Journal of Biochemistry and Molecular Biology, Vol. 32, No. 1, January 1999, pp. 67–71

JOURNAL OF
Biochemistry
and
Molecular Biology

Benzoyltransferase and Phenylacetyltransferase Activities in Cholestatic Rat Liver Induced by Common Bile Duct Ligation

Young Jin Kim and You Hee Kim†*

Department of Otorhinolaryngology, Dongguk University College of Medicine, 1090-1, Sugjaang-dong, Kungju-si, Kyungpuk-do 780-350, Korea

†Department of Biochemistry, Keimyung University School of Medicine, Taegu 700-712, Korea

Received 31 August 1998, Accepted 3 November 1998

We have investigated the effect of cholestasis on the closely related acyl-CoA:amino acid N-acyltransferase, benzoyltransferase, and phenylacetyltransferase activities in rat liver. Benzoyltransferase and phenylacetyltransferase activities in the liver cytosol, mitochondria, and microsome were investigated for a period of 42 d after common bile duct ligation. Both the mitochondrial and microsomal benzoyltransferases showed significant increase in their activities between the 1st and 7th day after common bile duct ligation, although the cytosolic benzoyltransferase activity did not show a significant change compared to the activities from the sham-operated control. The cytosolic phenylacetyltransferase activity showed a significant increase between the 1st and 2nd day, the mitochondrial activity showed a significant increase between the 2nd and 7th day, and microsomal activity showed a significant increase between the 1st and 7th day, respectively. Enzyme kinetic parameters of hepatic benzoyltransferase were analyzed using benzoyl coenzyme A as a substrate with the preparations from the 1st day post-ligation. Enzyme parameters of hepatic phenylacetyltransferase were also analyzed using phenylacetyl coenzyme A as a substrate with the preparations from the 2nd day post-ligation. The results indicated that although the K_m values of these enzymes were about the same as the sham-operated control, the V_{max} values of both enzymes increased significantly. These results, therefore, suggest that the biosynthesis of benzoyltransferase and phenylacetyltransferase has been induced in response to cholestasis.

Keywords: Benzoyltransferase, Cholestatic rat liver, Enzyme induction, Phenylacetyltransferase.

Introduction

Two closely related acyl-CoA:amino acid N-acyltransferases, benzoyltransferase (benzoyl coenzyme A: amino acid N-acetyltransferase) and phenylacetyltransferase (phenylacetyl coenzyme A: amino acid N-acetyltransferase) have been identified in liver mitochondria (Webster, 1981). They catalyze the conjugation of aromatic carboxylic acids with amino acids to form the corresponding amides (Webster *et al.*, 1976). Benzoyltransferase is specific for benzoyl-CoA, salicyl-CoA, and short straight and branched chain fatty acyl-CoA esters (Nandi *et al.*, 1979), as well as 2,4-dichlorophenoxyacetyl-CoA and 2,4,5-trichlorophenoxyacetatyl-CoA as substrates (Kelly and Vassey, 1986), while phenylacetyltransferase is specific for phenylacetyl-CoA, indoleacetyl-CoA, phenoxyacetyl-CoA, and 2,4-dichlorophenoxyacetyl-CoA (Killenberg *et al.*, 1980). Glycine is the preferred acyl acceptor for both enzymes, but either L-asparagine or L-glutamine may also serve as acyl acceptors (Nandi *et al.*, 1979).

The amino acid conjugates are more water soluble and usually less toxic than their precursor acids and are rapidly excreted by mammals in the urine or bile. Conjugation thus generally serves to protect the organism against a variety of cyclic acids in the form of xenobiotics, drugs, and dietary compounds (Webster *et al.*, 1976; Killenberg *et al.*, 1980). In mammals, the liver and kidney are involved in the conjugation of aromatic carboxylates, and this is coincident with the occurrence of these two enzymes.

