Biochemical Pharmacology, Vol. 42, No. 6, pp. 1189–1194, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00
Pergamon Press plc

RELATIONSHIP BETWEEN α-NAPHTHYLISOTHIOCYANATE-INDUCED LIVER INJURY AND ELEVATIONS IN HEPATIC NON-PROTEIN SULFHYDRYL CONTENT

LAWRENCE J. DAHM,* MARC B. BAILIE and ROBERT A. ROTH†
Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824, U.S.A.

(*Received* 2 *March* 1990; *accepted* 1 *April* 1991)

Abstract—Acute administration of α-naphthylisothiocyanate (ANIT) to rats has been used as a model of intrahepatic cholestasis. The mechanism of toxicity of ANIT is unknown, although recent evidence suggests a causal or permissive role for glutahione (GSH) (Dahm LJ and Roth RA, *Biochem Pharmacol* **42**: 1181–1188, 1991). In these studies, ANIT treatment elevated hepatic non-protein sulfhydryl (NPSH) content, an indicator of GSH content, when liver injury was evident. The purpose of the present study was to characterize the effects of ANIT on hepatic NPSH content and to relate these changes to the development of liver injury. In rats fasted for 24 hr, administration of ANIT (100 mg/kg, *per os* [p.o.]) did not change hepatic NPSH content, bile flow, or serum measurments of total bilirubin concentration, alanine aminotransferase (ALT) activity, or γ-glutamyltransferase (GGT) activity by 12 hr post-treatment relative to corn oil vehicle controls. However, by 24 hr after ANIT treatment, rats exhibited cholestasis and elevations in serum markers of liver injury. These markers were associated temporally with an increase in hepatic NPSH content, which consisted entirely of GSH. To determine whether the cholestasis caused by ANIT treatment might have caused the elevation of hepatic NPSH content, an extrahepatic cholestasis in rats was produced by ligation of the common bile duct. Bile duct ligation elevated hepatic NPSH content between 6 and 12 hr after ligation. Administration to rats of a non-hepatotoxic analog of ANIT, β-naphthylisothiocyanate, also elevated hepatic NPSH content 24 hr after treatment. Taken together, these results indicate that the elevation in hepatic NPSH content after ANIT treatment is associated temporally with the onset of liver injury, but this elevation does not appear to participate causally in the mechanism of injury.

The tripeptide glutathione (GSH)‡ is the major soluble intracellular thiol in mammalian cells, and it serves many functions. GSH supplies reducing equivalents to glutathione peroxidase for the reduction of hydrogen peroxide and organic hydroperoxides, which may be generated during oxidative stress. As a result, hydroperoxide-induced peroxidation of membrane lipids may be prevented. In addition, GSH functions as a coenzyme for glyoxalase and formaldehyde dehydrogenase [1]. GSH is also involved in the metabolism of electrophilic compounds, including xenobiotic agents and endogenous compounds, to mercapturic acids in the liver and other tissues.

In most instances, conjugation of a xenobiotic agent with GSH serves as a detoxification mechanism. For example, bromobenzene is bioactivated by hepatic, cytochrome P450-dependent mixed-function oxidases to an epoxide intermediate, which may cause hepatotoxicity by binding covalently to cellular macromolecules [2]. GSH provides protection presumably by conjugating to the epoxide. As expected, depletion of hepatic GSH content with diethyl maleate exacerbates the liver injury caused by bromobenzene [2].

Although GSH conjugation is usually a detoxification mechanism, certain agents become more toxic when conjugated to GSH. These include the nephrotoxicants trichloroethylene, 1,2-dibromoethane, and others [reviewed in Ref. 3]. Certain of these toxic GSH *S*-conjugates may cause injury by forming a reactive episulfonium ion. Other GSH *S*-conjugates may be metabolized ultimately by cysteine conjugate β-lyase to reactive thiols which are thought to cause injury [3].

