Activation of N-Hydroxyphenacetin to Mutagenic and Nucleic Acid-binding Metabolites by Acyltransfer, Deacylation, and Sulfate Conjugation

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ABSTRACT

N-Hydroxyphenacetin was activated to a mutagen in the Salmonella-Ames test by rabbit liver acyltransferase, rat liver cytosol, and rat liver microsomes. N-[ring-\(^3\)H]-Hydroxyphenacetin was bound to transfer RNA when activated by acyltransferase from rabbit or rat liver or rat liver microsomes. The acyltransferase-catalyzed binding was not inhibited by paraoxon, a deacetylase inhibitor. The use of N-hydroxyphenacetin radioactively labeled in the acetyl group, as well as the ring, indicated that deacetylation was involved in the microsometransferase catalyzed binding reaction. In addition, the microsome-catalyzed binding was inhibited 90% by paraoxon. p-Nitroso-phenacetone, a deacetylated derivative of N-hydroxyphenacetin, was synthesized and bound to transfer RNA without enzymatic activation. Activation of N-hydroxyphenacetin by sulfate conjugation was also found to lead to binding to transfer RNA. The data implicated acyl transfer, deacetylation, and sulfate conjugation as possible routes for the activation of N-hydroxyphenacetin.

INTRODUCTION

Phenacetin (p-ethoxyacetanilide) is an analgesic found in a number of nonprescription compounds (35) sold in the United States. The long-term consumption of phenacetin-containing analgesics has been found to lead to severe renal papillary necrosis and tumors of the renal pelvis and bladder in humans (5, 39). The abuse of these compounds has led to the removal of phenacetin from nonprescription analgesics in Sweden and Australia and the restriction of consumption elsewhere (5, 27, 39). Although conflicting data have appeared concerning the carcinogenicity of phenacetin in laboratory animals, several studies have now shown it to induce tumor formation in a number of tissues in the rat (18, 19), including the urinary bladder (18).

As an aromatic amide, phenacetin is known to undergo several metabolic reactions common to this group of compounds (10). These include N-hydroxylation (14), which is known to be an important step in the activation of aromatic amide carcinogens such as 2-acetylaminofluorene. N-OH-P\(^3\) is carcinogenic to rat liver (9). Recent studies have also shown N-OH-P to be mutagenic when activated by 9000 \(\times\) g rat liver supernatant (38) or rat liver microsomes (41).

A number of studies have been performed showing the activation of phenacetin to macromolecule-bound products (15, 16, 31, 33). Rat and hamster liver microsomes can activate phenacetin to bind to protein or glutathione by several different pathways. One of these pathways involves the formation of N-OH-P, which can be further activated to reactive metabolites by sulfation or glucuronidation (31). An earlier study by Nery (34) showed that phenacetin could be activated by microsomes to bind to nucleic acids as well as protein. These studies have not been extended. In addition, there have been no studies reported concerning the reaction of N-OH-P with nucleic acids.

Acyl transfer, deacylation, and sulfate conjugation are pathways believed to be involved in the activation of arylhydroxamic acids to mutagenic and carcinogenic metabolites (10). The present studies use mutagenicity and nucleic acid-binding data to further explore the metabolic activation of N-OH-P.

MATERIALS AND METHODS

Chemicals. tRNA was obtained from Calbiochem (La Jolla, Calif.). [1-\(^3\)H]Acetyl chloride was obtained from Amersham/Searle Corp. (Arlington Heights, Ill.). All other commercially obtained chemicals were reagent grade.

