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Ninety Day Toxicity Study of Chloroacetic Acids in Rats

HARI K. BHAT,* MARY F. KANZ,† GERALD A. CAMPBELL,† AND G. A. S. ANSARI*†

*Department of Human Biological Chemistry and Genetics, and †Department of Pathology, University of Texas Medical Branch, Galveston, Texas 77550

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Ninety Day Toxicity Study of Chloroacetic Acids in Rats. BHAT, H. K., KANZ, M. F., CAMPBELL, G. A., AND ANSARI, G. A. S. (1991). *Fundam. Appl. Toxicol.* **17**, 240–253. Chloroacetic acids are produced in drinking water as a result of disinfection processes. Chloroacetic acids are also metabolites of widely used and toxic halogenated hydrocarbons. Thus, chronic human exposure to these chemicals is likely to occur. The objective of the present study was to examine the toxic effects of monochloroacetic acid (MCA), dichloroacetic acid (DCA), and trichloroacetic acid (TCA) in a 90-day subchronic study in rats via oral exposure by drinking water. Chloroacetic acid solutions were prepared at concentrations which provided an approximate intake of ¼ the LD50 dose per day: MCA, 1.9 mM; DCA, 80.5 mM; TCA, 45.8 mM. Control rats received distilled water only. After 90 days, major organs were removed, fixed, paraffin embedded, and stained. Light microscopic examination of the major organs revealed variable degrees of alterations in the lung and liver of all three treated groups. In the liver, morphological changes were predominantly localized to the portal triads, which were mildly to moderately enlarged with random bile duct proliferation, extension of portal veins, fibrosis, edema, and occasional foci of inflammation. In the lungs, minimal alterations were observed as foci of perivascular inflammation on small pulmonary veins. Morphological changes in the testes and brain were seen only in the DCA treated group. Testes were atrophic with few spermatocytes and no mature spermatozoa. Focal vacuolation and gliosis were present in the forebrain and brainstem. The results of these studies indicate that, relative to their respective LD50 values, DCA given at 80.5 mM is more toxic than TCA given at 45.8 mM and MCA at 1.9 mM is least toxic.

Chloroacetic acids are chemically and environmentally important compounds. Mono-, di-, and trichloroacetic acids have been identified as by-products of chlorination processes for disinfection of drinking water (Christman *et al.*, 1983; Coleman *et al.*, 1984; Uden and Miller, 1983; Krasner *et al.*, 1989). Chloroacetic acids are also metabolites of several chlorinated hydrocarbons such as 1,1,2-trichloroethane, 1,2-dichloroethane, 1-chloroethene, and 1,1-dichloroethene (Yllner, 1971a,b; Rannug *et al.*, 1976; Liebler *et al.*, 1985; Liebler and Guengerich, 1983; Reichert *et al.*, 1979). Thus, the human population may be at a constant risk of exposure to these chlorinated acids.

Monochloroacetic acid (MCA), widely used as a herbicide and in the synthesis of various chemicals, is rapidly absorbed through skin and may cause death by systemic exposure (Woodard *et al.*, 1941; Berardi *et al.*, 1987). MCA penetrates the blood-brain barrier since mice surviving an oral LD50 or LD80 dose exhibit a peculiar anomaly after 24 hr in which the front paws are rigidly clasped together and the hind limbs splayed causing difficulty in walking (Berardi *et al.*, 1987). The mechanism by which MCA exerts its toxicity is not well understood. Chaiken and Smith (1969) ascribe the toxicity of MCA to the lability of the halogen, which allows this compound to react with sulfhydryl compounds. Direct inhibition of

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thiols in the kidney has been suggested to account for anuria in rats receiving toxic doses of MCA (Hayes *et al.*, 1973).

Dichloroacetic acid (DCA) has been used as a therapeutic agent for the treatment of several metabolic disorders; it also causes vasodilation, inhibits nerve impulse transmission in sympathetic ganglia, and protects the liver against carbon tetrachloride, cyanide, and arsenate toxicity (Stacpoole, 1969). DCA is neurotoxic to rats or dogs when administered orally at doses higher than 50 mg/kg/day for several weeks (Stacpoole *et al.*, 1979; Katz *et al.*, 1981; Spencer *et al.*, 1981; Yount *et al.*, 1982; Stacpoole *et al.*, 1984). Stacpoole *et al.* (1990) suggest that chronic DCA treatment might induce thiamine deficiency through an increased demand for this vitamin and they have found that coadministration of thiamine with DCA to rats significantly reduces the incidence of hind limb weakness and other behavioral changes typical of both DCA toxicity and thiamine deficiency.

Trichloroacetic acid (TCA) is used as a preemergence herbicide, a peeling agent for wrinkled, sun-damaged skin and tattoos, and as a common laboratory agent (Ayres, 1964; Collins, 1989; Piggot and Norris, 1988). Besides being present in drinking water as a result of chlorine disinfection, TCA is also a major metabolite of trichloroethylene (TCE) and tetrachloroethylene (Coleman *et al.*, 1976; Uden and Miller, 1983; Daniel, 1963; Dekant *et al.*, 1985). The metabolism of TCE results, in part, in the formation of TCA as a major metabolite and DCA as a minor metabolite. TCE is an organic solvent with wide industrial application as well as a contaminant of surface and ground water (Page, 1981; Love and Elero, 1982). TCA induces liver peroxisome proliferation (Odum *et al.*, 1988; Parnell *et al.*, 1986) and an increased incidence of adenomas and hepatocellular carcinomas are observed in mice exposed to TCA or DCA (Herren-Freund *et al.*, 1987). An increase in peroxisomal stimulating activity along with increased metabolic TCA formation, following TCE administration in mice compared to rats, has led to the speculation that TCA levels may be important in TCE carcinogenesis in mice (Prout *et al.*, 1985; Green and Prout, 1985).

