CARBON TETRACHLORIDE-INDUCED HEPATOTOXICITY IN RATS: EVIDENCE FOR DIFFERENT SUSCEPTIBILITIES OF RAT LIVER LOBES

Takashi MATSUBARA, Satoru MORI*, Akira TOUCHI, Yoko MASUDA and Yasuyoshi TAKEUCHI*

Shionogi Research Laboratories, Shionogi and Co. Ltd., Fukushima-ku, Osaka 553, Japan and *Aburahi Laboratories, Shionogi Research Laboratories, Shionogi and Co. Ltd., Koka-cho, Shiga 520-34, Japan

Accepted December 3, 1982

Abstract—The hepatotoxic effect of carbon tetrachloride (CCI₄), reflected by augmented blood aspartate aminotransferase and alanine aminotransferase activities and the extent of histological liver damage, was observed following oral administration of CCl₄ to rats. A marked increase of blood transaminase activities and severe degeneration of hepatocytes in the centrilobular region were detected 1–2 days after the administration, while the cytochrome P-450 content and the drug metabolizing activity in livers were depressed immediately after the administration. Based on these results, the effect of CCl₄ on hepatic cytochrome P-450 and the histological pattern of liver cells was observed using tissue samples obtained from various liver lobes of rats given CCl₄ 24 hr previously. Dose-dependent inactivation of cytochrome P-450 by the administration of CCl₄ was observed throughout the liver, with the most extensive decrease in the cytochrome content in the median lobe. The extent of liver damage (hydropic swelling degeneration and central necrosis in lobule) was also greater in the median and right liver lobes than in the left lobe. When a small amount of CCI₄ was administered, degeneration of liver cells was detected only in the median and right lobes with only slight degeneration in the left lobe. These results indicate different susceptibilities of rat liver lobes to CCl₄.

The hepatotoxic effects of carbon tetrachloride (CCI₄) have been extensively investigated. The results indicate that CCl₄ requires activation by hepatic cytochrome P-450 monooxygenase before its hepatotoxic effects are manifested (1–3). Morphological observation indicates that CCl₄ does not induce cell damage uniformly throughout the liver, but induces predominantly damage in the centrilobular region (2, 4, 5). On the other hand, several recent studies concerned with the distribution of various drug-metabolizing enzymes have revealed that cytochrome P-450 and NADPH-cytochrome c (P-450) reductase are localized or enriched in hepatocytes of the centrilobular region (6–9). These results indicate probably a close correlation between intralobular distribution of cytochrome P-450 monooxygenase and CCl₄-induced damage of hepatocytes in liver lobules, although the picture is more complex (10). In previous papers (11, 12), we reported that several enzymes are distributed heterogeneously not only within the lobule, but also in liver lobes: cytochrome P-450 monooxygenase activity is higher in the median and right lobes of liver, while the left lobe contains a higher concentration of mitochondrial enzymes. If the difference in the cytochrome P-450 content in various liver lobes causes different susceptibilities to CCl₄.
as in the case of liver lobules, CCl₄-induced cell damage should occur in restricted areas or lobes of livers. The present report offers evidence that CCl₄-induced cell damage and characteristic depression of cytochrome P-450-dependent monoxygenase activity occur preferentially in the median lobe and much more than in the left lobe of rat liver.

Materials and Methods

Animals and their treatments: Male Slc-Wistar strain rats (9-10 weeks old) were used exclusively. In some experiments, 16-week-old rats were also used. The animals were kept in an air-conditioned room (25±1 °C, 50-60% humidity) that was lighted 12 hr a day (8:00-20:00), and maintained on commercial rat chow (CA-1, Japan Clea, Tokyo) and water ad libitum. All animals were allowed at least 7 days to become acclimatized to the housing conditions prior to use in the experiments. CCl₄ (0.05-1.5 ml) was diluted with liquid paraffin to 5 ml, and the resulting solution was administered orally to rats at the volume of 5 ml/kg (dosage of CCl₄: 0.05-1.5 ml/kg body weight). The control group was given orally the same volume of liquid paraffin alone.

