Function of Reticuloendothelial System on CCl₄ Induced Liver Injury in Mice

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Abstract—Phagocytic activity as a function of the reticuloendothelial system (RES) has been studied in CCl₄-induced liver injury by using the carbon clearance test. Liver damage in mice was induced by administration of 20% CCl₄ in olive oil (p.o.). After a single administration of CCl₄, significant increases in liver/body weight ratio, serum GOT and GPT levels, α, β and γ-globulins and BSP retention, and decreases in serum albumin, an activity of the hepaplastintest and the correct phagocytic activity, α value, were found. After 15 administrations of CCl₄ (3 times a week), mild increases in serum GPT level and BSP retention and decreases in the activity of the hepaplastintest and both phagocytic indices, K and α values, were observed. However, zymosan treatment 3 days before sacrifice induced an increase in K value depressed by multiple administrations of CCl₄. The depression of carbon uptake by Kupffer cells can be seen by light microscopy after multiple administrations of CCl₄ compared with that of saline and olive oil. These findings indicate that the RES phagocytosis is suppressed more strongly in chronic liver injury by 15 CCl₄ administrations than in acute injury by a single one, although the biochemical parameters indicating liver injury are shown to have an opposite tendency. A clear correlation between the alteration of RES activity and the degree of liver injury was not noted.

The reticuloendothelial system (RES) usually plays the role of keeping the homeostasis of the human body and constitutes an important part of cellular host defense in the removal and degradation of bacteria, endotoxins, tumor cells and foreign material from the blood (1, 2). From the view of phagocytic activity as one of the many functions of the RES, the liver and spleen are very important organs. There were several clinical studies on the activity of the RES function in liver diseases, but these activities differed according to various conditions of liver diseases. In acute hepatitis (3, 4) and chronic hepatocellular inflammation (5), increased RES function has been reported; and in cirrhosis, alcoholics and other liver diseases, decreased RES function has been admitted (6–9). However, the relationship between hepatic damage and RES function in acute and chronic liver injury was not yet established using the same experimental conditions. Therefore, it seemed interesting to determine whether similar changes could be observed in experimental animals. The purpose of this paper is to examine the RES function in liver injury of mice by single and multiple treatments of carbon tetrachloride (CCl₄), which induces typical liver damage in animals (10). In other words, this study is undertaken to show the difference in RES function between acute and chronic liver injury and to discuss the relationship between RES function and liver injury. The phagocytic activity of RES was examined by a carbon clearance test. Serum GOT and GPT levels, liver/body weight ratio, BSP test and hepaplastintest were examined to set the index of liver damage. In addition, the change in serum protein fraction was examined by
electrophoresis. The histological changes and carbon uptake of liver and spleen were also observed by light microscopy after intravenous injection of colloidal carbon.

**Materials and Methods**

**Animal and drug administration:** Male ddY mice, 5 weeks of age at the start of experiments, were fed with a stock diet (MF, Oriental Kobo Co. Ltd.) and tap water ad libitum under standard laboratory conditions. The mice were divided into three groups: saline control group, olive oil treated group and CCl₄ treated group, in acute and chronic liver injury experiments, respectively.

CCl₄ and olive oil were purchased from Wako Pure Chemical Industries, Ltd. Zymosan A and calf serum were purchased from Sigma Chemical Co., Ltd. Each animal received 0.2 ml of 20% CCl₄ in olive oil (CCl₄: 0.04 ml) by a stomach tube to induce acute liver damage, and control animals received 0.2 ml of olive oil or 0.9% NaCl solution. To induce chronic liver damage, 0.04 ml CCl₄ per mouse was given to the animals 3 times a week (every other day) for 5 weeks; that is, the total number of 0.04 ml CCl₄ injections were 15. Twenty-four hr after single administration and 48 hr after multiple administrations, we examined all items of the experiment.

Zymosan was dissolved with saline containing 10% of calf serum and preincubated for 30 min at 37°C. Mice were given 50 mg/kg of zymosan by intraperitoneal administration 3 days before sacrifice in both single and multiple treatments of CCl₄.

