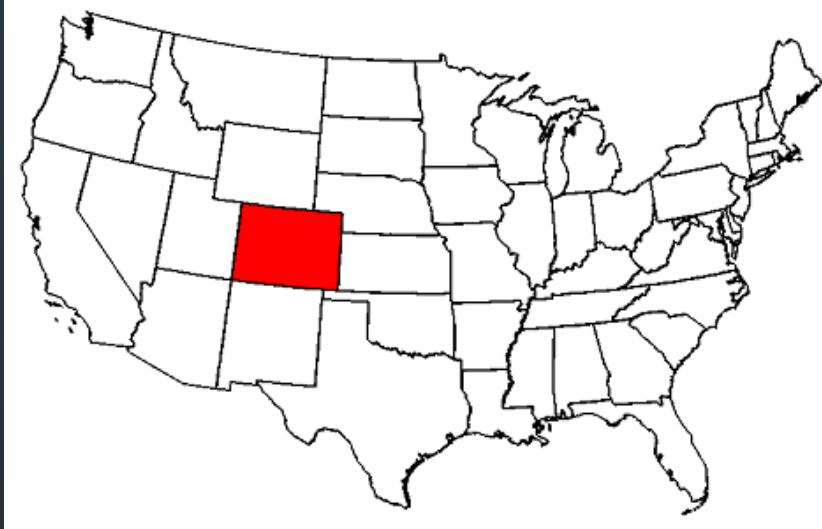


THE NAME OF THE STAINS: HISTOLOGY HISTORY PART 1

Jean Mitchell, BS, HT (ASCP)
Newcomer Supply – Middleton, Wisconsin
jmitchell@newcomersupply.com

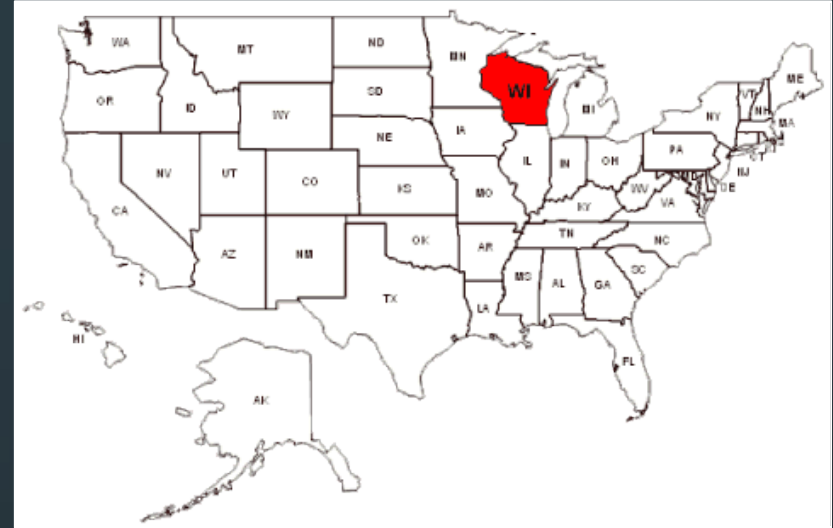
Jane Parr, BS, HT (ASCP)
National Jewish Health & University of Colorado
Anschutz Medical Center - Denver, Colorado
jane.parr@ucdenver.edu

ABOUT JANE



- 25+ Years in Histology
- NSH BOD
- Loves Dachshunds, Lemon Drop Martinis & the Beach

ABOUT JEAN



- 35+ Years in Histology
- NSH BOD
- Loves American Football, Travel, Wine, Books & Dogs

HISTOLOGICAL HISTORICAL PERSPECTIVE

1848: “Handbook of General & Special Histology” (Gerlach)

1858: “Cellular Pathology” (Virchow)

1865: Use of Hematoxylin in Published Works

1870’s: Histologists Using Chemicals as Fixatives/Stains

1873: Silver Impregnation - “Black Reaction” (Camillo Golgi)

1880: 1st Commercial Marketing of Dyes for Microscopy

1893: Published - Use of Formalin as a Fixative (Blum)

1900: Paraffin Embedding a Standard Procedure

LABORATORIES OF THE 1800-1900'S

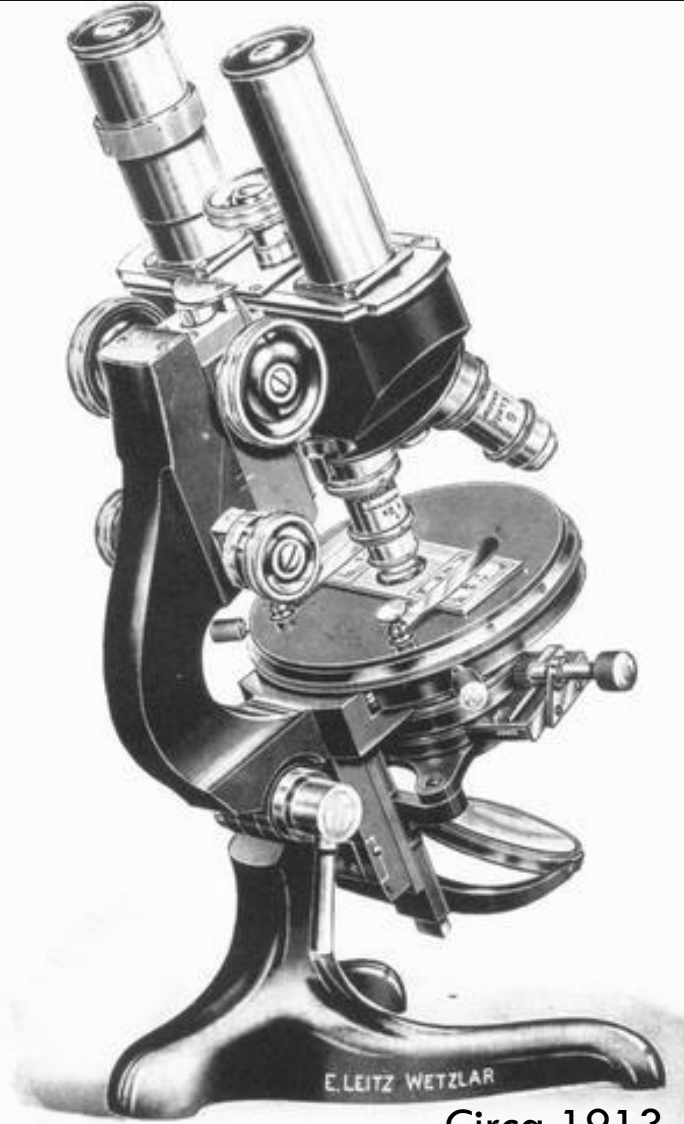
- 1. Supplies: Chemicals, Cassettes, Slides, Coverglass, Glassware, Paper Towels**
- 2. Exposure: Picric Acid, Silver, Osmium, Formaldehyde**
- 3. Safety: Gloves, Goggles, Fume Hoods, Disposal Methods**
- 4. Electricity: Overhead Lighting, Microscopes, Water Baths, Stir Plates, Hot Plates, Refrigerators**
- 5. Water: Running Water, Indoor Plumbing, Bathrooms**



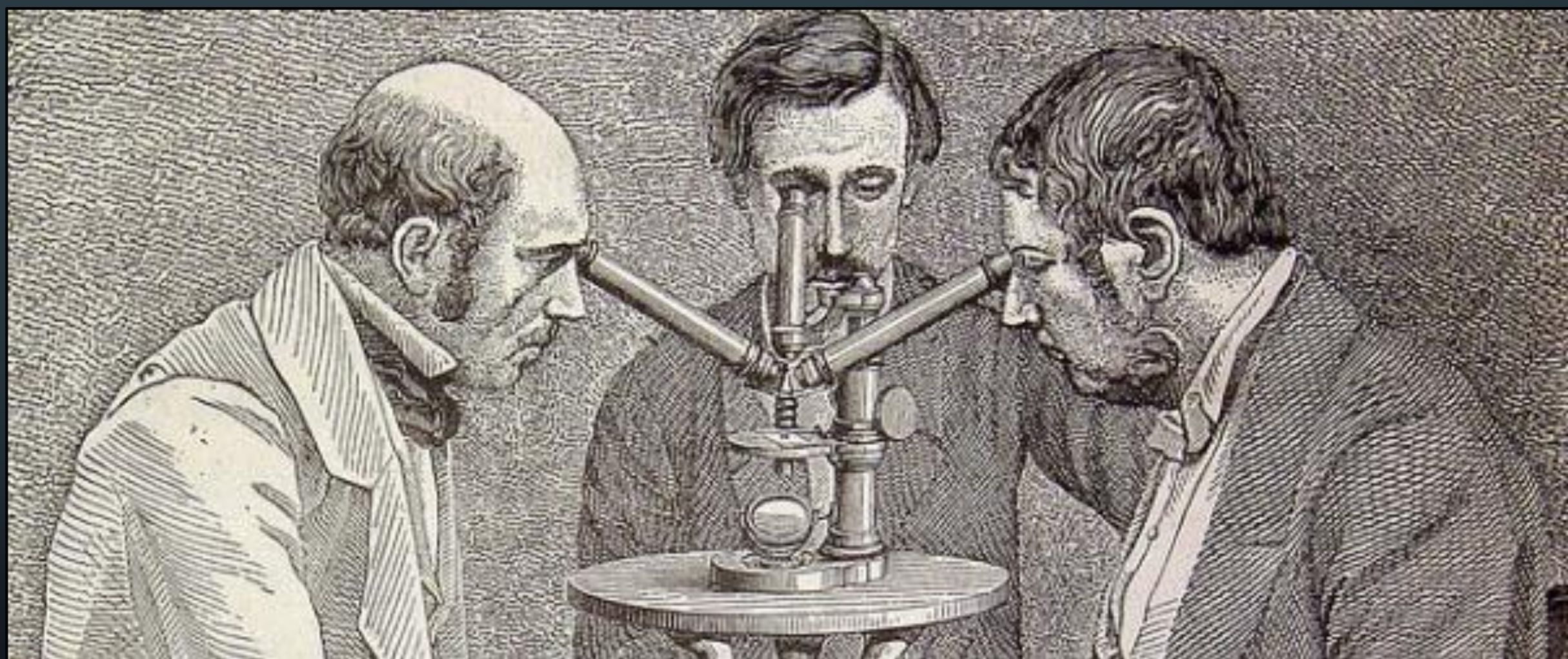
**British Monocular
Fixed-Mirror
Brass
Microscope
(circa 1800)**