The animals were killed by decapitation, and blood samples were obtained. Livers were quickly removed and blotted on filter paper, and tissue samples (400-600 mg wet weight) were obtained from various liver sites (Fig. 1). Liver homogenates and microsomes were prepared as described previously (12, 13).

Determination of enzyme activity: Hepatic drug-metabolizing activity (aniline hydroxylation and aminopyrine N-demethylation) was determined as described previously (14, 15). GOT (aspartate aminotransferase; EC 2.6.1.1) and GPT (alanine aminotransferase; EC 2.6.1.2) activities were detected essentially as described by La Due et al. (16) with slight modifications (17). Acid phosphatase (EC 3.1.3.2) activity was detected as reported previously (18) with slight modification (17). NAD(P)H-cytochrome c reductase activity was determined as described previously (19).

Cytochromes in the liver homogenate were detected by difference spectrophotometry using a Shimazu UV-300 spectrophotometer. Cytochrome P-450 was detected as described previously (12), and its concentration was calculated from the spectrum using the molar extinction difference of 104 mM⁻¹ cm⁻¹ (20) for the absorption difference between the peak position (at about 450 nm) and 490 nm. Cytochrome b₅ was determined with the NADH-reduced difference spectrum according to the method of Kajihara and Hagihara (21). Protein concentration was measured by the method of Lowry et al. (22) using bovine serum albumin as a standard.

Histological methods: Tissues for histological examination were fixed in neutral buffered 10% (v/v) formalin, dehydrated in alcohol and embedded in paraffin. Paraffin sections about 5μ thick were stained by a routine method with hematoxylin and eosin. Frozen sections were stained with oil red 0 for lipids. When required, tissues were fixed in Carnoy's fixative, and the sections were stained with periodic acid-Schiff (PAS). The entire slide was scanned, and the pathological changes were arbitrarily scored essentially as described by Bioulac et al.
DIFFERENT SUSCEPTIBILITIES OF RAT LIVER LOBES

(23): (a) necrosis: − to ++++ (−, no necrosis; ±, focal necrosis of 1–3 cells per lesion; +, focal necrosis of 5–8 cells per lesion; ++, zonal necrosis around central vein; ++++, zonal necrosis in central and intermediary zones; +++++, massive confluent necrosis); (b) ballooning or hydropic swelling degeneration: − to +++ (--, no ballooning; ±, few ballooned cells per lesion; +, more than 10 ballooned cells per lesion; ++, 1 or 2 rows of ballooned cells around the necrotic zone, per lesion; ++++, more than 2 rows of ballooned cells around the necrotic zone, per lesion); (c) steatosis or fatty degeneration: − to ++ (−, no steatosis; ±, slight steatosis; +, moderate steatosis; ++, severe steatosis); (d) decrease of PAS-positive substances: − to ++++ (−, no decrease; ±, more than 10 decreased cells per lesion; +, zonal appearance of the decreased cells in central zone; ++, zonal appearance of the decreased cells in central and intermediary zones of lobule; +++, appearance of the decreased cells from central to periportal zones; ++++, no detection of cells containing PAS-positive substances throughout the liver).

Results

Effect of CCl₄ administration upon several enzyme activities in blood and liver: Liver is known as a major target organ for CCl₄ toxicity. Alteration of plasma and liver enzyme activities in rats was then determined to characterize the pattern of CCl₄-induced hepatotoxicity. As shown in Fig. 2, activities of plasma GOT and GPT, characteristic enzymes indicating hepatocyte damage, increased gradually following administration of CCl₄, and the highest activities were observed 1–2 days after the administration. On the other hand, acid phosphatase activity in plasma was altered only slightly compared with that of transaminases.

