Age-Related Susceptibility to Chlordecone-Potentiated Carbon Tetrachloride Hepatotoxicity and Lethality Is Due to Hepatic Quiescence

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ABSTRACT

Previous studies revealed that postnatally developing rats are resilient to the lethal effects of chlordecone (CD) + carbon tetrachloride (CCl_4) combination. The objective of this study was to investigate the underlying mechanism. We hypothesized that ongoing cell division and cell cycle progression as well as additional toxicant-induced stimulation of tissue repair help in restraining the progression of injury on the one hand, and in recovery through speedy healing on the other. Postnatally developing (20- and 45-d) and adult (60-d) male Sprague-Dawley rats were challenged with a nontoxic single dose of CCl_4 (100 μL/kg, i.p.) or corn oil after pretreatment with either dietary CD (10 ppm) or normal diet (ND) for 15 d. Hepatocellular injury was assessed by measuring serum enzymes [alanine transaminase (ALT), sorbitol dehydrogenase (SDH)], and bilirubin, as well as by histopathologic examination of liver sections during a time course of 0–96 h after the administration of CCl_4 or corn oil. Hepatocellular regeneration was assessed by [^{3}H]thymidine ([^{3}H]T) incorporation into hepatic nuclear DNA. In CD + CCl_4 treatment, ALT, SDH, and bilirubin levels peaked between 36 and 48 h after CCl_4. All 20-d-old rats survived the challenge of CD + CCl_4. CD-potentiated hepatotoxicity and lethality of CCl_4 begin to be manifested in 45-d-old rats at 48 h and later times (25% mortality), whereas adult rats experience progressive hep-atotoxic injury and 100% mortality by 72 h. In contrast, regardless of pretreatment, 20-d-old rats recover fully from injury by 72 h after CCl_4 treatment. The rapid recovery of 20-d-old rats was associated with a combination of higher level of ongoing cell division and additional sustained stimulation of [^{3}H]T incorporation from 24 to 72 h after the administration of CCl_4. In the older rats (45- or 60-d-old) this response was significantly delayed and attenuated. Ongoing cell division and CCl_4-stimulated regeneration were inversely related to postnatal development (20-, 45-, or 60-d). These biochemical, histopathological, and [^{3}H]T incorporation studies suggest that the liver of younger rats has greater plasticity for repair after toxic injury whereas adult liver is much more quiescent in this regard. (*Pediatr Res* **38: 140–148, 1995**)

Abbreviations

CD, chlordecone
TGF, transforming growth factor
ND, normal diet
ALT, alanine transaminase
[^{3}H]T, [^{3}H]thymidine
SDH, sorbitol dehydrogenase
AUC, area under the curves

A greater understanding of the mechanisms underlying the influence of age on the susceptibility to chemical injury is critical in dealing with hepatotoxicity in newborn, young, adult, and elderly patients. Early studies showed that chemical-induced hepatic injury and disease can either increase or decrease with age (1). Recent studies have demonstrated that neonates and young rats are resilient to a number of hepato-toxicants such as CCl_4 (2-5), allyl alcohol (2), galactosamine (6), and acetaminophen (7). Most interestingly, young rats survive exposure to individually nontoxic combination of CD + CCl_4 (5), known to cause 100% mortality in adult rats (8, 9). Because there is a need to understand the mechanisms underlying this age-related difference in toxicity, and because the CD + CCl_4 model offers a unique opportunity to investigate these mechanisms, this model was used in the present studies.

CCl_4 is bioactivated by cytochrome P450 to $\cdot CCl_3$ free radical, which is further converted to a peroxy radical, $CCl_3O_2^\cdot$ (10). These free radicals readily react with polyunsaturated fatty acids of the endoplasmic reticulum and other hepatocel-

lular membranes to initiate the formation of organic lipid peroxides. In the presence of cellular O_2, these organic peroxy radicals in turn can react with other polyunsaturated fatty acids to perpetuate a series of self-propagating chain reactions. Substantial experimental data suggest that stimulation of hepatocellular regeneration and tissue repair mechanisms plays a critical role in the recovery from the limited toxic injury inflicted by a low dose of CCl_4 (11, 12). Rats receiving a low dose of CCl_4 (100 $\mu L/kg$) alone exhibit very limited liver injury as evidenced by histopathologic observations at 6 h after the administration of CCl_4. This injury is progressive up to 12 h, followed by recovery by 24 h (11, 13). Prior exposure to a nontoxic dose of CD (10 ppm in diet for 15 d) is known to amplify hepatotoxicity and lethality of CCl_4 in male (8) and female (9) adult rats. Studies have revealed that the CD + CCl_4 combination inhibits CCl_4-induced liver cell division and tissue repair, thereby permitting unrestrained progression of injury leading to hepatic failure and death (8, 9, 13–17).

Newborn and young animals are commonly assumed to be more sensitive to many drugs and chemicals (18, 19). However, experimental data suggest that newborns are less sensitive to a number of chemicals such as CCl_4 whose toxicity depends upon metabolic activation to toxic species (20). In these studies, lower cytochrome P450 levels is offered as a mechanism for resiliency of newborns to toxicity of CCl_4. In contrast to the above studies, Cai and Mehendale (5) reported that postnatally developing rats exhibit resiliency not only to CCl_4 alone, but also to the deadly combination of CD + CCl_4. These studies have also shown that this resiliency is not related to differences in cytochrome P450 levels or bioactivation-dependent mechanisms but is more likely due to greater plasticity in liver cell division and tissue repair mechanisms in younger animals, in contrast to the quiescent nature of adult liver.

The objective of this study was to investigate the mechanisms underlying the resiliency of rats to CCl_4 or to CD + CCl_4 hepatotoxicity and lethality during early postnatal development. To this end, the temporal aspects of the magnitude of injury inflicted by CCl_4 and the role of ongoing as well as stimulated tissue repair during the course of injury and recovery was investigated. We report here that the remarkable plasticity of growing livers during early postnatal development enables the rats to restrain toxic injury and restore liver function, leading to recovery from injury.

METHODS

Chemicals. Unless otherwise stated, all chemicals and enzyme kits used in this study were purchased from Sigma Chemical Co. (St. Louis, MO). [^{3}H]T (sp act, 2 Ci/mmol) was purchased from Moravek Biochemicals (Brea, CA).

