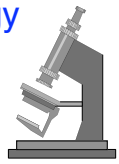
Chapter 1

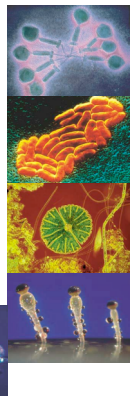
History & Scope of Microbiology



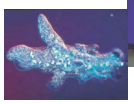
What is microbiology?

✓ study of organisms too small to be clearly seen by the unaided eye (i.e., microorganisms)

What are Microorganisms

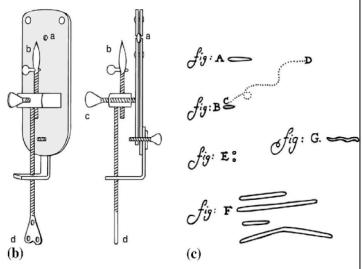


- ✓ Viruses - need to invade cells
- ✓ Bacteria - simple single celled organisms
- ✓ Algae - photosynthetic
- ✓ Fungi - yeasts and molds
- ✓ Protozoa - complex single celled organisms
- ✓ Others
  - ✓ *Helminths* - worms
  - ✓ Arthropods - insects

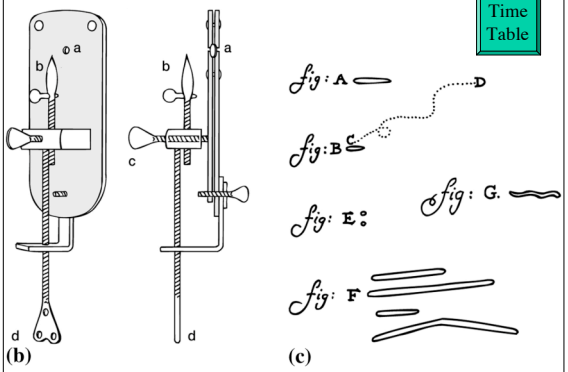


Discovery of Microorganisms

- ✓ Robert Hooke (1665) - compound microscope - cells of cork
- ✓ Antony van Leeuwenhoek (1676)
  - ✒ first person to observe and describe micro-organisms accurately



Antony van Leeuwenhoek



Some Historical Figures

✓ Schleiden and Schwann (1839) - Cell theory (Cells are the fundamental units of life and carry out all the basic functions of living things.)

History: The Conflict over Spontaneous Generation

✓ spontaneous generation
- ✏ living organisms can develop from nonliving or decomposing matter

✓ Francesco Redi (1668)
- ✏ disproved spontaneous generation for large animals
- ✏ showed that maggots on decaying meat came from fly eggs

Francesco Redi

Back

But could spontaneous generation be true for microorganisms?

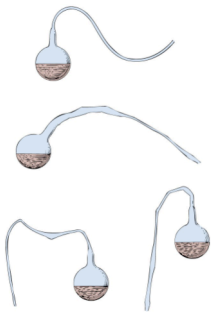
✓ John Needham (1713-1781)
- ✏ his experiment: mutton broth in flasks → boiled → sealed
- ✏ Results: broth became cloudy and contained microorganisms

✓ Lazzaro Sallanzani (1729-1799)
- ✏ Broth in flasks →sealed → boiled
- ✏ Results: not growth of microorganisms

Louis Pasteur (1861)

✓ his experiments
- ✏ placed nutrient solution in flasks
- ✏ created flasks with long, curved necks
- ✏ boiled the solutions
- ✏ left flasks exposed to air

✓ results: no growth of microorganisms



Louis Pasteur

Figure 1.11
Louis Pasteur (1822–1895), one of the founders of microbiology, is pictured here viewing a sample. Few microbiologists can match the scope and impact of his contributions to the science of microbiology.

