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Drug Actions

Lecture Notes

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McGill

McGill Drug Action Lectures (PHAR 300)

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Foreword

Principles of pharmacology and toxicology. Frequently encountered drugs will be used as a focus to illustrate sites and mechanisms of action, distribution, metabolism, elimination and adverse side effects. This note includes all the lectures for fall 2024.

This note includes a few terminologies in order to structurize the material. The following is the brief description of each terms:

1. Definition: An explanation on the meaning of a word
2. Observation: A further analysis of a word or idea through observing.
3. Method: Description of an experiment or usage of an idea in an experiment.
4. Concept: An important idea that's obtained through scientific testing
5. Notion: A less important idea (that could be realized after a concept)
6. Explanation: A more detailed analysis on a concept or notion
7. Example: A specific case used to demonstrate a general concept/notion
8. Mechanism of Action: A large stepwise explanation.

Prerequisites: BIOL 200, BIOL 201, PHGY 209 and 210.

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Fundamentals of Pharmacology

Chapter

Remark 1.1. In lecture 1, we began with only talking a bit about the usage and history of pharmacology so notes of it will not be included.

1.1 Pharmacodynamics

There are many ways to name a drugs, however there's only a few convention that's widely know:

Definition 1.1. **Chemical name** of a drug refers to the name that documents the chemical formula and molecular structure of said drug. There's only 1 chemical name for every drug

Definition 1.2. The **generic name** of a drug is a universal name assigned by the United States Adopted Name (USAN) Council and by WHO international Nonproprietary Names (INN) program. There's only 1 generic name for every drug

Definition 1.3. The **trade (brand) name** of a drug is a registered trademark, use is restricted to the owner of the patent, usually the manufacturer. There are various trade name for every drug.

Remark 1.2. *In this class, we'll be only using generic name!*

Example 1.1.1. One of the drug use to reduce pain and swelling has the trade name of **aspirin** which has generic name of **acetylsalicylic acid** and chemical names: $C_9H_8O_4$.

The reason we use generic name is because, firstly, it's universally recognized and second, there's a basic rule in giving generic name to a drug depending on what the drug is or acting on etc.

Example 1.1.2. Generic names of drugs that ends (suffix) with **-vir** are typically antiviral. Suffix like **-mab** indicates the drug is a monoclonal antibody or **-clone** for hypnotic and tranquilizer.

Definition 1.4. **Pharmacology** is the study of how drug act on a biological system. The words stems from the Greek word *pharmakon* (means "drug") and the suffix *-ology* (means "the study of") so together it's "the study of drug".

Definition 1.5. **Therapeutics** is a term to describe how you use a drugs clinically. This encompasses 2 concepts: **indications** (what you'd use the drug for) and **contraindications** (what you'd not use the drug for).

Definition 1.6. **Pharmacokinetics** is the study of what your body does to the drugs. This encompasses 4 concepts: absorption, distribution, metabolism and elimination.¹

Definition 1.7. **Pharmacodynamics** is the study of what a drug does to the body i.e. the biologica' effects of a drug, how and where it acts.

1.1.1 General Concept of Pharmacodynamics

We'll begin with pharmacodynamics. We can look at drugs effect on different level of the body e.g. what a drug do to your organ system, your tissues, your cells and even subcellular targets.

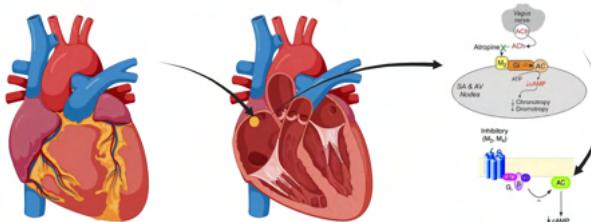


Figure 1.1: drug effect seen at different level of the body.

Example 1.1.3. A particular drug can have effects on the cardiovascular system; to be more specific, the site of action will be on the SA node. Focusing more in, we can see the specific SA and AV nodal cells the drug acts on. Finally, we can see the subcellular target of the drug to be on this GPCR (a receptor). See Figure 1.1.

¹One way to remember this is the abbreviation: ADME.

Notion 1.1 Drug design can be highly specific or even have very low specificity

Example 1.1.4. **Antiviral drugs** can bind specifically to an active site of a virus while leaving everything else in the body alone. Contrarily, **analgesic drugs** are less specific and thus can have multiple targets however, **they will have more side effects.**

Concept 1.1 *There are many ways of treating a disease i.e. Drugs that target different pathways could ultimately lead to a disease treatment.*

Example 1.1.5. The family of **hypertensive drugs** includes different categories drugs whose goal is to decrease blood pressure however they can be differ in its mechanism of action. See Figure 1.2.

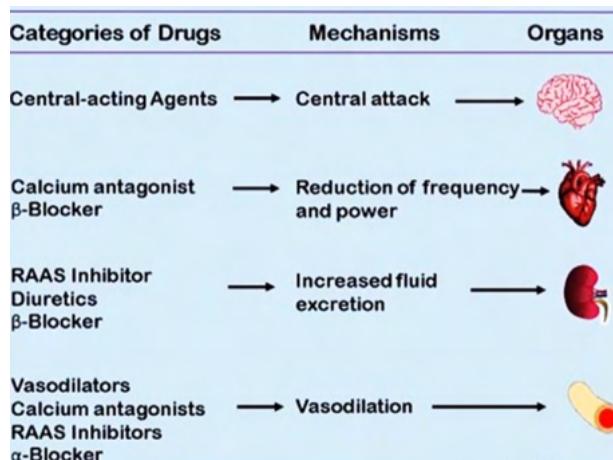


Figure 1.2: Categories of antihypertensive drugs.

Notion 1.2 Like specificity, a drug can either be highly selective or generalized

Example 1.1.6. ^{125}I odine intravenous injection are selective to only the thyroid while epinephrine intravenous injection have generalized targets like blood vessels, heart and kidneys.

Now that we've done looking at the general concept of pharmacodynamics, we'll look at it at the molecular level. When looking at drugs' actions, we're mainly looking at the receptor site that they act on.

1.1.2 Receptor-Based Drugs

Definition 1.8. A **drug receptor** is a protein target that can be activated by an endogenous/exogenous **ligand** which will ultimately yield a cellular response.

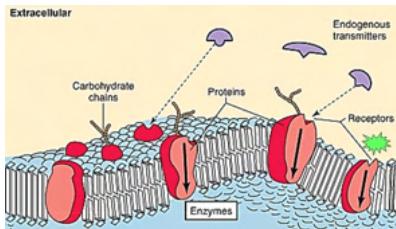


Figure 1.3: Illustration of drug receptors sitting on the cell membrane. These receptors are being targeted by endogenous ligands (purple shape) and can be identified and isolated by scientists using radioligand binding (green shape).

There are different categories of drug receptors. Majority is on the plasma membrane, called **membrane receptors**, while the rest few are located inside the cell, called **intracellular receptors**.

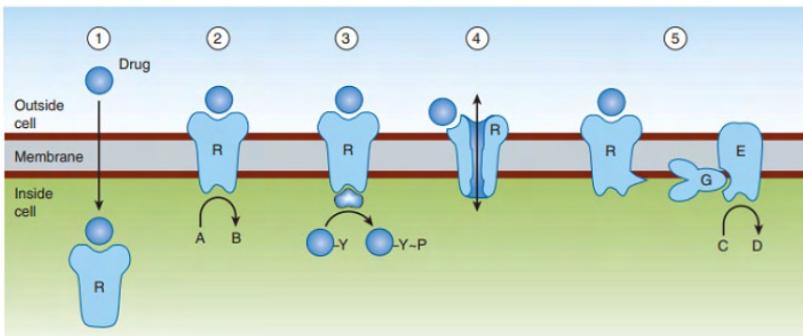


Figure 1.4: Receptors categories: intracellular (1) and membrane (2–5).

Membrane receptors can also further subcategorize into: **ion channels**, **tyrosine kinase**, **G-protein linked**, **enzymes-linked**, etc.

The purpose of having these receptors is to receive chemical signals for either cell-cell communication or just normal cell function. These chem-

ical signals have different classes depending on the way the signal is convey.

Definition 1.9. When a cell releases a ligand to act on itself the signalling is called **autocrine**. When the signal is release from a neighbouring cells through gap junction it's called **juxtacrine** signalling. When signalling is slightly further (not through gap junction) but still nearby, it's called **paracrine** signalling. Lastly, if distant cells signal via the blood stream it's called **endocrine** signalling.

Ion Channels

Definition 1.10. An **ion channel** is a transmembrane-spanning proteins that open to allow passage of specific ions.

Observation 1.1 Ion channels span the entire cell membrane. They consist of 3 states: open (allow ion to enter), closed (prevent ion from enter) and inactivated. They can be either voltage- (open at specific potential) or ligand-gated (open when a specific ligand binds).

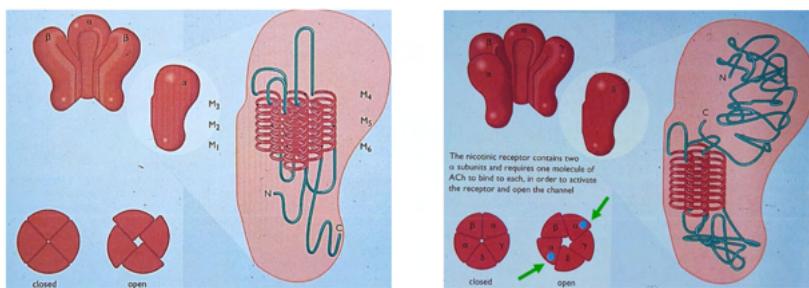


Figure 1.5: 4 units voltage-gated Ca^{2+} channel (left) and 5 units ligand-gated ion channel [nicotinic receptor] (right).

Remark 1.3. *The nicotinic receptor in figure 1.5, actually has lots of binding site which can either be binding site for the specific ligand or even allosteric site to modulate it.*

G-Protein Coupled Receptors (GPCRs)

Definition 1.11. **G-protein coupled receptors (GPCRs)** is a large class of surface receptors with an intracellular component and transmembrane com-

ponents.²

Observation 1.2 Upon looking at the structure of GPCRs, we can see that it has 7 transmembrane subunits whose extracellular surface allow ligands to bind; while, intracellularly, we find attaching G-protein, which are made up of the α , β and γ units. Upon activation GDP is replaced by GTP on the α units, of which will migrate and activate an effector protein which then induce a cellular response.

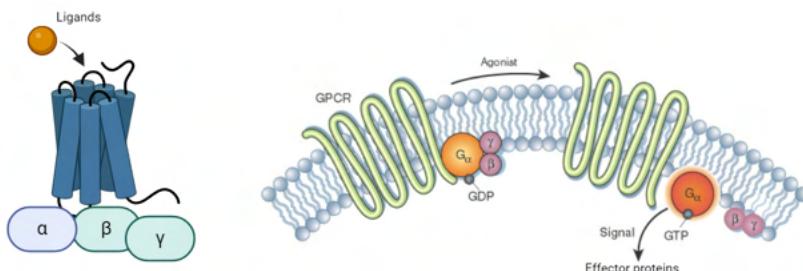


Figure 1.6: GPCRs structure (right) and mechanism (left).

Example 1.1.7. Epinephrine can bind to the binding site of a GPCRs thus activating it. With this activation, a second messenger chemical is produced called **cyclic AMP (cAMP)** by adenylyl cyclase that can modulate glucose release.

Remark 1.4. GPCRs are not only stimulatory but can also have inhibitory effects of the same pathway i.e. multiple receptors can modulate the same pathway.

Example 1.1.8. For the production of cAMP, you can have GPCRs that stimulate it while others inhibit it. The net effect (which depends on cAMP level) will be depending on if the stimulation is larger or smaller than the inhibition.

Another important aspect of GPCRs is that it can couple together to form either *hetero-* or *homo-oligomers* GPCRs (see Figure 1.8) which will have different purposes (we'll look at it later on in the course).

²The reason that this is a large class because we can have different combinations of GPCRs components to produce different receptors. So far, we've discovered > 800.

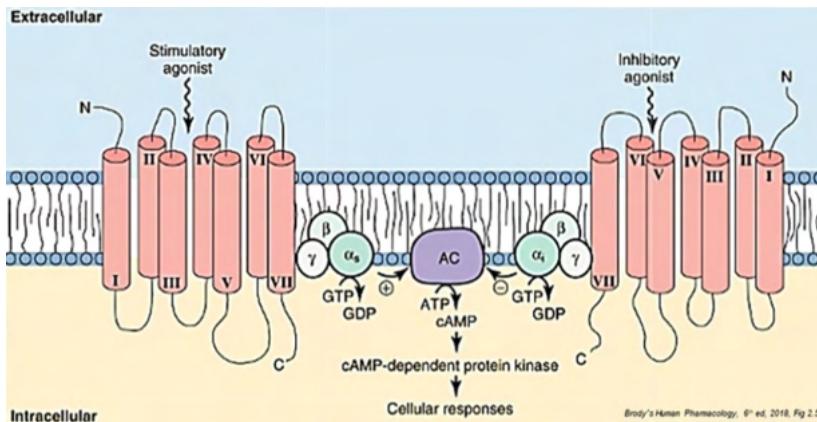


Figure 1.7: GPCRs modulation effect.

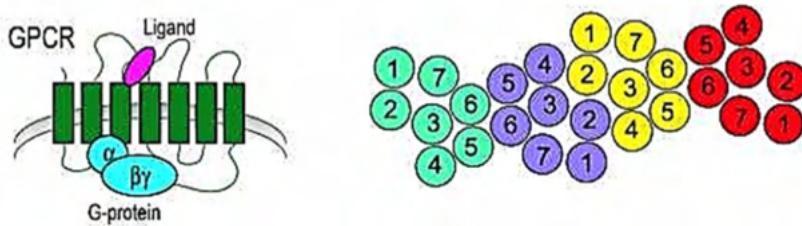


Figure 1.8: GPCRs coupling

Enzyme-Linked Receptors

Definition 1.12. **Enzyme-linked receptors** are receptors whose upon activation with will lead an activation of an enzyme.

Some of the important receptors in this class include: **tyrosine kinase receptors** (TKR), **cytokine receptors** and **natriuretic peptide receptors** (NPR). Though they're different receptors, they share similar structure and activations.

Observation 1.3 When a ligand binds to each monomer of the receptor, the 2 halves will come together and dimerize into 1 unit. This unit will have its intracellular subunit phosphorylated which then activate proteins and enzymes to carry out a cellular response. (See Figure 1.9)

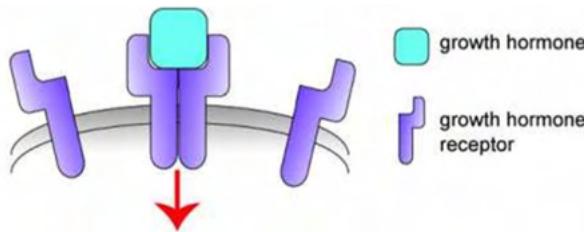


Figure 1.9: Enzyme-linked receptors activation.

Example 1.1.9. When TKR dimerize, its tyrosine will be phosphorylated to activate proteins for cellular response. When cytokine receptors dimerizes, its *Janus Kinase (JAK)* and *signal transducers & activators of transcription (STAT)* will be phosphorylated and carry out transcription. When NPsRs dimerize, an enzyme is activated to make cGMP which can control many cellular processes. See Figure 1.10.

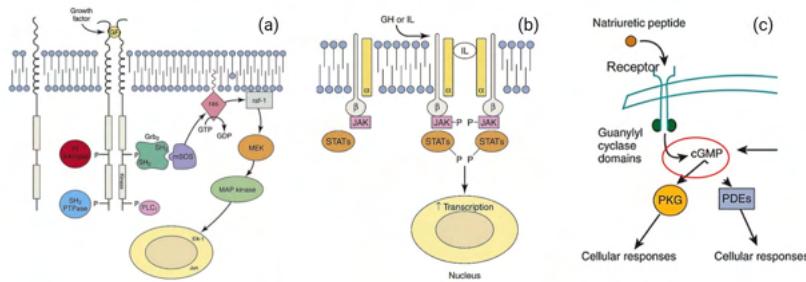


Figure 1.10: Types of enzyme-linked receptors: (a) TPR, (b) cytokine receptors and (c) NPR.

Though not specifically part of enzyme-linked receptors, there's an important concept we should know of.

Concept 1.2 (Receptors Turnover) *Receptors are continuously recycled by the cell.*

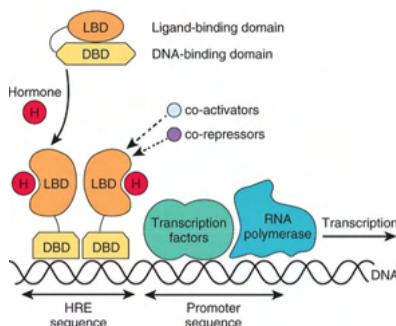
Intracellular Receptors

Definition 1.13. **Intracellular Receptors** are receptors found inside the cell rather than in its membrane.

Example 1.1.10. Some of the commonly known intracellular receptors includes: estrogen, progesterone and glucocorticoid receptor. All 3 of these receptors can alter the transcription of certain genes.

Observation 1.4 When a ligand enter the cell and bind to the intracellular receptor, the entire complex will translocate into the nucleus. Here, it will interact with the transcription machinery to either increase or decrease transcription of a certain gene.

Figure 1.11: Ligand such as hormone can bind to the LBD which will cause 2 receptors dimerize and then bind onto the DNA region. This DNA binding action will recruit transcription factors and RNA polymerase to begin RNA synthesis which subsequently protein synthesis.



1.1.3 Enzymes-Based Drugs

Definition 1.14. **Enzymes-based drugs** are drugs that can block or stimulate which then alter the synthesis or degradation of certain chemicals like transmitters, cytokines, hormones, and etc.

1.1.4 Miscellaneous Drugs

Obviously the 3 categories mentioned above will not cover every drug type which lead to the "miscellaneous" section. An important miscellaneous drug is **anticancer drugs** which are used to reduced the proliferation of cancerous cells.

1.1.5 Drug Response

Time response to drugs depends on the type of receptors drugs are acting on e.g. Ion channel will have the fastest response while DNA-linked drugs will be the slowest:

$$t_{\text{ion}} < t_{\text{GPCR}} < t_{\text{enzyme}} < t_{\text{DNA}}$$
 (1.1)

Concept 1.3 (Adaptations) Receptors tends to adapt when a particular ligands is present repeatedly.

Explanations. Repeated chronic usage of a particular drugs, used to activate a receptor, will lead to a decrease in number of said receptor which counteract the drug's effect. On the other hand, repeated use of a drug that block a receptor will lead to an increase of that said receptor. □

These effects are not apparent until the patient stop taking the drug because by then, the cellular response will require a large amount of that ligand that the body cannot naturally keeps up. This leads to patient experiencing **withdrawal effects**.

Another concept we will talk later is drugs can bind to receptors and etc. to either activate or block it. Furthermore, you also have site for drug to comes and bind and modulate the activity of a receptor.

1.1.6 Magnitude of Drug Effect

So now, we can ask ourselves, how do we quantify drug's effect? First off, why would we need to know that? Well...because we need to know the effective dosage of a drug without inducing an toxic effect on a patient. The process of quantification can be done by testing on cell/tissues.

Methods 1.1 Administer a particular drug with increasing concentration and records the percentage of maximal response for every increased increments.

This testing will yield the **concentration vs max response** in the typical **arithmetic scale**. This is still comprehensible but hard to compare between drugs. This is because we tend to compare drug at concentration where 50% of the maximal response is achieved. A way around this is changing the graph to a **logarithmic scale**. See Figure 1.12.

Not only does this helps us to compare drugs, it also helps to understand the range of dose to be used between human, since we're all a bit different from each other.

Biological Variation

To account for biological variation between humans, we can perform the following experiment.

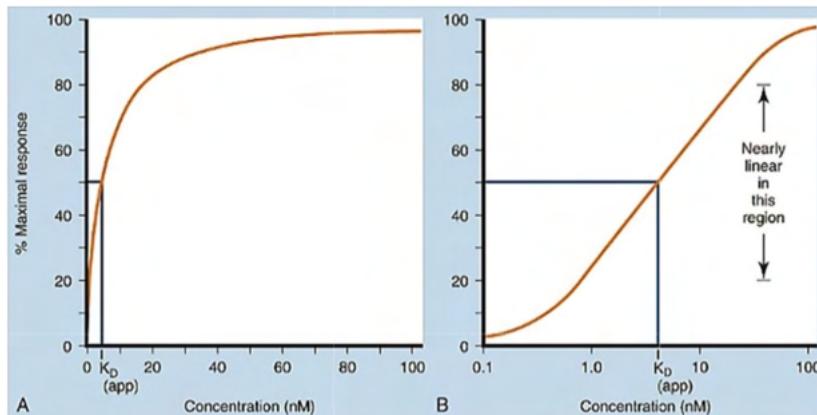


Figure 1.12: Arithmetic (A) vs logarithmic scale (B) of concentration vs % maximal response curve

Methods 1.2 Take 100 people and administer an increasing dose of a drug. At the same time, records the minimal dose at which they respond to.

We can take the information from the experiment and plot it into a histogram curve of dose vs number of response. However, we can then re-plot this into the **cumulative response vs dose** curve on a logarithmic scale. This new curve will allow us to determine the dose at which 50% of the population will respond to.

Definition 1.15. **Median effective dose (ED₅₀)** is the dosage of a drug which will induce a therapeutic effect for 50% of the population.

Example 1.1.11. We can use the ED₅₀ to compare different drugs. Some drugs can give a response at a lower dose than others which is more beneficial as it lowers the adverse effect in favour for therapeutic ones.

Not only it's good to compare drugs, it also helps us with designing better drugs to further minimize adverse effects.

Dose-Response Curve Studies

You can use dose-response curve for different organism level studies like:

- **Single Cell:** Studying the response of a single cell before and after 3 weeks of exposure to a particular drug. From this particular study, we see the cell becomes supersensitive post-drug exposure.

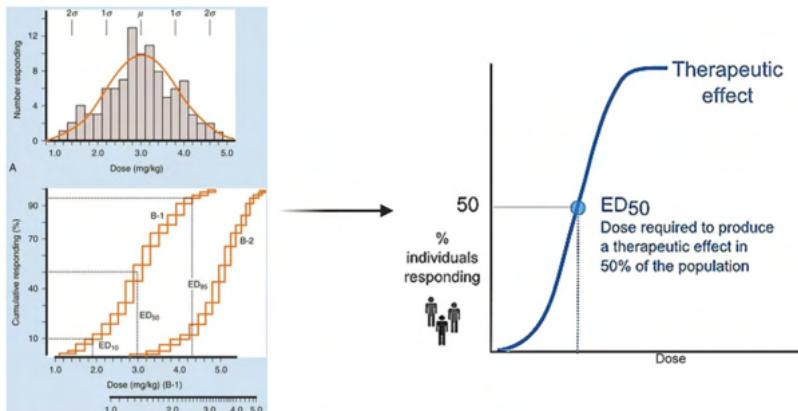


Figure 1.13: cumulative response curve and ED₅₀

- **Tissue/Organ:** Studying response of a tissue/organ by giving different dosage to it. In this particular study, an increase in acetocholine leads to increase contraction for ileum. See Figure 1.14

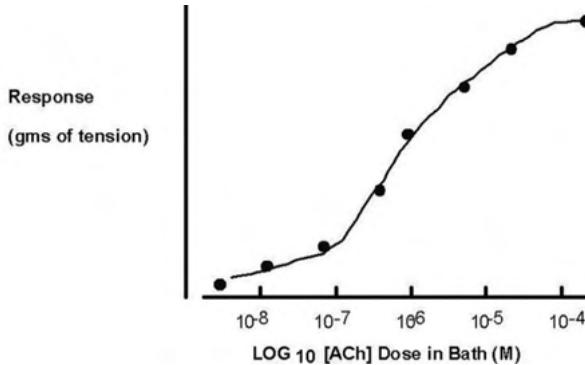


Figure 1.14: Study at the tissue and organ level

- **Individuals:** Studying response of an individual, e.g. human, mice, etc., involved an increasing dose of a drug and map out the response. In this particular study, we look at drugs that depresses the CNS. We found that an increasing dose of drug B will have an anesthetic effect while that of drug A will lead to a coma and even death for this individual. See Figure 1.15

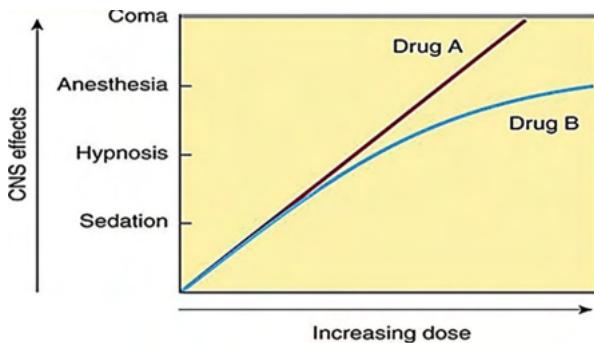


Figure 1.15: Study at the individual level.

- **Groups:** Studying response of a group involved taking groups of individuals and observe their response with an increasing dose of a drug. In this study, we look at how an increasing dose of alcohol can make a particular group to experience slowed reaction time to **ataxia** then finally coma and death. See Figure 1.16

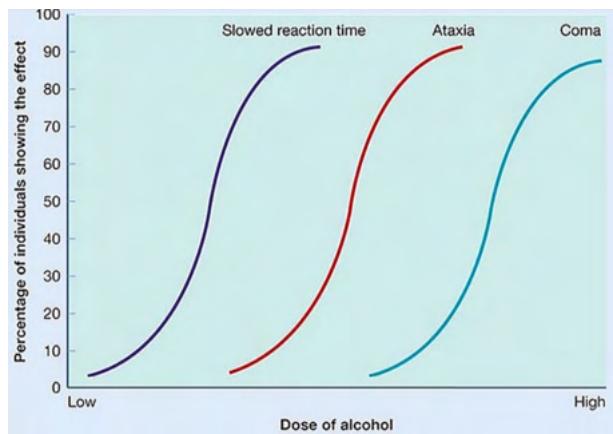


Figure 1.16: Study at the group level

1.1.7 Drug Actions

When it comes to drug actions, we can look at different properties that the drug possesses.

Definition 1.16. An **agonist** is a type of drug that activate/stimulate a receptor.

When looking at the dose–response curve of an agonist, there are a few things, we'll be focusing on.

Definition 1.17. A **threshold dose** the maximum dosage of a drug at which there's no response. A **ceiling (saturation) dose** is the maximum dosage of a drug at which it yields a response that begins to plateau i.e. going higher than this dose does not increase the effect.

Definition 1.18. **Potency** refers to any effective concentration between the threshold dose (yield no response) and the ceiling dose.

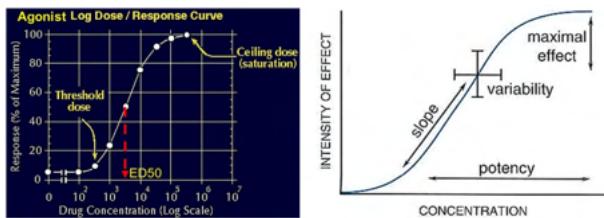


Figure 1.17: Dose–response curve with terminologies.

Example 1.1.12. A potency that we've looked at is the ED₅₀.

Definition 1.19. **Receptor occupancy** refers to the population of a receptor is occupied by a drug at a specific concentration.

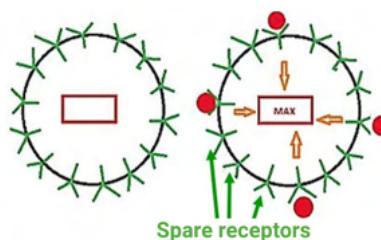


Figure 1.18: Cell at max response with low occupancy.

Typically, when a receptor occupancy is at its highest i.e. all receptors are occupied, we get the maximal response. Nevertheless, this is not so straightforward because of spare receptors.

Definition 1.20. **Spare receptors** are unbound receptors where the cell can still generate a maximal response with only other receptors bounded.

Concept 1.4 *Cells may still achieve a maximal response with low receptor occupancy.*

Definition 1.21. **Drug affinity** refers to the strength of attraction between a drug and its receptors. Typically, affinity will be measured by either the **drug association constant**, denoted as k_1 that measures the likelihood it will bind to the receptor; or the **drug dissociation constant**, denoted as k_{-1} that measures the likelihood the drug will separate from the receptor.

Definition 1.22. **Efficacy** refers to the drug ability to activate a receptor it binds to.

Because of this, drugs, or particularly agonists, can be differentiated depending on their affinity and efficacy.

Definition 1.23. A **full agonist** refers to an agonist that activates the receptor completely. Meanwhile, a **partial agonist** refers to an agonist that partially activates the receptor.

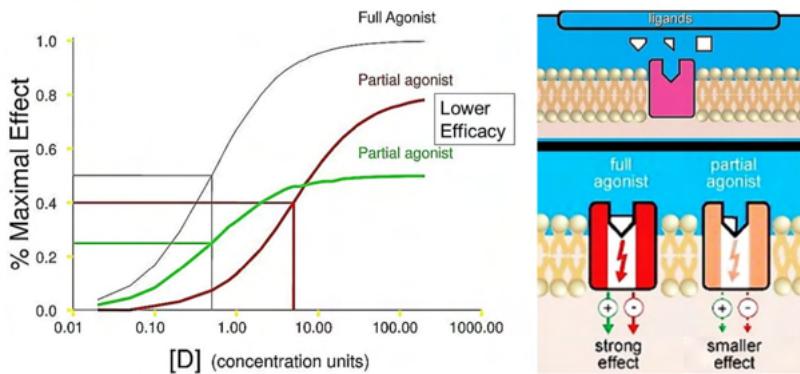


Figure 1.19: Full and partial agonist effect and dose-response curve.

When plotting this on the dose-response curve, we can see that the ceiling dose of a full agonist goes all the way to maximal effect while that of a partial agonist is much lower. Now, **why would we need a partial agonist if it doesn't induce max response?** Well...because the adverse effect of a partial agonist can be different.

Remark 1.5. *The response of a partial agonist is small but it might have to reach almost full receptor occupancy to achieve said effect.*

Observation 1.5 Now, seeing that a partial agonist can reach high receptor occupancy to achieve its effect. We can conclude that partial agonist can block the access of a full agonist.

Example 1.1.13. An opioid addicted patient can enter life-threatening withdrawal if we begin rehab by reducing the dose down to the minimum. However, if an opioid partial agonist is used, patient can still receive some of the opioid's effect but is not completely so that they cannot begin rehab.

Now, fully equipped with properties of agonist, we can compare the effects of each of them.

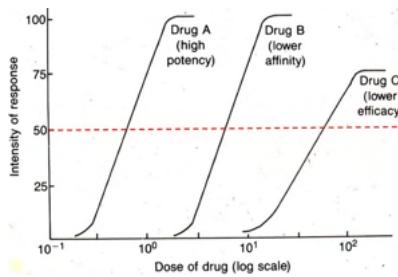


Figure 1.20: Drug A can achieve max response at low dosage thus it has high potency as compared to others. Drug B can also reach the same max response but at higher dosage hence it has low potency/affinity. Drug C cannot reach to max response hence it has low efficacy.

Antagonists

Definition 1.24. An **antagonist** is a type of drug that blocks a receptor from binding to an agonist thus it also blocks generation of cellular response.

Antagonist can have different based on its competitiveness and reversibility.

Definition 1.25. A **competitive antagonist** is an antagonist that competes with an agonist for the binding site of a receptor. A **noncompetitive antagonist** is an antagonist that binds at an allosteric site of a receptor such that only a partial response is yielded when an agonist binds to the active binding site. see Figure 1.21.

Concept 1.5 *Competitive antagonist will shift the dose-response curve to the right.*

Explanations. Since a competitive antagonist compete for the same binding site of an agonist, you'd need a higher dosage of agonist to overcome the effect of the antagonist. When we increase dose, the entire dose-response curve shift to the right. \square

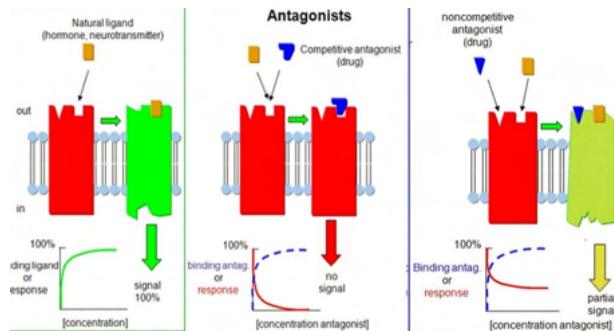


Figure 1.21: Competitive and noncompetitive antagonist effect.

Notion 1.3 In the presence of competitive antagonist, not only does the agonist dose increase, receptor occupancy will also increase

Explanations. Even though receptor occupancy is low for normal agonist, with the presence of antagonists, they can freely bind to the spare receptors which increase occupancy level. \square

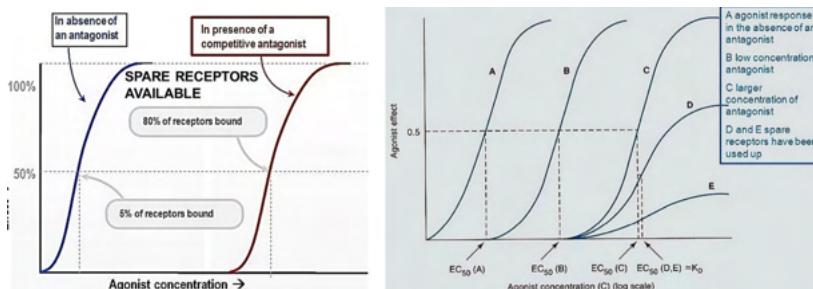


Figure 1.22: Competitive antagonist effects.

Remark 1.6. Competitive antagonist does not have any effect on the maximum effect however, with high enough antagonist concentration, max effect will obviously be affected.

Concept 1.6 Noncompetitive antagonist can lower the maximal response.

Explanations. By the definition of noncompetitive antagonist, the cell yield a partial response. By increases the noncompetitive antagonist, you further lower the maximal response since the agonist is now fully modulated by the noncompetitive antagonist. \square

This also makes an antagonist a sort of allosteric modulator

Definition 1.26. **Allosteric modulator** is a compound that bind to an allosteric site to either positively modulate the effect of a ligand or negatively modulate it (in the case of noncompetitive antagonist).

We can summarize the competitive and noncompeitive antagonistic effect in the figure below.

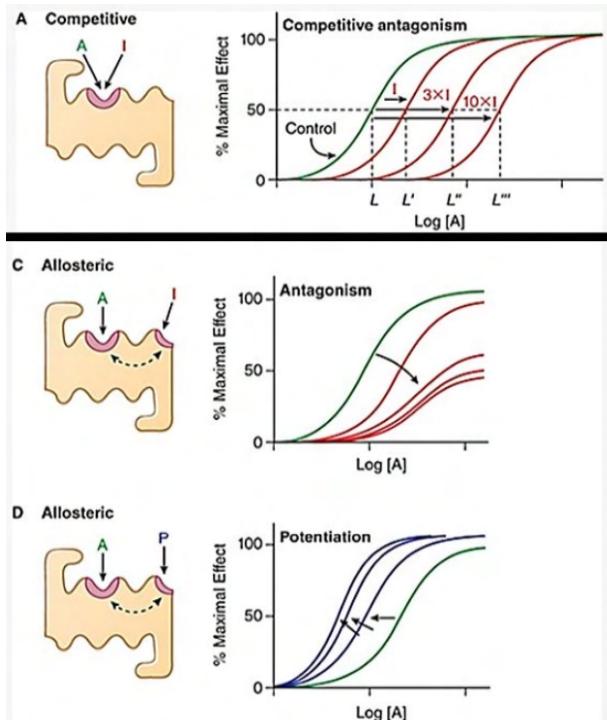


Figure 1.23: Summary of competitive and noncompetitive antagonist

Remark 1.7. It's possible to have potentiation of a dose-response curve via a positive allosteric modulator.

Alongside agonist, we can do another complete summary of its effect and effects of different combination.

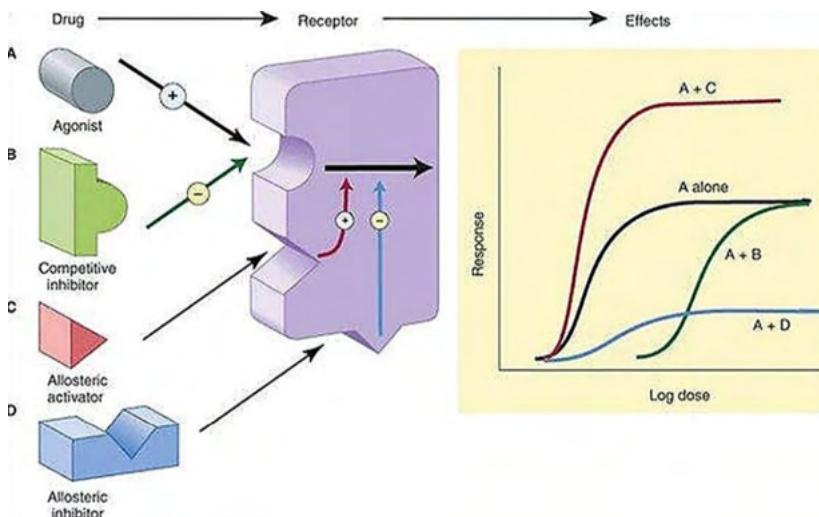


Figure 1.24: Summary of agonist and antagonist

Definition 1.27. A **reversible antagonist** is an antagonist which can be unbound by the body while a **irreversible antagonist** is an antagonist that once bind cannot be unbind.

1.1.8 Side Effects and Toxicity

For quantification of side effects and toxicity of a drug, we can use the same methods to quantify therapeutic effects. This will give us 2 quantities: TD_{50} and LD_{50} .

Definition 1.28. A **median toxic dose TD_{50}** is the dose that will be toxic for 50% of the population. Similarly, a **median lethal dose LD_{50}** is the dose that will be lethal for 50% of the population.

Definition 1.29. A **therapeutic window** is the dose range between the minimum and the maximum of the population that will experience the effect.

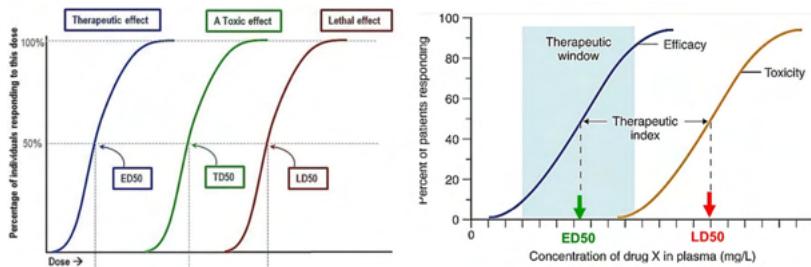


Figure 1.25: Different dose effect and therapeutic window.

Definition 1.30. A **therapeutic index** is defined as the following

$$\text{therapeutic index} = \frac{\text{LD}_{50}}{\text{ED}_{50}} \quad (1.2)$$

Example 1.1.14. For a drug with a therapeutic index of 4, it's not a safe drug as its therapeutic dose is too close to its lethal dose.

Sometimes, we can just use LD₁₀ to find the therapeutic index for drugs that are very unsafe. Normally, however, it's much more effective to know how toxic a drug is which lead to comparison between TD₅₀ and ED₅₀.

Remark 1.8. The therapeutic index would now be defined as $\frac{\text{TD}_{50}}{\text{ED}_{50}}$. ³

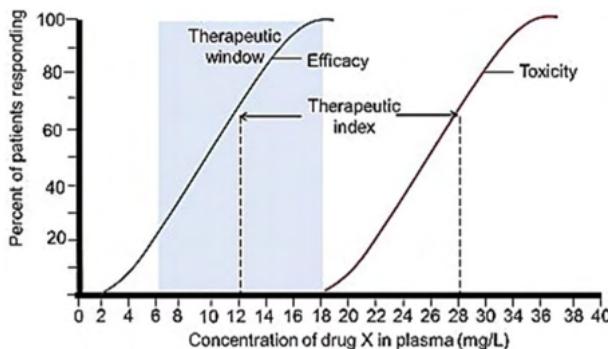


Figure 1.26: ideal therapeutic window.

³Some would call this the **protective index**.

In an ideal condition, you'd find the therapeutic window span until or even beyond the ceiling dose without crossing toward the toxic dose.

Example 1.1.15. **Warfarin** is a drug with a small therapeutic index which means its therapeutic window is smaller hence the effective dose that can induce a response for supposedly 100% of the population cross toward people getting toxic effect. On the other hand **penicillin** is a drug with a large therapeutic index which means larger therapeutic window hence nearly 100% of the population can experience the effect without generating toxicity.

Now, what we didn't take into account for the therapeutic index is that the slope of therapeutic and toxic dose can vary. See Figure 1.26

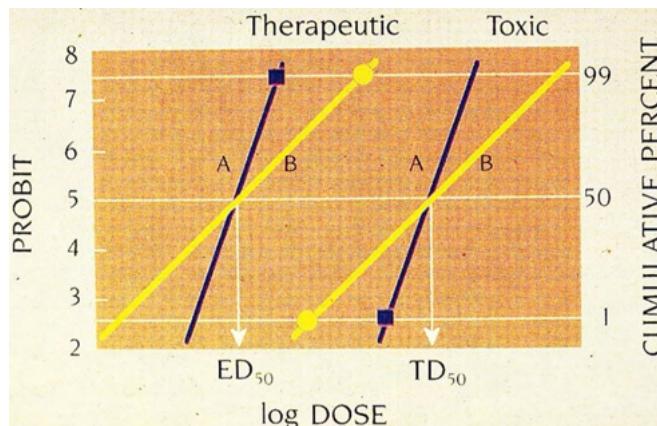


Figure 1.27: The therapeutic index for drug A and B are the same since its TD₅₀ and ED₅₀ stays the same. However, we see that the therapeutic window for drug A is much larger than drug B since it has a steeper slope i.e. drug A is safer than drug B.

Essentially, you cannot just rely on the therapeutic index to tell you the entire story but you also have to look at the curve to determine its safety.

Outliers Problems

We've mentioned before that people have variations between them hence drugs dose would be different. Typically, these variation are minimal however when you get large outliers, the analysis becomes more interesting.

For certain outliers, they may experience the toxic effect within the therapeutic window. Some may not experience the toxic effect even beyond the therapeutic window.

Remark 1.9. *The most dangerous of the 2 is the outliers that experience early toxicity.*

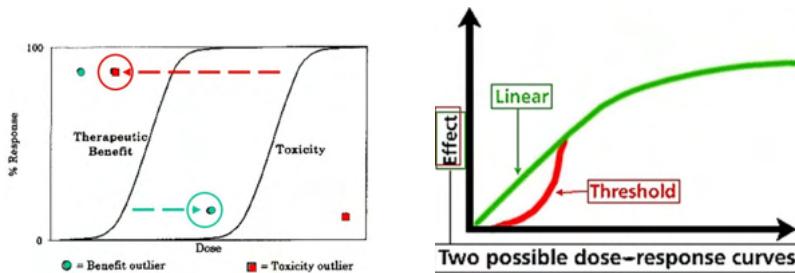


Figure 1.28: Outliers and toxicity (left); non threshold drug-response curve (right)

Last but not least for today's lecture, **certain drugs may not have a threshold dose** i.e. it's a linear relationship that the moment there's a dose, there will be a response. We will see later on that this is especially important to evaluate toxicity.

1.2 Pharmacokinetics I

In today's lecture, we will look at 2 of foundations of pharmacokinetics, namely, absorption and distribution. Before getting into knowing absorption, we're going to look at different way that we administer a drug.

1.2.1 Method of Administration

Definition 1.31. **Oral (enteral) route** are drugs administered as pills and taken up through the mouth.

Observation 1.6 Drugs under pills form are convenient and cheap to be made. Because it's easy to be made, we can also modify the action/release of the pill as it enters the body

Example 1.2.1. There are pills that can resist the stomach acid and only release + absorbed in the intestine; or even slow-releasing pills that will slowly be absorbed by the body.

The only "downside" to oral drugs is that it will involve interaction with the liver. This is because any drugs that pass through the intestines will be under control of first-pass effect of the liver.

Definition 1.32. Parental (by injection) route are drugs administered through injection.

Observation 1.7 The most evident advantage point of injection is that it will be much faster (do not require absorption by the intestine). Furthermore, it will bypass the liver and because of this, we can get a much more accurate dose right away (no more first-pass effect).

Nevertheless, it still has its downside and that is the requirement of equipments (syringes and needles). Not only that, these equipments need to be sterile.

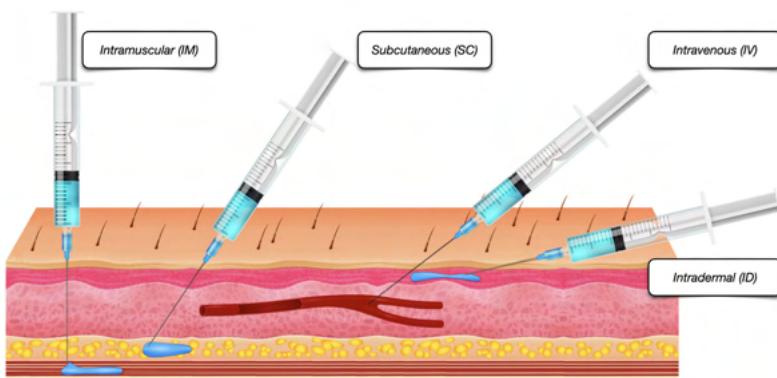


Figure 1.29: Different types of injection.

Definition 1.33. Within the injection categories, we can further subdivide it into smaller subcategories of injection:

- **Intradermal:** injection at the dermes layer of the skin.
- **Intravenous:** injection at the vein.
- **Subcutaneous:** injection at the fat layer of the skin (around the hypodermis).
- **Intramuscular:** injection at the muscle.

See Figure 1.29 for reference.

Some of these injection can be done at different sites on the body (to mainly avoid repeated injection of 1 particular area).

Observation 1.8 Evidently, the 4 mentioned above are only a few. There are other categories as well but are not as common (typically done in hospital/specialized clinics).

Example 1.2.2. Intraperitoneal injection is an injection at the peritoneum, **intrathecal injection** allows injection into the thecal space of the spinal cord, and **intracerebral injection** allows injection to the brain/ventricles.

All of these injection has a reason behind it e.g. intraperitoneal could be used if there's a tumour in the peritoneum and similarly for intracerebral injection (can also bypass the blood-brain barrier).

Definition 1.34. Inhalation route is drugs administered through the lung via inhaling.

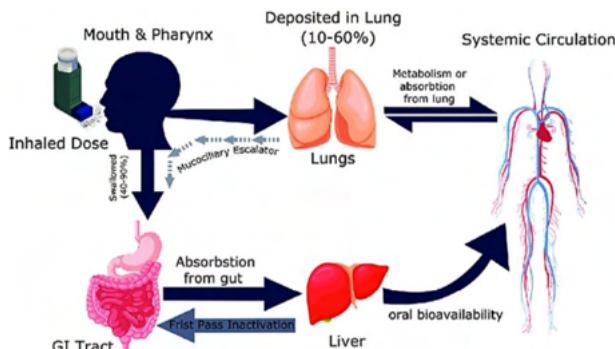


Figure 1.30: Inhalation route.

Observation 1.9 Interestingly, the inhalation route can not just only affect the lung but also the surrounding systems. This is because it's the site of gas exchange where blood is pass through with great intensity. In other words, inhaled drugs can have multiple sites of action (locally or systemically).

The effects of inhalation is very quick as it has large surface area but also great level of blood flow for gas exchange. Even for the systemics response, it could also be rapid too (though not as fast).

Remark 1.10. *Majority of the time, only 10 – 60% of inhaled drugs are deposited in the lung while the rest would be swallowed to the intestine.*

Definition 1.35. **Topical route** is a drug administration through application on the skin.

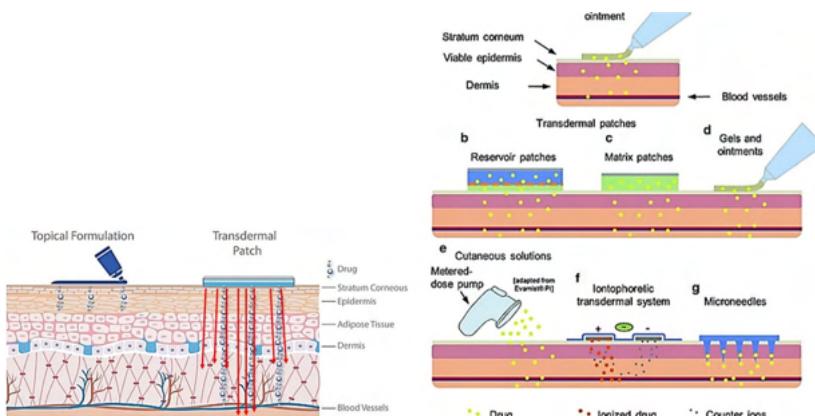


Figure 1.31: Topical route.

Observation 1.10 Topical route can be through transdermal patch (e.g. nicotine patch) or cream/ointment application. Once applied, drugs from these application will be slowly absorbed through the skin and enter the blood-stream.

We've so far developed lots of newer method for the topical route so that it could serve specifics purposes (or even replace injection if possible).

Definition 1.36. A type of topical route that specific localized for the eyes are called **intraocular route**.

Definition 1.37. Sublingual route is a drug administration at the bottom of the tongue.

Explanations. The reason we have sublingual route is because it can be absorbed very quickly to the systemics since there's high blood flow around the mouth area. Not only that, because it does not have to be swallowed, this drug effectively bypass the liver as well. □

Comparison between different routes

Methods 1.3 Obtain a patient and allow them to have a treatment of acetaminophen in 3 different route: intravenous (IV), pills (oral) and rectal with the equivalent concentration. After administration, measure the patient acetaminophen blood plasma concentration through time.

Observation 1.11

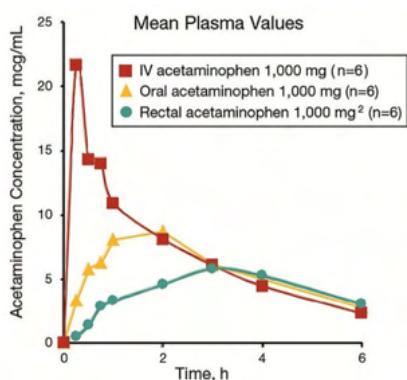


Figure 1.32: Acetaminophen concentration with different route

As of the time speaking, there has been continuous effort of developing new method for drug administration.

What you'd find is the curve in Figure 1.32. You can see that with IV injection, the patient will get the full directly then the drugs will be slowly distributed through the body then eliminated. With oral route, it follows a similar trend the end but the initial dose is lost because of the liver. Similarly for rectal though the dose will be even smaller.

Newer Methods of Administration

As of the time speaking, there has been continuous effort of develop-

Example 1.2.3. Some drugs are tested in different form of storage like micide, liposome, nanocapsule, etc. The nanocapsule/particle is in particular interest as its the form of storage we've used to store SARS-CoV-2 vaccine.

So now that we've looked at different ways of administering a drug, we can return to the pharmacokinetics of drugs.

1.2.2 Absorption

Definition 1.38. **Absorption** is the transfer of drug from site of administration to bloodstream.

Observation 1.12 In order for a blood to pass through the blood stream, it would have to pass through lots of cell membrane and gaps junction. Depending on the types of molecule the drug is, it may or may require the use of a **carrier** (a transmembrane protein used for transporting molecules).

Example 1.2.4. Gases, hydrophobic, and small polar molecules can pass through the membrane easily. Meanwhile, large molecules (like glucose) and charged ions won't be able to but will need the carrier (active transport).

Remark 1.11. *Most drugs are lipid soluble thus requiring some active transportation.*

We can look at the oral administration route and see how drugs can be absorbed at different level.

Stomach

1 thing about the stomach is that it's the main site to store food coming in but also sterilize it with strong acid (HCl).

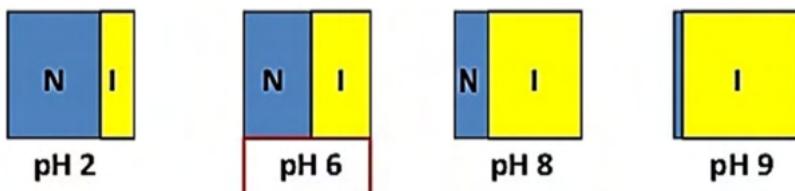


Figure 1.33: Ionization of acidic drugs at different pH

Observation 1.13 We can exploit this acidic environment to design a drug that can become non-ionized and thus can be absorbed in the stomach.

Example 1.2.5. Acidic drugs becomes non-ionized (more lipid soluble) when in contact with acidic medium. This means that drugs that are weak acids will be the best for stomach absorption while weak bases—drugs are less ideal and will be best for intestine absorption.

So to recap, for acidic drugs, its non-ionized form has the highest absorption while ionized form will have the worst absorption.

Example 1.2.6. We can see the effect ionization in real life with an antibiotic (acidic). In this case, when the drug: **tetracycline** is taken orally, it will become non-ionizing in the stomach and absorbed there directly. However, if the patient was to take antacid or milk beforehand, the environment is no longer as acidic as before which lead to lower plasma concentration of tetracycline (i.e. lower absorption).

Intestine

When it comes to the intestine, it has the highest surface area for absorption with lots of different absorptive mechanism. However, it will have a downside of have to pass through the liver.

Definition 1.39.

First pass metabolism is a concept of drugs absorbing in the intestine will pass through the liver to be metabolized even before they get to do their action.

Because of first pass metabolism, some drugs cannot be delivered orally. This is because some drugs can be heavily metabolized by the liver \Rightarrow we have to increase dose \Rightarrow toxicity. This is why we have different route to bypass the enteric system thus the liver too.

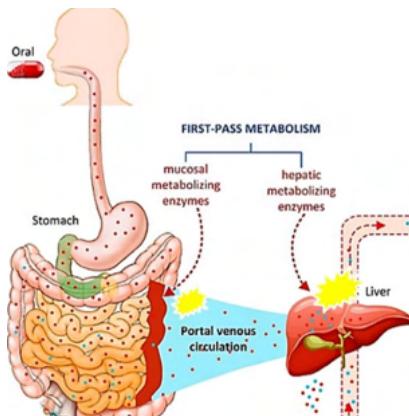


Figure 1.34: First pass metabolism.

Definition 1.40. **Bioavailability** is the fraction of administered drugs that can reach the systemic circulation after first pass metabolism

Concept 1.7 *Bioavailability allow correct dosage of drugs with high hepatic extraction (metabolized strongly by the liver).*

Notion 1.4 Bioavailability can differ from 1 types of administration to the next.

Explanations. The reason it differs is because certain route can just bypass the liver completely or has little breakdown once it reached to systemic. In this case, we have intravenous injection with the highest while oral route is the lowest bioavailability. \square

Notion 1.5 Different drugs can have varies in their bioavailability (even if they treat the same disorder).

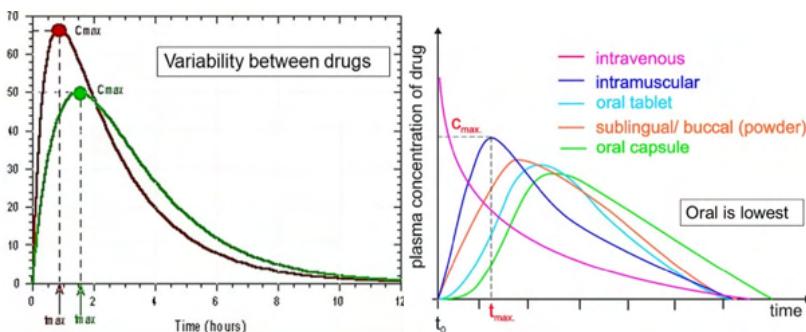


Figure 1.35: Drug bioavailability between different drugs (left), and between different route of administration (right).

1.2.3 Distribution

Definition 1.41. **Distribution** is the transfer of drug from blood stream to other sites of actions.

So now that we got the drug into circulation, it will not stay in circulation but will be delivered to sites that required the drug. These sites can serve many purposes like: **for drug binding, drug action, and drug transformation.**

Methods 1.4 To see this distribution, we can track the concentration a drug in a patient. Give a patient a dose of anesthetics via IV injection. Then, track the drug concentration in major tissues of the body.

Observation 1.14 What you'd find is the percentage of dose in the blood will begin to decrease as it gets distributed. As its concentration in blood

decreases, its concentration in the brain, viscera and lean tissues begin to increase. Once it reaches its peak, it will drop back down as drug are being metabolized and excreted.

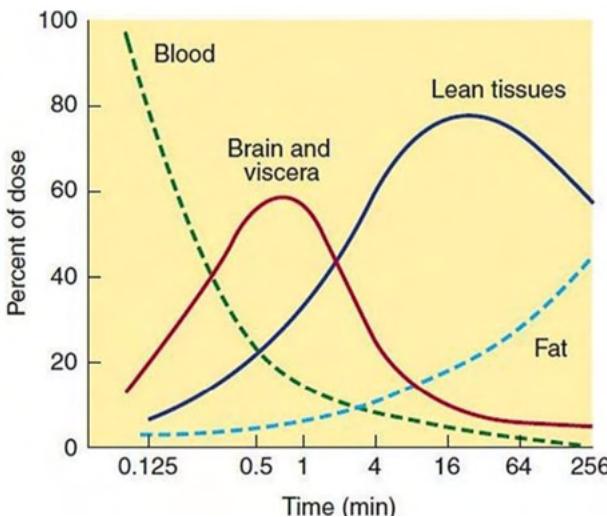


Figure 1.36: IV Anesthetics experiment.

Remark 1.12. *The concentration of drug in fat tissue increases very slowly as there's not much circulation to such tissues.*

With this experiment, we can realize the following

Concept 1.8 *Tissues that has high blood supply will have higher rate of distribution while the contrary for tissues with low blood supply.*

Example 1.2.7. When a patient is administered with halothane, it will distribute first to the brain, heart and liver. Later to muscle, fat, skin, etc.

When drugs enter systemic circulation, it will be in its active and free form or bounded form (bounded to another protein). The free/bounded drug can travel to sites of action where it can also bind to receptors. If not, it can travel to body tissue reservoirs and in the least wanted case, it will travel **unwanted site of action** which can induce adverse effect.

Lastly, drugs can then be metabolized to render it inactive and then excreted out of the body.

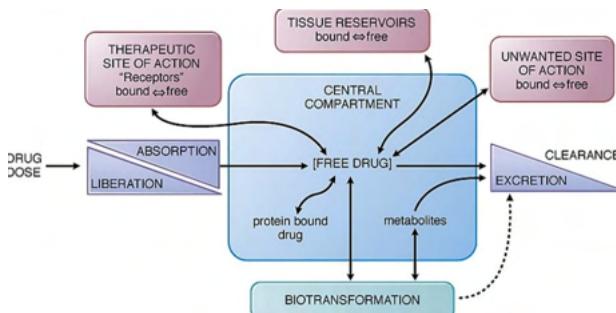


Figure 1.37: Drug distribution to tissues.

Drugs Distribution to Tissues

Drugs, either bound to protein or free, can cross the cell membrane or can bind to a receptor. Some of these drugs can be metabolized specialized cells e.g. liver cells will metabolize drugs into drug metabolite that can be released back into circulation for excretion.

The type of transportation through the cell membrane depends on the nature of drug (we've previously seen). The main 2 are passive and active transport (need not vs need energy respectively).

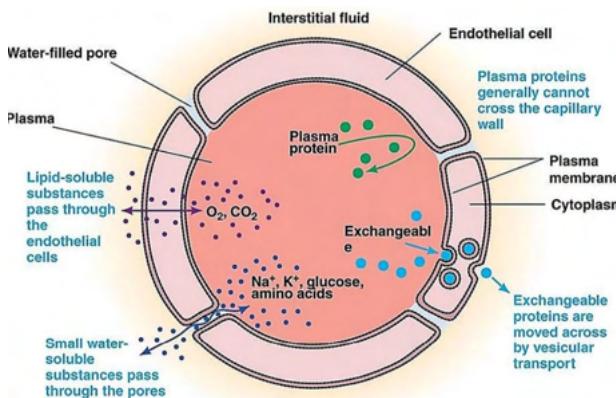


Figure 1.38: Transport across capillary wall.

Example 1.2.8. Drugs permeability through tissue and capillary walls de-

pends on the size of drug, lipophilicity, polar/ionic form and bounded to protein.

Observation 1.15 We can look closer at the peripheral capillary. What we'll see is that there are pores between these endothelial cells. These pores serve as openings for small water-soluble drug to pass through. When it comes to lipid soluble drug, it can simply pass through the endothelial cell. Along with many other transport. See Figure 1.38.

Notion 1.6 Diseases can alter the distribution of drug

Example 1.2.9. Tumour can cause blood flow to be excessive in the tissue it lies or even cut off circulation at those tissues completely.

Drug Concentration–Time Curve

We can plot the drug concentration vs the time elapsed to get the drug concentration–time curve. With this curve, we can see some important points and interval.⁴

Definition 1.42. Onset of effect is the time it takes for plasma concentration of drug to reach **minimum effective concentration (MEC)**. **Peak effect** is the maximum concentration of drug in the body.

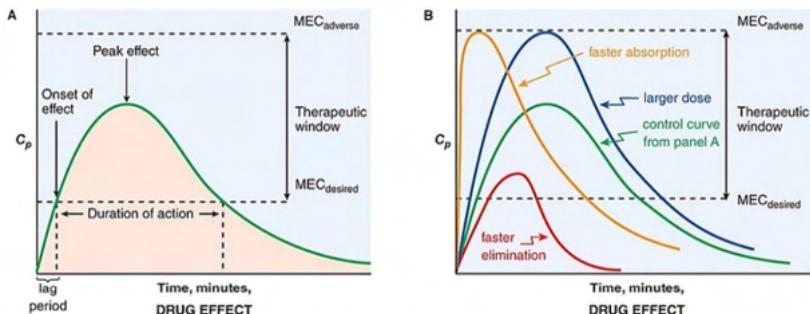


Figure 1.39: Drug concentration–time curve

Definition 1.43. Lag period is the time interval between the administration of drug and the onset of effect. **Duration of action** is the time interval between the first MEC point to the last MEC point.

⁴We've already talked about the therapeutic window from previous lecture.

Observation 1.16 There are many things that can alter the drug concentration-time curve (as compared to a control curve):

1. **Faster Absorption:** When drug has a fast absorptive rate, the peak effect can reach all the way to the MEC for adverse effect.
2. **Larger Dose:** When dose of a drug increase, it will also have as high of a peak effect as faster absorption however its lag period is longer.
3. **Faster Elimination:** When drug is eliminated quickly, the peak effect is not as high as the control and the duration of action is much shorter.

See Figure 1.39.B

The Blood Brain Barrier

Though the capillary are pretty much universal everywhere on the body, it's not the same in the brain. In the brain, the capillary is much more tightly packed together (i.e. no more pores). Not only that, there are tight junctions and **astrocytes'** foot processes that create a second enclosure around the capillary.⁵

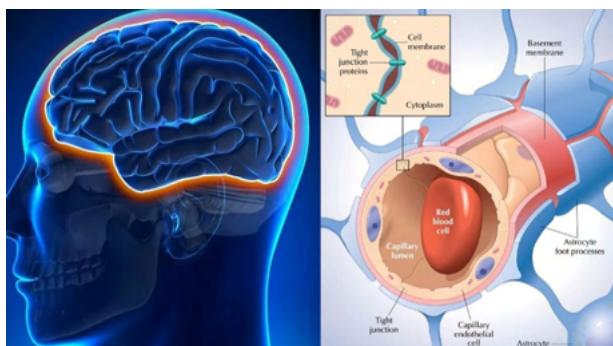


Figure 1.40: Blood brain barrier.

Explanations. Although seems overregulated, it's important to have this barrier so that only a important selected molecules that can pass and not just any. □

⁵Interestingly, the human brain capillaries can stretch up to 400mi or around 644km.

Remark 1.13. Though this barrier are quite apparent for adults, there are 2 cases where this barrier is not well-defined: **newborn and meningitis patients.**

Suppose that we've designed a drug that can cross the blood brain barrier, **how will be be eliminated?** Well...when it crosses the blood brain barrier, it can begin to be distributed in the brain and then later excreted to the CSF that circulate the entire central nervous system (CNS). This CSF will later drain out of the CNS into the **venous sinuses**. The venous sinuses can merge back to the systemic circulation then the drugs can be brought to the liver and metabolized there.

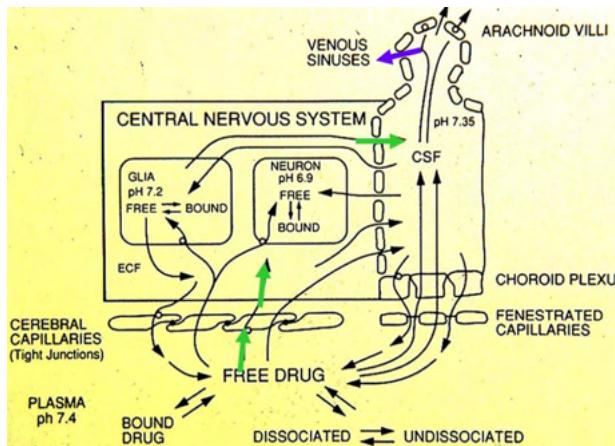


Figure 1.41: Drug after cross the blood brain barrier.

Drugs Action to the Placenta

Unfortunately, the **placenta** is not an effective drug barrier this means that lipid-soluble (rapidly) and water-soluble drug (slowly) can pass through. Not only that, **some drugs can concentrate in the fetus** which means there must be some **caution using it during pregnancy!**

Protein Binding In Circulation

Definition 1.44. The main protein that will be bound to drug in circulation is **albumin** along with other glycoprotein.

Observation 1.17 When drug is administered to the body, there will be some part that is unbound and allow to freely flow to their site of action/reservoir. On the other hand, protein-bound drug will remain in circulation (blood).

Notion 1.7 Normally, the level of albumin and glycoproteins are constants in circulation however there will be cases like liver disease and inflammation that can change the level of albumin and glycoproteins respectively.

When it comes to the distribution of drugs, there are 2 models we can look at.

Concept 1.9 *The one compartment model describes a drug distribution immediately distributed evenly through the body post-administration.*

Though this model is simple but it does not truly describe what happens in the body when drug is given. This is where the next model comes in.

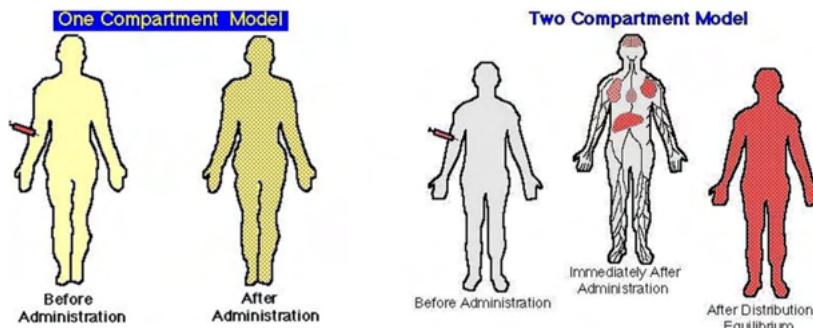


Figure 1.42: 1 compartment (left) vs 2 compartment (right) model

Concept 1.10 *The two compartment model describes a drug distribution through specific site of actions (organs) post-administration. Later, the drug will be re-distributed back in circulation evenly.*

Drug Distribution Back to Circulation

Now obviously, we've talked about how drugs can enter a cell which depending on its properties. Now, when drug is re-distributed back in circulation, it will depend on the properties of cell.

Definition 1.45.

P Glycoproteins is a trans-membrane transporter whose job is to transport protein out of the cell.

Observation 1.18

Interestingly, these P glycoproteins are very efficient at transporting drug out. In fact, it might be too efficient at getting rid of drug that for certain drug like anti-cancer, there might be problems.

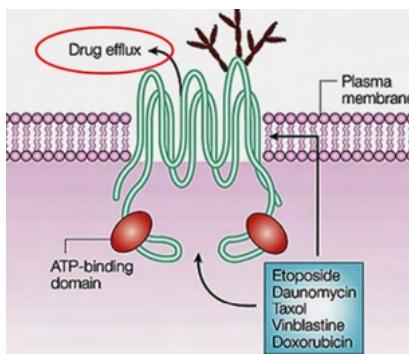


Figure 1.43: P glycoproteins

To see its effect, we can look at a drug plasma level with respect to time after doses. In the beginning, we see a drug level reaches its peak and then immediately drop down. This immediate drop highlights the effect of fast distribution. After the drug is distributed evenly in the body, it will now be slowly metabolized by the body.