Animals. Postnatally developing (20 and 45 d) and adult (60 d) male Sprague-Dawley rats were bred and maintained in our Central Animal Facilities (for the entire experiment) under controlled conditions of temperature (20 $\pm$ 1°C), relative humidity (50–80%), and illumination (12 h light, 12 h dark). All rats had free access to commercial powdered food (Teklad

4% crude fat rat/mouse diet no. 7001, Indianapolis, IN) and water.

Treatment. Rats (30 and 45 d) were maintained on the standard rodent powdered diet with or without 10 ppm CD for 15 d as described by Curtis and Mehendale (14). Newly parturent female rats were maintained on a similarly prepared diet (30 ppm CD) or ND for 15 d, starting from 5 d postparturition such that their 20-d-old offspring would have been exposed to CD for 15 d through milk feeding (5). This protocol has been previously validated to be equivalent to 10 ppm CD dietary exposure (5). On d 16, rats from each dietary protocol were challenged with single injection of CCl_4 (100 $\mu L/kg$, i.p.) in corn oil (1:1) or corn oil alone. Blood and liver samples were collected at 0, 6, 12, 24, 36, 48, 72, and 96 h after CCl_4 or corn oil treatment. The blood samples were used to determine serum enzymes (ALT and SDH) and serum total bilirubin. Liver samples were used to determine [^{3}H]T incorporation into hepatic nuclear DNA, and for histopathologic examination.

CCl_4-induced lethality. Any lethality induced by a single dose of CCl_4 (100 $\mu L/kg$, i.p., was assessed in 20-, 45-, and 60-d-old rats maintained on ND or CD diet for 15 d. The rats were observed twice daily for 14 d, and mortality was recorded. Results are reported for up to 72 h, because all rats surviving at 72 h survived thereafter.

Serum enzymes and bilirubin. ALT (EC 2.6.1.2) and SDH (EC 1.1.1.1.4) were measured as markers of liver injury by the methods of Bergmeyer et al. (21) and Asada and Galambos (22), respectively. Total bilirubin was measured as a marker of liver function as described by Michaelsson (23).

In vivo incorporation of [^{3}H]T into hepatocellular nuclear DNA. [^{3}H]T was administered at a dose of 50 $\mu Ci/300$ of g body weight (i.p.) 2 h before sacrifice. The procedure used for isolation of liver nuclear DNA was that described by Chang and Looney (24). DNA content of the supernatant fraction was measured with the diphenylamine reaction as described by Burton (25). [^{3}H]T incorporation data were expressed as cpm/mg DNA.

Hepatopathology. Immediately after the surgical removal of the liver, sections of the liver were washed in normal saline (0.9%) and fixed in phosphate buffered 10% formaldehyde, embedded in paraffin, and sectioned (5 μm thick). The sections were stained with hematoxylin and eosin for histopathologic examination under a light microscope.

Statistical analysis. The data are presented as the mean $\pm$ SEM. A completely randomized design and a general linear model of regression analysis from a statistical analysis system were used to conduct a multiple linear regression. Significant differences were calculated using Duncan's new multiple range comparisons. The criterion for significance was set at $p \leq 0.05$. The AUC for injury or tissue repair were calculated using a BASIC statistical program.

RESULTS

Lethality. The mortality induced by a single nontoxic dose of CCl_4 (100 $\mu L/kg$, i.p.) in 20-, 45-, and 60-d-old rats maintained on either ND or 10 ppm CD diet was observed and recorded for 14 d. Mortality did not occur in rats given ND +

CCl$_4$ combination. In CD-pretreated rats, 25 and 100% mortality occurred in 45- and 60-d-old rats, respectively, within 72 h after the administration of CCl$_4$. In contrast, mortality did not occur in 20-d-old rats regardless of pretreatment.

Hepatotoxicity of CCl$_4$. Hepatotoxicity was assessed by measuring serum ALT, SDH, and total bilirubin levels during a time course after the injection of CCl$_4$. It is evident from the ALT, SDH, and bilirubin data (Figs. 1–3) that regardless of age, CCl$_4$ treatment resulted in marginal elevation of ALT, SDH, and bilirubin levels in rats maintained on ND. Rats of all age groups promptly recovered from this injury. In contrast, hepatotoxicity was dramatically amplified in rats maintained on the CD diet. Significant elevations of serum enzymes and bilirubin were observed as early as 6 h, the peak occurring between 36 and 48 h in all age groups after CCl$_4$ (Figs. 1–3).
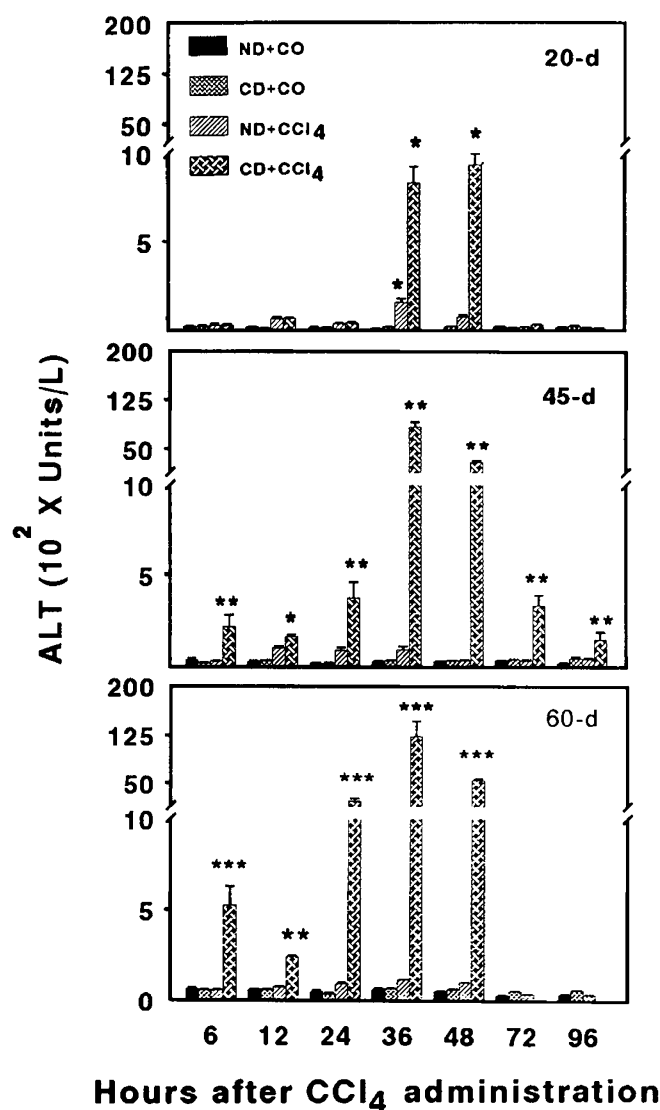

