EFFECTS OF PHENOBARBITAL ON DRUG METABOLIZING ENZYME ACTIVITIES AND OTHER BIOCHEMICAL PARAMETERS IN RATS WITH DL-ETHIONINE-INDUCED LIVER INJURY

Michishige NOGUCHI, Tatsuya FUJITSUKA, Katsuya HONDA and Yoshiaki KAWAI

Safety Research Laboratory, Tanabe Seiyaku Co., Ltd.,
16-89 Kashima-3-chome, Yodogawa-ku, Osaka 532, Japan

Received May 31, 1994 ; Accepted September 21, 1994

ABSTRACT — Phenobarbital (PB) was orally administered once at a dose of 100 mg/kg to the liver injury model rats treated with DL-ethionine (ET), and the effects of PB on the liver drug metabolizing enzymes (DME) were chiefly examined.

Liver weight, liver microsomal protein content, liver aniline 4-hydroxylase (ANH) activity, and aminopyrine N-dimethylase (AMD) activity were markedly increased in the ET-treated rats receiving PB. These findings suggested the induction of DME in the liver. However, the induction pattern of each enzyme was different. AMD activity at 48 hr after dosing of PB in the ET-treated rats was increased in the same degree as that in the control (normal). Whereas, ANH activity at 48 hr after dosing in the ET-treated rats was higher than that in normal rats. Liver lactate dehydrogenase (LDH) activity at 48 hr after dosing in the ET-treated rats was markedly increased, but such induction was not seen in normal rats.

These findings indicates that DME in the liver is induced by PB treatment in the ET-treated rats as well as in normal rats, and that the ET-treated rats have a function of physiological adaptation similar to that in normal rats. The induction pattern of liver or serum enzymes in the ET-treated rats receiving PB was different from that in normal rats. Furthermore, the induction pattern of these enzymes in the ET-treated rats receiving PB was different from that in the normal rats, which may be attributed to the difference of localization in liver cells of these enzymes affected by PB.

KEY WORDS: Drug metabolizing enzyme, Rat, Phenobarbital, Ethionine, Liver injury.

INTRODUCTION

Phenobarbital (PB) is a sedative and an antiepileptic drug, and it is also used for epileptics accompanied with liver diseases. The toxicity of drug is enhanced and efficacy lasts longer than usual in patients with liver diseases because of the disorder of the detoxication mechanism including liver drug metabolizing enzymes (DME) (Conney, 1967).

As part of the study on the effects of PB on epileptics with liver diseases, we previously reported the effects of PB on the toxicokinetic parameters after oral or intravenous treatment of PB using the experimental liver injury model.
(ethionine-treated rats), and we pointed out the disorder of excretion of PB from the blood, liver and kidney (Noguchi et al., 1993), but did not show the effects on the induction of DME.

Stengard (1984) reported that normal animals treated with an inducer such as PB show the induction of liver DME, proliferation of liver microsomes and increased liver weight. These changes in normal animals are considered to be caused by physiological adaptation of the liver (Conney, 1967). However, when PB is administered to animals with liver injury, it has not been clear whether the time-course change of the induction of liver DME is similar to that in normal animals. The examination of time-course changes of liver DME activity after dosing of PB in the ET-treated rats is useful to define the kinetics of PB in patients with a liver disease.

In the present study, we examined the effects of PB on liver DME activity using the liver injury model rats. After the rats were intraperitoneally administered ET 4 times at a dose of 500 mg/kg each, they received a single oral treatment with 100 mg/kg of PB. The time-course changes in liver microsomal DME activity and other biochemical parameters were examined, and were compared with those of normal animals (the control rats).

MATERIALS AND METHODS

1. Chemicals.

Sodium carboxymethylcellulose (CMC) was purchased from Katayama Chemical Industries. DL-ethionine (ET) was obtained from Nakalalai Tesque Ltd. (Kyoto, Japan). Sodium phenobarbital (PB) and aniline hydrochloride were purchased from Wako Pure Chemical Industries (Osaka, Japan). Aminopyrine was obtained from Aldrich Chemical Co. (Milwaukee, USA). All the other reagents were of special grade.

