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PROTECTION FROM CCl_4 TOXICITY BY PRESTIMULATION OF HEPATOCELLULAR REGENERATION IN PARTIALLY HEPATECTOMIZED GERBILS

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Abstract—The present investigation was undertaken to test our hypothesis that the slow responses of hepatocellular regeneration and tissue repair after CCl_4-induced liver injury are responsible for the high sensitivity of gerbils to the hepatotoxic and lethal effects of CCl_4. These studies were conducted in normal and actively regenerating livers using male gerbils 5 or 15 days after partial (2/3) hepatectomy (PH_5 and PH_{15}, respectively), or those undergoing sham operation (SH). An LD_{50} dose of CCl_4 (80 μL/kg, i.p.) resulted in a mortality (21%) significantly ($P < 0.05$) less than 50% in PH_5 gerbils 48 hr after CCl_4 administration, whereas the mortality observed in PH_{15} or SH gerbils was not significantly different from 50%. The elevations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were significantly ($P < 0.05$) less in PH_5 gerbils than in PH_{15} or SH groups after the administration of either the LD_{50} dose or a low dose (15 μL/kg) of CCl_4. Histopathological and histomorphometric examinations also indicated that CCl_4-induced liver injury was less severe in PH_5 gerbils than in the PH_{15} and SH groups. The hepatic microsomal cytochrome P450 content measured before CCl_4 administration in the PH_5 gerbils was decreased (26%) significantly ($P < 0.05$) as compared with the SH group, but was not significantly different from that of PH_{15} gerbils. *In vivo* metabolism of $^{14}CCl_4$ and lipid peroxidation in liver tissue were not significantly different among the various groups. Therefore, the protection against CCl_4 toxicity observed in PH_5 gerbils is unlikely to be due to decreased bioactivation of CCl_4 or lipid peroxidation in that group. [^{3}H]Thymidine incorporation into hepatocellular nuclear DNA was 4- to 5-fold higher in PH_5 gerbils than in the PH_{15} and SH groups, indicating active hepatocellular proliferation in PH_5 gerbils. [^{3}H]Thymidine incorporation was further increased significantly ($P < 0.05$) 24 hr after challenge with a low dose of CCl_4 in PH_5 gerbils, whereas it remained low until 48 hr after the CCl_4 injection in the PH_{15} or SH group. The protection against CCl_4 toxicity afforded by partial hepatectomy was closely associated with active hepatocellular regeneration. The overall results confirm the concept that the high sensitivity of gerbils to CCl_4 is due to very sluggish hepatocellular regeneration and tissue repair response to the CCl_4-induced liver injury.

The Mongolian gerbil (*Meriones unguiculatus*) has been reported to be highly sensitive to the toxicity of CCl_4 [1, 2] and other halomethanes [3, 4]. The high sensitivity of gerbils to CCl_4 could be attributed to their intensive metabolism of CCl_4 [2]. However, while the metabolism of CCl_4 is enhanced significantly in gerbils pretreated with powerful cytochrome P450 inducers such as phenobarbital, mirex, or chlordecone, the toxic effects of CCl_4 are not increased [1, 2], indicating that the final toxicological outcome of exposure to a toxicant requiring metabolic activation is not exclusively determined by metabolic activation. In our recent study, it was found that hepatocellular regeneration and tissue repair in response to CCl_4-induced liver injury in gerbils are very sluggish [4]. This observation led us to propose that the lack of an early phase of hepatocellular regeneration might play a significant role in predisposing the gerbil to a permissive progression of toxic effects inflicted by the intensive metabolism of CCl_4, thereby explaining their high sensitivity to CCl_4.

Partial hepatectomy is a well established model for stimulating hepatocellular regeneration. Following surgical partial (2/3) hepatectomy (PH‡) the liver is capable of total regeneration, and this phenomenon has been well characterized in rats. DNA synthesis begins approximately 12 hr post-surgery, with peak activity occurring at 24 hr; mitosis follows DNA synthesis. Ultimately, the original mass of the liver is regained within 7–14 days [5–7]. We employed partial hepatectomy in the present study to test the above-mentioned hypothesis. If our hypothesis holds, the prestimulated hepatocellular regeneration by PH should offer protection and reduce the sensitivity of gerbils to CCl_4 toxicity. When hepatocellular regenerative activity subsides, the original sensitivity of gerbils to CCl_4 should be restored.

However, PH is known to result in decreased

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‡ Abbreviations: PH, partial hepatectomy; PH_5, 5 days after partial hepatectomy; PH_{15}, 15 days after partial hepatectomy; SH, sham operated; and NS, gerbils without surgical manipulation.

microsomal cytochrome P450 levels [8–10]. Since the initiation of CCl_4 toxicity is dependent on the hepatic microsomal cytochrome P450-mediated bioactivation, the decreased cytochrome P450 level might result in attenuated CCl_4 metabolic bioactivation, which may lead to reduced toxicity of CCl_4. Although a 60% reduction of cytochrome P450 levels employing $CoCl_2$ treatment does not decrease metabolism of CCl_4 [11] and fails to provide any protection against the toxicity of a chlordecone + CCl_4 combination in rats [10, 12], the consequences of a possible decrease in cytochrome P450 content after PH on CCl_4 hepatotoxicity in gerbils should be directly tested. To validate the PH model for testing our hypothesis, *in vivo* metabolism of $^{14}CCl_4$ in gerbils with no surgical manipulation (NS), with sham operation (SH), and 5 or 15 days after the surgical partial hepatectomy (PH_5 and PH_{15}, respectively) was also investigated.

MATERIALS AND METHODS

Chemicals. Unless otherwise stated, all chemicals and enzyme kits used in this study were purchased from the Sigma Chemical Co. (St. Louis, MO). [^{3}H]Thymidine (sp. act. 20 Ci/mmol) and $^{14}CCl_4$ (99% purity, 2.8 mCi/mmol) were obtained from New England Nuclear (Boston, MA). $^{14}CCl_4$ was prepared with unlabeled CCl_4 in corn oil to achieve a concentration of CCl_4 (40 μL/mL) with a specific activity of 0.013 mCi/mmol. Analytical grade CCl_4 and scintillation fluid, Scintiverse E SX 16-4, were purchased from Fisher Scientific (Baton Rouge, LA).

Animals. Male Mongolian gerbils (40–60 g) were purchased from Tumblebrook Farm (West Brookfield, MA) and allowed to acclimatize for at least 1 week in our central animal facilities away from any contaminants. The gerbils were housed six per cage over untreated corn cob bedding under a 12-hr photoperiod, 50–80% humidity, at 21°. The gerbils were provided water and a standard rat chow (Rodent Laboratory Chow No. 5001, Ralston Purina Co., St. Louis, MO) *ad lib*.

