Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 1327–1331, March 1984
Biochemistry

N-acetyl-*p*-benzoquinone imine: A cytochrome P-450-mediated oxidation product of acetaminophen

(reactive metabolite/reduction/conjugation)

DAVID C. DAHLIN*, GERALD T. MIWA†, ANTHONY Y. H. LU†, AND SIDNEY D. NELSON*‡

*Department of Medicinal Chemistry, University of Washington, Seattle, WA 98195; and †Department of Animal Drug Metabolism, Merck, Sharp and Dohme Research Laboratories, Rahway, NJ 07065

Communicated by M. J. Coon, November 14, 1983

ABSTRACT ***N*-acetyl-*p*-benzoquinone imine (NAPQI) has been proposed as the toxic metabolite of acetaminophen for the past 10 years, although it has never been detected as an enzymatic oxidation product of acetaminophen. We report (*i*) direct detection of NAPQI formed as an oxidation product of acetaminophen by cytochrome P-450 and cumene hydroperoxide and (*ii*) indirect evidence that is compelling for NAPQI formation from acetaminophen by cytochrome P-450, NADPH, and NADPH–cytochrome P-450 reductase. Evidence is provided for the rapid reduction of NAPQI back to acetaminophen by NADPH and NADPH–cytochrome P-450 reductase such that steady-state levels of NAPQI were below our detection limits of 6.7×10^{-8} M. In mouse liver microsomal incubations, radiolabeled analogs of both NAPQI and acetaminophen bound covalently to microsomal protein with the loss of ≈20% of the acetyl group as acetamide. The binding in each case was decreased by glutathione with concomitant formation of 3-*S*-glutathionylacetaminophen. The binding also was decreased by L-ascorbic acid, NADPH, and NADH with reduction of NAPQI to acetaminophen. Results of partitioning experiments indicate that NAPQI is a major metabolite of acetaminophen, a considerable fraction of which is rapidly reduced back to acetaminophen.**

The widely used analgesic-antipyretic drug acetaminophen is known to cause serious liver necrosis at high doses in man and experimental animals (1, 2). An electrophilic product of cytochrome P-450 oxidation that depletes cellular glutathione (GSH) and covalently binds to tissue macromolecules has been implicated in this toxic reaction (3). Initially, the formation of the arylating metabolite was believed to involve *N*-oxidation of acetaminophen to *N*-hydroxyacetaminophen followed by dehydration to *N*-acetyl-*p*-benzoquinone imine (NAPQI) (4). However, subsequent kinetic studies and carrier pool trapping experiments *in vitro* (5, 6) and metabolism studies of *N*-hydroxyacetaminophen *in vivo* (7) have shown that if *N*-hydroxyacetaminophen is an intermediate, it must decompose at the enzymatic site of hydroxylation.

Although it appears that *N*-hydroxyacetaminophen is not significantly involved in acetaminophen toxicity, evidence still favors NAPQI as an ultimate toxin. We recently synthesized NAPQI in pure crystalline form, and studies have shown this reactive quinoneimine to be highly toxic in mice and to isolated hepatocytes (8). Other investigators, using aqueous solutions of NAPQI generated either electrochemically (9) or by dehydration of *N*-hydroxyacetaminophen (7, 10, 11), have shown NAPQI to be an electrophile and an oxidant. Similar properties were exhibited by stable benzene solutions of chemically synthesized NAPQI (12).

We present in this report direct evidence for the formation of NAPQI from acetaminophen in incubations of acetaminophen with cumene hydroperoxide (CHP) and hepatic cytochrome P-450 purified from phenobarbital-pretreated rats. Similar attempts to directly detect NAPQI in incubations of acetaminophen with either purified P-450, NADPH, and NADPH–cytochrome P-450 reductase, or with mouse liver microsomes and an added NADPH regenerating system, were unsuccessful. We provide evidence that the steady-state concentrations of NAPQI are diminished below the detection limits of our procedures ($\approx 7 \times 10^{-8}$ M) through rapid reduction of NAPQI back to acetaminophen by NADPH and NADPH–cytochrome P-450 reductase. However, further indirect evidence was provided for the formation of NAPQI in these incubations by comparing the disposition of radiolabeled derivatives of both synthetic NAPQI and acetaminophen.

