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Review

Formation and Metabolism of N-Nitrosamines

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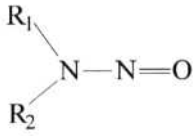
Abstract

The formation of nitrosamines and reactions of the first and second phase of their biotransformations were reviewed.

Keywords: nitrosamines, cytochrome P-450, xenobiotics, glucuronides

Introduction

Nitrosamines are compounds having the general structure:



where R| and R_2 are alkyl or aryl groups.

Nitrosamines are present in water, soil and air. They can be found contaminating food, feeding stuff (where they create the highest risk for health), drugs, cosmetics, and pesticides [1-4]. Nitrosamines are absorbed by skin, airways and the alimentary tract [5]. There is evidence that nitrosocompounds may be generated in vivo from nitrites or nitrates and primary, secondary and tertiary amines in organs of people who apparently were not exposed to these compounds [6-8].

Formation of Nitrosamines

N-nitrosamines are formed in the reaction of an electrophilic substitution of organic nitrogen with a nitrosating compound. Organic nitrogen is derived from I, **II** or **III** amines, hydroxylamine or amine peroxides, which are products of transformation of fertilizers and phenoxyacetic herbicides [9, 10].

A nitrosating agent (N_2O_3), can be formed from:

1) nitrites (nitrates III) - NO_2^-,
2) nitrates (nitrates V) - NO_3^-,
3) nitrocompounds - C - NO_2^-

In the case of amines, nitrosonium cation (NO^+) [11] derived from nitrogen trioxide (nitrogen **III** oxide) attacks a pair of electrons on the amine nitrogen, constituting nitrosoammonium cation and nitrite anion (Fig. 1 .a). Further reactions of the nitrosoammonium cation depends on its chemical structure and properties of nitrosocompound and conditions of the reaction. Nitrosoammonium cations, initially derived from primary amines, are subject to rearrangement by the transfer of two protons from amine nitrogen to oxygen (Fig. l.b) and elimination of water (Fig. l.c), producing a diazonium cation (Fig. 1 .d). Aliphatic diazocations proceed with the elimination of nitrogen and constitution appropriate carbonium ion (Fig. l.e). Desintegration is strongly exoenergetic on account of the high energy of bonds in the molecule of N_2.

Transformation of nitrosoammonium cations derived from secondary amines (Fig. 2) depends on the detaching of protons and production of N-dialkylnitrosoamines.

Transformation of nitrosoammonium cations derived from tertiary amines in low temperatures proceeds slowly with the production of aldehyde and secondary amines (Fig. 3) [5, 9, 10].

The formation of nitrosamines depends on the pH environment, alkalinity of amine, and temperature [8, 12, 13]. Primary aliphatic and aromatic amines at low pH and low temperature do not form nitrosocompounds, and reaction with nitrite proceeds by diazonium salts. The rate of formation of N-nitrosocompounds from secondary amines increases proportionally with a decrease in alkalinity of the amines. Tertiary aliphatic amines do not react with nitrous **III** oxide in strong acidic pH; however, increases in pH and temperature favour the process of

