PAPER

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'The heart less bounding': treating angina pectoris

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ABSTRACT Although one group of drugs (including amyl nitrite and glyceryl tinitrate) has been used in the treatment of angina for over 100 years, the mode of action became clear only after the endothelium-derived relaxing factor had been identified as nitric oxide in 1987. Originally sodium nitrite was included in this group of drugs but it rapidly fell out of favour. Recently, however, interest in its therapeutic use has been revived. The medical uses of saltpetre (potassium nitrate) may be due to the presence of nitrite as an impurity.

KEYWORDS Angina, nitric oxide, nitrite, saltpetre

LIST OF ABBREVIATIONS Cyanide (CN), endothelium-derived relaxing factor (EDRF), nitrate (NO_3^-), nitrogen dioxide (NO_2)

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INTRODUCTION

In 1867, Thomas Lauder Brunton was a newly qualified house surgeon at the Edinburgh Royal Infirmary. While treating one particular patient with severe angina pectoris, he found he could relieve the symptoms by removing blood, either by cupping or by venesection. This convinced him that the pain associated with angina could be treated by lowering arterial blood pressure. Clearly blood removal was not a convenient treatment for regular use, and so he turned to his friend at the Infirmary, Arthur Gamgee, who had noted that inhaling the vapours of amyl nitrite lowered the blood pressure of an animal.[1] Brunton impregnated a cloth with amyl nitrite for his patient to inhale, and observed that within seconds, the agonising chest pain had diminished. Relief lasted for many minutes. Trials with other patients confirmed these results. He reported his results in the *Lancet*[2] and amyl nitrite soon became established as the regular treatment for relieving anginal pain. Other nitrites were tried and some were found to have a similar effect but the best vasodilator of this type was not actually a nitrite (which contains an $-NO_2$ group) but a nitrate (which contains an $-NO_3$ group). That substance was nitroglycerine.

Nitroglycerine was first made by the Italian chemist Ascanio Sobrero at the University of Turin in 1849 and is, of course, an explosive.[3] It is surprising that Sobrero survived the discovery without blowing himself up. However, with careful handling, it is not dangerous as it explodes only on detonation. Brunton's work showed that, when inhaled, nitroglycerine lowers blood pressure dramatically, but can also cause severe headaches and so he did not pursue the matter any further. However, when others examined the effects of inhaling nitroglycerine, they found that headaches were not an inevitable consequence, and some confusion surrounded the medical potential of this substance.

The matter was examined in detail by William Murrell, a registrar at Westminster Hospital, and he concluded that headaches could be avoided if the dose was kept as low as one milligram, without any loss of vasodilator action. Further work established that, in the treatment of angina, nitroglycerine was superior to amyl nitrite as, although it took longer to produce its effect, the benefit lasted much longer.[4] So nitroglycerine gradually replaced amyl nitrite in medical practice and is now the routine medication given to those suffering from angina. Murrell found that the best delivery system was a tablet dissolved slowly in the mouth. Nowadays, nitroglycerine is often delivered via a 'puffer' or transdermally. Some concern was expressed initially that patients might hesitate to use a medication that was also a high explosive and so, for medical use, the name was changed to glyceryl trinitrate but the subterfuge was not entirely successful. However, there appear to be no reports of exploding patients.

While the medical value of nitroglycerine was being explored Alfred Nobel, the Swedish inventor was incorporating it into an absorbent mineral (*kieselguhr*) to make dynamite. It was from this invention that much of Nobel's fortune came. To emphasise the hazards of handling nitroglycerine, it is worth noting that on 3 September 1864, during the development of dynamite, there was a violent explosion in Nobel's laboratory in the Stockholm suburb of Heleneborg. Five people were killed, including Alfred Nobel's younger brother Emil. Alfred was very fond of his brother and the incident must have been a source of guilt as he never spoke of it throughout his life. A few years before he died, Nobel

developed angina and was treated with the very substance that had been the source of his immense wealth.[5]

During World War 1, many of the women recruited to work in munitions factories to pack explosives reported severe headaches, particularly on Mondays. The headaches were due to a substantial lowering of blood pressure from the inhalation of the vapours of nitroglycerine. As the working week progressed, they developed a resistance to the biological effect of nitroglycerine and the headaches disappeared. Over the weekend, when they were not packing munitions, the resistance disappeared and the headaches started again on Monday morning. Conditions in modern munitions factories have improved markedly and the environmental hazard is no longer present. However, the problem of resistance is still a difficulty in the medical use of nitroglycerine, particularly if the patient needs continuous medication.