Surgical manipulations. Partial hepatectomy procedures were carried out under light diethyl ether anesthesia following the method of Higgins and Anderson [13]. The median and left lateral lobes of the liver including gall bladder were removed through a 1- to 1.5-cm midventral abdominal incision. This results in approximately a two-third reduction in liver mass. SH animals underwent the same surgical manipulation procedure with the exception of the surgical removal of the liver lobes. Abdominal wall sutures and lobe ligations were made using chromic gut sutures (No. 1), and the skin wound was closed with silk sutures (No. 2). Survival of surgically manipulated gerbils was greater than 90% and the 10% deaths from surgery occurred during 36–96 hr after surgery. Our preliminary study indicated that 2 days post-PH the liver weights remained almost unchanged; 5 and 15 days post-PH, the liver weights were approximately 63 and 90% of the original liver weight. When surgical mortality occurred, it happened within the first 4 days after PH. The liver mass of the survivors was actively growing. Therefore, PH_5 was chosen to represent an active phase of hepatocellular regeneration. On the other hand, PH_{15} was chosen to represent a phase where hepatocellular regeneration stimulated by partial hepatectomy has virtually subsided. Hepatotoxicity and lethality of a low dose (15 μL/kg) and the LD_{50} dose (80 μL/kg) [2] of CCl_4 (corn oil as vehicle, 40 μL CCl_4/mL corn oil) administered i.p. were studied in PH_5, PH_{15} and SH gerbils. [^{3}H]Thymidine incorporation into hepatic nuclear DNA, as an index of hepatocellular regeneration, was also determined in these groups. *In vivo* metabolism of $^{14}CCl_4$ was estimated in NS, SH, PH_5, and PH_{15} gerbils.

Microsomal cytochrome P450. Hepatic microsomal protein and cytochrome P450 content in NS, SH, PH_5, and PH_{15} gerbils were determined using the method of Lowry *et al.* [14] and Omura and Sato [15], respectively.

Evaluation of CCl_4 hepatotoxicity. Hepatotoxicity was determined at a series of time points after challenge with a single low or LD_{50} dose of CCl_4 by assessing elevation of serum enzymes [alanine transaminase (ALT) and aspartate transaminase (AST)] and by histopathological examination of the liver tissue.

ALT and AST levels were quantified following the method of Reitman and Frankel [16]. Sigma Diagnostic Kits (Kit No. 505-OP) were used for assays.

For histopathological examination, liver sections were fixed in phosphate-buffered 4% formaldehyde, embedded in paraffin, stained with hematoxylin (H) and eosin (E), and examined under light microscope. Volume density of hepatocytes with one of the two morphologic features was determined separately according to Weibel *et al.* [17] as described previously [18–20]. The morphologic features under consideration were hepatocytes in metaphase and necrotic hepatocytes. Three or four gerbil livers were sampled for each group at each time point, and ten randomly selected areas from each of the two sections from each liver sample were counted.

Lethality. Lethality induced by an LD_{50} dose of CCl_4 was determined in PH_5, PH_{15}, and SH gerbils. After gerbils received a single dose of CCl_4 (80 μL/kg, i.p.), they were observed for 48 hr and lethality was recorded.

[^{3}H]Thymidine incorporation into liver nuclear DNA. [^{3}H]Thymidine incorporation into liver nuclear DNA was measured as an index of hepatocellular regeneration. The detailed procedures were described previously [4]. The procedure used for isolation of liver nuclear DNA was that described by Chang and Looney [21]. The DNA content was measured with the diphenylamine reaction as described by Burton [22]. [^{3}H]Thymidine incorporation data were expressed as cpm/mg DNA.

In vivo *metabolism of* $^{14}CCl_4$. The procedure for estimation of *in vivo* metabolism of $^{14}CCl_4$ in PH_5, PH_{15}, SH and NS gerbils was the same as that described earlier [2, 11, 23], except that the specific radioactivity of the $^{14}CCl_4$ in corn oil was 0.013 mCi/mmol instead of 0.04 mCi/mmol. Briefly, after injection of $^{14}CCl_4$ at a dose of 80 μL/kg, the gerbil was put in a glass metabolic chamber. The expired air was successively drawn at an approximate flow

Table 1. Liver regeneration in gerbils after partial hepatectomy

Days after surgery (N)	Liver weight as % of body weight	Liver weight as % of original weight*
0 (6)	1.41 ± 0.22	36.6 ± 5.6
2 (3)	1.53 ± 0.11	39.6 ± 2.8
5 (6)	2.41 ± 0.06	62.3 ± 1.6
8 (3)	2.98 ± 0.58	77.4 ± 3.7
11 (3)	3.16 ± 0.16	82.0 ± 4.1
15 (3)	3.46 ± 0.03	89.5 ± 0.7

N = the number of animals.

* The normal average liver weight reflected in the liver weight to body weight ratio was 3.86 ± 0.05% (mean ± SEM, N = 4). This mean value was used to calculate the liver weight expressed as per cent of the original weight.

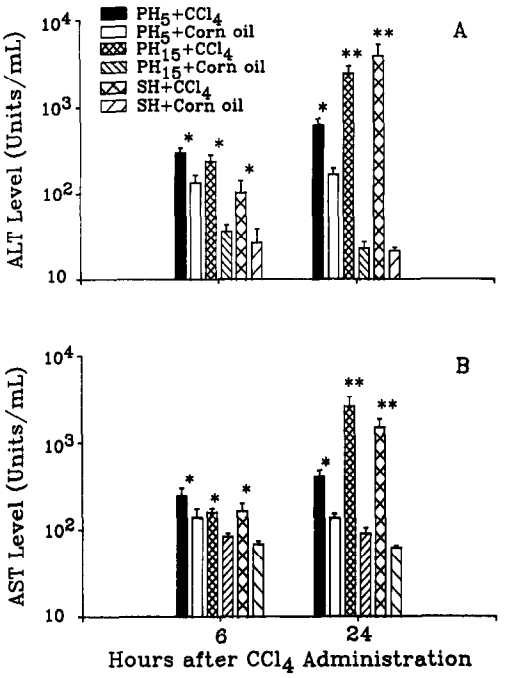

Fig. 1. Serum enzyme levels (panel A, ALT, and panel B, AST) in gerbils 5 or 15 days after partial hepatectomy (PH_5 and PH_{15}, respectively) and in sham-operated (SH) gerbils after challenge with CCl_4 (15 μL/kg). The results in each bar represent the means ± SEM of at least four gerbils. One asterisk indicates that the values are significantly ($P < 0.05$) different from the corn oil injected group with the same surgical manipulation at the corresponding time point. Two asterisks indicate a significant difference from $PH_5 + CCl_4$ gerbils at the corresponding time point ($P < 0.05$).

rate of 800 mL/min through two traps containing 10 mL of toluene to collect unmetabolized $^{14}CCl_4$ and a third trap containing 10 mL of NaOH to collect $^{14}CO_2$ derived from $^{14}CCl_4$. Each of the traps was immersed in an ice water bath maintained at 4°. Trap contents were removed for analysis at hourly interval for 6 hr. The radioactivity of collected $^{14}CCl_4$ and $^{14}CO_2$ was counted in a Packard 2200CA Tri-Carb Liquid Scintillation Analyzer and expressed as dpm. Upon completion of the 6-hr collection interval, the liver was surgically removed from the gerbil under diethyl ether anesthesia for the estimation of $^{14}CCl_4$ metabolites bound to liver tissue, hepatic lipid peroxidation and for histopathological observations.

