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Review

Evidence that Ingested Nitrate and Nitrite Are Beneficial to Health†

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ABSTRACT

The literature was reviewed to determine whether ingested nitrate or nitrite may be detrimental or beneficial to human health. Nitrate is ingested when vegetables are consumed. Nitrite, nitrate's metabolite, has a long history of use as a food additive, particularly in cured meat products. Nitrite has been a valuable antibotulinal agent in cured meats and may offer some protection from other pathogens in these products as well. Nitrite's use in food has been clouded by suspicions that nitrite could react with amines in the gastric acid and form carcinogenic nitrosamines, leading to various cancers. Nitrate's safety has also been questioned, particularly with regard to several cancers. Recently, and for related reasons, nitrite became a suspected developmental toxicant. A substantial body of epidemiological evidence and evidence from chronic feeding studies conducted by the National Toxicology Program refute the suspicions of detrimental effects. Recent studies demonstrate that nitrite, upon its ingestion and mixture with gastric acid, is a potent bacteriostatic and/or bactericidal agent and that ingested nitrate is responsible for much of the ingested nitrite. Acidified nitrite has been shown to be bactericidal for gastrointestinal, oral, and skin pathogenic bacteria. Although these are in vitro studies, the possibility is raised that nitrite, in synergy with acid in the stomach, mouth, or skin, may be an element of innate immunity.

HISTORY OF NITRITE

Sodium nitrite is recognized as a multifunctional food additive, especially useful in the preservation of meats. The history of use of nitrite (and nitrate) is intimately intertwined with that of salt and its long history as a meat and fish preservative, although the history of salt clearly precedes that of nitrate or nitrite. The date of the first use of salt as a preservative is not certain but may have been as early as 3000 BC *(3)*. The salt-rich Dead Sea facilitated food preservation in the Jewish Kingdom as early as 1600 BC *(3)*. The Romans learned to use salt as a preservative from the Greeks, and its use as a preservative was common practice by 900 BC. The preservation of meat and fish opened commerce, facilitated exploration, created trade routes, and facilitated the exchange of knowledge and culture *(19)*.

Even before it was proven by chemical analysis, the Romans recognized that salt containing "nitre" or saltpeter imparted a distinctive red color and flavor to meat *(3)*. Later, chemists demonstrated that, indeed, pure salt did not impart the "cured" flavor and color, but rather, that such was due to the presence of sodium or potassium nitrate as a contaminant of the salt *(18)*. It was further shown that nitrate was reduced by naturally occurring bacteria on the surface of meats to nitrite and nitric oxide and that the nitrite was responsible for the curing properties of distinctive flavor and color *(18)*.

ANTIBACTERIAL PROPERTIES OF NITRITE

From the 1920s through the 1940s, a significant amount of research was done on the antibacterial effects of nitrite. Nitrite was shown to inhibit anaerobic bacteria and exert bacteriostasis on bacteria associated with fish muscle, such as *Achromobacter, Aerobacter, Escherichia, Flavobacterium, Micrococcus,* and *Pseudomonas (18)*. It was also apparent to several investigators that there was a "pH effect" associated with nitrite's ability to inhibit or kill bacteria, in that nitrite was far more effective at acidic pH. For example, 1% nitrite was bactericidal to *Staphylococcus aureus* at pH 5.6, but that same concentration was not lethal at pH 7.2 *(20)*.

Nitrite is particularly effective against *Clostridium botulinum* (reviewed in *(18)*). Before the use of nitrite as a curing agent for meats, botulism was a serious problem associated with meats and sausages (in fact, *C. botulinum* derives its name from the Latin word for sausage, botulus). The antibotulinal properties of nitrite are multifactorial and involve the interaction of nitrite with other factors, such as salt, pH, heat treatment, spore level at outset, and original and residual nitrite level in the meat. Nitrite exerts its antibotulinal effect at two places in the life cycle of *C. botulinum* in heated systems; first, it inhibits the emergence of the vegetative cell from surviving spores, and second, it prevents cell division in any vegetative cells that do emerge

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(18). The result has been a remarkable record of safety for cured meats with regard to botulism compared with uncured, home-processed meats or cured meats in countries wherein nitrite is not used. Since 1899, only seven outbreaks of botulism with nine deaths in the United States and Canada have been attributed to commercially cured meat products, and in the majority of these outbreaks, underprocessing or gross temperature abuse have been cited *(18).* In the same period, of 51 home-processed meat outbreaks, 43 occurred in noncured meats *(18).*