The common bile duct-ligated rats have been widely used as an experimental model for human extrahepatic cholestasis (Kaplan and Righetti, 1970; Righetti and Kaplan, 1972; Kryszewski *et al.*, 1973). In humans,

* To whom correspondence should be addressed.
Tel: 82-53-250-7462; Fax: 82-53-252-1605
E-mail: Noel@dsmc.or.kr

anatomical or mechanical obstruction of the common bile duct occurs most commonly due to gallstones, neoplasms, or strictures, and less commonly due to primary biliary cirrhosis, cholangitis, or hepatitis (Mezey, 1976; Rosalki, 1976). In chronic cholestatic livers in humans and rats, the hepatocytes are particularly susceptible, thus allowing functional and morphological derangements to develop into pathological conditions such as necrosis, inflammation, fatty changes, biliary hyperplasia, fibrosis, and cirrhosis (Desmet, 1979; Kountouras *et al.*, 1984; Chang, 1987; Kim *et al.*, 1989).

To understand the biochemical alterations occurring during cholestasis, many laboratories have studied several liver enzymes in experimental animals (Kwak, 1985; Kwak *et al.*, 1988; Mun and Kwak, 1989; Kwon *et al.*,1990; Kwak and Lee, 1992; Mun, 1994; Ra *et al.*, 1994; Ihm *et al.*, 1995; Ihm and Kim, 1997). However, the possible changes of the benzoyltransferase and phenylacetyltransferase activities under cholestasis induced by common bile duct ligation have not been investigated. Previously, however, we have reported that cholestasis induced by common bile duct ligation affects the isozyme pattern of hepatic aryl sulfotransferase in rats (Ihm *et al.*, 1995). We have also reported that thiosulfate sulfurtransferase (rhodanese) and UDP-glucuronosyltransferase activities are reduced during experimental cholestasis in rats (Ihm and Kim, 1979). All the enzymes were shown to mediate detoxification. In the present study, we have systematically investigated the liver benzoyltransferase and phenylacetyltransferase activities with the subcellular fractions prepared from cholestatic rat liver induced by common bile duct ligation for a period of 42 d. In addition, K_m and V_{max} values for these enzymes were also analyzed with the 1st or the 2nd d post-ligated rat liver preparations and compared to those values obtained from the sham-operated control.

Materials and Methods

Chemicals Benzoyl coenzyme A lithium salt, phenylacetyl coenzyme A lithium salt, 5,5′-dithiobis (2-nitrobenzoic acid), tris (hydroxymethyl) aminotransferase, glycine, and bovine albumin standard (10 g/100 ml) were purchased from Sigma Chemical Co. (St. Louis, USA).

Animals Normal male Sprague-Dawely rats, weighing between 320 and 350 g, were used for the experiments. All animals were maintained on a pellet diet obtained commercially (Sam Yang Foods Co., Wonju, Korea) and tap water. During surgery, rats were anesthesized lightly with ether, and the abdomen was opened through a median line incision to the liver. The common bile duct was pulled out and then doubly ligated close to the liver and excised just below the confluence of the lobular ducts. Control animals were subjected to a sham operation (midline laparatomy) as a control. Each experiment was carried out with a group of 5 rats. Rats were sacrificed after 0.5, 1, 2, 3, 7, 14, 28, and 48 d following the operation. The livers were excised following perfusion (see below), and blood collected from the abdominal aorta. The serum was separated by centrifugation and stored at −20°C until use. All animals had been fasted for 12 h prior to sacrifice or surgery.

Subcellular fractionation The livers were perfused via the portal vein with cold 0.25 M sucrose and then excised, blotted, weighed, minced, and homogenized in 9 vol of 0.25 M sucrose. Each homogenate was subjected to cell fractionation. Cytosol, mitochondria, and microsomes were isolated by the sucrose linear density gradient centrifugation method (Kwak and Kwak, 1986), and stored at −80°C. All the isolation procedures were performed at 2 to 4°C. The cytosolic, mitochondrial, and microsomal fractions (hepatic subcellular fractions) were used for the enzyme assays.