The objective of this study was to determine the subchronic toxicity of MCA, DCA, and TCA in rats following oral exposure to these chloroacetic acids in water at a standardized equitoxic dose regimen. In any subchronic exposure, the manifested toxicity of a compound may be in direct relationship to its LD50 value. Although human exposure to these acids is likely to occur from chlorinated drinking water, few comparative toxicity studies using the three chloroacetic acids have been done. One previous toxicity study comparing MCA, DCA, and TCA was short in length (14 days) and used equimolar dose regimens (DeAngelo *et al.*, 1989). Other subchronic studies using equimolar dose regimens have examined the effects of DCA in dogs and rats (Katz *et al.*, 1981) or DCA and TCA in rats (Mather *et al.*, 1990). Therefore, we decided to investigate the toxicities of the chloroacetic acids based on an approximate intake of ¼ of the LD50 dose per day. In addition, the equitoxic dose regimens utilized in this study may more nearly approximate human exposure because chlorinated drinking water or surface water near industrial areas contain much greater concentrations of DCA or TCA than MCA (Uden and Miller, 1983; Coleman *et al.*, 1984).

MATERIALS AND METHODS

MCA (purity > 99%) and DCA (purity > 99%) were purchased from Aldrich Chemical Co., (Milwaukee, WI). TCA (purity > 99%) was obtained from Sigma Chemical Co. (St. Louis, MO).

Male Sprague–Dawley rats (Harlan, Indianapolis, IN) were acclimatized in our animal facility for one week after arrival with free access to water and food (Purina Rat Chow) and housed in a 12 hr light/dark cycle room. The rats were divided into four groups with five rats in each group. At the start of the study, rat body weights ranged from 220–243 g.

Aqueous solutions of MCA (1.9 mM), DCA (80.5 mM), and TCA (45.8 mM) were prepared in double distilled water. Our initial studies showed that a rat weighing ~240 g consumed ~25 ml of water each day. Solutions were prepared such that each rat would receive approximately

¼ of LD50 of one of the chloroacetic acids in drinking water per day. LD50 of MCA, DCA, and TCA are 76 mg/kg, 4.4 g/kg, and 3.3 g/kg, respectively (Stecher, 1976; Woodard *et al.*, 1941). These solutions were neutralized to pH 7.2–7.4 with 1 *N* sodium hydroxide. Each solution was given in glass bottles and solutions were freshly prepared and changed on alternate days. Chloroacetic acids are stable for more than 1 week under these conditions (Herren-Freund *et al.*, 1987; NTP TR 396). Group I received MCA, Group II received DCA, and Group III received TCA in water for 90 days. Group IV (controls) received distilled water only.

Fixation procedure. Rats were anesthetized with ether and the body cavity opened. The inferior vena cava was severed and major organs were perfused via the heart with 0.9% saline (5 min) and 1% glutaraldehyde (5 min) (Poly Sciences, Warrington, PA) in 0.1 M Pipes buffer (pH 7.35, 365 mOsm) (Sigma Chemical Co., St. Louis, MO). Perfusions were carried out at 37°C to maximize blood removal with saline prior to the initiation of fixation with glutaraldehyde.

Different organs were removed, blotted, and immediately weighed. Slices of liver, lung, heart, spleen, thymus, kidney, testes, and pancreas were postfixed in 10% neutral buffered formalin, dehydrated, and paraffin embedded. Evaluation of tissues for histopathology was conducted on hematoxylin and eosin stained sections. In addition, liver sections from all animals were stained with Masson's trichrome for evaluation of differences in collagen deposition. Dilation and extension of portal veins were assessed according to the following criteria: none, minimal (enlargement or extension of portal veins in less than one-fourth of portal triads), mild (enlargement or extension of portal veins in one-fourth to one-third of portal triads), or moderate (enlargement or extension of portal veins in one-third to one-half of portal triads). Differences in collagen deposition were assessed in cross sections of portal triads between 500 and 800 μm diameter according to the following criteria: none, minimal (collagen width external to portal veins, hepatic arteries or major bile ducts approximately the thickness of one hepatocyte), mild (collagen width greater than 1 but less than the thickness of 2 hepatocytes), or moderate (collagen width greater than the thickness of 2 hepatocytes).

Rat brains which had been postfixed in 10% neutral buffered formalin were sliced coronally using standard landmarks (optic chiasm, infundibulum, cerebral peduncles) as reference points to ensure consistency between animals in the levels examined. The number of slices produced varied between seven and eight per animal and included frontal, frontoparietal, parietal, and occipital levels of the forebrain and midbrain, and pons, medulla, and cerebellum in the hindbrain. Although the plane of section was not stereotaxically determined, the sections closely matched the coronal sections shown in the atlas of Paxinos and Watson (1986). Slices were marked with a punch to identify left and right sides. The slices were then processed through graded alcohols and clearing agent (HistoClear), infiltrated with paraffin, and embedded by standard histologic techniques. The resulting blocks were sectioned at 7 microns and slides of all blocks were stained with hematoxylin and eosin, (H & E), Luxol fast blue for myelin with H & E counterstain, and cresyl echt violet for nuclei and Nissl substance. Immunohistochemical staining for glial fibrillary acidic protein (GFAP), an astrocyte intermediate filament marker, was also performed on selected sections of DCA-treated and control rats to assess the extent of gliosis. GFAP was stained by the avidin-biotin method using a kit from Vector (Burlingame, CA).

Statistics. Significant differences were determined by analysis of variance using ABSTAT (Anderson Bell, Boulder, CO), a statistical program for personal computers. A level of $p < 0.05$ was considered to be significant.

