Peroxidative Metabolism of Carcinogenic N-Arylhydroxamic Acids: Implications for Tumorigenesis

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Peroxidative oxidations of chemical carcinogens including N-substituted aryl compounds could result in their metabolic activation because the products react with cellular molecules and lead to cytotoxicity, mutagenicity, and carcinogenicity. In vivo, peroxidative activities are chiefly of neutrophilic leukocyte origin. Neutrophils may be attracted to the sites of exposure to carcinogen and, via phagocytosis and respiratory burst, release oxidants that catalyze carcinogetic activation and/or cause DNA damage. Our studies, presented herein, concern oxidations of carcinogenic N-arylhydroxamic acids, N-hydroxy-N-2-fluorenlyacetamide (N-OH-2-FAA), and N-hydroxy-N-2-fluorenylbenzamide (N-OH-2-FBA), by enzymatic and chemical systems simulating those of neutrophils, myeloperoxidase and hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) ± halide, and hypohalous acid and halide at the physiologic concentrations (0.1 M Cl\textsuperscript{-} and/or 0.1 mM Br\textsuperscript{-}) and the pH (4–6.5) of phagocytosis. Studies also concern oxidations of the hydroxamic acids by rat peritoneal neutrophils stimulated to undergo respiratory burst and release myeloperoxidase in medium-containing 0.14 M Cl\textsuperscript{-} ± 0.1 mM Br\textsuperscript{-}. The metabolites formed in the presence of exogenous H\textsubscript{2}O\textsubscript{2} are consistent with two peroxidative mechanisms: one electron–oxidation to a radical that dismutates to equimolar 2-nitrosofluorene (2-NOF) and the ester of the respective hydroxamic acid and halide-dependent oxidative cleavage, especially efficient in the presence of Br\textsuperscript{-} to equimolar 2-NOF and the respective acyl moiety. 2-NOF and the esters undergo further enzymatic and nonenzymatic conversions to unreactive products and/or may bind to cellular macromolecules. The results suggest that peroxidative metabolism of N-arylhydroxamic acids by neutrophils, yielding the potent direct mutagen 2-NOF and the electrophilic esters, occurs \textit{in vivo} and is involved in the activation and thus local tumorigenicities of the hydroxamic acids at the site(s) of application. — Environ Health Perspect 102(Suppl 6):75–81 (1994)

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\textbf{Peroxidase-dependent Metabolism of \textit{N} \textit{Aryl}amines: Significance of Oxidizing Systems of Leukocyte Origin}

