Acetaminophen-induced Oxidation of Protein Thiols

CONTRIBUTION OF IMPAIRED THIOL-METABOLIZING ENZYMES AND THE BREAKDOWN OF ADENINE NUCLEOTIDES

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The administration of a hepatotoxic dose of acetaminophen (250 mg/kg) to mice induced the loss of protein thiols in mouse liver. Our data suggest that a significant portion of this loss was due to protein thiol oxidation. The administration of the nonhepatotoxic regioisomer, 3'-hydroxyacetanilide (600 mg/kg) did not produce a similar decrease in liver protein thiols despite producing similar levels of covalent binding. Mice treated with acetaminophen exhibited decreased glutathione peroxidase activity, decreased thioltransferase activity, and decreased adenine nucleotide concentrations in the liver. The increase in urinary allantoin after the administration of acetaminophen suggests that the decrease in adenine nucleotides was due to their degradation in the liver. Acetaminophen also promoted the conversion of the enzyme xanthine dehydrogenase to the oxidase form, and pretreatment of mice with allopurinol, an inhibitor of xanthine oxidase, significantly decreased acetaminophen-mediated hepatotoxicity. The conversion of xanthine dehydrogenase to the oxidase form may lead to a transient increase in the production of activated oxygen species. The increase in activated oxygen species coupled with decreases in glutathione peroxidase and thioltransferase activity may be responsible in part for the increased levels of oxidized protein thiols observed following acetaminophen administration.

Acetaminophen (4’-hydroxyacetanilide, APAP) is commonly administered for the treatment of pain and fever. Overdoses of the drug can produce acute hepatic necrosis in humans (1, 2) and experimental animals (3). Acetaminophen is metabolized to a reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI), by the enzyme cytochrome P-450 (4, 5). Once formed, this metabolite conjugates and depletes cellular glutathione (GSH) levels and then binds extensively to the sulfhydryl groups of cellular proteins (6). Despite removal of reactive metabolites to critical cellular macromolecules (7) and the production of oxidative stress (8, 9) have been proposed to account for the hepatotoxicity of APAP. The regioisomer of APAP, 3'-hydroxyacetanilide (AMAP), is metabolized by cytochrome P-450 to reactive metabolites. These metabolites also react with GSH and the sulfhydryl groups of proteins (10). However, AMAP, in contrast to APAP, is not hepatotoxic to mice (11). In experiments conducted in this laboratory (12), the administration of AMAP produced similar levels of covalent binding to mouse liver homogenates as did a hepatotoxic dose of APAP, yet no hepatotoxicity occurred. In addition, both AMAP and APAP depleted cytosolic GSH levels in the liver at these doses (12). An analysis of subcellular binding profiles indicates that APAP arylates mitochondrial proteins and depletes mitochondrial GSH to a greater extent than AMAP.

APAP administration disrupted calcium homeostasis in the liver while no disruption was observed with AMAP (12). For example, plasma membrane calcium-ATPase activity was lower in APAP-treated animals and their ability to sequester calcium in mitochondria was impaired. These changes apparently lead to mitochondrial calcium cycling 1 h after APAP administration. In contrast, the nonhepatotoxic regioisomer, AMAP, did not significantly affect either the ability of mitochondria to sequester calcium or the activity of the plasma membrane calcium-ATPases.

The mechanism responsible for the hepatotoxicity of APAP is examined further in the present paper. We report that APAP administration depletes cellular protein thiols by both arylation and oxidation. In addition, APAP leads to the conversion of the enzyme xanthine dehydrogenase to xanthine oxidase. This conversion, in conjunction with decreases in the activities of enzymes that affect cellular thiol homeostasis and the degradation of adenine nucleotides, may lead to a transient production of activated oxygen species and promote the oxidation of protein thiols.

EXPERIMENTAL PROCEDURES

Materials—APAP and the alanine aminotransferase determination kit (ALT 59-10) were purchased from Sigma. AMAP was obtained from Aldrich and then recrystallized from water prior to use. Sodium phenobarbital was acquired from Spectrum Chemical Manufacturing Co. (Gardena, CA). NADPH (Type I), NAD+ (Grade III-C), and glutathione reductase (Type VII) were purchased from Sigma. Sulforhodamine was synthesized according to Segel and Johnson (13). The identity of this product was confirmed by fast atom bombardment mass spectrometry.

