Chemistry 259

Medicinal Chemistry of Modern Antibiotics

Spring 2012

Lecture 2: ***History of Antibiotics***

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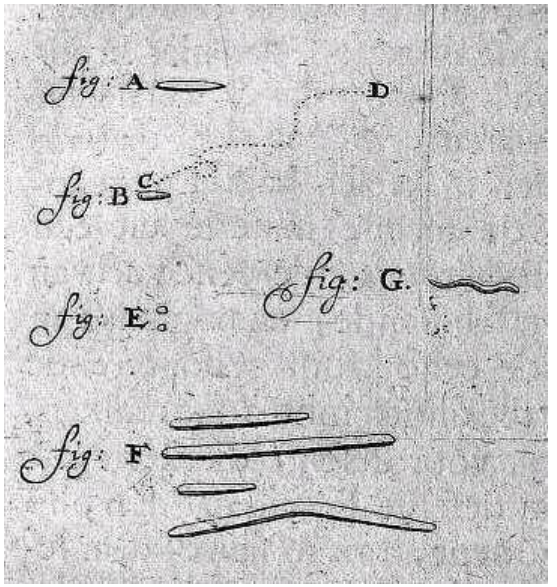
University of California, San Diego

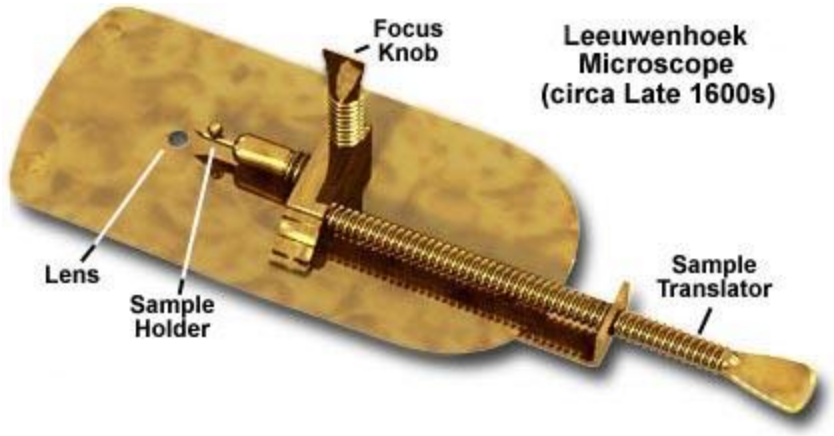
Prelude to Antibiotics: Leeuwenhoek & The Birth of Microbiology



Bacteria in tooth plaque (1683)

Antonie van Leeuwenhoek
(Delft, 1632-1723)

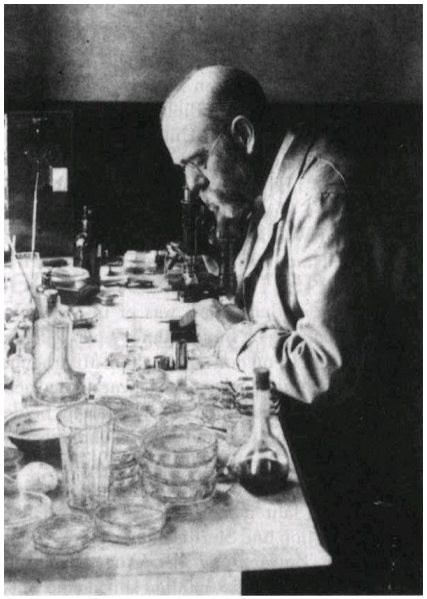
First to observe and describe single celled organisms which he first referred to as *animalicula*, and which we now know to be microorganisms (*protozoa, bacteria*).



Prelude to Antibiotics: Pasteur, Koch & The Germ Theory of Disease

Louis Pasteur
(Strasbourg, 1822-1895)

Showed that some microorganisms contaminated fermenting beverages and concluded that microorganisms infected animals and humans as well.



Robert Koch
(Berlin, 1843-1910)

Discovered *Bacillus anthracis*, *Mycobacterium tuberculosis*, *Vibrio cholerae* and developed “Koch’s Postulates".

Nobel Price in Medicine 1905 for work on tuberculosis.

Koch’s Postulates: (1890)

To establish that a microorganism is the cause of a disease, it must be:

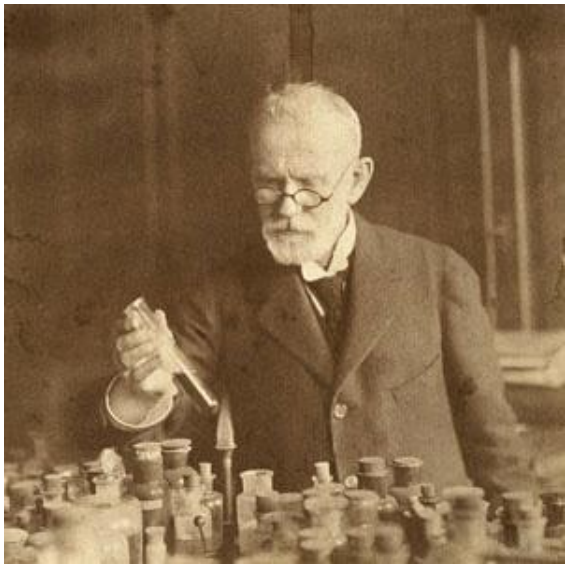
1) found in all cases of the disease.

2) isolated from the host and maintained in pure culture.

3) capable of producing the original infection, even after several generations in culture.

4) recoverable from an experimentally infected host.

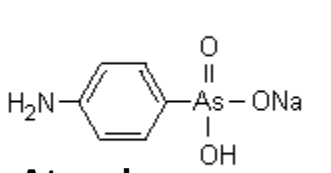
Invention of Modern Drug Discovery: Ehrlich & The Magic Bullet



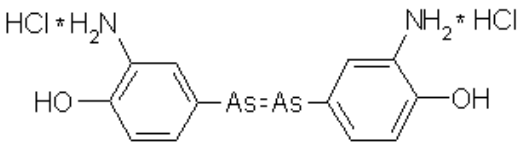
Paul Ehrlich
(Frankfurt, 1854-1915)

Synthesized and screened hundreds of compounds to eventually discover and develop the first modern chemotherapeutic agent (**Salvarsan**, 1909) for the treatment of syphillis (*Treponema pallidum*).

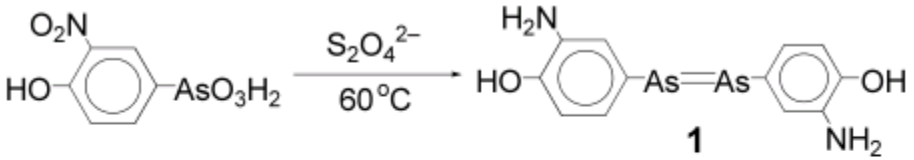
Nobel Price in Medicine 1908 for work on immunity (with Mechnikov).



Atoxyl
(Bechamp 1859)



Salvarsan
(Compound **606**, Hoechst 1910)



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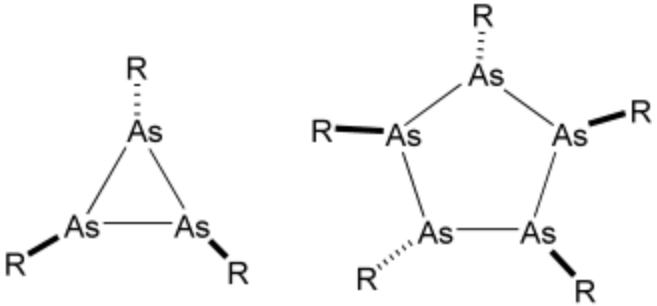

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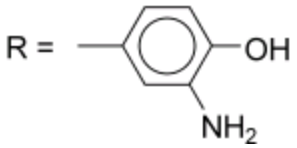
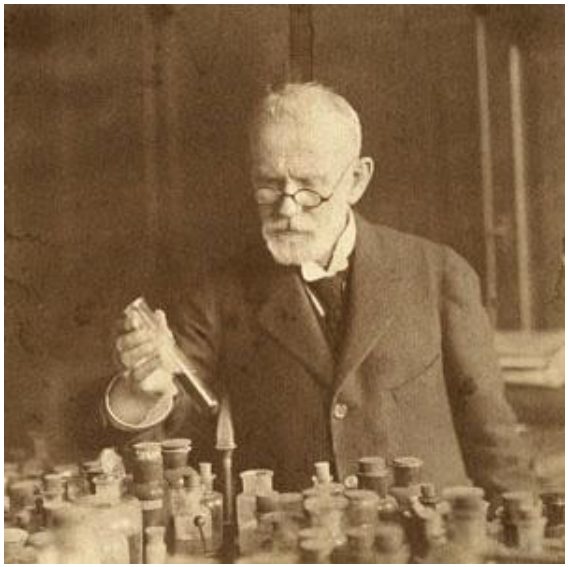


Salvarsan in solution consists of cyclic species (RAs)*n*, with *n*=3 (**2**) and *n*=5 (**3**) as the preferred sizes.

Lloyd *et al.* (2005) *Angewandte Chemie* **117** (6), 963.

Magic Bullet: Compound that selectively targets a disease causing organism while having no negative effect on human tissue.