**Measurements of liver damage and phagocytic activity:** The degree of liver damage was judged to examine liver/body weight ratio and serum GOT and GPT levels using the transaminase C-test (Wako Pure Chemical Industries, Ltd.). The total level of serum protein was determined by the refractometer (Atago Optical Works Co., Ltd.). Analysis of serum protein fractions was carried out by electrophoresis, and the A/G ratio was calculated from these results. The BSP test was determined from the disappearance rate of BSP from mouse blood 15 min after intravenous injection of 5 mg/kg body weight of BSP. The hepaplastin test was conducted by utilizing a specially prepared rabbit brain thromboplastin (Hepaplastin test: Eisai Co., Ltd.) with venous blood mixed with 3.2% sodium citrate (blood : citrate = 9:1) (11).

The carbon clearance test, which measured the intravascular removal rate of colloidal carbon (Pelikan, Germany), was used to examine the phagocytic capacity of RES. The mice received an intravenous injection of 1.6 mg/10 g body weight of prewarmed (37°C) colloidal carbon, prepared in sterilized physiological saline. Blood samples were obtained at 7 min intervals from the retro-orbital venous plexus using heparinized capillary tubes. These samples were hemolyzed in 0.1% Na₂CO₃, and the colloidal carbon concentration was determined by a spectrophotometer at 600 nm. The global phagocytic index K and the corrected phagocytic index α were calculated according to the following formulas (12):

\[
K = (\log C - \log C') / t - t', \quad \alpha = \frac{3K}{P_c} \times \frac{P_o}{P_c}
\]

Here:
- \(t\): time of first blood collection
- \(t'\): time of second blood collection
- \(c\): carbon concentration at the time of first blood collection
- \(c'\): carbon concentration at the time of second blood collection
- \(P_c\): animal body weight
- \(P_o\): liver and spleen weight

The global phagocytic activity and corrected phagocytic index are shown by the K and α values, respectively. These values are parameters indicating the phagocytic activity at 7 min after intravenous injection into the tail vein.

PEC (peritoneal exudate cells) was obtained from the peritoneal cavity of the mice after an intraperitoneal injection of 3 ml of sterilized saline containing 1 U of heparin per ml and the mice were shaken for 1 min. After decapitation and blood letting, we collected the solution of the saline from the abdominal cavity of the mice using an injection pump. The solution was centrifugated at 800 r.p.m. for 10 min, and the residue was considered as PEC. After 0.1% Triton X-100 treatment of PEC, the lysosomal enzyme activity of PEC was determined. Acid phosphatase was obtained by the modified
method of Marie (13), and β-glucuronidase was obtained by Kato's method (14).

For histologic estimate of liver damage caused by CCl₄, animals were sacrificed 7 min after carbon injection, and then their liver lobes were fixed in formalin, sectioned and stained with Hematoxylin and Eosin. Histologic estimates of the carbon uptake, Kupffer cells in the liver and macrophages in the spleen were observed by the same staining.

Statistical significance was evaluated using Student's t-test.

Results

1. The ratio of liver/body weight: After a single administration of CCl₄, there was an increase in the ratio of liver/body weight as compared to the ratios of the saline control. After 15 CCl₄ administrations, there was an increase in the ratio of liver/body weight as compared with that of the olive oil control (Fig. 1).

2. Serum protein level and serum protein fraction by electrophoresis: As shown in

<table>
<thead>
<tr>
<th>Table 1. Effect of single and multiple administrations of CCl₄ on the serum protein fraction by electrophoresis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>Single dose</td>
</tr>
<tr>
<td>(x1)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Multiple dose</td>
</tr>
<tr>
<td>(x15)</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Each value represents the mean % value with S.D. **:** significantly different from the olive oil value with P<0.05, P<0.01, respectively (Student's t-test). For details, see Fig. 1.
Fig. 2, no change was found in the total protein level in serum by the refractometer after the single dose and after 15 administrations (Fig. 2). The decrease in albumin and increase in α, β and γ-globulins were clearly found after single administration of CCl₄. After 15 CCl₄ administrations, no change was found except for the tendency of an increase in γ-globulin (Table 1). A decrease in A/G ratio was found according to the results on the serum protein fraction after a single administration of CCl₄ (Fig. 3).

3. Level of serum transaminase: The levels of GOT and GPT in serum were very high after the single administration of CCl₄, but after 15 administrations, the level of GOT did not change, while the level of GPT showed a very significant increase (Fig. 4).

4. BSP test: The result of the BSP test was shown by % ratio compared with the saline control. Retention of BSP was 852.4% after a single administration of CCl₄, while olive oil was 317% and CCl₄ was 284% after 15 administrations (Fig. 5).

