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ALTERED HEPATIC ENERGY STATUS IN CHLORDECONE (KEPONE®)-POTENTIATED CCl_4 HEPATOTOXICITY*

PRASADA RAO S. KODAVANTI,† URMILA P. KODAVANTI and HARIHARA M. MEHENDALE‡§
Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, MS 39216-4505, U.S.A.

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Abstract—Previous studies have demonstrated that increased intracellular calcium, depletion of glycogen, and suppressed hepatocellular division resulting in progression of hepatic lesion without recovery are associated with chlordecone (CD)-potentiated CCl_4 hepatotoxicity. Since these phenomena are indicative of compromised hepatic energy status, the present studies were designed to investigate this possibility. Neither hepatic ATP content nor mitochondrial Mg^{2+}-ATPase was altered significantly in rats maintained on diets contaminated with either CD (10 ppm), mirex (10 ppm) or phenobarbital (PB; 225 ppm) alone for 15 days. Similarly, CCl_4 (100 μL/kg) administration alone did not alter hepatic ATP levels or mitochondrial Mg^{2+}-ATPase activity in rats maintained on a normal diet. However, CCl_4 administration to CD pretreated rats resulted in significantly decreased hepatic ATP content as early as 1 hr (36%), and this decrease was irreversibly progressive with time (81% at 6 hr). Oligomycin-sensitive Mg^{2+}-ATPase was decreased significantly only starting at 6 hr (21%) after CCl_4 administration, indicating that depletion of ATP at early time points was most likely due to rapid utilization consequent to toxic events. CCl_4 administration to mirex or PB pretreated rats resulted in a smaller decrease in ATP levels (18–24%) only at 24 hr, returning to normal levels by 36–48 hr, in accord with rapid recovery from limited liver injury. These findings indicate that CCl_4 administration to CD but not to PB or mirex pretreated rats results in a severely compromised energy status of the liver. The progressive and early depletion of liver ATP and the inhibition of Mg^{2+}-ATPase in CD + CCl_4 treated rats indicate the association of compromised energy status with altered Ca^{2+} homeostasis, depletion of glycogen, and suppressed cell division in CD-potentiated CCl_4 toxicity.

The highly unusual chemical interaction exemplified by chlordecone (Kepone®; CD‖; Fig. 1) potentiation of CCl_4 hepatotoxicity has been well characterized [1–4]. The interaction results in the potentiation of halomethane hepatotoxicity at very small, nontoxic levels of CD and CCl_4. Although the association between induction of the hepatic microsomal cytochrome P450 (MFO) system and potentiation of halomethane hepatotoxicity is widely accepted, this interaction could not be explained by increased bioactivation of CCl_4 or increased lipid peroxidation [4], because our earlier experiments indicated that phenobarbital (PB; Fig. 1) does not potentiate CCl_4 hepatotoxicity or lethality to nearly the extent that CD does, despite a greater induction of the hepatic MFO system [5, 6]. Furthermore, close structural relatives of CD such as mirex (Fig. 1) or photomirex do not greatly potentiate CCl_4 toxicity [7]. These studies indicated that CCl_4 hepatotoxicity cannot be associated solely with increases in total cytochrome P450 and certain other drug-metabolizing enzymes or with increased lipid peroxidation [8, 9], and suggest the prevalence of some unidentified mechanisms in this toxicity.

Our previous experiments indicated an irreversible accumulation of Ca^{2+} in a biphasic manner, and the toxic sequel included complete liver failure followed by death between 36 and 48 hr after a single administration of CCl_4 (100 μL/kg) to 10 ppm CD pretreated rats [10–12], whereas these effects were not observed after treatment with either of these chemicals alone. Furthermore, hepatocellular glycogen stores are depleted precipitously [13–15], a result which is associated with an increase in intracellular Ca^{2+} [10–12]. Histomorphometric studies [13, 14] revealed stimulation of hepatocellular regeneration at 6 hr after the administration of a low dose of CCl_4 alone which normally allows animals to recover from the limited injury. Such a stimulation of hepatocellular regeneration is not expressed in animals receiving 10 ppm dietary CD and CCl_4, indicating that the suppressed hepatocellular regeneration, besides bioactivation, is responsible for the dramatic amplification of what is normally recoverable injury [4, 16].

