

N-Nitroso compounds in the diet

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Received 7 October 1998; received in revised form 12 February 1999; accepted 12 February 1999

Abstract

N-Nitroso compounds were known almost 40 years ago to be present in food treated with sodium nitrite, which made fish meal hepatotoxic to animals through formation of nitrosodimethylamine (NDMA). Since that time, *N*-nitroso compounds have been shown in animal experiments to be the most broadly acting and the most potent group of carcinogens. The key role of nitrite and nitrogen oxides in forming *N*-nitroso compounds by interaction with secondary and tertiary amino compounds has led to the examination worldwide of foods for the presence of *N*-nitroso compounds, which have been found almost exclusively in those foods containing nitrite or which have become exposed to nitrogen oxides. Among these are cured meats, especially bacon—and especially when cooked; concentrations of 100 $\mu\text{g kg}^{-1}$ have been found or, more usually, near 10 $\mu\text{g kg}^{-1}$. This would correspond to consumption of 1 μg of NDMA in a 100-g portion. Much higher concentrations of NDMA (but lower ones of other nitrosamines) have been found in Japanese smoked and cured fish (more than 100 $\mu\text{g kg}^{-1}$). Beer is one source of NDMA, in which as much as 70 $\mu\text{g l}^{-1}$ has been reported in some types of German beer, although usual levels are much lower (10 or 5 $\mu\text{g l}^{-1}$); this could mean a considerable intake for a heavy beer drinker of several liters per day. Levels of nitrosamines have been declining during the past three decades, concurrent with a lowering of the nitrite used in food and greater control of exposure of malt to nitrogen oxides in beer making. There have been declines of *N*-nitroso compound concentrations in many foods during the past two decades. The small amounts of nitrosamines in food are nonetheless significant because of the possibility—even likelihood—that humans are more sensitive to these carcinogens than are laboratory rodents. Although it is probable that alkylnitrosamides (which induce brain tumors in rodents) are present in cured meats and other potentially nitrosated products in spite of much searching, there has been only limited indirect evidence of their presence. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: *N*-Nitroso; Diet; Nitrosodimethylamine

1. Introduction

In their examination of the causes of human cancer, Doll and Peto [1] ascribe a major role (60%) to ‘diet’, based on epidemiological observations; they did not particularize which agents in the food might be responsible, or those that might be more important than others. Among the numerous chemical carcinogens that have been detected in human food and drink, *N*-nitroso compounds are among the most

recent and are distinguished by being very potent. They are seldom present by deliberate (if coincidental) addition. Since *N*-nitroso compounds are easily formed by interaction of a secondary amino compound with a nitrosating agent (commonly a nitrite salt, but also ‘nitrous gases’), it might have been expected that foods treated with nitrites for colouring, flavouring and preservation would likely contain *N*-nitroso compounds, but this apparently was not thought of.

The first inkling of such a connection was early in the 1960s when some sheep in Norway died of liver toxicity after feeding on fish meal that had been treated with sodium nitrite [2–4]. It would not have been expected that a nitrosamine, nitrosodimethylamine (NMDA), would be present in the nitrite-treated fish meal, since nitrosation of amines (in this case, dimethylamine and trimethylamine) was considered to take place only in acid solution, and the fish meal milieu was neutral or alkaline. Much later, Keefer and Roller [5] demonstrated that interaction of a secondary amine with nitrite readily took place in alkaline medium in the presence of a carbonyl compound (especially an aldehyde–formaldehyde in the case of fish meal). The presence of considerable quantities of nitrosamines in cured tobacco is also a consequence of nitrosation in non-acid medium [6]. It is noteworthy that the amount of NMDA in the fish meal was large, even considering the potent hepatotoxicity of NDMA, because some of the sheep died.

These observations followed closely the first report of toxicity and carcinogenicity of NDMA by Magee and Barnes [7], after which there was an explosion of interest in the toxicology of the *N*-nitroso compounds, which are among the simplest of chemical carcinogens. Most of the experiments done subsequently involved the testing of *N*-nitroso compounds of various structures (several hundred compounds), the results of which provided considerable insight into the mechanisms by which *N*-nitroso compounds were activated and induced cancer [8,9]. Among the findings were that 40 or more species that have been treated with, e.g., nitrosodiethylamine (NDEA), were all susceptible to its carcinogenic action [10], suggesting strongly that, unlike other types of carcinogen, there was probably no non-susceptible species (e.g., humans). Another finding was that many species differed as to which organ(s) responded to a particular *N*-nitroso compound, which also depended on the chemical structure of the carcinogen. There were some *N*-nitroso compounds that appeared to be inactive (i.e., non-carcinogenic), which also gave insight into mechanisms of carcinogenesis by these compounds. It would, however, be misleading to claim that the mechanism by which any *N*-nitroso compound induces tumors is understood (or, indeed, any other carcinogen), particularly

