

# Thiosulfate sulfurtransferase and UDP-glucuronosyltransferase activities in cholestatic rat liver induced by common bile duct ligation

Jong-Sool Ihm<sup>1</sup> and You-Hee Kim<sup>1,2</sup>

<sup>1</sup> Department of Biochemistry, Keimyung University  
School of Medicine, Taegu, 700-712, Korea

<sup>2</sup> Corresponding author

Accepted 10 September 1997

Abbreviations: UDP-GT, UDP-glucuronosyltransferase; CBD, common bile duct

## Abstract

We have investigated the effect of cholestasis on the hepatic thiosulfate sulfurtransferase (rhodanese) and UDP-glucuronosyltransferase (UDP-GT) activities in rats. Rhodanese activities in the liver cytosol, mitochondria and microsomal fractions as well as in the rat serum, and UDP-GT activity in the microsome have been investigated for a period of 42 days after common bile duct (CBD) ligation. The cytosolic rhodanese activity showed a significant decrease between the first through the 42nd day, and the mitochondrial activity showed a significant decrease between the 7th through the 42nd day after CBD ligation compared to the activities from the sham operated control, respectively. In the case of microsomal preparation, both rhodanese and UDP-GT also showed significant decrease in their activities after the ligation for the former enzyme between the 14th and the 42nd days, and for the latter enzyme between the third and 42nd days, respectively. On the other hand, the serum rhodanese activity increased markedly soon after the ligation, exhibiting the peak activity after 1 day of CBD ligation with about 4.6-fold increment. The activity subsequently decreased gradually reaching to the control level at the 42nd day post-ligation. Enzyme kinetic parameters of hepatic rhodanese and UDP-GT were analyzed using sodium thiosulfate and p-nitrophenol as substrates, respectively, with the preparations from the 28th 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 the both enzymes decreased significantly. These results, therefore, suggest that the biosynthesis of rhodanese and UDP-GT have

been reduced in response to cholestasis, and that the elevation of rhodanese activity in the serum is most likely due to leakage from the liver subsequent to CBD ligation.

**Keywords:** thiosulfate sulfurtransferase (rhodanese), UDP-glucuronosyltransferase, cholestatic rat liver

## Introduction

Thiosulfate sulfurtransferase (thiosulfate: cyanide sulfurtransferase, EC 2.8.1.1, rhodanese) is one of the conjugating enzyme which converts cyanides, sulfites, organic sulfonates and dithiols to less toxic sulfur compounds (Westly, 1973, 1980, 1981; Kim, 1979). The enzyme is mainly present in the mammalian liver (Westly, 1973, 1980), and in the blood (Drawbaugh and Marrs, 1987). UDP-glucuronosyltransferase (UDP-glucuronate  $\beta$ -D-glucuronosyltransferase, acceptor unspecific, EC 2.4.1.17, UDP-GT) is another class of conjugating enzymes which is mainly localized in endoplasmic reticulum of the liver (Kim, 1979; Kasper and Henton, 1980) and known to convert phenols, alcohols, amines and fatty acids to less harmful and more water-soluble metabolites by glucuronate conjugation, although its acceptor molecule is not clearly defined (Kim, 1979; Kasper and Henton, 1980).

The common bile duct (CBD) ligated rats have been widely used as an experimental model in human extrahepatic cholestasis (Kaplan and Righetti, 1970, Righetti and Kaplan, 1972; Kryszewski *et al.*, 1973). In human, anatomical or mechanical obstruction of CBD occurs most commonly due to gallstones, neoplasms, or strictures, and less commonly due to primary biliary cirrhosis, cholangitis, hepatitis (Mezey, 1976; Rosalki, 1976). In chronic cholestatic liver in humans and rats, the hepatocytes are particularly susceptible, thus yielding 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 *et al.*, 1987; Kim *et al.*, 1989).