Final blow to theory of spontaneous generation

- ✓ John Tyndall (1877)
  - ✏ demonstrated that dust carries microorganisms
  - ✏ showed that if dust was absent, nutrient broths remained sterile, even if directly exposed to air
  - ✏ also provided evidence for the existence of exceptionally heat-resistant forms of bacteria

The Role of Microorganisms in Disease

- ✓ was not immediately obvious
- ✓ establishing connection depended on development of techniques for studying microbes
- ✓ once established, led to study of host defenses - immunology

Recognition of the Relationship between Microorganisms and Disease

- ✓ Agostini Bassi (1835)
  - ✏ showed that a disease of silkworms was caused by a fungus
- ✓ M. J. Berkeley (ca. 1845)
  - ✏ demonstrated that the Great Potato Blight of Ireland was caused by a fungus
- ✓ Heinrich de Bary (1853)
  - ✏ showed that smut and rust fungi caused cereal crop diseases

More evidence…

- ✓ Louis Pasteur
  - ✏ showed that the pébrine disease of silkworms was caused by a protozoan

Other evidence…

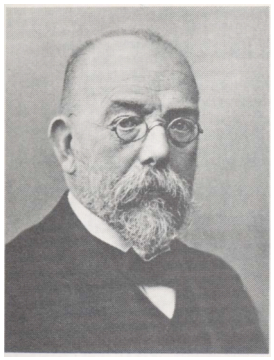
- ✓ Lister & Semmelweis - aseptic techniques in medicine
- ✓ Ignaz Philipp Semmelweis
  - ✏ Autopsies and disease (puerperal fever)
  - ✏ Nervous breakdown
- ✓ Joseph Lister
  - ✏ provided indirect evidence that microorganisms were the causal agents of disease
  - ✏ developed a system of surgery designed to prevent microorganisms from entering wounds
    - ⇨ Spray phenol in the instruments and over the wounds
  - ✏ his patients had fewer postoperative infections

Final proof…

- ✓ Robert Koch (1880s)
  - ✏ established the relationship between Bacillus anthracis and anthrax
  - ✏ used criteria developed by his teacher Jacob Henle (1809-1895)
  - ✏ these criteria now known as Koch's postulates
    - ⇨ still used today to establish the link between a particular microorganism and a particular disease

1876 - Robert Koch

- ✓ First to isolate and identify *B. anthracis* as the cause of anthrax
  - ✏ German Doctor
  - ✏ Country practice
  - ✏ Worked in his home
  - ✏ Transmitted the disease through 20 mice

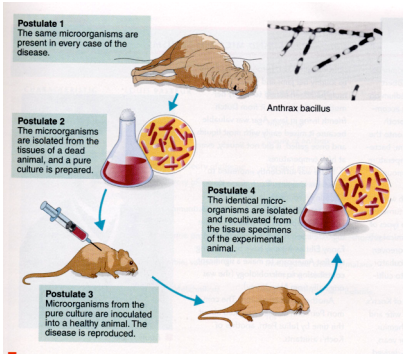


Koch's postulates

- ✓ The microorganism must be present in every case of the disease but absent from healthy individuals.
- ✓ The suspected microorganism must be isolated and grown in a pure culture.
- ✓ The same disease must result when the isolated microorganism is inoculated into a healthy host.
- ✓ The same microorganism must be isolated again from the diseased host.

Koch's Postulates

Postulates were originally outline by Jakob Henle in 1940



The Development of Techniques for Studying Microbial Pathogens

- ✓ Koch's work led to discovery or development of:
  - ✏ Agar (with the help of Fannie and Walter Hesse)
  - ✏ Petri dish (Richard Petri)
  - ✏ nutrient broth and nutrient agar
  - ✏ methods for isolating microorganisms- pure culture
  - ✏ anthrax
  - ✏ TB
  - ✏ cholera

Fannie and Walter Hesse

Other developments…

- ✓ Charles Chamberland (1851-1908)
  - ✏ developed porcelain bacterial filter
  - ✏ used to isolate first viruses studied
- ✓ Martinus Beijerinck - Characterized viruses

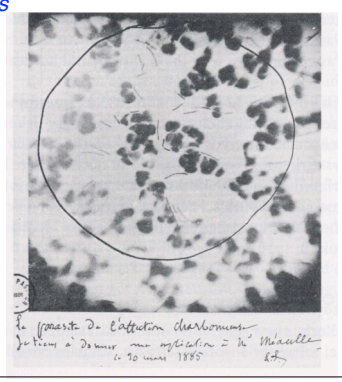
Immunological Studies

- ✓ Edward Jenner (ca. 1798)
  - ✏ used a vaccination procedure to protect individuals from smallpox
    - NOTE: this preceded the work establishing the role of microorganisms in disease

