Gram-Positive Bacterial Infections

Gram-positive bacteria like Bacillus anthracis and Listeria monocytogenes are of high clinical significance but are often barely regarded despite their ubiquitous occurrence and high resistance towards infaust conditions. But also diseases from atypical mycobacteria increase nowadays. Therefore, this article creates an overview over the symptoms and diagnostics of the different clinical pictures and their therapy.

Corynebacteria

The most important corynebacteria is *C. diphtheriae* which produces the diphtheria toxin and causes a serious, life-threatening disease called diphtheria. *C. ulcerans* and *C. pseudotuberculosis* are 2 other important species that are potentially toxigenic and can cause diphtheria. Recently, many non-toxigenic corynebacteria infections have been implicated as opportunistic infections in immunosuppressed patients.

Morphology of Corynebacteria

Corynebacteria are small prokaryotes that owe their name to the swollen end of their cell. On microscopy, this swollen end resembles a club (coryne = old greek for 'club'). Nevertheless, corynebacteria are pleomorphic which means that they can take on other shapes during their growth and appear more coccoid then.
Another important attribute of Corynebacteria is a V-shaped connection between mother and daughter cell after cell division. This form appears due to the so-called snapping post-fission movement. This snap division occurs because only the inner cell wall of corynebacteria participates in the cell division.

The outer wall surrounds the mother and daughter cell afterward and splits on one side due to cell growth. Both cells drift apart there but are still connected on one side so that the V-shape appears. This is also known as an array taking the shape of Chinese letters.

Laboratory diagnosis of Corynebacteria

Corynebacteria are aerobes, amastigotes and, for this reason, immobile rod cells that are not able to form spores or capsules. In the cell plasm of the bacteria, so-called polar bodies that include polyphosphate and calcium can be found. These polar bodies are barely visible on Gram stain so the Neisser stain is used for this purpose.

After staining, the bacteria are colored yellow to rose, while the polar bodies are dark blue to black. This kind of staining is still widely used in the detection of \textit{C. diphteriae} and \textit{C. pseudodiphteriticum}. Culture takes place on blood agar and can be made with sputum, gastric juice, laryngeal smear, urine, and even ejaculate or menstrual blood.

By putting a fosfomycin platelet onto the blood agar, the standard pharyngeal flora can be inhibited in growth. Corynebacteria are visible as greyish colonies with a slight flare of hemolysis. Tinsdale agar (natriumtellurite agar or Clauberg agar) works as an indicator medium. Due to the reduction of the included tellurium, Corynebacteria grow black with a blue flare on this medium. The tellurite also suppresses the growth of some bacteria of the pharyngeal flora.

It takes about 18–24 hours before the colonies become visible macroscopically. Afterward, the differentiation between pathogenic and apathogenic corynebacteria is achieved through biochemical reactions. Pathogenic species are characterized by their positive catalase reaction, negative urease reaction, the fermentative breakdown of
glucose (but not of saccharose), and nitrate reduction.

By their different colony morphology, hemolysis behavior, and ability to break down dextrin and glucose, the 3 biovars of *C. diphtheriae*: *mitis*, *intermedius*, and *gravis* can be distinguished. Different in vitro methods like gel immunodiffusion or PCR and in vivo tests like the Draize test, where serum of the patient or culture solution are put onto the shaved skin of a rabbit, are used to detect the diphtheria toxin.

The Elek test is a particular type of gel immunodiffusion test. In this test, a stripe of filtering paper drained with antitoxin is applied. Additionally, the stem to be analyzed is applied crosswise. If the stem includes diphtheria toxin, antigen-antibody complexes occur which can be seen as S-shaped lines. Nevertheless, the diagnosis of diphtheria is primarily clinical, with the laboratory diagnosis only for identifying specifics.

**Epidemiology and incidence of Corynebacteria**

Infection with *C. diphtheriae* is exclusively via human-to-human by droplet infection, other secretions, or direct contact. Due to the good vaccination coverage in western countries, contamination often takes place in subtropical areas where diphtheria is endemic. The incidence of diphtheria in Middle Europe is about 0.001/100,000 per year. Dogs and cats are a natural reservoir for toxigenic strains of *C. ulcerans*. Non-pathogenic species are part of the normal flora of the skin and mucosa.