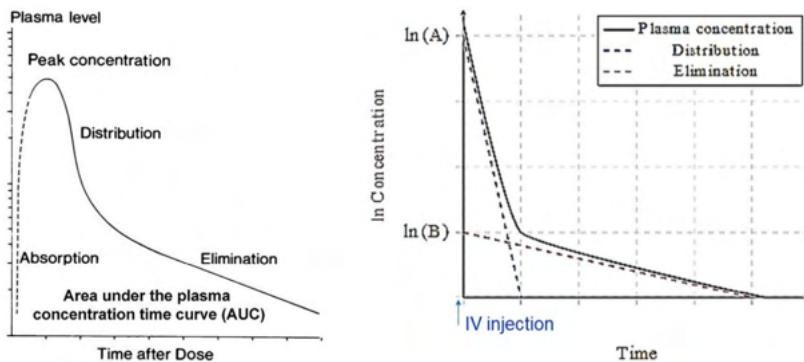


Figure 1.44: Drug plasma level vs time after administration graph.

We can also focus on the drug distribution and elimination portion and rescale it in a natural log graph. This will allow us to see the immediate shift in drug concentration drop between the 2 phase more apparent (exaggerated).

Volume of Distribution

Now, we will look at a way to quantify that overtime, drug at a specific central volume will be distributed evenly though the body or even the opposite.

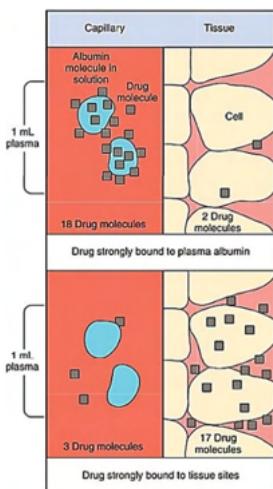
Definition 1.46. A **volume of distribution (v_d)** or **apparent volume of distribution (AVD)** is a term to quantifies distribution and is defined mathematically as

$$\text{AVD} = v_d = \frac{\text{amount of drug in body}}{\text{plasma concentration}} = \frac{X}{c_p} \quad (1.3)$$

i.e. it gives some understanding on the amount of drug distributed into the tissues.

Observation 1.19 This volume of distribution can vary because of the properties of the drug, protein and tissue binding.

Example 1.2.10.



If you administer a measurable amount of drug to the body (i.e. this will be a known value and is fixed). Now, we will measure its AVD. If its $\text{AVD} \downarrow \Rightarrow C_p \uparrow \Rightarrow$ there are high concentration of drug in circulation i.e. drug is strongly bound to albumin.

On the other hand, if $\text{AVD} \uparrow \Rightarrow C_p \downarrow \Rightarrow$ there are low concentration of drug in circulation i.e. drug is strongly bound to tissues and site of action.

Let's look at another example.

Example 1.2.11.

Figure 1.45: Small vs large AVD.

Suppose we take a breaker of water (of 500mL) and fill it with a specific dosage of 10mg then we measure its concentration in the beaker. Let's say that the measured concentration is 20mg/L then, we can calculate our AVD as 500mL. If we added in some charcoal, suppose that the measured concentration is now 2mg/L.

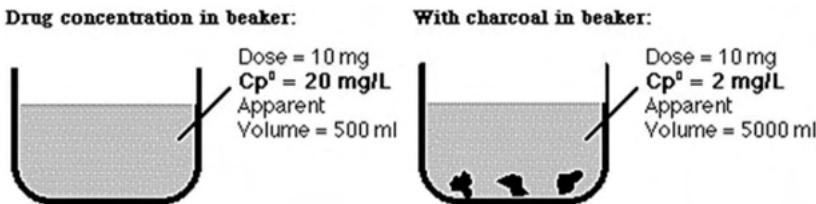


Figure 1.46: AVD of normal vs with activated charcoal.

Our AVD now increased to 5000mL. So we can see that $\text{AVD} \uparrow \Rightarrow$ the drug has great affinity to the charcoal.

Notion 1.8 Using AVD,⁶ we can determine that:

1. More drug is protein bound = plasma concentration \uparrow = low AVD.
2. More drug is tissue bound = plasma concentration \downarrow = high AVD.

Example 1.2.12. Different drugs will have very different AVD relative to the weight of the patient taking the dose i.e. AVD/kg . For the drug ibuprofen, it has an AVD/kg or $0.15\text{L}/\text{kg}$ which means the drug is mostly protein bound compared to azithromycin with $31\text{L}/\text{kg}$ (mostly tissue bound).

1.3 Pharmacokinetics II

When you give a drug, it will be distributed at different region of the body and then it will be eliminated. Before we can enter elimination phase, we must first deactivate the drug through biotransformation or metabolism phase.

1.3.1 Metabolism: General Consideration

Definition 1.47. **Drug metabolism** its the conversion of drug to less active/inactive metabolite and more water soluble. Sometimes it can convert inactive prodrug to its active form.

⁶We will use AVD to calculate another value called loading dose later on in this course.

Observation 1.20 Looking at the [parent drug] vs [metabolite], we can see that during administration, there's a sharp rise of [parent drug] while its [metabolite] also rises quite quickly. As the [parent drug] reached its peaked and begin to drop, the [metabolite] now rises slowly.

The liver is the organ that does most drug metabolism. Evidently, nearly everywhere on our body has drug metabolizing enzyme, even the brain; however, it's not as much as compared to the liver.

Observation 1.21 When you swallow a drug, it will first be absorbed in the intestine which will bring it to the hepatic portal veins into the liver. Once in the liver, part of it will be metabolized (known as **first-pass metabolism**). The rest of the fraction will continue in circulation and be bound or free to move to its site of action.

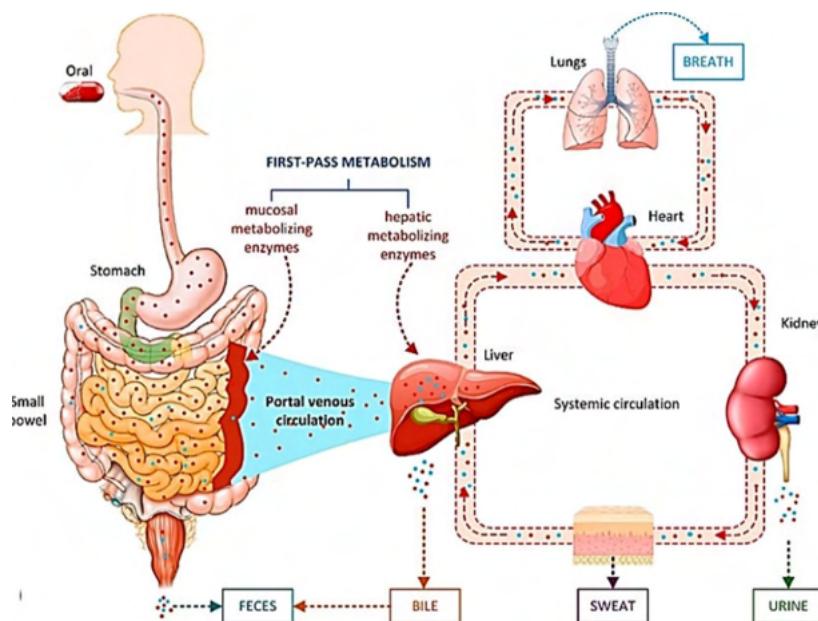


Figure 1.47: Drug metabolism scheme.

The Liver

Let's look at the liver for a bit as it's the main organ the will be doing this metabolism phase.

Observation 1.22 The liver is the second largest organ, has regenerative ability and can detoxify any compound entering the body. It's vital for life and has large functional reserve. Interestingly, disorders of the liver are very common and symptoms can be diverse too.

Another key aspect to look at also is how blood flow through the liver and how said blood can be detoxified.

Mechanism of Action (Hepatic Circulation): We begin with the blood arteries that will enter the liver.

1. As the arteries enter the livers, it begins to branch deeper in the liver which we call the **liver sinusoids**.
2. The wall between these sinusoids and the **hepatocytes** are very thin which allow for an incredible amount of exchange as well as activation of enzymes.
3. Lastly, the sinusoids converge back to a vein carrying out detoxified blood or even deactivated–drug carrying blood.

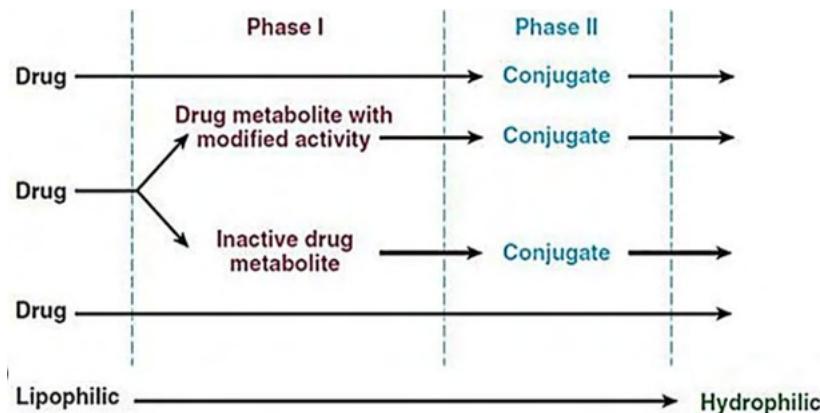


Figure 1.48: 2 phases of drug metabolism

The liver is also the primary site of metabolism of exogenous compounds such as food and drug. There are 2 phase of drug metabolism.

- Phase I: oxidation/reduction/hydrolysis. Here, the drug will be inactivated (majority of the time, rarely it will be activated).
- Phase II: conjugation. Here, something will be attached to the drug.

Remark 1.14. You begin with a lipophilic drug and ends up with it being hydrophilic at the end of metabolism.

1.3.2 Metabolism: Phase I

Now, we will look at an enzyme that contribute the most in phase I of metabolism

Definition 1.48. **Cytochrome P450 (CYP450)** is a large class (superfamily) of enzymes that can participate in lots of chemical reactions in the liver

Nomenclature of CYP Enzymes: Since we've said that CYP450 is the large class of enzymes, this means that there are specific enzymes that can be found in said class. This is how we give each of these enzymes a name:

<u>1st holder</u> superfamily	<u>2nd holder</u> family	<u>3rd holder</u> subfamily	<u>4th holder</u> gene
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Example 1.3.1. The enzyme CYP3A4 indicates a cytochrome P450 enzymes of family 3, subfamily A and gene 4.

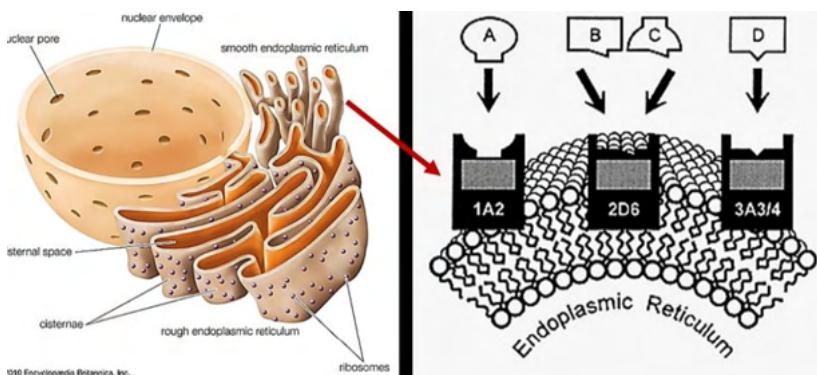


Figure 1.49: CYP450 on ER.

Observation 1.23 The most common reaction of CYP450 is **oxidation of the drug which will inactivate its biological activity**. There are obviously

other reaction but oxidation is the most common. The P450 enzyme will act on the drug which will lead to an oxygen atom incorporated into the drug structure. P450 are present in virtually every life on earth.

These CYP450 can be found on ER of hepatocytes where then can catalyze a reaction with a drug molecule. Certain CYP450 can catalyze reaction to more than 1 drugs at a time.

In human, there are 3 main isoform of CYP450 that are specifically to drug and **xenobiotics** metabolism: **CYP1, CYP2, and CYP3** (other CYP450 can metabolize drug but not as significant). Within these 3 mains, there are subtypes which are dedicated as drug metabolizing enzymes which include: **CYP1A2, CYP2D6, and CYP3A4**.

Remark 1.15. When looking at these enzymes, we would notice that they're polymorphic i.e. there are more than 1 structure of that enzymes depending on each person.

Notion 1.9 These enzymes are subjected to induction (similar to receptor adaption)

Explanations. When a person is taking a drug chronically, it can cause the body to recruit more and more of these enzymes which lead to the metabolism of drugs much faster. When the metabolism of drugs are too fast, patient will not get the full effect of said drug. □

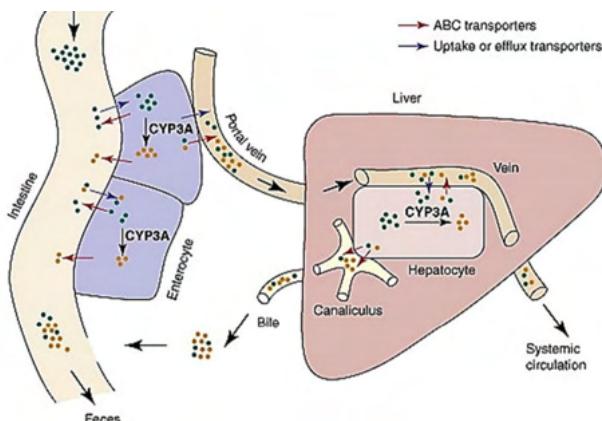


Figure 1.50: Drugs journey through first-pass metabolism.

For the drug to get to the liver, it has to enter the intestine first (if let's say the method of administration is through the mouth). On the intestine wall, there are some CYP3A4 that can metabolize tiny portion of the drug before reaching the liver. Not only that, some of these drugs may not even be able to pass through due to the existence of glycoproteins along its epithelium. (See figure 1.50)

Let's look at the full journey of a drug at a specific dose entering the intestine, through the kidney and ends up in circulation.

Example 1.3.2. When 150mg of drug administered orally, only around 120 mg is absorbed through the intestine. This 120mg will pass through the portal vein into the liver which will breakdown 90mg thus it removes ~ 75% and only 30mg enter the systemic circulation. So compared to the original dose, we say the *bioavailability* of this drug is 20%.

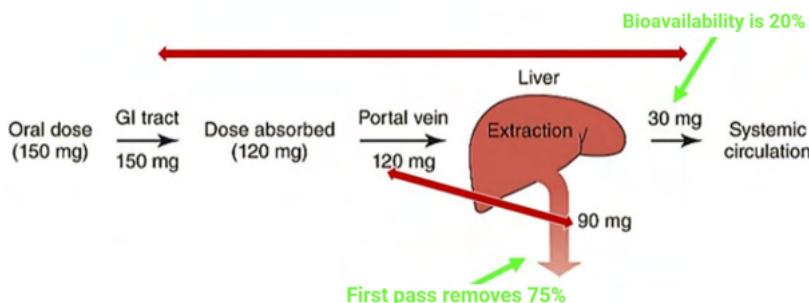


Figure 1.51: Drugs bioavailability.

Definition 1.49. **bioavailability** is the measure of the amount drug administered with respect to the amount of drug in circulation given as the following equation:

$$\text{Bioavailability} = \frac{\text{Original dose}}{\text{Dose in circulation}} \quad (1.4)$$

The brain has the smallest amount of drug metabolising enzymes and its P450 are quite different to that in the liver (specific to the need of the brain).

Observation 1.24 The factors affecting drug metabolizing enzymes can vary from individuals according to their age, gender, diseases, etc. Even envi-

ronment can have an effect to our drug metabolism like infection, fever, stress, etc.

Concept 1.11 CYP450s can be induced or inhibited.

Methods 1.5 A study look at the variability in levels of individual P450s in 18 human liver samples. Here we separate them into 3 groups with according to the presence of a specific CYP450. How do you check for the presence? By verifying the metabolism level of a specific drug that can be metabolized by said enzymes.

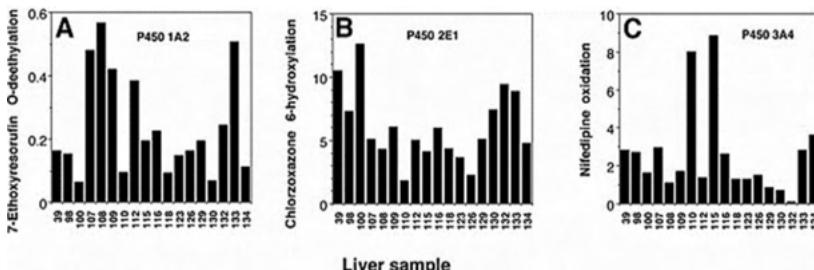


Figure 1.52: Variation in liver sample's P450 between 18 individual.

In the end, what you'd find is that there are no correlation between the variability of any of the enzymes i.e. an increase in activity or presence of 1 enzymes does not increase nor decrease the activity of another.

Notion 1.10 Enzyme induction can be lead to rapid metabolism

Example 1.3.3.

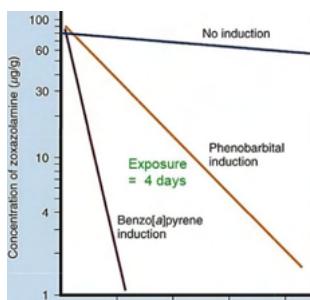


Figure 1.53: inductive effect

When looking at the action of on P450 without any inducer, we can see its metabolic rate to breakdown **zoxazolamine** is fairly slow (its concentration decreases slowly). When exposing said P450 to an inducer, like *phenobarbital*, the rate increases significantly. And in the case of *benzo[a]pyrene*, the rate sharply increases.

Since enzymes can be induced or inhibited. Induction of enzymes will come with a higher breakdown of drug. On the other hand, inhibition of enzymes will come with drug toxicity as the active drug cannot be broken down.

Remark 1.16. *This means it's important to check for drug interaction whether 1 drug can cause induction or inhibition of that enzymes when giving another drug.*

Enzymes	Substrates	Inhibitors	Inducers
CYP 3A4	amlodipine, simvastatin, warfarin, amiodarone, sildenafil, midazolam, fluoxetine, haloperidol, codeine, oxycodone, methadone, fentanyl	ciprofloxacin, ketoconazole, ritonavir, methylprednisolone, imatinib, tamoxifen, cimetidine, grapefruit juice	simvastatin, efavirenz, pentobarbital, carbamazepine, phenobarbital, phenytoin, valproic acid, caffeine
CYP 1A2	alosetron, caffeine, duloxetine, melatonin, ramelteon, tacrine, tizanidine	ciprofloxacin, enoxacin, fluvoxamine, oral contraceptives, phenylpropanolamine	montelukast, phenytoin, smoking components of cigarettes
CYP 2C8	repaglinide, paclitaxel, methadone	gemfibrozil, fluvoxamine, ketoconazole, trimethoprim	rifampin
CYP 2C9	celecoxib, warfarin, phenytoin	amiodarone, fluconazole, miconazole, oxandrolone, capecitabine, etravirine, fluvastatin, metronidazole, sulfisopyrazone, tigecycline	carbamazepine, rifampin, aprepitant, bosentan, phenobarbital, St. John's wort
CYP 2D6	lidocaine, metoprolol, haloperidol, fluoxetine, amitriptyline, metoclopramide, codeine, oxycodone, tramadol	amiodarone, chlorpromazine, citalopram, bupropion	rifampin, dexamethasone

Figure 1.54: Drug interaction with P450 enzymes

Observation 1.25 When looking at the plasma half-life of control subjects vs geriatric subjects, we can see that there's seem to be an increase in plasma half-life. However, we need to note that there are still a few outliers where older patient has the same drug metabolic rate as those who are younger
 ⇒ We cannot jump into conclusion based on the patient age.

Example 1.3.4. **Warfarin** is a anticoagulant drug that can prevent patients from heart attack. The downside to said drug is that it has a very narrow margin of safety

When tracking the metabolism of warfarin over a range of patient of different age. We can definitely see a trend of decrease metabolism however the variation is gigantic between individuals.

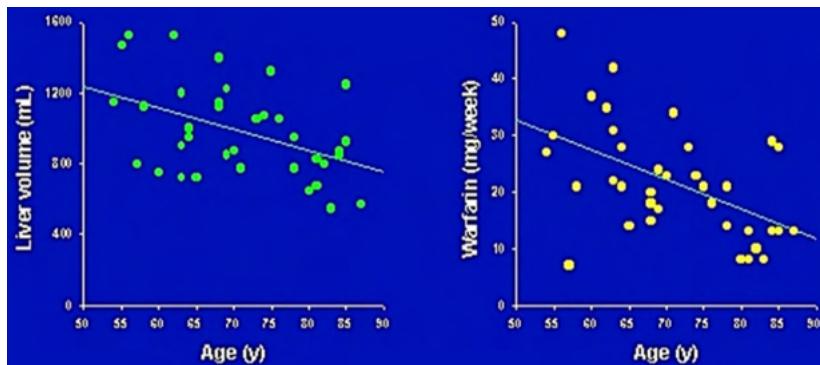


Figure 1.55: Warfarin breakdown through age.

The individual variations we've just shown above can be due to varieties of different reason. One of the main is **genetic variation**.

Example 1.3.5. Normally on average, the drug concentration at a specific timeline for a group of individual will follow graph A with the normal distribution. However, when you begin to consider individual variation or change a something about the environment, you'd get a shift to either extremities on graph B.

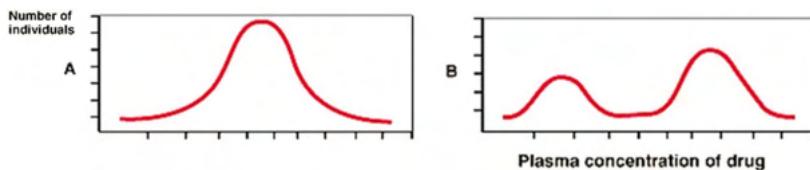


Figure 1.56: Graph A with the population of a drug concentration at a specific time (distribute normally) while Graph B with distribution in each extremities.

Observation 1.26 If we look more into the genetic variation of individuals, we would find some individual who has duplicated genes on one of the alleles which are **ultrarapid metabolizer** which metabolize at a very high rate. Then, you have majority of people with normal/extensive metabolizer with both normal alleles (or even a slight defect on 1). Then, as one or two of the alleles is defected, the their metabolic enzymes are worsened hence they're **intermediate metabolizer**. Lastly, the worst case is when

both alleles are completely null making them a **poor metabolizer**

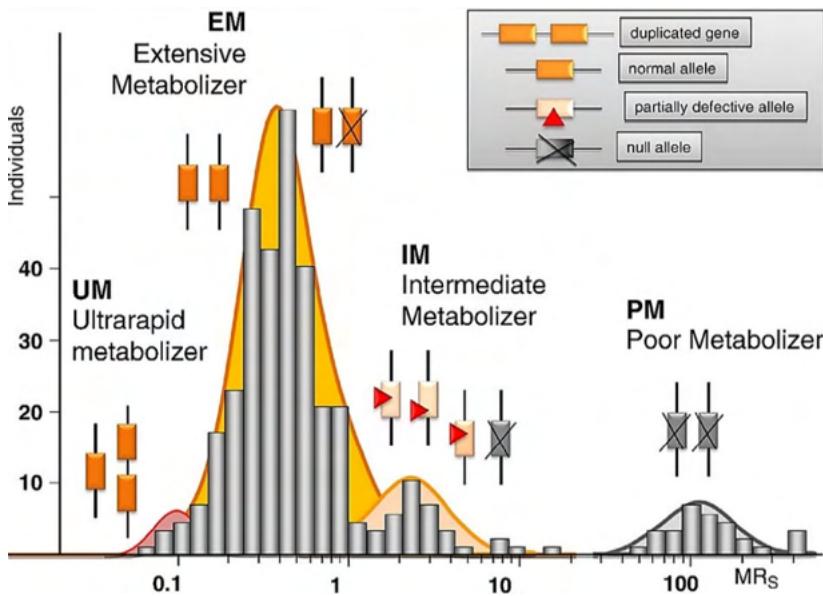


Figure 1.57: Population of different metabolizer

Example 1.3.6. P4502D6 is a P450 that can metabolize some common drug. This specific enzyme is polymorphic i.e. it can be different from 1 individual to the next.

For this fact, we can see that it's missing in around 7% of the population meaning that they can still metabolize the drug it's just it will take a longer time via other enzymes. On the other hand, it can be hyperactive in 30% of population.

Remark 1.17. *Interestingly, if a person does not have 2D6, when taking codeine, the body cannot break it down to morphine in order to yield the effect.*

Observation 1.27 The biological variations of the enzymes can affects the metabolism of a drug. We can see that the homozygous wild type enzymes will breakdown drug at a safe margin. For the heterozygous variant type,

you'd get a slight toxicity. In the worst scenario, you'd get very high toxicity of drug due to inefficient metabolism of said drug .

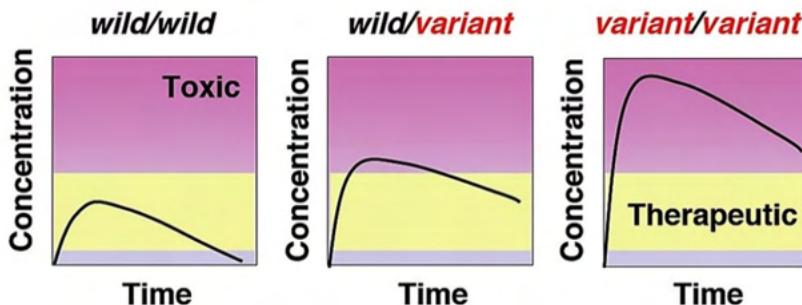


Figure 1.58: Biological variation of enzymes

Observation 1.28

When a metabolizer are poor, the plasma level of drug keeps increasing with every dose uptaked. This is because they can hardly breakdown the original dose which lead to toxicity.

Meanwhile, a metabolizer who are extensive or normal, the level of drug in plasma will drop at the beginning of new dose administration.

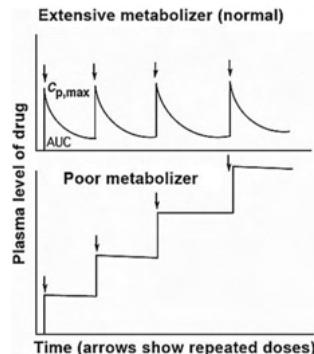


Figure 1.59: Poor vs extensive metabolizer

Pharmacogenomics

Definition 1.50. **Pharmacogenomics** is a specific field in pharmacology that investigate the genetic variation between people in terms of their ability to metabolize drug.

Example 1.3.7. If for a specific drug, we can know that there could be 5–6 variation of metabolizing enzymes for it, we can then test the patient for it in advanced.

On the other hand, if a specific genetic variant only show up 1 in 1000 patient, it would be unnecessary to test for it.

Other Drug Metabolizing Enzymes

Evidently, beside the P450s, there could be other enzymes to metabolize xenobiotics and drugs.

Example 1.3.8. We have enzymes in the liver that breakdown alcohol. To be more specific we have **alcohol dehydrogenase** (in the cytosol) that turn alcohol into acetaldehyde. Next, in the mitochondria, you have **acetaldehyde dehydrogenase** to turn acetaldehyde to acetate.

1.3.3 Metabolism: Phase II

We've just looked at Phase I, now we're focusing on phase II which can facilitate elimination of drug via the kidney.

In phase II, we have transferase instead of CYP. These transferases will act on the phase I metabolites by transferring a substrate it carry onto it. 2 of the most common transferase substrates are: **glutathione and glucuronic acid**.

Example 1.3.9. After aspirin undergo hydrolysis, it will take on its active form. Some of these active form can be eliminated by urine. Majority will enter phase II of metabolism where most of it will be deactivated by adding a glucuronide on it.

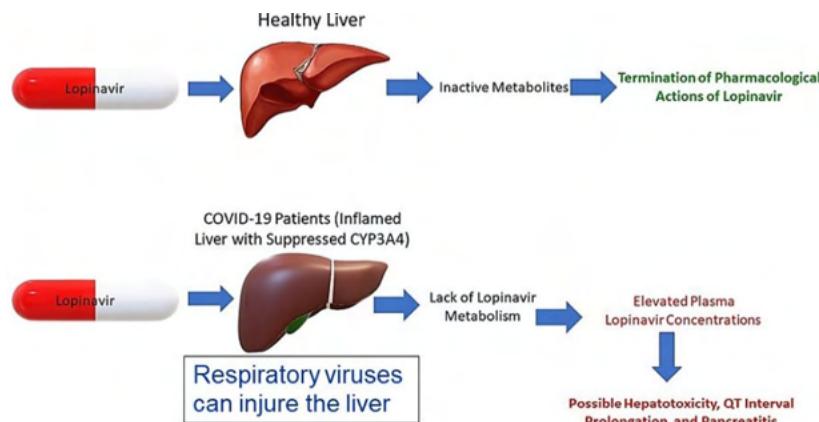


Figure 1.60: Healthy liver vs inflamed liver on Lopinavir metabolism.

Remark 1.18. Some drug during metabolism can damage the liver leading to some toxic consequences like necrosis, apoptosis, oxidative stress and etc.

Example 1.3.10. The drug **lopinavir** can be metabolized into inactive metabolites in a normal healthy liver. However, it was seen in cases of COVID-19 patients with inflamed liver that their CYP3A4 is suppressed. This leads to less metabolism of Lopinavir. This leads to elevated Lopinavir plasma concentration \Rightarrow Possible **hepatotoxicity**, **QT interval prolongation** and etc.

1.3.4 Excretion

Definition 1.51. **Excretion** is the last step of pharmacokinetics which is the removal of drug from the body.

Observation 1.29 The **kidney** is very effective at getting rid of exogenous compound because it can filter, secrete and reabsorb.

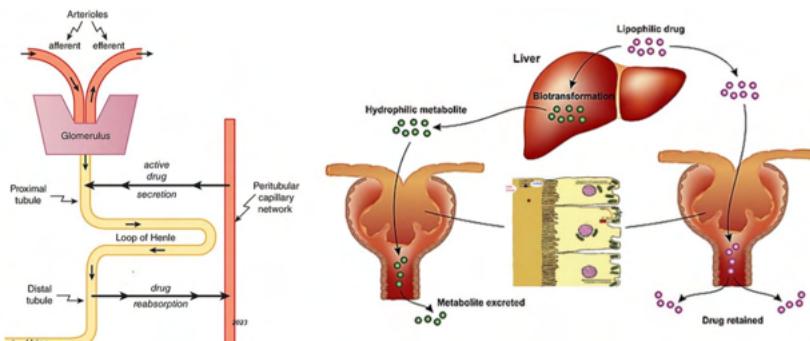


Figure 1.61: Drug elimination in the kidney.

Mechanism of Action (Drug Excretion in Kidney): After drug has been metabolized in the liver, it will be brought to the kidney.

1. Free drug will be filtered through the glomerulus and enter the tubules. If it's bound to albumin, it cannot go through.
2. At the proximal tubules, there are some secretions of active drug from the **peritubular capillary network** to it.
3. Then after it completes the loop of Henle, in the distal tubule, some drug might be absorbed back to the peritubular capillary.

This also highlights the importance of phase II metabolism where conjugation will make the drug water soluble. When it's more water soluble it can be excreted and sometimes reabsorbed easily.

Example 1.3.11. Starting with a drug, around 66% will be metabolically degraded in the liver while 33% will be eliminated by the kidney unchanged. Then from the 66%, half of it will be eliminated by the liver while the other half is eliminated by the kidney.

Remark 1.19. Some drugs can also be eliminated through exhalation. This is essentially how **breathalyzer** measure alcohol level

Similarly, some drugs can be secreted in the salivary glands e.g. cannabis, or even from the sweat gland.

Drug Clearance

Definition 1.52. **Clearance (CL)** quantifies the elimination of the drug. It's the volume of body fluid cleared per time unit and is typically constant for 1 individual.

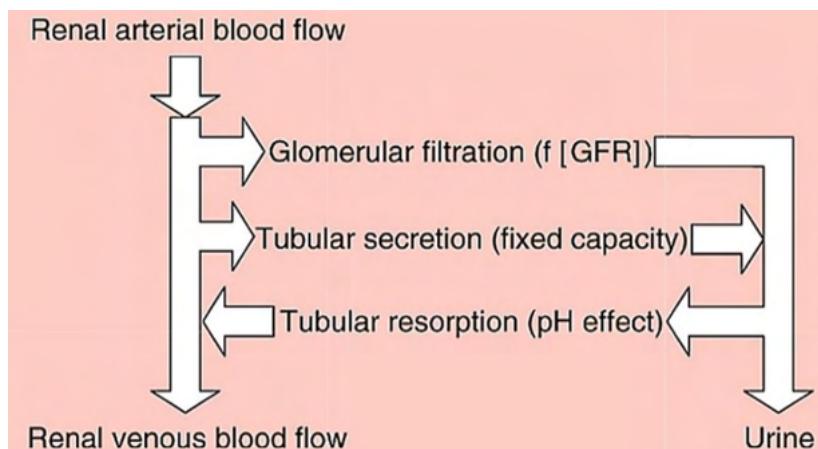


Figure 1.62: Renal clearance.

Example 1.3.12. **Renal clearance** looks at how much blood is cleared by the kidney per unit of time. It's defined as the following equation

$$\text{Renal CL} = \text{filtration} + \text{secretion} - \text{reabsorption} \quad (1.5)$$

Likewise, we can also define a clearance for the liver i.e. **hepatic clearance**.

Definition 1.53. The **total body clearance** is defined as the sum of individual organ clearances and is given as

$$CL_{\text{total}} = CL_{\text{hepatic}} + CL_{\text{renal}} + CL_{\text{pulmonary}} + CL_{\text{other}} \quad (1.6)$$

Time Course of Drug Elimination

We can now go back to the time course of drug elimination i.e. we're trying to quantify the rate of drug elimination.

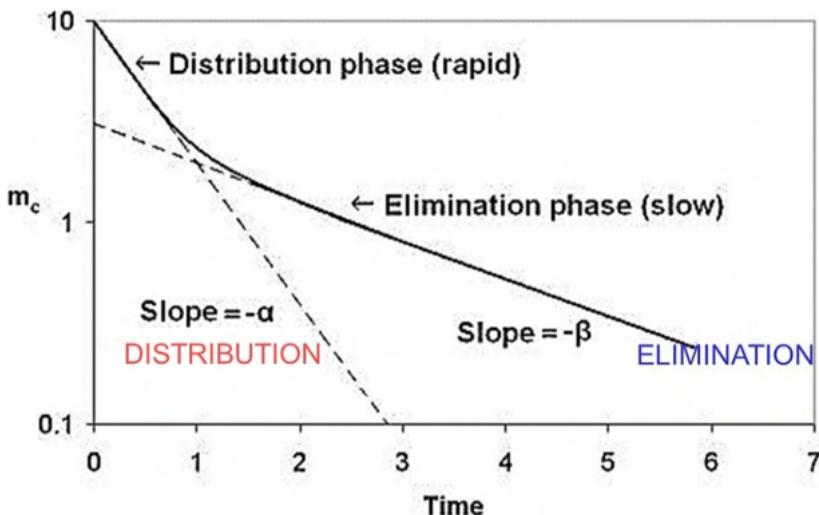


Figure 1.63: Distribution and elimination graph

Observation 1.30 We can look at the curve of distribution and elimination phase. In the beginning, after drug is redistributed in circulation, the distribution phase falls rapidly. But then, it will slow down as drugs are being metabolized and removed in the kidney.

Focusing on the elimination phase, we can see that it's a fairly straight line i.e. it's getting rid of the drug at a constant rate of time.

Definition 1.54. The **half-life** is the time it takes to get rid of half of the original concentration of the drug.

Definition 1.55. **First-order kinetics**, in this context, is a model of drug elimination where the amount of drug in eliminated is proportional to time elapsed i.e. constant fractions is eliminated per unit time.

Example 1.3.13. Looking at the plot of first-order kinetics of a drug, we can see calculate its half-life to be around 8 hours. WE can see that every 8h, the concentration kept dropping down by half each step. Until it reaches around 6% of the original concentration, we will regard this as fully cleared as at this concentration it's no longer clinically effective.

This also means from the graph, it takes around 4 half-lives to eliminate the drug (32 hours).

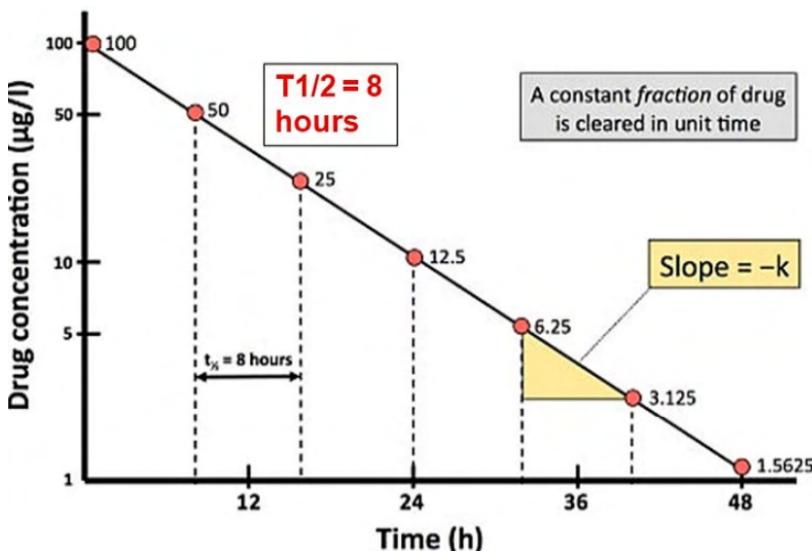


Figure 1.64: Drug elimination and half-life.

Remark 1.20. This means that drugs are relatively fully eliminated after 4 half-lives.

Remark 1.21. The half-life does not depend on the dose of the administered dose as you enzymes can metabolize them very effectively.

Not all drugs follow first order kinetics. Some drugs are eliminated at constant amount per unit of time instead of fractions. However, for a drug

follow first order, with a high enough dose, it can saturate enzymes and turn it into zero order.

Example 1.3.14. Aspirin follows first-order kinetics and has a $t_{1/2} = 3\text{h}$. However, at higher dosage of aspirin, the enzymes are fully saturated turning it into zero order where $t_{1/2} = 15\text{h} \Rightarrow$ longer to eliminate aspirin.

Dosage Scheduling

With the half-life we've just talked about, let's look at an effect scheduling of drug so that the amount of drug is at an effect level in the plasma.

Essentially, we want to give a drug a dose where its plasma concentration stay above in the minimal level and within the therapeutic window (below the minimal toxic level). Typically, this treatment will reach a plateau after 4 half-lives since drug is eliminated after 4 half-lives. This is for oral but the same can be observed with IV injection.

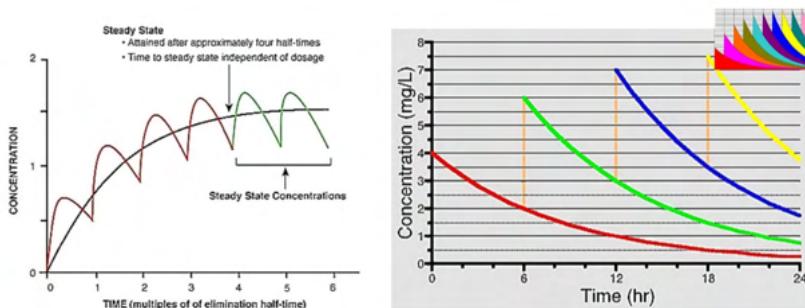


Figure 1.65: Oral vs IV drug scheduling plateau.

Observation 1.31 The dose is also important as we don't want too large of a dose that can lead to toxicity nor too little of a dose that can lead to under therapeutic. Not only that, you need to give it frequently enough to stay in the therapeutic range.

This also means it's much more effective to give small dose at high frequency than large dose every now and then.

Remark 1.22. Like any other, there are outliers such as people with slow metabolism where a normal frequency of drug can lead to toxicity.

Observation 1.32 In emergency situation, we need to have the drug to be immediately shoot up to the therapeutic range instead of slowly build up normally. For this, we will do **dose loading** where we give the patient very high dose of said drug then give maintenance dose so patient will stay in the therapeutic range.

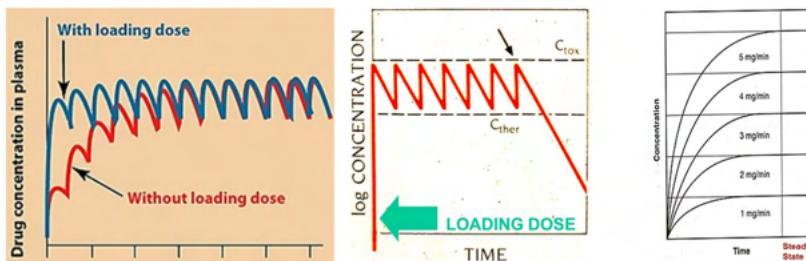


Figure 1.66: Loading dose and infusion rate plateau.

Remark 1.23. *It does not matter what infusion rate of a drug, they will all plateau at the same time.*

End of Lecture —

1.4 Special Topics In Pharmacokinetics

In the first 20 minutes of this lecture, it was simply a re-iteration of what the previous 4 lectures have been talking about hence no notes were taken.

Now, we will begin with how drug discovery and development happens in pharmaceutical companies and research labs.

1. **Discovery:** First, you have to scan through thousands of compounds in order to select good candidates to target a specific site you're after.
2. **Preclinical–Phase 0:** From preclinical up to phase 0, you'll mainly be testing the selected compounds for its toxicity, pharmacokinetics and dynamics.

3. **Phase 1–2B:** In phase 1, the compound will be tested on healthy individuals (typically from 20-40 yo) for its safety. In phase 2, the compound will be tested on patients with the disease where the drug is supposed to treat.
- Remark 1.24.** *If there's an emergency for this drug e.g. pandemic, we will skip phase 3, send to market and monitor its action strictly.*
4. **Phase 3:** In this phase, the drug will now be tested on larger population of patients to see its effects and maybe compare it to existing treatments.
 5. **Phase 4:** This is the final phase where the drug is marketed and monitored.

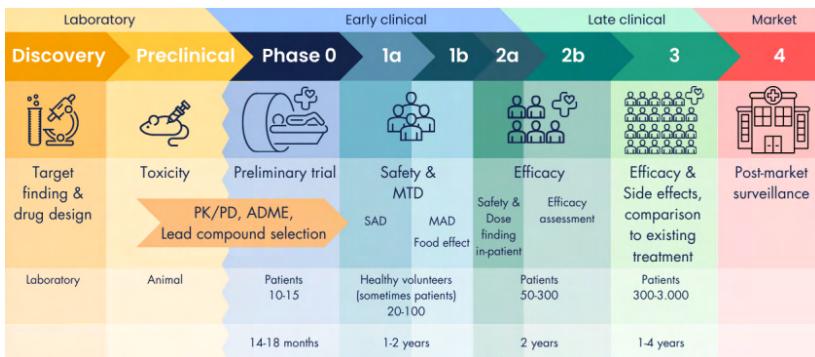


Figure 1.67: Phases of drug development.

1.4.1 Adverse Drug Reaction and Individual Variability in Drug Response

Definition 1.56. **Adverse drug reaction (ADR)** is a non-desired reaction that can happen when taking a drug. This non-desired reaction can be negative, positive but also changes nothing.

Definition 1.57. **Individual variability in drug response (IVDR)** is a concept where a drug may trigger different kind of response in different individuals.

Remark 1.25. These 2 concepts are not necessarily mutually exclusive i.e. one does not have to require another.

The reason there is such thing as IVDR is because we're all different from one another. Different people can have different physiological or even pathological response associated with specific pharmacokinetics/ pharmacodynamics. Evidently, another factor that can affect this is age

Example 1.4.1. In clinical trial, we tend to look at the difference between men and women as the 2 sexes are physiological different.

1.4.2 Pharmacokinetics During Pregnancy

Observation 1.33

First, let's look at the absorption. During pregnancy, we see an decrease in gastric emptying, intestinal mobility and gastric acid secretion. Most notably, gastric acid pH↑ ⇒ certain oral drugs that need acidic environment to be activated/absorbable will not work.

Interestingly, after a consumption of coffee, it was found to last in the body much longer if you're pregnant than if you're not.

Observation 1.34 When it comes to distribution during pregnancy, we see an increase in plasma volume and total body water ⇒ lower amount of drug bound to albumin (since it's less⁷) ⇒ more free drug to bind to site of actions.

Observation 1.35 Metabolism during pregnancy is quite contradictory, at least for drugs. This is because we will see a large decrease in hepatic drug metabolism yet there's a large hepatic blood flow.

For elimination, there's an increase in both glomerular filtration and renal secretion i.e. you're excreting drugs/things much higher than before.

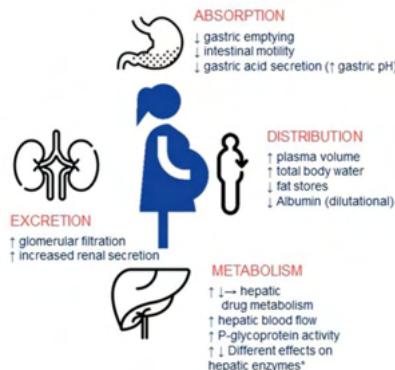


Figure 1.68: Changes in PK during pregnancy

⁷proportionally to the plasma volume

Effects on Fetus

Misconception. For a very long time, it was widely believed that the placenta is a barrier that protects the fetus from any harms originate from the mother. This was a definitive false as we've found there to exists many transporters and passage to the mother.

Example 1.4.2. The **Zika virus** is a strain of virus that can cause major birth defects. Once a pregnant mother is manifested with said virus, she will not be affected but her baby would.

Concept 1.12 *Different period of embryonic/fetal development will lead to different effects when it comes to exposure of the fetus to drug.*

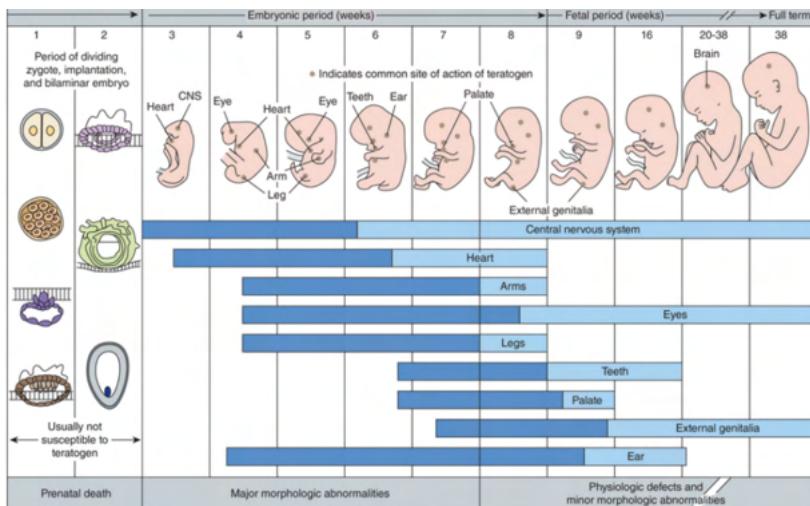


Figure 1.69: Stage specific effects during development.

Example 1.4.3. **Thalidomide** is a drug administered to pregnant women, mainly to treat morning sickness, was found to cause serious birth defects, namely, **thalidomide embryopathy**.

Exposure of the fetus to this drug can lead to Both of which are classified as **phocomelia** (defects of the extremities). To be more specific, exposure of around days 27–30 will lead to arm defects while days 30–33 will lead to leg defects.

From the fact that lots of things from the mother body can be easily transferred to the fetus, we can also realize the following.

Notion 1.11 A vaccinated mother, during pregnancy, can also transfer said antigen to the fetus and immunize it too.

Remark 1.26. *These vaccines must be non-living e.g COVID-19, flu mRNA vaccine.*

1.4.3 Pharmacokinetics in Infants and Aged Population

Observation 1.36 For a newborn babies, we see an increase in gastric pH, gastric emptying, respiration and even metabolism. This can then highly effect drug delivery.

Most strikingly, when an infant of ≤ 3 months old gets an infection of any sickness, they are required to be taken to the hospital. This is because it's the age where we're unsure how drug might be metabolize orally. i.e. drugs given to these ≤ 3 months old are IV injections.

Factors Contributing to Altered Drug Effects in Older Adults
Altered Drug Absorption and Disposition
<ul style="list-style-type: none">• Decreased gastric acid• Decreased lean body mass• Increased percentage of body fat• Decreased liver mass and blood flow• Reduced renal function
Altered Response to Drug
<ul style="list-style-type: none">• Altered receptor and/or postreceptor properties• Impaired sensitivity of homeostatic mechanisms• Common diseases: diabetes, arthritis, hypertension, coronary artery disease, cancer, glaucoma
Social and Economic Factors
<ul style="list-style-type: none">• Inadequate nutrition• Multiple-drug therapy• Noncompliance

Figure 1.70: Factors in elderly leading to drug effects alteration.

Observation 1.37 Contrary to the infants, where most of everything is increased for growth; in the elderly, most of everything is slowed down.

What's needed to be noted is the aged population are more vulnerable to chronic illnesses \Rightarrow an increase in medication taken \Rightarrow an increase complexity of possible drug interactions.

Not only that, in the aged population, the response to drugs can also has a large variation compared to the youngs. See Figure 1.70 for more factors that can alter drug effects.

Pharmacogenomics

We'll expand a little on pharmacogenomics from the previous lecture. We know that some of us may have variations in P450 enzymes which can lead to different response to drug

Example 1.4.4. CYP2D6 is an enzyme that metabolizes almost 25% of the drug we use (codeine is one of them) \Rightarrow an impairment and mutation will disable the person from taking 25% of the drug. What's more staggering is that variations of CYP2D6 can differ from just 1 nucleotide or even a large difference.

In certain individuals, you'd have an extra copy of the CYP2D6 copies \Rightarrow Ultrarapid CYP2D6 \Rightarrow metabolizes codeine to morphine too rapidly \Rightarrow overdose.

On the other hand, when losing a copy or has a dysfunctional copy \Rightarrow poor CYP2D6 \Rightarrow barely metabolizes codeine \Rightarrow no significant therapeutic effect from drug.

Observation 1.38 It is remarkable that we have such extensive knowledge about the genetic differences between individual and even biological variants between enzymes so...**why is that some drugs has high response rate while others are quite low?**⁸

Well...there are 2 reasons why. First, we do not actually apply everything we know from pharmacogenomics to account into making and giving the drug. This is because in order to get a complete sequence of a person, it will be quite costly.

Second, which is more important, is because of our **microbiome** and the stuffs we eat.

⁸The given drug was **COX-2** which has a response rate of 80%

1.4.4 Pharmacobiomics

Definition 1.58. A **microbiome** or **gut microbiota** is a bifunctional and heterogeneous ecosystem, often described as a ‘metabolic organ’, contains over **100 trillion microbes and 5 million genes**, making it much larger than human gene count (around 150 times).

Definition 1.59. **Pharmacobiomics** is a term used to describe the interaction between the microbiome and drug response which alter pharmacokinetics.

Notion 1.12 Lots of things can contribute to the building of a person microbiome

Explanations. It is evident that when a person repeatedly eat a particular food, live in a specific region or even having a routine/way of living. Their microbiome would be altered. □

Concept 1.13 *The nature of the microbiome can alter the body physiology.*

Immunotherapy and Pharmacobiomics

Example 1.4.5. A research paper from 2017 in Texas outlined the fact that gut microbiota has some correlation with the responsiveness of patient undergoing immunotherapy.

Just a little recap, some of our T cells have maintenance duty where they would check every cells in the body to see if it's defective or not. If it is, T cell will eliminate it. This detection is through the binding of the T cell receptor, known as **checkpoints**. If cell has the right complementation, T cell will leave it alone, otherwise \Rightarrow death.

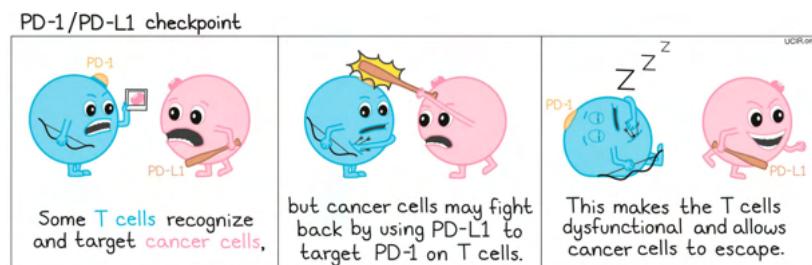


Figure 1.71: PD1/PD-L1 checkpoints

In cancer cells, it can fight back with its own receptor called **PD-L1** that can bind to the T cell PD-1 \Rightarrow dysfunction T cells and escape the check-point regulation.

Immunotherapy, in this case, is to administer anti-PD-1 or anti-PD-L1 to block said interaction between cancer cells and T cell \Rightarrow T cell is not dysfunctional and will check with its other receptors for complementation \Rightarrow cancer cells have none \Rightarrow death to cancer cells.

Now, let's get into the details of that experiment and how it was carried out.

Methods 1.6 First, obtain the fecal matter⁹ of individuals that responds to immunotherapy, we shall call **R**, and individuals that do not respond to immunotherapy, we shall called **NR**.

Grow a population of **germ-free mice** in the lab and separate them into 2 groups. In 1 group, we will perform a **fecal matter transplant (FMT)** of the R individuals to the mice; on the other group, FMT of NR is done.

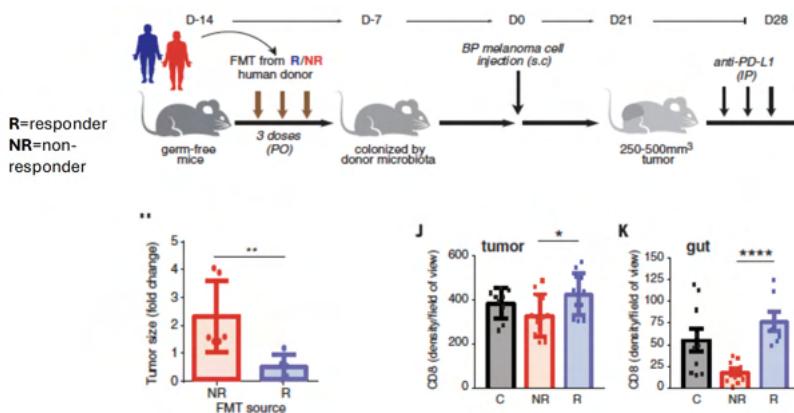


Figure 1.72: Mouse R and NR FMT experiment.

After, induce the same type of cancer/tumor on the all of the mouse and treat them with the similar immunotherapy and see which is more effective.

⁹A more scientific way of saying...poop

Remark 1.27. *For any scientific experiment, it's evident to have another population for control group as a reference*

Explanations. Now, in the end, what we'd find is that the mice with the R-FMT will recruit more T cells toward the tumour but also the gut than the controlled . Most importantly, the tumour size of the R-FMT mice are significantly smaller than that of NR-FMT mice.

What's interesting is that looking at the diet of the R and NR individual, scientist found that the NR individual takes a high amount of probiotics. This is not necessarily bad but in this situation, taking probiotics will promote the growth of 1 variants/family of bacteria while ignoring the rest \Rightarrow **no diversity**. The R group did better since their diet is mainly high in fiber which promotes microbiome growth as a whole \Rightarrow diversify microbiome.

□

Personalized Medicine

All of what we've said about pharmacogenomics and pharmacobiomics all lead back to the same and new beginning called **personalized medicine** i.e. medication with high specificity for each individuals.

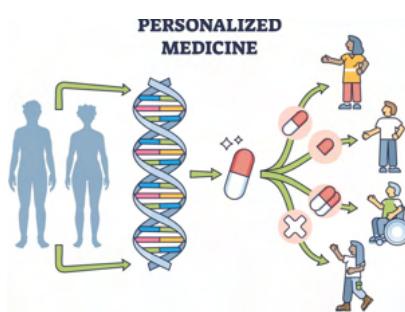


Figure 1.73: Personalized medicine banner

these matter, bringing us ever so close to achieve our goal of personalized medicine.

We've obviously discuss about the costly price of doing genetic sequencing thus it wouldn't be a great choice for personalized medicine ¹⁰. We could also try to take fecal matter from individuals to test for it. But this also comes with many people find it to be, more or less repulsive and will be dismissive in doing so.

In all cases, as we speak today, there are continuous research on

¹⁰At least for now, who knows what awaits us!

1.4.5 Pharmacokinetics of Biologic Drugs

In the last part of today's lecture, we will look at biologics drugs.

Definition 1.60. A **biologic drug** is a that's originated from an organism or their cells. e.g. **monoclonal antibodies (mAb)**.

Brief Overview of Antibodies

Definition 1.61. **Antibodies** or **immunoglobulins** are proteins that are produced by the immune cells, specifically B cells.

Observation 1.39 There are 5 different types of antibodies produced in our bodies: IgG, IgM, IgA, IgD and IgE.¹¹ The PD-1 immunotherapy we've discussed above uses antibodies in the form of IgG.

Types and characteristics of antibodies		Distribution in the body
IgG		<ul style="list-style-type: none"> Highest opsonization and neutralization activities. Classified into four subclasses (IgG1, IgG2, IgG3, and IgG4).
IgM		<ul style="list-style-type: none"> Produced first upon antigen invasion. Increases transiently.
IgA	 or or 	<ul style="list-style-type: none"> Expressed in mucosal tissues. Forms dimers after secretion.
IgD		<ul style="list-style-type: none"> Unknown function.
IgE		<ul style="list-style-type: none"> Involved in allergy.

Figure 1.74: Different Ig in the body.

When a person underwent vaccination, it's mainly the IgG that help with the long term immunity. IgA and IgM are more transient i.e. they're produced extremely large volume in a short period (during infection) but is also quite unstable.

¹¹the "Ig" stands for immunoglobulins.

Observation 1.40 (Structure of IgG). IgG is made from 2 light chain and 2 heavy chain forming a Y-shaped antibody. On each tips of this anti-body exists the **variable region** bind to an epitope. Note that this **variable region** can be design so that it's high specific for 1 epitope.

On the bottom of this antibody, you have the constant region that can bind to the Fc region and recruit white blood cells (such as basophil, eosinophil and neutrophil) to the site.

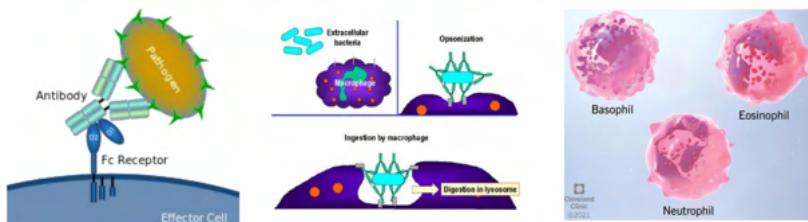


Figure 1.75: Fc region actions and different white blood cells

Mechanism of Action (Fc region): Briefly go through, when you have an infection, IgG will be produced a lot.

1. The release specific IgG will come and bind to the epitope of the bacteria/pathogen from the infection.
- 2a. Once bound, it can either bind directly to the Fc region of a white blood cells.
- 2b. Or, it can bind to the Fc of an effector cells which recruit white blood cells.
3. In the end, the recruited white blood cells will perform its jobs and destroy the pathogen.

So now, back to the immunotherapy, by introducing an antibody that block PD-1 or PD-L1, we're essentially inhibiting blocking 2 interaction at once. In either case, one of them will be phagocytosed which happened to be the cancer cell.

Antibodies vs Drugs

When it comes to the pharmacokinetics of these biologic drugs such as antibodies, we have to note the size differences between them \Rightarrow highly

likely that pharmacokinetics will change. Not only that, because they're too large (150kDa), navigating through endothelial cells would pose a challenge \Rightarrow inadequate distribution.

This leads us to the next things to consider, route of administration. Because of the size, it cannot be easily absorbed in the GI \Rightarrow best via IV injection \Rightarrow limiting methods and requires physician/medical practitioner. Furthermore, its weight also disable it to be cleared from circulation via renal clearance.

Another thing is that these antibodies might have some chemical modification, be glycosylated which marked it for clearance through the liver. All of which alter the pharmacokinetics and the therapy as a whole.

Summary

1. Properties of drugs and those of patients can affect the distribution of drug.
2. Patient factors can influence the rate and degree drug distribution.
3. Disease states and drug interactions can affect the degree of protein binding and/or transport.
4. Multiple person to person differences in pharmacokinetics results in altered.
5. Variables that effect drug efficacy are age, sex, microbiome composition.

1.5 Biological Adoptions

Within the last 20 years, The number of drug overdosing in the USA has increased in size by 6-fold. This crisis is mediated by lots of different drug but the main perpetrator is **fentanyl**.

Observation 1.41 In Canada, we're also seeing a rising trend from the past 2 years, projected to be nearly 10,000 deaths within the next few years.

What we've also realized is this statistics is more skewed toward the West Coast of Canada with the highest being British Columbia, follows by the provinces around the Prairie.

Interestingly, not just only for heroin/fentanyl that we've seen a rise in consumption and overdose. We've also found that the consumption of cannabis, alcohol and even tobacco during COVID-19. What's worst is that even when the pandemic slowed down the statistics of these consumption is still high and continues to rise.

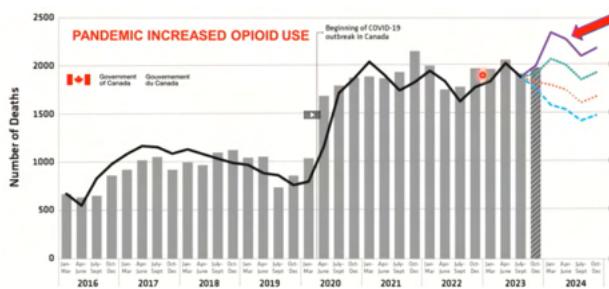


Figure 1.76: Canada statistics on opioid use.

Now, we will dive into some biological adaptions to drugs by the body. When it comes to this adaption, we'll be looking at 4 concepts: tolerance, addiction, dependence and withdrawal.

Definition 1.62. **Tolerance** is a person's decline response to an original dose i.e. it requires a higher dose to reach the same therapeutic effects.

Definition 1.63. **Withdrawal** is the physical/psychological reaction (could be severe) when drug is not taken.

Definition 1.64. **Dependence** is when the body physically relies on a drug to avoid withdrawal. Extending from this, **addiction** is simply dependence but for also the psychological effect.

Notion 1.13 A drug do not need to be addictive to induce tolerance, withdrawals and dependence.

Example 1.5.1. Certain hypertension drugs can cause dependence (blood pressure cannot go down without it) but is not addictive as it does not provide any psychological effect that patients need.

Observation 1.42 So now you might wonder, if a drug can cause dependency would it be highly controlled instead of OTC¹²? Well...not really there are some non-controlled drug that can cause dependency e.g. long-acting nasal spray can cause dependency which is why it has restriction of usage maximum in 1 week.

1.5.1 Tolerance

Knowing what tolerance is, how do you actually study it? Well...we can use some rat model experimentation.

Methods 1.7 A rat is put in an enclosed environment. It is then put on an IV line that's connected to a syringe of addictive drug. Within the enclosed space, there's a lever where the rat can press to self-inject with the drug. We then track how often the rat press the lever (or even the dose of injection).

Observation 1.43 Giving any normal drug, the rat will press the lever not so often. On the other hand, giving drugs like opioid, heroin, morphine etc., the frequency increases significantly.

Historical Background. In 1952, a man was admitted to an institute for overdosing on opioid. After treatment, he stated that he will go out and get more of said opioid...again. Researchers and doctors at the institute decided to invite the man to stay back so that they can give him some opioid + study his behaviour.

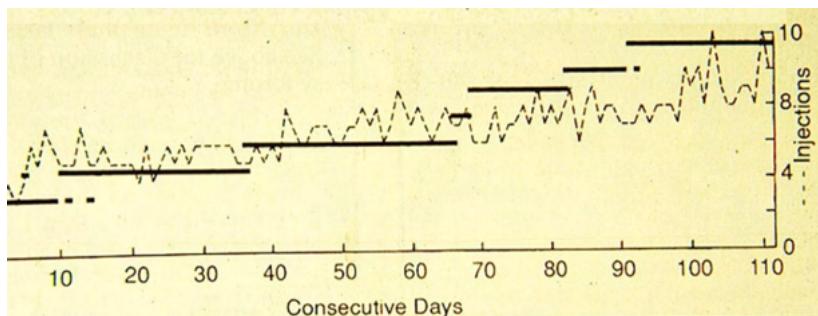


Figure 1.77: Human opioid experimentation in 1952.

¹²OTC is over-the-counter

Methods 1.8 The man was allowed to inject opioid at whatever dose he wanted and at however frequent he wanted. Researcher are not to intervene as the dose and frequency get higher but only to collect the data.

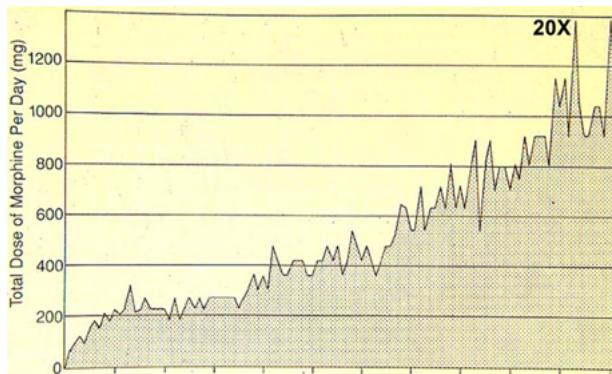


Figure 1.78: Total dose graph of the 1952 experimentation.

Observation 1.44 In the first week, we can see that he's injecting around 40mg of opioid twice daily. After several months of this experiment going, the dose tripled to nearly 120mg and 5-fold the frequency of 10 times per day (see Figure 1.77). If we were to record the total dose he took per day, we see an almost linear increase and by the time it reaches its max, the total dose was 20 times that of the original. See Figure 1.78

Observation 1.45

If we look at another experimentation on rat analgesia effect vs morphine concentration. What you'd see is that analgesic effect will decrease more and more while the morphine concentration gets higher and higher (over a long period of time).

This brings us to subcategories of tolerance as the following definition.

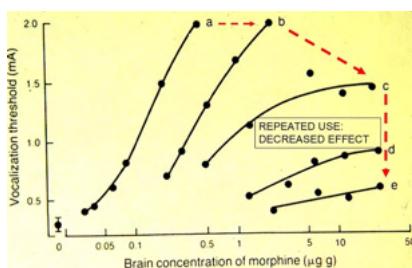


Figure 1.79: Mouse analgesia with morphine concentration.

Definition 1.65. Tolerance can be divided into 2 types relating to the period of time of drug administration:

1. **Acute:** a decrease in response with only a few (some cases 1) exposure to the drug (even at the same dose).
2. **Chronic:** repeated administration over a long period of time which cause a decreased response to a test (original) dose.

Definition 1.66. **Innate tolerance** refers to variability between each person i.e. the difference in metabolizing enzymes and various other things.

Definition 1.67. Beside chronic and acute tolerance, we can look at further subcategories within it such as:

- **Pharmacokinetics (PK) tolerance** is when you're metabolizing drug much faster than usual \Rightarrow [drug]↓ at site of action.
- **Pharmacodynamics (PD) tolerance** is when there's a decrease in effect due to biological adaptions.
- **Behavioral Tolerance** is when a drug has a decreased effect due to a conditioned response either conscious or unconscious.

Mechanism

Concept 1.14 With PD tolerance, the dose-response graph shifts down while for PK tolerance, the dose-response graph shifts to the right.

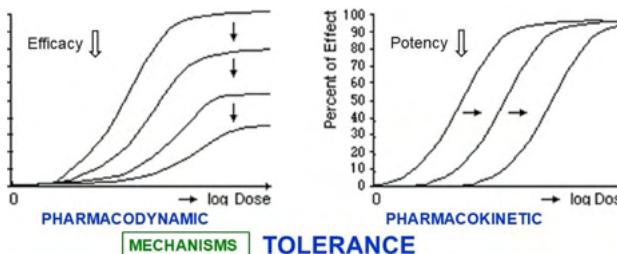


Figure 1.80: PD vs PK tolerance graph.

Explanations. Since PD tolerance lead to fast drug metabolism \Rightarrow efficacy of the drug would decrease thus the dose-response graph shifts down.

For PK tolerance, it's due to a decrease in effects from an original dose \Rightarrow need higher dose to reach normal effects as before \Rightarrow dose-response graph shifts to the right. \square

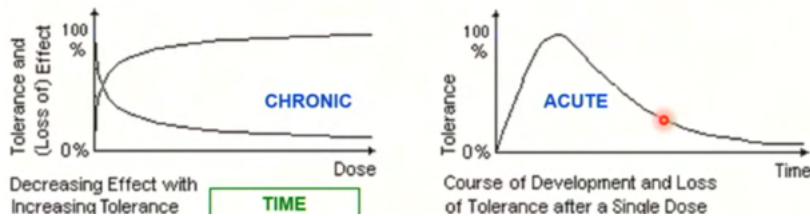


Figure 1.81: Chronic vs acute tolerance graph.

Sometimes, these mechanisms are not separated but can be combined together.

Example 1.5.2. Tolerance to anti-cancer drug is due to various mechanisms e.g. activation of DNA repair of cancerous cell will decrease the effect of drug (PK tolerance), activation of more P450 will increase metabolism of drug (PD tolerance), etc.

