Principles of Clinical Pharmacology and Therapeutics

and the maximum dosage whereby no toxic symptoms occur is called the therapeutic width.

Finally, administration of a substance can also induce a number of effects which have nothing whatsoever to do with the pharmacological properties of the substance; these are the placebo effects.

GENERAL PHARMACOLOGY

Nomenclature

Pharmacology deals with the knowledge of drugs. Drugs are chemical substances which affect living organisms and are used by the clinician to diagnose, prevent or cure diseases. So the safe use of drugs needs sound knowledge of their various aspects such as mechanism of action, doses, routes of administration, adverse affects, toxicity, drug interactions etc.

A health professional is also interested to know the chemical agents that are commonly responsible for household and industrial poisoning as well as environmental pollution so that he may prevent, recognize and treat such toxicity or pollution.

The word pharmacology is derived from the Greek words pharmakon (drug) and logos (study). The word drug has also a French origin—‘drouge’ (dry herb). In clinical practice, drug is a chemical substance that is used for the diagnosis, prevention and treatment of diseases in appropriate doses. WHO (1966) definition of a drug is any substance or product that is used or intended to be used to modify or to explore physiological system or pathological states for the benefit of the recipient.

Following are the major subdivisions of pharmacology:

- Pharmacy is a branch of pharmacology that deals with identification, selection, preservation, combining, analyzing, standardization, preparing, compounding and dispensing of medicines for administration to the patient. A pharmacist prepares compounds and dispenses medicines to the patient upon a written order of a licensed medical practitioner.

- Pharmacognosy is a term derived from the Greek word ‘gnosis’ which means knowledge. It is a branch of pharmacology that deals with the sources of drugs derived from plants and animals. It is also a study of physical and
chemical properties of such substances.

- Pharmacokinetics is a term derived from the Greek word ‘kinesis’ meaning a movement. It deals with the time course of drug absorption, distribution, metabolism and excretion. In other words, it means “What the body does to the drug”. It provides a rational basis for doses of a drug and helps in dosage adjustment in altered physiological and pathological states like aging, renal or hepatic impairment.

- Pharmacodynamics (Greek ‘dynamics’ means force) is the study of physiological and biochemical effects of drugs, mechanisms of action and the relationship of the plasma concentration of the drug with its response and the duration of action. In other words, it means “What the drug does to the body”.

- Pharmacotherapeutics (Greek ‘therapia’ means medical treatment) deals with the use of drugs in the diagnosis, treatment or prevention of a disease or their purposeful use in alteration of physiological functions for the benefit of the recipient. In other words, it is the clinical application of the pharmacokinetic and pharmacodynamic knowledge of the drug.

- Therapeutics deals with the science and art of treatment of diseases. When therapy is based on clinical evidence it is called Empirical Therapeutics. It means the drug is effective, although its mode of action is unknown.

- Chemotherapy deals with the use of chemotherapeutic agents to inhibit or destroy invading microbes, parasites or cancer cells with minimal effect on healthy living tissues.

- Toxicology (Greek ‘toxicon’ means poison) is the science of poisons. It deals with the adverse effects of drugs and poisonous effects of various chemicals (household, environmental, industrial or homicidal). It is also concerned with their source, chemical composition, action, tests for detection and antidotes. Clinical toxicology is the science of detection, diagnosis and treatment of poisoning.

- Pharmacogenetics is a relatively new field. It deals with genetically mediated variations in drug responses.

- Clinical Pharmacology is a branch of pharmacology that deals with the pharmacological effects of drugs in man. It gives useful data about the potency, usefulness, doses and toxicity of new drugs for their safe clinical use.

- Biopharmaceutics deals with the development of new drug delivery systems and new dosage forms. It also provides information how these dosage forms can influence the pharmacodynamic and pharmacokinetic properties of a drug.

- Medicinal Chemistry is the science of designing and synthesis of a new drug. It is based on the structure activity relationship data of existing drugs belonging to one generic group.

**DRUG INFORMATION SOURCES**

Most useful drug information sources are textbooks, drug reference books, drug compendia and journal articles. They provide information about established drugs and furnish information for understanding newer ones.

However, they do not include many other details such as trade names, physical and chemical properties, identification criteria, standards of purity and strength, methods of storage and dosage range for therapeutic use which are necessary from a legal point of view for drug control.

All these details of drugs are provided by Pharmacopoeias and Formulary. They are collectively known as Drug Compendia.

Pharmacopoeias are prepared by a committee, which usually has predominance of physicians. Formulary is prepared by a committee, which usually has predominance of pharmacists. Then there are non-official sources of information.

- Pharmacopoeia: It is an official code containing a selected list of the established drugs and medicinal preparations with descriptions of their physical properties, identification, purity, potency and the minimum standard required and the average dose for adults. Each country has its own pharmacopeia. 
  
  For example:
  
  - British Pharmacopeia
  - United States Pharmacopeia
  - Indian Pharmacopeia
  - European Pharmacopeia
  - Russian Pharmacopeia
Synthetic Sources

At present majority of drugs used in clinical practice are prepared synthetically, such as aspirin, oral antidiabetics, antihi-stamines, amphetamine, chloroquine, chlorpromazine, general and local anaesthetics, paracetamol, phenytoin, synthetic corticosteroids, sulphonamides and thiazide diuretics.

Advantages of synthetic drugs are:
- They are chemically pure.
- The process of preparing them is easier and cheaper.
- Control on the quality of the drug is excellent.
- Since the pharmacological activity of a drug depends on its chemical structure and physical properties, more effective and safer drugs can be prepared by modifying the chemical structure of the prototype drug.

Natural Sources

Drugs are obtained from the following natural sources.

Plants: Following categories of drugs are derived from roots, leaves or barks of plants.

Alkaloids

- These are nitrogenous heterocyclic bases, which are pharmacologically active principles of plants.
- They are composed of carbon, hydrogen, nitrogen and oxygen.
- They are bitter in taste and are often poisonous. These are, therefore, used in small doses.
- They are insoluble in water. However, they form salts with acids which are soluble in water.

<table>
<thead>
<tr>
<th>Alkaloid</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atropine</td>
<td>Atropa belladonna</td>
</tr>
<tr>
<td>Quinine</td>
<td>Cinchona bark</td>
</tr>
<tr>
<td>Morphine</td>
<td>Papavurum somniferum</td>
</tr>
</tbody>
</table>
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- They do not have marked pharmacological activity and have little pharmacological use except castor oil (purging) or arachis oil (demulcent).
- They may be of vegetable origin e.g. olive oil, castor oil, croton oil and peanut oil or of animal origin e.g. cod liver oil, shark liver oil and lard.
- *Mineral Oils* are mostly petroleum products and extracted by fractional distillation.
- These are mixtures of hydrocarbons of the methane and related aliphatic series.
- These are extracted in various consistencies - hard paraffin, soft paraffin and liquid paraffin.
- Hard and soft paraffins are used as vehicles for preparation of ointments while liquid paraffin is employed as a purgative.

**Gums**

Gums are colloidal exudates from plants which are polysaccharides chemically and yield simple sugars on hydrolysis.

- Upon addition of water, some of them swell or dissolve or form adhesive mucilage or remain unchanged.
- *Uses*: In gut agar and psyllium seeds act as hydrophilic colloids and function as bulk purgatives.
- Gum acacia and gum tragacanth are used as suspending agents in making emulsions and mixtures.

**Resins**

Resins are ill-defined solid substances found in plants, and are polymers of volatile oil.

- They are produced by oxidation and polymerization of volatile oils.
- They are insoluble in water but soluble in alcohol, chloroform and ether.
- *Examples*: Oleoresins (aspidium); gum resins (asafoetida); oleogum resin (myrrh); balsams (benz-oin, tolu, peru); benzoin shellac, podophyllum.
- *Uses*: Benzoin is used as inhalation in common cold.
- Tincture benzoin is applied as antiseptic protective sealing over bruises.
Minerals or their salts are useful pharmacotherapeutic agents. 
*For example:*
- Ferrous sulfate is used in iron deficiency anaemia.
- Magnesium sulfate is employed as purgative.
- Magnesium trisilicate, aluminium hydroxide and sodium bicarbonate are used as antacids for hyperacidity and peptic ulcer.
- Kaolin (aluminium silicate) is used as adsorbent in antidiarrheal mixtures.
- Radioactive isotopes of iodine, phosphorus, gold are employed for the diagnosis/treatment of diseases particularly malignant conditions.

**SEMISYNTHETIC SOURCES**

Sometimes semi-synthetic processes are used to prepare drugs when the synthesis of drugs (complex molecules) may be difficult, expensive and uneconomical or when the natural sources may yield impure compounds. Some examples are semisynthetic human insulin and 6-aminopenicillanic acid derivatives.

**BIOSYNTHETIC SOURCES (genetically engineered drugs)**

This is relatively a new field which is being developed by mixing discoveries from molecular biology, recombinant DNA technology, DNA alteration, gene splicing, immunology and immunopharmacology.

Some of the recent developments are genetically engineered novel vaccines (Recombivax HB - a hepatitis-B vaccine), recombinant DNA engineered insulins (Humulin- human insulin) for diabetes.

**DRUG NOMENCLATURE**

Three types of names are assigned to every drug:

1. **Chemical Name:** This name is given according to the chemical constitution of a drug. It indicates the precise arrangement of atoms and atomic groups in the molecule. However, chemical names are too complex and cumbersome to be used in prescription.
2. **Non-proprietary/Generic Name:** When a drug has been found therapeutically useful, it is given a non-proprietary name by
the United States Adopted Name (USAN) council. These names are used uniformly all over the world by an international agreement through the W.H.O. Non-proprietary name is called official when included in official books such as Indian, British, United States or International pharmacopeia. The non-proprietary name is often referred to as generic name.

3. **Proprietary/Trade/Brand Name:** The pharmaceutical company, which sells the non-proprietary drug selects the proprietary name and gets it registered. The trade name then becomes the sole property of the pharmaceutical company. Thus a non-proprietary drug may be marketed under many proprietary names by different firms. Proprietary name is usually smaller than the non-proprietary name and it is most widely used by medical practitioners.

Some examples are:

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>Non-Proprietary Name</th>
<th>Proprietary Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-(4-chlorobenzenesulphonyl-3-propylurea)</td>
<td>Chlorpropamide</td>
<td>Diabinese</td>
</tr>
<tr>
<td>-chlorodihyromethyl phenyl benzodiazepin-2-one.</td>
<td>Diazepam</td>
<td>Valium (Roche, India)</td>
</tr>
<tr>
<td>-2-one.</td>
<td></td>
<td>Calmpose (Ranbaxy, India)</td>
</tr>
</tbody>
</table>

**Prototype Drug:** Usually, attention is focussed on one or at the most two drugs belonging to a group in order to comprehend and co-relate their pharmacological effects with the other drugs of the same group. Drugs selected for this purpose are called prototype drugs e.g. chlorpromazine is a prototype drug for anti-psychotic drugs and morphine is a prototypal drug for narcotic analgesics.

### CATEGORIES OF DRUGS

Drugs may be divided into two categories:

1. **Prescription Drugs:** These drugs are used under medical supervision because these are considered to be unsafe. So they are dispensed only by an order of registered physician. e.g. antibiotics, anxiolytics, antidepressant etc.

2. **Non-Prescription Drugs:** These drugs are considered relatively safe and can be sold without physician’s prescription over the counter (OTC) e.g. vitamins, antacids, paracetamol etc.

### ISSUES IN PHYTOTHERAPY

The part of pharmacognosy focusing on use of crude extracts or semi-pure mixtures originating from nature, namely phytotherapy, is probably the best known and also the most debated area in pharmacognosy.

Although phytotherapy is sometimes connected to alternative medicine, when critically conducted, it may be considered the scientific study on the effects and clinical use of herbal medicines.

### Constituents and Drug Synergyism

One characteristic of crude drug material is that constituents may have an opposite, moderating or enhancing effect. Hence, the final effect of any crude drug material will be a product of the interactions between the constituents and the effect of each constituent on its own. To effectively study the existence and affect of such interactions, scientific studies must examine the affect that multiple constituents, given concurrently, have on the system.

Herbalists assert that as phytopharmaceuticals rely upon synergy for their activities, plants with high levels of active constituents like ginsenosides or hypericin may not correlate with the strength of the herbs. In phytopharmaceutical or herbal medicine, the therapeutic effects of herbs cannot be determined unless its active ingredient or cofactors are identified or the herb is administered as a whole.

One way manufacturers have attempted to indicate strength is to engage in standardization to a marker compound. Companies use different markers, or different levels of the same markers, or different methods of testing for marker compounds. Many herbalists believe that the active ingredient in a plant is the plant itself.

### Herb and Drug Interactions

The Sloan Kettering Memorial Cancer Centre stated, in a review of a juice product, which had been marketed as preventing cancer, that antioxidants could theoretically interfere with chemotherapy.

A recent review of the effect of antioxidants on chemotherapy, however, found no evidence for any deleterious effects of antioxidants on chemotherapy. A study of herb drug interactions...
indicated that the vast majority of drug interactions occurred in four classes of drugs, the chief class being blood thinners, but also including protease inhibitors, cardiac glycosides and the immuno-suppressant ciclosporin.

**Natural products chemistry**

Most bioactive compounds of natural origin are secondary metabolites, i.e., species-specific chemical agents that can be grouped into various categories. A typical protocol to isolate a pure chemical agent from natural origin is bioassay-guided fractionation, meaning step-by-step separation of extracted components based on differences in their physicochemical properties, and assessing the biological activity, followed by next round of separation and assaying.

Typically, such work is initiated after a given crude drug formulation (typically prepared by solvent extraction of the natural material) is deemed “active” in a particular *in vitro* assay. If the end-goal of the work at hand is to identify which ones of the scores or hundreds of compounds are responsible for the observed *in vitro* activity, the path to that end is fairly straightforward:

1. Fractionate the crude extract, e.g. by solvent partitioning or chromatography.
2. Test the fractions thereby generated with *in vitro* assay.
3. Repeat steps 1) and 2) until pure, active compounds are obtained.
4. Determine structure(s) of active compound(s), typically by using spectroscopic methods.

In *vitro* activity does not necessarily translate to activity in humans or other living systems. The most common means for fractionation are solvent-solvent partitioning and chromatographic techniques such as high-performance liquid chromatography (HPLC), medium-pressure liquid chromato-graphy, “flash” chromatography, open-column chromato-graphy, vacuum-liquid chromatography (VLC), thin-layer chromatography (TLC), with each technique being most appropriate for a given amount of starting material.

Countercurrent chromatography (CCC) is particularly well-suited for bioassay-guided fractionation because, as an all-liquid separation technique, concern about irreversible loss or denaturation of active sample components is minimized.

After isolation of a pure substance, the task of elucidating its chemical structure can be addressed. For this purpose, the most powerful methodologies available are nuclear magnetic resonance spectroscopy (NMR) and mass spectroscopy (MS). In the case of drug discovery efforts, structure elucidation of all components that are active *in vitro* is typically the end goal.

In the case of phytotherapy research, the investigator may use *in vitro* BAGF as a tool to identify pharmacologically interesting or important components of the crude drug. The work does not stop after structural identification of *in vitro* actives, however. The task of “dissecting and reassembling” the crude drug one active component at a time, in order to achieve a mechanistic understanding of how it works in phytotherapy, is quite daunting.

This is because it is simply too difficult, from cost, time, regulatory, and even scientific perspectives, to study experimental fractions of the crude drug in humans. *In vitro* assays are therefore used to identify chemical components of the crude drug that may rationally be expected to have a given pharmacological effect in humans, and to provide a rational basis for standardization of a crude drug formulation to be tested in [and sold/marketed to] humans.

**Loss of Biodiversity**

Farnsworth for example, has found that 25% of all prescriptions dispensed from community pharmacies in the United States from 1959 to 1980 contained active ingredients extracted from higher plants. In some countries in Asia and Africa 80% of the population relies on traditional medicine (including herbal medicine) for primary health care.

 Constituents of substances used by traditional healers, have rarely been incorporated into modern medicine. Quinine, physostigmine, d-tubocurarine, pilocarpine and ephedrine, have been demonstrated to have active effects Knowledge of traditional medicinal practices is fast disappearing, particularly in the Amazon, as native healers die out and are replaced by more modern medical practitioners. Botanists and pharmacologists are racing to learn these ancient practices, which, like the forest plants they employ, are also endangered.

An explanation for some species loss is habitat lost due to invasive species introduction. Herbalist David Winston has suggested that a high proportion of nonnative species seen as invasive (kudzu, Japanese knotweed, mimosa, lonicera, St. Johnswort and purple
For instance, ginseng which is field farmed may have significant problems with fungus, making contamination with fungicides an issue.

This may be remedied with woods grown programme, but they are insufficient to produce enough ginseng to meet demand. The wildcrafted echinacea, black cohosh and American ginseng often rely upon old growth root, often in excess of 50 years of age and it is not clear that younger stock will have the same pharmaceutical effect. Black cohosh may be adulterated with the related Chinese actea species, which is not the same. Ginseng may be replaced by ginseniodes from Jiaogulan which has been stated to have a different effect than the full panax root.

The problem may be exacerbated by the growth of pills and capsules as the preferred method of ingesting medication as they are cheaper and more available than traditional, individually tailored prescriptions of raw medicinals but the contents are harder to track. Seahorses are a case in point: Seahorses once had to be of a certain size and quality before they were accepted by practitioners and consumers.

But declining availability of the preferred large, pale and smooth seahorses has been offset by the shift towards prepackaged medicines, which make it possible for TCM merchants to sell previously unused juvenile, spiny and dark-coloured animals. Today almost a third of the seahorses sold in China are prepackaged.

The farming of plant or animal species, used for medicinal purposes has caused difficulties. Rob Parry Jones and Amanda Vincent write:

- One solution is to farm medicinal animals and plants. Chinese officials have promoted this as a way of guaranteeing supplies as well as protecting endangered species. And there have been some successes—notably with plant species, such as American ginseng—which is used as a general tonic and for chronic coughs. Red deer, too, have for centuries been farmed for their antlers, which are used to treat impotence and general fatigue. But growing your own is not a universal panacea. Some plants grow so slowly that cultivation in not economically viable. Animals such as musk deer may be difficult to farm, and so generate little profit. Seahorses are difficult to feed and plagued by disease in captivity. Other

Sustainable Sources of Plant and Animal Drugs

As species face loss of habitat or overharvesting, there have been new issues to deal with in sourcing crude drugs. These include changes to the herb from farming practices, substitution of species or other plants altogether, adulteration and cross-pollination issues.
species cannot be cultivated at all. Even when it works, farming usually fails to match the scale of demand. Overall, cultivated TCM plants in China supply less than 20 per cent of the required 1.6 million tonnes per annum. Similarly, China’s demand for animal products such as musk and pangolin scales far exceeds supply from captive-bred sources.

- Farming alone can never resolve conservation concerns, as government authorities and those who use Chinese medicine realise. For a start, consumers often prefer ingredients taken from the wild, believing them to be more potent. This is reflected in the price, with wild oriental ginseng fetching up to 32 times as much as cultivated plants. Then there are welfare concerns. Bear farming in China is particularly controversial. Around 7600 captive bears have their bile “milked” through tubes inserted into their gall bladders. The World Society for the Protection of Animals states that bear farming is surrounded by “appalling levels of cruelty and neglect”. Chinese officials state that 10 000 wild bears would need to be killed each year to produce as much bile, making bear farming the more desirable option. The World society for the Protection of Animals, however, states that “it is commonly believed in China that the bile from a wild bear is the most potent, and so farming bears for their bile cannot replace the demand for the product extracted from wild animals”.
- One alternative to farming involves replacing medical ingredients from threatened species with manufactured chemical compounds. In general, this sort of substitution is difficult to achieve because the active ingredient is often not known. In addition, most TCM users believe that TCM compounds may act synergistically so several ingredients may interact to give the required effect. Thus TCM users often prefer the wild source. Tauro ursodeoxycholic acid, the active ingredient of bear bile, can be synthesised and is used by some Western doctors to treat gallstones, but many TCM consumers reject it as being inferior to the natural substance from wild animals.

CHEMICAL PROPERTIES

Amphetamine is a chiral compound. The racemic mixture can be divided into its optical isomers: levo- and dextro-amphetamine. Amphetamine is the parent compound of its own structural class, comprising a broad range of psychoactive derivatives, from empathogens, MDA (3,4-Methylenedioxyamphetamine) and MDMA (3,4-Methylenedioxy-N-methamphetamine) known as ecstasy, to the N-methylated form, methamphetamine known as ‘meth’, and to decongestants such as ephedrine (EPH). Amphetamine is a homologue of phenethylamine. At first, the medical drug came as the salt racemic-amphetamine sulfate (racemic-amphetamine contains both isomers in equal amounts). Attention disorders are often treated using Adderall or a generic equivalent, a formulation of mixed amphetamine and dextroamphetamine salts that contain

- 1/4 dextroamphetamine saccharate
- 1/4 dextroamphetamine sulfate
- 1/4 (racemic dextro/laevo-amphetamine) aspartate monohydrate
- 1/4 (racemic dextro/laevo-amphetamine) sulfate

Pharmacodynamics

Amphetamine has been shown to both diffuse through the cell membrane and travel via the dopamine transporter (DAT) to increase concentrations of dopamine in the neuronal terminal. Amphetamine, both as d-amphetamine (dextroamp-hetamine) and l-amphetamine (or a racemic mixture of the two isomers), is believed to exert its effects by binding to the monoamine transporters and increasing extracellular levels of the biogenic amines dopamine, norepinephrine (noradrenaline) and serotonin.

It is hypothesized that d-amphetamine acts primarily on the dopaminergic systems, while l-amphetamine is comparatively norepinephrinergic (noradrenergic). The primary reinforcing and Behavioural-stimulant effects of amphetamine, however, are linked to enhanced dopaminergic activity, primarily in the mesolimbic dopamine system. Amphetamine and other amphetamine-type stimulants principally act to release dopamine into the synaptic cleft. The increased amphetamine concentration releases endogenous stores of dopamine from vesicular monoamine transporters (VMATs), thereby increasing intra-neuronal concentrations of transmitter. This increase in concentration effectively reverses transport of dopamine via the dopamine transporter (DAT) into the synapse.

In addition, amphetamine binds reversibly to the DATs and
blocks the transporter’s ability to clear DA from the synaptic space. Amphetamine also acts in this way with norepinephrine (noradrenaline) and to a lesser extent serotonin. In addition, amphetamine binds to a group of receptors called TrAce Amine Receptors (TAAR). TAAR are a newly discovered receptor system which seems to be affected by a range of amphetamine-like substances called trace amines.

Physical Effects of Pharmacology

Physical effects of amphetamine can include reduced appetite, increased/distorted sensations, hyperactivity, dilated pupils, flushing, restlessness, dry mouth, erectile dysfunction, headache, tachycardia, increased breathing rate, increased blood pressure, fever, sweating, diarrhea, constipation, blurred vision, impaired speech, dizziness, uncontrollable movements or shaking, insomnia, numbness, palpitations, arrhythmia. In addition, increased awareness of one’s body’s sensations can cause the user to perceive certain physical effects, such as sudden hot or coldness, a flushed face, or very rapid heart rate. In high doses or chronic use convulsions, dry or itchy skin, acne, pallor can occur.

Occasionally amphetamine use in males can cause an odd and sometimes startling effect to occur in which the penis when flaccid appears to have shrunk. The reason this occurs is because amphetamine is a potent vasoconstrictor or an agent that constricts blood vessels. The rigidity of the erection and the size of the penis are in part by affected by the amount of blood flow to the penis. When amphetamine constricts the blood vessels enough it reduces blood flow to the penis and then can produce a penis that is slightly smaller and this effect is often coupled along with impotence and erectile dysfunction. Upon erection the penis returns to normal size.

PSYCHOLOGICAL EFFECTS

Psychological effects of amphetamine can include anxiety and/
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Amphetamines are used by college and high-school students as a study and test-taking aid. Amphetamines work by increasing energy levels, concentration, and motivation, thus allowing students to study for an extended period of time. These drugs are often acquired through ADHD prescriptions to students and peers, rather than illicitly produced drugs. Amphetamines have been, and are still, used by militaries around the world. British troops used 72 million amphetamine tablets in the second world war and the RAF used so many that “Methedrine won the Battle of Britain” according to one report.

American bomber pilots use amphetamines (“go pills”) to stay awake during long missions. The Tarnak Farm incident, in which an American F-16 pilot killed several friendly Canadian soldiers on the ground, was blamed by the pilot on his use of amphetamine. A nonjudicial hearing rejected the pilot’s claim.

Amphetamine is also used by professional, collegiate and high school athletes for its strong stimulant effect. Energy levels are perceived to be dramatically increased and sustained, which is believed to allow for more vigorous and longer play. However, at least one study has found that this effect is not measurable. The use of amphetamine during strenuous physical activity can be extremely dangerous, especially when combined with alcohol and athletes have died as a result, for example, British cyclist Tom Simpson. Amphetamine use has historically been especially common among Major League Baseball players and is usually known by the slang term “greenies”. In 2006, the MLB banned the use of amphetamines. The ban is enforced through periodic drug-testing. If a player tests positive for amphetamine, the consequences are significant.

However, the MLB has received some criticism because the consequences for amphetamine use are dramatically less severe than for anabolic steroid use, with the first offense bringing only a warning and further testing. Truck drivers, especially long-haul drivers, often take amphetamine to combat symptoms of somnolence and to increase their concentration on driving.

**ACETOACETIC ESTER SYNTHESIS OF AMPHETAMINE**

Amphetamine can be synthesized by the sequential alkylation
of methyl acetoacetate with dimethyl sulfate and benzyl chloride, followed by hydrolysis and deacetylation to give 2-phenylpropionic acid which through reaction with thionyl chloride and ammonia forms 2-phenylpropionamide. Upon treatment with aqueous sodium hypochlorite, this amide undergoes Hofmann rearrangement to form racemic amphetamine (phenyl-2-aminopropane). The order in which methyl acetoacetate is alkylated with dimethyl sulfate and benzyl chloride is of utmost importance to produce the desired dialkylacetoacetate isomer. If methyl acetoacetate is benzylated before it is methylated, the methyl group adds to the benzylic carbon instead of on the acetoacetate alpha-carbon.

By first methylating the sodium salt of methyl acetoacetate and then benzylate the sodium salt of the formed alpha-methylacetoacetic ester, the formation of the desired isomer is ensured. Methyl acetoacetate was prepared from methyl acetate in good yields. Results of experiments using dimethyl sulfate or methyl iodide to alkylate methyl acetoacetate indicated that it was possible to get a higher yield of methyl methyl acetoacetate using methyl iodide, but its higher cost do not warrant its use.

Instead of using the route going through intermediates 5-6, tests indicate that methyl benzyl methyl acetoacetate will be transformed to 2-Phenylpropionamide in 25% aqueous ammonia to the extent of approximately 50% in two weeks, standing at room temperature.

![Chemical diagram](image)

**Methyl Methyl Acetoacetate**

4440 grams of methyl acetate, containing 2% methyl alcohol, was weighed into a 12L flask provided with a reflux condenser. 230g of sodium metal in the form of small pieces (~1 cm$^3$) was added to the methyl acetate at once. Heat was applied to bring the reaction mixture to reflux. After 11h all of the sodium dissolved. Excess methyl acetate was then distilled from the reaction mixture until all of the methanol azeotrope distilled off.

5 L of toluene was then added and distillation continued until the last of the methyl acetate was recovered. 1200g of dimethyl sulfate was then added over a period of 2h at refluxing temperature. Refluxing was continued until reaction was neutral. The reaction mixture was then cooled to room temperature, and 1400 mL of water added to dissolve the sodium methyl sulfate. The oil layer was separated, washed with 2 x 1000 mL water and then fractionately distilled to give 882g methyl methyl acetoacetate, bp 76-76.5°C/20mmHg. 1700g of methyl acetate was recovered as constant boiling mixture, balance was recovered with the toluene.

**Methyl Benzyl Methyl Acetoacetate**

750 grams of methyl methyl acetoacetate and 1690 mL of methanol were placed in a 3 L 3-neck flask provided with a reflux. 125 g of sodium metal was added, keeping the temp of the solution at 50°C. The solution was then added to 657g of benzyl chloride in a 5-liter flask. 2 h were required for the addition, keeping the temperature between 48-53°C. After several hours standing, allowing reaction to reach room temp, a test portion indicated that the reaction was 99.5% complete. Excess alcohol was then distilled off until a liquid temp of 83°C was reached. The reaction product was then cooled to 20°C, and 1400 mL of water was added to dissolve out salt. The oil was shaken with 10% NaOH for 10 min and then washed with 500 mL portions of water until neutral. The residual oil was then fractionately distilled to give 855g of methyl benzyl methyl acetoacetate and recovery of 165g benzyl chloride.

**2-Phenylpropionic Acid**

855 grams of methyl benzyl methyl acetoacetate from the above run was refluxed with a sodium methoxide solution (17g Na in 321mL methanol) for 3-4h, and then the constant boiling mixture of methyl acetate/methanol was slowly distilled off in the course of another 1.5h. The resulting benzyl methyl acetic acid methyl ester was then hydrolyzed by the addition of 120g of 30% aqueous NaOH. The sodium salt was given two extractions, using 200 mL of xylene each time. The methyl benzyl acetic acid was liberated from the sodium salt by the addition of 50% H$_2$SO$_4$ solution. The oil was washed with water, the water washes were combined, extracted with xylene, and then added to the methyl benzyl acetic acid. The xylene was distilled from the acid under vacuum. A yield of 567g of 2-
phenylpropionic acid was obtained, bp 150-155°C/8mmHg.

2-Phenylpropionyl Chloride

502g of thionyl chloride was weighed into a 2-liter 3-neck flask provided with a thermometer, agitator, dropping funnel and reflux condenser. 472g of the above described methyl benzyl acetic acid was then added over a period of one hour. The temperature during addition varied between 30-40°C. The excess thionyl chloride was then distilled off, and the acid chloride vacuum distilled. Yield 420g of 2-phenylpropionyl chloride, bp 118-120°C/15mmHg.

2-Phenylpropionamide

420g of methyl benzyl acetyl chloride, formed as above, was converted to the amide by adding the chloride slowly to 4260mL of toluene (or ether) saturated with NH₃ at 20°C, the NH₃ always being in excess. After all of the chloride was in the reaction product was heated on a steam bath to 62°C, and the separated out ammonium chloride filtered off. The filtrate was then cooled to 10°C, and the crystals of the 2-phenylpropionamide filtered and dried. Yield 336g methyl 2-phenylpropionamide. Upon recrystallization from toluene there was obtained 286g of amide having a mp of 108.4°C.

Phenyl-2-Aminopropane

230g of 2-phenylpropionamide prepared as above (mp 107-108.4°C) was added to sodium hypochlorite solution, made by passing 109g of chlorine into a solution of 277g of sodium hydroxide in 453 mL of water. The reaction mixture was held at 0°C for one hour. It was then slowly heated to 18°C, at which point considerable heat was given off and the solid went into solution. The flask, at this stage, had to be immersed in a freezing bath to prevent the temperature from getting too high. After the temperature was under control, the solution was heated to 58°C, whereupon the rearrangement occurred. The heating was continued until 70°C was reached. The solution was cooled; the oil layer separated and the solution extracted with toluene, using 60 mL each time.

The toluene solution was washed twice with 50 mL portions of water and 148g of conc HCl slowly added to it. The aqueous solution was extracted with 2x30 mL toluene. The amine was then liberated with 30% NaOH. The water from the precipitated amine was extracted with 3x60 mL portions of toluene. The toluene solution was washed with 2x100 mL water and then vacuum distilled. Yield: 131g (69%) of purified amine, bp 105°C/30mmHg. The bp at atmospherical pressure was 205-206°C, and the HCl salt had mp 146°-150°C.

Amphetamine psychosis

Amphetamine psychosis is a form of psychosis which can result from amphetamine or methamphetamine use. Typically it appears after large doses or chronic use, although in rare cases some people may become psychotic after relatively small doses. Other chemicals or drugs which similarly increase dopamine function. Amphetamine psychosis can include delusions, hallucinations and thought disorder. This is thought to be largely due to the increase in dopamine and perhaps serotonin activity in the mesolimbic pathway of the brain caused by amphetamine-like drugs, although other factors such as chronic sleep deprivation may also play a part.

The link between amphetamine and psychosis is one of the major sources of evidence for the dopamine hypothesis of schizophrenia. The link between amphetamine and psychosis was first made by Young and Scoville in 1938 and was originally considered to be a rare condition.

As amphetamine use increased after World War II, largely due to the widespread use of amphetamine compounds in nasal decongestant and dieting preparations, it became clear that chronic amphetamine use often led to psychotic symptoms. Hallucinations are frequently reported in chronic amphetamine users, with over 80% of users reporting the presence of hallucinatory experiences, typically as visual or auditory experiences. Delusions, paranoia, fears about persecution, hyperactivity and panic are also reported as the most common features.

Concurrent to having delusions and hallucinations, chronic amphetamine users may also display stereotyped, repetitive and seemingly purposeless movements, known as ‘motor stereotypies’ or more commonly as ‘knick knacking’, ‘tweaking’ or being ‘hung-up’. These may include examining, sorting, disassembling, and cleaning. The article on punding gives a more complete description of this Behaviour. This Behaviour may appear similar to the symptoms of OCD. One particular manifestation of psychosis associated with amphetamine use is delusional parasitosis or Ekbom’s syndrome, where a person falsely believes themselves to be infested with parasites.

However, related behaviour may occur in non-psychotic
conditions, where users will realise they are not infested by parasites but will pick at their skin anyway. This more closely resembles obsessive-compulsive disorder. However, it is important to note that in the above account, the Behaviour may be similar but the ideation is radically different.

There is no ideational connection between compulsive self-grooming and a delusional belief that one is infested with parasites - the “coke horrors” is William S. Burroughs called it.

**DRUG DISTRIBUTION**

When a drug is introduced into the body, where it ends up depends on a number of factors:

- Blood flow, tissues with the highest blood flow receive the drug first,
- Protein binding, drugs stuck to plasma proteins are crippled, they can only go where the proteins go (and that’s not very far!), 3) lipid solubility and the degree of ionisation, this describes the ability of drugs to enter tissues (highly lipid soluble/ unionised drugs can basically go anywhere).

**Protein Binding**

Most drugs bind to proteins, either albumin or alpha-1 acid glycoprotein (AAG), to a greater or lesser extent. Drugs prefer to be free, it is in this state that they can travel throughout the body, in and out of tissues and have their biological effect. The downside of this is that they are easy prey for metabolising enzymes.

As you would expect, more highly bound drugs have a longer duration of action and a lower volume of distribution. Generally high extraction ratio drugs’ clearance is high because of low protein binding and, conversely, low extraction ratio drugs’ clearance is strongly dependent on the amount of protein binding.

Why is this important? If a drug is highly protein bound, you need to give loads of it to get a therapeutic effect; as so much is stuck to protein. But what happens if another agent comes along and starts to compete with the drug for the binding site on the protein? Yes, you guessed it, the amount of free drug is increased. This is really important for drugs that are highly protein bound: if a drug is 97% bound to albumin and there is a 3% reduction in binding (displaced by another drug), then the free drug concentration doubles; if a drug is 70% bound and there is a 3% reduction in binding, this will make little difference.

The drugs that you really need to keep an eye on are: warfarin, diazepam, propranolol and phenytoin. For example, a patient on warfarin is admitted with seizures, you treat the patient with phenytoin, next thing you know - his INR is 10.

The amount of albumin does not appear to be hugely relevant. In disease states such as sepsis, the serum albumin drops drastically, but the free drug concentration does not appear to increase.

**Degree of ionization**

This is really important with regard to local anaesthetics. The essential fact to know is that highly ionized drugs cannot cross lipid membranes (basically they can’t go anywhere) and unionised drugs can cross freely. Morphine is highly ionised, fentanyl is the opposite. Consequently the latter has a faster onset of action. The degree of ionisation depends on the pKa of the drug and the pH of the local environment. The pKa is the the pH at which the drug is 50% ionised. Most drugs are either weak acids or weak bases. Acids are most highly ionised at a high pH (i.e. in an alkaline environment). Bases are most highly ionised in an acidic environment (low pH). For a weak acid, the more acidic the environment, the less ionised the drug, and the more easily it crosses lipid membranes. If you take this acid, at pKa it is 50% ionised, if you add 2 pH points to this (more alkaline), it becomes 90% ionised, if you reduce the pH (more acidic) by two units, it becomes 10% ionised. Weak bases have the opposite effect.

Local anaesthetics are weak bases: the closer the pKa of the local anaesthetic to the local tissue pH, the more unionised the drug is. That is why lignocaine (pKa 7.7) has a faster onset of action than bupivacaine (pKa 8.3). If the local tissues are alkalinised (e.g. by adding bicarbonate to the local anaesthetic), then the tissue pH is brought closer to the pKa, and the onset of action is hastened.

**PHARMACOGENOMICS**

The term pharmacogenomics (or pharmacogenetics, the two terms can be used interchangeably) is derived from pharmacology (study of pharmaceuticals) and genetics, so it is the study of how a person’s body reacts to pharmaceuticals, given that person’s specific genetic make-up. Widespread application of pharmacogenetics is not
done at present, but medical scientists believe that it has great potential to improve current therapies. By knowing an individual’s genetic profile, a doctor would be able to prescribe the correct medication, at the correct dosage. The risk of adverse reactions, side effects and overdosage would therefore be minimal.

The basis of pharmacogenomics is the identification of SNPs (pronounced as ‘snips’, derived from the abbreviation for ‘single-nucleotide polymorphisms’). SNPs are differences between individual human beings of a single base pair in their DNA. In the past, the sequencing of a person’s DNA was a lengthy and expensive procedure, but with the development of the DNA microarray (or DNA chip, as it is also called) the sequencing can be done quickly. SNPs can be used to map and identify specific genes that play a role in diseases such as diabetes, cancer and arthritis.

The proteins that these genes encode for can become targets for new therapies. As such, pharmacogenomics can play an important role in oncology, treatment of high blood cholesterol levels, tailoring treatment for people with psychiatric disorders, treatment for people with cardiovascular diseases.

However, the use of pharmacogenomics to ‘individualise’ treatment is still in its infancy. Pharmacogenetic testing to determine an individual’s possible reaction against treatment is currently most advanced in Scandinavian countries. Here it is mainly used for CYP2D6 genotyping (this word used for the first time), which aids individual dose selection to treat psychiatric illness.

**The Role of Biotechnology in Developing Vaccines**

The word ‘vaccine’ was derived from the Latin for ‘cow’ (vacca)–referring to Edward Jenner’s discovery in 1796 that milkmaids, who were in frequent contact with cowpox were immune against the dreaded smallpox. A vaccine is a harmless biological preparation that is given to humans to make them immune against a specific disease. The human body’s immune system recognises the vaccine as being ‘foreign’, destroys it, but also ‘remembers’ what this foreign matter looked like. When the body then actually encounters the ‘real’ disease (or virulent form), the immune system recognises it and will be ready to fight off the infection. Scientists may take one of several routes to develop a vaccine, depending on how the disease-causing microbe infects body cells, how the body’s immune system reacts, physical characteristics of the microbe and also where the vaccine is going to be used. The various approaches are the following:

**Live, Attenuated Vaccines**

These vaccines contain a version of the disease-causing microbe that has been weakened (attenuated), so that it cannot cause disease but only prompt the immune system to remember it. Live attenuated vaccines cause a very strong immune reaction, so that only one or two doses generally give lifelong immunity. It is mostly used against viral diseases, such as measles, mumps and chickenpox. It is not safe to use a live attenuated vaccine on a person with a weakened immune system (HIV-positive individuals or patients receiving chemotherapy). These vaccines also need to be refrigerated to stay potent, limiting their use in some developing countries. There is also the remote possibility that the weakened microbe might mutate back to its virulent form and cause disease.

**Inactivated Vaccines**

In this case, the disease-causing microbe is killed with chemicals, heat or radiation, and not just weakened. The microbe can therefore not mutate back to its virulent form. However, this type of vaccine does not produce such a strong immune reaction, so additional immunisation (‘booster shots’) are necessary. Inactivated vaccines are freeze-dried, so they can be stored easily–making them better for use in developing countries. Examples of inactivated vaccines are those against cholera, bubonic plague and hepatitis A.

**Subunit Vaccines**

These vaccines do not include the entire disease-causing microbe, but only the antigens that stimulate the immune system the most. Antigens are ‘markers’ on the surface of a microbe, and this is the part that is recognised by the immune system’s T-cells, and to which the T-cells bind. Scientists can make the antigens from the microbes in the laboratory using recombinant DNA technology. In this case the vaccine is called a recombinant subunit vaccine, such as the vaccine against the hepatitis B virus.

**Toxoid Vaccines**

These are vaccines that are used against bacteria that secrete toxins, for example diphtheria and tetanus. A toxoid vaccine is made by treating the toxin with formalin, rendering the toxin harmless. The vaccine causes the immune system to produce antibodies against the
The conference concluded that research on recombinant DNA should be ruled by strict guidelines, which were then issued by the National Institutes of Health. According to these guidelines, all experiments and trials concerning human gene transfer ('gene therapy trials') making use of recombinant DNA technology must be reviewed by the Recombinant DNA Advisory Committee of the NIH. Researchers who receive funding from the NIH for their work (many South African scientists receive funding from this source), or who conduct their research at facilities receiving funding from the NIH, are bound by these guidelines. However, researchers who receive private funding, and who conduct their research at privately funded institutions, are not bound by the guidelines.

Locally, research institutions such as the Medical Research Council has an Ethics Committee that is registered with the Office for Human Research Protection in the USA. The mandate of the MRC's Ethics Committee is to review all applications for funding of medical research to ensure that the goals of the project do not violate the sanctity of life and obey all rules. The Committee has developed a set of research guidelines on various forms of research, including human genetic research. In addition, every tertiary institution where such research is conducted, also has its own ethics committee that oversees research experiments.