**Pathomechanism of Corynebacteria**

Only Corynebacteria that are infected with a specific phage trigger the disease. This phage carries genes for the exotoxin that inhibits the translation during the phase of elongation in the protein biosynthesis. First, the B-part of the bacterium docks to the host cell, afterward the A-toxin is absorbed into the cell in a vacuole. The diphtheria toxin A inhibits elongation factor 2 by ADP ribosylation of a specific amino acid residue (diphthamide).

Also, some stems of *C. ulcerans* carry these phages and are, consequently, pathogenic. With the destruction of the infected cells, the typical pseudomembrane appears, consisting of necrotic cell components.

**Clinical features of Corynebacteria infection**
Two to 6 days after infection, symptoms like fatigue, sickness and swallowing pain first occur. With the typical pharyngeal diphtheria, a yellowish-white fur and musty-sweet mouth odor emerge. Infants are often afflicted with diphtheria up to the nose so that a nose discharge characterized by blood pus occurs.

**Laryngeal diphtheria** is a dreaded condition as stridor or even asphyxiation can occur. A resulting ‘diphtheritic croup’ or ‘true croup’ could occur with a symptom triad of barking cough, hoarseness, and aphonia.

With other corynebacteria, clinical features like wound infections, otitis, and urinary tract infections can also result.

**Therapy for Corynebacteria infection**

The early administration of antitoxin is of priority in diphtheria. Simultaneously, antibiotic therapy with penicillin G should be started. Persons who have contacted infected people and asymptomatic carriers should receive penicillin V or clarithromycin prophylactically. Other Corynebacteria are often multi-resistant and are only sensitive to vancomycin, teicoplanin, and linezolid. Immunization with diphtheria toxin is also helpful. Suspicion of disease, infection, and death by diphtheria are reportable.

**Listeria**

*Listeria monocytogenes* is an opportunistic pathogen that causes foodborne infectious disease, mostly seen in immunocompromised individuals, and vulnerable groups, such as pregnant women, neonates, and the elderly, with a high mortality rate up to 20-30% of clinical cases. It may cause mild and self-limiting febrile gastroenteritis in healthy people.

**Attributes of Listeria**

Listeriae are gram-positive rod cells that are mastigotes at low temperatures under 20°C (68°F) and have a typical end-to-end movement. The fermentative catalase-positive listeria can grow aerobically and anaerobically but do not form spores.
*L. monocytogenes* secretes a pore-forming toxin called **listeriolysin O**. On blood-containing agars, this toxin causes β-hemolysis; this characteristic allows the differentiation of virulent and avirulent strains. This toxin is analogous to streptolysin O from A-streptococci and pneumolysin from Pneumococci.

![](image)

Listeria are very resistant to outer influences as they can even survive the pasteurizing of milk. This special attribute is used in diagnostics in the form of **cold enrichment**. Also, in the body, listeria protect themselves from the host's immune defense by an **intracellular stay**.

In infected cells, listeria causes an evagination of the host cell by actin increase. Due to this evagination, the bacteria can enter neighbor cells without any contact with the extracellular defense.

**Incidence and epidemiology of Listeria**

Listeria can be found in the intestine of domestic and wild animals as well as humans. It also occurs in samples of soil, water, and waste. Very often it can be isolated from milk and milk products.

Typically, occupationally exposed persons like butchers, farmers, or veterinary medicals are infected. Moreover, pregnant women and unborn children, who have relative immunosuppression, hare at risk of listeria infection. About one-third of listeriosis affects pregnant women and newborns. Next to rubella and toxoplasmosis, listeriosis is the most frequent prenatal infection in Germany. Due to contaminated food, local outbreaks with several infected persons can occur. In Germany, the prevalence is at 1–4 cases per 1,000,000 inhabitants per year.

**Pathomechanism of listeriosis**

*L. monocytogenes* mostly finds its way into the host organism from the intestine over the M cells of the **Peyer plaques** and from there into local lymph nodes. From there, further spread takes place over the thoracic duct into the blood. Afterward, they are
absorbed from macrophages and leave the phagosome via pore-forming listeriollysin.

In the cytoplasm of the macrophages, they can reproduce unimpededly, leading to a release of chemotactic factors and monocytosis. The specific immune defense leads to the formation of granulomas.

