MICROBIOLOGY 345

Dr. Craig Moyer
Dr. Marion Brodhagen

Course text: Brock Biology of Microorganisms 11th or 12th Ed.
(Prentice Hall)

Today's Lecture

I. What is Microbiology?

- A. Classification of microorganisms
- B. Types of cells
  - 1. Bacteria
  - 2. Archaea
  - 3. Eukarya
- C. Roles of microbes in the biosphere
- D. Microbiology careers

Today's Lecture

II. The History of Microbiology

- A. Invention of the microscope
  - 1. Hooke
  - 2. Leeuwenhoek
- B. Spontaneous generation
  - 1. Redi - animals
  - 2. Needham - microbes
  - 3. Spallanzani - microbes
  - 4. Pasteur - microbes
- C. Fermentation (Pasteur)
- D. Contagious disease
- E. Disease prevention
- F. Modern history

Four major questions that drove the study and progress of microbiology in the past 3 centuries

What is Microbiology?

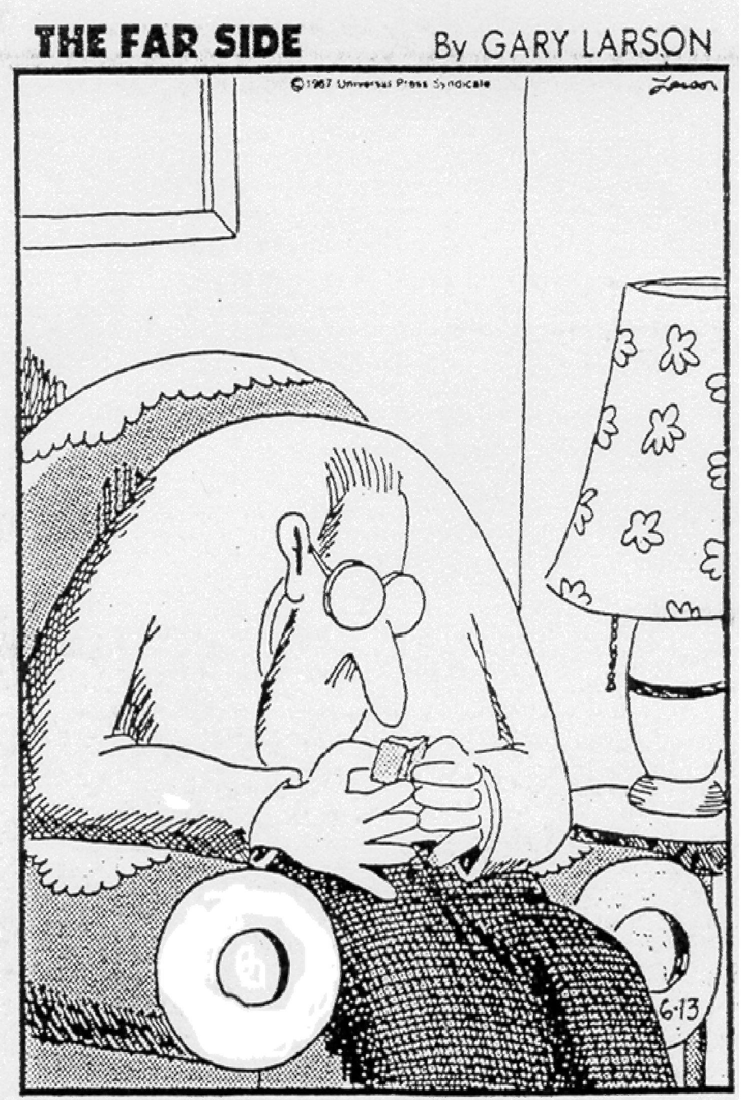
"micro" = small

"bio" = life

"-logy"(logos) = discourse

The study of organisms too small to be seen clearly with the unaided eye.

(e.g. without a microscope)...



Roger crams for his microbiology midterm.

What is a microorganism?

"There is no simple answer to this question. The word 'microorganism' is not the name of a group of related organisms, as are the words 'plants' or 'invertebrates' or 'fish'. The use of the word does, however, indicate that there is something *special* about small organisms; we use no special word to denote large organisms or medium-sized ones.

- Sistrom (1969)

Many organisms are microorganisms

‘Prokaryotes’ = no nucleus

Bacteria	most are beneficial; very few are pathogens
Archaea	no known pathogens; many extremophiles

‘Eukaryotes’ = nucleus

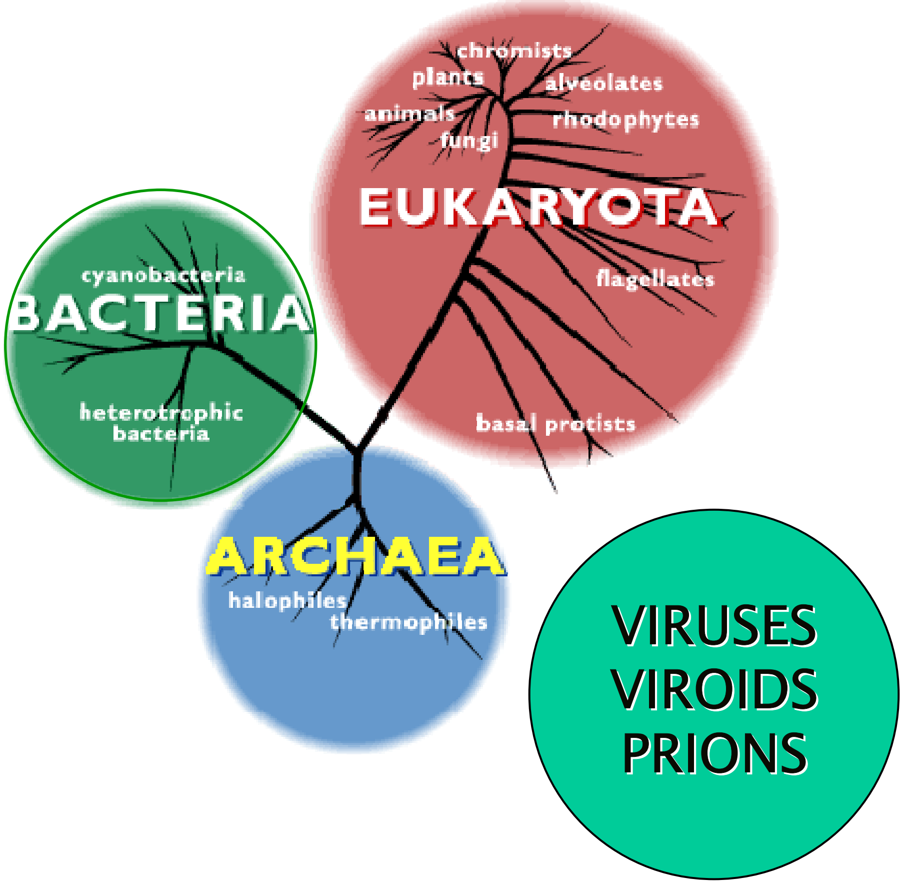
Algae	photosynthetic eukaryotes (autotrophs)
Fungi	heterotrophs; yeasts, molds, mushrooms
Protozoa	single-celled eukaryotes
Nematodes	microscopic unsegmented worms; protostomes, ubiquitous; ~80,000 spp. and ~15,000 are parasitic

Acellular

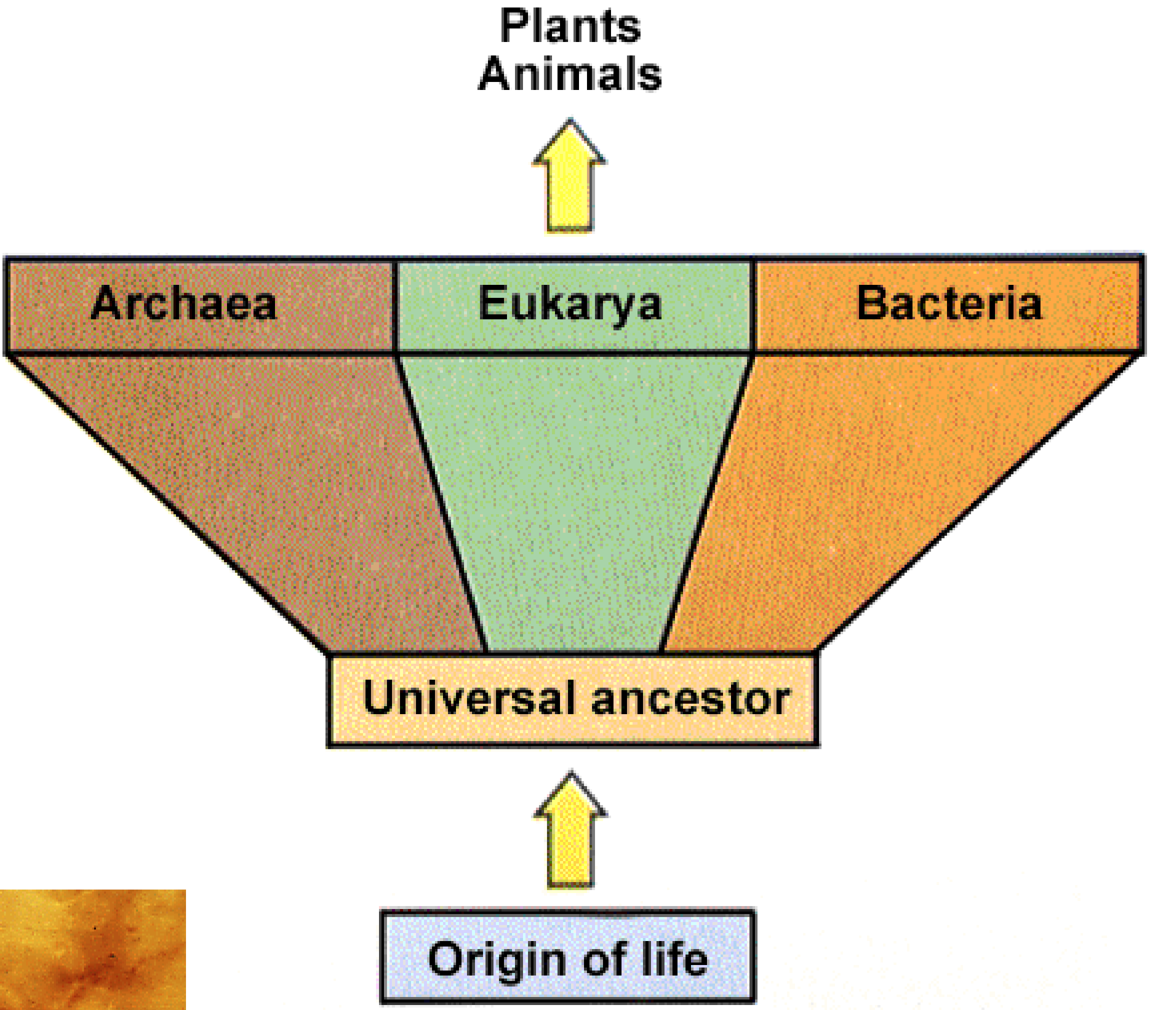
Viruses	nucleic acid (DNA or RNA) surrounded by a protein coat
Viroids	naked nucleic acid only (RNA); infect plants but not animals
Prions	naked protein only: infect animals but not plants

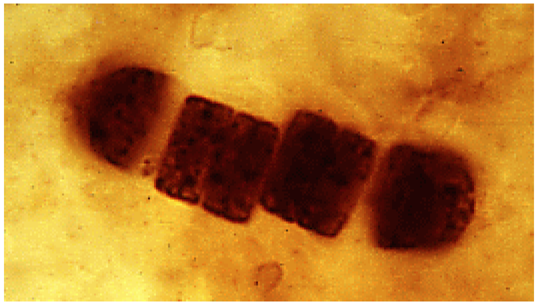
Many organisms are microorganisms

Algae, fungi, protozoa, nematodes



All three domains of life include microbes



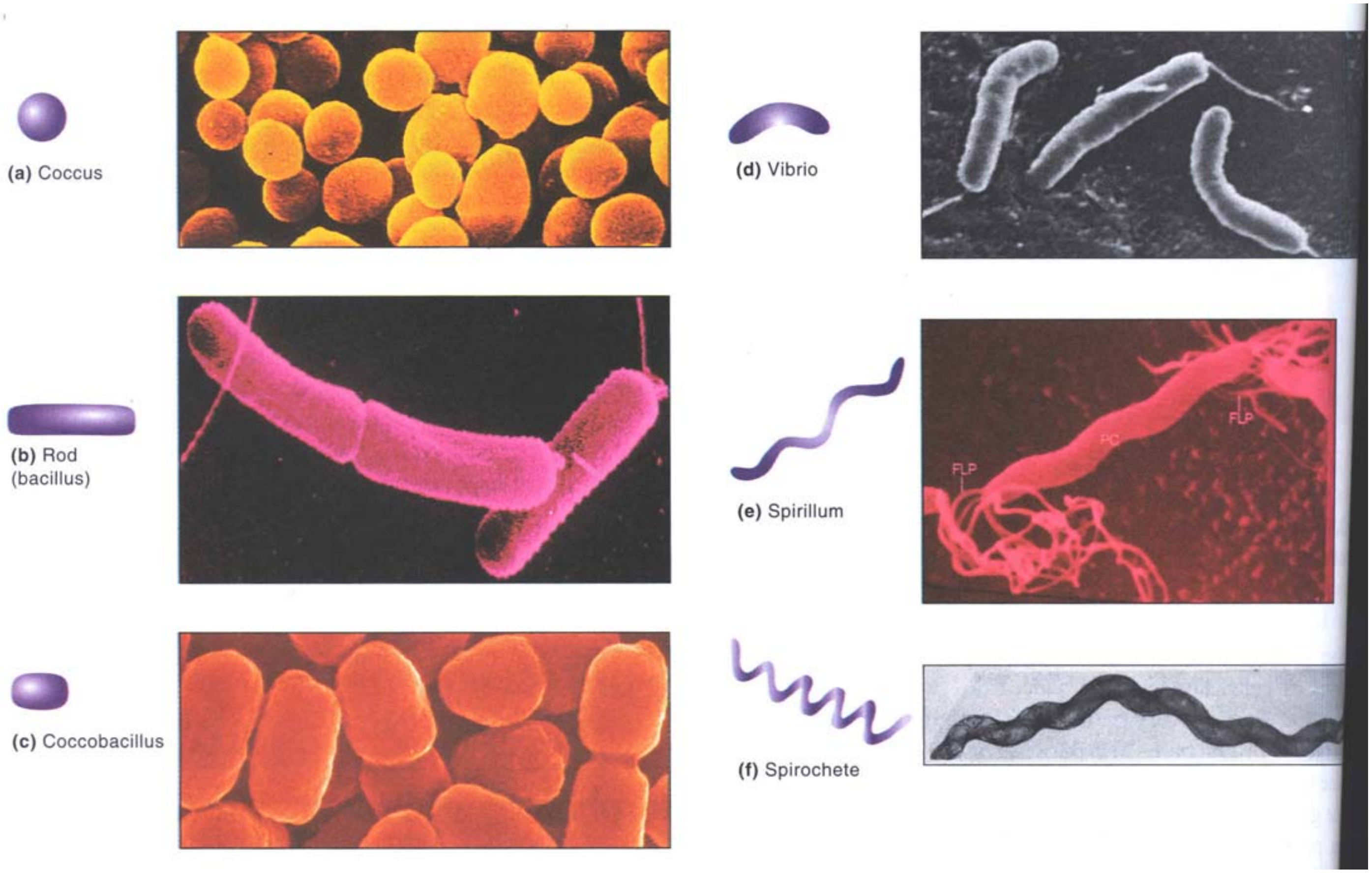


Fossil cyanobacteria in Bitter Springs chert (Australia); 1 million years old
Oldest bacterial fossils = 3.5 billion years old

‘Prokaryotic’ cells:

Size = microscopic (up to ~4 mM in length)

Shape = due to rigid cell wall



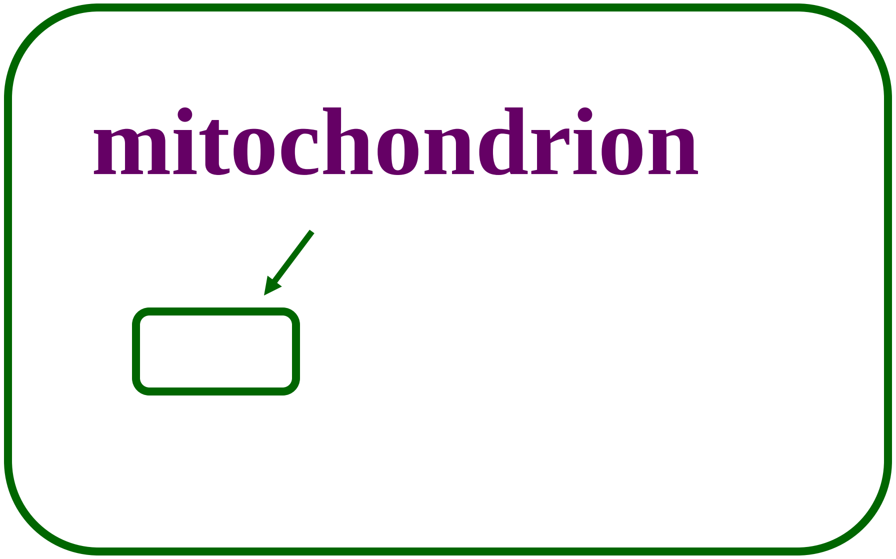
(*Vibrio cholerae* - cholera)

(*Helicobacter* - ulcers)

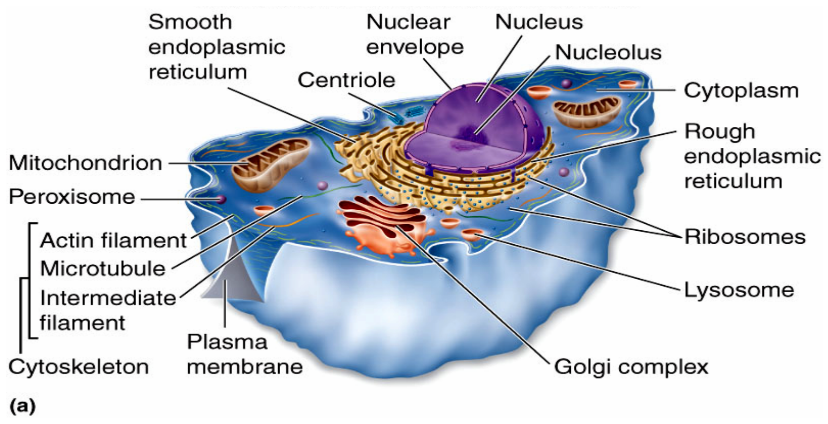
(*Borrelia* – Lyme *disease*)

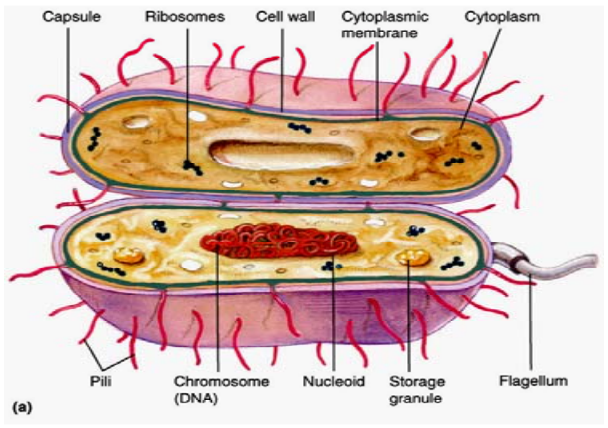
Eukaryotic cell

‘Prokaryotic’ cell

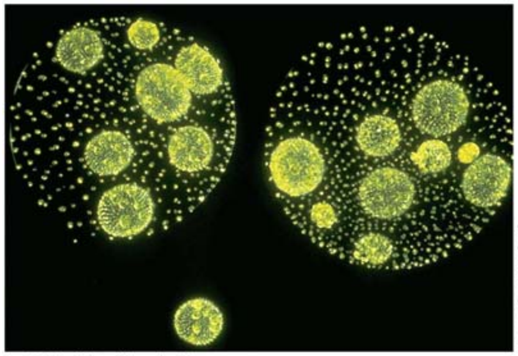


mitochondrion





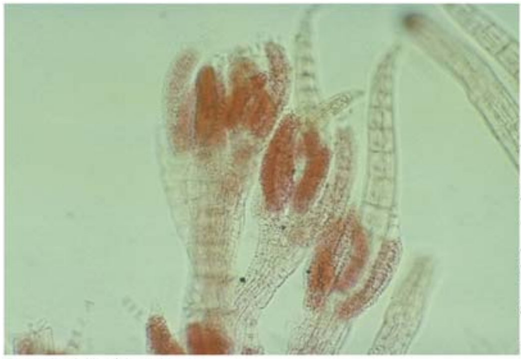
Algae: photosynthetic protists



Dennis Kunkel

Figure 14-35b Brock Biology of Microorganisms 11/e

Chlorophyta: *Volvox*



Carolina Biological Supply Co.