SAFETY ISSUES ABOUT NITRITE

Over time, nitrates (and nitrites) have been suspected of playing a role in methemoglobinemia in infants, cancer in humans, and even reproductive toxicities including birth defects, although epidemiological studies did not support the suspicions *(12).*

Dietary methemoglobinemia in infants is also called "Blue Baby syndrome." The oxidation of hemoglobin to methemoglobin in erythrocytes is caused by nitrites, not nitrates. If the level of methemoglobin exceeds 10 to 20% of hemoglobin in the erythrocytes, cyanosis may result. The disorder only occurs in infants under 6 months of age, whose protective enzyme system, NADH-cytochrome b5 reductase, is not yet fully developed *(13).* Methemoglobinemia in infants was due to nitrates in baby bottles, principally from water, being transformed to nitrites by bacteria that grew in the infant formula when proper hygiene was not employed. The lack of clinical symptoms after numerous feedings of foods containing significant levels of nitrate (carrot soup and spinach) strongly suggests that dietary sources of nitrate are not responsible for methemoglobinemia *(13).*

Ingested nitrites may react with various amines in the stomach to form nitrosamines, most of which are carcinogenic in animals *(13).* The concern over nitrite in cured meats came about when nitrosopyrrolidine was found in bacon. Subsequently, it has also been found in other cured meats, but usually at lower levels than in bacon *(18).* Nitrite levels are regulated in cured meat products to levels (not more than 156 ppm in the finished meat product or 120 ppm in bacon) at which nitrosamines are not likely to form. Finished product residual nitrite levels in cooked meat products are 10 ppm or less *(6).* The meat industry no longer uses nitrates, and this has been confirmed by a survey *(6).* Nevertheless, the suspicion of a link between nitrites in meats and other sources of ingested nitrites persisted in spite of the lack of epidemiological evidence *(5, 16).*

Numerous case-control studies have been conducted worldwide to determine if there is a link between gastric cancer and nitrate intake *(4, 5, 12, 16).* Elevated nitrate intake would lead to elevated salivary nitrate levels and, after reduction by oral bacteria, higher levels of ingested nitrite. Studies in Canada, Italy, Sweden, and Germany involving thousands of study subjects have failed to show an association or have demonstrated a negative association between estimated nitrate intake and gastric cancer, perhaps because much of the nitrate was from vegetables *(16).* Occupational exposure to very high levels of nitrate occurs in nitrate fertilizer workers. Although these workers have elevated body burdens of nitrate and elevated salivary nitrate and nitrite levels, no increase in gastric cancers has been observed *(16).* Case-control studies attempting to link nitrates and nitrite consumption to brain, esophageal, and nasopharyngeal cancers have also been inconclusive *(12).* Case-control studies suffer from several shortcomings, among which is the recall of food consumed, and although cohort studies are less prone to that source of bias, no prospective studies of this type have been reported to date *(12).* In other studies, for two decades, the relationship between the consumption of cured meats during pregnancy and the risk of brain and other tumors in offspring was examined *(4).* In a review of 14 epidemiological studies, 13 of which were case-control studies, Blot et al. *(4)* could not conclude that there was a relationship between cured meat consumption during pregnancy and brain or any other cancers *(4).* It may be that in the limited number of epidemiological studies linking nitrate, nitrite, or cured meat to a specific cancer, other as-yet uncharacterized factors are involved.

In 2000, the results of a comprehensive study by the National Toxicology Program and a multiyear rodent study using rats and mice were presented to the National Toxicology Program Technical Reports Review Subcommittee. This subcommittee determined that the study showed no evidence of carcinogenicity in male and female rats and male mice and only equivocal evidence (insufficient evidence) in female mice *(2).* In short, the suspicion of nitrite's carcinogenicity was not supported by the study. The report, TR-495, can be found at the National Toxicology Program's Web site *(17).*

Suspicions about nitrite being a developmental or reproductive toxicant were examined in 2000 by California's Developmental and Reproductive Toxicant Identification Committee. A review of 99 studies on sodium nitrite led this committee to conclude that sodium nitrite should not be listed as a developmental toxicant under California's Proposition 65 law *(2).*

METABOLISM OF NITRATE AND NITRITE

In humans, there are endogenous and exogenous sources of nitrite. Nitric oxide is the product of enzymatic synthesis in mammals and has profound physiologic effects, including control of blood pressure, immune response, wound repair, and neurologic function. Normal nitric oxide synthesis is at the level of 1 mg/kg body weight per day, and as such, there is an endogenous flux of at least 70 mg of nitric oxide per day for adult humans through the conversion of nitrite to nitrate *(13, 22).* Nitrite can be considered a natural metabolite.