N-OH-P was synthesized by a modified procedure as follows. p-[ring-\(^3\)H]-Nitrophenol (specific activity, 352 mCi/mmol) was synthesized by Midwest Research Institute (Kansas City, Mo.) and used as the starting material in the synthesis of N-OH-P. p-[ring-\(^3\)H]-Nitrophenetole (p-ethoxynitrobenzene) was prepared by refluxing [\(^3\)H]nitrophenol with ethyl iodide and K\(_2\)CO\(_3\) in acetone (1). p-[ring-\(^3\)H]-Nitrophenetole was reduced with zinc dust in acetic anhydride in the presence of triethylamine in tetrahydrofuran to give a mixture of [\(^3\)H]phenacetin and N-[\(^3\)H]acetoxycyclohexan. This mixture was treated with NH\(_3\) gas at \(-20^\circ\) to give a mixture of N-OH-P and phenacetin. The dried mixture was extracted with ether and 1 N NaOH, giving N-OH-P in the base layer and phenacetin in the ether layer. The base layer was acidified with 4 N HCl and the N-OH-P was extracted with ethyl acetate. The ethyl acetate was evaporated and the residue was dissolved in ether. The potassium salt of N-OH-P was formed and precipitated with a minimum amount of 1 N KOH in methanol. The salt was washed in ether and then dissolved in 1 N HCl and extracted into ether. The ether was evaporated and the N-OH-P was recrystallized from tetrahydrofuran: hexanes. The final product had a melting point of 102° (literature value, 103–104°) (8). Electron-impact mass spectroscopy showed a molecular ion at m/z 195 and fragments at m/z 179, 153, and 137. These correspond to losses of O, COCH\(_2\), and O + COCH\(_2\), respectively. Nuclear magnetic resonance of unlabeled N-OH-P prepared by this same method gave \(\delta\) 1.4 (triplet, 3H, ethoxymethyl), 2.0 (singlet, 3H, N-acetyl), 4.0 (quartet, 2H, ethoxymethylene), 7.2 (quartet, 4H, aromatic), and 8.8 (broad singlet, 1H, OH). A UV spectrum of the labeled compound gave the following: \(\lambda_{\max}\) 253 nm; and \(\lambda_{\max}\) 217 nm. Thin

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1 The studies in this report from the A. Alfred Taubman Facility were supported by USPHS Grants CA25904 and CA23800 from the National Cancer Institute through the National Bladder Cancer Project and an institutional grant from the United Foundation of Detroit.

2 To whom requests for reprints should be addressed.

3 The abbreviation used is: N-OH-P, N-hydroxyphenacetin.

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4 R. Roth, personal communication.
layer chromatography in 2 systems (ether, Rs 0.4; or ethyl acetate, Rs 0.3) gave a single spot which reacted with FeCl3 to give a red color and contained about 98% of the radioactivity on the plate. The specific activity of the compound was determined by calculating the concentration of a methanol solution from its absorbance at 253 nm (ε = 10,000) and then counting several aliquots of the solution.

[acetil-1-14C]ON-OH-P was prepared from unlabeled p-nitrophenol and [1-14C]acetylechloride following the published method (14) except NaOH was used to extract the product and HCl to reprecipitate it. Thin-layer chromatography in benzene:ethyl acetate (1:1) gave a single spot (Rf 0.35), containing >98% of the radioactivity on the plate.

p-[[ring-3H]Nitrosophenetole was prepared by oxidation of N-[ring-3H]hydroxyphenetidine as described previously (3, 7). The resulting mixture was chromatographed on a 10-g silica gel column and eluted with hexanes. An emerald-green band was collected and gave 25.1 mg of the nitroso compound (35% yield). Thin-layer chromatography of the nitroso derivative was carried out in ether:hexanes (5:95). The nitroso compound was >98% pure with no visible impurities and melted at 29–31° (literature value, 33°) (25). The UV spectrum was as follows: λmax, 340 nm (ε = 19,060); and λmax, 255 nm. The specific activity was determined as described above.

Preparation of Rat Liver Enzyme Fractions. Female or male Sprague-Dawley CD rats (Charles River Breeding Laboratories, Wilmington, Mass.) were sacrificed, and livers were removed and homogenized with a Polytron (Brinkmann Instruments, Inc., Westbury, N. Y.) in 4 volumes 0.05 M sodium pyrophosphate: HCl buffer, pH 7.0. The homogenate was centrifuged for 10 min at 10,000 × g, 4°. The resulting supernatant was centrifuged for 1 hr at 105,000 × g, 4°. The 105,000 × g supernatant, or microsomes suspended in buffer, were used as activating systems in the RNA-binding and mutagenicity assays. Enzyme preparations for use in mutagenicity assays were prepared under sterile conditions. Protein concentrations for the binding and mutagenicity assays were determined by the method of Lowry et al. (26).

