Metabolism of N-Nitrosodialkylamines by Human Liver Microsomes

Jeong-Sook H. Yoo, F. Peter Guengerich, and Chung S. Yang

ABSTRACT

The metabolism of N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine, N-nitrosobenzylmethylamine, and N-nitrosobutylmethylamine was investigated in incubations with human liver microsomes. All of the 16 microsomal samples studied were capable of oxidizing NDMA to both formaldehyde and nitrite at NDMA concentrations as low as 0.2 mM; the rates of product formation of the samples ranged from 0.18 to 2.99 nmol formaldehyde/min/mg microsomal protein (median, 0.53 nmol). At a concentration of 0.2 mM NDMA, the rates of denitrosation (nitrite formation) were 5 to 10% (median, 6.3%) of those of demethylation (formaldehyde formation); the ratio of denitrosation to demethylation increased with increases in NDMA concentration, in a similar manner to that obtained with rat liver microsomes, even though most of the human samples had lower activities than did the rat liver microsomes. The high affinity Km values of the four human samples ranged from 27 to 48 μM (median, 35 μM), which were similar to or slightly lower than those observed in rat liver microsomes, indicating that human liver microsomes are as efficient as rat liver microsomes in the metabolism of NDMA. The human liver microsomes also catalyzed the dealkylation and denitrosation of other nitrosamines examined. The rates of product formation and the ratios of denitrosation to dealkylation varied with the structures and concentrations of the substrates as well as with the microsomal samples tested. The results indicate that human liver microsomes are capable of metabolizing N-nitrosodialkylamines via the pathways that have been established with rat liver microsomes.

INTRODUCTION

Nitrosamines, carcinogenic compounds occurring widely in the environment, require metabolic activation for their cytotoxic and carcinogenic actions. The major activation step for N-nitrosodialkylamines is believed to be the oxygenation of the α-carbon catalyzed by a P-450-dependent enzyme system. In previous work, we have demonstrated that rat liver microsomes catalyze the metabolism of NDMA and exhibit at least three different Km values for NDMA (1, 2). The high affinity Km (KmI) form has been shown to be inducible by various factors such as fasting, diabetes, consumption of ethanol, and pretreatment with acetone or isopropanol (1, 3–5). The P-450 enzyme corresponding to the KmI form of NDMA dehydrochloride was purified and characterized (6). This form, referred to as P-450c (6) or P-450m (7) in our laboratory, is probably identical to P-450k (8). P-450k has been shown to be responsible mainly for the activation of NDMA to a mutagen for mammalian cells (9).

Because of the potent carcinogenicity of nitrosamines and their frequent exposure to humans as well as the species variability in nitrosamine tumorigenicity, knowledge of nitrosamine metabolism in human liver is of considerable importance. Information concerning the P-450k orthologue in human liver microsomes is of particular interest because this form is likely to be the enzyme responsible for the metabolism of the low level of NDMA present in the human body due to environmental exposure and endogenous synthesis. Recently, the cDNA for a human P-450k orthologue (referred to as human P-450c) was isolated and sequenced (10). The human P-450c cDNA shared 75% nucleotide and 78% predicted amino acid similarities to rat P-450c cDNA; the calculated molecular mass of the predicted human P-450k was slightly greater (56,916 daltons) than that of rat P-450k (56,634 daltons). It was not known whether this human enzyme would display the same catalytic activities as the rat enzyme. Although the metabolism of nitrosamines in human tissues and microsomes has been studied previously (11–14), the enzymology has not been thoroughly investigated. Also of great interest is the possible existence in human tissues of the metabolic pathway which leads to the denitrosation of nitrosamines. This metabolic pathway, which presumably leads to the inactivation of N-nitrosodialkylamines, has been previously established in rats (15–17) but has not been documented for humans. The present study was thus undertaken to investigate the enzymology of NDMA metabolism as well as the metabolism of other nitrosamines such as NDEA, NBzMA, and NBuMA in human liver microsomes. We report herein that the KmI value of human liver microsomes is similar to or slightly lower than that observed in rat liver microsomes, suggesting that human liver microsomes are as efficient as rat liver microsomes in the metabolism of NDMA. The metabolism of NDEA, NBzMA, and NBuMA varied with the structures and concentrations of the substrates as well as with the microsomal samples tested.