RESULTS

General. Rats treated with DCA and TCA showed a loss in body weight compared to the control group (Fig. 1). On Day 90 the mean body weights of the different groups were: control 448.2 ± 22.8; MCA 426.8 ± 22.1 (95.2% of control); DCA 295.8 ± 9.5 (66% of control, $p < 0.0001$); and TCA 370.8 ± 17.7 (82.7% of control, $p < 0.0001$). Organ weight differences among the four groups were found only for liver and testes. The DCA group showed hepatomegaly with increased liver weights but decreased testes weights compared to the other groups and controls. Mean liver weights for the four groups were: control 14.68

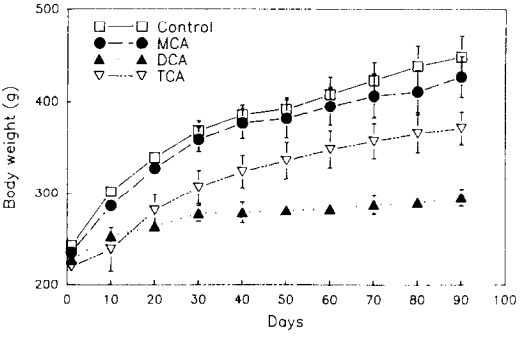


FIG. 1. Body wt profiles of control rats (□) and rats drinking water containing 1.9 mM MCA (●), 80.5 mM DCA (▲) and 45.8 mM TCA (▽) for 90 days. Each point represents the mean body weight of five rats plus or minus the standard deviation.

$\pm$ 0.78; MCA 13.25 $\pm$ 0.64 (90.3% of control, $p < 0.03$); DCA 16.35 $\pm$ 0.41 (111.4% of control, $p < 0.01$); and TCA 12.6 $\pm$ 0.72 (85.8% of control, $p < 0.002$). The percentage liver/body weight ratios for the different groups were: control 3.3, MCA 3.1, DCA 5.5 ($p < 0.0001$), and TCA 3.4. Mean testes weights of the different groups were: control 3.73 $\pm$ 0.2, MCA 3.70 $\pm$ 0.13, DCA 2.47 $\pm$ 0.97 ($p < 0.01$), and TCA 3.56 $\pm$ 0.10. The percentage testes/body weight ratios for the groups were: control 0.87, MCA 0.84, DCA 0.84, and TCA 0.96.

Light microscopy. At necropsy, no gross lesions were observed. Light microscopic examination of the major organs revealed variable degrees of alterations in the lung and liver of all three treated groups. In addition, changes in testes and brain were observed only in the DCA group.

Liver sections from control rats showed a typical architecture with several central veins and portal triads outlining the lobular substructure (Fig. 2a). One control rat showed minimal increases in collagen deposition in a minor number of portal triads (Table 1). In DCA-exposed rats, however, portal triads showed alterations ranging from mild to moderate. In the liver of one moderately affected animal, several portal triads contained greatly enlarged portal veins which were surrounded by increased numbers of bile ducts and ductules, areas of edema, and variable numbers of inflammatory cells (Fig. 2b). In two other moderately affected livers, portal veins were less dilated but were more tortuous with 6–10 branches per triad (Fig. 3a). Minimal to moderate increases in collagen deposition were observed in random portal triads (Fig. 3b) and around larger central veins. Small foci of inflammation were scattered within the periportal and midzonal parenchyma and were also located peripheral to endothelial cells of central veins; necrotic hepatocytes were rarely observed. Morphological alterations in the livers of MCA- and TCA-exposed rats were similar but generally ranged from minimal to mild (Fig. 4) in the two groups, respectively (Table 1).

In the lungs, foci of perivascular inflammation were observed in all three treatment groups. Such foci were extremely rare in the lungs of control rats which showed normal alveolar architecture (Fig. 5a). These foci were generally found on the periphery of small pulmonary veins (Fig. 5b) and were occasionally present at the bifurcations of veins into venules (Fig. 5c). Lymphocytes and macrophages were typically identified in these foci (Fig. 5d). An increase in the thickness of the adventitial layer of the adjacent venous walls was also frequently seen around these foci. The degree of perivascular inflammation in the lungs of treated rats was comparable to the degree of liver injury seen in the three treatment groups: DCA > TCA > MCA.

Morphological changes in the testes were seen only in the DCA-treated group. Variable

FIG. 2. Light micrographs of the livers of a control rat (a) and a rat drinking 80.5 mM DCA for 90 days (b). Trichrome stain, original magnification, (a,b) 60×. (a) In the control liver, typical portal triads (P) with normal portal veins (PV) are present at the margins of lobules containing central hepatic veins (CV). (b) The liver of the DCA-treated rat shows an enlarged portal triad with a dilated portal vein (PV) and increased amounts of collagen in the perivascular region (blue staining). Arrowheads indicate increased number of bile ducts and ductules. Clear areas within the portal triad represent edema (asterisk).

FIG. 3. Light micrographs of the livers of rats drinking 80.5 mM DCA for 90 days (a,b). Trichrome stain, original magnification, (a) 55×, (b) 100×. (a) The portal triad is enlarged and extended with several cross sections (arrows) of the portal vein (PV). (b) Increased amounts of collagen (indicated by the blue staining) are present around the portal triad. Deposition of collagen is beginning to occur within the hepatic cords, shown on the upper left side (arrows) of the portal vein (PV).

FIG. 4. Light micrograph of the liver of a rat drinking 45.8 mM TCA for 90 days. The enlarged portal triads (P) contain dilated portal veins (PV) and increased amounts of collagen. Trichrome stain, original magnification, 60×.