2. Animals.

Female Sprague-Dawley (SD) rats (Japan SLC) each weighing 130 to 179 g at approximately 6 weeks of age were used for the study. Rats were given rodent chow (CRF-1, Oriental Yeast Co.) and tap water ad libitum. All animals were individually housed in a stainless steel cage and the animal room was maintained at a temperature of 23±1°C, with a relative humidity of 55±5% and a 12 hr light-dark cycle (lights on from 6:30 to 18:30). Each experimental group consisted of 5 rats.

3. Experimental design and performance.

Liver DME and liver and serum biochemical parameters in the liver injury model (ET-treated rats): ET dissolved in physiological saline was intraperitoneally given at a dose of 500 mg/kg/day to the rats for 4 consecutive days. Fifteen rats (5 rats each at 3 time points; 24, 48 and 72 hr after the final dosing of ET) in the ET-treated groups and 5 rats in the control (non treated rats) group were killed under ether anesthesia at each time point. After blood sampling, the rats were killed, and the liver was removed and weighed.

Liver DME activities and liver and serum biochemical parameters after dosing of phenobarbital to the liver injury model: PB dissolved in physiological saline was orally administered once at a dose of 100 mg/kg to the liver injury model (ET-treated rats) 24 hr after the final dosing (500 mg/kg/day, 4 days, i.p.) and the control rats (normal rats). Of 35 (or 25) rats in the ET-treated and control groups 5 rats each were anesthetized with diethyl ether for taking blood and liver samples at 7 (or 5) time points (0, 0.5, 1, 4, 7, 16 and 48 hr, or 0, 1, 4, 28 and 48 hr after dosing of PB). After blood sampling, the rats were killed, and the liver was removed and weighed.

Preparation of liver microsomal suspension: For determination of liver DME activities, one g of liver sample was homogenized with 4 ml of ice-cold 1.15% KCl solution using a motor driven Warrin-Blender (Nihon Seiki Co.). The homogenate was centrifuged at 9,000×g for 15 min at 4°C. Eight mM of CaCl₂ was added to 4 ml of the supernatant (Cinti et al., 1972) and centrifuged at 25,000×g for 15 min at 4°C. To the pellet obtained was added 4 ml of 1.15% KCl, mixed by Ultra-turrax (Janke & Kunkel Co.), and centrifuged at 25,000×g for 15 min at 4°C. To the pellet was added 4 ml of 1.15% KCl, mixed by Ultra-turrax. The suspension of liver microsomes was prepared. It is well known that 50-75% of the protein contents are lost during the preparation of microsomal fraction (Schoene et al., 1971). If cytochrome P-450 or microsomal enzyme activities are determined in a whole liver homogenate, no reduction in the activities will be noted. Cinti and Schenkman (1971) have reported that the amounts of cytochrome P-450 were 29.1 nmoles/g liver in the liver homogenates and 0.56 nmoles/mg microsomal protein in microsomal fractions, and that the microsomal protein content was 52 (29.1 nmoles/g liver/0.56 nmoles/mg microsomal protein)±6 mg per g liver weight (male rats). The yield of liver microsomal protein obtained on the method of Cinti et al. (1972). The liver microsomal suspension was used for determination of protein content, aniline 4-hydroxylase (ANH) or aminopyrine N-demethylase (AMD) activity.

Assay of liver microsomal DME activity: Liver ANH and AMD activities in microsomal suspension were measured spectrophotometrically using p-aminophenol (p-AP) as the substrate (Imai et al., 1966) and by the quantitation of formaldehyde as described by Nash method as modified by Cochin and Axelrod (1959), respectively. Protein in the liver microsomal suspension was determined by the Lowry method with the use of BSA as a standard. Liver DME activity was expressed as nmol per mg microsomal protein.

Assay of serum and liver biochemical parameters: Blood was withdrawn from the abdominal aorta, blood was transferred into a polyethylene tube at 4°C and centrifuged at 900×g for 15 min at 4°C and stored at -70°C until assay. The liver samples were homogenized with 4 volumes of ice-cold 1.15% KCl solution using a motor driven Warrin-Blender. The liver homogenate was centrifuged at 900×g for 15 min at 4°C. Tissue extract supernatants were used as the crude liver fraction for measurement of biochemical parameters. Serum and liver (the crude fraction) GOT, GPT and LDH activities and triglycerides, total cholesterol, phospholipid, glucose and/or total protein levels were determined by an autoanalyzer (TECIRON XA18).

