Hepatic Lesions in Mice after Continuous Inhalation Exposure to 1,1,1-Trichloroethane

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Male CF-1 mice (24 to 34 gm.) were exposed to either 250 p.p.m. or 1000 p.p.m. of 1,1,1-trichloroethane in air continuously for 14 weeks. Control mice were exposed to room air. Serial sacrifice of exposed and control mice from 1 to 14 weeks demonstrated significant changes in the centrilobular hepatocytes of animals in the 1000 p.p.m. group. Moderate liver triglyceride accumulation was evident in the 1000 p.p.m. group and peaked at 40 mg. per gm. of tissue (wet weight) after 7 weeks of exposure. Partial recovery was indicated by a decrease in the hepatic triglyceride level to 16 mg. per gm. by 14 weeks of exposure to 1000 p.p.m.

Electron microscopic evaluation revealed that cytoplasmic alterations were most severe in centrilobular hepatocytes in the 1000 p.p.m. group and were mild to minimal in the 250 p.p.m. group. These alterations consisted of vesiculation of the rough endoplasmic reticulum, with loss of attached polyribosomes, increased smooth endoplasmic reticulum, microbodies, and triglyceride droplets. Some cells had ballooned cisternae of the rough endoplasmic reticulum.

Necrosis of individual hepatocytes occurred in 40 per cent of the mice exposed to 1000 p.p.m. for 12 weeks. This necrosis was associated with an acute inflammatory infiltrate and hypertrophy of Kupffer cells.

Comparison of these findings to the results obtained by other investigators studying dichloromethane indicates that the pathologic alterations observed with 1,1,1-trichloroethane were similar to those observed with dichloromethane, except for different time courses of the effects and different degrees of recovery. The toxic effects of 1,1,1-trichloroethane were of a type similar to those produced by carbon tetrachloride, but they appeared to be much less severe.

Additional key words: Endoplasmic reticulum, Liver, Toxicology, Methylchloroform.

1,1,1-Trichloroethane (methylchloroform) is a common industrial solvent that, in screening tests, has been significantly less toxic than carbon tetrachloride and one of the least toxic of the chlorinated hydrocarbons (1, 24). Most testing protocols have used intermittent exposures for determining vapor toxicity. In this study, mice were examined at intervals during continuous inhalation exposure to 1,1,1-trichloroethane, to determine the nature of pathologic alterations caused by exposures to low levels of this compound and to obtain data that would be useful in establishing acceptable levels for continuous exposures. Continuous exposures are of interest in particular closed environments, such as space cabins, since 1,1,1-trichloroethane may be present continuously at low levels in the atmosphere.

Previous data on intermittent exposures to 1,1,1-trichloroethane have indicated that the most prominent toxic effects are on the liver and central nervous system (1, 22, 24). Intermittent exposure to 1000 p.p.m. in air (3.0 hours per day, 5 days per week, for 3 months) produced centrilobular fatty change in the livers of guinea pigs. Intermittent inhalation exposure to 500 p.p.m. (7 hours per day, 5 days per week, for 6 months) produced no effect on rats, guinea pigs, rabbits, and monkeys, when compared with controls, in terms of growth, organ weights, hematologic values, gross pathology, and histopathology (24).

In the present experiment, mice were chosen as the test animal, since other workers (27) studying dichloromethane demonstrated that mice are quite sensitive to chlorinated hydrocarbon exposure. Two levels of exposure to 1,1,1-trichloroethane were chosen: 1000 p.p.m. to give a definite, mild, toxic effect, and 250 p.p.m. as an estimate of a concentration that might give a threshold effect or possibly no detectable effect.

MATERIALS AND METHODS

ANIMALS

Six hundred male CF-1 strain mice (Carworth Division; Becton, Dickinson and Company, Portage, Michigan) were used. At the beginning of the experiment, each rat weighed 24 to 34 gm. The animals were fed a standard laboratory diet (Purina laboratory chow, Ralston Purina

Company, St. Louis, Missouri) and were given water *ad libitum*. The Purina laboratory chow was stored at ambient temperatures and was used within 60 days of its milling date. All animals were placed into groups at random. At the termination of control and exposure periods, all mice were sacrificed by cervical dislocation at the same time of day.

Chemicals

1,1,1-Trichloroethane was obtained as Chlorothene (Dow Chemical Company, Midland, Michigan), which is technical grade and contains 94 to 97 per cent 1,1,1-trichloroethane, 2.4 to 3.0 per cent dioxane, 0.12 to 0.30 per cent butanol, and small amounts of ethylene dichloride, water, and other materials (24). Since large volumes of this solvent (880 liters) were necessary for the experiment, reagent grade compounds were not used. Also, many chlorinated hydrocarbons react readily with aluminum and aluminum alloys, so that the additives listed are necessary to inhibit the corrosion of these metals. In previous toxicity studies, no difference was detected between pure 1,1,1-trichloroethane and Chlorothene (24).

Exposure Chambers

All inhalation exposures were performed in large, controlled environment chambers called Thomas domes; these have been described elsewhere (14, 23). Three domes were used: control, 250 p.p.m. trichloroethane, and 1000 p.p.m. trichloroethane. All domes were maintained at a pressure of 725 mm. Hg to avoid leakage of gas. Other operating specifications were: air flow, 40 cu. feet per minute; carbon dioxide level, less than 0.2 per cent; temperature, 24 ± 2° C., and relative humidity, 50 ± 10 per cent. The 1,1,1-trichloroethane concentration was monitored six times per hour with a Beckman model 109A hydrocarbon analyzer. Trichloroethane concentrations were maintained at ±5 per cent of the stated concentration by an air pressure-activated induction system with a flow meter and evaporator. The hydrocarbon analyzer was standardized daily.

Exposure Procedure

The trichloroethane concentrations were established and were at equilibrium before introduction of the test animals. The domes were maintained at these concentrations continuously for 14 weeks. Each week, a cage containing 10 mice was removed from each dome. The mice were sacrificed within 5 to 45 minutes after removal from the domes for electron microscopic studies and within 5 to 75 minutes for light microscopy and liver triglyceride determinations. While the animals were in the domes, water and the standard laboratory diet were available *ad libitum*. The solid food was changed daily to avoid accumulation of adsorbed trichloroethane. Water was dispensed by lick-activated elixir valves.

Rats, dogs, and monkeys were also housed within each dome area. These animals were not sequentially examined for histopathology during the 14 weeks and are not considered here.

Following the 14-week exposure period, 20 mice per dome were placed in an animal-holding facility, were fed a comparable diet, and breathed room air. Ten mice per dome group were sacrificed at 2-week intervals postexposure.

Liver Sampling Procedures

After cervical dislocation, each mouse was weighed quickly. The liver was removed rapidly and weighed separately. All livers were sampled for routine light microscopy. Frozen sections of formalin-fixed tissue were prepared on three livers per dome group and stained for fat with oil red O.

For the electron microscopic portion of the study, three mice per dome group were selected at random and sacrificed by cervical dislocation; small biopsies were immediately taken of these livers with a razor blade, before any other procedures were begun.

For triglyceride analysis, the remainder of the livers within a dome group were pooled, placed into three bags, frozen in liquid nitrogen, and stored at −20° C. Just prior to analysis, the pooled livers in each bag were thawed and diced. One gram of tissue (wet weight) was removed from each bag and was analyzed for triglyceride (2).

All animals were autopsied. Samples of heart, lung, brain, intestine, liver, kidney, and pancreas were fixed in 10 per cent formalin (4 per cent formaldehyde) in neutral phosphate buffer and were processed for routine paraffin sections stained with hematoxylin and eosin.