When rats were given a large amount of CCl₄, the histologically detectable cell damage was observed throughout the liver. Then, cytochrome P-450-dependent drug-metabolizing activity was determined using liver tissues obtained from the left lobe (site 2 in Fig. 1). A marked decrease in cytochrome P-450 content and aminopyrine N-demethylase activity was detected 1 hr after the administration of CCl₄ (1.0 ml/kg), and both values remained low for 1–3 days after the treatment. After this, the activity recovered gradually, and both the activity and cytochrome P-450 content were at normal levels 7 days later (Fig. 3). Aniline hydroxylase activity in rat livers showed a similar pattern after CCl₄ administration (data not shown). On the other hand, transaminase activities in liver tissues decreased 10–20% at 1 day after the administration and then
Fig. 3. Alteration of hepatic enzyme activities in rats following administration of CCl₄. The animals were treated as shown in the legend of Fig. 2, and tissue samples were obtained from the left lobe (site 22) of livers. Enzyme activities and cytochrome content were detected using homogenate as the enzyme source. Enzymes determined were cytochrome P-450 (○), aminopyrine N-demethylase (●), GOT (△), GPT (□), and acid phosphatase (□). Data for each time point came from three rats. Statistical analyses (*P<0.05, **P<0.01) were carried out against the control group.

continued to decrease during the 7-day observation period. Acid phosphatase activity in the liver was not altered much by this treatment (Fig. 3).

Hepatic drug-metabolizing enzyme activity was also determined using isolated liver microsomes to obtain further information on the inhibitory effect of CCl₄. Microsomes for this study were prepared from whole livers of control and CCl₄-treated rats. Immediately after administration of a high dose of CCl₄ (1.0 ml/kg), cytochrome P-450 content and aniline hydroxylase and aminopyrine N-demethylase activities in liver microsomes began to decrease. By 24 hr, the content and the activities had fallen to 3-30% of the control levels (Table 1). Cytochrome b₅ content and NAD(P)H-cytochrome c reductase activity were also depressed immediately after (15-30 min) CCl₄ administration, but the depressions were very small compared with that of cytochrome P-450 content or drug-metabolizing activities. However, the cytochrome b₅ content and reductase activities were very low at 24 hr after the treatment (Table 1). Administration of liquid paraffin alone resulted in a slight decrease of the microsomal drug-metabolizing activity (Table 1), but plasma GOT and GPT activities were unaffected by this treatment (data not shown). Administration of various amounts of CCl₄ (0.05-1.5 ml/kg) led to dose-dependent increases in plasma GOT and GPT activities 24 hr later, while hepatic cytochrome P-450 content and aminopyrine N-demethylase activity showed dose-dependent decrease (data not shown). These results indicate clearly that the CCl₄ administration specifically affects cytochrome P-450, then depresses cytochrome P-450-dependent monooxygenation reactions. Decreases of cytochrome b₅ content and NAD(P)H-cytochrome c reductase activity were considered to be the secondary effect of CCl₄ intoxication.

Histological observation of CCl₄-induced hepatotoxicity: Histological observation of liver cells was carried out using tissue samples obtained from the left lobe (site 22 in Fig. 1). Cell necrosis in the centrilobular region appeared 6 hr after the CCl₄ administration, and a severe necrotic change was observed 1-2 days later. On the other hand, PAS-positive substances (glycogen) disappeared immediately after the treatment. Fatty degeneration of liver cells or deposit of lipids in cells was also detectable 1 hr after the administration. Mitotic liver cells were detected at 2 and 3 days after CCl₄ administration, indicating that recovery of injured cells occurred during this period. The pattern of necrotic cell damage shown in Table 2 was closely correlated to the alteration of plasma transaminase activity (Fig. 2). Decrease of PAS-positive substances and deposit of lipids in liver cells appear prior to necrotic cell damage.