South Africa has no specific law that governs the biotechnology industry or research field, but according to Prof. Tony Bunn and Dr. Michelle Mulder of the MRC's Innovation Centre, has legislation that governs different aspects of the subject. The Department of Science and Technology published a National Biotechnology Strategy in 2001, which outlines the government's plans to build the biotechnology industry in South Africa. Legislation mainly covers safety and ethical issues; as well as intellectual property rights. Safety and ethical issues are dealt with in the National Health Act No 61 of 2003 (Department of Health), which contains a chapter on the use of blood, blood products, tissue and human reproductive cells (sperm and ova) during medical research.

The Act also addresses the issue of human cloning. Intellectual property rights are covered from two fronts. The Companies and Intellectual Properties Registration Office (CIPLO), which forms part of the South African Department of Trade and Industry has laws that are applicable to biotechnology (for example Section 25 of the Patent Act, Act 57 of 1978 applies to scientific discoveries). The Department

Conjugate Vaccines

Many harmful bacteria have an outer coating of sugar molecules known as polysaccharides. This coating hides the antigens (markers) on the surface of the bacteria so that the immature immune system of a child or baby cannot recognise it. Scientists link antigens or toxoids from a microbe that an immature immune system can recognise to the polysaccharides, thereby making a conjugate vaccine. The vaccine against Haemophilus influenzae type B (Hib) is a conjugate vaccine.

DNA Vaccines

These vaccines are still in the experimental phase, but several types are already being tested in humans. A DNA vaccine only uses the genes of the microbe that code for the antigens of that microbe. When those genes enter the body, they are taken up by body cells, and then instruct the body cells to produce antigens. The antigens then stimulate the immune response. DNA vaccines are easy to produce and store. Vaccines against herpes and influenza are currently being tested.

Recombinant Vector Vaccines

Recombinant vector vaccines are similar to DNA vaccines, but they use an attenuated virus or bacterium as a vector to carry the DNA of the disease-causing microbe into the body. The vector virus then ‘infects’ body cells, thereby delivering the DNA to the body cells. Researchers are working on viral-based and bacterium-based recombinant vector vaccines against HIV, rabies and measles. A vaccine can be monovalent (immunizes against one disease), or multivalent (immunizes against more than one disease, or two or more strains of the same disease-causing microbe).

Regulation and Legislation of Biotechnology

The regulatory process ruling the use and development of biotechnology started soon after Boyer and Cohen’s discovery of the recombinant DNA technique. In mid-1974, scientists called for a voluntary moratorium on certain experiments with recombinant DNA. This was followed by the Asilomar Conference, during which scientists from all over the world, lawyers and government officials debated the way forward.

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exactly whether genes have more than one function. So if genes are replaced during gene therapy, this might influence other body processes.

- Most genetic disorders involve more than one gene, as well as interaction with the environment. Diet, lifestyle and other environmental factors play an important role. For example, genetic tests can show whether a woman carries a gene mutation at position BRCA1 (BRCA1 is the name of the gene, not a location: rewrite it as follows: “For example, genetic tests can show whether a woman carries a mutation in the BRCA1 gene.” This puts her at risk for breast cancer. But not everybody that carries this mutation develops breast cancer. Conversely, if genetic tests reveal that a person, for example, does not have a gene putting him at risk for cardiovascular disease, this might lead to carelessness.

BEHAVIORAL PHARMACOLOGY OF MUSCARINIC RECEPTORS

There is substantial pharmacological evidence that central cholinergic neurons are important in the acquisition and post-acquisition performance of a variety of learned behaviors. Many studies have demonstrated that antimuscarinic agents such as scopolamine and atropine have deleterious affects on such behaviors. Similarly, compounds (such as physostigmine) that enhance central cholinergic tone by inhibiting the catabolic enzyme acetylcholinesterase (AChE) can, under certain circumstances, enhance performance in learning and memory tasks. In addition, at appropriate doses a variety of muscarinic receptor agonists can enhance performance on tests of learning and memory. This body of pharmacological research has provided strong evidence that unspecified cholinergic systems in the brain play important roles in the acquisition and performance of learned tasks.

At present, there is no consensus about the psychological mechanisms underlying antimuscarinic-induced deficits. Disruptions of behavioral inhibition, working (short term) memory, retrieval from reference (long term) memory, attention, decisional processes, movement and strategy selection, and altered sensory processing are among the variables that have been proposed as mediating the
sources that implicate the hippocampus in short-term memory functions. In contrast to its hippocampal effects, when Dunnett et al. injected scopolamine into the prefrontal cortex it produced dose-dependent but delay-independent deficits in performance, suggesting a nonmnemonic, possibly attentional, basis to this disturbance.

CHOLINERGIC BASAL NUCLEAR COMPLEX: LESION STUDIES

Although various aspects had been considered earlier, the formal presentation of the “cholinergic hypothesis of geriatric memory dysfunction” was proposed by Bartus et al. in 1982.

The two central notions of the hypothesis were that:

- Forebrain cholinergic systems provide an essential substrate for a variety of cognitive processes, particularly those involved in learning and memory, and
- The learning and memory deficits of aging are attributable, at least in part, to a decline in the functional integrity of those forebrain cholinergic systems.

In its original formulation, this hypothesis was considered in the context of normal aging. However, several lines of evidence, including the loss of cortical cholinergic markers in Alzheimer’s disease (AD), the discovery of a correlation between these biochemical measures and mental test scores in AD patients, and the loss/atrophy of the basal forebrain neurons themselves, led Coyle et al. (9) to extend the cholinergic hypothesis to Alzheimer’s dementia—that is, to suggest that the more profound memory deficits of AD may also be attributable to extensive degeneration of the same forebrain cholinergic systems.

However, many other neuroanatomical and neurochemical systems also degenerate in AD, so that it is extremely difficult to establish a causal relationship with the cholinergic decline specifically. With this issue in mind, the formulation of the cholinergic hypothesis in 1982 stimulated a large number of studies in the subsequent decade which sought to investigate whether the explicit destruction of magnocellular cholinergic neurons in the basal forebrain by axon-sparing excitotoxins would produce a profile of deficits in experimental animals similar to the changes observed in the aged animal or comparable to more profound changes in learning and memory capacity that is such a distinctive feature of human dementia.
The expectation was that reproduction of comparable deficits by an explicit and selective experimental intervention would provide direct evidence in favor of the cholinergic systems having a truly causal role in their genesis. Unfortunately, the early enthusiasm that accompanied the introduction of this strategy has been tempered by subsequent findings that have identified a number of its shortcomings. These issues have been discussed extensively elsewhere and are therefore not reviewed in detail here. Suffice it to say that the most serious limitation of the lesion strategy derives from the fact that wherever they occur in the basal forebrain, cholinergic neurons are intermingled with populations of noncholinergic cells. This being the case, it is uncertain that the deficits in behavior that are produced by excitotoxin lesions of the basal forebrain are due specifically to the loss of cholinergic neurons.

What is needed for lesion-based strategies to contribute definitive information concerning the functions of central cholinergic systems is a toxin that is selective for cholinergic neurons. Unfortunately, at present no such compound exists. Despite the above-mentioned limitation of excitotoxin-lesion-based strategies, it is increasingly clear that animals with extensive lesions of the cholinergic neurons that form the nucleus basalis magnocellularis (nBM) (Structure and Function of Cholinergic Pathways in the Cerebral Cortex, Limbic System, Basal Ganglia, and Thalamus of the Human Brain) can perform normally in a variety of learning and memory tests (18, 22).

This is particularly evident in studies that have used quisqualic acid or α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) to lesion the basal forebrain in the region of the nBM. On the basis of such evidence, Dunnett et al. (18) have proposed that the mnemonic deficits described in the earlier studies that utilized ibotenic acid lesions of the basal forebrain are not attributable to destruction of cholinergic neurons but occur as a result of damage to corticostriatal output systems that course through the globus pallidus. If this is correct, what then are the functional consequences of damage to the telencephalic projections of the nBM? Recent data suggest that impaired attentional function may be one important consequence.

One of the first studies to demonstrate this was conducted by Robbins et al. (52), who used a 5-choice serial reaction time task to show that quisqualic acid lesions in the region of the nBM produced deficits in visual attentional function in rats. Subsequently this group of investigators has demonstrated that AMPA-induced lesions of the nBM produce similar impairments in this test of visual attention and that these deficits can be ameliorated by low doses of the AChE inhibitor physostigmine (40).

While reversibility by procholinergic drugs does not prove that a lesion-induced deficit is due to a damaged cholinergic system (22), it is clearly the most straightforward explanation for such a finding. In addition, and as will be seen below, data from a variety of other sources are compatible with the hypothesis that cortical projections of the cholinergic basal nuclear complex are neural substrates for some attentional functions.

**FUNCTIONAL CORRELATES OF CORTICAL ACETYLCHOLINE RELEASE**

It has been known for nearly 30 years that acetylcholine (ACh) release in the cortex increases markedly during EEG desynchronization. This is consistent with data from other approaches showing that the activity of neurons in the region of the nBM is increased during EEG desynchrony and that lesions of the nBM produce EEG slowing (Day et al. have shown that behavioral measures of arousal such as locomotor activity also correlate positively with cortical ACh release. In the context of the attentional hypothesis of cortical cholinergic function, recent data obtained with brain microdialysis have provided significant support.

Specifically, Day and Fibiger have shown that d-amphetamine potently increases ACh release in the cortex of awake, behaving animals. Methylphenidate has similar actions (Day and Fibiger, unpublished observations). Inasmuch as these compounds are known to improve attention in humans and are the treatment of choice in attention deficit disorder, their positive actions on cortical ACh release are entirely consistent with this hypothesis.

Subsequent pharmacological analysis has shown that stimulation of D1 dopamine receptors is the primary mechanism through which d-amphetamine produces these effects and raises the possibility that D1 receptor agonists may have therapeutic applications in the treatment attention deficit disorders (11, 12). The fact that scopolamine impairs performance on some attentional tasks in humans, particularly those that require active allocation of attentional capacity, is also consistent with the attentional hypothesis (16, 46).

Similarly, Sahakian et al.) recently demonstrated that
tetrahydroaminoacridine (THA), which among other actions is an AChE inhibitor, improves performance on certain tests of attentional function in patients with mild to moderate Alzheimer’s disease. It is noteworthy that this occurred in the absence of significant effects on tests of mnemonic function.

While these results suggest that central cholinergic mechanisms are involved in the regulation of attentional processes, they do not of course provide any information about the anatomical locus of such effects. At present, the only data pointing to a role for the nBM-cortical projection are the above-mentioned excitotoxin lesion studies in rodents, and as indicated earlier the interpretation of these results is far from straightforward.

In summary, while existing evidence is compatible with a role for this cholinergic projection in attention, definitive evidence regarding the validity of this hypothesis awaits the results of future research.

Clinical significance of cholinergic degeneration in Alzheimer’s disease: implications for cholinergic-based pharmacotherapies

As indicated above, the functions of the telencephalic projections of the cholinergic basal nuclear complex are not yet firmly established in animals. Similarly, the behavioral consequences of the degeneration of this complex in Alzheimer’s disease remain unknown (also Biological Markers in Alzheimer’s Disease, Experimental Therapeutics, and Cognitive Impairment in Geriatric Schizophrenic Patients: Clinical and Postmortem Characterization). Despite this, over the past decade many drug discovery programs have been based on the assumption that the cholinergic hypothesis of Alzheimer’s dementia will eventually be validated and that pharmacological restoration of central cholinergic tone will therefore be of significant therapeutic value in the treatment of this condition. There are, however, a number of reasons to be skeptical about this line of reasoning. Perhaps the most important is that this strategy assumes that postsynaptic targets of degenerating cholinergic terminals remain relatively intact in Alzheimer’s disease.

While postsynaptic muscarinic receptors are generally considered to be unaffected in the hippocampus and cortex of Alzheimer’s patients (1), there is abundant evidence that these structures undergo marked degeneration during the course of the disease. Indeed, it is possible that the primary pathological processes in Alzheimer’s disease occur in these telencephalic structures and that the damage to the cholinergic neurons in the basal forebrain is secondary and represents retrograde degeneration.

Given this, the question arises as to whether pharmacological enhancement of cholinergic transmission in these cytoarchitecturally damaged target structures would be expected to reduce the cognitive deficits in Alzheimer’s disease. Unless the loss of basal forebrain neurons in Alzheimer’s disease is the earliest degenerative event and unless there is a significant period during which the hippocampus and cortex remain relatively intact, hopes for successful cholinomimetic replacement therapies are poorly founded. In addition, the fact that the neuropathology of Alzheimer’s disease is increasingly being understood to involve many noncholinergic, chemically defined systems adds to the concern that procholinergic drugs may not benefit Alzheimer’s patients.

In this context, it is perhaps understandable that this pharmacological strategy has not yet produced clinically significant improvements in these patients. This is not to say that the development of procholinergic drugs may not eventually find important applications in other patient populations. As discussed above, there is evidence that central cholinergic systems may be important neural substrates for attention, an important component of the larger process termed cognition. This being the case, cholinomimetic drugs may prove to be useful in the treatment of attention deficit disorder.

In addition, they may also find applications in the treatment of more mildly impaired, more neurologically intact geriatric individuals such as those suffering from age-related memory loss. A better understanding of the normal functions of central cholinergic systems will be a key step in exploring these possibilities.
3

Applied Pharmacokinetics

The main focus of this course is the practical application of pharmacokinetic principles in day-to-day pharmacy practice. Therefore, the tutorial will cover only those aspects of basic pharmacokinetic theory which apply to dosing of 1 and 2 compartment drugs. This cursory review is not meant to be a substitute for a course in clinical pharmacokinetics. For more in-depth coverage of this complex subject, please refer to the publications listed in the reference section of this review.

- Define pharmacokinetics and clinical pharmacokinetics and differentiate between them.
- Explain the property of kinetic homogeneity.
- Define pharmacodynamics and relate it to pharmacokinetics.
- Define tolerance and relate it to pharmacokinetics.
- Describe the concept of the therapeutic concentration range.
- Define narrow therapeutic index.
- Identify factors that cause interpatient variability in drug disposition and drug response.
- Describe situations in which routine clinical pharmacokinetic monitoring would be advantageous.
- Define both one- and two-compartment models and list the assumptions made about drug distribution patterns in each.
- List the assumptions made when using a one-compartment model to describe the pharmacokinetics of a single intravenous dose.
- Represent graphically the typical natural log of plasma drug concentration versus time curve for a one-compartment model after an intravenous dose.

Basic Pharmacokinetics

- Identify the components of body fluids that make up extracellular and intracellular fluids and know the percentage of each.
- Describe the difference between whole blood, plasma, and serum.
- Define drug clearance and show how it is related to organ blood flow.
- Describe the difference between first- and zero-order elimination and how each appears graphically.

Half-life, Elimination Rate Constant and AUC

- Calculate the elimination rate constant given a natural log of plasma drug concentration versus time curve.
- Define half-life and calculate a drug’s half-life given a natural log of plasma drug concentration versus time curve.
- Define the relationship between half-life and elimination rate constant.
- Define drug clearance and relate it to the area under the plasma drug concentration curve and drug dose.
- Calculate a drug’s volume of distribution, concentration at time zero, and area under the plasma concentration versus time curve, given plasma concentration data after an intravenous drug dose.

Multiple Drug Administration

- Describe the principle of superposition and how it applies to multiple drug dosing.
- Define steady state and describe how it relates to a drug’s half-life.
- Provide the equation that estimates peak plasma concentration after multiple drug dosing and the equation that estimates trough concentration after multiple drug dosing (both at steady state).
- Understand the equation for accumulation factor at steady state.
Relationships of Pharmacokinetic Parameters
- Describe how changes in dose or dosing interval affect plasma concentrations after multiple dosing.
- Describe the relationship between the rate of continuous intravenous drug infusion, drug clearance, and steady-state plasma concentration.
- Calculate plasma drug concentrations during and after continuous IV infusion.
- Calculate an appropriate loading dose to achieve therapeutic range at onset of infusion.
- Calculate peak and trough concentrations at steady state after intermittent IV infusions.

Two Compartment Models
- Describe when to use back-extrapolation versus method of residuals.
- Calculate a residual line.
- Calculate alpha ($\alpha$), beta ($\beta$), and intercepts A and B for a drug conforming to a two-compartment model.
- Describe when to use a monoexponential versus a biexponential equation.
- Calculate $V_C$, $V_{area}$ (also known as $V_B$), and $V_{ss}$ (using both methods) for a two-compartment model.

Biopharmaceutics: Absorption
- Define and understand the factors that comprise the term biopharmaceutics.
- Understand the effects of the extent and rate of absorption of a drug on plasma concentrations and area under the curve (AUC).
- Name factors that can affect a drug’s oral bioavailability.
- Define and understand the relationship of bioavailability to drug absorption and AUC.
- Define and be able to calculate an $F$ factor for a drug given its intravenous (IV) and oral absorption time vs. concentration AUCs.
- Define and understand the factors involved in the oral absorption model.
- Understand the pharmacokinetic differences and clinical utility of controlled-release products.
- Name several techniques used in formulating controlled-release drugs.

Drug Distribution and Protein Binding
- Understand the major factors that affect drug distribution.
- Know the relative perfusion (i.e., high or low) characteristics of various body compartments (e.g., kidneys, fat tissue, lungs).
- Understand the physiochemical properties that affect drug distribution.
- Know the three main proteins that bind various drugs and their characteristics.
- Know the major factors that affect drug protein binding.
- Understand the dynamic processes involved in drug protein binding.
- Understand the difference between perfusion-limited distribution and permeability-limited distribution.

Drug Elimination Processes
- Understand the impact of disease and altered physiologic states on the clearance and dosing of drugs.
- Know the various routes of drug metabolism and excretion.
- Understand the two general types (phase I and II) of drug metabolism.
- Understand the methods of hepatic drug metabolism and the approaches used to quantitate and characterize this metabolism.
- Understand the effects of a drug’s hepatic extraction ratio on that drug’s removal via the liver’s first-pass metabolism.
- Understand the various processes involved in renal elimination (i.e., filtration, secretion, and reabsorption).
- Understand both the physiologic and mathematical relationship of drug clearance to glomerular filtration.

Nonlinear Processes
- Describe the relationship of both drug concentration and the plasma drug concentration versus time curve (AUC) to the dose for a nonlinear, zero-order process.
Explain the various biopharmaceutic processes that can result in nonlinear pharmacokinetics.

Describe how hepatic enzyme saturation can result in nonlinear pharmacokinetics.

Use the Michaelis-Menten model for describing nonlinear pharmacokinetics.

Describe V_max and K_m.

Use the Michaelis-Menten model to predict plasma drug concentrations.

Use the t_90% equation to estimate the time required for 90% of the steady-state concentration to be reached.

**Variation and Model Independent Relationships**

Identify the various sources of pharmacokinetic variation.

Explain how the various sources of pharmacokinetic variation affect pharmacokinetic parameters.

Describe how to apply pharmacokinetic variation in a clinical setting.

Name the potential sources of error in the collection and assay of drugs samples.

Explain the clinical importance of correct sample collection, storage and assay.

Describe ways to avoid or minimize errors in the collection and assay of drug samples.

Explain the basic concepts and calculations of the model independent pharmacokinetic parameters of total body clearance, mean residence time (MRT), volume of distribution at steady-state, and formation clearance.

**PHARMACOKINETICS**

This is the branch that is concerned with how a pharmacon acts after it is introduced into the body. This is firstly dependent on the way in which it was administered;

*This can be:*

- **Enteral:** By way of the intestine
- **Oral, per os:** By way of the mouth, swallowing sublingual: Beneath the tongue, sucking
- **Rectal:** By way of the anus (suppository) parenteral: by injection through the skin
- **Intracutaneous:** Within the skin
- **Subcutaneous:** Beneath the skin
- **Intramuscular:** Within the muscles intravenous: within a vein
- **Intraperitoneal:** Within the peritoneal cavity intracardiac: within the heart
- **Inhalation:** Drawing into the lungs (smoking)
- **Transcutaneous:** Absorption through the skin (bandage)

The most important aspect of administration per os is that when the substance has been absorbed into the blood of the gastrointestinal tract it goes on to the liver. One important function of the liver is to break down foreign substances and/or prepare them for elimination by the kidneys by changing them chemically. This process is called biotransformation. Biotransformation reduces the effect of many substances when taken per os. This is not the case with smoking or shooting up, which means that in these cases the effect is much stronger. Another way to avoid biotransformation in the liver is by rectal or sublingual administration (the nitrorate in angina pectoris) as the blood vessels from the mouth and rectum do not first lead to the liver.

Another factor influencing the strength of the effect is that it is not so much the amount of the substance in the blood (concentration in the blood) that determines the severity of the effect, but rather how quickly that concentration rises. A substance administered per os is absorbed slowly by the blood; its concentration in the blood rises slowly. With shooting up or smoking, concentration in the blood rises rapidly to very rapidly, causing a much stronger effect.

Blood transports the substance administered to the place where it is to have its effect. Whether the substance is easily soluble in water, or in fat is also important. If a substance is easily soluble in water, it mixes well with the blood; if it is easily soluble in fat, it mixes less easily, but stores well in the adipose tissue for later use.

*Substances are also broken down:* Although the liver is often mentioned in this context, the breakdown of enzymes also takes place elsewhere in the body. An enzyme is a substance that causes a chemical reaction to occur without being changed itself. Outside the medical world this reaction is called catalysis; a substance that has such a function is called a catalyst. Of importance in this connection is that some substances (also medicines) can speed up or
slow down the breakdown process. In cases of combined usage this can lead to a lowered or heightened effect. Examples of this are:

- Rifampicine (an antibiotic which is also used in tuberculosis cases): speeds up methadone breakdown, which reduces its effect; methaqualone (a sleep-inducing drug): slows down the breakdown of many substances (including opiates), which heightens their effect. An important concept with this is half-life, which is the amount of time the body needs to eliminate half of the substance present as measured by its concentration in the blood.

The substances, whether biotransformed or not, are then excreted again, usually with the urine. Substances can also be excreted with the feces, sweat, saliva, tears and mother’s milk.

If they are gasforming, they can be exhaled by the lungs:

- Agonist antagonist: Pharmaca are administered for their effect. They have that effect because they react with special molecules, called receptors. The pharmacon is then agonist with regard to a specific receptor. Other substances can have the opposite effect; these latter are referred to as the antagonists. An example of this is the agonist morphine of which nalorphine is the antagonist that counteracts the effects of morphine. It often ‘happens that the antagonists ‘fight’ with the agonists for an effect on the receptor; we then speak of competitive antagonists. This in contrast to situations in which the antagonist blocks the receptor just like that: we then speak of noncompetitive blocking. In the first case, the receptor blockade can be lifted again by a higher dosage of the agonist, in the second case it cannot.

**ROUTES OF DRUG ADMINISTRATION**

- Intravenous
- Buccal
- Rectal
- Transdermal
- Inhalational
- Oral
- Sublingual
- Intramuscular
- Subcutaneous
- Topical

Of all of these routes you are most likely to be asked about the transdermal, as it is fashionable. Otherwise, most other basic pharmacology questions tend to concern the pharmacology of intravenous agents; that is what is discussed below.

**First Order Kinetics**

A constant fraction of the drug in the body is eliminated per unit time. The rate of elimination is proportional to the amount of drug in the body. The majority of drugs are eliminated in this way.

What follows concerns drugs which follow first order kinetics.

The Volume of Distribution (Vd) is the amount of drug in the body divided by the concentration in the blood. Drugs that are highly lipid soluble, such as digoxin, have a very high volume of distribution (500 litres). Drugs which are lipid insoluble, such as neuromuscular blockers, remain in the blood, and have a low Vd.

The Clearance (Cl) of a drug is the volume of plasma from which the drug is completely removed per unit time. The amount eliminated is proportional to the concentration of the drug in the blood.

The fraction of the drug in the body eliminated per unit time is determined by the elimination constant (kel). This is represented by the slope of the line of the log plasma concentration versus time.

\[
\text{Cl} = \text{kel} \times \text{Vd}
\]

Rate of elimination = clearance \times concentration in the blood.

Elimination half life (t1/2): The time taken for plasma concentration to reduce by 50%. After 4 half lives, elimination is 94% complete.

It can be shown that the kel = the log of 2 divided by the t1/2 = \(0.693/t1/2\).

Likewise, \(Cl = \text{kel} \times \text{Vd}\), so, \(Cl = 0.693\text{Vd}/t1/2\).

And \(t1/2 = 0.693 \times \text{Vd}/\text{Cl}\)

The rate of elimination is the clearance times the concentration in the plasma

\[
\text{Roe} = \text{Cl} \times \text{Cp}
\]

Fraction of the total drug removed per unit time = Cl/Vd.

If the volume of distribution is increased, then the kel will decrease, the t1/2 will increase, but the clearance won’t change.

**Example**

You have a 10ml container of orange squash. You put this into a litre (ok 990ml!) of water. The Vd of the orange squash is 1000ml. If, each minute, you empty 10ml of the orange liquid into the 10ml container, discard this, and replace it with 10ml of water. The clearance is 10 ml per minute. The elimination half life is: 70 minutes. The kel is Cl/Vd = 10/1000 = 0.01. Shown the other way, 0.693/50 = 0.01.
Bioavailability
This is the fraction of the administered dose that reaches the systemic circulation. Bioavailability is 100% for intravenous injection. It varies for other routes depending on incomplete absorption, first pass hepatic metabolism etc. Thus one plots plasma concentration against time, and the bioavailability is the area under the curve.

Zero Order Elimination
Why if I have 10 pints of beer before midnight will I fail a breathalyser test at 8 am the following morning? Either this is due to alcohol having a very long half life (which it does not) or that alcohol is cleared in a different way. What happens is that the metabolic pathways responsible for alcohol metabolism are rapidly saturated and clear is determined by how fast these pathways can work. The metabolic pathways work to their limit. This is known as zero order kinetics: a constant amount of drug is eliminated per unit time. This form of kinetics occurs with several important drugs at high dosages concentrations: phenytoin, salicylates, theophylline, and thiopentone (at very large doses). Because high dose thio is very slow to clear, we no longer use it in infusion for status epilepticus (as it takes ages for the patient to wake up!).

Dosage regimens
The strategy for treating patients with drugs is to give sufficient amounts that the required therapeutic effect arises, but not a toxic dose.

The maintenance dose is equal to the rate of elimination at steady state (i.e., at steady state, rate of elimination = rate of administration):

Dosing rate = clearance × desired plasma concentration.

Drugs will accumulate within the body if the drug has not been fully eliminated before the next dose. Steady state concentration is thus arrived at after four half lives. This is all very well if you are willing to wait 4 half lives for the drug to be fully effective, but what if you are not? What you may need to do is to “load” the volume of distribution with the drug to achieve target plasma concentrations rapidly: the loading dose.

The loading dose = the volume of distribution × the desired concentration (i.e., the concentration at steady state).

You can figure this out by Loading dose = usual maintenance dose × usual dosage interval × kel (t1/2/0.693).
Hepatic Drug Clearance

Many drugs are extensively metabolised by the liver.

*The rate of elimination depends on:*
  - The liver’s inherent ability to metabolise the drug,
  - The amount of drug presented to the liver for metabolism.

This is important because drugs administered orally are delivered from the gut to the portal vein to the liver: the liver gobbles up a varying chunk of the administered drug (pre-systemic elimination) and less is available to the body for therapeutic effect. This is why you have to give a higher dose of morphine, for example, orally, than intravenously.

Hepatic drug clearance (i.e. the amount of each drug gobbled up by the liver) depends on:
  - The Intrinsic clearance (Cl int).
  - Hepatic blood flow.

These two factors are independent of one another, and their combined effect is the proportion of drug gobbled up: the extraction ratio.

For drugs that have a low intrinsic clearance, this effect can be increased by giving a second agent that boosts the effect of the liver’s enzyme system; these are enzyme inducers. Examples of such drugs are cigarettes, antiepileptics (carbamazepine & phenytoin), rifampicin, griseofulvin, alcohol and spironolactone (CAR GAS) [also barbiturates]. Consequently if a drug addict is given rifampicin or tuberculosis, a higher dose of heroin is required for the same effect. Enzyme inhibitors have the opposite effect: examples are flagyl, allopurinol, cimetidine, erythromycin, dextropropoxyphene, imipramine, (the) pill (FACE DIP).

Likewise, if the blood flow increases, the liver has less chance to gobble up the drug, and the extraction ratio falls. This is particularly the case, as you would expect, of the intrinsic clearance is low.

*Illustration: Think of factory workers picking bad apples out of a pile on a conveyor belt, if only one person (low intrinsic clearance) is doing the picking and the speed of the conveyor belt is increased, more bad apples get through. If there are several pickers (high intrinsic clearance) then they are much more able to cope with an increase in the speed of the conveyor belt, but there will come a rate at which they will become overwhelmed, and bad apples will get through.*
If we can determine the relationship between $CL_{cr}$ and $Kel$ from a number of patients, we can then determine the creatinine clearance in a new patient and estimate the elimination rate constant. We can then calculate an optimum dose and dosing interval for this patient.

How do we calculate $Kel$ for a particular drug and patient? For this we need to rely on data previously obtained and published in the literature. With this information we can construct a plot of $CL_{cr}$ versus $Kel$. This plot may be built into a computer program or nomogram:

$$Kel = Knr + (b \times CL_{cr})$$

The y-intercept ($Knr$) is the nonrenal elimination rate, or that which occurs with essentially no renal function. The slope ($b$) of the regression line is the linear relationship between $Kel$ and $CL_{cr}$.

### Calculating Creatinine Clearance

There is considerable controversy as to which equation is best for estimating $CL_{cr}$, what weight to use, and whether to round up the serum creatinine in the elderly. Please refer to the publications listed in the reference section for more in-depth discussion of these areas.

*The advantages of calculating $CL_{cr}$ from serum creatinine are:*

- Simple, convenient and reproducible.
- Requires only a single blood sample.
- Avoids the errors inherent in a timed urine collection.

*You should be aware of some pitfalls and precautions when calculating $CL_{cr}$ from serum creatinine:*

- Liver dysfunction = associated with a significant overprediction of $CL_{cr}$. Most authorities state that these equations should not be used in patients with liver disease.
- Emaciated = have low serum creatinine concentrations secondary to decreased muscle mass, resulting in a significant overprediction of $CL_{cr}$.
- Elderly = may have low serum creatinine concentrations secondary to decreased muscle mass, leading to a possible overprediction of $CL_{cr}$. Some authorities round up the serum creatinine in the elderly to avoid a falsely elevated $CL_{cr}$.
- Unstable renal function = C&G’s method is not reliable in patients with unstable renal function. Jelliffe’s multi-step method, which corrects for rising serum creatinine, may be more accurate in these patients.

### Jelliffe Method

Because Jelliffe’s method includes a step to correct for rising serum creatinine, it is more accurate than the Cockroft and Gault method in patients with unstable renal function.

- Estimate urinary creatinine excretion rate
  
  $$E_{Males} = Wt \times (29.305 - [0.203 \times (age)])$$
  $$E_{Females} = Wt \times (25.3 - [0.18 \times (age)])$$

  where:
  
  $Wt$ = Lean body weight, or Adjusted body weight if obese

- Correct for rising serum creatinine
  
  $$E = E - [4 \times ABW \times (SCr1 - SCr2)]/ D$$

  where:
  
  $Wt$ = Lean body weight, or Adjusted body weight if obese
  $SCr1$ = the latest serum creatinine
  $SCr2$ = the earlier serum creatinine
  $D$ = the number of days between

- Calculate corrected creatinine clearance ($CL_{cr}$)
  
  $$CL_{cr} = (E \times 0.12)/ (SCr \times BSA)$$

  where:
  
  $SCr$ = most recent serum creatinine

### Cockroft and Gault Method

$$CL_{cr} \text{ Males} = Wt(140 \text{ - Age})/ (SCr \times 72)$$

$$CL_{cr} \text{ Females} = 85\% \text{ of male value}$$

where:

$SCr$ = most recent serum creatinine
$Wt$ = Lean body weight, or Adjusted body weight if obese

### Drug Regimen Design

**Pharmacodynamics**
Pharmacodynamics is the study of the relationship between serum concentration and therapeutic effect. For antibiotics, the time course of antimicrobial activity must be understood before one can design a rational drug regimen.

**Rational dosing of Antimicrobial Agents**

From the viewpoint of pharmacodynamics, antimicrobial agents may be divided into three major groups.

**Group I**

β-lactams:

These agents have a kill rate that is concentration-independent as long as the concentration is above the minimum inhibitory concentration (MIC). Also, these agents have no significant post-antibiotic effect (PAE). PAE is the persistent suppression of bacterial growth following antibiotic exposure.

*Strategy for best results*: Maximize exposure time during which the plasma concentration exceeds MIC. This may be achieved by giving smaller doses more frequently; ultimately, continuous IV infusion of β-lactams would achieve the best results. In fact, when penicillin was first introduced, it was administered by continuous infusion; only later was it given intermittently, largely for the sake of convenience.

**Group II**

Vancomycin, Carbapenems, Macrolides, and Clindamycin

These agents have a kill rate that is concentration-independent as long as the concentration is above the MIC. Also, these agents also have an intermediate post-antibiotic effect, therefore serum levels may be allowed to drop below the MIC for a short period.

*Strategy for best results*: Maximize exposure time during which the plasma concentration exceeds MIC. This may be achieved by giving smaller doses more frequently (i.e., 500 mg 6 hrs is better than 1000 mg q 12 hrs).

**Group III**

Aminoglycosides, Fluoroquinolones, and Metronidazole

These agents have concentration-dependent kill rate and a significant post-antibiotic effect. The PAE exhibited by this group will prevent bacterial regrowth when tissue levels fall below the MIC for an extended period of time.

*Strategy for best results*: Aim for a “good peak” to maximize the ratio of Peak to MIC (or the 24 hour AUC to MIC ratio). This is achieved by giving larger doses less frequently. In the case of aminoglycosides, “give the kidney a break” too by allowing for a short (2 - 4 hrs) drug-free period to minimize nephrotoxicity.

**Some Practical Considerations**

- **Choose practical, convenient doses and administration schedules**: Odd doses and schedules may lead to errors in administration.
- **Consider a loading dose**: Some drugs, which are usually administered without a loading dose, may require a loading dose in order to quickly attain therapeutic levels.
- **Measure SDC’s when indicated**: Accurate assessment requires steady-state SDC’s (4-5 times the estimated half-life).
- **Closely monitor the clinical status of the patient**: Careful observation for signs of drug toxicity is imperative.

**Aminoglycosides**

**Introduction**

The aminoglycosides are the mainstay in the treatment of serious gram-negative systemic infections. A disadvantage of the aminoglycosides is their association with nephrotoxicity and ototoxicity, both of which are associated with elevated trough levels and sustained elevated peak levels.

**Antimicrobial Spectrum**

Aminoglycosides have bactericidal activity against most gram-negative bacteria including Acinetobacter, Citrobacter, Enterobacter, E. Coli, Klebsiella, Proteus, Providencia, Pseudomonas, Salmonella, Serratia and Shigella. The MIC’s of gram negative bacteria are usually less than 2 mcg/ml for gentamicin and tobramycin and 8 mcg/ml for amikacin.

Aminoglycosides are active against most strains of *Staphylococcus aureus* and *S. epidermidis*. Most strains of enterococcus are resistant to aminoglycosides alone, however when used in combination with penicillins they are often effective in enterococcal endocarditis due to synergistic antimicrobial mechanisms. Anaerobic bacteria are universally resistant because aminoglycoside transport into cells is
Principles of Clinical Pharmacology and Therapeutics

For gentamicin, tobramycin and netilmicin risk of ototoxicity and nephrotoxicity is increased if trough levels consistently exceed 2 mcg/ml. For amikacin, trough levels consistently greater than 10 mcg/ml have been associated with a higher risk of ototoxicity and nephrotoxicity.

Concentration-efficacy Relationships

The pharmacodynamic properties of aminoglycosides are:

- Concentration-dependent killing
- Significant post-antibiotic effect

Aminoglycosides eliminate bacteria quickest when their concentration is appreciably above the MIC for an organism, this is referred to as concentration dependent activity. The aminoglycosides also exhibit a significant post-antibiotic effect (PAE). PAE is the persistent suppression of bacterial growth following antibiotic exposure. Practically speaking this means that trough levels can drop below the MIC of targeted bacteria for a sustained period without decreasing efficacy.

For AG’s the ideal dosing regimen would maximize concentration, because the higher the concentration, the more extensive and the faster is the degree of bactericide. Therefore, the Peak/MIC ratio is an important predictor of efficacy. It has been shown that aminoglycosides eradicate bacteria best when they achieve a Peak/MIC ratio of at least 8-10. Therefore it is important to give a large enough dose to produce a peak level 8 to 10 times greater than the MIC.

<table>
<thead>
<tr>
<th>Initial serum peak level</th>
<th>Died</th>
<th>Survived</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 5mcg/ml</td>
<td>21%</td>
<td>79%</td>
</tr>
<tr>
<td>&gt;= 5mcg/ml</td>
<td>2%</td>
<td>98%</td>
</tr>
</tbody>
</table>

Table. Aminoglycoside Pharmacodynamics in Vivo

*Moore et al, J Infect Dis 149: 443, 1984*

Dosing Methods

Achieving therapeutic serum levels of aminoglycosides early in the course of treatment is critical to therapeutic success. Dosing error on the high side is preferable to the risks of under-treatment. An adequate loading dose is critical for rapid attainment of therapeutic peak levels.
The method of Sarrubi and Hull utilizes serum creatinine, lean body weight, age, and sex to estimate creatinine clearance. This method considers more patient variables, which may improve the estimation of aminoglycoside elimination. Lesar et al found that the Sarrubi and Hull nomogram achieved therapeutic concentrations in 78% of patients. Tsubaki and Chandler evaluated 5 methods for determining initial dosing requirements for gentamicin. They concluded that the Sarrubi and Hull method was the most accurate. However, dosing nomograms are initial guidelines only. They can produce substantial variations in serum concentrations and should be subsequently adjusted based on serum level determinations and clinical response.

Dosage regimens necessary to achieve therapeutic aminoglycoside serum concentrations can be quantitatively determined by using simple pharmacokinetic principles. Individualized pharmacokinetic parameters are determined from the patient’s serum concentration versus time data. Sawchuk and Zaske have described a method for establishing multiple infusion regimens based on individually calculated pharmacokinetic parameters. Lesar, et al found that this individualized method achieved therapeutic concentrations in 90% of patients.

For evaluation of serum level data, methods incorporating Bayesian principles appear to give the best overall predictive performance compared with traditional methods of vancomycin dosage adjustment. The Bayesian approach combines both population and patient-specific information (i.e., serum level data) in predicting dosage requirements.

Extended-interval (or “once-daily”) aminoglycoside dosing has gained popularity in recent years. The pharmacodynamic properties of AG’s form the basis of EI dosing. The concentration dependent activity of AG’s demonstrates that a large dose (5mg/kg) is needed to maximize killing. The persistent (post-antibiotic) effect of AG’s allows a dosing interval of 24-36 hours. This extended interval provides a beneficial wash out period during the gamma (tissue-release) phase, thus decreasing the incidence of toxicity.

This simplified dosing method is appropriate in young, otherwise healthy patients with sepsis. However, there are many patients who are not candidates for the Extended-interval dosing methodology, including those with the following conditions:

- Elderly
- Creatinine clearance less than 30
- Dialysis
- Pregnancy
- Endocarditis
- Cystic fibrosis
- Ascites
- Pediatrics
- > 20% BSA burns
- History of hearing loss or vestibular dysfunction
- Gram positive infections (when aminoglycoside is used for synergy)
- Mycobacterial infections

Population Model Parameters

Volume of Distribution

The average Vd of AG’s in otherwise healthy adults is 0.26 L/kg (range: 0.2-0.3). Although AG’s do not distribute into adipose tissue, they do enter the extracellular fluid contained therein. Therefore, obese patients require a correction in the weight used for Vd calculation: LBW + 40% of weight above LBW. Patients with cystic fibrosis have a markedly increased Vd of 0.35 L/kg due to increases in extracellular fluid brought about by the disease process. Patients with ascites have additional extracellular fluid because of accumulation of ascitic fluid, which increases the Vd to approximately 0.32 L/kg. Also, ICU patients may have a Vd 25-50% above normal.

Elimination Rate

AG elimination exhibits a close linear correlation with creatinine clearance, the average value for slope is between 0.0024 and 0.0029 and y-intercept of 0.01 to 0.015. Cystic fibrosis patients show a 50% increase in elimination rate. A major body burn increases the basal metabolic rate resulting in a marked increase in AG elimination. ICU patients are often hypermetabolic and therefore eliminate AG’s more rapidly.

Monitoring parameters

Careful observation for signs of drug toxicity is imperative.
- The following patient parameters should be monitored during
Dr. Bob: Aminoglycoside therapy:

- Aminoglycoside peak and trough levels Obtain levels 24 hours after initiating therapy, at steady state (approximately four half-lives), and every 2 to 3 days.
- BUN and serum creatinine
  Measure every two days, or every day in unstable renal function.
- Weight
  Weigh patient every two to seven days.
- Urine output
  Measure and monitor urine output daily
- Baseline and weekly audiograms, and check for tinnitus or vertigo daily.

- Therapeutic serum concentrations (mcg/ml) Below are some general guidelines, however, target serum concentrations should be individualized.
  - Gentamicin, Tobramycin, Netilmicin
    Peak:  Serious infection: 6-8
    Life-Threatening infection: 8-10
    Trough:  Serious infection: 0.5-1.5
    Life-Threatening infection: 1-<2
  - Amikacin, Kanamycin
    Peak:  Serious infection: 20-25
    Life-Threatening infection: 25-30
    Trough:  Serious infection: 1-4 Life-Threatening infection: 4-8

Precautions
- Proper timing of serum sampling is critical. The trough sample should be obtained 30 minutes prior to the dose. Measure the peak level 15 to 30 minutes after completion of the IV infusion to avoid the distributive phase. Measure the peak level 90 minutes after an IM injection. Drawing the peak too soon will result in inaccurate analysis.