Figure 1. Serum ALT activity during a time course of 96 h after CCl$_4$ (100 μL/kg, i.p.) administration to 20-, 45-, and 60-d-old rats maintained on either ND or 10 ppm CD diet for 15 d. Values are means ± SEM of four rats. * = Significant increase in ALT level compared with the control of same time point; ** = significantly higher ALT level than in 20-d-old CD + CCl$_4$ rats; *** = significantly higher ALT level compared with that in 20- and 45-d-old CD + CCl$_4$ rats.
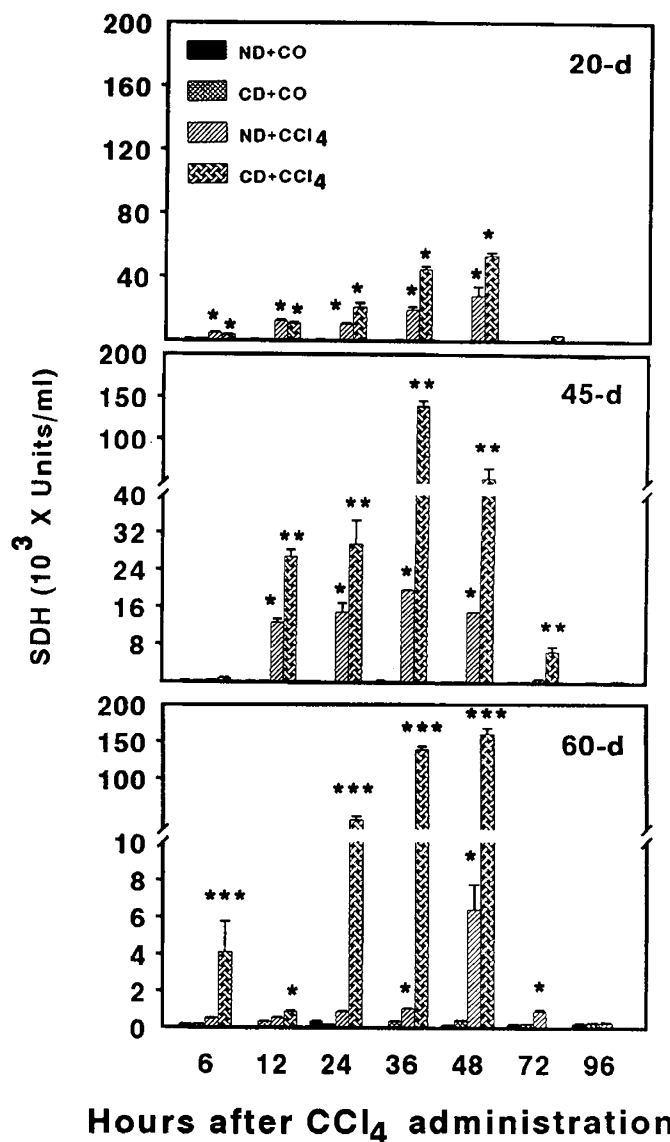

Figure 2. Serum SDH activity during a time-course of 96 h after CCl$_4$ (100 μL/kg, i.p.) administration to 20-, 45-, and 60-d-old rats maintained on either ND or 10 ppm CD diet for 15 d. Values are means ± SEM of four rats. * = Significant increase in SDH level compared with the control of same time point; ** = significantly higher SDH level than in 20-d-old CD + CCl$_4$ rats; *** = significantly higher SDH level compared with that in 20- and 45-d-old CD + CCl$_4$ rats.

In 60-d-old CD-pretreated rats, the elevation of these markers of liver injury progressed with time until 100% mortality occurred by 72 h. In 45-d-old rats toxicity and mortality (25%) was observed beginning at 48 h and later time points after CCl$_4$. As expected, there was no mortality in 20-d-old CD + CCl$_4$ rats. After reaching a peak, the serum enzymes fell to the background levels by 72 h, indicating a rapid recovery of the 20-d rats from CCl$_4$ liver injury.

Hepatopathology. Histopathologic alterations in 20-, 45- and 60-d-old rats were assessed by examining liver sections under a microscope for necrotic, swollen (balloon), and lipid-laden hepatocytes (Figs. 4–6). Necrotic cells were identified by pyknotic nuclei and ruptured plasma membrane, whereas, swollen cells were identified by enlargement and pale staining
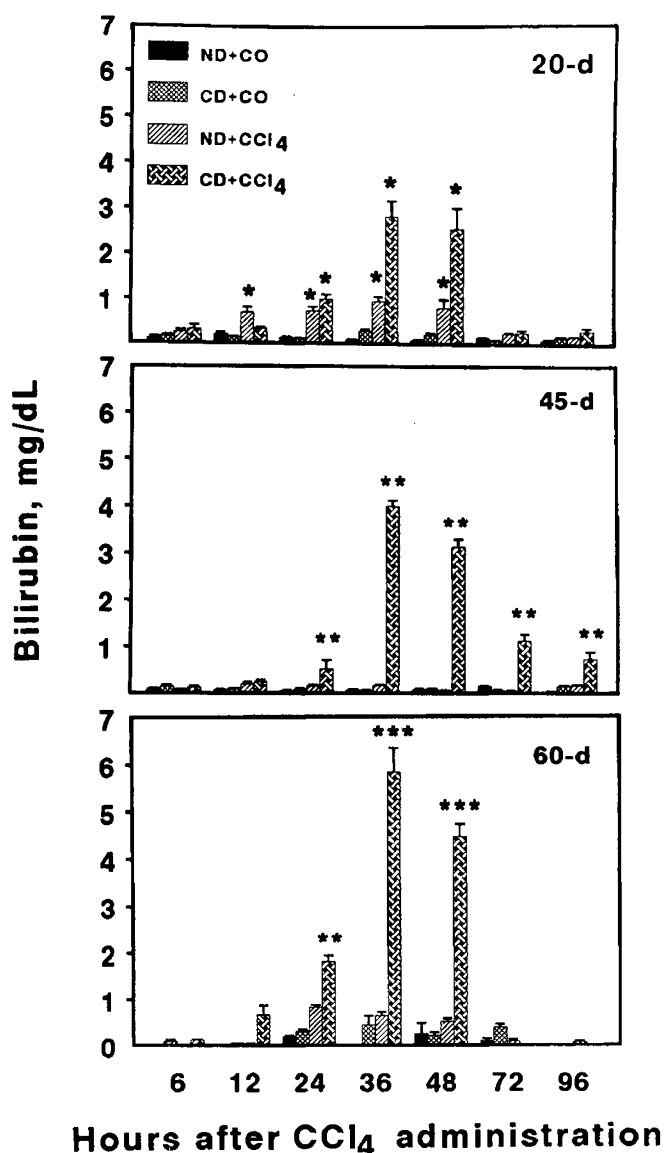
ND+CO
CD+CO
ND+CCl$_4$
CD+CCl$_4$

