

Circulation, 2008, 117(16) 2151-9

Published online 2008 April 22, doi:<http://dx.doi.org/10.1161/CIRCULATIONAHA.107.753814>

Therapeutic Uses of Inorganic Nitrite and Nitrate: From the Past to the Future

Anthony R. Butler, Ph.D. and Martin Feelisch, Ph.D.

From the Bute Medical School, University of St. Andrews, Scotland and the Clinical Sciences
Research Institute, Warwick Medical School, University of Warwick, England

Short Title: Therapeutic Uses of Nitrite and Nitrate

Total Word Count: 8,998 (Text without Refs: 6,353)
(incl. Title & Refs)

Journal Subj. Codes: [95] Endothelium/vascular type/nitric oxide
[118] Cardiovascular Pharmacology
[138] Cell signalling/signal transduction
[101] Nutrition

Key Words: Nitric oxide, pharmacology, history of medicine, cardiovascular
diseases, drugs

Correspondence to:

Anthony R. Butler
Bute Medical School
University of St. Andrews
St. Andrews
Fife, KY16 9ST
Scotland

or

Martin Feelisch
Clinical Sciences Research Institute
Warwick Medical School
Gibbet Hill Road
Coventry, CV4 7AL
England

Phone: +44-1334-463842
Fax: +44-1334-463808
e-mail: arb3@st-andrews.ac.uk

Phone: +44 -24-7652-8372
Fax: +44-24- 7615-0589
e-mail: mf@warwick.ac.uk

Introduction

The presence of nitrite (NO_2^-) and nitrate (NO_3^-) in bodily fluids has been known for some time. Dietary studies carried out by Mitchell *et al*¹ at the beginning of the twentieth century established that the amounts of nitrate excreted in the urine are higher than those ingested with the food, suggesting that the excess nitrate must be a product of endogenous biosynthesis. Later metabolic balance studies by Tannenbaum *et al*^{2,3} showed that this assumption was correct and provided unequivocal evidence for mammalian nitrate biosynthesis. Griess⁴, using his eponymous chemical test, showed that human saliva contains small quantities of nitrite and the detection of very high levels of nitrite in the urine of a volunteer, who happened to have contracted a fever, was the first indication that endogenous production of nitric oxide (NO) is part of the immune response. Nitrite is not normally present in urine, and it was Cruickshank and Moyes⁵ who realized that it originated from bacterial reduction of urinary nitrate, an observation that forms the basis of today's dipstick tests for urinary tract infection. Shortly after the discovery by Moncada's group that vascular endothelial cells produce NO from L-arginine⁶, Stuehr and Marletta reported that the same pathway accounts for the production of nitrite and nitrate by activated macrophages,⁷ and countless investigators have since used nitrite and nitrate to assess NO production in basic and translational research studies. More recently, the ease with which nitrate is reduced to nitrite and nitrite converted into NO has occasioned interest in the role of plasma nitrite in vascular smooth muscle relaxation and the control of blood pressure and flow⁸, and possible therapeutic uses of nitrite.^{9,10} Subsequent animal experimental studies revealed that a number of organs are protected against ischemia/reperfusion-related tissue injury following systemic application of small amounts of nitrite¹¹, suggesting further therapeutic uses. Strangely, this renewed interest in nitrite/nitrate, together with emerging data suggesting possible new roles for these anions in physiology, coincides with the conclusion by the International Agency for

Research on Cancer that ‘ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic for humans’.¹² The purpose of this review is neither to consider the physiological role of naturally occurring nitrite and nitrate in organs and bodily fluids or their usefulness as biomarkers of NO activity, nor to discuss their possible role as carcinogens, but to explore the uses of inorganic nitrite and nitrate in medicine, not only modern medicine but also medicine of the past. It transpires that medical interest in these oxyanions of nitrogen is not new.

Discovery and chemical properties

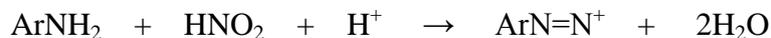
Nitrates, particularly potassium nitrate (known also as niter or nitre and salpeter), have been known since prehistoric times and, in the Middle Ages, natural deposits were commercially exploited. The Chinese invented gunpowder around CE 800 and, with its appearance in Europe during the thirteenth century, potassium nitrate became strategically important. Demand increased further with the Agricultural Revolution of the nineteenth century and the use of nitrates as fertilizers. Natural sources were eventually supplemented by synthetically produced nitrate at the beginning of the last century.¹³

Nitrite is present at trace levels in soil, natural waters, and plant and animal tissues. In pure form, nitrite was first made by the prolific Swedish chemist Scheele working in the laboratory of his pharmacy in the market town of Köping. He heated potassium nitrate at red heat for half an hour and obtained what he recognised as a new ‘salt’.¹⁴ The two compounds (potassium nitrate and nitrite) were characterised by Péligré.¹⁵



The release of oxygen from a substance known to alchemists as “aerial niter” since the times of Paracelsus¹⁶ explains the role of nitrates in gunpowder, rocket propellants and other explosives. Sodium nitrite rapidly gained importance in the development of organic

chemistry during the nineteenth century when it was discovered that nitrous acid (HNO_2) reacts with aromatic amines (ArNH_2) to produce diazonium ions¹⁷, a highly important synthon for the dyestuffs industry and for synthetic organic chemistry generally:



The mechanism of such diazotisation reactions has been subject to extensive study¹⁸ and diazotisation may be responsible, in part, for the carcinogenic role of nitrite under certain conditions, in particular drug-nitrite interactions.¹⁹

Nitric acid (HNO_3) is a strong acid and completely ionised at all biologically interesting pHs. Although nitrous acid (HNO_2) is a weak acid, with a pK_a of 3.15, it is also, at physiological pHs, completely dissociated, except in the stomach, on the surface of airways, within select cellular compartments such as the mitochondrial intermembrane space, endosomes, secretory vesicles, lysosomes and other acidic organelles, and on the skin.

Nitrite as a vasodilator

The scope of this review is limited to inorganic nitrite and nitrate, but interest in a medical role for inorganic nitrite was first aroused because of the spectacular success of organic nitrites, and related compounds, in the treatment of angina pectoris. Butter²⁰, writing about the treatment of angina in 1791, gave no drug treatment and had little more to offer than recommending a tranquil life style. However, while working at the Edinburgh Royal Infirmary in the 1860s, Brunton noted that the pain of angina could be lessened by venesection and wrongly concluded that the pain must be due to elevated blood pressure. As a treatment for angina the reduction of circulating blood by venesection was inconvenient. Therefore, he decided to try the effect on a patient of inhaling amyl nitrite, a recently synthesised compound and one that his colleague Gamgee²¹ had shown lowered blood pressure in animals. The result was dramatic.²² Pain associated with an anginal attack

