

Design for Biosecurity
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Lecture 28
Botulism Toxin

Welcome back. This is our third class of the sixth week, and today we will be focusing on botulism toxin. This toxin is notable for its diverse pathological effects, yet it is also utilized in Botox therapy, a treatment many of you might be familiar with for its cosmetic benefits. We will explore how this toxin works and its applications in today's class.

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Lecture 28

BOTULISM TOXIN

Botulism mainly affects wild and domesticated animals and begins with the growth of toxigenic clostridia in decaying anaerobic material, followed by release of the toxin. This infected material is consumed by botulinum neurotoxin (BoNT)-insensitive invertebrates (such as maggots), which disseminate the bacterium and the toxin to vertebrates. The cadavers of intoxicated animals provide an anaerobic environment that enables the bacterium to proliferate and release the toxin. The deposition of insect eggs (for example, from flies) leads to the growth of many intoxicated larvae, which are eaten by birds (or fish), generating a self-amplifying cycle that may rapidly involve many birds and/or fish. b | There are five forms of human botulism. The two most common forms are food-borne botulism (which occurs following the ingestion of BoNT-containing foods — typically canned foods) and infant botulism, which is caused by the ingestion of food contaminated with spores that germinate into neurotoxic clostridia in the gastrointestinal tract. In the infant gut, the bacterium has the potential to proliferate, owing to a lack of competition from the resident microbiota, which tends to be less robust in infants. The other three forms of human botulism are much rarer and include inhalational botulism (owing to inhalation of BoNT-containing aerosols), iatrogenic botulism (which is caused by the injection of excessive clinical doses of BoNT) and wound botulism (which is almost exclusively associated with drug injection). Following transcytosis across the intestinal epithelium and subsequent entry into the general circulation, the toxin eventually enters peripheral cholinergic nerve terminals, which causes the flaccid paralysis of botulism.

The diagram illustrates the life cycle of botulism. It starts with 'Decomposition of organic material' where 'Growth of C. botulinum, toxin production and deposition of insect eggs' occurs. 'Ingestion of insect eggs' leads to 'Death of intoxicated vertebrate'. A 'Diverse' cycle is shown where 'Ingestion of insect eggs' leads to 'Growth of C. botulinum, toxin production and deposition of insect eggs'. The diagram also shows 'Human botulism' with five forms: 'Infant botulism' (Ingestion of spores), 'Food-borne botulism' (Ingestion of toxin), 'Wound botulism' (Injection of toxin), 'Iatrogenic botulism' (Injection of toxin), and 'Inhalational botulism' (Inhalation of toxin). The toxin enters the body and 'Transcytosis across intestinal epithelium' leads to 'Toxin in general circulation', which then 'Enters into peripheral cholinergic nerve terminals'.

TOXIC TERRORISM.

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Let's start by focusing on what botulism toxin actually is. Botulism primarily affects both wild and domesticated animals. It begins with the growth of toxigenic Clostridium botulinum, a bacterium found in decaying anaerobic material, which then releases the toxin. The process starts with the decomposition of organic matter harboring Clostridium

botulinum. This material can be consumed by insensitive invertebrates, such as maggots, which then carry the bacterium and toxin to vertebrates.

The cadavers of intoxicated animals create an anaerobic environment that supports the proliferation of the bacterium. This bacterium thrives in low-oxygen conditions and continues to release toxins. For instance, the deposition of insect eggs, such as those of flies, leads to the growth of many intoxicated larvae, which are subsequently consumed by birds or fish, creating a self-perpetuating cycle of contamination. That is why you often see images of affected fish, which can quickly spread the toxin to birds and other animals.

In humans, there are five recognized forms of botulism. The two most common forms are:

1. Foodborne Botulism: This occurs when an individual ingests food contaminated with botulinum neurotoxin, typically from improperly canned foods.

