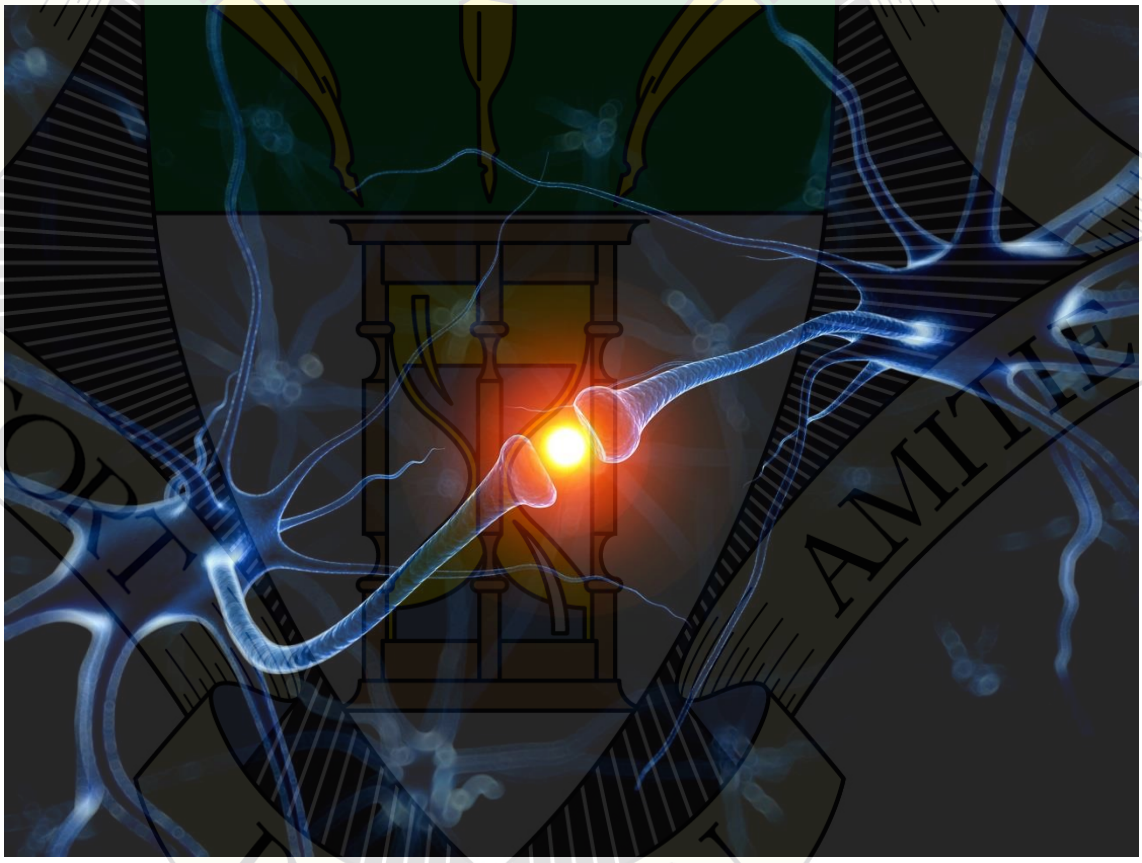
LYCÉE ERMESINDE MERSCH

The Applications of Neurotoxins

Using nature's poisons to our advantage



AN INDIVIDUAL PAPER

Author: Joanna Easter

Class: 2MB

Supervisor: François Mersch

Members of the Jury: Nathalie Jacoby and Julia Pruy

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I confirm that the work presented in this essay is my own and that I have written everything by myself.

Joanna Easter, 19th April 2015

Author's signature:

This essay has been defended and accepted on the _____

Signatures of the jury members:

Abstract

This paper will focus on the various uses mankind has found for some of nature's most poisonous substances: neurotoxins.

In an introduction to the subject, the meaning of the word "neurotoxin" will be explored. Then, in the following two chapters, the function of the nervous system will be described, as will the effects that neurotoxins can have on it.

Next, four examples of "useful" neurotoxins will be discussed in detail, with subchapters for each individual toxin treating aspects such as its mechanism of action, its origins, its historical uses, and its modern-day use in medicine. These four neurotoxins will be curare, botulinum toxin, morphine and lithium.

Finally, there will be a discussion of the ethics of using neurotoxins in medicine, followed by a conclusion.

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I. Introduction

What is a neurotoxin? Simply put, a neurotoxin is a poison which affects the body's nervous system.

The term "poison", however, is less easily defined. According to the Collins online English dictionary, a poison is "any substance that can impair function, cause structural damage, or otherwise injure the body"[1]. This definition, however, overlooks a major problem in the definition of a poison.

In the 16th century, the Swiss-German physician Paracelsus, sometimes known as "the father of toxicology", discovered that the body's reaction to a substance does not only depend on the nature of the substance, but also on the dose. Thus he claimed that "All things are poison and nothing [is] without poison; only the dose makes that a thing is no poison."

Accordingly, a poison must be defined as a substance that damages living tissue and has harmful or even fatal consequences when introduced into the body of a living organism under "appropriate conditions"[2]. These "appropriate conditions" include a sufficient dosage.

While this definition is quite correct, it does pose certain difficulties. For example, even a seemingly innocuous and essential substance such as water can be harmful if ingested in very large quantities. Does this make water a poison? Surely it can't be one, as it is essential to every living being's survival.

Due to this problem, it becomes necessary to measure a substance's toxicity. To do this, the **median lethal dose**, LD_{50}, is used. The LD_{50} is determined experimentally, typically through tests on laboratory rats or mice. It expresses the dose required to kill 50% of the tested population during a specified amount of time, and is usually expressed in mg/kg (milligrams of substance per kilogram of body mass).

It should be noted that the LD_{50} still leaves some problems unsolved. Firstly, not all the individuals tested are killed by the same dose. Some die when exposed to a far smaller dose than the LD_{50}, while others survive even this dosage. Secondly, the LD_{50} measures acute toxicity, but does not measure chronic toxicity. In other words, it doesn't allow for the fact that a substance can be fatal when an individual is exposed to low doses over a longer period of time. Also, it doesn't take non-fatal but nonetheless serious damage, for example brain damage, into account. Lastly, the difference between species is such that the LD_{50}, as tested on

[1] http://www.collinsdictionary.com/dictionary/english/poison
[2] http://www.britannica.com/EBchecked/topic/466463/poison#toc28075

mice or rats, cannot reliably be converted to apply to humans, as some substances are more toxic to rats than to humans, and vice versa.

While a substance with a relatively high LD_{50} is not fatal in lower doses, this does not mean that it is harmless. Even in small doses, such a substance can still have toxic effects on the body.

Likewise, a neurotoxin can affect the body's nervous system long before its LD_{50} is reached. These effects are often sought after, for both recreational and medicinal purposes.

Neurotoxins can be found in many different places in nature, as they are produced by many different living organisms including various animals, plants and bacteria. Some chemical elements and compounds, too, have neurotoxic properties, for example mercury and lead. While there are many natural neurotoxins there are also synthetic ones, which are man-made and can be synthesised in laboratories.

Both legal and illegal drugs contain neurotoxins, including those which are used very commonly.

Caffeine, for example, is consumed on a daily basis by a large portion of humanity. It is readily available to adults and children alike in almost every part of the world, and it is included in many popular beverages such as coffee, tea and energy drinks. Due to its high LD_{50} (estimated around 150-200 mg/kg, the equivalent of about 120 cups of coffee for the average adult) and low health risks, we generally don't think of it as a poison, but the stimulant properties of caffeine are nonetheless due to its neurotoxicity.

Alcohol and nicotine are also both used for their neurotoxic effects, and while their health risks are widely recognised, they are still legal. The same goes for illegal drugs: they are sought out for their effects on the body's nervous system, which are caused by their neurotoxicity.

No big secret is made of the fact that drugs can be extremely harmful, and from a young age we are taught by our parents, by our teachers and by the media that in using them, we seriously put our health at risk. What we are not so often taught is that the effects of neurotoxins can be turned to our advantage.

While a healthy individual's nervous system doesn't benefit from – and can easily be damaged by – being interfered with by the use of neurotoxic substances, this very interference can be useful in the treatment of numerous diseases affecting the nervous system. This means that certain neurotoxins, when used in lower than fatal doses, can be beneficial to us when used in medicine.

Poisons, as it turns out, have their uses too, and not all their applications are detrimental to human life. This paper will explore the positive side of neurotoxins, focusing on those that have been used to benefit mankind and examining both their current and their historical uses.

The subjects covered in this paper will include an introduction to the nervous system, the mechanisms of action of different types of neurotoxins, and a number of examples looked at in greater detail –including their origins, effects and uses. These examples will be followed by a discussion of the ethics involved in the use of neurotoxins in medicine, and an overall conclusion to the work presented.

II. An introduction to the nervous system

In order to understand how neurotoxins affect our bodies, it is first necessary to familiarise oneself with the functioning of the healthy nervous system.

The nervous system is the "control centre" of the body, responsible for perceiving our environment using our five senses, relaying the information obtained back to the brain, examining it, taking a decision based on it, and then conveying the order to act to whatever muscle is appropriate.

Not only does it control our voluntary movements, but it is also responsible for the involuntary muscular movements that assure the continued functioning of our vital organs – for example, it keeps our heart beating without the need for a conscious decision or effort.

The nervous system consists of two main parts: the brain and the spinal cord, forming what is known as the **central nervous system** (CNS), and the **peripheral nervous system** (PNS), which consists of the nerves that connect the CNS to the rest of the body.

