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

Anthony R. Butler, PhD; Martin Feelisch, PhD

**Abstract**—Potential carcinogenic effects, blue baby syndrome, and occasional intoxications caused by nitrite, as well as the suspected health risks related to fertilizer overuse, contributed to the negative image that inorganic nitrite and nitrate have had for decades. Recent experimental studies related to the molecular interaction between nitrite and heme proteins in blood and tissues, the potential role of nitrite in hypoxic vasodilatation, and an unexpected protective action of nitrite against ischemia/reperfusion injury, however, paint a different picture and have led to a renewed interest in the physiological and pharmacological properties of nitrite and nitrate. The range of effects reported suggests that these simple oxyanions of nitrogen have a much richer profile of biological actions than hitherto assumed, and several efforts are currently underway to investigate possible beneficial effects in the clinical arena. We provide here a brief historical account of the medical uses of nitrite and nitrate over the centuries that may serve as a basis for a careful reassessment of the health implications of their exposure and intake and may inform investigations into their therapeutic potential in the future. (*Circulation*. 2008;117:2151-2159.)

**Key Words:** cardiovascular diseases ■ drugs ■ history of medicine ■ nitric oxide ■ pharmacology

The presence of nitrite ( $\text{NO}_2^-$ ) and nitrate ( $\text{NO}_3^-$ ) in bodily fluids has been known for some time. Dietary studies carried out by Mitchell et al<sup>1</sup> at the beginning of the 20th 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 Green et al<sup>2,3</sup> showed that this assumption was correct and provided unequivocal evidence for mammalian nitrate biosynthesis. Griess,<sup>4</sup> 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 Moyes<sup>5</sup> 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 Palmer et al<sup>6</sup> that vascular endothelial cells produce NO from L-arginine, Marletta et al<sup>7</sup> reported that the same pathway accounts for the production of nitrite and nitrate by activated macrophages, 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 is converted into NO has occasioned interest in the role of plasma nitrite in

vascular smooth muscle relaxation,<sup>8</sup> the control of blood pressure and flow,<sup>8</sup> and possible therapeutic uses of nitrite.<sup>9,10</sup> Subsequent animal experimental studies revealed that a number of organs are protected against ischemia/reperfusion-related tissue injury after systemic application of small amounts of nitrite,<sup>11</sup> 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."<sup>12</sup> 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; rather, it is 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 saltpeter), have been known since prehistoric times, and in the Middle Ages, natural deposits were commercially exploited. The Chinese invented gunpowder around 800 CE, and with its appearance in Europe during the 13th century,

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potassium nitrate became strategically important. Demand increased further with the Agricultural Revolution of the 19th century and the use of nitrates as fertilizers. Natural sources were eventually supplemented by synthetically produced nitrate at the beginning of the last century.<sup>13</sup>

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<sup>14</sup> 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 recognized as a new "salt." The 2 compounds (potassium nitrate and nitrite) were characterized by Péligot<sup>15</sup> and the reaction established as  $2\text{KNO}_3 \rightarrow 2\text{KNO}_2 + \text{O}_2$ .

The release of oxygen from a substance known to alchemists as "aerial niter" since the times of Paracelsus explains the role of nitrates in gunpowder, rocket propellants, and other explosives.<sup>16</sup> Sodium nitrite rapidly gained importance in the development of organic chemistry during the 19th century, when it was discovered that nitrous acid ( $\text{HNO}_2$ ) reacts with aromatic amines ( $\text{ArNH}_2$ ) to produce diazonium ions,<sup>17</sup> a highly important synthon for the dyestuffs industry and for synthetic organic chemistry generally:  $\text{ArNH}_2 + \text{HNO}_2 + \text{H}^+ \rightarrow \text{ArN}=\text{N}^+ + 2\text{H}_2\text{O}$ .