5. Hepaplastintest: As shown in Table 2, the activity of hepaplastintest was suppressed significantly after a single dose and after 15 administrations of CCl₄, at rates of about 68% and 40% compared to those of saline control, respectively.

6. The phagocytic activity: After a single administration of CCl₄, the K value did not
change, but after 15 administrations of CCl₄, the decrease in K value was remarkable. This result points to the suppression of phagocytic activity. In addition, the α value which describes the level of phagocytic activity in liver and spleen also decreased significantly after single and multiple administrations of CCl₄. The increase in activity of the phagocytic one was acknowledged after 15 administrations of olive oil (Fig. 6).

7. The effect of zymosan in phagocytic activity: Table 3 shows the effect of zymosan in phagocytic activity. Zymosan treatment caused K values to increase but not the α value in all experimental groups after single and multiple administrations of CCl₄. It especially enhances the phagocytic activity that was suppressed by multiple administrations of CCl₄. In addition, the ratio of spleen/body weight increased remarkably.

8. Lysosomal enzyme activity of PEC: Table 4 shows the activity of acid-phosphatase and β-glucuronidase as a lysosomal enzyme of PEC. After a single administration of CCl₄, no apparent change except a mild decrease of acid-phosphatase activity was found.

9. Light-microscopical observations of carbon uptake in the liver and spleen of CCl₄ treated mice: In the liver, the severe fatty degeneration and often the swelling of hepatic cytoplasm, the disappearance of nuclei, the centrilobular necrosis and haemorrhage could be seen 24 hr after single CCl₄ administration. On the other hand, the carbon uptake rate by Kupffer cells in the liver of CCl₄ treated mice was nearly the same compared with that of saline treated mice (Fig. 7). In the case of 15 administrations of CCl₄, the condensation or disappearance of nuclei in the liver cells can be found; and simultaneously, a marked massive haemorrhage, necrosis and steatosis are frequently found. Furthermore, the Kupffer cells which took up particles of carbon were scarce in contrast with the saline treated mice (Fig. 8). In the spleen of olive oil mice, the increasing tendency of carbon uptake by macrophages around the white pulp could be observed after multiple administrations, but in the spleen of control and CCl₄ mice, neither significant difference in the carbon uptake was found.

### Table 2. Effect of single and multiple administrations of CCl₄ on the activity of heaplastinstest

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single dose</td>
<td>67.8± 5.7</td>
</tr>
<tr>
<td>CCl₄ (x1)</td>
<td>72.5± 8.8**</td>
</tr>
<tr>
<td></td>
<td>21.6±16.1</td>
</tr>
<tr>
<td>Multiple dose</td>
<td>72.8± 7.0</td>
</tr>
<tr>
<td>CCl₄ (x15)</td>
<td>68.8± 5.7</td>
</tr>
<tr>
<td></td>
<td>44.5± 9.9**</td>
</tr>
</tbody>
</table>

Each value represents the mean % value with S.D. **: significantly different from the control and olive oil values, respectively, with P<0.01 (Student’s t-test). For details, see Fig. 1.
Fig. 6. Effect of single and multiple administrations of CCl₄ on the phagocytic activity. Upper panel shows the K value, the global phagocytic indices and the lower panel shows the α value, the corrected phagocytic index. *, **: significantly different from the control value with P<0.05, P<0.01, respectively. $: significantly different from the olive oil value with P<0.01 (Student’s t-test). For details, see Fig. 1.

Table 3. Effect of zymosan on phagocytic activity in single and multiple administrations of CCl₄

