EFFECT OF DIETARY PROTEIN AND SULFUR-CONTAINING AMINO ACIDS ON URINARY METABOLITES IN RATS INJECTED WITH CHLOROBENZENE

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ABSTRACT — Rats were fed for 10 days a 10 or 20% casein diet, or the diets with 0.3% DL-methionine or L-cystine. Rats fed the 20% casein exceeded those fed the 10% casein in growth, hepatic glutathione (GSH) levels, and microsomal drug-metabolizing activities including cytochrome P-450, aminopyrine *N*-demethylase, and aniline hydroxylase. Supplement with methionine or cystine had significantly elevated hepatic GSH, regardless of the casein content. After the feeding, rats were intraperitoneally injected with chlorobenzene (0.5 mmol/kg body weight), and the urinary metabolites (4-chlorophenylmercapturic acid (4-CPMA), 2-, 3- and 4-chlorophenol (CPs), 4-chlorocatechol (4-CC), and 2-, 3- and 4-chlorophenylmethylsulfide (CPMSs)) were measured for 24 hours post-injection. Rats fed the 20% casein exceeded those fed the 10% casein in 4-CPMA, CPs, and in total urinary metabolites. Supplement with methionine or cystine to the 10% casein significantly increased 4-CPMA and decreased 4-CC. Supplement with methionine or cystine to the 20% casein also significantly increased 4-CPMA excretion, but had no effect on urinary 4-CC. The highest urinary excretion of CPMSs was observed in rats fed the 10% casein. Both increase of dietary protein and addition of the sulfur-containing amino acids decreased urinary CPMSs. These results indicate that total urinary metabolites are strongly associated with the microsomal drug-metabolizing activity, formation of the mercapturic acid is dependent on the hepatic GSH level, and the urinary CPMS level is independent on the mercapturic acid formation.

KEY WORDS : Chlorobenzene, Glutathione, Mercapturic acid, Methylthio-metabolite, Nutritional condition, Sulfur-containing amino acid.

INTRODUCTION

Chlorobenzene has been used extensively as an organic solvent and as a chemical intermediate for dyes and pesticides in the chemical industry. There has been a potential risk of exposure for humans engaged in the handling of this com-

pound. Animals exposed to chlorobenzene metabolize this xenobiotics to chlorobenzene-epoxide *via* microsomal mixed function oxidase in the liver. The epoxide compounds formed are further metabolized through three main pathways, in which the epoxide is converted to 4-chlorophenylmercapturic acid (4-CPMA) *via* glutathione (GSH)-conjugate, to three isomers of chlorophenol (CP) non-enzymatically, and to 4-

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chlorocatechol (4-CC) by epoxide hydrazine. The major urinary metabolites of chlorobenzene are glucuronate- or sulfate-conjugates of the CPs and 4-CC, and 4-CPMA (Spencer and Williams, 1950; Smith et al., 1972). In addition to these metabolites, three isomers of chlorophenylmethylsulfides (CPMSs), which are methylthio-derivatives of chlorobenzene, have been found as minor urinary metabolites in rats or mice injected with chlorobenzene (Kitamura et al., 1977; Yoshida and Hara, 1985a).

When an electrophilic compound, such as chlorobenzene, is administered to experimental animals, hepatic GSH is transiently decreased, due to the formation of mercapturic acid (Mitchell et al., 1973). The transient loss of GSH results in an acceleration of hepatic GSH synthesis activity, and then following this GSH levels increase to higher than the initial level (Yoshida and Hara, 1984). Hepatic cysteine shows a similar change to GSH and oxidative metabolic pathway of cysteine to taurine is lowered in rats administered with chlorobenzene (Yoshida and Hara, 1985b and c). These results indicate a strong association of chlorobenzene metabolism with sulfur-containing amino acids.

It has been reported that dietary sulfur-containing amino acid levels are an important factor influencing hepatic GSH and cysteine levels (Tateishi and Sakamoto, 1983). Thus, metabolism and toxicity of chlorobenzene could be altered by changes in dietary sulfur-containing amino acid levels. In fact, addition of 0.3% DL-methionine to a 10% casein diet prevented liver necrosis induced by chlorobenzene (Yoshida and Hara, 1985d). Gluten, the main protein in wheat and rich in cystine, is more effective in suppressing the hepatotoxicity of chlorobenzene than soybean protein, although the nutritive value as a protein is higher in the latter (Yoshida and Hara, 1986). The present study investigated the effect of dietary protein and sulfur-containing amino acids on the urinary excretion of chlorobenzene metabolites.