Zeiss Microscope from 1877



Circa 1913



TO CONSIDER: 1800-1900'S

- 1. 1835: 1st American Hotel w/Indoor Bathrooms**
 - 1860: Launceston – Underground Sewers. 1st in Australia; 3rd in the World
 - 1885: Chicago - 1st Comprehensive Sewer Project
- 2. 1879: Thomas Edison - Long Lasting Light Bulb**
 - Late 1880's some US/Australian cities had small electrical stations
 - 1895: Launceston – 1st Australian city w/electric street lights
 - 1930's: Larger towns/cities had electricity.
- 3. 1914-18: World War I – Blockade of German Products**
 - 1920: Supply of US Pre-War Dyes Exhausted
- 4. 1922: Commission on the Standardization of Biological Stains**
- 5. 1939-1945: World War II & Influence**
- 6. Health Conditions: Diseases, Vaccines, Antibiotics**
 - Plague, Typhoid, Malaria, Cholera, Yellow Fever, Polio

LABORATORY ACCESSORIES

Bunsen Burner (1855)

Erlenmeyer Flask (1860)

Coplin Jar (1897)

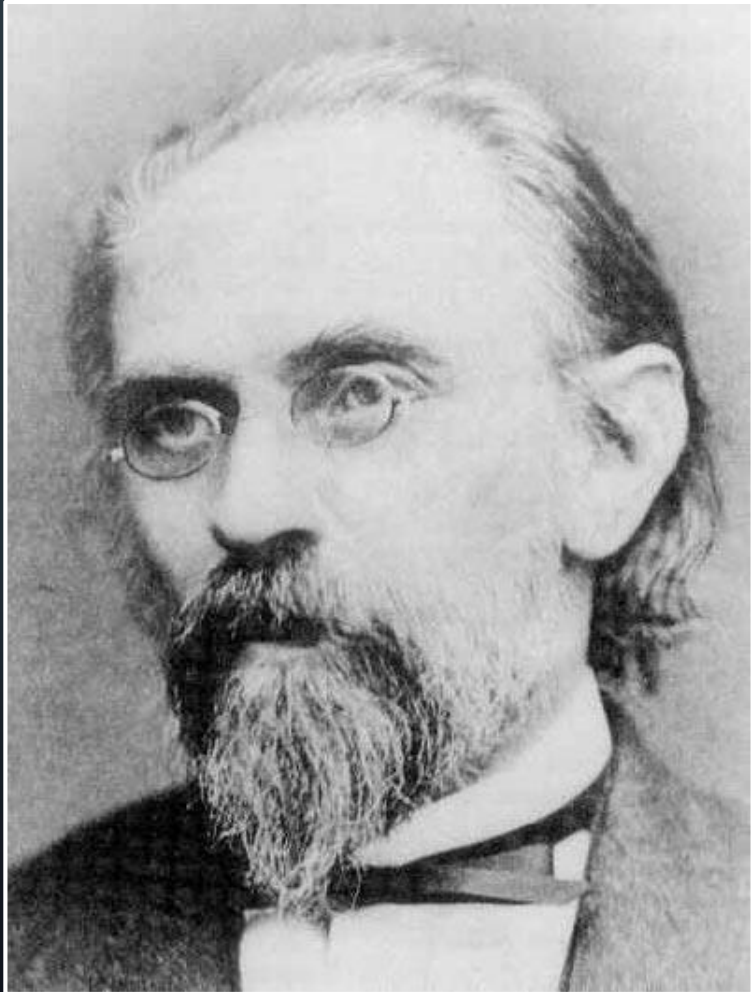


ROBERT BUNSEN: 1811-1899



1. German Chemist - Heidelberg
2. Investigated Spectrum of Heated Elements (flame spectroscopy)
 - Discovered: Caesium & Rubidium
 - Studied Arsenic and Antidote
 - Cacodyl Derivatives
3. 1855: Developed Bunsen Burner w/lab Assistant Peter Desaga
 - Never Patented

EMIL ERLENMEYER: 1825-1909



1. **German Pharmacist/Organic Chemist**
 - **Richard August Carl Emil Erlenmeyer**
2. **1857 Thesis: “On The Manufacture of the Artificial Manure Known as Superphosphate”**
 - **Worked on Fertilizers in Bunsen’s Lab**
3. **1860: Published a Description of a Conical Flask**

WILLIAM COPLIN: 1864-1928



1. Irish-Scottish/American Pathologist:
Jefferson Medical College, Philadelphia
 - 1896: H.F. Harris 1st Path Resident
2. Director of Philadelphia Public Health
3. Instrumental in Hospital Based Labs
4. Expert in Contagious Diseases
5. Curator - Museum of Pathology
6. Served in WWI in France – Colonel



HEMATOXYLIN & PATHOLOGY

1863: Combined w/Alum

1865: 1st Mentioned in Published Literature

1866: Ehrlich Added Acetic Acid

Components:

- **Haematoxylin → Haematein**
- **Oxidant: Oxygen → Mercuric Oxide → Sodium Iodate**
- **Mordant: Alum, Iron, Tungsten**
- **Other: Alcohol, Glacial, Glycerin, Ethylene/Propylene Glycol**

HEMATOXYLINS

Alum Mordant

- **Delafield (1885)**
- **Ehrlich (1886)**
- **Harris (1900)**
- **Mayer's (1903)**
- **Gill's (1972)**

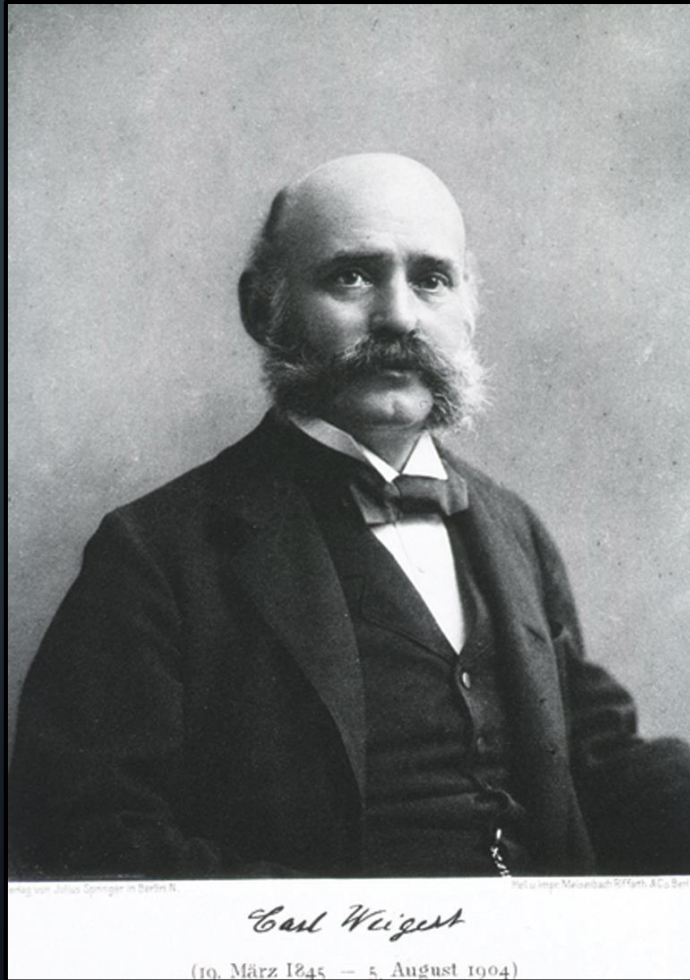
Iron Mordant

- **Heidenhain (1892)**
- **Weigert (1904)**
- **Verhoeff (1908)**

Tungsten Mordant

- **Mallory (1900)**

CARL WEIGERT: 1845-1904

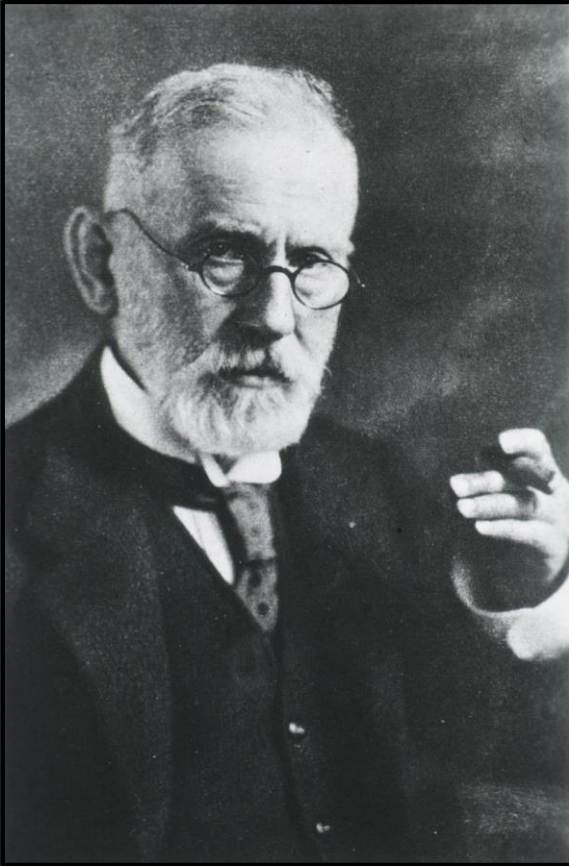


1. German Pathologist
2. Senckenberg Path Institute - Frankfurt
 - Taught Bielschowsky Histology Techniques
3. Cousin to Paul Ehrlich
4. 1878: Introduced Bismarck Brown
5. 1884: Method for Staining Myelin Sheath
6. 1898: Classic Elastic Fiber Staining Method