Enzyme assays Phenylacetyltransferase activity was assayed by monitoring the amino acid-dependent release of CoA thiol from the CoA ester substrate in the presence of 5,5′-dithiolbis (2-nitrobenzoic acid) according to the method of Webster (1981). Reaction mixtures contained in a final volume of 1 ml at 30°C contained the following: 25 μmol of Tris·HCl/10 μmol KCl buffer, pH 8.0, 0.2 mg of bovine serum albumin, 0.1 μmol of 5,5′-dithiobis (2-nitrobenzoic acid), 100 nmol of CoA ester, enzyme, and 100 μmol of glycine adjusted to pH 8.0. The final order of addition was CoA ester, enzyme, and amino acid. The initial linear rate was then recorded continuously at 412 nm with full-scale deflection on the recorder set at 0.1 optical density unit. With benzoyl-CoA as substrate, amino acid-dependent continuous disappearance of thioester absorbance at 280 nm was used for the enzymatic assay (Webster, 1981). Reaction mixtures were identical except that 5,5′-dithiobis (2-benzoic acid) was omitted. Units of enzymatic activity are given in micromoles of amino acid-dependent thiol formed or benzoyl-CoA cleaved per min at 30°C. Specific activities are defined as units of activity per units of absorbance of protein measured at 280 nm.

Determination of protein The protein concentrations of each subcellular fraction were determined by the biuret reaction (Gornall *et al.*, 1949), using bovine albumin as the reference protein.

Statistical analysis Values were expressed as mean ± SD. Statistical evaluation of the experimental data was done by the Student's t-test. P values of ≤ 0.05 were considered to be significant.

Results

The activity of benzoyltransferase of cholestatic rat liver increased significantly in the mitochondrial and the microsomal preparations after common bile duct ligation compared to the sham-operated control groups (Table 1). The activity of benzoyltransferase of cholestatic rat liver in the cytosolic preparation did not change after the operation compared to the control (Table 1). Cytosolic, mitochondrial, and microsomal phenylacetyltransferase activities in cholestatic rat liver increased significantly compared to the control groups (Table 2). V_{max} values of

Table 1. Activities of cytosolic, mitochondrial, and microsomal benzoyltransferase in cholestatic rat liver after common bile duct ligation.

Day(s) following ligation	Benzoyltransferase activities (nmol coenzyme A min^{-1} mg $protein^{-1}$)					
	Cytosol		Mitochondria		Microsome	
	liver of control	cholestatic liver	liver of control	cholestatic liver	live of control	cholestatic liver
0.5	7.8 ± 2.87	9.2 ± 3.13	54.6 ± 14.27	64.2 ± 18.85	33.5 ± 7.23	35.6 ± 6.29
1	7.5 ± 2.92	9.3 ± 3.25	55.2 ± 13.96	85.3 ± 20.26*	31.8 ± 6.85	52.9 ± 9.62**
2	7.7 ± 2.84	10.5 ± 3.14	53.4 ± 14.13	80.7 ± 21.34*	32.3 ± 7.18	55.4 ± 10.73**
3	7.5 ± 2.96	8.5 ± 2.87	53.0 ± 14.82	81.2 ± 18.84*	30.7 ± 6.94	53.0 ± 9.48**
7	7.3 ± 2.74	7.1 ± 2.92	51.8 ± 14.12	76.9 ± 19.07*	28.7 ± 6.42	42.3 ± 8.91*
14	7.1 ± 2.63	6.5 ± 2.74	49.2 ± 13.52	58.8 ± 16.15	27.8 ± 6.02	30.0 ± 7.83
28	7.2 ± 2.58	6.6 ± 2.83	49.6 ± 13.42	48.8 ± 15.41	26.4 ± 5.93	28.3 ± 6.42
42	7.0 ± 2.6	6.4 ± 2.96	48.5 ± 12.67	43.8 ± 14.18	26.7 ± 6.14	25.7 ± 5.52

The data are expressed as mean ± SD with 5 rats in each group.
Significant difference from sham-operated rat livers (*, $P<0.05$; **, $P<0.01$).

Table 2. Activities of cytosolic, mitochondrial, and microsomal phenylacetyltransferase in cholestatic rat liver after common bile duct ligation.