The mechanism of such diazotization reactions has been subject to extensive study.<sup>18</sup> Diazotization may be responsible, in part, for the carcinogenic role of nitrite under certain conditions, particularly in the context of drug-nitrite interactions.<sup>19</sup>

Nitric acid ( $\text{HNO}_3$ ) is a strong acid that is completely ionized at all biologically interesting pHs. Although nitrous acid ( $\text{HNO}_2$ ) is a weak acid, with a  $\text{pK}_a$  of 3.15 ( $\text{pK}_a$  is the pH at which the acid is 50% dissociated), it is also, at physiological pHs, completely dissociated, except in the stomach, on the surface of airways, within select cellular compartments (eg, 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. Butter,<sup>20</sup> writing about the treatment of angina in 1791, gave no drug treatment and had little more to offer than the recommendation of a tranquil lifestyle. However, while working at the Edinburgh Royal Infirmary in the 1860s, Brunton<sup>21</sup> 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 synthesized compound and one that his colleague had shown lowered blood pressure in animals (A. Gamgee, unpublished observation). The result was dramatic.<sup>21</sup> 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 favored 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 were less volatile. The most popular replacement was glyceryl trinitrate (GTN), an organic nitrate better known as nitroglycerin.<sup>22</sup> The fact that this compound is highly explosive and a component of dynamite appears not to have been a problem. In his 1894 textbook, Phillips<sup>23</sup> lists a number of chemically related compounds that can be used in the treatment of angina. The list includes not only amyl nitrite but also propyl, ethyl, and isobutyl nitrites, as well as GTN. A similar list is provided by White<sup>24</sup> in his 1899 textbook. GTN, a drug introduced into allopathic medicine thanks to extensive homeopathic studies by Hahnemann,<sup>25</sup> occasioned greatest favor among practicing physicians, and by 1956, in a symposium on hypotensive drugs,<sup>26</sup> it was the only drug of this type that was 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.<sup>27</sup> 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 that the Nobel family fortune is derived. Tragically, Nobel's younger brother Emil was killed while working with GTN, 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.<sup>28</sup> 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 the way they do. Each is a substrate for  $\geq 1$  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 characterized.<sup>29</sup> However, the contribution of this or other enzyme systems to the overall vasodilation by these drugs is difficult to assess because multiple metabolic pathways appear to act in concert.<sup>30</sup>

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 Mitchell<sup>31</sup> published a very full account of the biological action of potassium nitrite on humans and animals. At that time, the value of amyl nitrite in the treatment of angina was severely compromised by the short duration of its effect, so 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 ( $\approx 30$  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 on the conversion of organic nitrites to nitrous acid. Observations similar to those of Reichert and Mitchell were made by Atkinson<sup>32</sup> and Densham.<sup>33</sup> Practicing physicians, including Hay<sup>34</sup> and Leech,<sup>35</sup> examined the therapeutic value of inorganic nitrites as hypotensive drugs and noted that, although of slower onset, their 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 \$1,<sup>36</sup> and by the mid-1920s, an injectable solution of sodium nitrite became available (Nitrosklerin, E. Tosse & Co, Hamburg, Germany) for the treatment of hypertension and vasospasm.<sup>37</sup> Instructions for using sodium nitrite to treat angina are given in *Martindale's Additional Pharmacopoeia* and in the US *National Standard Dispensatory* of 1905.<sup>38</sup> A textbook on *Materia Medica* for medical students in 1921 gives details of the appropriate dose,<sup>39</sup> but by the middle of the 20th century, its medicinal use had essentially ceased, largely because of adverse side effects. Blumgarten<sup>40</sup> noted that sodium and potassium nitrites often caused nausea, belching, stomachache, and diarrhea. Although these side effects may have caused physicians to hesitate in prescribing sodium nitrite for angina, another event precipitated the fall of inorganic nitrite from favor (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 after conversion to NO by nonenzymatic mechanisms<sup>41,42</sup> and an oxygen-sensitive nitrite-reductase<sup>43</sup> and S-nitrosothiol-synthase<sup>44</sup> function of hemoglobin. Like NO, inhaled nebulized nitrite has been shown to be an effective pulmonary vasodilator<sup>45</sup> and, along with organic nitrites,<sup>46</sup> suggested for potential use in neonatal pulmonary hypertension. Although there is no doubt that appropriate pharmacological doses of nitrite can normalize elevated blood pressure,<sup>47</sup> the question of whether physiological concentrations of nitrite are vasodilator active is still a matter of debate.<sup>48,49</sup>

### 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:  $2\text{HNO}_2 \rightarrow \text{H}_2\text{O} + \text{N}_2\text{O}_3$  and  $\text{N}_2\text{O}_3 \rightarrow \text{NO} + \text{NO}_2$ .

