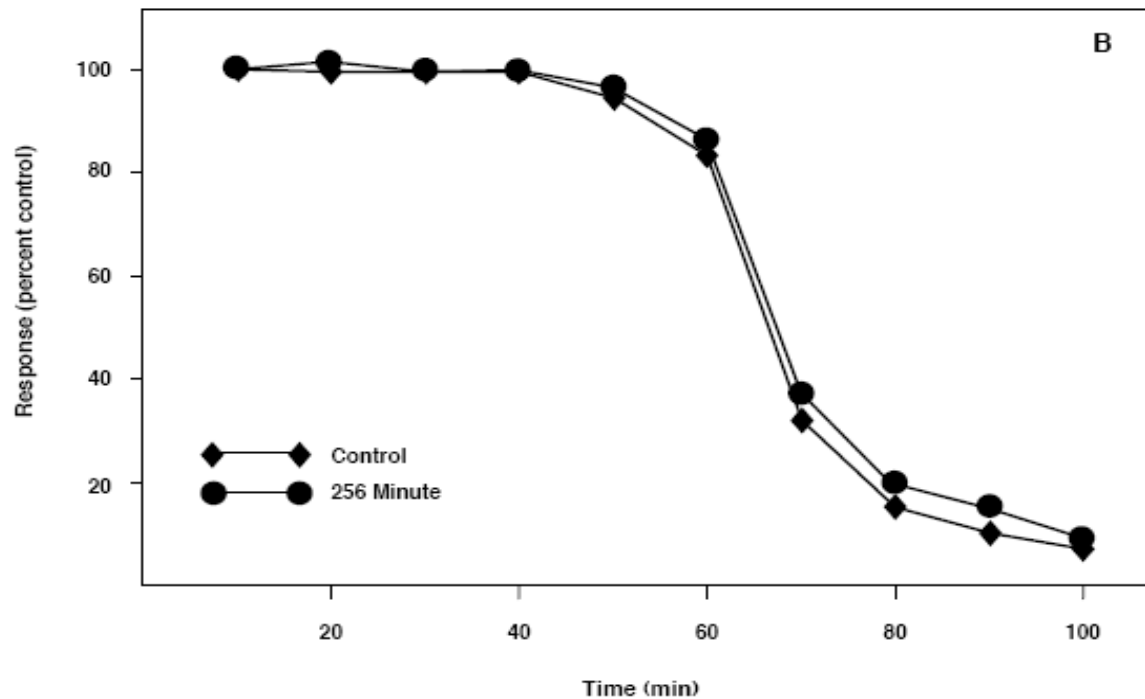
Stability in Blood



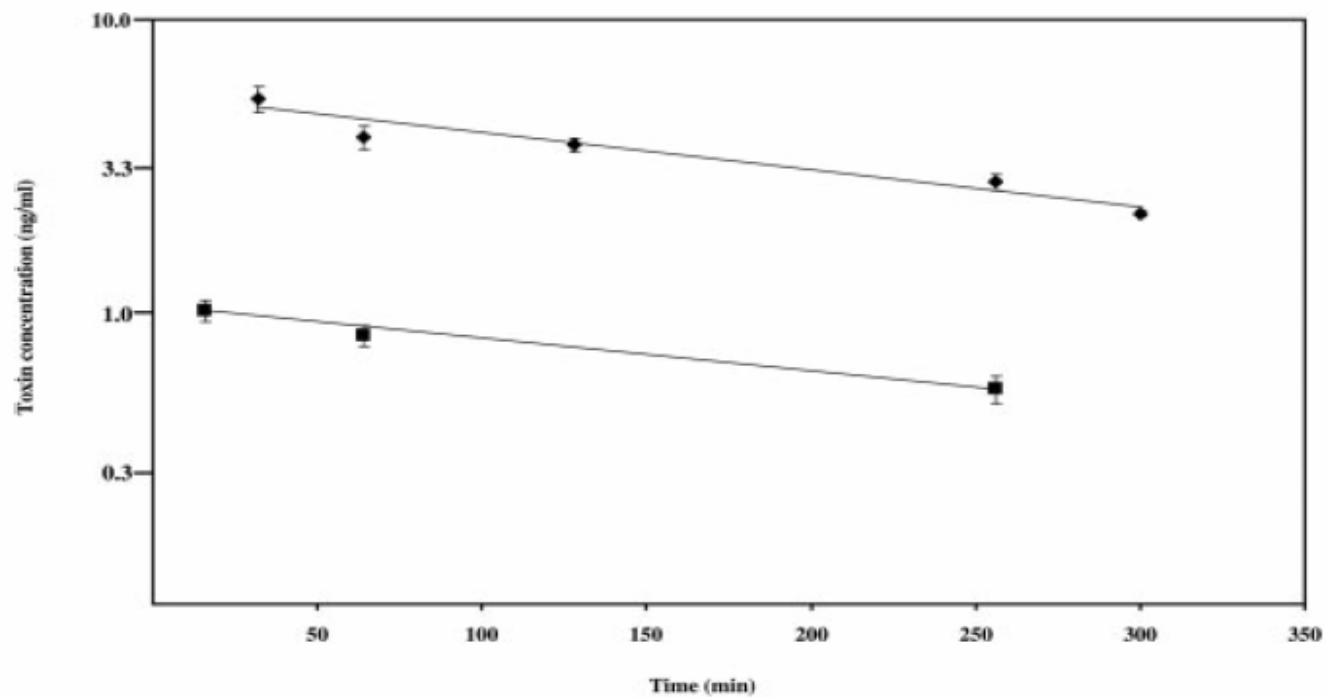
Residual Catalytic Activity



Neuromuscular Blocking Activity



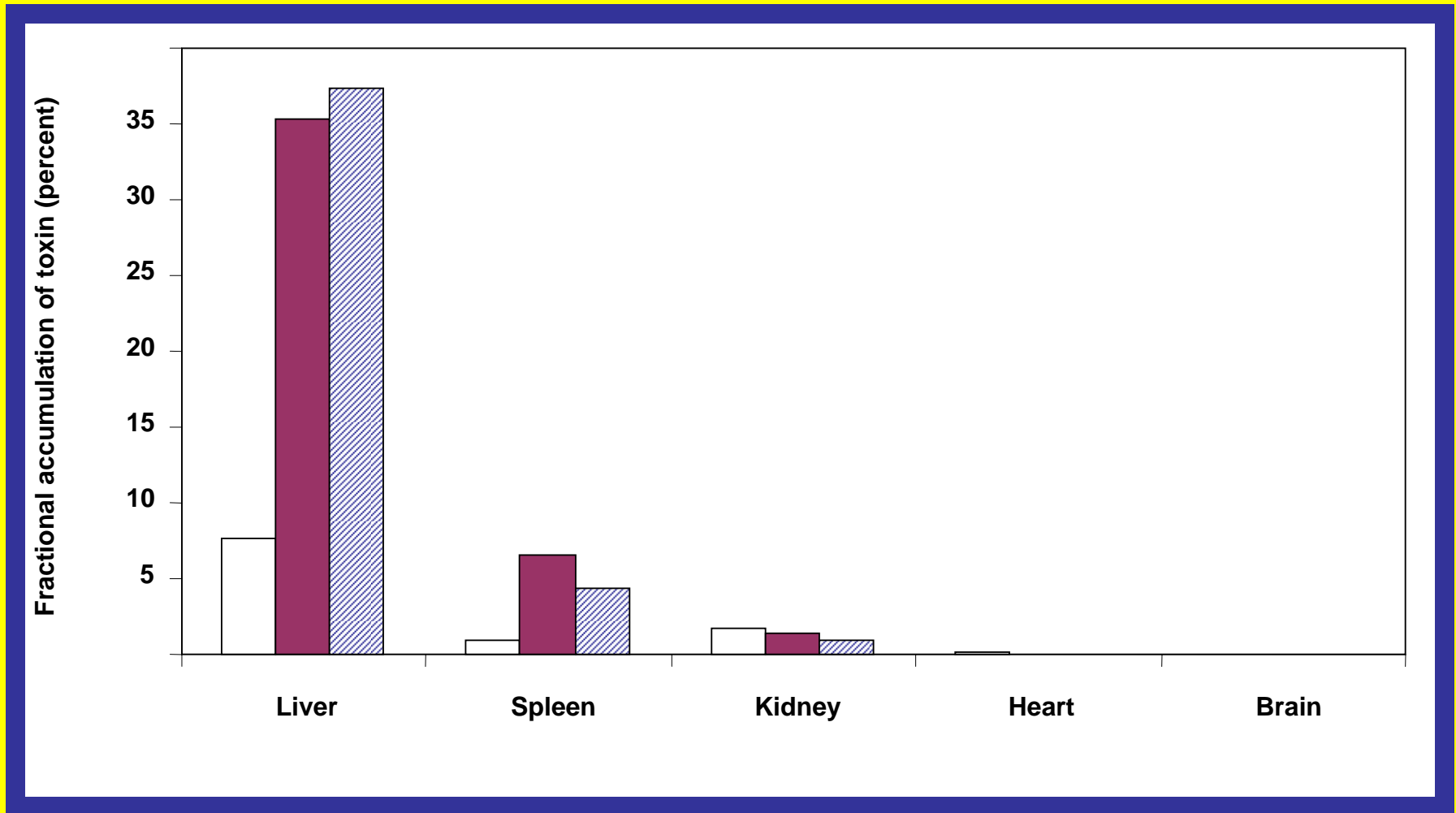
Biologic Half Life



Analyzing Tissue Distribution

- 1. As a naturally-occurring phenomenon**
- 2. As an induced phenomenon (i.e., in the presence of neutralizing antibodies)**

