



Pathology Associates International

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**PATHOLOGY REPORT
(ANCILLARY STUDY)**

**ELECTRON MICROSCOPIC EVALUATION
OF LIVER IN CYNOMOLGUS MONKEYS**

**26-WEEK CAPSULE TOXICITY STUDY WITH PERFLUOROOCTANE
SULFONIC ACID POTASSIUM SALT (PFOS;T-6295) IN CYNOMOLGUS
MONKEYS**

**CLIENT STUDY NUMBER 6329-223
PAI STUDY NUMBER EM 99.76**

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I. Pathology Narrative

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SUMMARY

Treatment of cynomolgus monkeys with 0.75 mg PFOS/kg/day for 26 weeks resulted in hepatocellular vacuolation and hypertrophy by light microscopy. Hepatocellular vacuolation and hypertrophy were primarily centrilobular in distribution and did vary in incidence and severity between individual animals. Ultrastructurally, there were increased lipid droplets within hepatocytes. Increased lipid droplets were present in both the high-dose males examined and in two of four high-dose females examined. Increased hepatocellular glycogen was present in the remaining two high-dose females. These ultrastructural changes correlated with hepatocellular hypertrophy and vacuolation noted by light microscopy and with increased liver weight parameters in the high-dose animals. The no-effect level for hepatocellular vacuolation (lipid droplet accumulation) was 0.15 mg/kg/day of PFOS (mid-dose group).

Quantitation of peroxisomes within hepatocytes by electron microscopy indicated no remarkable differences between control livers and livers treated with 0.03, 0.15, or 0.75 mg PFOS/kg/day. Ultrastructural differences in cellular membranes or mitochondria were not discernible between control and treated animals in the samples examined.

PROCEDURES

The objective of this study was to examine by electron microscopy the livers from selected animals in a 26-week capsule toxicity study with PFOS in cynomolgus monkeys. In addition to a general qualitative evaluation for differences between control and treated animals, the ultrastructural endpoints included quantitation of peroxisomes within hepatocytes and membrane and mitochondrial changes in hepatocytes. Light microscopy of paraffin-embedded liver sections was also performed.

For the toxicity study, 22 male and 22 female cynomolgus monkeys were given the test article or the vehicle once daily for at least 26 weeks according to Text Table 1.

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Text Table 1. Group Designations and Dosage Levels

Dosage Group	Dose Level (mg/kg/day) ^a	Total Material Dose Level (mg/kg/day) ^b	Number of Males	Number of Females
I	0 ^a	30 ^a	6 ^d	6 ^d
II	0.03	15 ^b	4	4
III	0.15	6 ^c	6 ^d	6 ^d
IV	0.75	30 ^c	6 ^d	6 ^d

^a Dose levels were provided. The control group (Group 1) received an equivalent amount of lactose in gelatin capsules as the total material administered to Group 4.

^b The low-dose group (Group 2) received the test material diluted with lactose (1:499, w:w).

^c The mid-dose (Group 3) and high-dose (Group 4) groups received the test material diluted with lactose (1:39, w:w).

^d Two animals in Groups 1, 3, and 4, designated as recovery animals, were treated for at least 26 weeks, then treatment was discontinued and the animals were observed for reversibility, persistence, or delayed occurrence of toxic effects for at least 13 weeks posttreatment.

Animals to be sacrificed at scheduled terminal and recovery sacrifice times were anesthetized with ketamine and xylazine, weighed, exsanguinated, and necropsied. Multiple tissues were collected and preserved in 10% neutral-buffered formalin according to the protocol. Additionally, pieces of liver from each animal were thin-sliced and placed in ½ strength Karnovsky's fixative (2.0% paraformaldehyde/2.5% glutaraldehyde in 0.1M phosphate buffer) for subsequent ultrastructural examination. These livers were processed into Epon blocks and submitted to this laboratory for subsequent electron microscopy processing and evaluation. Hematoxylin and eosin (H&E)-stained slide sections of formalin-fixed, paraffin-embedded livers were submitted for light microscopic examination.

Per sponsor request, tissue samples from terminal sacrifice control and high-dose males and females were initially processed and evaluated ultrastructurally (Text Table 2). Two high-dose males, Animals Nos. I05506 and I05509, died or were sacrificed in a moribund condition prior to scheduled terminal sacrifice. Ultrastructural examination of livers from these two unscheduled death animals was not performed. Following evaluation of the electron micrographs from the control and high-dose livers, the sponsor requested that electron microscopy be performed on the low- and mid-dose livers to evaluate whether increased lipid droplet accumulation was evident in these dose groups.

Text Table 2. Animals Selected for Electron Microscopy

0 mg/kg/day PFOS (Group 1 Control)		0.03 mg/kg/day PFOS (Group 2 low-dose)		0.15 mg/kg/day PFOS (Group 3 mid-dose)		0.75 mg/kg/day PFOS (Group 4 High-Dose)	
Males	Females	Males	Females	Males	Females	Males	Females
I05508	I05530	I05514	I05537	I05510	I05532	I05507	I05534
I05517	I05531	I05515	I05541	I05518	I05538	I05512	I05536
I05519	I05535	I05516	I05547	I05524	I05545		I05540
I05527	I05544	I05521	I05550	I05528	I05548		I05551

Thin sections (approximately 90 nm) from the Epon-embedded livers were cut, mounted on 200-mesh copper grids, stained with 5% methanolic uranyl acetate and Reynold's lead

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citrate, and examined on a Zeiss EM10C/CR, a Zeiss EM10, or a JEOL 100S transmission electron microscope. Centrilobular hepatocytes, where clearly identifiable in liver sections, were preferentially examined. Representative electron photomicrographs of liver 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, the H&E slides from the same animals selected for electron microscopy were evaluated and significant features were summarized on a light microscopic evaluation form.

RESULTS AND DISCUSSION

Individual interpretations of light microscopy slides and electron micrographs for each animal are shown in Section II. Representative electron photomicrographs were selected as report figures, labeled, and are shown in Section III. All remaining electron micrographs are maintained in the archived study file.

Light Microscopy

A summary of the incidence and severity of light microscopic findings in the control and high-dose livers is shown in Text Table 3. There was an increased incidence of vacuolation of the cytoplasm of hepatocytes in high-dose treated animals compared with control animals. The distribution of this vacuolation was predominantly centrilobular, but for one high-dose female, Animal No. I05534, there was diffuse slight hepatocellular vacuolation. Affected animals generally also had enlargement (hypertrophy) of centrilobular hepatocytes. This enlargement appeared to be related to the presence of the small to large vacuoles with the cytoplasm. Some sporadic vacuolation noted in low- and mid-dose animals did not appear to be treatment-related and was not clearly associated with hepatocellular hypertrophy.

Text Table 3. Incidence and Severity of Light Microscopic Liver Findings

Microscopic Finding	0 mg/kg/day (Control)		0.03 mg/kg (Low-Dose)		0.15 mg/kg (Mid-Dose)		0.75 mg/kg/day (High-Dose)	
	Males	Females	Males	Females	Males	Females	Males	Females
(number examined)	(4)	(4)	(4)	(4)	(4)	(4)	(2)	(4)
Vacuolation, hepatocellular, centrilobular								
minimal	-	-	1	-	-	-	-	2
slight	-	-	-	-	-	-	-	-
moderately-severe	-	-	-	-	-	-	1	1
Vacuolation, hepatocellular, diffuse								
slight	-	-	-	-	-	-	-	1
Vacuolation, hepatocellular, multifocal								
minimal	-	-	1	1	-	-	-	-

Text Table 3 Continued. Incidence and Severity of Light Microscopic Liver Findings

Microscopic Finding	0 mg/kg/day (Control)		0.03 mg/kg (Low-Dose)		0.15 mg/kg (Mid-Dose)		0.75 mg/kg/day (High-Dose)	
	Males	Females	Males	Females	Males	Females	Males	Females
(number examined)	(4)	(4)	(4)	(4)	(4)	(4)	(2)	(4)
Hypertrophy, hepatocellular, centrilobular								
minimal	-	-	-	-	-	-	1	2
slight	-	-	-	-	-	-	-	-
moderate	-	-	-	-	-	-	1	1
Clearing, cytoplasmic, centrilobular								
minimal	1	2	-	2	1	-	-	-
slight	-	-	3	1	1	2	1	2
Clearing, cytoplasmic, diffuse								
minimal	-	-	-	-	1	-	-	-
slight	-	-	-	-	1	-	-	-
Infiltrate, lymphohistiocytic								
minimal	4	4	4	4	3	3	1	3
slight	-	-	-	-	-	1	-	-
Inflammation, eosinophilic- granulomatous								
minimal	-	-	1	-	-	-	-	-
slight	-	1	-	-	-	-	-	-
Pigment, hepatocellular, centrilobular								
slight	-	-	1	-	-	-	-	-
Ectasia, biliary								
minimal	-	-	-	1	-	-	-	-
Angiectasis								
minimal	1	-	-	-	-	-	-	-

Electron Microscopy

Control (0 mg/kg/day). Ultrastructural features of control hepatocytes are demonstrated in Figures 1 - 3. Hepatocytes characteristically contain numerous mitochondria and abundant endoplasmic reticulum. Peroxisomes are common organelles composed of a fine granular matrix surrounded by a membrane and normally vary in size from 0.1 - 1.5 microns in diameter. Hepatocytes exhibit a microvillous border which protrudes into the perisinusoidal space. Tight junctions border bile canaliculi between hepatocytes. Glycogen is common in the cytoplasm and may be in the form of small granules or slightly larger rosettes. Residual bodies are secondary lysosomes (phagolysosomes) containing electron dense degenerative cytoplasmic material. Lipid metabolism is a normal liver function, and it's normal to see random lipid droplets within hepatocytes.