Animals—Male Swis Webster mice from Charles River Breeding Laboratories (Wilmington, MA) weighing 20–25 g were used throughout the course of this study. Mice were given drinking water containing 0.1% sodium phenobarbital for five days prior to the administration of AMAP or APAP. Food was withheld for 16 h immediately prior to and after the injection of the drug. At the indicated times, mice were killed by cervical dislocation, gallbladders were removed, and livers were utilized for subsequent procedures. Blood samples for plasma enzyme assays were collected by cardiac puncture. Adenine nucleotide and phosphate determinations were conducted on freeze-clamped samples.

Administration of Chemicals—In all experiments, APAP was administered at a dose of 250 mg/kg and AMAP at 600 mg/kg body
weights. Both drugs were dissolved in saline and injected intraperitoneally at a volume of 16.7 ml/kg body weight. It was necessary to warm AMAP solutions prior to injections. Allopurinol was administered 3 h prior to APAP at a dose of 125 mg/kg (14). Allopurinol was dissolved in warm saline adjusted to pH 8.0. The drug was then injected intraperitoneally at a volume of 20 ml/kg.

Adenine Nucleotide Determinations—Samples were prepared according to the procedures of Dills and Klasean (15) with slight modifications. Mice were anesthetized intraperitoneally with urethane solutions that were prepared the same day as the experiment. Livers were then quickly freeze-clamped, powdered, and mixed with acid as described (16). Following homogenization, the acidified homogenate was centrifuged at 16,000 × g for 2 min. The supernatant was removed and mixed with 5 M potassium carbonate until the pH was approximately 5.0. The resulting solution was centrifuged at 16,000 × g for 6 min. The supernatant was retained for phosphate and adenine nucleotide determinations. Adenine nucleotides were analyzed by anion-exchange-high-performance liquid chromatography (16) on a Partisil 10-SAX column (Alltech Associates Inc., Deerfield, IL) with UV detection.

Enzyme Assays—Excised livers were rinsed in cold 250 mM sucrose, 10 mM Tris, pH 7.4, and then suspended in 5 volumes of this buffer at 4 °C. Livers were cut into small pieces and homogenized with six strokes of a Potter-Elvehjem tissue grinder. Aliquots of this buffer at 4 °C. Livers were then quickly freeze-clamped, powdered, and mixed with DTT to a final concentration of 5 mM. The homogenate was then either sonicated for about 1 min to disrupt the cell and its constituents or treated with sodium dodecyl sulfate (1% final concentration), and then incubated at room temperature with DTT for 15 min. Following these procedures, the DTT-treated homogenate was assayed for protein thiols as before. Urine allantoin was measured following the administration of the drug. This decrease, however, was not determined to be significant. Protein thiol levels returned to control levels in the AMAP-treated animals at 6 h.

Significant decreases were observed in protein thiol levels at 1 and 6 h following APAP administration. At 1 h, protein thiol levels were 78.5% of controls. These levels remained depressed at the 6- h time point.

In a previous report (12), we quantified the levels of covalent binding to liver homogenates obtained from animals treated with these same doses of AMAP and APAP. Values of about 0.9 and 0.8 nmol bound per mg protein were detected in liver homogenates at 1 and 6 h, respectively, after APAP administration. Similar values were obtained with AMAP. Levels of 1.0 and 0.6 nmol bound per mg protein were measured at 1 and 6 h, respectively, with this drug. Based on protein thiol determinations, a loss of 14.9 nmol of sulfhydryl groups/mg protein occurred 1 h after APAP administration. This value is about 15 times the loss expected from covalent binding alone.

Further experiments were conducted to determine the contribution of sulfhydryl group oxidation to the observed loss of protein thiols. To assess this contribution, liver homogenates were either sonicated or treated with sodium dodecyl sulfate in the presence of the sulfhydryl-reducing reagent DTT. These treatments were used to disrupt cellular membranes and expose disulfide groups. DTT was removed prior to protein thiol determinations. Following these procedures, no significant decreases from control values were observed in the levels of protein thiols in the liver homogenates of either APAP- or AMAP-treated animals at 1 or 6 h (data not shown). This experiment suggests that oxidation accounts for a large fraction of the observed decrease seen in protein thiol levels following APAP administration.