GSH may be involved in tissue injury by mechanisms other than formation of toxic GSH *S*-conjugates of xenobiotic agents. For example, GSH is required in the formation of leukotriene C_4 (LTC_4) from LTA_4. LTC_4 and other thiol ether leukotrienes such as LTD_4 and LTE_4 comprise slow reacting substance of anaphylaxis which has been implicated in the adult respiratory distress syndrome, asthma, and inflammatory disorders [4]. Thiol ether leukotrienes have also been implicated in liver injury [5–7].

Studies in our laboratory indicate that GSH may be involved in α-naphthylisothiocyanate (ANIT)-

* Present address: Department of Biochemistry, Emory University School of Medicine, Atlanta, GA 30322.

† Send reprint requests to: Dr. Robert A. Roth, Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824.

‡ Abbreviations: GSH, glutathione; ANIT, α-naphthylisothiocyanate; NPSH, non-protein sulfhydryl; GSSG, gluathione disulfide; CO, corn oil; GGT, γ-glutamyltransferase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BNIT, β-naphthylisothiocyanate; LT, leukotriene; and DTNB, 5,5′-dithio-bis-(2-nitrobenzoic acid).

induced liver injury to rats, although the exact mechanism of toxicity is not known [8]. We observed that ANIT treatment elevated hepatic non-protein sulfhydryl (NPSH) content, an indicator of GSH content, when liver injury was evident. The purpose of the present study was to characterize elevations in hepatic NPSH content after ANIT treatment and to relate these changes to the development of liver injury.

MATERIALS AND METHODS

Materials. ANIT, GSH, 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB), purified glutathione reductase, Kit 605-D for bilirubin determination, and Kit 505 for aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were purchased from the Sigma Chemical Co. (St. Louis, MO). β-Naphthylisothiocyanate (BNIT) was obtained from the Aldrich Chemical Co. (Milwaukee, WI). All other reagents were of the highest grade commercially available. Polyethylene (PE) 10 tubing was purchased from Clay Adams (Parsippany, NJ). Ethilon® surgical suture was obtained from Ethicon (Somerville, NJ).

Animals. Male, Sprague–Dawley rats (CF:CD(SD)BR) (Charles River, Portage, MI) weighing 220–320 g were housed in plastic cages on aspen chip bedding under conditions of controlled temperature (18–21°) and humidity (55 ± 5%). A 12-hr light/12-hr dark cycle was maintained. Rats were allowed tap water and rat chow (Wayne Lab Blox, Allied Mills, Chicago, IL) *ad lib.* prior to experimentation.

Treatment protocol in studies with ANIT and BNIT. Rats were fasted for 24 hr prior to experimentation and for the remainder of the study. They were treated with ANIT (100 mg/kg, *per os* [p.o.]), BNIT (100 mg/kg, p.o.), or an equivalent volume of corn oil (CO) vehicle. At 0–24 hr after treatment, rats were anesthetized with an intraperitoneal (i.p.) injection of sodium pentobarbital (50 mg/kg) and placed on a heating pad to maintain body temperature. Following a midline laparotomy, the common bile duct was cannulated with PE 10 tubing, and bile flow was measured as described previously [8].

A blood sample was taken from the descending aorta for measurements of serum markers of liver injury. Total bilirubin concentration (Sigma Kit 605-D), γ-glutamyltransferase (GGT) activity, and AST activity were measured as described previously [8]. Serum ALT activity (Sigma Kit 505) was measured spectrophotometrically by monitoring formation of the phenylhydrazone of pyruvate. Hepatic non-protein sulfhydryl (NPSH) content was measured as an indicator of GSH content by the method of Ellman [9] as described by Costa and Murphy [10]. Total GSH equivalents (GSH + 2 glutathione disulfide [GSSG]) were measured by the glutathione reductase-coupled recycling method of Tietze [11].

