

Pathology Associates International

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**PATHOLOGY REPORT
(ANCILLARY STUDY)**

**ELECTRON MICROSCOPIC EVALUATION OF LIVER AND
LUNG AND LIGHT MICROSCOPIC EVALUATION OF
LIVER IN CRL:CD@BR VAF/PLUS® RATS)**

ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS

**CLIENT STUDY NUMBER 418-013
PAI STUDY NUMBER 99-11 (EM 99.39)**

Prepared by:

James B. Nold, D.V.M., Ph.D., Diplomate, A.C.V.P.

**Pathology Associates International
4915-D Prospectus Drive
Durham, NC 27713**

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**PREPARED FOR 3M CORPORATE TOXICOLOGY,
3M CENTER, ST. PAUL, MN 55144-1000**

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I. Pathology Narrative

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SUMMMARY

Ultrastructural evaluation of liver and lungs of rat pups from dams treated with 0 (control), 0.4, 1.6, or 3.2 mg/kg/day (high-dose) PFOS identified increased numbers of peroxisomes within hepatocytes of high-dose rat pup livers. Quantitation of peroxisomes indicated at least twice the number of peroxisomes in animals treated with 0.4 mg/kg/day and triple the number in those given 1.6 or 3.2 mg/kg/day. Differences in cellular membranes or mitochondria were not discernible between control and treated animals in the samples examined; however, suboptimal fixation precluded a detailed evaluation of these organelles. In lung samples, the presence of type II pneumocytes and lamellar bodies was evaluated. No significant ultrastructural differences were identified between lung from control and treated rats. Random type II pneumocytes containing lamellar bodies were identified in all lungs examined ultrastructurally.

PROCEDURES

The objective of this study was to examine by electron microscopy the livers and lungs from selected day-21 gestation rat fetuses in an oral (gavage) pharmacokinetic study of PFOS. In addition to a general qualitative evaluation for differences between control and treated animals, the ultrastructural endpoints included quantitation of peroxisomes within hepatocytes, membrane and mitochondrial changes in hepatocytes, and the quantitation of surfactant lamellar bodies in the lung. Light microscopy of paraffin-embedded liver sections was also performed.

For the pharmacokinetic study, 90 female Crl:CD@BR VAF/PLUS@ rats were given the test article or the vehicle once daily beginning 42 days prior to cohabitation with a male through day 20 postpartum according to Text Table 1.

Text Table 1. Dosage Levels, Concentrations and Volumes				
Dosage Group	Number of Mated Female Rats	PFOS Dosage (mg/kg/day)	PFOS Concentration (mg/mL)	Dosage Volume (mL/kg)
I	16	0 (Vehicle)	0	5
II	16	0.1	0.02	5
III	16	0.4	0.08	5
IV	16	1.6	0.32	5
V	16	3.2	0.64	5

At Caesarean-sectioning on day 21 of presumed gestation, lung and liver samples were collected from three fetuses per litter from five litters per dosage group. Samples of lung and liver from selected fetuses for Groups I, III, IV, and V were thin-sliced and placed in McDowell-Trump fixative (McDowell EM, 1976) and submitted to this laboratory for electron microscopy processing and evaluation. Sections of liver from these same animals were immersion-fixed in neutral buffered formalin and submitted along with electron microscopy samples.

Per sponsor request, tissue samples from selected pups were processed and evaluated (Text Table 2). The five digit number is the dam number; the suffixes 1-4 denotes the pup number from that dam's litter.

Text Table 2. Animals Selected for Electron Microscopy			
Group I Control (0 mg/kg/day)	Group III 0.4 mg/kg/day	Group IV 1.6 mg/kg/day	Group V 3.2 mg/kg/day
19504-3	19534-1	19560-1	19568-2
19505-1	19536-3	19560-2	19569-1
19506-4	19543-4	19560-3	19572-1
19509-1	19546-1	19562-2	19577-4
19516-2	19546-2	19562-3	19579-3

The lungs and livers were then processed into Spurr's resin for ultrastructural examination. Thick (1 μ) sections were cut, stained with toluidine blue, and examined to select representative areas for further electron microscopy processing. Thin sections (approximately 90 nm) were cut, mounted on 200-mesh copper grids, stained with 5% methanolic uranyl acetate and Reynold's lead citrate, and examined on a Zeiss 900 transmission electron microscope. Centrilobular hepatocytes, where clearly identifiable in liver sections, were preferentially examined. Representative electron photomicrographs of liver and lung were taken and significant ultrastructural features were summarized for each photograph and animal on a designated transmission electron micrograph interpretation form. The number of peroxisomes in hepatocytes was manually counted for each center photographed hepatocyte and recorded.

For light microscopy, formalin-fixed liver samples were processed and embedded in paraffin, cut at approximately five microns, stained with hematoxylin and eosin, and evaluated.

RESULTS AND DISCUSSION

Individual interpretations of light microscopy slides and electron micrographs for each animal are shown in Section II. Selected representative electron photomicrographs were labeled and are shown in Section III.

Light Microscopy

A summary of the incidence and severity of light microscopic findings in the liver is shown in Text Table 3. There was an increased incidence and severity of eosinophilic granularity of the cytoplasm of hepatocytes in treated rats compared with control rat livers. The severity of this finding increased in a dose-related fashion. Hepatocellular eosinophilic granularity was diffusely distributed in most animals given 1.6 or 3.2 mg/kg/day of PFOS, but was predominantly periportal in 1 animal given 1.6 and in 3 animals given 0.4 mg/kg/day. Cytoplasmic clearing of hepatocytes was prominent in control livers. This morphological description is usually associated with the normal accumulation of glycogen in hepatocytes. In this study, the incidence and severity of cytoplasmic clearing was almost inversely proportional to the occurrence of eosinophilic granularity.

The liver is a hematopoietic organ in rat fetuses and neonates. Thus, large numbers of hematopoietic cells are present within the sinusoids throughout the liver. Extramedullary hematopoiesis (EMH) occurred in all animals, including controls, and was uniformly graded as moderate to moderately-severe. The severity of EMH was increased in the treated groups. Random mitoses of hepatocytes are normal in rat livers, especially in fetuses and neonates. There appeared to be a slight increase in hepatocellular mitotic figures in the animals given 3.2 mg/kg/day PFOS.

Text Table 3. Incidence and Severity of Light Microscopic Liver Findings					
Microscopic Finding		PFOS Dose Levels (mg/kg/day)			
		0	0.4	1.6	3.2
(number examined)		(5)	(5)	(5)	(5)
Eosinophilic granularity, cytoplasmic, increased	minimal	2	2	1	-
	slight	-	3	3	1
	moderate	-	-	1	4
Cytoplasmic clearing	minimal	-	1	2	-
	slight	-	4	1	-
	moderate	5	-	1	-
Extramedullary hematopoiesis	moderate	5	4	1	-
	moderately-severe	-	1	4	5
Mitotic figures, increased	minimal	2	1	2	2
	slight	-	-	-	2

Electron Microscopy

Fixation of the tissues was less than optimal for electron microscopy. This was especially evident in the liver compared with the lung. Lung is inherently less dense than liver and allows better infiltration of the fixative into the tissue. Suboptimal fixation was characterized by clumping of large organelles, discontinuous plasma membranes, loss of detail of cytoplasmic organelles, dilatation of endoplasmic reticulum and the nuclear envelope, granular deposits in the mitochondrial matrix, and dilatation of mitochondrial cristae. The poor fixation of membranes made ultrastructural assessment of these structures impossible. Fixation, however, was still considered adequate to assess for the presence of peroxisomes in the hepatocytes and lamellar bodies in the lung and to make general comparisons between control and treated samples.

LIVER

Control (0 mg/kg/day). Subcellular features of control hepatocytes are demonstrated in Figures 1 and 2. Hepatocytes characteristically contain numerous mitochondria and prominent endoplasmic reticulum. Clumping of the large organelles (mitochondria, rough endoplasmic reticulum and peroxisomes) is indicative of poor fixation. In these cells rough endoplasmic reticulum was evident, but smooth endoplasmic reticulum was not because of suboptimal fixation. Mitochondria generally appeared dark and contained scattered dense deposits and some dilated cristae, both artifacts due to suboptimal fixation. Dilatation of endoplasmic reticulum and the nuclear envelope were additional artifacts of poor fixation. The fine granular material in the cytoplasm is consistent with glycogen particles. Peroxisomes were identified and quantitated in from representative hepatocytes. For the control livers, the average number of peroxisomes counted per hepatocyte photographed was 4.2 peroxisomes per cell (Text Table 4). Fetal and neonatal rat liver is a hematopoietic tissue, and numerous hematopoietic cells were evident within the liver sinusoids.

Text Table 4. Quantitation of Hepatocellular Peroxisomes							
0 mg/kg		0.4 mg/kg		1.6 mg/kg		3.2 mg/kg	
Animal Number	Number ¹ of Peroxisomes	Animal Number	Number of Peroxisomes	Animal Number	Number of Peroxisomes	Animal Number	Number of Peroxisomes
19504-3	3.2	19534-1	12.0	19560-1	11.6	19568-2	6.0
19505-1	3.0	19536-3	12.2	19560-2	12.8	19569-1	10.6
19506-4	4.4	19543-4	8.0	19560-3	10.2	19572-1	13.2
19509-1	5.2	19546-1	13.0	19562-2	10.6	19577-4	11.2
19516-2	5.0	19546-2	5.4	19562-3	20.0	19579-3	21.8
Mean =	4.2	Mean =	10.1	Mean =	13.0	Mean =	12.6

¹ Average number of peroxisomes per hepatocyte for five hepatocytes counted per animal.

0.4 mg/kg/day. The average number of peroxisomes per hepatocyte (10.1) was higher in the animals given 0.4 mg/kg/day PFOS compared with controls (Text Table 4). A representative hepatocyte is shown in Figure 3. Qualitatively, there were no identifiable

ultrastructural differences between the hepatocytes treated with 0 (control) and 0.4 mg/kg/day.

1.6 mg/kg/day. For animals given 1.6 mg/kg/day, the average number of peroxisomes per hepatocyte was 13.0. Figure 4 illustrates a representative hepatocyte from this treatment group. Otherwise, general ultrastructural morphology was similar in high-dose livers compared with control livers.

High-Dose (3.2 mg/kg/day). The average number of peroxisomes per hepatocyte (12.6) was higher in the high-dose animals compared with controls (Text Table 4). Figures 5 and 6 illustrate representative hepatocytes from high-dose animals. Differences in mitochondria and cellular membranes were not discernible between treated and control livers. Suboptimal fixation, however, of the tissues precluded detailed evaluation of these organelles.

LUNG

0 mg/kg/day (Control). Lung samples were atelectatic and not inflated with fixative. Therefore, most alveoli and alveolar septa were collapsed with poor delineation of alveolar and airway spaces. Figures 7-9 illustrate some normal cellularity and ultrastructural pulmonary anatomy. Type II pneumocytes (PN2) were randomly identified lining alveoli, terminal bronchioles, or within collapsed septa. Type II pneumocytes were identified by the presence of lamellar bodies, which are cytoplasmic vacuoles containing electron-dense laminated membrane-like profiles. Lamellar bodies represent the material secreted into the alveolar spaces as surfactant. Occasionally some lamellar body vacuoles were empty, as if the lamellar material had washed out or been secreted. Surfactant or lamellar material within airway spaces was only occasionally identified in control lungs or treated lungs. The type I pneumocyte (PN1) is characteristically flattened and lines the alveolar space as a thin margin of cytoplasm. Type I pneumocytes differentiate from type II pneumocytes. Many pneumocytes appeared to be cuboidal without distinctive lamellar bodies, and may represent a transition stage between PN2s and PN1s, especially since these lungs were fetal and had not been expanded with air. Additionally, some cuboidal epithelial cells represented terminal bronchiolar epithelium.

Type II pneumocytes and lamellar bodies were randomly identified in these control lungs. Some lamellar-like bodies were also present within capillary lumina, although in the control sections examined no lamellar bodies were clearly identified in the alveolar lumina.

0.4 mg/kg/day. Qualitatively, the lung samples from animals given 0.4 mg/kg/day appeared essentially the same as control samples. A representative sample is shown in Figure 10. Random type II pneumocytes containing lamellar bodies were identified, and only occasionally some lamellar material was seen within alveolar lumina.

1.6 mg/kg/day. Figures 11 and 12 illustrate some representative ultrastructural features from rat fetal lung samples given 1.6 mg/kg/day. These were considered essentially similar to the control and other treated samples. Lamellar bodies were identified within type II pneumocytes and, occasionally, within alveoli.

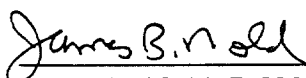
3.2 mg/kg/day (High-Dose). Ultrastructural pathology of the high-dose lungs was essentially similar to control, low- and mid-dose samples. The collapsed nature of most of the alveoli and septa made definitive quantitation impractical. Some lamellar bodies were identified within capillary lumina and only a small amount was seen within alveoli. Figures 13 and 14 illustrate some representative lung fields from the high-dose animals.

CONCLUSION

Ultrastructural evaluation of liver and lungs of rat pups from dams treated with 0 (control), 0.4, 1.6, or 3.2 mg/kg/day (high-dose) PFOS identified increased numbers of peroxisomes within hepatocytes of rat pup livers from dams given 1.6 or 3.2 mg/kg/day. Quantitation of peroxisomes indicated at least twice the number of peroxisomes in animals treated with 0.4 mg/kg/day and triple the number in those given 1.6 or 3.2 mg/kg/day. Differences in cellular membranes or mitochondria were not discernible between control and treated animals in the samples examined; however, suboptimal fixation precluded a detailed evaluation of these organelles.

In lung samples, the presence of type II pneumocytes and lamellar bodies was evaluated. No significant ultrastructural differences were identified between lung from control and treated rats. Random type II pneumocytes containing lamellar bodies were identified in all lungs examined ultrastructurally. Although some lamellar material (surfactant) was morphologically identifiable within alveolar lumina, no significant differences were noted between control and treated samples.

SIGNATURE OF AUTHOR



James B. Nold, D.V.M., Ph.D.,
Diplomate, A.C.V.P.

April 19, 1999
Date

REFERENCES

McDowell, EM and Trump, BF: Histologic fixative for routine diagnostic light and electron microscopy. *Arch. Pathol. Lab. Med.*, 100:405-414, 1976.