2. Infant Botulism: This results from the ingestion of food contaminated with spores of *Clostridium botulinum*, which then germinate into neurotoxic bacteria in the gastrointestinal tract.

We will discuss these forms in more detail, along with other types such as iatrogenic botulism (which results from medical treatments), inhalation botulism, and botulism from ingestion of spores. For instance, one common precaution in the United States is advising against giving honey to infants. Honey may contain botulinum toxin spores, which can pose a risk to young children.

Returning to the slide, in infants, the bacterium has the potential to proliferate due to the limited competition from resident microbiota, which is not yet fully developed in young children. The other three forms of human botulism are much rarer and include:

1. Inhalational Botulism: This occurs due to the inhalation of aerosols containing botulinum toxin. Such aerosols could potentially be used as bioterrorism agents.

2. Iatrogenic Botulism: This results from the injection of excessive clinical doses of botulinum toxin.

3. Wound Botulism: This form is almost exclusively associated with drug injection practices.

Botulism follows a specific path: after transcytosis across the intestinal epithelium, the toxin spreads through the general circulation and enters the peripheral nervous system, causing flaccid paralysis.

To summarize, *Clostridium botulinum* is a bacterium that produces a highly dangerous toxin known as botulinum toxin under low oxygen conditions. As an anaerobic bacterium, it thrives in environments with little to no oxygen. Botulinum toxins are among the most lethal substances known; they block nerve function and can lead to severe respiratory and muscular paralysis.

Human botulism can manifest in several forms, including:

Foodborne Botulism: Caused by the ingestion of improperly processed foods that contain the toxin. This rare but potentially fatal disease requires rapid diagnosis and treatment with antitoxin, essentially an antibody against the toxin. Homemade canned, preserved, or fermented foods are common sources, and careful preparation is crucial.

Infant Botulism: Occurs when infants ingest food contaminated with spores that germinate into neurotoxic *Clostridium botulinum* in the gastrointestinal tract.

Wound Botulism: Linked to drug injection practices.

Inhalational Botulism: Resulting from inhaling botulinum toxin-containing aerosols.

It's important to note that botulism is not transmitted from person to person. The spores of *Clostridium botulinum* are heat-resistant and exist widely in the environment. In the absence of oxygen, these spores germinate, grow, and produce the toxin.

There are seven distinct forms of botulinum toxin, labeled from types A to G. Among these, types A, B, E, and F are known to cause botulism in humans. Types C, D, and G primarily affect other mammals, including birds and fish. The botulinum toxin is ingested through improperly processed foods where bacteria or their spores have survived. These bacteria

grow and produce toxins under anaerobic conditions, meaning they thrive in environments devoid of oxygen.

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CASE STUDY OF BOTULISM TOXIN: KEY POINTS

- Clostridium botulinum is a bacterium that produces dangerous toxins (botulinum toxins) under low-oxygen conditions.
- Botulinum toxins are one of the most lethal substances known.
- Botulinum toxins block nerve functions and can lead to respiratory and muscular paralysis.
- Human botulism may refer to foodborne botulism, infant botulism, wound botulism, and inhalation botulism or other types of intoxication.
- Foodborne botulism, caused by consumption of improperly processed food, is a rare but potentially fatal disease if not diagnosed rapidly and treated with antitoxin.
- Homemade canned, preserved or fermented foodstuffs are a common source of foodborne botulism and their preparation requires extra caution.

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Human botulism, while predominantly a foodborne intoxication, can also result from intestinal infections with *Clostridium botulinum* in infants, wound infections, or inhalation of the toxin. The botulinum toxin is highly neurotoxic and affects the nervous system. Foodborne botulism is marked by descending flaccid paralysis, which can lead to respiratory failure. Early symptoms include significant fatigue, weakness, and vertigo, often followed by blurred vision, dry mouth, and difficulty swallowing and speaking.