disappeared rapidly and the effect lasted for several minutes, generally long enough for the patient to recover by resting. For a time amyl nitrite was the favoured treatment for angina but its volatility made it troublesome to administer and it was soon replaced by chemically related compounds that had the same effect but are less volatile. The most popular replacement was glyceryl trinitrate (GTN), an organic nitrate better known as nitroglycerin.²³ The fact that this compound is highly explosive and a component of dynamite appears not to have been a problem. In his textbook of 1897 Brunton²⁴ lists a number of chemically related compounds that can be used in the treatment of angina. The list includes not only amyl nitrite but propyl, ethyl and isobutyl nitrites, as well as GTN. A similar list is provided by White²⁵ in his textbook of 1899. GTN, a drug introduced into allopathic medicine thanks to extensive homeopathic studies by Hahnemann,²⁶ occasioned greatest favour amongst practising physicians and by 1956, in a symposium on hypotensive drugs,²⁷ it is the only drug of this type that is listed. GTN was first synthesized by Sobrero at the University of Torino in 1812 and, considering the way in which he handled it, he was fortunate not to cause a fatal accident.²⁸ He thought it too explosively violent to have any practical use. Nobel, the highly successful Swedish entrepreneur, was able to moderate its action by incorporating it into kieselguhr to form dynamite. It is largely from this invention the Nobel family fortune is derived. Tragically, Nobel's younger brother Emil was killed while working with GTN and this was a dark episode in Nobel's life. Sobrero bitterly resented Nobel's commercial success with what he saw as his invention, although Nobel always acknowledged his debt to Sobrero.²⁹ It is a curious coincidence that by 1895 Nobel had developed angina and was prescribed GTN as treatment, but it is a happier coincidence that the 1998 Nobel Prize for Physiology or Medicine was awarded for the discovery of the role of NO as a signaling molecule in the cardiovascular system. Now that NO is known to be an important vasorelaxant it is easy to see why drugs of this type act in the way they do. Each is a substrate

for one or more enzyme systems, possibly located in the vascular wall, that convert it into nitrite and subsequently to NO. One such enzyme, a mitochondrial aldehyde dehydrogenase, has been purified and partially characterised.³⁰ However, the contribution of this or other enzyme systems to the overall vasodilation by these drugs is difficult to assess since multiple metabolic pathways appear to act in concert.³¹

In view of the range of organic nitrites and related compounds that act as vasodilators it is not surprising that potassium and sodium nitrites were tested in this regard. In 1880, Reichert and Mitchell³² published a very full account of the biological action of potassium nitrite on humans as well as animals. At that time the value of amyl nitrite in the treatment of angina was severely compromised by the short duration of its effect and the search for an improved drug had begun. The effect of potassium nitrite on the nervous system, brain, spinal cord, pulse, arterial blood pressure and respiration of healthy human volunteers was noted, as was the variability between individuals. The most significant observation was that even a small dose of < 0.5 grains (~30 mg) given by mouth caused, at first, an increase in arterial blood pressure followed by a moderate decrease. With larger doses pronounced hypotension ensued. They also noted that potassium nitrite, however administered, had a profound effect on the appearance and oxygen carrying capacity of the blood. They compared the biological action of potassium nitrite with that of amyl and ethyl nitrites and concluded, rather interestingly, that the similarity of action depends upon the conversion of organic nitrites to nitrous acid. Similar observations to those of Reichert and Mitchell were made by Atkinson³³ and by Densham.³⁴ Practicing physicians, including Hay³⁵ and Leech³⁶, examined the therapeutic value of inorganic nitrites as hypotensive drugs and noted that, although of slower onset, the therapeutic effect lasts much longer and they might be seen as superior drugs. They soon appeared in the *Materia Medica* of the time. In 1906 the drug supplier Squibb sold a 1 lb bottle of sodium nitrite (*sodii nitris*) for one dollar³⁷, and by the mid 1920s an injectable

solution of sodium nitrite became available (Nitroskleran; E.Tosse & Co., Hamburg) for the treatment of hypertension and vasospasm.³⁸ Instructions for the use of sodium nitrite in the treatment of angina are given in Martindale's Additional Pharmacopoeia and in the US National Dispensatory of 1905.³⁹ A textbook on *Materia Medica* for medical students of 1921 gives details of the appropriate dose⁴⁰ but, by the middle of the twentieth century, its medicinal use had essentially ceased, largely because of adverse side effects. Blumgarten⁴¹ noted that sodium and potassium nitrites often caused nausea, belching, stomach ache and diarrhoea. While these side effects may have caused physicians to hesitate in prescribing sodium nitrite for angina another event precipitated inorganic nitrite's fall from favour (see below).

Interest in the vasodilator properties of nitrite enjoyed a renaissance with the notion that nitrite may be involved in the regulation of local blood flow following conversion to NO by non-enzymatic mechanisms^{42,43} and an oxygen-sensitive nitrite-reductase⁴⁴ and S-nitrosothiol-synthase⁴⁵ function of hemoglobin. Like NO, inhaled nebulized nitrite has been shown to be an effective pulmonary vasodilator⁴⁶ and, along with organic nitrites⁴⁷, suggested for potential use in neonatal pulmonary hypertension. While there is no doubt that appropriate pharmacological doses of nitrite can normalize elevated blood pressure⁴⁸, the question as to whether or not physiological concentrations of nitrite are vasodilator-active is still a matter of debate.^{49,50}

Conversion of nitrite into NO and NO-related products

In view of the close chemical connection between nitrite and NO it is tempting to assume that nitrite acts as a source of NO when functioning as a vasodilator. However, such conversion requires either strongly acidic conditions or enzymatic catalysis. At low pH nitrous acid can give rise to the spontaneous generation of NO:





While solutions of acidified nitrite have been used successfully to generate NO and induce vasorelaxation in isolated blood vessel studies,⁵¹ the same reaction mechanism has been proposed to explain nitrite's biological action.^{52,53} However, pHs at which this occurs are not generally found within living systems,⁵⁴ with the exceptions mentioned above. On the other hand, the enzyme xanthine oxidoreductase converts nitrite into NO when oxygen levels are low and this is a more likely course of action⁵⁵ in the vascular system, at least under ischemic conditions. In fact, recent data suggests that hypoxic NO formation from nitrite is carried out by multiple enzyme systems¹⁰ and occurs in virtually all tissues and organs (Feelisch *et al.*, 2006; unpublished data). Independent of its reduction to NO, nitrite is converted into NO-related products including S-nitrosothiols and NO-heme species at normal physiological pH and oxygen levels.⁵⁶ While it cannot be excluded that some of the biological effects of nitrite may be mediated by nitrite itself, it is fair to assume that the majority of nitrite's physiological and therapeutic actions that require conversion into NO and NO-related products involve enzymatic catalysis.

Nitrite as an antidote for cyanide and hydrogen sulphide poisoning

In popular literature cyanide (CN⁻) is considered to be the acme of human poisons. In fact it is by no means the most poisonous substance generally available but it acts very rapidly and it is upon this that its reputation rests. Large doses cause instant death and even with low doses the characteristic symptoms of cyanide poisoning (loss of consciousness, motionless eyes, dilated pupils, cold skin and sluggish pulse and respiration) appear within seconds. In spite of the catastrophic consequences of an overdose, potassium cyanide was over many years used in medicine as a treatment for chest complaints,⁵⁷ particularly a dry cough.⁵⁸ It was not removed from the British Pharmacopoeia until 1945.

