Nitrosamines as Environmental Carcinogens

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Human cancer might be caused by nitrosamines formed in the body from ingested nitrites and secondary amines. Cooking could be a source of secondary amines.

ALTHOUGH several groups of chemicals have been demonstrated to be carcinogenic during the past few decades, there are only few cases in which exposure to such chemicals has been causally related to cancer in man. For example, skin cancer in workers in the coal tar and creosote industries is clearly associated with exposure to polynuclear compounds; similarly, bladder cancer among workers in the dyestuff and rubber industries is related to exposure to 2-naphthylamine, benzidine and other aromatic amines. Apart from such restricted cases of occupationally induced cancer, there is little evidence relating the high incidence of human cancer to particular chemical carcinogens.

The recent discovery of the highly carcinogenic aflatoxins prompted the speculation that liver cancer in the tropics might be caused by ingestion of nuts and grains contaminated with mycotoxin as a result of improper harvesting and storage. Even so, mycotoxins can hardly be implicated in cancer in modern industrialized society.

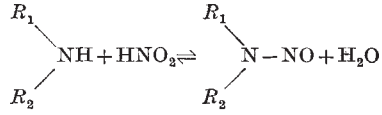
In our view, any group of chemical carcinogens significantly implicated in human cancer must be both widespread and multipotent. Such chemicals are the nitrosamines, intensively studied since the first report of hepatic cancer induced by nitrosodimethylamine in rats[1]. In a large series of nitrosamines recently tested, most were carcinogenic in a wide range of organs of various species[2]. The most interesting attributes of carcinogenic nitrosamines are their systemic action and organ specificity. Thus, nitrosopiperidine produces tumours of the oesophagus in rats, as well as in other sites, whether administered orally, intravenously or subcutaneously[3]. Nitrosoheptamethyleneimine and nitrosooctamethyleneimine both induce squamous carcinomas of the lung and oesophagus when given to rats in drinking water[4]; by the same route in hamsters, however, nitrosoheptamethyleneimine produces cancer of the forestomach only (unpublished results of W. L.).

Nitrosamines in the Environment

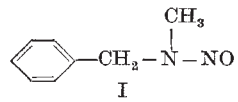
It thus seemed logical to look for nitrosamines in the environment in an attempt to account for some epidemiological aspects of human cancer. Unfortunately, nitrosamines are very difficult to detect, especially in low concentrations. They neither fluoresce nor have well defined absorption spectra, and have a wide range of physical characteristics, particularly in volatility and solubility. A group analysis is of limited value, for not all nitrosamines are carcinogenic. Nitrosamines have been found in tobacco smoke, grains and alcoholic beverages in concentrations of less than 5 p.p.m.[5]. The significance of such low concentrations to man is unknown[6]. Higher concentrations of nitrosamines have been found in nitrite-preserved fish meal intended for animal feeds; these fish meals were highly toxic to ruminants[7].

Precursors of Nitrosamines

There has been increasing interest in the possibility that nitrosamines can be formed *in vivo* and so give rise to tumours in individuals not apparently exposed to nitrosamines *per se*. Secondary amines have recently been shown to react with nitrite to form nitrosamines in conditions of defined *p*H and in other conditions similar to those in the mammalian stomach[8], and also *in vitro* in gastric juice[9,10].



Rats given nitrite and a secondary amine, methylbenzylamine, together in their diet developed oesophageal tumours[11], which are also induced by nitrosomethylbenzylamine (I), the corresponding nitrosamine[3].



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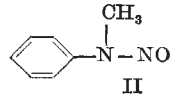


II

It has been suggested that such *in vitro* and *in vivo* interactions might be significant to man[6,9]. These considerations have directed our attention to the environmental distribution of nitrites and secondary amines as essential precursors of nitrosamines.