Electron Microscopy

Immediately after removal, each biopsy of liver for electron microscopy was diced into 1-cu. mm. blocks and fixed for 6 hours in 2 per cent formaldehyde-2 per cent glutaraldehyde-0.001 per cent picric acid buffered at pH 7.4 with 0.08 M sodium cacodylate (11). The blocks were placed in 0.1 M cacodylate buffer overnight and postfixed in 2 per cent osmium tetroxide in 0.1 M cacodylate buffer for 2 hours. All samples were dehydrated in ethanol-water solutions and embedded in Epon 812.

One-micrometer plastic sections were cut from each block with glass knives, stained with toluidine blue, and examined with a light microscope. Since it was evident by light microscopy that the toxic alterations occurred preferentially in a centrilobular distribution, those blocks with clearly identifiable central veins were chosen for electron microscopy. Ultrathin sections of centrilobular regions were cut with diamond knives, stained with uranyl acetate and lead citrate, and examined in a JEM-100B transmission electron microscope (Jeolco U. S. A., Medford, Massachusetts).

RESULTS

General Observations

During the course of the experiment, there were no obvious differences between control and exposed mice in terms of spontaneous activity, food and water intake, and general appearance of their hair coats.

Liver weight was increased in exposed mice, particularly in mice exposed to 1000 p.p.m. of 1,1,1-trichloro-

TABLE 1. EFFECTS OF 1,1,1-TRICHLOROETHANE EXPOSURE ON MOUSE LIVER WEIGHT AND TRIGLYCERIDE CONTENT

Wk. of exposure	Liver wt.			Liver wt./100 gm. body wt.			Liver triglyceride		
	Control	250 p.p.m.	1000 p.p.m.	Control	250 p.p.m.	1000 p.p.m.	Control	250 p.p.m.	1000 p.p.m.
	gm.						*mg./gm.*		
1	1.74	1.82	1.98	5.80	6.14	7.34[b]	4.01	6.04[a]	
2	1.71	1.97[a]	2.38[b]	5.59	6.27[a]	8.05[b]	5.57	5.94	22.54[b]
3	1.94	1.98	2.15	5.68	5.83	6.98[b]	6.24	3.75[a]	29.66[b]
4	1.96	1.94	2.31[b]	5.88	5.84	7.50[b]	4.90	7.53[b]	29.47[b]
5	1.93	1.88	2.15	5.55	5.76	6.54[a]	7.53	7.33	38.77[b]
6	1.93	2.02	2.07	5.49	5.98	6.45[b]	6.90	9.50	20.80[b]
7	1.95	2.05	2.55[b]	5.57	5.92	7.42[b]	7.47	6.30	40.90[b]
8	1.84	2.22[b]	2.28	5.32	6.62[b]	7.07[b]	6.93	10.43	21.40[a]
9	2.01	2.26	2.60[b]	5.52	6.21[a]	7.72[b]	4.37	6.20	28.73[a]
10	2.10	2.16	2.26	5.72	6.21	7.06[b]	5.37	6.23	24.73[b]
11	2.43	2.29	2.50	6.05	6.17	7.26[b]	6.40	4.53	19.67[b]
12	2.05	2.20	2.78[b]	5.83	5.99	7.66[b]	3.87	3.93	18.33[b]
13	2.35	2.45	2.53	6.03	6.56	7.31[b]	3.93	6.30[b]	12.50
14	2.28	2.34	2.46	5.89	6.24	7.20[b]	4.83	6.30	16.27[a]
Postexposure									
2	2.38	2.60	2.19	6.05	6.38	5.91	3.83	4.10	5.00
4	2.27	2.37	2.54	6.22	6.34	7.09[a]	3.40	3.45	2.60

[a] Significant at the 0.05 level.
[b] Significant at the 0.01 level.

ethane (Table 1). Comparison of liver weights by the Student *t*-test indicated that the increase at 1000 p.p.m. was significant ($p < 0.01$) at five sampling periods during the exposure. Presumably, the number of animals was too small for the weight differences to be significant consistently at all sampling periods. When liver weight was corrected for variation in body weight, the liver weight per 100 gm. body weight was elevated significantly at most of the sampling periods, even at 1 week (Table 1). At the 250-p.p.m. exposure level, liver weight per 100 gm. body weight was not generally elevated significantly, but it was once, at the 8-week sampling period. Liver triglyceride levels were elevated significantly in those mice exposed to 1000 p.p.m. but not in mice exposed to 250 p.p.m. of 1,1,1-trichloroethane (Table 1).

LIGHT MICROSCOPY

Standard histopathologic examination of paraffin sections of organs obtained at autopsy indicated that the principal morphologic alterations had occurred in the liver. From 1 to 14 weeks of exposure, livers from animals exposed to 1000 p.p.m. of 1,1,1-trichloroethane had prominent swelling of centrilobular hepatocytes. This swelling usually was associated with the presence of numerous small cytoplasmic vacuoles that did not displace the nucleus from its central location within the cell. The extent of this vacuolation in the liver lobule varied somewhat from animal to animal, but often vacuolation extended to the midpoint between central and portal veins, which in this report is considered the edge of the centrilobular region. Occasional cells, usually near central veins, showed so-called vacuolar degeneration (21) or "balloon degeneration" (25), *i.e.*, entensive enlargement due to vacuolation of the cytoplasm (Figs. 1 and 2). In these ballooned cells, the nuclei usually were pyknotic and occasionally were displaced by a large vacuole. In some cells, a portion of the cytoplasm remained free of vacuolar degeneration and resembled a condensed mass of very eosinophilic cytoplasm (Fig. 2).

After 12 weeks of exposure to 1000 p.p.m. of 1,1,1-trichloroethane, vacuolation of centrilobular hepatocytes was reduced, particularly in those cells adjacent to the central vein. However, occasional ballooned cells were observed in some animals after 14 weeks of exposure to 1000 p.p.m.

Necrosis of individual hepatocytes in the centrilobular zone became evident after 10 weeks of exposure to 1000 p.p.m. of 1,1,1-trichloroethane. By 12 weeks of exposure to 1000 p.p.m., 40 per cent of the animals had necrosis of hepatocytes, with an associated focal acute inflammatory infiltrate.

In the 1000 p.p.m. group, frozen sections stained with oil red O indicated that the fine vacuolation of the cytoplasm of centrilobular hepatocytes was associated with a parallel increase in the lipid content of these cells (Fig. 3). Some clearing of the fat content of centrilobular hepatocytes became evident after 12 weeks of exposure and was most pronounced in those cells adjacent to central veins. Similarly, the hepatic triglyceride content increased in the livers of animals exposed to 1000 p.p.m. of 1,1,1-trichloroethane (Fig. 3). The triglyceride levels correlated well with the evaluation of oil red O stains.

In animals exposed to 250 p.p.m. of 1,1,1-trichloroethane, fat accumulation was not elevated significantly above control values (Fig. 4). In two mice, centrilobular balloon degeneration of hepatocytes was noted, and one of these mice had necrosis of individual hepatocytes with a focal infiltrate of neutrophilic leukocytes.