Because extravascular tissues are frequent targets of chemical induction of cancer and have low levels of cytochrome P450 monoxygenases, alternative systems involved in metabolic activation of carcinogens have been sought. Marnett et al. (1) discovered an enzyme system, prostaglandin H synthase (PHS), containing cyclooxygenase and peroxidase activities that effected cooxidation of xenobiotics during oxidation of arachidonic acid to prostaglandins. Oxidations of these compounds, termed cosubstrates, were catalyzed by the peroxidase activity of PHS. Investigations from several laboratories on activation of carcinogenic \textit{N}-arylamines via PHS-mediated oxidations have recently been reviewed (2,3). PHS catalyzed one electron (1e\textsuperscript{-}) oxidation of \textit{N}-2-fluorenamine (2-FA) to nitrogen-centered free radicals, which yielded dimeric and polymeric products and 2-nitrofluorene (2-NO\textsubscript{2}F). However, the pathway to 2-NO\textsubscript{2}F still remains obscure because its expected precursors, \textit{N}-hydroxy-2-FA and 2-nitrosofluorene (2-NOF), were not detected among the products (4). The oxidation of 2-FA by PHS yielded two, as yet uncharacterized, adducts with DNA (5) that were different from \textit{N}-deoxyguanosin-7,8-yl)-BZ (6, 7). This adduct may be oxidized in vivo to \textit{N},\textit{N}-3-(deoxyguanosin-7,8-yl)-BZ. Adducts consistent with PHS-mediated oxidation of BZ, 2-naphthylamine, and 2-FA were found in dog bladder epithelia, and may be involved in bladder cancer induction (8). In addition to ubiquitous PHS, peroxidative activities in human or animal tissues also may be derived from endogenous peroxidase in various cells such as glandular acinar cells, epithelial and endothelial cells, and leukocytes (10). Neutrophils, which constitute 95% of polymorphonuclear leukocytes (PMNL), contain myeloperoxidase (MPO) (11). MPO is also found in monocytes or mononuclear phagocytes (MNL). Eosinophils (3.8% of total PMNL) and, presumably, mast cells contain eosinophil peroxidase. In various tissue eosinophilias, eosinophils may be the predominant infiltrating leukocytes (12) Their peroxidase activity per cell is approximately 2.5-fold greater than that of neutrophils. During phagocytosis, the leukocytes undergo degranulation, releasing peroxidase, and a respiratory burst generating superoxide anion (O\textsubscript{2}\textsuperscript{-}) from the reduction of O\textsubscript{2} by NAPD oxidase (13). O\textsubscript{2} is, in turn, converted to hydrogen peroxide.
MPO-dependent oxidations were implicated in activation of N-arylalines to DNA-bound products by PMNL (Table 1). In these experiments, incubations of 14C-labeled BZ, dimethylaminobenzene, 2-FA, or acetaminophen with phorbol myristate acetate (PMA)-treated guinea pig or human PMNL (15–17) or neutrophils of human leukemic cell line origin (18,19) resulted in covalent binding of radioactivity to leukocytic DNA. The release of MPO activity apparently was accomplished by treatment of leukocytes with PMA in buffers containing Ca** or solubilization with a cationic detergent. The leukocyte oxidizing system was simulated by MPO and H2O2/Cl–, which yielded covalently bound radioactivity to exogenous DNA (15–17). When 2-FA was reacted with human neutrophil lysates supplemented with H2O2, a single DNA adduct, N-(deoxyguanosin-8-yl)-2-FA, was detected by 32P-postlabeling (20). Thus, even though the mechanism(s) of oxidation leading to the electrophile reacting with DNA is unknown, MPO-dependent metabolism of carcinogenic N-arylalines leads to their activation and a DNA adduct associated with initiation of carcinogenesis. The leukocytes, both PMNL and MNL, also were used in studies of metabolism of drugs containing free –NH2 in para position to other substituents (Table 1) (21–23). The –NH2 group was oxidized to the hydroxylamine (detected only in the presence of ascorbic acid) and then to the nitro-compound. However, the presumed intermediary nitroso compound was not detected from any of the drugs investigated. Simulations of these oxidations with MPO and H2O2/Cl– yielded the same metabolites as above and also N-chloroamines that rearranged to o-chloro-compounds (24). The MPO-derived oxidation products from drugs containing the free –NH2 group have been implicated in adverse effects such as agranulocytosis and generalized hypersensitivity reactions during drug therapy (25). The detection of the hydroxylaminoderivatives from these drugs may be the result of peroxxygenation, the mechanism recently elucidated for chloroperoxidase-catalyzed N-oxidation of p-substituted N-arylamines (26). It is thus becoming increasingly evident that the structure of N-arylamine and the type of the substituent on the aromatic ring are factors in determining the oxidation products. The latter may also depend on the type of peroxidase present and the environment in a particular tissue.

The above evidence linking leukocytic peroxidative systems to activation of N-arylamines suggested that these systems also might be involved in the activation of the carcinogenic N-aryloxidamic acids. Thus, we investigated the peroxidative metabolism of N-hydroxy-N-2-fluorenylacemide (N-OH-2-FAA) and N-hydroxy-N-2-fluorenylbenzamide (N-OH-2-FBA) (Figure 1).

### Table 1. Metabolism of carcinogens and drugs containing –NH2 group by oxidizing systems of leukocytes.

| Compound | Oxidizing system* | Metabolite | References |
|----------|-------------------|------------|------------|
| 14C-Benzidine | PMNL + PMA or | 14C covalently bound to leukocytic or exogenous DNA | (15) |
| 14C-Methylaminobenzenzene | PMNL granules or | | |
| 14C-N2-Fluorenamine | MPO/H2O2/Cl–+ DNA | | |
| 14C-N2-Fluorenamine | PMNL + PMA or | 14C covalently bound to leukocytic or exogenous DNA | (16–19) |
| 14C-Acetaminophen | MPO/H2O2/Cl– + DNA or | | |
| N2-Fluorene | PMNL lyase + H2O2+ | N-deoxyguanosin-8-yl-2-fluorenamine* | (20) |
| 4-Amino-N-(2-diethyaminoethyl)benzamide (Procainamide) | PMNL or MNL + PMA | 4-hydroxylamo- and 4-nitro-derivatives | (21,22) |
| 4,4-Diaminodiphenylsulfone (Dapson) | | | |
| 4-Amino-N(3-methyl-5-isoxazolyl)benzenesulfonamide (Sulfamethoxazole) | PMNL or MNL + PMA | 4-hydroxylamo- and 4-nitro-derivatives | (23) |