In pregnant women, symptom-poor bacteremia usually occurs, which causes severe sepsis by placental transmission to the unborn child (granulomatosis infantiseptica). Typical symptoms in the fetus are infections of the liver, lungs, kidneys, and brain. Infections with Listeria can occur in every stage of the pregnancy but are most common in the 3rd trimester.

**Laboratory diagnosis for Listeria**

As testing material, liquor, blood, forewater, and tissue samples can be used. The cold resistance of listeria is useful for isolation because only a few other bacteria can grow at 4°C (39.2°F) (so-called cold enrichment). The propagation takes place on blood agar or in trypticase-soy-bouillon.

The colonies are small and white; in virulent stems, they are additionally surrounded by a flare of ß-hemolysis. In liquid culture mediums, they show a typical somersault-like movement. In microscopy, they often appear coccoid.

![Image: Listeria monocytogenes – Columbia Horse Blood Agar](Image: Listeria monocytogenes – Columbia Horse Blood Agar. By Nathan Reading, License: CC BY 2.0)

**Therapy for listeriosis**

The drug of choice is ampicillin in combination with an aminoglycoside.
Bacilli

Attributes of bacilli

Bacteria of the genus Bacilli are big aerobic, immobile, spore-forming rod cells that are mostly gram-positive but also slightly gram-variable. The species *Bacillus anthracis*, *cereus*, and *subtilis* are relevant to humans.

*Bacillus anthracis*

The rod cells of *B. anthracis* are stringed together like a chain and feature a capsule made of D-glutamic acid. This capsule is only formed on nutrient agar if increased CO$_2$ tension is present. Under unfavorable culture conditions, endospores are formed, which are extremely environmentally resistant.

During the Second World War, some islands in the Atlantic were infested with anthrax in biological weapon tests and, therefore, are still uninhabitable. *B. anthracis* is a biological agent that can be used in bioterrorism attacks. It is ubiquitous in the ground. The plasmid-coded exotoxin, also known as anthrax toxin, consists of 3 parts: the edema factor, the protective antigen, and the lethal factor.

Anthrax is a zoonosis. The human is infected by direct contact with sick or deceased grazers or indirectly by animal products like wool, bone meal, or saddle cloth. That is why anthrax is also called Woolsorter’s disease. Through skin lesions, the spores enter the human body and pass into the vegetative, toxin-building form.

Pathomechanism of *Bacillus anthracis*

The edema factor and lethal factor invade leukocytes under protection by their protective antigen. In the leukocytes, they increase the concentration of cAMP, which inhibits the phagocytosis of the infected cells. Since leukocytes are infested, the infected tissue typically seems indifferent. The lethal factor induces the necrosis of granulocytes and masses of anthrax bacteria are released. An uninhibited reproduction of bacilli and proliferation via lymph vessels and bloodstream follows.
Clinical picture of infections with *Bacillus anthracis*

- **Skin anthrax:** The most common localizations are the hands, forearms, and face. After an incubation time of 2-5 days, a painless but itchy papule (*pustula maligna*) with an edematous edge appears at the infection site. After a short period, necrosis of the center with black coloration occurs. At the edge, serous cysts emerge. Without treatment, the infection is fatal in about 20% of the cases due to toxemia and bacteremia. After a survived infection, humoral immunity of unknown duration occurs.

- **Pulmonary anthrax:** After initial symptoms, massive edemas appear in the neck and thorax regions. The patients suffer from dyspnea and fever. Pulmonary anthrax is the most dangerous clinical form and is notably difficult to treat.

- **Intestinal anthrax:** By ingestion of anthrax spores, severe enteritis can occur. This ends lethally in most cases due to the toxemia.

All 3 forms of anthrax can lead to sepsis with lethal outcome.

**Laboratory diagnosis of anthrax**

Depending on the kind of infection, the content of serous cysts in skin anthrax, sputum in pulmonary anthrax, and *feces* in intestinal anthrax can be used as test material. Tests must only take place in S3 laboratories. Simple culture mediums with aerobic conditions are being used.
The colony morphology of Bacillus anthrax taking the form of harsh colonies with curly branches at the edges, which are also called ‘head of Medusa’ is typical. In microscopy, the bacteria appear as box-like rod cells with central endospores which are arranged in long chains (‘bamboo stick’).