Different susceptibilities of rat liver lobes
Table 1. Alteration of liver microsomal enzymes in rats following administration of CCI₄

| Enzyme                        | Intact | Liquid paraffin alone | 15 min | 30 min | 24 hr |
|-------------------------------|--------|-----------------------|--------|--------|-------|
| Cytochrome P-450₁               | 0.92±0.04 | 0.83±0.02            | 0.53±0.02** | 0.46±0.01** | 0.12±0.03** |
| Cytochrome b₅₆            | 0.70±0.02 | 0.66±0.03            | 0.63±0.01*    | 0.61±0.01** | 0.23±0.03** |
| NADPH-cytochrome c reductase² | 0.24±0.01 | 0.21±0.01            | 0.20±0.01**  | 0.21±0.01    | 0.07±0.01** |
| NADH-cytochrome c reductase² | 2.02±0.08 | 1.97±0.24            | 1.74±0.10    | 1.93±0.12    | 0.67±0.16** |
| Aniline hydroxylase³        | 1.29±0.04 | 1.19±0.08            | 0.42±0.03**  | 0.32±0.03**  | 0.04±0.01** |
| Aminopyrine N-demethylase³   | 19.09±0.46 | 17.35±0.86           | 7.12±0.27**  | 7.03±0.41**  | 5.57±0.49** |

Dose of CCI₄ was 1.0 ml/kg. The animals given CCI₄ were killed 15 min, 30 min and 24 hr after the administration, and the control rats given liquid paraffin alone were killed 24 hr after the administration. The content or the activity of microsomal enzymes is expressed in the table as follows: ¹) nmoles/mg protein, ²) µmoles/min/mg protein, ³) nmoles/min/mg protein. The values in the table represent the mean±S.E. *Statistically significant (P<0.05) against the control (intact) group. **Statistically significant (P<0.01) against the intact group.

Table 2. Histological findings of rat livers following administration of CCI₄

| Time after administration | Number of rats | Central necrosis | Decrease of PAS-positive substances | Fatty degeneration | Mitotic liver cell |
|---------------------------|----------------|------------------|-------------------------------------|-------------------|-------------------|
| 0 (untreated)             | 3              | -(3)             | -(3)                                | -(3)              | -(3)              |
| 1 hr                      | 3              | -(3)             | ++(1), ++(2)                        | -(1), -(2)        | -(3)              |
| 6 hr                      | 3              | ±(3)             | ++(3)                               | +(3)              | -(3)              |
| 1 day                     | 3              | +++(3)           | ++(3)                               | +(3)              | -(3)              |
| 2 days                    | 3              | +++(3)           | +++(3)                              | +(3)              | ±(3)              |
| 3 days                    | 3              | +(3)             | +(3)                                | +(1), +(2)        | +(3)              |
| 7 days                    | 3              | -(3)             | +(3)                                | -(1), +(2)        | -(3)              |

The animals were given orally CCI₄ at 1.0 ml/kg. Histological changes in tissues were graded arbitrary as explained in "Methods". Number in parentheses indicates the number of the animals showing histological changes.
Table 3. Effect of CCl₄ administration on cytochrome P-450 content in various liver lobes of rats

|                  | Left lobe | Median lobe | Right lobe |
|------------------|-----------|-------------|------------|
|                  | Site #1   | Site #2     | Site #3    |
| Control          | 28.0±1.5  | 31.6±0.7    | 30.4±1.5   |
| CCl₄ administration |          |             |            |
| 0.05 ml/kg       | 22.6±1.3  | 23.7±0.9    | 26.0±1.4   |
| 0.1 ml/kg        | 20.4±1.7  | 20.0±2.3    | 22.2±2.1   |
| 1.0 ml/kg        | 2.6±0.8   | 1.8±0.8     | trace      |

The animals (16 weeks old) were given orally various amounts of CCl₄, with the rats in control group receiving liquid paraffin alone. Liver samples were obtained 24 hr after the administration. The values in the table represent the mean±S.E. of 3 animals.

* Statistically significant (P<0.05) against the content in site #1.
** Statistically significant (P<0.01) against the content in site #1.