Effects of APAP and AMAP on Cytosolic GSH-metabolizing Enzymes—We have shown previously that both AMAP and APAP deplete cytosolic GSH levels 1 h after administration and that these levels rebound by 6 h (12). However, little is known about the effects of these agents on those GSH-metabolizing enzymes involved in the protection of the cell against oxidative stress. The effects of AMAP and APAP on cytosolic glutathione peroxidase activity are shown in Table II. Some inhibition of the enzyme was detected after AMAP administration, but much greater decreases in activity occurred in APAP-treated animals. One h after APAP administration, glutathione peroxidase activity decreased to about 60% of control values. At 6 h, activity levels returned to about 70% of controls.

### Table I

| Treatment | nmol sulfhydryl/mg protein |
|-----------|---------------------------|
| Control   | 69.3 ± 8.4\(^a\)          |
| APAP      | 63.3 ± 6.2                |
| AMAP      | 54.4 ± 6.8\(^d\)          |

\(^a\) APAP and AMAP were administered at doses of 250 and 600 mg/kg body weight, respectively.
\(^b\) Mean ± S.D. (n = 12 for all values).
\(^c\) Numbers in parentheses, percentage of control values.
\(^d\) Statistically significant (p < 0.001) as determined by unpaired Student’s t test.
\(^e\) Statistically significant (p < 0.002) as determined by unpaired Student’s t test.
In contrast, neither AMAP nor APAP treatment significantly inhibited cytosolic glutathione reductase activity (data not shown). The effects of AMAP and APAP on thioltransferrase activity are shown in Table III. Both compounds inhibited this enzyme activity 1 h after drug administration, and APAP was the better inhibitor. The AMAP treatment decreased thioltransferase activity in 83% of controls, whereas APAP decreased activity to about 74% of controls. In both cases, thioltransferase activity returned to control levels by 6 h after administration of the drug.

Effects of AMAP and APAP on Conversion of Xanthine Dehydrogenase to Xanthine Oxidase—Xanthine dehydrogenase is a widely distributed enzyme which is involved in the degradation of all purines. Studies indicate that sulfhydryl reagents and the oxidation of disulfides can convert this enzyme into an oxidase form (28). Following this conversion, the enzyme utilizes oxygen instead of NAD+ as an electron acceptor during the metabolism of purines. The conversion of the enzyme to the oxidase form with the subsequent production of superoxide anion and hydrogen peroxide has been implicated in the pathogenesis of ischemia-reperfusion injury to tissues (28).

The effects of AMAP and APAP on the conversion of xanthine dehydrogenase to the oxidase form are shown in Table IV. It is widely accepted that under normal circumstances the enzyme exists almost entirely in the NAD+-dependent dehydrogenase form (29). Our results indicate that only about 10% of the total xanthine dehydrogenase-oxidase activity exists in the oxidase form in control animals. This value agrees with the values obtained by other researchers (29). AMAP produced no significant alterations in the relative ratio of the two forms of the enzyme. However, there was a significant increase in the amount of the oxidase form present 1 h after APAP administration. As much as 42% of the total enzyme activity was detected as the oxidase form. The magnitude of this conversion exceeds that reported to occur in an ischemia-reperfusion injury (29). After 6 h, the fraction of the enzyme present as the oxidase form returned to control levels.

The total amounts of the xanthine oxidase-dehydrogenase enzyme activity remained fairly constant between treatments. There was a slight rise in the total activity in the livers of APAP-treated animals, but this rise was not found to be significant.

Effects of AMAP and APAP on Hepatic Adenine Nucleotide and Inorganic Phosphate Pools—The main endogenous substrates of the enzyme xanthine dehydrogenase-oxidase are the breakdown products of purine metabolism. Since adenine nucleotides comprise the major labile purine pool of the cell, the status of this pool was examined. The effects of AMAP and APAP on the hepatic levels of adenine nucleotides and inorganic phosphate are reported in Table V. In control animals, the bulk of the adenine nucleotide pool exists as ATP. ADP was also measured as well as minor amounts of AMP. The total adenine nucleotide pool was about 3.4 μmol/g of liver, wet weight. AMAP treatment moderately increased this total adenine nucleotide pool and lowered hepatic inorganic phosphate levels.