By the end of the 19th century it was established that the toxicity of cyanide was due to interference with the process of cellular respiration.⁵⁹ Keilin⁶⁰ showed that cyanide reacts with the ferric heme of the enzyme cytochrome c oxidase, a vital link between the tricarboxylic acid cycle and formation of metabolic water causing inhibition of mitochondrial respiration. As cyanide also reacts with methemoglobin⁶¹ it should be possible to prevent the reaction of cyanide with cytochrome c oxidase by massively increasing the concentration of methemoglobin in the blood. Nitrite oxidizes the central iron atom of hemoglobin from the ferrous (Fe^{2+}) to the ferric (Fe^{3+}) state, producing methemoglobin and is, therefore, a potential antidote for cyanide poisoning. The clinical use of nitrite was first proposed by Hug⁶² and is now universally used. Sodium thiosulfate is also included in the antidote to provide a source of sulfur to aid the conversion of cyanide into thiocyanate by rhodanese. The first cases of acute cyanide poisoning in humans to be treated with nitrite and thiosulfate were reported in 1934. One patient had ingested 5g potassium cyanide but recovered after being given 1.5g sodium nitrite and 18g sodium thiosulphate.⁶³ In many countries nitrite is part of the cyanide antidote kit. Nowadays patients are given an ampoule of amyl nitrite by inhalation or an intravenous injection of 3% sodium nitrite, followed by a slow injection of 50% sodium thiosulfate.⁶⁴

Although formation of methemoglobin is generally accepted as the explanation of the efficacy of nitrite as an antidote, there is evidence to suggest that this is not the complete explanation.^{65,66} There may be alternative or additional routes whereby nitrite detoxifies but no details are available.⁶⁷ Compounds that promote NO release *in vivo* (like bradykinin) modify cyanide toxicity. Whether this is nitrite's alternative mode of action in detoxification or just another source of nitrite from endogenous NO is, at this time, difficult to assess.

Nitrite is also an efficacious antidote to poisoning by hydrogen sulphide (H_2S), an occupational hazard with high lethality and long-term neurological sequelae in survivors. Like

NO and CO, low concentrations of H₂S are produced endogenously and have vasodilator properties, but the physiological significance of its formation is currently unknown.⁶⁸ Supra-physiological concentrations of sulphide, as experienced after H₂S inhalation, lead to rapid inhibition of mitochondrial respiration by reversible binding to the central iron atom of cytochrome c oxidase in place of oxygen, explaining why H₂S poisoning shares many similarities with cyanide intoxication.⁶⁹ Nitrite administration, which is superior to that of oxygen alone⁷⁰ and often combined with hyperbaric oxygen therapy, is most effective when given immediately after H₂S exposure.⁷¹ It is thought to act via induction of methemoglobinemia and subsequent binding of hydrosulfide anions (HS⁻) to the oxidized blood pigment, leading to inhibition of cytochrome c oxidase and re-institution of aerobic respiration in the tissues. While this mode of action appears reasonable the rather slow rate of methemoglobin formation by nitrite is inconsistent with the rapid recovery typically observed in the clinical setting suggesting, as with the treatment of cyanide poisoning, the involvement of additional mechanisms. Although nitrite has been known to have protective and antidotal effects against experimental sulphide poisoning in rodents for many years⁷² nitrite administration for H₂S intoxication was introduced into human therapy only in the mid 1970s.⁷³ The recommended dosage regimen for nitrite in sulphide intoxication is identical to that established for the treatment of cyanide poisoning, i.e. initiation with inhalations of amyl nitrite followed by intravenous injection of 10 mL of a 3% solution of sodium nitrite.⁷⁴

Other medical uses of inorganic nitrite

In view of nitrite's success with angina it was tried for the treatment of other medical conditions. Law⁷⁵ recommended the administration of very large doses (20 grains or 1.3g) of sodium nitrite for the treatment of epilepsy. Other physicians tried this and found the side effects were far too serious to continue with the treatment, with considerable consequences

for the therapeutic use of inorganic nitrite. The toxic nature of such high doses was confirmed by Ringer and Murrell⁷⁶ and they concluded that Law had been using an impure sample of sodium nitrite that was largely sodium nitrate. They attempted to establish a safe dose but the reputation of sodium nitrite had suffered and, in view of the success of GTN, it disappeared from widespread use. The final blow came when Magee¹⁹ reported that certain nitrosamines, which could be formed in the stomach by reaction between nitrite and naturally-occurring secondary amines in food, are strongly carcinogenic in rodents. Although these findings were quickly confirmed by others and have been extended to other animal species, a causal relationship between nitrite and nitrate exposure and human cancer has not been unequivocally demonstrated.⁷⁷ Nevertheless, further medical use of nitrite ceased for decades, except as an antidote in emergencies, and maximal contaminant levels of nitrite and nitrate levels in drinking water and foods soon became strictly regulated in most countries worldwide. In light of the negative image nitrite has acquired over the years, it is somewhat surprising that the use of nitrite as an antibacterial agent in canned food has continued. More recently, the antimicrobial properties of nitrite that form the basis for its use in food preservation, have been explored for potential benefit in lung and skin diseases.

Acidified nitrite

Acidification is a prerequisite for nitrite to act as antimicrobial agent, suggesting (albeit not proving) that the active principle is NO. It has been known for some time that the nitrite found in human saliva originates from nitrate that is actively secreted into the oral cavity and gets partially reduced there by the local commensal bacterial flora.⁷⁸ After swallowing nitrite ends up in the acidic environment of the stomach and the NO thus produced is thought to contribute to the antibacterial effects of gastric juice. Similarly, the nitrite produced from nitrate in sweat is believed to exert antimicrobial effects on the surface of the skin.⁷⁹ Thus,

acidified nitrite may be a component of innate immunity at several locations on and within the body. Some of the attempts to capitalize on this insight point into potentially promising therapeutic directions, although few of these findings have made their way into the clinic.

The effectiveness of acidified nitrite in killing antibiotic-resistant *Pseudomonas* bacteria might offer a possibility to eradicate a major cause for chronic lung infections in cystic fibrosis patients,⁸⁰ provided a safe mode of administration was found. The antimicrobial properties of NO can be exploited by dermal application of crèmes containing nitrite and an acidifying agent, e.g. ascorbic acid, for the treatment of a number of skin diseases.⁸¹ The same concept has been demonstrated to increase microcirculatory blood flow in Raynaud patients^{82,83} and accelerate wound healing.⁸⁴ While the effects of acidified nitrite are typically ascribed to the generation of NO the possibility that part of the nitrite applied is absorbed and converted into NO-related products in the tissue cannot be excluded.

Use of inorganic nitrate in medicine

Although modern manuals of *Materia Medica* and pharmacopoeias state that potassium nitrate has no drug action other than acting as a diuretic (see below), historical records show that it has been used extensively in medicine over the years for the treatment of a number of conditions. In view of the close chemical relationship between nitrite and nitrate we suggest that the value of inorganic nitrate in medicine is due, at least in part, to its conversion into nitrite during administration or contamination with nitrite because of the manner in which it was manufactured.

Niter occurs in natural deposits in desert regions. Fairly large amounts are found in the north western provinces of China and it was well known to early Chinese alchemists. They called it *xiao shi* (salve-stone) and it was first recognised in the fourth century CE. It was a component of some of the elixirs of immortality concocted by Daoist savants as they searched for a means of realising the Daoist ideal of life without death.⁸⁵ Entirely by chance

they mixed it with sulfur and charcoal and thus created gunpowder, which was used by the Chinese not only for fireworks but also for civil engineering and warfare. The first printed formula for gunpowder occurs in a Chinese manual of war that appeared in CE 1044.