As described above, CCl₄ administration specifically causes a decrease in cytochrome P-450 content followed by histological changes of hepatocytes in the centrilobular region. Since the cytochrome P-450 monooxygenase is distributed heterogeneously within the liver (5-10), the effect of administering various amounts of CCl₄ on cytochrome P-450 content was determined using tissue samples obtained from various lobes of CCl₄-treated rat livers. As reported previously (9, 10), cytochrome P-450 content in the median and the right lobes of control rat livers were significantly higher than that in the left lobe (Table 3). With administration of various amounts of CCl₄, a dose-dependent decrease in the cytochrome content was observed throughout the liver. Interestingly, the decrease in cytochrome P-450 content was much greater in the median lobe, especially in sites #4 and #5, compared with that in the left lobe (sites #1 and #2), and only a trace amount of cytochrome P-450 was detected in the median and right lobes when a higher amount (1.0 ml/kg) of CCl₄ was administered to the rats (Table 3). Aminopyrine N-demethylase activity in various liver lobes showed a change similar to that in cytochrome content (data not shown).

Histological observation of tissue samples obtained from various lobes was performed to compare the effect of CCl₄ with the decrease of cytochrome P-450. The histological structure of the liver in control rats did not vary very much between the lobes (Fig. 4, A and B). Only weak alteration in the histological pattern was observed in the left lobe with administration of a lower dose of CCl₄ (0.05 ml/kg), but strong hydropic swelling degeneration of liver cells in the centrilobular region was observed in the median lobe (D) than in the left lobe (C). E and F: Liver obtained from CCl₄-administered rat (0.05 ml/kg). Hydropic swelling degeneration and central necrosis in the lobules are evident in both sections. The area of degeneration and necrosis is larger in the median lobe (D) than in the left lobe (C).

Fig. 4. Histological observations of rat liver cells. Rats were administered orally various amounts of CCl₄ (0.05-1.0 ml/kg) and livers were obtained 24 hr later. Tissue samples obtained from site #2 (A, C, E and G) and site #5 (B, D, F and H) were fixed and then stained with hematoxylin and eosin. ×75. A and B: Liver from untreated rats. C and D: Liver obtained from CCl₄-administered rat (0.05 ml/kg). Slight hydropic swelling degeneration and central necrosis in the lobule are larger in the median lobe (D) than in the left lobe (C). E and F: Liver obtained from CCl₄-administered rat (0.05 ml/kg). Hydropic swelling degeneration and central necrosis in the lobules are evident in both sections. The area of degeneration and necrosis is larger in the median lobe (D) than in the left lobe (C).
median and right lobes (Fig. 4, C and D). When rats were given CCl₄ at 0.1 ml/kg, the hydropic swelling degeneration of hepatocytes in the centrilobular region was observed throughout the livers, but the extent of degeneration was much greater in the median lobe than in the left lobe (Fig. 4, E and F). With administration of a high dose (1.0 ml/kg), necrotic cells in the centrilobular region were observed in both the left and median lobes. Also the extent of the lesions were worse in the median lobe (Fig. 4, G and H). Oil red 0 staining showed the fatty degeneration or deposit of lipids in liver cells, and the generation in the centrilobular region was more extensive in the left lobe when lower amounts of CCl₄ were administered. With administration of a high dose of CCl₄, the fatty degeneration was not very different among the lobes. Histological changes in all animals employed are shown in Table 4. Although the extent of the damage differed slightly among the animals, a similar pattern was obtained for the same experimental group. Repeated experiments always showed the site or lobe-specific lesions of liver cells, but the extent of the damage was slightly variable.