A much different effect was seen following APAP administration. In APAP-treated animals, adenine nucleotide pools were significantly decreased below control levels. This decrease was greatest at 1 h following the administration of the drug.
drug. At this time point, total adenine nucleotide pools were decreased to 53.4% of controls. The major nucleotide which contributed to this loss was ATP. At 6 h, the total adenine nucleotide pool recovered to some degree, but it was still significantly depressed. Hepatic inorganic phosphate levels were also affected by APAP administration, and these levels reflected the changes seen in the adenine nucleotide pool in that inorganic phosphate levels were elevated at 1 and 6 h after the APAP treatment.

Effects of AMAP and APAP on Urinary Allantoin Levels—The final breakdown product of purine metabolism in mice is allantoin. The enzyme urate oxidase metabolizes uric acid to allantoin, and this compound is excreted in the urine. Control urinary allantoin levels were determined to be 16.4 pmol of allantoin excreted per 100 g body weight in 5 h (Table VI). The APAP treatment elevated urinary allantoin levels over control values while the AMAP treatment had no significant effect.

Effects of Allopurinol on APAP-induced Hepatotoxicity—The xanthine oxidase inhibitor, allopurinol has been shown to inhibit APAP toxicity (14, 30). Fig. 1 indicates that allopurinol is also effective in inhibiting the APAP-induced toxicity observed in the present study. Serum alanine aminotransferase values increased from control values of 40 units/liter to 22,225 units/liter 24 h after APAP administration. Administration of allopurinol prevented this large increase. Serum alanine aminotransferase values were about 480 units/liter 24 h after APAP administration in allopurinol-pretreated animals.

**FIG. 1.** The effects of allopurinol on acetaminophen-induced increases in serum alanine aminotransferase levels. A, control animals (n = 5); B, APAP-treated animals (n = 8). Animals were given APAP at a dose of 250 mg/kg. After 24 h, serum samples were collected and serum ALT levels were measured. C, APAP + allopurinol-treated animals (n = 5). Allopurinol was administered 3 h prior to APAP at a dose of 125 mg/kg. APAP was administered as before. Serum ALT levels were measured at 24 h after APAP administration.

**DISCUSSION**

The data in the present study suggest that APAP administration produces a complex sequence of events which may lead to the transient production of activated oxygen, the loss of protein thiols, and eventually to cell death. The proposed sequence of events are outlined in Fig. 2. APAP is first oxidized by cytochrome P 450 to the reactive metabolite NAPQI (4). Following APAP administration, both the mitochondrial and cytosolic pools of GSH are depleted (12). Once GSH is depleted, cellular proteins are arylated, presumably, by the reactive metabolite NAPQI (6). In addition, cytosolic glutathione peroxidase activity is inhibited in APAP-treated animals. The inhibition of this enzyme as well as the depletion of GSH may make the cell very vulnerable to the deleterious effects of activated oxygen species. Even under normal circumstances, hydrogen peroxide and superoxide anion are generated in the cell from a variety of sources (31). This background production of activated oxygen may become significant if the GSH-glutathione peroxidase detoxification pathway is compromised. Under such circumstances, the pro-
Calcium as a result of this plasma membrane calcium-ATPase displayed decreased plasma membrane calcium-ATPase activity and impaired mitochondrial calcium sequestration 1 h after receiving the drug (12). The influx of extracellular calcium as a result of this plasma membrane calcium-ATPase inhibition in addition to the loss of the ability of mitochondria to sequester calcium may lead to large-scale calcium cycling by mitochondria.

This calcium cycling may have several important consequences for cell function. Thomas and Reed (37) suggest that calcium cycling may be involved in the production of oxidative stress in isolated hepatocytes by an unknown mechanism. Calcium has also been shown to increase hydrogen peroxide production by isolated rat heart mitochondria (38). In addition, calcium cycling decreases the rate of ATP synthesis by cell mitochondria (39). The uptake of calcium by mitochondria is known to require respiratory energy and to take precedence over ATP formation (40). This decreased ATP synthesis may lead to the increased breakdown of adenosine nucleotides in response to energy requiring cellular metabolism.