so, since the size of the dose, the frequency of the dose and the route of administration to a certain species (e.g., rats) can change the affected organ from lung to kidney to liver, even changing the target cell from which tumors arise from hepatocytes to endothelial cells [11]. These modulations make it difficult—if not impossible—to predict, based on experiments in rats or mice, which would be the target organ of a particular *N*-nitroso compound in humans (or, in fact, in any other species). On the other hand, NDEA has induced liver tumors in most of the species in which it has been tested, including rodents, snakes, birds, molluscs and monkeys [10,12].

Of the several hundred *N*-nitroso compounds that have been examined, only a few are likely to be encountered by humans outside the laboratory and almost all are nitrosamines, which are stable and not directly acting. As a group, nitrosamines induce tumors in a variety of organs [13], including liver, lung, kidney, bladder, pancreas, esophagus and tongue—depending on the species—but not in skin, brain, colon or bone. On the other hand, the unstable and directly acting nitrosamides (alkylnitrosoureas or alkylnitrosocarbamates) induce tumors of the nervous system (in rats but not in hamsters), stomach, gastrointestinal tract and bone. Many of the tumors induced by *N*-nitroso compounds are similar to the analogous human tumors and it is tempting to conclude that the latter might be caused by human exposure to *N*-nitroso compounds. Such human exposures occur in certain work environments, as a consequence of some habits (smoking and chewing tobacco), in the use of particular household products (cosmetics and shampoos), but probably the most widespread exposure is in food and drink. A related exposure (which will not be gone into here) is through the formation of *N*-nitroso compounds by interaction in the stomach of secondary and tertiary amino compounds (in food or medicines) with nitrite (in food and in saliva) or other nitrosating agents. This endogenous nitrosation, probably an important source of *N*-nitroso compounds, has been extensively discussed for many years [14] and has been demonstrated experimentally [15–17]. It has also been studied epidemiologically in connection with human cancer [18]. Most human cancers, with the exception of those caused by tobacco use, have no known cause, and widespread exposure to *N*-nitroso

compounds in food provides one link that should not be ignored.

2. Methods of analysis for *N*-nitroso compounds

At a meeting in Jamaica in 1968 sponsored by the World Health Organization dealing with distribution and occurrence of carcinogens, it was concluded that, while trace analysis of polynuclear hydrocarbons was possible to hundredths of a part per million or less, detection of nitrosamines was unlikely to be feasible even at 1 ppm, except in unusual circumstances. At about the same time, there was under development by the Thermo-Electron Corporation (Waltham, Massachusetts, USA), a device (thermal energy analyzer or TEA) for detecting *N*-nitroso compounds (although not entirely specific to them) using the electronic measurement of the infrared light emitted by NO liberated by pyrolysis of nitroso compounds. Prior to this, analysis and detection of *N*-nitroso compounds involved working up a batch of material by preferential extraction, concentration of the extract and chromatographic identification of the *N*-nitroso compound in a particular band, often using a colour reagent. As mentioned, the sensitivity of these methods was not great, nor was the specificity. This resulted in many erroneous detections (and measurements) of nitrosamines in cheeses, vegetable oils and other foods in which they were not present, or present at much lower concentrations than those reported. In one series of experiments by this author, acetic acid was confused with NDMA because both had the same retention time on a particular chromatographic column packing. The TEA [19] was not only more specific in detecting *N*-nitroso compounds (a few other chemical types also produced a response, but they were different in their behaviour in other respects, e.g., chromatographically [20]) but it was highly sensitive, enabling concentrations of fractions of a part per billion to be detected. Yet more recently, it has been required that a chromatographic column (gas-liquid or high-pressure liquid) be combined with a mass spectrometer as detector to definitively identify a particular component as a *N*-nitroso compound. More recent data, therefore, tend to be more reliable than earlier reports, e.g., the large concentrations of NDEA and

methylnitroso-benzylamine in some foods in China that were associated with a high incidence of esophageal cancer in inhabitants of those areas [21]; these claims were, in some instances, later shown to be inaccurate [22].