The nervous system's defining characteristic is the presence of **neurons** – cells responsible for the efficient transmission of information throughout the body. Thus, every nerve is in fact a long bundle of individual neurons. Neurons can be classified according to their function: there are **afferent** or **sensory neurons**, responsible for sending information from the organs into the CNS; **efferent** or **motor neurons**, which relay signals from the CNS to the rest of the body; and **interneurons**, which connect neurons within the CNS.

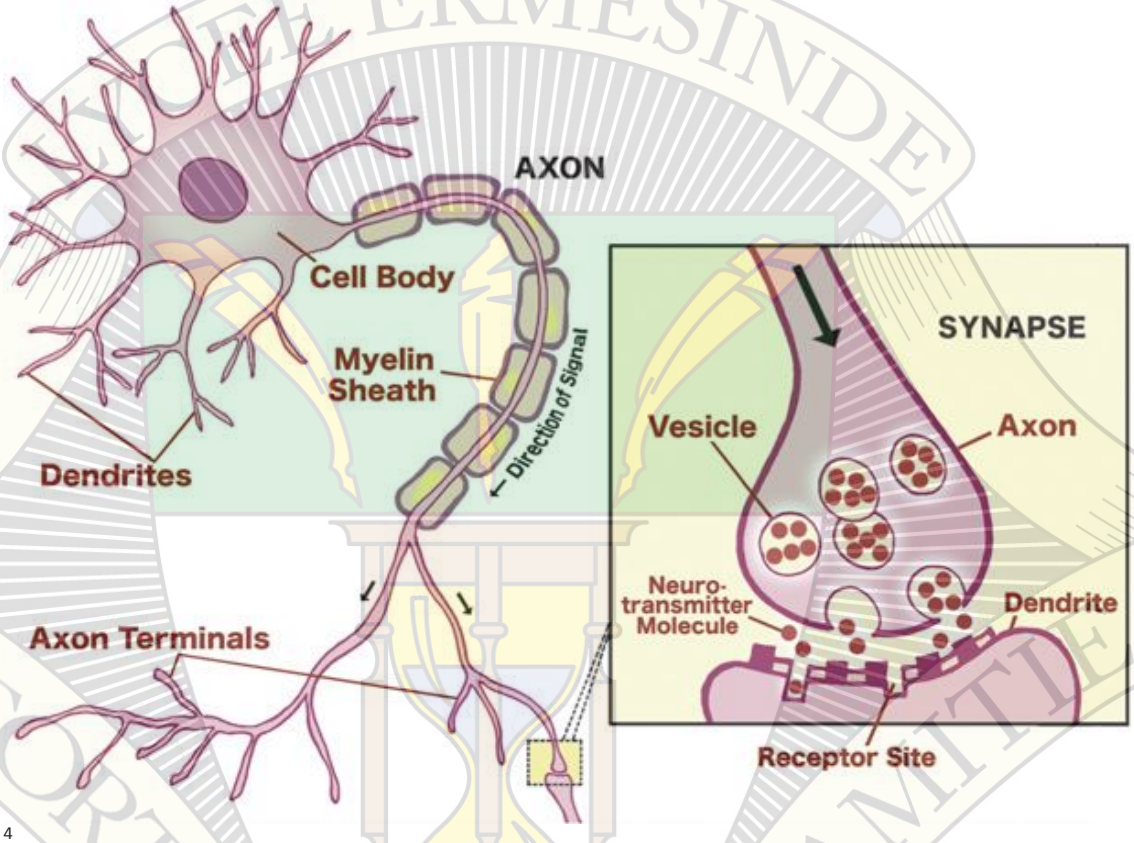
These neurons can connect to each other in two different ways:

- Electrically: The neurons are directly touching, letting an electrical current, called an **action potential**, pass from one to the next.
- Chemically[3]: The neurons are separated by a small gap called the **synaptic cleft**. Information is passed from one neuron to the next, across the synaptic cleft, in the form of a chemical signal. The chemicals which enable this communication between one neuron and the next are called **neurotransmitters**. They are stored in the **synaptic vesicles** of the first neuron, referred to as the **presynaptic** neuron, and are released from its **synapses** into the synaptic cleft. On the other side, the neurotransmitters bind to **neurotransmitter receptors** on the **dendrites** of the next neuron, referred to as the **postsynaptic** neuron.

[3] See figure I

This causes an action potential to be transmitted through the cell body of the neuron (the **soma**) and the **axon** until it finally reaches the **axon terminals**, which contain the synapses. Here, the action potential causes the release of more neurotransmitters. These then cross the synaptic cleft to the next neuron, and the process repeats itself.

Common neurotransmitters include dopamine, acetylcholine, norepinephrine (also known as noradrenaline), and serotonin.



[4]

Figure I: Synaptic transmission

How is an action potential created?

The inside of a neuron contains a number of large, negatively charged proteins as well as positively charged potassium ions (K^+). From the outside, the neuron is bathed in a fluid containing sodium ions (Na^+) and chloride ions (Cl^-). This is called the **extracellular fluid**.

It should be noted that while the K^+ concentration inside the cell is higher than it is on the outside, the extracellular fluid does contain some potassium ions. Likewise, the concentration of Na^+ is higher in the extracellular fluid, but the inside still contains a small amount of Na^+.

[4] Source for figure I: http://www.urbanchildinstitute.org/sites/all/files/databooks/2011/ch1-fg2-communication-between-neurons.jpg

Just like every other human cell, the neuron is surrounded by a selectively permeable membrane, separating the inside of the cell from the extracellular fluid. Made mainly of a lipid bilayer, a double layer of largely impermeable lipid cells, the cell membrane also contains a number of protein molecules embedded in it. These can either allow ions to pass from the inside of the cell to the outside and vice versa, in which case they are referred to as **ion channels**, or they can actively transport them in or out of the cell, in which case they are called **ion transporters** or **ion pumps**. The ion channels can either be **gated**, meaning that they only open and allow ions to pass under a specific circumstance, for example when the neuron is stimulated by a neurotransmitter, or ungated, in which case the ions can pass freely.

These ion channels and pumps are usually limited to specific ions – for example the **sodium-potassium pump**, which transports three Na^+ ions out of the cell for every two K^+ ions it transports in. This plays a large role in establishing the aforementioned difference in ion concentrations between the intra- and extracellular fluid.

Though the concentration of K^+ ions is higher inside the neuron, there are **potassium channels** which allow the K^+ to leave the cell. The potassium ions do so, following the general rule that substances tend to move from areas of high concentration to areas of lower concentration. This process is called **diffusion**. However, as the K^+ ions leave the cell, an electrical force pulls them back inside, as they are attracted by the negatively charged proteins. In the end the electrical force and the diffusion reach an equilibrium, and the cell reaches what is called its **resting membrane potential** – a difference in electrical potential between the inside and the outside of the cell. The value of this resting potential is around -70 mV.

Then there is the **action potential**, which occurs when a neuron is excited. When the neuron is stimulated, for example by a neurotransmitter, some of the **sodium channels**, which are gated, open. This allows Na^+ ions to enter the cell, making the inside of the neuron less negative. This process is called **depolarization**.

If the membrane potential reaches a **threshold** level of -55mV, the remaining sodium channels open, allowing more positively charged sodium ions to enter the cell and causing the membrane potential to shoot up to around +40mV. This happens all along the length of the axon, sending the now positive charge from one end of the neuron to the other. Once the action potential reaches the axon terminals, it causes calcium channels to open, allowing Ca^+ ions to enter the cell. This in turn causes neurotransmitters to be released from the synapse.

Then the sodium channels close and the potassium channels open, letting the K^+ ions leave the cell and causing the membrane potential to sink once more. This is called **repolarisation**.

However, so many potassium ions leave the cell that it is then more negative than it was before the process began, and the membrane potential reaches around -90mV: **hyperpolarisation** occurs. In order to bring the membrane potential back to -70mV, the sodium-potassium pump exchanges the K^+ ions, which are now outside the neuron, for the Na^+ ions on the inside, bringing the ion concentration back to where it was before the action potential occurred. This process takes a small amount of time, called the **refractory period**. During this period, no new action potential can occur.

What happens to the neurotransmitter after it binds to the receptors on the postsynaptic neuron?

Once the post-synaptic receptors have recognised the neurotransmitter bound to them, it is released back into the synaptic cleft. There, it must quickly be deactivated to prevent it from binding to the receptors again and firing surplus action potentials. This is achieved in one (or a combination) of two main ways.

- Reuptake: The neurotransmitter is brought back to the presynaptic cell by transporter proteins. There, they are "recycled" by being stored in a vesicle until they can be used to transmit a new chemical signal.
- Degradation: Neurotransmitter-specific enzymes located in the synaptic cleft quickly break the neurotransmitter down into its components, which can no longer stimulate the post-synaptic neuron. These components then either diffuse into the extracellular fluid where they no longer play a part in synaptic transmission, or they are "recycled" by being used to produce more of the neurotransmitter they originally came from. Some neurotransmitters are also taken back into the presynaptic neuron and broken down there.

III. How do neurotoxins work?

Neurotoxins can affect the nervous system in a variety of ways, interfering with neuronal transmission during any phase. This can include influencing either the electrical signal passed through the neuron, or the synaptic transmission of a chemical signal between neurons. This interference can be achieved in a number of different ways, some of the most important of which are listed below.