Discussion

CCl₄-induced hepatic damage is thought to be related to the formation of toxic metabolite(s), with the drug-metabolizing enzyme system, particularly NADPH-cytochrome c (P-450) reductase and cytochrome P-450, being involved in the conversion of CCl₄ (1-3, 24). As described previously (25, 26), cytochrome P-450 content depressed remarkably and specifically immediately after CCl₄ administration (Fig. 3 and Table 1). The results indicate cytochrome P-450 is affected immediately and followed by cell degeneration. On the other hand, an increase of blood transaminase activity and the appearance of liver cell damage began several hours after the administration. A close correlation between the alteration of blood transaminase activity and the centrilobular damage of hepatocytes was noted during the 7-day observation period (Fig. 2 and Table 1). Increase of blood transaminase activity is generally considered to indicate hepatocyte damage, and the above results agree with this.

Interestingly, the hepatotoxic effect of CCl₄ differed significantly among the various lobes of rat liver (Table 4 and Fig. 4). CCl₄-induced damage of hepatocytes was predominantly observed in the median lobe, although patterns of damage (hydropic swelling degeneration and necrosis in the centrilobular region) did not differ among the lobes (Fig. 4). Differences in the susceptibility of rat liver lobes to CCl₄ were also reported by Lawson and Pound (27), and the present histological results agree with theirs. In previous papers (9, 10), we reported that cytochrome P-450 monooxygenase is high or enriched in the median and right lobes of rat livers. Thus, the more extensive lesions in the median and right lobes due to CCl₄ administration should be related to the higher concentrations of cytochrome P-450 in these lobes. Recently, Smith et al. (28) demonstrated different responsibilities of various lobes of rat liver in hexachlorobenzene-induced porphyria, although the chemical distributed homogeneously within the liver, and this suggested that differences in drug-metabolizing activities among different lobes causes the differences. When liver samples were obtained 1-3 min after the oral administration of CCl₄, the decrease of cytochrome P-450 was observed throughout the liver, and the rate of decrease was almost the same in the various liver lobes (data not shown). These results suggest that CCl₄ distributed very rapidly within the liver, and CCl₄ concentration was not so different among the lobes, similar to the results of Smith et al. (28).

CCl₄ reacts extremely rapidly with cyto-
Table 4. Effect of administration of various amounts of CCl₄ on histological findings of hepatocytes

| Treatment of rat | Rat No. | Central necrosis | Hydropic swelling degeneration | Fatty degeneration |
|------------------|---------|-----------------|-------------------------------|-------------------|
|                  |         | #1  #2  #3  #4  #5  #6 | #1  #2  #3  #4  #5  #6 | #1  #2  #3  #4  #5  #6 |
| Control          | 1       | -    -    -    -    -    - | -    -    -    -    -    - | -    -    -    -    -    - |
|                  | 2       | -    -    -    -    -    - | -    -    -    -    -    - | -    -    -    -    -    - |
|                  | 3       | -    -    -    -    -    - | -    -    -    -    -    - | -    -    -    -    -    - |
| CCl₄ (0.05 ml/kg)| 4       | ±    ±    ±    -    -    ± | ±    ±    ±    ±    ±    ± | ±    ±    ±    ±    ±    ± |
|                  | 5       | -    -    -    ±    ±    - | -    -    -    -    -    - | -    -    -    -    -    - |
|                  | 6       | -    -    -    ±    ±    - | -    -    -    -    -    - | -    -    -    -    -    - |
| CCl₄ (0.1 ml/kg)| 7       | -    -    -    -    -    - | -    -    -    -    -    - | -    -    -    -    -    - |
|                  | 8       | ±    ±    ±    ±    ±    ± | ±    ±    ±    ±    ±    ± | ±    ±    ±    ±    ±    ± |
|                  | 9       | -    -    -    -    -    - | -    -    -    -    -    - | -    -    -    -    -    - |
| CCl₄ (1.0 ml/kg)| 10      | -    -    -    -    -    - | -    -    -    -    -    - | -    -    -    -    -    - |
|                  | 11      | ±    ±    ±    ±    ±    ± | ±    ±    ±    ±    ±    ± | ±    ±    ±    ±    ±    ± |
|                  | 12      | ±    ±    ±    ±    ±    ± | ±    ±    ±    ±    ±    ± | ±    ±    ±    ±    ±    ± |