Solutions of acidified nitrite have been used successfully to generate NO and to induce vasorelaxation in isolated blood vessel studies,<sup>50</sup> and the same reaction mechanism has been proposed to explain the biological action of nitrite.<sup>51,52</sup> However, pHs at which this occurs are generally not found within living systems,<sup>53</sup> 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 action<sup>54</sup> in the vascular system,

at least under ischemic conditions. In fact, recent data suggest that hypoxic NO formation from nitrite is carried out by multiple enzyme systems<sup>10</sup> and occurs in virtually all tissues and organs (Feelisch et al, unpublished data, 2006). Independently 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.<sup>55</sup> Although it cannot be excluded that some of the biological effects of nitrite may be mediated by nitrite itself, it is fair to assume that most of the physiological and therapeutic actions of nitrite that require conversion into NO and NO-related products involve enzymatic catalysis.

### Nitrite as an Antidote for Cyanide and Hydrogen Sulfide Poisoning

In popular literature, cyanide (CN<sup>-</sup>) is considered 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 on this rapid action that its reputation rests. Large doses cause instant death; 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. Despite the catastrophic consequences of an overdose, potassium cyanide was used in medicine for many years as a treatment for chest complaints,<sup>56</sup> particularly a dry cough.<sup>57</sup> 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.<sup>58</sup> Keilin<sup>59</sup> 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. Because cyanide also reacts with methemoglobin,<sup>60</sup> 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<sup>2+</sup>) to the ferric (Fe<sup>3+</sup>) state, producing methemoglobin, and is therefore a potential antidote for cyanide poisoning. The clinical use of nitrite in this setting was first proposed by Hug<sup>61</sup> and is now universally used. Sodium thiosulfate also is 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 5 g potassium cyanide but recovered after being given 1.5 g sodium nitrite and 18 g sodium thiosulfate.<sup>62</sup> 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.<sup>63</sup>

Although formation of methemoglobin is generally accepted as the explanation of the efficacy of nitrite as an antidote, evidence suggests that this is not the complete explanation.<sup>64,65</sup> There may be alternative or additional routes whereby nitrite detoxifies, but no details are available.<sup>66</sup> Compounds that promote NO release in vivo (like bradykinin) modify cyanide toxicity. Whether this is an alternative



mode of action of nitrite in detoxification or just another source of nitrite from endogenous NO is, at this time, difficult to assess.

Nitrite also is an efficacious antidote to poisoning by hydrogen sulfide (H<sub>2</sub>S), an occupational hazard with high lethality and long-term neurological sequelae in survivors. Like NO and CO, low concentrations of H<sub>2</sub>S are produced endogenously and have vasodilator properties, but the physiological significance of its formation is currently unknown.<sup>67</sup> Supraphysiological concentrations of sulfide, as experienced after H<sub>2</sub>S 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 H<sub>2</sub>S poisoning shares many similarities with cyanide intoxication.<sup>68</sup> Nitrite administration, which is superior to that of oxygen alone<sup>69</sup> and often is combined with hyperbaric oxygen therapy, is most effective when given immediately after H<sub>2</sub>S exposure.<sup>70</sup> It is thought to act via induction of methemoglobinemia and subsequent binding of hydrosulfide anions (HS<sup>-</sup>) to the oxidized blood pigment, leading to inhibition of cytochrome c oxidase and reinstatement of aerobic respiration in the tissues. Although 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 for many years to have protective and antidotal effects against experimental sulfide poisoning in rodents,<sup>71</sup> nitrite administration for H<sub>2</sub>S intoxication was introduced into human therapy only in the mid-1970s.<sup>72</sup> The recommended dosage regimen for nitrite in sulfide intoxication is identical to that established for the treatment of cyanide poisoning, ie, initiation with inhalations of amyl nitrite followed by intravenous injection of 10 mL of a 3% solution of sodium nitrite.<sup>73</sup>