Purification of Liver Acyltransferase. Rabbits were obtained from the colony of Dr. W. Weber, Department of Pharmacology, University of Michigan, and were of the fast-acetylator phenotype (13). The liver of a rat or rabbit was removed and homogenized in 4 volumes 0.05 M sodium pyrophosphate: HCl buffer, pH 7.0. The liver acyltransferase was partially purified essentially as described by Glowinski et al. (13). Briefly, this involved centrifugation of the homogenate at 105,000 × g and column chromatography of the cytosol on DEAE-cellulose, resulting in a 15-fold purification. Acyltransferase was assayed (21) using N5-[ring]3H]hydroxy-2-acetylaminofluorene (0.04 μCi/μmol) as substrate. The DEAE-column fractions containing maximum rabbit liver acyltransferase activity were combined and concentrated by ultrafiltration. Aliquots of the concentrated enzyme were frozen at −20°. No loss in activity occurred for several weeks when the rabbit liver enzyme was stored in this manner. The rabbit liver acyltransferase peak column fraction was used immediately, as this enzyme is less stable than that of the rabbit.

RNA Binding Assay. Binding to RNA was studied with rat or rabbit liver acyltransferase [equivalent to 1.4 to 1.8 nmol fluorocynamine bound per mg tRNA in the standard assay (21)], rat liver microsomes, or cytosol (1 mg protein) as the activating enzyme source. The incubation mixture included the following in a total volume of 0.8 ml: tRNA (15 A260 units); 0.05 M sodium pyrophosphate: HCl buffer (pH 7.0); an activation system; and [ring-3H]ON-OH-P (0.04 μCi; 70 μCi/μmol) in 10 μl dimethyl sulfoxide as substrate. The reaction was carried out at 37° for 30 min. The reactions described increased linearly for at least 30 min. Control mixtures received tRNA at the end of the incubation period. The binding to RNA was determined by precipitating and washing the RNA extensively on glass fiber filters as described above. In experiments in which doubly labeled N-OH-P was used, the 3H- and 14C-labeled components were mixed to give a 3H:14C ratio of about 5:1.

Activation of N-OH-P by sulfate conjugation was measured as described by King and Olive (23), using 105,000 × g adult male rat liver supernatant (1 mg protein) as the enzyme source. The incubation system consisted of Tris-HCl buffer and ATP, MgCl2, and Na2SO4 as the sulfate-generating system in a final volume of 1 ml. Binding to tRNA was measured as described above.

Mutagenicity Testing. Mutagenicity assays were performed with Salmonella typhimurium strain TA100, a gift from Dr. Bruce Ames. Assays were conducted by the method of Ames et al. (2), with some modifications, in the presence of rabbit liver acyltransferase, rat liver cytosol, or rat liver microsomes. The assay mixtures, preincubated at 37° for 30 min, contained the following: test compound, dissolved in 0.1 ml dimethyl sulfoxide; 0.1 ml bacterial culture (1 to 2 × 10^8 cells); and 0.5 ml of either rat liver microsomes (2 mg protein) or cytosol (11 mg protein) or 0.5 ml of 0.1 M sodium phosphate buffer, pH 7.4. Assays with 30 or 200 μl purified rabbit liver acyltransferase were adjusted to 0.7 ml with the same buffer. Two ml of top agar (at 45°), containing 0.1 μmol biotin and 0.1 μmol L-histidine-HCl, were added, and the mixture was vortexed and poured onto 10 ml minimal glucose agar. The plates were inverted and incubated at 37° for 72 hr. The number of revertant colonies was determined with an Artek Model 880 colony counter (Artek Systems Corp., Farmingdale, N. Y.) and corrected with an appropriate calibration factor determined for this instrument. The spontaneous mutability of tester strain TA100 was in the range of 98 to 135 revertants/plate. N-Hydroxy-2-acetylaminofluorene at 20 nmol/plate served as a positive control. Two independent tests were performed for each experimental series, using 2 or 3 replicate plates in each test.

RESULTS

Our studies began in an attempt to assess the mutagenic activity of phenacetin and some of its metabolites. Phenacetin (data not shown) was not mutagenic for TA100, as was found in previous studies (38). N-OH-P, however, was significantly mutagenic to TA100, when activated by rabbit liver acyltrans-
and unstable. However, p-nitrosophenetole, the expected tRNA in certain experiments was an additional precaution to bind to tRNA without enzymatic activation. The nitroso product of spontaneous oxidation of the hydroxylamine, did hydroxyphenetidine, was synthesized but was relatively impure was to nucleic acid rather than protein. The late addition of precipitations in order to be certain that the observed binding protein (data not shown), as well as tRNA, binding. For these found that microsomal activation of N-OH-P led to extensive group was lost in the formation of the RNA adduci. We also indicated by the data shown in Table 1. The microsome-catalyzed binding was inhibited 91% by paraxoxon, a deacetylase inhibitor. In addition, the 2H:14C ratio shows that the acetyl group was lost in the formation of the RNA adduct. We also found that microsomal activation of NOH-P led to extensive protein (data not shown), as well as tRNA, binding. For these reasons, we purified the RNA extensively through 2 ethanol activations in order to be certain that the observed binding was to nucleic acid rather than protein. The late addition of tRNA in certain experiments was an additional precaution to monitor the effectiveness of our tRNA purification procedure.

