



Botulism

肉毒桿菌

A Bioterrorism Agent

Dr. Lai Kang Yiu

Intensive Care Unit

Queen Elizabeth Hospital



肉毒桿菌感染 婦食滷豆乾亡 。東方日報 17/4/2010 桃園衛生局在大溪鎮扣查五百多包「大溪小印乾」



真空包裝的素肉、素蹄筋、豆乾

An outbreak of foodborne botulism in Taiwan. Tseng CK. Tsai CH. Tseng CH. Tseng YC. Lee FY. Huang WS. International Journal of Hygiene & Environmental Health. 212(1):82-6, 2009 Jan.

真空或罐頭包裝的食品，以及醃漬食品，容易提供缺氧環境，倘生產、包裝、保存不當，食物可能被肉毒桿菌污染。臭豆腐最易遭肉毒桿菌感染。專家建議，民眾購買食品應留意包裝、保存是否完整，即使是真空食品也應冷藏。此外，這種毒素極不耐熱，在攝氏一百度的沸水中煮十分鐘，就可輕易殺死，因此民眾最好先加熱再食用。

THE ODYSSEY ~ HOMER ~



Blood Sausages



Sausage making is a very old food preservation technique using salt, herbs and spices

It is often assumed that sausages were invented by Sumerians in what is Iraq today, around 3000 BC. Chinese sausage *làcháng* (臘腸/腊肠), which consisted of goat and lamb meat, was first mentioned in 589 BC. Homer, the poet of The Ancient Greece, mentioned a kind of blood sausage in the Odyssey (book 20, verse 25) , and Epicharmus (ca. 550 BC - ca. 460 BC) wrote a comedy titled *The Sausage* .

Evidence suggests that sausages were already popular both among the ancient Greeks and Romans

During the reign of the Roman emperor Nero, sausages were associated with the Lupercalia festival. The early Catholic Church outlawed the Lupercalia Festival and made eating sausage a sin. For this reason, the Roman emperor Constantine banned the eating of sausages. Early in the 10th century in the Byzantine Empire, **Leo VI the Wise outlawed the production of blood sausages following cases of food poisoning.**

One man's poison ... [is another's unfurrowed brow].

Aronson SM. Medicine & Health, Rhode Island. 91(12):359, 2008 Dec.

History

- © 1793, Justinus Kerner (1786-1862)
 - Outbreak in Wildebad, Germany
 - “Botulus” = Latin for sausage
 - Kerner’s disease
 - “Wurstgift” , “**Sausage**” or “**fatty**” poison
- © 1895, Emile Pierre Marie van Ermengem
 - Isolated organism during an epidemic in Ellezelles, Belgium, involving preserved ham
 - *Bacillus botulinus*, later *Clostridium botulinum*.
- © U.S. outbreaks in early 1920s led to improved industry processing
 - U.S. canning industry adopt a very conservative heat treatment, known as the 12D process, that reduces the probability of survival of the most heat resistant *C. botulinum* spores to one in a billion (10⁻¹²).
 - While commercially canned goods are required to undergo a "botulinum cook" at 121 °C (250 °F) for 3 minutes, and so rarely cause botulism (while home pressure canning equipment only can reach 240 °F),
- © 1951: Wound botulism
- © 1976 (Midura and Arnon and Pickett) : Infant botulism



Recent Outbreaks



- © 1987: NYC and Israel, Kapchunka, contained E-type botulism
 - Kapchunka also called "Faseikh" "moloha" "rybetz", "ribeyza," or "rostov." in the Middle Eastern community is an noneviscerated, salt-cured, air-dried, whole fish, which may or may not be smoked. It is consumed without further preparation, such as cooking.
- © 1995: Italy, eggplant in oil, B-type botulism
- © April 17th, 2007: Italy, olives made by Charlie Brown di Rutigliano & Figli S.r.l
- © May 20th, 2007: Lake Erie, Sheephead, Burbot and Perch had E-type botulism



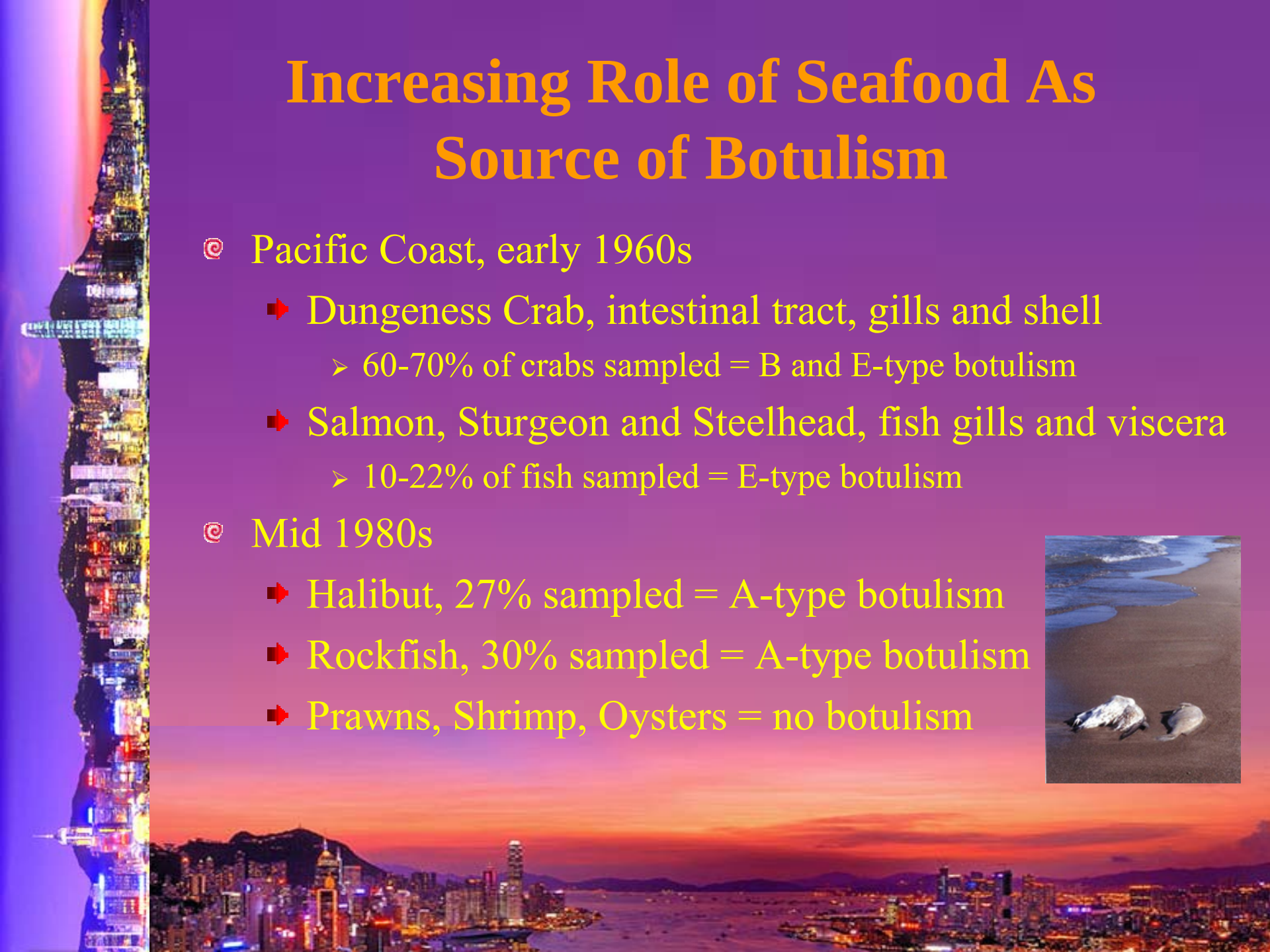
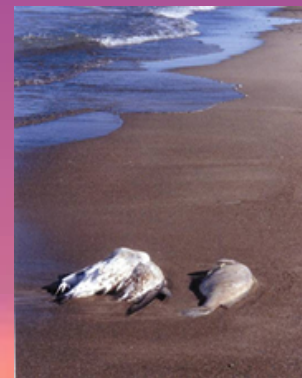
Increasing Role of Seafood As Source of Botulism

© Pacific Coast, early 1960s

- Dungeness Crab, intestinal tract, gills and shell
 - 60-70% of crabs sampled = B and E-type botulism
- Salmon, Sturgeon and Steelhead, fish gills and viscera
 - 10-22% of fish sampled = E-type botulism

© Mid 1980s

- Halibut, 27% sampled = A-type botulism
- Rockfish, 30% sampled = A-type botulism
- Prawns, Shrimp, Oysters = no botulism

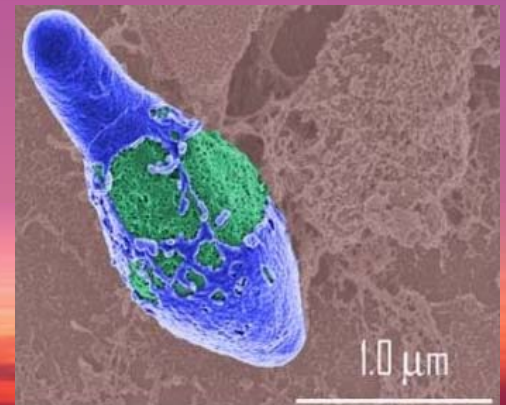


Biological Warfare

- © Lethal dose
 - 1 gram crystalline toxin dispersed evenly and inhaled = 1 million deaths
 - 70 μ g orally = lethal (70kg person) (LD50 ~ 0.005–0.05 μ g/kg)
 - Median lethal dose of about 1 ng/kg when introduced intravenously and 3 ng/kg when inhaled
- © Weaponized by several nations including the U.S., Japan, and Soviet Union, beginning in the 1930's
- © World War II – Stanley Lovell
 - gelatin capsules with a lethal dose
 - slipped into food or drink
 - tested on donkeys

Clostridium Botulinum

- © Obligate anaerobic, toxin producing, gram positive bacillus
- © Spores
 - Ubiquitous
 - Resistant to heat, light, drying and UV radiation
 - can remain dormant for 30 years or more
 - **Specific conditions for germination**
 - Anaerobic conditions
 - Warmth (10-50°C)
 - Mild alkalinity



Survival Characteristics

© Bacterial Growth inhibition:

- Salt: $> 5\%$
- pH: < 4.6
- A_w (water activity): < 0.85
 - Liquid malt extract ~ 0.60 , Honey 0.55 and 0.60, Dried malt extract 0.20.
- Temp: $< 4^\circ\text{C}$

The English word, sausage, is derived directly from the French word, *saucisse*, which in turn has descended from the Latin, *salsus*, meaning to salt and thus to preserve.

© Spores survive cooking

- 121°C , for at least 20 minutes and 116°C for 2 hrs
- Fortunately, ingestion of the spores is safe, except in infants, as the highly oxygenated and highly acidic environment of the digestive system prevents the spores from growing and producing the botulinum toxin.
- While commercially canned goods are required to undergo a "botulinum cook" at 121°C (250°F) for 3 minutes, and so rarely cause botulism (while home pressure canning equipment only can reach 240°F),

© Toxins colourless and odourless

- Heat labile
 - be destroyed if heated at 80°C for 10 minutes or 85°C for 5 minutes
 - LD₅₀ $\sim 0.005\text{--}0.05\ \mu\text{g/kg}$ with a median lethal dose of about 1 ng/kg when introduced intravenously and 3 ng/kg when inhaled



Neurotoxin	A	B	C	D	E	F	G
Human	X	X			X	X	
Horses		X	X				
Cattle		X	X	X			
Sheep			X				
Dogs			X	X			
Avian			X		X		
Mink & Ferret	X		X		X		

Neurotoxins: Can be destroyed by boiling

Seven different types of neurotoxin: A through G

Binds neuromuscular junctions

Different types affect different species

All cause flaccid paralysis

Only a few nanograms can cause illness



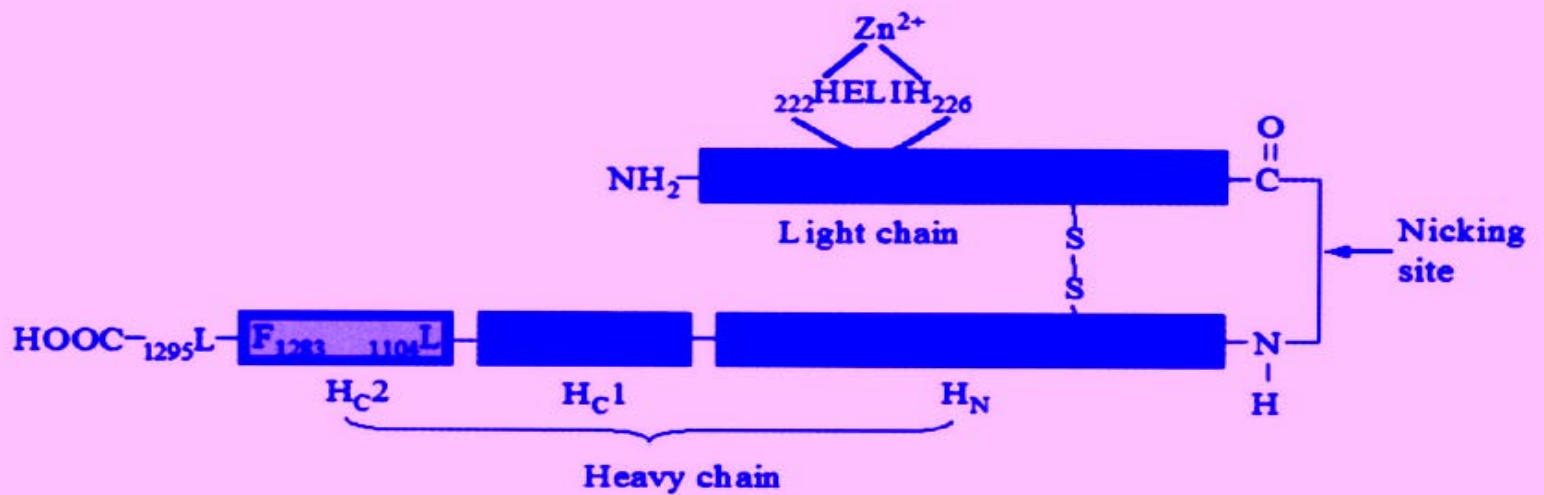
The possibility of type F illness should be suspected if onset is rapid and paralysis is severe.