Another, chemically related, vasodilator that was invented in the nineteenth century,[6] although its vasodilator action was not discovered until the 1950s,[7] is sodium nitroprusside (Nipride: $Na_2(Fe(CN)_5NO)$). The invention was made by a chemist, Lyon Playfair, who spent his childhood in St Andrews, while working at the laboratories of the Geological Survey in London. As the formula shows, it contains the –NO group (wherein lies its similarity to amyl nitrite and nitroglycerine) but also the five cyanide groups, causing grave concern amongst those considering its use. It is sometimes used in hypotensive anaesthesia[8] as an infusion but cases of death from cyanide poisoning have been reported.[9] The chemistry involved in the use of Nipride is complex[10] and, as cyanide release is possible, only very low doses of Nipride should be used. The body can tolerate low doses of cyanide as there is an enzyme that converts it into the non-toxic thiocyanate.

MODE OF ACTION

Both amyl nitrite and nitroglycerine (and some other, closely related compounds) had been used for well over a century for the treatment of the symptoms of angina without any clear idea of why they worked. The presence in all of them of one of the groups –NO, $–NO_2$ and $–NO_3$ should have alerted researchers to the mode of action but this did not occur until the 1980s. At that time, the distinguished muscle physiologist Robert Furchgott, working at the Downstate Medical Center in New York City, reported[11] the presence of a previously undetected 'messenger molecule' in the sequence of events leading to the relaxation of vascular muscle and consequent blood vessel dilation. The unknown 'messenger molecule' was produced, he discovered, by the endothelial cells lining the lumen of the vessel. He called it the 'endothelium-derived relaxing factor'. In spite of considerable endeavours, both by Furchgott and others, the chemical identity of the EDRF remained a mystery but it was generally considered to be a large, hormone-like molecule. The resolution of the mystery started at a conference on vasodilation at the Mayo Clinic in the USA in 1985, at which Furchgott was scheduled to speak. He had concluded, from purely circumstantial evidence, that the EDRF might be the gas nitric oxide (NO).[12] This should not be confused with nitrous oxide (N_2O), well-known in medical circles as an anaesthetic gas. This suggestion was a bold one as no-one had ever contemplated, let alone suggested, a normal biological role for nitric oxide. It was known outside the chemical community only as a dangerous pollutant, produced by the internal combustion engine. The catalytic converter on a car removes nitric oxide from the exhaust gases, converting it into nitrogen and water. Nitric oxide in the atmosphere can give rise to photochemical smog, a major problem in cities all over the world. Furchgott's suggestion, made public during his presentation at the conference, provoked great interest but there was no compelling evidence to support the idea.

Two scientists present at the Mayo Clinic conference set out to provide the experimental evidence that nitric oxide was, indeed, the EDRF. They were Salvador Moncada, then working at the Wellcome Research Laboratories in Beckenham, and Lou Ignarro from the University of California at Los Angeles. From *ex vivo* experiments they both, working independently, obtained strong evidence for the identity of nitric oxide with the EDRF. Two papers appeared simultaneously in 1987 announcing this result.[13, 14] At first, physiological and medical circles expressed grave doubts; it seemed so unlikely that this substance, a tiny molecule and normally a gas, could play a major role in as significant a process as vascular muscle relaxation. Also, nitric oxide is a radical and it is well-known that radicals are very reactive and destructive. However, the experimental evidence produced by Moncada and by Ignarro has stood the test of time, and subsequent work by others has confirmed beyond all reasonable doubt the identity of nitric oxide as the EDRF. The radical nature of nitric oxide is not really a problem; it is, for good chemical reasons, an unreactive radical. The discovery of a role for nitric oxide in vasodilation, however, was not the end of the story. Nitric oxide production is now known to be part of the immune system (which is why immune activity during a urinary tract infection results in nitrite in the urine) and is also a neurotransmitter in the brain and peripheral nervous system. There is now a sub-discipline of nitric oxide biology (with conferences in exotic places) and an International Nitric Oxide Society. Some mammalian physiology has had to be rewritten as a result of the discovery of a biological role for nitric oxide.

In 1998, the Nobel Prize for Physiology or Medicine was awarded for the discovery of biological nitric oxide. The recipients were Furchgott, Murad (who studied the enzyme responsible for vascular muscle relaxation) and

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Ignarro. The omission of Moncada from the award caused great consternation and outrage. The papers of the Nobel Committee are not made public for 50 years and so most of those living now will never know the reasons for this sad and puzzling omission. It has been criticised by at least one of those who received the award.

