Comparative Study on *in vitro* Inhibitory Effects of Heavy Metals on Rabbit Drug-Metabolizing Enzymes

Takayuki Nakahama,*,[a] Yoshio Inouye,[a] and Morio Fukuhara[b]

[a]School of Pharmaceutical Sciences, Toho University, Funabashi, Chiba 274–8510, Japan and [b]Department of Pharmaceutical Sciences, National Institute of Public Health, Tokyo 108–8638, Japan

(Received June 9, 2000; Accepted September 18, 2000)

The *in vitro* inhibitory effects of heavy metals, *i.e.*, mercury, cadmium, lead, nickel and beryllium in their chloride forms, on rabbit pulmonary drug-metabolizing enzymes were studied comparatively. Microsomal and cytosolic fractions prepared from rabbit lungs were incubated in the presence of heavy metals prior to enzyme assays. Mercury was the most potent in reducing cytochrome P450 content and mixed-function oxidase activities including NADPH-cytochrome *c* reductase and benzo[*a*]pyrene hydroxylase. The addition of mercury to pulmonary microsomal preparations resulted in a spectral shift from 450 nm to 420 nm in an absorption maximum of cytochrome P450-carbon monoxide complex with mercury chloride being more potent than methylmercury. Cadmium was also inhibitory, while the effects of nickel were noted only at higher concentrations. Neither lead nor beryllium was inhibitory. Among second-phase drug-metabolizing enzymes, UDP-glucuronyltransferase and glutathione *S*-transferase were found to be susceptible to the adverse effects of mercury and lead, respectively. The extent of inhibition of the latter activity by mercury were highly dependent on the concentration of glutathione, implying the complex formation between them. Cadmium was slightly inhibitory to both enzyme activities, though the other metals had no effect. The results indicate that the pulmonary toxicities of airborne heavy metals could be inferred using simple *in vitro* assays.

Key words —— heavy metal, drug-metabolizing enzyme, *in vitro*, lung, liver

INTRODUCTION

It is well known that heavy metals are widely distributed in an atmosphere and some of them can cause lung disorders in industrial fields.[1] Cadmium (Cd),[2–4] beryllium (Be),[5] mercury (Hg) and other metals[6] uptaken by inhalation have been reported to cause lung injuries in both human beings and experimental animals. Therefore, the evaluation of toxic potentials of metals is important for the risk assessment of human beings ordinarily exposed to these substances in an ambient air. Despite a number of studies concerning their toxicities, they have rarely been investigated comparatively. For this purpose and application to airborne chemicals in general, it is preferable to establish a simple *in vitro* method to estimate pulmonary toxicity. In the present study, the authors attempted to use *in vitro* biochemical parameters as surrogate markers for the toxicities on pulmonary cells or tissues.

Both phases 1 and 2 drug-metabolizing enzymes might play an important role in the protection of lung from dysfunctions caused by inhaled chemicals, as was the case of their hepatic counterparts. Therefore, the adverse effects on their activities *in vitro* could be candidate markers of pulmonary toxicity. Although drug-metabolizing enzymes of hepatic origin were proved to be suppressed by heavy metals,[7,8] it remains to be determined whether the pulmonary enzymes are susceptible to the suppressive effects of heavy metals. In fact, the distinct properties of drug-metabolizing enzymes were observed between lung and liver.[9]

We demonstrated previously that Cd fumes exhibited an inhibitory action on the activity of mixed-function oxidases in rabbit lung under short-term inhalation conditions (Fukuhara *et al.*, 1981),[10] and these *in vivo* effects of Cd fumes were in good correlation with the *in vitro* inhibitory effects of Cd on mixed-function oxidase activities in isolated pulmo-

*To whom correspondence should be addressed: School of Pharmaceutical Sciences, Toho University, 2–2–1 Miyama, Funabashi, Chiba 274–8510, Japan. Tel.: +81-47-472-2548; Fax: +81-47-472-2548; E-mail: nakahama@phar. toho-u. ac.jp

nary tissue fractions (Fukuhara and Takabatake, 1982).[11]

In this paper, the *in vitro* effects of a series of heavy metals known as atmospheric pollutants on the activities of pulmonary drug-metabolizing enzymes were studied comparatively in order to estimate their relative potentials to cause pulmonary disorders in exposed human beings.