Other developments…

- ✓ Pasteur and Roux
  - ✏ discovered that incubation of cultures for long intervals between transfers caused pathogens to lose their ability to cause disease
- ✓ Pasteur and his coworkers
  - ✏ developed vaccines for chicken cholera, anthrax, and rabies

Louis Pasteur's original picture of *Bacillus anthracis*



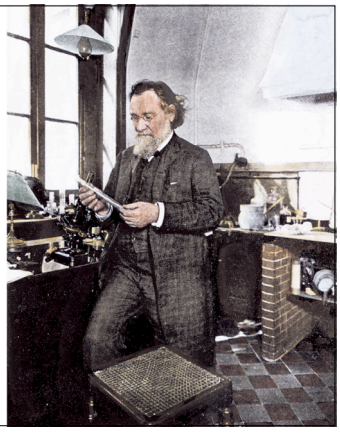
Some Historical Figures

- ✓ Hershey and Chase - Genetic material in some viruses is DNA
- ✓ Watson and Crick (1953) - Determined the structure of DNA
- ✓ Paul Ehrlich - Chemotherapy "magic bullet"
- ✓ Alexander Fleming (1928) - Discovered Penicillin

More developments…

- ✓ Emil von Behring (1854-1917) and Shibasaburo Kitasato (1852-1931)
  - ✏ developed antitoxins for diphtheria and tetanus
  - ✏ evidence for humoral immunity
- ✓ Elie Metchnikoff (1845-1916)
  - ✏ discovered bacteria-engulfing, phagocytic cells in the blood
  - ✏ evidence for cellular immunity

Elie Metchnikoff



Industrial Microbiology and Microbial Ecology

- ✓ Louis Pasteur
  - ✏ demonstrated that alcohol fermentations and other fermentations were the result of microbial activity
  - ✏ developed the process of pasteurization to preserve wine during storage

Industrial Microbiology and Microbial Ecology

- ✓ Sergei Winogradsky (1856-1953) and Martinus Beijerinck (1851-1931)
  - ✏ studied soil microorganisms and discovered numerous interesting metabolic processes (e.g., nitrogen fixation)
  - ✏ pioneered the use of enrichment cultures and selective media

Members of the Microbial World

- ✓ two types of cells
  - ✏ procaryotic cell
    - ⇨ relatively simple morphology
    - ⇨ lacks a true membrane-delimited nucleus
    - ⇨ Bacteria and Archaea
  - ✏ eucaryotic cell
    - ⇨ morphologically complex
    - ⇨ has a true membrane-delimited nucleus
    - ⇨ protozoa, algae, fungi, plants and animals
  - ✏ Viruses?

The Scope and Relevance of Microbiology

- ✓ importance of microorganisms
  - ✏ first living organisms on planet
  - ✏ live everywhere life is possible
  - ✏ more numerous than any other kind of organisms
  - ✏ global ecosystem depends on their activities
  - ✏ influence human society in many ways

Microbiology is a basic science

- ✓ Microbiologists study the basic biology of microorganisms
  - ✏ e.g., microbial morphology
  - ✏ e.g., microbial physiology
  - ✏ e.g., microbial genetics
- ✓ understanding microorganisms has improved the understanding of other organisms

Microbiology is also an applied science

- ✓ medical microbiology
- ✓ immunology
- ✓ food and dairy microbiology
- ✓ public health microbiology
- ✓ industrial microbiology
- ✓ agricultural microbiology

The Future of Microbiology:
Challenges and opportunities for future microbiologists

- ✓ infectious disease
- ✓ new and improved industrial processes
- ✓ microbial diversity and microbial ecology
  - ➨ less than 1% of earth's microbial population has been cultured

More challenges and opportunities…

- ✓ biofilms
- ✓ genome analysis
- ✓ microbes as model systems
- ✓ assessment of implications of new discoveries and technologies

Why use microbes in research?