Treatment protocol for bile duct ligation study. Rats were fasted for 24 hr prior to experimentation and for the remainder of the experiment. They were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and a midline incision was made. The common bile duct was isolated and ligated at two sites with 3–0 surgical silk. In sham-operated, control rats, the bile duct was isolated but not ligated. Abdominal muscles were closed with 4–0 Ethilon®, and the skin was closed with surgical staples.

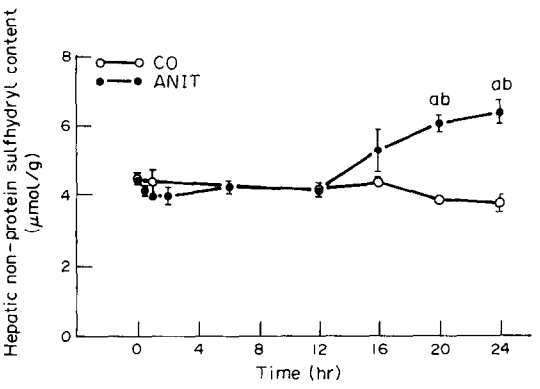

Fig. 1. Effect of ANIT on hepatic NPSH content. Rats were fasted prior to treatment with either ANIT (100 mg/kg, p.o.) or an equivalent volume of CO. Hepatic NPSH content was measured 0–24 hr later. Values are means ± SEM, N = 3–4. Key: (a) significantly different from CO group at the same time point, $P < 0.05$; and (b) significantly different from 0 hr time point, $P < 0.05$.

At 6–48 hr after surgery, rats were anesthetized with sodium pentobarbital (50 mg/kg,i.p.). A blood sample was taken from the descending aorta for determination of serum total bilirubin concentration to confirm cholestasis. Hepatic NPSH content was measured as described above.

Statistical analysis. Results are expressed as means ± SEM. Homogeneity of variance was tested using the F-max test. Log transformations were performed on nonhomogenous data. If the variances were homogeneous, data were analyzed using Student's *t*-test or a completely randomized analysis of variance, as appropriate. Individual comparisons between treatment means were made with Tukey's ω test [12]. When the variances were nonhomogeneous after log transformation of data, the data were analyzed with the nonparametric, distribution-free, multiple comparison test [13]. The criterion for significance was $p < 0.05$ for all comparisons.

RESULTS

Effects of ANIT on hepatic NPSH content and markers of liver injury. ANIT administration to rats did not change hepatic NPSH content up to 12 hr after treatment when compared to CO controls (Fig. 1). Bile flow and serum values for total bilirubin concentration, ALT activity, and GGT activity were also unchanged 12 hr after ANIT treatment (Fig. 2). By 20–24 hr, however, hepatic NPSH content was elevated (Fig. 1), and this increase appeared to result specifically from an increase in glutathione (Table 1). By 24 hr, ANIT-treated rats exhibited cholestasis and had elevations in serum of total bilirubin concentration and ALT and GGT activities (Fig. 2). ANIT treatment of rats caused cholestasis