To understand biochemical alteration(s) under the cholestasis, many laboratories have studied several hepatic enzymes in experimental animals (Kwak, 1985; Kwak *et al.*, 1988; Mun and Kwak, 1989; Kwon *et al.*, 1990; Kwak and Lee, 1992; Mun 1994; Park *et al.*, 1994; Ra *et al.*, 1994; Ihm *et al.*, 1995). However, the possible changes of the rhodanese and the UDP-GT activities under the cholestasis induced by CBD ligation have not yet been investigated. Previously, however, we have reported

that cholestasis induced by CBD ligation affects isozyme pattern of hepatic aryl sulfotrasferase in rats, and aryl sulfotransferase was shown to mediate detoxication (Ihm *et al.*, 1995). In the present study, we have systematically investigated the liver rhodanese and UDP-GT activities with the subcellular fractions prepared from the cholestatic rat liver induced by CBD ligation for a period of 42 days. In addition,  $K_m$  and  $V_{max}$  values for these enzymes were also analyzed with the 28th day post-CBD ligated rat liver preparations and compared to those values obtained from the sham-operated.

## Materials and Methods

### Chemicals

Potassium cyanide, sodium thiosulfate pentahydrate, ferric nitrate nonahydrate, ferric thiocyanate, UDP-glucuronic acid sodium, *p*-nitrophenol, *p*-nitrophenyl- $\beta$ -D-glucuronide, bovine serum albumin, Triton X-100, glycine, rhodanese (thiosulfate sulfurtransferase, type II, from bovine liver), UDP-GT (type III, from bovine liver) and bovine albumin standard (10 ml/100 mg) were purchased from Sigma Chemical Co. (St. Louis, USA).

### Animals

Normal male rats of the Sprague-Dawley strain, weighing between 320 and 350 g, were used for the experiments. All animals were maintained on a pellet diet obtained commercially (Sam Yang Food Co., Wonju, Korea) and tap water. During surgery, rats were anesthetized lightly with ether, and the abdomen was opened through a median line incision. The CBD 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 sham operation (midline laparectomy). Each experiment was carried out with a group of 5 rats. Rats were sacrificed after 0.5, 1, 2, 3, 7, 14, 28 and 42 days following the operation. The livers were excised following perfusion (see below), and blood was collected from the aorta. The serum was separated by centrifugation and was 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 enzyme assay.

### Enzyme assays

The hepatic subcellular preparations and serum rhodanese activities were measured in a spectrophotometer (Varian, Cary 210) according to the method of Westly (1981) using potassium cyanide and sodium thiosulfate as substrate. Ten  $\mu$ l of each subcellular preparation and/or 50  $\mu$ l of serum were incubated with 0.2 ml of 0.24 M potassium cyanide, 0.2 ml of 0.2 M potassium phosphate monobasic, and 0.2 ml of 0.25 M sodium thiosulfate pentahydrate for 20 min. The incubations were terminated by the addition of 0.5 ml of 15% (v/v) formaldehyde. The amount of ferric thiocyanate formed by the addition of 1.5 ml ferric nitrate reagent (10 g of ferric nitrate nonahydrate and 20 ml of 65% nitric acid per 150 ml) was read by absorbance change at 460 nm. The enzyme activity was expressed as amount of ferric thiocyanate formed per min per mg of protein for liver preparations or that formed per min per ml of serum.

The hepatic microsomal UDP-GT activity was measured according to the method of Reinke *et al.* (1986) with *p*-nitrophenol and UDP-glucuronic acid as substrate. Incubations were performed in 15 ml test tube with 2 ml of 50 mM phosphate buffer, pH 7.0, containing 3 mM UDP-glucuronic acid sodium salt, 1 mM *p*-nitrophenol, 1 mM magnesium chloride, 0.02 % bovine serum albumin, 0.05% (v/v) Triton X-100 solution and 0.2 ml of hepatic microsome. The incubations were initiated by the addition of UDP-glucuronic acid and were terminated after 20 min by the addition of 0.5 ml of 0.6 M perchloric acid. The precipitated proteins were removed by centrifugation. *p*-Nitrophenol remaining was determined by diluting 0.5 ml of the supernatant fraction with 2 ml of 1.6 M glycine buffer, pH 10.3, and reading absorbance at 436 nm ( $\epsilon_{436} = 7.11 \text{ mM}^{-1} \text{ cm}^{-1}$ ). The enzyme activity was expressed as amount of *p*-nitrophenyl- $\beta$ -D-glucuronide formed per min per mg of hepatic microsomal protein.