MATERIALS AND METHODS

Chemicals —— NADP, glucose 6-phosphate, glucose 6-phosphate dehydrogenase, cytochrome *c*, UDP-glucuronic acid and glutathione were obtained from Boehringer-Mannheim (Mannheim, Germany). Benzo[*a*]pyrene, Cd chloride ($CdCl_2$), Hg chloride ($HgCl_2$), methyl-Hg chloride (MeHgCl), nickel (Ni) chloride ($NiCl_2$), lead(Pb)chloride ($PbCl_2$) and Be chloride ($BeCl_2$) were products of Wako Pure Chemicals (Osaka, Japan). Aniline and aminopyrine were purchased from Sanko Pharmaceut.Indust. (Tokyo, Japan).

Animals and Preparation of Subcellular Fractions of Lung and Liver —— Male albino rabbits (Japanese White) weighing 2.5–3.0 kg were obtained from Japan Laboratory Animals Co. (Tokyo, Japan). Microsomal and cytosolic fractions were prepared from rabbit liver and lung as described previously.[10] Briefly, homogenates of these organs were centrifuged at 9,000 *g* for 10 min and the pellets were further centrifuged at 105,000 *g* for 1 hr to give microsomal (precipitate) and cytosolic (supernatant) fractions. Protein concentrations of these fractions were determined by Lowry's method[12] using bovine serum albumin as a standard.

Enzyme Assays and *in vitro* Studies —— Amounts of cytochrome P450 proteins in microsomal fractions from liver and lung were determined by the method of Omura and Sato[13] and Johannesen and DePierre,[14] respectively. NADPH-cytochrome *c* reductase, aminopyrine *N*-demethylase, and aniline hydroxylase were assayed by the method of Mazel[15] and NADH-dependent cytochrome b_5 reductase according to the method of Takesue and Omura.[16] NADPH- and NADH-benzo[*a*]pyrene hydroxylases[17] and UDP-glucuronyltransferase[18] were also assayed in microsomal fractions. Cytosolic fraction was tested for glutathione *S*-transferase activity.[19]

The effects of heavy metals were studied *in vitro* as described previously[11] on the levels of cytochrome P450 proteins and the activities of NADPH-cytochrome c reductase and NADPH-benzo[*a*]pyrene hydroxylase. These enzymes were chosen as the representative enzymes of the mixed-function oxidase system. The effects of heavy metals on the activities of second-phase drug-metabolizing enzymes, UDP-glucuronyl transferase and glutathione *S*-transferase, were studied in the same manner. Briefly, the metals were added to the reaction mixtures consisting of pulmonary subcellular fractions, co-factors and substrates, and the whole was kept at 37°C for 20 min in advance of the enzyme reactions. The 20 min-preincubation was found to be long enough to obtain the maximum inhibition rates for most of the pulmonary drug-metabolizing enzymes.[11]

RESULTS

Comparison of the Activities of Drug-Metabolizing Enzyme in Rabbit Lung and Liver

To characterize rabbit lung from a biochemical viewpoint, the basal levels of activities of mixed-function oxidases and second-phase drug-metabolizing enzymes as well as the protein content of cytochrome P450s were compared with those for liver. The enzyme activities were expressed on a protein basis and the protein levels of cytochrome P450s on the basis of wet organ weight. The results are summarized in Table 1. The protein content of cytochrome P450s in pulmonary microsomal fraction was approximately one third that of hepatic origin. The specific activities of aminopyrine *N*-demethylase, aniline hydroxylase and NADPH-cytochrome c reductase activities in pulmonary microsomes were higher than 50% of their hepatic counterparts. Concerning NADH-cytochrome b_5 reductase, there was no distinct difference between the enzyme activities of lung and liver. However, NADPH- and NADH-benzo[*a*]pyrene hydroxylases of pulmonary microsomes were of rather low specific activities compared with the respective hepatic enzymes. It is noteworthy that the activities of pulmonary second-phase drug-metabolizing enzymes were 2 to 3 orders of magnitude lower than the hepatic ones, as shown by the results for cytosolic glutathione S-transferase (1.2%) and microsomal UDP-glucuronyltransferase (0.2%) activities.