Additional symptoms may include vomiting, diarrhea, constipation, and abdominal swelling. As the disease progresses, weakness can extend to the neck and arms, eventually impacting the respiratory muscles and the muscles of the lower body. Notably, botulism does not present with fever or loss of consciousness. The absence of fever is a key distinguishing feature of botulism. The symptoms are caused by the toxin produced by the bacteria, not the bacteria itself. As previously mentioned, the bacteria germinate, grow, and

produce toxins in low-oxygen environments.

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The image shows a video player interface for a lecture titled "Lecture 28". The main content is a slide titled "OVERVIEW" with three bullet points. Handwritten annotations in black ink are present on the slide:

- A large bracket on the left side encompasses all three bullet points.
- The first bullet point is underlined.
- The second bullet point has "Clostridium botulinum" circled, "heat-resistant" circled, and "exist widely in the environment" circled. The words "A-G" are written below the text "types A-G".
- The third bullet point has "improperly processed food" underlined.

The video player controls at the bottom show a play button, a progress bar at 7:42 / 21:26, and various icons for volume, full screen, and subtitles.

Symptoms typically appear within 12 to 36 hours after exposure, with a possible range of 4 hours to 8 days. Although the incidence of botulism is relatively low, the mortality rate is high. Prompt diagnosis and immediate treatment are crucial; early administration of antitoxin and intensive respiratory care are essential. Without timely intervention, the disease can be fatal in 5 to 10 percent of cases.

Regarding foodborne botulism, it's important to remember that *Clostridium botulinum* is an anaerobic bacterium, meaning it only grows in the absence of oxygen. Botulism occurs when this bacterium grows and produces toxins in food before it is consumed. The spores of *Clostridium botulinum* are widespread in the environment, including in soil, rivers, and seawater. The bacteria thrive and produce toxins in foods that have low oxygen content, combined with specific storage temperatures and preservative conditions.

It's important to be cautious because some preservatives can actually promote the growth of botulinum toxins. This issue often arises with lightly preserved foods and inadequately

processed home-canned or home-bottled items.

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SYMPTOMS OF FOODBORNE BOTULISM

- Botulinum toxins are neurotoxic and therefore affect the nervous system. Foodborne botulism is characterized by descending, flaccid paralysis that can cause respiratory failure. Early symptoms include marked fatigue, weakness and vertigo, usually followed by blurred vision, dry mouth and difficulty in swallowing and speaking. Vomiting, diarrhoea, constipation and abdominal swelling may also occur. The disease can progress to weakness in the neck and arms, after which the respiratory muscles and muscles of the lower body are affected. There is no fever and no loss of consciousness.
- The symptoms are not caused by the bacterium itself, but by the toxin produced by the bacterium. Symptoms usually appear within 12 to 36 hours (within a minimum and maximum range of 4 hours to 8 days) after exposure. Incidence of botulism is low, but the mortality rate is high if prompt diagnosis and appropriate, immediate treatment (early administration of antitoxin and intensive respiratory care) are not given. The disease can be fatal in 5 to 10% of cases.

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Clostridium botulinum will not thrive in acidic conditions, so you won't find this bacterium in the acidic parts of your gastrointestinal tract, where the pH is less than 4.5. Therefore, botulinum toxin is not produced in acidic foods. However, while a low pH prevents bacterial growth, it does not degrade any preformed toxins. The bacteria themselves will not grow in low pH environments, but the toxin remains unaffected. To prevent bacterial growth and toxin formation, a combination of low storage temperatures, salt content, and pH levels is employed.

Botulinum toxin has been detected in a range of foods, including low-acid preserved vegetables such as green beans, spinach, mushrooms, and beets; various types of fish, including canned tuna and fermented, salted, or smoked fish; and meat products like ham and sausages. The specific foods involved can vary by region, reflecting local dietary habits. Sometimes, commercially prepared foods are implicated as well.