One of the oldest accounts of the use of niter in Chinese medicine is a treatment of what appears to be angina in an eighth century Chinese manuscript uncovered at the Buddhist grotto of Dunhuang.⁸⁶ The patient is instructed to take niter, hold it under the tongue for a time and then swallow the saliva. The significance of the instructions is that under the tongue, even in a healthy mouth, there are nitrate-reducing bacteria that convert some of the nitrate into nitrite.^{78,87} So, if the patient follows the physician's instructions fully, he or she will be taking in nitrite known to be a treatment for the alleviation of anginal pain.

Arab physicians were amongst the most advanced of the medieval period but there is no mention of niter in a book on cardiac drugs by Avicenna, born CE 980. The first extant Arabic mention of niter occurs in a book by Kitab al-Jami' fi al-Adwiya al-Mufrada (Book of the Assembly of Medical Simples) finished by Abu-Muhammad al-Malaqi Ibn al-Baitar around CE 1240. Niter was called Thalji al-Sin (Chinese snow), indicating the contact between Chinese and Arab civilisations. It was about this time that Arabs started to use niter in gunpowder, as well as a component of prescriptions.

Niter does not occur naturally to any great extent in Europe and the efficacious use of niter in early European medicine is easier to understand if one realizes how the niter was produced. When gunpowder became known in Europe (Roger Bacon mentions it in CE 1240) there was enormous demand for niter and much was shipped to Europe from India, where it occurs in natural deposits. But the demand outstripped supply and indigenous manufacture was started. It was made in plantations or 'nitriaries', particularly in France and Germany. Natural conditions were simulated by exposing heaps of decaying organic matter mixed with lime to atmospheric action.⁸⁸ Nitrates appeared as efflorescences and were converted into the

potassium salt by reaction with potassium carbonate (potash). There are two groups of bacteria responsible for this process: *Nitrosomonas* convert ammonia into nitrite and *Nitrobacter* convert nitrite into nitrate.⁸⁹ It is quite possible that niter from nitriaries contained some nitrite, thus giving it medicinal value. This is unlikely in niter from natural deposits as they are old and aerial oxidation will, over time, convert all the nitrite into nitrate. So, the eighth century Chinese physician mentioned previously had to instruct the patient on how to generate nitrite but European physicians of the 14-17th centuries, using niter from a different source, could prescribe it without further refinement as there was nitrite there already.

However, such a prescription was rather hit-or-miss as the amount of nitrite present was a matter of chance. In one of the most comprehensive accounts of the use of niter, methods of making it more effective are described. The book, by Challoner, was printed in London in 1584 and entitled '*A short discourse of the most rare and excellent vertue of nitre*'.⁹⁰ The spelling of the English is idiosyncratic (rather like that of modern students) as spelling was not fully standardised until the publication of Johnson's dictionary in 1775. Challoner's book is concerned mainly with the value of niter in treating various dermatological conditions ('diseases of the skinne'), including 'tawnie steynings, freckles, duskness and flegmatike evaporations'. It will, he claims, 'restore the skinne and complexion to the native bewtie'. The key to understanding this claim lies in the first section of the book where the author tells his readers how to make niter more effective ('yet more sharpe and subtile'). He describes three ways and they all involve heating (called 'calcination' by Challoner). That, of course, converts some of the niter into potassium nitrite and so, without realising it, Challoner anticipated the discovery of potassium nitrite by Scheele by nearly 200 years. As discussed above, nitrite has an antibacterial effect and accelerates wound healing, hence its effectiveness on infected skin blemishes ('skales, scrabbes, skurffe, dandruffe,

pimples, tetters, bytes' and so on). Naturally-occurring nitrite in saliva has the same effect and explains, in part, why most animals instinctively lick a wound.⁹¹

Challoner does not stop with the application of niter to the skin. He claims that it can be used 'for uncumbring and clensing of the lunges' and for the 'remedie of hoarnesses, olde coughe and toughe coughe, weising in the windpipes' and so on. For this use he suggests making the niter into a pill and then 'hold one of those pilles lounge under the tongue, to mixe thereof as much as may be with the moisture of the mouth ... and lastlie swallow it', a procedure curiously reminiscent of the Chinese prescription and anticipating some of the work of Lundberg, Weitzberg, Cole and Benjamin.⁷⁸

Nitrate and the treatment of lung diseases

For a time amyl nitrite was used for relieving patients suffering an asthma attack and, in a paper⁹² of 1891, other nitrites, including sodium nitrite, were suggested for this. The author remarks that the use of nitrites is not the treatment of choice but that it is said to be beneficial, probably by virtue of its smooth muscle relaxing effects. However, relief could be delivered even better by a procedure using nitrate rather than nitrite. Blotting paper was soaked in a solution of niter and allowed to dry. Squares of the paper were burnt in a jar and the patient inhaled the fumes. Apparently, this procedure was frequently successful in relaxing a bronchial spasm. It was first published as a patent in 1867⁹³, is described in detail in the Encyclopædia Britannica of 1911⁹⁴ and occurred as recently as 1926 in the US Dispensary.⁹⁵ The products of thermal decomposition of niter include NO, NO₂ and O₂⁹⁶. As NO is a poor bronchodilator and NO₂ is toxic it is difficult to see how inhalation of this mixture brings relief. Possibly the combination has an effect greater than the sum of its parts.

In addition to its use in asthma, sodium nitrate was given orally for the treatment of chronic bronchitis.⁹⁷ It is unclear whether the apparent effectiveness of this treatment was

secondary to its conversion to nitrite causing bronchial relaxation and antibacterial effects or due to an effect of nitrate itself.

Nitrates as diuretics

Nitrates have been used as diuretics for centuries. One of the first descriptions of the medical use of potassium nitrate for the treatment of dropsy (edema) is found in Thomas Willis' 'Pharmaceutice Rationalis' of 1674.⁹⁸ While it was long known that relatively large amounts (grams) were required to achieve effective diuresis, the dose-response relationship was first established in systematic "homeopathic provings" in 1825.⁹⁹ Clear differences in potency exist between various nitrate salts,¹⁰⁰ with ammonium nitrate being the most effective. Their mode of action was revealed by studies in dogs demonstrating an enhanced excretion of urinary chloride and sodium, resulting in a net loss of salt and water, due an increased glomerular filtration without an equivalent increase in tubular reabsorption.^{101,102} Whether these effects are mediated by formation of nitrite or NO is unknown.