5. Statistical analysis.

The results are presented as the mean values ±S.D. Statistical processing was done by parametric analysis of variance using a Statistical Analysis Package (Y.K.D. CO., Ltd.) and a personal computer (NEC PC-9801; MUSCOT computer program).

RESULTS

1. Changes in liver DME activities and levels of others in the liver injury model (ethionine-treated rats).

Table 1 shows the time-course changes of body weight, liver weight and liver DME (ANH, AMD) activities in rats after receiving ET intraperitoneally at a dose of 500 mg/kg for 4 days.

The body weight 24, 48 and 72 hr after dosing of ET were significantly lower than that in the control non-treated rats). The liver weight and hepatic AMD activity 24 hr after dosing of ET were significantly lower than those in the control (non-treated rats). However, there were no significant changes in hepatic ANH activity after dosing of ET.

Table 2 shows the results of liver and serum biochemical parameters in the control (non-treated rats) and ET-treated rats.

In the liver biochemistry, the liver GPT activity and TC level were significantly lower, and TG level was significantly higher. The liver LDH activity and PL and GLU levels tended to be lower in the ET-treated rats than those in the control (non-treated rats).

In serum biochemical examination, TG, TC, PL, GLU, and TP levels in the ET-treated rats were significantly lower than those in the control (non-treated rats). The GOT and GPT activities in the ET-treated rats tended to be higher than
Table 1. Changes of body weight, liver weight, aniline 4-hydroxylase and aminopyrine N-dimethylase activities in rats treated with ethionine intraperitoneally at a dose of 500 mg/kg for 4 days.

<table>
<thead>
<tr>
<th>Time after the final treatment</th>
<th>body weight (g)</th>
<th>liver weight (g)</th>
<th>aniline 4-hydroxylase (nmol p-AP/mg protein/min)</th>
<th>aminopyrine N-dimethylase (nmol HCHO/mg protein/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control (non treated rats)</td>
<td>182 ± 9.9</td>
<td>8.49 ± 1.25</td>
<td>0.162 ± 0.021</td>
<td>1.37 ± 0.18</td>
</tr>
<tr>
<td>24 hr</td>
<td>150 ± 16.0**</td>
<td>5.57 ± 1.08**</td>
<td>0.168 ± 0.076</td>
<td>0.75 ± 0.26**</td>
</tr>
<tr>
<td>48 hr</td>
<td>160 ± 9.6**</td>
<td>6.56 ± 0.79</td>
<td>0.156 ± 0.064</td>
<td>0.97 ± 0.34*</td>
</tr>
<tr>
<td>72 hr</td>
<td>158 ± 19.9*</td>
<td>6.82 ± 1.64</td>
<td>0.224 ± 0.071</td>
<td>1.65 ± 0.24</td>
</tr>
</tbody>
</table>

Values are means ± S.D. (n=5)
* ; Significantly different (P<0.05) from control (non treated rats).
** ; Significantly different (P<0.01) from control (non treated rats).
*** ; Significantly different (P<0.001) from control (non treated rats).

