Chemical Substances of Antibiotics

Jeremiah Roberson

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Antibiotics

Modern therapy by chemicals, chemotherapy, is attributed to Paul Ehrlich who synthesised the first antibiotic, an arsenic compound patterned after an azo dye that he found to stain a microorganism selectively. The compound first called compound 606 was introduced commercially in 1910 as a treatment of syphilis under the name of Sylvarsan.

Arsphenamine
(sylvarsan)
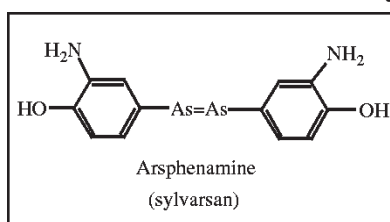
Many compounds were investigated over the next 50 years as antibacterial agents. Some structures are very complex and necessitated the development of new synthetic organic reactions in order to prove their structures. An intense effort to develop medicinal agents against malaria was required because many soldiers were afflicted with the disease.

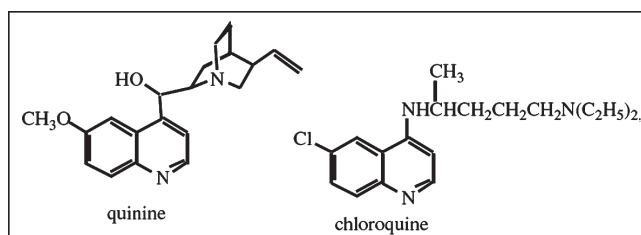Quinine, discovered active against malaria in 1990, was reasonably toxic and other agents were required. Chloroquine (1946) was synthesised and found to be highly effective against malaria.

quinine

chloroquine

Careful studies of the antibacterial compound known as Prontisil, a dye that strongly stains proteins, showed that it was metabolised to sulfanilamide, a more effective antibiotic. Prontosil is therefore termed a prodrug as it is not the substance responsible for the biological effect but it does produce the effective drug.

Prontisil
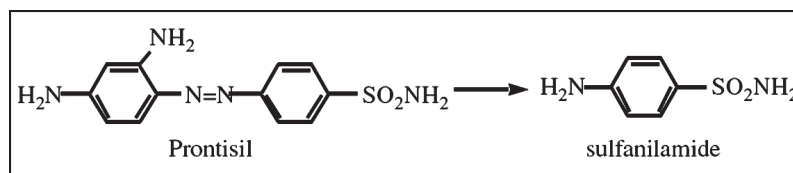
sulfanilamide

These studies led to the investigation of compounds containing the sulfonamide function as potential antibiotics and led to a number of very effective antibiotic agents. Many of these compounds are sulfanilamides that have an organic group in place of one of the hydrogens of the sulfonamide. Sulfathiazole proved to be a highly effective antibiotic.

sulfathiazole
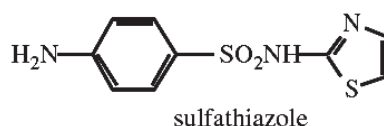
As the search for better antibiotics continued, a revolutionary drug was developed by H. W. Florey and E.

Chain in 1938. Penicillin was produced by microorganisms of mold and was found to inhibit the growth of other microorganisms. Penicillin was so important in the treatment of bacterial infections that many research groups from all over the world combined their research efforts in the isolation, purification, testing and synthesis of penicillin drugs. The structurally related cephalosporin antibiotics were also discovered during this time.
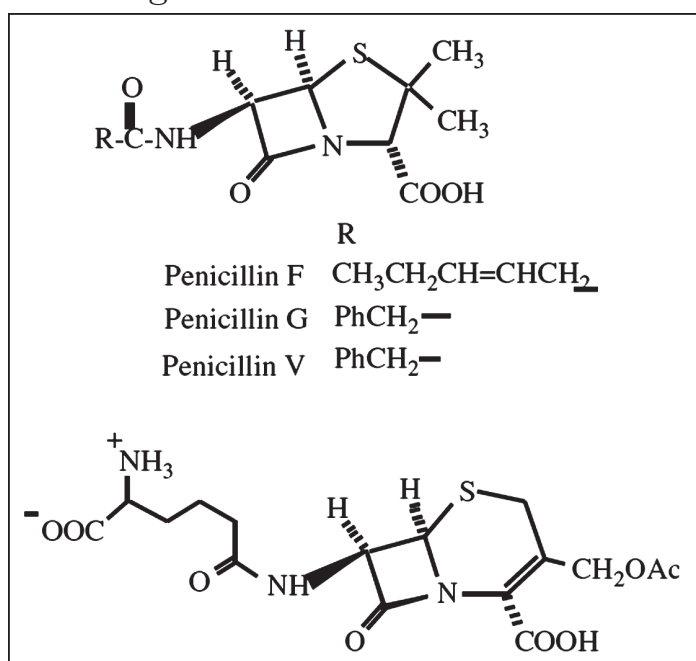
The search for antibiotics continued to be successful with the isolation and structure determination of the complex antibiotics streptomycin and tetracycline. Chloramphinacol, isolated in 1947, possesses a relatively simple structure. With its two chiral carbons, it has four stereoisomers. Studies at Parke, Davis and Co. showed that only the R, R stereoisomer is active against microorganisms. This finding began the study of stereoisomers as medicinal agents.

chloramphenicol
treatment of typhoid fever
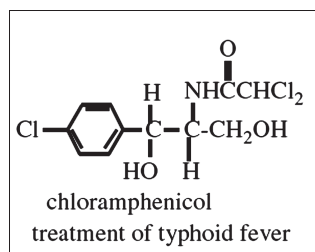
Antibiotic agents with large ring systems were also isolated. Erythromycin, a macrocyclic compound, is effective against penicillin resistant bacteria. Another large ring system that contains only amino acids is Tyrocidine A, a component of gramicidin antibacterial agents. Some cyclic peptides are used in the treatment of other diseases such as Hodgkin's disease.

OPIATES

Opiates belong to a class of compounds known as alkaloids that are nitrogen containing substances found in several plant species. The naturally occurring nitrogen compounds found in dried opium seeds are called opiates and are well-known for their strong analgesic effects and for their highly addictive nature.

Opium extracts have been used for many years to treat pain, but only in the early 1900's was the seed analysed and found to contain over twenty alkaloids. Morphine accounts for about 10 per cent of the extract by weight. Scientists studied morphine and similar compounds for many years until its structure was determined by synthesis in 1954. Two other pain-killing alkaloids with highly addictive properties are also found in opium, heroin and codeine.

Heroin is perhaps the most abused substance in today's society. Morphine, in medicinal chemistry, represents a very important lead compound for the derivation of new pain

alleviating drugs. Morphine and many other naturally occurring biological substances, contains the well-recognised b-phenethylamine structure outlined with heavy lines. This structural unit is also found in lysergic acid diethyl amide (LSD), another widely abused substance derived from an alkaloid.
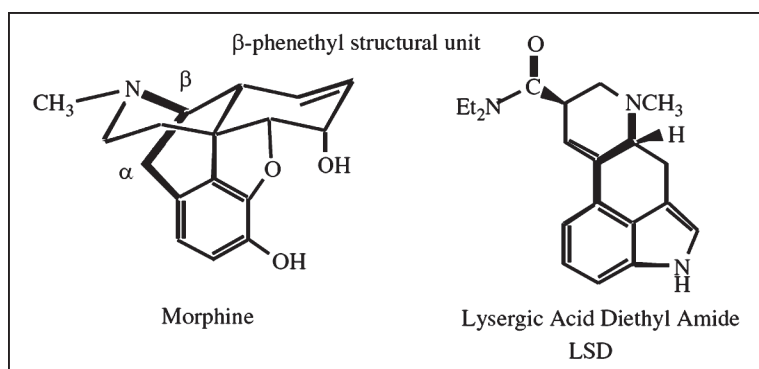
The many studies of morphine-like compounds have given some interesting facts about the structural requirements for the drug's activity. For example, the N-methyl group, the secondary alcohol and the alkene functions are unnecessary for drug activity.

Replacement of the N-methyl with N-allyl produces nalorphine that acts against the activity of morphine and serves as a treatment for overdoses of morphine, but it has hallucinogenic side effects. Modifications of the ring system are also possible and produce some interesting morphine-like drugs. Removal of the ether ring, along with the alcohol and alkene, produces after methylation, dextromethorphane a popular ingredient in cough syrup. Removal of both the ether ring and the cyclohexenol ring, with addition of a cyclopropyl function on the nitrogen give bremazocine that is 200 times more active than morphine.

Further modification with removal of the ether ring, the cyclohexenol ring and the cyclopentane ring to leave a phenyl substituted piperidine ring produces the drug that is about six times as active as morphine. Finally removal of all the rings except phenyl produces the well-known methadone. Methadone is used in the treatment of morphine addiction because its side effects are less severe than that of morphine, but it is just as addictive.

Methadone
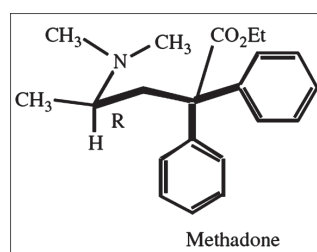
DRUG MECHANISM

The mechanism of how a drug exerts its effect is a very significant finding in the determination of new and improved drugs. Early medicinal chemists had to rely on screening and drug modification for new drugs, but the inclusion of modern mechanistic theories into drug searches advances the field considerably.

However, the fortuitous discovery of a drug without much knowledge of how or why it works still plays an important role in medicinal chemistry. A major finding in drug mechanism is that many drugs react with a receptor protein that occurs in cell walls or in the cytoplasm of cells. The interaction between drug and receptor is similar to the enzyme-substrate interaction in which the drug must fit into a receptor cite by its molecular shape.

The drug in its interaction with the receptor may cause an enhanced biological response (agonist), a decreased biological response (antagonist) or be delivered to a site in the cell where the drug effects beneficial changes. The body contains many receptor proteins that are present as receptor proteins for natural processes. The drug must compete with the natural chemicals for the protein in order to be effective. The study of histamine and its receptor permitted rational design of new drugs.
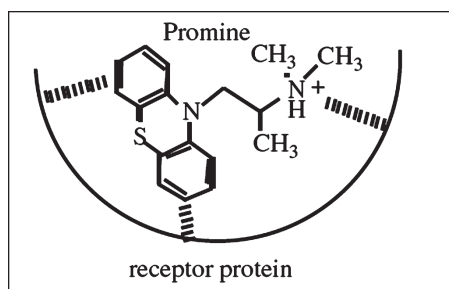
In this case, histamine was found to bind with a receptor protein-called the histamine-2 receptor. New drugs were deigned that would mimic histamine binding but would not permit histamine to bind. The effects of these types of drugs were to act as antihistamines. A novel approach to the treatment of breast cancer is the use of agents that bind with the estrogen receptor protein. Mammary cancers are known to have a buildup of estrogen receptor protein and estradiol, the primary female hormone. An estradiol analog with a radioactive fluorine atom in the 16a position competes very strongly for the estrogen receptor protein. Thus the mammary cancer becomes labelled for diagnostic purposes and the radiation produced from the 18F can destroy the nearby cancer cells. The radioactive fluorine compound is called a radiopharmaceutical.

17β-estradiol   16α-[18]Fluoro-17β-estradiol
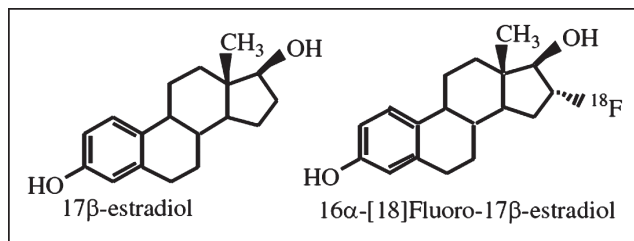
A very important type of drug is one that interacts with an enzyme. The enzyme is fooled into using the drug as a natural substrate, but the drug does not continue the function of the enzyme and its activity is inhibited. Such drugs are called mechanism-based suicide inhibitors. The reaction mechanism is used to design the drug and the enzyme commits suicide when it accepts the drug. An example of this behaviour is found with many antibiotic drugs that inhibit the growth of bacterial cells. Bacterial cells must synthesise folic acid whereas humans obtain folic acid from food. An enzyme named dihydropteroate synthetase is responsible for the synthesis of folic acid in a process that uses paraaminobenzoic acid. The enzyme also accepts sulfanila-mide because of its similarity in structure to paraaminobenzoic acid, but the sulfanilamide binds tightly with the enzyme and prevents the enzyme from participating in further synthesis of folic acid. The cell dies without the folic acid. The mechanism of action of most drugs involves receptor binding and enzyme inhibition. The study of enzymes and the structure of active sites are thus major efforts in medicinal studies.

Folic Acid

p-aminobenzoic acid
substrate

sulfanilamide
inhibitor

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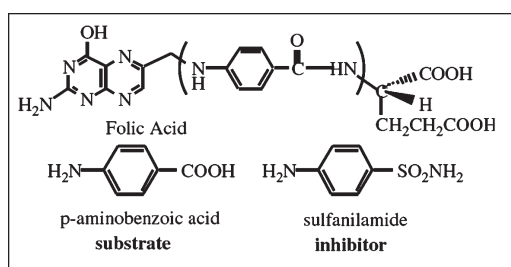
DRUG ALTERATION

Once a lead compound with medicinal properties is discovered changes are done synthetically in the compound. Such changes were observed in the sulpha drugs above in which different substituents were placed on the nitrogen. Many changes in the structure of morphine-related compounds (opiates) were shown in which alkyl groups were changed and rings were removed systematically. The important finding is the lead compound, but very often the altered drug shows better properties or less toxicity. Another alteration of drugs is the incorporation of fluorine.

Fluorine atoms allow the original structure to retain its molecular size because fluorine is a small atom. Also, the fluorine is very electronegative and thus introduces polarity into the molecule. The change can sometimes be very beneficial in drug discovery. Once a drug is discovered many experiments are conducted to determine the pharmokinetics of the drug. These studies show how the drug is delivered to the site of action, how the drug is distributed throughout the body and how the drug is metabolised.

Acyclovir   amino-acyclovir
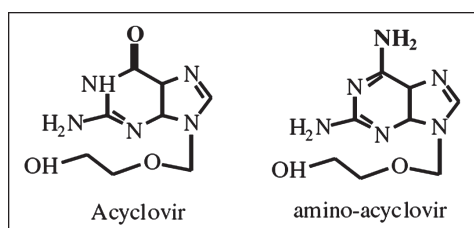
The results of the studies sometimes provide clues as to how the drug could be altered to improve its pharmokinetic properties and effectiveness. Biodistribution studies of the drug acyclovir, used in the treatment of herpes simplex virus, show that it is only absorbed about 15 per cent when taken

orally. A better absorption is found when the carbonyl group is replaced by an aminogroup.

L-Dopa is a well-know drug for the treatment of Parkinson's disease. It is actually a prodrug because it is decarboxylated to the amine that is the active drug. Only about 1 per cent of L-dopa actually enters the central nervous system because it is decarboxylated by enzymes in the liver. A solution to improving the uptake of L-dopa is to add another drug that inhibits the decarboxylation enzyme thus permitting more L-dopa to reach the active site. The administered dose of Ldopa can be reduced more than 50 per cent when used in conjunction with the enzyme inhibitor.

AROMATHERAPY AND THE MEDICAL COMMUNITY

Aromatherapy is slowly making its way into mainstream medical establishments in areas where it is not regarded as a direct threat to pharmaceutical medicine. Unfortunately, many times the first step into complementary medicine is as a last resort, when orthodox medicine has all but given up on a positive outcome for a client. Some remarkable examples of wound management and ongoing care of the aged. Future areas of focus will be in the use of essential oils in the field of infectious control and even palliative care, when quality of life is one of the most important issues to be encountered. Worldwide it is nurses, in particular, who are championing the cause of aromatherapy as a complementary medicine.

Nurses recognise the therapeutic and holistic issues surrounding quality of life. By using aromatherapy they can

provide a level of personal care that is often missing in a clinical environment. As the broader knowledge and scientific understanding of essential oils increase, there is likely to be an increase worldwide in the use of essential oils in hospital settings to mediate pain, reduce stress, assist in wound-care management, pain management, reduce nausea and reduce rates of infections.

The 'English' or 'popular aromatherapy' is the most prevalent style of aromatherapy worldwide but aromatherapy practices do vary from country to country. This variation is in part due to the fact that the practice of aromatherapy is dependent on each country's legal and educational requirements for certification. It is in France, where it more closely follows the biomedical medical model in its administration that aromatherapy is best accepted by the orthodox medical community.

LIMITATIONS OF TREATMENT

Aromatherapy in all its forms has many uses and applications; it is not, however, the cure-all that it is sometimes purported to be. Popular aromatherapy can provide real benefits in many areas of stress management and in dealing with emotional issues. Clinical aromatherapy can be helpful in many areas including infectious disease control, chronic-condition care, pain management and wound care. Yet aromatherapy in all its forms should not be viewed as a substitute but as a complement to orthodox health care.

A prime benefit of aromatherapy knowledge is that it can be shared with patients to enable patients themselves to obtain relief from symptoms of some minor conditions. As more practitioners in all forms of medicine gain greater understanding of what is available there will be closer cooperation and networking, which will only improve patient outcomes and quality of life.

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Chemical Drug Substances

SUBSTANCES

Many pharmacologically active substances are totally synthetic organic chemicals, which are produced in bulk quantities by the active pharmaceutical ingredients (APIs) manufacturers to comply with good manufacturing practices (GMPs). Some are also highly purified and well-characterized, naturally occurring active substances. Chemical purity of a synthetic API, a characteristic with a significant impact on the drug product quality, is accomplished only if impurities are each present at a nominal concentration less than or equal to a predefined limit.

Impurities are unwanted coexisting components in bulk pharmaceutical chemicals that arise during manufacture and/or subsequent storage. According to the definition given by the International Conference on Harmonization (ICH) of

Technical Requirements for Registration of Pharmaceuticals for Human Use, impurity is any component of a substance for pharmaceutical use that is not the chemical entity defined as the substance. Excluding enantiomers, polymorphic forms and extraneous contaminants — the presence of the last is inconsistent with GMP — ICH classifies impurities associated with a chemical API as inorganic, organic (process- and drug-related) and residual solvents.

However, some medicinal substances are mixtures of closely related compounds. These compounds have similar activity; they contribute to the assay result and are not regarded as impurities (such an example is cefamandole free acid in the pure form of cefamandole nafate). Budesonide epimer A is not regarded as an impurity although it must be controlled to ensure batch-to-batch consistency and uniformity among different manufacturers. Wherever a substance is supplied in pure form as an organic or inorganic salt, the organic acid or the inorganic counter ion are not considered impurities (examples include maleic acid in enalapril maleate, benzene sulfonate in amlodipine besilate and chlorides in substances supplied as hydrochloride salts). Coexisting water in pure APIs is not an impurity either.

ORGANIC IMPURITIES IN SYNTHETIC APIS

ICH further classifies organic impurities as starting materials, by-products, intermediates, degradation products, reagents, ligands and catalysts. Enantiomers, which are not included in the previous classifications, are stereoisomers with the same molecular formula as the drug substance,

differing only in the spatial arrangement of atoms within the molecule and having a nonsuperimposable mirror image relation (chirality). Enantiomers have the same physical and — in an achiral environment — chemical properties, except the optical rotation.

Diastereoisomers (isomers of drugs with more than one chiral centre) and geometric isomers are both chemically distinct, pharmacologically different and readily separated. Many of the marketed drugs are chiral and are often supplied as mixtures of enantiomers (racemates) rather than single enantiomers. However, certain single enantiomeric forms of chiral drugs are regarded as improved chemical entities with a better pharmacological profile. Some of the chiral drug substances offered as pure enantiomers are naturally biosynthetic products. In these cases, the presence of the enantiomeric impurities is excluded because of the high level of enantioselectivity of their biosyntheses.

However, technological advances such as chiral separation or assymetric synthesis permit commercial production of several single enantiomer drugs. In the manufacture and control of these drugs the other enantiomer (antipode) is an undesirable organic impurity. For this reason, chiral chromatographic tests are included in the pharmacopoeial monographs of some single enantiomeric drugs. In the future such tests will become more common. A presentation somewhat different from that of ICH, but very detailed, which also gives useful information on the chemical and analytical characterization of the related organic impurities in drug substances and drug products, has recently been published. Each impurity must be investigated with respect to both

chemistry and safety aspects. The former include identification (structural characterization), reporting and quantitation using suitable analytical procedures, while the latter include a process of acquiring and evaluating data concerning the biological safety of an impurity (qualification). Individually listed impurities, limited with specific acceptance criteria, are referred to as specified and they can be either identified or unidentified. Unspecified impurities are limited by a general acceptance criterion. A decision tree for the identification and qualification along with the corresponding thresholds, which are dependent on the maximum permitted daily dose (MDD), is given by ICH.

Summing up, the following list of organic impurities must be presented in the specification of a synthetic drug substance:

- ❖ Each specified identified or unidentified impurity
- ❖ Any unspecified impurity
- ❖ Total impurities.

Specified unidentified impurities are referred to by an appropriate qualitative analytical description (*e.g.*, relative retention time).

CONTROL OF ORGANIC IMPURITIES

A description of the identified and unidentified existing impurities in a chemical drug substance is referred to as the impurity profile (IP). Impurity profiling includes the procedure aimed at the detection, structure elucidation/identification and the quantitative determination of these impurities. Efforts are mainly focussed on the profiling of the organic impurities as the other possible groups, such as inorganic impurities and residual solvents, are easily identified and their toxicity is known. The presence of organic impurities

in a drug substance is closely dependent on the process of manufacture. A different route of synthesis will tend to lead to a different IP.

In pharmaceutical research and development, IP is often decided by using high performance liquid chromatography (HPLC), mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectrometry. Direct coupling or multiple hyphenation of these techniques along with the use of modern software for spectral/ chromatographic searching is a valuable tool for the detection of impurities at trace levels. In case of volatile, but thermally stable compounds gas chromatography (GC) coupled with various detection systems still plays an important role. Investigation of the impurities in complex natural products by using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) MS has been proposed. Capillary electrophoresis and solid phase microextraction/GC–MS have also been successfully used. Normally, more than one analytical system is applied for the confirmation of an IP.

Isocratic and gradient reversed-phase HPLC with ultraviolet-visible (UV-Vis) detection remains the most suitable analytical procedure for routine impurity testing. Baseline separation of all the potential organic impurities and the active substance should be performed. Better specificity is established by using photodiode array detectors, when the method is under development. In certain applications ion pairing offers better peak separation and post-column derivatization lowers detection limits. GC and thin layer chromatography (TLC) are often applied in the industrial quality control (QC) laboratories for impurity

testing. TLC determinations have a semi-quantitative nature, but allow the detection of impurities completely retained or those not retained at all by the stationary phase.

DRUGS TREATING HEART FAILURE

Heart failure is a chronic disease needing lifelong management. However, with treatment, a failing heart can become stronger and signs and symptoms of heart failure can improve.

Doctors sometimes can correct heart failure by treating the underlying cause. For example, repairing a heart valve or controlling a fast heart rhythm may reverse heart failure. But for most people, the treatment of heart failure involves a balance of the right medications, and in some cases, devices that help the heart beat properly.

MEDICATIONS

Doctors usually treat heart failure with a combination of medications. Depending on your symptoms, you might take one, two or more of these drugs. Several types of drugs have proved useful in the treatment of heart failure. They include:

ANGIOTENSIN-CONVERTING ENZYME (ACE) INHIBITORS

These drugs help people with heart failure live longer and feel better. ACE inhibitors are a type of vasodilator, a drug that widens or dilates blood vessels to lower blood pressure, improve blood flow and decrease the workload on the heart. Examples include enalapril (Vasotec), lisinopril (Prinivil, Zestril) and captopril (Capoten). ACE inhibitors also blunt some of

the effects of hormones that promote salt and water retention. ACE inhibitors can cause an irritating cough in some people. It may be best to put up with the cough, if you can, to gain the medication's benefits. But be sure to discuss this with your doctor. Switching to another ACE inhibitor or an angiotensin II receptor blocker (ARB) may relieve the problem.

ANGIOTENSIN II (A-II) RECEPTOR BLOCKERS (ARBS)

These drugs, which include losartan (Cozaar) and valsartan (Diovan), have many of the beneficial effects of ACE inhibitors, but they don't cause a persistent cough. They may be an alternative for people who can't tolerate ACE inhibitors.

DIGOXIN (LANOXIN)

This drug, also referred to as digitalis, increases the strength of your heart muscle contractions. It also tends to slow the heartbeat. Digoxin reduces heart failure symptoms and improves your ability to live with the condition.

BETA BLOCKERS

This class of drug slows your heart rate and reduces blood pressure. Examples include carvedilol (Coreg), metoprolol (Lopressor) and bisoprolol (Zebeta). These medicines also reduce the risk of some abnormal heart rhythms. Beta blockers may reduce signs and symptoms of heart failure and improve heart function.

DIURETICS

Often called water pills, diuretics make you urinate more frequently and keep fluid from collecting in your body.

Commonly prescribed diuretics for heart failure include bumetanide (Bumex) and furosemide (Lasix). The drugs also decrease fluid in your lungs, so you can breathe more easily. Because diuretics make your body lose potassium and magnesium, your doctor may also prescribe supplements of these minerals. If you're taking a diuretic, your doctor will likely monitor levels of potassium and magnesium in your blood through regular blood tests.

ALDOSTERONE ANTAGONISTS

These drugs include spironolactone (Aldactone) and eplerenone (Inspra). They're primarily potassium-sparing diuretics, but they have additional properties that help the heart work better, may reverse scarring of the heart and may help people with severe heart failure live longer.