20-d

45-d

60-d

Bilirubin, mg/dL

6   12   24   36   48   72   96

Hours after CCl$_4$ administration

Figure 3. Serum bilirubin concentration during a time course of 96 h after CCl$_4$ (100 μL/kg, i.p.) administration to 20-, 45-, and 60-d-old rats maintained on either ND or 10 ppm CD diet for 15 d. Values are means ± SEM of four rats. * = Significant increase in bilirubin level as compared with the control of same time point; ** = significantly higher bilirubin level than in 20-d-old CD + CCl$_4$ rats; *** = significantly higher bilirubin level compared with that in 20- and 45-d-old CD + CCl$_4$ rats.

of the cytoplasm. Lipid-laden cells were identified by round vacuoles (droplets) within the cytoplasm. Histopathologic changes were complementary to the serum enzyme and bilirubin elevations, as well as [^{3}H]T incorporation data. Photomicrographs representing maximal liver injury inflicted by CCl$_4$ in each age group for both ND and CD-treated rats are depicted in the middle panels of Figs. 4–6. Liver photomicrographs depicting recovery from CCl$_4$ injury are shown in the bottom panels (Figs. 4–6). No histopathologic alterations were evident in the livers of control rats (0-h time point, upper panels) regardless of dietary treatment and age. Morphologic changes were evident in 20-d-old ND- and CD-pretreated rats as early as 6 h after CCl$_4$ treatment. In 20-d-old ND rats, maximum

injury was observed at 24 h after CCl$_4$ as evidenced by the extent of necrotic, ballooned, and lipid-laden hepatocytes in the centrilobular areas (Fig. 4C). In the 20-d CD rats, the injury progressed with time, the maximum injury being evident at 48 h (Fig. 4D). Liver injury was resolved in both ND and CD-pretreated rats by 72 h after the administration of CCl$_4$ (Fig. 4, E and F), indicating recovery from CCl$_4$-induced liver injury by replacement of dead cells with newly divided resilient cells, thereby restoring the centrilobular architecture.

In 45- and 60-d ND rats, liver injury was evident at 6 h with the maximum being at 12 h (Figs. 5C and 6C, respectively) after the administration of CCl$_4$, followed by recovery at 48 (Fig. 5E) and 24 h (Fig. 6E), respectively. In 45- and 60-d-old CD rats, necrotic, ballooned, and lipid-laden cells were evident as early as 6 h after CCl$_4$ treatment. Maximum injury was evident in both groups at 48 h (Figs. 5D and 6D). These alterations were progressive with time until 25 and 100% mortality occurred by 72 h in 45- and 60-d-old rats, respectively. In the surviving 75% of 45-d-old rats the morphologic alterations in the livers were minimal at 96 h after the administration of CCl$_4$ (Fig. 5F), indicating resolution of the injury. The mitotic index was significantly higher in 20-d rats from 24–48 h after the treatment with CCl$_4$ regardless of dietary protocol, compared with those for 45- and 60-d rats (data not presented).

In vivo incorporation of [^{3}H]T into hepatic nuclear DNA. [^{3}H]T incorporation was measured as an index of S-phase activity. In control rats (0-h time point not treated with CCl$_4$), there were no significant differences in the incorporation of [^{3}H]T among the two older age groups. Likewise, CD treatment had no significant influence on the incorporation of [^{3}H]T among the three age groups. However, the baseline values in 20-d-old rats were significantly higher than those in older age groups (Fig. 7). Regardless of the dietary pretreatment, 20-d-old rats exhibited a higher rate of [^{3}H]T incorporation than the older age groups. In both dietary groups, the wave of significantly increased [^{3}H]T incorporation began as early as 24 h and continued through 72 h after the administration of CCl$_4$ in 20-d-old rats. In contrast, in the older rats this response was delayed and diminished. In 45- and 60-d-old ND rats, increased [^{3}H]T incorporation occurred between 12 and 36 h after CCl$_4$ treatment and then returned to the baseline, whereas CD + CCl$_4$-treated rats exhibited slower and diminished [^{3}H]T incorporation, the maximum being between 48 and 72 h after CCl$_4$ treatment for 45-d-old rats and 48 h for 60-d-old rats (Fig. 7). The 75% survival in 45-d-old CD rats is presumably due to a very strong response in tissue repair activity particularly between 48 and 72 h after the administration of CCl$_4$. The delayed and diminished [^{3}H]T incorporation in adult rats (60-d-old) is a reflection of the suppressed S-phase stimulation and tissue repair, presumably leading to a loss of restraint on the progression of liver injury and consequently leading to 100% mortality (Fig. 6F).