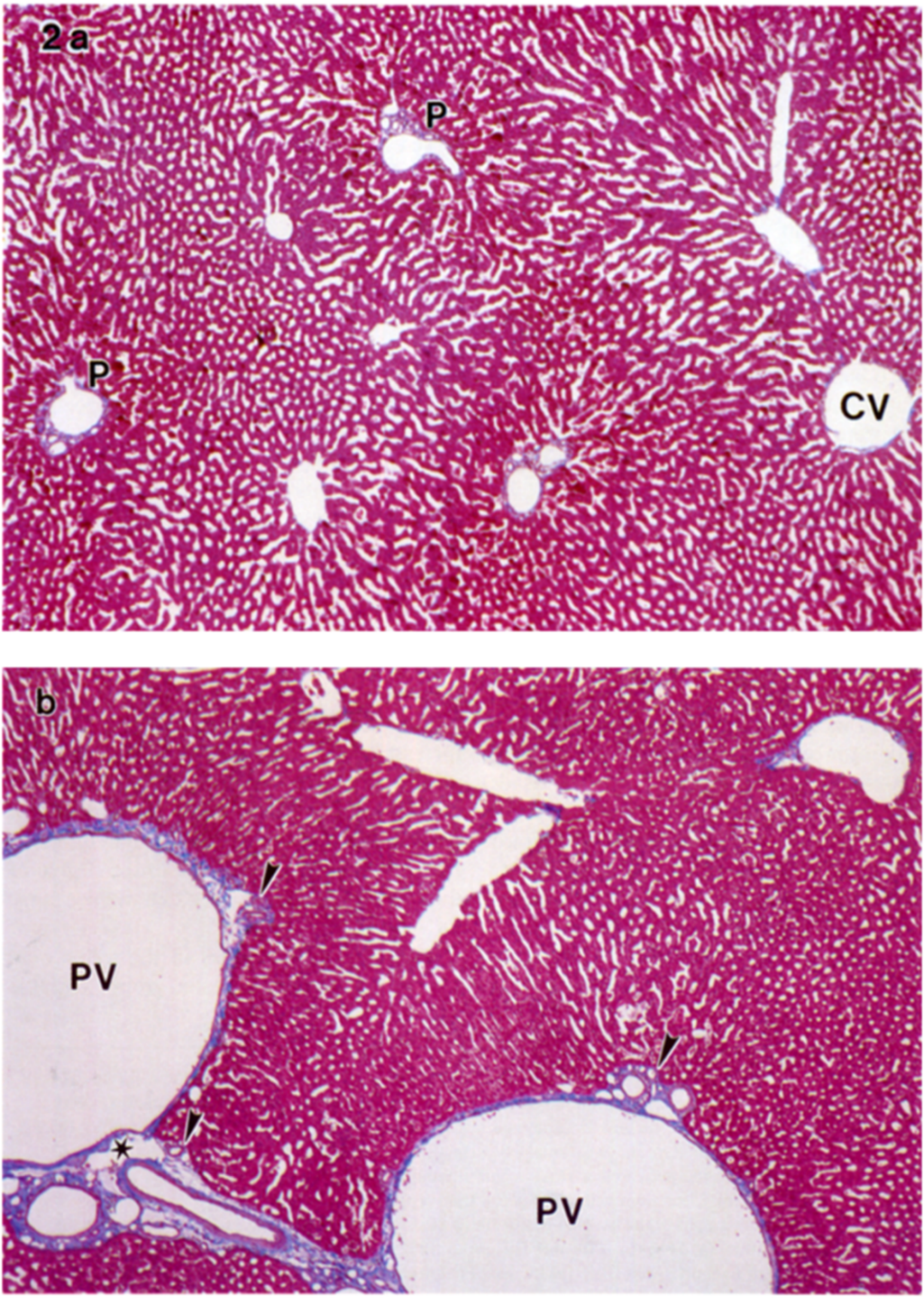
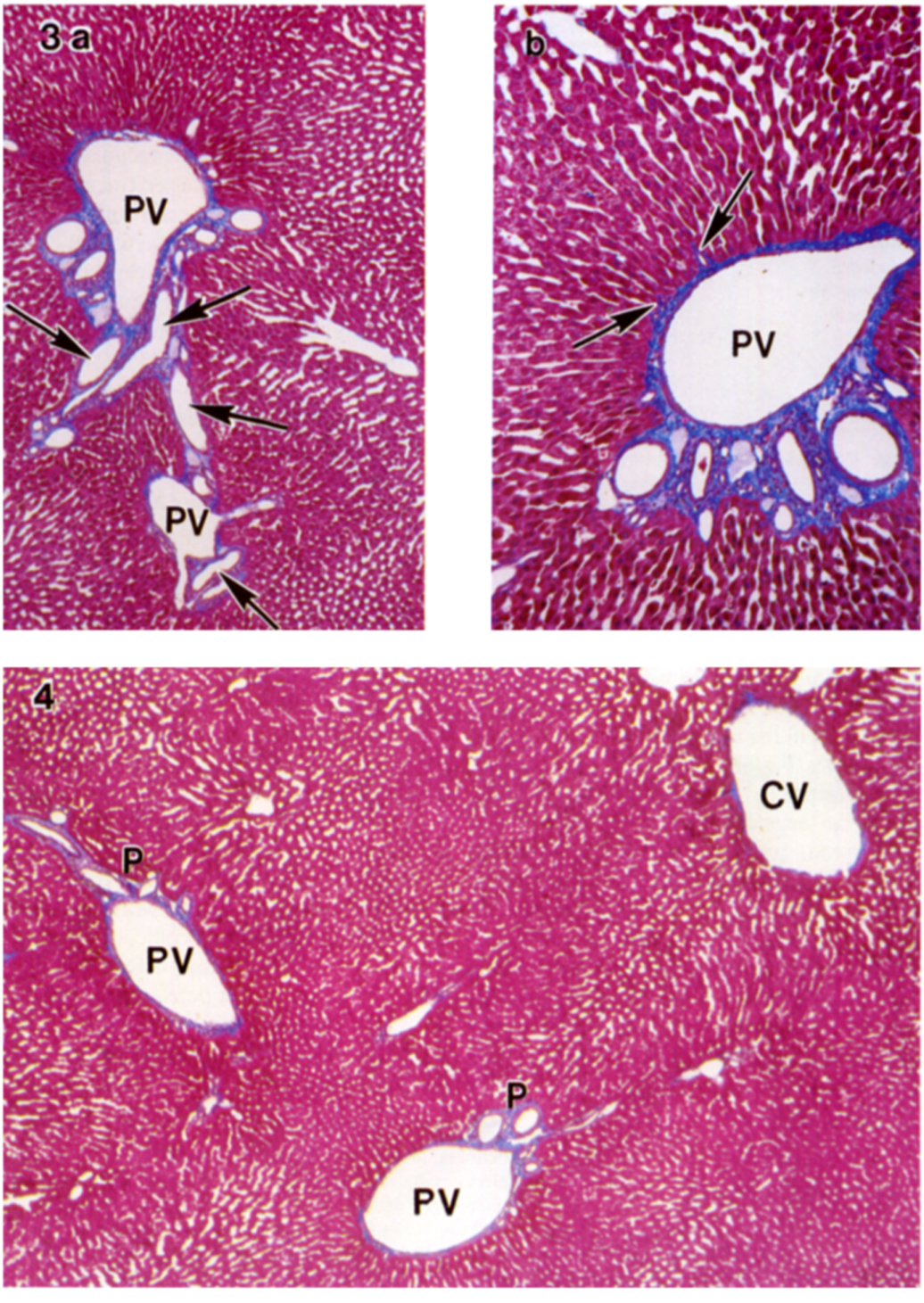