Unlike some other diuretics, spironolactone can raise the level of potassium in your blood to dangerous levels. A medication called BiDil is a single pill that combines hydralazine and isosorbide dinitrate — both of which dilate and relax the blood vessels. BiDil increases survival when added to standard therapy in black people with advanced heart failure. This is the first drug studied and approved for a specific racial group. Further studies will be necessary to determine if this combination medicine will be helpful for others with heart failure. You'll probably need to take two or more medications to treat heart failure. Your doctor may prescribe other heart medications as well — such as nitrates for chest pain, a statin to lower cholesterol or blood-thinning medications to help prevent blood clots - along with heart failure medications. You may be hospitalized for a few days

if you have a flare-up of heart failure symptoms. While in the hospital, you may receive additional medications to help your heart pump better and relieve your symptoms. You may also receive supplemental oxygen through a mask or small tubes placed in your nose. If you have severe heart failure, you may need to use supplemental oxygen long term.

SURGERY AND MEDICAL DEVICES

In some cases, doctors recommend surgery to treat the underlying problem that led to heart failure. For example, a damaged heart valve may be repaired or, if necessary, replaced with a new one. Doctors recommend coronary bypass surgery to treat severely narrowed coronary arteries that are contributing to heart failure. Researchers continue to search for new and better ways to treat heart failure. Some treatments being studied and used in certain people include:

IMPLANTABLE CARDIOVERTER-DEFIBRILLATORS (ICDS)

An ICD is a device implanted under the skin and attached to the heart with small wires. The ICD monitors the heart rhythm. If the heart starts beating at a dangerous rhythm, the ICD shocks it back into normal rhythm. Sometimes a biventricular pacemaker is combined with an ICD for people with severe heart failure.

CARDIAC RESYNCHRONIZATION THERAPY (CRT)
OR BIVENTRICULAR PACING

A biventricular pacemaker sends timed electrical impulses to both of the heart's lower chambers (the left and right

ventricles), so that they pump in synchrony and in a more efficient, coordinated manner. As many as half the people with heart failure have abnormalities in their heart's electrical system that cause their already-weak heart muscle to beat in an uncoordinated fashion. This inefficient muscle contraction wastes the heart's limited energy and may cause heart failure to worsen. Sometimes a biventricular pacemaker is combined with an ICD for people at greatest risk of rhythm problems.

HEART PUMPS

These mechanical devices, called left ventricular assist devices (LVADs), are implanted into the abdomen and attached to a weakened heart to help it pump. Doctors first used heart pumps to help keep heart transplant candidates alive while they waited for a donor heart. LVADs are now being considered as an alternative to transplantation. Implanted heart pumps can significantly extend and improve the lives of some people with end-stage heart failure who aren't eligible for or able to undergo heart transplantation or are waiting for a new heart. Some people have such severe heart failure that surgery or medications don't help. They may need to have their diseased heart replaced with a healthy donor heart. Heart transplants have dramatically improved the survival and quality of life of people with severe heart failure. However, candidates for transplantation often have to wait years before a suitable donor heart is found. Some transplant candidates improve during this waiting period through drug treatment or device therapy and can be removed from the transplant waiting list.

TREATMENTS

CARDIAC WRAP SURGERY

Researchers are studying a technique that wraps a failing heart in a mesh bag, helping to prevent further failure. A surgeon pulls the mesh wrap over the base of the heart and attaches it with stitches. The goal is to prevent a weakened heart from enlarging (dilating) and failing further. Studies are ongoing.

VENTRICULAR RESTORATION SURGERY

This surgery is being used experimentally to treat some people with heart failure caused by a heart attack. During the surgery, doctors remove scar tissue in the ventricular muscle caused by a heart attack and reshape the remaining healthy tissue to restore a more normal elliptical left ventricle shape. Reducing the size of and reshaping the left ventricle help restore normal function to the pumping mechanism.

ENHANCED EXTERNAL COUNTERPULSATION (EECP)

This noninvasive technique has been used as a treatment for heart-related chest pain, and researchers are studying this treatment to see if it's beneficial for people with heart failure. Inflatable pressure cuffs are placed on the calves, thighs and buttocks. These cuffs are inflated and deflated in sync with your heartbeat. The theory is that EECP increases blood flow back to the heart.

CHEMOTHERAPEUTIC SPECTRA

The chemotherapeutic spectrum of a drug refers to the species of microorganisms affected by that drug:

❖ Narrow spectrum (chemotherapeutic agents acting only on a single or a limited group of microorganisms, *e.g.* isoniazid is active only against mycobacteria).

❖ Extended spectrum (agents that are effective against gram-positive organisms and also against a significant number of gram-negative bacteria (*e.g.*, ampicillin is considered to have an extended spectrum because it acts against gram-positive and gram-negative bacteria).

❖ Broad spectrum (drug such as tetracycline and chloramphenicol affect a wide variety of microbial species and are referred to as broad spectrum antibiotics. Administration of broad-spectrum antibiotics can drastically alter the nature of the normal bacterial flora and can precipitate a superinfection of an organism, *e.g.*, candida whose growth is normally kept in check by the presence of other microorganism).

COMBINATIONS OF ANTRIMICROBIAL DRUGS

Therapeutically advisable to treat with the single agent that is most specific for the infecting organism. This strategy reduces the possibility of superinfection and decreases the occurrence of resistant organisms. However, situations in which combinations of drugs are employed do exist (*e.g.* the treatment of tuberculosis). Treatment with a combination of drugs may lead to the emergence of superinfection antagonism between the drug, or an increased incidence of toxicity.

DRUGS TREATING

CHRONIC DISEASE

Rheumatoid arthritis (RA) is a chronic autoimmune disease that causes inflammation of the joints and may cause inflammation of other tissues in the body. The immune system consists of the cells and proteins in our bodies that fight infections. An autoimmune disease occurs when our immune system doesn't recognize part of our body and attacks it as if it were an invader such as a bacteria or virus. In rheumatoid arthritis, the immune system targets synovial membrane and attacks it. The synovial membrane is secretes synovial fluid into the joint. Synovial fluid is the joint fluid that lubricates and nourishes the joint. Other tissues can also be targeted by the immune system in rheumatoid arthritis, but the synovium, or synovial membrane, is generally the primary target. When the synovial membrane is attacked, it becomes inflamed (synovitis) and can thicken and erode. As the synovial membrane is destroyed, the synovial fluid fluid is also destroyed because it is not being secreted. The surrounding structures can also become involved leading to the joint deformities that can be seen in rheumatoid arthritis.

Rheumatoid arthritis is a debilitating and common disease, affecting approximately 1 per cent of the population. Women are affected three times as often as men. It is not clear exactly what causes or triggers the autoimmune response. Many researchers believe that a bacterial, viral, or fungal infection may trigger the autoimmune response. Other researchers believe there is a genetic role in the development of

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rheumatoid arthritis. Despite active research, the question of what causes rheumatoid arthritis remains a debated topic. Whatever the trigger or underlying cause, it has been shown that rheumatoid arthritis is an autoimmune disease. The course of rheumatoid arthritis is often relapsing and remitting, meaning that a person can suffer with symptoms for a prolonged period of time (perhaps months or years) and then the symptoms go away for a while (perhaps years) only to recur again at a later date. The severity and chronicity of rheumatoid arthritis varies from person to person. Most people who develop rheumatoid arthritis will do so between the ages of 20 and 60. In general, the earlier that symptoms develop, the more severe the disease will be.

DIAGNOSED

There is no single test that can diagnose rheumatoid arthritis. Instead, your physician must look at your entire history, physical examination, laboratory tests and radiographs when making the diagnosis of rheumatoid arthritis.

The first step your doctor will take when diagnosing rheumatoid arthritis is take a complete medical history from you. Your age is an important factor when considering the diagnosis. Because most people who develop rheumatoid arthritis are between the ages of 20 and 60, if you fall outside of this age range it makes the diagnosis less likely. Common symptoms from rheumatoid arthritis include morning stiffness that lasts for longer than one hour, bilateral symmetrical involvement of small joints (*e.g.* both hands), and multiple joints being involved (often 3 or more). The most common joints to be involved are the fingers, hands, wrists,

and feet. Often, people with rheumatoid arthritis may also complain of general fatigue, weakness, low-grade fever without an obvious source of infection, decreased appetite, weight loss, and/or muscle pain. People with rheumatoid arthritis typically will complain of difficulty performing tasks of daily living such as writing, preparing food, grasping a cup, getting dressed, and turning a doorknob.

Occasionally, rheumatoid arthritis can affect the heart (pericarditis) in which case the person may have chest pain that is worse when taking a deep breath or when lying down. Almost any organ in the body can become affected by rheumatoid arthritis. When the blood vessels themselves are involved, a vasculitis develops that can be debilitating and dangerous. These complications are more common with long-standing chronic rheumatoid arthritis.

Patients with rheumatoid arthritis will typically not have pain or swelling in the finger joint closest to the fingertip (the distal interphalangeal joint). The other joints in the hand are often affected. The affected joints are often tender to the touch. Depending on how long the person has been having symptoms, and the severity of the rheumatoid arthritis, there may also be some deformity in the fingers, hands, wrists, and other joints. Rheumatoid nodules may develop and be felt as firm lumps beneath the skin. These occur most commonly at points of pressure under the elbow and on the fingers. Usually rheumatoid nodules do not cause pain but they can become infected or put pressure on a nerve in which case they would need to be treated.

Your doctor will likely order several tests. Blood tests will reveal an anemia (decreased hemoglobin) in 80 per cent of

patients with rheumatoid arthritis. The erythrocyte sedimentation rate (ESR) is a general marker of inflammation and is elevated in 90 per cent of patients. In 70 per cent of rheumatoid arthritis a marker called rheumatoid factor (RF) will be present. However, all of these tests are nonspecific and may be positive in people without rheumatoid arthritis. The results of these tests need to be integrated by your physician with your other findings to make the diagnosis of rheumatoid arthritis. On radiographs (x-ray), the typical findings of rheumatoid arthritis include cysts, osteopenia, swelling, bony erosions, narrowed joint space, deformities, and fractures. Your physician may perform an arthrocentesis of the affected joint or joints. This procedure involves putting a needle under sterile conditions into the affected joint and aspirating the fluid. The fluid is then sent for analysis. This procedure is used in this setting to rule out other possible etiologies of the pain and swelling such as infection, gout, and pseudogout.

In a person with suspected rheumatoid arthritis and neck pain or neurologic symptoms (pain, numbness, weakness radiating into the arms or legs), the physician should perform tests to ensure that there is no instability of the cervical spine (vertebrae in the neck) because people with rheumatoid arthritis are more susceptible to this type of instability than the general population.

TREATMENT

There is no cure for rheumatoid arthritis. However, several treatments exist to decrease the symptoms of rheumatoid arthritis and greatly improve quality of life.

❖ *Early diagnosis*: The sooner that rheumatoid arthritis is treated, the better. If you think you might have rheumatoid arthritis, or other type of arthritis, don't delay in getting to your doctor. Prolonging treatment only makes it more difficult to treat later.

❖ *Diet*: One of the simplest treatments, and yet a treatment that has been found to be effective for many people, is consuming an anti-inflammatory diet. Primarily, this consists of consuming omega-3 fatty acid containing foods, including small cold water fish, fruits, and vegetables.

❖ *Weight loss*: As with other forms of arthritis, if you are overweight then losing weight can make a large impact on decreasing pain and stiffness in your joints by taking some of the pressure off of them.

❖ *Exercise and rest*: Rest is an important part of treating rheumatoid arthritis. One of the symptoms of rheumatoid arthritis can be fatigue as well as a sense of generalized malaise. It is important to get adequate rest so that your body does not get run down, This is true for everyone, but particularly true for people with rheumatoid arthritis. People with rheumatoid arthritis should not exercise to the point of exhaustion. Rather, they should take frequent breaks and rest before they get too tired.

While rest is important, it is also important to stay active and participate in an exercise regimen. The exercise regimen should include cardiovascular exercise (bicycling, swimming, etc), stretching, and strengthening exercises. Your physician can provide you with a structured exercise programme

tailored to your individual needs. Ideally, the exercise programme should include exercises such as swimming that do not load the joints with too much pressure. It is very important to promote flexibility in the joints and move each joint through its full range of motion at least once a day.

- ❖ *Physical therapy*: It is important to be enrolled in a well-structured physical therapy programme. In addition, physicians and physical therapists can fit you for gait aids (*e.g.* cane) when necessary.

- ❖ *Occupational therapy*: Because rheumatoid arthritis affects the small joints of the wrist, hand, and fingers, as well as other joints, it can make activities of daily living difficult. Occupational therapists specialize in helping patients overcome these difficulties and perform their activities of daily living. A structured programme with an occupational therapist can include learning proper body biomechanics, developing strategies to perform activities despite limitations, the use of splints to aid in tasks (*e.g.* hand splints, wrist splints), and other ways to use healthier body parts to compensate for body parts that are more affected by the disease process. Splints are also helpful in maintaining proper joint alignment.

- ❖ *Modalities*: Physicians, chiropractors, physical therapists, and occupational therapists may use a variety of modalities to help reduce symptoms. These modalities include ice, heat, massage, ultrasound, warm wax, and electrical stimulation.

- ❖ *Oral medications*: There are two basic lines of oral medication treatment for rheumatoid arthritis. First-

line treatment is used for acute inflammation and pain. These medications include non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, ibuprofen, naproxen, and etodolac. The most common side effect from this class of medication is abdominal problems such as stomach pain, ulcers, and bleeding in the gastrointestinal tract. Combining these medications with stomach protecting medicines like sucralfate and misoprostol can reduce the risk of stomach problems. A protonpump inhibitor medication such as omeprazole or pantoprazole (*e.g.* Prevacid, Protonix), can also be helpful to reduce the risk of stomach problems.

Steroids are also considered a first line treatment for rheumatoid arthritis. Steroids can be taken orally or injected directly into an affected joint. Long-term corticosteroid treatment carries a significant number of risks including easy bruising, cataracts, increased risk of infection, skin necrosis, osteoporosis and muscle wasting. The risk of osteoporosis can be decreased by taking supplements of calcium and vitamin D. The risk of increased infection rates can be reduced by gradually tapering off steroids instead of abruptly stopping taking high-dose steroids (which can also result in an acute flare up of the rheumatoid arthritis). Second-line medications include disease-modifying anti-rheumatic drugs (DMARDs). These medications are not anti-inflmmatory medications but they do promote remission of the disease. DMARDs are taken chronically for months or years. They generally take longer to be effective than first-line drugs. Depending on the response of the individual, more than one DMARD may be

used at a time. While reserved as second-line therapy, many physicians believe that the sooner DMARDs are used, the better the response.

DMARDs include hydroxycholoquine, sulfasalzine, methotrexate, D-penicillamine, gold salts, azathioprine, and cyclophosphamide. Plaquenil is an antimalarial medication that has been found to be helpful. Patients taking plaquenil should be monitored by an opthamologist because there is a small but increased risk of developing vision changes. Other side effects include skin rashes, muscle weakness, stomach problems. Sulfasalzine is a medication that is typically used for inflammatory bowel disease (*e.g.* ulcerative colitis, Crone's disease). It has been found to be useful for rheumatoid arthritis. Skin rash, headache, nausea, fever, and stomach problems are the most common side effects. People with sulfa allergy should not take sulfasalzine. People with gastrointestinal or genitourinary obstruction should also not take this drug.

Methotrexate is an immunosuppressive drug that has also been shown to effective in rheumatoid arthritis. It is better tolerated than some of the other DMARDs but side effects include stomach problems, malaise, fatigue, fever, increased infection rate, and chills. Because of the small risk of bone marrow or liver dysfunction, all patients on this drug require regular blood testing. Azathioprine, cyclophosphamide, and other immunosuppressive drugs are generally reserved for severe, recalcitrant cases of rheumatoid arthritis because of the potential for serious side effects. D-penicillamine is also used to treat rheumatoid arthritis. Side effects include fever, chills, mouth sores, skin rash, and kidney and bone marrow

damage. Gold salts used to be used more frequently than at present. The large number of side effects of gold salts have made them less attractive than other DMARDs. Nevertheless, in select patients they may be helpful.

Anti-TNF alpha factor medications have recently emerged as a newer class of medication for the treatment of rheumatoid arthritis. In many patients, this class of drug has been extremely helpful. Etanercept, infliximab, and adalimurnab are all members of this class of drug. TNF alpha is a potent inflammatory mediator. TNF alpha is released from damaged cells and essentially acts like a beacon calling other inflammatory cells to come to the site. Anti-TNF alpha medications stop TNF alpha from calling to other inflammatory cells and so they help break the inflammatory cycle. Etanercept is injected under the skin either once or twice a week. Infliximab is given intravenously, and adalimumab is injected weekly or every other week. Anti-TNF alpha medications may cause stomach problems, nausea, vomiting, upper respiratory infection symptoms, and skin rash. Anti-TNF alpha medications are typically reserved for patients who have not responded to DMARD therapy. Once anti-TNF alpha therapy is begun, DMARDs are also often used in conjunction.

Injections: Local injections of cortisone are often effective in treating symptomatic joints. The bursae that cover and protect joints are also more commonly inflamed in rheumatoid arthritis. These bursae also can be injected with cortisone to reduce the inflammation and pain. Research is currently underway at academic institutions into injecting anti-TNF alpha medication directly into the affected joints.

However, this is currently an investigational treatment. Surgery: When joints become very symptomatic, interfere with activities of daily living and quality of life, and when these joints do not respond to more conservative treatments, surgical intervention can be considered. Depending on the joint and degree of involvement, multiple surgical options are available. A thorough discussion of all the potential risks and benefits should be pursued with your doctor. Total hip replacement and total knee replacement are considered when the hip and knee are involved, respectively.

GOUT

Gout, a painful and potentially debilitating form of arthritis, has afflicted such famed figures. Today it affects roughly two million Americans. This disorder develops after tiny, needle-like crystals of uric acid (a biological waste product) accumulate in joints, causing swelling and extreme sensitivity, sometimes to the point where even the slight touch of a sheet is unbearable. The same crystals may cause kidney stones if they accumulate in the kidneys.

Gout usually affects one joint at a time, most often the big toe, but sometimes a knee, ankle, wrist, foot, or finger. If gout persists for many years, uric acid crystals may collect in the joints or tendons and under the skin, forming whitish deposits known as tophi. About 90 per cent of people with gout are men older than 40, and African American men are twice as likely as Caucasian men to be affected. Gout tends not to occur in women until at least 10 years after menopause.

CAUSES

For many people, gout develops after a combination of factors contributes to the buildup of excessive levels of uric

acid in the body. Abnormally high levels of uric acid may result from a diet that is rich in purines, chemicals that are broken down into uric acid by the body. Purines can be found in anchovies, nuts, and organ foods such as liver, kidney, and sweetbreads.

Sometimes, for unknown reasons, the body will produce too much uric acid regardless of diet. Gout can also develop when the kidneys excrete too little uric acid, which can happen in people with some types of kidney disease and in those who drink too much alcohol. In addition, obesity or sudden weight gain can cause elevated levels of uric acid.

Some medications, particularly diuretics, also contribute to high uric acid levels. People at risk for developing gout include those with a family history of the disease and those with hypertension, hyperlipidemia, or diabetes. Symptoms of gout pain and swelling within a joint, especially the big toe often, an initial episode that occurs at night shiny red or purple skin around the affected joint extreme tenderness around the joint.

DIAGNOSING

To reach a diagnosis, your doctor will ask you about your diet, your medication use, your alcohol consumption, and whether you have a family history of gout. During a physical exam, your doctor will inspect your inflamed joints and look for tophi on your skin. Your doctor may also use a needle to withdraw a small fluid sample from your affected joint. This fluid will be examined under a microscope to determine whether uric acid crystals are present. Your doctor may also order a blood test to determine your uric acid level, but this test is not definitive because — for a variety of different

reasons — many people without gout have an elevated uric acid level, and even in people with gout, the results may be normal.

TREATMENT

Gout is usually treated with a two-prong medication strategy: The first goal is to ease attacks of joint pain and inflammation, while the second, longer-term goal is to decrease blood uric acid level and prevent further attacks. Usually a doctor begins by prescribing a nonsteroidal anti-inflammatory drug (NSAID) to control pain and inflammation. Aspirin may raise your uric acid level; for this reason aspirin (and aspirin containing NSAIDs such as salsalate) is not a good NSAID choice to treat an attack of gout. However, many people take low dose aspirin to reduce the risk of heart attack, stroke or other serious health problems. If you've been instructed to take low dose aspirin, don't stop taking it without discussing it with your doctor. If you cannot tolerate an NSAID or if these drugs are ineffective, your doctor may suggest a corticosteroid. Less often, high dose oral colchicine is prescribed, but be aware that this drug tends to cause unpleasant side effects (nausea, vomiting, cramps, diarrhea) and is not well tolerated in about 80 per cent of people.

For people with attacks that respond poorly to therapy, involve multiple joints, or occur frequently, or when kidney stones or tophi are present, a second type of drug may be prescribed to prevent future gout attacks. It's important to keep taking this drug even after you feel better. The first choice is usually allopurinol (Aloprim, Zyloprim), which decreases your body's production of uric acid. Other options include probenecid (Benemid) and sulfinpyrazone (Anturane), which help the kidneys to eliminate uric acid.

An investigational medication, febuxostat, is not yet approved by the FDA, but has shown promise as a potential new treatment for gout. You can help prevent further attacks by avoiding diuretics, limiting your alcohol intake, drinking plenty of water, and maintaining a healthy weight. You may also want to reduce your consumption of foods that seem to trigger gout attacks, such as meat and certain types of seafood and vegetables — although many people find that strict dietary restrictions are of limited benefit.

PSEUDOGOUT

Pseudogout is a form of arthritis that occurs when a particular type of calcium crystal accumulates in the joints. As more of these crystals are deposited in the affected joint, they can cause a reaction that leads to severe pain and swelling. The swelling can be either short-term or long-term and occurs most frequently in the knee, although it can also affect the wrist, shoulder, ankle, elbow, or hand. The pain caused by pseudogout is sometimes so excruciating that it can incapacitate someone for days.

As its name suggests, the symptoms of pseudogout are similar to those of gout (see "Gout"). Pseudogout can also resemble osteoarthritis or rheumatoid arthritis. A correct diagnosis is vital, as untreated pseudogout can lead to joint degeneration and osteoarthritis. Pseudogout is most common in the elderly, occurring in about 3 per cent of people in their 60s and as many as half of people in their 90s.

CAUSES

The cause of this condition is unknown. Because risk increases significantly with age, it is possible that the physical

and chemical changes that accompany aging increase susceptibility to pseudogout. Certain medical conditions also make people more susceptible to pseudogout. These include an underactive thyroid (hypothyroidism), a genetic disorder of iron overload (hemochromatosis), or excessive blood levels of calcium (hypercalcemia).

Pseudogout also can be triggered by joint injury, such as joint surgery or a sprain, or the stress of a medical illness. If the underlying condition causing pseudogout can be identified and treated, it may be possible to prevent future attacks. Frequently, however, there is no identifiable trigger. Symptoms of pseudogout pain, swelling, and stiffness around a single joint, especially the knee or wrist occasionally, more then one joint affected at a time fever, usually low-grade.

DIAGNOSIS

It may be difficult to diagnose pseudogout because it shares so many symptoms with gout, infection, and other causes of joint inflammation. In fact, pseudogout often occurs in people with other joint problems, such as osteoarthritis. Therefore, even when pseudogout is correctly identified, it is important to investigate whether there are other conditions present as well.

Doctor may order an x-ray of the inflamed joint in order to look for calcium deposits in the cartilage, although these deposits are sometimes present in healthy elderly people who do not experience the swelling that characterizes pseudogout. To verify the presence of calcium crystals, your doctor may remove a small amount of fluid from the affected joint. This is done with a needle, after applying a numbing medication

to the joint. This joint fluid is then analysed for evidence of calcium crystals, inflammation, or infection. Your doctor may also order tests for other conditions that can trigger pseudogout, including tests of calcium and thyroid function.

TREATING PSEUDOGOUT

To combat joint pain and swelling, your doctor may prescribe NSAIDs such as indomethacin and naproxen, or may give you glucocorticoid injections to keep the swelling down. Your doctor may also remove fluid from the inflamed joint, a procedure called aspiration, as this may help to ease the pressure and inflammation. The combination of joint aspiration and medication usually eliminates symptoms within a few days, although the doctor may also recommend treatment with oral corticosteroids over a short period of time. Daily use of a low-dose NSAID or colchicine, a medicine that is also used in the treatment of gout, may help to prevent further attacks. Unfortunately, there is no treatment available that can dissolve the calcium crystal deposits, although the joint degeneration that often goes along with pseudogout may be slowed by treatments that decrease joint swelling.

3

Antidiabetic Drug

Anti-diabetic drugs treat diabetes mellitus by lowering glucose levels in the blood. With the exceptions of insulin, exenatide, and pramlintide, all are administered orally and are thus also called oral hypoglycemic agents or oral antihyperglycemic agents. There are different classes of anti-diabetic drugs, and their selection depends on the nature of the diabetes, age and situation of the person, as well as other factors. Diabetes mellitus type 1 is a disease caused by the lack of insulin. Insulin must be used in Type I, which must be injected or inhaled. Diabetes mellitus type 2 is a disease of insulin resistance by cells.

Treatments include:

- ❖ Agents which increase the amount of insulin secreted by the pancreas,
- ❖ Agents which increase the sensitivity of target organs to insulin,

❖ Agents which decrease the rate at which glucose is absorbed from the gastrointestinal tract.

Several groups of drugs, mostly given by mouth, are effective in Type II, often in combination. The therapeutic combination in Type II may include insulin, not necessarily because oral agents have failed completely, but in search of a desired combination of effects. The great advantage of injected insulin in Type II is that a well-educated patient can adjust the dose, or even take additional doses, when blood glucose levels measured by the patient, usually with a simple meter, as needed by the measured amount of sugar in the blood.

Insulin: Insulin is usually given subcutaneously, either by injections or by an insulin pump. Research is underway of other routes of administration. In acute care settings, insulin may also be given intravenously. There are several types of insulin, characterized by the rate which they are metabolized by the body.