Suspicion of illness, disease, and death by anthrax are subject to mandatory reporting. Moreover, reporting is mandatory in case of the detection of *Bacillus anthrax*.

**Therapy for anthrax infections**

Upon suspicion of anthrax, **high-dose antibiotic therapy with penicillin G, ciprofloxacin, or tetracyclines** should be started. In bioterrorist assaults, ciprofloxacin is preferred because resistances can be transferred from *B. cereus* to anthrax in the laboratory.

New antibiotics found to be effective against anthrax include levofloxacin, daptomycin, gatifloxacin, and dalbavancin. Newer therapies like peptides, bacteriophages enzymes, monoclonal antibodies, etc. are being evaluated.

Farm animals should be vaccinated against anthrax. Exposed persons can profit from new **vaccines** that are based on the blockade of the protective antigen. Carcasses have to be burned.

**Bacillus cereus**
In humans, Bacillus cereus causes invasive local infections and self-limiting food intoxications.

**Food intoxications from *Bacillus cereus***

*B. cereus* produces 3 toxins: the **emetic toxin** and 2 enterotoxins. While the enterotoxins are heat-labile and cannot be activated proteolytically, the emetic toxin is heat-stable and cannot be inactivated by proteolysis.

The enterotoxins are often absorbed from **cooked rice or meat**. About 1–6 hours after the intake of contaminated food, vomiting occurs. After 10–12 hours, stomachache and watery diarrhea with tenesmus and vomiting appear. The symptoms last for about 24 hours. The intoxication is self-limiting. Two forms of intestinal illness, diarrheal and emetic, have been described and are attributed to different toxins. Treatment is symptomatic and most patients recover within 24 hours after symptom onset.

**Local infections with *Bacillus cereus***

Since *B. cereus* produces numerous tissue-destructing virulence factors, the wound infection leads to **gas gangrene-like myonecrosis** that is only of superficial character. Therapy can be surgical and/or antibiotic. In contrast to *B. anthracis*, *B. cereus* often forms β-lactamases, therefore antibiosis with *vancomycin* or *clindamycin* is preferable.

**Laboratory diagnosis of *Bacillus cereus***

Feces and food can be investigated. The most important difference from *B. anthracis* is the motility of *B. cereus*. 
**Propionibacteria**

![Image: Propionibacterium acnes. By CDC/Bobby Strong, License: Public Domain]

**Attributes of Propionibacteria**

*Propionibacteria* are anaerobic, oxygen-tolerant, non-spore-forming, immobile, gram-positive rod cells. They stand out by their slow growth and high requirements for culture. They owe their name to propionic acid fermentation where especially carbohydrates like glucose and fructose serve as a substrate for the main product—propionic acid. On blood agar, β-hemolysis can be detected. Some propionibacteria have probiotic properties and are being used in dairy probiotic products.

**Clinical picture of pathogenic Propionibacteria**

Non-pathogenic propionibacteria mostly live on the skin as normal flora and commensals. *Propionibacterium acnes* can be found primarily in the sebum of hair follicles where it can cause inflammation by induction of chemokine and cytokine production. The leukocytes attracted by this lead to the formation of pus-filled pustules.

In the hair follicles, optimal conditions for proliferation are provided because there is an anaerobic milieu and the propionibacteria have a lipase available which they can use to harvest the fat contained in sebum for energy production.

Furthermore, propionibacteria have been described as the cause of endocarditis and spondylodiscitis. *P. acnes* can additionally lead to the development of circulating immune complexes which can be deposited on bones and joints. This clinical picture is especially relevant to young adults and is called the SAPHO syndrome which stands for synovitis, acne, pustulosis, hyperostosis, and osteitis.
Actinomycetes

Image: Actinomyces naeslundii. By CDC/Dr. Lucille Georg, License: Public Domain

Attributes and detection of actinomycetes

For humans, the **anaerobic, fermentative actinomycetes** are of particular importance. They are characterized by a positive Gram stain and their elongated, branched form. They are **immobile** and do **not form spores**. Actinomycetes can often be found as pathogens or commensals on the mucosa of endotherms and metabolize carbohydrates and organic acids there.
The radial-filamentous branches of actinomycetes are visible under the microscope. They are called ray fungus and owe their name to their appearance (Greek aktis for ray and mykes for fungus). Moreover, macroscopically, a radial structure shows in the druses formed by the actinomycetes. Druses are nodular conglomerates surrounded by a wall of lymphocytes where the bacteria hyphae spread radially on the inner side.