© **Botulism type F**

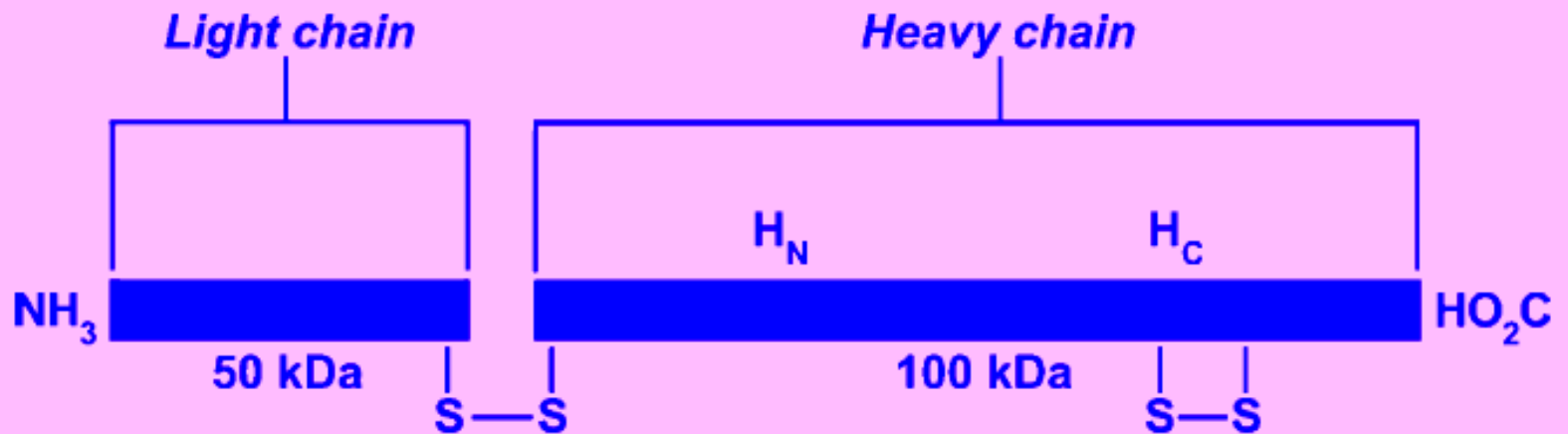
- causes $\approx 1\%$ of botulism cases in the United States
- Two outbreaks have been reported, 1 in the United States, the other in Europe.
- A recent review described all 13 cases of botulism type F from the USA between 1981 and 2002. Clinical signs were respiratory failure within 24 hours of symptom onset, complete or near complete quadriplegia by the fifth day, and neuromuscular recovery beginning on the eighth day. On average, patients received mechanical ventilation for 24 days and were hospitalized for 30 days. These features represent a more precipitous initial course than is typical for type A or type B botulism but a more rapid recovery.

Adult botulism type F in the United States. Gupta A, Sumner CJ, Castor M, Maslanka S, Sobel J Neurology 2005;65:1694-700

Clinical recovery and circulating botulinum toxin type F in adult patient. Sobel J, Dill T, Kirkpatrick CL, Riek L, Luedtke P, Damrow TA. Emerging Infectious Diseases. 15(6):969-71, 2009 Jun.



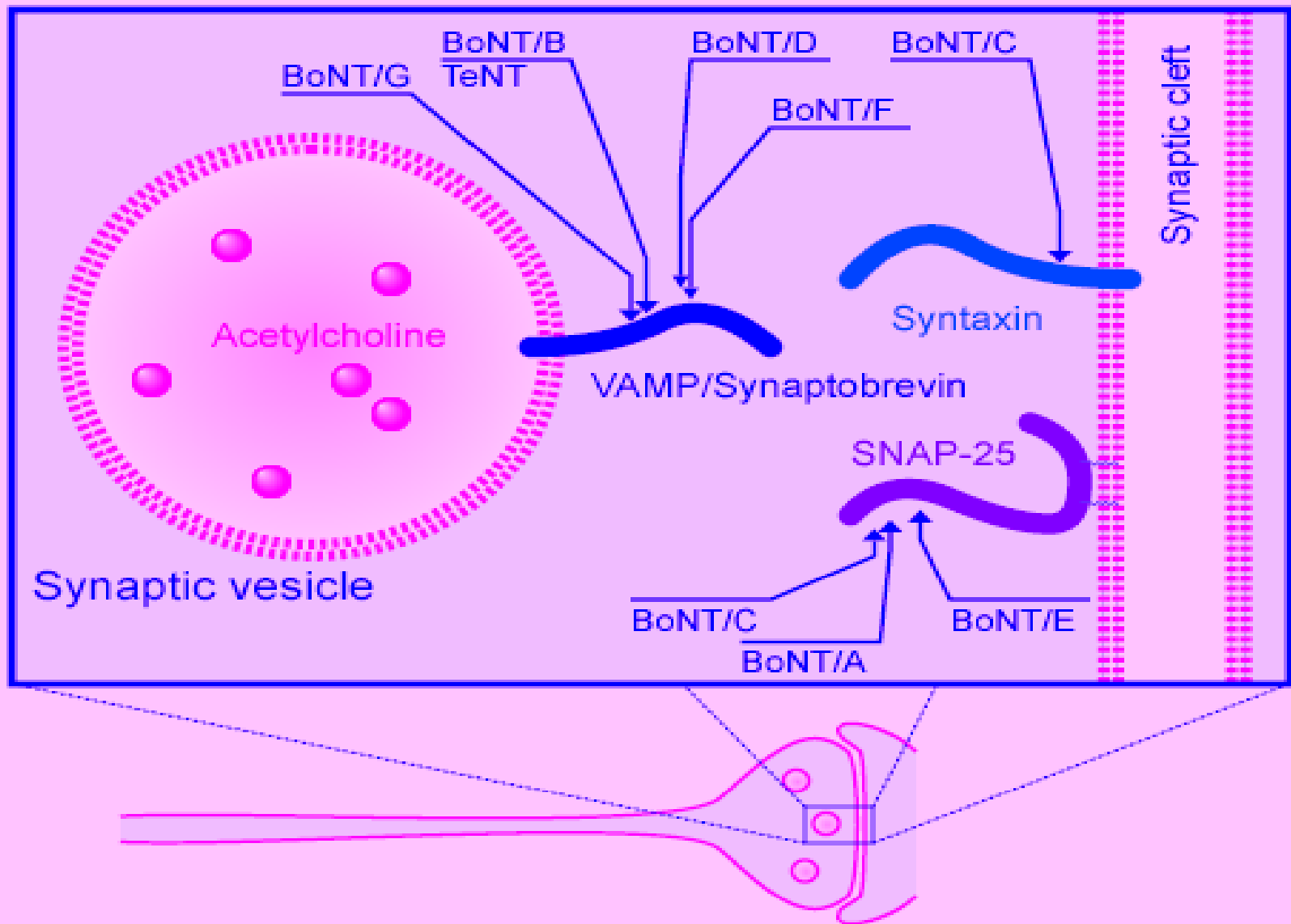
Schematic diagram of a BoNT (A) showing different domains.

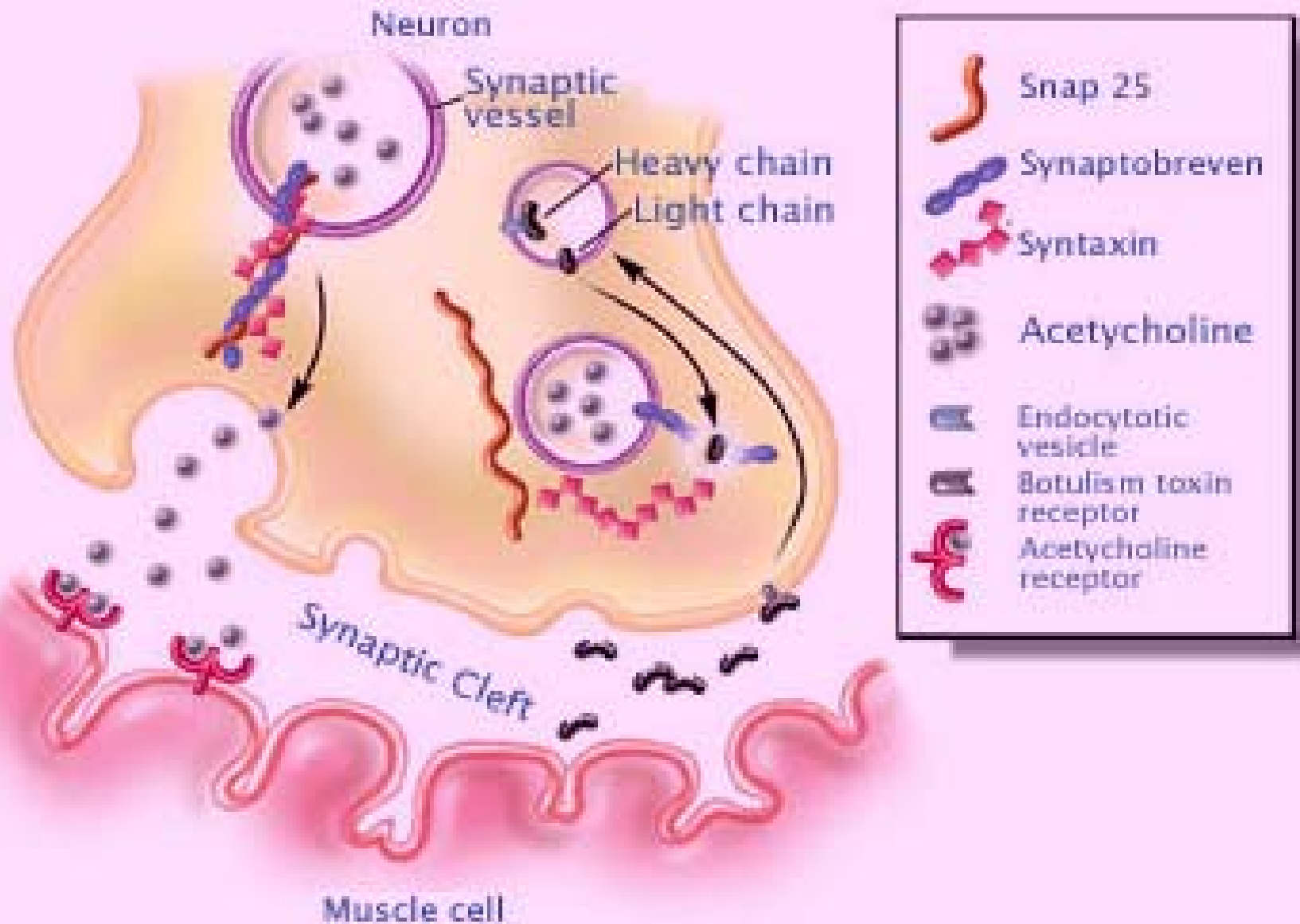


Structure of botulinum neurotoxin.