A major deficiency in our information about the distribution of *N*-nitroso compounds in foods is the carcinogenic non-volatile compounds. These comprise two important groups, the alkylnitrosamides (including alkylnitrosoureas) and hydroxylated nitrosamines [including nitrosodiethanolamine and nitrosobis-(2-hydroxypropyl)amine]. These are important carcinogens of considerable potency and can be isolated chromatographically only by high-pressure liquid chromatography (HPLC), not gas chromatography; identification at low concentrations of alkylnitrosamides is not easy, because they are not very stable [23], but they are important because they are among the few carcinogens that induce tumors of the nervous system in experimental animals [24] and are suspected of responsibility for brain tumors in children [18]. Methylnitrosourea has not been identified directly in food (cured meats), but its presence has been inferred by the identification of a methylated receptor (nitroso-*t*-butylglycine) formed by direct methylation [25]; alkylnitrosamides are powerful, directly acting alkylating agents, which have been linked to their mutagenic and carcinogenic activities. Another group of non-volatile *N*-nitroso compounds, almost all of which are non-carcinogenic or weakly carcinogenic, is the nitrosamino acids, some of which are quite widespread in foods.

3. Occurrence of *N*-nitroso compounds in food and beverages

The use of sodium nitrite to colour, flavour and preserve (prevention of botulism) meat and fish, replacing sodium nitrate which had been used since time immemorial, led to the search for *N*-nitroso compounds in such foods, once nitrosamines were classified as carcinogens in 1956 [7]. Many investigators in the field of food toxicology developed a great interest in this topic, as did the meat packing industry. There was a special interest in bacon, because it is so widely consumed. There developed in the 1970s quite a controversy over the use of nitrite in bacon, which was often vacuum-packed with a

consequent risk of infection with the anaerobic *Clostridium botulinum*. The regulation then (not always enforced) was that the maximum level of sodium nitrite in bacon should be 200 ppm; a similar regulation applied to other nitrite-cured meats in the USA, such as ham, sausages (many kinds), corned beef, etc. Certain kinds of smoked fish, such as smoked salmon, were also treated with sodium nitrite, and the concentration was less well-regulated than in meat.

Analysis of bacon and other cured meats for nitrosamines in the early years (1960s) was not very accurate nor specific. The most common *N*-nitroso compounds identified in foods were NDMA and NDEA, but there were ‘identifications’ of some nitrosamines which were distinctly dubious [26]. The tendency was for investigators to examine the foods in their own countries, so there is a wealth of information about nitrosamine contamination worldwide, even if the accuracy of some of the reports is suspected.

Because the isolation and identification of volatile nitrosamines is relatively easy, most of the attention have been given to them and there has been much less interest in non-volatile nitrosamines or in nitrosamides, although the latter are by no means to be ignored. Some non-volatile nitrosamines have been studied and are of little interest because they appear to be non-carcinogenic, including nitrosoproline, nitrosohydroxyproline, nitrosopipelic acid, nitrosothiopropine and nitroso-4-methyl-thiopropine [27,28]; they have been detected and quantified in cured meats, but their main toxicological interest lies in their ability to act as nitrosating agents, for transnitrosation [29], and they can be thermally decarboxylated [14] to produce cyclic nitrosamines which are potent carcinogens.

Nitrosopyrrolidine was discovered in the vapours from fried bacon and also in fried bacon itself, mainly in the fat, at concentrations approximating 100 ppb, which is considered quite significant; 100 g of such fried bacon would contain 10 µg of nitrosopyrrolidine, which is one of the more potent carcinogenic nitrosamines. The nitrosopyrrolidine might have arisen, at least in part, by decarboxylation of nitrosoproline, or by some other mechanism; as suggested by Loepky et al. [30]; there was also NDMA in cooked bacon, although at lower levels.

Nitrosopyrrolidine was absent from uncooked bacon. The same was true of other cured meats; nitrosamino acids were present in the uncooked products, together with some moderate levels of nitrosamines (mainly NDMA), but some nitrosopyrrolidine and higher levels of NDMA in the cooked products. The amount of nitrosopyrrolidine formed in cooked bacon depends on the duration and temperature of frying, much more at higher temperatures, very little in microwave cooking. Another nitrosamine, nitrosothiazolidine, was discovered in bacon and other cured meats, and appears to be derived from interaction of cysteine, formaldehyde and nitrite. Nitrosothiazolidine was at first thought to be carcinogenic on the basis of analogy with the potent carcinogen, nitrosooxazolidine [31], and because some of its preparations were mutagenic in the Ames test. Subsequently, it was found that purified preparations of nitrosothiazolidine were not mutagenic and a chronic feeding study in rats was negative [32], showing that its presence in cured meats was not a carcinogenic risk.

