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Treatment of Infectious Disease: Drugs and Drug Resistance

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Section A

History and Principles of Chemotherapy

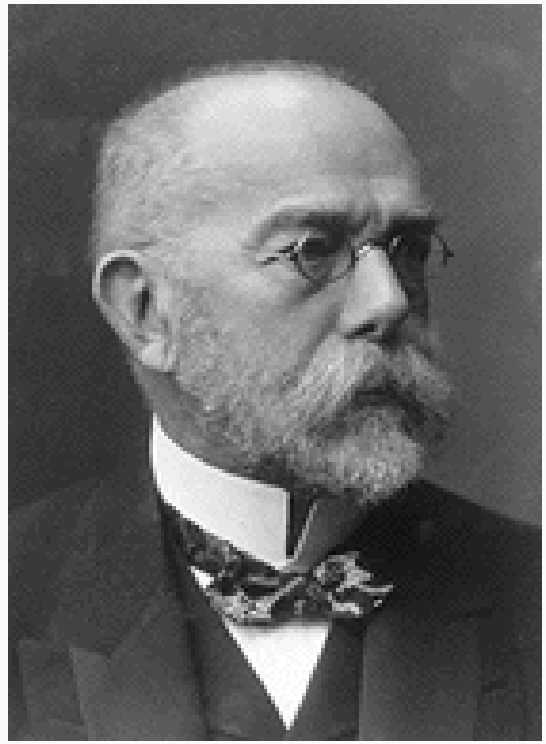
Chemotherapy of Infectious Disease

- Use of chemicals—natural, synthetic, or semi-synthetic—to control replication of pathogens in an infected individual
- Chemotherapy generally depends upon selective toxicity—the chemicals must be toxic to the pathogen but not toxic (or less toxic) for the host
- Chemotherapy is used medically but can also form an element of a public health effort to control spread of a disease in a population

Cinchona succyruba

History of Chemotherapy: Robert Koch

- Traditional remedies contain active chemotherapeutic agents—some still in use
- Robert Koch (1860s): the germ theory of disease
  - Provided a target for chemo-therapeutic agents
  - Introduced the concept of selectivity for pathogens of chemicals (dyes)

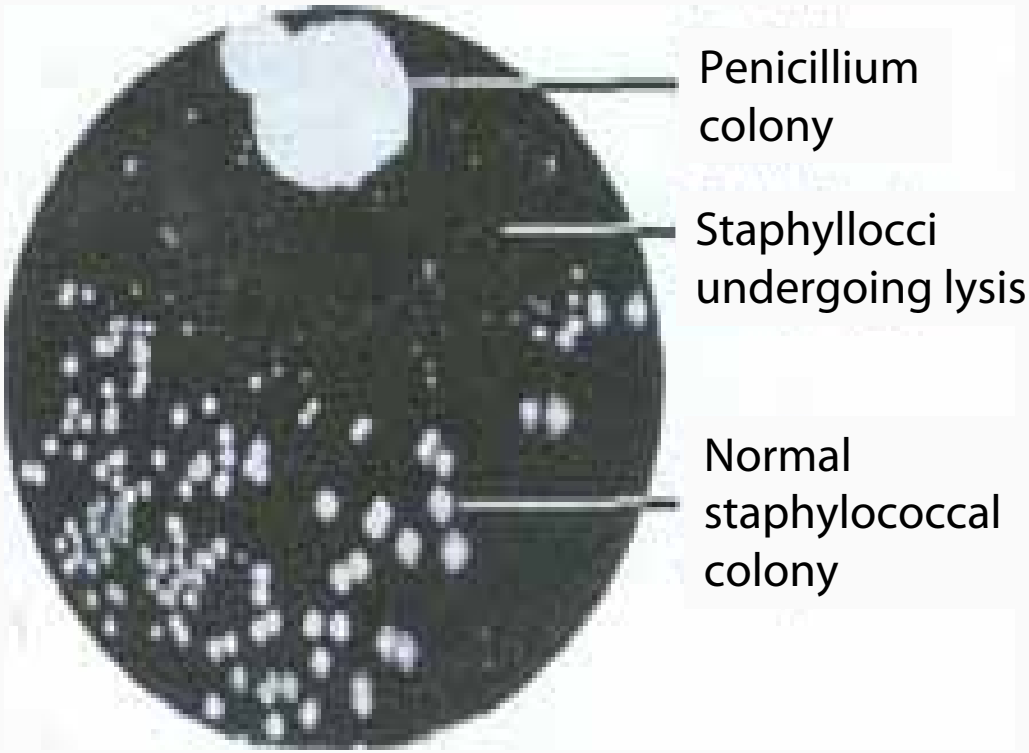


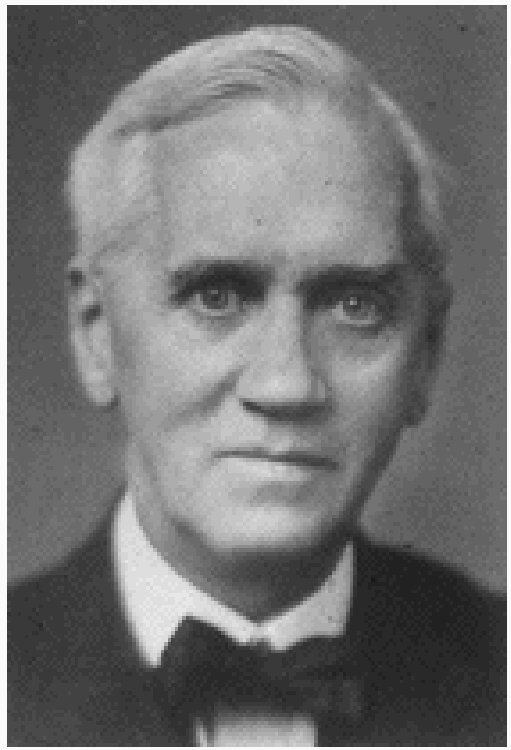
History of Chemotherapy: Paul Erlich

- Paul Ehrlich (1908)
  - Discovered Salvarsan (606), organic arsenic-containing anti-syphilitic
  - Invented a drug discovery approach still used: systematic chemical modification of a "lead" compound