Culture is via Columbia agar but this usually takes several days or weeks. The colonies appear yellowish and dry with small streaks.

### Actinomycosis

The main cause of the actinomycosis is *Actinomyces israelii*. This bacterium belongs to the normal oral flora but can intrude into deeper layers in mucosal injuries and lead to the formation of fistulas and granulation tissue. Via these fistulas, actinomycetes can reach the blood circulation. Actinomycosis may mimic malignancy in various anatomical sites.
In 95% of the cases, only cervicofacial actinomycosis occurs where inviscid pus with sulfur-yellow druses drains from the fistulas. Hormonal factors are likely to play a role in the emergence of actinomycosis because men are distinctly more often affected, in contrast to children who are never affected.

Therapy should be a combination of surgery and antibiosis. Because of the β-lactamase-forming collateral flora, a combination of amoxicillin and clavulanic acid has been established as standard therapy.

Other clinical features of Actinomycetes

An important ophthalmologic differential diagnosis portrays the lacrimal canaliculitis caused by A. israelii.

Furthermore, actinomycetes can appear sensitizing and, by the formation of immune complexes, can cause a 3rd-grade allergy. In this context, they are a typical cause of the so-called farmer’s lung, a form of exogenous allergic alveolitis.

Clostridia
Clostridia are **obligately anaerobic, spore-forming rod cells**. This species is ubiquitous in nature and can often be found in the intestinal tract of humans. The endospores formed under unfavorable living conditions are resistant to heat and exsiccosis and are able to survive in an aerobic milieu.

**Clostridium perfringens**

This bacterium is the main (but not the only) cause of gas gangrene, also called **clostridial myonecrosis**. Especially in times of war, this infection frequently occurs because *C. perfringens* spores ubiquitously exist in the ground and can get into wounds from there.

**Attributes of Clostridium perfringens**

*C. perfringens* is a box-shaped, gram-positive, immobile rod cell without granulocytes. It is frequently referred to as having a clay brick-shape. Additionally, in most isolates, a polysaccharide capsule can be found. Due to its toxins, *C. perfringens* is divided into the types A–E.

**Pathophysiological mechanism of gas gangrene**

*C. perfringens* type A strains are the predominant cause of gas gangrene. The **alpha-toxin** is a **lecithinase** that destroys cell membranes by splitting lecithin.
A decrease of the redox potential of the infected tissue is **required** for the **sprouting of Clostridia spores**. For example, this is the case in circulatory disorders or necroses. This is why the cell destruction and emergence of edemas by further occurring toxins from *C. perfringens* can be seen as a vicious cycle because it facilitates its own growth. Due to fermentation, the eponymous gas is formed.

**Clinical picture of gas gangrene**

After an incubation time of about 2 days, a swelling and brownish-livid discoloration of the extremely painful infection area occurs. Crepitations due to the CO$_2$ gasification can be detected by palpation. A serous and, due to volatile fatty acids, foul-smelling fluid drains from the wounds. The muscles undergo necrotic changes. Toxin-induced shock in gas gangrene can lead to death within hours.

**Diagnosis of clostridial myonecrosis**

The diagnosis has to be made **clinically** based on symptoms and **radiographs**. In the radiograph, a typical **pinnation of the affected muscles** shows. For microscopic examination, wound secretions and muscle excisions are suitable.

The detection of the alpha-toxin takes place with the help of **Nagler's test**. In this test, culture Clostridia is made on agar with egg yolk where the formation of lecithinase can be seen as a haze around the bacteria colony.

Blood-containing agar or nutritious bouillons like **liver bouillon** could also be used. *C. perfringens* grow relatively fast at 45°C (113°F).
Suspected clostridial infection and gastroenteritis caused by *C. perfringens* are subject to mandatory reporting.

Therapy for infections with *Clostridium perfringens*

**Surgical wound revision** with eventual amputation of the infected extremity is essential because antibiotics often does not reach the necrotic tissue. A combination of **penicillin G** and **metronidazole** is often used because in most cases, it is a combined infection with other anaerobic bacteria.

Furthermore, **hyperbaric oxygenation** in hyperbaric oxygen chambers is the standard solution for gas gangrene infections to eliminate these obligately anaerobic bacteria.