Antagonists

- Preventing the creation of an action potential through inhibition of the ion channels: The neurotoxin blocks the Na^+ or K^+ channels essential for the creation and transmission of an action potential. The presynaptic cell can receive information, but cannot fire an action potential to pass it on.
- Preventing the release of neurotransmitters: The neurotoxin blocks the Ca^+ channel, and thus prevents the presynaptic cell from releasing any neurotransmitters. The action potential is fired in the presynaptic neuron, but the information never reaches the postsynaptic cell.
- Influencing neurotransmitter production: The neurotoxin interferes with the production of neurotransmitters within the neuron. A lack of neurotransmitters prevents a chemical signal being sent from one neuron to the next.
- Influencing neurotransmitter transport: Some neurotransmitters which are produced within the neuron are transported to the synapse inside microtubules. Here, the neurotoxin inhibits the proper functioning of these microtubules, resulting in a lack of neurotransmitters to be released into the synaptic cleft.
- Inhibiting the release of neurotransmitters: The neurotoxin prevents the neurotransmitters from being released from the synaptic vesicles. No chemical signal can be sent.
- Blocking receptor molecules: There are two ways for a neurotoxin to block receptor molecules.

  1) It binds to the receptor in the same place as the neurotransmitter should, occupying the space and thus blocking the receptor molecule. This is called competitive binding.

  2) The neurotoxin binds to a different part of the receptor. This does not block the space where the transmitter should bind, however the neurotoxin causes the receptor to change shape or otherwise to reject the neurotransmitter. This is called non-competitive binding.

In both cases, the neurotoxin prevents the postsynaptic neuron from receiving a signal.

Looking at the mechanisms of action above, it becomes apparent that they all result in a lack of communication between neurons. The neurotoxins which reduce or inhibit synaptic transmission are collectively known as **antagonists**.

Agonists

- Preventing reuptake: The transporter molecules responsible for recapturing "used" neurotransmitters and bringing them back to the presynaptic cell for "recycling" are disabled. The neurotransmitter will then keep on binding and re-binding to the receptor molecules on the post-synaptic cell, increasing its effect.
- Preventing degradation: The enzymes responsible for breaking "used" neurotransmitters down are disabled. The effects are as above.
- Imitating neurotransmitters: The neurotoxin binds to the receptor molecules on the postsynaptic membrane in the neurotransmitter's place. The receptor molecules then mistake it for the transmitter and react accordingly. This results in a "false" signal being sent.

These mechanisms of action increase synaptic transmission. The neurotoxins which act this way are known as **agonists**.

IV. Curare

1) Introduction

It is often assumed that curare is the name of one specific toxin. This is incorrect: in fact, curare is a collective term for a number of different toxins. What these toxins have in common is that they are all toxic organic compounds of botanical origin. They also all originate from South America, where they have long been used as arrow poison in hunting by indigenous peoples. The name "curare" is derived from the South American word "woorari", which means "poison".

Figure II : *Strychnos toxifera*

Curare is made from a large variety of plants, most of which come from the family Menispermaceae (e.g. *Chondrodendron tomentosum*, a type of indigenous liana) or the *Strychnos* genus of the family Loganiaceae (e.g. *Strychnos toxifera*). [5]

These were prepared in various combinations, together with other plants which were added to create the right consistency. Sometimes, poisonous insects and spiders or the fangs of venomous snakes were also added to the preparation in hope that these would increase the potency of the poison. It is possible that these ingredients did have some effect, but the main toxic components that have been identified in curare are those of botanical origin.

The resulting substance is a dark brown resinous paste with an aromatic, tarry odour and a bitter taste. This was then added to the tips of blow darts, which the natives shot through hollow bamboo canes.

2) Classification and chemical description

In 1895, the German pharmacologist Professor Rudolf Boehm decided to classify different types of curare by the containers in which the South American natives stored and transported them. He came up with the following three main categories:

- Tube curare, which was kept inside hollowed-out bamboo canes
- Pot curare, which was stored inside earthenware jars or pots

[5] Source for figure II: http://en.wikipedia.org/wiki/File:Strychnos_Toxifera_by_Koehler_1887.jpg

- Calabash curare, which was stored inside hollowed-out gourds

Variety of Curare	Type of Alkaloid	Name (Boehm)	Composition	Activity
Tube curare	Amorphous quaternary	Tubocurarine	$C^{19}H^{21}NO^4$	++++
	Crystalline tertiary	Curine	$C^{18}H^{19}O^3N$	+
Pot curare	Amorphous quaternary	Protocurarine	$C^{19}H^{20}O^2N$	+++
	Crystalline tertiary	Protocurine	$C^{20}H^{23}O^3N$	+
	Crystalline tertiary	Protocuridine	$C^{19}H^{21}O^3N$	+
Calabash curare	Amorphous quaternary	Curarine	$C^{19}H^{26}N^2O$	++++

Figure III: Boehm's table of curare classification and the chemical composition of the active substances

[6]

Each one of these types of curare has a different chemical composition, though they all contain neurotoxic **alkaloids**[7].

In this table, "crystalline" and "amorphous" refer to the structure of the substance. An amorphous substance is a substance that isn't crystalline, and can also be called non-crystalline. "Tertiary" and "quaternary" refer to the number of carbon atoms to which the nitrogen atoms are bound.

The last column on the right indicates the paralysing activity shown by each substance. The amorphous quaternary alkaloids are highly active in this respect, while the crystalline tertiary amines are hardly paralysing at all. Some of the latter have nonetheless proved to be toxic in other ways.

The most well-known and also the most toxic of these alkaloids is tubocurarine, also known as d-tubocurarine, which was later found to be useful in medicine.

Note: The above table was sourced from a paper written in 1942. It has been included here as it gives a good overview of the different alkaloids contained in each variety of curare. However, there is at least one error in the molecular formulas listed, as other, newer sources consulted[8] give $\mathbf{C_{37}H_{41}N_2O_6}$ as the molecular formula of tubocurarine.

[6] Source for figure III: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1940167/pdf/annrcse00004-0029.pdf

[7] Organic compound with basic chemical properties and containing at least one nitrogen atom (N). Many alkaloids are pharmacologically active. Thus they are often toxic, and some are used as recreational or medicinal drugs.

[8] E.g. http://www.chemspider.com/Chemical-Structure.5778.html

This second formula is the accurate one. It can only be assumed that the discovery of the correct formula relatively recent.

Because of this inaccuracy in the table, we may also wonder whether the other molecular formulas listed in the table are correct, but this question remains

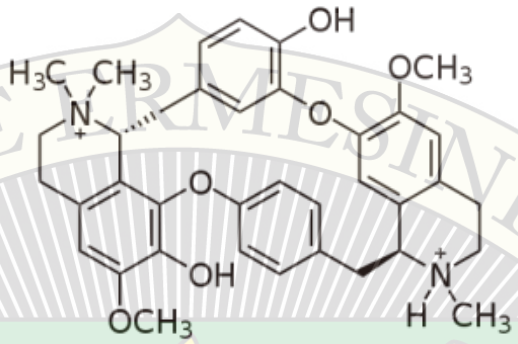


Figure IV: Tubocurarine molecule

unanswered, as there is only very limited information available on the subject.

[9]

3) Toxicity and mechanism of action

Though there is more than one form of curare, they all have one specific property in common: their paralysing effect.

As the nervous system has to send information in order for a muscle to move, it logically follows that these substances that cause paralysis do so by obstructing the necessary communication between neurons. This is achieved through the blocking of the nicotinic acetylcholine receptors (nAChR), one of the two subtypes of acetylcholine (Ach) receptor, through competitive binding. The nAChR are found at the neuromuscular junction, where the nervous system and the muscular system are connected. Thus the order for the muscles to move doesn't arrive at its destination, and this results in paralysis.

This muscular paralysis begins at the toes, the eyes and the ears before spreading to the neck and limbs, and finally reaching the muscles involved in respiration, notably the diaphragm. Once the diaphragm is paralysed respiration ceases, and death by asphyxiation ensues.

As curare only affects skeletal muscle, the cardiac muscle is not paralysed. This means that the heart is not immediately affected, though it will stop beating several minutes after respiratory paralysis sets in, due to oxygen deprivation.

[9] Source for figure IV: http://naturespoisons.com/2014/05/13/curare-from-paralyzed-to-anesthetized-tubocurarine/

The human LD_{50} of curare has not been established, but it is thought to vary from one variety to the next.

Interestingly, as death is caused purely by asphyxiation, it is possible to keep the subject alive purely through artificial respiration. The paralysing effects of the curare eventually wear off, and normal breathing begins again, leaving the subject with no ill effects whatsoever.

It is also interesting to note that animals shot by curare-poisoned arrows are still edible. This means that curare is only dangerous to humans when it enters the bloodstream, and is harmless when ingested. Research shows that this is due to our slow absorption and rapid excretion of the substance, as the lining of the digestive tract is difficult for curare toxins to pass through and they are excreted fairly quickly through the urine.

As the paralysing alkaloids in curare bind competitively to the nAChR rather than causing the receptors to reject ACh molecules, their effect can be counteracted with substances that increase the amount of ACh. This excess ACh then binds to the receptors that are not yet blocked, counteracting the paralysing effect of the toxins. For example physostigmine, an alkaloid derived from the Calabar bean, is an antidote to curare as it inhibits the enzymes responsible for breaking down acetylcholine molecules in the synaptic cleft.