History of Chemotherapy: Alexander Fleming

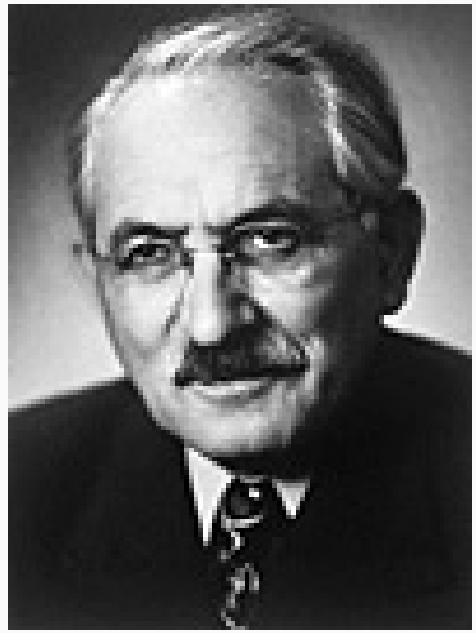
- Alexander Fleming (1929) discovered penicillin
- Florey and Chain (1939) purified penicillin and demonstrated its use in humans





History of Chemotherapy: Domagk and Waksman

- Gerhard Domagk
  - Sulfanilamide (synthetic) (1934)
- Selman Waksman
  - Streptomycin (1939)

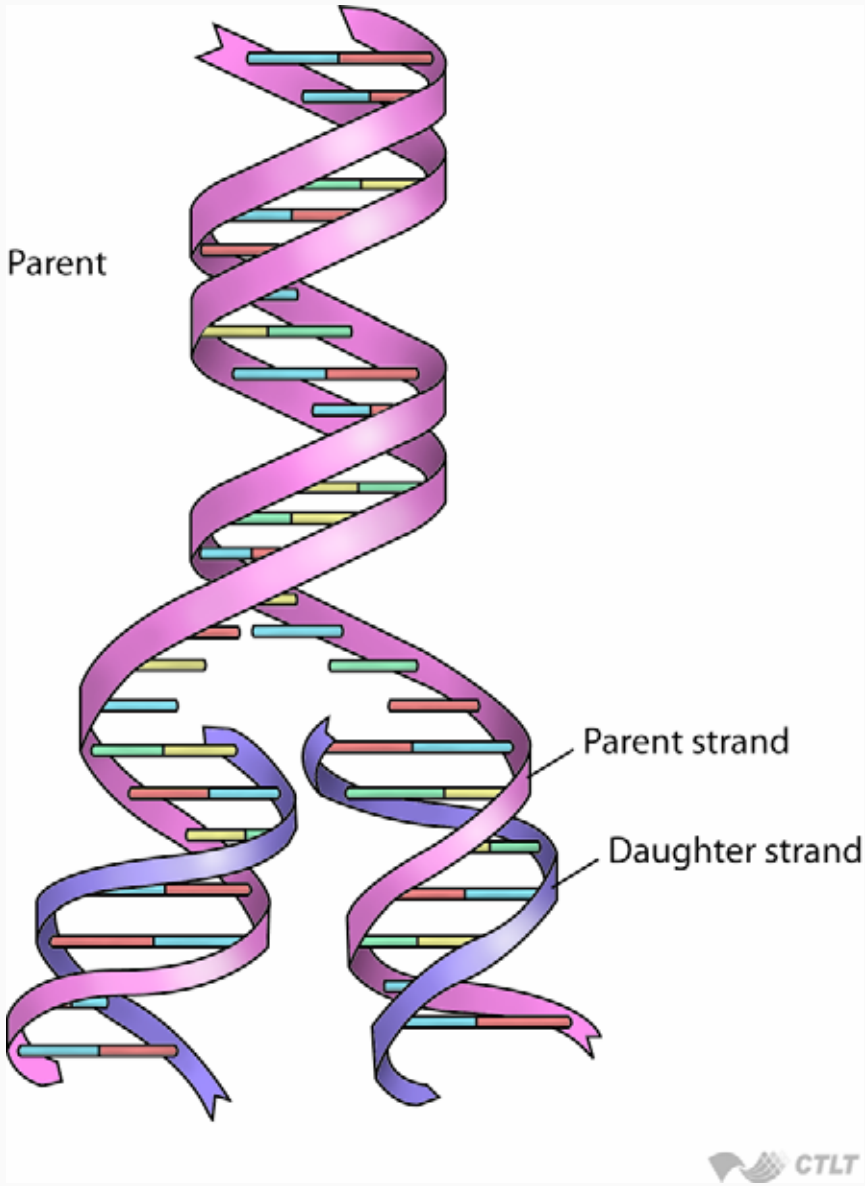


Mechanisms of Drug Action

- Drugs are poisons: they work by interfering with an essential process in a pathogen (for example, DNA, protein, cell wall synthesis)
  - This generally is done by inhibition of function of a specific protein, preventing a particular chemical reaction or other event
  - Action must be specific for the pathogen
- Several drugs, including pyrimethamine, target a step in utilization of the vitamin folic acid, which indirectly prevents DNA synthesis

dTMP and DNA

- DNA synthesis is essential for propagation of most pathogens
- DNA synthesis depends on a supply of small molecule precursor compounds, including the nucleoside monophosphate dTMP