Abbreviations: PMA, phorbol myristate acetate; PMNL, polymorphonuclear leukocytes (guinea pig peritoneal); MPO, myeloperoxidase; MNL, mononuclear leukocytes (monocytes) (human blood). *In buffers containing 0.1 to 0.15 M NaCl and 0.05 mM CaCl2 or complete Hank’s balanced salt solution, pH 7 to 7.4. **Solubilized with cetyltrimethylammonium bromide. From human blood. Derived from human leukemic cell line (HL-60). Isolated by 32P-postlabeling. From human and canine blood.

(H2O2) through spontaneous or superoxide dismutase-catalyzed dismutation. Using H2O2, MPO and eosinophil peroxidase catalyze oxidation of halides, preferentially chloride (Cl–) and bromide (Br–), respectively, to generate the powerful oxidants hypohalous and hypobromic acids (HOCl and HOBr) (13,14). The hypohalous acids are capable of halogenation of endogenous amines such as taurine to N-chloro- and N-bromotaurine, which are more stable oxidants.
of carcinogen deposits may be involved in their oxidations and yield products relevant to their local carcinogenicities.

To investigate this possibility, we first examined oxidations of the hydroxamic acids by chemical and enzymatic oxidants simulating those generated by leukocytes. Two peroxidative pathways in oxidation of the hydroxamic acids were considered (Figure 1): 1e– oxidation to nitroxyl free radicals, which dismutate to equimolar C-nitroso compound and the ester of the hydroxamic acid, the reaction initially reported by Bartsch et al. (28–30) for chemical and enzymatic oxidations of N-arylacetoxyhydroxamic acids; and hypohalous acid-catalyzed oxidative cleavage of the hydroxamic acid to a C-nitroso compound and the respective acid, the reaction that we reported for halide-dependent enzymatic oxidations of N-OH-2-FBA (31). Our earlier investigation also showed that enzymatically or chemically generated 

\[ \text{HOCl, HOBr, or N-bromotaurine} \]

but not N-chlorotaurine, were capable of oxidation of N-OH-2-FBA via both 1e– and oxidative cleavage (32). Accordingly, N-OH-2-FBA would undergo 1e– oxidation to a nitroxyl free radical that would dismutate to 2-NOF and the ester \( \text{N-benzyloxy-2-FBA} \) (N-BzO-2-FBA) or halide-dependent oxidative cleavage or both to 2-NOF and benzoic acid (BA). Although evidence for

\[ \text{1e– oxidation of N-OH-2-FBA by alkaline} \]

\[ \text{K}_2\text{Fe(CN)}_6 \]

in benzene was reported by Bartsch et al. (28), the presumed N-BzO-2-FBA was not quantified, and 2-NOF accounted for only 14%, rather than the theoretical 50%, of the substrate. Hence, we reexamined the oxidation of N-OH-2-FBA by \( \text{K}_2\text{Fe(CN)}_6 \) and confirmed the presence of a nitroxyl free radical by electron spin resonance (ESR) spectroscopy (Figure 2). However, the area and maximum amplitude of the signal produced within 1 min from N-OH-2-FBA were approximately 50 and 60%, respectively, of those from N-OH-2-FAA, indicating that N-OH-2-FBA was more slowly oxidized. Separation of the radical dismutation products by HPLC and estimation of their amounts in the oxidation mixtures based on the synthesized standards (33) showed that 2-NOF and the ester accounted for 44 and 40% of the substrate, respectively. This result confirmed the originally postulated mechanism of 1e– oxidation of N-OH-2-FBA by a chemical oxidant in nonaqueous media (28).