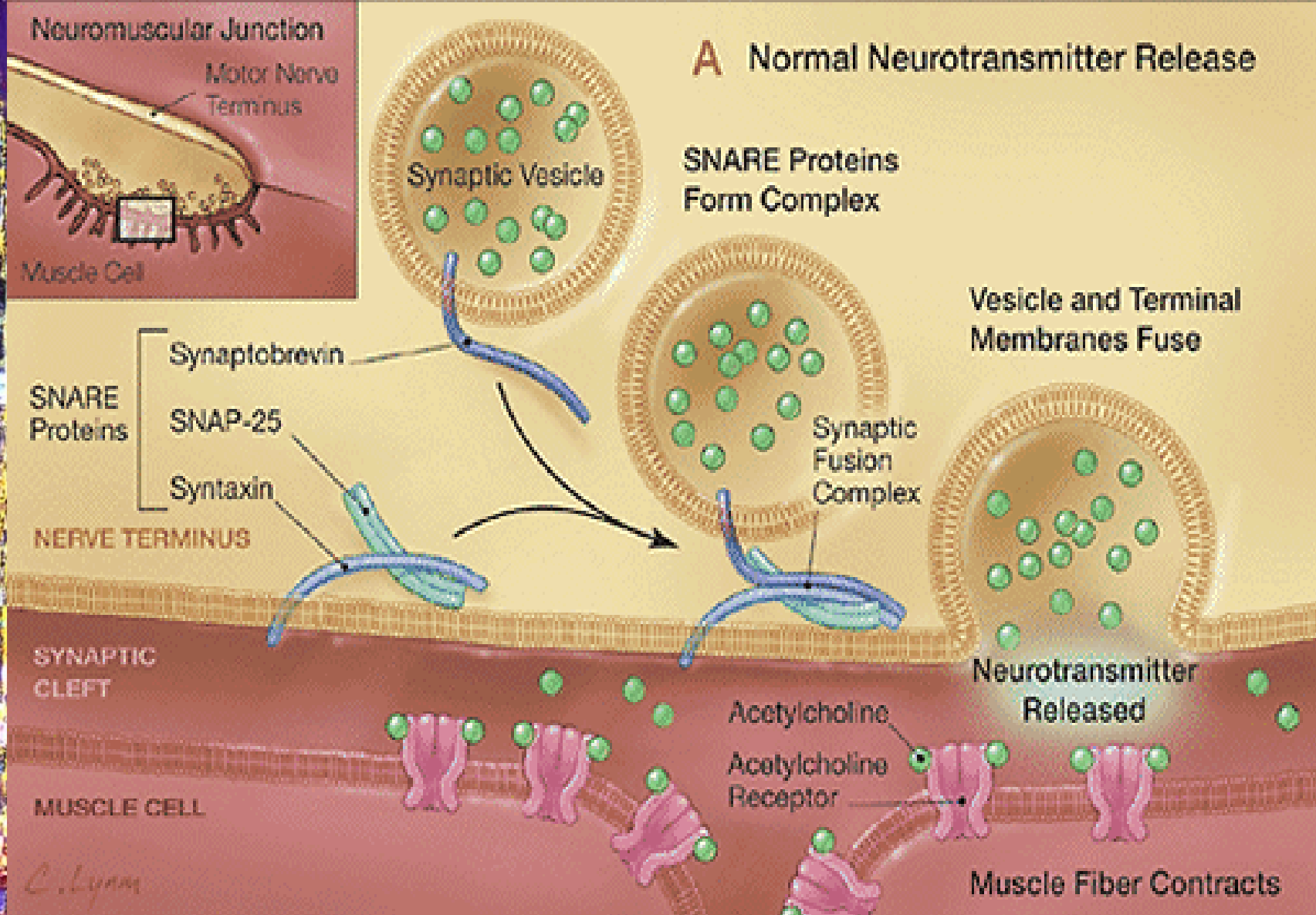
Botulinum Neurotoxin

100-kDa heavy chain joined by a disulphide bond to a 50-kDa light chain.



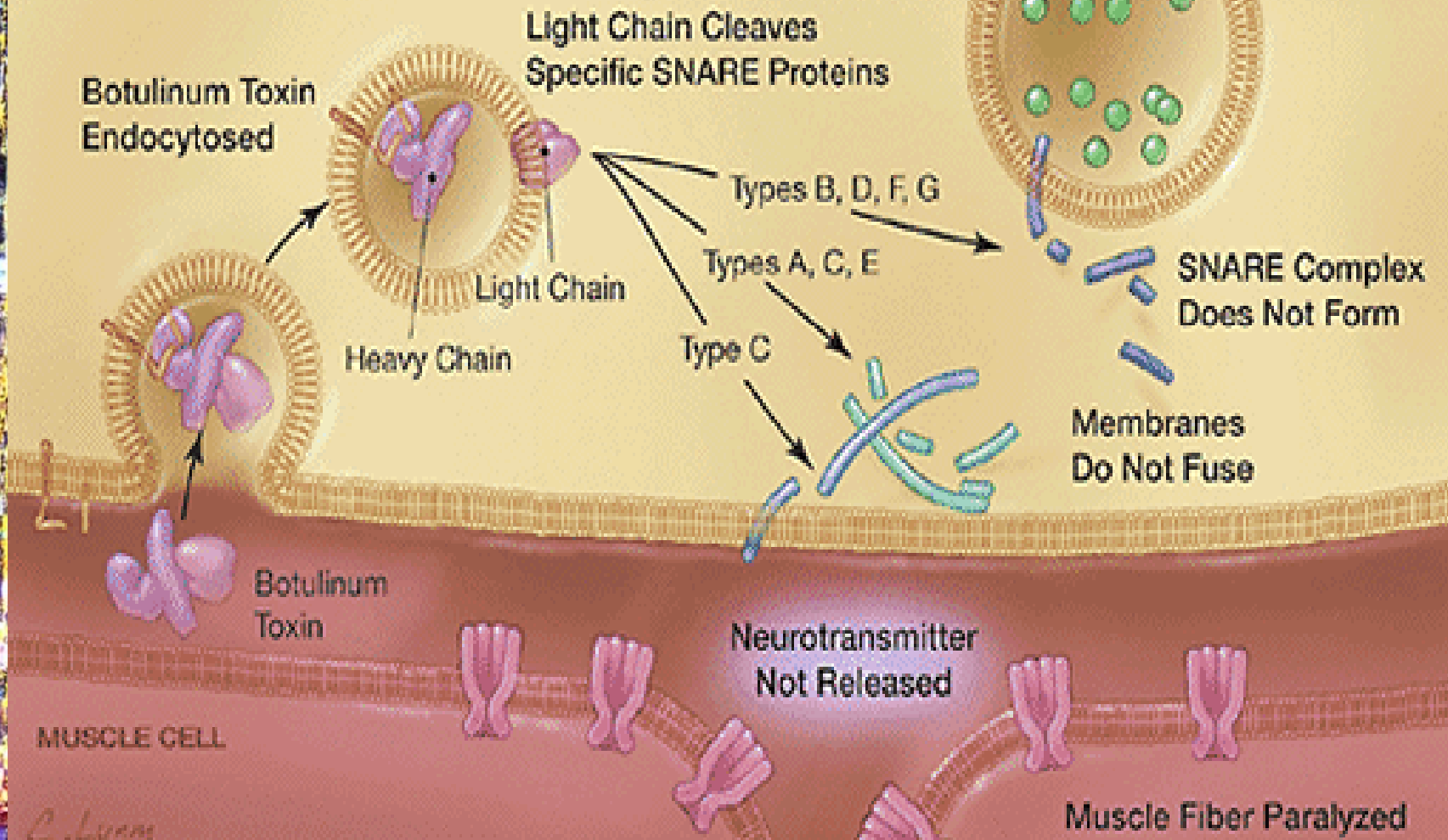


Finkelstein A: Channels formed in phospholipid bilayer membranes by diphtheria, tetanus, botulinum and anthrax toxin. J Physiol 1990; 84:188.



Arnon SS, Schechter R, Inglesby TV, et al; Working Group on Civilian Biodefense. Botulinum toxin as a biological weapon: medical and public health management. JAMA. 2001;285:1059-1070.

B Exposure to Botulinum Toxin



Arnon SS, Schechter R, Inglesby TV, et al; Working Group on Civilian Biodefense. Botulinum toxin as a biological weapon: medical and public health management. JAMA. 2001;285:1059-1070.

Only types A, B, E, and F cause human disease. Types A and E bind to synaptosomal-associated protein 25, type C binds to syntaxin, and types B, D, and F bind to vesicle-associated membrane protein.



Turkey with sausage meat stuffing

“The well-intentioned cook decides to make the dressing for the turkey the night before. This gives her more time to do many other important things the next day. She stuffs the turkey the night before, and places it in the refrigerator to be cooked the next day.

*Unfortunately, she doesn't know she is creating ideal conditions for food poisoning. Obviously, the stuffing that she put into the turkey is somewhere between **40 ° and 140 ° F**. Because the various parts of dressing have some sort of liquid in them, the **moisture** is also there. Lastly, she sews up the turkey to create a **lack of oxygen** in its cavity”.*

Ideal condition for germination of *C. botulinum* spores

- | | |
|----------------------------|----------------------------|
| (1) Temperature 40°-140° F | (4) Low-acid (ie, pH >4.6) |
| (2) Moisture | (5) Low-solute environment |
| (3) Anaerobic condition | |



The problem is even more pronounced when smoking meats

- The important role of nitrate

- ① 1. First of all, meats contain a lot of moisture. Water is then also added to sausages to facilitate stuffing. Hams and other meats are pumped up with water
- ② 2. Lack of oxygen – when smoking we intentionally decrease the amount of available air (and in turn oxygen). This allows our sawdust or wood chips to generate lots of smoke. If they had more air, they would burst into flames.
- ③ 3. Temperatures between 40° and 120° F– most smoking is done at this temperature range.
- ④ The most dangerous range is from 78° – 95° F (26 °– 35° C), and that fits into “warm smoking” method. Bacteria thrive at this temperature.
- ⑤ **Nitrates/nitrites prevent the transformation of *C. botulinum* spores to toxins.** Nitrate and nitrite are converted to nitric oxide by microorganisms and combine with the meat pigment myoglobin to give the cured (腌渍) meat color. **Nitrates are cheap, commonly available, and completely safe in amounts recommended by the Food and Drug Administration.** Addition of Alpha-tocopherol, ascorbic acid, and erythorbic acid can inhibit nitrosamine production.

Federal regulations permit a maximum addition of 2.75 ounces of sodium or potassium nitrate per 100 pounds of chopped meat, and 0.25 ounce sodium or potassium nitrite per 100 pounds of chopped meat. Potassium nitrate (saltpeter) was the salt historically used for curing.

遠古以前，「臘」是指祭祀儀式，祭祀後的剩餘肉類往往會醃製起來，以備食物短缺時食用。按中國傳統，人們通常約在農曆十二月（「臘月」）製備，作為慶祝春節的一種食品，而這種食品因而稱為「臘味」。



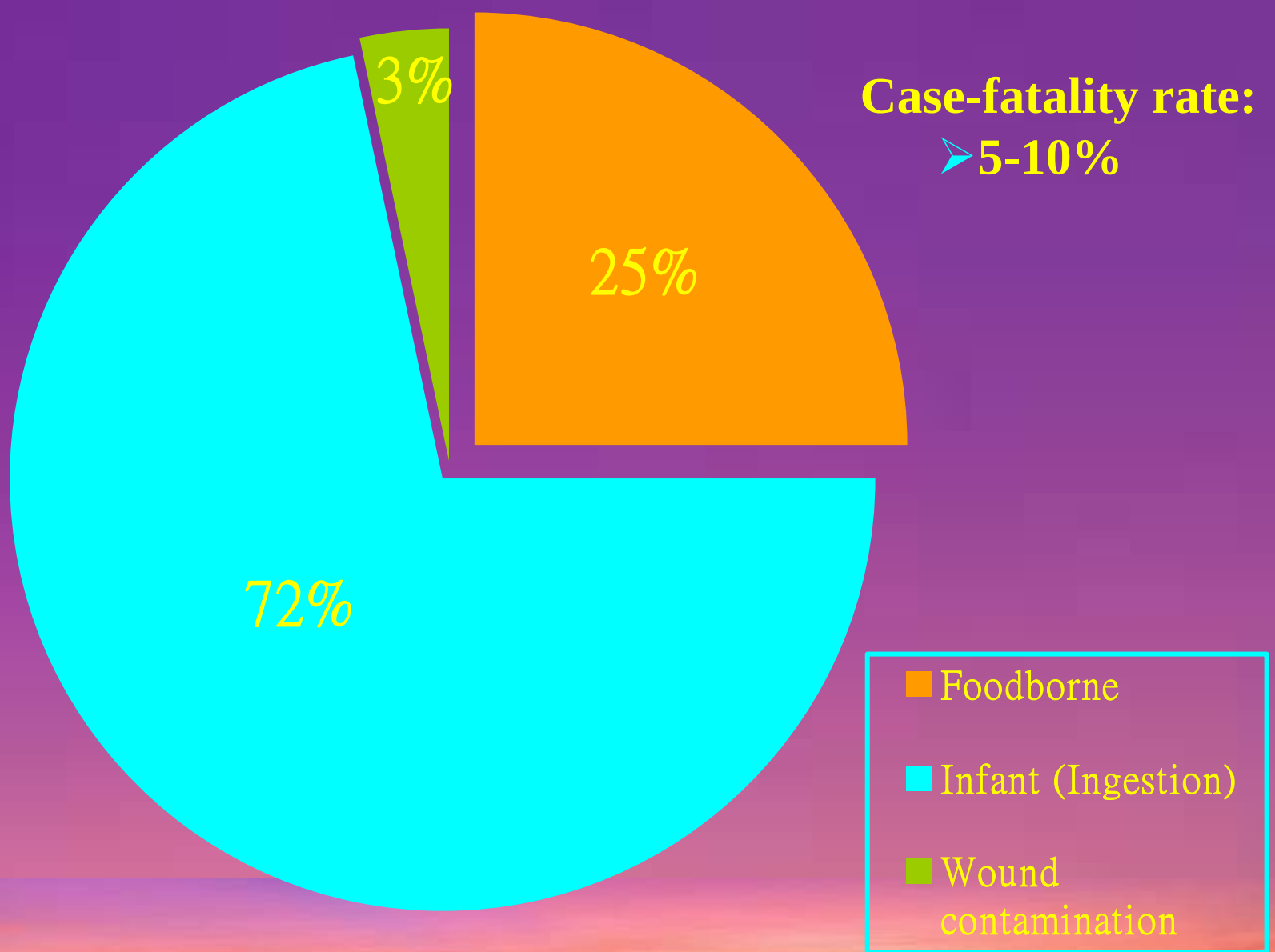
- All forms fatal and are medical emergency
- Incubation period: 12-36 hours

The challenge of diagnosis and treatment. Brook I. Botulism: Rev Neurol Dis 2006;3:182-9.