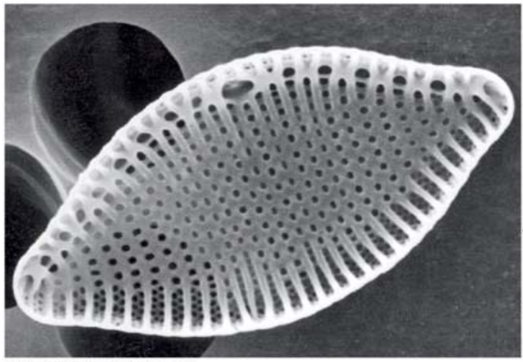
Figure 14-36b Brock Biology of Microorganisms 11/e

Rhodophyta: *Polysiphonia*

Rita R. Colwell

Figure 14-37a Brock Biology of Microorganisms 11/e

Dinoflagellata: *Gonyaulax* **(**red tide)



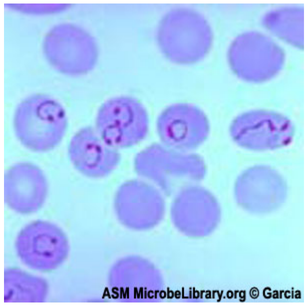
Irena Kaczmarska

Figure 14-36c Brock Biology of Microorganisms 11/e

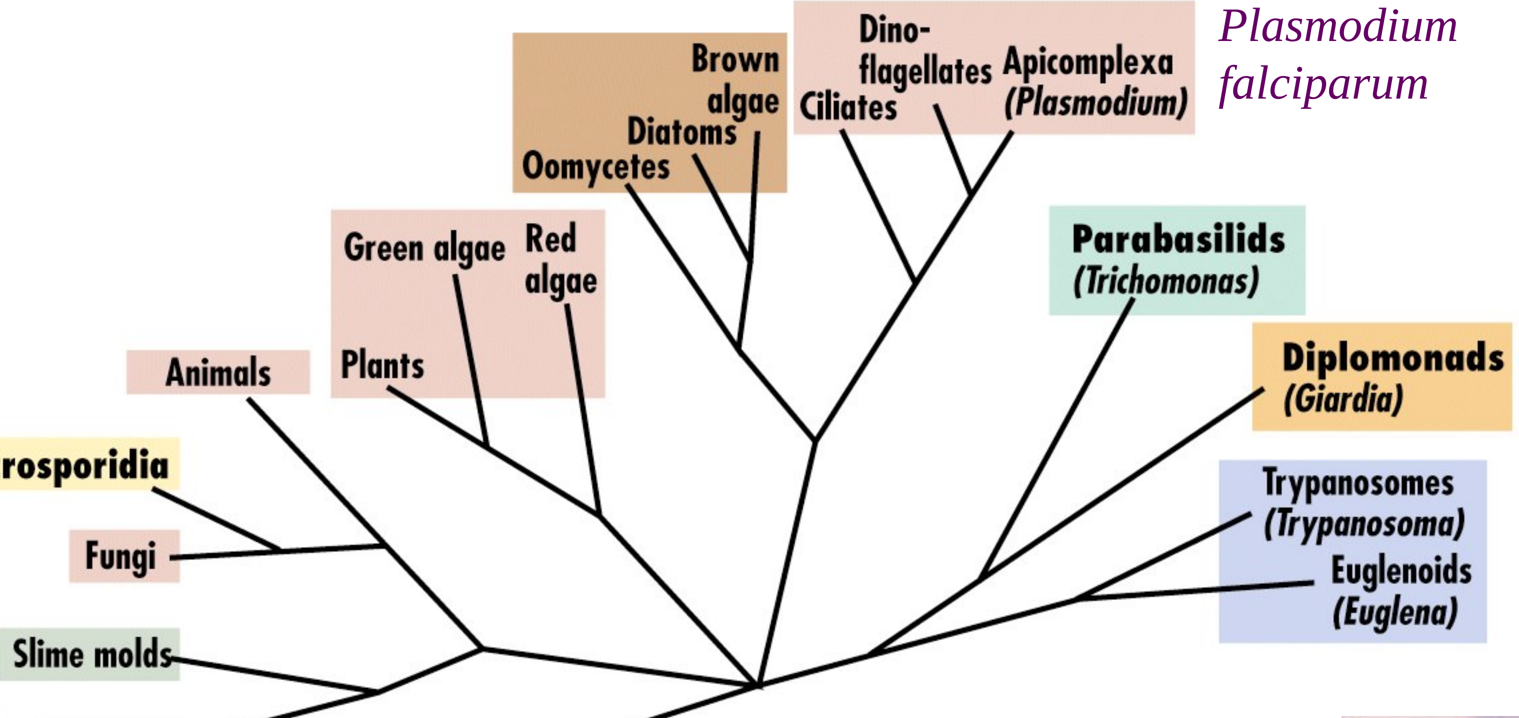
Chrysophyta: *Nitzschia*

Protists

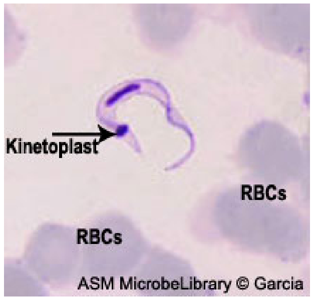
Paramecium

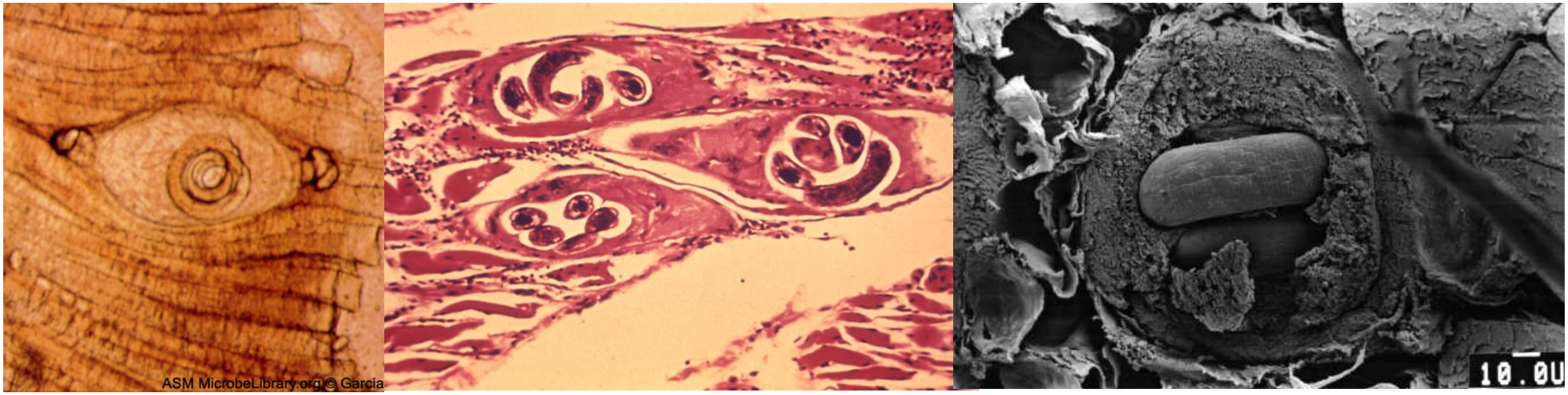


Plasmodium falciparum



Trypanosoma cruzi





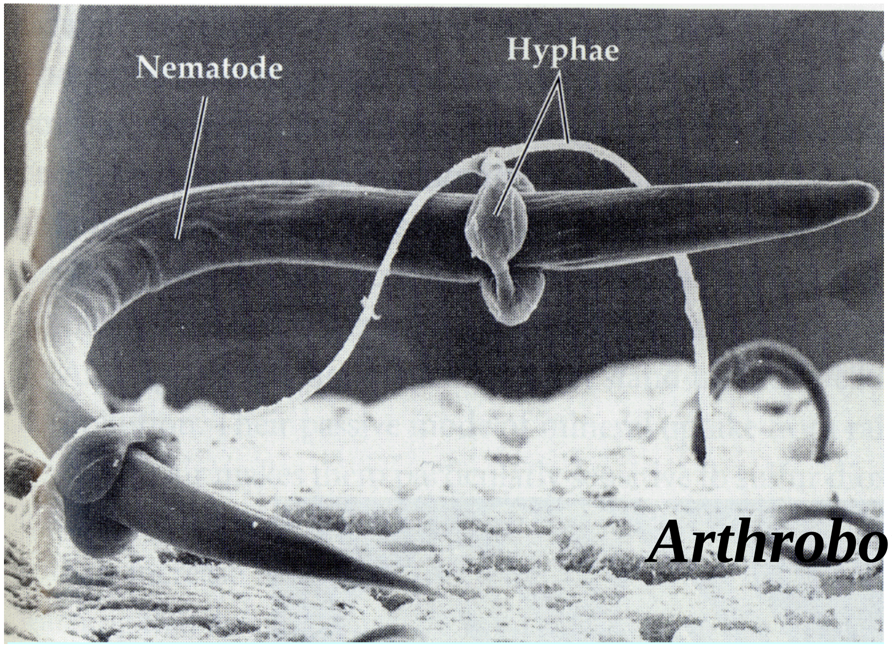
Developing *Trichinella* cysts within human muscle tissue

Parasitic worms (Kingdom Animalia, Class Nematoda)

Human hookworms

Fungi

Eukaryotic
Spore-bearing
Heterotrophic
- -live in their food
- -produce extracellular enzymes



Arthrobotrys

http://www.microbelibrary.org/microbelibrary/files/ccImages/Articleimages/Loynachan/Nem-TrapFungi.mov

-*Armillaria ostoyae*, or the honey mushroom: largest & oldest organism on Earth?
-Malheur National , Oregon
-3.5 miles across (1,665 football fields)
-2,400 - 7,200 years old
-Causes *Armillaria* root disease, which kills swaths of conifers in many parts of the U.S. and Canada

-Fusion (sexual compatibility) is a sign that isolates are from the same genetic individual.
-Researchers paired fungal samples in Petri dishes to see if they fused. →
-DNA fingerprinting determined where one individual fungus ended.

…read the whole story:
http://tomvolkfungi.net/

"All the News That's Fit to Print"

The New York Times

National Edition

VOL.CXLI . . . . No. 48,924

THURSDAY, APRIL 2, 1992

World's Biggest, Oldest Organism Twin Crowns for 30-Acre Fungus:

By NATALIE ANGIER

Scientists have discovered what could be the largest and oldest living organism on earth, an individual mightier than the blue whale, the giant sequoia tree or such past pretenders to size supremacy as the dinosaur.

The organism is a giant fungus, an interwoven filagree of mushrooms and rootlike tentacles spawned by a single fertilized spore 1,500 to 10,000 years ago and now extending for more than 30 acres in the soil of a forest near Crystal Falls, Mich., along the Wisconsin border.

The fungus, called Armillaria bulbosa, has many tiny breaks in it but is genetically uniform from one end of its expanse to the other, which is why scientists say it rightfully deserves to be called a single individual. They suggest it has been growing possibly since the end of the last Ice Age, making it older than any other known organism on earth. If all its mushrooms and tendrils are considered together, the fungus weighs about 100 tons, about as much as a blue whale.

Dr. Myron L. Smith and Dr. James B. Anderson of the University of Toronto in Mississauga, Ontario, and Dr. Johann N. Bruhn of Michigan Technological University in Houghton report their discovery of the mammoth Armillaria in today's issue of the journal *Nature*.

"This is a fascinating report," said Dr. Thomas D. Bruns, an assistant professor of plant pathology and a fungal researcher at the University of California at Berkeley. "The catchy part of it is, when you really begin to appreciate how large this thing is, it's mind-boggling. People usually think of a mushroom as a little creature, but most of the action of a fungus is underground."

The organism survives by feeding on dead wood and other detritus, spreading outward right beneath the surface as it senses the presence of nutrients nearby. But scientists believe that the fungus has probably reached its maximum dimensions; at one, and possibly several, of its borders, the Armillaria is bumping up against competing fungi, which are blocking the older giant's further colonization of the forest.

Researchers said the finding will force biologists to rethink their assumptions about what constitutes an individual, a fundamental problem in the study of the natural world and its ecosystems. Scientists normally view a single organism as something bound by a type of skin, whether of animal flesh or plant cellulose. But fungi, along with other organisms like coral and some types of grasses, grow as a network of cells and threadlike elements whose boundaries are not always clear.

What is more, some elements of the newly discovered Armillaria grow independently, thus straining the idea that the entire fungal patch can truly be considered an individual. Nevertheless, biologists said that given its uniform genetic makeup the mold merited its ranking as a one giant creature.

"The individual is the basic unit of biology," said Dr. Rytas Vilgalys, an assistant professor of botany at Duke University. "Fungi like Armillaria offer us an opportunity for re-examining what the basic unit might be."

Scientists said the new work was particularly significant because it used detailed genetic analysis, similar to the techniques of DNA fingerprinting, to prove that the 30-acre fungus was a discrete being, which had grown over the years by sending out clonal shoots of itself. Other extremely large fungal growths have been identified in the past, but researchers could never be sure that the growths represented individual fungi, rather than populations of smaller molds whose edges had become smeared together.

The New York Times

Giant fungus was found in forest near Crystal Falls, Mich.

"We used genetic markers to distinguish between these two possibilities," Dr. Anderson said. "It shocked us to have found such a large fungal entity that is so ancient."

"A lot of people have asked us if this is an April Fool's joke," he continued. "I've assured them it is not."

Larger Growths Are Possible

As startled as they were to discover the colossal patch of fuzz, the researchers said their Armillaria may not even be the largest fungal clone around.

"It's the most successful one we're aware of, but this is in a mixed forest with many kinds of trees," Dr. Bruhn said. "We would think where there was a stand of pure trees like birch or aspen, a single fungus might be more successful still." In that case a fungus with a taste for a particular type of tree might be able to proliferate especially quickly and over the entire area before encountering any competitors.

The new discovery also underscores the ubiquity and power of the planet's fungi, a kingdom of organisms quite distinct from the plant and animal kingdoms.

"Fungi are the base of all terrestrial ecosystems," Dr. Bruns said. "No ecosystem on the planet would continue to operate without fungi to decompose and recycle wood and plants."

But fungi are not always innocuous; they sometimes attack healthy tissue. A few virulent fungal species, like the Dutch elm pathogen, have man devastate entire populations of

Mapping a Discovery

The scientists came upon the described mega-fungus in 198 studying tree pathogens for the Walking over a couple of acres a on the Michigan-Wisconsin they collected samples of Arm mushrooms, familiarly known a ton or honey mushrooms, togethe the underlying shoestringlike tures called rhizomorphs, which nourishment for the fungus. Th assessed 12 genes from the fungal and realized that all their sa were the clonal offshoots of a being.

Going back over the next years and collecting even more laria samples, they continued p back the borders of that fungal at last realizing that they had thing enormous on their hands.

Michigan Technological Univer

Mushrooms growing through a tree stump are only tips of an organ iceberg. A 100-ton giant fungus, extending through 30 acres Michigan forest, is believed to be the largest organism in the world.

Through experiments measuring the growth rate of the fungus on wooden stakes, they were able to estimate how long it would have taken the clone to reach its current dimensions.

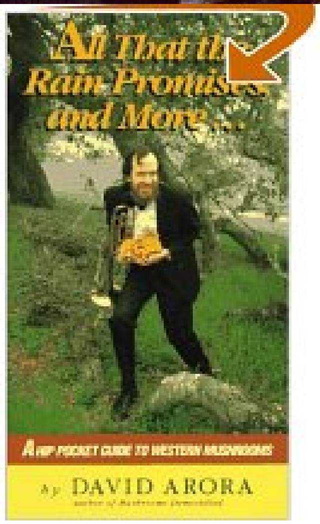
Feeding Off Rivals

The scientists now believe that at some point in the distant past a fertilized spore, blown from a parent A laria mushroom, settled onto the germinated and extended re brown rootlike rhizomorphs o ward, seeking wood debris to fe Eventually, the fungal webbing began sprouting mushrooms to s above ground and disperse new spores to the wind.

Learn more about fungus!

The reason Stradivarius violins sound sublime… fungi??

http://schaechter.asmblog.org/
(see August 28 2008 post)

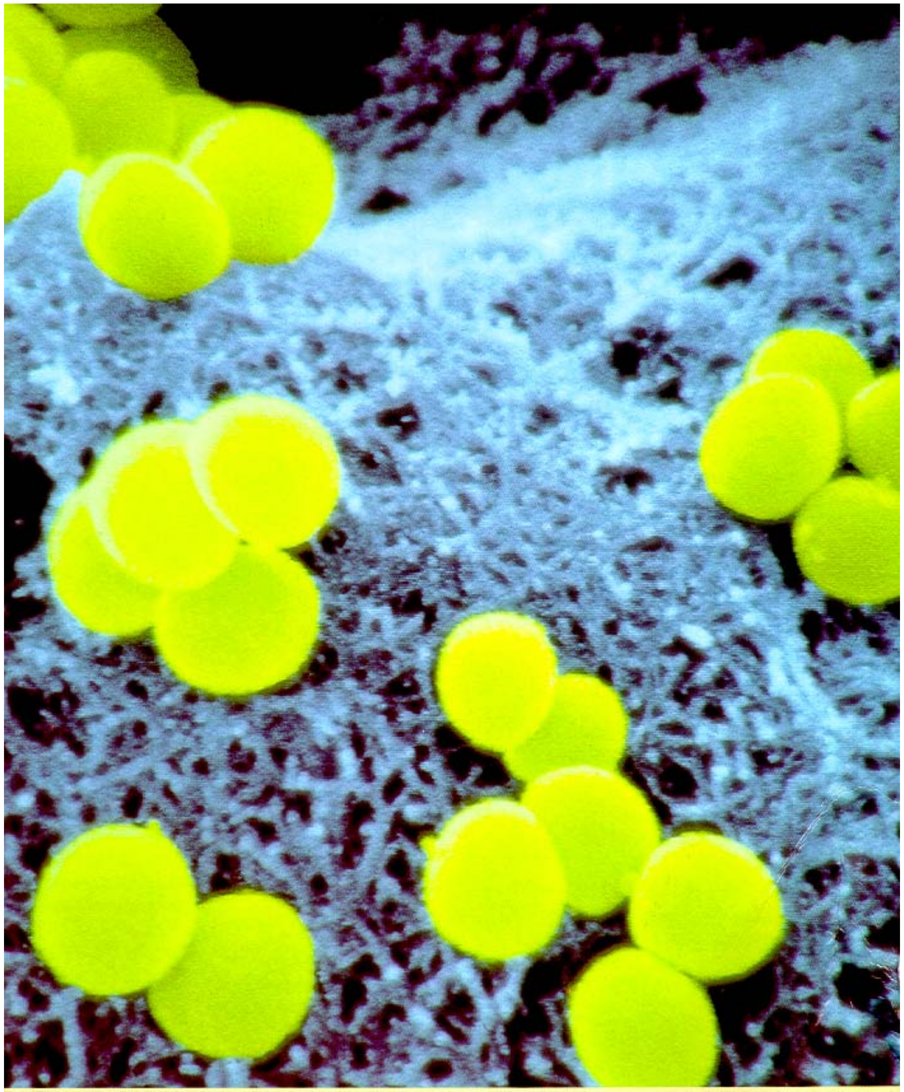


http://www.mycolog.com/index.html

Great PNW mushroom guide

Three Fundamental Kinds of Cells:

Bacteria



- **No Nucleus (DNA = "Nucleoid")**
- **Mostly circular chromosomes**
- **No membranous cytoplasmic organelles**
- **70s ribosomes**
- **Reproductively haploid Asexual reproduction**
- **Small (usually 1-10 microns)**
- **Autonomous**

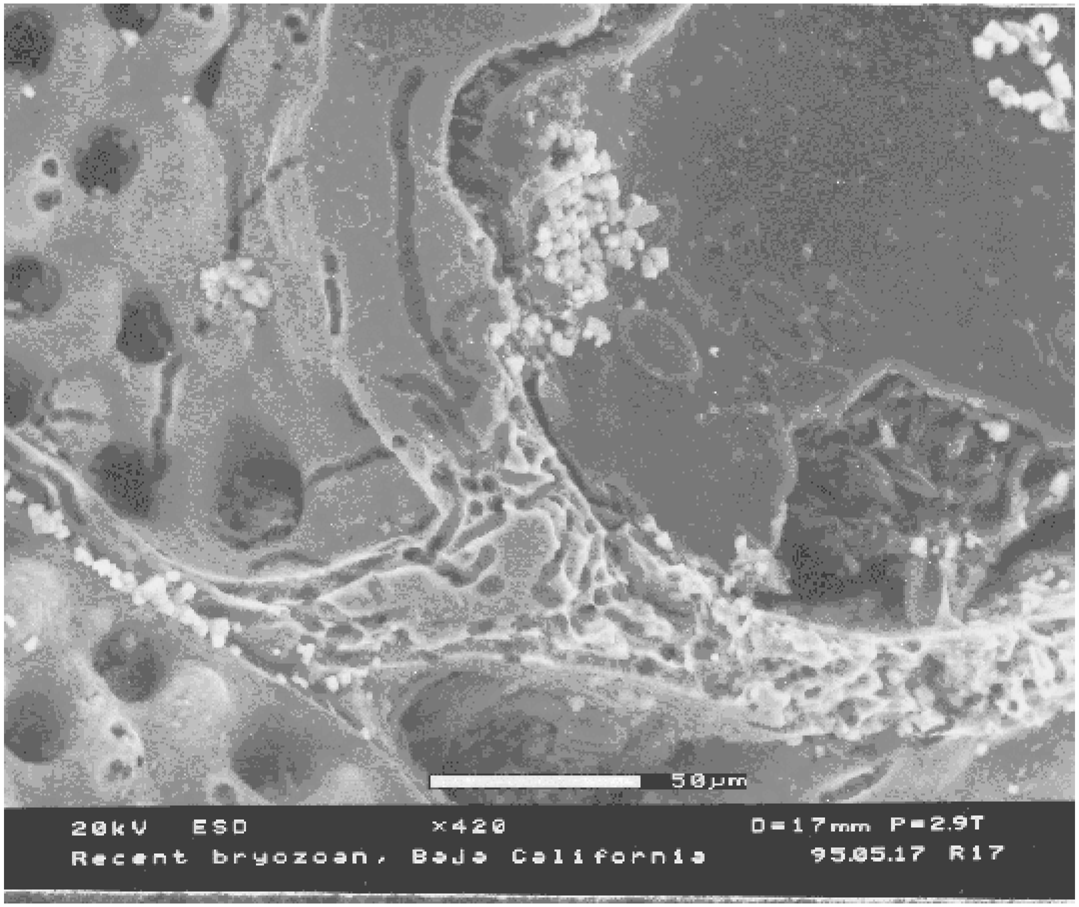
Molecular differences from Archaea:

- **4-subunit RNA polymerase**
- **Peptidoglycan cell wall**
- **Ester-linked fatty acid membrane lipids**
- **You'll learn others later**

Three Fundamental Kinds of Cells:

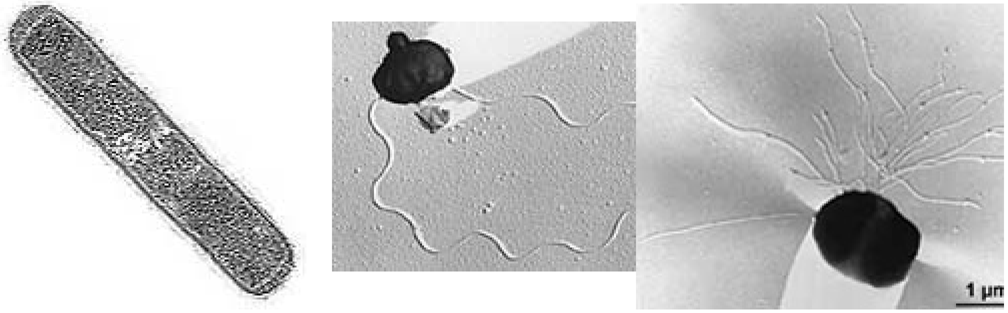
Archaea

- No Nucleus (DNA = “Nucleoid”)
- Mostly circular chromosomes
- No membranous cytoplasmic organelles
- 70s ribosomes
- Reproductively haploid
  Asexual reproduction
- Small (usually 1-10 microns)
- Autonomous



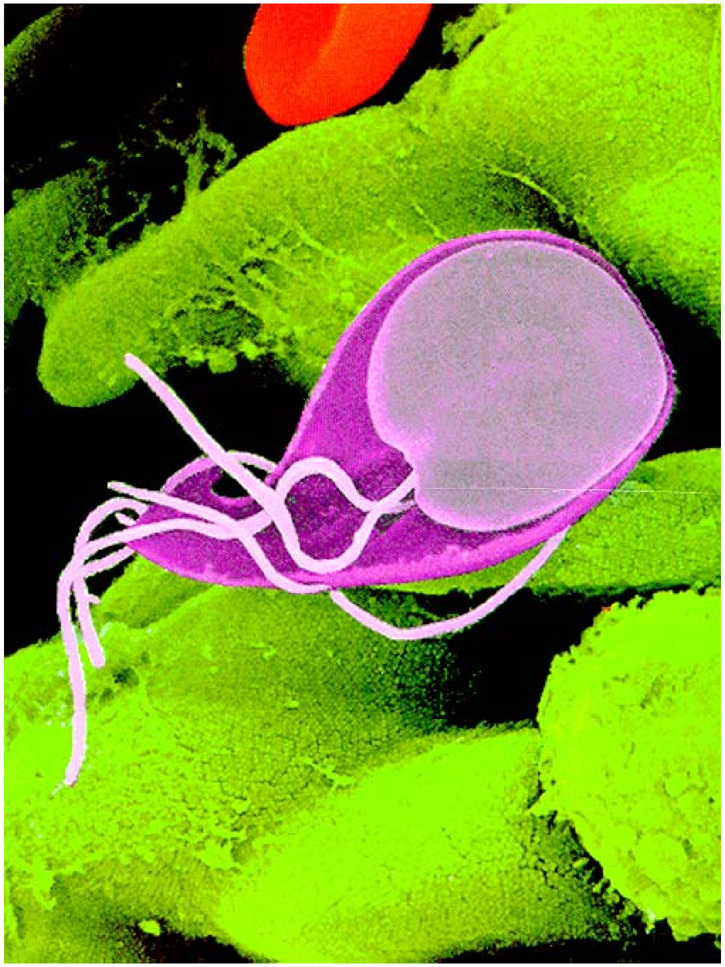
Molecular differences from Bacteria:

- 8-subunit RNA polymerase
- Non-peptidoglycan cell wall
- Ether-linked isoprenoid cell membra
- You’ll learn others later



Three Fundamental Kinds of Cells:

Eukaryotic



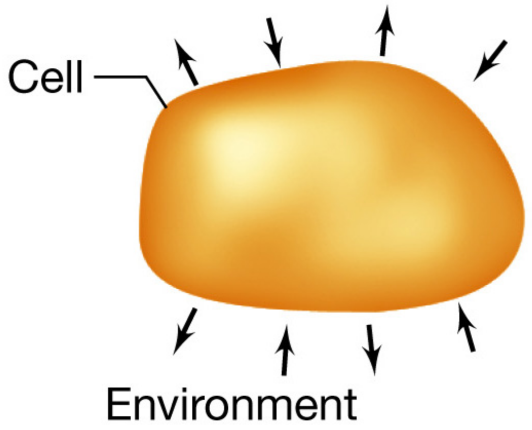
- Nucleated
- Mostly linear chromosomes
- Membranous organelles
  (mitochondria, chloroplasts, Golgi apparatus, endoplasmic reticulum)
- 80s ribosomes
- Reproductively diploid
  sexual reproduction
- Large (usually 8-10 microns)
- *Usually* multicellular

But what is a cell?

Hallmarks of cellular life

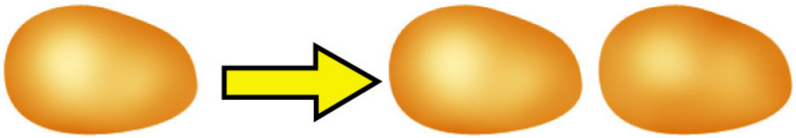
1. Metabolism

Uptake of chemicals from the environment, their transformation within the cell, and elimination of wastes into the environment. The cell is thus an *open* system.



2. Reproduction (growth)

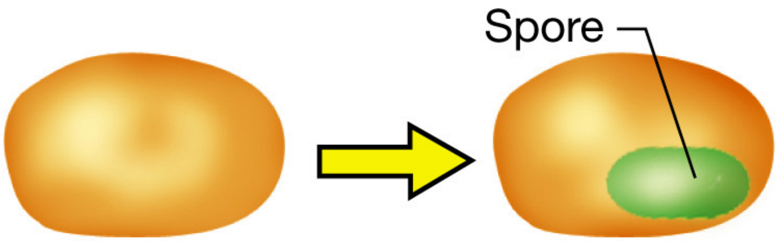
Chemicals from the environment are turned into new cells under the direction of preexisting cells.



Hallmarks of cellular life

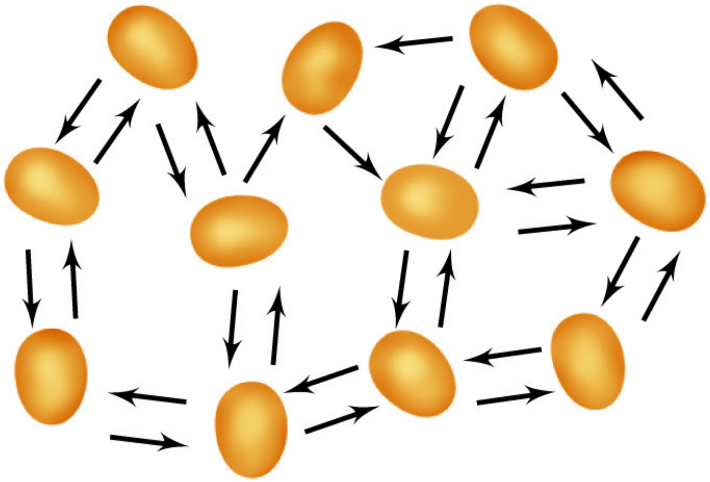
3. Differentiation

Formation of a new cell structure such as a spore, usually as part of a cellular *life cycle*.



4. Communication

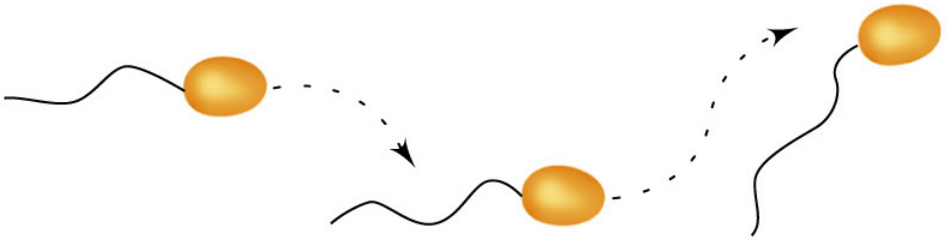
Cells *communicate* or *interact* primarily by means of chemicals that are released or taken up.



Hallmarks of cellular life

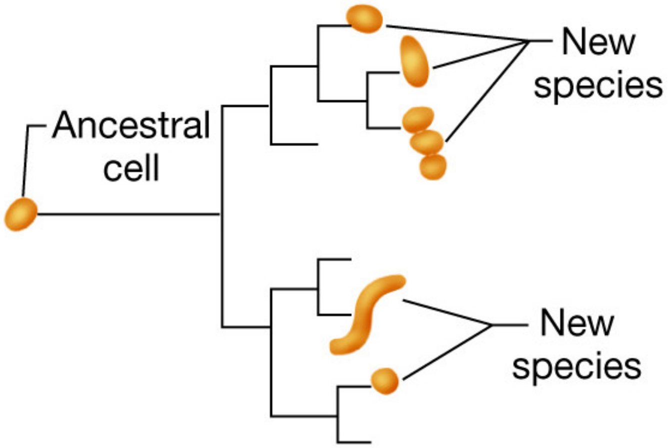
5. Movement

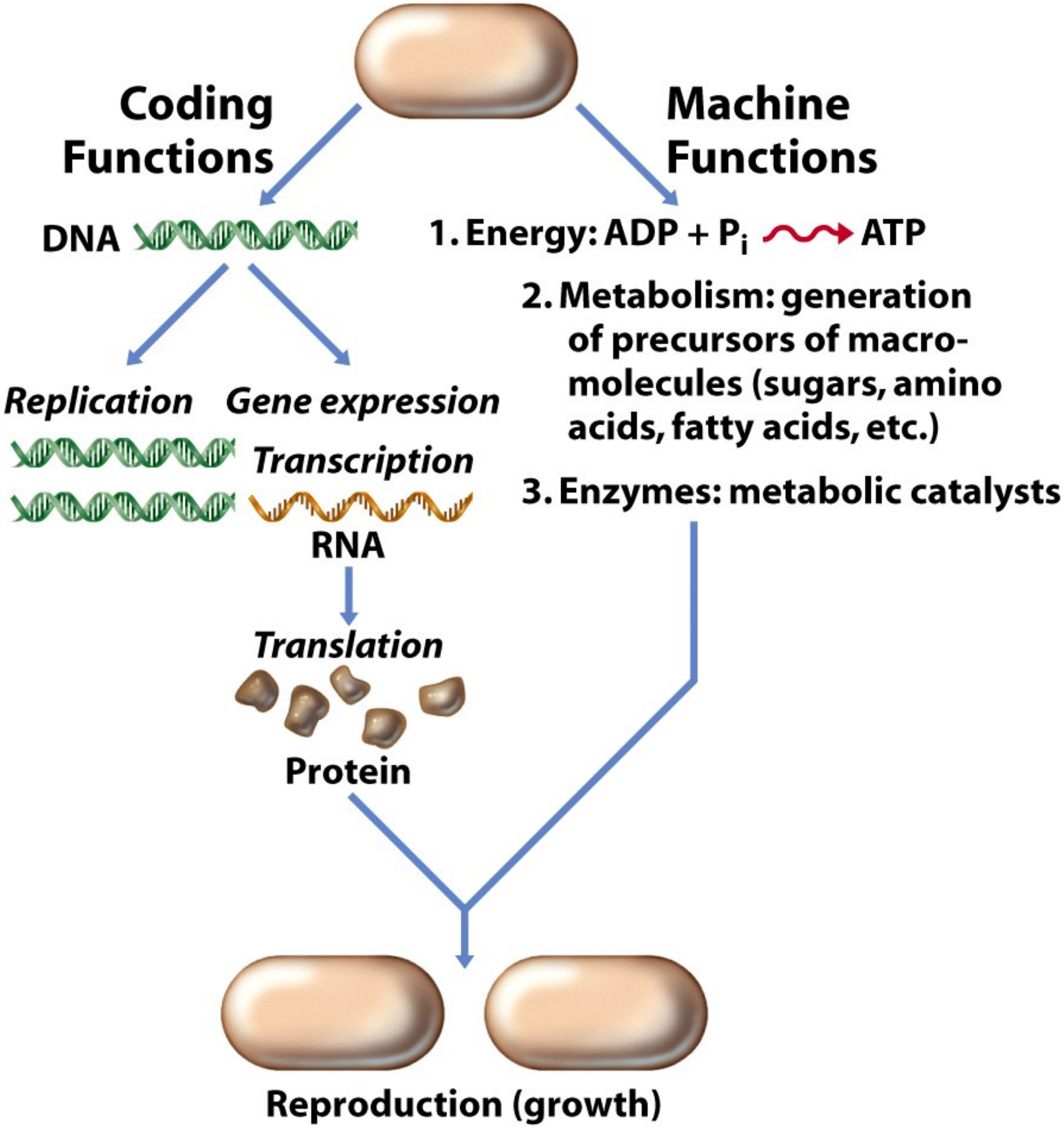
Living organisms are often capable of self-propulsion.



6. Evolution

Cells *evolve* to display new biological properties. Phylogenetic trees show the evolutionary relationships between cells.





Coding Functions

Machine Functions

DNA

Replication

Gene expression

Transcription

RNA

Translation

Protein

1. Energy: ADP + P_i $\rightsquigarrow$ ATP

2. Metabolism: generation of precursors of macromolecules (sugars, amino acids, fatty acids, etc.)

3. Enzymes: metabolic catalysts

Reproduction (growth)

Figure 1-4 Brock Biology of Microorganisms 11/e

Microbiology is a basic life science

- microbes must solve the same basic life problems as the rest of life

- all three domains of life include microbes

- to understand the rest of life and the biosphere (geosphere and atmosphere) we must understand the microbes

Microbes

The planet's smallest organisms are responsible for the most important geochemical processes on earth…

- Composition of atmosphere (air)
- Quality of hydrosphere (water)
- Nature of lithosphere (rocks)
- Maintain chemistry of the biosphere

Microbes = 50% of the Earth's biomass

Estimated 5 x 10^{30} bacterial and archaeal cells on earth
Microbial carbon equals or exceeds carbon fixed in plants!

At least **50% of the O_2** in the air today is a result of microbial photosynthesis (other 50% from plant photosynthesis… really microbial too – chloroplasts are endosymbionts)

Aside from synthetic nitrogen fertilizers, prokaryotic nitrogen fixation is the only source of **utilizable N**. Without this, it’s estimated that plants would run out of N in **7 days**.

Prokaryotes: The unseen majority

Whitman et al., 1998 PNAS

	Total C (Pg)	Total N (Pg)	Total P (Pg)
Plants:	560	12-20	1-2
Prokaryotes:	350-550	70-120	7-12

Take Home Message: Prokaryotes contain 60 to 100% the cellular carbon of all plants along with ~10x the N and P of plants!

Prokaryotes: The unseen majority

Whitman et al., 1998 PNAS

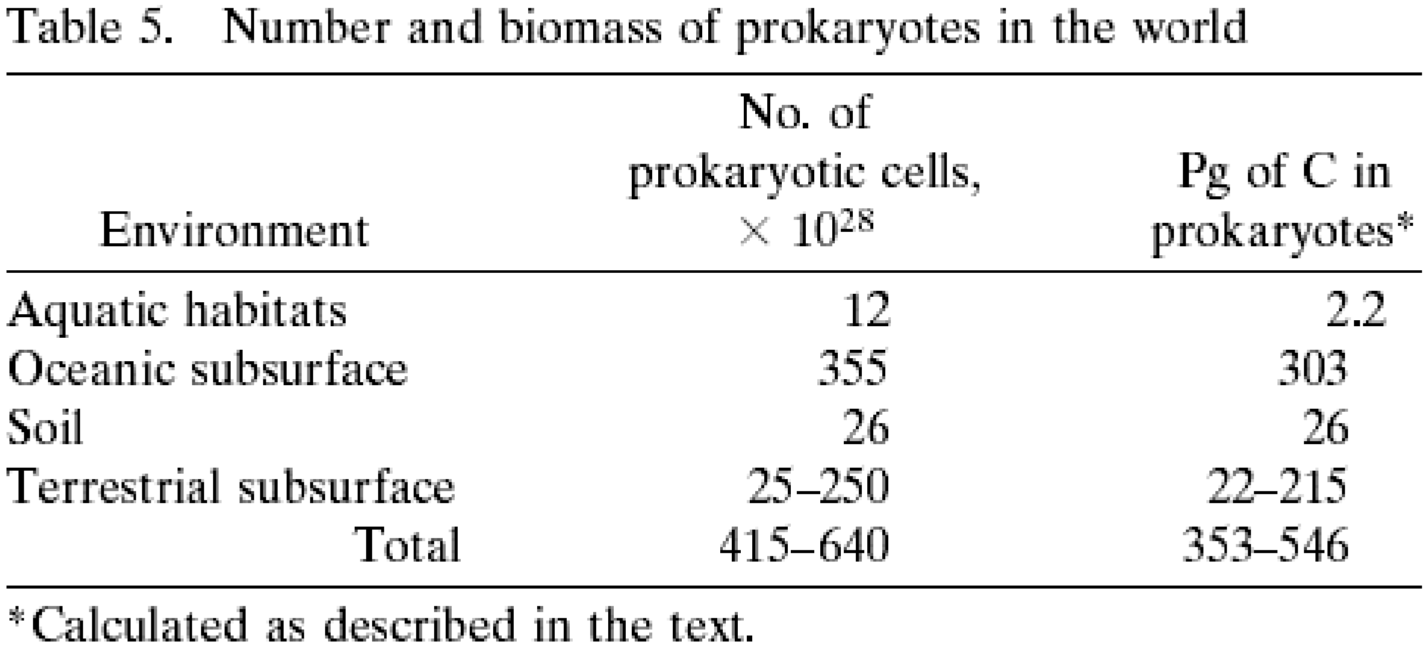


Table 5. Number and biomass of prokaryotes in the world

Environment	No. of prokaryotic cells, × 10^{28}	Pg of C in prokaryotes*
Aquatic habitats	12	2.2
Oceanic subsurface	355	303
Soil	26	26
Terrestrial subsurface	25–250	22–215
Total	415–640	353–546

*Calculated as described in the text.

Pg = Petagram or 10^{15}grams

Natural Microbial Populations

- Typical soil: ~10^9 MO's per gram
- Typical fresh water: ~10^6 to 10^7 MO's per ml
- Open Ocean: ~10^5 to 10^6 MO's per ml

- Complexity (soil): 10^4 to 10^5 different prokaryote-sized genomes per gram

WHY STUDY MICROBIOLOGY?

> “The role of the infinitely small is infinitely large.”
>
> - Louis Pasteur (1862)

Reasons to study microbiology:

1. Bacteria and Archaea are part of us (human "supraorganism").
2. Some Bacteria cause disease for us and for other organisms.
3. Microbes are involved in mineral cycling of elements like N, S, Fe, etc. and can be used in bioremediation to break down toxins and industrial waste products or synthesize biofuels.
4. Microbes are exploited for their utility in agriculture and industry.
5. Microbes help us to study evolution (including the origin of life), genetics, cell and molecular biology, and ecology.