MATERIALS AND METHODS

Materials. Materials and their sources were: acetaminophen, silver (I) oxide, Florisil, *p*-aminophenol, and L-ascorbic acid, from Aldrich; GSH, bovine serum albumin, NADPH, NADH, NADP, glucose 6-phosphate, and glucose-6-phosphate dehydrogenase, from Sigma; CHP and [1-^{14}C]acetic anhydride, from ICN, Plainview, NJ, and Irvine, CA, respectively; [*ring*-U-^{14}C]acetaminophen, from Pathfinder Laboratories, St. Louis, MO; Aquasol-2 scintillant, from New England Nuclear; ultrapure dimethyl sulfoxide, from Alfa Products, Danvers, MA; male BALB/c mice, from Tyler Laboratories, Bellevue, WA; and butylated hydroxytoluene, from Nutritional Biochemicals. Chloroform and all HPLC solvents were chromatography grade from Burdick and Jackson. All aqueous buffers were passed through a Chelex-100 ion-exchange column (Bio-Rad) to remove trace metal ion impurities. Hepatic cytochrome P-450 and NADPH–cytochrome P-450 reductase were purified from phenobarbital-pretreated rats and assayed as described elsewhere (13, 14). HPLC analyses were performed on a component system consisting of a Waters Associates model 6000 A solvent delivery system and model U6K injector with a 2-ml sample loop, with detection and column conditions as described for the various assays. Liquid scintillation spectrometry was carried out on a Beckmann LS-7500 instrument. NAPQI was synthesized as reported (8). The syntheses of [*acetyl*-^{14}C]NAPQI and [*ring*-^{14}C]NAPQI were identical except that [*acetyl*-^{14}C]acetaminophen (0.41 mCi/mmol; 1 Ci = 37 GBq) and [*ring*-^{14}C]acetaminophen (0.20 mCi/mmol) were used as reactants. Radiochemical purity of all compounds was >98%, as determined by HPLC as described in the legend to Fig. 1.

Incubation Conditions. Reactions of purified cytochrome P-450 (0.5 μM), CHP (2.0 mM), and acetaminophen (0.5 mM) were conducted in 200 μl of sodium phosphate buffer (0.05 M, pH 7.4) with shaking in a water bath at 37°C. HPLC

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: NAPQI, *N*-acetyl-*p*-benzoquinone imine; CHP, cumene hydroperoxide; EC, electrochemical; GSH, glutathione.

‡To whom reprint requests should be addressed.

analyses for steady-state levels of enzymatically generated NAPQI were performed 6.0 min after reaction initiation. In some experiments, CHP was replaced by varying concentrations of purified rat liver NADPH–cytochrome P-450 reductase and NADPH or an NADPH regenerating system and 20 μg of phosphatidylcholine per ml. The regenerating system was added to attain final concentrations of 5 mM glucose 6-phosphate, 1 mM NADP, and 3 mM $MgCl_2$ along with 0.2 unit of glucose-6-phosphate dehydrogenase/200-μl incubation volume. Incubations with mouse liver microsomes were carried out as described (3) in a volume of 3 ml with 2 mg of microsomal protein per ml. Concentrations of substrates and other compounds were as described in the figure legends.

Assays. The electrochemical detection of NAPQI generated in purified rat liver cytochrome P-450 incubations of acetaminophen with CHP was carried out essentially by the method of Miner and Kissinger (9). The reverse-phase HPLC analysis was performed on 25-μl samples of the incubations injected directly onto a μBondapak C_{18} column (3.9 mm × 30 cm) 6.0 min after reaction initiation. Details of the assay are described in the legend to Fig. 1. The concentration of NAPQI generated from the P-450 oxidation of acetaminophen, as well as the determination of detection limits (1.1×10^{-7} M NAPQI), were established by generating standard curves with NAPQI and calculating limits of detection [$c_{L(k=3)}$] as defined by IUPAC using the propagation of errors approach (15).

CHP-supported incubations of [*ring*-^{14}C]-acetaminophen (0.5 mM, 9.78 mCi/mmol) with purified rat liver cytochrome P-450 were carried out as described above. Six minutes after the incubations were started, 25-μl samples were injected directly onto the HPLC. Details of the assay are described in the legend to Fig. 1. The detection limit with this method was calculated to be 6.7×10^{-8} M, as determined by the method described above using [*ring*-^{14}C]NAPQI standards.