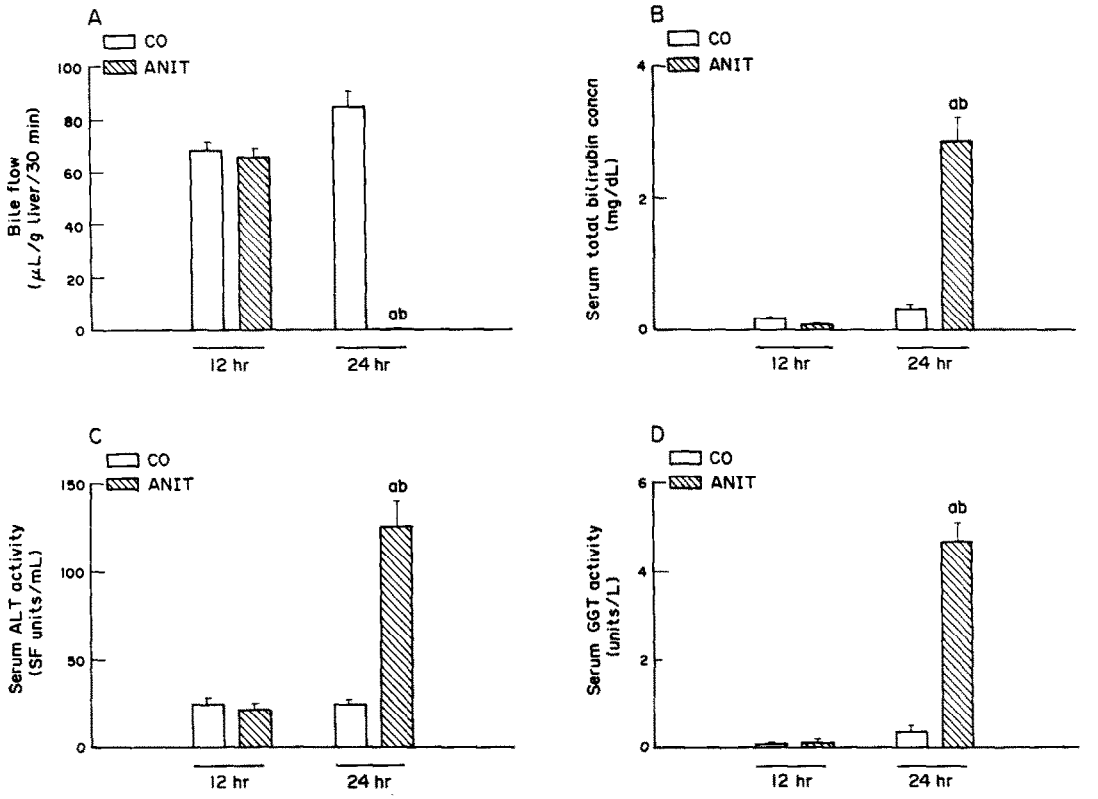

Fig. 2. Liver injury caused by ANIT 12 and 24 hr after treatment. Rats were fasted prior to treatment with either ANIT (100 mg/kg, p.o.) or an equivalent volume of CO. At 12 or 24 hr, bile flow (A), and serum determinations of total bilirubin concentration (B), ALT activity (C), and GGT activity (D) were made. Abbreviations: concn., concentration; and SF, Sigma Frankel. Values are means ± SEM, N = 3–4. Key: (a) significantly different from group treated with CO at 12 hr, $P < 0.05$; and (b) significantly different from group treated with CO at 24 hr, $P < 0.05$.

Table 1. Effect of ANIT on hepatic NPSH and glutathione content

Treatment*	NPSH†	Glutathione equivalents† (GSH + 2 GSSG)
CO	3.4 ± 0.2	3.2 ± 0.3
ANIT	7.0 ± 0.6‡	7.5 ± 0.6‡

* Rats were fasted for 24 hr and were treated with either ANIT (100 mg/kg, p.o.) or CO vehicle. Hepatic NPSH and glutathione content were measured 24 hr later. Values are means ± SEM, N = 3–4.

† Values are expressed as μmol/g liver.

‡ Significantly different from respective CO group, $P < 0.05$.

and elevations in serum markers of liver injury as early as 18 hr after treatment (data not shown). Thus, markers of liver injury and the elevation of hepatic NPSH content changed temporally together after ANIT treatment.

Effects of BNIT on hepatic NPSH content and markers of liver injury. BNIT is a non-hepatotoxic isomer of ANIT [14, 15], and it was used to assess whether ANIT-induced changes in hepatic NPSH content were linked to hepatotoxicity. Administration of BNIT to rats at a dose equivalent to that of ANIT (100 mg/kg, p.o.) did not cause cholestasis or elevate serum markers of liver injury 24 hr later (Table 2). However, BNIT treatment did elevate hepatic NPSH content (Table 2).

