Corynebacterium and Rhodococcus

)

HISTORY

Bretonneau 1821
Clinical characterisation of diphtheria

Klebs 1883
Detecting the bacterium

- Loeffler 1884

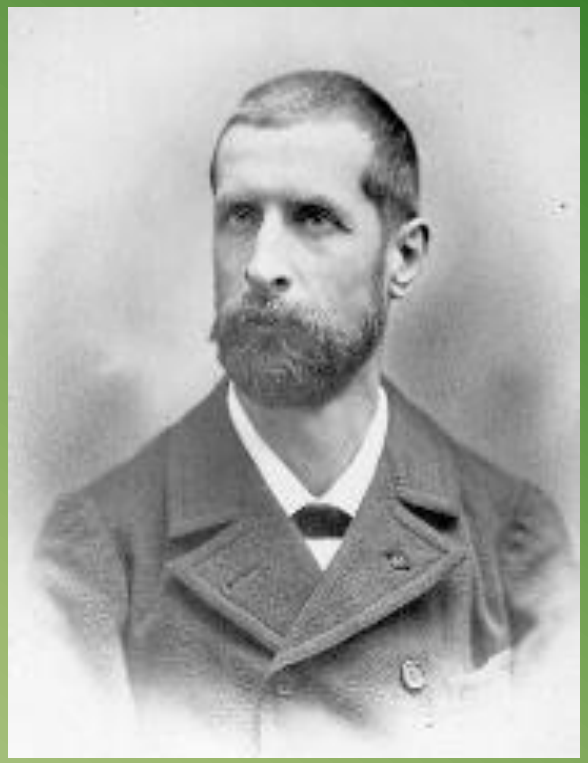
  Isolating the bacterium
- Media

Loeffler's coagulated serum-agar

Roux and Yersin 1888

Discovering the diphtheria toxin

Pierre Paul Émile Roux
1853 – 1933



Alexandre Yersin
1863 – 1943

Behring and Kitasato

1890-1892

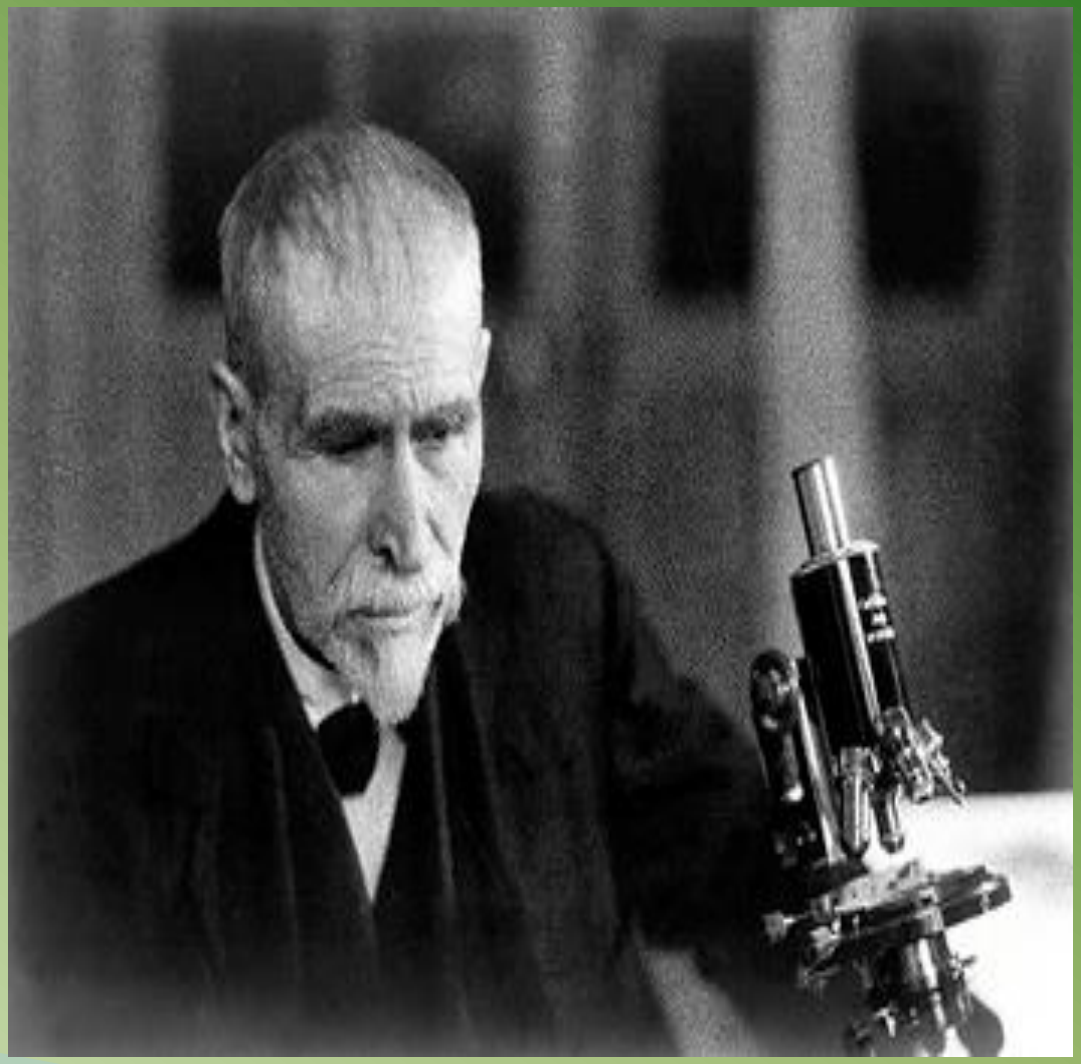
- Discovering the diphtheria antitoxin
- Antitoxic immunity

Emil Adolph von Behring
1854 – 1917

Shibasaburo Kitasato
1852 – 1931

Roux 1894

Treatment with antitoxin



Emil von Behring
1901
Nobel prize

Behring 1913
Active immunisation I. with toxin-antitoxin mix

Schick 1913

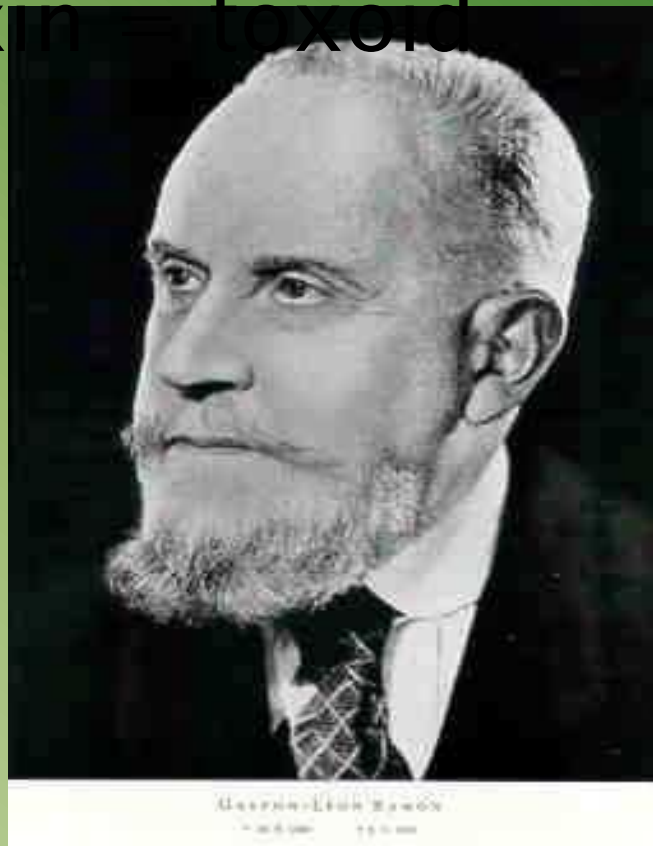
Skin test

Ramon 1923

Active immunisation

II.

Anatoxin – toxoid



INTRODUCTION

Previously,the genus *Corynebacterium* contained bacteria,which had the one Common characteristic that they showed typical V forms.such bacteria are Characterised as coryneforms.however, in other characteristics the member of The traditional *Corynebacterium* genus very greatly from one another. More recent systematics have defined *Corynebacteria* as coryneform, Facultatively anaerobic,catalase positive,nonmotile rods,while former members Not fulfilling these criteria have been placed in other genera.

EXAMPLES

Arcanobacterium	catalase nagative
Rodococcus	obligatory aerobic
Oerskovia	form filaments
Eubacterium	obligatory anaerobic

TAXONOMY

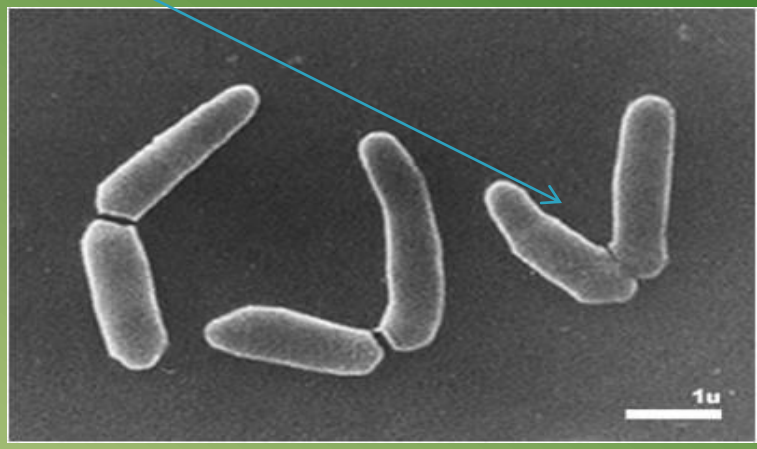
Kingdom: Bacteria
Phylum: Actinobacteria
Order: Actinomycetales
Suborder: Corynebacterineae
Family: Corynebacteriaceae
Genus: *Corynebacterium*

Lehmann & Neumann 1896

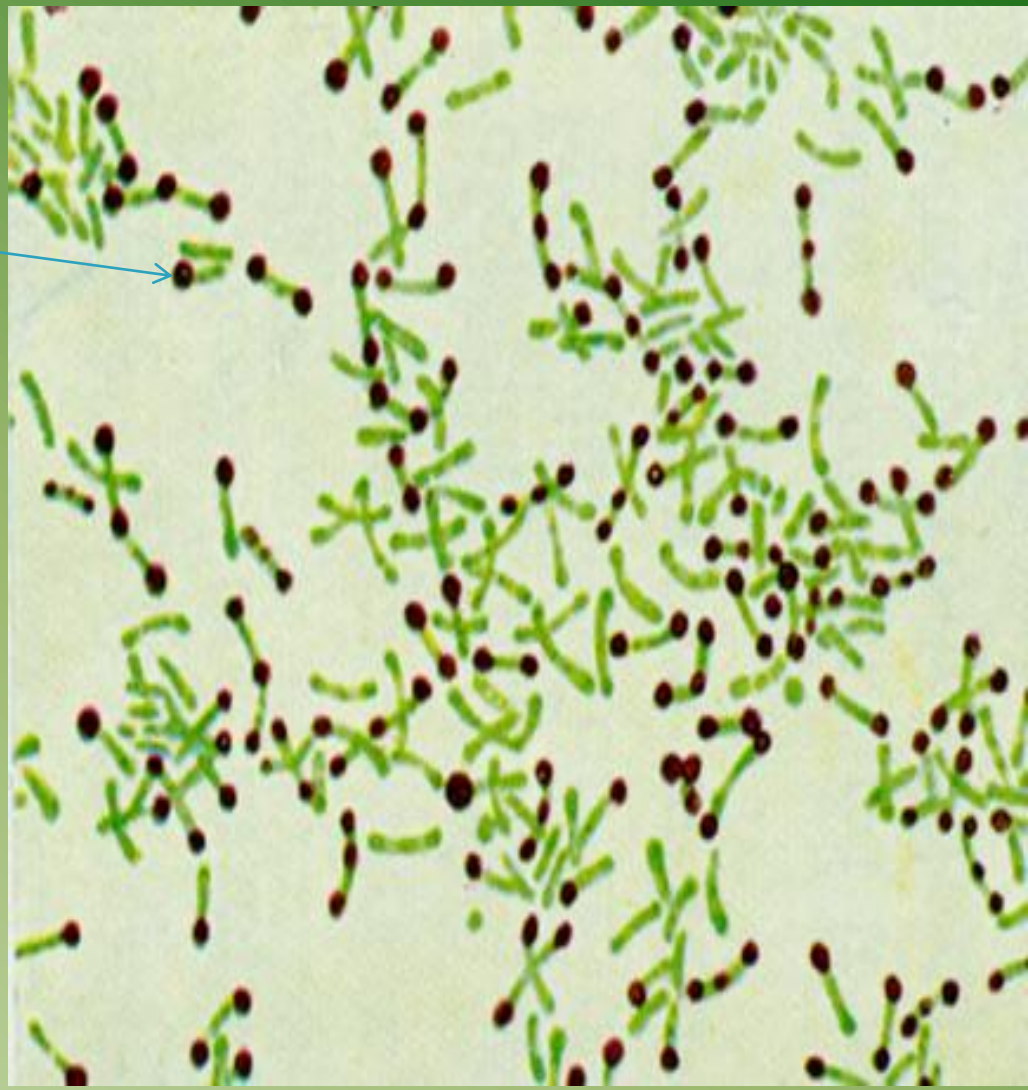
GENERAL CHARACTERITCS

- *Corynebacterium sp*. contain Lipid-rich cell wall – (mycolic acids with 22 to 36 carbon atoms),meso-diaminopimelic acid, arabino-galactan polymers.
- 16S r-RNA gene sequences indicate a close relationship of *Corynebacterium* to *Mycobacterium*, *Nocardia*, and *Rhodococcus.*
- *Corynebacterium sp*. have high DNA G+C content.
- *Corynebacterium* composed of 59 species of which 36 are medically relevant