Day(s) following ligation	Phenylacetyltransferase activities (nmol coenzyme A min^{-1} mg $protein^{-1}$)					
	Cytosol		Mitochondria		Microsome	
	liver of control	cholestatic liver	liver of control	cholestatic liver	live of control	cholestatic liver
0.5	9.8 ± 2.83	10.4 ± 2.72	60.8 ± 14.62	57.0 ± 5.26	38.2 ± 9.84	36.5 ± 11.63
1	9.7 ± 2.62	14.4 ± 3.08*	61.2 ± 13.74	77.6 ± 20.78	30.1 ± 10.23	68.9 ± 18.12**
2	9.6 ± 2.54	13.5 ± 3.15*	60.5 ± 14.35	98.2 ± 27.15*	37.2 ± 9.76	60.3 ± 17.35*
3	9.6 ± 2.68	10.7 ± 2.74	60.9 ± 14.52	92.3 ± 20.46*	35.7 ± 9.28	56.4 ± 15.17*
7	9.4 ± 2.61	9.0 ± 2.64	58.2 ± 13.62	87.5 ± 22.35*	36.3 ± 8.92	55.2 ± 15.85*
14	9.5 ± 2.57	8.8 ± 2.26	56.6 ± 12.85	70.1 ± 17.23	32.3 ± 8.86	44.5 ± 14.68
28	9.4 ± 2.49	9.0 ± 1.86	56.0 ± 12.29	65.6 ± 14.9	33.4 ± 9.14	34.8 ± 9.18
42	9.3 ± 2.44	8.6 ± 2.72	56.3 ± 13.44	60.4 ± 11.76	32.6 ± 8.96	30.6 ± 9.09

The data are expressed as mean ± SD with 5 rats in each group.
Significant difference from sham-operated rat livers (*, $P<0.05$; **, $P<0.01$).

benzoyltransferase in cholestatic rat liver increased significantly compared to the control, although K_m values in these preparations did not change (Table 3). Similarly, V_{max} values of phenylacetyl transferase in the cytosolic, mitochondrial, and microsomal preparations of cholestatic liver increased significantly but K_m values of all the hepatic preparations did not change significantly after common bile duct ligation (Table 4).

Table 3. Benzoyltransferase kinetic parameters from cholestatic rat liver determined with benzoyl-coenzyme A.

Cell fraction	K_m (mM)		V_{max} (nmol coenzyme A min^{-1} mg $protein^{-1}$)	
	liver of control	cholestatic liver	liver of control	cholestatic liver
Mitochondria	0.31 ± 0.08	0.33 ± 0.09	65.4 ± 15.31	113.2 ± 23.27**
Microsome	0.30 ± 0.06	0.28 ± 0.04	38.9 ± 7.23	64.2 ± 12.35**

Michaelis-Menten constants for phenylacetyltransferase were determined using benzoyl-coenzyme A and glycine at 30°C for mitochondrial and microsomal fractions of sham-operated male rat livers (liver of control) and of cholestatic male rat livers at first day after common bile duct ligation. The data are expressed as mean ± SD with 5 rats in each group. Significant difference from sham-operated rat livers (**, $P<0.01$).

Discussion

The effects of cholestasis on the activities of several xenobiotic biotransforming enzymes have been studied in cholestatic rat liver. We have previously reported that

Table 4. Phenylacetyltransferase kinetic parameters from cholestatic rat liver determined with phenylacetyl-coenzyme A.

Cell fraction	K_m (mM)		V_{max} (nmol coenzyme A min^{-1} mg $protein^{-1}$)	
	liver of control	cholestatic liver	liver of control	cholestatic liver
Cytosol	0.64 ± 0.15	0.66 ± 0.18	12.0 ± 2.78	17.8 ± 3.43*
Mitochondria	0.29 ± 0.08	0.27 ± 0.06	74.1 ± 16.47	116.3 ± 30.24*
Microsome	0.32 ± 0.07	0.29 ± 0.09	48.4 ± 10.36	79.3 ± 20.16*