In our attempts to examine enzymatic oxidations of N-OH-2-FBA, we encountered problems related to the poor solubility of the hydroxamic acid and instability of N-BzO-2-FBA, which, in part, underwent \textit{ortho} rearrangement (Figure 1) (33). Therefore, our estimates of the ester formed from N-OH-2-FBA include both N- and o-BzO-2-FBA. Comparison of 1e– oxidation of N-OH-2-FAA and N-OH-2-FBA by horseradish peroxidase [HRP]/\( \text{H}_2\text{O}_2 \) at pH 7.0 showed that the nitroxyl-free radical signal was easily detectable from N-OH-2-FAA, but barely detectable from N-OH-2-FBA. The relative magnitudes of the radical signals from the hydroxamic acids corresponded to the levels of the dismutation products formed.
under similar conditions (Figure 3). Whereas 2-NOF and N-AcO-2-FAA accounted for 41 and 29%, respectively, of N-OH-2-FAA, the respective products 2-NOF and N-BzO-2-FBA accounted for only 5 and 2.2% of N-OH-2-FBA and indicated that N-OH-2-FBA was much less susceptible to 1e⁻-oxidation in an aqueous medium or was inhibitory to the enzyme. Because decreasing the concentration of N-OH-2-FBA from 30 to 10 μM only slightly decreased the amounts of the products (Figure 3), it appears that the lower concentration was near saturating for the enzyme.

We also compared oxidations of the two hydroxamic acids by MPO and H₂O₂ ± halide under conditions optimal for product formation from N-OH-2-FAA (Figure 4). At pH 6.5, the presence of halide did not affect the amounts of 2-NOF formed from N-OH-2-FAA. However, in the absence of halide, the amounts of N-AcO-2-FAA were greater, indicating increased level of 1e⁻-oxidation. Under these conditions, the presence of halide increased the amounts of 2-NOF formed from N-OH-2-FBA but had no effect on the small amounts of its ester. A similar product profile was determined from N-OH-2-FBA oxidized by MPO and H₂O₂/halide at pH 4 and 5 and suggested that the major source of 2-NOF was halide-dependent oxidation of N-OH-2-FBA. However, N-OH-2-FBA yielded approximately 10 times less 2-NOF than did N-OH-2-FAA, which was nearly completely converted to 2-NOF. In the presence of the less easily oxidized N-OH-2-FBA, H₂O₂ may have led to inactivation of MPO or destroyed HOB₃, the product of oxidation of Br⁻ by MPO and H₂O₂. As with HRP and H₂O₂, oxidations of N-OH-2-FAA at 30 or 10 μM by MPO and H₂O₂ ± halide yielded similar amounts of the products, suggesting saturation or inhibition of MPO or both.

Oxidations of the hydroxamic acids by hypohalous acids also were compared (Table 2). More 2-NOF, the major product, was produced from both compounds by equimolar HOB₃ at pH 4.0 than by excess HOCl at pH 5.0, reflecting the greater oxidizing potential of HOB₃. The determination of small amounts of the esters from oxidations of the hydroxamic acids by hypohalous acids indicated relatively low levels of 1e⁻-oxidation. The ESR signal of nitrosoyl-free radical, indicative of 1e⁻-oxidation, was detected only during oxidation of N-OH-2-FAA by HOCl (Figure 2). With the controlled rate of oxidation of N-OH-2-FAA by HRP and H₂O₂, a detectable level of radical was maintained while HOCl-catalyzed radical generation was rapid and brief. With the more powerful oxidant HOB₃, no radical signal from N-OH-2-FAA and only a faint signal from N-OH-2-FBA were detected. Based on the relative amplitudes of the radical signals and the amounts of the esters, excessive amounts of 2-NOF were produced from hypohalous acid-catalyzed oxidations (Table 2). This supported our original proposal that the major source of 2-NOF from the oxidations of the hydroxamic acid by HOCl or HOB₃ is via oxidative cleavage (32). Likewise, the determinations of acetate (31) and BA (Table 2) from these oxidations supported oxidative cleavage of the hydroxamic acid. BA also was found from MPO and H₂O₂ and Br⁻-catalyzed oxidation of N-OH-2-FBA (33), which supported oxidative cleavage as the major reaction for enzymatically generated HOB₃. We also found that the esters did not contribute to 2-NOF formation. 2-NOF occasionally yielded small amounts of 2-NO₂F.

The elucidation of the conditions for enzymatic or chemical oxidations of the N-arylhdroxamic acids, by oxidants simulating those of leukocyte origin, prompted our investigation of metabolism of N-OH-2-FAA and N-OH-2-FBA by rat PMNL in vitro.