- Small, Gram positive
- Arranged as single cells, pairs, V, L, and Y forms, palisades, and "Chinese letters"
- Irregular swellings at one end -(club shaped).
- Non acid fast
- Non-motile
- Non- sporeforming
- Aerobic or facultative anaerobic
- Catalase positive, oxidase negative



- Contains metachromatic granules (Babes-Ernst granules)
- Loeffler‘s agar slant contains serum & egg that enhance the formation of metachromatic granules (polymerized polyphosphoric acid) in *C. diphtheriae*



SNAPPING DIVISION

- Cell wall contains 2 layers of peptidoglycans

inner wall grows inward to divide

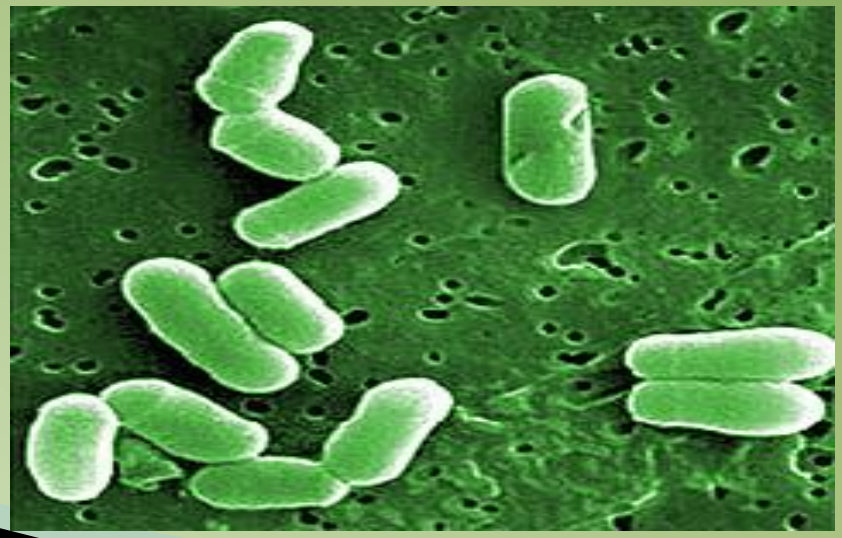
2 new cells formed & as it thickens

put tension on outer wall

SNAPPING DIVISION

- Outer wall apart except at one end

which holds 2 cells together and cells appear in V & L shape



Clinically important Gram positive bacilli

Spore forming

1. *Bacillus sp*
2. *Clostridium sp*

Non spore forming

1. *Corynebacterium sp*
2. *Listeria sp*
3. *Lactobacillus sp*

Bacilli with branching filaments

1. *Actinomyces sp*
2. *Nocardia sp*

LIPOPHILICITY

- Most species-non-lipophilic, some- lipophilic.
  - Nonlipophilic

fermentative non-fermentative:

C. diphtheriae	*C. auris*
C. xerosis and *C. striatum*	*C. afermentans subsp. afermentans*
C. amycolatum	*C.pseudodiphtheriticum*
C. argentoratense	*C. propinquum*
C. glucuronolyticum	

Lipophilic

- *C. jeikeium*
- *C. urealyticum*
- *C. afermentans subsp. lipophilum*
- *C. accolens*
- *C. bovis*

Species

- *Corynebacterium diphtheriae*, the cause of diphtheria in humans.

Nondiphtheriae Corynebacteria (diphtheroids)

- *Corynebacterium aquaticum*
- *Corynebacterium haemolyticum*
- *Corynebacterium glutamicum*
- *Corynebacterium minutissimum*
- *Corynebacterium amycolatum*
- *Corynebacterium striatum*
- *Corynebacterium bovis*
- *Corynebacterium ovis (C.pseudotuberculosis)*
- *Corynebacterium hofmannii [Corynebacterium psudodipthericum]*

- *Corynebacterium urealyticum*
- *Corynebacterium ulcerans*
- *Corynebacterium xerosis*

- *C.renale* group:-
- *Corynebacterium renale*
- *Corynebacterium pilosum*
- *Corynebacterium cystitidis*

HOSTS AND DISEASES

PATHOGEN	HOST	DISEASE
C.bovis	cattle	Subclinical mastitis
C.kutscheri	Laboratory rodents	Superficial abcesses,caseopurulant foci in liver ,lung and lymph nodes
C.pseudotuberculosis **1.Non nitrate reducing biotype** **2.Nitrate reducing biotype**	Sheep ,goats Horse cattle	Caseous lymphadenitis Ulcerative lymphangitis,abcesses
C.renale group *1.C.renale* *2. C.pilosum* *3. C.cystitidis*	Cattle Sheep and goats Cattle Cattle	Cystitis,pyelonephritis **Ulcerative balanoposthitis (pizzle rot)** Cystitis,pyelonephritis Severe Cystitis,rare pyelonephritis
C. ulcerans	cattle	mastitis

Virulence Factors in Corynebacterium Species

C. diphtheriae	Diphtheria exotoxin
C. jeikeium	Antibiotic resistance
C. urealyticum	Antibiotic resistance; urease production
C. pseudotuberculosis	Diphtheria exotoxin; phospholipase D
C. ulcerans	Diphtheria exotoxin; phospholipase D

Corynebacterium: Modes of Infection

C. diphtheriae	**respiratory droplets**
C. ulcerans	**raw milk**
C. pseudotuberculosis	**close animal contact or drinking raw milk**
C. jeikeium, ***C. amycolatum***	**Nosocomial infection -wounds**
C. urealyticum	**urethral commensal**

Important Pathogenic Corynebacterial Species

- *Corynebacterium diphtheria*
- *Corynebacterium jeikeium*
- *Corynebacterium urealyticum*

C.renale group:-

- *Corynebacterium renale*
- *Corynebacterium pilosum*
- *Corynebacterium cystitidis*

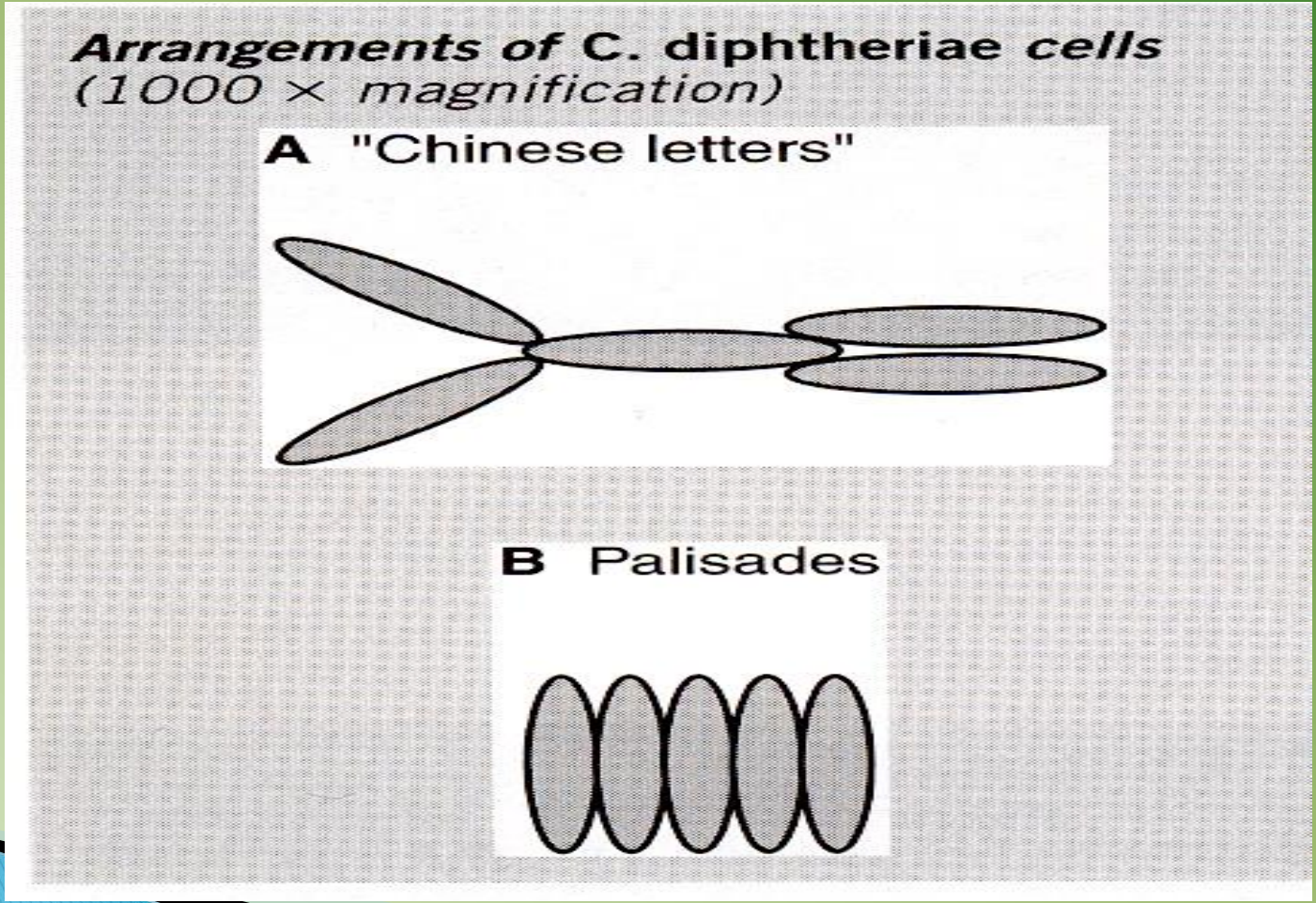
Corynebacterium diphtheriae

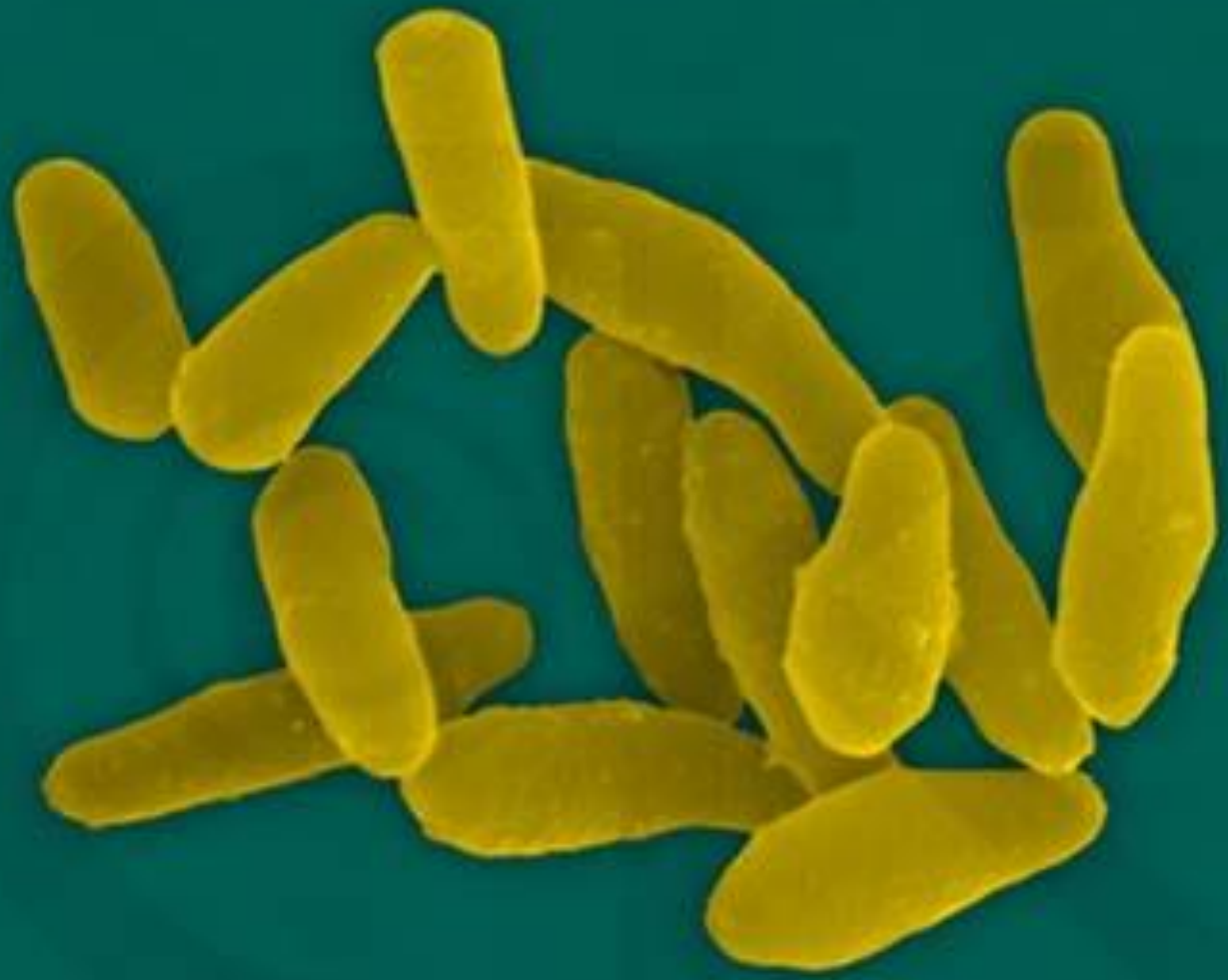
- pathogenic bacterium that causes diphtheria.
- Also known as the **Klebs-Löffler bacillus**, because it was discovered in 1884 by Klebs and Löffler.
- Binomial name *Corynebacterium diphtheriae* was given by **Kruse, 1886.**
- Nonmotile, noncapsulated, Non spore forming club-shaped, Gram-positive bacillus.

