

General Disclaimer

One or more of the Following Statements may affect this Document

- This document has been reproduced from the best copy furnished by the organizational source. It is being released in the interest of making available as much information as possible.
- This document may contain data, which exceeds the sheet parameters. It was furnished in this condition by the organizational source and is the best copy available.
- This document may contain tone-on-tone or color graphs, charts and/or pictures, which have been reproduced in black and white.
- This document is paginated as submitted by the original source.
- Portions of this document are not fully legible due to the historical nature of some of the material. However, it is the best reproduction available from the original submission.

U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

AD-A020 124

HEPATIC PATHOLOGY IN MICE AFTER CONTINUOUS INHALATION
EXPOSURE TO 1,1,1-TRICHLOROETHANE

AEROSPACE MEDICAL RESEARCH LABORATORY

PREPARED FOR
NATIONAL AERONAUTICS AND SPACE

AUGUST 1975

ADA020124

SPACE MEDICAL RESEARCH LABORATORY
RESEARCH REPORT
SPACE MEDICAL RESEARCH LABORATORY

SPACE MEDICAL RESEARCH LABORATORY

REPORT



Approved for public release; restrictions are unlimited.

U.S. GOVERNMENT PRINTING OFFICE
1975 O 150-150
C

SPACE MEDICAL RESEARCH LABORATORY
RESEARCH REPORT
SPACE MEDICAL RESEARCH LABORATORY
Wright-Patterson Air Force Base, Ohio

Reproduced by
**NATIONAL TECHNICAL
INFORMATION SERVICE**
U S Department of Commerce
Springfield VA 22151

NOTICES

When US Government drawings, specifications, or other data are used for any purpose other than specifically stated Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever, and the fact that the Government may have furnished, furnished, or in any way supplied the said drawings, specifications, or other data, is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

Do not return this copy. Retain or destroy.

Please do not request copies of this report from Aerospace Medical Research Laboratory. Additional copies may be purchased from:

National Technical Information Service
5285 Port Royal Road
Springfield, Virginia 22151

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," DHEW 73-23.

This report has been reviewed and cleared for open publication and/or public release by the appropriate Office of Information (OI) in accordance with AFR 190-17 and DODD 5250.9. There is no objection to unlimited distribution of this report to the public at large, or by DDC to the National Technical Information Service (NTIS).

This technical report has been reviewed and is approved for publication.

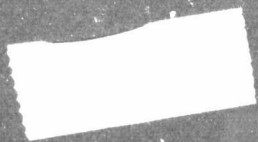
FOR THE COMMANDER

Anthony A. Thomas

ANTHONY A. THOMAS, M.D.
Director, Toxic Hazards Division
6570th Aerospace Medical Research Laboratory

AIR FORCE - 30-9-75 - 150

SEARCHED	INDEXED
SERIALIZED	FILED
BY	
CONTINUATION/AVAILABILITY	
DATE	
AVAIL. STATE	
A	



REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER AMRL-TR-74-60	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) HEPATIC PATHOLOGY IN MICE AFTER CONTINUOUS INHALATION EXPOSURE TO 1,1,1-TRICHLOROETHANE		5. TYPE OF REPORT & PERIOD COVERED Final Report
7. AUTHOR(s) Neil S. McNutt, Major, USAF, MC Robert I. Amster, Major, USAF, VC Ernest E. McConnell, Major, USAF, VC Fred Morris, TSgt, USAF		6. PERFORMING ORG. REPORT NUMBER
9. PERFORMING ORGANIZATION NAME AND ADDRESS Aerospace Medical Research Laboratory Aerospace Medical Division, AFSC Wright-Patterson Air Force Base, Ohio		8. CONTRACT OR GRANT NUMBER(s) In part under contract F33615-73-C-4059
11. CONTROLLING OFFICE NAME AND ADDRESS Aerospace Medical Research Laboratory Aerospace Medical Division, AFSC Wright-Patterson Air Force Base, Ohio		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62202F; 6302; 630206; 63020620
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		12. REPORT DATE August 1975
		13. NUMBER OF PAGES 58
		15. SECURITY CLASS. (of this report) Unclassified
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release; distribution unlimited.		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report) PRICES SUBJECT TO CHANGE		
18. SUPPLEMENTARY NOTES This study was partially supported by the National Aeronautics and Space Administration/Manned Spacecraft Center under Project Order NASA T-9035B.		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Endoplasmic reticulum Liver triglyceride Fatty liver Methylchloroform Hepatic necrosis 1,1,1-Trichloroethane Inhalation toxicology Liver		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Male CF-1 mice (24-34g) were exposed to either 250ppm or 1,000ppm 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 1,000ppm group. Moderate liver triglyceride accumulation was evident in the 1,000ppm group and peaked at 40mg/gm of tissue (wet weight) after 7 weeks of exposure. Partial recovery was indicated by a drop in the hepatic triglyceride		

20. Abstract (Continued)

level to 16mg/gm by 14 weeks of exposure to 1,000ppm. Electron microscopy revealed that cytoplasmic alterations were most severe in centrilobular hepatocytes in the 1,000ppm group, and were mild to minimal in the 250ppm group. These alterations consisted of vesticulation of the rough endoplasmic reticulum with loss of attached polyribosomes, increased smooth endoplasmic reticulum, microbodies, and triglyceride droplets. Some cells had balloned cisternae of the rough endoplasmic reticulum. Focal hepatocyte necrosis occurred in 40% of the mice exposed to 1,000ppm for 12 weeks. This necrosis was associated with an acute inflammatory infiltrate and hypertrophy of Kupffer cells. These findings indicate that the pathological alterations observed with 1,1,1-trichloroethane are 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 are of similar type to those produced by carbon tetrachloride but appear much less severe.

PREFACE

This study was partially supported by the National Aeronautics and Space Administration/Manned Spacecraft Center under Project Order NASA T-9035B. The research was performed at the Toxic Hazards Division, Pathology Branch, Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio from July, 1973 to May, 1974. The authors gratefully acknowledge the valuable assistance of the staff from the University of California at Irvine who performed the exposures under contract F33615-73-C-4059.

INTRODUCTION

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 (Adams et al., 1950; Torkelson et al., 1958). 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 pathological 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 (Adams et al., 1950; Torkelson et al., 1958; Rowe et al., 1963). Intermittent exposure to 1,000ppm in air (3.0 hrs/day, 5 days/wk, 3 months) produced centrilobular fatty change in the livers of guinea pigs. Intermittent inhalation exposure to 500ppm (7 hrs/day, 5 days/wk, 6 months) produced no effect on rats, guinea pigs, rabbits, and monkeys, when compared to controls, in terms of growth, organ weights, hematologic values, gross pathology, and histopathology (Torkelson et al., 1958).

In the present study, mice were chosen as the test animal since a previous experiment with dichloromethane demonstrated that mice are quite sensitive to chlorinated hydrocarbon exposure (Weinstein and Diamond, 1972). Two levels of exposure to 1,1,1- trichloroethane were chosen: 1,000ppm to give a definite mild toxic effect, and 250ppm as an estimate of a concentration that might give a threshold effect or possibly no effect.

MATERIALS AND METHODS

Animals

Six hundred male CF-1 strain mice (Carworth Division; Becton, Dickinson and Co.; Portage, Mich.) were used. At the beginning of the experiment, the body weights were from 24 to 34 grams each. The animals were fed a standard laboratory diet (Purina Laboratory Chow, Ralston Purina, Co., St. Louis, Missouri) and were given water ad libitum. The Purina Laboratory Chow was used within 60 days of its milling date, and was stored at ambient temperatures. 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^R (Dow Chemical Company, Midland, Mich.) which is technical grade and contains 94 to 97% 1,1,1- trichloroethane, 2.4 to 3.0% dioxane, 0.12 to 0.30% butanol, and small amounts of ethylene dichloride, water and other materials (Torkelson et al., 1958). 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 (Torkelson et al., 1958).

Exposure Chambers

All inhalation exposures were performed in large controlled environment chambers called Thomas domes, that have been described elsewhere (McNerney and Mac Ewen, 1965; Thomas, 1965). Three domes were used: control, 250ppm trichloroethane, and 1,000ppm trichloroethane. All domes were maintained at 725mmHg pressure to avoid leakage of gas. Other operating specifications were: airflow 40 cubic feet/minute, carbon dioxide level less than 0.2%, temperature $24 \pm 2^\circ$ C., and relative humidity $50\% \pm 10\%$. The 1,1,1- trichloroethane

concentration was monitored 6 times per hour with a Beckman Model 109A hydrocarbon analyzer. Trichloroethane concentrations were maintained at \pm 5% of the stated concentration by an air pressure - activated induction system with a flowmeter 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.

Within each dome area, rats, dogs, and monkeys were also housed. These animals were not sequentially examined for histopathology during the 14 weeks and will not be considered in this manuscript.

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

Liver Sampling Procedures

After cervical dislocation, each mouse was quickly weighed. The liver was rapidly removed 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, sacrificed by cervical dislocation, and immediately small biopsies were taken of these livers with a razor blade before any other procedures.

For triglyceride analysis, the remainder of the livers were pooled within a dome group and 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 by the method of Butler et al. (1961).

All animals were autopsied. Samples of heart, lung, brain, intestine, liver, kidney, and pancreas were fixed in 10% formalin (4% 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 1mm^3 blocks and fixed for 6 hours in 2% formaldehyde - 2% glutaraldehyde - 0.001% picric acid buffered at pH 7.4 with 0.08M sodium cacodylate (Ito and Karnovsky, 1968). The blocks were placed in 0.1M cacodylate buffer overnight and post-fixed in 2% osmium tetroxide in 0.1M cacodylate buffer for 2 hours. All samples were dehydrated in ethanol-water solutions and embedded in Epon 812.

One-micron 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 were 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 (JOEL, USA, Medford, Mass.).

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 1,000ppm 1,1,1- trichloroethane (Table I). Comparison of liver weights by the students T-test indicated that the increase at 1,000ppm was significant ($P < 0.01$) at 5 sampling periods during the exposure. Presumably the number of animals was too small for the weight differences to be consistently significant at all sampling periods. When liver weight was corrected for variation in body weight, the liver weight per 100 grams body weight was significantly elevated at most of the sampling periods, even at 1 week (Table I). At the 250ppm exposure level, liver weight per 100 grams body weight was not generally elevated significantly but was once at the 8 week sampling period. Liver triglyceride levels were significantly elevated in those mice exposed to 1,000ppm but not in mice exposed to 250ppm 1,1,1- trichloroethane (Table I).

TABLE I

Effects of 1,1,1-Trichloroethane
Exposure on Mouse Liver Weight and Triglyceride Content

Week of Exposure	Liver Weights, Gms.			Liver weight/100 gms (gms) body weight			Liver Triglyceride mg/gm		
	Control	250 ppm	1000 ppm	Control	250 ppm	1000 ppm	Control	250 ppm	1000 ppm
1	1.74	1.82	1.98	5.80	6.14	7.34**	4.01	6.04*	-
2	1.71	1.97*	2.38**	5.59	6.27*	8.05**	5.57	5.94	22.54**
3	1.94	1.98	2.15	5.68	5.83	6.98**	6.24	3.75*	29.66**
4	1.96	1.94	2.31**	5.88	5.84	7.50**	4.90	7.53**	29.47**
5	1.93	1.88	2.15	5.55	5.76	6.54*	7.53	7.33	38.77**
6	1.93	2.02	2.07	5.49	5.98	6.45**	6.90	9.50	20.80**
7	1.95	2.05	2.55**	5.57	5.92	7.42**	7.47	6.30	40.90**
8	1.84	2.22**	2.28	5.32	6.62**	7.07**	6.93	10.43	21.40*
9	2.01	2.26	2.60**	5.52	6.21*	7.72**	4.37	6.20	28.73*
10	2.10	2.16	2.26	5.72	6.21	7.06**	5.37	6.23	24.73**
11	2.43	2.29	2.50	6.05	6.17	7.26**	6.40	4.53	19.67**
12	2.05	2.20	2.78**	5.83	5.99	7.66**	3.87	3.93	18.33**
13	2.35	2.45	2.53	6.03	6.56	7.31**	3.93	6.30**	12.50
14	2.28	2.34	2.46	5.89	6.24	7.20**	4.83	6.30	16.27*
Post-Exposure									
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*	3.40	3.45	2.60

*Significant at the 0.05 level.

**Significant at the 0.01 level.

Light Microscopy

Standard histopathological examination of paraffin sections of organs obtained at autopsy indicated that the principle morphological alterations were in the liver. From one week to 14 weeks of exposure, livers from animals exposed to 1,000ppm 1,1,1- trichloroethane showed prominent swelling of centrilobular hepatocytes (figs. 1, 2, and 3)*. Usually this swelling 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 vacuolization in the liver lobule varied somewhat from animal to animal, but often vacuolization extended out to the midpoint between central and portal veins, which in this report will be considered the edge of the centrilobular region. Occasional cells, usually near central veins, showed so-called "balloon degeneration", i.e. extensive enlargement due to vacuolization of the cytoplasm (fig. 2). In these ballooned cells, the nuclei were usually pyknotic and occasionally were displaced by a large vacuole.