Michaelis-Menten constants ( $K_m$  and  $V_{max}$ ) of the enzymes were determined with subcellular fractions of sham operated rat livers and/or cholestatic rat livers at the 28th day after operation, at variable concentrations of sodium thiosulfate (for rhodanese) and *p*-nitrophenol (for UDP-GT). The enzyme kinetic constants were calculated using Lineweaver-Burk plot.

### 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  $\pm$  SD Statistical evaluation of the experimental data was evaluated by Student's t-test. *P* values of  $\leq 0.05$  were considered to be significant.

## Results and Discussion

The effects of the cholestasis on activities of several xenobiotic biotransforming enzymes, such as xanthine oxidase (Kwak, 1985), alcohol dehydrogenase, catalase, microsomal ethanol oxidizing system (Kwak *et al.*, 1988), monoamine oxidase (Mun and Kwak, 1989), glutathione S-transferase, glutathione peroxidase (Kwon *et al.*, 1990), arylesterase, carboxylesterase, cholinesterase (Kwak and Lee, 1992) and aryl sulfotransferase (Ihm *et al.*, 1995) have been previously studied in cholestatic rat liver. Especially, the rhodanese and the UDP-GT are xenobiotic biotransforming enzymes (Kasper and Henton, 1980; Westly, 1980). Nevertheless the changes of the rhodanese and UDP-GT have not been studied under the cholestasis induced by CBD ligation. In order to understand the effects of cholestasis on these enzyme activities, we have determined the activities of cytosolic, mitochondrial and microsomal rhodanese and microsomal UDP-GT in cholestatic rat liver induced by CBD ligation for a period of 42 days. The activity of rhodanese in serum was also measured. Values of  $K_m$  and  $V_{max}$  for these hepatic rhodanese and UDP-GT at the 28th day after CBD ligation were determined using sodium thiosulfate and *p*-nitrophenol as substrate, respectively.

The cytosolic rhodanese activity in the rat liver showed a significant decrease from the first day to the 42nd day subsequent to CBD ligation (Table 1). The activity of mitochondrial rhodanese also decreased in the cholestatic rat liver, beginning from seventh day after the ligation, and continued to decrease until 42nd day compared to the sham operated liver activity (Table 1). In the case of microsomal rhodanese, the rate of decrease of the activity is least responsive, showing a decrease of the activity at 14th day (Table 1). However, quite contrary to it, the serum rhodanese activity increased markedly soon after

the ligation; about 4.6-fold increase was observed at 1 day after the ligation, and then, the activity gradually decreased to the control level at 42nd day of post-ligation (Table 2). In the case of microsomal UDP-GT, the reduced activity was seen on the third day which further decreased on the 42nd day (Table 3).

In order to investigate whether the changes of the rhodanese and UDP-GT activities in the cholestatic rat liver are possibly due to alteration in their catalytic property of the enzyme,  $K_m$  and  $V_{max}$  values were determined with 28th day post-CBD ligation preparations. As shown in Table 4,  $K_m$  values of rhodanese did not change significantly compared to those from sham-operated liver in all three preparations of cytosol, mitochondria and microsome. However,  $V_{max}$  significantly decreased in all preparations; 3.3  $\mu\text{mol}$  ferric thiocyanate  $\text{min}^{-1}$   $\text{mg}$  protein $^{-1}$  vs. 1.35 for cytosol; 4.64  $\mu\text{mol}$  ferric thiocyanate  $\text{min}^{-1}$   $\text{mg}$  protein $^{-1}$

**Table 2.** Activities of serum thiosulfate sulfurtransferase (rhodanese) after ligation of CBD in rats. The data are expressed as mean  $\pm$  SD with 5 rats in each group. Sham: sham operation.