<table>
<thead>
<tr>
<th>Treatment</th>
<th>K index</th>
<th>α index</th>
<th>% Body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>Single dose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont.</td>
<td>0.068±0.014</td>
<td>8.62±1.20</td>
<td>4.37±0.59</td>
</tr>
<tr>
<td>Zymosan (×1)</td>
<td>0.086±0.022*</td>
<td>8.95±0.89</td>
<td>4.63±0.32</td>
</tr>
<tr>
<td>CCl₄</td>
<td>0.069±0.025</td>
<td>7.03±0.95*</td>
<td>5.50±0.37**</td>
</tr>
<tr>
<td>CCl₄+Zymosan</td>
<td>0.082±0.014</td>
<td>8.13±1.20</td>
<td>5.62±0.40**</td>
</tr>
<tr>
<td>Multiple dose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont.</td>
<td>0.057±0.006</td>
<td>9.19±0.90</td>
<td>3.91±0.32</td>
</tr>
<tr>
<td>Zymosan (×15)</td>
<td>0.148±0.081*</td>
<td>10.05±1.97</td>
<td>4.55±0.41*</td>
</tr>
<tr>
<td>Olive oil</td>
<td>0.072±0.038</td>
<td>9.79±1.36</td>
<td>3.84±0.31</td>
</tr>
<tr>
<td>Olive oil+Zymosan</td>
<td>0.165±0.087*</td>
<td>9.68±2.16</td>
<td>4.78±0.41###</td>
</tr>
<tr>
<td>CCl₄</td>
<td>0.052±0.002**</td>
<td>6.74±1.00**</td>
<td>5.04±0.78**</td>
</tr>
<tr>
<td>CCl₄+Zymosan</td>
<td>0.099±0.045*Δ</td>
<td>7.25±1.10</td>
<td>5.34±0.80</td>
</tr>
</tbody>
</table>

Each value represents the mean % value with S.D. *, **: significantly different from the control value with P<0.05 and P<0.01, respectively. ###: significantly different from the olive oil value with P<0.01. Δ, ΔΔ: significantly different from the CCl₄ value with P<0.05 and P<0.01, respectively (Student’s t-test).

Table 4. Effect of single administration of CCl₄ on lysosomal enzyme activity of PEC

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Acid-phosphatase</th>
<th>β-Glucuronidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont.</td>
<td>352.95±166.65</td>
<td>387.86±176.07</td>
</tr>
<tr>
<td>Olive oil</td>
<td>187.25± 78.68</td>
<td>382.74± 58.17</td>
</tr>
<tr>
<td>CCl₄</td>
<td>156.48± 16.79</td>
<td>409.56± 41.39</td>
</tr>
</tbody>
</table>

Each value represents the mean mmol with S.D. as p-nitrophenol/10 cells.
uptake rate nor histological difference were found.

**Discussion**

It is reported that the RES function increases in acute hepatitis (3, 4), and decreases in alcoholism and in certain forms of liver disease (5-9) and the susceptibility to infections increases. However, there are only a few reports on the RES function in animals with hepatic injury induced by toxic agents. We examined phagocytic activity, which is
one of the most important roles among many RES functions, in CCl₄ treated mice using the carbon clearance test. Since Souich et al. reported that the disposition of low doses of colloidal carbon was governed by several first-order parallel processes using male and female white rabbits (15), the low dose (16 mg/kg) of colloidal carbon was intravenously administered into mice.

CCl₄ is an agent, typical of the haloaliphatic compounds, which exerts hepatotoxic effects by its active metabolite. After a single injection of CCl₄, a decrease in the α index, but not in the K index, was seen, although many biochemical parameters strongly indicated hepatic injury. The decrease in the α index is considered due to the apparent increase in liver weight after a single treatment of CCl₄, because the α value is calculated by K x (animal body/liver and spleen weights). After 15 administrations of CCl₄, both phagocytic indices significantly decreased with the increase in liver and spleen weights, although the changes in biochemical parameters indicating liver injury were mild.

In general, the increase in plasma levels of cytoplasmic and mitochondrial enzymes accurately reflect liver injury by CCl₄ treatment (16). Single treatment of CCl₄ in mice induced the increase in serum transaminase as the parameter of liver injury. The degree of this increase was about 5 to 6 times that of the saline control. On the other hand, in animals treated with 15 administrations of CCl₄, the GOT level did not change and the GPT level increased to 3.5 times that of the saline control. Busuttil et al. (17) reported that biochemical manifestation of hepatic injury during the development of cirrhosis was prominent only in cases of acute injury from intermittent doses in rats. Our results also seemed to show a more apparent change in liver injury by a single treatment of CCl₄ than that by multiple treatments.

There are many reports on the injury mechanism of CCl₄. According to the current hypothesis, the cause of CCl₄ induced liver injury is trichloromethyl free radical (·CCl₃); that is, CCl₄ is metabolized to its hepatotoxic products by cytochrome P-450 (18). However, CCl₄ caused a decrease in the activity of cytochrome P-450 and aminopyrine demethylase at the same time, both being main drug metabolizing enzymes in rats (19, 20). Also, high dosage of CCl₄ after a single treatment does not show high toxicity for cells because of the decrease in ·CCl₃ formation in rats (20). Glende reported that protection against a lethal dose of CCl₄ is complete at 24 hr when aminopyrine demethylase and cytochrome P-450 are 25 to 35% of the normal activity. This protection continues for 4 days in rats (21). In the case of GOT after multiple administrations of CCl₄, the biosynthesized amount of this enzyme decreased and this enzyme was considered to be released entirely from the liver cells. In addition, Rees and Sinha reported that an examination of the serum enzymes in liver injury did not give a direct measure of the extent of the lesion in rats (22).

