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An explosive story

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Gunpowder was developed in the 13th century, as a result of the discovery of how to purify saltpetre. This led to the isolation of amyl nitrite, which turned out to be an effective treatment for angina pectoris. Nitroglycerine is not only a high explosive but also effective in treating angina. The search for an understanding of how it works in the body has led to the astonishing discovery that nitric oxide gas is actually produced in the body and is involved in the control of a number of important functions, including blood pressure.

The discovery of gunpowder in the 13th century led to a revolution in warfare. To begin with, its explosive capacity would be used in 'shells' that could be lobbed onto the enemy host by various means and would cause panic as well as loss of life; later, when the explosive had been tamed, it could be used as a propellant and so the gun was created. Gunpowder was a simple mixture of three components: wood charcoal, sulphur and saltpetre. It was on the availability of the latter that the creation of gunpowder had to wait. Mixtures of charcoal and sulphur, often with asphalt or fat, had been used in warfare for centuries, usually known as Greek Fire, but they were not explosive. So what was the problem with saltpetre?

Saltpetre was mostly derived from efflorescences on the walls of caves or in cellars and was recognized as a salt, but later was distinguished from common salt and given names such as nitron or natron. Perhaps it was recognized as being different as it burns when thrown into a fire. The material was a crude mixture of various salts but contained the nitrates of potassium and sodium. The addition of it to sulphur and charcoal did not make a reliable explosive mixture. However, the 13th century was a period when Alchemy, the study of natural materials and their properties, was developing. This was a secret and illegal occupation because of the assumption that it was associated with necromancy. Nevertheless, it was the beginnings of modern science and its name became incorporated into chemistry (Chemie, chimique). One of the Alchemists found out how to purify

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crude saltpetre, by boiling it with water, removing the scum and the insoluble residue, and then harvesting the crystals that were deposited on cooling.

An important feature of this purification was that it produced the nitrate of potassium free from that of sodium. The latter was fatal to any use in explosives because it was deliquescent, i.e. attracted water and would make the gunpowder damp.

But there had to be a second discovery, namely that this new material, when added to charcoal and sulphur, changed it from a fiery fuel into an explosive. It is believed that these two discoveries could have been made by the 13th century monk, Roger Bacon. Bacon was one of the leading scholars of his time, a polymath who wrote on a variety of subjects, including philosophy and mathematics. He was also an Alchemist and, some time before 1249, published a work entitled *Epistola de Secretis Operibus Artis et Naturae et de Nullitate Magiae* where the statement occurs of 'an explosive powder which causes hideous crackling and is able to destroy armies'. The problem is that his claim to have discovered the preparation of saltpetre and gunpowder seems to be written in code, apparently a practice among Alchemists because of the secret and illegal nature of their practices. Henry Hime[1] presents the case and a possible decipherment that provides the full prescription, but it has to be admitted that this supposition is far from universally accepted.

However, the availability of pure saltpetre led to the discovery of nitric acid, a powerful acid which, when combined with hydrochloric acid, was known as *acqua regia*, which could dissolve gold, the queen of metals. An early account of *acqua regia* is given by Vanoccio Biringuccio in his *Pirotechnica*, and also by Gianbattista della Porta, by Agricola and others. The next advance came much later from the study of the action of nitric acid on other substances, notably alcohols. Antoine-Jerome Balard[2] in 1844 isolated amyl alcohol from fusel oil, the oil that does not distil from an alcoholic mixture such as from the fermentation in which whisky is made. He prepared a number of esters, including that with nitric acid, which we will return to later. The young Italian chemist, Ascari Sobrero[3] formed compounds by the action of nitric acid on sugar, lactose, dextrine and other biological materials and found that they were explosive. However, in 1847, he reacted nitric acid with glycerol and produced a highly explosive oil which was glycerol trinitrate (also called nitroglycerine), which he called *pyroglycerine.* On heating a small amount in a tube it exploded violently and the fragments of glass cut his face and even injured an eye. He did no more work with it, although he continued to have a distinguished career in chemistry.