Prelude to Modern Drug Discovery: Ehrlich & The Magic Bullet



Paul Ehrlich
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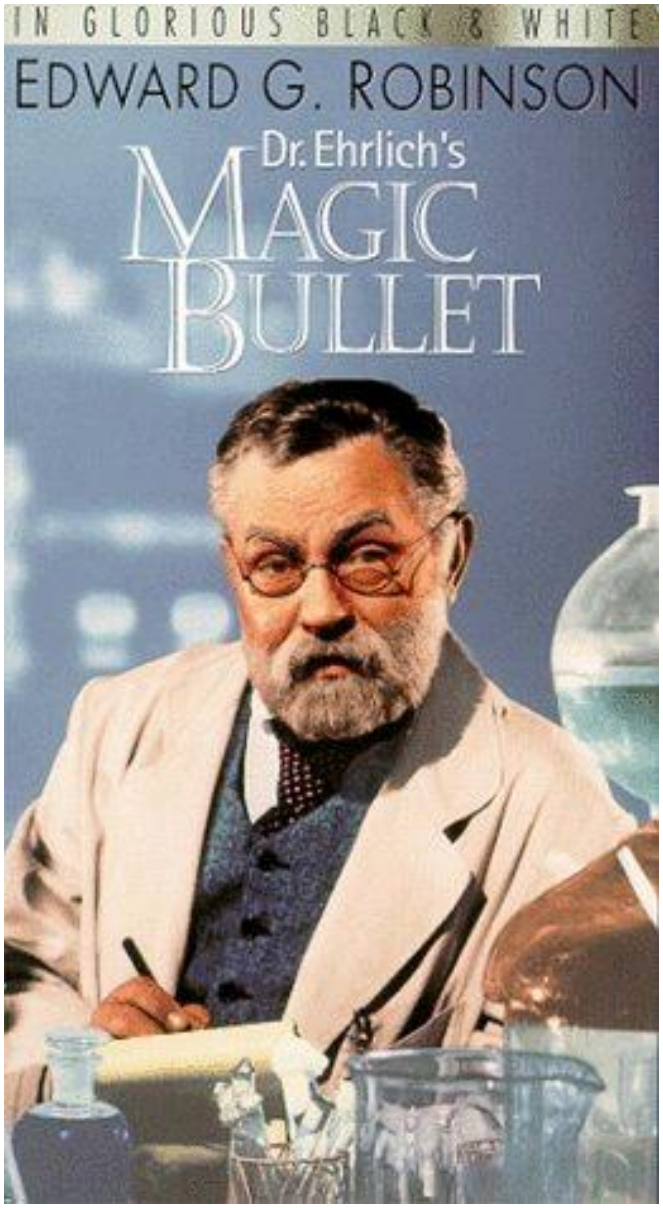


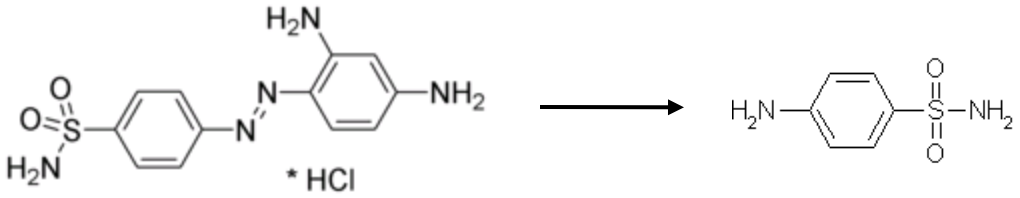
1940 (USA)

First Antibiotics: Domagk Discovers Sulfonamides ("Sulfa-Drugs")

Gerhard J. P. Domagk
(Wuppertal, 1895-1964)

Worked at Bayer (IG Farben) where he discovered and developed sulfonamides (**Prontosil**), the first drugs effective against bacterial infections.

Nobel Price in Medicine 1939 for discovery of sulfonamides.



Prontosil (red azo dye)
(Bayer 1935)

Sulfanilamide
(1936)

Prontosil is a prodrug that is not active *in vitro*. Cleavage in the gastrointestinal tract leads to the active compound sulfanilamide which is competes with *p*-aminobenzoic acid, the substrate of dihydropteroate synthetase in the bacterial synthetic pathway to folic acid.

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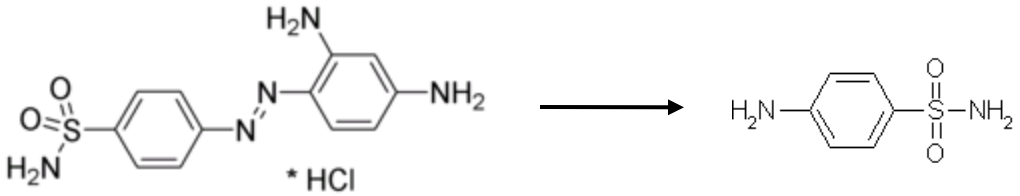


Prontosil (red azo dye)
(Bayer 1935)

Sulfanilamide
(1936)

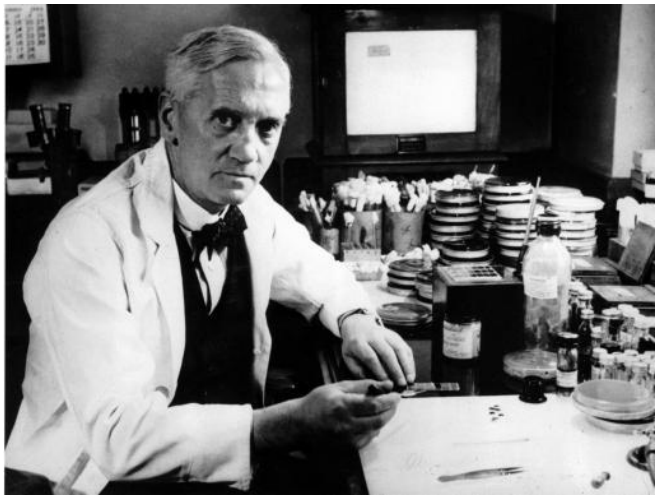
Domagk treated his own daugther with prontosil to fight a severe streptococcal infection and eventually saved her life.

He was forced by the Nazi regime to refuse the Nobel Price and was finally able to receive the price in 1947 (but not the monetary portion).

Sulfanilamide had a central role in preventing infection during WW II.

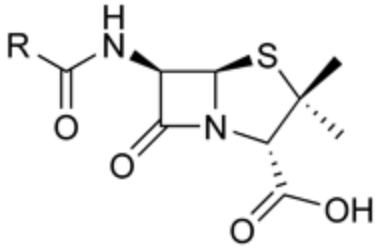
A toxic preparation of sulfanilamide in diethylene glycol (Elixir Sulfanilamide) killed over 100 children in the US, and led to the passage of the Federal Food, Drug, and Cosmetic Act (FD&C) by Congress in 1938 giving authority to the Food and Drug Administration (FDA) to oversee the safety of drugs.

First Antibiotic from a Natural Source: Fleming Discovers Penicillin

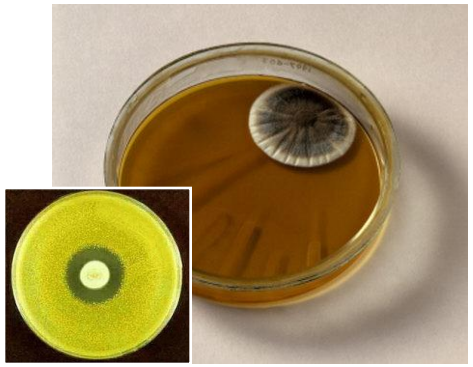


Alexander Fleming
(London, 1881-1955)

Nobel Price in Medicine 1945 for discovery of penicillin (with Chain and Florey).



Penicillin
(R= benzyl, alkyl, etc.)



Penicillins are β-lactam antibiotics that act as suicide substrates for peptidoglycan transpeptidases involved in crosslinking the bacterial cell wall.

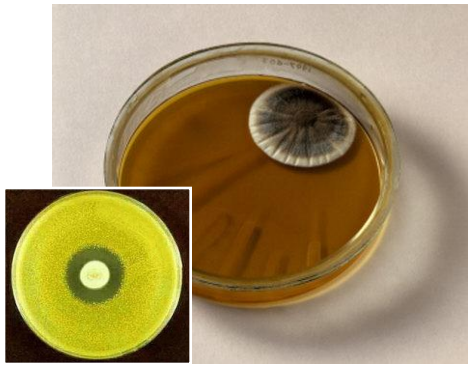
Alexander Fleming observed, working at St. Mary's Hospital in London in 1928, that a plate culture of *Staphylococcus* had been contaminated by a blue-green mold (*Penicillium*) and that colonies of bacteria adjacent to the mold were being dissolved. The mold grown in pure culture produced a substance that killed bacteria. Naming the substance penicillin, Fleming in 1929 published the results of his investigations (*Brit. J. Exper. Path.* 1929, 10, 226), noting that pencillin might have therapeutic value if it could be produced in quantity (which he was unable to do).

(In 1896, the French medical student Ernest Duchesne originally discovered the antibiotic properties of *Penicillium*, but failed to report a connection between the fungus and a substance that had antibacterial properties, and *Penicillium* was forgotten in the scientific community until Fleming's rediscovery.)

Antibiotic Susceptibility Screening by Disk Diffusion Assay

Agar plate with lawn of bacterial growth.

Paper disks impregnated with antibiotic



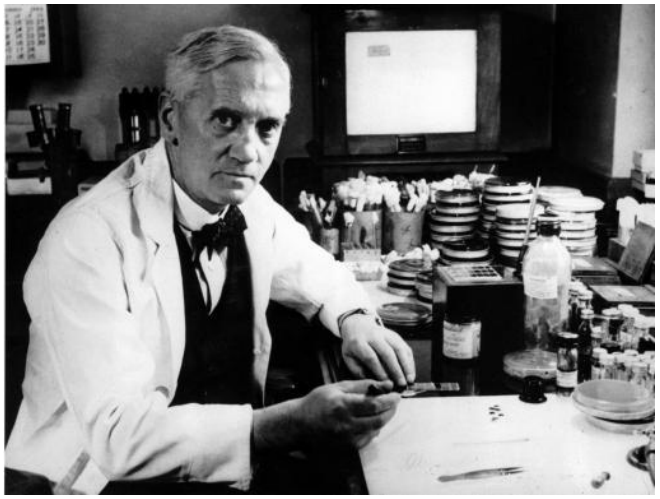
When bacterial multiplication proceeds more rapidly than the drug can diffuse, the bacterial cells that are not inhibited by the antimicrobial will continue to multiply until a lawn of growth is visible and no zone of inhibition appears around the disk.

When the antimicrobial is present in inhibitory concentrations, no growth will appear in the zone around the disk. The more susceptible the bacterial strain tested, the larger the zone of inhibition.

The diameter of the zone of inhibition is indirectly proportional to the minimal inhibitory concentration (MIC).

(The MIC assay is readily adapted to liquid cultures. Bacterial growth is measured as optical density at 600 nm, in the absence and presence of different amounts of drug.)