PAUL MAYER: 1848-1923



1. German/Italian: Histologist at Zoological Station - Naples
2. 1870's: Began Research & Systemized Staining Methods
3. 1891: Haematoxylin Must Ripen Before Use
 - Demonstrated Oxidized Compound → Haematein
4. Greatest Authority on the Subject of Haematoxylin (per H.F. Harris)
5. 1896: Published Mucicarmine Techniques



PAUL EHRLICH: 1854-1915

1. German Biochemist - Cousin to Carl Weigert
2. Coined the term “Magic Bullet”
3. Work W/Toxins & Antitoxins
4. 1878: 1st Described Mast Cells
5. 1882: 1st Documented Use of Fluorescent Dyes
6. 1886: Naturally Ripened Alum Hematoxylin
7. 1908: Nobel Prize for Immune Response Theory
8. 1909 - 1st Treatment for Syphilis (salvarsan)
 - 1940s & Penicillin



Howard Walter Florey (1898-1968)



ABOUT PENICILLIN

- 1928: Alexander Fleming Discovered Anti-Bacterial Properties of the Mold Penicillin
- 1938-42: Florey Developed Penicillin as a Medicine (Pharmacologist/Pathologist)
- 1941: Conducted 1st Clin Trial in Oxford
- 1945: Florey, Fleming, Chain – Nobel Prize in Medicine
- One of Australia's Greatest Figures
- Credited for Saving 200+ Million Lives

(HENRY FAUNTLEROY) H.F. HARRIS: 1867-1926

1888: Jefferson Medical College, Philadelphia

1889: Professor of Chem: Southern Med College, Atlanta, GA

1897: Associate of Pathology/Bacteriology w/Coplin

1900: Journal of Applied Microscopy Publication

- On the Rapid Conversion of Haematoxylin into Haematein in Staining

1902: Reported 1st Case of Pellagra in the U.S.

- The “Red Death” ; The 4 D’s”

1905-17: Secretary GA State Board of Health &
Director of Labs

- Stance on Prohibition & Drug Addiction



THE TRIAL OF LEO FRANK

ATLANTA 1913-1915



DR. H. F. HARRIS, CHIEF STATE'S WITNESS AS
TO CONDITION OF MARY'S BODY.

1. Leo Frank: Manager of Atlanta National Pencil Company Manager

2. Mary Phagan: 13yo Company Employee

- Found Assaulted & Murdered April 26, 1913 in Company Basement

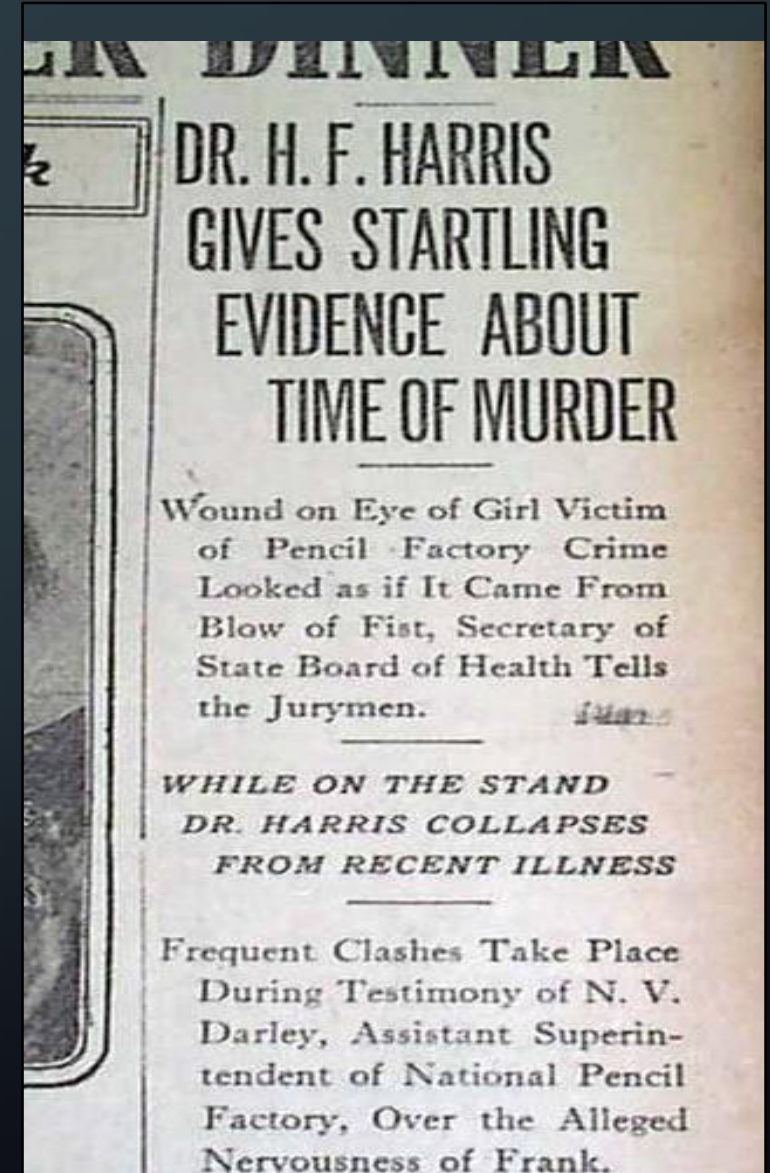
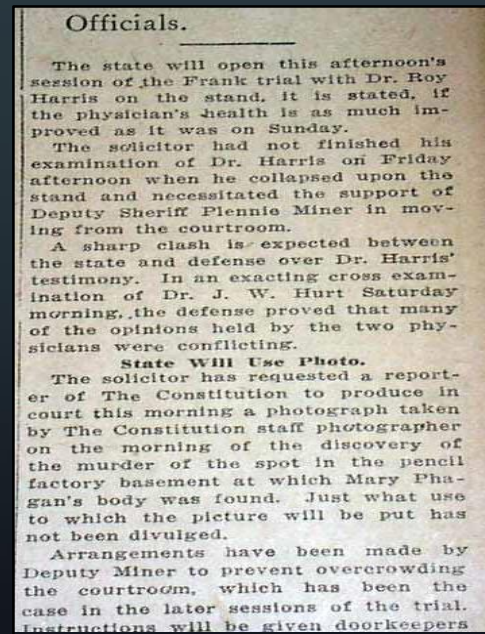
3. Evidence & Considerations:

- Jim Conley, Janitor: Contradictory Statements of Whereabouts
- Newt Lee, Night Watchman: Found the Body & Bloody Shirt @ Home
- Leo Frank: No Hard Evidence
- Extreme Anti-Semitism in 1913

4. Leo Frank: Arrested for Rape/Murder of Mary Phagan

5. Harris Conducted the Autopsy (body exhumed 3 times)

- Testified to the State of the Body
- Determined Time of Death
- No Evidence of Rape but Some Assault
- Testimony Refuted & Denounced





Leo Frank Lynching

Near this location on August 17, 1915, Leo M. Frank, the Jewish superintendent of the National Pencil Company in Atlanta, was lynched for the murder of thirteen-year-old Mary Phagan, a factory employee. A highly controversial trial fueled by societal tensions and anti-Semitism resulted in a guilty verdict in 1913. After Governor John M. Slaton commuted his sentence from death to life in prison, Frank was kidnapped from the state prison in Milledgeville and taken to Phagan's hometown of Marietta where he was hanged before a local crowd. Without addressing guilt or innocence, and in recognition of the state's failure to either protect Frank or bring his killers to justice, he was granted a posthumous pardon in 1986.