DISCUSSION

Literature reports indicate that rats during early postnatal development are resilient to structurally and mechanistically
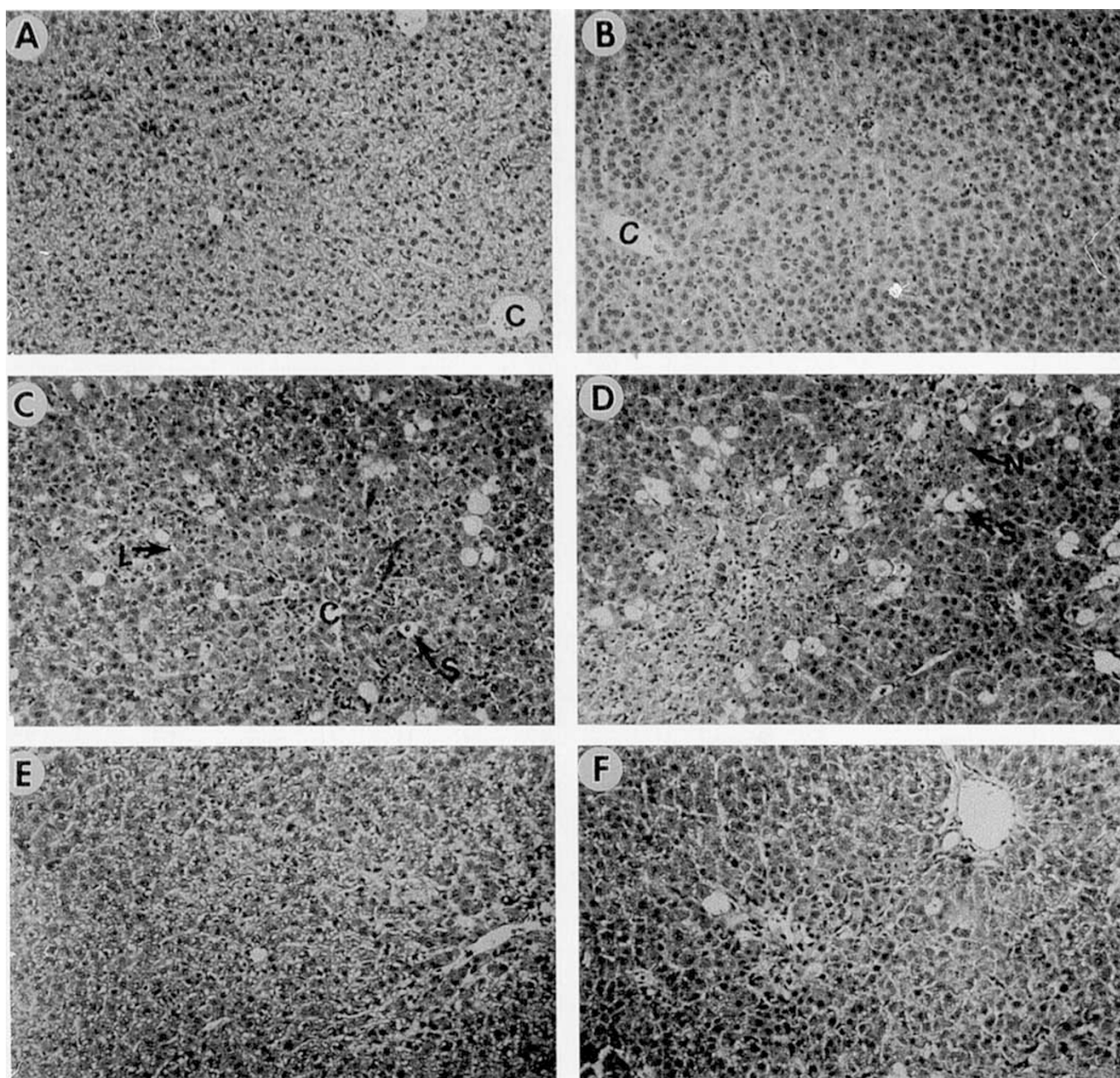
Figure 4. Photomicrographs of liver sections from 20-d-old rats maintained on either ND or 10 ppm CD diet for 15 d. On d 16, they were challenged with a single nontoxic dose of CCl_4 (100 μL/kg, i.p.). *A*, Control for ND; *B*, control for CD diet; *C*, time of maximum injury (24 h) in ND + CCl_4 group; *D*, time of maximum injury (48 h) in CD + CCl_4 group; *E*, time of recovery (72 h) in ND + CCl_4 group; *F*, Time of recovery (72 h) in CD + CCl_4 group. Magnification, X120. *C* = central vein; *N* = necrosis; *L* =lipid; *S* = swollen cells.

dissimilar hepatotoxicants (2–7). Early studies by Dawkins (3) and Cagen and Klaassen (4) showed that newborns are resilient to the toxic effect of CCl_4. In the same studies, 4-, 7-, 10-, 14-, and 21-d-old rats were reported to be as sensitive as adult rats. These studies used high doses of CCl_4 (1–2 mL/kg). It is known that cell division and tissue repair mechanisms are inhibited at high doses of toxic chemicals (26, 27). The mechanism of resiliency in newborns to CCl_4 toxicity was suggested to be due to lower amounts of cytochrome P450 required to catalyze the bioactivation of CCl_4 (3, 4).

In adult male rats, preexposure to 10 ppm CD is known to result in 67-fold amplification of CCl_4 hepatotoxicity and

