Nitrosation of Food Amines under Simulated Stomach Conditions

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Many *N*-nitrosamines and *N*-nitrosamides are carcinogenic to a number of different species of animals (Schmahl & Osswald, 1967), and the metabolism of *NN*-dimethylnitrosamine in human liver is very similar to that in the corresponding organ of the rat (Montesano & Magee, 1970). The synthesis of nitrosamines from secondary amines and nitrite in gastric juice from various species has been reported (Sen *et al.*, 1969), as well as the induction of malignant tumours as a result of feeding with nitrite and amines such as morpholine and *N*-methylbenzylamine in high doses (Sander & Burkle, 1969). Of large amounts of nitrite administered to dogs concurrently with dimethylamine, only a small proportion was recovered from the stomach contents, although the formation of *NN*-dimethylnitrosamine was very small (G. Kazantsis, P. N. Magee & C. L. Walters, unpublished work). In cases of anacidity the concentration of nitrite in the human stomach can be as high as 24 mg/100 ml of contents (Sander & Burkle, 1969). Apart from specialized instances such as nitrate-rich spinach stored without refrigeration, the concentration of nitrite in foods is usually less than 100 p.p.m.

The reaction with nitrite of a secondary amine at the optimum pH for nitrosation, namely 2.5–3.0, is very greatly dependent on the basicity of the amine (Mirvish, 1972). Further, the rate of nitrosation of a secondary amine is proportional to the square of the nitrite concentration (Mirvish, 1970). Tertiary amines and quaternary ammonium compounds can also react with nitrite to yield nitrosamines (Fiddler *et al.*, 1972). Therefore the extent of nitrosation of various foods has been studied, by making use of their detection as a group by use of the Eisenbrand & Preussmann (1970) denitrosation procedure. No compounds containing a nitrogen–oxygen bond other than *N*-nitrosamines and *N*-nitrosamides have been found to respond to this sensitive test (Johnson & Walters, 1971).

After incubation *in vitro* for 3 h at 37°C, akin to the normal maximum residence time in the stomach, and at pH values in the range 1.0–3.4 and a nitrite concentration of 1.45 mM, little or no nitrosation occurred within the dichloromethane-extractable fraction of foods such as meat, eggs and bread. After the nitrite concentration had been raised 100-fold to a value (0.15 M) far in excess of that likely to be

encountered in the stomach as the result of food consumption, nitrosation did occur in the dichloromethane-extractable fraction, suggesting that the amine precursors were of such basicity to resist attack by nitrous acid under the mild conditions ascribed to the stomach.

Nitrosation did take place in the fractions of food that entered aqueous solution in incubations under 'stomach' conditions, but again the extent of reaction within a period of 3 h at 37°C at a nitrite concentration of 1.45 mM was less than 10% of that when the nitrite concentration was raised 100-fold to ensure the nitrosation of as many amines as possible. The fractionation on Sephadex G-10 of material retained in the aqueous portion of such incubations of various foods has indicated that nitrosation of a range of peptides occurs to different extents. The amino acid composition of these peptides is under study.

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Gaseous Products of the Interaction of Sodium Nitrite with Porcine Skeletal Muscle

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It is well established that NaNO₂ in contact with skeletal muscle forms nitrosyl-myoglobin (Haldane, 1901), and muscle mitochondria are believed to be implicated in this process (Walters *et al.*, 1967). It has also been reported that NO gas is formed from the interaction of nitrite with skeletal muscle