4) History

We do not know how long curare has been used by native South Americans, but reports of similar or identical arrow poisons have reached Europe since 1596, when Sir Walter Raleigh described his travels through what is today known as Guyana. Later, missionaries also described the lethal arrow poisons used for hunting by the natives. However, very little was known about the substance until the early nineteenth century, when Alexander von Humboldt and Aimé Bonpland travelled South America and upon their return described for the first time how they had seen natives living near the Orinoco River prepare the poison. Also during the first half of the nineteenth century, Sir Benjamin Collins Brodie and Charles Waterton both experimented with curare, proving that an animal subjected to the poison could be kept alive using artificial respiration, and a little later the botanist Robert Hermann Schomburgk identified and named a vine commonly used in curare, *Strychnos toxifera*.

It was the British chemist Harold King, however, who first isolated an active ingredient from curare. In 1935, he obtained a sample of tube curare from a museum, and from that sample he extracted tubocurarine.

5) Use in medicine

After King's discovery of tubocurarine became known, the substance quickly came to the attention of medical professionals. Under the form of d-tubocurarine chloride, the substance was found to be very useful in anaesthesia, where it was used especially in situations that required absolute muscular relaxation, for example during abdominal surgery. Though it is rarely used today, the use of d-tubocurarine chloride in anaesthesia that began in the 1940s paved the way to the invention of the synthetic muscle relaxants used in anaesthesia today, for example rocuronium and pancuronium.

Tubocurarine can also be used to control the muscle spasms in people suffering from tetanus.

V. Botulinum toxin

1) Introduction

Botulinum toxin is another paralytic, this time of bacterial origin. It is produced by the bacterium *Clostridium botulinum*. Botulinum toxin has seven well-known subtypes, designated with the letters A through to G, as well as an eighth type, known as type H, discovered in 2013.

Of these eight types, four (types A, B, E and F) are associated with illness in humans. The properties of type H remain, as yet, relatively unknown.

These subtypes are extremely toxic. In fact, these forms of botulinum toxin are the most deadly toxins known to man, with a human LD_{50} of 1 ng/kg if administered intravenously, subcutaneously or intramuscularly, and 3 ng/kg if inhaled. Thus it may come as a surprise to many that the subtypes A and B have numerous medical and even cosmetic applications, and are available under various brand names, one of which is certainly known to the vast majority of us – the trade name "Botox".

2) *Clostridium botulinum*

Clostridium botulinum, which produces botulinum toxin, is a rod-shaped, spore-forming bacterium. These spores are not, as it would be natural to assume, involved in reproduction – they are instead a way for the bacterium to survive in unfavourable conditions. Thus, the spores can lie dormant for years until the conditions become favourable and the bacterium grows active once more. It is also an obligate anaerobic, meaning that oxygen is toxic to the bacterium and that it cannot survive in normal atmospheric oxygen concentrations. However, in spore form, *C. botulinum* can survive these oxygen concentrations, merely remaining dormant during this time.

There are four known phenotypic groups of *C. botulinum* bacteria, known as groups I to IV, each producing one or more subtypes of botulinum toxin.

Phenotypic group		I	II	III	IV
Toxin subtype		A, B, F	B, E, F	C, D	G
Growth temperature (°C)	**Optimum**	35 – 40	18 – 25	40	37
	Minimum	12	3.3	15	N.A.

Figure V: phenotypic groups of C. botulinum

Furthermore, two other bacteria of the *Clostridium* genus have been found to produce botulinum toxin: *C. butyricum*, producing toxin E, and *C. baratii*, producing toxin F. Nonetheless, *C. botulinum* remains the main cause of botulinum toxin poisoning.

C. botulinum is found in the soil and marine sediments all over the world, and can thus contaminate vegetables growing in the soil or colonise the gastrointestinal tract of various animals.

3) Chemical description

Botulinum toxin is a protein consisting of two polypeptide chains – a heavy chain and a light chain – linked by a disulphide bond. A polypeptide chain is an unbranched chain of amino acids linked by covalent bonds.

4) Mechanism of action

Just like curare, botulinum toxin is an antagonist, and causes paralysis. Unlike curare, it does this not by blocking ACh receptors, but by preventing the release of the neurotransmitter from the presynaptic cell.

Both the heavy chain and the light chain contribute to the protein's toxicity. The heavy chain binds to proteins on the outside of the neuron, from where the toxin is taken into the cell. Once inside, the light chain acts like a protease[10] and breaks down a protein responsible for the release of neurotransmitters into the synaptic cleft. The particular protein affected here is known as a SNAP-25 protein, a type of SNARE protein. Without this protein, the neurotransmitters, particularly ACh, cannot be released, and the signal that results in a muscular contraction cannot be sent. The result of this is a flaccid paralysis.

5) Toxicity and botulism

Exposure to botulinum toxin results in a potentially fatal paralytic illness called botulism. In severe cases, death can be caused by respiratory arrest. Treatment, which involves the use of specific antitoxins, drastically lowers the mortality rate. In severe cases respiratory support may be needed, sometimes for several months.

There are three main forms of botulism, which will be described in detail below.

[10] Enzyme which breaks down proteins

- Infant botulism

  This form of botulism occurs when infants swallow *Clostridium botulinum* spores, which then colonise the gastrointestinal tract and germinate there, becoming active due to the oxygen-free conditions. The bacterium then begins producing botulinum toxin.

  In adults and older children ingested spores do not generally pose a health risk, as the body's immune system prevents them from germinating. Infants, however, have a less well-developed immune system. Therefore infant botulism generally only occurs in children under the age of one year.

  The main cause of infant botulism is the consumption of contaminated honey. For this reason, it is recommended not to feed honey to babies under one year of age.

  The incubation period for infant botulism is unknown, and estimates range from 3 to 30 days.

  The first sign of infection is often constipation. Further symptoms include weakness, lack of appetite, a weak cry, a floppy head and neck, resulting in "a striking loss of head control"[11], and, in severe cases, respiratory paralysis.

  With proper treatment, the mortality rates of infant botulism are less than 2%.

  Note: Botulism caused by the ingestion of *C. botulinum* spores and the subsequent colonisation of the gastrointestinal tract can also affect adults and older children, though extremely rarely. The individuals affected usually have a history of bowel disease, abdominal surgery or immunodeficiency, or they have recently taken antibiotics.

- Food-borne botulism

  The illness is caused through ingestion of foods contaminated by *Clostridium botulinum*. As the bacterium only flourishes in very low-oxygen or oxygen-free environments, the main sources of food-borne botulism are improperly preserved foods. For this reason, food-borne botulism cases became more frequent during the early twentieth century when canned foods first became widely available. Today the commercial canning process is strictly controlled, so the main sources of food-borne botulism tend to be home-canned foods or freshly prepared, tightly wrapped but improperly refrigerated foods. Unrefrigerated baked potatoes, for example, are a relatively high-risk food.

  Generally, storage at room temperature and low acidity content make a food more susceptible to *Clostridium botulinum* spores germinating, and thus producing botulinum

[11] http://www.who.int/mediacentre/factsheets/fs270/en/

toxin. Though *C. botulinum* spores are heat-resistant, the toxin itself is not, so the risk can largely be neutralised by thoroughly heating the food above 85°C for over five minutes.

The symptoms of food-borne botulism include fatigue, blurred or double vision, slurred speech, drooping eyelids, dryness of the mouth, difficulty swallowing, muscular weakness, and paralysis, beginning at the neck and shoulders and descending from there. Vomiting, diarrhoea or constipation, and abdominal pains can also occur. Severe cases lead to respiratory paralysis.

The incubation period for foodborne botulism is about 12 - 36 hours. Mortality rates are around 5 - 10% with treatment, and 60% without treatment.

- Wound botulism

  This is a rare form of botulism in which the toxin enters the body through a wound. This can happen when a wound comes into contact with contaminated soil or other contaminated substances, but it is often connected to intravenous drug use. It is particularly associated with the injection of black tar heroin.

  The symptoms are similar to those of food-borne botulism, though the gastrointestinal symptoms generally aren't present. The incubation period can be as long as two weeks.

  Wound botulism is often treated surgically by removing the source of the toxin. The treatment also includes the use of the previously mentioned antitoxins. The mortality rate of properly treated wound botulism is around 7%.

6) History

The first full description of food-borne botulism was published by the German medical officer and poet Justinus Kerner between 1817 and 1822. Though he did not succeed in finding the origin of the toxin he suspected was responsible for the condition, he did find that the illness was related to the consumption of badly-preserved meat products, particularly sausages. Thus, he simply referred to the cause of the illness as "sausage poison". Kerner then went on to animal experiments as well as clinical experiments on himself, trying to find out exactly how the toxin worked. The effects he observed led him to envision the possible therapeutic use of the toxin.

In 1870, another German physician by the name of Muller named and further described botulism. The name he chose comes from the Latin *botulus*, meaning sausage.

A further development was made in 1895 when an outbreak of botulism occurred in the small Belgian village of Ellezelles, where the guests of a funeral dinner all fell ill some days after

attending. The poisoning was thought to have been caused by the smoked ham which had been served. From this ham, the Belgian bacteriologist Emile van Ermengem managed to extract *C. botulinum*, the bacterium that causes botulism.

In 1944, the American biochemist Dr Edward Schantz succeeded in isolating not only the bacterium, but the toxin itself – more specifically, botulinum toxin type A. He began his research on the subject during World War II, when he was stationed at Fort Detrick, Maryland, to explore toxins and bacteria for use in warfare. After President Nixon signed the Biological and Toxin Weapons Convention in 1972, the research facility at Fort Detrick was closed. Schantz, however, continued his research of botulinum toxin from the University of Wisconsin, switching his interest in the toxin from military to medicinal.

During his time at Fort Detrick, Schantz was contacted by Alan B. Scott, an eye surgeon who was interested in botulinum toxin for the possible treatment of strabismus, a condition colloquially known as "crossed eyes", where the eyes are unable to both focus on the same point in space. Scott researched the idea extensively, conducting tests first on monkeys, then on humans, and came to the conclusion that the treatment was effective.

