

Invited commentary

Nitrate toxicity: myth or reality?

Nitrate has long been one of the more emotive anions, always being talked about, whether with pride or horror. It is abundantly present in soil and in water (Hill, 1991a), and is taken up by plants, for which it is a major source of utilizable N. It has a remarkably low toxicity in adults (Hill, 1991b). It is regularly administered as NH_4NO_3 at doses of 10 g/d for 4–5 d as a diuretic without any acute toxic side-effects (Bruijns, 1982). The only chronic toxic effects are those resulting from the nitrite formed by its reduction by bacterial enzymes. If we could all live in a germ-free world, therefore, there would be no hazard. Unfortunately we have to accept the world as it is, and that means with its nitrate-reducing bacteria.

Nitrate is the end-product of the microbial breakdown of nitrogenous organic material in the soil and in sewerage. It is a valuable fertilizer. In the earlier days of municipal sewerage disposal the amount of nitrate produced was a matter of pride because it indicated the completeness of the breakdown of potentially harmful material. Much of this nitrate used to end up in drinking water either as a result of being pumped into local rivers, or after percolating down through the soil to the aquifers tapped by wells, but that caused little concern, because nitrate was good. Until 1945 there was little interest in the amount of nitrate in the human diet. Then came the first convincing report (Comly, 1945) of infant methaemoglobinaemia (Met-Hb) associated with consumption by babies of feeds made up in nitrate-rich drinking water, and attitudes changed.

In the literature survey by Walton (1951), Roe (1933) is credited with being the first to associate nitrate exposure with fatal infant Met-Hb. However, there had been many earlier reports from animal and *in vitro* studies of the role of nitrate and nitrite in methaemoglobin formation (e.g. Gamgee, 1868; Binz & Geringer, 1901). It was only a problem with high nitrate concentrations, and in 1970 the WHO first set upper limits for the nitrate content of drinking water (Oakes, 1991). Following the report by Comly (1945) there was a major attempt to decrease the number of people drinking water from private wells, and to supply them with piped water (which was easier to control).

In the early weeks of life, before the establishment of the gastric acid barrier, the infant gut is colonized by bacteria throughout its length, and so nitrate in the feed is readily reduced in the stomach and small intestine by bacterial nitrate reductase (*EC* 1.6.6.1) to nitrite. This is then absorbed and reacts with haemoglobin to form methaemoglobin; this latter has greatly reduced O_2 -binding capacity, and so the infant tissues are starved of O_2 and cyanosis results. If 60% of the haemoglobin is in the form of methaemoglobin this is generally accepted as a lethal level. Later in life the infant

develops a gastric acid barrier and so the colonization of the upper gut is decreased to very low levels, minimizing nitrite formation. In addition, an erythrocyte enzyme is formed which destroys nitrite and so protects the haemoglobin from nitrite toxicity. In consequence the lethal dose of nitrite needed for an adult is 2–9 g (World Health Organization, 1985). It has now been confirmed that the problem of infant Met-Hb is greater if the well water is bacterially contaminated. This is hardly surprising since it is the nitrite produced by bacterial action that is the problem. Whether the bacteria are in the well or in the gut, if high levels of nitrate are present in the water then high levels of nitrite will be produced, and this will result in Met-Hb in the infant. The measures to decrease nitrate levels in drinking water have been very successful and fatal Met-Hb is now rare in Western countries. However in Hungary, for example, where the great majority of people live in rural areas and the cost of piped water supplies would be prohibitive, serious Met-Hb was common as late as the 1980s (M Borszonyi, personal communication). Nitrate in drinking water is therefore a health hazard to the unprotected infant, and WHO recommendations and EU legislation recognize that.

The next concern to arise was that of human cancer as a result of the bacterial production of *N*-nitroso compounds (NNC) in the colonized hypochlorhydric stomach. There is no doubt that bacteria can catalyse the reaction between nitrite and nitrosatable N compounds to yield NNC. This was reviewed by Leach (1988), who demonstrated that some gastric juice bacteria were very potent *N*-nitrosating species. NNC have been conclusively demonstrated in the stomach of persons with gastric hypochlorhydria (Reed *et al.* 1996). Gastric hypochlorhydria is known to predispose to human gastric cancer (Hill, 1994), and Correa *et al.* (1975) postulated that the mechanism for the progression from chronic atrophic gastritis to gastric cancer is via bacterial production of NNC. NNC have been shown to be carcinogenic in more than forty animal species tested. These include mammals, birds, reptiles and fish, and there is no reason to suspect that human beings are uniquely resistant. Vanzant *et al.* (1932) showed that more than 20% of people over 50 years old were achlorhydric. It is probable that the value is lower now in Western countries, and perhaps the rapid decline in gastric cancer incidence in Western countries is due to a similar decrease in the prevalence of hypochlorhydria. However, it is likely that the conditions in the USA in the 1930s reflect the current conditions in much of the world. In addition, of course, there are the tens of thousands of people who have decreased gastric acidity because of gastric surgery. Thus, the proportion of the population with hypochlorhydria is far from negligible.

We have long known that nitrite is a potent antimicrobial agent and it is this that makes it such a good food preservative (Roberts & Dainty, 1991), so how can hypochlorhydria be a risk factor for bacterial production of nitrite and of NNC? In food, the nitrite kills the small numbers of potentially infecting organisms and the same is perhaps true for the small numbers entering the acid stomach (though the acidity itself is a potent anti-microbial agent). What is clear, though, is that at pH values > 4 nitrite is no protection whatsoever. In a review of studies of pH, gastric juice bacteria and nitrite (Hill, 1988), in every study where there was an elevated gastric juice pH there was both a rich bacterial flora and a high nitrite concentration. In not one of the twenty-four studies was there any problem in growing live bacteria from the gastric juice, despite the high nitrite concentrations.

So the situation remains the same as it has been for the last few decades. If we were all germ-free and lived in a germ-free environment then our babies could consume nitrate-rich diets with impunity. Unfortunately, in the real world bacteria are everywhere. Under those circumstances high nitrate exposures present a potential hazard to babies from Met-Hb, not because of the nitrate itself but because of what the bacteria produce from it, and because of the lack of the protective mechanisms that will develop later in the baby's life. Similarly, if only we could all maintain optimum levels of gastric acidity then nitrate would present no risk of gastric cancer. Unfortunately we are not all as we would wish to be, and a significant proportion of the normal population has decreased gastric acidity and, therefore, a resident bacterial flora in the stomach. In such human subjects we know that there is a risk of bacterial *N*-nitrosation in the stomach. We do not know the dose needed to cause gastric cancer in man, but we know that the rate of the reaction increases with increasing nitrite (and therefore nitrate) concentration. Do we need to take the risk?

It was in this light that the legislation on human nitrate exposure from food and water was passed by the WHO and EU. I find it very reasonable.

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