Total hepatic ^{14}C, $^{14}CCl_4$ metabolites bound to hepatic lipid and non-lipid components, and free $^{14}CCl_4$ in hepatic tissue were determined as previously described [2] and expressed as dpm/g liver. *In vivo* lipid peroxidation was assessed by the method described by Davis and Mehendale [24]. Diene conjugation of the chloroform–methanol (2:1) extracts from the whole liver homogenate was measured at 243 nm in a Gilford 2200 response spectrophotometer. Values were expressed as O.D./mg lipid.

Statistics. Data of measured variables of surgical groups at the corresponding time points were subjected to conventional one-way ANOVA. Duncan multiple comparison was used to determine the significance of differences between groups when one-way ANOVA indicated a significant F value. The χ^2 was used to test the significance of the differences between the observed and the expected lethality (50%) 48 hr after the administration of an LD_{50} dose of CCl_4 in each group. The level of statistical significance was set at $P \leq 0.05$.

RESULTS

Liver regeneration following partial hepatectomy. The liver weight changes expressed as a percentage of body weight and as a percentage of the original liver weight after PH are shown in Table 1. As compared to rats [5], liver regeneration after PH in gerbils was much slower. In the first 2 days, there was no significant increase in liver weight. Even by 15 days after PH, the liver regained weight only to about 90% of the original weight, indicating a very sluggish response of hepatocellular regeneration and tissue repair.

Serum enzymes. Regardless of surgical manipulation, both ALT and AST levels in PH_5, PH_{15} and SH gerbils were increased significantly as compared with the respective corn oil-injected group 6 hr after the administration of a low dose of CCl_4 (Fig. 1), but there were no significant differences in elevation of ALT or AST between CCl_4-treated PH_5, PH_{15} and SH gerbils at that time, indicating that the initial injury was similar in each group. However, the elevation of ALT or AST activity was significantly higher in PH_{15} and SH gerbils than in PH_5 gerbils 24 hr after CCL_4 administration, suggesting that the PH_5 group was more resistant to progression of CCl_4-induced liver injury. The LD_{50} dose of CCl_4 resulted in even higher elevation of ALT and AST levels in all of the corresponding surgically manipulated groups as compared with the effects of a low dose (Fig. 2). Again, the elevation of either the ALT or AST level was not significantly different between CCl_4-treated PH_5, PH_{15} and SH gerbils 6 hr after the administration of CCl_4, an indication of

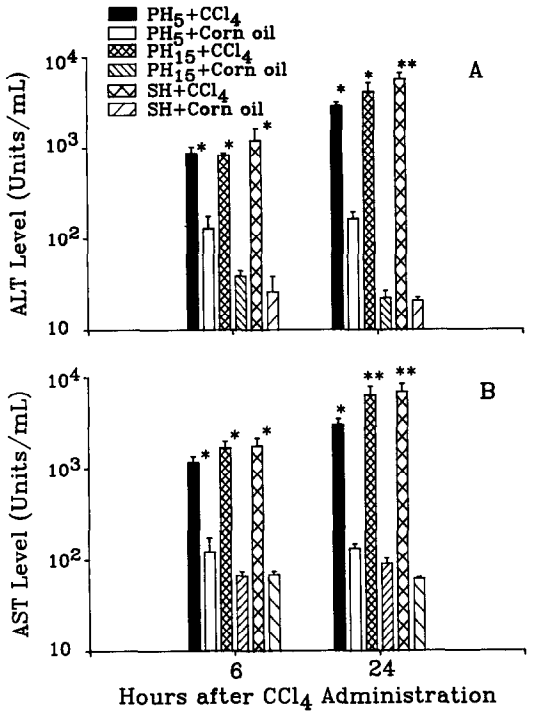

Fig. 2. Serum enzyme levels (panel A, ALT, and panel B, AST) in gerbils 5 or 15 days after partial hepatectomy (PH_5 and PH_{15}, respectively) and in sham-operated (SH) gerbils after challenge with CCl_4 (80 μL/kg). The results in each bar represent the means ± SEM of at least four gerbils. One asterisk indicates that the values are significantly ($P < 0.05$) different from the corn oil injected group with the same surgical manipulation at the corresponding time point. Two asterisks indicates a significant difference from $PH_5 + CCl_4$ gerbils at the corresponding time point ($P < 0.05$).

Table 2. Effect of surgical manipulation on lethality induced by a 48-hr LD_{50} of CCl_4 in gerbils

Surgical manipulation	Days after surgery	% Lethality (N)
Partial hepatectomy	5	21* (14)
Partial hepatectomy	15	58 (12)
Sham operation	5	50 (10)
Sham operation	15	64 (11)

* Significantly lower than the expected value (50%) $P < 0.05$.

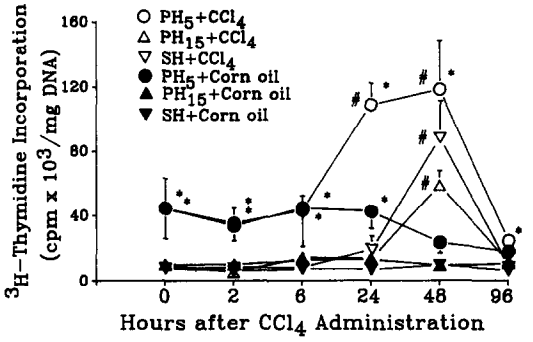

Fig. 3. [^{3}H]Thymidine incorporation in the hepatic nuclear DNA of gerbils 5 or 15 days after partial hepatectomy (PH_5 and PH_{15}, respectively) and in sham-operated (SH) gerbils after challenge with CCl_4 (15 μL/kg). The results of each point represent the means ± SEM of at least four gerbils. One asterisk indicates that the values are significantly ($P < 0.05$) different from PH_{15} or SH gerbils injected with corn oil at the corresponding time point. The # symbol indicates a significant difference from the respective surgically manipulated gerbils administered with corn oil at the corresponding time point ($P < 0.05$).

similar liver injury. The AST level in PH_{15} and SH gerbils and the ALT level in SH gerbils became significantly higher than in the PH_5 group at 24 hr, indicating again that PH_5 gerbils were more resistant to CCl_4 toxicity. Because of significant variation, ALT levels were not significantly different between PH_5 and PH_{15} gerbils at 24 hr. However, the actual increment of the mean ALT level between 6 and 24 hr in $PH_{15} + CCl_4$ gerbils (840–4023 units/mL) was significantly greater than that in the $PH_5 + CCl_4$ group (591–2815 units/mL). It was observed that the ALT level in the PH_5 + corn oil group was higher than in the PH_{15} + corn oil and SH + corn oil groups, which was an indication of surgical injury (Figs. 1 and 2). In the presence of this surgical liver injury symptom, the resistance of PH_5 gerbils to progression of CCl_4-induced liver injury was more impressive.

Lethality. The mortality in each group of gerbils 48 hr after the administration of an LD_{50} dose of CCl_4 is presented in Table 2. Lethality in PH_5 gerbils was significantly lower than the expected value (50%), whereas it was not significantly different from 50% in other groups. These data indicate that at 5 days after partial hepatectomy, the lethal effects of CCl_4 were substantially attenuated by PH.