Now, remember the dosage scheduling of a specific drug where its concentration increases incrementally over time until it reaches a stabilization period. Now, look at the graph below, we can see that after a period of time, the concentration drop incrementally instead. So obviously, there's a tolerance but then **how can you tell whether it's PK or PD tolerance?**

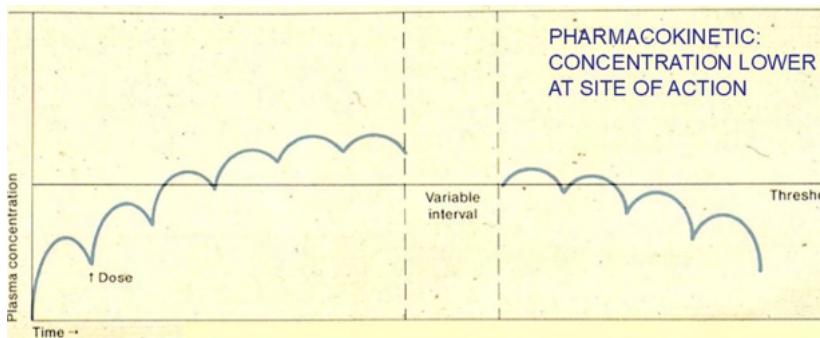


Figure 1.82: PK tolerance dose scheduling graph.

Well, remember that PD tolerance is when the [drug] decreases at the

site of action, while PD tolerance is when the effect of a drug is decreased. In the case above, we see a decrease in [drug] in the plasma even though dosage stays the same \Rightarrow body is metabolizing and getting rid of drug much faster \Rightarrow PK tolerance.¹³

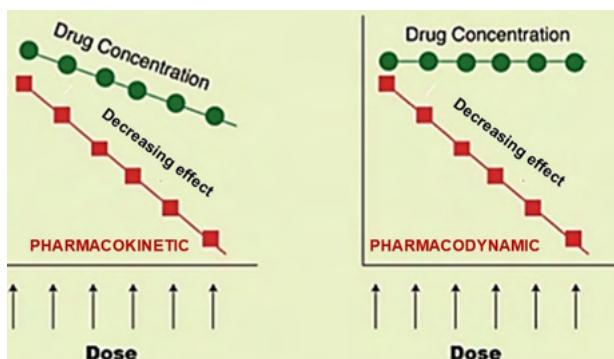


Figure 1.83: PK vs PD tolerance.

So, in both PK and PD tolerance you'd get a decrease in effect however the [drug] does not decrease in PD but only in PK. But in either case, to reach back to maximal effect, you need higher dose.

Remark 1.28. There are lots of drug that can develop tolerance and there's no universal structure for any of them.

Model to Study Tolerance

There are many ways to study tolerance listed below

- Whole animal
- Isolated organs
- Tissue culture
- Subcellular fractions

Another more modern way to study is using PET scan to look at patient's brain in response to specific drugs etc.

¹³In the case of PD tolerance, you'd expect the same graph as the normal (since this graph does not describe effect but only concentration).

Acute Tolerance

We will now look at the acute tolerance of cocaine.

Observation 1.46 A person is given a first controlled dose of cocaine, we see that after few minutes, the dopamine response to the drug rise to its maximal (700) then slowly diminish in the next couple of minutes.

When we wait a little after then give a second dose (same as first) of cocaine. Surprisingly, we see a decrease in effect by almost half. This is due to acute tolerance.

Basically the neurons are rapidly adapting to the effect of cocaine.

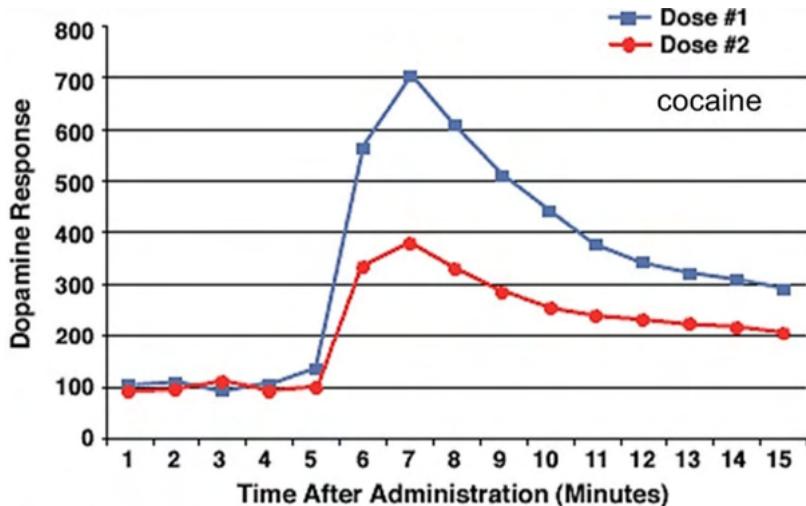


Figure 1.84: Acute tolerance of cocaine.

Observation 1.47 We're looking at the plasma concentration of the cocaine and the level of intoxication through the span of administration to elimination.

First, as cocaine is administered, its plasma concentration starts rising as well as the intoxication level. Eventually the intoxication level will plateau out. Then, as the plasma concentration keep rises (re-distribution), the intoxication level also drops. It will reach peak distribution level while the intoxication level still drop quickly. Eventually, the intox. level drops to 0 when the plasma concentration still dropping.

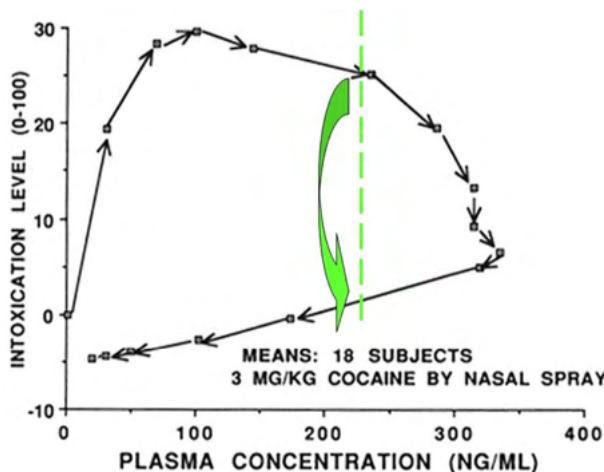


Figure 1.85: Cocaine intox level vs plasma concentration.

Essentially, when you're looking at the green concentration on the graph, we see a lower effect as compared to before and this is the acute tolerance.

1.5.2 Drug Dependence

The major phenomenon of drug dependence is PD tolerance. In drug dependence, we need drugs to avoid the withdrawal effects.

Observation 1.48 In the brain, there will be balance between its inhibition and excitation. If a drug causes inhibition, it will disturb this balance and tip the scale more to inhibition. The tolerance mechanism is simply the body counter response to push back toward excitation.

After a prolonged taking of this drug, so when we stop taking the drug, the body will keep doing the imbalance itself by increasing excitation. This is essentially what withdrawal is.

Mechanism of Action (Drug Dependence Cycle): Supposed that a person is taking a CNS depressant.

1. After administration of the drug, it will induce its effect and cause imbalance of the inhibition and excitation.

2. The body adaption mechanism activated and restore the balance (drug tolerance).
 3. When a person stop taking the drug, the body continue to do its adaptation rebalance which causes imbalance on the opposite side
=> withdrawal.
 4. If the person starts to go into rehab, they will recover from this neuroadaptation mechanism. Thus, they're no longer dependence on the drug.
- Unfortunately, some people will go back to administering the drug and the cycle restarts.

Neuroplasticity

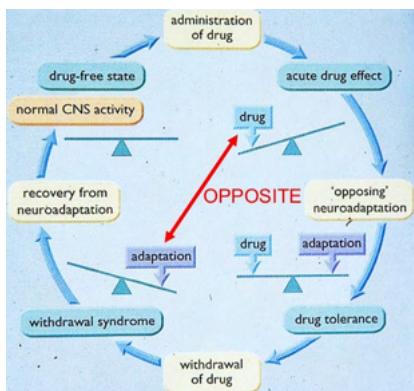


Figure 1.86: Drug dependence cycle.

by the amount of substrate, enzymes and cofactors. Additionally, you also have neurons adapting to taking agonist by making less of the receptors or more if it was an antagonist.

Definition 1.68. Neuroplasticity involves looking at all of these changes as a whole. It basically is the ability of neurons to change its activity in accordance with whatever stimuli it's receiving.

More Tolerance Types

Definition 1.69. Surmountable tolerance is a tolerance that you can overcome by increasing the dose. **insurmountable tolerance** is a tolerance that

An important concept that lead to biological adaption in the brain for drug is neuroplasticity. Before getting into the subject, let's take a brief walk on neurons. At the presynaptic neurons, we can see that there are many sites for neurotransmitter to propagate, synthesize, store, metabolize, release, etc.

All of the actions of these site can be altered in the presence of a drug e.g. the neurotransmitter enzymes for synthesis can be altered

you cannot overcome (as the efficacy is much lower now).

Explanations. The reason behind surmountable tolerance is a reduce in postsynaptic receptors i.e. it requires higher dose to reach the same effect. For insurmountable tolerance, the presynaptic neurons does not produce as much transmitter as before. □

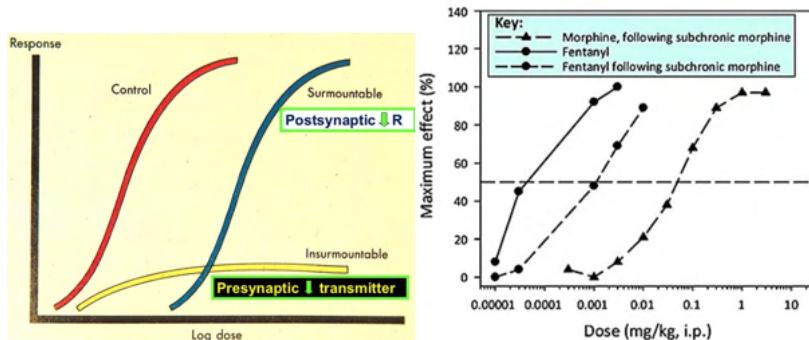


Figure 1.87: Surmountable vs insurmountable tolerance (left) and cross tolerance (right).

Definition 1.70. **Cross tolerance** is the body having tolerance to 1 drug would also have the same tolerance for another drug of the same family.

Example 1.5.3. When a person develop tolerance to fentanyl i.e. now they need a higher dose than before. When we inject the patient it morphine, a drug in the same family as fentanyl, the patient also have tolerance to it.

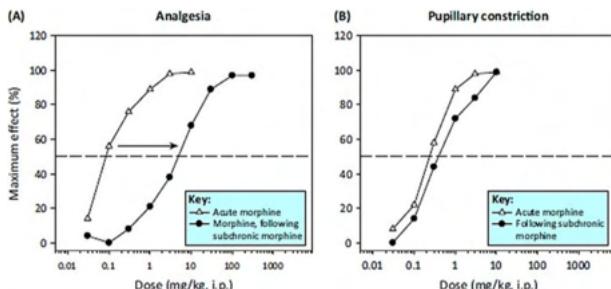


Figure 1.88: Differential tolerance.

Definition 1.71. **Differential tolerance** is the body tolerance does not happen at the same speed for different parts of the body.

Example 1.5.4. When taking morphine over a long period of time, the patient will develop tolerance for it. To be specific, it's the analgesic effect of morphine that will change i.e. requires a higher dose to reach the same analgesic effect as before.

On the other hand, when we look at the pupillary constriction of the patient, a typical response to taking morphine, there's only a slight change in dose i.e. it does not develop tolerance because of differential tolerance.

Observation 1.49 Differential tolerance can be dangerous as 1 site of the body may experience large tolerance while other experience only a little bit. Suppose that the other site is vital for functionality, that little nudge in tolerance would be enough to be fatal.

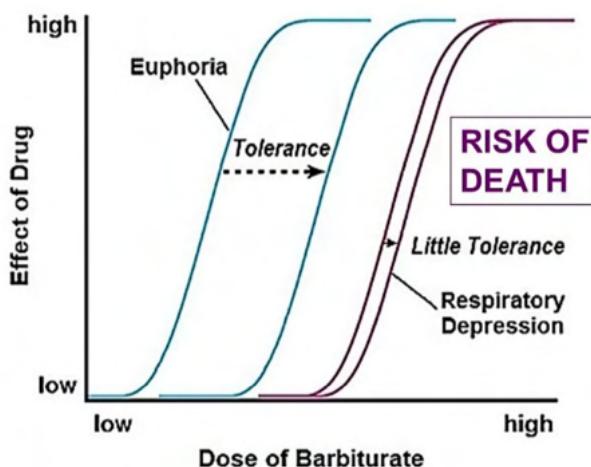


Figure 1.89: Differential tolerance of barbiturate.

Example 1.5.5. **Barbiturate** is a drug that can make the body develop tolerance to it. To be specific, it's the euphoric feeling that it provides will be heavily affected by tolerance. Though it only cause small tolerance to the respiratory rate but this small changes can lead to respiratory depression and possibly death.

Definition 1.72. **Behavioural tolerance** is the phenomenon where an individual alters their responsive to a drug due to repeated exposure, and this change in responsiveness is influenced by learned behaviors or environmental cues.

Observation 1.50 First, study a group of rat to find their lethal dose for heroin on first exposure. Now, rats are made to be addicted to heroin in a specific environment. As we increase the dose to possible lethality, the percentage of rat death decreases significantly. Now, if we were just put the same rats in a different environment, the lethality rises more again.

Essentially, there's some kind of drug adaption that's related to the environment they live in.

Methods 1.9 (Classical Conditioning). Gets a group of subject to test for tolerance of a drug. The subject is separated into 2 groups: 1, injection of the drug is made before performing a test (that can measure the defect of ability [to do some measurable tasks] through time); 2, injection is made after performing a test.

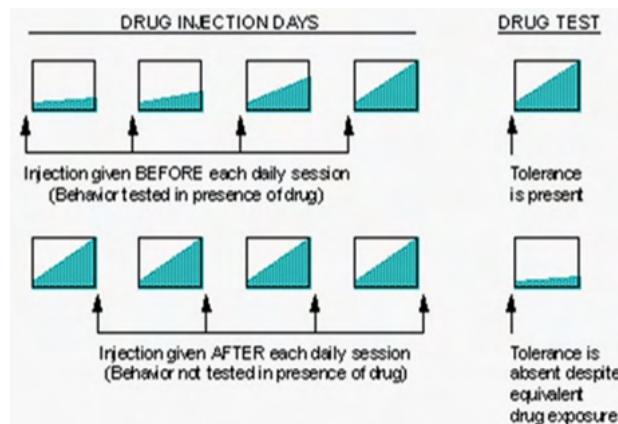


Figure 1.90: Classical conditioning

In the end of the experimentation, we see in the first group every passing days of injection, there's a rise in defect of ability \Rightarrow tolerance is present.

Contrarily, in the second group, who's already have tolerance developed in

the system will not experience this increase in deficit when having injection before the test instead. This is because they never have to adapt to doing the test after drug exposure.

Remark 1.29. This is also known as **conditioned tolerance**.

Observation 1.51 When injecting alcohol to the system of a person before vs after a training session, we will see that the amount of people impaired from injection before is less than those injected after. \Rightarrow No conditioned tolerance

Observation 1.52 We can compare 3 subjects that are either: abstain from alcohol, drink moderately or drink heavily; with increasing level of alcohol consume vs the perform of a task.

What you'd see is that abstainers will lose their ability to do task, like doing subtraction or walking properly, much faster since they've never consumed alcohol while doing these. With heavy drinkers, they're used to doing these tasks while under the influence which means their ability to do them with increasing alcohol level won't be affected much.

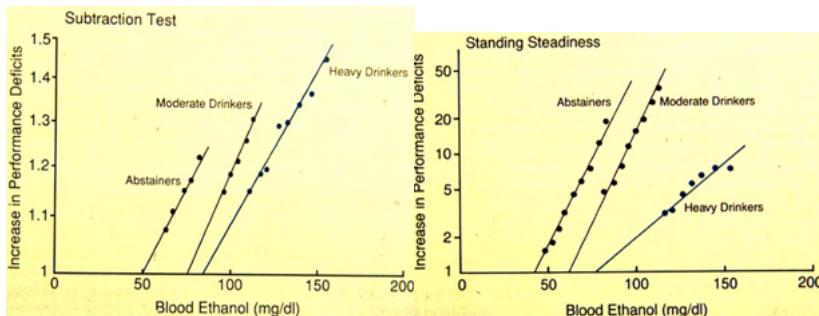


Figure 1.91: Behaviour tolerance of alcohol.

1.5.3 Withdrawal

When it comes to withdrawal, there can be many different syndromes

Example 1.5.6. When a rat, under self-injected heroin, stops taking the drug; some common withdrawal effect that it experience include increase sensitivity to pain, irritability and dysphoria.

We can also test this in guinea pig and even measure the contraction of their ileum during withdrawal. We can see that there will be a peak of intensity of the reaction and then slowly decreases.

Remark 1.30. Duration of these withdrawals can differ from 1 person to the next but also depends on the drug.

Either case, in general, you can classify withdrawal syndromes into 2 kinds: acute (short-term) and chronic (long-term).

Observation 1.53 For opiates, some common acute withdrawal syndromes includes: chills, nausea, tremors, diarrhea, irritability, etc. Meanwhile, for heroin, the person may experience their peak of acute syndrome however they will also experience a lot of symptoms post-acute.

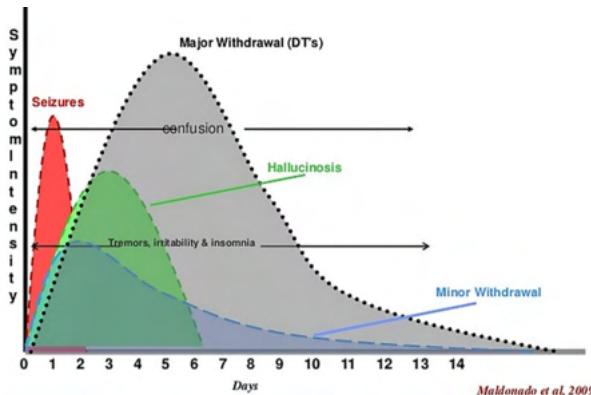


Figure 1.92: Alcohol withdrawal syndromes and duration.

Observation 1.54 We can also look at the withdrawal syndromes of alcohol usage. In the beginning after stop drinking, an addicted individual will experience lots of different syndromes including seizures, hallucination and even confusion. Some of these syndromes peak in the first week and will gradually decrease in the second week.

It's important to note that yes, these are some major syndromes but you can also have syndromes that are very subtle that could last for years like craving.

Concept 1.15 The intensity of the withdrawals relates to the stabilization dose (dose that allow the individual to experience the effect normally).

Concept 1.16 *The severity of withdrawals relates to the drugs half-life.*

Explanations. With drugs that have a longer half-life, the drug is getting metabolized and eliminated from the body while giving time to neurons to adjust back thus eliminating tolerance mechanism thus decrease withdrawal severity. For fast-acting drugs, we would see a much severe withdrawal syndrome. □

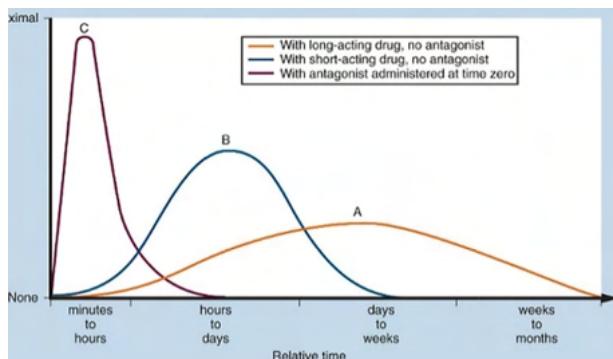


Figure 1.93: Withdrawal severity and drugs half-time.

Definition 1.73. **Rebound** is a appearance of symptoms that may or may not be absent while taking the drug but will appear at high severity when stopping the drug.

Example 1.5.7. When taking hypnotic drugs, it will decrease the amount of REM. As you take more of it the body start to become tolerant to it. Now if you suddenly stop taking it, the rebound effect will occur where the there's a significant increase in REM sleep. It will actually take quite a while to return to normal level. ¹⁴

Notion 1.14 Withdrawals can promotes continued use of the drug

Explanations. This is self-explanatory as when you stop taking the drug, the body develop withdrawals which pushes you to take more drug to sub-sides the withdrawals, etc. □

¹⁴Interestingly, some people can get nightmares because of this.

How do you treat drug dependency?

Well... The usual method is to decrease the dose gradually then substitute it for a safer drug and decrease the dose of said drug too. Other methods that were found to prevent relapse are with antagonist or partial agonists. Finally, to prevent craving, the addict can go through therapy and changing their lifestyle.

The reason behind drug dependence is because of the gain in reward (which activate the dopaminergic pathway) while avoid the punishment by the body (withdrawals). This obviously **do not stop at just psychoactive drugs but also some antihypertensive drugs can lead to dependency but not addiction necessarily.**

This leads to the next important concept that is the addiction cycle.

Mechanism of Action (Addiction Cycle):

1. It begins with some kind of emotional trigger that lead to the craving.
2. Craving will cause the individual to go to their usual ritual or location becoming rewarding.
3. Now that the individual is at the location, they will begin to use the substance or engage in whatever action they're addicted to.
4. After the usage, the individual will experience guilt.
5. Lastly, this guilt feelings will lead to an emotional trigger and the cycle repeats.

1.5.4 Addiction

Like we've previously discussed, addiction is simply dependency in addition with the psychological rewards it provides. A more rigorous definition of addiction follows:

Definition 1.74. **Addiction** is a state of in which an organism engages in compulsive behaviour that is reinforcing (rewarding or pleasurable). Not only that said organism loses control when there's a limiting intake.

Because of this definition, it's not necessarily have to do with drugs which lead to the next definition.

Definition 1.75. **Behavioural addiction** is a type of addiction that's not related to substance uses but more behaviour-based.

Example 1.5.8. Internet and gaming can be addictive. Working can be addictive. Sex can be addictive. And of course, gambling can be addictive. In the recent years, the most observed kind of addiction that has little talk about is phone addiction.

Observation 1.55 There are lots of signs of addictions like mood swings, changes in activity, secretive behaviour, excessive absent from work or school due to engaging in behaviours that causes addiction, etc.

Pharmacokinetics of Substance Addiction

When a drug have rapid onset time and presence in high blood concentration, it has an addiction potential. Addictive drugs that are IV administered will actually facilitate it to absorbed faster and thus get a bigger "hit". In-transal is another way drugs are absorbed rapidly hence it's quite easy to get addicted to smoking.

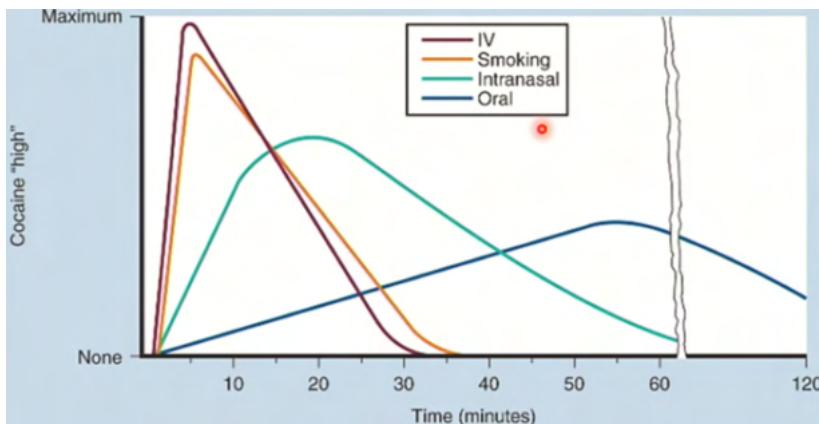


Figure 1.94: Pharmacokinetics of cocaine consumption.

Pharmacodynamics

In pharmacodynamics of addiction, we're mainly looking at the **dopamine-ergic pathway**. The best way to look at this pathway is in rats.

Observation 1.56 Here, we can see the **nucleus accumbens** which is where most of released dopamine would get to which we can collect and record when we give some stimuli to the brain.

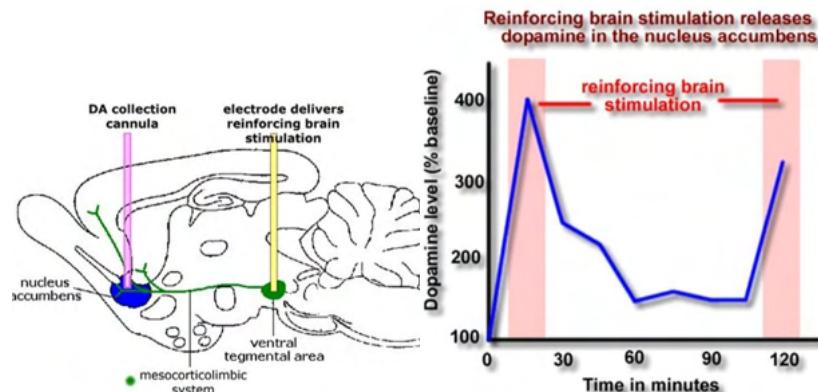


Figure 1.95: Nucleus accumbens rats model

Even though rat's brain has been the model for addiction, it's in fact very close to that of human. To be more specific in human, you have the **ventral tegmental area** as well as **substantia nigra** that releases dopamine throughout the brain. These pathways make up the **dopaminergic pathway**. Certain sites of this pathway release would be the keypoints to addiction. In all cases, what these drugs, like heroin, alcohol, etc., do is that it activates dopaminergic neurons leading to activation of the dopaminergic pathway thus the rewarding system.

As for the neurons themselves, obvious with more release of these dopamine it would lead to neuroplasticity which is the one of underlying cause of addiction. All of this lead to the final, more scientific way, of describing addiction.

Mechanism of Action (Addiction): Suppose that you take an addictive drug.

1. This leads to the activation of the reward system (circuits) which is connected to your memory system that allows you to remember the feeling and the drug you've taken.
2. When you continuously take the drug, it will activate craving pathway in the brain which lead to binging of the drug i.e. you've lost control and now the reward pathway is at an all time high pushing you to take more and more.
3. When you stop taking the drug, you'd experience withdrawal (al-

ready talked about from above). During this time, lots of pathways are affected + the reward system is at an all low.

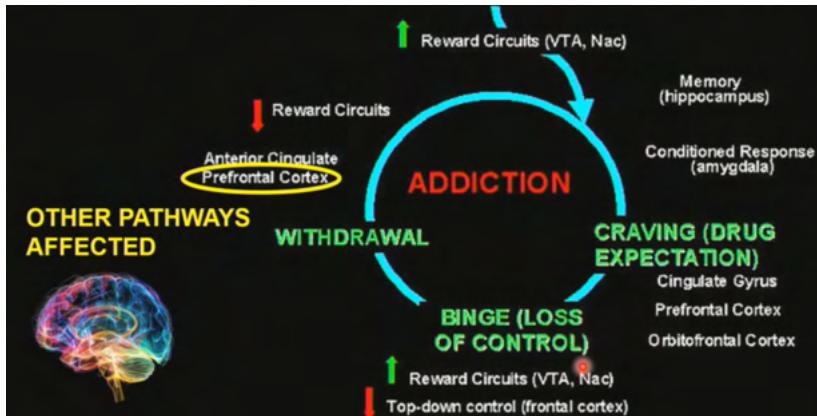


Figure 1.96: Cycle of drug addiction.

Concept 1.17 *The relative addictive properties of psychoactive substances.*

Even though nicotine and alcohol are not as illegal as compared to other drug, its relative addictive properties are still as high as cocaine and other drug.

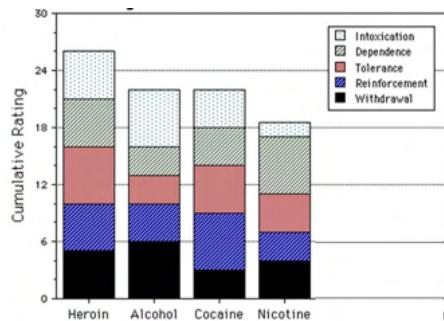


Figure 1.97: Relative addictive properties chart.

In this chapter, we will be discussing about psychoactive drugs.

2.1 Nicotine

Definition 2.1. **Nicotine** is a dangerous and highly addictive chemical that is the main active ingredients in cigarettes

Observation 2.1 Nicotine is in fact naturally found in lots of plant acting as its own botanical insecticide. 1 plants in particular that has a high concentration of nicotine that we can extract is **tobacco**.

This extraction can be done in 2 ways: smoking, where we burn the tobacco plant to get the nicotine from the smoke; vaping, where the nicotine liquid is pre-extracted from tobacco to then be turn into smoke that you can inhale.

2.1.1 Epidemiology of Nicotine

Compared to all other addictive drugs, nicotine addiction is the highest globally. It kills more than 7 million people per year.

Observation 2.2 Knowing this dangerous effect caused by nicotine and smoking, the US, UK and Canada has decided to go against the use of smoking. This incredible transformation reduced the amount of cigarette consumption in Canada by the 2010. Nevertheless, this is only a regional changes, **but the global consumption of cigarette is still at an all time high.**

In Canada specifically, our leaders have been campaigning on the usage of imageries and waronings on cigarette packages (of which these companies are very resentful of).

Observation 2.3 Because of this movement against the usage of cigarette, these companies decided to find create a new way out which is the introduction of **e-cigarette/vaping**. Even though it's a different kind of brand-

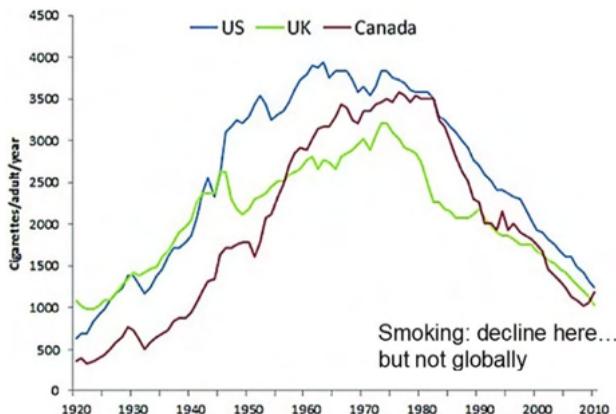


Figure 2.1: Cigarette consumption in UK, US and Canada.

ing, it still have the addictive nicotine and can cause death.

We can see that through the years, there has been significant increase of e-cig purchase by the public. Like in the past, e-cig has been heavily advertised without any scrutiny.

Whether or not it's cigarette or e-cig, both of their purpose is to deliver nicotine to circulation and brain in the fastest way possible. In this case, the **route of administration used is through inhalation** which would be absorbed in the lung.

Remark 2.1. *Compared to IV injection, inhalation of nicotine will be much faster and take only 7 seconds.*

This is also the reason why it's addictive since it has rapid absorption rate. Interestingly, we've also found that it's not only nicotine that's delivered into the system but **at least 4000 – 7000 other chemicals**.

2.1.2 Toxicity of Nicotine

Like we've discussed, cigarettes cause more death than any other reason. It was estimated that it will kill at least 600 million people currently alive on earth.

Observation 2.4 Smoking has strong correlation to many cancer. Not only that it decreases the **mucociliary clearance** of the lung with increasing us-

age of smoking. Because of this decrease in mucociliary clearance, there will be an increase built-up of dangerous exogenous particle that can further damage the lung.

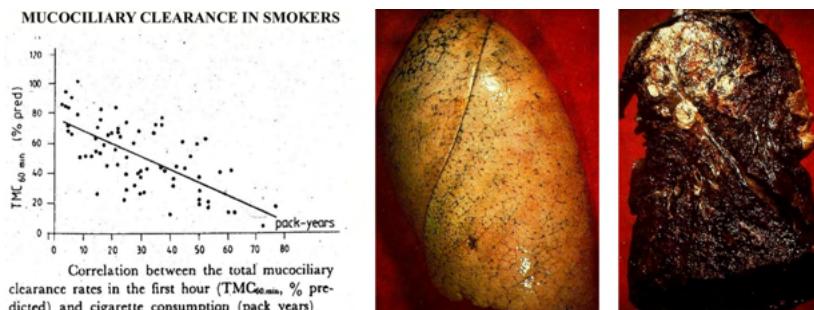


Figure 2.2: Mucociliary clearance of smokers and their lung before vs after

Observation 2.5 Specifically here in Quebec, where we have asbestos mine, it was determined that the effect of smoking and asbestos inhalation will act together and multiply each other effect leading to a significant increase risk of lung cancer.

Observation 2.6 Roughly 1/3 of deaths from **coronary heart disease** are caused by smoking. It can also cause stroke which can either kill or impair any motor functions. Furthermore, it damages the blood vessels around the body.



Figure 2.3: Vasoconstriction in the hand through 1 cigarette.

Observation 2.7 Interestingly, researchers also found that nicotine can cause **vasoconstriction**, which is a constriction of blood vessels, in the skin, hair, eyes and even gums. This is why it's the underlying cause behind skin damage and heavy wrinkles for aging population that consume nicotine.

Methods 2.1 A subject was told to place their hand in front of a sensor that detect blood flow. The subject was then asked to smoke 1 cigarette.

By the end of the experiment, we found that just 1 cigarette along is enough to cut off lots of blood flow in the hand. See figure 2.3.

Remark 2.2. *Cigarette kills at least 50% of smokers.*

Second- and Thirdhand Smoke

Definition 2.2. **Secondhand smoke** are by-product smoke that are indirectly produced from a person smoking.

Observation 2.8 Secondhand smoke kills roughly 900 thousands people every year. At lot of dangerous and carcinogens are faound a much higher concentration in the secondhand smoke than that of the primary one.



Figure 2.4: Second- and thirdhand smoke.

Definition 2.3. **Thirdhand smoke** is secondhand smoke, which contains lots of health hazardous materials, that begin to fall down and settles on the environment.

Observation 2.9 Thirdhand smoke are especially dangerous for children and pets. Most of the time, younger children and pets do not wear as much clothing but also spend much more time on the floor and other furniture which can easily expose to the thirdhand smoke, given that there's a smoker in the family.

For children specifically, the thirdhand smoke increases the risk of brain tumor, other cancers, asthma, serious infection and worst of all **sudden infant death (SID)**.

Observation 2.10 Now, like we've said that there's been a raise consumption of e-cig; but what's much more concerning is that the consumption of these e-cig also increases for teenagers. Not only that, it's been observed that there's an increase in e-cig and liquid nicotine poisoning within the last 10 years.

2.1.3 Pharmacokinetics of Nicotine

Majority of time, nicotine is administered to the lung and absorbed there + it's the fastest form for absorption to the body. Nevertheless, nicotine can be absorbed almost everywhere in the body: transdermally, orally and even through the GI tract.

Absorption

Observation 2.11 The rate of absorption depends on the pH of the drug. A typical cigarette will have ingredients that make the more much more alkaline which makes the nicotine in its unionized form and thus absorbed instantly.

Because it can be inhaled, it does not have to go through first-pass metabolism

Another reason that nicotine is rapidly absorbed to the body is because it enters the lung where lots of alveoli that has blood vessel circulating them for gas exchange.

Observation 2.12 We can test for the time of absorption of 2 cigarette at different concentration of nicotine. Amazingly, regardless of the concentration, they will both reach their peak concentration at the same time i.e. the rate of absorption is the same.

Methods 2.2 Obtain 3 types of cigarette: normal, high and low concentration of nicotine. Divided subjects into 3 groups with their respective cigarette and let them smoke + measure the blood nicotine level.

At the end of the test, what we found is that the plasma concentration of nicotine for all 3 groups are relatively the same.

Explanations. The reason this happened is because the group that has the high [nicotine] tends to take smaller puffs of smoke, while for the low [nicotine] group will take a longer and stronger puff of smoke. □

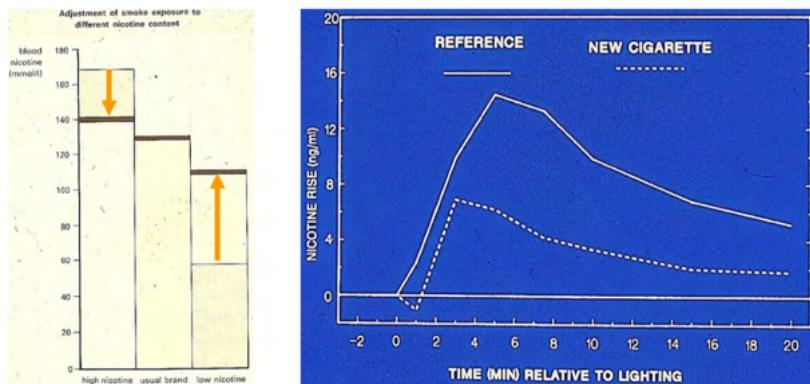


Figure 2.5: Nicotine absorption rate and controllable bioavailability.

Concept 2.1 From the above experiment we see that, smokers can control the bioavailability of nicotine.

Observation 2.13 Comparing the nicotine absorption of inhalation with other forms of administration like oral: nicotine gum, oral snuff and chewing tobacco, we see that its absorption rate are not quite as rapid but is still fast ⇒ nicotine is readily absorbed in the mouth and GI tract. See Figure 2.6 (next page).

Remark 2.3. The GI tract absorption of nicotine is in fact too good which can be potentially lethal.

Distribution

As we've said above, nicotine is very lipid-soluble, it can be distributed everywhere in the body and can even cross the blood brain barrier. It can also affect the fetus by crossing through the placenta but also be present in breast milk.

Observation 2.14 Because it can cross the blood brain barrier easily, it can also trigger the **chemoreceptor trigger zone (CTZ)** which lead to vomiting. This helps the body in case of ingestion of lethal dose of nicotine.

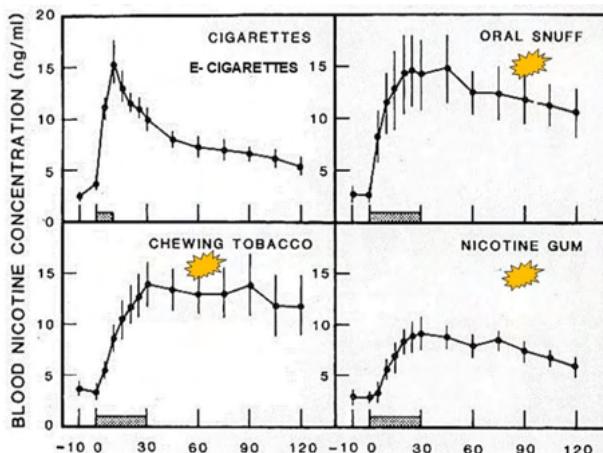


Figure 2.6: Blood concentration of nicotine through various method of administration.

Metabolism

The main enzyme that metabolize nicotine is CYP2A6. In the system, nicotine has a half-life of 2 hours.

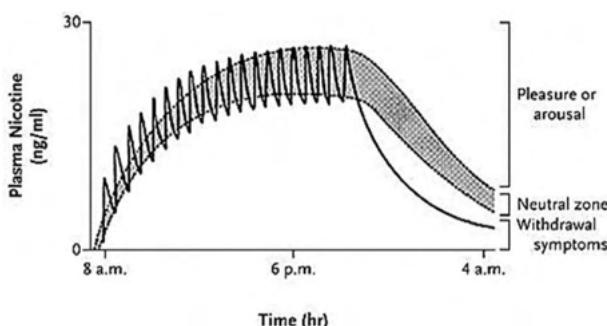


Figure 2.7: Plasma of nicotine of a smoker in a day .

Observation 2.15 We notice that a smoker go through the day will have a similar effect of how an IV injection scheduling looks like, that is, it has sharp increase every new dose and then reach a steady-state.

Because the nicotine has a short half-life, once the smoker goes to sleep, the nicotine level will drop to the point there's none left by the next morning \Rightarrow Withdrawals begin thus need to smoke again.

Observation 2.16

For CYP2A6, it will break nicotine into **cotinine** which can then be further conjugated and then eliminated.

Like most other enzymes, there are variations of these enzymes which will lead to people having slower metabolism of nicotine. This also means that a person can be addicted to nicotine even though they smoke very little since their plasma nicotine is still high.

Observation 2.17

We can see this through testing different CYP2A6 alleles and its nicotine/cotinine level. With the normal allele (active CYP2A6), nicotine will reach a peak and then decrease right away while the cotinine level sky-rocket then decrease.

On the other hand, inactive CYP2A6 variant will lead to a significantly higher plasma nicotine that barely decreases while the cotinine level only rises ever so slightly.

It must be noted that **CYP2A6 can activate some procarcinogens that forms cytotoxic and genotoxic metabolites** i.e. by having defective CYP2A6 is actually a good thing as it decreases carcinogenicity.

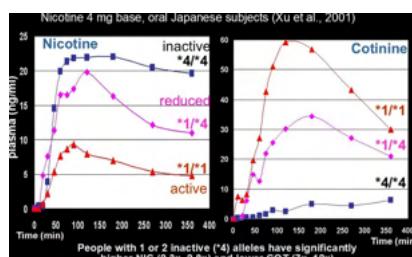
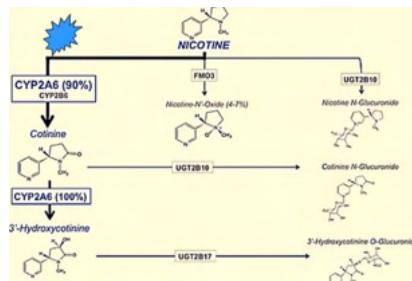


Figure 2.8: Nicotine to cotinine by CYP2A6 (top). Testing of nicotine and cotinine level of different CYP2A6 level (bottom).

Elimination

Nicotine is eliminated/excreted through the urine. Now, even though metabolites are excreted here, some of its parent compound (in this case: nicotine) will also be excreted. But because urine is alkaline, nicotine will be in its lipid soluble form and thus can be reabsorbed more easily \Rightarrow decrease in nicotine excretion.

2.1.4 Pharmacodynamics of Nicotine

Nicotines can act on the **autonomous nervous system (ANS)** by binding to its own receptors located in many ganglia. Here, in the illustration, we can see that it can has power effect on the periphery which includes the sympathetic and the parasympathetic system.

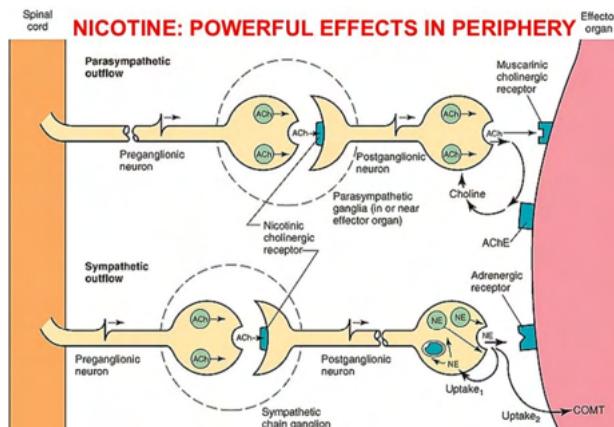


Figure 2.9: Nicotine affect the ANS.

Observation 2.18 The reason that it can do so because of an existence of a receptor called **nicotinic receptor**. In fact, the heart and the vasculature also have these receptors which explain why consumer of nicotine tend to have higher heart rate and blood pressure.

Nicotinic Receptor

The nicotinic receptors are found everywhere in the body. It has so much effects, complex activity and connection to the point that there are books

and research journals dedicated just for it.

Observation 2.19 Structurally, it is a ligand-gated ion channel that are made from 5 proteins subunits (pentamer). Because it's made from 5 proteins, there are lots of variants to it which include a combination of any of 9 α and/or 3 β subunits.¹ We can separate the receptor according to its **muscle-type and neuronal**, of which we will focus on is neuronal one.

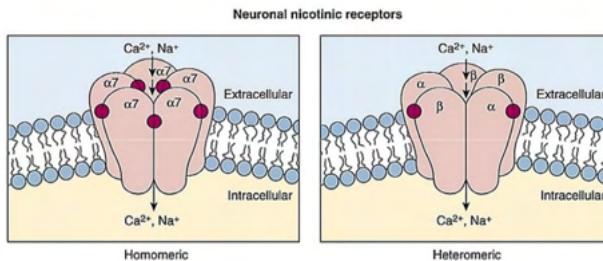


Figure 2.10: Neuronal nicotinic receptors.

For the neuronal nicotinic receptor, it can either made from 5 same (homomeric) or different subunits (heteromeric). For the homomeric variant, the most common is made from 5 $\alpha 7$ subunits. For the heteromeric variant, the most common is made from 2 $\alpha 4$ and 3 $\beta 2$ subunits. It is the $\alpha 4\beta 2$ variant that would be upregulated by nicotine.

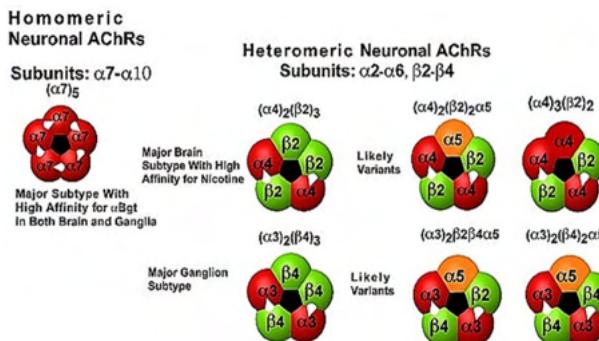


Figure 2.11: Different variant of neuronal nicotinic receptors.

¹Different combination could also lead to different ions that it can allow to pass through too.

Mechanism of Action (Upregulation of Nicotinic Receptor): Nicotine is brought into the system.

1. It binds to a stand-by nicotinic receptor and open it allow ions to travel in or out.
2. After the ion channel closes, the receptors will be desensitized however, this desensitization will last much longer as compared to other ligand like acetylcholine.
3. Longer desensitization will lead to long term inactivation of these receptors.
4. The body's adaption mechanism will upregulate the amount of nicotinic receptors.

Observation 2.20 Like we've mentioned, these nicotinic receptors exist everywhere in the brain and in fact it's one of the main key modulator of the releases of many neurotransmitter (that can lead to inhibition or stimulation) e.g. dopamine, glutamate, acetylcholine, opiate peptide and etc.

Upon binding to nicotinic receptor, nicotine will stimulate it for acetylcholine but then will block them. Not only that, it will cause the release of epinephrine from the medulla and norepinephrine frp, the sympathetic nerves. As a result, **it will lead to a subtle improvement in performance.** Then it follows with **sedation** where skin temperature decreases, skeletal muscle relaxes, salivation increases even though hunger decreases etc.

Observation 2.21 (Poisoning Potential). It must be noted because nicotine cause excitation follows by inhibition. At high/dangerous dose (like accidental consumption by children), it can first cause tremor, convulsion then complete paralysis. Finally, if at lethal dose, individual can die from respiratory failure.

Notion 2.1



Smokers have more nicotinic receptors than non-smokers.

Explanations. This is quite self-evident as we've said above with the upregulation of nicotinic receptor, the presence of nicotine will lead to its increase. We can test

Figure 2.12: Presence of nicotinic receptor in non-smoker vs smoker.

this by looking at the amount of these receptors in smokers vs non-smokers using some kind of detectors. See figure 2.12. \square

Observation 2.22 What we've found also that in mice with knockout gene of either $\beta 2$ or $\alpha 2$, they do not have a dopamine increase when given nicotine nor will they develop any addiction.

Until today, there has been continuous discovery on the widespread effects on presynaptic nicotinic receptors as well as its modulating ability.

Remark 2.4. *Because it has so much ties to the brain, it was found that the smoking rate of people with psychological illness is much higher than normal.*

Example 2.1.1. The prevalence of people smoking with schizophrenia is around 88% while 75% for depression and 80% for alcoholism.

2.1.5 Addiction of Nicotine

Like we've said before, nicotine has the highest number of addicts than any other addictive substances. In fact, its addictive potential is so much more compared to typical addictive substances like heroin or cocaine.

Observation 2.23 It was found that a typical smoker will take around 11 attempts to quit over a 19 years span to stop.

Explanations. It's hard and takes so long to quit because the brain, especially the reward pathways, is litter with these nicotinic receptors. \square

Observation 2.24

Normally, we have the right amount of $\alpha 4\beta 2$ nicotinic receptors on **GABA cells** that can inhibit the activities of dopaminergic neurons and $\alpha 7$ on glutamate that can stimulate them. In the case of a smoker, the circulating nicotine will lead to an upregulation of $\alpha 4\beta 2$ receptor \Rightarrow desensitize/decrease stimulation from GABA inhibition. Meanwhile, $\alpha 7$ are hardly affected but will be stimulated strongly by nicotine \Rightarrow increase stimulation from glutamate

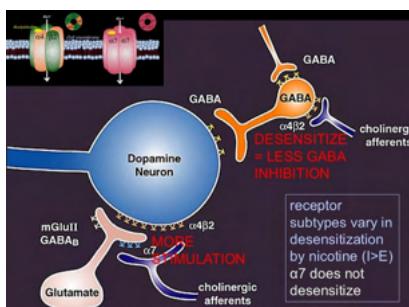
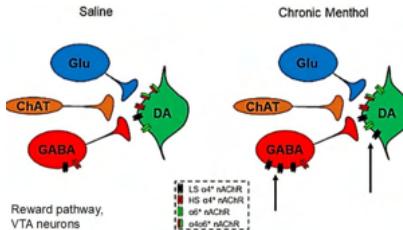


Figure 2.13: $\alpha 7$ and $\alpha 4\beta 2$ nicotinic receptors on the activities of dopaminergic neurons

excitation. In the end, you'd get more excitation and less inhibition for the dopaminergic neurons.

Observation 2.25



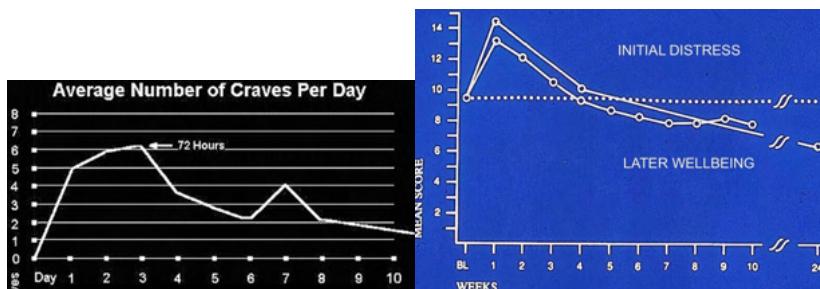


Figure 2.16: Nicotine craving peaks and diminishes (left), nicotine withdrawals (right)

rehab reported to have better sleeps and improved well-being around this time as well.

The withdrawals goes down because the body is slowly undoing its up-regulation of nicotinic receptors and by the end smokers who successfully quit will have the same nicotinic receptor count as normal non-smokers.



Figure 2.17: Nicotinic receptor count.

Observation 2.27

Nevertheless, this also comes with its own cost. Because of the long lasting withdrawals that patients have to endure, it's also quite easy for them to relapse. Here, we can see the amount of abstainer of smoker drop from 100% to 30% within just 3 months of treatment and further drop to 10% by the end

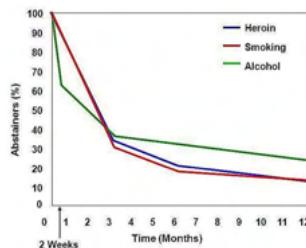


Figure 2.18: Relapse of nicotine (and other substances)

of the year. This is also a similar trend for other drug which is why we've mentioned above that it takes on average 19 years for some to fully stop.

Treatment to Nicotine Addiction

There are currently lots of treatment to treat nicotine addiction.

Treatment 1. Instead of taking in nicotine through inhalation, we can use a different method of taking it in which has lower absorptive rate but also at lower dose e.g. OTC patch, gum, or even prescribed spray and inhaler.

Treatment 2. Instead of giving nicotine, we can give blockers or partial agonist to the nicotinic receptors. In this case, **bupropion** is an antagonist that will target the nicotinic receptors of the VTA dopaminergic neurons. Or **Varenicline** is a partial agonist that can binds and induce partial effect at the $\alpha 4\beta 2$ nicotinic receptors.

Cessation rates of the two randomized controlled outcome trials (5,6)

	end of treatment		one year follow up	
	study one	study two	study one	study two
Varenicline	44	44	22	23
Bupropion	30	30	16	15
Placebo	18	18	8	10

Figure 2.19: Controlled trial of bupropion and varenicline

In the case of bupropion, by the end of the treatment around 44% has quit with a diminish of 22% by a year follow-up. On the other hand, for varenicline, around 30% is treated with a diminish down to 15% by a year follow-up. Evidently, it's not the best method but we're still trying to find ways to improve.

Treatment 3. We could try to lower the metabolism of CYP2A6. The logic here is by lowering its metabolism, there will be more circulating nicotine \Rightarrow patient will smoke less. The medication that was used to do this is **methoxsalen**.

Treatment 4. Another way is to instead decrease dopamine release using

the drug **topiramate**. What we found is that the amount of dopamine decreases when a patient is given some doses of topiramate prior to nicotine as compared to just nicotine alone.

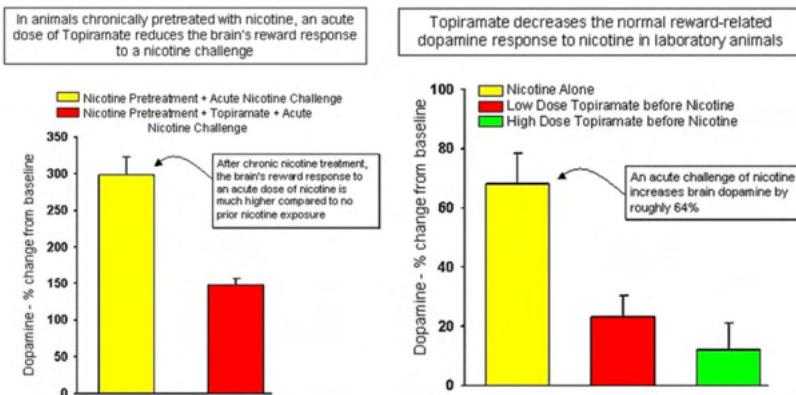


Figure 2.20: Topiramate treatment.

Comparing to a placebo, we can also see the amount of non-smokers, by the end of 12 weeks study, is half that of the topiramate.

There has been regular testing for nicotine vaccine but we will see where future takes us on this one.

The Danger with E-Cigarette

It was often advertised that E-cig are not that harmful but this couldn't be further from the truth. With E-cig, not only you're getting the typical 7000 different chemical in your body, but also the artificial flavour coming in, which is supposed to be in your GI tract and not your lung.

What's worse is that these chemicals are heated up by e-cig which can lead to subreactions happening with these chemicals that **generate lots of derivatives** \Rightarrow **you're intaking even more than the normal 7000 chemical!** Not only that, the secondhand smoke coming from e-cig vs cig are practically identical and even containing some dangerous unknown carcinogens i.e. the long term effects are unavoidable no matter how much "better" it is compared to normal cigarette.

In the end, the best way to treat nicotine would be to avoid it from the beginning altogether. Nonetheless, we're still finding newer methods that

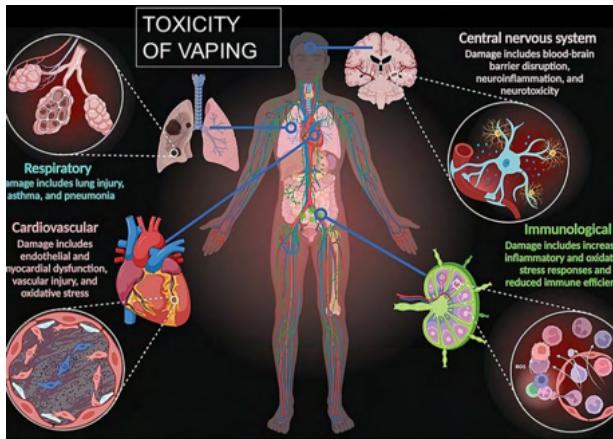


Figure 2.21: Toxicity of vaping.

aide with its treatment and prevention.

Lecture 8: September 24th, 2024.

2.2 Ethanol

Definition 2.4. **Ethanol (EtOH)**, under the form of beer and alcohol, is a powerful and legal drug. Not only that it's advertised heavily.

Observation 2.28 A lot of people has disorder that related to alcohol usage. It has lots of side effects for long term and short term. Worldwide there are around 240 million people that consume alcohol, in a problematic ways. This is much higher compared to drug usage which is only 15 million.

CNS Depressants

EtOH is a general depressant of the CNS. At low dose, it can depress some excitatory area but as the dose increase the person can be sedated, then reaching coma which can finally lead to death.

Definition 2.5. **CNS depressant** is a class of drug used to reduced brain activity which lead to muscle relaxation.

Looking at CNS depressants, there are 5 main concepts we have to focus on.

Concept 2.2 (Addition). CNS depressants have additive effects. This includes any sedatives, antidepressants, anticonvulsants, antianxiety and opioids.

Explanations. When a person take antidepressant and consume EtOH at the same, the effect of them can add which lead to toxicity. This is also the reason why you cannot take sleeping pill after drinking alcohol (which lead to death). □

Concept 2.3 (Irreversible). Alcohol cannot be reversed with a stimulants

Explanations. This completely debunk a myth that you can give a person coffee alcohol to wake them up. Caffeine does not reverse its effects. □

Concept 2.4 (Non-general). General depressant are not totally general

Explanations. Evidently, at higher dose, these CNS depressants will knock out everything but still each depressants can differs from each other. □

Concept 2.5 (Rebound). Chronic use of CNS depressant will lead to rebound excitation if stop.

Concept 2.6 (Tolerance). All general CNS depressant will cause tolerance. There are also frequent cross tolerance

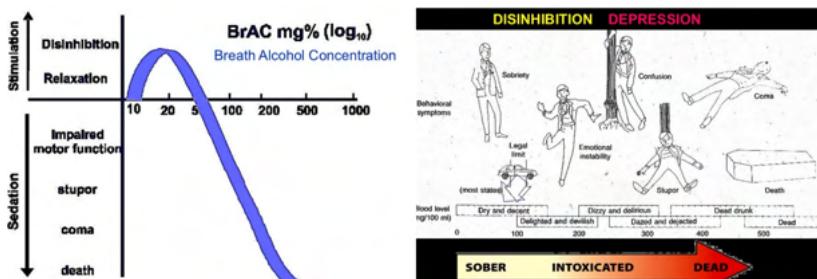


Figure 2.22: Alcohol level and sobriety.

Observation 2.29 As the dose of EtOH increases, it starts affect different part of the brain. More specifically, at around 0.03 – 0.06%, it affects our emotions and judgement. From 0.05 – 0.39%, it affects the voluntary motor

skills and at the final stage of 0.4 – 0.6%, it affects the involuntary system.

Interestingly, at low dose, EtOH acts as a stimulant but as the dose increases, its CNS depressant effects start to manifest that can worsen till death.

Remark 2.5. *When a person is passing out via alcohol, it's a must to tender to their care as the person GI tract is still digest more alcohol which could be fatal.*

A common symptoms of EtOH consumption is amnesia and delusions

Definition 2.6. **Amnesia** is a term to describe memory loss. **Partial amnesia** is a fragmentary loss of memory i.e. they have gaps in what has transpired. On the other hand, a **blackout** is a total loss of memory for specific time.²

Observation 2.30 EtOH is responsible 1/3 of dead drivers (responsible for half of dead pedestrians) and around 1/4 of private plane crashes and home accidents. Remarkably, half of accidents related to boating has something to do with ethanol.

Observation 2.31 For driving, ideally, you should not have any drink. As of 2024, Most provinces in Canada has a EtOH limit of 0.05% and even then you'd get your license suspended. 34% of Canadian road accident is due to EtOH. What needs to be noted that, the risk of fatal crashes at 0.05% will double and triple at 0.08% compared to 0%.³

Remark 2.6. *For every death of EtOH origin, 10 others are seriously injured.*

Interestingly, the consumption of ethanol can vary from different regions though almost all of them are for **recreational purposes**. We see the highest consumption in the north. Not only that, the days of the week also effect when it comes to the amount of accident that are alcohol related (higher in weekend). See Figure 2.23.

²Blackouts do not happen to everyone but is possible.

³At 0.08%, the driver will have criminal charges against them.

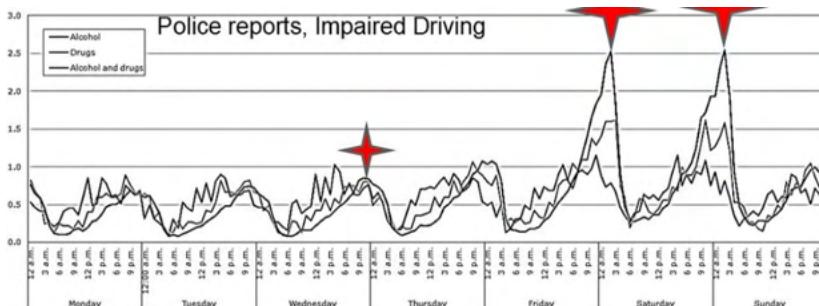


Figure 2.23: Impaired driving throughout a week.

2.2.1 Epidemiology of Ethanol

In a Harvard studies of over 10,000 students through > 100 campuses, they've found that drinking by college students causes > 1000 deaths and > 500k assaults. Worst of all, 1 in 5 college women experiences sexual assault because of drinking.

In Canadian university, most students consume alcohol from the previous month (on average 4.5 drinks) and half of them drink frequently. On their heaviest drinking, they can consume up to 7 drinks. The highest problem to this physical harms to their health. Additionally, they also report to miss lectures and showing signs of dependency.

Observation 2.32 It must be realized that the brain continues develop until somewhere in the mid 25s. So by drinking earlier in the age, it can negatively affect the brain development.

Methods 2.3 Compare the of 2 15-year-olds with either high drinking or no drinking. Allow the 2 teens to complete some auditory verbal learning test and a logical memory test.

In the end, for the non-binge drinker, they score 7.84 as compared to the heavy drinker that score 6.97 out of 15 for the auditory verbal learning test. For the logical memroy test, the non-binge drinker score around 54.72 while the heavy drinker score only 51.35. (These test were made when they're still sober).

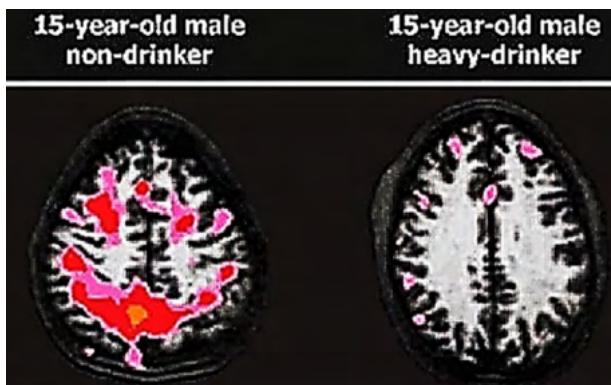


Figure 2.24: Brain activity during a memory task of a non-drinker vs heavy-drinker 15 years old male.

2.2.2 Pharmacodynamics of Ethanol

EtOH acts in a variety of area in the brain e.g. cerebellum for coordination, cortex for judgement, but also the reward pathway.

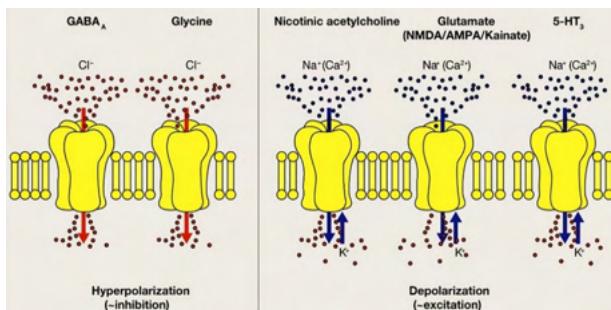


Figure 2.25: EtOH targets mainly GABA_A and Glutamate receptors.

EtOH targets ion channel type receptors and ion channels themselves. It acts on inhibitory pathways like GABA, glycine and excitatory pathways like glutamate. To be specificm, it inhibits the excitation and activating the inhibition.

Definition 2.7. **GABA_A receptor** is a ligand-gated ion channel. When GABA comes and bind to it, it will open and allow Cl⁻ to flow through which leads to inhibition.

Observation 2.33 Strikingly, there are lots of allosteric sites on GABA_A receptors where drugs can bind to and this includes one for EtOH.

The GABA_A does not just sit on the postsynaptic site but they also exist in the **extrasynaptics**, places beyond the synapse. When GABA are released to bind at the postsynapse, some can travel to the extrasynapses to bind to these receptors. Here, they're not broken down quickly which lead to a continuous tonic current present i.e. **there's always a tonic level of inhibition coming from GABA at all time** though not enough to show significant changes.

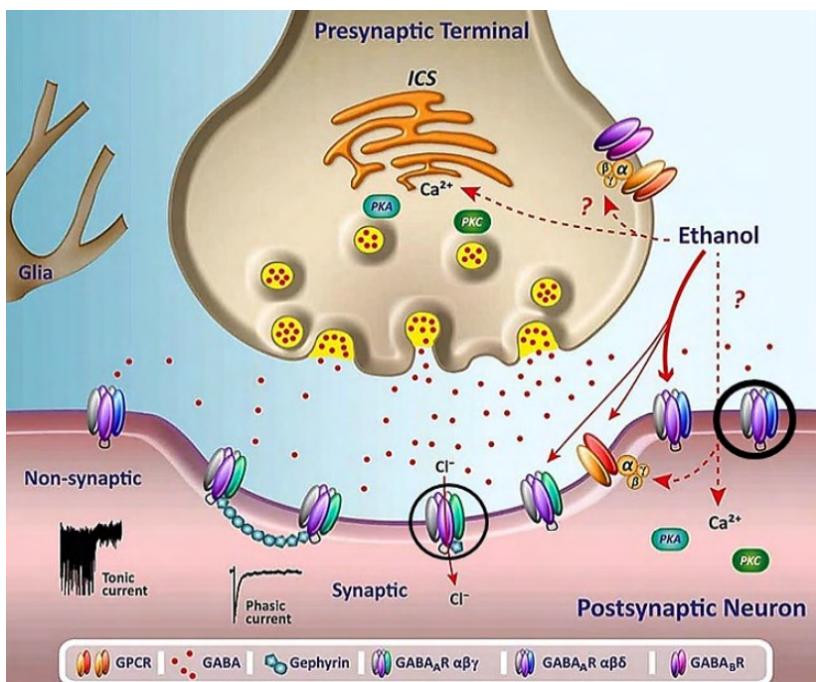


Figure 2.26: GABA_A receptors.

Now, EtOH acts on both of these GABA_A receptors \Rightarrow there are now 2 sources of inhibition. We've also theorized that it may have some alteration effects on the [Ca²⁺] in the presynaptic but also postsynaptic neurons (but are not experimentally tested yet). Additionally, EtOH leads to the release of glycine that enhance the postsynaptic response.

Observation 2.34 Now we said that it also blocks excitation. EtOH can block glutamate receptors. This blockage is the reason behind your have intellectual impairment under the influence.

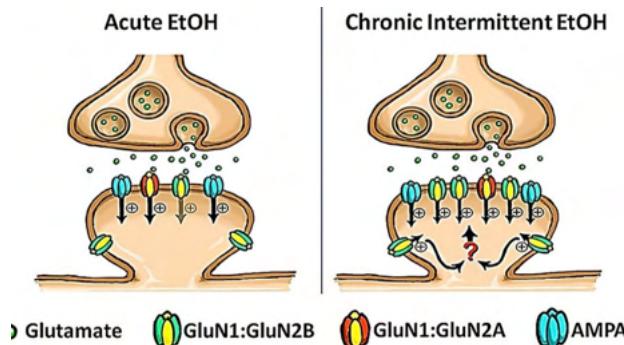


Figure 2.27: Acute vs chronic consumption of EtOH on glutamate receptors.

For acute consumption, it only blocks the excitation of these neurons. On the other hand, with chronic consumption, the neurons will increase the production of these receptors which lead to excess excitation when there's no EtOH in circulation.

Summary

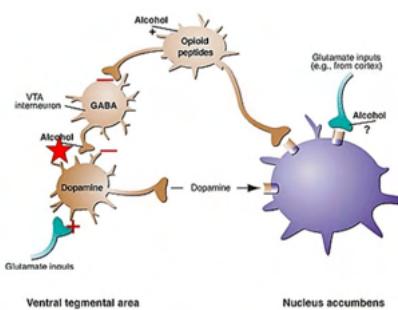


Figure 2.28: EtOH facilitate dopamine release-

leaser with amnesia and cognitive impairment while drinking.

EtOH can inhibit excitation via blocking of glutamate receptors, while potentiate inhibition via stimulation of GABA_A and glycine receptors.

It also have an effect on Ca²⁺ and Na⁺-channel which can have an effect on the release of neurotransmitters. Its effects on serotonin can be relates to the a increase in impulsiveness and aggression. Furthermore, the blocking of acetylcholine release has a probable factor with amnesia and cognitive impairment while drinking.

When looking at the model of cells acting together that can lead to addiction. You can see EtOH facilitate the release of dopamine from dopaminergic neurons in the VTA to the nucleus accumbens.

2.2.3 Pharmacokinetics of Ethanol

Absorption

EtOH is a simple, small molecule and lipid soluble meaning it can be absorbed quickly in the GI tract within just 5 minutes from drinking. The blood level of EtOH will peak around $1/2 - 1\text{h}$ where it absorbs most of it.

This rate can be changes according to the condition in the stomach and GI tract. The absorption will decrease with consumption of high fat food.⁴

Explanations. This is because fat delay gastric emptying. Evidently there will be absorption in the stomach but majority is in the GI.