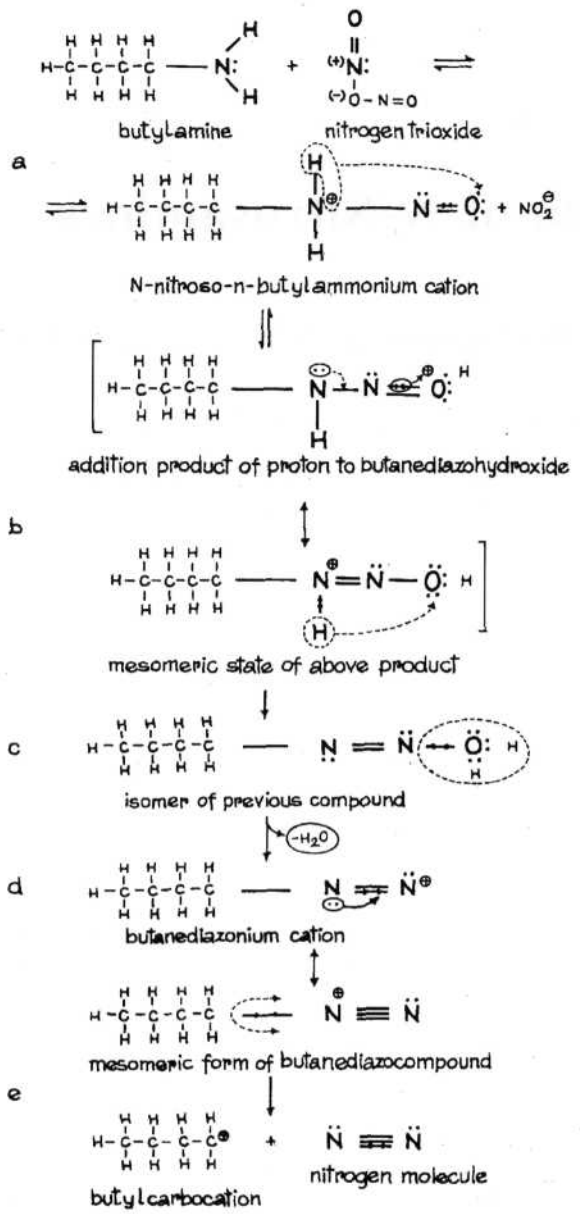


Fig. 1. Formation of nitrosamines from primary amines and their transformations [9, 19] (modified).

nitrosation. Tertiary aromatic amines undergo C-nitrosation at the para position [14].

Autotrophic and heterotrophic nitrification and denitrification bacterial biochemical processes can perform the formation of the nitrosamines, by numerous microorganisms such as: *Arthrobacter, Bacillus, Pseudomonas, Mycobacterium, Nocardia, Streptomyces* and soil fungi such as *Aspergillus, Fusarium, Penicillium, Candida, Cephalosporium* and many others [1]. One believed that microorganisms reduce nitrate to nitrite, degrade proteins to secondary amines and create an appropriate environment (slightly acidic) [3, 13, 15]. Archer *et al.* [16] suggested that hydrophobic interactions between hydrocarbon radicals of alkylamines with lipid (hydrophobic) compound of cell localized mainly in bacterial cell wall, increase the rate of formation of nitrosamines by releasing an electron pair of amine nitrogen. Presumably, cation surfaces of bacterial walls attract nitrate III anions and create suitable high con-

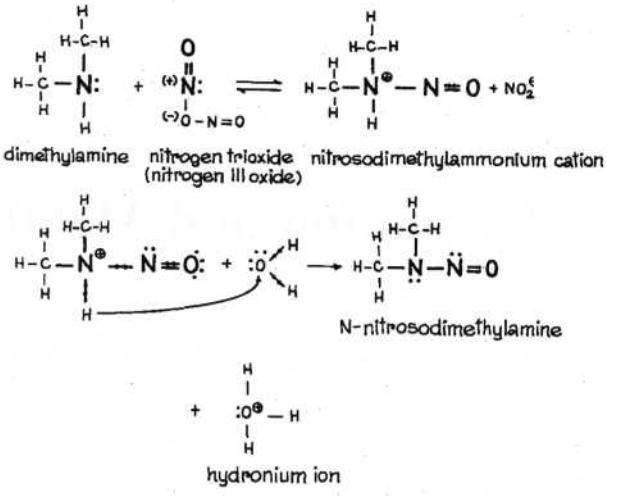


Fig. 2. Formation of nitrosamines from secondary amines [9, 19] (modified).

centrations of the nitrosating agent in the presence of amines [8].