Extensive animal and human studies by Keith, Whelan and Bannick¹⁰³ confirmed the superiority of the ammonium over the sodium salt of nitrate. They also demonstrated that nitrates can potentiate the effects of other diuretics and that toxic symptoms are remarkably rare, even when administered in doses of 10-15 g daily for several weeks. Thus, ammonium nitrate was introduced as a new more effective diuretic in 1926 and used with great success for the treatment of various forms of edema in North America, in particular at the Mayo Clinic. After a time of exaggerated emphasis on possible toxic effects of nitrates during the preceding two decades, which lead physicians to use lower, inadequate doses, it looked as whether ammonium nitrate was there to stay as the diuretic of choice. What had triggered the fear of inducing severe cyanosis when employing potassium or sodium nitrate as diuretics before was the toxicity associated with the use of massive amounts of bismuth subnitrate for

diagnostic purposes,¹⁰⁴ which is somewhat surprising since the toxicity of large amounts of nitrate was well known for a long time.¹⁰⁵ Concerns about the safety of nitrates reached a new height with the appearance of case reports about transient methemoglobinemia following administration of ammonium nitrate.^{106,107} The reasons for these rare complications (which disappeared on discontinuation of nitrate therapy in most cases) remain unclear, but may have been due to either contamination of the nitrate salt with nitrite, renal insufficiency causing elevated circulating levels of nitrate or gastrointestinal disorders with enhanced reduction of nitrate to nitrite by the bacterial gut flora.¹⁰⁸ With alternative diuretics in the form of organic mercurials available, the therapeutic use of nitrates as diuretics was abandoned by the mid 1930s.

Nitrate in other medicinal preparations

The fact that most nitrate salts are readily water-soluble has been exploited to produce medicines that require quick dissolution or application in liquid form. Although the effects of most of these drugs (e.g., cerium and silver nitrate) have little to do with the amounts of nitrate they contain, application of large enough quantities can cause methemoglobinemia.¹⁰⁹ Presumably, the same holds true for the excessive use of toothpastes aimed at treating dental hypersensitivity, some of which contain up to 10% potassium nitrate, although no intoxication from this source is documented in the literature.

Conclusions and outlook

In spite of the widespread use of sometimes astonishing amounts of nitrite and nitrate for different indications in medicine of the past, little use is made of them in contemporary medicine (except as antidote and solubility-enhancer). This is a result of several factors, some of which we have described in this review. Apart from the replacement by more modern and

effective medicines in some cases, the major driving force for this development appears to have been the fear fostered by discussions, both in the lay press and in the scientific literature, about the purported health risks of exposure to nitrite and nitrate. Reports about methemoglobinemia in infants caused by drinks or food prepared with nitrate-rich (and bacterially contaminated) well water and vegetables such as spinach, celery and carrots (“blue baby syndrome”), intentional and occupational intoxications in adults, increasing nitrate levels in soil and lakes due to fertilizer overuse and the formation of potentially carcinogenic N-nitrosamines all contributed to the negative image nitrite and nitrate have held in recent years. As a result, major efforts have been made to remove as much nitrite and nitrate as possible from our drinking water, to advocate nitrite substitution against other (often less effective) food preservatives and to establish cultivation conditions that result in crops with reduced levels of nitrate. While possible long-term consequences of a chronically reduced intake of nitrite and nitrate on human health are unknown, doubts have been raised about the general health risk of nitrite/nitrate intake.^{77,110-112} Interestingly, the average dietary intake of nitrate roughly equals that produced by the endogenous production of NO.¹¹³ Thus, if nitrite was truly of concern to human health because of its propensity to form carcinogenic nitrosamines, then the human body would have a significant evolutionary design flaw because ~5% of all ingested and endogenously produced nitrate eventually ends up as nitrite in the stomach, as pointed out by Archer¹¹⁰ (so far about “intelligent design”). In spite of the critical voices nitrite and nitrate’s image remains stigmatized.

What appears to have the greatest potential to change our current conception about the risk and value of nitrite and nitrate is the most recent emergence of data on the physiological and pharmacological effects of relatively low concentrations/doses of nitrite. Previously considered a biologically inert oxidative decomposition product of NO, nitrite has been proposed to be a signalling molecule in its own right.⁵⁶ Given its propensity for conversion

into NO and related species, unequivocal evidence for this role may be difficult to provide unless nitrite-specific signalling pathways were identified. Although speculative, it is possible that the nitrite-based reaction channels of contemporary mammalian cells are a vestige of earlier bacterial pathways and that the evolutionary more recent L-Arg:NO pathway utilizes signalling cascades originally evolved for nitrite, not the other way round. Regardless, surprisingly low amounts of nitrite have been demonstrated to exert potent cytoprotective effects against ischemia/reperfusion-related tissue damage *in vivo*^{10,11}, an action possibly mediated by modulation of mitochondrial function.¹¹⁴ Nitrate, which has been proposed to contribute to the health-promoting effects of the Mediterranean diet¹¹⁵ has been demonstrated to inhibit platelet aggregation,¹¹⁶ to mildly lower blood pressure,¹¹⁷ to enhance gastric mucosal defense mechanisms¹⁰⁸ and to reduce the oxygen cost of exercise.¹¹⁸ The latter is perhaps one of the most surprising of the more recent findings across the spectrum of nitrate actions. This particular observation may explain why an enhanced production of NO, which not only elevates blood flow and thus oxygen transport to tissues but leads to increased levels of circulating nitrite and nitrate, is crucial for the adaptation of life to the chronic hypoxia experienced at high altitude.¹¹⁹ Taken together, these results have shifted the attention away from toxic and vasodilator properties to a focus on metabolic effects. Moreover, they make one wonder to what extent inorganic nitrate may contribute to the effectiveness of organic nitrates in the setting of heart failure, for example.

Although efforts are underway to assess the potential usefulness of inorganic nitrite in a number of clinical research studies at the US National Institutes of Health none of these are likely to wet the appetite of the pharmaceutical industry to invest substantial amounts of money into drug development because not only are intellectual property claims related to simple inorganic compounds legally difficult to defend, but the material itself is cheap and readily available. The situation may change if medicinal chemists came up with new prodrugs

that allowed targeted delivery of nitrite to specific tissues or organs or nitrite/nitrate was intelligently used as adjuvant to current therapeutics. Which of the many facets of nitrite and nitrate action is likely to form the basis for future pharmaceutical exploitation is difficult to predict at present. Although rational approaches to the pharmacological treatment of medical problems have a tendency to ridicule the wisdom of century-old folk medicine and condemn alchemist's doing as quackery, there is much to learn from the past. In reviewing nitrite and nitrate's therapeutic use over centuries it appears that some of the potential these simple compounds may hold for medical use have not been realized, often because the basis for some unwanted drug effects were not understood and thus could not be controlled at the time. But even if the scare factor continues to dominate the mainstream thinking for some more time, there is an obvious need for a careful reassessment of the health risks of nitrite and nitrate. If initiated soon, such activity may provide the necessary "activation energy" to overcome the fear and stimulate the development of new therapeutic principles that utilize pathways regulated by nitrite and nitrate.

Funding Sources

This work was supported by funds from the Guthrie Trust (a travel grant for visiting the Wellcome History of Medicine Library in London, ARB) and the Medical Research Council (Strategic Appointment Scheme, MF).

Disclosures

The authors have nothing to disclose.