A Biochemical Pathway: Production of dTMP

- dTMP is produced by a series of linked bio-chemical reactions (a biochemical pathway)
  - Each arrow represents a chemical reaction
  - Starting material and products of the reaction are indicated at the tails and heads of the arrows
  - Reactions are conducted by enzymes, noted beside the arrow
  - Fused arrows indicate reactions that happen in concert

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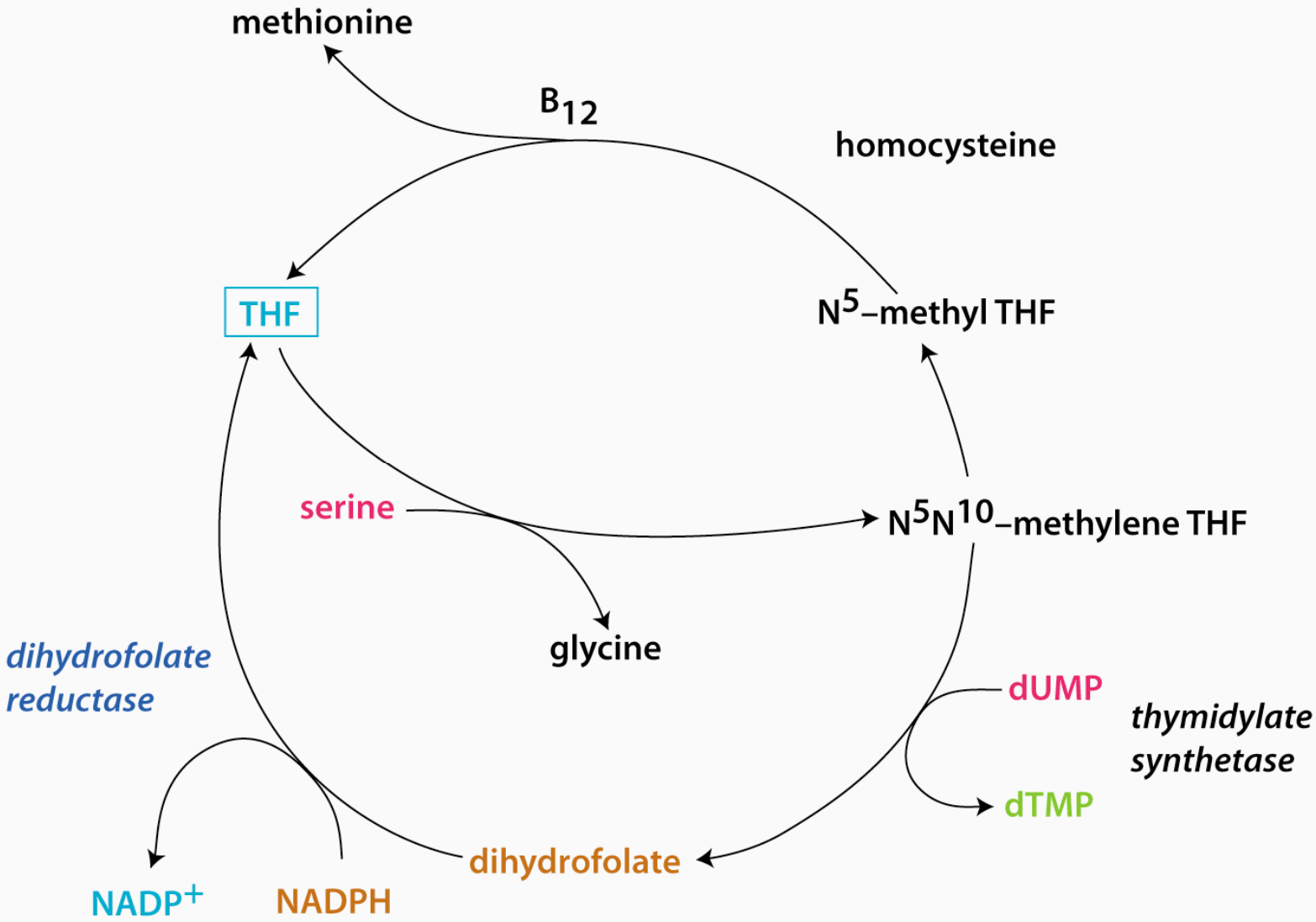
A Biochemical Pathway: Production of dTMP