Potential for A Good Biological Weapon:

- Botulinum toxin is lethal at a femtomolar dose of 10^{-9} g/kg, making botulinum toxin 15,000-100,000 times more potent than sarin gas.
- Isolated fairly easily from soil
- Could be released as an aerosol or as a contaminant in the food supply
- High lethality: 1 aerosolized gram could potentially kill 1 million people
- Expensive, long-term care needed for recovery



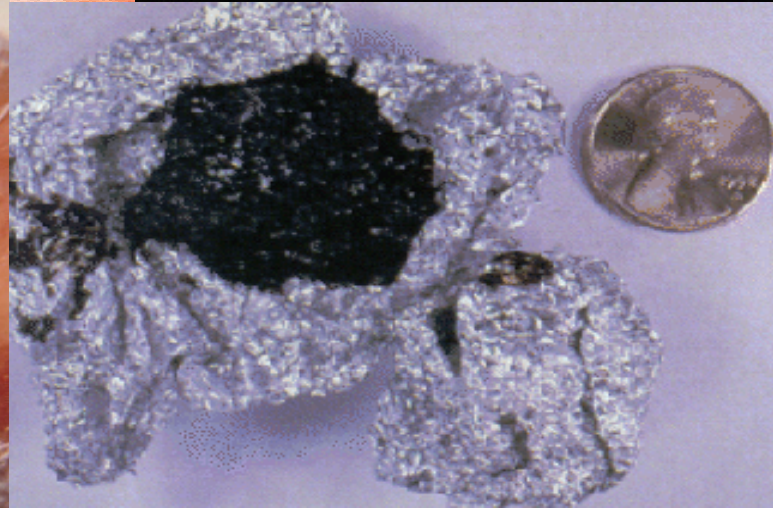
Botulism in U.S.A.



Characteristics of the natural forms of botulism				
Type	Cause	Examples of sources	Clinical manifestations*	Confirmation of the diagnosis†
Foodborne	Ingestion of food contaminated by preformed botulinum toxin	- Canned food - Fermented fish	Beginning 12–36 h after ingestion: - Nausea, vomiting, abdominal pain, diarrhea and dry mouth - Cranial nerve involvement (blurry vision, diplopia, nystagmus, ptosis, dysphagia, dysarthria and facial weakness) - Descending muscle weakness	- Detection of botulinum neurotoxin in serum, stool, gastric aspirate or food - Isolation of <i>C. botulinum</i> from stool or gastric aspirate - Electrophysiologic investigations may be useful
Infant (1 wk–12 mo)	Ingestion of spores that colonize the host's gastro-intestinal tract and release toxins	- Honey - Environmental dust	- Constipation - Weakness - Difficulty feeding - Descending or global hypotonia - Drooling - Anorexia - Irritability - Weak cry	- Isolation of <i>C. botulinum</i> - Detection of botulinum neurotoxin in feces - Presumptive diagnosis should be made based on clinical presentation and electrophysiologic results
Wound	Infection of a wound by <i>Clostridium botulinum</i>, which produces a neurotoxin	- Puncture wounds - Abscess - Abrasions - Drug use (e.g., "black tar" heroin, inhaled cocaine)	Similar to the manifestations of foodborne botulism but no gastrointestinal symptoms	- Confirmed by electrophysiologic results - Presence of toxins in the wound - Fever - Leukocytosis
Colonization	Production of toxin in the gastro-intestinal tract of an infected adult host	- Ingestion - Source usually unknown (no preformed toxin found in suspect foods)	Similar to the manifestations of infant and foodborne botulism	- Isolation of <i>C. botulinum</i> - Detection of botulinum neurotoxin in feces - Presumptive diagnosis should be made based on clinical presentation and electrophysiologic results

*Clinical manifestations common to all forms of botulism include: absence of a fever, hemodynamic stability (early), generally symmetric neurological deficits, and responsiveness with a lack of sensory deficits except potentially blurry vision.

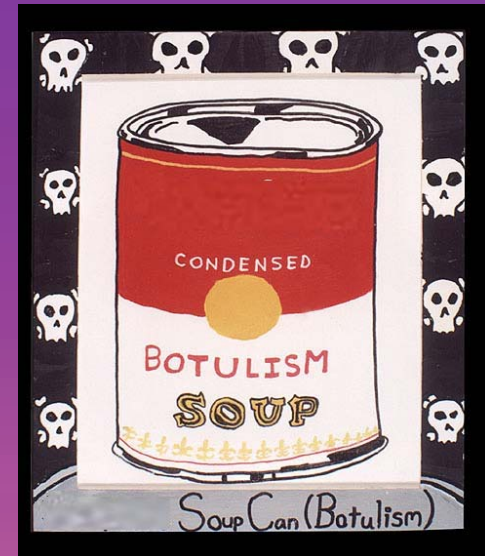
†Once any type is suspected, careful monitoring and treatment should be initiated immediately, including clinical evaluation of respiratory system compromise, including pulse oximetry, spirometry and arterial blood gas measurement. Intubation may be required. Antitoxin or immune globulin should be started immediately. Antibiotics are not indicated, except for wound botulism.



Black Tar Heroin

Food-borne Botulism

- © Second most common form
- © Caused by eating food containing the toxin produced by *C. botulinum*
- © Antigenic variations types A/E
- © 18-36 hours after ingestion (range, 2 h to 8 d)
- © Most common from home-canned foods
 - Asparagus, green beans, beets, corn, baked potatoes, garlic, chile peppers, tomatoes; type A
 - Improperly fermented fish (Alaska); type E
- © Dangerous because most contaminated foods cannot be detected until too late





Food-borne Botulism

Signs and Symptoms

- ⊙ 12 – 36 hrs after
- ⊙ Muscle weakness,
- ⊙ Diarrhea
- ⊙ Blurred vision
- ⊙ Vomiting
- ⊙ Eventual Paralysis

Treatment and Recovery

- ⊙ Equine Botulinum Antitoxin
- ⊙ Lingering symptoms are fatigue and respiratory difficulty
- ⊙ With early detection, proper treatment, no long term effects observed

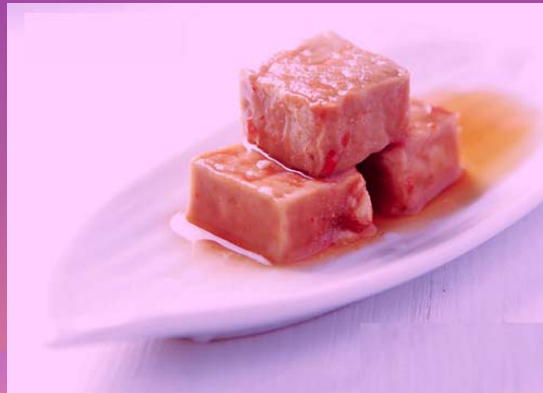
Prevention

- ⊙ Inspect canned food for
 - Bulging
 - Loose lids
 - Mold
 - Odor
- ⊙ Proper home canning procedures:
 - Hygiene
 - Time schedule,
 - Proper processing method
 - Equipment
- ⊙ Avoiding home canning or cheaply produced commercial food





Canned Bamboo shoot



**Truffle oil
or sauce**

Botulism associated with commercially canned chili sauce--Texas and Indiana, July 2007.

CDC. MMWR - Morbidity & Mortality Weekly Report. 56(30):767-9, 2007 Aug 3.

Signs and symptoms predictive of respiratory failure in patients with foodborne botulism in Thailand. Wongtanate M. Sucharitchan N. Tantisiriwit K. Oranrigsupak P. Chuesuwan A. Toykeaw S. Suputtamongkol Y. American Journal of Tropical Medicine & Hygiene. 77(2):386-9, 2007 Aug.

Foodborne botulism from home-prepared fermented tofu--California, 2006. Centers for Disease Control and Prevention (CDC). MMWR 56(5):96-7, 2007 Feb 9.



Drinks	pH
Lemon Juice	2.3
Vinegar	2.4-3.4
Grape Fruit Juice	3-3.3
Orange Juice	3-4
Apple Juice	2.9-3.3
Tomato and tomato juice (Russia outbreak)	4.2
Sour milk	4.3-4.5
Low pH reduced botulism through two mechanisms: enhanced susceptibility of C. botulinum spores to heat sterilization, and inhibition of germination of surviving spores.	

Popugaila VM, et al. Cases of botulism caused by preserved tomato juice. Gig Sanit 1972; 37:97-199

Shtein A, et al. Cases of botulism caused by domestically preserved tomatoes. Vopr Pitan 1969; 28: 92-93

Loufty MR, et al. An outbreak of foodborne botulism in Ontario. Can J Infect Dis 2003; 14:206-209.

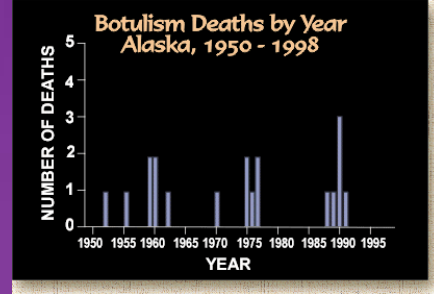
Bacterial food poisoning and botulism, in: Wilson GS, Miles A. Topley and Wilson's Principles of Bacteriology, Virology and Immunity, 6th ed, London, Edward Arnold, 1975. pp. 2104-2105.

Outbreak of botulism in Kenyan nomads. Smith DH, Timms GL, Refai M Ann Trop Med Parasitol. 1979 Apr; 73(2):145-8.

pH Values of Common Items: <http://www.jackeden.com/tips/phmat.html>



Alaska Delicacies Causing Botulism



Fermented
Seal/walrus flipper

Fermented seal oil

Stink eggs
(fermented fish eggs)
(salmon-egg cheese)

Muktuk
(fermented whale)

Stinky tail
(fermented beaver tail)
(海狸,水獭)

Stinkheads
(fermented fish head)



Fermented whale skin with blubber.



Wooden barrel with plastic lid used to ferment salmon heads.

Botulism among Alaska Natives. The role of changing food preparation and consumption practices.

Shaffer N. Wainwright RB. Middaugh JP. Tauxe RV. Western Journal of Medicine. 153(4):390-3, 1990 Oct.
http://www.epi.alaska.gov/pubs/botulism/bot_03.htm



Traditional method of a grass-lined hole in the ground allow the air to circulate.

Stinkheads, fermented salmon heads, are a traditional Alaskan delight and delicacy invented by the Yup'ik Eskimos. After the heads are lopped off, they're buried in the ground for at least a few weeks. During this time the heads begin to rot. Before they have a chance to disintegrate completely, they're dug up, mashed into a sort of pudding and served cold in bowls. Getting it past your nose is a serious problem, but the result is reportedly somewhat hallucinogenic. Some say the finished taste might be compared to a very-aged cheese. Stinkheads are often used as a rite of passage to test "gussaks" (foreigners) who claim to want know more about native culture.

A customary way of preparing them was to place fish heads and guts in a wooden barrel, cover it with burlap, and bury it in the ground for about a week. For a short while in modern times, **plastic bags and buckets replaced the barrel**, however this **increased the risk of botulism**. the Yupik who had switched to more modern methods of fish rotting using plastic buckets are now being encouraged to go back to barrels because fish rotting in plastic is more likely to generate botulism.

Foods implicated in confirmed botulism outbreaks; Alaska, 1950 - 1997

The result of changing food preparation and consumption practices e.g. using plastic bag in fermentation.

Type of Food	Number Implicated	Number Toxin Positive
Sea Mammal		
Seal	37	23
Whale	11	5
Fish		
Salmon eggs	24	12
Salmon Heads	8	2
Salmon, other	2	1
Whitefish	8	4
Herring	1	0
Land Mammal		
Beaver tail	4	4
Other	5	2
Unknown	5	0
Total	105	53

Alaska Native Americans have one of the highest rates of food-borne botulism in the world.
Traditional aerobic, slow, low-temperature fermentation can prevent botulism

2. Wainwright RB, Heyward WL, Middaugh JP, Hatheway CL, Harpster AP, Bender TR. Food-borne botulism in Alaska, 1947--1985: epidemiology and clinical findings. J Inf Dis 1988; 157:1158--62.

3. Chion L, Hennessy T, Horn A, Carter G, Butler J. A survey of knowledge, attitudes, and practices related to fermented foods known to cause botulism among Alaska Natives of southwest Alaska. [Abstract]. Presented at the 2nd International Conference on Emerging Infectious Diseases, Atlanta, Georgia, July 2000.

4. Eisenberg MS, Bender TR. Plastic bags and botulism: a new twist to an old hazard of the north. Alaska Med, July 1976;47:-9.

5. Shaffer N, Wainwright RB, Middaugh JP. Botulism among Alaska Natives: the role of changing food preparation and consumption practices. West J Med 1990;153:390--3.

Ferment food at a cold temperature.



Keep food cold by fermenting it in the ground and away from the sun.

Ideally, the temperature of the food should be below 37 degrees Fahrenheit. It might take longer to ferment the food, but it may be safer because if you keep food cold, you prevent botulism poison from developing.



Use proper methods when you prepare your Native foods.



Eating fermented food has some risk of botulism. To reduce your risk of botulism, ferment food by using the traditional method of a grass-lined hole in the ground. Use methods that allow the air to circulate.



Because botulism grows better when fresh air is not moving around, do **NOT** use plastic or glass containers. Even a plastic container without a lid may allow the botulism germ to grow in the bottom of the container.

Use salt when drying fish. Salt is a natural preservative and reduces the chance that botulism poison will grow.

Boil your food before eating it.



Botulism toxin is destroyed by high heat. Boiling fermented food for 10 minutes can destroy the botulism poison. It might change the taste from what you are used to, but it is one way to protect you and your family from botulism.