MATERIALS AND METHODS

Chemicals. NDMA, sulfanilamide, and N-(1-naphthyl)ethylene-diamine dihydrochloride were purchased from Aldrich Chemical Co. (Milwaukee, WI). NDEA, NBuMA, cytochrome c, NADP, NADPH, glucose-6-phosphate (disodium salt), glucose-6-phosphate dehydrogenase (Type XV), and semicarbazide-HCl were obtained from Sigma Chemical Co. (St. Louis, MO). NBzMA was from Ash Stevens, Inc. (Detroit, MI). Freund's complete and incomplete adjuvants were purchased from Difco Laboratories (Detroit, MI). Nitrocellulose membranes were obtained from Bio-Rad Laboratories (Richmond, CA). Phosphatase-labeled goat anti-rabbit IgG, 5-bromo-4-chloro-3-indolylphosphate, and nitroblue tetrazolium were purchased from Kirkegaard & Perry Laboratories, Inc. (Gaithersburg, MD). Reagents for electrophoresis were obtained from sources described previously (18), and all other chemicals were reagent grade from commercial sources.

Human Liver Microsomes. Human liver samples were obtained, through the Nashville Regional Organ Procurement Agency, from...
organ donors who met accidental deaths and donated other tissues for transplant. Livers were removed, perfused, and chilled on ice within 15–30 min of death, and small portions (1–6 cm³) were frozen in liquid nitrogen and stored at −70°C (19). Individual microsomal fractions were prepared from these liver samples and stored at −70°C as described (20). Glycerol in the microsomal preparations was removed by dialysis against 0.25 M sucrose before use. Subject code numbers refer to individual livers for which donor age, sex, and cause of death have been given elsewhere in many cases (20–23).

Enzyme Assays. Protein and P-450 contents were determined as described previously (24). NADPH-P-450 reductase was assayed at room temperature using cytochrome c as an artificial electron acceptor (25). One unit of reductase activity corresponds to the NADPH-dependent reduction of 1 nmol cytochrome c/min/mg protein. The metabolism of NDMA was measured as formation of formaldehyde and nitrite for demethylation and denitrosation, respectively (2, 4, 26). In brief, the assay mixture contained (in a total volume of 1.0 ml) 50 mM Tris-HCl (pH 7.0 at 37°C), 10 mM MgCl₂, 150 mM KCl, an NADPH-generating system (0.4 mM NADP+, 10 mM glucose-6-phosphate, and 0.4 units glucose-6-phosphate dehydrogenase), human liver microsomes (0.7–0.8 mg protein), and NDMA as indicated. Blanks were prepared in the absence of either NDMA or the NADPH-generating system. For standards, HCHO and NaNO₂ (66 and 10 nmol/ml incubation, respectively) were added, at around the midpoint of the incubation, to the assay mixture in the absence of either NDMA or the NADPH-generating system. At the termination of the 20-min reaction, the assay mixture was centrifuged, and 0.35 ml of supernatant was used for the determination of formaldehyde for NDMA activity (4). Another 0.35 ml of the supernatant was used for the determination of nitrite (2) as modified from the method of Appel and Graf (27). The 0.35-ml aliquot was mixed with 0.075 ml of 100 mM sulfanilamide in 3 N HCl. After 5 min, 0.075 ml of 1 mM N-(1-naphthyl)ethylenediamine dihydrochloride in 3 N HCl was added, followed by immediate mixing with a vortex. Absorbance at 546 nm was measured after the mixture was kept at room temperature for 10 min.