Corynebacterium diphtheriae

- Grows well under strict aerobic condition.
- Resistant in environment due-
  lipid in cell wall like mesodiaminopimelinic acid ,arabinogalactane and mycolic acid.
- Very much variable in size.
- Lysogenic bacteriophage encodes for potent exotoxin in virulent strains.

Arrangement of *C.diphtheriae*





Corynebacterium diphtheriae. Rod,club-shaped Bacterium

DIFFERENCE BETWEEN DIPTHERIA & DIPHTHEROID

S. N	DIPTHERIA	DIPTHEROID
1	LONG AND STAIN UNEVENLY WITH METYLENE BLUE	SMALL AND STAIN UNIFORMALLY WITH METHYLENE BLUE
2	METACHROMATIC GRANULES PERSENT	ABSENT
3	WEAKLY GRAM POSITIVE	STRONGLY GRAM POSITIVE
4	RESISTANT TO ACTION OF NaF	SUSCEPTIBLE
5	PURELY AEROBIC	FACULTATIVE ANAEROBE
6	HIGHLY PATHOGENIC	LESS OR NON-PATHOGENIC

Epidemiology of Diphtheria

DISEASE/BACTERIAL FACTORS

Diphtheria exotoxin disrupts peptide formation in ribosomes

Phospholipase D increases vascular permeability and promotes spread of organism

TRANSMISSION

Person to person by inhalation or skin contact

Asymptomatic carriage maintains bacteria in population

WHO IS AT RISK?

Unvaccinated people

People in crowded, poor urban areas

Children

GEOGRAPHY/SEASON

Worldwide, where vaccination programs are not in place

No seasonal incidence

MODES OF CONTROL

Early use of diphtheria antitoxin to neutralize exotoxin

Penicillin or erythromycin effective for infected patients and asymptomatic carriers

Active immunization with diphtheria toxoid during childhood (DPT vaccine), then booster shots every 10 years for life

Antimicrobial prophylaxis for close contacts of patients with diphtheria

Pathogenicity

The pathogenicity of *Corynebacterium diphtheriae* includes two distinct phenomena:

1. **Invasion of the local tissues of the throat**, which requires colonization and subsequent bacterial proliferation. Nothing is known about the adherence mechanisms of this pathogen.

2. **Toxigenesis**: bacterial production of the diphtheria toxin. The virulence of *C. diphtheriae* cannot be attributed to toxigenicity alone, since a distinct invasive phase apparently precedes toxigenesis. However, it cannot be ruled out that the diphtheria toxin plays a role in the colonization process due to its short-range effects at the colonization site.

Three strains of Corynebacterium diphtheriae are recognized, gravis, intermedius and mitis.
They are listed here by falling order of the severity of the disease that they produce in humans.
All strains produce the identical toxin and are capable of colonizing the throat.
The differences in virulence between the three strains can be explained by their differing abilities to produce the toxin in rate and quantity, and by their differing growth rates.

The gravis strain has a generation time (in vitro) of 60 minutes; the intermedius strain has a generation time of about 100 minutes; and the mitis stain has a generation time of about 180 minutes.

The faster growing strains typically produce a larger colony on most growth media. In the throat (in vivo), a faster growth rate may allow the organism to deplete the local iron supply more rapidly in the invaded tissues, thereby allowing earlier or greater production of the diphtheria toxin.

Also, if the kinetics of toxin production follow the kinetics of bacterial growth, the faster growing variety would achieve an effective level of toxin before the slow growing varieties.

Toxigenicity

Two factors have great influence on the ability of *Corynebacterium diphtheriae* to produce the diphtheria toxin:
(1)low extracellular concentrations of iron.
(2) The presence of a lysogenic prophage in the bacterial chromosome. The gene for toxin production occurs on the chromosome of the prophage, but a bacterial repressor protein controls the expression of this gene.

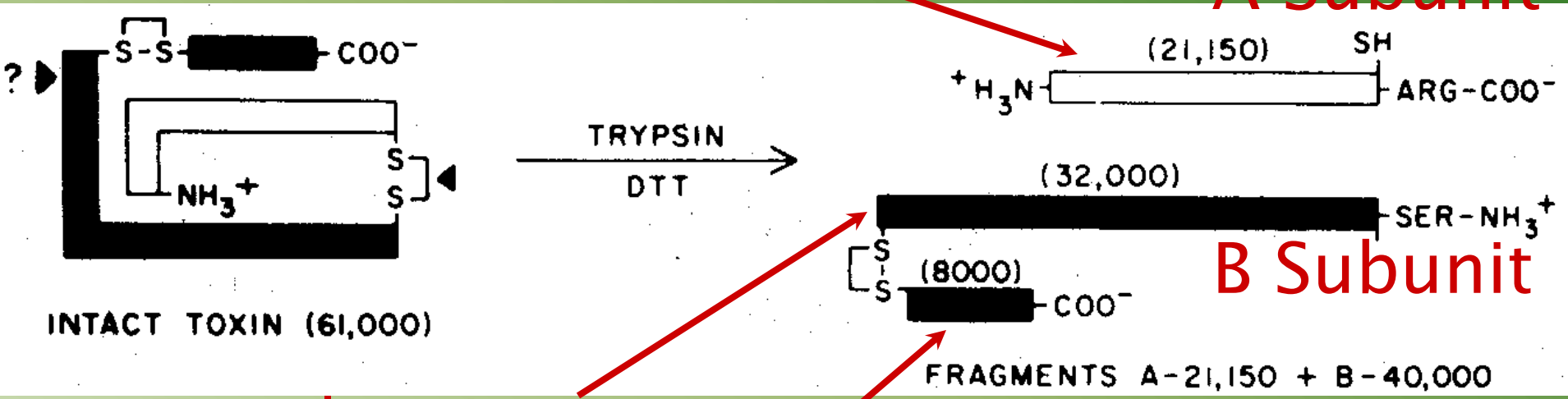
The repressor is activated by iron, and it is in this way that iron influences toxin production. High yields of toxin are synthesized only by lysogenic bacteria under conditions of iron deficiency.

The role of iron.

In artificial culture the most important factor controlling yield of the toxin is the concentration of inorganic iron (Fe++ or Fe+++) present in the culture medium.

Toxin is synthesized in high yield only after the exogenous supply of iron has become exhausted (This has practical importance for the industrial production of toxin to make toxoid. Under the appropriate conditions of iron starvation, *C. diphtheriae* will synthesize diphtheria toxin as 5% of its total protein!). Presumably, this phenomenon takes place in vivo as well.

Molecular Structure of Diphtheria Toxin



Catalytic Region

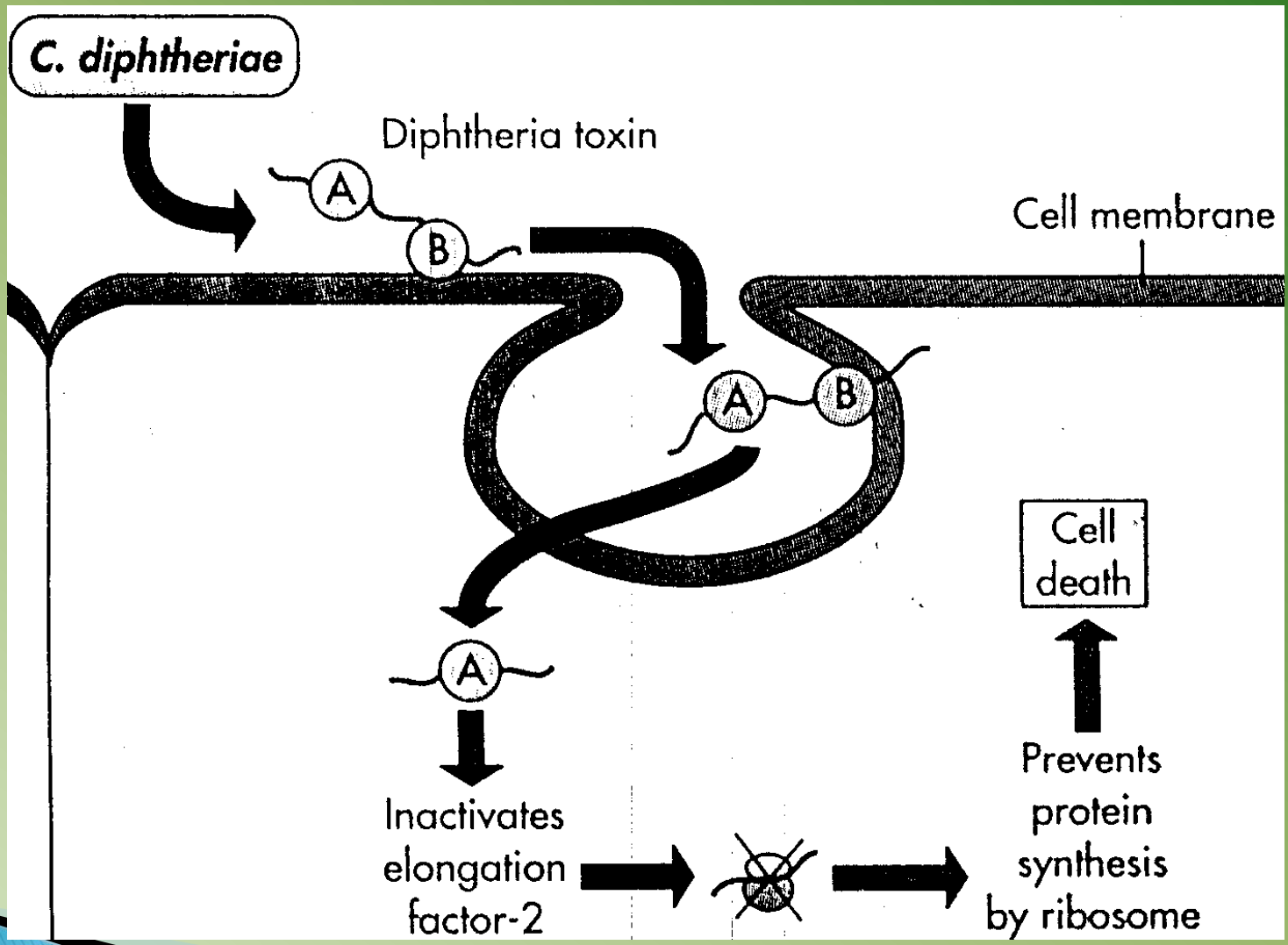
A Subunit

B Subunit

Translocation Region

Receptor-Binding Region

Mechanism of Action of Diphtheria Toxin: Inhibition of Protein Synthesis



C. diphtheriae

Clinical Diseases

Respiratory diphtheria

Incubation period: 2-6 days.

Inflammation begins in the respiratory tract, causing sore throat, exudative pharyngitis that develops into pseudomembrane, and low grade fever. Prostration and dyspnea soon follow, which may lead to suffocation if not promptly relieved by intubation or tracheotomy.

Damage to the heart causes irregular cardiac rhythm.

Visual disturbance, difficulty in swallowing and paralysis of the arms and legs also occur but usually resolve spontaneously.

Death may be due to asphyxia or heart failure.

Cutaneous diphtheria: mild (papule → ulcer with grayish membrane) with little toxigenic effects. Stimulates antitoxin production.

DIAGNOSIS

Lab diagnosis

Samples collected:

- Nasal swabs, Exudate from the lesion, pseudomembrane .
- Exudate from the lesion should be inoculated onto blood agar and selective media: cysteine-tellurite agar; serum tellurite agar; Loeffler's slant .

Microscopy

- Methylene blue stain shows metachromatic granules
- Gram stain shows Gram-positive pleomorphic rods arranged in perpendicular, parallel, and pallisade formations

Cultural characteristics

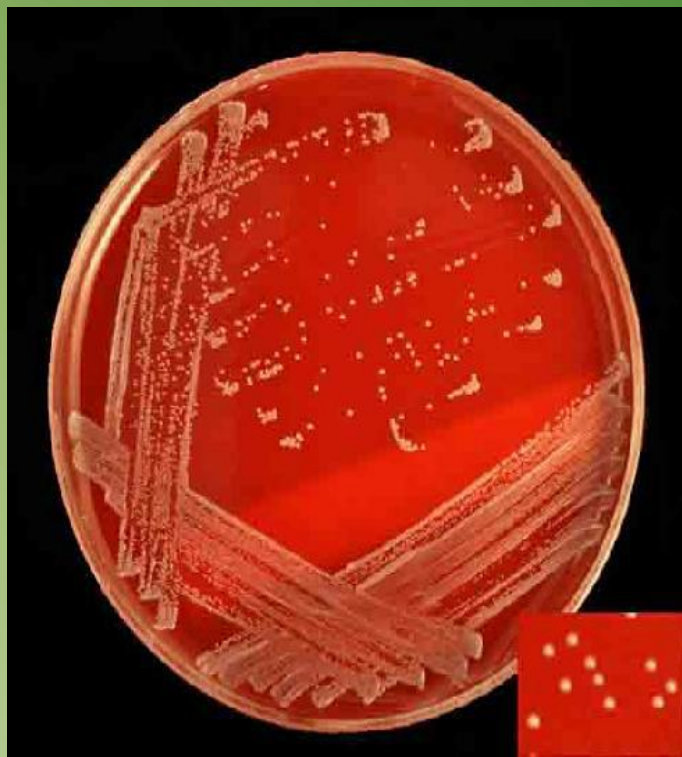
- 4 morphological types of *C. diphtheriae* are found on tellurite containing media:
  - Gravis – large, gray colonies
  - Intermedius – small, dull gray to black.
  - Mitis – black colonies with a gray periphery
  - Belfanti- larger white opaque colonies
- Biotype *intermedius* rarely occurs in clinical infection and *belfanti* rarely contains the tox+ gene
  - Incubation -35-37^0 C for 24 hours.
  - They prefer a pH of 7.8-8.0 for good growth.
  - They require access to oxygen for growth.

