

The Cancer Phantom Nitrates, Nitrites, and Nitrosamines

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The role of nitrite, and indirectly nitrate, in the food supply as a public health hazard is under investigation. The problem of nitrate in water is well known to you; nitrate serves as a source of nitrite, via reduction, which may be a cause in infants of cyanosis or methemoglobinemia. Also significant, however, is the recent claim that nitrite may react with secondary amines in food or drugs to form nitroso compounds that are carcinogenic, and possibly mutagenic and teratogenic as well.

While these undesirable effects have been demonstrated in animal tests, I want to emphasize at the outset that cancer in humans has not been related to the nitroso compounds. Since animals are susceptible to these carcinogens, however, it is very possible that humans, too, are sensitive to them.

Nitrate is relatively nontoxic; doses as high as one gram a day have been given for diuretic purposes. The legal limit in potable water is 10 mg nitrate-N/liter. Nitrate enters the water supply by leaching from the surface soil or from sewage disposal areas. In special situations, such as heavily fertilized soil in arid or semiarid areas, or the land around animal feed lots, very high concentrations of nitrate can be detected in the water.

Nitrate is absorbed by growing plants which, under normal conditions, metabolize it rapidly through nitrite and several other forms of nitrogen to produce protein. Some species of plants normally accumulate nitrate, and most plants under abnormal growth conditions also build up a reservoir of nitrate. This nitrate can be reduced to nitrite by enzymes in plant tissue after harvesting or by contaminating bacteria.

Nitrite is about 10 times more toxic than nitrate and can be lethal. Nitrite is absorbed rapidly from the stomach, combining with iron in blood hemoglobin. The oxygen carrying capacity of the blood is reduced, which can be fatal when about 80% of the hemoglobin is immobilized. Higher stomach pH and the presence of bacteria, particularly during digestive upset, make infants more susceptible to methemoglobinemia than adults. Thus, if water contains nitrates it can be potentially dangerous to drink or to use for preparing formulas and food.

Many cases of infant methemoglobinemia have been reported in the last 30 years, principally from water high in nitrate. Other factors have to be considered, however. It has been suggested that infants fed orange juice — and thus Vitamin C — might be protected from developing methemoglobinemia. Home-prepared spinach purees have also been implicated as the source of nitrite for methemoglobinemia, especially in Europe. The relatively high concentration of nitrate in spinach, as in some other vegetables, can be converted to nitrite on maceration and improper storage of the fresh vegetable material.

Virtually all cases of food-caused methemoglobinemia in infants have been caused by home-prepared foods; only one recorded case was traced to a commercial product in which a strained beet preparation caused illness in a one-month old infant. Several cases of methemoglobinemia occurred in older children who had eaten frankfurters and other sausage products containing illegal, grossly high concentrations of nitrite due to human error. In New York, several



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years ago, a mysterious case of eleven "blue" men who developed methemoglobinemia within a few hours of each other baffled the Department of Public Health until it was found all had eaten oatmeal at the same restaurant, putting sugar on the cereal. Unfortunately, one of the kitchen staff had accidentally filled some of the sugar containers from a bag of nitrite which was used for curing meat.

The chief concern over nitrite these days, among some segments of the public, lies with the nitroso compounds formed as a result of the chemical reaction of nitrite with secondary amines under conditions that favor the reaction. The amines are found in minute quantities in

meat, fish, fruits, and vegetables, or they may occur as precursors convertible into amines under the proper conditions. Nitroso compounds have been used industrially for many years but it has been only 18 years since the carcinogenic nature in animals was recognized, and a relatively recent 10 years since nitrosamines were found in food.

Until 1970, however, analysis for these compounds was limited. The tests were either insufficiently sensitive to detect trace quantities of nitrosamines, or not specific for these compounds. In the latter case, many substances normally found in food or reagents reacted in such a manner as to give false positive results for nitroso compounds. There is doubt today that many of the early investigations of nitrosamines in various foods actually measured these compounds.

Since about 1970, however, a number of procedures using sophisticated, highly sensitive instrumentation, or more specific chemical interactions, have permitted us to measure nitrosamines down to the 1 $\mu\text{g}/\text{Kg}$ level or 1 part per billion. Such sensitivity has taxed the ingenuity of the analytical chemist in recovering trace quantities of nitrosamines from the food matrix in good yields.

Contaminating materials are an even greater problem at such low levels of nitrosamines. Fortunately, however, the presence of nitrosamines that may be indicated by instruments such as the gas chromatograph can be confirmed by the use of the mass spectrometer. The fragments of the nitrosamine produced in the mass spectrometer are fingerprints that lead to the identification of the original structure. It is now generally accepted that any report of a nitroso compound in the food supply must have confirmation by mass spectrometry.