SECRETAGOGUES

SULFONYLUREAS

Sulfonylureas were the first widely used oral hypoglycemic medications. They are *insulin secretagogues*, triggering insulin release by direct action on the K_{ATP} channel of the pancreatic beta cells. Eight types of these pills have been marketed in North America, but not all remain available. The "second-generation" drugs are now more commonly used. They are more effective than first-generation drugs and have fewer side effects. All may cause weight gain. Sulfonylureas bind strongly to plasma proteins. Sulfonylureas are only

useful in Type II diabetes, as they work by stimulating endogenous release of insulin. They work best with patients over 40 years old, who have had diabetes mellitus for under ten years. They can not be used with type I diabetes, or diabetes of pregnancy. They can be safely used with metformin or -glitazones. The primary side effect is hypoglycemia.

Typical reductions in A1C values for second generation sulfonylureas are 1.0-2.0 per cent.

- ❖ First-generation agents

  - ♦ Tolbutamide (Orinase)
  - ♦ Acetohexamide (Dymelor)
  - ♦ Tolazamide (Tolinase)
  - ♦ Chlorpropamide (Diabinese)

- ❖ Second-generation agents

  - ♦ Glipizide (Glucotrol)
  - ♦ Glyburide (Diabeta, Micronase, Glynase)
  - ♦ Glimepiride (Amaryl)
  - ♦ Gliclazide (Diamicron)

MEGLITINIDES

Meglitinides help the pancreas produce insulin and are often called "short-acting secretagogues." Their mode of action is original, affecting potassium channels. By closing the potassium channels of the pancreatic beta cells, they open the calcium channels, hence enhancing insulin secretion. They are taken with or shortly before meals to boost the insulin response to each meal. If a meal is skipped, the medication is also skipped.

Typical reductions in A1C values are 0.5-1.0 per cent.

❖ Repaglinide (Prandin)

❖ Nateglinide (Starlix)

Adverse reactions include weight gain and hypoglycemia.

SENSITIZERS

BIGUANIDES

Biguanides reduce hepatic glucose output and increase uptake of glucose by the periphery, including skeletal muscle. Although it must be used with caution in patients with impaired liver or kidney function, metformin, a biguanide, has become the most commonly used agent for type 2 diabetes in children and teenagers. Amongst common diabetic drugs, metformin is the only widely used oral drug that does not cause weight gain.

Typical reductions in A1C values for metformin is 1.5-2.0 per cent.

❖ *Metformin (Glucophage)*: Metformin may be the best choice for patients who also have heart failure. Should be temporarily discontinued before any radiographic procedure involving intravenous iodinated contrast as patients are at an increased risk of lactic acidosis.

❖ *Phenformin (DBI)*: Used from 1960s through 1980s, withdrawn due to lactic acidosis risk.

❖ *Buformin*: Also withdrawn due to lactic acidosis risk.

Metformin is usually the first-line medication used for treatment of type-2 diabetes. It is generally prescribed at initial diagnosis in conjunction with exercise and weight loss

as opposed to in the past, where Metformin was prescribed after diet and exercise had failed. Initial dosing is 500 mg once daily, then if need be increased to 500 mg twice daily up to 1000 mg twice daily. It is also available in combination with other oral diabetic medications.

There is an extended release formulation available, but it is typically reserved for patients experiencing GI side effects.

THIAZOLIDINEDIONES

Thiazolidinediones (TZDs), also known as "glitazones," bind to PPARā, a type of nuclear regulatory proteins involved in transcription of genes regulating glucose and fat metabolism.

These PPARs act on Peroxysome Proliferator Responsive Elements (PPRE). The PPREs influence insulin sensitive genes, which enhance production of mRNAs of insulin dependent enzymes. The final result is better use of glucose by the cells.

Typical reductions in A1C values are 1.5-2.0 per cent.

- ❖ Rosiglitazone (Avandia)
- ❖ Pioglitazone (Actos)
- ❖ Troglitazone (Rezulin): used in 1990s, withdrawn due to hepatitis and liver damage risk.

As a result of multiple retrospective studies, there is a concern about rosiglitazone's safety, although it is established that the group, as a whole, has beneficial effects on diabetes. The greatest concern is an increase in the number of severe cardiac events in patients taking it. The ADOPT study showed that initial therapy with drugs of this type may prevent the progression of disease, as did the DREAM trial. Concerns about the safety of rosiglitazone arose when a retrospective

meta-analysis was published in the *New England Journal of Medicine.* There have been a significant number of publications since then, and a Food and Drug Administration panel voted, with some controversy, 20:3 that available studies "supported a signal of harm," but voted 22:1 to keep the drug on the market.

The meta-analysis was not supported by an interim analysis of the trial designed to evaluate the issue, and several other reports have failed to conclude the controversy. This weak evidence for adverse effects has reduced the use of rosiglitazone, despite its important and sustained effects on glycemic control. Safety studies are continuing.

In contrast, at least one large prospective study, PROactive 05, has shown that pioglitazone may decrease the overall incidence of cardiac events in people with type II diabetes who have already had a heart attack.

4

Antibiotic Resistance

Antibiotic resistance is a specific type of drug resistance when a microorganism has the ability of withstanding the effects of antibiotics. Antibiotic resistance evolves via natural selection acting upon random mutation, but it can also be engineered by applying an evolutionary stress on a population. Once such a gene is generated, bacteria can then transfer the genetic information in a horizontal fashion (between individuals) by plasmid exchange.

If a bacterium carries several resistance genes, it is called multiresistant or, informally, a superbug. The term antimicrobial resistance is sometimes used to explicitly encompass organisms other than bacteria.

Antibiotic resistance can also be introduced artificially into a microorganism through transformation protocols. This can aid in implanting artificial genes into the microorganism. If

the resistance gene is linked with the gene to be implanted, the antibiotic can be used to kill off organisms that lack the new gene.

CAUSES

The widespread use of antibiotics both inside and outside of medicine is playing a significant role in the emergence of resistant bacteria. They are often used in animals but also in other industries which at least in the case of agricultural use lead to the spread of resistant strains to human populations.

In some countries antibiotics are sold over the counter without a prescription which compounds the problem. In human medicine the major problem of the emergence of resistant bacteria is due to misuse and overuse of antibiotics by doctors as well as patients. Other practices contributing towards resistance include the addition of antibiotics to the feed of livestock.

Household use of antibacterials in soaps and other products, although not clearly contributing to resistance, is also discouraged (as not being effective at infection control). Also unsound practices in the pharmaceutical manufacturing industry can contribute towards the likelihood of creating antibiotic resistant strains.

Certain antibiotic classes are highly associated with colonisation with superbugs compared to other antibiotic classes.

The risk for colonisation increases if there is a lack of sensitivity (resistance) of the superbugs to the antibiotic used and high tissue penetration as well as broad spectrum activity

against"good bacteria". In the case of MRSA, increased rates of MRSA infections are seen with glycopeptides, cephalosporins and especially quinolones. In the case of colonisation with C difficile the high risk antibiotics include cephalosporins and in particular quinolones and clindamycin.

IN MEDICINE

The volume of antibiotic prescribed is the major factor in increasing rates or bacterial resistance rather than compliance with antibiotics.

Inappropriate prescribing of antibiotics has been attributed to a number of causes including: people who insist on antibiotics, physicians simply prescribe them as they feel they do not have time to explain why they are not necessary, physicians who do not know when to prescribe antibiotics or else are overly cautious for medical legal reasons.

A third of people for example believe that antibiotics are effective for the common cold and 22% of people do not finish a course of antibiotics primarily due to that fact that they feel better (varying from 10% to 44% depending on the country). Compliance with once daily antibiotics is better than with twice daily antibiotics.

Sub optimum antibiotic concentrations in critically ill people increase the frequency of antibiotic resistance organisms. While taking antibiotics doses less than those recommended may increase rates of resistance, shortening the course of antibiotics may actually decrease rates of resistance. Poor hand hygiene by hospital staff has been associated with the spread of resistant organisms and an

increase in hand washing compliance results in decreased rates of these organisms.

ROLE OF OTHER ANIMALS

Drugs are used in animals that are used as human food, such as cows, pigs, chickens, fish, etc, and these drugs can affect the safety of the meat, milk, and eggs produced from those animals and can be the source of superbugs. For example, farm animals, particularly pigs, are believed to be able to infect people with MRSA.

The resistant bacteria in animals due to antibiotic exposure can be transmitted to humans via three pathways, those being through the consumption of meat, from close or direct contact with animals, or through the environment.

The World Health Organization concluded that antibiotics as growth promoters in animal feeds should be prohibited (in the absence of risk assessments).

In 1998, European Union health ministers voted to ban four antibiotics widely used to promote animal growth (despite their scientific panel's recommendations).

Regulation banning the use of antibiotics in European feed, with the exception of two antibiotics in poultry feeds, became effective in 2006. In Scandinavia, there's evidence that the ban has led to a lower prevalence of antimicrobial resistance in (non-hazardous) animal bacterial populations.

In the USA federal agencies do not collect data on antibiotic use in animals but animal to human spread of drug resistant organisms has been demonstrated in research studies. Antibiotics are still used in U.S. animal feed-along with other ingredients which have safety concerns.

Growing U.S. consumer concern about using antibiotics in animal feed has led to a niche market of"antibiotic-free" animal products, but this small market is unlikely to change entrenched industry-wide practices.

In 2001, the Union of Concerned Scientists estimated that greater than 70% of the antibiotics used in the US are given to food animals (e.g. chickens, pigs and cattle) in the absence of disease. In 2000 the US Food and Drug Administration (FDA) announced their intention to revoke approval of fluoroquinolone use in poultry production because of substantial evidence linking it to the emergence of fluoroquinolone resistant campylobacter infections in humans. The final decision to ban fluoroquinolones from use in poultry production was not made until five years later because of challenges from the food animal and pharmaceutical industries. Today, there are two federal bills (S. 549 and H.R. 962) aimed at phasing out"non-therapeutic" antibiotics in US food animal production.

MECHANISMS

The top section represents a population of bacteria before exposure to an antibiotic. The middle section shows the population directly after exposure, the phase in which selection took place. The last section shows the distribution of resistance in a new generation of bacteria. The legend indicates the resistance levels of individuals.

Antibiotic resistance can be a result of horizontal gene transfer, and also of unlinked point mutations in the pathogen genome and a rate of about 1 in 10^8 per chromosomal replication.
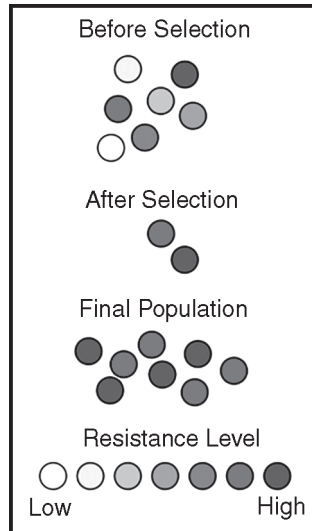
Fig. Schematic Representation of How Antibiotic Resistance Evolves Via Natural Selection.

The antibiotic action against the pathogen can be seen as an environmental pressure; those bacteria which have a mutation allowing them to survive will live on to reproduce. They will then pass this trait to their offspring, which will result in a fully resistant colony.

The four main mechanisms by which microorganisms exhibit resistance to antimicrobials are:

- *Drug inactivation or modification*: e.g. enzymatic deactivation of *Penicillin* G in some penicillin-resistant bacteria through the production of β-lactamases.

- *Alteration of target site*: e.g. alteration of PBP—the binding target site of penicillins—in MRSA and other penicillin-resistant bacteria.

- *Alteration of metabolic pathway*: e.g. some sulfonamide-resistant bacteria do not require para-aminobenzoic acid (PABA), an important precursor for the synthesis of folic acid and nucleic acids in

bacteria inhibited by sulfonamides. Instead, like mammalian cells, they turn to utilizing preformed folic acid.

- *Reduced drug accumulation*: By decreasing drug permeability and/or increasing active efflux (pumping out) of the drugs across the cell surface.

There are three known mechanisms of fluoroquinolone resistance. Some types of efflux pumps can act to decrease intracellular quinolone concentration. In gram-negative bacteria, plasmid-mediated resistance genes produce proteins that can bind to DNA gyrase, protecting it from the action of quinolones.

Finally, mutations at key sites in DNA gyrase or Topoisomerase IV can decrease their binding affinity to quinolones, decreasing the drug's effectiveness. Research has shown that the bacterial protein LexA may play a key role in the acquisition of bacterial mutations giving resistance to quinolones and rifampicin.

RESISTANT PATHOGENS

STAPHYLOCOCCUS AUREUS

Staphylococcus aureus (colloquially known as "Staph aureus" or a *Staph infection*) is one of the major resistant pathogens. Found on the mucous membranes and the human skin of around a third of the population, it is extremely adaptable to antibiotic pressure.

It was the first bacterium in which penicillin resistance was found—in 1947, just four years after the drug started being mass-produced. Methicillin was then the antibiotic of

choice, but has since been replaced by oxacillin due to significant kidney toxicity. MRSA (methicillin-resistant *Staphylococcus aureus*) was first detected in Britain in 1961 and is now "quite common" in hospitals. MRSA was responsible for 37% of fatal cases of blood poisoning in the UK in 1999, up from 4% in 1991. Half of all *S. aureus* infections in the US are resistant to penicillin, methicillin, tetracycline and erythromycin.

This left vancomycin as the only effective agent available at the time. However, strains with intermediate (4-8 ug/ml) levels of resistance, termed GISA (glycopeptide intermediate *Staphylococcus aureus*) or VISA (vancomycin intermediate *Staphylococcus aureus*), began appearing in the late 1990s.

The first identified case was in Japan in 1996, and strains have since been found in hospitals in England, France and the US. The first documented strain with complete (>16 ug/ml) resistance to vancomycin, termed VRSA (Vancomycin-resistant *Staphylococcus aureus*) appeared in the United States in 2002. A *new* class of antibiotics, oxazolidinones, became available in the 1990s, and the first commercially available oxazolidinone, linezolid, is comparable to vancomycin in effectiveness against MRSA. Linezolid-resistance in *Staphylococcus aureus* was reported in 2003.

CA-MRSA (Community-acquired MRSA) has now emerged as an epidemic that is responsible for rapidly progressive, fatal diseases including necrotizing pneumonia, severe sepsis and necrotizing fasciitis. Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most frequently identified antimicrobial drug-resistant pathogen in US hospitals. The epidemiology of infections caused by MRSA is rapidly changing. In the

past 10 years, infections caused by this organism have emerged in the community. The 2 MRSA clones in the United States most closely associated with community outbreaks, USA400 (MW2 strain, ST1 lineage) and USA300, often contain Panton-Valentine leukocidin (PVL) genes and, more frequently, have been associated with skin and soft tissue infections.

Outbreaks of community-associated (CA)-MRSA infections have been reported in correctional facilities, among athletic teams, among military recruits, in newborn nurseries, and among men who engage in frequent homosexual activities. CA-MRSA infections now appear to be endemic in many urban regions and cause most CA-S. aureus infections.

STREPTOCOCCUS AND ENTEROCOCCUS

Streptococcus pyogenes (Group A Streptococcus: GAS) infections can usually be treated with many different antibiotics. Early treatment may reduce the risk of death from invasive group A streptococcal disease.

However, even the best medical care does not prevent death in every case. For those with very severe illness, supportive care in an intensive care unit may be needed. For persons with necrotizing fasciitis, surgery often is needed to remove damaged tissue. Strains of S. *pyogenes* resistant to macrolide antibiotics have emerged, however all strains remain uniformly sensitive to penicillin.

Resistance of *Streptococcus pneumoniae* to penicillin and other beta-lactams is increasing worldwide. The major mechanism of resistance involves the introduction of mutations in genes encoding penicillin-binding proteins.

Selective pressure is thought to play an important role, and use of beta-lactam antibiotics has been implicated as a risk factor for infection and colonization. Streptococcus pneumoniae is responsible for pneumonia, bacteremia, otitis media, meningitis, sinusitis, peritonitis and arthritis.

Penicillin-resistant pneumonia caused by *Streptococcus pneumoniae* (commonly known as *pneumococcus*), was first detected in 1967, as was penicillin-resistant gonorrhea. Resistance to penicillin substitutes is also known as beyond *S. aureus*. By 1993 *Escherichia coli* was resistant to five fluoroquinolone variants.

Mycobacterium tuberculosis is commonly resistant to isoniazid and rifampin and sometimes universally resistant to the common treatments. Other pathogens showing some resistance include *Salmonella*, *Campylobacter*, and *Streptococci.*

Enterococcus faecium is another superbug found in hospitals. Penicillin-Resistant Enterococcus was seen in 1983, vancomycin-resistant enterococcus (VRE) in 1987, and Linezolid-Resistant Enterococcus (LRE) in the late 1990s.

PSEUDOMONAS AERUGINOSA

Pseudomonas aeruginosa is a highly prevalent opportunistic pathogen. One of the most worrisome characteristics of *P. aeruginosa* consists in its low antibiotic susceptibility.

This low susceptibility is attributable to a concerted action of multidrug efflux pumps with chromosomally-encoded antibiotic resistance genes (e.g. *mexAB-oprM*, *mexXY* etc) and the low permeability of the bacterial cellular envelopes.

Besides intrinsic resistance, *P. aeruginosa* easily develop acquired resistance either by mutation in chromosomally-encoded genes, or by the horizontal gene transfer of antibiotic resistance determinants. Development of multidrug resistance by *P. aeruginosa* isolates requires several different genetic events that include acquisition of different mutations and/or horizontal transfer of antibiotic resistance genes.

Hypermutation favours the selection of mutation-driven antibiotic resistance in *P. aeruginosa* strains producing chronic infections, whereas the clustering of several different antibiotic resistance genes in integrons favours the concerted acquisition of antibiotic resistance determinants.

Some recent studies have shown that phenotypic resistance associated to biofilm formation or to the emergence of small-colony-variants may be important in the response of *P. aeruginosa* populations to antibiotics treatment.

CLOSTRIDIUM DIFFICILE

Clostridium difficile is a nosocomial pathogen that causes diarrheal disease in hospitals world wide. Clindamycin-resistant *C. difficile* was reported as the causative agent of large outbreaks of diarrheal disease in hospitals in New York, Arizona, Florida and Massachusetts between 1989 and 1992. Geographically dispersed outbreaks of *C. difficile* strains resistant to fluoroquinolone antibiotics, such as Cipro (ciprofloxacin) and Levaquin (levofloxacin), were also reported in North America in 2005.

SALMONELLA AND E. COLI

E. coli and Salmonella come directly from contaminated food. Of the meat that is contaminated with E. coli, eighty

per cent of the bacteria are resistant to one or more drugs made; it causes bladder infections that are resistant to antibiotics ("HSUS Fact Sheet").

Salmonella was first found in humans in the 1970s and in some cases is resistant to as many as nine different antibiotics ("HSUS Fact Sheet"). When both bacterium are spread, serious health conditions arise. Many people are hospitalized each year after becoming infected, and some die as a result.

ACINETOBACTER BAUMANNII

On November 5, 2004, the Centers for Disease Control and Prevention (CDC) reported an increasing number of *Acinetobacter baumannii* bloodstream infections in patients at military medical facilities in which service members injured in the Iraq/Kuwait region during Operation Iraqi Freedom and in Afghanistan during Operation Enduring Freedom were treated. Most of these showed multidrug resistance (MRAB), with a few isolates resistant to all drugs tested.

ALTERNATIVES

PREVENTION

Rational use of antibiotics may reduce the chances of development of opportunistic infection by antibiotic-resistant bacteria due to dysbacteriosis. In one study the use of fluoroquinolones are clearly associated with *Clostridium difficile* infection, which is a leading cause of nosocomial diarrhea in the United States, and a major cause of death, worldwide. There is clinical evidence that topical dermatological preparations containing tea tree oil and thyme

oil may be effective in preventing transmittal of CA-MRSA. Vaccines do not suffer the problem of resistance because a vaccine enhances the body's natural defenses, while an antibiotic operates separately from the body's normal defenses. Nevertheless, new strains may evolve that escape immunity induced by vaccines.

While theoretically promising, anti-staphylococcal vaccines have shown limited efficacy, because of immunological variation between *Staphylococcus* species, and the limited duration of effectiveness of the antibodies produced. Development and testing of more effective vaccines is under way. The Australian Commonwealth Scientific and Industrial Research Organization (CSIRO), realizing the need for the reduction of antibiotic use, has been working on two alternatives. One alternative is to prevent diseases by adding cytokines instead of antibiotics to animal feed.

These proteins are made in the animal body "naturally" after a disease and are not antibiotics so they do not contribute to the antibiotic resistance problem. Furthermore, studies on using cytokines have shown that they also enhance the growth of animals like the antibiotics now used, but without the drawbacks of non-therapeutic antibiotic use.

Cytokines have the potential to achieve the animal growth rates traditionally sought by the use of antibiotics without the contribution of antibiotic resistance associated with the widespread non-therapeutic uses of antibiotics currently utilized in the food animal production industries. Additionally, CSIRO is working on vaccines for diseases.

PHAGE THERAPY

Phage therapy, an approach that has been extensively researched and utilized as a therapeutic agent for over 60 years, especially in the Soviet Union, is an alternative that might help with the problem of resistance. Phage therapy was widely used in the United States until the discovery of antibiotics, in the early 1940s. Bacteriophages or "phages" are viruses that invade bacterial cells and, in the case of lytic phages, disrupt bacterial metabolism and cause the bacterium to lyse. Phage therapy is the therapeutic use of lytic bacteriophages to treat pathogenic bacterial infections.

Bacteriophage therapy is an important alternative to antibiotics in the current era of multidrug resistant pathogens. A review of studies that dealt with the therapeutic use of phages from 1966–1996 and few latest ongoing phage therapy projects via internet showed: phages were used topically, orally or systemically in Polish and Soviet studies.

The success rate found in these studies was 80–95% with few gastrointestinal or allergic side effects. British studies also demonstrated significant efficacy of phages against *Escherichia coli*, *Acinetobacter* spp., *Pseudomonas* spp and *Staphylococcus aureus*. US studies dealt with improving the bioavailability of phage. Phage therapy may prove as an important alternative to antibiotics for treating multidrug resistant pathogens.

RESEARCH

NEW MEDICATIONS

Until recently, research and development (R&D) efforts have provided new drugs in time to treat bacteria that became

resistant to older antibiotics. That is no longer the case. The potential crisis at hand is the result of a marked decrease in industry R&D, and the increasing prevalence of resistant bacteria. Infectious disease physicians are alarmed by the prospect that effective antibiotics may not be available to treat seriously ill patients in the near future.

The pipeline of new antibiotics is drying up. Major pharmaceutical companies are losing interest in the antibiotics market because these drugs may not be as profitable as drugs that treat chronic (long-term) conditions and lifestyle issues.

The resistance problem demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibiotics. One of the possible strategies towards this objective is the rational localization of bioactive phytochemicals. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins. Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. In many cases, these substances serve as plant defence mechanisms against predation by microorganisms, insects, and herbivores.

Many of the herbs and spices used by humans to season food yield useful medicinal compounds including those having antibacterial activity.

Traditional healers have long used plants to prevent or cure infectious conditions. Many of these plants have been investigated scientifically for antimicrobial activity and a large number of plant products have been shown to inhibit growth

of pathogenic bacteria. A number of these agents appear to have structures and modes of action that are distinct from those of the antibiotics in current use, suggesting that cross-resistance with agents already in use may be minimal. For example the combination of 5'-methoxyhydnocarpine and berberine in herbs like Hydrastis canadensis and Berberis vulgaris can block the MDR-pumps that cause multidrug resistance. This has been shown for Staphylococcus aureus.

Archaeocins is the name given to a new class of potentially useful antibiotics that are derived from the Archaea group of organisms. Eight archaeocins have been partially or fully characterized, but hundreds of archaeocins are believed to exist, especially within the haloarchaea. The prevalence of archaeocins is unknown simply because no one has looked for them.

The discovery of new archaeocins hinges on recovery and cultivation of archaeal organisms from the environment. For example, samples from a novel hypersaline field site, Wilson Hot Springs, recovered 350 halophilic organisms; preliminary analysis of 75 isolates showed that 48 were archaeal and 27 were bacterial.

In research published on October 17, 2008 in *Cell*, a team of scientists pinpointed the place on bacteria where the antibiotic myxopyronin launches its attack, and why that attack is successful. The myxopyronin binds to and inhibits the crucial bacterial enzyme, RNA polymerase.

The myxopyronin changes the structure of the switch-2 segment of the enzyme, inhibiting its function of reading and transmitting DNA code. This prevents RNA polymerase from delivering genetic information to the ribosomes, causing the

bacteria to die. One of the major causes of antibiotic resistance is the decrease of effective drug concentrations at their target place, due to the increased action of ABC transporters. Since ABC transporter blockers can be used in combination with current drugs to increase their effective intracellular concentration, the possible impact of ABC transporter inhibitors is of great clinical interest.

ABC transporter blockers that may be useful to increase the efficacy of current drugs have entered clinical trials and are available to be used in therapeutic regimes.

APPLICATIONS

Antibiotic resistance is an important tool for genetic engineering. By constructing a plasmid which contains an antibiotic resistance gene as well as the gene being engineered or expressed, a researcher can ensure that when bacteria replicate, only the copies which carry along the plasmid survive. This ensures that the gene being manipulated passes along when the bacteria replicates.

The most commonly used antibiotics in genetic engineering are generally "older" antibiotics which have largely fallen out of use in clinical practice.

These include:
- Ampicillin
- Kanamycin
- Tetracycline
- Chloramphenicol

Industrially the use of antibiotic resistance is disfavored since maintaining bacterial cultures would require feeding them large quantities of antibiotics. Instead, the use of

auxotrophic bacterial strains (and function-replacement plasmids) is preferred.