FIGURE 2



FIGURES 3 AND 4

TABLE 1

HEPATIC HISTOPATHOLOGY FOLLOWING SUBCHRONIC EXPOSURE TO CHLOROACETIC ACIDS

	Control	MCA	DCA	TCA
Collagen deposition				
Normal	4	1	—	1
Minimal	1	3	1	2
Mild	—	1	2	1
Moderate	—	—	2	1
Portal vein dilation/ extention				
Normal	5	1	—	—
Minimal	—	2	1	1
Mild	—	2	1	3
Moderate	—	—	3	1

Note. Criteria for grading histopathology are described under Materials and Methods. Collagen deposition was assessed on trichrome-stained sections; portal vein dilation/ extension was assessed on H & E-stained sections. Each group contained five animals.

degrees of atrophy, from mild to severe, were found within the group of five animals. In control animals, oval to elongated seminiferous tubules in the testes showed normal spermatogenesis (Fig. 6a). Thin layers of interstitial cells were present between the tubules (Fig. 6b). In the testes of two DCA-treated rats, the seminiferous tubules were severely atrophic and contained enlarged Sertoli cells, very few spermatocytes, and no mature spermatozoa (Fig. 6c). Interstitial hyperplasia, characterized by increased numbers of interstitial cells, was most pronounced in areas of severe atrophy (Fig. 6d). In three animals with mild to moderate testicular atrophy, individual seminiferous tubules showed disrupted spermatogenesis and the formation of multinucleated giant cells. Testicular alterations were not observed in MCA- and TCA-treated animals.

The primary alteration seen in the brains of DCA-treated rats included vacuolation and gliosis in major white matter tracts and in structures with mixed gray and white matter. In addition, focal areas with neuronal eosinophilia and shrinkage and nuclear pyknosis were observed in gray matter (cerebral cortex, hippocampus, and cerebellum). Although the neuronal changes seemed subjectively more severe in some of the DCA-exposed animals, they did not differ significantly in distribution from similar changes seen in controls. Focal vacuolation and gliosis, on the other hand, were observed only in the DCA-treated animals. The structures most severely affected in the forebrain were the heavily-myelinated superior-lateral portions of the corpus callosum and contiguous hemispheric white matter, the deep layers of cerebral cortex, and the cerebral peduncles. Brain stem structures that showed selective involvement included the medial lemniscus, inferior colliculus, ventral cochlear nucleus, and medial vestibular nucleus. Other myelinated white matter structures (cervical spinal tracts, pons, cerebellum, anterior commissure) and basal ganglia were less severely involved. An example of the vacuolation in an area with mixed gray and white matter (inferior colliculus) is shown in Fig. 7a. Such structures (including inferior colliculus, basal cortex, pons, and other brain stem nuclei) also had extensive reactive gliosis, as demonstrated by GFAP immuno-

FIG. 5. Light micrographs of the lungs of a control rat (a) and rats drinking 80.5 mM DCA (b), 1.9 mM MCA (c), and 80.5 mM DCA (d) for 90 days. Hematoxylin and eosin stain, original magnification, (a,b) 110×, (c) 275×, (d) 450×. (a) A typical small pulmonary vein (V) is seen branching into a venule in a control lung. Normal alveoli surround the vein and a bronchus (B) is present in the lower right corner. (b) Two perivascular inflammatory foci (arrows) are seen on the margin of a pulmonary vein in a DCA-treated rat. A third focus of inflammation may also be present (arrowhead). (c) An inflammatory focus is seen at the junction of a pulmonary vein into a smaller vein (arrow) in an MCA-treated rat. Another possible focus of inflammation occurs on the opposite wall of the vein (arrowhead). (d) An enlargement of the inflammatory focus at center right in micrograph b shows the presence of lymphocytes (arrows) and macrophages (arrowheads).