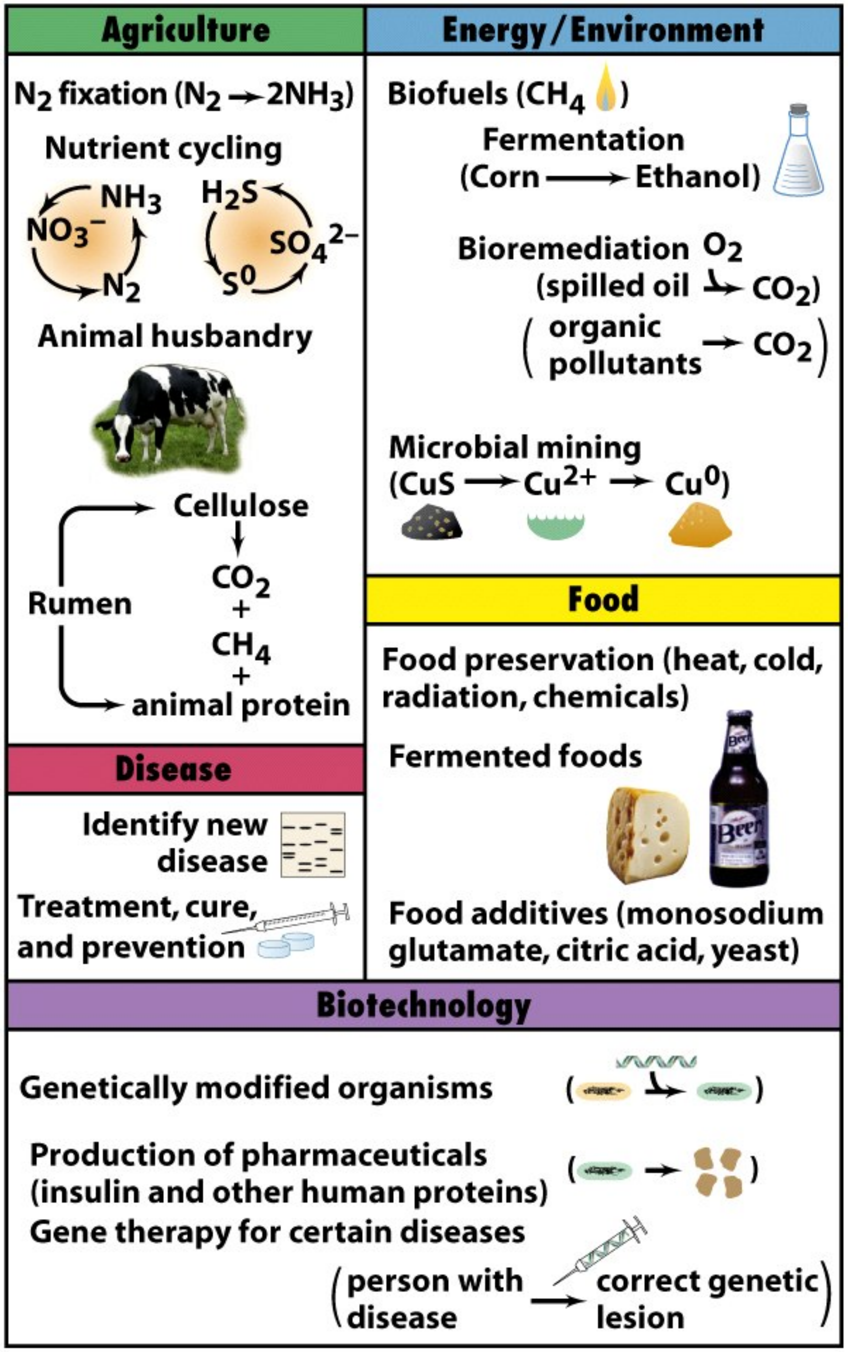


Figure 1-6 Brock Biology of Microorganisms 11/e
© 2006 Pearson Prentice Hall, Inc.

Basic and applied research

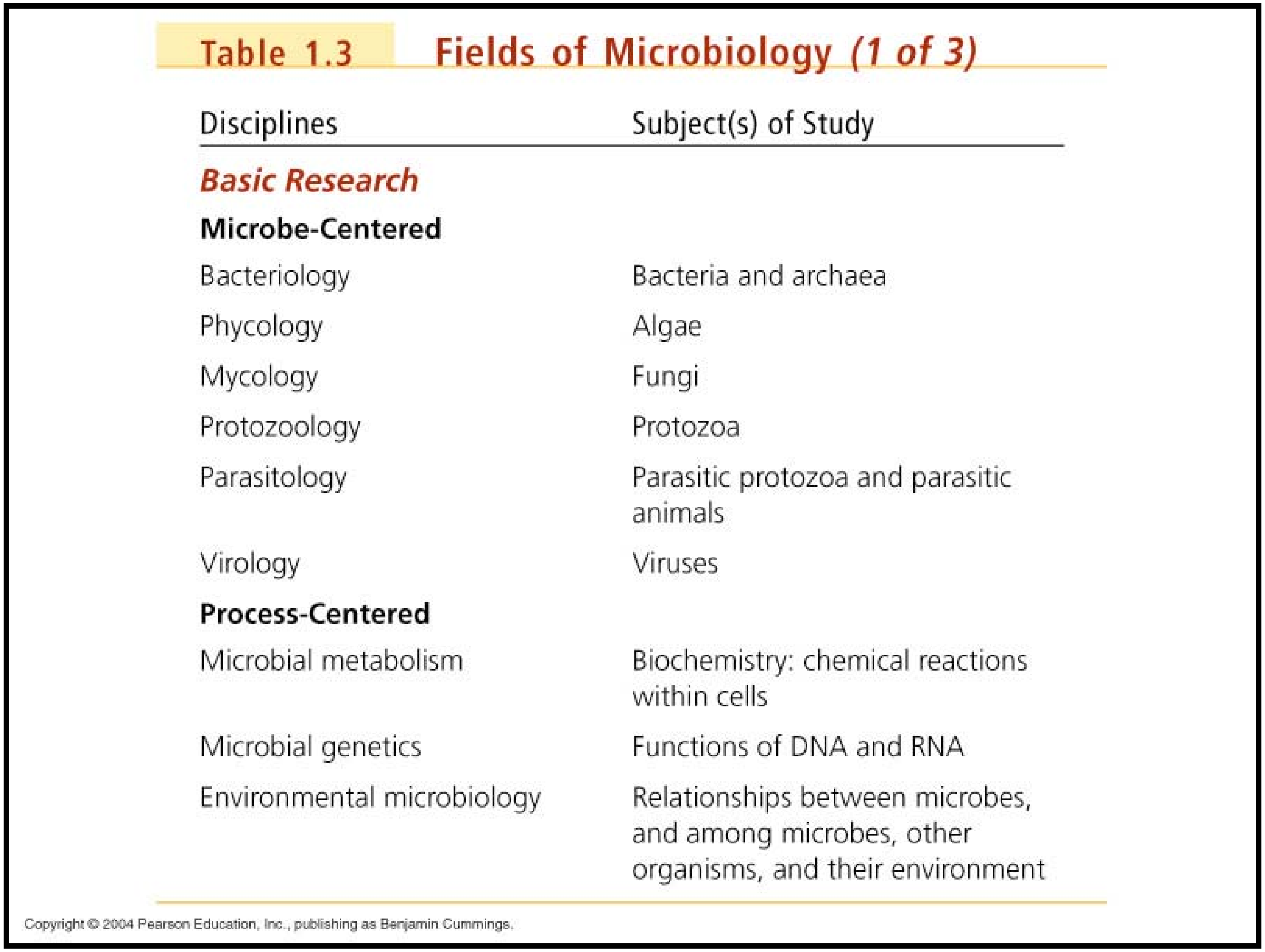


Table 1.3 Fields of Microbiology *(1 of 3)*

Disciplines	Subject(s) of Study
Basic Research	
Microbe-Centered	
Bacteriology	Bacteria and archaea
Phycology	Algae
Mycology	Fungi
Protozoology	Protozoa
Parasitology	Parasitic protozoa and parasitic animals
Virology	Viruses
Process-Centered	
Microbial metabolism	Biochemistry: chemical reactions within cells
Microbial genetics	Functions of DNA and RNA
Environmental microbiology	Relationships between microbes, and among microbes, other organisms, and their environment

Basic and applied research

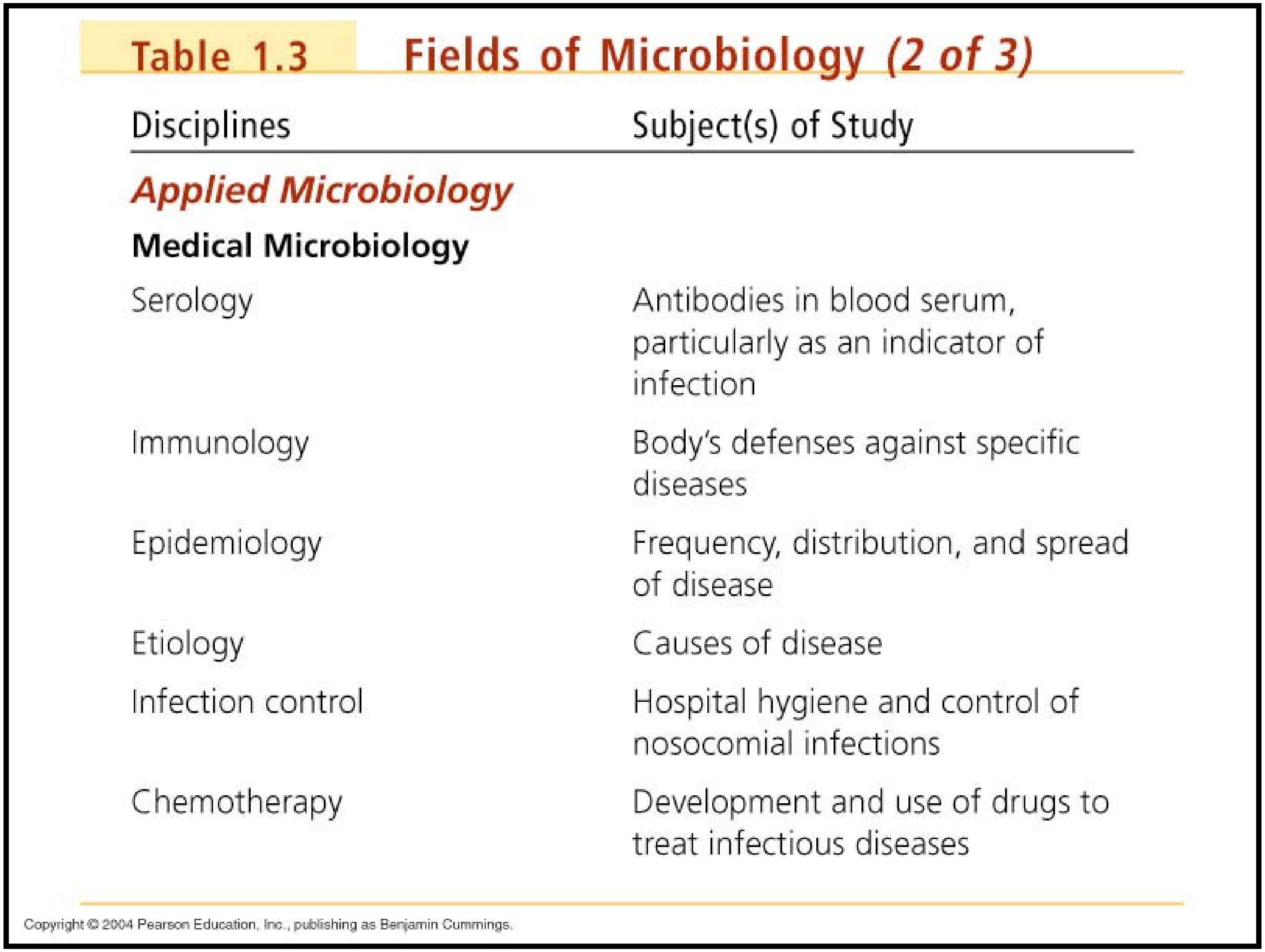


Table 1.3 Fields of Microbiology *(2 of 3)*

Disciplines	Subject(s) of Study
Applied Microbiology	
Medical Microbiology	
Serology	Antibodies in blood serum, particularly as an indicator of infection
Immunology	Body's defenses against specific diseases
Epidemiology	Frequency, distribution, and spread of disease
Etiology	Causes of disease
Infection control	Hospital hygiene and control of nosocomial infections
Chemotherapy	Development and use of drugs to treat infectious diseases

Basic and applied research

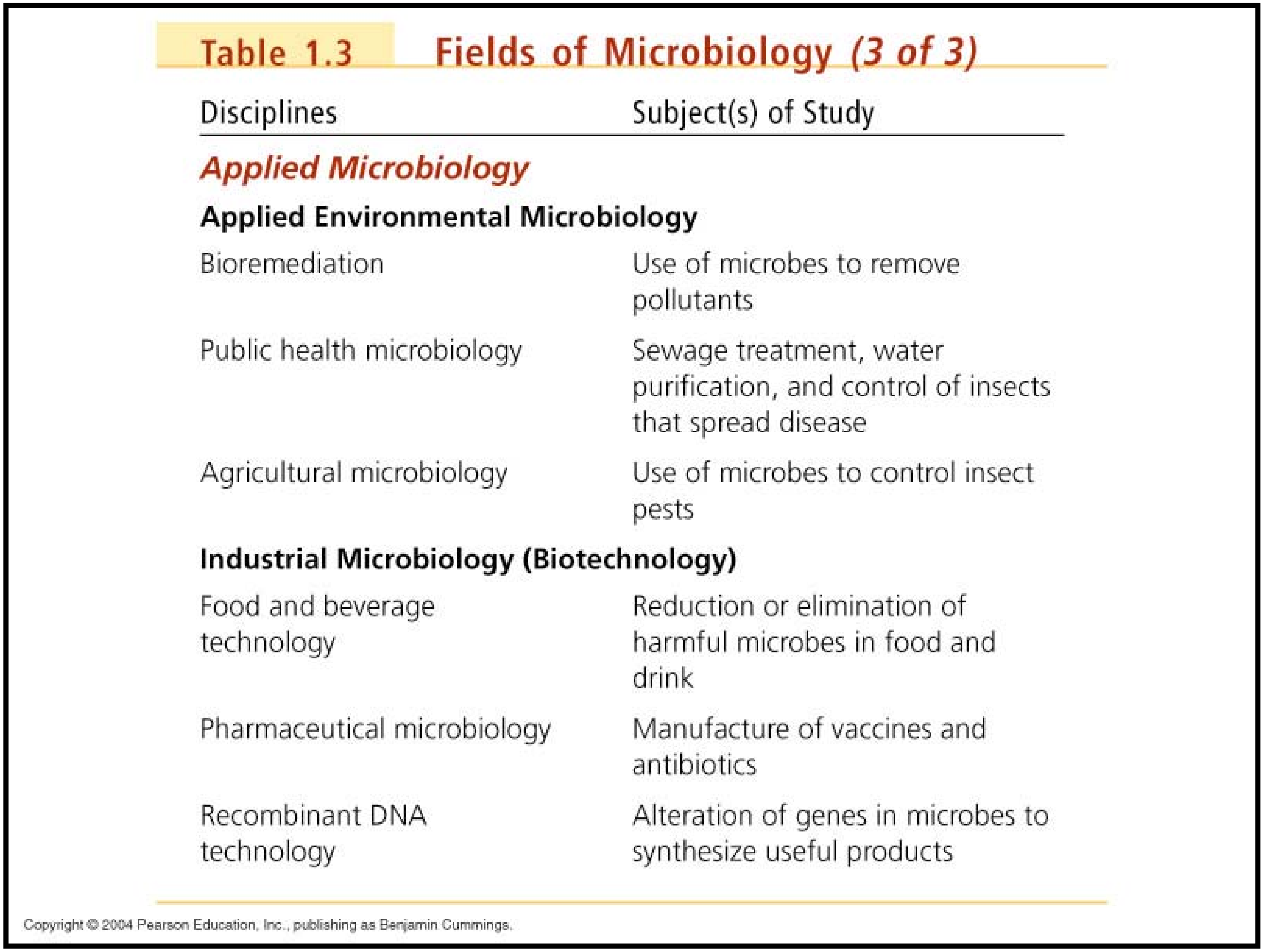


Table 1.3 Fields of Microbiology *(3 of 3)*

Disciplines	Subject(s) of Study
Applied Microbiology	
Applied Environmental Microbiology	
Bioremediation	Use of microbes to remove pollutants
Public health microbiology	Sewage treatment, water purification, and control of insects that spread disease
Agricultural microbiology	Use of microbes to control insect pests
Industrial Microbiology (Biotechnology)	
Food and beverage technology	Reduction or elimination of harmful microbes in food and drink
Pharmaceutical microbiology	Manufacture of vaccines and antibiotics
Recombinant DNA technology	Alteration of genes in microbes to synthesize useful products

Research is spurred by practical applications

Table 1.1 Some Industrial Uses of Microbes

Product or Process	Contribution of Microorganism	Product or Process	Contribution of Microorganism
Foods and Beverages		***Other Products***	
Cheese	Flavoring and ripening produced by bacteria and fungi; flavors dependent on the source of milk and the type of microorganism	Antibiotics	Produced by bacteria and fungi
		Human growth hormone, human insulin	Produced by genetically engineered bacteria
Alcoholic beverages	Alcohol produced by bacteria or yeast by fermentation of sugars in fruit juice or grain	Laundry enzymes	Isolated from bacteria
		Vitamins	Isolated from bacteria
		Diatomaceous earth (used in polishes and buffing compounds)	Composed of cell walls of microscopic algae
Soy sauce	Produced by fungal fermentation of soybeans		
Vinegar	Produced by bacterial fermentation of sugar	Pest control chemicals	Insect pests killed or inhibited by bacterial pathogens
Yogurt	Produced by bacteria growing in skim milk	Drain opener	Protein-digesting and fat-digesting enzymes produced by bacteria
Sour cream	Produced by bacteria growing in cream		
Artificial sweetener	Amino acids synthesized by bacteria from sugar		
Bread	Rising of dough produced by action of yeast; sourdough results from bacterial-produced acids		

Microbes are used in many processes

Applied and basic research are intertwined

History of Microbiology

Four questions that drove microbiology

1. **What is the origin of life (does spontaneous generation occur)?**

2. **Fermentation: Why does wine go bad (acidic)? What causes fermentation?**

3. **What causes contagious disease?**

4. **OK, microbes cause disease (germ theory).**

How do we prevent and treat disease?

1. What is the origin of life (does spontaneous generation occur)?

Spontaneous generation : *living organisms arising from non-living substances*

Proposed by **Aristotle** based on his observations of nature: continuum between non-living and living matter

- rotting meat --> maggots
- Conclusions bases on untested observation
  - Observation: flies found on rotting meat
  - Conclusion: flies originate from rotting meat
  - Observation: Nile river floods forming mud --> frogs appear
  - Conclusion: frogs originate from mud
  - Observation: silos with moldy grain have lots of rats
  - Conclusion: rats come from moldy grain
  - Observation: lots of rats in the sewers and streets with garbage
  - Conclusion: rats come from sewage and garbage

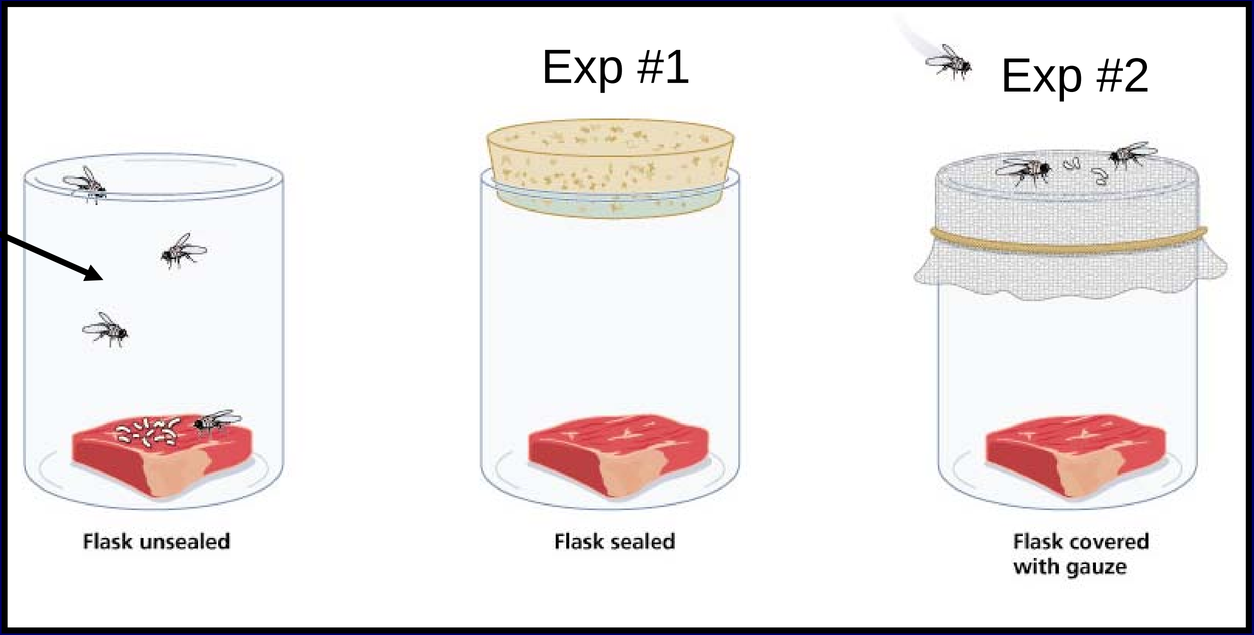
The Scientific Method

Debate over spontaneous generation led to development of scientific method:

- A group of observations leads scientist to ask question about some phenomenon
- The scientist generates hypothesis (potential answer to question)
- The scientist designs and conducts experiment to test hypothesis
- Based on observed results of experiment, scientist either accepts, rejects, or modifies hypothesis

Francesco Redi
(1626-1697)

Control



One variable:
Sealed or not

Observation:	Flies and maggots are found on rotting meat.
Question:	Does rotting meat make maggots and flies?
Hypothesis:	Rotten meat does not turn into flies (falsifiable). Flies make more flies.
Prediction:	Rotting meat kept away from flies will not form flies or maggots.
Test:	Put meat in a sealed jar.
Observation:	No flies, no maggots. (meticulous, rigorous, *repeatable*)
Conclusion:	Flies originate from other flies, not rotten meat.