THE ACQUISITION AND SPREAD OF ANTIBIOTIC RESISTANCE IN BACTERIA

The development of resistance is inevitable following the introduction of a new antibiotic. Initial rates of resistance to new drugs are normally on the order of 1%. However, modern uses of antibiotics have caused a huge increase in the number of resistant bacteria. In fact, within 8-12 years after widespread use, strains resistant to multiple drugs become widespread. Multiple drug resistant strains of some bacteria have reached the proportion that virtually no antibiotics are available for treatment. Antibiotic resistance in bacteria may be an inherent trait of the organism (*e.g.* a particular type of cell wall structure) that renders it naturally resistant, or it may be acquired by means of mutation in its own DNA or acquisition of resistance-conferring DNA from another source.

MANUFACTURE OF ANTIBIOTIC

Antibiotics are chemical substances that can inhibit the growth of, and even destroy, harmful microorganisms. They are derived from special microorganisms or other living systems, and are produced on an industrial scale using a fermentation process.

Although the principles of antibiotic action were not discovered until the twentieth century, the first known use of antibiotics was by the Chinese over 2,500 years ago. Today, over 10,000 antibiotic substances have been reported.

Currently, antibiotics represent a multibillion dollar industry that continues to grow each year.

Antibiotics are used in many forms-each of which imposes somewhat different manufacturing requirements. For bacterial infections on the skin surface, eye, or ear, an antibiotic may be applied as an ointment or cream. If the infection is internal, the antibiotic can be swallowed or injected directly into the body. In these cases, the antibiotic is delivered throughout the body by absorption into the bloodstream.

Antibiotics differ chemically so it is under-standable that they also differ in the types of infections they cure and the ways in which they cure them. Certain antibiotics destroy bacteria by affecting the structure of their cells. This can occur in one of two ways.

First, the antibiotic can weaken the cell walls of the infectious bacteria, which causes them to burst. Second, antibiotics can cause the contents of the bacterial cells to leak out by damaging the cell membranes. Another way in which antibiotics function is by interfering with the bacteria's metabolism. Some antibiotics such as tetracycline and erythromycin interfere with protein synthesis. Antibiotics like rifampin inhibit nucleic acid biosynthesis. Still other antibiotics, such as sulfonamide or trimethoprim have a general blocking effect on cell metabolism.

The commercial development of an antibiotic is a long and costly proposal. It begins with basic research designed to identify organisms, which produce antibiotic compounds. During this phase, thousands of species are screened for any sign of antibacterial action.

When one is found, the species is tested against a variety of known infectious bacteria. If the results are promising, the organism is grown on a large scale so the compound responsible for the antibiotic effect can be isolated.

This is a complex procedure because thousands of antibiotic materials have already been discovered. Often, scientists find that their new antibiotics are not unique. If the material passes this phase, further testing can be done.

This typically involves clinical testing to prove that the antibiotic works in animals and humans and is not harmful. If these tests are passed, the Food and Drug Administration (FDA) must then approve the antibiotic as a new drug. This whole process can take many years.

The large-scale production of an antibiotic depends on a fermentation process. During fermentation, large amounts of the antibiotic-producing organism are grown. During fermentation, the organisms produce the antibiotic material, which can then be isolated for use as a drug.

For a new antibiotic to be economically feasible, manufacturers must be able to get a high yield of drug from the fermentation process, and be able to easily isolate it. Extensive research is usually required before a new antibiotic can be commercially scaled up.

HISTORY

While our scientific knowledge of antibiotics has only recently been developed, the practical application of antibiotics has existed for centuries. The first known use was by the Chinese about 2,500 years ago. During this time, they discovered that applying the moldy curd of soybeans to

infections had certain therapeutic benefits. It was so effective that it became a standard treatment.

Evidence suggests that other cultures used antibiotic-type substances as therapeutic agents. The Sudanese-Nubian civilization used a type of tetracycline antibiotic as early as 350 A.D.

In Europe during the Middle Ages, crude plant extracts and cheese curds were also used to fight infection. Although these cultures used antibiotics, the general principles of antibiotic action were not understood until the twentieth century.

The development of modern antibiotics depended on a few key individuals who demonstrated to the world that materials derived from microorganisms could be used to cure infectious diseases. One of the first pioneers in this field was Louis Pasteur. In 1877, he and an associate discovered that the growth of disease-causing anthrax bacteria could be inhibited by a saprophytic bacteria.

They showed that large amounts of anthrax bacilli could be given to animals with no adverse affects as long as the saprophytic bacilli were also given. Over the next few years, other observations supported the fact that some bacterially derived materials could prevent the growth of disease-causing bacteria.

In 1928, Alexander Fleming made one of the most important contributions to the field of antibiotics. In an experiment, he found that a strain of green *Penicillium* mold inhibited the growth of bacteria on an agar plate. This led to the development of the first modern era antibiotic, penicillin. A few years later in 1932, a paper was published which

suggested a method for treating infected wounds using a penicillin preparation. Although these early samples of penicillin were functional, they were not reliable and further refinements were needed. These improvements came in the early 1940s when Howard Florey and associates discovered a new strain of *Penicillium,* which produced high yields of penicillin. This allowed large-scale production of penicillin, which helped launch the modern antibiotics industry.

After the discovery of penicillin, other antibiotics were sought. In 1939, work began on the isolation of potential antibiotic products from the soil bacteria streptomyces. It was around this time that the term antibiotic was introduced. Selman Waxman and associates discovered streptomycin in 1944.

Subsequent studies resulted in the discovery of a host of new, different antibiotics including actinomycin, streptothricin, and neomycin all produced by *Streptomyces.* Other antibiotics that have been discovered since include bacitracin, polymyxin, viomycin, chloramphenicol and tetracyclines. Since the 1970s, most new antibiotics have been synthetic modifications of naturally occurring antibiotics.

RAW MATERIALS

The compounds that make the fermentation broth are the primary raw materials required for antibiotic production. This broth is an aqueous solution made up of all of the ingredients necessary for the proliferation of the microorganisms. Typically, it contains a carbon source like molasses, or soy meal, both of which are made up of lactose and glucose

sugars. These materials are needed as a food source for the organisms. Nitrogen is another necessary compound in the metabolic cycles of the organisms. For this reason, an ammonia salt is typically used. Additionally, trace elements needed for the proper growth of the antibiotic-producing organisms are included. These are components such as phosphorus, sulfur, magnesium, zinc, iron, and copper introduced through water soluble salts. To prevent foaming during fermentation, anti-foaming agents such as lard oil, octadecanol, and silicones are used.

THE MANUFACTURING PROCESS

Although most antibiotics occur in nature, they are not normally available in the quantities necessary for large-scale production. For this reason, a fermentation process was developed. It involves isolating a desired microorganism, fueling growth of the culture and refining and isolating the final antibiotic product. It is important that sterile conditions be maintained throughout the manufacturing process, because contamination by foreign microbes will ruin the fermentation.

STARTING THE CULTURE

- Before fermentation can begin, the desired antibiotic-producing organism must be isolated and its numbers must be increased by many times. To do this, a starter culture from a sample of previously isolated, cold-stored organisms is created in the lab. In order to grow the initial culture, a sample of the organism is transferred to an agar-containing plate. The initial culture is then put into shake flasks along

with food and other nutrients necessary for growth. This creates a suspension, which can be transferred to seed tanks for further growth.

- The seed tanks are steel tanks designed to provide an ideal environment for growing microorganisms. They are filled with the all the things the specific microorganism would need to survive and thrive, including warm water and carbohydrate foods like lactose or glucose sugars. Additionally, they contain other necessary carbon sources, such as acetic acid, alcohols, or hydrocarbons, and nitrogen sources like ammonia salts. Growth factors like vitamins, amino acids, and minor nutrients round out the composition of the seed tank contents. The seed tanks are equipped with mixers, which keep the growth medium moving, and a pump to deliver sterilized, filtered air. After about 24-28 hours, the material in the seed tanks is transferred to the primary fermentation tanks.

FERMENTATION

- The fermentation tank is essentially a larger version of the steel, seed tank, which is able to hold about 30,000 gallons. It is filled with the same growth media found in the seed tank and also provides an environment inducive to growth. Here the microorganisms are allowed to grow and multiply. During this process, they excrete large quantities of the desired antibiotic. The tanks are cooled to keep the temperature between 73-81° F (23-27.2 ° C). It

is constantly agitated, and a continuous stream of sterilized air is pumped into it. For this reason, anti-foaming agents are periodically added. Since pH control is vital for optimal growth, acids or bases are added to the tank as necessary.

ISOLATION AND PURIFICATION

- After three to five days, the maximum amount of antibiotic will have been produced and the isolation process can begin. Depending on the specific antibiotic produced, the fermentation broth is processed by various purification methods. For example, for antibiotic compounds that are water soluble, an ion-exchange method may be used for purification. In this method, the compound is first separated from the waste organic materials in the broth and then sent through equipment, which separates the other water-soluble compounds from the desired one. To isolate an oil-soluble antibiotic such as penicillin, a solvent extraction method is used. In this method, the broth is treated with organic solvents such as butyl acetate or methyl isobutyl ketone, which can specifically dissolve the antibiotic. The dissolved antibiotic is then recovered using various organic chemical means. At the end of this step, the manufacturer is typically left with a purified powdered form of the antibiotic, which can be further refined into different product types.

REFINING

- Antibiotic products can take on many different forms. They can be sold in solutions for intravenous bags

or syringes, in pill or gel capsule form, or they may be sold as powders, which are incorporated into topical ointments. Depending on the final form of the antibiotic, various refining steps may be taken after the initial isolation. For intravenous bags, the crystalline antibiotic can be dissolved in a solution, put in the bag, which is then hermetically sealed. For gel capsules, the powdered antibiotic is physically filled into the bottom half of a capsule then the top half is mechanically put in place. When used in topical ointments, the antibiotic is mixed into the ointment.

- From this point, the antibiotic product is transported to the final packaging stations. Here, the products are stacked and put in boxes. They are loaded up on trucks and transported to various distributors, hospitals, and pharmacies. The entire process of fermentation, recovery, and processing can take anywhere from five to eight days.

QUALITY CONTROL

Quality control is of utmost importance in the production of antibiotics. Since it involves a fermentation process, steps must be taken to ensure that absolutely no contamination is introduced at any point during production. To this end, the medium and all of the processing equipment are thoroughly steam sterilized. During manufacturing, the quality of all the compounds is checked on a regular basis. Of particular importance are frequent checks of the condition of the microorganism culture during fermentation.

These are accomplished using various chromatography techniques. Also, various physical and chemical properties of the finished product are checked such as pH, melting point, and moisture content. In the United States, antibiotic production is highly regulated by the Food and Drug Administration (FDA). Depending on the application and type of antibiotic, more or less testing must be completed. For example, the FDA requires that for certain antibiotics each batch must be checked by them for effectiveness and purity. Only after they have certified the batch can it be sold for general consumption.

THE FUTURE

Since the development of a new drug is a costly proposition, pharmaceutical companies have done very little research in the last decade. However, an alarming development has spurred a revived interest in the development of new antibiotics.

It turns out that some of the disease-causing bacteria have mutated and developed a resistance to many of the standard antibiotics. This could have grave consequences on the world's public health unless new antibiotics are discovered or improvements are made on the ones that are available. This challenging problem will be the focus of research for many years to come.

ANTIBIOTIC RESISTANCE

The emergence of antibiotic resistance is an evolutionary process that is based on selection for organisms that have enhanced ability to survive doses of antibiotics that would have previously been lethal.

Antibiotics like Penicillin and Erythromycin which used to be one-time miracle cures are now less effective because bacteria have become more resistant. Antibiotics themselves act as a selective pressure which allows the growth of resistant bacteria within a population and inhibits susceptible bacteria.

Antibiotic selection of pre-existing antibiotic resistant mutants within bacterial populations was demonstrated in 1943 by the Luria-Delbrück experiment. Survival of bacteria often results from an inheritable resistance.

Any antibiotic resistance may impose a biological cost and the spread of antibiotic resistant bacteria may be hampered by the reduced fitness associated with the resistance which proves disadvantageous for survival of the bacteria when antibiotic is not present. Additional mutations, however, may compensate for this fitness cost and aids the survival of these bacteria.

The underlying molecular mechanisms leading to antibiotic resistance can vary. Intrinsic resistance may naturally occur as a result of the bacteria's genetic makeup. The bacterial chromosome may fail to encode a protein which the antibiotic targets.

Acquired resistance results from a mutation in the bacterial chromosome or the acquisition of extra-chromosomal DNA. Antibiotic-producing bacteria have evolved resistance mechanisms which have been shown to be similar to and may have been transferred to antibiotic resistant strains.

The spread of antibiotic resistance mechanisms occurs through vertical transmission of inherited mutations from previous generations and genetic recombination of DNA by

horizontal genetic exchange. Antibiotic resistance is exchanged between different bacteria by plasmids that carry genes which encode antibiotic resistance which may result in co-resistance to multiple antibiotics.

These plasmids can carry different genes with diverse resistance mechanisms to unrelated antibiotics but because they are located on the same plasmid multiple antibiotic resistance to more than one antibiotic is transferred. Alternatively, cross-resistance to other antibiotics within the bacteria results when the same resistance mechanism is responsible for resistance to more than one antibiotic is selected for.

ANTIBIOTIC MISUSE

Inappropriate antibiotic treatment and overuse of antibiotics have been a contributing factor to the emergence of resistant bacteria. The problem is further exacerbated by self-prescribing of antibiotics by individuals without the guidelines of a qualified clinician and the non-therapeutic use of antibiotics as growth promoters in agriculture.

Antibiotics are frequently prescribed for indications in which their use is not warranted, an incorrect or sub-optimal antibiotic is prescribed or in some cases for infections likely to resolve without treatment.The overuse of antibiotics like penicillin and erythromycin which used to be one-time miracle cures were associated with emerging resistance since the 1950s.

Therapeutic usage of antibiotics in hospitals has been seen to be associated with increases in multi-antibiotic resistant bacteria.

Common forms of antibiotic misuse include excessive use of prophylactic antibiotics in travelers, failure to take into account the patient's weight and history of prior antibiotic use when prescribing, since both can strongly affect the efficacy of an antibiotic prescription, failure to take the entire prescribed course of the antibiotic, failure to prescribe or take the course of treatment at fairly precise correct daily intervals (e.g."every 8 hours" rather than merely"3x per day"), or failure to rest for sufficient recovery to allow clearance of the infecting organism.

These practices may facilitate the development of bacterial populations with antibiotic resistance. Inappropriate antibiotic treatment is another common form of antibiotic misuse. A common example is the prescription and use of antibiotics to treat viral infections such as the common cold that have no effect.

One study on respiratory tract infections found"physicians were more likely to prescribe antibiotics to patients who they believed expected them, although they correctly identified only about 1 in 4 of those patients". Multifactorial interventions aimed at both physicians and patients can reduce inappropriate prescribing of antibiotics.

Delaying antibiotics for 48 hours while observing for spontaneous resolution of respiratory tract infections may reduce antibiotic usage; however, this strategy may reduce patient satisfaction.

Several organizations concerned with antimicrobial resistance are lobbying to improve the regulatory climate. Approaches to tackling the issues of misuse and overuse of antibiotics by the establishment of the U.S.

Interagency Task Force on Antimicrobial Resistance which aims actively address the problem antimicrobial resistance are being organised and coordinated by the US Centers for Disease Control and Prevention, the Food and Drug Administration (FDA), and the National Institutes of Health (NIH) and also includes several other federal agencies.

An NGO campaign group is Keep Antibiotics Working. In France, an"Antibiotics are not automatic" government campaign starting in 2002 led to a marked reduction of unnecessary antibiotic prescriptions, especially in children.

In the United Kingdom, there are NHS posters in many doctors surgeries indicating that'unfortunately, no amount of antibiotics will get rid of your cold', following on from many patients specifically requesting antibiotics from their doctor inappropriately, believing they will help treat viral infections.

In agriculture, associated antibiotic resistance with the non-therapeutic use of antibiotics as growth promoters in animals resulted in their restricted use in the UK in the 1970.

Currently there is a EU wide ban on the non-therapeutic use of antibiotics as growth promoters. It is estimated that greater than 70% of the antibiotics used in U.S. are given to feed animals (e.g. chickens, pigs and cattle) in the absence of disease.

Antibiotic use in food animal production has been associated with the emergence of antibiotic-resistant strains of bacteria including Salmonella spp., Campylobacter spp.,

Escherichia coli, and Enterococcus spp. Evidence from some US and European studies suggest that these resistant bacteria cause infections in humans that do not respond to commonly prescribed antibiotics. In response to these

practices and attendant problems, several organizations (e.g. The American Society for Microbiology (ASM), American Public Health Association (APHA) and the American Medical Association (AMA)) have called for restrictions on antibiotic use in food animal production and an end to all non-therapeutic uses. However, delays in regulatory and legislative actions to limit the use of antibiotics are common, and may include resistance to these changes by industries using or selling antibiotics, as well as time spent on research to establish causal links between antibiotic use and emergence of untreatable bacterial diseases. Two federal bills (S.742 and H.R. 2562) aimed at phasing out non-therapeutic antibiotics in US food animal production were proposed but not passed. These bills were endorsed by public health and medical organizations including the American Holistic Nurses' Association, the American Medical Association, and the American Public Health Association (APHA). The EU has banned the use of antibiotics as growth promotional agents since 2003.

RESISTANCE MODIFYING AGENTS

One solution to combat resistance currently being researched is the development of pharmaceutical compounds that would revert multiple antibiotic resistance. These so called resistance modifying agents may target and inhibit MDR mechanisms, rendering the bacteria susceptible to antibiotics to which they were previously resistant.

These compounds targets include among others:

- Efflux inhibition(Phe-Arg-β-naphthylamide)
- Beta Lactamase inhibitors - Including Clavulanic acid and Sulbactam

BEYOND ANTIBIOTICS: TREATING NON-BACTERIAL INFECTIONS

The comparative ease of identifying compounds which safely cured bacterial infections was more difficult to duplicate in treatments of fungal and viral infections. Antibiotic research led to great strides in the knowledge of biochemistry, establishing large differences between the cellular and molecular physiology of the bacterial cell and that of the mammalian cell. This explained the observation that many compounds that are toxic to bacteria are non-toxic to human cells. In contrast, the basic biochemistries of the fungal cell and the mammalian cell are much more similar. This restricts the development and use of therapeutic compounds that attack a fungal cell, while not harming mammalian cells.

Similar problems exist in antibiotic treatments of viral diseases. Human viral metabolic biochemistry is very closely similar to human biochemistry, and the possible targets of antiviral compounds are restricted to very few components unique to a mammalian virus.

5

## Chemistry of Antibiotics

METHODS OF DRUG DISCOVERY

The discovery of new drugs is exemplified in the above sections on antibiotics and analgesics. The procedure is much more refined now than nearly a hundred years ago, but the steps in discovering new drugs or lead compounds to drugs are still similar.

DRUG SCREENING

Perhaps the largest effort of the pharmaceutical industry is the search for new drugs. Many compounds from many sources, both natural and synthetic, are tested for effectiveness against many types of medical problems. Screening requires many involved tests in both test tubes and in live subjects. Analysts need to be very astute in their observations in order not to miss a new drug or to improperly evaluate an effective drug. Once a compound is found to be an effective medicinal agent, then it is the lead compound. New compounds are

synthesized in the laboratory in order to find perhaps a better medicine or one with less toxicity. The process takes many years of intense work. Some examples of compounds found in natural sources have been seen as the antibiotics or the analgesics. Scientists then follow up on the lead compounds and attempt to synthesize better compounds. An older drug, ephedrine, was discovered in 1924 from the herbs of Chinese medicine as a treatment for breathing problems such as asthma. Medicinal chemists have modified the structure to provide a wide base of bronchodilator agents.
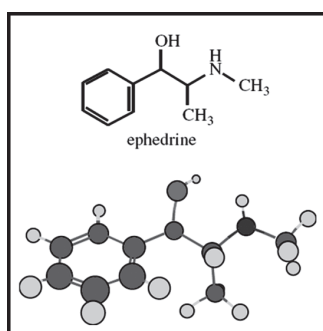
Taxol, is a very complicated substance found in the California yew tree bark. It is a very important new anti-cancer treatment, but it is in very short supply. Chemists are very active in the synthesis of taxol and related compounds in order to produce more effective and more available materials.
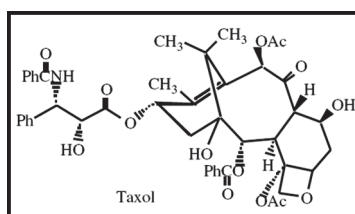
Other interesting drugs have been synthesized in the laboratory but their effectiveness as medicines was not

discovered immediately. AZT, an important agent against HIV, was first prepared many years ago but was later screened as an anti-HIV agent because the AIDS virus was discovered after AZT was prepared.

3"-adido-3'-deoxythymidine
AZT
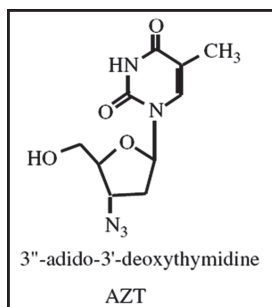
Librium, an important tranquilizer and somewhat abused drug, was tested two years after its first laboratory synthesis because it was not originally thought to be an important structure. As a lead compound Librium has led to several other important tranquilizers.
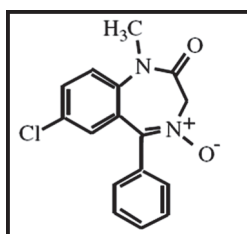
The examples shown above serve to illustrate the beginning of a process. Many other cases are known where drug discovery is based on unusual natural products, or materials based on herbal remedies, or from fortuitous accidents.

# COMPUTATIONAL METHODS

HANSCH EQUATION

A computational method to predict drug effectiveness based on physical properties instead of molecular structure was

introduced by Hansch in 1960. In this method parameters on the hydrophobic (water dislike) character of the compound (P), the hydrophobic contributions of the substituents (p), electronic characteristics of the substituents (s, known as Hammett constants) and steric factors (Es, known as Taft constants) are correlated with drug effectiveness. The general Hansch equation is shown below.

$$\text{drug effectiveness} = k_1 \log P + k_2\, p + k_3\, s + k_4\, E_s + k_5$$

The k values indicate the significance of each term. When applied to a number phenethylamine compounds related to ephedrine, the drug effectiveness was found to depend only on substituent (X and Y) hydrophobic and electronic properties.
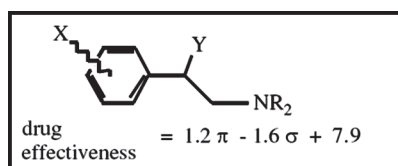
The equation says that compounds with highly hydrophobic (positive p values) and electron donation (negative s values) will prove to have greater effectiveness.

The Hansch equation for the above compounds is relatively simple, but for some drugs a very complicated relationship is observed. The Hansch study of drugs requires that many compounds be evaluated and correlated with the various parameters, not a simple task.

MOLECULAR MODELING

Modern high-speed computers have the capability of carrying out many complicated calculations in a very short time. The structural parameters for a specific protein receptor

(or enzyme) can be placed in a computer along with structural parameters for a drug molecule (real or hypothetical) and the computer will calculate and graph the fit of the drug with the protein active site.

The molecules may be manipulated in three dimensions to enhance the view and show the best fit of the "docking" of the molecules. Such molecular modeling can be used to predict the structure and effectiveness of new medicinal agents. Below is a model of captopril, a blood pressure medication.

This model is produced by drawing the structure with a ChemDraw programme, and placing it into a Chem 3D programme.

The structure is presented in colour and may be manipulated in 3D with the programme. Examination of the structure when one has the shape of the enzyme active site can give information about the desired location of substituents.

Captopril
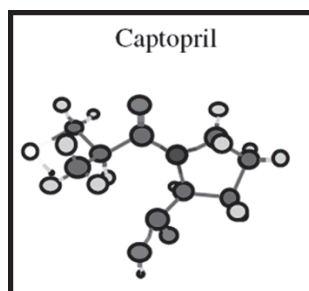
SUCCESSFUL DRUGS

Medicines have been developed to treat almost every disease. The development of medicinal agents takes many years of intense work by many scientists skilled in different fields. A few of the commonly prescribed medications are shown below.
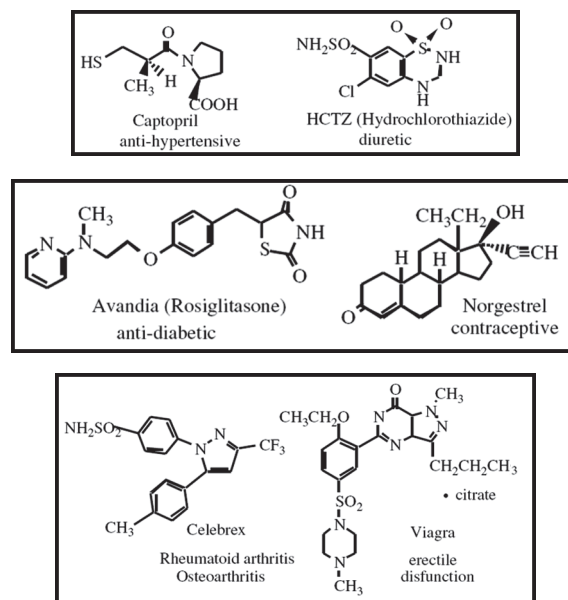
Although various treatments for diseases have been used for centuries, the early history of chemotherapy, chemical treatment of disease, started with *Paul Ehrlich in the 1900′ and his discovery of antibiotic compounds.*

Many antibiotics with a wide variety of applications and structures are now known. Drugs known as p*rodrugs* that release the true drug have become common through the studies of the mechanism of drug action.

The *opiate family* of drugs is used in the treatment of pain. Although, many are highly addictive, the systematic study of their mode of action has led to a modern approach for drug discovery. Now known drugs are modified according to structural features thought to be responsible for the drug activity. The wellknown *β-phenethyl amine structural unit of opiates* is identified in many structures with known chemotherapeutic properties. Drugs are often *discovered by extensive screening tests* of materials synthesized in the laboratory or obtained from natural sources.