Nitrates and Nitrites

Nitrates are widely distributed in nature, particularly in plants and forage, and nitrites are readily formed from them. Excessive use of nitrate fertilizers, use of the herbicide 2,4-dichlorophenoxyacetic acid, and molybdenum deficiency in soil result in accumulation of nitrate in plants and forage[12] and methaemoglobinaemia in cattle as a consequence of nitrite poisoning. A close association has been demonstrated recently between molybdenum deficiency in garden plants and the occurrence of oesophageal cancer in localized areas of the Transkei; dimethylnitrosamine has been isolated from food plants in these areas[13]. Nitrates can occur in significant concentrations in water, particularly in agricultural areas, and produce toxic or fatal methaemoglobinaemia in infants after conversion to nitrite[14]. Contamination of water with nitrates results from sewage discharges, intensive use of nitrate fertilizers, and rising water tables which leach sub-soil nitrates into well waters. Nitrites are intermediate in the reduction of nitrates in plants and can be produced from nitrates in plants and other sources by nitroreductases present in a wide range of saprophytic and parasitic bacteria[15]. Large concentrations of nitrites are found in stored green vegetables, especially spinach, celery and green salad, as a result of bacterial reduction of nitrate[16]. Furthermore, nitrates and nitrites are widely used as preservatives in meat and fish, in permissible concentrations of 500 and 200 p.p.m. in the United States[17]. It seems likely, therefore, that nitrite ions are not infrequently present in the stomach of man. The average daily intake of nitrite has been estimated as 22 μmoles[9], equivalent to 1·5 mg of $NaNO_2$; some individuals undoubtedly consume much more nitrite.

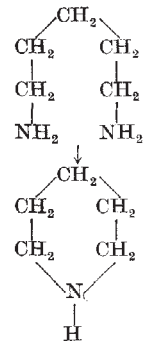
Secondary Amines

The limiting factor, then, in the formation of nitrosamines seems to be the availability of nitrosatable secondary amines. Because secondary amines have seldom been sought in the environment, information about their distribution is scanty. They occur, particularly as dimethylamine and diethylamine, in fish meal and fish products, cereals and tea[18] and in tobacco and tobacco smoke[19]. In such sources, secondary amines are usually found together with primary and tertiary amines.

Methods of identifying secondary amines are not well developed and those usually measured are diethylamine and dimethylamine; the remaining bases are conventionally grouped as other unidentified amines. Conversion of dimethylamine and diethylamine into nitrosamines is not favoured in dilute acid conditions, as prevail in the stomach, because of the relatively strong basicity of these amines[11,20]; this conversion has, however, been demonstrated with diethylamine[10]. In this connexion, rats fed a mixture of dimethylamine or diethylamine and sodium nitrite failed to yield tumours[11,20]. Conversion of other secondary amines to nitrosamines is favoured when the nitrosamine, such as nitrosomethylbenzylamine (I) and nitrosomethylaniline (II), tends to be less soluble. Cyclic amines, such as piperidine (III), are in this category and the derived nitrosamines are potent carcinogens[3]. Because the feeding of nitrite alone did not give rise to tumours[11,20], the formation of nitrosamine from either endogenous secondary amines, such as epinephrine or proline, or from secondary amines in rat food is unlikely.

Secondary Amines in Cooking

Man is the only species which cooks and preserves its food, and cooking possibly affords a source of secondary amines. Pyrolysis of protein and cooking of protein food might produce free amino-acids such as proline, arginine and hydroxyproline, and nitrosatable secondary amines such as pyrrolidine and piperidine. The diamines cadaverine and putrescine present in partially decayed meat and fish are converted by heating into piperidine (III) and pyrrolidine (IV), respectively; these two amines could therefore be formed when meat or fish is cooked.



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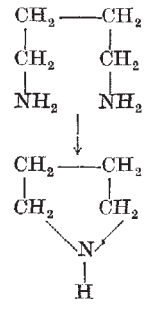
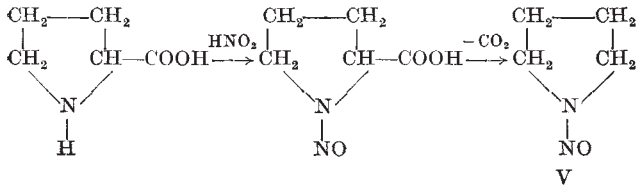