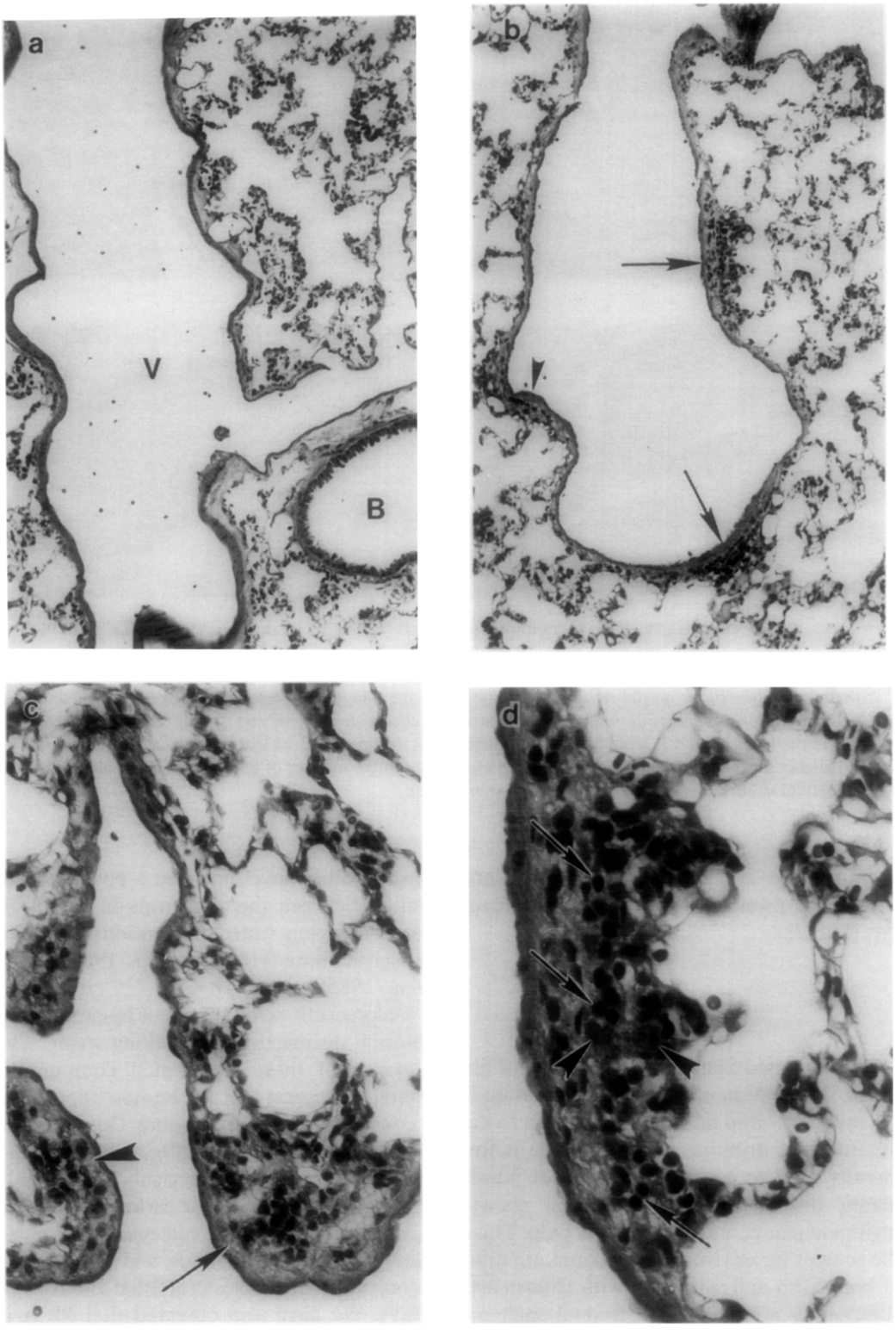


FIGURE 5

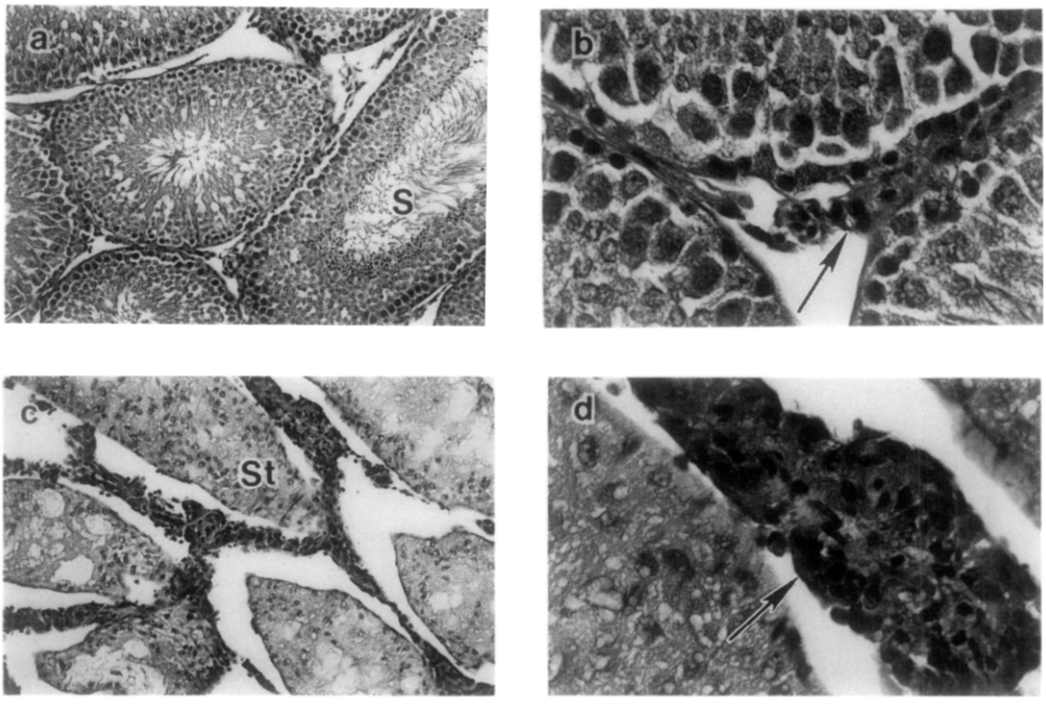


FIG. 6. Light micrographs of the testes from a control rat (a,b) and a rat drinking 80.5 mM DCA for 90 days (c,d). Hematoxylin and eosin stain, original magnification, (a,c) 75×, (b,d) 325×. (a) Normal spermatogenesis is occurring within seminiferous tubules and mature spermatozoa (S) are seen in a majority of the tubules. (b) Higher magnification micrograph shows the thin layer of interstitial cells (arrow) present between normal seminiferous tubules. (c) Seminiferous tubules are atrophic and contain large Sertoli cells (St) in the testes of a DCA treated rat. Spermatogenesis is disrupted and no mature spermatozoa are seen. (d) Higher magnification micrograph indicates an increase in the number of Leydig cells in the abnormal interstitial tissue (arrow) of the testes of a DCA-treated rat.

staining (Fig. 7b). Neither vacuolation nor gliosis was present in the brains of control rats (Fig. 7c).

DISCUSSION

In the United States and other parts of the world, disinfection of water by chlorination is the most common practice. In addition to the treatment of drinking water, chlorine is frequently used to control biofouling at power plants, disinfect waste water from sewage treatment plants, and bleach paper pulp. Thus, the sources for environmental contamination of both fresh and salt water with chlorinated compounds are ubiquitous. Several epidemiologic studies have indicated a possible association between the consumption of chlorinated drinking water and various forms of cancer in humans (Cantor *et al.*, 1985; Cragle *et al.*, 1985).