Studies are then conducted to gain information on the *mechanism of the drug action.* Drug *interactions with enzymes* as substrates or inhibitors then lead to the structural features required in designing new drugs.

The drug structure is modified synthetically according to the drug mechanism in order to provide new drug molecules. *Computational methods* are used to show the correlation of drug structure with physical and biochemical properties of the drug, and to determine the efficacy of the drug.

The computations lead to drug modifications for changing the required properties. Computers are often used to *model drug chemistry.* Computer programs will allow the observation in *three-dimensions of a drug interaction with a protein, or permit the determination of the stereochemistry of the drug.*

CLASSIFICATIONS OF ANTIBIOTIC

Chemical substance that in dilute solutions can inhibit the growth of microorganisms or destroy them with little or no harm to the infected host.

Early antibiotics were natural microbial products, but chemists have modified the structures of many to produce semisynthetic and even wholly synthetic ones. Since the discovery of penicillin (1928), antibiotics have revolutionized the treatment of bacterial, fungal, and some other diseases.

They are produced by many actinomycetes (e.g., streptomycin, tetracycline) and other bacteria (e.g., polypeptides such as bacitracin) and by fungi (e.g., penicillin). Antibiotics may be broad-spectrum (active against a wide range of pathogens) or specific (active against one, or one class). Drawbacks include activity against beneficial

microorganisms, often causing diarrhea; allergies; and development of drug-resistant strains of the targeted microorganisms.

Antibiotics may be informally defined as the sub-group of anti-infectives that are derived from bacterial sources and are used to treat bacterial infections. Other classes of drugs, most notably the sulfonamides, may be effective antibacterials. Similarly, some antibiotics may have secondary uses, such as the use of demeclocycline (Declomycin, a tetracycline derivative) to treat the syndrome of inappropriate antidiuretic hormone (SIADH) secretion. Other antibiotics may be useful in treating protozoal infections.

Although there are several classification schemes for antibiotics, based on bacterial spectrum (broad versus narrow) or route of administration (injectable versus oral versus topical), or type of activity (bactericidal vs. bacteriostatic), the most useful is based on chemical structure.

Antibiotics within a structural class will generally show similar patterns of effectiveness, toxicity, and allergic potential.

- *Penicillins*: The penicillins are the oldest class of antibiotics, and have a common chemical structure which they share with the cephalopsorins. The two groups are classed as the beta-lactam antibiotics, and are generally bacteriocidal—that is, they kill bacteria rather than inhibiting growth. The penicillins can be further subdivided. The natural pencillins are based on the original penicillin G structure;

penicillinase-resistant penicillins, notably methicillin and oxacillin, are active even in the presence of the bacterial enzyme that inactivates most natural penicillins. Aminopenicillins such as ampicillin and amoxicillin have an extended spectrum of action compared with the natural penicillins; extended spectrum penicillins are effective against a wider range of bacteria. These generally include coverage for *Pseudomonas aeruginaosa* and may provide the penicillin in combination with a penicillinase inhibitor.

- *Cephalosporins*: Cephalosporins and the closely related cephamycins and carbapenems, like the pencillins, contain a beta-lactam chemical structure. Consequently, there are patterns of cross-resistance and cross-allergenicity among the drugs in these classes. The "cepha" drugs are among the most diverse classes of antibiotics, and are themselves subgrouped into 1st, 2nd and 3rd generations. Each generation has a broader spectrum of activity than the one before. In addition, cefoxitin, a cephamycin, is highly active against anaerobic bacteria, which offers utility in treatment of abdominal infections. The 3rd generation drugs, cefotaxime, ceftizoxime, ceftriaxone and others, cross the blood-brain barrier and may be used to treat meningitis and encephalitis. Cephalopsorins are the usually preferred agents for surgical prophylaxis.

- *Fluroquinolones*: The fluroquinolones are synthetic antibacterial agents, and not derived from bacteria.

They are included here because they can be readily interchanged with traditional antibiotics. An earlier, related class of antibacterial agents, the quinolones, was not well absorbed, and could be used only to treat urinary tract infections.

The fluroquinolones, which are based on the older group, are broad-spectrum bacteriocidal drugs that are chemically unrelated to the penicillins or the cephaloprosins. They are well distributed into bone tissue, and so well absorbed that in general they are as effective by the oral route as by intravenous infusion.

- *Tetracyclines*: Tetracyclines got their name because they share a chemical structure that has four rings. They are derived from a species of *Streptomyces* bacteria. Broad-spectrum bacteriostatic agents, the tetracyclines may be effective against a wide variety of microorganisms, including rickettsia and amebic parasites.

- *Macrolides*: The macrolide antibiotics are derived from *Streptomyces* bacteria, and got their name because they all have a macrocyclic lactone chemical structure. Erythromycin, the prototype of this class, has a spectrum and use similar to penicillin. Newer members of the group, azithromycin and clarithyr-omycin, are particularly useful for their high level of lung penetration. Clarithromycin has been widely used to treat *Helicobacter pylori* infections, the cause of stomach ulcers.

- *Others*: Other classes of antibiotics include the aminoglycosides, which are particularly useful for their effectiveness in treating *Pseudomonas aeruginosa* infections; the lincosamindes, clindamycin and lincomycin, which are highly active against anaerobic pathogens. There are other, individual drugs which may have utility in specific infections.

RECOMMENDED DOSAGE

Dosage varies with drug, route of administration, pathogen, site of infection, and severity. Additional considerations include renal (kidney) function, age of patient, and other factors. Patients should consult manufac turers' recommendations or ask their doctors.

SIDE EFFECTS

All antibiotics cause risk of overgrowth by non-susceptible bacteria. Manufacturers list other major hazards by class; however, the health care provider should review each drug individually to assess the degree of risk.

Generally, breastfeeding is not recommended while taking antibiotics because of risk of alteration to infant's intestinal flora, and risk of masking infection in the infant. Excessive or inappropriate use may promote growth of resistant pathogens.

- *Penicillins*: Hypersensitivity may be common, and cross allergenicity with cephalosporins has been reported. Penicillins are classed as category B during pregnancy.

- *Cephalosporins*: Several cephalosporins and related compounds have been associated with seizures.

Cefmetazole, cefoperazone, cefotetan and ceftriaxone may be associated with a fall in prothrombin activity and coagulation abnormalities. Pseudomembranous colitis (inflammation of the colon) has been reported with cephalosporins and other broad spectrum antibiotics. Some drugs in this class may cause renal toxicity. Pregnancy category B.

- *Fluoroquinolones*: Lomefloxacin has been associated with increased photosensitivity. All drugs in this class have been associated with convulsions. Pregnancy category C.

- *Tetracyclines*: Demeclocycline may cause increased photosensitivity. Minocycline may cause dizziness. hildren under the age of eight should not use tetracyclines, and specifically during periods of tooth development. Oral tetracyclines bind to anions such as calcium and iron. Although doxycycline and minocycline may be taken with meals, patients are advised to take other tetracycline antibiotics on an empty stomach, and not to take the drugs with milk or other calcium-rich foods. Expired tetracycline should never be administered. Pregnancy category D; use during pregnancy may cause alterations in bone development.

- *Macrolides*: Erythromycin may aggravate the weakness of patients with myasthenia gravis. Azithromycin has, rarely, been associated with allergic reactions, including angioedema, anaphylaxis, and dermatologic reactions, including Stevens-Johnson syndrome and toxic epidermal necrolysis.

Oral erythromycin may be highly irritating to the stomach and may cause severe phlebitis (inflammation of the vein) when given by injection. These drugs should be used with caution in patients with liver dysfunction. Pregnancy category B: Azithromycin, erythromycin. Pregnancy category C: Clarithromycin, dirithromycin, troleandomycin.

- *Aminoglycosides*: This class of drugs causes kidney and hearing problems. These problems can occur even with normal doses. Dosing should be based on renal function, with periodic testing of both kidney function and hearing. Pregnancy category D.

INTERACTIONS

Use of all antibiotics may temporarily reduce the effectiveness of birth control pills; alternative birth control methods should be used while taking these medications. Antacids should be avoided while on tetracyclines as the calcium can impair absorption of this antibiotic class.

For this reason, tetracyclines should not be taken just before or after consuming foods rich in calcium or iron. Consult specialized references for additional interactions to specific antibiotics.

RECOMMENDED USAGE

To minimize risk of adverse reactions and development of resistant strains of bacteria, antibiotics should be restricted to use in cases where there is either known or a reasonable presumption of bacterial infection. The use of antibiotics in viral infections is to be avoided. Avoid use of fluroquinolones for trivial infections. In severe infections, presumptive therapy

with a broad-spectrum antibiotic such as a third generation cephalosporin may be appropriate. Treatment should be changed to a narrow spectrum agent as soon as the pathogen has been identified. After 48 hours of treatment, if there is clinical improvement, an oral antibiotic should be considered.

When the pathogen is known or suspected to be *Pseudomonas,* a suitable beta-lactam drug is often prescribed in combination with an aminoglycoside. A single agent cannot be relied upon for treatment of *Pseudomonas.* When the patient has renal insufficiency, azactam should be considered in place of the aminoglycoside. In treatment of children with antibiotic suspensions, caregivers should be instructed in use of oral syringes or measuring teaspoons. Household teaspoons are not standardized and will give unreliable doses.

ANTIMICROBIAL PHARMACODYNAMICS

The assessment of the activity of an antibiotic is crucial to the successful outcome of antimicrobial therapy. Non-microbiological factors such as host defence mechanisms, the location of an infection, underlying disease as well as the intrinsic pharmacokinetic and pharmacodynamic properties of the antibiotic.

Fundamentally, antibiotics are classified as either having lethal or bactericidal action against bacteria or are bacteriostatic, preventing bacterial growth. The bactericidal activity of antibiotics may be growth phase dependent and in most but not all cases the action of many bactericidal antibiotics requires ongoing cell activity and cell division for the drugs' killing activity.

These classifications are based on laboratory behaviour; in practice, both of these are capable of ending a bacterial infection.

'In vitro' characterisation of the action of antibiotics to evaluate activity measure the minimum inhibitory concentration and minimum bactericidal concentration of an antimicrobial and are excellent indicators of antimicrobial potency.

However, in clinical practice, these measurements alone are insufficient to predict clinical outcome. By combining the pharmacokinetic profile of an antibiotic with the antimicrobial activity, several pharmacological parameters appear to be significant markers of drug efficacy.

The activity of antibiotics may be concentration-dependent and their characteristic antimicrobial activity increases with progressively higher antibiotic concentrations. They may also be time-dependent, where their antimicrobial activity does not increase with increasing antibiotic concentrations; however, it is critical that a minimum inhibitory serum concentration is maintained for a certain length of time.

A laboratory evaluation of the killing kinetics of the antibiotic using kill curves is useful to determine the time- or concentration-dependence of actimicrobial activity.

ANTIBIOTIC CLASSES

Antibiotics are commonly classified based on their mechanism of action, chemical structure or spectrum of activity. Most antibiotics target bacterial functions or growth processes.

Antibiotics which target the bacterial cell wall (penicillins, cephalosporins), or cell membrane (polymixins), or interfere

with essential bacterial enzymes (quinolones, sulfonamides) usually are bactericidal in nature. Those which target protein synthesis, such as the aminoglycosides, macrolides and tetracyclines, are usually bacteriostatic. Further categorization is based on their target specificity: "narrow-spectrum" antibiotics target particular types of bacteria, such as Gram-negative or Gram-positive bacteria, while broad-spectrum antibiotics affect a wide range of bacteria. In the last few years three new classes of antibiotics have been brought into clinical use. This follows a 40-year hiatus in discovering new classes of antibiotic compounds. These new antibiotics are of the following three classes: cyclic lipopeptides (daptomycin), glycylcyclines (tigecycline), and oxazolidinones (linezolid). Tigecycline is a broad-spectrum antibiotic, while the two others are used for Gram-positive infections. These developments show promise as a means to counteract the bacterial resistance to existing antibiotics.

PRODUCTION

Since the first pioneering efforts of Florey and Chain in 1939, the importance of antibiotics to medicine has led to much research into discovering and producing them. The process of production usually involves the screening of wide ranges of microorganisms, and their testing and modification. Production is carried out using fermentation, usually in strongly aerobic form.

ADMINISTRATION

Oral antibiotics are simply ingested, while intravenous antibiotics are used in more serious cases, such as deep-seated systemic infections. Antibiotics may also sometimes be administered topically, as with eye drops or ointments.

SIDE EFFECTS

Although antibiotics are generally considered safe and well tolerated, they have been associated with a wide range of adverse effects. Side effects are many, varied and can be very serious depending on the antibiotics used and the microbial organisms targeted. The safety profiles of newer medications may not be as well established as those that have been in use for many years. Adverse effects can range from fever and nausea to major allergic reactions including photodermatitis and anaphylaxis. One of the more common side effects is diarrhea, sometimes caused by the anaerobic bacterium *Clostridium difficile*, which results from the antibiotic disrupting the normal balance of the intestinal flora, Such overgrowth of pathogenic bacteria may be alleviated by ingesting probiotics during a course of antibiotics.

An antibiotic-induced disruption of the population of the bacteria normally present as constituents of the normal vaginal flora may also occur, and may lead to overgrowth of yeast species of the genus *Candida* in the vulvo-vaginal area. Other side effects can result from interaction with other drugs, such as elevated risk of tendon damage from administration of a quinolone antibiotic with a systemic corticosteroid.

DRUG-DRUG INTERACTIONS

CONTRACEPTIVE PILLS

Hypothetically, interference of some antibiotics with the efficiency of birth control pills is thought to occur in two

ways. Modification of the intestinal flora may result in reduced absorption of estrogens.

Secondly, induction of hepatic liver enzymes causing them to metabolize the pill's active ingredients faster may affect the pill's usefulness.

However, the majority of studies indicate that antibiotics do not interfere with contraception. Even though a small percentage of women may experience decreased effectiveness of birth control pills while taking an antibiotic, the failure rate is comparable to those taking the pill.

Moreover, there have been no studies that have conclusively demonstrated that disruption of the gut flora affects contraception.

Interaction with the combined oral contraceptive pill through induction of hepatic enzymes by the antifungal medication griseofulvin and the broad-spectrum antibiotic rifampicin has been shown to occur. It is recommended that extra contraceptive measures are applied during antimicrobial therapy using these antimicrobials.

ALCOHOL

Alcohol can interfere with the activity or metabolization of antibiotics. It may affect the activity of liver enzymes, which break down the antibiotics.

Moreover, certain antibiotics, including metronidazole, tinidazole, cephamandole, ketoconazole, latamoxef, cefoperazone, cefmenoxime, and furazolidone, chemically react with alcohol, leading to serious side effects, which include severe vomiting, nausea, and shortness of breath. Alcohol consumption while taking such antibiotics is therefore not recommended. Additionally, serum levels of doxycycline and erythromycin succinate may, in certain circumstances, be significantly reduced by alcohol consumption.

6

Applications of Monoclonal Antibodies

HEMATOLOGIC MALIGNANCIES

There are a number of antigens and corresponding monoclonal antibodies for the treatment of B cell malignancies. One of the most popular target antigens is CD20, found on B cell malignancies. The CD52 antigen is targeted by the monoclonal antibody alemtuzumab, which is indicated for treatment of chronic lymphocytic leukemia.

The CD22 is targeted by a number of antibodies, and has recently demonstrated efficacy combined with toxin in chemotherapy-resistant hairy cell leukemia. Two new monoclonal antibodies targeting CD20, tositumomab and ibritumomab, have been submitted to the Food and Drug Administration (FDA). These antibodies are conjugated with radioisotopes.

The first monoclonal antibody to receive FDA approval was rituximab. Rituximab is a chimeric unconjugated monoclonal antibody directed at the CD20 antigen, a signature B cell antigen. CD20 has an important functional role in B cell activation, proliferation, and differentiation. This antigen is a transmembrane protein composed of 297 amino acids. The intracellular portion contains phosphorylation sequences for protein kinase C, calmodulin, and casein kinase.

CD20 is thought to act as a calcium channel as well, given the great structural homology between the CD20 protein and the calcium channels. When CD20 was introduced into cell lines by transfection, an increase in intracellular calcium was observed within the transfected cells. With monoclonal antibody stimulation, we see calcium influx within the cells. Calcium chelators blocked apoptosis induced by CD20 stimulation by monoclonal antibodies.

When monoclonal antibodies attach and particularly cross-link CD20 antigen, an increase in intracellular calcium is again observed. This increase appears to activate the SER family of tyrosine kinases, resulting in further phosphorylation of the CD20 inner cytoplasmic chain and also phospholipase C-gamma. At the same time there is an upregulation of C-myc and myb messenger ribonucleic acid (RNA), an increase in adhesion molecule expression and an upregulation of MHC class II proteins. The ultimate result is caspase 3 activation, causing cell apoptosis.

As previously mentioned, CD20 is a natural focus for monoclonal antibody therapy because of its relatively high degree of expression in B cell malignancies, perhaps as high as 95 per cent in follicular lymphomas, even with the

heterogeneity discussed earlier. The monoclonal antibody rituximab was designed specifically to target CD20. Rituximab is predominantly human (95 per cent).

The variable light and heavy chain portion of rituximab is murine, but the remainder is humanized so the formation of human anti-mouse antibody is not significant. Rituximab is thought to induce cell apoptosis by inducing calcium influx, releasing caspase activity. In addition, evidence of indirect effects through ADCC and CDC has been observed.

Rituximab is indicated for treatment of low-grade lymphomas refractory to conventional chemotherapy. Based upon this work it has been evaluated for first-line and combination therapy. Results of studies using rituximab as first-line treatment of low-grade non-Hodgkin lymphoma have been encouraging.

Patients who had not received any prior therapy were treated with rituximab 375 mg/m^2 on a weekly basis for 4 weeks and then re-evaluated 2 weeks post-therapy. The patients who achieved a complete or partial response, or who had stable disease received rituximab maintenance therapy (weekly for 4 weeks every 6 months). Patients who showed evidence of progression were taken off maintenance therapy.

At the time of initial re-evaluation at 6 weeks, 54 per cent of the patients showed objective response to treatment. An additional 36 per cent had stable disease or minor response. At the time of publication 13 patients had undergone a second course of treatment, and 4 additional responses were documented. Four patients improved from partial to complete response. Treatment with rituximab was well tolerated, with only 1 of the 39 patients experiencing grade 3-4 infusion

related toxicity. These responses were durable as well. For patients who achieved partial or complete response, one-year follow-up showed no evidence of disease progression. One-year survival was 69 per cent; survival at two years 67 per cent. While overall survival is not an unusual finding in low-grade lymphoma, the duration of response remains relatively impressive. Rituximab has been combined with conventional chemotherapy for patients with intermediate grade or diffuse large cell non-Hodgkin lymphoma.

CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) is standard therapy for this type and stage of disease. In a multi-institutional study, 33 patients with newly diagnosed large cell lymphoma received six infusions of rituximab 375 mg/m^2 on day 1 of each cycle combined with six doses of CHOP on day 3 of each cycle.

The overall response rate was 94 per cent, with 20 patients (61 per cent) achieving a complete response. Eleven patients (33 per cent) experienced partial response, and 2 patients were found to have progressive disease. Median duration of response and time to progression had not been reached after a median follow-up time of 26 months. Twenty-nine patients remained in remission during this observation period. The most frequent adverse events associated with rituximab were fever and chills, primarily during the first infusion.

The investigators concluded that rituximab did not appear to increase the toxicity of therapy. hese results were confirmed in a phase III randomized trial of CHOP and rituximab in elderly patients conducted by the French Lymphoma Cooperative Group (GELA). These patients had stage II to IV diffuse large cell lymphoma, were newly

diagnosed and therapy-naïve. This study focused on elderly patients (60 to 80 years), because the efficacy of CHOP is decreased in the elderly. Patients had Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2. Patients were randomized to receive CHOP alone with cytokine support or CHOP with rituximab, given on day 1. These regimens were administered every 21 days for 6 to 8 cycles.

Interim results were reported at the 2000 annual meeting of the American Society of Hematology. Patients who received CHOP plus rituximab showed a statistically significant (P < 0.0005) advantage in event-free survival over CHOP alone. This advantage translated into an improvement in overall survival of 83 per cent with rituximab (P < 0.01) versus 68 per cent in these 400 patients. A similar study is now in progress, with an additional arm to evaluate the value of rituximab as maintenance therapy for responders.

SOLID TUMORS

Compared to hematologic malignancies, solid tumors do not have as many specific targets for monoclonal antibodies that are not cross-reactive with antigens on normal tissues. Two significant monoclonal antibodies have been used in solid tumors: edrecolomab and trastuzumab.

Edrecolomab targets the 17-1A antigen seen in colon and rectal cancer, and has been approved for use in Europe for these indications. Its antitumor effects are mediated through ADCC, CDC, and the induction of an anti-idiotypic network. In an initial study of 189 patients with resected stage II colorectal cancer, treatment with edrecolomab reduced the relative risk of mortality by 32 per cent compared with

observation alone (P < 0.01). Edrecolomab is undergoing investigation in two large phase III trials in patients with stage III colon cancer, either as monotherapy or in combination with fluorouracil-based chemotherapy.

In the US, the most commonly used monoclonal antibody for the treatment of solid tumors is trastuzumab, which targets the HER-2/neu antigen. This antigen is seen on 25 per cent to 35 per cent of breast cancers. Trastuzumab is thought to work in a variety of ways: downregulation of HER-2 receptor expression, inhibition of proliferation of human tumor cells that overexpress HER-2 protein, enhancing immune recruitment and ADCC against tumor cells that overexpress HER-2 protein, and downregulation of angiogenesis factors. This last mechanism may be very important in terms of metastatic disease.

In phase I and II trials of patients with metastatic breast cancer, treatment with a combination of trastuzumab and cisplatin resulted in prolongation of survival and higher response rates than that seen with cisplatin alone.

Trastuzumab plus chemotherapy when compared to chemotherapy alone was associated with longer time to disease progression (median 7.4 months vs. 4.6 months, P<0.001), a higher rate of objective response (50 per cent vs. 32 per cent, P<0.01), a longer duration of response (median 9.1 vs. 6.1 months, P<0.001), a lower rate of death at 1 year (22 per cent vs. 33 per cent, P=0.008), longer survival (median survival 25.1 vs. 20.3 months, P=0.01) and a 20 per cent reduction in the risk of death.

This trial evaluated 469 patients with metastatic breast cancer that overexpressed HER-2/neu. Patients were

randomly assigned to receive chemotherapy alone (n=234) or chemotherapy plus trastuzumab (n=235). The only significant adverse event was cardiac dysfunction, which was managed with standard medical treatment.

Trastuzumab has also been studied as monotherapy. This study involved 214 patients with relapsed metastatic breast cancer who had been heavily pre-treated: 90 per cent had received prior anthracyclines and 65 per cent had received taxane therapy. The majority had either lung or liver metastasis. All patients received a loading dose of trastuzumab (4 mg/kg), followed by weekly maintenance therapy of 2 mg/kg until evidence of disease progression.

Primary endpoints were tumor assessment relative to response. Secondary endpoints were duration of response, time to tumor progression, time to treatment failure, and quality of life.

Eight complete and 26 partial responses were identified for an objective response rate of 15 per cent in the intent-to-treat population (95 per cent CI; 11 per cent to 21 per cent). Median duration of response was 9.1 months; median duration of survival was 13 months. Toxicity was minimal, although cardiac dysfunction occurred in 4.7 per cent of patients. Patients who had higher overexpression of HER-2/neu had a better overall response rate. As first-line monotherapy, trastuzumab has demonstrated efficacy and safety in patients with metastatic breast cancer. This study included 114 women with HER-2-overexpressing metastatic breast cancer with no prior chemotherapy. Patients were randomized to receive a loading dose of trastuzumab 4 mg/kg followed by 2 mg/kg weekly, or an 8 mg/kg loading dose

followed by 4 mg/kg weekly. Primary endpoint was overall response rate. Secondary endpoints were disease relapse, time to tumor progression, and overall survival.

Complete response rates were relatively low (7/114) regardless of loading and maintenance doses. Partial response rates were nearly identical at 19 per cent vs. 21 per cent, for an overall response rate of 24 per cent vs. 28 per cent, which was not statistically significant. Seventeen (57 per cent) of 30 patients with an objective response and 22 (51 per cent) of 43 patients with clinical benefit had not experienced disease progression at 1-year follow-up.

These investigators found no benefit to the higher versus lower dose of trastuzumab. However, they noted a difference in response that correlated to HER-2 overexpression. Overexpression was measured by immunohistochemistry (IHC) and then rechecked with fluorescent *in situ* hybridization (FISH). Interestingly, patients who were positive for overexpression by IHC were not necessarily positive by FISH. Overexpression as confirmed by FISH was strongly correlated to response, and these patients appeared to have garnered the most clinical benefit from treatment with trastuzumab.

When we look at these data together first-line monotherapy with trastuzumab appears to have better overall response. However, median time to disease progression remains disappointingly short. The incidence of cardiac toxicity cannot be minimized, particularly in patients with prior anthracycline therapy or cardiac disease. These patients had a 10 per cent incidence of severe myocardial toxicity and one death from ventricular arrhythmia. Still, these data suggest the

possibility of significant response rates, including improvement in overall survival. While early reports on the use of monoclonal antibodies may have been overenthusiastic, the results of these studies show there is still cause for cautious optimism as we go forward. Cure may yet elude us, but stable disease is an attainable, highly desirable goal.

MONOCLONAL ANTIBODY TECHNOLOGY

Substances foreign to the body, such as disease-causing bacteria and viruses and other infectious agents, known as antigens, are recognized by the body's immune system as invaders. Our natural defenses against these infectious agents are antibodies, proteins that seek out the antigens and help destroy them.

Antibodies have two very useful characteristics. First, they are extremely specific; that is, each antibody binds to and attacks one particular antigen. Second, some antibodies, once activated by the occurrence of a disease, continue to confer resistance against that disease; classic examples are the antibodies to the childhood diseases chickenpox and measles.

