Nitrates in the human diet - good or bad?

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Review article

Nitrates in the human diet – good or bad?

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Abstract — Although the nitrate and nitrite have been used for centuries, it has only recently been discovered that nitrate is manufactured in mammals by the oxidation of nitric oxide and that the nitrate formed also has the potential for disinfecting the food we eat. The mechanisms by which nitric oxide and other nitrogen oxides provide selective toxicity towards pathogens is not yet completely understood, and it is likely that the mechanisms will be different with different organisms. Whereas it is clear that acidified nitrite is produced on mucosal surfaces, and that this combination is effective in killing a variety of human gut and skin pathogens, there is no definite evidence as yet that this mechanism is truly protective in humans exposed to a contaminated environment. Further understanding of the complex chemistry of nitrogen oxides may also help develop new antimicrobial therapies based on augmenting what seems to be a simple and effective host defence system.

nitrates / nitrites / human diet / health

Résumé — **Les nitrates dans l'alimentation humaine : une bonne ou une mauvaise chose ?** Bien que les nitrates soient connus depuis des siècles, c'est seulement depuis peu qu'il a été découvert que les nitrates sont synthétisés chez les mammifères à partir du monoxyde d'azote, et que les nitrates formés ont la capacité de tuer les bactéries présentes dans les aliments consommés. Les mécanismes par lesquels le monoxyde d'azote et d'autres oxydes d'azote manifestent une toxicité sélective vis-à-vis de bactéries pathogènes ne sont pas encore totalement élucidés. Il est probable que ces mécanismes dépendent des organismes considérés. Alors qu'il est clair que les nitrites acidifiés sont produits à la surface des muqueuses, et que cela permet de tuer de nombreux agents pathogènes de la peau et de l'intestin de l'Homme, une incertitude demeure quant aux mécanismes de protection réels de l'Homme exposé à un environnement contaminé. Une meilleure compréhension de la chimie complexe des oxydes d'azote permettra d'aider au développement de nouvelles thérapies antibactériennes fondées sur l'accroissement de ce qui semble être un système de défense de l'hôte simple et efficace.

nitrates / nitrites / alimentation humaine / santé humaine

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1. INTRODUCTION

There has been considerable controversy concerning the possible harmful effects of nitrate in our diet and in the environment. Nitrate has, in general, been considered harmful, although the evidence for this has become less convincing with time. Recently, since the discovery of nitric oxide as an important biological molecule which regulates many bodily functions and provides host defence against numerous micro-organisms, the image of nitrate has, to some extent been rehabilitated. This is because of the discovery of a novel biochemical pathway which can lead to the formation of large, possibly protective, amounts of nitric oxide in mammals from the sequential reduction of inorganic nitrate. The purpose of this paper is to consider the possible harmful and beneficial properties of inorganic nitrates which are an essential component of biological systems and which are encountered in large amounts in our diet every day.

Inorganic nitrates (NO_3^-) have been added as a food preservative, especially pig meat, to make ham and bacon for centuries. As well as its beneficial effect to limit the growth of serious pathogens such as *Clostridium botulinum* [30], nitrate, or more specifically its reduction product nitrite (NO_2^-) has also the dubious benefit of rendering muscle tissue a bright pink colour, by the formation of nitrosomyoglobin. It has subsequently become clear that nitrate is generally non-reactive with organic molecules and has to be chemically or enzymatically reduced to nitrite to be effective as an antimicrobial agent [5]. Nitrate is also found in large quantities in green, leafy vegetables such as lettuce and spinach, particularly when grown under low light conditions (see below and Tab. I).

Despite its long use, there have been considerable concerns about the use of nitrate and nitrite as a preservative in food and about the content of these ions in vegetables and drinking water. There are two reasons for this.

Table I. Contribution (%) of various foodstuffs to dietary intake of nitrate and nitrite (after Committee on Nitrite and Alternative Curing Agents in Food [7]).

Food [30]	Nitrate	Nitrite
Cured meats	1.6	39
Fresh meats	0.8	7.7
Vegetables	87	16
Fruit / juices	6	1.3
Baked foods / cereals	1.6	34
Milk / milk products	0.2	1.3
Water	2.6	1.3

Firstly, the theoretical possibility of forming carcinogenic N-nitroso compounds in food to which these ions are added and in humans in vivo, due to nitrosation of secondary amines also present in the diet or ingested as drug therapy [37]. Nitrosation of amines and other chemicals will occur rapidly under acidic conditions (such as in the human stomach) when nitrite is present, due to the formation of nitrous acid [42]. It will also occur in the stomach under more neutral pH when there are bacteria present. The mechanism of this presumably enzymatic nitrosation is not understood.

Nitrous acid is an effective nitrosating agent due to its ability to donate an NO^+ group. These nitrosation reactions are catalysed by halide ions (such as chloride) and thiocyanate due to the formation of nitrosohalide (such as NOCl) and nitrosothiocyanate (NOSCN) respectively, and these intermediates will more effectively donate NO^+ groups. Both chloride and thiocyanate ions are present in gastric juice in high concentrations, the latter is derived from the diet (particularly brassicas such as cabbage) and is concentrated in saliva. Nitrate in the diet is similarly concentrated in saliva following absorption and reduced to nitrite in the mouth. It therefore seems that the stomach is an ideal reaction chamber for the nitrosation of susceptible swallowed chemicals.

This scheme of human metabolism of nitrate and nitrite seems to be counterproductive for human health and has prompted a reconsideration of exactly how these ions are handled following ingestion. We have formulated a scheme whereby the formation of nitrogen oxides in the stomach and on the skin surface, derived from dietary nitrate and nitrite, may protect against bacterial, and possibly viral, pathogens.