There were no discernible differences between control males and females in this study, and all were considered normal. A summary of ultrastructural findings is shown in Text Table 4. One male, Animal No. I05527, appeared to have increased numbers of peroxisomes compared with the other control animals. However, because so few animals and individual hepatocytes were examined, this value may not be significantly increased.

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Text Table 4. Summary of Ultrastructural Findings

Treatment Group (mg/kg/day)/Sex	Animal No.	Comments
(0) Male	I05508	Normal
(0) Male	I05517	Normal
(0) Male	I05519	Normal
(0) Male	I05527	Normal. Increased peroxisomes
(0.03) Male	I05514	Mild increase in lipid droplets
(0.03) Male	I05515	Slight increase in lipid droplets
(0.03) Male	I05516	Normal
(0.03) Male	I05521	Normal
(0.15) Male	I05510	Normal
(0.15) Male	I05518	Normal
(0.15) Male	I05524	Normal, slight increase in peroxisomes
(0.15) Male	I05528	Normal
(0.75) Male	I05507	Lipid droplet accumulation, hepatocellular, moderately severe. Increased glycogen
(0.75) Male	I05512	Lipid droplet accumulation, moderate, hepatocellular. Increased peroxisomes
(0) Female	I05530	Normal
(0) Female	I05531	Normal
(0) Female	I05535	Normal
(0) Female	I05544	Normal
(0.03) Female	I05537	Normal
(0.03) Female	I05541	Normal
(0.03) Female	I05547	Normal
(0.03) Female	I05550	Normal
(0.15) Female	I05532	Normal
(0.15) Female	I05538	Increased glycogen
(0.15) Female	I05545	Normal
(0.15) Female	I05548	Normal
(0.75) Female	I05534	Increased glycogen
(0.75) Female	I05536	Lipid droplet accumulation, moderately severe
(0.75) Female	I05540	Lipid droplet accumulation, mild to moderate
(0.75) Female	I05551	Increased glycogen

Peroxisomes were identified and quantitated in representative hepatocytes. For the control livers, the average number of peroxisomes counted per hepatocyte photographed was 11.50 for control males and 10.94 peroxisomes per cell for control females. (Text Table 5). Depending upon fixation and staining, peroxisomes may be difficult to differentiate in routine electron photomicrographs from primary lysosomes or marginal cuts through the ends of mitochondria. Cytochemical and immunocytochemical methods are available for specifically identifying peroxisomes by light and electron microscopy, and utilization of these methods would provide a more specific and accurate method of peroxisome quantitation (Beier K, 1992; Reddy UN, 1988).

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Text Table 5. Quantitation of Hepatocellular Peroxisomes

0 mg PFOS/kg/day (Control)				0.03 mg PFOS/kg/day (Low-Dose)			
Male		Female		Male		Female	
Animal Number	Number ¹ of Peroxisomes	Animal Number	Number of Peroxisomes	Animal Number	Number of Peroxisomes	Animal Number	Number of Peroxisomes
I05508	7.50	I05530	12.75	I05514	6.00	I05537	8.80
I05517	12.25	I05531	13.75	I05515	15.00	I05541	12.00
I05519	3.50	I05535	7.00	I05516	7.20	I05547	10.00
I05527	22.75	I05544	10.25	I05521	10.80	I05550	17.60
Mean =	11.50	Mean =	10.94	Mean =	9.75	Mean =	12.10

0.15 mg PFOS/kg/day (Mid-Dose)				0.75 mg PFOS/kg/day (High-Dose)			
Male		Female		Male		Female	
Animal Number	Number ¹ of Peroxisomes	Animal Number	Number of Peroxisomes	Animal Number	Number of Peroxisomes	Animal Number	Number of Peroxisomes
I05510	10.60	I05532	4.20	I05507	11.0	I05534	5.50
I05518	18.40	I05538	7.20	I05512	23.25	I05536	3.75
I05524	23.20	I05545	9.00			I05540	12.50
I05528	14.60	I05548	17.00			I05551	9.50
Mean =	16.70	Mean =	9.35	Mean =	17.12	Mean =	7.81

¹ Average number of peroxisomes per hepatocyte for four hepatocytes counted per animal for control and high-dose groups and five hepatocytes counted per animal for low- and mid-dose groups.

High-Dose (0.75 mg/kg/day). Lipid droplets were increased in animals given 0.75 mg PFOS/kg/day (Text Table 4). This increase was present in both high-dose males examined (Animal Nos. I05507 and I05512) and in two high-dose females (Animal Nos. I05536 and I05540). These are illustrated in Figures 4 - 7. Lipid droplets were randomly distributed in the cytosol and varied in size from less than .1 micron to greater than several microns in diameter. They generally were clear, but occasionally contained wispy lamellar material or faint smooth electron density. They were not membrane-bound, but appeared to form a defined margin with surrounding cytoplasm. These lipid droplets correlate with hepatocellular vacuolation described in these animals by light microscopy.

Increased cytoplasmic glycogen was evident in two high-dose females (Animal Nos. I05534 and I05551), and often formed distinct "pockets" of glycogen, resembling vacuoles or clear droplets, but containing glycogen particles (Figure 8). The accumulation of glycogen apparently correlates with the hepatocellular vacuolation described by light microscopy in these two animals. Lipid droplets did not appear increased in these two animals.

For high-dose animals, the average number of peroxisomes per hepatocyte was 17.12 for males and 7.81 for females (Text Table 5). One male (Animal No. I05512) averaged 23.25 peroxisomes per hepatocyte, however, this is not remarkably higher than that found in the control male Animal No. I05527. Animal No. I05507 averaged 11.0 peroxisomes per hepatocyte, similar to the average control value for males. Thus, administration of 0.75 mg/kg/day of PFOS to monkeys for 26 weeks did not appear to induce peroxisome proliferation in the liver.

Differences in mitochondria and cellular membranes were not discernible between high-dose and control livers. Mitochondria from control and high-dose livers did not exhibit remarkable differences ultrastructurally (Figures 3, 9, 10).

Low-Dose (0.03 mg/kg/day). Electron microscopic examination of the livers from monkeys given 0.03 mg/kg/day of PFOS did not identify any atypical ultrastructural features compared with control sample. Lipid droplets were identified in some hepatocytes, and two low-dose males appeared to have slightly increased lipid droplet accumulation (Animal Nos. I05514 and I05515). However, increased lipid droplet accumulation was not seen in the low-dose females or in the mid-dose males and females, and this observation in the two low-dose males was believed to represent normal biological variance and sampling. As noted earlier, lipid metabolism is a normal liver function, and it's normal to see random lipid droplets within hepatocytes. Figure 11 illustrates a hepatocyte from the low-dose group.

For low-dose animals, the average number of peroxisomes per hepatocyte was 9.75 for males and 12.10 for females (Text Table 5).

Mid-Dose (0.15 mg/kg/day). There was no consistent ultrastructural evidence of increased lipid droplet accumulation in the livers of the mid-dose monkeys given 0.15 mg/kg/day of PFOS. This mid-dose level, 0.15 mg/kg/day, is the no-effect level for the PFOS-related effect of increased hepatocellular lipid accumulation (vacuolation) in monkeys given PFOS. One female, Animal No. I05538, had increased glycogen, but a marked increase in glycogen was not seen in any other mid-dose livers examined.