The second characteristic of antibodies makes it possible to develop vaccines. A vaccine is a preparation of killed or weakened bacteria or viruses that, when introduced into the body, stimulates the production of antibodies against the antigens it contains.

It is the first trait of antibodies, their specificity, that makes monoclonal antibody technology so valuable. Not only can antibodies be used therapeutically, to protect against disease; they can also help to diagnose a wide variety of illnesses,

and can detect the presence of drugs, viral and bacterial products, and other unusual or abnormal substances in the blood. Given such a diversity of uses for these disease-fighting substances, their production in pure quantities has long been the focus of scientific investigation. The conventional method was to inject a laboratory animal with an antigen and then, after antibodies had been formed, collect those antibodies from the blood serum (antibody-containing blood serum is called antiserum). There are two problems with this method: It yields antiserum that contains undesired substances, and it provides a very small amount of usable antibody. Monoclonal antibody technology allows us to produce large amounts of pure antibodies in the following way: We can obtain cells that produce antibodies naturally; we also have available a class of cells that can grow continually in cell culture. If we form a hybrid that combines the characteristic of "immortality" with the ability to produce the desired substance, we would have, in effect, a factory to produce antibodies that worked around the clock.

In monoclonal antibody technology, tumor cells that can replicate endlessly are fused with mammalian cells that produce an antibody.

The result of this cell fusion is a "hybridoma," which will continually produce antibodies. These antibodies are called monoclonal because they come from only one type of cell, the hybridoma cell; antibodies produced by conventional methods, on the other hand, are derived from preparations containing many kinds of cells, and hence are called polyclonal. An example of how monoclonal antibodies are derived is described below.

A myeloma is a tumor of the bone marrow that can be adapted to grow permanently in cell culture. When myeloma cells were fused with antibody-producing mammalian spleen cells, it was found that the resulting hybrid cells, or hybridomas, produced large amounts of monoclonal antibody. This product of cell fusion combined the desired qualities of the two different types of cells: the ability to grow continually, and the ability to produce large amounts of pure antibody.

Because selected hybrid cells produce only one specific antibody, they are more pure than the polyclonal antibodies produced by conventional techniques.

They are potentially more effective than conventional drugs in fighting disease, since drugs attack not only the foreign substance but the body's own cells as well, sometimes producing undesirable side effects such as nausea and allergic reactions. Monoclonal antibodies attack the target molecule and only the target molecule, with no or greatly diminished side effects.

ANTIBODY THERAPY IN MONOCLONAL ANTIBODIES

Antibodies are proteins produced by the B lymphocytes of the immune system in response to foreign proteins, called antigens. Antibodies function as markers, binding to the antigen so that the antigen molecules can be recognized and destroyed by phagocytes. The part of the antigen that the antibody binds to is called the epitope. The epitope is thus a short amino acid sequence that the antibody is able to recognize.

Structurally antibodies are proteins consisting of four polypeptide chains. These four chains form a quaternary structure somewhat resembling a Y shape. Each B cell in an organism synthesizes only one kind of antibody. In an organism, there is an entire population of different types of B cells and their respective antibodies that were produced in response to the various antigens that the organism had been exposed to. However to be useful as a tool, molecular biologists need substantial amounts of a single antibody (and that antibody alone).

Therefore we need a method to culture a population of B cells derived from a single ancestral B cell, so that this population of B cells would allow us to harvest a single kind of antibody.

This population of cells would be correctly described as monoclonal, and the antibodies produced by this population of B cells are called monoclonal antibodies. In contrast, antibodies obtained from the blood of an immunized animal are called polyclonal antibodies.

Antibodies or immunoglobulins are a crucial component of the immune system, circulating in the blood and lymphatic system, and binding to foreign antigens expressed on cells. Once bound, the foreign cells are marked for destruction by macrophages and complement. In the context of cancer immunotherapy, monoclonal antibodies have brought to light a wide array of human tumor antigens. In addition to targeting cancer cells, antibodies can be designed to act on other cell types and molecules necessary for tumor growth. For example, antibodies can neutralize growth factors and thereby inhibit tumor expansion.

Monoclonal antibodies are made by injecting human cancer cells, or proteins from cancer cells, into mice so that their immune systems create antibodies against foreign antigens. The murine cells producing the antibodies are then removed and fused with laboratory-grown cells to create hybrid cells called hybridomas. Hybridomas can indefinitely produce large quantities of these pure antibodies.

PRODUCTION OF MONOCLONAL ANTIBODIES

The production of monoclonal antibodies was pioneered by Georges Kohler and Cesar Milstein in 1975. Let us see how their method, now tried and tested for over 20 years, would be applied in a particular case.In order for us to isolate a B lymphocyte producing a certain antibody, we first have to induce the production of such a B cell in an organism.

For example, if we need an antibody for avian SERCA2 protein, we would inject the protein into a mouse. This is typically done in two doses, an initial "priming" dose and a second "booster" dose 10 days later. Since the protein is of foreign origin, the mouse immune system recognizes it as such and soon some of the B cells in the mouse would begin production of the antibody to avian SERCA2.

A sample of B cells is extracted from the spleen of the mouse and added to a culture of myeloma cells (cancer cells). The intended result is the formation of hybridomas, cells formed by the fusion of a B cell and a myeloma cell. The fusion is done by using polyethylene glycol, a virus or by electroporation.
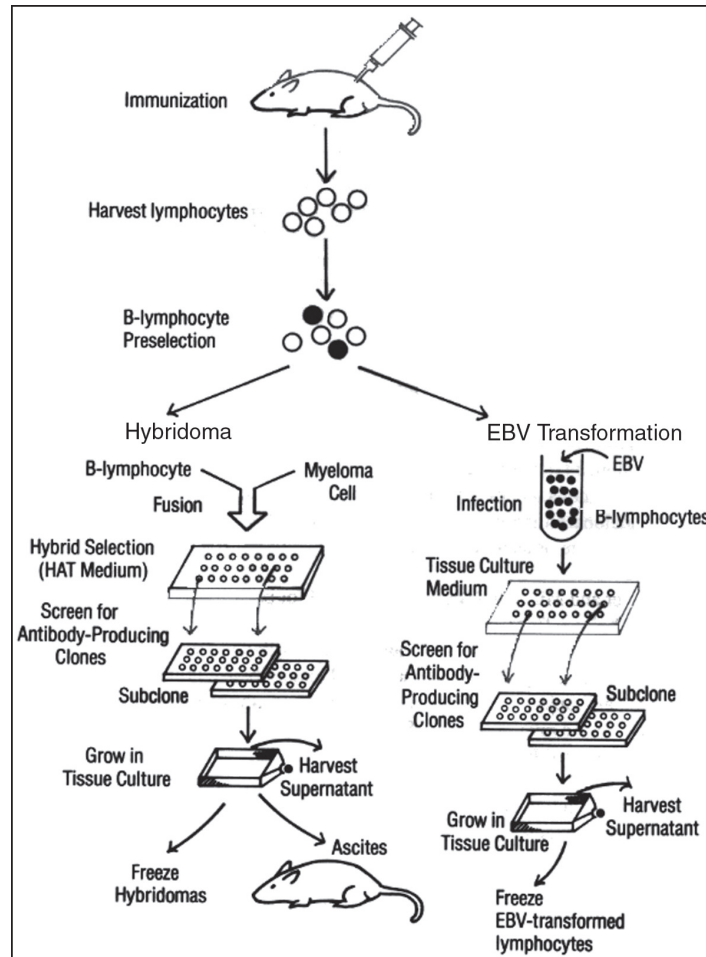
Fig. Monoclonal Antibodies Production

The next step is to selct for the hybridomas. The myeloma cells are HGPRT- and the B cells are HGPRT. HGPRT is hypoxanthine-guanine phosphoribosyl transferase, an enzyme involved in the synthesis of nucleotides from hypoxanthine, an amino acid. The culture is grown in HAT (hypoxanthine-aminopterin-thymine) medium, which can sustain only HGPRT cells. The myeloma cells that fuse with another myeloma cell or do not fuse at all die in the HAT medium since they are HGPRT-. The B cells that fuse with another B cell or do not fuse at all die because they do not

have the capacity to divide indefinitely. Only hybridomas between B cells and myeloma cells survive, being both HGPRT+ and cancerous.

The initial collection of B cells used is heterogenous, i.e. they do not all produce the same antibody. Therefore the hybridoma population too does not produce a single antibody. There is also another complication.

A hybridoma cell is initially tetraploid, having been formed by the fusion of two diploid cells. However the extra chromosomes are somehow lost in subsequent divisions in a random manner.

This means that we cannot be certain that the hybridomas will all produce the desired antibody or even any antibody at all. Screening is required to decide which hybridoma cells are actually producing the desired antibody.

Each hybridoma is cultured and screened after doing SDS (sodium dodecyl sulfate - polyacrylamide gel electrophoresis) and Western blots. The probe used is the epitope of the antibody that is desired, which may be labeled by radioactivity or immunofluorescence. Once we are sure that a certain hybridoma is producing the right antibody, we can culture that hybridoma indefinitely and harvest monoclonal antibodies from it.

USES OF MONOCLONAL ANTIBODIES

Monoclonal bodies have a variety of academic, medical and commercial uses. It would be impossible to list all of these here. But the following list should indicate how ubiquitous monoclonal antibody technology has become in biotechnology.

- Antibodies are used in several diagnostic tests to detect small amounts of drugs, toxins or hormones, e.g., monoclonal antibodies to human chorionic gonadotropin (HCG) are used in pregnancy test kits. Another diagnostic uses of antibodies is the diagnosis of AIDS by the ELISA test.

- Antibodies are used in the radioimmunodetection and radioimmunotherapy of cancer, and some new methods can even target only the cell membranes of cancerous cells. A new cancer drug based on monoclonal antibody technology is Ritoxin.

- Monoclonal antibodies can be used to treat viral diseases, traditionally considered "untreatable". In fact, there is some evidence to suggest that antibodies may lead to a cure for AIDS.

- Monoclonal antibodies can be used to classify strains of a single pathogen, e.g. Neisseria gonorrhoeae can be typed using monoclonal antibodies.

- Researchers use monoclonal antibodies to identify and to trace specific cells or molecules in an organism, e.g., developmental biologists at tyhe University of Oregon use monoclonal antibodies to find out which proteins are responsible for cell differentiation in the respiratory system.

- OKT3, an antibody to the T3 antigen of T cells, is used to alleviate the problem of organ rejection in patients who have had organ transplants.

DEVELOPMENT OF MONOCLONAL ANTIBODIES

Monoclonal antibodies have several roles in cancer therapy. Monoclonal antibodies have been used in a variety of ways

in the management of cancer including diagnosis, monitoring, and treatment of disease. They aid in diagnosis, such as the application of flow cytometry in the identification of different subsets of non-Hodgkin's lymphoma. We can use monoclonal antibodies to monitor disease progression, such as the measurement of carcinoembryonic antigen in colon cancer. Most importantly, we can utilize monoclonal antibodies directly as therapy.

Relative to treatment, monoclonal antibodies can react against specific antigens on cancer cells and may enhance the patient's immune response. Monoclonal antibodies can be programmed to act against cell growth factors, thus blocking cancer cell growth. We can conjugate or link monoclonal antibodies to anticancer drugs, radioisotopes, other biologic response modifiers, or other toxins.

When the antibodies bind with antigen-bearing cells, they deliver their load of toxin directly to the tumor. Monoclonal antibodies may also be used to preferentially select normal stem cells from bone marrow or blood in preparation for a hematopoietic stem cell transplant in patients with cancer. There are a number of considerations when using monoclonal antibodies for therapy. First, a target antigen must be selected. It is important that this antigen is presented uniquely by the tumor cells and not on normal tissues.

The immunogenicity of the monoclonal antibody itself is a concern because of how they are derived. As they are often derived from non-human monoclonal antibodies, they are capable of eliciting an immune response themselves. Half-life is another factor. Will it be long enough to have the desired effect? There are also logistical problems such as cost and

availability. Anti-idiotype monoclonal antibodies are a good example of this, as their development has been prohibited by cost. Finally, a decision as to whether or not the monoclonal antibody will be used alone or if it will be conjugated (i.e., attach radioisotopes, toxins, or chemotherapy) in order to get the desired therapeutic effect.

MECHANISM OF ACTION

Monoclonal antibodies achieve their therapeutic effect through various mechanisms. They can have direct effects in producing apoptosis or programmed cell death. They can block growth factor receptors, effectively arresting proliferation of tumor cells. In cells that express monoclonal antibodies, they can bring about anti-idiotype antibody formation.

In this situation Rituximab (IDEC-C2B8), a chimeric antibody, targets the CD20 antigen. This antigen is expressed on a significant number of B cell malignancies. Rituximab is an IgG monoclonal antibody, and has an Fc receptor. The Fc fragment of the monoclonal antibody binds the Fc receptors found on monocytes, macrophages, and natural killer cells. These cells in turn engulf the bound tumor cell and destroy it. Natural killer cells secrete cytokines that lead to cell death, and they also recruit B cells.

Indirect effects include recruiting cells that have cytotoxicity, such as monocytes and macrophages. This type of antibody-mediated cell kill is called antibody-dependent cell mediated cytotoxicity (ADCC). Monoclonal antibodies also bind complement, leading to direct cell toxicity, known as complement dependent cytotoxicity (CDC).

ANTIBODY THERAPY

Antibody therapy can be used in a variety of ways to treat cancer.

As described above, they may act through ADCC or CDC. An alternative approach is to conjugate the monoclonal antibody to a toxin, a cytotoxic agent, or a radioisotope. With conjugated monoclonal antibodies a toxin is actually bound to the antibody, which then attaches to the antigen. The antibody conjugate is absorbed into the cell itself, resulting in cell death.

We can attach a radioisotope such as iodide-131 to directly infuse the cancer cell with radiotherapy, and also mitigate the effects to normal surrounding tissue. Finally, we can attach chemotherapy. The chemotherapeutic agent is taken directly into the targeted malignant cell, rather than being systemically absorbed.

OBSTACLES TO SUCCESSFUL THERAPY

There are a number of obstacles to successful therapy with monoclonal antibodies. The antigen distribution of malignant cells is highly heterogeneous, so some cells may express tumor antigens while others do not. Antigen density can vary as well, with antigens expressed in concentrations too low for monoclonal antibodies to be effective.

Tumor blood flow is not always optimal. If monoclonal antibodies need to be delivered via the blood, it may be difficult to reliably get the therapy to the site. High interstitial pressure within the tumor can prevent the passive monoclonal antibodies from binding. Sometimes the tumor

antigen is even released, so the antibody binds to a free-floating antigen and not the tumor cell. Since monoclonal antibodies are derived from mouse cell lines, the possibility of an immune response to the antibodies exists. This response not only decreases the efficacy of monoclonal antibody therapy, but also eliminates the possibility of re-treatment.

Very rarely do we see cross-reactivity with normal tissue antigens – in general target antigens that are not cross reactive with normal tissue antigens are chosen. Despite these obstacles, there has been tremendous success in the clinical application of monoclonal antibodies in hematologic malignancies and solid tumors.

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Production of Antibiotics

The mass production of antibiotics began during World War II with streptomycin and penicillin. Now most antibiotics are produced by staged fermentations in which strains of microorganisms producing high yields are grown under optimum conditions in nutrient media in fermentation tanks holding several thousand gallons. The mold is strained out of the fermentation broth, and then the antibiotic is removed from the broth by filtration, precipitation, and other separation methods.

In some cases new antibiotics are laboratory synthesized, while many antibiotics are produced by chemically modifying natural substances; many such derivatives are more effective than the natural substances against infecting organisms or are better absorbed by the body, e.g., some semisynthetic penicillins are effective against bacteria resistant to the parent

substance. Antibiotic, any of a variety of substances, usually obtained from microorganisms, that inhibit the growth of or destroy certain other microorganisms.

TYPES OF ANTIBIOTICS

The great number of diverse antibiotics currently available can be classified in different ways, e.g., by their chemical structure, their microbial origin, or their mode of action. They are also frequently designated by their effective range.
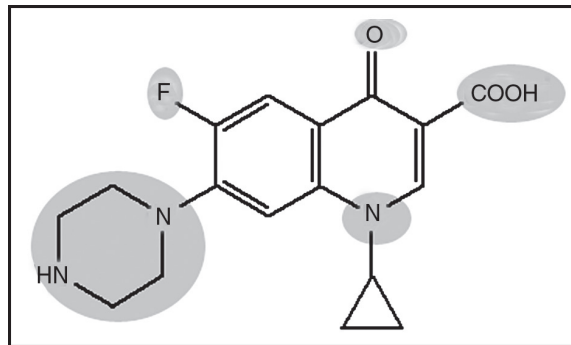
Fig. Ciprofloxacin

Tetracyclines, the most widely used broad-spectrum antibiotics, are effective against both Gram-positive and Gram-negative bacteria, as well as against rickettsias and psittacosis-causing organisms. Ciprofloxacin (Cipro) is another broad-spectrum antibiotic, effective in the treatment of mild infections of the urinary tract and sinuses. The medium-spectrum antibiotics bacitracin, the erythromycins, penicillin, and the cephalosporins are effective primarily against Gram-positive bacteria, although the streptomycin group is effective against some Gram-negative and Gram-positive bacteria. Polymixins are narrow-spectrum antibiotics effective against only a few species of bacteria. Antibiotics are either injected, given orally, or applied to the skin in

ointment form. Many, while potent anti-infective agents, also cause toxic side effects. Some, like penicillin, are highly allergenic and can cause skin rashes, shock, and other manifestations of allergic sensitivity. Others, such as the tetracyclines, cause major changes in the intestinal bacterial population and can result in superinfection by fungi and other microorganisms. Chloramphenicol, which is now restricted in use, produces severe blood diseases, and use of streptomycin can result in ear and kidney damage.

Many antibiotics are less effective than formerly because antibiotic-resistant strains of microorganisms have emerged. Antibiotics have found wide nonmedical use. Some are used in animal husbandry, along with vitamin B_{12}, to enhance the weight gain of livestock.

However, some authorities believe the addition of antibiotics to animal feeds is dangerous because continuous low exposure to the antibiotic can sensitize humans to the drug and make them unable to take the substance later for the treatment of infection. In addition low levels of antibiotics in animal feed encourage the emergence of antibiotic-resistant strains of microorganisms.

Drug resistance has been shown to be carried by a genetic particle transmissible from one strain of microorganism to another, and the presence of low levels of antibiotics can actually cause an increase in the number of such particles in the bacterial population and increase the probability that such particles will be transferred to pathogenic, or disease-causing, strains. Antibiotics have also been used to treat plant diseases such as bacteria-caused infections in tomatoes, potatoes, and fruit trees. The substances are also

used in experimental research. Although for centuries preparations derived from living matter were applied to wounds to destroy infection, the fact that a microorganism is capable of destroying one of another species was not established until the latter half of the 19th cent. when Pasteur noted the antagonistic effect of other bacteria on the anthrax organism and pointed out that this action might be put to therapeutic use.

Meanwhile the German chemist Paul Ehrlich developed the idea of selective toxicity: that certain chemicals that would be toxic to some organisms, e.g., infectious bacteria, would be harmless to other organisms, e.g., humans. In 1928, Sir Alexander Fleming, a Scottish biologist, observed that *Penicillium notatum,* a common mold, had destroyed staphylococcus bacteria in culture, and in 1939 the American microbiologist René Dubos demonstrated that a soil bacterium was capable of decomposing the starchlike capsule of the pneumococcus bacterium, without which the pneumococcus is harmless and does not cause pneumonia.

Dubos then found in the soil a microbe, *Bacillus brevis,* from which he obtained a product, tyrothricin, that was highly toxic to a wide range of bacteria. Tyrothricin, a mixture of the two peptides gramicidin and tyrocidine, was also found to be toxic to red blood and reproductive cells in humans but could be used to good effect when applied as an ointment on body surfaces. Penicillin was finally isolated in 1939, and in 1944 Selman Waksman and Albert Schatz, American micro-biologists, isolated streptomycin and a number of other antibiotics from *Streptomyces griseus.*
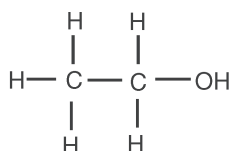
ETHANOL

Fig. Ethanol

Ethanol fermentation is the biological process by which sugars such as glucose, fructose, and sucrose are converted into cellular energy and thereby producing ethanol and carbon dioxide as metabolic waste products. Yeasts carry out ethanol fermentation on sugars in the absence of oxygen. Because the process does not require oxygen, ethanol fermentation is classified as anaerobic. Ethanol fermentation is responsible for the rising of bread dough, the production of ethanol in alcoholic beverages, and for much of the production of ethanol for use as fuel.

Ethanol (ethyl alcohol, grain alcohol) is a clear, colorless liquid with a characteristic, agreeable odor. In dilute aqueous solution, it has a somewhat sweet flavour, but in more concentrated solutions it has a burning taste. Ethanol, CH_3CH_2OH, is an alcohol, a group of chemical compounds whose molecules contain a hydroxyl group, –OH, bonded to a carbon atom. The word *alcohol* derives from Arabic *al-kuhul*, which denotes a fine powder of antimony used as an eye makeup. *Alcohol* originally referred to any fine powder, but medieval alchemists later applied the term to the refined products of distillation, and this led to the current usage.

Ethanol melts at –114.1°C, boils at 78.5°C, and has a density of 0.789 g/mL at 20°C. Its low freezing point has made it useful as the fluid in thermometers for temperatures

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below –40°C, the freezing point of mercury, and for other low-temperature purposes, such as for antifreeze in automobile radiators.

Ethanol has been made since ancient times by the fermentation of sugars. All beverage ethanol and more than half of industrial ethanol is still made by this process. Simple sugars are the raw material. Zymase, an enzyme from yeast, changes the simple sugars into ethanol and carbon dioxide. The fermentation reaction, represented by the simple equation

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$$
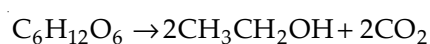
Is actually very complex, and impure cultures of yeast produce varying amounts of other substances, including glycerine and various organic acids. In the production of beverages, such as whiskey and brandy, the impurities supply the flavour. Starches from potatoes, corn, wheat, and other plants can also be used in the production of ethanol by fermentation.

However, the starches must first be broken down into simple sugars. An enzyme released by germinating barley, diastase, converts starches into sugars. Thus, the germination of barley, called malting, is the first step in brewing beer from starchy plants, such as corn and wheat. The ethanol produced by fermentation ranges in concentration from a few per cent up to about 14 per cent. Above about 14 per cent, ethanol destroys the zymase enzyme and fermentation stops. Ethanol is normally concentrated by distillation of aqueous solutions, but the composition of the vapour from aqueous ethanol is 96 per cent ethanol and 4 per cent water. Therefore, pure ethanol cannot be obtained

by distillation. Commercial ethanol contains 95 per cent by volume of ethanol and 5 per cent of water. Dehydrating agents can be used to remove the remaining water and produce absolute ethanol.

Much ethanol not intended for drinking is now made synthetically, either from acetaldehyde made from acetylene, or from ethylene made from petroleum. Ethanol can be oxidized to form first acetaldehyde and then acetic acid. It can be dehydrated to form ether. Butadiene, used in making synthetic rubber, may be made from ethanol, as can chloroform and many other organic chemicals. Ethanol is used as an automotive fuel by itself and can be mixed with gasoline to form gasohol.

Ethanol is miscible (mixable) in all proportions with water and with most organic solvents. It is useful as a solvent for many substances and in making perfumes, paints, lacquer, and explosives. Alcoholic solutions of nonvolatile substances are called tinctures; if the solute is volatile, the solution is called a spirit. Most industrial ethanol is denatured to prevent its use as a beverage. Denatured ethanol contains small amounts, 1 or 2 per cent each, of several different unpleasant or poisonous substances. The removal of all these substances would involve a series of treatments more expensive than the federal excise tax on alcoholic beverages.

These denaturants render ethanol unfit for some industrial uses. In such industries undenatured ethanol is used under close federal supervision. When an alcoholic beverage is swallowed, it passes through the stomach into the small intestine where the ethanol is rapidly absorbed and distributed throughout the body.

The ethanol enters body tissues in proportion to their water content. Therefore, more ethanol is found in the blood and the brain than in muscle or fat tissue. The ethanol is greatly diluted by body fluids. For example, a 1-ounce shot of 100-proof whiskey, which contains 0.5 fluid ounces of ethanol (about 15 mL), is diluted 5000-fold in a 150-pound human, producing a 0.02 per cent blood alcohol concentration.

THE CHEMICAL PROCESS OF FERMENTATION

The chemical equation below summarizes ethanol fermentation, in which one hexose molecule is converted into two ethanol molecules and two carbon dioxide molecules:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$
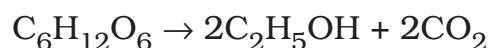
The process begins with a molecule of glucose being broken down by the process of glycolysis into pyruvate:

$$C_6H_{12}O_6 \rightarrow 2CH_3COCOO^- + 2H^+$$
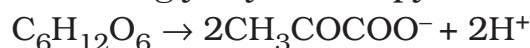
This reaction is accompanied by the reduction of two molecules of NAD^+ to NADH and a net of two ADP molecules converted to two ATP plus the two water molecules.