Laboratory Detection of *Corynebacterium diphtheriae*

Culturalcharacterictics:

On Blood Agar

- Translucent, gray, or white non-hemolytic colonies on blood agar up to 2 mm in size after 18-24 hours in 5% CO_2 at 37°C
- No growth on enteric agar (MacConkey)

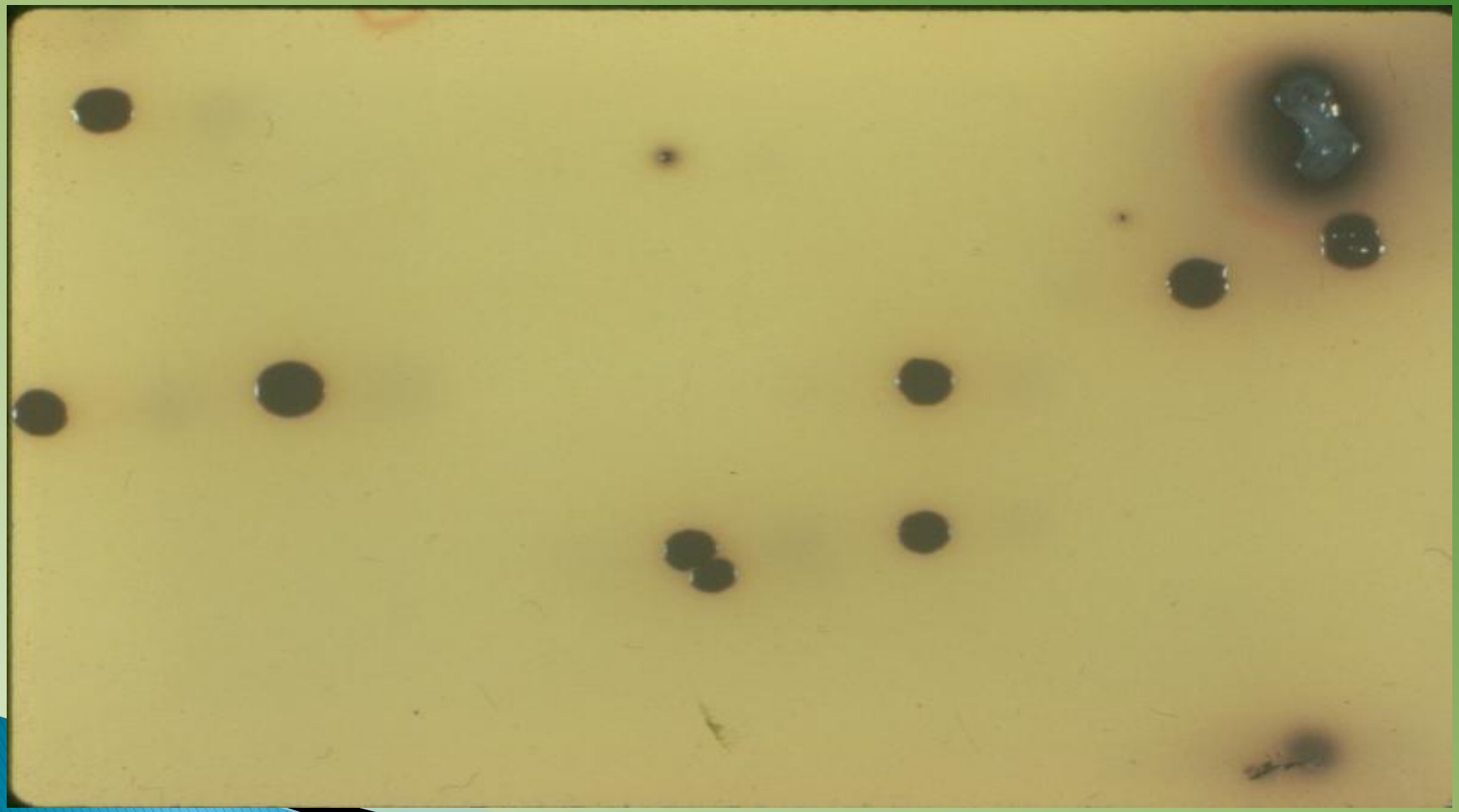


- On Loeffler's serum slant or Pai's slant (heat inspissated serum and whole egg medium): Deep blue or red metachromatic granules (accumulated inorganic polyphosphates) by methylene blue stain after incubation

- On Tinsdale agar :Black colonies (tellurite reductase) are surrounded by a brown halo (cystinase)

- Tinsdale cystine-tellurite blood agar is selective and differential by containing potassium tellurite. *Staphylococcus* and *Proteus* can produce black colonies, but the colonies lack a brown halo, and the colonies demonstrate gram-positive cocci (*Staphylococcus*) or gram-negative rods (*Proteus*) by Gram's stain.

Tinsdale cystine-tellurite blood agar



- Biochemistry
  - Catalase +
  - Oxidase -
  - Nitrate+

- ***In vivo* test:**

  Shick test

  Romer -test

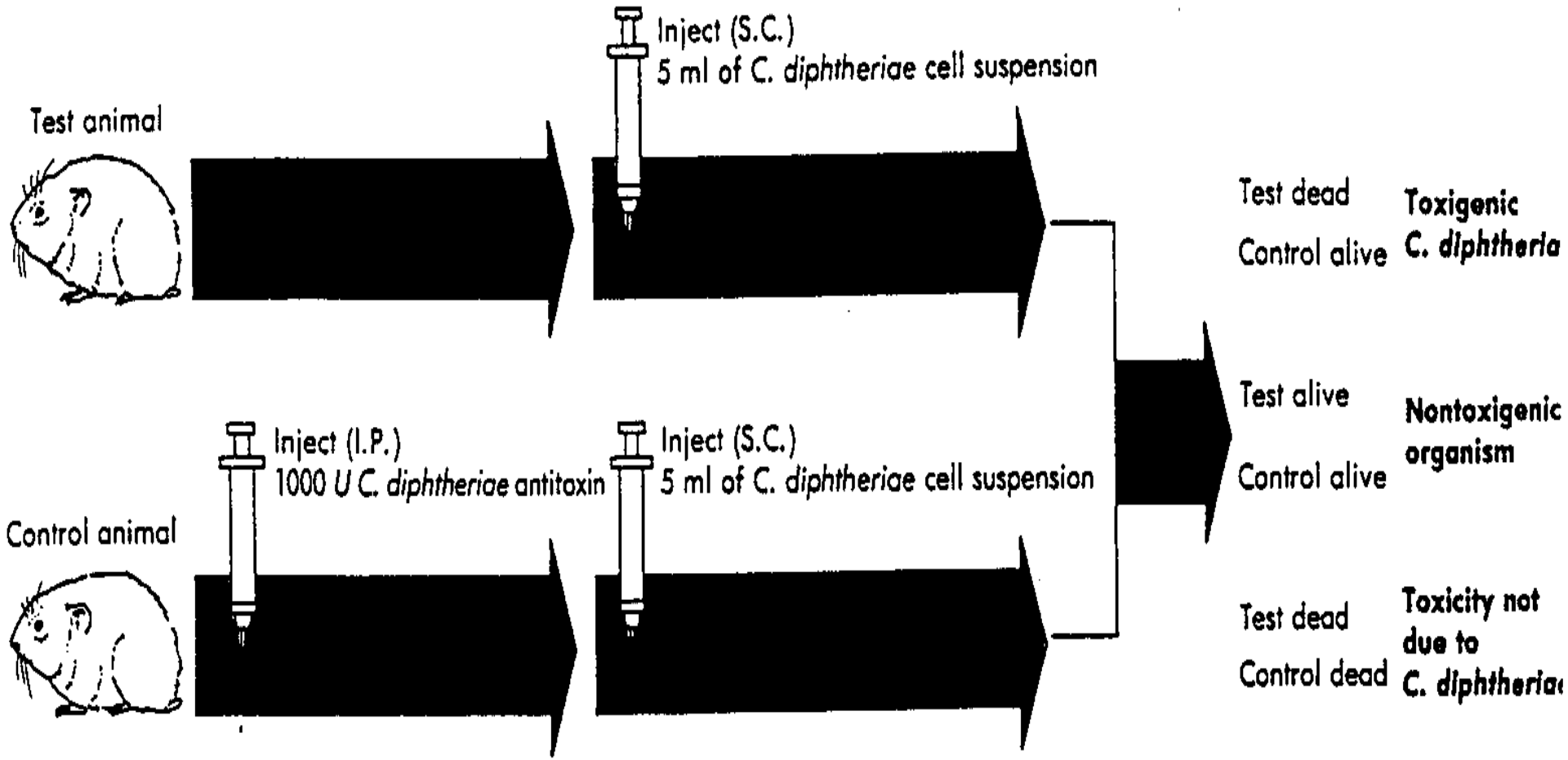
- ***In vitro* test:**

  Elek test (immunodiffusion)

- Detection of toxin gene by PCR

- Schick Test: introduced by Schick in 1913.
- it enables us to distinguish between individuals who are susceptible and those who are resistant (i.e., immune) to diphtheria toxin and to test for sensitivity to toxoid.
-
  - Intracutaneous injection of 1/50 MLD (minimal lethal dose) (for a guinea pig) of diphtheria toxin produces a strong, but tolerable, reaction in individuals having no antitoxin.
  - Individuals having 1/30 unit or more of antitoxin per ml of blood neutralize this test dose and show no reaction. Such individuals are also usually resistant to diphtheria.

IN VIVO DETECTION OF DIPHTHERIA EXOTOXIN (Römer – test)

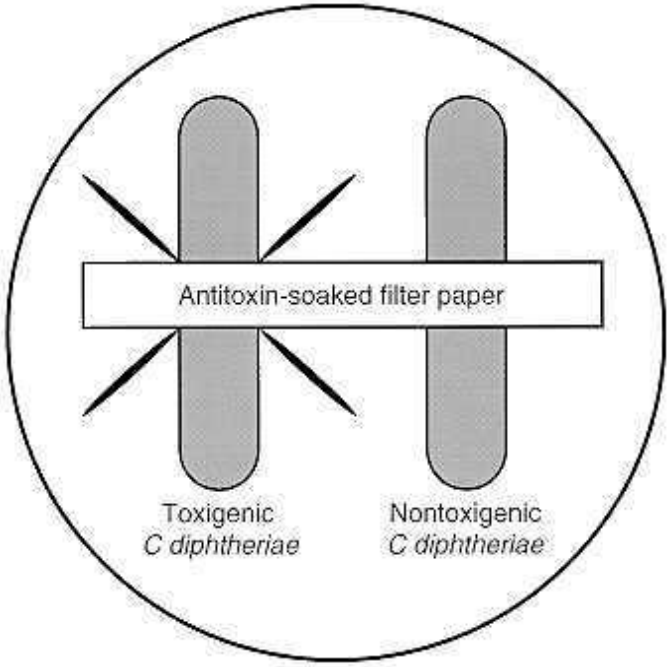


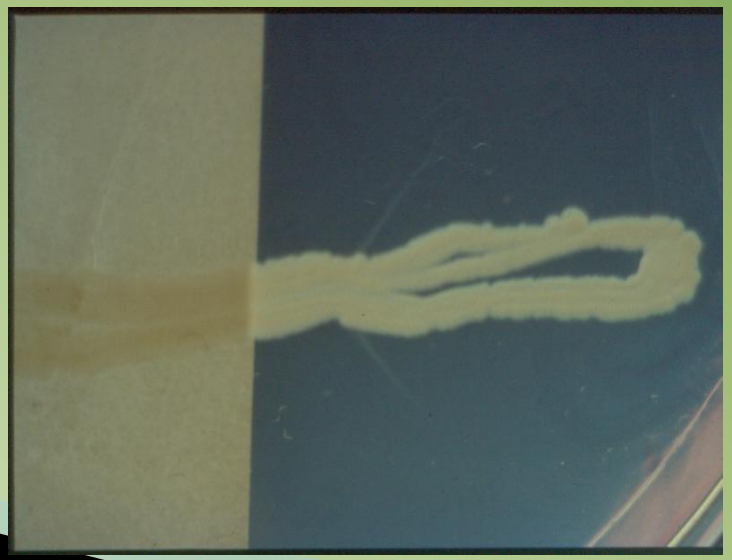
ELEK ASSAY FOR DIPHTHERIA TOXIN

- Paper strip or disk saturated with diphtheria antitoxin is placed in molten agar at 55°C, and allowed to sink to the bottom of the plate.
- The agar is allowed to solidify by cooling to room temperature.
- Streaks of unknown test organisms are placed at a right angle to the strip, or around the periphery of the disk.

ELEK ASSAY FOR DIPHTHERIA TOXIN

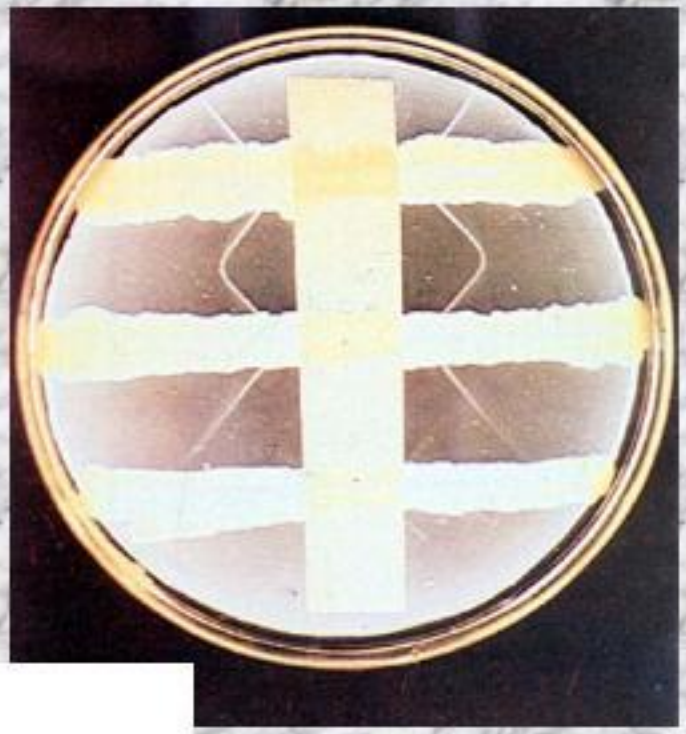
- Development of an agar precipitin line within 1–2 days of incubation at 35°C is a positive result for diphtheria toxin.
- In an Elek test using a strip, the precipitin line forms at a 45° angle to the strip.
- In an Elek test using a disk, the precipitin line forms between the disk and test organism.