Biotransformation of N-Nitrosamines

Nitrosamines are comparatively stable under conditions present in organisms, before being subjected to degradation to biologicallly active derivatives [17-19], Nitrosamines reach the liver by the blood stream. In the liver microsomes, enzymes are present which are responsible for reactions of the first and second phase of biotransformation of nitrosocompounds. The main purpose of both phases of biotransformation of exogenic compounds, among them xenobiotics, is to increase their solubility in water (polarity), which facilitates their excretion from the organism. In the first

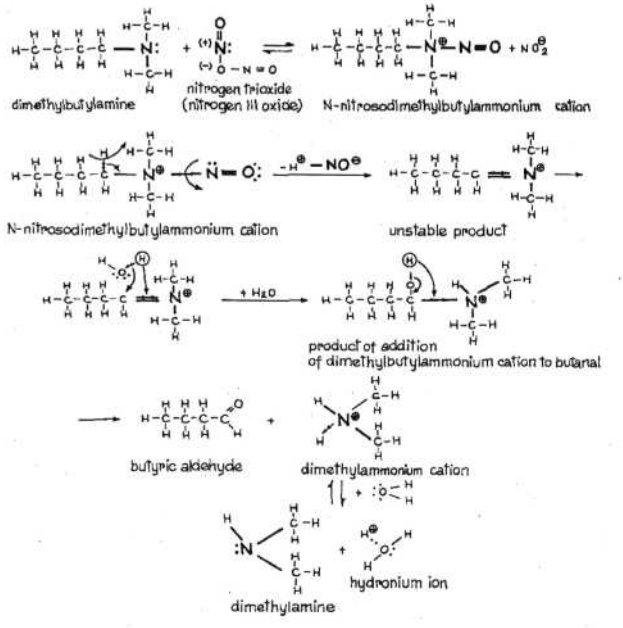
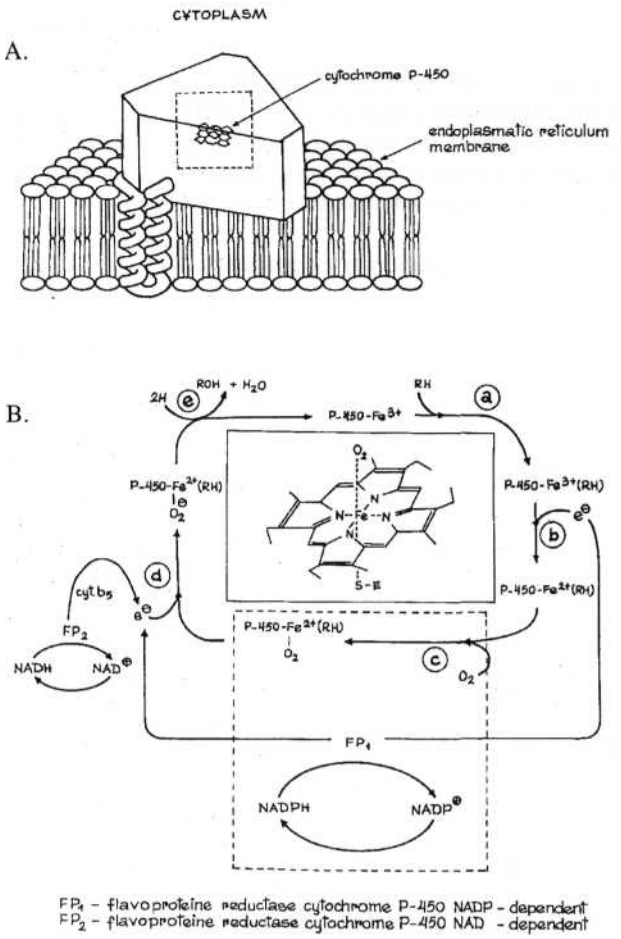


Fig. 3. Formation of nitrosamines from tertiary amines and their transformation [9, 10, 19] (modified).