The dealkylations of NDEA, NBeMA, and NBuMA were measured as formation of acetaldehyde, benzaldehyde, and formaldehyde, respectively. The denitrosation of these nitrosamines was assayed as nitrite formation. The formation of aldehydes was measured by a modification of the method of Farrelly (28). Incubation conditions were identical to that used for the NDMA metabolism except that after initiation of the incubation, test tubes were placed in a water bath at 37°C. The use of cytochrome c (Sigma, St. Louis, MO) to minimize evaporation of the metabolites (aldehydes). The incubation was terminated by injecting 0.1 ml of a 1:1 mixture of 50% ZnSO₄ and 0.1 M semicarbazide into the tubes, followed by mixing with a vortex; this procedure served to quench the reaction as well as to trap the aldehydes as their semicarbazone derivatives. To the tubes 0.1 ml saturated Ba(OH)₂ was then added and mixed, preceding centrifugation.

For aldehyde determination, a 0.35-ml aliquot of the resulting supernatant was added to a 16×125-mm tube containing 1 ml H₂O, 0.1 ml of 0.25% 2,4-dinitrophenylhydrazine reagent in 6 n HCl, and 1.5 ml hexane, and the tube was capped and shaken for 1 h. One ml of the hexane layer was then added to 0.35 ml acetonitrile followed by mixing with a vortex for 20–30 sec. Finally, 0.125 ml of the acetonitrile layer was transferred to sample vials which were subsequently loaded onto a WISP autoinjector (Waters Associates, Milford, MA) and 50 μl of sample were injected onto a high performance liquid chromatography column. The column (5 × 100 mm) was Rad-Pak C₈ on 5-μm silica fitted in an RCM-100 Module (Waters). The mobile phase was 65% methanol/acetonitrile (in H₂O) at a flow rate of 1.1 ml/min. The peaks due to the dinitrophenylhydrazine derivatives of the aldehydes had retention times of 3.5, 4.5, 8.2, and 9.5 min for formaldehyde, acetaldehyde, butyraldehyde, and benzaldehyde, respectively. They were monitored by a model 440 UV detector (Waters) at a wavelength of 340 nm and quantified by their peak areas which were determined with an integrator.

RESULTS

NDMA Metabolism and P-450α Orthologue in Human Liver Microsomes. It was recently observed that glycerol was a competitive inhibitor of the Km for rat liver microsomal NDMA (26). Sixteen human liver microsomal samples originally stored in 20% glycerol (20) were, therefore, dialyzed against 0.25 M sucrose shortly before the initiation of the present study and the dialyzed samples were used throughout the study. The history of some of the 16 subjects has been reported elsewhere (20–23). Based on our experience obtained from the study of NDMA metabolism in rat liver microsomes, which exhibit multiple Km values for NDMA, three substrate concentrations (0.2, 4, and 100 mM NDMA) were selected to determine the profiles of NDMA metabolism, and the results are presented in Table 1. All 16 microsomal samples were able to metabolize NDMA to produce both formaldehyde and nitrite at NDMA concentrations as low as 0.2 mM; the oxidation rates of the 16 samples ranged from 0.18 to 2.99 nmol HCHO/min/mg protein (median, 0.53 nmol) and from 0.01 to 0.25 nmol NO₂⁻/min/mg protein (median, 0.03 nmol). At 0.2 mM NDMA, the rates of denitrosation (NO₂⁻ formation) were 6 to 10% (median, 6.3%) those of demethylation (HCHO formation). The ratio of denitrosation to demethylation increased with increases in NDMA concentration; e.g., the median values of the ratios were 8 and 24% at 4 and 100 mM NDMA, respectively. These properties of NDMA metabolism in human liver microsomes are in general similar to those in rat liver microsomes (2), although the rates of metabolism for both demethylation and denitrosation in most of the former were about half those in the latter.