lethality (8, 9, 14). Cai and Mehendale (5) used a single nontoxic dose of CCl_4 (100 μL/kg, i.p.) with or without 10 ppm CD diet to show that rat pups exhibit a remarkable resiliency to a combination of CD + CCl_4, which normally leads to 100% mortality in adult male (8) and female (9) rats. Further studies revealed that this is more likely related to ongoing liver growth and a promptly stimulated tissue repair mechanism rather than to differences in total cytochrome P450 levels or bioactivation-dependent mechanisms (5). In the present study, we were interested in investigating the temporal aspects of liver injury as well as tissue repair during a time course after a single low dose of CCl_4 with or without prior
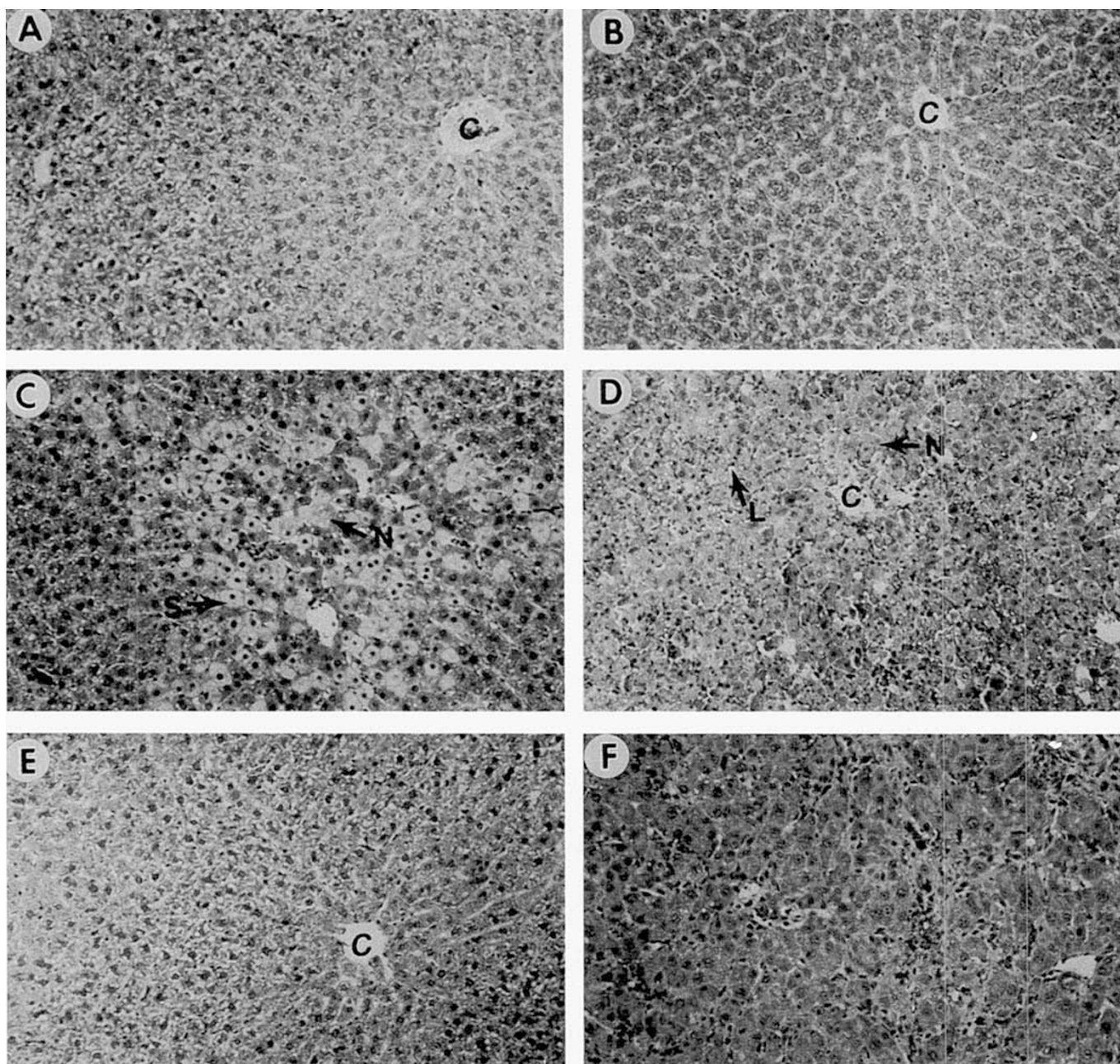
Figure 5. Photomicrographs of liver sections from 45-d-old rats maintained on either ND or 10 ppm CD diet for 15 d. On d 16, they were challenged with a single nontoxic dose of CCl_4 (100 μL/kg, i.p.). A, control for ND; B, control for CD diet; C, time of maximum injury (12 h) in ND + CCl_4 group; D, time of maximum injury (48 h) in CD + CCl_4 group; E, time of recovery (48 h) in ND + CCl_4 group; F, time of recovery (96 h) in CD + CCl_4 group. Magnification, ×120. C = central vein; N = necrosis; L = lipid; S = swollen cells.

exposure to nontoxic level of CD in rats during postnatal development.

After a low dose of CCl_4 (100 μL/kg), transient liver injury occurs regardless of age between 6 and 36 h as evidenced by elevation of serum ALT, SDH, total bilirubin (Figs. 1–3), and histopathologic observations (Figs. 4–6; A, C, and E). During this period of injury, ongoing (20 and 45 d) as well as stimulated hepatocellular regeneration were evident until 48 h as evidenced by [^{3}H]T incorporation data (Fig. 7). This response was much stronger in 20-d rats compared with that in 45- and 60-d rats. Consequently, progression of tissue injury in 20-d-old rats was restrained much more effectively. In CD-