It is clear now why the substances mentioned in this article are effective in the treatment of angina. Because of their chemical constitution, they are capable of being metabolised, once in the body, into nitric oxide. They all have nitrogen and oxygen atoms in the molecule making this possible. At least one enzyme, located in vascular walls, that carries out this reaction has been characterised.[15] The nitric oxide thus produced causes very general vessel dilation. Exactly why this so effective in relieving the pain of angina without causing problems elsewhere in the circulatory system is complex[16] and further discussion would be out of place in this article. One would have thought that as major a discovery as that of nitric oxide in the vasculature would have been responsible for new cardiovascular drugs, particularly for the treatment of angina but, so far, this has not been the case. Delivering nitric oxide exactly where it is required has proved difficult. The only new drug that affects nitric oxide production developed since 1987 is Viagra and the discovery of its use in the treatment of erectile dysfunction was serendipitous.

INORGANIC NITRITE

There is one related anti-anginal drug of historical importance which soon disappeared from *Material Medica* but which has now made a sudden reappearance in research circles for reasons that will be described later. It is the simple inorganic salt potassium nitrite (KNO_2) and differs from the other anti-anginal drugs mentioned previously, in that it is an ionic compound rather than a covalent one. Potassium nitrite should be distinguished from its better-known cousin potassium nitrate (saltpetre, KNO_3), a component of gunpowder.

Potassium nitrite was first made by the remarkable Swedish chemist Carl Wilhelm Scheele (1742–86) in 1774.[17] He made many of his discoveries in a primitive laboratory attached to his pharmacy in the small Swedish town of Köping. He heated saltpetre at dull red heat for about half an hour and obtained a new salt that we now know as potassium nitrite. Its properties are very different from those of saltpetre and it rapidly gained importance in the manufacture of azo-dyes, in the preservation of tinned meat and in medicine.

The vasodilator action of potassium nitrite was first examined in 1880[18] by Edward Reichert of the University of Pennsylvania, in association with the distinguished neurologist Weir Mitchell. The effect of an intravenous injection or oral administration of potassium nitrite was found to be similar to that of inhaling amyl nitrite but the lowering of blood pressure lasted much longer, although the onset was slower. The result of this study was that, for a time, an infusion of potassium or sodium nitrite or administration by mouth, was a recognised treatment for the relief of anginal pain and for the treatment of other cardiovascular conditions. Under the entry '*sodii nitris*' the *National Standard Dispensatory* of 1905 says: '…potassium nitrite exerts the same influences upon the human organism as do glyceryl trinitrate and amyl nitrite. On account of the slowness of its absorption and elimination its effect is more prolonged. It is given in the dose of 1 to 3 grains'.[19] Throughout the early twentieth century, potassium and sodium nitrite appeared in *Materia Medica* and in the 1906 edition of Squibb's *Materia Medica* sodium nitrite was on offer at one dollar for 1 lb with a recommended dose of one grain. However, it was never really popular as an anti-anginal drug, in spite of its superiority to amyl nitrite and the absence of danger in handling it.

There are several reasons for its lack of acceptance as a drug. Firstly, an infusion is more difficult to administer than the procedures used for amyl nitrite and nitroglycerine. Secondly, even at fairly low doses, potassium and sodium nitrite are toxic because of reaction with haemoglobin in blood to givemethaemoglobin, which renders it incapable of carrying oxygen.[20] Thirdly, things were spoiled for potassium nitirite by WT Law, a doctor in private practice, who claimed that potassium nitrite was effective in the treatment of epilepsy.[21] However, Law gave very large doses (20 grains) which, he claimed, gave rise to no adverse side effects. Others could not replicate this and patients complained of unacceptable symptoms, such as severe headaches, nausea and giddiness. The explanation of the inconsistency was that Law was using a particularly impure sample of potassium nitrite that was largely potassium nitrate, a substance with little biological action.[22] However, harm had been done to the reputation of potassium nitrite and, as other vasodilators were available, its use declined. Fourthly the reputation of inorganic nitrite was irreparably damaged by the discovery that in acid conditions, as in the stomach, in reacts with secondary amines to produce secondary nitrosamines, shown in 1956 to be potent carcinogens.[23] Although sodium nitrite has never been shown as a cancer-causing agent in humans, the danger is always there. Its continued use in food preservation is surprising. Unsurprisingly, pharmacies no longer stock sodium or potassium nitrite. In the proceedings of a symposium on hypotensive drugs held in 1956, neither sodium nor potassium nitrite is even mentioned.[24]