First Antibiotic from a Natural Source: Fleming Discovers Penicillin

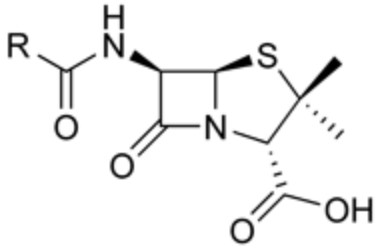


Alexander Fleming
(London, 1881-1955)

Nobel Price in Medicine 1945 for discovery of penicillin (with Chain and Florey).

Ernst B. Chain
(1806-1979)

Howard W. Florey
(1898-1968)



Penicillin
(R= benzyl, alkyl, etc.)

In 1939, Australian H. W. Florey, German E. B. Chain, and others, working at the University of Oxford, reinvestigated penicillin and demonstrated its *in vivo* efficacy (*Lancet* 1940, 239, 226).

They started to work on the larger scale production of penicillin. In 1941, laboratories at the US Department of Agriculture and Merck & Co joined into penicillin research.

A high yield producing strain (*Penicillium chrysogenum*) was isolated from a moldy cantaloupe that a scientist brought to the laboratory.

Within a short period, large scale production of pencillin was established to produce antibiotic for the Allied forces.

First Antibiotic from a Natural Source: Industrial Production of Penicillin

1940: priceless
1943: $20 /dose
1946: $0.55 /dose

Photos: Penicillin production in the 1940s

Despite efforts of mass production, availability of penicillin was severely limited. Rapid renal clearance of the drug required frequent administration at high doses. In the early days of penicillin therapy it was common practice to re-isolate the drug from excreted urine of patients for reuse.

First Antibiotic from a Natural Source: Industrial Production of Penicillin

Drug advertising from the 1940s.

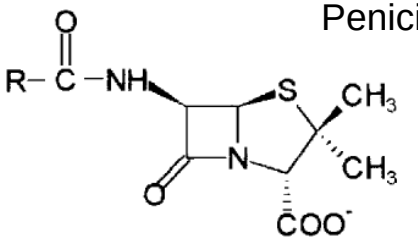
1949 (Great Britain)

First Antibiotic from a Natural Source: Penicillin & β-Lactams

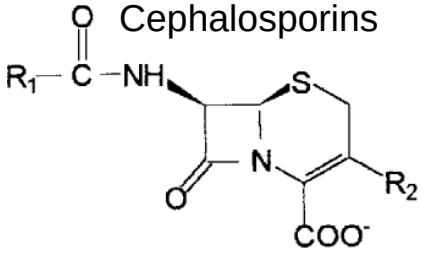
Dorothy M. C. Hodgkin
(Oxford, 1910-1994)

Nobel Price in Chemistry 1964 for X-ray crystallographic structure determination of important biochemical molecules.

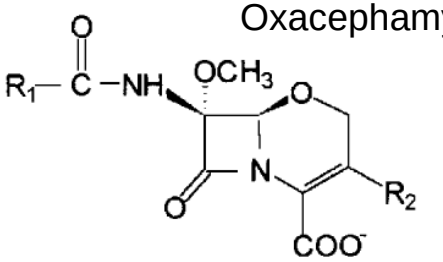
Penicillins



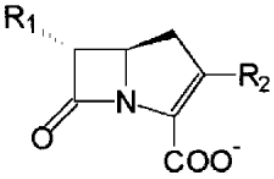
Cephalosporins



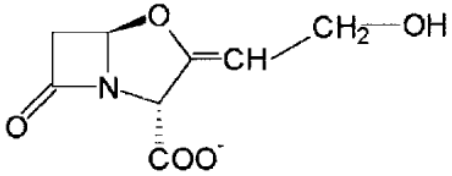
Oxacephamycins



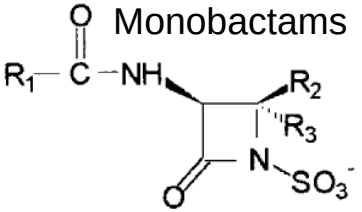
Carbapenems



Clavulanate



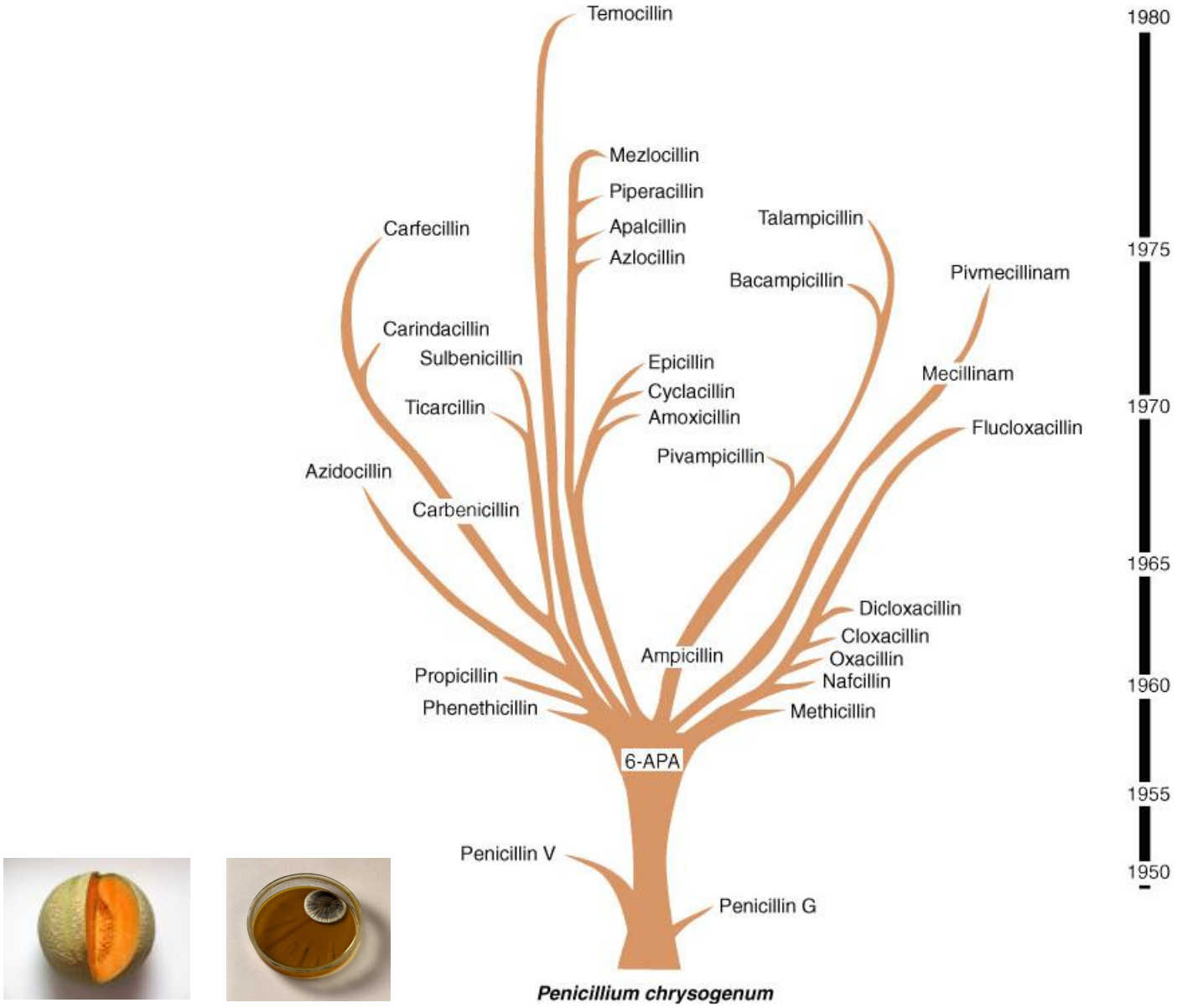
Monobactams



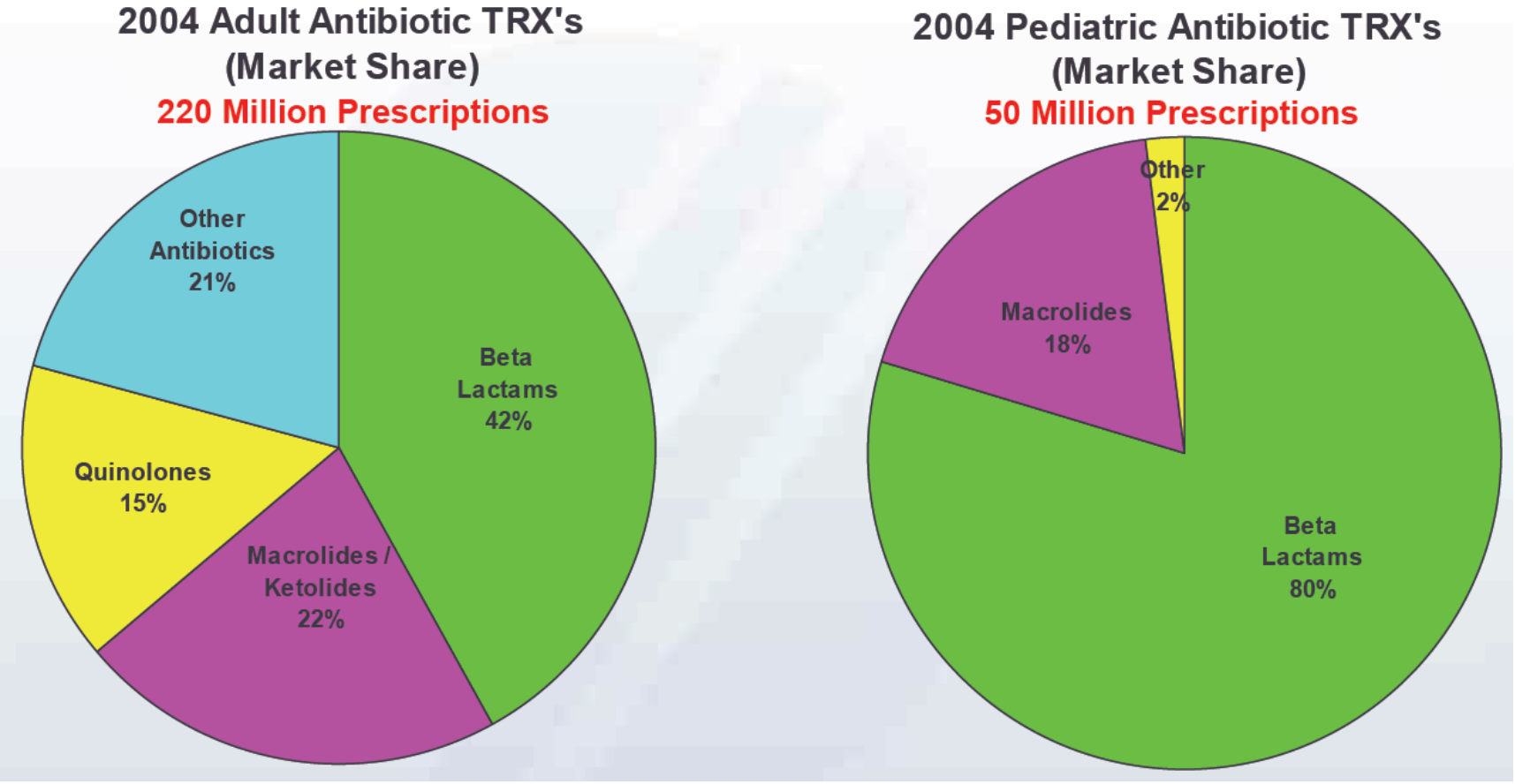
In 1944, Hodgkin crystallized penicillin and determined its structure by X-ray crystallography, solving a controversy around the chemical constitution of the antibiotic and paving the way for discovery of improved antibiotics by modification of the natural products and total synthetic approaches.