Chloroacetic acids are major by-products in chlorine disinfection of drinking water. The presence of these chlorinated compounds, therefore, suggest that the human population is constantly at risk of exposure. Our objective was to compare the toxic effects of mono-, di-, and trichloroacetic acid in a subchronic study in drinking water. In our earlier studies, we have shown that MCA can react with phospholipids and neutral lipids, and that it forms a conjugate with cholesterol (Bhat and Ansari, 1989). We have also observed that MCA is

distributed into hydrophilic tissues at earlier time points and accumulates into lipophilic tissues at later time periods using whole-body autoradiography (Bhat *et al.*, 1990).

In this study using equitoxic doses, alterations in body weight and organ weights were most severe with oral DCA followed by less severe changes with TCA and minor changes with MCA. DCA (80.5 mM) decreased body weight by 34% and increased liver weight by 11%; testes weight was reduced to 66% of control values by 90 days. Katz *et al.* (1981) noted decreased body and liver weights in rats gavaged with 500 and 2000 mg DCA/kg/day for 13 weeks; however, qualitatively smaller testes were observed only in the 2000 mg/kg group. DeAngelo *et al.* (1989) found that concentrations of MCA, TCA, or DCA in drinking water from 1 to 5 g/liter decreased body weight in rats after 14 days; liver weight decreases occurred only in the MCA group. The MCA-exposed rats in this study exhibited the least toxicity, but our results indicate that 1.9 mM MCA is sufficient to cause a 10% reduction in liver weight.

Morphological changes observed in brain and testes were limited to DCA-exposed rats only. These results are consistent with the findings of Yount *et al.* (1982) that DCA is neurotoxic and with the studies of Katz *et al.* (1981) who indicated that brain and testes are the principal target organs of DCA intoxication. Katz *et al.* (1981) found that brain lesions at all doses occurred primarily in the cerebrum and to a lesser extent in the cerebellum and were characterized by vacuolation of the myelinated white tracts; lesions were not observed in optic or sciatic nerves. In the rats of this study, focal vacuolation was observed not only in the cerebrum and to a lesser degree in the cerebellum, but also in brain stem, cervical spinal cord, pons, and basal ganglia. In addition, reactive gliosis was seen in mixed white and gray matter. The doses of TCA and MCA used in this study produced no alterations in brain, although MCA is known to be neurotoxic at higher acute doses (Berardi *et al.*, 1987; Quick, 1983).

The presence of small inflammatory foci in hepatocellular zones 1 through 3 and the variable alterations in portal veins and portal triads among animals suggests chronic mild loss of injured cells with continual removal of damaged cellular components and initiation of nonspecific portal fibrosis and bile duct proliferation (Fuller, 1985). Subchronic administration of the model toxin, carbon tetrachloride, at 33 mg/kg/day for 12 weeks leads to extensive degenerative changes prior to cirrhosis; these changes include bile duct hyperplasia, portal fibrosis, inflammatory foci, necrotic hepatocytes, lobular distortion, and hyperplastic nodules (Bruckner *et al.*, 1986). Many different types of liver injury (chemical, viral, and parasitic) can lead to increased deposition of collagen and eventually to cirrhosis; the mechanism(s) which allows various forms of injury to progress to the same endpoint is unknown (Diegelmann and Linblad, 1985).

The perivascular inflammation observed in the lungs of all three treated groups has cell components characteristic of chronic inflammation. Since the rats were in our animal facility for <100 days, and since the control rats showed very minor signs of inflammation, infection in the animals at the beginning of the study is unlikely to be the underlying cause of the inflammation. The possibility exists that exposure of these rats to chloroacetic acids for 90 days made them less resistant, and thus more susceptible to infection, but infection in the lung is most commonly present in airways or lung parenchyma. Furthermore, the progressive effects of these compounds (MCA < TCA < DCA) on portal triads in the liver were paralleled by the degree of perivascular inflammation of the lung which suggests systemic toxicity of the chloroacetic acids.

Katz *et al.* (1981) observed no hepatotoxic effects of subchronic administration of DCA at 500 and 2000 mg/kg/day in rats, although alterations in liver Kupffer cells and gall bladder mucosal hyperplasia were seen in dogs at doses of 50 to 100 mg/kg/day. In addition, secondary effects of DCA exposure in dogs were increases in pulmonary inflammatory le-