### Other Medical Uses of Inorganic Nitrite

In view of the success of nitrite with angina, it was tried for the treatment of other medical conditions. Law<sup>74</sup> recommended the administration of very large doses (20 grains or 1.3 g) of sodium nitrite to treat epilepsy. Other physicians tried this dose and found that the side effects were far too serious to continue the treatment, with considerable consequences for the therapeutic use of inorganic nitrite. The toxic nature of such high doses was confirmed by Ringer and Murrell,<sup>75</sup> who 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 because of the success of GTN, nitrite disappeared from widespread use. The final blow came when Magee and Barnes<sup>19</sup> 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.<sup>76</sup> 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 an 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.<sup>77</sup> 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.<sup>78</sup> Thus, acidified nitrite may be a component of innate immunity at several locations on and within the body. Some attempts to capitalize on this insight point in 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,<sup>79</sup> provided a safe mode of administration can be found. The antimicrobial properties of NO can be exploited by dermal application of creams containing nitrite and an acidifying agent, eg, ascorbic acid, to treat a number of skin diseases.<sup>80</sup> The same concept has been demonstrated to increase microcirculatory blood flow in Raynaud patients<sup>81,82</sup> and to accelerate wound healing.<sup>83</sup> Although 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 pharmacopeias state that potassium nitrate has no drug action other than as a diuretic (see below), historical records show that it has been used extensively in medicine over the years to treat 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 northwestern provinces of China, and it was well known to early Chinese alchemists. They called it xiao shi (solve stone), and it was first recognized in the 4th century CE. It was a component of some of the elixirs of immortality concocted by Daoist savants as

they searched for a means of realizing the Daoist ideal of life without death.<sup>84</sup> 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 1044 CE.

One of the oldest accounts of the use of niter in Chinese medicine is as a treatment for what appears to be angina in an 8th century Chinese manuscript uncovered at the Buddhist grotto of Dunhuang.<sup>85</sup> 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, nitrate-reducing bacteria convert some of the nitrate into nitrite.<sup>77,86</sup> 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 among the most advanced of the medieval period, but there is no mention of niter in a book on cardiac drugs by Avicenna, born 980 CE. The first extant Arabic mention of niter occurs in a book by Kitab al-Jami' fi al-Adwiyā 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 civilizations. It was about this time that Arabs started to use niter in gunpowder and 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 1240 CE), 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.<sup>87</sup> Nitrates appeared as efflorescences and were converted into potassium salt by reaction with potassium carbonate (potash). Two groups of bacteria are responsible for this process: *Nitrosomonas* convert ammonia into nitrite, and *Nitrobacter* convert nitrite into nitrate.<sup>88</sup> It is quite possible that niter from nitriaries contained some nitrite, thus giving it medicinal value. This is unlikely in niter from natural deposits because they are old and aerial oxidation will, over time, convert all the nitrite into nitrate. So, the 8th century Chinese physician mentioned previously had to instruct the patient on how to generate nitrite, but European physicians of the 14th to 17th centuries, using niter from a different source, could prescribe it without further refinement because nitrite was there already.

However, such a prescription was rather hit-or-miss in that 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*.<sup>89</sup> The spelling of the English is idiosyncratic (rather

like that of modern students) because spelling was not fully standardized 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 in which the author tells his readers how to make niter more effective ("yet more sharpe and subtile"). He describes 3 ways, all involving heating (called "calcination" by Challoner). Heat, of course, converts some of the niter into potassium nitrite, and so, without realizing 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, tetteres, bytes" and so on). Naturally occurring nitrite in saliva has the same effect and explains, in part, why most animals instinctively lick a wound.<sup>90</sup>

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 hoarshesses, olde coughes and toughe coughes, 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 et al.<sup>77</sup>

### Nitrate and the Treatment of Lung Diseases

For a time, amyl nitrite was used for relieving patients suffering an asthma attack. In an article<sup>91</sup> in 1891, other nitrites, including sodium nitrite, were suggested for this purpose. 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 1867,<sup>92</sup> is described in detail in the *Encyclopedia Britannica* of 1911,<sup>93</sup> and occurred as recently as 1926 in the *US Dispensatory*.<sup>94</sup> The products of thermal decomposition of niter include NO, NO<sub>2</sub>, and O<sub>2</sub>.<sup>95</sup> Because NO is a poor bronchodilator and NO<sub>2</sub> is toxic, it is difficult to see how inhalation of this mixture brings relief. The combination possibly has an effect that is greater than the sum of its parts.