Since then, penicillins and other β-lactam drugs have become the single most important class of antibiotics.

First Antibiotic from a Natural Source: Penicillin & β-Lactams



First Antibiotic from a Natural Source: Penicillin & β-Lactams Today



(Source: IMS Health, National Prescription Audit)

(The comparatively larger share of β-lactams in the pediatric prescriptions reflects the good tolerance and safety profile of these antibiotics.)

Impact of Penicillin and Sulfonamides on Mortality

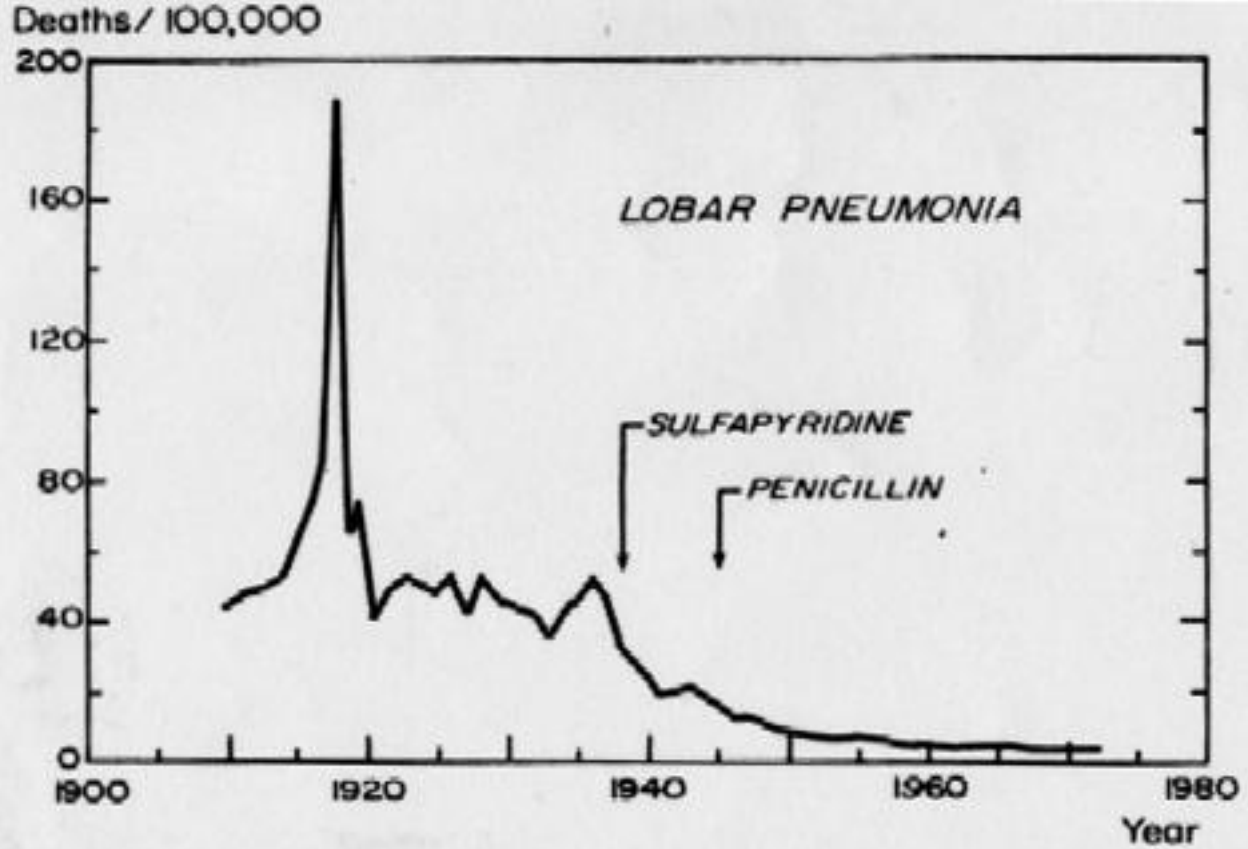


FIGURE 2. Lobar Pneumonia: Deaths per 100,000 Population—Impact of Sulfapyridine and Penicillin

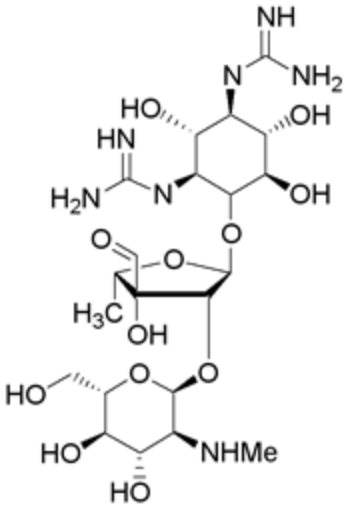
SOURCE: See figure 1.

Antibiotics from Systematic Screening: Waksman & Aminoglycosides

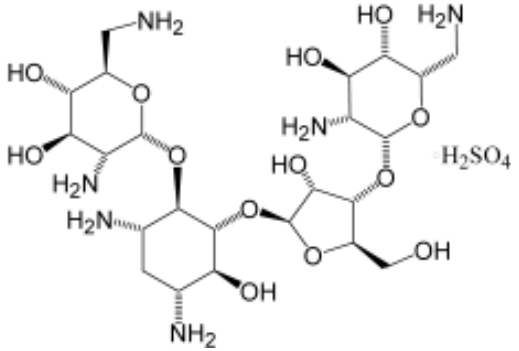
Selman A. Waksman
(New Jersey, 1888-1973)

Worked at Rutgers University where he systematically screened soil bacteria and fungi as source of antibiotics. Discovered a number of new antibiotics, including actinomycin, clavacin, streptothricin, streptomycin, grisein, neomycin, fradicin, candicidin, candidin, and others.

Nobel Price in Medicine 1952 for discovery of streptomycin, the first antibiotic effective against tuberculosis.



Streptomycin
(1943)
from *Streptomyces griseus*



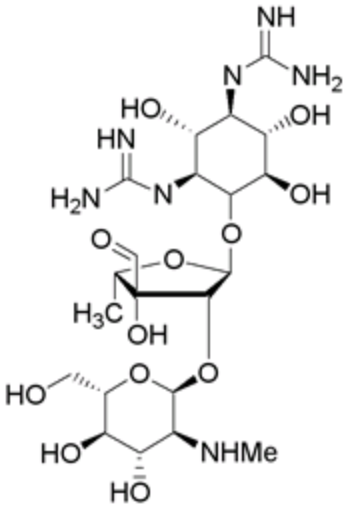
Neomycin B
(1948)
from *S. fradiae*

Aminoglycoside antibiotics bind to the decoding region of bacterial ribosomal RNA and interfere with the accuracy of protein synthesis.

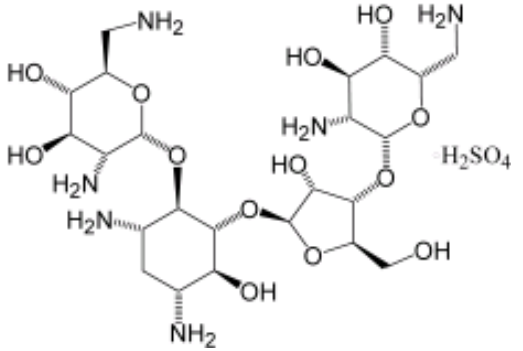
Funded by Merck & Co, Waksman adopted methods of mass screening that had been successful in the German dye industry (also -> Salvarsan) to identify new antibacterial natural products.
Waksman's laboratory performed the microbiological screening, and Merck scientists took over chemistry, pharmacology and large scale production.

