TOXICOLOGY AND APPLIED PHARMACOLOGY 76, 437–453 (1984)

Dose–Response Relationships in Ketone-Induced Potentiation of Chloroform Hepato- and Nephrotoxicity

ESTHER M. BROWN* AND WILLIAM R. HEWITT*,†,[1]

*Department of Veterinary Biomedical Sciences, College of Veterinary Medicine, University of Missouri, Columbia, Missouri 65211, and †Department of Pharmacology, School of Medicine, University of Missouri, Columbia, Missouri 65212

Received January 9, 1984; accepted July 24, 1984

Dose–Response Relationships in Ketone-Induced Potentiation of Chloroform Hepato- and Nephrotoxicity. BROWN, E. M., AND HEWITT, W. R. (1984). *Toxicol. Appl. Pharmacol.* **76**, 437–453. Chloroform ($CHCl_3$)-induced hepato- and nephrotoxicity was evaluated in male, Fischer 344 rats pretreated with various dosages (1.0 to 15.0 mmol/kg, po) of acetone (Ac), 2-butanone (Bu), 2-pentanone (Pn), 2-hexanone (Hx), or 2-heptanone (Hp). The $CHCl_3$ challenge dosage (0.5 ml/kg, ip) produced slight centrilobular hydropic degeneration and patchy degeneration and necrosis in the proximal tubules of corn oil-pretreated rats. Each of the ketones studied produced a dose-related potentiation of $CHCl_3$ liver and kidney injury. $CHCl_3$ produced extensive tubular and centrilobular necrosis when administered to ketone-pretreated rats. The relationship between ketone dosage and the magnitude of the potentiated response was nonlinear. Maximum potentiation of $CHCl_3$ toxicity occurred in the dosage range of 5.0 to 10.0 mmol ketone/kg. Ketone dosages greater than 10.0 mmol/kg were associated with a reduction in the degree of $CHCl_3$ injury. At the lowest ketone dosage (1.0 mmol/kg), potentiating capacity appeared to be related to ketone carbon skeleton length. No differences in potentiating capacity were discernable between the ketones at dosages of 5.0 to 10.0 mmol/kg. Thus, whether or not there is a relationship with carbon chain length and potentiation depends upon the dosage of the ketone.

Relatively few studies have focused on structure–activity relationships which could provide a basis for identifying chemical classes capable of potentiating hepato- and nephrotoxic insults or predicting the relative potentiating capacity of a given chemical. However, recent reports have indicated that (1) ketonic solvents and ketogenic chemicals can potentiate the toxicity of a number of toxicants; and (2) the relative potentiating capacity of ketones may be influenced by carbon skeleton length. For example, five ketones (acetone (Ac), 2-butanone (Bu), 2-pentanone (Pn), 2-hexanone (Hx), 2-heptanone (Hp)) potentiated $CHCl_3$-induced hepatotoxicity in Sprague–Dawley rats when administered at a dosage of 15.0 mmol/kg (Hewitt *et al.*, 1983). In addition, a weak but significant correlation between carbon skeleton length and the severity of the liver injury produced was observed. Similarly, a significant linear correlation existed between the increase in ketone chain length and the magnitude of the nephrotoxic response to $CHCl_3$ in Sprague–Dawley rats (Hewitt *et al.*, 1982). Thus a structure–function relationship appeared to exist in which the length of the ketone carbon skeleton was a determinant of the relative potentiating capacity of the ketone.

However, a subsequent investigation (Hewitt and Brown, 1984) found no relationship

[1] Present address: Pathology/Toxicology Department, Smith Kline & French Laboratories, Philadelphia, Pa.

0041-008X/84 $3.00

between ketone chain length and potentiating ability when $CHCl_3$ was administered to ketone (15.0 mmol/kg)-pretreated Fischer 344 rats; with this strain, the ketones were approximately equivalent in potentiating ability. Furthermore, MacDonald *et al.* (1982) and Hewitt and Plaa (1983) demonstrated that the potentiation of 1,1,2-trichloroethane and 1,1-dichlorethylene hepatotoxicity by acetone followed a nonlinear dose–response curve. Low dosages of acetone potentiated the toxic effects of these halocarbons whereas higher acetone dosages were without effect. These observations suggest that the linear relationship between ketone chain length and potentiating capacity may have been fortuitous; simply reflecting a strain difference or the usage of a less than optimal ketone dosage. Therefore, dose–toxic response curves were generated for each of five ketones by utilizing a single challenge dose of $CHCl_3$ to determine if a relationship between ketone chain length and relative potentiating capacity exists. As in previous studies (Hewitt *et al.*, 1982, 1983; Hewitt and Brown, 1984), the series of five ketones examined ranged in chain length from three to seven carbons.

METHODS

Male, Fischer 344 rats (150 to 300 g) were purchased from Harlan Industries, Inc. (Indianapolis, Ind) and were maintained on Purina Lab Chow and water *ad libitum*. Animals were acclimatized for 7 to 10 days with a light/dark cycle of 12 hr each. Acetone, 2-butanone, 2-pentanone, 2-hexanone, and 2 heptanone were solubilized in corn oil and administered by gavage. Rats were given a single po dosage of a ketone (diluted to a final volume of 10 ml/kg) at 1, 5, 7.5, 10, 12.5, or 15 mmol/kg; control rats received 10 ml/kg of corn oil. Eighteen hours after pretreatment, rats were challenged with $CHCl_3$ (0.5 ml/kg, ip) diluted in corn oil (final volume of solution, 4 ml/kg). Control animals were treated with corn oil (4 ml/kg, ip) in place of $CHCl_3$. Twenty-four hours after $CHCl_3$ administration, the animals were lightly anesthetized with ether and blood was removed via cardiac puncture, with heparin as the anticoagulant. The liver and kidneys were rapidly excised, rinsed in ice-cold 0.9% NaCl, and weighed. Sections from the liver and one kidney were immediately removed for histological processing; the remainder of the renal cortical tissue was used for determination of organic ion transport capacity.

Ac (99+%), Bu (99+%), Pn (97%), Hx (99+%), Hp (98%), and $CHCl_3$ (99+%) were purchased from Aldrich Chemical Company, Inc. (Milwaukee, Wisc.) and used as supplied. [1-^{14}C]Tetraethylammonium bromide (sp act 4.5 mCi/mmol) and [^{14}C]toluene were purchased from New England Nuclear (Boston, Mass.). All other chemicals were obtained at the highest commercial purity and used as supplied.

Hepatic and renal slices (3 mm) were fixed in a mercury–Formol solution for 3 days and then washed in running water for 12 hr. The tissues were trimmed, dehydrated in tetrahydrofuran, infiltrated with paraffin under partial vacuum, and embedded in paraffin. All sections were cut at 5 μm. One set of sections was stained with hematoxylin and eosin whereas a second set was treated with periodic acid–Schiff's reagent (PAS) to better visualize the basement membrane and the brush border of the renal tubular epithelium.

TABLE 1

DOSE–RESPONSE RELATIONSHIPS IN KETONE-INDUCED POTENTIATION OF $CHCl_3$ TOXICITY: 24-hr MORTALITY[a]

Ketone dosage (mmol/kg)	$CHCl_3$ challenge (0.5 ml/kg)	24-hr mortality (No. of dead/No. of treated)				
		Acetone	2-Butanone	2-Pentanone	2-Hexanone	2-Heptanone
1.0	Yes	0/6	0/6	0/6	0/6	0/8
5.0	Yes	0/6	0/6	0/6	0/6	2/8
7.5	Yes	0/6	0/6	0/6	0/6	4/8
10.0	Yes	0/6	0/6	0/6	0/6	7/9
12.5	Yes	0/6	0/6	1/6	2/6	2/8
15.0	Yes	0/6	0/6	0/6	1/6	0/8
15.0	No	0/6	0/6	0/6	0/6	0/7

[a] Male, F344 rats received (ip) the challenge dose of $CHCl_3$ or vehicle 18 hr following a single po dose of one of the ketones at a dosage ranging from 1.0 to 15.0 mmol/kg. Mortality was monitored during the 24-hr period following $CHCl_3$ administration. No deaths occurred in 32 control rats or in 32 rats treated with vehicle + $CHCl_3$.