Light microscopic examination of 1-μm. plastic sections confirmed the fat accumulation in centrilobular hepatocytes indicated by the other methods. In addition,

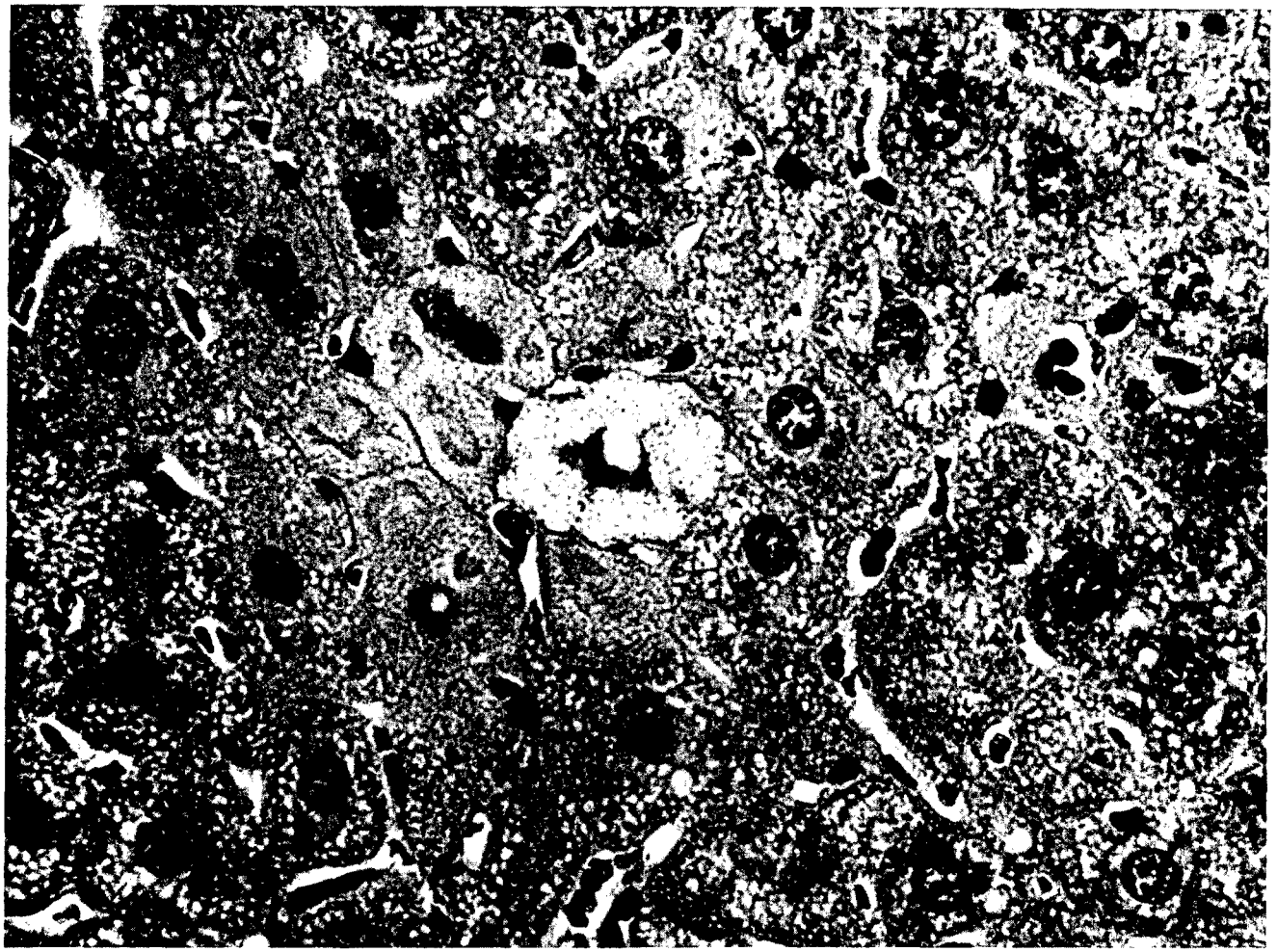

FIG. 1. Light micrograph showing an individual cell that has undergone balloon degeneration. The nucleus is pyknotic and deformed by a central vacuole. Other nearby cells show less severe cytoplasmic vacuolation attributed to triglyceride accumulation; 1000 p.p.m. of 1,1,1-trichloroethane, 4 weeks of exposure. Hematoxylin and eosin; ×770.

occasional, empty appearing vacuoles in centrilobular hepatocytes were noted that were distinctly different from the very osmiophilic fat droplets. Such empty appearing vacuoles were present in centrilobular hepatocytes from animals exposed to 1000 p.p.m. and in a few animals exposed to 250 p.p.m. of 1,1,1-trichloroethane.

In the postexposure samples, there was no evidence of significant fatty change in hepatocytes, and hepatic triglycerides were at control levels (Figs. 3 and 4). In some of the livers in the 1000 p.p.m. group, aggregates of hypertrophied Kupffer cells that contained a green pigment marked sites of prior hepatocyte necrosis. Rarely, a few centrilobular regions had a disordered architecture, suggesting collapse accompanied with hypertrophy of regenerating hepatocytes.

In those animals observed in the postexposure period, it became evident that many control and experimental mice had enlarged spleens and evidence of granulocytopoiesis in the hepatic sinusoids. Megakaryocytes rarely were present in the livers, but were abundant in the spleens. In a few animals, focal collections of mature granulocytes were indistinguishable from areas of acute inflammation. Tissue Gram stains (MacCallum-Goodpasture stains) were negative for bacteria.

ELECTRON MICROSCOPY

The electron microscopic studies were restricted to an evaluation of the regions in which damage was visible by light microscopy, *i.e.*, the centrilobular zone.

Control livers from unexposed mice were examined at each sampling time but were indistinguishable from each other. Centrilobular hepatocytes in control animals were rather large cells, occasionally binucleate, with abundant cytoplasm (Fig. 5). The cytoplasm typically contained rod-shaped mitochondria, a few cisternae and tubules of rough endoplasmic reticulum, and a small amount of smooth endoplasmic reticulum generally associated with aggregates of glycogen. Polyribosomes were readily observed attached to the membranes of the rough endoplasmic reticulum. The Golgi complex tended to be small. Occasional microbodies (peroxisomes) were present. Small triglyceride droplets were present.

In those animals exposed to 1000 p.p.m. of 1,1,1-trichloroethane, centrilobular hepatocytes contained a moderate increase in triglyceride droplets after 1 week of exposure, but often had few other indications of damage. After 4 weeks of exposure, the majority of centrilobular