- ✓ Simple structures
  - ➨ Unicellular
- ✓ Large numbers
  - ➨ Growing 1 billion microbes costs less than maintaining 10 rats
- ✓ Rapid growth
  - ➨ *E. coli* divides every 20 minutes
  - ➨ Excellent for genetic engineering

Table 1.1 Some Important Events in the Development of Microbiology

Date	Microbiological History	Other Historical Events
1546	Fracastoro suggests that invisible organisms cause disease	Publication of Copernicus's work on the heliocentric solar system (1543)
1590–1608	Jansen develops first useful compound microscope	Shakespeare's *Hamlet* (1600–1601)
1676	Leeuwenhoek discovers "animalcules"	J. S. Bach and Handel born (1685)
1688	Redi publishes work on spontaneous generation of maggots	Isaac Newton publishes the *Principia* (1687)
		Linnaeus's *Systema Naturae* (1735)
		Mozart born (1756)
1765–1776	Spallanzani attacks spontaneous generation	
1786	Müller produces first classification of bacteria	French Revolution (1789)
1798	Jenner introduces cowpox vaccination for smallpox	Beethoven's first symphony (1800)
		The battle of Waterloo and the defeat of Napoleon (1815)
		Faraday demonstrates the principle of an electric motor (1821)
1838–1839	Schwann and Schleiden, the Cell Theory	England issues first postage stamp (1840)
1835–1844	Bassi discovers that silkworm disease is caused by a fungus and proposes that many diseases are microbial in origin	Marx's *Communist Manifesto* (1848)
1847–1850	Semmelweis shows that childbed fever is transmitted by physicians and introduces the use of antiseptics to prevent the disease	Velocity of light first measured by Fizeau (1849)
		Clausius states the first and second laws of thermodynamics (1850)
1849	Snow studies the epidemiology of a cholera epidemic in London	Graham distinguishes between colloids and crystalloids
		Melville's *Moby Dick* (1851)
		Otis installs first safe elevator (1854)
		Bunsen introduces the use of the gas burner (1855)
1857	Pasteur shows that lactic acid fermentation is due to a microorganism	
1858	Virchow states that all cells come from cells	Darwin's *On the Origin of Species* (1859)
1861	Pasteur shows that microorganisms do not arise by spontaneous generation	American Civil War (1861–1865)
		Mendel publishes his genetics experiments (1865)
		Cross-Atlantic cable laid (1865)
1867	Lister publishes his work on antiseptic surgery	Dostoevski's *Crime and Punishment* (1866)
1869	Miescher discovers nucleic acids	Franco-German War (1870–1871)

continued...

Table 1.1 Some Important Events in the Development of Microbiology

Date	Microbiological History	Other Historical Events
1876–1877	Koch demonstrates that anthrax is caused by *Bacillus anthracis*	Bell invents telephone (1876)
		Edison's first light bulb (1879)
1880	Laveran discovers *Plasmodium*, the cause of malaria	
1881	Koch cultures bacteria on gelatin	Ives produces first color photograph (1881)
	Pasteur develops anthrax vaccine	
1882	Koch discovers tubercle bacillus, *Mycobacterium tuberculosis*	First central electric power station constructed by Edison (1882)
1884	Koch's postulates first published	Mark Twain's *The Adventures of Huckleberry Finn* (1884)
	Metchnikoff describes phagocytosis	
	Autoclave developed	
	Gram stain developed	
1885	Pasteur develops rabies vaccine	First motor vehicles developed by Daimler (1885–1886)
	Escherich discovers *Escherichia coli*, a cause of diarrhea	
1886	Fraenkel discovers *Streptococcus pneumoniae*, a cause of pneumonia	
1887	Petri dish (plate) developed by Richard Petri	
1887–1890	Winogradsky studies sulfur and nitrifying bacteria	Hertz discovers radio waves (1888)
1889	Beijerinck isolates root nodule bacteria	Eastman makes box camera (1888)
1890	Von Behring prepares antitoxins for diphtheria and tetanus	
1892	Ivanowsky provides evidence for virus causation of tobacco mosaic disease	First zipper patented (1895)
1894	Kitasato and Yersin discover *Yersinia pestis*, the cause of plague	
1895	Bordet discovers complement	Röntgen discovers X rays (1895)
1896	Van Ermengem discovers *Clostridium botulinum*, the cause of botulism	
1897	Buchner prepares extract of yeast that ferments	Thomson discovers the electron (1897)
	Ross shows that malaria parasite is carried by the mosquito	Spanish-American War (1898)
1899	Beijerinck proves that a virus particle causes the tobacco mosaic disease	
1900	Reed proves that yellow fever is transmitted by the mosquito	Planck develops the quantum theory (1900)
1902	Landsteiner discovers blood groups	First electric typewriter (1901)

continued...