Stimulation of hepatocellular regeneration within 6 hr after a low dose of CCl_4 [4, 16] and the Ca^{2+} extrusion mechanisms are events dependent on the energy status of the liver. Severely compromised energy status might be expected to incapacitate the

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† Present address: Department of Neurology, University of Mississippi Medical Center, Jackson, MS 39216.

‡ Recipient of the 1988 Burroughs Wellcome Toxicology Scholar Award.

§ Address correspondence to: Dr. H. M. Mehendale, Department of Pharmacology and Toxicology, University of Mississippi Medical Center, 2500 North State St., Jackson, MS 39216-4505.

‖ Abbreviations: CD, chlordecone; CCl_4 carbon tetrachloride; PB, phenobarbital; MFO, mixed-function oxidase; and G-6-PDH, glucose-6-phosphate dehydrogenase.

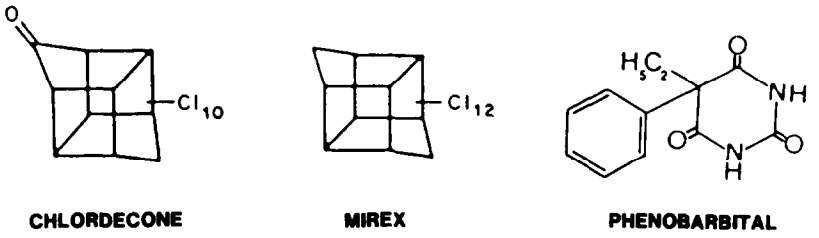


Fig. 1. Structural formulae of chlordecone, mirex and phenobarbital.

function of hepatocytes. Therefore, the present study was designed to investigate if hepatic ATP and hepatomitochondrial Mg^{2+}-ATPase are altered in CD pretreated rats upon subsequent administration of a small dose of CCl_4. PB and mirex pretreated rats were also included in the present study as positive and negative controls, respectively, for the potentiation of CCl_4 hepatotoxicity. We report here that the energy status of liver was not altered after a 15-day dietary treatment with 10 ppm CD, 10 ppm mirex, or 225 ppm PB alone or after a single dose of CCl_4 (100 μL/kg) alone. However, the same dose of CCl_4 decreased hepatic ATP levels as early as 1 hr after administration to CD pretreated rats, and this decline was irreversibly progressive with time. Such a severe perturbation was not observed in rats treated with PB or mirex in combination with CCl_4.

MATERIALS AND METHODS

Chemicals. Chlordecone (99% purity) was purchased from Chem Service (West Chester, PA). Mirex (100% purity) was obtained from the U.S. Environmental Protection Agency, Research Triangle Park, NC. Sodium phenobarbital was purchased from Mallinckrodt, Inc. (Paris, KY), carbon tetrachloride from J. T. Baker (Phillipsburg, NJ). All the other chemicals used in the present study were reagent grade and purchased from commercial sources.

Animals and treatment. Male Sprague–Dawley rats (150–175 g) were obtained from the Charles River Breeding Laboratories (Wilmington, MA). They were maintained in central animal facilities (room temperature, 21°; relative humidity, 50%) away from any known contaminants, under a 12-hr dark–light cycle. Rats were housed four per cage on crushed corn cob bedding, and received a commercial powdered rat chow (Ralston Purina Co., St. Louis, MO) containing 0 (normal diet), 10 ppm CD, 10 ppm mirex or 225 ppm PB and water *ad lib.* for a period of 15 days. The control and treatment diets were prepared as described previously [7].

On day 15 of dietary treatment, CCl_4 (100 μL/kg, in corn oil) was administered intraperitoneally. Proper controls were maintained by injecting an equivalent amount of corn oil (1 mL/kg). At 1, 2, 6, 24, 36, or 48 hr after CCl_4 administration, the rats were anesthetized with ether, and liver samples were collected quickly for analysis of ATP and for isolation of mitochondria. Of the four groups of rats studied, CD pretreated rats began to die starting at 36 hr after CCl_4 administration, as shown previously [6], and, therefore, only surviving rats were used for analysis at the 36- and 48-hr time points (36 hr = 10% mortality; 48 hr = 50% mortality). While this was the only practical way by which we could continue the time–course studies for this group, the findings clearly will tend not to represent progression of toxicity, since they came from relatively resistant animals.