SALTPETRE

Although potassium nitrite was discovered in 1774, there is documentary evidence that it was used, unknowingly,

before that time in the treatment of angina. Why this occurs is due to the strange relationship between potassium nitrate (a natural product) and potassium nitrite. Saltpetre (potassium nitrate) is made naturally by the action of bacteria on nitrogenous material from animal and vegetable sources and, in a few places, occurs as large deposits. The largest of these (it is sodium nitrite rather than the potassium salt) is in Chile, where it was mined as a fertiliser, and there are deposits of potassium nitrate in India and China. The Chinese used these deposits to produce gunpowder, invented in the Tang Dynasty (CE 618–907). Precise instructions for making gunpowder are given in a printed book *Wu Jing Zong Yao* (*Collection of the Most Important Military Techniques*) of CE 1044. In the production of potassium nitrate by bacterial action, potassium nitrite is an intermediate, but aerial oxidation to nitrate over time means there is no contamination of nitrate by nitrite in the old deposits. The arrival of gunpowder in Europe eventually changed the science of warfare radically. By the sixteenth century, much was shipped to Europe from China and India by the East India Company, but there was also an indigenous method of manufacture. Heaps of soil and nitrogenous waste, known as saltpetre plantations or nitriaries, were allowed to stand; nitrate gradually appeared as an efflorescence and was harvested. As a 'young' product, it almost certainly contained potassium nitrite as an impurity, a matter of some significance in the use of potassium nitrate as a medicament.

There are few references to potassium nitrate in ancient pharmacopoeia and we may conclude from this that it was thought to be of no medical value. However, by Tudor times, it was being used in the treatment of angina, undoubtedly because the material being used came from nitriaries and contained some potassium nitrite. As the amount of impurity would have varied from sample to sample it is not surprising that it did not establish itself as an infallible treatment for angina, but it seems likely that Tudor physicians had anticipated the discoveries of Reichart and Mitchell.[12]

There is a very precise account given of the use of potassium nitrate in the treatment of what appears to be angina in a Chinese eighth century medical manuscript discovered in the Buddhist grotto at Dunhuang.[21] The potassium nitrate used would have been obtained from old desert deposits, containing no potassium nitrite as an impurity. However, the physician instructs the patient to hold the potassium nitrate under the tongue and then swallow the saliva. There are colonies of bacteria in the mouth that can reduce nitrate to nitrite[22] and so, if the patient followed the instruction carefully enough, he or she would swallow a dose of the vasodilator potassium nitrite.

Some of the finest physicians of the Middle Ages were, of course, Arabic. There is no mention of the use of saltpetre in cardiovascular medicine in the text on cardiovascular drugs by Abu'Ali al-Husayn ibn 'Abdallah ibn Sina, latinised as Avicenna (born CE 980).[27] This may have been because there is no naturally occurring potassium nitrate in Arabia. The first mention of it occurs in *Kitab al-Jami'fi al-Adwiya al-Mufrada* (*Book of the Assembly of Medical Simples*), finished by Abu Muhammad al-Malaqi Ibn al-Baiþar about CE 1240, and this saltpetre was probably obtained from China.

There is one rather curious use of potassium nitrate in the treatment of asthma. Paper was soaked in a solution of potassium nitrate and dried. It was then ignited in a jar and the jar covered. The contents of the jar were then inhaled by the sufferer. The ignition of potassium nitrate produces several oxides of nitrogen, including nitric oxide, which would have relaxed bronchial smooth muscles and aided patients suffering from asthmatic spasms. This procedure was still in use in 1926.

Why has interest in potassium nitrite as a vasodilator blossomed to such an extent that there was an international conference on the subject at the National Institute of Health in Maryland in September 2005? There is a fair amount of potassium nitrite in blood plasma and in tissue, and Mark Gladwin, a chest physician at the National Institute of Health, has suggested that this is the source of some of the nitric oxide that controls the dilation of blood vessels.[28] The enzymatic conversion of nitrite into nitric oxide occurs readily and the role of xanthine oxidoreductase in this regard has been fully explored.[29] If serum nitrite is a natural source of nitric oxide and a supply of nitric oxide is essential for optimum blood flow, possibly supplementing serum nitrite levels, either by medication or by a modified diet, might enhance cardiovascular health. Equally, nitrite therapy might be beneficial when extra nitric oxide is required in a crisis. This latter matter has already been explored in relation to ischemia-reperfusion damage.[30] Possibly, Scheele's serendipitous discovery of 1774 has a role to play in twenty-first century medicine after a century of neglect.

Author's note: The first words of the title of this article are from line 139 of a poem by Matthew Arnold, entitled *Thyrsis* (1866), describing the effects of age.

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