http://biology.clc.uc.edu/courses/bio114/spontgen.htm

History of Microbiology

Robert Hooke publishes his discovery of cells in cork (1665)

Antony van Leeuwenhoek observes “animacules” using his homemade microscope (Netherlands; 1676)

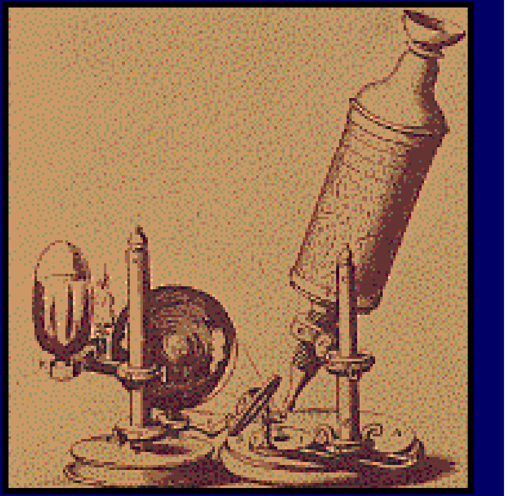
1600’s

1700’s

1800’s

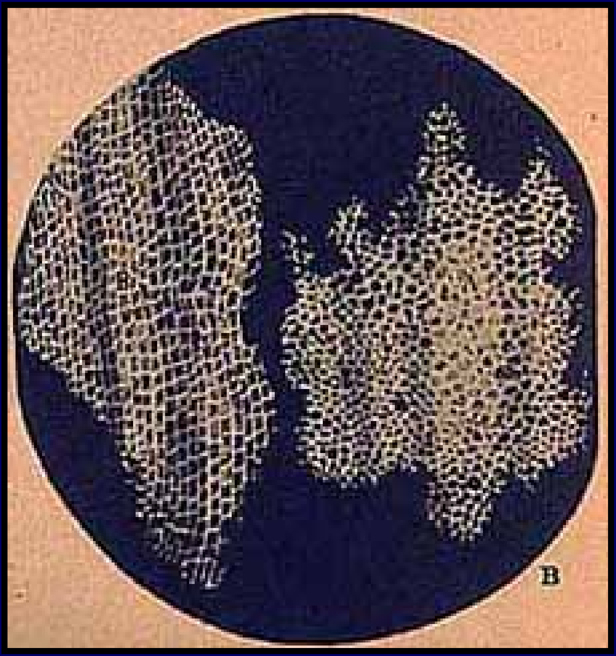
1900’s

Cell theory



- Robert Hooke (1665): publishes paper, “*Micrographia*”
  - Calls holes in slices of cork “cells” (Latin: small rooms)
- Cell theory:
  - All living things are composed of cells
  - All cells come from other cells

Cork slices



http://www.ucmp.berkeley.edu/history/hooke.html

> *. . . I could exceedingly plainly perceive it to be all perforated and porous, much like a Honey-comb, but that the pores of it were not regular. . . . these pores, or cells, . . . were indeed the first microscopical pores I ever saw, and perhaps, that were ever seen…*

Robert Hooke, Micrographia

1665 (typo in text): Robert Hooke using a primitive microscope, is the first to describe a microbe (mold spores).

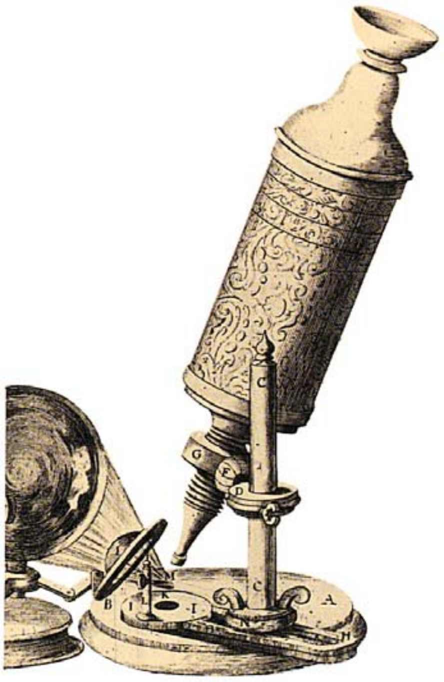


Figure 1-8a Brock Biology of Microorganisms 11/e
© 2006 Pearson Prentice Hall, Inc.

Later, when fungi had been extensively studied, these drawings were accurate enough to identify spores from common bread mold.

Figure 1-8b Brock Biology of Microorganisms 11/e
© 2006 Pearson Prentice Hall, Inc.

Leeuwenhoek and microscopy
1676

- 1st observation of microorganisms

- Meticulous observation and recordkeeping

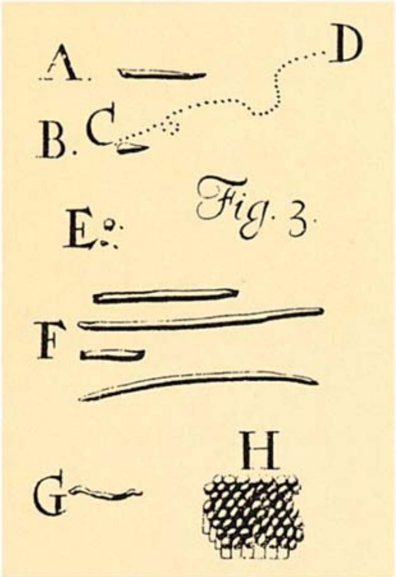


Figure 1-9b Brock Biology of Microorganisms 11/e

- 1st observation of microorganisms

- Plaque from teeth: “so many that I believe them to exceed the number of men in a kingdom” – AvL

- Protozoa, bacteria

John Needham's Experiments (1745)

100 years later, scientists did not believe that animals (e.g. maggots, mice) could arise spontaneously, but did believe that **microbes** could.

History of Microbiology

1600's

Francesco Redi argues against spontaneous generation of animals (maggots/flies)

Leeuwenhoek and Hook observe microbes (1676)

1700's

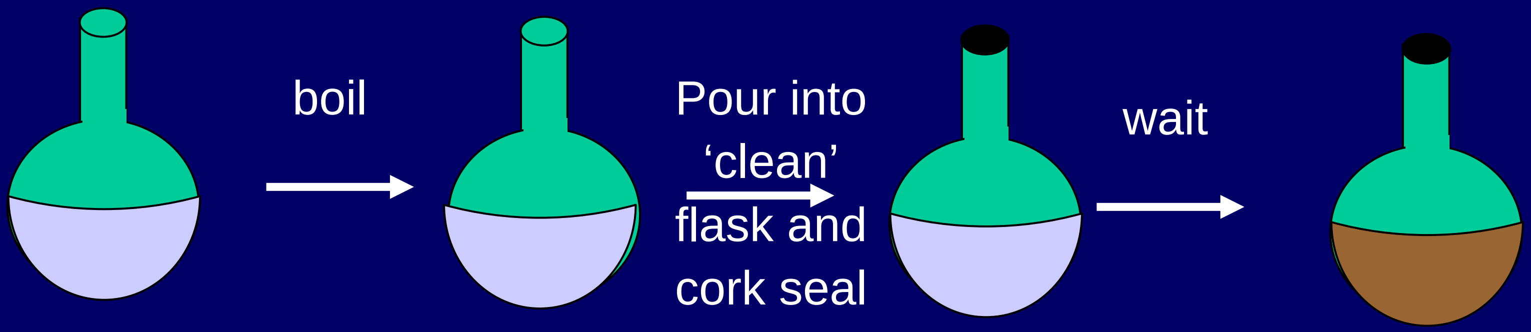
John Needham argues for spontaneous generation of microbes (1745)

Spallanzani argues against spontaneous generation of microbes (1768)

1800's

1900's

John Needham
(1713-1781)



boil

Pour into ‘clean’ flask and cork seal

wait

Observation:	Microbes grow in liquid ‘soup’ (meat and plant materials).
Question:	Do **microbes** spontaneously generate?
Hypothesis:	Microbes do not originate from dead material.
Prediction:	Microbes will not form from dead materials in ‘soup’.
Test:	Boil ‘soup’ in flask to kill all microbes, then seal and wait.
Observation:	Sealed flasks contain microbes.
Conclusion:	Microbes originate spontaneously from soup….**WHY???**

http://biology.clc.uc.edu/courses/bio114/spontgen.htm

John Needham’s Experiments (1745)

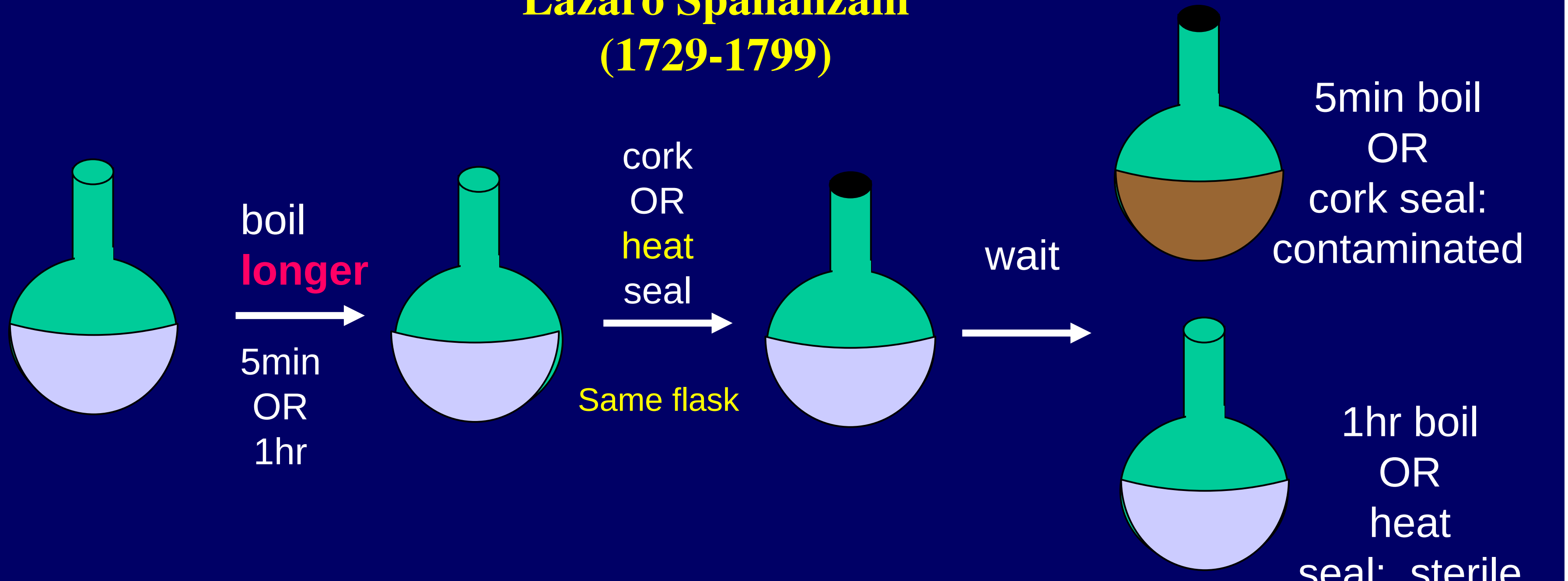
Needham’s experiments with beef gravy and infusions of plant material reinforced the idea that microbes arise via spontaneous generation

Spallanzani’s Experiments (1768)

Spallanzani hypothesized that:

- Needham failed to heat vials sufficiently to kill all microbes or had not sealed vials tightly enough
- Microorganisms exist in air and can contaminate experiments
- Spontaneous generation of microorganisms does not occur

Lazaro Spallanzani
(1729-1799)



boil
longer
5min
OR
1hr

cork
OR
heat
seal

Same flask

wait

5min boil
OR
cork seal:
contaminated

1hr boil
OR
heat
seal: sterile

Observation:	Microbes grow in liquid ‘soup’ (meat and plant materials).
Question:	Do microbes spontaneously generate?
Hypothesis:	Microbes do not originate from dead material.
Prediction:	Microbes will not form in ‘soup’ if properly sealed and prepared.
Test:	Boil ‘soup’ in flask to kill all microbes, then heat seal and wait.
Result:	Heat sealed flasks do not contain microbes.
Conclusions:	Microbes contaminants in air; not all microbes dead in Needham exp; microbes arise from other microbes.

http://biology.clc.uc.edu/courses/bio114/spontgen.htm

Spallanzani's Experiments (1768)

Evidence **against** spontaneous generation: better sterilization = no growth

Critics said sealed vials did not allow enough air for organisms to survive and that prolonged heating destroyed "life force"

History of Microbiology

1600’s

Francesco Redi argues against spontaneous generation of animals (maggots/flies)

Leeuwenhoek and Hook observe microbes (1676)

1700’s

John Needham argues for spontaneous generation of microbes (1745)

Spallanzani argues against spontaneous generation of microbes (1768)

1800’s

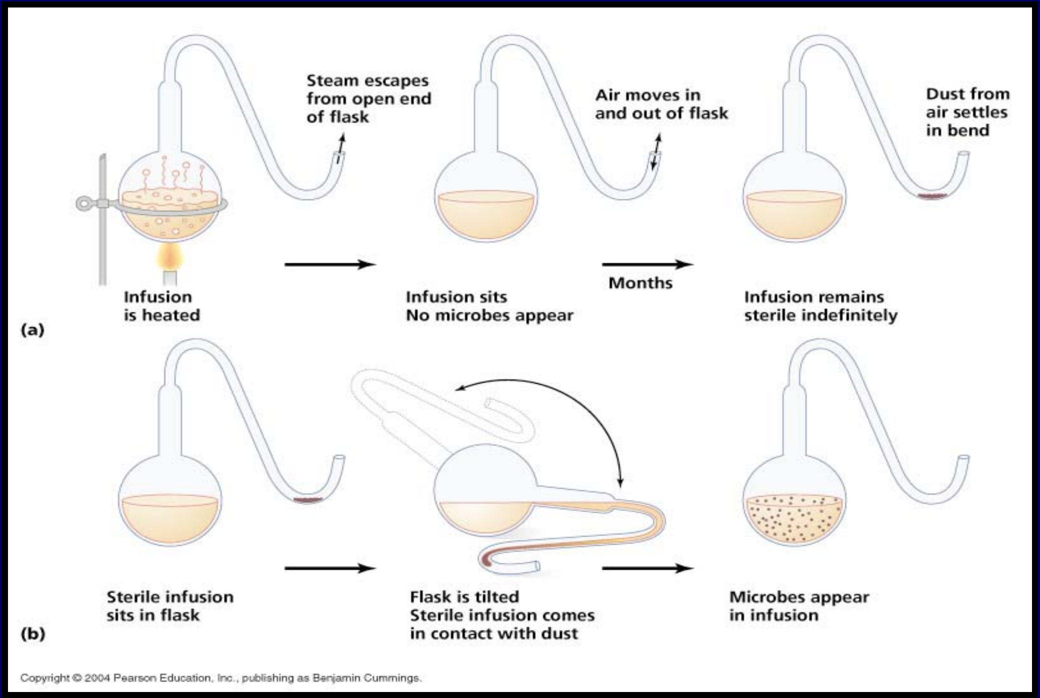
Darwin’s Origin of the Species is published and the spontaneous generation debate re-ignites (1859)

Louis Pasteur publishes experiments that refute the theory of spontaneous generation (France; 1861)

Robert Koch, studying anthrax, validates the germ theory of disease (Germany; 1876); later establishes Koch’s postulates (1884)

1900’s

Louis Pasteur
(1822-1895)



Paris Academy of Sciences

offered prize to resolve conflict

Observation:	Microbes grow in liquid 'soup' (meat and plant materials).
Question:	Do microbes spontaneously generate?
Hypothesis:	Microbes do not originate from dead material.
Prediction:	Microbes will not form in 'soup' if protected from airborne contaminants.
Test:	Boil 'soup' in swan-necked flask to kill all microbes, wait (18 months!), expose to dust.
Observation:	Flasks do not contain microbes until exposed to the dust.
Conclusions:	Microbes arise from microbes present in dust (wins prize - 1864).

http://biology.clc.uc.edu/courses/bio114/spontgen.htm

2. Fermentation : Why does wine go bad (acidic)?
What causes fermentation?


Photo: IntMedCom

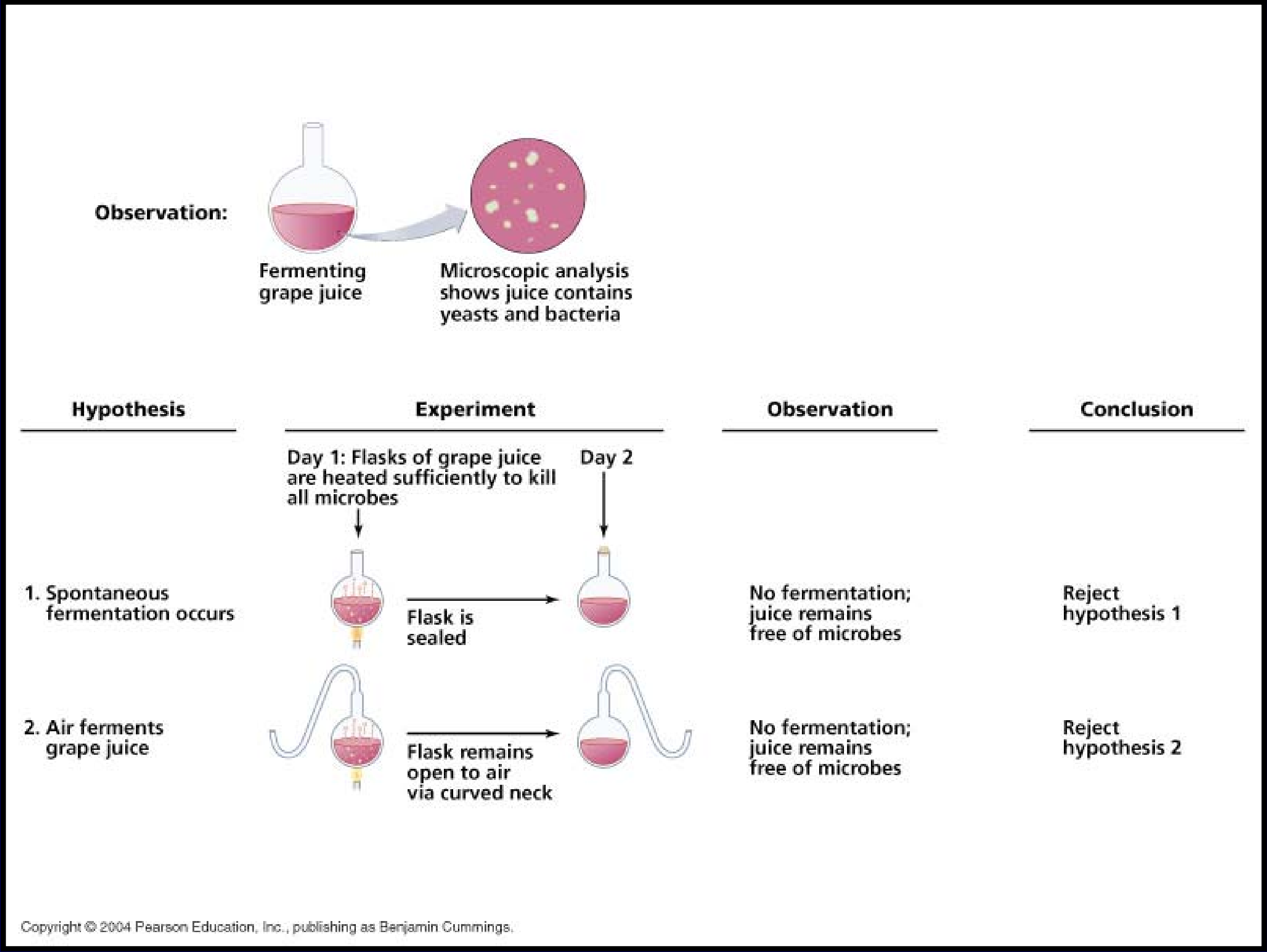
Spoiled (acidic) wine threatening livelihood of vintners, so they funded research into how to promote production of alcohol, but prevent spoilage by acid during fermentation

People argued for four sources of fermentation:

1. Spontaneous fermentation
   (hmmm… sounds like spontaneous generation)
2. Air
3. Living organism: Yeast
4. Living organism: Bacteria