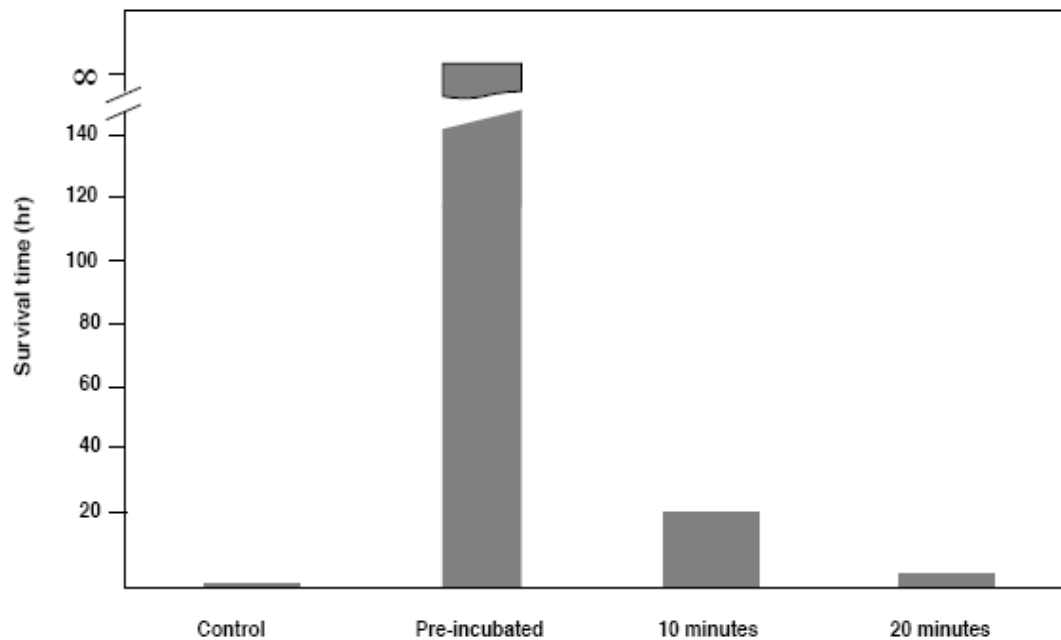
Tissue Accumulation (\pm Neutralizing Antiserum)