After 12 weeks exposure to 1,000ppm 1,1,1- trichloroethane, vacuolization of centrilobular hepatocytes became somewhat reduced, particularly in those cells adjacent to the central vein. However, occasional ballooned cells were observed in some animals after 14 weeks exposure to 1,000ppm.

Focal necrosis of hepatocytes in the centrilobular zone became evident at 10 weeks exposure to 1,000ppm 1,1,1- trichloroethane. By 12 weeks exposure to 1,000ppm, 40% of the animals had focal necrosis of hepatocytes with an associated acute inflammatory infiltrate (fig. 4).

In the 1,000ppm group, frozen sections stained with Oil-Red-O indicated that the fine vacuolization of the cytoplasm of centrilobular hepatocytes was associated with a parallel increase in lipid content of these cells (fig. 5). Similarly, hepatic triglyceride content also increased in the livers of animals exposed to 1,000ppm 1,1,1- trichloroethane (fig. 5). 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.

* Figures are located in Appendix beginning on page 18.

In animals exposed to 250ppm 1,1,1- trichloroethane, fat accumulation was not significantly elevated above control values (fig. 6). In two mice, centrilobular balloon degeneration of hepatocytes was noted and one of these mice had focal hepatocyte necrosis with infiltration of neutrophilic leukocytes.

An interesting feature of the balloon degeneration, observed particularly in livers from animals in the 1,000ppm group, was that a portion of the cytoplasm often remained free of the ballooning change and resembled a condensed mass of very eosinophilic material (fig. 3). This appearance was strikingly similar to the light microscopic image of Mallory bodies or "alcoholic hyalin" often described in liver specimens from humans with toxic hepatic necrosis associated with alcohol abuse (Popper and Schaffner, 1957).

Light microscopic examination of one micron plastic sections confirmed the fat accumulation in centrilobular hepatocytes indicated by the other methods. In addition, occasional empty-appearing vacuoles were noted in centrilobular hepatocytes that were distinctly different from the very osmiophilic fat droplets. Such empty-appearing vacuoles were present in centrilobular hepatocytes from animals exposed to 1,000ppm and in a few animals exposed to 250ppm 1,1,1- trichloroethane.

In the post-exposure samples, there was no evidence of significant fatty change in hepatocytes and hepatic triglycerides were at control levels (figs. 5 and 6). In some of the livers in the 1,000ppm group, aggregates of hypertrophied Kupffer cells containing a green pigment marked sites of prior hepatocyte necrosis. Rarely, a few centrilobular regions had a disordered architecture suggesting collapse accompanied by hypertrophy of regenerating hepatocytes.

In those animals observed in the post-exposure period, it became evident that many mice had enlarged spleens and evidence of granulocytopenia in the hepatic sinusoids. Megakaryocytes were rarely 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 (Mac Callum - 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. Control centrilobular hepatocytes were generally rather large cells, occasionally binucleate, with abundant cytoplasm (fig. 7). This 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 glycogen aggregates. 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 1,000ppm 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 (fig. 8). After 4 weeks of exposure, the centrilobular 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 (figs. 9 and 10). Polyribosome configurations were rare. Within the lumen of the vesicles, there were small triglyceride droplets (fig. 10) and occasionally small, very osmiophilic whirls of lamellae (fig. 11). These osmiophilic whirls were often 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. 11). Microbodies were markedly increased in number (fig. 11). Lysosomal vesicles were more prominent than in control cells and frequently contained degenerated organelles and osmiophilic membranous whirls ("myelin figures") (fig. 12).

In occasional cells from animals exposed to 1,000ppm 1,1,1-trichloroethane for 4 weeks, the rough endoplasmic reticulum was dilated to form large vesicles filled with a flocculent material of low electron density (figs. 9 and 10). In extreme form, this type of vesiculation represented the balloon degeneration seen by light microscopy.

After 4 to 14 weeks exposure to 1,000ppm 1,1,1-trichloroethane, centrilobular hepatocytes often had extensive cytoplasmic alterations of the type described above for 4 weeks of exposure (figs. 13 to 16). Although the severity of the changes decreased in many centrilobular hepatocytes at 14 weeks (compared to 4 weeks), triglyceride droplets, microbodies, and smooth endoplasmic reticulum remained increased compared to control levels.

Mallory bodies were not definitely identified within ballooned cells. Abnormal aggregates of filamentous material were observed rarely, but they were not comparable in size to the Mallory bodies visible by light microscopy. This discrepancy may be due to the sampling problem inherent in studies using electron microscopy.

In animals exposed to 250ppm 1,1,1-trichloroethane, centrilobular hepatocytes frequently were indistinguishable from control hepatocytes by the methodology used (fig. 17). 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 250ppm group were evident after 10 weeks of exposure, but were not as dramatic as those observed at 1,000ppm.

DISCUSSION

The hepatic toxicity of chlorinated hydrocarbons has been investigated for many years with particular emphasis on the toxicology of carbon tetrachloride (reviewed by Recknagel, 1967). 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.

In the case of carbon tetrachloride, hepatotoxicity is dependent on metabolic conversion of the carbon tetrachloride to a toxic intermediate whose exact nature has yet to be determined. Strong evidence for this concept has been provided by Dawkins (1963) who found that newborn rat liver is much less sensitive to carbon tetrachloride than at 7 days after birth. This implies that certain of the enzymatic changes in hepatocytes at the time of birth are necessary to convert carbon tetrachloride to its toxic metabolite. Chopra et al., (1972) have suggested that the enzyme necessary for this conversion is NADPH-cytochrome-c-reductase. It has been suspected for some time, that the toxic metabolite is a free radical, perhaps $\cdot\text{CCl}_3$. 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 and coworkers (reviewed by Recknagel, 1967) have 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 beta-lipoproteins. The loss of ability to secrete lipoproteins, and the depression of protein synthesis 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 (Recknagel, 1967). In this study, 40% of the animals showed evidence of focal necrosis of 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 (Popper and Schaffner, 1957).

Unfortunately, relatively little is known about the metabolism of 1,1,1- trichloroethane and its resemblance to chloroform. Hake et al., (1960) prepared 1,1,1- trichloroethane-1-¹⁴C and found that, after a single injection intraperitoneally in the rat, 99% of the radioactivity was recovered as unchanged 1,1,1- trichloroethane 1-¹⁴C in the expired air. Most of the remaining label (1%) was recovered in the urine, 0.25% was volatile on air drying and 0.75% was present as 2,2,2- trichloroethanol excreted as the glucuronide. Less than 0.01% 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. 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. (Remmer and Merker, 1965; Jones and Fawcett, 1966). The present study indicates that, by 4 weeks exposure to 1,000ppm 1,1,1- trichloroethane, there was a considerable increase in the volume of membranous tubules

resembling smooth endoplasmic reticulum. Reynolds, et al (1972), 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 from this highly chlorinated compound. On the other hand, judging stimulation of smooth endoplasmic reticulum by morphological techniques alone may not be reliable. Reynolds and Ree (1971) have found that aggregates of smooth-surfaced membranous tubules appear by 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 1,000ppm group. Levels of hydroxylase activity should be measured and surface areas of endoplasmic reticulum membranes should be quantitated in making such a distinction.

A dramatic increase in the number of microbodies was found at 4 to 14 weeks of exposure to 1,000ppm 1,1,1- trichloroethane. Microbodies are membrane-bounded organelles that are found in a large number of cell types (Hruban et al., 1972; de Duve, 1973). 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 is usually associated with certain oxidase enzymes (McGroarty and Tolbert, 1973). 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 (de Duve, 1966, 1969).

Microbodies have also been associated with lipid metabolism in hepatocytes (Reddy, 1973) but the exact nature of this association has not been clearly delineated. Observations by these other workers suggests 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 the catalase content of the cytoplasm. A relationship between microbodies and lipid turnover seems the most likely possibility since Gordon and Lough (1972) have found that microbodies become plentiful by the fifth day of regression (following cessation of ethanol induction) of a fatty liver in rats.

In a previous study from this laboratory (Weinstein et al., 1971, 1972), the toxicity of dichloromethane was investigated after continuous inhalation of 5,000ppm 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 dilatation of cisternae of rough endoplasmic reticulum and perinuclear cisternae (Weinstein et al., 1971). 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. Hepatocyte necrosis was reported in dichloromethane study (Weinstein et al., 1972). 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 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 (Popper and Schaffner, 1957).

An incidental finding was the presence of occasional ballooned cells with prominent eosinophilic masses in the cytoplasm that resembled, by light microscopy, the appearance of Mallory bodies or "alcoholic hyalin" (Popper and Schaffner, 1957). Since these bodies were relatively rare, they were not encountered in samples for electron microscopy. Structures resembling Mallory bodies have been observed in other studies of halocarbon induced toxic hepatic necrosis (Popper and Schaffner, 1957).

Caution should be exercised in interpreting the differences between the toxic effects of dichloromethane (Weinstein et al., 1972) 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 elucidate the nature of adaptive responses to these compounds and their relation to the structure of the halocarbon.

REFERENCES

- Adams, E.M., H.C. Spencer, V.K. Rowe, and D.D. Irish. 1950. "Vapor toxicity of 1,1,1-trichloroethane (methylchloroform) determined by experiments on laboratory animals." A.M.A. Arch. Ind. Hyg. and Occup. Med. 1: 225.
- Butler, V.M. Jr., H.M. Maling, M.G. Horning, and B.B. Brodie. 1961. "The direct determination of liver triglycerides." J. Lipid Res. 2: 95.
- Chopra, P., S. Roy, V. Ramalingaswami, and N.C. Nayak. 1972. "Mechanism of carbon tetrachloride hepatotoxicity. An in vivo study of its molecular basis in rats and monkeys." Lab. Invest. 26: 716.
- Dawkins, M.J.R. 1963. "Carbon tetrachloride poisoning in the liver of newborn rat." J. Pathol. Bacteriol. 85: 189.
- de Duve, C. 1966. "Peroxisomes (microbodies and related particles)." Physiol. Rev. 46: 323.
- de Duve, C. 1969. "The peroxisomes: a new cytoplasmic organelle." Proc. Roy. Soc. Lond. (B) 173: 71.
- de Duve, C. 1973. "Biochemical studies on the occurrence, biogenesis and life history of mammalian peroxisomes." J. Histochem. Cytochem. 21: 941.
- Gordon, E.R. and J. Lough. 1972. "Ultrastructural and biochemical aspects during the regression of an ethanol-induced fatty liver." Lab. Invest. 26: 154.
- Hake, C.L., T.B. Waggoner, D.N. Robertson, and V.K. Rowe. 1960. "The metabolism of 1,1,1-trichloroethane by the rat." Arch. Environ. Health 1: 101.
- Hruban, Z., E.L. Vigil, A. Slesers, and E. Hopkins. 1972. "Microbodies constituent organelles of animal cells." Lab. Invest. 27: 184.
- Ito, S. and M.J. Karnovsky. 1968. "Formaldehyde-glutaraldehyde fixatives containing trinitro compounds." J. Cell Biol. 39: 168a.
- Jones, A.L. and D.W. Fawcett. 1966. "Hypertrophy of the agranular endoplasmic reticulum in hamster liver induced by phenobarbital." J. Histochem. Cytochem. 14: 215.
- McGroarty, E. and N.E. Tolbert. 1973. "Enzymes in peroxisomes." J. Histochem. Cytochem. 21: 949.
- Popper, H. and F. Schaffner. 1957. "Toxic Hepatic injury." Chapter 41 In: Liver: Structure and Function. McGraw Hill Co., Blakiston Division, N.Y. pg 391.

- Recknagel, R.O. 1967. "Carbon tetrachloride hepatotoxicity." Pharmacol. Rev. 19: 145.
- Reddy, J.K. 1973. "Possible properties of microbodies (peroxisomes). Microbody proliferation and hypolipidemic drugs." J. Histochem. Cytochem. 21: 967.
- Remmer, H. and H.J. Merker. 1967. "Effect of drugs on the formation of smooth endoplasmic reticulum and drug metabolizing enzymes." Ann. N.Y. Acad. Sci. 123: 79.
- Reynolds, E.S. and H.J. Ree. 1971. "Liver parenchymal cell injury. VII. Membrane denaturation following carbon tetrachloride." Lab. Invest. 25: 269.
- Reynolds, E.S., H.J. Ree, and M.T. Moslen. 1972. "Liver parenchymal cell injury. IX Phenobarbital potentiation of endoplasmic reticulum denaturation following carbon tetrachloride." Lab. Invest. 26: 290.
- Rowe, V.K., T. Wujkowski, M.A. Wolf, S.E. Sadek, and R.D. Stewart. 1963. "Toxicity of a solvent mixture of 1,1,1- trichloroethane and tetrachloroethylene as determined by experiments on laboratory animals and human subjects." Amer. Ind. Hyg. Assoc. J. 24: 541.
- Torkelson, T.R., F. Dyen, D.D. McCollister, and V.K. Rowe. 1958. "Toxicity of 1,1,1- trichloroethane as determined on laboratory animals and human subjects." Amer. Ind. Hyg. Assoc. J. 19: 353.
- Weinstein, R.S., D.D. Boyd, and K.C. Back. 1971. "Dichloromethane hepatotoxicity in mice with continuous and intermittent inhalation exposures." In: Proceedings on 2nd Annual Conference on Environmental Toxicology, Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio AMRL-TR-71-120, pg 147.
- Weinstein, R.S., D.D. Boyd, and K.C. Back. 1972. "Effects of continuous inhalation of dichloromethane in the mouse: morphologic and functional observations." Toxicol. Appl. Pharmacol. 23: 660.
- Weinstein, R.S. and S.S. Diamond. 1972. "Hepatotoxicity of dichloromethane (methylene chloride) with continuous inhalation exposure at a low dose level." In; Proceedings of 3rd Annual Conference on Environmental Toxicology; Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio: AMRL-TR-72-130, pg 209.