The research of Edward J. Schantz and that of Alan B. Scott laid the groundwork for the many current applications of botulinum toxin, both in medicine and in cosmetics.

7) Medical and cosmetic applications

Botulinum toxins type A and B are used in the treatment of various medical conditions involving abnormal muscular contractions, and type A is also used for cosmetic purposes.

The toxin is administered by injection in minute doses, which allows the effect to remain local. However, the effects are temporary and the injections have to be renewed regularly.

Botulinum toxin type A is available under the brand names of BOTOX, Dysport and Oculinum, while type B is marketed as Myobloc or Neurobloc.

Medicinal uses

Botulinum toxin is used in the treatment of the following medical conditions:

- Strabismus
- Blepharospasm, an involuntary twitch of the eyelid.
- Hemifacial spasm, a condition characterised by involuntary muscular contractions on only one side of the face.
- Cervical dystonia, also known as spasmodic torticollis, a painful disorder which causes involuntary neck movement and eventually spasms in the neck muscles.

- Achalasia, a condition that prevents the oesophagus from moving food into the stomach. This is due to a lack of muscular relaxation.
- Cerebral palsy
- Hyperhidrosis, excessive sweating.
- Overactive bladder syndrome
- Migraines

Cosmetic uses

Only botulinum type A, sold under the well-known brand name BOTOX, is used for cosmetic purposes. It is used to treat wrinkles, for example glabellar frown lines (vertical lines between the eyebrows that are caused by frowning or squinting), horizontal lines on the forehead, crow's feet, or horizontal wrinkles on the bridge of the nose.

[12]

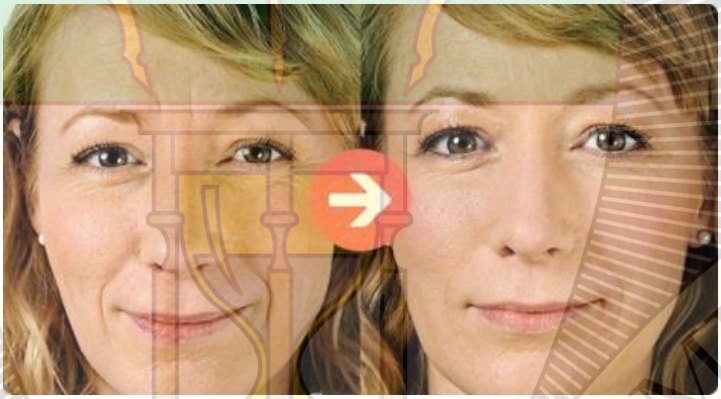


Figure VI: Woman before and after having her glabellar frown lines treated with BOTOX

As facial wrinkles are caused by muscular contraction, injecting small quantities of diluted botulinum toxin can temporarily stop the process by causing local paralysis.

The effect becomes visible 24-72 hours after the treatment. In order to maintain it, the injections have to be repeated every 2 to 6 months, as their effects are only temporary.

BOTOX has become more and more popular, with many people seeing the non-surgical, minimally invasive procedure as an easy way to improve their looks. A study conducted by the International Society of Aesthetic Plastic Surgery showed that 5,145,189 BOTOX treatments had been performed in 2013, 1,271,739 of which took place in the USA.

Risks and possible side effects

The side effects following the use of botulinum toxin can include the following symptoms:

[12] Source for figure VI: http://www.skinclinicwales.co.uk/results/

- bruising, soreness or swelling at the injection site
- dry eyes, drooping eyelids, blurred or double vision
- dryness of the mouth, difficulty swallowing or articulating
- headaches, neck pain

Allergic reactions including itchy skin, rashes, welts, dizziness, fainting, wheezing and asthma-like symptoms have also been reported.

Many of these side effects are caused by the toxin spreading beyond the injection site and affecting muscles elsewhere. For example, the use of Botox to treat glabellar frown lines may result in drooping eyelids, as they are fairly close to the injection site. In large doses, the botulinum toxin could spread to the respiratory muscles, causing potentially fatal respiratory paralysis.

When used for cosmetic purposes, the botulinum toxin is injected in minute doses. This means that any side effects that may occur are relatively harmless and also temporary, just like the desired effect.

When used to treat medical conditions, however, the dose of toxin administered is usually significantly larger. Accordingly, this can result in much more serious side effects. The risk seems particularly high when the toxin is used to treat children with cerebral palsy, and a number of deaths have been associated with the treatment.

No deaths have been reported in connection with botulinum toxin used for cosmetic purposes.

8) Botulinum toxin as a biological weapon

Due to its extreme toxicity, botulinum toxin has often been considered for use as a biological weapon.

The use of botulinum in warfare goes back to the 1930s, when the Japanese gave their prisoners food contaminated with *C. botulinum* following their invasion of Manchuria. At this time, of course, it was the bacterium they used rather than the toxin, as this had not been isolated yet.

This was achieved by Edward Schantz during the Second World War, when he himself was researching the use of botulinum as a bioweapon for the USA. The Allies feared that the Germans, too, had weaponised the bacterium, and set about creating a vaccine for their troops. Thankfully neither the Allies nor the Axis powers used the toxin.

In 1991, after the Gulf War, a United Nations inspection team reported that Iraq had admitted to having produced 19000 litres of botulinum toxin, more than half of which was used in

weapons. These weapons included 13 missiles with a 600km range, as well as about 100 bombs. The total amount of botulinum toxin produced was three times the amount needed to kill the current world population by inhalation. Claims by the Iraqi government that the toxin was destroyed immediately after the war have not been confirmed, though further investigations by the USA have found no evidence to the contrary.

The former USSR had also developed botulinum toxin as a biological weapon. To the present date, three other countries are believed to have done so: Iran, North Korea and Syria.

Not only governments have envisaged the use of botulinum as a weapon, however. It has also been used in at least three attempted terrorist attacks by the Japanese cult Aum Shinrikyo between 1990 and 1995. The organisation dispersed aerosols in Tokyo as well as in US military installations in Japan. However, the attacks failed for an unknown reason. Had they succeeded, they would have been catastrophic, especially in densely populated areas such as Tokyo. A point-source aerosol diffusing botulinum toxin is estimated to kill or incapacitate 10% of the population within 500m downwind of it.

The main difficulty involved in the weaponisation of botulinum toxin is the stabilisation of the toxin.

VI. Morphine

1) Introduction

Morphine is an opiate – a narcotic alkaloid derived from the opium poppy, *Papaver somniferum*. It is named after Morpheus, the mythological Greek god of sleep and dreams.

It is the main active ingredient of opium, and the substance from which heroin is derived. It's a powerful analgesic, relieving pain by affecting the central nervous system. It is also highly addictive.

The lethal dose of morphine varies quite a bit depending on the user and the route of exposure, as frequent users quickly develop a tolerance for the substance which significantly raises the lethal dose. Also, the LD_{50} of morphine taken orally is much higher than if it is injected intravenously, as a smaller percentage of the substance is actually absorbed when it is ingested. In spite of these difficulties, the human LD_{50} of orally taken morphine has been established to be around 200mg. It is important to note that this figure refers to 200mg of substance per person, not per kilogram of body mass, as the latter would place its toxicity on par with that of caffeine. Needless to say, morphine is significantly more toxic.

2) The opium poppy

The opium poppy, with its greyish-green leaves and red, white, pink or mauve flowers, has long been prized as an ornamental garden plant.

It is also the source of poppy seeds, which are an important foodstuff, being used in baking and cooking all over the world, not only in the form of seeds but also in the form of poppy seed oil.

It may therefore seem surprising that the very same plant is the source of some of the most addictive substances known to man: opium, and its derivative heroin.

[13]

Figure VII : Opium poppy (*Papaver somniferum*)

The Latin name of the plant already indicates its properties: *Papaver somniferum* literally translates as "sleep-bringing poppy".

[13] Source for figure VII: http://www.pfaf.org/Admin/PlantImages/PapaverSomniferum.jpg

The opium poppy contains latex, milky white in colour when fresh, which leaks out when the plant is damaged or cut. This is present in every part of the plant except for the seeds, especially the seed pods. This latex contains various alkaloids, including morphine. When it dries, it becomes resinous and brown in colour. This dried latex is raw opium.

Traditionally, opium is produced by scoring the seed pods of the opium poppy and letting the latex dry there before scraping it off.

The latex from *Papaver somniferum* generally has a morphine content of roughly 12% and also contains a large number of other alkaloids including codeine and papaverine, both of which have medical applications. The concentration of all these alkaloids can be significantly raised through selective breeding, meaning that many crops of opium poppy grown for their narcotic or medicinal properties have a morphine content that is higher than 12%.

The opium poppy contains the highest concentration of narcotic alkaloids, but other species of the *Papaver* genus do contain smaller amounts.

Though the seeds of *Papaver somniferum* do not contain the latex from which opium is made, they do contain very small amounts of narcotics. These amounts are so small that they do not have any noticeable effect on the human body. However, it has been demonstrated that a person who has recently consumed poppy seeds – for example two poppy seed bagels – can test positive for narcotics.

3) Chemical description

The molecular formula for morphine is $C_{17}H_{19}NO_3$. It is an alkaloid based on benzylisoquinoline, but it has two additional ring closures.

Its structure is similar to that of other opiates.