TABLE 2

DOSE-RESPONSE RELATIONSHIPS IN KETONE-INDUCED POTENTIATION OF $CHCl_3$ NEPHROTOXICITY: PAH S/M RATIO[a]

Ketone dosage (mmol/kg)	$CHCl_3$ challenge (0.5 ml/kg)	PAH S/M ratio				
		Acetone	2-Butanone	2-Pentanone	2-Hexanone	2-Heptanone
1.0	Yes	19.16 ± 2.09 (6)	10.43 ± 2.49 (6)	13.37 ± 2.18 (6)	11.03 ± 3.52 (6)	18.68 ± 2.01 (8)
5.0	Yes	6.17 ± 3.30[b] (6)	2.85 ± 0.55[b] (6)	5.02 ± 0.64[b] (6)	6.99 ± 1.24[b] (6)	4.74 ± 1.08[b] (6)
7.5	Yes	7.10 ± 2.45[b] (6)	2.45 ± 0.31[b] (6)	5.98 ± 0.40[b] (6)	5.47 ± 1.38[b] (6)	3.96 ± 1.51[b] (4)
10.0	Yes	5.63 ± 1.98[b] (6)	4.01 ± 1.02[b] (6)	5.40 ± 1.28[b] (6)	5.53 ± 1.52[b] (6)	4.89 ± 2.55 (2)
12.5	Yes	11.38 ± 3.05 (6)	4.70 ± 1.67[b] (6)	14.61 ± 3.3 (6)	11.00 ± 2.80 (4)	12.94 ± 2.23 (6)
15.0	Yes	8.40 ± 2.37[b] (6)	6.08 ± 2.30[b] (6)	15.33 ± 4.60 (6)	18.18 ± 4.34 (5)	18.35 ± 5.77 (8)
15.0	No	26.24 ± 0.96 (6)	26.08 ± 0.81 (6)	29.62 ± 1.37 (6)	25.79 ± 1.92 (5)	29.84 ± 1.57 (7)

[a] Refer to Table 1 for a description of the treatment regimen. The mean slice PAH S/M ratio was 26.90 ± 0.81 (25.25 to 28.55) in 32 control rats and 17.83 ± 1.24 (15.30 to 20.36) in 32 rats treated with vehicle + $CHCl_3$. Numbers in parentheses indicate the number of animals treated.

[b] Significantly different than the group receiving vehicle + $CHCl_3$ ($p < 0.05$).

TABLE 3

DOSE-RESPONSE RELATIONSHIPS IN KETONE-INDUCED POTENTIATION OF $CHCl_3$ NEPHROTOXICITY: PLASMA CREATININE CONTENT[a]

Ketone dosage (mmol/kg)	$CHCl_3$ challenge (0.5 ml/kg)	Plasma creatinine (mg/100 ml)				
		Acetone	2-Butanone	2-Pentanone	2-Hexanone	2-Heptanone
1.0	Yes	0.58 ± 0.11 (6)	1.37 ± 0.29 (6)	0.91 ± 0.18 (6)	1.51 ± 0.36 (6)	1.01 ± 0.19 (8)
5.0	Yes	1.59 ± 0.22[b] (6)	1.87 ± 0.23[b] (6)	2.12 ± 0.20[b] (6)	1.80 ± 0.24[b] (6)	1.85 ± 0.27[b] (6)
7.5	Yes	1.38 ± 0.29 (6)	2.03 ± 0.13[b] (6)	1.79 ± 0.12[b] (6)	1.86 ± 0.16[b] (6)	2.02 ± 0.42 (4)
10.0	Yes	1.66 ± 0.27[b] (6)	1.94 ± 0.24[b] (6)	2.55 ± 0.40[b] (6)	2.07 ± 0.11[b] (6)	1.81 ± 0.66 (2)
12.5	Yes	1.32 ± 0.24 (6)	2.04 ± 0.16[b] (6)	1.43 ± 0.37 (5)	1.91 ± 0.24 (4)	1.86 ± 0.17[b] (6)
15.0	Yes	1.49 ± 0.18[b] (6)	2.11 ± 0.29[b] (6)	1.49 ± 0.52 (6)	1.70 ± 0.32 (5)	1.59 ± 0.32 (8)
15.0	No	0.44 ± 0.06 (6)	0.35 ± 0.05 (6)	0.32 ± 0.06 (6)	0.27 ± 0.07 (6)	0.48 ± 0.04 (7)

[a] Refer to Table 1 for a description of the treatment regimen. The mean PCr was 0.39 ± 0.03 (0.34 to 0.44) in 31 control rats and 0.79 ± 0.08 (0.62 to 0.96) in 32 rats treated with vehicle + $CHCl_3$. Numbers in parentheses indicate the number of animals treated.

[b] Significantly different than the group receiving vehicle + $CHCl_3$ ($p < 0.05$).

All histologic sections were read without prior knowledge of the functional data to minimize any bias in judging the type and severity of the lesions. The types of cellular changes, e.g., degeneration, necrosis, and the area affected, were recorded for all hepatic and renal sections from all surviving rats. Attempts to evaluate kidney necrosis as reported by others (Hewitt *et al.*, 1979, 1980; McMurtry and Mitchell, 1977; Mitchell *et al.*, 1973; Soderlund *et al.*, 1980) did not prove to be beneficial because the necrotic tubules (S_1 and S_2) were present throughout the renal cortex without any gradual progression from focal necrotic areas to total involvement within the dosage range examined.

The various cellular changes in the liver were recorded according to their juxtaposition to the central vein. An evaluation of the amount of hepatic tissue involved in the lesion was performed on hepatic sections from rats challenged with $CHCl_3$ following pretreatment with each of the five ketones at dosages of 1, 10, and 15 mmol/kg. The liver lesions were measured at 100× with a calibrated ocular reticle. Ten microscopic fields containing a central vein were selected, and the abnormal tissue extending peripherally from the margin of the central vein was measured. Care was taken to select only central veins cut in cross section to minimize error. The widths of the lesions (in μm) for the 10 fields per section were averaged to yield a representative value for each animal.

Plasma glutamic-pyruvic transaminase (GPT) and blood urea nitrogen (BUN) were determined as previously described (Hewitt *et al.*, 1980). Plasma creatinine (PCr) was determined by the method of Lustgarten and Wenk (1972). Uptake of *p*-aminohippurate (PAH) and tetraethylammonium (TEA) ions by renal cortical slices (Cross and Taggart, 1950) was assessed in the presence of sodium lactate (10 mM) as previously described (Hewitt *et al.*, 1980). Accumulation of PAH and TEA was expressed as the slice-to-medium (S/M) ratio, where S = milligrams (dpm) of PAH (TEA) per gram wet weight of tissue and M = milligrams (dpm) of PAH (TEA) per milliliter of incubation medium.

Data were expressed as $\bar{x} \pm$ SE. The 95% confidence limits were calculated for the mean of each treatment group. Treatment means were considered to be significantly different if no overlap in the 95% CL occurred.

FIG. 1. These figures illustrate the effect of corn oil (CO) alone and the variable effects of corn oil and chloroform, and the lesions produced by a ketone alone. (a) Kidney (CO only). Normal proximal tubules (A) and one distal tubule (B) in the cortex from rat given only CO. PAS (×624). (b) Kidney (CO + $CHCl_3$). Normal uriniferous tubules. The microvilli of proximal tubules stain dark because they are PAS positive. PAS (×192). (c) Kidney (CO + $CHCl_3$). Epithelia of the proximal tubules display degeneration with loss of the apical portion of the cells (A). Nuclei are viable. A few hyaline bodies (arrows) are visible. PAS (×624). (d) Kidney (CO + $CHCl_3$). An example of necrotic proximal tubular epithelia (B) and hyaline bodies (arrows) in a tubule with epithelial degeneration (A). PAS (×624). (e) Kidney (Ac + CO). Proximal tubular epithelia with apical degeneration (A) produced by Ac alone. PAS (×624). (f) Kidney (Hx + CO). Severe epithelial degeneration in proximal tubules (A) with hyaline bodies (arrows) produced by Hx alone. PAS (×624).