□

On the other hand, rate of absorption will increase by drinking carbonated liquids i.e. rate of absorption of EtOH is much higher when drinking beer or champagne.

Observation 2.35 Normally 20% can be absorbed in the stomach due to the presence of some alcohol dehydrogenase in the gastric mucosa turning it to aldehyde. As a result, the side effect of this is **gastritis** (inflammation of the stomach).

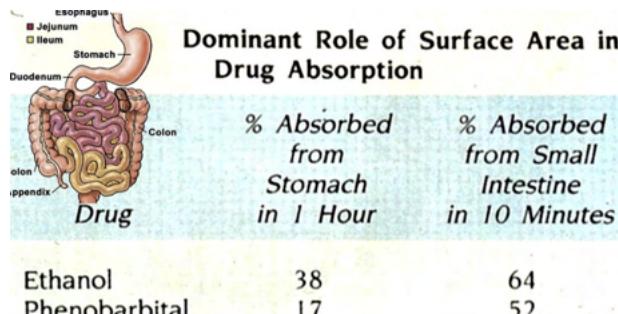


Figure 2.29: Absorption of EtOH in the stomach vs small intestine.

In the small intestine, within 10min, you've absorbed almost twice that of stomach in an hour.

⁴This is also why you don't get drunk as fast/much with a full stomach.

Distribution

Ethanol is distributed in the total body water. In male vs female, there's a difference in total body water (male higher) \Rightarrow More diluted in male but said difference will get smaller as people age.

Due to the high blood flows to the brain, EtOH gets to the brain quickly. At the brain, it can stimulates the CTZ which lead to vomitting. This is good since it enables the body to remove any excess dose of EtOH that can lead to toxicity in the GI tract.

Metabolism

EtOH saturates very rapidly which means as the dose increase, the model of metabolism changes from first-order immediately to zero-order i.e. the rate of absorption is still high while the its metabolic rate is decreasing.

With first-order, it's removing a fraction of EtOH over a period of time. On the other hand, because of saturation, the EtOH now follows zero-order which will take a much longer time to be metabolized (at around 20mg/ 100mL/h or half a drink is metabolized per hour).

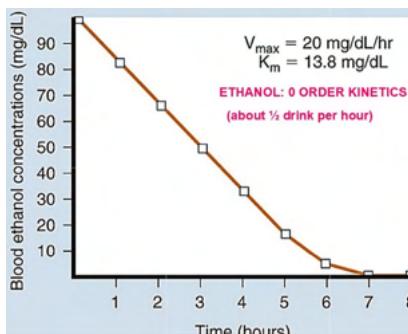


Figure 2.30: Zero-order of EtOH.

Mechanism of Action (Metabolism of EtOH): EtOH is absorbed and distributed through the body. It will then pass through the liver.

1. In the liver (and gastric mucosa), ethanol is turned into acetaldehyde via **alcohol dehydrogenase (ADH)**.

2. Acetaldehyde is quite toxic to the body so it will be further converted to acetate via **acetaldehyde dehydrogenase (ALDH)**.

There are variations in these 2 enzymes. This is because there are 7 genes involved in encoding the ADH and 19 for ALDH. Thus there's a greater extensive genetic variation and in some population, 50% of them have a less active form of the ALDH.

Explanations. For these individuals, you'd get lots of effects from acetaldehyde which include flushing, headache, vomiting, hypotension and sweating. □

Now, not only ADH and ALDH are the only enzymes that breakdown alcohol. In cases of chronic usages, you have certain P450 enzymes (like CYP2E1) that can be induced to help EtOH metabolism. This also explain while these people tend to be fine at higher dose than other patient.

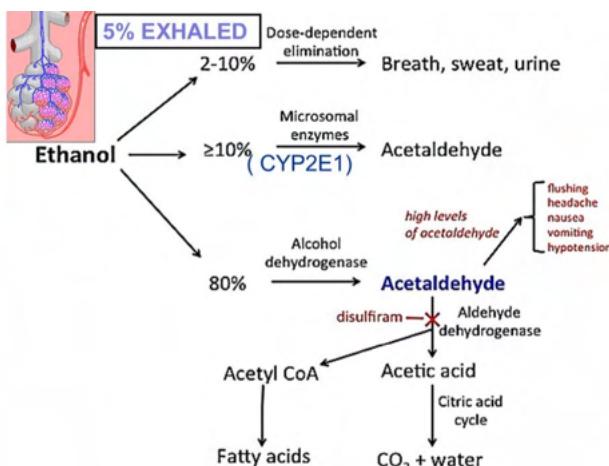


Figure 2.31: Metabolism of EtOH.

Observation 2.36 For the consumed EtOH, around 80% is broken down in the ADH and ALDH pathway, $\geq 10\%$ are from enzymes (depending if you're chronic drinker or not) and $\sim 5\%$ of the drug is exhaled unchanged. The exhaled one is the logic behind breath test.

It must be noted that there's differences in male and female (in term of pharmacokinetics). In female, there is a lower number of ADH which lead to slower metabolism, and lower body size and body water will lead to higher alcohol level in blood. i.e. a female can drink below the limit and still be considered higher (with respect to male).

Excretion

EtOH is excreted by the kidney. Because it's lipid-soluble, there will be some reabsorption of it.

2.2.4 Toxicity of Ethanol

We first look at the acute toxicity of EtOH. The therapeutic index of ethanol is only around 4 which means that it only takes around 4 dose from recreation use to reach lethality. If EtOH was to put through a clinical trial, it would never even stand a chance in phase I let alone getting in.

Observation 2.37 You can actually compare EtOH to other CNS depressants like benzodiazepine and barbiturate. We can see that ethanol has an even steeper curve meaning that it has a smaller therapeutic index \Rightarrow dangerous. Its steepness is a little more severe than that of **barbiturate**, a CNS depressant that were banned for easily lethal.⁵ As of now, we're using a much more safe CNS depressant like **benzodiazepine** which has a therapeutic index of 500 \Rightarrow safer over a range of dosage.

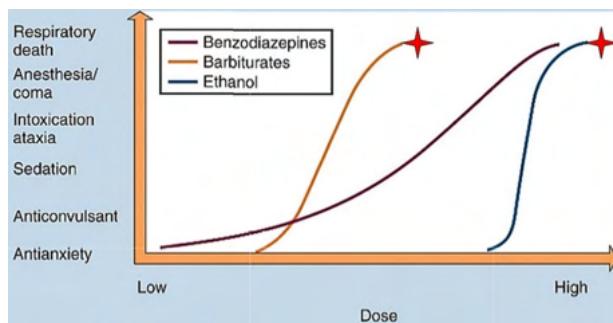


Figure 2.32: Acute toxicity of EtOH and other CNS depressant.

Remark 2.7. Note that these acute overdose of EtOH or barbiturate can be fatal.

Example 2.2.1. The Montreal children's hospital treats an average of 1 severely intoxicated teen per week! Sometimes the drinking that lead to intoxication

⁵Funny that this drug is made illegal yet a substance that's as lethal as it (or even slightly more), is still widely consumed.

was because of dare or to "look like an adult".

In Quebec, a 14-year-old drowned to death after accidentally falling into a local stream after consuming three 568mL can of alcoholic beverage with an alcohol content of nearly 11.9%. This is an equivalent of 12 glasses of wine!

Teratogenic

We can look at EtOH in the lense of a **teratogen**, which is a substance that a pregnant mother can ingest which lead to fetal abnormalities.

Observation 2.38 EtOH can be transferred to the fetus from a mother drinking. As the EtOH plasma level raises and reaches its peak, some of it will slowly distribute into the fetus, which we can measure in the amniotic fluid. This build up of EtOH can induce fetal alcohol syndrome in babies.

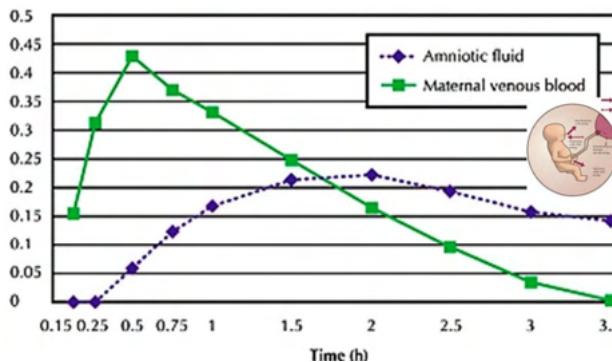


Figure 2.33: Build up of alcohol level in the mother plasma and amniotic fluid.

Definition 2.8. **Fetal alcohol syndrome** is a spectrum of disorder that a person can develop when their mother has consumed alcohol during pregnancy.

This syndrome affect around 300,000 people in Canada alone and what's sad is that this is a preventable disease.

Chronic

Obviously, with chronic drinking of EtOH, you can die.

Observation 2.39 EtOH can affect lots of bodily function but the 2 main targets are the brain and the liver (for chronic EtOH induced toxicity). Chronic usage can lead to **hepatitis**, then fatty liver and eventually **cirrhosis**, which is a severe scarring disorder of the liver.

We can actually see that the mortality from cirrhosis sharply increases as the amount consumption increases.

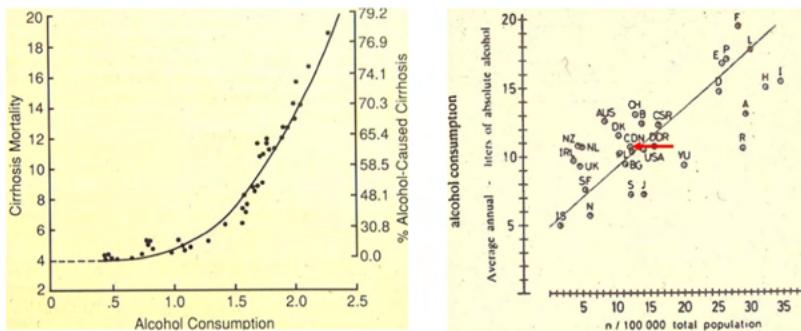


Figure 2.34: Mortality of Cirrhosis and consumed amount.

Beside the liver and the brain are affected, it also affect CVS, lungs, sexual response, GI, muscle, etc.

Remark 2.8. *Ethanol is a carcinogen and can cause 7 different kinds of cancer.*

Misconception. Low doses of alcohol can protect against heart disease.
Reality. Low dose with regular use will actually increases the chance of having a heart attack and strokes. In fact, there is no health benefit at any dose of alcohol. It does not prolong life expectancy (this is through a meta-analysis of around 5 million participants).

Observation 2.40 Usage of EtOH can lead to neurodegeneration which includes dementia, cerebellar ataxia, depression and etc. For the CVS, it causes arrhythmias, hypertension, strokes and many others. Through the lifetime of a drinker, they can develop chronic pancreatitis.

Impressively, we can combine chronic drinking with smoking, it also substantially increases the risk for **esophageal cancer**.

Furthermore, EtOH can damage the liver not only through chronic usage but also through metabolism. This is because its metabolism will generate aldehyde intermediate metabolite which is quite cytotoxic that can lead to lipid accumulation, inflammation and even fibrosis.

Observation 2.41 Now, let's say that you have cirrhosis, the liver will be damaged and thus blood flow through it is impaired. Alcohol entering orally will be taken effects by first-pass metabolism but because the liver blood flow is damaged, there will be an increase pressure in the portal circulation. With **surmounting pressure**, fluid will begin to leak out into the abdominal cavity which head toward **ascites**.

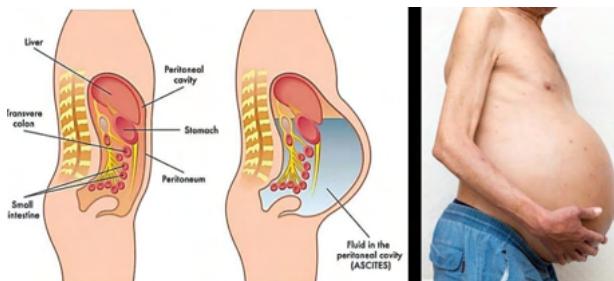


Figure 2.35: Ascites.

Another problem with cirrhosis is that the liver can no longer detoxify substances which mean they would be able to freely flow in circulation, reaching the brain, heart and lung. All of these are called **hepatic encephalopathy** u.e. injury to the brain that's due to the liver not detoxifying.

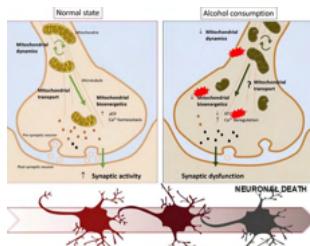


Figure 2.36: EtOH impairing mitochondria.

EtOH also seems to impair the mobility of mitochondria which lead to the impair function of neuron and death. Thus **it has irreversible brain damage** due to the loss of many neurons or even decrease in its functionality. See Figure 2.36

Of course, if you stop drinking, on average, it will increase survivability of that person.

2.2.5 Addiction of Ethanol

The effect of EtOH is less as blood level is falling than it's rising at any given concentration. This is the acute tolerance which is less pronounced than that of chronic.

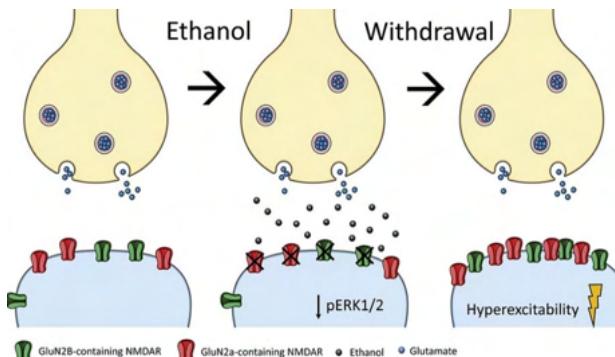


Figure 2.37: Upregulation of glutamate receptors leading to hyperexcitability.

Chronic Tolerance

With chronic tolerance of EtOH, there will be an upregulation of the glutamate receptors leading to **hyperexcitability** (see Figure 2.37).

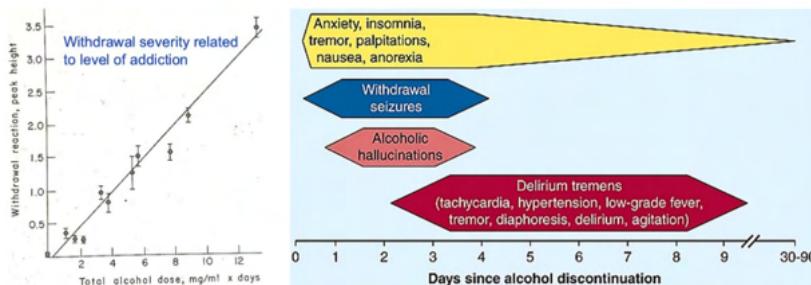


Figure 2.38: Withdrawals of EtOH.

When EtOH is taken away, patients can experience withdrawal seizures, hallucination and even delirium tremens that can last for days. The severity of withdrawal is linear to the level of addiction. This is because the hyperexcitability can kill off some of these neurons which is why it's best to treat

them slowly with CNS depressant.

Treatment 1. A way to stop a drinker from keep drinking is blocking the ALDH so that they get the full symptoms of acetaldehyde.

Treatment 2. There are drugs (like **acamprosate**) that can acts as glutamate receptor antagonist which can lower the abstinence symptoms. Or drug, like **naltrexone**, that can block opioid receptors to lower EtOH rewarding effect.

There are still continuous research on drugs that treat addiction of EtOH. However, so far what we've found to be the most effective is through psychological method via the **Alcoholic Anonymous (AA) meetings**. The last thing to comment is that we're still trying to figure out if there's an increased level of alcoholism as a result of the pandemic.

2.3 Cocaine, Amphetamine and Caffeine

We'll be looking at some stimulants drugs, namely: **cocaine, amphetamine and caffeine**.

Definition 2.9. **CNS stimulants** is a type of drug that can increase or stimulate the activity of the CNS.

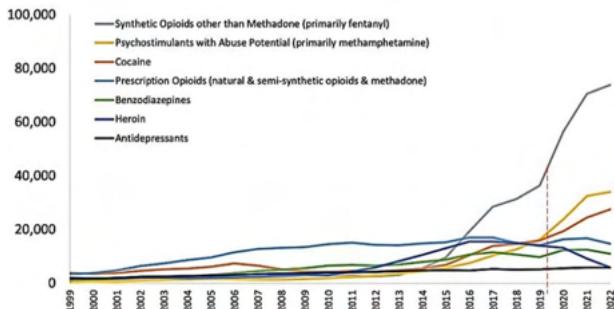


Figure 2.39: Danger of CNS stimulants.

Along with some dangerous opioid that's the leading cause of overdosing in the USA, we can see that cocaine and other CNS stimulants drugs

also place second and third to this. Thus, we can evidently see that CNS stimulants are also a major problem.

Definition 2.10. **Cocaine** is a CNS stimulant that originates from the extract of a plant called *Erythroxylum coca*.



Figure 2.40: Coca plant and cocaine molecule.

Observation 2.42 Originally, this plant was used recreationally by South American through chewing which will yield some mild effect such as numbing and decrease hunger. It was until the 1880s that the first extraction of cocaine from the plant.

The cocaine extract was mainly used for local anesthetics such as remedies for toothache but also used recreationally in drinks for its stimulating effects. Unfortunately, in the past, people believe that cocaine would be the cure to opium addiction as it's a stimulants while opium are depressants (which as we've seen, does not matter).

Observation 2.43 During this time, because of its title for being used recreationally, cocaine was also being mixed with wine and most notably, a company name **Coca-Cola** has it as an ingredient in their drink.

At first, coca-cola was advertising their drinks as a remedies to cure fatigueness and sleeplessness. Slowly, they turns into making their drink as recreational consumption. Because of this, some children was able to obtain and consume it which lead to death. Thus, by the 1900s, the cocaine extract were completely removed.

Nevertheless, cocaine plantations are still a thing (mostly in South America) and are still exporting it out (some might be illegal too).

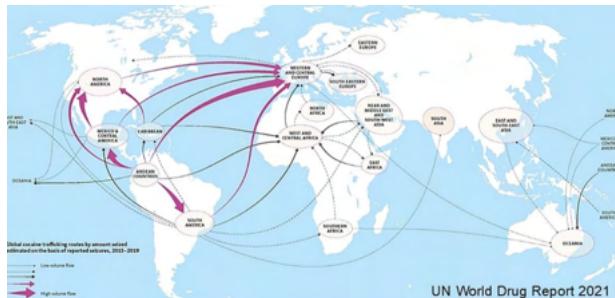


Figure 2.41: Cocaine trafficking.

Observation 2.44 Cocaine can exist in 2 forms: cocaine HCl and free-base (crack). Cocaine HCl is cocaine in powder form that can be sniff directly or injected. In its free-base form, cocaine can withstand higher temperature which allow it to be smoked.



Figure 2.42: Cocaine HCl and free base form.

2.3.1 Pharmacodynamics of Cocaine

The pharmacodynamics of cocaine has been studied extensively.

Observation 2.45 First, it can **blocks voltage-gated Na⁺ channel**. This also means that the axon conduction would be blocked as well. Not only in the brain but it can also affect other excitable tissues like the heart, nerves, etc.

At high/dangerous dose, cocaine can cause convulsion then respiratory arrest. If the administration was made rapid, it can bypass its excitation effect and go to respiratory arrest directly then death. In the heart, this dose can block the SA node or even conduction of the His-Purkinje System
⇒ **cardiac arrest.**

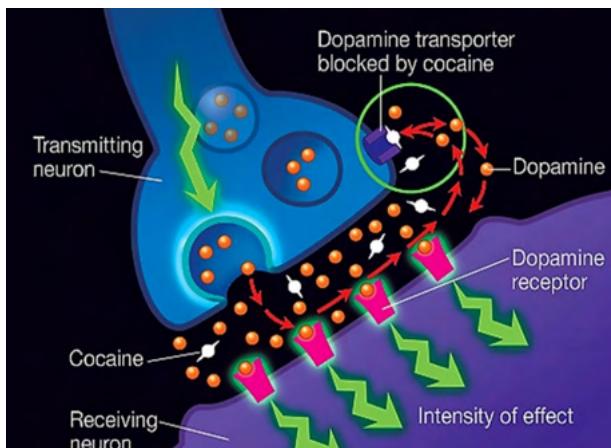


Figure 2.43: Cocaine block neurotransmitter reuptake.

Observation 2.46 Next, cocaine was found to also block the uptake of neurotransmitter, in particular: noradrenaline, 5-HT and dopamine. Because it blocks the reuptake of dopamine ⇒ higher extracellular dopamine than usual ⇒ higher activation of dopaminergic neurons ⇒ addictive potential.

Remark 2.9. *This does not just happen in the brain but in the periphery which is why it has local anesthetics effects.*

Methods 2.4 We can study the effects of cocaine using mouse model. Study the movement of 2 mouse of which 1 is normal while the other has injected doses of cocaine.

In the end of the experiment, we will see that the cocaine-injected mouse will have noticeably more movement than that of the normal.

Methods 2.5 Using the mouse model again, this time we get it addicted to cocaine. Then, we remove its dopamine neurons in the mesolimbic reward pathway and allow the mouse to self-inject cocaine.

What we'll find is the mouse will stop taking cocaine as it will not provide any "high" due to the lack reward pathway. Notice that there are 3 dopaminergic pathways in the brain (not just reward) which means that the effects of cocaine can be widespread too.⁶

Remark 2.10. *Cocaine is a complex drug and there are still more effects that it can induce that we've yet to discover.*

2.3.2 Pharmacokinetics of Cocaine

Absorption

Regardless of the way cocaine is absorbed in the body, it will quickly spread through the body, mainly the brain and heart to induce its effects.

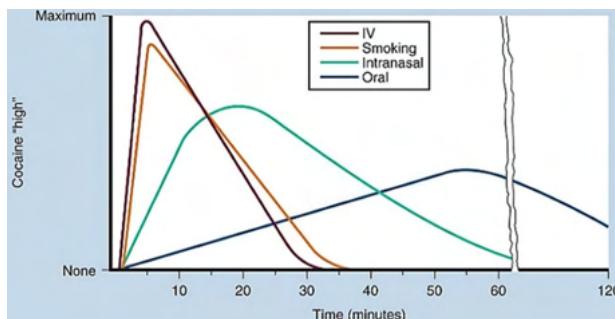


Figure 2.44: Absorption of cocaine.

Observation 2.47 The injection and smoking of cocaine has the fastest rate of absorption to the system. This follows by intranasal i.e. sniffing the powder. Lastly, the oral route will have the slowest rate of absorption but also a lower "high".

Explanations. The oral route is much slower compared to the rest because cocaine is heavily metabolized in the liver after the first-pass (75% of it is metabolized in first-pass). □

In summary, oral has the highest first-pass, slow absorptions with little euphoric feeling; intranasal route will not be well-absorbed because it can cause vasoconstriction in the nose which can lead to potential injuries.

⁶This is also because cocaine is a non-selective drug.

Next, for IV injection it's faster but require syringe; for the most common method, smoking would be the fastest, cheapest and easier way to be absorbed.

Distribution

Like we've mentioned, when cocaine is absorbed, regardless of method, it will be distributed everywhere in the brain, heart as well as everywhere else in the body that have excitable tissues.

Metabolism

When it comes to the metabolism of cocaine, there are lots of enzymes to do this but all are under the same type of enzymes and that is **carboxyl esterase**. The metabolism takes place in the liver and because it has so much enzymes \Rightarrow half-life is short.

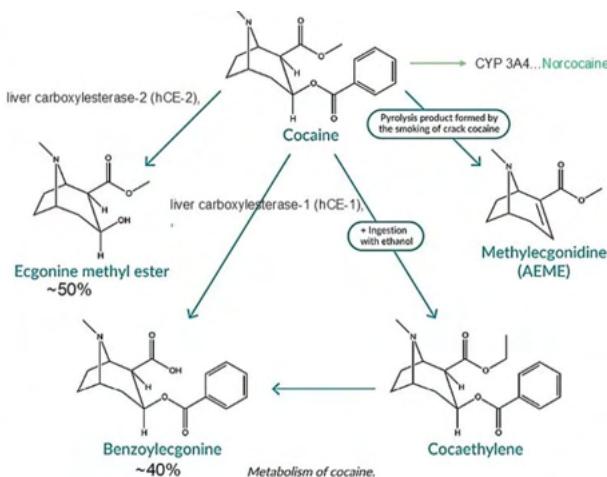


Figure 2.45: Cocaine metabolism.

Observation 2.48 Normally, cocaine is metabolized into **benzoylecggonine** that can then be eliminated. When taking cocaine with ethanol however, the metabolism will produce a compound called **cocaethylene**.⁷

⁷Typically, usage of cocaine can have some side effects and people drink alcohol to reduce said effects.

Cocaethylene is a very toxic compound that's even worst than cocaine. Its half-life is $3.5\times$ that of cocaine, lethal dose is much lower and can cause cardiac arrest more commonly. With chronic usage, it can lead to severe liver damage, risk of stroke. All in all, it's $20\times$ more risky for death than cocaine alone.

Elimination

Lastly, cocaine metabolites can be eliminated/excreted in urine for days.

Remark 2.11. *Interestingly, these metabolites can be deposited in hair as well which we can test for any prior use.*

2.3.3 Toxicity of Cocaine

When consuming lots of cocaine, there would be many toxic consequences.

Observation 2.49 We've previously said that it would have some negative effects regarding the CVS this includes cardiac arrhythmia and **myocardial infarction (heart attack)**.

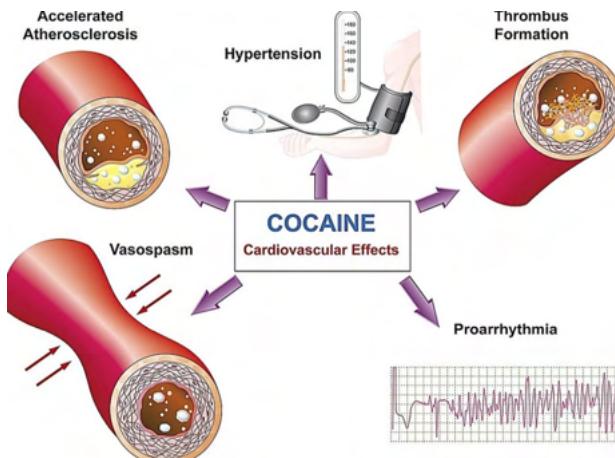


Figure 2.46: Cocaine effects on the CVS.

In particular, it will cause vasospasm and hypertension. With vasospasm of vessels on the heart muscle, it can lead to inconsistent heart rhythm hence arrhythmia. For hypertension, it can risk damaging the blood vessel

which can lead to **atherosclerosis** and which create blockage and if this was a coronary artery \Rightarrow heart attack.

It does not just limit at the heart, hypertension and vasospasm is widespread through the body and if ends up in vessels of the brain, it risks of having **cerebrovascular accident (stroke)**. Another this that is very dangerous is the development of an **aneurysm** (bulging out of weakened areas of blood vessels).

Observation 2.50 It must be noted too that cocaine is rapidly absorbed in the heart. We can see this by tracking cocaine concentration in the heart with the concentration in the lung in the graph below.

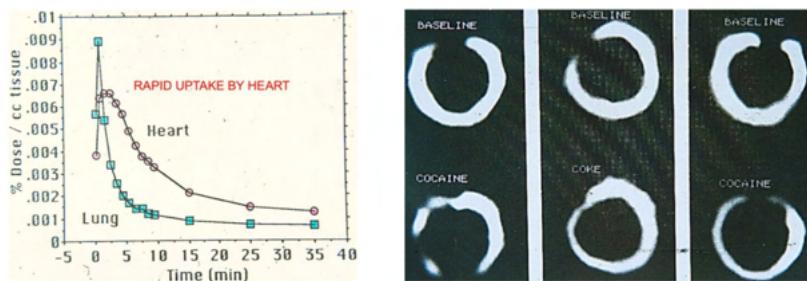


Figure 2.47: Cocaine absorbed rapidly in the heart and decrease blood flow there.

Furthermore, cocaine can cause a decrease blood flow in the heart. We can track this by looking at blood flow in the heart of dog before and after cocaine administration.

Observation 2.51 Abuse of cocaine can also lead to **seizure**. With the abuse, there's a particular group of people can experience **agitated delirium**. Patients with this particular syndrome can be intensely delirious with lots of aggressiveness. They also experiences hyperthermia prior to this (which probably has something to do with a variant of D2 receptor).

Unfortunately, subgroups that experience this do not need high dosage, its mechanism are not yet understood as there's no animal model available. Lastly, it is fatal as post-delirious state can lead to respiratory arrest and ultimately, death.

Observation 2.52 Next, consumption of cocaine can lead to **acute pul-**

monary injury (crack lung). Furthermore, because of its stimulating effects, it can create death from aggression towards other which includes, but not limited to, murder, accidents and trauma. When the stimulating effects are over, it can lead to depression and then suicide.

Chronic use of cocaine can lead neuronal injury since it blocks the re-uptake of dopamine, which at high concentration can be neurotoxic, especially for infants. This also means that pregnant mothers, when consume cocaine, can cause fetal death and abnormalities ("crack babies"). It's not as dramatic as the fetal alcohol syndromes but it includes some dangerous conditions too, includes: fetal hypoxia, premature labour, impaired brain development and limb abnormalities.

2.3.4 Addiction of Cocaine

Observation 2.53

With repeated use of cocaine, we've seen that there will be a depletion of dopamine which can lead to addiction due to biological adaption of neurons. In fact, this changes can be long-lasting even after withdrawals. In fact, when observing rats activity weeks after stopping cocaine, it's not back to normal yet.

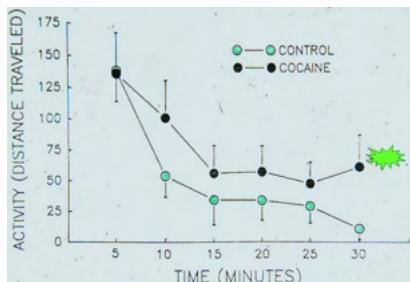


Figure 2.48: Rats activity weeks after stopping cocaine administration.

On top of that, cocaine repeated use can change the nature of glutamate receptors i.e. glutamate receptors normally can only allow Na^+ in but with the repeated use it modified into allowing Ca^{2+} . This implies that **long term usage of cocaine can modify the nature and the number of glutamate receptors.** See Figure 2.50

Methods 2.6 This long lasting effects can be tested and seen and is more severe than alcohol supposedly. Obtain 3 groups of individuals who are either: alcoholic, cocaine-user and normal. For the alcoholic and cocaine group, they will be made to stop their usage for 3 months. Now, we will test for their reaction time and resting tremor.

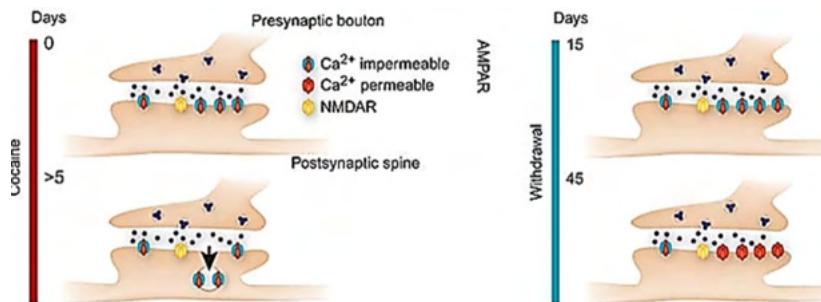


Figure 2.49: Changes in glutamate receptors with cocaine usage.

Observation 2.54 As we can see alcohol group has reaction time that is getting closer to that of the normal group i.e. there are some progress of reverting back. However, when looking at the cocaine group, even after stopping for 3 months, their reaction time is still noticeably higher than the rest.

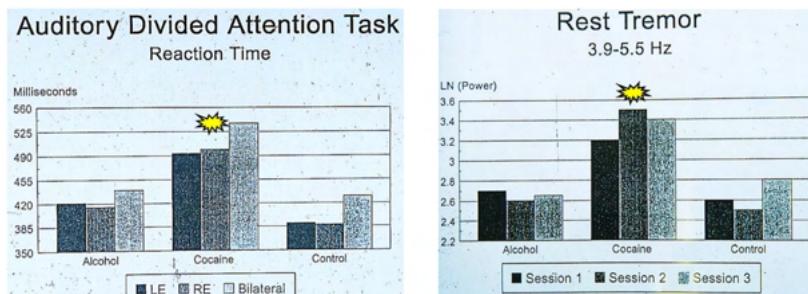


Figure 2.50: Reaction time and resting tremor of cocaine user.

Similarly, cocaine users after 3 months stopping will still have higher resting tremor than that of normal and alcohol users.

Observation 2.55 Looking at the cell density of primate that were put on 1 year of cocaine then stop for 2 years, we see that there are still continuous changes in the cell numbers. This changes has high correlation to long-term cognitive impairment as well.

Not only in primates, we also see this in human where there are still changes in after withdrawals. And if you were to look at the dopamine

receptor count of cocaine user, we can see its availability start to decline more and more overtime.

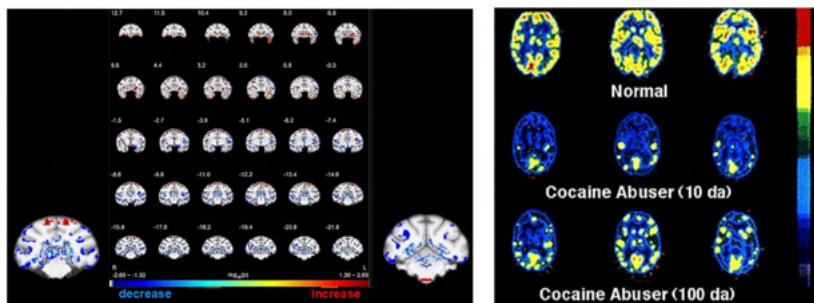


Figure 2.51: Changes in neurons of primates (left) and human (right).

Observation 2.56

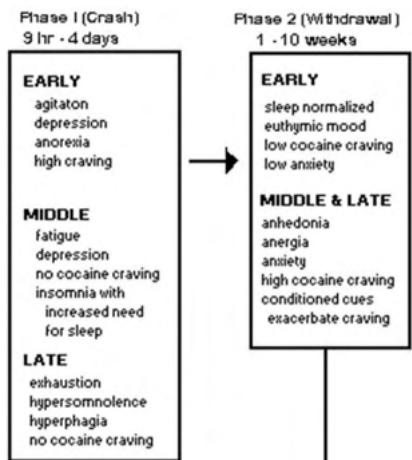


Figure 2.52: 2 phases of cocaine stopping.

When it comes to addiction of cocaine, the most common pattern is binging on it, could be 1 dose every couple days or even hours. They're tend to be more irritable which could be due to cross usage of other drugs like alcohol and heroin. Repeated usage may damage sexual function, kidney and can produce anxiety, depression, psychosis and etc. We can become tolerant to cocaine as well.

When a person stop taking cocaine, they will experience 2 different phases: phase I and phase II. In phase I, it's known as the crash, that last 9h to 4 days which includes agitation, fatigue and exhaustion, etc. In the phase II, it's the withdrawals, that last 1-10 weeks which can have positive effects like normalized sleeps but also negative

like anxiety.

An remarkable aspect of withdrawal from cocaine in **severe anxiety** which could be caused by the changing of noradrenergic pathway.

Observation 2.57 Interestingly, this addiction is also related behaviour too. In the experiment, we let a cocaine addict watch a video that's either related to nature or cocaine. What we've found is that the amygdala of the addict lit up when the video about cocaine showed up.

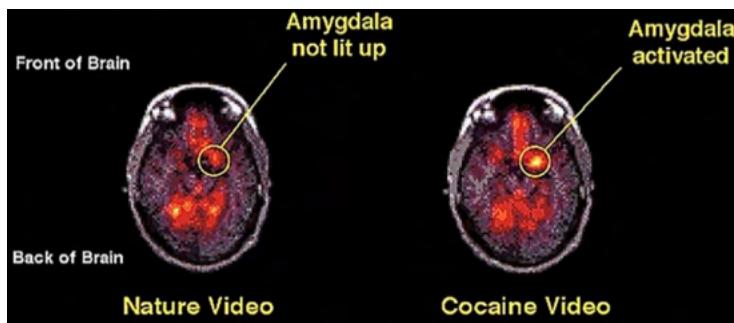


Figure 2.53: Cocaine addiction through visual cues.

Remark 2.12. *Chronic usage of cocaine can also have lots of effect on neurons dendrites.*

Now, we will look at some possible treatment that's being develop to this moment.

Treatment 1. We can administer to patient **dopamin transporter blocker** (like GBR 12909) which can decrease the amount of dopamine in the reward system when cocaine is administered.

Treatment 2. This is a way we're still developing and that is a **vaccine against drugs**. So far, this is still a theoretical idea but we're trying to find ways to bring it to reality.

2.3.5 Amphetamines

Definition 2.11. **Amphetamines** are CNS stimulant that can increase dopamine and NAD release whie blocking their reuptake thereby affecting the nora-drenergic pathways in the brain.

Observation 2.58 A combination of amphetamine with another drug called **dextroamphetamine** is used to treat ADHD as well as **narcolepsy**, which is the inability to stay awake.⁸

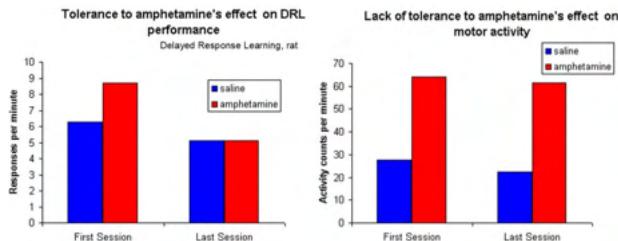


Figure 2.54: Amphetamine tolerance effects.

Observation 2.59 Typically, this therapeutic treatment is made with low doses but with higher doses it can have lots of addictive potential. Like cocaine, people that take amphetamine will develop tolerance especially for learning. Meanwhile, when it comes to motor activity, there's no tolerance effect i.e. still high with the next session of administration.

Observation 2.60

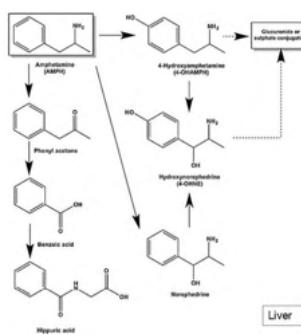


Figure 2.55: Metabolism of amphetamine in the liver.

Regarding the metabolism of amphetamine, some of its metabolites are neurotoxic. Not only that, certain amphetamine derivatives can also induce neurotoxicity.

Definition 2.12. "Designer" amphetamine or amphetamine derivatives are drugs that are made to have the same original amphetamine base structure with some addition and/or modification. See Figure 2.56.

Regardless of whichever derivatives, they all have similar effect as

⁸The combination of amphetamine and dextroamphetamine is sold under the name *Adderall*

amphetamine which are increasing the release of dopamine, NAD and block their reuptake \Rightarrow addictive.

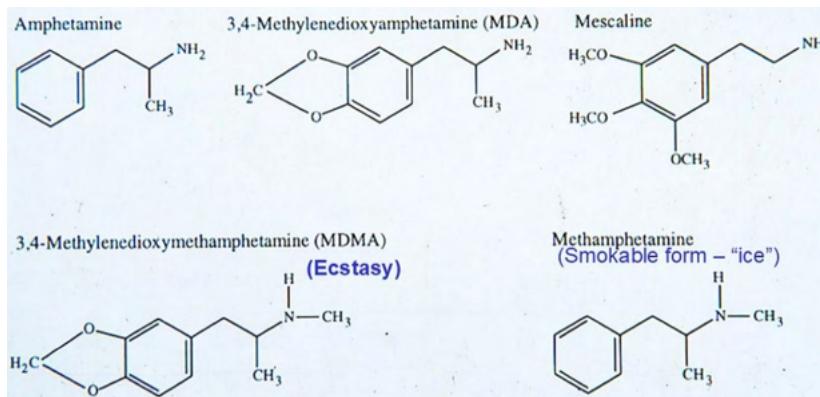


Figure 2.56: Amphetamine derivatives.

Methphetamines

Observation 2.61 Methphetamines can damage dopaminergic neurons and its pathways to do so is quite complex.

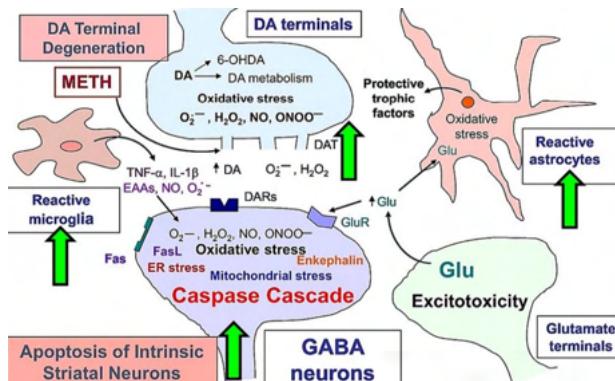


Figure 2.57: Methamphetamine neurotoxicity.

First, highly addictive drugs can cause oxidative stresses and creates radicals in dopaminergic neurons' terminals which also will damage its

postsynapses. These oxidative stresses will lead to their degeneration which trigger the brain in recruiting **microglia** and **astrocytes** that releases cytokines and reactive compounds to these neurons. All in all, lots of pathways are involved and active which lead to death of some neurons in the brain.

In the end you'd get an increase in neuronal loss as well as a decrease of dopamine receptors. Now, if a meth abuser were to stop, we would see a recovery of the dopamine transporter however cognitive function will not recover.

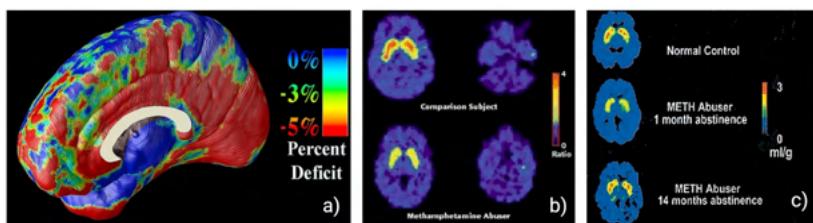


Figure 2.58: a) neuronal loss b) decrease in dopamine receptors and c) recovery from meth with no cognitive function recovery.

Remark 2.13. Because of the loss of dopaminergic neurons with the use of meth, users of this drug are actually high risk of having **Parkinson's disease**.

Ecstasy (MDMA)

Observation 2.62

Ecstasy (MDMA) is a stimulant that has hallucinogenic effects. It can act on a variety of receptors but most prominently is **serotonin (5-HT) receptors**.

Remark 2.14. MDMA can release toxic metabolites that can increase free radical production and lead to 5-HT axonal degeneration.

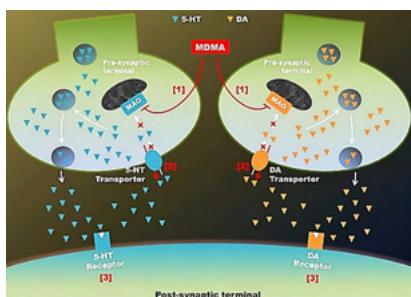


Figure 2.59: MDMA effects on 5-HT receptors and DA transporter.

Observation 2.63 There are lots of area in the brain that can be affected by MDMA: neocortex, basal ganglia, amygdala, hippocampus and hypothalamus. Usage of MDMA can induce some acute effects like heightened perception, stimulation, reduced appetite and elevated mood. On the other hand, there are some adverse effects like clouded thinking, disturbed behaviour and jaw-clenching and even life-threatening effects like hyperthermia, arrhythmia and renal failure.

When looking at its long term effects of its usage, there will be a reduction in serotonin and its metabolites. Simultaneously, there's reduction in 5-HT transporter and terminals (due to degeneration).

Methods 2.7 MDMA can also lead to permanent damage to memory control. We can look at primates again that were using ecstasy chronically and then stop. We will see that after 7 years of stopping, there will be some recovery of 5-HT neurons but the net change is a permanent loss.

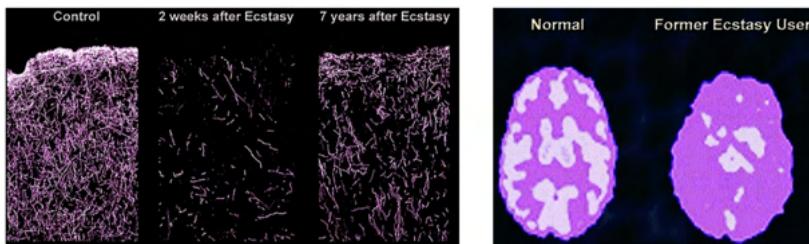


Figure 2.60: Presence of 5-HT neurons in primates study (left), Loss of 5-HT transporters (right)

Not only that, we will see a loss of some 5-HT transporters as well.

2.3.6 Caffeine

Definition 2.13. **Caffeine** is a CNS stimulant that presents in coffee, tea and lots of other beverages. It's part of a family of drugs called **xanthine** which also include varieties that are present in chocolate.

Observation 2.64 Depending on the kind of beverage you're drinking, the caffeine contents can vary wide a lot. Nevertheless, it's quite a universal knowledge that coffee has the highest content of caffeine compared to any other drinks.

Pharmacokinetics

We'll now briefly look at the pharmacokinetics of caffeine

- **Absorption:** The absorption of caffeine is rapid and reach peak onset around 0.5 – 2h.
- **Distribution:** Caffeine is distributed in all tissues rapidly (also include the fetus).
- **Metabolism:** Its metabolism is also as rapid with a half-life of 4 – 5h and even more in smokers.
- **Excretion:** Caffeine can be excreted through the urine via the kidney.

Observation 2.65 What's remarkable is that neonates do not have enzymes to metabolize caffeine of which its half-life is around 65-102h premature at birth or around 82h at normal birth. This number will decrease down to 14.4h and will reach to relative normal level of 2.6h by 5-6 months old where they have the enzymes.

Pharmacodynamics

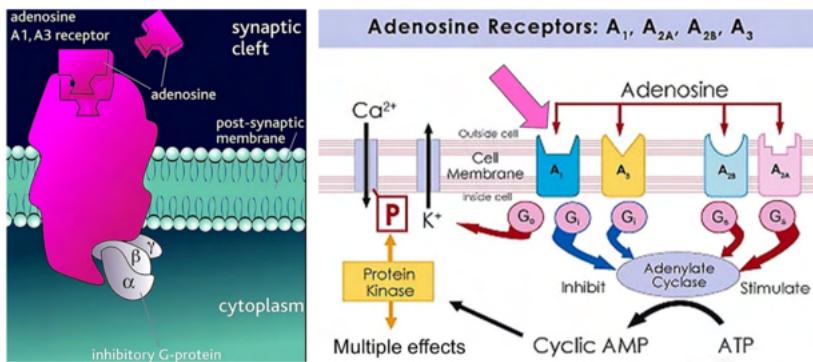


Figure 2.61: A1 receptors and pathways.

Observation 2.66 Caffeine is a competitive antagonist of **adenosine (A1) receptors**. Adenosine is a neuromodulator that can inhibit the release of

lots of different neurotransmitters (like dopamine, noradrenaline, acetylcholine, glutamate,). This means by if caffeine is a antagonist to the receptor of an inhibitor, it can thus allow a little reactivation of these neurotransmitter which is the origin of the awkeness feeling it gives i.e. **adenosine lowers the activity of cholinergic pathway \Rightarrow sleepiness; while caffeine will block adenosine \Rightarrow cholinergic pathwat reactivate \Rightarrow awkeness.**

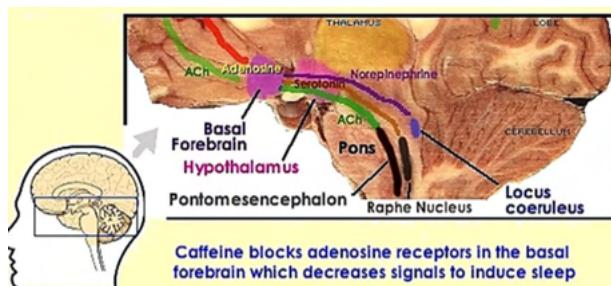


Figure 2.62: Caffeine affects the A1 receptors.

Observation 2.67 Caffeine can have effects on the brain, heart (increase heart rate), cerebral vessels, gastric acid (increase secretion) and even diuresis (increase urine formation).

When consuming caffeine daily, you'd build rapid tolerance which lead to an increase of A1 receptors and people can experience some mild withdrawals. Luckily, we've found no link to cancer with the usage of caffeine. Nevertheless, do not consume it during a panic attack and it has a lethal dose of 100 cups.

Remark 2.15. *All in all, moderate consumption of caffeine is the best.*

2.4 Benzodiazepines and Cannabinoids

In today's lecture, we will mainly look at drugs that can treat certain mental diseases/disorders.

Definition 2.14. **Psychopharmacology** is the study of drug that can affect and possibly change the brain behaviour.

Lecture 10: October 1st, 2024.

Evidently, there's been observation of many mental illnesses throughout history. Pharmacologists have done lots of research to figure out of the neuronal connection and communication, it's not done yet but we're still getting there.

2.4.1 Schizophrenia

Definition 2.15. **Schizophrenia** is a severe mental illness that are characterized by a combination of hallucination, delusion and even disorganized thinking and behaviour.

Observation 2.68 Schizophrenia is a mental illness that we have little knowledge about its mechanism. There's been a number of studies on the genetic vulnerability of this illness and so far, it shows that identical twins have a near 50% risk of developing schizophrenia. Obviously, there are other underlying factors and not just genetics.

Looking at monozygotic twins of which one was affected by schizophrenia while the other is unaffected. We see that there are changes in the brain structures between them.

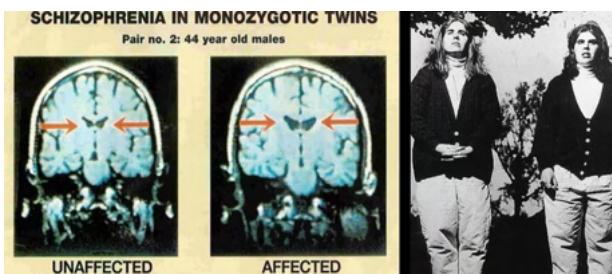


Figure 2.63: Schizophrenia in monozygotic twin.

Through psychopharmacology, researchers of the past has developed drugs that can treat schizophrenia. These drugs were found to mainly target the D2 receptors for dopamine.

Observation 2.69 The number of patient that suffer from schizophrenia reaches its all time high in the early 1950s. By the mid 1950s, pharmacologists have begin to develop psychoactive drugs to attempt and treat this. Remarkably, from then, we see a fast decrease of in number of patient and by the 1980s, we've only got around 200 patient (as compared to 500+).

Schizophrenia underlying cause was an increase in dopamine release. When $[dopamine] \uparrow$ in the extracellular space of the brain will lead to delusion and hallucination (symptoms of schizophrenia). Not only that, it can cause impaired cognition.

Remark 2.16. *With lots of testing and comparison different antipsychotic drugs, we've found that its effectiveness correlates to its ability to block D2 receptors.*⁹

Observation 2.70 The first drug that was designed to do so is called **Chlorpromazine** that can mostly block D2 receptors. Evidently, for first time, this drug is not selective enough \Rightarrow it has adverse effects however, the fact that it treat schizophrenia outweigh the adverse effects it brings (according to patients).

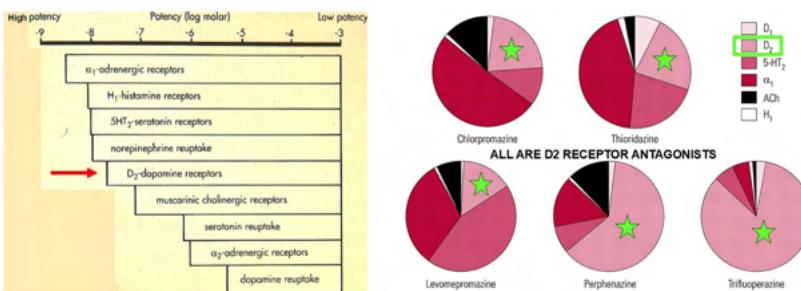


Figure 2.65: Selectivity of chlorpromazine compared to other drugs.

Now, we can compare chlorpromazine to other drugs that were developed later like **thioridazine** to **trifluoperazine**. There's an increase in selectivity of the drug to the D2 receptors.

With the development of newer antipsychotic drugs that are more selective to D2, it will lead to a reduction of adverse effects. Clearly, just being

⁹In other words, the more selective it is to D2 receptors, the better it is.

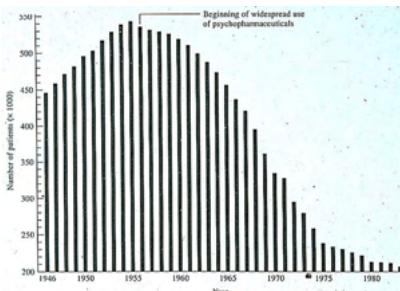


Figure 2.64: Decrease in schizophrenia cases with the development of drug.

selective to D₂ is not enough, we also have to study the neuronal communication in order to further reduce the adverse effects and increase efficacy of the drug. Nevertheless, this is more challenging in the brain than in the periphery.

Explanations. This is because neurons are connected in a hierarchical fashion in the periphery while in the brain, it's a more diffused hierarchical order i.e. 1 neurons at the top can induce its effects only multiple different neurons which can also induce their effects to others and etc. □

2.4.2 Depression

Definition 2.16. **Depression** is a fairly common mood disorder that is characterized by feeling of prolonged sadness (most of the time without reasons), loss of interest and low mood in general.

Observation 2.71 Drugs that can treat depression are called **antidepressant drugs** and they can have different mechanism of actions depending on the type. Nonetheless, all of them will lead to an increase release of neurotransmitters or decrease its metabolism in the presynapses. Mostly, **these drugs are designed to target either noradrenergic or serotonergic neurons.**

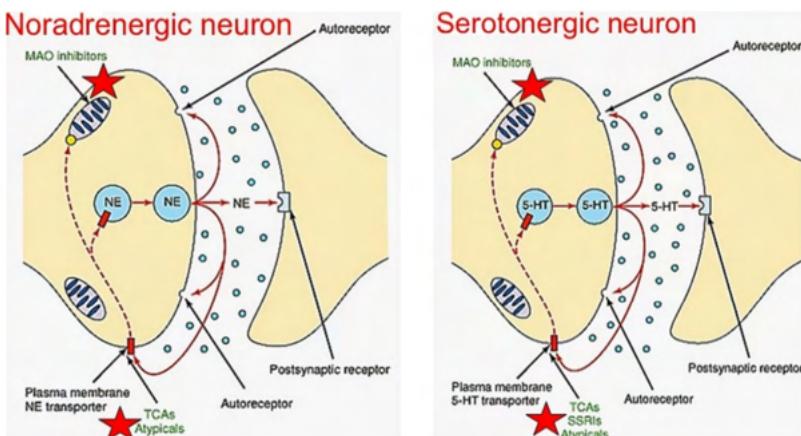


Figure 2.66: Antidepressants effects on noradrenergic and serotonergic neurons.

Example 2.4.1. **tricyclic antidepressants (TCAs)** can treat depression through blocking the reuptake of noradrenaline as well as 5-HT. For **selective serotonin reuptake inhibitors (SSRIs)**, it only blocks 5-HT reuptake to treat depression. Also, you have **monoamine oxidase inhibitor** that can decrease the metabolism of noradrenaline and 5-HT.

Observation 2.72

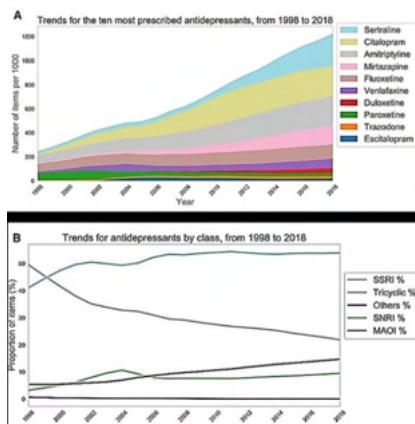


Figure 2.67: Trend in antidepressant drug studies on these kind of drugs.

Looking at the trend of drug development for depression but also usage, we're seeing an increase in usage and design or SSRI such as sertraline and citalopram.

It must be noted that 5-HT pathway in the brain is fairly extensive. We've found that there are around 7 families of 5-HT receptor (with all of them being GPCRs beside 1 which is ligand-gated) and are located at different location. Thus, there's been continu-

So to summarize the idea of these drugs a bit, you have drugs that are specifically designed to decrease reuptake of noradrenaline, others are for decrease 5-HT reuptake, and others for decrease in metabolisms

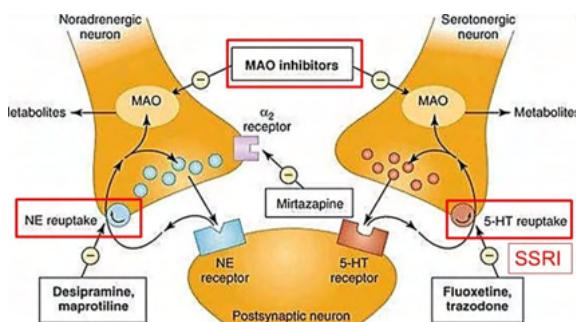


Figure 2.68: Antidepressant drugs mechanisms.

As of 2023, SSRIs are still at the top of most usage for antidepressant effect. We also see lots of usage **serotonin noradrenaline reuptake inhibitors (SNRIs)** but not as significant as SSRIs. Evidently, the ranges in usage will depend on the need of each person and their physiology.

Observation 2.73 SSRIs are not perfect and can induce some adverse effect. One of the major one is its inhibition of some CYP enzymes. This can be dangerous as patients can be taking other drugs which lead to drug interaction where said drug cannot be broken down.

MULTIPLE ACTIONS OF ANTIDEPRESSANTS

Class	Drugs	Re-uptake Inhibition			Receptor Blockade			Potent CYP enzyme Inhibition		
		SRI	NRI	DRI	Hist	Musc	Alpha1	2D6	2C19	1A2
SSRIs	Fluoxetine	+++	-					✓		
	Fluvoxamine	+++	-					✓	✓	✓
	Sertraline	+++	-	+		-	-		✓	
	Paroxetine	++++	+	+		++		✓		
	Citalopram	++	-					(✓) weak		
	Escitalopram	++	-							
TCAs	Amitriptyline	+++	++	-	++	++++	++	✓	✓	
	Nortriptyline	++	++++	-	+	+	+			
NDRI	Bupropion	-	-	+						
SNRI	Venlafaxine	++	-							
	Duloxetine	++	-							
NaSSA	Mirtazapine				++++	-	-			

Figure 2.69: Antidepressants function and inhibition of CYP enzymes.

When it comes to the side effects, it can vary from 1 person to the next but in general, they could experience some GI problems, weight gain, sexual function, hypotension and altered sleep.

Observation 2.74 Another thing to consider is that people will have different alleles for metabolizing enzymes. For normal individual, they would experience some side effects of the drug but also its therapeutic effects. For intermediate metabolizer, they will metabolize antidepressants slower \Rightarrow more side effects.

In the worst case, poor metabolizer will have a hard time metabolizing it \Rightarrow potential to overdose. Additionally, this can be confusing as people will think this overdosing effects is a symptoms of depression and ignore it.

Lastly, it must be noted that not all depression required drug and can simply a little therapy session is enough.

Example 2.4.2. People that suffer from **seasonal affective disorder** will experience depression. This can be due to change in light settings such as going from a tropical environment with lots of sunlight to that of tundra or polar environment where sunlight is at minimals. For said individual, the best way is through **light therapy** i.e. staying in a room with bright artificial light that has the same frequency as the sun in the tropics.

2.4.3 Benzodiazepines

Before talking about benzodiazepine, let's talk about the psychological condition that it treats: anxiety.

Definition 2.17. **Anxiety** is an emotion that refers to the anticipation of future concern or even just an unpleasant inner conflict.

Observation 2.75 Anxiety is not a rare condition that people experience. Through practice, people tend to overcome their anxiety because it's a mechanism that forces you to prepare something you're about to do.

From that observation alone, we can tell that **anxiety, to a certain level, have good adaptive function**. However, with excess or too little anxiety can be bad too.

Definition 2.18. **Anxiety states** are combinations of psychological and mental manifestation of anxiety. It has nothing to do with real danger and more to do with sporadic anxiety attack or as a persistent low level of anxiety state.

Observation 2.76 Though generally treatment of anxiety would be through therapy or self-reevaluation however certain cases of anxiety would required one to be medicated.

Example 2.4.3. The followings are anxiety conditions that are medicated:

- Stress-related
- Generalized anxiety disorder
- Panic attacks
- Phobias

- Secondary anxiety, disease
- Drug-induced anxiety.

Definition 2.19. **Benzodiazepines** are a class of drug used to mainly treat anxiety (anti-anxiety).

Observation 2.77 The name derived from its own structure of having a benzene ring (benzo-) and a 7-membered ring with 2 nitrogen (diazepine). Not only that, there are possibly R-group that can be attached to this basal structure allow specific uses.

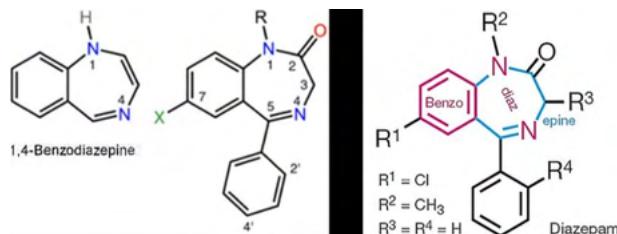


Figure 2.70: Structure of Benzodiazepine.

Like we've mentioned, benzodiazepines are treatment to anxiety. In certain forms, they're useful to induce sleep, act as anticonvulsant, muscle relaxant and can even impair memory function i.e. inducing amnesia.¹⁰

Remark 2.17. *Nevertheless, they're still most useful in acute, stress-related anxiety.*

Observation 2.78 As compared to other psychoactive drugs, they're much more safer. This is because its lethal dose is over $1000\times$ greater than the normal therapeutic dose.

Pharmacodynamics

Benzodiazepines' main targets are GABA_A receptors and their subtypes (which is why the drug can be designed with good specificity).

Definition 2.20. **GABA_A Receptors** are receptors that allow GABA to enter and hyperpolarize the cell, specifically neurons \Rightarrow they will be inhibited.

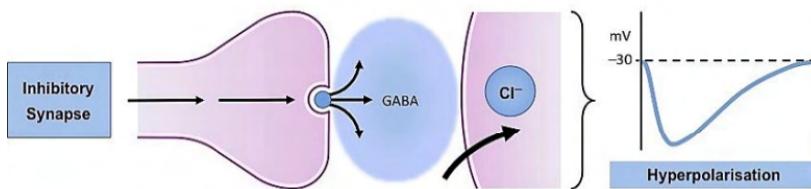


Figure 2.71: GABA and its inhibitory nature.

Observation 2.79 GABA_A receptors are found everywhere in the brain and their system can induce inhibitory effects on other types of neurotransmitter system like 5-HT, dopamine, etc.

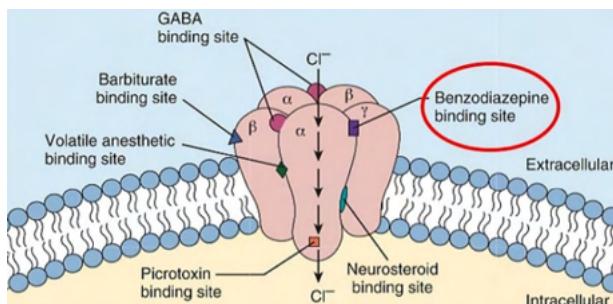


Figure 2.72: GABA_A receptors subunits.

Looking at the structure of GABA_A receptors, we can see that it's made up of different subunits. In particular, it can be a pentamer with a combination from either of the followings: 5 α subunit, 4 β subunits and 3 γ subunits.¹¹ These subunits would hold a specific allosteric site and benzodiazepine can bind to.

Observation 2.80 At the postsynapses, some GABA_A receptors can site directly at the synapse and some will be located in the extrasynapses. Typically, released GABA will first bind to the GABA_A receptors at the synapses to induce the true phasic inhibition. On the contrary, some GABA can move to the extrasynapses and activate these GABA_A receptors to induce

¹⁰Inducing amnesia would be useful if the patient have to go through an unpleasant medical procedure.

¹¹There exists also the δ , ε , π and θ subunits but rare.

the tonic inhibition. These extrasynapses GABA_A receptors can be positively modulated by neurosteroids produced by astrocytes as well.

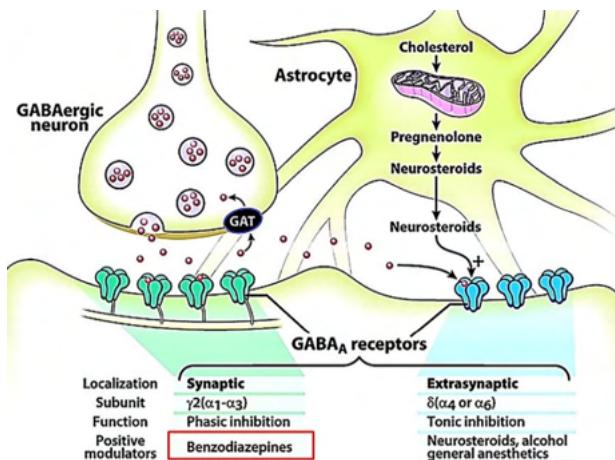


Figure 2.73: GABA_A at synapses and extrasynapses.

Benzodiazepines are positive modulators of GABA_A receptors. Unlike other drug that bind to a receptors will induce the effect, benzodiazepines will come and potentiate the effects made by GABA on its receptor. In this instance, upon binding to its allosteric site on GABA_A receptors, benzodiazepines will push the dose-response curve toward the left \Rightarrow we're now requiring less GABA to reach the same maximal response.

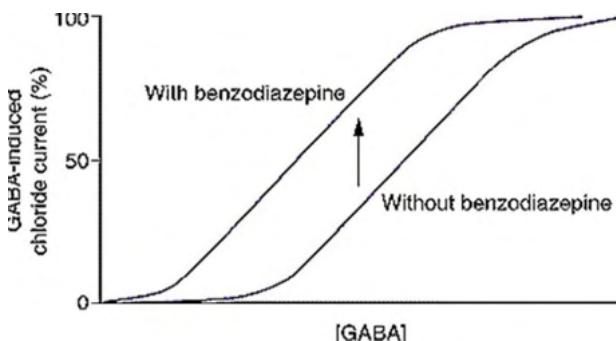


Figure 2.74: Modulation effects from benzodiazepines.

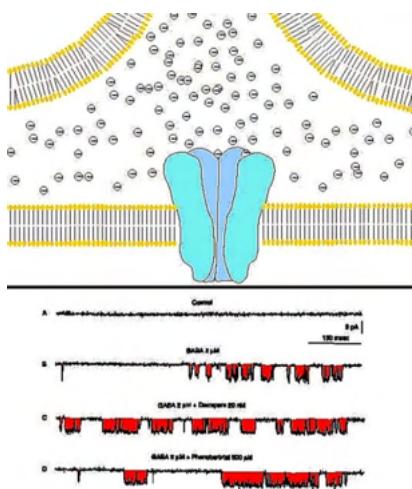
Observation 2.81

Figure 2.75: Benzodiazepines vs barbiturates.

non-selective \Rightarrow benzodiazepines are more safe. \square

Now, we can compare benzodiazepines with barbiturate in term of their ability in induce inhibition. What we can see is the activity in barbiturate is less frequent but will last for quite a long time contrasted to benzodiazepines that have short repeated burst on activity for GABA inhibition.

Explanations. The reason that this happens is because benzodiazepines will modulate the frequency of the opening while barbiturate modulate its duration of opening. Another thing is that benzodiazepines do not act directly and is more selective on its receptors while barbiturate will act more direct and is

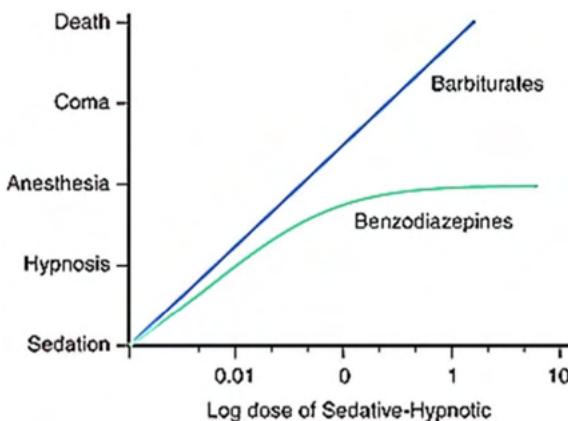


Figure 2.76: Increasing dose of benzodiazepines vs barbiturates.

Observation 2.82 In fact, the above explanation can be seen through the

effects of benzodiazepines and barbiturate have as we increase the dose. What we'll see is an increase in dosage in benzodiazepines will eventually plateau at its anesthetics effects. Contrarily, with increasing dose of barbiturates, it will lead to death.

Now, the location that benzodiazepine can acts will vary a lot:

- **Antianxiety:** hippocampus and amygdala.
- **Sedative/Hypnotic:** cerebral cortex.
- **Amnesia:** cerebral cortex and hippocampus.
- **Muscle Relaxant:** spinal cord, cerebellum and brainstem.
- **Antiepileptic:** cerebellum and hippocampus.
- **Abuse Potential:** midbrain–dopamine

Remark 2.18. Another thing we've found about the GABA_A subunits are binding to α_1 subunits would lead to sedation while α_2 would lead to antianxiety effects.