Table 2. Liver and serum biochemical parameters in the ethionine treated rats.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>GOT (U/g liver)</th>
<th>GPT (U/g liver)</th>
<th>LDH (mg/g liver)</th>
<th>triglyceride (mg/g liver)</th>
<th>total cholesterol (mg/g liver)</th>
<th>phospholipid (mg/g liver)</th>
<th>glucose (mg/g liver)</th>
<th>total protein (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Liver)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control (non treated rats)</td>
<td>93 ± 20</td>
<td>33 ± 15</td>
<td>209 ± 173</td>
<td>8.6 ± 2.2</td>
<td>1.9 ± 0.4</td>
<td>5.2 ± 1.3</td>
<td>18.8 ± 3.8</td>
<td></td>
</tr>
<tr>
<td>ethionine-treated rats</td>
<td>73 ± 14**</td>
<td>14 ± 6</td>
<td>169 ± 75</td>
<td>18.0 ± 3.2</td>
<td>1.1 ± 0.5</td>
<td>3.9 ± 1.2</td>
<td>13.6 ± 11.4</td>
<td></td>
</tr>
<tr>
<td>(Serum)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control (non treated rats)</td>
<td>53.2 ± 2.1</td>
<td>31.7 ± 4.2</td>
<td>152 ± 37</td>
<td>84.2 ± 16.9</td>
<td>56.0 ± 25.3</td>
<td>131 ± 10</td>
<td>184 ± 12</td>
<td>5.99 ± 0.10</td>
</tr>
<tr>
<td>ethionine-treated rats</td>
<td>74.1 ± 4.3</td>
<td>41.3 ± 4.2</td>
<td>164 ± 35</td>
<td>54.0 ± 15.1</td>
<td>29.1 ± 15.4</td>
<td>86 ± 28</td>
<td>138 ± 11</td>
<td>5.12 ± 0.33</td>
</tr>
</tbody>
</table>

Values are means ± S.D. (N=5)
* ; Significantly different (P<0.05) from control (non treated rats).
** ; Significantly different (P<0.01) from control (non treated rats).
*** ; Significantly different (P<0.001) from control (non treated rats).

2. Effect of phenobarbital on liver DME activities and others in ethionine-treated rats.

Figure 1 shows the liver weight; Figure 2 shows the time-course changes of liver ANH and AMD activities. Figure 3 shows the time-course changes of serum GOT, GPT and LDH after dosing of PB in the control (normal rats) and the ET-treated rats.

The liver weight in the control and ET-treated groups was significantly higher 48 hr after dosing of PB than the initial mean weight in each group. The liver microsomal protein content in the control and the ET-treated group after dosing of PB were significantly higher than that of the initial level in each group (control rats: the initial level vs. the level 48 hr after dosing of PB; 24.8 ± 5.4 vs. 30.6 ± 5.9 mg/g liver, mean ± S.D. and ET-treated rats: the initial level vs. the level 7 or 48 hr after dosing of PB; 22.8 ± 4.6 vs. 27.0 ± 2.5
Drug-metabolizing-enzyme induction by phenobarbital in ethionine-treated rats.

**Fig. 1.** The time-course changes in liver weights and microsomal protein content after a single oral administration of phenobarbital at 100 mg/kg in control (normal rats) and ethionine-treated rats. Vertical bars represent the mean and S.D.

* ; Significantly different ($P<0.05$) from the initial level in control (normal rats).

# ; Significantly different ($P<0.05$) from the initial level in ethionine-treated rats.

## ; Significantly different ($P<0.01$) from the initial level in ethionine-treated rats.

**Fig. 2.** The time course changes in hepatic aniline 4-hydroxylase (ANH) and aminopyrine N-demethylase (AMD) activities after a single oral administration of phenobarbital at 100 mg/kg in control (normal rats) and ethionine-treated rats.

Vertical bars represent the mean and S.D.

* * ; Significantly different ($P<0.05$) from the initial level in control rats (normal rats).

* * * ; Significantly different ($P<0.01$) from the initial level in control rats (normal rats).

* * * * ; Significantly different ($P<0.001$) from the initial level in control rats (normal rats).

# ; Significantly different ($P<0.05$) from the initial level in ethionine-treated rats.

## ; Significantly different ($P<0.01$) from the initial level in ethionine-treated rats.

## ## ; Significantly different ($P<0.001$) from the initial level in ethionine-treated rats.
or 32.1±5.0 mg/g liver, mean±S.D.), judging from the yield of microsomal protein during preparation of microsomes.

The liver microsomal ANH activity was significantly lower 0.5 hr after dosing of PB in the control (normal rats) and ET-treated rats, but it was significantly higher 4, 16 and 48 hr after dosing in the control rats, and 16 and 48 hr after dosing in the ET-treated rats than the initial level. The liver ANH activity 48 hr after dosing of PB in the ET-treated rats was significantly higher than that in the control. The liver microsomal AMD activity was significantly increased 7, 16 and 48 hr after dosing in the ET-treated rats and 48 hr after dosing in the control as compared with the initial level. The liver microsomal AMD activity 48 hr after dosing of PB in the control was significantly higher than that in the ET-treated rats. However, the degree of increment of AMD activity from 0 to 48 hr after dosing in the ET-treated group was similar to that in the control group.