II. Light Microscopy and Transmission Electron Micrograph Interpretation Forms ¹

¹ Forms are typographically corrected versions of signed/dated raw data sheets maintained in archived study file.

LIGHT MICROSCOPIC EVALUATION

Study No.: 418-013 (PAI 99-11)

Species/Strain: Crl:CD®BR VAF/Plus®
Rats, Term Fetuses

Animal No: see below

Tissue: Liver

Sex: n/a

Interpreting Pathologist (signature and date): _____

Dose	Animal Number	Microscopic Findings and Comments
0 mg/kg	19504-3	1. Extramedullary hematopoiesis (EMH), diffuse, moderate 2. Cytoplasmic clearing, diffuse, moderate 3. Mitotic figures, minimal, multifocal
	19505-1	1. EMH, diffuse, moderate 2. Cytoplasmic clearing, diffuse, moderate
	19506-4	1. EMH, diffuse, moderate 2. Cytoplasmic clearing, diffuse, moderate 3. Eosinophilic granularity, cytoplasmic, increased, periportal, minimal
	19509-1	1. EMH, diffuse, moderate 2. Cytoplasmic clearing, diffuse, moderate 3. Eosinophilic granularity, cytoplasmic, increased, periportal, minimal
	19516-2	1. EMH, diffuse, moderate 2. Cytoplasmic clearing, diffuse, moderate 3. Mitotic figures, minimal, multifocal
0.4 mg/kg	19534-1	1. EMH, diffuse, moderate 2. Cytoplasmic clearing, diffuse, minimal 3. Eosinophilic granularity, cytoplasmic, increased, diffuse, minimal
	19536-3	1. EMH, diffuse, moderate 2. Cytoplasmic clearing, diffuse, slight 3. Eosinophilic granularity, cytoplasmic, increased, periportal, slight
	19543-4	1. EMH, diffuse, moderate 2. Cytoplasmic clearing, diffuse, slight 3. Eosinophilic granularity, cytoplasmic, increased, periportal, slight
	19546-1	1. EMH, diffuse, moderate 2. Cytoplasmic clearing, diffuse, slight 3. Eosinophilic granularity, cytoplasmic, increased, diffuse, minimal
	19546-2	1. EMH, diffuse, moderately-severe 2. Cytoplasmic clearing, diffuse, slight 3. Eosinophilic granularity, cytoplasmic, increased, periportal, slight 4. Mitotic figures, minimal, multifocal

Dose	Animal Number	Microscopic Findings and Comments
1.6 mg/kg	19560-1	1. Eosinophilic granularity, cytoplasmic, increased, moderate, diffuse 2. EMH, diffuse, moderately-severe
	19560-2	1. EMH, diffuse, moderately-severe 2. Cytoplasmic clearing, diffuse, minimal 3. Eosinophilic granularity, cytoplasmic, increased, diffuse, slight 4. Mitotic figures, minimal, multifocal
	19560-3	1. EMH, diffuse, moderate 2. Cytoplasmic clearing, diffuse, moderate 3. Eosinophilic granularity, cytoplasmic, increased, periportal, minimal 4. Mitotic figures, minimal, multifocal
	19562-2	1. EMH, diffuse, moderately-severe 2. Cytoplasmic clearing, diffuse, slight 3. Eosinophilic granularity, cytoplasmic, increased, diffuse, slight
	19562-3	1. EMH, diffuse, moderately-severe 2. Cytoplasmic clearing, diffuse, minimal 3. Eosinophilic granularity, cytoplasmic, increased, diffuse, slight
3.2 mg/kg	19568-2	1. Eosinophilic granularity, cytoplasmic, increased, moderate, diffuse 2. EMH, diffuse, moderately-severe 3. Mitotic figures, slight, multifocal
	19569-1	1. Eosinophilic granularity, cytoplasmic, increased, slight diffuse 2. EMH, diffuse, moderately-severe 3. Mitotic figures, slight, multifocal
	19572-1	1. Eosinophilic granularity, cytoplasmic, increased, moderate, diffuse 2. EMH, diffuse, moderately-severe 3. Mitotic figures, minimal, multifocal
	19577-4	1. Eosinophilic granularity, cytoplasmic, increased, moderate, diffuse 2. EMH, diffuse, moderately-severe 3. Mitotic figures, minimal, multifocal
	19579-3	1. Eosinophilic granularity, cytoplasmic, increased, moderate, diffuse 2. EMH, diffuse, moderately-severe

Incidence and Severity of Light Microscopic Liver Findings				
Microscopic Finding	PFOS Dose Levels (mg/kg/day)			
	0	0.4	1.6	3.2
(number examined)	(5)	(5)	(5)	(5)
Eosinophilic granularity, cytoplasmic, increased				
minimal	2	2	1	-
slight	-	3	3	1
moderate	-	-	1	4
Cytoplasmic clearing				
minimal	-	1	2	-
slight	-	4	1	-
moderate	5	-	1	-
Extramedullary hematopoiesis				
moderate	5	4	1	-
moderately-severe	-	1	4	5
Mitotic figures, increased				
minimal	2	1	2	2
slight	-	-	-	2

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013; EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 19504-3

Tissue: Liver

Treatment Group: 0 mg/kg, Control

Block No(s).: 99.39-1
Z15778 -

Sex: non applicable

Photo/Negative No(s).: Z15782

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): _____ 3-10-99

Features: Z15778. Liver. Hepatocyte adjacent to central vein at left and bottom. Bile canaliculus at top. Cytoplasmic organelles are clumped together with intervening spaces of fine granular material. Mitochondria appear dense and have some dilated cristae. Five (5) peroxisomes counted.

Z15779. Liver. Hepatocyte exhibiting similar cellular details as Z15778. Zero (0) peroxisomes clearly identified.

Z15780. Liver. Hepatocyte appears similar to above examples with clumped organelles. A hematopoietic cell is in the top right sinusoid and a bile canaliculus is present in the lower left. Mitochondria appear dense and have some dilated cristae. Three (3) peroxisomes counted.

Z15781. Liver. Hepatocyte and adjacent sinusoid containing two nucleated red blood cells. Mitochondria appear dense with some granular deposits and dilated cristae. Few numbers of peroxisomes identified, although poor fixation makes their clear identification difficult. Four (4) peroxisomes counted.

Z15782. Liver. Center hepatocyte is surrounded by several other hepatocytes, with a sinusoid containing a nucleated erythrocyte in bottom right and a bile canaliculus in top portion of photograph. Mitochondria appear dense with some granular deposits and dilated cristae. Four (4) peroxisomes counted.

Conclusions: Normal hepatocyte. Suboptimal fixation. Average 3.2 peroxisomes per hepatocyte.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013; EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 19505-1

Tissue: Liver

Treatment Group: 0 mg/kg, control

Block No(s).: 99.39-3
Z15788 -

Sex: non applicable

Photo/Negative No(s).: Z15792

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z15788. Liver. Hepatocyte contains abundant fine granular material and some clumping of mitochondria and endoplasmic reticulum. Mitochondria are dense and contain some dilated cristae. Few peroxisomes are noted, four (4) counted.

Z15789. Liver. Hepatocyte appears similar to Z15788, with fine granular cytoplasmic material and clumping of larger organelles. Four (4) peroxisomes counted.

Z15790. Liver. Hepatocyte. Ventrally is a large sinusoid with a Kupffer cell among several erythrocytes. A hematopoietic cell is on the lower right. Some dilated blebs of rough endoplasmic reticulum (RER) are evident in the center hepatocyte. . Mitochondria appear dense with some granular deposits and dilated cristae. One (1) peroxisome counted.

Z15791. Liver. Hepatocyte appear partially detached from adjacent cells and suboptimally fixed. . Mitochondria appear dense with some granular deposits and dilated cristae. Two (2) peroxisomes counted.

Z15792. Liver. Hepatocyte appears essentially similar to those described above.

Abundant fine granular material in cytoplasm. . Mitochondria appear dense with some granular deposits and dilated cristae. Some dilated blebs of RER noted. Four (4) peroxisomes counted.

Conclusions: Normal hepatocyte. Suboptimal fixation. Average 3.0 peroxisomes per hepatocyte.

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TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013; EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 19505-1

Tissue: Lung

Treatment Group: 0 mg/kg, Control

Block No(s).: 99.39-4

Z15793 -

Sex: non applicable

Photo/Negative No(s).: Z15797

Significant Lesions (check one): _____ Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z15793. Lung. Alveolus with lining cells. Two cuboidal pneumocytes with fine granular cytoplasm. Top cell has an extended flattened portion that contains a lamellar-like structure, and may be a PN2 that is becoming a PN1. Normal, more flattened PN1s are present adjacent to these two central cells.

Z15794. Lung. Two of four cuboidal lining cells have lamellar bodies characteristic of PN2. Smaller and darker cells has a microvillous border than contains a lamellar structure, probably alveolar surfactant.

Z15795. Lung. Alveolus with lining cells. Dark cuboidal cell is a PN2 and contains several lamellar bodies. Some flattened PN1s are present as well as another cuboidal pneumocyte. A capillary containing erythrocytes is within the alveolar septum.

Z15796. Lung. Alveolus and area of collapsed septa. Two PN2s are present, both containing two to six lamellar bodies. No alveolar luminal lamellar structures are evident.

Z15797. Lung. Alveolus and lining cells. The dark columnar cell is a PN2 and contains several lamellar bodies. The left adjacent pneumocytes appear not to have flattened out as more differentiated PN1s. A second PN2 is just to the right of the central PN2, and contains several small lamellar bodies. Subjacent to the alveolus is a capillary containing a leucocyte and endothelial cell.

Conclusions: Normal lung. A moderate number of type II pneumocytes were identifiable, and contained lamellar bodies.

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TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013; EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 19506-4

Tissue: Liver

Treatment Group: 0 mg/kg, control

Block No(s).: 99.39-5
Z15808 -

Sex: non applicable

Photo/Negative No(s).: Z15812

Significant Lesions (check one): _____ Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z15808. Liver. Hepatocyte contains abundant fine granular cytoplasmic material and some clumping of predominantly mitochondria and rough endoplasmic reticulum. Five (5) peroxisomes counted.

Z15809. Liver. Hepatocyte appears similar to Z15808. Some dilated blebs of RER are noted. Six (6) peroxisomes counted.

Z15810. Liver. Hepatocyte has a washed-out appearance to much of its cytoplasm, and is surrounded by hematopoietic cells ventrally. Endothelial cells are incompletely defined. At the top left of the photograph is a bile canaliculus. Two (2) peroxisomes are counted.

Z15811. Liver. Hepatocyte with prominent bile canaliculus at lower left and sinusoid on right margin of photograph. Mitochondria are dense and contain some dense deposit and dilated cristae. Six (6) peroxisomes counted.

Z15812. Liver. Hepatocyte contains abundant washed-out cytoplasm and clumping of RER and mitochondria. At the top are two hematopoietic cells and a bile canaliculus is present in lower right of photograph. Three (3) peroxisomes counted.

Conclusions: Normal liver. Suboptimal fixation. Average 4.4 peroxisomes per hepatocyte

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013; EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 19506-4

Tissue: Lung

Treatment Group: 0 mg/kg, control

Block No(s).: 99.39-6
Z15818 -

Sex: non applicable

Photo/Negative No(s).: Z15822

Significant Lesions (check one): _____ Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z15818. Lung. Dark cell with round nucleus in the center is a PN2 which partially lines the alveolar space. Several lamellar bodies are within its cytoplasm. The large clear cytoplasm represents an adjacent PN1. A capillary containing an erythrocyte within the septum is immediately adjacent the PN2. The remainder of the tissue is collapsed alveoli and septa.

Z15819. Lung. A thin-margin of PN1 surrounds a protruding septum and capillary in the middle of the photograph. Two endothelial cells appear to line the capillary, which contains an erythrocyte. To the right is a somewhat darker PN2, which contains several lamellar bodies. In the central bottom is a large type I pneumocyte, with a large polygonal nucleus and mostly clear cytoplasm.

Z15820. Lung. PN1s line several septa in this photograph. Dorsally, the septal capillary contains a lymphocyte and a nucleated erythrocyte. In the center capillary a granulocytic cell is evident. No discernible PN2s or lamellar bodies are evident.

Z15821. Lung. Alveolus and lining cells. A PN2 is present lining the alveolus. It is the darker cell with several dilated lamellar bodies. Most of the lamellar material in these bodies is missing. Two PN1s are evident on the right margin of this alveolar space. Large nucleus on the left appears to belong to an endothelial cell lining a septal capillary.

Z15822. Lung. Alveolus and several lining cells. Most appear to be PN1s with abundant somewhat clear cytoplasm, which hasn't completely flattened out. No definitive PN2s are evident, although two small vacuoles in a lining pneumocyte at the bottom of the photograph may represent lamellar bodies. A capillary containing two nucleated cells occupies the right side of the photograph.

Conclusions: Normal lung. Occasional type II pneumocytes containing lamellar bodies identified. No lamellar material noted within alveolar spaces.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013; EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 19509-1

Tissue: Liver

Treatment Group: 0 mg/kg, control

Block No(s).: 99.39-7

Sex: non applicable

Z15823 -
Photo/Negative No(s).: Z15827

Significant Lesions (check one): _____ Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z15823. Liver. Hepatocyte bordered by sinusoid containing nucleated erythrocyte on left and other hepatocytes on bottom and right. An endothelial cell is in the upper left portion of the photograph. There is clumping of the mitochondria and RER with an intervening open area or areas filled with fine granular material. The RER is dilated. Mitochondria contain some granular deposits. Five (5) peroxisomes counted.

Z15824. Liver. Hepatocyte. At the top is a nucleated erythrocyte. Sinusoidal endothelium is poorly delineated. The RER shows some blebbing and dilatation, and mitochondria have some dense deposits and some dilatation of cristae. Five (5) peroxisomes counted.

Z15825. Liver. Hepatocyte surrounded by hematopoietic cells. The RER and nuclear envelope shows some dilatation and blebbing. Mitochondria appear dense and have some granular deposits and dilated cristae. Seven (7) peroxisomes counted.

Z15826. Liver. Hepatocyte appears essentially similar to above described cells with clumping of major organelles. Five (5) peroxisomes counted.

Z15827. Liver. Hepatocyte surrounded by hematopoietic cells. A single larger vacuole is present in the cytoplasm and contains a flocculent like material. Four (4) peroxisomes counted.