Table 1.1 Some Important Events in the Development of Microbiology

Date	Microbiological History	Other Historical Events
1903	Wright and others discover antibodies in the blood of immunized animals	First powered aircraft (1903)
1905	Schaudinn and Hoffmann show *Treponema pallidum* causes syphilis	Einstein's special theory of relativity (1905)
1906	Wassermann develops complement fixation test for syphilis	
1909	Ricketts shows that Rocky Mountain spotted fever is transmitted by ticks and caused by a microbe (*Rickettsia rickettsii*)	First model T Ford (1908)
		Peary and Hensen reach North Pole (1909)
1910	Ehrlich develops chemotherapeutic agent for syphilis	Rutherford presents his theory of the atom (1911)
1911	Rous discovers a virus that causes cancer in chickens	Picasso and cubism (1912)
		World War I begins (1914)
1915–1917	D'Herelle and Twort discover bacterial viruses	Einstein's general theory of relativity (1916)
		Russian Revolution (1917)
1921	Fleming discovers lysozyme	
1923	First edition of *Bergey's Manual*	Lindberg's transatlantic flight (1927)
1928	Griffith discovers bacterial transformation	
1929	Fleming discovers penicillin	Stock market crash (1929)
1931	Van Niel shows that photosynthetic bacteria use reduced compounds as electron donors without producing oxygen	
1933	Ruska develops first transmission electron microscope	Hitler becomes chancellor of Germany (1933)
1935	Stanley crystallizes the tobacco mosaic virus	
	Domagk discovers sulfa drugs	
1937	Chatton divides living organisms into procaryotes and encaryotes	Krebs discovers the citric acid cycle (1937)
		World War II begins (1939)
1941	Beadle and Tatum, one-gene-one-enzyme hypothesis	
1944	Avery shows that DNA carries information during transformation	The insecticide DDT introduced (1944)
	Waksman discovers streptomycin	
		Atomic bombs dropped on Hiroshima and Nagasaki (1945)
1946	Lederberg and Tatum describe bacterial conjugation	United Nations formed (1945)
		First electronic computer (1946)
1949	Enders, Weller, and Robbins grow poliovirus in human tissue cultures	

continued...

Table 1.1 Some Important Events in the Development of Microbiology

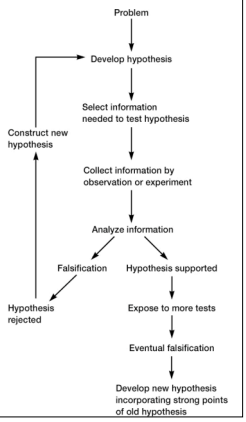
Date	Microbiological History	Other Historical Events
1950	Lwoff induces lysogenic bacteriophages	Korean War begins (1950)
1952	Hershey and Chase show that bacteriophages inject DNA into host cells	First hydrogen bomb exploded (1952)
		Stalin dies (1952)
	Zinder and Lederberg discover generalized transduction	First commercial transistorized product (1952)
1953	Phase-contrast microscope developed	U.S. Supreme Court rules against segregated schools (1954)
	Medawar discovers immune tolerance	
	Watson and Crick propose the double helix structure for DNA	
1955	Jacob and Wollman discover the F factor is a plasmid	Montgomery bus boycott (1955)
	Jerne and Burnet propose the clonal selection theory	Sputnik launched by Soviet Union (1957)
1959	Yalow develops the radioimmunoassay technique	Birth control pill (1960)
1961	Jacob and Monod propose the operon model of gene regulation	First humans in space (1961)
1961–1966	Nirenberg, Khorana, and others elucidate the genetic code	Cuban missile crisis (1962)
		Nuclear test ban treaty (1963)
1962	Porter proposes the basic structure for immunoglobulin G	Civil Rights March on Washington (1963)
	First quinolone antimicrobial (nalidixic acid) synthesized	President Kennedy assassinated (1963)
		Arab-Israeli War (1967)
		Martin Luther King assassination (1968)
		Neil Armstrong walks on the moon (1969)
1970	Discovery of restriction endonucleases by Arber and Smith	
	Discovery of reverse transcriptase in retroviruses by Temin and Baltimore	
1973	Ames develops a bacterial assay for the detection of mutagens	Salt I Treaty (1972)
	Cohen, Boyer, Chang, and Helling use plasmid vectors to clone genes in bacteria	Vietnam War ends (1973)
1975	Kohler and Milstein develop technique for the production of monoclonal antibodies	President Nixon resigns because of Watergate cover-up (1974)
	Lyme disease discovered	
1977	Recognition of archaea as a distinct microbial group by Woese and Fox	Panama Canal Treaty (1977)

continued...