Michaelis-Menten constants for phenylacetyltransferase were determined using phenylacetyl-coenzyme A and glycine at 30°C for cytosolic, mitochondrial and microsomal fractions of sham-operated male rat livers (liver of control) and of cholestatic male rat livers at second day after common bile duct ligation. The data are expressed as mean ± SD with 5 rats in each group. Significant difference from sham-operated rat livers (*, $P<0.05$).

enzyme activities were increased in xanthine oxidase (Kwak *et al.*, 1985; Mun, 1994), the microsomal ethanol oxidizing system, aldehyde dehydrogenase (Kwak *et al.*, 1988), cytosolic arylsulfotransferase (Ihm *et al.*, 1995), glyoxalase I (Byun *et al.*, 1995), and arylamine acetyltransferase (Rhee and Kwak, 1988) under cholestasis induced by common bile duct ligation. We have also reported that enzyme activities were decreased in catalase, alcohol dehydrogenase (Kwak *et al.*, 1988), monoamine oxidase (Mun and Kwak, 1989; Chung and Kwak, 1992), glutathione S-transferase, glutathione peroxidase (Kwon *et al.*, 1990; Kwak *et al.*, 1990), arylesterase, carboxylesterase, cholinesterase (Kwak and Lee, 1992), microsomal arylsulfotransferase, mitochondrial arylsulfotransferase (Ihm *et al.*, 1995), catechol-O-methyltransferase (Mun, 1996), UDP-glucuronosyltransferase, and thiosulfate sulfurtransferase (Ihm and Kim, 1997) in cholestatic rat liver. In particular, benzoyltransferase and phenylacetyltransferase are xenobiotic biotransforming enzymes (Killenberg & Webster, 1980). Nevertheless, the changes of the benzoyltransferase and phenylacetyltransferase have not been studied under cholestasis induced by common bile duct ligation. In order to understand the effects of cholestasis on these enzyme activities, we have determined the activities of cytosolic, mitochondrial, and microsomal benzoyltransferase and phenylacetyltransferase in cholestatic rat liver induced by common bile duct ligation for a period of 42 d. Enzyme kinetic parameters (K_m and V_{max}) for benzoyltransferase were determined with the hepatic preparations from the first day post-ligation using benzoyl-CoA as a substrate. The values of K_m and V_{max} for hepatic phenylacetyltransferase were also analyzed at the second day post-ligation with phenylacetyl-CoA as a substrate.

The mitochondrial and microsomal benzoyltransferase activities in cholestatic rat liver showed significant increase from the first day to the seventh day subsequent to common bile duct ligation (Table 1). However, the activity of cytosolic benzoyltransferase did not show a significant change compared to the activity from the sham-operated control (Table 1). The cytosolic phenylacetyl transferase activity showed a significant increase in the early days of cholestasis; the increase was observed from the first day to the second day of post-ligation (Table 1). The activity of mitochondrial phenylacetyltransferase also increased in cholestatic rat liver beginning from the second day after the ligation continued to decrease until the 7th day compared to the sham-operated liver activity (Table 2). The microsomal phenylacetyltransferase activity increased markedly soon after the ligation; about a 2-fold increase was observed at the first day after ligation, and then the activity gradually decreased to the control level by the 28th day of post-ligation (Table 2).

In order to investigate whether the changes of the benzoyltransferase and phenylacetyltransferase activities in the cholestatic rat liver were due to alteration in their catalytic activity or not, K_m and V_{max} values were determined with the first and second day post-ligation preparations, respectively. As shown in Table 3, the K_m values of benzoyltransferase did not change significantly compared to those from the sham-operated liver in the preparations of mitochondria and microsome. However, V_{max} values significantly increased in both preparations; 65.4 nmol coenzyme A/min/mg protein vs. 113.5 for mitochondria and 38.9 nmol coenzyme A/min/mg protein vs. 64.2 for microsomal fractions, respectively. Similarly, the V_{max} values of phenylacetyltransferase also increased in all three preparations compared to the control; 12.0 nmol coenzyme A/min/mg protein vs. 17.8 for the cytosol, 74.1 nmol coenzyme A/min/mg protein vs. 116.3 for the mitochondria, and 48.4 nmol coenzyme A/min/mg protein vs. 79.3 for the microsomal fractions, although the K_m values did not change significantly in all hepatic fractions (Table 4).