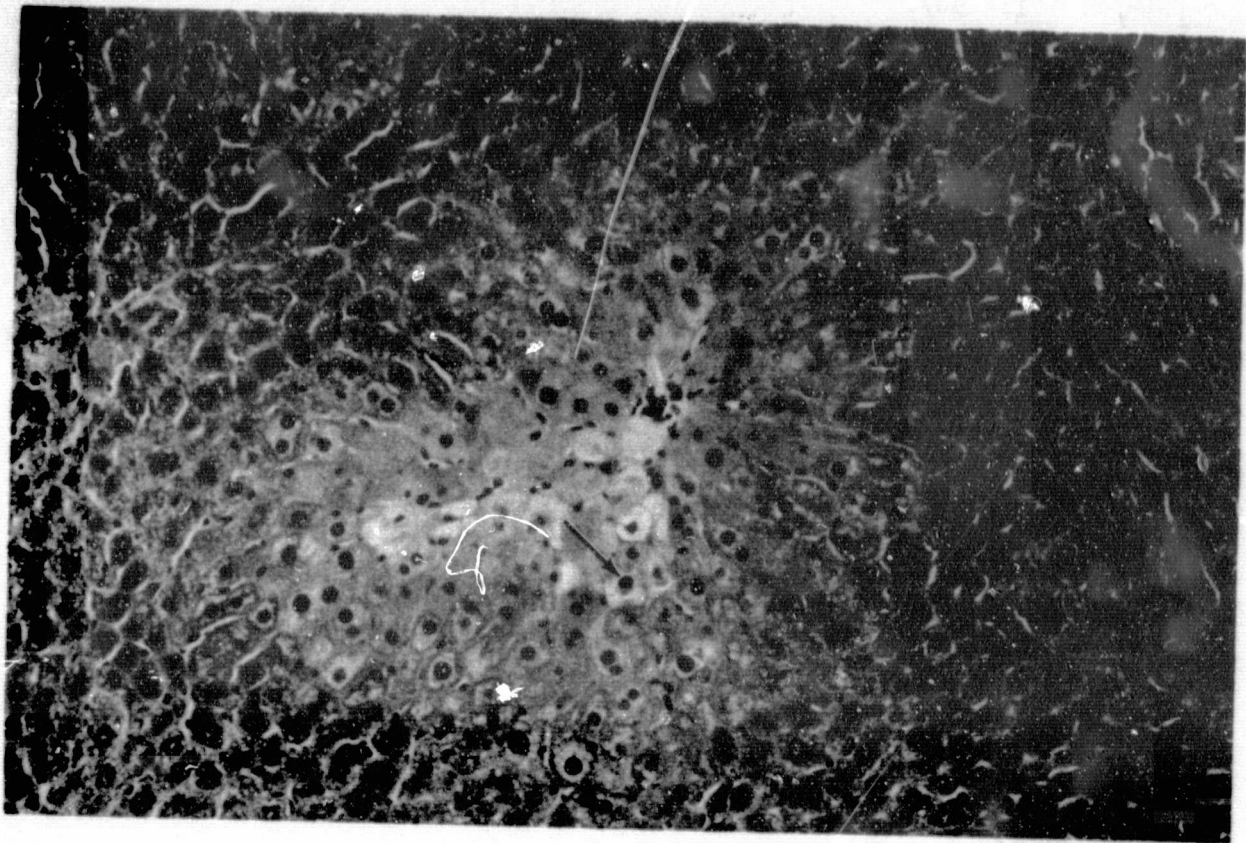


Figure 1: Light micrograph of liver from a mouse exposed to 1,000ppm 1,1,1- trichloroethane for 4 weeks. This micrograph demonstrates a centrilobular region containing enlarged hepatocytes as well as cells undergoing balloon degeneration with nuclear pyknosis (at arrow). Paraffin section; hematoxylin and eosin. (X 190)

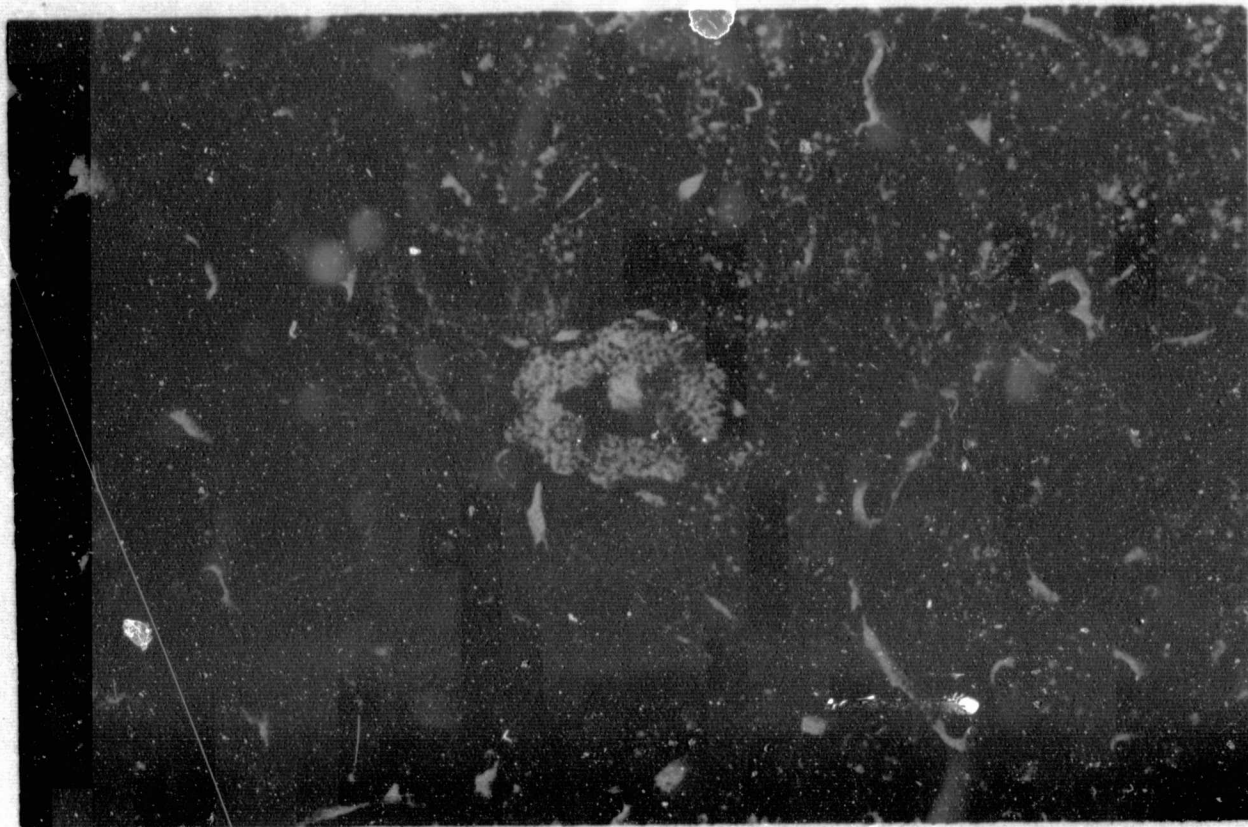


Figure 2: Light micrograph showing an individual cell that has undergone balloon degeneration. The nucleus is pyknotic and deformed by a central vacuole. Other nearby cells show less severe cytoplasmic vacuolization attributed to triglyceride accumulation. 1,000ppm 1,1,1- trichloroethane; 4 weeks exposure; hematoxylin and eosin. (X 770)

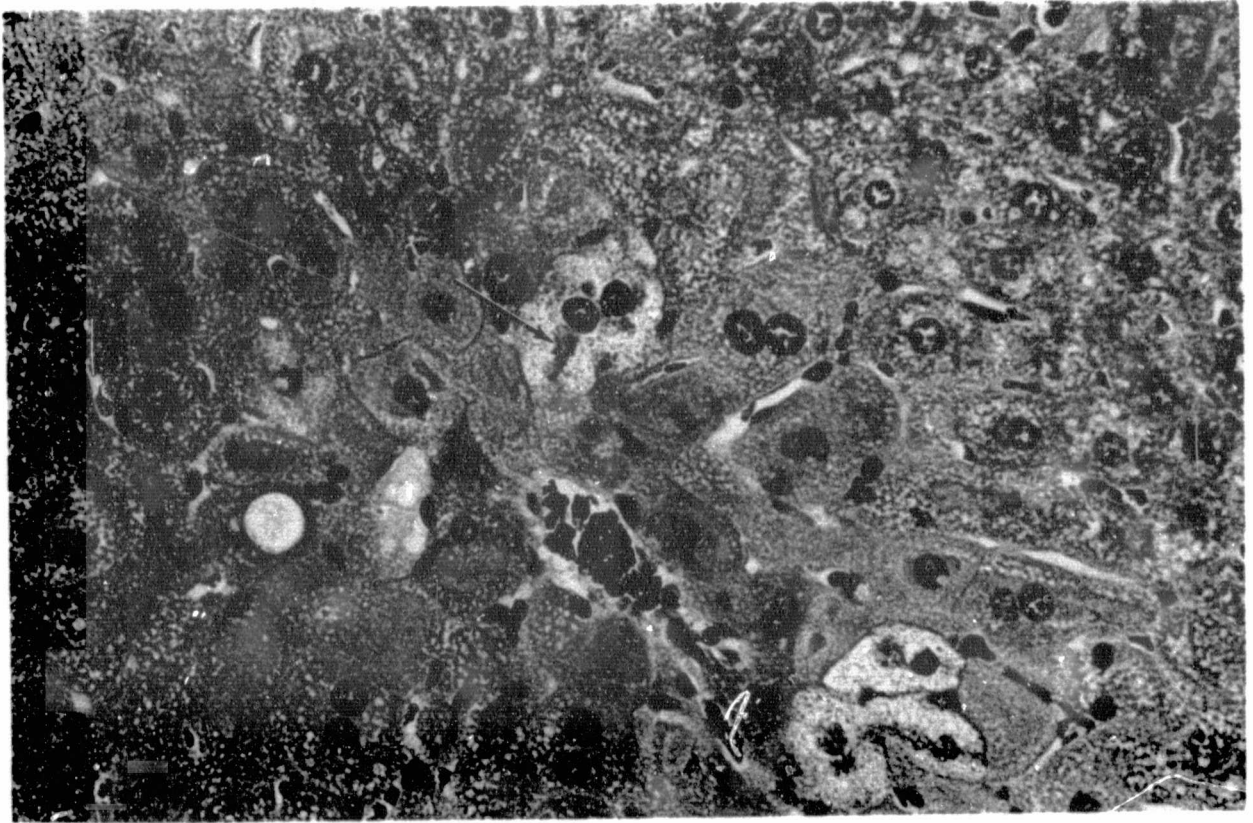


Figure 3: Light micrograph of a centrilobular hepatocyte with balloon degeneration and a prominent eosinophilic cytoplasmic mass that resembles a Mallory body ("alcoholic hyalin") (at arrow). 1,000ppm 1,1,1- trichloroethane; 4 weeks exposure; hematoxylin and eosin. (X 480)