In earlier work,
Pasteur had isolated both from wine

Pasteur and fermentation



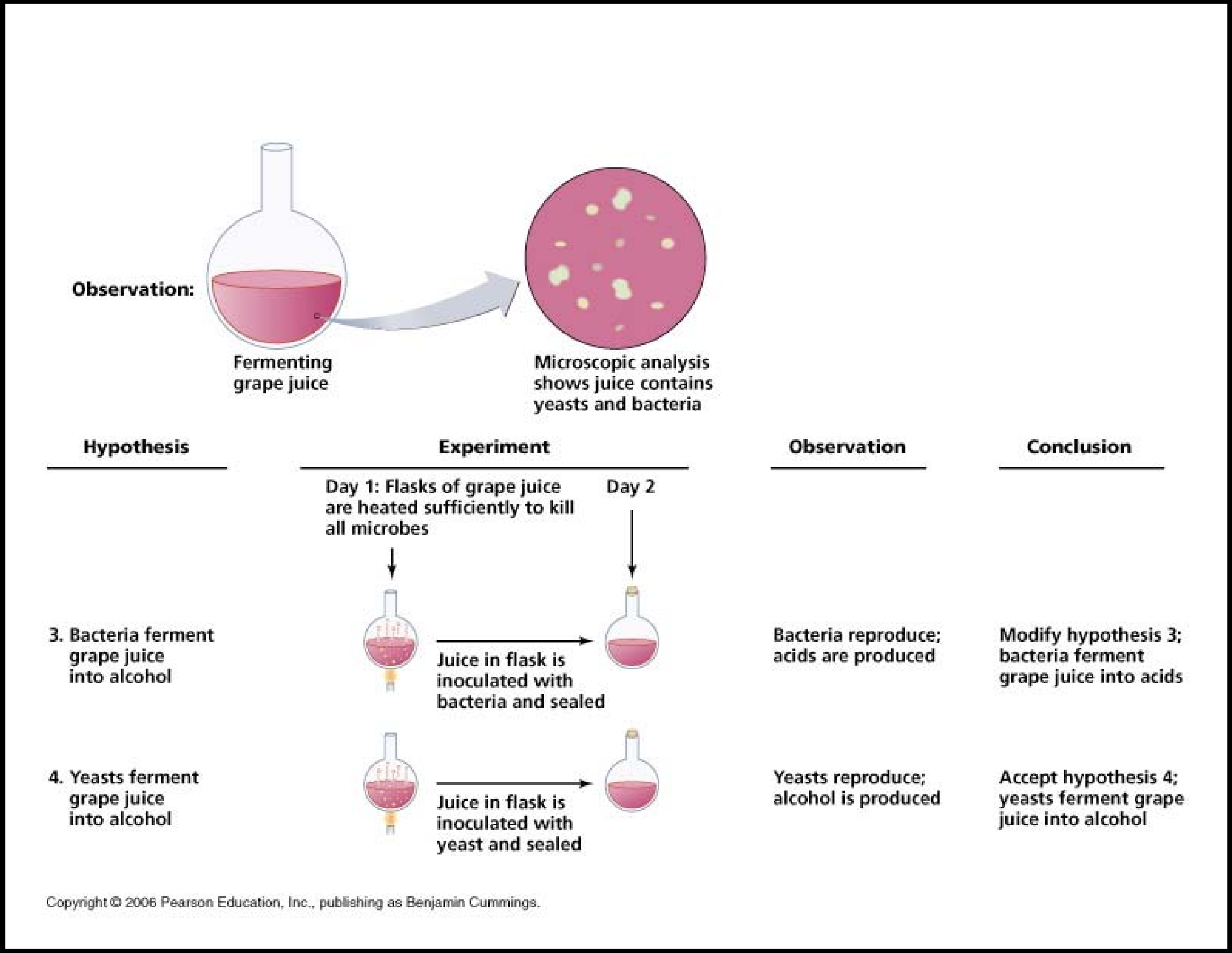
Observation:

Fermenting grape juice

Microscopic analysis shows juice contains yeasts and bacteria

Hypothesis	Experiment	Observation	Conclusion
	Day 1: Flasks of grape juice are heated sufficiently to kill all microbes Day 2		
1. Spontaneous fermentation occurs	Flask is sealed	No fermentation; juice remains free of microbes	Reject hypothesis 1
2. Air ferments grape juice	Flask remains open to air via curved neck	No fermentation; juice remains free of microbes	Reject hypothesis 2

Pasteur and fermentation



Observation:

Fermenting grape juice

Microscopic analysis shows juice contains yeasts and bacteria

Hypothesis	Experiment			Observation	Conclusion
	Day 1: Flasks of grape juice are heated sufficiently to kill all microbes		Day 2		
3. Bacteria ferment grape juice into alcohol		Juice in flask is inoculated with bacteria and sealed		Bacteria reproduce; acids are produced	Modify hypothesis 3; bacteria ferment grape juice into acids
4. Yeasts ferment grape juice into alcohol		Juice in flask is inoculated with yeast and sealed		Yeasts reproduce; alcohol is produced	Accept hypothesis 4; yeasts ferment grape juice into alcohol

- Pasteurization: fast low (55^{o}C) heat, kills organisms but retains flavor
- Allows inoculation with specific organism (yeast)

Louis Pasteur
(1822-1895)
"Father of microbiology"

Refuted the concept of spontaneous generation
- -swan necked flask experiment

Fermentations (changes in organic matter) were due to microbes
"diseases" of wines→ "Pasteurization"
- -aseptic technique
- -sterilization

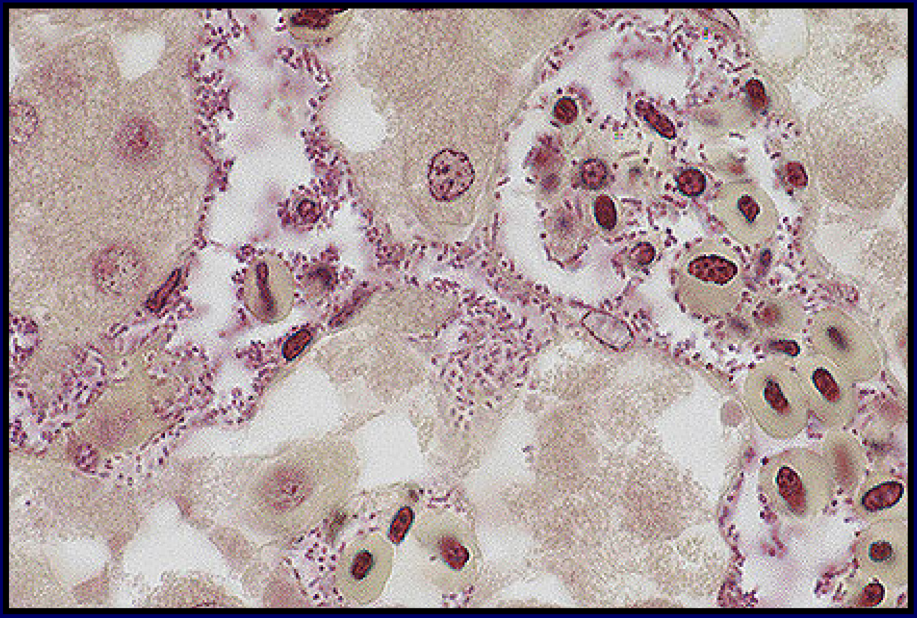
Discovered attenuation of disease-causing microbes induces immunity.
Vaccines for anthrax, rabies, fowl cholera.

For more, see: http://www.pasteur.fr/pasteur/histoire/histoireUS/Pasteur.html

Attenuated vaccines

Attenuate: To make thin, to dilute, to weaken or lessen

- *Pasteurella multocida*
    - Chicken cholera
    - 5-15% fatal
- Pasteur left cultures on bench over vacation and they stopped causing disease (oxygen)
- Chickens challenged with virulent form were protected!
- Chemical methods used to attenuate anthrax also worked



http://www.afip.org/vetpath/WSC/WSC95/images/c18c3.jpg

"Chance favors the prepared mind."
- Louis Pasteur

Rabies vaccine

1880-1885: Pasteur masters his experimental method. He studies rabies. He tries to isolate the germ but cannot find it. Rabies is a disease affecting the nervous system. He grows an “invisible micro-organism'' on rabbit marrow and thereby attenuates its virulence.

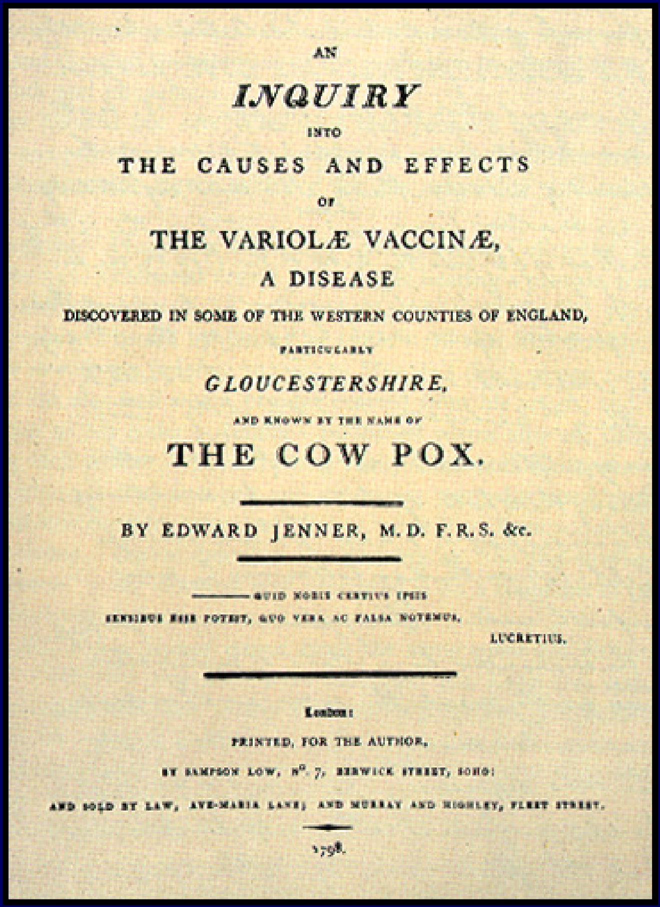
He applies this method of attenuation of virulent rabies to human beings for the first time on July 6th, 1885, when he treats Joseph Meister.

“Pasteur oversaw injections of the child Joseph Meister with "aged" spinal cord allegedly infected with rabies virus. Pasteur used the term "virus" meaning poison, but had no idea of the nature of the causative organism. Although the treatment was successful, the experiment itself was an ethical violation of research standards. Pasteur knew he was giving the child successively more dangerous portions.”

http://dwb.unl.edu/Teacher/NSF/C10/C10Links/www.asmusa.org/mbrsrc/archive/SIGNIFICANT.htm

Edward Jenner
(1749-1823)

- Vaccination (*Vaccinia* virus)
- Observation: milkmaids who suffered cowpox infections rarely contracted smallpox. *Latin “vacca” = cow.*
- No viruses or bacteria discovered yet…
- 14 May 1796: cowpox inoculation from pustule of Sarah Nelmes’ arm to James Phipps (8yrs old)
- 1 Jul 1796: when exposed to disease, James remained healthy.


AN
INQUIRY
INTO
THE CAUSES AND EFFECTS
OF
THE VARIOLÆ VACCINÆ,
A DISEASE
DISCOVERED IN SOME OF THE WESTERN COUNTIES OF ENGLAND,
PARTICULARLY
GLOUCESTERSHIRE,
AND KNOWN BY THE NAME OF
THE COW POX.

BY EDWARD JENNER, M. D. F. R. S. &c.

——QUID NOBIS CERTIUS IPSIS
SENSIBUS ESSE POTEST, QUO VERA AC FALSA NOTEMUS.
LUCRETIUS.

London:
PRINTED, FOR THE AUTHOR,
BY SAMPSON LOW, Nº. 7, BERWICK STREET, SOHO:
AND SOLD BY LAW, AVE-MARIA LANE; AND MURRAY AND HIGHLEY, FLEET STREET.
1798.

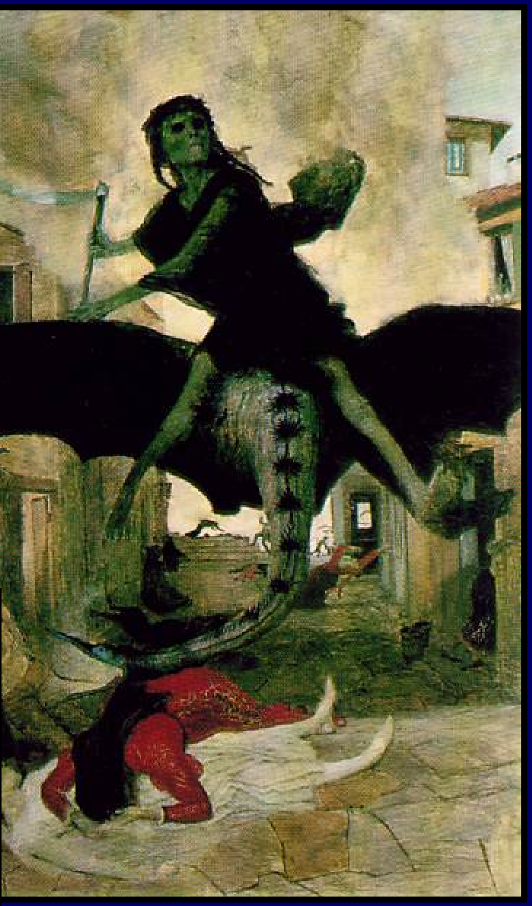
http://www.sc.edu/library/spcoll/nathist/icevv.jpg

1798: **Edward Jenner** introduces the concepts of vaccination using cowpox material to prevent small pox, unfortunately many questions left unanswered.

1804-1806: The Lewis and Clark Expedition.

3. What causes contagious disease?

- Bad vapors?
- Evil spirits?
- Sin?
- State of medicine: dismal



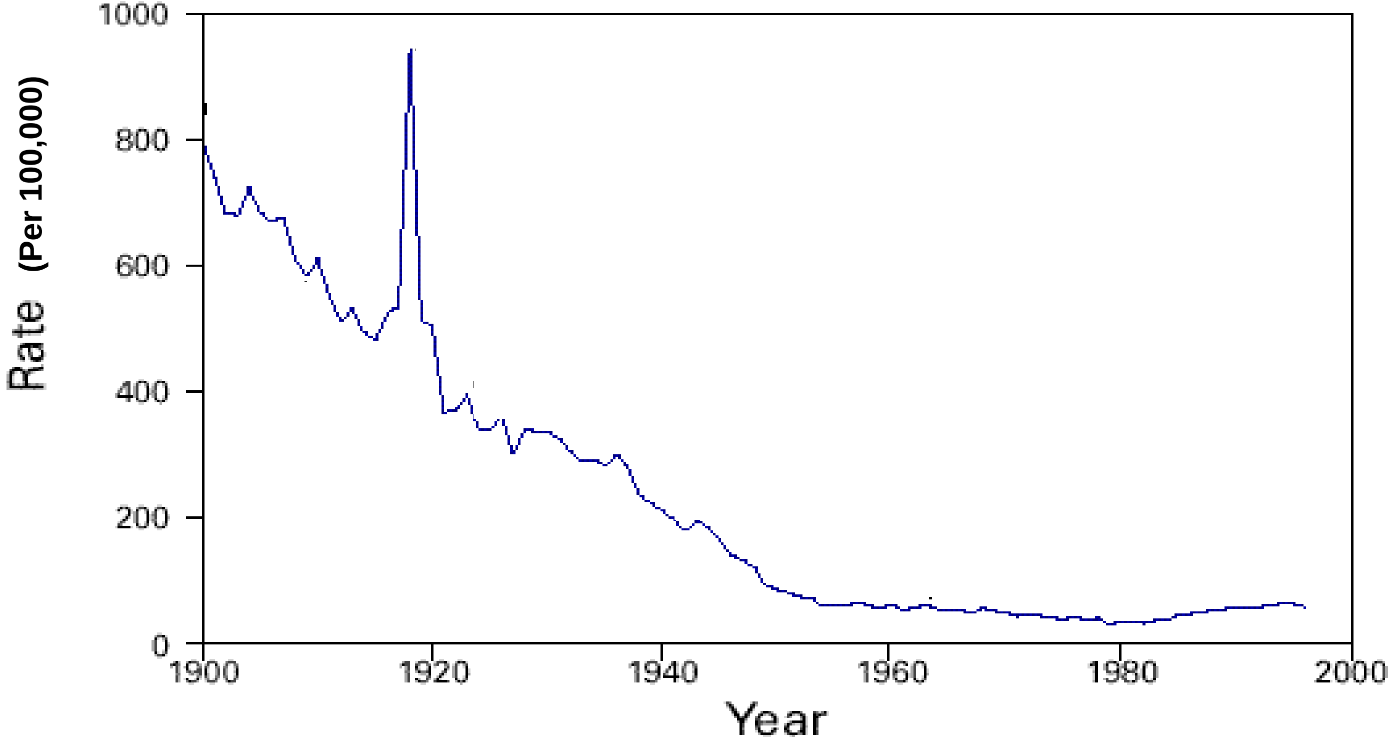
“Plague”, by Arnold Boecklin
(Bubonic plague/Black Death)
Yersinia pestis

http://www.artchive.com/artchive/B/boecklin/plague.jpg.html

http://www.nlm.nih.gov/exhibition/cesarean/cesarean_1.html

http://info.med.yale.edu/library/gifs/art-metallic-300.jpg

FIGURE 1. Crude death rate* for infectious diseases — United States, 1900–1996†



History of Microbiology

1600's

Francesco Redi argues against spontaneous generation of animals (maggots/flies)

Leeuwenhoek and Hook observe microbes (1676)

1700's

John Needham argues for spontaneous generation of microbes (1745)

Spallanzani argues against spontaneous generation of microbes (1768)

1800's

Darwin's Origin of the Species is published and the spontaneous generation debate re-ignites (1859)

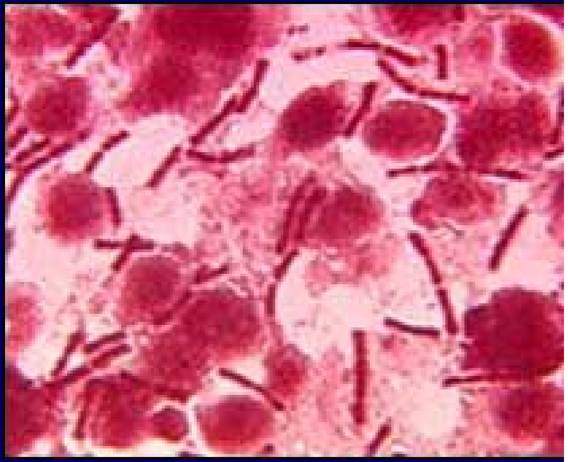
Louis Pasteur publishes experiments that refute the theory of spontaneous generation (France; 1861)

Robert Koch, studying anthrax, validates the germ theory of disease (Germany; 1876); later establishes Koch's postulates (1884)

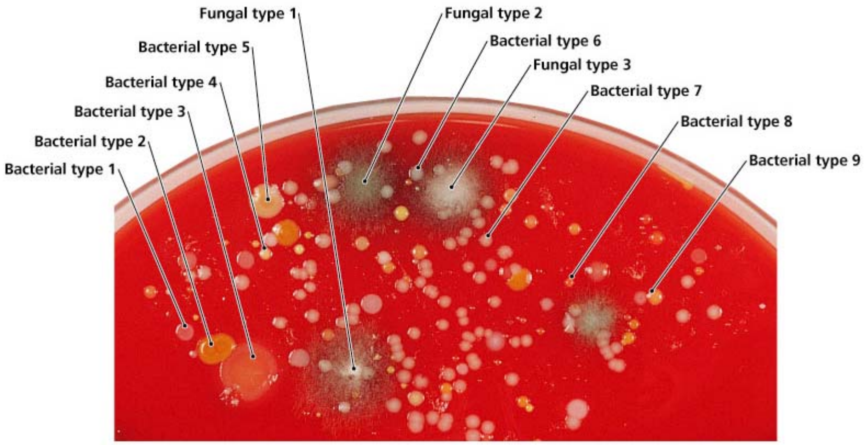
1900's

Robert Koch
(1843-1910)
Contemporary of Pasteur

- Because of the work of Pasteur and others, it was widely believed that germs cause disease
- Koch gave definitive experimental support to the germ theory of ‘infectious’ disease
- Koch noted *Bacillus anthracis* cells and spores in blood of infected animals; proved that they were the causal agent of disease via Koch’s Postulates



http://www.rppi.org/images/anthrax.jpg



Robert Koch
(1843-1910)
Pure cultures

Isolation of single colonies (pure cultures)

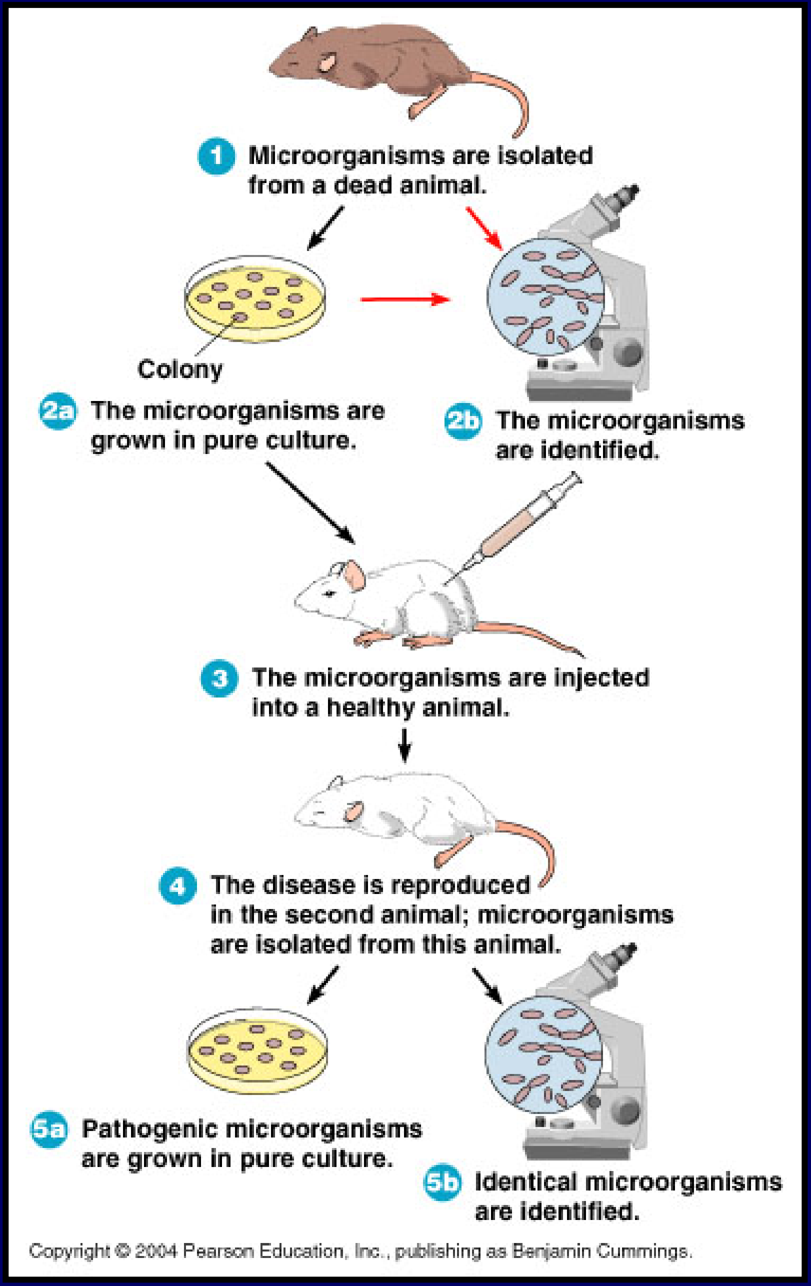
- Grow on solid nutrient source: first potato slices, then gelatin, finally agar (from seaweed; Fannie Hesse’s suggestion)
- Inferred that each colony arose from a single cell that had fallen onto the surface
- Note characteristic shapes and colors of each colony

- This technique allowed isolation and testing of purified bacterial agents

- One problem: bacteria rarely exist in pure cultures in nature so pure cultures are largely artificial!