References

1. Mitchell HH, Shonle HA, Grindley HS. The origin of nitrates in urine. *J Biol Chem.* 1916; 24: 461-490.
2. Green LC, Tannenbaum SR, Goldman P. Nitrate synthesis in the germfree and conventional rat. *Science.* 1981; 212:56-8.
3. Green LC, Ruiz de Luzuriaga K, Wagner DA, Rand W, Istfan N, Young VR, Tannenbaum SR. Nitrate biosynthesis in man. *Proc Natl Acad Sci USA.* 1981; 78:7764-8.
4. Griess P. Über Metadiamidobenzol als Regens auf Salpétrige Säure. *Chem Ber.* 1878; 11:624-627.
5. Cruickshank J, Moyes, JM. The presence and significance of nitrites in urine. *Br Med J.* 1914; 2:712-713.
6. Palmer RM, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature.* 1988; 333:664-6.
7. Marletta MA, Yoon PS, Iyengar R, Leaf CD, Wishnok JS. Macrophage oxidation of L-arginine to nitrite and nitrate: Nitric oxide is an intermediate. *Biochemistry.* 1988; 27: 8706-8711.
8. Gladwin MT, Shelhammer JH, Schechter AN, Pease-Fye ME, Wacziarg MA, Panza JA, Ognibene FP, Cannon RO. Role of circulating nitrite and S-nitrosohemoglobin in the regulation of regional blood flow in humans. *Proc Natl Acad Sci USA.* 2000; 97: 11482-11487.
9. Dejam A, Hunter CJ, Tremonti C, Pluta RM, Hon YY, Grimes G, Partovi K, Pelletier MM, Oldfield EH, Cannon RO, Schechter AN, Gladwin MT. Nitrite infusion in humans and nonhuman primates. Endocrine effects, pharmacokinetics, and tolerance formation. *Circulation.* 2007; 116: 1821-1831.

10. Lundberg JO, Weitzberg E, Gladwin MT. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. *Nat Rev Drug Discov.* 2008; 7:156-167.
11. Dezfulian C, Raat N, Shiva S, Gladwin MT. Role of the anion nitrite in ischemia-reperfusion cytoprotection and therapeutics. *Cardiovasc Res.* 2007; 75: 327-338.
12. IARC Monographs, Volume 94: Ingested Nitrates and Nitrites; International Agency for Research on Cancer, Lyon, France; 2007
(<http://monographs.iarc.fr/ENG/Meetings/94-nitratenitrite.pdf> ; accessed Sept 4, 2007)
13. Barnum DW. Some history of nitrates. *J Chem Ed.* 2003; 80: 1393-1396.
14. Scheele CW. *Chemische Abhandlung von der Luft und dem Feuer.* Upsala & Leipzig: M Swederus; 1777.
15. Pélégot E. Sur l'acide hypoazotique et sur l'acide azoteux. *Ann Chim Phys.* 1841; 2: 58-68.
16. Debus AG. The Paracelsian aerial niter. *Isis.* 1964; 55: 43-61.
17. Williams DLH. *Nitrosation Reactions and the Chemistry of Nitric Oxide.* London: Elsevier; 2004.
18. Ridd JH. Nitrosation, diazotisation and deamination. *Quart Rev* 1961; 15: 418-436.
19. Magee PN, Barnes JM. The production of primary hepatic malignant tumours in the rat by feeding dimethylnitrosoamine. *Brit. J. Cancer.* 1956; 10:114-122.
20. Butter W. *A Treatise on the Disease Commonly Called Angina Pectoris* London: J Johnson; 1791.
21. Gamgee A. unpublished observation.
22. Brunton TL. On the use of nitrite of amyl in angina pectoris. *Lancet* 1867; 2: 97-98.
23. Murrell W. Nitro-glycerine as a remedy for angina pectoris. *Lancet* 1879; 1: 80-81, 113-115.

24. Phillips CDF. *Materia Medica, Pharmacology and Therapeutics Inorganic Substances*. London; J & A Churchill; 1882.
25. White WH. *Materia Medica Pharmacy, Pharmacology and Therapeutics*. London: J & A Churchill; 1899.
26. Fye WB. Nitroglycerin: a homeopathic remedy. *Circulation*. 1986; 73: 21-29.
27. Harrington M. *Hypotensive Drugs* London: Pergamon Press; 1956.
28. Bellamy A. The development of nitroglycerine as an explosive. *Atti del convegno in celebrazione del centenario della morte di Ascanio Sobrero*. University of Turin, 1989.
29. Fant K. Alfred Nobel; En Biografi. Norstedts Förlag, Stockholm 1991, p.302.
30. Chen Z, Zhang J, Stamler. Identification of the enzymatic mechanism of nitroglycerine bioactivation. *Proc Natl Acad Sci USA*. 2002; 99: 8306-8311.
31. Janero DR, Bryan NS, Saijo F, Dhawan V, Schwalb DJ, Warren MC, Feelisch M. Differential nitros(yl)ation of blood and tissue constituents during glyceryl trinitrate biotransformation in vivo. *Proc Natl Acad Sci USA*. 2004; 101: 16958-16963.
32. Reichert ET, Mitchell SW. On the physiological action of potassium nitrite. *Amer J Med Sc*. 1880; 156: 158-180.
33. Atkinson GA. The physiology of the nitrites and nitro-glycerine. *J Anat Physiol*. 1888; 22: 225-239, 351-371.
34. Densham B. The adjuvant action of the lactate ion on the vaso-dilator effect of sodium nitrite. *J Physiol* 1927; 63:175-179.
35. Hay M. Nitrite of sodium in the treatment of angina pectoris. *Practitioner*. 1883; 30: 170-194.
36. Leech DJ. On the duration of the action of medicines. *Brit Med J*. 1885; 2: 1005-1011.
37. Squibbs, ER. *Squibb's Materia Medica*, 1906.

38. Deusch G, Liepelt A. Die Hautkapillaren beim arteriellen Hochdruck und ihre Beeinflussung durch Nitrite. *Deut Arch Klin Med* 1928;160:207-211.
39. Hare HA, Caspari C, Rusby HH. *National Standard Dispensatory*. Lea Bros & Co: Philadelphia; 1905.
40. Bennett RG, *Materia Medica and Pharmacy for Medical Students*. London; HK Lewis: 1921.
41. Blumgarten AS. *Textbook of Materia Medica and Therapeutics* MacMillan, New York, 1934.
42. Modin A, Bjorne H, Herulf M, Alving K, Weitzberg E, Lundberg JO. Nitrite-derived nitric oxide: a possible mediator of 'acidic-metabolic' vasodilation. *Acta Physiol Scand*. 2001; 171: 9-16.
43. Zweier JL, Wang P, Samouilov A, Kuppusamy P. Enzyme-independent formation of nitric oxide in biological tissues. *Nat Med*. 1995; 1: 804-809.
44. Cosby K, Partovi KS, Crawford JH, Patel RP, Reiter CD, Martyr S, Yang BK, Waclawiw MA, Zalos G, Xu X, Huang KT, Shields H, Kim-Shapiro DB, Schechter AN, Cannon RO, Gladwin MT. Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. *Nat Med*. 2003; 9: 1498-1505.
45. Angelo M, Singel DJ, Stamler JS. An S-nitrosothiol (SNO) synthase function of hemoglobin that utilizes nitrite as a substrate. *Proc Natl Acad Sci USA*. 2006; 103:8366-71.
46. Hunter CJ, Dejam A, Blood AB, Shields H, Kim-Shapiro DB, Machado RF, Tarekegn S, Mulla N, Hopper AO, Schechter AN, Power GG, Gladwin MT. Inhaled nebulized nitrite is a hypoxia-sensitive NO-dependent selective pulmonary vasodilator. *Nat Med*. 2004; 10:1122-7.