The result that human microsomes metabolized NDMA effectively at a low concentration (0.2 mM NDMA) strongly suggested the possible existence of a P-450α orthologue in human microsomes. We, therefore, subjected the microsomal samples to sodium dodecyl sulfate-polyacrylamide slab-gel electrophoresis and then transferred the proteins electrophoretically to nitrocellulose paper. When the resolved proteins were treated with anti-P-450α IgG in an immunoblot analysis, only one major band (human P-450α) was detected (Fig. 1). The intensity of the human P-450α bands was measured using a densitometer. The peak area of sample number 15 was arbitrarily set at 100 units and relative values of the other samples were obtained. The human P-450α content was highly correlated with the NDMA activity at 0.2 mM NDMA (r = 0.971; P < 0.001), whereas the NDMA activity was rather weakly correlated with the total P-450 content (r = 0.769; P < 0.01) or the reductase activity (r = 0.539; P < 0.05) (Table 1).
**Table 1** NDMA metabolism in human liver microsomes

The assay mixture contained (in a total volume of 1.0 ml) buffer, an NADPH-generating system, human liver microsomes (0.7–0.8 mg protein), and NDMA as indicated. After a 20-min reaction at 37°C, HCHO and NO₂⁻ formed were measured and expressed as nmol/min/mg protein.

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<th>Sample no.*</th>
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<th>Reductase activity (units)</th>
<th>P-450&lt;sub&gt;me&lt;/sub&gt; (units)</th>
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<th>NO₂⁻</th>
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* Each sample number has its own subject code and the information for some of the subjects has been reported (20–23): sample numbers 1 through 16 correspond to subject codes 29, 33, 34, 35, 39, 93, 94, 95, 97, 98, 99, 100, 103, 104, 105, and 106, respectively.

**Fig. 1.** Immunoblots of human liver microsomal proteins. Lanes A and B, purified rat P-450<sub>aa</sub> (0.25 μg/lane); lanes 1 to 16, human liver microsomal sample numbers 1 to 16 (9.3 μg protein/lane) corresponding to Table 1.

Microsomal sample number 15 had 0.20 nmol P-450<sub>me</sub>/mg microsomal protein, which is equivalent to 27% of total P-450 content (0.74 nmol/mg protein). It can be thus estimated that 1 nmol of human P-450<sub>me</sub> can oxidize NDMA (when present at 0.2 mM) to produce 15 nmol HCHO/min.

Kinetic Parameters of NDMA Metabolism in Human Liver Microsomes. The results obtained in the preceding section led us to examine in detail the enzymology of NDMA metabolism in four selected microsomal samples. Fig. 2 shows NDMA metabolism in one microsomal sample (sample no. 15): a, demethylation and b, denitrosation. The data are expressed in Lineweaver-Burke double-reciprocal plots. The kinetic constants were obtained by linear regression analyses of the double-reciprocal plots and were very similar to those obtained from Eadie-Hofstee plots. Because the K<sub>m</sub> values of NDMA metabolism are known to be affected by a host of experimental conditions, the presently observed parameters can only be considered apparent K<sub>m</sub> and V<sub>max</sub> values. The high affinity K<sub>a</sub> values of demethylation and denitrosation were 30 and 24 μM, respectively. Judging from the experimental variation of the denitrosation assay, these values may be considered to be similar rather than different. In previous studies with rat and hamster microsomes, we have demonstrated that the high affinity K<sub>a</sub> (K<sub>m</sub>) values for demethylation and denitrosation are the same (2, 30). At higher NDMA concentrations the rate of demethylation was quite different from that of denitrosation, resulting in different low affinity K<sub>m</sub> values between the two reactions; this property is similar to that observed in rat liver microsomes (2).