Dr. Smith: Drawing at exactly the right time is not as important as having the lab note the exact times that the samples were drawn. Also, have the nurse note the exact times that the sample infusion was started and when it ended. Please be aware of the widespread policy of nursing personnel to record a dose as having been given exactly as ordered if it is given within 30 minutes of the recorded time. This will lead to significant errors in analysis, please ensure that all those involved record the exact times.

This issue cannot be stressed enough. Inaccurate recording of drug administration times and lab draw times are the greatest source of calculation error, having a greater effect than pharmacy preparation error or lab assay error.

- Outliers: In general the Bayesian approach to the determination of individual drug-dosage requirements performs better than other methods. However, outlying patients in a population (ie, those patients whose pharmacokinetic parameters lie outside of the 95th percentile of the population) may be put at risk. As is always the case, computerized algorithms can only assist in the decision-making process and should never become a substitute for informed clinical judgement.

PRINCIPLES OF PHARMACOKINETIC

The subtitle, Understanding the Basics, accurately describes the author’s approach in writing this introductory text. In the preface, he states that the goal of the text is “to provide the concepts used to formulate approaches but was not intended to be a clinical guide to dosing adjustments.” This text serves as an introduction for the student, or a re-introduction for the practicing pharmacist, to the basic pharmacokinetic concepts that form the foundation for calculating pharmacokinetic dose adjustments.

The book is divided into four sections: Basic Concepts, Parenteral Dosing Adjustments, Oral Dosing, and Advanced Considerations. Each section is further divided into a total of ten chapters. The Basic Concepts section contains chapters on Pharmacokinetic Processes, Kinetic Processes Applied to the Whole Body, Disposition Parameters, and Parameters Used in Adjusting Doses. The lone chapter in the
Parenteral Dosing Adjustments section is entitled Infusion. Chapters in the Oral Dosing section include Important Parameters, Bioavailability/Bioequivalence, and Multiple Dosing Regimens. Finally, the Advanced Considerations section introduces readers to the Two-Compartment Model and Non-Linear Pharmacokinetics.

Each chapter begins with a set of study guide questions. These questions are clear, well written, and help readers focus on the most important concepts presented. At the end of each chapter is a set of study problems. The problems allow readers to apply the concepts presented, and with the answers found in the appendix, serve as a means of self-assessment.

With the focus of the book in mind, concepts are presented in a simple but scientifically sound manner. Equations are presented in their most universal form, and are not altered to perform dosing calculations for specific drugs or drug classes. Rather than introducing equations through extensive mathematical derivations, Schoenwald employs more intuitive and physiologic explanations. The examples illustrating pharmacokinetic concepts are easily understood, even for readers whose calculus classes are but a distant memory. This seems to be especially true of the chapters in the Basic Concepts section. Figures and tables are used efficiently, and they are labeled in a way that quickly acclimates readers.

This book’s strength is also its weakness. While it provides an easy to understand introduction to the concepts and skills needed to calculate dose adjustments for patients, it does not go beyond that. Those looking for a text covering the latest techniques in pharmacokinetics research or to provide in-depth instruction on dose optimization of clinically monitored drugs should probably look elsewhere. Therefore, it would probably not be appropriate as the lone text used in advanced clinical pharmacokinetics courses or graduate level pharmacokinetics courses.

Practicing pharmacists needing to recall basic pharmacokinetic concepts will find this to be a helpful reference, but there are a number of clinical pharmacokinetics references available that provide more of the information needed to calculate specific pharmacokinetic dose adjustments. With its concise presentation and user-friendly examples, this text would best be utilized in introductory pharmacokinetics courses at the undergraduate level. It would also be a valuable addition to the reference collection of any medical library or drug information center.
Vancomycin, Carbapenems, Macrolides, and Clindamycin

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DRUGS DISTRIBUTION

Drug enters the body by absorption. Inside the body, drugs move in the blood to different parts of the body. Distribution of drugs can be defined as: “The process by which a drug reversibly leaves the blood stream and enters the interstitium (extracellular fluid) and/or
the cells or tissues.” The drugs are present in free or bound form and different processes or mechanisms affect their distribution.

Distribution is the process by which a drug diffuses or is transferred from intravascular space to extravascular space (body tissues). These spaces are described mathematically as volume(s) of distribution. In the simplest of terms, a drug’s volume of distribution is that volume of bodily fluid into which a drug dose is dissolved. Therefore, if we know the dose that was given, and we can measure the serum level (concentration), then we can calculate a volume:

\[ \text{Volume of distribution} = \frac{\text{Dose}}{\text{drug concentration}} \]

Of course, the human body is not a glass beaker. Drug is distributing in and out of many tissue compartments while it is simultaneously being eliminated. This complex and continually changing environment must be simplified in order to mathematically model the human body. Therefore, the body is usually divided into two spaces, a central and a tissue compartment.

**Central Volume (Vc)**

The central volume of distribution (Vc) is a hypothetical volume into which a drug initially distributes upon administration. This compartment can be thought of as the blood in vessels and tissues which are highly perfused by blood.

**Drugs Elimination**

Drugs are cleared primarily by the liver and kidneys. Excretion into the urine is a major route of elimination for metabolites and unchanged drug.

Most drugs are eliminated by a first-order process. With first-order elimination, the amount of drug eliminated is directly proportional to the serum drug concentration (SDC).

With first order elimination, at a certain point in therapy, the amount of drug administered during a dosing interval exactly replaces the amount of drug excreted. When this equilibrium occurs (rate in = rate out), steady-state is reached.

**Clearance (CL)**

Clearance is a descriptive term used to evaluate efficiency of drug removal from the body. Clearance is not an indicator of how much drug is being removed; it only represents the theoretical volume of blood which is totally cleared of drug per unit time. Because clearance is a first-order process, the amount of drug removed depends on the concentration.

Clearance can be thought of as the proportionality constant that makes the average steady-state drug level equal to the rate of drug administration. Clearance (rate out) can be calculated from the dose (rate in) and average steady-state concentration:

\[ \text{Cl} = \frac{(\text{Dose}/\text{interval})}{\text{Cpss ave}} \]

**DRUG ACTING ON AUTONOMIC GANGLIA**

Conventionally, anticholinergic drugs are those which block actions of Ach on autonomic effectors and in the CNS exerted through muscarinic receptors. Though nicotinic antagonists also block certain actions of Ach, they are generally referred to as ‘ganglion blockers’ and ‘neuromuscular blockers’. Atropine, the prototype drug of this class, is highly selective for muscarinic receptors, but some of its synthetic substitutes do possess significant nicotinic blocking property in addition. The selective action of atropine can easily be demonstrated on a piece of guinea pig ileum where Ach induced contractions are blocked without affecting those evoked by histamine, 5-HT or other spasmogens. The selectivity is, however, lost at very high doses. All anticholinergics are competitive antagonists.

**The Influence of Drugs on the ANS**

Drugs that affect the ANS can have important therapeutic value, and they can be used to treat certain diseases because they can increase or decrease activities normally controlled by the ANS. Drugs that affect the ANS can also be found in medically hazardous substances such as tobacco.

Direct-acting drugs bind to ANS receptors to produce their effects. For example, stimulating agents bind to specific receptors and activate them, and blocking agents bind to specific receptors and prevent them from being activated. The main topic of this essay is direct-acting drugs. It should be noted, however, that indirect-acting drugs can also influence the ANS.

For example, some drugs indirectly produce a stimulatory effect by causing the release of neurotransmitters or by preventing the metabolic breakdown of neurotransmitters. Other drugs indirectly produce an inhibitory effect by preventing the biosynthesis or release of neurotransmitters.
Drugs that Bind to Alpha and Beta receptors

Drugs that activate adrenergic receptors are adrenergic (a-dri-ner’jik) agents or sympathomimetic (sim-tha-pom-im-et’ik) agents.

Drugs such as phenylephrine stimulate alpha receptors, which are numerous in the smooth muscle cells of certain blood vessels, especially in the digestive tract and the skin. These drugs increase blood pressure by causing vasoconstriction.

On the other hand, isoproterenol is a drug that selectively activates beta receptors, that are found in cardiac muscle and bronchiolar smooth muscle. Beta-adrenergic-stimulating agents are sometimes used to dilate bronchioles in respiratory disorders such as asthma and are occasionally used as cardiac stimulants.

Drugs such as phenoxybenzamine that bind to and block the action of alpha-receptors are alpha-adrenergic (a-dre-ner’jik) blocking agents. The therapeutic uses of these drugs are limited, but phenoxybenzamine is used for the treatment of pheochromocytoma (fe’o-kro-mo-si-to’mah). This disorder is usually caused by a non-cancerous tumor of the adrenal medulla that results in the excessive release of norepinephrine. The norepinephrine stimulates vasoconstriction, causing high blood pressure. Phenoxybenzamine can help to lower blood pressure by blocking the effects of norepinephrine.

Propranolol (pro-pran’o-lol) is an example of a beta-adrenergic blocking agent. These drugs are sometimes used to treat high blood pressure, some types of cardiac arrhythmias, and patients recovering from heart attacks. Blockage of the beta receptors within the heart prevents sudden increases in the heart rate and thus decreases the probability of arrhythmic contractions.

Drugs that Bind to Nicotinic Receptors

Drugs that bind to nicotinic receptors and activate them are nicotinic (nik’-o-tin-ik) agents. Although these agents have little therapeutic value and are mainly of interest to researchers, nicotine is medically important because of its presence in tobacco. Nicotinic agents bind to the nicotinic receptors on all postganglionic neurons within the autonomic ganglia and produce stimulation.

Responses to nicotine are variable and depend on the amount taken into the body. Because nicotine stimulates the postganglionic neurons of both the sympathetic and parasympathetic divisions, much of the variability of its effects results from the opposing actions of these divisions.

For example, in response to the nicotine contained in a cigarette, the heart rate may either increase or decrease; and its rhythm tends to become less regular as a result of the simultaneous actions on the sympathetic division, which increase the heart rate, and the parasympathetic division, which decreases the heart rate.

Blood pressure tends to increase because of the constriction of blood vessels, which are almost exclusively innervated by sympathetic neurons.

In addition to its influence on the ANS, nicotine also affects the central nervous system; therefore not all of its effects can be explained on the basis of action on the ANS. Nicotine is extremely toxic, and small amounts can be lethal.

Drugs that bind to and block nicotinic receptors are called ganglionic blocking agents because they block the effect of acetylcholine on both parasympathetic and sympathetic postganglionic neurons.

The effect of these substances on the sympathetic division, however, overshadows the effect on the parasympathetic division. For example, trimethaphan camsylate can be used to treat high blood pressure.

It blocks sympathetic stimulation of blood vessels, causing the blood vessels to dilate, which decrease blood pressure. Ganglionic blocking agents have limited uses because they affect both sympathetic and parasympathetic ganglia. Whenever possible, more selective drugs are now use.

Drugs that Bind to Muscarinic Receptors

Drugs that bind to and activate muscarinic receptors are muscarinic (mus’ka-rin-ik) agents, or parasympathomimetic (par-a-sim’pa-tho-mi-met’ik) agents.

These drugs activate the muscarinic receptors of target tissues for both divisions of the ANS. Most observable responses are parasympathetic because all the structures innervated by the parasympathetic division have muscarinic receptors, whereas almost all the structures innervated by the sympathetic division have adrenergic receptors.
Muscarine causes increased sweating; increased secretion of glands in the digestive system; decreased heart rate; constriction the pupils; and contraction of respiratory, digestive, and urinary system smooth muscles.

Bethanechol chloride is a parasympathomimetic agent used to stimulate the urinary bladder following surgery, because the general anesthetics used for surgery can temporarily inhibit a person’s ability to urinate. The drug helps to prevent the accumulation of urine until normal urinary bladder function returns.

Drugs such as atropine that bind to and block the action of muscarinic receptors are muscarinic blocking agents, or parasympathetic blocking agents.

These drugs dilate the pupil of the eye and are used during eye examinations to allow the examiner to see the retina through the pupil. They also decrease salivary secretion and are used during surgery to prevent patients from choking on excess saliva while they are anesthetised.

**EFFECT OF BRAIN ON DRUGS**

Here are summaries of the effect of select street drugs on the brain. Some of the introductory information is derived from About.com. Select authoritative references for information about effects of drugs on the brain.

**Heroin**

Heroin is a highly addictive opiate (like morphine). Brain cells can become dependent (highly addictive) on this drug to the extent that users need it in order to function in their daily routine.

While heroin use starts out with a rush of pleasure, it leaves the use in a fog for many hours afterwards. Users soon find that their sole purpose in life is to have more of the drug that their body has become dependant on.

**Marijuana**

The parts of the brain that control emotions, memory, and judgement are affected by marijuana. Smoking it can not only weaken short-term memory, but can block information from making it into long term memory. It has also been shown to weaken problem solving ability.

**Alcohol**

Alcohol is no safer than drugs. Alcohol impairs judgement and leads to memory lapses. It can lead to blackouts. It distorts vision, shortens coordination, and in addition to the brain can damage every other organ in the body.

**Cocaine**

Cocaine, both in powder form and as crack, is an extremely addictive stimulant. An addict usually loses interest in many areas of life, including school, sports, family, and friends. Use of cocaine can lead to feelings of paranoia and anxiety. Although often used to enhance sex drive, physical effect of cocaine on the receptors in the brain reduce the ability to feel pleasure (which in turn causes the dependency on the drug).

**Inhalants**

Inhalants, such as glue, gasoline, hair spray, and paint thinner, are sniffed. The effect on the brain is almost immediate. And while some vapors leave the body quickly, others will remain for a long time.

The fatty tissues protecting the nerve cells in the brain are destroyed by inhalant vapors. This slows down or even stops neural transmissions. Effects of inhalants include diminished ability to learn, remember, and solve problems.

**Ecstasy**

Extended use of this amphetamine causes difficulty differentiating reality and fantasy, and causes problems concentrating. Studies have found that ecstasy destroys certain cells in the brain. While the cells may re-connect after discontinued use of the drug, they don’t re-connect normally. Like most drugs, this one impairs memory and can cause paranoia, anxiety, and confusion.

**LSD**

While some people use LSD for the sense of enhanced and vivid sensory experience, it can cause paranoia, confusion, anxiety, and panic attacks. Like Ecstasy, the user often blurs reality and fantasy, and has a distorted view of time and distance.

**Steroids**

Anabolic steroids are used to improve athletic performance and
gain muscle bulk. Unfortunately, steroids cause moodiness and can permanently impair learning and memory abilities.

**Tobacco**

Tobacco is a dangerous drug, putting nicotine into your body. Nicotine affects the brain quickly, like other inhalants, producing feelings of pleasure, like cocaine, and is highly addictive, like heroin.

**Methamphetamine**

Known on the street as meth, speed, chalk, ice, crystal, and glass, methamphetamine is an addictive stimulant that strongly activates certain systems in the brain.

**Ritalin**

This drug is often prescribed to treat attention deficit disorder. It is becoming an illicit street drug as well. Drug users looking for a high will crush Ritalin into a powder and snort it like cocaine, or inject it like heroin. It then has a much more powerful effect on the body. It causes severe headaches, anxiety, paranoia, and delusions.

**DRUG-RECEPTOR INTERACTIONS**

- Drugs typically exert their effects by interacting with a macromolecule (receptor)
- Drug-receptor interactions have been important in:
  - New drug development
  - Therapeutic decisions
- Major roles of receptors:
  - Determines quantitative relationships between drug dose and pharmacological effect.
  - Determines drug action selectivity
  - Mediates antagonist (blocking) as well as agonist (activating) effects
- Molecular characteristics of receptors:
  - Usually proteins, specifically regulatory proteins – mediating effects of:
    - Neurotransmitters
    - Autacoids (histamine, serotonin, endogenous peptides, prostaglandins, leukotrienes)
  - Hormones
    - Some receptors are enzymes — may be inhibited or occasionally activated by drugs
      - e.g. dihydrofolate reductase (receptor) — methotrexate (drug inhibitor)
    - Other receptors are transport proteins:
      - e.g. Na/K ATPase (receptor for digitalis glycosides [digoxin (Lanoxin, Lanoxicaps), digitoxin (Crystodigin)])
    - Still other receptors are structural proteins:
      - Tubulin — receptor for certain anticancer drugs and anti-inflammatory drugs

**Signal Transduction**

- Signal transduction is the process by which extracellular inputs (drug-receptor interactions) leads to intracellular messages that modulate cellular physiology.
- Molecular mechanisms of signal transduction:
  - Five basic mechanisms:
    - Drug crosses the cellular membrane: activates an intracellular receptor
    - Transmembrane receptor protein: intracellular enzyme activity affected by drug binding to a site on the enzyme that can alter its activity.
    - Drug-transmembrane receptor protein complex binds and stimulates a second protein, such as a protein tyrosine kinase.
      - A tyrosine kinase enzyme promotes phosphorylation of proteins (at the aminoacid tyrosine site)
    - Drug binding to a transmembrane ion channel changes the ion channel conductance property — affecting membrane potential
v. Agonist drug binding to a transmembrane receptor causes stimulation of a GTP-binding signal transducer protein (G protein) — leading to an increase in intracellular second messenger that results in many secondary intracellular responses.

Drug (ligand)-regulated Transmembrane Enzymes

• Mediate signaling first step by:
  - Insulin
  - Epidermal growth factor (EGF)
  - Platelet-derived growth factor (PDGF)
  - Atrial natriuretic factor (ANF)

Intracellular Receptors

• Lipid-soluble drugs, after crossing the cell membrane barrier, interact with intracellular receptors. Example: nitric oxide (NO) — stimulates guanylyl cyclase, increasing cGMP levels
• The agents below bind to DNA response elements that control transcription:
  - Thyroid hormone
  - Corticosteroids
  - Mineralocorticoids
  - Sex steroids
  - Vitamin D

Cytokine Receptors

• Activated by many diverse peptide ligands:
  - Growth hormone
  - Erythropoietin
  - Some interferons
  - Other growth and differentiation regulators

Ligand-gated Channels

• Introduction: Many drugs mimic or block the action of normally occurring (endogenous) agents that effect ion conductance of membrane integrated ion channels.
• Introduction: Many drugs mimic or block the action of normally occurring (endogenous) agents that effect ion conductance of membrane integrated ion channels.
• Endogenous ligand include:
  - Acetylcholine
  - Gamma amino butyric acid (gaba, inhibitory action)
  - Excitatory amino acids:
    i. Glycine
    ii. Aspartate
    iii. Glutamate
• Receptor example: nicotinic acetylcholine receptor:
  - Activation:
    i. Acetylcholine binds
    ii. Receptor channel opens
    iii. Na⁺ enters (down its concentration and electrical gradient)
    iv. Depolarisation occurs (EPSP)
• Other multisubunit ligand-gated examples:
  - Glutamate receptor
  - GABA_A receptor
  - Benzodiazepines (diazepam [Valium] enhance chloride conductance by allosteric modification of the GABA_A receptor
    - Glycine receptor
    - 5-HT_3 receptor

G proteins and Second Messengers

• Second messenger effects:
  - Increases in cAMP
  - Ca²⁺ concentration changes
  - Phosphoinositides effects
Four steps:
1. Drug binding
2. G protein activation (cytoplasmic side)
3. Activity of effector (ion channel or enzyme) changed
4. Intracellular second messenger concentration changes
   - cAMP: effector enzyme — adenylyl cyclase, converting ATP to cAMP
   - Adenylyl cyclase activated by a G protein
   - G proteins may be activated by many neurotransmitters and hormones

Receptor desensitisation:
- The magnitude of receptors-mediated responses decrease with repeated drug administration.
- Desensitisation is often reversible.

Concentration-Response Relationship
- Drug effect (assuming the drug acts reversibly with the receptor) is thought proportional to the number of occupied receptors.
- Drug (D) + Receptor (R) « DR leads to Effect (equation 1)
- Observed Drug Effect = (maximal drug effect · )/ K_d + [D] (equation 2)
  - Where [D] is the free drug concentration;
  - K_d is the dissociation constant for the drug-receptor (DR) complex
  - Equation 2 describes drug potency — the dependency of drug effect on drug concentration

Drug antagonists bind either to the receptor itself or to some component of the effector mechanism to prevent the agonist action.
- Antagonists themselves have no effect.
- If the antagonist-mediated inhibition can be overcome by increasing agonist concentration ultimately reaching the same maximal effect, the antagonist is termed competitive.
  i. Competitive inhibition is based on reversible binding at receptor sites.
  ii. With competitive inhibition, the dose-effect curve will be shifted to the right.
  iii. With competitive inhibition, the maximal drug effect will not be affected.
- By contrast, a non-competitive antagonist will prevent the agonist from producing a maximal effect (and any agonist concentration)
  - If the antagonist binds at the active site and is a reversible antagonist, the inhibition will be competitive.
  - If the antagonist binds that the active site and is an irreversible antagonist, the inhibition will be non-competitive.

Concepts for signaling mechanisms and drug action
- Intracellular receptors:
  - Lipid-soluble drugs, after crossing the cell membrane barrier, interact with intracellular receptors.
    Example: nitric oxide (NO) — stimulates guanylyl cyclase, increasing cGMP levels
  - Numerous agents can bind to DNA response elements, thus controlling transcription.
- Hormones that act through gene transcription may take thirty minutes to several hours lag time before effect begins and may take a long time to dissipate.

G Protein Coupling
- G-protein coupled receptors are involved in signal
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transduction for:
- Biogenic amines
- Eicosanoids
- Peptide hormones

• G-Protein systems influence other important regulatory molecules, such as:
  - Adenyl cyclase (cAMP)
  - Phospholipases A2, C and D.
  - Ca²⁺, K⁺, Na⁺ channels
  - Transport proteins

second messenger systems: cAMP, calcium and phosphoinositides, cGMP

• cAMP: intracellular second messenger
  - Hormone response mediator:
    i. Carbohydrate breakdown (liver)
    ii. Triglyceride breakdown (fat cells)
    iii. Conservation of water (renal — vasopressin)
    iv. Calcium homeostasis
    v. Cardiac chronotropic (rate) and inotropic (contractility) state
    vi. Adrenal and sex steroids regulation (responding to corticotropin and follicle stimulating hormone)
    vii. Smooth muscle relaxation
    viii. Other endocrine/neural effects

• Specificity:
  - due to the presence of different protein substrates, associated with different cell types:
    i. Liver:
    ii. Fat cells

• Termination of effect:

  - Proteins which were phosphorylated by cAMP dependent processes are dephosphorylated by the action of specific and non-specific enzymes (phosphatases).
  - cAMP is degraded to 5'-AMP (inactive) by cyclic nucleotide phosphodiesterases.
    i. Some pharmacological effects of caffeine, theophylline, and other methylxanthsines may be due to competitive inhibition of cAMP degradation

• Calcium and Phosphoinositides
  - G protein or tyrosine kinase receptor linked
  - Central Steps:
    i. Stimulation of phospholipase C
    ii. Subsequent cascade of steps results in: increased intracellular calcium enhances calcium binding to calmodulin
    iii. Calmodulin regulates enzyme activities, including calcium-dependent protein kinases.

• cGMP:
  - cGMP-based signal transduction may be more limited than cAMP-based systems.
  - Intestinal mucosa and vascular smooth muscle:
  - Vascular smooth muscle

• Signaling mechanisms – interrelationships:
  - activation of calcium-phosphoinositide and cAMP signaling systems may produce complementary or opposing results:
    i. Opposition: vasopressor induced smooth
Acetylcholine binds
Receptor channel opens
Na⁺ enters (down its concentration and electrical gradient)
Depolarisation occurs (EPSP)

**Nitric Oxide**
- Blood vessel endothelium is required for ACh-mediated smooth muscle relaxation.
- The endothelial cell layer modulates vessel responsiveness to autonomic and hormonal influences.
- Endothelial cell elaborate endothelium-derived relaxing factor (EDRF, NO) and a contracting factor.
  - Pharmacological actions of:
    - Serotonin
    - Histamine
    - Bradykinin
    - Purines
    - Thrombin are mediated to some degree by stimulation of NO release.
- EDRF is nitric oxide.
- Endothelial-released nitric oxide:
  - Diffuses into vascular smooth muscle
  - Increases cGMP
  - Facilitates vascular smooth muscle relaxation
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5

The Nervous System

WORKING

To be able to understand how psychopharmaca works (the pharmaca that effect our brains thereby influencing the mind), it is necessary to have some insight into the structure of the brain. So, first something about the brain. The brain is often compared to a computer. One common denominator is, for example, that in both information processing systems the information is sent by electrical impulse. However, the elements that make up a computer are connected to each other: the computer is a continuous system.

Our brain, on the other hand, is made up of nerve cells, neurons, which are not connected to each other: our brain is discontinuous. The brain has about 10,000,000,000 of these neurons as well as many more supporting cells, called glial cells. Each nerve cell is connected to approx. 10,000 others and together they form a network which makes even the most advanced supercomputer seem a mere rudiment. Neurons consist of a cell body with a long process (called an axon) that makes contact with the dendrites of other neurons.

What is special about neurons is that there is a potential difference between the inside and the outside of the cell resulting from differences in the concentration of the sodium ions and the potassium ions. The chief extracellular ion is sodium, the chief intracellular ion, potassium. Disruption of this pattern results in a change in the potential difference. This disruption rushes like a wave along the surface of the neuron. This disrupted conduction is called an action potential. Action potentials are conducted along the long processes of the neurons, the axons, and transfer messages in this way. When an action potential reaches the end of the axon, an impulse does not jump over to the dendrite, but releases a chemical stored in a little sac at the end of the axon.

This chemical, a neurotransmitter, passes the presynaptic membrane, pushes through the narrow cleft between the axon and the dendrite of the next neuron, the synaps, and reacts with special molecules in the cell wall of the next neuron. These molecules are receptors located in the postsynaptic membrane.

Each nerve cell has about 10,000 synapses, a number of which are always active. This activity continually causes such disturbances in that tension difference. In each case when the result of all these disturbances go beyond a specific limit, the neuron generates a new action potential which is then transmitted along the process of that cell to the next synapses.

- Presynaptic neuron with sac
- Synaptic cleft
- Postsynaptic membrane

After the neurotransmitter has ‘opened the gates’, it is thrust from the receptor and either broken down or taken up again in the axonal terminal for re use.

This latter process is called re uptake. Chemicals that block re uptake intensify the effect of the neurotransmitter because they remain in the synaptic cleft and continue to stimulate the receptors.

The neuron is, thus, a kind of small calculator that can only add (if the imbalances reinforce each other) or subtract (if the imbalances work against each other) and in this can be compared with a transistor in a computer.

An example might clarify this process. Everyone knows about the kneejerk reflex: a tap on the knee tendon makes a bent knee contract. The underlying mechanism is that the hammer tap on the knee tendon stimulates a signal in that tendon whereby one or a series of action potentials are passed on along the nerve to a synaps in the spinal cord. The neurotransmitter released there stimulates another neuron whose process sends the action potential to the upper leg muscles which then contract and extend the leg. Although it is easy for the doctor to elicit this reflex, it is difficult to elicit oneself. This is because the concentration needed by an individual to elicit the reflex is also in the form of a series of opposing action potentials.
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various ways. First the production, the synthesis, of the neurotransmitter can be stimulated by administering its building blocks.

Next, the neurotransmitter is stored (storage) in small sacs. Drugs can have an effect on this; the most clear example here is the drugs that break open the sacs that protect the transmitter against enzymes which are breaking down, so that these enzymes destroy the transmitter and the effect of the neurotransmitter concerned is lost.

The third phase is separation in the synaptic cleft: the release. Some drugs enhance separation or have the opposite effect and inhibit it, which results in either an increased or reduced effect. The fourth phase is stimulation of the receptor: psychopharmaca often imitates the neurotransmitter by stimulating the receptors itself.

After having stimulated the receptor, the neurotransmitter returns to the synaptic cleft. Two things can happen here: either enzymatic breakdown of the neurotransmitter or reuptake takes place. In reuptake, the neurotransmitter molecules are taken up again by the presynaptic neuron for reuse. Drugs can either enhance or block both breakdown and reuptake.

Finally, there are drugs that interfere with the intracellular mechanism after stimulation by a neurotransmitter, thereby blocking or enhancing the effect. This is then manipulation of the postreceptor mechanisms.

ORGANIZATION OF THE NERVOUS SYSTEM

Our nervous system evolved from a simple string of nerve cells that could only bring about the simplest reflex movements such as e.g. in the lancelet. This tiny animal is about the simplest vertebrate and is not much more complicated than a worm. At the other end of this developmental line is the human with the most complicated nervous system. The most important principle underlying this development is that new elements are continually being added to the existing system; they do not displace it. Quite the reverse in fact, as the older elements are, as it were, manipulated by the newly evolved higher elements. Our nervous system consists of superimposed control circuits that continually make more complicated behavior possible.

A distinction is made between two subdivisions: the central nervous system (CNS), which consists of the brain and spinal cord,
and the peripheral nervous system (PNS), that part of the nervous system outside the CNS which consists mainly of the nerves that extend from the CNS.

Another distinction made is of functional character, namely between the voluntary and the involuntary nervous systems. The first allows us to control our skeletal muscles consciously. The second, also called the autonomic nervous system (ANS), regulates all events not under conscious control: the activity of the internal organs, such as the heart, stomach, etc. The ANS itself has two parts: the sympathetic part that activates, and the parasympathetic part that de-activates.

The central nervous system is built up of the following parts:

**Spinal Cord**

The ‘simplest’ part of our nervous system is the spinal cord. In theory, it can still be compared with the nervous system of the lancelet. As noted earlier, the knee-jerk reflex travels along the spinal cord. All our motor activity happens by manipulation of such reflex centers by the higher nerve centers. All communication with the brain travels through the spinal cord. Two thick tracks of axons run from the brain to these reflex centers for motor control: the pyramidal tracts. In addition, thick bundles of axons travel to the brain from the sensory receptors in the skin, muscles, etc. and control sensory input of the brain.

**Brain Stem**

This is actually the first part of the original brain stem that continued to develop because the most important sensory organs (in order of their evolutionary development: for smell, taste, vision and hearing) are localized at the front side of the body. Information processing from these organs led to continual enlargement of the brain.

Control of the vital functions is organized in the brain stem. This is where breathing and blood pressure are regulated; where the awake/sleep cycle is regulated. All (passive) vital functions necessary to stay alive are controlled from here.

**Little Brain (Cerebellum)**

The cerebellum plays an important role in body movement. While the brain stem can only regulate simple reflexes ‘independently’, in the cerebellum complicated patterns of movement, ‘movement melodies’, are initiated and stored as fixed patterns. The cerebellum is present even in primitive fish and its importance increases along the evolutionary scale. The more complicated the motor activity of an animal, the larger the cerebellum.

**Limbic System**

The limbic system can best be described as the first beginning of the great brain. Research has given us some idea of how this system works. Both the nature and the results of such research can best be illustrated by the following famous example. The Spanish neurophysiologist DEGADO planted electrodes into a specific part of the limbic system of a Spanish fighting bull and fitted it with a receiver so that when a signal was sent by a transmitter a current would flow through one of the electrodes and stimulate the cells concerned. Once the wound from the operation had healed, the bull was taken to the arena and, in the traditional way, provoked fury by the toreadors. Delgado then entered the arena and provoked the bull which then charged him, head down to take Delgado on his horns. When the bull was only a few meters away from Delgado, he pressed the button of the transmitter in his pocket which stimulated the bull’s brain and the bull calmed down immediately. Efforts by the toreadors to enrage the bull again were unsuccessful as long as Delgado kept stimulating the bull’s brain. Only by pressing another button which stimulated a different part of the limbic system could the bull again be brought to a state of rage without the help of the toreadors. Evidently, the cells in which Delgado planted his electrodes regulate rage and tranquillity. The cells concerned are part of the limbic system and are found in the amygdaloid body, the amygdala. In a way similar to this rats and other animals can, by selective stimulation of other parts of the limbic system, be motivated to eat, resp. refuse food, to sexual arousal resp. be made immune to the usual sexual stimulations. In short, the limbic system regulates a number of very vital emotions. This effect can also be elicited in humans:

‘The first time we were able to demonstrate that systems in the limbic brain that both start and stop attack behaviour was with patient Thomas R. Thomas’ chief problem was his violent rage.... Electrodes were implanted in his amygdala and the daily stimulation of specific parts of it (the lateral) kept him free from attacks of rage for two months. Since it is not possible to continue this regimen throughout a patient’s life, those parts of his amygdala which elicited an attack
punishment center is stimulated if you then continue to eat. Stimulation of this center could, then, give a feeling of reward, without there necessarily being any ‘rewardable’ behavior.

These subcortical centers, together with some parts of the cerebral cortex which other mammals also have, can be referred to as the ‘horse’ in us.

**Cortex Cerebri (the Cerebral cortex)**

The cerebral cortex is the most complicated part of the nervous system. It is divided into four parts: the frontal lobe, the parietal lobe, the temporal lobe and the occipital lobe. All sensory perceptions are ‘projected’ onto the cerebral cortex and the outside world is represented by these projection fields. For example, each point in the visual field is represented by a point in the visual projection field in the occipital lobe. This is, incidently, where the story comes from that you can go blind if you fall on your tailbone. The shock is conducted along the spinal cord to the back of the head and the skull knocks against the visual projection field. This if damaged, causes blindness although nothing is wrong with the eyes. In the same way, all sounds, from high to low, are represented in the auditory projection field in the temporal lobe, the entire body surface is represented in the sensory cortex in the parietal lobe and all muscles in the frontal lobe. In addition to these projection fields, we also have association fields where the connections between this sensory input and motor output are made. That part of the brain which ‘seats’ language is particularly large even compared with our closest relative, the chimpanzee. In this association field the different aspects of language - hearing, seeing (both objects and letters) and speaking (larynx muscles) coordinate. The slightest damage to this area can lead to abnormalities such as aphasia, dyslexia, etc.

**The cerebral cortex is, mainly because of the latter mentioned association field, the ‘knight’ in us. Every psychosocial worker should realize that we are not people, but rather knights sitting on a horse, with the horse standing on a crocodile. We pay close attention to the horse, but if the crocodile in us suddenly turns left or right, we tumble to the ground: we call it a life crisis then.**

**CENTRAL NERVOUS SYSTEM**

Within the central nervous system (CNS) there are a number of physiological/biochemical loci where genetic influences and drug
been demonstrated, the demonstrable hereditary variation in neurosensitivity to alcohols seems likely to involve these membrane mechanisms. The direct action of anesthetics on membranes is thought to occur via alteration of the proteins found there; these proteins are likely to be those of the channels. One investigation in this area has attempted to use genetic variation as a tool for studies of possible commonality of action of ethanol and halothane, a gaseous anesthetic.

The LS and SS mice described earlier were shown to be similarly affected by halothane, even though their ethanol response is so strikingly different. It was concluded that the two drugs act via different mechanisms. In the present context, it would appear that different classes of membrane proteins are being affected by the two drugs. Alternatively, the differential LS and SS response to ethanol may not take its origin in membrane effects.

Receptors

In recent years it has become increasingly apparent that the functioning of the brain is critically dependent on highly specialized receptor molecules, protein in nature, and thus potentially subject to genetically controlled variation. These receptors are usually thought of as specific for particular neurotransmitters, neuromodulators, or hormones. Several clear examples of the involvement of such receptors exist in the psychopharmacogenetic literature. We deal here with drug effects and receptor studies of three types: benzodiazepines, opiates, and barbiturates.

Benzodiazepines have recently been shown to bind to specific sites in CNS membrane fragments. Thus it appears that the nervous system may possess endogenous neurotransmitter systems that are similar in function to the functions affected by this class of tranquilizers. The clinical efficacy of benzodiazepines (i.e., their anxiolytic effects) tends to correlate well with their affinity for these specific receptors.

It has been long known that the narcotic effectiveness of depressant agents such as alcohol is related to the degree of lipid solubility. Thus the degree to which a compound can distribute itself into neural membranes is a direct predictor of its anesthetic potency. Because it is well accepted that anesthetics and many sedative hypnotic drugs can act in this way, it is a parsimonious assumption that genetic variation in membrane construction could result in differing lipid solubility of the same drug in different genotypes.

Although such genetic variation has, to our knowledge, not yet been demonstrated, the demonstrable hereditary variation in neurosensitivity to alcohols seems likely to involve these membrane mechanisms. The direct action of anesthetics on membranes is thought to occur via alteration of the proteins found there; these proteins are likely to be those of the channels. One investigation in this area has attempted to use genetic variation as a tool for studies of possible commonality of action of ethanol and halothane, a gaseous anesthetic.

The LS and SS mice described earlier were shown to be similarly affected by halothane, even though their ethanol response is so strikingly different. It was concluded that the two drugs act via different mechanisms. In the present context, it would appear that different classes of membrane proteins are being affected by the two drugs. Alternatively, the differential LS and SS response to ethanol may not take its origin in membrane effects.
The Maudsley non-reactive rats (MNR) had a higher specific binding of diazepam in every brain region examined than did the Maudsley reactive (MR) rats. The largest differences were found in limbic structures. The nature of the differences was primarily one of receptor number rather than affinity. In hypothalamic tissue the number of binding sites was 1000 fmol/mg protein in MR rats and 1385 fmol/mg protein in MNR rats. The affinity for the ligand was only slightly higher in the MNR strain: $K_D = 3.64$ nmol for MR and 3.24 nmol for MNR.

These authors suggest this issue of receptor density as a plausible basis for the reactivity differences in the strains. Presumably the more reactive MR rats have a less extensive endogenous “tranquilizing” system. In another study from the same laboratory similar results were found in four mouse strains. “Emotional” BALB/c mice had a lower density of benzodiazepine receptors than did three “less emotional” strains. Both these studies reinforce the notion that endogenous benzodiazepine systems play a role in emotional behaviour. A more complete analysis in the future will, we hope, combine this type of study with actual examination of benzodiazepine effects on “emotionality.”

Another type of receptor, the opiate receptor, has been similarly studied. Considerable genetic diversity has been demonstrated for behavioural effects of opiate compounds in mice. Again, a parsimonious prediction might be that opiate receptor density or affinity could serve as the basis for varied behavioural effects of opiates. Two groups of investigators have directly addressed this issue.

Baran et al. investigated naloxone binding and analgesic response to morphine in $C_{57}$BL/6By, BALB/cBy, their reciprocal $F_1$ hybrids, and seven recombinant inbred strains derived from those parental stocks. Considerable strain differences for total stereospecific naloxone binding in whole brain and also analgesic response were reported. Even though the strain with the lowest naloxone binding (CxBK) also had the lowest analgesic response, the correlation between binding and analgesia across strains was non significant.

The relatively low statistical power for this correlation suggests that a real, but minor relationship may exist. Crabbe and Belknap point out that genotypic variation in behavioural response may well arise in part from dispositional factors, and thus the relative importance of receptor characteristics would diminish.

We would suggest two additional possibilities. First, the Baran et al. study did not examine specific brain regions. To the extent that analgesic effectiveness of morphine is mediated by specific brain regions (e.g., the periaqueductal grey), analysis of a heterogeneous whole brain could well obscure important predictability from specific regions.

The notion of nonhomogeneous opiate receptor system distributions raises a second issue. Present conceptions of opiate systems within the brain assume not only anatomical topographies, but biochemical heterogeneity of opiate receptor subtypes. It is conceivable then, in this context, that naloxone specific binding may not reflect a more specific receptor subtype, which holds importance for the genetic variation in analgesic potency. Use of another ligand might reveal a correlation with the genetic effects on analgesia. A recent study has examined the role of subpopulations of opiate receptors in genotype influenced morphine sensitivity. This work with $C_{57}$BL/6 and DBA/2 strains of mice found that strain differences in opiate receptor number depended on both ligand (and thus subpopulation) and brain region.

The $C_{57}$BL strain had considerably more striatal opiate receptors when D-Ala$_2$-Met-enkephalin or Leu-enkephalin were used as ligands. Such differences did not occur when naloxone or dihydromorphine were ligands, nor did they occur in three other regions examined. These results suggested that the locomotor response to morphine in $C_{57}$BL mice could take its origin in this striatal subpopulation of opiate receptors. It is interesting that neither of these studies on strain differences in opiate receptor characteristics reported differences among genotypes in affinity ($K_D$) of the ligand for the receptor.

This suggests that the kind of genetic variation present may not be that of structural gene mutation but rather complex regulation of the number of receptors inserted into neural membranes, or perhaps cell number. In this context, it may well be that control of receptor number in neural tissue could serve as a prime research area for regulation of gene action in higher organisms. A developmental perspective on this issue would probably also be of value.

As a third example of receptor characteristics and genetic control of drug response, the work of Waddingham, Riffey, Belknap, and...
Marijuana:
- Absorption: Marijuana may be inhaled or ingested.
- Metabolism/Elimination: THC (delta-9-tetrahydrocannabinol) is highly fat-soluble and may take up to three months to be fully eliminated from the body by the liver and kidneys. One joint affects the body for a period of two to four hours.
- Brief Overview: Marijuana is the most frequently used illicit drug in America and has been linked to harming a developing fetus. It has the same or similar effects as depressants, stimulants, and hallucinogens. Marijuana cigarettes yield almost four times as much tar as tobacco, creating a higher risk of lung damage.
- Short-term Effects
  - Increases in heart rate, body temperature, and appetite.
  - Drowsiness.
  - Dryness of the mouth and throat.
  - Reddening of the eyes and reduction in ocular pressure.
- Long-term Effects
  - Special Hazards Involving the Driving Task: Marijuana has been linked to the impairment of the ability to drive a vehicle. Concentration is affected and there is difficulty in perceiving time and distance, which can lead to the following: bad judgment, impaired reaction time, poor speed control, an inability to accurately read signs, drowsiness, and distraction.
  - Effects with Other Drugs: When marijuana is combined with alcohol it creates greater impairment in areas such as reaction time and coordination. When combined with sedatives and opiates, it can cause an increase in anxiety and even hallucinations, along with an increase in heart rate and blood pressure when used with amphetamines. On the other hand, effects are somewhat unpredictable when marijuana is combined with stimulants, such as nicotine, caffeine, amphetamines, and cocaine.