47. Moya MP, Gow AJ, Califf RM, Goldberg RN, Stamler JS. Inhaled ethyl nitrite gas for persistent pulmonary hypertension of the newborn. *Lancet*. 2002; 360:141-3.
48. Beier S, Classen HG, Loeffler K, Schumacher E, Thöni H. Antihypertensive effect of oral nitrite uptake in the spontaneously hypertensive rat. *Arzneimittelforschung* 1995; 45:258-261.
49. Lauer T, Preik M, Rassaf T, Strauer BE, Deussen A, Feelisch M, Kelm M. Plasma nitrite rather than nitrate reflects regional endothelial nitric oxide synthase activity but lacks intrinsic vasodilator action. *Proc Natl Acad Sci USA*. 2001; 98: 12814-12819.
50. Dalsgaard T, Simonsen U, Fago A. Nitrite-dependent vasodilation is facilitated by hypoxia and is independent of known NO-generating nitrite reductase activities. *Am J Physiol Heart Circ Physiol*. 2007; 292: H3072-H3078.
51. Furchgott RF, Bhadrakom.. Reactions of strips of rabbit aorta to epinephrine, isopropylarterenol, sodium nitrite and other drugs. *J Pharmacol Exp Ther*. 1953; 108: 129-143.
52. Farrari R, Cargoni A, Bernocchi P, Pasini E, Curello S, Ceconi C, Ruigrok TJC. Metabolic adaptation during a sequence of no-flow and low-flow ischemia. *Circulation*. 1996; 94: 2587-2596.
53. Samloulilov A, Kuppusamy P, Zweier JI. Evaluation of the magnitude and rate of nitric oxide production from nitrite in biological systems. *Arch Biochem Biophys*. 1998; 357:1-7.
54. Butler AR, Ridd JH. Formation of nitric oxide from nitrous acid in ischemic tissue and skin. *Nitric Oxide*. 2004; 10: 20-24.
55. Webb A, Bond R, McClean P, Uppal R, Benjamin N, Ahluwalia A. Reduction of nitrite to nitric oxide during ischemia protects against myocardial ischemic-reperfusion damage *Proc Natl Acad Sci USA*. 2004; 101:13683-13688.

56. Bryan NS, Fernandez BO, Bauer SM, Garcia-Saura MF, Milsom AB, Rassaf T, Maloney RE, Bharti A, Rodriguez J, Feelisch M. Nitrite is a signaling molecule and regulator of gene expression in mammalian tissues. *Nat Chem Biol.* 2005; 1: 290-297.
57. Granville AB. *Further Observations on the Internal Use of the Hydro-cyanic (Prussic) Acid.* London: Butgess & Hill; 1820.
58. Magendie F. Sur l'emploi de l'acide prussique dans le traitement de plusieurs maladies de poitrine, et particulièrement dans la phtisie pulmonaire. *Ann Chim Phys* 1817; 6:347-360.
59. Commoner, B. Cyanide inhibition as a means of elucidating the mechanism of cellular respiration. *Biol Rev (Cambridge Phil Soc).* 1940, 15:168-201.
60. Keilin D, Hartree EF. Cytochrome c and cytochrome oxidase. *Proc Royal Soc London Series B.* 1939; 127:167-191.
61. Kobert R. Über Cyanmethämoglobin und den Nachweis der Blausäure. Stuttgart, Enke: Germany; 1891; cited in Keilin D. *The History of Cell Respiration and Cytochrome* Cambridge: CUP; 1966.
62. Hug E. Accion del nitrite de sodio del hiposulfate de sodio en el tratamiento de la intoxication provocada por el ciannuro de potassio en al conejo. *Rev Soc Argent Biol* 1932; 3: 270-276.
63. Viana C, Cagnoli H, Cendan J. L'action du nitrite de sodium dans l'intoxication par les cyanures. *C R Seances Soc Biol Fil.* 1934; 115:1649-1651.
64. Matthew H & Lawson AAH. *Treatment of Common Acute Poisonings*, Edinburgh: 4 ed Churchill Livingstone: 1979.
65. Cucinell SA, Groff WA, Vick JA, Weger N. Treatment of cyanide poisoning *Fed Proc.* 1974; 33: 234.

- 66 Isom GE, Way JL. Lethality of cyanide in the absence of inhibition of liver cytochrome oxidase. *Biochem Pharmacol.* 1976; 25: 605-608.
- 67 Baskin SI, Froehlich HL, Groff WA The dissociation of reversal of cyanide (CN) toxicity and methemoglobin formation by nitrite (N) in the isolated heart *Fed Proc.* 1986; 45: 196
- 68 Ali MY, Ping CY, Mok YY, Ling L, Whiteman M, Bhatia M, Moore PK. Regulation of vascular nitric oxide in vitro and in vivo; a new role for endogenous hydrogen sulphide? *Br J Pharmacol.* 2006; 149:625-634.
- 69 Smith RP, Gosselin RE. Hydrogen sulfide poisoning. *J Occup Med.* 1979; 21: 93-97.
- 70 Smith RP, Kruszyna R, Kruszyna H. Management of acute sulfide poisoning. Effects of oxygen, thiosulfate, and nitrite. *Arch Environ Health.* 1976; 31: 166-169.
- 71 Beck JF, Bradbury CM, Connors AJ, Donini JC. Nitrite as antidote for acute hydrogen sulfide intoxication? *Am Ind Hyg Assoc J.* 1981; 42: 805-809.
- 72 Scheler W, Kabisch R. On the antagonistic alteration of acute H₂S poisoning in mice with methemoglobin forming agents. *Acta Biol Med Ger.* 1963; 11: 194-199.
- 73 Stine RJ, Slosberg B, Beacham BE. .Hydrogen sulfide intoxication. A case report and discussion of treatment. *Ann Intern Med.* 1976; 85:756-758.
- 74 Chen KK, Rose CL Nitrite and thiosulfate therapy in cyanide poisoning. *J Am Med Assoc.* 1952; 149: 113-119.
- 75 Law WT. Sodium nitrite in the treatment of epilepsy. *Practitioner.* 1882; 28: 420-424.
- 76 Ringer S, Murrell W. Nitrite of sodium as a toxic agent *Lancet* 1883; 2: 766-767.
- 77 van Grinsven HJM, Ward MH, Benjamin N, de Kok TM. Does the evidence about health risks associated with nitrate ingestion warrant an increase of the nitrate standard for drinking water? *Environ Health.* 2006; 5: 26-30.