Pyruvate is then converted to acetaldehyde and carbon dioxide. The acetaldehyde is subsequently reduced to ethanol by the NADH from the previous glycolysis, which is returned to NAD^+:

$$CH_3COCOO^- + H^+ \rightarrow CH_3CHO + CO_2$$
$$CH_3CHO + NADH \rightarrow C_2H_5OH + NAD^+$$
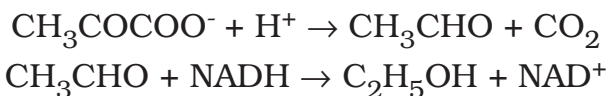
Yeast will perform the above two reactions only if oxygen is excluded from the environment. Otherwise yeast will oxidize pyruvate completely to carbon dioxide and water.

| Glucose | Pyruvate | Acetaldehyde | Ethanol |
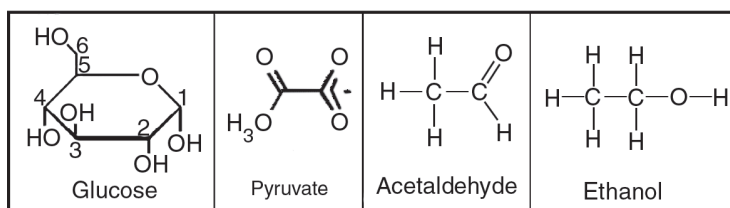
USES

Ethanol fermentation is responsible for the rising of bread dough. Yeast organisms consume sugars in the dough and produce ethanol and carbon dioxide as waste products. The carbon dioxide forms bubbles in the dough, expanding it into something of a foam. Nearly all the ethanol evaporates from the dough when the bread is baked.

sssThe production of all alcoholic beverages employs ethanol fermentation by yeast. Wines and brandies are produced by fermentation of the natural sugars present in fruits, especially grapes. Beers, ales, and whiskeys employ fermentation of grain starches that have been converted to sugar by the application of the enzyme, amylase, which is present in grain kernels that have been germinated.

Amylase-treated grain or amylase-treated potatoes are fermented for the production of vodka. Fermentation of cane sugar is the first step in producing rum. In all cases, the fermentation must take place in a vessel that is arranged to allow carbon dioxide to escape, but that prevents outside air from coming in, as exposure to oxygen would prevent the formation of ethanol.

Similar yeast fermentation of various carbohydrate products is used to produce much of the ethanol used for fuel.

SINGLE CELL PROTEIN

Single-cell proteins (SCP) refer to the dried cells of microorganisms. SCP's are used as protein sources in human foods or animal feeds. Many raw materials have been considered as carbon and energy sources for SCP production.

In many cases, these raw materials have been hydrolyzed by physical, chemical and enzymatic methods before use. Ram horns are significant waste products of the meat industry in Turkey.

For example, the slaughterhouse in Erzurum directly discharges about 25 tons a year. Fibrous proteins such as horn, feather, nail and hair are also abundant waste products. These waste products can be converted to biomass, protein concentrate or amino acids using proteases derived from certain microorganisms.

Horns consist of a-keratin, which has a very high content of cysteine and they also contain most of the common amino acids. Horns have the components of bone and blood tissues and are rich in some growth factors required by microorganisms. The main aim of this study was to investigate the suitability of horn hydrolysate as a substrate in SCP production.

SCP is the name given to a variety of microbial products, that are produced by fermentation. When properly produced, this materials make satisfactory proteinaceous ingredients for animal feed or human food. The production of protein from hydrocarbon wastes of the petroleum industry is the most recent microbiological industry.

Yeast, fungi, bacteria, and algae are grown on hydrocarbon wastes, and cells are harvested as sources of protein. It has been calculated that 100 lbs of yeast will produce 250 tons of proteins in 24 hours, whereas a 1000 lbs steer will synthesize only 1 lb of protein 24 hours and this after consuming 12 to 20 lbs of plant proteins. Similar, algae grown in ponds can produce 20 tons (dry weight) of protein, per

acre, per year. This yield is 10 to 15 times higher than soybean and 25 to 50 times higher than corn. There are both advantages and disadvantages in using microorganisms for animal or human consumption. Bacteria are usually high in protein (50 to 80 per cent) and have a rapid growth rate. The principal disadvantages are as follows:

- Bacterial cells have small size and low density, which makes harvesting from the fermented medium difficult and costly.
- Bacterial cells have high nucleic acid content relative to yeast and fungi This can be detrimental to human beings, tending to increase the uric acid level in blood. This may cause uric acid poising or gout. To decrease the nucleic acid level additional processing step has to be introduced, and this increases the cost.
- The general public thinking is that all bacteria are harmful and produce disease. An extensive education programme is1required to remove this misconception and to make the public accept bacterial protein.

Yeasts have as advantages their larger size (easier to harvest), lower nucleic add content, high lysine content and ability to grow at acid pH. However the most important advantage is familiarity and acceptability because of the long history of its use in traditional fermentations. Disadvantages include lower growth rates, lower protein content (45 to 65 per cent), and lower methionine content than in bacteria.

Filamentous fungi have advantages.in ease of harvesting, but have their limitations in lower growth rates, lower protein content, and acceptability. Algae have disadvantages of having cellulosic cell walls which are not digested by human beings. Secondly, they also concentrate heavy metals.

RAW MATERIALS

The compounds that make the fermentation broth are the primary raw materials required for antibiotic production. This broth is an aqueous solution made up of all of the ingredients necessary for the proliferation of the microorganisms. Typically, it contains a carbon source like molasses, or soy meal, both of which are made up of lactose and glucose sugars. These materials are needed as a food source for the organisms. Nitrogen is another necessary compound in the metabolic cycles of the organisms.

For this reason, an ammonia salt is typically used. Additionally, trace elements needed for the proper growth of the antibiotic-producing organisms are included. These are components such as phosphorus, sulfur, magnesium, zinc, iron, and copper introduced through water soluble salts. To prevent foaming during fermentation, anti-foaming agents such as lard oil, octadecanol, and silicones are used.

STARTING THE CULTURE

Before fermentation can begin, the desired antibiotic-producing organism must be isolated and its numbers must be increased by many times.

To do this, a starter culture from a sample of previously isolated, cold-stored organisms is created in the lab. In order to grow the initial culture, a sample of the organism is transferred to an agar-containing plate. The initial culture is then put into shake flasks along with food and other nutrients necessary for growth. This creates a suspension, which can be transferred to seed tanks for further growth.

The seed tanks are steel tanks designed to provide an ideal environment for growing microorganisms.

They are filled with the all the things the specific microorganism would need to survive and thrive, including warm water and carbohydrate foods like lactose or glucose sugars. Additionally, they contain other necessary carbon sources, such as acetic acid, alcohols, or hydrocarbons, and nitrogen sources like ammonia salts.

Growth factors like vitamins, amino acids, and minor nutrients round out the composition of the seed tank contents. The seed tanks are equipped with mixers, which keep the growth medium moving, and a pump to deliver sterilized, filtered air. After about 24-28 hours, the material in the seed tanks is transferred to the primary fermentation tanks.

FERMENTATION

The fermentation tank is essentially a larger version of the steel, seed tank, which is able to hold about 30,000 gallons. It is filled with the same growth media found in the seed tank and also provides an environment inducive to growth. Here the microorganisms are allowed to grow and multiply. During this process, they excrete large quantities of the desired antibiotic. The tanks are cooled to keep the temperature between 73-81° F (23-27.2°C).

It is constantly agitated, and a continuous stream of sterilized air is pumped into it. For this reason, anti-foaming agents are periodically added. Since pH control is vital for optimal growth, acids or bases are added to the tank as necessary.

ISOLATION AND PURIFICATION

After three to five days, the maximum amount of antibiotic will have been produced and the isolation process can begin. Depending on the specific antibiotic produced, the fermentation broth is processed by various purification methods. For example, for antibiotic compounds that are water soluble, an ion-exchange method may be used for purification. In this method, the compound is first separated from the waste organic materials in the broth and then sent through equipment, which separates the other water-soluble compounds from the desired one.

To isolate an oil-soluble antibiotic such as penicillin, a solvent extraction method is used. In this method, the broth is treated with organic solvents such as butyl acetate or methyl isobutyl ketone, which can specifically dissolve the antibiotic. The dissolved antibiotic is then recovered using various organic chemical means. At the end of this step, the manufacturer is typically left with a purified powdered form of the antibiotic, which can be further refined into different product types.

REFINING

Antibiotic products can take on many different forms. They can be sold in solutions for intravenous bags or syringes, in pill or gel capsule form, or they may be sold as powders, which are incorporated into topical ointments. Depending on the final form of the antibiotic, various refining steps may be taken after the initial isolation.

For intravenous bags, the crystalline antibiotic can be dissolved in a solution, put in the bag, which is then hermetically sealed. For gel capsules, the powdered antibiotic

is physically filled into the bottom half of a capsule then the top half is mechanically put in place. When used in topical ointments, the antibiotic is mixed into the ointment.

From this point, the antibiotic product is transported to the final packaging stations. Here, the products are stacked and put in boxes. They are loaded up on trucks and transported to various distributors, hospitals, and pharmacies. The entire process of fermentation, recovery, and processing can take anywhere from five to eight days.

QUALITY CONTROL

Quality control is of utmost importance in the production of antibiotics. Since it involves a fermentation process, steps must be taken to ensure that absolutely no contamination is introduced at any point during production. To this end, the medium and all of the processing equipment are thoroughly steam sterilized.

During manufacturing, the quality of all the compounds is checked on a regular basis. Of particular importance are frequent checks of the condition of the microorganism culture during fermentation. These are accomplished using various chromatography techniques. Also, various physical and chemical properties of the finished product are checked such as pH, melting point, and moisture content.

In the United States, antibiotic production is highly regulated by the Food and Drug Administration (FDA). Depending on the application and type of antibiotic, more or less testing must be completed. For example, the FDA requires that for certain antibiotics each batch must be checked by them for effectiveness and purity. Only after they

have certified the batch can it be sold for general consumption. Since the development of a new drug is a costly proposition, pharmaceutical companies have done very little research in the last decade. However, an alarming development has spurred a revived interest in the development of new antibiotics. It turns out that some of the disease-causing bacteria have mutated and developed a resistance to many of the standard antibiotics.

This could have grave consequences on the world's public health unless new antibiotics are discovered or improvements are made on the ones that are available. This challenging problem will be the focus of research for many years to come.
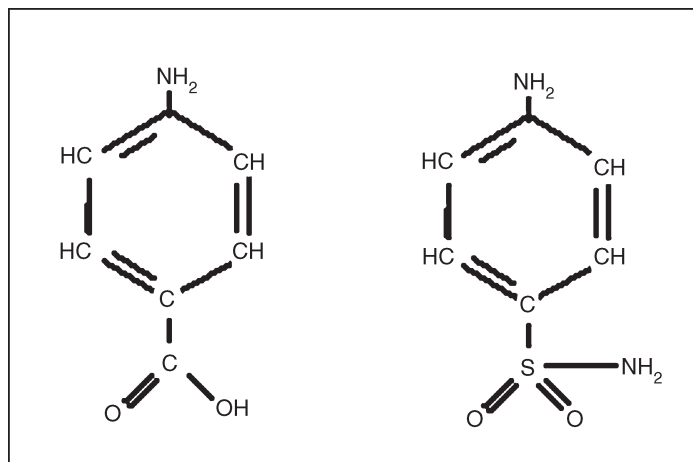
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Synthetic Antibiotics

ANTIBIOTICS

SULFA DRUGS

Sulfanilamide was the first antibacterial agent. Many other sulfa drugs (such as sulfamethoxazole) have since come into use.

THE CHINK IN THE ARMOR

Both bacteria and their human hosts require folic acid for:
- Nucleic acid synthesis (it is converted into purines and thymidine) as well as
- Protein synthesis (precursor of the amino acids methionine and glycine)

However,
- Bacteria synthesize their folic acid starting with para-aminobenzoic acid (PABA), while
- We must ingest our folic acid already formed; that is, for us it is a vitamin.

Sulfanilamide, and the other sulfa drugs, are analogs of PABA; they compete with PABA and, when chosen, block the synthesis of folic acid. Mammals ignore PABA and its analogs and thus can tolerate sulfa drugs.

FOLIC ACID ANALOGS

These synthetic molecules block the final step in the conversion of PABA to folic acid so they, too, block nucleotide and protein synthesis in bacteria but not in mammals.

Trimethoprim is one of several in current use. These folic acid analogs are often used in combination with a sulfa drug.
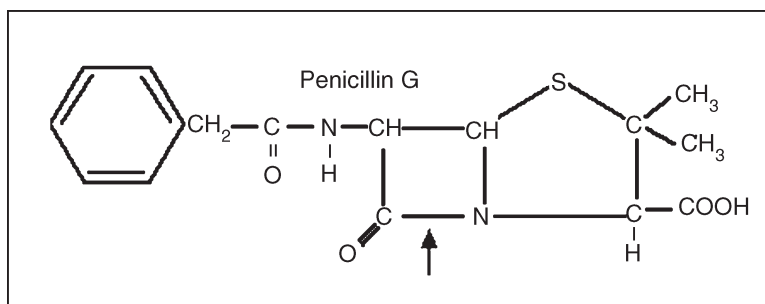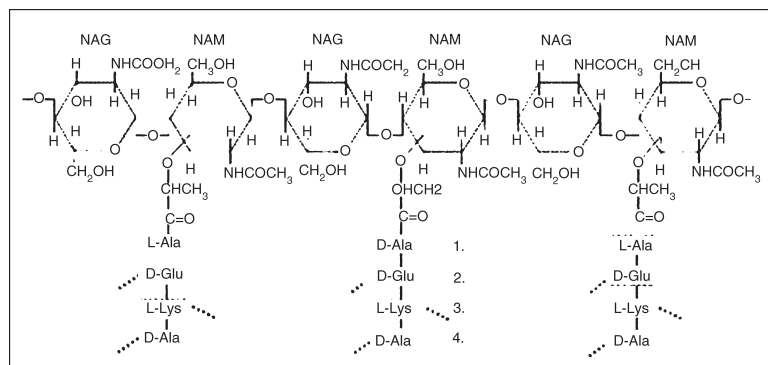
THE BETA-LACTAMS

The beta-lactams get their name from the characteristic ring structure — shown here in blue — that they all share. (The green arrow shows the bond that is broken by the beta-lactamases that are synthesized by many penicillin-resistant bacteria.)

They include the:

- Penicillins such as
  - Penicillin G (a natural product) produced by the fungus Penicillium chrysogenum
  - Ampicillin (a semi-synthetic)
  - Amoxicillin (semi-synthetic)
- Cephalosporins There are over two dozen of them in current use. Most are semi-synthetics derived from the secretion of the mold Cephalosporium. Some examples:
  - Cephalexin (*e.g.*, Keflex®)
  - Cefaclor (*e.g.*, Ceclor®)
  - Cefixime (*e.g.*, Suprax®)
- Carbapenems such as
  - Meropenem (Merrem®)
  - Ertapenem (Invanz®)

The Chink in the Armor = the Bacterial Cell Wall

The beta-lactams all work by interfering with the synthesis of the bacterial cell wall — a structure that is not found in eukaryotes. The walls of bacteria are made of a complex polymeric material called peptidoglycan. It contains both amino acids and amino sugars.

The amino sugars are of two kinds:

1. N-acetylglucosamine (NAG) and its close relative
2. N-acetylmuramic acid (NAM).

These two form a linear polymer of NAG alternating with NAM. They are linked by aglycosidic bond between the #1 and #4 carbons and are oriented in the same way they are in cellulose.

Side chains containing 4 or 5 amino acids are attached to each NAM. These form covalent bonds with amino acids in adjacent chains.

● =NAM   ● =NAG   〰〰 = Peptide Bridage
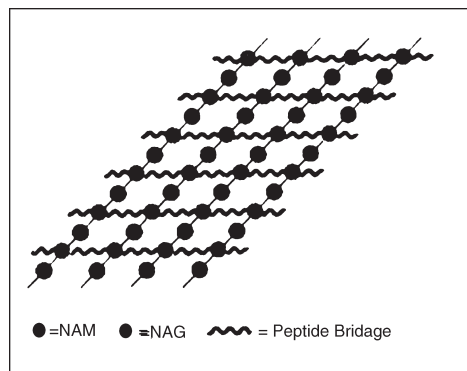
The bonds may:

- Be direct to the next chain or
- Include additional peptide cross bridges (*e.g.*, 5 glycine residues) which
- Extend to chains in the same plane (shown here) as well as to chains above and below.

This elaborate, covalently cross-linked structure provides the great strength of the cell wall. It also leads to the

remarkable conclusion that the bacterial cell wall meets the definition of a single molecule!

The beta-lactam antibiotics bind to and inhibit enzymes needed for the synthesis of the peptidoglycan wall. While they have little effect on resting bacteria, they are lethal to dividing bacteria as defective walls cannot protect the organism form bursting in hypotonic surroundings.

AMINOGLYCOSIDES

These are products of actinomycetes (soil bacteria) or semi-synthetic derivatives of the natural products.

EXAMPLES ARE

- Streptomycin
- Kanamycin
- Neomycin
- Gentamycin

The Chink in the Armor = the bacterial ribosome

The 70S bacterial ribosome differs in several ways from the 80S eukaryotic ribosome. The aminoglycosides bind to the 30S subunit of the bacterial ribosome and

- Interfere with the formation of the initiation complex
- Cause misreading of the mRNA.

Although the eukaryotic ribosome in the cytosol is relatively unaffected by these drugs, ribosomes in the mitochondria are 70S and sensitive to their effects.

TETRACYCLINES

These are natural products derived from soil actinomycetes or their semi-synthetic derivatives.

Examples:

- Chlortetracycline (trade name = "aureomycin")
- Oxytetracycline (trade name = "terramycin")
- Doxycycline

The Chink in the Armor = the bacterial ribosome

Tetracyclines bind to the 30S subunit of the bacterial ribosome. They prevent the transfer of activated amino acids to the ribosome so protein synthesis is halted.

MACROLIDES, LINCOSAMIDES, STREPTOGRAMINS

The Chink in the Armor = the bacterial ribosome

All these antibiotics bind to the 23S rRNA molecule in the large (50S) subunit of the bacterial ribosome where they block the elongation of the growing peptide chain. Because of their similar action, the development of antibiotic resistance to one usually extends to all the others.

MACROLIDES

Macrolides are also products of actinomycetes (soil bacteria) or semi-synthetic derivatives of them.

Erythromycin, azithromycin (Zithromax®), and clarithromycin (Biaxin®) are a commonly-prescribed macrolides.

LINCOSAMIDES

The first member of this group was also isolated from a soil actinomycete (found near Lincoln, Nebraska). A semi-synthetic derivative, called clindamycin(Cleocin®), is now widely used against Gram-positive bacteria.

STREPTOGRAMINS

Quinupristin and dalfopristin are examples. As of 1 October 1999, they will be sold as a mixture under the trade

name Synercid. Combined, they show great promise in treating certain infections resistant to vancomycin — currently the antibiotic of last resort for some hospital-acquired infections.

FLUOROQUINOLONES

Ciprofloxacin (Cipro®), levofloxacin and norfloxacin are examples. Cipro is the preferred antibiotic for people who have been intentionally exposed to anthrax, although some other antibiotics appear to be equally effective.

The Chink in the Armor = DNA topoisomerases

The fluoroquinolones block the action of two bacterial topoisomerases — enzymes that relieve the coils that form in DNA when the helix is being opened in preparation for,

- Replication or
- Transcription or
- Repair

The topoisomerases in eukaryotes are not affected.

POLYPEPTIDES

The most common of these are the polymixins.

They behave as detergents, increasing the permeability of the membranes that encase bacteria and causing the contents of the bacterial cell to leak out.

RIFAMPIN

This semi-synthetic antibiotic binds to the bacterial RNA polymerase and prevents it from carrying out its role in transcription. Its affinity for the equivalent eukaryotic enzyme is much lower. Rifampin is also known as rifampicin.

MUPIROCIN

This antibiotic blocks the action of the bacterial isoleucine tRNA synthetase, the enzyme responsible for attaching the amino acid isoleucine (Ile) to its tRNA in preparation for protein synthesis, so protein synthesis is inhibited. It spares the equivalent eukaryotic enzyme.

CYCLOSERINE

Cycloserine inhibits synthesis of the bacterial cell wall but by a different mechanism than the beta-lactam antibiotics discussed above. Cycloserine is an analog of D-alanine and blocks the incorporation of D-alanine into the peptide bridges in the bacterial cell wall. It is derived from an actinomycete.

AMINOCYCLITOLS

These products of another actinomycete achieve their effect by interfering with the 30S subunit of the bacterial ribosome. Spectinomycin (trade name = Trobicin®) is an example. It is particularly effective against the gonococcus, the bacterium that causes the sexually-transmitted disease (STD) gonorrhea.

GLYCOPEPTIDES

Glycopeptides also interfere with the synthesis of the bacterial cell wall but by a different mechanism than the beta-lactams. Vancomycin is a widely-used glycopeptide in the U.S. It binds to the D-alanines on the precursors of the peptidoglycan cross bridges preventing their cross-linking. It has become the antibiotic of last resort as resistance to the other antibiotics has become more and more common.

OXAZOLIDINONES

The first of these new antibiotics, linezolid, was approved by the U.S. Food and Drug Administration on 19 April 2000. It is effective against many Gram-positive bacteria that have developed resistance to the older antibiotics.

Linezolid attacks a previously-unexploited chink in the bacterium's armor: the proper assembly of the two ribosomal subunits (30S and 50S). It does not affect eukaryotic ribosomes — and thus translation of mRNAs in the cytosol. However, it does affect the bacterial-like mitochondrial ribosomes and can interfere with the synthesis of those mitochondrial proteins synthesized by them.

LIPOPEPTIDES

These are natural compounds derived from a species of Streptomyces. The one now in clinical use is daptomycin (Cubicin®). It is effective against Gram-positive bacteria. It attacks another previously-unexploited chink in the bacterial armor — the integrity of its cell membranes.

So far there is no evidence of bacteria developing resistance against it.

RESISTANCE TO ANTIBIOTICS

None of the antibiotics discussed above is effective against all bacterial pathogens.

INTRINSIC RESISTANCE

Some bacteria are intrinsically resistant to certain of the antibiotics. Example: Gram-positive bacteria are much less susceptible to polymixins than Gram-negative bacteria. [The "Gram" designations refer to the behaviour of the bacteria

when stained with the Gram stain; this behaviour is a reflection of the very different organization of their cell walls.]

ACQUIRED RESISTANCE

Many bacteria acquire resistance to one or more of the antibiotics to which they were formerly susceptible. In the U.S. in the decade from 1985–1995, resistance of Shigella (which causes gastrointestinal illness) to ampicillin grew from 32% to 67%. And, while only 7% of these isolates were resistant to the combination of sulfamethoxazole and trimethoprim at the start of the decade, that figure had grown to 35% by the end of the decade.

Bacteria develop resistance by acquiring genes encoding proteins that protect them from the effects of the antibiotic. In some cases the genes arise by mutation; in others, they are acquired from other bacteria that are already resistant to the antibiotic. The genes are often found on plasmids which spread easily from one bacterium to another — even from one species of bacterium to another.

EXAMPLES

·Synthesis of the enzyme penicillinase — or other beta-lactamases — provides protection from the beta-lactam antibiotics. These enzymes break the beta-lactam ring at the position shown with the green arrow in the diagram of penicillin G.

- Likewise synthesis of cephalosporinases defeats the cephalosporins.
- Defeating quinolones:
  - Some bacteria do this by modifying their DNA gyrase.

- Others, *e.g.*, Mycobacterium tuberculosis, develop quinolone resistance by synthesizing a protein that resembles a short length of DNA. This protein binds the gyrase so it cannot form the DNA/gyrase complex that is the target of quinolone action.

- Some bacteria synthesize "pumps" in their plasma membrane through which they remove antibiotics like tetracyclines from the interior of the cell.

- Bacteria may methylate their ribosomes obscuring the target of antibiotics (*e.g.*, erythromycin) that ordinarily bind to and inactivate the ribosome — or conversely

- They may enzymatically modify the antibiotic (*e.g.*, kanamycin) so it can no longer "see" its ribosomal target.

- Bacteria may modify the structure of their peptidoglycan wall and thus avoid the inhibitory effects of antibiotics like cycloserine.

An alarming number of human pathogens have acquired genes to combat all the presently-used antibiotics except vancomycin and recently vancomycin-resistant bacteria have appeared. These multidrug-resistant strains are particularly common in hospitals where antibiotic use is heavy, and the patients often have weakened immune systems.

ANTIBIOTIC RESISTANCE IN GENETI-CALLY MODIFIED CROPS

Antibiotic-resistance genes are used as "markers" in genetically modified crops. The genes are inserted into the

plant in early stages of development to in order to detect specific genes of interest. *e.g.* herbicide-resistant genes or insecticidal toxin genes. The antibiotic-resistance genes have no further role to play, but they are not removed from the final product. This practice has met with criticism because of the potential that the antibiotic-resistance genes could be acquired by microbes in the environment. In some cases these marker genes confer resistance to front-line antibiotics such as the beta-lactams and aminoglycosides.

INAPPROPRIATE USE OF ANTIBIOTICS IN THE MEDICAL ENVIRONMENT

One problem is the casual use of antibiotics in medical situations where they are of no value. This is the fault of both health care workers and patients. Prescribers sometimes thoughtlessly prescribe 'informed' demanding patients with antibiotics. This leads to use of antibiotics in circumstances where they are of not needed, *e.g.* viral upper respiratory infections such as cold and flu, except when there is serious threat of secondary bacterial infection. Another problem is patient failure to adhere to regimens for prescribed antibiotics. Patients and doctors need to realise their responsibility when they begin an antibiotic regimen to combat an infectious disease.

There are several measures that should be considered:

- Patients should not take antibiotics for which there is no medical value (corollary: doctors should not prescribe antibiotics for which there is no medical value).

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- Patients should adhere to appropriate prescribing guidelines and take antibiotics until they have finished.
- Patients should be give combinations of antibiotics, when necessary, to minimize the development of resistance to a single antibiotic (as in the case of TB).
- Patients need to be given another antibiotic or combination of antibiotics if the first is not working.

COMBATING ANTIBIOTIC RESISTANCE

The following are recommendations to combat the development of antibiotic resistance in bacteria and other microorganisms.

Search for new antibiotics. To combat the occurrence of resistant bacteria, biotechnology and pharmaceutical companies must constantly research, develop and test new antimicrobials in order to maintain a pool of effective drugs on the market.