For mid-dose animals, the average number of peroxisomes per hepatocyte was 16.70 for males and 9.35 for females (Text Table 5). Figure 12 illustrates a hepatocyte from the mid-dose group.

CONCLUSION

Ultrastructural evaluation of livers from cynomolgus monkeys treated with 0.75 mg PFOS /kg/day for 26 weeks identified increased lipid droplets within hepatocytes. Increased lipid droplets were present in both treated males examined and in two of four treated females examined. Increased hepatocellular glycogen was present in the remaining two high-dose females. These ultrastructural changes correlated with hepatocellular hypertrophy and vacuolation noted by light microscopy and with increased liver weight parameters in the high-dose animals. The no-effect level for hepatocellular vacuolation (lipid droplet accumulation) was 0.15 mg/kg/day of PFOS (mid-dose group). Quantitation of peroxisomes within hepatocytes by electron microscopy indicated no remarkable differences between control livers and livers treated with 0.03, 0.15 or 0.75 mg/kg/day. Ultrastructural differences in cellular membranes or mitochondria were not discernible between control and treated animals in the samples examined.

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July 13, 1999
Date

REFERENCES

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Reddy, UN et. al: Tissue specificity and species differences in the distribution of urate oxidase in peroxisomes. *Lab. Invest.*, 58(1)100-111, 1988.

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.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkeys

Animal No: see below

Tissue: Liver

Sex: see below

Interpreting Pathologist (signature and date): _____

Dose/Group	Animal Number	Microscopic Findings and Comments
0 mg/kg (Control) Males	I05508	1. Infiltrate, lymphohistiocytic, multifocal, minimal
	I05517	1. Infiltrate, lymphohistiocytic, multifocal, minimal
	I05519	1. Infiltrate, lymphohistiocytic, multifocal, minimal 2. Clearing, cytoplasmic, centrilobular, minimal
	I05527	1. Infiltrate, lymphohistiocytic, multifocal, minimal 2. Angiectasis, focal, minimal
0 mg/kg (Control) Females	I05530	1. Infiltrate, lymphohistiocytic, multifocal, minimal
	I05531	1. Infiltrate, lymphohistiocytic, multifocal, minimal 2. Clearing, cytoplasmic, centrilobular, minimal
	I05535	1. Infiltrate, lymphohistiocytic, multifocal, minimal 2. Clearing, cytoplasmic, centrilobular, minimal
	I05544	1. Infiltrate, lymphohistiocytic, multifocal, minimal 2. Inflammation, eosinophilic-granulomatous, focal, slight
0.75 mg/kg (High-dose) Males	I05507	1. Infiltrate, lymphohistiocytic, focal, minimal 2. Vacuolation, hepatocellular, centrilobular, moderately-severe 3. Hypertrophy, hepatocellular, centrilobular, moderate
	I05512	1. Clearing, cytoplasmic, centrilobular, slight 2. Hypertrophy, hepatocellular, centrilobular, minimal
0.75 mg/kg (High-dose) Females	I05534	1. Infiltrate, lymphohistiocytic, focal, minimal 2. Vacuolation, hepatocellular, diffuse, slight 3. Hypertrophy, hepatocellular, centrilobular, minimal
	I05536	1. Vacuolation, hepatocellular, centrilobular, moderately-severe 2. Hypertrophy, hepatocellular, centrilobular, moderate
	I05540	1. Infiltrate, lymphohistiocytic, multifocal, minimal 2. Vacuolation, hepatocellular, centrilobular, minimal 3. Clearing, cytoplasmic, centrilobular, slight 4. Hypertrophy, hepatocellular, centrilobular, minimal
	I05551	1. Infiltrate, lymphohistiocytic, multifocal, minimal 2. Vacuolation, hepatocellular, centrilobular, minimal 3. Clearing, cytoplasmic, centrilobular, slight

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Dose/Group	Animal Number	Microscopic Findings and Comments
0.03 mg/kg (Low-dose) Males	I05514	1. Infiltrate, lymphohistiocytic, multifocal, minimal 2. Inflammation, eosinophilic-granulomatous, focal, minimal 3. Pigment, hepatocellular, multifocal, centrilobular, minimal 4. Clearing, cytoplasmic, centrilobular, slight
	I05515	1. Infiltrate, lymphohistiocytic, multifocal, minimal 2. Vacuolation, hepatocellular, centrilobular, minimal
	I05516	1. Infiltrate, lymphohistiocytic, multifocal, minimal 2. Clearing, cytoplasmic, centrilobular, slight
	I05521	1. Infiltrate, lymphohistiocytic, multifocal, minimal 2. Pigment, hepatocellular, multifocal, centrilobular, slight 3. Vacuolation, hepatocellular, multifocal, minimal 4. Clearing, cytoplasmic, centrilobular, slight
0.03 mg/kg (Low-dose) Females	I05537	1. Infiltrate, lymphohistiocytic, multifocal, minimal 2. Clearing, cytoplasmic, centrilobular, slight
	I05541	1. Infiltrate, lymphohistiocytic, multifocal, minimal 2. Clearing, cytoplasmic, centrilobular, minimal
	I05547	1. Infiltrate, lymphohistiocytic, multifocal, minimal 2. Ectasia, biliary, focal, minimal
	I05550	1. Infiltrate, lymphohistiocytic, multifocal, minimal 2. Clearing, cytoplasmic, centrilobular, minimal 3. Vacuolation, hepatocellular, multifocal, minimal
0.15 mg/kg (Mid-dose) Males	I05510	1. Infiltrate, lymphohistiocytic, multifocal, minimal 2. Clearing, cytoplasmic, diffuse, minimal
	I05518	1. Infiltrate, lymphohistiocytic, multifocal, minimal 2. Clearing, cytoplasmic, centrilobular, minimal
	I05524	1. Clearing, cytoplasmic, centrilobular, slight
	I05528	1. Infiltrate, lymphohistiocytic, multifocal, minimal 2. Clearing, cytoplasmic, diffuse, slight
0.15 mg/kg (Mid-dose) Females	I05532	1. Infiltrate, lymphohistiocytic, multifocal, minimal
	I05538	1. Infiltrate, lymphohistiocytic, multifocal, minimal 2. Clearing, cytoplasmic, centrilobular, slight
	I05545	1. Infiltrate, lymphohistiocytic, multifocal, minimal
	I05548	1. Infiltrate, lymphohistiocytic, multifocal, slight 2. Clearing, cytoplasmic, centrilobular, slight

Interpreting Pathologist (signature and date): _____

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TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05508

Tissue: Liver

Treatment Group: 0 mg/kg (Control)

Block No(s): 99.76-1

Sex: Male

43401 -
Photo/Negative No(s): 43405

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): _____

Features: 43401: Liver. Hepatocyte exhibiting large central nucleus. The cytoplasm contains many mitochondria and abundant endoplasmic reticulum. Bile canaliculi are evident at the lower right and left margins of this hepatocyte. A number (approximately 20-25) of small to medium-size clear lipid droplets are present, principally in the upper portion of the cell. Mitochondria appear normal; normal matrix granules are evident in most. Several lysosomes and peroxisomes are present. Five (5) peroxisomes counted.

43402: Liver. Hepatocyte is possibly bi-nucleate, but this may be function of cut through the nucleus. Some small to medium-sized clear lipid droplets present, similar to 43401. Microvillous border in space of Disse evident at top margin of cell. Several lamellar myelin-like bodies present within lysosomes. Five (5) peroxisomes counted.

43403: Liver. Hepatocyte with bile canaliculus at lower right. Only a few small to medium-size clear lipid droplets are evident. Numerous mitochondria and abundant endoplasmic reticulum are evident. Clear structure at the bottom may be dilated Golgi, reticulum or artifact. Nine (9) peroxisomes counted.

43404: Liver. Hepatocyte with adjacent endothelial cell and sinusoid at the bottom of the photograph. Hepatocyte contains several small to medium-sized clear lipid droplets. One droplet contains some lamellar material. Eleven (11) peroxisomes counted.

43405: Liver. Hepatocyte with higher magnification of mitochondria. Seven mitochondria present with intervening rough endoplasmic reticulum and cytosol. Outer membrane, cristae, matrix and granules appear normal.