Rat livers were obtained from the same animals used for the experiment of Table 3. Tissue samples obtained from six different sites (#1-#6) were examined histologically and graded as described in "Methods".
chrome P-450, with consequent rapid inactivation of the hemoprotein in vivo (Table 1 and Fig. 3). Inactivation of cytochrome P-450 in different lobes may be related differently to the extent of the lesion in the different lobes (Table 3). However, differences in the depression of cytochrome content among the lobes were not large compared to the differences in the cell damage among the lobes. Thus, some factor(s) other than a drug-metabolizing enzyme may participate in the degeneration of hepatocytes in various lobes. Copher and Dick demonstrated the unequal distribution of the portal blood in dog liver lobes (29), which suggests different oxygen tension in various lobes. If this was the case in rat liver, an unequal distribution of the portal blood might be one of the factors to produce the different susceptibilities of rat liver lobes to xenobiotics. Further studies are thus required to elucidate the different susceptibilities of liver lobes in regard to the function of cytochrome P-450.

In conclusion, the present results show that the liver cannot be regarded as a homogeneous organ in toxicological studies and that the heterogeneous distribution of cytochrome P-450 monooxygenase in the different lobes may be one of the factors causing different susceptibilities of liver lobes to xenobiotics. However, no satisfactory explanation for the differences can be given at present.

References

1) Slater, T.: Necrogenic action of carbon tetrachloride in the rat: A speculative mechanism based on activation. Nature 209, 36-40 (1960)
2) Davis, D.C., Schroeder, D.H., Gram, T.E., Reagen, R.L. and Gillette, J.R.: A comparison of the effects of halothane and CCl₄ on the hepatic drug metabolizing system. J. Pharmacol. Exp. Ther. 177, 556-566 (1971)
3) Reynolds, E.S. and Moslen, M.T.: Free-radical damage in liver. In Free Radicals in Biology. Edited by Pryor, W.A., Vol. IV, p. 49-94, Academic Press, New York (1980)
4) Ashworth, C.T., Luibbel, F.J., Sanders, E. and Arnold, N.: Hepatic cell degeneration. Arch. Pathol. 75, 212-225 (1963)
5) Rouiller, Ch.: Experimental toxic injury of the liver. The Liver, Edited by Rouiller, Ch., Vol. II, p. 325-445, Academic Press, New York (1964)
6) Gooding, P.E., Chayer, J., Sawyer, B. and Slater, T.F.: Cytochrome P-450 distribution in rat liver and the effect of sodium phenobarbitone administration. Chem. Biol. Interact. 20, 299-310 (1978)
7) Taia, Y., Redick, J.A. and Baron, J.: An immunohistochemical study on the localization and distribution of NADPH-cytochrome c (P-450) reductase in rat liver. Mol. Pharmacol. 17, 374-381 (1980)
8) Redick, J.A., Kawabata, T.T., Guengerich, F.P., Krieter, P.A., Shires, T.K. and Baron, J.: Distribution of monooxygenase components and epoxide hydratase within the livers of untreated male rats. Life Sci. 27, 2465-2470 (1980)
9) Baron, J., Redick, J.A. and Guengerich, F.P.: An immunohistochemical study on the localization and distributions of phenobarbital- and 3-methylcholanthrene-inducible cytochrome P-450 within the livers of untreated rats. J. Biol. Chem. 256, 5931-5937 (1981)
10) Sweeney, G.D.: Functional heterogeneity among liver cells: implications for drug toxicity and metabolism. Trends in Pharmacol. Sci. 2, 141-144 (1981)
11) Matsubara, T., Touchi, A., Ogawa, M. and Mori, S.: Heterogenous distribution of cytochrome P-450 monooxygenase in rat liver lobes. In Microsomes, Drug Oxidations and Drug Toxicity, Edited by Sato, R. and Kato, R., p. 433-434, Univ. of Tokyo Press. Tokyo (1982)
12) Matsubara, T., Touchi, A. and Ogawa, A.: Heterogeneous distribution of the cytochrome P-450 monooxygenase system in rat liver lobes. Japan. J. Pharmacol. 32, 999-1011 (1982)
13) Matsubara, T., Yoshiihara, E., Iwata, T., Tochino, Y. and Hachino, Y.: Biotransformation of coumarin derivatives. (1) 7-alkoxyxocoumarin O-dealkylase in liver microsomes. Japan. J. Pharmacol. 32, 9-21 (1982)
14) Matsubara, T. and Tochino, Y.: Inhibitory action of cyanide on aniline hydroxylase system. FEBS Lett. 52, 77-80 (1976)
15) Matsubara, T., Touchi, A. and Tochino, Y.: Hepatic aminopyrine N-demethylase system. Further studies on assay procedures. Japan. J. Pharmacol. 27, 127-138 (1977)
16) La Due, J.S., Wróblewski, F. and Kamen, A.: Serum glutamic oxaloacetic transaminase activity in human acute transmural myocardial
infarction. Science 120, 497-499 (1954)