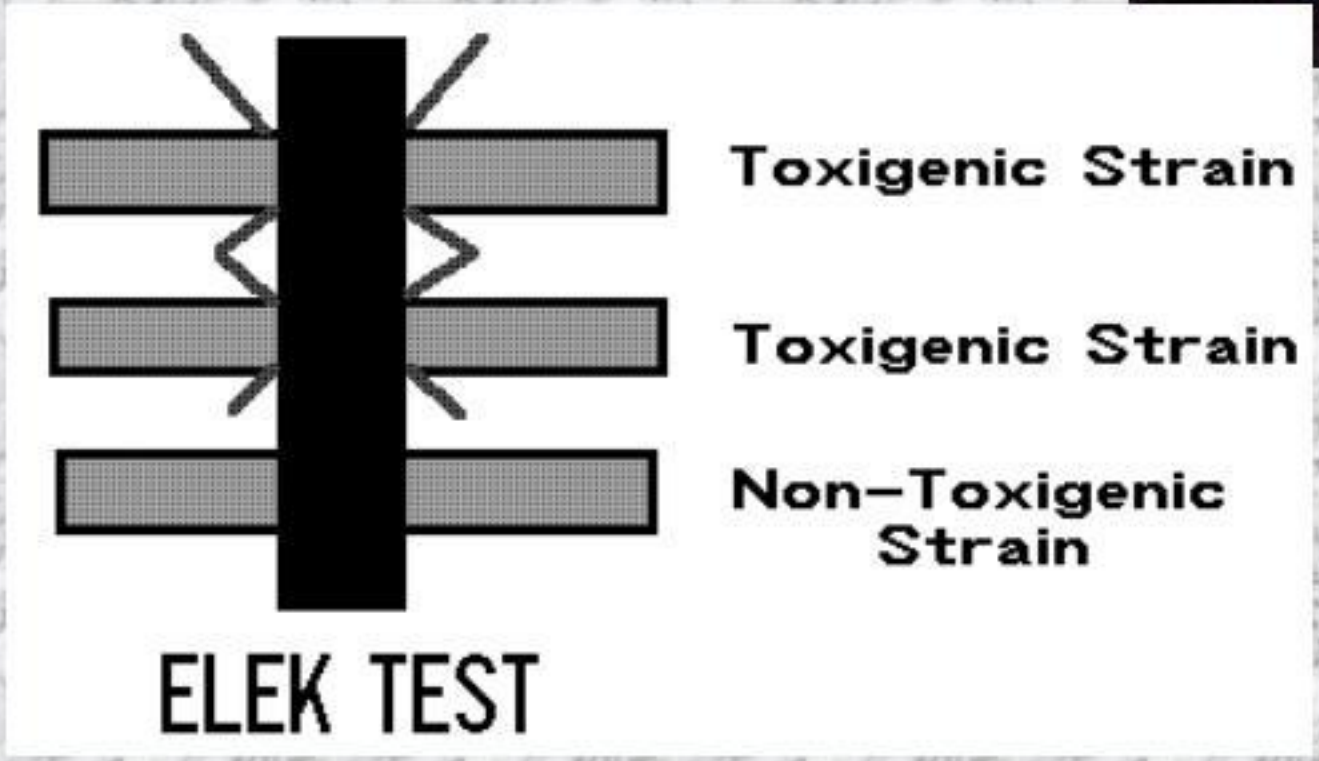

Antitoxin-soaked filter paper
Toxigenic
C diphtheriae
Nontoxigenic
C diphtheriae



Elek Plate Toxigenicity Test

Filter paper saturated with antitoxin placed across an agar plate with *Corynebacterium* streaked at right angles. Precipitation of toxin by antitoxin.





Immunity to Diphtheria

Acquired immunity to diphtheria is due primarily to toxin-neutralizing antibody (antitoxin).

Passive immunity in utero is acquired transplacentally and can last at most 1 or 2 years after birth.

In areas where diphtheria is endemic and mass immunization is not practiced, most young children are highly susceptible to infection.

Probably active immunity can be produced by a mild or inapparent infection in infants who retain some maternal immunity, and in adults infected with strains of low virulence (inapparent infections).

Individuals that have fully recovered from diphtheria may continue to harbor the organisms in the throat or nose for weeks or even months.

In the past, it was mainly through such healthy carriers that the disease was spread, and toxigenic bacteria were maintained in the population.

Before mass immunization of children, carrier rates of *C. diphtheriae* of 5% or higher were observed.

Because of the high degree of susceptibility of children, artificial immunization at an early age is universally advocated.

Toxoid is given in 2 or 3 doses (1 month apart) for primary immunization at an age of 3 – 4 months.

A booster injection should be given about a year later, and it is advisable to administer several booster injections during childhood.

Usually, infants in the United States are immunized with a trivalent vaccine containing diphtheria toxoid, pertussis vaccine, and tetanus toxoid (DPT or DTP vaccine).

Immunity

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Treatment

- Antibiotics:Penicillin G
  Erythromycin
- Antitoxin: Used for neutralizing exotoxin
  Effective in conjunction with antibiotic therapy
- Toxoid:Toxoid preparations are used for vaccines as active immunization for diphtheria. Usually given in conjunction with pertussis and tetanus vaccines (DPT vaccine) or as a booster with tetanus (TD)

DIPHTHERIA

DIAGNOSIS

Clinical suspicion
Swab for culture
Toxin production

PREVENTION

Immunization
(toxoid)

TREATMENT

Penicillin
Anti-diphtheretic serum
Maintaining airway
Supportive

Corynebacterium jeikeium

- Opportunistic infections in immunocompromised (e.g., patients with blood disorders, bone marrow transplants, intravenous catheters)

- Multiple antibiotic resistance common (MDR)

Corynebacterium urealyticum

- Urinary tract infections (UTI's); rare but important.

- Urease hydrolyzes urea; release of NH_4^+, increase in pH, alkaline urine, renal stones

Corynebacterium renale Group

Pathogenesis

- Corynebacterial attachment to urethral epithelium
- Ascending growth to bladder ==> cystitis
- Ascending growth to kidney
  - spreads chronically, relentlessly
- Virulence factors
  - Pili
    - piliated organisms more resistant to phagocytosis than nonpiliated
  - Renalin (*C. renale*): extracellular cytolytic protein

Clinical Presentation

- Posthitis (pizzle rot, sheath rot): preputial ulcerative dermatitis
  - high protein diets ==> alkaline urine, excretion of urea-action of urease ==> ammonia
  - result: ulceration of preputial epithelium, secondary infections
  - lesions spread to preputial mucosa ==> crusting, swelling, pain
  - purulent exudate inside prepuce ==> necrosis
    - sinus tracts drain through prepuce to skin
    - chronic scarring of preputial orifice

DIFFERENTIATION IN *C.RENALE* GROUP

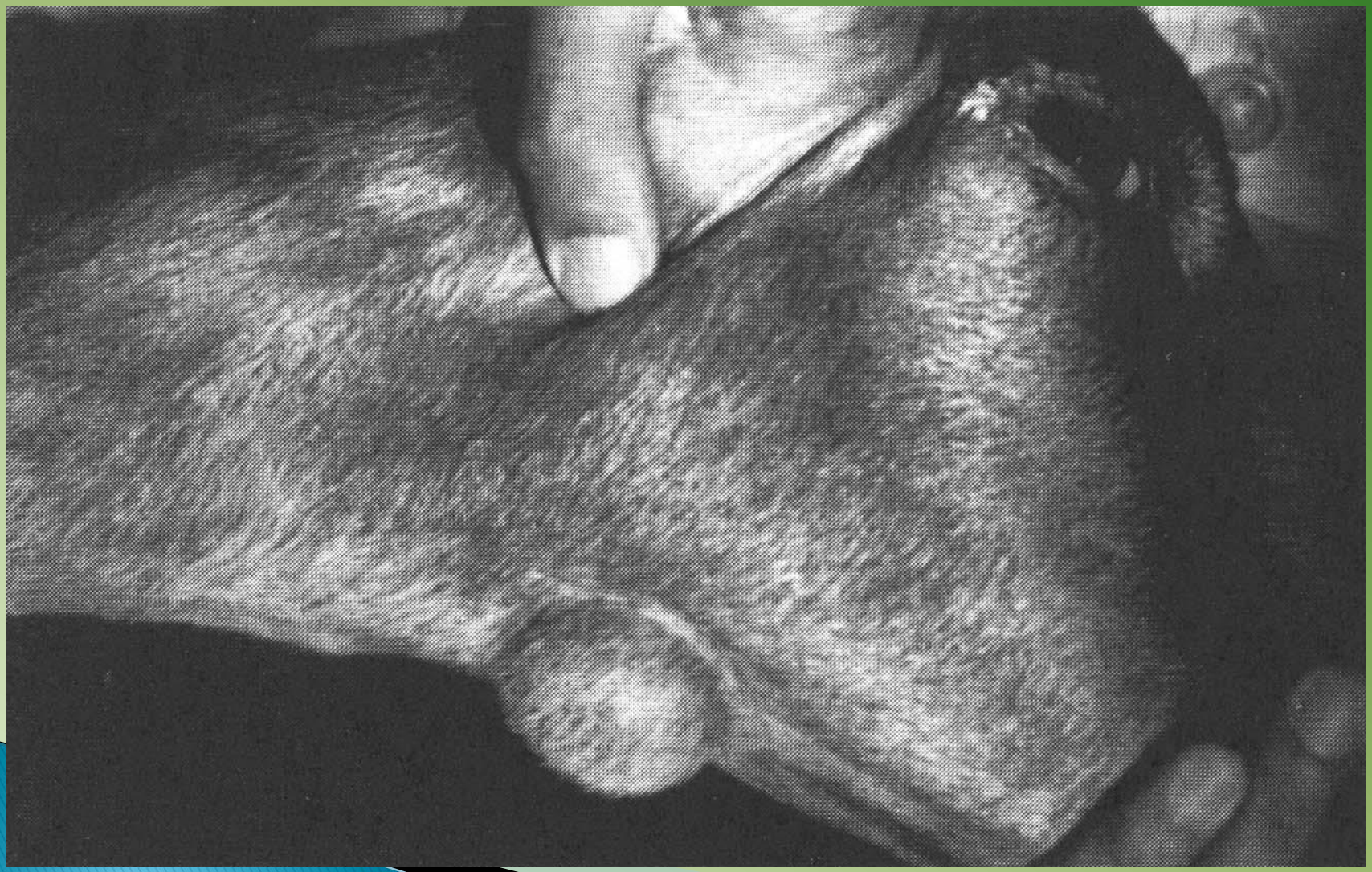
FEATURE	*C.renale*	*C.pilosum*	*C.cystitidis*
Colour of colonies	Pale yellow	Yellow	white
Growth in broth of pH 5.4	+	–	–
Nitrate reduction	–	+	–
Acid from xylose	–	–	+
Acid from starch	–	+	+
Casein digestion	+	–	–
Hydrolysis of tween 80	–	–	+

Corynebacterium pseudotuberculosis

Caseous lymphadenitis (CLA) or cheesy gland in sheep and goats (pigeon breast or breastbone fever);

- Chronic abscessation: peripheral LN
- Thick caseous exudate, slightly greenish
- Spreads rapidly within flock
- Visceral lesions more common in sheep
- Human infection – uncommon

Caseous lymphadenitis in submadibular lymph node



Corynebacterium pseudotuberculosis – Caseous Lymphadenitis

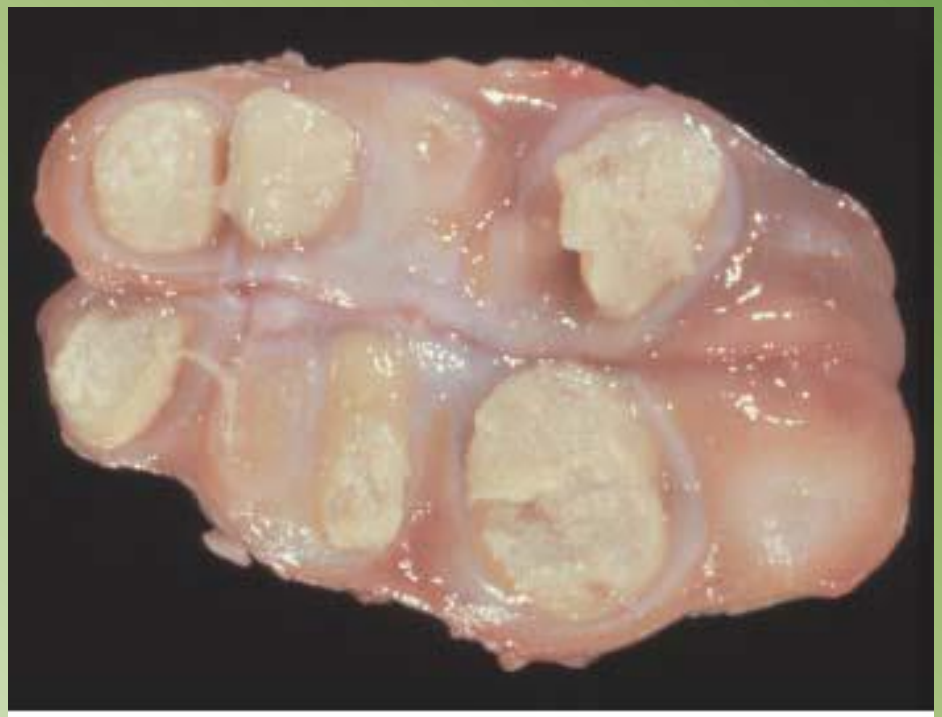


Fig. 13-72 Chronic caseous lymphadenitis, *Corynebacterium pseudotuberculosis*, lymph node, sheep. The lymph node has been sliced longitudinally exposing three chronic abscesses enclosed by thick fibrous capsules and containing yellowish caseous pus.