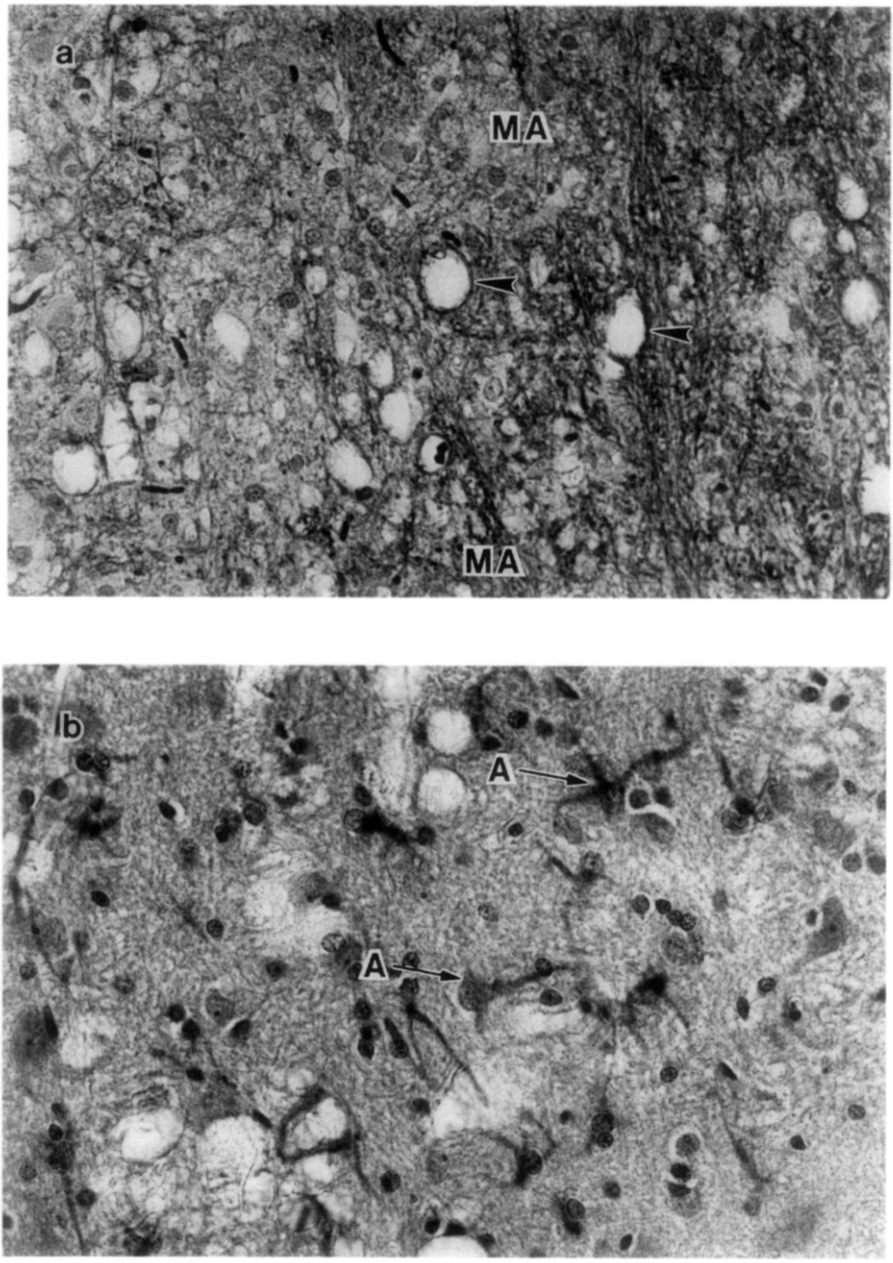


FIG. 7. Light micrographs of the inferior colliculus region of the brain of a rat drinking 80.5 mM DCA for 90 days (a,b) and a control rat (c). Hematoxylin and eosin/Luxol fast blue stain (a) and (c); avidin-biotin immunohistochemical stain for glial fibrillary acidic protein (GFAP) (b); original magnifications, (a–c) 335×. (a) The lesion shows extensive vacuolation, neuronal loss, and gliosis. Vacuoles are associated with myelinated axons (MA) and myelin-staining rims surround other vacuoles (arrows). (b) The GFAP immunohistochemical stain demonstrates cell bodies and processes of reactive astrocytes (A) in the same area. (c) Normal architecture of the control brain shows no vacuolation or gliosis.

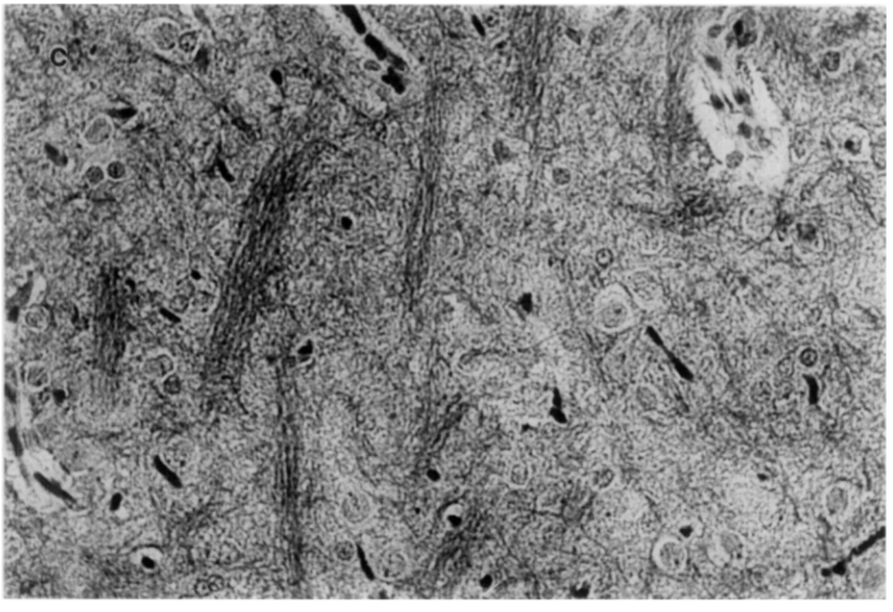


FIG. 7—*Continued*

sions and ocular keratitis. Differences between the results of Katz *et al.* (1981) and the present study may be attributed to the method of exposure (gavage vs drinking water) and the initial weight of the rats (150 vs 240 g). Age and body weight are known to influence toxicity of halogenated hydrocarbons (Bruckner *et al.*, 1986).

Recently, Mather *et al.* (1990) reported decreased body weight gains and increased organ to body weight ratios for liver and kidney in rats exposed to 500 and 5000 ppm DCA in drinking water for 90 days. Increased organ to body weight ratios for liver and kidney were also found in rats exposed to 5000 ppm TCA for 90 days. Histologically, livers of DCA and TCA exposed rats had focal areas of intracellular swelling or pockets of proteinaceous fluid which occasionally disrupted normal liver architecture in the DCA group. Glycogen accumulation was significant in enlarged hepatocytes in both DCA- and TCA-exposed rats. Cells of the tubular epithelium and glomeruli showed degenerative changes in the kidneys of only DCA-exposed rats.

Mather *et al.* (1990) found no alterations in testes and brain of rats exposed to 5000 ppm DCA for 90 days but indicated that testicular atrophy and neurological lesions were manifest by 6 months at 500 and 5000 ppm (unpublished observations). These authors conclude that liver and kidney are the major targets of subchronic exposure to DCA but only at doses greater than those present in the environment or in chlorinated drinking water. The results of our study are generally consistent with the findings of Mather *et al.* (1990) with the exception of kidney toxicity. Current experiments in our laboratory investigating the specific ultrastructural alterations induced in liver by subchronic exposure to MCA, DCA, and TCA will provide further evidence concerning the differences in toxicity among the chloroacetic acids.

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