FIG. 2. (a) Kidney (Ac 1.0 mmol/kg + $CHCl_3$). Ac pretreatment produced degeneration in epithelium of the proximal tubules (A) and early necrosis (B) in others. PAS (×192). (b) Kidney (Bu 1.0 mmol/kg + $CHCl_3$). Bu pretreatment caused extensive epithelial necrosis in the proximal tubules (B) and some casts (C). PAS (×192). (c) Kidney (Pn 1.0 mmol/kg + $CHCl_3$). Necrotic proximal tubules (B) and casts (C) make up most of the cortex. PAS (×192). (d) Kidney (Hx 1.0 mmol/kg + $CHCl_3$). Hx pretreatment produced severe proximal tubular necrosis (B) and casts (C). PAS (×192). (e) Kidney (Hp 1.0 mmol/kg + $CHCl_3$). Widespread tubular necrosis (B) and casts (C) produced by Hp pretreatment. PAS (×192). (f) Kidney (Hp 1.0 mmol/kg + $CHCl_3$). High power view of area marked in e. The epithelium of the proximal tubule is gone leaving only the intact basement membrane (arrow heads). Macula densa (arrow) in normal distal tubule. PAS (×164).

FIG. 3. (a) Kidney (Hx 1.0 mmol/kg + $CHCl_3$). Necrotic proximal tubular epithelia (B) and hyaline casts (C) are typical lesions. Notice the two proximal tubules (A) with slight apical epithelial degeneration. PAS (×192). (b) Kidney (Hx 1.0 mmol/kg + $CHCl_3$). High power view illustrating the denuded tubules with intact basement membranes (arrow heads), hyaline bodies (arrows) and casts (C). PAS (×624). (c) Kidney (Hx 5.0 mmol/kg + $CHCl_3$). Proximal tubular epithelial necrosis (B) is widespread and of such severity that only basement membranes remain (arrow heads). PAS (×192). (d) Kidney (Hx 10.0 mmol/kg + $CHCl_3$). Severe proximal tubular necrosis (B) with intact denuded basement membranes (arrow heads). PAS (×192). (e) Kidney (Hx 15.0 mmol/kg + $CHCl_3$). Necrotic proximal tubules (B) comprise most of the cortex. PAS (×192). (f) Kidney (Hx 15.0 mmol/kg + $CHCl_3$). High power view of area marked in e. The basement membranes (arrow heads) remain intact in the necrotic tubules. Notice the cellular debris in the lumen. PAS (×624).