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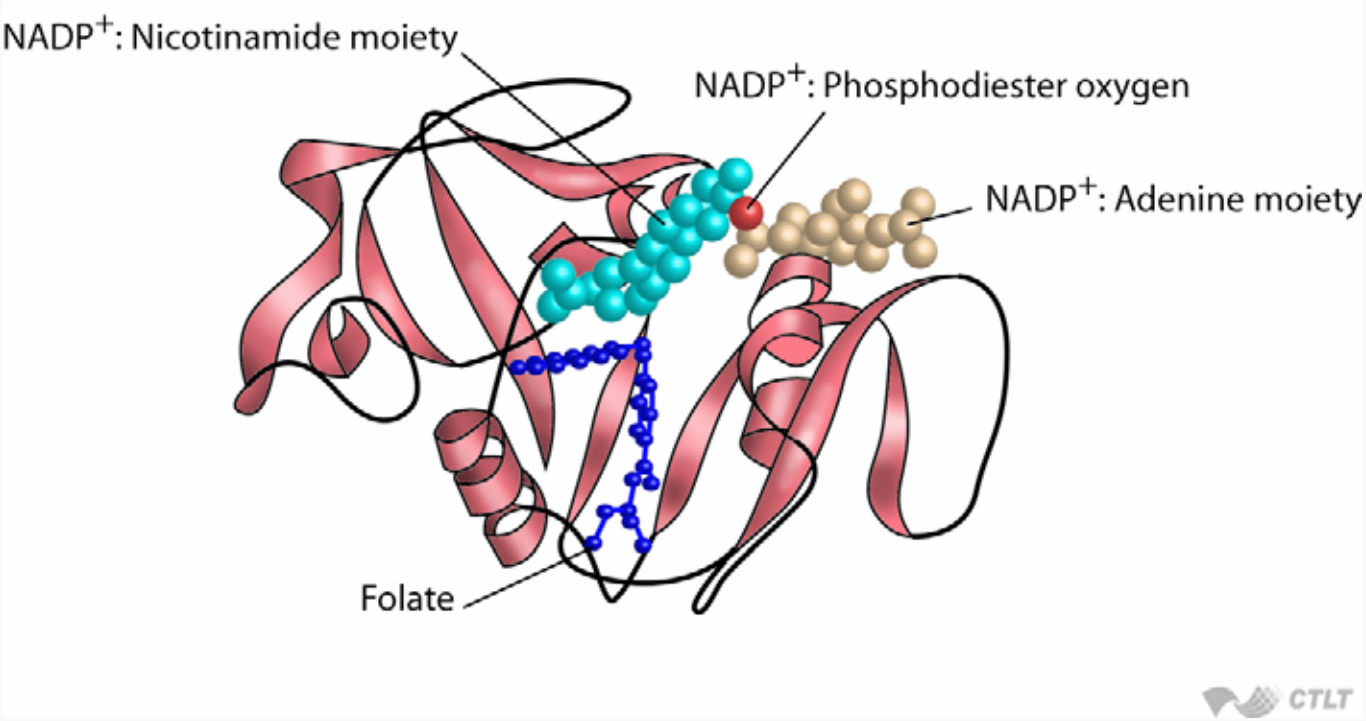
A Biochemical Pathway: Production of dTMP

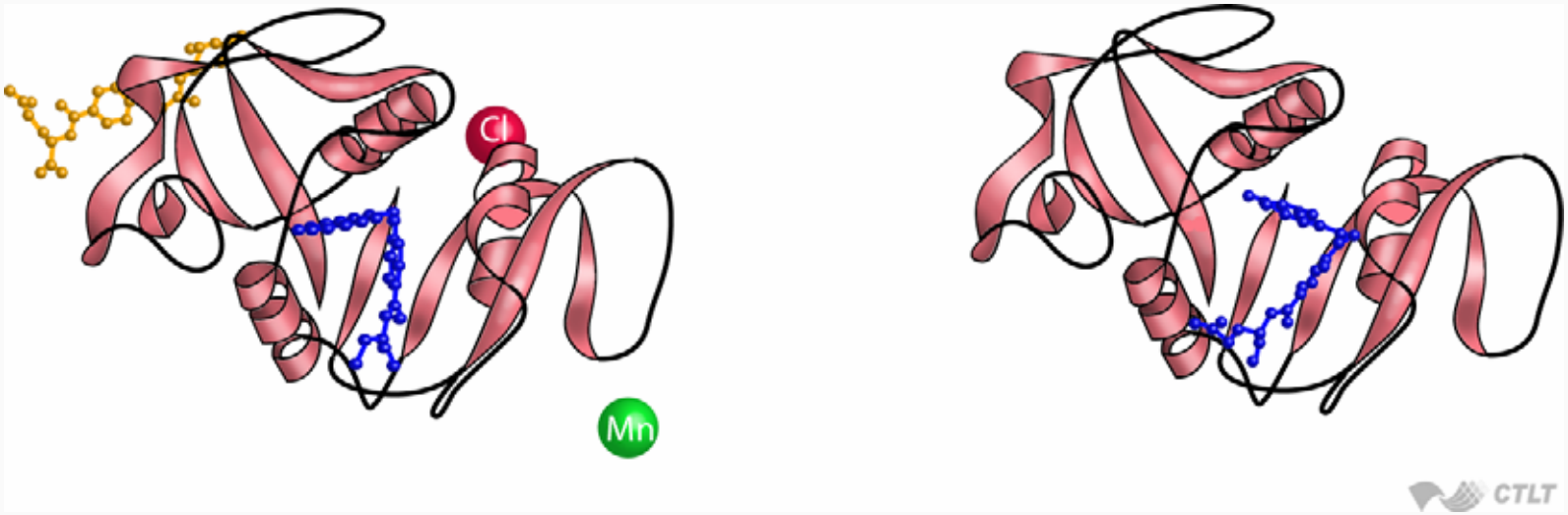
- dTMP is produced from dUMP by a reaction that also converts NN-methylene THF to dihydrofolate (DHF)
- The NN-methylene THF is regenerated by two-step process: DHF-> THF -> NN-methylene THF
- Step 1 is mediated by dihydrofolate reductase and requires NADPH
- Note that a supply of NN-methylene THF is required for continued dTMP synthesis
- If DHFR function is prevented, dTMP and DNA synthesis ceases
- A variety of drugs, including pyrimethamine and trimethoprim, blocks DHFR and kills their targets by inhibiting DNA synthesis