MATERIALS AND METHODS

Seventy-two male Wistar 6-week-old rats weighing 140 to 160 g were housed in hanging

stainless-steel wire-meshed cages in a temperature (23°C)- and humidity (60%)-controlled room on a 12 hr light (08:00–20:00)-dark cycle. Animals were allowed free access to experimental diets and drinking tap water. The rats were divided into six groups of twelve rats and fed 10 or 20% casein diet or diets supplemented with 0.3% DL-methionine or 0.3% L-cystine. The composition of the 10% or 20% casein diets were as follows (%): casein, 10 or 20; potato starch, 58 or 48; sucrose, 15; soybean oil, 8; salt mixture (composition (g/kg diet): CaHPO₄, 2H₂O, 8.736; KH₂PO₄, 15.432; NaH₂PO₄, 5.61; NaCl, 2.796; Ca-lactate, 21.054; Fe-citrate, 1.908; MgSO₄, 4.302; ZnCO₃, 0.066; MnSO₄·4-6H₂O, 0.203; CuSO₄·5H₂O, 0.018; KI, 0.006; Na₂SeO₃, 0.00022), 6; vitamin mixture (obtained from Oriental Yeast Co. (Tokyo) and the composition has been previously described (Yoshida and Hara, 1985d)), 1; and cellulose powder, 2. Methionine or cystine was added to the casein diets at the expense of potato starch.

On the 11th day after the start of feeding, six rats of each group were killed by decapitation with surgical scissors and the livers were excised. The remaining animals were intraperitoneally injected with chlorobenzene (0.5 mmol/kg body weight) that was dissolved in olive oil, and housed in metabolic cages and given the experimental diets for an additional 2 days. Urine samples of the rats injected with chlorobenzene were collected for 24 hours post-injection and used for analysis of urinary metabolites. Excision of the livers and the injection with chlorobenzene were performed between 10:00 and 12:00 AM.

The liver portions (about 1 g) were homogenized in 4 volumes of cold 10% trichloroacetic acid (TCA). The TCA-soluble fraction obtained by centrifugation was used for the GSH assay. GSH was measured by the colorimetric method based on the reaction with 5,5'-dithiobis-(2-nitrobenzoic acid) (Ellman, 1959), specifically, the value obtained by GSH analysis is defined as the concentration of TCA-soluble sulfhydryls. However, our results confirmed that the level of sulfhydryls corresponded to reduced GSH levels by an enzymatical assay for GSH (Tietze, 1969; Yoshida and Hara, 1984).

Another portion (about 3 g) of livers were homogenized in 3 volumes of Tris-HCl buffer (10 mM, pH 7.4) containing 1% KCl. The homogenate was centrifuged at $10,000\times g$ for 20 min, and the supernatant obtained was further centrifuged at $105,000\times g$ for 60 min. The precipitate obtained was used as the microsomal pellet after washing with buffer. Cytochrome P-450 content (Omura and Sato, 1964), aniline hydroxylase activity (Imai et al., 1966), and aminopyrine *N*-demethylase activity (La Du et al., 1955) of the pellet were measured. These enzyme activities and cytochrome P-450 content were expressed as unit and nmol per mg microsomal protein, respectively.

One ml of the urine was boiled with 3.3 M HCl for 3 hours, and the CPs and 4-CC released were extracted with ether. The ether extract was dried up and dissolved in methanol and then analyzed with High Performance Liquid Chromatography (HPLC) by a modified method as described previously (Yoshida and Hara, 1985b). Methanol/water/acetic acid ($v/v/v=30/69/1$) was used as the mobile phase instead of methanol/water ($v/v=30/70$). Analytical concentrations of CPs were expressed as a sum of 2-, 3-, and 4-CP.

Another 1 ml portion of the urine was adjusted to pH 1.0 with HCl and was extracted with ether. The extract was dried up and heated with 1 ml of 2 N NaOH at 95°C for 30 min. After neutralization with 10% HCl, 4-chlorothiophenol formed from 4-CPMA by alkaline hydrolysis was determined by HPLC (Yoshida and Hara, 1985b).

One ml of the remaining urine was adjusted

to pH 11.0 with NaOH and kept at a room temperature for 20 hours. CPMSs in this alkalinized urine were extracted with ether and directly determined by HPLC (Yoshida and Hara, 1985a). Results were expressed as a sum of 2-, 3-, and 4-CPMS.