(Courtesy Dr. W. Crowell, College of Veterinary Medicine, The University of Georgia; and Noah's Arkive, College of Veterinary Medicine, The University of Georgia.)

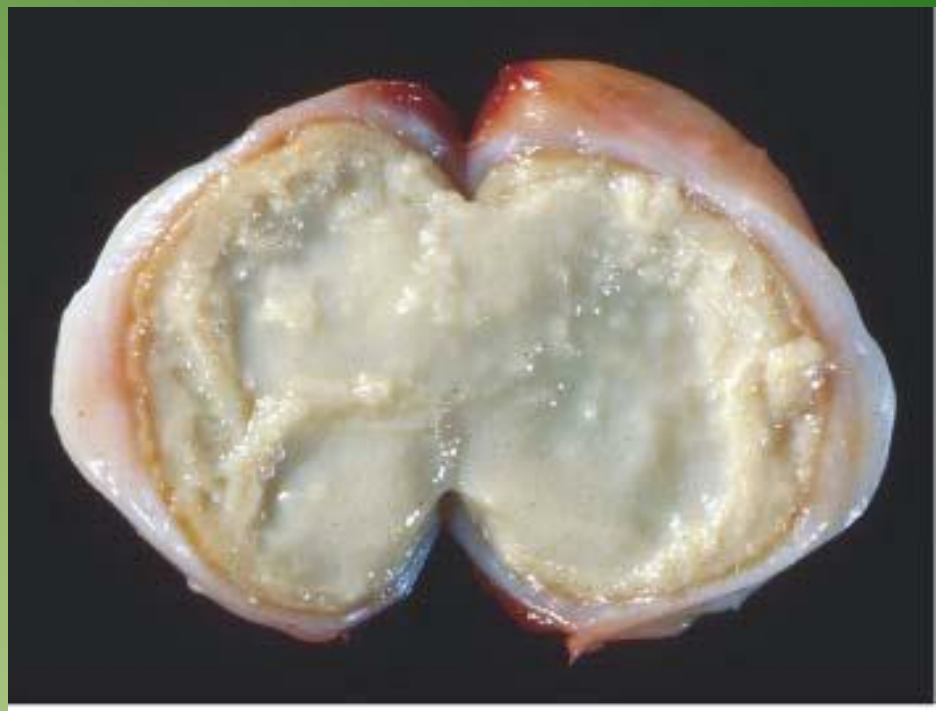
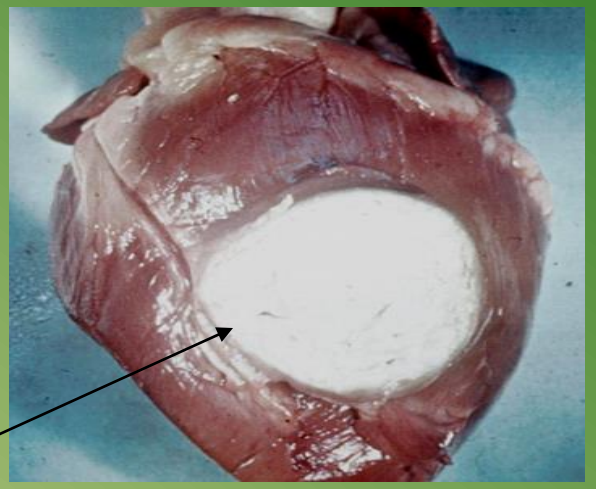


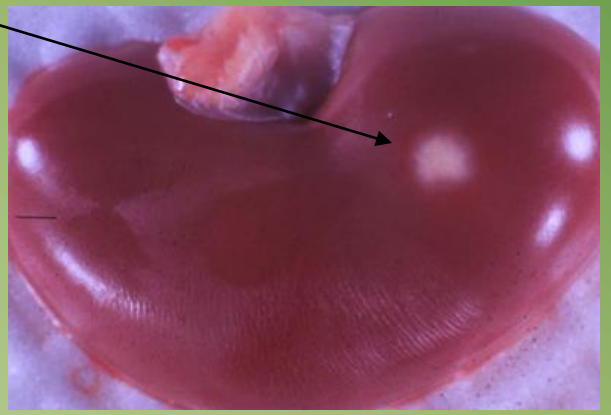
Fig. 13-71 Caseous lymphadenitis, *Corynebacterium pseudotuberculosis*, lymph node, sheep. The whole lymph node has been replaced by an abscess containing mostly semifluid yellowish pus. This is an early stage of caseous lymphadenitis, before the pus has become inspissated and caseous.

(Courtesy Dr. K. Read, College of Veterinary Medicine, Texas A&M University; and Noah's Arkive, College of Veterinary Medicine, The University of Georgia.)

Pathogenesis

- Phagocytosed (facultative intracellular parasite)
- Multiplies in phagolysosome, phagocytes die
- Permeability increases, spread to regional LN
- Abscesses: primary, secondary sites
- May become metastatic ("thin ewe syndrome")





Hepatic Abscess – *Corynebacterium pseudotuberculosis*

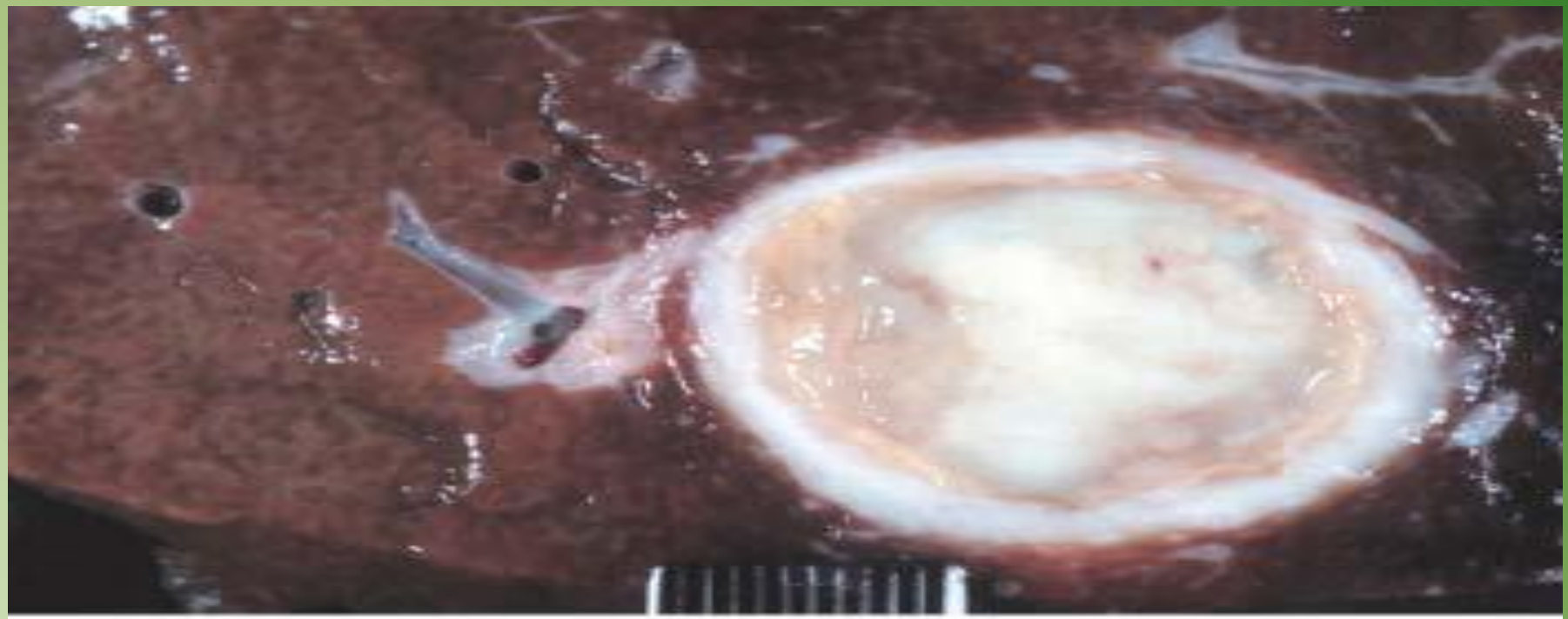


Fig. 8-52 Chronic hepatic abscesses, *Corynebacterium pseudotuberculosis*, liver, sheep. Note the thick fibrous capsule and the pale caseous exudate characteristic of pus produced by *Corynebacterium pseudotuberculosis* in sheep.

(Courtesy College of Veterinary Medicine, North Carolina State University.)

Vaccination

- Early vaccines – inactivated whole cell preparations
- Toxoid vaccines – Phospholipase D (PLD) formalin inactivated
- Glanvac™ – Australian combined clostridial/CLA vaccine offers significant protection in sheep and goats but not authorised for use in Britain or Ireland
- Modern recombinant PLD vaccine

Rhodococcus equi

Rhodococcus equi

Scientific classification

Kingdom:	Bacteria
Phylum:	Actinobacteria
Order:	Actinomycetales
Suborder:	Corynebacterineae
Family:	Nocardiaceae
Genus:	*Rhodococcus*
Species:	*Rhodococcus equi*

Goodfellow & Alderson 1977

Introduction

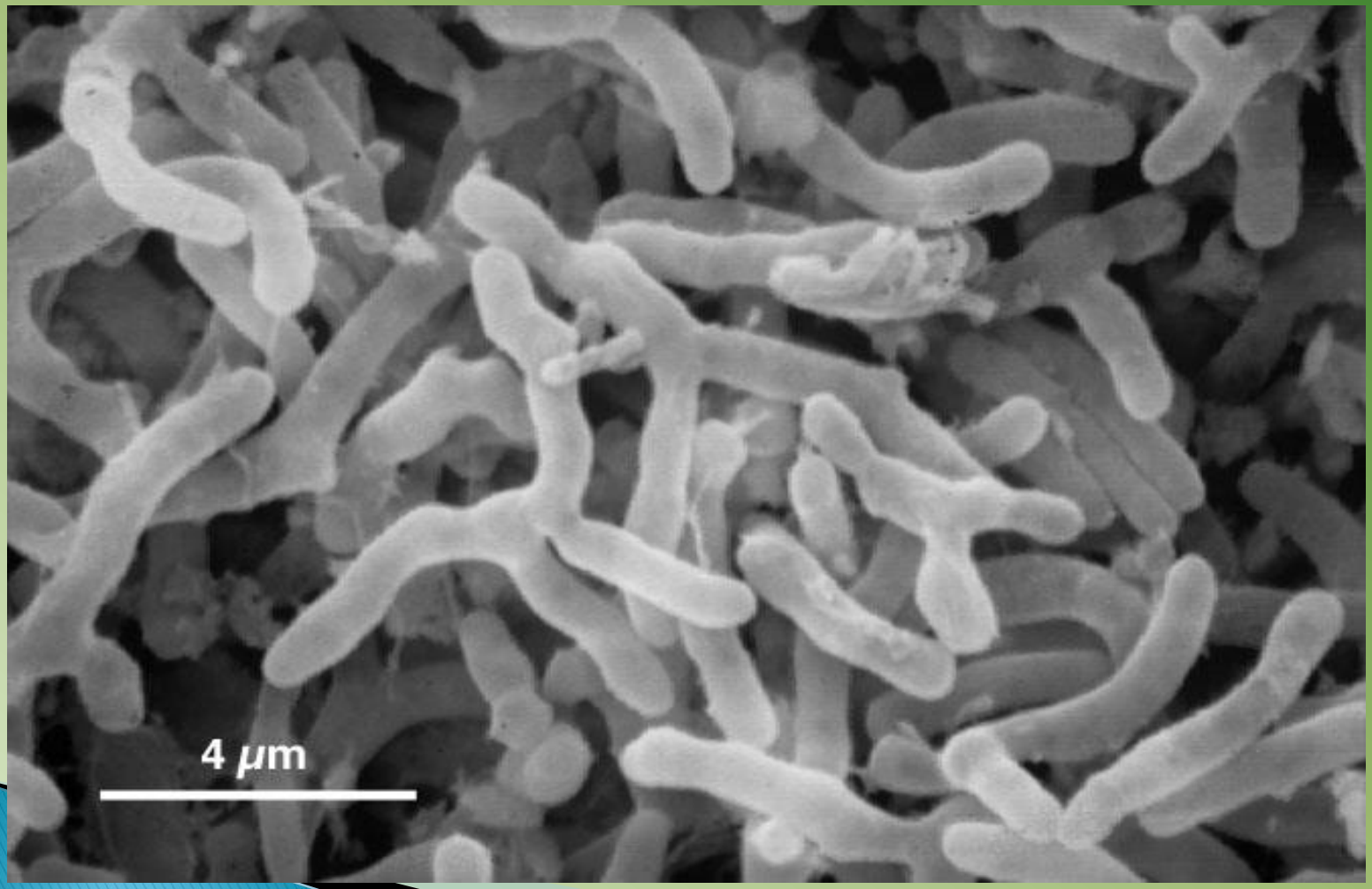
- *Rhodococcus equi* was first recognized as a zoonotic pathogen
- Original isolation as *Corynebacterium equi* in 1923 from foals with granulomatous pneumonia (Magnusson)
- First documented human infection in 1923 in a 29 year male with plasma cell hepatitis on immunosuppresants
- Only 12 more human cases recorded over the next 15 years