Recently, there has been some concern about the finding of nitrosodi-*n*-butylamine (NDBA) in some meat products, albeit at low concentrations [50]. This unusual nitrosamine, which induces tumours of the urinary bladder in rats and other species [9], was traced to rubber netting used to tie up the cured meat after processing; derivatives of dibutylamine are used in rubber manufacture and apparently become nitrosated at some stage and the NDBA then migrates into the meat. Any time food comes into contact with a rubber product, it can be expected that nitrosamines in the rubber will migrate (slowly) into the food. The process of manufacture of rubber invariably results in the formation of nitrosamines as by-products, which can attain considerable concentrations (many parts per million or more). Another unusual nitrosamine, nitrosodiphenylamine, has been reported in apples (which had been treated with diphenylamine). The concentration of the nitrosamine was 2 to 6 µg kg⁻¹, there being a higher concentration in the skins [33]. This is probably of minor importance, because of the weakness of nitrosodiphenylamine as a carcinogen, although it does cause bladder tumours in rats [34].

The pattern of nitrosamine content was similar in nitrite or nitrate-treated products throughout the

world, although in Japan and East Asia, where relatively little meat is eaten, the focus has been on fish, nitrite-treated, smoked or otherwise processed. There have been persistent reports, e.g., from Hong Kong [35], of the relation between the high incidence of nasopharyngeal cancer and the consumption of nitrite-containing smoked fish as a major component of the (Chinese) diet. NDMA has been identified in samples of the fish (reminiscent of Ender and Ceh's story of the Norwegian fish meal) and, although NDMA is considered mainly an inducer of liver tumors and lung or kidney tumors in rats, there have been experiments in which it has induced tumors of the nasal cavity in rodents, albeit these are morphologically different from human nasopharyngeal tumors. Because of the well-known differences between species in response to *N*-nitroso compounds [8], particularly in target organ, it is unwise to assume that a particular *N*-nitroso compound would induce the same tumors in humans as in any of the common experimental animals.

It was discovered a long time ago that alkylnitrosoureas are potent inducers of tumours of the brain and nervous system in rats and mice (but not in hamsters, gerbils or Guinea pigs, Kleihues et al. [36]) especially when administered transplacentally via pregnant animals in the latter stages of pregnancy. This has led to the search for a source of alkylnitrosoureas in the human diet as an explanation of brain tumours in humans, especially children; there are few known nervous system carcinogens and none as effective as alkylnitrosoureas. There have been a number of epidemiological studies of cured meat consumption by pregnant women who bore children that later developed tumours of the nervous system and none have been conclusive, although some were suggestive. In particular, there seems to be a relation between the consumption of 'hot dogs' (Frankfurter sausages) in the USA and pediatric nervous system tumors, the incidence of which is significantly elevated in children who consume five or more sausages a week [18].

In spite of much searching, there has been little or no evidence of the presence of methylnitrosourea or any other alkylnitrosourea in cured meats, although Mende et al. [25] have inferred the presence of such compounds because of the methylating capacity of the material. Creatinine (a component of meat) was

once thought to be a source of methylnitrosourea after nitrosation (and therefore likely to be present in cured meats) but there has been no good evidence that the yield of MNU from a reaction of creatinine with nitrite is substantial. A similar alkylnitrosamide, methylnitrosourethane (methylnitrosocarbamic acid ethyl ester), has induced tumours of the nervous system transplacentally in rats [37]. Since many widely used pesticides are esters of *N*-methylcarbamic acid and are very easily nitrosated [38], it is feasible that residues of their *N*-nitroso derivatives could be ingested (or formed *in vivo*) and induce nervous system tumours in children. However, there seem to have been no transplacental studies in rats or other animals of any such nitrosated pesticide.