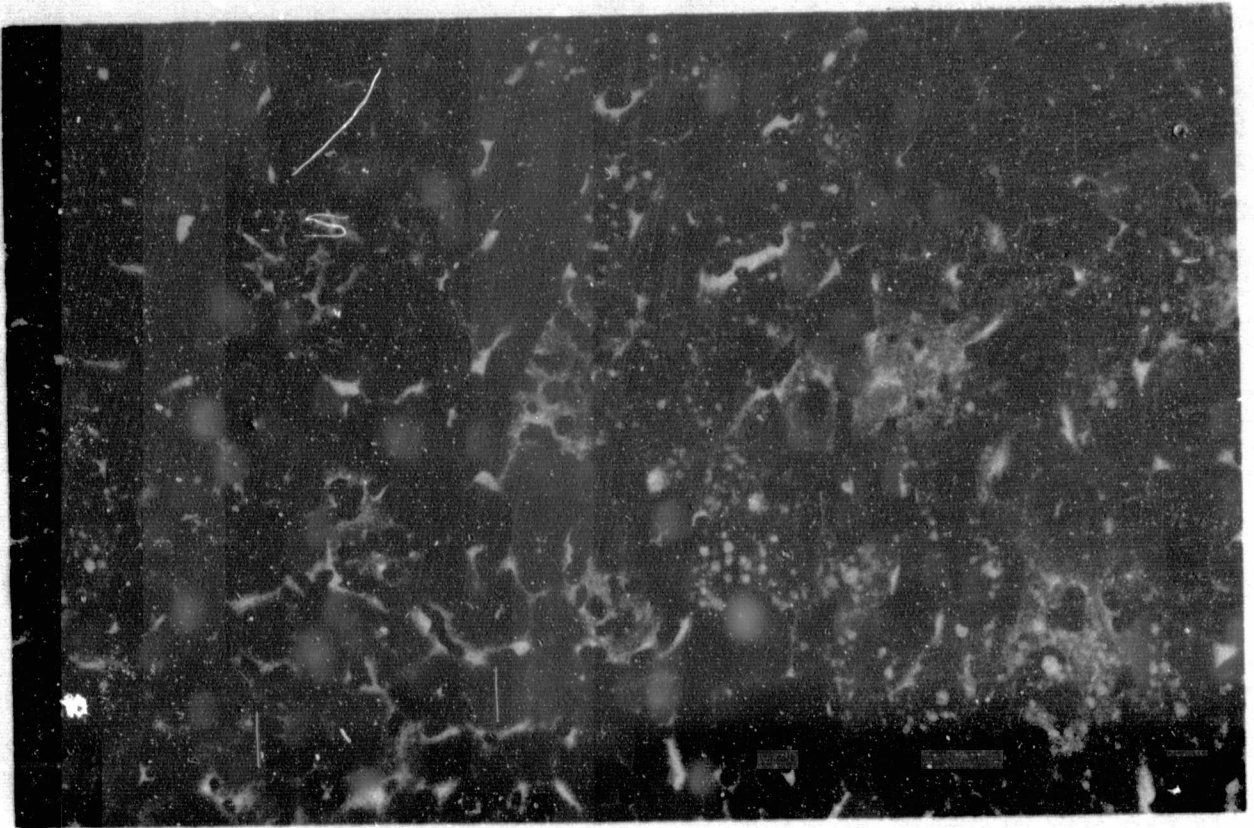


Figure 4: Light micrograph of the liver of a mouse exposed to 1,000ppm 1,1,1- trichloroethane for 12 weeks. Several hepatocytes (at the border between centrilobular and periportal zones) have undergone coagulation necrosis and have attracted focal accumulations of neutrophilic leukocytes. Tissue gram stains were negative for bacteria. Nearby centrilobular hepatocytes have enlarged cytoplasm and fine vacuolization that stained positively for fat with Oil-red-O. Paraffin section; hematoxylin and eosin. (X 460)

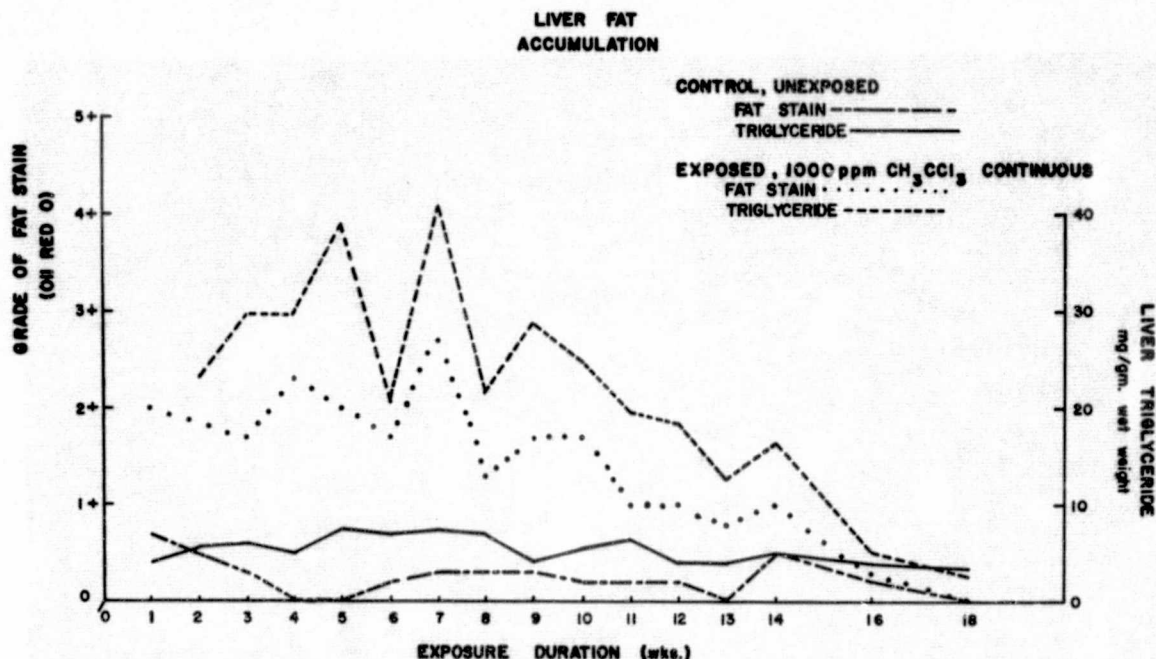


Figure 5: Composite graph of data from Oil-red-O stains for fat (which were graded from one to five in severity of fatty change) and from triglyceride determinations on both control mice and mice exposed to 1,000ppm 1,1,1- trichloroethane. After 14 weeks, all animals were exposed only to room air. The graph shows a close correspondence between the results of Oil-red-O stain and triglyceride analysis. In the 1,000ppm group, fat accumulation peaked at approximately 7 weeks; there was partial recovery from 7 to 14 weeks and full recovery from fat accumulation after the exposure to 1,1,1- trichloroethane was stopped at 14 weeks.

Grading of Oil-red-O stained sections was according to the following criteria: 1+ = Fatty change, microglobular marked, up to 5 cell diameters from border to central vein; 2+ = Fatty change, microglobular marked, up to 10 cell diameters from border of central vein; 3+ = Fatty change, marked, greater than 10 cell diameters from central vein with many confluent areas and some tendency for macroglobular fat.

The graph connects adjacent means (of 3 determinations) by a straight line without an attempt at curve fitting. 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.

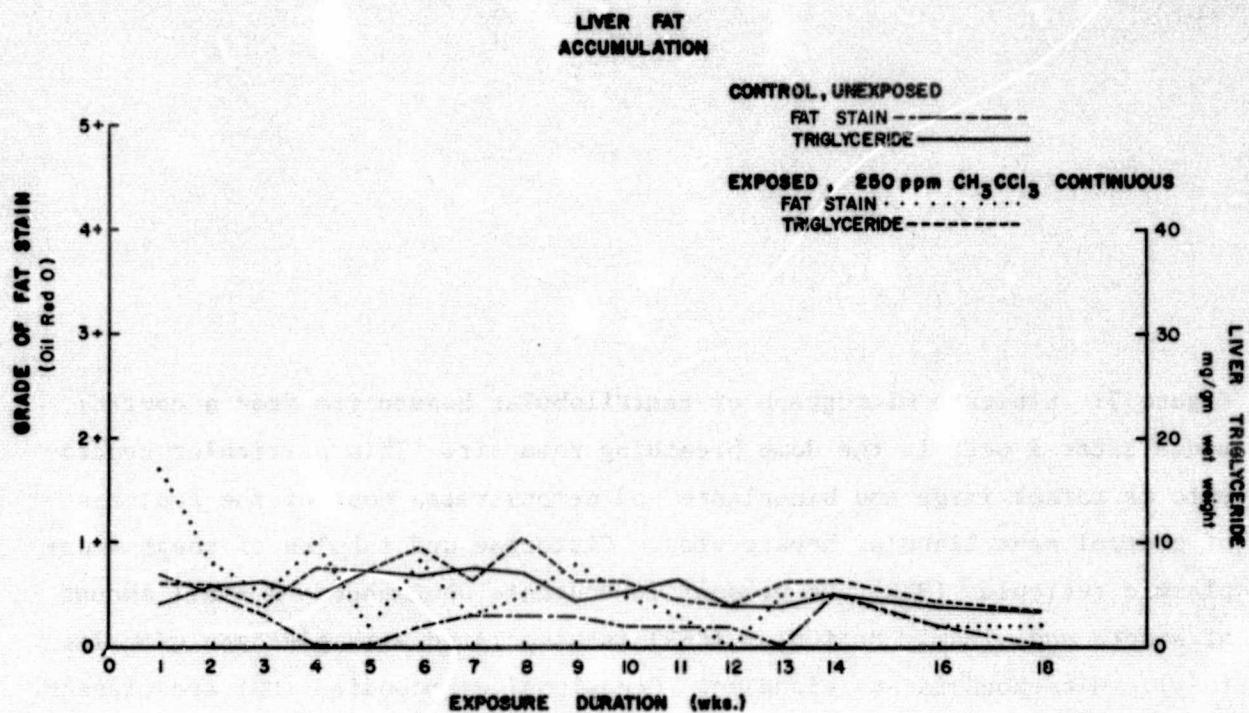


Figure 6: Composite graph similar to Fig. 5 that shows the close correspondence of fat stains and triglyceride values in both control mice and mice exposed to 250ppm 1,1,1- trichloroethane from 1 to 14 weeks. This indicates that 250ppm is near the threshold dose for toxic effects in terms of hepatic triglycerides.

Figure 7: Electron micrograph of centrilobular hepatocyte from a control mouse after 1 week in the dome breathing room air. 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. A small Golgi region is visible. (X 17,700)



Figure 7

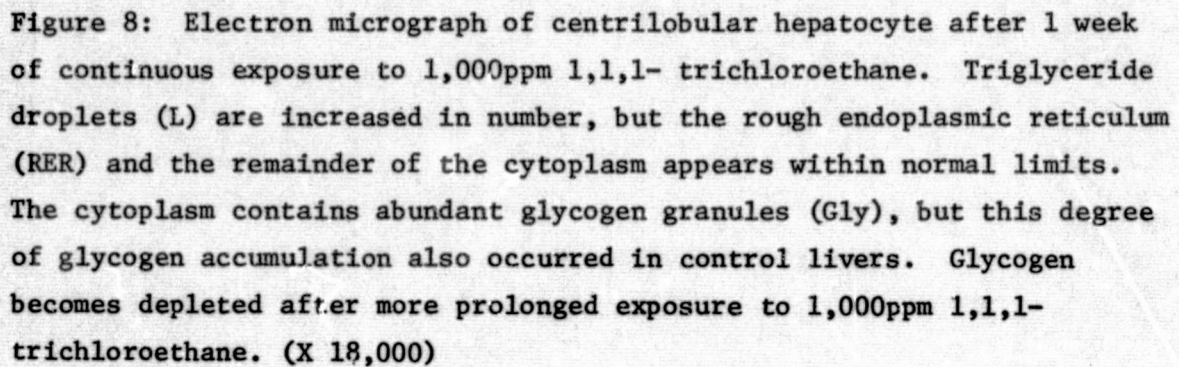


Figure 8: Electron micrograph of centrilobular hepatocyte after 1 week of continuous exposure to 1,000ppm 1,1,1- trichloroethane. Triglyceride droplets (L) are increased in number, but the rough endoplasmic reticulum (RER) and the remainder of the cytoplasm appears within normal limits. The cytoplasm contains abundant glycogen granules (Gly), but this degree of glycogen accumulation also occurred in control livers. Glycogen becomes depleted after more prolonged exposure to 1,000ppm 1,1,1- trichloroethane. (X 18,000)

