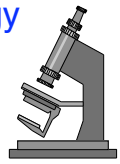
Scope and History of Microbiology

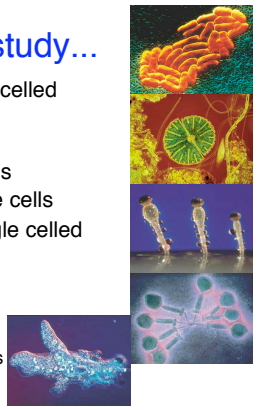


Living organisms…

- ✓reproduce
- ✓require a carbon and energy source
- ✓form new cell structures and components
- ✓excrete wastes
- ✓are irritabl
- ✓mutate

Microbiologists study...

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

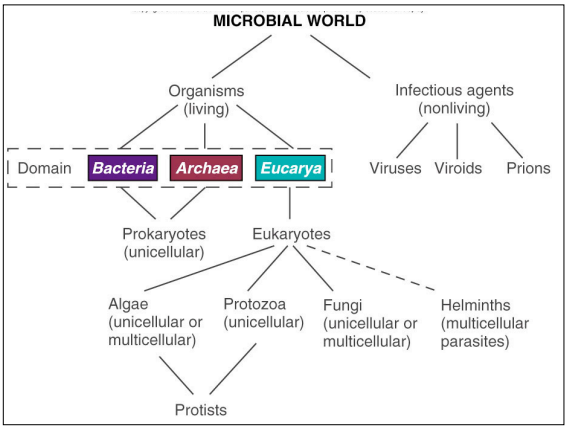


Domain System (Carl Woese, 1977)

- ✓Based on the rRNA sequences
  - ✏Bacteria
    - ✑Single cell prokaryotes (no membrane organelles)
    - ✑Contain peptidoglycan in the cell wall
  - ✏Archaea
    - ✑ Single cell prokaryotes
    - ✑ No peptidoglycan in the cell wall
    - ✑ Typically grow in extreme environments (ex. hot springs)
  - ✏Eucarya
    - ✑Single or multicellular eukaryotes
    - ✑ - Members include algae, fungi, and protozoa
    - ✑ - Contain membrane organelles (ex. mitochondria)

"Non-living Microbes"

- ✓Viruses
  - ✏Nucleic acid surrounded by a protein coat
- ✓Viroids
  - ✏Single short RNA molecules that infect plants
- ✓Prions (Prusiner, 1982)
  - ✏Protein only; causes different neurodegenerative diseases



Fields of Microbiology

- ✓ Organism
  - ✓ Bacteriology
  - ✓ Phycology
  - ✓ Mycology
  - ✓ Protozoology
  - ✓ Parasitology
  - ✓ Virology
- ✓ Process or function
  - ✓ Microbial metabolism
  - ✓ Microbial Genetics
  - ✓ Microbial Ecology
- ✓ Health related
  - ✓ Immunology
  - ✓ Epidemiology
  - ✓ Etiology
  - ✓ Infection control
  - ✓ Chemotherapy
- ✓ Application of knowledge
  - ✓ Food and beverage technology
  - ✓ Environmental microbiology
  - ✓ Industrial microbiology
  - ✓ Pharmaceutical Microbiology
  - ✓ Genetic engineering

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

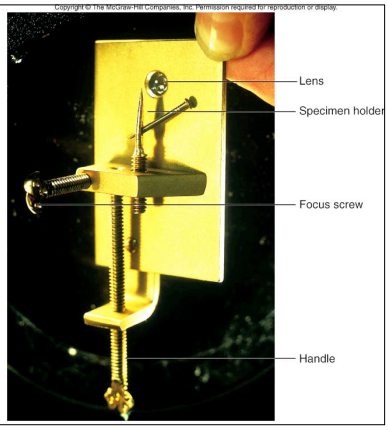
Some Historical Figures

- ✓ Robert Hooke (1665) - compound microscope - cells of cork
- ✓ Anton van Leeuwenhoek (1670s) - observed first living cells
- ✓ Schleiden and Schwann (1839) - Cell theory (Cells are the fundamental units of life and carry out all the basic functions of living things.)
- ✓ Francesco Redi (1668) - Offered evidence to disproved spontaneous generation
- ✓ Louis Pasteur and John Tyndall (1861) - Successfully disproved spontaneous generation
- ✓ Louis Pasteur - Pasteurization, Silkworms, rabies vaccine, Cholera vaccine, anthrax

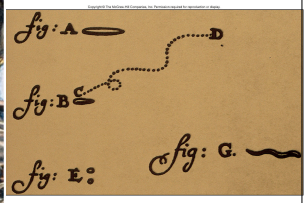
Some Historical Figures

- ✓ Robert Koch (1880s)- Koch's postulates, pure culture, anthrax, TB, cholera
- ✓ Semmelweis and Lister - aseptic techniques in medicine
- ✓ Edward Jenner - Vaccination for smallpox
- ✓ Martinus Beijerinck - Characterized viruses
- ✓ Hershey and Chase - Genetic material in some viruses is DNA
- ✓ Watson and Crick (1953) - Determined the structure of DNA
- ✓ Paul Ehrlich - Chemotherapy "magic bullet" (salvarsan)
- ✓ Alexander Fleming (1928) - Discovered Penicillin

Antony van Leeuwenhoek's microscope



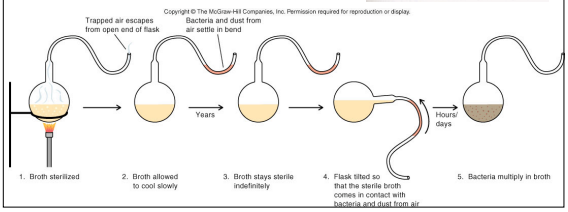
Leeuwenhoek's drawings



Francesco Redi

Back

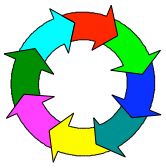
Pastuer's experiment



Koch's postulates

1. A specific microorganism must be found in ever case of the disease.
2. The microorganism must be isolated and grown in pure culture (laboratory).
3. If introduced into a susceptible host the host will develop the disease.
4. The infectious microorganism must be recovered from the host and grown in the laboratory.

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.

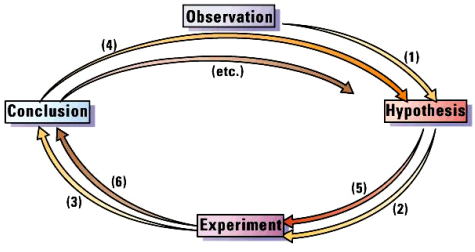
Collecting and Evaluating Results

- ✓ 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.

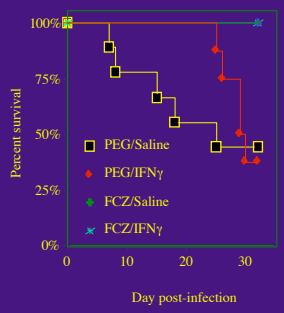
Methods

- 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

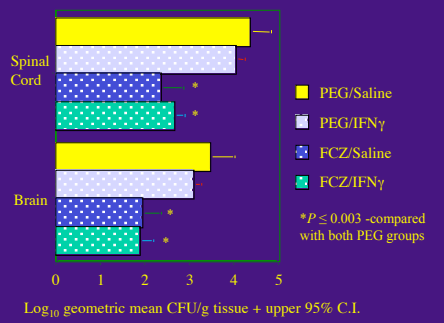
Time to Euthanasia

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.

End