Our results agree with this hypothesis. The total liver adenine nucleotide pool declined to about 50% of control levels 1 h after APAP administration, and this decrease coincided with the loss in the ability of mitochondria to sequester calcium (12). The degradation of this pool is also supported by the increase in the amount of the terminal purine breakdown product, allantoin, excreted in the urine in APAP-treated mice. Our results also indicate that APAP administration promotes the conversion of the enzyme xanthine dehydrogenase to the oxidase form. This conversion in conjunction with the degradation of the adenine nucleotide pool may lead to the production of activated oxygen species and the subsequent oxidation of protein thiols as seen in this study. This loss in protein thiols may in turn lead to cell death. Studies with a variety of toxic agents have correlated the loss of protein thiols with the development of cellular toxicity (41-43).

The mechanism we have proposed for the development of APAP hepatotoxicity is supported by several lines of evidence. Incubation of isolated hepatocytes with DTT after APAP (44, 45) or NAPQI (35, 45) exposure decreased toxicity. The DTT treatment had no effect on total covalent binding levels, and both studies concluded that the oxidation of protein thiols by APAP and NAPQI may be an important event in the development of toxicity. Also, fructose has been shown to potentiate APAP toxicity (46). Previous studies have shown that fructose promotes the degradation of liver adenine nucleotides (47), as was observed in the present report with APAP. In addition, Beales et al. (48) showed that calcium-EDTA protects isolated hepatocytes against APAP-induced toxicity. One mechanism proposed by these authors to account for this protection involved the buffering of cytosolic calcium. Buffering cytosolic calcium may also prevent calcium cycling.

Finally, we have demonstrated that the xanthine oxidase inhibitor allopurinol protects against the hepatotoxicity of APAP. These data agree with observations of other researchers (14), and in a recent abstract (30) allopurinol was shown to protect against APAP-induced toxicity without altering APAP-reactive metabolite formation. It was concluded that APAP promotes neutrophil accumulation in the liver of APAP-treated mice. The data presented here suggest that allopurinol also may protect against APAP toxicity by inhibiting liver xanthine oxidase. The magnitude and the time course of the conversion of the enzyme from the dehydrogenase to the oxidase form cannot be explained solely by the accumulation of neutrophils in the liver.

A major argument against the occurrence of oxidative stress in APAP toxicity in vivo is the lack of GSSG that is excreted in the bile in response to APAP toxicity (49). Our results suggest a possible explanation for this observation. We propose that oxidative stress occurs shortly after APAP administration in mice. At this time, activated oxygen species are produced in liver cells which are substantially depleted of GSH and that have impaired cytosolic glutathione peroxidase activity.
and thioltransferase activities. However, we observed no inhibition of cystolic glutathione reductase in APAP-treated mice. It is, therefore, likely that the small amounts of GSSG formed in the cytosol during this period will be rapidly reduced back to GSH. Since mitochondria contain their own glutathione peroxidase and reductase activity, these observations may not apply to this organelle. Indeed, high levels of GSSG have been observed in mitochondria after APAP administration (12).

It is important to note that the oxidative stress that we suggest occurs in APAP toxicity is transient in nature, and is complete before the onset of the APAP 6-h time point. Both mitochondrial and cytosolic GSH levels have returned to near control levels 6 h after receiving APAP, and the ability of liver mitochondria to sequester calcium has returned to normal (12). In addition, the fraction of the xanthine dehydrogenase-oxidase enzyme present in the oxidase form returned to control levels at this time point. However, hepatic protein thiols did not return to control levels despite the increase in cellular GSH and the return to control levels of thioltransferase activity. This may relate to the inaccessability of certain protein disulfides to GSH. Studies have indicated that protein disulfide bonds are frequently resistant to reduction by GSH under physiological conditions (50).