Table 1. Comparison of Drug-Metabolizing Enzymes in the Microsomal Fractions of Lung and Liver of Rabbit

Enzyme	Lung	Liver	Ratio (Lung/liver)
Organ wet weight (g/100 g-body.weight)	0.38 ± 0.03	2.30 ± 0.20	(0.16)
Cytochrome P450 (nmol/mg-protein)	0.33 ± 0.03	1.00 ± 0.10	(0.33)
Enzyme activity (nmol/min/mg-protein)			
Aminopyrine *N*-demethylase	2.9 ± 0.3	4.9 ± 0.3	(0.59)
Aniline hydroxylase	0.26 ± 0.05	0.40 ± 0.03	(0.65)
NADPH-cyt. *c* reductase	19.2 ± 1.6	33.8 ± 2.1	(0.57)
NADH-cyt. b_5 reductase	2.8 ± 0.2	2.9 ± 0.2	(0.96)
Benzo[*a*]pyrene hydroxylase			
NADPH-dependent	0.09 ± 0.01	1.60 ± 0.10	(0.06)
NADH-dependent	0.06 ± 0.01	0.40 ± 0.05	(0.15)
Glucuronyl transferase	0.03 ± 0.01	17.2 ± 1.3	(0.002)
Glutathione *S*-transferase	67 ± 5	5660 ± 150	(0.012)

Each value represents the mean ± S.E.M. of 5 animals.

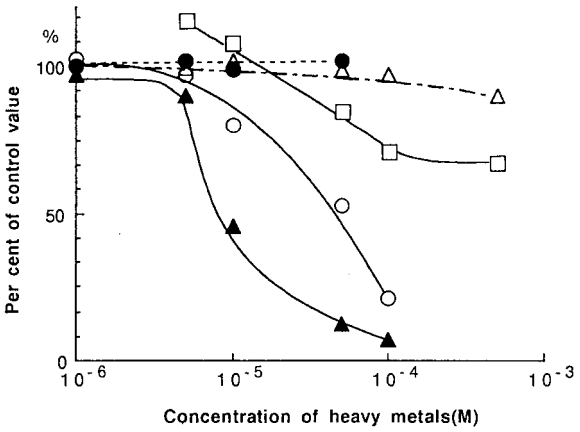

Fig. 1. *In vitro* Effects of Heavy Metals on Cytochrome P450 of Rabbit Lung Microsomes

Hg (▲), Cd (○), Ni (□), Be (△) and Pb (●) were added to the incubation mixtures containing lung microsomes at the concentrations indicated and the whole was incubated at 37°C for 20 min. Decreases in cytochrome P450 contents were measured. Each point represents the percentage of the level vs. that in the microsomes incubated without heavy metals.

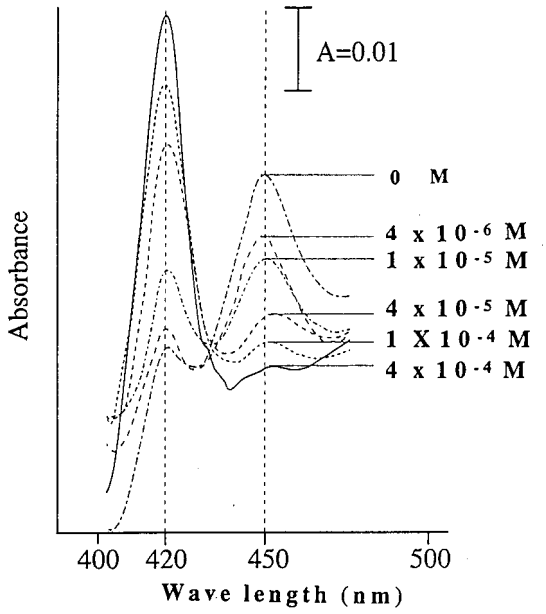

Fig. 2. Spectral Shift of Maximum Absorption of Cytochrome P450-CO Complex in the Presence of Hg

Spectra of the reduced form of cytochrome P450-CO complex were determined after the incubation of pulmonary microsomes at 37°C for 20 min in the presence of different concentrations of Hg.