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EXPOSURE AND TRANSMISSION

- **Foodborne botulism**

$-O_2$

C. botulinum is an anaerobic bacterium, meaning it can only grow in the absence of oxygen. Foodborne botulism occurs when C. botulinum grows and produces toxins in food prior to consumption. C. botulinum produces spores and they exist widely in the environment including soil, river and sea water.

The growth of the bacteria and the formation of toxin occur in products with low oxygen content and certain combinations of storage temperature and preservative parameters. This happens most often in lightly preserved foods and in inadequately processed, home-canned or home-bottled foods.

C. botulinum will not grow in acidic conditions (pH less than 4.6) and therefore the toxin will not be formed in acidic foods (however, a low pH will not degrade any pre-formed toxin). Combinations of low storage temperature and salt contents and/or pH are also used to prevent the growth of the bacteria or the formation of the toxin.

LOW pH → X degrade the TOXIN

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Lecture 28

FOODBORNE BOTULISM

The botulinum toxin has been found in a variety of foods, including low-acid preserved vegetables, such as green beans, spinach, mushrooms, and beets; fish, including canned tuna, fermented, salted and smoked fish; and meat products, such as ham and sausage. The food implicated differs between countries and reflects local eating habits and food preservation procedures. Occasionally, commercially prepared foods are involved.

Though spores of C. botulinum are heat-resistant, the toxin produced by bacteria growing out of the spores under anaerobic conditions is destroyed by boiling (for example, at internal temperature greater than 85 °C for 5 minutes or longer). Therefore, ready-to-eat foods in low oxygen-packaging are more frequently involved in cases of foodborne botulism.

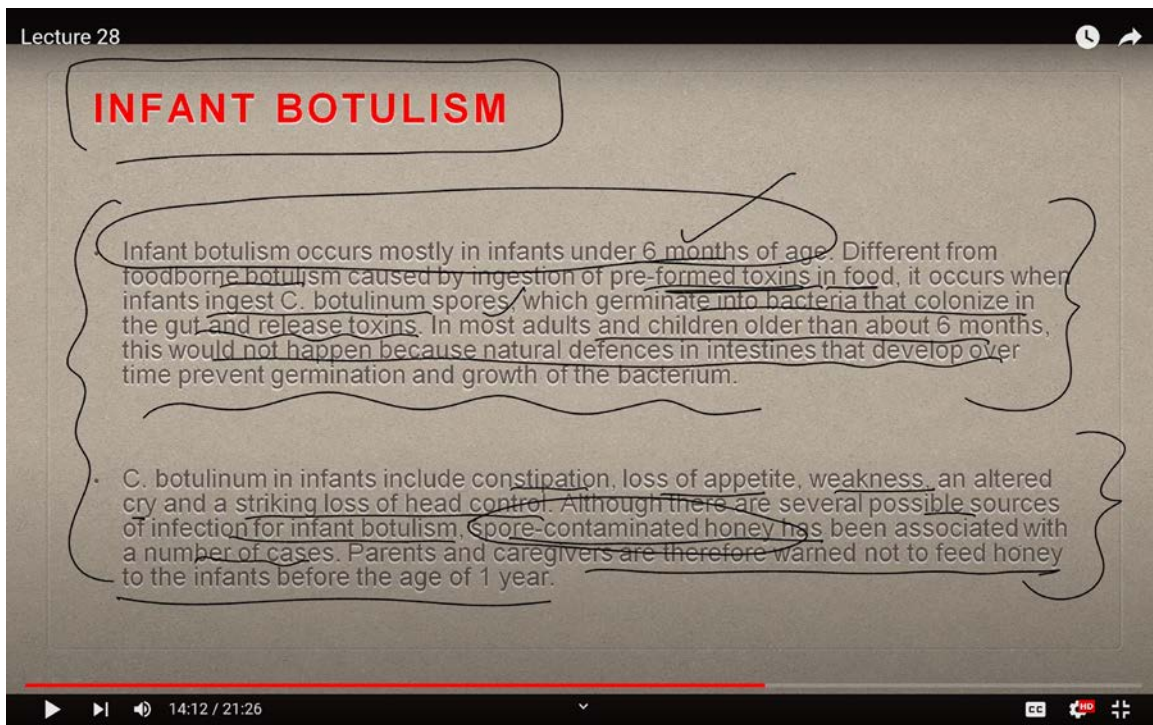
Food samples associated with suspect cases must be obtained immediately, stored in properly sealed containers, and sent to laboratories in order to identify the cause and to prevent further cases.