Kinetics. All kinetic measurements were done at 25.0 ± 0.2°C with a Shimadzu UV 250 spectrophotometer. To a 1-ml solution of NADPH (0.5 mM) in 0.1 M sodium phosphate buffer (pH 7.4) in a cuvette was added a 10-μl solution of NAPQI or *p*-benzoquinone in freshly distilled tetrahydrofuran. The reaction of NADPH and NAPQI was followed by monitoring the disappearance of NADPH at λ = 340 nm. A pseudo-first-order rate constant was determined at limiting concentrations of NAPQI (0.05 mM) in the presence of excess NADPH (0.5 mM). Values were obtained from the least squares slope of semilogarithmic plots of NADPH concentration vs. time. Second-order rate constants for reactions of NADPH with NAPQI and *p*-benzoquinone were determined at 0.5 mM concentrations of both reactants.

Covalent Binding and Metabolite Determinations. [*ring*-^{14}C]-Acetaminophen (1.0 mM, 0.48 mCi/mmol), [*ring*-^{14}C]NAPQI (0.05 mM, 0.20 mCi/mmol), [*acetyl*-^{14}C]acetaminophen (1.0 mM, 0.41 mCi/mmol), or [*acetyl*-^{14}C]NAPQI (0.05 mM, 0.21 mCi/mmol) was incubated with mouse liver microsomes for 20 min in a shaking incubator at 37°C. Reactions were terminated by the addition of 1.5 ml of ice-cold acetone and centrifugation to separate the precipitated protein. Covalent binding determinations were carried out on the isolated protein by published procedures (3). Metabolites were assayed by injecting 75-μl aliquots of the supernatant fraction on a 5-μm Ultrasphere ODS column (4.6 mm × 25 cm) and elution with a solvent gradient from 5% to 30% methanol over 12 min in 0.01 M KH_2PO_4 containing 1% acetic acid (nonlinear program 10, Waters Associates model 660 solvent programmer). Retention times of standards by using UV detection at 254 nm were: hydroquinone, 4.4 min; benzoquinone, 9.1 min; acetaminophen, 12.4 min; and 3-*S*-glutathionylacetaminophen, 15.5 min. Between injections the column was flushed with 95% methanol. Fractions were collected at 1-min intervals for 20 min in 10 ml of Aquasol-2 scintillant by using an LKB 2112 Redirac fraction collector. Quantities of metabolites were then determined by the eluted radioactivity. Acetamide was assayed as described (16).

RESULTS

NAPQI was detected by reductive electrochemical (EC) methods as an oxidation product of acetaminophen in CHP-supported incubations of cytochrome P-450 that was purified from phenobarbital-treated rats. HPLC chromatograms of incubations containing acetaminophen and CHP with cytochrome P-450 showed only the presence of acetaminophen and CHP by UV detection (Fig. 1*A*). Furthermore, NAPQI was not observed by reductive EC detection in control incubations in which cytochrome P-450 was omitted (Fig. 1*B*). However, a peak was consistently observed by HPLC/EC detection at the retention time of NAPQI in incubations of acetaminophen and CHP containing cytochrome P-450 (Fig. 1*C*). The mean (±SD) NAPQI concentration in four complete incubations was $(2.6 \pm 0.3) \times 10^{-7}$ M. Benzoquinone, a hydrolysis product of NAPQI, was also detected (retention time, 6.2 min) in these incubations, but the high background noise level surrounding this peak precluded its quantitation.

Similarly, NAPQI was not detected either by UV techniques in complete incubations (Fig. 1*D*) or by radiochemical techniques in incubations lacking cytochrome P-450 but containing [*ring*-^{14}C]-acetaminophen (Fig. 1*E*). However, NAPQI was detected as a radiolabeled product in incubations of [*ring*-^{14}C]acetaminophen, CHP, and purified cytochrome P-450 (Fig. 1*F*). The mean (±SD) concentration of NAPQI in four complete incubations was $(3.4 \pm 0.8) \times 10^{-7}$ M, as determined from the specific activity of the substrate.

As further evidence that the ^{14}C-containing metabolite was NAPQI, the radiolabeled peak and 1.0-min fractions before and after were collected directly into vials that contained 5 μl of a solution of L-ascorbic acid (1.0 mM). Ascorbate reduced NAPQI to acetaminophen, which was then reanalyzed by HPLC. Approximately 88% of the radioactivity collected in the NAPQI peak was accounted for as acetaminophen. The samples derived from collections 1.0 min before and after the NAPQI peak eluted no radioactivity with acetaminophen.

The same methods were used in attempts to detect NAPQI formation from acetaminophen in incubations of purified cytochrome P-450 with NADPH and NADPH–cytochrome P-450 reductase and in mouse liver microsomes with an added NADPH regenerating system. NAPQI concentrations in all cases were below detection limits.