Stop the use of antibiotics as growth-promoting substances in farm animals. Of major concern is the use of antibiotics as feed additives given to farm animals to promote animal growth and to prevent infections rather than cure infections. The use of such antibiotics contributes to the emergence of antibiotic-resistant bacteria that threaten human health and decreases the effectiveness of the same antibiotics used to combat human infections.

Use the right antibiotic in an infectious situation as determined by antibiotic sensitivity testing, when possible. Stop unnecessary antibiotic prescriptions. Unnecessary

antibiotic prescriptions have been identified as causes for an enhanced rate of resistance development. Unnecessary prescriptions of antibiotics are made when antibiotics are prescribed for viral infections (antibiotics have no effect on viruses). This gives the opportunity for indigenous bacteria (normal flora) to acquire resistance that can be passed on to pathogens.

Finish antibiotic prescriptions. Unfinished antibiotic prescriptions may leave some bacteria alive or may expose them to sub-inhibitory concentrations of antibiotics for a prolonged period of time. *Mycobacterium tuberculosis* is a slow growing bacteria which infects the lung and causes tuberculosis. This disease kills more adults than any other infectious disease. Due to the slow growing nature of the infection, treatment programmes last for months or even years. This has led to many cases on unfinished prescriptions and 5% of strains now observed are completely resistant to all known treatments and hence incurable.

Several other possible solutions have been proposed or implemented to combat antibiotic resistance.

In the pharmaceutical industry, past and current strategies to combat resistance have not been effective. Pharmaceutical companies are seeking new, less costly strategies to develop antibiotics. A decrease in the number of prescriptions for antibiotics, especially in small children, is occurring.

Several countries such as the UK have regulations concerning the use of antibiotics in animal feed. Large scale public health education efforts are underway to stress the

importance of finishing prescriptions. Indeed, in many places, failure to finish tuberculosis prescriptions can result in jail time.

BACTERIAL RESISTANCE TO ANTIBIOTICS

INTRODUCTION

In the past 60 years, antibiotics have been critical in the fight against infectious disease caused by bacteria and other microbes. Antimicrobial chemotherapy has been a leading cause for the dramatic rise of average life expectancy in the Twentieth Century. However, disease-causing microbes that have become resistant to antibiotic drug therapy are an increasing public health problem. Wound infections, gonorrhea, tuberculosis, pneumonia, septicemia and childhood ear infections are just a few of the diseases that have become hard to treat with antibiotics. One part of the problem is that bacteria and other microbes that cause infections are remarkably resilient and have developed several ways to resist antibiotics and other antimicrobial drugs. Another part of the problem is due to increasing use, and misuse, of existing antibiotics in human and veterinary medicine and in agriculture. In 1998, in the United States, 80 million prescriptions of antibiotics for human use were filled. This equals 12,500 tons in one year. Animal and agricultural uses of antibiotics are added to human use. Agricultural practices account for over 60% of antibiotic usage in the U.S., so this adds an additional 18,000 tons per year to the antibiotic burden in the environment.

Nowadays, about 70 per cent of the bacteria that cause infections in hospitals are resistant to at least one of the drugs most commonly used for treatment. Some organisms are resistant to all approved antibiotics and can only be treated with experimental and potentially toxic drugs. An alarming increase in resistance of bacteria that cause community acquired infections has also been documented, especially in the staphylococci and pneumococci (*Streptococcus pneumoniae)*, which are prevalent causes of disease and mortality. In a recent study, 25% of bacterial pneumonia cases were shown to be resistant to penicillin, and an additional 25% of cases were resistant to more than one antibiotic.

Microbial development of resistance, as well as economic incentives, has resulted in research and development in the search for new antibiotics in order to maintain a pool of effective drugs at all times. While the development of resistant strains is inevitable, the slack ways that we administer and use antibiotics has greatly exacerbated the process.

Unless antibiotic resistance problems are detected as they emerge, and actions are taken immediately to contain them, society could be faced with previously treatable diseases that have become again untreatable, as in the days before antibiotics were developed.

HISTORY OF ANTIBIOTICS AND EMERGENCE OF ANTIBIOTIC RESISTANCE

The first antibiotic, penicillin, was discovered in 1929 by Sir Alexander Fleming, who observed inhibition of

staphylococci on an agar plate contaminated by a *Penicillium* mold. Fleming was searching for potential antibacterial compounds. He noticed that a patch of the mold *Penicillium notatum* had grown on a plate containing the bacterium *Staphylococcus* and that around the mold there was a zone where no *Staphylococcus* could grow. After more research, he was able to show that culture broth of the mold prevented growth of the *Staphylococcu* seven when diluted up to 800 times. He named the active substance penicillin but was unable to isolate it.

While Fleming was working on penicillin, Gerhard Domagk, a German doctor, announced the discovery of a synthetic molecule with antibacterial properties. He named the compound Prontosil, and it became the first of a long series of synthetic antibiotics called sulfonamides or sulfa drugs. Prontosil was introduced to clinical use in the 1930s and was used to combat urinary tract infections, pneumonia and other conditions. While sulfa drugs in many cases are not as effective as natural antibiotics, they are now in widespread use for the treatment of many conditions. Gerhard Domagk was awarded the Nobel prize in 1939 for his discovery of Prontosil. In 1946, penicillin became generally available for treatment of bacterial infections, especially those caused by staphylococci and streptococci. Initially, the antibiotic was effective against all sorts of infections caused by these two Gram-positive bacteria. Penicillin had unbelievable ability to kill these bacterial pathogens without harming the host that harbored them. It is important to note that a significant fraction of all human infections are caused by these two bacteria (*i.e.*, strep throat,

pneumonia, scarlet fever, septicemia, skin infections, wound infections, etc.). In the late 1940s and early 1950s, new antibiotics were introduced, including streptomycin, chloramphenicol and tetracycline, and the age of antibiotic chemotherapy came into full being. These antibiotics were effective against the full array of bacterial pathogens including Gram-positive and Gram-negative bacteria, intracellular parasites, and the tuberculosis bacillus. Synthetic antimicrobial agents such as the "sulfa drugs" (sulfonamides) and anti-tuberculosis drugs, such as para aminosalicylic acid (PAS) and isoniazid (INH), were also brought into wider usage.

THE FIRST SIGNS OF ANTIBIOTIC RESISTANCE

There has probably been a gene pool in nature for resistance to antibiotic as long as there has been for antibiotic production, for most microbes that are antibiotic producers are resistant to their own antibiotic. In retrospect, it is not surprising that resistance to penicillin in some strains of staphylococci was recognized almost immediately after introduction of the drug in 1946. Likewise, very soon after their introduction in the late 1940s, resistance to streptomycin, chloramphenicol and tetracycline was noted. By 1953, during a *Shigella* outbreak in Japan, a strain of the dysentery bacillus (*Shigella dysenteriae*) was isolated which was multiple drug resistant, exhibiting resistance to chloramphenicol, tetracycline, streptomycin and the sulfonamides. Over the years, and continuing into the present almost every known bacterial pathogen has developed resistance to one or more antibiotics in clinical use. Evidence also began to accumulate that bacteria could

pass genes for drug resistance between strains and even between species. For example, antibiotic-resistance genes of staphylococci are carried on plasmids that can be exchanged with *Bacillus, Streptococcus* and *Enterococcus* providing the means for acquiring additional genes and gene combinations. Some are carried on transposons, segments of DNA that can exist either in the chromosome or in plasmids. In any case, it is clear that genes for antibiotic resistance can be exchanged between strains and species of bacteria by means of the processes of horizontal gene transmission (HGT).

MULTIPLE DRUG RESISTANT ORGANISMS

Multiple drug resistant organisms are resistant to treatment with several, often unrelated, antimicrobial agents as described above in *Shigella*.

Some of the most important types of multiple drug resistant organisms that have been encountered include:

- MRSA - methicillin/oxacillin-resistant *Staphylococcus aureus*
- VRE - vancomycin-resistant enterococci
- ESBLs - extended-spectrum beta-lactamases (which are resistant to cephalosporins and monobactams)
- PRSP - penicillin-resistant *Streptococcus pneumoniae*

MRSA and VRE are the most commonly encountered multiple drug resistant organisms in patients residing in non-hospital healthcare facilities, such as nursing homes and other long-term care facilities. PRSP are more common in patients seeking care in outpatient settings such as physicians' offices and clinics, especially in pediatric settings.

ESBLs are most often encountered in the hospital (intensive care) setting, but MRSA and VRE also have a significant nosocomial ecology.

METHICI LLIN-RESISTANT STAPH AUREUS

MRSA refers to "methicillin-resistant *Staphylococcus aureus*", which are strains of the bacterium that are resistant to the action of methicillin, and related beta-lactam antibiotics (*e.g.* penicillin and cephalosporin). MRSA have evolved resistance not only to beta-lactam antibiotics, but to several classes of antibiotics. Some MRSA are resistant to all but one or two antibiotics, notably vancomycin-resistant. But there have been several reports of VRSA (Vancomycin-Resistant Staph Aureus) that are troublesome in the ongoing battle against staph infections. MRSA are often sub-categorized as Hospital-Associated MRSA (HA-MRSA) or Community-Associated MRSA (CA-MRSA), depending upon the circumstances of acquiring disease. Based on current data, these are distinct strains of the bacterial species. HA-MRSA occurs most frequently among patients who undergo invasive medical procedures or who have weakened immune systems and are being treated in hospitals and healthcare facilities such as nursing homes and dialysis centers. MRSA in healthcare settings commonly causes serious and potentially life threatening infections, such as bloodstream infections, surgical site infections or pneumonia.

In the case of HA- MRSA, patients who already have an MRSA infection or who carry the bacteria on their bodies but do not have symptoms (colonized) are the most common

sources of transmission. The main mode of transmission to other patients is through human hands, especially healthcare workers' hands. Hands may become contaminated with MRSA bacteria by contact with infected or colonized patients. If appropriate hand hygiene such as washing with soap and water or using an alcohol-based hand sanitizer is not performed, the bacteria can be spread when the healthcare worker touches other patients.

MRSA infections that occur in otherwise healthy people who have not been recently (within the past year) hospitalized or had a medical procedure (such as dialysis, surgery, catheters) are categorized as community-associated (CA-MRSA) infections. These infections are usually skin infections, such as abscesses, boils, and other pus-filled lesions. About 75 per cent of CA-MRSA infections are localized to skin and soft tissue and usually can be treated effectively. However, CA-MRSA strains display enhanced virulence, spread more rapidly and cause more severe illness than traditional HA-MRSA infections, and can affect vital organs leading to widespread infection (sepsis), toxic shock syndrome and pneumonia. It is not known why some healthy people develop CA-MRSA skin infections that are treatable whereas others infected with the same strain develop severe, fatal infections.

Studies have shown that rates of CA-MRSA infection are growing fast. One study of children in south Texas found that cases of CA-MRSA had a 14-fold increase between 1999 and 2001.

CA-MRSA skin infections have been identified among certain populations that share close quarters or experience

more skin-to-skin contact. Examples are team athletes, military recruits, and prisoners. However, more and more CA-MRSA infections are being seen in the general community as well, especially in certain geographic regions.

Also, CA-MRSA are infecting much younger people. In a study of Minnesotans published in The Journal of the American Medical Association, the average age of people with MRSA in a hospital or healthcare facility was 68. But the average age of a person with CA-MRSA was only 23.

More people in the U.S. now die from MRSA infection than from AIDS. Methicillin-resistant *Staphylococcus aureus* was responsible for an estimated 94,000 life-threatening infections and 18,650 deaths in 2005, as reported by CDC in the Oct. 17, 2007 issue of The Journal of the American Medical Association. The national estimate is more than double the invasive MRSA prevalence reported five years earlier. That same year, roughly 16,000 people in the U.S. died from AIDS, according to CDC. While most invasive MRSA infections could be traced to a hospital stay or some other health care exposure, about 15% of invasive infections occurred in people with no known health care risk. Two-thirds of the 85% of MRSA infections that could be traced to hospital stays or other health care exposures occurred among people who were no longer hospitalized. People over age 65 were four times more likely than the general population to get an MRSA infection. Incidence rates among blacks were twice that of the general population, and rates were lowest among children over the age of 4 and teens.

Extended-Spectrum beta-lactamase (ESBL) - producing Gram-negative bacteria Extended-spectrum beta-lactamases

(ESBLs) are plasmid-associated beta lactamases that have recently been found in the *Enterobacteriaceae*. ESBLs are capable of hydrolyzing penicillins, many narrow spectrum cephalosporins, many extended-spectrum cephalosporins, oxyimino-cephalosporins (cefotaxime, ceftazidime), and monobactams (aztreonam). Beta-lactamase inhibitors (*e.g.* clavulanic acid) generally inhibit ESBL producing strains. ESBL producing isolates are most commonly *Klebsiella ssp*, predominantly *Klebsiella pneumoniae*, and *E. coli*, but they have been found throughout the *Enterobacteriaeae.*

Because ESBL enzymes are plasmid mediated, the genes encoding these enzymes are easily transferable among different bacteria. Most of these plasmids not only contain DNA encoding ESBL enzymes but also carry genes conferring resistance to several non-ß-Lactam antibiotics. Consequently, most ESBL isolates are resistant to many classes of antibiotics. The most frequent coresistances found in ESBL-producing organisms are aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol, and sulfamethoxazole-trimethoprim. Treatment of these multiple drug-resistant organisms is a therapeutic challenge.

ESBL producing strains have been isolated from abscesses, blood, catheter tips, lung, peritoneal fluid, sputum, and throat cultures. They apparently have a world-wide distribution. Rates of isolation vary greatly worldwide and within geographic areas and are rapidly changing over time. In the United States, between 1990 to 1993, a survey of the intensive care units of 400 hospitals recorded an increase from 3.6% to 14.4% in ESBL producing strains of *Klebsiella*. In 1994, the CDC reported that 8% of *Klebsiella*

spp from a few large centers produced ESBLs. In Europe, as of 1995, ESBLs occurred in 20%-25% of *Klebsiella ssp* from patients in ICUs, although they were found in patients up to 30%-40% frequency in France.

Known risk factors for colonization and/or infection with organisms harboring ESBLs include admission to an intensive care unit, recent surgery, instrumentation, prolonged hospital stay and antibiotic exposure, especially to extended-spectrum beta-lactam antibiotics. Use of extended-spectrum antibiotics exerts a selective pressure for emergence of ESBL producing strains. The resistance plasmids can then be transferred to other bacteria, not necessarily of the same species, conferring resistance to them. The lower GI tract of colonized patients is the main reservoir of these organisms. Gastrointestinal carriage can persist for months. In some cities in the United States, nursing homes may be an important reservoir of ESBL producing strains. Nursing home patients are more likely to be treated empirically with antibiotics, and thus on admission to a hospital to be more likely to possess an ESBL producing strain. Patient to patient transmission of ESBL producing organisms occurs via the hands of hospital staff. It is known that ESBL producing strains can survive in the hospital environment. Nosocomial infections in patients occur through the administration of extended spectrum beta-lactam antibiotics or via transmission from other patients via health care workers, who become colonized with resistant strains via exposure to patients or other health care workers. Spread of ESBL producing strains can be minimized by good infection control practices, especially by good hand washing technique.

BACTERIAL MECHANISMS OF ANTIBIOTIC RESISTANCE

Several mechanisms have evolved in bacteria which confer them with antibiotic resistance. These mechanisms can either chemically modify the antibiotic, render it inactive through physical removal from the cell, or modify target site so that it is not recognized by the antibiotic.

The most common mode is enzymatic inactivation of the antibiotic. An existing cellular enzyme is modified to react with the antibiotic in such a way that it no longer affects the microorganism. An alternative strategy utilized by many bacteria is the alteration of the antibiotic target site. These and other mechanisms are shown in the the figure and accompanying table below.
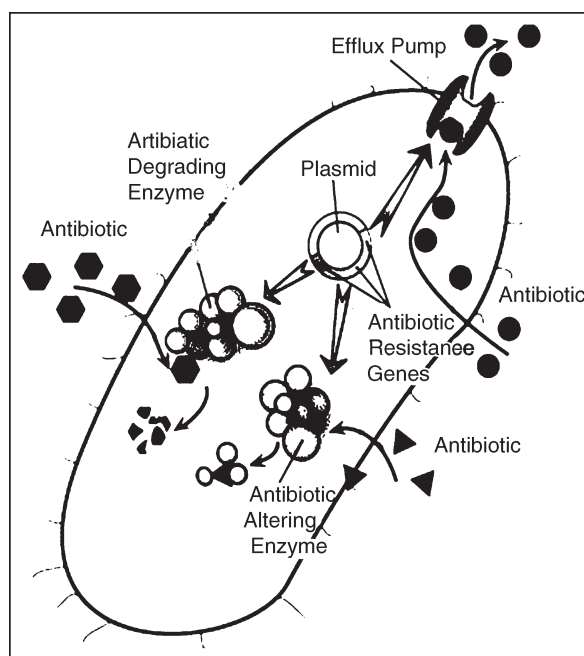
MECHANISMS OF ANTIBIOTIC RESISTANCE IN BACTERIA

Antibiotic	Method of resistance
Chloramphenicol	Reduced uptake into cell
Tetracycline	Active efflux from the cell
β-lactams, Erythromycin, Lincomycin	Eliminates or reduces binding of antibiotic to cell target
β-lactams, Aminoglycosides, Chloramphenicol	Enzymatic cleavage or modification to inactivate antibiotic molecule
Sulfonamides, Trimethoprim	Metabolic bypass of inhibited reaction
Sulfonamides, Trimethoprim	Overproduction of antibiotic target (titration)

THE ACQUISITION AND SPREAD OF ANTIBIOTIC RESISTANCE IN BACTERIA

The development of resistance is inevitable following the introduction of a new antibiotic. Initial rates of resistance to new drugs are normally on the order of 1%. However, modern uses of antibiotics have caused a huge increase in the number of resistant bacteria.

In fact, within 8-12 years after wide-spread use, strains resistant to multiple drugs become widespread. Multiple drug resistant strains of some bacteria have reached the proportion that virtually no antibiotics are available for treatment. Antibiotic resistance in bacteria may be an inherent trait of the organism (*e.g.* a particular type of cell wall structure) that renders it naturally resistant, or it may be acquired by means of mutation in its own DNA or acquisition of resistance-conferring DNA from another source.

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INHERENT (NATURAL) RESISTANCE

Bacteria may be inherently resistant to an antibiotic. For example, an organism lacks a transport system for an antibiotic; or an organism lacks the target of the antibiotic molecule; or, as in the case of Gram-negative bacteria, the cell wall is covered with an outer membrane that establishes a permeability barrier against the antibiotic.

ACQUIRED RESISTANCE

Several mechanisms are developed by bacteria in order to acquire resistance to antibiotics. All require either the modification of existing genetic material or the acquisition of new genetic material from another source.

VERTICAL GENE TRANSFER

The spontaneous mutation frequency for antibiotic resistance is on the order of about of about $10^{-8} - 10^{-9}$. This means that one in every every $10^8 - 10^9$ bacteria in an infection will develop resistance through the process of mutation. In *E. coli*, it has been estimated that streptomycin resistance is acquired at a rate of approximately 10^{-9} when exposed to high concentrations of streptomycin. Although mutation is a very rare event, the very fast growth rate of bacteria and the absolute number of cells attained means that it doesn't take long before resistance is developed in a population.

Once the resistance genes have developed, they are transferred directly to all the bacteria's progeny during DNA replication. This is known as vertical gene transfer or vertical evolution.

The process is strictly a matter of Darwinian evolution driven by principles of natural selection: a spontaneous mutation in the bacterial chromosome imparts resistance to a member of the bacterial population. In the selective environment of the antibiotic, the wild type (non mutants) are killed and the resistant mutant is allowed to grow and flourish

HORIZONTAL GENE TRANSFER

Another mechanism beyond spontaneous mutation is responsible for the acquisition of antibiotic resistance. Lateral or horizontal gene transfer (HGT) is a process whereby genetic material contained in small packets of DNA can be transferred between individual bacteria of the same species or even between different species.

There are at least three possible mechanisms of HGT, equivalent to the three processes of genetic exchange in bacteria. These are transduction, transformation or conjugation. Conjugation occurs when there is direct cell-cell contact between two bacteria (which need not be closely related) and transfer of small pieces of DNA called plasmids takes place. This is thought to be the main mechanism of HGT. Transformation is a process where parts of DNA are taken up by the bacteria from the external environment. This DNA is normally present in the external environment due to the death and lysis of another bacterium.

Transduction occurs when bacteria-specific viruses (bacteriophages) transfer DNA between two closely related bacteria.
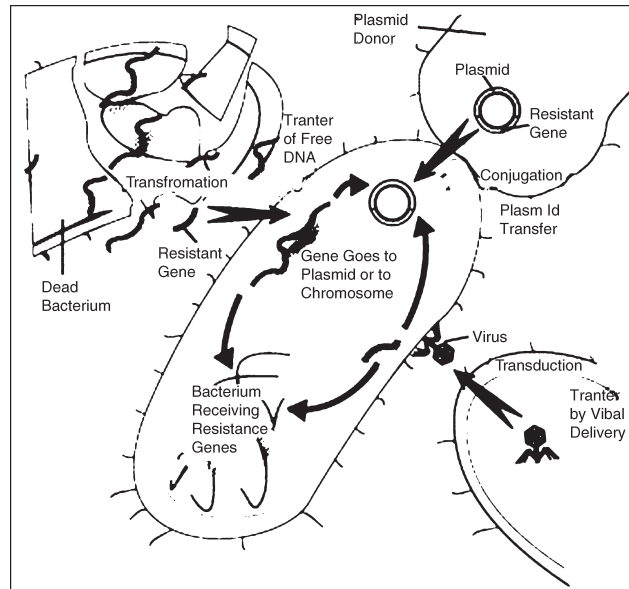
MECHANISMS OF HORIZONTAL GENE TRANSFER (HGT) IN BACTERIA

The combined effects of fast growth rates to large densities of cells, genetic processes of mutation and selection, and the ability to exchange genes, account for the extraordinary rates of adaptation and evolution that can be observed in the bacteria. For these reasons bacterial adaptation (resistance) to the antibiotic environment seems to take place very rapidly in evolutionary time. Bacteria evolve fast!
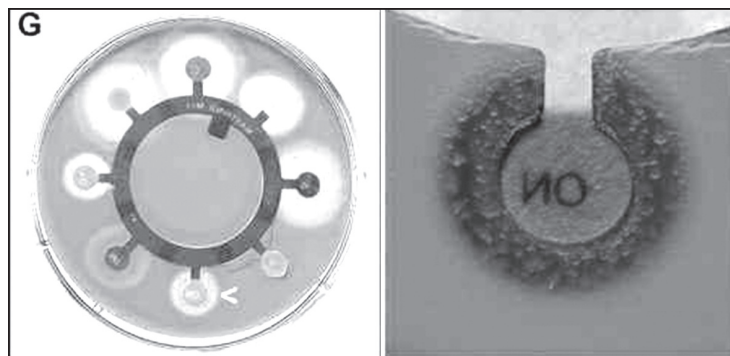
ANTIBIOTICS IN FOOD AND WATER

Prescription drugs are not the only source of antibiotics in the environment. In the United States, antibiotics can be found in beef cattle, pigs and poultry. The same antibiotics then find their way into municipal water systems when the run-off from housing facilities and feedlots contaminates streams and groundwater. So it's a double hit: we get antibiotics in our food and drinking water, and we meanwhile promote bacterial resistance. Routine feeding of antibiotics to animals is banned in the European Union and many other industrialized countries. Maybe they know something we don't.

INDISCRIMINATE USE OF ANTIBIOTICS IN AGRICULTURE AND VETERINARY PRACTICE

The non-therapeutic use of antibiotics in livestock production makes up at least 60 per cent of the total antimicrobial production in the United States. Irresponsible use of antibiotics in farm animals can lead to the development of resistance in bacteria associated with the animal or with people who eat the animal. Such resistance can then be passed on to human pathogens by mechanisms of HGT.

Of major concern is the use of antibiotics as feed additives given to farm animals to promote animal growth and to prevent infections (rather than cure infections). The use of an antibiotic in this way contributes to the emergence of antibiotic-resistant pathogens and reduces the effectiveness of the antibiotic to combat human infections.

SUMMARY

The discovery of antibiotics was a leap in modern medicine. They have been able to stop the growth or kill many different kinds of microorganisms. However, bacteria have proven to be much more innovative and adaptive than we imagined and have developed resistance to antibiotics at an ever increasing pace. Bad practices and mismanagement have only exacerbated the situation. We could soon return to a state of medical health that was as dire as that which occurred prior to antibiotic use. However, with more research, education of the public, and well thought out regulations, the problems can be solved. Several strategies are currently used to find new antibacterial compounds and new strategies are in development and trial.

Not only is there a problem in finding new antibiotics to fight old diseases (because resistant strains of bacteria have emerged), there is a parallel problem to find new antibiotics to fight new diseases. In the past three decades, many "new" bacterial diseases have been discovered (*E. coli* O157:H7 gastric ulcers, Lyme disease, toxic shock syndrome, "skin-eating" streptococci). Already broad patterns of resistance exist in these pathogens, and it seems likely that we will soon need new antibiotics to replace the handful that are effective now against these bacteria, especially as resistance begins to emerge among them in the selective environment antibiotic chemotherapy.

It is said that the discovery and use of antibiotics and immunization procedures against infectious disease are two developments in the field of microbiology that have

contributed about twenty years to the average life span of humans in developed countries where these practices are employed. While the greater part of this span in time is probably due to vaccination, most of us are either still alive or have family members or friends who are still alive because an antibiotic conquered an infection that otherwise would have killed them. If we want to retain this medical luxury in our society we must be vigilant and proactive We must fully understand how and why antimicrobial agents work, and why they don't work, and realise that we must maintain a stride ahead of microbial pathogens that can only be contained by antibiotic chemotherapy.