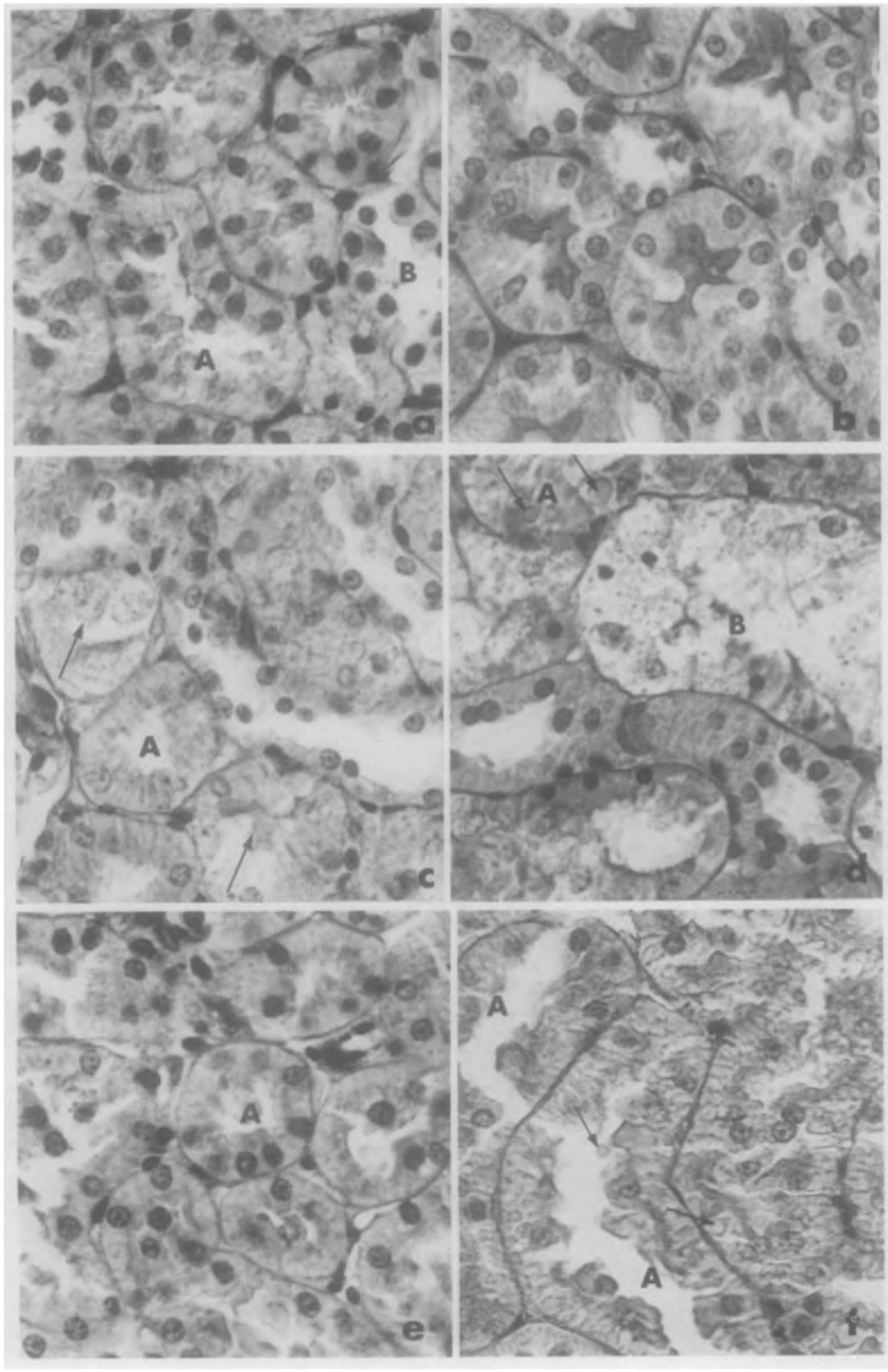

FIGURE 1

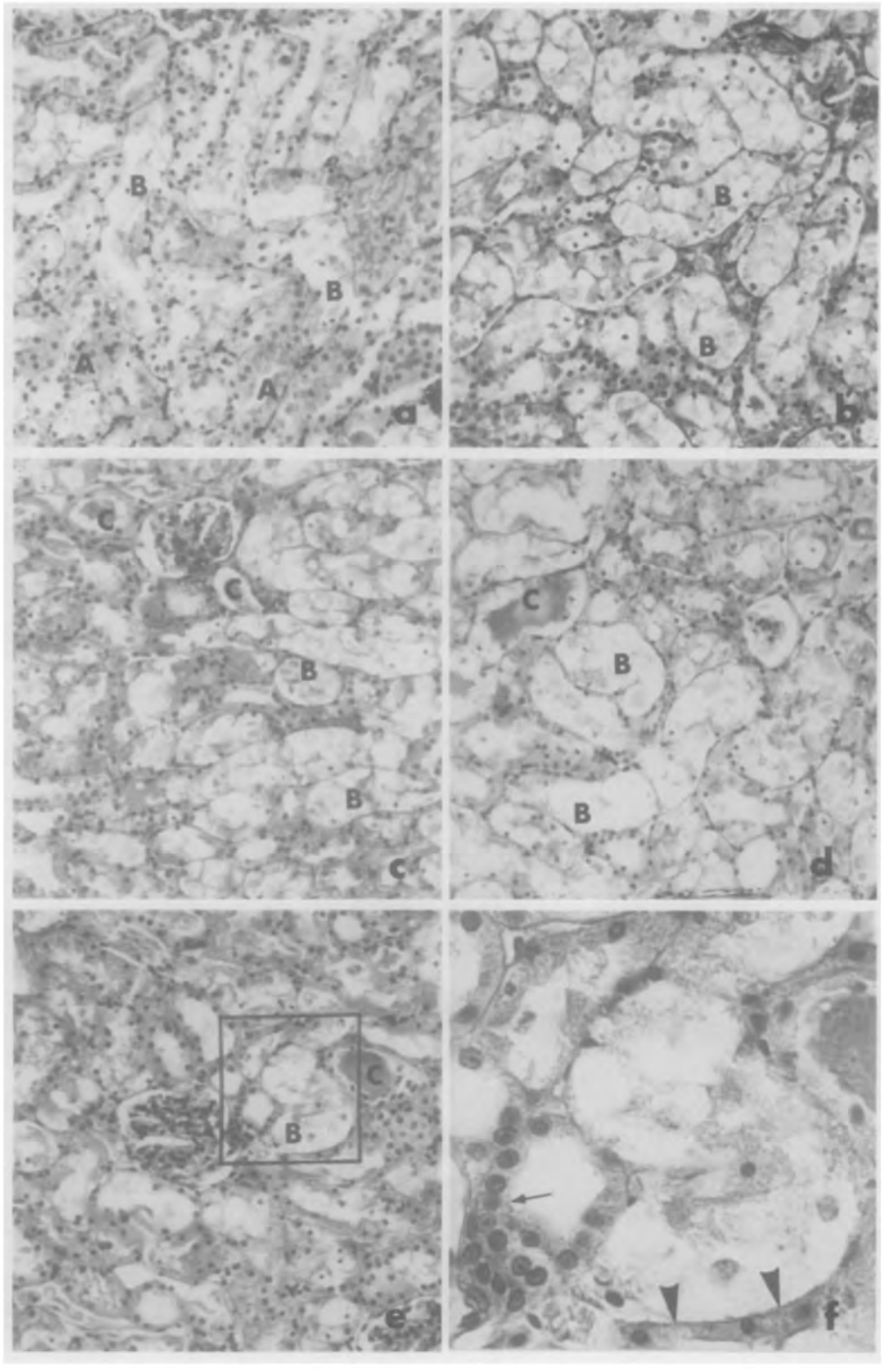

FIGURE 2

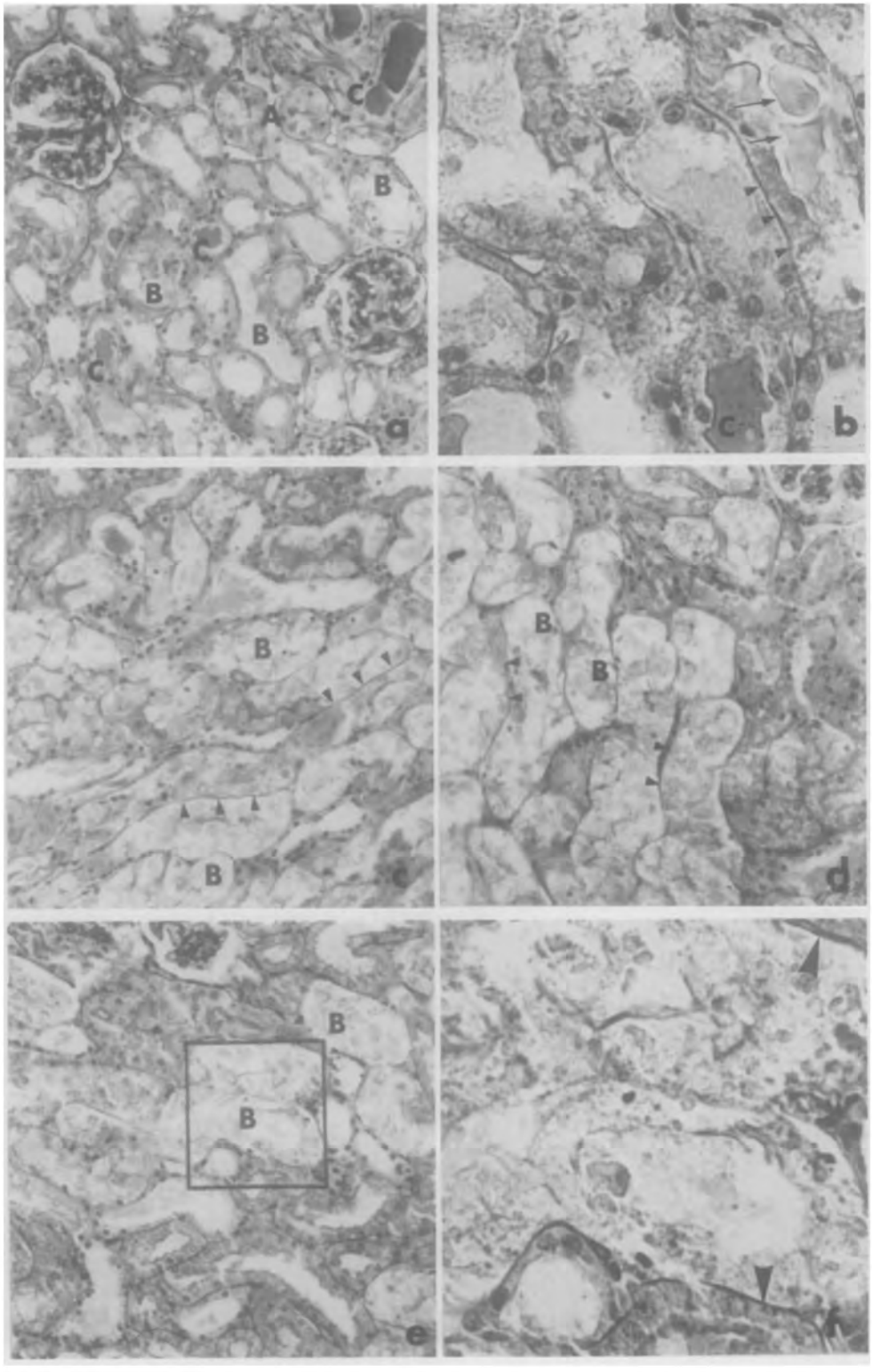

FIGURE 3

RESULTS

Deaths that occurred at unscheduled times were observed in rats receiving the $CHCl_3$ challenge dosage following pretreatment with Pn (12.5 mmol/kg), Hx (12.5 and 15.0 mmol/kg), or Hp (5.0 to 12.5 mmol/kg) (Table 1). $CHCl_3$-induced mortality increased with the Hp dosage administered up to a peak at 10 mmol/kg (78% mortality); larger Hp dosages were associated with reduced mortality rates. Other treatment combinations did not result in deaths that occurred at unscheduled times (Table 1).

Treatment of rats with a single dosage (15 mmol/kg) of Ac, Bu, Pn, Hx, or Hp alone did not alter renal cortical slice PAH accumulation (Table 2) or plasma creatinine content (Table 3). The $CHCl_3$ challenge dosage produced a moderate degree of functional impairment in vehicle-pretreated rats as indicated by a 34% depression in slice PAH accumulation (Table 2) and a twofold increase in PCr content (Table 3).

Each of five ketones examined potentiated $CHCl_3$-induced nephrotoxicity. However, the severity of the dysfunction produced was not linearly related to ketone dosage (Tables 2 and 3). Regardless of the ketone chosen, maximum potentiation of $CHCl_3$ kidney injury was achieved at the 5.0 to 10.0 mmol/kg dosage range. For example, slice PAH accumulation was reduced 69% and PCr elevated 2.6-fold in rats receiving Hx (10.0 mmol/kg) + $CHCl_3$ as compared to rats treated with vehicle + $CHCl_3$. In contrast, Hx dosages of 12.5 and 15.0 mmol/kg did not significantly alter $CHCl_3$-related changes in PAH uptake or PCr content. Only Ac and Bu significantly increased $CHCl_3$ renal injury when given at 15.0 mmol/kg. None of the ketones altered the severity of $CHCl_3$ kidney dysfunction when administered at 1.0 mmol/kg. Similar results were obtained when BUN content and slice TEA accumulation were used as indices of nephrotoxicity (data not included).