Effects of Heavy Metals on the Cytochrome P450 Content and the Enzyme Activities of Mixed-Function System in Pulmonary Microsomal Fractions

Effects of five inorganic metals on the cytochrome P450 content and the activities of pulmonary microsomal mixed-function oxidases were studied comparatively in the concentration range of 4×10^{-7} to 4×10^{-4} M.

As can be seen in Fig. 1, the addition of Hg to microsomal preparations resulted in a marked reduction in cytochrome P450 content in a concentration-dependent manner. The spectral shift of the absorption maximum of cytochrome P450-carbon monoxide complex from 450 to 420 nm was observed to be a function of Hg concentration (Fig. 2), and the absorption maximum at 450 nm completely disappeared at the highest concentration tested. In the presence of Cd, the reduction in cytochrome P450 content was also noted to a lesser extent. The suppressive effect of Ni was marginal at higher con-

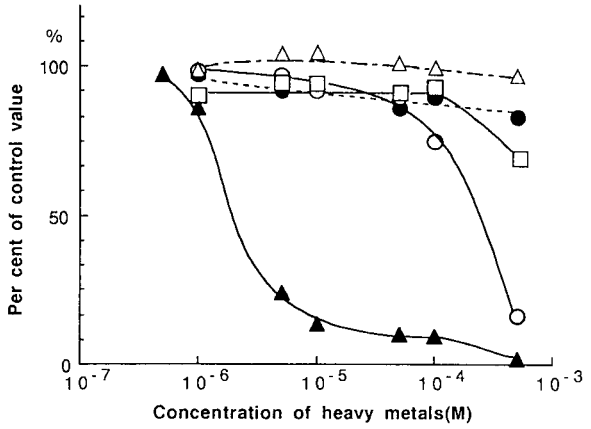

Fig. 3. *In vitro* Effects of Heavy Metals on the NADPH-Cytochrome *c* Reductase Activity of Rabbit Lung Microsomes

Microsomal preparations were incubated in the presence of different concentrations of heavy metals at 37°C for 20 min prior to the enzyme assays. Hg (▲), Cd (○), Ni (□), Be (△), Pb (●)

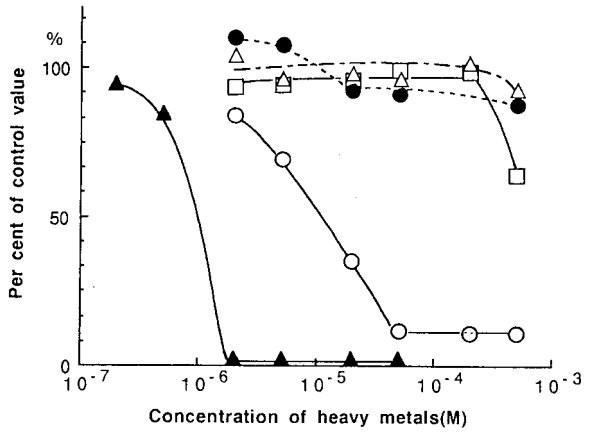

Fig. 4. *In vitro* Effects of Heavy Metals on the Arylhydrocarbon Hydroxylase Activity of Rabbit Lung Microsomes

The assay procedures and the symbols for metals are the same as those in Fig. 3.

centrations, whereas Be and Pb did not affect cytochrome P450 content.

For NADPH-cytochrome *c* reductase, the highest inhibition of enzyme activity was recognized in the presence of Hg (Fig. 3). The inhibitory effect of Cd was observed only at higher concentrations and the other metals were substantially of no effect on the enzyme activity.