Nitroglycerine is forever associated with the name of Alfred Nobel[4] and the following is how he became involved with it. His father, Immanuel, was an engineer and inventor and when he heard of the plans to build the Suez Canal, he became enthusiastic about using explosives in the building of canals, tunnels

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and roads and started experiments in his backyard. The result of an explosion there so frightened his neighbours that he was prohibited from carrying on with his work. So, in 1837, he moved from Sweden to Finland and continued his experiments and tried to interest the Russians. This led to him setting up a factory making armaments in St Petersburg and the whole family, including young Alfred, moved there in 1842. In 1862, Alfred with his brother Robert started experiments with nitroglycerine on the advice of the Russian scientist, Nikolai Zinin. It was after much experimentation and many disastrous explosions that, in 1867, Nobel hit on the idea of absorbing the explosive in the diatomaceous earth, *kieselguhr* and so produced *Dynamite*, a safely handlable high explosive.

We will now introduce a new strand to our story. A Scottish physician, Thomas Lauder Brunton was working in London. He had studied physiology and pharmacology in Germany with Brücke, Kühne and Ludwig and was interested in the application of these sciences to clinical medicine, particularly to the circulation. The established, rather ineffective treatment for angina pectoris was bleeding, on the assumption that lowering the blood pressure would cause relief. Brunton had heard from a colleague in Edinburgh, the physiologist Arthur Gamgee, that amyl nitrite lowered the blood pressure in animals. Gamgee had been led to do his experiments as a result of reading a report by Frederick Guthrie who had been following up the synthesis of amyl nitrite by Balard referred to earlier. Guthrie[6] reported of amyl nitrite that 'Das Einalten einer kleiner Menge seines Dampfes bewirkt vor übergehend Blutdrang nach dem Hals und sehr vermehrten Herzschlag' (Inhalation of a small amount of the vapour caused a rush of blood to the neck and greatly increased palpitations). Brunton[5] reported in 1867,

> I had the opportunity of constantly observing a patient who suffered from angina pectoris and of obtaining numerous sphygmographic tracings, both during the attack and during the interval. These showed that during the attack the blood pressure rose and the form of the pulse was such as could only be caused by contraction of the arterioles. The pain which came on every night lasted for one and a half to two hours… On pouring five to ten drops of amyl nitrite on a cloth and giving it to the patient to inhale, the physiological action took place in thirty to sixty seconds and simultaneously with the flushing of the face, the pain completely disappeared and generally did not return until its wonted time the next night.

He found the same effect in other patients and it became an established and safe treatment. The treatment had two drawbacks; the duration of action might be too brief and it could be rather embarrassing socially to have to inhale in public. It was soon found that other nitrites and nitrates had a similar action and it became clear that nitroglycerine had advantages. Although it could not be given by inhalation, it could be incorporated in a tablet, which was placed under the tongue

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and produced longer lasting relief; this tablet remains in use to this day as a first line treatment for angina.

The moral is that even substances designed to kill can also reduce the suffering of others! There is the curious fact that when Alfred Nobel was terminally ill he wrote from Paris 'I have come here to consult a famous heart specialist… it seems an irony of fate that they should be prescribing nitroglycerine internally for me! They call it trinktin, to avoid terrifying the chemists and the public'.[7]

Scientists are never satisfied in finding just a useful result such as we have described, they want to know the means by which the results are achieved and, in this instance, the quest led to a whole new aspect of biology, which we will now follow.