Erected by the Georgia Historical Society, the Jewish American
Society for Historic Preservation, and Temple Kol Emeth

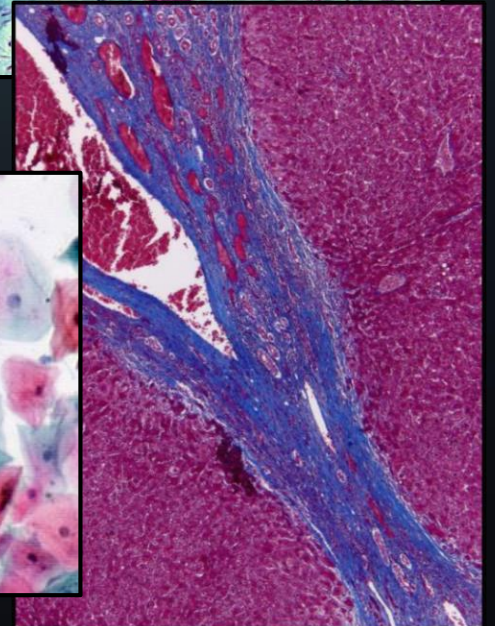
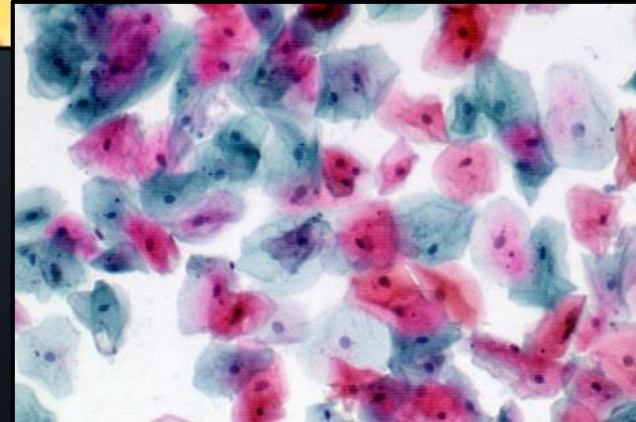
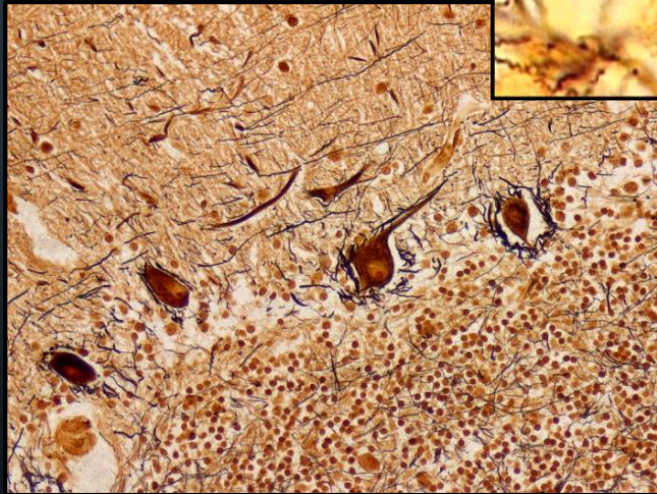
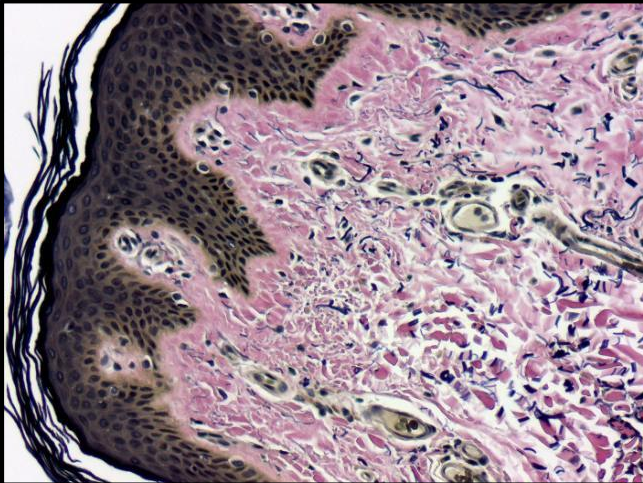
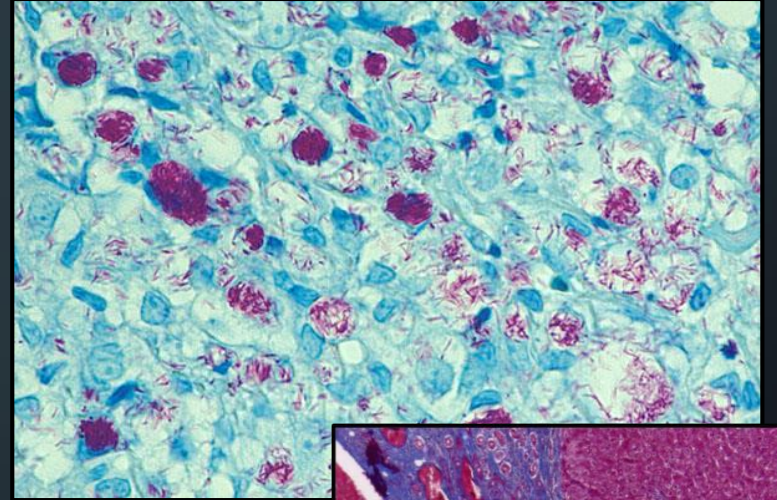
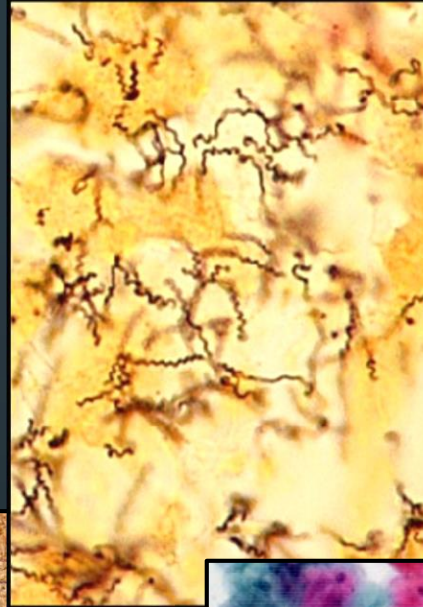
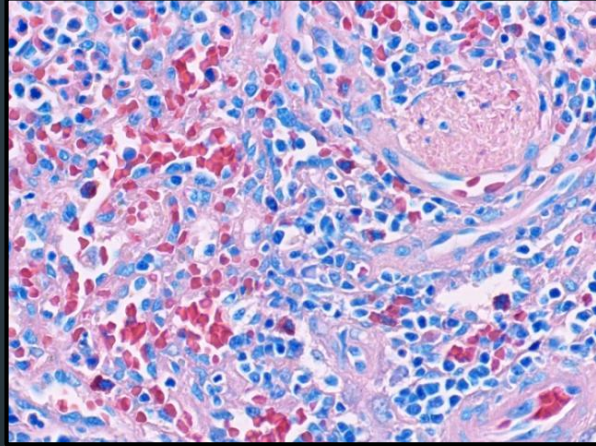
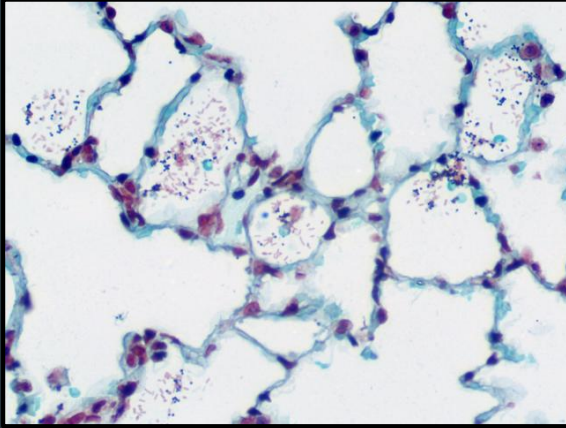


