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contents, although the formation of *NN*-dimethylnitrosamine was very small (G. Kazantsis, P. N. Magee & C. L. Walters, unpublished work).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 24mg/100ml 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 100p.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 3h 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 1.45mM, 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 (0.15M) 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 3h at 37°C at a nitrite concentration of 1.45mM 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 $NaNO_2$ 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

(Walters & Taylor, 1964). The present communication describes analyses of head-space gases produced from anaerobic incubations of nitrite (2.9mM) and minced porcine skeletal muscle at pH6.0 at 37°C in the presence of the antibiotic chloramphenicol to restrict bacterial involvement in the experiments. Analyses were performed by using medium-resolution mass spectrometry and g.l.c. and employed nitrite labelled with the stable isotope ^{15}N. With $Na^{14}NO_2$ major peaks were obtained in the mass spectrum at *m/e* values 28, 30 and 44; when $Na^{15}NO_2$ was employed the only new peaks observed were at *m/e* 31 and 46. This indicates the formation of NO and N_2O respectively. NO production increased linearly with nitrite concentration up to 14mM, and the output of N_2O, although much less than that of NO, also increased.

Peaks were also observed at *m/e* values 26 and 27 and were considered to be part of the fragmentation pattern of ethylene, the molecular ion of which ($m/e = 28$) would have been obscured by background $^{14}N_2$. Confirmation of the formation of C_2H_4 has been provided by g.l.c. and high-resolution mass spectrometry. C_2H_4 production increased with nitrite concentration up to 50mM, and, as the bacterial count of the muscle with nitrite present was very low, the formation of C_2H_4 was apparently not associated with bacterial action. CO_2 has also been detected (at $m/e = 44$) as a product. A markedly decreased output of NO and C_2H_4 that was observed in the presence of the respiratory-chain inhibitor Amytal (Ernster *et al.*, 1963) suggested that gas evolution was associated with the respiratory oxidation–reduction systems of the tissue. This view is supported by the earlier observation that NO production was enhanced by the inclusion of the electron donor reduced Methylene Blue (Walters & Taylor, 1964).

The reduction of nitrite to NO involves a redox potential change of +0.99V (Charlot *et al.*, 1958), close to that for the reduction of O_2. The combination of two nitrogen atoms as in N_2O could proceed through the labile dimerization of any nitroxyl (NOH) formed, followed by elimination of H_2O from the resultant hyponitrous acid. N_2O may also be formed from the action of nitrous acid with quaternary ammonium compounds and tertiary amines (Fiddler *et al.*, 1972) and with NADH (Evans & McAuliffe, 1956) or thiol groups (Möhler & Ebert, 1971); C_2H_4 is possibly produced by loss of HNO_2 from ethyl nitrite, which has been found in meat products preserved with nitrite.

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A Microsomal Catechol *O*-Methyltransferase from Rat Liver

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A microsomal enzyme capable of *O*-methylating catechols differs from cytoplasmic catechol *O*-methyltransferase in its response to 3,4-benzopyrene and to cold stress (Inscoe *et al.*, 1965). The two enzymes also have different pH optima.

We have studied the properties of catechol *O*-methyltransferase in rat liver microsomal preparations and in the fraction obtained on solubilization of the microsomal membrane. Microsomal preparations were obtained by differential centrifugation of rat liver homogenates in iso-osmotic KCl, and the washed microsomal pellets were finally suspended either in $0.2\text{M}-Na_2HPO_4$ adjusted to pH6.5 with $0.2\text{M}-KH_2PO_4$ or in iso-osmotic KCl. Alternatively rat liver microsomal preparations were obtained by the technique described by Dallner (1963) and finally suspended either in $0.2\text{M}-Na_2HPO_4$ adjusted to pH6.5 with $0.2\text{M}-KH_2PO_4$ or in 0.25M-sucrose.

The microsomal fractions were incubated at 37°C with L-adrenaline bitartrate (640μM) and *S*-adenosyl-[*Me*-^{14}C]methionine (75μM) and the reaction was terminated by the addition of borate buffer, pH10.0. The [*Me*-^{14}C]methylcatechol formed in the reaction was extracted from the incubation mixture with toluene–pentanol (3:2, v/v) and its radioactivity measured by liquid-scintillation counting.

Initial observations of microsomal catechol *O*-methyltransferase show that the pH optimum is about 6.5 whereas that of the cytoplasmic enzyme is approx. 7.9. The microsomal enzyme is not released from the membranes in the presence of saturated NaCl, which suggests that catechol *O*-methyltransferase is not ionically bound to the microsomal membrane. Optimal solubilization was