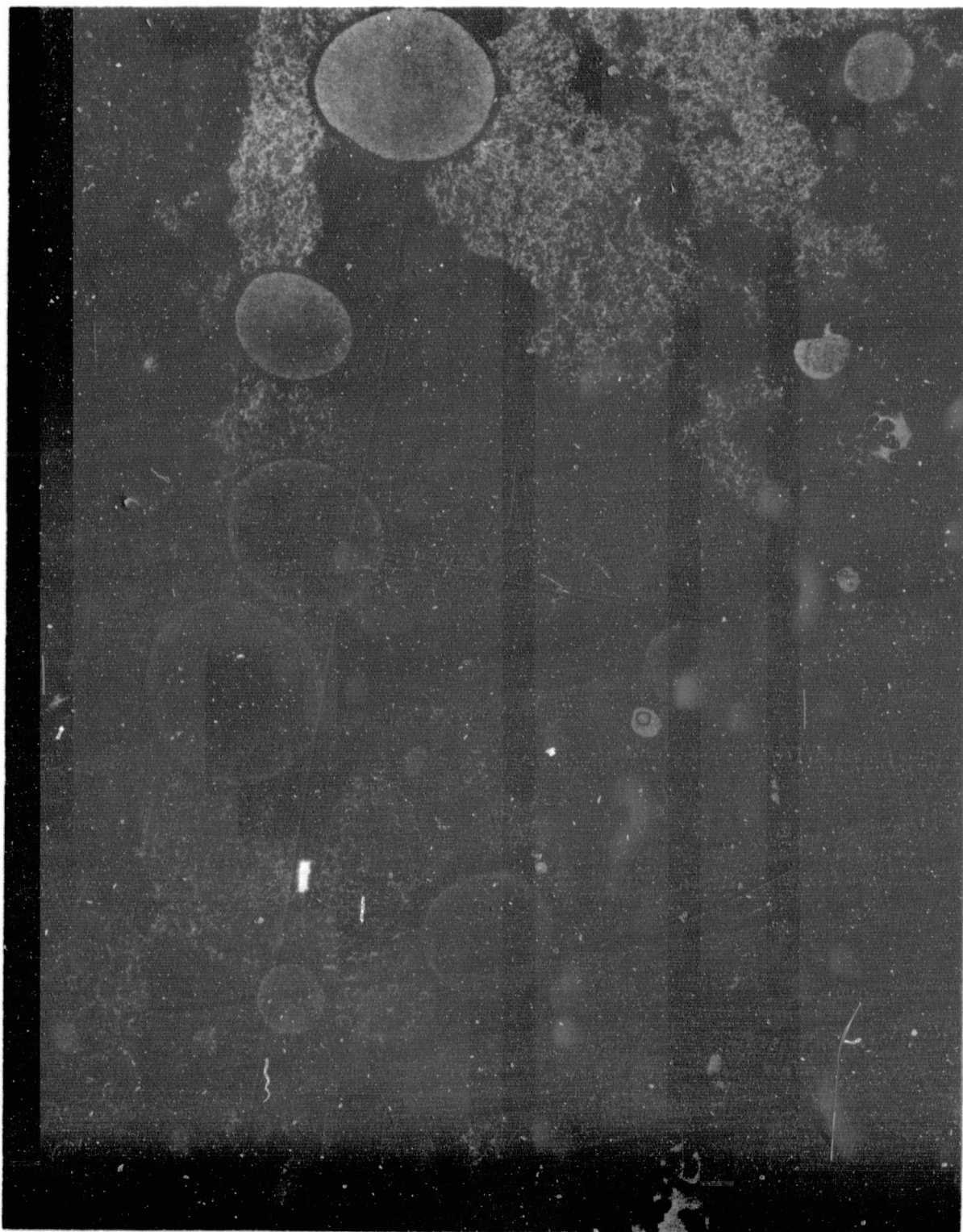


Figure 8

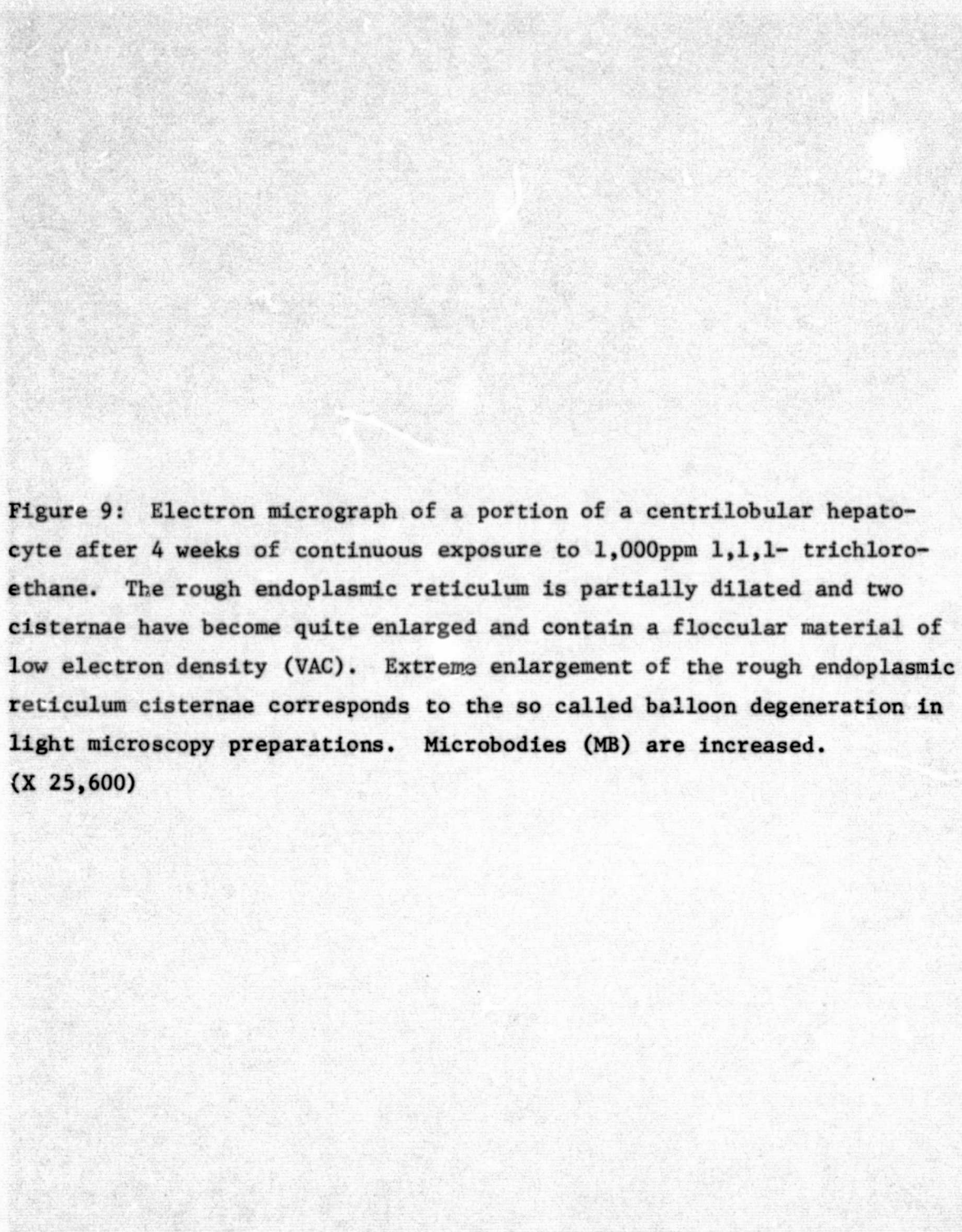


Figure 9: Electron micrograph of a portion of a centrilobular hepatocyte after 4 weeks of continuous exposure to 1,000ppm 1,1,1- trichloroethane. The rough endoplasmic reticulum is partially dilated and two cisternae have become quite enlarged and contain a floccular material of low electron density (VAC). Extreme enlargement of the rough endoplasmic reticulum cisternae corresponds to the so called balloon degeneration in light microscopy preparations. Microbodies (MB) are increased.
(X 25,600)

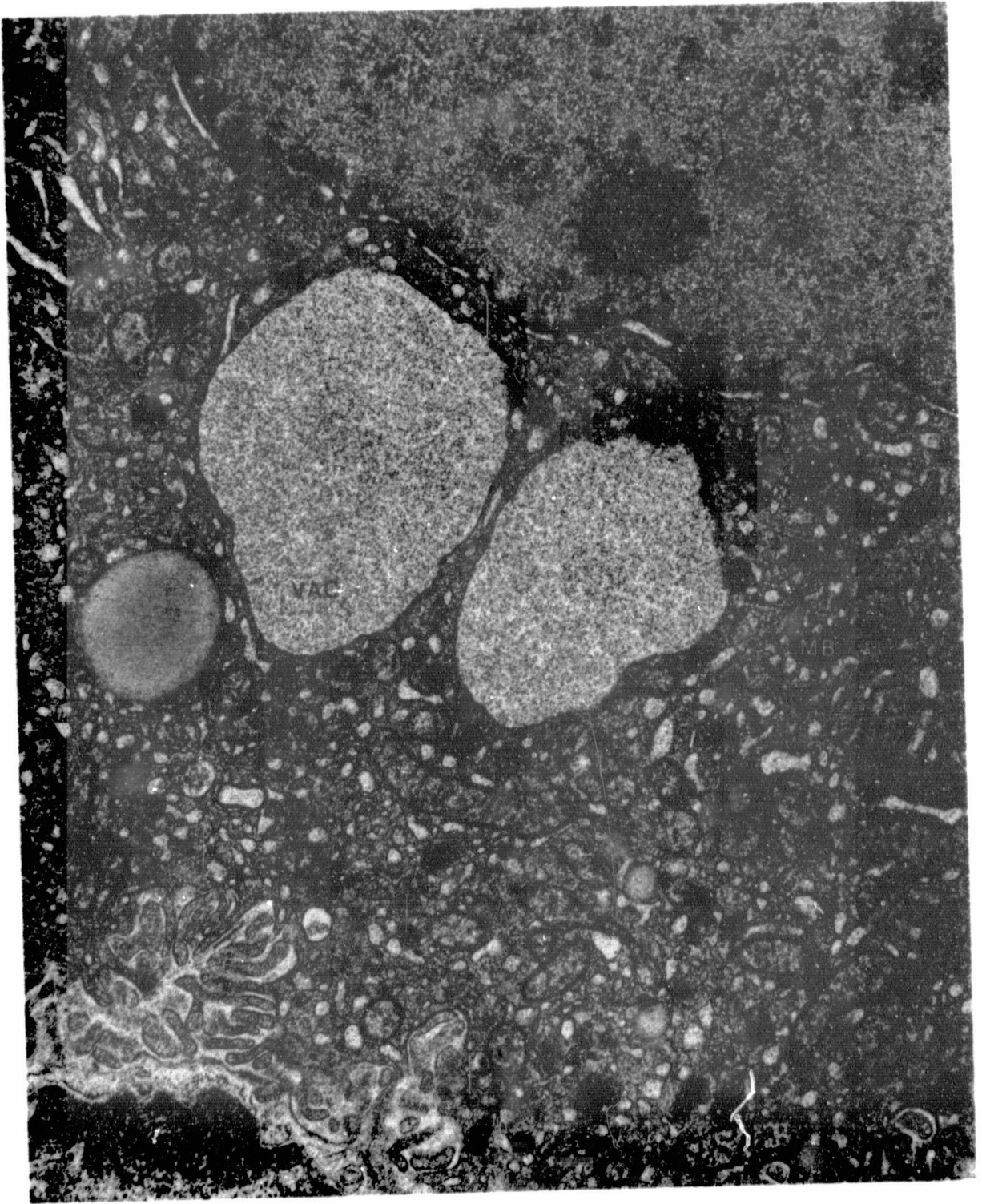


Figure 9

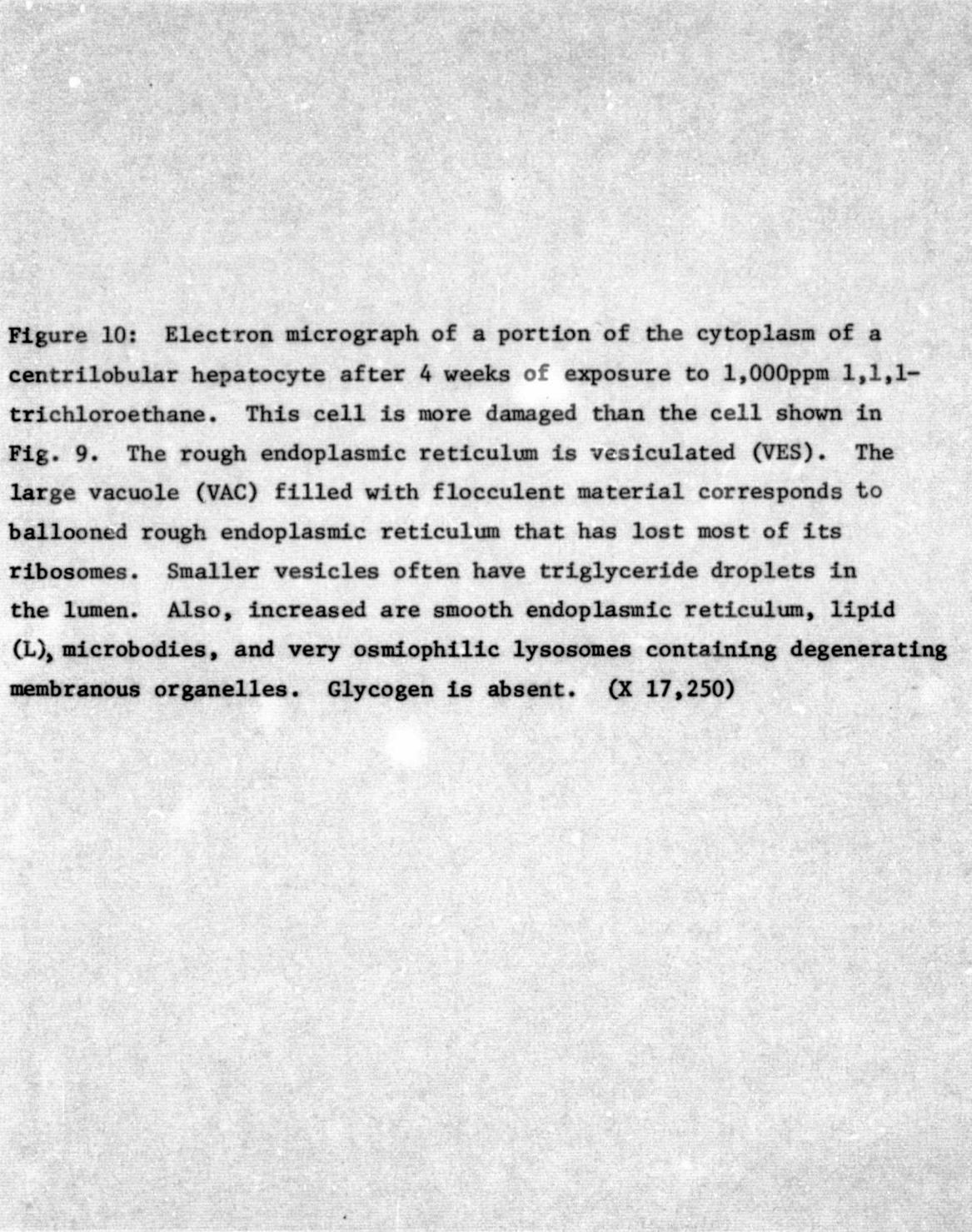


Figure 10: Electron micrograph of a portion of the cytoplasm of a centrilobular hepatocyte after 4 weeks of exposure to 1,000ppm 1,1,1-trichloroethane. This cell is more damaged than the cell shown in Fig. 9. The rough endoplasmic reticulum is vesiculated (VES). The large vacuole (VAC) filled with flocculent 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. (X 17,250)