Day(s) following ligation	Rhodanese activities (nmol ferric thiocyanate $\text{min}^{-1}$ $\text{ml}^{-1}$ )	
	Sham	CBD ligation
0.5	204 $\pm$ 55	887 $\pm$ 225 <sup>c</sup>
1	210 $\pm$ 50	1,167 $\pm$ 378 <sup>c</sup>
2	207 $\pm$ 52	958 $\pm$ 264 <sup>c</sup>
3	205 $\pm$ 52	926 $\pm$ 200 <sup>c</sup>
7	207 $\pm$ 53	637 $\pm$ 120 <sup>c</sup>
14	209 $\pm$ 50	543 $\pm$ 125 <sup>c</sup>
28	202 $\pm$ 48	313 $\pm$ 81 <sup>a</sup>
42	204 $\pm$ 48	205 $\pm$ 74

<sup>a</sup>  $P < 0.05$ ; <sup>b</sup>  $P < 0.01$ ; <sup>c</sup>  $P < 0.01$ ; <sup>a,c</sup> values were compared with that of sham operated control.

**Table 1.** Activities of cytosolic, mitochondrial and microsomal thiosulfate sulfurtransferase (rhodanese) in cholestatic rat liver after CBD ligation. The data are expressed as mean  $\pm$  SD with 5 rats in each group; Sham: sham operation.

Day(s) following ligation	Rhodanese activities ( $\mu\text{mol}$ ferric thiocyanate $\text{min}^{-1}$ $\text{mg}$ protein $^{-1}$ )					
	Cytosol		Mitochondria		Microsome	
	Sham	CBD ligation	Sham	CBD ligation	Sham	CBD ligation
0.5	3.06 $\pm$ 0.34	2.85 $\pm$ 0.33	4.35 $\pm$ 0.91	4.18 $\pm$ 0.81	0.24 $\pm$ 0.07	0.26 $\pm$ 0.07
1	3.10 $\pm$ 0.33	2.61 $\pm$ 0.29 <sup>a</sup>	4.31 $\pm$ 0.91	4.02 $\pm$ 0.76	0.25 $\pm$ 0.07	0.35 $\pm$ 0.10
2	3.10 $\pm$ 0.32	2.50 $\pm$ 0.32 <sup>a</sup>	4.30 $\pm$ 0.92	3.76 $\pm$ 0.81	0.25 $\pm$ 0.07	0.31 $\pm$ 0.09
3	3.08 $\pm$ 0.31	2.27 $\pm$ 0.30 <sup>b</sup>	4.30 $\pm$ 0.92	3.25 $\pm$ 0.94	0.25 $\pm$ 0.07	0.31 $\pm$ 0.07
7	3.09 $\pm$ 0.30	1.83 $\pm$ 0.32 <sup>c</sup>	4.25 $\pm$ 0.90	2.44 $\pm$ 0.76 <sup>b</sup>	0.25 $\pm$ 0.06	0.25 $\pm$ 0.06
14	3.07 $\pm$ 0.29	1.11 $\pm$ 0.29 <sup>c</sup>	4.26 $\pm$ 0.86	2.18 $\pm$ 0.73 <sup>b</sup>	0.24 $\pm$ 0.06	0.12 $\pm$ 0.05 <sup>b</sup>
28	3.09 $\pm$ 0.29	1.06 $\pm$ 0.27 <sup>c</sup>	4.26 $\pm$ 0.86	1.14 $\pm$ 0.56 <sup>c</sup>	0.24 $\pm$ 0.06	0.10 $\pm$ 0.04 <sup>b</sup>
42	3.07 $\pm$ 0.28	1.03 $\pm$ 0.31 <sup>c</sup>	4.25 $\pm$ 0.85	1.08 $\pm$ 0.53 <sup>c</sup>	0.24 $\pm$ 0.06	0.09 $\pm$ 0.04 <sup>b</sup>

<sup>a</sup>  $P < 0.05$ ; <sup>b</sup>  $P < 0.01$ ; <sup>c</sup>  $P < 0.01$ ; <sup>a,b,c</sup> values were compared with that of sham operated control.

vs. 1.68 for mitochondria; and 0.289  $\mu\text{mol}$  ferric thiocyanate  $\text{min}^{-1} \text{mg protein}^{-1}$  vs. 0.159 for microsomal fractions, respectively. Similarly, the  $V_{\max}$  value of microsomal UDP-GT also decreased compared to the control (29.2  $\text{nmol } p\text{-nitrophenyl } \beta\text{-D-glucuronide min}^{-1} \text{mg protein}^{-1}$  vs. 11.5), although the  $K_m$  value did not change significantly (Table 5).