Confirmed findings of nitrosamines have been reported principally in fish and meat products treated with nitrite. A few reports of nitrosamines at 1 g/Kg in raw meat or fish have not been explained. Nitrite is used in curing or "corning" the meat product. Dating back to antiquity, this process was used to preserve meat,

initially by the liberal application of salt. The pink color and flavor that has become characteristic of cured products developed at the same time. At some point in history it was found that the salt used was contaminated with "saltpeter" or potassium nitrate, so this compound was added separately for its desired effect.

In the 1890's it was recognized that bacteria in the curing solution reduced nitrate to nitrite, and that it was actually the nitrite that was responsible for the cure effect. In 1926 the Department of Agriculture established regulations for the use of nitrite in curing meat, based on the amount of nitrite found in then-conventional nitrate cures in which acceptable products were produced. Nitrate is still included in most cure formulae, but principally as a reservoir for nitrite.

Although salt, and possibly nitrate (and/or nitrite) were originally used to preserve food products, we now have refrigeration, canning, and other techniques for preserving meat. Why then, the question is asked, do we still use nitrite? This compound develops the characteristic color and flavor of cured meats that are produced during cooking of nitrite-treated products. There are also antioxidative effects which prevent breakdown of fats, thus extending the shelf life of the product, and some effect on texture. Most important, however, is the role of nitrite in preventing growth of *Clostridium botulinum* in canned or vacuum packaged products, thus preventing formation of the deadly toxin, botulin.

Botulism was first recognized in a case of poisoning from sausage, hence the name, which means "sausage" in Latin. In some as-yet-unknown fashion, nitrite and salt concentrations, pH and temperature of cooking are jointly effective so that under proper conditions the growth of clostridia is inhibited. The same effect could be achieved by increasing salt concentrations or by heating the product at sterilization temperature, but the nature of the product would be changed. To date there are no acceptable substitutes available for nitrite.

Reports of nitrosamines in meat products have not been frequent.

Hams appear to be free of those nitrosamines for which they were tested. In our laboratory 3 samples of frankfurters out of some 40 tested contained low levels of N-nitrosodimethylamine. There are other reports of nitrosamines in Hungarian salami and other sausage products. It is now believed that these items may have been contaminated by preformulated cure mixtures that contained nitrite and spices and in which nitrosamines had formed. Use of such premixed preparations is now restricted by the USDA and FDA.

The most significant finding is that bacon contains N-nitrosopyrrolidine after frying. If all of the reports in the literature are combined, about 70% of the bacon tested contained this nitrosamine, generally in the 15 to 30 g/Kg range, but concentrations as high as 108 g/Kg have been reported. The mechanism of nitrosopyrrolidine formation is still unknown; it is not present in raw bacon. We believe a precursor amino acid, proline, forms nitrosoproline. This compound has been shown in our laboratory to change into nitrosopyrrolidine at the temperature at which bacon is fried.

We have been studying the effect of various conditions on nitrosopyrrolidine formation — processing, storage time, temperature, age of belly, etc. — but to date these do not appear to play a major role. However, we did demonstrate that the salt of ascorbic acid, or isoascorbic acid, could reduce, or even prevent, formation of nitrosamines. This is now undergoing tests by the American Meat Institute, in which one of the major meat products producers is preparing bacon with various levels of nitrite and ascorbate. The results are being watched closely by both FDA and USDA, and the nitrosamine analyses are being done in FDA laboratories.

In addition to formation in foods, there has been concern over the possibility of nitrosamines forming in the gastrointestinal tract following ingestion of amines, or their precursors, and nitrite or nitrate. Not only are foods implicated, but a number of drugs also have chemical structures that could degrade to nitrosatable secondary amines.

Model experiments with gastric juice in test tubes and with rats have shown formation of nitrosamines from some amines or drugs. However, the quantities used have been unrealistically high and the rat may not be the best test animal. The human stomach contains very few bacteria under normal conditions, whereas the stomach of the rat is loaded with bacteria that can reduce ingested nitrate to nitrite and decompose the amine precursors to these compounds. Since nitrite and nitrate are absorbed very quickly from the stomach very little, if any, reaches the intestines where similar bacteria can be found in the human. pH conditions are also different in the human and rat stomach, and the nitrosation reaction is very sensitive to pH.