In vitro *studies with CD and mirex.* Stock solutions of these insecticides were prepared in ethanol. This test solution (1–2 μL) was added to the reaction mixture to obtain the desired final concentration. Ethanol (1–2 μL) had no effect on mitochondrial Mg^{2+}-ATPase.

Preparation of mitochondria. After isolation, liver was washed with ice-cold homogenizing medium (0.32 M sucrose, 1 mM EDTA and 10 mM imidazole, pH 7.5). The fractionation was done according to the procedure of Green *et al.* [17]. The liver tissue (4 g) was homogenized in cold homogenizing medium and centrifuged at 750 *g* for 10 min to remove nuclei and cellular debris. The supernatant fraction was centrifuged at 9000 *g* for 20 min. The resultant pellet was suspended in homogenizing medium and again centrifuged at 9000 *g* for 20 min. The pellet containing mitochondria was suspended in the homogenizing medium, quick-frozen in liquid nitrogen, and stored at −80° until used.

Determination of ATP. ATP content was measured spectrophotometrically using the enzymatic method described by Lamprecht and Trautschold [18]. Liver was removed quickly by freeze-clamping, homogenized in 4 vol. of 0.75 M perchloric acid (PCA), and centrifuged at 5000 *g* for 20 min. The supernatant fraction obtained after centrifugation at 5000 *g* was adjusted to pH 7.4 with 1 N NaOH. One milliliter of the above sample was added to 1 mL buffer (50 mM Tris, 50 mM KCl, 0.2 mM EDTA, 6.7 mM $MgCl_2$), 0.015 mL NADP (0.1 M), 0.03 mL glucose (1 M), 0.01 mL hexokinase (10 mg/mL) and 5 μL glucose-6-phosphate dehydrogenase (G-6-PDH, 2 units). Absorbance changes were measured before and after the addition of hexokinase, G-6-PDH and glucose at 340 nm using a Gilford (Response 2200) spectrophotometer with the temperature controlled at 37°. The changes in absorbance at 340 nm were observed until the absorbance value became constant. The difference between the absorbance change before and after the addition of hexokinase, G-6-PDH and glucose was taken to represent ATP and was expressed as micromoles per gram liver.

Table 1. Hepatic ATP content and mitochondrial Mg^{2+}-ATPase activities in rats maintained on normal, chlordecone, mirex and phenobarbital diets for 15 days

Diet	ATP (μmol/g liver)	Mg^{2+}-ATPase (μmol P_i/mg protein/hr)
Normal	1.879 ± 0.107	18.61 ± 0.81
Chlordecone (10 ppm)	1.622 ± 0.061	17.84 ± 0.66
Mirex (10 ppm)	1.695 ± 0.088	19.41 ± 0.95
Phenobarbital (225 ppm)	1.653 ± 0.067	18.06 ± 1.11

Values are means ± SE of four or more preparations, and each preparation was assayed in duplicate.

Mg^{2+}-ATPase assay. Mg^{2+}-ATPase activity in rat liver mitochondrial fraction was measured according to the method described by Fritz and Hamrick [19] and as reported earlier [20]. The reaction medium contained 5 mM ATP, 1 mM ouabain, 5 mM $MgCl_2$, 20 mM KCl, 100 mM NaCl, 135 mM imidazole–HCl buffer (pH 7.5), 0.2 mM NADH, 0.5 mM phosphoenol pyruvate, and approximately 9 units of pyruvate kinase and 12 units of lactic acid dehydrogenase. A 50- to 60-μL mitochondrial fraction, equivalent to a protein content of 40–60 μg, was used. Absorbance changes in the reaction mixture were measured at 340 nm using a Gilford Response spectrophotometer with the temperature controlled at 37°. The change in absorbance at 340 nm over a period of 10 min was used in calculating the specific activity. Enzyme activities were expressed as micromoles P_i per milligram of protein per hour. Protein was determined by the method of Lowry *et al.* [21], using bovine serum albumin as a standard. The Mg^{2+}-ATPase activity was delineated into oligomycin-sensitive and -insensitive Mg^{2+}-ATPases, using 5×10^{-6} M oligomycin in the reaction mixture.