[^{3}H]*Thymidine incorporation*. The [^{3}H]thymidine incorporation data in PH_5, PH_{15} and SH gerbils challenged with a low or an LD_{50} dose of CCl_4 are presented in Figs. 3 and 4. Hepatocellular regeneration, as indicated by the [^{3}H]thymidine incorporation, was stimulated significantly by PH. The stimulated [^{3}H]thymidine incorporation in the PH group was about 5-fold higher than in SH gerbils on day 5 (Fig. 3, PH_5 + corn oil, 0 hr), whereas it was not significantly different from that in SH gerbils on day 15 (Fig. 3, PH_{15} + corn oil, 0 hr), indicating that the stimulated increase of hepatocellular regeneration was subsiding. This subsidence was also demonstrable by the [^{3}H]thymidine incorporation data in PH_5 + corn oil group at 96 hr (Fig. 3), when the radioactivity incorporated into nuclear DNA in that group returned almost to the prestimulation level. In response to a challenge with a low dose of CCl_4, [^{3}H]thymidine incorporation was further significantly increased in PH_5 gerbils at 24 hr. In contrast, a significant increase of [^{3}H]thymidine incorporation was not observed in PH_{15} and SH gerbils until 48 hr. The increase of [^{3}H]thymidine

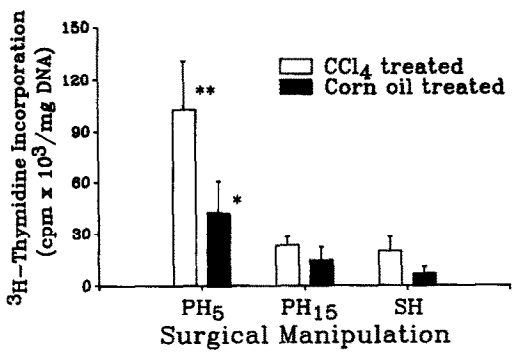

Fig. 4. [^{3}H]Thymidine incorporation in the hepatic nuclear DNA of gerbils 5 or 15 days after partial hepatectomy (PH_5 and PH_{15}, respectively) and in sham-operated (SH) gerbils 24 hr after challenge with CCl_4 (80 μL/kg). The results of each point represent the means ± SEM of four gerbils. One asterisk indicates that the value is significantly ($P < 0.05$) different from the PH_{15} or SH group injected with corn oil. Two asterisks indicate a significant difference from the other groups ($P < 0.05$).

incorporation induced by CCl_4 vanished in each group by 96 hr after CCl_4 challenge.

To investigate the stimulation of hepatocellular regeneration after challenge with a high dose of CCl_4, [^{3}H]thymidine incorporation in PH_5, PH_{15} and SH gerbils 24 hr after the administration of an LD_{50} dose of CCl_4 was assessed (Fig. 4). Similarly, [^{3}H]thymidine incorporation in PH_5 gerbils 24 hr after the administration of CCl_4 was further significantly increased over the prestimulated level (PH_5 + corn oil), which was significantly higher than that in PH_{15} or SH gerbils. In contrast, hepatocellular regeneration, as indicated by [^{3}H]thymidine incorporation data, in PH_{15} and SH gerbils remained unchanged at that time. These data suggest that the observed stimulation of hepatocellular regeneration and tissue repair in response to CCl_4-induced liver injury was not affected by the dose range used in the current study.

Histopathology and histomorphometry. The photomicrographs of liver sections from PH_5, PH_{15} and SH gerbils 24 hr after the administration of a low dose of CCl_4 are shown in Fig. 5. Morphometric examination of the liver sections provided a quantitative comparison of liver injury and mitotic activities among groups (Fig. 6). The combined results from histopathological and morphometric examinations of liver sections were consistent with the serum enzyme elevation and [^{3}H]thymidine incorporation data. Volume density of necrotic hepatocytes in gerbils injected with corn oil only never exceeded 1% regardless of any surgical manipulations. Six hours after CCl_4 administration, volume density of necrotic hepatocytes was similar between PH_5, PH_{15} and SH gerbils (Fig. 6A). The liver injury at 24 hr after CCl_4 administration, as indicated by vacuolation and necrotic hepatocytes, was less severe in PH_5 gerbils than in PH_{15} and SH groups (Fig. 5). Although volume density of necrotic hepatocytes was increased in PH_5, PH_{15} and SH gerbils 24 and 48 hr after CCl_4 injection, the progress of liver injury as indicated by increased volume density of necrotic cells was more prominent in PH_{15} and SH groups than in PH_5 gerbils. Volume density of necrotic hepatocytes in PH_{15} or SH gerbils 24 or 48 hr after CCl_4 administration was significantly greater than in PH_5 gerbils (Fig. 6A).

Hepatocytes in metaphase could be occasionally observed in control PH_5 gerbils, though not at high frequency, indicating the existence of prestimulated hepatocellular regeneration (Fig. 6B). Hepatocytes in metaphase were not evident in control PH_{15} and SH gerbils at any time points examined in this study. After the administration of CCl_4, volume density of hepatocytes in metaphase in PH_5 gerbils first declined a little by 6 hr and then increased rapidly. The appearance of hepatocytes in metaphase was observed more frequently in PH_5 than in PH_{15} and SH groups 24 and 48 hr after the administration of CCl_4 (Figs. 5 and 6). Although the increase of volume density of hepatocytes in metaphase in CCl_4-administered PH_5 gerbils versus control PH_5 gerbils was not significant at 24 hr, this difference became significant at 48 hr. In contrast, the increased volume density of hepatocytes in metaphase stimulated by CCl_4 was not significant in PH_{15} and SH gerbils even at 48 hr after CCl_4 administration.

The photomicrographs of liver sections from PH_5, PH_{15} and SH gerbils 24 hr after the administration of an LD_{50} dose of CCl_4 are presented in Fig. 7. Although differences in liver injury between groups can rarely be found, significant cellular regenerative activity, as indicated by mitosis, was frequently observed only in PH_5 gerbils. Mitosis was not evident in PH_{15} and SH gerbils at that time. The coincidence between the less severe liver injury and prestimulated hepatocellular regeneration and tissue repair by PH as well as the earlier appearance of augmented regenerative activity induced by CCl_4 observed in PH_5 gerbils suggests that resistance to CCl_4 toxicity in gerbils is closely associated with the timing of the onset as well as with the extent of hepatocellular regenerative responses.

Microsomal cytochrome P450. Hepatic microsomal cytochrome P450 content in SH gerbils was significantly higher than that in PH_5 or PH_{15} gerbils and that in NS gerbils was higher than all other groups (Table 3). These data indicate that PH surgery results in decreased hepatomicrosomal cytochrome P450 content. However, since there were no significant differences in cytochrome P450 content between PH_5 and PH_{15} groups, the observed protective effects of PH are unlikely to be due to decreased cytochrome P450 content. The decrease in cytochrome P450 content resulting from the PH necessitated the *in vivo* $^{14}CCl_4$ metabolism study in order to test the possibility that the mechanism underlying the protection against CCl_4 toxicity might be related to decreased bioactivation of CCl_4.