Antibiotics from Systematic Screening: Waksman & Aminoglycosides

Selman A. Waksman
(New Jersey, 1888-1973)



Streptomycin



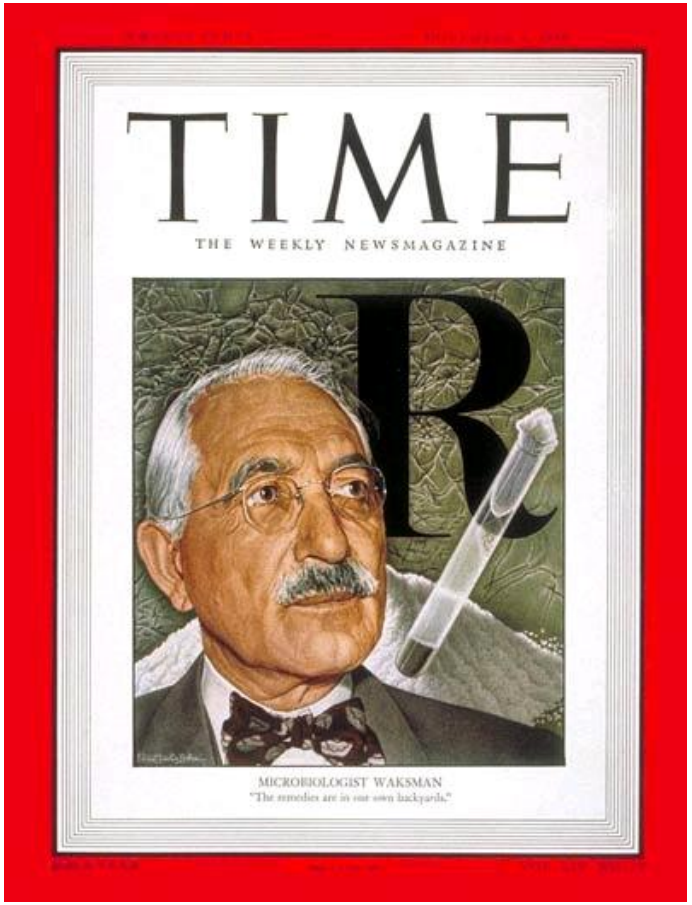
Neomycin B

Thus, in the Summer of 1943, the shelves in the New Brunswick laboratory began to fill up with jars of soil being enriched by the addition of pathogens; and the incubator became crowded with petri dishes in which individual bacteria were being pitted against those same pathogens....

About three months after the start of this program, two strains of *Streptomyces griseus* were isolated that showed great promise. They were found by the agar plate method (there either had not yet been time for the soil enrichment process to show any results or that method failed in this instance), and only after several thousand microbial types had been tested.

Sam Epstein and Beryl Williams, *Miracles from Microbes: The Road to Streptomycin* (New Brunswick, N.J.: Rutgers University Press, 1946), p. 136.

Antibiotics from Systematic Screening: Waksman & Aminoglycosides



Nov. 1949

The Merck company agreed to turn over the rights for streptomycin to Rutgers University, which in turn licensed companies for production. In the late 1940s eight pharmaceutical companies began mass producing streptomycin.

An estimated $1 million worth of the drug was provided for one the largest clinical studies of a drug ever undertaken, a study involving several thousand tuberculosis patients.

Streptomycin production at Merck in the 1940s

Two years after World War II, the two antibiotics commercially developed by Merck & Co, penicillin and streptomycin, account for half the sales of all drugs.

Timeline of Antibiotics Discovery: Introduction Into the Clinic

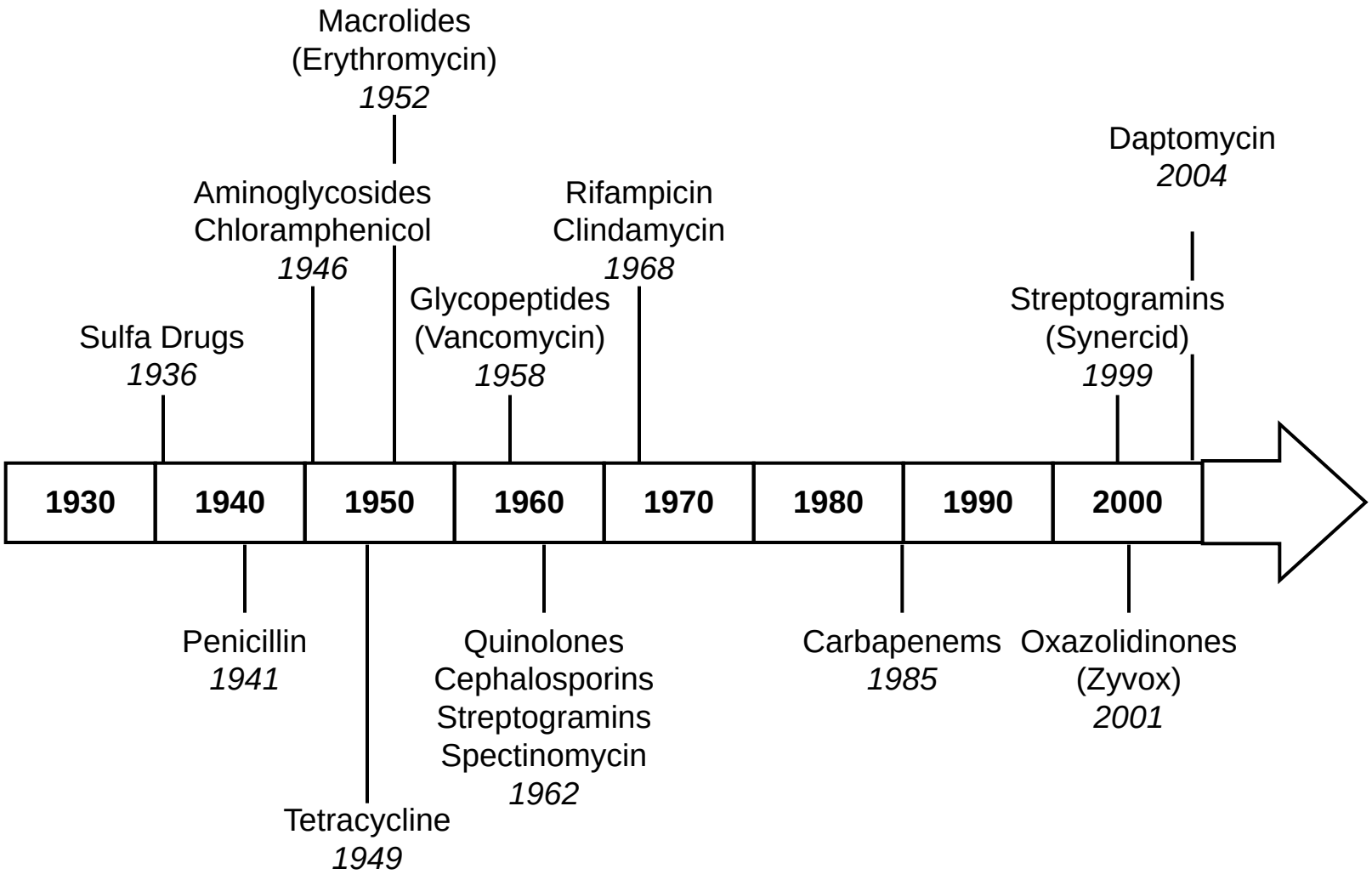
Table 1 – Antibiotic class with approximate year of clinical introduction, lead derivation, example of drug and mechanism of action

Antibiotic class	Introduction	Derivation	Example	Mechanism
Sulphonamide	1935	Synthetic	Sulfapyridine	Antifolate
β-Lactam	1941	NP-derived	Penicillin	Bacterial cell wall
Bacterial peptide	1942	NP-derived	Bacitracin	Bacterial cell wall
			Polymixin	Bacterial cell membrane
Aminoglycoside	1944	NP-derived	Streptomycin	Protein synthesis
Cephalosporin	1945	NP-derived	Cephalosporin	Bacterial cell wall
Nitrofuran	1947	Synthetic	Nitrofurantoin	Various
Hexamine	1947	Synthetic	Methenamine mandelate	Release of formaldehyde
Chloramphenicol	1949	NP-derived	Chloramphenicol	Protein synthesis
Tetracycline	1950	NP-derived	Chlortetracycline	Protein synthesis
Isoniazid	1951	Synthetic[a]	Isoniazid	Fatty acid biosynthesis
Viomycin	1951	NP-derived	Viomycin	Protein synthesis
Macrolide	1952	NP-derived	Erythromycin	Protein synthesis
Lincosamide	1952	NP-derived	Lincomycin	Protein synthesis
Streptogramin	1952	NP-derived	Virginiamycin	Protein synthesis
Cycloserine	1955	NP-derived	Cycloserine	Bacterial cell wall
Glycopeptide	1956	NP-derived	Vancomycin	Bacterial cell wall
Novobiocin	1956	NP-derived	Novobiocin	DNA synthesis
Ansamycin	1957	NP-derived	Rifamycin	RNA synthesis
Nitroimidazole	1959	Synthetic	Tinidazole	DNA synthesis
Ethambutol	1962	Synthetic	Ethambutol	Bacterial cell wall
Quinolone	1962	Synthetic	Nalidixic acid	DNA synthesis
Fusidane	1963	NP-derived	Fusidic acid	Protein synthesis
Diaminopyrimidine	1968	Synthetic	Trimethoprim	Antifolate
phosphonate	1969	NP-derived	Fosfomycin	Bacterial cell wall
Pseudomonic acid	1985	NP-derived	Mupirocin	Protein synthesis
Oxazolidinone	2000	Synthetic	Linezolid	Protein synthesis
Lipopeptides	2003	NP-derived	Daptomycin	Bacterial cell membrane

[a] Isoniazid is based on the structure of nicotinamide (vitamin B_2).