Conclusions: Normal hepatocyte. Suboptimal fixation. Average 5.2 peroxisomes per hepatocyte.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013; EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 19509-1

Tissue: Lung

Treatment Group: 0 mg/kg, control

Block No(s).: 99.39-8
Z15828 -

Sex: non applicable

Photo/Negative No(s).: Z15832

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z15828. Lung. Alveolus and lining cells. The darker cuboidal cell is a PN2 and contains several lamellar bodies. An erythrocyte is immediately to the left of the PN2 and appears to be within the alveolus. At top left is a pneumocyte which has two small lamellar-like bodies and is probably a PN2 in transition to a PN1. A flattened PN1 is immediately on top and to the right of the dark PN2. Beneath these cells the septa are collapsed together.

Z15829. Lung. Alveolus and lining cells. The smaller dark cell is a PN2 with several lamellar bodies. To its right is a flattened PN1 and to its left is a cuboidal PN1. The lamellar or myelin-like material to the right of the PN2 appears to be within a capillary lumen. At the bottom right is a thin-walled septum containing a capillary and erythrocyte.

Z15830. Lung. Two erythrocytes are within the capillary lumen. The lining pneumocytes are flattened and appear to contain several vacuoles that may have contained lamellar bodies. Subjacent are collapsed septa and a mixture of septal cells including pneumocytes and endothelial cells.

Z15831. Lung. Area of collapsed alveolar septa. At the left middle and right middle-top are dark cells which contain lamellar bodies and are PN2s. Other cells are compressed pneumocytes, endothelial cells, and blood cells within capillaries. Some lamellar debris appears to be within capillary lumina.

Z15832. Lung. Area of collapsed septa. The large round clear cell is a cuboidal pneumocyte. Dorsal to this are endothelial cells lining a capillary. The lamellar structure appears to be within the lumen of the capillary.

Conclusions: Normal lung. Scattered type II pneumocytes present. Some lamellar material also present within capillaries. No lamellar bodies noted in alveolar spaces.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013; EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 19516-2

Tissue: Lung

Treatment Group: 0 mg/kg, control

Block No(s).: 99.39-10
Z15838 -

Sex: non applicable

Photo/Negative No(s).: Z15842

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z15838. Lung. Two pneumocytes at the bottom are PN2 and contain some lamellar bodies. Dorsal to them are collapsed septa containing primarily pneumocytes and endothelial cells.

Z15839. Lung. Several pneumocytes are present. Central pneumocyte contains several lamellar bodies and is a PN2. Flattened PN1s line the protruding septum which contains a capillary, endothelial cell and leucocyte within the capillary.

Z15840. Lung. Single septum lined by PN1s on either side. On the right top is a PN2 with several lamellar bodies. The right lower pneumocyte also contains a single lamellar body. The septal capillary and endothelium are also evident.

Z15841. Lung. Area of collapsed septa. At right are two pneumocytes which contain multiple lamellar bodies. Other compressed septal cells appear to be pneumocytes and endothelial cells.

Z15842. Lung. Area of collapsed septa. Several cuboidal pneumocytes are present. Centrally is a capillary containing an erythrocyte. Next to it is a couple lamellar structures that appear to be within the capillary lumen.

Conclusions: Normal lung. Random type II pneumocytes containing lamellar bodies were identified. No lamellar bodies were clearly identified within alveolar spaces.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013; EM-99.39

Species/Strain: Crl:CD®BR VAF/Plus®
Rat

Animal No.: 19516-2

Tissue: Liver

Treatment Group: 0 mg/kg, control

Block No(s).: 99.39-9

Sex: non applicable

Z15833 -
Photo/Negative No(s).: Z15837

Significant Lesions (check one): _____ Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z15833. Liver. Hepatocyte with a sinusoid at lower right and a bile canaliculus on the left. Cell has abundant washed-out cytoplasm and clumping of mitochondria and RER. There is dilatation of RER and the nuclear envelope. Mitochondria contain some granular deposits and some dilated cristae. Plasma membranes and sinusoidal endothelial cells are poorly delineated. Five (5) peroxisomes counted.

Z15834. Liver. Dilation and blebbing of RER. Some granular deposits present in mitochondria. Nine (9) peroxisomes counted.

Z15835. Liver. Mitochondria contain some dense deposits and dilated cristae. There is blebbing of the RER. Plasma membranes are poorly defined. Five (5) peroxisomes counted.

Z15836. Liver. Hepatocellular morphology essentially similar as those described above. Four (4) peroxisomes counted.

Z15837. Liver. Mitochondria contain some dense deposits and dilated cristae. Plasma membranes are poorly defined. A multivesicular body is present to the right of the nucleus. Cell at lower left appears to be an endothelial cell, but may be a hematopoietic cell in the sinusoids. Two (2) peroxisomes counted.

Conclusions: Normal liver. Suboptimal fixation. Average 5.0 peroxisomes per hepatocyte.

001564

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013 EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 19534-1

Tissue: Lung

Treatment Group: 0.4 mg/kg

Block No(s).: 99.39-32
Z15941 -

Sex: non applicable

Photo/Negative No(s).: 15945

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z15941. Lung. Alveolus and lining cells. All the lining cells appear to be type I pneumocytes (PN1s). Beneath the PN1s is a round to polygonal cell containing 5 lamellar bodies in its cytoplasm; this is a type II pneumocyte (PN2). This is probably a lining cell, and the plane of section is somewhat flat through the septal wall.

Z15942. Lung. Alveolar space and lining cells. The dark and somewhat flattened lining cell is a PN2 and contains several vacuoles empty of contents. These appear to be “empty” lamellar bodies. The lighter-staining pneumocyte at the far left also contains two similar-appearing vacuoles.

Z15943. Lung. Alveolus and lining cells. The dark nucleus is part of a flattened pneumocyte, but also attaches with two adjacent more lucent PNIs. No lamellar bodies noted in this photograph, although a vacuole adjacent to the top left nucleus may be an “empty” lamellar body. Septal walls and alveoli are collapsed and normal pulmonary architecture is indistinct.

Z15944. Lung. The dark lining cell on the right contains several lamellar bodies and is a PN2. The other lining cells, although cuboidal, appear to be pneumocytes which have not flattened out in the characteristic type I pneumocyte fashion.

Z15945. Lung. Alveolus and lining cells. The two dark lining cells both appear flattened along the septal wall and are PN1s. No lamellar bodies are seen in these lining cells. The top cell in the photograph appears detached and surrounded by some extracellular lipid-like material. Its identity is unclear, but may be a macrophage or endothelial cell. A capillary is present at the top left.

Conclusions: Essentially normal fetal lung. Random type II pneumocytes with lamellar bodies identified. No remarkable abnormalities noted.

001566

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013 EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 19536-3

Tissue: Liver

Treatment Group: 0.4 mg/kg

Block No(s).: 99.39-33

Sex: non applicable

Photo/Negative No(s).: Z15950

Significant Lesions (check one): X Yes No

Interpreting Pathologist (signature and date): _____

Features: Z15946. Liver. Hepatocyte. There is dilatation of RER and of the nuclear envelope. Some granular deposits are evident within some mitochondria. Four (4) peroxisomes counted.

Z15947. Liver. Hepatocyte. There is some clumping of organelles. The RER and nuclear envelope are dilated. Seventeen (17) peroxisomes counted.

Z15948. Liver. Hepatocyte. There is poor definition of the plasma membrane. RER and the nuclear envelope are dilated. Fourteen (14) peroxisomes counted.

Z15949. Liver. Hepatocyte appears similar to those described above. Eighteen (18) peroxisomes counted.

Z15950. Liver. Hepatocyte. At the top is an endothelial cell and several adjacent erythrocytes and one nucleated erythrocyte. The plasma membrane of the hepatocyte is indistinct and organelles are clumped in the cytoplasm. RER is dilated. Eight (8) peroxisomes counted.

Conclusions: Increased number of peroxisomes per hepatocyte compared with control samples. Average 12.2 peroxisomes per hepatocyte. Suboptimal fixation.

001567

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013 EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 19536-3

Tissue: Lung

Treatment Group: 0.4 mg/kg

Block No(s).: 99.39-34

Z15951 -

Sex: non applicable

Photo/Negative No(s).: Z15955

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z15951. Lung. Alveolus and area of collapsed septa. The bottom alveolus contains debris including some lamellar material consistent with secreted lamellar bodies (surfactant) from PN2s. The lining pneumocytes are all flattened and appear to be PN1s. No PN2s are identified. At least two capillaries are present in the collapsed septal area.

Z15952. Lung. Alveolus and lining cells. PN1s line the alveolar space and are flattened. On the right the nucleus and cell beneath the PN1 contains a single dark lamellar body, which suggest this cell maybe a PN2. Other cells in the photograph are endothelial cells, granulocyte in a capillary and some nonidentified cells. No lamellar bodies are present in the alveolar lumen.

Z15953. Lung. The prominent dark lamellar or myelin-like body in the center of the photograph is within a capillary space. Type 1 pneumocytes are flattened and line the alveolar space. At the bottom of the photograph in the collapsed septal area is a cell which contains two lamellar bodies, a PN2. No abnormalities noted.

Z15954. Lung. Alveolus and lining cells. A small central dark cell contains 3-4 lamellar bodies and is a PN2. The adjacent cell is cuboidal, but appears to be a pneumocyte which has not flattened out. A capillary containing several erythrocytes and a lining endothelial cell is prominent in the center of the photograph. Some debris is present in the alveolar lumen, and some may be fine lamellar material. The nucleated cell at the top contains two lamellar bodies and is also a PN2.

Z15955. Lung. Lamellar bodies within a PN2 line the alveolar space. Collapsed alveoli and septa fill the top of the page. No abnormalities noted.

Conclusions: Essentially normal fetal lung. Some type II pneumocytes identified containing lamellar bodies. Some lamellar bodies present within alveoli.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013 EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 19546-1

Tissue: Liver

Treatment Group: 0.4 mg/kg

Block No(s).: 99.39-37
Z15966 -

Sex: non applicable

Photo/Negative No(s).: Z15970

Significant Lesions (check one): X Yes No

Interpreting Pathologist (signature and date): _____

Features: Z15966. Liver. Hepatocyte. Plasma membrane is indistinct and cytoplasmic organelles are clumped together. Mitochondria show some dense deposits and dilated cristae. Fifteen (15) peroxisomes counted.

Z15967. Liver. Hepatocyte appears morphologically similar to Z15966, with clumping of organelles, indistinct plasma membranes, and mitochondrial changes. RER appears dilated. Seven (7) peroxisomes counted.

Z15968. Liver. Hepatocyte. A prominent bile canaliculus is present in the bottom right corner. Organelles are clumped with large intervening spaces of fine granular material. Mitochondria show some dense deposits and dilated cristae. Twenty (20) peroxisomes counted.

Z15969. Liver. Hepatocyte appears essentially similar to Z15968. Fixation is poor. Eleven (11) peroxisomes counted.

Z15970. Liver. RER is dilated and plasma membranes are indistinct. An endothelial cell is not present at sinus at top. Mitochondria show some dense deposits and dilated cristae. Twelve (12) peroxisomes counted.

Conclusions: Liver exhibits poor fixation. There appears to be increased numbers of peroxisomes per hepatocyte. Average 13.0 peroxisomes per hepatocyte.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013 EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 19546-1

Tissue: Lung

Treatment Group: 0.4 mg/kg

Block No(s).: 99.39-38
Z15971 -

Sex: non applicable

Photo/Negative No(s).: Z15975

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z15971. Lung. Alveolus and lining cells. Several lining cells contain lamellar bodies, most predominantly the center cell with at least nine lamellar bodies. This is a distinctive PN2. The three cells at the top of the photograph appear to be pneumocytes whose alveoli are out of plane of section or are collapsed.

Z15972. Lung. Photograph slightly out of focus. An unattached PN2 is present in the alveolar space. Both sides of the septum are lined by flattened PN1s. A capillary and endothelial cell are evident within the septum. No lamellar material is present in the alveoli.

Z15973. Lung. Alveoli and septum. The dark cell on the top left is a PN2 with several “empty” lamellar bodies. The other lining cells are PN1s. A capillary with a luminal erythrocyte is present in the center of the septum. No lamellar material is present in the alveoli.

Z15974. Lung. Alveolus and lining cells. A flattened PN1 lines the bottom margin of the alveolus. Next to it is a cuboidal pneumocyte with several clear vacuoles, a PN2. The vacuoles are “empty” lamellar bodies. The central cell with a microvillous border, dilated nuclear envelope and distended endoplasmic reticulum is a cuboidal pneumocyte exhibiting some elements of suboptimal fixation. No lamellar bodies noted in the alveoli.

Z15975. Lung. Cuboidal lining cell in center right and cell at center left both contain one or two vacuoles which appear to be lamellar bodies. These cells are probably PN2s converting to PN1s after expressing their lamellar (surfactant) material. No lamellar material noted in the alveoli.

Conclusions: Essentially normal fetal lung. Scattered type II pneumocytes with lamellar bodies identified. No lamellar material is present in the alveoli.

001572

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013 EM-99.39

Species/Strain: Crl:CD®BR VAF/Plus®
Rat

Animal No.: 19546-2

Tissue: Liver

Treatment Group: 0.4 mg/kg

Block No(s).: 99.39-39
Z15976 -

Sex: non applicable

Photo/Negative No(s).: Z15980

Significant Lesions (check one): _____ Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z15976. Liver. Hepatocyte exhibits a poorly-defined plasma membrane. The organelles are clumped and the mitochondria show dilated cristae. At top right is an interstitial cell surrounded by collagen, probably a fibroblast. At the lower left are some hematopoietic cells within a sinusoid. Four (4) peroxisomes counted.

Z15977. Liver. Two hepatocytes with an intervening bile canaliculus. Hematopoietic cells are present in the upper right and lower left sinusoids. The larger cytoplasmic organelles are clumped together and the cytosol appears washed-out or contains a fine granular material. The mitochondria show some dilated cristae. The hepatocyte (bottom cell) contains seven (7) peroxisomes.

Z15978. Liver. Central hepatocyte in photograph appears similar to those described above, fixation is poor. Two (2) peroxisomes counted.

Z15979. Liver. Hepatocyte, whose morphology is similar to those described above. Five (5) peroxisomes counted.

Z15980. Liver. Hepatocyte exhibits a poorly-defined plasma membrane. The organelles are clumped and the mitochondria show dilated cristae. RER is dilated. Nine (9) peroxisomes counted.