Experimental data were statistically tested by analysis of variance followed by the Least Significant Difference test (Snedecor and Cochran, 1967).

RESULTS

Growth and Hepatic GSH: Table 1 shows body weights and hepatic GSH contents of rats fed the experimental diets for 10 days. During the feeding period, there were no differences in feed consumption among the six groups. Body weights of rats fed the 10% casein diet were significantly lower than rats fed the 20% casein diet. Supplement with methionine or cystine to the 10% casein diet caused a small increase in growth. However, there were no differences in body weights between rats fed the 20% casein diet and those fed the 20% casein diet supplemented with methionine or cystine.

Hepatic GSH levels of rats fed the 10% casein diet were remarkably lower. Supplement with methionine or cystine to this diet increased GSH. Hepatic GSH levels in the rats fed the 10% casein diet supplemented with methionine or cystine were almost the same as those fed the 20% casein diet. In addition, supplement with sulfur-containing amino acids to the 20% casein diet further increased GSH levels.

Table 1. Body and liver weight and hepatic glutathione concentration in rats.

Diet		Body weight	Liver weight	Glutathione
Casein	Supplement	(g)	(g/100g body weight)	($\mu\text{mol}/100\text{g body weight}$)
10%	None	171 ± 2^a	4.39 ± 0.12^a	6.8 ± 0.1^a
10%	Methionine	187 ± 4^b	4.54 ± 0.16^a	24.5 ± 1.8^b
10%	Cystine	185 ± 3^b	4.54 ± 0.11^a	24.6 ± 1.8^b
20%	None	201 ± 5^c	4.42 ± 0.17^a	25.1 ± 0.1^b
20%	Methionine	200 ± 6^c	4.50 ± 0.15^a	36.6 ± 0.7^c
20%	Cystine	199 ± 6^c	4.42 ± 0.20^a	38.1 ± 1.6^c

Values are means $\pm$ S. E. ($n=6$). Means in the same column not sharing a common superscript differ significantly ($P<0.05$).

Drug-metabolizing enzyme activity: Table 2 shows cytochrome P-450 content and the activities of two drug-metabolizing enzymes in the liver microsomes of rats. These three parameters were similarly influenced by the dietary condition. Elevation of dietary protein level significantly increased the P-450 content and the drug-metabolizing activities. Addition of methionine or cystine to the 10% casein diet had no effect on the P-450 or enzyme activities. However, additions to the 20% casein diet showed increases in all parameters, the increase by methionine was particularly significant.

Urinary excretion of chlorobenzene metabolites: Excretion of urinary metabolites of chlorobenzene during 24 hr post-injection period are summarized in Table 3.

Rats fed the 20% casein diet had significantly

higher urinary excretions of 4-CPMA and CPs, than rats fed the 10% casein diet, and the sum of the urinary metabolites was also significantly higher in the rats fed the 20% casein diet.

Supplement with methionine or cystine to the 10% casein diet significantly increased urinary 4-CPMA levels. However, the total excretion of metabolites was not changed by the supplement, because urinary 4-CC excretion was significantly decreased in the rats fed the methionine- or cystine-supplemented 10% casein diet.

Supplement with methionine or cystine to the 20% casein diet also significantly increased the 4-CPMA excretion, but had no effect on urinary 4-CC. Excretion of CPs was slightly decreased by the supplement, but this change was not significant. The methionine-supplement to the 20% casein diet significantly increased the

Table 2. Cytochrome P-450 content and drug metabolizing activity.

Diet		Cytochrome P-450	Aniline hydroxylase	Aminopyrine <i>N</i> -demethylase
Casein	Supplement			
10%	None	0.57 ± 0.03 ^a	1.82 ± 0.17 ^a	1.51 ± 0.04 ^a
10%	Methionine	0.53 ± 0.05 ^a	1.75 ± 0.11 ^a	1.45 ± 0.04 ^a
10%	Cystine	0.54 ± 0.02 ^a	1.73 ± 0.18 ^a	1.43 ± 0.05 ^a
20%	None	0.66 ± 0.04 ^b	2.67 ± 0.13 ^b	1.66 ± 0.06 ^b
20%	Methionine	0.71 ± 0.03 ^b	3.29 ± 0.11 ^c	1.82 ± 0.04 ^c
20%	Cystine	0.71 ± 0.03 ^b	2.79 ± 0.19 ^b	1.75 ± 0.08 ^{bc}

Cytochrome P-450 is expressed as nmol per mg microsomal protein. Aniline hydroxylase activity is expressed as nmol *p*-aminophenol formed per minute per mg microsomal protein. Aminopyrine *N*-demethylase activity is expressed as nmol formaldehyde formed per minute per mg microsomal protein. Values are means ± S. E. (n=6). Means in the same column not sharing a common superscript differ significantly (P<0.05).