GARY GILL: 1944 -

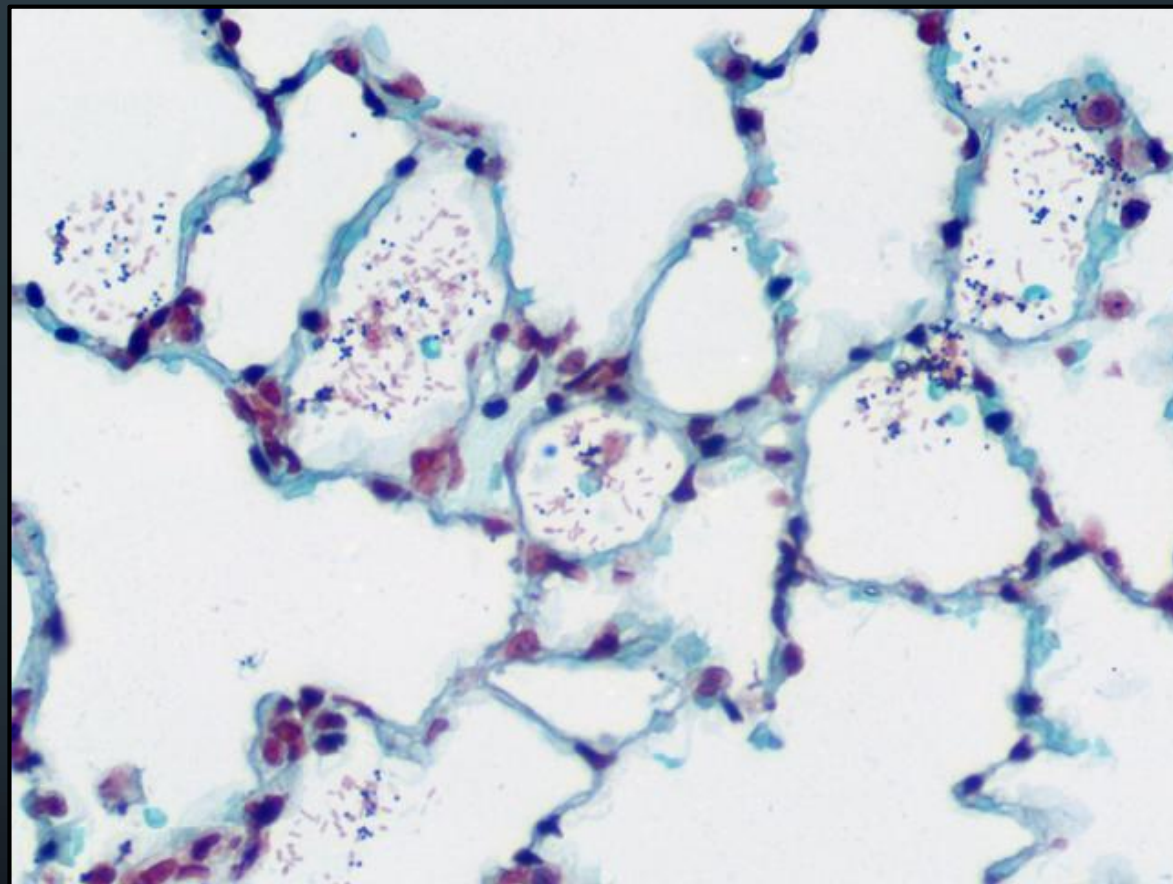
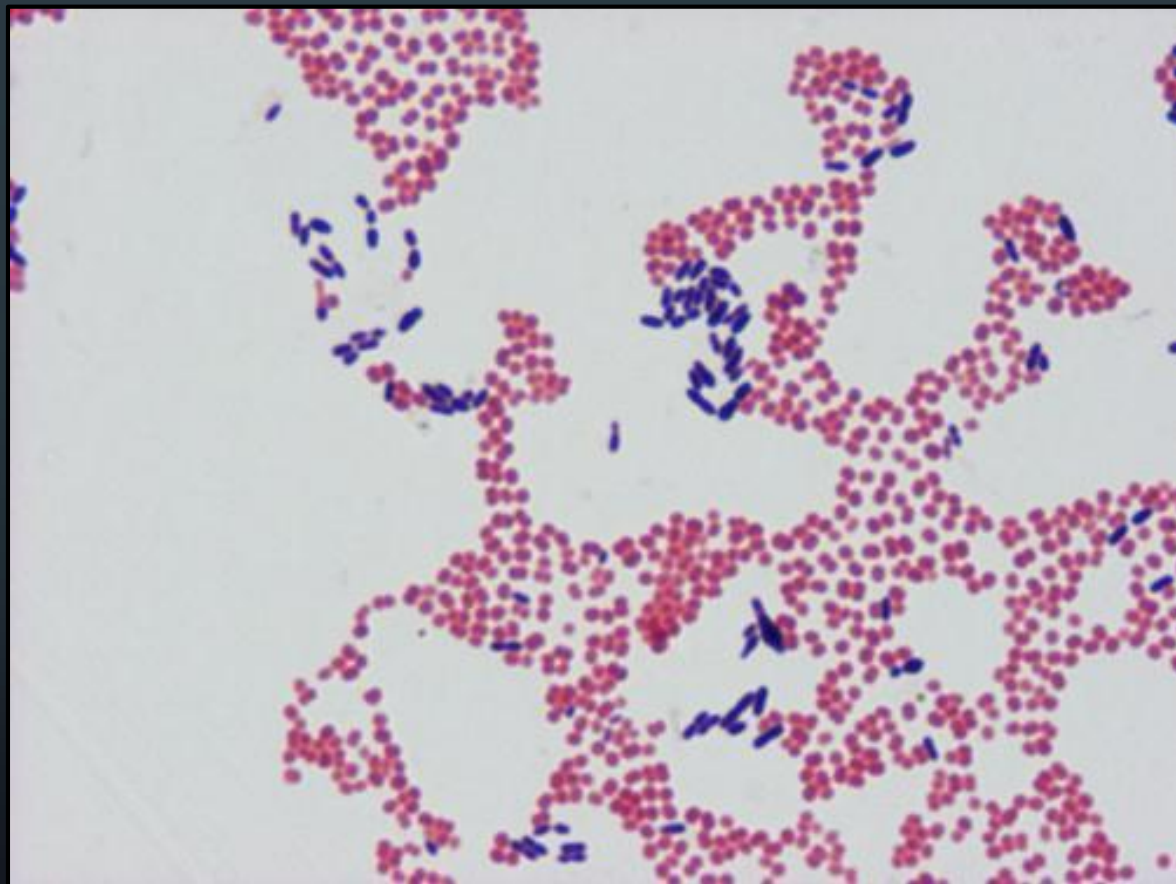
1. 1964: Johns Hopkins School of Cytology
2. 1964-87: Johns Hopkins Employee
 - Pap Stain - Lack of Standardization
 - Progressive Staining w/out Differentiation
 - Gill 1, 2, 3 for Varied Specimens
3. 1972: No Royalties Involved
 - “thanks to bad advice in 1972 from corporate counsel for Johns Hopkins Medical School.”
4. 1973: “Gill” Surname Registered w/US Patent/Trademark Office w/out Permission.



THE NAMES OF THE STAINS



WHAT'S MY NAME?



HANS CHRISTIAN GRAM: 1853-1938



1. Danish Physician and Bacteriologist
2. Development of a Method of Staining Bacteria
 - Only Stained Gram+ Organisms
 - Weigert Added Gram- Staining

"I have therefore published the method, although I am aware that as yet it is very defective and imperfect; but it is hoped that also in the hands of other investigators it will turn out to be useful."

JAMES HOWARD BROWN: 1884-1956

LENA BRENN (BROWN/BRENN GRAM STAIN)



1. Brown: American Bacteriologist/Professor
 - Streptococci Differentiation/Characterization
 - 100+ Publications on Organisms & Techniques
 - 1923-43: Johns Hopkins University
 - Colleague of Fite's
2. Brenn: Johns Hopkins University
 - Not Listed in Faculty/Resident Directory
 - Possible Researcher



1929-30 JOHNS HOPKINS PATHOLOGY DEPARTMENT



James Howard
Brown

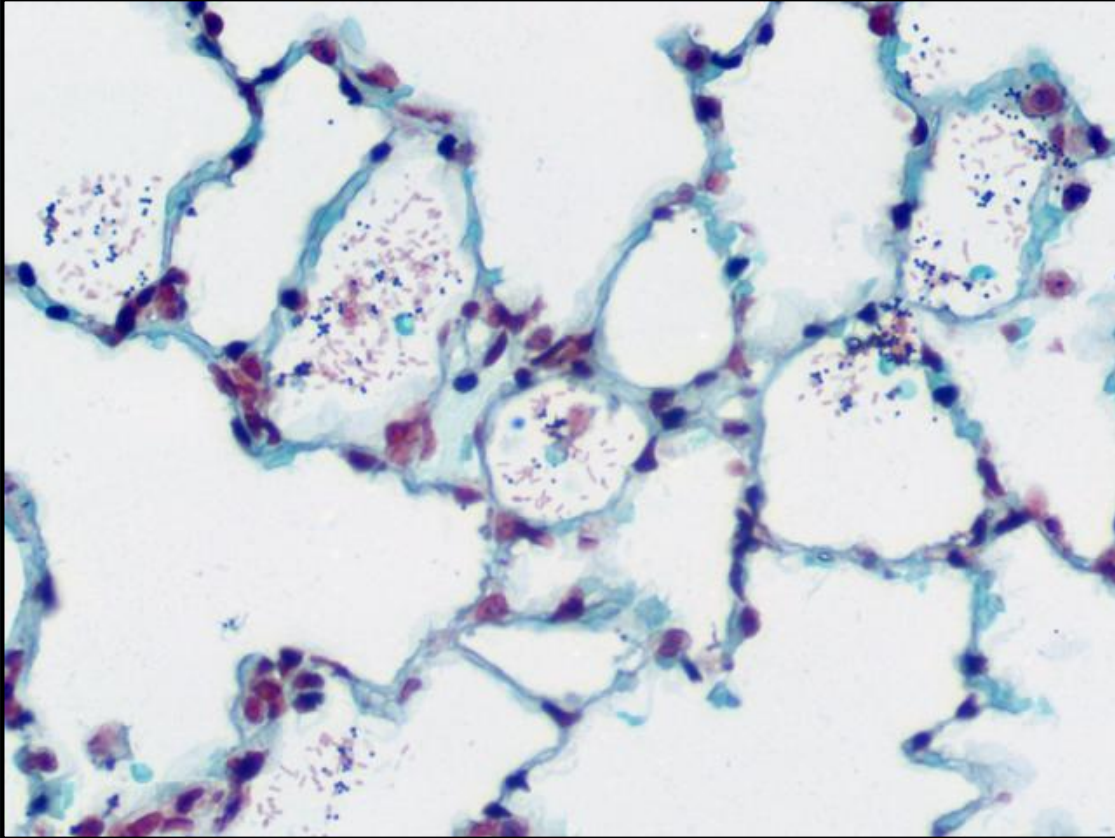
ROBERT C BROWN (BROWN/HOPPS GRAM STAIN)

HOWARD CARL HOPPS: 1914-1999

1. Hopps: Professor of Pathology - U of Missouri
 - Author of Many Publications: “Principles of Pathology”
 - 1973: “Staining of Bacteria in Tissue Sections: A Reliable Gram Stain Method” w/ Robert C. Brown, MD
2. Brown: U of North Carolina
 - Director of Cancer Research
 - Currently (as of 2015) Pathologist/Emeritus at Chapel Hill, NC