**Peroxidative Metabolism of Carcinogenic N-Arylhydroxamic Acids by Rat Peritoneal Neutrophils in Vitro**

The carcinogenicities for rat peritoneum of N-OH-2-FAA, and especially N-OH-2-FBA, administered i.p. in aqueous suspensions (27), suggested influx of leukocytes in response to the foreign compound deposits. Hence, neutrophils, the predominant leukocytes, were elicited into rat peritoneum through i.p. injections of proteose peptone and harvested within 4 hr for
studies of metabolism of N-OH-2-FAA and N-OH-2-FBA. Neutrophils were suspended in Hank's balanced salt solution (HBSS) (without Ca\(^{2+}\) and Mg\(^{2+}\)), pH 7.2. In the absence of Ca\(^{2+}\), azurophil granules containing MPO remained intact when the cells were treated with PMA to effect the respiratory burst. Addition of a cationic detergent, cetyltrimethylammonium chloride (Cetac) at 0.002% or 0.02%, prompted loss of granule structure. These microscopic changes, observed with the stained cell preparations, were reflected in the spectrum of the oxidants produced by neutrophils (Figure 5). Thus, treatment with PMA in the absence of Ca\(^{2+}\) effected the respiratory burst as shown by O\(_2\) and H\(_2\)O\(_2\) production. Some hypohalous acid but no MPO activity was detected. Addition of Cetac caused immediate release of MPO activity, and the level of the activity was higher at the higher concentration of Cetac.

Metabolism of N-OH-2-FAA by neutrophils was determined before and after release of MPO (Figure 6). Because Cl\(^-\) or Br\(^-\) or both had a significant effect on the formation of 2-NOF by MPO and H\(_2\)O\(_2\) (Figure 4), we examined metabolism of N-OH-2-FAA by neutrophils in HBSS (Figure 6A) and HBSS + 0.1 mM Br\(^-\) (Figure 6B). Both the substrate depletion and metabolite generation were monitored. In these experiments, N-OH-2-FAA was added 30 min after initiation of respiratory burst by PMA. However, no substrate depletion or metabolite formation occurred in the presence of O\(_2\) or H\(_2\)O\(_2\) or both, the products of the respiratory burst. Release of MPO activity by 0.002% Cetac and addition of 50 \(\mu\)M H\(_2\)O\(_2\) initiated metabolism which was augmented by the addition of the second aliquot of H\(_2\)O\(_2\) (no metabolism was detectable in the absence of H\(_2\)O\(_2\)). The metabolites included N-AcO-2-FAA, 2-NOF, 2-FAA, and 2-NOF. The amounts of 2-NOF, and especially 2-NOF, were significantly greater in the presence of Br\(^-\). These data were consistent with our previous findings that N-OH-2-FAA was oxidized chiefly to 2-NOF by MPO and H\(_2\)O\(_2\) in the presence of Br\(^-\) or Cl\(^-\)+Br\(^-\) (32,33). The increased level of oxidation of N-OH-2-FAA was likely because of the formation of Br\(^-\)-derived oxidants. However, Br\(^-\) had no apparent effect on the level of 1e\(-\) oxidation because the amounts of N-AcO-2-FAA and 2-FAA were similar.

We also have investigated the fate of 2-NOF and N-AcO-2-FAA because their reactivities in cellular systems might affect the amounts detected in metabolism studies. The recovery of 2-NOF was only 22% and decreased with time, especially in active cells (34). Formation of small amounts of 2-NOF that was H\(_2\)O\(_2\)-dependent and both enzymatic and nonenzymatic, could not account for all the losses of 2-NOF. The low recoveries of the latter were likely because of reactivity with cellular components such as glutathione or protein binding or both (27). The recovery of N-AcO-2-FAA decreased from 75 to 8% in 24 min (34). In the absence of H\(_2\)O\(_2\), 73% of the ester was recovered as 4-OH-2-FAA (Figure 1). Similarly, large amounts of 4-OH-2-FAA were formed from N-AcO-2-FAA incubated with heat-inactivated cell lysates or in HBSS. The data were consistent with the nonenzymatic conversion of N-AcO-2-FAA to 4-OH-2-FAA in buffers containing 0.14 M Cl\(^-\), reported by Scribner (35). Addition of H\(_2\)O\(_2\) disrupted the time-dependent increase in the formation of 4-OH-2-FAA, which probably was oxidized to a phenoxy radical. However, no 4-
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Figure 7. Deacetylation of N-AcO-2-FAA or N-OH-2-FAA by rat peritoneal neutrophils. Cells suspended in Hank's balanced salt solution containing 0.1 mM Br- and 10 nM PMA (10^6 cells/ml) were incubated at 37°C for 30 min before the addition of 0.002% Cetac with (—) or without (— —) 0.1 mM paraoxon. After 15 min, N-AcO-2-FAA (■) or N-OH-2-FAA (△) at 10 μM was added (0 point on the graph) and incubation continued for 24 min. Aliquots (0.9 ml) of the incubation mixtures were analyzed for unreacted compounds and metabolites as described previously (34). Values are the mean ± SD from two experiments, each carried out in duplicate. ▲ denotes N-OH-2-FAA formed from N-AcO-2-FAA.