Among the serum enzymes, the GOT activity was significantly increased in the control (normal rats) 0.5, 1, 4, 7 and 16 hr after dosing of PB, and in the ET-treated rats 0.5 hr after dosing of PB. The activity returned to the initial level 48 hr after dosing. The serum GPT activity in the ET-treated rats was significantly increased 0.5 and 1 hr after dosing of PB, but there were no changes in the control. The ET-treated rats showed a significant increase in serum LDH activity 0.5, 1, 4, 16 and 48 hr after dosing of PB as compared with the initial level. However, the control showed no significant changes in serum LDH activity.

Tables 3 show the results of time-course changes of liver GOT, GPT and LDH activities after dosing of PB in the control (normal rats) and ET-treated rats.

The GOT and GPT activities in the control and ET-treated rats after dosing of PB were not changed. The LDH activity of the supernatant centrifuged at 900×g in the ET-treated rats 48 hr after dosing of PB was significantly higher than that at the initial level. The liver LDH activity in the control without PB dosing did not increase.
Table 3. The time-course changes of liver GOT, GPT and LDH activities in the ethionine-treated rats receiving phenobarbital orally at a dose of 100 mg/kg.

<table>
<thead>
<tr>
<th>item</th>
<th>group</th>
<th>time after dosing of phenobarbital</th>
<th>times after without dosing of phenobarbital</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 hr</td>
<td>1 hr</td>
</tr>
<tr>
<td></td>
<td>control rats</td>
<td>0</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>(normal rats)</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>ethionine-</td>
<td>129</td>
<td>140</td>
</tr>
<tr>
<td>GOT</td>
<td>treated rats</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>(U/g liver)</td>
<td></td>
<td>25</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>control rats</td>
<td>41.1</td>
<td>37.2</td>
</tr>
<tr>
<td>GPT</td>
<td>(normal rats)</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>(U/g liver)</td>
<td></td>
<td>8.3</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td>ethionine-</td>
<td>23.8</td>
<td>21.0</td>
</tr>
<tr>
<td>LDH</td>
<td>treated rats</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>(U/g liver)</td>
<td></td>
<td>8.7</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>control rats</td>
<td>457</td>
<td>498</td>
</tr>
<tr>
<td>(normal rats)</td>
<td></td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>ethionine-</td>
<td>493</td>
<td>585</td>
</tr>
<tr>
<td>treated rats</td>
<td></td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70</td>
<td>65</td>
</tr>
</tbody>
</table>

Values are means± S.D. (n=5)

* ; Significantly different (P<0.05) from initial level in the ethionine-treated rats.

DISCUSSION

Pharmacokinetic studies after administration of some drugs to patients with liver disease or experimental liver injury animals (Alvin et al., 1975; Iga et al., 1977; Williams, 1983; Stengard, 1984; Loeser and Siegers, 1985) have been reported. Several studies on hepatic DME or metabolism of drug after dosing of drugs (Fukuhara and Takabatake, 1975; Tavoloni et al., 1983) using normal animals have also been reported. However, only a few studies on liver DME using animals or patients (Krähleubühl et al., 1990) with liver injury have been reported.

The liver injury model induced by treatment with ET used for this study is known to have characteristics such as increased liver TG and decreased liver TP (Platt and Cockrill, 1969; Yoshitake et al., 1991). Further, Berry and Friedman (1977) reported that female animals show a high sensitivity in hepatotoxicity of ET and are associated with synthesis of liver lipids and protein. Our data were in agreement with these findings.