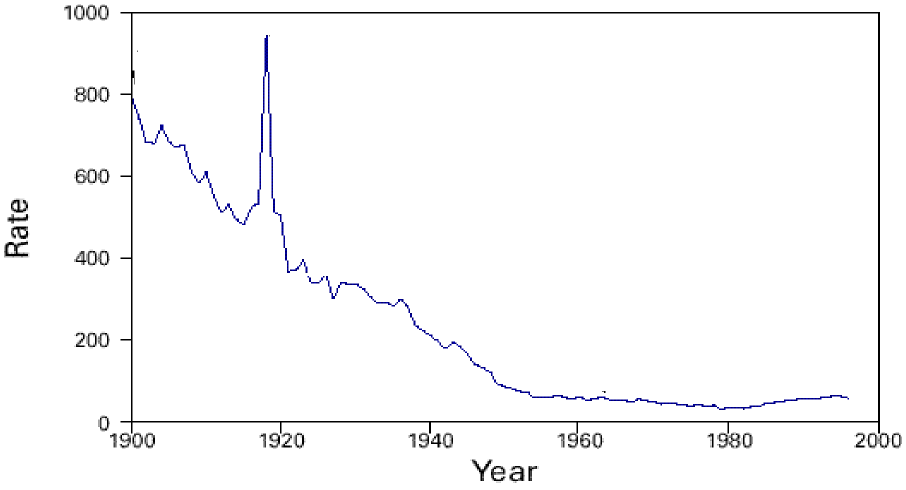
Koch's Postulates

1. Causative agent found in every case of disease and NOT found in healthy hosts.
2. The agent must be isolated and grown outside the host.
3. When introduced into a healthy, susceptible host, the agent must cause the same disease.
4. The same agent must be reisolated from the infected host.



http://diverge.hunter.cuny.edu/~weigang/Images/14-03_koch_1.jpg

FIGURE 1. Crude death rate* for infectious diseases — United States, 1900–1996†



“Consumption”, or TB, killed 1 in 7 (more if you only count young and middle-aged patients). Cough, wasting, death. Cause unknown.

St. Francis of Assisi
John Keats
Elizabeth Barrett Browning
Henry David Thoreau
Emily Brönte
Anton Chekhov
Frederic Chopin

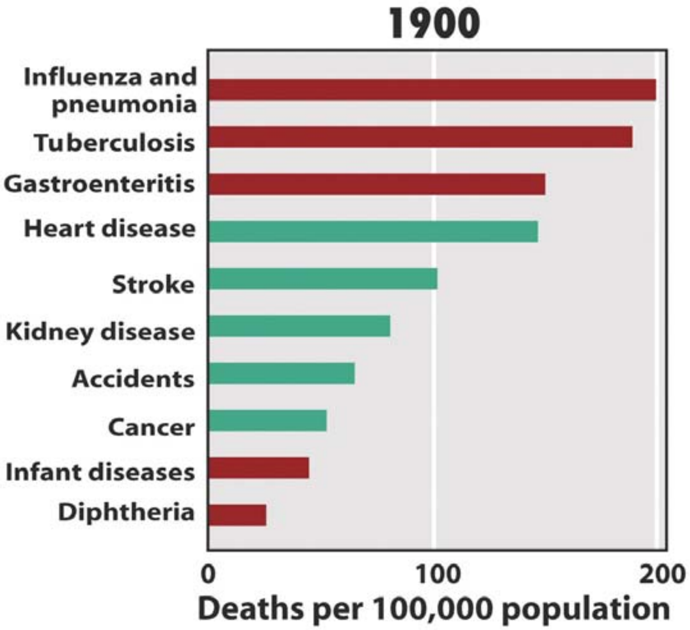


Figure 1-7 part 1 Brock Biology of Microorganisms 11/e
© 2006 Pearson Prentice Hall, Inc.

1882: Koch demonstrated that the causal agent of tuberculosis is *Mycobacterium tuberculosis*:

-isolated from patients
-cultured on blood agar
-pure colonies used to sicken guinea pigs
-selective staining of mammalian tissues (brown) and a bacillus-shaped “parasite” (blue)

-Nobel prize 1905

Learn more!

Pathology of *Mycobacterium tuberculosis:*

http://www.nature.com/nrmicro/animation/imp_animation/index.html

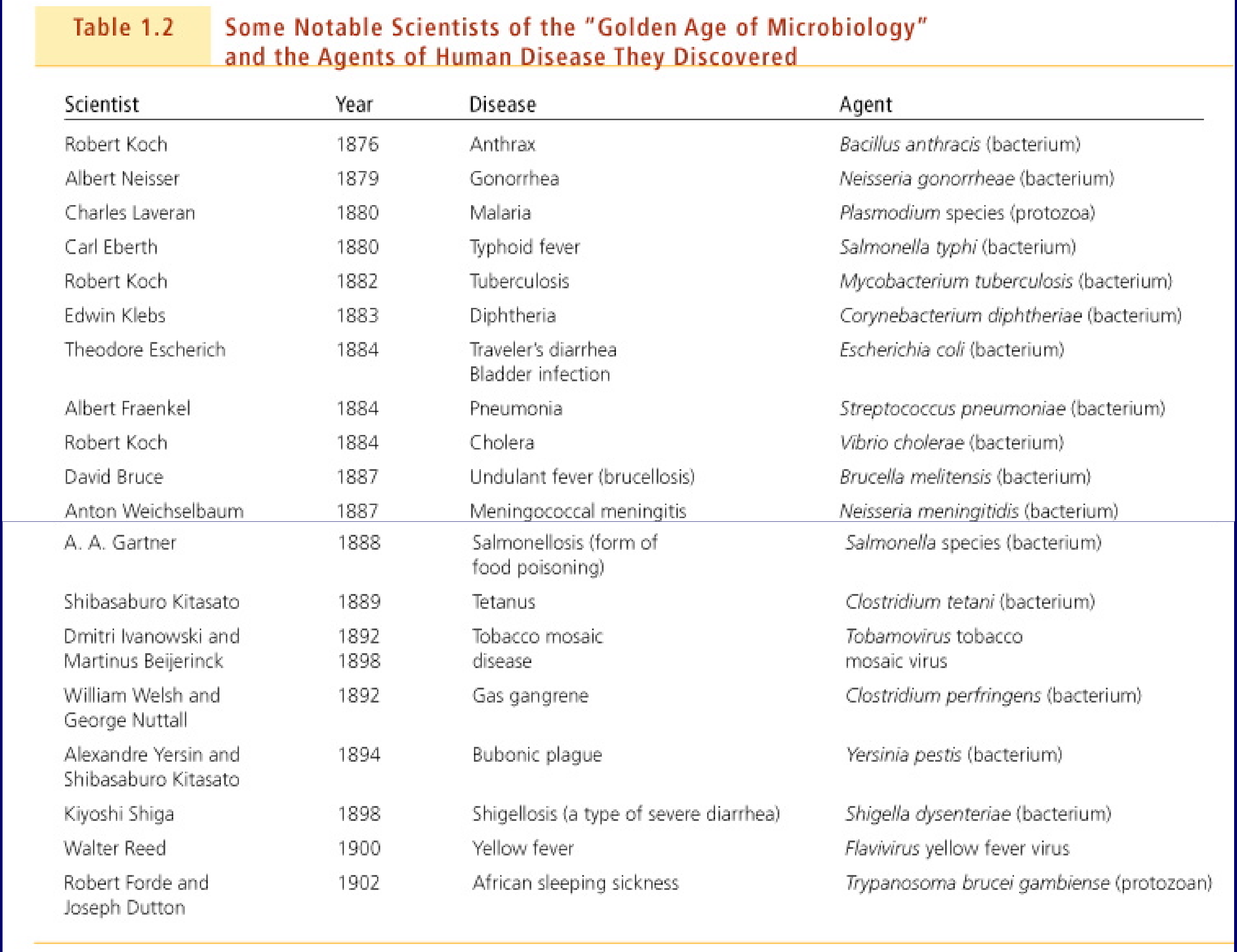


Table 1.2 Some Notable Scientists of the "Golden Age of Microbiology" and the Agents of Human Disease They Discovered

Scientist	Year	Disease	Agent
Robert Koch	1876	Anthrax	*Bacillus anthracis* (bacterium)
Albert Neisser	1879	Gonorrhea	*Neisseria gonorrheae* (bacterium)
Charles Laveran	1880	Malaria	*Plasmodium* species (protozoa)
Carl Eberth	1880	Typhoid fever	*Salmonella typhi* (bacterium)
Robert Koch	1882	Tuberculosis	*Mycobacterium tuberculosis* (bacterium)
Edwin Klebs	1883	Diphtheria	*Corynebacterium diphtheriae* (bacterium)
Theodore Escherich	1884	Traveler's diarrhea Bladder infection	*Escherichia coli* (bacterium)
Albert Fraenkel	1884	Pneumonia	*Streptococcus pneumoniae* (bacterium)
Robert Koch	1884	Cholera	*Vibrio cholerae* (bacterium)
David Bruce	1887	Undulant fever (brucellosis)	*Brucella melitensis* (bacterium)
Anton Weichselbaum	1887	Meningococcal meningitis	*Neisseria meningitidis* (bacterium)
A. A. Gartner	1888	Salmonellosis (form of food poisoning)	*Salmonella* species (bacterium)
Shibasaburo Kitasato	1889	Tetanus	*Clostridium tetani* (bacterium)
Dmitri Ivanowski and Martinus Beijerinck	1892 1898	Tobacco mosaic disease	*Tobamovirus* tobacco mosaic virus
William Welsh and George Nuttall	1892	Gas gangrene	*Clostridium perfringens* (bacterium)
Alexandre Yersin and Shibasaburo Kitasato	1894	Bubonic plague	*Yersinia pestis* (bacterium)
Kiyoshi Shiga	1898	Shigellosis (a type of severe diarrhea)	*Shigella dysenteriae* (bacterium)
Walter Reed	1900	Yellow fever	*Flavivirus* yellow fever virus
Robert Forde and Joseph Dutton	1902	African sleeping sickness	*Trypanosoma brucei gambiense* (protozoan)

…and see Table 1.1 in your text for a broader (not only medical) view!

4. OK, microbes cause disease (germ theory).

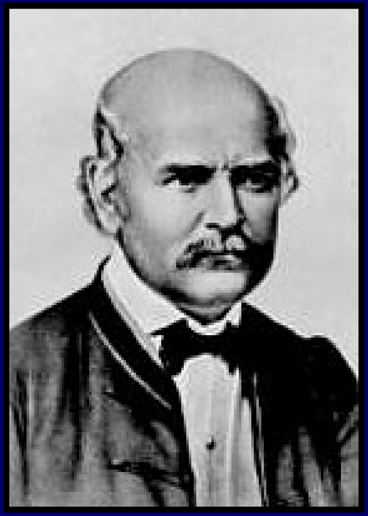
How do we prevent and treat disease?

Early medicine:

- Long hours
- No handwashing
- Dirty clothes
- No sanitation systems

Warren, P. (2005). http://www.umanitoba.ca/faculties/medicine/units/history/notes/surgery/surgery2.html

Ignaz Semmelweis: aseptic technique to halt germ spread (1818-1865)



http://www.m-ww.de/persoenlichkeiten/semmelweis.html

Childbed fever:

- endometrium infection by Group A *Streptococcus*

Spread by med students but not midwives

Wash hands frequently:

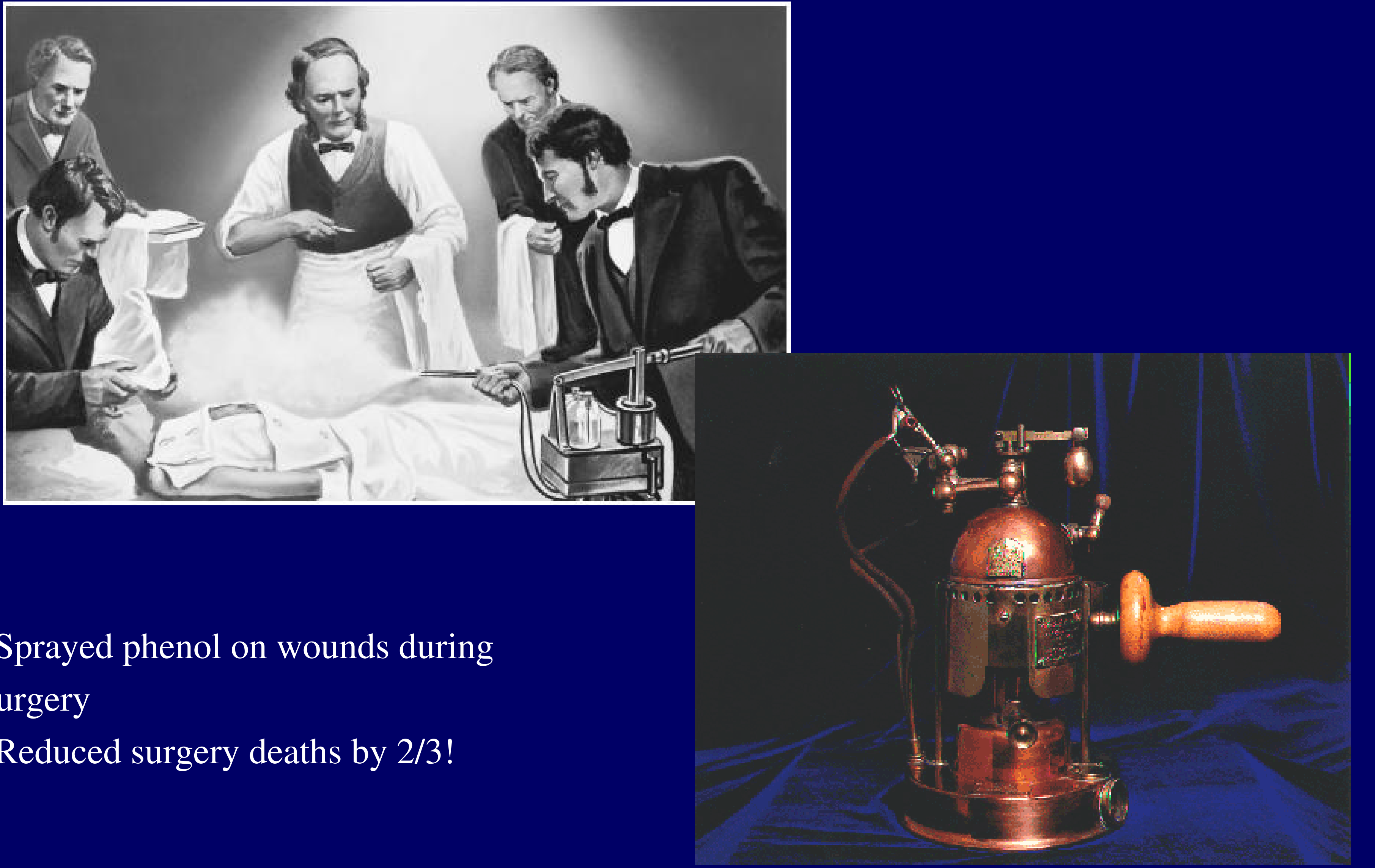
- Reduced death rate from 18.3 to 1.3% in one year

Irony:

Semmelweis died of *Streptococcus* infection in hospital

In the late 1840's, Dr. Ignaz **Semmelweis** was an assistant in the maternity wards of a Vienna hospital. There he observed that the mortality rate in a delivery room staffed by medical students was up to three times higher than in a second delivery room staffed by midwives. In fact, women were terrified of the room staffed by the medical students. **Semmelweis** observed that the students were coming straight from their lessons in the autopsy room to the delivery room. He postulated that the students might be carrying the infection from their dissections to birthing mothers. He ordered doctors and medical students to wash their hands with a chlorinated solution before examining women in labor. The mortality rate in his maternity wards eventually dropped to less than one percent.

Joseph Lister: antiseptic technique to kill germs
(1827-1912)

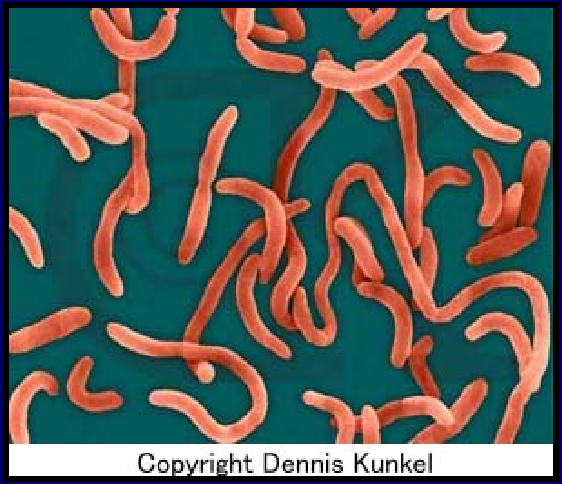


- Sprayed phenol on wounds during surgery
- Reduced surgery deaths by 2/3!

http://www.cf.ac.uk/hisar/people/kw/lister.jpg

John Snow: immunology (1818-1858)

- *On the mode of communication of cholera* (1849)
  - Cholera is an infectious agent transferred in stools and vomit
  - Waterborne disease, rare in US, but still exists in warm coastal climes (India, S. America, sub Saharan Africa)
  - *Vibrio cholerae*
- Epidemiology
- Public health
- Cholera epidemics in London linked to a single well by Snow's research (Broad street pump)



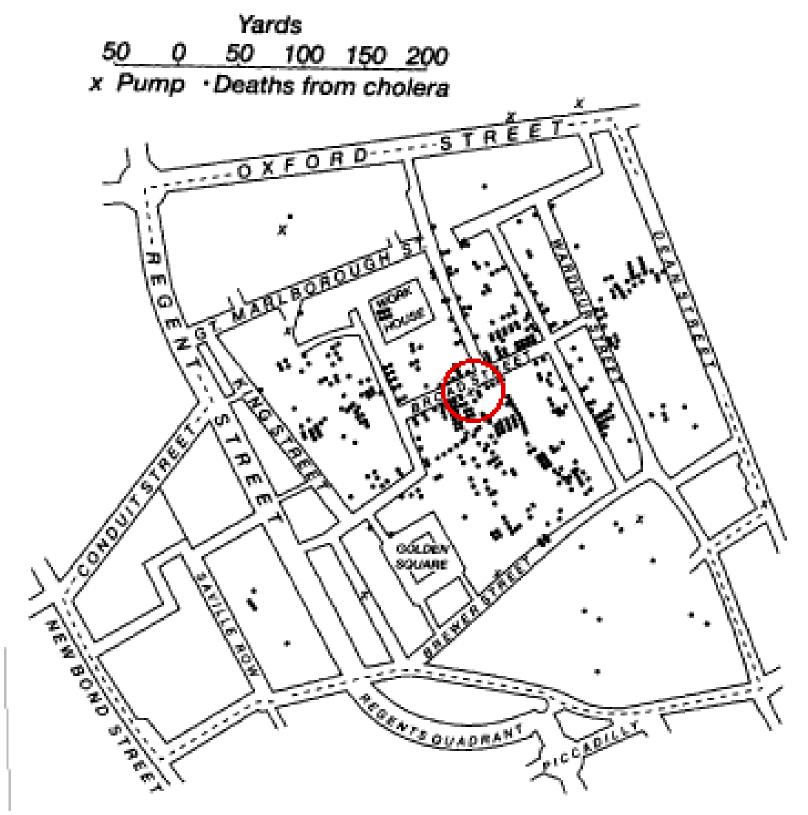
Copyright Dennis Kunkel

Vibrio cholera

http://www.ph.ucla.edu/epi/snow/watermap1856/watermap_1856.html

http://www.buddycom.com/bacteria/gnr/gnrgluox.html

John Snow: first epidemiology (1854)



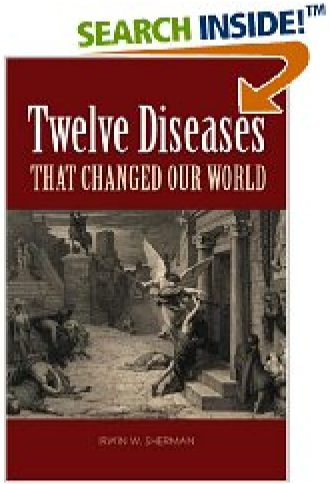
Tracked cholera episodes and observed that homes served by one of two major water systems were markedly (10X) more susceptible to cholera, regardless of standard of living

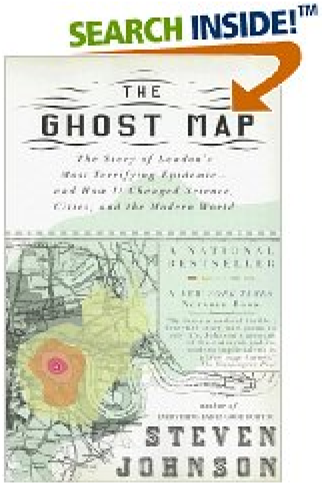
Cholera epidemics in London were linked by Snow's research to a single well (Broad Street pump) – it drew water from the Thames in an area where untreated sewage entered the river.

http://www.ph.ucla.edu/epi/snow/watermap1856/watermap_1856.html

http://www.buddycom.com/bacteria/gnr/gnrgluox.html

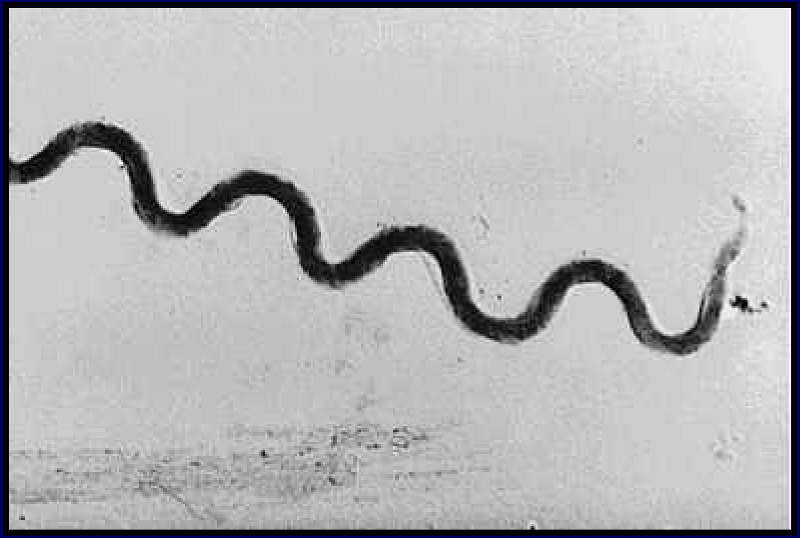
Learn more!