Table 3. Composition of urinary metabolites of chlorobenzene.

Diet		Urinary metabolites (μmol/24 h/kg body weight)				
Casein	Supplement	4-CPMA	CPs	4-CC	CPMSs	Total
10%	None	48.6 ± 2.5 ^a	76.5 ± 3.4 ^{ab}	19.8 ± 3.3 ^b	3.2 ± 0.7 ^b	148.1 ± 9.0 ^a
10%	Methionine	55.8 ± 3.0 ^b	70.0 ± 5.0 ^a	11.7 ± 2.6 ^a	2.2 ± 0.8 ^{ab}	139.7 ± 9.3 ^a
10%	Cystine	62.3 ± 3.6 ^{bc}	75.9 ± 4.0 ^{ab}	9.2 ± 1.3 ^a	2.1 ± 0.6 ^{ab}	149.5 ± 9.5 ^a
20%	None	68.1 ± 5.9 ^c	88.4 ± 2.9 ^c	17.1 ± 1.8 ^b	2.1 ± 0.6 ^{ab}	175.7 ± 10.2 ^b
20%	Methionine	90.2 ± 5.9 ^d	85.5 ± 4.5 ^{bc}	18.1 ± 1.6 ^b	1.6 ± 0.4 ^a	195.4 ± 10.0 ^c
20%	Cystine	86.7 ± 3.7 ^d	80.8 ± 4.2 ^{bc}	18.1 ± 2.6 ^b	1.5 ± 0.5 ^a	187.1 ± 8.9 ^{bc}

Values are means ± S. E. (n=6). Means in the same column not sharing a common superscript differ significantly (P<0.05).

4-CPMA : 4-Chlorophenylmercapturic acid. CPs : Sum of 2-, 3- and 4- chlorophenol. 4-CC : 4-Chlorocatechol. CPMSs : Sum of 2-, 3- and 4-chlorophenylmethylsulfide. Total : Sum of 4-CPMA, CPs, 4-CC and CPMSs.

total excretion of the metabolites, but the cystine-supplement did not cause a significant effect on overall excretion.

Changes due to dietary condition in urinary CPMSs were unique compared to other metabolites. Rats fed the 10% casein diet excreted the most CPMSs. Both increases in dietary protein and addition of the sulfur-containing amino acids decreased urinary CPMSs. CPMS levels excreted by rats fed the 20% casein diet supplemented with methionine or cystine were half the amount excreted by rats fed the 10% casein diet.

DISCUSSION

It is well established that the activities of liver microsomal drug-metabolizing enzymes increase as dietary protein levels are augmented (Kato et al., 1962; Campbell and Hayes, 1974). This was further confirmed by the present study, in which elevation of the dietary casein level significantly increased the content of cytochrome P-450 and the activities of aniline hydroxylase and aminopyrine *N*-demethylase in the liver microsomes (Table 2).

Dietary protein quality is also a known factor affecting drug metabolism; low protein quality decreases drug-metabolizing activity (Campbell and Hayes, 1976; Miranda and Webb, 1973). Supplement of methionine or cystine to casein-based diets improves the quality of the dietary protein by enriching the amino acid composition. In fact, addition of sulfur-containing amino acids to the 10% casein diet increased the body weight. However, a significant effect on drug-metabolizing activity was observed only by addition of methionine to the 20% casein diet. Kato et al. (1981) reported that rats fed various diets, different in protein source, for 3 weeks experienced body weight gain that positively correlated with activity of the microsomal enzyme system, including aminopyrine *N*-demethylase, aniline hydroxylase, and cytochrome P-450. Feeding period in the present study was only 10 days. Thus, a further feeding period may have been necessary to demonstrate a clear effect of the supplement with methionine or cystine, on the microsomal drug-metabolizing activity.

Since chlorobenzene is a highly volatile compound, considerable amounts of this compound are excreted by expiration; and about one-third of the dose is metabolized in liver and excreted through the urine (Smith et al., 1972; Yoshida and Hara, 1985b). In the present study, the sum of all the urinary chlorobenzene metabolites was increased when the casein level was elevated or methionine was added to the 20% casein diet. This variation, due to the dietary condition, paralleled an increase in the microsomal drug-metabolizing activities. The total amounts of urinary metabolites were strongly associated with drug-metabolizing activities. Thus, microsomal drug-metabolizing activity is an important factor determining the amounts of chlorobenzene metabolized.