Direct evidence was sought for the binding of deacetylated N-OH-P to tRNA. The product of deacetylation of NOH-P, as indicated by the data shown in Table 1. The microsome-catalyzed binding assay. Table 2 shows that binding was increased almost 5-fold over controls in the presence of purified rat liver acyltransferase. In addition, the use of N-OH-P showed that the acetyl group was lost, another characteristic of an acyltransferase-catalyzed reaction. Rabbit liver acyltransferase also activated N-OH-P to a tRNA-binding metabolite (Table 2), again with loss of the acetyl group. In some experiments (data not presented), N-hydroxy-2-acetylaminofluorene was compared to N-OH-P as a substrate in the rabbit acyltransferase-catalyzed binding to tRNA. The former compound is a substrate commonly used in the acyltransferase assay (21). N-Hydroxy-2-acetylaminofluorene was a more effective substrate by a factor of 20. Neither reaction was inhibited by paraxoxon, indicating that cytosolic deacetylase was not present in the purified enzyme preparation.

Additional evidence that the activity presented in Table 2 was due to acyltransferase was found by using N-OH-P as a substrate in the acetyl transfer assay of Booth (6). This colorimetric assay measures the loss of 4-aminoazobenzene due to transacetylation by aryldiamidonic acids. This acetyl transfer activity has been found to correspond to acyltransferase activity in the most purified preparations of the latter enzyme (22). N-OH-P was found to be as active as N-hydroxy-4-acetylamino benzyl in the acetyl transfer assay.

![Graph showing mutagenicity of N-OH-P in S. typhimurium TA100 without (L) and with rat liver 105,000 x g microsomes (M) or cytosol (C). Microsomal and cytosolic protein concentrations were about 2 and 11 mg/plate, respectively. Points, mean number of revertants per plate from 6 replicate plates; bars, S.D.](chart)

Chart 2. Mutagenicity of N-OH-P in S. typhimurium TA100 without (L) and with rat liver 105,000 x g microsomes (M) or cytosol (C). Microsomal and cytosolic protein concentrations were about 2 and 11 mg/plate, respectively. Points, mean number of revertants per plate from 6 replicate plates; bars, S.D.

The data in Table 1 indicate a high level of tRNA binding by N-OH-P. For this reason, acyltransferase was purified from rat and rabbit liver and used in the tRNA-binding assay. Table 2 shows that binding was increased almost 5-fold over controls in the presence of purified rat liver acyltransferase. In addition, the use of N-OH-P showed that the acetyl group was lost, another characteristic of an acyltransferase-catalyzed reaction. Rabbit liver acyltransferase also activated N-OH-P to a tRNA-binding metabolite (Table 2), again with loss of the acetyl group. In some experiments (data not presented), N-hydroxy-2-acetylaminofluorene was compared to N-OH-P as a substrate in the rabbit acyltransferase-catalyzed binding to tRNA. The former compound is a substrate commonly used in the acyltransferase assay (21). N-Hydroxy-2-acetylaminofluorene was a more effective substrate by a factor of 20. Neither reaction was inhibited by paraxoxon, indicating that cytosolic deacetylase was not present in the purified enzyme preparation.

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Since sulfate activation is involved in the formation of rat liver

<table>
<thead>
<tr>
<th>Assay conditions</th>
<th>pmol bound/mg tRNA</th>
<th>pmol bound (blanks subtracted)</th>
<th>Ring: acetyl moieties bound to tRNA</th>
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<td>Control</td>
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<td>Control (rabbit AT)</td>
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<td>Standard (rat AT)</td>
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<td>10.8 ± 2.0</td>
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* Mean ± S.D.

Table 2

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</thead>
<tbody>
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<td>Control</td>
<td>1.33 ± 0.13</td>
<td>1.13 ± 0.16</td>
<td>1.2</td>
</tr>
<tr>
<td>Control + paraoxon</td>
<td>0.45 ± 0.03</td>
<td>1.01 ± 0.16</td>
<td>0.4</td>
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<tr>
<td>Standard</td>
<td>4.43 ± 1.23</td>
<td>1.24 ± 0.08</td>
<td>28.2</td>
</tr>
<tr>
<td>Standard + paraoxon</td>
<td>0.74 ± 0.06</td>
<td>1.35 ± 0.08</td>
<td>0.9</td>
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</table>

* Mean ± S.D.