[14]

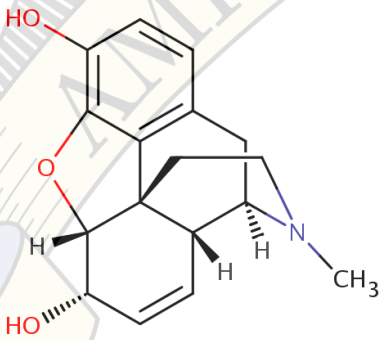


Figure VIII: Morphine molecule

4) Mechanism of action and toxicity

When one talks about opioids[15], one automatically thinks of substances the body is exposed to or receives from the outside. In truth, however, there are also endogenous opioids, which the

[14] Source for figure VIII: http://moldb.wishartlab.com/molecules/DB00295/image.png
[15] Chemical with similar pharmacological properties to morphine or other opiates

body produces itself and which play an important role in its functioning, acting as neurotransmitters.

They bind to the body's opioid receptors, of which there are three main types:

- μ receptors or mu receptors
- κ receptors or kappa receptors
- δ receptors or delta receptors

Each receptor, once activated, is responsible for a different set of effects on the body. Stimulated μ receptors, for example, cause analgesia and euphoria, amongst other effects.

Different endogenous opioids have a larger affinity for a different opioid receptor. This way, a specific opioid might bind, for example, only to δ receptors.

One such endogenous opioid is endorphin, which is a contraction of the term "**endo**genous mo**rphin**e". Endorphin is released during periods of stress and pain to alleviate the discomfort, as well as being responsible for feelings of pleasure. Thus, it is also released during sex and after a good meal, for example.

Endorphin has a strong affinity for μ receptors, to which it binds once it is released. There, it stimulates the receptors, causing pain relief and euphoria. These effects are similar to those of morphine – hence the name "endorphin".

When morphine enters the body, it binds to these very same μ receptors, mimicking endorphin and causing stimulation of the receptors.

While in small doses this results in feelings of euphoria, larger doses can be fatal. When activated, the μ receptors also cause respiratory depression, which can in turn cause potentially fatal asphyxia.

5) History

Ancient uses

The opium poppy is one of the oldest medicinal plants known to man, with records of its use dating back to the 34th century B.C., when it was already cultivated in Mesopotamia by the Sumerians, who called it *Hul Gil*, literally meaning "the joy plant". Soon the Assyrians and the Egyptians also became aware of its properties. In India, too, it was well-known.

It is thought that opium was brought to Europe by Egyptian traders. In Greece, its medicinal properties were recognised by Hippocrates during the 4th or 5th century B.C.

Opium use in China

Interestingly, though opium is often associated with China, it was only introduced there around the 7th or 8th century A.D. Even then, it was not particularly popular. This started to change in the 16th century, when European traders discovered the drug and began importing it into China. However, it remained a very expensive commodity, available only to the extremely wealthy.

For a long time opium use had been restricted to oral intake. As raw opium is very bitter, it was often added to various beverages in an attempt to mask the unpleasant taste. The Europeans changed the way opium was taken by introducing the practice of smoking it.

Opium smoking was introduced to China during the 18th century, where it was mixed with tobacco to make *madak*. This became quite popular, and the Chinese emperor Yongzheng banned the sale of *madak* in 1729 as he wanted to decrease the tobacco addiction that was becoming widespread. To the opium itself he did not object, and the sale of the pure product for medicinal purposes was still permitted. This ban only encouraged the smoking of pure opium, however.

In the mid-18th century, the British East India Company assumed control of Bengal and other opium-growing areas of India, eventually establishing a monopoly on the opium trade by forbidding Indian opium growers from selling their product to any other company. This opium they then began importing into China, where they sold it or traded it for goods such as tea and silk. With opium becoming more readily available, addiction, too, became widespread. In response to this, the Chinese government outlawed opium completely in 1799.

The British, however, continued to supply China with opium illegally. In an attempt to stop this, the imperial commissioner Lin Zexu was sent to the port of Guandong in late 1838. There, he ordered all foreign traders to surrender their stocks of opium, eventually seizing and disposing of a vast amount of British product. This was a significant economic loss for the British, and brought the two countries into direct conflict.

The friction caused by China's attempts to supress the British opium trade resulted in the Opium Wars (1839-1842 and 1856-1860), both of which the British won. China was forced to lift their ban on opium and grant the British various trading privileges.

As opium was readily available from the mid-19th century onwards, addiction became even more common. At the beginning of the 20th century, it was estimated that 27% of the adult male population were opium users. By 1950, about 5% of the overall population were addicted.

The problem of opium addiction persisted in China throughout the first half of the 20th century until the Communist Party of China came into power in 1949 and Mao Zedong's government succeeded in eradicating the opium trade during the 1950s.

Laudanum – the opium of the western world

Though well-known in ancient Greece, for a long time the medicinal properties of the opium poppy were overlooked in the western world.

It was reintroduced by the Swiss-German physician Paracelsus in the 16th century. He discovered that opium was more soluble in alcohol than in water, and created a tincture of opium to which he added crushed pearls, musk, amber, spices and other ingredients. This substance he named laudanum, derived from the Latin word *laudare*, meaning "to praise", and recommended for use as a painkiller. Little is known about this early form of laudanum, with some sources claiming it was not a tincture but a solid, fashioned into little pills.

While Paracelsus' laudanum remained relatively unknown, a later variation on the formula became exceedingly popular. In the 1660s, an English physician named Thomas Sydenham dissolved opium in alcohol and flavoured the resulting tincture with saffron and cinnamon. He named this mixture laudanum, presumably after Paracelsus's concoction, and promoted it for use as an analgesic, cough suppressant and anti-diarrheal. This laudanum typically contained about 1 part of powdered opium dissolved in 9 parts of alcohol.

Considering the other medication available at the time, it is no surprise that such a medicine quickly became very popular. By the 18th century, the tincture of opium that had become known as Sydenham's laudanum was in use all over the country. By the 19th century it was being used to treat everything from tuberculosis and cholera to migraine headaches, menstrual cramps and insomnia. It was even used to treat colicky infants.

Not only was laudanum easily obtainable, as its sale was not regulated until the 20th century, but it was also cheaper than ordinary alcoholic beverages, presumably due to its different taxation. As a consequence, addiction became very common, both among the working classes, who used it as an alternative to the more expensive alcohol, and the upper classes. Its use was also rampant in literary circles, Samuel Taylor Coleridge and Lord Byron being among the drug's illustrious users. The most well-known account of opium addiction is most probably Thomas De Quincey's autobiographical *Confessions of an English Opium Eater*, first published anonymously in the *London Magazine* in 1821.

The discovery of morphine

Morphine, the main active ingredient of the opium poppy, was discovered by the German chemist Friedrich Sertürner, who first isolated it in 1804. This discovery was celebrated throughout the medical community, and many scientists were convinced that morphine was safer than opium. Morphine was promoted for use as an analgesic. It was also used to treat alcohol and opium addiction, with doctors erroneously believing that it was less damaging to the human body as well as less addictive than the two aforementioned substances.

Forty years after the discovery of morphine, the hypodermic needle was used successfully for the first time, allowing medication to be injected straight into the patient's bloodstream. This had a significant advantage over oral administration, as the effect of the drug was instantaneous. Soon morphine, too, was being injected rather than taken orally.

The combination of these two ground-breaking inventions provided one of the most effective methods of pain relief known to man, but also set the stage for the horrors caused by intravenous drug use and addiction. During the American Civil War, morphine was used extensively for anaesthesia during surgery and as pain relief for the wounded. By the end of the war, over 400 000 people were addicted to the substance. This condition became known as "soldier's disease". Soon it was realised that far from being a less problematic version of opium, morphine was in fact far more addictive.

6) Modern-day use in medicine

Though the potential for addiction is very high, morphine remains to this day a valuable source of pain relief.

Today, morphine is used:

- To relieve the pain of patients who have suffered a myocardial infarction (heart attack). Not only is morphine an analgesic, but it also has a calming effect which relieves any anxiety the patient might have, preventing any further damage to the heart which could be caused by this stress.
- As a general anaesthetic during surgery, and as an analgesic after surgery.
- As pain relief for cancer patients, particularly as part of the palliative care offered to terminal cancer patients.

This list is by no means comprehensive, with morphine being used to treat patients suffering from many different conditions with symptoms including severe pain.

Morphine is generally administered intravenously, though oral administration is still used occasionally.

Side effects

The most serious side effect of morphine use is the addiction which can rapidly develop. The addiction is usually psychological at the beginning, with the user feeling unable to face daily life without the drug. Physical dependence takes somewhat longer to develop. Once this has set in, the user will feel physical symptoms of withdrawal when not on morphine.

An addict attempting to quit will experience withdrawal symptoms including sweating, chills, a runny nose, muscle aches, goose bumps, sleeplessness, nausea, stomach cramps, vomiting and diarrhoea. This period of withdrawal symptoms is commonly known as "cold turkey", and is also suffered by heroin addicts.

Tolerance also builds quite quickly, meaning that if a patient has to take morphine over an extended period of time, they will eventually need larger doses in order to feel the drug's effects.

Other possible side effects of morphine use include drowsiness, constipation, nausea, vomiting, and, in large doses, potentially fatal respiratory depression.

7) Production

Though morphine can be extracted from opium this method of production is fairly laborious, as opium is traditionally harvested by scoring the opium poppy's seed pods and collecting the dried latex by hand. Then, the morphine itself would have to be extracted from the opium.

Therefore, most of the morphine used medicinally today is extracted not from opium, but from "poppy straw", which is simply the dried plant. This is often obtained after the poppy seeds have been harvested, meaning that the same crop can be used to produce two different products.