Conclusions: Essentially normal liver. Poor cellular fixation. Average 5.4 peroxisomes per hepatocyte.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013 EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 19546-2

Tissue: Lung

Treatment Group: 0.4 mg/kg

Block No(s).: 99.39-40
Z15981 -

Sex: non applicable

Photo/Negative No(s).: Z15985

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z15981. Lung. Alveolus and lining cells. A partially detached pneumocyte is present in the air space. Large right central cell contains a single lamellar body near its top margin. The other dark organelles appear to be predominantly mitochondria. The cytoplasm of these cuboidal pneumocytes contains a fine granular material suggestive of glycogen.

Z15982. Lung. Central cell contains multiple lamellar bodies and is the cytoplasm of a PN2. This is in an area of collapsed alveoli and septa. A capillary and endothelial cell are at the left of the photograph. The two dark cells on either side of the PN2 are not clearly identifiable. The bottom right cell is a PN1. No abnormalities described.

Z15983. Lung. Two columnar cells on the right both contain lamellar bodies, although the majority of their cytoplasm consist of fine granular material. PNIs appear to be at the bottom of the photograph and a capillary and endothelial cell are at the left.

Z15984. Lung. An area of collapsed alveoli and septa. At the top and bottom are two round to polygonal cells that contain some lamellar bodies consistent with type II pneumocytes (PN2s). Some lamellar material appears to be extracellular adjacent to the bottom PN2. The central cells all appear to be PN1s. At the bottom right is a capillary, endothelial cell and erythrocyte. No lamellar bodies noted in alveoli.

Z15985. Lung. At the top right is a cuboidal PN2 lining the alveolus. This cell contains several lamellar bodies, and sits next to a more flattened PN1. Centrally is a capillary containing two large endothelial cell nuclei and an erythrocyte.

Conclusions: Essentially normal fetal lung. Random type II pneumocytes (PN2s) containing lamellar bodies were identified. Some extracellular lamellar material identified outside one type II pneumocyte.

001574

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013 EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 19560-1

Tissue: Liver

Treatment Group: 1.6 mg/kg

Block No(s).: 99.39-21
Z15884 -

Sex: non applicable

Photo/Negative No(s).: Z15888

Significant Lesions (check one): X Yes No

Interpreting Pathologist (signature and date): _____

Features: Z15884. Liver. Hepatocyte. Plasma membranes and adjacent endothelial cells are indistinct, consistent with suboptimal fixation. Dilation of RER with some blebs. Some dilated cristae in mitochondria. A single clear vacuole is present in the cytoplasm on the right side of the cell. Fifteen (15) peroxisomes counted.

Z15885. Liver. Some granular deposits present in mitochondria and dilatation of RER. Twenty-one (21) peroxisomes counted.

Z15886. Liver. Hepatocyte in center of photograph with hematopoietic cells at top. Some clumping of mitochondria and RER. Dilated RER. Six (6) peroxisomes counted.

Z15887. Liver. Hepatocyte containing a single large lipid droplet in dorsal cytoplasm. At top right is a hematopoietic cell and at lower left is a bile canaliculus. There is clumping of cytoplasmic organelles. RER and nuclear envelope appear dilated. Five (5) peroxisomes counted.

Z15888. Liver. Hepatocyte similar to those described above. Some stain precipitate is present on photograph. Eleven (11) peroxisomes counted.

Conclusions: There appears to be increased numbers of peroxisomes per hepatocyte compared with control samples. Average 11.6 peroxisomes per hepatocyte. Suboptimal tissue fixation.

001575

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013 EM-99.39

Species/Strain: Crl:CD®BR VAF/Plus®
Rat

Animal No.: 19560-1

Tissue: Lung

Treatment Group: 1.6 mg/kg

Block No(s).: 99.39-22
Z15889 -

Sex: non applicable

Photo/Negative No(s).: Z15893

Significant Lesions (check one): _____ Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z15889. Lung. Alveolus and lining cells. Top cell with darker cytoplasm is a PN2 and contains two lamellar bodies. The lower cell also contains two prominent vacuoles, which are probably expressed lamellar bodies even though the lamellar material is no longer evident. The central cell is a PN1. Beneath the PN1 is an extension of an unidentified cell out of plane of section. A capillary is present at the bottom left of the photograph. No lamellar material is present in the alveolar space.

Z15890. Lung. Three lining cells and a capillary are evident. The central dark cell with an oblong nucleus is a PN2, and it contains 3 discernible lamellar bodies. At the top left and to the right of the PN2 are PN1s with more lucent cytoplasm. The capillary and lining endothelial cell are at the top right. A dark erythrocyte is present in the capillary.

Z15891. Lung. Alveolus, lining cells, and capillaries. Centrally is a PN2 somewhat sandwiched in between two flattened PN1s lining the alveolus. The PN2 contains several lamellar bodies within its cytoplasm. Capillaries containing dark erythrocytes and lined by endothelium are present at the upper left and lower right of the photograph. No lamellar material is present in the alveolar space.

Z15892. Lung. Features are similar to those described for Z15891. A single PN2 is present between two PN1s lining the alveolus. The PN2 contains several lamellar bodies. The cell beneath the PN2 has an irregular nucleus, some prominent RER and is surrounded by some collagen; this appears to be a fibroblast.

Z15893. Lung. Alveolus with several cuboidal pneumocytes. There are not clearly distinctive PN2s in this photograph. The capillary at the bottom contains a lamellar myelin-line structure within its lumen. No lamellar material is present in the alveolar space.

Conclusions: Essentially normal lung. Several type II pneumocytes with lamellar bodies were identified. Fixation was considered acceptable.

001576

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013 EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 19560-2

Tissue: Liver

Treatment Group: 1.6 mg/kg

Block No(s).: 00.39-23

Sex: non applicable

Z15894 -
Photo/Negative No(s).: Z15898

Significant Lesions (check one): X Yes No

Interpreting Pathologist (signature and date): _____

Features: Z15894. Liver. Hepatocyte with surrounding hematopoietic cells. This cell appears partially detached from adjacent hepatocytes, although two hepatocytes are present in the lower right of photograph, all three sharing a common bile canaliculus. There is clumping of mitochondria, RER and some peroxisomes. Some dilatation of cristae is noted. Thirteen (13) peroxisomes counted.

Z15895. Liver. Hepatocyte appears similar to cell described in Z15894 with clumping of organelles. Some granular deposits noted in mitochondria. Fourteen (14) peroxisomes counted.

Z15896. Liver. Hepatocyte has clumping of organelles with intervening cytoplasm filled with fine granular material, probably predominantly glycogen. Mitochondria appear dense and contain some granular deposits and dilated cristae. Ten (10) peroxisomes counted.

Z15897. Liver. Hepatocyte appears similar to those described above. Plasma membranes are indistinct. A hematopoietic cell is at the bottom of the photograph. Fourteen (14) peroxisomes are counted.

Z15898. Liver. Hepatocyte morphology essentially same as above. A bile canaliculus serves four attached hepatocytes in the top center of the photograph. Thirteen (13) peroxisomes counted.

Conclusions: There appear to be increased numbers of peroxisomes per hepatocyte. Average 12.8 per hepatocyte. Suboptimal fixation of tissue.

001577

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013 EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 19560-3

Tissue: Liver

Treatment Group: 1.6 mg/kg

Block No(s).: 99.39-25

Sex: non applicable

Z15904 -
Photo/Negative No(s).: Z15908

Significant Lesions (check one): X Yes No

Interpreting Pathologist (signature and date): _____

Features: Z15904. Liver. Hepatocyte bordered by hepatocytes on three sides and sinusoid with hematopoietic cell on right. Bile canaliculi at top and on left. There is clumping of larger organelles within the cytoplasm. Mitochondria contain some dilated cristae. Ten (10) peroxisomes counted.

Z15905. Liver. Hepatocyte with clumping of organelles. The RER appears dilated. Seven (7) peroxisomes counted.

Z15906. Liver. Hepatocyte with hematopoietic cells along top, left, and bottom. There is clumping of the larger organelles. Mitochondria contain some dense deposits and dilated cristae. Fifteen (15) peroxisomes counted. Some peroxisomes exhibit marked degeneration.

Z15907. Liver. Hepatocyte morphology essentially same as described above. Seven (7) peroxisomes counted.

Z15908. Liver. Hepatocyte adjacent to central vein. Appears similar to previously described cells with clumping of mitochondria and RER. Twelve (12) peroxisomes counted.

Conclusions: Cellular fixation suboptimal to poor. There appears to be slightly increased numbers of peroxisomes compared with control liver. Average 10.2 peroxisomes per hepatocyte.

001579

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013 EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 19560-3

Tissue: _____ Lung

Treatment Group: 1.6 mg/kg

Block No(s).: 99.39-26
Z15910 -

Sex: non applicable

Photo/Negative No(s).: Z15914

Significant Lesions (check one): _____ Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z15910. Lung. Alveolus and lining cells. All pneumocytes are cuboidal in this photograph. The second cell from the right contains several lamellar bodies and is a PN2. The third cell also contains two small lamellar bodies. No other remarkable features noted.

Z15911. Lung. The dark cuboidal lining cell contains multiple cytoplasmic vacuoles and appears to be a PN2 although definitive lamellar structures are not within the vacuoles. The pneumocyte at top right also contains 1-2 vacuoles which probably are/were lamellar bodies.

Z15912. Lung. Alveolus and lining cells. Along the alveolus pneumocytes all appear flattened and to be PN1s. At bottom of photograph in collapsed septal area is a cell containing multiple lamellar structures, cytoplasm of a PN2. No lamellar structures are present in the alveolar space.

Z15913. Lung. At top center a large PN2 contains multiple lamellar bodies. A dark thin PN1 lines the remainder of the airway space. At bottom right a capillary contains a large white blood cell. Dorsal to this is an unidentified cell with dilated endoplasmic reticulum and nuclear envelope.

Z15914. Lung. Alveolar space and lining cells. Within the alveolus are multiple extracellular lamellar bodies. Lining pneumocytes in this region all appear to be PN1s. Several capillaries are present in the photograph.

Conclusions: Essentially normal fetal lung. Some type II pneumocytes and lamellar bodies identified. Some lamellar bodies were occasionally present in alveoli.

001580

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013 EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 19562-2

Tissue: Liver

Treatment Group: 1.6 mg/kg

Block No(s).: 99.39-27
Z15915 -

Sex: non applicable

Photo/Negative No(s).: Z15919

Significant Lesions (check one): X Yes No

Interpreting Pathologist (signature and date): _____

Features: Z15915. Liver. Hepatocyte and surrounding hematopoietic cells. Sinusoidal endothelium and plasma membranes are indistinct, but cytoplasmic organelles are fairly evenly dispersed. Dilated cristae are present in the mitochondria. Nine (9) peroxisomes counted.

Z15916. Liver. Hepatocyte and surrounding hematopoietic cells. Dilated cristae are present in the mitochondria. Sinusoidal endothelium and plasma membranes are indistinct. Dilatation of RER. Eleven (11) peroxisomes counted.

Z15917. Liver. Hepatocyte with clumping of organelles and washed-out appearance of much of the cytoplasm. Plasma membrane is indistinct. Twelve (12) peroxisomes counted.

Z15918. Liver. Hepatocyte morphology similar to those described above. Mitochondria contain some dense deposits and dilated cristae. Eight (8) peroxisomes counted.

Z15919. Liver. Hepatocyte contains multiple clear vacuoles and marked dilation of RER. Mitochondria contain some dense deposits and dilated cristae. Thirteen (13) peroxisomes counted.

Conclusions: Poor cellular fixation of liver tissue. There appears to be a slight increase in the number of peroxisomes per hepatocyte. Average 10.6 peroxisomes per hepatocyte.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013 EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 15962-3

Tissue: Liver

Treatment Group: 1.6 mg/kg

Block No(s).: 99.39-29
Z15925 -

Sex: non applicable

Photo/Negative No(s).: Z15929

Significant Lesions (check one): X Yes No

Interpreting Pathologist (signature and date): _____

Features: Z15925. Liver. Hepatocyte surrounded by other hepatocytes and a hematopoietic cell on the right. There is clumping of the cytoplasmic organelles and large areas of washed out cytoplasm. Mitochondria appear to have some granular densities and some dilated cristae. There is dilatation and blebbing of RER. Twenty-six (26) peroxisomes counted.

Z15926. Liver. Hepatocyte appears similar to hepatocyte in Z15925. There are hematopoietic cells on the left and bottom. Twenty-three (23) peroxisomes counted.

Z15927. Liver. Hepatocyte with indistinct cytoplasmic borders. There is dilatation and blebbing of RER. Mitochondria appear to have some granular densities and some dilated cristae. Twenty-one (21) peroxisomes counted.

Z15928. Liver. Hepatocyte appears essentially similar to above cells. Fixation is suboptimal. Twelve (12) peroxisomes counted.

Z15929. Liver. Hepatocyte with indistinct cytoplasmic borders. There is dilatation of RER. Mitochondria appear to have some granular densities and some dilated cristae. Eighteen (18) peroxisomes counted.

Conclusions: Increased numbers of peroxisomes per hepatocyte. Average 20.0 peroxisomes per hepatocyte. Poor, suboptimal fixation.

001583

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013 EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 19562-3

Tissue: Lung

Treatment Group: 1.6 mg/kg

Block No(s).: 99.39-30

Z15930 -

Sex: non applicable

Photo/Negative No(s).: Z15934

Significant Lesions (check one): _____ Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z15930. Lung. Alveolus and lining cells. Two PN2s with lamellar bodies are present. One PN2 exhibits pseudopodia of cytoplasm extending into the alveolus. The other PN2 contains several large cytoplasmic vacuoles. Some small tubular surfactant bodies are present in the alveolar lumen. Capillaries with endothelial cells and erythrocytes are present beneath the lining cells.

Z15931. Lung. Alveolus and lining cells. Centrally is a dark PN2 which contains one distinct lamellar body dorsal to its nucleus. The cell immediately on top of this dark cell contains an open-faced nucleus but also contains several lamellar bodies, a PN2. The other lining cells appear to be PN1s.

Z15932. Lung. Alveolus and lining cells. Within the alveolus is a free and unattached cell which is not clearly identified as to cell type. All the lining pneumocytes appear to be flattened, but the one on the lower and middle right appears to contain several vacuoles and one distinct lamellar body, i.e., a PN2 which has differentiated into a PN1. No lamellar bodies noted in alveolar lumen.