If fermented foods are stored in plastic or glass containers before they are eaten, they should be boiled because the botulism poison cannot be seen, smelled, or tasted. Boiling will kill the poison that is in the food.

Diagnostic Pentad

- © Botulism should be strongly considered if a patient has a history of consuming traditional Alaska Native food and three or more of the following five signs or symptoms are present:
 - Nausea or vomiting
 - Dysphagia
 - Diplopia
 - Dilated, fixed pupils
 - Dry throat
- © In a study of 705 patients who had botulism, 68% of patients had at least three symptoms on admission, whereas only 2% had all five symptoms

Food-borne botulism in Alaska, 1947-1985: epidemiology and clinical findings. Wainwright R.B., Heyward W.L., Middaugh J.P., et al: *J Infect Dis* 157. (6): 1158-1162.1988;
Signs and symptoms predictive of death in patients with foodborne botulism—Republic of Georgia, 1980-2002. Varma J.K., Katsitadze G., Moiscrafishvili M., et al: *Clin Infect Dis* 39. (3): 357-362.2004;

Infant Botulism

Global occurrence of infant botulism, 1976-2006. Koepke R. Sobel J. Arnon SS. Pediatrics. 122(1):e73-82, 2008 Jul.

- © Most common form in U.S.
 - 2 per 100,000 live births in US
 - Antigenic variations types A/B
- © **The incubation period is 2-4 weeks.**
The peak age of incidence is 2-4 months.
- © Spore ingestion
 - Germinate then toxin released and colonize large intestine
- © Infants < 1 year old
 - 94% < 6 months old
- © Spores from varied sources
 - Honey, food, dust, corn syrup
- © Lack of breast milk



Breastfeeding may protect infants from lethal fulminant infant botulism, but exclusive breastfeeding is a risk factor for the disease, presumably because the relatively pristine bowel flora of the exclusively breastfed infant is more permissive for spore germination and toxin production.

Vehicles of Infant Botulism

- ⊙ *Honey
 - 16% of samples positive for spores
 - Accounts for ~15% of cases
- ⊙ Corn syrup
 - 28% of samples positive for spores
- ⊙ Food
- ⊙ Carpet, soil/dust, dust from new construction, etc.
- ⊙ Powdered infant milk formulae !!!
 - *C botulinum* type B spores in a PIF in the United Kingdom with an evident link to a case of laboratory-confirmed type B infant botulism in 2001
 - Presence of soil-dwelling clostridia in commercial powdered infant formulas in U.S.A.

A case of infant botulism with a possible link to infant formula milk powder: evidence for the presence of more than one strain of *Clostridium botulinum* in clinical specimens and food, M.M. Brett, J. McLauchlin and A. Harris et al., J Med Microbiol 54 (2005), pp. 769–776.

Characterization of *Clostridium botulinum* strains associated with an infant botulism case in the United Kingdom, E.A. Johnson, W.H. Tepp, M. Bradshaw, R.J. Gilbert, P.E. Cook and E.D.G. McIntosh, J Clin Microbiol 43 (2005), pp. 2602–2607.

Presence of soil-dwelling clostridia in commercial powdered infant formulas. Barash JR, Hsia JK, Arnon SS. Journal of Pediatrics. 156(3):402-8, 2010 Mar.

Wound Botulism



- © Least common, but on the rise
- © Occurs when spores inoculate a wound and anaerobic conditions allow germination and subsequent production of the toxin
- © Associated with IV drug use (rise in black tar heroine use), and medical equipment ex. catheters
- © Often misdiagnosed as other neurological syndromes



Outbreak of wound botulism in injecting drug users. Schroeter M. Alpers K. Van Treeck U. Frank C. Rosenkoetter N. Schaumann R. Epidemiology & Infection. 137(11):1602-8, 2009 Nov.
Wound Botulism in Injection Drug Users Wiltrud Maria Kalka-Moll, Ute Aurbach, Reiner Schaumann, Rosemarie Schwarz, and Harald Seifert Emerg Infect Dis. 2007 June; 13(6): 942-943

Adult Intestinal Toxemia Botulism

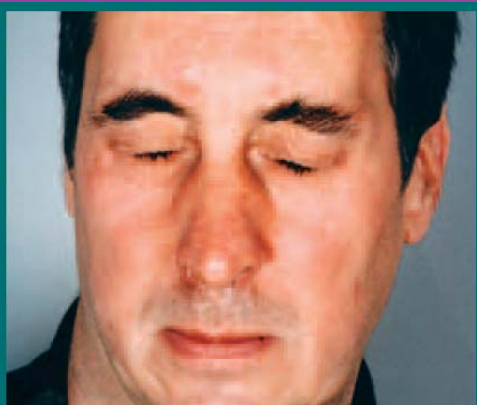
- © Rare
- © Organism colonizes the lumen of the intestinal tract and produces botulinum neurotoxin in vivo.
- © Risk factors include bowel abnormality, previous bowel surgery, Meckel diverticulum, Crohn disease and long-term antimicrobial therapy.
- © With specific regard to botulism caused by *C. botulinum*, trimethoprim–sulfamethoxazole and nalidixic acid are recommended because they have been shown to have no effect on the botulin agent. *C. butyricum* botulism are resistant to trimethoprim–sulfamethoxazole.

Intestinal toxemia botulism in Italy, 1984-2005. Fenicia L, Anniballi F, Aureli P. European Journal of Clinical Microbiology & Infectious Diseases. 26(6):385-94, 2007 Jun.

Susceptibility of *C. botulinum* to thirteen antimicrobial agents. Swenson JM, Thornsberry L, McCroskey L, Hatheway CL, Dowell VR (1980) Antimicrob Agents Chemother 18:13–19

Therapeutic Use of botulinum Toxin

Blepharospasm



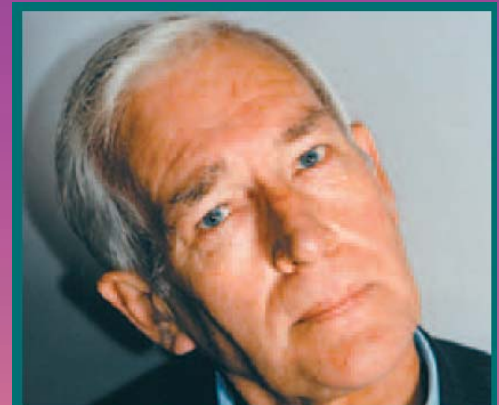
Blepharospasm (focal dystonia of the orbicularis oculi; early symptoms may include, uncontrollable blinking [especially in bright light], eye irritation, photosensitivity)

Hemifacial Spasm



Hemifacial spasm (spasm of the face; tends to affect the left side of the face more frequently than the right, with the muscles on the side of the mouth particularly affected)

Torticollis



Torticollis (affects the muscles of the neck, leading to inclination of the neck).

The good, bad and ugly aspects of the *Clostridium botulinum* neurotoxins
Professor Mike Peck

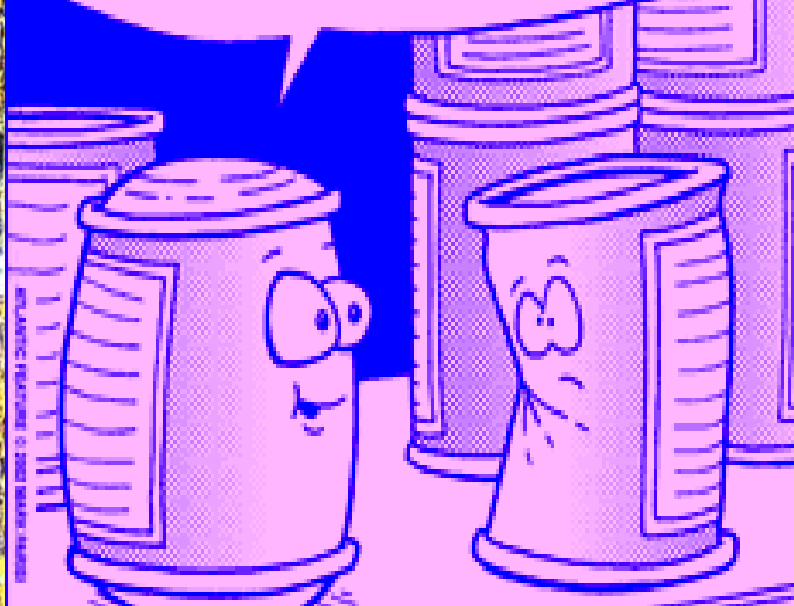


Focal Dystonias	Innapropriate Muscle Contractions
Blepharospasm	Achalasia/pyloric stenosis
Cervical dystonias	Anismus
Laryngeal dystonias	Bruxism (TMJ)
Occupational cramps (ie, writer's cramp)	Nystagmus
Other focal dystonias	Tension headaches
Involuntary movements	Back spasm
Hemifacial spasm	vaginismus
Tics	Strabismus
Voice, head, and limb tremors	Spasticity
Palatal myoclonus	Lower urinary tract
Cosmetic	Detrusor overactivity incontinence
Axillary hyperhidrosis	Detrusor sphincter dyssynergia
Glabellar facial lines	Urinary retention after suburethral sling

Current indications for botulinum toxin therapy (FDA and non-FDA approved)

**Botulinum toxin: from life-threatening disease to novel medical therapy. Mahajan ST.
Brubaker L. American Journal of Obstetrics & Gynecology. 196(1):7-15, 2007 Jan.**

DON'T WORRY, THAT HAPPENED TO ME TOO, BUT BOTULISM GOT RID OF THE WRINKLES...



New Form of Botulism

The wrinkle preventing effect of Botox lasts for approximately 3-6 months

BOTOX IS INTRODUCED



2007



2006

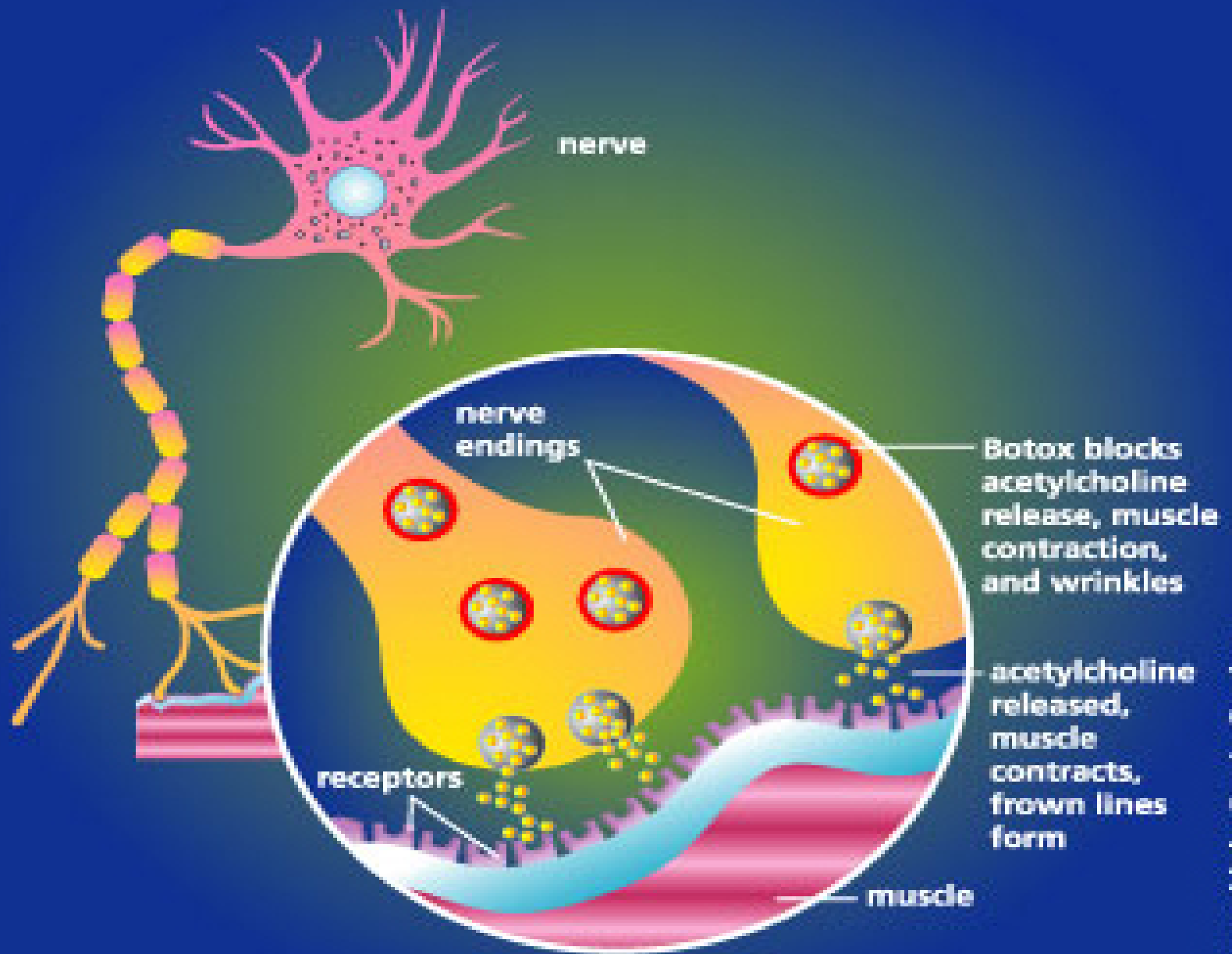
Has Hillary recruited Botox to her campaign? By PAUL THOMPSON 2007 Mail On-line



Overdose of botulinum toxin type A (Botox and Botox Cosmetic) and botulinum toxin type B (Myobloc) may experience adverse reactions, including respiratory failure and death.