Conclusions: Normal hepatocyte. Average 7.5 peroxisomes counted per hepatocyte.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05517

Tissue: Liver

Treatment Group: 0 mg/kg (control)

Block No(s).: 99.76-2

Sex: Male

43406 -
Photo/Negative No(s).: 43410

Significant Lesions (check one): _____ Yes X No

Interpreting Pathologist (signature and date): _____

Features: 43406: Liver. Hepatocyte surrounded by other hepatocytes with bile canaliculi in the lower right and upper left margins of the cell. There are numerous mitochondria and abundant endoplasmic reticulum. The ER and nuclear envelope appear slightly dilated. Only a couple small clear lipid droplets evident. Several small lysosomes or vacuoles with microvesicles in them are present. Five (5) peroxisomes counted.

43407: Liver. Hepatocyte with perisinusoidal microvillous border evident in lower right and upper left of photograph. A bile canaliculus is present on the right middle margin of the cell. Several small to medium-sized clear lipid droplets are evident. One larger one contains some lamellar profiles. Four (4) peroxisomes counted.

43408: Liver. Hepatocyte with sinusoid containing erythrocytes at the top. A lipid-containing perisinusoidal cell (Ito cell) is present at the top left and a bile canaliculus is present at the bottom. Random small clear lipid droplets are present in the hepatocellular cytoplasm and some of the ER appears mildly dilated. Eleven (11) peroxisomes counted.

43409: Liver. Hepatocyte contains numerous dense lysosomes and/or peroxisomes intermixed with mitochondria and abundant ER. Several small clear lipid droplets are evident. Twenty-nine (29) peroxisomes counted.

43410: Liver. Enlarged photograph of mitochondria. Ten mitochondria are clustered together in this photograph. Although the image is a little out of focus, these organelles appear essentially normal. There may be mild dilatation of the ER in the cytoplasm.

Conclusions: Normal hepatocyte. Average 12.25 peroxisomes per hepatocyte.

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TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05519

Tissue: Liver

Treatment Group: 0 mg/kg (control)

Block No(s): 99.76-3

43421 - 43425,

Sex: Male

Photo/Negative No(s): 43431

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): _____

Features: 43421: Liver. Hepatocyte. At lower left is a prominent perisinusoidal fat-storing cell (Ito cell) containing numerous large clear lipid droplets. The centered hepatocyte contains one large clear lipid droplet and several smaller ones. The nuclear envelope and ER is mildly dilated. Moderate numbers of glycogen granules present. Scattered residual bodies noted. Four (4) peroxisomes counted.

43422: Liver. Mitochondria and surrounding cytosol and ER. Membranes of ER are not very distinct. Glycogen in cytosol. Mitochondria appear essentially normal. Lamina of membranes and cristae appear parallel. Several normal granules in the matrix of the mitochondria.

43423: Liver. Mitochondria. Same view as 43422 but slightly less magnification. Some dilatation of ER. Mitochondria appear normal. A peroxisome is present in the lower left of the photograph.

43224: Liver. Hepatocyte with several medium-sized and one large clear lipid droplets. Small granules within cytosol and around ER appear to be glycogen particles or ribosomes and polyribosomes. Four (4) peroxisomes counted. Slightly out of focus.

43425: Liver. Hepatocyte surrounded by other hepatocytes and a sinusoid at lower right. Several bile canaliculi evident between hepatocytes. A single medium-sized clear lipid droplet is present in the hepatocyte. Four (4) peroxisomes counted. A couple of larger dense lysosomes also identified.

43431: Liver. The centered hepatocyte is oblong diagonally in the photograph, and doesn't contain any distinct lipid droplets. A few lipid droplets are present in adjacent hepatocytes. There is mild dilation of the ER and nuclear envelope. Only a few lysosomal structures noted. Two (2) peroxisomes counted.

Conclusions: Normal hepatocyte. Average 3.5 peroxisomes per hepatocyte.

003631

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05527

Tissue: Liver

Treatment Group: 0 mg/kg (control)

Block No(s).: 99.76-4

Sex: Male

43426 -
Photo/Negative No(s).: 43420

Significant Lesions (check one): X Yes No

Interpreting Pathologist (signature and date): _____

Features: 43426: Liver. Hepatocyte with several small to medium-sized lipid droplets (approximately 20). The adjacent lower left hepatocyte shows significant dilatation of its ER. At top left and right margin, the extracellular interstitium has some collagen fibers. Some residual bodies noted. Twenty-one (21) peroxisomes counted.

43427: Liver. Mitochondria. Membrane, cristae and matrix appear normal. Several normal granules present matrix. Some rough ER present adjacent to mitochondria. Lysosome present at left of center mitochondrion.

43428: Liver. Hepatocyte. There is mild dilation of the ER and nuclear envelope. Scattered small to medium-size clear lipid droplets (approximately 18). Scattered residual bodies noted. Twenty-five (25) peroxisomes counted.

43429: Liver. Hepatocyte. Bile canaliculi are evident at the lower right and top of the cell. The ER is mildly dilated. Scattered clear lipid droplets are evident. Abundant numbers of dense bodies (peroxisomes)/lysosomes and residual bodies are present. Thirty (30) peroxisomes counted.

43430: Liver. Hepatocyte appears similar to above described cells. There are some scattered small to medium-size clear lipid droplets. Some residual bodies are present. Fifteen (15) peroxisomes counted.

Conclusions: Normal hepatocyte. Appear to be increased numbers of peroxisomes compared with other control samples. Average 22.75 peroxisomes per hepatocyte.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05530

Tissue: Liver

Treatment Group: 0 mg/kg (control)

Block No(s): 99.76-5

Sex: Female

L4756 -
Photo/Negative No(s): L4760

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): _____

Features: L4756: Liver. Hepatocyte contains three small lipid droplets. There are bile canaliculi at the top right at top left, and a perisinusoidal microvillous border at the bottom. The nuclear envelope and ER shows some mild artifactual (fixation) dilation. Numerous mitochondria are present. Five (5) peroxisomes counted.

L4757: Liver. Mitochondria. Higher magnification of five mitochondria. Matrix and dense granules appear normal. ER between mitochondria is slightly dilated.

L4758: Liver. Hepatocyte. A sinusoid is present at the top of the photograph, lined by an endothelial cell. Four (4) medium-sized lipid droplets are present in this cell. The nuclear envelope and ER shows some mild artifactual (fixation) dilation. Numerous mitochondria are present. Four (4) peroxisomes counted.

L4759: Liver: Hepatocyte containing scattered small to medium-sized lipid droplets (approximately 14). Scattered primary lysosomes and/or peroxisomes present. There is a perisinusoidal microvillous border at the top beneath an endothelial cell and sinusoid. Twenty-five (25) peroxisomes counted.

L4760: Liver. Hepatocyte containing approximately nine (9) small to medium-sized lipid droplets. Seventeen (17) peroxisomes counted.

Conclusions: Normal hepatocyte. Average 12.75 peroxisomes per hepatocyte.

003633

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05531

Tissue: Liver

Treatment Group: 0 mg/kg (control)

Block No(s).: 99.76-6

L4761 -

Sex: Female

Photo/Negative No(s).: L4765

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): _____

Features: L4761: Liver. Hepatocyte with large central nucleus. Two margins are straight-edged by the grid bar. A few small to medium-sized clear to slightly shaded lipid droplets are present (approximately 32). ER profiles and glycogen granules are scattered throughout the cytosol. A moderate number of primary lysosomes and/or peroxisomes noted. Nine (9) peroxisomes counted.

U4762: Liver. Mitochondria. One oval and one club-shaped mitochondria present. Both appear normal with cristae, membranes, and matrix. The club-shaped one contains several normal dense granules and some slightly dilated cristae, probably fixation artifact. Some RER and smooth ER (SER) are evident in the cytosol.

L4763: Liver. Hepatocyte surrounded by interstitium containing some collagen fibers. Cells has slightly dilated ER and nuclear envelope. A single small lipid droplet is present in this cell. Four (4) peroxisomes counted.

L4764: Liver. This hepatocyte shows some dilation of ER and several lamellar residual bodies in the cytoplasm. Four (4) small lipid droplets are evident. Numerous scattered glycogen granules are present. Eight (8) peroxisomes counted.

L4765: Liver. Hepatocyte containing 5 small to medium-sized clear lipid droplets. Bile canaliculi are evident at the lower left and top right of the cell margins. A cluster of RER is present in the lower left side of the nucleus. Some larger dark lysosomes/peroxisomes are present among some of the mitochondria. Thirty-four (34) peroxisomes counted.

Conclusions: Normal hepatocyte. Average 13.75 peroxisomes per hepatocyte.