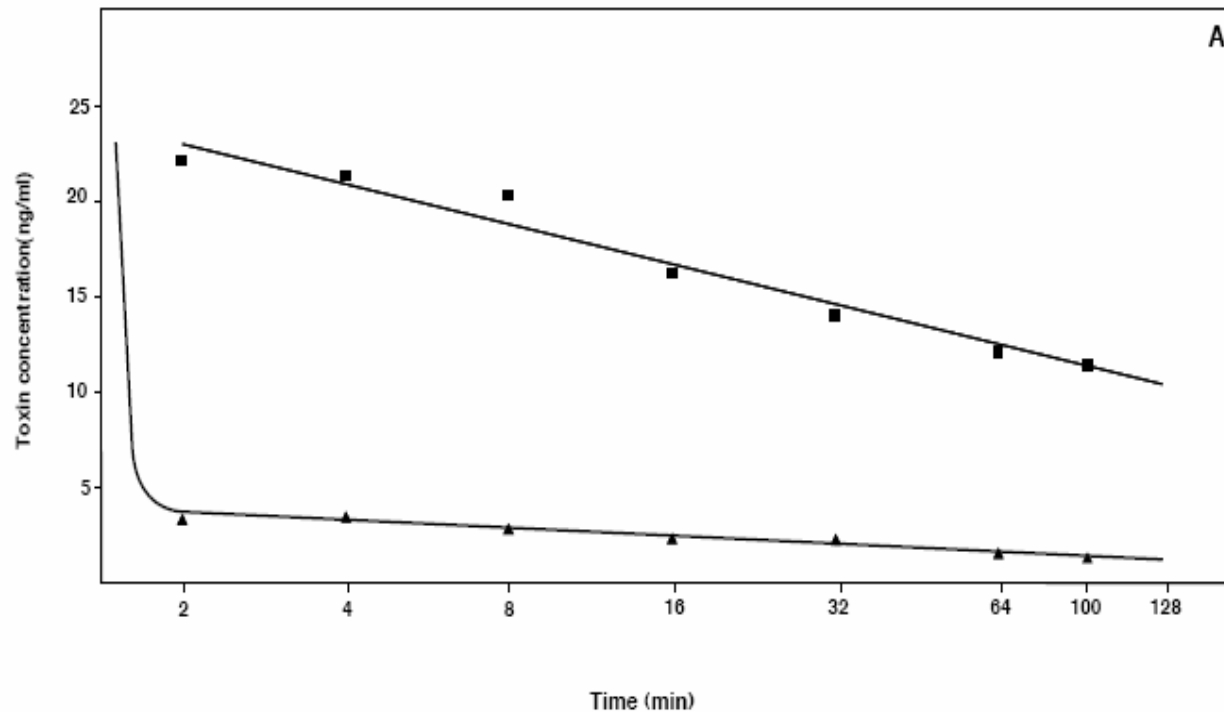
Analyzing Tissue Distribution

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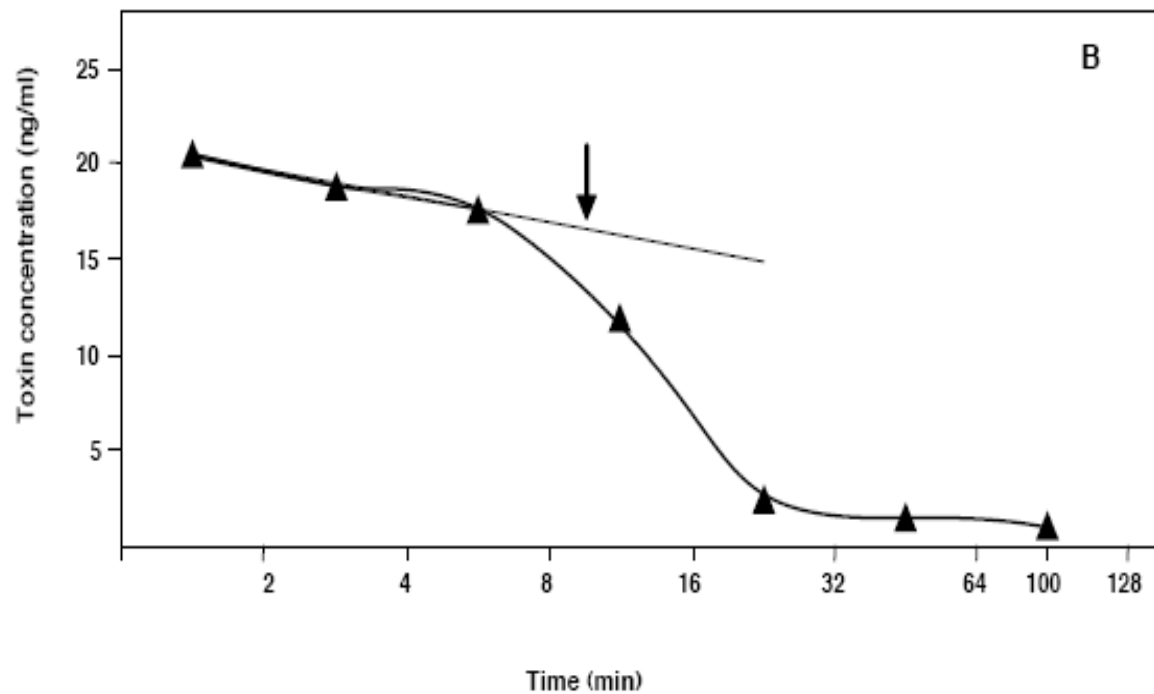
Window of Opportunity



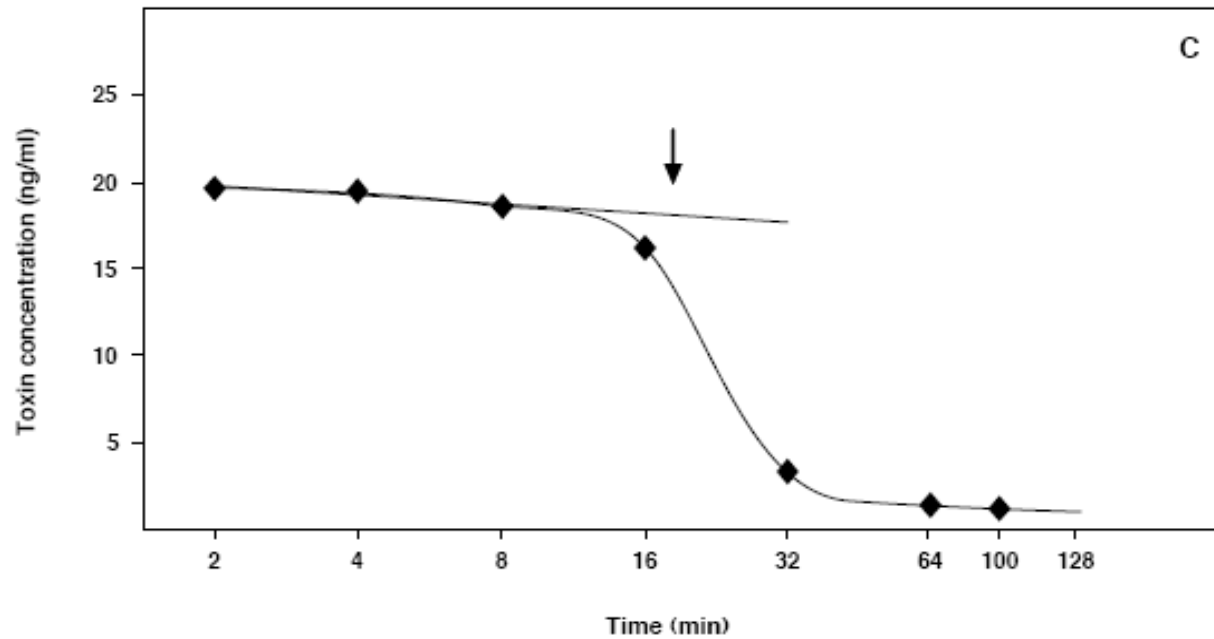
Clearance from Circulation (t = 0 min)