Example 2.4.4. Alprazolam and diazepam are both benzodiazepine i.e. they both have the same core. However, they're different since the added R-group are different which is why alprazolam can deal with panic attacks while diazepam can deal with anxiety.

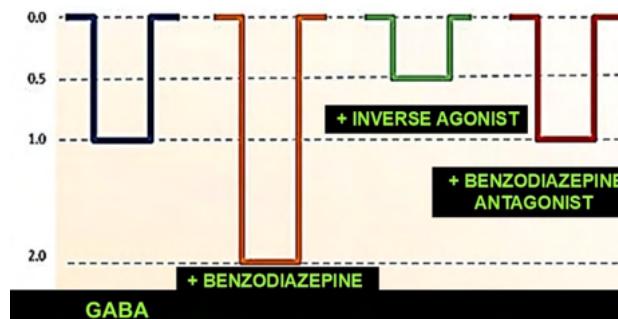


Figure 2.77: Inhibition effects of GABA release alone, + benzodiazepine, + inverse agonist, and + antagonist.

Observation 2.83 Now, benzodiazepines act like an agonist which shift the dose-response curve to the left. On the other hand, there are **inverse agonist** such as **β -carbolines** that will shift the dose-response curve to the right \Rightarrow harder for inhibition. Of course, we also have antagonist for GABA_A receptors that block it such as **flumazenil**. ¹²

Once again, just to drive home the point, we can see the usage of benzodiazepine will increase the alertness of patient. As well as the effects vs doses of drug of different kind of agonist and antagonist.

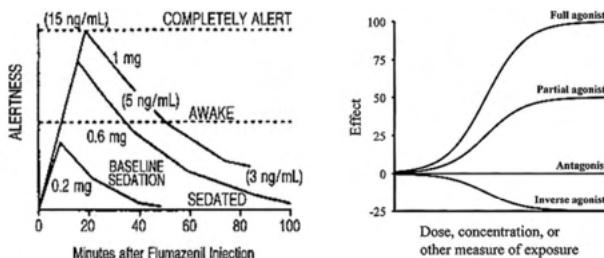


Figure 2.78: Benzodiazepines antagonists (left), and effect vs dose graph of different agonists and antagonist (right).

There has been continuous development of newer drugs than can have effects on the GABA_A receptors as well.

Example 2.4.5.

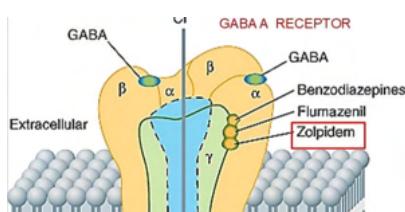


Figure 2.79: Zolpidem binding location on GABA_A.
on GABA_A.
decrease in fear of the animal when it's introduced to newer locations.

Zolpidem is a new kind of anxiety that have a slight different binding site to benzodiazepines. It's a more selective agonist and could bind to receptor subtypes.

For these kinds of drugs, animal studies have been used. We can test it by seeing if the drug can decrease in fear of the animal when it's introduced to newer locations.

¹²In cases of overdose, an antagonist to these receptors are important as it can help the patient recover more rapidly from the effect.

Just end this part, benzodiazepines can also act as a muscle relaxant by binding to presynaptic receptors (G-coupled K⁺ channel) in the spinal cord which will inhibit the release of many transmitters.

Pharmacokinetics

We'll now go through quickly the pharmacokinetics of benzodiazepines

- Absorption:** Good absorption through oral administration. It's highly lipid soluble and have a peak onset ~ 1h.
- Distribution:** It's distributed widely and has variable proteins binding. It can enter the brain rapidly.
- Metabolism:** It's metabolized mainly by CYP3A into active intermediates with a long half-life.
- Excretion:** It's eliminated via the kidney in the form of urine.

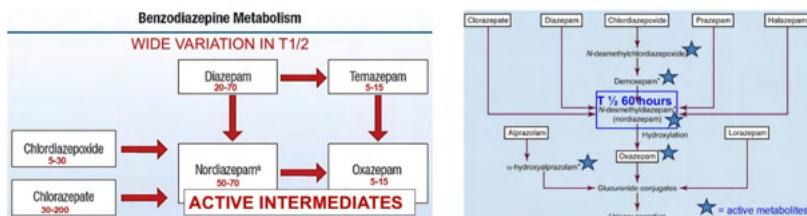


Figure 2.80: Wide variation of benzodiazepine half-life turning to their active metabolites with a significant half-life.

Observation 2.84 CYP3A can metabolize lots of drug which also introduce potential drug interaction if there is any. All of the different forms of benzodiazepines will have different half-life however, when it's broken down into its active metabolite, they all have a significant half-life.

Here, you can see that for either the IV injection or oral consumption of diazepam, they will all eventually plateau at a certain concentration. Meanwhile, diazepam will be metabolized into nordiazepam which continue to rise and stay in the system for a longer period of time.

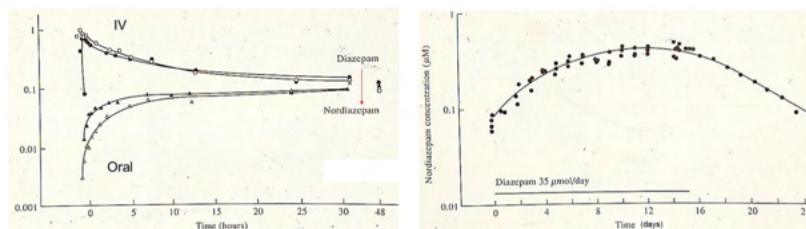


Figure 2.81: Administration of diazepam and its active metabolite (nordiazepam) level.

Remark 2.19. *Half-life will also vary depending on the age of patient but will be of greater concern for people that cannot metabolize it correctly e.g. young children and during pregnancy.*

Side Effects

Benzodiazepines have a variety of side effects depending on the types of receptor they act on. But in total, here's some general consideration:

- It can cause sedation \Rightarrow do not combine it with other CNS depressants.
- It can cause muscle relaxation \Rightarrow do not drive.
- It can cause tolerance if taken for a long time which can lead to dependency and experiencing withdrawals.
- It can cause amnesia \Rightarrow cognitive impairment and chronic high blood level.

Example 2.4.6. It was found that in Quebec, the chance of getting into a motor vehicle accident increases when you take benzodiazepines with a longer half-life as compared to the ones with shorter half-life.

Observation 2.85 When benzodiazepines alone, there could be low toxicity present. Taking it in combination with other drug, on the other hand, can be fatal. It has an antagonist (in case of an overdose).

Tolerance

Tolerance can occur with benzodiazepine but people are mainly taking it to subdue the withdrawal symptoms. When it comes to the withdrawal symptoms, it would be the opposite of what the drug do and that is hyperactivity of the nervous system e.g. headache, seizures, sweating, difficulty falling asleep, etc.

Observation 2.86 Taking it to treat insomnia for a long time can lead to a rebound when suddenly stop which can lead to nightmares that last for around 1 week i.e. this drug needs to be taken wisely and carefully.

Evidently, it can treat insomnia but we have to be using the short acting form and not repeatedly abuse it. Typically, a treatment of insomnia is not necessarily have to be drug but could be a change in lifestyle, sleeping pattern and avoid stimulants.

Observation 2.87 We've already mentioned before that there has been development of newer drugs to act on GABA_A receptors that are non- benzodiazepine e.g. Zolpidem. This new drug can bind selectively to only $\alpha 1$ subunits which means it can induce sedation and treat insomnia without altering REM sleep which lead to nightmares and a whole host of problems.

Not only that we've been designing benzodiazepines with faster onset and shorter duration.

Stage Fright

It must be noted that most performers and speakers do not take antianxiety medication for stage fright. They tend to take β -blocker which can reduce palpitation and tremors.

2.4.4 Cannabinoids

Definition 2.21. **Cannabinoids** is a class of compounds/drug that can bind to the cannabinoid receptors in the body.

So far, we've seen some strong evidence with cannabinoid being good for chronic pain in adults but also to treat chemotherapy-induced nausea/vomiting. There are also other moderately but also less substantial evidence with the treatment of cannabinoid.

Strength of evidence	Indications
Conclusive/Substantial	Chronic pain in adults Chemotherapy-induced nausea/vomiting
Moderate	Severe forms of epilepsy in children including Dravet syndrome and Lennox-Gastaut syndrome AIDS-associated cachexia Patient-reported spasticity associated with multiple sclerosis Sleep disturbances associated with obstructive sleep apnea, fibromyalgia, chronic pain, and multiple sclerosis
Limited to insufficient	Posttraumatic stress disorder, social anxiety disorder, Tourette syndrome, traumatic brain injury, addiction, amyotrophic lateral sclerosis, cancer, dystonia, Huntington disease, irritable bowel syndrome, Parkinson disease, schizophrenia, spasticity related to spinal cord injury

Figure 2.82: Therapeutic significance of cannabinoids.

Pharmacodynamics

Observation 2.88 Cannabinoids, like we've said above, will act on the cannabinoids receptors which includes 2 types: CB1 and CB2. CB1 are mostly found in the brain (in fact, it's everywhere in the brain) while CB2 are mostly found in the immune system.

In the body, we produce **endocannabinoids**, under the form of **anandamide (AEA)** and **2-arachidonoylglycerol (2-AG)**, that bind to these receptors. Similarly, there are plants that produce **phytocannabinoids**, under the form of **THC** and **cannabidiol (CBD)**, or we can synthetically make the cannabinoids, under the form of **nabilone**, to bind to these receptors.

Observation 2.89 CB1 receptors are found mainly in the presynapses and it can inhibit the release of different neurotransmitter such as GABA, glutamate, dopamine and etc. Meanwhile, CB2 are mainly found in the postsynapses and could have some potential role in neural/psychiatric disorders.

Mechanism of Action (CB1 Action):

1. In the postsynapse, precursor of cannabinoids begin to synthesize AEA and 2-AG.
2. The newly made endocannabinoids, will go, bind to and activate

the CB1 receptor.

3. Upon activation, it will block the Ca^{2+} -channel while activate K^+ -channel (which hyperpolarize the cell). Not only that, neurotransmitter release as well as cAMP will inhibited.

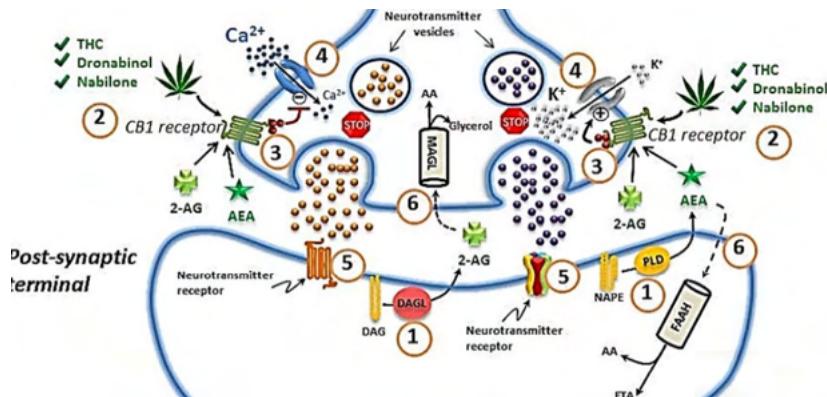


Figure 2.83: Pharmacodynamics of cannabinoids with CB1.

Pharmacokinetics

Cannabinoids will be distributed everywhere in the body.

Observation 2.90 When you take an exogenous cannabinoids, it could changes the some P450 enzymes activity. In this instance, consumption of THC will lead to the induction of CYP1A2 while that of CBD will lead to CYP3A4 inhibition. In either case, it can potentially affect the metabolism of many other drugs. In fact, there are around 333 drugs that can interact with cannabis with 24 of them having major interactions while the rest are moderate.

Toxicity of Cannabinoids

When you smoke anything (cigarettes included), there is a high chances that it will produce carcinogen and thus consumption cannbinoids via smoke (from cannabis) will have said chance too. Not only that, it also have risks of cancers, heart attacks and even strokes. What's worst is that we have yet to discover any long term effects that it might induce on the body.

Observation 2.91

Because it has effects on neurotransmitters, including dopamine, it has addictive potential. Chronic use of this saw changes in GABA and glutamate receptors. Though again, the long term consequences are still unknown but so far we know that users could experience **cannabis use disorder (CUD)**, which involves tolerance

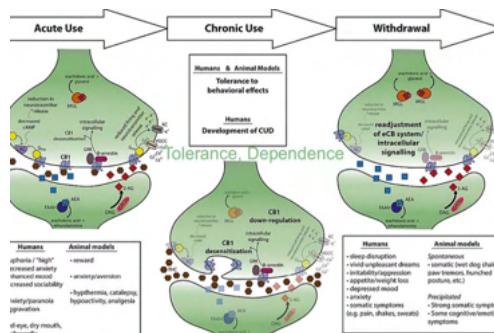


Figure 2.84: Chronic use of cannabinoids.

Remark 2.20. *It must be noted that consumption of cannabis while driving is dangerous as it can impair driving for 5 hours straight.*

As of the current moment, it must be noted that there are continuous studies of cannabinoids. Remarkably, at McGill, there's a whole department for its research ¹³

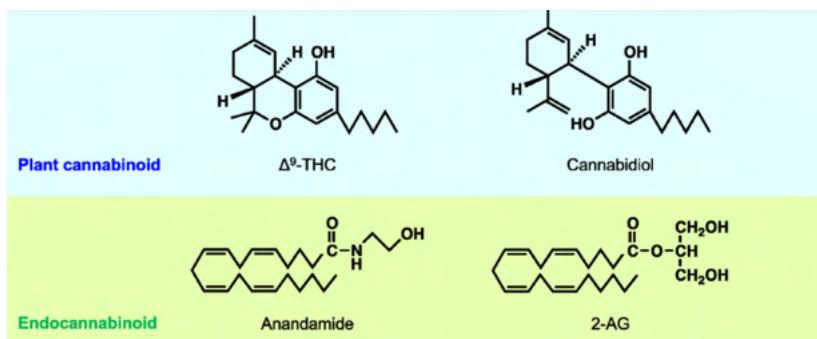


Figure 2.85: Structure of endocannabinoids and phytocannabinoids.

¹³The image below is not from class and its just to illustrate the structure of the cannabinoids.

Anti-inflammatories, Analgesics and Surgery Drugs

3.1 Corticosteroids

In today's lecture, we will look at what corticosteroids are, what their signalling are, side effects and some pharmacokinetics.

3.1.1 Brief on Inflammation and Immunity

Definition 3.1. An **inflammation** is an immune response to an **irritant**, which could be germs or even any foreign objects.

Example 3.1.1. When you have a splinter in your finger, your skin will begin to swell up i.e. inflammation, and this is because your immune cells are targeting that area. These cells produce chemokines and cytokines that lead to accumulation of fluids and cells.

Essentially, inflammation is a defense response to localize, isolate and eliminate an infection.

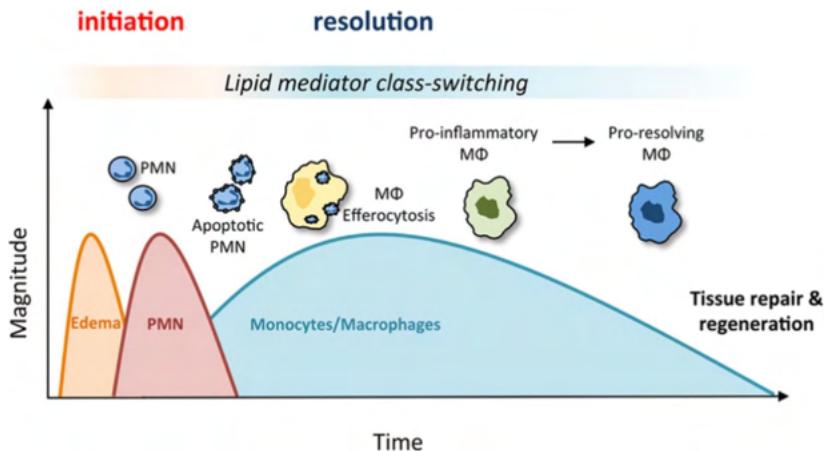


Figure 3.1: Stages of inflammation .

Observation 3.1 Based of past observation, we've determined 5 "cardinal" signs of an inflammation and this includes: **pain, heat, redness, swelling a loss of function** at the site of inflammation. Though loss of function isn't always the case but it will be present.

At the beginning of an injury, the site will be isolated that results in edema. Later, immune cells will be recruited to the site of injury to possibly clear out cells underwent apoptosis. You also have recruitment of pro-inflammatory mediators that releases different cytokines. In the end-stage, pro-resolving mediators will be presents and begin to clear out the inflammation.

Basically, all of these steps are highly complex and rapid to protect the body from foreign materials. Nevertheless, this is an acute event and the body resolves this by producing *glucocorticoids*.

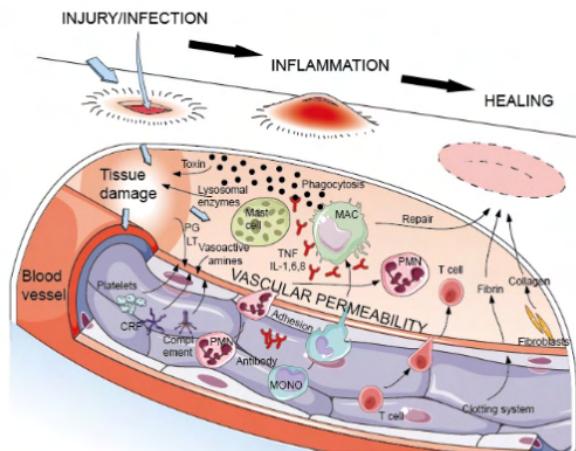


Figure 3.2: Inflammation.

Inflammation Associated Diseases

Definition 3.2. **Inflammation associated diseases** are diseases that lead to the activation of inflammatory responses even though there's no injury nor infection.

Example 3.1.2. Arthritis, inflammatory bowel and gut are all inflammation-based diseases.

So in order to treat these diseases, we need to have a drug that to reduce this constant activation of immune cells. This is where a class of drug called **immunosuppressant drugs** come in and they have, in general, 3 classifications: **antiproliferative, immunophilin-binding agents and glucocorticoids**; all of which we will talk about today but with a higher emphasis of glucocorticoids. Evidently, these drugs are not the only ones, there are biopharmaceuticals methods like antibodies and fusion proteins that also help too.

Brief on Immunity

Before talking about the drugs, we want to take a brief walk through the immune system.

Observation 3.2 When talking about the immune system, we're talking about 2 things: **innate and adaptive immunity**.

Cells that are from the innate immune system include: macrophages, neutrophils, eosinophils, basophils and dendritic cells. They are immune cells that will be first recruited to the site of injury and have low specificity (they target all foreign particles). On the other hand, adaptive immunity have 2 kinds: B and T cells; of which will take time to produce destructive mechanism with high specificity against pathogens.¹

Autoimmune diseases happen when the specificity of these immune cells is turned off and they start targeting cells-cells.

Mechanism of action (Activation of Adaptive Immunity): Suppose that a foreign pathogen just enters your body through an injury.

1. The innate immune system is activated and all of its cells will be present at the site to fight the infection.
2. A class of innate immune cells called **antigen presenting cells (APC)** will engulf and kill the pathogen while leaving a piece of it (antigen) to present to T cells.
3. Upon binding to T cell and presenting it the antigen, T cells will develop and proliferate into **cytotoxic T cell** and **T helper cell**.
4. T helper cells can enter the lymph nodes and release cytokines

¹We also have another cells called natural killer T cell that fits in both categories of innate and adaptive.

to activate B-cells that turn into **plasma cells**. These plasma cells produce antibodies with high specificity to the pathogens.

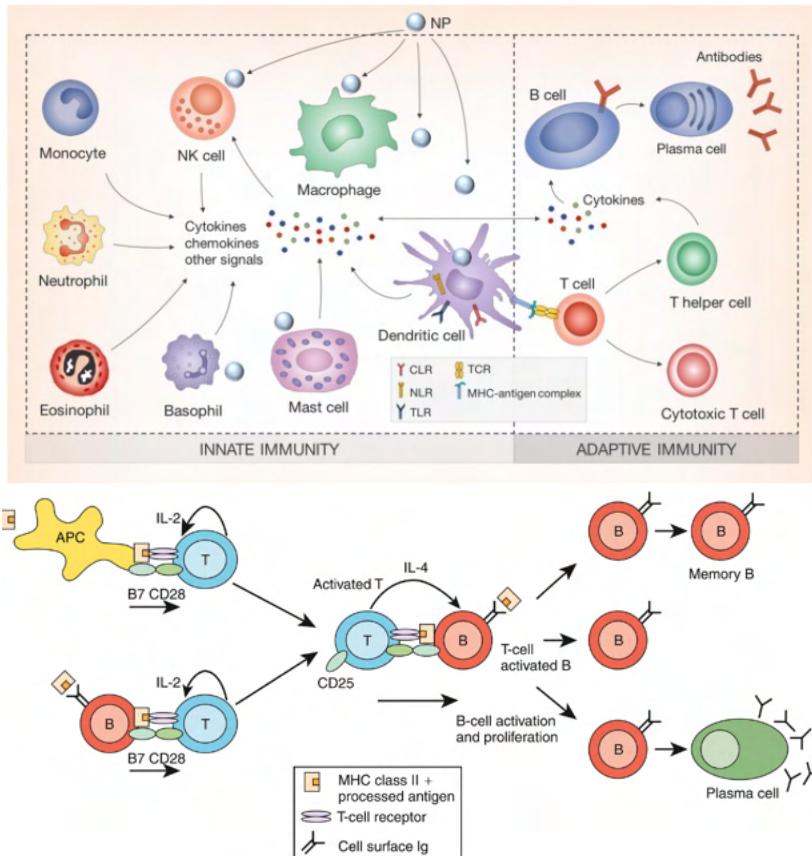


Figure 3.3: Innate and adaptive immunity during an infection.

Remark 3.1. *The key step that some of the drugs we're studying is to target the proliferation of T-cells.*

Definition 3.3. **Cytokines** are signaling proteins that can initiate inflammation by activating immune cells or even recruit them (specifically chemokines).

Observation 3.3 This also means that when there's an excessive amount of cytokines \Rightarrow excess activation and recruitment of immune cells \Rightarrow excess inflammation and autoimmune diseases.

There are lots of cytokines but the ones that we've mainly looked at to target includes IL-12 and TNF- α .

The table below includes the differences between innate and adaptive (acquired) immunity

Characteristic	Innate Immune Response	Acquired Immune Response
Onset	Immediate	Days to weeks
Mechanism of antigen recognition	Pattern recognition receptors recognize common molecules on microbes and viruses	Antigen-specific receptors (T-cell receptor, B-cell receptor)
Cell types involved	Macrophages, neutrophils, mast cells, natural killer cells, NK T cells, innate lymphoid cells	Dendritic cells, T cells, B cells
Soluble factors	Complement, type I interferon, select cytokines and chemokines	Select cytokines and chemokines

3.1.2 Immunosuppressant Drugs

We will now talk about the immunosuppressant drugs we've previously mentioned above². In all three drugs, we need to selectively eradicate immuno-competent cells without touching the target cells. But it has to have a balance i.e. you cannot completely shut down the immune system as it still needed to fight actual infections.

Antiproliferative and Antimetabolite Drugs

Definition 3.4. **Antiproliferative drugs** are cytotoxic drugs that inhibit the proliferation of both T and B cells. They include alkylating agents like cyclophosphamide, nitrogen mustard and nitrosoureas.

Observation 3.4 This kind of drugs, as the name suggested, will stop the proliferation of T cell upon activation by APCs. This is done by blocking DNA replication. Wait...**but isn't all cells in the body replicating?** Well...yes but you need to remember that at specific times, the highest replication rate is made by T cells in individuals with autoimmune diseases (after all

²Another class used to treat inflammation are NSAIDs but we will discuss them in the next lecture.

these drugs are for them) \Rightarrow therapeutic effects will overshadow the adverse.

Definition 3.5. **Antimetabolic agents** are compounds that resembles the typical metabolites and can block B and T cell proliferation by inhibiting the synthesis of substrates necessary for replications.

Example 3.1.3. **Methotrexate** is a antimetabolic agents that competitively inhibit dihydrofolate reductase \Rightarrow decreases the synthesis of folic acids that are required in purine and pyrimidine synthesis.

Immunophilin-Binding Agents (Calcineurin and mTOR Inhibitors)

Definition 3.6. **Immunophilins** are proteins that have chaperone³ and enzymatic activity which makes them have lots of functions from neurotrophic actions to regulation of cell proliferation.

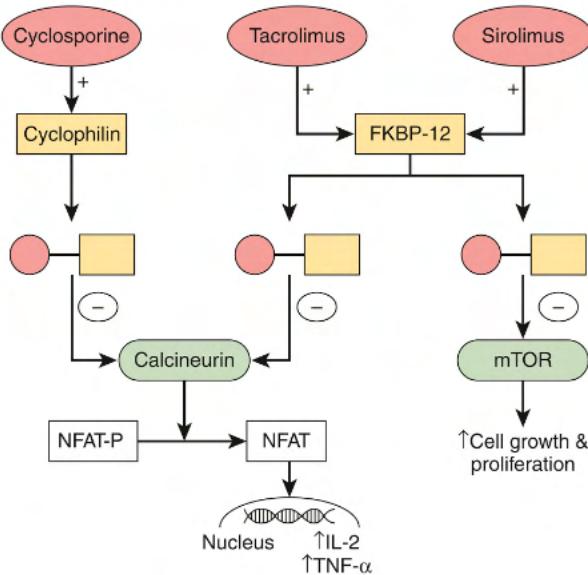


Figure 3.4: Immunophilin-binding agents.

³Proteins that assist other proteins folding post-synthesis

Observation 3.5 2 important immunophillin that we mainly target include: cyclophilin and FKBP-12. The **immunophilins-binding drugs** will bind to immunophillin and block the production of cytokines, specifically IL-2 and TNF- α .

To be brief, cycloporine will bind to cyclophilin A to form a complex that ultimately inhibit calcineurin which is an essential complex for synthesis of IL-2 and TNF- α .

Glucocorticoids

Lastly, it's glucocorticoids which is the most widely used immunosuppressive drugs e.g. **prednisone**.

Observation 3.6 Glucocorticoids are pleiotropic (it has multiple effects) and will block anything related to immune cells proliferation and they're very potent.

They can inhibit vasodilation during an inflammatory insult and thus prevent leukocytes migration. They decrease T cell activation and the activation of IL-2, 1, 6 and NK- κ B. Lastly, they bind to glucocorticoids response elements on DNA which activate anti-inflammatory genes.

3.1.3 Hormones and Signalling

Now, as our focus is more toward glucocorticoids, we need to ask ourselves, **what is glucocorticoids?** Well...it's a type of steroids...then **what is steroids?** Well...

Definition 3.7. **Steroids** are biologically active organic compounds with 4 rings in specific molecular configuration. They have 2 main functions: can alter cell membrane fluidity and are signalling molecules i.e. they're also a class *hormones*.

Definition 3.8. **Hormones** are signalling molecules that can be chemically and structurally diverse compounds. Chemical-wise, they're divided into: amino acid, peptide and steroid hormones.

Observation 3.7 Amino acids hormones are derivatives of tyrosine which include **epinephrine** and **thyroid hormones**. Peptide hormones can be further sub-classified based on the size, glycosylation, single- or double-chain peptides. Steroids hormones are cholesterol derivatives and can be

subclassified into adrenal steroids and sex steroids which are made in the adrenal glands and ovaries or testes, respectively.

Definition 3.9. The **Endocrine system** is a messenger system consisting of feedback loops of hormones that are released by internal glands into circulation. These hormones will ultimately alter the body metabolism, energy level, growth, response to injury and etc.

Remark 3.2. *What's most important for today's lecture is the ability of hormones to control response to injury, stress, resolution of inflammation.*

With all of this in mind, we can put together a complete picture of what glucocorticoids are.

Definition 3.10. **Corticosteroids** are a class of steroid hormones produced in the adrenal gland. They include 2 kind of steroids: **glucocorticoids** and **mineralocorticoids**.

Observation 3.8 At the end of an inflammation, the adrenal gland will produce glucocorticoids that will be sent to the site and suppresses inflammation. While glucocorticoids are important for immunosuppression, mineralcorticoids are important for Ca^{2+} and Na^+ uptake. The primary mineralcorticoids are **aldosterone**.

Steroid Synthesis

Observation 3.9 All secreted steroids are made from cholesterol which can come from the diet or made de novo. In all organ, the metabolic pathways that mediate steroid synthesis are similar. Organ-specific steroids are made depending on the presence of a specific catalytic enzymes. Ultimately, the action of steroids is mediated largely altering gene transcription through interaction with DNA of the promoter region.

Remark 3.3. *Looking at figure 3.5, it's important that you remember that the enzyme that mediate the transformation of pregnenolone and progesterone into glucocorticoids is **17 α -hydroxylase (CYP17)***

Hormones Signaling

We will now look at how the body signal to turn cholesterol into cortisol (a glucocorticoids). The overview of the pathway is that: stresses, illness and

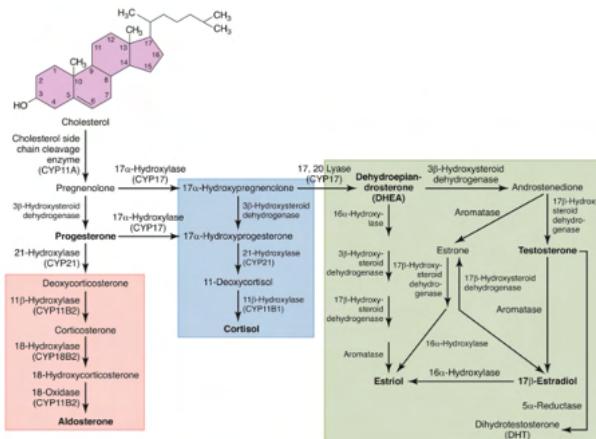


Figure 3.5: Synthesis of Steroids.

other physiological condition will signal to the hypothalamus which produce **corticotropin-releasing hormones (CRH)** and **arginine vasopressin (AVP)**. These 2 will act on the pituitary gland which lead to the synthesis of **adrenocorticotropic hormone (ACTH)** which can act the adrenal gland to produce cortisol.

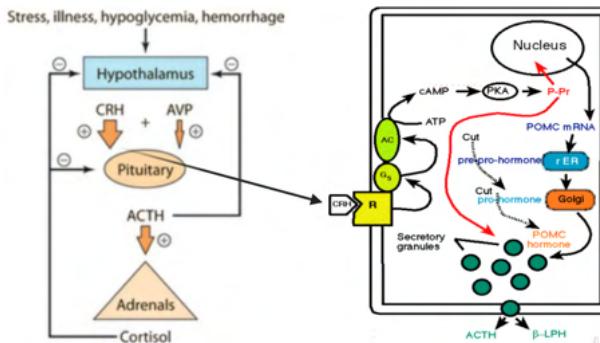


Figure 3.6: Production of ACTH from CRH.

Observation 3.10 First, CRH is a peptide of 41 aa⁴ and it's a *prohormone* produced in the hypothalamus. In the anterior pituitary gland, CRH pro-

⁴aa: amino acids

motes the production of **proopiomelanocorticotropin (POMC)** by binding to GPCRs called **CRHR1 and 2**. POMC can be cleaved sequentially by **pro-hormone convertase 1/3 (PC1/3)** to make pro-ACTH that become ACTH.

Observation 3.11 Once ACTH is made, it's carried out of the adrenal gland, into circulation, and ends up in the adrenal gland. The adrenal gland is made up of different zone (Zona) and the one that ACTH regulate is called **zona fasciculata/reticularis**.

Here, it will increase the delivery of cholesterol into the mitochondria and the transcription of steroidogenic enzymes (important to make **pregnenolone**). Not only that, it promotes CYP11B1 and CYP17 to take cholesterol to make cortisol.

The following table shows all the enzymes that present in different tissues which can mediate formation of steroid hormones.

Tissue	CYP11A	CYP11B	CYP17	CYP21	Aromatase*	5α-Reductase*
Adrenal glands						
Zona glomerulosa	+	++	-	+	-	-
Zona fasciculata	+	+	+	+	-	-
Zona reticularis	+	+	+	+	-	-
Testes						
Sertoli cells	-	-	-	-	+	-
Leydig cells	+	-	+	-	-	-
Ovary						
Glomerulosa	+	-	-	-	+	-
Theca	+	-	+	-	-	-
Corpus luteum	+	-	-	-	+	-
Adipose tissue	-	-	-	-	+	-
Prostate	-	-	-	-	-	+

As we look more into the synthesis of cortisol, we first see CYP17 making a precursor to cortisol from progesteron and pregnenolone. Then, in the last step, the enzyme CYP11B1 will convert it fully to cortisol.

Observation 3.12 Remember, this is part of the endocrine system of which we've mentioned that there are feedback loops. In this instance, under stressful condition, the hypothalamus produces CRH which acts on the pituitary gland to make ACTH. ACTH can trigger the production of cortisol in the adrenal gland.

In return, cortisol can then travel up to the hypothalamus and adrenal gland to shut down the production CRH and ACTH, respectively. This is negative feedback loop of product inhibition.

3.1.4 Glucocorticoids

Now, that we've known the production of glucocorticoids (GC), let's see how it functions in the body. Nevertheless, before that, let's check with ourselves first...**how do hormones function?** Well...hormones, like other ligands, will bind to their respective receptors which trigger a whole host of secondary messaging in the cell that will ultimately lead to modulation of cellular physiology \Rightarrow organism physiology in large scale.

Since, we're focusing on GC, the receptors that it will bind to are *steroid hormones receptors*.

Definition 3.11. **Steroid hormones receptors** are receptors that steroid hormones can bind to. They're generally found in the intercellular space e.g. cytoplasm or nucleus. Upon activation, they can lead to changes in gene expression over a period of time.

Remark 3.4. *The reason that hormones can travel easily into the intracellular to bind to its receptors is because they're lipophilic \Rightarrow diffuse easily through the membrane.*

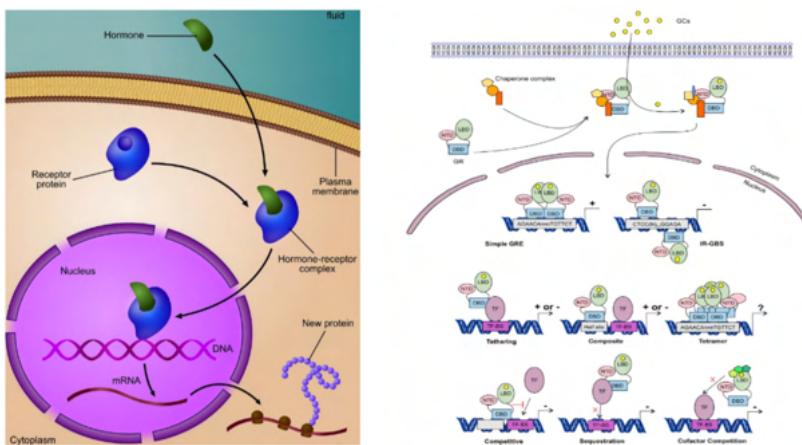


Figure 3.7: Theoretical mechanism of GR and steroid hormones receptors.

Observation 3.13 It must be noted that the mechanism of action of GC on its receptors is still in debate and is unclear. All of that is clear to use is that:

GC can bind to its receptors called **GC receptor (GR)**⁵, which also bind to a chaperon complex. This whole new megacomplex can enter the nucleus to either *transactivate* or *transrepress* a **GC response element (GRE)**.

This will link with the suppression of inflammation as GC can stop T-cell replication, cytokines production and a lot more immune responses i.e. it changes cell proliferation, survival, differentiation while decrease pro-inflammatory chemicals like cytokines.

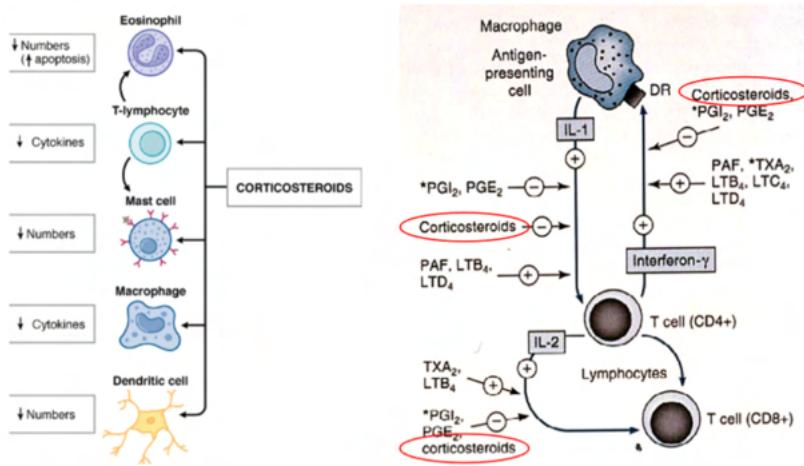


Figure 3.8: GC inhibiting immune cells (innate and adaptive) and inhibit immune signaling.

This makes them a great drug since you do not need to understand the pathology of the inflammatory disease, it would just shut it down anyway.

Observation 3.14 Though we've mostly shown that GC is very effective at controlling inflammation, it can also have some metabolic consequences. Generally, GC can increase blood glucose to the brain and heart by:

1. Increase glycogenolysis
2. Increase gluconeogenesis
3. Increase lipolysis
4. Increase protein catabolism and decrease its synthesis.

⁵GRα is the ligand-bound form of GR.

3.1.5 Pharmacokinetics of Glucocorticoids

Observation 3.15 GC can be administered through many route but it's best to locally administer it to minimize adverse effect. Around 80 – 90% of circulating GC is bounded by plasma proteins called *corticosteroid-binding protein (CBG)*, 5 – 10% are loosely bound to albumin while 3% – 10% are free and active.

Note that CBG can also bind to synthetic GC like prednisolone or prednisone but **not dexamethasone** \Rightarrow 100% of plasma dexamethasone is bioactive.

Definition 3.12. **Corticosteroid-binding protein (CBG)** is a protein that transport GC in the blood and thus modulate its tissue availability.

Observation 3.16 Though normally, GC is bound to CBG in blood, but in stressful or inflammatory condition, a molecule called **elastase** will release GC from the complex. Because CBG strongly bound to GC, it needs when giving GC as drug, we also need to consider the CBG binding and loss of bioavailability!

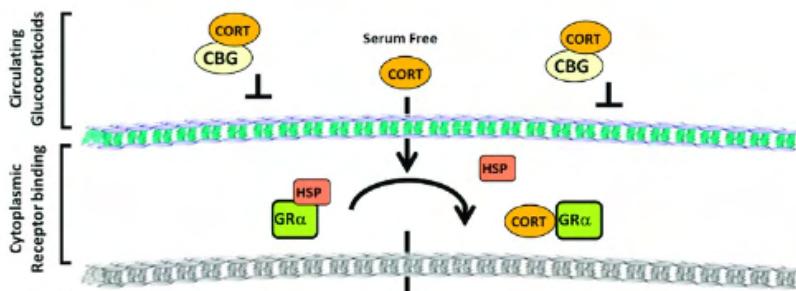


Figure 3.9: CBG and GC binding.

Observation 3.17 Most GCs are absorbed readily and rapidly from the GI tract, synovial and conjunctival fluid while slowly absorbed through the skin. It must be noted that when the body has elevated estrogen e.g. pregnancy or contraceptive usage, there would be an increase in CBG biosynthesis \Rightarrow requires higher GCs concentration for the same therapeutic effect. And almost like for all other lipophilic drugs, body weight matters!

Interestingly, the level of GC fluctuate throughout of the circadian rhythm with an upshift during sleep and decreases while awake.

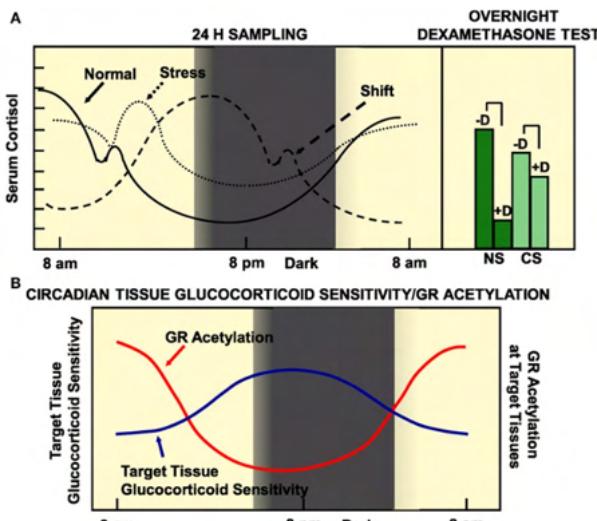


Figure 3.10: GC fluctuation with circadian rhythm.

GCs as Drugs

We've managed to find ways to modify GCs in order to make it last longer in the system. Not only that GCs and mineralcorticoids (MCs) have very similar biosynthesis and activation which allow us to do interesting modifications.

Example 3.1.4. Addition of F at position 9 will enhance activity of both GCs and MCs.

Addition of methyl group at position 16, e.g. betamethasone or dexamethasone, will increase GR activation while eliminates MR activation.⁶ This makes these drugs long lasting as compared to prednisone, prednisolone and methylprednisolone, which only have intermediate plasma half-lives.

⁶MR: mineralcorticoids receptors

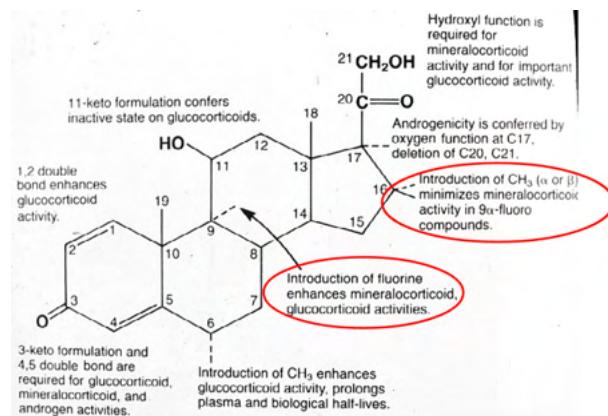


Figure 3.11: Modification of GCs.

Observation 3.18 Evidently, we've said that GCs are absorbed rapidly in the stomach thus there are pills form exist for it. Not only that, we also have topical cream to treat any surface inflammation (e.g. eczema) and inhalation pump for asthma (which is a type of inflammatory response).

Replacement Therapy

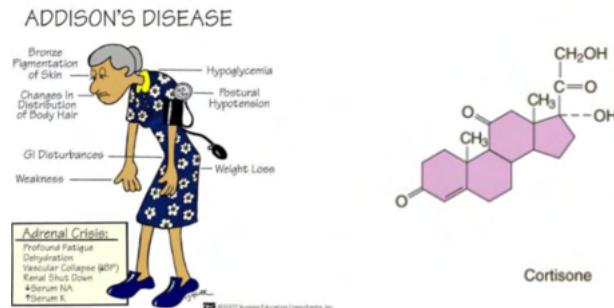


Figure 3.12: Addison's disease and treatment.

Definition 3.13. **Addison's disease** is a rare autoimmune disease characterized by the insufficient production of cortisol and aldosterone by the adrenal gland.

Treatment: Treatment of Addison's disease include hormone replacement therapy in order to correct the level of steroid hormones that were not producing. This treatment include: oral hydrocortisone (Cortef), prednisone or methyprednisolone⁷ to replace cortisol while **fludocortison acetate** to replace aldosterone.

Definition 3.14. Myeloproliferative disorders is a rare type of blood cancer that begin with an abnormal mutation of stem cells resulting in an over-production of any combination of WBCs, RBCs and platelets.

Treatment: GCs are used to reduce the side effects of excess WBCs.

3.1.6 Adverse Effects of Glucocorticoids

Observation 3.19 Although GCs have very good therapeutic usage, they also have its side effect. When cortisol is too low, it could induce hairiness, acne, irregular periods, reduced fertility and etc.

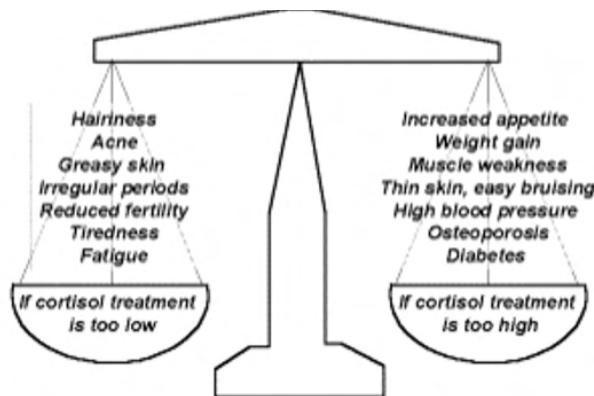


Figure 3.13: Effects of GCs.

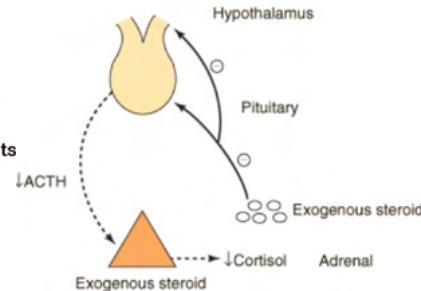
These side effects all have to do with the fact that GCs share similar biosynthetic pathway to sex hormones. Now, if cortisol is too high, there also adverse effects like increased appetite, weight gain, muscle weakness, etc.

⁷These hormones are given at specific schedule to mimic fluctuation in a 24-hours period.

Prolonged Adverse Effects

When using GCs chronically, there would be more serious adverse effects shown below:

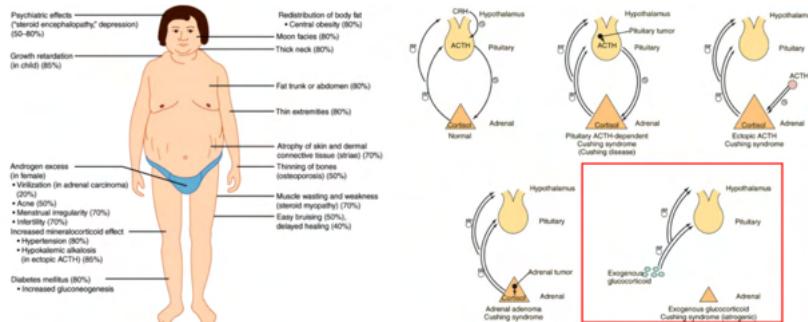
- Osteoporosis and fracture**
≥ 3 months - increase in fracture risk
- Glucose intolerance and diabetes**
doubled in rheumatoid arthritis patients taking ≥ 7.5 mg prednisone
- Central obesity**
- Muscle wasting**
- Increased risk of infections**
- Depression**
- Cataracts**



Concept 3.1 Chronic exposure of high dose of exogenous steroid can shut down the hypothalamus-pituitary-adrenal (HPA) axis and will lead to adrenal atrophy.

The reason that this happens is because the amount of ACTH begin sent to adrenal gland decreases which lead to its deactivity.

Observation 3.20 Another adverse effect of inhaling GCs is **oral candidiasis (thrush)**. This is because during inhalation, some of these GCs can come into contact with mouth which can immunosuppress it.



Definition 3.15. Cushing's Syndrome is a syndrome where an individual is taking high dose of hormone cortisol over a long period of time.

Observation 3.21 These individuals will end up having a moon face, thickening neck, weight gain and also all the mentioned adverse effects of high dose of cortisol.

Now, Cushing's syndrome can be caused by altered body's hormone signaling or from overuse of steroids. e.g. if there's a tumor in the pituitary gland, produced cortisol wouldn't be able to go back and inhibit production ACTH. Using concept 3.1, when overusing cortisol hormone, the HPA axis shuts down and the adrenal gland began to atrophy. This lead to the body require an ever higher amount of cortisol \Rightarrow manifestation of Cushing's syndrome.

3.2 NSAIDs and Acetaminophen

Definition 3.16. Non-steroidal anti-inflammatory drugs (NSAIDs) are drugs used to decrease pain and inflammation that do not are not steroids e.g. glucocorticoids.

Commonly Available NSAIDs	
Classification of NSAIDs	Examples
<i>Salicylates</i>	<u>Aspirin</u> [*]
Acetylated	Salsalate, trisalicylate
Nonacetylated	
<i>Nonsalicylates</i>	
Nonselective COX-1/COX-2s or traditional NSAIDs	<u>Ibuprofen</u> [*] , indomethacin, naproxen [*] , sulindac, ketoprofen, ketorolac, fenoprofen, diclofenac, piroxicam, diflunisal, oxaprozin, tolmetin
Semiselective NSAIDs*	Meloxicam, etodolac, nabumetone
COX-2 selective inhibitor	<u>Celecoxib</u>

Greater COX-2 inhibition than COX-1; ^{}Available over the counter.
NSAIDs = nonsteroidal anti-inflammatory drugs; COX = cyclooxygenase.

Observation 3.22 Though NSAIDS is the large family of drugs, it can be subdivided into smaller one. In this lecture, we will focus more on: aspirin, traditional NSAIDS and COX-2 selective inhibitor.

Remark 3.5. *Acetaminophen is not an NSAID but we will talk about it in this lecture.*

Before talking about NSAIDS and their properties in general, let's first look at the origin of NSAIDS.

Observation 3.23 Back in the day, people were trying different remedies from plants in order to reduce pain. One of the things they've found is that crushed up **white willow** (*salix alba*) bark will reduce pain (with lots of side effects). Similarly, leaves from the **meadowsweet** (*spirea ulmaria*) also have similar effect.

In 1839, scientists were intrigued by this and thus tried to isolate the active ingredient from these plants and they've found **salicin**. Nevertheless, salicin still has some serious side effects even though it reduces pain.

In 1899, a scientist named Felix Hoffmann decided to create a derivative from salicin by adding an acetyl group which makes **acetylsalicylic acid**. Acetylsalicylic acid was sold under the name **aspirin**⁸ by his pharmaceutical company, Bayer. Even so, he was not sure what was the mechanism behind the action of aspirin.

In 1971, an English pharmacologist named John Robert Vane discovered that aspirin blocks the synthesis of prostaglandin which reduces pain and inflammation. With this, he won the 1982 Nobel Prize.

3.2.1 Pharmacodynamics of NSAIDs

Concept 3.2 *NSAIDs blocks the synthesis of prostaglandin*

In order to understand the above concept, we need to ask ourselves what is prostaglandin or even how is it synthesized.

Observation 3.24 **Prostaglandin (PG)** is a derivative of **arachidonic acid**. In specific circumstances, phospholipase will break some of the phospholipid and releases arachidonic acid which can be converted into PG by **cyclooxygenases (COXs)**.

The reason that it was called "prostaglandin" was because it was first discovered in semen and scientist thought that it was only produced in semen i.e. produced by the prostate gland \Rightarrow prostaglandin. This is further from the truth as **prostaglandin can be produced by almost all cells and is used for autocrine and paracrine communication**.

⁸The name was a combination of words with: "a" from acetyl, "spir" from the plant *spirea ulmaria* and "-in" a drug suffix.

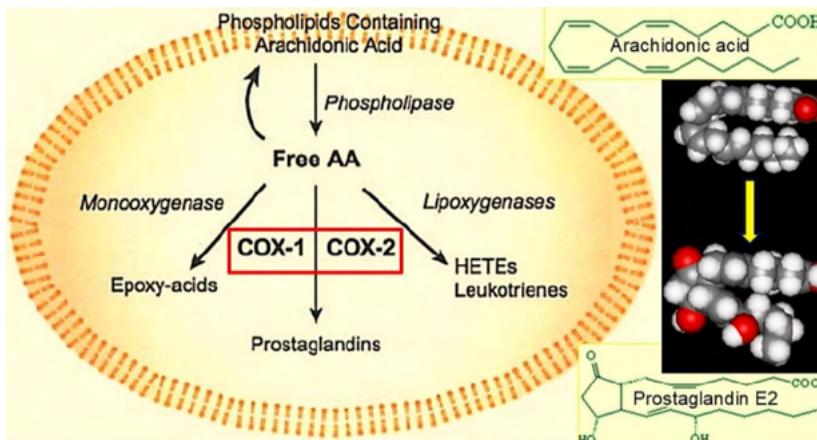


Figure 3.15: Synthesis of prostaglandin.

Remark 3.6. PG consists of different specific types of prostaglandin with the most common being **PGE₂**.

It must be noted that COXs is not the only enzymes that can break arachidonic acid down but there are 2 other enzymes.

Definition 3.17. In all 3 cases of enzymes, when arachidonic acid is broken down, its products will all belong to a class of molecule called **eicosanoids**⁹

Observation 3.25 But here in this lecture, we're mainly looking at PG thus focus more of COXs. The mechanism of action of COXs is fairly simple, all it does is adding 2 oxygens to the 8 and 12 position of arachidonic acid. This addition will cyclize the 2 position forming a ring and thus PG is formed.

We tend to find COXs dimerize together and sit directly on the membrane e.g. the surface of the ER. COXs will have active site where arachidonic acid can enter and leave as PG. The newly synthesized PG can then travel to their respective PG-receptor (which are GPCRs mostly) and cause a change in cellular function.

The 3 main classes of prostaglandins¹⁰ consists of prostacyclin, prostaglandins

⁹ *eicosa* means 20 in Greek.

¹⁰Though not mentioned in the lecture, I found that the class of molecule produced by COXs are called *prostanoids* (subclass of eicosanoids) instead of being called prostaglandins which can cause confusions

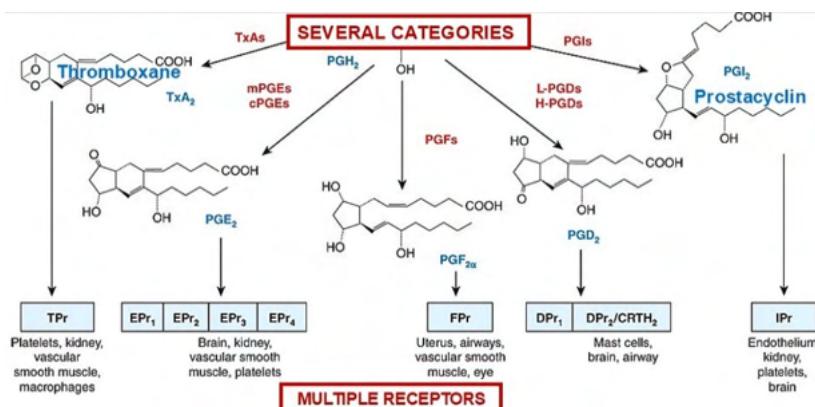


Figure 3.16: Different effects of prostaglandins resulting from prostaglandins binding to their receptors which are found at different parts of the body.

and thromboxanes.

- Both prostaglandins and prostacyclins can cause pain and inflammation. With fever is only prostaglandins.
- For thromboxane, it's mostly have to do with platelet aggregation and vasoconstriction.

Since we're focusing on prostaglandin, here's more role that prostaglandin can play:

Pathologic	Physiologic
Fever	Temperature control
Asthma, ulcers	Bronchial tone
Diarrhea	Cytoprotection
Dysmenorrhea	Intestinal mobility
Pain, inflammation	myometrial tone
Bone erosion	Semen viability

So now, it's become evident that the blocking of prostaglandins synthesis i.e. blocking COXs would reduce pain, inflammation and fever. To be specific, blocking we'd either block COX-1 or COX-2; with COX-2 being actually more predominantly induced during injuries.

Observation 3.26 NSAIDs have **analgesic** (relieve pain), **anti-inflammatory** (relieve inflammation) and **antipyretic** effects (relieve fever).

Analgesic Effects of NSAIDs

To see the analgesia of NSAIDs, we need to understand a little about the physiology of pain.

Observation 3.27 Suppose that you have a cut on your finger, the pain signal will travel through your nerves that will end up in the spinal cord that will travel up to the thalamus and then into one of the cortex. Once in the cortex, there will be a mechanism going back to the finger.

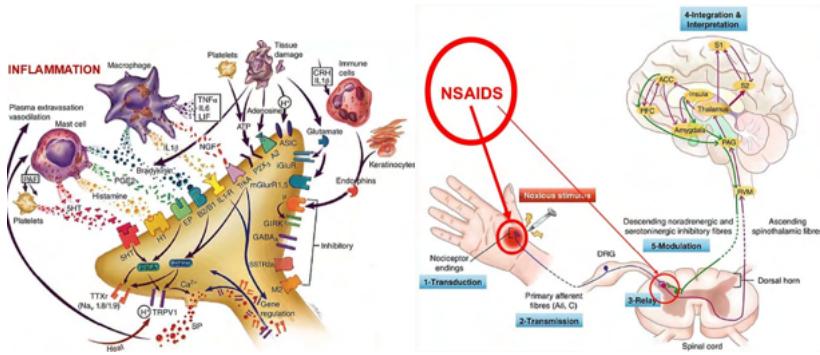


Figure 3.17: Signalling of pain and NSAIDs action.

So then...**What's generating this signal that will be sent up the spinal cord?** Well...when cells are injured, they (along side with immune cells) will release different mediators including PG, histamine, etc. that will activate nerve endings of **nociceptors** that will lead to the sensation of pain.

If we look specifically at PG's action, it will alter different ion channel
⇒ alter the signalling of nerve ending and generate a propagating action potential.

Observation 3.28 Now, NSAIDs can act directly at the nerve ending of the injured site or it can have some activity in the spinal cord.

Anti-Inflammatory Effects of NSAIDs

We've already spoken about the 5 signs of inflammation includes: **heat, redness, swelling, pain, and sometimes loss of function** at the site of injury.

Observation 3.29 During an inflammation, you have an increase in cells that come to the site of injury and releases a wide range of mediators which

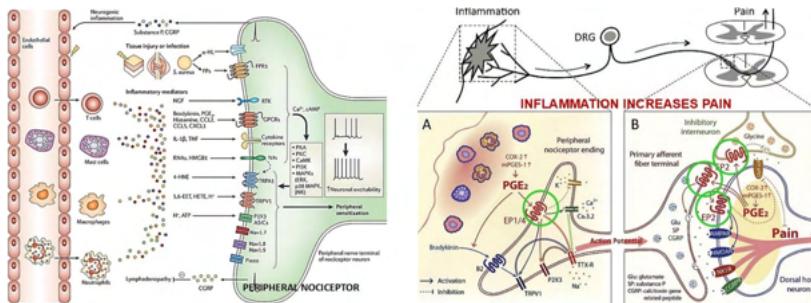


Figure 3.18: Inflammation and pain.

could further increases pain. To be specific, when this happens, PGE₂ is released and bind to the PG receptors which lead to a signal generated and move to the spinal cord. At the spinal cord, there will be more synthesis of PGE₂ as well as EP2 receptors on neighbouring interneurons.

When it comes to NSAIDs, as well as acetaminophen, they have have the following effects:

- Relief mild-to-moderate somatic pain including headache, toothache, myalgia, and athralgia.
- Relief in inflammatory disorders including rheumatoid arthritis, osteoarthritis, gout (beside acetaminophen).
- Reduce fever
- Prophylaxis (prevention) of heart attack and stroke (only aspirin).

Remark 3.7. NSAIDs play important roles in treating many inflammation but interestingly, it can even decrease the incidence of certain cancer.

Beside preventing heart attacks and inflammation, NSAIDs are actually widely used in North America for relieving joints and bone pain caused by rheumatoid arthritis and osteoarthritis (but also other diseases).

Observation 3.30 Because of how effective NSAIDs are, we actually used to relieve pain for other animal too that use their joint a lot e.g. Canine arthritis is a major problem in dog that can be relieved by NSAIDs. It must be noted that there are biological difference between species thus a drug for 1 species can be toxic for the other thus we must be cautious of this.

Remark 3.8. DO NOT GIVE aspirin nor acetaminophen to a cat as it can be toxic for them!

Antipyretic Effects of NSAIDs

Definition 3.18. **pyrogens** are chemicals that can induce a fever.

Observation 3.31 During an infection, exogenous pyrogens. (substances) are released by pathogens which will activate monocyte-macrophage and T-cells. In return, they will release endogenous pyrogens that can travel to the **hypothalamic temperature-regulating center**. In this center, PGE₂ is produced which raise the body's temperature set point. The autonomic system will respond through trying to match that set point by generate (e.g. shivering) and conserve (e.g. decrease sweating) heat. This is what a fever is.

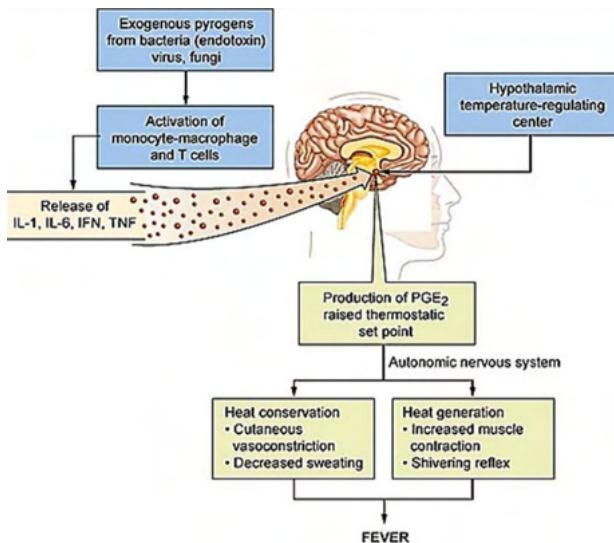


Figure 3.19: Mechanism of a fever.

Once again, because PGs are involved in this process, our NSAIDs can interfere with its production which ultimately reduce fever.

Side Effects of NSAIDs

There are many side effects to NSAIDs but the most prominent are that in the upper GI i.e. the stomach which includes:

1. **Dyspepsia:** discomfort of the stomach, similar to indigestion \Rightarrow loss of appetite,
2. **Erosion:** mucosal linings of the stomach is disrupted.
3. **Anemia:** GI bleeding.
4. **Ulcers:** when GI bleeding becomes severe, there can be formation of ulcers and even perforation.

Another side effects it could have includes: renal dysfunction, failure (chronic/acute), changes in blood pressure and heart failure. Not only that, because of its anti-platelet effects, it can cause blood loss during injury.

Remark 3.9. These side effects are mostly observed for people taking NSAIDs chronically at high dose.

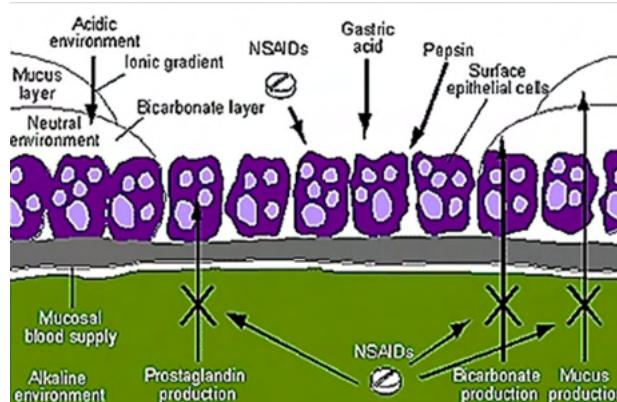


Figure 3.20: NSAIDs blocking mucus and bicarbonate production in the stomach.

Observation 3.32 The reason that NSAIDs can cause an ulcer is because it blocks the production of not only PG but also bicarbonate and mucus for the stomach \Rightarrow the protective layer of stomach against its gastric acid is removed. The acid can then come in direct contact with the epithelial cells and cause an ulcer.

3.2.2 Aspirin

Aspirin is the most commonly used drugs as it's the cheapest to make. Along with its NSAIDs effects, it also have an extra effect of anti-thrombotic which is why it's the most commonly used drug for heart disease in North America.

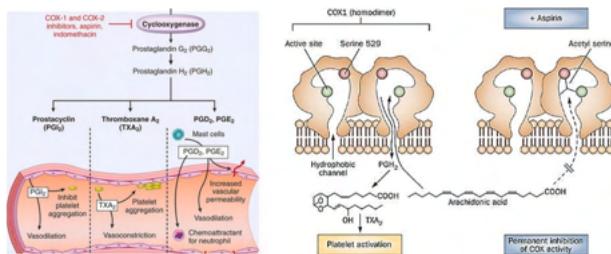


Figure 3.21: Mechanism of action of aspirin.

Observation 3.33 When looking at a COX-1 homodimer, aspirin can come into its active site and acetylate it permanently \Rightarrow irreversible inhibition of COX-1 and 2 (the body would need to make more to reverse this effect). This inhibition of COXs blocks the conversion of arachidonic acid to PGs.

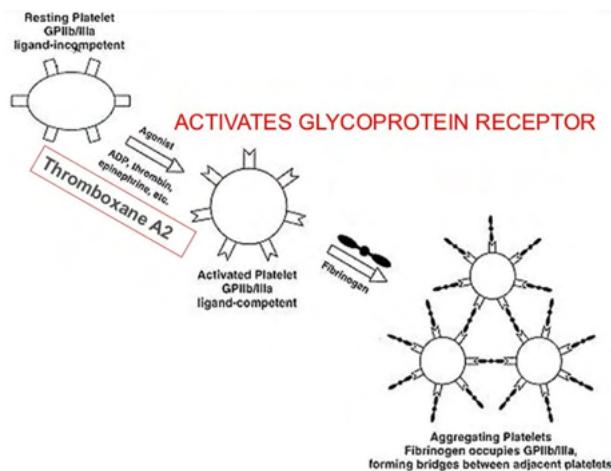


Figure 3.22: Thromboxane A2 and platelet aggregation.

When there's an injury along the blood vessel, platelets will start producing thromboxane A2 which can activate other platelet and consolidate together. Aspirin can block COX-1 which is the major enzyme to produce thromboxane A2 \Rightarrow aspirin is also anti-thrombotic. Because it's anti-thrombotic, it prevents the formation of plaque that block blood vessels \Rightarrow prevent strokes and heart attacks.

Remark 3.10. Interestingly, through a long-term study of aspirin usage, these patients have lower colon cancer risk than other.¹¹

Pharmacokinetics of Aspirin

We've seen this in previous lecture but at low dose aspirin, it follows first kinetics while at high dose, it follows zero order kinetics which leading it having longer half-life.

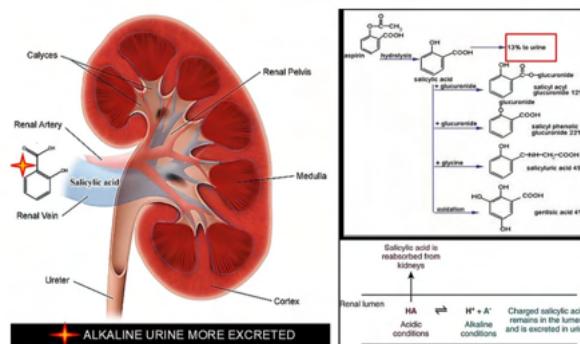


Figure 3.23: Excretion of aspirin.

Observation 3.34 Aspirin can enter the body and hydrolyze into its salicylic acid form which are typically removed through urine (13%). If not, salicylic acid can then undergo type II reaction to become other forms that it can be easily excreted by the kidney.

Interestingly, if in some circumstances that the urine is acidic, the salicylic acid will be reabsorbed while under basic condition will be remain and excreted out,

¹¹This also means that inflammation has something to do with **carcinogenesis** which is the transformation of normal cells into cancerous ones.

Toxicity of Aspirin

Definition 3.19. **Salicylism** is a term used to describe aspirin poisoning or toxicity.

Observation 3.35 The major side effect of people experiencing salicylism (due to long term usage at high dose) includes headache, tinnitus (hearing of a ringing noise), dizziness, hearing impairment and even dim vision.

In more extreme side effect if the patients have an overdose

- Confusion and drowsiness
- Sweating and hyperventilation
- Nausea and vomiting
- Marked acid-base disturbance
- Hyperpyrexia
- Dehydration
- cardiovascular and respiratory collapse, coma convulsions and ultimately death.

Remark 3.11. *We do not give aspirin to children as, even though rare, there's a chance that it can cause **Reye's syndrome**¹². Furthermore, we still have no clue on its pathogenesis.*

3.2.3 Traditional NSAIDs

The traditional NSAIDs includes different drugs that will block COX-1 and 2 at different ratio. A candidate to represent the traditional NSAIDs we will be focusing on is **ibuprofen**¹³

Ibuprofen also go into COXs to inhibit it. It must be noted that there will be drug interaction between ibuprofen and aspirin. Specifically, when taking ibuprofen before aspirin, ibuprofen will come and block COXs before aspirin \Rightarrow aspirin is useless in this case.

And like any other NSAIDs, it will all be processed in the liver and excreted in its inactive form in urine.

¹²A syndrome characterized by the swelling of liver and brain.

¹³Ibuprofen is an acronym of: **iso-butyl-propenoic-phenolic acid**.

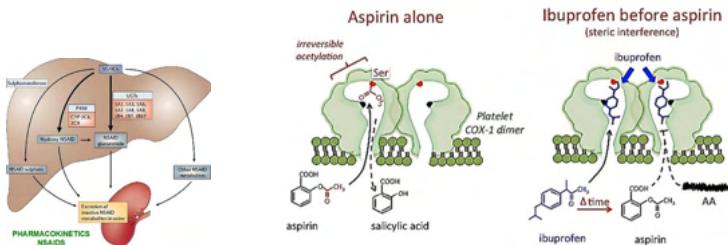


Figure 3.24: Metabolism and excretion of NSAIDs. Interaction of aspirin and ibuprofen.