DHF and NADPH Bind to DHFR

- *E. coli* DHFR



Inhibitors of DHFR Prevent Dihydrofolate Binding



Dihydrofolate

Trimethoprim

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Section B

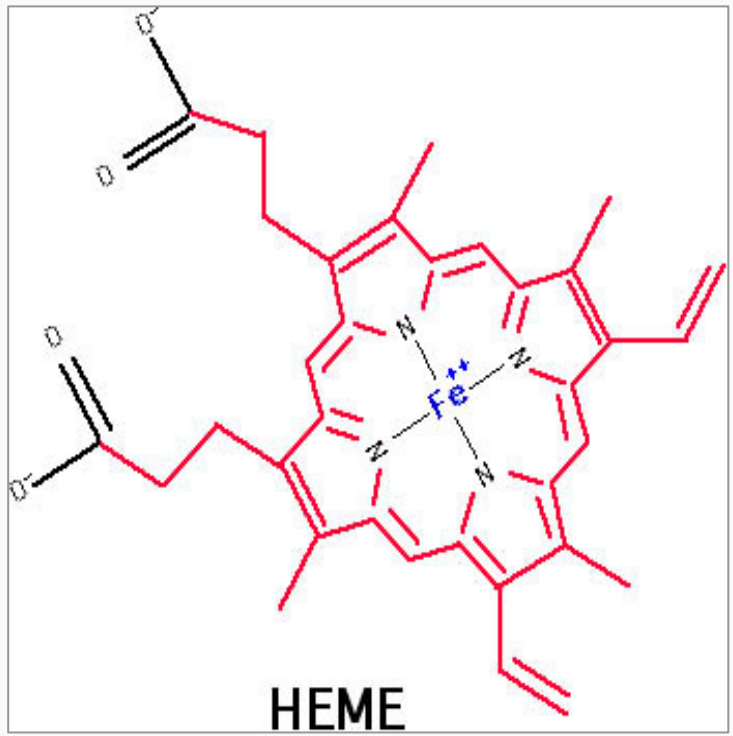
Mechanisms of Selective Toxicity

Selective Toxicity

- Inhibit a reaction in the pathogen that is not present in the host
- Inhibit a common reaction but act specifically on the pathogen's enzyme
- Accumulate specifically in the pathogen

Selectivity: Chloroquine

- Malaria parasites live in red blood cells (RBCs; erythrocytes)
- They degrade the major RBC protein, hemoglobin, for energy, and biochemical precursors
- Heme, the iron-containing component of hemoglobin, is left over



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Selectivity: Chloroquine

- Heme is toxic
- Malaria detoxified heme by converting it to insoluble (and inert) hemozoin
- Chloroquine inhibits conversion of heme to hemozoin
- Free heme kills the parasite

Selectivity: Pyrimethamine

- Pyrimethamine inhibits an enzyme (DHFR) that occurs in most organisms, including humans
- Although all DHFRs are related, those of humans and plasmodium are not identical
- Small differences in the amino acid sequences of the human and plasmodium enzymes give them slightly different shapes—and, consequently, different abilities to bind pyrimethamine
- It takes about 1,500 times as much pyrimethamine to inhibit mammalian DHFR 50% than to inhibit the same amount of *plasmodium* DHFR

Selectivity: Chloroquine

- Different organisms accumulate chemicals differently
- Chloroquine concentrations in plasmodium can be as much as 1,000 times as high as serum levels