Botox Linked To Respiratory Failure And Death, FDA Feb. 10, 2008 Botox Risks Under Review Bridget M. Kuehn JAMA. 2008;299(12):1417.
 Coté TR, Mohan AK, Polder JA, Walton MK, Braun MM (September 2005). "Botulinum toxin type A injections: adverse events reported to the US Food and Drug Administration in therapeutic and cosmetic cases". *J. Am. Acad. Dermatol.* 53 (3): 407-15.
 Le Canada met en garde contre les effets dangereux du Botox (AFP) - 13 janv. 2009
 Iatrogenic botulism due to therapeutic botulinum toxin a injection in a pediatric patient. Crowner BE, Brunstrom JE, Racette BA. *Clinical Neuropharmacology*. 30(5):310-3. 2007 Sep-Oct.

How Botox Works



Potential Bioterrorism Threat

- © Aum Shinriky cult
- © Extremely potent and lethal
- © Easily produced and transported
- © Signs of deliberate aerosol or foodborne release of toxin
 - No common source
 - Large number of acute cases clustered
- © Uncommon toxin type (C, D, F, G)
- © Point source aerosol release
 - Incapacitate or kill 10% of persons within 0.5 km downwind





Presenting Symptoms

© Adult

- Dysphagia - 96%
- Dry mouth - 93%
- Diplopia - 91%
- Dysarthria - 84%
- Extremity weakness - 73%
- Constipation - 73%
- Blurred vision - 65%
- Nausea - 64%
- Dyspnea - 60%
- Vomiting - 59%
- Abdominal cramps - 42%
- Diarrhea - 19%

© Infant

- Poor ability to suck - 96%
 - Poor head control - 96%
 - Hypotonia - 93%
 - Weak crying - 84%
 - Constipation - 83%
 - Lethargy - 71%
 - Facial weakness - 69%
 - Irritability - 61%
 - Hyporeflexia - 52%
 - Sluggish pupils - 50%
 - Respiratory difficulty - 43%
- 

When do symptoms usually begin?



JUNE						
SUN	MON	TUE	WED	THUR	FRI	SAT
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30					

Symptoms usually
begin

18 to 36 hours
after eating food containing the
botulism poison

Symptoms can show up
as early as

6 hours or as late as 10 days, depending
on how much
poison is in the food



What are the symptoms?



Blurry vision



Double vision



Drooping eyelids



Dry mouth



Trouble swallowing



Slurred speech



Trouble breathing



Muscle weakness

Common Presenting Symptoms

Adult Clinical Signs

- @ Nausea, vomiting, diarrhea
- @ Double vision
- @ Difficulty speaking or swallowing
- @ Descending weakness or paralysis
 - Shoulders to arms to thighs to calves
- @ Symmetrical flaccid paralysis
- @ Respiratory muscle paralysis



A



B



Patient was asked to have biggest smile

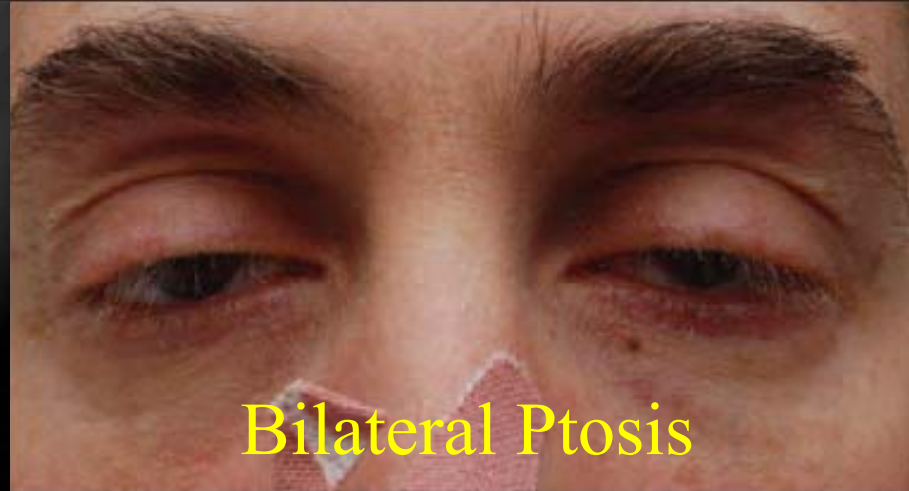
Botulinum Toxin as a Biological Weapon: Medical and Public Health Management Arnon et al JAMA Volume 285(8), 28 February 2001, pp 1059-1070

A. Note bilateral mild ptosis, dilated pupils, disconjugate gaze, and symmetric facial muscles.

B. Note absent periorbital smile creases, ptosis, disconjugate gaze, dilated pupils, and minimally asymmetric smile



Suspected small bowel obstruction
with negative laparotomy



Bilateral Ptosis



Pupil unreactive to light

Adult Intestinal Toxaemia due to Botulism

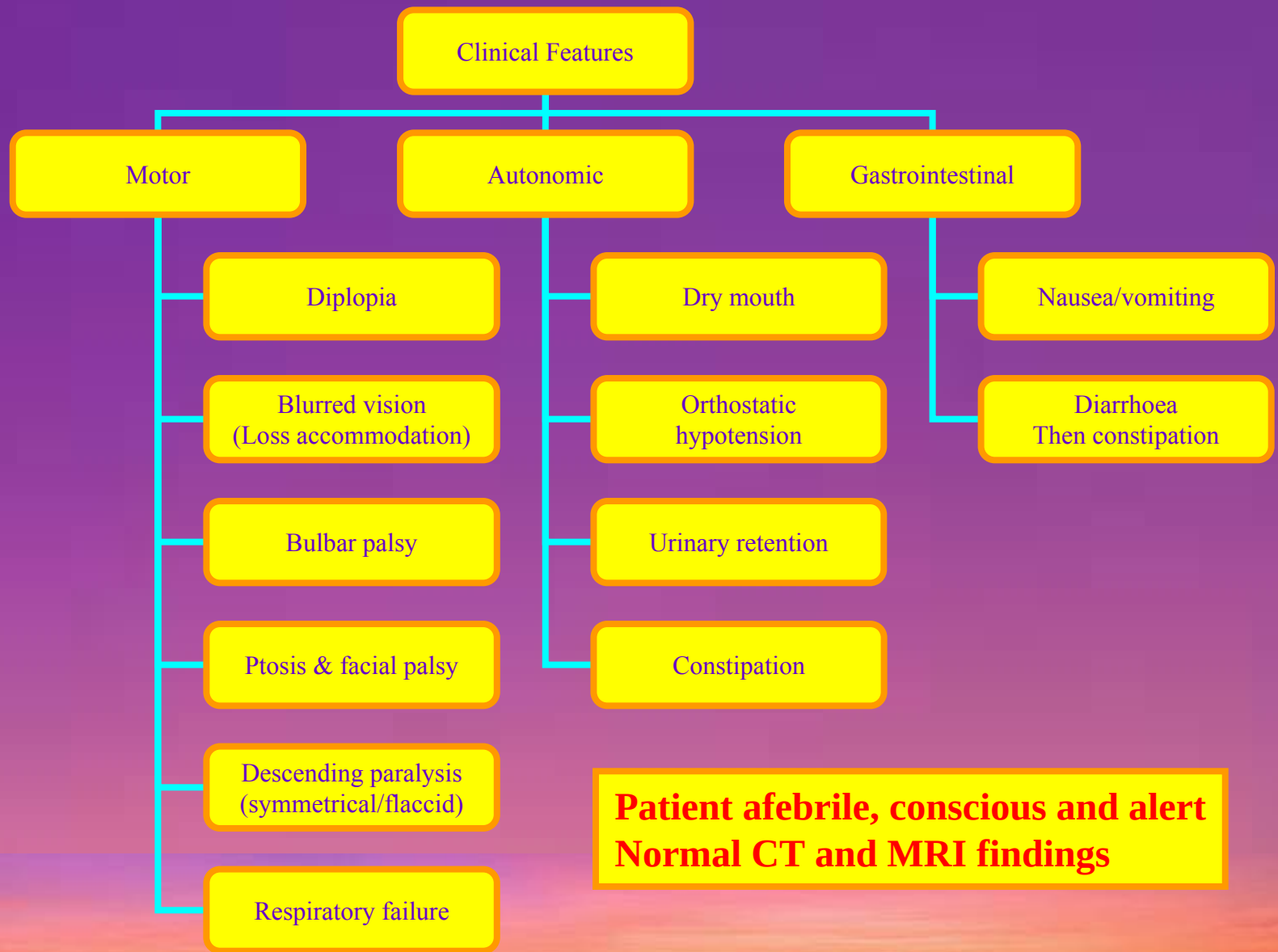
Bowel loops and eyelid droops. Spiegelman J. Cescon DW. Friedman Y.
Mazza BV. Austin JW. Rachlis A. Murray BJ.
CMAJ Canadian Medical Association Journal. 179(9):927-9, 2008 Oct 21.

Don't fall in to this diagnostic pitfall

Wound botulism in an intoxicated intravenous drug abuser presenting with respiratory failure



Diagnostic pitfall: wound botulism in an intoxicated intravenous drug abuser presenting with respiratory failure. Roysl G. Katchanov J. Stachulski F. Schultze J. Ploner CJ. Endres M. Intensive Care Medicine. 33(7):1301, 2007 Jul. Botulism: a rare complication of injecting drug use. Wenham TN. Emergency Medicine Journal. 25(1):55-6, 2008 Jan.



Botulism

In clusters

Descending
Flaccid Paralysis
No anticholinergic sign



No Fever

Conscious
and alert

High Index of Suspicion for Bioterrorism by Botulinum Toxin



Clinical Predictors of Respiratory Failure

Patients who did not have either nausea or vomiting and did not have urinary retention that required Foley catheterization was less likely to develop respiratory failure. This clinical predictor rule had a sensitivity of 75.5% and a specificity of 90.7%.

The clinical syndrome most predictive of respiratory failure was nausea or vomiting and any cranial neuropathy with urinary retention or difficulty swallowing. This clinical syndrome had a sensitivity of 69.8% and a specificity of 93.6%.

Signs and symptoms predictive of respiratory failure in patients with foodborne botulism in Thailand.
Wongtanate M, Sucharitchan N, Tantisiriwit K, Oranrigsupak P, Chuesuwan A, Toykeaw S, Suputtamongkol Y. American Journal of Tropical Medicine & Hygiene. 77(2):386-9, 2007 Aug.



Infant showing "floppy head", typical symptoms of infant botulism. (Photo courtesy of Dr. Stephen Arnon, California Department of Health Services).

Infant Clinical Signs

- @ Constipation
- @ Lethargy
- @ Poor feeding
- @ Weak cry
- @ Bulbar palsies
- @ Failure to thrive
- @ Poor muscle tone, “floppy” baby syndrome



Infant Botulism

Signs and Symptoms

- ⊙ Poor feeding (weak sucking)
- ⊙ Weak gag
- ⊙ Weak cry
- ⊙ Decreased movement
- ⊙ Appearing lethargic
- ⊙ Flat, blunted facial expression
- ⊙ Trouble swallowing
- ⊙ Excessive drooling
- ⊙ Muscle weakness
- ⊙ Breathing problems
- ⊙ Ptosis (Drooping eyelids)
- ⊙ Poor head control
- ⊙ Decreased anal sphincter tone
- ⊙ Decreased deep tendon reflexes

Treatment and Recovery

- ⊙ New drug: **BabyBIG®**, Botulism Immune Globulin Intravenous (Human) (BIG-IV)
- ⊙ Drastically reduces lethargy, IV feeding and overall hospital stay
- ⊙ With early detection, proper treatment, no long term effects observed



Hypotonia
(decreased
muscle tone)



Floppy Baby Syndrome



Botulism Differential Diagnoses

- © Guillain-Barré syndrome
- © Myasthenia gravis
- © Stroke
- © Tick paralysis
- © Lambert-Eaton syndrome
- © Psychiatric illness
- © Poliomyelitis
- © Diabetic Complications
- © Drug intoxication
- © CNS infection
- © Overexertion