**Clostridium tetani**
Attributes of *Clostridium tetani*

The structure of *C. tetani* corresponds to those of other Clostridia. The **tennis racket** shape is characteristic.

**Clinical picture of tetanus**

Newborns are most likely to be affected by this bacterium, where *C. tetani* leads to the so-called disease of the 8th day (or **tetanus neonatorum**). This is an infection of the umbilicus since this provides an optimal anaerobic milieu.

At the portal of entry, *C. tetani* proliferates and forms **tetanospasmin** which is released by autolysis and retrogradely spreads down the nerves up to the anterior horn cells in the spinal cord. There, it proteolytically divides **synaptobrevins**. The synaptobrevins are involved in the release of GABA into the synaptic cleft. So a neutralization of the inhibitory effect takes place.

Due to the excessive activity of the spinal motoneurons, spasticity with strychnine-like, tonic-clonic convulsions occurs.

The first symptoms appear after an incubation time of a few days to 3 weeks. The tension of the masticatory muscles (trismus) and a permanent contraction of the facial muscles (**risus sardonicus**) are typical.
Laboratory diagnosis of *Clostridium tetani*

Since culture often fails, toxin detection is important. For this detection, an animal experiment is necessary. In most cases, mice or guinea pigs are vaccinated with different amounts of the patient’s serum. The experiment is rated as positive when the animals die in seal position, which means with catalepsy of the rear legs.

Control animals are simultaneously vaccinated with the patient’s serum and antitoxin. These animals should survive for a positive mice protection experiment.

**Therapy for tetanus infections**

In addition to the administration of anti-tetanus toxin antibodies, the therapy consists of anticonvulsant drugs and artificial respiration to prevent the paralysis of the respiratory muscles.

Due to the high lethality of tetanus, active vaccination with formalinized toxins (toxoids) is of great importance.

**Atypical mycobacteria**

The atypical mycobacteria which are potentially pathogenic for humans are often summarized as MOTT (mycobacteria other than tuberculosis).

**Attributes of atypical mycobacteria**

The structure of MOTT does not differ from those of other mycobacteria. They also have an acid-proof capsule and are extremely resistant to environmental influences, heat, and many disinfectants. They ubiquitously occur in the ground and in water samples, some of them only in particular areas like *Mycobacteria ulcerans* for example.
which is only prevalent in Africa.

MOTT is classified into 4 groups. Slow-growing mycobacteria that only produce a pigment during light exposure, belong to group I. The so-called scotochromogenic mycobacteria of group II also produce pigment in the dark. Slowly growing MOTT that does not produce pigment belongs to group III, whereas all fast-growing ones belong to group IV.

Diseases due to atypical mycobacteria

As an opportunistic pathogen, the atypical mycobacteria especially cause infections in immunosuppressive patients.

Pulmonary infections that do not differ clinically from tuberculosis frequently occur. Further, granulomatous infections of the skin and lymphangitis of the cervical lymph nodes are frequent. *M. scrofulaceum* causes ‘scrofula”—a granulomatous cervical adenitis which is usually seen in children.

![Image](https://example.com/lady-windermere-syndrome.png)

*M. marinum* is the cause of the so-called swimming pool granuloma. After bathing in contaminated water and an incubation time of 2–3 weeks, granulomas that ulcerate after some time appear at entry portals like the elbows and knees. *M. ulcerans* causes ‘Buruli ulcer’, primarily affecting the lower extremities.

Laboratory diagnosis of atypical mycobacteria

As with *M. tuberculosis*, culture usually succeeds in Löwenstein-Jensen agar. Fast-growing mycobacteria already exhibit colonies after 3–4 days, other ones only after an incubation period of several days to weeks. Differentiation of different MOTT organisms especially depends on the growth rate, culture morphology, and pigment formation. The differentiation is complemented with restriction fragment length polymorphism.
In contrast to tuberculosis, it is not obligatory to report the disease.

**Therapy for atypical mycobacteria**

Most MOTT are highly resistant to tuberculostatics which is why the therapy could be **very difficult**. In infection with *M. avium* or *M. intracellulare*, a combination of **clarithromycin, ethambutol, and rifabutin** is recommended. In other atypical mycobacteriosis, up to 6 antibiotics may be necessary.

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