12:12 / 21:26

This wide range of potential sources underscores the importance of understanding where infections can occur and highlights the extensive potential zone of contamination. Despite the heat resistance of *Clostridium botulinum* spores, the toxin produced by bacteria growing from these spores under anaerobic conditions can be destroyed by boiling. Boiling at an internal temperature above 85 degrees Celsius (185 degrees Fahrenheit) for five minutes or more is effective in neutralizing the toxin. Therefore, foods packaged in low-oxygen environments are more frequently associated with foodborne botulism cases.

For suspected cases of botulism, it is crucial to obtain food samples immediately, store them in properly sealed containers, and send them to laboratories for analysis to identify the cause and prevent further cases.

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The image shows a video lecture slide titled "Lecture 28" with the main heading "INFANT BOTULISM" in red. The slide contains two bullet points describing the condition. The first bullet point explains that infant botulism occurs in infants under 6 months of age, where they ingest *C. botulinum* spores that germinate and release toxins in the gut, unlike foodborne botulism. The second bullet point lists symptoms such as constipation, loss of appetite, weakness, and loss of head control, and notes that spore-contaminated honey is a common source of infection, leading to a warning for parents not to feed honey to infants under 1 year old. The video player interface at the bottom shows the current time is 14:12 out of 21:26.

Lecture 28

INFANT BOTULISM

- Infant botulism occurs mostly in infants under 6 months of age. Different from foodborne botulism caused by ingestion of pre-formed toxins in food, it occurs when infants ingest *C. botulinum* spores which germinate into bacteria that colonize in the gut and release toxins. In most adults and children older than about 6 months, this would not happen because natural defences in intestines that develop over time prevent germination and growth of the bacterium.
- *C. botulinum* in infants include constipation, loss of appetite, weakness, an altered cry and a striking loss of head control. Although there are several possible sources of infection for infant botulism, spore-contaminated honey has been associated with a number of cases. Parents and caregivers are therefore warned not to feed honey to the infants before the age of 1 year.