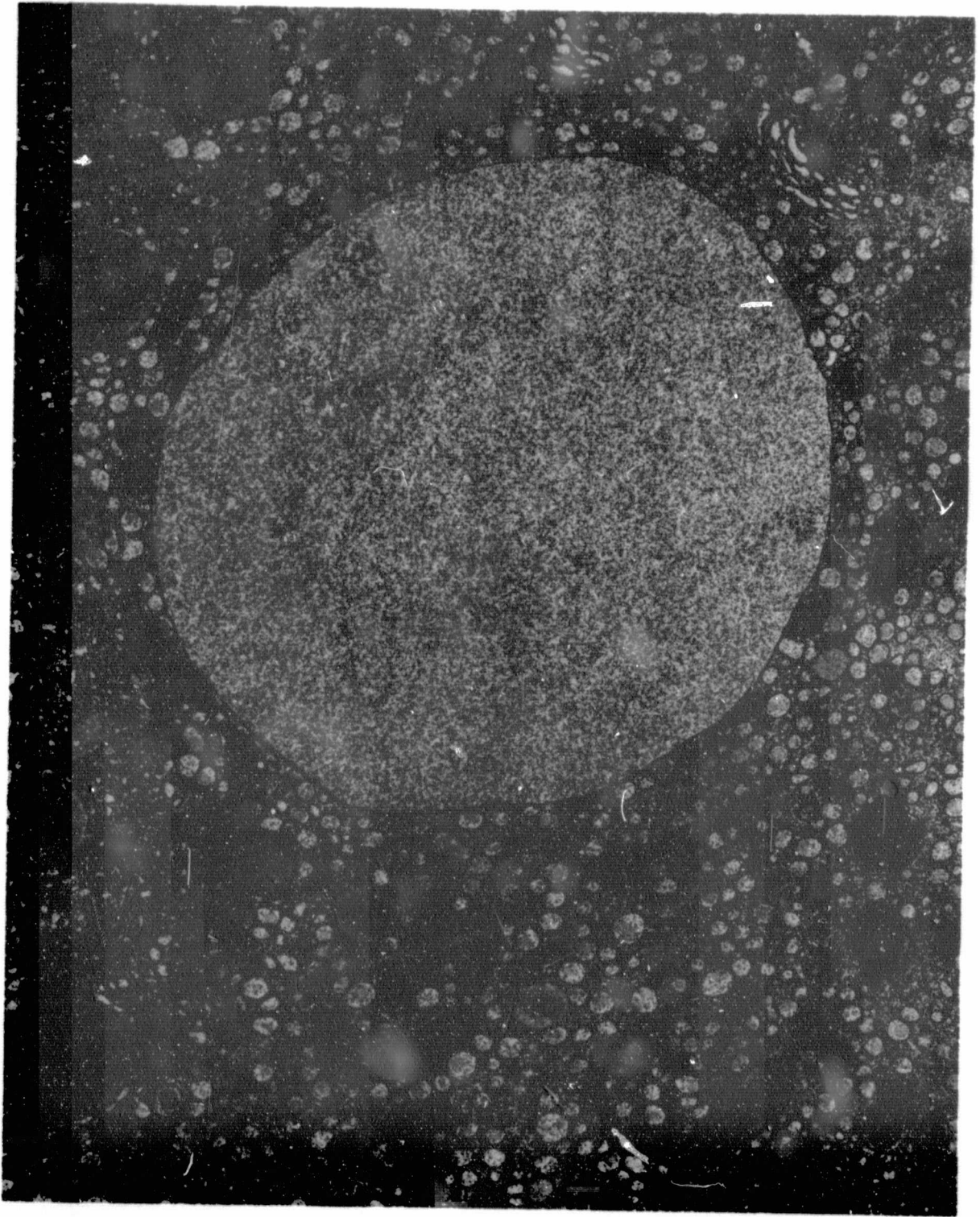


Figure 10

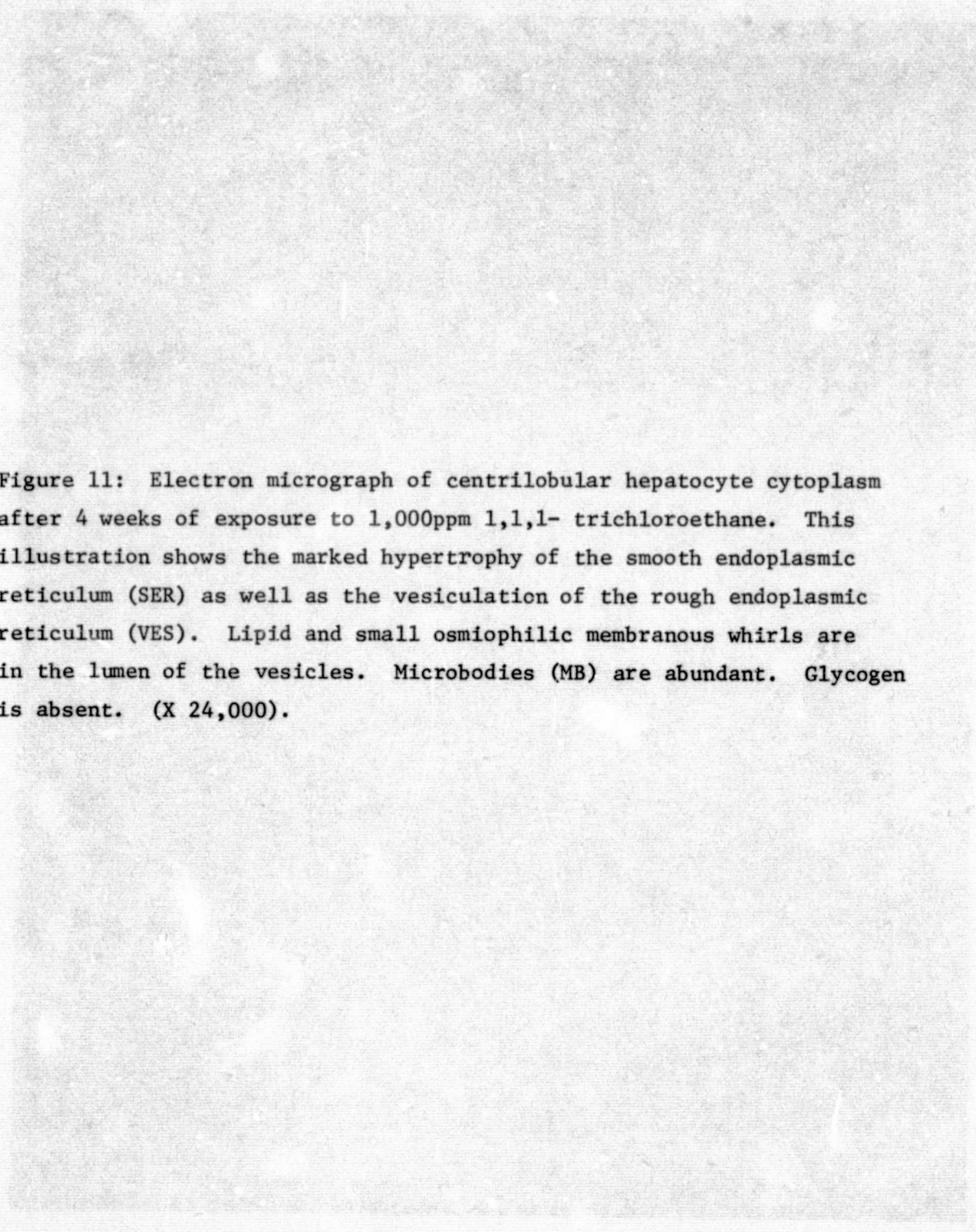


Figure 11: Electron micrograph of centrilobular hepatocyte cytoplasm after 4 weeks of exposure to 1,000ppm 1,1,1- trichloroethane. This illustration shows the marked hypertrophy of the smooth endoplasmic reticulum (SER) as well as the vesiculation of the rough endoplasmic reticulum (VES). Lipid and small osmiophilic membranous whorls are in the lumen of the vesicles. Microbodies (MB) are abundant. Glycogen is absent. (X 24,000).

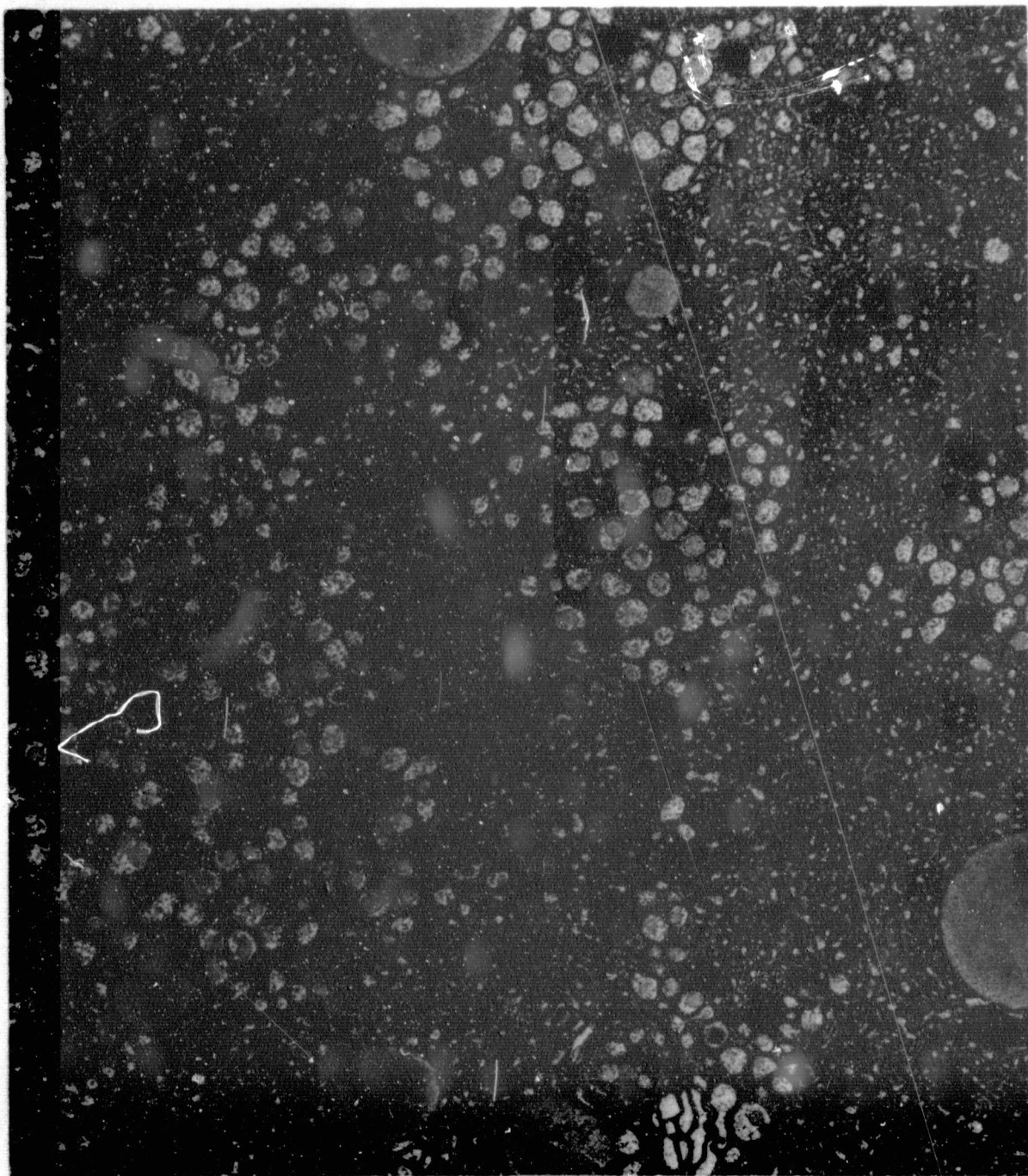


Figure 11

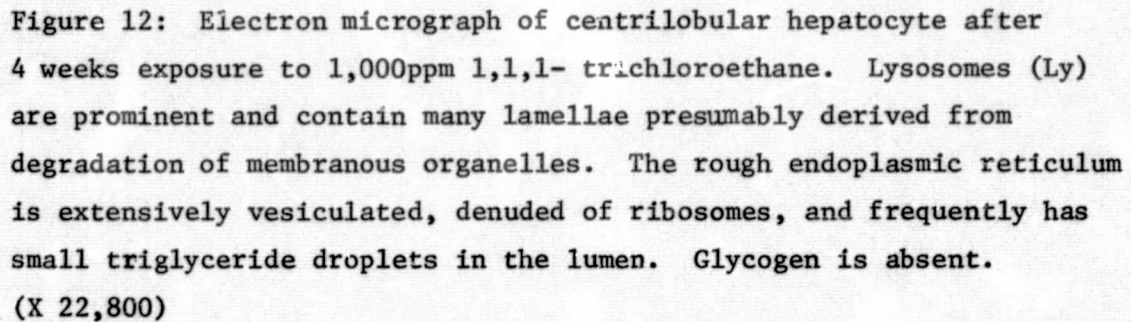


Figure 12: Electron micrograph of centrilobular hepatocyte after 4 weeks exposure to 1,000ppm 1,1,1- trichloroethane. Lysosomes (Ly) are prominent and contain many lamellae presumably derived from degradation of membranous organelles. The rough endoplasmic reticulum is extensively vesiculated, denuded of ribosomes, and frequently has small triglyceride droplets in the lumen. Glycogen is absent. (X 22,800)