The inability to detect NAPQI in incubations containing NADPH and NADPH–cytochrome P-450 reductase in contrast to CHP-supported reactions is due to reduction of NAPQI back to acetaminophen. NADPH itself is rapidly oxidized by NAPQI with a pseudo-first-order rate constant of 0.57 min^{-1} at 25°C. At equimolar concentrations (0.5 mM) in 0.1 M phosphate buffer (pH 7.4), the second-order rate constant for oxidation of NADPH by NAPQI (3.81 $mM^{-1} \cdot min^{-1}$) is >10 times greater than that for oxidation of NADPH by *p*-benzoquinone (0.32 $mM^{-1} \cdot min^{-1}$). The major product (≈90%) of the reaction between NADPH and NAPQI is acetaminophen (Fig. 2). Competing imine hydrolysis yields small amounts of *p*-benzoquinone (≈2%) under these conditions. NADH also efficiently reduces NAPQI to acetaminophen at a rate virtually identical to the reduction of NAPQI by NADPH (data not shown). Enzyme-catalyzed reduction of NAPQI by purified NADPH–cytochrome P-450 reductase has been previously examined (17). NAPQI is rapidly reduced by the reductase at a rate that is almost 3 times the nonenzyme-mediated rate under the same conditions and is as fast as that observed for reduction by NADPH–cytochrome P-450 reductase of other rapidly metabolized quinones (18).