17) Matsubara, T. and Touchi, A.: Inhibitory action of cefamandole on glutamic pyruvic transaminase activity in rats. Chemotherapy 27, Supp. 5, 740-748 (1979) (in Japanese)

18) Bessey, O.A., Lowry, O.H. and Brock, M.J.: A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. J. Biol. Chem. 164, 321-329 (1946)

19) Matsubara, T. and Tochino, Y.: Electron transport systems of lung microsomes and their physiological functions. I. Intracellular distribution of oxidative enzymes. J. Biochem. (Tokyo) 70, 981-991 (1971)

20) Matsubara, T., Koike, M., Touchi, A., Tochino, Y. and Sugeno, K.: Quantitative determination of cytochrome P-450 in rat liver homogenate. Anal. Biochem. 75, 596-603 (1976)

21) Kajihara, T. and Hagihara, B.: Crystalline cytochrome b5. I. Purification of cytochrome b5 from rabbit liver. J. Biochem. (Tokyo) 63, 453-461 (1968)

22) Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265-275 (1951)

23) Bioulac, P., Despujols, L., Bedin, C., Iron, A., Saric, J. and Balabaud, C.: Decreased acute hepatotoxicity of carbon tetrachloride and bromobenzene by cholestyramine in the rat. Gastroenterology 81, 520-526 (1981)

24) Tuchwerber, B., Werringloer, J. and Kourounakis, P.: Effect of phenobarbital or pregnenolone-16a-carbonitrile (PCN) pretreatment on acute carbon tetrachloride hepatotoxicity in rats. Biochem. Pharmacol. 23, 513-518 (1974)

25) Recknagel, R.O., Glende, E.A., Jr. and Huszkevycz, A.M.: Chemical mechanisms in carbon tetrachloride toxicity. In Free Radicals in Biology. Edited by Pryor, W.A., Vol. III, p. 97-132, Academic Press, New York (1977)

26) Fander, U., Haas, W. and Kroner, H.: The damage of the hepatic mixed function oxygenase system by CCl₄: Significance of incorporation of ¹⁴C CCl₄ metabolites in vivo. Exp. Mol. Pathol. 36, 34-43 (1982)

27) Lawson, T.A. and Pound, A.W.: The different susceptibility of rat liver lobes to carbon tetrachloride and dimethylnitrosamine. Br. J. Exp. Pathol. 55, 583-586 (1974)

28) Smith, A.G., Francis, J.E. and De Matteis, F.: Lobes of rat liver respond at different rates to challenge by dietary hexachlorobenzene. Biochem. Pharmacol. 29, 3127-3131 (1980)

29) Copher, G.H. and Dick, B.M.: "Stream line" phenomena in the portal vein and the selective distribution of portal blood in the liver. Arch. Surg. 17, 408-419 (1928)