$^{14}CCl_4$ expiration. The percentage of ^{14}C recovered as $^{14}CCl_4$ from PH_5, PH_{15}, SH and NS gerbils is depicted in Fig. 8A. No significant differences in expiration of $^{14}CCl_4$ as unmetabolized parent compound were found among PH_5, PH_{15}, SH, and NS gerbils over a period of 6 hr. The percentage of the administered dose of ^{14}C that was expired as $^{14}CCl_4$ ranged from 81 to 94%. Despite the reduced liver

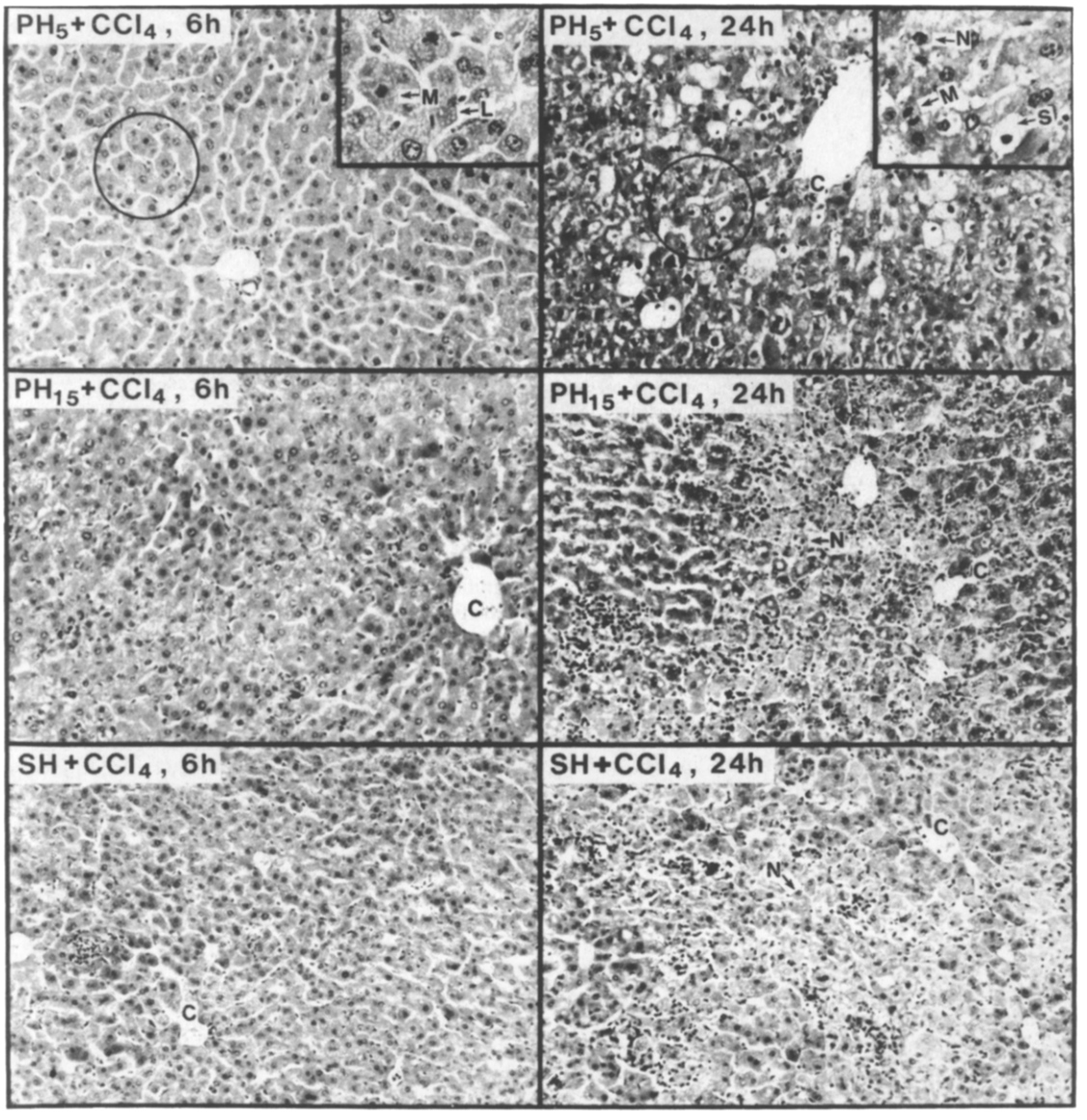

Fig. 5. Photomicrographs (H and E stain) of liver sections from PH_5, PH_{15} and SH gerbils injected with 15 μL CCl_4/kg (×200). The left panels show that severity of liver injury was similar in each group 6 hr after CCl_4 administration and that mitotic activity was observed only in PH_5 gerbils. The right panels show liver injury in each group 24 hr after CCl_4 administration. Liver injury was less severe in PH_5 gerbils and mitosis could be found only in PH_5 gerbils. Insets show magnified areas (×400). C = central vein; L = cells with lipid droplets; M = mitotic cell; N = necrotic cell; and S = swollen cell.

mass and total cytochrome P450 content, surgical manipulation did not alter significantly the ventilatory elimination of unaltered $^{14}CCl_4$.

$^{14}CO_2$ *derived from* $^{14}CCl_4$. The percent of ^{14}C recovered as $^{14}CO_2$ from PH_5, PH_{15}, SH and NS gerbils is shown in Fig. 8B. The mean values of $^{14}CO_2$ production derived from $^{14}CCl_4$ for PH_5, PH_{15}, SH, and NS gerbils over the period of 6 hr were 1.04, 1.18, 1.30 and 1.37%, respectively. Regardless of the significant differences in cytochrome P450 contents between these groups, no significant differences in $^{14}CO_2$ production were found among them.

Radioactivity in hepatic fractions. Free $^{14}CCl_4$ retained in hepatic tissue and binding of $^{14}CCl_4$-derived label to hepatic tissue fractions were measured as additional parameters of CCl_4 bioactivation (Fig. 9). When the data for hepatic ^{14}C-label were expressed on a per gram liver basis, the total radioactivity in liver of PH_5 gerbils was significantly higher than in SH and NS groups. Although the mean value of ^{14}C-label in liver of PH_5 gerbils was higher than in PH_{15} gerbils, the differences were not statistically significant. The significantly elevated total radioactivity in liver of PH_5 gerbils could be attributed to the significantly

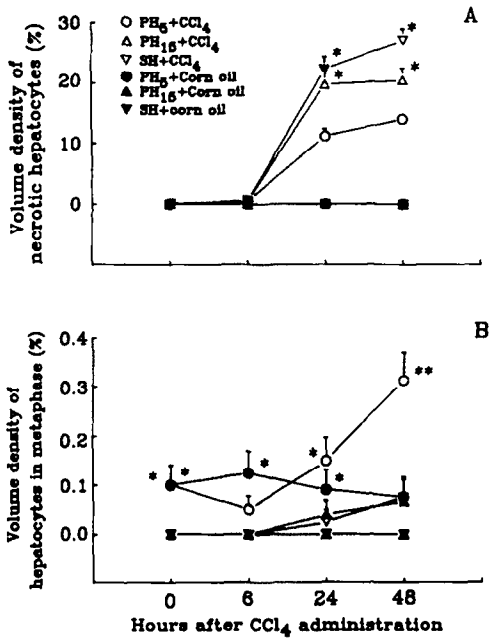

Fig. 6. Volume densities of necrotic hepatocytes (A) and hepatocytes in metaphase (B) measured in gerbils 5 or 15 days after partial hepatectomy (PH_5 and PH_{15}, respectively) and in sham-operated gerbils at various time points after the administration of CCl_4 (15 μL/kg) or corn oil. Each point represents the mean ± SEM of at least sixty randomly selected areas. In panel A, asterisks indicate significant ($P < 0.05$) differences from PH_5 gerbils challenged with CCl_4 at the corresponding time point. In panel B, one asterisk indicates that the values are significantly ($P < 0.05$) different from PH_{15} and SH gerbils with the same treatment at the corresponding time point. Two asterisks indicate a significantly higher value than observed in other groups ($P < 0.05$).