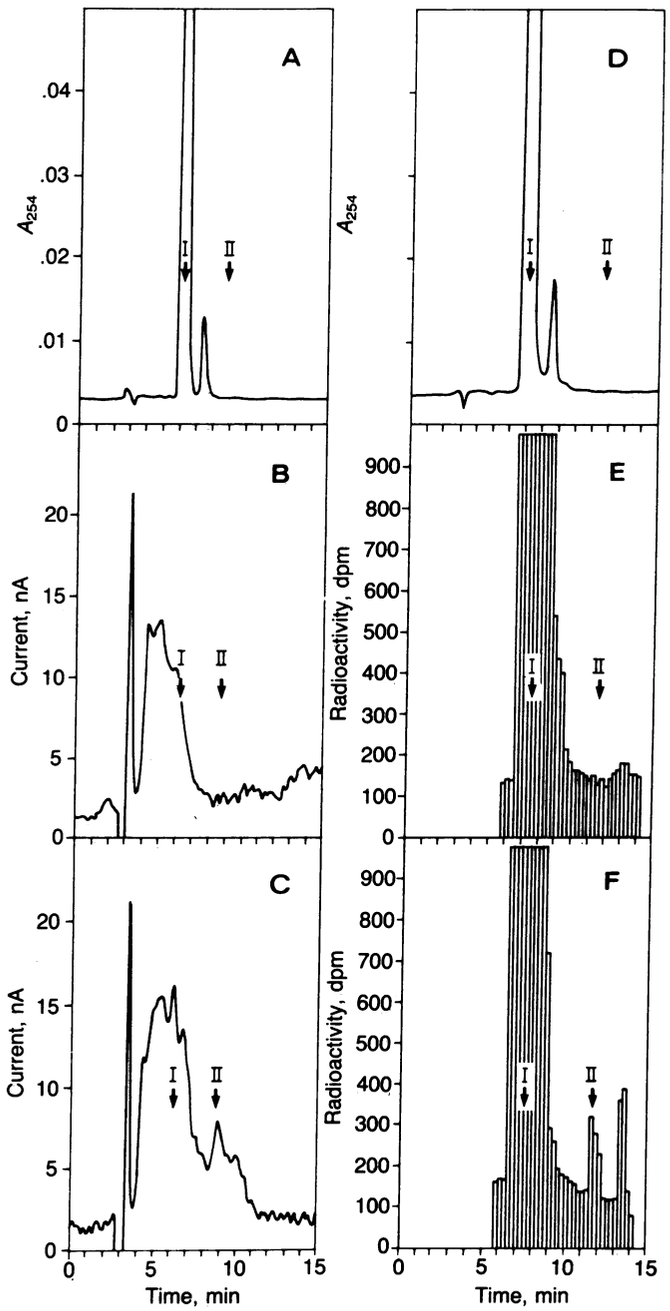

FIG. 1. HPLC chromatograms of NAPQI generated in acetaminophen incubations with purified cytochrome P-450 and CHP. (*A–C*) Performed on a μBondapak C_{18} column with 83% 0.1 M citrate buffer (pH 6.8) and 17% methanol with tandem detection by UV and EC methods. Retention times were acetaminophen (I), 6.2 min, and NAPQI (II), 8.8 min. The chromatogram in *A* resulted from detection by UV at 254 nm, whereas those in *B* and *C* were from EC detection at −0.24 V. *A* and *C* represent complete incubations of acetaminophen with P-450 and CHP. The chromatogram in *B* is from an incubation omitting P-450; those in *D–F* were from incubations with [^{14}C]acetaminophen (9.78 mCi/mmol) as substrate and were performed with the same column as above with a mobile phase of 80% 0.1 M phosphate buffer (pH 7.4) and 20% methanol. The retention times were acetaminophen (I), 7.4 min, and NAPQI (II), 11.8 min. The chromatogram in *D* resulted from detection by UV at 254 nm, whereas those in *E* and *F* are bar plots of ^{14}C recovered in 15-sec fractions of the eluants. *D* and *F* are from a complete incubation of [^{14}C]acetaminophen with P-450 and CHP, whereas *E* is from a representative control (P-450 omitted). In *E* and *F* the radioactivity collections near the [^{14}C]acetaminophen retention time are off-scale. The peak recorded in the UV spectrum between acetaminophen and NAPQI is due to CHP.

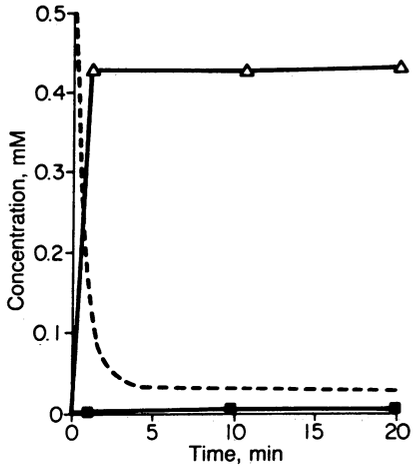

FIG. 2. Time course of reaction between NADPH and NAPQI; initial concentration of both reactants was 0.5 mM. The dashed line (---) shows the time course of oxidation of NADPH as determined by continuous UV monitoring at 340 nm. Concentrations of NADPH remaining were calculated based on a standard curve of absorbance vs. known concentrations of NADPH. Values were determined every 0.2 min for the first 2 min of reaction between NADPH and NAPQI and every 1 min thereafter. Concentrations of acetaminophen (△) and *p*-benzoquinone (■) were determined by the HPLC-radiochemical assay for metabolite determinations.

Further evidence to support the contention that both nonenzymatic and enzymatic reduction of a reactive metabolite of acetaminophen occurs comes from titration experiments (Fig. 3). Covalent binding of reactive metabolites of acetaminophen was decreased by increasing concentrations of NADH (Fig. 3*A*). In incubations supported by NADPH alone, binding increased up to a maximal concentration of NADPH, reflecting the cofactor requirement for oxidation of acetaminophen to the reactive metabolite. Binding thereafter decreased as the rate of reduction of reactive metabolite exceeded its rate of formation. Finally, in incubations supported by an NADPH regenerating system, increasing the P-450 reductase/P-450 ratio produced a parabolic effect on binding (Fig. 3*B*), presumably reflecting the involvement of reductase in both activation of acetaminophen to its reactive metabolite and deactivation of the metabolite by reduction back to acetaminophen.

Although NAPQI was not directly detectable as a metabolite of acetaminophen in incubations that contained NADPH because of its rapid reduction back to acetaminophen, evidence for its formation in these systems was obtained by comparing the metabolic fates of acetaminophen and synthetic NAPQI. Because microsomes from mouse liver are significantly more active in the generation of reactive metabolites of acetaminophen than microsomes from rat liver, we used mouse liver microsomes for comparisons.

In incubations fortified with an NADPH regenerating system, [*ring*-^{14}C]acetaminophen covalently bound to microsomal protein to a slightly lesser extent than [*ring*-^{14}C]-NAPQI at substrate concentrations of 1 mM and 50 μM, respectively (Table 1). Omission of the NADPH regenerating system virtually eliminated the binding of acetaminophen, whereas the binding of NAPQI was increased ≈4-fold. Addition of either L-ascorbic acid or GSH decreased the binding of both NAPQI and acetaminophen. It has been shown previously that the aromatic ring of a reactive metabolite of acetaminophen is bound to a greater extent than the acetyl group and that the acetyl group is released as acetamide (16, 19). Both [*acetyl*-^{14}C]acetaminophen and [*acetyl*-^{14}C]-NAPQI bound to microsomal protein to a lesser extent than their ring-labeled analogs, with approximately the same de-