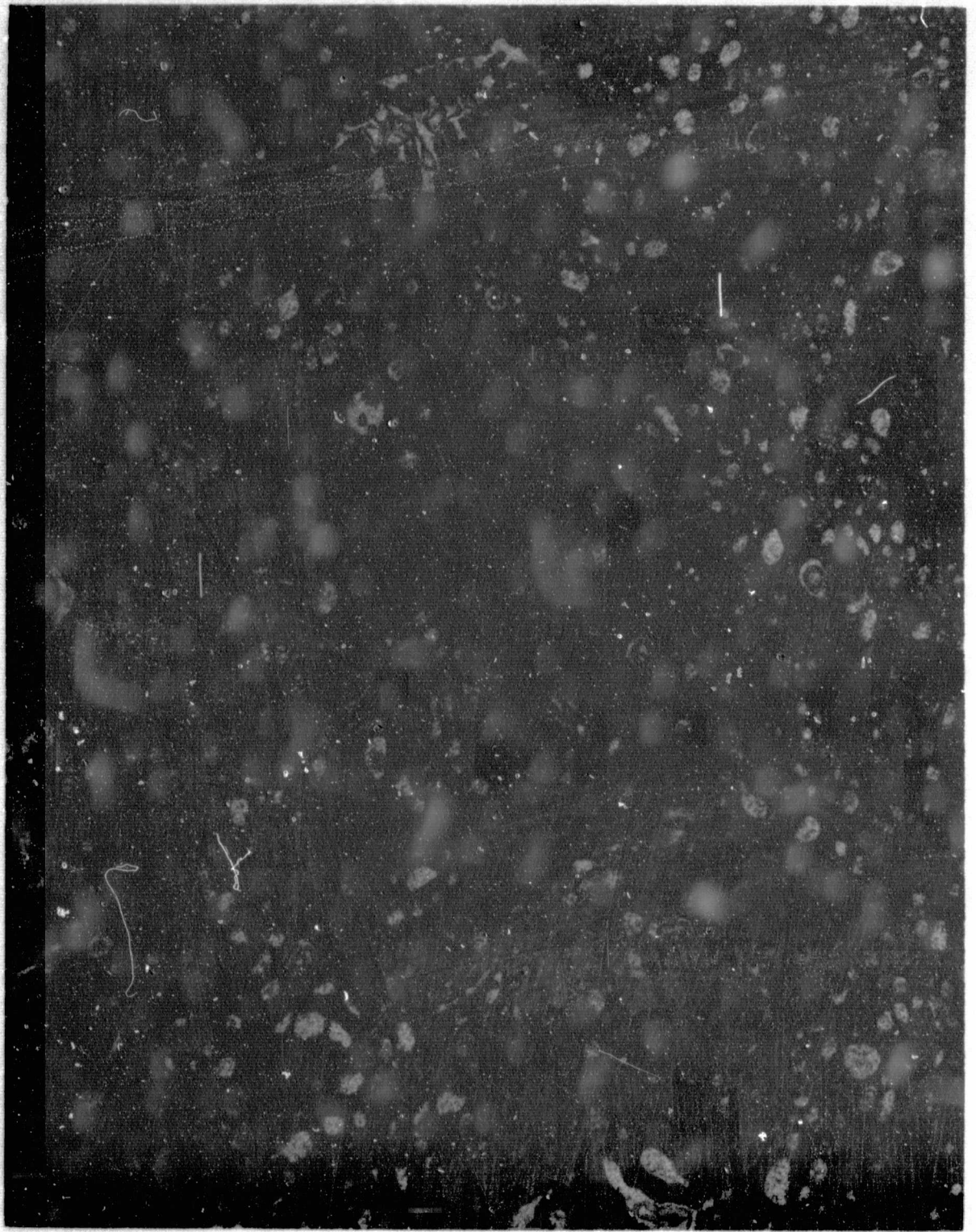


Figure 12

Figure 13: Centrilobular hepatocyte cytoplasm after 7 weeks exposure to 1,000ppm 1,1,1- trichloroethane. This electron micrograph demonstrates the marked increase in microbodies (MB) observed particularly near the "blood front" of the cell. Lipid droplets (L) are increased and vesicles of rough endoplasmic reticulum contain lipid. The smooth endoplasmic reticulum (SER) is very hypertrophied and small aggregates of glycogen granules are present. (X 16,700).

Reproduced from
best available copy.



Figure 13

Figure 14: Electron micrograph of a centrilobular hepatocyte after 14 weeks exposure to 1,000ppm 1,1,1- trichloroethane. The lipid accumulation has decreased in some cells, such as this one, but hypertrophy of the smooth endoplasmic reticulum (SER) persists. The rough endoplasmic reticulum is vesiculated and contains small lipid droplets in the lumen. Microbodies (MB) are abundant and often contain a central nucleoid. (X 21,200)

Reproduced from
best available copy.

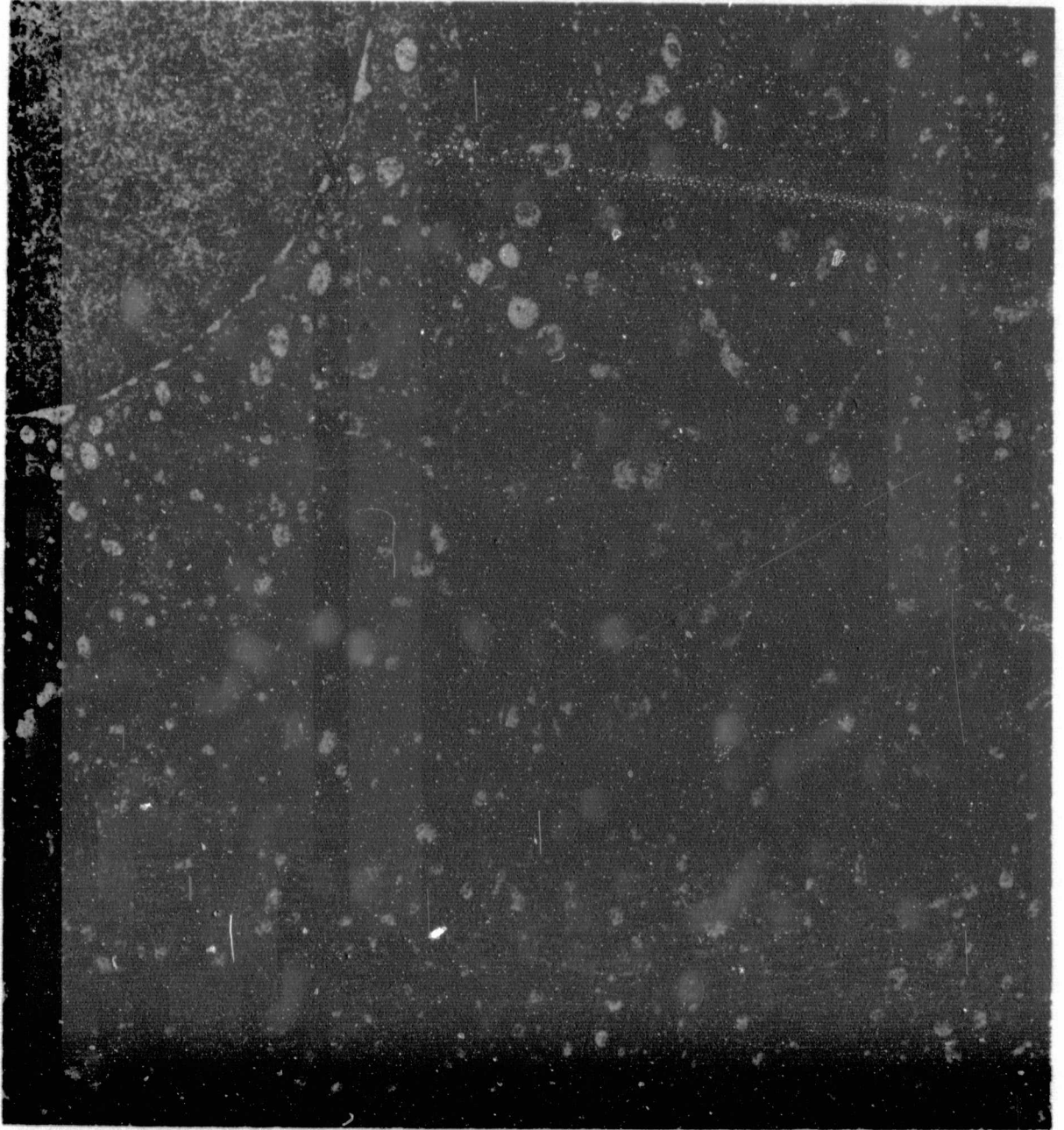


Figure 14

Figure 15: Electron micrograph of centrilobular hepatocyte after 14 weeks exposure to 1,000ppm 1,1,1- trichloroethane at higher magnification to demonstrate the vesiculation of the rough endoplasmic reticulum and the lipid droplets within the vesicles (VES). Also, Hypertrophied smooth endoplasmic reticulum (SER) and microbodies (MB) are illustrated. (X 32,400)