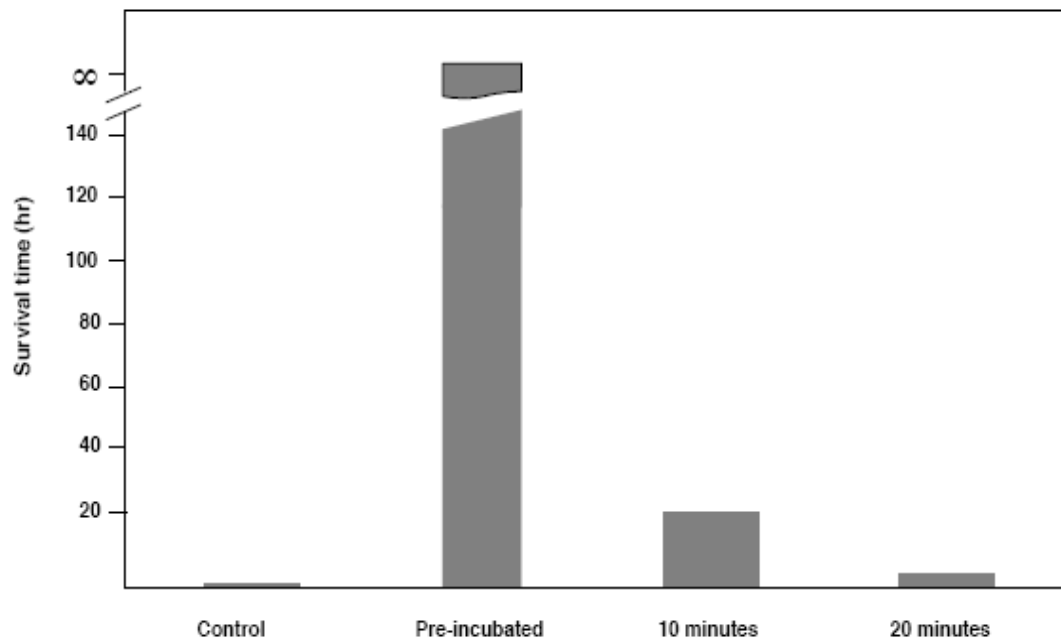
Clearance from Circulation ($t = 10$ min)



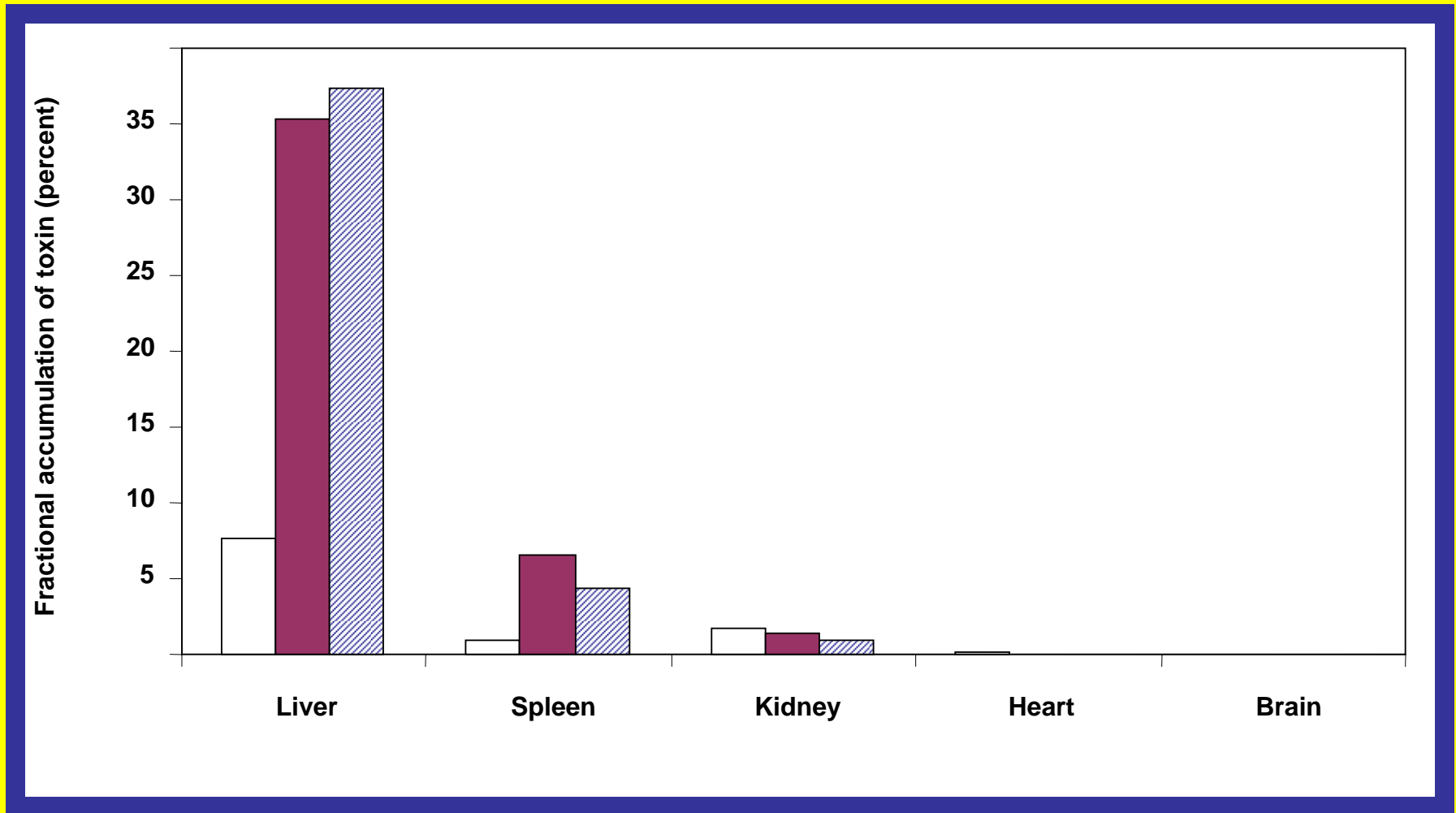
Clearance from Circulation (t = 20 min)