- Originally named *Corynebacterium equi* based on morphology
- 1980 – reclassified in genus *Rhodococcus* after cell wall composition & biochemical reactions found to be more closely related to *Nocardia & Mycobacteria*
- Organism found in areas with domesticated animals
- *R. equi* present in animals that graze on soil.
- soil organism widespread in the environment
- Environmental presence magnified by fecal contamination.
- Organism multiplies in horse manure
- Disease incidence correlated with environmental contamination

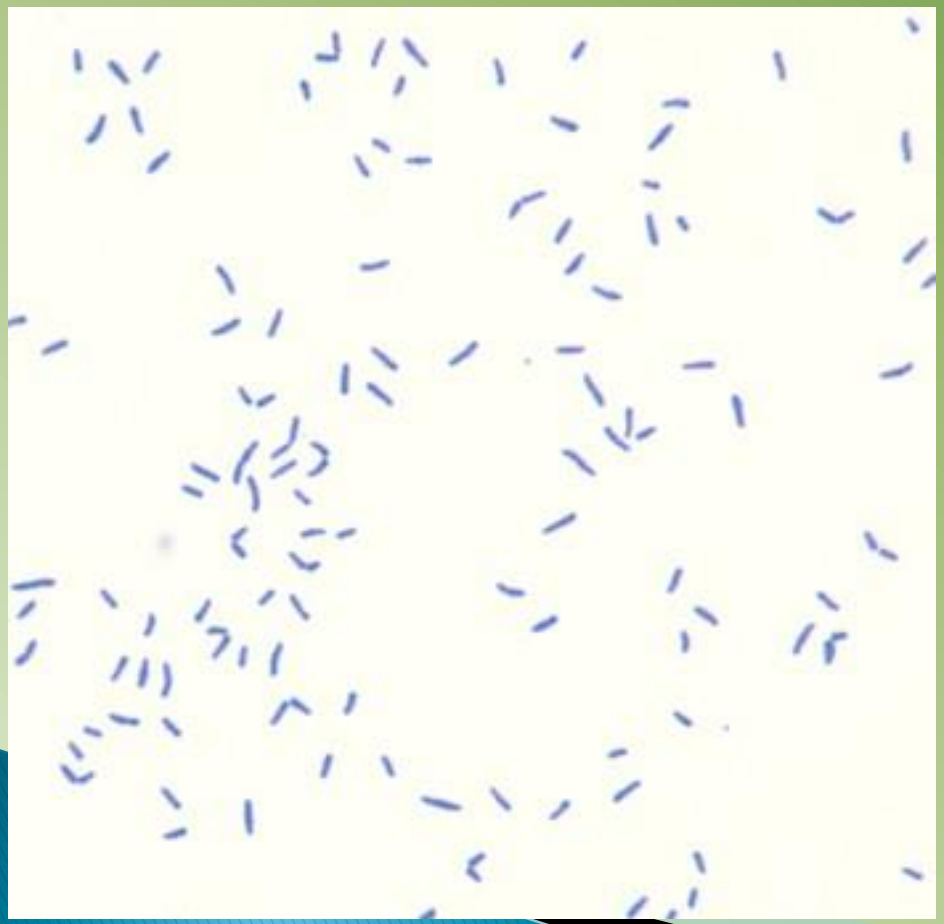
Morphology

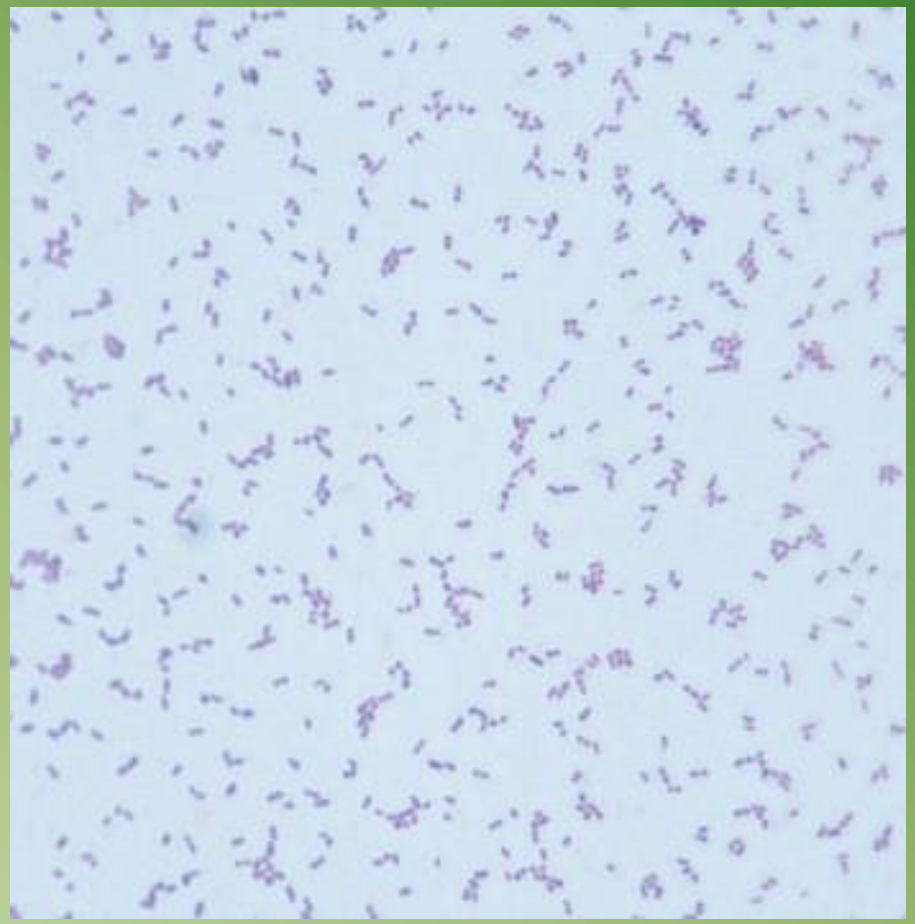
- Facultative intracellular, obligatory aerobic nonmotile, non-fermenting, non-spore forming, pleomorphic gram positive coccobacilli
- Usually has polysaccharide capsule
- Pleomorphic: typically appears coccoid but in liquid media forms long rods or short filaments
- Rudimentary branching may be present unlike Mycobacteriaceae)
- Characteristic rod to coccus growth cycle variation

Electron Micrograph



Gram-positive cells with a pronounced rod-coccus cycle. Cells in early exponential phase are rod-shaped and cells in stationary phase are coccoid.





- Named *Rhodococcus* for its production of red pigment (actually salmon pink) after four days.
- Inconsistently acid fast or partially acid fast; tends to be true on direct smears and fresh clinical isolates, but lost on subculture
- Presence of tuberculostearic acid and cell wall mycolic acids
- At least 27 different polysaccharide capsular serotypes (no relationship to virulence)
- Distinguishing factors:
  - Urease positive: unlike *Corynebacterium*
  - Non-fermenting: unlike *Corynebacterium*
  - Lacks aerial hyphae: unlike *Nocardia, Streptomyces*
  - + rudimentary branching: unlike Mycobacerteriaceae

Virulence Factors

- Strains isolated from foals have a large plasmid
- Waxy components in cell wall enhances intracellular survival
- Plasmid coding for VAP (virulence associated protein, *R.equi* factor) also enhances survival in macrophages
- Plasmid only expressed at temperature of 30 C or greater
  - Vap A (encodes a surface Lipoprotein)
  - Vap C – H genes (C, D, E =secreted proteins)
- Large Plasmid Negative strain (mutant) –causes no lesions in host.
- Large Plasmid Expressing strain (wild type) – causes lesions in lungs
- *R. equi* produces cholesterol oxidase that damages mammalian cell membranes.

Virulence

- Associated with specific surface antigens encoded in the DNA of a large plasmid.
- Production of these antigens is temperature dependent. (34–41 C)
- Other factors enhancing virulance are
  - ✓ capsular polysaccharides
  - ✓ mycolic acid cell wall
- these factors retard phagocytosis and various exoenzymes.

▪Pneumonia caused by *R. equi* has been reported in patients with human immunodeficiency virus infection.

•Only virulent strains of *R. equi* harbouring a virulence plasmid of 85 to 90 kd .

Clinical conditions associated with *R.equi*

Host	Clinical condition
1. Foals up to 1 to 4 moth of age	Suppurative bronchopneumonia and pulmonary abscessation
2. Horses	Superficial abscessation
3. Pigs,cattle	Mild cervical lymphadenopathy
4. Cat	Subcutaneous abscesses and mediastinal granulomas

Symptoms in Equines

- Abd. Pain in acute phase
- Fever
- Nasal discharge
- Coughing
- Diarrhea
- Wt. Loss

- diffuse bronchial sounds, cough, wheeze.
- Sub acute form more rare but devastating – acute respiratory distress, high fever high mortality.
- high respiratory rate (over 40 resp per minute).
- Untreated foals develop progressive crackling sounds heard all over the lung and harsh inspiratory sounds which can be heard without a stethoscope
- Chronic Pyogranulomatous bronchopneumonia with extensive lung abscesses.

Lungs of foal with extensive antero-ventral pyogranulomatous abscesses typical of the lesions of *R. equi* pneumonia.

Rhodococcus equi enteritis

- Organisms coughed up and swallowed Enters intestinal M cells overlying GALT[Gut Associated Lymphoid Tissue]
- – Pyogranulomatous mesenteric lymphadenitis
- – Ulcerative colitis

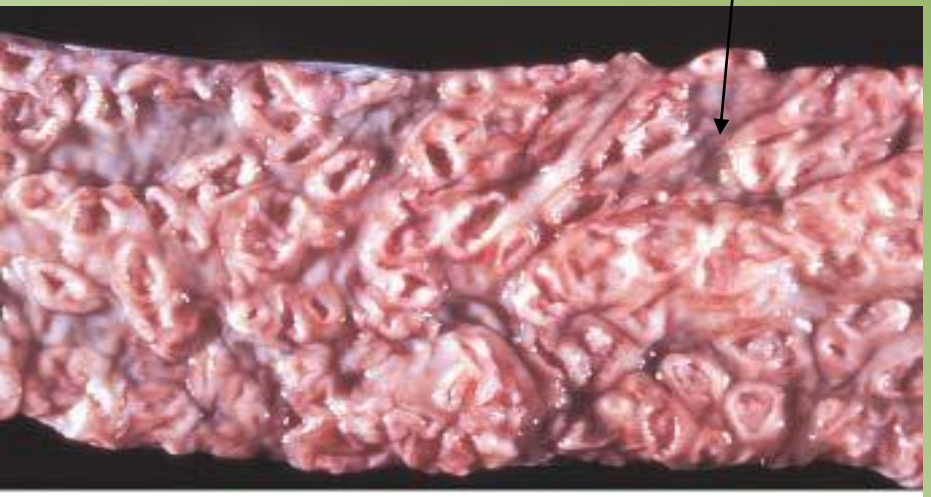


Fig. 7-145 Multifocal ulcerative colitis, colon, horse. *Rhodococcus equi* infection causes multiple mucosal ulcers centered over gut-associated lymphoid tissue.

(Courtesy Dr. H. Gelberg, College of Veterinary Medicine, Oregon State University.)

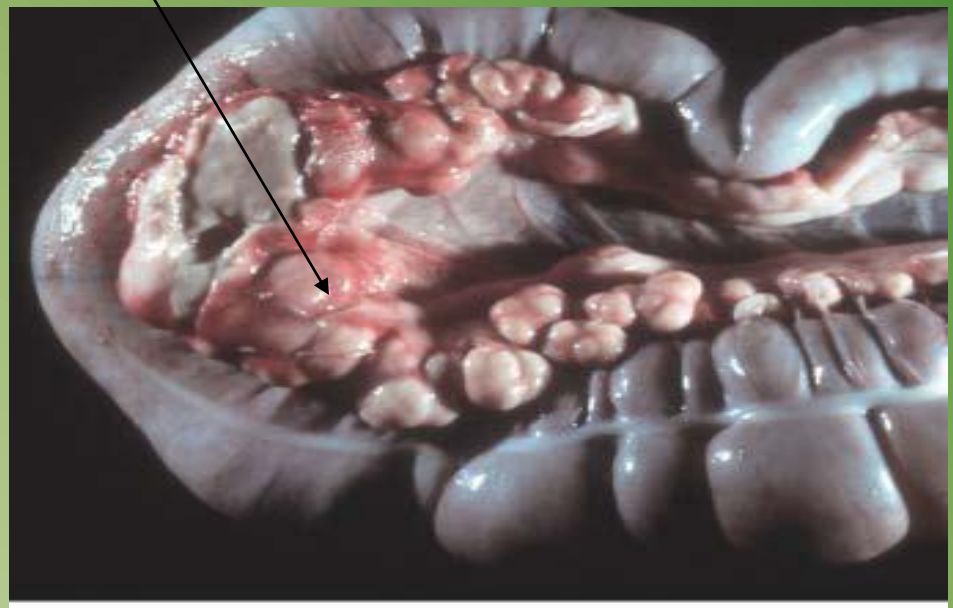


Fig. 7-146 Mesenteric lymphadenitis, colon, horse. Infection of colic lymph nodes with *Rhodococcus equi* causes pyogranulomatous lymphadenomegaly.