Today, the main legal growers of opium poppy used for medicinal purposes such as the extraction of morphine are India, Turkey and Australia.

Papaver somniferum is also grown for the production of illegal opiates such as heroin. At present Afghanistan is the world's largest producer of heroin. Other countries growing opium poppies used for the production of heroin include Columbia, parts of Mexico and an area of south-eastern Asia (including Burma, Vietnam and Thailand) which is known as the Golden Triangle.

8) Derivatives of morphine

Morphine can be used to produce other opiates, notably heroin and codeine.

Heroin, also known as diacetylmorphine, was first synthesised by the British chemist C. R. Wright in 1874. However, it was not commercialised until 1898, when Bayer, a German pharmaceuticals company began marketing it as a cough suppressant and a supposedly non-addictive alternative to morphine.

Much as morphine was used in an attempt to treat opium addiction, heroin was in turn used to treat morphine addiction. It was later found out that is was simply a more potent, more addictive, faster-acting form of morphine.

During the 20th century heroin was more and more strictly regulated, but this did not prevent heroin addiction from becoming a very serious social problem.

Today, the personal possession of heroin is illegal in almost every country. However, in some countries it is still used medicinally as an analgesic for patients in severe pain as well as in palliative care.

Codeine can also be derived from morphine. Though it is also present in raw opium, its concentration is much lower than that of morphine. This makes it easier to obtain in sufficient quantity by synthesising it from morphine.

Codeine is used primarily as a cough suppressant and as an analgesic, though it is occasionally used as an antidiarrhoeal as well. It is usually administered orally. Common preparations containing codeine include cough syrups and preparations which contain codeine in combination with another painkiller such as ibuprofen. Though in some countries codeine is available solely by prescription, in others it is available over the counter in low doses.

There are also a large number of other morphine derivatives used in medicine, such as dihydromorphine and hydromorphone. These drugs are usually referred to as semi-synthetic opioids, as they are synthesised from a naturally-occurring opioid.

VII. Lithium

1) Introduction

The neurotoxins we have studied in detail so far have been of vegetable or bacterial origin, which may lead some to believe that neurotoxic substances always originate from some type of lifeform. This is not the case, however. Many chemical elements are neurotoxic, including lead, manganese, and lithium.

Lithium is an alkali metal, and like all elements belonging to the group of the alkali metals it his highly reactive. Because of this, it doesn't naturally occur in its pure form. Instead, it appears in the form of compounds. Lithium is present in seawater in concentrations of roughly 0.18 ppm[16] by weight, as well as in the earth's crust, where the concentration is about 20 ppm by weight. The highest concentrations of lithium are present in igneous rocks such as granite – which is where the name "lithium" comes from, as it is derived from the Greek word *lithos*, meaning stone.

Lithium in its pure form is corrosive, causing severe burns if it comes into contact with skin. Lithium compounds, however, are not, though they do have neurological effects. These effects can be very valuable in medicine, and some of these compounds, for example lithium carbonate (Li_2CO_3) and lithium citrate ($Li_3C_6H_5O_7$), are used to treat mental disorders.

2) Mechanism of action

Though it is widely known that lithium compounds are psychoactive, their mechanism of action remains to be discovered. It is believed that its effects are caused by decreasing the release of the neurotransmitter norepinephrine (also known as noradrenaline) and increasing the production of serotonin. How precisely this is achieved is currently unknown.

3) History

Lithium was first used in medicine in the mid-19th century by the English physician Sir Alfred Baring Garrod, who discovered uric acid in patients suffering from gout. Garrod, who had observed that lithium carbonate (Li_2CO_3) was capable of dissolving uric acid isolated from the human kidneys in in vitro situations, recommended it for use in the treatment of gout, as well as other conditions associated with an excess of uric acid, including rheumatism and renal calculi, more commonly known as kidney stones – though today it is known that uric acid stones make up only 5-10% of kidney stones, most being calcium-based, usually containing

[16] Parts per million

calcium oxalate (CaC_2O_4). Garrod also believed that gout might be the cause of certain mental disorders including mania and depression. He described as these phenomena as “brain gout” and postulated that this, too, could be treated with lithium carbonate.

In the 1870s, the use of lithium to treat mania and depression was further researched by both the American physician William Hammond, and two Danish scientists: the neurologist Carl Lange, and his brother Frederick “Fritz” Lange, a psychiatrist.

William Hammond, who worked at the Bellevue Hospital in New York, described a mental disorder characterised by periods of depression alternating with periods of acute mania – a condition we now know to be manic depression, more commonly known as bipolar disorder. This he treated using lithium bromide. However, for unknown reasons his later writings make no further reference to lithium, suggesting he may have abandoned the treatment, possibly due to lithium bromide’s toxicity.

The Lange brothers further characterised manic depression and both treated it with lithium carbonate, as they still believed to condition to be caused by high levels of uric acid in the blood. Carl Lange wrote a paper on the subject, which he submitted to the Danish Medical Society in 1886. As this paper was in Danish, it was only available to a small audience, and as there was quite a strong opposition to the Lange brothers’ theory at the time there were no translations, apart from some German ones which did not become internationally known.

Thus, the possibility of lithium use in the treatment of mania and bipolar disorder was shelved for the moment and to all intents and purposes forgotten about.

During the second half of the 19th century, excessive uric acid was held responsible for a large variety of ailments, and the virtues of lithium were extolled extensively in relation to their treatment. This led to a wide selection of supposedly lithium-laced products being put on the market, including lithium tablets and waters said to contain large amounts of lithium. These waters, generally called “lithia waters”, were marketed as something close to a cure-all, and they sold very well until it was proven that if they contained any lithium at all, it was in such small quantities that any therapeutic benefit was impossible.

A testament to the popularity of lithium is that when 7 Up was launched in 1929, it contained lithium citrate ($Li_3C_6H_5O_7$) and was sold as "Bib-Label Lithiated Lemon-Lime Soda".

During the 1940s, research connecting a high sodium chloride intake to heart disease and high blood pressure prompted researchers to find salt substitutes for sufferers. One such salt substitute contained significant amounts of lithium chloride (LiCl). It was prescribed to heart patients for a short period of time before concerns were raised about its harmful side effects. After a number of cases of poisoning, including several deaths, were reported, the prescription

and sale of lithium was banned completely in the USA in 1949. The substance was also removed from 7 Up in 1948.

The same year that lithium was banned in the USA, an Australian psychiatrist named John Cade, who worked in a psychiatric hospital, published a paper documenting his successful use of lithium carbonate in the treatment of manic depressive patients. Interestingly though, his discovery was coincidental.

Cade initially put forward the idea that mania was caused by an excess of a substance that was naturally produced by the body, and that depression was caused by an insufficiency of the same substance. To test this hypothesis, he injected guinea pigs with the urine of manic, schizophrenic, melancholic (depressive) and healthy subjects, and discovered that the lethal dose of urine of manic subjects was smaller than that of any of the other urine types. The next step was to inject guinea pigs with the separate substances found in urine to determine which one had toxic effects. He found that injections of urea ($CO(NH_2)_2$) produced the same effects as he had observed in guinea pigs injected with the urine of a manic subject. However, when analysing the concentration of urea in the different urines, he found no difference. Therefore he reasoned that there must be some other substance present in the urine of people suffering from mania which increases the toxicity of urea.

His research on the subject of urea was complicated by the fact that urea is relatively insoluble in water. To overcome this problem, he used lithium urate ($C_5H_3LiN_4O_3$) in his subsequent experiments as it was a particularly soluble urate. When he injected lithium urate into guinea pigs together with urea, Cade was surprised to find that the toxicity was lower than that of pure urea. In order to determine the precise effect of lithium in this experiment, he injected the guinea pigs with lithium carbonate and urea, once again observing a reduction in toxicity. Finally, he simply injected them with lithium carbonate. This produced a remarkable effect: the guinea pigs became tranquil, lethargic and unresponsive.

Cade immediately saw the potential of the sedative properties of lithium which he had discovered. In order to ascertain the safety of his newly-discovered drug he tested it on himself before using it to treat patients suffering from mania, depression and schizophrenia, all of whom were hospitalised where Cade worked. He found it to have no effect on the depressive patients and an only slightly calming effect on schizophrenic patients, but the results of the treatment of manic patients were remarkable. In fact, one of the most troublesome manic patients had calmed down significantly after only three weeks of lithium treatment, and within twelve weeks he had recovered sufficiently to be discharged from the hospital.

Cade's paper on the subject was published in 1949, which was rather unfortunate, as very little interest was shown in the USA due to the recent salt substitute scandal and the subsequent ban on lithium. However the subject was taken up by various individual scientists. During this period the toxicity of lithium proved to be a difficulty, as the technology necessary to monitor the level of lithium in the blood was not yet available. This meant that a few incidents of lithium poisoning occurred before the researchers could find the necessary balance between the ineffective dose and the harmful dose. The two of the most notable scientists who studied lithium as a psychiatric medication during this period were the Australians C.H. Noack and E.M. Trautner who jointly published a paper in 1951 which confirmed Cade's findings.

Cade's paper did not go completely unremarked upon internationally, however. Erik Strömgren, the head of a Danish psychiatric clinic, read it and was sufficiently interested in the ideas it proposed to encourage the psychiatrist Mogens Schou, who worked at the clinic, to carry out a randomised control trial [17] on the effects of lithium in manic patients. Schou's findings, too, were positive, and in 1954 he published them in a British journal.