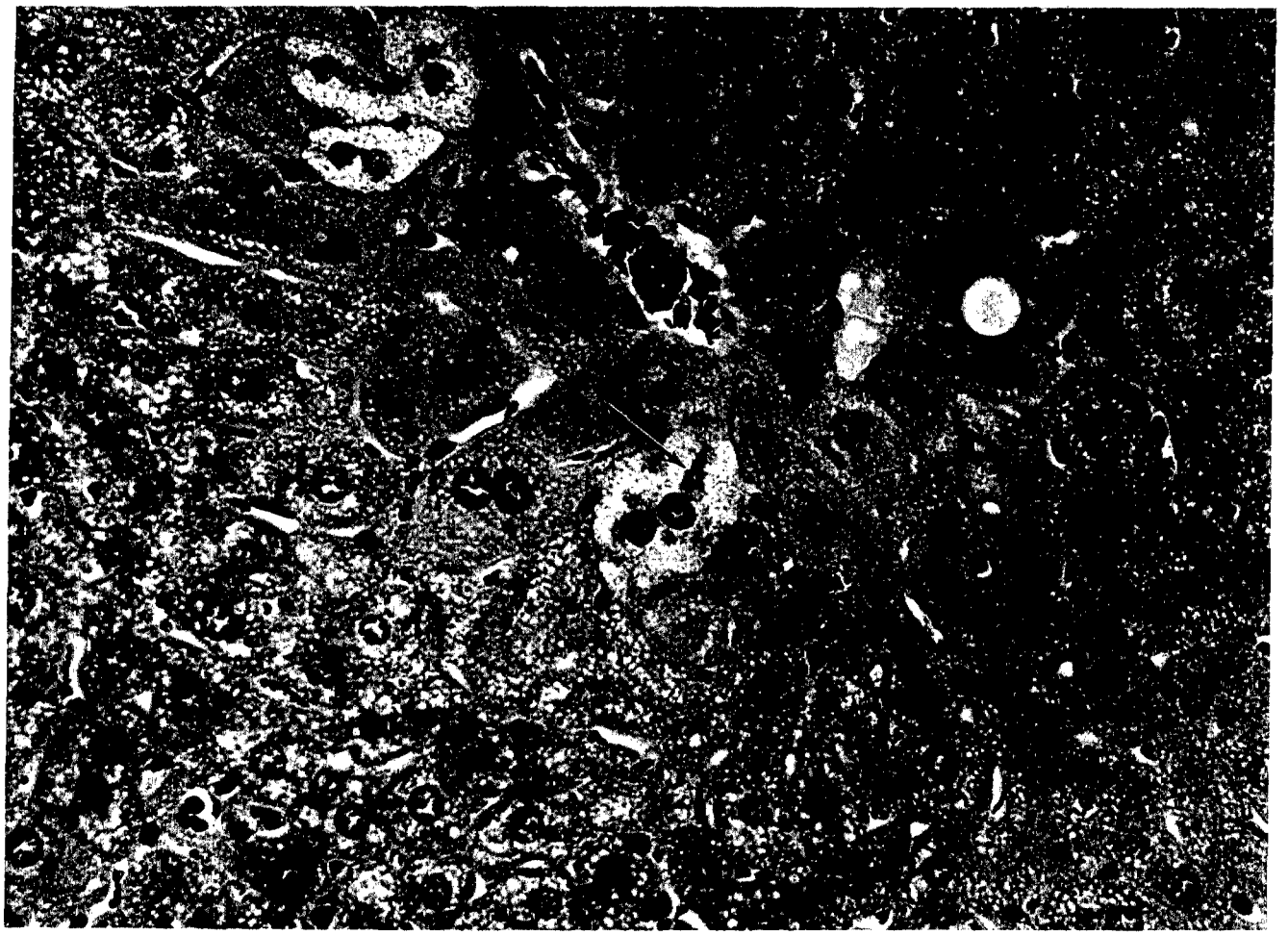

FIG. 2. Light micrograph of a centrilobular hepatocyte with balloon degeneration and a prominent eosinophilic cytoplasmic mass (*arrow*); 1000 p.p.m. of 1,1,1-trichloroethane, 4 weeks of exposure. Hematoxylin and eosin; ×480.

hepatocytes had extensive cytoplasmic alterations, although the severity of these alterations varied from cell to cell. The rough endoplasmic reticulum was vacuolated and tended to have only a few ribosomes attached to the membrane (Fig. 6). Polyribosome configurations were rare. Within the lumen of the vesicles, there were small triglyceride droplets (Fig. 6) and occasionally small, very osmiophilic whorls of lamellae. These osmiophilic whorls often were seen at the periphery of the triglyceride droplets. There was a striking increase in the volume of cytoplasm containing tubules, resembling smooth endoplasmic reticulum in a tight meshwork (Fig. 7). Microbodies were markedly increased in number (Fig. 7). Lysosomal vesicles were more prominent than in control cells and frequently contained degenerated organelles and osmiophilic membranous whorls ("myelin figures").

In occasional cells from animals exposed to 1000 p.p.m. of 1,1,1-trichloroethane, centrilobular hepatocytes often had extensive cytoplasmic alterations of the type described above for 4 weeks of exposure (Fig. 8). Although the severity of the changes was decreased in many centrilobular hepatocytes at 14 weeks (compared with 4 weeks), triglyceride droplets, microbodies, and smooth endoplasmic reticulum remained increased, compared with control levels. In some cells, severe alterations persisted (Fig. 8).

In animals exposed to 250 p.p.m. of 1,1,1-trichloroethane, centrilobular hepatocytes frequently were indistinguishable from control hepatocytes by the methodology used. Occasional cells had vesiculated rough endoplasmic reticulum containing small lipid droplets. These cells also had an increase in smooth endoplasmic reticulum and an increased number of microbodies and triglyceride droplets. Rarely, ballooned vesicles of rough endoplasmic reticulum were noted. The cytoplasmic alterations in the 250-p.p.m. group were evident after 10 weeks of exposure, but were not as dramatic as those observed at 1000 p.p.m.

DISCUSSION

The hepatic toxicity of chlorinated hydrocarbons has been investigated for many years; particular emphasis has been placed on the toxicology of carbon tetrachloride (16). Gradually, other solvents have been developed to replace carbon tetrachloride in many industrial uses, but the investigation of these other solvents has received relatively little attention.

The hepatotoxicity of carbon tetrachloride is dependent on its metabolic conversion to a toxic intermediate,