How much nitrosamine do we have to eat before tumor formation occurs? There is no definite answer to this question at present, which is the crux of the arguments about nitrosamines in the food supply. At this time, there have been no complete dose-response studies carried out, to the best of my knowledge. We do not know whether there is a concentration of nitrosamine below which cancer formation does not occur. We do not know whether the action of nitrosamines is cumulative. We do not know anything about synergistic or antagonistic actions of other compounds in the environment, or about the effects of bodily health and metabolism on the action of nitrosamines. We don't know these things about cancer formation in the animals, let alone in the human body.

To demonstrate the carcinogenic action of nitrosamines, investigators have used excessive quantities of material. Their rationale is that if, under normal conditions, only 1 case of cancer occurs in 10,000 individuals, they would have to use at least that many animals, and probably many more, to get statistically significant data. Since this is economically and practically unfeasible, these investigators claim that comparable results will be obtained by using higher doses on fewer animals. The pharmacological activity of many compounds in large quantities, however, is different from that of

trace amounts. Sodium chloride, for instance, is toxic in large concentrations. Furthermore, unknown impurities in the synthesized nitrosamines tested could have influenced the results in the concentrations used.

There has been only one "reasonable" study to date in which rats were fed daily a normal diet containing 40% cured meat. The meat had 60 g/Kg of several nitrosamines that formed naturally in the curing process. After two years, the rats showed no unusual symptoms. More studies of this type are needed to evaluate the carcinogenic potential of nitrosamines.

There are a few dose-response studies that can be mentioned. Dimethylnitrosamine produced tumors in 1 of 26 rats fed 2 mg/Kg rations daily; at 5 mg/Kg and higher, as many as 70% developed liver tumors. Therefore, 1 mg/Kg could be considered a "threshold dose" for this nitrosamine under these conditions. In another study, a single dose of 20 mg dimethylnitrosamine/Kg body weight was carcinogenic. Dimethylnitrosamine fed daily to rats led to a "marginal effect" dose of about 0.5 mg/Kg. Preussmann stated that, applying the generally accepted practice of using a safety margin of 100 in extrapolating animal data to humans, these results suggest that 5 to 10 g/Kg (parts per billion) of the low molecular weight nitrosamines might be considered a "tolerable" dose. Such calculations are still highly theoretical; there are too many unknown factors that can modify the results.

Similarly, calculations on the consumption of the dietary nitrosation reactants are almost meaningless. The quantity of amines or amine precursors consumed in either foods or drugs cannot be estimated. We don't know all that are present, or the concentrations of those that are known. The major amount of nitrite would come from cured meats. Assuming an average of 50 mg/Kg, which is the residual nitrite content after processing and some storage, two frankfurters or 4 ounces of bacon or ham would furnish about 5 mg of nitrite.

Nitrite has also been detected in the saliva of all people tested and, while insufficient information is available on the nitrite content in

saliva after eating high nitrate-containing foods and other factors, 12 mg nitrite per day appears to be a reasonable average. The nitrite content of vegetables and water is negligible and nitrate should not be reduced to nitrite under normal stomach conditions. Thus, there is a daily intake of about 17 mg nitrite, or 34 mg if the value is doubled to allow for unforeseen occurrences. Over a 24-hour period, this concentration could be too small to be of importance; however, a concentrated dose of nitrite could occur during a meal. The 5 to 10 mg of nitrite ingested in meat might form a nitrosamine if it contacted a nitrosatable amine in the food bolus in the stomach, if the concentration ratios were correct, if the pH and other factors were favorable, and if the reaction occurred quickly enough, since nitrite is readily absorbed from the stomach.

The multiplicity of important beneficial effects achieved by the use of nitrite in certain foods seems currently to outweigh presumed risks, since cancer in humans has not been related to ingestion of nitroso compounds. The Department of Agriculture, the Food and Drug Administration, and laboratories around the world are trying to eliminate, or reduce, any hazard that might exist from the use of nitrite in the food supply without too great a change in the nature of cured meat products as we know them. Complete prohibition of nitrite could lead to an increase in home curing or curing by small, neighborhood meat processors, with the greater danger of uncontrolled use of sodium nitrite by untrained people or increased potential for botulin poisoning.

Present research efforts are directed toward determining the minimum concentration of nitrite needed for each class of cured meat, toward modifications in processing that would reduce the possibility of nitrosamine formation, toward the use of compounds such as ascorbate that will interfere with the formation of nitrosamine but not with the desirable activities of nitrite, and toward development of substitutes for nitrite. Given the opportunity to accomplish this, our investigators should soon provide us with food free from nitrosamine hazards.