It seemed possible that the action of nitroglycerine was due to it breaking down in solution and indeed it does so, readily providing both nitrates and nitrites. However, these materials are only one hundredth of the activity of the starting material, so this seemed an unlikely explanation, but we will return to this matter later. The first glimmerings of the truth came from studies on cyclicGMP, a substance that is formed in cells from a parent compound GTP by an enzyme called *guanyl cyclase*. The activation of this enzyme with the consequent increased production of cGMP was implicated in the control of the activity of cells, through various mediators but notably acetylcholine. In some instances, cGMP stimulated cells, in others it reduced activity. Ferid Murad and his collaborators undertook a wide-ranging exploration of the effect of a variety of substances on the enzyme. In the course of this they found, in 1975, that three nitrogen compounds – azide, hydroxylamine and nitrite – activated the enzyme. There followed a period in which they were able to unravel the way in which these assorted compounds produced these effects and were then led to examine nitroglycerine, as well as other nitrogen compounds that could also dilate blood vessels. They found that all of these compounds activated the enzyme; they also made the significant observation that the blood pigment, haemoglobin, or the equivalent substance in muscle, myoglobin, would prevent activation. This observation led to the proposal that all these compounds acted by producing the gas, nitric oxide (NO), which was the real active entity. There is an interesting bit of history here. Saltpetre had been used for centuries to preserve meat, and also giving it a healthy looking pinky-red colour. Ludimar Hermann had shown in 1865 that NO reacted with haemoglobin and, in 1868, Arthur Gamgee also investigated this very comprehensively. The NO combined with the iron in haemoglobin and, later, it was found to react with iron in myoglobin and other iron complexes in the body. Murad had no difficulty in showing that NO activated the enzyme and that the inhibitory effect of haemoglobin was due to it sidetracking the NO, although it does also accelerate the breakdown of NO to nitrite. This observation was followed by Louis Ignarro showing that NO caused relaxation of smooth muscle

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and that, for nitroglycerine to produce a similar action, it had first to be activated by other tissue constituents.

Now these days we know that when a drug produces an action, it is frequently found that it is imitating the action of a normal tissue regulating substance. So the big question that arose was the possibility that NO was a normal regulator. It seemed so unlikely that this rather toxic gas, NO, was a normal body constituent that this possibility was approached with considerable diffidence.

The time has come for us to direct our attention to a quite different kind of study. Robert Furchgott had a long standing interest in the control of the circulation and, in 1978, he was studying the relaxation of the muscle in blood vessels by the substance acetylcholine. This substance had been shown in 1914 to cause a great fall in blood pressure due mainly to relaxation of the small arteries (arterioles); it was also later found to be the agent that transmitted the action of the parasympathetic nerves to the blood vessels. Pharmacologists often use 'isolated' pieces of tissue to study drug action to eliminate some of the complications of studies in a whole animal. Furchgott was using a preparation of rings cut from the aorta (main artery) of a rabbit. When these rings were made to contract by some other drug, such as noradrenaline, the contraction could be opposed by acetylcholine. Furchgott started to get very strange irregular results: in some preparations acetylcholine it would not relax it at all. After a good deal of investigation the cause was found; it depended on how careful one had been in preparation of the rings. If care was taken not to rub the inner (intimal) surface of the rings all was well, but even gentle rubbing of the surface led to the loss of the acetylcholine response. Microscopic examination showed that even this gentle rubbing had led to some damage to the intimal surface. This suggested that it was these intimal cells that responded to acetylcholine and somehow communicated the effect of this response to the muscle cells in the artery wall. In 1980 a critical test reminiscent of the famous experiment by which Otto Loewi established that acetylcholine was the transmitter of the vagus nerve in the heart was carried out. Furchgott recorded the contraction of an artery ring whose intima has been rubbed and placed in close proximity another piece of artery whose intima was intact; acetylcholine now produced contraction! The intact intima had released a substance that caused the damaged ring to contract; the nature of the substance was at this stage was totally unknown, so it was simply called the *endothelium derived relaxing factor* (EDRF). It was noteworthy that the action of nitrites, nitroglycerine and isoprenaline, which could also produce relaxation of the rings, did so equally well whether or not there was intimal damage. The evidence gradually built up that EDRF was NO. The release of NO from the intima was detected spectroscopically, and EDRF was sensitive to the presence of haemoglobin. This is not the place to elaborate on the details through which this was convincingly established, but an important step was the demonstration that