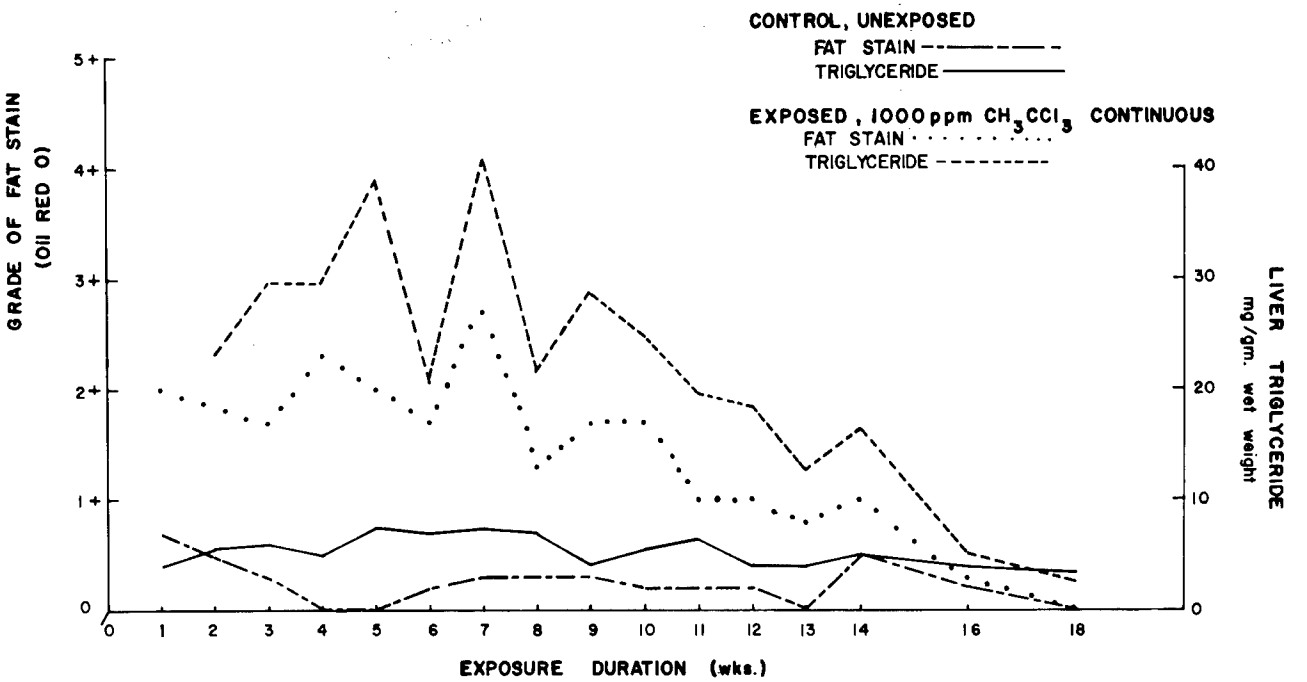

FIG. 3. Liver fat accumulation. Composite graph of data from oil red O stains for fat (which were graded from 1 to 5 in severity of fatty change) and from triglyceride determinations on both control mice and mice exposed to 1000 p.p.m. of 1,1,1-trichloroethane. After 14 weeks, all animals were exposed only to room air. The graph shows a close correspondence between the results of oil red O stain and triglyceride analysis. In the 1000 p.p.m. group, fat accumulation peaked at approximately 7 weeks; there was partial recovery after 7 to 14 weeks, and full recovery from fat accumulation after the exposure to 1,1,1-trichloroethane was stopped at 14 weeks. Oil red O-stained sections were graded according to the following criteria: *1+*, fatty change, microglobular marked, up to five cell diameters from border to central vein; *2+*, fatty change, microglobular marked, up to 10 cell diameters from border to central vein; *3+*, fatty change, microglobular marked, greater than 10 cell diameters from central vein, with many confluent areas and some tendency for macroglobular fat. Higher grades (*4+*, *5+*) were not encountered. The graph connects adjacent means (of three determinations) by a straight line; no attempt to fit curves was made. The close correspondence between fluctuations in triglyceride level and fat stains suggests that the fluctuations may be due to variations in the status of the animal response, possibly artifactual.

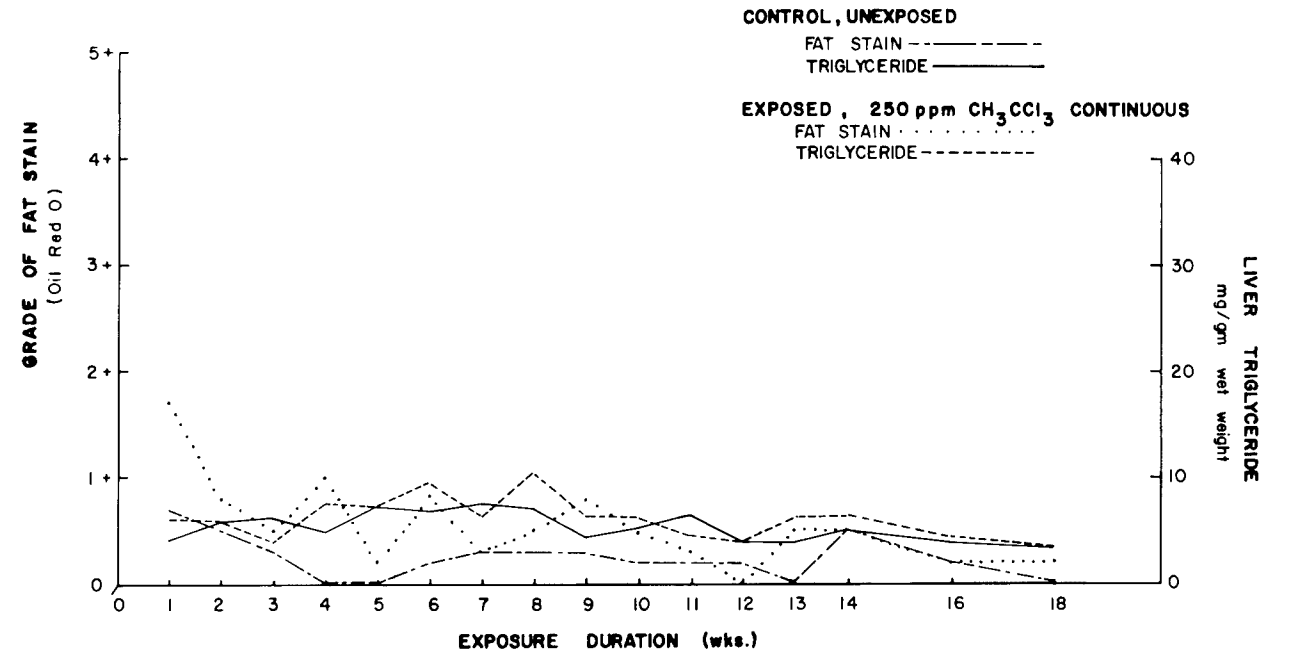

FIG. 4. Liver fat accumulation. Composite graph, similar to that shown in Figure 5, shows the close correspondence of fat stains and triglyceride values in both control mice and mice exposed to 250 p.p.m. of 1,1,1-trichloroethane for 1 to 14 weeks. This indicates that 250 p.p.m. is near the threshold dose for toxic effects on hepatic triglycerides.