3.2.4 COX-2 Inhibitor

CoX-2 inhibitors are NSAIDs that are designed to specifically target COX-2. The idea here is that instead of blocking everything including the production of platelets, why don't we just design a drug that specific block pain, inflammation and fever only. This could reduce the amount of side effects.

Observation 3.36 Basically, blocking COX-2 only is the way as it reduces PG but does not effect prostacyclin nor thromboxane A2 (produced by COX-1). Remarkably, blocking COX-2 does not only reduces pain, fever and inflammation but it also have some effects on constitutive functions.

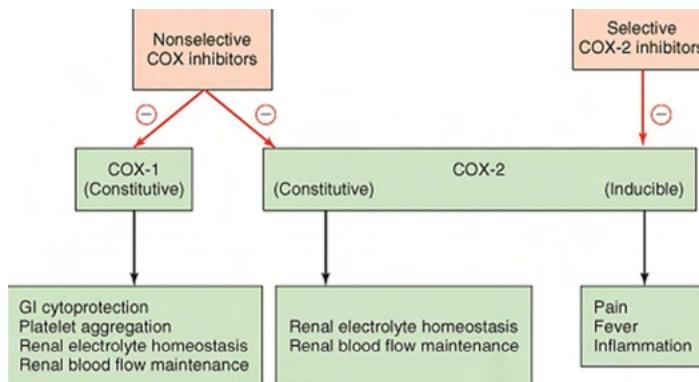


Figure 3.25: Non-selective vs selective COX-2 inhibitors.

How can we design a drug that only block COX-2? Well...this we only need to look at the structure of COX-2. Specifically, COX-2 has a wider

channel that COX-1 \Rightarrow a selective COX-2 inhibitors can just be designed to be larger than other NSAIDs \Rightarrow it can enter COX-2 but not COX-1.

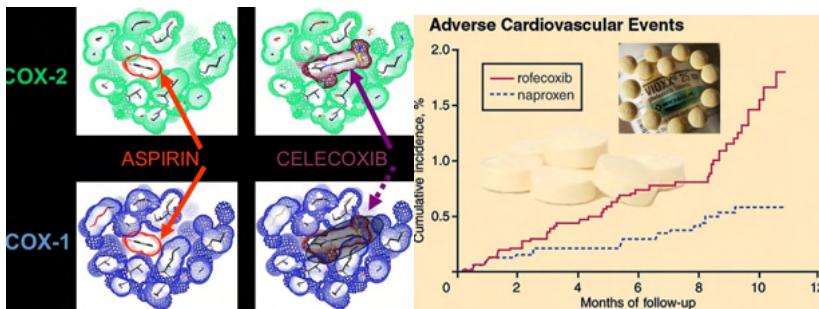


Figure 3.26: Aspirin (non-selective) vs celecoxib (COX-2 selective inhibitor) [left]. Adverse cardiovascular events study between naproxen and rofecoxib [right].

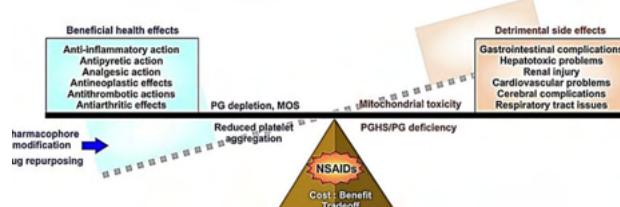
You can see from the figure above that **celecoxib**, a selective COX-2 inhibitor, cannot enter COX-1 because it's too big.

Observation 3.37 COX-2 also have a slightly different distribution than COX-1 but still it has some type of normal function.

Even if its distribution was different, when testing against a control drug, if it cause the number of incidence of a particular disorder in question to increase out of proportion, it will be taken out of market.

Example 3.2.1. When looking at adverse cardiovascular incidence between naproxen (non-selective) and **rofecoxib** (selective). There's a significant increase of incidence in rofecoxib as compared to naproxen \Rightarrow it's taken out of market as of 2004 by Merck.

Essentially, for an NSAIDs (and even ideally for all drugs) to stay in market, its therapeutic effects must outweigh its adverse effects!



3.2.5 Acetaminophen

Acetaminophen¹⁴ is the most commonly used over-the-counter drug worldwide. It exists in many form and combination with other drugs.

Remark 3.12. *It has analgesic and antipyretic effect BUT NOT anti-inflammatory! \Rightarrow it's not an NSAID.*

What's the mechanism of acetaminophen? Well...there's been lots of speculations of its mechanism but to be frank...we're not sure but hey, if it works, it works!

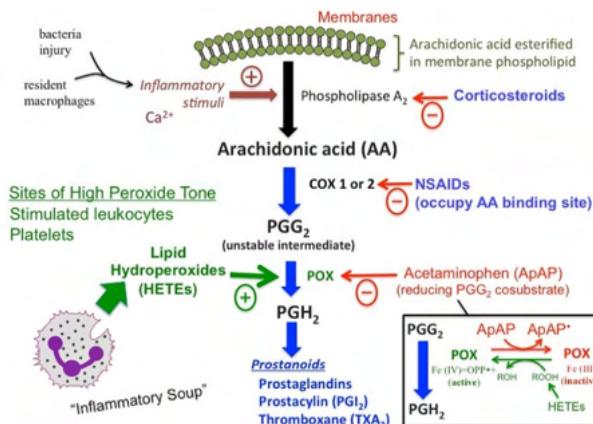


Figure 3.27: Theoretical mechanism of acetaminophen. Furthermore, apparently acetaminophen only acts in some tissues.

Pharmacokinetics of Acetaminophen

Acetaminophen has a half-life of around 2-3 hours. Nevertheless, what's much more significant (in a bad way) is that when it's metabolized in the liver, there's a potential to form **NAPQI** (by P450) which is a toxic metabolite to the liver.

Essentially, during an overdose of acetaminophen, a large amount of NAPQI is made and this can lead to apoptosis and necrosis of hepatocytes; and this happens gradually \Rightarrow the effect of liver death will slowly come

¹⁴Like other drugs, its name is an acronym of *para-acetylaminophenol*. Similarly, for its brand name **Tylenol**: *para-acetylaminophenol*

out instead of immediate. In fact, when looking at the fatal cases of poisonings in 2023, acetaminophen is quite high on the list.

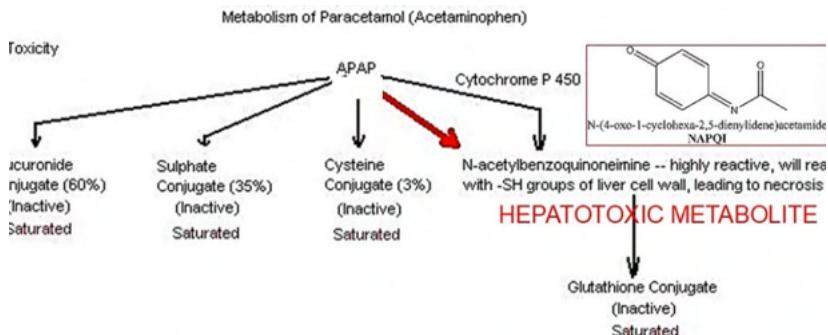


Figure 3.28: NAQPI production

It is certainly lethal so then **how do we know if we had an overdose?** Well...because of the effects not immediate, it will happen in the following stages.

1. 0-24 hours: Anorexia, nausea and vomiting.
2. 24-72 hours: abdominal pain, right upper region, elevated serum enzymes indicating liver injury.
3. 72-96 hours: Vomiting and symptoms of liver failure, and even sometimes renal failure along with pancreatitis.
4. > 5 days: resolution of hepatotoxicity or progression of multiple organ failure, even fatal.

How can we "reverse" this overdosing? Well...when the body made NAPQI, it can be conjugated with **glutathione** that will yield a harmless metabolite \Rightarrow the antidote to acetaminophen overdosing is increasing glutathione. This can be done by using a drug called **N-acetylcysteine**.

Ethanol Interaction

When taking acetaminophen, you should avoid ethanol as it can increase the risk of acetaminophen hepatotoxicity. **Why is that?** Well...from previous lecture, EtOH can induce P450 \Rightarrow P450 will make more NAQPI that cause liver injury. Not only that, EtOH will depletes glutathione storage \Rightarrow the NAQPI conjugation to harmless metabolite is slowed down.

3.3 Opioid Analgesics

In today's lecture, we will look at opioids but before that we're going to discuss some recent breakthrough in the field of chronopharmacology.

Definition 3.20. **Chronopharmacology** is a branch of pharmacology that study how the effects of drugs can vary depending on the rhythms of the body e.g. circadian cycles, hormone fluctuation. i.e. absorption, distribution, metabolism and excretion vary according to the time of day.

Observation 3.38 Chronopharmacology are poorly understood. This is because we base a lot of our studies on mice and animal models. The problem is that they have different cycles compared us e.g. mice are nocturnal.

Nevertheless, we were able to made some major advancement in this field in 2024. A group a research was able to grow human hepatocytes and observe its circadian rhythm like usual. They found that the activity of major P450s depends on the time of the cycle and this could alter the drug effects, toxicity and possible interaction.

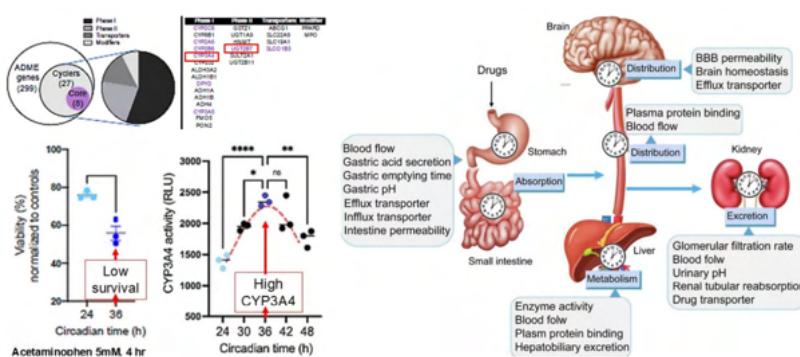


Figure 3.29: Chronopharmacology and the effect on acetaminophen metabolism by CYP3A4.

Example 3.3.1. CYP3A4 is the main liver enzyme that can degrade acetaminophen into its toxic metabolites that's dangerous to the liver. What researcher did was to give the hepatocytes the acetaminophen at the start or the middle of the daily cycle of CYP3A4. Remarkably, those that were given

in the middle of daily cycle of CYP3A4 has lower survival rate. When tracking the CYP3A4 activity through out the cycle, we also see that it peaks at the middle of the cycle.

⇒ acetaminophen toxicity relates to the CYP3A4 cycle and activity. However, this does not just stop for only acetaminophen since almost 50% of clinical drugs are metabolized by CYP3A4. Thus, on-going studies about this are still being made.

Not only that, when it comes to treating pains, chronopharmacokinetics are highly relevant as another enzymes called **UGT2B7** that can metabolize opioids ⇒ its cycle and activity can change the active analgesic given.

3.3.1 General Consideration of Opioids

Opioids are drugs used to deal against pain in the clinical setting as it can affect almost all level. However, it's also being abused by people for its effects and is also highly addictive. More than 14,000 people have died due to opioid overdose.



Figure 3.30: *Papaver somniferum*.

Definition 3.21. **Opioids** are a class of drugs derived from or even mimics alkaloids, called **opiates**, found in the opium poppy, *Papaver somniferum*. i.e. it includes opiates along with any synthetic or endogenous derivatives that are similar.

Observation 3.39 There are 3 main area that the production of opium, specifically, **heroin**, is mostly concentrated and that is **Afghanistan, Myanmar and Mexico**. In 2023, Afghanistan has significantly decreased its production. This created a ripple effect leading to the other places to increase its own production. Furthermore, with the rise of **fentanyl**, another derivative more potent, it can replace heroin as a whole for many addicts.

Brief History of Opioids

Observation 3.40 The power effect by the opiates was recognized > 3000 years ago. The production of said opiates are simple: stab the plant so that white extract pour out. This extract will be left on the plant until it hardens into a brown resin that can be scraped off.

The major drug that's in the brown resin is called **morphine**¹⁵. Another active substance can be found along with morphine in the natural extraction is **codeine**.

Morphine can be added with 2 acetyl group to form **diacetylmorphine (heroin)**. The main difference is that heroin can cross the blood-brain barrier. Bayer pharmaceutical was the first to discover and commercialize heroin as an over-the-counter medication in 1898. By 1860, we've learned that injection of heroin would yield a very strong response.

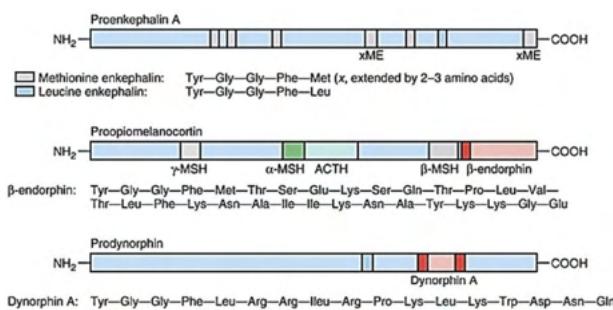


Figure 3.31: Endorphins, enkephalins and dynophins.

In 1975, Professor Hans Kosterlitz discovered the **endogenous opioids** which are opioids found and made by our body. This includes: **endorphins**,

¹⁵It got its name from the Greek God of dreams: Morpheus

enkephalins and dynorphins that are distributed throughout the central nervous system.

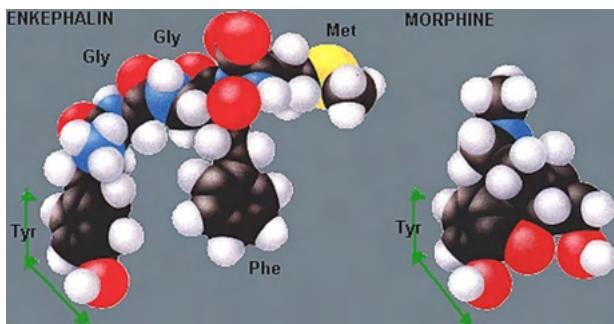


Figure 3.32: Enkephalin and morphine structural similarities.

Remark 3.13. Looking at the structure of enkephalin and morphine, they are quite similar which is why morphine can bind to its receptors.

Types of Opioids

Like we've just said, we have the naturally occurring opioids like: morphine and codeine. Then, we have the synthetically made which are most of the time, have morphine-like action and they can vary in potency and effects.

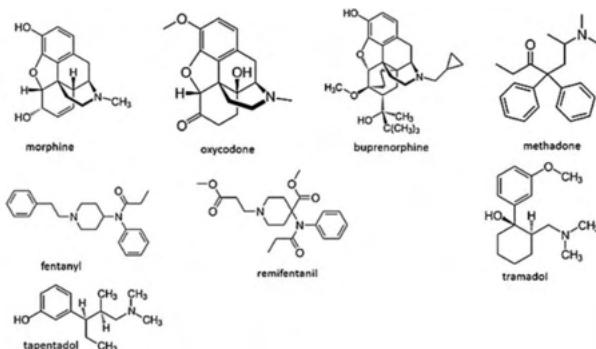


Figure 3.33: Different types of opioids.

Observation 3.41 Like many other drugs, opioids' structure correlate to its activity \Rightarrow modification of its structure will yield a modification in activity.

Example 3.3.2. Morphine is an agonist to the μ -receptor which can be modified into **buprenorphine**, a partial agonist to the μ -receptor. This can be a good derivative as it would help some addict to recover. With further modification, you can get **naloxone** which is a antagonist to the receptor and can help with treating overdosage.

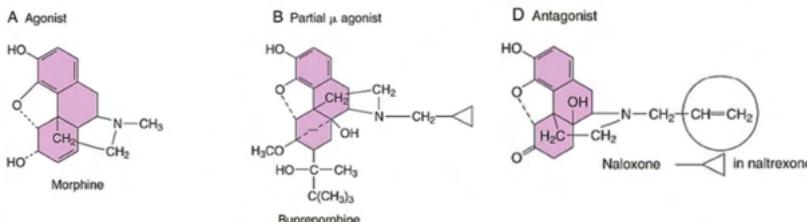


Figure 3.34: Morphine and its structural modification.

3.3.2 Pharmacodynamics of Opioids

Before talking about the pharmacodynamics, let's briefly go through the nociceptive system (pain). First, when there's an injury, nociceptors will fire signals from the area to the spinal cord. Here, the signal travel up to the cortex and we feel pain. Not only that, another signal travel down and will affect the intensity of pain. Previously on NSAIDs, they only act at the site of injury and maybe a little at the crossing in the spinal cord. For opioids, they act almost everywhere along that pathway.

Observation 3.42 First, opioids can decrease pain signal and this is

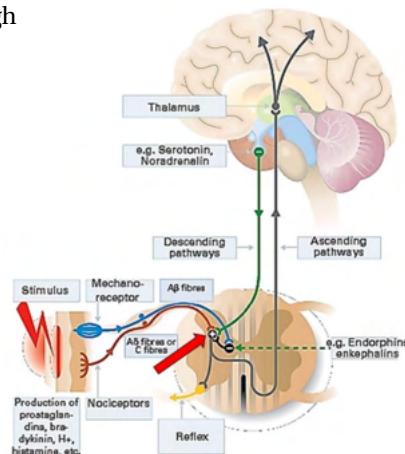


Figure 3.35: Nociceptive system.

dony by binding to its receptor that subsequently block Ca^{2+} channel \Rightarrow transmitter release \downarrow in the presynaptic neurons. In the post synaptic neurons, the opioid can bind to its receptor that act on the K^+ channel that hyperpolarize the cell. Ultimately, intensity of pain signal travel up \downarrow .

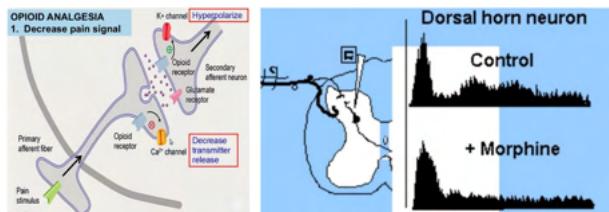


Figure 3.36: Opioids analgesia and experimentation.

We can in fact, see this by activate those neurons and trace its activity of the control group vs morphine treated group (see Figure 3.36).

Second, opioids can have **supraspinal analgesic** effect which is the ability to induce analgesia from the descending pathway from the cortex down the the site. This is done through increasing pain inhibition. the opioids can bind to its receptors on GABAergic neurons and inhibit it from relaying signal down.

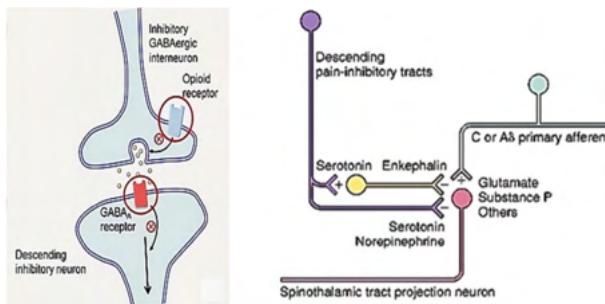


Figure 3.37: Supraspinal analgesia by opioids.

Basically, opioids are decreasing the transmission of pain in the spinal cord. They act on both the ascending and the descending pathway. They also act on the presynaptic and postsynaptic neurons.

Another strong effect that opioids have is on the reaction to pain. According to patient, the pain is less and they can still feel it but somehow it stop bothering them.

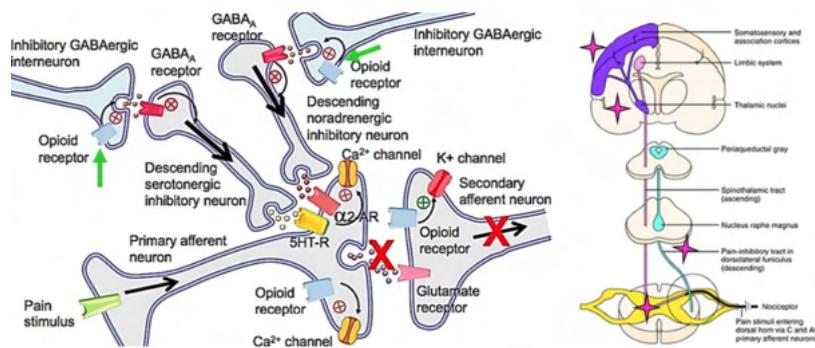


Figure 3.38: Complete pathway by opioids (left) and opioids effect on pain sensation and emotion (right), star-area are the parts where opioids have effects.

Opioid Receptors

Opioids receptors can be found throughout the entire nervous system. There are mainly 3 types: μ , δ and κ – receptors. There is also a speculation of another called **opioid receptor-like (ORL/NOP)** but we will not get into that today. We're mainly going to focus on the μ –opioid receptor.

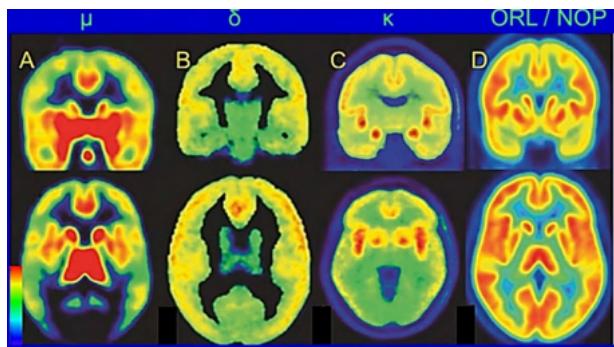


Figure 3.39: Opioid receptor distribution in the brain.

Observation 3.43 μ -receptors are a type of GPCRs. When an opioid binds to it, it can induce various effect like activation of K^+ channel, inhibition of Ca^{2+} channel and even deactivation of adenylyl cyclase (make cAMP). All of this is to cater to the effect of hyperpolarizing the cell and thus decrease its neurotransmitters release.

Because it's a type of GPCRs, its cycle is also the same: after activation, it will be brought into the cell by endocytosis. Then, it can either be degraded or recycle back in its inactive form waiting to bind to opioid.

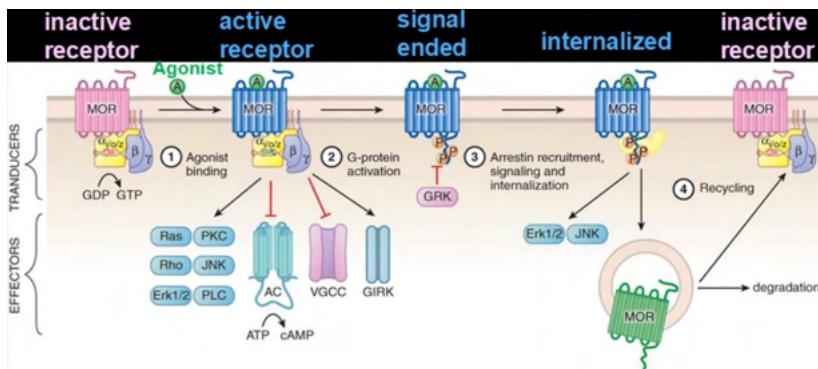


Figure 3.40: Activation of μ -opioid receptor and its recycling.

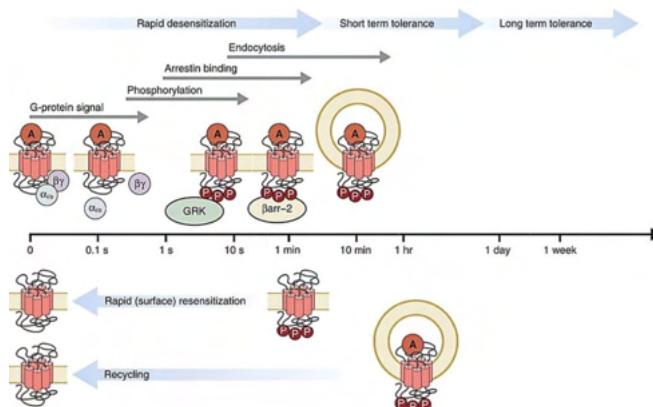


Figure 3.41: Time course of μ -opioid receptor activation and recycle

When μ -opioid receptors is activated it will have effect on the following effectors: GTPase, kinases, phospholipase C, adenylyl cyclase, voltage-gated Ca^{2+} channel, G protein-coupled K^+ channel. Not only that, the time course of this activation until its recycling or degradation is very rapid.

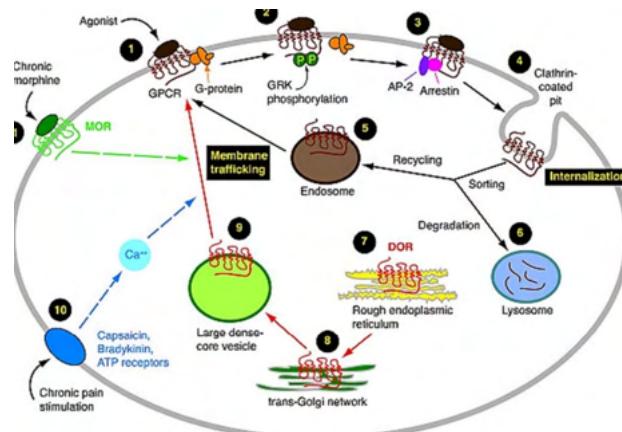


Figure 3.42: A complete illustration of μ -opioid receptor plasticity.

Remark 3.14. *Chronic usage of morphine will also alter the amount of synthesized μ -opioid receptor to be sent to the cell surface.*

Interestingly, these receptors can also form dimer at the surface of the cell. We found that when they form heterodimer is when the effect is at its highest while no response in homodimer. This could be a possible drug target

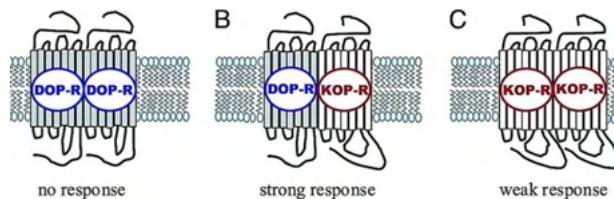


Figure 3.43: Dimerization of opioid receptors.

Now, we've mentioned before but will say it again, opiates can act on many places in the brain and nervous system. But probably more important is it's ability to induce analgesia.

Observation 3.44 Remarkably, the opioids can relieve pain like analgesics drugs but there won't be no impairment in other sensory modalities i.e. you block the pain but can still feel touch, taste and see. This is also the reason that it was used in the First World War to relieve pain and even stress and anxiety while can still fight.

You also have opioid receptors in your GI tract and their activation will lead to suppression of cough, inducing euphoria, depression of respiration, pupillary constriction (**miosis**) and effect seizures.

Does each of the 3 opioid receptors involve in different effect? Well... unfortunately no, each of them will have some play in each effects e.g. analgesia involves all 3 receptors, GI and sedation is both μ and κ while depression of respiration involves only μ . This means that if you target 1 receptor, you'd get other side effects mentioned.

3.3.3 Pharmacokinetics and -Dynamics of Morphine

Evidently, we cannot look at the pharmacokinetics of a class of drug, so we'll shift our focus to the "representative" of opioids which is morphine.

Observation 3.45 Morphine can be taken orally, rectally, via injection and inhalation. With the oral and rectal route, its absorption in the GI tract is usually slow and incomplete. Because of this inefficiency, the most common way is through injection (IM, IV and SC) and inhalation¹⁶ which is much more rapid.

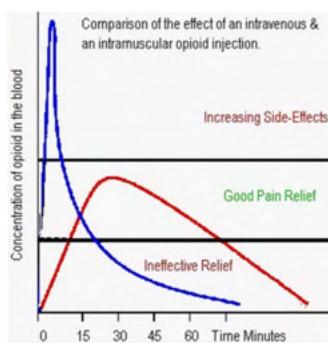


Figure 3.44: IV vs IM injection of morphine.

Even though oral route is not ideal, it can help with relieving GI pains. Now, when it comes to injection, therapeutically it's done via IM injection. This is because IV injection will rapidly overshoot the therapeutic window. However, for drug addicts, IV injection is ideal for them as it will give them the "high". Furthermore, because of the rapid overshoot and immediate drop after peak, it's highly addictive.

¹⁶Inhalation is typically not a therapeutic way to take it

Observation 3.46 Only 20% of morphine can cross the blood-brain barrier. It's metabolized in the liver and has a half-life of 2-4 hours. After metabolism, it can be eliminated in urine.

It must be noted that **metabolites of morphine will differ depending on the route of administration**. To be specific, when taking morphine orally, the metabolite produced would be quite psychoactive and neurotoxic. On the other hand, with injection, there majority would be converted into inactive metabolite with half-life 4h. The rest of the metabolites are highly active with half-life of 3h (**morphine-6-glucuronide**). If left in the body for > 50h, it can cause renal failure.

Pharmacodynamics of Morphine

Like other opioids, morphine is used to control pain but it also have a big side effect of impairing motility of the GI tract. Normally this side effect can be severe and dangerous but it can be useful in certain situation.

Observation 3.47

The GI tract is controlled by its own nervous system called the enteric nervous system. Along side that, there are lots of μ -opioid receptors all over the GI thus it can have multiple action on the GI tract. In particular, when morphine bind to these receptors, it can decrease peristalsis by damp down longitudinal contraction and cause sphincter spasm. Additionally, there will be decrease water secretion and increase water absorption of stool in the tract. All of this will total in a severe constipation.

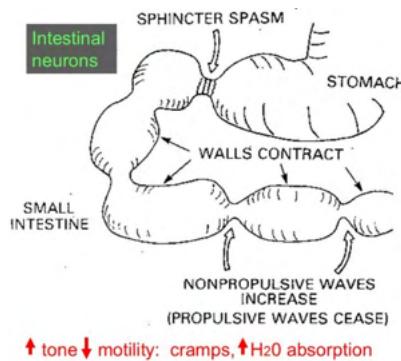


Figure 3.45: Opioid induced constipation.

Remark 3.15. Although with this severe constipation, it also help with patient suffering from serious diarrhea like though who's infected with cholera.

Observation 3.48 Morphine can also make people nauseous and there can

be multiple stimuli to the CTZ¹⁷. Other serious side effect includes respiratory depression which is simply a decrease in breathing rate by decreasing sensitivity to CO_2 in the brain. In fact, many overdose death is resulting in the person completely stop breathing. Interestingly, morphine can cause pupil constriction and even the person is in the dark, it will continue to persist. Because of the sphincter spasm, there would be urinary retention i.e. even when the urinary bladder is full, the person cannot release it. Also, there will be a decrease in urine due to the changes in ADH.

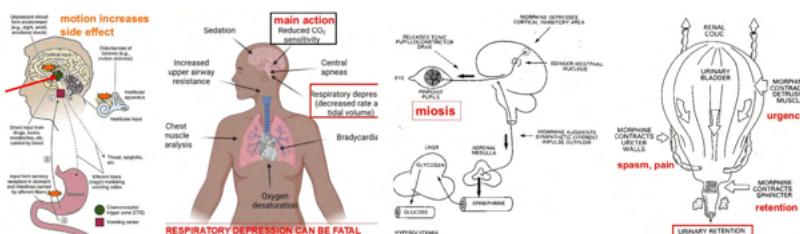


Figure 3.46: Side effects of morphine.

Differential Tolerance

High Degree of Tolerance	Moderate Degree of Tolerance	Minimal or No Tolerance
Analgesia Euphoria, dysphoria Mental clouding Sedation Respiratory depression Antidiuresis Nausea and vomiting Cough suppression	Bradycardia	Miosis Constipation Convulsions Antagonist actions

The body can develop tolerance to some of the effects induced by morphine. Unfortunately, the body will become tolerant to an important effect like analgesia and in the case of drug addicts: euphoria. Bradycardia would be moderately tolerated while miosis and constipation has no tolerance at all.

Figure 3.47: Differential tolerance of morphine.

3.3.4 Codeine

Observation 3.49 Codeine is another opioid that can be taken orally. It can have moderate analgesic effect but can work with NSAIDs to produce an even better effect. In the diagram below you can see the pain relief by

¹⁷Motion can increase this side effect

NSAIDs and codeine alone and with them combined (best result). This is also why codeine and aspirin is often used together in dental works.

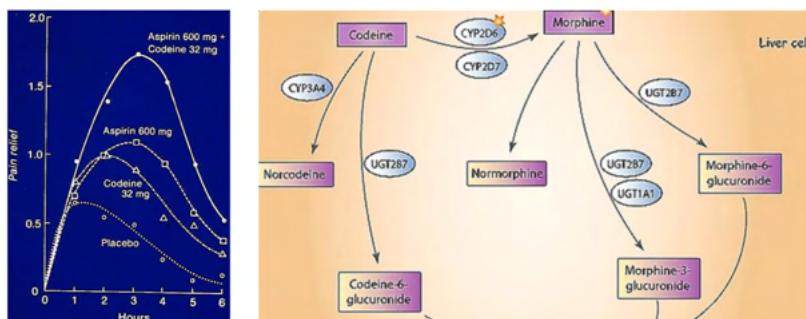


Figure 3.48: Synergy of codeine and aspirin (left). Codeine metabolism (right).

It must be noted codeine alone is not an active form that can do produce the analgesia. When entering the body, it's metabolized by CYP2D6 into morphine which is the active metabolite. Codeine can be broken down into norcodeine which is inactive and eliminated by the bile. The metabolized morphine can then be broken down later on by its own enzymes mentioned before.

As we've previously mentioned on the genetic variation of P450 enzymes, there are also genetic variation of CYP2D6 that can cause the person to be unable to metabolize codeine.

Remark 3.16. *Tamoxifen is a drug used to treat breast cancer but it needs to be activated by CYP2D6 which means anyone have defective CYP2D6, they cannot be treated.*

3.3.5 Other Opioids

Like we've seen before, opioid is a large family of morphine-like drug that can act differently from one to the other.

Example 3.3.3. Morphine is a full agonist of μ -opioid receptor as compared to buprenorphine, a partial agonist or even naloxone, an antagonist. Obviously, each of them will have different purpose in a treatment.

These drugs will have different half-life which is for different effect (see table below):

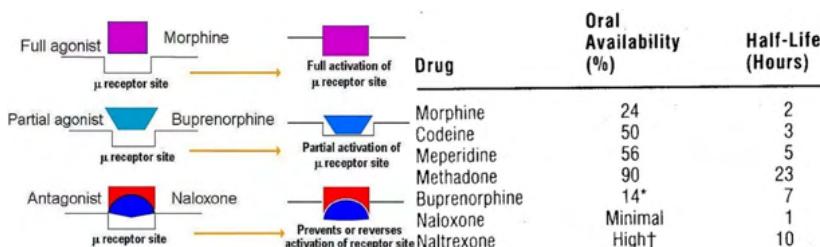


Figure 3.49: Activation of μ -opioid receptor by different opioids.

Example 3.3.4. **Hydromorphone** is μ agonist and is used often for chronic severe pain. Its structure is quite similar to that of morphine. **Meperidine** is a good analgesic with side effects differ from morphine however it would require a higher dose to reach the same efficacy of morphine.

A well-known drug that is newly considered to be replacing heroin is **fentanyl**. Its time to peak effect is only 5 min (compared to morphine's 20 min) but its duration of action is much shorter of 45 min (all IV injection). Interestingly, it's lipid-soluble which means we can put it in the form of patches or even lollipops (for children). It's actually been used extensively in clinical setting however it's also been responsible to kill lots of people (addicts).

Loperamide is another drug that is part of opioid and is used to treat people with chronic diarrhea. It cannot cross the blood-brain barrier and only effect the GI. All in all, we have a whole range of opioids for different purposes.

Also, we've found that a mix of agonist and antagonist has demonstrated to have a lower abuse potential however it would give less pain relief. Thus, this is best in case of lower but chronic pain in patients.

3.3.6 Clinical Uses and Addiction

The main clinical uses of opioids are of the followings:

1. **Analgesia:** This is mainly to relieve pain while having other sense non-impaired. They're typically given at a specific schedule.
2. **Cough Suppression:** Decreases coughing like **dextromethorphan**.

WEAK AGONISTS:	
methadone – oral absorption, long T½ , block WD in opioid addict stabilize maintenance therapy, (like nicotine replacement)	
Pure Agonists	Morphine, codeine, meperidine, fentanyl , remifentanil, propoxyphene , hydrocodone , oxycodone
Agonist-Antagonist	Nalbuphine, butorphanol, Buprenorphine
Pure Antagonists	Naloxone, Naltrexone
Partial Agonists	Pentazocine
ANTAGONISTS:	
naloxone binds μ ; must inject, T½ minutes - treat opioid OD naltrexone - orally absorbed, T½ ~1 day – former opioid addicts prevents drug effect	

Figure 3.50: Summary of opioids.

3. Anti-diarrhea: treatment of severe diarrhea like loperamide.

Addiction

Definition 3.22. Narcotics¹⁸ is a large class of psychoactive drug that can have numbing or paralyzing properties. They tend to first induce euphoric feeling and then tranquilize and sleepiness; of which can last about 3-5h.

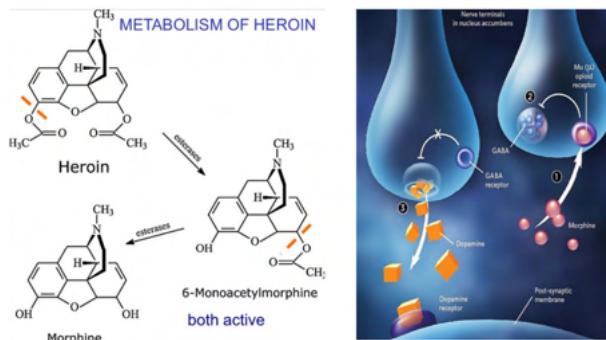


Figure 3.51: Heroin metabolism and its inhibition of GABA.

¹⁸The words have Greek origin which means "stupor"

Observation 3.50 Heroin is the prototype drug for injection. It is much more potent than morphine and can cross the blood-brain barrier more easily; it used to be sold legally. Although it can be taken orally, for those seeking a drug effect or a high, heroin is typically injected. When metabolized, heroin breaks down into two derivatives of morphine that are both active, giving it a slightly longer duration of effect.

As with other addictive substances, heroin stimulates the reward pathway in the brain. It blocks the release of GABA, which then disinhibits the release of dopamine, enhancing the feeling of euphoria.

Observation 3.51 The problem we're facing with opioids addiction right now is the lethal dose of the drug become ever more lower e.g. **carfentanil** is the newer derivative whose lethal dose is much lower than heroin (5000 times more potent than heroin).

Furthermore, this problem has been increasing with more death as more of these derivatives are released on the street. Here, we can see the combination of fentanyl and a sedative called **xylozine** also increases the overdose death in the US.

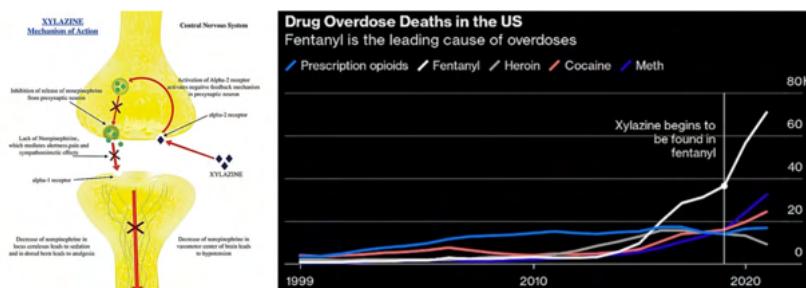


Figure 3.52: Xylozine mechanism (left) and drug overdose in the US.

Xylozine is a sedative and its action with fentanyl is to extend its duration thus it lasts longer. However, it also makes it more difficult to deal with an over dose.

In Canada alone in 2024, there are roughly 1,906 deaths from opioid overdose in just the first 3 months. 81% is caused by fentanyl and carfentanil. 61% of them were actually in accompany with other stimulants. The province with the highest death related to opioid overdose in Canada is British Columbia and it has been increasing ever since COVID-19.

What could we do? Well...so far, all we could do is figure out more ways to treat the overdose against these newer recreational drugs. We're trying to provide some maintenance to these addicts by giving methadone (something we can control with drug treatment facility and pharmacy).

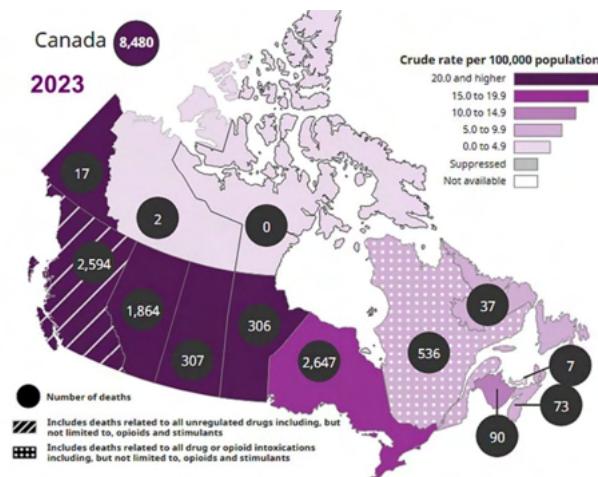


Figure 3.53: Opioid overdose in Canada.

In this chapter, we will look at various aspect of toxicology and environmental pollution effects on health.

4.1 Toxicology

Definition 4.1. **Toxicology** is the study of the adverse effects of chemical, physical, or biological agents on living organisms and the ecosystem, including the prevention and amelioration of the effects i.e. the science of poisons.

Observation 4.1 Similar to pharmacology, there are many branches in toxicology which include, but not limited to, medical toxicology, analytical toxicology, applied toxicology, foreign toxicology, industrial toxicology, immune toxicology, genetic toxicology, environmental toxicology, reproductive toxicology, etc.

The 2 basic functions of toxicology is: **to study the effects nature and mechanism of adverse effects, and assess the likelihood of adverse effects.**

Concept 4.1 *The dose make the poison*

Explanations. The concept above is a famous quote by Swiss physician **Paracelsus** (1493-1541) talking about how a dose of any particular substance would lead to its poisonous effects. This is true as any substance at a really dose will cause some poisonous effects e.g. drinking too much water will lead to water intoxication. Essentially, when looking at poisons and toxicants, we'll be looking at their doses. □

Definition 4.2. A **poison** is a chemical that can cause harm and even death to living organisms. When a poison is naturally occurring (e.g. made by organisms), it's called a **toxin**.

Example 4.1.1. **myotoxins** and **phytotoxins** are toxins produced by fungi and plants respectively. Both of which are poisonous. **Venom** is a type of toxin produced by animal (by some definition, venom are toxin that produced by animal and is delivered by said animal to another)

Definition 4.3. A poison that can be either synthetically made or naturally made is called **toxicant**¹.

4.1.1 Neurotoxicants

Observation 4.2 The largest class of toxicant is the ones that effect the nervous system, **neurotoxicants**. This is because neurons are the largest cells in the body (it can extend from the brain all the way to the peripheries). Not only that, it requires lots of energy in order to generate the membrane potential but also propagating action potential.

Toxins are made for either defense or attack by the animal or plant the synthesize them. Many of these toxins target nicotinic receptors, of which they can either acts as agonist or antagonist.

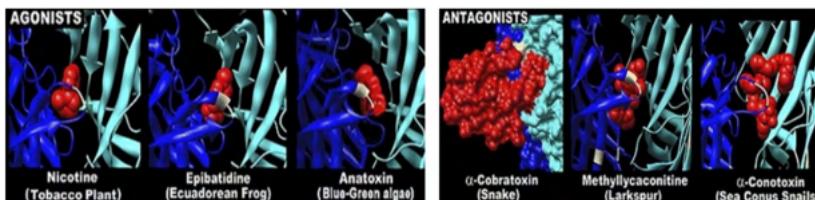


Figure 4.1: Neurotoxins targeting nicotinic receptors.

Observation 4.3



Figure 4.2: Galantamine and plants that synthesize it.

Many toxins can also target the post-synaptic enzymes such as the **acetylcholinesterase (AChE)** e.g. the flower *Narcissus* (daffodil) and *Galanthus nivalis* (snowdrop) can make a toxin called **galantamine**², which is a **AChE inhibitor**.

Remark 4.1. Lower dose of galantamine has been looked at as potential drug for treating certain CNS disorders.

¹Basically an umbrella term

²It's name after *galanthus* as they produce the highest concentration of this toxin

Observation 4.4 There are many ways to evaluate effects of toxins. To be particular, you can see its effect in neuron cultures, and most recently, you have (cerebral) **organoids** which is a way to study drugs in a lab without killing animal. This is because organoids is almost like a system of cells interact with each other.

4.1.2 Cardiotoxicants

As the name suggested, cardiotoxicants are toxicants that mainly affect the cardiovascular system. The most common category of this kind of toxins made by plants are called **cardiac glycosides**.

Observation 4.5 This type of toxin is effective since the heart is also vulnerable just as the nervous system is. First off, the heart requires lots of energy (for both electrical and mechanical properties) as it has to beat continuously and has low energy reserve.

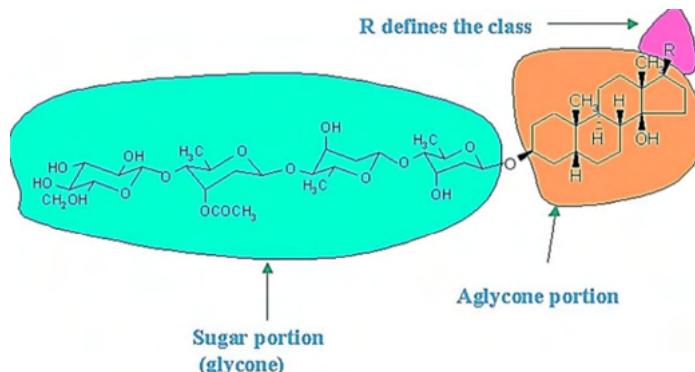


Figure 4.3: General structure of cardiac glycoside.

Cardiac glycosides blocks the Na^+/K^+ pump in order to create membrane potential for the heart to beat correctly. In general, it will first block said pump and which lead to a build up of Ca^{2+} (due to another exchange mechanism linking to the pump). This leads to more forceful contraction of the heart and ultimately lead to cardiac arrhythmia.

Notion 4.1 In general, these toxins have toxic effects to the conduction system. They can lower pacemaker activity, conduction velocity and promotes ectopic impulse generation.

Example 4.1.2.

***Convallaria majalis* (Lily of the valley)** is a plant that can produce up to 20 different cardiac glycosides. Many of our garden plants contain different cardiac glycosides such as **digitoxin**. We also have household plants that are poisonous such as *Kalanchoe delagoensis* and the most vulnerable to them are children and pets.

In Montreal especially, we have ***Nerium oleander*** which can be found almost everywhere and can produce different cardiac glycosides and **oleandrin**.



Figure 4.4: Oleander.

Example 4.1.3. *Ricinus communis* (castor bean) is a plant that produces well-known toxin called **ricin**. It consists of 2 sites: 1 of which is the toxin site while the other is the binding site. Its mechanism is to bind to ribosome and block protein synthesis (very powerful).

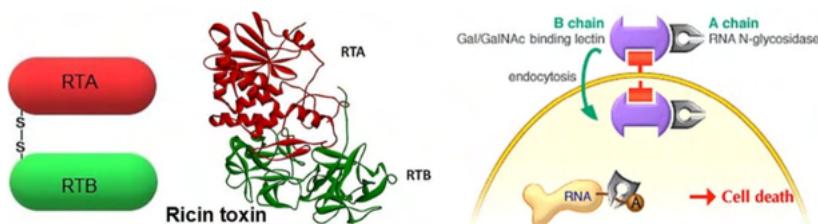


Figure 4.5: Ricin structure and mechanism.

Observation 4.6 Once again, we need to mention that the dose matters e.g. apple seeds contain **amygdalin** which is a toxin but it's not dangerous for us to eat. This is because we need to consume a really large amount to induce the toxic effect. Similarly to most food we eat, it's not just the chemical but more importantly is the amount of it present.

4.1.3 Electronic Waste as Toxicants

Now, what we've just talked about are toxins which are naturally made by organisms. But we also have toxicants such as many components in electronics in waste nowadays e.g. lead is a toxicant that can create oxidative stress and affect neurotransmission.

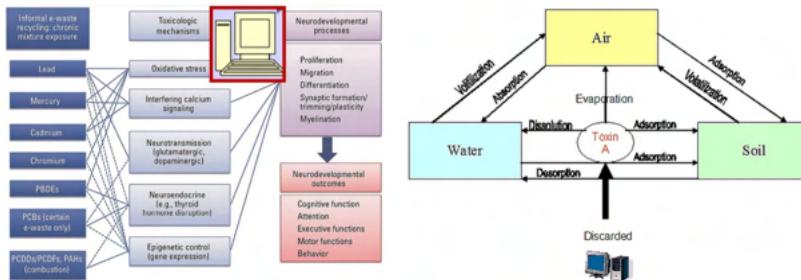
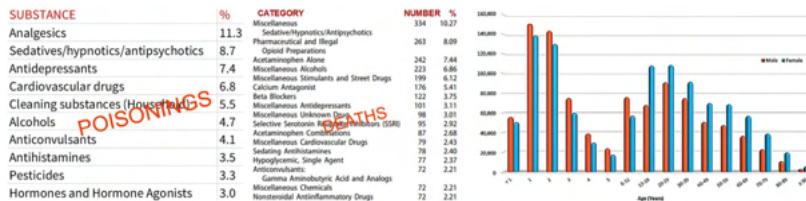


Figure 4.6: Electronic waste as toxicants.

When throwing away electronic waste to dumping grounds, these toxicants can leak out into the soil and even water if there is. If they're volatile enough, they can go up into the air and circulate everywhere.



In the above tables, we can see some of the substance involved in human poisoning and the number of deaths that occur with the poisoning of these drugs. We can also see that the most vulnerable group to poisoning is children of the age of 1 – 2.

Pollution

Pollution can also affect different system in the body but the most vulnerable system are the heart, lungs and brain.

Observation 4.7 When you breath in air particles, it will likely be trapped by the mucus in the lung which will be empty out via the mucociliary clear-

ance but also captured by phagocytes. Nevertheless, some of these air pollutant particles can be so small that it by-pass these defense systems and enter deeper into the lung, and even diffuse through the blood vessels and enter circulation.

Not only that it can enter through the lung, some of these particles can even enter through the olfactory bulb in the nose.

Air pollution has been linked to many impaired development and even dementia.

4.2 Environmental Cardiology

Definition 4.4. **Environmental cardiology** is the study of cardiovascular disease and its relevance/causes by the environment e.g. pollution, toxins. Basically, it analyzes what we're breathing and swallowing can affect the cardiovascular system.

Remark 4.2. *The particles we're mostly concerned with are those that is less than $5\mu\text{g}/\text{m}^3$.*

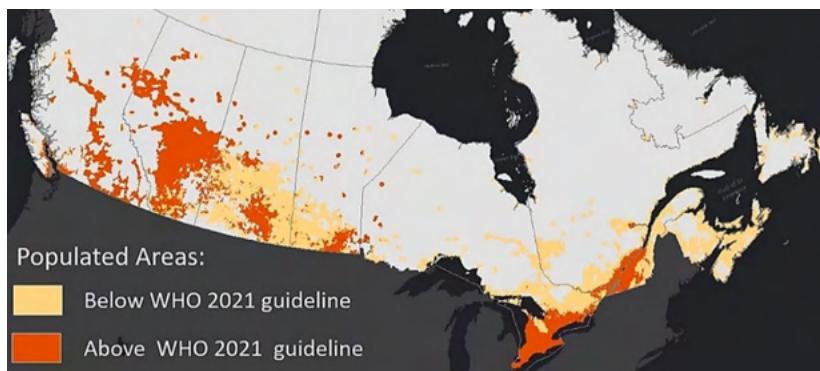


Figure 4.7: WHO PM 2.5 guidelines. Almost 86% of Canadians live in areas where air pollution exceed this guideline.

Observation 4.8 These microparticles can have serious effects on the heart, blood vessels and even the autonomic nervous system that can modulate the heart. As an end result, exposure to them can lead to manifestation of

acute heart attack, stroke and even chronic atherosclerosis.

There are many ways to do researches on these microparticles. A methods we've previously discussed, that's recently developed, is using organoid. In this case, cardiac organoid.

Even as research are being done, it's recorded that more than 15,000 deaths/year is due to acute and chronic exposure to air pollution, which is mostly through diesel and gas engine by-product. **70% of these death are due to cardio- and cerebrovascular disease.**

Definition 4.5. **Stenosis** is the abnormal narrowing of the blood vessels.

Observation 4.9 It's been determined that stenosis can be severe even before symptoms occur i.e. the blood vessel can narrow a lot and there will not be any symptoms until later on.

In all cases, these microparticles can cause atherosclerosis which, as a consequence, can be a thrombus or an embolus that will occlude the blood vessel. You could also experience hemorrhage and even abnormal spasm. In fact, 20% of people with these problems, where there's an occlusion in the coronary artery, will experience sudden death.

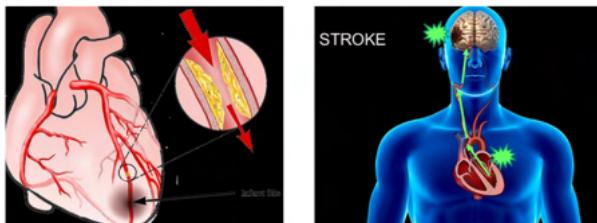


Figure 4.8: Sudden death by cardiovascular occlusion and stroke.

Another dangerous condition can manifest if the formed thrombus in the heart vessel is pumped directly up to the brain vessel which can cause a stroke.

4.3 Environmental Toxicology

Definition 4.6. **Environmental toxicology** is a multidisciplinary field of science concerned with the study of the harmful effects of various chemical, biological and physical agents on living organisms

Observation 4.10 While traffic-related air pollutants are a significant focus, many other exposures exist, including those in water, soil, and air. This field establishes regulations to limit harmful exposures.

Notion 4.2 The environment in which a toxin is released influences its potential absorption.

Example 4.3.1. Some toxins may settle in the soil and become inaccessible to humans, while others may lead to extensive exposure e.g. toxin released in water can get absorbed by fish which will be caught by human and thus we absorb the toxin to us.

Concept 4.2 (*Biomagnification*). When pollutants and toxins get absorbed by organisms at the bottom of the food chain, it can move up with ever increasing concentration.

Concept 4.3 (*Bioaccumulation*). A pollutant or toxin can build up in an organism when its rate of absorption is higher than its rate of elimination.

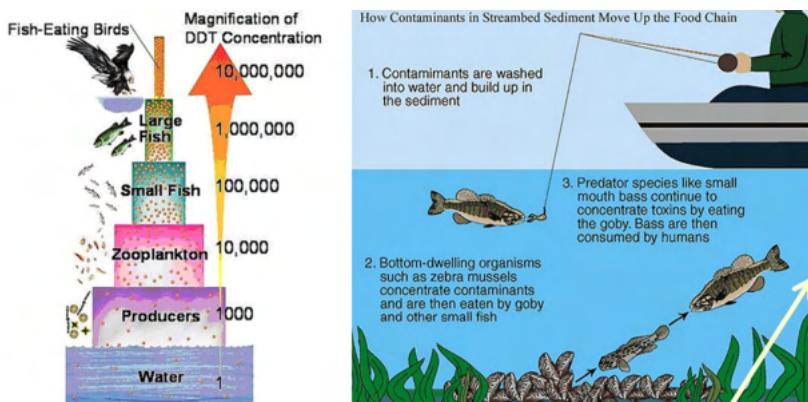


Figure 4.9: Biomagnification and its process moving up the food chain.

Example 4.3.2. DDT and other pesticides can enter the water. Then, it can accumulate in plankton and move up the food chain. Due to biomagnification, each step up the food chain, this toxin will become more and more concentrated. In fact, because of the biomagnification of DDT and pesticides, it nearly wipes out the bald eagle population. Luckily, there are

efforts to ban DDT in some developed countries thereby allowing many species population to recover.

Definition 4.7. **Lethal dose (LD)** is the identification of the dose that is lethal for an organism. Typically, for substances, we look at the **LD₅₀** which is the dose that can be lethal for 50% of the population.

Example 4.3.3. Sugar has an LD₅₀ of more than 10,000mg/kg compared to botulinum toxin which is only 0.0001mg/kg.

Definition 4.8. To evaluate the safety of a substance, we use the **therapeutic index** which for animals is defined as LD₅₀/ED₅₀. For humans, it's TD₅₀/ED₅₀. Where ED₅₀ is the dose that is effective for 50% of the population while TD₅₀ is the dose that is toxic for 50% of the population.

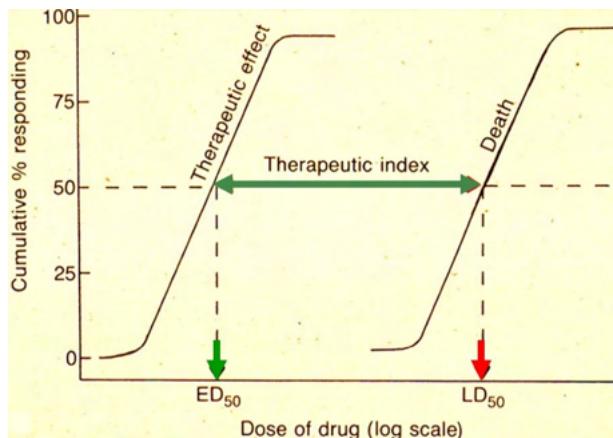


Figure 4.10: Therapeutic index.

Remark 4.3. *The smaller therapeutic index, the more dangerous the drug is and v.v.*

Definition 4.9. The **Margin of safety** is measured by LD₀₁/ED₉₉.

Observation 4.11 We've previously seen so far that most of the toxic and therapeutic drug effect curve are parallel but sometimes they may not be like that. There would be an overlap of the curve where the toxic range can lie within the therapeutic range. Sometimes, the rise of the toxicity by

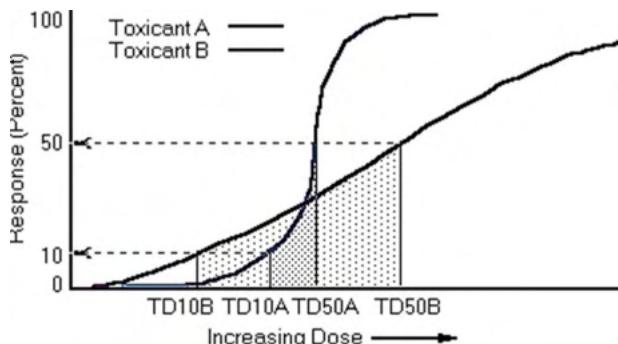


Figure 4.11: Comparison of 2 toxicants based on the dose increase and the yield effect. In such situation, toxicant A is more dangerous as a small increase in dose can result in a massive toxicity event.

dosage can be mild and sometimes it can be severe i.e. slight increase in dose result in an extreme case of toxicity.

Notion 4.3 There is no safe lower dose (threshold) for carcinogens.

Definition 4.10. When there's such threshold, we describe it through the following: **No observed adverse effect level (NOAEL)**, which is the highest data point at which there was not an observed toxic/adverse effect; and **lowest observed adverse effect level (LOAEL)**, which is the lowest data point at which there was an observed toxic/adverse effect.

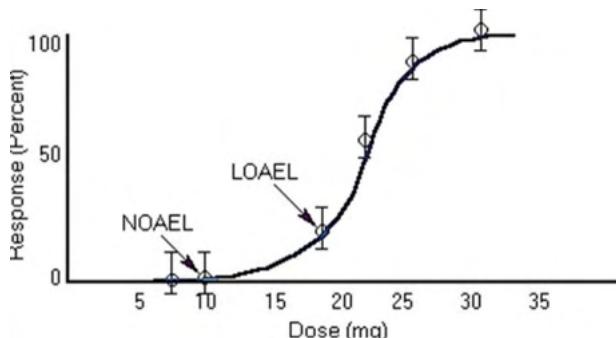


Figure 4.12: NOAEL and LOAEL.

Remark 4.4. Interestingly, lower dose of vitamin can have a negative effect for therapeutic and this is because you're deprived from vitamin.

Concept 4.4 (Hormesis). At a specific dosage, certain drug can have a positive effect but then will have the opposing effect when the dose keep increasing.

Example 4.3.4. Pesticide is a drug used to decrease the growth/population of pest in order to save crop yield. At a specific concentration, pesticide will have do its effect whereby the population of pest decrease. However, when its concentration increases, there might not be any effect or even worst increase the population of the pest.

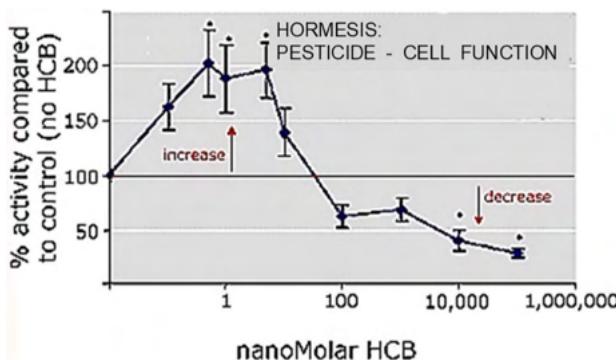


Figure 4.13: Hormesis of pesticide.

Animal Studies for Toxicology

Animal studies remain vital for understanding toxic effects, as they provide insights into how toxins interact with whole organ systems. However, ethical considerations about animal use are important. For perspective, far more animals are killed as roadkill than in research labs.

Remark 4.5. While research animals are monitored closely, cruel practices like glue traps for pests remain a concern, underscoring the need for humane methods.

For instance, rat poison stations using anticoagulants like warfarin provide a less painful way to manage rodent populations.

4.4 Epidemiology

Definition 4.11. **Epidemiology** is the study of how often diseases occur in different groups of people and why.

In toxicology, epidemiology plays a complementary role, studying population-level impacts of toxins to inform public health interventions. This multidisciplinary approach allows us to understand, manage, and mitigate the risks posed by environmental toxicants.

There are many types of studies in epidemiology of which we can look at in particular is **observational studies** which can be split into 3 different studies: **cross-sectional surveys, cohort studies and case-control studies**.

Definition 4.12. A **cohort study** is a type of observational study that follows a group of participants (*cohort*) over a period of time, examining how certain factors (like exposure to a given risk factor) affect their health outcomes.

Observation 4.12 A cohort study can be done **prospectively** which is to measure all of their initial functions and then monitor them through time with respect to the control group. Or, it can be done **retrospectively** which is to measure all of their functions after the fact (+ ask them how long was the exposure) then compare it right away to a control group.

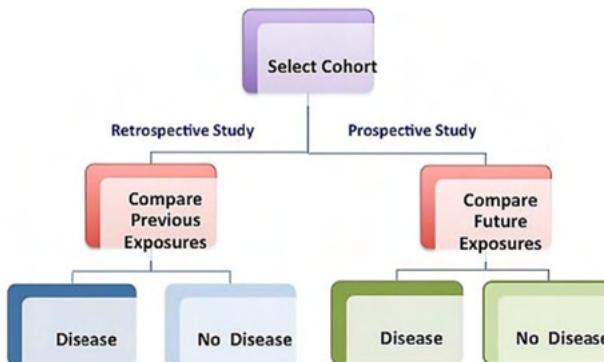


Figure 4.14: Cohort study.

Definition 4.13. A **case-control study** is an observational study that compares two groups of people: those with the disease or condition under

study (cases) and a very similar group of people who do not have the disease or condition (controls).

Remark 4.6. *Case-control study is always retrospective,*

Definition 4.14. A **Cross-sectional study design** is a type of observational study that involves collecting data from a group of people at a single point in time.

Example 4.4.1. This can be done through national census, which are sent through mail.

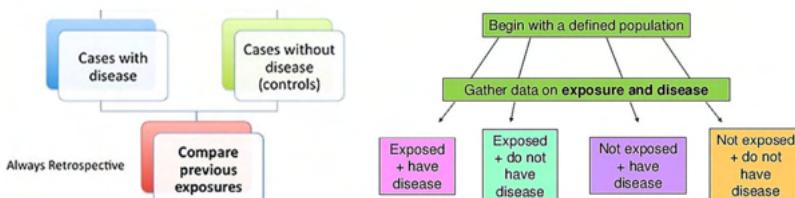


Figure 4.15: Case-control (right) and cross-sectional study design (left).

4.4.1 Statistical Values for Epidemiology

Definition 4.15. An **odds ratio (O/R)** is the ratio of the risk of disease in a case-control study for an exposed vs control group

Example 4.4.2. If the O/R is 2 \Rightarrow the exposed group has twice the risk, easy enough.

Definition 4.16. A **standard mortality ratio (SMR)** is the relative risk of death based on a comparison of an exposed and a control group i.e. it says what's the increased risk of death.

Example 4.4.3. When SMR is 150 it indicates that there's a 50% greater risk.

Definition 4.17. A **relative risk (RR)** is the ratio expressing the occurrence of disease in an exposed vs control population.

Example 4.4.4. When RR is 175, it indicates that there's a 75% increase in risk for said population.

Observation 4.13 Epidemiological studies measure the risk of illness or death in an exposed population compared to that risk in an identical un-exposed control population. This also means that these 2 population we're comparing must ideally have the same age, sex, race, social status, geographical area, environmental and even lifestyle influences, etc.

4.4.2 Biases and Confounding Factors

Suppose that you have a hypothesis that looks pretty good and then you do the analysis of the data from humans. You later find out that your study didn't confirm what the evidence in advance looked like, then several things may have gone wrong and they are **biases**.

Definition 4.18. A **selection bias** is if the two groups are not really comparable. So when select the controls versus the test, the exposed group, they really aren't the same age, background or whatever.

Definition 4.19. An **information bias** is when the information you're collecting is not really accurate.

Definition 4.20. A **recall bias** is when individuals cannot recall a particular events or details they've been exposed to.

Example 4.4.5. If you're asking people, to tell you what they ate or what they were exposed to or how much of something they did that wasn't necessarily a wise choice last year, the year before, or the year before, they may not be able to recall it very accurately or they may not really want to recall it all that accurately.

Definition 4.21. Confounding factors occur when the study and control population differ with respect to factors which might influence the occurrence of the disease i.e. you didn't set it up properly

Example 4.4.6. you forgot to ask one of the groups if they smoked or not or how much they drank.

4.4.3 Risk Assessment

Definition 4.22. Risk assessment is how you basically characterize the risk of something

You need to have the dose-response data and also need to know how much someone's or the population is going to be exposed to it. So risk assessment has to be done for governments and municipalities to decide what's safe for the population, what isn't, and what laws they're going to make.

Definition 4.23. When the risk is identified. Then, laws and regulations are made to mitigate it is called **risk management**



Figure 4.16: Risk assessment and management.

Remark 4.7. *Risk management not only involve on mitigating the risk but also allocating appropriate funding to "undo" certain problems.*

Example 4.4.7. Looking at the clean up of a particular beach from **dioxin**, a persistent organic pollutant, in the soil. We can see that the greater the clean up becomes, the smaller the concentration of dioxin will be. However, the cost will also significantly increase higher. Thus, risk management will need to make appropriate decision on the budget of this project.

So it's not a straightforward set of decisions, but **decisions are also made based on who's likely to be affected.**

Example 4.4.8. **Bisphenol A** is a toxic chemical used to make various food containers. it was banned some time ago here in Canada from baby bottles because of the understanding that babies are more vulnerable than anybody else. So again, the population that is at risk will be considered when governments make these kinds of decisions.

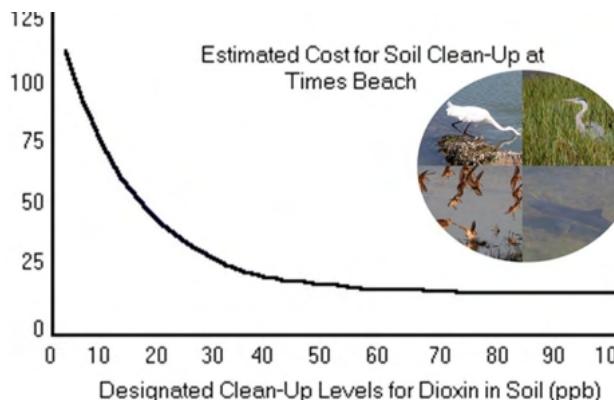


Figure 4.17: Clean up cost of dioxin.

4.5 Lead

Definition 4.24. **Lead poisoning** occurs when lead builds up in the body, often over months or years. Even small amounts of lead can cause serious health problems.

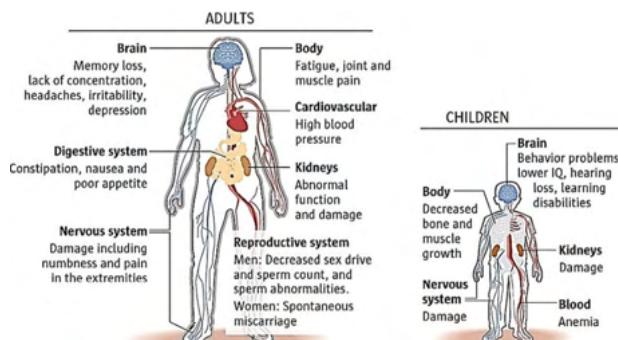


Figure 4.18: Lead poisoning.