Table 2 summarizes the kinetic parameters of NDMA de-
methylation in four selected microsomal samples. Each of the four samples exhibited at least three different $K_m$ values. The $K_m$ values of the four human samples ranged from 27 to 48 $\mu M$ (median, 35 $\mu M$), which are similar to or slightly lower than the $K_m$ value (40-50 $\mu M$) observed in rat liver microsomes (26). The results suggest the existence of a P-450e orthologue in human microsomes. It is not known, however, why the $K_m$ values vary among different microsomal samples. Although there were experimental errors in such determinations, the variations among different experiments with the same sample were usually less than the range (27-48 $\mu M$) observed. The variation, therefore, may reflect some unknown inherent differences among different microsomes. The $K_{I}$ form, ranging from 0.59 to 0.99 $\mu M$, displayed 24-64% of the activity of the $K_{II}$ form as judged by corresponding $V_{max}$ values. The $K_{III}$ form ranging from 20 to 50 $\mu M$ displayed $V_{max}$ values from 0.99 to 2.21 nmol/min/mg and these values were independent of the $V_{max}$ values for the $K_{II}$ form. The $K_{II}$ and $K_{III}$ values reflect a composite of different P-450 enzymes existing in the microsomes, and can only be considered operational terms rather than real kinetic constants. The presently observed $K_{II}$ values were slightly higher than the value (0.22-0.36 mm) observed in rat liver microsomes, but the $K_{III}$ values for human and rat microsomes were in the same range.

Metabolism of Other Nitrosamines by Human Liver Microsomes. Previous studies of nitrosamine metabolism in rat liver microsomes have demonstrated that acetone- or ethanol-induced microsomes have a high activity for demethylation and deethylation and that phenobarbital-induced microsomes have a high activity for denitrosation and debutylation (31), suggesting that P-450e and P-450, (a major phenobarbital-inducible form of P-450) have different affinities for different alky groups of nitrosamines. In the present study, the metabolism of NDEA, NBzMA, and NBuMA in four selected human liver microsomes was examined using three substrate concentrations (0.2, 2, and 20 mM). The results for NDEA are presented in Table 3. When assayed at 0.2 mM NDEA the rates of deethyl- ation with different microsomes were parallel with, and were about 60% of, the rates of demethylation obtained with 0.2 mM NDMA. The rates of deethylation with sample numbers 8, 15, and 16 increased as NDEA concentration increased to 2 mM, and then leveled off or slightly decreased at 20 mM NDEA, probably due to the saturation of substrate binding to P-450ne and substrate inhibition. In comparison, the activity of sample number 14 was lower at 0.2 mM NDEA, but the activity increased by 1.6- to 2.7-fold when assayed at 2 and 20 mM, respectively. The results suggest that, in addition to P-450e, NDEA is also metabolized by other P-450 forms which display higher $K_m$ values. The denitrosation of NDEA followed a similar pattern to that of the deethylatation. The ratios of denitro- sation to deethylatation at 0.2 and 2 mM NDEA were about 0.2, approximately 3-fold higher than the denitrosation to demethylation ratio in NDMA metabolism.

For the metabolism of NBzMA, sample number 15 was still most active among the four microsomal samples studied (Table 4), but other features of the metabolism were quite different from those of NDMA and MDEA. The rates of the demethyl- ation increased by 1.6- to 2-fold upon increasing the substrate concentration from 0.2 to 2 mM, but further increase in the demethylation rate was not observed upon elevating the NBzMA concentration to 20 mM. In contrast, such a saturation behavior was not seen with the debenzylation reaction. Upon increasing NBzMA concentration from 0.2 to 2 mM, the deben- zylation rates were enhanced 3- to 4-fold; and at 20 mM NBzMA, the rates were increased about 10-fold for sample numbers 8, 15, and 16 and 20-fold for sample number 14. As a consequence, the ratio of the rates of debenzylation to demethyla- tion was 2.7 at 0.2 mM NBzMA and increased about 7-fold upon increasing NBzMA concentration to 20 mM. The sub- strate saturation behavior of the denitrosation reaction fell in between the patterns of the demethylation and debenzylation reactions. The ratios for the denitrosation rates of 0.2, 2, and 20 mM NBzMA were 1, 1.6-1.9, and 3.7-6.6, respectively. The ratio of denitrosation to dealkylation was 0.24 at 0.2 mM NBzMA and decreased gradually with increases in NBzMA concentration due to rather marked elevation of debenzylation rate.