Liver DME has been reported to show different responses in sex (Kato and Gillette, 1965) or pathological conditions such as alloxan diabetes, fasting (Salazar et al., 1988), adrenectomy and morphine administration (Miranda and Webb, 1973; Campbell and Hayes, 1974) and difference of immunological type in cytochrome P-450 (Thomas et al., 1976). It is not always clear whether the changes in DME activity after drug treatment are toxic or physiological. Fukuhara and Takabatake (1975) reported that a drug was hepatotoxic when liver DME activity was inhibited by treatment with the drug even if there was no histological changes in liver injury. On the other hand, Conney (1967) reported that the liver changes accompanied by liver enlargement and induction of liver DME were caused by physiological adaptation to the drugs. The results of this liver injury model showed hepatotoxic.

In hormone-related DME changes, Kato and
Gillette (1965) reported that alloxan diabetic rats and thyroxine-treated rats had decreased liver AMD (androsterone dependent enzyme) activity and unchanged liver ANH (androsterone independent enzyme) activity in the males, but that these activities were unchanged in the females. On the other hand, the rat with liver injury induced by treatment with CCl₄ are known to have decreased ANH and AMD activities in both sexes (Kato et al., 1967). Since only the liver AMD activity was decreased and the liver ANH activity was unchanged in the female rats with our liver injury model, this change was considered to belong to the type in which androsterone-dependent enzyme activity alone decreased. Furthermore, this finding is probably related to the sex difference described above (Berry and Friedman, 1977).

The yield of liver microsomal protein in female rats obtained by the method of Cinti et al. (1972) modified in our laboratory was 25–30 mg per g liver weight. The result was in agreement with the data (20–35 mg per g liver weight) of Mazel (1974).

PB was administered to the rats with liver injury induced by treatment with ET in this study. Microsomal protein level, liver DME activity, and the liver weight in the ET-treated rats were markedly increased after dosing and they were increased in the manner similar to those in the control (normal rats). Thus, the ET-treated rats after dosing of PB showed physiological adaptation such as induction of liver DME and liver enlargement similar to normal rats. However, the time-course changes in DME activities were different between the ET-treated and normal rats. Namely, the liver ANH activity in each group 0.5 hr after dosing of PB was lower than that in each of the initial level, respectively. That in the ET-treated rats 48 hr after dosing of PB was higher than that in the control. The liver AMD activity in the ET-treated rats 48 hr after dosing of PB was lower than that in the control. Also, the time-course changes in liver LDH activity in the ET-treated rats were markedly increased 48 hr after dosing, but no changes appeared in the control. The change in liver LDH activity was not based on rebound in ET treatment to rats because the liver LDH activity in the ET-treated rats without PB dosing did not increase. The increase of LDH activity after dosing of PB was a prominent characteristic in the ET-treated rats.

Furthermore, these findings indicated that increases in serum GOT and GPT activities after dosing of PB were due to even leakage in the damage of hepatocellular membrane since enzyme activities in the serum increased but those in the liver did not change. However, in both the serum and liver, the LDH activities in the ET-treated rats 1 or 48 hr after dosing of PB increased, respectively. Such differences in the induction pattern of each serum GOT, GPT and LDH, or liver LDH, ANH and AMD may be due to the disparity of the intracellular localization of the enzymes affected by PB in the ET-treated and control groups. These changes suggested that the increases in serum enzymes were caused not only by the damage of hepatocellular membrane but also the level of the intrahepatocellular enzyme.

Concerning localization of hepatic enzymes, Egashira and Waddell (1984) showed the differences in the localization of alcohol dehydrogenases in the mouse liver. Wolfson et al. (1958) reported that the sites of liver fraction damaged with CCl₄ can be judged by the increase in enzyme activity because there is a time lag between elevations in each enzyme. Our datas showed that there are difference between the ET-treated and control groups in an induction pattern and a time lag among increases in liver enzyme activities after dosing of PB.

In conclusion, the ET-treated rats after dosing of PB showed physiological adaptation such as liver enlargement and liver DME induction similar to normal rats, but the induction pattern was different from that in normal rats in the effect of PB on the liver fraction.

ACKNOWLEDGMENTS

We wish to express our thanks to Dr. Yoshio Nakayama, general manager of Safety Research Laboratory, Tanabe Seiyaku, for his many important suggestions and encouragement on this work, and our thanks to Mr. Shinji Minamide and Koki Tanaka for expert technical assistance.
REFERENCES