Nitrates in blood plasma have two sources, endogenous and exogenous. Endogenous sources are described above. Exogenous sources are food (principally vegetables) and water that contribute about 80% and 10 to 15% of alimentary nitrate, respectively. In fact, when the average daily human ingestion of nitrate and nitrite was estimated in 1997, 95.3% of nitrate was calculated to be from vegetables and 0% from cured meats *(22).* Conversely, only 2.2% of nitrite was calculated to be from vegetables and 4.8% from

cured meats. The largest source of ingested nitrite was from saliva, 92.8% *(6).*

Nitrate entering the gastrointestinal tract in food is absorbed into the plasma via the proximal small intestine *(22).* About 65 to 70% of plasma nitrate is lost to passive urinary excretion. Of the two active secretion mechanisms, colonic and salivary, salivary appears to be more important, with 25% of ingested nitrate being recycled via secretion in saliva *(22).* The recycling of ingested nitrate through plasma to salivary nitrate has been called "enterosalivary circulation of dietary nitrate" *(8).* Of the 25% ingested nitrate recycled through salivary secretion, approximately one-fifth (ca. 5% of the total ingested nitrate) is converted to nitrite by oral cavity microorganisms *(13, 22).* Although this may seem like a small amount, it has been established that this salivary source of nitrite accounts for approximately 93% of the total ingested nitrite *(6).* Studies in the rat have shown that the reduction of salivary nitrate to nitrite occurs on a specialized area on the posterior of the tongue that is colonized by nitrate-reducing bacteria *(8).* The bacteria responsible for reducing nitrate to nitrite on the tongue of rats and pigs have been characterized, and a high concentration of these nitrate-reducing bacteria on the posterior portion of the tongue has been confirmed *(14).*

Thus, those who still believe nitrite contributes to carcinogenesis must explain a paradox, in that the bulk of ingested nitrite comes from a metabolic pathway, and the level of nitrite ingestion is partially controlled by the level of ingested nitrates. Nitrates cannot be totally avoided if vegetables are eaten; thus, one would have to conclude that vegetable consumption contributes indirectly to carcinogenesis, and there is no evidence for this relationship. However, a recent study suggested that it may be beneficial to modulate the oral nitrate-to-nitrite conversion in order to lessen the induction of methemoglobinemia and the formation of carcinogenic N-nitroso compounds *(21).* In these studies, the authors attempted to modulate the nitrate-to-nitrite conversion by affecting the nitrate-reducing bacteria with chemicals, such as mouthwashes with various active ingredients and toothpastes. Thus, there is still a suspicion of the negative effect of nitrate (and thus nitrite) on human health.

NITRITE AND HUMAN PATHOGENS

Numerous studies have focused on the putative toxicity of nitrate and nitrite and the possible role of dietary nitrate and nitrite in carcinogenesis via the formation of N-nitroso compounds *(5, 9).* As previously stated, recent chronic toxicity studies in two rodent species failed to confirm sodium nitrite as a carcinogen *(2, 17).* Additionally, while some N-nitroso compounds are carcinogens, epidemiologic evidence has failed to link nitrate consumption and cancer risk *(4, 5, 12, 16).*

Curiously, while papers attempting to link dietary nitrate and nitrite were continuing to be published, other studies began to appear that cast a positive light on dietary nitrate and nitrite. Duncan et al. *(8)* reported that, upon acidification in the stomach, nitrite in saliva derived from dietary nitrate generates nitrogen oxides that may afford protection from swallowed pathogenic microorganisms. They further demonstrated that nitrite is formed from nitrate by bacteria on the tongue. Dougall et al. *(7)* conducted studies in human volunteers; they demonstrated that administration of the broad-spectrum antibiotic amoxycillin destroyed nitrate reductase-producing bacteria in the mouth and speculated that this might explain the observed increased incidence of certain infections in persons taking broad-spectrum antibiotics.