higher ^{14}C-binding to the non-lipid fraction of PH_5 gerbil liver. No significant differences in ^{14}C-bound to hepatic lipid fraction were found between the groups. Free $^{14}CCl_4$ present in PH_{15} gerbil liver was significantly higher than in SH or NS gerbils. However, owing to the decreased liver mass in PH gerbils (especially PH_5), the differences in radioactivity bound to hepatic tissue between the groups became insignificant when the data of total hepatic ^{14}C-label were expressed as a percentage of the $^{14}CCl_4$ dose administered. The total hepatic ^{14}C-label was 0.81 ± 0.15, 0.84 ± 0.24, 0.58 ± 0.05 and 0.56 ± 0.13% of $^{14}CCl_4$ administered for PH_5, PH_{15}, SH and NS gerbils, respectively.

Total $^{14}CCl_4$ metabolism. Combining the values for $^{14}CO_2$ production derived from $^{14}CCl_4$ and the ^{14}C-label bound to hepatic tissue (total ^{14}C radioactivity minus free $^{14}CCl_4$) allowed for an assessment of total CCl_4 metabolism (expressed as percent of dose administered). The comparison of total CCl_4 metabolism among PH_5, PH_{15}, SH and NS (Table 3) suggests that the total metabolism of the administered $^{14}CCl_4$ in 6 hr was not decreased significantly by surgical manipulation and that the observed protection against CCl_4 toxicity in PH_5 gerbils was unlikely to be due to the alteration of CCl_4 bioactivation.

Lipid peroxidation. As determined by diene conjugation of extracted hepatic lipids, lipid peroxidation at 6 hr post-CCl_4 challenge was not significantly different among PH_5, PH_{15}, SH and NS gerbils (0.012, 0.011, 0.016 and 0.014 O.D./mg lipid, respectively). The hepatic lipid contents were also not significantly different among the groups (67.5, 70.7, 66.0 and 74.0 mg lipid/g liver for PH_5, PH_{15}, SH and NS gerbils, respectively).

DISCUSSION

Gerbils are almost 35-fold more sensitive to CCl_4 toxicity than rats, as indicated by the 48-hr LD_{50} values [2]. Although the intensive metabolism of CCl_4 may contribute to their high sensitivity to this compound, enhanced metabolism of CCl_4 by cytochrome P450 inducers, such as phenobarbital, chlordecone and mirex, fails to increase CCl_4 toxicity [2]. Our previous study [4] provided two lines of evidence to suggest that hepatocellular regeneration and tissue repair may have an important role in recovery from CCl_4-induced liver injury. First, the significantly increased cell division and tissue repair stimulated by a low dose of CCl_4 led to the complete recovery from the limited liver injury, whereas an LD_{50} dose of CCl_4 resulted in not only more severe hepatic tissue damage but also complete ablation of the repair process, leading to a progression of liver injury culminating in roughly 50% lethality. Similar contrasting consequences of a low dose versus a high dose of CCl_4 also have been reported in rats [25]. Second, as compared with the rat data [18, 19], gerbils start hepatocellular regeneration in response to a low dose of CCl_4 stimulation much slower (6 hr vs 42 hr), and they are much more sensitive to CCl_4 toxicity. This relationship suggests that the sluggish cell division observed in gerbils may be the basis of their high susceptibility to CCl_4 toxicity. We were interested in testing this concept in the following experimental premise. If the high sensitivity of gerbils to CCl_4 toxicity is due to their sluggish or delayed cell division and tissue repair, the prestimulated hepatocellular regeneration by PH should provide protection. Administration of either a low or high dose of CCl_4 at the time when hepatocellular regeneration is actively undergoing should result in much less toxicity. Conversely, the high sensitivity of gerbils to CCl_4 should be restored when the PH-stimulated hepatocellular regeneration phases out.

The results of the present studies indicate that PH provides protection against CCl_4 hepatotoxicity and lethality, if CCl_4 is administered at the time of active hepatocellular regeneration. This conclusion is supported by the significantly decreased serum enzyme levels (Figs. 1 and 2) as well as decreased histopathological alterations (Figs. 5 and 6) after the administration of either a low or a high dose of CCl_4 to PH_5 gerbils. The significantly reduced lethality in PH_5 gerbils after the administration of an LD_{50} dose of CCl_4 also provides evidence in corroboration of this conclusion. This conclusion is further supported by the results from the PH_{15} gerbils, wherein