SPECIFIC EFFECTS ON THE CENTRAL NERVOUS SYSTEM

Drugs affect the various areas of the brain and change normal brain activity. It is important to know what specific functions are located in each of the major brain areas, to better understand the effects of drugs and alcohol on behavior and functioning.

Hypothalamus
The hypothalamus regulates homeostasis, the body’s system for keeping itself balanced. This includes: sleep and wake cycles, hunger, thirst, sexual behavior, blood pressure, and temperature. Also, the hypothalamus determines what parts of the body are affected by analgesics and regulates hormonal impulses and emotions.

Medulla
The medulla is responsible for head balance, movement, and assisting the hypothalamus in regulating automatic body functions.

Cerebral Cortex
The cerebral cortex contains half of the nervous system’s cells, which regulates the speed and vomiting reflexes. It is also responsible for language, abstract thinking, personality, and interpretation of emotion and sensory information, including judgment.

Cerebellum: The cerebellum is responsible for coordination of muscles, maintenance of balance, and specific memory and learning system functions that are not to one part of the brain.
Cocaine

- **Absorption**: Cocaine enters the body in one of three ways: injection, smoking, or snorting.
- **Metabolism/Elimination**: Cocaine is a strong stimulant to the central nervous system. Its effects can last anywhere from 20 minutes to several hours, depending on the content, purity, administration, and dosage of the drug.

**Brief Overview**
- Cocaine users become dependent on the drug.
- Crack is a form of the drug that is highly addictive.
- Exposure to the drug can harm a developing fetus.
- It produces short-lived senses of euphoria, the length depends on how the drug was administered.

**Short-term Effects**
- May cause extreme anxiety and restlessness.
- May experience the following medical conditions: twitches, tremors, spasms, coordination problems, chest pain, nausea, seizures, respiratory arrest, and cardiac arrest.

**Long-term Effects**
- May cause extreme alertness, watchfulness, impaired judgment, impulsiveness, and compulsively repeated acts.
- May cause stuffiness, runny nose, tissue deterioration inside the nose, and perforation of the nasal septum.

**Special Hazards Involving the Driving Task**
- Cocaine may successfully mask fatigue, however, high dosages impair judgment and interfere with the ability of the driver to concentrate.
- Coordination and vision are impaired.
- There is an increase in impulsive behaviors with tendencies to take more, risks and create confusion within the user.

**Effects with Other Drugs**
- Additive effects are noted when cocaine is combined with over-the-counter products, such as diet pills or antihistamines.

- Cocaine taken with psychotropic drugs, especially antidepressants, can be extremely detrimental.
- A person who has extremely high blood pressure and uses cocaine may suffer from a stroke or heart attack.
- Some users combine cocaine with alcohol and sedatives to cushion the “crash” or feeling of depression and agitation that sometimes occurs as the effects of cocaine wear off.
- A person using cocaine maintains the illusion of being alert and stimulated, although physical reactions are impaired.
- Further research indicates that additive and antagonistic effects can be produced when cocaine is mixed with alcohol.
- If cocaine is used in high doses, as in the case of overdose, alcohol will probably have an additive effect on the symptoms that eventually contribute to death.
- When cocaine is injected in combination with heroin, sometimes called “speedballing,” there is an increased risk of toxicity, overdose, and death.

**Sedative Hypnotics**

- **Absorption**: Sedative Hypnotics are absorbed through ingestion.
- **Metabolism/Elimination**: Sedative Hypnotics are eliminated by the liver and excreted in urine. Their effect can last anywhere from two to ten hours.

**Brief Overview**
- Antianxiety tranquilizers are among the most commonly prescribed drugs in the world.
- Driving under the influence of tranquilizers is dangerous.
- A person can become dependent on tranquilizers and depressant drugs, which make them feel calmer, more relaxed, and drowsy.

**Short-term Effects**
- Short-term effects can occur with low to moderate use.
- May experience moderate relief of anxiety and a
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• There may be temporary memory impairment, confusion, and impaired thinking.
• A person could be in a stupor, and have altered perception and slurred speech.

**Long-term Effects**

• Special Hazards Involving the Driving Task
  - The use of tranquilizers produces drowsiness, incoordination, altered perceptions, memory impairment, poor control of speech, and slower reaction time.
  - Effects on driving include: poor tracking, difficulty in maintaining lane position, and neglecting roadside instructions.
  - When combined with alcohol, the effects may be more hazardous.

• Effects with Other Drugs
  - Some people in methadone treatment programs use benzodiazepines to enhance the effects of methadone.
  - When tranquilizers are combined with alcohol or other central nervous system depressants, synergistic effects may be produced, which may be fatal.
  - Alcohol increases the absorption of benzodiazepines, slows their break down in the liver and can cause cardio vascular and respiratory depression.
  - People who take stimulants sometimes take tranquilizers to off set agitation and sleepiness.

**Opiates**

• Absorption: Opiates are normally absorbed though injection.
• Metabolism/Elimination: Opiates are metabolized by the liver and may have a lengthy metabolism due to excessive half-lives of the drugs.

• Brief overview
  - Opiates can cause sedation and euphoria.
  - They are often used to relieve pain, suppress coughs, and control physical conditions such as diarrhea.
  - Respiratory depression and death can occur from overdoses of opiates.
  - Opiates may impair a person’s ability to drive.
  - A person can become physically and psychologically addicted to opiates.

• Short-term Effects
  - Include drowsiness, dizziness, mental confusion, constriction of pupils, and euphoria.
  - Some opiate drugs, such as Codeine, Demerol, and Darvon, also have stimulating effects.
  - Stimulating effects include: central nervous system excitation, increased blood, elevated blood pressure, increased heart rate, tremors, and seizures.

• Long-term Effects
  - May include impaired vision, pulmonary complications, and menstrual irregularity.
  - A person may experience nightmares, hallucinations, and mood swings.

• Special Hazards Involving the Driving Task
  - Opiates can cause drowsiness, mental confusion, and visual impairment even at lower, moderate doses.
  - A driver may have difficulty keeping the vehicle in the correct lane and may make errors in judgment.

• Effects with Other Drugs
  - Alcohol greatly increases the present effects of
Amphetamines

- Absorption: Amphetamines are absorbed by the body in one of three ways: snorting, swallowing, or injection.
- Metabolism/Elimination: Amphetamines are eliminated through the liver.
- Brief Overview
  - Amphetamines have a strong central nervous system stimulant which can increase alertness and induce a sense of well-being.
  - If used while driving, amphetamines are dangerous.
  - The use of amphetamines reduces a person’s resistance to disease.
- Short-term Effects
  - A person may experience a loss of appetite, increased alertness, and a feeling of well-being.
  - A person’s physical condition may be altered by an increase in breathing and heart rate, elevation in blood pressure, and dilation of pupils.
- Long-term Effects
  - Anxiety and agitation.
  - Sleeplessness.
  - Higher blood pressure and irregular heart beat.
  - Increased susceptibility to disease.
- Special Hazards Involving the Driving Task
  The use of amphetamines can interfere with concentration, impair vision, and increase the driver’s tendencies to take risks.

Effects with Other Drugs

- Amphetamines should never be taken with a class of antidepressants known as MAO inhibitors, because of potential hypertensive crisis.
- Amphetamine users sometimes use marijuana and depressant drugs in order to avoid the adverse side effects of the “crash,” therefore creating multiple drug dependencies.

Effect with Other Drugs

- A person injecting heroin mixed with cocaine or methamphetamine, known as “speedballing,” produces a stimulant effect.
- The listed drug combinations increase the risk of toxicity, overdose, and death.

OVERVIEW OF THE AUTONOMIC NERVOUS SYSTEM

It was stated at the beginning that the peripheral autonomic system has a prominent place as a site of drug action. We will now look at the organization of this system, and at the distribution of transmitter receptors within it. This will enable us to understand the effects of drugs acting upon this system and rationales behind their usage.

The autonomic nervous system consists of two functionally distinct parts that frequently exert antagonistic effects on their target organs. These are referred to as the sympathetic and the parasympathetic system, respectively. Figure depicts some essential features. The parasympathetic system, for the most part, emerges from the central nervous system at the level of the medulla oblongata, which is the lowermost part of the brain. These neurons reach some nerve centers in the periphery, which are named ganglia (singular: ganglion), where they trigger activity in secondary neurons that in turn reach out to the target organs. The sympathetic system mostly emerges at the thoracic portion of the spinal cord. It too has relay neurons in peripheral ganglia (which are connected with each other in the so-called ‘sympathetic chains’, located on either side of the spine). The parasympathetic and sympathetic ganglia are outside the
central nervous system, and therefore readily accessible to drugs that do not cross the blood brain barrier.

The target tissues that are controlled by the secondary neurons (the ones originating in the ganglia) include:

- Secretory cells in various glands, both exocrine and endocrine;
- Heart conduction system and muscle cells;
- Smooth muscle cells in the intestine, other hollow organs (bronchi, urinary tract, sexual organs, etc.) and in the blood vessels.

Figure also shows the major types of neurotransmitter receptors found within the autonomic nervous system:

- The nicotinic acetylcholine receptor occurs in both the sympathetic and the parasympathetic ganglia. The receptors found in the neuromuscular synapse are of the nicotinic type as well. However, the subtype is different, and therefore selective drug action is possible.
- Muscarinic acetylcholine receptors occur in the target tissues. They are mostly found in parasympathetic synapses, but they also occur in the sympathetically innervated sweat glands.
- Adrenergic receptors are always related to sympathetic activity, either within synapses (as shown here), or diffusely distributed and by responding to circulating epinephrine.
- Dopamine D₁ receptors are less widespread than adrenergic receptors. One prominent occurrence is in the kidney arteries. Accordingly, dopamine and related agonists are being used in intensive care treatment of acute kidney failure to improve kidney perfusion.

Very commonly, a target tissue will be stimulated by the sympathetic system and inhibited by the parasympathetic system, or vice versa. Examples are found in table Among the parasympathetic responses listed there, we find stimulation of smooth muscle in the bronchi, and relaxation of smooth muscle in the arterioles; both are mediated by muscarinic acetylcholine receptors (cf. Figure. Here, we have an example of diverse effecter mechanisms triggered from similar receptors. Similarly, the adrenergic receptors can operate different intracellular switches as needed. These different effecter mechanisms are covered in some more detail in the chapter on G protein-coupled receptors. A ‘take-home’ message from table is that, by and large, muscarinic receptors mediate the parasympathetic effects, whereas the sympathetic ones are mediated by adrenergic receptors.

\[ M: \text{Muscarinic,} \]
\[ N: \text{Nicotinic cholinergic receptors;} \]
\[ D: \text{Dopaminergic, } \alpha, \beta: \text{Adrenergic receptors.} \] (The innervation of skeletal muscles by \( \alpha \)-motoneurons is shown for comparison but not part of the autonomic system.) BBB: Blood brain barrier. It protects the entire central nervous system, i.e. both the brain and the spinal cord.

Table. Examples of Organ Responses to Autonomic Innervation.

From the effects of the autonomic nervous system on the various target organs (table), we can easily understand several applications of drugs that cause synaptic stimulation or inhibition:

- In patients having undergone abdominal surgery, quite frequently the activity of the intestine is sluggish. Drugs that stimulate muscarinic receptors will help to correct this.
- As we have seen, drugs that block \( \alpha \)-adrenergic receptors (e.g., phenoxybenzamine) will help to lower the resistance in arterioles and therefore reduce blood pressure.
- Blockers of \( \beta_1 \)-adrenoceptors help to reduce the workload of the heart, but they sometimes slow down the generation or propagation of excitation too much, resulting in slow and occasionally irregular heartbeat.
- Drugs that stimulate \( \beta_2 \)-adrenoceptors will help to dilate the bronchi (by reducing the smooth muscle tone there) will be useful in asthma, which basically consists in impeded air flow due to a spastic narrowing of the bronchi.
- If the effect of \( \beta_2 \) agonists in asthma proves insufficient, one additional therapeutic option is to add a drug that will inhibit the cholinergic (parasympathetic) stimulation of the bronchial smooth muscle, such as ipratropium bromide.

A peculiar element within the autonomic nervous system is the medulla (inner part) of the adrenal gland. This is the site of production for epinephrine and norepinephrine that are released into the circulation. It is directly controlled by cholinergic neurons emerging from the spinal cord, so it assumes the place of a sympathetic ganglion. In fact, the cells in the adrenal medulla are of neural origin - they are nerve cells turned gland cells. In contrast, the cortex (outer part of the adrenal gland) is a ‘proper’ gland tissue not of neural
Definition

The nervous system is made up of CNS (Central Nervous System) & PNS (Peripheral nervous System). The CNS is made up of brain and spinal cord. And the PNS is made up of SNS & ANS.

- Autonomic nervous system (ANS) is mostly innervates visceral organs of the body.
- Two neurons are present in the ANS pathway. One is called as preganglionic neuron and other is called as post ganglionic neuron. The junction between two neurons is called as ganglion or synapse.

Preganglionic neurons

Its cell body is present in the brain or spinal cord and its axon is elongated from inside to outside of CNS. The axon is myelinated. Actually, the cell body is present in the lateral horn of gray matter in thoracolumbar division (sympathetic). And cell body of cranio-sacral division is present in the cranial nerves.

Postganglionic neurons

It is the second neuron present after the ganglia. Its cell body and dendrites are located in the autonomic ganglion. It is unmyelinated.

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The Evolving Medical Paradigm

The particular phase of evolution facing twenty-first century medicine is unique but not entirely new. Global mythology reveals that the quest for a world-view that yields health and wellbeing is timeless. In Central African mythology there is a dangerous one-legged, one-armed half man known as the 'Chiruwi', or 'mysterious thing'. This creature challenges anyone he encounters to a fight with the promise that if vanquished he will reveal many medicines and the lucky victor will become skilled in the healing arts. Perhaps western medicine is currently in the process of dealing with a cultural Chiruwi.

Another mythical figure relevant to the medical situation of the third millennium comes in the form of the Greek God of the mountainside, Pan. This playful and energetic god could be fearsome and he instilled in humans who ventured carelessly into his domain a sudden and groundless fright, or 'pan-ic'. On the other hand Pan offered bounty to those who honoured him. To these worshippers he would offer health and the wisdom to reach the universal source.

In an unconscious homage to Pan and the Chiruwi there is a distinct global movement to embrace something beyond the biomedical model of medicine that has been the beloved child of science. Global organisations, governments, science and individuals are turning in ever greater numbers to the complementary paradigm of medicine. In a sense, there is a metaphoric move toward honouring the nature god Pan in the hope that perhaps he will offer the wisdom...
that will lead to truly fundamental healing. This is not to say that there is a mood to discard totally the advances of biomedical medicine. A Luddite yearning for simpler days past will not move medicine forward. Rather what is occurring is the shaping of a post-modern medical paradigm.

**TERMS OF ENGAGEMENT**

To begin a discussion of the changes that are taking place in medicine it is necessary to define terms. For much of the twentieth century the biomedical model was the dominant medical paradigm and we will refer to it as ‘orthodox medicine’. It features a drug and surgical approach to disease management. Underpinning these curative tools is a scientific and reductionist attitude toward the human body that tracks disease to its biochemical roots.

Essentially the orthodox philosophy sees medical science as applied biology and its practitioners as diagnostic biochemists. It is based on the principle of Cartesian Dualism which implies that, even if there were no mind in it, the nerves, muscles and blood vessels of the body would have the same functions. This separation of mind and body was espoused by Rene Descartes in the seventeenth century and has served as the underpinning of the orthodox approach since that time.

The separation of mind and body paved the way for the development of a medical approach based on measurable quantities. This was what allowed medicine to become a science and possess directly predictive properties.

The dualist notion was born just as the scientific revolution of the seventeenth century was under way. Isaac Newton’s mechanistic theories were emerging and it was only natural that the orthodox science of medicine came to view the human body as a machine.

Orthodox medicine has been incredibly successful. It has prolonged lives and, along with improvements in hygiene, been part of a social and medical milieu that has seen many diseases eradicated. Yet the latter part of the twentieth century and now the twenty first century has seen a widespread movement toward another medical model.

‘Complementary medicine’ is a term that encompasses a range of practices and philosophies. Some are ancient and some are relatively new but what they have in common is that they are based on theories or explanatory mechanisms that are not in keeping with the orthodox biomedical model.

Some of the leading medical modalities that come under the heading of ‘complementary medicine’ including among their number herbal medicine, homoeopathy, acupuncture, nutrition, massage and counselling. These modalities are diverse but they hold common central attitudes.

Complementary medicine rejects the dualism of orthodox philosophy while accepting the science of medicine. Fundamentally, complementary modalities embrace ‘a concept of medicine as a science of the human person and insist on an understanding of disease as something that involves a systemic dislocation of the whole person, not just of the body’. Within complementary medical modalities there are variations on this philosophy yet the holistic view that health is a dynamic interplay between mind, body and spirit is common to all. Certainly the premises of complementary medicine are finding increasing support with scientists, governments and people the world over.

**The People**

On a global scale there is substantial and justifiable acceptance of the many benefits that orthodox medicine brings. Simultaneously, however, the western world, birthplace of orthodox medicine, is embracing complementary medicine in an emphatic way.

The World Health Organization (WHO) estimates that the global market for complementary therapies stands at 60 billion US dollars a year and is growing steadily. This represents a worldwide trend that is reflected in individual countries in significant ways.

Data from the British parliament support the idea that complementary medicine use in Britain is high and is increasing. In 1999 in Britain 93 million pounds was spent on complementary medicine and five million Britons visited the 50 000 complementary practitioners who operate there. By 2002 the amount that Britons spent on complementary medicines had risen to 126 million pounds. Australia is also experiencing an exponential increase in the use of complementary medicine. It is estimated that Australians spent 2.3 billion Australian dollars on complementary therapies and medicine in the year 2000, which is a 120 per cent increase on what they spent in 1993.

Overall in 2000 Australian people spent four times more money
BODIES OF INTELLIGENCE

Complementary medicine treats the body as a holistic organism with dynamic processes that operate to keep that body in balance. Implicit to this paradigm is the acceptance that all parts of a human being are connected and that disease in one area can result from imbalance in another area. This conflicts with the traditional approach of orthodox medicine that targets symptoms in discrete body areas. Evolving scientific theory, however, is finding ways to encompass the holistic philosophy. One of the newer scientific world-views is complexity theory.

This theory is applied to a variety of fields including ecology, physics, economics and computing where complex structures exist. In essence, complexity theory holds that once the rules governing these systems are found, it is possible to make effective predictions and even to control apparently complex entities. One salient point that is also emerging from complexity theory is that the properties of the system cannot necessarily be tracked to individual components but arise from the whole system. The implications of this notion for medicine are clear.

If complexity theory holds true then disease arises from the whole body, not its discrete parts. Effectively, pathology arises ‘when the body self-organises in response to some disturbance but becomes confused and ends up worse at self-regulation than before’. In this light the mechanistic notions of orthodox medicine cannot hope to achieve cure but can only patch holes in the network that is the human body. Under complexity theory, genes, lifestyle and environment all interact to impact on the network that is the human body and the result is either disease or health. This paradigm is coincident with theories of holistic healing and reflects that at the true sharp end of science, the underpinnings of holistic medicine are being reinforced.

The complete coalescence of science and complementary theory will not happen immediately. Nevertheless the signs and symptoms...
that science is actively considering complementary medicine are manifold. Deliberate investigation of complementary medicines is taking place within the orthodox scientific establishment in many ways. There is a plethora of studies in the medical and scientific journals examining the components of complementary medicine from acupuncture to herbal medicine to yoga.

A striking illustration of modern science meeting traditional methods is research in Wales that may result in a 600-year-old yielding new drugs. In a village called Myddafai in Wales in the early thirteenth century a physician called Rhiwallon founded a line of healers that spread throughout the Welsh land. Over 500 remedies using more than 200 different plants. Modern pharmacists are engaged in examining the Myddafai cures to see what chemicals they may yield that can be turned into pharmaceutical drugs. Similar pursuits are occurring in the southern hemisphere.

In Australia the Commonwealth Scientific and Industrial Research Organisation (CSIRO) has announced that it will be turning considerable attention to the investigation of the healing properties of herbs. In addition to examining traditional Western herbs such as garlic, the CSIRO also intends to examine native plants for their economic and medicinal potential. The dilemma facing scientific inquiry into herbs is that isolating so-called ‘active ingredients’ from herbs violates holistic principles and is likely to have a negative effect on efficacy. Still, interest is the first step toward understanding.

Herbs, however, are not the only ancient medicines that are experiencing a rising tide of scientific interest. If ever there was a symbol through which the orthodox medical establishment has expressed its disdain for the healing wisdom that had passed before, it was the leech.

The image of patients festooned with leeches has been emblematic of the supposed naivety of historical healing. Indeed, the leech was a popular medical tool throughout much of history. Babylonian writings suggest that the leech was being used medicinally as long as 3500 years ago.

The Egyptians also embraced leeches as healing tools and the Europeans took up the annelids with equal verve. So popular was leech therapy with medieval European healers that physicians of this time were actually known as ‘leeches’. It is estimated that by 1850 French physicians were using 100 million leeches per year so it is not surprising that in the nineteenth century the European leech was dancing along the edge of extinction.

Medicinal leeches were used over the centuries for bloodletting and were applied to congested or inflamed parts of the human body. Many ailments were subjected to the application of leeches. In America in the nineteenth century, leeches were used as a common home remedy to treat gum disorders and haemorrhoids, and to relieve the pain of large bruises.

The advent of improved understanding of bacteriology and infection in the latter nineteenth century led to a significant focus on hygiene and a drop in popularity for the leech. Thus in the twentieth century, orthodox medicine scoffed at the apparently farcical adherence to the leech therapy of earlier centuries. In the last decade of the second millennium CE, however, leeches have made a medical comeback.

It has been found that in cases of venous congestion, where re-establishing the flow of blood is essential, leeches have great therapeutic value. As they consume their meal of blood, leeches promote blood flow through the tissue. Even after a leech is full of blood and detaches from the body, the anticoagulants it secretes into the tissue allow the wound to ooze blood for hours afterwards.

These effects cannot be replicated by application to the site of anticoagulants such as heparin. Evidence is also growing to support the pain-relieving effects of leeches for conditions such as arthritis.

One study has evaluated the effectiveness of leeches in relieving arthritis of the knee. This study took place at the University of Essen, Germany, and involved sixteen patients with confirmed osteoarthritis of the knee. Ten of the patients were treated with leeches and six were controls. For those in the leech group, four leeches were applied around the knee joint for a period of 80 minutes each day. Both the control and the leech group were given conventional treatment for pain excluding non-steroidal anti-inflammatories. Those in the leech group experienced a rapid relief of pain which was faster than those in the control group, with sustained improvement after four weeks and no major complications. This is just one of many studies reporting that leech therapy can reduce pain and improve joint mobility in arthritis.

To testify that orthodox medicine is genuinely interested in leeches, there are moves afoot to develop mechanical leeches that
establishing health. Nevertheless this immensely popular sector of health management reflects clearly that orthodox science and the medicine that it inspires has embraced one of the premises of complementary medicine, which is the simple notion that lifestyle, including diet, has real and significant impacts on health.

Thus on many levels orthodox medicine and complementary medicine are already moving together. At government and bureaucratic levels, this move is happening at an even more rapid pace.

**THE BUREAUCRACY**

Simon Mills, respected British medical herbalist, has put the situation succinctly: ‘Public demand for complementary medicine has grown to a level where communication and co-operation with orthodox health services is necessary’. Perhaps the most significant recognition of this fact has come from the World Health Organization (WHO).

In light of the sweeping interest in complementary medicine the WHO has released a detailed policy paper on this topic. As a starting point the WHO defines ‘traditional medicine’ as including diverse medical practices incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques and exercises.

The WHO observes that in the western world where orthodox medicine has been the dominant paradigm, complementary medicine is the manifestation of traditional medicine. Going the WHO indicates that the reason for the western world’s turn toward complementary medicine is ‘concern about the adverse effects of chemical medicines, a desire for more personalised health care and greater public access to health information’.

In light of these trends the WHO has made several significant recommendations. First, the WHO has observed that as of the year 2000, 25 countries reported having a national traditional medicine policy. These policies are recommended to other countries with recommendations to create legal mechanisms that will promote equity in access to traditional/complementary medicines and to ensure

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**Nutrients or Nutraceuticals**

At the most basic level food is the root of health and, perchance, disease. The axiom that food should be synonymous with medicine has now passed into popular consciousness and parlance.

Perhaps the most tangible manifestation of the acceptance by orthodox science and medicine of the curative power of nutrition is the development of what are known as ‘functional foods’ or ‘nutraceuticals’. Since the British navy realised that limes could ward off scurvy, three centuries of investigation has revealed that food contains substances that are vital to health. In that time many nutrients and minerals have been discovered. In the early part of the twenty-first century, however, another revolution is taking place in the foods available to human beings.

The modern diet is based on a few central plants that have been domesticated and bred to be increasingly bland. This narrowing and blanching of the human food chain has resulted in a relative deficiency of health-promoting substances in the average western diet.

The missing substances are neither nutrients nor toxins but lie somewhere in between and their lack is resulting in modern humans being ‘pharmacologically impoverished’. To make up for this deficit, modern science has turned its attention to the development of ‘nutraceuticals’.

These nutraceuticals are foods that richly supply the consumer with substances that are generally missing from modern food but which are health-promoting. Leading among these are yoghurts containing Lactobacillus bacteria and margarines fortified with plant sterols.

It is estimated that by the year 2006 the market in nutraceuticals will be worth 35.4 billion US dollars annually. This is not to advocate nutraceuticals, as the option of consuming a greater diversity of fruit and vegetables would be a premium, and far preferable, strategy in establishing health. Nevertheless this immensely popular sector of health management reflects clearly that orthodox science and the medicine that it inspires has embraced one of the premises of complementary medicine, which is the simple notion that lifestyle, including diet, has real and significant impacts on health.
Post-Modern Medicine

There was a time when the term ‘modern’ was synonymous with ‘good’ or ‘best’. Possibly as a result of the Darwinian revolution in thought, the ‘modern’ was conceived to be at the apex and what had passed before was, by definition, lesser. Such a view is increasingly holding less credence.

There is a rising perception that the philosophical milieu must itself evolve to a new level if the planet and the individuals on it are to survive. In essence, a yearning for something beyond the modern is emerging. Hence in many spheres post-modern schools of thought are arising.

‘Modernity’ could be said to correspond to the changes of the Industrial Age and perhaps to have begun in the mid-eighteenth century. Under the ‘modern’ paradigm science provides the ultimate model for understanding.

Science is theoretically neutral and objective and scientists are ideally supposed to operate through their unbiased, rational capacities. Of course, whether such a ‘disembodied scholar’ can ever exist is problematic and a debate unto itself. The post-modern world by contrast coincides roughly with the Information Age and its parameters are fundamentally different from that which has passed before. In the post-modern reality, knowledge cannot be divorced from ethical considerations.

The mechanical dictums of the Industrial Revolution are no longer enough and methodologies that encompass more than the strictly measurable are in demand. In medicine this is manifesting as the turn away from reductionist biochemistry toward the holistic notions of complementary healing.

The exact shape of post-modern medicine remains to be elucidated, yet a few elements of it are already clear. The health paradigm of the twentieth century treated the human body as if it were a car that could simply be repaired with a quick trip to the garage, otherwise known as the doctor’s surgery.

The post-modern view is that visible symptoms both mask and
Principles of Clinical Pharmacology and Therapeutics

Principles of Clinical Pharmacology and Therapeutics

Biopsychosocial medicine was developed by Dr George Engel and was first clearly enunciated by him in 1977. Engel began as a devoted student of the biomedical model of medicine but soon came to an appreciation of the role of the psyche in illness. His understanding of ulcerative colitis is illustrative of his philosophy. Through his own work Engel observed that ulcerative colitis was a disease of the bowel mucosa with implication of the vascular system.

In addition, however, Engel examined 700 cases of ulcerative colitis and found personality traits that predisposed to the disease. He found that ulcerative colitis patients tended to be compulsive, dependent and had problems with relationships that stemmed from a retention of the mother-child relationship dynamic.

Engel found that this type of personality predisposed the bowel tissue to changes that occur in ulcerative colitis. Thus he was elucidating the inseparability of mind and body as well as the relationship of that mind and body unit to the environment in which it exists.

Engel embellished this model over the decades until he enunciated the ‘biopsychosocial model’, which is an approach to the management of disease that factors in the biological, psychological and sociological aspects of the individual.

Whatever the name given to the discipline, the uniting factor of post-modern medicine is that it recognises the importance of relationship: both the relationship of the mind and body and of the whole person to the environment. The name gaining popularity as the descriptor for this post-modern paradigm is ‘integrated medicine’.

An Integrated Model

The newly installed President of the American Medical Association, Dr Wendell C. Phillips, made a bold statement. He said: ‘The chief role of the physician of the future will be to keep his patients well rather than treat them when they are sick’. These commendable sentiments were somewhat before their time as they were expressed in 1926.

Dr Phillips’ Utopian vision has not come to pass and orthodox medicine wavers perilously on the precipice of being simply the technical management of disease. Orthodox medicine has focused on specific disease intervention and has ignored concepts of self-healing and holism.

Complementary healing has blossomed into this vacuum, yet the
way forward is not simply to tack elements of complementary healing onto the orthodox edifice. Similarly, to discard all that orthodox medicine and science has achieved would be farcical. Advocates of orthodox and complementary medicine alike are acknowledging that an integrated model is the future of medicine.

Integrated medicine will have as its premise a search for health and healing rather than disease and treatment. Patients will be viewed as whole beings with minds and spirits as well as bodies and not just as a biochemical puzzle to be solved. Technology will sit alongside lifestyle factors as primary instruments of healing.

Most importantly, integrated medicine will encourage greater involvement of the patient in the healing process. It will recognise that information is an individual’s right and that health is an individual’s responsibility.

‘Integration’ in this context can be seen to have a broad application to the medical future. Future medicine will not only be an integration of the orthodox and complementary paradigms but will depend on an integration at the individual level.

This will involve an integration of the mind, spirit and body as well as an integration of the individual into their larger environment. Individuals will be more informed about and responsible for their own health.

The medical community will treat people as unique and individuals will embrace a notion of health that goes beyond the absence of disease. Health will be acknowledged as a dynamic process that arises from the internal balanced integration of the individual and that person’s interactions with their community.

The challenge of the integrated model is dealt with in the Conclusion to this volume but suffice it to say that the integrated medical future is one of individual empowerment and immense potential. The philosophies and methods outlined in the rest are contributing forces that shape the present and future of medicine. They are diverse yet unified and their blend is a significant part of the rich mixture that is the future of healing and, therefore, of the human race.

Without the advent of steam distillation there would be no aromatherapy today. Steam distillation uses water in its vapour form, steam, to vapourise the volatile aromatic compounds found in various parts of aromatic plants. Once vapourised the steam and volatile aromatic oil vapours are channelled into a condenser (heat exchanger), where cold water flows around the hot vapour-filled pipes. This quick cooling of the vapour returns both the steam and volatile oil back to liquids. Volatile oil is predominantly composed of hydrocarbons and oxygenated hydrocarbons; these very non-polar components and the polar water do not mix. By placing both within a separator the two are easily separated, usually with the volatile oil floating on top of the water (due to lower specific gravity) where it can be easily drawn off.

This simple form of extraction allowed human beings to produce highly concentrated aromatic substances from plants. These volatile aromatic substances are called ‘essential oils’. Essential oils have many advantages over their parent plant material, in that being highly concentrated they can be more easily transported, stored and used in a wide variety of ways, from culinary to medicinal.

Essential oils are derived from the fragrant components of aromatic flowers, grasses and trees. They are also referred to as volatile oils because if left in the open air they will quickly evaporate.

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The Essence of Aromatherapy

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Plant families rich in essential oils include the Lamiaceae (mint), Apiaceae (carrot and parsley), Asteraceae (daisy), Myrtaceae (eucalyptus and tea tree) and Rutaceae (citrus fruits). It is important to note that essential oils are not the only components of modern aromatherapy practice. Other aromatic substances commonly used within aromatherapy include:

- **Expressed oils**: These are citrus oils that are produced by maceration of the fruit, which ruptures the cells containing oil. These cells are called flavedo and are found within the outer layers of the rind.
- **Absolutes**: These are aromatic substances usually extracted from flowers such as jasmine, rose, neroli, mimosa and boronia for the fragrance and perfumery industry, using petrochemical solvents which do not damage the heat-sensitive aromatics.
- **Carbon dioxide extracts**: Some aromatics are extracted from dried plant material using hypercritical carbon dioxide at great pressure (20 atmospheres) and low temperature. This is a form of ‘solvent’ extraction that requires expensive technology to produce very pure and highly concentrated extracts.

Today, within complementary therapies, aromatherapy has a broad base. Unfortunately the term ‘aromatherapy’ has also been subverted by the corporate world to market products with often less than natural origins.

This subversion has been one of the major reasons why the orthodox medical community and the general public alike do not take aromatherapy seriously. Such a view is unfortunate, as modern aromatherapy is in fact a rigorous medical modality.

Much has been discovered since Gattefosse’s time and there is now a greater understanding of the chemical structures and the therapeutic properties of the constituents of essential oils. This understanding has emerged from the empirical use of the oils by practitioners to treat a wide variety of conditions and diseases, combined with knowledge gained from analysis of the oils by chemists. Arising from this understanding are several distinct modern approaches to aromatherapy.

**CONTEMPORARY AROMATHERAPY**

The first of these approaches came from the work of a French doctor, Jean Valnet, who used essential oils during the Crimean War to treat the wounded, when medical supplies were exhausted. His work built upon the discoveries of Gatefosse and showed the effective antimicrobial and wound-healing properties of essential oils.

The tradition of using essential oils in this very medical sense continues in France to this day. The French doctor Daniel Pene’l has done much to popularise the French method of aromatherapy in the English-speaking world and is thought of as the father of the worldwide ‘Aromatic Medicine’ movement.

Across the channel in England, however, a different approach to aromatherapy exists. The predominant paradigm of aromatherapy in England began when the work of Valnet inspired Marguerite Maury, a French cosmetologist, to pursue the uses of essential oils in beauty therapy.

It was this work that set the stage for using low concentrations of essential oils in vegetable oils as carriers to nurture the skin. Later in 1959 at a beauty therapist conference Maury met Micheline Arcier, who went on to develop the popular massage technique used by aromatherapists.

The ‘Micheline Arcier technique’ (MR), as it is known, combines the modalities of Swedish massage, lymphatic drainage, polarity therapy and acupressure with aromatic oils.

When performed correctly, the MR massage technique is gentle yet has profound effects in lowering client stress levels. MR massage is the cornerstone of English aromatherapy but the final part of the English puzzle came when Robert Tisserand published his fundamental text The Art of Aromatherapy in 1977.

This work not only popularised aromatherapy with the general public, but also catalysed the formation of the English aromatherapy industry as a whole. The English ‘popular aromatherapy’ model quickly spread to America and Australia where it was initially taken up by beauty therapists as a method for introducing a pleasant smelling relaxation massage to their clients.

Later various schools and colleges started to teach certificate and diploma courses in aromatherapy, predominantly following the English model.

The third style of aromatic use comes from Germany and involves...
the inhalation of aromatics to treat psychological and emotional disorders and diseases. ‘Aromachology’, as it has become known, has shed light upon the powerful effects aromatics have upon the unmediated pathway that olfaction has to the human brain.

With the turn of the twenty-first century there has been much progress around the world in the reintegarion of all forms of aromatherapy. Today in countries like Australia, England, New Zealand and the USA popular aromatherapy is being practised alongside clinical aromatherapy.

As acceptance by orthodox practitioners and regulatory authorities grows and industry experience and the practitioner knowledge base expands, there will be the advent of ‘aromatic medicine’ in these countries.

Many aromatherapy practitioners are already upgrading their skills and knowledge in the areas of anatomy and physiology, chemistry, pharmacology, toxicology and the uses of new essential oils.

Networking with other complementary therapists, such as remedial massage therapists, reflexologists, Bowen Technique therapists, naturopaths, herbalists and flower essence practitioners to name a few, is also becoming more commonplace as practitioners realise the benefits of these strategic alliances.

Orthodox medicine is also beginning to use the knowledge and skills of aromatherapists in areas such as childbirth, palliative care and aged care. Integration of aromatherapy with other medical practice has seen improvement in patient outcomes by relieving physiological and psychological stresses as well as improving quality of life.

THE SCIENCE OF AROMATHERAPY

The explanatory theories of how and why aromatics work on human physiology and psychology closely parallel the development of orthodox medical rationales and discoveries.

The evolution of this understanding began with the earliest studies of gross anatomy in the Renaissance by Leonardo da Vinci and has continued through to the current advances in cellular research.

Scientific techniques and methods of analysis have provided many useful tools in discovering the complex chemical interactions between human physiological systems and essential oils.

Organic chemistry, for example, has provided essential oil researchers with a chemical roadmap to understanding the chemical constituents of essential oils: ‘A more detailed understanding of the actual chemical components of essential oils began with the work of chemist Otto Wallach between the years 1880 and 1914. Wallach was an assistant to the eminent F.A. Kekule, the first to accurately describe the chemical structure of the benzene molecule. Benzene is the basic structure of many cyclic aromatic compounds within aromatic chemistry such as phenols and ethers Wallach’s work is part of a rich vein of research into the essential oils of plants throughout the world.

To take just one example, in Australia there has been an exponential growth in the cataloguing and analysis of native Australian plants and their constituents, including essential oils, over the past century by many scientists including:

These plant chemists used empirical analytical techniques to determine the constituent makeup of many Callitris and Eucalyptus species before the advent of gas chromatography and mass spectrometry.

- Penfold in the 1940s to 1950s: a major researcher in essential oils at the then Museum of Technology, Sydney, Penfold and his colleagues were responsible for discovering and analysing many new Australian aromatic species.
- Boland, J.J. Brophy, E. Lassak and I.A. Southwell have been a few of today’s productive researchers who have since the late 1970s been rechecking much of the original research conducted by previous scientists. They have also contributed research on many new species in the Myrtaceae family and other Australian genera.

Other scientific and historical antecedents have also provided foundational and empirical knowledge to the field of aromatherapy. These contributions come from activities ranging from indigenous use of plant materials to herbalism.

Activity in these fields, in combination with the ability to analyse chemically the constituents found within aromatic substances, has advanced theories of aromatherapy exponentially. Two important examples of the use of modern scientific method being applied to aromatherapy oils are gas chromatography (GC) and mass spectrometry (MS).
GC separates the essential oil into individual chemical constituents and MS identifies those constituents by their molecular structure. ‘In combination gas chromatography and mass spectrometry is a very powerful, complex and expensive tool for essential oil research’.

Increasingly, however, GC-MS analysis is not overly expensive as there are many private and government laboratories which will conduct analysis under contract for a modest fee, thus allowing growers of aromatic plants to benefit from this modern analytical technique.

These methods of investigation have led to greater understanding of the constituents found within aromatic compounds and how those constituents may affect human physiology.

Current knowledge of human anatomy and physiology allied with the chemical constituents of essential oils make it possible to postulate and attempt to prove clinically the efficacy of aromatherapy as a healing tool.

**Aromatic chemistry**

Aromatic chemistry is concerned with explaining the actions of functional groups and their interaction with human physiology. Functional groups are small parts of aromatic molecules which give that molecule its effect. Take for instance the OH (hydroxyl) group of alcohols and phenols which is anti-bacterial.

Aromatic constituents are categorised by the number of carbon atoms in the molecule and by their functional groups. Strictly speaking monoterpenes (C 10) and sesquiterpenes (C 15) are not functional groups but the hydrocarbon chains to which functional groups are attached.

They do, however, have therapeutic actions and are included in discussions on this subject. Most aromatic constituents found within essential oils can be placed within one of the following groups: By using the example of lavender it is possible to illustrate functional groups and the biological impact of aromatic chemistry.

From this analysis it will be evident that understanding essential oils from this perspective allows practitioners to choose an oil based on its physiological and/or psychological effects on the body.

**Lavender**

To appreciate the overall effects and the actions of the components of essential oils, it is helpful to look at one plant as a case study. In this instance, lavender is a wise choice as it is a herb and a fragrance that has never gone out of fashion and has enjoyed widespread use from historical times to the present day. There are many species of lavender grown worldwide, each with unique properties, but there are some broad conclusions that can be drawn. The chemical constituents and major functional groups of lavender respectively.

The research supporting the physiological effects of essential oils and their constituents is extensive. For lavender it is appropriate to begin with a now famous piece of research from the University of Vienna wherein mice were exposed to Lavender essential oil and two of its main constituents, linalool and linalyl acetate, to ascertain their sedative effects. The researchers found that mice that were exposed to and inhaled lavender and its constituents showed a significant decrease in activity, and this decrease was closely related to exposure time to the oils.

The sesquiterpenes of lavender, with several double bonds, are reputed to have anti-inflammatory actions that are effective in reducing inflammation caused by stings and bites. Monoterpenols have been shown to have strong anti-bacterial and antifungal properties. Carson and Riley found that terpinen-4-ol, linalool and alpha terpineol are active against Escherichia coli, Staphylococcus aureus and Candida albicans.

Terpinen-4-ol was the only constituent tested which was active against Pseudomonas aeruginosa. Bowles also reports studies that show linalool from Lavender oil has a spasmylytic effect on smooth muscle while lavender ketones can kill papilloma and the herpes virus.

Tisserand states that lavender inhibits mycobacterium Tuberculosis, Staphylococcus, Gonococcus, Eberth’s Bacillus (typhoid) and Loeffler’s Bacillus(diphtheria) and when vaporised is effective against Pneumococcus and haemolytic Streptococcus.