The addition of the sulfur-containing amino acids to the diets significantly increased the hepatic GSH and the urinary excretion of 4-CPMA, regardless of the dietary protein level. This indicated that a formation of mercapturic acid is dependent upon the hepatic GSH level. The addition of sulfur-containing amino acids to the 10% casein diet decreased urinary 4-CC while increasing 4-CPMA. This inverse relation between 4-CC and 4-CPMA was also previously observed (Yoshida and Hara, 1986). The main metabolic pathway of chlorobenzene in rats is formation of mercapturic acid, and the formation of catechol may be increased when the hepatic GSH level is insufficient for the mercapturic acid formation.

In the present study, we also measured urinary CPMSs, methylthio-metabolites of chlorobenzene. Two different pathways have been proposed for the formation of methylthio-metabolites, namely transformation from mercapturic acid or GSH conjugate (Tateishi et al., 1978; Kimura et al., 1984), or direct addition of a methylthio group from the methionine residue of available protein (Scribner et al., 1965; Wong et al., 1976). According to the former hypothesis, the formation of methylthio-metabolites are dependent on mercapturic acid. However, rats fed the 10% casein diet excreted the most CPMSs, and both increases in dietary protein and addition of the sulfur-containing amino acids decreased urinary CPMSs. Urinary excretion of CPMSs did not parallel excretion of mercapturic acid.

In addition, only 4-CPMA was found as mercapturic acid in urine, while three isomers of CPMS were detected. Thus, transformation *via* mercapturic acid or GSH conjugate seems unlikely. Since chlorobenzene-epoxide, a common intermediate of chlorobenzene metabolism, is highly reactive, it is likely that this intermediate may readily bind to methylthio groups on methionine residues in protein. The urinary CPMSs may be derived from a protein-adduct of chlorobenzene. In rats fed the 10% casein diet, the highest urinary CPMSs, and the lowest hepatic GSH and urinary mercapturic acid levels were observed. Decreased hepatic GSH levels raise the hepatotoxicity of chlorobenzene (Yoshida and Hara, 1985d and 1986), and protein-adducts may be readily formed in this dietary condition.

In the view of industrial toxicology, the detection of a protein- or a DNA-adduct of xenobiotics is more important than the measurement of the urinary metabolites, because the adducts are directly associated with manifestation of the toxicity and carcinogenicity (Lohman, 1988). The measurement of urinary CPMSs may serve as a detection method for protein-adducts of chlorobenzene metabolites in workers handling this organic solvent.