In vitro studies of acidified nitrite's effect on several important human enteric pathogens were conducted by Dykhuizen et al. *(9, 11). Yersinia enterocolitica, Salmonella* Enteritidis, *Shigella sonnei,* and *Escherichia coli* O157 were exposed to pH levels from 2.1 to 5.4 with and without various concentrations of nitrite and for varying times from 30 min to 2 h. The synergistic relationship between acid pH and nitrite was apparent for all pathogens studied *(9).* Acid alone was relatively ineffective, and in some instances, the bacteria continued to grow. The combination of acid and nitrite (acidified nitrite) was highly effective in killing the pathogens. The relative susceptibility of the pathogens to acidified nitrite was reported as follows: *Y. enterocolitica* > *Salmonella* Enteritidis > *Salmonella* Typhimurium = *S. sonnei* > *E. coli* O157 *(11).*

Helicobacter pylori is able to survive in the human stomach and has been associated with ulcers and gastric cancers *(9, 10). H. pylori* was able to survive at pH 2, but not when nitrite was added at a concentration of 1 mM *(10).* Dykhuizen et al. *(9)* speculate that ingestion of foods rich in nitrate may protect against colonization of the stomach by *H. pylori,* although there is no epidemiologic evidence at present to support this speculation. No investigations have specifically been done to determine any relation between nitrate intake and *H. pylori* survival *(9).*

The enterosalivary circulation of nitrate may have additional beneficial physiological effects. The nitrite formed from the oral reduction of salivary nitrate is swallowed and acidified in the stomach to form nitric oxide and other oxides of nitrogen *(15).* Besides the antimicrobial effect of acidified nitrite against ingested pathogens, nitrogen oxide species formed in the stomach may demonstrate vasodilator activity and modulate platelet activity, and they may even play a role in gastrointestinal motility and microcirculation *(15).* Thus, the role of dietary nitrate may be of vital importance, particularly in the immunocompromised and those routinely exposed to gastrointestinal pathogens. The bactericidal effect of nitrite was ascribed primarily to nitrous acid and possibly other unidentified nitrogenous metabolites, but not to nitric oxide or nitrogen dioxide *(24),* in studies conducted to mimic the stomach.

Studies on the effects of acidified nitrite on gastrointestinal pathogens have led to studies on other microbial ecosystems. The skin is an acidic environment, and nitrite is excreted in sweat. The addition of nitrite to acid enhanced the killing of common cutaneous pathogens such as *Propionibacterium acnes, S. aureus, Streptococcus pyogenes,* and *Trichophyton mentagrophytes (23).* The concentrations of nitrite used in these in vitro bactericidal studies were higher than the concentrations measured in sweat, but the authors conclude that other cofactors in sweat may po-

tentiate the effect of acidified nitrite on the skin *(23)*. The antimicrobial effects of nitric oxide on periodontal bacteria have also been studied. Nitric oxide is generated in the oral cavity from nitrite derived from salivary nitrate. At pH levels <5.0, low concentrations of nitrite (0.2 mM) were effective in killing the periodontal bacteria *Fusobacterium nucleatum, Eikenella corrodens,* and *Porphyromona gingivalis (1)*. The authors suggest that under appropriate conditions, nitrite in saliva may affect the growth and survival of bacteria implicated in periodontal disease *(1)*.

CONCLUSIONS

Although nitrite is ingested by humans as a food additive (sodium nitrite), it is mainly ingested as a product of the enterosalivary cycling of nitrate from plasma to saliva and the subsequent reduction of nitrate to nitrite in the mouth. About 7% of ingested nitrite comes from food and 93% from nitrate in saliva.

Nitrite has been suspected to be a carcinogen for several decades, but numerous epidemiologic studies have failed to support consistently a link between nitrate or nitrite and cancer. Recent chronic feeding studies in two rodent species failed to link nitrite, even at extremely high oral dose levels, to cancer. Recent suspicions that nitrite might be a developmental toxicant were also found to lack foundation. Since 93% of ingested nitrite comes from normal metabolic sources, if nitrite caused cancers or was a reproductive toxicant, it would imply that humans have a major design flaw. Despite all evidence to the contrary, questions about the safety of nitrate and nitrite will likely persist until definitive prospective studies in humans are conducted.

Data from many studies demonstrate that nitrite in the presence of acid is a powerful bacteriostatic and bactericidal agent, depending on the pH, the concentration of nitrite, and the target microorganism. To date, all studies on the bactericidal effects of nitrite on gastrointestinal pathogens have been performed in vitro, and although it would add significant difficulties, in vivo experiments, either epidemiologic or clinical, should be designed and conducted within feasibility. If it is demonstrated conclusively that nitrite acts in synergy with acid as a barrier to gastrointestinal pathogens and that it also has a role in the defense of the skin, teeth, and gums from pathogenic bacteria, then nitrite would seem to fit the necessary criteria for being an element of innate immunity.

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