OH-2-FAA was detected in the peroxidative metabolism of N-OH-2-FAA (Figure 6), probably because the levels of N-AcO-2-FAA were too low for its conversion to detectable 4-OH-2-FAA. Small amounts of N-AcO-2-FAA were found to be O-deacetylated to NOH-2-FAA, especially in the absence of H_2O_2 (Figure 7). O-Deacetylation was inhibited by paraoxon. The data were indicative of O-esterase activity in the neutrophils, which might lead to regeneration of the substrate from the product N-AcO-2-FAA (Figure 1). On the other hand, we found no evidence for N-deacetylase activity (Figure 7) that would catalyze formation of N-hydroxy-2-FAA from N-OH-2-FAA and thus lead to 2-NOF via oxidation of the N-hydroxy-2-FAA.

Metabolism of N-OH-2-FAA by rat peritoneal neutrophils was examined under somewhat modified conditions (Figure 8). Because of its low solubility, N-OH-2-FAA was used at 10 μM. During the 30-min incubation no metabolism took place. After addition of Cetac (0.02%), negligible amounts of O2-2-FBA and 2-NOF, and small amounts of 2-FBA and 2-NOF were found, suggesting the presence of low levels of H_2O_2. The data were consistent with nearly quantitative recovery of the unchanged substrate (Figure 8) that was more efficiently extracted in the presence of the detergent. Additions of H_2O_2 significantly increased consumption of the substrate and formation of metabolites, especially of 2-FBA and 2-NOF. The latter was formed, in part, from 2-NOF because in a separate experiment, approximately 15% of the added 2-NOF was found to be metabolized to 2-NO_F in the presence of H_2O_2.

Comparison of the metabolite ratios (2-NOF + 2-NO_F) and the amounts of the amides obtained from peroxidative metabolism of N-arylhydroxamic acids by neutrophils (Figures 6, 8) suggested a higher level of 1e-oxidation for N-OH-2-FBA than N-OH-2-FAA relative to the oxidative cleavage. Thus, as with the N-arylamines, the extent of oxidation and the product profile are determined by the structure and, hence, physico-chemical properties of the N-arylhydroxamic acids. It is possible that less soluble compounds such as N-OH-2-FBA remain longer at the site of application and cause prolonged infilltrations and oxidant release by leukocytes. Under such circumstances, in addition to DNA damage from reactive metabolites of carcinogens, leukocytic oxidants may cause direct damage to DNA. Demonstrating that these oxidative processes occur in vivo is a complex undertaking. Based on our studies, several approaches appear viable: a) demonstration of the presence of the more stable derivates (e.g., 4-OH-2-FBA, 2-NO_F, o-BzO-2-FBA (Figure 1)); b) characterization of both carcinogen- and oxidant-induced DNA damage in target tissues (e.g., peritoneal serosa); c) determination of the effect of increasing physiologic concentration of Br⁻, a significant environmental pollutant (36), on the tumorigenicity of the hydroxamic acids; and d) demonstration of the interaction products of the major metabolite 2-NOF with glutathione, protein, and unsaturated lipids. Because of the exceptional direct mutagenicity of 2-NOF in the bacterial systems (27), it especially merits investigation. Recent evidence that interaction of C-nitroso compounds with unsaturated lipids is a source of O_2 (37) or genotoxic products via lipid peroxidation (38) may be pertinent to the biologic significance of 2-NOF.

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