Window of Opportunity

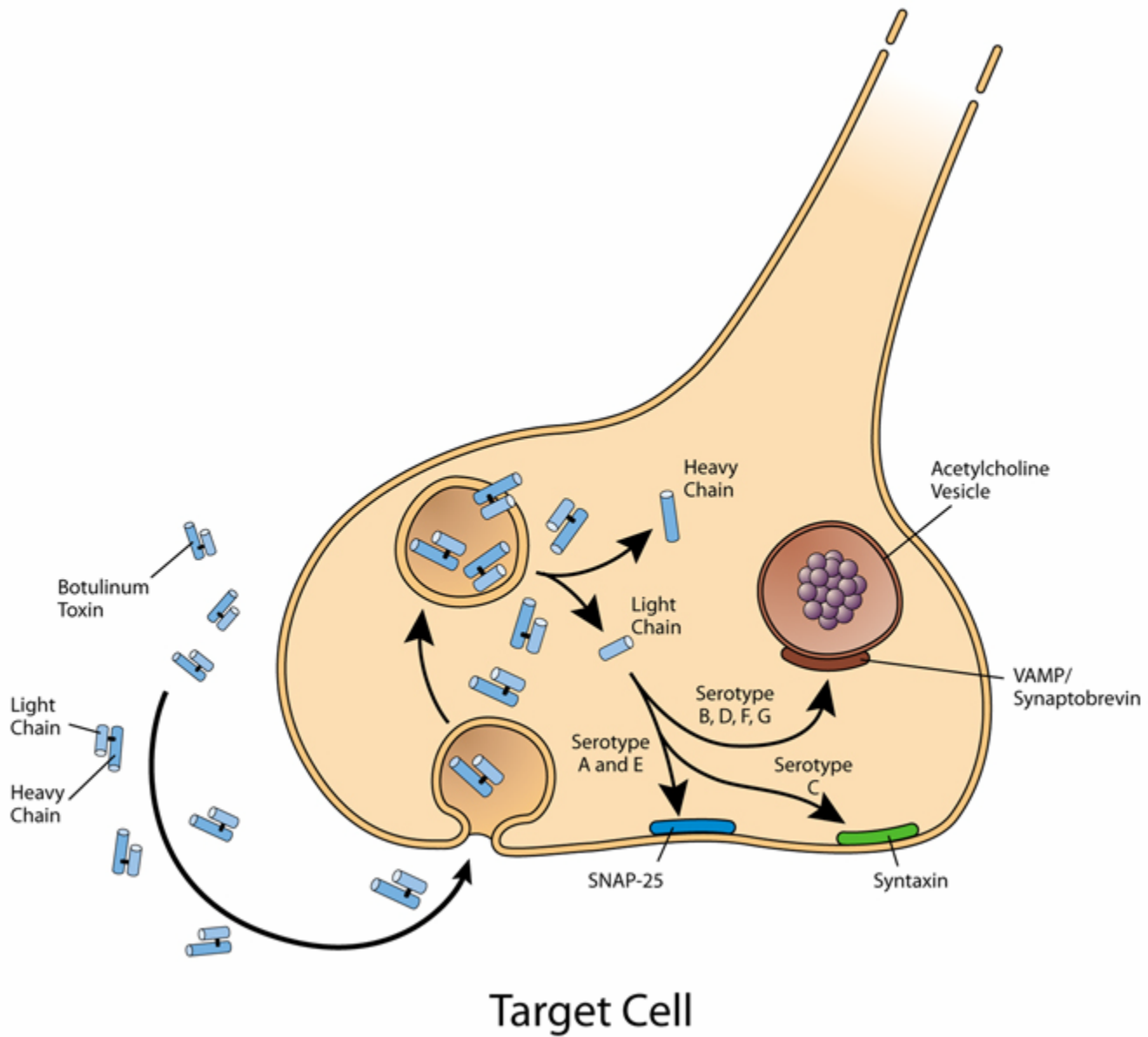


Tissue Accumulation (\pm Neutralizing Antiserum)