003634

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05535

Tissue: Liver

Treatment Group: 0 mg/kg (control)

Block No(s).: 99.76-7

Sex: Female

L4766 -
Photo/Negative No(s).: L4770

Significant Lesions (check one): _____ Yes X No

Interpreting Pathologist (signature and date): _____

Features: L4766: Liver. Hepatocyte contains some mild dilatation of the nuclear envelope and ER (fixation artifact). On the left and right sides are bile canaliculi, and ventrally is the sinusoidal margin showing the perisinusoidal microvillous border. Several dense residual bodies are present in the cytoplasm. Three small lipid droplets, one containing a lamellar body, are evident. One (1) peroxisome counted.

L4767: Liver. Mitochondria. Higher magnification of four mitochondria show normal morphology. Membranes, cristae and matrix appear normal. Scattered dense granules are present. The ER in the cytosol is mildly dilated.

L4768: Liver. Hepatocyte. There is moderate dilatation of the ER and nuclear envelope. Several scattered small to medium-sized clear lipid droplets are evident, as are some scattered residual bodies. Sinusoids are present at the bottom right and upper left of the photograph, and a bile canaliculus is present on the right margin of this centered hepatocyte with its neighbor hepatocyte. Two (2) peroxisomes counted.

L4769: Liver. Hepatocyte with sinusoid in upper right corner and canaliculi prominent along the bottom border. The cell exhibits dilated ER and nuclear envelope like the previously described cells for this animal – indicating suboptimal fixation. Multiple residual bodies are present. Nineteen (19) peroxisomes counted.

L4770: Liver. Hepatocyte with similar overall morphology as L4769. Six (6) peroxisomes counted.

Conclusions: Normal hepatocyte. Suboptimal fixation. Average 7.0 peroxisomes per hepatocyte.

003635

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05544

Tissue: Liver

Treatment Group: 0 mg/kg (control)

Block No(s).: 99.76-8

L4771 -

Sex: Female

Photo/Negative No(s).: L4775

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): _____

Features: L4771: Liver. Along left margin of hepatocyte are some interstitium and collagen fibers. Hepatocyte contains approximately 17 small to medium-sized clear lipid droplets. A few residual bodies are evident. There is some minimal to mild dilatation of ER. Eight (8) peroxisomes counted.

L4772: Liver. Enlarged photograph of three mitochondria. No abnormalities noted.

L4773: Liver. Hepatocyte with one large and several small to medium-sized lipid droplets in the cytoplasm. Eight (8) peroxisomes counted.

L4774: Liver. Hepatocyte with prominent microvillous border along bottom sinusoid. An endothelial cell nucleus is evident along this border. In the hepatocyte are several medium-sized clear lipid droplets. The ER does show some mild dilatation and there are abundant glycogen granules in the cytoplasm. Several dark residual bodies are present. Eight (8) peroxisomes counted.

L4775: Liver. Hepatocyte with sinusoid on the right and prominent canaliculus on top and left margins. No lipid droplets present in centered hepatocyte, although one medium-sized lipid droplet is present in top adjacent hepatocyte. Cytoplasm contains some RER but not much SER, possibly a periportal hepatocyte. Random peroxisomes and/or primary lysosomes noted. Seventeen (17) peroxisomes counted.

Conclusions: Normal hepatocyte. Average 10.25 peroxisomes per hepatocyte.

003636

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05514

Tissue: Liver

Treatment Group: .03 mg/kg (low-dose)

Block No(s).: 99.76-15

Sex: Male

Z16232 -
Photo/Negative No(s).: Z16236

Significant Lesions (check one): X Yes No

Interpreting Pathologist (signature and date): _____

Features: Z16232. Liver. Hepatocyte. Hepatocyte contains multiple small to medium-sized lipid droplets scattered throughout its cytoplasm. At the top margin is a microvillous border and some collagen fibers in the space of Disse. Smooth endoplasmic reticulum (SER) and rough endoplasmic reticulum (RER) are present throughout the cytoplasm with many mitochondria also throughout. Mitochondria are well-preserved and cristae, granules and matrix are clearly evident. Several irregular dark staining residual bodies are present. One (1) peroxisome counted.

Z16233. Liver. Hepatocyte. A moderate number of small to medium-sized lipid droplets are present in the cytoplasm, as well as several prominent residual bodies. The upper left and lower left borders show microvilli and collagen fibers adjacent to a sinusoid or central vein. The RER forms a distinct palisade at the bottom of the cell. Nine (9) peroxisomes counted.

Z16234. Liver. Hepatocyte. Centered hepatocyte shows minimal fixation artifact with some slight dilatation of SER and the nuclear envelope. There are a few small to medium-sized lipid droplets present. Four (4) peroxisomes counted.

Z16235. Liver. Hepatocyte. Cell appears similar to those described above. There are random lipid droplets and multiple residual bodies. Five (5) peroxisomes counted.

Z16236. Liver. Hepatocyte. Multiple small to medium-sized lipid droplets present in the cytoplasm. Only a small nuclear profile is evident in this cell. Eleven (11) probable peroxisomes counted.

Conclusions: Tissue appears well-fixed for ultrastructural examination. There appears to be a mild increase in numbers of lipid droplets. Average 6.0 peroxisomes per hepatocyte.

003637

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05515

Tissue: Liver

Treatment Group: .03 mg/kg (low-dose)

Block No(s).: 99.76-16

Sex: Male

Photo/Negative No(s): Z16241

Significant Lesions (check one): X Yes No

Interpreting Pathologist (signature and date): _____

Features: Z16237. Liver. Hepatocyte. A central vein is present at the top margin and contains a large amount of collagen extracellularly. A bile canaliculus is present on the left between three hepatocytes. the endoplasmic reticulum and nuclear envelope are slightly dilated – this is a fixation artifact. Several lipid droplets are present. Prominent glycogen granules are present throughout the cytoplasm. Twenty-two (22) peroxisomes.

Z16238. Liver. Hepatocyte. A bile canaliculus is present at the top border with another hepatocyte. One large and several smaller lipid droplets are present. Mitochondria appear normal and have fine cristae, matrix, and granules. Twenty-one (21) peroxisomes.

Z16239. Liver. Hepatocyte. Large central nucleus is adjacent to a large lipid droplet. A few smaller lipid droplets are present. There is artifactual dilatation of the RER and the nuclear envelope. Several residual bodies are present. Fourteen (14) peroxisomes counted.

Z16240. Liver. Hepatocyte. The centered hepatocyte contains several medium-sized lipid droplets. There is marked dilatation of the RER and the nuclear envelope. A bile canaliculus is present on the left margin of this cell and extracellular collagen is evident in the upper right. Ten (10) peroxisomes counted.

Z16241. Liver. Hepatocyte. Hepatocyte contains two larger lipid droplets and several smaller ones. The RER and nuclear envelope are dilated. The ventral margin shows a microvillous border next to an endothelial cell at the bottom. A prominent bile canaliculus is present on the left, surrounded by dark desmosomes. Eight (8) peroxisomes counted.

Conclusions: Slight (equivocal) increase in lipid droplets. Average 15.0 peroxisomes per hepatocyte.

003638

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05516

Tissue: Liver

Treatment Group: .03 mg/kg (low-dose)

Block No(s): 97.76-17

Sex: Male

Z16242 -
Photo/Negative No(s): Z16246

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z16242. Liver. Hepatocyte. Hepatocyte is adjacent to a cell containing a large lipid droplet, probably a perisinusoidal fat-storing cell (Ito cell). The hepatocyte contains numerous normal-appearing mitochondria and scattered dark irregular residual bodies. Smooth and RER are present throughout the cytoplasm. One (1) probable peroxisome counted.

Z16243. Liver. Hepatocyte. Hepatocyte has a bile canaliculus present along its right margin with an adjacent hepatocyte. The cytosol contains abundant smooth and RER and numerous mitochondria. A few residual bodies are present. Two (2) peroxisomes seen.

Z16244. Liver. Hepatocyte. Hepatocyte exhibits very slight dilatation of the endoplasmic reticulum and nuclear envelope. Glycogen granules are present throughout the cytoplasm, and scattered residual bodies are present. Two (2) probable peroxisomes identified.

Z16245. Liver. Hepatocyte. A clear vacuole with a central round density is present to the left of the nucleus of the hepatocyte. This appears to be a cytoplasmic invagination from the plasma membrane. Normal-appearing mitochondria are present throughout the cytoplasm. A few residual bodies are present. Fifteen (15) peroxisomes counted.

Z16246. Liver. Hepatocyte. The centered hepatocyte appears essentially similar to above-described cells. There are glycogen granules throughout the cytoplasm. Sixteen (16) peroxisomes counted.