Z15933. Lung. Alveolus and lining cells. The middle lining cell with only a small portion of nucleus showing contains several vacuoles, which appear to be “empty” lamellar bodies. The angular nucleus above it is a PN1 nucleus. The large dorsal clear pneumocyte has not flattened and may contain a couple empty lamellar vacuoles. The oblong nucleus beneath the PN2 is an endothelial cell nucleus. Some small tubular structures in the lumen of the alveolus may be released lamellar surfactant.

Z15934. Lung. Alveolus, lining cells and collapsed alveolar septa. The lining pneumocytes all appear flattened. No lamellar bodies are present. At the top is a large endothelial cell nucleus and erythrocyte adjacent to it. No unusual findings noted.

Conclusions: Several type II pneumocytes with lamellar bodies seen. Also, some lamellar substance within alveoli noted.

001584

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013; EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 19568-2

Tissue: Liver

Treatment Group: 3.2 mg/kg, high-dose

Block No(s).: 99.39-11
Z15799 - Z15802,

Sex: non applicable

Photo/Negative No(s).: Z15909

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z15799. Liver. Hepatocyte with sinusoid containing hematopoietic cell at top and longitudinal segment of bile canaliculus at bottom right. Hepatocyte contains one distinct multivesicular body. Numerous mitochondria show some slight dilation of cristae, and there is some dilated blebbing of RER. Sinusoidal endothelial cell at top is poorly defined, suggestive of suboptimal fixation. Five (5) peroxisomes counted.

Z15800. Liver. Hepatocyte surrounded by hematopoietic cells. Six (6) peroxisomes counted.

Z15801. Liver. Hepatocyte surrounded by hematopoietic cells. Plasma membrane of the hepatocyte is indistinct and fixation is poor. . Mitochondria appear dense with some granular deposits and dilated cristae. Dilated blebs of RER are noted. Three (3) peroxisomes counted.

Z15802. Liver. Similar overall cellular morphology to Z15801. Plasma membrane is indistinct. . Mitochondria appear dense with some granular deposits and dilated cristae. Seven (7) peroxisomes counted.

Z15909. Liver. Hepatocyte with granular cytoplasm intermixed with mitochondria, RER, and random peroxisomes. . Mitochondria appear dense with some granular deposits and dilated cristae. Nine (9) peroxisomes counted.

Z15798: Liver. No negative; no print developed.

Conclusions: Normal hepatocyte. Suboptimal fixation. Average 6 peroxisomes per hepatocyte.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013; EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 19568-2

Tissue: Lung

Treatment Group: 3.2 mg/kg, high-dose

Block No(s).: 99.39-12
Z15803 -

Sex: non applicable

Photo/Negative No(s).: Z15807

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z15803. Lung. Air space lined by several cuboidal epithelial cells. These cells appear to be pneumocytes which have not flattened out into differentiated PNIs. They have fine granular cytoplasm without many distinct organelles. Dorsally are two blood vessels lined by endothelial cells. No lamellar bodies noted.

Z15804. Lung. Alveolus and lining cells. Two cuboidal pneumocytes are PN2s and contain lamellar bodies. Two adjacent PN1s are associated with these cells. Adjacent tissue is collapsed septa and cell types are less clearly definable.

Z15805. Lung. A cluster of cuboidal pneumocytes similar to those described in Z15803. To the left is a capillary lined by an endothelial cell. Within the capillary are two lamellar-like structures that appear to be extracellular. No PN2s or alveolar lamellar bodies noted.

Z15806. Lung. Alveolus with lining cells. Pneumocytes are low cuboidal and appear to be PN1s, although not completely flattened out. No PN2s noted. The cell to the right with an oblong nucleus appears to be a fibroblast. It contains numerous RER and some collagen fibrils are present extracellularly.

Z15807. Lung. Alveolus with two PN1s, although cells are not flattened. Adjacent tissue is collapsed septa and interstitium. No PN2s or alveolar lamellar bodies noted.

Conclusions: Occasional type II pneumocytes noted. Some intravascular lamellar-like material also present. Many type I pneumocytes have apparently not flattened out, probably normal fetal lung.

001586

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013; EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 19569-1

Tissue: Lung

Treatment Group: 3.2 mg/kg, high-dose

Block No(s).: 99.39-14

Sex: non applicable

Photo/Negative No(s).: Z15852

Significant Lesions (check one): _____ Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z15848. Lung. Alveolus with lining cells. Several cuboidal pneumocytes are present. The central cell has two basilar vacuoles which probably represent lamellar bodies, although the lamellar material is absent in them. A small portion of a septal capillary is present at left.

Z15849. Lung. Section shows several cuboidal to columnar epithelial cells. The central cell appears to contain two small vacuoles, one with some lipid-like material, possibly a lamellar body. To the lower right of this cell is a cell with an irregular nucleus and several lipid or lamellar vacuoles; this cell appears to be a macrophage. A capillary is in the center of the photograph with an endothelial lining cells.

Z15850. Lung. A cluster of cuboidal pneumocytes, with mostly cytoplasm containing a fine granular material. No characteristic lamellar bodies are present in these pneumocytes or in the alveolar space. An interstitial cell contains a single myelin-like or lamellar body, but this cell is not clearly identified as to cell type.

Z15851. Lung. Two PN2s are present and they contain multiple lamellar bodies. A fibroblast appears to contain the elongated nucleus to the right of these PN2s.

Z15852. Lung. A cluster of pneumocytes or epithelial cells as described in above photographs. The top cell contains a couple lamellar bodies and is a PN2. The cells appear to be pneumocytes (PN1s) which haven't flattened out, or PN2s in transition to PN1s.

Conclusions: Essentially normal lung. Some random PN2s were identified. There are numerous cuboidal pneumocytes which appear to not have flattened out.

001588

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013; EM-99.39

Species/Strain: Crl:CD®BR VAF/Plus®
Rat

Animal No.: 19572-1

Tissue: Liver

Treatment Group: 3.2 mg/kg, high-dose

Block No(s).: 99.39-15

Sex: non applicable

Z15853 -
Photo/Negative No(s).: Z15857

Significant Lesions (check one): X Yes No

Interpreting Pathologist (signature and date): _____

Features: Z15853. Liver. Plasma membranes are poorly defined and many cytoplasmic details are apparently lost. Membranes and cristae within mitochondria are indistinct. Nine (9) peroxisomes counted.

Z15854. Liver. Similar to Z15853, plasma and cellular membranes are poorly defined due to poor fixation. RER appears markedly dilated. Thirteen (13) peroxisomes counted.

Z15855. Liver. Hepatocyte with hematopoietic cells to the right margin and another hepatocyte and bile canaliculus at the top. RER profiles are dilated and plasma membranes are indistinct. Twelve (12) peroxisomes counted.

Z15856. Liver. Hepatocellular morphology is similar to cells described above. Fourteen (14) peroxisomes counted.

Z15857. Liver. Hepatocyte and surrounding hematopoietic cells. Plasma and cellular membranes are poorly defined. Eighteen (18) peroxisomes counted.

Conclusions: Hepatocytes poorly fixed. There are higher numbers of peroxisomes within hepatocytes as compared with controls. Average 13.2 peroxisomes per hepatocyte.

001589

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013; EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 19572-1

Tissue: Lung

Treatment Group: 3.2 mg/kg, high-dose

Block No(s).: 99.39-16

Sex: non applicable

Photo/Negative No(s).: Z15863

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z15858. Lung. Alveolus and lining cells. Central lining cell appears to contain one lamellar body to the right of its nucleus, a PN2. To its left is a capillary containing an erythrocyte and lining endothelial cell. Plasma and cellular membranes are poorly defined due to suboptimal fixation. Some cellular debris is present in the alveolar space.

Z15859. Lung. Alveolus and predominantly cuboidal lining cells. Lining cell on right and bottom right both contain some lamellar body vacuoles and appear to be PN2s. The other two pneumocytes appear to be PN1s. No specific abnormalities noted.

Z15860. Lung. Collapsed septum and lining cells. Most appear to be type I pneumocytes, although not flattened out. No lamellar bodies identified. Fixation is poor.

Z15861. Lung. Large top center cell is a pneumocyte. At its dorsal margin is vacuole that may be an "empty" lamellar body. Other lining cells appear to be type I pneumocytes. At bottom right is a small portion of a cell with some lysosomes that appear to contain some crystals or possibly lamellar debris. This cell may be a PN2 or a macrophage. The cell at top right with numerous cytoplasmic inclusions is a granulocyte within a capillary, its endothelial nucleus directly below the granulocyte.

Z15862. Lung. Collapsed septa. Lining this are several flattened pneumocytes, both dark and light. The collapsed area contains several light-staining pneumocytes and endothelial cells. A granulocyte is present in the capillary at the right. The oval nucleus next to this vessel appears to belong to a fibroblast. No abnormalities noted. No lamellar bodies clearly evident in this photograph.

Z15863. Lung. Collapsed septal area. The lamellar myelin-like material in the top center is within a capillary lumen. The top air space is lined by a flattened PN1. Some lamellar material may be present in the alveolus. At the bottom left is a darker cell which contains a lamellar body.

Conclusions: Essentially normal fetal lung. Some lamellar bodies noted in type II pneumocytes. Fixation was suboptimal.

001590

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013; EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 19577-4

Tissue: Liver

Treatment Group: 3.2 mg/kg, high-dose

Block No(s).: 99.39-17

Sex: non applicable

Z15864 - Z15868
Photo/Negative No(s).: Z15940

Significant Lesions (check one): X Yes No

Interpreting Pathologist (signature and date): _____

Features: Z15864. Liver. Hepatocyte with surrounding erythrocytes in sinusoids. Plasma and cellular membranes are indistinct due to poor fixation. Mitochondria show some granular deposits and dilated cristae. Three (3) peroxisomes counted.

Z15865. Liver. Hepatocyte surrounded by adjacent hepatocytes and hematopoietic cells. A bile canaliculus is present at the bottom of the photograph. Eleven (11) peroxisomes counted.

Z15866. No negative.

Z15867. Liver. The plasma membrane is indistinct. Eleven (11) peroxisomes counted.

Z15568. Liver. Hepatocyte and adjacent hematopoietic cells. Dilation and blebbing of RER. Plasma membranes are indistinct. Sixteen (16) peroxisomes counted. Some of the peroxisomes show advanced degeneration.

Z15940. Liver. Hepatocyte with poorly-defined plasma membranes. Mitochondria show dilatation of cristae. Fifteen (15) peroxisomes counted.

Conclusions: Hepatocytes exhibit features of poor fixation. There appears to be a slight increase in the number of peroxisomes per hepatocyte. Average 11.2 peroxisomes per hepatocyte.

001598

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013; EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 19577-4

Tissue: Lung

Treatment Group: 3.2 mg/kg, high-dose

Block No(s).: 99.39-18

Sex: non applicable

Z15869 -
Photo/Negative No(s).: Z15873

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z15869. Lung. Alveolar space and lining cells. Central pneumocyte on right contains several lamellar bodies and is a PN2. A detached cell is just above this one and may be a pneumocyte or possibly a lymphocyte. At the top left is a capillary with an oblong endothelial cell nucleus.

Z15870. Lung. Area of collapsed alveolar septa. Centrally is a dark cell that contains several cytoplasmic vacuoles, two of which contain lamellar body material. Two cell beneath this cell also contain similar cytoplasmic vacuoles and may be PN2s. Other cells with abundant more clear cytoplasm appear to be type I pneumocytes, although they are not flattened.

Z15871. Lung. Ventrally a PN1 lines the airway space. The smaller dark cell adjacent to and covered by it contains multiple small clear vacuoles, but lamellar material is not clearly evident. Dorsally the tissue contains collapsed septa with a mixture of endothelial cells, capillary lumen, pneumocytes, and interstitium. No abnormalities noted.

Z15872. Lung. Area of collapsed alveoli and septa. Centrally are two PN2s containing vacuoles and lamellar bodies. Ventrally is a capillary containing a lamellar-type body within its lumen.

Z15873. Lung. Ventrally are several cuboidal pneumocytes lining an alveolar space. The central cell contains a couple small lamellar-type structures in its cytoplasm. To the right and top right are capillaries containing some lamellar material in their lumina. At the top is a flattened pneumocyte.

Conclusions: Normal lung. Some type II pneumocytes identified. Some lamellar structures present within capillary lumina.

001592

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013 EM-99.39

Species/Strain: CrI:CD®BR VAF/Plus®
Rat

Animal No.: 19579-3

Tissue: Liver

Treatment Group: 3.2 mg/kg, high-dose

Block No(s).: 99.39-19

Sex: non applicable

Photo/Negative No(s).: Z15878

Significant Lesions (check one): X Yes No

Interpreting Pathologist (signature and date): _____

Features: Z15874. Liver. Hepatocyte surrounded by hematopoietic cell at lower left and sinusoid containing erythrocytes at upper right. Endothelial cell is poorly delineated and hepatocellular plasma membranes are indistinct. Seventeen (17) peroxisomes counted.

Z15875. Liver. Hepatocyte mixed with some hematopoietic cells. Hepatocellular boundaries are incomplete on this cell, and peroxisomes are not counted for this cell. Granular deposits are present in some mitochondria. There appears to be abundant rough endoplasmic reticulum.

Z15876. Liver. Hepatocyte with indistinct plasma membranes, but contains abundant mitochondria, endoplasmic reticulum, and peroxisomes. Thirty (30) peroxisomes counted.

Z15877. Liver. Hepatocyte, similar in overall morphology to those described above. Some granular deposits are present within some mitochondria. Endoplasmic reticulum and nuclear envelope appear dilated. Twenty-four (24) peroxisomes counted.

Z15878. Liver. Hepatocyte, similar in overall morphology to those described above. Some granular deposits are present within some mitochondria. Endoplasmic reticulum and nuclear envelope appear dilated. Sixteen (16) peroxisomes counted.

Conclusions: Tissue fixation suboptimal, but better than some previous animals. There appears to be increased numbers of peroxisomes per hepatocyte. Average 21.75 per hepatocyte (four cells counted). No other changes noted compared with control livers.