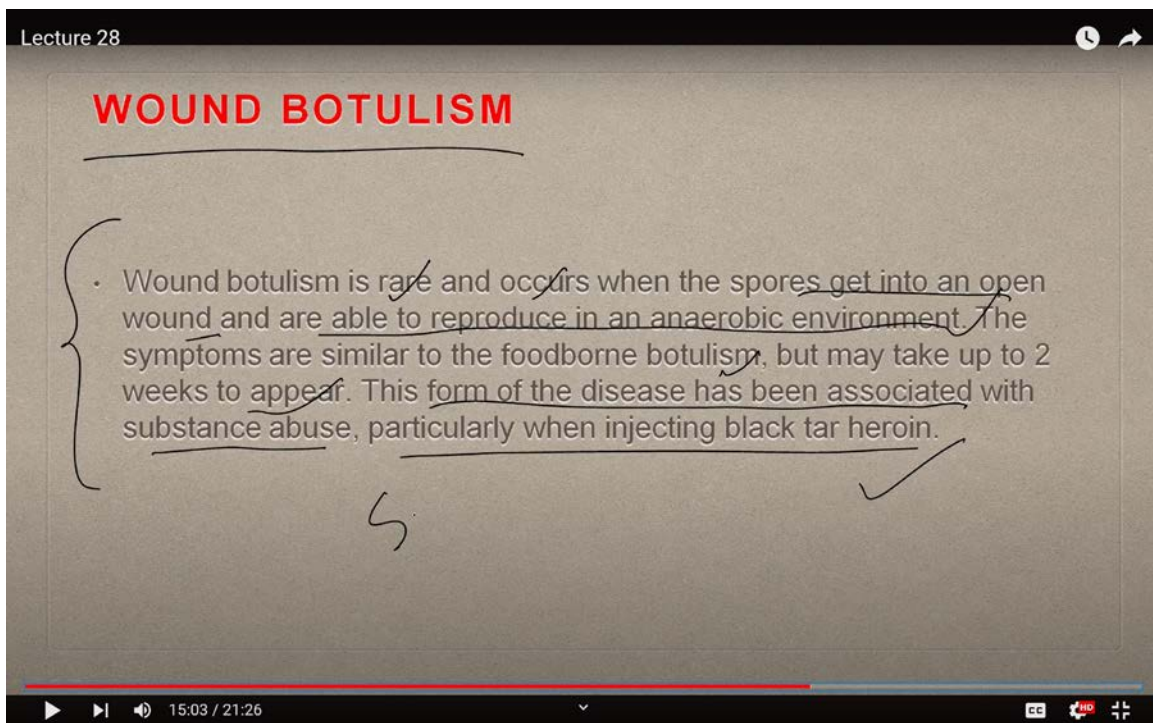
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Regarding infant botulism, this condition predominantly affects infants under six months of age. At this age, the microbial defenses in the gut are not yet fully developed, making it easier for *Clostridium botulinum* to grow. Infants are less exposed to diverse foods and are typically reliant on breast milk or infant formula. Unlike foodborne botulism, which is

caused by ingesting preformed toxins, infant botulism occurs when infants ingest *Clostridium botulinum* spores. These spores then germinate into bacteria that colonize the gut and produce toxins. In most older children and adults, the natural defenses in the intestine prevent the germination of these bacteria. However, in infants under six months, these defenses are not yet established. Symptoms of infant botulism include constipation, loss of appetite, weakness, altered crying patterns, and a notable loss of head control. One significant source of infection in infants is spore-contaminated honey, which has been linked to several cases of infant botulism.

Parents and caregivers are strongly advised not to give honey to infants before they reach one year of age. This caution arises from multiple cases where honey has been found to contain *Clostridium botulinum* spores. In many Western countries, this recommendation is widely enforced to prevent botulism.

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The image shows a video player interface for a lecture slide. The slide title is "WOUND BOTULISM" in red text. Below the title, there is a list of bullet points describing the condition. The text in the slide is as follows:

- Wound botulism is rare and occurs when the spores get into an open wound and are able to reproduce in an anaerobic environment. The symptoms are similar to the foodborne botulism, but may take up to 2 weeks to appear. This form of the disease has been associated with substance abuse, particularly when injecting black tar heroin.

The video player interface includes a progress bar at the bottom showing the current time as 15:03 out of 21:26. There are also icons for play, volume, and other video controls.

Wound botulism, the third form of botulism we discussed, is a rare condition that occurs when *Clostridium botulinum* spores enter an open wound and proliferate in an anaerobic

environment. The symptoms of wound botulism are similar to those of foodborne botulism but may take up to two weeks to manifest. This form of botulism is often associated with substance abuse, particularly in individuals injecting black tar heroin. Thus, wound botulism is frequently observed in cases of drug addiction.

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The image shows a video player interface for a lecture slide. The slide title is "INHALATION BOTULISM" in red. The text on the slide is as follows:

- Inhalation botulism is rare and does not occur naturally, for example it is associated with accidental or intentional events (such as bioterrorism) which result in release of the toxins in aerosols. Inhalation botulism exhibits a similar clinical footprint to foodborne botulism. The median lethal dose for humans has been estimated at 2 nanograms of botulinum toxin per kilogram of bodyweight, which is approximately 3 times greater than in foodborne cases.
- Following inhalation of the toxin, symptoms become visible between 1–3 days, with longer onset times for lower levels of intoxication. Symptoms proceed in a similar manner to ingestion of botulinum toxin and culminate in muscular paralysis and respiratory failure.
- If exposure to the toxin via aerosol inhalation is suspected, additional exposure to the patient and others must be prevented. The patient's clothing must be removed and stored in plastic bags until it can be washed thoroughly with soap and water. The patient should shower and be decontaminated immediately.