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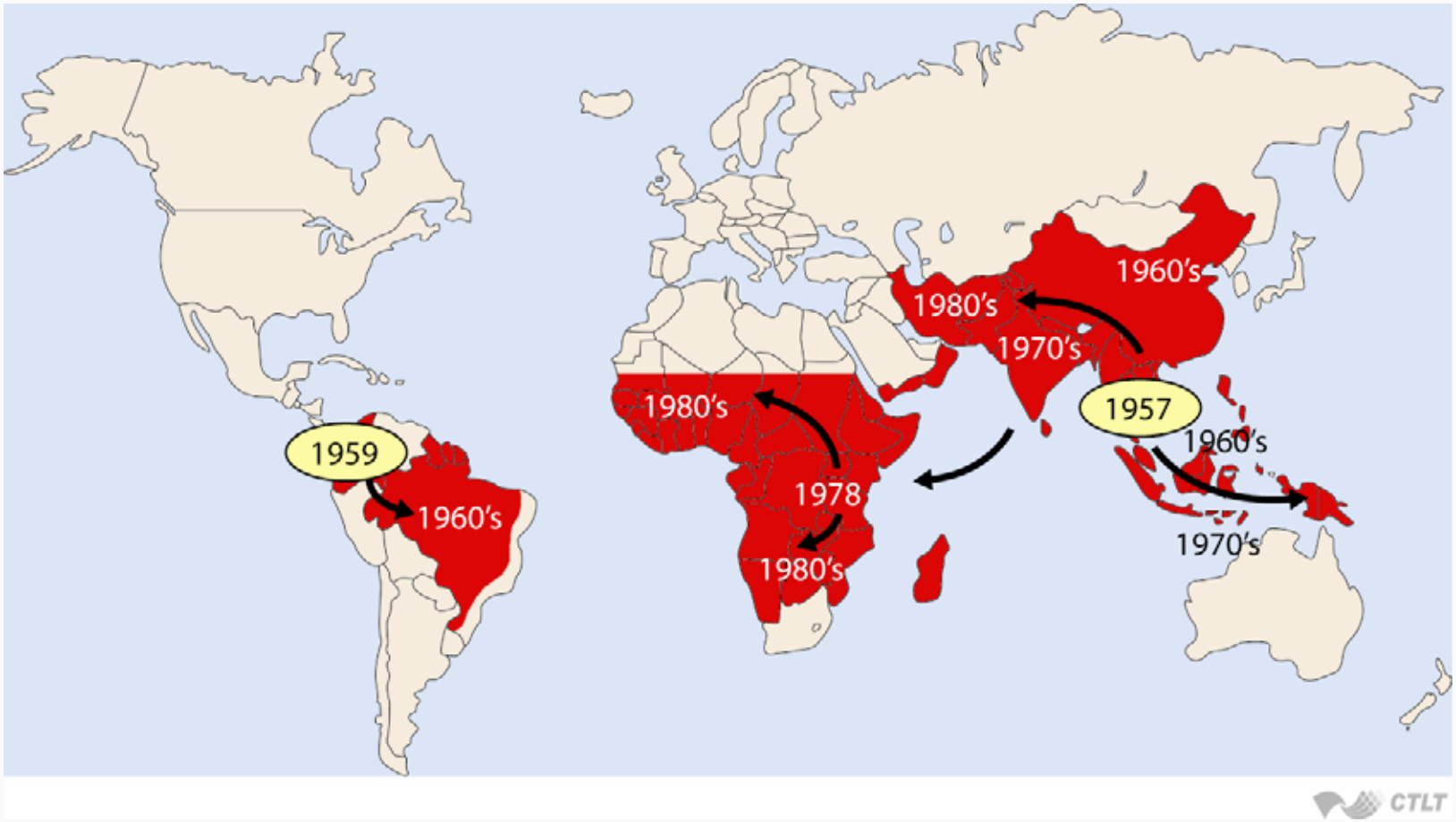
Section C

Drug Resistance in Public Health

Spread of Drug-Resistant Malaria

- Spread of chloroquine-resistant *P. falciparum*



Three Mechanisms of Drug Resistance

- Alter the target enzyme so that chemotherapy is no longer effective
- Reduce the intracellular concentration of a chemotherapeutic agent
- Destroy drugs by enzymatic methods

Drug Resistance Mechanisms: Reduced Target Binding

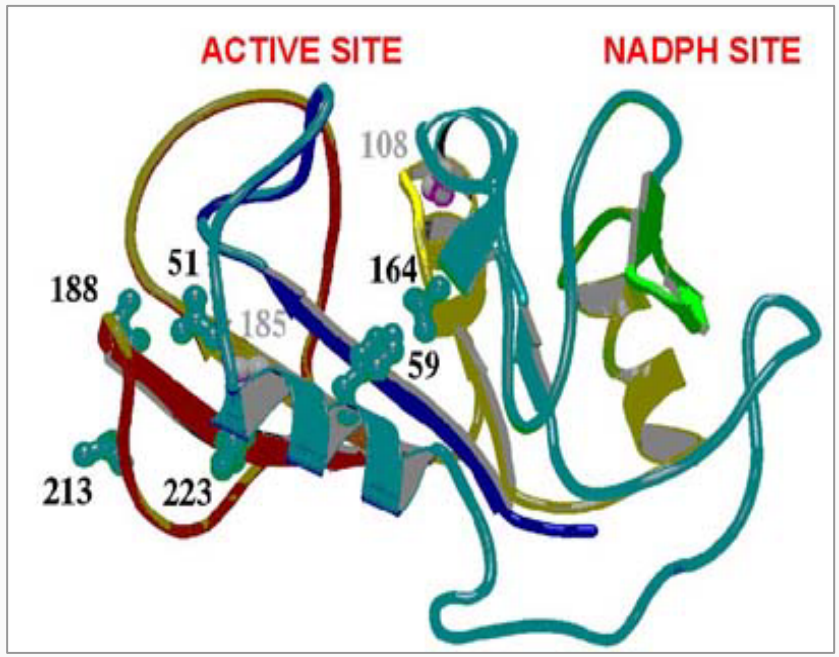
- Drug (e.g., pyrimethamine) and substrate (e.g., DHF) binding depends on interactions with specific amino acids in a protein
- These interaction are not always with the identical amino acids
- Changes in amino acid sequence in an enzyme can reduce drug binding without altering substrate binding

Continued

Drug Resistance Mechanisms: Reduced Target Binding

- As few as three or four amino acid changes in DHFR can prevent pyrimethamine binding without affecting folate binding