The metabolism of NBuMA in human liver microsomes was somewhat similar to that of NBzMA but with substantial differences (Table 5). The demethylation rate was several-fold higher than the deethylatation rate at 0.2 and 2 mM NBuMA. However, the former reaction appeared easily saturable but the latter was not. Upon increasing the substrate concentration to 20 mM, the demethylation rate changed only slightly whereas the deethylatation rate increased markedly and surpassed the demethylation rate. The substrate saturation pattern of the denitrosation, again, fell in between the demethylation and deethylatation reactions.

**DISCUSSION**

During the past decades the properties of hepatic NDMA metabolism have been extensively studied. It has been demonstrated that at least two NDMAe enzyme systems, one with a $K_m$ value of 0.2
to 0.3 mM and the other with a $K_m$ value of 44 to 51 mM, are responsible for the demethylation of NDMA (32). Recently, one of our laboratories has demonstrated the presence of an even lower $K_m$ (higher affinity) form of NDMAc ($K_m = 40$ to $50 \mu M$) in rat liver microsomes and this form has been shown to be important in the bioactivation of NDMA (1, 2, 9, 26, 33). The P-450 species responsible for this high affinity ($K_m$) form, P-450ac, has been purified from rat liver microsomes and characterized (6). The cDNA for both the rat and human P-450ac genes have been sequenced (10) the human P-450ac cDNA shares 75% nucleotide and 78% predicted amino acid sequence identities to rat P-450ac cDNA. Wrighton et al. (34) reported that a human P-450ac orthologue resembled P-4505 and P-450LM3, (an ethanol-inducible form of rabbit P-450) in its NH$_2$-terminal amino acid sequence. From this information, it might be expected that the enzymeology of NDMA metabolism in human liver microsomes is similar to that in rat liver microsomes. The present study showed that human liver microsomal samples catalyzed NDMA metabolism efficiently at low NDMA concentrations and that the activity correlates well ($r = 0.971$) with the quantity of P-450ac as determined by immunoblot analysis using anti-P-450ac IgG. This result is similar to recent reports of Wrighton et al. (34) showing that the NDMAc activity (measured at 1 mM NDMA) was well correlated ($r = 0.87$) with the level of a P-450ac orthologue in human liver microsomes and that this enzyme activity was inhibited (70–80%) by anti-P-450ac, IgG.

In the present work we further characterized the kinetic parameters and demonstrated the existence of the $K_m$ form of NDMAc in human liver microsomes. The observed value of $K_m$ is similar to or slightly lower than the $K_m$ value of rat liver microsomal NDMAc. This indicates that human liver microsomes contain an enzyme similar to that in rat liver microsomes for the metabolism of NDMA. The human microsomal NDMAc activity, however, was not inhibited by a monoclonal antibody preparation (designated as 1-91-3 in Ref. 35) which has been shown previously (35) and in the present work to inhibit more than 90% of the NDMAc activity in rat liver microsomes (data not shown). This result suggests that the rat P-450ac possesses an antigenic site which is absent or inaccessible in human microsomal P-450ac. Judging from the possible low cellular level of NDMA that humans are most likely to be exposed to under normal conditions, the high affinity $K_m$ form of NDMAc is probably the most important enzyme to consider in the metabolism of this carcinogen. By assaying the activity with a substrate concentration of 0.2 mM, we believe that we have accurately assayed the activity of this enzyme with little interference from the activity of the low affinity $K_m$ II form.

Using rat liver microsomes, we have previously demonstrated the specificity of P-450 enzymes in the metabolism of different alkyl groups of nitrosamines (31). The present study demonstrated that human liver microsomes have similar properties. The rates of demethylation and deethylation of NDMA, NDEA, NBzMA, and NBU MA were rather high at low substrate concentrations and easily saturable; whereas the rates of the debenzylations and debutylation of NBzMA and NBU MA increased with increasing substrate concentrations and were not saturated at 20 mM. In both human and rat liver microsomes, the rate of NDEA deethylation was about half that of NDMA demethylation and, in NBzMA metabolism, the debenzylase rate was several-fold higher than the demethylation rate. On the other hand, the metabolism of NBU MA in human liver microsomes appeared to be quite different from that observed in rat liver microsomes; in the former the rate of demethylation was several-fold greater than that of debutylation at substrate concentrations $<2$ mM whereas the latter showed only slightly higher rates of demethylation than debutylation.