Statistics. Comparisons between values were made using analysis of variance for unbalanced design, followed by Scheffe's multiple comparisons [22]; $P < 0.05$ was considered to be significant.

RESULTS

Hepatic energy status after a 15-day dietary treatment with CD, mirex or PB. Previous studies have shown that dietary CD treatment for 15 days at doses of 50 ppm and higher results in a decrease in Mg^{2+}-ATPase activity, suggesting interference by CD with ATP metabolism [23, 24]. This raised the possibility that CD pretreated rats with low hepatic energy levels may become more sensitive to CCl_4 hepatotoxicity. Although the dose of CD (10 ppm) used for potentiation of CCl_4 hepatotoxicity was smaller in comparison to the doses used in the above-referenced studies, we wished to investigate whether 10 ppm CD had any effect on ATP metabolism. Hepatic ATP content and mitochondrial Mg^{2+}-ATPase activity levels in normal rats and in rats treated with CD, mirex or PB for 15 days were determined (Table 1). There was no significant difference in either hepatic ATP levels or in Mg^{2+}-ATPase activities among normal, CD, mirex or PB treated rats at the levels used in this investigation, indicating a lack of effect from these dietary treatments alone on the energy status of the liver.

In vitro *effects of CD and mirex on mitochondrial Mg^{2+}-ATPase.* Since CD has been reported to be an inhibitor of mitochondrial ATP metabolism, we tested the effectiveness of CD and mirex *in vitro* on Mg^{2+}-ATPases. Attempts were made to correlate the effects of CD concentration *in vivo* after 15 days of treatment with *in vitro* incubation studies. Although several factors may influence the pharmacokinetics and distribution of this compound [25, 26], we chose to consider the worst possible case in estimating the CD and mirex concentrations in mitochondria. Using a treatment protocol identical to the one used in the present study, Curtis and Mehendale [5] reported that the levels of CD and mirex were 52 and 19 μg/g liver respectively. As an approximation, the mitochondrial content of these compounds can be estimated as 0.73 μg of CD or 0.27 μg of mirex per mg mitochondrial protein (mitochondrial protein in 1 g of liver employing our isolation procedure is 14 mg, N = 5). We wished to test whether the estimated levels of CD or mirex in mitochondria after *in vivo* treatment would alter the mitochondrial Mg^{2+}-ATPase activity levels *in vitro*. Oligomycin-sensitive and oligomycin-insensitive Mg^{2+}-ATPase activities were determined *in vitro* (Table 2) in the presence of various concentrations of CD or mirex. CD *in vitro* did not inhibit oligomycin-sensitive Mg^{2+}-ATPase significantly at the levels found in the livers of 10 ppm CD treated rats. The lowest concentration at which a significant inhibition could be demonstrated was 2.5 μg CD/mg mitochondrial protein. This is 3- to 4-fold higher than the estimated mitochondrial CD content *in vivo*. Since it would require more than a 3-fold higher level of CD to inhibit oligomycin-sensitive Mg^{2+}-ATPase, our conclusions based on the estimated mitochondrial CD content normalized to the average mitochondrial protein should still be valid. Mirex did not alter oligomycin-sensitive Mg^{2+}-ATPase activity at any concentration studied. These *in vitro* observations are in agreement with the *in vivo* results (Table 1). Oligomycin-insensitive Mg^{2+}-ATPase activity was not altered at any concentration studied either with CD or mirex, suggesting its insensitive nature; this is in agreement with earlier reports [23, 24].