Renal histologic alterations were confined to the S_1 and S_2 portions of the proximal tubule; no involvement of S_3 cells or the renal corpuscles was detected (Figs. 1 to 3). Kidney sections from 32 control (vehicle + vehicle) rats were normal (Fig. 1a). The $CHCl_3$ challenge dosage produced no changes in 9/32 (29%), tubular degeneration in 9/32 (29%), and isolated areas of tubular necrosis in 13/32 (41%) of vehicle-pretreated rats, respectively (Figs. 1b–d). Degenerative changes were observed in S_1 and S_2 segments of several rats that received a ketone (15.0 mmol/kg) + vehicle. Although these lesions were isolated and did not involve all proximal tubules, there was a progression in the severity of tubular degeneration related to the length of the ketone carbon skeleton. Tubular degeneration in Hx-pretreated rats (Fig. 1f) was so severe that the basal portion of the cell containing the nucleus was all that remained, while in kidneys from rats receiving Ac alone (Fig. 1e) only the apical microvilli were degenerated.

Kidneys from rats treated with a ketone + $CHCl_3$ had tubular necrosis of varying severity, numerous hyaline bodies, and tubular casts (Figs. 2 and 3). A general trend existed in that the severity and/or incidence of the renal lesions in rats treated with a ketone + $CHCl_3$ increased with the dosage of ketone administered, up to and including the 10.0 mmol/kg dosage, and subsequently decreased (Table 4). At the 1.0 mmol/kg dosage, Ac-pretreated rats had fewer severe tubular changes and fewer tubules affected (Fig. 2a). Conversely, kidneys from Hx- and Hp-pretreated rats had the most severe tubular lesions and the greatest tubular mass involved (Figs. 2e and f). Renal lesions in Bu- and Pn-pretreated rats were of intermediate severity; sections from the Pn-pretreated group (Fig. 2b) appeared to be more severely affected than those taken from the Bu-pretreated group (Fig. 2c). Thus, at this dosage (1.0 mmol/kg) ketone potentiating ability increased with the length of the carbon skel-

TABLE 4

ANIMALS WITH RENAL NECROSIS[a]

Ketone dosage (mmol/kg)	$CHCl_3$ challenge (0.5 mg/kg)	No. with renal necrosis/No. of survivors				
		Acetone	2-Butanone	2-Pentanone	2-Hexanone	2-Heptanone
1.0	Yes	3/6	6/6	6/6	5/6	5/8
5.0	Yes	4/6	6/6	6/6	6/6	6/6
7.5	Yes	4/6	6/6	6/6	6/6	4/4
10.0	Yes	5/6	6/6	6/6	6/6	2/2
12.5	Yes	4/6	6/6	4/5	4/4	6/6
15.0	Yes	5/6	6/6	3/6	4/5	5/8

[a] Refer to Table 1 for a description of the treatment regimen.

eton up to and including the 6-carbon ketone, Hx. It was difficult to discriminate between the severity of $CHCl_3$-induced kidney injury in the Hx- and Hp-pretreated rats (Figs. 2d–f).

A somewhat similar relationship between carbon chain length and potentiating capacity was observed at the 10.0 mmol/kg dosage. Rats receiving Ac + $CHCl_3$ had the least severe renal lesions, whereas the greatest degree of injury appeared to occur in rats treated with Hx + $CHCl_3$. Sections from the latter group exhibited complete loss of tubular epithelium, large hyaline bodies, and numerous granular casts (Fig. 3). Renal lesions in the group treated with Hp + $CHCl_3$ were also extremely severe; the high mortality rate in this group rendered any comparisons of kidney injury between the Hx- and Hp-pretreated groups difficult. Similarly, no attempt was made to rank the kidney lesions in Bu- or Pn-pretreated rats at this dosage due to the marked severity and extent of the S_1 and S_2 lesions.

Pretreatment of rats with Ac, Bu, Pn, Hx, or Hp resulted in an appreciable potentiation of $CHCl_3$-induced hepatotoxicity (Tables 5 and 6). None of the ketones produced a marked degree of hepatic dysfunction when administered alone at a dosage of 15.0 mmol/kg; the $CHCl_3$ challenge produced a small but significant increase in plasma GPT activity when administered to rats pretreated with Ac, Bu, Pn, or Hx in the dosage range of 5.0 to 15.0 mmol/kg (Table 5). Within this range there was no apparent relationship between the magnitude of the elevation in plasma GPT activity and either ketone dosage or ketone carbon chain length. Although the combination of 5.0 mmol/kg HP + $CHCl_3$ resulted in a significant increase in GPT activity, larger dosages of this ketone did not significantly exacerbate the $CHCl_3$-induced elevation in GPT activity. This discrepancy probably reflects the high mortality rate observed when larger dosages of Hp preceded $CHCl_3$ administration (Table 1). None of the ketones potentiated the $CHCl_3$-medicated GPT increase when administered at 1.0 mmol/kg (Table 5).

The major hepatic lesions were hydropic degeneration (balloon cells) and necrosis. Hydropic degeneration was differentiated from fatty degeneration in a separate experimental series with Oil Red O. Necrosis and/or hydropic degeneration were centrilobular, usually involving the cells in zones 3 and 2; however, some lesions extended into zone 1 as well. In those sections in which hydropic degeneration was the only change, it was confined to the innermost portion of zone 3, sometimes involving only the first one or two cells around the central vein. Whenever necrotic changes were seen, these cells were

TABLE 5

DOSE–RESPONSE RELATIONSHIPS IN KETONE-INDUCED POTENTIATION OF $CHCl_3$ HEPATOTOXICITY: PLASMA GPT ACTIVITY[a]

Ketone dosage (mmol/kg)	$CHCl_3$ challenge (0.5 ml/kg)	GPT activity (units/ml)				
		Acetone	2-Butanone	2-Pentanone	2-Hexanone	2-Heptanone
1.0	Yes	218 ± 46 (6)	1683 ± 514 (6)	956 ± 415 (6)	1198 ± 343 (6)	879 ± 400 (8)
5.0	Yes	4055 ± 1317[b] (6)	7033 ± 1086[b] (6)	6528 ± 1314[b] (6)	6434 ± 779[b] (6)	4488 ± 712[b] (6)
7.5	Yes	3860 ± 1293[b] (6)	5785 ± 819[b] (6)	5717 ± 722[b] (6)	8619 ± 540[b] (6)	4984 ± 2149 (4)
10.0	Yes	4799 ± 1261[b] (6)	6827 ± 1148[b] (6)	4492 ± 1445[b] (6)	7835 ± 509[b] (6)	2608 ± 2412 (2)
12.5	Yes	4814 ± 1063[b] (6)	5382 ± 1131[b] (6)	5142 ± 843[b] (5)	7778 ± 829[b] (4)	1543 ± 838 (6)
15.0	Yes	4624 ± 430[b] (6)	3773 ± 1315[b] (6)	4532 ± 2062 (6)	7739 ± 2242[b] (5)	2106 ± 850 (8)
15.0	No	27 ± 4 (6)	36 ± 11 (6)	26 ± 2 (6)	33 ± 6 (6)	29 ± 3 (7)

[a] Refer to Table 1 for a description of the treatment regimen. The mean SGPT was 30 ± 4 (22 to 38) in 31 control rats 265 ± 55 (153 to 377) in 31 rats treated with vehicle + $CHCl_3$. Numbers in parentheses indicate the number of animals treated.

[b] Significantly different than the group receiving vehicle + $CHCl_3$ ($p < 0.05$).

either dispersed among the balloon cells or large necrotic areas were circumscribed by balloon cells. Occasional evidence of glycogen depletion was observed but there was no apparent ketone or dosage relationship.

Liver sections taken from 32/32 rats receiving vehicle + vehicle were normal, as were the majority of sections prepared from rats receiving a ketone (15.0 mmol/kg) plus a vehicle challenge; mild hydropic degeneration was observed in 2/31 and glycogen depletion in 3/31 of the rats in this group. Normal livers were found in 19/32 rats (59%) treated with vehicle + $CHCl_3$; 13/32 (41%) exhibited moderate liver injury characterized by hydropic degeneration without necrosis (Fig. 4a).