AMAP is not hepatotoxic to mice despite considerable covalent binding to cellular proteins by its reactive metabolites (12). In contrast to APAP, AMAP does not induce calcium cycling or decreases in liver adenine nucleotides. Thus, AMAP would not be expected to generate large-scale oxidative stress according to the mechanism proposed for APAP. In agreement with this hypothesis, AMAP did not significantly affect liver protein thiols. APAP inhibited hepatic protein thiols but APAP did not. In agreement with this hypothesis, AMAP did not deplete mitochondrial GSH to a greater extent than AMAP. Both AMAP and APAP deplete cytosolic GSH, but APAP depletes mitochondrial GSH to a greater extent than AMAP. This difference may be important for the development of toxicity. Recently, we observed that AMAP is hepatotoxic if mice are pretreated with the GSH synthesis inhibitor buthionine sulfoximine. This effect may relate to the combined ability of buthionine sulfoximine and AMAP to deplete mitochondrial GSH. Depletion of mitochondrial GSH may in turn promote the oxidation of critical cellular thiols. Further research is required to examine this hypothesis.

We present results in the present study which suggest that APAP can induce oxidative stress by a novel mechanism. Additional studies are required to examine this mechanism and to determine if it may be applicable for other hepatotoxic agents.

Acknowledgment—We would like to acknowledge Cheryl Peters for her assistance in preparing this manuscript.

REFERENCES

1. Davidson, D. G. D., and Eastham, W. N. (1966) Br. Med. J. 2, 497–499
2. Prescott, L. F., Wright, N., Roscoe, P., and Brown, S. S. (1971) Lancet I, 519–522
3. Davis, D. C., Potter, W. Z., Jollow, D. J., and Mitchell, J. R. (1974) Life Sci. 14, 2059–2100
4. Dahlin, D. C., Miwa, G. T., McDaniel, A. Y. H., and Nelson, S. D. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 1327–1331
5. Harvison, P. S., Guengerich, F. P., Rashed, M. S., and Nelson, S. D. (1988) Chem. Res. Toxicol. 1, 47–52
6. Hoffman, K., Streeter, A. J., Axworthy, D. B., and Baillie, T. A. (1986) Mol. Pharmacol. 27, 566–573
7. Jollow, D. J., Mitchell, J. R., Potter, W. Z., Davis, D. C., Gillette, J. R., and Brodie, B. B. (1973) J. Pharmacol. Exp. Ther. 187, 195–202
8. Wendel, A., and Feuerstein, S. (1981) Biochem. Pharmacol. 30, 2513–2520
9. Gerson, R. J., Casini, A., Gilfor, D., Serroni, A., and Farber, J. L. (1985) Biochem. Biophys. Res. Commun. 126, 1129–1137
10. Streeter, A. J., Sjogren, S. M., Axworthy, D. B., Nelson, S. D., and Baillie, T. A. (1984) Drug Metab. Dispos. 12, 565–576
11. Nelson, E. B. (1980) Res. Commun. Chem. Pathol. Pharmacol. 28, 447–456
12. Tirmenstein, M. A., and Nelson, S. D. (1989) J. Biol. Chem. 264, 9814–9819
13. Segel, I. H., and Johnson, M. J. (1963) Anal. Biochem. 5, 390–397
14. Gale, G. R., and Smith, A. B. (1988) Res. Commun. Chem. Pathol. Pharmacol. 50, 305–320
15. Dills, R. L., and Kraassen, C. D. (1985) J. Pharmacol. Methods 14, 189–197
16. Pogolotti, A. L., and Santi, D. V. (1982) Anal. Biochem. 126, 335–349
17. Lowry, O. H., and Burk, R. F. (1976) Biochem. Biophys. Res. Commun. 71, 952–958
18. Worthington, D. J., and Rosemeyer, M. A. (1974) Eur. J. Biochem. 48, 167–177
19. Schenkenrath, L., and Cinti, D. L. (1978) Methods Enzymol. 52, 83–89
20. Mannervik, B., Axelsson, K., and Larsson, K. (1981) Methods Enzymol. 77, 281–285
21. Row, P. B., and Wyngaard, J. B. (1966) J. Biol. Chem. 241, 5571–5576
22. Stirpe, F., and Della Corte, E. (1969) J. Biol. Chem. 244, 3855–3863
23. Heinonen, J. K., and Lahiri, R. J. (1981) Anal. Biochem. 113, 313–317
24. Lawry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
25. Peterson, G. L. (1977) Anal. Chem. 39, 346–356
26. Di Monte, D., Ross, D., Dellouo, G., Eklow, L., and Orrenius, S. (1984) Arch. Biochem. Biophys. 235, 334–342
27. Borchers, R. (1977) Anal. Biochem. 79, 612–613
28. Parks, D. A., and Granger, D. N. (1986) Acta Pharmacol. Scand. 548, (suppl.) 87–99
29. Chambers, D. E., Parks, D. A., Patterson, G., Roy, R., McCord, J. M., Yoshida, S., Parmley, L. F., and Downey, J. M. (1985) J. Mol. Cell. Cardiol. 17, 145–152
30. Jasek, H., and Mitchell, J. R. (1989) FASEB J. 3, A920
31. Chance, B., Sies, H., and Boveris, A. (1979) Physiol. Rev. 59, 557–608
32. Blair, I. A., Robb, A. R., Davis, D. S., and Cump, T. M. (1980) Tetrahedron Lett. 21, 4947–4950
33. Albano, E., Rundgren, M., Harvison, P. J., Nelson, S. D., and Moldeus, P. (1986) Mol. Pharmacol. 28, 506–511
34. Coles, B., Wilson, I., Wardman, P., Hinson, J., A., Nelson, S. D., and Ketterer, B. (1988) Arch. Biochem. Biophys. 264, 253–260
35. Runnem, M., Porubek, D. J., Harvison, P. J., Cote, G., Cote, G., I. A., Moldeus, P., and Nelson, S. D. (1988) Mol. Pharmacol. 34, 566–572
36. Nicotera, P., Rundgren, M., Porubek, D. J., Cote, G., Cote, G., I., Moldeus, P., Orrenius, S., and Nelson, S. D. (1989) Chem. Res. Toxicol. 2, 45–50
37. Thomas, C. E., and Reed, D. J. (1988) J. Pharmacol. Exp. Ther. 245, 501–507
38. Cadenas, E., and Boveris, A. (1988) Biochem. J. 258, 31–37
39. Richter, C., and Frei, B. (1988) Free Rad. Biol. Med. 4, 365–375
40. Vercesi, A., Reynafarje, B., and Lehniger, A. L. (1978) J. Biol. Chem. 253, 6279–6285
41. KU, R. H., and Billings, R. E. (1986) Arch. Biochem. Biophys. 247, 183–189
Acetaminophen-induced Oxidation of Protein Thiols