Table 1

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<td>0.9</td>
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tumors by N-hydroxy-2-acetylaminofluorene (10), the ability of sulfate to activate N-OH-P to a rRNA-binding metabolite was investigated. Table 3 shows that, in the presence of a sulfate-generating system, N-OH-P bound to rRNA. Approximately a 3-fold increase in binding is observed when the "standard + sulfate" system is compared to the "control + sulfate." There was a greater than 5-fold increase in binding in the sulfate-activated system over that of the cytosol alone (standard assay mixture). The control mixture with sulfate resulted in higher binding than control without sulfate, probably due to the persistence of a reactive sulfate conjugate. The use of [acetyl-14C]N-OH-P in the sulfate-generating system revealed that, in contrast to the microsome- and acyltransferase-catalyzed reactions of N-OH-P with rRNA, the acetyl group was retained in the adduct (ring:acetyl, 0.7).

**DISCUSSION**

The mutagenicity and binding data presented in this report indicate that N-OH-P is metabolized by acetyl transfer, deacetylation, and sulfate conjugation. Chart 3 illustrates these reactions as they would occur with N-OH-P as the substrate. N-OH-P has been shown previously (38, 41) to be mutagenic in the presence of 9000 x g supernatant or microsomes. Studies by Wirth et al. (41) have provided evidence that microsomal activation of N-OH-P to a mutagen is inhibited by paraaxon. In addition, their study found p-nitrosophenetole to be a potent direct-acting mutagen. Our data show that N-OH-P is also activated by acetyl transfer to a metabolite mutagenic to Salmonella TA100. Acyltransferase has been found previously to activate N-hydroxy-2-acetylaminofluorene (40) and N-hydroxydiacetylbenzidine (29) to mutagens in the Salmonella TA1538 system. The present studies used strain TA100 bacteria and used highly purified rabbit liver acyltransferase to show that N-OH-P is activated by this pathway, similarly to the other arylhydroxamic acids mentioned above.

Although Nery (34) described the binding of labeled phenacetin to DNA, RNA, and protein when activated by rat liver microsomes, our data provide the first evidence for nucleic acid binding of activated N-OH-P. The microsome-catalyzed binding of phenacetin found by Nery could have been due to N-hydroxylation although N-OH-P was not synthesized and tested in his studies. [acetyl-3H]Phenetidine, the deacetylated derivative of phenacetin, did not bind to nucleic acids in the absence of a microsomal activating system (34). In contrast, deacetylation appears to be involved in the microsome-catalyzed binding of phenacetin to microsomal protein observed by Nelson et al. (33) and in the binding of N-OH-P to rRNA in our system.

Phenacetin has been found previously to be N-hydroxylated in the presence of hamster liver microsomes (14). Studies of the further metabolism of N-OH-P (30) have shown the N,O-sulfate and N,O-glucuronide conjugates to be the major products. These conjugates bind to protein, possibly through the formation of the intermediate N-acetylbenzoquinoneimide (30, 31). While interaction of these conjugates with nucleic acids had not been studied directly in the previous studies, competition experiments with nucleosides indicated that reaction with nucleic acids did not occur (31). In contrast, in direct experiments, our data indicate that N-OH-P activated by sulfate conjugation reacts with rRNA. Competition studies with nucleosides are not necessarily predictive of binding reactions of polynucleotides with arylamines, as shown by Kadlubar et al. (20) in studies of naphthylamine derivatives, although such studies were useful in elucidating the structures of adducts derived enzymatically from N-hydroxy-2-acetylaminofluorene (24). Sulfation of N-hydroxy-2-acetylaminofluorene by a liver cytosol system does lead to binding to nucleic acid (23), a process believed to be involved in the hepatocarcinogenicity of this compound (10). The reaction of sulfate conjugates of N-OH-P with protein is believed to occur through a different intermediate (N-acetylbenzoquinoneimide) (30, 31), which may react directly with macromolecules. This intermediate is also a possibility in the reaction of sulfate-conjugated N-OH-P with rRNA in our experiments, since the acetyl group is retained in the rRNA adducts (Table 3). However, our data are insufficient to confirm this possibility.