Conclusions: Essentially normal hepatocytes. Average 7.2 peroxisomes per hepatocyte.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05521

Tissue: Liver

Treatment Group: 0.03 mg/kg (low-dose)

Block No(s).: 99.76-18

Sex: Male

Photo/Negative No(s).: Z16251

Significant Lesions (check one): _____ Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z16247. Liver. Hepatocyte. Several large irregular electron-dense residual bodies are present in the centered hepatocyte and the subjacent hepatocyte. The SER and nuclear envelope are slightly dilated. Also, the mitochondria appears slightly swollen, although this appears to be artifactual along with the dilated SER. A few small lipid droplets are present. Fourteen (14) peroxisomes counted.

Z16248. Liver. Hepatocyte. Clusters of fine granules, probably glycogen, are present in the cytoplasm. The mitochondria appears slightly dilated, as does the SER in an adjacent hepatocyte to the top left. The smooth ER is poorly defined. Several small lipid droplets are present. Two erythrocytes are present at the lower right of the photograph. Eleven (11) peroxisomes counted.

Z16249. Liver. Hepatocyte. The RER, nuclear envelope, and SER in the hepatocyte are slightly dilated, consistent with some fixation artifact. Several small lipid droplets and residual bodies are present. A small segment of a perisinusoidal fat-storing cell is present at the lower right, adjacent to an endothelial cell. Sixteen (16) peroxisomes identified.

Z16250. Liver. Hepatocyte. Several small lipid droplets are present in the hepatocyte. Multiple dark and irregular residual bodies are also present. The ER is slightly dilated throughout. Three (3) probable peroxisomes counted.

Z16251. Liver. Hepatocyte. There is mild dilatation of the nuclear envelope and the endoplasmic reticulum throughout. Mitochondria have a paler matrix and may be slightly enlarged. On the left is the hepatocyte's microvillous border, with an adjacent endothelial cell and erythrocyte in the sinusoid. A bile canaliculus is present in the lower right. Ten (10) peroxisomes counted.

Conclusions: Essentially normal hepatocytes. Some minimal to slight fixation artifacts. Average 10.8 peroxisomes per hepatocyte.

003640

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05537

Tissue: Liver

Treatment Group: .03 mg/kg (low-dose)

Block No(s): 99.76-19

Sex: Female

Z16252 -
Photo/Negative No(s): Z16256

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z16252. Liver. Hepatocyte. Fixation appears good. Dorsal to the hepatocyte is a central vein or sinusoid area. Several medium-sized lipid droplets are present in the cytoplasm. Fine glycogen granules are scattered throughout the cytoplasm. A desmosome is present at the lower left margin with an adjacent hepatocyte. Ten (10) peroxisomes counted.

Z16253. Liver. Hepatocyte. Hepatocyte shows very slight dilatation of endoplasmic reticulum. There is one large lipid droplet and several small droplets within the cytoplasm. A bile canaliculus is present at the top right margin of the cell. Five (5) peroxisomes counted.

Z16254. Liver. Hepatocyte. This cell appears essentially similar to Z16253, although there are lesser numbers of lipid droplets. Two irregular dark-staining residual bodies are present on the left and right side of the nucleus. The one on the right is somewhat vacuolated. Eleven (11) peroxisomes counted.

Z16255. Liver. Hepatocyte. A medium-sized lipid droplet containing some lamellar material is present to the right of the nucleus. Just a few smaller lipid droplets are present elsewhere in the cytoplasm. The endoplasmic reticulum is slightly dilated. At the bottom is the microvillous border extending into the space of Disse. A few small residual bodies are present. Six (6) probable peroxisomes counted.

Z16256. Liver. Hepatocyte. In this hepatocyte two mitochondria appear paler and somewhat swollen. The rest appear normal. Twelve (12) probable peroxisomes counted.

Conclusions: Essentially normal hepatocytes. Quantity of lipid appears within normal expectations. Average 8.8 peroxisomes per hepatocyte.

003641

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05541

Tissue: Liver

Treatment Group: .03 mg/kg (low-dose)

Block No(s).: 99.76-20

Sex: Female

Photo/Negative No(s).: Z16261

Significant Lesions (check one): _____ Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z16257. Liver. Hepatocyte. Microvillar borders are present along the lower left and upper right margins of this hepatocyte. A bile canaliculus is present at the lower right. Lamellar material within a lipid droplet is present to the lower right of the central nucleus. There is abundant SER throughout the cell, and the RER appears slightly dilated. Seven (7) probable peroxisomes counted.

Z16258. Liver. Hepatocyte. Several medium-size lipid droplets are present, as well as approximately eight residual bodies, in the cytoplasm. The lower left margin is a microvillous border, and a bile canaliculus is present in the mid and upper right margins of this hepatocyte. Eight (8) peroxisomes counted.

Z16259. Liver. Hepatocyte. This cell is surrounded by several other hepatocytes, and contains approximately ten medium-sized lipid droplets in its cytoplasm. Several very dark round to oval residual bodies are present at the top of the cell. Several dark mitochondria noted; these are often difficult to differentiate from lysosomes or peroxisomes. Fourteen (14) peroxisomes counted.

Z16260. Liver. Hepatocyte. Hepatocyte appears essentially similar to those described above. Two lipid droplets noted. There is slight dilatation of the endoplasmic reticulum and nuclear envelope. Twenty-three (23) peroxisomes counted.

Z16261. Liver. Hepatocyte. Cell contains approximately 5 small to medium-sized lipid droplets. At the bottom is a sinusoid containing a platelet or segment of a granulocyte in the circulation. Another sinusoidal margin and microvillous border is present at the top. There is slight dilatation of the endoplasmic reticulum. Eight (8) peroxisomes counted.

Conclusions: Essentially normal hepatocytes. Some lipid droplets noted, but within normal expectations for hepatocytes. Average 12.0 peroxisomes per hepatocyte.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05547

Tissue: Liver

Treatment Group: 0.03 mg/kg (low-dose)

Block No(s): 99.76-21

Sex: Female

Photo/Negative No(s): Z16262 -
Z16266

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z16262. Liver. Hepatocyte. Photograph shows a hepatocyte with just a small nuclear profile. A microvillous border is present at the top right. Some artifactual knife marks are present. Several dark residual bodies are present, and only about six small lipid droplets are evident in the cytoplasm. Abundant SER and RER are throughout the cytoplasm intermixed with numerous mitochondria. Four (4) peroxisomes identified.

Z16263. Liver. Hepatocyte. Numerous collagen fibers are present along the dorsal extracellular margin. At the lower left this hepatocyte's microvillous border extends in the space of Disse. There is slight dilatation of the nuclear envelope and endoplasmic reticulum. Several residual bodies and lipid droplets are present. Fine glycogen granules are randomly scattered throughout the cytoplasm. Six (6) peroxisomes clearly identified.

Z16264. Liver. Hepatocyte. There is slight dilatation of the endoplasmic reticulum and nuclear envelope. A bile canaliculus is present at the dorsal right margin of the cell, and another hepatocyte is present along the ventral margin. The general morphology is similar to the above described cells. Only one lipid droplet is evident in this cell. Several residual bodies are evident. Fourteen (14) probable peroxisomes identified.

Z16265. Liver. Hepatocyte. A central vein and sinusoid surround this hepatocyte on the lower and upper left margins. Erythrocytes are present in the vascular lumens. Within the hepatocyte there is slight dilatation of the endoplasmic reticulum and nuclear envelope. Two small lipid droplets are present. Nine (9) peroxisomes counted.

Z16266. Liver. Hepatocyte. Hepatocyte appears essentially similar to cells described above. There is slight dilatation of the endoplasmic reticulum and nuclear envelope. A large irregularly-shaped residual body is present to the lower left of the nucleus. Seventeen (17) probable peroxisomes counted.

Conclusions: Essentially normal hepatocytes. Average 10.0 peroxisomes per hepatocyte.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05550

Tissue: Liver

Treatment Group: .03 mg/kg (low-dose)

Block No(s): 99.76-22

Sex: Female

Photo/Negative No(s): Z16267 -
Z16272

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z16267. Liver. Hepatocyte. Abundant numbers of glycogen granules are present throughout the cytoplasm of this hepatocyte. Bile canaliculi are evident on the right, left and lower left margins. A large dark residual body is present in an adjacent hepatocyte at the top left. Several smaller residual bodies are present in the cytoplasm of the centered hepatocyte. Mitochondria appear normal, having cristae, sporadic granules and a fine grainy matrix. Five (5) probable peroxisomes counted.