Effects of bile duct ligation on hepatic NPSH content. To determine whether the cholestasis caused by ANIT treatment might have elevated hepatic NPSH content, an extrahepatic cholestasis was produced by bile duct ligation. Elevations in serum total bilirubin concentration by 6 hr after bile duct ligation in rats confirmed cholestasis (Fig. 3A). Ligation of the common bile duct caused elevations in hepatic NPSH content compared to sham-operated controls starting between 6 and 12 hr after ligation (Fig. 3B). Hepatic NPSH content as well as total bilirubin concentration remained elevated for the duration of the study (48 hr).

DISCUSSION

ANIT is a cholestatic agent which causes injury to bile duct epithelial cells and hepatocytes, primarily in periportal regions of the liver [16, 17]. In the present study, ANIT caused liver injury as assessed

Table 2. Effects of BNIT on hepatic NPSH content and markers of liver injury

Treatment*	Hepatic NPSH content (μmol/g)	Bile flow (μL/g liver/30 min)	Serum total bilirubin concentration (mg/dL)	Serum AST activity (SF units/mL)	Serum GGT activity (U/L)
CO	3.3 ± 0.1	63 ± 5	0.12 ± 0.01	174 ± 21	ND
BNIT	4.7 ± 0.2†	71 ± 7	0.13 ± 0.02	184 ± 21	ND

* Rats were fasted for 24 hr and were treated with either BNIT (100 mg/kg, p.o.) or CO vehicle. Hepatic NPSH content and markers of liver injury were measured 24 hr later. Values are means ± SEM, N = 4–8. ND = not detectable.
† Significantly different from respective CO group, $P < 0.05$.

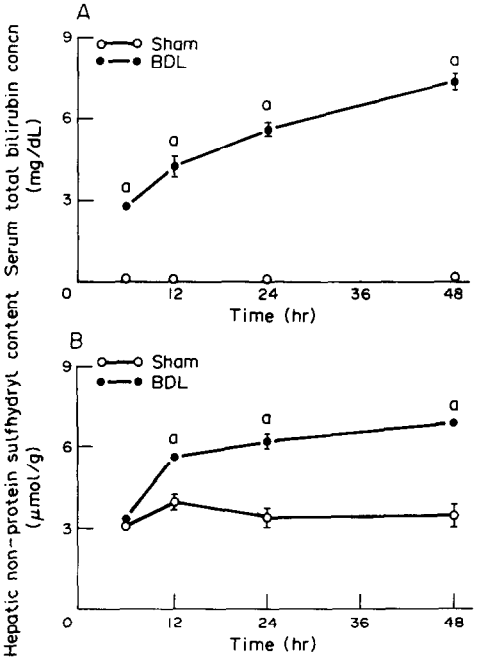

Fig. 3. Effect of bile duct ligation (BDL) on hepatic NPSH content. Rats were fasted prior to BDL or sham operation, and serum total bilirubin concentration (A) and hepatic NPSH content (B) were measured 6–48 hr later. Values are means ± SEM, N = 4–6. Key: (a) significantly different from sham-operated group at the same time point, $P < 0.05$.

by cholestasis and elevations in serum of total bilirubin concentration, transaminase (AST and ALT) activities, and GGT activity. Injury to bile duct epithelial cells and hepatocytes was assessed by serum GGT and transaminase activities, respectively [18–20]. Changes in markers of liver injury at various times after ANIT treatment were of a magnitude similar to those obtained by others [19, 21, 22]. However, Drew and Priestly [22] reported that ANIT caused elevations in plasma ALT activity at 2 and 12 hr after treatment. We did not observe any elevation in serum ALT activity at 12 hr. This difference in results may relate to the higher dose of ANIT (200 mg/kg) that they used.

In this study, hepatic NPSH content was elevated after ANIT treatment. The glutathione reductase-coupled recycling method, which specifically measures glutathione [11], was used to show that the elevation of hepatic NPSH content was due to glutathione. Although this method measures total glutathione equivalents (i.e. GSH + 2 GSSG), the results suggest that the elevation was due to increases in GSH, since NPSH content was increased to a similar extent and since the NPSH assay does not detect GSSG. Changes in hepatic NPSH content were not the result of any nutritional differences caused by ANIT treatment, since all rats were fasted throughout the study. The lack of a diurnal variation in hepatic NPSH content in this study was likely the result of fasting.