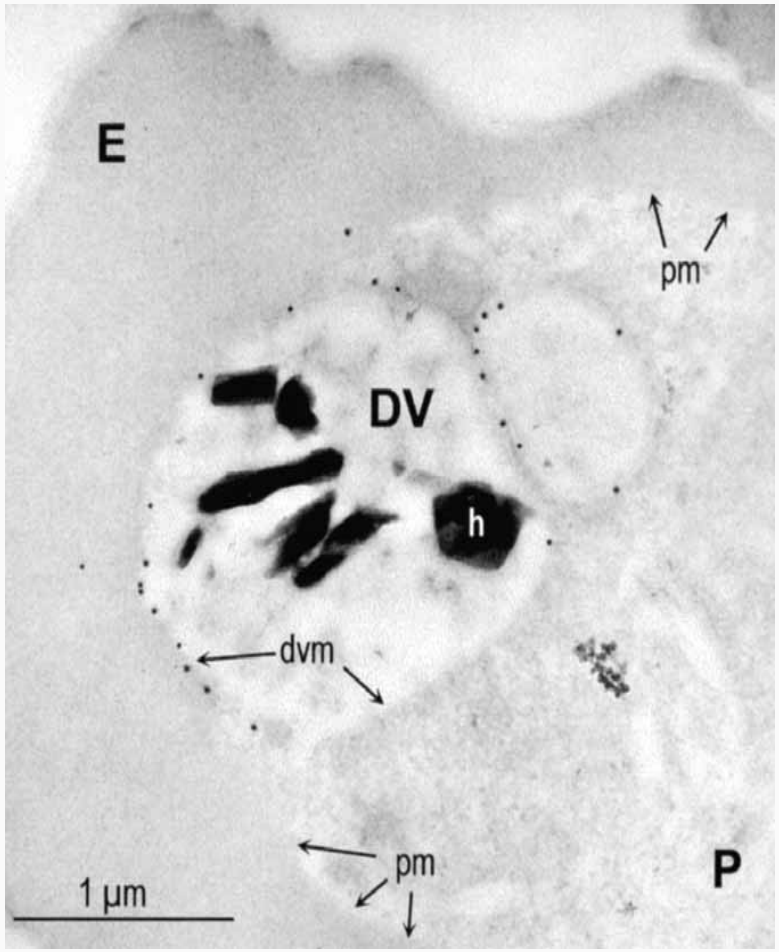
Model of Malaria Dihydrofolate Reductase Enzyme with Mutations that Confer Resistance to Antimalaria Drugs



Source: Adapted from Lemcke, Christenson, and Jorgenson, *Bioorg. Med. Chem.* 7:1003. 1999.

Drug Resistance Mechanisms: Reduced Concentration

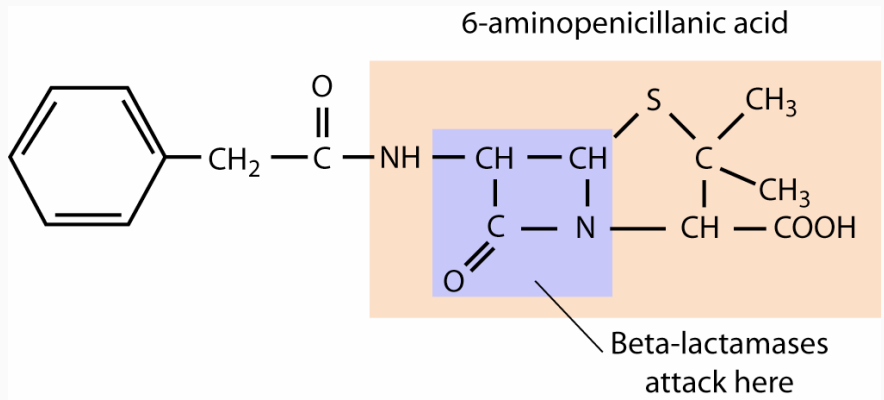
- Cells control the concentrations of intracellular solutes by regulating the activities of pumps that take up and expel individual chemicals
- Chloroquine resistant strains of *P. falciparum* show reduced intracellular levels of the drug and have mutations in a gene (PfCRT) that may be a chemical pump



Source: Cooper et al. *Mol. Pharmacology* 61:35 (2002)

Drug Resistance Mechanisms: Drug Destruction

- Some drug-resistant organisms produce enzymes that attack and destroy drugs
  - For example, β-lactamases break a specific chemical bond in penicillin and its derivatives and inactivate them
- A related mechanism is drug modification
- Many of the genes for these enzymes are carried on mobile genetic elements that can be transmitted from one bacterium to another and sometimes across species lines



Penicillin G: High activity against most gram-positive bacteria

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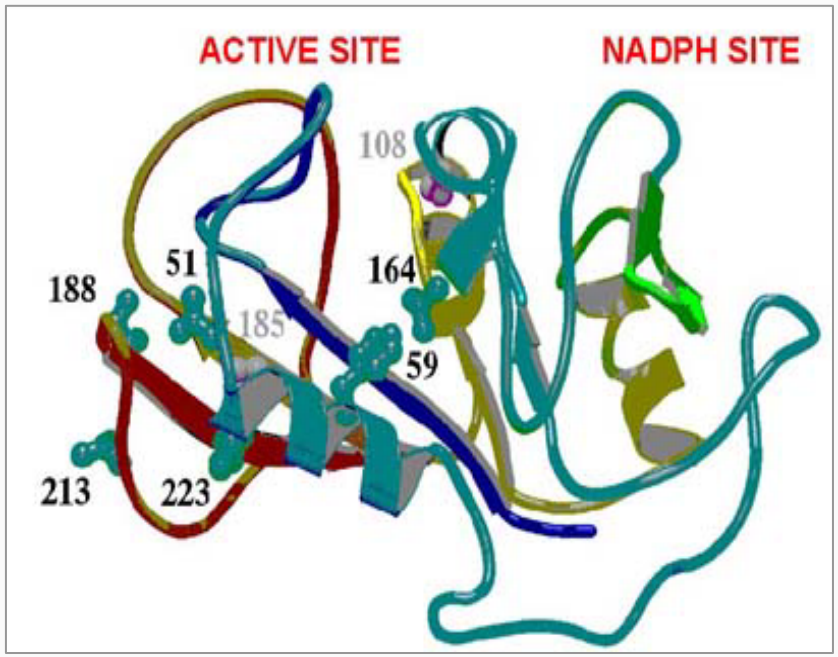
Section D