001593

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 418-013 EM-99.39

Species/Strain: Crl:CD®BR VAF/Plus®
Rat

Animal No.: 19579-3

Tissue: Lung

Treatment Group: 3.2 mg/kg, high-dose

Block No(s).: 99.39-20

Sex: non applicable

Photo/Negative No(s).: Z15883

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z15879. Lung. Alveolus, lining cells and collapsed alveolar septa area. The lining cell contains several lamellar bodies and is a PN2. Some miscellaneous cellular debris appears to be in the alveolar lumen, but no lamellar material. To the right is a cell containing several cytoplasmic vacuoles and an irregular nucleus. This may be a PN2, but also may be a macrophage. Other cells appear to be compressed pneumocytes.

Z15880. Lung. Centrally is a fine granular material containing multiple lamellar bodies. This appears to be extracellular, although it appears mixed with other cellular debris and is probably a ruptured (artificially?) PN2. Other cells lining the airway appear to be PN1s.

Z15881. Lung. Area of collapsed alveoli and septa. Centrally are several cells which contain large vacuoles containing some scant material. These may be PN2s, but also may be macrophages. At the top an elliptical vacuole appears to contain some lamellar material. Three dorsal nuclei have dilated nuclear envelopes, suggesting suboptimal fixation.

Z15882. Lung. Thickened, collapsed septal wall, lined by pneumocytes. Some free lamellar material appears in the alveolar lumen on the left mixed with some cellular debris. The central cell in the "septum" appears to be a PN1. The right margin is lined by a PN1. The dark septal cell at the lower right contains some lamellar bodies and is probably a PN2.

Z15883. Lung. Area of collapsed alveoli and septa. Similar to Z15880, this might represent a ruptured PN2. At top in the lumen is multiple lamellar bodies. The collapsed septa and capillaries appear essentially normal.

Conclusions: Essentially normal lung. Some normal type II pneumocytes identified. Some lamellar material identified within alveoli.

001594

III. Transmission Electron Micrographs

NOTE:

The original glossy figure photographs (Figures 1 - 14) were delivered to Dr. Marvin T. Case with the draft pathology report on March 19, 1999.

Figure Legends

Magnification. Print magnification is indicated on the back of each photograph. All report figures are at a magnification of 11,200X. The measurement bar on each photomicrograph equals one micron (0.001mm).

Figure 1. Liver. Hepatocyte showing normal cytoplasmic organelles. Clumping of mitochondria and rough endoplasmic reticulum reflects suboptimal fixation. Some peroxisomes are identified. Hematopoietic cells are present ventrally. A bile canaliculus is present at the junction of three hepatocytes in the top left. Animal No. 19506-4, 0 mg/kg/day (control), 11,200X magnification.

Figure 2. Liver. Hepatocyte. Dilatation of the rough endoplasmic reticulum, an indistinct plasma membrane, and organelle clumping indicate suboptimal fixation. Mitochondria are dense and show some granular deposits and dilated cristae. Some representative peroxisomes are labeled. An endothelial cell nucleus is present lining the sinusoid in the upper left corner of the photograph. Animal No. 19509-1, 0 mg/kg/day (control), 11,200X magnification.

Figure 3. Liver. Cellular morphology is similar to Figure 2. Some peroxisomes are labeled and appear more numerous than in controls. The rough endoplasmic reticulum and nuclear envelope are dilated. A bile canaliculus is present in upper left of the photograph. Animal No. 19534-1, 0.4 mg/kg/day, 11,200X magnification.

Figure 4. Liver. Mitochondria show dilatation of cristae. Some peroxisomes, increased in number, are labeled. Some hematopoietic cells are present in the adjacent sinusoid. Animal No. 19560-2, 1.6 mg/kg/day, 11,200X magnification.

Figure 5. Liver. Hepatocyte with an indistinct plasma membrane. Cristae are dilated within mitochondria. Several peroxisomes are labeled. Animal No. 19577-4, 3.2 mg/kg/day (high-dose), 11,200X magnification.

Figure 6. Liver. Hepatocyte whose plasma membrane is indistinct. Representative peroxisomes are labeled. Animal No. 19579-3, 3.2 mg/kg/day (high-dose), 11,200X magnification.

Figure 7. Lung. Alveolus and lining cells. Two type II pneumocytes containing lamellar bodies are present. The central dark cell may be a type II pneumocyte which has expressed its lamellar bodies. Type I pneumocytes can be seen lining the alveolus at the top and bottom margin of the air space. Subjacent alveoli are collapsed and the represented cells are pneumocytes and septal cells at different functions of cut. Animal No. 19505-1, 0 mg/kg/day (control), 11,200X magnification.

Figure 8. Lung. Alveoli and septum. A type II pneumocyte is present lining the right alveolus. This cell contains several lamellar bodies containing faint laminated material. The other lining cells are type I pneumocytes. An endothelial cell and capillary are present in the septum at the top of the photograph. Animal No. 18915-1, 0 mg/kg/day (control), 11,200X magnification.

Figure 9. Lung. Alveolus and septa. The central narrow alveolus is bounded by two septa containing capillaries with luminal erythrocytes. A lamellar myelin-like structure is present in the lumen of the upper capillary. Flattened type I pneumocytes line the alveolus. No type II pneumocytes or lamellar bodies are evident in this photograph. Animal No. 19504-3, 0 mg/kg/day (control), 11,200X magnification.

Figure 10. Lung. Alveolus and lining cells. A type II pneumocyte containing multiple lamellar bodies is present centrally. Much of the lamellar material has washed-out of the lamellar bodies. A second type II pneumocyte is present near the bottom, and contains a single lamellar body. The remaining cells appear to be type I pneumocytes. Animal No. 19546-1, 0.4 mg/kg/day, 11,200X magnification.

Figure 11. Lung. Alveolus and lining cells. At the top is a cuboidal type II pneumocyte containing several lamellar bodies. The adjacent lining cells appear to be pneumocytes which have not flattened out. The lamellar myelin-like structures are extracellular within capillary spaces. Animal No. 19560-2, 1.6 mg/kg/day, 11,200X magnification.

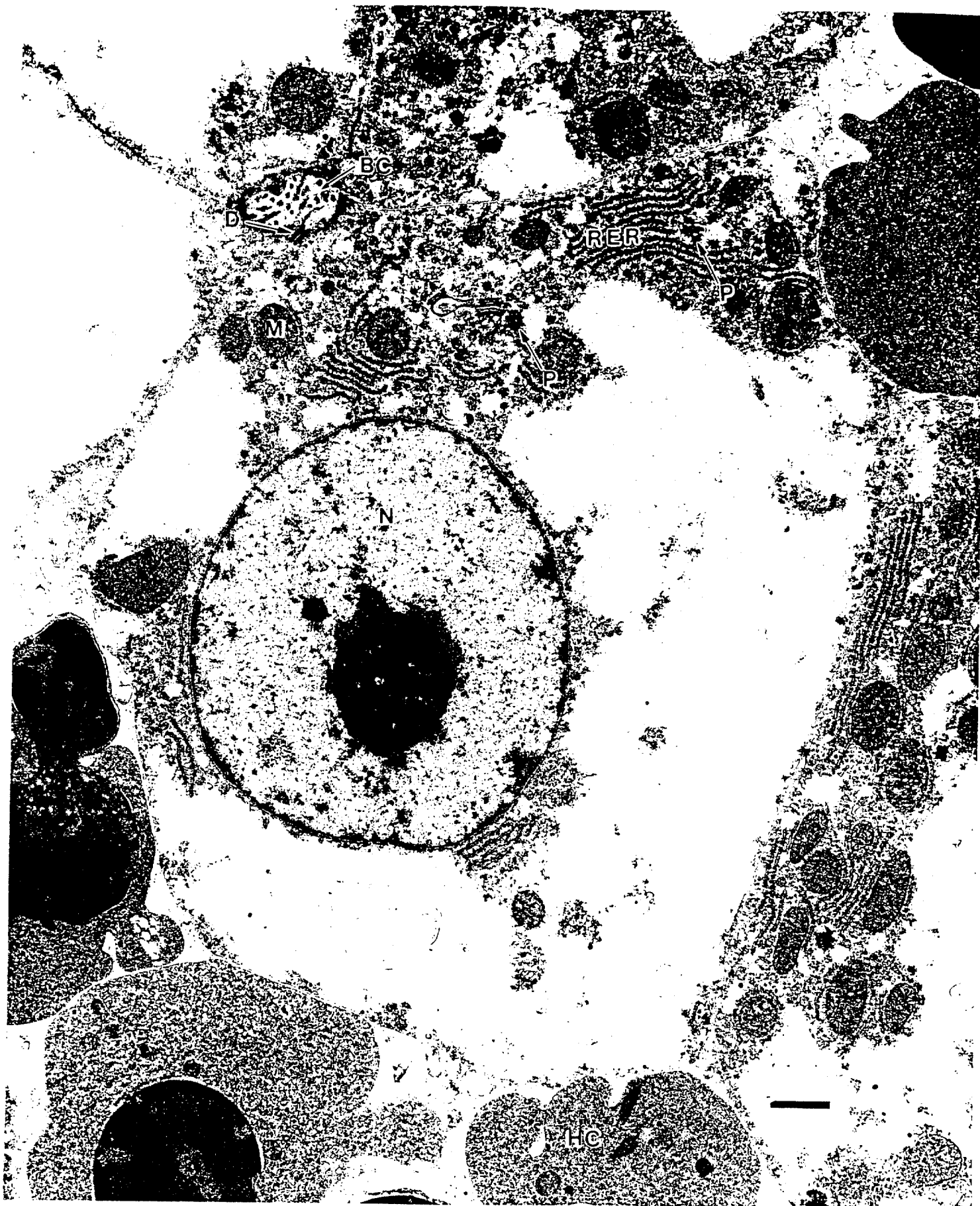
Figure 12. Lung. Alveolus and lining cells. Alveolar space contains abundant lamellar-like material, which is consistent with surfactant secreted from type II pneumocytes. The dark cellular segment near the bottom may be an extension of a type II pneumocyte. The remaining lining cells appear to be type I pneumocytes or cuboidal pneumocytes. Capillaries with lining endothelial cells are present dorsally and at the lower right. Animal No. 19560-3, 1.6 mg/kg/day (high-dose), 11,200X magnification.

Figure 13. Lung. Alveolus and lining cells. Two type II pneumocytes containing lamellar bodies are present. A type I pneumocyte is present lining the alveolus ventral to the type II pneumocytes. Within the septal wall collagen fibers and a fibroblast are evident. The material in the alveolus is nondescript cellular debris. Animal No. 19569-4, 3.2 mg/kg/day (high-dose), 11,200X magnification.

Figure 14. Lung. Alveoli and septum. Both sides of the septum are lined by flattened pneumocytes. The large central cell in the septum appears to be a type I pneumocyte. Two cells ventrally contain lamellar bodies and are type II pneumocytes. Animal No. 19579-3, 3.2 mg/kg/day (high-dose), 11,200X magnification.

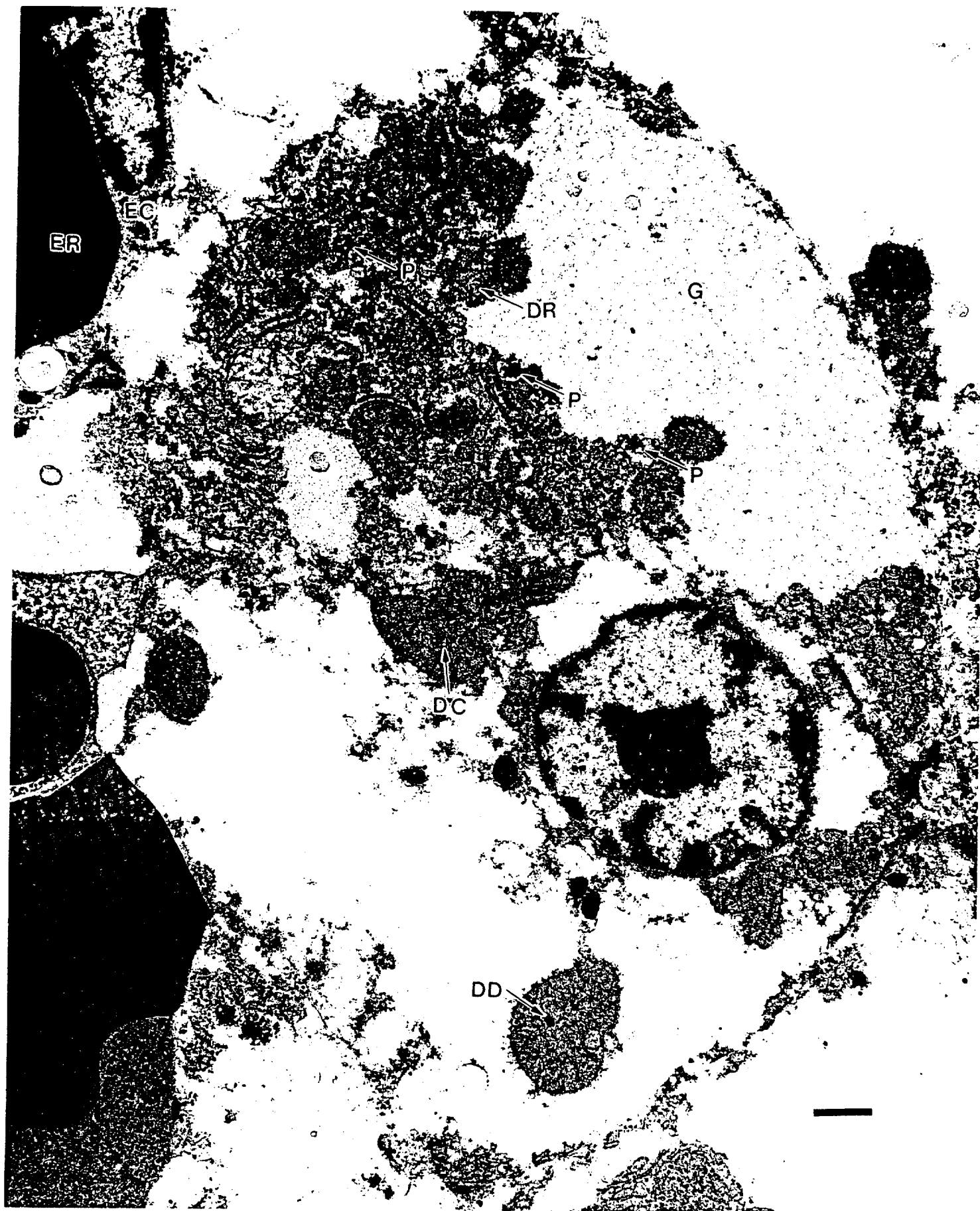
Index of Labels for Ultrastructural Structures