In addition to its use in asthma, sodium nitrate was given orally to treat chronic bronchitis.<sup>96</sup> 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.<sup>97</sup> Although 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.<sup>98</sup> Clear differences in potency exist between various nitrate salts,<sup>99</sup> 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 caused by increased glomerular filtration without an equivalent increase in tubular reabsorption.<sup>100,101</sup> Whether these effects are mediated by formation of nitrite or NO is unknown.

Extensive animal and human studies by Keith et al<sup>102</sup> 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 to 15 g daily for several weeks. Thus, ammonium nitrate was introduced as a new, more effective diuretic in 1926 and was used with great success to treat various forms of edema in North America, particularly at the Mayo Clinic. After a time of exaggerated emphasis on possible toxic effects of nitrates during the preceding 2 decades, which led physicians to use lower, inadequate doses, it looked as though ammonium nitrate was here to stay as the diuretic of choice. What had triggered the fear of inducing severe cyanosis when potassium or sodium nitrate was used as a diuretic before was the toxicity associated with the use of massive amounts of bismuth subnitrate for diagnostic purposes,<sup>103</sup> which is somewhat surprising because the toxicity of large amounts of nitrate was well known for a long time.<sup>104</sup> Concerns about the safety of nitrates reached a new height with the appearance of case reports about transient methemoglobinemia after administration of ammonium nitrate.<sup>105,106</sup> The reasons for these rare complications (which disappeared on discontinuation of nitrate therapy in most cases) remain unclear but may have been due to 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.<sup>107</sup> 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 (eg, cerium and silver nitrate) have little to do with the amounts of nitrate they contain, application of large enough quantities can cause methemoglobinemia.<sup>108</sup> 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

Despite 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, in both the lay press and 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 as a result of fertilizer overuse, and the formation of potentially carcinogenic N-nitrosamines all contributed to the negative image that 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 replacement of nitrite by other (often less effective) food preservatives, and to establish cultivation conditions that result in crops with reduced levels of nitrate. Although 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.<sup>76,109–112</sup> Interestingly, the average dietary intake of nitrate roughly equals that produced by the endogenous production of NO.<sup>113</sup> Thus, if nitrite truly were 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  $\approx 5\%$  of all ingested and endogenously produced nitrate eventually ends up as nitrite in the stomach, as pointed out by Archer<sup>109</sup> (so far about "intelligent design"). Despite the critical voices, the image of nitrite and nitrate remains stigmatized.

What appears to have the greatest potential to change our current perception of 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 signaling molecule in its own right.<sup>55</sup> Given its propensity for conversion into NO and related species, unequivocal evidence for this role may be difficult to provide unless nitrite-specific signaling pathways are 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 evolutionarily more recent L-arginine/NO pathway uses signaling 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,<sup>10,11</sup> an action possibly mediated by modulation of mitochondrial function.<sup>113</sup> Nitrate, which has been proposed to contribute to the health-promoting effects of the Mediterranean diet,<sup>114</sup> has



been demonstrated to inhibit platelet aggregation,<sup>115</sup> to mildly lower blood pressure,<sup>116</sup> to enhance gastric mucosal defense mechanisms,<sup>107</sup> and to reduce the oxygen cost of exercise.<sup>117</sup> The last 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.<sup>118</sup> 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 whet 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 come up with new prodrugs that allow targeted delivery of nitrite to specific tissues or organs or if nitrite/nitrate is intelligently used as an 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 to condemn the alchemist's doing as quackery, there is much to learn from the past. In reviewing the therapeutic use of nitrite and nitrate over centuries, it appears that some of the potential that these simple compounds may hold for medical use have not been realized, often because the basis for some unwanted drug effects was not understood and thus could not be controlled at the time. But, even if the scare factor continues to dominate mainstream thinking, 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 to stimulate the development of new therapeutic principles that use pathways regulated by nitrite and nitrate.

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