Metabolism and Elimination

1. There is no information on mechanisms for systemic metabolism of toxin, or on mechanisms for elimination of metabolites.
2. There is little information on intraneuronal metabolism of toxin, or on the fate of metabolites.



Compartments of Interest (Metabolism and/or Elimination)

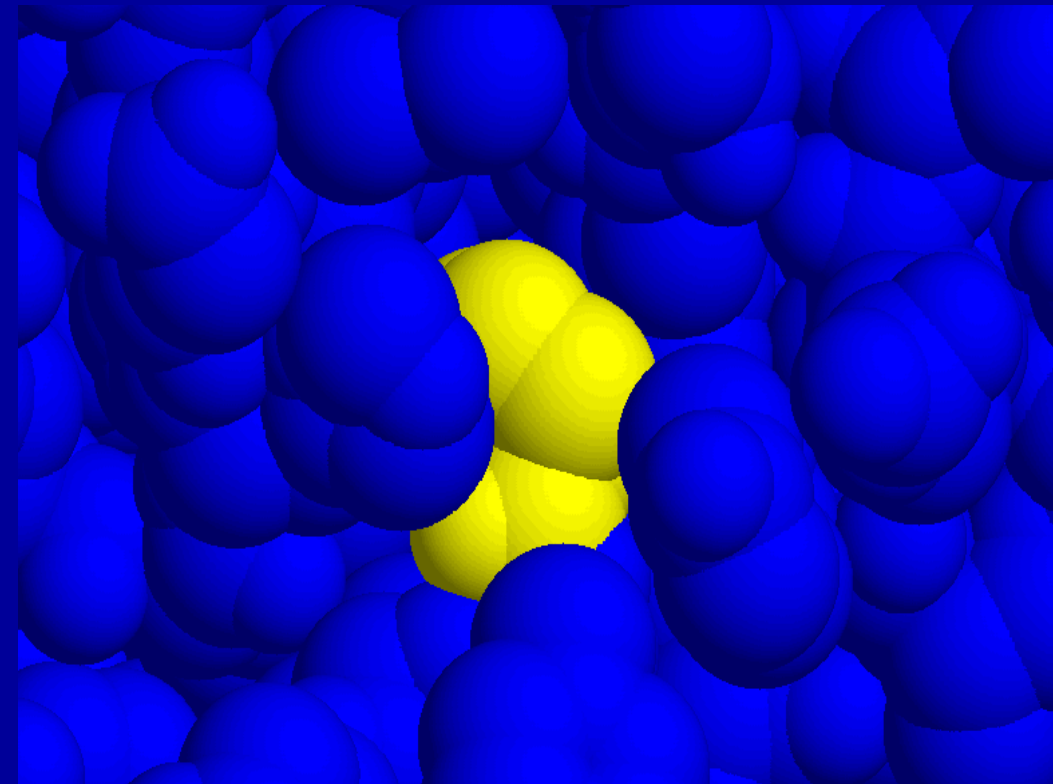
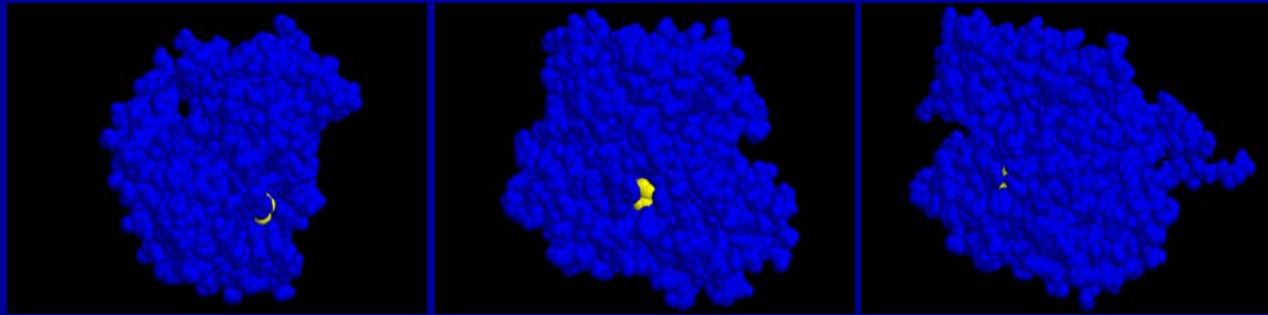
Export