<u>Abbreviation</u>	<u>Structure</u>
A	Alveolar space
BC	Bile canaliculus
BE	Bronchiolar epithelium
CL	Capillary lumen
Cap	Capillary
CN	Centrioles
CO	Collagen
CP	Cuboidal pneumocyte
D	Desmosome
DD	Dense deposits
DC	Dilated cristae
DN	Dilated nuclear envelope
DR	Dilated RER
EC	Endothelial cell
ER	Erythrocyte
FB	Fibroblast
G	Glycogen
HC	Hematopoietic cells
L	Lipid droplet
LB	Lamellar body
M	Mitochondria
N	Nucleus
P	Peroxisome
PN1	Type I pneumocyte
PN2	Type II pneumocyte
PM	Plasma membrane
RER	Rough endoplasmic reticulum



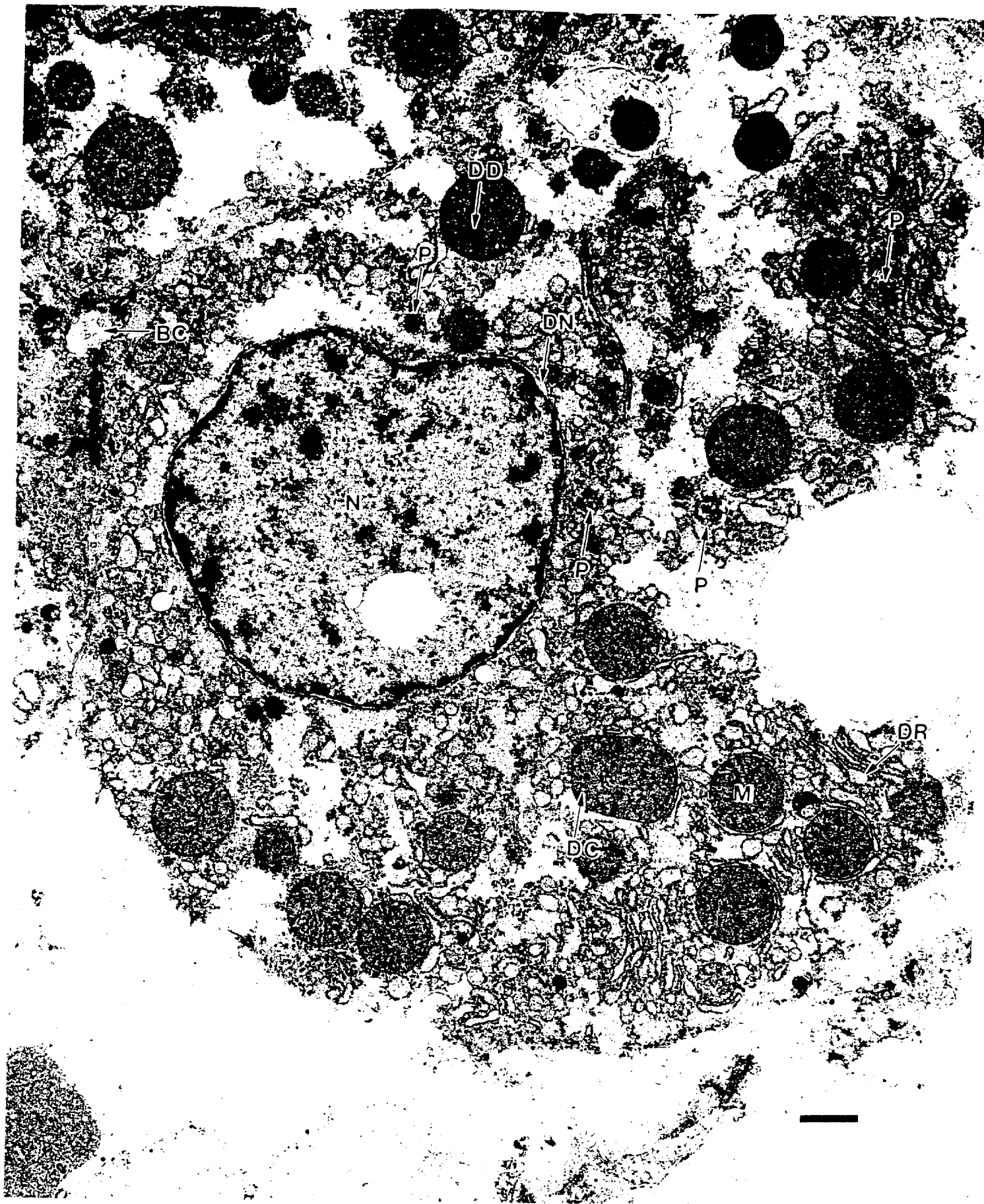
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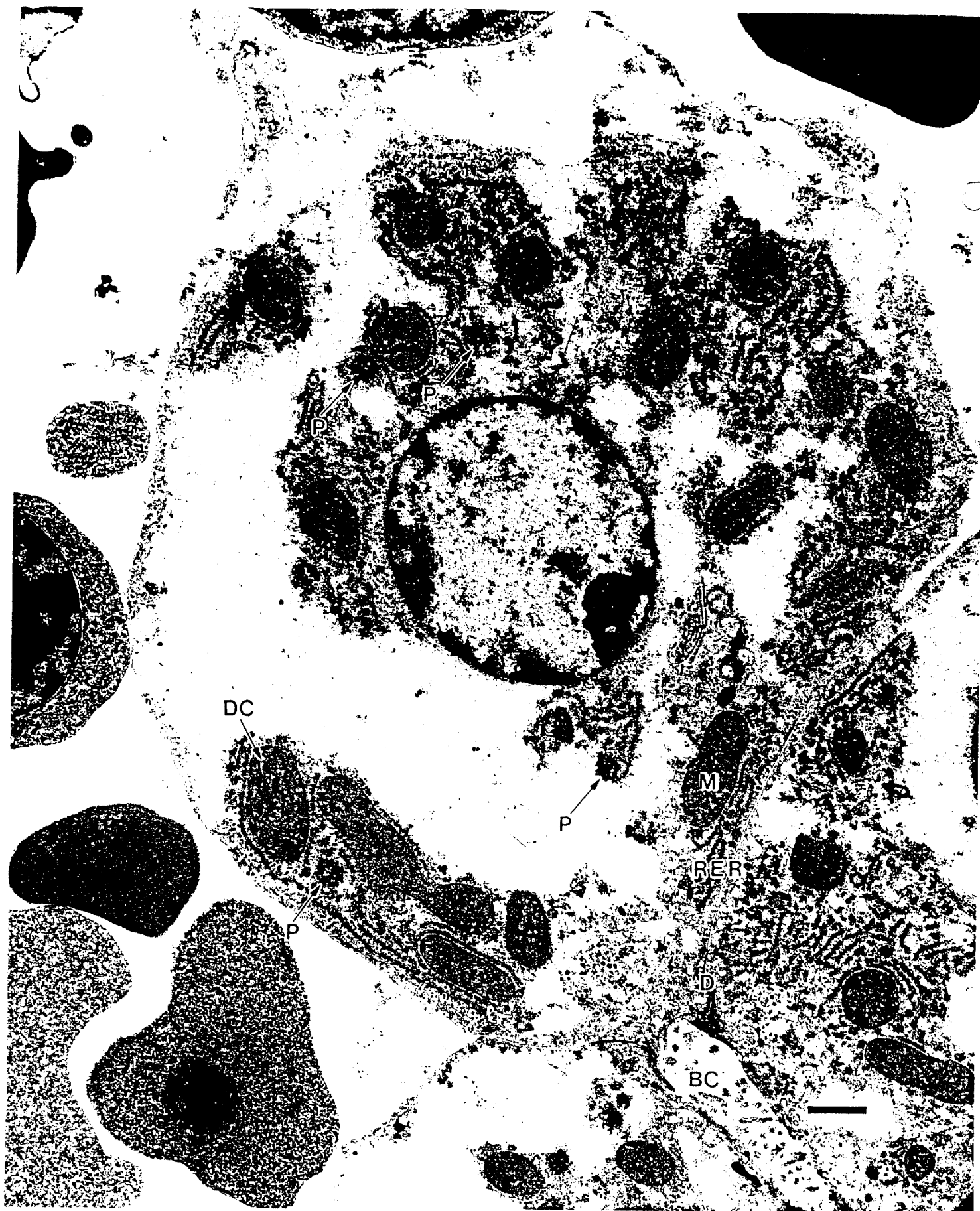
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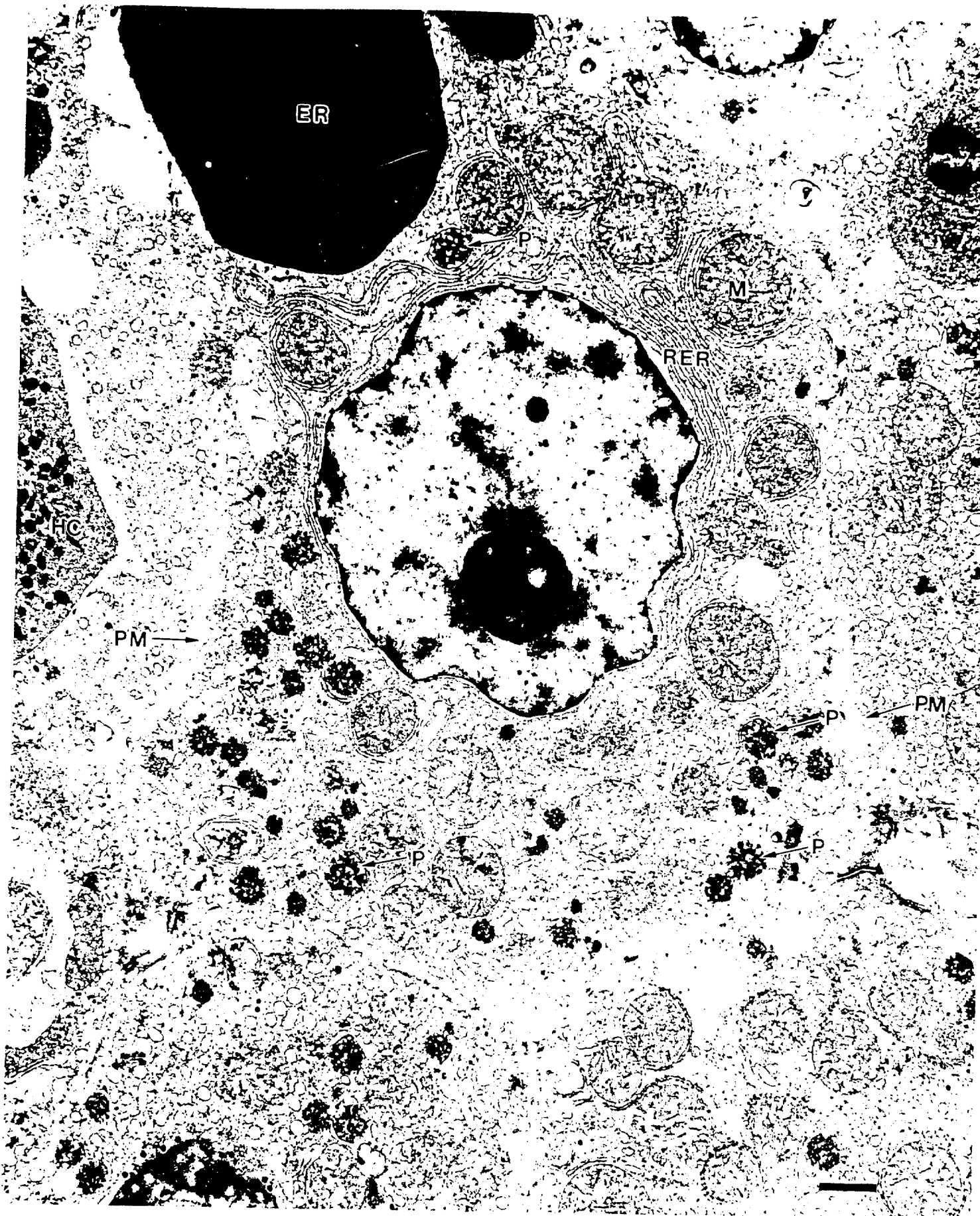
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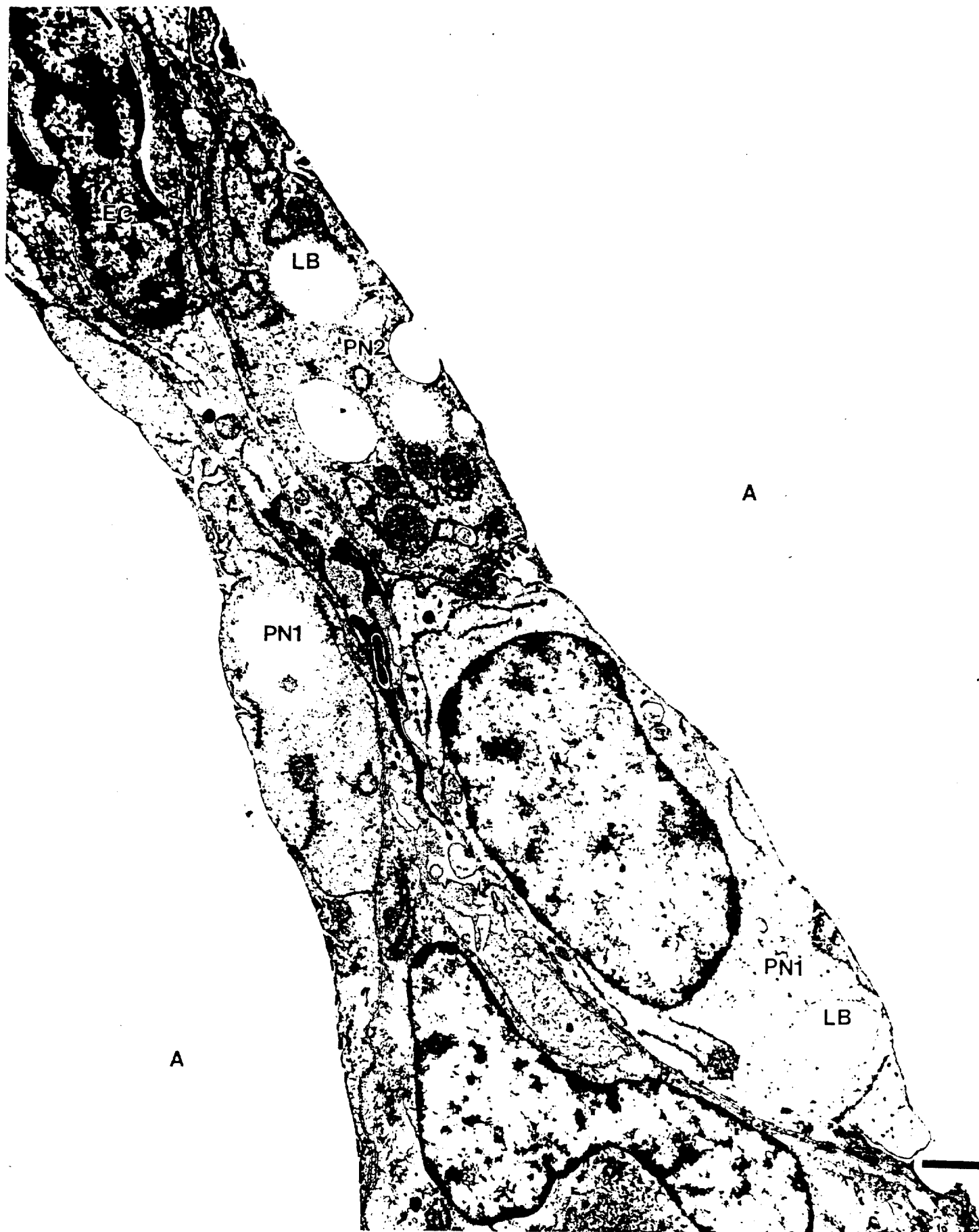
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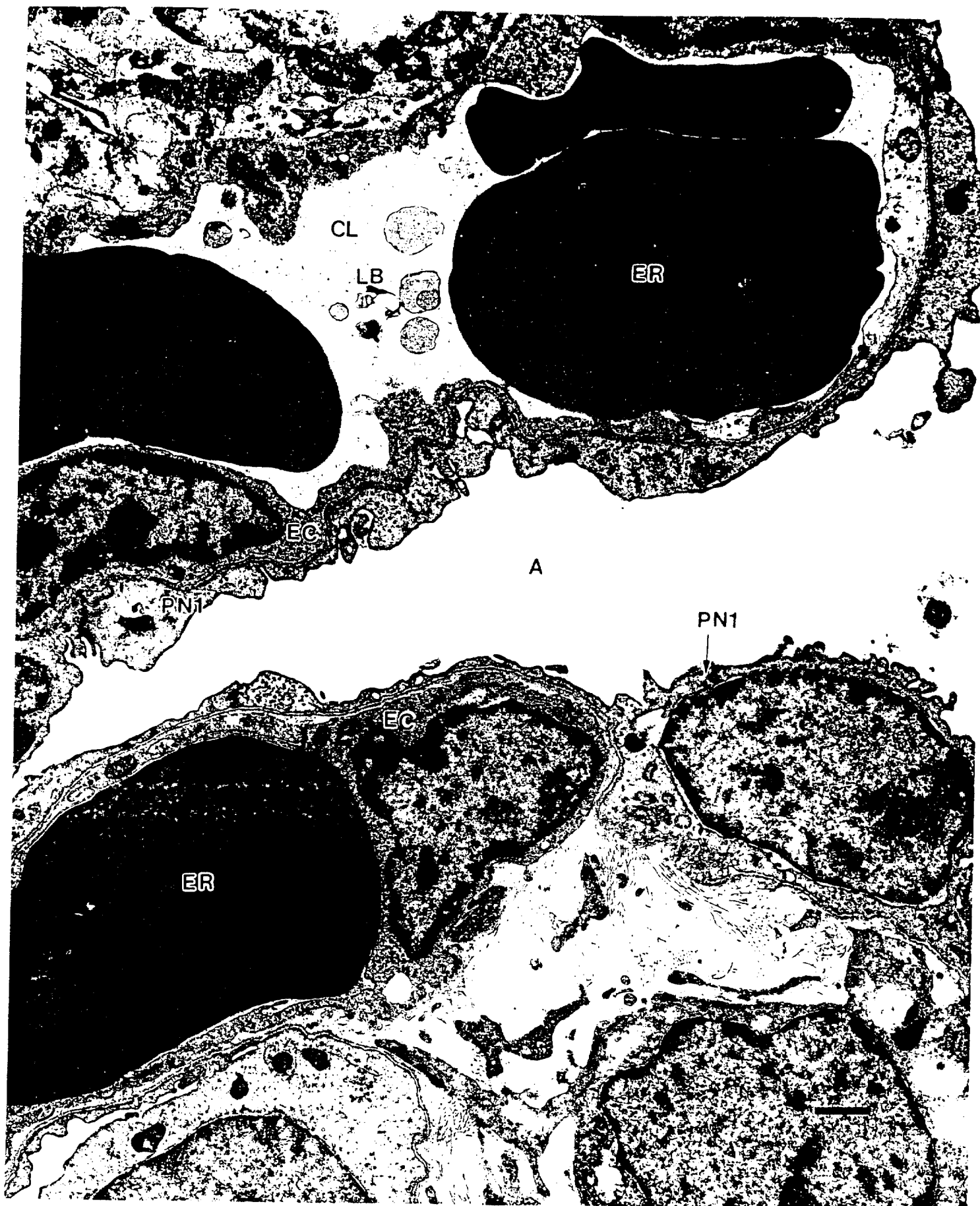
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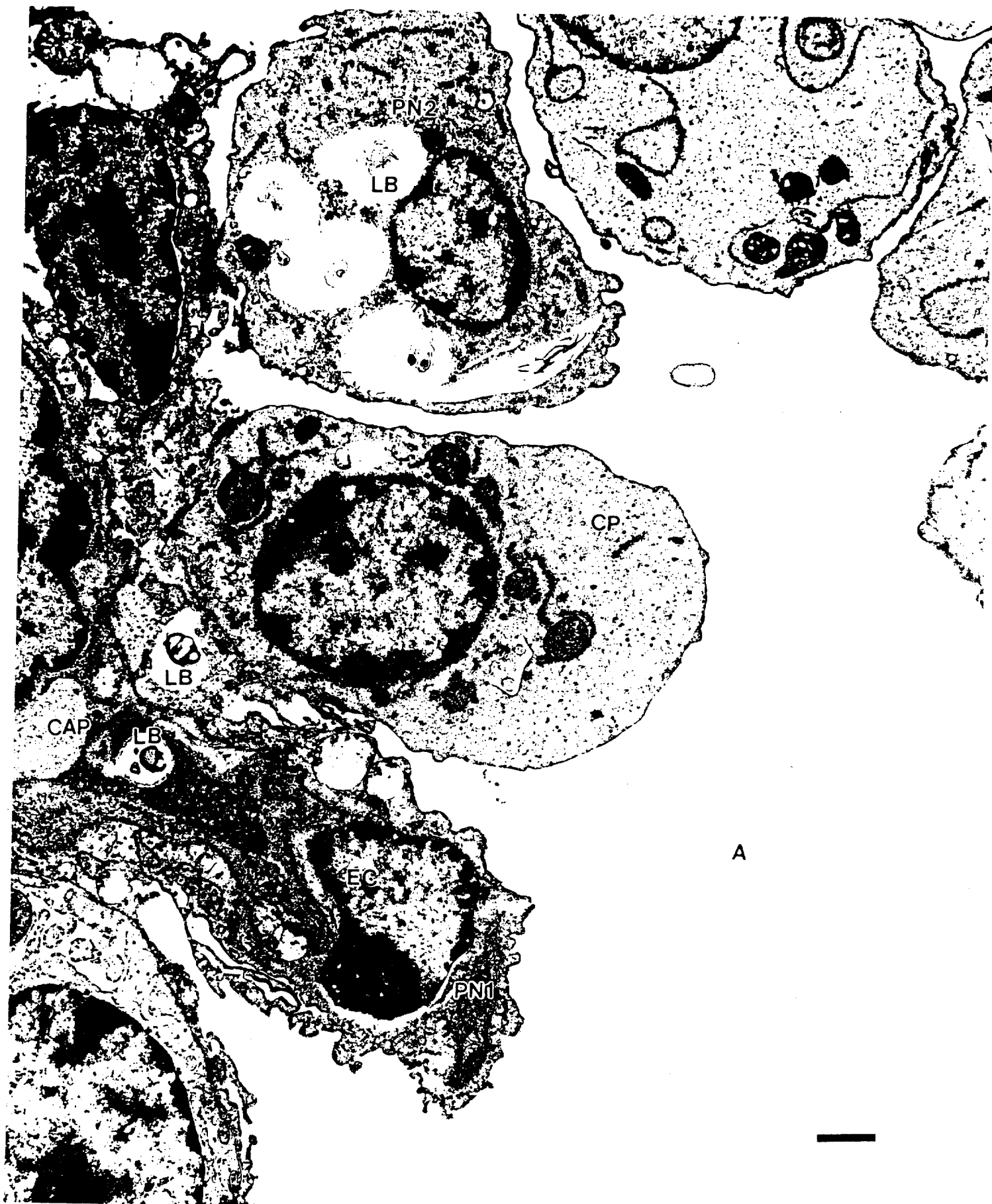
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001608