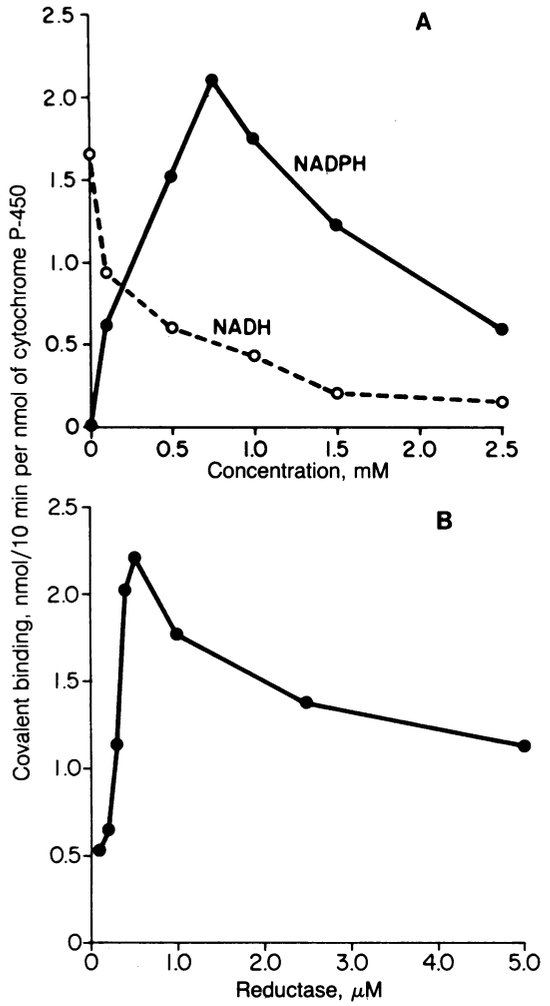

FIG. 3. Effects of increasing concentrations of NADPH and NADH (*A*) and increasing concentrations of NADPH–cytochrome P-450 reductase (*B*) on covalent binding of acetaminophen to bovine serum albumin. Incubations contained 0.5 nmol of cytochrome P-450, 20 μg of phosphatidylcholine, 5 μmol of [*ring*-^{14}C]acetaminophen (0.98 mCi/mmol), and 10 mg of bovine serum albumin in a final volume of 1 ml of 0.05 M potassium phosphate buffer (pH 7.4). Points represent the average results from three separate incubations. Individual results did not vary by >13% from the averages.

gree of retention of label (Table 1). In both cases [^{14}C]acetamide was recovered as the released product.

Partitioning of [*ring*-^{14}C]NAPQI to its reduction product, acetaminophen, and conjugation products, 3-*S*-glutathionylacetaminophen and protein-bound material, was determined by HPLC for the various incubation conditions that are described in Table 1. One of the major products under all conditions was acetaminophen (Fig. 4). Reduction is significantly enhanced in incubations containing ascorbic acid (Fig. 4), with a concomitant decrease in conjugation products, whereas conjugation with GSH increases dramatically in the presence of exogenously added GSH at the expense of reduction and binding to microsomal protein (Fig. 4).

Table 1. Effects of NADPH, L-ascorbic acid, GSH, and site of radiolabel on covalent binding of reactive metabolites of acetaminophen and NAPQI to mouse liver microsomal protein

Substrate	Covalent binding, nmol/incubation*	% of control
[*ring*-^{14}C]Acetaminophen, 1 mM	12.5 ± 1.0	100.0
−NADPH regenerating system	0	0
+L-Ascorbic acid, 1 mM	1.9 ± 0.2	14.8
+GSH, 1 mM	1.5 ± 0.1	12.0
[*acetyl*-^{14}C]Acetaminophen, 1 mM	10.1 ± 1.4	80.8
[*ring*-^{14}C]NAPQI, 50 μM	18.3 ± 1.2	100.0
−NADPH regenerating system	70.5 ± 3.2	385.0
+L-Ascorbic acid, 1 mM	2.6 ± 0.2	14.2
+GSH, 1 mM	2.7 ± 0.1	14.7
[*acetyl*-^{14}C]NAPQI, 50 μM	14.8 ± 1.9	80.9

*Values are means ± SD of four incubations.

DISCUSSION

Results of our experiments with NAPQI and acetaminophen can be explained by the scheme diagramed in Fig. 5. Acetaminophen is oxidized by cytochrome P-450 to NAPQI, a highly reactive quinone-like compound that can be rapidly reduced back to acetaminophen by a variety of reductants. This yields levels of NAPQI in NADPH-supported incubations that are below our detection limits. Such redox cycling of acetaminophen has been previously suggested based on measurements of NADPH oxidation (10, 19).