Another possible directly acting *N*-nitrosamide that might be present in meat (and thereby related to tumours of the nervous system) is methylnitrosoguanidine, because guanidine is a constituent of meat. Its derivative, methylnitrosoguanidine (MNNG—an artificial compound), is one of the most common compounds in carcinogenesis and mutagenesis studies. Alas, methylnitrosoguanidine is a very unstable compound and has yet to be isolated, although evidence has been obtained for its presence in solution. Little is known of its toxic, mutagenic or carcinogenic properties (it could be formed transiently in solution) and feeding tests of methylguanidine and nitrite in rats to provide evidence of its formation in the stomach failed to give rise to any tumours. So evidence that any particular *N*-nitroso compound (or, indeed, any other known carcinogen) in the diet is responsible for brain tumours in children (or in adults) is bleak, and research activity in this area is rare.

Although for many years there was slow progress in understanding the magnitude of the problem of nitrosamines in food, it could not match the intensity of effort following the discovery of NDMA in beer, as a result of a comprehensive survey of nitrosamines in food and beverages [39,40]. No doubt beer contamination (Table 1) was considered a more serious matter than other foods regarding contamination with carcinogens, especially in Germany, so the problem was solved with some haste. The highest concentrations of NDMA were found in 'Rauchbier' (dark or smoky beer), high in malt content, so the origin of the NDMA was traced to alkaloids, horde-

Table 1
Nitrosamines (NDMA) in beer

Country	Type of beer	Maximum concentration ($\mu\text{g l}^{-1}$)
Austria	Lager	10
Belgium	Strong lager	12
Canada	Beer	5
Czechoslovakia	Lager	2.5
Denmark	Lager	0.5
France	Lager	7
Germany	Lager	1
	Rauchbier	68
Sweden	Lager	1
Switzerland	Strong lager	9
United Kingdom	Strong pale ale	8
USA	Beer	8

nine and gramine (and perhaps others) present in barley and hence, in the malt. These alkaloids were derivatives of dimethylamine and were easily nitrosated, in this case, by nitrogen oxides as the nitrosating agent [41]. The nitrogen oxides were in the flue gases from the burning of the fuel used to heat the malt and allowed to come in contact with the latter. The solution to the problem was to sequester the malt from the gases, which was the reason some beers seemed to be free of NDMA (as was proclaimed in their advertising). Since that time, beers have had a greatly reduced nitrosamine content; before that time, beer in Germany and several other countries was a major source of exposure of people to carcinogenic nitrosamines, because of the very large quantities of beer that were drunk. One liter of beer containing 50 ppb of NDMA (by no means the highest concentration found), for example, provided 50 μg to the drinker, who might drink several liters a day. NDMA was also present in whiskies of various kinds, no doubt arising from the same source (barley malt), but at lower concentrations than in beer and posing a lower cancer risk, because whisky is consumed by most people in smaller amounts than beer. Although changes in beer manufacture, and increased regulation, have reduced the level of nitrosamines in beer to 5 ppb (which is generally 'accepted'), it must be remembered that 1 l of beer containing 5 ppb of NDMA provides 5 μg of NDMA to the drinker, who might well drink several liters in a day. On the other hand, someone who eats 100 g of bacon (a good serving) containing 10 ppb

of NDMA and 100 ppb of nitrosopyrrolidine would consume 1 μg of NDMA and 10 μg of NO-Pyrr.

Intake of nitrosamines from other foods are similar, but might be repeated daily among people who are fond of cured meats. Japanese people and other Eastern peoples often eat fish treated with nitrite and/or smoked, in which the levels of nitrosamines are often somewhat higher than in Western cured meats, and the intake of nitrosamines in these regions might be more than traces. In the case of Hong Kong and other coastal Chinese cities, it is tempting to believe that the high incidence of nasopharyngeal cancer in these societies is due to consumption of NDMA (and possibly other nitrosamines) in smoked fish, which is eaten in large quantities; in some experiments in rats and hamsters, NDMA has induced tumours of the nasal cavity. The nitrosopiperidine in some samples of cured meats (up to 50 or 250 ppb) almost certainly arises from nitrosation of piperine (and other piperidine derivatives) in the spices used to flavour the meats. These concentrations of nitrosamines in selected cured meats provide the consumers substantial exposure to these potent carcinogens.