**Physiological Principles**

In discussing human physiology and aromatherapy the primary concern is with the pharmacokinetics of essential oils. This is the study of the absorption, distribution, metabolism and excretion of essential oils in the body. It can incorporate issues such as the physiological and physiochemical factors which influence the rate of absorption,
distribution, metabolism and excretion.

Take, for example, Thyme and Thuja essential oils (Thuja being a highly toxic oil). Oral ingestion of large amounts of Thyme or smaller amounts of Thuja oil instantly places a huge burden on the liver, due to their hepatotoxic constituents. However, dermal absorption of a similar dose may cause little concern and olfactory application will have no toxic effects whatsoever.

**Olfaction Theory**

The most obvious piece of human anatomy connected with aromatherapy is the nose. The sense of smell and the process of olfaction is one of the most interesting aspects in the use of aromatics therapeutically. Olfaction has been described thus:

The sense of smell is a primal sense for most humans as well as animals. From an evolutionary standpoint it is one of the most ancient senses. Olfaction allows for vertebrates ... to identify food, mates, predators and provides both sensual pleasures as well as warning of dangers. For both humans and animals it is one of the important means by which our environment communicates to us.

Bowles describes the process of olfaction as being when odorous substances are breathed in through the nose, dissolve in the olfactory mucosa and may stimulate the olfactory nerves which have fine hair-like endings called cilia embedded in the mucus. They may stimulate the trigeminal nerve, which is a branched nerve responsible for detecting pain, touch, temperature in many parts of the face and head, including the nasal epithelium.

The olfactory system is classified as a chemoreceptor because it responds to chemicals in solution. This is similar to the sense of taste and in fact contributes greatly to that sense. When a person has a head cold for instance, their sense of taste diminishes owing to the corresponding reduction in the sense of smell. To understand this complex process it is best to examine what happens physiologically when an odour is perceived.

The receptors for olfaction are found in the nasal epithelium (Regio olfactoria) of the superior and medial nasal conchae. The olfactory receptor cells are modified neurons with fine hair-like extensions called cilia. The human olfactory system contains approximately 100 million of these specialised cells.

The cilia, which cover the conchae, are themselves continuously covered in a layer of mucus which is exuded from the anterior glands.

In order for an odour/aroma to be detected it must be dissolved in this watery mucus and this is why the sense of smell is recognised as chemoreception.

The cilia detect these dissolved chemicals and initiate nerve signals. The olfactory nerve (first cranial nerve) transmits this signal up through the cribiform plate, which has approximately twenty holes (foramina) to facilitate this passage. The cribiform plate forms part of the ethmoid bone of the cranium.

The structure of these olfactory nerves is similar to the general anatomy of nerve structures. They possess dendrites and cell bodies but differentiate and terminate in the highly specialised olfactory vesicle. Each odour/aroma or sensation of odour/aroma is then transmitted by an unmyelinated nerve fibre (axon) which connects with other receptor cells. These nerves then synapse with nerves within the olfactory bulbs. The olfactory bulbs are ovoid in shape and are extensions of the brain that are positioned superior to the nasal cavity. This connection to the brain is known as the olfactory tract. Each set of olfactory nerves connects to specialised cells in the olfactory bulb called mitral cells. One hundred axons from the olfactory nerve contact with each mitral cell in groupings known as glomeruli. There is a complex interplay within the olfactory bulbs because interneurons link the glomeruli with each other and they also connect the two olfactory bulbs with each other. These form two areas of the brain called the medial and lateral olfactory areas. These areas are connected to the limbic system in the brain.

**Olfaction and the limbic system**

Anatomically, the human sense of smell is strongly linked to the generation of emotion. The olfactory bulbs are directly connected to the hippocampus and the amygdala in the limbic system, which is important for memory and emotion.

This is why smells are evocative of past places and feelings. Some smells stimulate the limbic system to activate the hypothalamus and pituitary gland, which triggers the release of hormones associated with appetite and emotional responses including pleasure, fear and sexual drive.

The human response to aromas is associated with olfaction naturally. The neurons in the olfactory system, which are the chemical sense of the body. The structures of the limbic system extend from the midbrain through the hypothalamus into the basal forebrain,
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

which is not only concerned with visceral functions but also with emotional expression.

The cortical and medial nuclei of the amygdala, a body situated within this system, receives information from the olfactory system. The basolateral nuclei are involved with the expression of emotion.

The limbic system is a collection of nuclei and tracts in the brain that are involved in the creation of emotions, sexual behaviours, fear and rage, motivation and processing of memory. These components of the limbic system are primarily located as a border point where the cerebrum is connected to the midbrain. This includes the rim of gyri around the corpus callosum.

Also forming part of the limbic system are the mamillary bodies, which are reflex centres for olfaction and protrude from the base of the hypothalamus posterior to the pituitary gland. The hypothalamus, which is found at the base of the diencephalon, is responsible for visceral regulations such as body temperature and homoeostasis. The hypothalamus is also the part of the limbic system that regulates many motivations, drives and emotions.

The amygdala is another vital part of the limbic system and is located in the temporal lobe just anterior to the hippocampus. The amygdala is connected to the ability to process chemical signals from the olfactory system, hippocampus, cerebral cortex and hypothalamus in the expression of emotions.

In turn, the hippocampus is found deeply interior in the temporal lobe and is connected to the cortex on the interior base of the cerebral cortex. The hippocampus’s function is the formation of new memories. Damage to this region of the brain renders people unable to form new memories but old memories remain undamaged.

Lastly, the septal area of the limbic system is believed to be responsible for the recognition of pleasure and reward. It is located beneath the anterior of the corpus callosum and is connected to the hypothalamus, hippocampus and amygdala.

These components comprise the limbic system which is thought to be a primitive area of the brain because it is responsible for many autonomic visceral functions. However, the interconnectedness of this system and its role in mood, behaviour, emotions, motivations, sexual drive and memory make the limbic system an integral aspect of human physiology and psychology.

That this brain system is linked to the olfactory system in many ways makes the use of essential oils via olfaction a fascinating area of research. The mapping of the neural pathways from olfaction throughout the limbic system is just beginning.
Being.

Here is the seat of our sexuality, the impulse of attraction and aversion, our motivation and our moods, memory and creativity as well as our autonomic nervous system. The brain centre for smell is closely related and connected to the limbic system.

This centre is in turn closely in communication with the hypothalamus which is of course a reflex point through the nerve pathways with the autonomic nervous system (ANS) and via chemical messengers with the pituitary gland and other glands in the body.

The interaction of the olfactory and limbic systems may go some way to explaining the powerful relationship between human physiology and essential oils. Much research is still needed to be done to ascertain, map and understand this complex aspect of human brain chemistry.

It is known that the entire body is capable of receiving chemical information from essential oils but the olfactory system and limbic system are the only parts of the central nervous system that allow for direct contact with the external environment.

Hence these systems are integral to individual personalities and that makes these systems extremely important in a therapeutic context. As a therapist it is important to know and understand the complex structure and chemical communications that comprise olfaction.

However, as with all aspects of human physiology there are variables that can impact on the sense of smell in a client presenting for aromatherapy treatment. Has the client had a stroke or other nervous system injury that may have damaged the region of the brain relating to smell?

Does the client smoke cigarettes or use other medical or illegal inhalants which can also impair this brain region? If the client is female, she will have a heightened olfactory sense during ovulation. Additionally, not all aromas will evoke a positive response. The limbic system being associated with both olfaction and memory may, on some occasions, trigger a negative physiological/psychological response. Jane is a 45-year-old corporate executive who, due to her busy lifestyle and work schedule, is suffering from a stress-related skin disorder. An aromatherapist may decide to use Lavender essential oil (for its well-documented calming and sedative properties) 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 healthcare.

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.

**IMPLICATIONS OF OLFACTION FOR AROMATHERAPY**

Unlike other human senses smell has no gatekeepers and in effect has an unmediated impact on the brain. As the olfactory system is linked physiologically with the limbic system, the lexicon of aromas collected over a lifetime can have a profound effect. For example, the smell of freshly baked bread may cause the physiological response of an increase in saliva production. This response to aroma is unmitigated by the higher functions of the brain. So it can be concluded that essential oils, being highly aromatic substances, will have an effect on the human physiology. This may be for two reasons. First, that the person smelling an essential oil has that aroma already in their memory via the limbic system and has associated it with a pleasurable experience.

Therefore it is an act of recollection activating long-term memory and exciting neural pathways in the limbic system, specifically the septal region. Second, the aromatic molecules having passed through the million cilia in the nasal passage, through the cribiform plate and epithelium, have chemically interacted with the nerves, synapses and neurotransmitters and entered the limbic system.

Thereby the oils have caused an alteration of brain chemistry which elicits the brain’s own neurotransmitters, producing sedating, arousing, pleasurable or excitatory responses.

Odour stimuli in the limbic system or olfactory brain release neurotransmitters among them encephalin, endorphins, serotonin and noradrenaline within the limbic system resides the regulatory mechanisms of our highly explosive inner life, the secret core of our being.

Here is the seat of our sexuality, the impulse of attraction and aversion, our motivation and our moods, memory and creativity as well as our autonomic nervous system. The brain centre for smell is closely related and connected to the limbic system.

This centre is in turn closely in communication with the hypothalamus which is of course a reflex point through the nerve pathways with the autonomic nervous system (ANS) and via chemical messengers with the pituitary gland and other glands in the body.

The interaction of the olfactory and limbic systems may go some way to explaining the powerful relationship between human physiology and essential oils. Much research is still needed to be done to ascertain, map and understand this complex aspect of human brain chemistry.

It is known that the entire body is capable of receiving chemical information from essential oils but the olfactory system and limbic system are the only parts of the central nervous system that allow for direct contact with the external environment.

Hence these systems are integral to individual personalities and that makes these systems extremely important in a therapeutic context. As a therapist it is important to know and understand the complex structure and chemical communications that comprise olfaction.

However, as with all aspects of human physiology there are variables that can impact on the sense of smell in a client presenting for aromatherapy treatment. Has the client had a stroke or other nervous system injury that may have damaged the region of the brain relating to smell?

Does the client smoke cigarettes or use other medical or illegal inhalants which can also impair this brain region? If the client is female, she will have a heightened olfactory sense during ovulation. Additionally, not all aromas will evoke a positive response.

The limbic system being associated with both olfaction and memory may, on some occasions, trigger a negative physiological/psychological response. Jane is a 45-year-old corporate executive who, due to her busy lifestyle and work schedule, is suffering from a stress-related skin disorder. An aromatherapist may decide to use Lavender essential oil (for its well-documented calming and sedative properties)
in the treatment of this condition.

Upon offering Jane a sample of Lavender essential oil to smell, the therapist discovers that she cannot stand the smell, in fact it causes the opposite response expected. What is evidenced is an increase in Jane’s heart rate and blood pressure. After calming down Jane reveals that as a small child she was often forced to stay with her grandmother, who mistreated her. The grandmother wore lavender and the aroma immediately transported her back to being that small, scared child.

The sense of smell is, as this example illustrates, a profound memory key that can strip away years of cognitive thought in an instant. Conversely, if Jane’s grandmother had been a sweet, loving person Jane would probably have enjoyed the smell of lavender, as there would be many positive associations attached to this scent.

This is just one of the many reasons why taking a thorough medical history from a client is very important. Aside from the various physical contraindications there may be psychological and/or emotional issues that aromatic stimuli may trigger when used within a treatment.

**Dermal Absorption Theory**

The olfactory route is not the only way in which essential oils can impact on human physiology. The integumentary system is a highly complex body system and it is one of the traditional methods of applying essential oils via massage.

Skin protects the deeper tissue from either mechanical (bumps) or chemical (acids and bases) damage, bacterial damage, ultraviolet radiation, thermal damage and desiccation. Skin aids the body in heat loss and retention, excretion of urea and uric acid and synthesis of vitamin D. This multifunctional organ is composed of three layers: the epidermis, dermis and subcutaneous.

In an aromatherapy massage the application of essential oils in a base oil/cream can be used for a number of possible outcomes. The practitioner may be treating a disorder of the skin itself but more commonly it is used as a method for essential oil uptake into the bloodstream.

Essential oil constituents can in turn circulate through the entire body exerting physiological effects before being excreted via urine, faeces, lungs, lymph or the integumentary system itself.
Subjects who inhaled Lavender essential oil showed increased beta power on EEG patterns, suggesting increased drowsiness; they reported feeling more relaxed with less depressed mood. Their maths computations were faster and more accurate upon testing after the inhalation.

Subjects who were tested with Rosemary oil showed decreased alpha and beta power on EEG patterns, suggesting increased alertness. They also reported less anxiety, and felt more relaxed and alert. Their maths computations were faster but not more accurate following the inhalation.

**Dermal aromatherapy research**

There have been claims made by researchers investigating aromatherapy that dermal absorption does not occur and that the effects of essential oils are mainly due to olfactory effects or absorption into the bloodstream via the lungs.

These theories have since been proven to be incorrect. Clinical studies on dermal application of essential oils are few but some evidence is emerging in support of dermal absorption as an effective approach to aromatherapeutic treatment.

In a trial conducted at the University of Bradford, England, Williams and Barry tested the theory that various terpene compounds would enhance the dermal penetration of a polar molecule 5-fluorouracil (5-FU). Their finding revealed that various essential oil constituents had differing effects upon the rate of absorption, with 1, 8-cineole (oxide) increasing absorption by 95 times.

A later study from Bradford by Cornwell and Barry showed that sesquiterpenes also increased dermal absorption, with nerolidol (found in Nerolina oil) being the most effective with an increased absorption of twentyfold.

The sesquiterpenes were also noted for being extremely difficult to remove from the skin once applied, which adds to their use as fixatives/base compounds within blends. For stress-related hypertension, for instance, the therapist may use dermal application as part of a treatment regime. One study conducted in Vienna found that the topical application of Western Australian Sandalwood (Santalum spicatum) essential oil has been clinically proven to lower stress levels and systolic blood pressure.

(Note: face masks were worn by the test subjects to prevent the olfactory pathway from influencing the results.) A 1998 randomised, double-blind, controlled, seven-month trial (with followups at three and seven months) was conducted in Scotland to investigate the effects of essential oils upon alopecia areata (hair loss).

The test group had essential oils of Thyme, Rosemary, Lavender and Cedarwood diluted in jojoba and grapeseed oils massaged into their scalp daily. The control group had just the carrier oils jojoba and grapeseed massaged into their scalp daily.

The study showed an improvement of 44 per cent for the test group compared to 6 per cent for the control group. The authors concluded that the essential oils were a safe and effective treatment for hair loss.

**Methods of application**

There is a wide variety of application methods for the use of aromatics from personal perfumery to massage, aromatic bathing, inhalation or even in foods and beverages. The different methods are diverse and within each of these are variations that depend largely upon the expertise of the practitioner and the desired outcome. For example, the method of aromatic bathing ranges from simple to advanced in its application. At the simple end of the scale is the ‘English’ low-dose method using a few drops of essential oils for a soothing, relaxation bath.

Alternatively, higher doses (up to 50 drops, when properly emulsified in the water) are used. Additionally, application procedures for essential oils are always evolving. Aromatic profusion is a relatively new technique drawn from the clinical and aromatic medicine area of aromatherapy, whereby the therapist applies a single, relatively large dose (10–15 mL) of a neat essential oil blend to the client’s body in the treatment of acute viral or bacterial infections.

This is far removed from the low-dose model found in popular aromatherapy and relies on considerably more skill in areas of aromatic chemistry, pharmacology and therapeutic blending. As diverse as the methods of applying essential oils are, the conditions for which they are effective are even more so. Some of the main ailments that will benefit from aromatherapy treatment.

**Wounds**

As modern pharmaceutical medications become less effective
Research into the biochemical pathways responsible has revealed that Western Australian Sandalwood oil appears to inhibit inflammatory response by blocking enzyme 12-lipoxygenase and both cyclooxygenases COX1 and COX2.

**Pain**

Pain management has recently become a favoured topic among aromatherapists, as many are now working in areas other than relaxation massage and are finding that client health needs revolve around quality of life, which for some is based upon pain management.

Aromatherapy massage is particularly suited to the treatment of pain due to its soothing and calming qualities. Massage and the application of oils via this method can be localised to concentrate on the area of pain or to achieve general relaxation.

While traditionally oils such as Wintergreen, Rosemary and Lavender have been used to help mediate pain, there are also newly discovered and commercially released oils available. One example of these is the previously little known Australian Kunzea (Kunzea ambigua) essential oil.

This oil is showing promising effects in relieving the pain of arthritis and muscular sprains and strains. Ongoing research into how this and other oils mediate pain is currently being conducted by the author.

**Respiratory illness**

Aromatherapy is particularly appropriate to ease the discomfort of respiratory illnesses. Colds, flu, sinus infections and bronchitis respond to the anti-inflammatory, decongestant and antimicrobial qualities of certain oils.

The inhalation method and aromatic bathing methods are both suitable for the treatment of conditions of the respiratory tract. Thyme and Eucalyptus oils are often employed in treatment of respiratory illnesses but there is a wide range of essential oils that can be used and blended to alleviate symptoms in such conditions.

**Rheumatic conditions**

Aromatic bathing may be used to treat a variety of conditions such as rheumatic joint complaints and aching muscles. Many commercial bath products utilise the beneficial effects of essential oils...
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in their formulations.

Lavender and Wintergreen are known for the relief they can yield in arthritic states. Sports people will relate to the smells of certain essential oils like Peppermint, Wintergreen and Basil that can be used in ointments or oil blends for sore muscles.

Other Conditions: Just a few conditions that respond well to aromatherapy treatment include:

- Bruises
- Colic
- Cracked skin
- Dandruff
- Flatulence
- Fluid retention
- Foot odour
- Genitourinary infections (cystitis and vaginal thrush)
- Headache
- Insomnia
- Ringworm
- Stress and tension
- Tinea

Practice Issues

When essential oils enter the human body they have varying effects, depending upon the pathway and dosage. Paracelsus, the father of toxicology, stated that 'The dose makes the poison.' By this he meant that all substances are poisonous depending on the dosage given; for some the toxic amount is quite low and for others very high.

Oral ingestion of essential oils such as Thuja or Thyme essential oils, for instance, places a huge burden upon the liver, due to their hepatotoxic constituents, while dermal application of a similar amount may cause no concern whatsoever.

Understanding the key differences between dermal and oral toxicity and between the therapeutic dose and the toxic dose forms the necessary knowledge base in aromatic pharmacology. With this knowledge therapists can safely prescribe and use higher concentrations and doses of essential oils in treatments than are normally used in popular aromatherapy.

In aromatherapy today there are two levels of treatment dosage rates. Clinical aromatherapy uses high-concentration essential oils (up to 100 per cent) and doses measured in millilitres for the treatment of physical diseases and illnesses such as acute influenza.

By contrast popular aromatherapy employs low-concentration oils (1–5 per cent) and doses measured in drops for the treatment of psychological/emotional conditions such as stress.

At high dosages essential oils act like any other drug, with all of the benefits and associated dangers. At lower dosages, however, essential oils appear to have neurotransmitter and/or hormonal-like properties, for example the apparent phytoestrogen effects upon women of anethole-rich essential oils such as Sweet Fennel or Aniseed Myrtle.

When these oils are applied topically to the lower abdomen and back in a relatively low dosage of 2–5 per cent they have been found to ease menstrual cramping. For older female clients the same dosage will, over a period of a few months, help to mediate peri-menopausal signs and symptoms.

In a clinical setting knowledge of olfaction, the limbic system and brain chemistry is a valuable asset for the therapist. This is especially true for acute conditions, which require immediate attention, such as the lowering of high blood pressure or the reduction of a bipolar/depressive or anxious psychological state.

Bearing in mind that referral may be necessary in extreme cases, the inhalation of the appropriate essential oils which are known to have sedating or calming effects are particularly useful in these conditions. Again, a well-trained therapist who is knowledgeable about the chemical constituents of essential oils will be able to select those essential oils most suited to the outcome required.

In the case of a client suffering depression, for example, inhalation of essential oils to interact positively with the limbic system can form part of a treatment regime. An oil blend in this instance may include Bergamot, Spikenard, Lavender and Rose. In a sound aromatherapy practice, these oils will have been chosen with the chemistry and effect of each oil clearly understood by the therapist.

Contraindications

Aromatherapy where contraindications have been listed for essential oils when none were warranted, based on the chemical constituents and toxicology of the oils involved. Many of these
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Warnings were based upon the incorrect interpretation of herbal lore and lack of clear scientific data.

Today there is a far better understanding of the issues surrounding aromatic toxicology thanks to the work of researchers such as Cuba, Tisserand and Balacs. In their seminal work Essential Oil Safety, Tisserand and Balacs clearly discuss many relevant issues within this important area of aromatherapy.

Ron Cuba has also done much to refute and debunk unwarranted fear-based warnings and his article on toxicity myths is of equal importance. Some important and basic examples of toxicity issues in aromatherapy are:

- **Anticoagulants** Using Wintergreen oil (98 per cent methyl salicylate) with clients taking anticoagulant medication, such as warfarin, has the ability to potentiate the anticoagulant effects, thus causing internal haemorrhaging.
- **Pregnancy** There has been much misinformation regarding the potential dangers to both mother and child during pregnancy.

Much of this caution has been ethically based, but much is also not based upon science. Reviewing the toxicological data it appears that very few commonly used essential oils are dangerous at any stage of pregnancy. Nevertheless, oils with a toxicity rating of 2 (LD50 between 1 and 2 g/kg) should be avoided in the first trimester; this is dose dependent and oils such as Basil (rich in ethers) should be avoided, due to the detoxification load placed upon the liver by such oils.

Having said this, certain oils are used with good effect to relieve pregnancy symptoms like fluid retention and nervous tension. The emphasis in pregnancy must therefore be that aromatherapy should always be administered either by, or in consultation with, a qualified practitioner.

- **Epilepsy** There are many listings for essential oils that may trigger an epileptic fit; when the data are reviewed there are no documented cases of essential oils causing an epileptic fit. Oils high in toxic ketones should be avoided, but commonly accused oils such as Rosemary appear to be safe at popular aromatherapy dosage rates.
- **Dermal irritation** Certain essential oil constituents are known to be irritant to dermal and mucous membrane tissues.

Aldehydes such as cinnamon aldehyde have a reputation for being irritant in this manner. The effects of most essential oil constituents causing dermal irritation can be negated to a large degree by blending with other constituents known to ‘quench’ the irritancy effects.

- **Phototoxicity** Certain expressed citrus oils such as Bergamot, Bitter Orange, Lemon, Lime and Angelica contain a particular type of furocoumarin, bergaptene, which can cause serious phototoxic effects to skin when applied with subsequent exposure to sunlight or ultraviolet radiation.

**Quality Issues**

The quality of the aromatics on offer within the essential oil, food and flavouring and aromatherapy industries varies greatly depending upon their end use. How does an aromatherapist choose their aromatics and determine whether an aromatic has been stretched, adulterated or reconstituted from synthetic constituents? As part of their professional training aromatherapists should be exposed to many varying qualities of aromatics.

They need to experience synthetic and isolated constituents to allow them to build an aromatic memory (in a similar way to wine appreciation) for what makes an outstanding, therapeutic-grade aromatic.

Most aromatherapists rely upon trust, trust in their teachers, peers and specialist industry suppliers who themselves are often working aromatherapists, to keep them informed and supplied with quality aromatics. Reliable suppliers of aromatics to the aromatherapy industry will generally have years of experience, be informative and have quality assurance standards in place. Items to look for on quality aromatic packaging and literature include:

- Common and botanical names;
- Specific chemotype (CT) for some species such as thyme and rosemary;
- Country of origin;
- Part of plant used and extraction technique;
- Batch coding and best before or use-by date; and
- Dark glass bottle (for neat aromatics) with appropriate sized flowrestriction dripper.

Percentage constituent analysis charts (from GCMS) should also
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%.

- **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%.

- **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%.

- **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 PPARα, a type of nuclear regulatory proteins involved in transcription of genes regulating glucose and fat metabolism.

These PPARs act on Peroxisome 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%.

- 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.

**Alpha-glucosidase Inhibitors**

Alpha-glucosidase inhibitors are “diabetes pills” but not technically hypoglycemic agents because they do not have a direct effect on insulin secretion or sensitivity. These agents slow the digestion of starch in the small intestine, so that glucose from the starch of a meal enters the bloodstream more slowly, and can be matched more effectively by an impaired insulin response or sensitivity.

These agents are effective by themselves only in the earliest stages of impaired glucose tolerance, but can be helpful in combination with other agents in type 2 diabetes.

Typical reductions in A1C values are 0.5-1.0%.

- Miglitol (Glyset)
- Acarbose (Precose/Glucobay)

These medications are rarely used in the United States because of the severity of their side effects (flatulence and bloating). They are more commonly prescribed in Europe. They do have the potential to cause weight loss by lowering the amount of sugar metabolized.

Research has shown the culinary mushroom Maitake (*Grifola frondosa*) has a hypoglycemic effect, possibly due to the fact the mushroom naturally acts as an alpha-glucosidase inhibitor.

**ALTERNATIVE MEDICINE**

A recent review article presents the profiles of plants with hypoglycaemic properties, reported in the literature from 1990 to 2000 and states that “Medical plants play an important role in the management of diabetes mellitus especially in developing countries where resources are meager.”

The first registered use of anti-diabetic drugs was as *herbal extracts* used by Indians in the Amazon Basin for the treatment of type 2 diabetes, and today promoted as *vegetable insulin* although not formally an insulin analog.

The major recent development was done in Brazil around *Myrcia sphaerocarpa* and other *Myrcia* species. The usual treatment is with concentrated (root) *Myrcia* extracts, commercialized as “Pedra hume de káu”.

Phytochemical analysis of the *Myrcia* extracts reported kinds of flavanone glucosides (myrciacitrins) and acetophenone glucosides (myrciaphenones), and inhibitory activities on aldose reductase and alpha-glucosidase.

Walnut leaf can significantly reduce fasting blood glucose levels in rats with alloxan-induced diabetes, and rats thus treated show some
evidence of regeneration of the beta cells. Garlic also significantly reduces fasting blood glucose levels in rats with alloxan-induced diabetes. At least two studies have shown that cinnamon can act significantly reducing some effects of diabetes. One study on people used fine ground cassia (Cinnamomum aromaticum) for oral consumption. Another study used an extract (MHCP) on laboratory rats.

The study on people published in 2003 conducted in the Department of Human Nutrition, NWFP Agricultural University, Peshawar, Pakistan concluded “that the inclusion of cinnamon in the diet of people with type 2 diabetes will reduce risk factors associated with diabetes and cardio-vascular diseases.” The study on laboratory rats at Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University published in 2001 used purified hydroxychalcone (MHCP) from cinnamon.

Part of the study’s conclusion stated that “the MHCP is fully capable of mimicking insulin” and recommended further studies. The Food and Drug Administration has not yet evaluated the use of cinnamon for the management of diabetes. It should be noted that the spice sold as cinnamon is often obtained from C. verum (true cinnamon), not C. aromaticum (cassia).

Research has shown the Maitake mushroom (Grifola frondosa) has a hypoglycemic effect, and may be beneficial for the management of diabetes. The reason Maitake lowers blood sugar is due to the fact the mushroom naturally acts as an alpha glucosidase inhibitor.

Other mushrooms like Reishi, Agaricus blazei, Agrocybe cylindracea and Cordyceps have been noted to lower blood sugar levels to a certain extent, although the mechanism is currently unknown.

Cinnamon

Though not yet evaluated by the Food and Drug Administration, at least two studies have shown that cinnamon can act significantly reducing some effects of diabetes. One study on people used fine ground cinnamon (Cinnamomum cassia) for oral consumption. Another study used an extract (MHCP) on laboratory rats. The study on people published in 2003 conducted in the Department of Human Nutrition, NWFP Agricultural University, Peshawar, Pakistan concluded:

The results of this study demonstrate that intake of 1, 3, or 6 g of cinnamon per day reduces serum glucose, triglyceride, LDL cholesterol, and total cholesterol in people with type 2 diabetes and suggest that the inclusion of cinnamon in the diet of people with type 2 diabetes will reduce risk factors associated with diabetes and cardiovascular diseases.

The study on laboratory rats at Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University published in 2001 used purified hydroxychalcone from cinnamon.

The extract was named “MHCP”. Part of the study’s conclusion stated that “the MHCP is fully capable of mimicking insulin” and recommended further studies. Other studies have failed to reproduce these results, and, because large doses of cinnamon are not innocuous, some experts advise against treatment of diabetes with cinnamon.

Chromium and Vanadium

- **Chromium**: Cholesterol and triglycerides are risk factors in heart disease and diabetes, and studies show that chromium lowers levels of total cholesterol, LDL cholesterol, and triglycerides. Chromium supplements such as chromium picolinate have been shown to improve glucose tolerance in people with type 2 diabetes, although other studies have not replicated this result. A meta analysis of these trials concluded that chromium supplements had no beneficial effect on healthy people, but that there might be an improvement in glucose metabolism in diabetics, although the authors stated that the evidence for this effect remains weak.

- **Vanadium**: A form of vanadium, vanadyl sulfate, seems to improve glucose control in people with type 2 diabetes. A pilot study has also found evidence that Tai Chi and Qigong reduce the severity of type 2 diabetes.

Benfotiamine, a pro-vitamin of vitamin B1 which has been in use in Europe as an over-the-counter medicine for alcoholic neuropathy for the past half century with no significant side-effects or toxicity, has recently been found to block the major metabolic pathways by which excess blood glucose in the body is transformed into the advanced glycation endproducts (AGEs) which cause diabetic complications.

Studies have shown that taking oral benfotiamine can prevent diabetic retinopathy, diabetic neuropathy, and diabetic nephropathy independently of any affect on the blood sugar levels of the patient.
In theory, taking benfotiamine might allow patients to be less scrupulous in trying to normalize blood sugar levels and thus free them from the danger of hypoglycemia and the stress of stringent blood sugar monitoring, while still protecting them against the negative effects of hyperglycemia.

Research is ongoing to establish the full significance of benfotiamine in the treatment of diabetes.

**Traditional Plant Treatments for Diabetes**

A study was made of the effects on glucose homeostasis in normal and streptozotocin (induced) diabetic mice of eleven plants that have been used as traditional treatments for diabetes.

The mice were given diets containing dried leaves from the following plants: agrimony (Agrimonia eupatoria), alfalfa (Medicago sativa), blackberry (Rubus fructicosus), celandine (Chelidonium majus), eucalyptus (Eucalyptus globulus), lady’s mantle (Alchemilla vulgaris), and lily of the valley (Convallaria majalis); seeds of coriander (Coriandrum sativum); dried berries of juniper (Juniperus communis); bulbs of garlic (Allium sativum) and roots of liquorice (Glycyrrhiza glabra). The study concluded that “The results suggest that certain traditional plant treatments for diabetes, namely agrimony, alfalfa, coriander, eucalyptus and juniper, can retard the development of streptozotocin diabetes in mice”.

**Mushrooms**

Research has shown the Maitake mushroom (Grifola frondosa) has a hypoglycemic effect, and may be beneficial for the management of diabetes. The reason Maitake lowers blood sugar is due to the fact the mushroom naturally acts as an alpha glucosidase inhibitor.

Other mushrooms like Reishi, *Agaricus blazei*, *Agrocybe cylindracea* and *Cordyceps* have been noted to lower blood sugar levels to a certain extent, although the mechanism is currently unknown.

**Aloe Vera**

Oral administration of aloe vera might be a useful adjunct for lowering blood glucose in diabetic patients as well as for reducing blood lipid levels in patients with hyperlipidaemia. Ten controlled clinical trials were found to reach that conclusion in four independent literature searches. However, caveats reported in each study led the researchers to conclude that aloe vera’s clinical effectiveness was not yet sufficiently defined in 1999.

**MEDICAL EQUIPMENT APPROACH**

**Development of Continuous Blood Glucose Monitoring**

Technology for continuous blood glucose monitoring supports the mission of the artificial pancreas by:

- Automatically providing a blood glucose reading every few minutes without finger sticks from the user,
- Monitoring trends pertaining to rising and falling blood sugars, which is helpful in the prediction of blood glucose levels in the immediate future,
- Comparing blood sugar levels and predictions against a high blood sugar threshold, and then prompting the user that a correction bolus from an insulin pump is needed immediately,
- Comparing blood sugar levels and predictions against a low blood sugar threshold, and then prompting the user to reduce the basal insulin from the pump or to eat something.

These capabilities suggest that a stream of real-time data can be used to “close the loop” and control the insulin pump directly.

Some issues with the present performance of continuous sensing technology suggest that additional study is needed for application to the artificial pancreas:

- Continuous sensors require calibration a few times a day, by performing a manual blood glucose test with a finger stick, and then entering the blood glucose data into the continuous system for a sensor correction,
- Continuous sensors are measuring interstitial glucose, so there is a time delay between the sensor data and the true blood glucose,
- Automatic control removes the intellect of the user, which can be an additional safeguard when the data is subject to error and must be verified before taking action.

As the state of the art for blood glucose monitoring continues to advance, so does the promise of the artificial pancreas.

**Feedback of Real-time Blood Glucose Data to an Insulin Pump for Basal Control**

The first step in controlling an insulin pump based on continuous
blood glucose data is to automatically control the basal rate of the insulin pump. When a bolus has not recently been performed, the pump can manage the blood glucose level by adjusting the basal rate as needed:

- When the blood sugar is increasing, a small correction bolus can be automatically delivered and a higher basal rate can be set;
- When the blood sugar is decreasing, the basal rate can be halted to deny the quantity of insulin needed to bring the blood glucose level back up until the basal rate can be continued at a new lower rate;
- And with adaptive filtering techniques, the pump can “learn” the unique basal rates for the person as a function of the time of day.

When controlling the basal rate alone, the closed loop can still correct a meal bolus error that was too large or small for the food consumed by:

- Recognizing an imbalance between the bolus “insulin on board” and the level of blood glucose,
- Automatically bolusing to correct a shortage of insulin,
- Automatically reducing or interrupting the basal rate to correct an abundance of insulin,
- And using adaptive filtering techniques to “learn” the carbohydrate to insulin ratios for each meal bolus.

First Clinical Tests: Implantable Insulin Pumps and Continuous Glucose Sensors

In France, a human clinical trial of an artificial pancreas is underway. The system is fully automated by combining Medtronic MiniMed’s long-term glucose sensor and its implantable insulin pump. A summary of the project shows promise as well as some present limitations:

- The implantable sensor is inserted into a neck vein leading to the heart.
- The sensor is connected, via an electrical-type wire under the skin, to the implantable insulin pump: as blood sugar levels fluctuate, a signal tells the pump how much insulin to deliver.
- The sensor accurately measured glucose in 95% of cases when compared with values obtained by fingersticks.

Insulin and Amylin Combination

When pramlintide (brand name Symlin or synthetic amylin) is used in combination with insulin, the benefits for post-prandial glycemic control are substantial. Pramlintide is a relatively new treatment for diabetes. The treatment involves:

- A separate injection of pramlintide before a meal,
- A reduction in insulin bolus by 50% for that meal.

Pramlintide can be infused using an insulin pump. At the present time, the mixing of pramlintide and insulin in the same cartridge is not an approved practice, so two infusion pumps are used simultaneously.

Since insulin and amylin are co-secreted by the pancreatic beta cells in response to raising blood glucose levels, using pramlintide and insulin together more closely duplicates the function of the pancreas.

Symlin has potential to support the artificial pancreas project because:

- Insulin and pramlintide may in the future be automatically infused together
  - At a mixture from a single automatic insulin pump, or
  - Two infusion pumps could be used automatically with the insulin pump acting as master and the similin pump acting as slave, or
  - A dual system in one pump machine (two cartridges, a dual infusion set tube, and two
This could include:
- A prebolus of pramlintide (optional perhaps, but resolves issue with insulin timing)
- Initiation of a combination bolus with the initial spike sized in proportion to the present blood glucose level and trends in the change of blood glucose level,
- Modification to the square wave portion of the bolus, increasing or extending if blood sugar is increasing, and decreasing or limiting in duration when blood sugar is decreasing.

The benefits of an automatic bolus delivery might include:
- Increased accuracy in the total insulin delivered relative to what was needed,
- Freedom to the user of the artificial pancreas,
- Elimination of glycemic excursions due to user error (such as forgetting to bolus in conventional pump therapy),
- Adaptability to changes in digestion of carbohydrates based on food choices,
- Adaptability to variable metabolic needs due to stress, illness, or exercise.

Glucagon Combination

The purpose of glucagon is to raise blood sugar, primarily by promoting release of stored glucose in the liver. Human glucagon has been synthesized by recombinant DNA technology and is available in a dry powder form in the glucagon rescue kit.

Glucagon injection pens are also sometimes provided to diabetics in the UK along with insulin. This is useful for rescue of unconscious diabetics from a severe state of hypoglycemia.

In healthy pancreatic function, glucagon production is initially suppressed by beta cell production of insulin and amylin when blood sugar is high, and then is later produced by low or falling blood sugar.

The natural pancreatic function uses glucagon at the end of an insulin cycle to release glucose from the liver, with two advantages:
- To prevent low blood sugar, and
- To speed the overall insulin action by cancelling the insulin tail.

If an artificial pancreas was to simulate the natural endocrine pancreas to the maximum extent, then insulin and amylin would be...
used at the beginning of an insulin cycle and glucagon would be used at the end of the insulin cycle.

Research with diabetic pigs given insulin-glucagon combination via separate subcutaneous infusion pumps demonstrated closed loop control without incidence of hypoglycemia.

While the copycat endocrine function including glucagon seems desirable, the benefits relative to the cost and complexity of an artificial pancreas without glucagon are not yet known.

Drug Discovery and Development

9

Microcalorimetry can be utilized throughout small molecule drug discovery. Shown below is the small molecule drug discovery pipeline. Please run your cursor over the pipeline click on a section to discover how microcalorimetry can be applied to your area of interest.

Biotherapeutics and Vaccines

Microcalorimetry can be utilized throughout the discovery and development of biotherapeutics and vaccines. Shown below is the biotherapeutics and vaccines discovery and development pipeline. Please run your cursor over the pipeline and click on a section to discover how microcalorimetry can be applied to your area of interest.

Drug discovery and development process

Novartis drug discovery and development efforts begin and end with the patient. Our R&D efforts are guided by two principles:

Do we understand the underlying mechanism or cause of the disease? Does this disease represent a significant unmet medical need
in patients?

If the answer to both questions is “yes,” then we develop a research program aimed at better understanding the disease and finding an effective therapy.

Drug Discovery

Pharmaceutical Discovery Services

SRI’s pharmaceutical discovery teams bring more than 40 years of interdisciplinary scientific expertise to our clients. Our goal is to assist government and private clients at all stages of the drug discovery cycle as they seek to rapidly identify and optimize new drug leads and move them into preclinical development.

Scope of Services

We provide discovery contract research and services to clients worldwide, delivering the experience and resources needed to bridge the gap from drug discovery to development. Our interdisciplinary teams combine expertise in molecular biology, biochemistry, immunology, medicinal chemistry, synthetic and process chemistry, pharmacology, computer-aided drug design, molecular modeling, and assay design and implementation to address client needs. From basic biological research and target identification to lead discovery and optimization to in vitro screening and in vivo efficacy testing, we work with clients to help them rapidly iterate through the discovery process as they pursue promising leads.

Our capabilities in both in vitro and in vivo testing provide a seamless transition effectively complementing a client’s in-house capabilities. SRI’s customized drug screening capabilities can provide valuable insights into the potential of new compounds. We design each study to optimize the client’s time and resources, and incorporate a broad range of capabilities to ensure the best efficacy evaluation of the client’s compounds. Post-discovery, we are available to work with clients to move from the lab into clinical formulations, testing, and eventually to market.

BIOASSAYS FOR DRUG DISCOVERY

The first biological assays involved measuring the effect of a chemical substance on a living organism. The results of these experiments are essential for assessing potential activity, toxicity and side effects of drugs. Projects can be divided into those with known molecular targets and those with a defined phenotypic effect in a cell or tissue-based assay. Setting up a bioassay that is suitable for discovering initial hits involves the following steps:

Known Molecular Target Projects

- Express and purify the protein target from a suitable expression system in bacterial, mammalian, yeast or insect cells.
- Characterise the protein in terms of protein folding, functional effects ie enzyme activity etc.
- Analyse the structure of the protein via crystallography or other biophysical techniques, if possible.
- Develop an assay which enables quantitative measurement of the activity for which the target protein is essential. This may involve measuring enzyme activity and kinetics, receptor binding or more downstream functions of the target. The main assay to be used for compound screening activities needs to be highly reproducible; validated with any available known tool compounds; work robustly with compounds in solvents such as DMSO; and amenable to medium throughput assay formats ie 96/384 well automated assay systems. The DDF screening group can support this development.

Targets where the cellular/tissue effects are known, but the molecular target is not defined. It can be acceptable to progress projects where a desirable cellular or tissue effect is observed but the defined molecular target is not known. In this case a different assay path is likely such as:

- Characterise the biology of the cellular effect and its link to the disease of interest. Ideally this will implicate the effect in a human based cellular/tissue system.
- Develop an assay using a readily accessible, appropriate and amenable cell line or primary culture to quantitatively measure the desired biological effect e.g. levels of cytokines, calcium flux etc.
- As above, this assay needs to be highly reproducible, validated with any available known tool compounds, work robustly with compounds in solvents such as DMSO and amenable to medium throughput assay formats ie 96/384 well automated assay systems. The DDF screening group can
Bioassay of drugs

Mouse model has been used for testing new drugs for years although it is costly. Recently, zebrafish has been identified as an important vertebrate model for studying the development of embryos and human diseases. When compared with mammalian models, experimental results showed that zebrafish embryos exhibited similar responses to drugs for cardiovascular diseases, anti-angiogenesis and anti-cancer. This suggests that zebrafish model can be used as the bridge between in vitro model and in vivo model in the drug discovery and screening process. In addition, pollutants can induce similar pathological responses in zebrafish as well as in other mammalian models. Based on these discoveries, we have developed a set of proprietary tools for drug screening and pollutant testing. The bioassay, combining these proprietary tools and zebrafish embryos with unique advantages, provides a cost-effective, economical and speedy solution for the testing on drug action, toxicity and environmental pollutants.