NAPQI is also an electrophile that reacts with nucleophiles such as GSH to produce 3-*S*-glutathionylacetaminophen. In addition, NAPQI is hydrolyzed to *p*-benzoquinone and acetamide. *p*-Benzoquinone further reacts by binding to protein, as evidenced by small amounts of protein binding of the aromatic ring of both acetaminophen and NAPQI without the acetyl group (Table 1).

The most conclusive indirect evidence to support the intermediacy of NAPQI in acetaminophen oxidation by cytochrome P-450 comes from comparison of the partitioning of

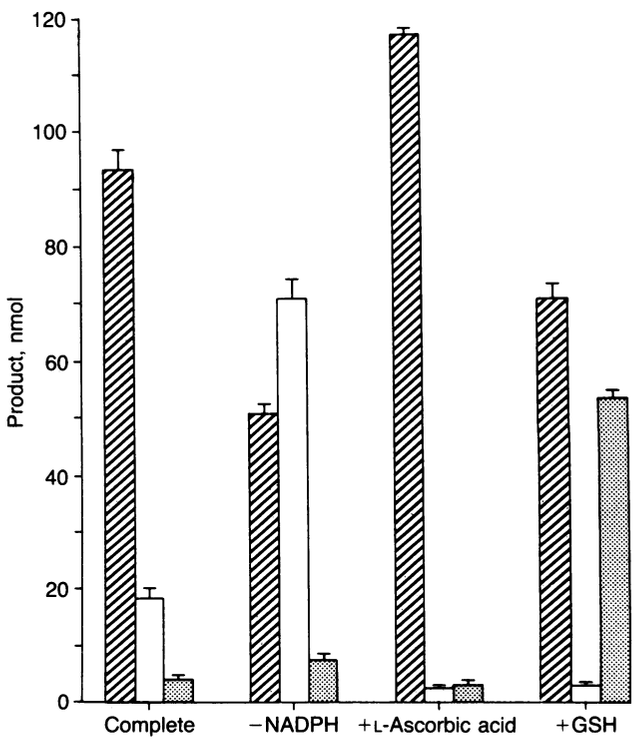

FIG. 4. Acetaminophen (▨), protein-bound product (□), and 3-*S*-glutathionylacetaminophen (▥) that were formed in incubations of [*ring*-^{14}C]NAPQI and mouse liver microsomes with an NADPH regenerating system (Complete), without an NADPH regenerating system, with an NADPH regenerating system and 1 mM L-ascorbic acid, and with an NADPH regenerating system and 1 mM GSH. Assays were performed by a combined HPLC-radiometric method. Error bars represent 1 SD from the mean as calculated from four incubations.

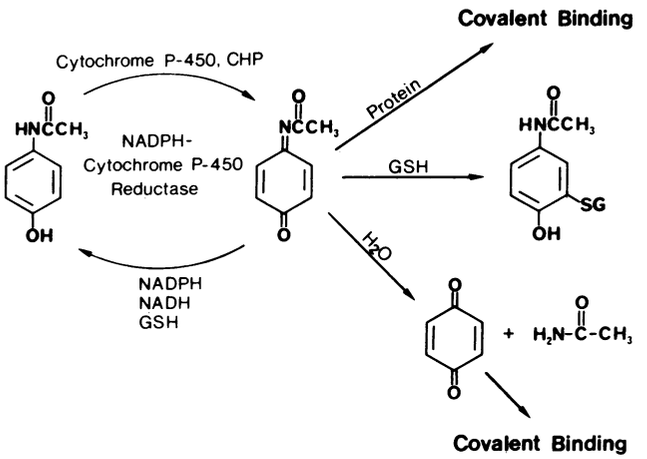

FIG. 5. Scheme depicting formation and decomposition pathways for NAPQI.

NAPQI and the reactive metabolite of acetaminophen under various conditions of incubation (Table 1). For example, the amount of covalently bound metabolite after addition of L-ascorbic acid relative to covalently bound metabolite in incubations lacking ascorbate was approximately the same for both acetaminophen and NAPQI (14.8% and 14.2% of controls, respectively). Similar relative decreases in covalent binding were observed for both acetaminophen and NAPQI with addition of GSH (12% and 14.7% of controls, respectively).