After a single CCl₄ administration, the results of serum protein fractionation by electrophoresis showed a decrease in albumin, an elevation of α, β and γ-globulins and a decrease in A/G ratio. After 15 administrations of CCl₄, there was no decrease in albumin, a slight elevation in β-globulin (P<0.1) and no change in the A/G ratio. Since all serum proteins except immune globulin are biosynthesized in liver cells, it seems that the damage of liver cells by CCl₄ administration induced a decrease in albumin content. Our results also showed a significant decrease in albumin content after single administration of CCl₄, but not after multiple administrations. No decrease in serum total protein after both treatments was observed. It is considered that severe liver damage induces the decrease in protein catabolism and the elongation of protein's half time in order to supplement the decrease in the protein synthesis in the animal body. The levels of serum proteins are known to vary with age, sex, pregnancy and treatment with CCl₄ in rats (23, 24). It is also said that hypergammaglobulinaemia is common in patients with liver disease (7, 25). It is widely believed that many antigens are phagocytised, and their antigenicity is lost by the Kupffer cells. However, in damaged liver, many antigens have a low chance of being phagocytised by Kupffer cells because these cells must phagocyte many degraded cells. Therefore, foreign materials have a high
chance of remaining in contact with antibody forming cells in blood, and a high-γ-globulin content in serum is observed. Our result also showed a high-γ-globulin content in serum after a single administration of CCl₄, but a mild tendency after multiple administrations. It seems that the phagocytosis of Kupffer cells depends on the amounts of endogenous components and exogenous materials.

BSP is conjugated with glutathione in the liver cells and is eliminated into the bile. It is considered that the elimination of BSP is delayed in liver injury. In the case of a single treatment of CCl₄, a very significant BSP accumulation and an increase in serum γ-globulin component with a decrease in α value were obtained at the same time. In the case of multiple treatments of CCl₄, a mild accumulation of BSP with an apparent decrease in phagocytic activity was seen. Since the BSP test is associated with the liver cell’s activity for uptake of pigments and of its elimination into the bile, it seems that a single CCl₄ treatment causes more severe damage to liver cells than multiple treatments. In our experimental conditions, the decrease in RES function is considered to be not always consistent with liver parenchymal cell function. Experiments using micro-aggregated human serum albumin for a Kupffer cell phagocytic capacity in patients with liver and inflammatory bowel diseases were reported by Wardle et al. (5). Kupffer cell clearances are reduced in proportion to BSP clearance in obstructive jaundice and in secondary biliary cirrhosis, but are increased in patients with chronic hepatocellular inflammation. Conversely, Kupffer cell phagocytosis is often depressed in patients with alcoholic hepatitis. They considered that the Kupffer cell function as measured by BSP was clearly independent of liver parenchymal cell function in some kinds of liver diseases except obstructive jaundice.

In patients with cirrhosis and chronic active hepatitis (7), the tendency of an increase in RES function except in the liver was acknowledged. The spleen, an organ of RES, is an important one besides liver. Their reticulocytes work to phagocytize and take part in the production of the specific antibody. Souhami (26) reported that the depression of hepatic phagocytosis was associated with the stimulation of the splenic uptake of antigen and of the immunal response by colloidal carbon in mice. We observed the carbon uptake state of spleen and liver tissues under the light microscope in CCl₄ treated mice. In the spleen, many macrophages took up carbon particles in the marginal zone between white and red pulps. However, it was difficult to show the difference in carbon uptake rates between control and mice with liver injury.