Z16268. Liver. Hepatocyte. Cytoplasm has a somewhat light appearance due to slight dilatation of the endoplasmic reticulum. The nuclear envelope is also slightly dilated. These are artifactual fixation-related changes. Prominent bile canaliculi are present on the left and lower right cellular margins. No lipid droplets evident. Fifteen (15) peroxisomes counted.

Z16269. Liver. Hepatocyte. Cell appears similar to Z16267 described above. There appears to be abundant glycogen granules in the cytosol. A microvillous border is present at the upper right extending in to the space of Disse, while bile canaliculi are evident along the lower left and upper left margins. There are many dark mitochondria which are difficult to differentiate from peroxisomes. Twenty-seven (27) probable peroxisomes counted.

Z16270. Liver. Hepatocyte. This hepatocyte appears to be binucleate. An endothelial cell is present lining the sinusoid ventrally and two erythrocytes are present dorsal to this cell. The endoplasmic reticulum and nuclear envelope is slightly dilated. Twenty (20) peroxisomes seen.

Z16272. Liver. Hepatocyte. Two large and multiple smaller lipid droplets are present in this hepatocyte. Dorsally, the microvillous border extends into the space of Disse and is adjacent to an endothelial cell. Ventrally are a small segment of a lipid-containing cell, probably a perisinusoidal fat-storing cell and an adjacent hepatocyte. Twenty-one (21) probable peroxisomes counted.

Conclusions: Essentially normal hepatocytes. Although one cell (Z16272) had a lot of lipid droplets, the overall impression is that lipid content in this liver was within expected normal limits. Average 17.6 peroxisomes per hepatocyte.

003644

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05510

Tissue: Liver

Treatment Group: .15 mg/kg (mid-dose)

Block No(s).: 99.76-23

Sex: Male

Photo/Negative No(s).: Z16277

Significant Lesions (check one): _____ Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z16273. Liver. Hepatocyte. Numerous glycogen granules are present randomly throughout the SER and cytosol. Fixation appears good. One swollen "barbell-shaped" mitochondria is present, but all other mitochondria appear normal. Scattered dark residual bodies are present. Thirteen (13) peroxisomes counted.

Z16274. Liver. Hepatocyte. Several small to medium-sized lipid droplets are present in the cytoplasm of the hepatocyte. Along its right margin its microvillous border faces another hepatocyte. A poorly-defined bile canaliculus is present at the lower left. The cytoplasm is filled with SER, RER, mitochondria and glycogen granules throughout. Some peroxisomes are noted. Scattered dark residual bodies are present. Ten (10) peroxisomes counted.

Z16275. Liver. Hepatocyte. Centered hepatocyte has only a small nuclear profile. At the bottom is a central vein surrounded by an endothelial cell and a moderate number of extracellular collagen fibers. An endothelial cell is the lighter shaded cell at the top right. Three lipid droplets present in the cytoplasm. Thirteen (13) peroxisomes counted.

Z16276. Liver. Hepatocyte. This cell appears similar to Z16273. Approximately 10 small to medium-sized lipid droplets are present in the cytoplasm. Numerous glycogen granules are evident in the cytosol. A lamellar body is present at the bottom margin of the cell. Fourteen (14) peroxisomes counted.

Z16277. Liver. Hepatocyte. Approximately a dozen small to medium-sized lipid droplets are present in the cytoplasm. The cluster of 5 droplets at the far right are in another cell. The cytoplasm contains abundant fine glycogen granules. Several residual bodies are noted. Three (3) probable peroxisomes counted.

Conclusions: Essentially normal hepatocytes. Moderate amount of glycogen in several cells. Number of lipid droplets appears normal. Average 10.6 peroxisomes per hepatocyte.

003645

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05518

Tissue: Liver

Treatment Group: .15 mg/kg (mid-dose)

Block No(s): 99.76-24

Sex: Male

Z16278 -
Photo/Negative No(s): Z16282

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z16278. Liver. Hepatocyte. At the top right this hepatocyte is bordered by a perisinusoidal fat-storing cell containing several prominent lipid droplets. Four small lipid droplets are present in the hepatocyte. A bile canaliculus, adjacent to a large residual body, is present on the left margin of the cell. There is slight dilatation of the nuclear envelope and endoplasmic reticulum. Multiple irregular residual bodies are evident. Thirteen (13) peroxisomes clearly identified.

Z16279. Liver. Hepatocyte. The centered hepatocyte is bordered by a darker hepatocyte on the right. On its left is a band of interstitial collagen. The nuclear envelope and endoplasmic reticulum are slightly dilated. Several endocytotic vesicles are present along the right cellular membrane. Sixteen (16) probable peroxisomes counted.

Z16280. Liver. Hepatocyte. Hepatocyte has prominent bile canaliculi along its right and left margins with the adjacent hepatocytes. One small lipid droplet noted. Mitochondria stain somewhat more pale in this cell and contrast with darker peroxisomes. Multiple clusters of RER evident. Twelve (12) peroxisomes counted.

Z16281. Liver. Hepatocyte. This hepatocyte appears to be binucleate. At the top left is an erythrocyte within the central vein or sinusoid. Several small lipid droplets are present in the cytoplasm. There is slight to moderate dilatation of the endoplasmic reticulum and nuclear envelope. Some shrunken mitochondria are difficult to differentiate from lysosomes or peroxisomes. Thirty-two (32) probable peroxisomes counted.

Z16282. Liver. Hepatocyte. General cellular morphology is similar to cell described above. There is slight dilatation of the endoplasmic reticulum and nuclear envelope. Nineteen (19) peroxisomes counted.

Conclusions: Essentially normal hepatocytes. Only small numbers of lipid droplets seen. Average 18.4 peroxisomes per hepatocyte.

003646

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05524

Tissue: Liver

Treatment Group: .15 mg/kg (mid-dose)

Block No(s).: 99.76-25

Sex: Male

Photo/Negative No(s).: Z16287

Significant Lesions (check one): X Yes No

Interpreting Pathologist (signature and date): _____

Features: Z16283. Liver. Hepatocyte. Hepatocyte contains two medium-sized lipid droplets. The nuclear envelope and endoplasmic reticulum are slightly dilated. At the top right is a bile canaliculus between this cell and an adjacent hepatocyte. Desmosomes are present around the canaliculus. Thirty-one (31) peroxisomes counted.

Z16284. Liver. Hepatocyte. This centered hepatocyte is surrounded by other hepatocytes on all margins in this photograph. Bile canaliculi are evident at the top left and bottom left margins. No lipid droplets are evident in this cell. Mitochondria, RER and SER appear normal. Twenty-nine (29) probable peroxisomes counted.

Z16285. Liver. Hepatocyte. Two erythrocytes are present in the sinusoid adjacent to the left of this hepatocyte. Collagen fibers are present in the space of Disse. Along the right margin of this cell is a prominent bile canaliculus. A single medium-sized lipid droplet is present in this cell. Twenty-seven (27) peroxisomes counted.

Z16286. Liver. Hepatocyte. Cell appears essentially similar to cells described above. At top left and bottom right are erythrocytes within sinusoids. No distinct lipid droplets are present in this cell. There is slight dilatation of the endoplasmic reticulum and nuclear envelope. Several dark residual bodies are present. Twenty-one (21) peroxisomes counted.

Z16287. Liver. Hepatocyte. Cell has two large and several smaller lipid droplets present in its cytoplasm. There is slight dilatation of the endoplasmic reticulum and nuclear envelope. Several dark residual bodies are present. Glycogen granules are present throughout the cytoplasm. Eight (8) peroxisomes counted.

Conclusions: Essentially normal hepatocytes. Slight increase in average number of peroxisomes. No increase in lipid noted. Average 23.2 peroxisomes per hepatocyte.

003647

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05528

Tissue: Liver

Treatment Group: .15 mg/kg (mid-dose)

Block No(s): 99.76-26

Sex: Male

Photo/Negative No(s): Z16288 -
Z16292

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z16288. Liver. Hepatocyte. This hepatocyte contains about five medium-sized and approximately fifteen small lipid droplets in its cytoplasm. Microvillous border beneath endothelial cells are evident at the upper right and lower left margins. A bile canaliculus surrounded by desmosomes is present at the lower right margin. Scattered dark-staining residual bodies are present in the cytoplasm. There is slight dilatation of the endoplasmic reticulum and the nuclear envelope. Five (5) peroxisomes clearly identified.