It is defined as the determination of the potency of chemical and biological agents such as drugs, hormones, vitamins, toxins, and antitoxin by means of biological indicators (in a live animals, isolated tissues or cell line).

Biological Indicators:
- Blood pressure
- Blood glucose
- Muscle contraction
- Inhibition of growth of microorganisms

Application of Bioassay in Pharmacology
- Determination of drugs potency.
- Screening of new agents isolated from plants, animals or chemical labs and find their field of activities.
- Determine the therapeutic advantage of one drug over another treatments.
- Determination of the pharmacological activities of a new drug.
- Establishment of SAR.

Comparison between Bioassay and Chemical assay

Screening of Drugs

It means thorough investigation of substance for pharmacological activity and evaluation of this activity i.e. scanning and evaluation.

The main purposes of screening are to determine whether the new substance are worthy for further attention and to indicate which among them have the most interesting pharmacological properties.

Types of Screening

A- Simple Screening

It involves the use of one or two simple tests to find substances having a particular property. For example, a single test for conc. of glucose in blood can be used to screen compound for hypoglycemic activity.

B- Blind Screening

It provides clues to potential activities of new drugs and indicates the fields of activity if they exist. It also shows pharmacological inertness if it exists. The chief purposes of the blind screening are to demonstrate whether these new drugs are worthy of further attention and to indicate which among them have the most interesting pharmacological activities.

C- Programmed Screening

It is used when a new drug of specific type is to be screened for some pharmacological effects. Examples are screening of certain drugs on the cardiovascular system, CNS, kidney, blood etc.

It includes the use of quantitative assay of the most interesting compounds and their comparison with drugs known to be quite active representative members of their pharmacological class.

It also provides indications of potential side effects.

Steps for Biological Screening of a Drug

Neuropharmacological Tests
To detect sedative, hypnotic, tranquillizer, psychomotor stimulant, muscle relaxant, analgesic, antipyretic, and peripheral vasodilator activities. Mice are usually used here because they are small, easy to handle, economic, mammals and have long life span. Signs to be observed include consciousness, awareness, motor activity, pain response and excitability. Other responses should be evaluated like pupil size secretions, heart rate, respiration, and writhing.

**Cardiovascular Tests**

To study the action of new drug on blood pressure and heart rate usually on anesthetized rat or cat. A group of 4 tests is performed after recording normal blood pressure including; electrical stimulation of vagus for 2 seconds, injection of 5 mcg/kg of Ach, occlusion of both carotid arteries for 45 seconds and injection of 5 mcg/ml of epinephrine.

**The Cat Nictitating Membrane**

It is an additional eye lid that composed of smooth muscle innervated by post-ganglionic sympathetic nerve fibers. It has alpha and beta subtype of receptors. Nictitating membrane is attached to a lever for recording its contraction. By the aid of this test we can determine whether the new drug is ganglionic blocker, adrenergic neuron blocker or alpha receptors blocker.

Tests on isolated organs like guinea pig ileum, intestine, rat uterus, aorta etc. This is usually carried out to illustrate the type of receptors the drug act on.

**Factors Affecting Pharmacological Response to drugs**

*They include the following:*

- Species and strain of animals; differ in biochemical, functional and morphological features thus their response to the drug will vary quantitatively or qualitatively. Example, morphine in CNS depressant in rabbit and human and CNS stimulant in cats and dogs.
- Sex; difference in drug response among different sex is attributed to difference in the level of metabolizing enzymes.
- Age; the level of metabolizing enzymes varies with age and some enzymes may be lacked in newly born animal.
- Diseases; the hypersensitivity to catecholamine action on the

**Biological standardization**

**Biological Standardization of Functional Modules**

My exploration into genetic engineering for my M.Sc. thesis has led me to an interesting path of new exciting research in gene synthesis. For those of you that have not been in contact with this topic before, it can simply be described as the synthesis of gene-length DNA from chemically derived oligonucleotides, which in turn are short Feb 23 posting in McKinsey&Company: What matters: “Over the past few decades, most new jobs, wealth, and growth were created in the knowledge and digital realm. And while venture capital represented only about 0.2 percent of US GDP, the companies it created generated about 17 percent of economic activity. The Internet changed virtually every industry. Yet as far-reaching as the digital revolution was, the ability to code life will likely reach even further.

**Bio Brick Standardization Process**

DNA cannot currently be fabricated purely using an in vitro process (it still requires an intermediate step using a host organism, e.g. yeast or E.Coli). Nevertheless, a transition period has certainly begun. An interesting model in regards to this is the BioBrick Foundation that was introduced (according to Wikipedia) by Tom Knight (MIT), Drew Endy (Stanford) and Christopher Voigt (UCSF). The trademarked words BioBrick and BioBricks refer to a specific brand of open source genetic parts, defined via an open technical standards setting process;

- You develop some scheme for standardizing some aspect of synthetic biology work.
- You convince at least one other person, at a different location from you, that the scheme would help them with something that they care about.
- You each demonstrate that the proposed standard works for each of you (i.e., the standard must work and be good for something).
Value Extraction

It will be interesting to see what type of interesting value extraction models that may arise from this open standard. Some early models related to education include; To paraphrase Barry Schuler in his TED talk Genomics 101, about the fact that it is a fine line between playing god and learning the laws of nature. We are not creating anything artificial we are only changing around the already existing building blocks in nature to understand what the rules of the game are.

FACTORS MODIFYING DRUG ACTIONS

Individuals vary in drug effect from time to time & from other individuals Nature of systemic effects of drugs depends on following factors:

Physiological factors (age, sex, pregnancy, lactation, body wt., food) Pathological state (kidney or liver disease) Environmental factors cont. Psychological/emotional state Interaction with other drugs (drug-drug interactions)

Physiological factors

Age

Extreme of age show extreme drug sensitivity Newborn babies & elderly= greater & more prolonged effect of drugs b/c of less efficient drug metabolism & renal functions

Infants

Premature infants= poor renal & hepatic functions more sensitive to various drugs E.g., Chloramphenicol = Gray baby syndrome (inadequate metabolism) Ampicillin & morphine = GIT absorption (less acidity) Tetracycline = staining of teeth Corticosteroids = retardation of growth in children

Elderly

Renal & hepatic function decline slowly after middle age Activity of hepatic microsomal enzymes decline with age Vd of lipid soluble drugs increases Elderly require less due to degenerative changes in kidney, liver, brain, heart Cont., E.g., Diazepam & benzoiazepines = t1/2 Digoxin = Vd Benzodiazeines= more confusion & less sedation in elderly Hypotensive drugs= postural hypotension in
Pathological state

- Disease can cause pharmacokinetic or pharmacodynamic variation

PK Variation

- Variation in absorption
  - Gastric stasis—in migraine
  - Malabsorption—ileal or pancreatic disease
- Variation in distribution
  - Altered PPB of phenytoin in chronic renal failure (binding of phenytoin to PPB?)
- Variation in metabolism
  - Hepatic cirrhosis & portal HTN
- Variation in excretion
  - Acute and/or chronic renal failure
- Pharmacodynamic alterations
  - Variation in receptors
  - In myasthenia gravis, nephrogenic diabetes insipidus, familial hypercholesterolemia

Genetic factors

- It affects drug action due to genetic differences among the races & certain persons in same population
- Genetic variation is an important source of PK variability

Examples:
- Genetic polymorphism= fast/slow acetylators (hydralazine, procainamide, isoniazid)
- Plasma choline esterase variant (suxamethonium)
  - Hydroxylase polymorphism (extensive or poor metabolism of debrisoquine)
  - Ethnic differences in drug metabolism: propranolol, hemolytic anemia due to some oxidizing agents (primaquine, sulphonamides)

Environmental factors

- Microsomal enzyme inducers
  - e.g., Hydrocarbons in tobacco smoke, charcoal broiled meat induce CYP1A
- Smokers metabolize drugs more rapidly than non smokers
Psychological state

- General anesthetics required in ?dose for nervous & anxious patients
- Higher doses of chlorpromazine needed in schizophrenics
- Placebos (inert dosage form) produce therapeutic benefits in psychomotor angina pectoris & bronchitis in asthma

Interaction with other drugs

- Administration of one drug (A) can alter action of another drug (B) by PK or PD mechanisms
- This is c/d drug-drug interaction
- May be desired or beneficial like multidrug treatment of tuberculosis
- Or undesirable or harmful

FACTORS MODIFYING THE DOSE

The Nature Of The Disease

In great pain, as in peritonitis, morphine may be borne in doses that would ordinarily be poisonous. On the other hand, in cyanosis or conditions with bad breathing, morphine should be used with caution because of its tendency to depress the respiration. In malaria, quinine can be borne in larger doses than when it is used for other purposes.

Again, in Bright’s disease or other conditions involving the eliminating organs drugs may more readily accumulate in the system and cause cumulative poisoning; and in functional or organic disturbance of the liver certain substances, like phenol or morphine, may have a more pronounced poisonous effect than otherwise.

The Object Of The Medication

Quinine as a bitter appetizer may be given in doses of one or two grains, while quinine for malaria is given in a single large dose of 15 or 20 grains, followed by 5 grains three times a day for a month. In a cough mixture for a child syrup of ipecac is given in dose of 2 to 5 minims, but in croup, where an emetic effect is desired, a whole teaspoonful is administered.

It is to be noted that preparations for local action are active according to their percentage strength rather than according to the actual amount of drug employed.

The Form Of The Remedy

As a rule, this makes but little difference; yet, other things being equal, liquids are more rapidly active than solids, and alcoholic liquids more than aqueous. Active principles are more rapid than crude drugs, powders and dry-filled capsules than pills, fresh-made pills than coated pills. Some cathartic drugs, like aloes and cascara, are more effective cathartics than their active principles. This is because of the extractive matter present, which retards absorption and keeps the active principles in the alimentary tract until they reach the colon.

The Channel of Administration.

It has usually been taught that the hypodermatic dose should be half, and the dose by rectum twice, that by mouth. In a number of instances, however, it has been demonstrated that drugs are as quickly absorbed from the rectum as from the stomach, or even more quickly; and also that, in ordinary circumstances, most drugs are absorbed from the stomach or duodenum with sufficient rapidity to give the full effect of the drug in a short time. Therefore, since rectal and hypodermatic medication are resorted to only under special circumstances, their dose is the same as that by mouth. In rectal medication the strength of the preparation rather than the total dose is usually desired, for the rectum is seldom resorted to for any but local medication. In intravenous medication the dose is a special one for the few drugs that may be so administered, and is usually comparatively small. In conditions of edema, hypodermatic medicaments may be retarded in their absorption, and in congestive conditions of the stomach and bowels, mouth doses may be retarded.

The Time of Administration

After meals the dose is diluted and absorption delayed by the admixture with the stomach contents; so if a rapid effect is desired, a larger dose must be given. On the contrary, the empty stomach allows immediate local action and more ready absorption, as commonly observed in the greater activity of alcoholic drinks taken before meals.

The Frequency of Administration

It goes without saying that the dose of a powerful drug is less if
**DRUG DEVELOPMENT**

The dynamics of drug development is one of the defining characteristics of the pharmaceutical industry. Despite its importance to the industry, there is little information on how long it takes for particular drugs to go through human clinical trials and the probabilities of successful completion. Recently, a number of authors have started making use of historical data on the development of drugs through human clinical trials in the US and elsewhere in the world (for example, Abrantes-Metz, 2003, Kyle, 2002, Danzon et al, 2003). These authors are using this data examine determine the relationship between drug characteristics and successful durations, market entry, and the use of licensing arrangements, respectively. This type of historical data has the potential to provide industry analysts with a much clearer picture of late stage pharmaceutical development and new drug entry. The current paper presents some summary statistics on duration and frequencies of successful completion of the human clinical trials. While this analysis is not sophisticated or detailed enough to provide answers to many of the questions researchers and practitioners are interested in, it does provided readers with some stylized facts to guide future work.

The paper analyzes a sample of drugs that have entered human clinical trials somewhere in the world between 1989 and 2002. The data provides information on entry and exit dates from the three different stages of the human clinical trials for the first indication that the drug was being developed (post-1989). The data also provides information on drug characteristics such as primary indication, chemical composition, route of administration and originating company. The analysis provides frequencies with which drugs with different characteristics successfully complete the different stages of the human clinical trials. For example, drugs that have been originally developed by one of the 10 largest drug companies have a higher than average probability of getting to market. The analysis also provides mean durations for drugs that successfully complete the different stages of the human clinical trials. For example, AIDS drugs are in human clinical trials for an average of 5 years, which is 3 years shorter than the average drug in the sample. In general, the results presented should not be interpreted as causal effects of drug characteristics on success rates or successful durations. Rather these results should be interpreted as central tendencies or simply as statistical observations of the drug development process.

Analysis of drug development and new drug entry must address four major questions. First, do “important” new drugs get through the regulatory process quicker than other drugs? In the US, the FDA offers a number of programs aimed to encourage development of important life-saving drugs, including prioritizing drugs at registration and offering fast tracks through human clinical trials and registration for specified drugs (particularly AIDS drugs). According to the FDA, priority drugs that successfully complete the review process have significantly shorter durations than standard drugs (FDA, 2003). Dranove and Metzler (1994) analyze the FDA’s role in drug development durations by analyzing successful duration from discovery to market for US drugs.

The authors find that economic indicators seem to be more important in determining durations than “scientific” indicators. This paper and Abrantes-Metz (2003) use more detailed data on the durations and failure rates for drugs in human clinical trials. This
development was discontinued between 1981 and 1992, the sponsors cited “economic reasons” as the explanation for why development was discontinued. These results suggest that expected market return is an important determinant of success probabilities and durations. The results presented below show that the probability of entry tends to increase with market size, except for drugs destined for very large markets. It is not clear how to interpret such results. One issue is that companies do not randomly choose which drugs to develop, and simple risk/return analysis suggests that companies may try to develop drug with lower probability of getting to market if those drugs are expected to have a higher return.

In fact, Danzon et al (2003) find that drugs with a higher expected return have a lower probability of getting to market and argue that this result is consistent with equilibrium behavior. The analysis presented in this paper is not detailed enough to account for such endogeneity issues. The results also show that drugs destined for larger markets tend to spend longer in development. This result seems as odds with our expectation; however it is again not obvious how such results should be interpreted given that durations are heavily influenced by the drug companies.

Fourth, how do the drug’s characteristics affect success frequencies and durations? Dranove and Metzler (1994) have some information on how some characteristics affect durations. However, the data is not detailed enough to determine how characteristics affect particular phases of the human clinical trials. The analysis presented in DiMasi (2001) is similar to this paper, however it is done on drugs in development prior to 1995. A recent change in the industry has been the introduction of biotechnology drugs into human clinical trials.

The results show that biotech drugs tend to have higher probabilities of getting to market although their average durations are similar to the average durations over all drugs. The results also suggest significant differences between drugs with different routes of administration (ROA). Oral drugs seem to be quicker to market but with a lower probability of successful completion of human clinical trials.

This result is consistent with an equilibrium story that oral drugs have higher expected returns, however these results are not based on a structural estimation so should be interpreted with care. For
example, it may simply be the case that it is easier to conduct trials on oral drugs. The paper proceeds as follows. Section II presents a brief description of the drug development process. Section III describes the data used in the analysis, and provides definitions of the variables used. Section IV presents and discusses the results. Section V concludes.

**Human Clinical Trials**

The process of drug discovery to market can be decomposed into six distinct periods. The first period is commonly known as Preclinical. In general, after preclinical analysis, a company wishing to launch a drug on the US market, for example, files an Investigatory New Drug (IND) application with the FDA. If accepted, the drug goes into human clinical trials, which has three basic phases, called Phase 1, Phase 2 and Phase 3 (the second, third and fourth periods, respectively). An IND may be filled for one or more phases.

Generally, the phases are completed sequentially and after the Phase 3 trials have been completed, a company wishing to launch a drug on the US market will file a New Drug Application (NDA) with FDA and move into the fifth period. A drug that passes FDA review successfully is registered in the “Orange Book”. Once registered, the drug moves into the sixth period and the company can launch the drug on to the US market. A similar process occurs in other countries. In preclinical trials, the pharmaceutical company uses genetic analysis, pharmacological tools and “animal models” to test for the safety and the effectiveness of the drug for particular disease indications. Unfortunately, because the data set analyzed below is based on information that is voluntarily given to the public by the drug’s sponsor, the information on preclinical trials is not very accurate.

Note that according to the FDA, only 1 in 1,000 drugs pass the preclinical stage and are proposed for testing in humans (FDA, 2002). However, almost half of the R&D expenditures occur in the preclinical stage of development (Levy, 1999) The first phase of the human trials is called Phase 1. Phase 1 trials are generally carried out on a healthy volunteer population of between 20 and 80.

According to the FDA, “These studies are designed to determine the metabolic and pharmacological actions of the drug in humans, the side effects associated with increasing doses, and, if possible, to gain early evidence on effectiveness” (FDA, 2003). Phase 2 trials involve several hundred patients with the disease condition, and are designed to give an early indication of the drugs effectiveness. Phase 3 trials are larger with patient numbers between several hundred and a few thousand, and are designed to give information on the balance between safety and effectiveness.

**Data**

Pharmaprojects contains information on 27,987 new branded drug entities that have reached the late stage development from 1980 to 2002. For the purposes of this study, we limited the sample size to the 3,328 drugs that have entered either Phase I, or Phase II, or Phase III of the human clinical trials somewhere in the world for the first time since 1989. Note that information on every stage of development is available for only a limited number of drugs. The data is based on information that is voluntarily provided by the pharmaceutical companies in the form of press releases and academic conferences.

Table:

(1) in the appendix presents information on the number of drugs for which we have information on the different phases of development. Note that of the drugs for which the data provides information on Phase 3, just less than half have no information on the previous phases. It is thus necessary to be careful about interpreting results for drugs in Phase 1 and Phase 2 as there may be substantial self-selection bias in the sample. Although not reported, the good news is that most of the censoring of earlier phases occurs in the earlier years of the study (prior to 1994) suggesting that the censoring is not necessarily related to the expected success of the drug, but related to the standard left censoring problem in duration data.

The length of time in each phase is determined by the time between the entry date of the particular phase and the entry date of the next phase. However, for Phase 3, the entry date of the next phase is the date on which the drug was launched somewhere in the world (for the first time). It should be noted that this phase explicitly includes time spent in government review after the Phase 3 clinical trials have ended. The measure of “success” is the probability of completing each phase of development, where successful completion of Phase 1 is defined as entry into Phase 2, similarly for Phase 2. For successful completion of Phase 3, we assume entry on to the US market.
A number of measures are used to provide some information related to the topics discussed in the introduction. In relation to the drug’s importance, the major measure is the drug’s indication. The indication of the drug is generally its “primary indication”, which is defined as the indication for which the drug is further along in its development. Most drugs are taken through human clinical trials for one indication prior to being tested for other indications. However, it should be noted that in the U.S., for example, doctors are free to prescribe approved drugs for any indication. Given this, it may not always be the case that the drug is intended for its “primary indication”.

The measure of company size is “Big Pharma”. A drug is categorized as either being originally developed by a big pharma firm or a non-big pharma firm. The drug’s firm is a big pharma firm if the company’s world revenue for 2001 was one of the top ten in the pharmaceutical industry. One concern with using a measure of revenue is that it is endogenously determined, with successful drugs getting to market and creating revenue for the firm. In the results we also report success probabilities and successful durations by individual company for the 8 companies with the largest number of drugs in the data base.

In the life of a drug from discovery to market, there are many companies that are involved in its development, human clinical trials and marketing. In the results presented below the only company discussed is the drug’s “originator”. This is the company, according to Pharmaprojects, that discovered the drug. However, it may not be the company that sponsors the drug through the human clinical trials or takes the drug on to the market. One advantage of using the drug’s originator is that to some extent it is exogenous to the likely success of the drug in human clinical trials, particularly as only 1 in 1000 drugs ever makes it from discovery to human clinical trials.

A disadvantage is that the originator, particularly a small company, is likely to license the drug to a large company in order for the larger company to take the drug through the trials and on to the market. We therefore may be underestimating the advantage to a drug of being sponsored by a large firm.

The measure of market size is the current world revenue for the drug’s therapeutic class and pharmacological description. For example, the market size for the arthritis drug, Celebrex, is equal to the world revenue for arthritis drugs based on the Cox-2 inhibitor. The market size is then categorized into five discrete groups. This is a very crude measure of expected return, particularly as it does not account for the number of drugs in the market. Unfortunately, we don’t have more direct measures of market size, such as the actual revenue earned by the drug.

We also don’t have any information on the cost of drug development. However, one advantage of this measure is that it provides some indication of the market size for drugs that have not yet reached the market.

Finally, the data provides a number of other measures of drug characteristics including the drug’s route of administration and the drug’s original material. The drug’s route of administration is categorized by a number of degrees of specificity. For example, a pill is categorized as “alimentary”, and then “oral”. We report results as specifically as possible while having enough drugs in the category for sensible statistics.

The drug’s original material is similarly categorized, so a particular biotech drug may be categorized as “biological”, and then “recombinant protein”. We report the statistics at the highest category level. Table (2) represents the number of drugs in each phase of development according to their company size, material, route of administration and market size. Since 1989, first time entry drugs number 1,796 for Phase I, 1,879 for Phase II, and 1,025 for Phase III. Of the 398 drugs that have been launched worldwide, only 217 of them have been launched into the US market. 1,465 of the 3,328 drugs in the sample have been withdrawn or discontinued from development.

**Results**

**Do Important Drugs Get to Market Faster?**

In the US, the FDA has instituted policies that give pharmaceutical companies the opportunity to get “important” drugs to market. These policies include faster review of “priority” drugs and fast-tracking of human clinical trials for certain drugs. Priority drugs are defined by the FDA at the time of registration (generally after the completion of the Phase 3 clinical trials). The FDA also offers the opportunity for some drugs to shorten their time in human clinical trials and in this way, “fast-tracking” drugs to market. Time in
development is calculated by adding together the average duration that drugs in the sample spend in each stage of development.

On average, it takes just under 8 years for a drug to go from Phase I of human clinical trials to market launch in the US. The same figures for Phase II and Phase III drugs are 6.1 and 3.7 years respectively. More specifically, an average drug spends 1.7 years in Phase I, 2.4 years in Phase II, and 3.7 years in Phase III before launch. Graph I presents a graph showing the estimated duration for the drugs in the data set by their primary indication. While it takes just 5.5 years on average for HIV/AIDS drugs to get from Phase I to the market, it takes drugs for Parkinson’s disease almost twice that long to go through the same process.

Drugs for arthritis also spend more than 9 years, and asthma drugs spend more than 8 years in clinical trials on average. HIV/AIDS, anti-hypertension, and leukemia cancer drugs are some drugs that spend less than 7 years in clinical development. Again, this result is suggestive, but more sophisticated analysis is necessary to determine whether more important drugs get to market faster, and why.

Are there Economies of Scale or Scope in Drug Development?

While the data and the analysis is not nearly detailed enough to get at this question, we can present some summary statistics on the relationship between firm size (as measured by revenue) and success probabilities and successful durations. The probabilities are calculated by multiplying together the estimated probabilities of a drug moving from one particular stage in development to the next stage.

The method of calculation can be expressed by the following equation:

$$ Pr(\text{Launch}=1 \mid \text{Phase I}=1) = Pr(\text{Launch}=1 \mid \text{Phase III}=1) \times Pr(\text{Phase III}=1 \mid \text{Phase II}=1) \times Pr(\text{Phase II}=1 \mid \text{Phase I}=1) $$

In words: probability of drugs being launched onto the market when they enter Phase I equals the product of the probability of drugs getting from Phase I to Phase II multiplied by the probability of the drugs in Phase II advancing to Phase III, multiplied by the probability of drugs in Phase III being launched onto the US market.

The reason behind this method is that information on all stages of clinical development is available for only a limited number of drugs. By studying this group of drugs exclusively, we would significantly reduce the sample size, and thereby, potentially exclude important information. Instead, we calculate the probabilities of the drugs in each phase of development getting to the next phase from the time they entered Phase I clinical trial until their launch to the market, and then multiplying the results together. The probabilities of drugs moving from a particular stage to the next are calculated using the number of drugs that have advanced to the next stage as numerator, and the sum of drugs that have been suspended, withdrawn or discontinued from that particular stage, or moved on to the next stage as the denominator. Drugs that are still active in that particular stage of development are not used in this calculation.

The results presented in Tables (4) through (9), show that drugs originally developed by Big Pharma firms are more likely to get to market, especially from Phase 3, where Big Pharma drugs have a 47% probability of getting to market, compared to 36% for non-Big Pharma drugs. Tables (5) and (6) show that this pattern holds for particular types of drugs such as drugs indicated for arthritis and drugs indicated for hypertension. In regards to successful durations, overall Big Pharma drugs are slightly quicker to market, but this pattern does not hold for the two subsets of drugs presented in Tables (8) and (9). We should be very careful interpreting such results as suggesting that there are economies of scale or scope in pharmaceutical development, given both the discussion above on endogeniety and the heterogeneity in both success rates and successful durations for some of the larger companies.

Graphs (2) and (3) suggest that different companies may have different strategies in relation to drug development. It is particularly noteworthy that drugs from Company H have the lowest probability of getting to market, just 5% from Phase 1, and one of the longest successful durations at almost 8 years. On the other hand drugs invented by Company E have very high probabilities of entering the US market at 30% from Phase 1. Again these types of statistics are simply suggestive. We cannot conclude that the heterogeneity is due to such development strategies. We can however conclude that it will be difficult to empirically estimate economies of scale or scope given that company specific development strategies may influence observed probabilities of success.

Conclusion

Drug development is one of the salient characteristics of the
pharmaceutical industry. However, it is not an area of the industry for which we have a lot of information. Recently, a number of authors have started to make use of data on success rates and durations for human clinical trials. This study analyzes the probability of success and the length of successful durations for 3,328 branded drugs that had entered either Phase I, Phase II or Phase III of the human clinical trials somewhere in the world between 1989 and 2002. Our basic summary is that approximately 1 in 8 drugs that entered Phase I are launched on the US market. On average, this part of the development process takes just under 8 years.

This number is close to the FDA’s own figure of 8.5 years in their tracking U.S. human clinical trials. The complete process of getting a drug to the market can be substantially longer. Bosch and Lee (1994) report that it takes a total of 12 years to get a new drug approval from the FDA. We excluded the preclinical period from our analysis since the Pharmaprojects data set is based on public information, and so focuses on drugs that have already made it to the late stage development.

There are four major questions, that studies like this one, may be able to answer. Do more important drugs get to market quicker? Are there economies of scale or scope in drug development? What effect does the expected return have on the drug’s development? What effect do characteristics of the drug have on the drug’s development? We do find that HIV/AIDS drugs get to market quicker than the average drug.

We find that drugs originally developed by the 10 largest pharmaceutical companies have slightly lower probabilities of US entry from Phase I, but spend substantially less time in all clinical development phases than the average drug. Drugs with the potential for extremely lucrative markets of US $10 billion or more tend to spend more time in development, and have a lower probability of actually reaching the market.

Biological drugs have somewhat higher probabilities of making it to the US market, but this may vary across indications. The results give, at best, partial answers to these questions. In some cases the results seem unintuitive, but as discussed above, answering these questions is quite complicated and requires careful analysis of these newly available data sets. It is hoped that the results discussed above increase our knowledge of the industry and create interest in more sophisticated econometric analysis such as that presented in Abrantes-Metz et al.

ADVERSE DRUG REACTIONS

Such a drug would be aimed precisely at a disease site and would not harm healthy tissues. Although many new drugs are aimed more accurately than their predecessors, none of them, as of yet, hit the target precisely.

Most drugs produce several effects, but usually only one effect—the therapeutic effect—is wanted for the treatment of a disorder. The other effects may be regarded as unwanted, whether they are intrinsically harmful or not. For example, certain antihistamines cause drowsiness as well as control the symptoms of allergies. When an over-the-counter sleep aid containing an antihistamine is taken, drowsiness is considered a therapeutic effect. But when an antihistamine is taken to control allergy symptoms during the daytime, drowsiness is considered an annoying, unwanted effect.

Most people, including health care practitioners, refer to unwanted effects as side effects; another term used is adverse drug event. However, the term adverse drug reaction is technically more appropriate for drug effects that are unwanted, unpleasant, noxious, or potentially harmful.

Not surprisingly, adverse drug reactions are common. Most adverse drug reactions are relatively mild, and many disappear when the drug is stopped or the dose is changed. Some gradually subside as the body adjusts to the drug. Other adverse drug reactions are more serious and last longer. About 3 to 7% of all hospital admissions in the United States are for treatment of adverse drug reactions. Adverse drug reactions occur during 10 to 20% of hospital admissions, and about 10 to 20% of these reactions are severe.

Digestive disturbances—loss of appetite, nausea, a bloating sensation, constipation, and diarrhea—are particularly common adverse drug reactions, because most drugs are taken by mouth and pass through the digestive tract. However, almost any organ system can be affected. In older people, the brain is commonly affected, often resulting in drowsiness and confusion.

Severity of Adverse Drug Reactions

There is no universal scale for describing or measuring the severity of an adverse drug reaction. Assessment is largely subjective.
FDA Definition

Any adverse event associated with the use of drugs in humans whether or not considered drug related including the following:

- An adverse event occurring in the course of the use of drug in professional practice,
- An adverse event from drug overdose whether accidental or intentional,
- An adverse event occurring from drug abuse,
- An adverse event from drug withdrawal,
- Any significant failure of expected pharmacological action.

Probability

- Definitive
- Probable
- Possible
- Doubtful

Determination based on the presence of conclusive reports in literature, a negative dechallenge, positive rechallenge, alternate causes, a positive dose responserelationship, a temporal relationship, and presence of toxic drug concentration.

Severity

- Mild: Symptomatic treatment, alter dose, no change of drug
- Moderate: Change in drug therapy
- Severe: Unexpected untoward leading to possible debility, or death.

Gender Effects of Pharmacology

Results of various animal studies illustrated the fact that significant SEX difference in drug metabolism and elimination did provide an impetus for sex based research in humans.

Recent advances in the characterisation of specific isoenzymes of drug metabolism paved the way for preliminary identification of enzyme systemaffected by sex. Limited current studies showed apparent CYT P450 (CYP 3A4) activity higher in females than males while other enzymes are increased in males. Men and woman show different pharmacodynamic response to various drugs probably so with drugs having low therapeutic range.

In addition to sex gender differences in drug metabolism may
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drug. Similar clearance for Alprazolam in females are higher than men. In contrast clearance of Oxazepam, Temazepam, Chlordiazepoxide increased in males. Whereas no sex difference were observed with Nitrazepam, Bromazepam, Triazolam, and Lorazepam.

the above research findings have the following lacunae:

- There can be significant sex difference in systemic drug metabolism.
- Sex differences in drug absorption and first pass metabolism (because majority of studies are conducted with po administration).
- Change due to ageing can be gender specific.

Drugs of same pharmacological class (with similar structure) may show marked sex differences – as illustrated by:

- Temazepam and Oxazepam metabolised through conjugation - produces greater in males than females.
- Nitrazepam is metabolized by nitro reduction group - No sex difference.
- Other benzodiazepine metabolized primarily via oxidative metabolism with CYP isoenzymes which are greater in females than males.

Cytochrome P450:

Metabolism of phenazone (antipyrine) shows significant sex difference. In humans liver enzyme CYP3A4 is responsible for more than 50% of drugs including cyclosporine, quinine, niridazole, dapsone, erythromycin, lidocaine, troleandomycin and young females have 1-4 times.

Enzyme Specific by CYP3A4 mediate N-demethylation. CYP3A4 is also involved in OH lation of steroid hormones and therefore rapid elimination of predisolone. (difference due to interhepatic hepatic clearance).

CYP3A4: Tirimlad, verapamil, diazepam 20% more in males Midazolam inactivation by CYP3A4 20-40% more faster in females. Sex difference in steroid hormone levels. Progesterone activates CYP3A4. Steroid hormones activate CYP isoenzymes through gene expression.

CYP2D6 involved in the metabolism of propranolol, timolol, mexilitine, flecainide, codeine, dextromethorphan all of which show

qualitative and quantitative gender differences: body compartment

male have increase weight and differences in weight are due to body water space, muscle mass, organ blood flow and organ function which can alter pharmacokinetic profiles. females tend to have more percent of body fat; (Vd of lipophilic substance like trazodone, sufentanil)

in extreme obesity significant PCK drug alteration are observable Vd in females - Low Vd for ethanol, High Vd for diazepam.

Even after correction made for weight difference, lean body mass body surface area, significance sex difference to exist in drug metabolism which are attributable to factors like; Hepatic metabolism - Benzodiazepines - complex role of sex in drug metabolism and PCK.

Granblatt et al & Ochs et al observed in young females higher total and unbound hepatic clearance of diazepam than young males. Higher Vd for diazepam.

Ageing reduces total diazepam hepatic clearance in men but not in women.

The clearance can be influenced by factors like smoking and other drugs. Similar clearance for Alprazolam in females are higher than men. In contrast clearance of Oxazepam, Temazepam, Chlordiazepoxide increased in males. Whereas no sex difference were observed with Nitrazepam, Bromazepam, Triazolam, and Lorazepam.

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play a role in increasing adverse drug reactions in women.

Female specific issues such as pregnancy, menopause, menstruation may have profound drug effects in man. A few clinically significant ones like increased elimination of anti-epileptics decreasing their efficacy in pregnancy, oral contraceptives interfering with metabolism of many drugs and conversely certain drugs can impair contraceptive efficacy.

In the past women were under represented in the participation of clinical studies. Women were initially excluded for clinical studies during their child bearing potential period. FDA extended them from Phase I to early Phase II.

The assumption that women and men metabolise and respond to drugs similarly is no longer tenable and it is also clear sex specificity occurrences like pregnancy, menopause, oral contraceptives etc., profoundly influence drug metabolism and therefore the response. On getting back to animal studies now it is known that there are large sex gender differences in that female rats show differences in levels of CYP3A4 isoenzyme. This and other observations should provide an impetus for gender based research in human.

Qualitative and quantitative gender differences: body compartment

Male have increase weight and differences in weight are due to body water space, muscle mass, organ blood flow and organ function which can alter pharmacokinetic profiles. Females tend to have more percent of body fat; (Vd of lipophilic substance like trazodone, sufentanil).

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Ageing reduces total diazepam hepatic clearance in men but not in women.

The clearance can be influenced by factors like smoking and other
genetic polymorphism – but no sex differences OH lation of clomipramine is greater in males. Ring oxidation is mediated by CYP2D6 and side chain cleaving by CYP1A, CYP2C. Ondonsetron by CYP2D6 is faster in males and significant difference in propranolol is observed.

CYP2C19 with mephenytoin, mephobarbital, omeprazole, propranolol is greater or higher in males whereas with piroxicam is higher in females.

CYP1A2 is involved oxidation of Theophylline and women have low CYP1A2 and smoking induces this enzyme and therefore thiouixene is faster in males.

Therefore sex, smoking and age influence theophylline metabolism. Because conflicting reports in CYP1A2, its implication in gender difference cannot be attributed at this stage.

**Conjugation**

By CYP mediated hydroxylation step is rate limiting. Temozapam, oxazepam cleared faster in males because of conjugation M:F clearance ration is 1.5:1.

Digoxin clearance is 12% less in females.

Males have high glucoronyl transferase activity and therefore clearance paracetamol is 22% greater in males.

Dihydrouracil dehydrogenase metabolism, flurourcil is reduced in females.

Renal elimination:
- **Filtration:** Greater in males
- **Ssecretion:** Greater in males
- **Reabsorption:** No diff.

**Protein Binding**

Less significant alpha 1 acid glycoprotein is reduced by estrogen and therefore females have low levels of protein binding e.g. warfarin.

**Absorption**

Sex influences gastric emptying time and intestinal transition time. Stomach alc dehydrogenase is reduced in females per oral absorption of aspirin is more rapid and IM aspirin is slower in females. Bio-availability is higher in females (aspirin) reduced Cu absorption in males.

**Pulmonary Route**

Females show reduced pulmonary absorption of cromolyn na, and ribavirin. Females show reduced pulmonary absorption of cromolyn na, and ribavirin

**Pharmacodynamics**

**Antipsychotics:** Women greater impairment & increased ADR and therefore much lower doses are advocated.

**Imipramine:** Men respond better.

**Panic attacks:** better with tricyclics in males - MAO inhibitors in women.

Platelets of men have fewer receptors sites than females in binding paroxetine (5HT antagonist)

CVS drugs: Increased ADR in women and increased antithrombic effect.

**Atracurium:** Increased response in omen.

Mehtyl prednisolone gender based differ, in PCK is offset by pharmacodynamic response because if increased sensitivity in females with methyl prednisolone.

Antiinflammatory activity with naproxen, piroxicam women show greater ADR.

**Clinical Significance**

Drugs with wide T.I because of greater magnitude does not necessitate dose adjustment and only with narrow TI dose adjustment is necessary.

**Sex Specific Disease:** Menopause estrogen and/or progesterone therapy reduces risk of osteoporosis, reduce cardio-vascular disease and reduce risk of endometrial cancers. Aging in women is significant than men. Alfentanil clearance in female has inverse correlation (CYP3A4 is reduced menopause).

Reduced estrogen metabolism in old age and reduced prednisolone clearance in postmenopausal women. Estrogen replacement in menopause does not reverse the enz status but affect PCK differently Ex: Prednisolone, anti-inflammatory steroids and reduced piroxicam clearance. CA ABSORPTION IS IMPAIRED BY MENOPAUSAL STATE.

Oral Contraceptives can Alter Metabolism of Other Drugs:

Drugs interfering with Oral Contraceptives:
- Those increase hepatic metabolism
- Decrease absorption from entero-hepatic circulation:
Alternation due to changes in steroids during pregnancy:
- Increased progesterone inhibits CYP1A2 and increases CYP3A4.

Clinical significance: In pregnancy, with oral contraceptives, menopause, and menstrual cycle phase there is alteration in both PCK and pharmacodynamic aspects of drug handling and therefore drugs with narrow therapeutic range dosage adjustment is necessary. A sudden change in drug efficacy or toxicity should raise suspicion of gender phenomenon.

It is anticipated that gender difference in drug metabolism may become an important factor in deciding the dosage of drug with narrow therapeutic range probably involving an increase in incidence of ADR in man.

Types of Adverse Drug Reactions

Many adverse drug reactions represent an exaggeration of the drug’s therapeutic effects (called type 1 or overdose reactions). For example, a person taking a drug to reduce high blood pressure may feel dizzy or light-headed if the drug reduces blood pressure too much. A person with diabetes may develop weakness, sweating, nausea, and palpitations if insulin or an oral antidiabetic drug reduces the blood sugar level too much. This type of adverse drug reaction is usually predictable but sometimes unavoidable. It may occur if a drug dose is too high, if the person is unusually sensitive to the drug, or if another drug slows the metabolism of the first drug and thus increases its level in the blood (Factors Affecting Response to Drugs: Drug Interactions). Type 1 reactions are usually not serious but are relatively common.

Some adverse drug reactions result from mechanisms that are not currently understood (called type 2 or idiosyncratic reactions). This type of adverse drug reaction is largely unpredictable. Examples of such adverse drug reactions include skin rashes, jaundice, anemia, a decrease in the white blood cell count, kidney damage, and nerve injury that may impair vision or hearing. These reactions tend to be more serious but typically occur in a very small number of people. Affected people may be allergic or hypersensitive to the drug because of genetic differences in the way their body metabolizes or responds to drugs.
Some adverse drug reactions are not related to the drug’s therapeutic effect but are usually predictable, because the mechanisms involved are largely understood. For example, stomach irritation and bleeding often occur in people who regularly use aspirin or other nonsteroidal anti-inflammatory drugs (NSAIDs—see Pain: Nonsteroidal Anti-Inflammatory Drugs). The reason is that these drugs reduce the production of prostaglandins, which help protect the digestive tract from stomach acid.

Treatment of poisoning

To Poison Proof your Home

Most poisonings occur when parents or caregivers are home but not paying attention. The most dangerous potential poisons are medicines, cleaning products, antifreeze, windshield wiper fluid, pesticides, furniture polish, gasoline, kerosene and lamp oil. Be especially vigilant when there is a change in routine. Holidays, visits to and from grandparents’ homes, and other special events may bring greater risk of poisoning if the usual safeguards are defeated or not in place.

- Store medicine, cleaners, paints/varnishes and pesticides in their original packaging in locked cabinets or containers, out of sight and reach of children.
- Install a safety latch – that locks when you close the door – on child-accessible cabinets containing harmful products.
- Purchase and keep all medicines in containers with safety caps. Discard unused medication.
- Never refer to medicine as “candy” or another appealing name.
- Check the label each time you give a child medicine to ensure proper dosage.
- Never place poisonous products in food or drink containers.
- Keep coal, wood or kerosene stoves in safe working order.
- Maintain working smoke and carbon monoxide detectors.

Because the primary goal should always be to prevent an adverse event, it makes sense to first discuss poison prevention in the home.

The following messages should be part of anticipatory guidance during prenatal and well-infant visits:

- Keep potential poisons out of sight and out of reach.
- Always reengage child-resistant closures in the locked mode immediately after using a pharmaceutical or consumer product.
- Never transfer a substance from its original to an alternate container.
- Safely dispose of all unused and no longer needed medications.
- Do not refer to medicines as candy.
- Post the poison control center number near the telephone. The universal telephone number in the United States is (800) 222-1222. Calls are routed to the local poison control center.

Additional information can be obtained from the AAP brochure “Protect Your Child From Poison.”

