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Nutrition and nitrosamine formation

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Nutrition and the formation of N-*nitroso compounds*

N-nitroso compounds are an important group of chemical carcinogens that could be involved in human cancer (Magee, 1982). Only a few examples have been found in food and beverages (Assembly of Life Sciences, 1981) but suitable precursors are common dietary constituents (Assembly of Life Sciences, 1981), and the mildly acidic conditions of the stomach are favourable for their formation (Challis, 1981). Over 90% of the *N*-nitroso compounds tested have proven to be carcinogenic, some in as many as thirty species ranging from mice to primates (Magee *et al.* 1976). Many are organ-specific carcinogens, producing tumours remote from the site of their administration (Magee *et al.* 1976). Nonetheless, unequivocal evidence that *N*-nitroso compounds cause human cancer has proven elusive.

Formation of N-*nitroso compounds*

N-nitroso compounds are usually formed by the interaction of a nitrosating agent (NOX) derived from either nitrite salts or nitrogen oxides with an amino substance (e.g. R_2NH) as in eqn (1). The amino substance can be one of several

$$R_2NH + NOX \rightleftharpoons R_2NNO + HX \qquad (1)$$
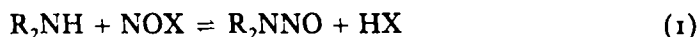
types of compound such as secondary and tertiary amines, secondary and tertiary amides, peptides, substituted ureas, guanidines and urethanes. In the case of amines, amides and ureas, the products are the *N*-nitrosamines, *N*-nitrosamides and *N*-nitrosoureas shown in Fig. 1. The mechanism and extent of formation of the *N*-nitroso derivative is dependent on both the structure of the amino substance and the source of the nitrosating agent, as well as the reaction conditions.

With nitrite salts, the most extensive reactions apply to weakly basic amino substances (e.g. aromatic amines, amides, ureas) under aqueous acidic conditions (Challis, 1981). For secondary amines and amino acids, formation of their *N*-nitroso derivatives usually passes through a maximum at pH 2·5–3·5 where the yield is inversely proportional to the basicity of the amino substance (Mirvish, 1975). These reactions are catalysed by substances such as halides (Douglass *et al.*
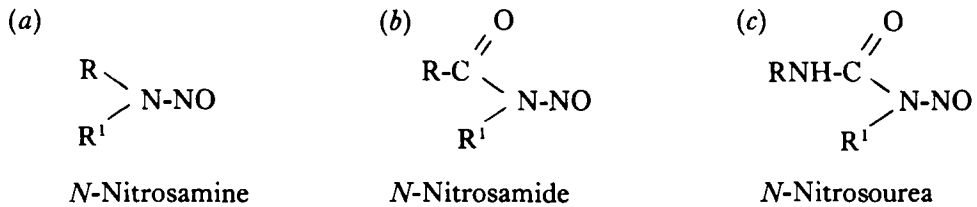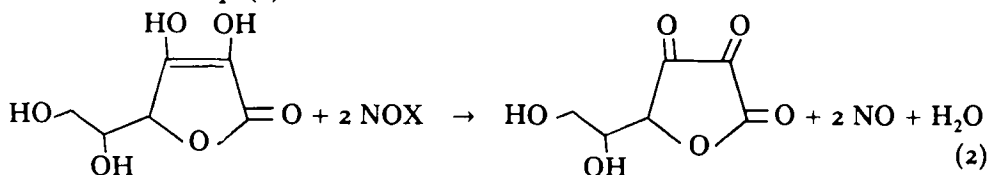
(a)

$$\begin{array}{c} R \\ \diagdown \\ \diagup N\text{-NO} \\ R^1 \end{array}$$

N-Nitrosamine

(b)

$$\begin{array}{c} O \\ \| \\ R\text{-}C \\ \diagdown \\ \diagup N\text{-NO} \\ R^1 \end{array}$$

N-Nitrosamide

(c)

$$\begin{array}{c} O \\ \| \\ RNH\text{-}C \\ \diagdown \\ \diagup N\text{-NO} \\ R^1 \end{array}$$

N-Nitrosourea

Fig. 1. *N*-nitroso compounds formed from (a) amines, (b) amides and (c) ureas. R, R^1 = alkyl, aryl or part of a cyclic structure.

1978), thiocyanate (Douglass *et al.* 1978), and formaldehyde (Casado *et al.* 1981). Tertiary amines react similarly to secondary amines, but usually to a much lower extent (Mirvish, 1975; Gowenlock *et al.* 1979). For amides, peptides, ureas and guanidines formation of their *N*-nitroso derivatives rarely occurs above pH 4, but increases steadily with increasing acidity (i.e. decreasing pH) (Mirvish, 1975; Challis *et al.* 1985). These reactions are not catalysed by other substances and their extent varies in a complex way with substrate structure and basicity (Mirvish, 1975; Challis, 1981).