Table 1.1 Some Important Events in the Development of Microbiology

Date	Microbiological History	Other Historical Events
	Gilbert and Sanger develop techniques for DNA sequencing	
1979	Insulin synthesized using recombinant DNA techniques	Hostages seized in Iran (1978)
	Smallpox declared officially eliminated	Three Mile Island disaster (1979)
1980	Development of the scanning tunneling microscope	Home computers marketed (1980)
1982	Recombinant hepatitis B vaccine developed	AIDS first recognized (1981)
1982–1983	Discovery of catalytic RNA by Cech and Altman	First artificial heart implanted (1982)
1983–1984	The human immunodeficiency virus isolated and identified by Gallo and Montagnier	Meter redefined in terms of distance light travels (1983)
	The polymerase chain reaction developed by Mullis	
1986	First vaccine (hepatitis B vaccine) produced by genetic engineering approved for human use	Gorbachev becomes Communist party general secretary (1985)
		Berlin Wall falls (1989)
1990	First human gene-therapy testing begun	Persian Gulf War with Iraq begins (1990)
		Soviet Union collapse; Boris Yeltsin comes to power (1991)
1992	First human trials of antisense therapy	
1995	Chickenpox vaccine approved for U.S. use	
	Haemophilus influenzae genome sequenced	
1996	*Methanococcus jannaschii* genome sequenced	Water found on the moon (1998)
	Yeast genome sequenced	
1997	Discovery of *Thiomargarita namibiensis*, the largest known bacterium	
	Escherichia coli genome sequenced	
2000	Discovery that *Vibrio cholerae* has two separate chromosomes	

Scientific method



The Scientific Method

- ✓Observation
- ✓Establishing a model
- ✓Formulate a hypothesis
- ✓Experimentation
- ✓Collecting and evaluating results
- ✓Drawing conclusions
- ✓Reporting what has been found

Observations

- ✓Looking
- ✓Hearing
- ✓Smelling
- ✓Touching
- ✓Measuring
- ✓Reading about previous studies
- ✓Serendipity

Models

- ✓A simplified view of how the components of a system operate.
  - ✓ Must be consistent with previous scientific knowledge.
  - ✓Must offer new insight.
- ✓May compare a process that is not understood to one that is.

Hypothesis

- Is NOT an educated guess.
- Someone can devise an experiment to disprove the hypothesis if it were incorrect.
  - Can you truly prove an hypothesis correct??
- A hypothesis is valuable only if it is testable.

Two forms of hypothesis

- ✓ Null hypothesis (H_o)
  - ✓ States that what is observed or measured is not unusual from what is usually observed, or from what is seen in the control experiment.
- ✓ Alternative hypothesis (H_a)
  - ✓ States that what is observed or measured is unusual from what is usually observed.

Experiments

- ✓ Procedures carried out under conditions controlled by the scientist.
- ✓ Experiments include controls and treatment variables designed and implemented to prove the hypothesis false if possible.

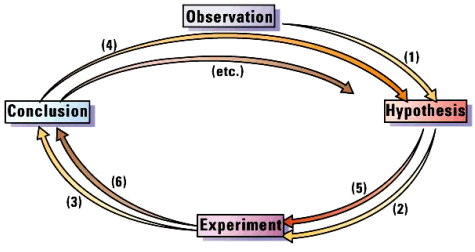
Collecting and Evaluating Results

- ✓ Collect experimental data.
- ✓ Computer and statistical analysis.

Conclusions

- ✓ Evaluate the results.
- ✓ Refine hypothesis or test alternative hypothesis.

Scientific method



Reporting

- ✓ Inform other scientists.
- ✓ Permits scrutiny of scientific community.
- ✓ Informs the public.

Theory

- ✓ A set of related hypotheses that consistently resists scientists efforts to disprove them.

Objective

- ✓ Determine the therapeutic efficacy of IFNγ alone and in combination with fluconazole for the treatment of experimental coccidioidal meningitis.