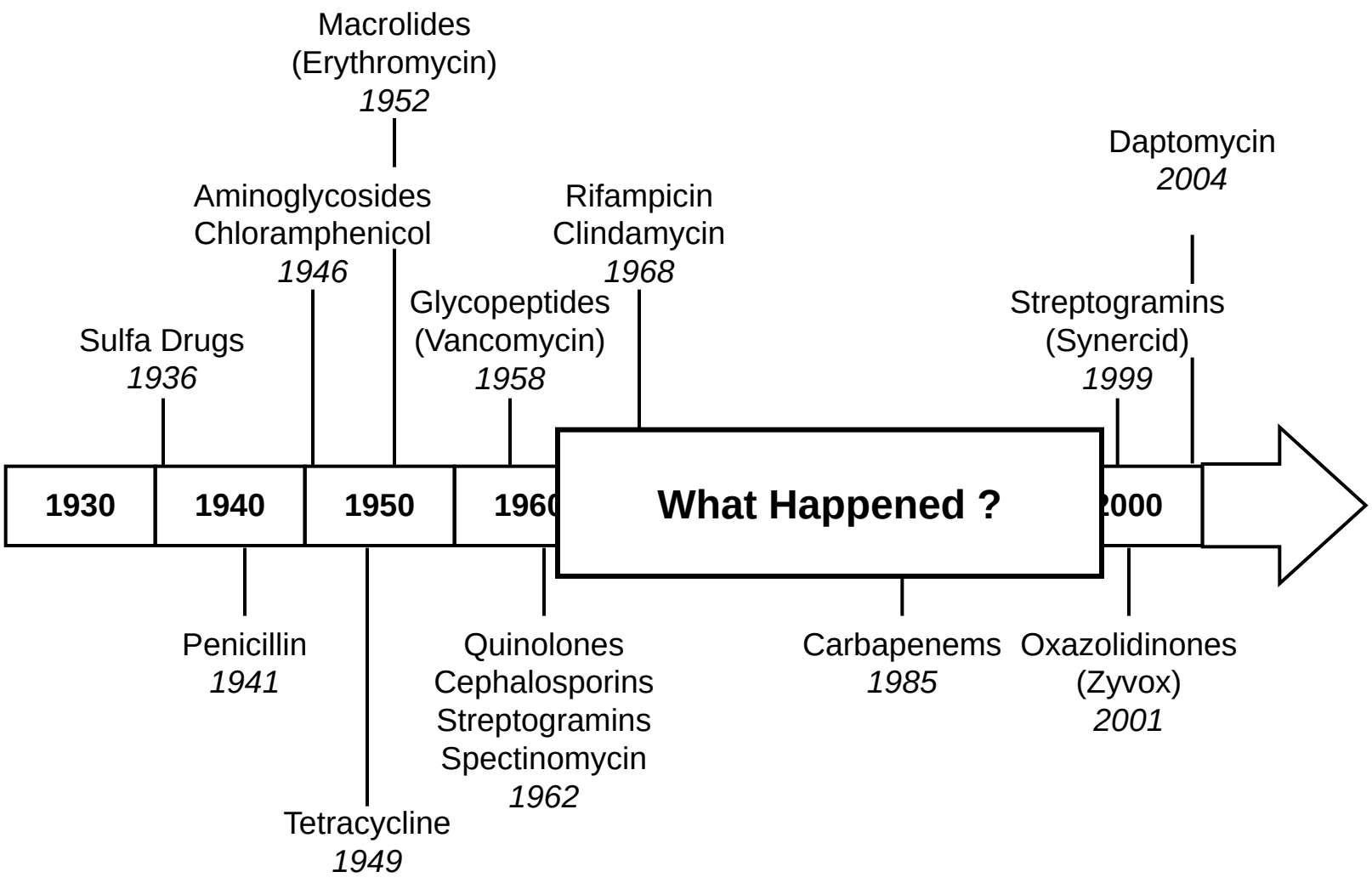
(Source: Biochem. Pharmacol. 2006, 71, 919)

Timeline of Antibiotics Discovery: Introduction Into the Clinic



(Antibiotics classes of distinct chemical constitution and mechanism of action)

Timeline of Antibiotics Discovery: The End of the Golden Age (1941-1962)



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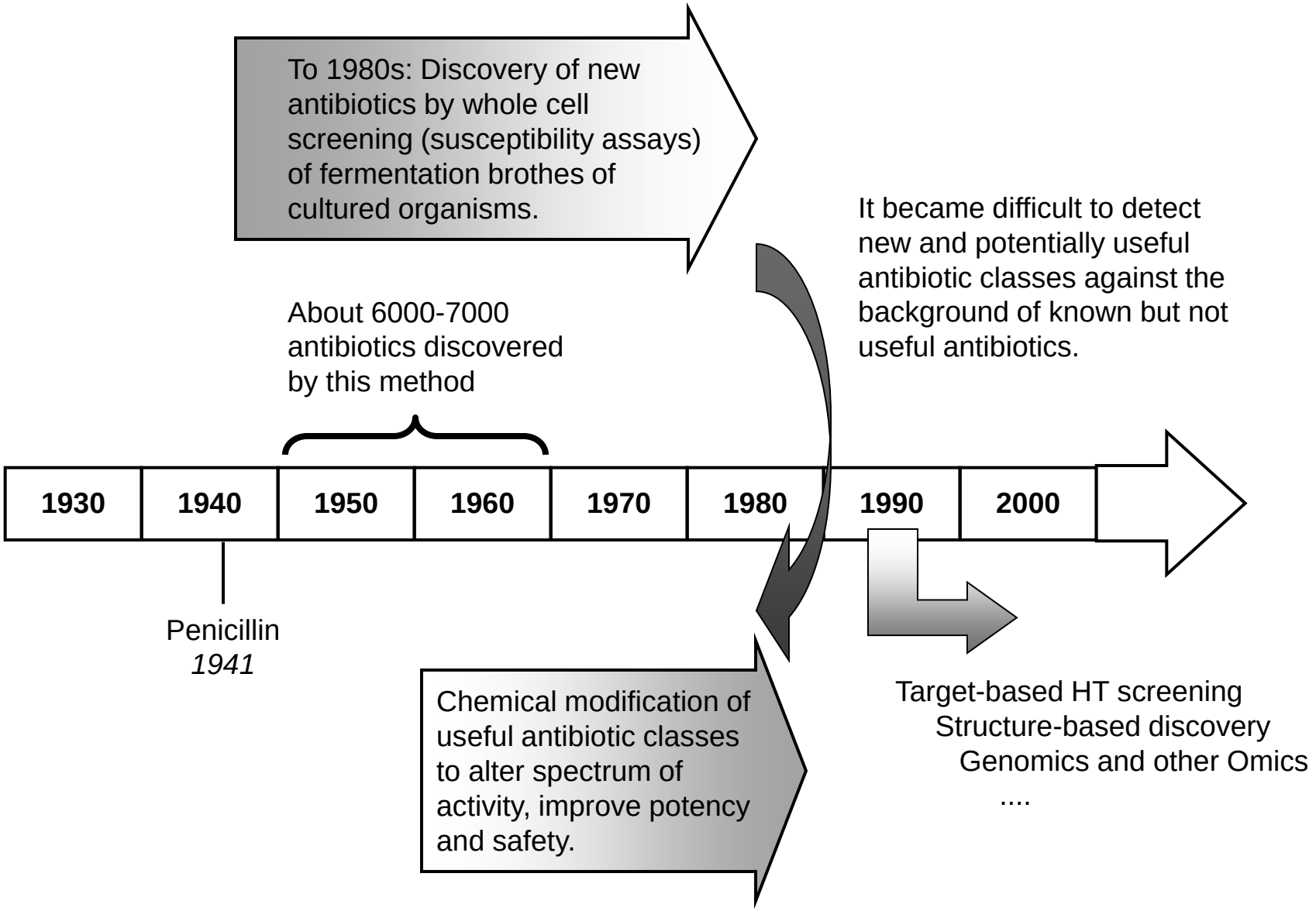


Timeline of Antibiotics Discovery: The End of the Golden Age (1941-1962)

• By the late 1980s a large number of antibiotics were available for medical use at the same time when the discovery of new drug classes by traditional methods was extraordinarily difficult.

• The traditional process of antibiotics dicovery by whole cell screening of natural sources, purification of individual compounds and structural characterization became an activity of diminishing returns.

and today ...

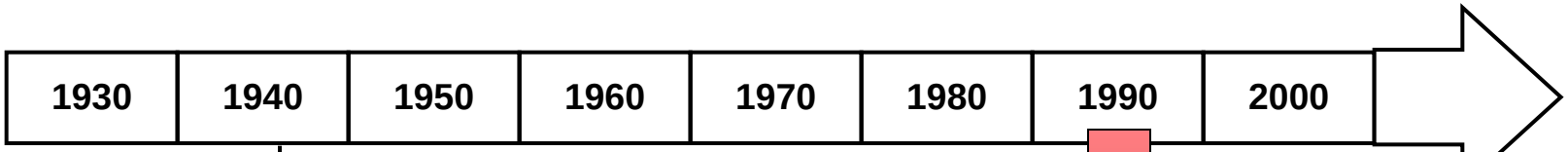
Table 1

Project therapeutic class	Risk adjusted NPV x$1,000,000
Musculoskeletal	1,150
Neuroscience	720
Oncology	300
Vaccines	160
Injectable Antibiotic (Gm+)	100
MS- Psoriasis	60
Liver Transplant	20
Oral Contraceptive	10

Timeline of Antibiotics Discovery: The End of the Golden Age (1941-1962)

To 1980s: Discovery of new antibiotics by whole cell screening (susceptibility assays) of fermentation brothes of cultured organisms.

What about these ?

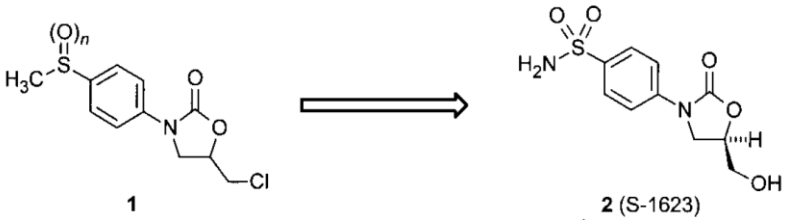


Penicillin
1941

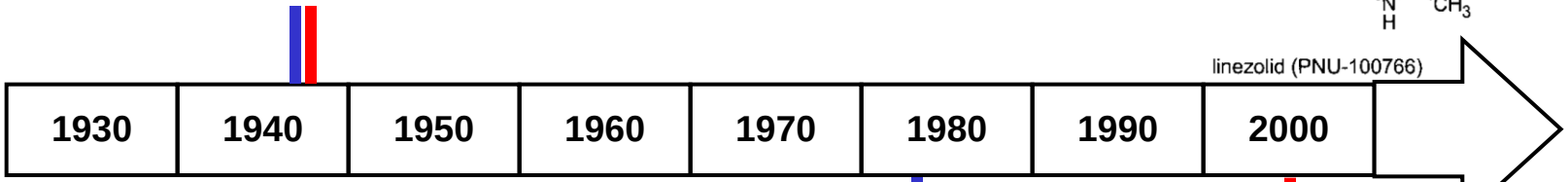
Chemical modification of useful antibiotic classes to alter spectrum of activity, improve potency and safety.

Target-based HT screening
Structure-based discovery
Genomics and other Omics
....