Clostridium
botulinum
Blood agar

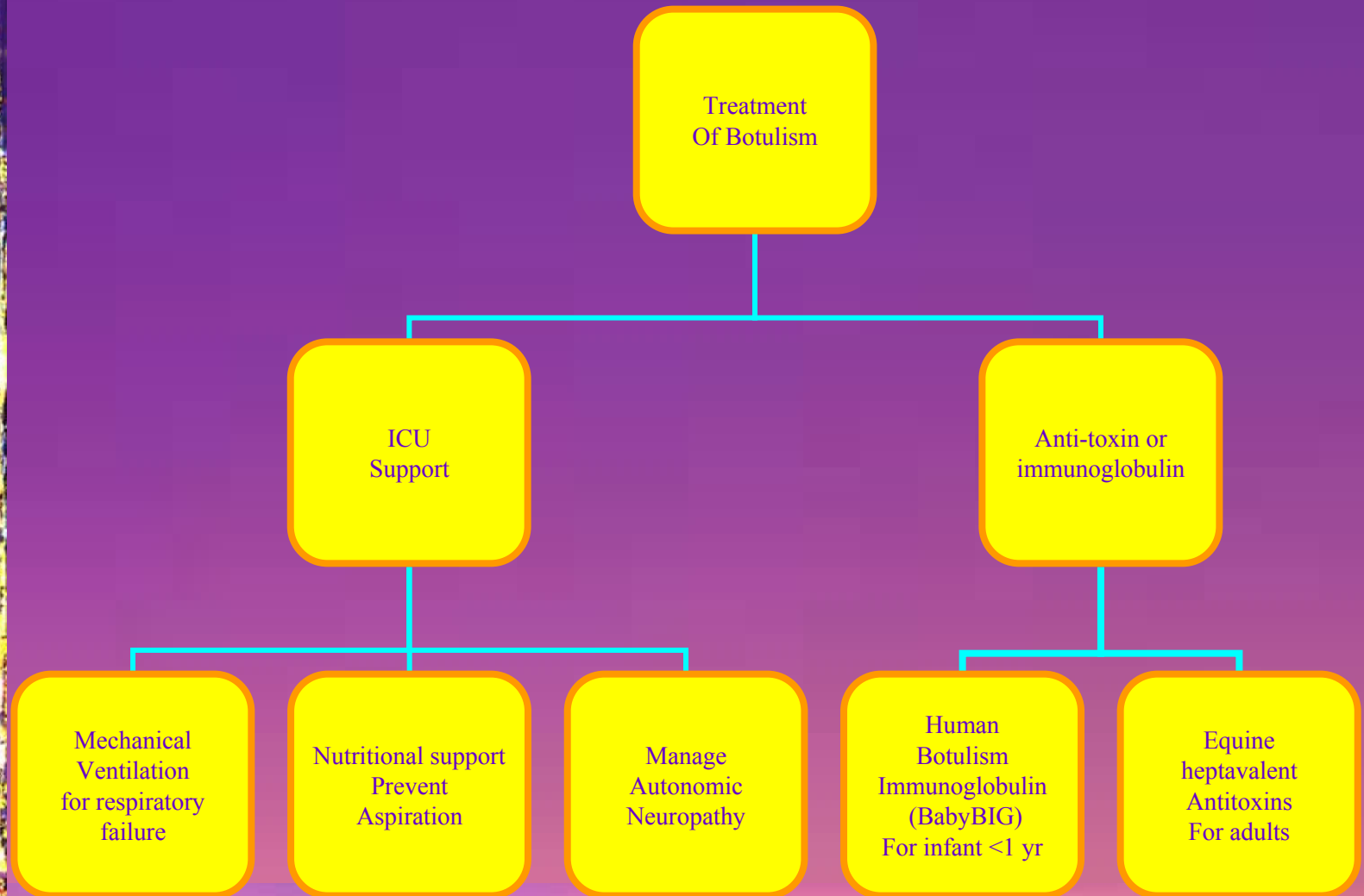
Diagnosis



- © Clinical signs
- © Toxin in serum, stool, gastric aspirate, suspected food (60% +ve food-borne)
 - Mouse neutralization test, result in 48 hours
- © Culture of stool or gastric aspirate, wound
 - Takes 5-7 days
- © Electromyography also diagnostic
 - Electromyography (EMG) demonstrates a nonspecific, decreased amplitude of action potentials. Rapid repetitive EMG at frequencies of 20-50 Hz is more specific for botulism and useful in excluding Guillain-Barré syndrome, but this response does not distinguish botulism from Lambert-Eaton syndrome. Infant botulism is characterized by a pattern known as brief, small, abundant motor-unit action potential on EMG in clinically affected muscles.
- © Lumbar puncture to exclude G.B.S.

Result of Some Ancillary Testing

- ⊙ Tensilon test may be slightly positive
- ⊙ Brain imaging (CT or MRI) normal
- ⊙ Lumbar puncture normal
- ⊙ Electromyography may show decreased amplitude of action potentials in involved muscle groups
- ⊙ Rapid repetitive electromyography (20-50 Hz) will result in facilitation (increasing pattern of action potential amplitude)



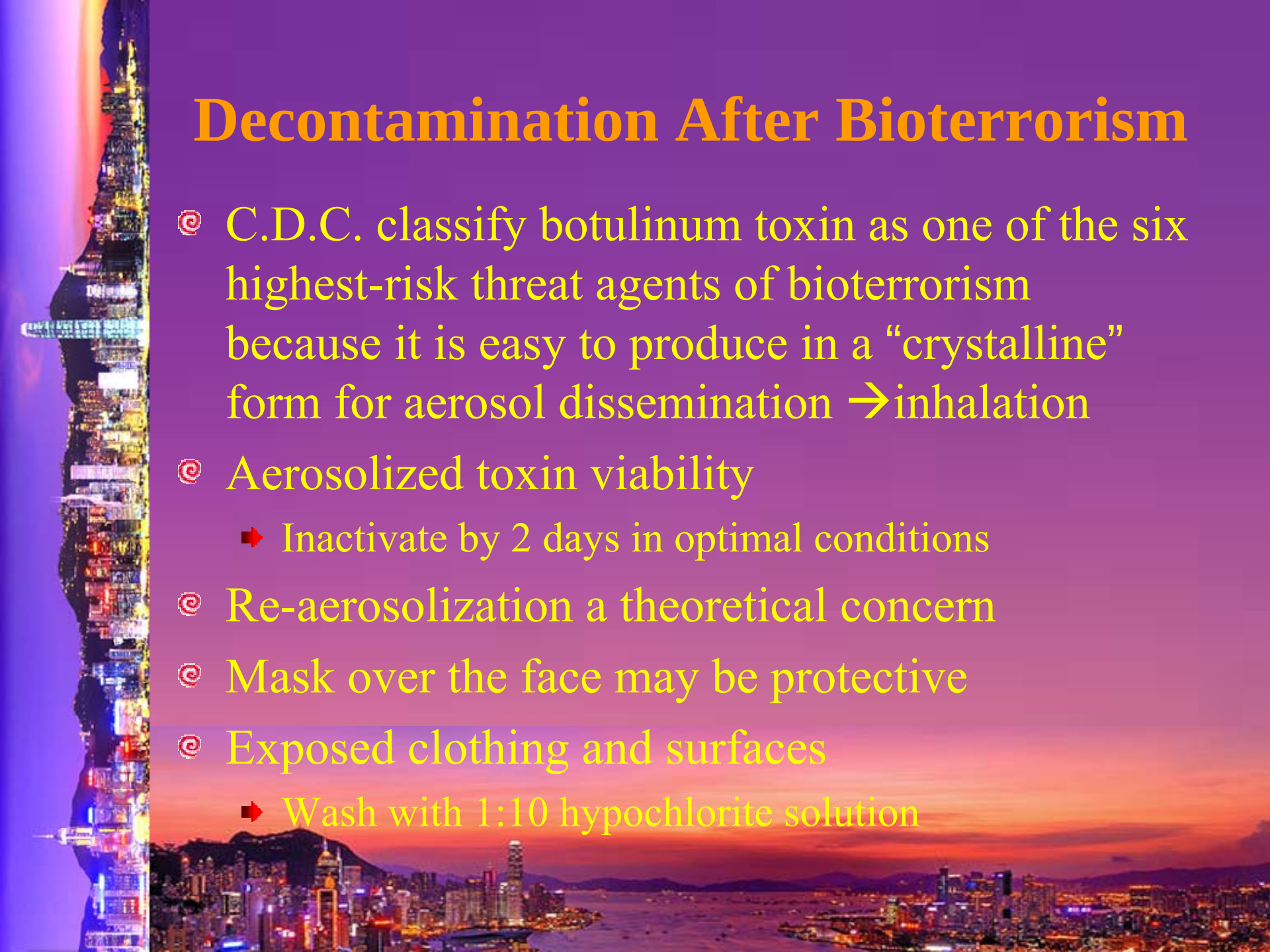
Treatment of Botulism

Treatment

- © Intensive care immediately
 - Ventilator for respiratory failure
 - Airway protection to prevent aspiration
 - Manage autonomic neuropathy
 - Adequate nutritional support (patient may have ileus)
 - Prevent DVT, bed sores and nosocomial sepsis etc
- © Heptavalent Botulinum Antitoxin (HBAT) for adults
 - Derived from equine source
 - CDC distributes
 - Used on a case-by-case basis
- © Human Botulism Immune globulin for < 1yr
 - Infant cases of types A and G
 - Human Botulism Immune Globulin Intravenous (BabyBIG) for infant < 1 years

Decontamination After Bioterrorism

- © C.D.C. classify botulinum toxin as one of the six highest-risk threat agents of bioterrorism because it is easy to produce in a “crystalline” form for aerosol dissemination → inhalation
- © Aerosolized toxin viability
 - ➡ Inactivate by 2 days in optimal conditions
- © Re-aerosolization a theoretical concern
- © Mask over the face may be protective
- © Exposed clothing and surfaces
 - ➡ Wash with 1:10 hypochlorite solution



Treatment of botulism:

New Pentavalent antitoxin 13/3/2010

@ Equine Pentavalent Antitoxin:

- Trivalent (A,B &E) and monovalent antitoxins is replaced by a pentavalent antitoxin since 13/3/2010.
- Effective in the treatment of foodborne, intestinal, and wound botulism: Passive immunity.
- Effectiveness for inhalation botulism has not been proven
- Does not reverse current paralysis, but may limit progression and prevent nerve damage if administered early
- Dosage: 1 vial
- Antitoxin need not be repeated since the circulating antitoxins have a half-life of 5 to 8 days. Repeat HBAT dosing might be indicated only for some wound or intestinal colonization patients if in situ botulinum toxin production continues after clearance of antitoxin.
- 9% of people experience some hypersensitivity to equine antitoxin



MMWR Weekly

March 19, 2010 / 59(10);299

CDC announces the availability of a new heptavalent botulinum antitoxin (HBAT, Cangene Corporation) through a CDC-sponsored Food and Drug Administration (FDA) Investigational New Drug (IND) protocol. HBAT replaces a licensed bivalent botulinum antitoxin AB and an investigational monovalent botulinum antitoxin E (BAT-AB and BAT-E, Sanofi Pasteur) with expiration of these products on March 12, 2010. As of March 13, 2010, HBAT became the only botulinum antitoxin available in the United States for naturally occurring noninfant botulism.

http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5910a4.htm?s_cid=mm5910a4_e



Heptavalent Botulinum Antitoxin (HBAT)

- © HBAT contains equine-derived antibody to the seven known botulinum toxin types (A--G) with the following nominal potency values: 7,500 U anti-A; 5,500 U anti-B; 5,000 U anti-C; 1,000 U anti-D; 8,500 U anti-E; 5,000 U anti-F; and 1,000 U anti-G.
- © HBAT is composed of <2% intact immunoglobulin G (IgG) and $\geq 90\%$ Fab and F(ab')₂ immunoglobulin fragments; these fragments are created by the enzymatic cleavage and removal of Fc immunoglobulin components in a process sometimes referred to as despeciation. Fab and F(ab')₂ fragments are cleared from circulation more rapidly than intact IgG (2)

Administration of HBAT

http://www.epi.alaska.gov/bulletins/docs/b2010_05.pdf

- ⊙ The recommended adult dosing is one 20 mL vial of H-BAT. Consult SOE for dosing children aged >12 months. Frozen HBAT can be thawed at 37°C (99°F) in a water bath.
- ⊙ Obtain patient consent prior to administration of H-BAT.
- ⊙ *Sensitivity testing prior to H-BAT administration is not required.*
- ⊙ Prepare infusion under aseptic conditions.
- ⊙ *Do not shake vial; avoid foaming.*
- ⊙ Visually assess for particulate matter and discoloration.
- ⊙ Do not infuse unless it is clear, is not turbid, and contains no particulate matter.
- ⊙ Dilute 1:10 in 0.9% Sodium Chloride, Injection, USP. The premixed, unused IV bag can be stored refrigerated for use within approximately 8 to 10 hours.



Administration of H-BAT

http://www.epi.alaska.gov/bulletins/docs/b2010_05.pdf

- Ⓢ Use of an in-line filter is optional. If chosen, an in-line filter (pore size 15 μ) is recommended; smaller pore sizes may slow the infusion rate.
- Ⓢ Have epinephrine, diphenhydramine and intubation capabilities immediately available to treat anaphylactic or anaphylactoid reactions.
- Ⓢ To minimize allergic reactions, administer slowly using a continuous infusion pump starting at 0.5 mL/min for the first 30 minutes.
- Ⓢ If no infusion-related safety concerns are evident, increase to 1 mL/min for the next 30 minutes.
- Ⓢ If no infusion-related safety concerns are evident, increase to 2 mL/min for the remainder of the infusion.
- Ⓢ Due to its IND status, H-BAT requires that providers track patient outcomes (paperwork is provided in the state-supplied H-BAT kit).

BabyBIG

Botulism Immune Globulin Intravenous (Human) (BIG-IV)

http://www.infantbotulism.org/babybig_package_insert.pdf

- © FDA approved Solvent-detergent-treated, sterile, lyophilized powder of human-derived botulism antitoxin antibodies for the treatment of infant botulism types A and B.
- © <1 year: 50 mg/kg (1 mL/kg) IV infusion; 25 mg/kg/h IV (0.5 mL/kg/h) initial infusion rate (0-15 min), not to exceed infusion rate of 50 mg/kg/h (1 mL/kg/h)
≥1 year: Not indicated
- © half-life of 28 days in vivo

Differences in Outcomes of Infants Treated with BIG-IV*

Randomized Placebo-Controlled Trial (129 infants)[†]

Duration of	Placebo	BIG-IV
Hospitalization	5.7 wk	2.6 wk
ICU care	5.0 wk	1.8 wk
Mechanical ventilation	4.4 wk	1.8 wk
Tube feeding	10.0 wk	3.6 wk
Total hospital charges	\$163,000	\$74,800
Open-Label Use (366 infants)		
Duration of hospitalization	BIG-IV @ 4–7 days hosp 2.9 wk	BIG-IV @ <4 days hosp 2.0 wk

*Data from reference 9. All differences statistically significant; P values ≤ 0.001 .