Possible AFIP (Armed Forces Institute of Path) Connection

GRAM TWORT STAIN



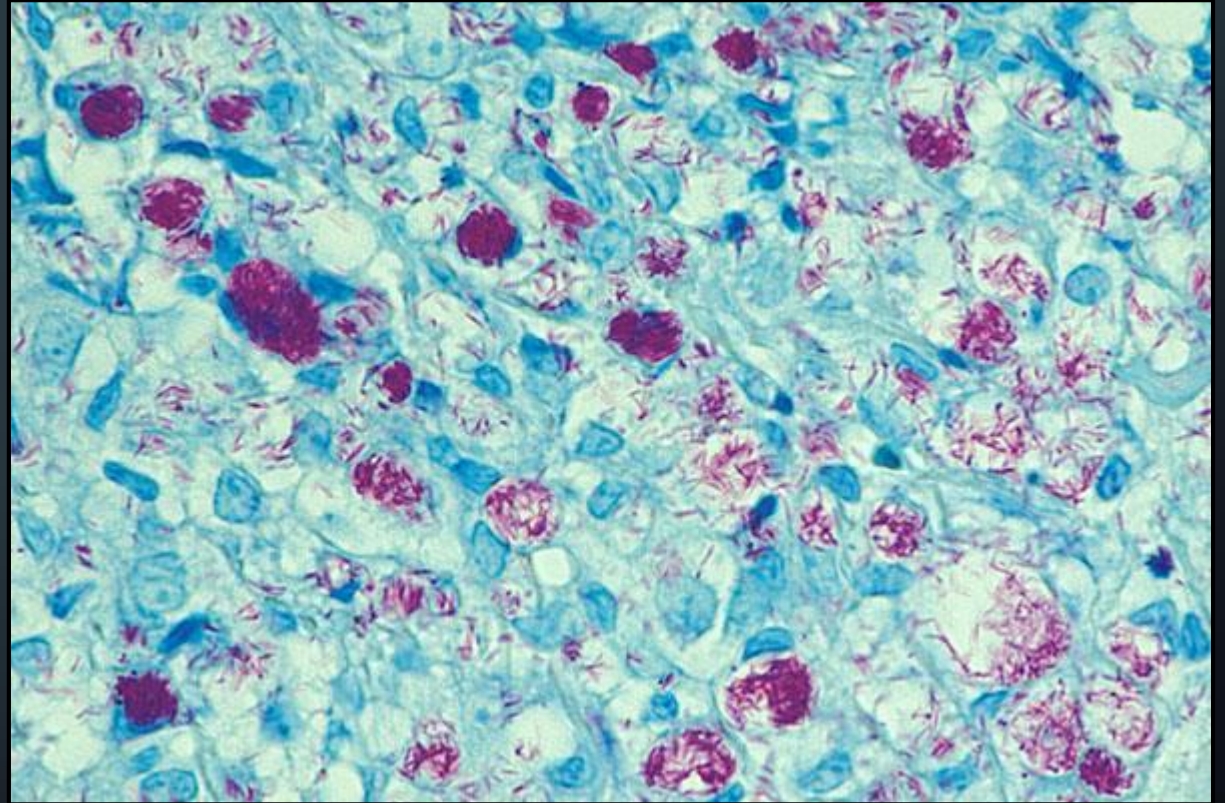
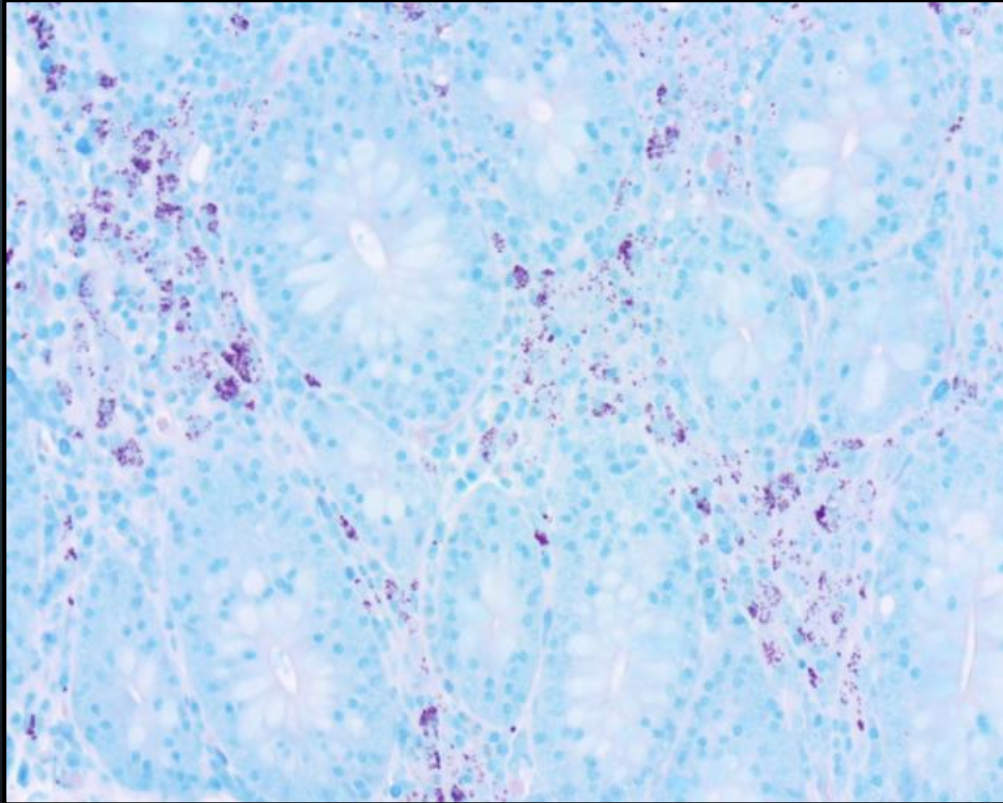
FREDERICK WILLIAM TWORT: 1877-1950



1. English Bacteriologist
2. 1909: Brown Animal Sanctuary Institute (London)
3. 1915: Discovered Bacteriophages
 - Leprosy, Bacteria & Virus Research
4. WWI: Served in Royal Army
5. 1924: “An Improved Neutral Red Light Green Double Staining for Animal Parasites, Microorganisms and Tissues”
6. 1944: Lab was Bombed & Destroyed
 - Lost Financial Support
 - Deprived of his Post



WHAT'S MY NAME?



FRANZ ZIEHL 1857-1926



1. German Bacteriologist/Professor - Lubeck
2. 1882: AFB 1st Described by Koch
3. 1882: Ehrlich AFB Stain (fuchsin/aniline blue stain)
4. 1882: Ziehl Modifications to the Carbol Fuchsin Stain
 - Basic Fuchsin w/Carbolic Acid
 - Carbolic Acid = Phenol