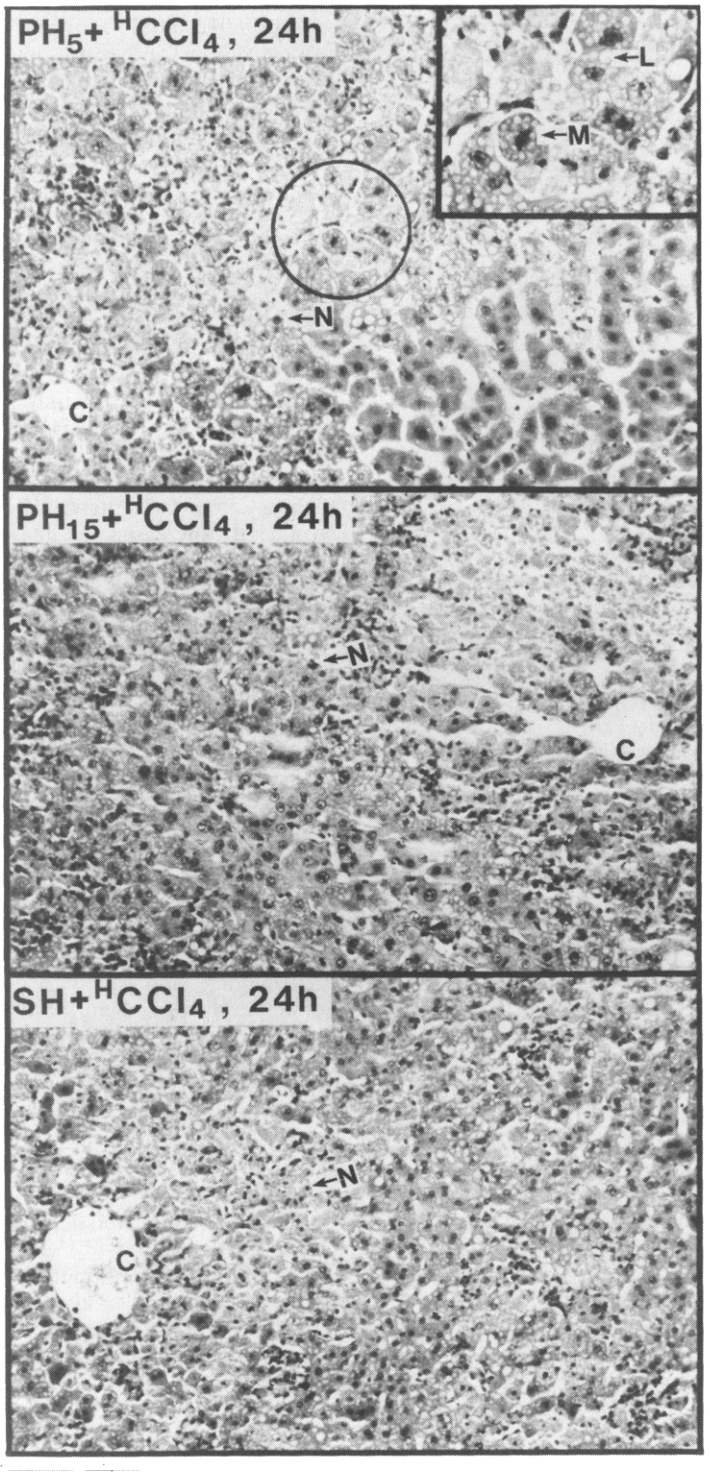

Fig. 7. Photomicrographs (H and E stain) of liver sections from PH_5, PH_{15} and SH gerbils 24 hr after an injection with 80 μL CCl_4/kg (×200). Inset shows magnified areas (×400). Gerbil liver was greatly damaged 24 hr after an LD_{50} dose of CCL_4 administration in all groups. Mitotic cells could still be found in PH_5 gerbils. C = central vein; L = hepatocytes with lipid droplets; N = necrotic cell; M = mitotic cell and $^{H}CCl_4$ = high dose of CCl_4.

Table 3. Effects of surgical manipulation on hepatic cytochrome P450 content and total $^{14}CCl_4$ metabolism*

Surgical manipulation	Hepatic microsomal cytochrome P450 (nmol/mg protein)	$^{14}CCl_4$ metabolized† (% of dose)
5 days after partial hepatectomy (PH_5)	0.46 ± 0.04	1.84 ± 0.23
15 days after partial hepatectomy (PH_{15})	0.40 ± 0.06	2.02 ± 0.37
Sham-operated (SH)	0.62 ± 0.04‡	1.89 ± 0.12
No surgical manipulation	0.78 ± 0.07§	1.90 ± 0.28

* Results are expressed as means ± SEM of 4–5 gerbils.
† Sum of ^{14}C as $^{14}CO_2$ + ^{14}C bound to hepatic tissue.
‡ Significantly higher than PH_5 and PH_{15}, $P < 0.05$.
§ Significantly higher than other groups, $P < 0.05$.

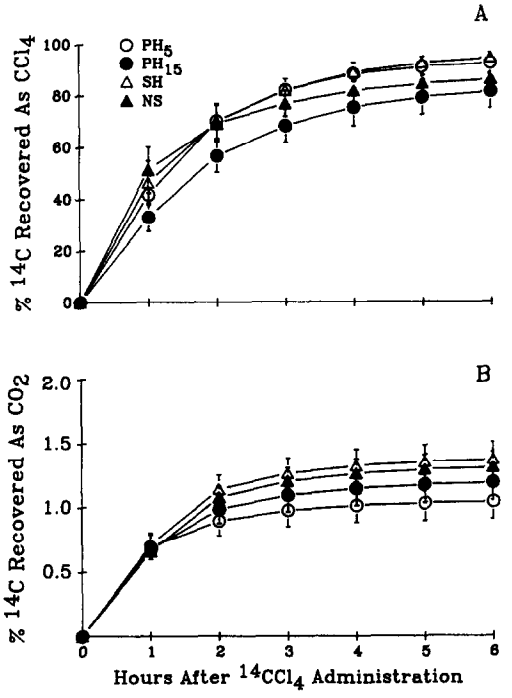

Fig. 8. Expiration of $^{14}CCl_4$ in the expired air (A) and expiration of $^{14}CCl_4$-derived $^{14}CO_2$ (B) from gerbils 5 or 15 days after partial hepatectomy (PH_5 and PH_{15}, respectively), sham operated (SH), and those with no surgical manipulation (NS). The results at each time point represent the means ± SEM of four gerbils.

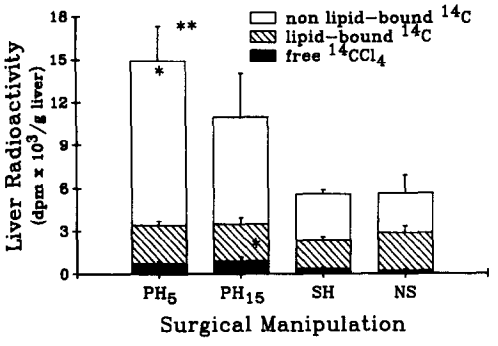

Fig. 9. Hepatic radioactivity derived from $^{14}CCl_4$ 6 hr after $^{14}CCl_4$ administration. Total radioactivity accountable in the hepatic fractions, free $^{14}CCl_4$, lipid-bound ^{14}C and non-lipid-bound ^{14}C were determined as described under Materials and Methods. Abbreviations are as in Fig. 8. The results in each bar represent the mean ± SEM of four gerbils. One asterisk indicates that ^{14}C radioactivity bound to non-lipid fraction in PH_5 gerbils and free $^{14}CCl_4$ in PH_{15} gerbil livers are significantly ($P < 0.05$) higher than that bound to the corresponding fraction in SH and NS gerbils. Two asterisks indicate that the total radioactivity accountable in the hepatic tissue of PH_5 gerbils was significantly different from that in the SH or NS group ($P < 0.05$).

hepatocellular regeneration stimulated by partial hepatectomy had essentially phased out. Our present findings show that PH_{15} gerbils become sensitive to the toxicity of CCl_4, as the stimulated hepatocellular regeneration phases out. The serum enzyme elevations, histopathological alterations and lethality were significantly greater in PH_{15} gerbils as compared to PH_5 gerbils and not significantly different from those observed in SH gerbils. The significantly decreased serum enzyme elevations and histopathological alterations observed in PH_5 gerbils 24 hr after CCl_4 administration were indicative of protection and not merely due to decreased liver mass available for the release of cytosolic enzymes. First, the elevation of serum enzymes induced by an LD_{50} dose of CCl_4 was much greater than that induced by a low dose of CCl_4, indicating that if greater injury were to occur, it would have been detected through serum enzyme elevations despite the reduced liver mass in PH_5 gerbils. Second, no downward adjustment in CCl_4 dose was made for PH animals, even though the ratio of liver weight to body weight in PH_5 was only about 60% of the normal liver (Table 1). Despite the administration

of a proportionately larger dose of CCl_4, the protective effect was still observed in PH_5 gerbils. Therefore, the magnitude of the protective effect observed at the time of maximally stimulated hepatocellular regeneration by PH observed here is in actuality somewhat underrepresented.