A second potential mechanism for nitrite toxicity is the formation of methaemoglobin due to oxidation of the iron in haemoglobin. This is due to the reaction of nitrite with oxyhaemoglobin, where it is oxidised to form nitrate. It should also be noted that nitric oxide also reacts quickly with oxyhaemoglobin to produce methaemoglobin and nitrate. This may be more important than nitrite in causing this problem in infants fed on well water.

Methaemoglobinaemia is generally only a problem in young infants, but has been the main reason for statutory limitations on the concentrations of nitrate in drinking water. For nitrate to cause methaemoglobinaemia it has to be reduced to nitrite in food, water or in the body. The mechanisms by which this may occur will be considered in the next section.

2. NITRATE METABOLISM BY BACTERIA AND PLANTS

In nature, nitrogen is continually cycled between nitrogen gas, and the fully oxidised nitrogen molecule – nitrate and the fully reduced nitrogen molecule – ammonia. Plants and bacteria have somewhat different uses for nitrogen molecules. In general, plants need nitrogen as ammonia as a precursor to protein synthesis. They have the appropriate enzymes to reduce nitrate through to ammonia, a process requiring energy provided by photosynthesis. This occurs in at least two distinct steps catalysed by separate enzymes – nitrate reduction to nitrite (nitrate reductase) and then nitrite reduction to ammonia (nitrite reductase). Nitrate is present in the soil due to organic decomposition or the fixing of atmospheric nitrogen by nitrogen-fixing bacteria which may be associated with roots of certain leguminous plants. Nitrate is commonly stored in the leaves of vegetables such as lettuce and spinach, and accumulates to very high concentrations under low light conditions. This is presumably because of a reduction in the flux of nitrate through to ammonia when insufficient energy is available from photosynthesis. As a result, especially in cool climates, green vegetables contribute to our nitrate intake more than any other single source [6].

Although some micro-organisms will also reduce nitrate to ammonia for protein synthesis, many also use nitrate reduction for the purpose of anaerobic respiration. The enterobacteriacae such as *E. coli* can switch from using oxygen to burn available fuel to using nitrate as an electron acceptor (or oxidant) which is then converted to nitrite. The facultative anaerobes which inhabit the human mouth allow little further reduction of nitrite, presumably because they lack the nitrite reductase enzyme.

3. NITRATE AND NITRITE METABOLISM IN MAN

It was first shown in 1916 that humans excrete more nitrate than they consume [27]. This was confirmed in careful studies by Tannenbaum's group in the 1970's and 1980's [16, 17] and is now known to be due, at least in part, to the formation of nitric oxide by mammalian cells from the amino acid L-arginine.

3.1. Nitric oxide synthesis via NO synthase

Following the demonstration in 1980 by Furchgott and Zawadski [15] that an intact vascular endothelium was necessary for blood vessels to relax when exposed to the neurotransmitter acetylcholine, it was clear that endothelial cells were able to synthesise a short-lived vasodilator substance. It took seven years to identify this substance which turned out to be the simple molecule nitric oxide (NO) [29]. It is now known that there are three distinct nitric oxide synthase enzymes.

Two of these enzymes, the endothelial isoform and the neuronal isoform, continually synthesise NO whereas the inducible isoform produces this molecule from arginine only when the appropriate cell is exposed to bacterial cell wall products or pro-inflammatory cytokines such as interferon gamma and tumour necrosis factor alpha.

The function of the endothelial isoform is to provide continual vasodilatation. Inhibition of NO synthesis with arginine analogues such as methylarginine causes high blood pressure in animals and man, and intriguingly, it has recently been shown that nitric oxide synthesis is impaired in patients with hypertension [14]. Although many central (brain) neurones contain the neuronal form of NO synthase, the precise function of NO in central nervous function is yet unclear. It is likely that these two isoforms contribute to the majority of NO synthesis which is rapidly converted to nitrate when this molecule encounters oxidised haemoglobin or superoxide.

Inducible nitric oxide synthase is easily demonstrated within a few hours in mouse macrophages exposed to bacterial lipopolysaccharide (LPS). Indeed if rodents are exposed to LPS or made septicaemic there is a large rise in plasma and urinary nitrate concentration. It has been much more difficult to demonstrate synthesis of NO in human cells exposed to LPS, although it is clear that overwhelming infection will increase nitrate synthesis in man [28]. Merely injecting killed bacteria, as in a vaccine, is not effective in enhancing NO synthesis [23]. The one infection which will cause a large increase in NO synthesis is gastroenteritis. We do not yet know that this is due to induction of NO synthase. The large rise in plasma nitrate in this condition may, however, be effective in preventing the recirculation of pathogens through the stomach by mechanisms discussed below.

4. NITRATE AND NITRITE IN THE DIET

4.1. Nitrate

Because of the potential for toxicity, there have been many studies in the last 20 years which have studied the effect of nitrate in the human diet and estimated the intake of this ion in different populations. For those people who eat a large amount of vegetables, then the main source of nitrate will be the green leaves of plants such as lettuce and spinach. Significant amounts are also found in root vegetables such as beetroot and carrots. For those who eat few vegetables then the nitrate concentration of tap water becomes an important factor determining nitrate intake. The concentration of nitrate in drinking water has been limited to 50 $mg \cdot l^{-1}$ in Europe, mainly because of concern about methaemoglobinaemia in infants (although this is now extremely rare). As it is now known that nitrite is the effective antimicrobial product of nitrate, it is less common that nitrate itself is used as a preservative for meat products such as sausage and ham, and these foods generally have little nitrate content, although nitrate is available from pharmacies to use as a meat preservative.

4.2. Nitrite

The average intake of nitrite is considerably less than that of nitrate. The main source is again vegetables which will convert nitrate to nitrite on storage and when contaminated by nitrate-reducing bacteria. Occasionally this can result in very high

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