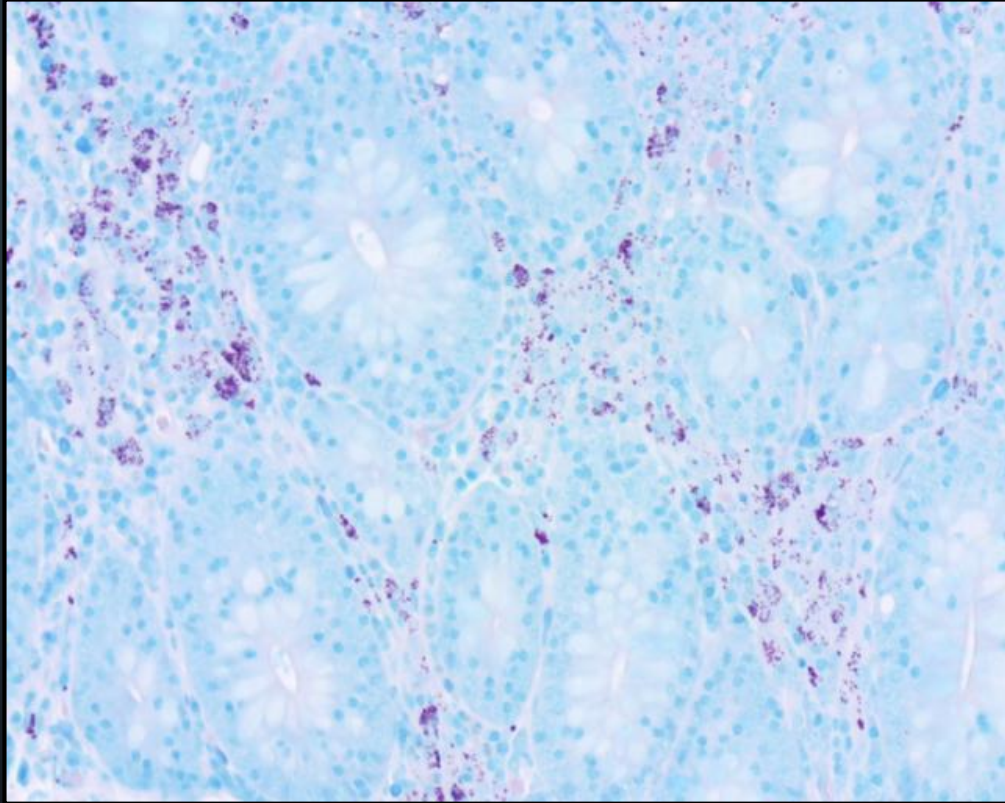
FRIEDRICH CARL ADOLF NEELEN

1854-1898

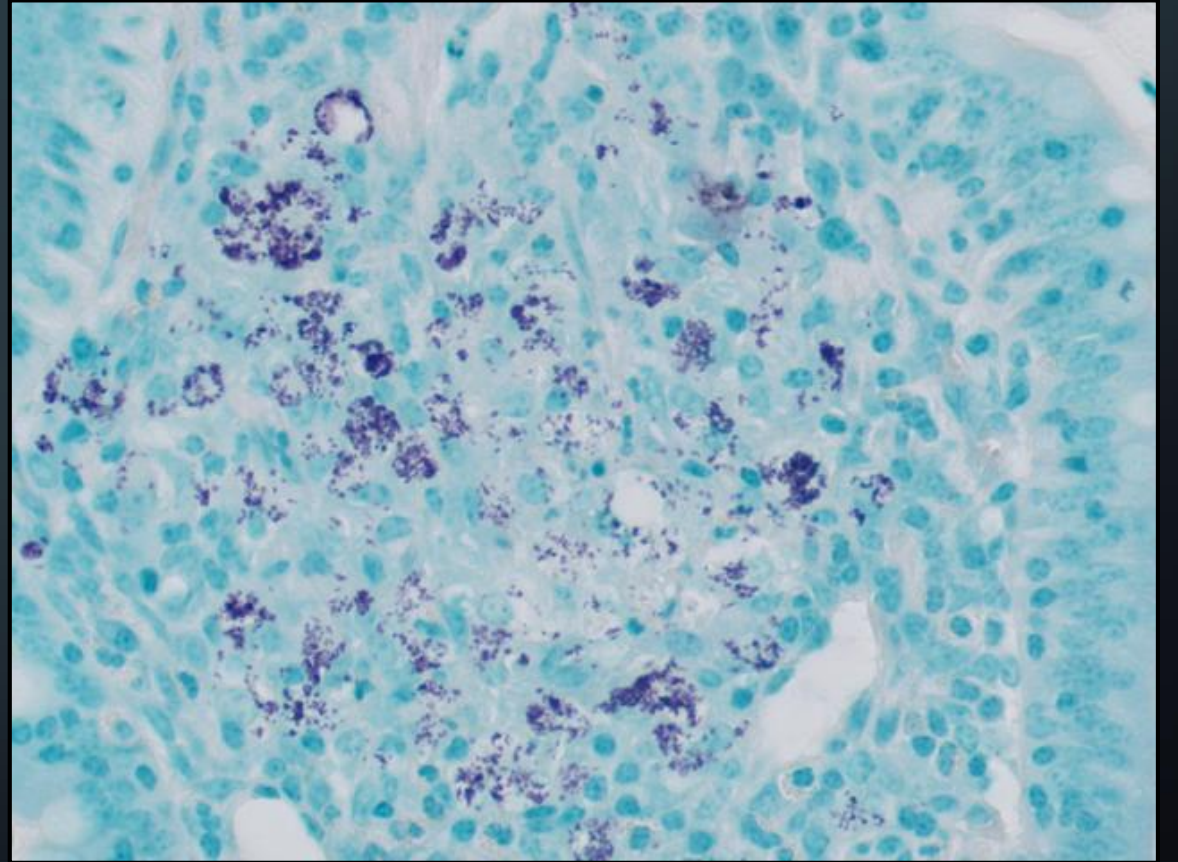
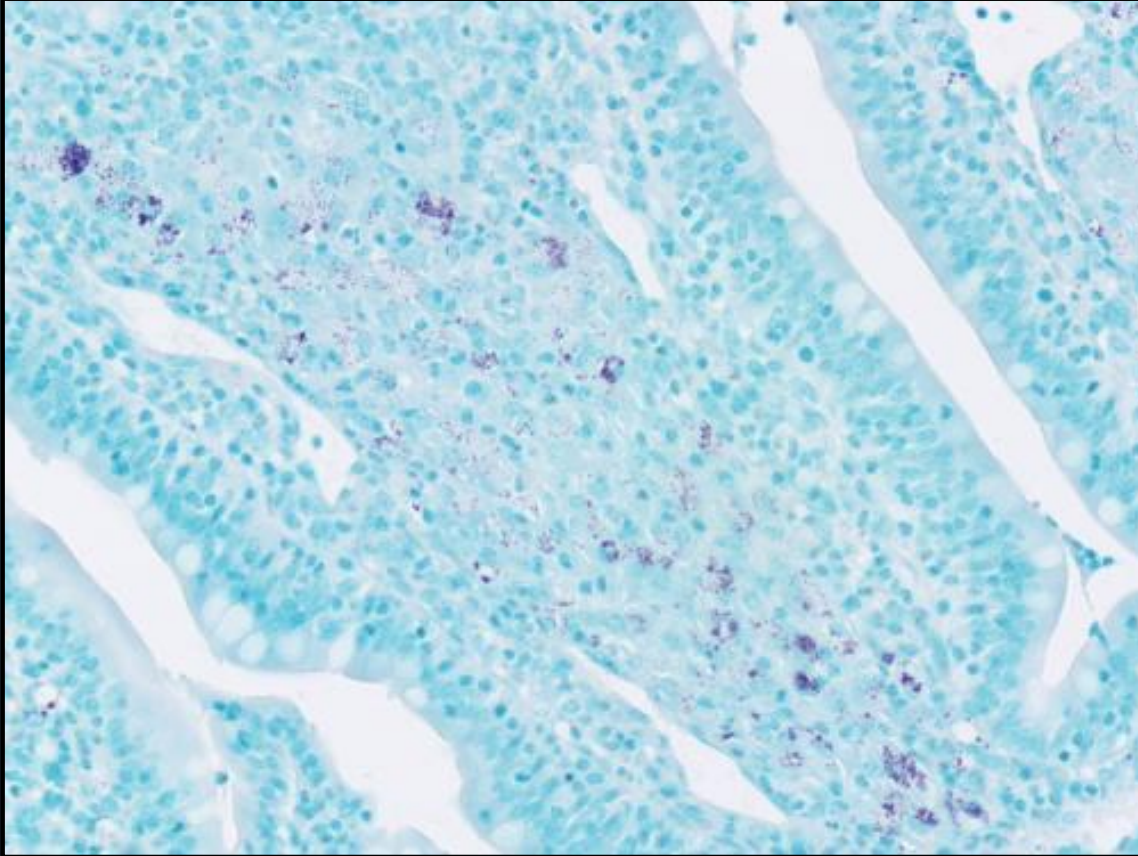


1. German Pathologist: Rostock/Dresden
2. 1883: Added Decolorizing Step to Ziehl's Modification
 - 25% Sulphuric Acid
3. Known as a Recluse
4. Died due to Pathogen Exposure After Years of Bacteriological Research

ZIEHL-NEELENSEN STAIN



WHAT'S MY NAME?



JOSEPH J. KINYOUN: 1860-1919



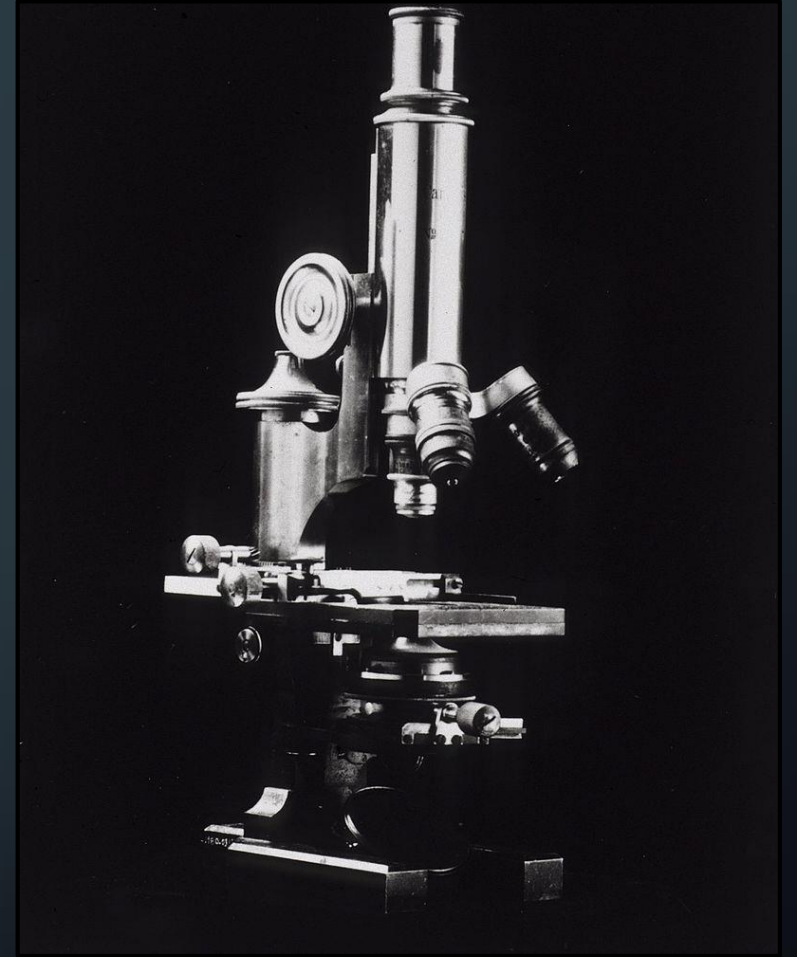
Born in North Carolina; Raised in Missouri

1882: Bellevue Medical College, NY

- **Self Taught in Histology, Pathology & Bacteriology**

1886-02: Marine Hosp Service Hygienic Lab

- **Research & Bacteriological Investigations**
- **Standardization of Biological Preparations**
- **Examination/Purification of Water Supplies**
- **Supported Quarantine Activities**
- **Cholera, Plague, Smallpox & Yellow Fever**



**MHS Laboratory of Hygiene in 1887 – Staten Island, NY
(to study the link between microscopic organisms & infectious diseases)**

1887: 1st Detection of Cholera in Western Hemisphere

- 2 Italian Ships w/Cholera Deaths En-Route

1899-1902: Sent to California for Plague Control

- Heavy Ship Traffic into San Francisco

1900: January - S.S. Australia Anchored in San Francisco

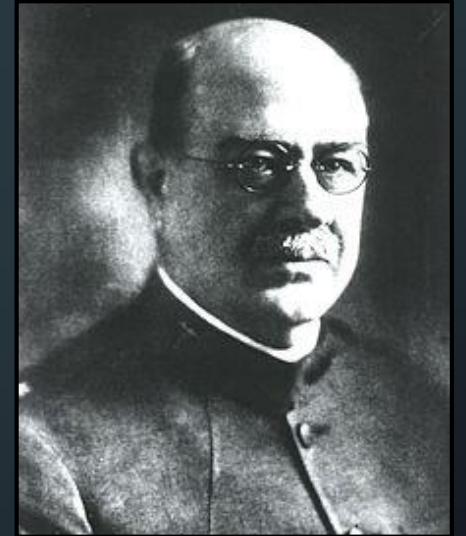
1900: March – 1st Case of Bubonic Plague in the US