The mechanism of ANIT-induced liver injury is

unknown, although recent evidence suggests a causal or permissive role for NPSHs. For example, agents which deplete hepatic NPSH content, such as buthionine sulfoximine, diethyl maleate, and phorone, afford protection against the liver injury caused by ANIT [8]. The observation that ANIT elevates hepatic NPSH content does not by itself implicate NPSHs in the mechanism of toxicity. However, since this elevation is associated temporally with the onset of ANIT-induced liver injury and since NPSHs appear to be involved in the mechanism of injury [8], we determined whether the elevation of hepatic NPSH content was linked to the development of liver injury.

Two lines of evidence suggest that the elevation of hepatic NPSH content is unrelated to the mechanism of toxicity. The first was the result of the study with BNIT. BNIT has some effects on the liver that are similar to those of ANIT, but it is not hepatotoxic [14, 15]. For example, like ANIT, acute administration of BNIT to rats alters hepatic mixed-function oxidase content and activity, but it does not affect markers of liver injury as does ANIT [14, 15]. As expected, BNIT administration to rats did not cause cholestasis or elevate any marker of liver injury. However, BNIT elevated hepatic NPSH content 24 hr after treatment. Since BNIT was not hepatotoxic yet caused an elevation in hepatic NPSH content, it seems unlikely that the elevation of NPSH content after ANIT treatment is critical to the mechanism of toxicity.

A second approach to assess the mechanism and significance of elevated hepatic NPSH content in ANIT hepatotoxicity was bile duct ligation. Although bile duct ligation probably does not reflect completely the liver injury caused by ANIT intoxication, it does produce some similar pathological changes in the liver [23, 24], and ANIT is thought to cause cholestasis in part by obstruction of bile ductules [17, 24, 25]. Therefore, certain changes in the liver after bile duct ligation may be applicable to the ANIT model. In this study, bile duct ligation elevated hepatic NPSH content. Although we have not specifically measured hepatic GSH content after bile duct ligation, it is likely that it accounts for the elevation of hepatic NPSH content, since buthionine sulfoximine, an inhibitor of GSH synthesis, prevented this increase (data not shown). That bile duct ligation elevated hepatic NPSH content suggests that the reduction in bile flow after ANIT administration may cause, in part, the elevation of hepatic NPSH content.

After bile duct ligation, serum total bilirubin concentration was greatly elevated by 6 hr (Fig. 3A), although hepatic NPSH content did not increase until 6–12 hr (Fig. 3B). This delay was not observed with ANIT. ANIT-induced cholestasis was manifested by 24 hr (Fig. 2A) and was evident as early as 18 hr (data not shown), and hepatic NPSH content was elevated between 16 and 20 hr (Fig. 1). Thus, these markers changed temporally together after ANIT treatment, whereas a lag of 6–12 hr occurred between bile duct ligation and elevation of hepatic NPSH content. This suggests that ANIT probably elevates hepatic NPSH content by mechanisms in addition to obstruction of bile ductules.

ANIT impairs the transport of cholephilic agents, i.e. bilirubin, across the canalicular membrane of hepatocytes [26], and it might similarly affect GSH transport. Thus, the mechanism by which ANIT-induced cholestasis increases hepatic NPSH content may relate to changes in hepatic glutathione turnover, which result almost entirely from efflux of glutathione across the sinusoidal and canalicular membranes of hepatocytes [27–30]. Studies *in vivo* in fasted rats have demonstrated that turnover of hepatic glutathione equivalents (i.e. GSH + 2 GSSG) is approximately 1.6 μmol/g/hr; efflux of glutathione across the basolateral membrane accounts for approximately 90% of this, whereas efflux across the canalicular membrane into bile comprises the remaining 10% (i.e. 0.17 μmol/g/hr) [30]. Since GSH accounts for approximately 80% of total glutathione equivalents in bile [31], one would expect the rate of biliary GSH efflux to be about 0.14 μmol/g/hr. Interestingly, this rate is very close to that for hepatic NPSH elevation (i.e. 0.19 μmol/g/hr) occurring 12–24 hr after ANIT administration (Fig. 1). Therefore, the increase in hepatic NPSH content may result from inhibition of GSH efflux across the canalicular membrane of hepatocytes.