No relationships between ketone carbon skeleton length and the incidence for severity of $CHCl_3$ liver injury were apparent at the 1.0 mmol/kg dosage. Treatment with Ac and $CHCl_3$ produced liver necrosis in 2/6 rats. The infrequent necrotic cells observed were interspersed with balloon cells and leukocytes (Fig. 4b); livers from 2 other similarly treated rats had only balloon cells. In contrast, hydropic degeneration and necrosis were found in all liver sections taken from rats (12) pretreated with 1.0 mmol/kg of Bu or Hx and subsequently given $CHCl_3$ (Figs. 4c and e). Moreover, the hepatic lesions in these animals involved the greatest mass of liver tissue (Table 6). Appreciable necrosis was observed in only 1/6 and 1/8 of the $CHCl_3$ challenged rats pretreated with Pn or Hp, respectively (Figs. 4d and f); however, livers from five rats in each of these groups had severe hydropic degeneration with occasional necrotic cells scattered among the balloon cells. The liver lesions produced in the Pn- and Hp-pretreated rats involved a smaller area than that observed in livers from the Bu- and Hx-pretreated groups (Table 6).

It was difficult to discriminate between the ketones when larger dosages were utilized for pretreatment. As a group, approximately 95% of the surviving animals pretreated with any of the ketones at a dosage of 5.0, 7.5, 10.0,

TABLE 6

LIVER LESION MEASUREMENTS (μm)[a]

Ketone dosage (mmol/kg)	$CHCl_3$ challenge (0.5 mg/kg)	Acetone	2-Butanone	2-Pentanone	2-Hexanone	2-Heptanone
1.0	Yes	24 ± 12 (6)	91 ± 29 (6)	54 ± 14 (6)	90 ± 24 (6)	18 ± 10 (8)
10.0	Yes	106 ± 22 (6)	173 ± 20 (6)	169 ± 40 (6)	440 ± 124 (6)	88 ± 76 (2)
15.0	Yes	156 ± 10 (6)	165 ± 32 (6)	96 ± 42 (6)	296 ± 50 (5)	129 ± 31 (8)

[a] Refer to Table 1 for a description of the treatment regimen. Data expressed as $\bar{x}$ ± SE determined in (n) rats.

or 12.5 mmol/kg exhibited hepatic necrosis, whereas only 71% of those treated with the 15.0 mmol/kg ketone dosage had necrotic lesions. A similar pattern was observed when the severity of the hepatic lesions was examined in that the width of the lesion seemed to plateau at the 10.0 mmol/kg dosage (Table 6, Fig. 5). The necrotic area in livers from rats pretreated with Hx included 2.5- to 3-fold more hepatic tissue than that from the Ac, Bu, Pn, or Hp groups. Because of the extensive mortality in the Hp-pretreated group, it was difficult to evaluate the severity of the liver damage compared to that observed in other groups at the 10.0 mmol/kg dosage. Increasing the ketone dosage to 15.0 mmol/kg did not appreciably alter the width of the $CHCl_3$ hepatic lesion in rats pretreated with Ac, Bu, or Pn; Hx increased the area of the $CHCl_3$ lesion to a lesser extent at 15.0 than at 10.0 mmol/kg although the severity of the damage within the injured area was comparable at the two Hx dosages (Figs. 5c and d).

The severity of the lesions produced by the interaction of a ketone with $CHCl_3$ was striking. For example, Hx at the 10.0 and Hp at the 15.0 mmol/kg dosages produced massive potentiation of $CHCl_3$-induced necrosis in which all cell boundaries were obliterated and only pycnotic nuclei remained (Fig. 6). These lesions included most of the liver lobule, and frequently the only viable hepatic cells remaining were those surrounding the portal areas (Figs. 5c and 6c). In some sections the central vein was obscured due to disruption of the endothelial lining (Figs. 6a and b).

DISCUSSION

The predominant conclusions that can be drawn from this investigation are (1) each of the ketones studied produced a dose-related potentiation of $CHCl_3$-induced renal and hepatic injury; (2) the relationship between ketone dosage and the magnitude of the potentiated response was nonlinear; and (3) the length of the ketone carbon skeleton did not significantly influence the relative potentiating capacity of the ketone.

The nonlinear nature of ketone dose–response curve was not surprising. Previous studies demonstrated that low dosages of acetone (3.0 to 7.0 mmol/kg) produced maximum potentiation of 1,1,2-trichloroethane hepatotoxicity. Greater dosages (13.0 to 20.4 mmol/kg) protected against 1,1,2-trichloroethane toxicity (MacDonald *et al.*, 1982). Similarly, acetone increased 1,1-dichloroethylene toxicity when utilized at 5.0 or 10.0 mmol/kg; higher dosages of acetone (15.0 and 30.0 mmol/kg) did not potentiate 1,1-dichloroethylene toxicity (Hewitt and Plaa, 1983). The present investigation extends these observations to the ketone-induced potentiation of $CHCl_3$ renal and hepatic injury. Maximum potentiation of $CHCl_3$ toxicity occurred at ketone dosages of 5.0 to 10.0 mmol/kg. Ketone dosages greater than 10.0 mmol/kg were associated with a reduction in the degree of potentiation.

Several investigators demonstrated that Ac, Hx, 2,5-hexanedione, or *n*-hexane exacerbated $CHCl_3$ (0.5 ml/kg)-induced toxicity in Sprague–Dawley rats (Hewitt *et al.*, 1980; Branchflower and Pohl, 1981). A subsequent

study, however, found no potentiation of $CHCl_3$ renal injury by Pn, Hx, or Hp until the $CHCl_3$ dosage was increased to 0.75 ml/kg. At this $CHCl_3$ dosage, Ac and Bu did not significantly alter $CHCl_3$ renal injury (Hewitt *et al.*, 1982). The results from this and other studies (MacDonald *et al.*, 1982; Hewitt and Plaa, 1983) suggested that the failure to observe a significant interaction between $CHCl_3$ and Ac or Bu was probably due to the selection of a less than optimum ketone dosage.

The mechanism(s) responsible for the nonlinear nature of the ketone dose–response curves is unknown. Hx potentiates $CHCl_3$-induced liver injury by increasing $CHCl_3$ bioactivation to a reactive intermediate (Branchflower and Pohl, 1981: Cowlen *et al.*, 1984). Ketonic solvents are metabolized by cytoplasmic as well as microsomal enzymes (Dietz *et al.*, 1981; Spencer *et al.*, 1980; Couri and Milks, 1982). At high dosages, ketones may reduce $CHCl_3$ biotransformation thereby reducing $CHCl_3$ toxicity. Alternatively, high dosages of these ketones may injure the cell in such a manner as to reduce $CHCl_3$ metabolism and toxicity. Support for this hypothesis can be drawn from the present study in which degenerative changes were observed in kidneys of rats treated with 15.0 mmol/kg of a ketone alone (Figs. 1e and f).

The relationship between ketone carbon skeleton length and potentiating capacity observed in previous studies (Hewitt *et al.*, 1980, 1982, 1983) was not observed in the current investigation. Although, $CHCl_3$-in-

FIG. 4. (a) Liver (CO + $CHCl_3$). Centrilobular hepatic hydropic degeneration (balloon cells) (A) some of which have pycnotic nuclei (arrows), surrounding the central vein (CV). Normal hepatocytes (C) are at outer margin of the lesion. PAS (×192). (b) Liver (Ac 1.0 mmol/kg + $CHCl_3$). Balloon cells (A), a few necrotic cells (B), and leukocytic infiltration located near the central vein (CV). Normal hepatocytes (C) surround the lesion. PAS (×192). (c) Liver (Bu 1.0 mmol/kg + $CHCl_3$). The central vein (CV) is surrounded by necrotic cells (B), and balloon cells (A) marginate the lesion adjacent to normal hepatocytes (C). PAS (×192). (d) Liver (Pn 1.0 mmol/kg + $CHCl_3$). A mixture of balloon (A) and necrotic (B) cells surrounding the central vein (CV). Normal hepatocytes (C) are adjacent to the balloon cells. Many balloon cells have pycnotic nuclei. PAS (×192). (e) Liver (Hx 1.0 mmol/kg + $CHCl_3$). Most of the hepatocytes in zones 3 and 2 are necrotic (B) with balloon cells scattered throughout the lesion. The lesion involves zones 3 and 2 and part of zone 1. The portal vein (PV) is at the top left and the central vein (CV) is at the lower right. PAS (×192). (f) Liver (Hp 1.0 mmol/kg + $CHCl_3$). The lesion consists primarily of balloon cells (A), clusters of necrotic cells (B), and leukocytes surrounding the central vein (CV). Normal hepatocytes (C) are adjacent to the lesion. PAS (×192).