IV

Nitrosoproline has been synthesized recently[21]. It has been further shown that both proline and hydroxyproline are nitrosated in dilute acid solution at 35°–40° C in the presence of an excess of sodium nitrite; in each case the yield exceeded 50 per cent of the theoretical value; the crystalline nitrosamino-acids were identified by mass and nuclear magnetic resonance spectrometry. It has not been possible to isolate a nitroso derivative of arginine. In the presence of dilute alkali at ambient temperature, nitrosoproline undergoes decarboxylation with formation of nitrosopyrrolidine (V), as identified by mass spectrometry. The preparation and properties of nitrosoproline

and nitrosohydroxyproline will be reported elsewhere (unpublished results of W. L.).



The possibility therefore exists that proline ingested in food can be converted into nitrosoproline by nitrite present in the stomach. The nitrosoproline could then be decarboxylated, possibly bacterially, in the alkaline conditions prevailing in the duodenum and small intestine, yielding the highly carcinogenic nitrosopyrrolidine. It is likely that hydroxyproline undergoes parallel reactions *in vivo*.

Uses of Secondary Amines

Secondary and other amines are formed during alcoholic fermentation. A mixture of crude organic bases was at one time made commercially by distillation of the residues of fermented molasses with lime; nitrogenous bases have been found in spirits and several amines, including pyrrolidine, in wine[22]. It is therefore possible that the high incidence of oesophageal cancer associated with the consumption of crude beers and other liquors in Zambia[5] is due to secondary amines, in addition to nitrosamines.

Various flavouring agents are prepared from secondary amines[23], including bread flavourings prepared from pyrrolidine or piperidine, and meat flavourings prepared from free amino-acids including proline. These additives could be nitrosatable themselves or contain the unreacted amines. Some toothpastes contain derivatives of sarcosine; nitrososarcosine is carcinogenic[3]. Some drugs are secondary amines, such as piperazine which is used in the treatment of worms; dinitrosopiperazine produces tumours of the oesophagus and liver when fed to rats[3].

Tobacco contains several secondary amines, particularly pyrrolidine to the extent of 0·01 per cent[19]. These amines could be released and ingested when tobacco is chewed, particularly when mixed with lime. Cigarette smoke contains many secondary amines, including pyrrolidine and piperidine[19]. These amines could dissolve in the saliva during smoking and be converted to nitrosamines in the stomach. Such nitrosamines could be absorbed and give rise to tumours of the lung systemically, a property of several nitrosamines.

In vivo formation of nitrosamines from ingested nitrite and certain secondary amines is potentially a serious problem, especially because nitrosamines are possibly more significant in human cancer in industrialized society than are polynuclear compounds, azo dyes or aflatoxins. Epidemiological data on the carcinogenicity of nitrosamines should be re-evaluated in the light of these considerations. It should, however, be stressed that the individual occurrence of nitrites and secondary amines is not necessarily hazardous; both reactants must be present simultaneously in the stomach to form nitrosamine. Even so, not all secondary amines react with nitrite to form nitrosamines in conditions prevailing in the mammalian stomach, and not all nitrosamines are carcinogenic.

Consideration should be given to the reduction or elimination of one or other nitrosamine precursors, nitrites and certain secondary amines, from the diet. For this purpose, more information is needed on the quantitative distribution of these compounds in the environment. On the immediate practical level, however, it should not be difficult to reduce the amount of nitrite and nitrate added as preservatives to food, particularly meat and fish.

Removal from Diet

In summary, nitrosamines seem to be a major candidate class of carcinogens that are likely to be causally related to human cancer in industrialized society. Nitrosamines act systemically and produce cancer in a wide variety of organs of many species. Additionally, individual nitrosamines exhibit marked organ specificity. Although methods for detection of nitrosamines lack sensitivity, their widespread environmental distribution has been demonstrated recently. Even more widespread and of possibly greater significance are the precursors of nitrosamines—nitrites and secondary amines. These precursors react in defined conditions *in vitro* and probably also *in vivo* to form nitrosamines. Reduction of human exposure to nitrites and certain secondary amines, particularly in foods, may result in a decrease in the incidence of human cancer.

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