treated rats, CCl_4-induced hepatotoxicity led to 25 and 100% mortality in 45- and 60-d-old rats, respectively. The role played by the quiescent nature of adult liver cells in restraining the progression of injury is amply evident here. Hepatocellular regenerative activity was not evident in 60-d rats until 48 h. This was too little and too late to restrain the progression of liver injury, consequently leading to liver failure and mortality in 60-d-old rats. In contrast to the quiescence of adult liver, remarkable plasticity of the growing livers enables the younger rats to restrain the progression of injury in an inverse relationship to age. Despite experiencing similar liver injury, 20- and 45-d rats differ in that this injury becomes progressive in 45-d
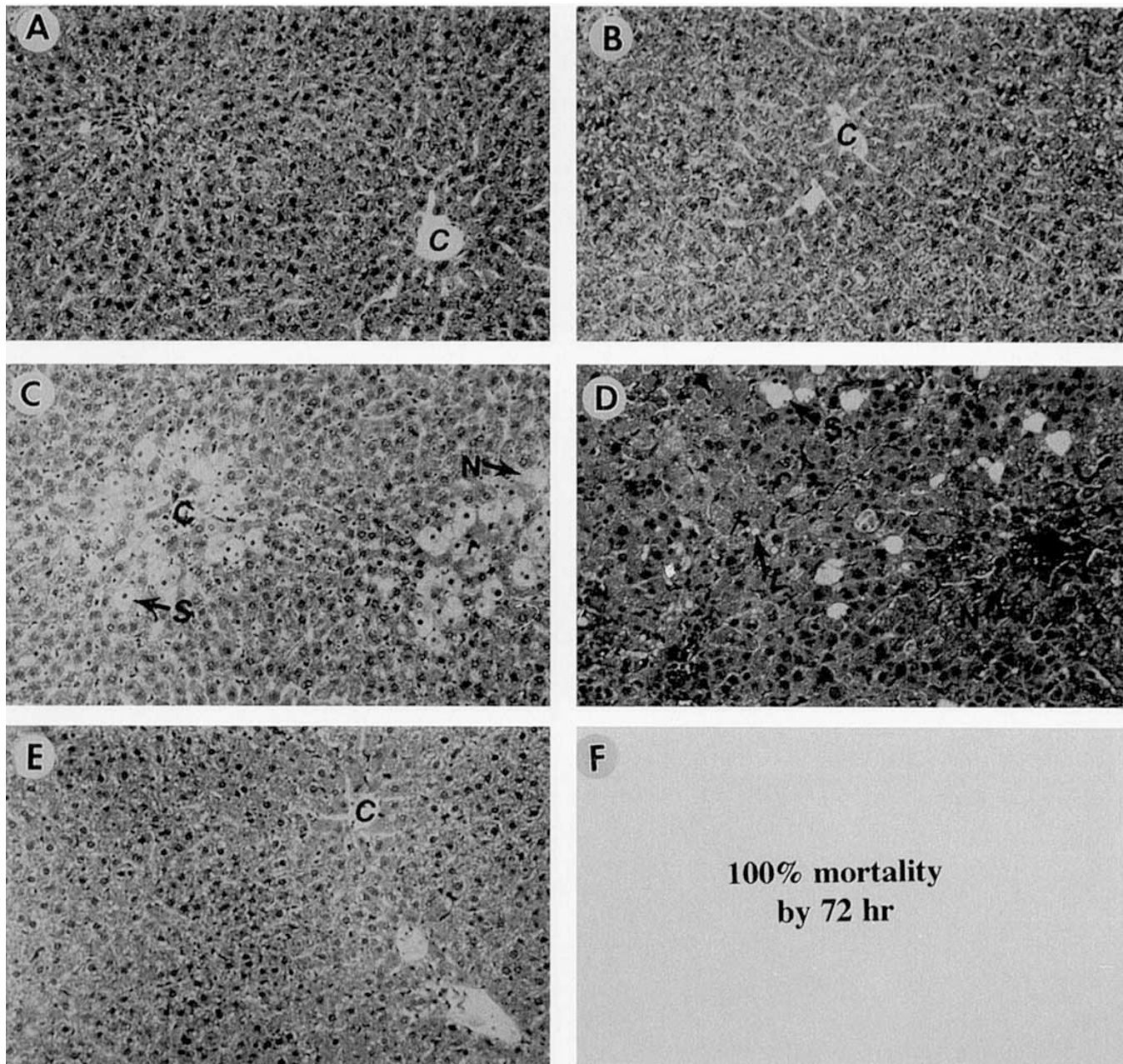
Figure 6. Photomicrographs of liver sections from 60-d-old rats maintained on either ND or 10 ppm CD diet for 15 d. On d 16, they were challenged with a single nontoxic dose of CCl_4 (100 μL/kg, i.p.). *A*, control for ND; *B*, control for CD diet; *C*, time of maximum injury (12 h) in ND + CCl_4 group; *D*, time of maximum injury (48 h) in CD + CCl_4 group; *E*, time of recovery (24 h) in ND + CCl_4 group; *F*, time at which 100% mortality occurred (72 h) in CD + CCl_4 group. Magnification, ×120. *C* = central vein; *N* = necrosis; *L* = lipid; *S* = swollen cells.

rats, leading to 25% mortality, whereas 20-d rats recover fully. In 45-d CD rats, hepatocellular regeneration and tissue repair increased significantly between 36 and 72 h after the administration of CCl_4, as indicated by [^{3}H]T incorporation and mitotic index. In contrast to the effect in 45- and 60-d CD-treated rats, CD treatment did not affect ongoing liver growth or CCl_4-stimulated hepatocellular regeneration and tissue repair in 20-d rats, which is in agreement with previous studies (5). In 20-d-old rats, regardless of diet, significant hepatocellular regeneration and tissue repair were evident at 24 h, continuing throughout 72 h after the administration of CCl_4, as indicated by increased [^{3}H]T incorporation and mitotic index.

These findings indicate that the remarkable plasticity of growing livers in younger rats enables them to recover from injury by additional prompt stimulation of hepatocellular regeneration and tissue repair.

The extent of CCl_4-induced liver injury in all CD treated rats was comparable regardless of age. This is clearly evident from histopathological observations of liver sections. However, serum enzyme elevations (ALT, SDH) are not equal because younger rat livers contain roughly 3-fold less ALT and SDH (our unpublished observations). Therefore, in age-related studies serum enzyme elevations should be used with caution for quantitative estimations of liver injury. Ongoing liver growth
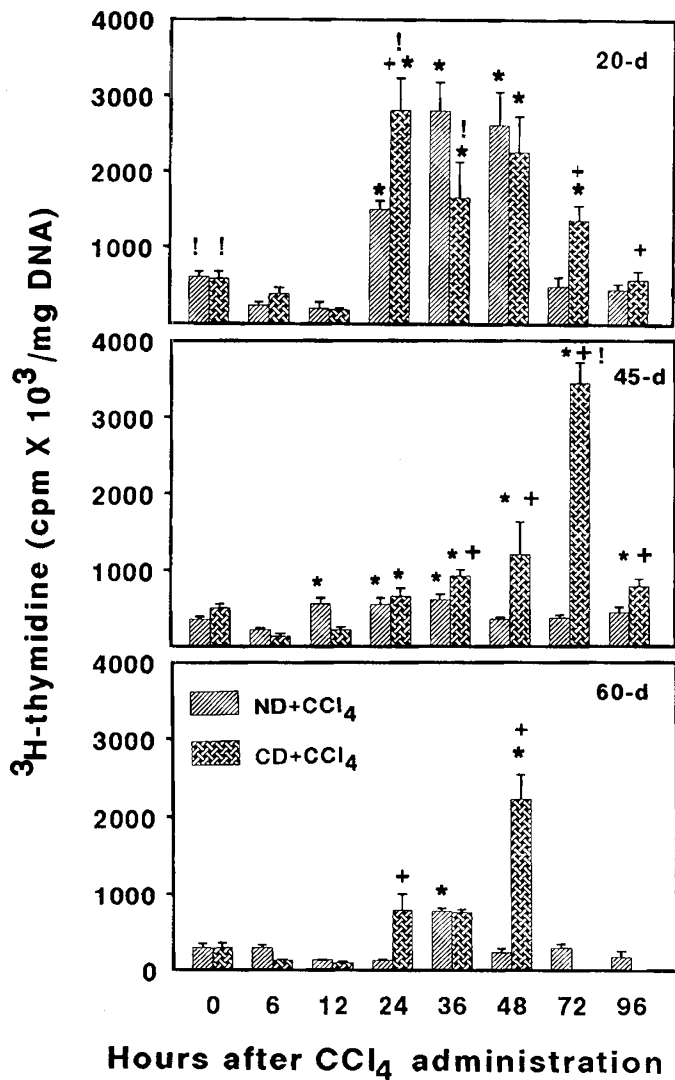
Figure 7. [^{3}H]thymidine incorporation into hepatic nuclear DNA during a time course after CCl$_4$ (100 μL/kg, i.p.) administration to 20-, 45-, and 60-d-old rats maintained on either ND or 10 ppm CD diet for 15 d. Values are means ± SEM of four rats. * = Significantly higher as compared with the respective control group (0 h time point); + = significantly higher as compared with ND + CCl$_4$ rats at the corresponding time period; ! = significantly higher as compared with 60-d-old CD + CCl$_4$ rats at the corresponding time.