It is possible that other pathways available to N-OH-P, such as deacetylation and acyltransfer, are important in its bioactivation. Deacetylation has been found to be an important reac-

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Table 3

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<tr>
<th>Sulfate activation of N-OH-P</th>
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</tr>
<tr>
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<tr>
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* Mean ± S.D.
tion in the mutagenicity of 2-acetylaminofluorene and N-hydroxy-2-acetylaminofluorene to Salmonella in the Ames test (11, 12). The rat liver nuclei-induced mutagenicity and nucleic acid binding of N-hydroxy-2-acetylaminofluorene are inhibited by paraoxon (37). Deacetylation appears to be involved in the in vivo carcinogenicity of some arylhydroxamic acids (10); however, some arylhydroxylamines are too unstable to synthesize in pure form and test adequately for carcinogenicity in the whole animal. Our data suggest that p-nitrosophenetole binds to tRNA without enzymatic activation. The nitroso analog would be expected to result from the spontaneous oxidation of N-hydroxyphenetidine, the expected product of deacetylation of N-OH-P. As mentioned above, this compound is also a direct-acting mutagen (41). The data of Wirth et al. (41), along with those presented here, indicate that deacetylation of N-OH-P to N-hydroxyphenetidine results in extensive interaction with nucleic acids.

Acyltransfer is another enzymatic pathway common to N-hydroxy-2-acetylaminofluorene and N-OH-P in the liver. Our data with enzyme preparations purified by ion-exchange chromatography, showed that both rabbit and rat liver were capable of performing this reaction. That this activity was acyltransferase and not deacetylase was confirmed by data showing that the same preparation was active in the colorimetric acetyl transfer assay of Booth (6) with N-OH-P as substrate and by the failure of paraoxon to inhibit the tRNA-binding activity when the purified enzyme was assayed with N-OH-P or N-hydroxy-2-acetylaminofluorene as substrate. It is unknown whether N-OH-P is carcinogenic to rabbit liver as it is to rat liver (9), and the role that acyl transfer plays in the carcinogenicity of this compound in rat liver is similarly unknown. Acyl transfer has been proposed as an alternative mechanism for carcinogen activation in extrahepatic tissues of the rat, such as mammary gland and ear duct (22), which do not have detectable sulfotransferase (17). Phenacetin has been found to be carcinogenic to female rat mammary gland and ear duct (19). Acyl transfer is another enzymatic pathway common to N-hydroxy-2-acetylaminofluorene and N-OH-P in the liver. Our data with enzyme preparations purified by ion-exchange chromatography, showed that both rabbit and rat liver were capable of performing this reaction. That this activity was acyltransferase and not deacetylase was confirmed by data showing that the same preparation was active in the colorimetric acetyl transfer assay of Booth (6) with N-OH-P as substrate and by the failure of paraoxon to inhibit the tRNA-binding activity when the purified enzyme was assayed with N-OH-P or N-hydroxy-2-acetylaminofluorene as substrate. It is unknown whether N-OH-P is carcinogenic to rabbit liver as it is to rat liver (9), and the role that acyl transfer plays in the carcinogenicity of this compound in rat liver is similarly unknown. Acyl transfer has been proposed as an alternative mechanism for carcinogen activation in extrahepatic tissues of the rat, such as mammary gland and ear duct (22), which do not have detectable sulfotransferase (17). Phenacetin has been found to be carcinogenic to female rat mammary gland and ear duct (19). Acyl transfer may, therefore, be a valid mechanism for further study with respect to phenacetin and N-OH-P carcinogenicity.

The target organs for phenacetin carcinogenesis in humans are those of the urinary tract. There is no apparent tumorigenic effect of phenacetin to human liver, although its deethylated metabolite, acetaminophen, is hepatotoxic at high dosages (28). The analgesic nephrotoxicity found in humans who abuse phenacetin to human liver, although its deethylated metabolite, acetaminophen, is hepatotoxic at high dosages (28). The analgesic nephrotoxicity found in humans who abuse phenacetin to human liver, although its deethylated metabolite, acetaminophen, is hepatotoxic at high dosages (28). The analgesic nephrotoxicity found in humans who abuse phenacetin to human liver, although its deethylated metabolite, acetaminophen, is hepatotoxic at high dosages (28). The analgesic nephrotoxicity found in humans who abuse phenacetin to human liver, although its deethylated metabolite, acetaminophen, is hepatotoxic at high dosages (28).


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