This article recieved much more attention worldwide than any of the previous papers, and international interest began to grow, though it only reached the USA in the 1960s. By the late 60s, many countries were already using lithium in psychiatry. In 1970, the FDA[18] finally approved lithium as a treatment for mania in the USA, and the ban was lifted.

4) Medical applications

Today, lithium carbonate and less frequently lithium citrate are used in psychiatry. Medications containing lithium include Camcolit in the UK and Eskalith in the USA.

Lithium is one of the most effective mood stabilisers currently available. It is principally used to treat bipolar disorder, helping sufferers with their manic episodes as well as lowering suicide rates significantly. It is often prescribed for long periods of time, even to be taken as maintenance therapy in between manic episodes. Lithium has also been found to lower suicide rates among depressive and schizophrenic patients.

It can also be helpful in the treatment of cluster headache, a type of severe and recurring headache affecting only one side of the head, particularly the area around the eye.

The main difficulty of lithium treatment is that the necessary dose is only slightly lower than the harmfully toxic dose. This means that the patient's blood levels of lithium have to be strictly monitored during the treatment and kept at a constant level.

[17] Trial in which subjects are randomly allocated one treatment or the other
[18] Food and Drug Administration

Lithium has various side effects including dehydration, as it inhibits vasopressin, an antidiuretic hormone, which is responsible for the body's water retention. Dehydration, in turn, results in higher blood levels of lithium, which can be toxic. Because of this, patients being treated with lithium need to consume a certain daily amount of water and salt. This fights dehydration and makes sure the blood levels of lithium remain stable.

Other side effects can include nausea, vomiting, diarrhoea, increased thirst and urination, weight gain, hand tremors, impaired memory, hair loss or hair thinning, and acne.

Rare side effects include hypo– and hyperthyroidism, both of which can be treated with additional medication, and the significantly more serious loss of renal function, which can result in permanent kidney damage.

Lithium is unsuitable for pregnant women as it has been associated with certain birth defects including Ebstein's anomaly, a congenital heart defect.

In the case of an overdose, symptoms include trembling, loss of voluntary muscle control, slurred speech, involuntary eye movements, muscle twitching, convulsions and renal failure. Patients who survive an overdose may retain permanent damage to the brain.

5) Lithium in drinking water

Lithium is a trace element in drinking water all over the world, but its concentration varies significantly.

Numerous studies been conducted on the correlation between lithium concentrations in drinking water and suicide rates in the local population, and the results have almost exclusively shown that high concentrations of lithium are associated with a lower suicide rate. Even when socio-economic factors affecting suicide rates have been taken into account, the result remains the same. This would indicate that the two factors are inversely proportional, even though the local population's average daily intake of lithium is considerably lower that the dose prescribed medicinally.

A separate study, undertaken in Texas, links high lithium concentrations in drinking water to a lower incidence of violent crime, specifically homicide and rape.

These findings have led to many people, scientists and politicians alike, suggesting that adding more lithium to our supply of drinking water would be a viable method of reducing crime and suicide rates.

VIII. The medical ethics of neurotoxins

In medicine, it is of the utmost importance that the patient can rely on their doctor to do the right thing and trust his or her advice completely. This places a huge responsibility on the shoulders of the medical practitioner, who will at times have to make incredibly hard decisions. To make sure these decisions are made as well as possible, there is a certain set of guidelines the practitioner must follow: the code of medical ethics.

Medical ethics are based on four main principles:

- Autonomy: The patient has a right to decide what happens to their body. This means that they can accept or refuse any treatment proposed, even if it goes against the doctor's recommendations, and their wish must be respected.
- Justice: The medical practitioner must be as fair as possible when choosing how to distribute rare or unreadily available medical resources amongst their patients.
- Beneficence: The medical practitioner must act in the patient's interest at all times and attempt to do as much good as possible, adapting the treatment to the individual as necessary to ensure the best possible effect.
- Non-maleficence: Derived from the Latin phrase "*primum non nocere*", meaning "first, do no harm", non-maleficence is arguably the most important principle of all in medical ethics. It states that the medical practitioner must avoid harming their patients at all costs. This includes situations where there is a risk of harming the patient but no certainty. In these situations, non-maleficence can come into conflict with beneficence, for example when a treatment that is likely to be beneficial might have a serious side effect that could cause harm to the patient. This is called the double effect. In this case, it is generally accepted that it is better to do nothing than to risk doing something that could do more harm than good.

Other important ethical values to be taken into account in medicine are:

- Respect: The patient – and the medical practitioner, though this is usually less of an issue – have the right to be treated with respect and to retain their dignity.
- Informed consent: The medical practitioner must be honest with the patient, truthfully disclose what is involved in the treatment proposed, and ensure that the patient has a sufficient understanding of the process and its implications to be able to make an informed decision. The patient is then free to give or withhold their permission for any of the treatments proposed.

When comparing these values to the histories of the four neurotoxins this paper has focused on, it quickly becomes apparent that the modern-day medical applications of these substances could probably not have been discovered if scientists had respected the guidelines of medical ethics during their research. Many discoveries in medicine generally have only been possible due to early experimentation on patients with little knowledge of the possible outcomes of the treatment. The use of morphine to treat opium addiction harmed the patients rather than helping them, for example, and Cade's human testing of lithium carbonate for bipolar disorder was problematic as very little was known about the compound and even less was known about its long-term effects. It is also debatable whether patients suffering from severe mania should be considered in possession of an adequate decision-making capacity. In cases in which the patient is judged incapable of making the decision, another person must make the decision for them, usually a close family member – something that may well not have been respected in cases such as Cade's. In addition, as neurotoxins can be extremely toxic, medical research has resulted in a number of deaths. In fact, some of the treatments still in use today can have potentially fatal side effects, such as the treatment of cerebral palsy with botulinum toxin.

Does this mean that these treatments, which have had problematic histories, should not be used today? No, of course not. While some of the practices used by researchers in the past were not in keeping with the code of medical ethics and would be unacceptable in modern society, there is no reason for us to reject the effective treatments they have provided us with.

The information that we have gathered in medicine over time is very valuable, as it not only helps us treat the illnesses of today, but also gives us a starting point from which we can research cures for the illnesses of tomorrow. The ever-advancing state of our technology is enabling us to do more and more research in vitro and to accurately and safely monitor in vivo experiments, and by learning from our past mistakes in the field of medicine and applying that knowledge to our future endeavours, we can carry out our research more safely and with more background information and resources available than ever before.

The fact remains, however, that the use of neurotoxins in medicine today is not risk-free, with many treatments that can have serious and even fatal side effects – an issue which proves both the importance and the difficulty of the "correct" application of medical ethics. Many treatments have a strong possibility of "double effect", but in dismissing them completely, the medical practitioner could withhold the patient's only hope of recovery. This is where autonomy and informed consent come into play: all that can be done is to inform the patient truthfully about the treatment's risks as well as its potential merits, and to assist them as well as possible in making an informed decision.

Although the side effects of many neurotoxic medications can be serious, it is important to remember that they will not necessarily occur at all, and to bear in mind what a significant improvement can be made in a vast number of people's lives – be they sufferers of cerebral palsy or bipolar disorder – by offering them the option of treatment.

IX. Conclusion

When we think about neurotoxins, the things that spring to mind often include horror visions of drugs, brain damage and death. This is not without reason, for the effects of neurotoxins can be very serious indeed. This fact has been known to mankind before the word "neurotoxin" was ever used, beginning with the discovery that certain plants are not to be eaten if one wants to live, and that the bites of certain animals can do much more damage than their size would imply. Neurotoxins are a fundamental part of nature, and given the threat they pose – and have posed more acutely to our forager ancestors in the past – wariness and even fear are the only natural reactions one can have.

Over time however, through curiosity, necessity, perseverance and coincidence, humanity has learned more about this natural threat, and has found methods to channel it and apply it in ways that are beneficial to certain individuals' health.

Neurotoxins have provided us with some of the most vital medicines known to man, such as anaesthetics and analgesics, which not only alleviate pain and severe discomfort, but also allow us to carry out innumerable life-saving surgical procedures. In addition, neurotoxins have also given us the possibility of improving the lives of millions suffering from debilitating conditions, be they primarily physically debilitating (such as cerebral palsy), psychologically debilitating (such as bipolar disorder) or socially debilitating (such as hyperhidrosis). In all of these cases, the use of neurotoxic treatments can significantly improve the sufferer's quality of life.

But the road to these medical achievements has been a rocky one. As many researchers have found out at the expense of their test subjects and even of their patients, the beneficial dose and the harmful one can be very similar, and many neurotoxins considered for use in medicine bring with them a plethora of possible side effects which can be more serious than the condition that is to be treated. Though neurotoxins can indeed be very beneficial in medicine, their effects have to be extensively researched before the treatment is used, and the patient must often be strictly monitored once the treatment has begun in order to make sure any side effects or symptoms of overdose or allergy are caught as early as possible.

Neurotoxins can provide us with extraordinarily effective treatments, but apart from their side effects they also bring other problems with them, such as possibilities for misuse. This can include use as recreational drugs or as biological weapons, for example.

In order to minimise all these negative aspects, we must rely on medical professionals to act in their patient's best interest by proposing the most suitable treatment for each particular case

and informing the patient of any possible risks or side effects involved. We must also rely, however, on the world's governments, trusting them to safely regulate the sale of neurotoxic substances and to have the sense to avoid the weaponisation of biological substances, including toxins.

Contrary to initial appearances, neurotoxins are not simply obstacles that nature has put in our way – in fact they could be viewed as a gift. But they are by no means an easy gift to make use of, and we must endeavour to apply them as safely and as ethically as possible.

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