The fact that the decreased rhodanese activity in the liver cytosol after the CBD ligation accompanied by the

**Table 3.** Activities of liver microsomal UDP-GT after ligation of CBD in rats. The data are expressed as mean  $\pm$  SD with 5 rats in each group. Liver of sham, sham operated rat liver.

Day(s) following ligation	UDP-GT activity ( $\text{nmol } p\text{-nitrophenyl } \beta\text{-D-glucuronide min}^{-1} \text{mg protein}^{-1}$ )	
	Liver of sham	Cholestatic liver
0.5	21.9 $\pm$ 2.8	19.5 $\pm$ 3.5
1	21.0 $\pm$ 2.9	18.6 $\pm$ 4.3
2	22.4 $\pm$ 3.1	18.4 $\pm$ 5.4
3	21.8 $\pm$ 2.7	11.2 $\pm$ 5.1 <sup>b</sup>
7	22.1 $\pm$ 3.0	10.3 $\pm$ 4.7 <sup>b</sup>
14	22.6 $\pm$ 2.8	9.9 $\pm$ 3.4 <sup>c</sup>
28	22.3 $\pm$ 2.5	8.9 $\pm$ 3.2 <sup>c</sup>
42	22.5 $\pm$ 2.6	5.7 $\pm$ 2.7 <sup>c</sup>

<sup>b</sup>  $P < 0.01$ ; <sup>c</sup>  $P < 0.001$ ; <sup>b,c</sup> values were compared with that of sham operated control.

increased activity in the serum poses an interesting proposition in that the liver rhodanese might have been leaked easily into the blood stream due to an increased permeability of the hepatocyte membrane, caused by cholestasis as pointed out previously (Park *et al.*, 1994; Ihm *et al.* 1995). It should be also noted that the lower  $V_{\max}$  values of rhodanese in all subcellular fractions of CBD-ligated liver as well as that of the microsomal UDP-GT might reflect the lower levels of the activities in CBD-ligated liver. In addition, it is also conceivable that the reduced enzyme levels of both rhodanese and UDP-GT accompanied by lower  $V_{\max}$  values in CBD-ligated liver preparations might have been to reduced biosynthetic capability of the cholestatic liver where functional abnormalities of the liver are expected to develop.

## References

- Chang, D. S., Kwak, J. S. and Shon, 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
- Desment, V. J. (1979) Cholestasis: extrahepatic obstruction and secondary biliary cirrhosis. In *Pathology of the Liver* (MacSween, R. M. N., Anthony, P. P. and Scheuer, P., eds.), pp. 272-305, Churchill Livingstone, New York
- Drawbaugh, R. B. and Marrs, T. J. (1987) Interspecies differences in rho-danese (thiosulfate sulfurtransferase, EC 2.8.1.1) activity in liver, kidney and plasma. *Comp. Biochem. Physiol. [B]* 86: 307-310
- 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

**Table 4.** Kinetic parameters of thiosulfate sulfurtransferase (rhodanese) from cholestatic rat liver. Thiosulfate sulfurtransferase activities were determined using sodium thiosulfate and potassium cyanide at 25°C for cytosolic, mitochondrial and microsomal fractions of sham-operated male rat livers and cholestatic male rat livers at the 28th day after CBD ligation. The data are expressed as mean  $\pm$  SD with 5 rats in each group. Liver of sham, sham operated rat liver.

Cell fraction	$K_m$ ( $\mu\text{M}$ )		$V_{\max}$ ( $\mu\text{mol ferric thiocyanate min}^{-1} \text{mg protein}^{-1}$ )	
	Liver of sham	Cholestatic liver	Liver of sham	Cholestatic liver
Cytosol	247 $\pm$ 42	236 $\pm$ 51	3.63 $\pm$ 0.35	1.35 $\pm$ 0.24 <sup>c</sup>
Mitochondria	265 $\pm$ 47	252 $\pm$ 55	4.64 $\pm$ 0.96	1.68 $\pm$ 0.52 <sup>c</sup>
Microsome	603 $\pm$ 126	581 $\pm$ 138	0.289 $\pm$ 0.066	0.159 $\pm$ 0.046 <sup>b</sup>

<sup>b</sup>  $P < 0.01$ ; <sup>c</sup>  $P < 0.001$ ; <sup>b,c</sup> values were compared with that of sham operated rat livers.