Lead is detrimental for both adults and children, but it's particularly damaging for children and it affects all of the organ systems. You can see for the last couple of years, Health Canada has been recalling toys because of the lead level. This is because kids are always putting something in their mouth including toys. So if the [lead] in the toy is high, the lead is going to

be absorbed in the saliva, the kid is going to swallow it \Rightarrow unsafe.

Observation 4.14 We had a decrease in particular in the [lead] in toys for a while, however it shot up in the last few years. The situation here is that hearing, growth, and intellectual development are affected at fairly low concentrations in children. However, They can be exposed to lead from a variety of things e.g. lead in the water if there are lead pipes entering the house, and there can be lead in flaking paint if the paint has not been changed or renewed in many, many years.

Remarkably, they used to be exposed to high levels of lead through car exhaust because it used to be in gasoline. Luckily, once it was realized how dangerous this was, it was banned. And so the lead level in the air and the gasoline came down. Now, thank heaven, it has been banned everywhere.

Observation 4.15 Lead poisoning is not good for anybody initially. It has CNS effects, GI effects and etc . These get worse through time and the particular risk is for children. So even at lower levels, there's impaired intellectual function in children. There is no safe exposure level for lead for children.

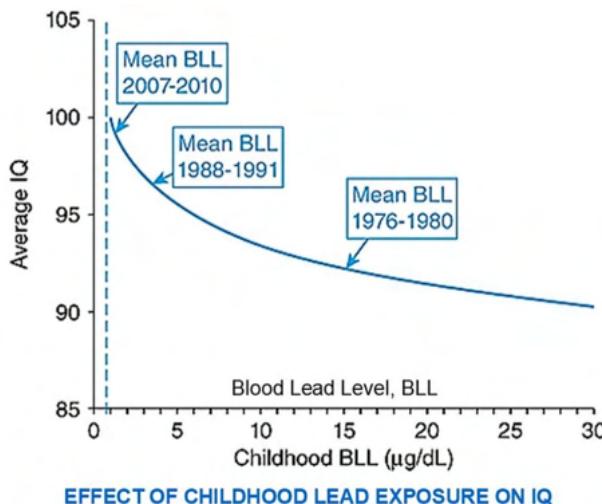


Figure 4.19: Average IQ and lead concentration in blood.

The higher levels of blood lead that were present 30-40 years ago had a

definite effect on the intellectual development of children that were living in areas where they were exposed, like in the middle of the city with a lot of cars. Hence, we can see its effects on children IQ level.

Lead can alter the function of divalent cations, mainly Ca^{2+} . It also affects ion channels and a lot of proteins. All you need to know is that it has a variety of effects, including on glutamate receptors, neurons, and in terms of the kinetics, the lead goes everywhere in the body.

4.5.1 Toxikinetcs of lead

Lead can stay in your blood, and it will come down in the blood when exposure stop, but it will stay in the body³, specifically, in the bone. Then it gets leaked out of the bone at different times in the future.

Observation 4.16

Lead is dangerous not just to humans, but to the other creatures in our environment. Birds, we have so much lead in the environment now and in the water, and it accumulates in the fish. The birds eat the fish, and so on. So we have a big problem with water birds all around the world.

4.6 Mercury

Mercury is another one, and it is again something that has prominent CNS effects. Mercury originates from factories, from natural sources like volcanoes. It can get in the soil which can leak out to the water, and then it can enter to the air/atmosphere i.e. It's going all over the

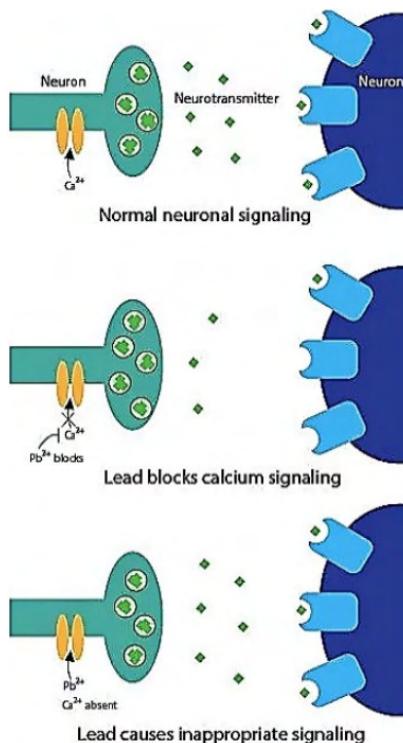


Figure 4.20: Lead alter signalling

³Basically, the cumulative lead exposure level stay constant at the end of exposure and does not decrease

place. Sometimes there's an organic form of **methyl mercury**, and it can bioaccumulate in many organism and then biomagnifies to us and other animals at the top.

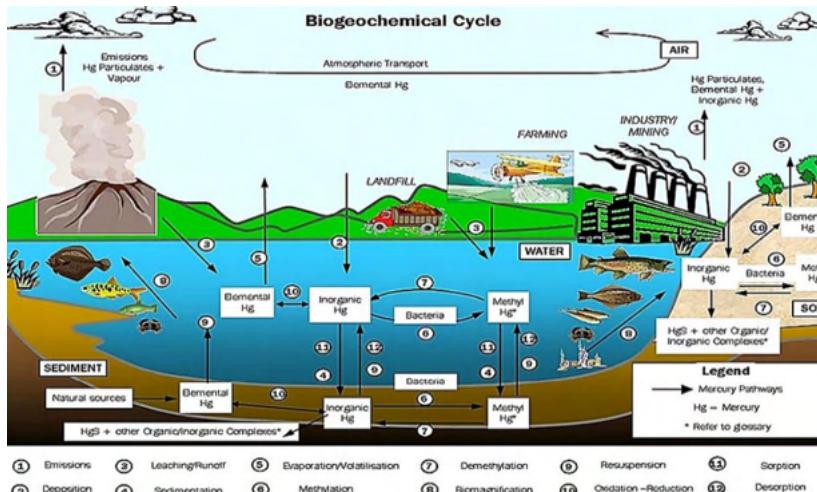


Figure 4.21: Biogeochemical cycle of mercury.

Observation 4.17 The short-term effect is CNS, memory loss and peripheral difficulties with the nervous system and all of these general functions due to deterioration of the nervous system.

Observation 4.18 (Grasshopper Effect). When mercury level rises from factory and etc. It will contaminate the soil then water then enter the air. It can land on the ground in the winter. In the spring, it rises from the ground and the air currents move it. Basically mercury is able to "hop" around further north for each season.

Remark 4.8. Many other organic pollutants also experience grasshopper effect which in the end they all concentrated further North.

4.7 Pesticides

And last but briefly, we will look at pesticides.

Definition 4.25. **Pesticides** are chemicals used to deal with weeds, diseases, and insects, mostly insects in our crops. **Herbicides** are chemicals specifically used to get rid of weeds

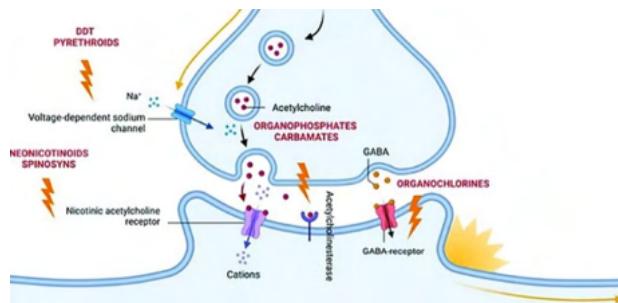


Figure 4.22: Insecticides action on nerves.

Observation 4.19 They're not that toxic to mammals and birds, but the insecticides are, as they target the nervous system of insects can sometimes affect humans too. Looking at a cholinergic synapse, pesticides can act either presynaptically or postsynaptically. We'll briefly look at several classes of pesticides. Insecticides, for instance, have saved millions of lives by preventing diseases spread by insects, especially in warmer climates where mosquitoes, ticks, lice, and fleas thrive.

4.7.1 Insecticides

Definition 4.26. **Insecticide** is a form of pesticide where it's specifically target insects.

Observation 4.20 Insecticides are used worldwide. In safer environments, they're applied with protective measures, but in poorer regions, they're often used without such precautions, affecting various creatures. Insecticides can run off fields into water, rising into the air, and impacting ecosystems.

4.7.2 Organochlorines Insecticides

Toxicity varies among different insecticides, with **organochlorines** being a classic example. These are chlorinated compounds, with **DDT** is a well-

known one. While DDT has saved millions of lives, it has severe environmental consequences.

Observation 4.21 It persists in the environment, accumulates in the body fat of exposed creatures, and has a long environmental half-life. This accumulation disrupts the food chain, particularly in birds, leading to fragile eggs and a decline in species like bald eagles and herring gulls.

Concept 4.5 (Ideal Pesticide Criteria). For a pesticide to be considered ideal, it needs to be:

1. Very low in toxicity.
2. Little to 0 persistence in the environment.
3. Little risk to non-target organisms.
4. Little development of resistance

Observation 4.22 The mechanism involves these insecticides acting on Na^+ channels, prolonging their opening⁴. This leads to the insect begin to convulse and die.

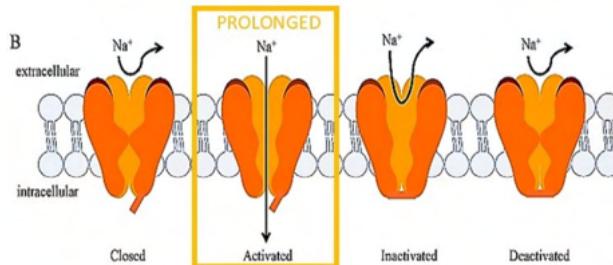


Figure 4.23: Na^+ channel prolongation.

4.7.3 Organophosphate and Pyrethroids

Observation 4.23 **Pyrethroids**, an insecticides, that also act on these Na^+ channels, causing insects to become hyperexcitable and die. Another group

⁴Insects are more vulnerable to this than mammals.

of insecticides, namely **organophosphates**, affects acetylcholinesterase, by binding to it irreversibly and leading to paralysis and respiratory failure. Some organophosphates are used as chemical warfare agents, but safer variants are employed in agriculture, where they can be metabolized quickly by mammals.

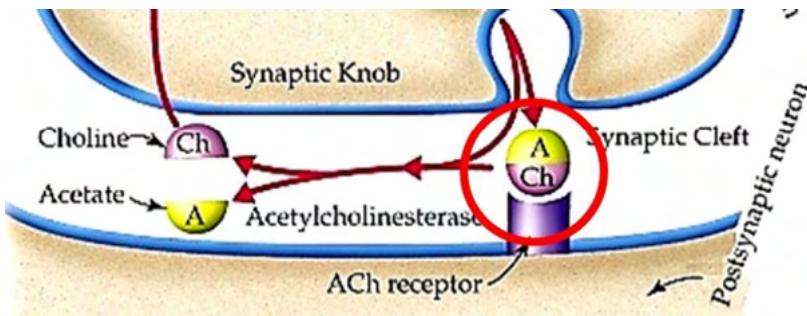


Figure 4.24: Effect of organophosphate insecticides.

Example 4.7.1. **Malathion** is an insecticide that is the least toxic to mammals since they have enzymes to metabolize it rapidly.

The use of pesticides is necessary to feed the global population, but their use should be minimized, focusing on safer alternatives. Research in toxicology remains crucial to improve their safety and effectiveness.

5

Special Topics

Chapter

Lecture 18: November 12th, 2024.

For the first 2 sections, we will cover the basic research, designing of new drugs and how they're tested.

5.1 Drug Design I: Basic Research.

First, we need to base our decisions about making drugs and how we stay healthy on the **evidence**. This evidence has allowed us to develop drugs and which have increased quality of life and longevity remarkably. When we consider the evidence for drugs, we have to think about a few key things:

1. Does it work? 2. Side effects? And 3. Comparison to available treatment?

Remark 5.1. *If we go through all of these key steps and the positive outweigh the negative then it will end up on market.*

5.1.1 Research Consideration: Now vs Future

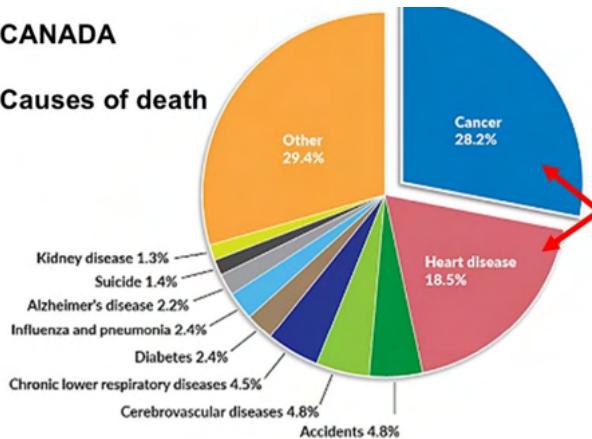


Figure 5.1: Leading cause of death in Canada.

When considering the big picture, it's crucial to think about **where we**

are now and where we're going because many changes are taking place and will continue to occur in the near future.

Observation 5.1 The major causes of death in Canada are heart disease and cancer, with cancer now being number one. Therefore, these areas are naturally a focus of much drug research.

Over the past century, our lifespans have doubled due to advancements like antibiotics, clean water, sanitation, and immunization.

Example 5.1.1. For instance, the low number of deaths in Canada compared to other countries during the COVID-19 pandemic can be attributed to immunization efforts, curfews, and protective measures. In contrast, in the U.S., excess mortality due to low immunization rates highlights the impact of misinformation and inadequate public health measures. Vaccination, masks, and travel limitations played a significant role in reducing our death rate during the pandemic.



Figure 5.2: Changes in lifestyle in North America (left) and excess death during COVID-19 (right).

The Future

Looking ahead, the future of pharmacology includes **personalized therapy**, where treatments are tailored to an individual's genetic makeup to maximize effectiveness and minimize side effects. We're also seeing new types of drugs, such as those involving **cells, cytokines, and antibodies**. In cancer treatment, advances in understanding different tumor types enable tailored therapies. Neurological diseases, like Parkinson's and Alzheimer's, are also receiving significant attention because they're caused by specific abnormalities, not aging itself. Better understanding these diseases will lead to improved prevention and treatment options.

Definition 5.1. **Drug discovery** is a big field of medicine and pharmacology emphasize the process whereby a new candidate medications are found.

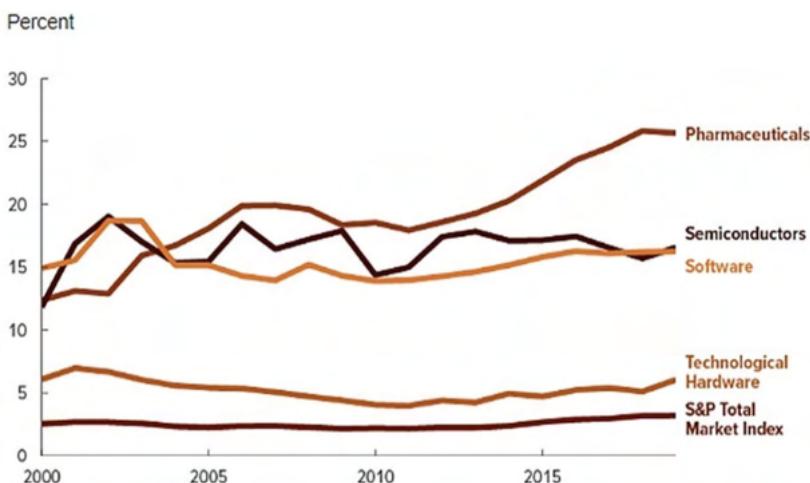


Figure 5.3: Pharmaceutical industry spending on drug discovery.

Observation 5.2 In order have a new drug discovery, we need to do lots of research. In fact, pharmaceutical industries invests heavily in research, even more than any other type of company, because understanding the fundamental problems of diseases is essential to designing and testing effective drugs. This process is getting more expensive because previous easier target has been identified so now we're tackling much more complicated problems.

Not only that, the type of drugs developed are also changing where we see an increase in **biologic drugs**, which are from living organisms and cells. We can also see different disease categories with new drug approved from 2023: with the highest to be cancer and neurological-related treatment, follow by infectious diseases.

5.1.2 Research Consideration: Challenges with AD

Observation 5.3 Drug development involves starting with thousands of potential compounds, narrowing these down through preclinical testing, and

eventually moving to clinical trials. For every 10,000 compounds, only one might make it to market after rigorous testing and billions of dollars spent. The costliest part of this process is clinical testing in humans, which must be done carefully to ensure safety and effectiveness.

Nevertheless, there's been many successes, in particular, in survival of childhood cancer. However, for some of the neurological diseases, there's still some difficulties.

Example 5.1.2. The challenges are significant because we still don't understand completely what goes wrong in **Alzheimer's disease (AD)** i.e. what's the pathogenesis? We know that the brain shrinks, there are abnormal deposits in there, but the whole process is still not understood. And because there's such an interest in this, many drugs have been studied and developed to try and treat Alzheimer's disease and they don't work.

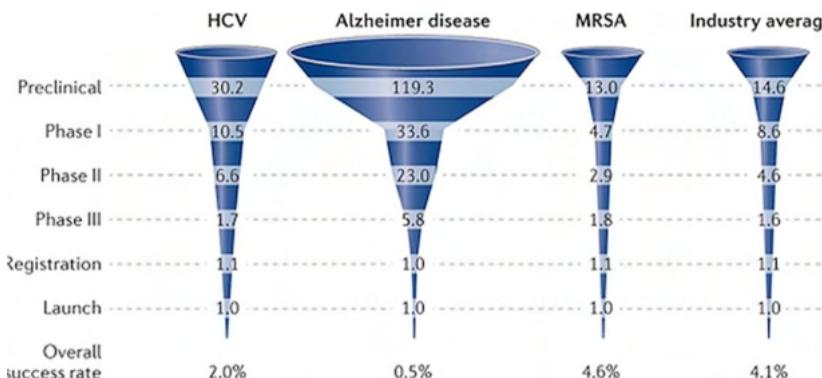


Figure 5.4: Drugs discovery for AD.

For 20 years, they tested about 150 new drugs for AD and not one of them was useful. In fact, for every 120 drugs that actually go into the pre-clinical stage and then into clinical testing for AD, you're lucky if you get one and a half of it approved. Whereas for the industry average, the success rate is not great, but a lot better than the success rate for AD. And here you see a company that spent a huge amount of money and they got all the way through to the last stage of clinical tests in humans but then it turned out it didn't work.

Concept 5.1 *AD is due to amyloid plaques formation and build up.*

Observation 5.4 Because of the above concept, a company developed monoclonal antibodies that they felt would stop this plaque formation called **Aducanumab**. It got through the clinical testing, but the benefit was so small compared to nothing at all that most countries wouldn't approve it because it costs a fortune.

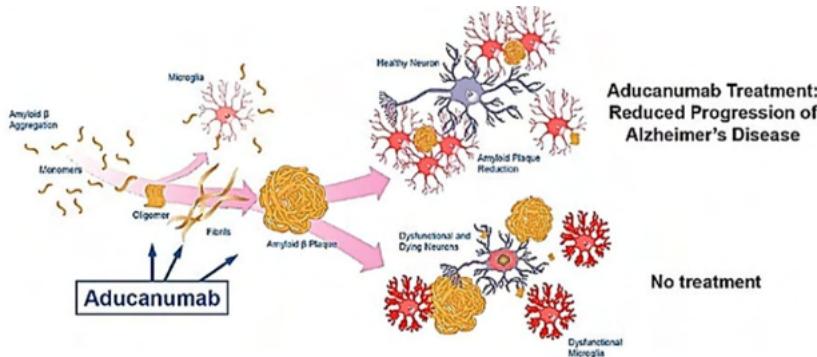


Figure 5.5: Proposed mechanism of aducanumab.

Remark 5.2. *Canada didn't approve it. It's very expensive and the benefit was minimal*

Nonetheless, the positive thing is the number of drugs that are now being tested for Alzheimer's. We can see different categories of drug and some of it is just basically hoping that one or more of these will at least be better than what we have now and will point us in the right direction to eventually solve this problem. There are other concerns besides some of the things like Alzheimer's that we don't really understand the pathogenesis for.

Observation 5.5 There's also concerns for antibiotics research as there's a gradual decline in it. This is because the antibiotics will cure the patient directly in a short period of time (2-3 weeks). So it's not cost effective to spend billions to develop a new antibiotic because you're going to lose money on it. Nevertheless, companies have been encouraged to think about the fact that there's drug resistance for many of our microorganisms now. So, as of currently, we can see a little increase in research of it again.

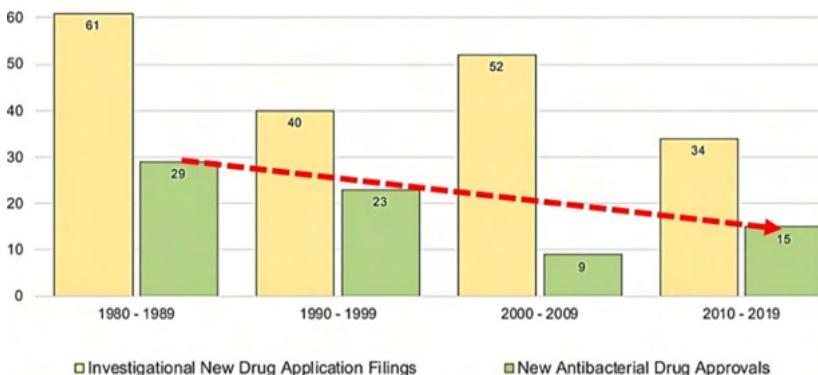


Figure 5.6: Decline in antibiotics research.

5.1.3 Drug Discovery

Observation 5.6 (Preclinical stages). The process of drug discovery begins with an idea, often inspired by basic research or observations in pathology labs. Then, they enter preclinical research, where scientists determine the drug's target and structure, test its effects in the lab, and evaluate its toxicity and pharmacology in animal models.



Figure 5.7: Government and industry on drug discovery. Basic research is more predominant by government and institutions, translational research are shared between them while clinical research are mostly by pharmaceutical industry.

The main challenges in healthcare, such as treating CNS diseases like

Parkinson's and Alzheimer's, arthritis, cancer and cardiovascular diseases (the big 2 problems), drive much of this research. Global concerns like emerging infections and the effects of pollution are also areas where drug development is crucial. Basic research is primarily done by governments and universities, while pharmaceutical industries focus mostly on translational and clinical research.

Drug Target

Definition 5.2. **Pathogenesis** is the process by which a disease and develop.

Example 5.1.3. The pathogenesis of coronary artery disease is due to the build up of plaque which eventually lead to the blockage of said artery and can lead to a heart attack.

Remark 5.3. *Evidently, to develop a drug, you need to identify target.*

Example 5.1.4. For diseases caused by microbes, the target might be the microbe's enzyme's active site that is vulnerable. For human diseases however, there are many potential targets, such as receptors on cell surfaces or organelle functions inside cells. Common drug targets include enzymes and receptors, but as research progresses, new drug targets emerge.

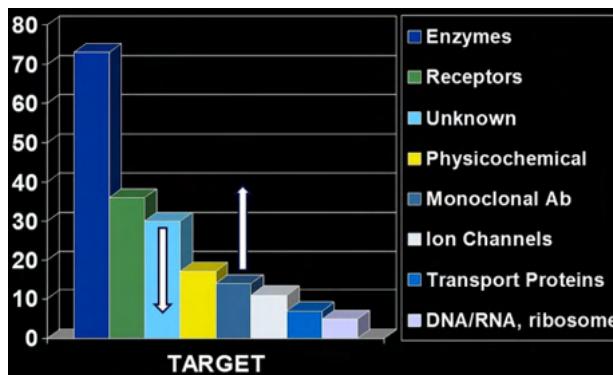


Figure 5.8: Drugs targets for human diseases.

Observation 5.7 Human biology is quite complex, with interconnected pathways and feedback mechanisms, therefore identifying specific targets remains challenging. As of currently, there are currently around 500 known

protein targets for existing drugs, but future research, particularly into genetic abnormalities and pharmacogenomics, will provide better-targeted therapies with fewer side effects.

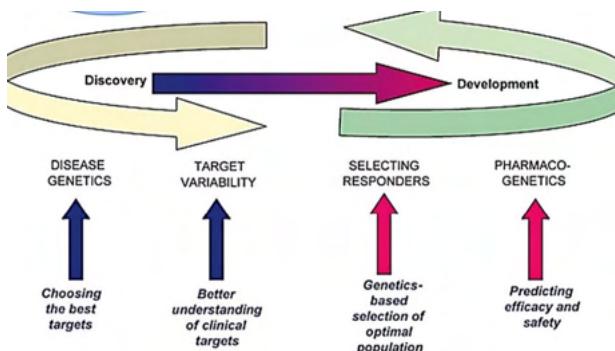


Figure 5.9: Genetics role on drug discovery.

5.1.4 Genomics

Definition 5.3. **Genomics** is the study of an organism's genome (its genetic material) and how that information is applied. **Functional genomics** is the study of how gene (and protein) will function and interact.

Observation 5.8 Genomics plays a crucial role in advancing drug development. Understanding the genetic variations in diseases such as cancer allows for more precise targeting. Functional genomics can also help validate drug targets or even improve drug development.

There's been research in gene therapy for cystic fibrosis and some attempts have done to deliver the missing or even replace the defective gene. However, gene therapy (particularly CRISPR-based treatment) has already seen breakthroughs from last year (2023), such as in **sickle cell disease** and **thalassemia**, where stem cells are treated outside the body to correct genetic defects.

However, drug development isn't straightforward, as most diseases involve multiple genetic factors i.e. due to genetics polymorphism, we have to reconsider the pharmacodynamics and kinetics as well. So far, we know that genomics will help us with understading the disease mechanism and

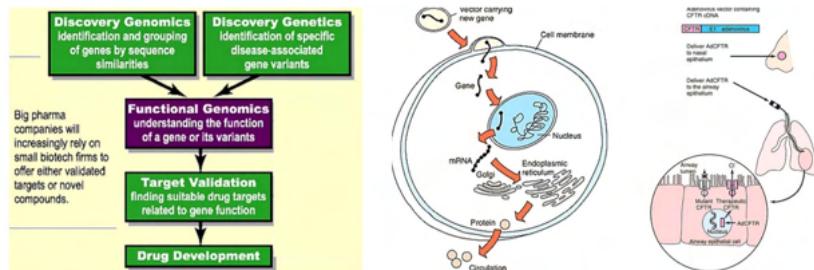


Figure 5.10: Genomics and gene therapy.

heterogeneity, individual variable and target therapy for that variation.

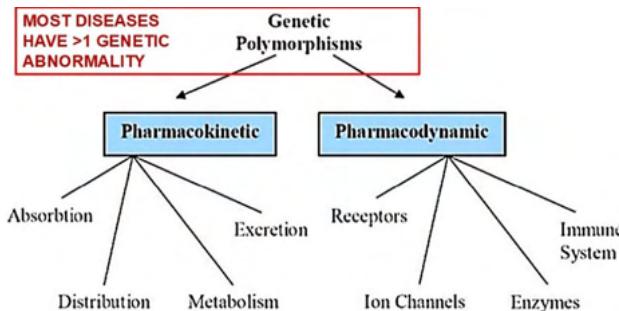


Figure 5.11: Genetics polymorphism.

As genomic technology improves, personalized medicine that accounts for genetic and environmental variations will become more feasible. This will allow for more targeted and effective therapies in the future.

Definition 5.4. **Bioinformatics** is a scientific field that involves using computational method to collect, store and analyze biological data e.g. DNA and amino acid sequence.

Observation 5.9 Bioinformatics enables extensive computation. A key application is using DNA microarrays to target specific abnormalities in diseases. By analyzing the genetic makeup, we can better understand and diagnose conditions, such as distinguishing between tumor cells and normal cells. This improves prognosis and allows for more precise targeting of molecular abnormalities, leading to personalized treatments.

So now, we've effectively determine what the target is. Now, we need to determine what can bind to it. This can be done by modelling the target site and keep on trying different molecule binding to it.

Example 5.1.5. We model the enzyme's structure and the drug that would effectively bind to it, particularly in the enzyme's active site. From this initial model, we refine the drug's structure to improve its efficacy and targeting capabilities. This process of design and iteration is a cornerstone of modern drug development.

5.1.5 Combinatorial chemistry

Definition 5.5. **Combinatorial chemistry** is a synthesis strategy that enables simultaneous production of large numbers of related compounds.

Observation 5.10 The basic idea is that you have a target, a therapeutic target, and you get some compound, which is the lead compound that binds to your target. You can then optimize this compound by creating thousands of structural derivatives that will also act on the target, but may be more effective and have fewer side effects elsewhere. **Combinatorial chemistry** facilitates this by enabling the creation of a wide range of derivatives.



Figure 5.12: Combinatorial chemistry.

Classically, if you were to make a compound, you would combine different segments of the chemical structure to create the final molecule. With

combinatorial chemistry, you take many variations of different sections of the molecule—such as segment A, segment B, and segment C—and combine them, generating thousands of variations, which makes up the **combinatorial library**. This is especially important for drug development and improvement.

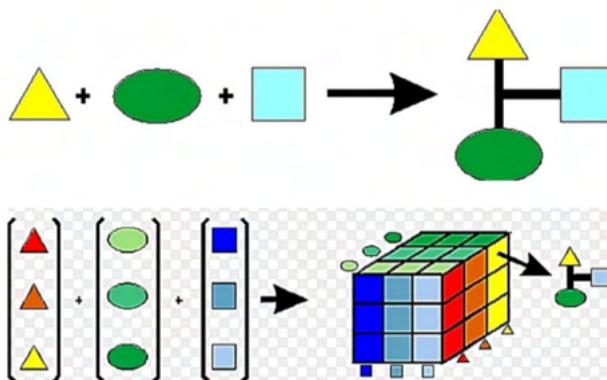


Figure 5.13: Classical (top) vs combinatorial synthesis (bottom).

Example 5.1.6. If a drug on the market already has some side effects, you can use combinatorial chemistry to make thousands of variations of that drug and test them to find a better option.

5.1.6 Robotics: High Throughput Screening

Definition 5.6. A **High Throughput screening (HTS)** is the use of automated equipment to rapidly test thousands of sample for biological activity.

Example 5.1.7. After thousands of compounds is made by combinatorial chemistry, they can be screened in a short period of time using robotics HST to test for each variation effectiveness.

Observation 5.11 Looking at the manual methods used in the past for antibiotic development, testing was done by hand in a laboratory using dishes to isolate and test each compound.

With HTS, it's possible to produce and screen up to 20,000 compounds a day, though the equipment is expensive and requires maintenance. The

system can identify both agonists and antagonists once a target has been identified. The test systems used for this screening can vary, including: mammalian cells, microbes, human hepatocytes, and synthetic membranes.

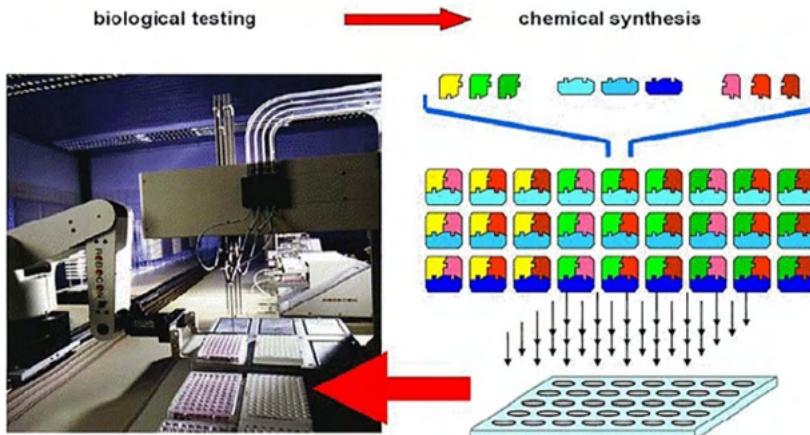


Figure 5.14: High throughput screening.

Now, when it comes to HTS, we can divide it into 2 types: **cell-free** or **cell-based**. In cell-free HTS, only a target enzyme is used to test. On the other hand, in cell-based HTS, actual cells are tested in small chambers. The quantification of these results is typically done using luminescence, radioactivity, or fluorescence, etc.

Observation 5.12 HTS involves four key components:

- A compound library with thousands of compounds
- An automatic assay system to detect the desired reactions
- Robotics for inserting and handling the compounds
- A sophisticated computerized system for analyzing the resulting data.

The system works with plates containing wells where compounds are added and tested with different systems, like antibody-based assays or reporter gene assays.

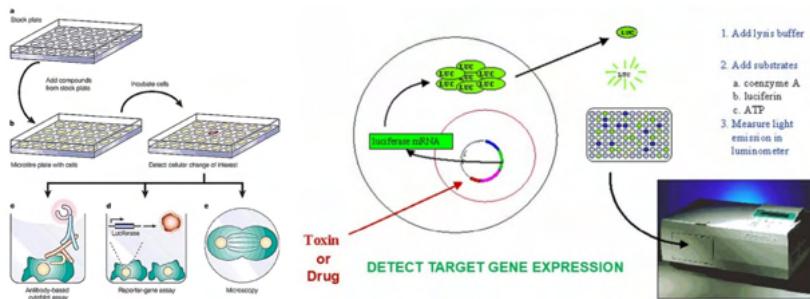


Figure 5.15: Reporter gene assay using Luciferase.

Example 5.1.8. Luciferase is an enzyme that will glow once it detect the target it was designed to bind to. From here, you can then detect the gene expression.

Example 5.1.9. A target protein can be combined with a mixture of compounds, and after the ones that don't bind are washed away, the remaining bound compounds can be identified using mass spectrometry.

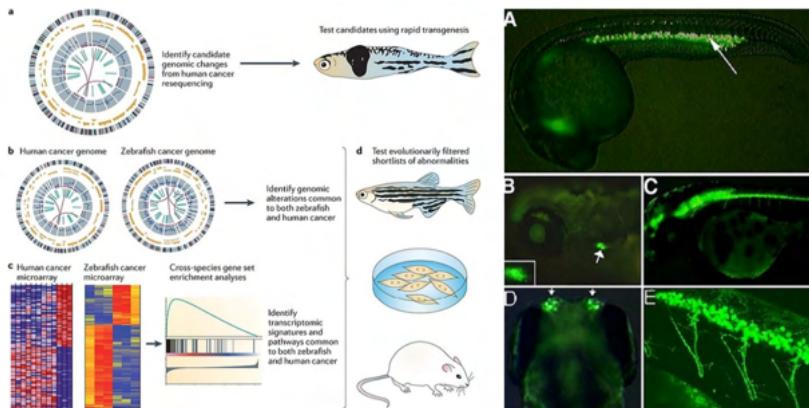


Figure 5.16: Zebrafish used in research.

Observation 5.13 Additionally, living creatures, such as **zebrafish**, are used in testing and particularly useful for studying drug effects, including how drugs affect the vasculature. By labeling the zebrafish's vasculature, researchers can observe how potential drug candidates might impact it. These

studies can be used to investigate potential cancer treatments, genetic alterations, and other conditions. The output from zebrafish studies can then inform further research in other creatures.

Remark 5.4. *HST is sophisticated but cannot evaluate bioavailability, pharmacokinetics, toxicity, or specificity¹.*

Mutagen Monitor

Some clever systems have been developed to assess **mutagenicity**², such as using yeast cells with a DNA repair gene, or through testing for toxicants in the air.

While HTS mainly focuses on drug development, identifying new toxicants or other harmful agents is also an area of concern. For example, more advanced tests are being developed to detect toxicants, both for drug safety and environmental testing.

5.1.7 Optimization and Evaluation

The drug discovery process is not only about creating new compounds but also improve the already existed ones.

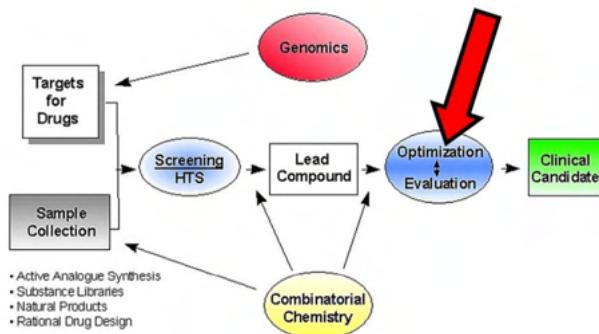


Figure 5.17: Drug discovery process.

After the final design is determined or (re-)optimized, the compound proceeds to clinical trials, though this stage can be challenging.

¹These factors must be tested in living systems.

²Mutagen is an agent that can permanently change genetic material e.g. DNA

In clinical trials, many drugs fail to make it through because humans are genetically diverse, and a drug that works in the lab doesn't always work in humans. This is why only a small fraction of drugs make it to market.

Software for Drugs Discovery

To help with drug development, computer software is used to handle the vast amounts of biological data involved. Bioinformatics plays a crucial role in managing and interpreting this data.

Definition 5.7. **Computational chemistry** is a branch of chemistry that uses computer simulation to solve chemistry-related problems.

Observation 5.14 Computational chemistry helps by modeling how drugs will bind to receptors, predicting how a drug will interact with an enzyme, and designing drugs that are more efficacious.

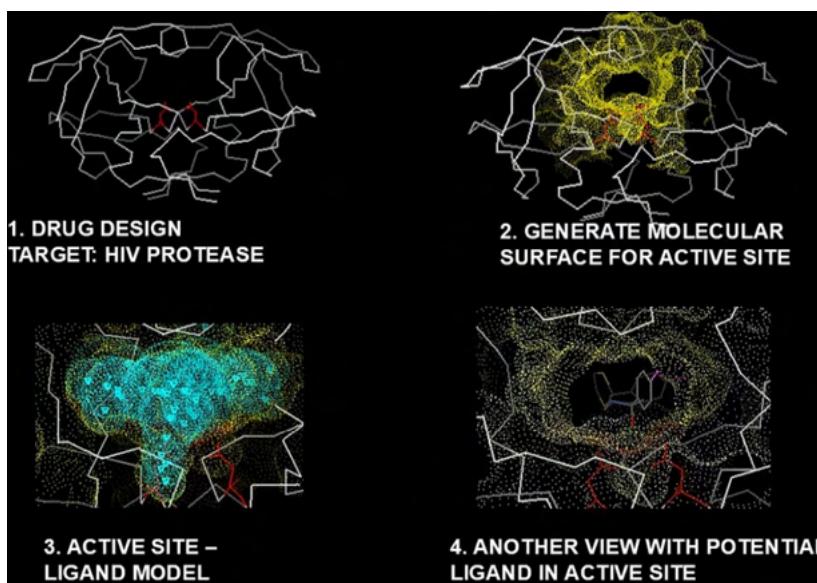


Figure 5.18: Softward aided with designing HIV protease inhibitors.

With the help of computer-aided drug design, drugs, such as **HIV protease inhibitors**, have been developed more efficiently, with the software

predicting how the drug will bind to the target enzyme. This software allows researchers to model the enzyme's active site, hydrophobic interactions, and other factors to design the most effective drug candidates.

Example 5.1.10. A very successful new drug used to treat **chronic lymphocytic leukemia** by inhibiting enzymed overexpressed in B-cell malignancies.

Here we have a new drug for heart disease, which is based on a straightforward understanding of how the body handles LDL cholesterol. This drug works by targeting a specific enzyme, called **PCSK9**. When this PCSK9 is blocked, it protects the LDL receptor, allowing the liver to remove more LDL cholesterol from the circulation. As a result, much less of it is available to form plaque inside the arteries, which can cause heart disease.

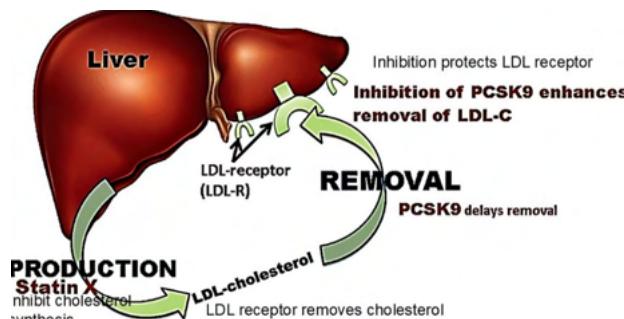


Figure 5.19: PCSK9 inhibitor vs statin action on cholesterol treating heart disease.

Before this, another category of drugs called **statin** was used to inhibit cholesterol synthesis, and these drugs have proven to be very beneficial for people at risk of heart attacks.

Supercompression of Drug Discovery

Putting all of what we've discussed (genomics, combinatorial chemistry, etc.) together will ultimately decrease the pre-clinical stage of drug development. Another emerging field is using **biosimulation** which are used to predict both negative and positive effect of a drug.

5.1.8 Monoclonal Antibodies

We have a lot of technology now that's developed to create **monoclonal antibodies (mAb)**, and there are different varieties of them:

- **Chimeric antibodies:** Antibodies with human constant regions.
- **Primatized antibodies:** Antibodies with primate-derived antibodies
- **Humanized antibodies:** Partially humanized antibodies
- **Transgenic mouse antibodies:** Fully humanized antibodies

We have had a lot of success with the ones that have been put out on the market such as treating **autoimmune disorder** (e.g. Crohn's disease and rheumatoid arthritis), **cancer** (e.g. B-cell malignancies and breast cancer), and **osteoporosis**.

Example 5.1.11. Osteoporosis occurs when osteoclast breaks down bone faster than osteoblast rebuild it. A mAb used to treat osteoporosis is called **denosumab** which can bind to RANKL and prevent it to bind to osteoclast and activate it i.e. it inhibit osteoclast activity.

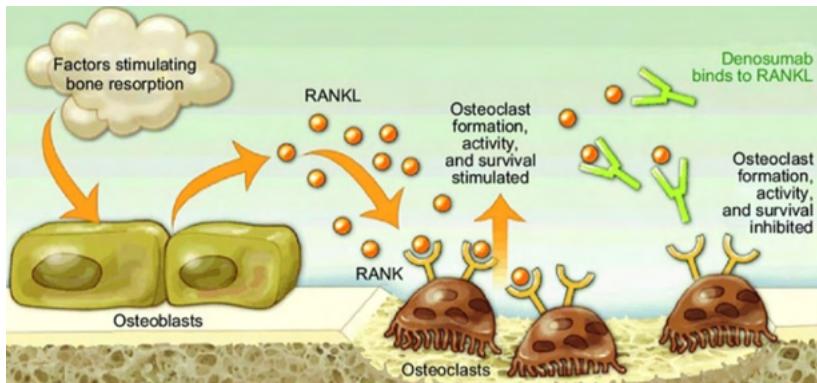


Figure 5.20: Mechanism of action of denosumab.

Here we have colorectal cancer and all the different potential targets for monoclonal antibodies in the target tissue. These are now being used clinically. We also have a drug that has been very beneficial in inhibiting one of the pathogenic molecules involved in autoimmune diseases, including different types of arthritis.

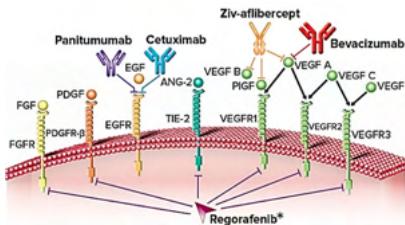


Figure 5.21: Colorectal cancer target.

5.1.9 Preclinical Evaluation

Observation 5.15 After the drug is found in the lab, we have to evaluate it in some type of living creature. **Preclinical evaluation is mostly done using lab rats.** This is because, we know a great deal about the biology of our laboratory rat, and also have transgenic and knockout mice. Rodents are a big benefit in terms of our preclinical testing. We can work out the detailed mechanism of action, look at the pharmacokinetics, and see how the rats respond to a particular drug. This gives us an idea of what humans will experience.

Different animals can be used for different types of studies. Some other animal species, like primates, are used at the very end of certain studies.

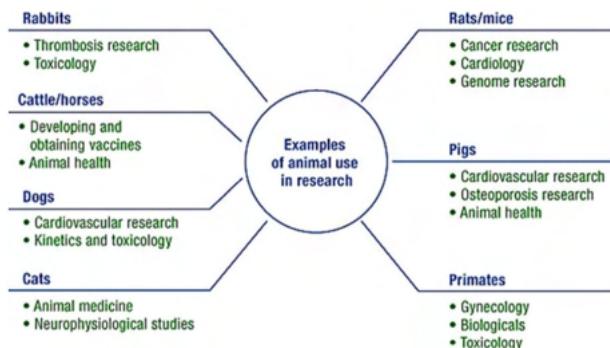


Figure 5.22: Animals used in research.

Remark 5.5. *Regardless of how good a drug is, if it causes toxicity in these test, we won't use it.*

Therefore, you need to do both acute and chronic toxicity tests. The AMES test can help to look for mutagen but we also have to look for carcinogens and teratogens.

Complete Picture of Basic Research and Optimization

In the big picture, drug discovery involves a lot of basic work in the lab. You need to find a target. Finding the target is based on understanding the pathogenesis of the disorder. Then, you find a **lead**, which is a molecule that interacts specifically with the target. You optimize that lead. You can go through thousands of compounds in the same family and test them all to see which would be the best to selectively target that particular thing you're interested in. Then, you need to work out the ADMET (absorption, distribution, metabolism, excretion, toxicity) to understand the pharmacokinetics, and test the drug's safety in some type of lab animal.

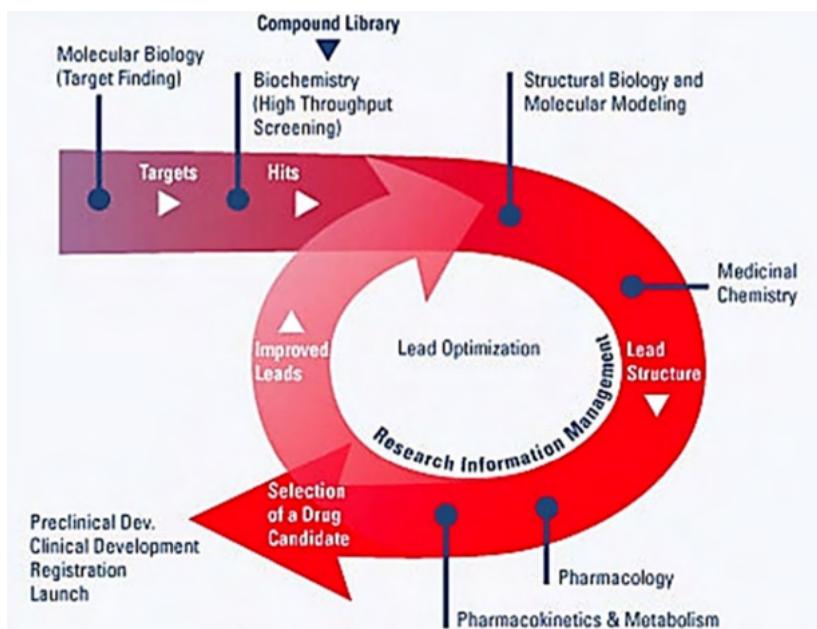


Figure 5.23: Cycle of lead optimization.

Sometimes, you need to go around the lead optimization cycle several times because you discover something that is both safe and effective, and

it will likely be more effective than anything available out there now, you apply to the government to allow it to be tested in people.

5.1.10 Newer Area of Development

There are newer areas of development in testing and even an expansion of what you may think of as a drug.

Example 5.1.12. Here we have the development of golden rice, a newer concept. We are changing the plant to include a vitamin, and this is protecting millions of children from vitamin deficiency in other parts of the world. This is a new take on drug development. We're actually changing a plant.

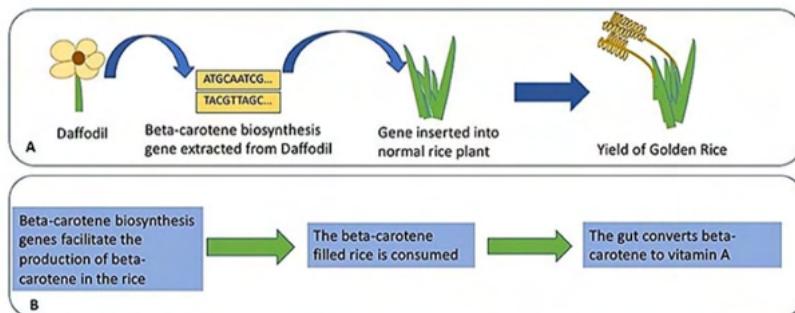


Figure 5.24: Golden rice.

5.2 Drug Design 2: Clinical Trials

In this lecture, we will be focusing on the clinical trial aspect when it comes to designing and introducing a new drug to market.

Observation 5.16 When you think about the kind of research that's done and also the kinds of clinical trials that are taking place, obviously cancer is the biggest problem. It's the area that we're putting the most effort into in terms of research and clinical studies and developing new drugs. Interestingly, heart disease is second in terms of the illness and death, but it's not second in terms of the new drug studies.

When you're looking at the situation, it is different in Canada and the US. For once, the death rate from cardiovascular disease has come way down in Canada and not down as much in the U.S. So these are just some background comments related to some of the things I said earlier.

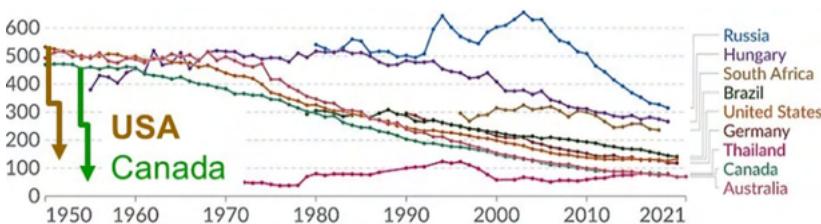


Figure 5.25: Cardiovascular disease between Canada vs US.

One of the things that you will all be aware of was the incredible value of the vaccine against COVID and the millions of lives that it saved just here in North America and many more around the world. We now have a variety of vaccines against COVID, and this is the latest one that's available now. This is all being accomplished through an "accelerated" form of clinical trials.

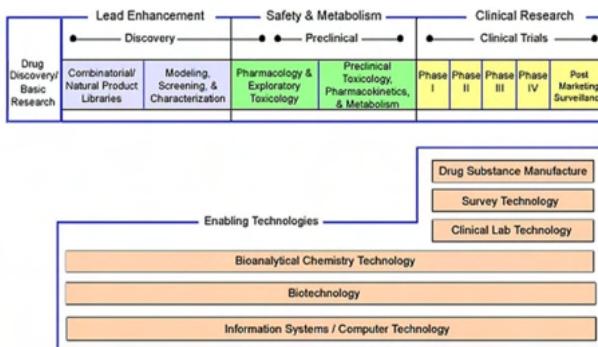


Figure 5.26: Drug development process.

So the drug development process, as we talked about last day, starts off in basic research labs around the world. Then with the company working out a lead drug and working out its safety and metabolism and so on. And then doing the clinical trials, which is what we're going to be looking at today.

And during the clinical trials, a lot of work has to be done behind the scenes by the chemists and technologists. They have to figure out how to

manufacture the test drug in some type of usable form like a pill. And so they have to do all of that background work and work out the biotechnology and all of that sort of thing. Remarkably, clinical trials is probably the most expensive part of drug development and it can cost up to 2.6 billions \$. This is because they have to spend around 40,000\$ per person/patient in the trial.

5.2.1 General Consideration

The main point of clinical trial is to ask some of the following questions:

- Is it safe?
- Does it work?
- How does it compared to existing drugs?

Clinical trials are typically under the control of Health Canada in Canada and under the FDA in the US. The trials are typically randomised but more importantly *double blind*.

Definition 5.8. A **double blind** study refers to clinical trial where neither the participants nor the researchers know which treatment or intervention the participants are receiving until the trial is over. Typically, the treatment is the actual drug vs placebo, or even the drug and an existing treatment.

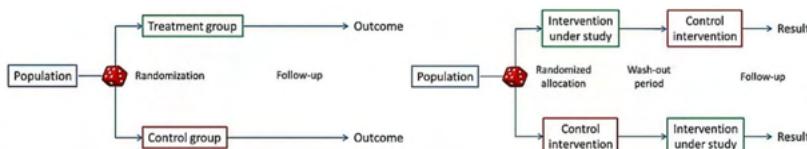


Figure 5.27: 2 strategies of clinical trials study: 1 simple randomization of normal treatment vs placebo/control. The other is the treatment vs control but later is swapped i.e. treatment becomes the control and v.v. In all cases, it will be double-blind to not introduce bias by patients and physician.

Example 5.2.1. For many cancer treatment and drug development, many units and hospitals can cooperate with each other in a large study. Supposedly, a new drug and they can test it in many types of cancer to see which is most effective.

Note that even when clinical trials is over, the pharmaceutical company will continue to regulate and monitor the drug post-marketing. This is called "phase IV" but not part of the clinical trials (3 phases).

5.2.2 Clinical Trials: Phase I

Observation 5.17 In Phase I of clinical trials, you're looking for the safety (doses, etc.) and pharmacokinetics of said drug. This typically consists of 20-80 participants, of which would need to be in their best health. This phase will take several months and have a 70% success rate.

5.2.3 Clinical Trials: Phase II

Observation 5.18 In phase II of clinical trials, you're trying to study the reliability and the side effects of the drugs. This phase include 100-300 participants and have around 33% success rate.

Definition 5.9. A **Placebo** is a treatment or substance that is intended to have no therapeutic value, but is given in the same way as an active treatment or drug.

Example 5.2.2. If you're testing a drug to relieve pain, the participant would have to rate their pain accordingly. Furthermore, this rating will be compared to another set of participants that takes, maybe a placebo, or a pre-existing drug. In order for the drug to proceed through phase II, it needs to score higher (more effective) than the placebo or the pre-existing drug.

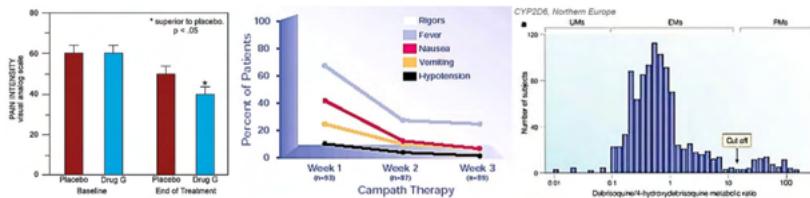


Figure 5.28: Comparison to placebo, side effects, and genetic polymorphisms

Observation 5.19 When it comes the side-effects, most of the time, they tend to go away in the first 2 weeks as the body begin to compensate. Another thing you'd get from this phase is understanding of genetic polymorphism and how it would effect to metabolize the drug in question.

5.2.4 Clinical Trials: Phase III

Observation 5.20. In phase III of clinical trials, we're looking for the efficacy of the therapy and monitor more of its adverse reaction (most of the time, it's the more serious ones). This includes 1000 – 3000 participants and new drugs have 25 – 30% success rate.

Remark 5.6. *Testing the drug efficacy isn't the only test in this trials but also maybe a change in lifestyle e.g. for Type 2 diabetes patient*

Remark 5.7. *It must be noted that this trials (result) also heavily rely on patient adherent to taking the drugs when sent home.*

Example 5.2.3. The rate of having atherosclerosis is much lower if the person actually stick to the drug plan as compared to them not taking it correctly to schedule.

Notion 5.1 Interpreting data is highly important in this phase.

Explanations. When the outcomes of the drug is finalized, it must be interpreted carefully to see if the drug should be marketed or not. e.g. if the drug's mortality rate is the same with the placebo, it's useless. □

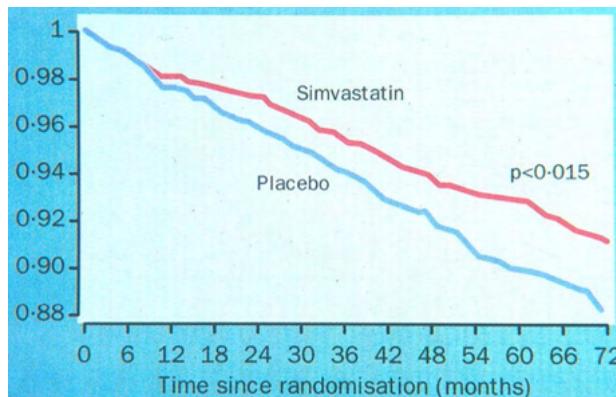


Figure 5.29: Simvastatin vs placebo.

Example 5.2.4. The treatment of **simvastatin** vs the placebo might looks similar in term of portion of people not having heart failure within the span of 1 year. However, when compared to a much longer effect over 2-3 years,

we can see that Simvastatin does pose a better therapeutic effect and increase the amount of people not experiencing heart failure (as compared to placebo).

Notion 5.2 Side effects can bias the reaction of patient

Explanations. This is because side effects in the initial stage might have some changes in the therapeutic effect but afterward when the body compromise, it will truly show if the drug is therapeutic for its intended use.
□

Example 5.2.5. Taking 2 drugs (Lorazepam vs Amitriptyline) that are potentially used to treatment pain and both of them has sedative side effect within the first 2 weeks. Initially, when given the drug, both patient group experience less pain. Now, as the trial continue, we will past the threshold of the side effect, Amitriptyline will have the continue decrease in pain experience by patients. However, for the Lorazepam group, patient will start experiencing pain again. Thus Lorazepam not being an analgesia, and the reason that it lower pain initially is because sedation can make patients response less to pain.

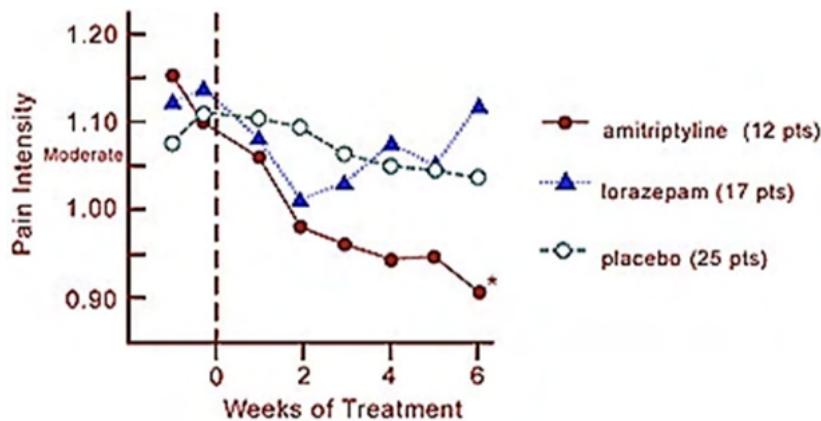


Figure 5.30: Lorazepam vs Amitriptyline.

5.2.5 Placebo Response

Definition 5.10. When a person takes a placebo, they may experience some positive effect to whatever the placebo is supposed to "treat". This re-

sponse is called the **placebo effect (response)**. Note that negative (adverse) effect can also occur called the "**nocebo effect**".

Explanations. The reason that there's such thing as placebo effect is because the psychology of the user but also social factors can influence their health and the "effect" itself. \square

Example 5.2.6. We can compare the therapeutic effect for analgesia that is given by an automated machine vs a physician. We can see that the pain relief is much higher when the physician was present and this increase was due to the placebo response.

Observation 5.21 In fact, if you were to look at the patient's brain when responding to a placebo, it shares many similarities to that of the actual drug.

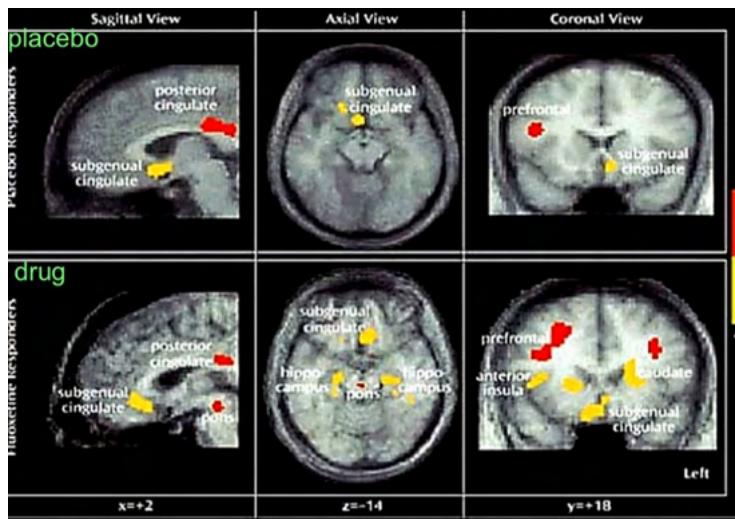


Figure 5.31: Placebo vs drug treated brain.

Concept 5.2 Placebo response can occur in all system but with the highest effect is on the CNS with mood etc.

Notion 5.3 Placebo response is much greater in younger people as compared to elders.

Remark 5.8. *Clinical trials have many challenges when it comes to cancer.*

Observation 5.22 We've mentioned that there's many research when it comes to cancer treatment and cure. One of those treatment is chemotherapy which is not the most ideal way.

To begin with, cancer cells are not homogeneous, each cells will have a different mutation one of which will be resistance to the treatment. When the patient is treated with chemo, all of the non-resistant cells die which leave out the resistant ones to strive. This ultimately lead to death as the resistant cells cannot be eradicated.



Figure 5.32: Ineffectiveness of chemotherapy.

Now let's just look at some examples of fairly recent developments for osteoporosis.

Example 5.2.7.

Here, the trial of the drug, called **Denosumab**, was just giving just one dose (injection) a year and it worked! These people were healthy and strong and had no bone loss at all, in fact, some strengthening of their bones. When you look at a problem like osteoporosis and the therapy, this is something, again, that you have to monitor with time. Because if someone's at risk, if they have small bones to begin with, and they're at risk of losing bone to the point where they're going to have

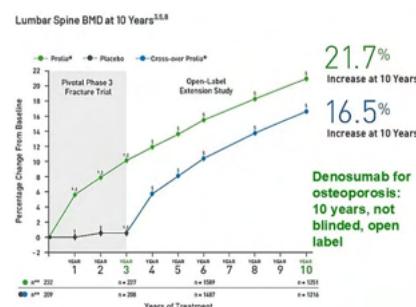


Figure 5.33: Denosumab treatment with osteoporosis.

fractures, you're going to have to treat them perhaps for the rest of their life.

In fact, when compared to the placebo, it has a big significant difference which is why they decided to get all of the people on placebo to begin the actual treatment. There are a few people who have some, but most people have no significant side effects.

5.2.6 Government Revision

Observation 5.23 Now that the clinical trials are over, the documents and experiment will be review by the government or whoever is in charge of that country drug and health development. They're many reviewing the safety data and its risk factors. After revision, the drug can begin to reach the market.

Remark 5.9. *The time it takes to bring the drug into clinic is around 14-17 years with almost 4 billion \$ invested and there's 95% chance of failing!*

Observation 5.24 Majority of these failure is due to unclear pathogenesis of the disease the drug is tasked to treat.

5.2.7 Drug Manufacturing

Observation 5.25 Once everything is set, pharmaceutical company will crank up its manufacturing of the drug and of course naming it. The manufactured drugs need to be safe and effective and ideally be orally absorbed.

Observation 5.26 In Canada, the pharmaceutical company is allowed to hold patent of their new drug (original drug) for a certain amount of year then any other company will be allowed to make biosimilar drugs (generic drug) to the original.

Explanations. The reason they're allowed to hold patent and basically "monopolize" that drug is to remake all of the money they've invested initially into the developing the drug. □

Observation 5.27 Many chemist and engineers from the pharmaceutical company has came up with pills delivery system for 12 hours. This slow released form of drug can be used to slowly releasing its content throughout the day. This enable the patient to take the drug maybe only once a day.

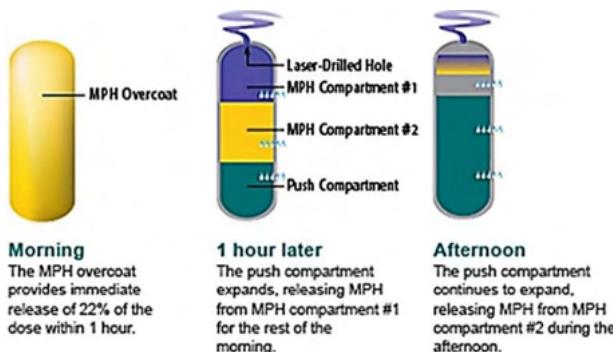


Figure 5.34: Drug delivery system.

Evidently, when these drugs are made, there will be heavy quality control to make them as sterile as possible.

5.2.8 Phase IV

Observation 5.28 In phase IV, we're mainly monitoring the long-term safety and efficacy of the therapy post-approval. This typically involved a very big population of participants over years. Drugs here have around 70 – 90% success rate.

Notion 5.4 Efficacy data are easier to obtain than safety data

Explanations. The reason that efficacy data are easier to obtain since we know what the intended use for the drug is. So from there, we can extrapolate its effect when using the drug. On the other hand, safety data is hard to come by since we're not certain when the side effect of the drug yet thus we need to carefully observe patients. □

Concept 5.3 *For uncommon drug interaction with the new drug, it will present maybe once every 1000 cases. For rare toxicity, sample size increases to maybe once every 100,000 cases.*³

Concept 5.4 *Drugs are much more vulnerable to infants and the elderly.*⁴

³And these cases will be noted and follow-up by pharmacist.

⁴Which is why they're not tested on during trials

Explanations. The reason that they're more vulnerable since they will have lower levels of P450 enzymes thus decrease the metabolic rate of drug in the system leading to more side effects. □

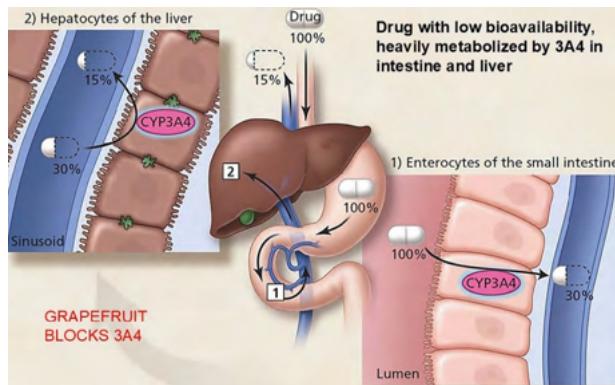


Figure 5.35: Metabolizing drugs interrupted by grapefruit.

Remark 5.10. *There's not only interaction of drugs but also with food, in particular, grapefruit juice.*

Concept 5.5 (Black Box Warning). *When a drug has a **black box warning** label, it means the drugs can be taken but with high precaution and is typically intended for specific individuals.*

Observation 5.29 During phase III and phase IV, researchers begin a process called **repurposing**. As the name suggested, the repurposing process allow researchers to discover if the new drug can have side effects that'll be useful to treat other diseases.

Special Cases of Clinical Trials

Observation 5.30 When the disease is potentially fatal or has some astounding breakthrough. The clinical trials phases will be shortened, which is a **fast-track**. e.g. COVID-19 vaccine is a drug that was fast-tracked as it will have significant impact for the then pandemic.

Definition 5.11. **Orphan drugs** are drugs where its usage are limited to only a small subset of individuals. i.e. they're drugs used to treat uncommon diseases.

Observation 5.31 Typically, pharmaceutical companies do not perform research on these orphan drugs since it will takes lots of money to invest but when marketed, only a few will buy it i.e. they lose money. This is also a reason why, government has begun to handing out grants and funds to research these drugs which can help those individuals.

5.2.9 Successes of Clinical Trials

Like we've mentioned, a lot of these successes after clinical trials are from cancer, follows by vaccines and CNS research. So, we will look at successes of many cancer treating drugs.

Observation 5.32 The Nobel Prize was recently awarded for the understanding that immunotherapy could be used against cancer. Tumor cells, as it turns out, are incredibly clever—they have developed mechanisms to inhibit T cells, which would typically recognize them as abnormal. Immune cells are always patrolling the body, identifying anything unusual and eliminating it. Tumor cells, which are by definition abnormal due to accumulated genetic mutations and irregular surface receptors, manage to block T cell recognition through specific interactions. A strategy in can-

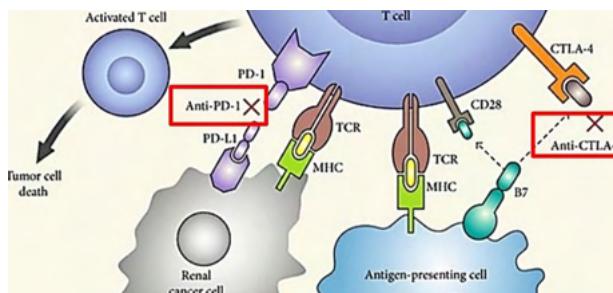


Figure 5.36: Immunotherapy.

cer therapy has been developed to block the tumor cells to evade immune detection. This is done through blocking the receptor using monoclonal antibodies. This cancer therapy is called **immunotherapy**

Example 5.2.8. **Lung cancer**, once considered a death sentence, now has significantly improved survival rates thanks to immunotherapy, more specifically the drug is called **Pembrolizumab**. Similarly, **melanoma**, a skin cancer that used to be almost universally fatal, now sees a majority of patients surviving due to combined antibody therapies (the best case having **Ipilimumab + PD-1 blockade and etc.**).