- 78 Lundberg JO, Weitzberg E, Cole JA, Benjamin N. Nitrate, bacteria and human health. *Nat Rev Microbiol.* 2004; 2: 593-602.
- 79 Weller R, Pattullo S, Smith L, Golden M, Ormerod A, Benjamin N. Nitric oxide is generated on the skin surface by reduction of sweat nitrate. *J Invest Dermatol.* 1996; 107: 327-331.
80. Yoon SS, Coakley R, Lau GW, Lyman SV, Gaston B, Karabulut AC, Hennigan RF, Hwang SH, Buettner G, Schurr MJ, Mortensen JE, Burns JL, Speert D, Boucher RC, Hassett DJ. Anaerobic killing of mucoid *Pseudomonas aeruginosa* by acidified nitrite derivatives under cystic fibrosis airway conditions *J Clin Invest.* 2006; 116: 436-46.
81. Weller R, Price RJ, Ormerod AD, Benjamin N, Leifert C. Antimicrobial effect of acidified nitrite on dermatophyte fungi, *Candida* and bacterial skin pathogens. *J Appl Microbiol.* 2001; 90: 648-652.
- 82 Tucker AT, Pearson RM, Cooke ED, Benjamin N. Effect of nitric-oxide-generating system on microcirculatory blood flow in skin of patients with severe Raynaud's syndrome: a randomised trial. *Lancet.* 1999; 354: 1670-1675.
- 83 Khan F, Pearson RJ, Newton DJ, Belch JFF, Butler AR. Chemical synthesis and microvascular effects of new nitric oxide donors in humans. *Clin Sci.* 2003; 195: 577-584.
- 84 Weller R, Finnen MJ. The effects of topical treatment with acidified nitrite on wound healing in normal and diabetic mice. *Nitric Oxide.* 2006; 15: 395-399.
- 85 Ts'ao TC, Ho PY, Needham J. An early mediaeval Chinese alchemical text on aqueous solutions. *Ambix* 1959; 7: 122-155.
- 86 Butler AR, Moffett J. in *Medieval Chinese Medicine. The Dunhuang medical manuscripts.* Ed Lo Y & Cullen C. London: Routledge; 2004.

- 87 Duncan C, Dougall H, Johnson P, Green S, Brogan R, Leifert C, Smith L, Golden M, Benjamin N. Chemical generation of nitric oxide from the enterosalivary circulation of dietary nitrate. *Nat Med.* 1995; 1: 546-551.
- 88 Pietsch G. *Dissertation sur la Generation du Nitre.* Berlin: Chez Haude et Spener; 1746.
- 89 Postgate J. *Nitrogen Fixation.* 3rd Edition Cambridge, CUP, 1998.
- 90 Challoner T. *A Short Discourse on the Most Rare and Excellent Vertue of Nitre.* London; G Dewes; 1584.
- 91 Benjamin N, Pattullo S, Weller R, Smith L, Omerod A. Wound licking and nitric oxide. *Lancet.* 1997, 349: 1776.
- 92 Pearse F. The treatment of paroxysmal nerve disorders. *Practitioner* 1891; 46: 29-32.
- 93 Cassan S. 'Relieving affections of the lungs or chest' UK Patent 2524; 1868.
- 94 Encyclopædia Britannica, 11th ed, Cambridge: CUP; 1911.
- 95 U.S. Dispensatory, 1926.
- 96 Oza TM. Thermal decomposition of sodium and potassium nitrites. *J Indian Chem Soc.* 1945; 22: 173-180.
97. Ziment I. History of the treatment of chronic bronchitis. *Respiration.* 1991; 58 (Suppl 1): 37-42.
- 98 Willis T. *Pharmaceutice Rationalis: Or an Exercitation of the Medicines in Humane Bodies.* T. Dring, C. Harper and J Leigh, London, UK; 1679.
- 99 Jörg JCG. *Materialien zu einer künftigen Heilmittellehre durch Versuche der Arzneyen an gesunden Menschen gewonnen u. gesammelt.* Carl Cnobloch, Leipzig; 1825.
- 100 Hiatt EP. Comparison of the diuretic effects of nitrate salts with other diuretic agents. *Am J Physiol.* 1957; 189:173-176.

- 101 Cahn A. Die Magenverdauung im Chlorhunger. *Ztschr. F. Physiol. Chem.* 1886; 10: 522-535.
- 102 Greene I, Hiatt EP. Renal excretion of nitrate and its effect on excretion of sodium and chloride. *Am J Physiol.* 1954; 180: 179-182.
- 103 Keith, NM, Whelan M, Bannick EG. The action and excretion of nitrates. *Arch Int. Med.* 1930; 46: 797-832.
- 104 Beck EG. Toxic effects from bismuth subnitrate: With reports of cases to date. *JAMA* 1909; 52:14-18.
- 105 Alexander W. *William Alexander's medizinische Versuche und Erfahrungen.* Leipzig, Germany: A.d.E.; 1773
- 106 Eustermann GB, Keith.NM. Transient methemoglobinemia following administration of ammonium nitrate. *Med Clin North Am.* 1929; 12: 1489-1496.
- 107 Tarr L. Transient methemoglobinemia due to ammonium nitrate. *Arch Int Med.* 1933; 51: 38-44.
- 108 Petersson J, Phillipson M, Jansson EA, Patzak A, Lundberg JO, Holm L. Dietary nitrate increases gastric mucosal blood flow and mucosal defense. *Am J Physiol Gastrointest Liver Physiol.* 2007; 292: G718-G724.
- 109 Humphreys SD, Routledge PA. The toxicology of silver nitrate. *Adverse Drug React Toxicol Rev.* 1998; 17: 115-43.
- 110 Archer DL. Evidence that ingested nitrate and nitrite are beneficial to health. *J Food Prot.* 2002; 65: 872-875.
- 111 Addiscott, T.M. and Benjamin, N. (2000) Are you taking your nitrate? *Food Sci Technol Today.* 2000; 14: 59-61.
- 112 J. L'Hirondel J, L'Hirondel. J-L. *Nitrate and Man: Toxic, Harmless or Beneficial?* CABI Publishing, Oxford, 2002.

- 113 Suschek CV, Schewe T, Sies H, Kroncke KD. Nitrite, a naturally occurring precursor of nitric oxide that acts like a 'prodrug'. *Biol Chem.* 2006; 387: 499-506.
- 114 Shiva S, Sack MN, Greer JJ, Duranski M, Ringwood LA, Burwell L, Wang X, MacArthur PH, Shoja A, Raghavachari N, Calvert JW, Brookes PS, Lefer DJ, Gladwin MT. Nitrite augments tolerance to ischemia/reperfusion injury via the modulation of mitochondrial electron transfer. *J Exp Med.* 2007; 204: 2089-2102.
- 115 Lundberg JO, Feelisch M, Bjorne H, Jansson EA, Weitzberg E. Cardioprotective effects of vegetables: is nitrate the answer? *Nitric Oxide.* 2006; 15: 359-362.
- 116 Richardson G, Hicks SL, O'Byrne S, Frost MT, Moore K, Benjamin N, McKnight GM. The ingestion of inorganic nitrate increases gastric S-nitrosothiol levels and inhibits platelet function in humans. *Nitric Oxide.* 2002; 7: 24-29.
- 117 Larsen FJ, Ekblom B, Sahlin K, Lundberg JO, Weitzberg E. Effects of dietary nitrate on blood pressure in healthy volunteers. *N Engl J Med.* 2006; 355: 2792-2793.
- 118 Larsen FJ, Weitzberg E, Lundberg JO, Ekblom B. Effects of dietary nitrate on oxygen cost during exercise. *Acta Physiol.* 2007; 191: 59-66.
- 119 Erzurum SC, Ghosh S, Janocha AJ, Xu W, Bauer S, Bryan NS, Tejero T, Hemann C, Hille R, Stuehr DJ, Feelisch M, Beall CM. Higher Blood Flow and Circulating Nitric Oxide Products Offset High-Altitude Hypoxia among Tibetans. *Proc Natl. Acad. USA.* 2007; 104: 17593-17598.