With nitrogen oxides, reactions are most extensive for strongly basic amino substances (e.g. aliphatic and heterocyclic amines) under non-aqueous or neutral and alkaline aqueous conditions (Challis & Kyrtopoulos, 1979). Oxygen (Challis & Kyrtopoulos, 1979), iodine (Challis & Outram, 1979), polyhydroxy compounds (e.g. carbohydrates) (Challis & Shuker, 1980) and several metal salts (Challis *et al.* 1978; Challis & Outram, 1978) have an enhancing effect, whereas acidic conditions are inhibitory (Challis & Kyrtopoulos, 1979). Amides, peptides, ureas and guanidines do not react with nitrogen oxides in aqueous media (Challis & Kyrtopoulos, 1979).

Many compounds and components of foods other than amino substances can react with nitrite salts and nitrogen oxides, and thus reduce the amounts available for the formation of *N*-nitroso compounds. Such compounds include ascorbic acid (vitamin C) (Mirvish, 1981), α-tocopherol (vitamin E) (Newmark & Mergens, 1981) and other natural and synthetic antioxidants (e.g. tannins, chlorogenic acid, butylated hydroxy anisole, sulphite) (Douglass *et al.* 1978; Challis, 1981; Mirvish 1981). These either bind the nitrosating agent (NOX) irreversibly or reduce it to nitric oxide (NO)—a relativly ineffectual nitrosating agent—as shown for ascorbic acid in eqn (2).

$$\begin{array}{c} HO \quad OH \\ \diagup\diagdown \end{array} \!=\! O + 2\,NOX \quad \rightarrow \quad \begin{array}{c} O \quad O \\ \| \| \end{array} \!=\! O + 2\,NO + H_2O$$

$$\tag{2}$$

Further, primary amines, most amino acids and peptides may undergo deamination (eqn (3)) (Ridd, 1961; Challis, 1981), which also reduces the amount of nitrosating agent without forming N-nitroso compounds.

$$RNH_2 + NOX \rightarrow RX + N_2 + H_2O \qquad (3)$$
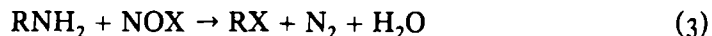
Dietary occurrence of N-nitroso compounds

Combustion generates nitrogen oxides and nitrite salts are added as a preservative to some foods. These can generate N-nitroso compounds during processing and cooking if conditions allow the nitrosating agents to interact with amino substances in the foodstuff. Low levels of volatile N-nitrosamines have been found in some foods and beverages (e.g. N-nitrosodimethylamine in beer (Assembly of Life Sciences, 1981; Spiegelhalder *et al.* 1979) and N-nitrosopyrrolidine in bacon (Assembly of Life Sciences, 1981)). Where such contaminants have been identified, changes in processing technology have reduced their levels (Assembly of Life Sciences, 1981; Preussmann *et al.* 1981). It is conceivable, however, that some foods and beverages contain higher levels of largely unidentified non-volatile N-nitroso compounds (Pollock, 1981). Their identification, origin and health risk are the subject of much current work.

Dietary occurrence of precursors

From a nutritional standpoint, the occurrence of precursors is of as much interest as N-nitroso compounds because the acidic conditions of the stomach are suitable for nitrosation reactions.

Amino substances of one sort or another are present in most foods. This information has been reviewed (Singer & Lijinsky, 1976; Neurath *et al.* 1977; Maga, 1978; Smith, 1981), but much is either too qualitative or incomplete for reliable health-risk assessment. Individual secondary and tertiary amines in most foods rarely exceed 10 ppm and more often are less that 2 ppm (Singer & Lijinsky, 1976; Neurath *et al.* 1977; Maga, 1978; Smith, 1981). One exception is fish, where up to 740 ppm dimethylamine (Singer & Lijinsky, 1976) and 1400 ppm trimethylamine (Maga, 1978) have been reported in some samples. The highest concentrations, however, may relate to spoilage (Gruger, 1972). Cocoa products and cheeses are usually rich in amines (Neurath *et al.* 1977; Maga, 1978; Smith, 1981), but most are primary compounds which should undergo deamination (eqn (3)) and therefore reduce the overall formation of N-nitroso compounds. Further, secondary and tertiary amines in foods are often associated with 1000 fold larger concentrations of ammonia (Neurath *et al.* 1977; Maga, 1978; Smith, 1981), which should also decompose nitrite and nitrogen oxides without the formation of N-nitroso compounds. Less information is available about the presence of amides, ureas and guanidines in the diet, despite forceful arguments that their N-nitroso derivatives could be especially important in gastric cancers (Mirvish, 1983). Alkylureas and alkylguanidines have been found in some fish and meat products (Fujinaka *et al.* 1976; Mirvish *et al.* 1980), and in fried bacon (Mirvish *et al.* 1980),
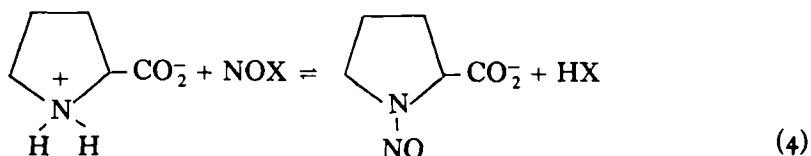
probably from the oxidation of creatine and creatinine. Further, proteins and peptides are the most abundant dietary nitrogen constituents and their behaviour towards nitrosating agents should be similar to amides and ureas (Challis, 1981). Until recently (Challis *et al.* 1985), their nitrosation and the chemical and biological properties of their *N*-nitroso derivatives have been largely overlooked.