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001609



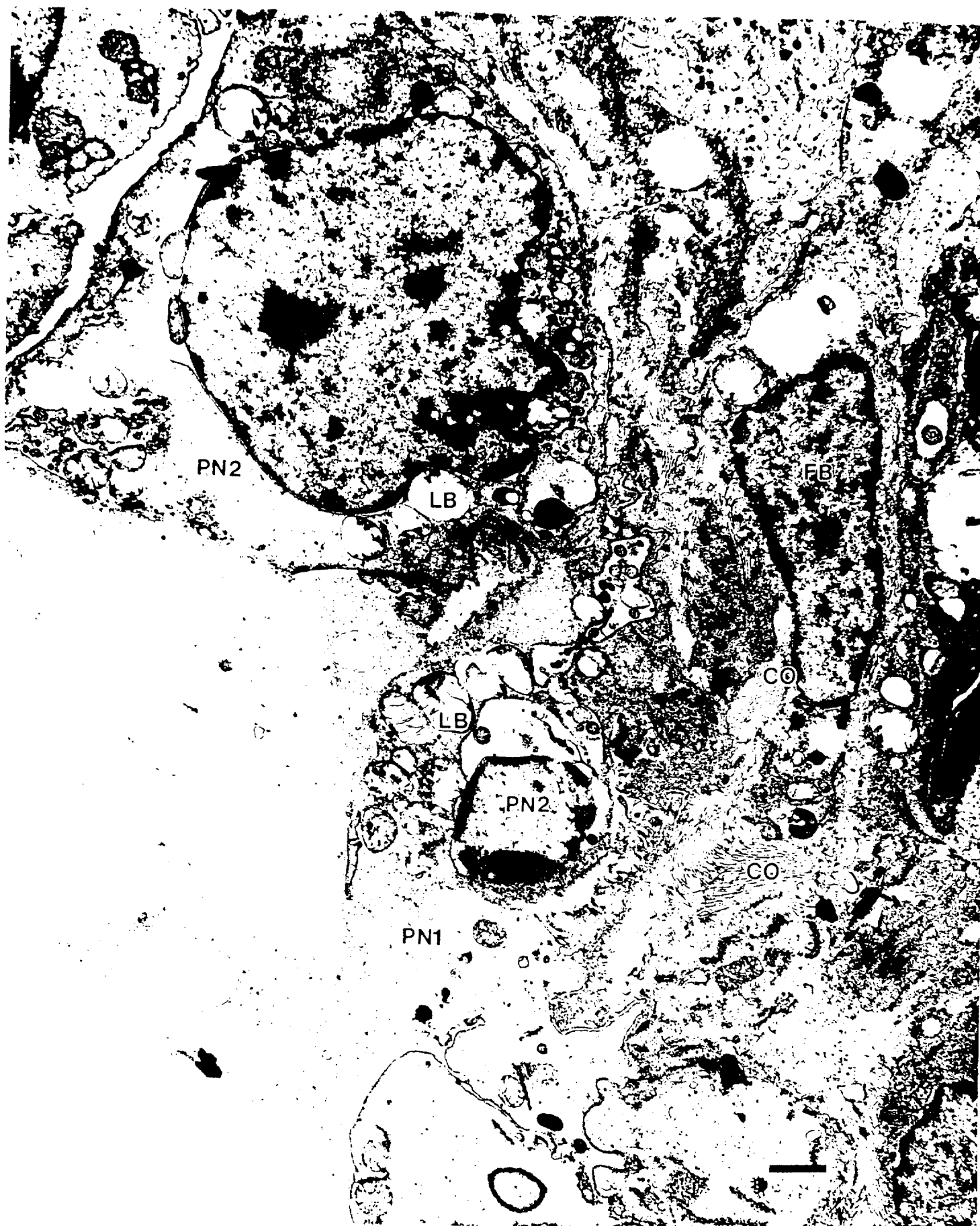
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001600



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001614



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001612

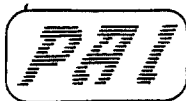


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001613

IV. Quality Assurance Statement

001614



Pathology Associates International

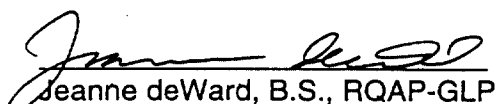
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QUALITY ASSURANCE STATEMENT

This electron microscopy study has been inspected and audited by the Quality Assurance Unit as required by the Good Laboratory Practices Regulations promulgated by the U.S. Food and Drug Administration. PAI has a functioning and responsive Quality Assurance Unit which reports directly to management. The following is a record of inspections/audits and their resulting reports:

<u>Date of Inspection</u>	<u>Phase Inspected</u>	<u>Date Findings Reported to Management and Study Director</u>
2/22/99	Processing and Embedding	2/22/99
3/22, 23, 24, 25/99	Data Audit	3/25/99
3/25/99	Draft Report Audit	3/25/99
4/5/99	Final Report Audit	4/5/99


Jeanne deWard, B.S., RQAP-GLP
Quality Assurance Specialist

4/5/99
Date

Sponsor: 3M Corporate Toxicology

Test Article: PFOS

Study Number: 418-013, PAI Study EM-99.39

001615