Handwritten annotations in black ink include:

- A checkmark above the first bullet point.
- A bracket on the left side of the first two bullet points.
- The number "2" in the first bullet point is circled, with "nanograms" written next to it.
- A bracket under "1–3 days" in the second bullet point.
- A bracket under "muscular paralysis and respiratory failure" in the second bullet point.
- A bracket under the entire third bullet point.

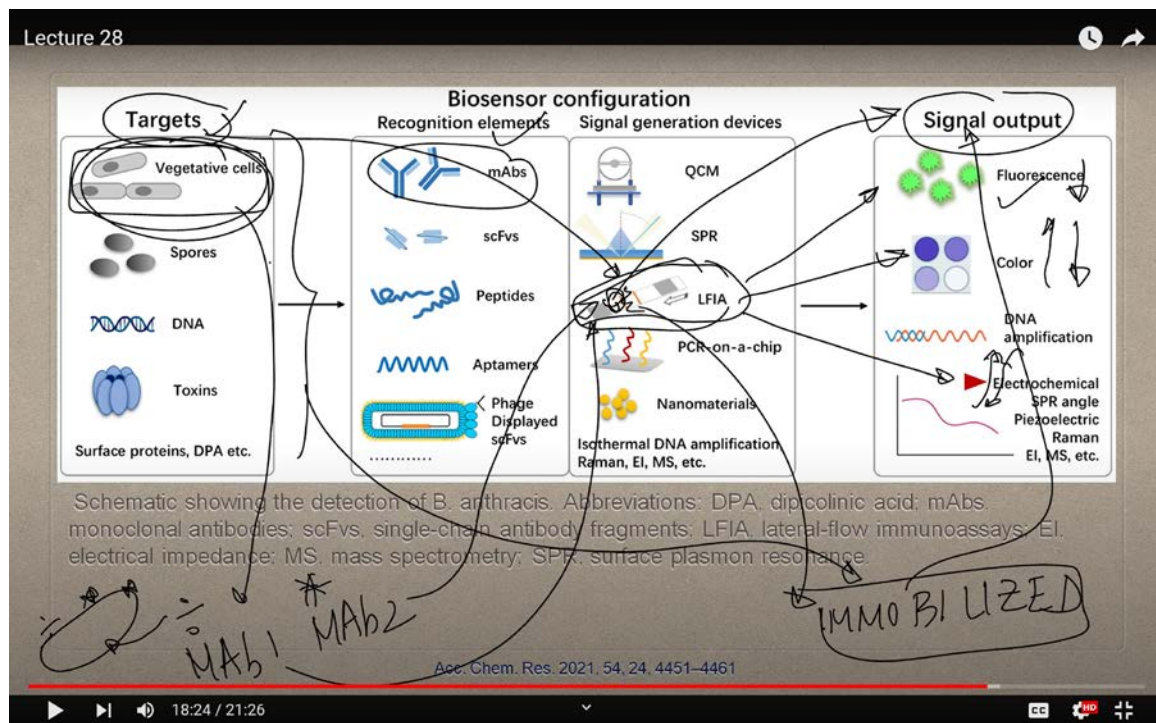
The video player interface at the bottom shows a progress bar at 16:31 / 21:26 and various control icons.