In summary, ANIT administration to rats causes liver injury by a mechanism which appears to require NPSHs, i.e. GSH [8]. An elevation of hepatic NPSH content, consisting entirely of GSH was associated with the onset of liver injury, although this elevation probably did not contribute to the mechanism of toxicity. Further work is required to clarify how ANIT elevates hepatic GSH content.

Acknowledgements—The authors thank Kathleen M. Vorick for valuable technical assistance. This work was supported by USPHS Grant ES04139. L. J. D. was supported in part by a Proctor and Gamble Fellowship administered by the Society of Toxicology.

REFERENCES

1. Meister A, Glutathione. In: *The Liver: Biology and Pathobiology* (Eds. Arias IA, Jakoby WB, Popper H, Schachter D and Shafritz DA), 2nd Edn, pp. 401–417. Raven Press, New York, 1988.
2. Jollow DJ, Mitchell JR, Zampaglione N and Gillette JR, Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. *Pharmacology* **11**: 151–169, 1974.
3. Anders MW, Lash L, Dekant W, Elfarra AA and Dohn DR, Biosynthesis and biotransformation of glutathione *S*-conjugates to toxic metabolites. *CRC Crit Rev Toxicol* **18**: 311–341, 1988.
4. Bach MK, *The Leukotrienes: Their Structure, Actions, and Role in Diseases*, pp. 26–36. The Upjohn Company, Kalamazoo, 1983.
5. Keppler D, Hagmann W, Rapp S, Denzlinger C and Koch HK, The relation of leukotrienes to liver injury. *Hepatology* **5**: 883–891, 1985.
6. Hagmann W, Steffan A-M, Kirn A and Keppler D, Leukotrienes as mediators in frog virus 3-induced hepatitis in rats. *Hepatology* **7**: 732–736, 1987.
7. Tiegs G and Wendel A, Leukotriene-mediated liver injury. *Biochem Pharmacol* **37**: 2569–2573, 1988.