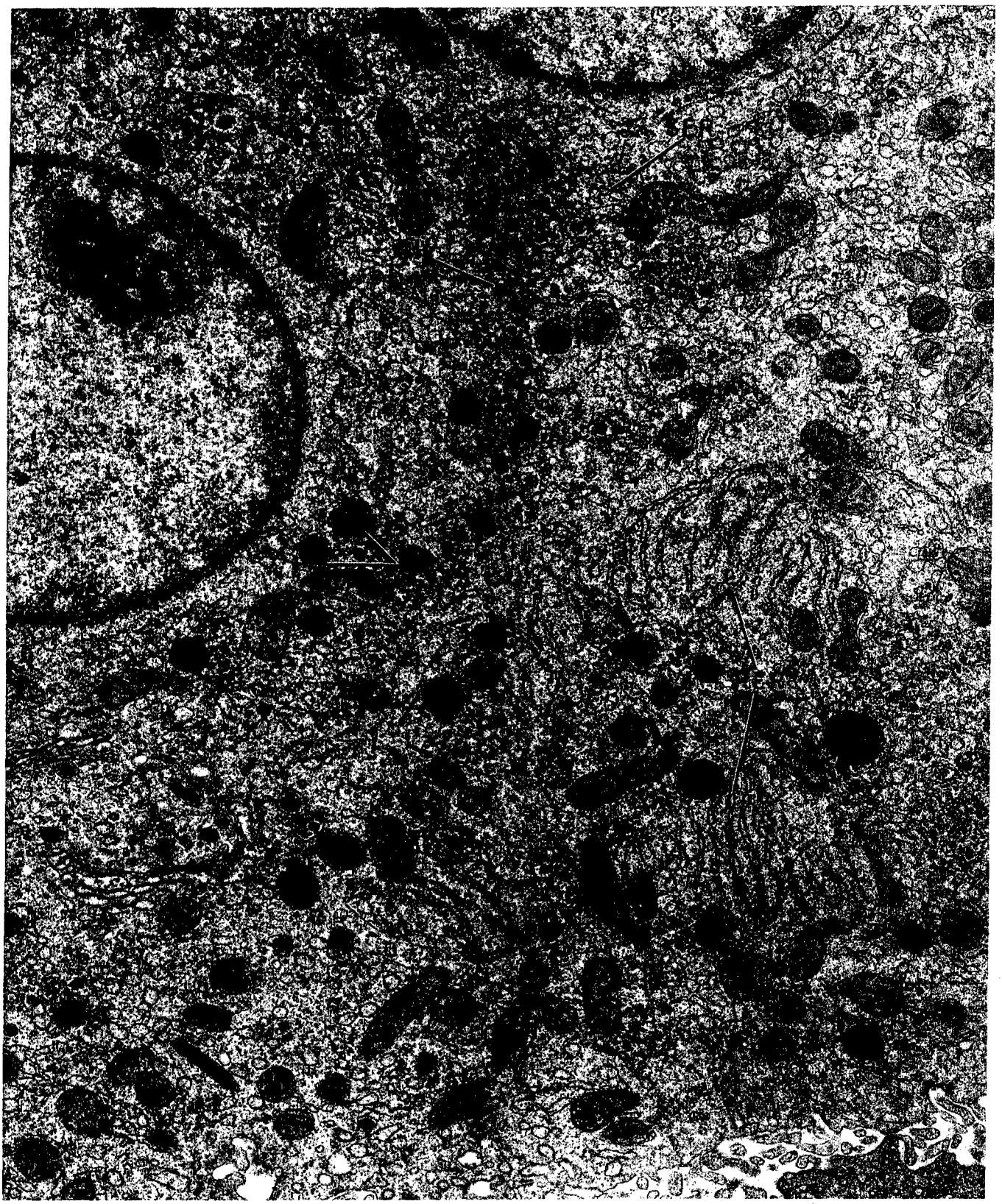

FIG. 5. Electron micrograph of a centrilobular hepatocyte from a control mouse that had breathed room air in the dome for 1 week. This particular hepatocyte is rather large and binucleate and demonstrates most of the features of control centrilobular hepatocytes. Cisternae and tubules of rough endoplasmic reticulum (*RER*) are present in moderate abundance. A small amount of smooth endoplasmic reticulum (*SER*) is associated with glycogen granules (*Gly*). Mitochondria are elongate, occasional microbodies (*MB*) are present, and a small Golgi region is visible. ×17,700.

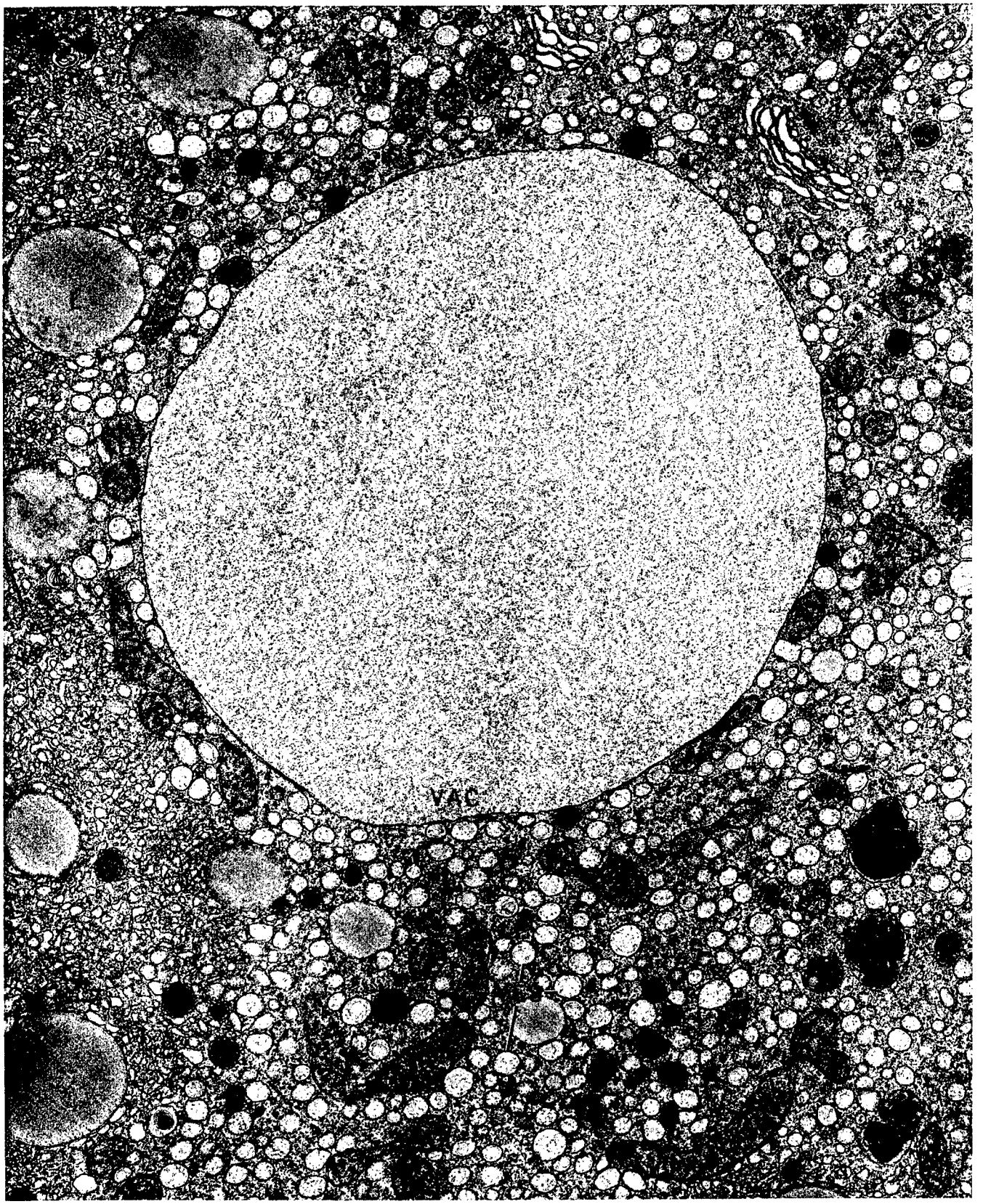

FIG. 6. Electron micrograph of a portion of the cytoplasm of a centrilobular hepatocyte after 4 weeks of exposure to 1000 p.p.m. of 1,1,1-trichloroethane. The rough endoplasmic reticulum is vesiculated (*VES*). The large vacuole (*VAC*) filled with flocculent material corresponds to ballooned rough endoplasmic reticulum that has lost most of its ribosomes. Smaller vesicles often have triglyceride droplets in the lumen. Also increased are smooth endoplasmic reticulum, lipid (*L*), microbodies, and very osmiophilic lysosomes containing degenerating membranous organelles. Glycogen is absent. ×17,250.