Figure 8. AUC for CCl$_4$-induced tissue injury (a) and tissue repair (b) during a time course of 96 h after CCl$_4$ (100 μL/kg, i.p.) administration to 20-, 45-, and 60-d-old rats maintained on either ND or 10 ppm CD diet for 15 d.

and differences in the promptness of the hepatocellular regenerative response may be of pivotal importance in explaining the age-related differences in hepatotoxicity. Furthermore, age-related differences in CD-potentiated CCl$_4$ hepatotoxicity and lethality are associated with the balance between two opposite biologic events occurring simultaneously. CCl$_4$-induced liver injury (Fig. 8a) and tissue repair (Fig. 8b), as demonstrated by AUC for ALT and [^{3}H]T incorporation, respectively, illustrate this point. For example, the AUC for CCl$_4$-induced liver injury in 20-d CD-treated rats is 1/10 of that observed in 45- and 60-d rats. The AUC for tissue repair induced by CCl$_4$ in 20-d CD-rats is about 5-fold larger than that observed in 60-d CD-rats. The AUC for tissue repair in 45-d rats is similar to that calculated for 20-d rats, correlating with the 75% survival of 45-d CD rats receiving CCl$_4$.

Why are rats so efficient in tissue repair during early postnatal development? One reason is much higher efficiency of
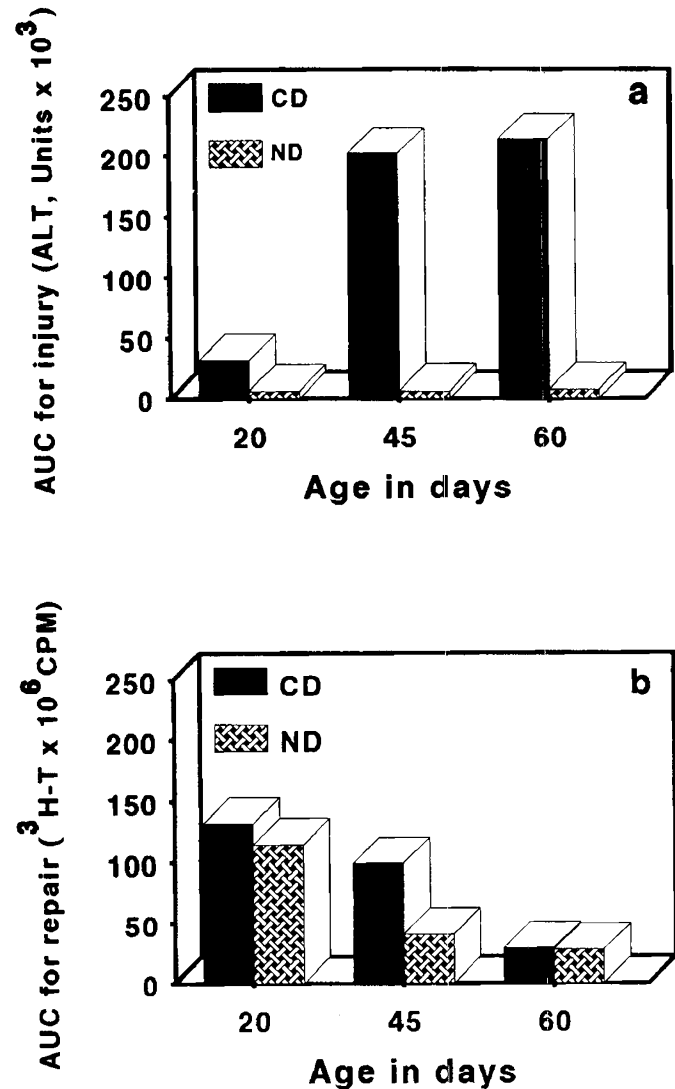
DNA synthesis, as evidenced by the shorter duration required for S phase and the cell cycle (28). In younger rats (21-d), both DNA synthesis and the cell cycle require about half the time required for 56-d rats. Therefore, the quiescence of adult rat livers appears to be responsible for lesser and delayed tissue repair response, allowing in the interim the injury to progress in an unrestrained manner.

Studies indicate that hepatocellular regeneration is subject to regulation by growth factors such as TGF-α and TGF-β (29, 30) whose expressions are developmentally regulated (31). TGF-α is known to be a powerful endogenous hepatocyte mitogen, whereas TGF-β inhibits mitogenic stimulation induced by TGF-α (29, 30). The expression of TGF-α increases upon tissue injury (32) and plays an important role in recovery (33). One other event that is thought to be essential for tissue regeneration is activation of specific proto-oncogenes which are known to influence the competency of surrounding cells to divide through "cell priming" (29, 34). Therefore, the possibility that the age-related differences observed in CCl$_4$ hepa-

totoxicity and its potentiation by CD is due to differences in the expressions of early proto-ocncogenes needs to be investigated. In addition to ongoing cell division, our findings also suggest that quicker and greater cell division response occurs during early postnatal development. This may either be due to quicker stimulation of TGF-α expression, or inhibition of TGF-β expression, upon toxicant challenge.

In conclusion, the findings of the present study indicate that the resiliency of postnatally developing rats to the hepatotoxic and lethal effects of CD + CCl_4 combination is due to the hepatic plasticity in responding to injury by stimulated tissue repair. In contrast, the hepatocellular quiescence acquired with age leads to a slower and lower tissue repair response, leading to unrestrained progression of injury.

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