A number of possible mechanisms including alteration in bioactivation of CCl_4 and lipid peroxidation might be considered to explain the protection against CCl_4 toxicity by PH. Since metabolic bioactivation of CCl_4 by cytochrome P450-mediated reaction is the first obligatory step for the toxicity of CCl_4 [26–28], *in vivo* metabolism of CCl_4 in PH_5, PH_{15}, SH and NS gerbil was investigated in this study. A somewhat decreased metabolism, as indicated by production of $^{14}CO_2$ derived from $^{14}CCl_4$ (Fig. 8B), was observed in PH_5 and PH_{15} gerbils as compared to SH and NS groups. Even though there was no significant difference between PH_5 and PH_{15} gerbils, CCl_4 was significantly more toxic to PH_{15} than PH_5. Furthermore, despite a decrease in cytochrome P450 content after PH as previously reported in rats [10, 29], the total metabolism of CCl_4 was not significantly different among PH_5, PH_{15}, SH and NS gerbils (Table 3). Therefore, changes in metabolism measured by the methods used in the current study cannot satisfactorily explain the protection against CCl_4 toxicity by PH. These findings are consistent with the report by Young and Mehendale [11], indicating that the metabolism and disposition of CCl_4 are unaltered in PH rats despite a decrease in cytochrome P450 content. Their study also demonstrated that even after a 60% decrease in hepatic microsomal cytochrome P450 achieved by $CoCl_2$ treatment, $^{14}CCl_4$ metabolism and toxicity are unaltered [10, 11]. Our results also imply that cytochrome P450 is not a limiting factor in bioactivation of the dose of CCl_4 used in this experiment and that despite slightly decreased cytochrome P450 content by PH, there is still sufficient cytochrome P450 to bioactivate the amount of CCl_4 required to initiate liver injury.

Reduced lipid peroxidation after partial hepatectomy has been reported [30] and correlated with regenerative hyperplasia in rats [31]. The present findings, however, reveal that *in vivo* lipid peroxidation was not affected significantly by PH. Although lipid peroxidation was decreased slightly in PH_5 as compared with SH, it was almost identical in PH_5 and PH_{15} gerbils in contrast to the significant differences in hepatotoxicity found between PH_5 and PH_{15} groups. Furthermore, our previous work showed that even in the presence of greater CCl_4-induced lipid peroxidation in gerbils pretreated with phenobarbital, chlordecone or mirex, the resultant hepatotoxicity is not enhanced [2]. Therefore, alteration of lipid peroxidation is unlikely to be the mechanism for the protection against CCl_4 toxicity observed in PH_5 gerbils.

The serum enzyme and histopathological data indicate that the liver injury was almost identical among PH_5, PH_{15} and SH gerbils 6 hr after the administration of either a low or an LD_{50} dose of CCl_4, when the majority of administered CCl_4 was expired as unmetabolized parent compound [2, 23, 26]. The liver injury progressed rapidly in PH_{15} and SH gerbils thereafter, whereas it developed slowly in PH_5 gerbils after the administration of a low dose of CCl_4. In the case of an LD_{50} dose of CCl_4, differences in liver injury among groups were hard to discern based on the histopathological examination even at 24 hr after the dose (Fig. 7). However, the mortality associated with the injection of an LD_{50} dose of CCl_4 was significantly less in PH_5 gerbils as compared to PH_{15} and SH groups. These findings suggest that events other than the initiation of liver injury play an important role in determination of the final outcome of hepatotoxicity. The preplaced tissue repair and further augmentation of cellular regeneration appear to be the mechanisms that promote tissue healing and recovery from liver injury in the case of PH_5 gerbils.

A remarkable species difference in the promptness of cellular regenerative response is evident among gerbils, mice and rats. In response to CCl_4-induced liver injury, tissue repair, as indicated by increased [^{3}H]thymidine incorporation into nuclear DNA and increased mitosis, starts as early as 2–6 hr after CCl_4 administration in rats [18, 19, 32, 33]. Similar responses are found in mice at 24–36 hr after CCl_4 [34] or $CHCl_3$ [20] treatment. In contrast, the repair response is not evident in gerbils until 42 hr after CCl_4 treatment [4]. The results from the present study indicate that not only is the chemical-induced tissue repair process in gerbils considerably sluggish, but PH-induced hepatic regeneration is also sluggish. Within a span of several hours to 2 days after the PH, rapid increases of DNA synthesis and cell proliferation are promptly observed in rats [5, 32, 33]. This response is fully expressed as indicated by a more than 40-fold increase of [^{3}H]thymidine incorporation into nuclear DNA and a significant increase of the mitotic index; no further increase of regenerative activity is stimulated by CCl_4 [32, 33]. In contrast, the stimulated hepatocellular regeneration by PH in gerbils is quite limited and occurs much more slowly. Based on the liver mass change (Table 1), the regenerative activity of gerbil liver during the first 2 days after PH was very low. The limited increase of [^{3}H]thymidine incorporation into nuclear DNA and mitotic index (Figs. 3 and 6) stimulated by PH, and an additional incremental increase of these two indices by CCl_4 administration, indicate that the tissue repair response is not fully expressed in gerbils after partial (2/3) hepatectomy alone. These data suggest that the sluggish repair response of gerbils is not an oddity specific to chemical-induced injury, but rather an intrinsic attribute of this species. The present findings reveal that the promptness of the cellular regenerative response to either chemical-induced or PH-induced liver injury in rats, mice and gerbils is in a descending order. The sensitivity of these species to the toxic and lethal effects of CCl_4 is in an ascending order [2, 35, 36]. These observations suggest that species differences in susceptibility to a particular toxicant may lie in their ability to respond to injury by stimulating tissue repair and healing.

The reasons for such significant species differences in promptness of tissue repair in response to liver injury are not known at this time. It has been reported recently that O^6-alkyl guanine

alkyltransferase, an important DNA repair enzyme, has high activity in rat liver, a somewhat lower activity in mouse liver, and the lowest activity in gerbil liver [37]. Recent investigations also reveal that some key factors such as epidermal growth factor (EGF), transforming growth factor α (TGFα), and TGFβ are involved in the regulation of hepatocellular regeneration after CCl_4 induced [38, 39] and surgically induced liver injury [40–42] in rats. Whether the differences in activity of some enzymes or in these regulatory factors are responsible for the sluggish response of hepatcellular regeneration and tissue repair needs to be investigated.

In summary, the present findings reveal that the protective effects of partial hepatectomy against CCl_4 toxicity observed in gerbils are closely related with the prestimulated hepatocellular regeneration. Despite the decreased microsomal cytochrome P450 content of the liver, bioactivation of CCl_4 and CCl_4-induced lipid peroxidation, measured by the methods used in the present study, were not affected by PH. Our hypothesis that the lack of an early response of cellular regeneration is a critical factor, apart from the intensive metabolic bioactivation, determining the high sensitivity of the gerbil to CCl_4 is supported by these findings. Our study also raises the possibility that species differences in susceptibility to chemical liver injury may be explained on the basis of their ability to repair tissue injury.

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