

SELECTIVE TOXICITY AND MICROBIAL RESISTANCE

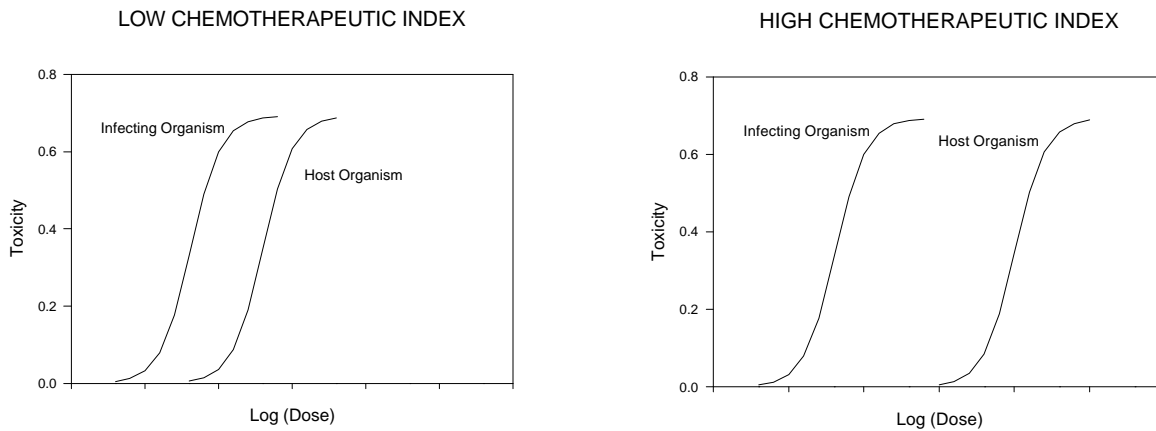
Definitions:

CHEMOTHERAPY: A term coined by Paul Ehrlich around 1900, referring to the use of chemical agents to kill infectious organisms.

CHEMOTHERAPEUTIC AGENT: A compound that kills an infectious organism by direct chemical action (not by enhancing host defense mechanisms).

SELECTIVE TOXICITY: An agent is selectively toxic if it harms the invading species without harming the host species.

CHEMOTHERAPEUTIC INDEX: Originally defined by Ehrlich as the ratio of (minimal curative dose/ maximal tolerated dose); A more modern definition is $[LD_{50} \text{ host}/ED_{50} \text{ disease}]$; proportional to (but not necessarily identical to) $[LD_{50} \text{ Host}/ LD_{50} \text{ Parasite}]$.



BIOCHEMICAL BASIS OF SELECTIVE TOXICITY

Original concept by Ehrlich was tissue uptake; parasites selectively took up certain stains - could they selectively take up toxins? First use of the concept of a "receptor".

Main bases of selective toxicity:

- 1) Functional differences on an organic level -- Red Squill
- 2) Functional differences on a cellular level -- rapid division and replication of tumor cells
- 3) Morphological differences -- cell wall in bacteria
- 4) Drug uptake and accumulation -- tetracycline
- 5) Drug metabolism -- Malathion
- 6) Specific subcellular binding sites (Ehrlich's receptor concept)
 - a) Ribosomal binding proteins -- streptomycin
 - b) Enzymes -- sulfonamides

**TABLE I
MECHANISMS OF ANTIBIOTIC RESISTANCE**

AGENT	MODE OF ANTIBACTERIAL ACTION	MICROBIAL RESISTANCE MECHANISM
Sulfonamides	Block synthesis of tetrahydrofolic acid	R plasmid-coded, sulfonamide-resistant dihydropteroate synthetase; Also overproduction of PABA
Trimethoprim	Competitive inhibition of dihydrofolate reductase; Block synthesis of tetrahydrofolic acid	R plasmid-coded, trimethoprim-resistant dihydrofolate reductase
Penicillins and Cephalosporins	Interfere with cell wall biosynthesis by inhibiting synthetic enzymes	Hydrolysis of beta-lactam ring by beta-lactamase
Tetracyclines	Inhibit protein synthesis (translation) by binding to 30S ribosomal subunits	Transport of drug out of cell; cell does not accumulate drug
Aminoglycosides	Bind to 30S (and 50S) ribosomal subunit, cause translational errors, inhibit peptide elongation	Modification of drug by R plasmid-coded enzyme; Reduced uptake of drug; Alteration of ribosomal binding site
Erythromycin and Clindamycin	Bind to 50S ribosomal subunit; inhibit peptide elongation	Enzymatic modification (methylation) of ribosomal drug-binding sites
Chloramphenicol	Inhibits protein synthesis (translation) by interacting with 50S ribosomal subunit	Inactivation by drug metabolism; Reduced rate of drug transport into cell
Rifampicin	Binds to bacterial RNA polymerase; blocks transcription	Altered drug binding affinity due to spontaneous mutation; Not R plasmid-coded
Quinolones	Binds to bacterial topoisomerase II; blocks transcription	Altered drug binding affinity due to spontaneous mutation; Not R plasmid-coded

MICROBIAL RESISTANCE TO CHEMOTHERAPEUTIC AGENTS

1) Clinical Significance

Rapid establishment of resistant microbial strains due to chemical selection

Sulfonamides: useful in 1930's vs. gonorrhea; by late 40's, 80% of all gonorrhea was sulfonamide resistant

Penicillin: first used widely in mid-40's; by 1946, 14% of *Staph. aureus* was pen. resistant; by 1947, 38%; by 1949, 59%; today essentially all *Staph. aureus* is penicillin resistant

Development of resistant *Shigella* strains in England, 1974-1982: Sulfonamide resistance - stayed constant at 80%; Streptomycin resistance - went from 40% to 70%; Tetracycline - from 15% to 60%; ampicillin and chloramphenicol - from 3% to 50%

Agents of choice learned now may not be useful in 5 years' time

2) Biochemical Mechanisms of Resistance -- Essentially a reversal of selective toxicity.