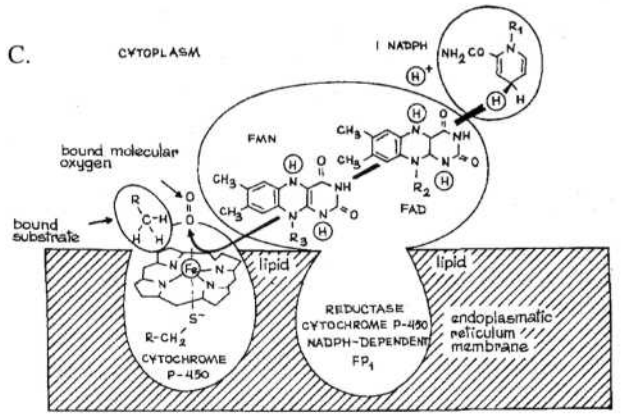


Fig. 4. Mechanism of action of cytochrome P-450 [25] (modified):
A. Localization of cytochrome P-450 in membrane of endoplas mic reticulum;
B. Action of cytochrome P-450;
C. Mechanism of electrone transfer with participation of reductase cytochrome P-450 NADP dependent.

phase of biotransformation of nitrosamines, hydroxylation and dealkylation are the main reactions [8]. In the second phase, the products of the first phase undergo transformation to polar metabolites by the action of specific enzymes which conjugate them with glucuronic or sulfuric acids or aminoacids or glutathione [1, 7]. Enzymes of the first and second phases of biotransformations were detected not only

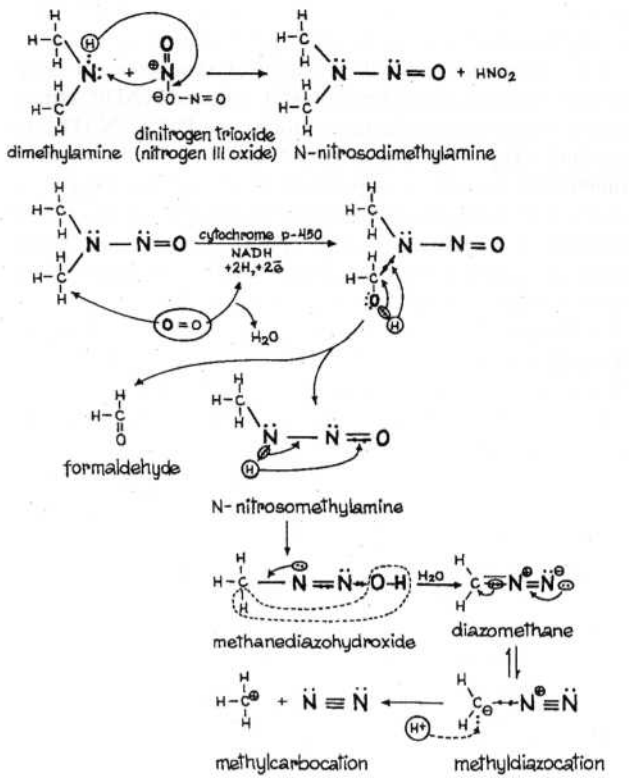


Fig. 5. Biodegradation of N-nitrosodimethylamine [20, 26] (modified).

in liver but also in the intestine, kidney, lungs, brain, skin and placenta [7, 19].

Biotransformation reactions are catalyzed by microsomal enzymes dependent on cytochrome P-450 (Fig. 4.1), i.e. a set of hemoproteins catalyzing the activation of molecular oxygen and transfer of oxygen to lipophilic molecule of the xenobiotic [21-24] (Fig. 4.1). Mechanism of cytochrome P-450 action is presented in Fig. 4.2.

Xenobiotics enter the cycle and combine with oxidized cytochrome P-450 with iron prostetic group remaining on +3 step of oxidation (Fig. 4.2.a). Cytochrome P-450 reductase (NADP-dependent) reduced complex xenobiotic cytochrome P-450 (Fig. 4.2.b). It allows association of the oxygen molecule (Fig. 4.2.c). The complex with an associated oxygen molecule accepts successive electron from cytochrome b_5 (Fig. 4.2.d) and desintegrate to hydroxylated