It should be noted that the higher V_{max} values of phenylacetyltransferase in all subcellular fractions of common bile duct-ligated livers as well as those in mitochondrial and microsomal benzoyltransferase might reflect the higher levels of activities in common bile duct-ligated liver. In addition, it is also conceivable that the increased enzyme levels of both benzoyltransferase and phenylacetyltransferase accompanied with higher V_{max} values in common bile duct-ligated liver preparations might have been due to increased biosynthetic capability of the cholestatic liver where functional abnormalities of the liver are expected to develop. All through the previous and present findings, however, the mechanism of changes in activities of several biotransforming enzymes in cholestatic hepatobiliary disease are still unknown. Further investigations should eventually resolve this issue.

References

Byun, Y. J., Kim, Y. H. and Kwak, C. S. (1995) Effect of common bile duct ligaton on liver and serum glyoxalase-I activities in ethanol intoxicated rats. *Keimyung Univ. Med. J.* **14**, 330–339.

Chang, E. S., Kwak, J. S. and Sohn, T. J. (1987) An ultrastructural study on the proliferative changes of bile ductules after ligation of common bile duct. *Kyungpook Univ. Med. J.* **28**, 113–122.

Chung, S. K. and Kwak, C. S. (1992) Effect of common bile duct ligation on liver monoamine oxidase activity in ethanol intoxicated rats. *Korean Biochem. J.* (presently *J. Biochem. Mol. Biol.*) **25**, 210–218.

Desmet, V. J. (1994) Cholestasis: extrahepatic obstruction and secondary biliary cirrhosis; in *Pathology of the Liver*, MacSween, R. M. N., Anthony, P. P., Scheuer, P. J., Burt, A. D. and Portman, C. B. (eds.), pp. 272–305, Churchill Livingstone, New York.

Gornall, A. G., Bardawill, C. J. and David, M. M. (1949) Determination of serum protein by means of biuret reaction. *J. Biol. Chem.* **177**, 751–766.

Ihm, J. S., Kim, Y. H. and Kwak, C. S. (1995) Aryl sulfotransferase activity in cholestatic rat liver induced by common bile duct ligation. *Korean J. Biochem.* **27**, 141–147.

Ihm, J . S. and Kim, Y. H. (1997) Thiosulfate sulfurtransferase and UDP-glucuronosyl-transferase activities in cholestatic rat liver induced by common bile duct ligation. *Korean J. Biochem.* **29**, 197–201.

Kaplan, M. M. and Righetti, A. B. (1970) Induction of rat liver alkaline phosphatase; the metabolism of the serum elevation in bile duct obstruction. *J. Clin. Invest.* **49**, 508–516.

Kelley, M. and Vessey, D. A. (1986) Interaction of 2,4-dichlorophenoxyacetate (2,4-D) and 2,4,5 trichlorophenoxyacetate (2,4,5-T) with the acyl-CoA; amino acid N-acetyltransferase enzymes of bovine liver mitochondria. *Biochem. Pharmacol.* **35**, 289–295.

Killenberg, P. G. and Webster, L. J. Jr. (1980) Conjugation by peptide bond formation; in *Enzymatic Basis of Detoxication*, Jacoby, W. B. (ed.), Vol. II, pp. 141–167, Academic Press, New York.

Kim, H. S., Park, J. Y., Kim, E. Y., Kwak, K. S., Choi, Y. H. and Chung, J. M. (1989) Morphologic changes of hepatocyte induced by common bile duct ligation. *Korean J. Int. Med.* **36**, 459–470.

Kountouras, J., Billing, B. H. and Scheuer, P. J. (1984) Prolonged bile duct obstruction: a new experimental model for cirrhosis in rats. *Br. J. Exp. Path.* **65**, 305–311.

Kryszewski, A. J., Neale, G., Whilfield, J. B. and Moss, D. W. (1973) Enzyme changes in experimental biliary obstruction. *Clin. Chim. Acta* **47**, 175–182.