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NO was formed in the endothelium from the amino acid arginine by an enzyme that was called *endothelial nitric oxide synthase* (eNOS). This enzyme could be inhibited by N-methylarginine, an analogue of the substrate and when this was present the effect of acetylcholine was decreased or abolished. Interestingly, one could now test whether NO was produced at an effective concentration even when not stimulated by acetylcholine; N-methylarginine raised the blood pressure and this could be suppressed by supplying more arginine. The NO mechanism was therefore part of the normal blood pressure regulation! We have discussed the formation of NO, but why does it not stay around? The answer is that in the presence of water and oxygen, NO is unstable and decays into nitrite and nitrate, its lifetime is only a few seconds, and this is why the experiments to form an active substance from hydrolysis of nitroglycerine failed. It is usually found in biology that nature is parsimonious and when a useful regulatory system has evolved it is put to more than an isolated use, so it would be surprising if the NO mechanism were restricted just to the endothelium. There was at least one other system on which Furchgott's suspicion alighted. John Gillespie had been trying to understand the effect of nerves that caused relaxation of the bull's retractor penis muscle. The relaxation was not due to either acetylcholine or noradrenaline and Gillespie had obtained in acid solutions an active extract which he called the NANC factor; this turned out to be NO! Furthermore, N-methylarginine blocks penile erection in the rat in response to electrical stimulation. So NO is concerned with penile erection and, even more interesting, *Viagra* is an inhibitor of an enzyme that degrades cGMP and so is connected with improving the cGMP response produced by NO. So there is even sex in this story!

I have summarized here, in simple terms, a very complex series of developments in which many scientists made significant contributions. In fact there is evidence of NO involvement in a wide variety of bodily systems including the nervous system, the immune system and blood clotting. The list goes on and cannot be elaborated here.

The biology of NO has become a major area of research and by 1998, when the Nobel Prize[8] for Physiology and Medicine was awarded to Furchgott, Murad and Ignarro for their part in these discoveries, more than 20,000 scientific papers on the subject had appeared.

The moral

This is fine example of how important science grows from improbable beginnings. Elsewhere in this issue of the *European Review* there is an article by J-C. Petit in which the merits of fundamental research are discussed. The present case is a splendid example of the way that fundamental and applied research interdigitate. I suppose that the Alchemists prepared pure saltpetre because of the pleasure of

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having turned some messy droppings into shiny crystals, but it became *applied* very soon to making gunpowder. Balard and Guthrie's work was pure chemistry making new compounds for interest's sake, but it led to application in the treatment of disease. Sobrero's discovery of nitroglycerine was applied research directed to finding explosives, but this also led to a treatment for disease which, in turn, stimulated pure, inquisitive research that is stimulating applied research looking for new drugs.

References and notes

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2. A. Balard (1844) Ueber den Amylalcohol. Ann. der Chemie und Pharmacie, **LII**, 311–316. Balard thought that he had made amyl *nitrate* but in fact it was amyl *nitrite*.
3. A. Sobrero (1848) Some new substances from the action of nitric acid on some organic vegetable substances. *Ann der Chemie und Pharmacie*, **64**, 398.
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6. F. Guthrie (1859) Beiträge zur kenntnis der Amylverbindungen. Ann. der Chemie und Pharmacie, **CXI**, 82–91.
7. N. Halasz (1960) *Nobel. A Biography* (Robert Hale), p. 167.
8. I have not given chapter and verse for the discoveries involved in the identification of NO as the active species produced by the nitro compounds or its physiological significance because the number and source of the contributions is so vast. If you want to find out more about it, it is suggested that in the first place you access the Nobel Lectures of Furchgott, Ignarro and Murad on the Web. These lectures give you an excellent summary of the position as seen in 1998, from three different points of view.

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