REFERENCES

- Campbell, T. C. and Hayes, J. R. (1974): Role of nutrition in the drug-metabolizing enzyme system. *Pharmacol. Rev.*, **26**, 171-197.
- Campbell, T. C. and Hayes, J. R. (1976): The effect of quantity and quality of dietary protein on drug metabolism. *Fed. Proc.*, **35**, 2470-2474.
- Ellman, G. L. (1959): Tissue sulfhydryl groups. *Arch. Biochem.*, **82**, 70-77.
- Imai, Y., Itoh, A. and Sato, R. (1966): Evidence for biochemically different types of vehicles in the hepatic microsome fraction. *J. Biochem.*, **60**, 417-428.
- Kato, N., Tani, T. and Yoshida, A. (1981): Effect of dietary quality of protein on liver mixed function oxidase system, plasma cholesterol and urinary ascorbic acid in rats fed PCB. *J. Nutr.*, **111**, 123-133.
- Kato, R., Chiesara, E. and Vassanelli, P. (1962): Factors influencing induction of hepatic microsomal drug-metabolizing enzymes. *Biochem. Pharmacol.*, **11**, 211-220.
- Kimura, R., Sano, H., Itagaki, K., et al. (1984): Identification of sulfur-containing metabolites of *m*-dichlorobenzene and their disposition and relationship with glutathione in rats. *J. Pharmacobiodyn.*, **7**, 234-245.
- Kitamura, S., Sumino, K. and Mio, T. (1977): New sulfur-containing metabolites of chlorobenzenes. *Jap. J. Hyg.*, **32**, 200. (abstract)
- La Du, B. N., Gaudette, L., Trousof, N., et al. (1955): Enzymatic dealkylation of aminopyrine (Pyrimidon) and other alkylamines. *J. Biol. Chem.*, **214**, 741-752.
- Lohman, P. H. M. (1988): Summary: Adducts, *In* Methods for detecting DNA damaging agents in humans: Applications in cancer epidemiology and prevention. (H. Bartsch, K. Hemminki and I. K. O'Neill eds.) IARC Scientific Publications No. 89, pp. 13-20, International Agency for Research on Cancer, Lyon.
- Miranda, C. L. and Webb, R. E. (1973): Effect of dietary protein quality on drug metabolism in the rat. *J. Nutr.*, **103**, 1425-1430.
- Mitchell, J. R., Jollow, D. J., Potter, W. Z., et al. (1973): Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. *J. Pharmacol. Exp. Ther.*, **187**, 211-217.
- Omura, T. and Sato, R. (1964): The carbon monoxide binding pigment of liver microsomes. *J. Biol. Chem.*, **239**, 2370-2378.
- Scribner, J. D., Miller, J. A. and Miller, E. C. (1965): 3-Methylmercapto-*N*-methyl-4-aminoazobenzene: An alkaline-degradation product of a labile protein-bound dye in the livers of rats fed *N, N*-dimethyl-4-aminoazobenzene. *Biochem. Biophys. Res. Commun.*, **20**, 560-565.
- Smith, J. R. L., Shaw, B. A. J. and Foulkes, D. M. (1972): Mechanism of mammalian hydroxylation: Some novel metabolites of chlorobenzene. *Xenobiotica*, **2**, 215-226.
- Snedecor, C. W. and Cochran, W. G. (1967): *Statistical Methods*, 6th. ed., Iowa State University Press, Ames.
- Spencer, B. and Williams, R. T. (1950): Studies in detoxication. 33. The metabolism of halogenobenzenes. A comparison of glucuronic acid, ethereal sulfate and mercapturic acid conjugations of chloro-, bromo- and iodo-benzenes and of the *o*-, *m*- and *p*-chlorophenols. Biosynthesis of *o*-, *m*- and *p*-chlorophenylglucuronides.

- Biochem. J., **47**, 279–284.
- Tateishi, M., Suzuki, S. and Shimizu, H. (1978): The metabolism of bromazepam in the rat — identification of mercapturic acid and its conversion *in vitro* to methylthio-bromazepam. *Biochem. Pharmacol.*, **27**, 809–810.
- Tateishi, N. and Sakamoto, Y. (1983): Nutritional significance of glutathione in rat liver. *In* Glutathione: Storage, Transport and Turnover in Mammals (Y. Sakamoto, T. Higashi and N. Tateishi, eds.). pp.13–38. Japan Sci. Soc. Press, Tokyo.
- Tietze, F. (1969): Enzymatic method for quantitative determination of nanogram amounts of total and oxidized glutathione: application to mammalian blood and other tissues. *Anal. Biochem.*, **27**, 502–522.
- Yoshida, M. and Hara, I. (1984): Effect of intraperitoneal injection with chlorobenzene on glutathione metabolism in rat liver. *Ind. Health*, **22**, 11–21.
- Yoshida, M. and Hara, I. (1985a): Analysis of chlorophenylmethylsulfides in the urine of rats injected with chlorobenzene by high performance liquid chromatography. *Ind. Health*, **23**, 283–287.
- Yoshida, M. and Hara, I. (1985b): Composition of urinary metabolites and variation of urinary taurine levels in rats injected with chlorobenzene. *Ind. Health*, **23**, 239–243.
- Yoshida, M. and Hara, I. (1985c): Variation of cysteine level by chlorobenzene-induced perturbation of glutathione metabolism in rat liver. *J. Nutr. Sci. Vitaminol.*, **31**, 69–76.
- Yoshida, M. and Hara, I. (1985d): Effect of dietary methionine on chlorobenzene-induced variation in hepatic glutathione and lipid peroxide levels in rats. *Nippon Eiyō Shokuryō Gakkaishi*, **38**, 279–283.
- Yoshida, M. and Hara, I. (1986): Effect of dietary protein quality on chlorobenzene metabolism and its toxicity in rats. *Jap. J. Hyg.*, **41**, 822–827.
- Wong, L. T., Solomonraj, G. and Thomas, B. H. (1976): Metabolism of [^{14}C] paracetamol and its interactions with aspirin in hamsters. *Xenobiotica*, **6**, 575–584.