FIG. 5. (a) Liver (Hx 1.0 mmol/kg + $CHCl_3$). Balloon (A) and necrotic (B) cells surround the central vein (CV). Some balloon cells have pycnotic nuclei (arrows). Normal hepatocytes (C) are visible at the upper and lower margins of the photograph. PAS (×192). (b) Liver (Hx 5.0 mmol/kg + $CHCl_3$). The necrotic tissue mass (B) extends well into zone 2 of the liver lobule with balloon cells (A) at the margin of the lesion. Normal hepatocytes (C) are in the upper right and lower left corners. PAS (×192). (c) Liver (Hx 10.0 mmol/kg + $CHCl_3$). The necrotic mass (B) extends throughout the hepatic lobule. The only normal (C) hepatocytes visible are those surrounding the portal vein (PV) and bile duct (bd) of the portal area. The central vein is completely obscured. PAS (×192). (d) Liver (Hx 15 mmol/kg + $CHCl_3$). Necrotic cells (B) surround the central vein (CV) with balloon cells (A) at the margin of the necrotic mass. Normal hepatocytes (C) are visible in the top one third of the picture. PAS (×192).

FIG. 6. (a) Liver (Hx 10 mmol/kg + $CHCl_3$). The necrotic tissue (B) extends throughout the liver lobule. A few scattered normal hepatocytes are seen around the bile duct (bd) of a portal area. PAS (×192). (b) Liver (Hx 10 mmol/kg + $CHCl_3$). A high power view of the area marked in Fig. 6a. The endothelial lining of the central vein (CV) is disrupted and the necrotic mass is infiltrated with leukocytes. PAS (×624). (c) Liver (Hp 15 mmol/kg + $CHCl_3$). The necrotic tissue extends throughout the liver lobule. Notice the leukocytic infiltration. PAS (×192). (d) Liver (Hp 15 mmol/kg + $CHCl_3$). A high power view of the area marked in c. illustrating a disruption of the endothelial lining of the central vein (CV) and the necrotic hepatocytes. PAS (×624).