We have previously studied the mechanisms of denitrosation of low concentrations of NDMA by rat liver microsomes and postulated that it may share the initial $\alpha$-oxygenation step with the dealkylation pathway (2, 7, 16, 17, 30). It is reasonable, therefore, to demonstrate that such a pathway exists in human tissues for the metabolism of N-nitrosodialkylamines and that the relationship between this pathway and the dealkylation pathway is similar to that observed in rat liver microsomes. However, the ratio of the rates of denitrosation to dealkylation increases upon increasing the substrate concentrations. It appears that other mechanisms may also be involved in the denitrosation of high concentrations of NDMA. Because denitrosation may be a detoxifying metabolic pathway for N-nitrosodialkylamines, it is important to consider the substrate concentration (or dosage) factor when we assess the relative importance of these pathways. The same considerations should also be applied when we weigh the relative importance of one dealkylation reaction versus another (e.g., demethylation versus...

### Table 4 Metabolism of NBzMA by human liver microsomes

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<th>Sample no.</th>
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<td>C$_2$H$_4$CHO</td>
<td>HCHO</td>
<td>NO$_2^-$</td>
</tr>
<tr>
<td>8</td>
<td>0.30</td>
<td>0.10</td>
<td>0.11</td>
</tr>
<tr>
<td>14</td>
<td>0.26</td>
<td>0.12</td>
<td>0.10</td>
</tr>
<tr>
<td>15</td>
<td>1.02</td>
<td>0.39</td>
<td>0.28</td>
</tr>
<tr>
<td>16</td>
<td>0.27</td>
<td>0.07</td>
<td>0.09</td>
</tr>
</tbody>
</table>

### Table 5 Metabolism of NBU MA by human liver microsomes

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>0.2 mM</th>
<th>2 mM</th>
<th>20 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C$_2$H$_4$CHO</td>
<td>HCHO</td>
<td>NO$_2^-$</td>
</tr>
<tr>
<td>8</td>
<td>0.06</td>
<td>0.59</td>
<td>0.05</td>
</tr>
<tr>
<td>14</td>
<td>0.07</td>
<td>0.33</td>
<td>0.04</td>
</tr>
<tr>
<td>15</td>
<td>0.59</td>
<td>1.92</td>
<td>0.15</td>
</tr>
<tr>
<td>16</td>
<td>0.03</td>
<td>0.30</td>
<td>0.04</td>
</tr>
</tbody>
</table>
debenzylation) in the metabolism of an asymmetrical N-nitroso-
dialkylamine.

These results are consistent with the postulate that human P-450\textsubscript{\textalpha} plays an important role in catalyzing the demethylation and denitrosation of NDMA as well as other N-nitrosodialkyl-
amines especially when they are present in low concentrations.

The regulation of this P-450 enzyme in humans is relatively
unknown. It may be speculated that human P-450\textsubscript{\textalpha} could be
induced by alcohol consumption, fasting, diabetes, and other
factors that are known to induce this enzyme in rats (36). This
postulate, however, remains to be substantiated. It was noted
in this work that sample number 15 had the highest content of
P-450\textsubscript{\textalpha} and metabolic activities. The case history of this donor
did not provide sufficient information for interpreting the high
activity. The reason for the individual variabilities in the me-
tabolism of nitrosamines is an important issue and remains to
be systematically investigated.

ACKNOWLEDGMENTS
The authors thank S. M. Ning for the immunoblot analysis
and M. J. Lee for the high performance liquid chromatography analysis.

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Metabolism of \( N \)-Nitrosodialkylamines by Human Liver Microsomes

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