Based on these results, NAPQI is subject to both reduction and conjugate addition reactions. Under our "complete" incubation conditions with microsomes from mouse liver and an NADPH regenerating system, the partition ratio between reduction and conjugation (GSH conjugate plus covalent binding) is 4.4 (Fig. 4). In other words, ≈80% of the NAPQI that is formed by microsomal P-450-mediated oxidation of acetaminophen is apparently reduced back to acetaminophen by both chemical and enzyme-mediated processes. Thus, partitioning of NAPQI between reduction and conjugation may change considerably under conditions *in vivo*, where cellular concentrations of reductants and nucleophiles are continually in flux and where other enzymes, such as the glutathione-*S*-transferases, may play significant roles in the disposition of NAPQI. Further investigations are necessary to examine the disposition of NAPQI in the cell matrix.

In conclusion, NAPQI has been directly detected as an oxidation product of cytochrome P-450 in CHP-supported reactions. Although the mechanism of this oxidation reaction may not be identical to that of P-450 reactions supported by NADPH and NADPH–cytochrome P-450 reductase (20, 21), other indirect evidence was obtained with synthetic radiolabeled analogs of both acetaminophen and NAPQI to clearly demonstrate the formation of NAPQI as a major metabolite of acetaminophen.

This work was supported by Grants GM 25418, GM 32165, and AM 30699 from the National Institutes of Health.

1. Boyd, E. M. & Bereczky, G. M. (1966) *Br. J. Pharmacol.* **26,** 606–614.
2. Prescott, L. F., Wright, N., Roscoe, P. & Brown, S. S. (1971) *Lancet* **i,** 519–522.
3. Mitchell, J. R., Jollow, D. J., Potter, W. Z., Gillette, J. R. & Brodie, B. B. (1973) *J. Pharmacol. Exp. Ther.* **187,** 211–217.
4. Jollow, D. J., Thorgeirsson, S. S., Potter, W. Z., Hashimoto, M. & Mitchell, J. R. (1974) *Pharmacology* **12,** 251–271.
5. Hinson, J. A., Pohl, L. R. & Gillette, J. R. (1979) *Life Sci.* **24,** 2133–2138.
6. Nelson, S. D., Forte, A. J. & Dahlin, D. C. (1980) *Biochem. Pharmacol.* **29,** 1617–1620.
7. Calder, I. C., Hart, S. J., Healey, K. & Ham, K. N. (1981) *J. Med. Chem.* **24,** 988–993.
8. Dahlin, D. C. & Nelson, S. D. (1982) *ibid.* **25,** 885–886.
9. Miner, D. J. & Kissinger, P. T. (1979) *Biochem. Pharmacol.* **28,** 3285–3290.
10. Corcoran, G. B., Mitchell, J. R., Vaishnav, Y. N. & Horning, E. C. (1980) *Mol. Pharmacol.* **18,** 536–542.
11. Gemborys, M. W., Mudge, G. H. & Gribble, G. W. (1980) *J. Med. Chem.* **23,** 304–308.
12. Blair, I. A., Boobis, A. R., Davies, D. S. & Cresp, T. M. (1980) *Tetrahedron Lett.* **21,** 4947–4950.
13. West, S. B., Huang, M. T., Miwa, G. T. & Lu, A. Y. H. (1979) *Arch. Biochem. Biophys.* **193,** 42–50.
14. Miwa, G. T., West, S. B., Huang, M. T. & Lu, A. Y. H. (1979) *J. Biol. Chem.* **254,** 5695–5700.
15. Long, G. L. & Winefordner, J. D. (1983) *Anal. Chem.* **55,** 713A–724A.
16. Nelson, S. D., Forte, A. J., Vaishnav, Y., Mitchell, J. R., Gillette, J. R. & Hinson, J. A. (1981) *Mol. Pharmacol.* **19,** 140–145.
17. Powis, G. A., Svingen, B. A., Dahlin, D. C. & Nelson, S. D. (1983) *Biochem. Pharmacol.*, in press.
18. Powis, G. & Appel, P. L. (1980) *Biochem. Pharmacol.* **29,** 2567–2572.
19. Hinson, J. A., Pohl, L. R., Monks, T. J. & Gillette, J. R. (1981) *Life Sci.* **29,** 107–116.
20. Fasco, M. J., Piper, L. J. & Kaminsky, L. S. (1979) *Biochem. Pharmacol.* **28,** 97–103.
21. Capdevila, J., Estabrook, R. W. & Prough, R. A. (1980) *Arch. Biochem. Biophys.* **200,** 186–195.