Effect of a low dose of CCl_4 on hepatic energy status. Table 3 shows hepatic ATP content and mitochondrial Mg^{2+}-ATPase activities in rats maintained

Table 2. Effects *in vitro* of chlordecone and mirex on rat hepatic mitochondrial oligomycin-sensitive and -insensitive Mg^{2+}-ATPases

Concentration (μg/mg mitochondrial protein)	Oligomycin-sensitive Mg^{2+}-ATPase (μmol P_i/mg protein/hr)	Oligomycin-insensitive Mg^{2+}-ATPase (μmol P_i/mg protein/hr)
Control	15.59 ± 0.17	4.08 ± 0.21
Chlordecone		
0.5	13.22 ± 0.70	4.11 ± 0.20
1.0	13.45 ± 1.04	4.10 ± 0.26
2.5	12.62 ± 0.73*	4.10 ± 0.44
5.0	9.48 ± 1.82*	4.17 ± 0.25
10.0	8.97 ± 1.23*	3.83 ± 0.37
Mirex		
2.7	15.75 ± 0.42	4.18 ± 0.31
5.4	15.84 ± 0.10	4.43 ± 0.38
11.0	15.71 ± 0.13	3.87 ± 0.33

The reaction mixture of 1 mL contained 135 mM imidazole–HCl buffer (pH 7.5), 5 mM ATP, 5 mM $MgCl_2$, 20 mM KCl, 100 mM NaCl, 0.2 mM NADH, 0.5 mM phosphoenol pyruvate, 9 units of pyruvate kinase, 12 units of LDH, and 50–60 μg of mitochondrial protein. Desired concentrations of chlordecone or mirex were included in the incubation medium and, after the protein determinations, the actual values were calculated as μg/mg mitochondrial protein. Values are means ± SE of four different preparations, and each preparation was assayed in duplicate.

* Significantly different from control at $P < 0.05$.

Table 3. Effect of CCl_4 (100 μL/kg) alone on hepatic ATP and mitochondrial Mg^{2+}-ATPase activities in rats maintained on a normal diet for 15 days

Hr after CCl_4 administration	ATP (μmol/g liver)	Mg^{2+}-ATPase (μmol P_i/mg protein/hr)
0	1.88 ± 0.11	18.6 ± 0.8
2	1.78 ± 0.10	15.7 ± 2.1
6	1.93 ± 0.08	18.5 ± 1.1
24	2.03 ± 0.09	18.9 ± 1.4
48	1.72 ± 0.04	17.8 ± 1.4

Values are means ± SE of four or more preparations, and each preparation was assayed in duplicate.

on a normal diet, followed by a single i.p. administration of CCl_4 (100 μL/kg). CCl_4 did not alter either ATP or Mg^{2+}-ATPase in the liver at any time up to 48 hr. This indicates that CCl_4 alone at the low dose used in our interaction studies did not interfere with the hepatic energy status.

Effect of CCl_4 on the hepatic energy status of CD, mirex or PB pretreated rats. Although CD and CCl_4 individually did not alter the hepatic energy status, we wished to investigate the possibility that a CD + CCl_4 combination might cause a severe decrease in energy level and that this might be related to the severe hepatic injury. CCl_4 administration to CD pretreated rats resulted in a significant decrease in hepatic ATP content as early as 1 hr; this decrease was irreversibly progressive with time (Fig. 2) to 81% by 6 hr and the ATP content remained approximately at that level thereafter. Oligomycin-sensitive Mg^{2+}-ATPase was decreased significantly only starting at 6 hr (and later) after CCl_4 administration (Fig. 3). Oligomycin-insensitive Mg^{2+}-ATPase was also inhibited at 24 hr (Fig. 4). The results obtained for

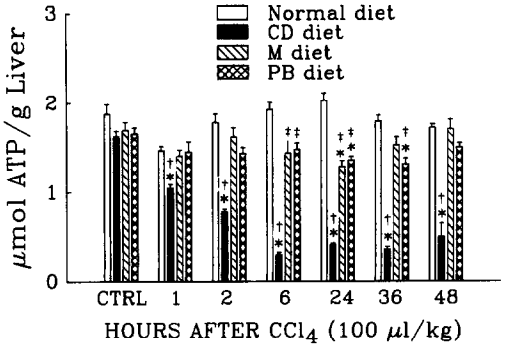


Fig. 2. Hepatic ATP levels at different time points after a single CCl_4 (100 μL/kg) administration to rats maintained on a normal, chlordecone (CD), mirex (M) or phenobarbital (PB) diet for 15 days. Values are the means ± SE of four to six rats. Key: (*) Significantly different from the respective control at $P < 0.05$; (†) Significantly different ($P < 0.05$) from normal, M and PB groups at that period of time, and (‡) Significantly different ($P < 0.05$) from the normal group at that period of time.