[†]Infants eligible only if <3 hospital days.

Infant botulism and treatment with BIG-IV (BabyBIG). Long SS Pediatric Infectious Disease Journal. 26(3):261-2, 2007 Mar.

Allergic reactions to the three types of antitoxin given in a botulism outbreak

Allergic reaction	Antitoxin type		
	Heptavalent (n = 20)	Bivalent (n = 61)	Quadrivalent (n = 10)
Skin test performed	0	29	6
Positive reaction	0	5	2
Anaphylaxis		0	1
Urticarial rash		0	0
Maculopapular rash		0	0
Negative reaction	0	24	4
Anaphylaxis		0	0
Urticarial rash		2	1
Maculopapular rash		3	1
Skin test not performed	20	32	4
Anaphylaxis	1	0	0
Urticarial rash	0	1	0
Maculopapular rash	1	5	0

Skin testing prior to antitoxin administration was not a useful predictor of allergic reaction
Anaphylaxis occurred in 5–10% of the patients

Signs and symptoms predictive of respiratory failure in patients with foodborne botulism in Thailand.
Wongtanate M, Sucharitchan N, Tantisiriwit K, Oranrigsupak P, Chuesuwan A, Toykeaw S, Suputtamongkol Y. American Journal of Tropical Medicine & Hygiene. 77(2):386-9, 2007 Aug.



	Mortality Rate	Length of Hospital Stay
Anti-toxin < 24 hours	10%	10 days
Anti-toxin > 24 hours	15%	41 days
No anti-toxin	46%	56 days

Patients who did not receive antitoxin within 12 h after presentation were **three times** more likely to develop respiratory failure in 20 patients with wound botulism after black tar heroin consumption

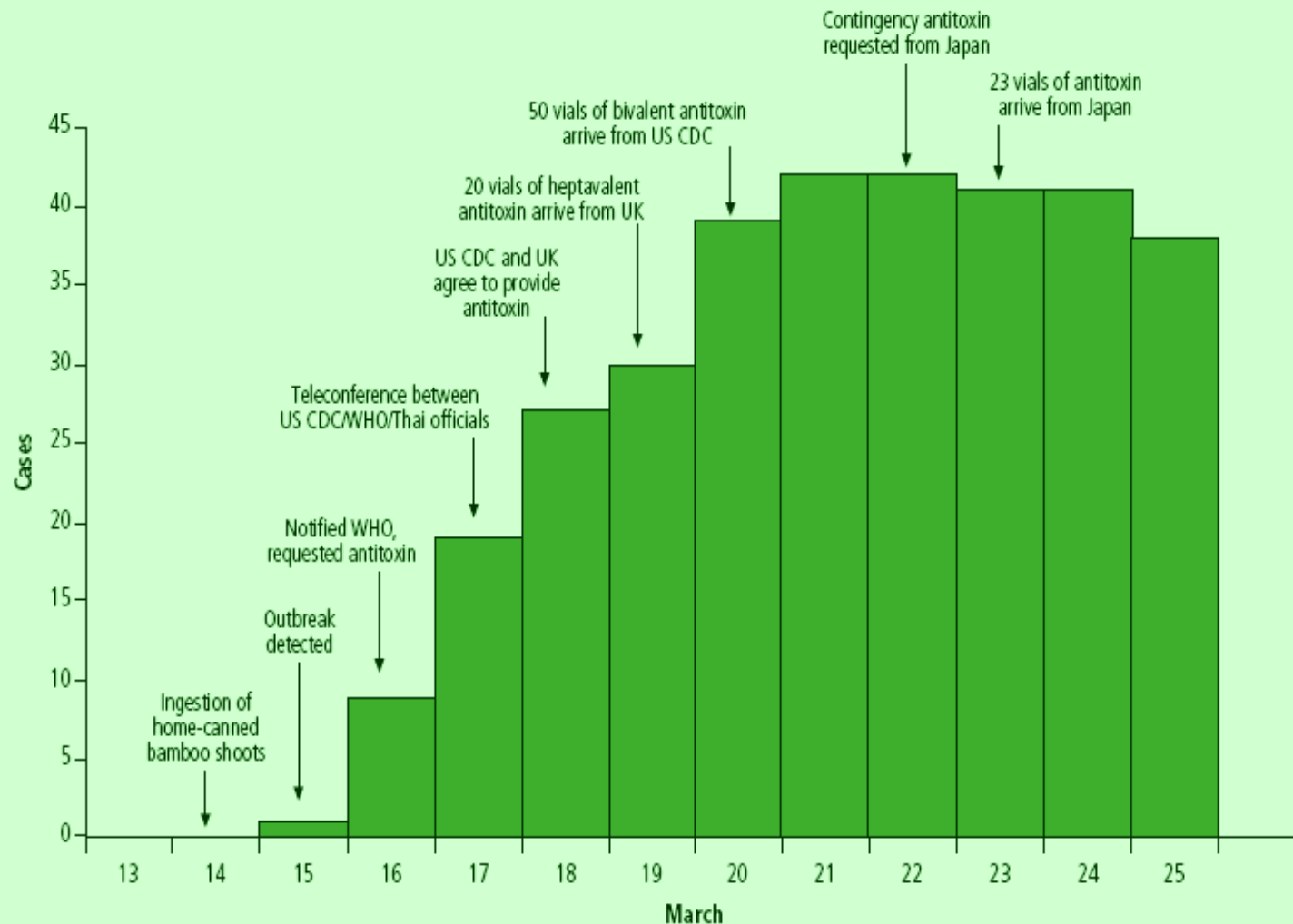
Clinical predictors of respiratory failure and long-term outcome in black tar heroin-associated wound botulism, Sandrock and Murin, 2001 C.E. Sandrock and S. Murin, Chest **120** (2001), pp. 562–566.

A shorter incubation period is likely to result in a significantly higher risk of death ($P=0.01$). The observed **case fatality** was more than 50% for those who developed symptoms within the first 18 h after exposure, possibly reflecting underlying dose-dependent mechanisms.

Incubation period as a clinical predictor of botulism: analysis of previous izushi-borne outbreaks in Hokkaido, Japan, from 1951 to 1965. Nishiura H. Epidemiology & Infection. 135(1):126-30, 2007 Jan.



Daily number of botulism outbreak patients on respirators and steps in managing the outbreak



Should Hong Kong Have Its Own Stock of Anti-toxin ?

The need for global planned mobilization of essential medicine: lessons from a massive Thai botulism outbreak. Ungchusak K. Chunsuttiwat S. Braden C. Aldis W. Ueno K. Olsen S. Wiboolpolprasert S. Bulletin of the World Health Organization. 85(3):238-40, 2007 Mar.

Human: Prevention

- Ⓢ Do not feed honey to children <1 yr of age
- Ⓢ Proper food preservation methods and canning
 - Proper time, temperature and pressure
 - 🌈 80°C for 30 min or 100°C for 10 min
- Ⓢ Prompt refrigeration of foods
- Ⓢ Proper heating of home canned foods (Boil foods for > 10 minutes)
- Ⓢ Decontamination
 - Boil suspected food before discarding
 - Boil or chlorine disinfect utensils used
- Ⓢ Seek medical care for wounds
- Ⓢ Avoid injectable street drugs
- Ⓢ Botulism vaccine for high risk group
 - A toxoid vaccine (antigen types A, B, C, D, and E) is available for laboratory workers at high risk of exposure

Botulinum Toxoid and Research

© Pentavalent botulinum toxoid

- Pentavalent (ABCDE) botulinum toxoid is a combination of aluminum phosphate-adsorbed toxoid derived from formalin-inactivated type A,B,C,D and E botulinum toxins, with formaldehyde and thimerosal used as preservatives.
- Botulinum toxoid is not licensed; it is distributed by the CDC under an IND protocol. The toxoid is used as an adjunct in the protection of laboratorians from accidental exposure to botulinum toxins.
- It should be administered only to at risk individuals who are actively working or expect to be working with cultures of *Clostridium botulinum* or the toxins
- Immunity from botulism, however, has the disadvantage of precluding an individual from realizing the potential benefits of therapeutic botulinum toxin, if such a need were to arise. (Resistant to botox !!!)

© Future research

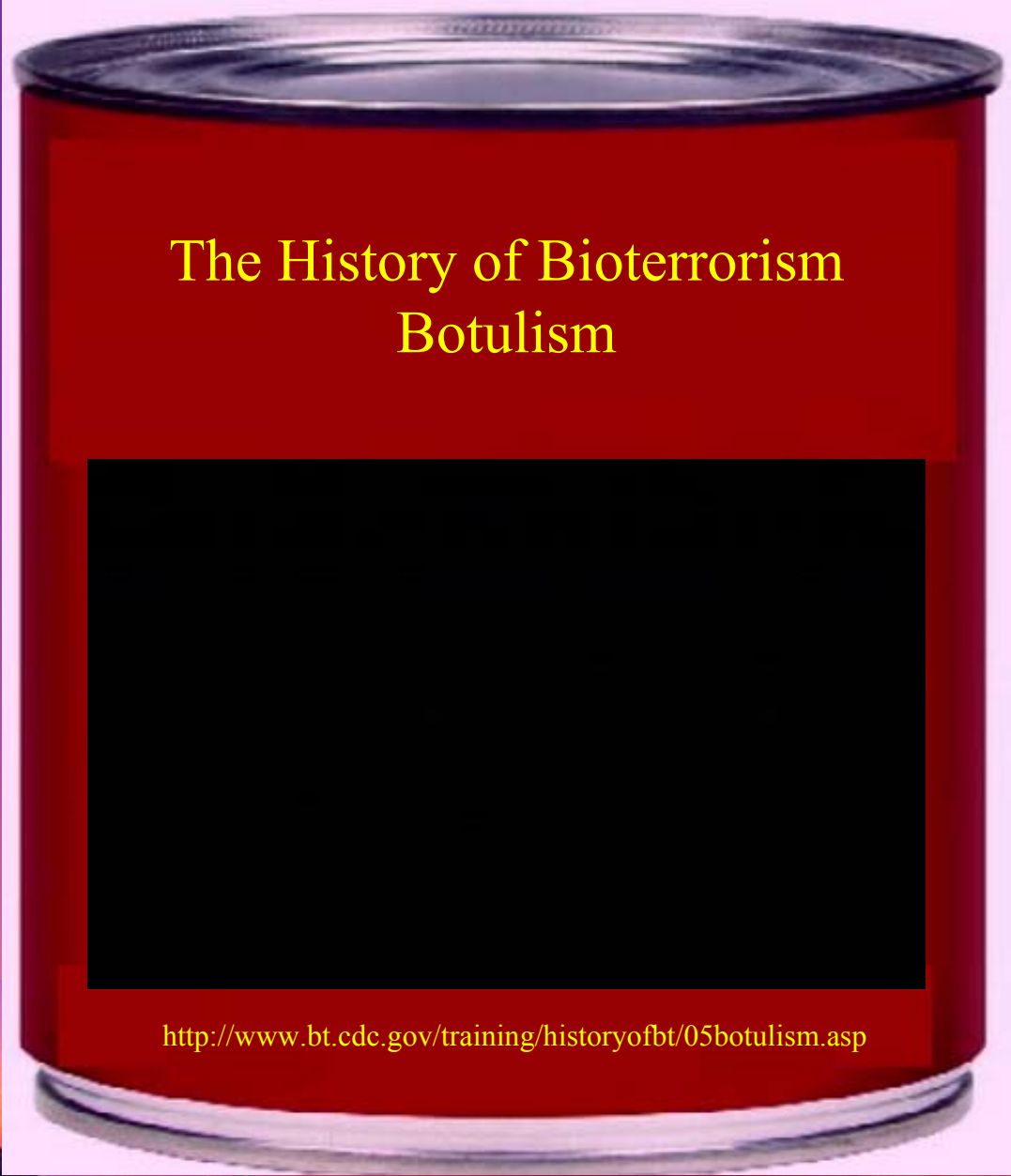
- Recombinant subunit vaccines are in development and a bivalent H(c) vaccine (rBV A/B (*Pichia pastoris*)) is presently being evaluated in a phase II clinical trial.
- ? Adenovial vector-based mucosal vaccine
- ? recombinant Hc subunit of *Clostridium botulinum* neurotoxin

Botulism and vaccines for its prevention. Smith LA. Vaccine. 27 Suppl 4:D33-9, 2009 Nov 5.

An adenoviral vector-based mucosal vaccine is effective in protection against botulism.

Xu Q, Pichichero ME, Simpson LL, Elias M, Smith LA, Zeng M. Gene Therapy. 16(3):367-75, 2009 Mar.

The recombinant Hc subunit of *Clostridium botulinum* neurotoxin serotype A is an effective botulism vaccine candidate. Yu YZ, Li N, Zhu HQ, Wang RL, Du Y, Wang S, Yu WY, Sun ZW. Vaccine. 27(21):2816-22, 2009 May 11.



The History of Bioterrorism Botulism

<http://www.bt.cdc.gov/training/historyofbt/05botulism.asp>



發酵食品



保證不含防腐劑！

Should Hong Kong Have Its Own Stock of Anti-toxin ?



保證不含防腐劑臘腸臘肉

Thank You For Your Attention !