Likewise, Hg was the most potent inhibitor of NADPH-benzo[*a*]pyrene hydroxylase (Fig. 4). Cd and Ni were also inhibitory but less potent than Hg, and the other metals had no effect on this enzyme activity. Since Hg was proved to be the most potent inhibitor of NADPH-benzo[*a*]pyrene hydroxylase

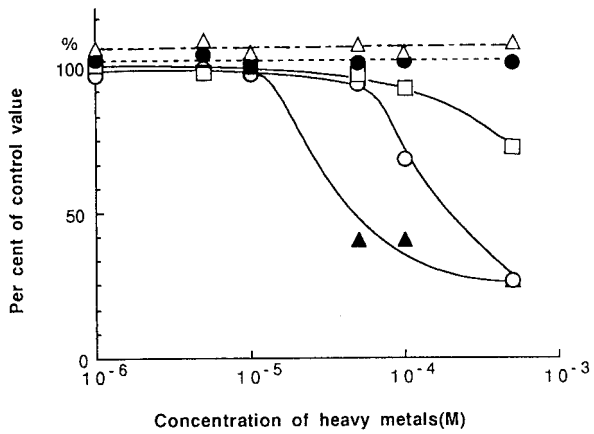

Fig. 5. *In vitro* Effects of Heavy Metals on the UDP-Glucuronyl Transferase Activity of Rabbit Lung Microsomes

The assay procedures and the symbols for metals are the same as those in Fig. 3.

among the metals tested, the effect of MeHg on the same enzyme activity was tested to see whether the observed inhibitory effect of Hg was dependent on the chemical form. The 50% inhibitory concentration (IC_{50}) of MeHg at 20×10^{-7} M was found to be higher than that of Hg at 8×10^{-7} M, suggesting that inorganic Hg was more potent than the organic form.

Effects of Heavy Metals on the Activities of Second-Phase Drug-Metabolizing Enzymes in Pulmonary Microsomal and Cytosolic Fractions

Among second-phase drug-metabolizing enzymes, UDP-glucuronyltransferase activity in the microsomal fraction and glutathione *S*-transferase in the cytosolic fraction were tested for their susceptibilities to heavy metals. In parallel with the results for the first-phase drug-metabolizing enzymes, Hg was the top-ranked inhibitor of UDP-glucuronyltransferase, followed by Cd and Ni (Fig. 5). No marked effects were noted for Be or Pb. In contrast, Pb and Cd were equally potent in the reduction of glutathione S-transferase activity (Fig. 6), while Be and Ni did not show any effect. The results for Hg are not included in Fig. 6 because the effect of Hg depends on the concentration of glutathione in the reaction mixture due to the complex formation with Hg.[20,21]

DISCUSSION

In the present study, it was shown that some of heavy metals present in the atmospheric environ-

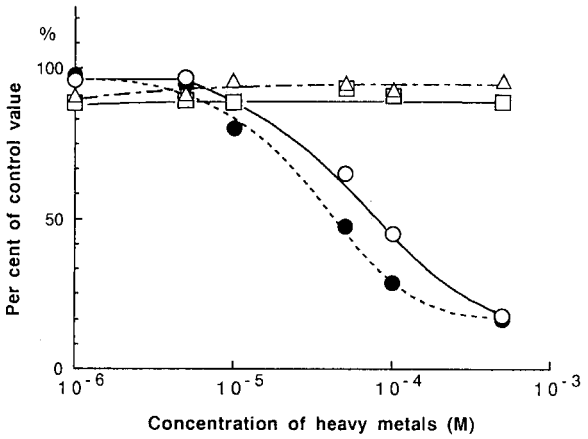

Fig. 6. *In vitro* Effects of Heavy Metals on the Glutathione *S*-Transferase Activity of Rabbit Lung Cytosol