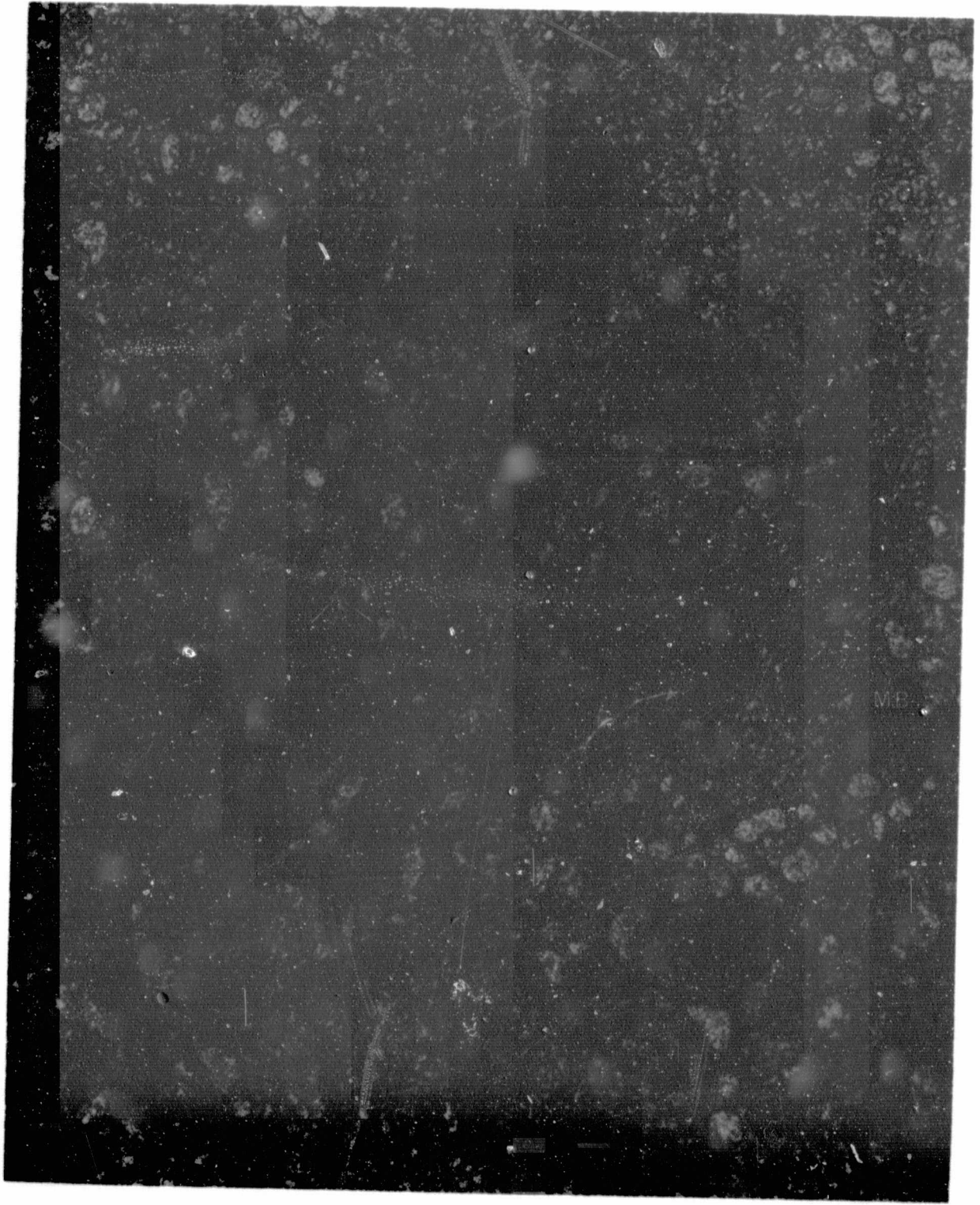


Figure 15

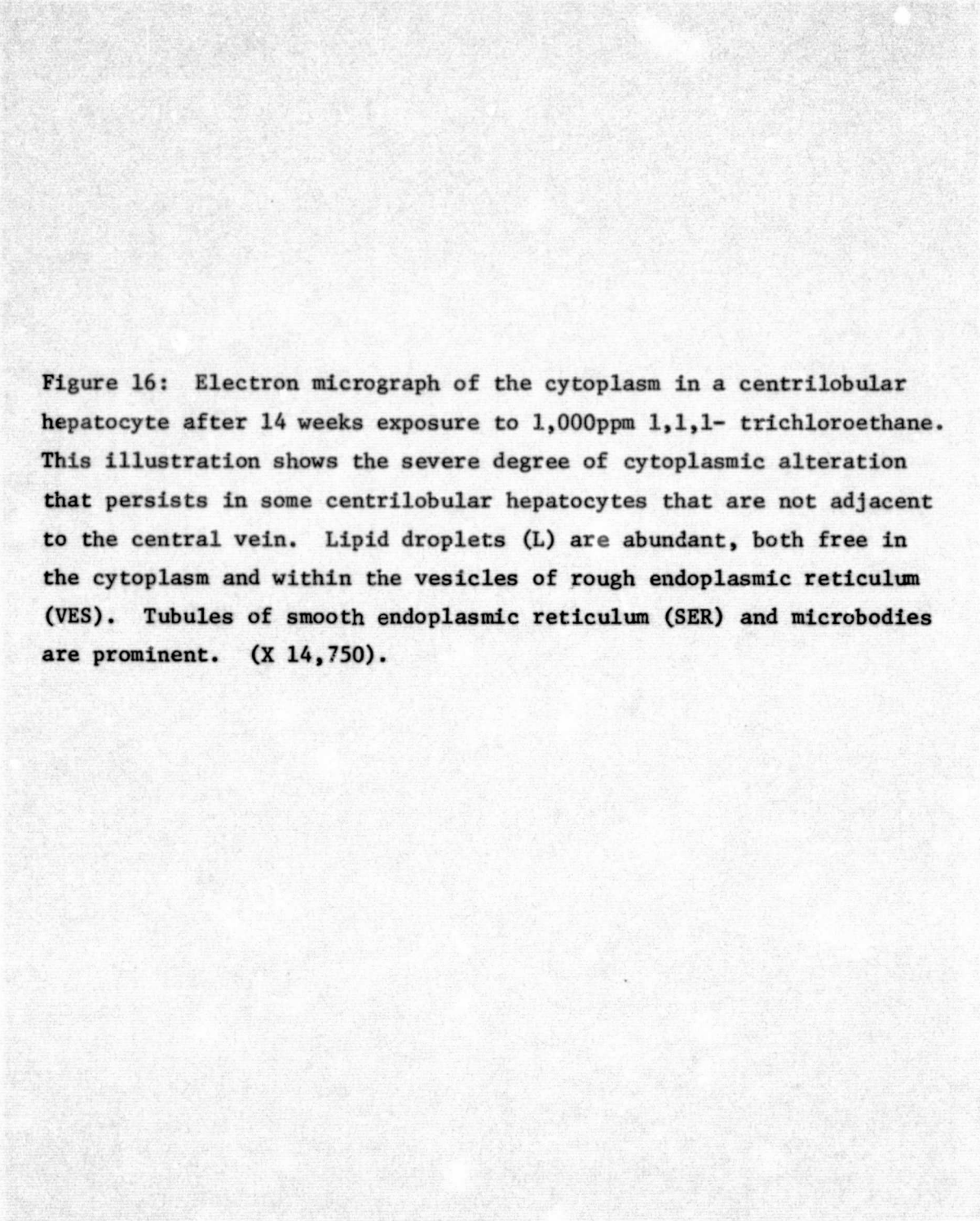
The image is a large, rectangular area that is mostly blank and has a grainy, light gray texture, characteristic of a low-quality scan of a document page. It appears to be the location of a figure, but the content is not visible.

Figure 16: Electron micrograph of the cytoplasm in a centrilobular hepatocyte after 14 weeks exposure to 1,000ppm 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. (X 14,750).

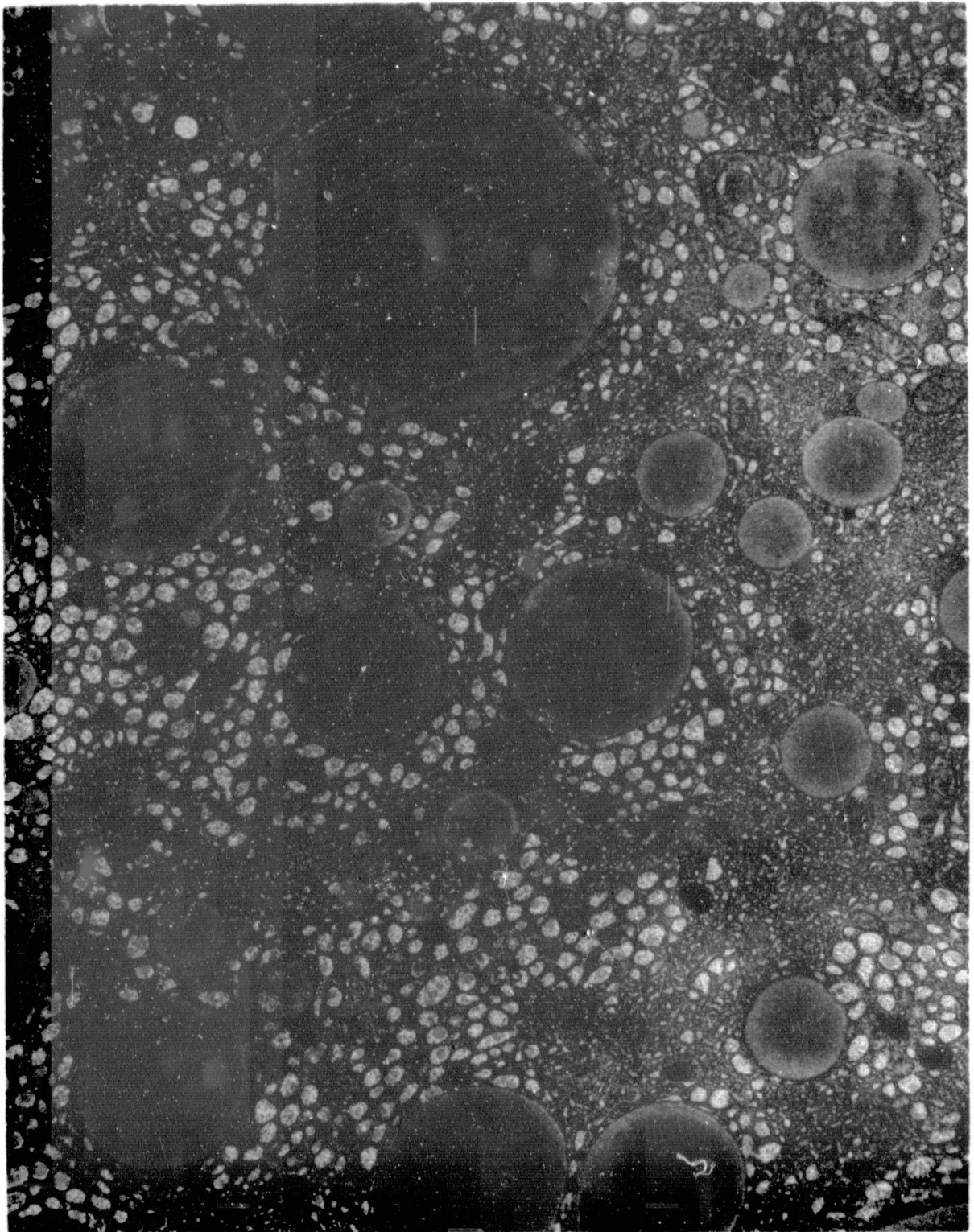


Figure 16

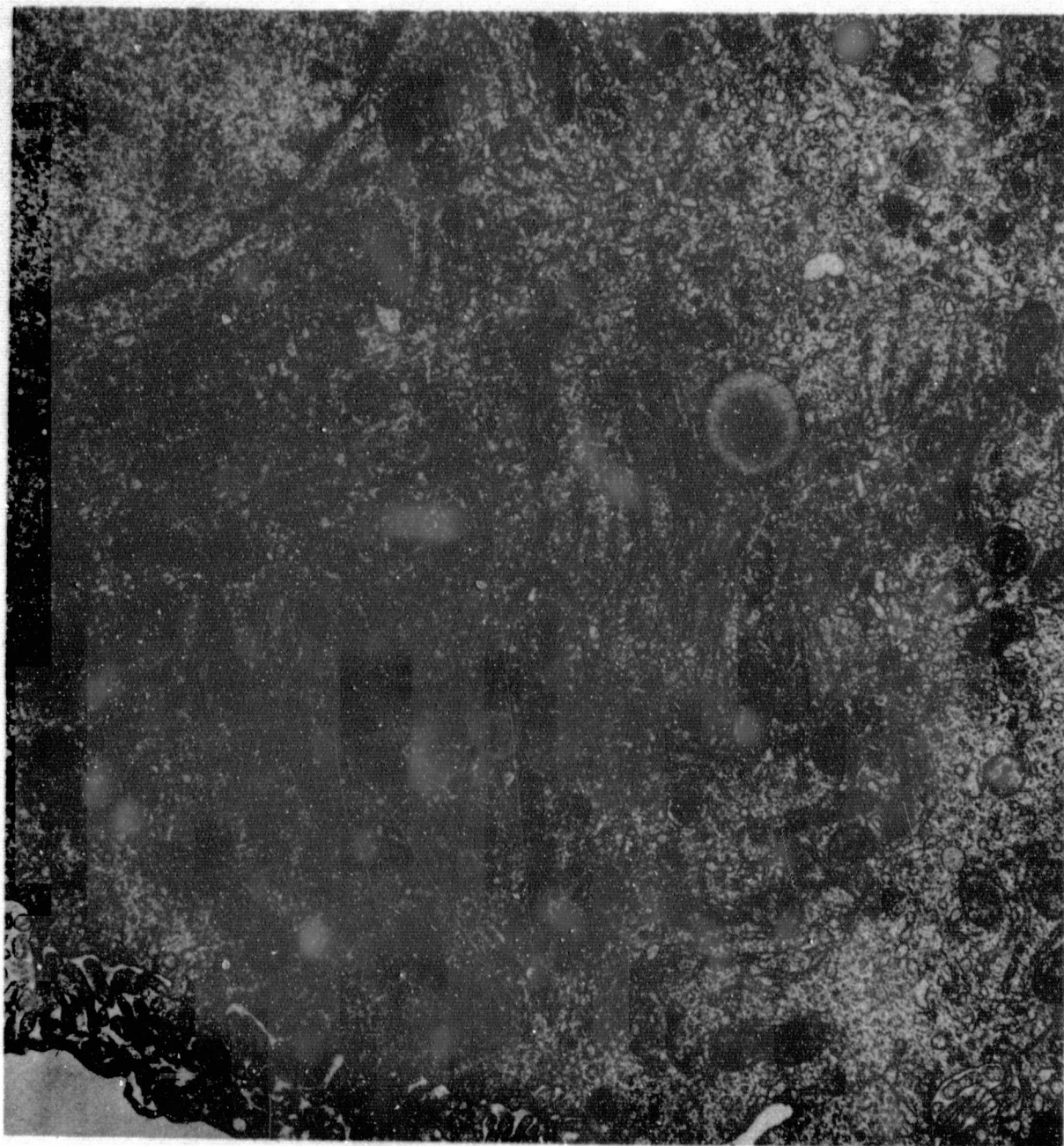


Figure 17: Electron micrograph of a centrilobular hepatocyte after 4 weeks continuous exposure to 250ppm 1,1,1- trichloroethane. This cell is indistinguishable from cells in control preparations. A few cells in the 250ppm group showed mild changes of the type illustrated previously for 1,000ppm. (X 20,000)