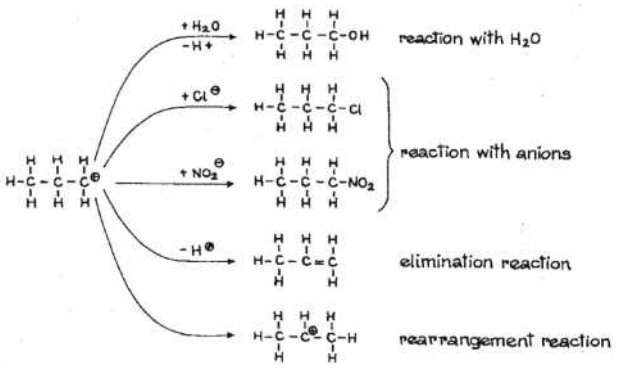


Fig. 6. Posible reaction of carbocation [9, 10] (modified).

product (R-OH) and water (Fig. 4.2.e). Cytochrome P-450 is regenerated (oxidized) and cycle returns to its initial status. Therefore for hydroxylation of xenobiotics it is necessary to have cytochrome P-450, reduced NADP, atmospheric oxygen and reductase cytochrome P-450 (NADP-dependent) (Fig. 4.3). The effect of action of the entire set of mentioned factors is activation of molecular oxygen in such a way as to allow utilization of one of its atoms for the creation of a hydroxyl group. The acceptor for the second atom of oxygen is hydrogen from NADP, which binds oxygen to create a molecule of water [4, 5, 20].

Efficiency of microsomal chain of electron transport depends mainly on the rate of regeneration of reduced NADP. It regeneration proceeds mainly by reaction of oxidation of glucose-6-phosphate to 6-phosphogluconic acid in the pentose cycle [19].

The results of investigation of the metabolism of dialkylnitrosamines (i.e. N-nitrosodimethylamine) suggest the formation of monoalkylnitrosoamine in the first phase of biotransformation as a transition product with simultaneous degradation to alkylating compounds (i.e. diazoalkane or carbonium ion) [19]. A scheme of biodegradation of nitrosamines using the example of N-nitrosodimethylamine is shown in Fig. 5.

The carbocation generated during biotransformations of nitrosamines is very reactive and, depending on conditions, is the subject of further reactions leading to the formation of different products (Fig. 6) [9].

As the effect of possible reactions of carbocation, one can obtain relatively complex mixture of products of nitrosamine metabolism. Some metabolites of nitrosamines generated in the first phase of biotransformation have a preserved nitroso group and in this form are conjugated with glucuronic acid, sulfuric acid or glycine and excreted with urine [14, 19]. Fig. 7 presents a scheme of glucuronides formation as an example of conjugation proceeded in the reactions of the second phase.

The reactions of the second phase of biotransformations of N-nitrosocompounds are characterized by the stereospecific attack of an electron pair of the xenobiotic on the carbocation of the conjugating factor. Synthesis of glucuronides is catalyzed by glucuronyl transferase localized in the endoplasmatic reticulum. Glucuronic acid is produced from uridine diphosphate glucuronic acid, which is produced from UDP-glucose, by action of UDP-glucose dehydrogenase. During conjugation of xenobiotic with glucuronic acid configuration of Q in glucuronic acid changes from a- to β-configuration [25].

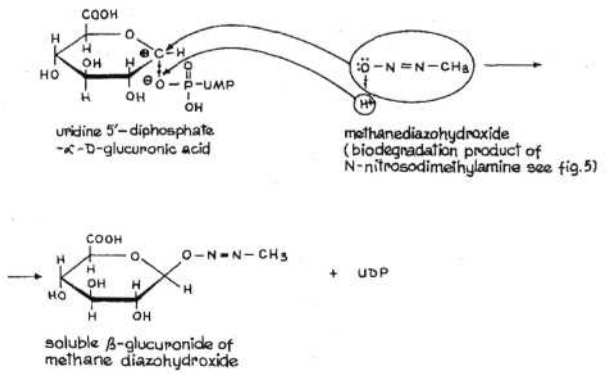


Fig. 7. Nitrosamine glucuronides [25],

Nitrosamines are excreted partially in urine and exhaled in air. Remaining nitrosamines are degraded to carbon dioxide by active intermediates [14]. Active intermediates are very important from the toxicological point of view as many of them have carcinogenic activity.

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