Kwak, C. S. (1985) Xanthine oxidase activity in the cholestatic rat liver. *Keimyung Univ. Med. J.* **4**, 125–130.

Kwak, C. S., Kim, Y. H. and Mun, K. C. (1988) Activities of alcohol metabolizing enzymes in the cholestatic rat liver. *Keimyung Univ. Med. J.* **7**, 64–75.

Kwak, C. S., Kim, Y. H. and Jo, J. S. (1990) Effect of common bile duct ligation on liver glutathione S-transferase, glutathione peroxidase and glutatione reductase activities in ethanol intoxicated rats. *Korean Biochem. J.* (presently *J. Biochem. Mol. Biol.*) **23**, 251–261.

Kwak, C. S. and Kwak, J. S. (1986) Cell fractionation method of the rat liver. *Keimyung Univ. Med. J.* **5**, 45–53.

Kwak, C. S. and Lee, S. H. (1992) Carboxylase, arylesterase and cholinesterase activities in cholestatic rat liver induced by common bile duct ligation. *Korean Biochem. J.* (presently *J. Biochem. Mol. Biol.*) **25**, 251-261.

Kwon, Y. C., Mun, K. C. and Kwak, C. S. (1990) Glutathione S-transferase, glutathione reductase and glutathione peroxidase activities in cholestatic rat liver. *Keimyung Univ. Med. J.* **9**, 159–170.

Mezey, E. (1976) Diagnosis of liver disease by laboratory methods and specific liver disease; in *The Laboratory in Clinical Medicine*, Halsted, J.A. (ed.), pp. 417–445, B. W. Saunders, Philadelphia.

Mun, K. C. (1994) Correlation between superoxide radical production and hepatic damage induced by bile duct ligation. *Korean Biochem. J.* (presently *J. Biochem. Mol. Biol.*) **27**, 346–349.

Mun, K. C. (1996) Catechol-O-methyltransferase activities in cholestatic rat's liver induced by common bile duct ligation. *J. Biochem. Mol. Biol.* (formerly *Korean Biochem. J.*) **29**, 142–145.

Mun, K. C. and Kwak, C. S. (1989) Monoamine oxidase activity in cholestatic rat liver. *Keimyung Univ. Med. J.* **8**, 69–77.

Nandi, D. L., Lucas, S, V. and Webster, L. T. Jr. (1979) Benzoyl-coenzyme A: glycine N-acetyltransferase and phenylacetyl-coenzyme A: glycine N-acetyltransferase from bovine liver mitochondria. Purification and characterization. *J. Biol. Chem.* **254**, 7230–7237.

Ra, C. Y., Mun, K. C. and Kwak, C. S. (1994) Effects of bile duct ligationon serum and hepatic 5′-nucleosidase activities in ethanol intoxicated rats. *Korean J. Biochem.* **27**, 117–123.

Righetti, A. B. and Kaplan, M. M. (1972) Disparate response of serum and hepatic alkaline phosphatase and 5′-nucleosidase to bile duct obstruction in the rat. *Gastroenterology* **62**: 1034–1039.

Rosalki, S. B. (1976) Enzyme tests in disease of the liver and hepatobiliary tract; in *The Principles and Practice of Diagnositic Enzymology*, Wilkinson, J. H. (ed.), pp. 303–360, Edward Arnold, London.

Webster, L. T. Jr. (1981) Benzoyl-coenzyme A: glycine N-acetyltransferase and phenylacetyl-coenzyme A: glycine N-acetyltransferase; in *Methods in Enzymology*, Jacoby, W. B. (ed.), Vol. 77, pp. 301–307, Academic Press, New York.

Webster, L. T. Jr., Siddiqui, U. A., Lucas, S. V., Strong, J. M. and Mieyal, J. J. (1976) Identification of separate acyl-CoA: glycine and acyl-CoA: L-glutamine N-acetyltransferase activities in mitochondrial fractions from liver of rhesus monkey and man. *J. Biol. Chem.* **251**, 3352–3358.