8. Dahm LJ and Roth RA, Protection against α-naphthylisothiocyanate-induced liver injury by decreased hepatic non-protein sulfhydryl content. *Biochem Pharmacol* **42**: 1181–1188, 1991.
9. Ellman GL, Tissue sulfhydryl groups. *Arch Biochem Biophys* **82**: 70–77, 1959.
10. Costa LG and Murphy SD, Effect of diethylmaleate and other glutathione depletors on protein synthesis. *Biochem Pharmacol* **35**: 3383–3388, 1986.
11. Tietze F, Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: Applications to mammalian blood and other tissues. *Anal Biochem* **27**: 502–522, 1969.
12. Steel RGD and Torrie JH, *Principles and Procedures of Statistics. A Biometrical Approach*, 2nd Edn, pp. 173–175, 185–186, 195–238, 336–347, and 390–393. McGraw-Hill, New York, 1980.
13. Gad G and Weil CS, *Statistics and Experimental Design for Toxicologists*, pp. 61–65. Telford Press, Caldwell, 1986.
14. Becker BA and Plaa GL, Hepatotoxicity of α-naphthylisothiocyanate cogeners with particular emphasis on phenylisothiocyanate. *Toxicol Appl Pharmacol* 7: 804–811, 1965.
15. El-Hawari AM and Plaa GL, Impairment of hepatic mixed-function oxidase activity by α- and β-naphthylisothiocyanate: Relationship to hepatotoxicity. *Toxicol Appl Pharmacol* **48**: 445–458, 1979.
16. Desmet VJ, Krstulović, B and van Damme B, Histochemical study of rat liver in alpha-naphthyl isothiocyanate (ANIT) induced cholestasis. *Am J Pathol* **52**: 401–421, 1968.
17. Goldfarb S, Singer EJ and Popper H, Experimental cholangitis due to alpha-naphthyl isothiocyanate (ANIT). *Am J Pathol* **40**: 685–697, 1962.
18. Moran E, Eliakim M, Suchowolski A and Ungar H, Serum vitamin B_{12} and glutamic-oxalacetic transaminase in experimental intrahepatic obstructive jaundice. *Gastroenterology* **49**: 408–415, 1961.
19. Leonard TB, Neptun DA and Popp JA, Serum gamma glutamyl transferase as a specific indicator of bile duct lesions in rat liver. *Am J Pathol* **116**: 262–269, 1984.
20. Zafrani ES, Bulle F, Mavier P, Préaux AM, Lescs MC, Siegrist S, Dhumeaux D and Guellaen G, Gamma-glutamyltranspeptidase in cholestasis. An histochemical, biochemical and molecular approach using two experimental models. *Hepatology* **10**: 682, 1989.
21. Ruwart MJ, Rush BD, Friedle NM, Stachura J and Tarnawski A, 16,16-Dimethyl-PGE_2 protection against α-naphthylisothiocyanate-induced experimental cholangitis in the rat. *Hepatology* **4**: 658–660, 1984.
22. Drew R and Priestly BG, Microsomal drug metabolism during α-naphthylisothiocyanate-induced cholestasis. *Toxicol Appl Pharmacol* **35**: 491–499, 1976.
23. Steiner JW, Phillips MJ and Baglio C, Electron microscopy of the excretory pathways in the liver in α-naphthylisothiocyanate intoxication. *Am J Pathol* **43**: 677–696, 1963.
24. Ungar H, Moran E, Eisner MN and Eliakim M, Rat intrahepatic biliary tract lesions from alpha-naphthyl isothiocyanate. *Arch Pathol* **73**: 427–435, 1962.
25. Woolley J, Mullock BM and Hinton RH, Reflux of biliary components into blood in experimental intrahepatic cholestasis induced in rats by treatment with α-naphthylisothiocyanate. *Clin Chim Acta* **92**: 381–386, 1979.
26. Roberts RJ and Plaa GL, Alteration of the plasma disappearance and biliary excretion patterns of exogenously administered bilirubin by α-naphthylisothiocyanate. *J Pharmacol Exp Ther* **15**: 330–336, 1966.
27. Sies H, Brigelius R and Akerboom TPM, Intrahepatic glutathione status. In: *Functions of Glutathione: Biochemical, Physiological, Toxicological, and Clinical Aspects* (Eds. Larsson A, Holmgren A, Orrhenius S and Mannervik B), pp. 51–64. Raven Press, New York, 1983.
28. Inoue MI, Kinne R, Tran T and Arias IM, The mechanism of biliary secretion of reduced glutathione. Analysis of transport process in isolated rat-liver canalicular membrane vesicles. *Eur J Biochem* **134**: 467–471, 1983.
29. Ookhtens M, Hobdy K, Corvasce MC, Aw TY and Kaplowitz N, Sinusoidal efflux of glutathione in the perfused rat liver. Evidence for a carrier-mediated process. *J Clin Invest* **75**: 258–265, 1985.
30. Lauterburg BH, Adams JD and Mitchell JR, Hepatic glutathione homeostasis in the rat: Efflux accounts for glutathione turnover. *Hepatology* **4**: 586–590, 1984.
31. Eberle D, Clarke R and Kaplowitz N, Rapid oxidation *in vitro* of endogenous and exogenous glutathione in bile of rats. *J Biol Chem* **256**: 2115–2117, 1981.