Zymosan, a material derived from yeast cell walls, has certain chemical similarities to and shares some of the biologic properties of endotoxins. However, zymosan shows no toxic signs and causes a marked increase in the rate of phagocytosis in rats and mice (27, 28). In multiple administrations of CCl₄, zymosan with calf serum treatment enhances the decreased K index, but not the α index because of the increase in ratios of liver/body and spleen/body weights. Also, the increased spleen weight produced by zymosan suggests that it causes an increase in the number of spleen cells and that the increased rate of phagocytosis may be at least partially due to a greater number of phagocytic cells. From the light microscopical observation of the liver tissues in CCl₄ treatment mice, severe necrosis and steatosis were observed. Furthermore, a few Kupffer cells took up carbon particles in mice treated with multiple CCl₄ injections compared to that of single treatment. These microscopical observations also indicate that the phagocytic activity is depressed after multiple treatments of CCl₄ in mice. There were many reports that the phagocytic function of the fixed macrophages may be depressed in acute and chronic alcoholic patients and rats (29–31). Moreover, the perfused and isolated liver from rats fed with chronic ethanol (20% alcohol ×3 weeks) showed an apparent reduction of the bactericidal and phagocytic activity, and this shows that the effects of chronic ethanol may contribute at least in part to a defective opsonization of bacteria (32). In our experiment of multiple administrations of CCl₄, one of the reasons for the depression of the RES function may have been the depletion of serum opsonins. This is because zymosan...
with calf serum increases the phagocytic activity depressed by multiple CCl₄ administrations. From histological observations, we can also admit that the carbon particle uptake is increased by Kupffer cells in zymosan treated mice compared to non-treated mice. In addition, mice with multiple administrations of CCl₄ had very severe conditions, and it seemed that hepatic blood flow was strongly depressed. As a result, the clearance rate of carbon particles administered intravenously may have been decreased.

In the case of a single treatment of CCl₄, even though there were very severe steatosis and variable degrees of necrosis, many Kupffer cells took up as many carbon particles as in the control group. Parry (33) studied the effect of CCl₄ induced liver necrosis on the mobilization of Kupffer cells in mice. In carbon prelabelled liver, the Kupffer cell does not mobilize from the surviving liver at 24 hr, but mobilizes 72 hr after CCl₄. When CCl₄ induced liver injury was confirmed immunohistochemically, the Kupffer cell's infiltration was most prominent 72 hr after injury in rats (34). It seems possible therefore that the enhancement of phagocytic activity by mobilization or the increase in numbers of Kupffer cells are observed at 72 hr or 96 hr after the single administration of CCl₄. CCl₄ has a biphasic effect on Kupffer cells in rats: a transient fall in the number of isolated macrophages is corrected by a rapid influx of mononuclear cells which quickly differentiate into Kupffer cells (35). Moreover, it is necessary for us to examine the Kupffer cell's activity.

Carbon particles clearance rates are affected by various causes such as concentration of the particles in the blood, intravascular coagulation and fibrinolysis, platelet aggregation and disaggregation, the number of phagocytic cells, phagocytic activity of each cell, blood flow through the RES system and loss of particles through endothelial gaps (36). Among them, we take notice of the phagocytic activity of PEC since the lysosomal enzyme activity is considered one criteria for the activation of phagocytes (37). In the previous paper, we showed the increase in the lysosomal enzyme activities of PEC after zymosan injection (28). However, in the PEC from CCl₄ injected mice, we observed only a decreasing tendency in acid-phosphatase activity as a lysosomal enzyme. There was no significant change because of the wide variety of all the values. Moreover, it is necessary for us to examine the lysosomal enzyme activity of the Kupffer cells (38) to know each cell's activity.

The heparplastintest is a convenient method for determining the true activity of coagulation factors (II, VII and X). Our results showed the depression of the productive ability of these factors in liver cells after single and multiple administrations of CCl₄. The degree of injury is stronger 24 hr after a single injection of CCl₄ than 48 hr after multiple CCl₄ injections. Also from this result of intravascular coagulation activity, RES function by the carbon clearance test was seen not to be associated directly with liver cell injury. However, when CCl₄ is given to the rat which is pretreated with lipopolysaccharide from one bacterial strain, protection from CCl₄ toxicity occurs, serum transaminase values decline and histologic lesions in the liver do not appear. It is suggested that endotoxin, which is rapidly detoxified by RES, takes part in CCl₄ liver injury (39).

In summarizing our experimental condition using mice, CCl₄ induced liver injury was found to suppress the RES phagocytosis, and its effect seemed stronger in chronic than acute injuries, although such biochemical parameters as liver injury changed more severely after a single CCl₄ administration than multiple administrations. These results suggest that the RES function as measured by the carbon clearance test is clearly independent of liver parenchymal cell function.

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