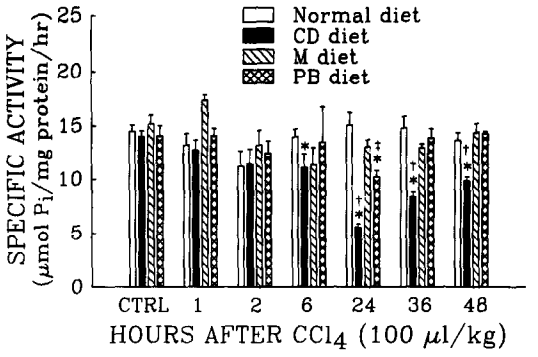


Fig. 3. Oligomycin-sensitive Mg^{2+}-ATPase activity levels in hepatic mitochondria at different time points after CCl_4 (100 μL/kg) administration to normal, chlordecone (CD), mirex (M) or phenobarbital (PB) pretreated rats. Values are the means ± SE of four to five preparations, and each preparation was assayed in duplicate. Key: (*) Significantly different from the respective control at $P < 0.05$; (†) significantly different ($P < 0.05$) from normal, mirex and PB groups at that period of time; and (‡) significantly different ($P < 0.05$) from the normal group at that period of time.

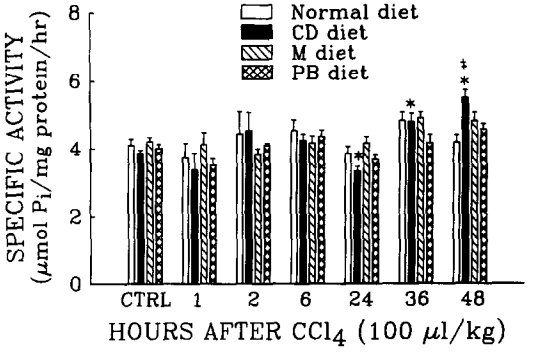


Fig. 4. Oligomycin-insensitive Mg^{2+}-ATPase activity levels in hepatic mitochondria at different time points after CCl_4 (100 μL/kg) administration to normal, chlordecone (CD), mirex (M), or phenobarbital (PB) pretreated rats. Values are the means ± SE of four to six preparations, and each preparation was assayed in duplicate. Key: (*) significantly different from the respective control at $P < 0.05$; and (‡) significantly different ($P < 0.05$) from the normal group at that period of time.

this group of rats at 36 and 48 hr after CCl_4 administration should be viewed with caution, since all these parameters were analyzed in surviving rats only. For example, where these assays were performed in surviving animals, oligomycin-sensitive and -insensitive Mg^{2+}-ATPase activities showed a slight recovery at 36–48 hr against the 24-hr time period instead of a progressive decrease.

Although hepatic ATP levels were decreased significantly only at 24 hr after CCl_4 administration to mirex or PB pretreated rats, this decrease was considerably less than noted for the CD + CCl_4 treated rats. Moreover, they were restored to control levels by 36 hr in mirex and by 48 hr in PB pretreated rats (Fig. 2). Similarly oligomycin-sensitive Mg^{2+}-ATPase was decreased significantly only at 24 hr in PB treated rats; the inhibition was considerably less than noted for the CD + CCl_4 treatment and returned to control level by 36 hr (Fig. 3). There was no significant change in oligomycin-sensitive Mg^{2+}-ATPase in mirex pretreated rats at any time point after CCl_4 administration. Oligomycin-insensitive Mg^{2+}-ATPase was not altered at any time point after CCl_4 administration to either mirex or PB pretreated rats (Fig. 4). These results indicate that CCl_4-induced decrease in hepatic ATP levels and synthesis of ATP recovered rapidly in PB or mirex pretreated rats, but not in CD pretreated rats.