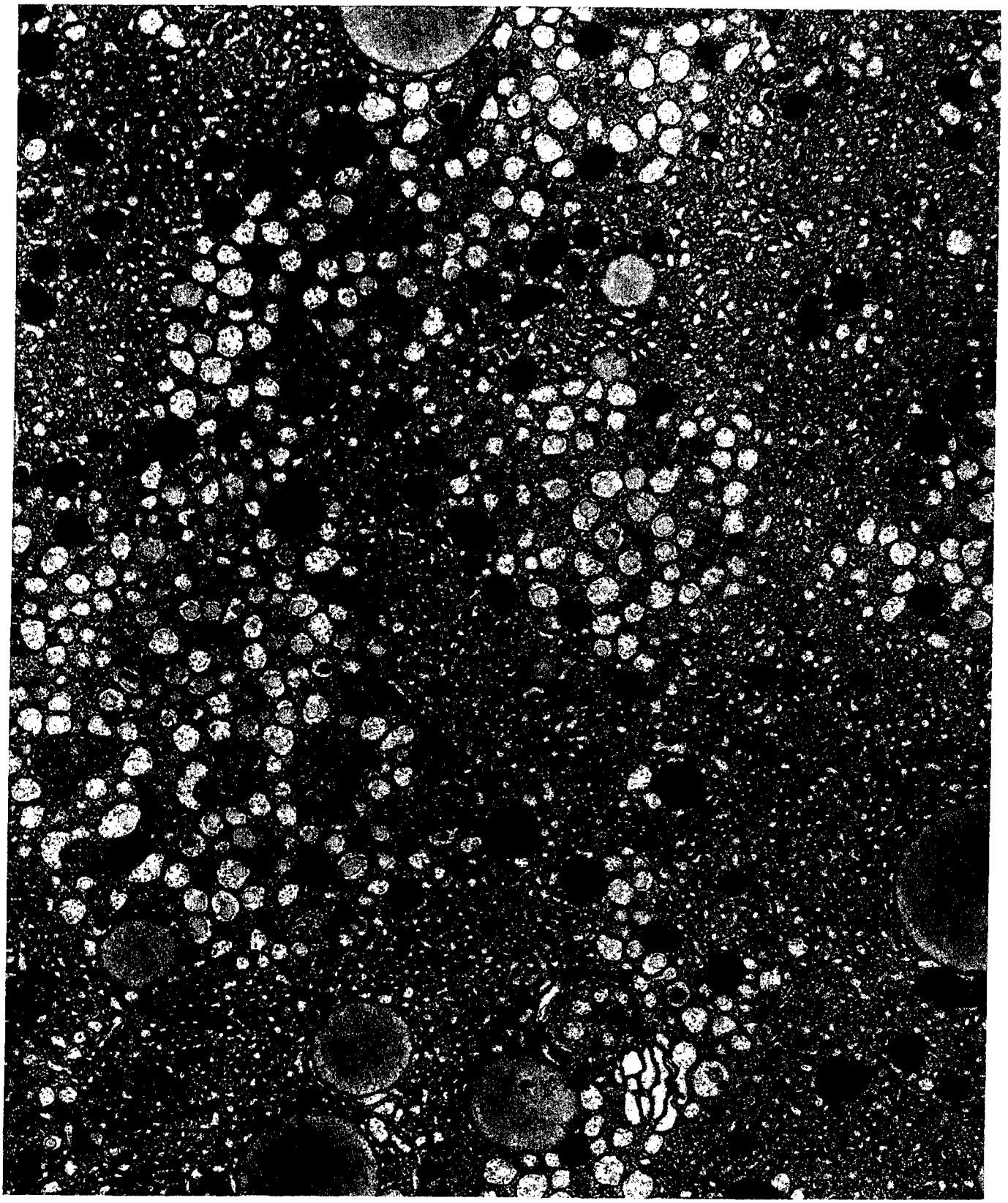

FIG. 7. Electron micrograph of centrilobular hepatocyte cytoplasm after 4 weeks of exposure to 1000 p.p.m. of 1,1,1-trichloroethane. This illustration shows the marked hypertrophy of the smooth endoplasmic reticulum (*SER*) as well as the vesiculation of the rough endoplasmic reticulum (*VES*). Lipid and small osmiophilic membranous whorls are in the lumen of the vesicles. Microbodies (*MB*) are abundant. Glycogen is absent. ×24,000.

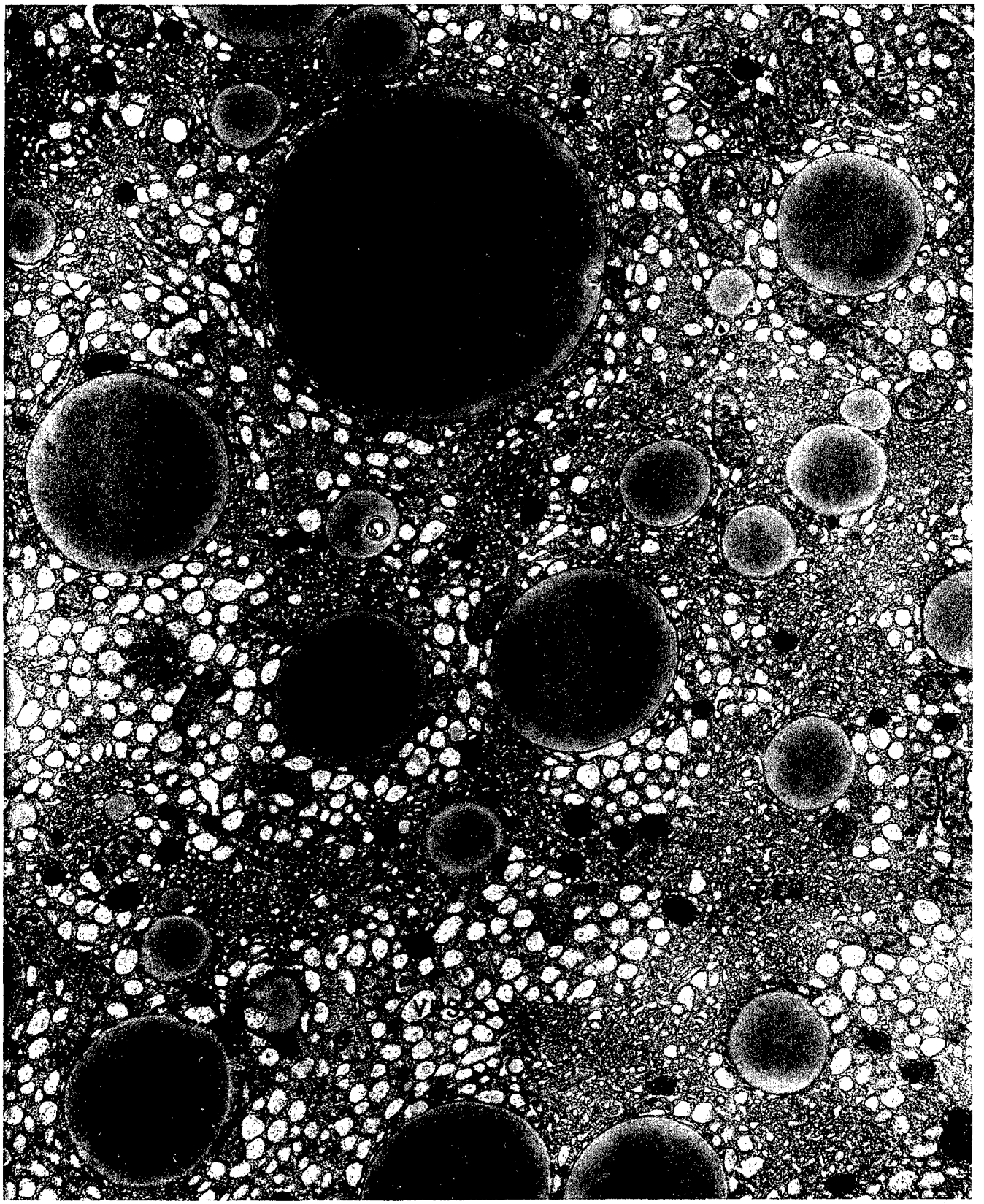

FIG. 8. Electron micrograph of the cytoplasm in a centrilobular hepatocyte after 14 weeks of exposure to 1000 p.p.m. of 1,1,1-trichloroethane. This illustration shows the severe degree of cytoplasmic alteration that persists in some centrilobular hepatocytes that are not adjacent to the central vein. Lipid droplets (*L*) are abundant, both free in the cytoplasm and within the vesicles of rough endoplasmic reticulum (*VES*). Tubules of smooth endoplasmic reticulum (*SER*) and microbodies are prominent. ×14,750.