Redistribution

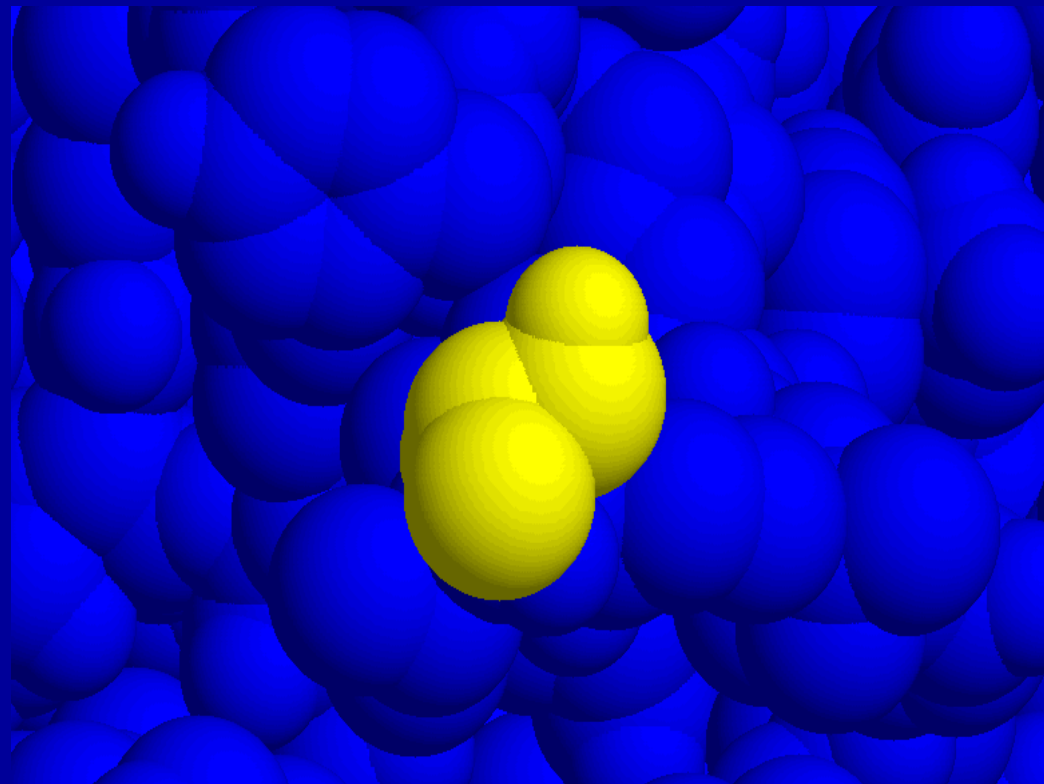
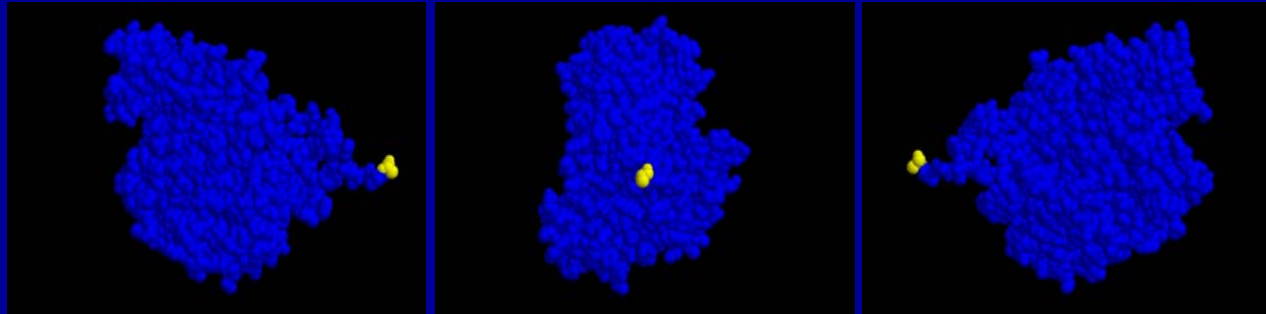
Cleavage- Lysosome
Cleavage- Proteasome

Nerve Cell

3-D Structure of LC Highlighting Aminoterminal Proline



3-D Structure of LC Highlighting Carboxyterminal Arginine 431



Endoprotease Activity

1. **Hirokozu Kouguchi manuscript**
2. **Lysosomal endoprotease (Cathepsin B, Cathepsin D)**
3. **Non-lysosomal endoprotease (7 enzymes; Calpain, CCK8-Generating Endoprotease, Brain Neuropsin, etc.)**
4. **Analogy with gut**

In Vitro Human Body

1. Human gut or airway epithelial cells in culture
2. Human vascular endothelial cells in culture
3. Human (or rodent model) neuromuscular junction



Pharmacokinetics of Toxin and Antibiotics