In Table 2 is shown the types of food commonly containing nitrosamines and the highest concentration reported of each nitrosamine. This list is not comprehensive and many of the references in this review catalogue extensive results from analytical surveys of diets [8,27,28,42]. The diets of many countries and societies vary in their content of nitrosamines, many are low and others are relatively high, depending on the types of food which contain nitrosamines. Food has been surveyed for nitrosamines in only selected countries, including Kashmir, Thailand, China, Japan, as well as the United States, Canada and countries of Europe. The amounts consumed by individuals vary widely, so that average consumption has little meaning in relation to carcinogenic risk of individuals. For example, there are people (especially children, who might be particularly susceptible to carcinogens) who eat bacon, ham or sausages daily and there are others (including this author and vegetarians) who consume none, and everything in between. The consumption of nitrosamines by those who eat a lot of cured meats will almost certainly be much greater than by vegetarians (unless the latter drink vast amounts of

Table 2
Nitrosamines found in foods

Nitrosamine	Food and highest concentration recorded ($\mu\text{g kg}^{-1}$)	Relative carcinogenicity
<i>N</i> -Nitroso-Dimethylamine	beer (8), bacon (17), cheese (5), cured meats (22), sausage (12), Thai fish (25), smoked pickled fish (32), dried milk (4.5), broiled squid Japan (300), salted meat Russia (54), etc.	+++ +
Diethylamine	corn bread, seafood China (4.8), sausages (10), cheese (20)	+++ +
Pyrrolidine	fried bacon (100), sausages, China, Germany (45), broiled squid Japan (10), smoked meat (10), ham (36)	+++
Piperidine	bologna, sausages (50), spiced smoked meat (9), Chinese pickles (14), Thai fish (23), Thai pork (6), Tunis stew base (43)	+++
Methylbenzylamine	corn bread, China (> 100)	+++ +
Thiazolidine	smoked pork (5), sausage (5), smoked oysters (109), fried bacon (240), cured meats (27), smoked fish (2)	–
Proline	fried bacon (68), cured meats (400), smoked pork (2100), sausage (940), smoked oyster (167)	–
Thiazolidine-Carboxylic acid	fried bacon (14000), cured meats (3900), smoked fish (1600)	–

beer). I heard it said at one time that one quarter of the meat consumed in the USA is cured with nitrite, illustrating the magnitude of the problem, even though the amount of nitrite used in meat curing is now more firmly controlled than one or two decades ago. There have been estimates published of the average intake of nitrosamines by people in a particular country or region and these have been of the order of $1 \mu\text{g person}^{-1} \text{ day}^{-1}$. However, as discussed above, this value can be close to zero in many people and considerably greater in others who consume a lot of beer or cured meats.

The main carcinogenic *N*-nitroso compounds found in food throughout the world are NDMA, NDEA, nitrosopyrrolidine and nitrosopiperidine, with nitrosodi-*n*-butylamine occasionally (derived from the food's contact with rubber). This is reflected in surveys of foods from a variety of countries. However, there have been reports (not always verified) of some unusual nitrosamines in some foods from China, Japan and other oriental countries. These nitrosamines include nitroso-1-methyl-acetyl-3-methylbutylamine, methylnitrosobenzylamine and nitrosodibenzylamine [21]. Most 'food nitrosamines' gave rise to tumours of the liver or esophagus when fed to rats, but would induce different tumors in hamsters, which have never been reported to bear tumours in the esophagus.

In spite of much searching, particularly in connection with pediatric brain tumours [43], there have

been few reports of the occurrence of alkylnitrosamides, especially alkylnitrosoureas, in foods. In rats, methylnitrosourea and ethylnitrosourea (and higher homologues) given transplacentally to pregnant females have induced tumours of the nervous system (including brain) in a high proportion of the offspring [44]. The doses have often been quite small to produce this effect.

One reason for the failure to detect alkylnitrosoureas in food is probably their instability, especially in alkaline solution. Mende et al. [25] have inferred the presence of methylnitrosourea in some foods by an indirect method involving the measurement of methylation of nitroso-*t*-butylglycine by diazomethane released from the directly acting nitroso-alkylurea.

4. Quantitative considerations

The concentrations of *N*-nitroso compounds in foods are often tiny, and human exposure to them from this source is quite small, but the consequences to people are not to be ignored for two reasons. Firstly, because of the great carcinogenic potency of this group of carcinogens (in animal studies), particularly some of the simpler ones and, secondly, because nitrosamines might well be more effective (dose-for-dose) in humans than they are in experimental rodents, as they are more effective in rats