The prevention of all potentially dangerous exposures to harmful substances can never be achieved; therefore, early and effective treatment after the event is a priority. In young children, the routes of exposure include ingestion, skin contact, eye contact, and inhalation. First aid treatment in the home for the latter 3 is straightforward and not controversial. This includes copious irrigation of the skin or eye with tap water for 15 to 20 minutes or safe removal from the potentially dangerous environment in the case of inhalation exposure. The next step is a call for help. If the victim is conscious and alert, call the local poison control center. If the victim has collapsed or stopped breathing, call 911 for emergency transportation to the hospital.

There is controversy regarding home treatment of the ingestion of a potentially toxic substance. Dilution by having the child drink 100 to 200 mL of water or another drink is a routine recommendation for the ingestion of a nonpharmaceutical; however, this is not recommended after the ingestion of a medication, because there is concern that it would hasten the drug’s absorption because of earlier exit from the stomach. The next decision is whether further in-home assessment or intervention at a hospital is required. The poison control center will advise the caregiver if this is necessary.

Treatment [SH]

If your child is unconscious, not breathing, or having convulsions or seizures due to poison contact or ingestion, call 911 or your local emergency number immediately. If your child has come in contact with poison, and has mild or no symptoms, call your poison control center at 1-800-222-1222.
Different types and methods of poisoning require different, immediate treatment:

- **Swallowed poison**: Remove the item from the child, and have the child spit out any remaining substance. Do not make your child vomit. Do not use syrup of ipecac.
- **Skin poison**: Remove the child’s clothes and rinse the skin with lukewarm water for at least 15 minutes.
- **Eye poison**: Flush the child’s eye by holding the eyelid open and pouring a steady stream of room temperature water into the inner corner.
- **Poisonous fumes**: Take the child outside or into fresh air immediately. If the child has stopped breathing, start cardiopulmonary resuscitation (CPR) and do not stop until the child breathes on his or her own, or until someone can take over.
- **In case of any doubt whatsoever**, go immediately to a doctor or hospital emergency room. If possible, have someone drive you there.
- **See that the doctor gets a copy of the pesticide label or at least knows what was being used. Your supervisor or the grower will be of help there.**
- **Doctors use massive doses of atropine sulfate to treat organophosphate and carbamate poisoning. They also use 2-PAM to treat organophosphate poisoning, but it should not be used for carbamate poisoning.**

## MECHANISMS OF DRUG ACTION

### Definition

The initial requirement for drug action is adequate drug delivery to the target site. This depends largely on blood flow in the tumor bed and the diffusion characteristics of the drug in tissue. However, delivery may also be influenced by the extent of plasma protein binding, the absorption of orally administered drugs, first-pass metabolism in the liver, and the requirement for activation by various mechanisms. Blood flow across a capillary bed is directly proportional to the arteriovenous pressure difference and inversely proportional to the geometric and viscous resistances. The geometric resistance to blood flow increases with increasing tumor size, a factor that may limit drug and oxygen delivery to large tumors and thereby diminish the effectiveness of treatment with chemotherapy or irradiation.

The most common route of drug administration for treatment of both localized and disseminated disease is by intravenous infusion, which by definition, makes 100% of the drug available in the blood. Drugs may be administered by a number of routes in addition to intravenous infusions, however, to achieve specialized pharmacologic and therapeutic goals. Regional administration may be employed to more directly target the drug to the principal tumor site and to achieve a higher drug concentration in the vicinity of the tumor. Intraperitoneal infusion of cisplatin for ovarian cancers, intrapleural administration of bleomycin in the treatment of solid tumors, and intrathecal administration of cytarabine (ara-C) as a means of treating leukemias are examples of intracavitary drug delivery. Alternatively, intravascular administration, such as intraarterial infusion of fluorodeoxyuridine into the hepatic artery for treatment of liver diseases, has been used to achieve a pharmacologic advantage. Although oral administration is the most convenient and least expensive route of drug administration, it is associated with problems of inconsistent drug bioavailability among and within patients. More consistent pharmacokinetic results are achieved with subcutaneous or intramuscular drug injections.

Delivery of the drug to the target cell is also dependent upon the rate of removal from the blood. Excretion, either by the kidneys or by the biliary route, constitutes a major clearance mechanism. In addition, many drugs are cleared by metabolism to less effective or inactive metabolites as the blood passes through large body organs. Drug binding to plasma proteins can also effectively lower the concentration of free drug, available for entry into target cells, to a small fraction of the total concentration in blood.
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insulin therapy

insulin therapy is the treatment of diabetes by administration of exogenous insulin.

insulin is used medically to treat some forms of diabetes mellitus. patients with type 1 diabetes mellitus depend on external insulin (most commonly injected subcutaneously) for their survival because the hormone is no longer produced internally.

patients with type 2 diabetes mellitus are insulin resistant, have relatively low insulin production, or both; certain patients with type 2 diabetes, on rare occasions, may eventually require insulin if other medications fail to control blood glucose levels adequately.

principles

insulin is required for all animal life (excluding certain insects). its mechanism of action is almost identical in nematode worms (e.g. c. elegans), fish, and mammals, and it is a protein that has been highly conserved across evolutionary time. insulin must be administered to patients who experience such a deprivation. clinically, this condition is called diabetes mellitus type 1. in addition, the effect of a sugar-rich versus a starch-rich meal is highlighted. the initial sources of insulin for clinical use in humans were cow, horse, pig or fish pancreases. insulin from these sources is effective in humans as it is nearly identical to human insulin (three amino acid difference in bovine insulin, one amino acid difference in porcine).

differences in suitability of beef-, pork-, or fish-derived insulin for individual patients have historically been due to lower preparation purity resulting in allergic reactions to the presence of non-insulin substances.

though purity has improved steadily since the 1920s ultimately reaching purity of 99% by the mid-1970s thanks to high-pressure liquid chromatography (hplc) methods, but minor allergic reactions still occur occasionally, although the same types of allergic reactions have also been known to occur in response to synthetic "human" insulin varieties.

insulin production from animal pancreases was widespread for decades, but very few patients today rely on insulin from animal sources, largely because few pharmaceutical companies sell it anymore.

biosynthetic "human" insulin is now manufactured for widespread clinical use using genetic engineering techniques using recombinant dna technology, which the manufacturers claim reduces the presence of many impurities, although there is no clinical evidence to substantiate this claim. eli lilly marketed the first such insulin, humulin, in 1982.

humulin was the first medication produced using modern genetic engineering techniques in which actual human dna is inserted into a host cell (e. coli in this case).

the host cells are then allowed to grow and reproduce normally, and due to the inserted human dna, they produce a synthetic version of human insulin.

however, the clinical preparations prepared from such insulins differ from endogenous human insulin in several important respects; an example is the absence of c-peptide which has in recent years been shown to have systemic effects itself.

genentech developed the technique lilly used to produce humulin, although the company never commercially marketed the product themselves.novo nordisk has also developed a genetically engineered insulin independently.

according to a survey that the international diabetes federation conducted in 2002 on the access to and availability of insulin in its member countries, approximately 70% of the insulin that is currently sold in the world is recombinant, biosynthetic 'human' insulin.

a majority of insulin used clinically today is produced this way, although the clinical evidence has provided conflicting evidence on whether these insulins are any less likely to produce an allergic reaction.
mixtures of insulin plus other substances including preservatives. These delay absorption of the insulin, adjust the pH of the solution to reduce reactions at the injection site, and so on.

Slight variations of the human insulin molecule are called insulin analogues, (technically “insulin receptor ligands”) so named because they are not technically insulin, rather they are analogues which retain the hormone’s glucose management functionality. They have absorption and activity characteristics not currently possible with subcutaneously injected insulin proper.

They are either absorbed rapidly in an attempt to mimic real beta cell insulin, or steadily absorbed after injection instead of having a ‘peak’ followed by a more or less rapid decline in insulin action, all while retaining insulin’s glucose-lowering action in the human body.

However, a number of meta-analyses, including those done by the Cochrane Collaboration in the United Kingdom in 2002, Germany’s Institute for Quality and Cost Effectiveness in the Health Care Sector [IQWiG] released in 2007, and the Canadian Agency for Drugs and Technology in Health (CADTH) also released in 2007 have shown no unequivocal advantages in clinical use of insulin analogs over more conventional insulin types.

Choosing insulin type and dosage/timing should be done by an experienced medical professional working closely with the diabetic patient.

The commonly used types of insulin are:

- Rapid-acting types are presently insulin analogues, such as the insulin analogues aspart or lispro. These begin to work within 5 to 15 minutes and are active for 3 to 4 hours. Most insulins form “clumps” which delay entry into the blood in active form; these analog insulins do not, but have normal insulin activity. Newer varieties are in now in Phase II clinical trials which are designed to work rapidly, but retain the same genetic structure as regular human insulin.

- Short-acting, such as regular insulin – starts working within 30 minutes and is active about 5 to 8 hours.

- Intermediate-acting, such as NPH – starts working in 1 to 3 hours and is active 16 to 24 hours.

- Long-acting, such as ultralente insulin – starts working in 4 to 6 hours, and is active well beyond 32 hours.

- Insulin glargine and Insulin detemir – both insulin analogs for veterinary usage in the treatment of companion animals with diabetes.

There are several problems with insulin as a clinical treatment for diabetes:

- Mode of administration.
- Selecting the ‘right’ dose and timing.
- Selecting an appropriate insulin preparation (typically on ‘speed of onset and duration of action’ grounds).
- Adjusting dosage and timing to fit food intake timing, amounts, and types.
- Adjusting dosage and timing to fit exercise undertaken.
- Adjusting dosage, type, and timing to fit other conditions, for instance the increased stress of illness.
- Variability in absorption into the bloodstream via subcutaneous delivery
- The dosage is non-physiological in that a subcutaneous bolus dose of insulin alone is administered instead of combination of insulin and C-peptide being released gradually and directly into the portal vein.
- It is simply a nuisance for patients to inject whenever they eat carbohydrate or have a high blood glucose reading.
- It is dangerous in case of mistake (most especially ‘too much’ insulin).

Types

Medical preparations of insulin (from the major suppliers – Eli Lilly, Novo Nordisk, and Sanofi Aventis – or from any other) are never just ‘insulin in water’. Clinical insulins are specially prepared...
which start working within 1 to 2 hours and continue to be active, without major peaks or dips, for about 24 hours, although this varies in many individuals.

- A mixture of NPH and regular insulin – starts working in 30 minutes and is active 16 to 24 hours. There are several variations with different proportions of the mixed insulins.
- A mixture of Semilente and Ultralente (typically in the proportion 30% Semilente to 70% Ultralente), known as Lente, is typically active for an entire 24 hour period. Beef Lente, in particular, has a very ‘flat’ profile.

Yeast-based

In late 2003, Wockhardt commenced manufacture of a yeast-based insulin costing $3.25 in India claiming it eliminated the risk of contracting diseases such as BSE and CJD associated with insulin derived from pigs and cattle. However, the company continues to manufacture insulin derived from pigs in the United Kingdom.

Modes of Administration

Unlike many medicines, insulin cannot be taken orally at the present time. Like nearly all other proteins introduced into the gastrointestinal tract, it is reduced to fragments (even single amino acid components), whereupon all ‘insulin activity’ is lost.

There has been some research into ways to protect insulin from the digestive tract, so that it can be administered in a pill. So far this is entirely experimental.

Subcutaneous

Insulin is usually taken as subcutaneous injections by single-use syringes with needles, an insulin pump, or by repeated-use insulin pens with needles. Patients who wish to reduce repeated skin puncture of insulin injections often use an injection port in conjunction with syringes.

Administration schedules often attempt to mimic the physiologic secretion of insulin by the pancreas. Hence, both a long-acting insulin and a short-acting insulin are typically used.

Insulin Pump

Insulin pumps are a reasonable solution for some. Advantages to the patient are better control over background or ‘basal’ insulin dosage, bolus doses calculated to fractions of a unit, and calculators in the pump that may help with determining ‘bolus’ infusion dosages.

The limitations are cost, the potential for hypoglycemic and hyperglycemic episodes, catheter problems, and no “closed loop” means of controlling insulin delivery based on current blood glucose levels.

Insulin pumps may be like ‘electrical injectors’ attached to a temporarily implanted catheter or cannula. Some who cannot achieve adequate glucose control by conventional (or jet) injection are able to do so with the appropriate pump.

As with injections, if too much insulin is delivered or the patient eats less than he or she dosed for, there will be hypoglycemia.

On the other hand, if too little insulin is delivered, there will be hyperglycemia. Both can be life-threatening. In addition, indwelling catheters pose the risk of infection and ulceration, and some patients may also develop lipodystrophy due to the infusion sets.

These risks can often be minimized by keeping infusion sites clean. Insulin pumps require care and effort to use correctly. However, some patients with diabetes are capable of keeping their glucose in reasonable control only with an insulin pump.

Inhalation

In 2006 the U.S. Food and Drug Administration approved the use of Exubera, the first inhalable insulin. It has been withdrawn from the market by its maker as of 3Q 2007, due to lack of acceptance.

inhaled insulin claimed to have similar efficacy to injected insulin, both in terms of controlling glucose levels and blood half-life.

Currently, inhaled insulin is short acting and is typically taken before meals; an injection of long-acting insulin at night is often still required. When patients were switched from injected to inhaled insulin, no significant difference was observed in HbA1c levels over three months.

Accurate dosing was a particular problem, although patients showed no significant weight gain or pulmonary function decline over the length of the trial, when compared to the baseline.

Following its commercial launch in 2005 in the UK, it was not recommended by National Institute for Health and Clinical Excellence for routine use, except in cases where there is “proven injection phobia diagnosed by a psychiatrist or psychologist”.
In January 2008, the world’s largest insulin manufacturer, Novo Nordisk A/S, also announced that the company was discontinuing all further development of the company’s own version of inhalable insulin, known as the AERx iDMS inhaled insulin system.

Similarly, Eli Lilly and Company ended its efforts to develop its Air inhaled insulin in March 2008. However, MannKind Corp. (whose majority owner, Alfred E. Mann), remains optimistic about the concept.

**Transdermal**

There are several methods for transdermal delivery of insulin. Pulsatile insulin uses microjets to pulse insulin into the patient, mimicking the physiological secretions of insulin by the pancreas.

Jet injection had different insulin delivery peaks and durations as compared to needle injection. Some diabetics find control possible with jet injectors, but not with hypodermic injection.

Both electricity using iontophoresis and ultrasound have been found to make the skin temporarily porous. The insulin administration aspect remains experimental, but the blood glucose test aspect of ‘wrist appliances’ is commercially available.

Researchers have produced a watch-like device that tests for blood glucose levels through the skin and administers corrective doses of insulin through pores in the skin. Intranasal insulin: Intranasal insulin is being investigated.

**Oral Insulin**

The basic appeal of oral hypoglycemic agents is that most people would prefer a pill to an injection. However, insulin is a protein, which is digested in the stomach and gut and in order to be effective at controlling blood sugar, cannot be taken orally in its current form.

The potential market for an oral form of insulin is assumed to be enormous, thus many laboratories have attempted to devise ways of moving enough intact insulin from the gut to the portal vein to have a measurable effect on blood sugar. As of 2004, no products appear to be successful enough yet to bring to market.

Novo Nordisk announced on December 7, 2009, that it had initiated its first phase 1 trial with oral insulin analogue (NN1952). The aim of the trial is to investigate the safety, tolerance, pharmacokinetics (exposure to drug) and pharmacodynamics (effect) of NN1952 in healthy volunteers and people with type 1 and type 2 diabetes. Results from the trial, which is planned to enroll about 80 people, are expected to be reported in the first half of 2011. NN1952 has been designed to address some of the key challenges relating to oral insulin delivery.

Furthermore, it uses the GIPET (R) formulation technology from Merrion Pharmaceuticals (IEX Quoted) to facilitate insulin absorption from the gut. In November 2008, Merrion entered into a development and license agreement to develop and commercialise oral formulations of Novo Nordisk’s proprietary insulin analogues, using Merrion’s proprietary GIPET (R).

A Connecticut-based biopharmaceutical company called Biodel, Inc. is developing what it calls VIAtab, an oral formulation of insulin designed to be administered sublingually.

This therapy is a tablet that dissolves in minutes when placed under the tongue. In a Phase I study, VIAtab delivered insulin to the bloodstream quickly and resembled the first-phase insulin release spike found in healthy individuals.

The company claims that an oral insulin therapy would be more convenient than currently available injectable or inhalable therapies, and they expect that convenience to result in increased insulin usage among the currently underserved early-stage patients with Type 2 diabetes, thus helping to create better long-term outcomes for that patient population.

Oramed Pharmaceuticals, Inc., a biotechnology company based in Jerusalem, Israel, is currently conducting Phase 2B clinical trials of its oral insulin capsule, ORMD-0801, on 30 patients diagnosed with type 2 diabetes.

An article published in the *Journal of Diabetes Science and Technology* indicates that Oramed’s platform technology has two components:

- A chemical make-up that protects insulin during passage through the gastrointestinal tract,
- Absorption enhancers so that insulin could be absorbed by the intestine.

Oramed Pharmaceuticals, Inc. through Phase 1 clinical trials, has demonstrated that its oral insulin is safe, well tolerated, and has consistently reduced glucose and c-peptide levels in patients.

Australian biopharmaceutical company Apollo Life Sciences plans to enter the phase I trial of its oral insulin tablet in mid-2008.
Biocon, Asia’s largest biopharmaceutical company, based in Bangalore, India, is also developing an oral insulin product. It has just completed phase IIa trials.

**PANCREATIC TRANSPLANTATION**

Another improvement would be a transplantation of the pancreas or beta cell to avoid periodic insulin administration. This would result in a self-regulating insulin source. Transplantation of an entire pancreas (as an individual organ) is difficult and relatively uncommon. It is often performed in conjunction with liver or kidney transplant, although it can be done by itself.

It is also possible to do a transplantation of only the pancreatic beta cells. However, islet transplants had been highly experimental (read ‘prone to failure’) for many years, but some researchers in Alberta, Canada, have developed techniques with a high initial success rate (about 90% in one group).

Nearly half of those who got an islet cell transplant were insulin-free one year after the operation; by the end of the second year that number drops to about one in seven. However, researchers at the University of Illinois at Chicago (UIC) have slightly modified the Edmonton Protocol procedure for islet cell transplantation and achieved insulin independence in diabetes patients with fewer but better-functioning pancreatic islet cells. Longer-term studies are needed to validate whether it improves the rate of insulin-independence.

Beta cell transplant may become practical in the near future. Additionally, some researchers have explored the possibility of transplanting genetically engineered non-beta cells to secrete insulin. Clinically testable results are far from realization at this time. Several other non-transplant methods of automatic insulin delivery are being developed in research labs, but none is close to clinical approval.

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**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), glycyclcyclines (tigecycline), and oxazolidiniones (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, cephalexin, lactamoxef, cefuroxime, 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.

**ANTIBIOTICS**

Many cures for infectious diseases prior to the beginning of the twentieth century were based on medicinal folklore. Cures for infection in ancient Chinese medicine using plants with antibiotic-like properties began to be described over 2,500 years ago. Many other ancient cultures, including the ancient Egyptians, ancient Greeks and medieval Arabs already used molds and plants to treat infections. Cinchona bark was a widely effective treatment of malaria in the 17th century, the disease caused by protozoan parasites of the genus *Plasmodium*.

Scientific endeavours to understand the science behind what caused these diseases, the development of synthetic antibiotic chemotherapy, the isolation of the natural antibiotics marked milestones in antibiotic development

Originally known as antibiosis, antibiotics were drugs that had actions against bacteria. The term antibiosis which means ‘against life’ was introduced by the French bacteriologist Vuillemin as a descriptive name of the phenomenon exhibited by these drugs.

Antibiosis was first described in 1877 in bacteria when Louis Pasteur and Robert Koch observed that an airborne bacillus could inhibit the growth of *Bacillus anthracis*. These drugs were later renamed antibiotics by Selman Waksman, an American microbiologist in 1942.

Synthetic antibiotic chemotherapy as a science and the story of antibiotic development began in Germany with Paul Ehrlich, a German medical scientist in the late 1880s.

Dr. Ehrlich noted that certain dyes would bind to and colour human, animal or bacterial cells, while others did not. He then extended the idea that it might be possible to make certain dyes or chemicals that would act as a magic bullet or selective drug that would bind to and kill bacteria while not harming the human host.

After much experimentation, screening hundreds of dyes against various organisms, he discovered a medicinally useful drug, the man-made antibiotic, Salvarsan. However, the adverse side-effect profile of salvarsan, coupled with the later discovery of the antibiotic penicillin, superseded its use as an antibiotic.

The work of Ehrlich, which marked the birth of the antibiotic revolution, was followed by the discovery of Prontosil by Domagk in 1932.

Prontosil, the first commercially available antibacterial antibiotic was developed by a research team led by Gerhard Domagk (who received the 1939 Nobel Prize for Medicine for his efforts) at the Bayer Laboratories of the IG Farben conglomerate in Germany.

Prontosil had a relatively broad effect against Gram-positive cocci
fungi.

**Purpose**

Many treatments for cancer destroy disease-fighting white blood cells, thereby reducing the body’s ability to fight infection. For example, bladder, pulmonary, and urinary tract infections may occur with chemotherapy. Single-celled organisms called protozoa are rarely a problem for healthy individuals. However, they can cause serious infections in individuals with low white blood cell counts. Because of the dangers that infections present for cancer patients, antibiotic treatment often is initiated before the exact nature of the infection has been determined; instead, the choice of antibiotic may depend on the site of the infection and the organism that is likely to be the cause. Often, an antibiotic that kills a broad spectrum of bacteria is chosen and several antibiotics may be used together.

The common antibiotics that are used during cancer treatment include:

- **Atovaquone (Mapren):** antiprotozoal drug used to prevent and treat a very serious type of pneumonia called *Pneumocystis carinii* pneumonia (PCP), in individuals who experience serious side effects with SMZ-TMP (Sulfamethoxazole/Trimethoprim, brand name Bactrim).
- **Aztreonam (Azactam):** monobactam antibiotic used to treat gram-negative bacterial infections of the urinary and lower respiratory tracts and the female organs, and infections that are present throughout the body (systemic infections or septicemia).
- **Cefepime (Maxipime), ceftazidime (Ceptaz, Fortaz, Tazicef, Tazidime), and ceftriaxone sodium (Rocephin):** members of a group of antibiotics called cephalosporins used to treat bacterial infections of the urinary and lower respiratory tracts, and infections of the skin, bones, joints, pelvis, and abdomen.
- **Ciprofloxacin (Cipro):** fluoroquinolone antibiotic used to treat certain gram-negative and gram-positive bacteria and some mycobacteria.
- **Clindamycin phosphate (Cleocin):** used to treat gram-positive and gram-negative bacterial infections and, in individuals who are allergic to sulfadiazine, toxoplasmosis caused by a parasitic protozoa.

The discovery and development of this first sulfonamide drug opened the era of antibiotics. The discovery of natural antibiotics produced by microorganisms stemmed from earlier work on the observation of antibiosis between micro-organisms. Pasteur observed that “if we could intervene in the antagonism observed between some bacteria, it would offer ‘perhaps the greatest hopes for therapeutics’”.

Bacterial antagonism of *Penicillium spp.* were first described in England by John Tyndall in 1875. However, his work went by without much notice from the scientific community until Alexander Fleming’s discovery of Penicillin in 1928.

Even then the therapeutic potential of penicillin was not pursued. More than ten years later, Ernst Chain and Howard Florey became interested in Fleming’s work following the earlier discovery of another natural antibiotic like substance named gramicidin from *B. brevis*.

In 1939, Rene Dubos isolated gramicidin, one of the first commercially manufactured antibiotics in use during World War II to prove highly effective in treating wounds and ulcers. Florey and Chain succeeded in purifying penicillin. The purified antibiotic displayed antibacterial activity against a wide range of bacteria. It also had low toxicity and could be taken without causing adverse effects.

Furthermore its activity was not inhibited by biological constituents such as pus, unlike the synthetic antibiotic class available at the time the sulfonamides. The discovery of such a powerful antibiotic was unprecedented. The development of penicillin led to renewed interest in the search for antibiotic compounds with similar capabilities.

Because of their discovery of penicillin Ernst Chain, Howard Florey and Alexander Fleming shared the 1945 Nobel Prize in Medicine. Florey credited Dubos with pioneering the approach of deliberately, systematically searching for antibacterial compounds. Such a methodology had led to the discovery of gramicidin, which revived Florey’s research in penicillin.

**ONCOLOGY ANTIBIOTICS**

Antibiotics are drugs that are used to treat infections caused by bacteria and other organisms, including protozoa, parasites, and fungi.
disappeared.

Furthermore, it is important to keep the level of antibiotic in the body at a constant level during treatment. Therefore, the drug should be taken on a regular schedule. If a dose is missed, it should be taken as soon as possible. If it is almost time for the next dose, the missed dose should be skipped. Doubling up doses is generally not recommended.

Average adult dosages of common antibiotics for cancer patients are as follows:

- **Atovaquone**: For PCP treatment, 750 mg oral suspension twice a day, or tablets three times per day, for 21 days; for PCP prevention, 1,500 mg oral suspension, once a day; must be taken with balanced meals.
- **Aztreonam**: 1–2 gm every 6–12 hours, injected into a vein, over a 20–60 minute-period.
- **Cefepime**: 500 mg to 2 gm, injected into a vein or muscle, every 8–12 hours for 7–10 days.
- **Ceftazidime**: 250 mg to 2 gm, injected into a vein or muscle, every 8–12 hours.
- **Ceftriaxone**: 1–2 gm, injected into a vein or muscle, every 24 hours.
- **Ciprofloxacin**: 500–750 mg of the tablet or suspension, every 12 hours, for 3–28 days, taken two hours after meals with 8 oz of water; bone and joint infections usually are treated for at least 4–6 weeks; 200–400 mg injected every 8–12 hours.
- **Clindamycin**: 150–300 mg of capsule or solution, every six hours; 300–600 mg every six to eight hours or 900 mg every eight hours, injected into a vein or muscle.
- **Gentamicin (gentamycin) sulfate (generic name product, Garamycin, G-Mycin, Jenamicin)**: amino-glycoside antibiotic used to treat serious infections by many gram-negative bacteria that cannot be treated with other medicines.
- **Metronidazole hydrochloride (Flagyl, Metric 21, Metro I.V., Protostat)**: used for anaerobic bacteria and protozoa.
- **Pentamidine (generic name product, Pentam 300)**: used to treat PCP if serious side effects develop with SMZ-TMP.
- **Pyrimethamine (Daraprim)**: antiprotozoal medicine used together with sulfadiazine to treat toxo-plasmosis; or in combination with other medicines for treating mild to moderate PCP, in individuals who cannot tolerate the standard treatment.
- **Sulfadiazine (generic name product)**: sulfonamide antibiotic used with pyrimethamine to treat toxoplasmosis.
- **Sulfamethoxazole**: Trimethoprim (SMZ-TMP) (generic name product, Bactrim, Cotrim, Septra, Sulfatrim): the sulfonamide antibiotic, sulfamethoxazole, used in combination with trimethoprim, to prevent and treat PCP and bacterial infections, such as bronchitis and middle ear and urinary tract infections.
- **Trimethoprim** (generic name product, Proloprim, Trimox): primarily used to prevent or treat urinary tract infections.
- **Vancomycin hydrochloride** (generic name product, Vancocin): glycopeptide antibiotic used to treat a variety of serious gram-positive bacterial infections for which other medicines are ineffective, including strains of *Staphylococcus* that are resistant to most oral antibiotics.

Most of these antibiotics kill bacteria by preventing them from making protein for their cell walls. Ciprofloxacin and metronidazole prevent bacteria from reproducing by interfering with their ability to make new DNA. All of these drugs are approved for prescription by the U.S. Food and Drug Administration.

**Recommended Dosage**

Dosages of antibiotics depend on the individual, the infection that is being treated, and the presence of other medical conditions.

For children, the dosage usually is based on body weight and is lower than the adult dosage. To be effective, an entire treatment with antibiotics must be completed, even if the symptoms of infection have disappeared.

Recommended dosages of common antibiotics for cancer patients are as follows:

- **Atovaquone**: For PCP treatment, 750 mg oral suspension twice a day, or tablets three times per day, for 21 days; for PCP prevention, 1,500 mg oral suspension, once a day; must be taken with balanced meals.
- **Aztreonam**: 1–2 gm every 6–12 hours, injected into a vein, over a 20–60 minute-period.
- **Cefepime**: 500 mg to 2 gm, injected into a vein or muscle, every 8–12 hours for 7–10 days.
- **Ceftazidime**: 250 mg to 2 gm, injected into a vein or muscle, every 8–12 hours.
- **Ceftriaxone**: 1–2 gm, injected into a vein or muscle, every 24 hours.
- **Ciprofloxacin**: 500–750 mg of the tablet or suspension, every 12 hours, for 3–28 days, taken two hours after meals with 8 oz of water; bone and joint infections usually are treated for at least 4–6 weeks; 200–400 mg injected every 8–12 hours.
- **Clindamycin**: 150–300 mg of capsule or solution, every six hours; 300–600 mg every six to eight hours or 900 mg every eight hours, injected into a vein or muscle.
- **Gentamicin**: Dosage determined by body weight, every 8–24 hours for at least 7–10 days, injected into a vein or muscle.
- **Metronidazole**: For bacterial infections, 7.5 mg per kg (3.4 mg per lb) of body weight up to a maximum of 1 gm, every six hours for at least seven days (capsules or tablets); 15 mg per kg (6.8 mg per lb) for the first dose, followed by half that dosage every six hours for at least seven days (injected into a vein); for protozoal infections caused by amebas, 500–750 mg of oral medicine, three times per day for 5–10 days; for trichomoniasis, 2 gm for one day or 250 mg three times per
day for seven days (oral medicine); extended-release tablets for vaginal bacterial infections, 750 mg once a day for seven days.

- **Pentamidine**: For treating PCP, 4 mg per kg (1.8 mg per lb) of body weight, once per day for 14–21 days, injected into a vein over one to two hours, while lying down.
- **Pyrimethamine**: For toxoplasmosis, 25-200 mg tablets, taken with other medicine, for several weeks.
- **Sulfadiazine**: For bacterial and protozoal infections, 2–4 gm for the first dose, followed by 1 gm every four to six hours (tablets).
- **SMZ-TMP**: 800 mg of sulfamethoxazole and 160 mg of trimethoprim, (tablet or oral suspension), every 12 hours for bacterial infections and every 24 hours for prevention of PCP; dosage based on body weight for PCP treatment; injections based on body weight, every six, eight or 12 hours for bacterial infections and every six hours for PCP treatment.
- **Trimethoprim**: 100 mg tablet every 12 hours for 10 days; for prevention of urinary tract infections, once a day for a long period.
- **Vancomycin**: 7.5 mg per kg (3.4 mg per lb) of body weight, or 500 mg–1 gram, injected or taken orally, every 6–12 hours.

### Precautions

- **Stomach or intestinal problems or colitis (inflammation of the colon) may affect the use of:**
  - Atovaquone
  - Cephalosporins
  - Clindamycin

- **Kidney or liver disease may affect the use of:**
  - Aztreonam
  - Cefepime
  - Ceftazidime
  - Ciprofloxacin
  - Clindamycin
  - Gentamicin
  - Metronidazole
  - Pentamidine

### Side Effects

- Some individuals may have allergic reactions to antibiotics. If symptoms of an allergic reaction (such as rash, shortness of breath, swelling of the face and neck), severe diarrhea, or abdominal cramping occur, the antibiotic should be stopped and the individual should seek medical advice.

Because antibiotics can affect bacteria that are beneficial, as well as...
as those that are harmful, women may become susceptible to infections by fungi when taking antibiotics. Vaginal itching or discharge may be symptoms of such infections. All patients may develop oral fungal infections of the mouth, indicated by white plaques in the mouth.

Injected antibiotics may result in irritation, pain, tenderness, or swelling in the vein used for injection. Antibiotics used in cancer patients may have numerous side effects, both minor and severe; however, most side effects are uncommon or rare.

The more common side effects of atovaquone, aztreonam, cephalosporins, ciprofloxacin, clindamycin, gentamicin, metronidazole, and SMZ-TMP include:

- Nausea and vomiting
- Diarrhea
- Loss of appetite Eating active cultured yogurt may help counteract diarrhea, but if a patient has low white blood cells, this remedy is not recommended. For mild diarrhea with cephalosporins, only diarrhea medicines containing kaolin or attapulgite should be taken. With clindamycin, diarrhea medicines containing attapulgite should be taken several hours before or after the oral antibiotic. Diarrhea following antibiotics like clindamycin may indicate a bacterial infection that needs additional therapy, and a physician should be consulted.

*Other side effects of atovaquone may include:

- fever
- skin rash
- cough
- headache
- insomnia

*Other side effects of ciprofloxacin may include:

- Abdominal pain
- Increase in blood tests for kidney function
- Dizziness or light-headedness
- Inflammation or tearing of a tendon
- Drowsiness
- Insomnia

Other common side effects of clindamycin include abdominal pain and fever. Side effects may occur up to several weeks after treatment with this medicine. Gentamicin and vancomycin may cause serious side effects, particularly in elderly individuals and newborn infants. These include kidney damage and damage to the auditory nerve that controls hearing.

*Other, more common side effects of gentamicin may include:

- Changes in urination
- Increased thirst
- Muscle twitching or seizures
- Headache
- Lethargy

When gentamicin is injected into a muscle, vein, or the spinal fluid, the following side effects may occur:

- Leg cramps
- Skin rash
- Fever
- Seizures Side effects from gentamicin may develop up to several weeks after the medicine is stopped.

*More common side effects of metronidazole include:

- Mouth dryness
- Unpleasant or metallic taste
- Dizziness or light-headedness
- Headache
- Stomach pain Sugarless candy or gum, bits of ice, or a saliva substitute may relieve symptoms of dry mouth.

Pentamidine, pyrimethamine, sulfonamides, SMZ-TMP, and trimethoprim can lower the number of white blood cells, resulting in an increased risk of infection. These drugs also can lower the number of blood platelets that are important for blood clotting. Thus, there is an increased risk of bleeding or bruising while taking these drugs.

*Serious side effects of pentamidine may include:

- Heart problems
- Low blood pressure
- High or low blood sugar
- Other blood problems
- Decrease in urination
- Sore throat and fever
- Sharp pain in upper abdomen Some of these symptoms may
not occur until several months after treatment with pentamidine.

Pyrimethamine and trimethoprim may lower the red blood cell count, causing anemia. Leucovorin or the vitamin folic acid may be prescribed for anemia.

Some individuals become more sensitive to sunlight when taking sulfonamides, SMZ-TMP, or trimethoprim. Other common side effects of sulfonamides and SMZTMP include:

- Dizziness
- Itching
- Skin rash
- Headache
- Mouth sores or swelling of the tongue
- Fatigue

If vancomycin is injected into a vein too quickly, it can cause flushing and a rash over the neck, face, and chest, wheezing or difficulty breathing, and a dangerous decrease in blood pressure.

**Interactions**

Many prescription and non-prescription medicines can interact with these antibiotics. Therefore, it is important to consult a complete list of known drug interactions. Among the more common or dangerous interactions:

- Antibiotics that lower the number of blood platelets, with blood thinners (anticoagulants), such as warfarin
- Aztreonam and metronidazole with alcohol; it is important not to consume alcohol until at least three days after treatment with these antibiotics
- Ciprofloxacin with antacids, iron supplements, or caffeine
- Pentamidine or pyrimethamine with previous treatments with x rays or cancer medicines (increased risk of blood cell damage)
- Trimethoprim with diuretics to remove excess fluid in the elderly

Many medicines can increase the risk of hearing or kidney damage from gentamicin.

*These include:*

- Cisplatin
- Combination pain medicine with acetaminophen and aspirin
- or other salicylates (taken regularly in large amounts)
- Cyclosporine
- Inflammation or pain medicine, except narcotics
- Lithium
- Methotrexate
- Other medicines for infection

**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
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 clarithromycin, 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 manufacturers' 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.

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. Children 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 fluoroquinolones 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.

**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
antibiotic is delivered throughout the body by absorption into the bloodstream.

Antibiotics differ chemically so it is understandable 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...
after the discovery of penicillin, other antibiotics were sought. in 1939, work began on the isolation of potential antibiotic products from the soil bacteria streptomycetes. 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 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 conducive 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
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.

**Beyond Antibiotics: Treating Multi-drug Resistant Bacteria**

Multi-drug resistant organisms (MDRO) generally refer to bacteria that are not affected by the clinical doses of classical antibiotics, particularly the antibiotics which were being used to treat them until recently. The rise of these organisms has created a need for alternative antibacterial therapies.

Phage therapy, the use of particular viruses to attack bacteria, was in use during the 1920s and 1930s on humans in the US, Western and Eastern Europe. Phage are commonly a part of the ecology surrounding bacteria and provide substantial population control of bacteria in the intestine, the ocean, the soil and other environments.

The success of these therapies are largely anecdotal or otherwise poorly controlled. The original publications are also generally inaccessible, even to persons with Russian language fluency. With the discovery of penicillin in the 1940s, Europe and the US changed therapeutic strategies to the use of antibiotics. However, in the former Soviet Union phage therapies continued to be studied.

In the Republic of Georgia, the Eliava Institute of Bacteriophage, Microbiology & Virology continues to research the use of phage therapies. Various companies (Intralytix, among others), universities, and foundations in North America and Europe are currently researching phage therapies.

However, concerns about genetic engineering in freely released viruses currently limit certain aspects of phage therapy.

One result is attempts to use phage in ways other than to directly infect the bacteria. While bacteriophage and related therapies provide a possible solution to aspects of antibiotic resistance, their place in clinical therapy is still in question.

Different classes of bacteriocins have different potential as therapeutic agents. Small molecule bacteriocins (microcins, for example, and lantibiotics) may be similar to the classic antibiotics; colicin-like bacteriocins are more likely to be narrow-spectrum, demanding new molecular diagnostics prior to therapy but also not raising the spectre of resistance to the same degree.

One drawback to the large molecule antibiotics is that they will have relative difficulty crossing membranes and travelling systemically throughout the body.

For this reason, they are most often proposed for application topically or gastrointestinally. Because bacteriocins are peptides, they are more readily engineered than small molecules. This may permit the generation of cocktails and dynamically improved antibiotics that are modified to overcome resistance.

Nutrient withdrawal is a potential strategy for replacing or
supplementing antibiotics. The restriction of iron availability is one way the human body limits bacterial proliferation. Mechanisms for freeing iron from the body (such as toxins and siderophores) are common among pathogens.

Building on this dynamic, various research groups are attempting to produce novel chelators which would withdraw iron otherwise available to pathogens (bacterial, fungal and parasitic). This is distinct from chelation therapy for conditions other than bacterial infections - including successful treatment for iron overload.

Vaccines are a commonly suggested method for combating MDRO infections. They actually fit within a larger class of therapies that rely on immune modulation or augmentation. These therapies either excite or reinforce the natural immune competency of the infected or susceptible host, leading to the activity of macrophages, the production of antibodies, inflammation, or other classic immune reactions. Just as the macrophage engulfs and consumes bacteria, various forms of biotherapy have been suggested which employ organisms to consume the pathogens. This includes the employment of protozoa and maggot therapy.

Probiotics are another alternative that goes beyond traditional antibiotics by employing a live culture which may in theory establish itself as a symbiont, competing, inhibiting, or simply interfering with colonization by pathogens.

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Points to Consider in Phytotherapy

STANDARDIZATION

In herbal medicine, standardization refers to providing processed plant material that meets a specified concentration of a specific marker constituent. Active constituent concentrations may be misleading measures of potency if cofactors are not present. A further problem is that the important constituent is often unknown.

For instance St John’s wort is often standardized to the antiviral constituent hypericin which is now known to be the “active ingredient” for antidepressant use. Other companies standardize to hyperforin or both, although there may be some 24 known possible constituents.

Only a minority of chemicals used as standardization markers are known to be active constituents. Standardization has not been standardized yet: different companies use different markers, or different levels of the same markers, or different methods of testing for marker compounds.

Herbalist and manufacturer David Winston points out that whenever different compounds are chosen as “active ingredients” for different herbs, there is a chance that suppliers will get a substandard batch (low on the chemical markers) and mix it with a batch higher in the desired marker to compensate for the difference.

Quality
The quality of crude drugs or plant medicines depends upon a variety of factors:

- Genetically strong seed,
- Use of correct species,
- Maturity of the plant at harvest,
- Soil and air quality,
- Climate,
- Organoleptic factors (i.e. sensory properties of a particular chemical, the taste, colour, odour and feel),
- Flavour and odor,
- Post-harvest processing and a variety of other factors.

These conditions have been noted in historical herbals such as Culpepper’s Complete Herbal or The Shen Nong or Divine Farmer’s Materia Medica. This was standard pharmacognosy curriculum for many years.

Storage after collection is a factor worthy of study; researchers in Nara, Japan have stored samples of ginseng root (Panax ginseng), licorice root (Glycyrrhiza glabra) and rhubarb root (Rheum emodi) that have been shown to retain their active properties for over 1,200 years.

In modern times the foregoing aspects are no less important, but have been neglected with the advent of laboratory testing, although it generally is true that only certain constituents are identified and measured.

Processes like HPLC (High performance liquid chromatography), GC (gas chromatography), UV/VIS (Ultraviolet/Visible spectrophotometry) or AA (Atomic Absorption spectroscopy) are used to identify species, measure bacteriological contamination, assess potency and eventually creating Certificates of Analysis for the material.

Quality should be overseen by either authorities ensuring Good Manufacturing Practices or regulatory agencies by the US FDA. In the United States one frequently sees comments that herbal medicine is unregulated, but this is not correct since the FDA and GMP regulations are in place. In Germany, the Commission E has produced a book of German legal-medical regulations which includes quality standards.

Safety

The safety and effectiveness of alternative medicines have not be been scientifically proven and remains largely unknown. A number of herbs are thought to be likely to cause adverse effects. Furthermore, “adulteration, inappropriate formulation, or lack of understanding of plant and drug interactions have led to adverse reactions that are sometimes life threatening or lethal.”