the exact nature of which has yet to be determined. Strong evidence for this concept has been provided by Dawkins (4), who found that liver of the newborn rat is much less sensitive to carbon tetrachloride than it is 7 days after birth. This implies that the increased levels of certain enzymes in hepatocytes after birth are necessary to convert carbon tetrachloride to its toxic metabolite. Chopra *et al.* (3) have suggested that the enzyme necessary for this conversion is reduced nicotinamide adenine dinucleotide phosphate-cytochrome *c* reductase. It has been suspected for some time that the toxic metabolite is a free radical, perhaps $\cdot CCl_3$ or $\cdot Cl$. Such a free radical would be expected to catalyze peroxidation of the unsaturated fatty acids in the nearby membranes, *i.e.*, the endoplasmic reticulum. Recknagel (16) has hypothesized that lipid peroxidation of the microsomal membranes causes morphologic alterations of the endoplasmic reticulum, loss of drug-metabolizing activity, loss of glucose-6-phosphatase activity, depression of protein synthesis, and loss of formation and excretion of low density β-lipoproteins. The depression of protein synthesis and the loss of ability to secrete lipoproteins are considered the most important mechanisms involved in the accumulation of triglyceride in hepatocytes exposed to carbon tetrachloride. Similar mechanisms would be expected to be operative for 1,1,1-trichloroethane.

It is interesting that the necrogenic effect of hepatotoxins can be separated to some extent from the effect of triglyceride accumulation. For example, ethionine, an analog of methionine, will induce severe fat accumulation in the hepatocytes of female rats without causing necrosis (16). In this study, 40 per cent of the animals showed evidence of necrosis of individual hepatocytes after 12 weeks of exposure to 1,1,1-trichloroethane. Carbon tetrachloride and chloroform can cause much more severe hepatocyte necrosis throughout the centrilobular zone (15).

Unfortunately, relatively little is known about the metabolism of 1,1,1-trichloroethane and its resemblance to chloroform. Hake *et al.* (9) prepared 1-^{14}C-1,1,1-trichloroethane and found that, after a single injection intraperitoneally in the rat, 99 per cent of the radioactivity was recovered as unchanged 1-^{14}C-1,1,1-trichloroethane in the expired air. Most of the remaining label (1 per cent) was recovered in the urine, 0.25 per cent was volatile on air drying, and 0.75 per cent was present as 2,2,2-trichloroethanol, excreted as the glucuronide. Less than 0.01 per cent was isolated from the liver tissue.

Since respiratory exchange appears to be the principal route of excretion of 1,1,1-trichloroethane, the inhalation toxicity studies performed are of particular interest. Administration of the compound by inhalation would effectively block the main path of excretion and allow the animal to achieve a tissue equilibrium concentration. Under these conditions, considerable amounts of 1,1,1-trichloroethane might be metabolized in the liver and large amounts of 2,2,2-trichloroethanol might be formed (although the metabolism has not been studied after inhalation exposure). The fact that 1,1,1-trichloroethane undergoes hydroxylation suggests that one important adaptive mechanism in the exposed animal may be the stimulation of drug-hydroxylating enzymes, such as those associated with the smooth endoplasmic reticulum (12, 18). The present study indicates that, within 4 weeks of exposure to 1000 p.p.m. of 1,1,1-trichloroethane, there was a considerable increase in the volume of membranous tubules resembling smooth endoplasmic reticulum. Reynolds, Ree, and Moslen (20) have demonstrated that hypertrophy of the smooth endoplasmic reticulum in response to phenobarbital administration potentiates the damage resulting from subsequent exposure to carbon tetrachloride. Therefore, it is possible that, if smooth endoplasmic reticulum formation is stimulated by 1,1,1-trichloroethane, this stimulation may be detrimental to the ability of the hepatocytes to resist damage resulting from this highly chlorinated compound. On the other hand, judging stimulation of smooth endoplasmic reticulum by morphologic techniques alone may not be reliable. Reynolds and Ree (19) have found that aggregates of smooth surfaced membranous tubules appear within 30 minutes after poisoning with carbon tetrachloride. This time course seems too rapid for membrane proliferation and suggests that some of the aggregates of tubules result from denaturation and condensation of membranes from rough and smooth endoplasmic reticulum. On the basis of the present study of 1,1,1-trichloroethane, a clear distinction is not possible between proliferation and denaturation as the source of the smooth surfaced tubules that were abundant in the centrilobular hepatocytes in the 1000 p.p.m. group. Levels of hydroxylase activity would have to be measured and surface areas of endoplasmic reticulum membranes would have to be determined quantitatively in making such a distinction.

A dramatic increase in the number of microbodies was found after 4 to 14 weeks of exposure to 1000 p.p.m. of 1,1,1-trichloroethane. Microbodies are membrane-bounded organelles that are found in a large number of cell types (7, 10). The functions of microbodies may differ in various cell types, depending on the exact enzymatic composition of the microbody matrix. The enzyme most consistently found in microbodies is catalase, and this usually is associated with certain oxidase enzymes (13). The concept has arisen that the oxidases may generate hydrogen peroxide that might be harmful to the cell if not broken down by the catalase packaged in the microbodies (5, 6). Microbodies have also been associated with lipid metabolism in hepatocytes (17), but the exact nature of this association has not been clearly delineated. Observations by Reddy (17) suggest that the microbody proliferation found in this study could be related either to the accumulation of hepatic triglyceride or possibly to a response of the hepatocyte to lipoperoxidation by increasing catalase content of the cytoplasm. A relationship between microbodies and lipid turnover seems the most likely possibility since Gordon and Lough (8) have found that microbodies become plentiful by the 5th day of regression (following cessation of ethanol ingestion) of a fatty liver in rats.

In a previous study from this laboratory by Weinstein, Boyd, and Black (25), the toxicity of dichloromethane was investigated after continuous inhalation of 5000 p.p.m. of dichloromethane in air. At this concentration, the hepatic triglycerides rose to peak levels by 3 days and promptly returned almost to control levels by the end of 7 days. With 1,1,1-trichloroethane, the peak of triglyceride accumulation was rather broad and occurred approximately at 7 weeks. Recovery was partial and gradual over the 7- to 14-week intervals of exposure. In the dichloromethane study, centrilobular cells underwent "balloon degeneration" associated with prominent dilation of cisternae of rough endoplasmic reticulum and perinuclear cisternae (25). In contrast, 1,1,1-trichloroethane caused only occasional centrilobular cells to undergo severe balloon degeneration, but many cells had a few ballooned cisternae of rough endoplasmic reticulum. Hepatocytic necrosis was reported in the dichloromethane study (25, 26). Similarly, 1,1,1-trichloroethane-exposed animals had occasional necrotic hepatocytes associated with focal infiltrates of neutrophilic leukocytes in the hepatic lobules. Such necrosis should be recognized as an important aspect of the toxicology of 1,1,1-trichloroethane, and possibly of dichloromethane as well, since it occurs in the critical centrilobular region of the lobule, even though it is not as impressive as the necrosis caused by chloroform or carbon tetrachloride (15).

Caution should be exercised in interpreting the differences between the toxic effects of dichloromethane (26) and of 1,1,1-trichloroethane. Although observed differences may be related to differences in the structure and metabolic fate of these compounds, other differences in the conduct of the experiments should be noted, *e.g.*, concentration of the compounds, exposure duration, and mouse strain. A further detailed comparison of the effects of dichloromethane and 1,1,1-trichloroethane may help to elucidate the nature of adaptive responses to these compounds and their relation to the structure of the halocarbon.

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