- Plague “Concocted” by Kinyoun
- \$7000 Contract Put Out on Kinyoun

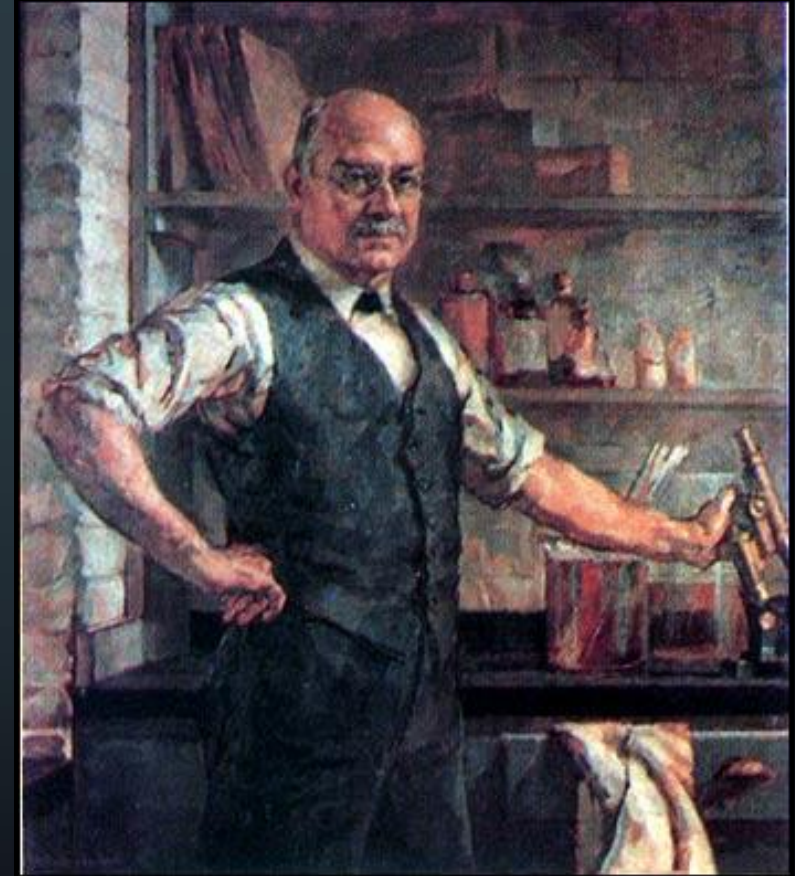
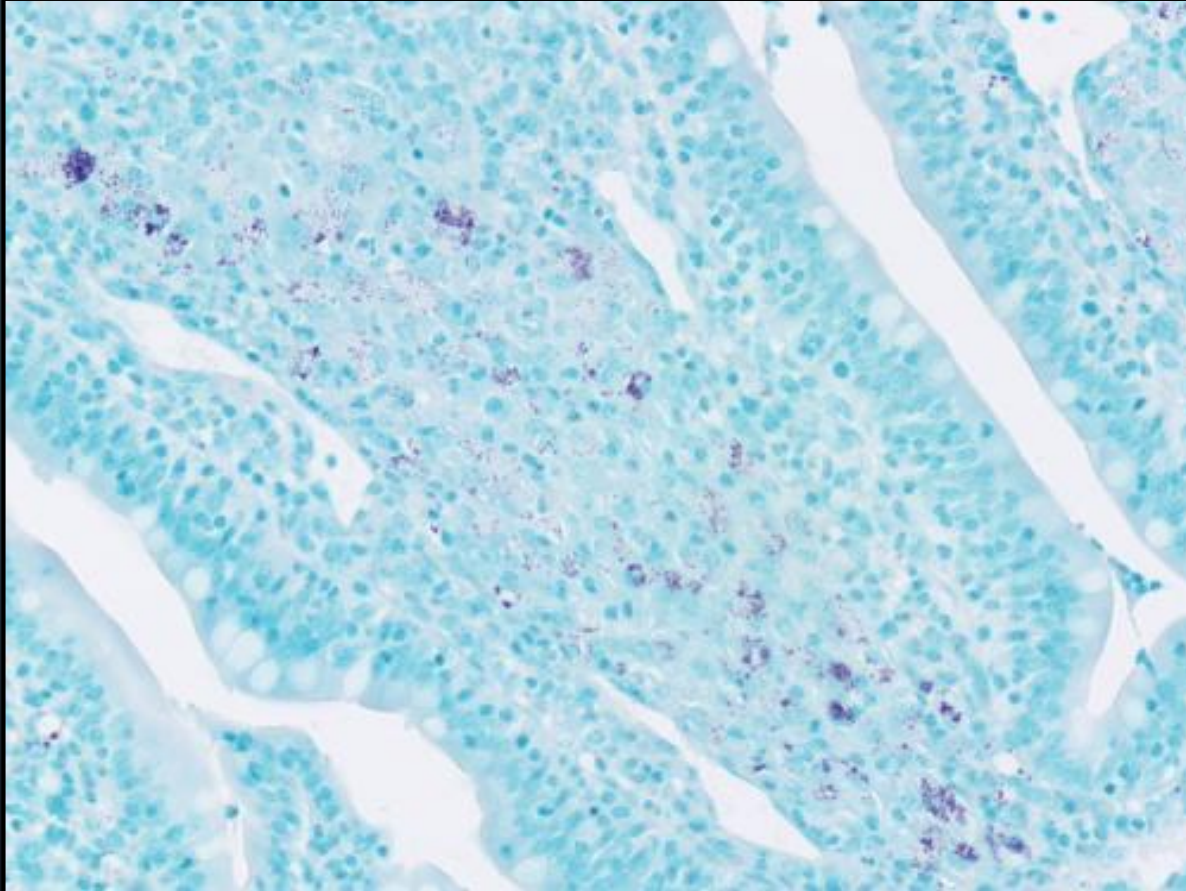
1902: Resigned Under Tremendous Stress/Pressure

1903-18: Worked on Water Quality, Public Sanitation, Vaccines

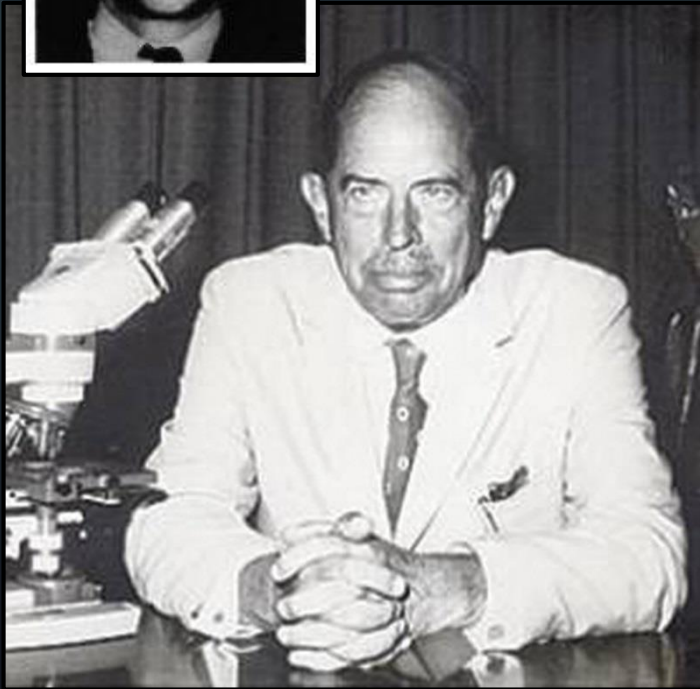
- 1915: Published Staining Method for Tubercle Bacilli



AFB KINYOUN STAIN



GEORGE LIDDLE FITE: 1904-1993



1. Born in Texas – Harvard Med School
2. 1929-31: NIH
 - Colleague of J.H. Brown (Brown/Brenn)
3. 1937-65: US Public Health Services
4. 1956-65: Hansen's Disease Center
 - Carville, Louisiana
 - Leprosy = Hansen's Disease (Gerhard Armauer Hansen)
5. Research: Histopathology of Leprosy
6. Prolific Writer & Renowned Speaker
7. Died from Pneumonia/Alzheimers

ABOUT LEPROSY



**Peel Island, Queensland
Lazaret/Leprosarium
1907-1959**

1. 1873: *M. leprae* Described by Hansen
 - Significant in Norway, Iceland, England
2. 1st Human Disease Bacterium Identified
3. leprae: “disease that makes skin scaly”
4. 4 Strains of *M. leprae*
 - Based on Geographic Regions
5. 1940s: 1st Effective Treatment
6. Current – Multidrug Therapy (MDT)

References Include:

- 1. Newcomer Supply for Stain Photo's: www.newcomersupply.com**
- 2. www.findagrave.com**
- 3. www.medscape.com**
- 4. American Society of Microbiology: www.asm.org**
- 5. American Journal of Clinical Pathology: <http://ajcp.oxfordjournals.org>**
- 6. Royal Society of Chemistry: www.rsc.org**
- 7. Thomas Jefferson University: www.Jefferson.edu**
- 8. Johns Hopkins University: www.pathology.jhu.edu**
- 9. Journal of Histotechnology: Reference Articles**
 - “A Short Histology of Histopathology Technique”, Michael Titford, Vol 29, No 2, 2006.**
 - “Harris Hematoxylin, What Harris Really Wrote and the Mechanism of Hemalum Stain”, Susan Meloan and Holde Puchtler, Vol 10, No 4, 1987.**