Proper double-blind clinical trials are needed to determine the safety and efficacy of each plant before they can be recommended for medical use. Although many consumers believe that herbal medicines are safe because they are “natural”, herbal medicines may interact with synthetic drugs causing toxicity to the patient, may have contamination that is a safety consideration, and herbal medicines, without proven efficacy, may be used to replace medicines that have a proven efficacy.

The political issues around the safety of crude drugs vary from considering natural remedies “safe” regardless of potential dangers to considering them a dangerous unknown.

Ephedra has been known to have numerous side effects, including severe skin reactions, irritability, nervousness, dizziness, trembling, headache, insomnia, profuse perspiration, dehydration, itchy scalp and skin, vomiting, hyperthermia, irregular heartbeat, seizures, heart attack, stroke, or death.

Poisonous plants which have limited medicinal effects are often not sold in material doses in the United States or are available only to trained practitioners, these include:

- Aconite
- Arnica
- Belladonna
- Bryonia
- Datura
- Gelsemium
- Henbane
- Male Fern
- Phytolacca
- Podophyllum and
- Veratrum

Furthermore, herbs such as Lobelia, Ephedra and Eonymus that cause nausea, sweating, and vomiting, have been traditionally prized for this action.
Plants such as Comfrey and Petasites have specific toxicity due to hepatotoxic pyrrolizidine alkaloid content. There are other plant medicines which require caution or can interact with other medications, including St. John’s wort and grapefruit.

**Recommended Standards for Random Clinical Trials**

The Consolidated Standards of Reporting Trials (CONSORT) group came up with standards for random clinical trials (RCT) of herbs in 1996, revised in 2002. This statement comprises a 22-item checklist and flow diagram to guide authors, peer reviewers, editors, and readers on the essential information required in reports of two-group parallel RCTs of natural products. The CONSORT statement is endorsed by leading medical journals, editorial groups, professional societies, and funding bodies.

- The title and/or abstract should include the Latin binomial for the plant species from which the herbal medicine(s) originated, the part(s) of the plant used in the preparation, and the type of preparation (e.g., dried crude herb, ethanolic extract). The background should include a statement explaining the rationale for investigation of the specific herbal medicinal product and whether the indication for which it is being tested is new or is based on traditional use. Participant eligibility criteria in a trial testing a traditional indication (e.g., in traditional Chinese herbal medicine, a trial may test the effects of an herbal medicine intervention for liver chi (Qi) deficiency) should describe the theories and concepts underlying this indication.

- The description of the intervention must include the herbal medicinal product name, manufacturer, plant part used, type of preparation, source and authentication of the herbal material, pharmaceutical quality (e.g., herbal drug-to-extract ratio, type and concentration of the extraction solvent, quantity of known active constituents per unit dose), and dosage regimen and qualitative testing (purity). Also, reporting of the rationale for the control/placebo used in the trial is recommended. For studies involving herbal medicine practitioners as part of the intervention, details of practitioners (e.g., training, registration status) should be reported. Not all recommendations are relevant for all types of herbal medicine interventions. Therefore, we begin this section with the words “where applicable.” For example, a report of an RCT of an herbal medicinal product comprising crude herbal material (e.g., leaves, stems, root) prepared as a tea or decoction does not require reporting of the “type and concentration of solvent used and the plant to plant extract ratio.” In addition, herbal interventions made by the investigators specifically for the study will not have a finished product or extract name or manufacturer. For such products, all methods used in preparing and formulating the product must be reported. Similarly, allegiance is only relevant for studies in which the practitioner is a part of the intervention. In other studies, the practitioner may serve a more neutral role and thus their characteristics need not be reported. With these exceptions, all information outlined in these recommendations are suggested to be reported for all herbal medicine interventions.

- Also, outcome measures should reflect the intervention and indications tested while considering their underlying theories and concepts. For the results section, it is recommended that in addition to other baseline data, RCTs of herbal medicine interventions report any concomitant medication, herbal medicinal product, or other CAM use. It is recommended that when interpreting the results there be consideration of the specific herbal product and dosage regimen tested. This includes an overview of evidence on this particular herbal medicinal product. When considering generalizability, it is suggested that authors report how the product used in the trial generalizes to products used in self-care and/or in clinical practice.

**Studies Performed without Proper Identification of Assay of Plant Materials**

With the decline in pharmacognosy education in the United States, it has been common for herbal research to be done on plants that have not been botanically identified, which have not been assayed for strength and which do not allow for proper understanding of the herb named in the research. For instance, Eleutherococcus senticosus is frequently identified as “ginseng” although it is not part of the Panax genus and has significantly different medicinal characteristics. As Jonathan Treasure, NIMH, has written:

“Inaccurate spelling of herb names is not uncommon in medical
It has a short underground stem, from which dark-colored tapering roots descend. The crown or upper portion of the root gives rise to new plants. When touched to one’s lip, the juice of the aconite root produces a feeling of numbness and tingling. This plant is used as a food plant by some Lepidoptera species including Dot Moth, The Engrailed, Mouse Moth, Wormwood Pug, and Yellow-tail.

The roots of *Aconitum ferox* supply the Nepalese poison called *bikh*, *bish*, or *nabee*. It contains large quantities of the alkaloid *pseudaconitine*, which is a deadly poison. *Aconitum palmatum* yields another of the *bikh* poisons. The root of *Aconitum luridum*, of the Himalaya, is said to be as virulent as that of *A. ferox* or *A. napellus*.

Several species of *Aconitum* have been used as arrow poisons. The Minaro in Ladakh use *A. napellus* on their arrows to hunt ibex, while the Ainu in Japan used a species of *Aconitum* to hunt bear. The Chinese also used *Aconitum* poisons both for hunting, and for warfare.

Many species of *Aconitum* are cultivated in gardens, having either blue or yellow flowers. *Aconitum lycoctonum* (Alpine wolfsbane) is a yellow-flowered species common in the Alps of Switzerland. As garden plants the aconites are very ornamental, hardy perennial plants. They thrive in the garden soils, and will grow in the shade of trees. They are easily propagated by divisions of the root or by seeds; care should be taken not to leave pieces of the root where livestock might be poisoned.

**Traditional Uses**

*Aconite* has long been used in the traditional medicine of Asia (India, China). In Ayurveda the herb is used to increase *pitta* (bile) and to enhance penetration in small doses. However more frequently the herb is detoxified according to the samskaras process and studies, cited in the detoxification section below show that it no longer possesses active toxicity.

It is used in traditional Chinese medicine as a treatment for Yang deficiency, “coldness”, general debilitation. The herb is considered hot and toxic. It is prepared in extremely small doses. More frequently ginger processed aconite, of lower toxicity, “fu zi” is used.

*Aconite* is one ingredient of Tribhuvankirti, an Ayurvedic preparation for treating a “cold in the head” and fever. *Aconite* was mixed with patrinia and coix, in a famous treatment for appendicitis described in a formula from the Jingui Yaolue *Aconite* was also
in poisoning by veratrine and colchicum.

The action of aconitine on the circulation is due to an initial stimulation of the cardio-inhibitory centre in the medulla oblongata (at the root of the vagus nerves), and later to a directly toxic influence on the nerve-ganglia and muscular fibres of the heart itself. The fall in blood-pressure is not due to any direct influence on the vessels. The respiration becomes slower owing to a paralytic action on the respiratory centre and, in warm-blooded animals, death is due to this action, the respiration being arrested before the action of the heart.

Aconite further depresses the activity of all nerve-terminals, the sensory being affected before the motor. In small doses, it therefore tends to relieve pain, if this is present. The activity of the spinal cord is similarly depressed. The pupil is at first contracted, and afterwards dilated.

The cerebrum is totally unaffected by aconite, consciousness and the intelligence remaining normal to the last. The antipyretic action which considerable doses of aconite display is not specific but is the result of its influence on the circulation and respiration and of its slight diaphoretic action.

**Toxicology**

Marked symptoms appear quickly following the ingestion of a poisonous dose of aconite. The initial signs are gastrointestinal including nausea, vomiting, and diarrhea. There is followed by a sensation of burning, tingling, and numbness in the mouth and face, and of burning in the abdomen. In severe poisonings pronounced motor weakness occurs and cutaneous sensations of tingling and numbness spread to the limbs.

Cardiovascular features include hypotension, bradycardia, sinus tachycardia, and ventricular arrhythmias. Other features may include sweating, dizziness, difficulty in breathing, headache, and confusion. The main causes of death are ventricular arrhythmias and asystole. The only post-mortem signs are those of asphyxia.

The treatment of poisoning is mainly supportive. All patients require close monitoring of blood pressure and cardiac rhythm. Gastrointestinal decontamination with activated charcoal can be used if given within 1 hour of ingestion.

The major physiological antidote is atropine, which is used to treat bradycardia. Other drugs used for ventricular arrhythmia include lidocaine, amiodarone, bretylium, flecainide, procainamide,
and mexiletine. Cardiopulmonary bypass is used if symptoms are refractory to treatment with these drugs. Successful use of charcoal hemoperfusion has been claimed in patients with severe aconite poisoning.

Poisoning may also occur following picking the leaves without wearing gloves; the aconitine toxin is absorbed easily through the skin. From practical experience, the sap oozing from eleven picked leaves will cause cardiac symptoms for a couple of hours. In this event, there will be no gastrointestinal effects.

Tingling will start at the point of absorption and extend up the arm to the shoulder, after which the heart will start to be affected. The tingling will be followed by unpleasant numbness. Treatment is similar to poisoning caused by oral ingestion.

Aconitine is a potent neurotoxin that blocks tetrodotoxin-sensitive sodium channels. Pretreatment with barakol 10 mg/kg IV reduces the incidence of aconitine-induced ventricular fibrillation and ventricular tachycardia, as well as mortality. Five ìg/kg IV of tetrodotoxin has the same effect. The protective effects of barakol are probably due to the prevention of intracellular sodium ion accumulation.

Canadian actor Andre Noble died during a camping trip on July 30, 2004 after the accidental consumption of aconite from monkshood.

Aconite was reported by the *Sunday Mirror* to have been used as a poison in the murder of Pakistan cricket coach Bob Woolmer during the 2007 Cricket World Cup. However there is now evidence that Bob Woolmer was not murdered but died of natural causes.

**Detoxification**

Both Chinese medicine and Ayurveda have methods of processing aconite to reduce its toxicity.

In Chinese medicine, the traditional pao zhi or preparation of aconite is to steam it with ginger in a fairly elaborate procedure. Due to the variable levels of toxicity in any given sample of the dried herb, there are still issues with using it. Most but not all cases of aconite toxicity in Taiwan were due to the consumption of unprocessed aconite.

According to an article by the Indian scientists Thorat and Dahanukar, “Crude aconite is an extremely lethal substance. However, the science of Ayurveda looks upon aconite as a therapeutic entity. Crude aconite is always processed i.e. it undergoes ‘samskaras’ before being utilized in the Ayurvedic formulations.

This study was undertaken in mice, to ascertain whether ‘processed’ aconite is less toxic as compared to the crude or unprocessed one.

It was seen that crude aconite was significantly toxic to mice (100% mortality at a dose of 2.6 mg/mouse) whereas the fully processed aconite was absolutely non-toxic (no mortality at a dose even 8 times as high as that of crude aconite). Further, all the steps in the processing were essential for complete detoxification.”

**ATROPA BELLADONNA**

Atropa belladonna, commonly known as belladonna or deadly nightshade, is a perennial herbaceous plant in the family Solanaceae, native to Europe, North Africa, and Western Asia. The foliage and berries are extremely toxic, containing tropane alkaloids. These toxins include scopalamine and hyoscyamine which cause a bizarre delirium and hallucinations. The drug atropine is derived from the plant.

It has a long history of use as a medicine, cosmetic, and poison. Before the Middle Ages, it was used as an anesthetic for surgery, and it was used as a poison by early men, ancient Romans, including the wives of two Emperors, and by Macbeth of Scotland before he became a Scottish King.

The genus name "atropa" comes from Atropos, one of the three Fates in Greek mythology (the one who cuts the thread of life), and the name "atropa bella donna" is derived from an admonition in Italian and Greek meaning "do not betray a beautiful lady".

**Description**

Atropa belladonna is a branching herbaceous perennial, often growing as a subshrub, from a fleshy rootstock. Plants grow to 1.5 metres (4.9 ft) tall with 18 centimetres (7.1 in) long ovate leaves. The bell-shaped flowers are dull purple with green tinges and faintly scented.

The fruits are berries, which are green ripening to a shiny black, and approximately 1 centimetre (0.39 in) in diameter. The berries are sweet and are consumed by animals that disperse the seeds in their droppings, even though the seeds contain toxic alkaloids. There is a pale yellow flowering form called Atropa belladonna var. lutea with pale yellow fruit.
Atropa belladona is rarely used in gardens, but when grown it is usually for its large upright habit and showy berries. It is naturalized in parts of North America, where it is often found in shady, moist locations with limestone-rich soils. It is considered a weed species in parts of the world, where it colonizes areas with disturbed soils.

Germination of the small seeds is often difficult, due to hard seed coats that cause seed dormancy. Germination takes several weeks under alternating temperature conditions but can be sped up with the use of gibberellic acid. The seedlings need sterile soil to prevent damping off and resent root disturbance during transplanting.

**Naming and Taxonomy**

The first botanical description was by Linnaeus in *Species Plantarum* in 1753. It is in the nightshade family (*Solanaceae*), which it shares with potatoes, tomatoes, eggplants, jimsonweed, tobacco, wolfray, and chili peppers. The common names for this species include belladonna, deadly nightshade, divale, dwale, banewort, devil’s cherries, naughty man’s cherries, black cherry, devil’s herb, great morel, and dwayberry.

The name Atropa is thought to be derived from that of the Greek goddess Atropos, one of the three Greek fates or destinies who would determine the course of a man’s life by the weaving of threads that symbolized their birth, the events in their life and finally their death; with Atropos cutting these threads to mark the latter.

The name “belladonna” comes from the Italian language, meaning “beautiful lady”; originating either from its usage as cosmetic for the face, or, more probably, from its usage to increase the pupil size in ladies.

**Toxicity**

Belladonna is one of the most toxic plants found in the Western hemisphere. All parts of the plant contain tropane alkaloids. The berries pose the greatest danger to children because they look attractive and have a somewhat sweet taste.

The consumption of two to five berries by children and ten to twenty berries by adults can be lethal. The root of the plant is generally the most toxic part, though this can vary from one specimen to another. Ingestion of a single leaf of the plant can be fatal to an adult.

The active agents in Belladonna, atropine, hyoscine (scopolamine), and hyoscyamine, have anticholinergic properties. Atropa belladonna has been mistaken for blueberries and ingestion of six berries resulted in acute anticholinergic syndrome.

The symptoms of belladonna poisoning include dilated pupils, sensitivity to light, blurred vision, tachycardia, loss of balance, staggering, headache, rash, flushing, dry mouth and throat, slurred speech, urinary retention, constipation, confusion, hallucinations, delirium, and convulsions.

The plant’s deadly symptoms are caused by atropine’s disruption of the parasympathetic nervous system’s ability to regulate nonvolitional/subconscious activities such as sweating, breathing, and heart rate. The antidote for belladonna poisoning is physostigmine or pilocarpine, the same as for atropine.

*Atropa belladonna* is also toxic to many domestic animals, causing narcosis and paralysis. However, cattle and rabbits seem to eat the plant without suffering harmful effects. Its anticholinergic properties will cause in humans the disruption of cognitive capacities like memory and learning.

**Uses**

**Cosmetics**

The common name *belladonna* originates from its historic use by women - *Bella Donna* is Italian for beautiful lady. Drops prepared from the belladonna plant were used to dilate women’s pupils, an effect considered attractive. Today it is known that the atropine in belladonna acts as an antimus-carinic, blocking receptors in the muscles of the eye that constrict pupil size.

Belladonna is currently rarely used cosmetically, as it carries the adverse effects of causing minor visual distortions, inability to focus on near objects, and increased heart rate. Prolonged usage was reputed to cause blindness.

**Medicine**

There is currently insufficient scientific evidence to recommend the use of *A. belladonna* in its natural form for any condition, although some of its components, in particular *l*-atropine which was purified from belladonna in the 1830s, have accepted medical uses.

Donnatal is a prescription pharmaceutical, approved in the United States by the FDA, that combines natural belladonna alkaloids...
in a specific, fixed ratio with phenobarbital to provide peripheral anticholinergic/antispasmodic action and mild sedation.

According to its labeling, it is possibly effective for use as adjunctive therapy in the treatment of irritable bowel syndrome (irritable colon, spastic colon, mucous colitis) and acute enterocolitis.

**Traditional and Alternative Medicine**

*A. belladonna* has been used in traditional treatments for centuries for an assortment of conditions including headache, menstrual symptoms, peptic ulcer disease, histaminic reaction, inflammation, and motion sickness, with at least one 19th century eclectic medicine journal explaining how to prepare a Belladona tincture for direct administration to patients.

Homeopathic remedies prepared from the belladonna plant have been sold as treatments for various conditions, although there is no scientific evidence to support the efficacy of this use. Clinically and in research trials, the most common preparation is diluted to the 30C level in homeopathic notation.

This level of dilution contains no molecules of the original plant, although preparations with lesser dilutions which statistically contain trace amounts of the plant are advertised for sale.

**Recreational Drug**

*Atropa belladonna*, along with related plants such as jimson weed (*Datura stramonium*), have occasionally been used as a recreational drug because of the vivid hallucinations and delirium that it produces. These hallucinations are most commonly described as very unpleasant, however, and recreational use is considered extremely dangerous because of the high risk of unintentional fatal overdose. In addition, the central nervous system effects of atropine include memory disruption, which may lead to severe confusion.

**Poison**

It was used by early men in poisonous arrows. In Ancient Rome, it was used as a poison by Agrippina the Younger, wife of Emperor Claudius, and Livia, who is rumored to have used it to kill her husband Emperor Augustus.

Macbeth of Scotland, when he was still one of the lieutenants of King Duncan I of Scotland, used it during a truce to poison the troops of the invading Harold Harefoot, King of England, to the point that the English troops were unable to stand their ground and had to retreat to their ships.

**Folklore**

In the past, it was believed that witches used a mixture of belladonna, opium poppy, and other plants, typically poisonous (such as monkshood and poison hemlock) in flying ointment they applied to help them fly to gatherings with other witches.

Carlo Ginzburg and others have argued that flying ointments were preparations meant to encourage hallucinatory dreaming; a possible explanation for the inclusion of belladonna and opium poppy in flying ointments concerns the known antagonism between tropane alkaloids of belladonna (*specifically scopolamine*) and opiate alkaloids in the opium poppy, *Papaver somniferum* (*specifically morphine*), which produces a dream-like waking state.

This antagonism was known in folk medicine, discussed in eclectic (botanical) medicine formularies, and posited as the explanation of how flying ointments might have actually worked in contemporary writing on witchcraft.

The antagonism between opiates and tropanes is the original basis of the Twilight Sleep that was provided to Queen Victoria to deaden pain as well as consciousness during childbirth, and which was later modified so that isolated alkaloids were used instead of plant materials. The belladonna herb was also notable for its unpredictable effects from toxicity.

**DRYOPTERIS FILIX-MAS**

*Dryopteris filix-mas* (Common Male Fern or Male Fern) is one of the most common ferns of the temperate Northern Hemisphere, occurring throughout much of Europe, Asia, and North America.

It favours damp shaded areas and is common in the understory of woodlands, but is also found in shady places on hedge-banks, rocks, and scree in. It is much less abundant in North America than in Europe. The half-evergreen leaves have an upright habit and reach a maximum length of 1.5 m, with a single crown on each rootstock. The bipinnate leaves consist of 20-35 pinnae on each side of the rachis. The leaves taper at both ends, with the basal pinnae about half the length of the middle pinnae. The pinules are rather blunt and equally lobed all around. The stalks are covered with orange-brown scales. On the abaxial surface of the mature blade 5 to 6 sori develop
in two rows. When the spores ripen in August to November, the indusium starts to shrivel, leading to the release of the spores. This species hybridises easily with *Dryopteris affinis* (Scaly Male Fern) and *Dryopteris oreades* (Mountain Male Fern).

### Cultivation and Uses

The root was used, until recent times, as an anthelmintic to expel tapeworms. It is sometimes referred to in ancient literature as Worm Fern. It is also grown as an ornamental fern in gardens.

**Pokeweed**

The pokeweeds, also known as poke, pokebush, pokeberry, pokeroat, polk salad, polk salat, inkberry or ombú, comprise the genus *Phytolacca*, perennial plants native to North America, South America, East Asia and New Zealand.

Pokeweed contains *phytolaccatoxin* and *phytolaccigenin*, which are poisonous to mammals. However, the berries are eaten by birds, which are not affected by the toxin because the small seeds with very hard outer shells remain intact in the digestive system and are eliminated whole.

Pokeweeds are herbs growing from 1 to 10 ft. tall, although specimens as tall as 14 ft. have been observed. They have single alternate leaves, pointed at the end, with crinkled edges. The stems are often pink or red. The flowers are greenish-white, in long clusters at the ends of the stems. They develop into dark purple berries.

*Phytolacca dioica*, the ombú, grows as a tree on the pampas of South America and is one of the few providers of shade on the open grassland. It is a symbol of Argentina and gaucho culture.

### Uses

All parts of pokeweed are toxic including the raw aboveground leaves sprouting in the early spring. The poisonous principles are found in highest concentrations in the rootstock, less in the mature leaves and stems, and least in the fruits.

Young leaves, if collected before acquiring a red colour, are edible if boiled for 5 minutes, rinsed, and reboiled. Berries are toxic when raw but cooked juice is edible (the seeds remain toxic after cooking).

Young pokeweed leaves can be boiled three times to reduce the toxin, discarding the water after each boiling. The result is known as *poke salit*, or *poke salad*, and is occasionally available commercially.

Many authorities advise against eating pokeweed even after thrice boiling, as traces of the toxin may still remain. It should never be eaten uncooked.

For many decades, poke salad has been a staple of southern U.S. cuisine, despite campaigns by doctors who believed pokeweed remained toxic even after being boiled. The lingering cultural significance of Poke salad can be found in the 1969 hit song “Polk Salad Annie,” written and performed by Tony Joe White, and famously covered by Elvis Presley, as well as other bands including the El Orbits of Houston, Texas.

Pokeberry juice is added to other juices for jelly by those who believe it can relieve the pain of arthritis. There are pokeweed festivals held annually in Blanchard, Louisiana; Gainesboro, TN; Harlan, KY; and, Arab, Alabama.

Since pioneer times, pokeweed has been used as a folk remedy to treat many ailments. It can be applied topically or taken internally. Topical treatments have been used for acne and other ailments. Internal treatments include tonsilitis, swollen glands and weight loss.

Grated pokeroat was used by Native Americans as a poultice to treat inflammations and rashes of the breast. Independent researchers are investigating *phytolacca*’s use in treating AIDS and cancer patients. Especially to those who have not been properly trained in its use, pokeweed should be considered dangerous and possibly deadly.

Ingestion of poisonous parts of the plant may cause severe stomach cramping, nausea with persistent diarrhea and vomiting, slow and difficult breathing, weakness, spasms, hypotension, severe convulsions, and death.

However, consuming fewer than 10 uncooked berries is generally harmless to adults. Several investigators have reported deaths in children following the ingestion of uncooked berries or pokeweed juice. Severe poisonings have been reported in adults who ingested mature pokeweed leaves and following the ingestion of tea brewed from one-half teaspoonful of powdered pokeroat.

Pokeweed berries yield a red ink or dye, which was once used by aboriginal Americans to decorate their horses. The United States Declaration of Independence was written in fermented pokeberry juice (hence the common name ‘inkberry’).

Many letters written home during the American Civil War were
written in pokeberry ink; the writing in these surviving letters appears brown. The red juice has also been used to symbolize blood, as in the anti-slavery protest of Benjamin Lay. A rich brown dye can be made by soaking fabrics in fermenting berries in a hollowed-out pumpkin.

Some pokeweeds are also grown as ornamental plants, mainly for their attractive berries; a number of cultivars have been selected for larger fruit panicles. Pokeweeds are used as food plants by the larvae of some Lepidoptera species including Giant Leopard Moth.

**Toxic Principle**
Toxic constituents have been identified including the alkaloid phytolaccine (and the alkaloid phytolaccotoxin), as well as a glycoprotein.

**Clinical Signs**
- **In humans:** The eating of limited quantities of poke, perhaps of the shoots, may cause retching or vomiting after two hours or more. These signs may be followed by dyspnea, perspiration, spasms, severe purging, prostration, tremors, watery diarrhea and vomiting (sometimes bloody) and, sometimes, convulsions. In severe poisonings, symptoms are weakness, excessive yawning, slowed breathing, fast heartbeat, dizziness, and possibly seizures, coma and death.
- **In horses:** Colic, diarrhea, respiratory failure.
- **In swine:** Unsteadiness, inability to rise, retching. Jerking movements of the legs. Below-normal temperature.
- **In cattle:** Same general signs plus a decrease in milk production.

**VERATRUM**

*Veratrum* is a genus of coarse, highly poisonous perennial herbs of the Melanthiaceae family. In English they are usually known as the False hellebores. Members of *Veratrum* are known both in western herbalism and traditional Chinese medicine as toxic herbs to be used with great caution.

It is one of the medicinals (“Li lu”) cited in Chinese herbal texts as incompatible with many other common herbs because of its potentiating effects. Especially, many root (and root-shaped) herbs, particularly ginseng, san qi, and hai seng, will create and or exacerbate a toxic effect. *Veratrum* species are used as food plants by the larvae of some Lepidoptera species including Sataceous Hebrew Character.

Birth defects in livestock grazing on *Veratrum californicum* (native to the western United States) led to the study of cyclopamine and jervine which are important in animal developmental biology including cancer treatment.

**Distribution**

*Veratrum* is found in many areas of the temperate Northern Hemisphere. Widely distributed in montane habitats throughout North America. *Veratrum* species prefer full sunlight and deep, wet soils, and are common in wet mountain meadows, swamps, and near streambanks.

*Veratrum* species occur from Alaska south into the mountains of California, and are widely distributed throughout the Rocky Mountains. *Veratrum* is very abundant in Eastern North America from Quebec southward through the Appalachians into North Carolina. Thirteen species are found in China.

**Toxicity**

*Veratrum* species contain highly toxic steroidal alkaloids (e.g. veratridine), that activate sodium ion channels and cause rapid cardiac failure and death if ingested. All parts of the plant are poisonous, with the root and rhizomes being the most poisonous parts of the plant. Following ingestion symptoms typically occur between 30 minutes and 4 hours after ingestion.

Symptoms include nausea and vomiting, abdominal pain, numbness, headache, sweating, muscle weakness, bradycardia, hypotension, cardiac arrhythmia, and seizures. Treatment for poisoning includes gastrointestinal decontamination with activated charcoal followed by supportive care including antiemetics for persistent nausea and vomiting, along with atropine for treatment of bradycardia and fluid replacement and vasopressors for the treatment of hypotension.

**Uses**

Native Americans used the juice pressed from the roots of this plant to poison arrows before combat. The dried powdered root of this plant was also used as an insecticide. Western American Indian
tribes have a long history of the use of this plant as an effective treatment for cancerous tumors, and combined minute amounts of the winter-harvested root of this plant with *Salvia dorii* to potentiate its effects and reduce the toxicity of the herb. The plants teratogenic properties and ability to induce severe birth defects were well known to Native Americans. *Veratrum* species produce highly toxic steroidal alkaloids only when the plants are in active growth. During the winter months, when the plant enters its dormant stage, it degrades and metabolizes most of its toxic alkaloids.

Herbalists and Native Americans who used this plant for medicinal purposes harvested the roots during the winter months when the levels of toxic constituents were at their lowest. The roots of *V. nigrum* and *V. schindleri* have been used in Chinese herbalism (where plants of this genus are known as “li lu”. Li lu is used internally as a powerful emetic of last resort, and topically to kill external parasites, treat tinea and scabies, and stop itching. Some herbalists refuse to prescribe li lu internally, citing the extreme difficulty in preparing a safe and effective dosage, and that death has occurred at a dosage of 0.6 grams.During the 1930s *Veratrum* extracts were investigated in the treatment of high blood pressure in humans. Patients treated often suffered side effects due to the narrow therapeutic index of these products.

Due to their toxicity and the availability of other less toxic drugs, use of *Veratrum* as a treatment for high blood pressure in humans was discontinued. *Veratrum* species are an important source of life-saving medications used in modern medical preparations which lower blood pressure, slow the heartbeat, and are used for cancer treatment.

Medicine Gene Therapy

The advancement made in medicine and genetics has changed the entire scenario of treatment processes in patients. For many years, the study of medical genetics was confined to determine whether a particular human disease had a genetic basis or not. Once the genetic nature of a disease was established, it was sometimes possible to devise a diagnostic test to identify the disease in newborns or prenatal conditions.

Biomedical researchers are currently redefining human geography. These modern explorers are elaborating a new human map, based on genes, that is likely to alter our views of the world—and our place in it—even more profoundly than did the maps generated by Columbus and other fifteenth and sixteenth-century explorers in the history. This has made a modern generation of therapeutic agents targeting the genetic diseases.

The human genetic maps are likely to alter our perceptions of self and other, of normality and abnormality, particularly in the area of procreation. This can be seen most clearly in the consideration of the impacts of prenatal genetic testing. Thanks to the researchers involved in work on the Human Genome Project and the media reports it has attracted over the past several years, the public has received a steady stream of information on the latest gene to be discovered by science. Such announcements are generally greeted with enthusiasm, and the hope that genetic solutions will save us from our human frailties. But, this hope may be overly optimistic and ambitious.

While genetic technologies may offer a few more keys to unlock
of genetic disorder is associated with liver and with haemopoietic organs. Genetic defect in many instances results into stillbirth or neonatal death. Those who survive with genetic diseases frequently have significant physical, mental, developmental or social problems. The researchers have long been imagined to curing heritable diseases by introducing the external healthy genes into the patients. The recent advances made in recombinant DNA technology and medicinal fields have made possible the isolation of defective genes containing cells and reintroduction of these cells into the patient again. The recombinant DNA technology has led to an amazing extension of the science of animal biotechnology. Through these advanced techniques, genes and genomes of a wide range of different organisms from microorganisms to animals are being manipulated for the benefit of mankind. The key technique in genetic engineering is gene transfer which has revolutionized the approaches of genetic analysis. It has enabled the isolation and cloning of genes from any organisms and introduces the same to virtually any living cell that may be prokaryotic or eukaryotic in nature. The ability to recombine and manipulate genes has made once impossible notion into feasible practice and lot of attention is focused in medicinal genetic engineering.

Hence, at present the treatment of genetic diseases by the introduction of gene or genes into the affected cells to provide gene functions that are deficient because of genetic or acquired deficiency is under focus of the medicine. Large numbers of gene therapy therapeutic agents are under clinical trials and still many more are on the way of development. The day will also come to practice of using the genes as prescribed drugs in the medical care.

DEFINITION OF GENE THERAPY

Gene therapy is a novel approach to treat, cure or ultimately prevent disease by changing the expression of a person’s gene. Or the treatment of any disease by the introduction of genes into affected cells to provide gene functions that are deficient because of genetic or acquired deficiency is known as gene therapy. Gene therapy is in its infancy, and current gene therapy is primarily experimental, with most human clinical trials only in research stages. The development of a therapeutic agent for humans usually passes through four clearly defined levels of study and testing before it can be sanctioned for use in regular practice.
This makes sure that any therapeutic agents should not show any harmful effects or side effects on the host or receiving organisms.

• Preclinical trials: Animal testing of the therapeutic agent to determine a rationale for therapeutic use, toxicity’s, dose details, and risks involved.
• Phase I: A small human trial generally involving dose escalation and focusing on safety and pharmacology of therapeutic agent.
• Phase II: An expanded human trial with the focus on efficacy in a particular patient population.
• Phase III: A large human trial intended to establish a definitive role for a drug or biologic.

The same things should also be followed by the gene therapy technology. Even though, there is no new or synthetic organics present in the gene therapy, the cells used for the process may create unexpected problems.

Current practices of symptomatic treatment for genetic disorders are:

• Replacement therapy for missing factor. Eg: coagulation factor for hemophilia.
• Long term blood transfusion for thalassaemia.
• Replacement with immunoglobulins for children with congenital hypogammaglobulinemia.
• Hormone therapy for certain diseases. Eg: Growth hormone for dwarfism, insulin injection for diabetics.
• Replacement of missing enzymes. Eg: Gauchers disease, ADA deficiency.
• Diet control prevents accumulation of toxic metabolites. Eg: Phenylketonuria, galactosemia etc.
• Bone marrow transplantation to correct blood disorders. Eg: SCID viii. Cofactor responsive metabolic disorders. Eg: methylmalonic acidemia, homocystinuria colatamine, multiple acyl-coA dehydrogen, deficiency-riboflavin etc.

Symptomatic disease treatments show very low rate of success. It is completely successful in eight diseases (12% of total), moderately successful in 14% of the total and not cured in rest. Majority of the genetic diseases are not treated by regular conventional therapeutic agents such as synthetic organic drugs or naturally isolated supplements. This makes the treatment process as only a short term solution or cure. This treatment can also cause a large number of side effects in an accumulative manner. And this will not be able to provide the complete curing effect and makes the patient to be under unrepairable damage to the various organs of the body.

Disadvantages of the Above Therapy Processes

• High cost is involved in the method of practice.
• Need of continuous treatment throughout the life.
• Danger of transmission of AIDS virus and other pathogenic agents during blood transfusion.
• High iron contents after repeated blood transfusions in the transgenic patients has side effects. Hence, at present lot of emphasis is given to find out a better and permanent solution for the genetic disorders that are found to be effective in all respect. For this purpose the gene therapy is found out to be the most effective therapeutic method.

Historical Highlights of the Evolution of Gene Therapy

Gene therapy can be described as the introduction of a fully functional and expressible gene into a target cell. This results in permanent curing of the specific genetic disease.

The evolution of gene therapy as a new method is followed the different phases as listed below:

• Phase I: Emergence and acceptance of the concept of gene event. Period: 1866-1953
• Phase III: Clinical development event. Period: 1975 onwards

On 14/9/1990 after exhaustive reviews by various regulatory panels, the first human gene therapy with severe combined immunodeficiency (SCID), which lacks enzyme adenosine deaminase was done. In this case cloned gene was introduced into lymphocytes (a type of white blood cell) that had been removed from the patient. After culturing the lymphocytes that were not having adenosine deaminase were transfused with the normal gene segment. This cell thus produced the adenosine deaminase enzyme in all the transfused cells. These cells were later returned or transplanted in to the patient.
Severe combined immunodeficiency (SCID), is an inherited genetic disease and in such individuals the blood lymphocytes lack enzyme adenosine deaminase (ADA). This is the key enzyme in purine nucleoside metabolism of the lymphocytes and thus could not synthesize the purines. In the absence of active enzyme adenosine deaminase (ADA), deoxyadenosine is phosphorylated to yield high levels of dATPs that are 50-fold greater than normal levels.

This high concentration of dATP inhibits ribonucleotide reductase and thus preventing the synthesis of the other deoxynucleoside triphosphate (dNTPs). This choking off DNA synthesis and thus cell proliferation of the lymphocytes. Thus the lymphocytes concentrations will become lesser and lesser. This condition leads to the increased fatal infections by the potential pathogens.

Since lymphocytes mediate much of the immune response and fatal infections cannot be avoided due to the lack of the fighting lymphocytes special care must be taken and isolated (protective measures should be taken) to avoid all types of potentially fatal infections.

The mutations in all eight known ADA variants obtained from SCID patients appear to structurally perturb the active site of adenosine deaminase. As mentioned earlier first breakthrough in gene therapy was carried out at the National Institute of Health by three medical professionals namely Dr. W. French Anderson, Dr. Micheal Blaese, and Dr. Kenneth Culver in 1990, September.

They treated a four year-old patient Miss. Ashanthi Desilva in the hospital. They took T lymphocytes and transfected with the normal adenosine deaminase gene. Four months later another patient eleven year old Cynthia Cutshall also underwent the similar treatment. Both the patients had been injected with 11 to 12 gene therapy processes. This process effectively treated the disease because even after three years their T lymphocytes showed positive for adenosine deaminase normal gene.

FOUR MAJOR APPROACHES TO GENE THERAPY

In gene therapy different methods are available to correct the defective version of the gene in an individual depending upon how exactly the changes have been made. By using gene therapy approaches large number of diseases are considered as potential source for treatment. There are four major approaches that can be made to obtain the proper function of the gene in the human and they are gene addition, gene replacement, gene switching and gene regulation.

Gene Addition

This approach involves addition of a normal gene capable of expressing a normal gene product thus restoring a genetically defective function. The pre-existing mutant (errant) gene is not altered or changed and continue to be present in the system.

Gene Replacement

In this method mutant gene sequence is altered that has an abnormal gene product. Here the specific excision or repair of a mutant gene is done and transduction by normal gene sequence is carried out. The pre-existing abnormal (errant) gene is no longer present in the cells that are used for the transplantation.

Gene Switching

In this type of gene therapy the establishment of alternative pathways to circumvent mutant gene function is carried out.

Gene Regulation or Alteration

By this method of gene therapy altering the regulation of normal or mutant gene is carried out. Here the repression or activation of the normal or mutant gene will be done.

TYPES OF GENE THERAPY

In human body mainly two types of cells are present. They are somatic cells and germ cells. These cells are used for correcting the diseased genes to function as normal cells. Based on the types of cells used in the therapy processes it can be two types in humans and they are as follow:

1. Germ line gene therapy
2. Somatic gene therapy

Germ Line Gene Therapy

The term indicates the introduction of transgene or transgenic cells into the germ line as well as into the somatic cell population in the next generation. This therapy should have ability to cure patient
but some gametes also carry corrected version of the gene for proper phenotype. Thus, the entire future generation gets cured from the genetic disease. The protocol is relevant for application in humans in the removal of an early embryo with a defective genotype from a pregnant woman and injection with transgenic cells containing the wild type gene.

These cells become part of many tissues of the body, often including the germ line, which will give rise to the gonads. This therapy is more ethically problematic. This approach alters both the somatic cell and germ cell lines of the individual and ensures that the genetic alterations will be passed onto the individual offspring. The ethical issues involved in this approach to gene therapy are currently prohibitive but provide a lively area for debate and discussion about the future of gene therapy. No human germ-line gene therapy has been performed to date.

**Somatic Gene Therapy**

The aim of the therapy is only to focus on the body (soma) or vegetative cells. This is intended to alter the individual genetic constitution without altering the genetic makeup of the children of that individual. Presently, it is impossible to render an entire body transgenic organism. In such cases it is likely that not all the cells of that tissue need to become transgenic that can ameliorate the overall disease symptoms. The method proceeds by removing some cells from a patient with a defective genotype and making these cells transgenic through the introduction of copies of the cloned wild type gene. The transgenic cells are then reintroduced into the patient’s body which in turn providing normal function. In addition, various strategies for implementing somatic cell gene therapy are beginning to emerge and can be grouped under the broad categories of ex-vivo gene therapy, in-vivo gene therapy and antisense therapy. The gene therapy can be done at two different levels of individual.

The first one is embryo gene therapy in this method genetic constitution of embryo is altered after zygote is formed. The second method is patient therapy, in this healthy gene is introduced into affected person tissues. The healthy gene overcomes the defect without affecting the inheritance. The difference between the germ line therapy and somatic gene therapy are listed.

**SOMATIC GENE THERAPY**

With the discovery of the molecular basis of DNA transformation in bacteria in which a gene from one strain can be transferred to and expressed in another strain, human genetic diseases might be cured by introduction of normal gene into the appropriate somatic cell type. During the 1980s, the prospects of human somatic gene therapy techniques were developed, eukaryotic expression vectors were generated. With this transgenic experiments with mice became routine practice. With this transgenic experiments with mice became routine practice.
The death of cells invariably takes place by a process called apoptosis. This is a programmed physiological process by which cells commit suicide in response to damage or the presence or absence of specific environmental stimuli, for example, depletion of nutrients. The Birmingham scientists have already characterised several stresses that trigger apoptosis in large-scale bioreactors.

We have also shown that ectopic expression of the anti-apoptotic cellular protein known as Bcl-2 inhibits apoptosis in NS0 myeloma, CHO and hybridoma cell lines. Bcl-2 can promote survival at very low dilution rate in continuous culture. In general, the group has found that by introducing anti-apoptotic genes into cells they can increase the robustness and survival of cells in culture. This shows that we can enhance the survival and productivity of cell lines by manipulating their “death pathway.”

This opens up interesting new directions in biochemical engineering. It was also possible to control cell proliferation by using our knowledge of how cell growth and the cell division cycle are regulated by an extended family of enzymes called cyclin-dependent kinases, or cdks.

The activity of cdks, and progress through the cell cycle, depends not only on the availability of cyclin regulatory subunits but also the levels of specific cdk inhibitory proteins (ckis). Two members of the KIP family of ckis, p21CIP1 and p27KIP1, inhibit the activity of the G1-phase cyclin E dependent kinase cdk2, and are normally involved in causing cell cycle arrest, for example in cells containing damaged DNA or in the presence of TGF-beta.

We have introduced an inducible copy of the p21CIP1 gene into cell lines to produce anchorage-independent, proliferation-controlled cell lines, which arrest in G1-phase of the cell cycle with enhanced secreted antibody production. The improved productivity is independent of the inducible promoter used to achieve this cytostasis (the non-dividing state).

We have extended the survival period of these cells by constitutively over-expressing Bcl-2 protein. This has inhibited apoptosis while maintaining the high productivity associated with cytostasis. We are now carrying out experiments to understand how cytostatic arrest increases productivity. We are employing genomics and metabolomics techniques to identify the genes that are involved in antibody synthesis and cell size; two main processes associated with the over-expression.