A) Alteration of a target binding site

Bacteria resistant to erythromycin and lincomycin methylate the 30S ribosomal rRNA antibiotic binding site to prevent binding

B) Alteration of a target enzyme

Sulfonamide resistant bacteria produce a dihydropteroate synthetase that is not sulfonamide inhibitable

C) Metabolism of the drug to a nontoxic species

Penicillin resistance due to β -lactamase activity

Chloramphenicol resistance due to acetyl transferase

D) Prevention of drug accumulation

Tetracycline-resistant bacteria pump the drug out of the cell

Multi-drug-resistant tumor cells pump a variety of drugs out of the cell

Aminoglycoside-resistant bacteria do not actively concentrate drug, as susceptible strains do

TRANSMISSION OF RESISTANCE PHENOTYPES; ESTABLISHMENT OF RESISTANT BACTERIAL POPULATIONS

Resistance phenotypes involve cellular proteins (enzymes, binding proteins, active transport proteins, etc.) Basis of resistance is mutation or acquisition of gene(s) coding for critical proteins

1) Spontaneous mutation of chromosomal DNA -- passed on to future generations but not directly transmitted to other bacteria

2) Transfer of extrachromosomal DNA elements (R plasmids) among bacteria

Main route of rapid spread of resistance in a previously susceptible bacterial population

A) R plasmids -- small pieces of extrachromosomal DNA; 1 to 5 megadaltons (about 1/1000th the size of the bacterial chromosome)

Stable genetic elements that are transcribed and replicated along with chromosomal DNA (not like viruses)

Code for 1 or more resistance phenotypes - can confer resistance to multiple drugs, even of different classes

B) Transfer of R plasmids among cells

Occurs among related or unrelated strains -- 3 mechanisms

1) transformation - cells pick up and incorporate shed plasmids - infrequent and inefficient

2) transduction - phage-mediated

3) conjugation - most common mechanism - direct DNA exchange by cell-cell contact

Plasmid can remain extrachromosomal, or be incorporated into chromosomal DNA

Resistance genes can be transferred to non-pathogenic strains, (e.g. enterobacteria, epidermal flora) which can act as "reservoirs" for genes that can later be transferred to pathogenic strains

C) Role of chemical selection in establishment of resistant bacterial populations

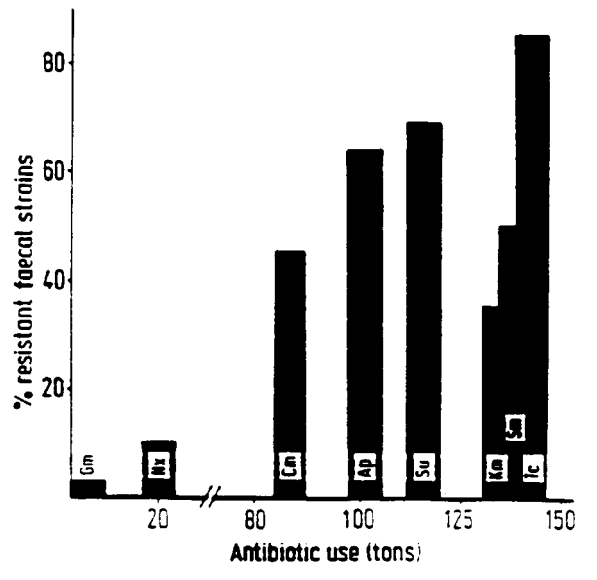
Darwinian selection - Presence of large amounts of antibiotic in bacterial environment kills off susceptible strains, providing space and food for resistant strains

1) Hospitals are perfect breeding grounds for resistant bacteria, especially when antibiotics are used for routine prophylaxis

"Nosocomial" infections are a major problem

Strict oversight is necessary to prevent overuse of antibiotics

2) Antibiotics in the environment (see figure)



Correlation between antibiotic use and resistance carried by 295 *E. Coli*, 198 *Proteus Mirabilis*, 63 indole-positive *Proteus* spp., and 39 *Salmonella* strains isolated from diarrhea specimens in Toluca, Mexico, in 1977. Gm = gentamicin. Nx = nalidixic acid. Cm = chloramphenicol. Ap = ampicillin. Su = sulfonamides. Km = kanamycin. Sm = streptomycin. Tc = tetracycline.

a) Over prescription of antibiotics, nonprescription availability of antibiotics leading to non-appropriate use

b) Inclusion of antibiotics in livestock feed

Produces larger, healthier livestock

Estimated that 50% of total tonnage of antibiotics produced each year go into livestock feed

Meat can contain traces of resistant nonpathogenic enterobacteria that can transfer resistance to pathogenic bacteria, either directly, or via human enterobacteria or dermal flora