Interferon Gamma

- ✓ IFNγ favors Th-1 responses
- ✓ IFNγ activates NO producing cells -- peripheral macrophages, microglia, and astrocytes
- ✓ IFNγ activates oxygen metabolite producing cells -- neutrophils, monocytes
- ✓ Therapy for certain intracellular infections
- ✓ Enhances in vitro effector-cell antifungal activity against *Coccidioides immitis*

Methods

- ✓ NZW Rabbits, 3-4 kg, male
- ✓ Hydrocortisone acetate, I.M. Days -1, 0, 1, 2, 3
- ✓ *Coccidioides immitis* challenge, 4.4 x 10^4 & 6.0 x 10^4 arthroconidia, intracisternally, day 0
- ✓ Treatment - 21 days starting on day 5
  - ⇨PEG-200 orally Q.D. & Saline SQ Q.O.D. (control)
  - ⇨PEG-200 orally Q.D. & IFNγ (1 x 10^6 U/kg) SQ Q.O.D.
  - ⇨FCZ (40 mg/kg/day) orally Q.D. & Saline SQ Q.O.D.
  - ⇨FCZ (40 mg/kg/day) orally Q.D. & IFNγ (1 x 10^6 U/kg) SQ Q.O.D.
  - ⇨Uninfected animals - PEG-200 orally Q.D. & IFNγ (1 x 10^6 U/kg) SQ Q.O.D.

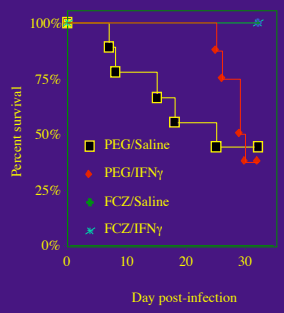
Methods

- Daily clinical evaluation
- CSF and serum sampled every 7 to 12 days
- Buprenorphine was given as needed to alleviate pain and discomfort
- Moribund animals were euthanized
- Euthanize surviving animals 7 or 8 days after last treatment

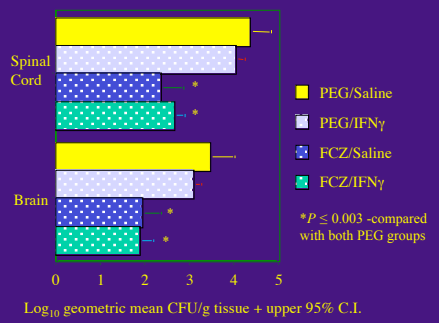
Time to Euthanasia

Treatment	n	Mean time to euthanasia (days)
PEG/Saline	9	22.3
PEG/IFNγ	8	29.4
FCZ/Saline	8	32.0*
FCZ/IFNγ	8	32.0*

*$P \leq 0.02$



Recovery of *C. immitis* from Spinal Cord and Brain



*$P \leq 0.003$ -compared with both PEG groups

Log_{10} geometric mean CFU/g tissue + upper 95% C.I.

Conclusions

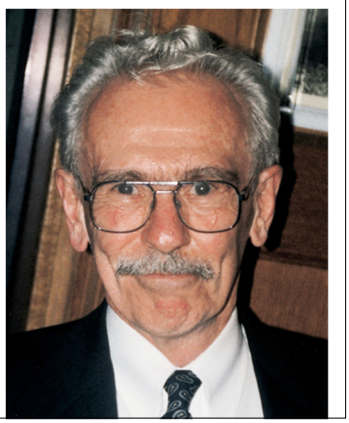
- ✓ IFNγ appeared to have a modest effect on survival and tissue CFU reduction, however, it was not significant.
- ✓ Fluconazole was effective at controlling coccidioidal meningitis and reducing *C. immitis* in the CSF and tissues.
- ✓ At the dosages tested, it is not clear if IFNγ-FCZ combination therapy has an advantage over fluconazole alone.

✓Rita R. Colwell-
✓Study and genetics of *Vibrio Cholerae*
✓Established the field of Marine Biotechnology

✓R.G.E Murray-
✓Bacterial envelops and taxonomy

✓Martha Howe-
✓Contributed greatly to the understanding of thebacteriophage Mu

✓Fredrerick C. Neidhardt-
✓Regulation of E. coli physiology and metabolism



✓Jean E. Brenchley-
✓Studied glutamate and glutamine metabolism
✓Biotechnoligical uses of phychrophilic microbes