One of the rarest forms of botulism is inhalation botulism. This type does not occur naturally and is typically linked to accidental or deliberate events, such as bioterrorism, which involves the release of botulinum toxin in aerosol form. Inhalation botulism presents clinical symptoms similar to those of foodborne botulism. The median lethal dose for humans has been estimated at just two nanograms, highlighting the extreme potency of the botulinum toxin. For instance, if an individual weighs 60 kilograms, approximately 120 nanograms, three times the dose typically required for foodborne botulism, would be needed to be lethal.

Symptoms of inhalation botulism become evident within one to three days after exposure, with a longer onset time and potentially lower levels of intoxication compared to foodborne

botulism. The progression of symptoms mirrors that of botulinum toxin injection, eventually leading to muscular paralysis and respiratory failure. If there is suspicion of exposure to botulinum toxin via inhalation, it is crucial to prevent further exposure. The patient's clothing should be removed and stored in plastic bags until it can be thoroughly washed with soap and water, and the patient should shower and be decontaminated immediately.

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Another type of botulinum intoxication is waterborne botulism, which theoretically could occur from ingesting preformed toxins. However, standard water treatment processes such as boiling and disinfection with a 0.1% hypochlorite bleach solution are effective in destroying the toxin, so the risk is considered low. Botulism of undetermined origin generally involves cases in adults where no specific food or wound source can be identified.

These cases are comparable to infant botulism and may arise when the normal gut flora is disrupted due to surgical procedures or antibiotic treatment. Adverse effects from pure botulinum toxin have been reported in medical and cosmetic applications, which leads us

to the topic of Botox therapy. It is essential to note that botulinum toxin is a fascinating substance because the bacteria itself does not cause harm until it produces the toxin.

The challenge with botulinum toxin is that its production requires an anaerobic environment and specific pH conditions. This makes detecting the bacteria and its toxin quite challenging. For instance, if you only detect the vegetative cells of *Clostridium botulinum*, you may not identify the problem since these cells do not exhibit effects until they produce the toxin. Toxin production only occurs in the absence of oxygen and under specific temperature and pH conditions.

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The image shows a video lecture slide titled "OTHER TYPE OF INTOXICATION" in red text. The slide contains three bullet points:

- Waterborne botulism could theoretically result from the ingestion of the pre-formed toxin. However, as common water treatment processes (such as boiling, disinfection with 0.1% hypochlorite bleach solution) destroy the toxin, the risk is considered low.
- Botulism of undetermined origin usually involves adult cases where no food or wound source can be identified. These cases are comparable to infant botulism and may occur when the normal gut flora has been altered as a result of surgical procedures or antibiotic therapy.
- Adverse effects of the pure toxin have been reported as a result of its medical and/or cosmetic use in patients, see more on 'Botox' below.

Handwritten notes in black ink are present on the slide:

- "BOTULINUM TOXIN" is written at the top right, with an arrow pointing to "Bacteria" below it.
- "infect" is written to the right of the first bullet point, with an arrow pointing to the text "ingestion of the pre-formed toxin".
- "+ drug" is written below "infect".
- "NEUROLOGICAL ASPE" is written at the bottom left.
- "BOTOX" is written in the center bottom.
- "fashion world" is written at the bottom right, with an arrow pointing to the third bullet point.

The video player interface at the bottom shows the time 20:47 / 21:26 and various control icons.

In practical terms, detecting vegetative cells alone would not be very useful. What we have developed is an antitoxin, which only works when the vegetative cells are actively producing the toxin. Thus, the challenge in developing assays or technologies for detecting botulinum toxin lies in the fact that we are targeting the toxin itself rather than the bacterial source. The *Clostridium botulinum* only becomes problematic once it starts producing the neurotoxin.

Moving forward, we will explore the fascinating applications of botulinum toxin, particularly its use in Botox therapy. To recap, we have discussed what botulinum toxin is, how it is generated by bacteria, and its various forms of infection. In the next class, we will delve into its applications in the fashion world, particularly focusing on Botox and its neurological effects. So I'll close in here. In the next class, we'll start with the Botox therapy. Thank you.