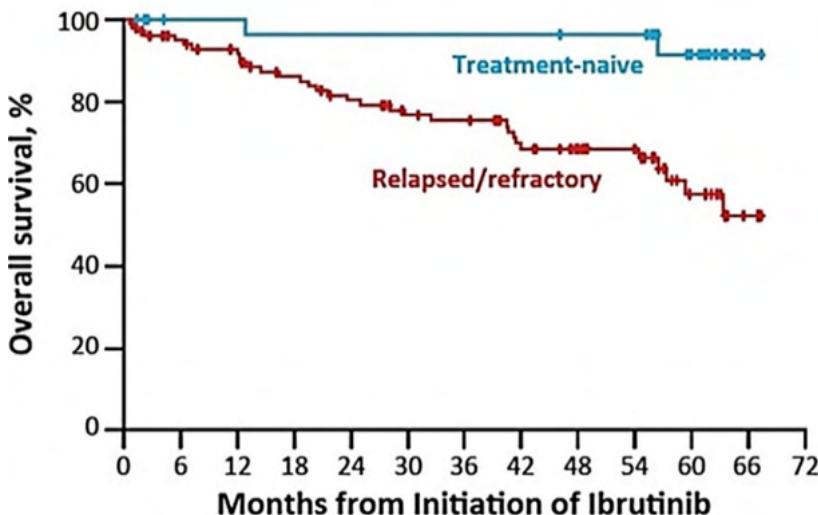


Figure 5.37: Survival rate of leukemic patient under treatment vs non-treatment.

Example 5.2.9. One example involves a form of **leukemia** where researchers identified an overactive enzyme, called **BTK⁵**, as a key factor in the disease. They developed a drug to block this enzyme, called **Ibrutinib**, which has proven far superior to traditional chemotherapy. This drug can be taken orally, has minimal side effects, and allows patients to lead normal lives.

New ways to treat cancer

Observation 5.33 Monoclonal antibodies have also opened new avenues for cancer treatment. These antibodies can **deliver anti-cancer drugs or**

⁵involved in controlling the proliferation of B-cells

radioactive isotopes directly to tumor cells, reducing side effects and enhancing efficacy. They can also target specific growth factors receptors on cancer cells, slowing or stopping tumor growth.

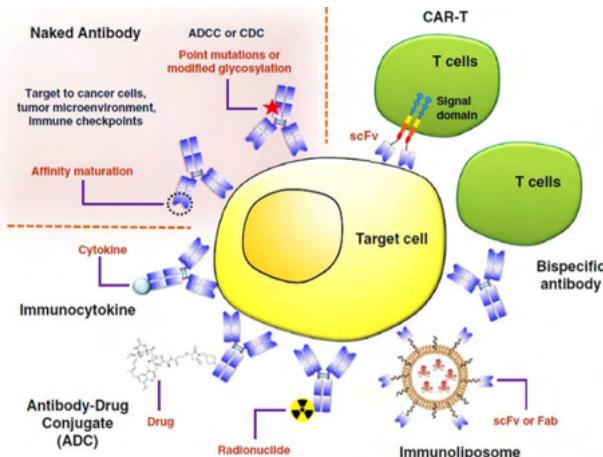


Figure 5.38: Different ways to treat cancer using monoclonal antibodies.

Observation 5.34

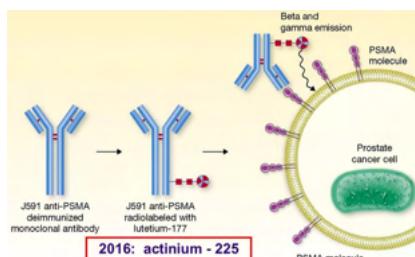


Figure 5.39: Diagnosis of prostate cancer with monoclonal antibodies.

Diagnostic advancements are equally remarkable. For example, prostate cancer can now be tracked and staged using labeled monoclonal antibodies, enabling precise targeting of tumor cells with radioactive compounds. Not only that, we can also attach radioactive compound to the antibodies which will bind to the cancer cell and destroy it.

Observation 5.35 We can also create cancer vaccine with this. The idea here is that you have tumor cells circulating blood all the time i.e. a blood sample would have a small amount of them. We can take this cell and infuse it with other cells in the body. This will trigger the T cells to attack the cancer cells.

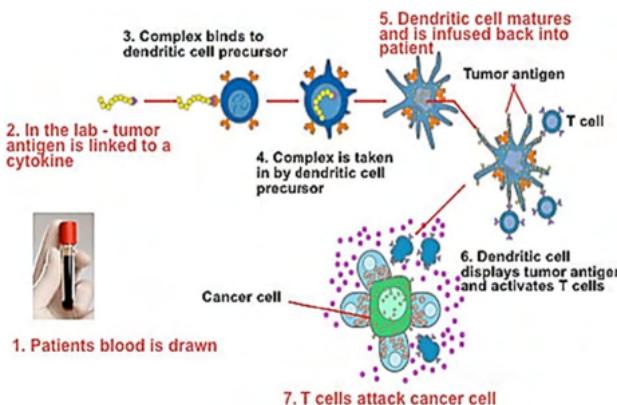


Figure 5.40: Tumor cell vaccine.

Treatment of Migraines

Observation 5.36 There's a particular compound that is known to be part of the pathogenesis of migraines, and there are three different antibodies now that will block it. In fact, there are four of them now that will block it, and three have already been approved and are on the market for treating migraines, to be specific: **erenumab**, **fremanezumab** and **galcanezumab**.

Treatment of Heart Disease

Observation 5.37 We also have breakthroughs in treating heart disease. This is through limit the amount of cholesterol floating around in your blood that's able to be taken in and accumulated in these plaques in the blood. Here we have the receptor that's able to internalize the cholesterol in the liver and get rid of it.

There are two types of drugs on the market now. **Statins** decrease the synthesis of cholesterol in the liver, and **PCSK9 inhibitors** to increase the breakdown by allowing more of the cholesterol to be taken up by these receptors and then broken down in the liver. They help to create a better situation for people with potential blockages in their arteries.

Observation 5.38 We also have newer drugs for treating **heart failure**, such as **neprilisin inhibitor**. You have natural peptides that will lower the blood

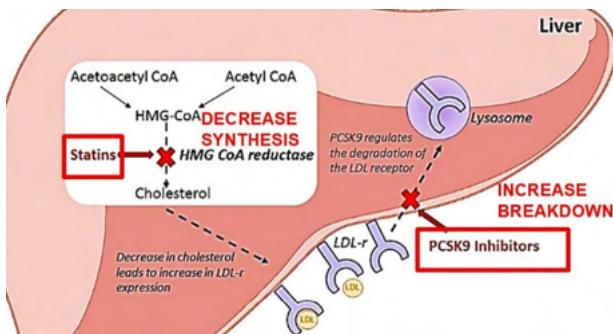


Figure 5.41: Drugs to lower cholesterol circulation.

pressure and are normally metabolized. By blocking their metabolism, you increase the natural peptides. This has been a straightforward mechanism of action, improving quality of life and survival.

Other Major Breakthrough

Example 5.2.10. We also have breakthroughs in treating viruses. **Hepatitis C** is now curable—it used to kill everybody, but it's now curable. We also have continuous research on vaccine which helps to immunize children early on.

Observation 5.39 As of currently, they're working out better ways all the time to give drugs orally so you don't need to inject them. This is pretty sophisticated. They're figuring out better targets. The intracellular targets for pharmaceutical drugs are harder ones to study, but there's a lot of work on that now.

Remark 5.11. *Nevertheless, CNS diseases are still a major site of challenge. We need to understand Alzheimer's and Parkinson's*

Remark 5.12. *The goal of pharmacological research is not only to extend our lifespan but to extend our health span.*

Remark 5.13. *These drugs also benefit pets as any drugs made for human, they're likely to be extrapolated onto dogs, cats and many other pets.*

5.3 Drug Interaction

Case Study. A 71-year-old woman with **paroxysmal atrial fibrillation** being treated with **warfarin** 3.75 mg/day presented at our hospital with a 5-day history of increasing swelling in her neck, sialorrhoea, and difficulty swallowing.

Week earlier, she had been started on **fluconazole** 200 mg once a day because of oral candidiasis. She had a **sublingual haematoma**.

Lab Values. Laboratory testing showed a C-reactive protein level of 65 mg/L (normal value < 5.0 mg/L), a white cell count of 12.5×10^9 per L with 83.3% neutrophils, and a prothrombin time of less than 5% (normal value > 70%) with an international normalised ratio (INR) of more than 9.0 (normal range 0.8 – 1.3).

What happened to her? Well...the fluconazole reduced the cytochrome P450-depedent metabolic clearance of warfarin. This means that the duration and magnitude of warfarin is now shot up which lead to the hypoprothrombinemic effect.

Observation 5.40 Adverse drug reaction (ADR) is the 1 of the leading causes of morbidity and mortality in health care. Around 7,000 deaths occurred to ADRs annually and 350,000 ADR occurred in nursing home each year⁶.

Observation 5.41 Drug-drug interaction can occur when:

1. Many drugs used for the same treatment and v.v.
2. Over-the-counter (OTC) medications and substances.
3. Mostly in elderly (as they're more likely to be on multiple meds).

Concept 5.6 (Consequences of Interaction). When drug-drug interaction occurs, it can have the following effects:

- *Intensification: Which can either be increasing therapeutic effects or adverse effects.*
- *Reduction of therapeutic effect*
- *Production of unique responses.*

⁶Though the exact number isn't exact due to different consideration.

Observation 5.42 We can classify the type of interaction as follows:

1. Additive: The effect of 2 drugs add up.
2. Antagonistic: The effect of 2 drugs reduce the overall effect.
3. Potentiation: The effect of 1 drug is enhanced by the other drug.
4. Synergistic/supraadditive: The overall effect is much higher than additive effect of 2 drugs.

Furthermore, we can classify the interaction according to its pharmacokinetics and pharmacodynamics modifications.

5.3.1 Pharmacokinetics Interactions

We will now look at pharmacokinetics interaction which can divide into ADME.

Absorption Interactions

Observation 5.43

Many things that can affect the GI tract absorption but here are some of them:

1. Agents that bind drugs.
2. Agents that increase GI motility.
3. Agents that change P-glycoprotein ⁷

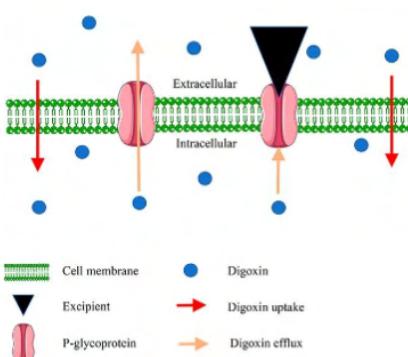


Figure 5.42: P glycoprotein and absorption.

Example 5.3.1. Usage of antacids can increase gastric pH however, it can also decrease GI absorption. Intestinal transporter could increase the net absorption which are usually expelled.

⁷An important transporter.

Example 5.3.2. Many vasoconstrictors can slow down the subcutaneous absorption of drugs. In particular, local anesthetics and EPI absorption, which are injected subcutaneously, will decrease by β -blockers (cardiac depressants).

Example 5.3.3. Iron can interact with levothyroxine by binding and forming a complex which reduce absorption. Meanwhile, antacides can interact with ciprofloxacin by reducing its absorption in the GI.

Distribution Interaction

Observation 5.44 The distribution of drugs can be affected by:

1. Competition of the same binding site.
2. Alteration of the compartment.

Example 5.3.4. **Sulfonamides** can displace methotrexate, phenytoin, sulfonylureas, and warfarin from binding sites on albumin.

Example 5.3.5. **Diuretics** can reduce total body water \Rightarrow increase [aminoglycosides + Li] in plasma \Rightarrow increase drug toxicities.

Metabolic Interaction

Observation 5.45 As we've known, P450 is the enzymatic family that process drugs thus anything that alter its activity will alter metabolism of drug. This means:

- Agents that increase P450 activity will increase metabolism of certain drugs. e.g. chronic administration of barbiturates, carbamazepine, etc.
- Agents that decrease P450 activity will decrease metabolism of certain drugs. e.g. cimetidine, disulfiram, erythromycin, etc.

Example 5.3.6. CYP2D6 can catalyze the active form of codeine, process many β -blockers and tricyclic antidepressants. However, it can be inhibited **fluoxetine, haloperidol, paroxetine and quinidine**.

Example 5.3.7. CYP3A is responsible for many metabolism but can be inhibited by many drugs such as: ketoconazole, itraconazole, fluconazole, cimetidine, grapefruit juice, etc.

Notion 5.5 The main thing that can alter the clearance of a drug is a drug that reduce hepatic blood flow.

Example 5.3.8. Propanolol can interact with morphine and verapamil by reducing hepatic blood flow and thereby reducing the clearance of metabolised morphine and verapamil.

Observation 5.46 Monoamine oxidase inhibitors (MAOIs) were the first type of antidepressant developed. Those who took this medication need to avoid food and drinks with **tyramine**.

Explanations. Tyramine triggers can increased heart rate, blood pressure, and vasoconstriction. It acts as an indirect sympathomimetic, causing these effects by releasing stored neurotransmitters. Tyramine is also broken down by MAO, MAOIs prevent this breakdown. This can lead to high tyramine levels, amplifying its effects and potentially causing dangerous spikes in blood pressure \Rightarrow fatal. \square

So, we summarize this part for metabolic interaction with 3 kinds of interactions:

- Inhibition: agents that can inhibit P450 enzymes will decrease drug metabolism.
- Induction: agents that can induce P450 enzymes can increase drug metabolism
- Competition: agents that can compete to be metabolized by the same P450 enzymes.

Excretion Interactions

Observation 5.47 Excretion of drugs through the kidney can be altered by the followings:

- Agents that reduce renal blood flow. e.g. β -blockers
- Agents that inhibit renal transport mechanism. e.g. Aspirin affects uric acid handling in the proximal tubule.

- Agents that alter urinary pH. e.g. drugs with specific ionization state may not be eliminated properly

Example 5.3.9. Probenicid can interact with penicillin by reducing its elimination and thereby alter the scheduling of penicillin.

5.3.2 Pharmacodynamics Interactions

Antagonistic Interactions

Observation 5.48 Antagonistic interaction occurs when 1 drug is an agonist while the other is an antagonist that is taken together.

Example 5.3.10. Antagonistic interaction can arise with a person taking anti-anxiety medication but also need to take medication for asthma. This is because anti-anxiety medications are generally β -blocker that blocks β -adrenergic receptor however, asthma medication is a β -adrenoreceptor activator. Thus interaction will occur.

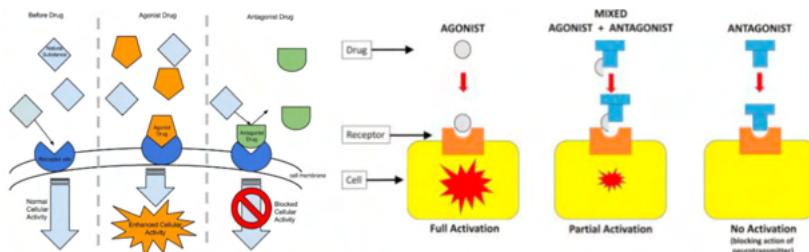


Figure 5.43: Antagonistic interaction.

Observation 5.49 Though this is a simple thing to see, however when you have a mixed agonist-antagonistic drug⁸ (such as **pentazocine**) or partial agonist (such as **pindolol**), the interaction occurred to the pure agonist is much harder to predict.

Remark 5.14. *It must be noted that not all antagonism is based on receptor interactions.*

⁸This class of drug includes drugs that can activate but at the same time inhibit certain receptors.

Example 5.3.11. NSAIDs can interact with ACE inhibitors by reducing renal elimination of Na^+ thereby decrease its antihypertensive action.

Additive Effect Interactions

Observation 5.50 Like we've discussed, interaction can lead to additive effect and interestingly, it does not necessarily have to be on the same receptors.

Example 5.3.12. Tricyclic antidepressants and interact with diphenhydramine to produce an additive effect that cause excessive atropine-like effects such as: mouth dryness, blurred vision, dry eyes, photophobia, etc. This is because they both have muscarinic receptor inhibitory actions.

Example 5.3.13. Aspirin, thrombolytics, and thyroid hormones can interact with warfarin leading to excessive bleeding complication. This is all due to additive effect of decreasing clotting factor in the blood vessel \rightarrow leads to excessive bleeding.

Synergistic Effect Interactions

Observation 5.51

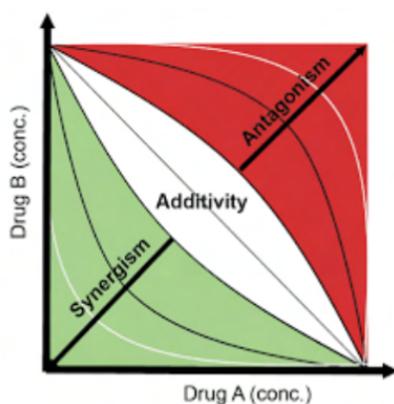


Figure 5.44: Synergistic interaction

Synergistic interactions occur when additive effect is potentiated to a higher level. It's much less common as compared to antagonistic or additive interaction.

Example 5.3.14. We can detect synergistic effect by taking sulfonamides and dihydrofolic acid reductase inhibitors like trimethoprim. Combination of these 2 will lead to a strong antibiotic therapy.

5.3.3 Antibiotics Interactions

Nevertheless, not all interactions are bad e.g. antibiotics interactions are found to be extremely useful.

Definition 5.12. **Antibiotics** are substances that can inhibit the growth and replication of bacteria. They're a type of *antimicrobials* that can attack bacteria on or in the body.

Definition 5.13. **Antimicrobials**⁹ are substances that are used to kill or inhibit microbes.

Observation 5.52 There are 2 widely used types of antimicrobials: **antiseptics** and **disinfectants**. These 2 are similar in that they will eliminate microbes, including bacteria. However, antiseptics can be applied on living tissue without damaging them, while disinfectants are solely used to apply on non-living surfaces.

Remark 5.15. Originally, antibiotics only denote substances that produced by microorganism to counter bacteria but now, it's used interchangeably with synthetic substances to fight bacteria therapeutically.

Definition 5.14. **Bacteria** are single celled microorganism with no nucleus nor membrane-bound organelles. They are classified into 5 different groups according to their shape: spherical (**cocci**), rod (**bacilli**), spiral (**spirilla**), comma (**vibrios**) and corkscrew (**spirochaetes**). These microorganisms can exist as a single cell or a cluster.

Observation 5.53 Not all bacteria are bad e.g. gut bacteria. However, many bacteria can cause diseases, of which are usually acute. This includes:

- *Escherichia coli* and *Salmonella* cause food poisoning.
- *Helicobacter pylori* cause gastritis and ulcers.
- *Neisseria gonorrhoeae* causes the sexually transmitted disease gonorrhea.
- *Neisseria meningitidis* causes meningitis.
- *Staphylococcus aureus* causes a variety of infections in the body, including boils, cellulitis, abscesses, wound infections, toxic shock syndrome, pneumonia, and food poisoning.
- *Streptococcal bacteria* cause a variety of infections in the body, including pneumonia, meningitis, ear infections, and strep throat.

Concept 5.7 Bacteria can also be classified according to their cell wall staining, which can either be: Gram-negative and Gram-positive.¹⁰

⁹This includes antibiotics, antivirals, antifungals, etc.

¹⁰Gram-positive appear purple under the stain and Gram-negative appear pink.

Observation 5.54 Antibiotics are often specific to either Gram-positive or Gram-negative bacteria, which allows targeted treatment without disturbing all the bacteria in your body (e.g., the gut microbiome). However, **broad-spectrum antibiotics** can target both types, which is sometimes necessary.

Remark 5.16. *Most of the time, gram-negative bacteria are bacilli while gram-positive are cocci.*

Observation 5.55

The introduction of antibiotics into medicine revolutionised the way infectious diseases were treated. Between 1945 and 1972, average human life expectancy jumped by eight years. It's especially important in today's world.

However, with the development of these antibiotics, bacteria has begin to build resistance to these drug. As a result more than 35,000 people died out of 2.8 millions infected with these antibiotic resistance bacteria.

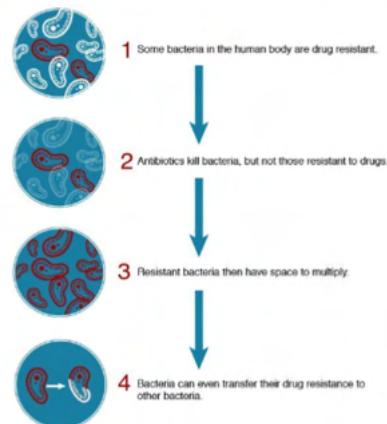


Figure 5.45: Development of antibiotic resistance in bacteria.

Definition 5.15. Drug resistance

is the ability of an organism (in this case, bacteria) to withstand the effects of a drug (antibiotics).

Observation 5.56 Though, no one can avoid the risk of resistant infections, there are subgroups of people that are in much greater risk such as people with chronic diseases. Nevertheless, we've begin to notice that the concurrent use of more than 1 antimicrobial agent lead to a positive effect in patient, even if the bacteria is antibacterial resistant.

Multi-antibacterial Agent Treatment

Like any other drugs, when antibiotics are taken together, they can have synergistic and antagonistic effects (but also indifferent effect i.e. com-

bined them doesn't change anything). However, we will focus on the synergistic effects.

Observation 5.57 The synergistic effect can be seen when taking **penicillin** with **aminoglycosides**. In particular, penicillin is an antibiotics that compromise its cell wall integrity which ultimately lead to its death (they can't survive in the osmotic environment of the body). On the other hand, aminoglycoside is an antibiotics by acting directly on its DNA and lead to inhibition of many protein synthesis. So, in either case, by taking them together, you're getting the absolute kill of the bacteria.

This is also useful for antibiotic resistance bacteria since these 2 drugs acts on 2 completely different pathway.

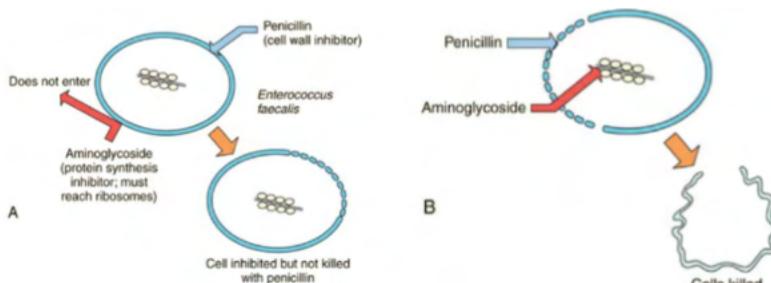


Figure 5.46: Synergistic interaction of penicillin and aminoglycosides.

Example 5.3.15. A bacteria that is resistant to penicillin can be treated with aminoglycosides. This lead to the destruction of major protein that give the bacteria penicillin-resistant ability. Thus, when combined with penicillin, it will further kill the bacteria, as it's no longer penicillin resistant.

5.3.4 Pseudomonas Aeruginosa

Definition 5.16. *Pseudomonase aeruginosa* is a gram-negative opportunistic bacteria that can cause **pneumonia**. They're part of the ESKAPE pathogens and are resistant to many antibiotics.

Observation 5.58 Upon infecting the body, it can use its **type 3 secretion system (T3S)** or **injectisome** to inject different virulence factors into host cells and thereby killing them. Furthermore inspection of this T3S injectisome is a subunit called **PcrV**. Furthermore, we also found a virulence

factor called **PS1 exopolysaccharide (EPS)** which can help with bacteria to aggregate together.

So, by targeting these factors, we can ultimately stop the infection of *P. aeruginosa*. This is where we develop and multifunctional antibody called **BiS4 α Pa** that can bind and inhibitor both of these factors. What this antibody can also do is open up the bacteria to be targetted by immune cells.

Methods 5.1 Begin with mice infected with *P. aeruginosa*, we will separate them into groups based on the treatment. First, you have the control group which only have the normal antibodies produced in the body. Next is the group treatment with **meropenem (MEM)**, a broad-spectrum antibiotic. Then, it's the group with only the Bis4 α PA. Lastly, we have the group in combination of MEM and BiS4 α Pa

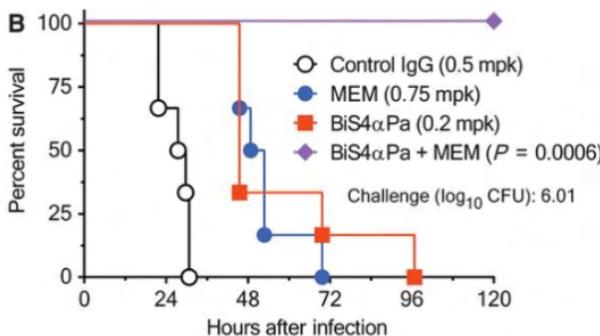


Figure 5.47: Treatment outcomes of mice infected with *P. aeruginosa*.

Observation 5.59 We can see that in all cases beside the combination treatment, they all eventually die within 3 days. With the combination treatment, virtually all of the mice in said group survived. This is mostly due to the synergistic interaction of the 2 treatment.

6.1 Cosmetics and Protectants

Definition 6.1. **Cosmetics** are products contain different mixtures of different chemical and even drug. They can be used to enhance beauty and/or alter one's appearance.

We will start off this lecture with talking about products for the skin but first, we need to know a bit about it.

Observation 6.1

Skin is the largest known organ in the human body. It consists of different layers of cells but also nerves ending and blood vessels. The skin color of 1 person may differ from another based on the ratio melanin by melanocytes. Not surprisingly, skin can be subjected to different changes in order to "entertain" one's view of beauty.

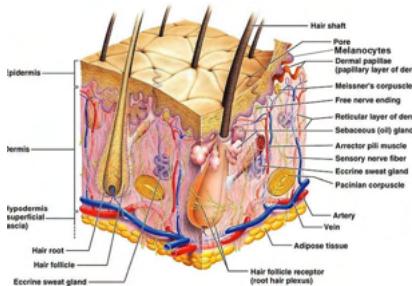


Figure 6.1: Skin layers.

Example 6.1.1. Many has viewed darker color skin to be more attractive than lighter which lead them to try to darken it through tanning under the sun's UV light. Under prolonged exposure of the sun, melanocytes will increase its melanin production and insertion leading to the tanning of skin color. However, overdoing tanning can lead to **sunburn** when there's an overexposure to UV light.

Example 6.1.2. Tattoo is also a major form of alteration of the skin for beauty and even symbolism. The mechanism behind a tattoo is simple. First, the ink will be pushed into the skin dermis where dermal macrophages are. Then, these macrophages will uptake these ink particles but cannot get rid of them and so the ink particles stay in place. When the macrophages

die, the ink particles will be reuptaken by another macrophages.¹

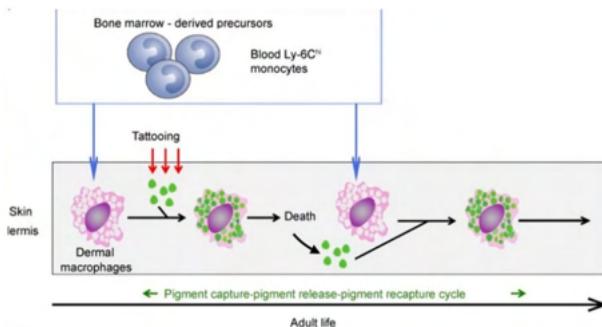


Figure 6.2: Tattoo mechanism.

Observation 6.2 Skin is also the major organ used for sensing the outside environment. Its structure can adapt based on where it is on the body e.g. skin of the foot sole is thicker than skin at the back of your ear. Not only that, its thickness and area will vary by age (obviously).

Remark 6.1. *This is also why many skin patches are placed behind the ear as with thinner skin there, absorption would be faster.*

Remark 6.2. *Many drugs such as antibiotics, diuretics, NSAIDs, etc. can cause **photosensitivity**, which is when the skin becomes sensitive to sun ray.²*

6.1.1 Skin-related Products

We will now look at some specific categories of skin-related products.

Sunscreens

Definition 6.2. **Sunscreen** is a topical product used to protect the skin from the sun. It's mainly used to block the UVB and UVA rays from the sun. Its efficacy is measured in **sun protective factor**, which is simply a factor before one gets a sunburn.

¹There will obviously be some loss of ink during these processes which is why tattoo tends to get fuzzier through time.

²Thus, increasing chances of sunburn

Example 6.1.3. Suppose that a person will get a sunburn with 5 minutes of direct exposure. If they were to apply a sunscreen with SPF 30, then, the exposure time will increase to $5 \times 30 = 150$ minutes before experiencing a sunburn.

Remark 6.3. *It's important to not get sunburns as experiencing 5 sunburns was shown to double the risk of having skin cancer.*

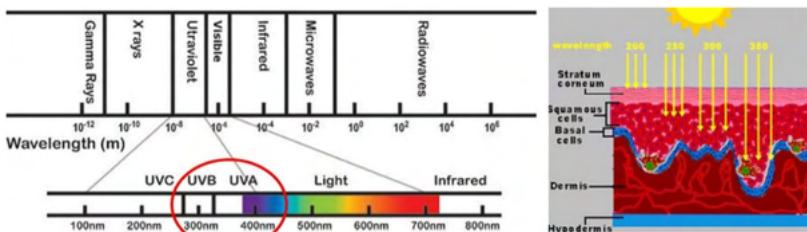


Figure 6.3: UVA and B and the wavelength that absorbed in the skin.

Observation 6.3 One might be fooled that on cloudy days, they do not need to wear sunscreen. This couldn't be further from the truth as 80% of sun's ray can get through cloud, mist and even fog. For snow, it reflects up to 85%! Thus, it's important to apply sunscreen, especially in children who are more vulnerable.

Interestingly, a lot of aging skin in the elderly are the result of **photoaging**, where it's the sun that cause the skin alteration through time.

Observation 6.4 As we've described in example 7.1.1., tanning is a common practice for people to "enhance their beauty". This also leads to the development of tanning bed. **This was shown to be even worse than tanning in the sun as it increases melanoma risk by 59%.** Which is why it's been deemed carcinogenic by Health Canada (complete banned) and even WHO.

Retinoids

Definition 6.3. **Retinoids** are a class of chemical derived vitamin A. It serves as an important product used to treat acne.

Definition 6.4. **Acne** is a skin condition (inflammation) that is a result of increase in production of sebum, keratin and proliferation of bacteria.

Observation 6.5 It occurs in several stages. First, there's an increase in sebum production which later lead to its oxidation and eventual trap. Then, bacteria in these region will feed on the sebum and proliferation. This will lead to recruitment of leukocytes that try to fight this infection. Ultimately, there's an inflammation.

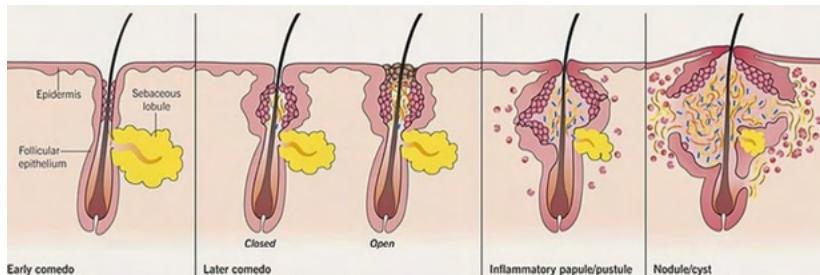


Figure 6.4: Stages of acne.

Definition 6.5. **Comedo** is a term refers to a clogged hair follicle due to the build up of keratin and sebum. We can classify comedo into 2 types: **blackhead**³ (open comedo) and **whitehead** (closed comedo).

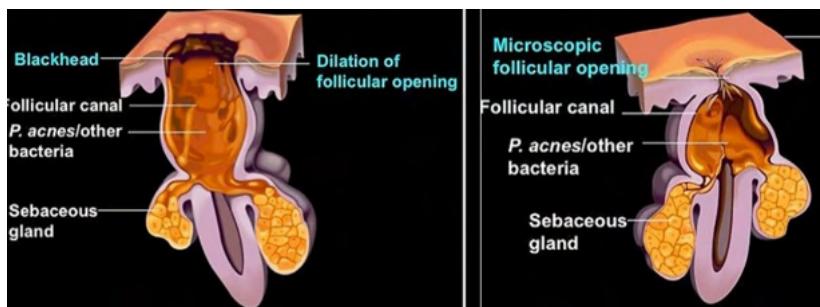


Figure 6.5: Comedo.

Remark 6.4. There's no animal model throughout history to describe acne i.e. we're the only one that have it.

³Blackhead looks black because of the pigment not dirt!

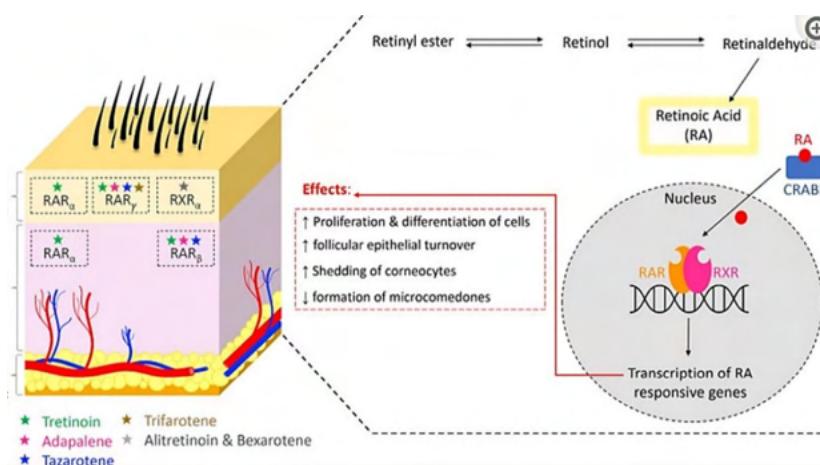


Figure 6.6: Different retinoids acting on RARs and RXRs.

Observation 6.6 This also means that to figure out the manifestation of acne is also difficult. Nevertheless, we were still able to develop and understanding which is just a chronic inflammation of the pilosebaceous unit. Even so, the pathogenesis is not completely understood.

So then, **What do retinoids do?** Well...they can normalize follicular keratinization i.e. inhibit excess deposit of kertin; they can also inhibit the excess production of sebum by the sebaceous gland (also other hormones and oral contraceptive). Reduction of bacterial growth be done by antibiotics and even steroids.

Observation 6.7 Now, we've previously define that retinoids are derivative of vitamin A. We also needs to recognize that there are a multitude of derivative of vitamin A, of which we're currently on the 4th generation derivative. **What this will do is increase selectivity and decrease toxicity.**

Observation 6.8 Retinoids can bind to **retinoic acid receptors (RARs)** and **retinoic X receptors (RXRs)**. These 2 will then form a dimer post-binding and stay in the nucleus to regulate gene transcription. They're documented to linked with 500 different genes including differentiation, proliferation, apoptosis and etc.

For the retinoids, they can vary in affinity for different subtypes of RARs i.e. we can personalize the drug to treat acne depending on each person's conditions and needs.

Observation 6.9 Isotretinoin is a retinoid that is sold legally to treat severe cases of acne. However, it will always come with labeling to prevent pregnant patients from taking it as it can lead to severe birth defects⁴ i.e. it's a teratogen.

Every drug will have its up and down. For isotretinoin, it's associated with inflammatory bowel disease in patients and possible interactions with NSAIDS/acetaminophen.

Beauty Aids

When it comes to beauty aids, some of them can be useful like soap which will help with disinfection however, here and there, there will be guilt-trip advertisement of "antibacterial" soap which does nothing compared to the regular.

Observation 6.10 For beauty aids (cosmetics), they tend to be divided into 3 categories based on the constituents: powder, grease and liquid. In most cases, a lot of these beauty aids boil down to its over the top advertisement to lure people in and buy its products; which, to be frank, does not do much or even none at all.

Example 6.1.4. Certain cosmetics advertise to control **cellulite**, which are just little bumps from excess fat. There's no such cream that reduce cellulite, all you have to do is just losing weight.

Definition 6.6. **Cosmeceutical** is a cosmetic product that is purported to have therapeutic action capable of affecting the skin positively beyond the time of its application.

So, what we've been discussing are cosmeceuticals. **Are they safe?** Well... yes and no. Yes since they have certain components that do what they're intended to do. No, since a lot of these products contains traces of heavy metals and other harmful chemicals which can be detrimental for health.

Example 6.1.5. We've found traces of **phthalates** in many perfumes, lotions, nail polish, etc. This chemical is a carcinogen and can lead to reproductive dysfunction, endocrine disruption, and etc.

⁴It will lead to malformations of face, kidney and heart. Lead to psychological and intellectual impairment.

Observation 6.11 You might think that plant product is a safer bet then. Well...not really, this is because plants also consist of tons of different chemicals, of which some can do more harm than good.

Antiviral Drugs

Definition 6.7. **Herpes simplex** is a herpes viral infection that can cause painful blisters on the lips.

Observation 6.12

At first, you have primary infection of the *herpes virus*, which will enter the skin, enter sensory nerves ending, then lives up in the ganglia. Sitting in the nervous cells, it will not be targeted by the immune system. Under various conditions, the dormant herpes virus will come back to the skin surface as a lesion where the nerves endings are.

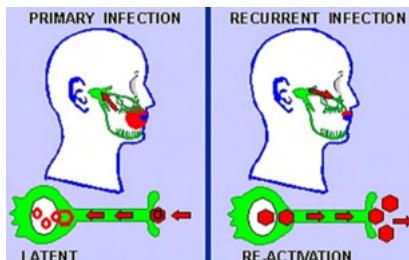


Figure 6.7: Herpes infection.

Observation 6.13 Although, it's not curable, we know a lot about its structure and function which means we have derived a myriad of drugs to treat it. In fact, we have an entire family of drug just to treat herpes. These medications work by targeting its viral DNA polymerase, can cause chain termination, all of which can inhibit viral reproduction.

Remark 6.5. *The person infected with it can feel its movement up and begin to form a lesion. When the drug is taken at this time, it can prevent lesion formation.*

Immunosuppressive Drugs

Definition 6.8. **Psoriasis** is a skin disease that causes a rash with itchy, scaly patches, most commonly on the knees, elbows, trunk and scalp. Basically, it's a disease where there's an increase in skin turn over and thickness.

Observation 6.14 Psoriasis is not curable but can be treated. In the past, there are treatment such as phototherapy accompanied with topical agents

/steroids. However, in recent time, there's been development of monoclonal antibodies that target cytokines and thereby block immunological and inflammatory response.

6.1.2 Hair and Modifications

Observation 6.15 In total of, we have around 10 million hair on our body with around 100k on our head. It will have a grow rate of 6in per year reaching a maximum length of around 3ft⁵.

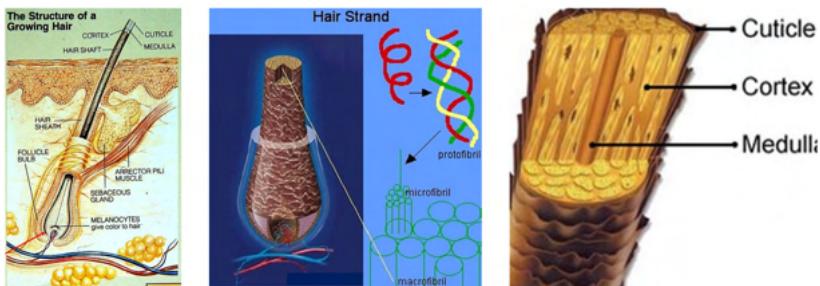


Figure 6.8: Hair structure.

Observation 6.16 Focus at a single hair, we can see that it starts growing at the follicle bulb with blood vessels supplying nutrients. It also has melanocytes to gives off the hair its color. Looking at its cross-section, we can see that it comprise of 3 parts: **medulla, cortex and cuticle**, in such order from the inside out. Zooming further at the hair cross-section, we can see individual fibers called **macrofibril**, which is made from multiple **microfibril**, which is made from multiple **protofibril**, and itself is made from the whinding of **keratin**.

Observation 6.17 The water content in hair will differ at different level of humidity. This is also why heat-dried hair will retain lower amount of moisture content and thus it will stay in the shape that it was heat-dried.

Observation 6.18 (Hair Growth Cycle). During the active growth phase, known as the **anagen phase** (2-6 years), hair grows continuously for about

⁵There are genetics disorders that lead to longer than 3ft hair growth.

five years on average. When the hair reaches the end of its growth phase, it transitions briefly into the **catagen phase**, where it begins to retract. Following this, the hair enters the resting phase, or **telogen phase**. During this phase, the base of the hair pulls upward, and eventually, the hair falls out. Afterward, the cycle starts anew. Stem cells activate to form a new hair, beginning the growth process all over again.

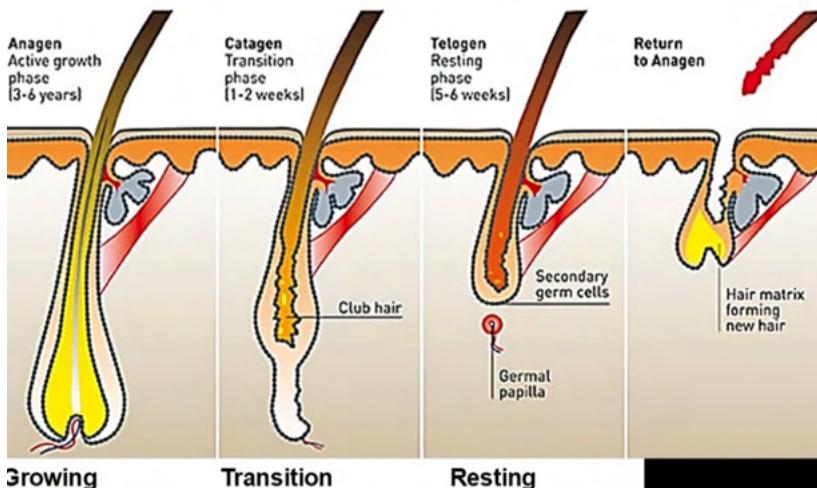


Figure 6.9: Hair growth cycle.

Remark 6.6. Beard growth peak in July, while scalp hair sheds the most during summer.

Observation 6.19 Hormones can influence hair growth. We can track the amount of anagen hair in pregnant patients during and after pregnancy. At the beginning, there's a sharp increase in anagen hair and stay their throughout pregnancy. Then, during post-partum, they will immediately lose all of said hair but immediately go back up to normal level.

Hair damages

Observation 6.20 You can have different damages to the hair strands. In normal condition, the cuticle all lay on top of each other and form a smooth

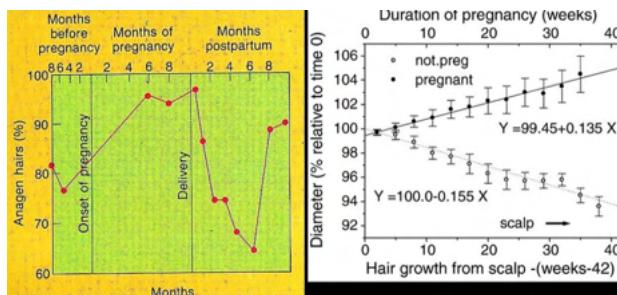
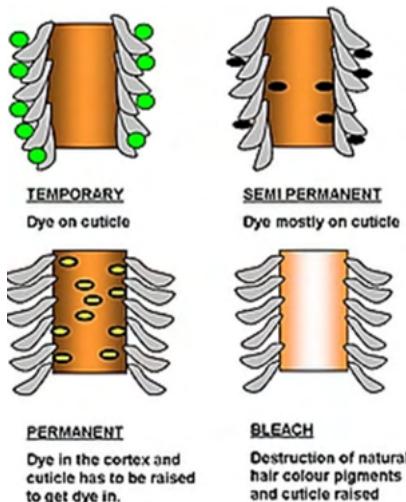


Figure 6.10: Hair growth of pregnant patients.

surface. When the hair is treated with different products and overheating, it can lead to formation of *split-end*. It can also be damaged by chlorine in swimming pools.

You can damage the cuticle by combing backward, using the curling iron, bleaching and hair-spray.

Hair Modifications



So now let's ask ourselves **how do we change our hairshape?** Well...if we remember our discussion above, hair consists of keratin. These keratin is held by disulfide bond. So, by breaking this bond, we can change the shape of the hair and make it stay in this shape by restoring the bond.

How about changing color? Well... we just use a combination of melanin for the desired color. But then **how do you actually put the dye in?** Well..it depends, if it's a temporary coloration, we added on top of the cuticle layer which

Figure 6.11: Hair dyeing.

can then be washed off. For a more permanent dye, we first have to "open up" the cuticle, then let the dye diffuse through to the cortex.

Hair Loss

Observation 6.21 You lose around 50 strands of hair per day. During hair loss in males, the terminal hair is replaced by a very small vellus hair or, eventually, nothing at all. This process occurs in different patterns and gradually over time. In women who carry the gene for hair loss, they typically don't lose all their hair, but it becomes thinner.

Solution (past): Wigs and hats are often used in the past to cover up baldness. You also have remedies for baldness during the 1700-1800s, which most do not work.

Solution (new): Hair transplants have become a viable option. **Minoxidil** is the active ingredient in Rogaine⁶, which can increase follicular size and prolong the growth phase of hair. Another drug, **finasteride**, blocks dihydrotestosterone (DHT) production by inhibiting the enzyme 5 α reductase, which converts testosterone into DHT.

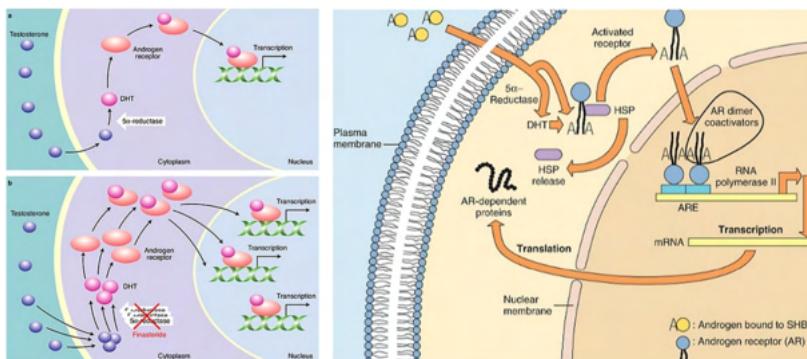


Figure 6.12: Mechanism of finasteride.

Observation 6.22 People with the gene for baldness tend to produce more DHT, which has a much greater effect and leads to hair loss. Blocking the excess DHT has proven effective, and for some individuals, it works very

⁶First cosmeceutical company

well. However, finasteride is teratogenic for male fetuses, so women should not use it. It's also advised that men planning to have children avoid it.

Observation 6.23 Hair repair products have also gained popularity, addressing damage caused by bleaching, heat drying, and other harsh treatments. Products like Olaplex and K18 can repair damaged keratin. Olaplex works by creating a cross-link between broken disulfide bonds in hair, forming a stronger connection. K18, named for its keratin peptides, relinks strands and restores hair integrity, effectively improving scruffy or damaged hair.

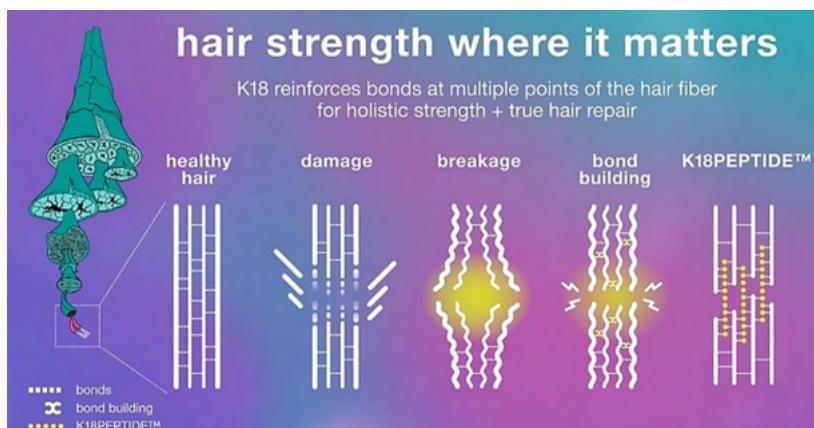


Figure 6.13: K18.

6.1.3 Teeth-related Products

When discussing overall grooming, it's essential to consider teeth as well.

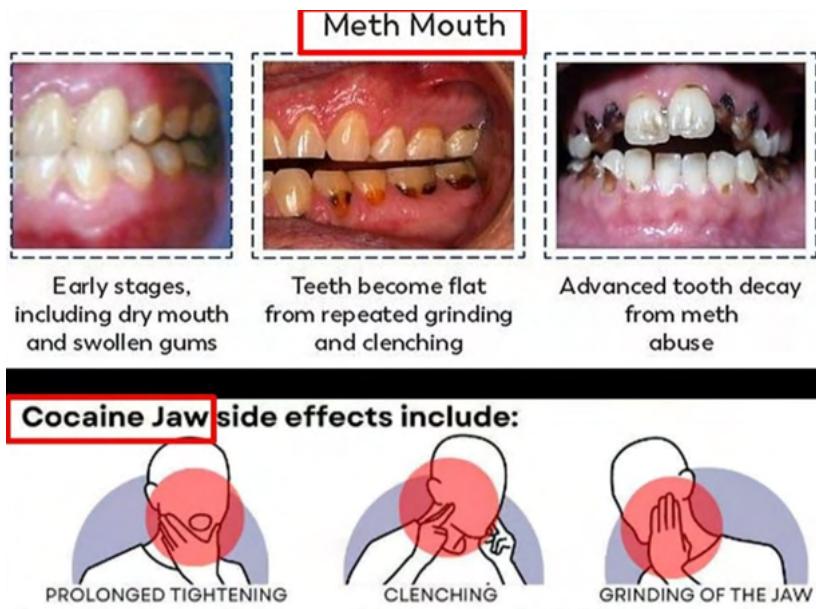
Observation 6.24 In our entire life, we will have 2 sets of teeth: the first is in our childhood while the second set is permanent in adulthood. Drugs can affect both of these sets during childhood and adulthood.

Example 6.1.6. Tooth discoloration can result from drugs like tetracycline, especially when exposed during fetal development or early childhood. While tetracycline saves lives, its cosmetic side effects can be addressed with modern dental procedures.

Example 6.1.7. **Gingival hyperplasia**, caused by certain anti-seizure medications, leading to excessive gum growth.

Remark 6.7. Stimulants used by drug addicts can also lead to many dental problems.

Example 6.1.8. For an addict, repeated methamphetamine abuse will lead to "**meth mouth**" which causes flattened teeth by grinding down of teeth, then severe dental decay. Similarly, Repeated abuse of cocaine will lead to "**cocaine jaw**" which leads to increase caries, tooth loss and etc.



TEETH: increased caries, tooth loss, periodontal disease

Figure 6.14: Meth mouth and cocaine jaw.

Observation 6.25 Fluoride is a vital component in maintaining dental health. Fluoride strengthens teeth by binding to the **hydroxyapatite** in enamel, making it more resistant to decay. Its benefits were discovered through studies showing reduced tooth decay in areas with fluoridated water. The opposite proponent to fluoride is sugar which can acidify the saliva and demineralize teeth.

Remark 6.8. *Water in Montreal is unfortunately not fluorinated.*

Observation 6.26 Gum disease, such as **gingivitis**, results in red, swollen gums that bleed easily and can lead to tooth loss if untreated. Regular brushing, flossing, and dental visits prevent gingivitis. Modern mouth-washes and toothpastes contain antibacterial, antifungal, and anti-inflammatory ingredients to combat gum disease and maintain oral health.

With proper care, you can keep your teeth well into old age. So, smile and take care of your teeth!

6.2 Vitamins

Definition 6.9. **Vitamins** are essential⁷ micronutrients required by the body to carry out a range of normal functions.

Observation 6.27 As the definition has stated, they're good for different bodily function, but also growth and maintenance of the body. Even as essential as they are, **we only need a small amount and they're not used as energy source.**

Notion 6.1 Food preparation can affects the vitamins in food.

Explanations. This is self-evident as when you prepare food such as cooking it, heating it, etc., you're creating a chemical reaction which affect vitamins. □

Observation 6.28 Food preparation maybe a way to affect vitamins but there are other factors that can affect vitamins' quantity:

- Source (e.g. plant vs animals).
- Sunlight.
- Moisture.
- Growing conditions.
- Packaging and storage.
- Plant's maturity pre-harvesting.

⁷By essential, we mean that we need to uptake this through the diet

Why aren't we getting enough vitamins? Well...if we were to eat many processed food, then by notion 7.1, the food's vitamins is heavily affected. Incorrect storage conditions can also rid of the vitamins in them. Drug-drug interactions may also affect the metabolism of vitamins.

Remark 6.9. *Because not all of us can get the necessary vitamins in life, OTC vitamins supplement was made for this specific reason.*

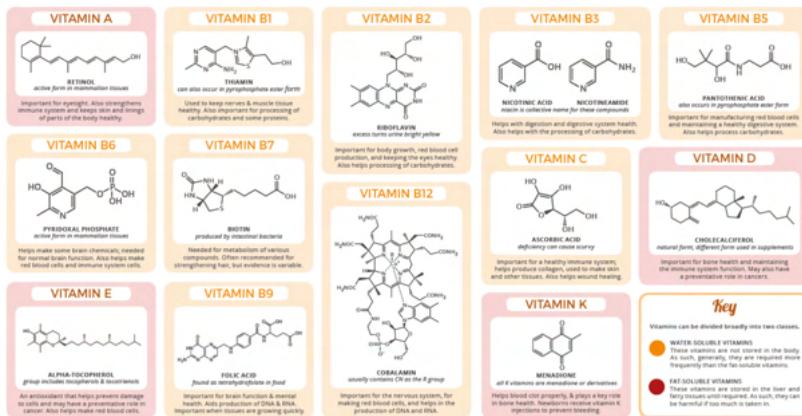


Figure 6.15: 13 vitamins that are essential for the body.

Definition 6.10. **Provitamins** are substances that can be converted to vitamins in the body i.e. they're the inactive preliminaries to vitamins.

Example 6.2.1. β -carotene is a provitamin that once enter the body, will be cleaved into vitamin A.

Observation 6.29 We can divide the 13 vitamin in Figure 7.15, into 2 groups based on their solubility: **fat-soluble and water-soluble**.

Before diving into each vitamins in these 2 groups, let's see the difference, physiological-wise, between the 2 groups.

Observation 6.30 For fat-soluble vitamins, upon consumption into the lumen of the intestines, they will be solubilized by bile and pancreatic enzymes to then form **micelles**. These micelles are then transported into the intestinal cells via transporters. Here, they're assembled into **chylomicrons** which will then directly go into the lymphatic systems.

On the other hand, water-soluble vitamins can directly pass through the cell and enter circulation.

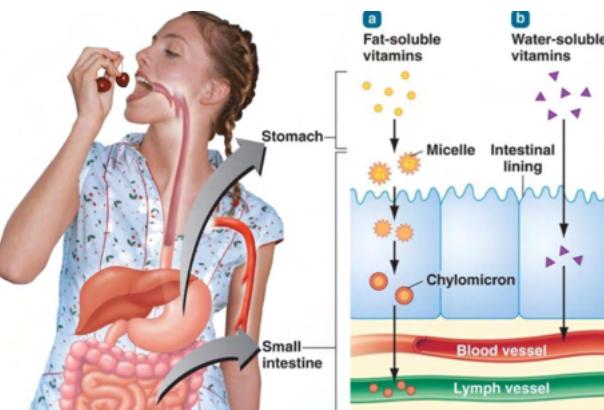


Figure 6.16: Absorption of fat-soluble vs water-soluble vitamins.

Remark 6.10. *High dose of vitamin A and E can be absorbed directly from the water-miscible emulsions.*

Remark 6.11. *Since they enter the lymphatic system first, fat-soluble vitamins takes longer to enter circulation.*

Remark 6.12. *Fat-soluble can be stored in fat deposits which can increase toxicity risk.*

6.2.1 Fat-Soluble Vitamins

There are 3 kinds of fat-soluble vitamins: **vitamin A, D, E and K**.

Vitamin A

Observation 6.31 Vitamin A has 2 forms: active forms (which are retinoids) and the precursors (carotenoids). For the retinoids, we've discussed that it's used mainly to treat acne, but we've also found that it's important for cell production and immune function. Furthermore, it's important for reproductive and bone health.

Concept 6.1 *Vitamin A is important for vision both day and night.*

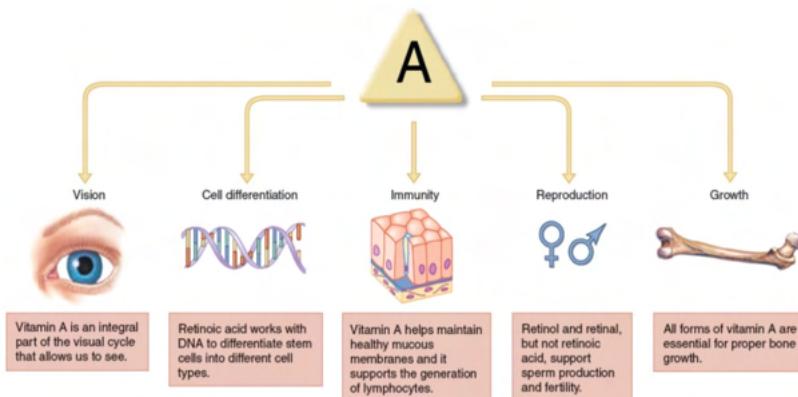


Figure 6.17: Vitamin A functions.

Explanations. Vitamin A is the precursor to rhodopsin which is an important photopigment in the rods of the eyes thus enable us to have slightly night vision. Thus, vitamin A has an impact on vision. □

Observation 6.32 We'll now briefly go through how we see things. First, it's due to 2 photoreceptors in the retina of our eyes: **rods and cones**. When light passes through the lens of the eyes, they now have to travel through the retina to reach the rods and cones. To keep things simple, the rods are useful for night vision and cones are for day vision. The rods have photopigment called rhodopsin which can be produced by vitamin A.

Observation 6.33 The best source of vitamin A is beef liver, carrots, sweet potato and chicken liver.

Observation 6.34 (Consequences). A deficiency of vitamin A can lead to **xerophthalmia, keratin**, vulnerable to infection, growth retardation, bone deformations, etc.

On the other hand, excess of vitamin A will lead to toxicity with symptoms: **fatigue, vomiting, abdominal pain, bone and joint pain, etc.** At the toxic level, it acts as a teratogen, cause discoloration of skin however can be used for acne treatment.

Remark 6.13. Furthermore, it has been associated with increased risk for hip fracture in postmenopausal women, lung cancer, CVS and total mortality.

Vitamin D

Observation 6.35 Vitamin D will have its active form once being processed by the liver and kidney, called **25-hydroxyvitamin D**. It's important for bone health and helps regulate insulin formation and secretion. It's also used as a ligand to control genes of different functions.

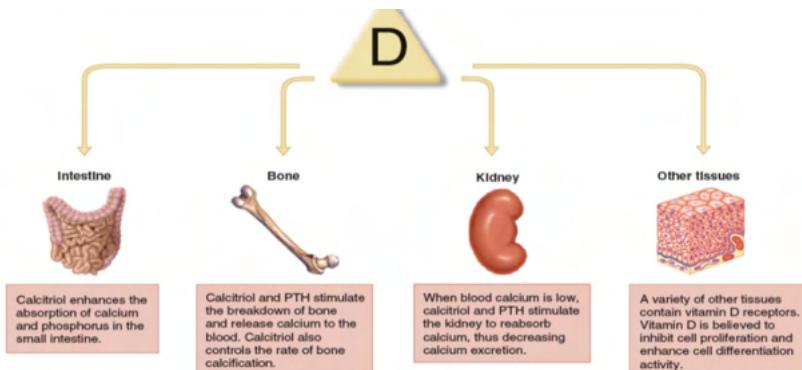


Figure 6.18: Vitamin D.

Observation 6.36 Some of the main sources of vitamin D includes cod liver oil, sardines, fortified milk and even sunlight exposure.

Explanations. For the sunlight exposure, **provitamin D₃** will absorb the UVB radiation in the skin which convert it to **previtamin D₃**, then immediately isomerize to vitamin D₃. Vitamin D₃ can then travel to the liver and kidney to form the main circulating form of 25-hydroxyvitamin D and **1,25-dihydroxyvitamin D** (biologically active). □

Observation 6.37 (Consequences). Deficiency of vitamin D leads to **rickets** in children, **osteomalacia** and **osteoporosis** in adults. Meanwhile, toxicity level of vitamin D will lead to **hypercalcemia**.

Remark 6.14. *It's false (inconclusive) to say that vitamin D has some chemoprevention effects.*

Vitamin E

Observation 6.38 There are 8 forms of compounds similar to vitamin E however only the **α -tocopherol** form is required by human. It's stored in

body fat and acts as antioxidant i.e. protects cells from free radicals and lower risk of certain chronic diseases.

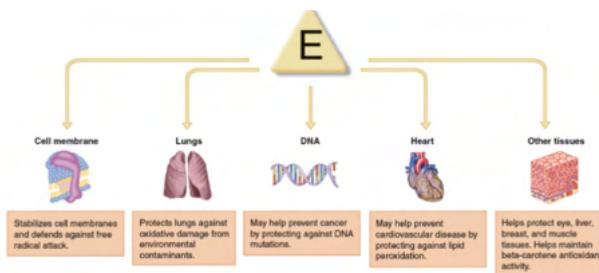


Figure 6.19: Vitamin E.

Observation 6.39 The recommended daily intake of vitamin E is 15mg/day for adults and 19mg/day for breastfeeding. The main sources of vitamin E includes: nuts, seeds, vegetable oil, fruit, etc.

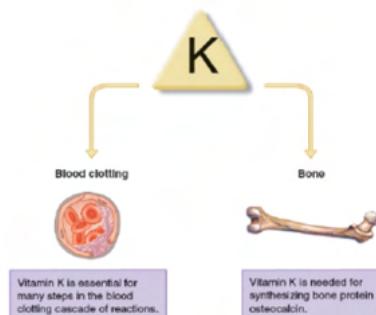
Observation 6.40 (Consequences). Deficiency in vitamin E is due to mal-absorption of fat or even rare genetic disorders.

Contrarily, for toxicity, we haven't found a lot of adverse effects however it can still interfere with blood clotting and cause lung injury if inhaled.

Vitamin K

Observation 6.41

There are 2 forms of vitamin K: **K1** and **K2**. They are used for blood clotting and bone health. The typical dietary recommendation is 120mg/day for men and 90mg/day for women. The main sources include green veggies, plant oils and intestinal bacteria.



Explanations. The reason that it's important for blood clotting is be-

Figure 6.20: Vitamin K.

cause it's needed in the synthesis of thrombin i.e. vitamin K promotes synthesis of coagulation factors. \square

Remark 6.15. *The drug **warfarin** inhibits the action of vitamin K synthesis which lead to its anticoagulant effect.*

Observation 6.42 (Consequences). Deficiency of vitamin K is rare in healthy people but higher risk in babies. Similarly, its toxicity is rare but can interact with anticoagulant medications.

6.2.2 Water-Soluble Vitamins

Water-soluble vitamins encompassed 8 vitamin B and vitamin C, all of which primarily acts as coenzyme and antioxidant respectively.

Definition 6.11. **Coenzymes** are organic non-protein molecules that is required by an enzyme to perform its catalytic activity.

Definition 6.12. **Antioxidants** are compounds that inhibit oxidation, a chemical reaction that produce free radicals.

Vitamin B1 (Thiamin)

Observation 6.43 Vitamin B1 functions as coenzyme for energy metabolism. In particular, it's the **thiamin pyrophosphate** which mainly breaks glucose to energy, makes RNA and DNA; power proteins, and help synthesize neurotransmitter.

The daily recommended dose of vitamin B1 includes 1.2mg/day for men, 1.1mg/day for women, 1.4mg/day during pregnancy and 1.5mg/day during breastfeeding.

Some of its main food sources includes: pork, legumes, nuts and seeds, fish and seafood, etc.

Remark 6.16. *Cooking will reduce vitamin B1 content.*

Observation 6.44 (Consequences). Deficiency of thiamin is called **beriberi** which is indicated through the overall muscle weakness and nerve destruction. Some milder symptoms include headache, irritation, depression and etc. Meanwhile, for toxicity, there's been no report.

Vitamin B2 (Riboflavin)

Observation 6.45 Vitamin B2 acts as coenzymes for energy metabolism but also helps antioxidants. It's mainly food in milk, dairy products, enriched grains and eggs.

Remark 6.17. *Light breaks down riboflavin easily which is why milk is stored in opaque container.*

Observation 6.46 (Consequences). Deficiency of riboflavin is called **ari-boflavinosis** which is characterized by **hyperemia** and **edema** in mouth and throat, **angular stomatitis**, **cheilosis**, etc. It occurs most often with alcoholic patient.

There's been no reported case of toxicity.

Vitamin B3 (Niacin)

Observation 6.47 Vitamin B3 acts as coenzymes for energy metabolism but also for FA synthesis. It can be found in whole and enriched grains, meat, poultry, fish but also can be synthesized from **tryptophan**⁸

Observation 6.48 (Consequences). Deficiency of niacin is called **pellagra** which is characterized by Dermatitis, diarrhea, dementia and death. It can be worsened with deficiency of vitamin B1, 6 and iron.

At toxic/high dose, niacin is used to treat high blood cholesterol with side effect of skin flushing and liver damage.

Vitamin B6 (Pyridoxine)

Observation 6.49 Vitamin B6 has an active form called **pyridoxal 5'-phosphate (PLP)**. It helps in producing non-essential amino acids, neurotransmitters, hemoglobin and even lower [homocysteine] in blood.

It can be found in meat, fish, poultry, potatoes, and etc.

Observation 6.50 (Consequences.) A deficiency of pyridoxine will leadn to **microcytic hypochromic anemia**, heart disease and even damage to nervous system such as depression, headaches and etc.

⁸An amino acid.

Toxicity of pyridoxine can cause subtle neurological damage with many other symptoms include upset stomach, headache, sleepiness and etc.

Vitamin B9 (Folate)

Observation 6.51 Vitamin B9 is an coenzyme for DNA synthesis, cell division and amino acid metabolism. It's needed for normal red blood cell maturation and can work with pyridoxine to control [homocysteine]. It can be found in fortified cereals, enriched grains, green leafy veggies, orange juice and etc.

Observation 6.52 (Consequences). A deficiency in folate will lead to **megaloblastic anemia, spina bifida⁹**, and heart disease.

A high level of folate can be used to mask the deficiency in vitamin B12. However, at this toxic level, it can lead to hypersensitive people experience hives and respiratory distress.

Vitamin B12 (Cobalamin)

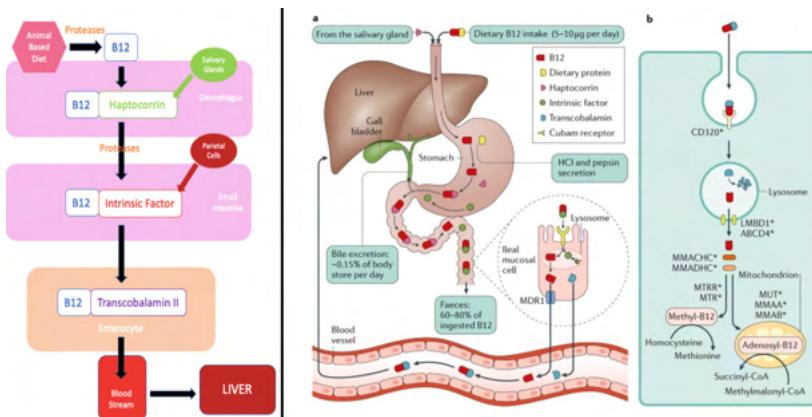


Figure 6.21: Vitamin B12 absorption.

Observation 6.53 Vitamin B12 is used for DNA, RBC synthesis and metabolism of homocysteine. It's also important to main the myelin sheath around

⁹A birth defect where the spinal chord is exposed outside of the spine.

nerves. It can be found in only animal products e.g. meats, milk, eggs, etc. and some fortified foods.

Observation 6.54 (Absorption of Vitamin B12). When vitamin B12 is absorbed, it will first be found to a carrier called **haptocorrin** and protect it from the acidic environment of the stomach. Next, when it's in the small intestine, it will be bound by **intrinsic factors** to help with absorption. Once inside the enterocyte, it will be bound by **transcobalamin II** and transport it in blood stream.

Observation 6.55 (Consequences). A deficiency of cobalamin can lead to **pernicious anemia** and **megaloblastic anemia**. On the other hand, there's no known toxicity.

Vitamin B7 (Biotin)

Observation 6.56 Vitamin B7 is a coenzyme and is essential for amino acid metabolism, FA and DNA synthesis and etc. It can be found in cauliflower, liver, peanuts, cheese.

Remark 6.18. *Avidin* is a protein (found in raw egg whites) that can bind to biotin and prevent its absorption.

Remark 6.19. Deficiency and toxicity are rare.

Vitamin C

Observation 6.57 Vitamin C functions as antioxidant. It's required for collagen synthesis, increase absorption of iron in food and is used to make different essential compounds. It can be found in fruits such as citrus, strawberries, kiwi; and in vegetables like broccoli, tomatoes, potatoes, etc. Interestingly, vitamin C cannot be bound by protein in blood and is thus cannot be stored.

Remark 6.20. Large dose of vitamin C may reduce the length of a cold however it does not protect against cold.

Observation 6.58 (Consequences). A deficiency of vitamin C will lead to **scurvy** while toxicity will lead to GI distress.



Principles of pharmacology and toxicology.
Frequently encountered drugs will be used as
a focus to illustrate sites and mechanisms of
action, distribution, metabolism, elimination
and adverse side effects.