DISCUSSION

The prevailing theory for the mechanism of hepatocellular injury by CCl_4 and $CBrCl_3$ states that these compounds are bioactivated to the $^{\cdot}CCl_3$ free radical by cytochrome P450 mediated reactions [27–29]. The $^{\cdot}CCl_3$ free radical produced initiates the peroxidation of adjacent membrane lipids [29]. There is also evidence for the formation of trichlorocarbon peroxy radical ($CCl_3OO^{\cdot}$), which may be the ultimate moiety initiating cellular injury [30]. The principal mechanisms put forth for the potentiation of CCl_4 hepatotoxicity by PB [31], 1,1,1-trichloro-2,2′-bis-(*p*-chlorophenyl)ethane (DDT) [32] and other modulators [33, 34] of toxicity are enhanced metabolic activation [29] followed by increased lipid peroxidation. However, considerable experimental evidence indicates that the capacity of CD to potentiate CCl_4 hepatotoxicity does not appear to be related to CD-induced MFO measured as total cytochrome P450 and its associated enzymes [5], to the slightly increased bioactivation of CCl_4 *in vitro* [8] or *in vivo* [9], or to the increased lipid peroxidation [2, 8]. This raised the possibility that there are some unidentified mechanisms involved in this interaction.

The time–course study of histopathological alterations and histomorphometric analysis [13, 14] have indicated that there are two dynamic aspects associated with CD sensitization of animals to CCl_4 toxicity [16]. First, the initial greater injury associated with CD pretreatment can be explained by slightly increased bioactivation of CCl_4 in livers of animals exposed to CD [8, 9]. Second, the progressive phase of the liver injury could be due to suppressed cellular regeneration and tissue repair [14, 35], leading to highly amplified liver toxicity. Hepatic regeneration is a complex process and dependent on ATP formation [36, 37]. For example, energy equivalent to 20 ATP molecules is required to move the chromatids to the poles [38]. The present investigations were prompted by the possibility that compromised hepatocellular energy metabolism would be expected to incapacitate cellular regeneration and other metabolic processes dependent on ATP.

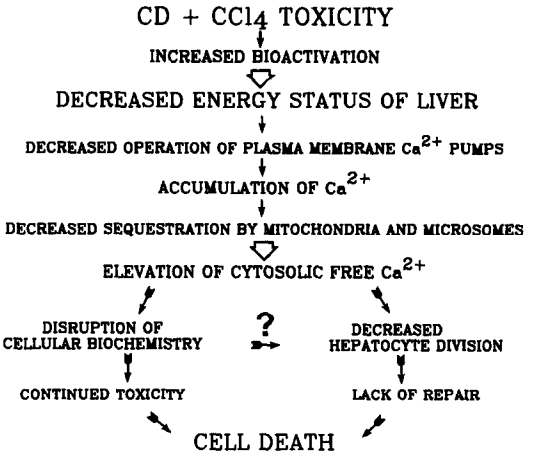
Previous studies have indicated that preexposure to much larger doses of CD causes inhibition of cellular energy production [23, 24] and subsequent impairment of hepatobiliary function [39]. Mitochondrial Mg^{2+}-ATPase, a key enzyme in oxidative phosphorylation leading to the synthesis of ATP molecules, was reported to be inhibited [23, 24].

There is also evidence that CD interferes with mitochondrial oxidative phosphorylation [40] and energy metabolism [41]. Based on this information, we wished to investigate if preexposure to dietary CD resulted in a compromised energy status in the liver. Although CD impairs energy production at higher doses, our present work demonstrates, through *in vivo* as well as *in vitro* studies, that the energy status of the liver was not altered at 10 ppm CD in the 15-day dietary protocol. These observations underscore the powerful nature of the toxic interaction of the CD + CCl_4 combination, since it occurred at levels of CD which cannot be associated with impairment of cellular energetics or with any animal toxicity [4].

The second logical question was whether the low dose of CCl_4 alone used in our CD + CCl_4 interaction affects energy status in the liver. The low dose of CCl_4 (100 μL/kg) did not alter either ATP levels or the synthesis of ATP in rats maintained on a normal diet, establishing that this dose of CCl_4 alone cannot be associated with any cytotoxic events affecting energy status. Stimulation of hepatocellular regeneration within 6 hr after a low dose of CCl_4 administration, resulting in recovery from the limited injury, is consistent with the undisturbed hepatocellular energy status.