Timeline of Antibiotics Discovery: Tale of Two Cases, Then and Now



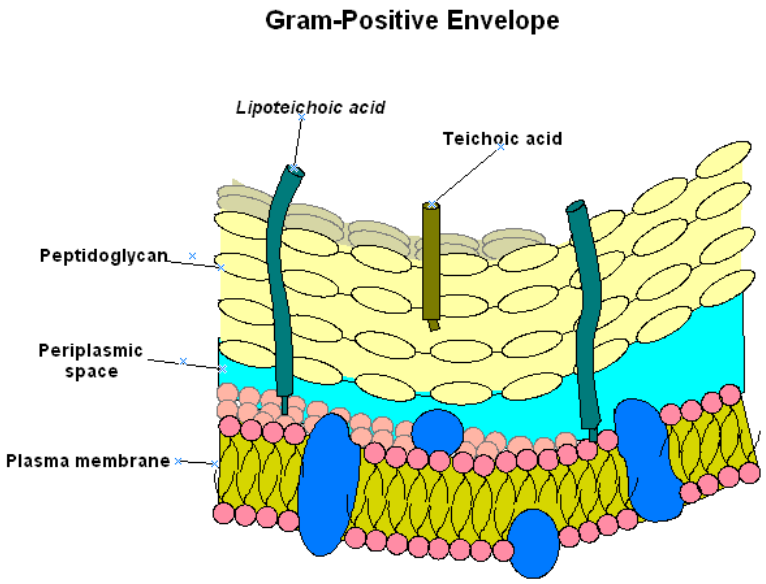
Streptomycin
discovered: 1943
in the clinic: 1944

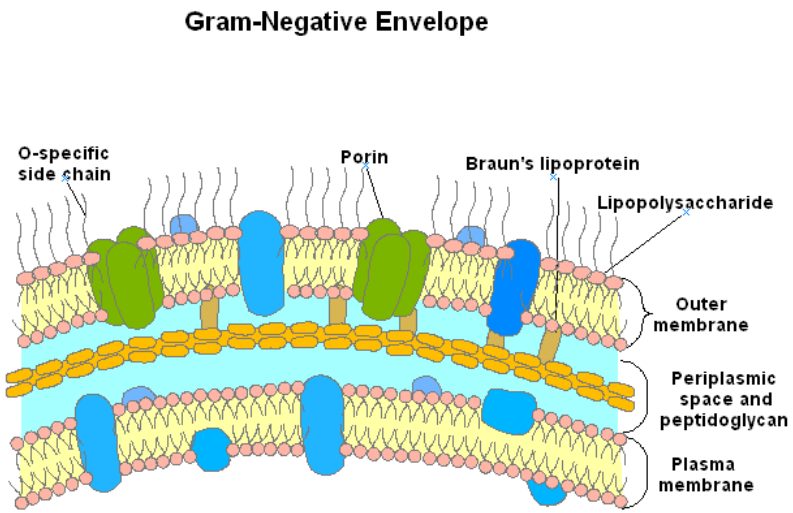


Oxazolidinones
(Zyvox, linezolid)
discovered: 1979 *in the clinic: 2001*

Du Pont (DuP 105, DuP721) -> stopped due to toxicity concerns
-> Upjohn (~1987)
-> Pharmacia (buys Upjohn, 1995)
-> Pfizer (buys Pharmacia, 2003)

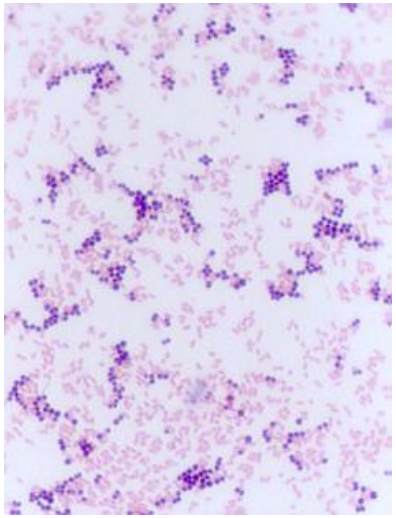
Classification of Bacteria by Gram Staining





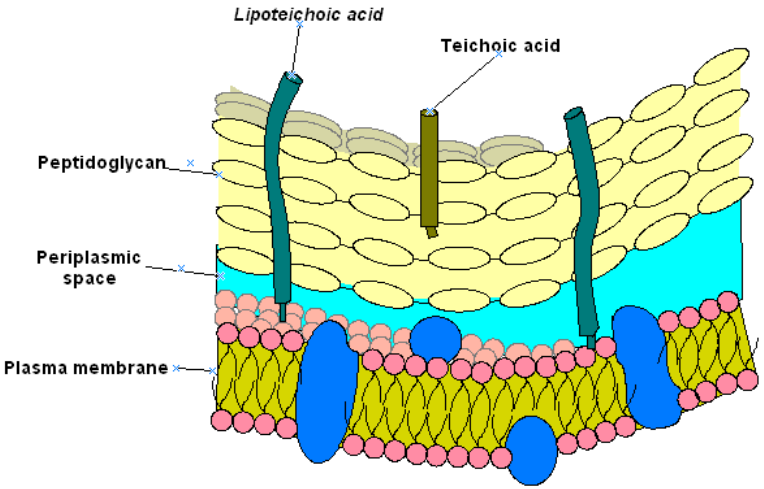
Gram staining is a technique that distinguishes between two groups of bacteria by the identification of differences in the structure of their cell walls (Christian Gram, Copenhagen 1853-1938): gram-positive bacteria remain colored after staining with crystal violet and subsequent washing; gram-negative bacteria do not retain dye.

Cells on a microscope slide are heat-fixed and stained with crystal violet. Then they are treated with an iodine-potassium iodide solution that allows the iodine to enter the cells and form a water-insoluble complex with the crystal violet dye. The cells are treated with alcohol or acetone solvent in which the iodine-crystal violet complex is soluble. Following solvent treatment, only gram-positive cells remain stained, possibly because of their thick cell wall, which is not permeable to solvent. After the staining procedure, cells are treated with a counterstain, i.e., a red acidic dye such as safranin or acid fuchsin, in order to make gram-negative (decolorized) cells visible. Counterstained gram-negative cells appear red, and gram-positive cells remain blue.

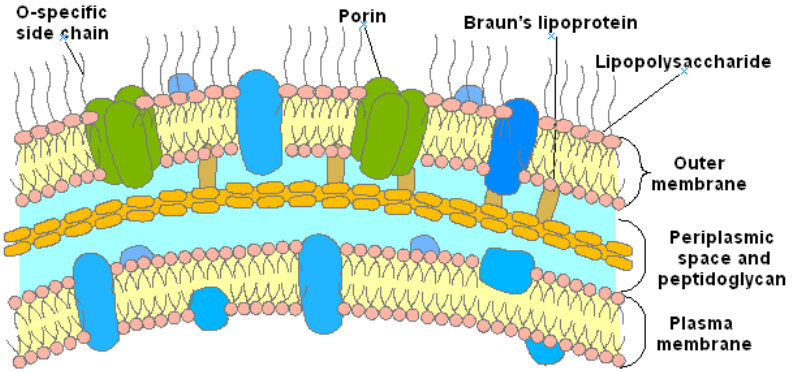


Classification of Bacteria by Gram Staining

Gram-Positive Envelope



Gram-Negative Envelope



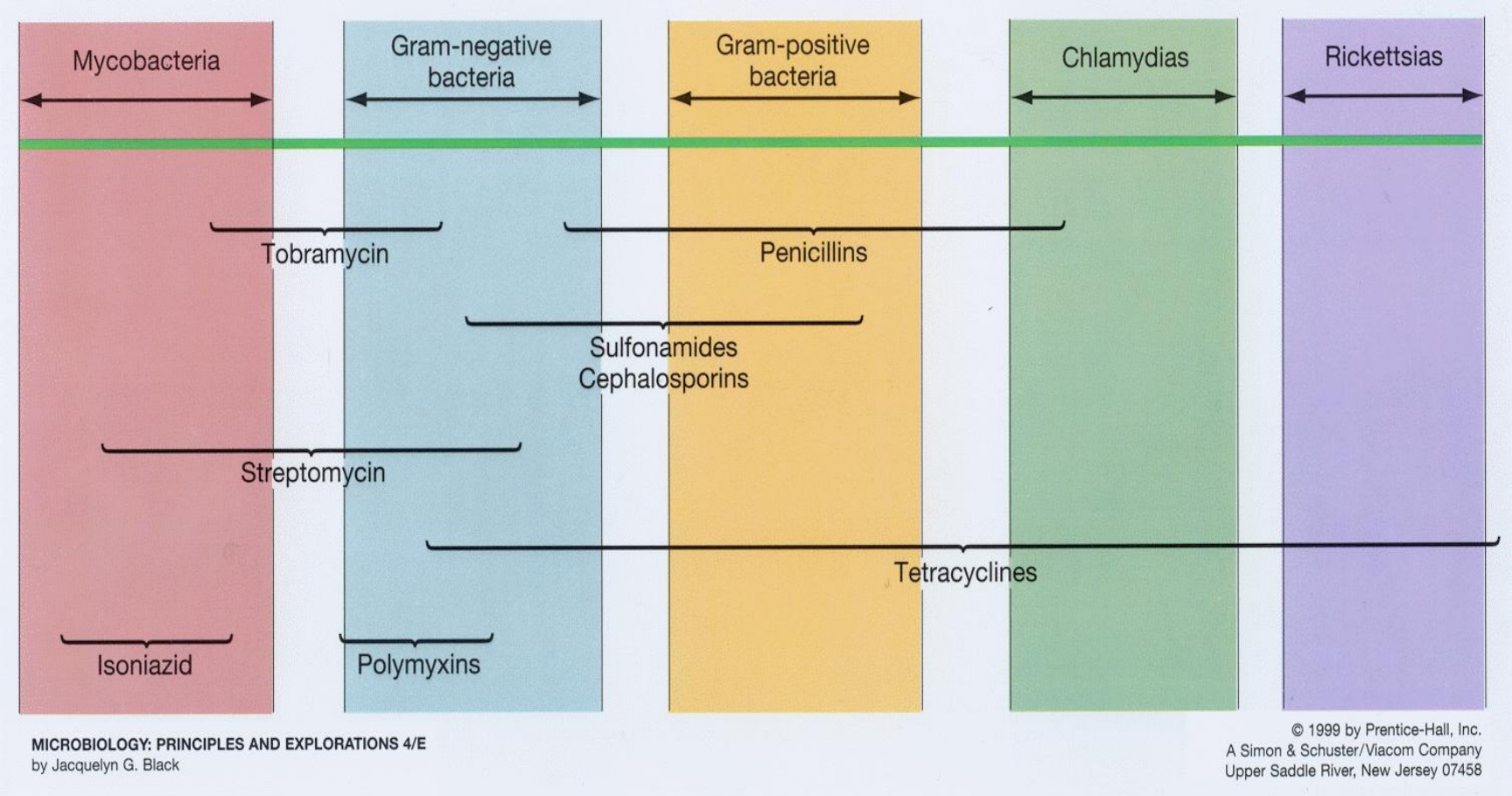
Gram positive:

Staphylococcus aureus
Staphylococcus epidermidis
Bacillus anthracis
Streptococcus spp.
Enterococcus spp.
Clostridium spp.
...