Because nitrate is reduced to nitrite by oral and gastric bacteria (Ralt & Tannenbaum, 1981) the dietary intake of both is relevant to gastric nitrosation. Both are present in food, especially vegetables (White, 1975; Assembly of Life Sciences, 1981), and in most water supplies at low level (Assembly of Life Sciences, 1981). Both form in the lungs on inhalation of the nitrogen oxides in polluted atmospheres and in tobacco smoke (Goldstein *et al.* 1980; Oda *et al.* 1981). Nitrite is added to some meats as a preservative against *Clostridium botulinum* (Assembly of Life Sciences, 1981). Thus, ingestion of nitrite and nitrate salts is unavoidable. A recent estimate puts the USA dietary nitrite intake at approximately 0·8 mg/d of which 40% derives from cured meat, 35% from cereal products and 15% from vegetables, and the dietary nitrate intake at approximately 75 mg/d of which 85% is from vegetables (Assembly of Life Sciences, 1981). Further, these intakes may be increasing in consort with the use of artificial fertilizers, which raise the concentration of nitrite and nitrate salts in both vegetables (White, 1975; Assembly of Life Sciences, 1981) and water supplies (Assembly of Life Sciences, 1981). Vegetables, however, often contain correspondingly high levels of antioxidants (e.g. ascorbic acid and polyphenols) (Assembly of Life Sciences, 1981; Fine *et al.* 1982) which are able to react with nitrosating agents (eqn (2)) and therefore reduce the formation of *N*-nitroso compounds.

Gastric nitrosation

The acidic conditions of the stomach are suitable for nitrosation by nitrite from food and swallowed saliva. About 25% of ingested nitrate is recirculated into the saliva of which about 20% (i.e. 5% of the ingested nitrate) is then reduced to nitrite by oral bacteria (Spiegelhalder *et al.* 1976; Tannenbaum *et al.* 1976). Of the nitrite entering the stomach, about 20% arises directly from food and about 80% from the reduction of salivary nitrate. Thus, gastric nitrosation is more dependent on nitrate than nitrite intake.

Ingestion of proline and vegetable juices rich in nitrate increases urinary yields of *N*-nitrosoproline (eqn (4)) (Ohshima & Bartsch, 1981), and human gastric aspirates contain small amounts of largely unidentified *N*-nitroso compounds (Reed *et al.* 1981; Milton-Thompson *et al.* 1982). This is good evidence that humans allow the reduction of nitrate to nitrite and sustain gastric nitrosation reactions.

$$\text{(structure)} \overset{+}{\underset{\underset{H\ \ H}{N}}{}}\!\!-CO_2^- + NOX \rightleftharpoons \text{(structure)} \underset{\underset{NO}{N}}{}\!\!-CO_2^- + HX \qquad (4)$$

In healthy humans, gastric nitrite concentrations oscillate and reach maxima of about 30 μM (with considerable individual variation) 1–2 h after the consumption of food (Milton-Thompson *et al.* 1982). Gastric acidity varies similarly from about pH 1 on fasting to about pH 3 after food (Milton-Thompson *et al.* 1982). Under these conditions, *N*-nitroso compounds are most likely to form from aromatic amines, ureas and peptides (Challis *et al.* 1982). In patients who have pernicious anaemia (Ruddell *et al.* 1978), or have undergone gastric surgery (Schlag *et al.* 1980), gastric pH is commonly above 5, nitrate-reducing bacteria multiply in the stomach and gastric nitrite concentrations are much higher. Whether these conditions produce enhanced levels of *N*-nitroso compounds is the subject of current debate.

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