than they are in mice or hamsters. Evidence that this is so lies in a comparison of the dose of nitrosamines to a heavy cigarette smoker [42], 20 cigarettes day⁻¹ or approximately 4 µg nitrosamines or 0.06 µg day⁻¹ kg⁻¹ body weight—consisting largely of the rather weak carcinogen, nitrosornicotine, and the almost inactive nitrosoanatabine, together with approximately 20% (corresponding to 0.012 µg kg⁻¹ day⁻¹) of the potent carcinogen NNK (4-methyl-nitrosamino-3-pyridylbutanone). Hoffmann and Hecht [45] assume that the nitrosamines play a major role in the increased cancer risk of tobacco chewers and tobacco smokers; this dose is responsible for a 10% incidence of cancer in heavy smokers. In comparison, in the large scale dose-response study of the more potent NDMA and NDEA in male rats carried out two decades ago, the minimal effective dose (causing a 1–4% tumor incidence) was 40 µg day⁻¹ kg⁻¹ body weight [46]. Even considering that exposure of the rats lasted 2–3 years, while exposure of humans could last 40–50 years, the difference of more than three orders of magnitude in nitrosamine exposure between the experimental animals and smokers indicates that humans are more responsive to carcinogenic nitrosamines than are rats.

It has been difficult, so far, to relate ingestion of food containing *N*-nitroso compounds with a particular incidence of cancer. The finding that certain foods contained carcinogenic nitrosamines (albeit at low concentrations) led to the suspicion that they might be responsible for the high incidence of stomach cancer in many parts of the world and in some societies among them. It was a natural assumption that direct contact with these carcinogens in the stomach was responsible. However, good evidence has been lacking connecting the exposure with the cancer. This is understandable because nitrosamines are prime examples of systemic carcinogens that do not act locally and very few of those tested in animals have induced tumours of the glandular stomach, such as humans possess; some nitrosamines do induce tumours of the squamous or non-glandular stomach which is present in some rodents.

Correlation of other types of cancer with consumption of foods containing *N*-nitroso compounds has not been very successful, although there have been several epidemiological investigations of a connection between brain tumours in children and the

large amount of cured meats eaten by their mothers while they were pregnant. The epidemiological studies are complicated by the formation of *N*-nitroso compounds from ingested secondary and tertiary amines while nitrite (from food or formed endogenously [47] and present in saliva) is in the stomach [14]. This, of course, adds to the total exposure to carcinogenic *N*-nitroso compounds.

An exception to this failure to find an epidemiological correlation appears to be the high incidence of esophageal cancer in certain parts of China and in other parts of the world, where nitrosamines have been found in substantial concentrations in native foods [48].

5. The future control of nitrites

The focal point of attempts to reduce the concentration of *N*-nitroso compounds in foods is the control of nitrites (or other nitrosating agents) which can come into contact with nitrosatable amino compounds. There has been notable success in reducing the concentration of NDMA in beer by controlling the exposure of malt to nitrogen oxides, effectively stopping nitrosation of the alkaloids in the malt. There has also been some success in reducing the amount of nitrite used in meat curing, which is the source of most of the *N*-nitroso compounds in cured meats. Since the rate of formation of nitrosamines is proportional to the square of the nitrite concentration, reduction of the latter has an enlarged effect in reducing the amount of nitrosamine formed. The reduction in nitrite and the virtual elimination of nitrate has been both by voluntary action by manufacturers and, in many countries, by government regulation. Among the regulations has been the reduction of nitrite permitted in bacon from 200 to 125 ppm and the elimination of nitrite from certain types of sausage (in Norway), as well as a reduction in the use of nitrite in fish. These controls have led, over the past 20 years, not only to a reduction in the nitrosamine content of many common cured meats, but also to a lessened exposure to nitrite which participates in endogenous formation of *N*-nitroso compounds. From the early 1970s, there was a gradual reduction of the amount of nitrosopyrrolidine in fried bacon from above 100 ppb to approximately 30

ppb or less in the late 1970s, but more recently, there has been an increase to as much as 65 ppb of NO-PYR, up to 110 ppb of all volatile nitrosamines in fried bacon and 85 ppb in the fried-out fat [49]. There is less evidence that there is action to reduce human exposure to *N*-nitroso compounds in fish, smoked or nitrite-cured, in those countries in which fish is a large part of the diet (Japan, China, Russia, etc.).

However, it is to be hoped that such action will be taken voluntarily, since governments seem to have been rather immobile on this issue, although information about the possible carcinogenic risk has been available for decades. This action will surely lower exposure of human populations to the broadly acting carcinogenic *N*-nitroso compounds and thereby reduce the risk of cancer to these people.

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