**Table 5.** Kinetic parameters of microsomal UDP-GT from cholestatic rat liver. Experimental conditions were as described in "Materials and Methods". Microsomal fraction of sham operated male rat livers and cholestatic male rat livers at the 28th day after CBD ligation were used for the enzyme assay.

$K_m$ ( $\mu\text{M}$ )	$V_{\max}$ ( $\text{nmol } p\text{-nitrophenyl } \beta\text{-D-glucuronide min}^{-1} \text{mg protein}^{-1}$ )	
	Liver of sham	Cholestatic liver
9.59 $\pm$ 1.61	9.23 $\pm$ 2.14	29.2 $\pm$ 2.6
		11.5 $\pm$ 2.8 <sup>c</sup>

<sup>c</sup>  $P < 0.001$  vs. sham operated control.

- 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
- Kaplan M. M. and Righetti, A. (1970) Induction of rat liver alkaline phosphatase: the mechanism of the serum elevation in bile duct obstruction. *J. Clin. Invest.* 49: 508-516
- Kasper, C. B. and Henton, A. (1980) Glucuronidation. In *Enzymatic Basis of Detoxication* (Jacoby, W. B., ed.), Vol. II, pp. 3-36, Academic Press, New York
- Kim, B. K. (1979) *Enzyme Nomenclature*, IUB, pp. 164-165, 228-229, Academic Press, New York
- Kim, H. S., Park, J. Y., Kawk, K. S., Choi, Y. H and Chung, J. M. (1989) Morphologic changes of hepatocytes 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 the rat. *Br. J. Exp. Pathol.* 65: 305-311
- Kryszewski, A. J., Neale, G., Whifford, 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 meta-bolizing enzymes in the cholestatic rat liver. *Keimyung Univ. Med. J.* 7: 64-75
- 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) Carboxylesterase, arylesterase and cholinesterase activities in cholestatic rat liver induced by common bile duct ligation. *Korean Biochem. J.* 25: 251-261
- Kwon, Y. C., Mun, K. C. and Kwak, C. S. (1990) Glutathione S-transferase, 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.* 27: 346-349
- Mun, K. C. and Kwak, C. S. (1989) Monoamine oxidase activity in cholestatic rat liver. *Keimyung Univ. Med. J.* 8: 69-77
- Park, E. M., Mun, K. C. and Kwak, C. S. (1994)  $\alpha$ -D-Mannosidase and  $\beta$ -D-mannosidase activities in cholestatic rat liver induced by bile duct ligation. *Korean J. Biochem.* 26: 197-202
- Ra, C. Y., Mun, K. C. and Kwak, C. S. (1994) Effects of bile duct ligation on serum and hepatic 5'-nucleosidase activities in ethanol intoxicated rats. *Korean J. Biochem.* 27: 117-123
- Reinke, L. A., Moyer, M. J. and Notley, K. A. (1986) Diminished rates of glucuronidation and sulfation in perfused rat liver after chronic ethanol administration. *Biochem. Pharmacol.* 35: 439-441
- Righetti, A. B. 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 Diagnostic Enzymology* (Wilkinson, J. H., ed.), pp. 303-360. Edward Arnold, London
- Westly, J. (1973) Rhodanese. *Adv. Enzymol. Areas Mol. Biol.* 39: 327-368
- Westly, J. (1980) Rhodanese and sulfate pool. In *Enzymatic Basis of Detoxication* (Jacoby, W. B., ed.), Vol. II, pp. 245-262, Academic Press, New York
- Westly, J. (1981) Thiosulfate: cyanide sulfurtransferase(Rhodanese). In *Methods in Enzymology* (Jacoby, W. B., ed.), Vol. II, pp. 245-262, Academic Press, New York