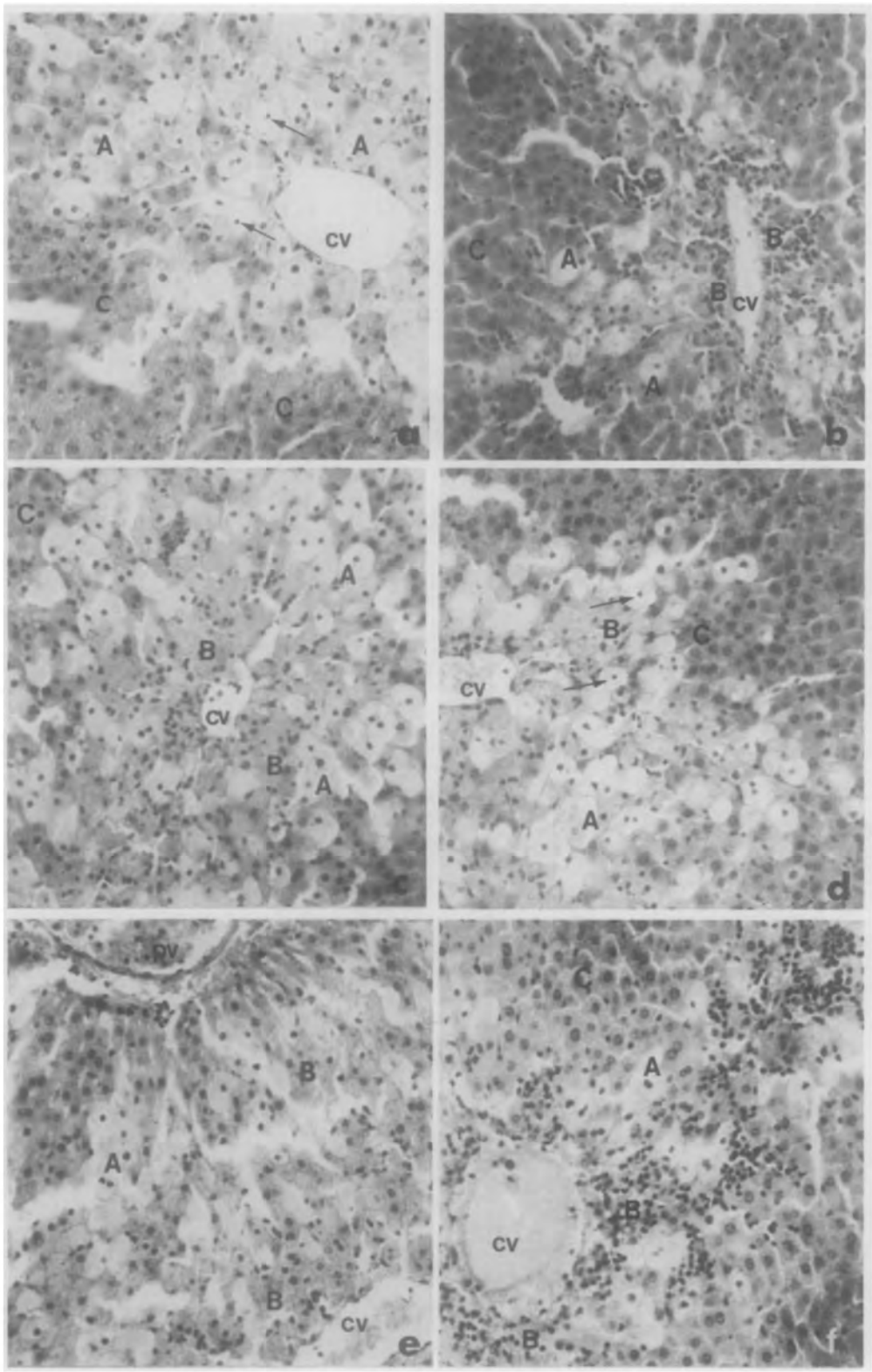

FIGURE 4

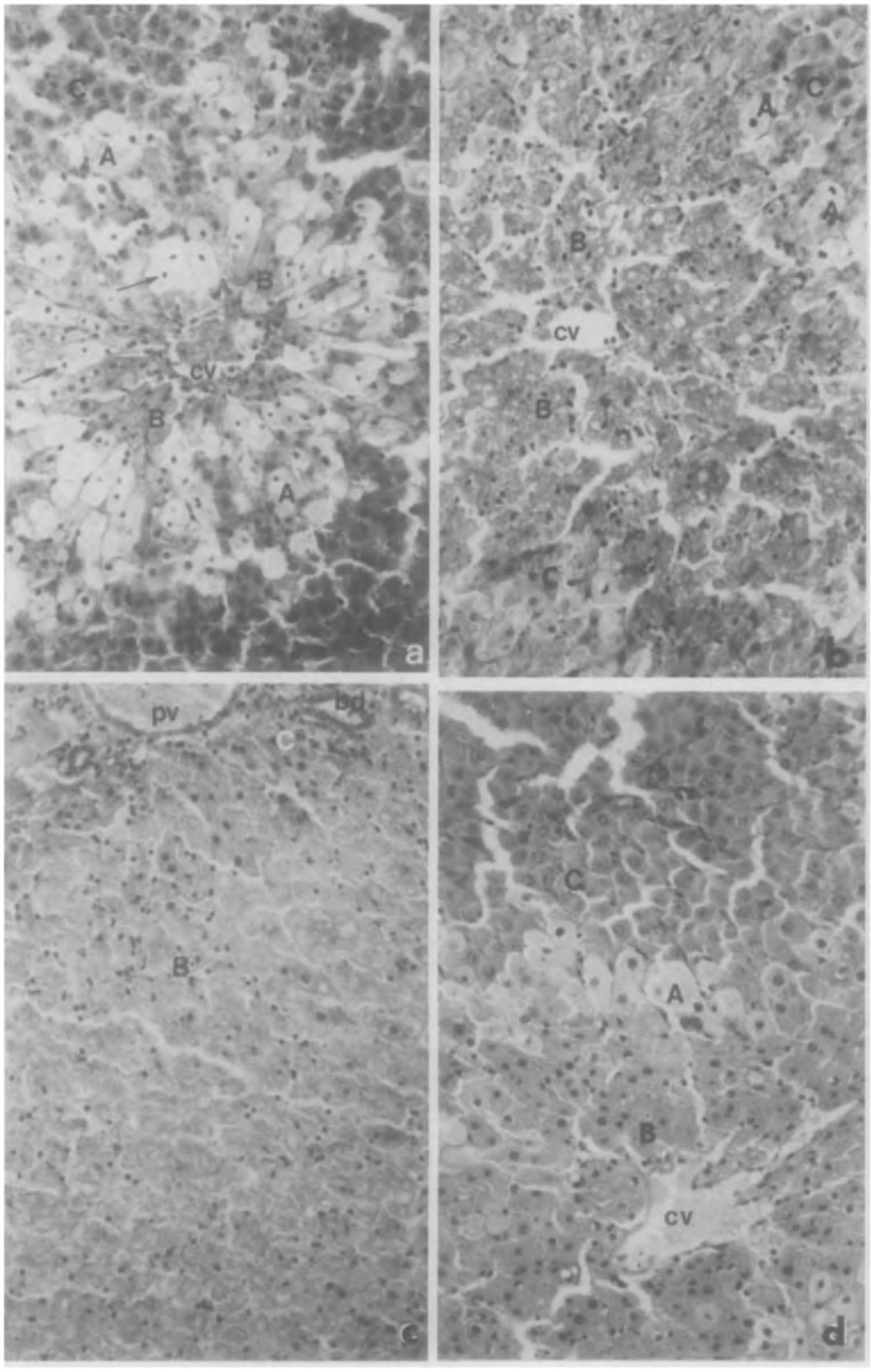

FIGURE 5

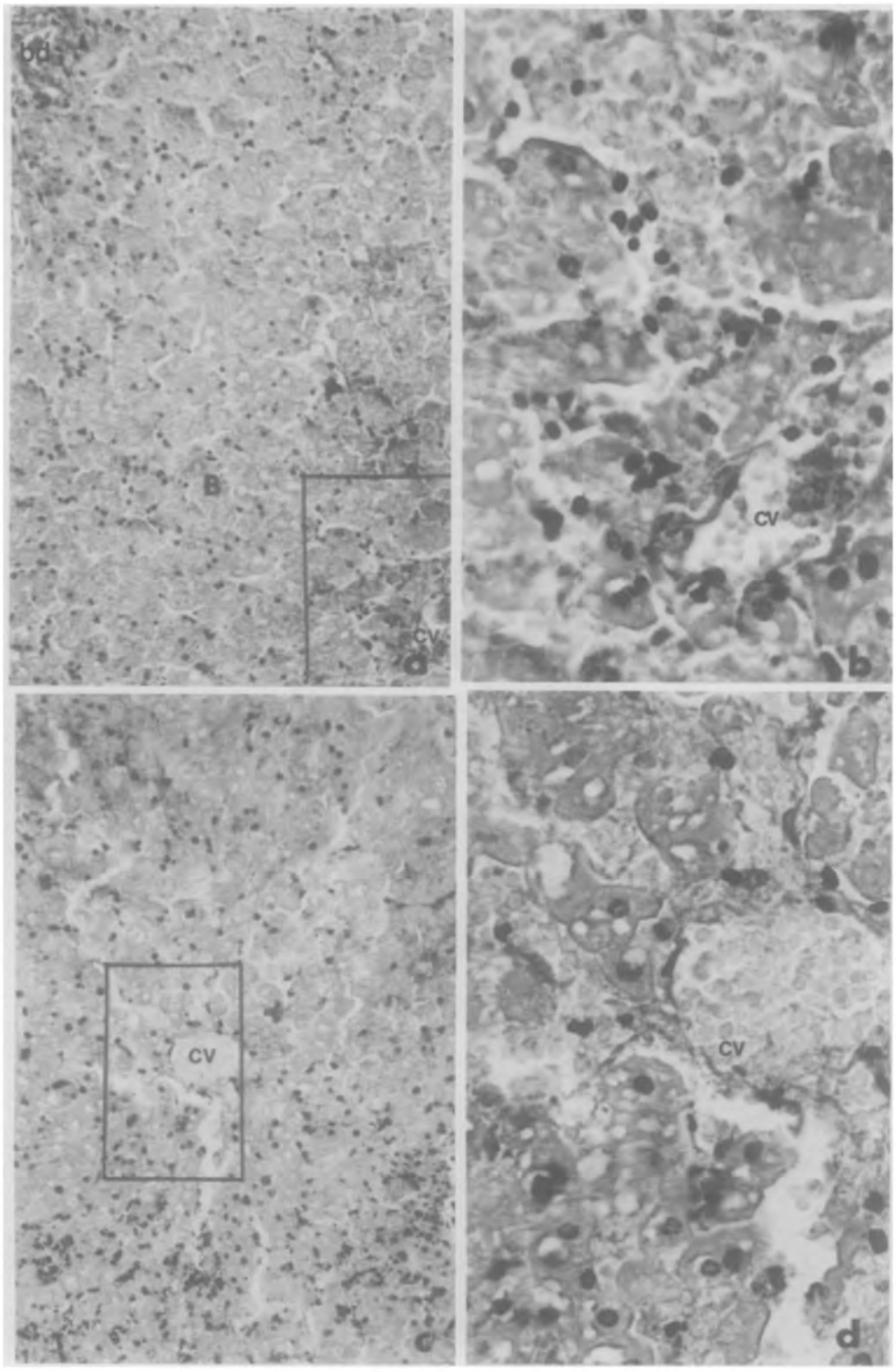

FIGURE 6

duced mortality (Table 1) increased in the order of Ac = Bu < Pn < Hx < Hp, the extent of hepatic and renal dysfunction was not related to ketone chain length. Regardless of the ketone used, all hepatic lesions were initially centrilobular in nature and progressed toward the periportal region as the ketone dosage/chain length, and thus the severity of damage, increased (Fig. 5). The severity of the renal lesion produced by $CHCl_3$ in ketone (1.0 mmol/kg)-pretreated rats also increased as the length of the carbon skeleton increased (Fig. 2). However, the relationship between the severity of the hepatic and renal lesions and ketone chain length was obscured at higher ketone dosages. The relative importance of this structural feature must be questioned particularly in view of the fact that the relationship can be eliminated by altering the degree of exposure to the ketones. The possibility that other structural characteristics may alter the efficacy of ketonic solvents as potentiating agents cannot be resolved at present.

The ketones utilized in this study were capable of producing renal tubular degeneration when administered alone at a dosage of 15.0 mmol/kg (Figs. 1e and f). Intraepithelial hyaline bodies in the S_1 and S_2 portions of the proximal tubules accompanied the degeneration and necrosis in the kidneys of Hx-pretreated rats (Fig. 3f). Similar lesions were reported in renal sections from rats after inhalation of petroleum hydrocarbons (Carpenter *et al.*, 1975, 1977) or po administration of JP-5 jet fuel (Parker *et al.*, 1981). 2-Hexanone and various odd carbon number methyl ketones have been isolated from the urine of Fischer 344 rats exposed to JP-4 jet fuel (Vernot and Pollard, 1983). In addition, Pitts *et al.* (1983) have recently implicated branched chain pentanes as causative agents in the nephrotoxic response to hydrocarbon fuel exposure. Recent ultrastructural studies of Hx-potentiated $CHCl_3$ renal damage seem to indicate that the initial renal lesions may be in the glomerular filtration apparatus and that the tubular degeneration, hyaline body formation, and necrosis are secondary responses (Kanchanapangka, 1983). These observations suggest that ketonic solvents, whether acting alone or as modifiers of the toxic properties of short chain alkanes, may be of marked importance in hydrocarbon fuel nephropathy.

ACKNOWLEDGMENTS

We gratefully acknowledge the expert technical assistance of Maxine Little and Mildred Floyd. This work was supported by DHHS Grant 5 ROI OH00986 04.

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