Cytosolic preparations were incubated in the presence of different concentrations of heavy metals at 37°C for 20 min prior to the enzyme assays. Cd (○), Ni (□), Be (△), Pb (●)

ment were inhibitory against pulmonary drug-metabolizing enzymes *in vitro*. The potentials of individual metals are variable. However, Hg was generally the most potent in the inhibition of tested enzyme activities. Although acute exposure to a high level of Hg vapor may cause pneumonitis and other symptoms of pulmonary dysfunction,[6] few biochemical studies have been done on the pulmonary toxicity of this metal. Based on the results obtained in this study, Hg might exert a more potent inhibitory effect on a pulmonary drug-metabolizing function than the other heavy metals simultaneously tested when inhaled through respiratory tracts. The *in vitro* inhibitory action of Hg on pulmonary drug-metabolizing enzymes was partly explained by the direct degradation of cytochrome P450s as can be seen by a marked shift in the absorption maximum of cytochrome P450-carbon monoxide complex from 450 to 420 nm in the presence of Hg at concentrations inhibitory to most enzymes (Fig. 2). Atmospheric Hg was reported to be at least 90% in the form of Hg vapor and the remainder consisted of inorganic Hg and MeHg compounds,[6] which were released from incinerators, extraction processes for gold, mining, *etc*. Furthermore, concern has recently focused on the Hg exposure caused by dental amalgam, which is believed to be the major background source of human exposure to this metal.[6,22] Due to its highly diffusible and lipophilic nature, about 80% of inhaled Hg is estimated to be retained in the body.[6] It is thus important to evaluate human risks to exposure to atmospheric Hg.

For the other metals, there have been a few studies demonstrating the inhibitory actions on pulmonary enzymes *in vivo* (inhalation experiment) and *in vitro*. In the present study, Cd was shown to be another potent inhibitor of pulmonary enzymes *in vitro*, though it could not exceed Hg, in good accordance with our published findings as well as those of other groups using a series of animals exposed to metals in aerosol[10,23] and an *in vitro* study.[11] Although the toxicities of Be and Pb to lungs have been reported in animal experiments,[1] they were found to be less potent than Hg and Cd in terms of the suppression of enzyme activities. The discrepancy in the results obtained *in vitro* and *in vivo* imply that Be and Pb might exert their *in vivo* effects by different mechanisms from those of Hg and Cd.

Concerning the effects of heavy metals on the hepatic drug-metabolizing enzyme activities, Cd was reported to be suppressive on mixed-function oxidases.[24–29] Hg,[30,31] Pb,[30–34] Be[35] and Ni[7] are also able to reduce the hepatic enzyme activities. Based on those findings and our new results, the organ specificities shown by liver and lung in the susceptibilities of drug-metabolizing enzymes to several heavy metals were attributable partly, if not entirely, to the organ specific nature of enzymes. The basal activities of mixed-function oxidases and second-phase drug-metabolizing enzymes in lung were generally lower than those in liver in terms of a specific activity (Table 1). The activities of second-phase enzymes, which play a crucial role in the detoxification of xenobiotics, were extremely poor in 1ung, indicating that the toxic metabolites resulting from first-phase oxidative reactions could confer more severe damage to lung than liver. The toxicities or pharmacological activities of some chemicals are known to depend largely on the characteristics and contents of metabolizing enzymes in target organs. This indicates that the biochemical background of pulmonary toxicities of certain chemicals could be estimated by the activities of drug-metabolizing enzymes of lung origin. The metals uptaken via respiratory tracts should be given attention because they would affect the functional integrity of drug-metabolizing enzymes as environmental factors modifying the toxicities or pharmacological actions of xenobiotics or drugs.

Since there were few studies on the correlation between pulmonary toxicities of metals[36] and their inhibitory activities on drug-metabolizing enzymes, the authors refrain from concluding that the inhibitory potentials of metals on enzyme activities shown in the present study represent their ability to cause

pulmonary toxicity. However, based on the results of our previous studies comparing the *in vivo* and *in vitro* activities of Cd in lung[10,11] and a series of metals in liver, the *in vivo* inhalation toxicities of metals might be inferred from their *in vitro* anti-enzymatic potentials. In conclusion, the authors propose the *in vitro* enzyme assay system using pulmonary fractions as a simple alternative to preliminarily estimate inhalation toxicities, though this remains to be further confirmed.