SEARCH INSIDE!™
Twelve Diseases
THAT CHANGED OUR WORLD
IRWIN W. SHERMAN


SEARCH INSIDE!™
THE
GHOST MAP
The Story of London's Most Terrifying Epidemic—and How It Changed Science, Cities, and the Modern World
A NATIONAL BESTSELLER
STEVEN
JOHNSON

Paul Ehrlich: Chemotherapy and antimicrobial drugs
(1854-1915)

- "Magic bullet"
- Tested over 900 arsenic compounds on mice
- One of these (Salvarsan) worked on *Treponema pallidum* (syphilis) and cured the disease without killing the patient
- First antimicrobial

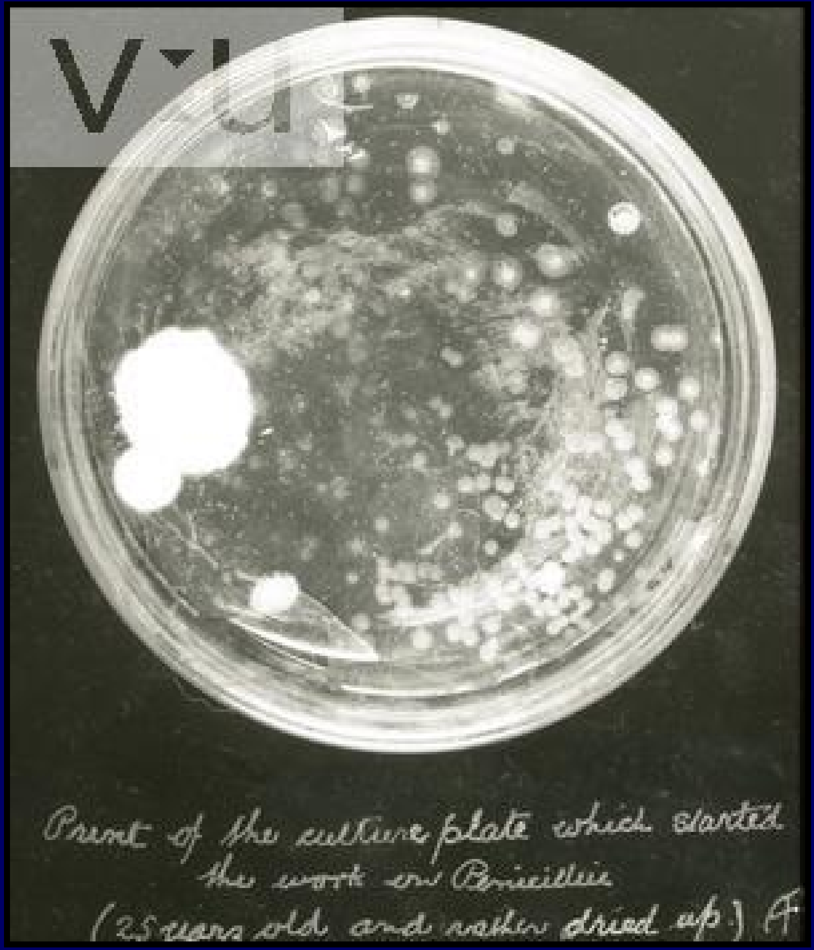


T. pallidum spirochete

http://www.pbs.org/wgbh/aso/databank/entries/dm09sy.html
http://www.uveitis.org/images/syphil1.jpg

Alexander Fleming
(1881-1955)

1928: Messy lab bench leads to penicillin discovery.



Print of the culture plate which started the work on Penicillin
(25 years old and rather dried up) AF

Penicillium

1938 - Flory and Chain (Oxford) purify penicillin. It is used to treat infections of soldiers in WWII.

http://www.chemsoc.org/timeline/pages/1928.html
http://www.visualsunlimited.com/browse/vu360/vu3606.html

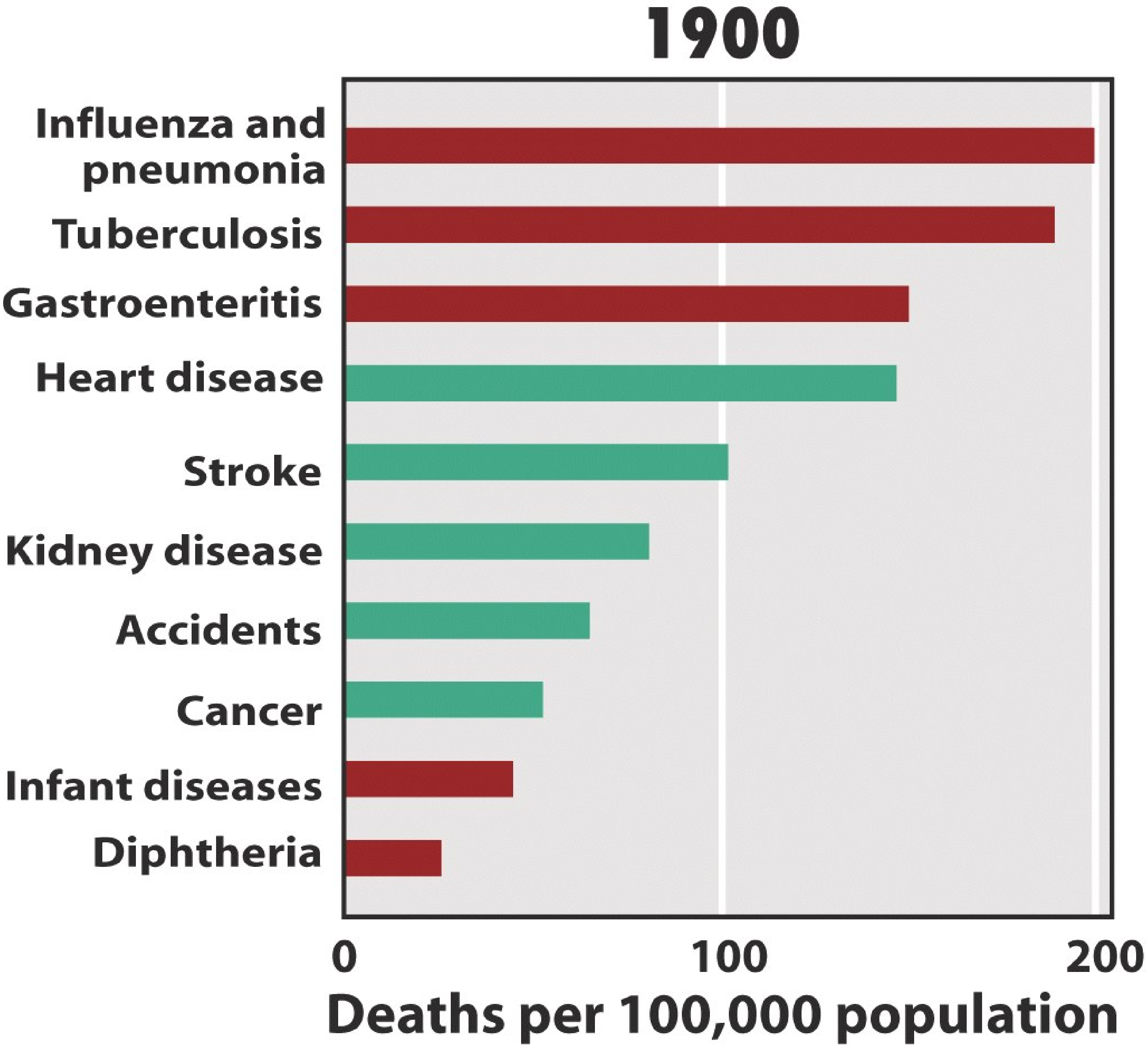


Figure 1-7 part 1 Brock Biology of Microorganisms 11/e

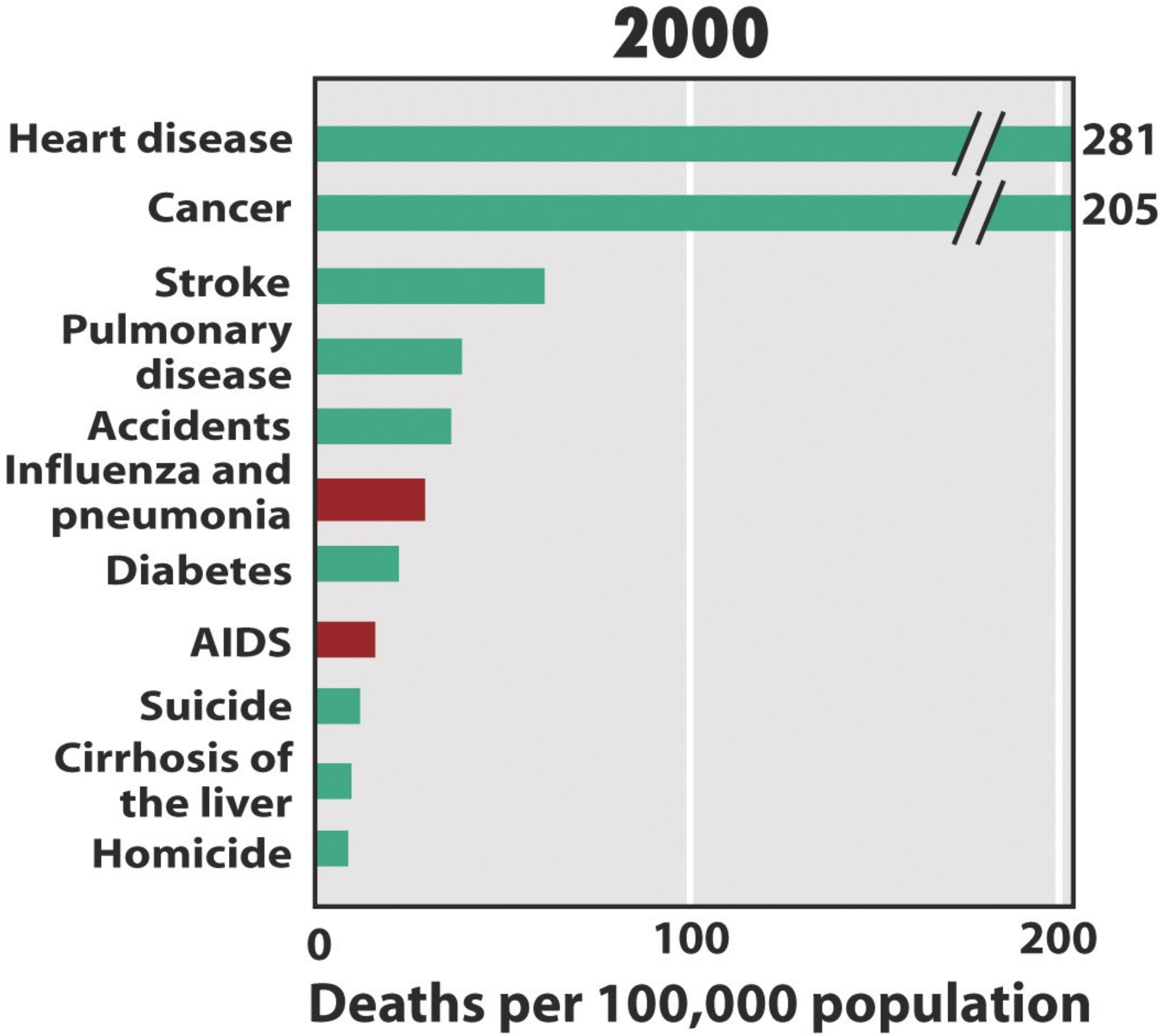


Figure 1-7 part 2 Brock Biology of Microorganisms 11/e

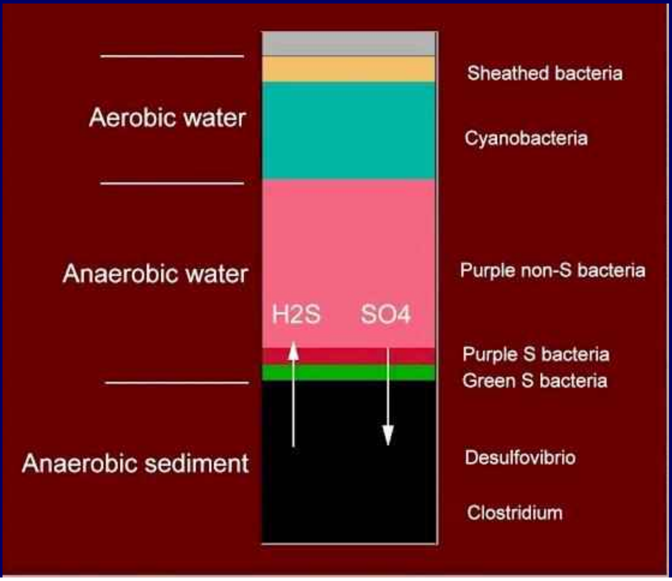
Sergei Winogradsky
(1856-1953)
Microbes in mixed communities

- Interested in nutrient cycling:
  - Nitrification, $NH_4 \rightarrow NO_3$
- Showed that bacteria can oxidize iron, sulfur, and ammonia to obtain energy; showed that bacteria can incorporate CO_2 into organic matter as do plants
- Proposed concept of chemolithotrophy (oxidation of inorganic compounds linked to energy conservation
- Described anaerobic bacterial N_2-fixation (*Clostridium pasteurianum*)

Sergei Winogradsky
(1856-1953)
Microbes in mixed communities

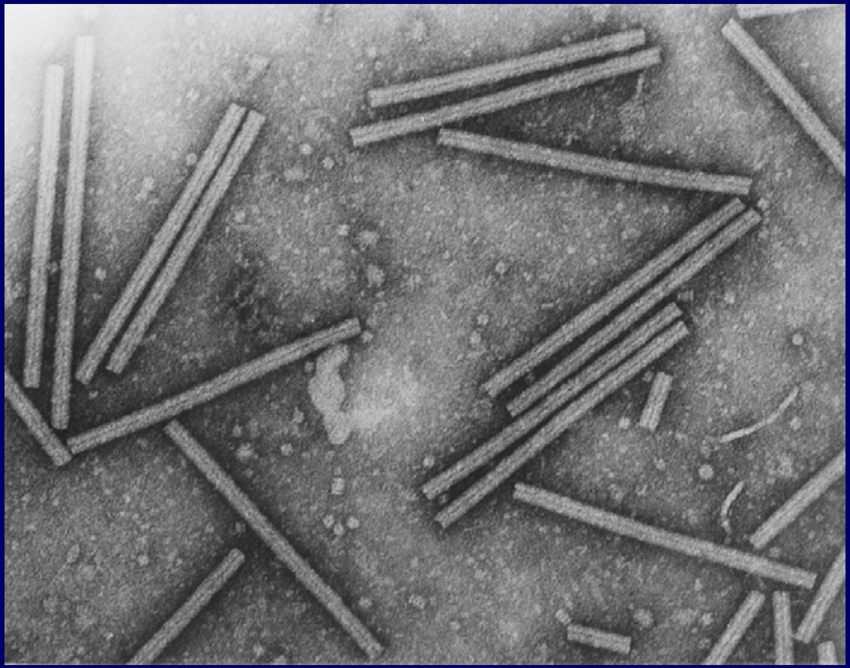
Showed that bacteria are biogeochemical agents

- **Winogradsky Column**: device for culturing a large diversity of microbes; pond sediment + carbon source + sulfur source + light; gradients of O_2 and sulfide form selecting for various metabolic abilities.



Martinus Beijerinck: Microbes in mixed communities (1851-1931)

- Described aerobic N_2 fixation (*Azotobacter, Rhizobium*)
- Found in 1892 that filtered plant extracts would transmit Tobacco Mosaic
- Filters didn’t allow bacteria to pass
- Called the filterable agent a ‘virus’ by Beijerinck



http://www.ncbi.nlm.nih.gov/ICTVdb/WIntkey/Images/a6.gif

Also introduced the idea of using **enrichment culture** to select for certain microbes: isolate natural samples in a selective fashion, with careful attention to nutrient and incubation requirements. Isolated:

-sulfate-reducers
-sulfur-oxidizers
-nitrogen fixers
-green algae
-etc.

History of Microbiology

1600's

Robert Hooke publishes his discovery of cells in cork (1665)

Antony van Leeuwenhoek observes "animacules" using his homemade microscope (Netherlands; 1676)

1700's

Edward Jenner developed a vaccination procedure for smallpox (England; 1796)

1800's

John Snow traces the spread of cholera to drinking water (England; 1855)

Louis Pasteur publishes experiments that refute the theory of spontaneous generation (France; 1861)

Robert Koch, studying anthrax, validates the germ theory of disease (Germany; 1876); later establishes Koch's postulates (1884)

1900's

Chain and Florey isolate penicillin and use it to treat a patient (England; 1940)

Carl Woese recognizes that Archaea are distinct from Bacteria (United States; 1977)

Barry Marshall demonstrates that a bacterium causes stomach ulcers (Australia; 1984)

James Watson, Francis Crick, Rosalind Franklin, and Maurice Wilkins determine the structure of DNA (England; 1953)

The first complete nucleotide sequence of a bacterial genome is completed (United States; 1995)

Modern History

- **1980's:**

Genetic manipulation and cloning (PCR)
AIDS and causative agent HIV
Ribozymes or catalytic RNAs
RNAs used historical documents – Molecular phylogeny
reshapes biological systematics to 3 domains of life

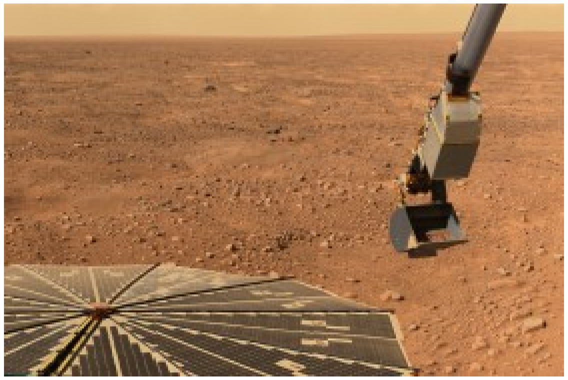
- **1990's:**

Genetically engineered microorganisms
Prions or specific protein pathogens
Full genome sequences

- **2000's:**

Ed DeLong's contributions to marine microbiology
Craig Venter's environmental genome shotgun sequencing
David Relman, Jeffrey Gordon, and others: description of the human supraorganism
Human metagenome sequencing project

What's next… ???



“The microbes will have the last word.”

- Louis Pasteur