Having established that dietary 10 ppm CD alone or a low dose of CCl_4 alone did not interfere with hepatic energy metabolism (Tables 1–3), we investigated whether the sequential combination of these two chemicals alters hepatic energy status. Interestingly, the energy status of the liver was greatly impaired when the same low dose of CCl_4 was administered to rats pretreated with 10 ppm CD. The depletion of ATP levels observed in the present study is closely associated with the rapid and precipitous depletion of hepatic glycogen levels between 1 and 4 hr after the administration of CCl_4 to CD treated rats [14]. Association of a rapid depletion in glycogen with decreased ATP stores of liver cells indicates a highly increased demand of ATP in response to the toxic insult. This decrease in ATP levels was not compensated for by increased ATP production in hepatocytes, since Mg^{2+}-ATPase was inhibited significantly starting 6 hr after CCl_4 administration to CD pretreated rats. Thus, CD + CCl_4 toxicity not only increased ATP utilization but also inhibited ATP synthesis. This combination exerted a dual effect and hence resulted in a dramatic drop in cellular ATP levels.

There is also a close association of these events with hepatocellular accumulation of excessive Ca^{2+} [10–12]. It is well known that there is an ATP-dependent mechanism to extrude intracellular Ca^{2+}. Accumulation of intracellular Ca^{2+} could also cause activation of cytosolic phosphorylase *a*, leading to increased glycogenolysis.* The demand on cellular energy would be increased with increasing accumulation of intracellular Ca^{2+}, since the Ca^{2+} extrusion mechanism of the plasma membrane is driven by cellular ATP [42]. Most interestingly, ATP levels



Scheme 1. Schematic illustration of how compromised hepatocellular energy may lead to disruption of Ca^{2+} homeostasis causing cell death. Compromised cellular energy also would be expected to halt normal homeostatic mechanisms, which normally should have stimulated neighboring hepatocytes to divide.

were depleted severely (81%) at 6 hr after CCl_4 administration to CD pretreated rats. In addition to the decrease in ATP levels, the enzymes involved in ATP synthesis were also inhibited, preventing restoration of ATP levels. In view of the failure to meet the increased cellular energy demand of the tissue, the cells are incapacitated, which may lead to failure of cell division as evidenced by Lockard *et al.* [14]. Furthermore, the hepatocytes may also become sensitive to cellular injury leading to excessive damage because of lack of ATP to govern the majority of cellular processes during CD + CCl_4 toxicity (Scheme 1).

The findings of marginal and reversible effects on ATP and Mg^{2+}-ATPase with mirex or PB pretreatments, which were used as negative and positive controls, respectively, for potentiation of CCl_4 toxicity, are also supportive of the concept that compromised energy status in CD pretreated rats is unique and leads to progressive liver injury. A mirex + CCl_4 combination, which does not show any potentiation [5, 7], or a PB + CCl_4 combination which shows marginal potentiation when compared to a CD + CCl_4 combination [6, 7], did not result in severely compromised hepatic energy status. Hepatic ATP levels were decreased only slightly (18–24%) at 24 hr after CCl_4 administration, and recovered to control levels by 36–48 hr. The PB + CCl_4 combination resulted in a greater decrease in ATP levels than mirex + CCl_4 in accord with the relative toxic effects [6, 7], and this fall in ATP was also recoverable. Furthermore, inhibition of Mg^{2+}-ATPase was observed with PB + CCl_4 treatment, whereas the mirex + CCl_4 treatment did not affect the enzyme activity. These observations are in agreement with the recovery of PB or mirex rats from the limited initial injury caused by CCl_4, when compared to CD rats, where the limited injury enters a progressive phase, culminating in death. The observed severely compromised energy status in CD but not in PB or

* Kodavanti PRS, Kodavanti UP and Mehendale HM, CCl_4-induced alterations of hepatic calmodulin and free Ca^{2+} levels in rats pretreated with chlordecone (Kepone®). Manuscript submitted for publication.

mirex pretreated rats given CCl_4 leads to chaotic derangements in cellular biochemistry. This results in progressive cell necrosis, and the progressive phase of injury is accelerated on the one hand by cell death (Scheme 1) and, on the other, by the absence of hepatocellular regeneration and a tissue repair mechanism.

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