Acknowledgement The authors appreciate the cooperation of Dr. G. Urakubo, School of Pharmaceutical Sciences, Toho University.

REFERENCES

1) Nemery, B. (1990) Metal toxicity and the respiratory tract. *European Respiratory Journal*, **3**, 202–219.
2) Lauwerys, R. R., Buchet, J. P. and Roels, H. A., Brouwers, J., Stanescu, D. (1974) Epidemiological survey of workers exposed to cadmium. *Archives of Environmental Health,* **25**, 145–148.
3) Bus, J. S., Vinegar, A. and Brooks, S. M. (1978) Biochemical and Physiologic changes in lungs of rats exposed to a cadmium chloride aerosol. *American Review of Respiratory Disease,* **118**, 573–580.
4) Henderson, R. F. A., Rebar, H., Pickrell, J. A. and Newton, G. J. (1979) Early damage indicators in the lung. III. Biochemical and cytological response of the lung to inhaled metal salts. *Toxicology and Applied Pharmacology*, **50**, 123–136.
5) Reeves, A. L. (1977) Beryllium carcinogenesis. *Advances in Experimental Medicine and Biology*, **91**, 13–27.
6) Clarkson, T. W. (1997) The toxicology of mercury. *Critical Reviews in Clinical Laboratory Sciences*, **34**, 369–403.
7) Arizono, K., Sakamoto, J., Mikajiri, M., Murashima, A. and Ariyoshi, T. (1993) Effects of various metals on hepatic biological responses in rat. *Trace Elements in Medicine*, **10**, 80–84.
8) Ueng, T. H., Ueng, Y. F., Chen, T. L. and Alvares, A. P. (1991) The relationship between induction of metallothionein and inhibition of monooxygenases by cadmium and lead. *Journal of Chinese Biochemical Society*, **20**, 87–98.
9) Philpot, R. M. and Smith, B. R. (1984) Role of cytochrome P-450 and related enzymes in the pulmonary metabolism of xenobiotics. *Environmental Health*, **55**, 359–367.
10) Fukuhara, M., Bouley, G., Godin, J., Girard, F., Boisset, M. and Boudene, C. (1981) Effects of short-term inhalation of cadmium oxides on rabbit pulmonary microsomal enzymes. *Biochemical Pharmacology*, **30**, 715–720.
11) Fukuhara, M. and Takabatake, E. (1982) *In vitro* inhibitory action of cadmium on microsomal monooxygenases of rabbit lung. *Biochemical Pharmacology*, **31**, 3425–3429.
12) Lowry, O. H., Rosebrough, N. J., Farr, A. and Randall, R. J. (1951) Protein measurement with the Folin phenol reagents. *Journal of Biological Chemistry*, **193**, 265–275.
13) Omura, T. and Sato, R. (1964) The carbon monoxide-binding protein of liver microsomes. *Journal of Biological Chemistry*, **239**, 2370–2378.
14) Johannesen, K. A. M. and DePierre, J. W. (1978) Measurement of cytochrome P-450 in the presence of large amounts of contaminating hemoglobin and methohemoglobin. *Analytical Biochemistry*, **86**, 725–732.
15) Mazel, P. (1972) In *Fundamentals of Drug Metabolism and Drug Disposition*, (LaDu, B. M., Mandel, H. G. and Way, E. L., Eds.), Williams and Wilkins, Baltimore, p.546.
16) Takesue, S. and Omura, T. (1970) Purification and properties of NADH-cytochrome b_5 reductase solubilized by lysosomes from rat liver microsomes. *Journal of Biochemistry*, **67**, 267–276.
17) Dehnen, W., Tomingas, R. and Roos, J. (1973) A modified method for the assay of benzo[*a*]pyrene hydroxylase. *Analytical Biochemistry*, **53**, 373–383.
18) Lilienblum, W., Walli, A., Bock, K. (1982) Differential induction of rat liver microsomal UDP-glucuronyltransferase activities by various inducing agents. *Biochemical Pharmacology*, **31**, 907–913.
19) Habig, W. H., Pabst, M. J. and Jokoby, W. N. (1974) Glutathione *S*-transferases. The first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry*, **249**, 7131–7139.
20) Osawa, M. and Magos L. (1974) The chemical form of the methyl mercury complex in the bile of the rat. *Biochemical Pharmacology*, **23**, 1903–1905.
21) Rabenstein, D. L. and Isab, A. A. (1982) A proton nuclear magnetic resonance study of the interaction of mercury with intact human erythrocytes. *Biochimica. et Biophysica. Acta*, **721**, 374–384.
22) Mackert, J. R. and Jr. Berglund, A. (1997) Mercury exposure from dental amalgam filings: absorbed dose and the potential for adverse health effects. *Critical Review in Oral Biology and Medicine*, **8**, 410–436.
23) Boisset, M. and Boudene, C. (1981) Effect of a single exposure to cadmium oxide fumes on rat lung microsomal enzymes. *Toxicology and Applied Pharmacology*, **57**, 335–345.