42. Pascoe, G. A., Olafsdottir, K., and Reed, D. J. (1987) Arch. Biochem. Biophys. 256, 150-158
43. Orrenius, S. (1985) Trends Pharmacol. Sci. (FEST suppl.) 1-4
44. Tee, L. B. G., Boobis, A. R., Huggett, A. C., and Davies, D. S. (1986) Toxicol. Appl. Pharmacol. 83, 294-314
45. Moore, M., Thor, H., Moore, G., Nelson, S., Moldeus, P., and Orrenius, S. (1985) J. Biol. Chem. 260, 13035-13040
46. Price, V. F., and Jollow, D. J. (1989) Biochem. Pharmacol. 38, 289-297
47. van den Berghe, G., Bronfman, M., Vanneste, R. and Hers, H.-G. (1977) Biochem. J. 162, 601-609
48. Beales, D., Hue, D. P., and McLean, A. E. M. (1985) Biochem. Pharmacol. 34, 19-23
49. Smith, C. V., and Mitchell, J. R. (1985) Biochem. Biophys. Res. Commun. 133, 329-336
50. Davidson, B. E., and Hird, F. J. R. Biochem. J. 104, 473-479
51. Hebbel, R. P., Sleczka, O., Filer, W., and Rank, B. H. (1986) Biochem. Biophys. Acta 862, 8-16
Acetaminophen-induced oxidation of protein thiols. Contribution of impaired thiol-metabolizing enzymes and the breakdown of adenine nucleotides.
M A Tirmenstein and S D Nelson

J. Biol. Chem. 1990, 265:3059-3065.

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