Sources and Consequences of Drug Resistance

Sources of Drug Resistance: Mutation

- For many drug targets, amino acid sequence changes can result in reduced drug binding and drug resistance
- Amino acid sequence changes are the consequence of changes in DNA sequence in the relevant gene
- Mutation is a common source of drug resistance

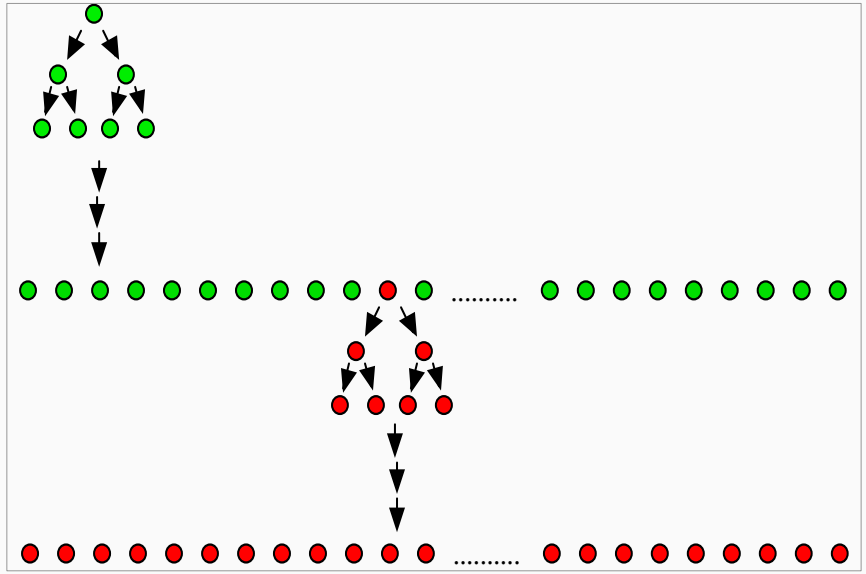
Model of Malaria Dihydrofolate Reductase Enzyme with Mutations that Confer Resistance to Antimalaria Drugs



Source: Adapted from Lemcke, Christenson, and Jorgenson, *Bioorg. Med. Chem.* 7:1003. 1999.

Mutation

- Mutations arise at random as organisms grow
- In a large population of pathogens, drug-resistant individuals will periodically arise when chance mutation alters some drug target
- If a drug is present, resistant individuals can continue to grow



Frequency of Drug-Resistant Mutants

- The frequency with which drug resistant organisms arise depends upon:
  - Mutation frequency
  - Number of mutations required to confer resistance
- Based on reasonable estimates of mutation frequency in bacteria or eukaryotes, something like 1 per 10^{10} or 10^{11} organisms will carry any particular mutation
  - The number is *much* lower with many viruses, including HIV

Continued

Frequency of Drug-Resistant Mutants

- Is one drug-resistant mutation per 10^{10} or 10^{11} organisms a problem?
  - It depends upon the drug, pathogen, and host
- For TB, a reasonable bacterial load is 10^{11} per cavity
  - Resistance to many TB drugs can be conferred by single mutations
  - Each cavity on the average will contain an organism resistant to any particular TB drug
- For malaria, parasite loads can be greater than 10^{13} per person
  - Each individual will carry hundreds of potentially resistant organisms (for single-substitution resistance)

Practical Consequences

- At least two factors mitigate this bleak picture
  - A few surviving parasites/bacteria after drug treatment generally will be dealt with by the immune system
  - High-level resistance to many drugs requires multiple mutations (three or four, for pyrimethamine), and these must arise independently
    - Frequencies of 1 per 10^{30}–10^{40}
    - Importantly, resistance to low doses of many antibiotics requires only single mutations

Sources of Drug Resistance: the Environment

- Drug-resistance genes are present in environmental organisms
  - Presumably, these arose due to selection by natural antibiotics
  - Artificial selection can increase frequency
  - Selection occurs in both humans and animals
    - In humans, in hospitals (for example)
    - Agricultural use of antibiotics is correlated with presence of resistant organisms in some cases
    - Impact on public health is unclear
    - Prudence in agricultural use of new classes of antibiotics seems warranted

Drug Resistance and Mobile Genetic Elements

- Plasmids (small circular, replicating DNA molecules)
- ICEs (integrating conjugative elements)
- Encode genes for resistance to antibiotics (sometimes several) and for transfer of themselves to other bacteria via physical contact
  - For example, Vibrio SXT—chloramphenicol, sulphamethoxazole, trimethoprim, and streptomycin
- Frequently can move across species and genus lines
  - Mobility can be induced by DNA-damaging agents including antibiotics (e.g., ciprofloxicin)

Management of Drug Resistance

- It is essential to maintain therapeutic doses of drugs when used
- Special care is required in immunocompromised individuals
- It is desirable to use multiple drugs for therapy when available
- These lessons have been applied to TB chemotherapy in the United States and have been very successful
- HAART treatment for HIV infection is multi-drug
- Malaria treatment is evolving slowly in this direction