24) Hadley, W. M. and Miya, T. S. (1974) Bousquet W.F. Cadmium inhibition of hepatic dray metabolism in the rat. *Toxicology and Applied Pharmacology*, **28**, 284–291.
25) Yoshida, T., Ito, Y., Suzuki, Y. and Uchiyma, M. (1976) Inhibition of hepatic UDP-glucuronyltransferase activity by organophosphate insecticides and by carbon disulfide in mice. *Bulletin of Environmental Contamination and Toxicology*, **15**, 402–424.
26) Hayes, J. A., Snider, G. L. and Palmer, K. C. (1976) The evolution of biochemical damage in the rat lung after cadmium exposure. *American Review of Respiratory Disease*, **113**, 121–130.
27) Teare, F. W., Jasansky, P., Renaud, L. and Read P. R. (1977) Acute effect of cadmium on hepatic drug-metabolizing enzymes in the rat. *Toxicology and Applied Pharmacology*, **41**, 57–65.
28) Aitio, A., Ahotupa, M. and Parkki, M. (1978) Inhibition of drug-metabolizing enzymes by heavy metals *in vitro*. *Biochem. Biophys. Res. Commun.*, **83**, 850–856.
29) Means, J. R., Carson, G. P. and Schnell, R. C. (1979) Studies on the Mechanism of cadmium-induced inhibition of the hepatic microsomal monooxygenase of the male rat. *Toxicology and Applied Pharmacology*, **48**, 293–304.
30) Alvares, A. P., Leigh, S., Cohn, J. and Kappas, A. (1972) Lead and methyl mercury; effects of acute exposure on cytochrome P-450 and the mixed function oxidase system in the liver. *Journal of Experimental Medicine*, **135**, 1406–1409.
31) Abbas, A. B. (1980) Effect of mercuric chloride on microsomal enzyme system in mouse liver. *Pharmacology*, **21**, 59–63.
32) Roomi, M. W., Columbano, A., Ledda-Columbano, G. M. and Sarma D. S. R. (1986) Lead nitrate induces certain biochemical properties characteristic of hepatocyte nodules. *Carcinogenesis*, **7**, 1643–1646.
33) Falke, H. E. and Zwennis, W. C. M. (1990) Toxicity of lead acetate to female rabbits after chronic subcutaneous administration. 1. Biochemical and clinical effects. *Archives of Toxicology*, **64**, 522–529.
34) Nehru, B. and Kaushals, S. (1992) Effect of lead on hepatic microsomal enzyme activity. *Journal of Applied Toxicology*, **12**, 401–405.
35) Teixeira, C. F. P., Yasaka, W. J., Silva, L. F., Oshiro, T. T. and Oga S. (1990) Inhibitory effects of beryllium chloride on rat liver microsomal enzymes. *Toxicology*, **61**, 293–301.
36) Camner, P., Curstedt, T., Jrstrand, C., Johannsson, A., Robertson, B. and Wiernik, A. (1985) Rabbit lung after inhalation of manganese chloride. A comparison with the effects of chlorides of nickel, cadmium, cobalt, and copper. *Environmental Research*, **38**, 301–309.