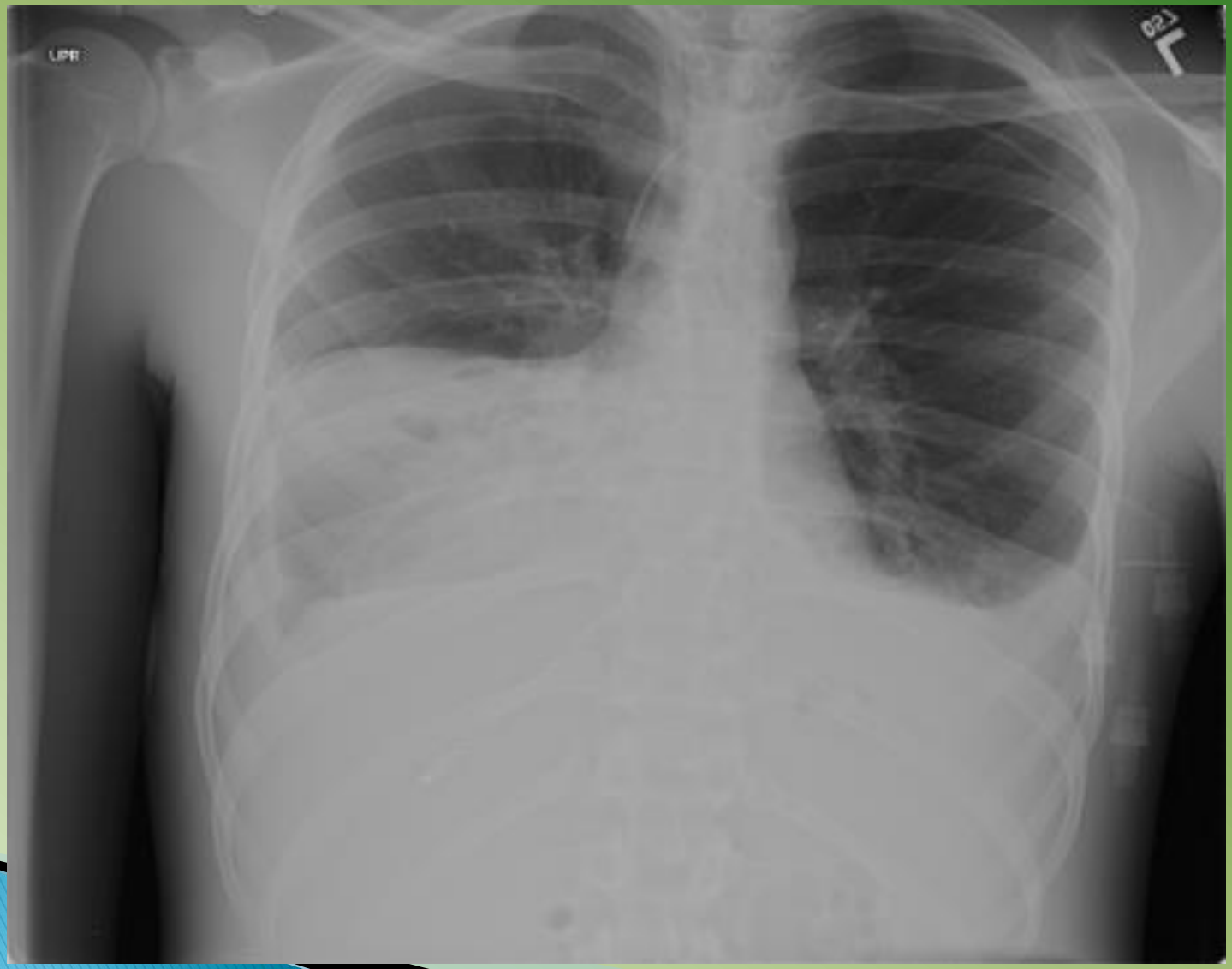
(Courtesy Dr. H. Gelberg, College of Veterinary Medicine, Oregon State University.)

Huma infection

- Of all documented infections: 80-90% immunocompromised
- Of these, 50-60% HIV, 15-20% hematopoietic or other malignancies, 10% transplant recipients
- Reported in at least 28 states, and 5 different continents
- At least 19 cases reported in immunocompetent hosts

- In radiographic findings multiple nodular infiltrates is most common.

Radiographic Findings



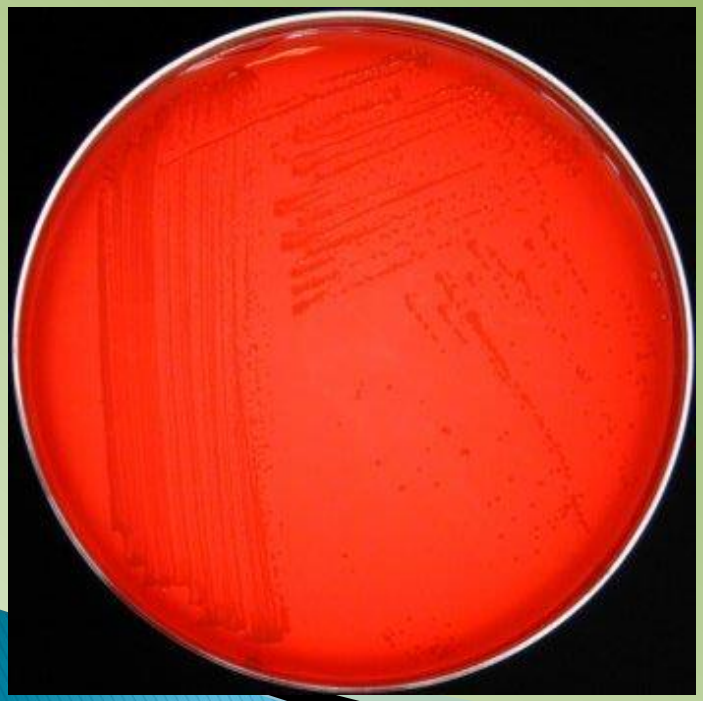
Extra pulmonary infection in human beings

- In declining frequency:
  - Bacteremia – very common
  - CNS (abscess, meningitis) – very common
  - Subcutaneous & other soft tissue abscesses
  - Wound infections
  - Septic arthritis
  - Endopthalmitis
  - Other sites (pharynx, middle ear, lymph nodes, bone, GI tract)

Colony and Culture Characteristics

- **Culture of TTA (transtracheal aspirates) or bronchial washes; direct smears – clusters of G+ve rods or cocci , extra- & intracellular**
- Easily cultured in nonselective media
- Forms large, smooth, **round**, irregular, highly mucoid colonies on blood agar by 48 hours **that tend to coalesce.**
- **Non-hemolytic.**
- **Various colors of pigment that tend to be reddish.**
- **Catalase test-positive.**

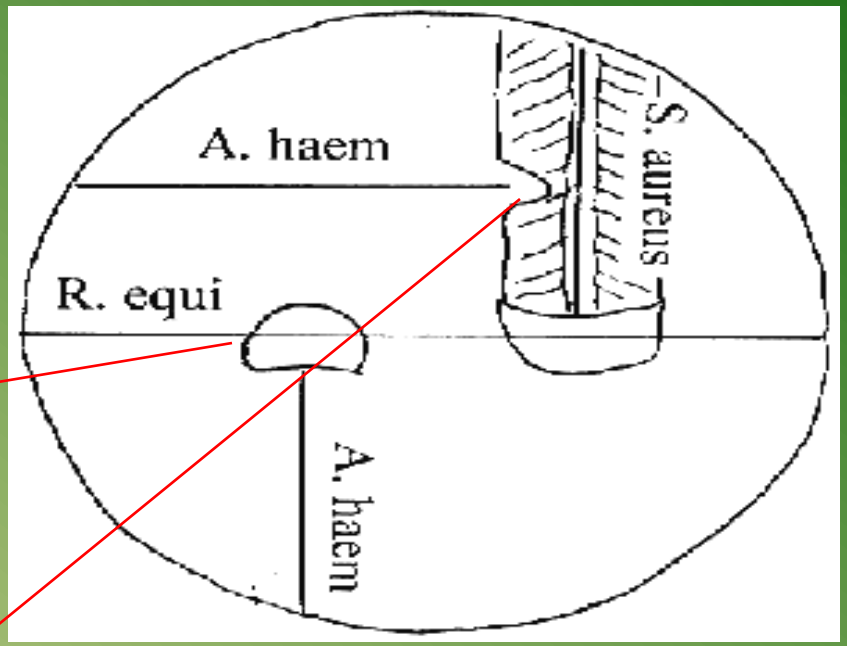
- Relatively large and mucoid colonies. Initially the colonies are greyish but after further incubation may appear slightly salmon in colour

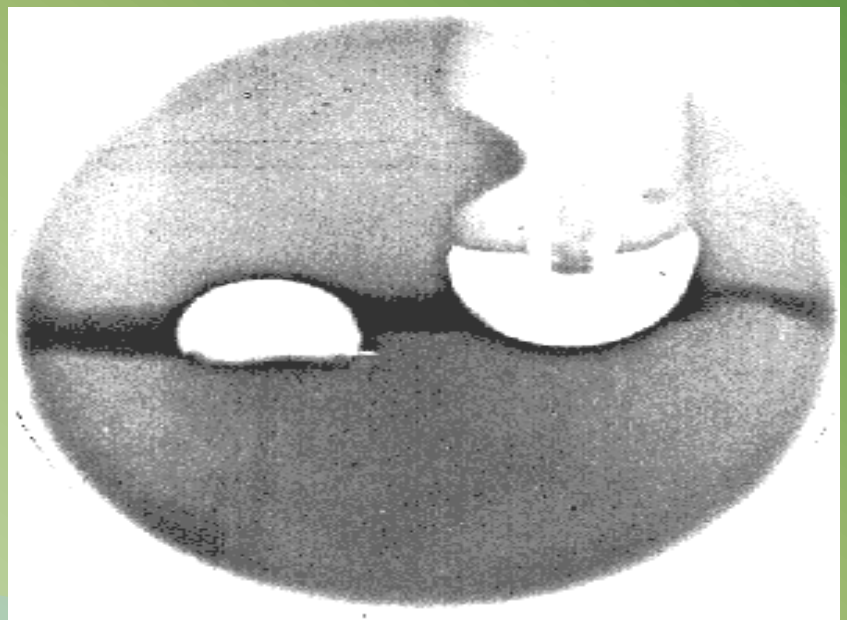


- The same Blood Agar plate examined with transmitted light. There is no haemolysis.

CAMP TEST

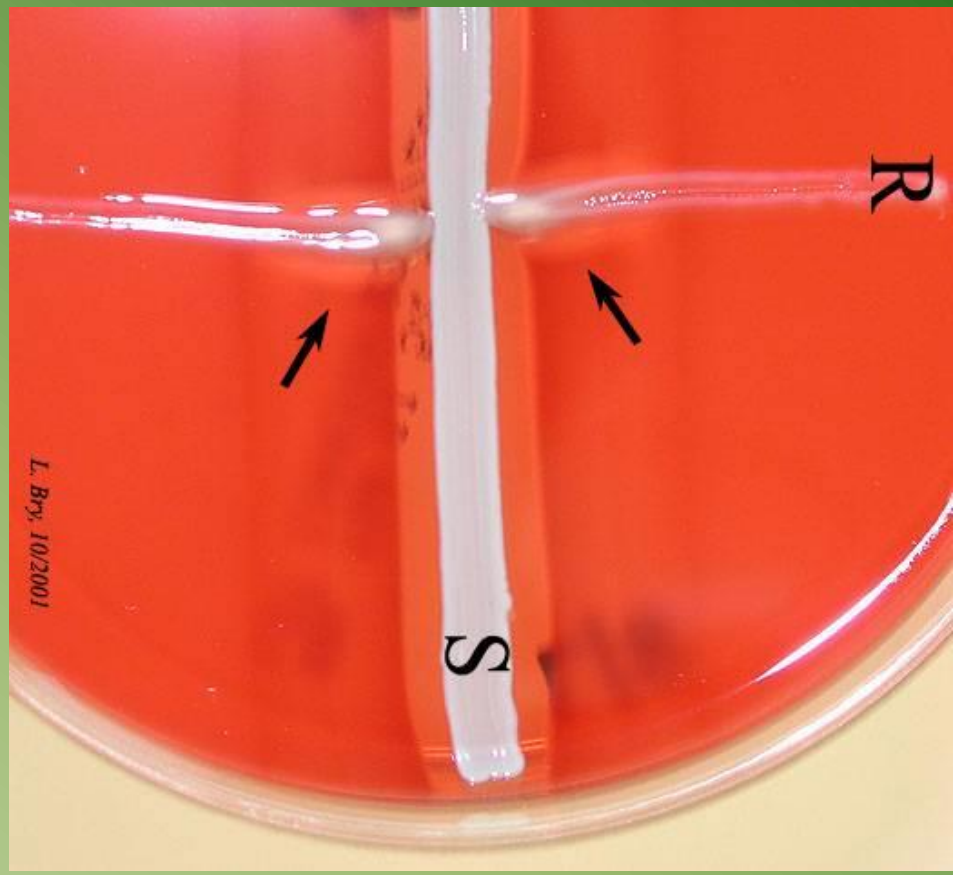
- Cooperative and antagonistic hemolytic reactions on sheep blood agar
- Demonstrating cooperative hemolysis between *Rhodococcus equi* and *Arcanobacterium haemolyticum*,
- Antagonistic hamolysis between *Arcanobacterium haemolyticum*, *and Staphylococcus aureus*. Partial hemolysis by *S. aureus* (cross-hatched on diagram) is inhibited in the proximity of *A. haemolyticum*.





Blood Agar plate showing *equi* factors where R= *Rhodococcus equi* and S= *S.aureus*

- CAMP test for cholesterol oxidase produced by *R. equi*. R = *Rhodococcus*; S = lecithinase producing, hemolytic *Staphylococcus aureus*.
- Arrows point to additional zones of beta-hemolysis where secreted cholesterol oxidase works with lecithinase secreted by the Staphylococci to cause hemolysis of sheep's RBCs in the media



Immunity and Treatment

- Most likely cell-mediated immunity.
- BUT humoral immunity involved since passive immunity aids in preventing disease and severity of disease is inversely related to abundance of circulating antibody.
- Antibiotics are effective. Erythromycin/Azithromycin in combination with Rifampin used in foals

THANK YOU.