Gram negative:

Escherichia coli
Enterobacter cloacae
Helicobacter pylori
Slamonella enteritidis
Salmonells typhi
Hemophilus influenzae
Pseudomonas aeruginosa
Klebsiella pneumoniae
Legionella pneumophila
...

Antibacterial Spectrum of Antibiotics



Timeline of Antibiotics Discovery: Chasing After Trends

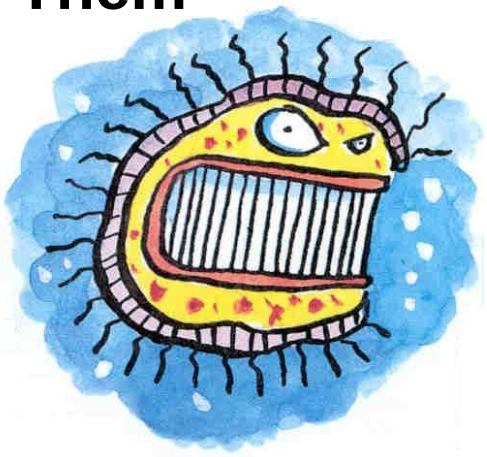
Us

1960s-1980s: focus on antibiotics for resistant **Gram-negative** pathogens (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *E. coli*)

after 1980s: focus on antibiotics for resistant **Gram-positive** pathogens (MRSA: methicillin-resistant *Staphylococcus aureus*)

1930	1940	1950	1960	1970	1980	1990	2000

Them

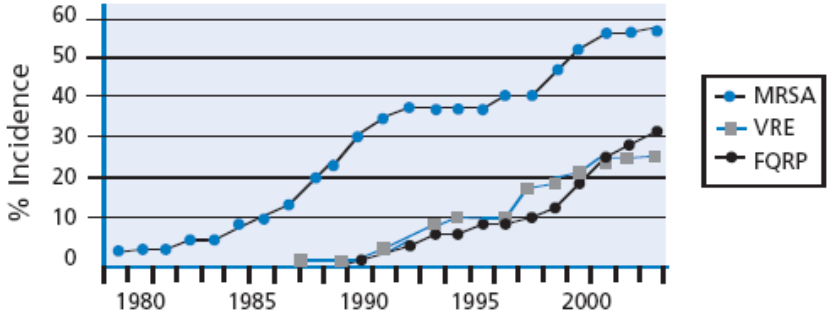


Resistance development in **Gram-positive** bacteria (*Staphylococci*, *Streptococcus pneumoniae*, *Enterococci*)

Resistance development in **Gram-negative bacteria** (*Pseudomonas aeruginosa*, *E. coli*, *Acinetobacter baumannii*)

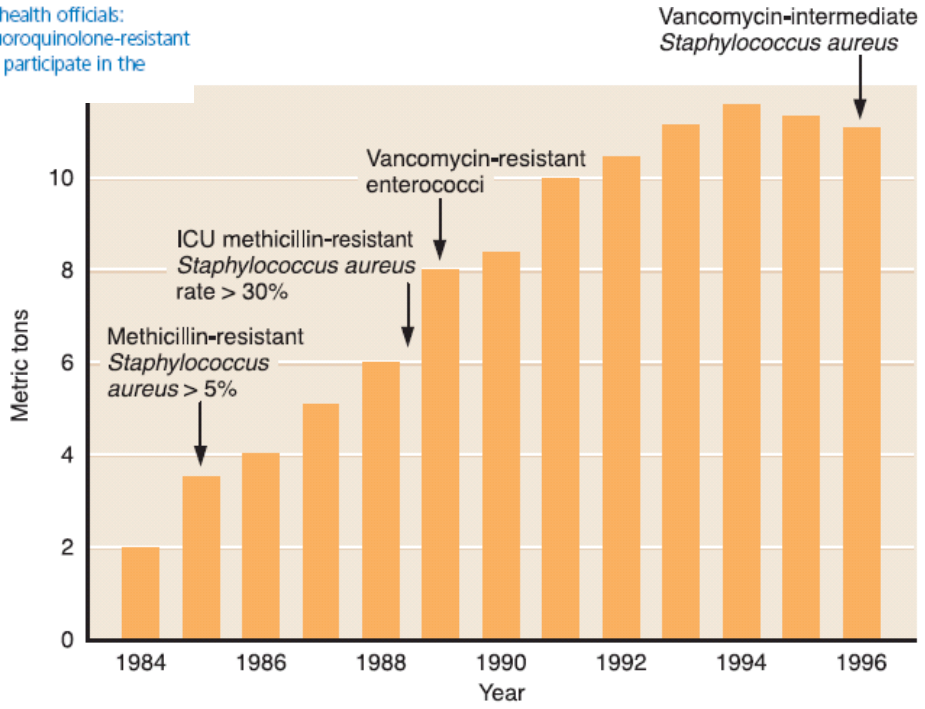
The Race Goes On

Chart 1: Resistant Strains Spread Rapidly



Source: Centers for Disease Control and Prevention

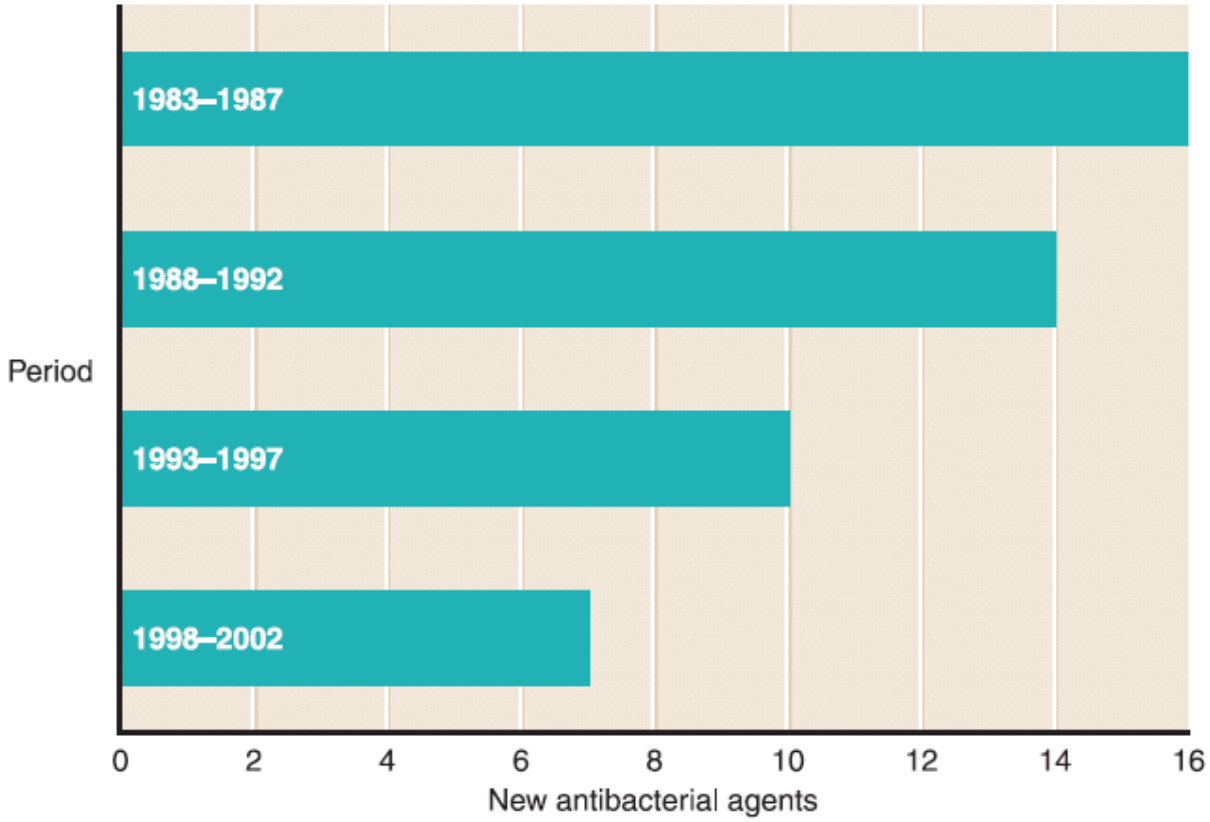
This chart shows the increase in rates of resistance for three bacteria that are of concern to public health officials: methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and fluoroquinolone-resistant *Pseudomonas aeruginosa* (FQRP). These data were collected from hospital intensive care units that participate in the National Nosocomial Infections Surveillance System, a component of the CDC.



Resistance creates markets, use creates resistance. Vancomycin use in the United States, 1984–1996.

The Future of Antibiotics?

In 2002, **out of 89** new medicines emerging on the market, **no new antibacterial drugs** were approved. Since 1998, only seven new antibacterials have been approved. Current annual reports for leading pharmaceutical companies (Merck, Pfizer, GlaxoSmithKline, Bristol-Myers Squibb, Aventis, Abbot, AstraZeneca, Lilly, Hoffmann-LaRoche, Johnson & Johnson, and Novartis) list only **4 new antibacterials in the drug pipeline out of 290 agents** listed (or 1.38% of the products in development).



New antibacterial agents approved in the United States per 5-year period from 1983 to 2002.

(Source: ASM News 2004, 70, 275)