Z16289. Liver. Hepatocyte. This hepatocyte's microvillous border extends into the space of Disse at the upper left margin of the photograph. At the lower left is a perisinusoidal fat-storing cell (Ito cell). There is slight dilatation of the endoplasmic reticulum and the nuclear envelope. Two small lipid droplets are evident. Sixteen (16) peroxisomes counted.

Z16290. Liver. Hepatocyte. This cell is a little darker staining than previous cells. At lower left a granulocyte is present in the sinusoid or central vein. At the top right a perisinusoidal fat-storing cell (Ito cell) is present. Several small lipid droplets are present in the hepatocyte and a few residual bodies are evident. There is slight dilatation of the endoplasmic reticulum and the nuclear envelope. Nineteen (19) peroxisomes counted.

Z16291. Liver. Hepatocyte. Much of the cytoplasm has a dense granular appearance, composed of SER, glycogen granules, and probable cytoskeletal components. Approximately eighteen small to medium-sized lipid droplets are present. Nineteen (19) probable peroxisomes counted.

Z16292. Liver. Hepatocyte. Hepatocyte appears similar to Z16291. There are several lipid droplets present in the cytoplasm. Fourteen probable (14) peroxisomes counted.

Conclusions: Essentially normal hepatocytes. Lipid droplet numbers within normal expectations. Average 14.6 peroxisomes per hepatocyte.

003648

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05532

Tissue: Liver

Treatment Group: .15 mg/kg (mid-dose)

Block No(s): 99.76-27

Sex: Female

Photo/Negative No(s): Z16293 -
Z16297

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z16293. Liver. Hepatocyte. This photograph has a centered hepatocyte. At the top are multiple lipid droplets which are in an adjacent perisinusoidal fat-storing cell (Ito cell), not in this hepatocyte. There are numerous glycogen granules in the cytosol intermixed with the endoplasmic reticulum. Two very small lipid droplets are present. Mitochondria appear normal. They show fine cristae, some granules and finely grainy matrix. Four (4) peroxisomes clearly identified.

Z16294. Liver. Hepatocyte. A single large lipid droplet is present in the cytoplasm beneath the nucleus. Similar to Z16293, there are many fine granules in the cytoplasm which appear to be glycogen granules. Three (3) peroxisomes identified in this hepatocyte.

Z16295. Liver. Hepatocyte. Several medium-sized lipid droplets are present in this hepatocyte. At the upper right is a "P"-shaped extracellular area containing collagen fibers. Moderate amounts of glycogen are present in the cytoplasm of the hepatocyte. Several residual bodies are also present. Five (5) probable peroxisomes counted.

Z16296. Liver. Hepatocyte. Centered hepatocyte appears similar to those described above. Three lipid droplets are evident in its cytoplasm. At the bottom is a longitudinal profile of a bile canaliculus and a cross-section of a canaliculus at the top margin. Fine glycogen granules are present throughout. Six (6) peroxisomes counted.

Z16297. Liver. Hepatocyte. . Centered hepatocyte appears similar to those described above. Two medium-sized and several small lipid droplets present. To the right is an area of interstitium containing some collagen fibers. The nucleus probably belongs to an endothelial cell. Three (3) peroxisomes counted.

Conclusions: Essentially normal hepatocytes. Moderate amounts of glycogen present in most cells. Lipid droplet numbers appear normal. Average 4.2 peroxisomes per hepatocyte.

003649

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05538

Tissue: Liver

Treatment Group: .15 mg/kg (mid-dose)

Block No(s): 99.76-28

Sex: Female

Z16298 -
Photo/Negative No(s): Z16302

Significant Lesions (check one): X Yes No

Interpreting Pathologist (signature and date): _____

Features: Z16298. Liver. Hepatocyte. Section has a prominent single knife mark transecting the cell beneath the nucleus. Numerous glycogen granules are present in the cytoplasm. A single small lipid droplet is present. Mitochondria and other organelles appear normal. Five (5) peroxisomes counted.

Z16299. Liver. Hepatocyte. Hepatocyte has a single large and several small lipid droplets. A sinusoid is present at the upper left margin where the microvillous border extends into the space of Disse. There are abundant glycogen granules in the cytoplasm. Some mitochondria are lighter staining than the majority of darker one. These light mitochondria are somewhat difficult to differentiate from lysosomes or peroxisomes. Eighteen (18) probable peroxisomes counted.

Z16300. Liver. Hepatocyte. Cell contains abundant glycogen often in irregular clear spaces or pockets in the cytosol. A single lipid droplet is present. Three (3) peroxisomes identified.

Z16301. Liver. Hepatocyte. This centered hepatocyte has adjacent hepatocyte dorsally and ventrally and a microvillous-sinusoidal border at the top right. Its cytoplasm contains multiple irregular pockets of glycogen granules. Other organelles appear somewhat clumped between the glycogen pockets. Nine (9) peroxisomes counted.

Z16302. Liver. Hepatocyte. This cell appears similar to Z16301, although it has more pockets of glycogen granules and has an overall light appearance. Other organelles appear clumped between the glycogen granules. One (1) peroxisome clearly identified.

Conclusions: Essentially normal hepatocytes. There is increased glycogen in some of these hepatocytes. Average 7.2 peroxisomes per hepatocyte.

603650

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05545

Tissue: Liver

Treatment Group: .15 mg/kg (mid-dose)

Block No(s): 99.76-29

Sex: Female

Photo/Negative No(s): Z16303 -
Z16307

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z16303. Liver. Hepatocyte. This cell is bordered by hepatocytes on the top right and bottom right and by an interstitial margin on the left. Prominent bile canaliculi are evident between adjacent hepatocytes. There is slight dilatation of the endoplasmic reticulum and nuclear envelope. No lipid droplets are evident. Several dark irregular residual bodies are present. Six (6) probable peroxisomes counted.

Z16304. Liver. Hepatocyte. Centered hepatocyte is surrounded by hepatocytes on all margins. Bile canaliculi are evident at the right and bottom margins. Its endoplasmic reticulum and nuclear envelope are slightly dilated. Several residual bodies are present. No lipid droplets are present. Fourteen (14) probable peroxisomes counted.

Z16305. Liver. Hepatocyte. Hepatocyte appears similar to Z16303 above. At top left is a central vein, lined by an endothelial cell and containing a segment of an erythrocyte. No lipid droplets are evident in this hepatocyte. Seven (7) peroxisomes clearly identified.

Z16306. Liver. Hepatocyte. This cell is somewhat lighter staining than previous hepatocytes, although the mitochondria still are staining fairly darkly. Cell on the far right may be an endothelial cell. Twelve (12) probable peroxisomes counted.

Z16307. Liver. Hepatocyte. All mitochondria in this cell are darkly staining. There is slight dilatation of the endoplasmic reticulum. Six (6) peroxisomes identified.

Conclusions: Essentially normal hepatocytes. No evidence of increased lipid droplets. Average 9.0 peroxisomes per hepatocyte.

003651

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05548

Tissue: Liver

Treatment Group: .15 mg/kg (mid-dose)

Block No(s): 99.76-30

Sex: Female

Z16308 - Z16309

Photo/Negative No(s): Z16311-

Z16313

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): _____

Features: Z16308. Liver. Hepatocyte. The centered hepatocyte is bordered by adjacent hepatocytes on its left and right margins. On the right is a prominent bile canaliculus. A microvillous border is evident at the top right. Fixation appears good and cytoplasmic organelles appear normal. No lipid droplets are present in the cytoplasm. Abundant SER is present in the cytoplasm. There are a number of shrunken dark, variably-sized mitochondria, which are difficult to clearly differentiate from possible lysosomes or peroxisomes. One large lysosome is present at about "4:40 o'clock" from the nucleus, and contains some linear debris. Twenty-nine (29) peroxisomes identified.

Z16309. Liver. Hepatocyte. This hepatocyte exhibits slight dilatation of the endoplasmic reticulum and nuclear envelope. Three small to medium-sized lipid droplets are present. An erythrocyte is evident in a sinusoid at the bottom left of the photograph. Random dark and irregular residual bodies are present. Seventeen (17) peroxisomes clearly identified.

Z16310. Liver. Hepatocyte. Crystalloid inclusions in some mitochondria, an occasional finding in mitochondria. Peroxisomes not counted in this hepatocyte.

Z16311. Liver. Hepatocyte. One large and several smaller lipid droplets are present in the cytoplasm. There is slight dilatation of the endoplasmic reticulum and nuclear envelope. Prominent bile canaliculi are present on the left and right margins with adjacent hepatocytes. Sixteen (16) probable peroxisomes counted.

Z16312. Liver. Hepatocyte. This hepatocyte is bordered by adjacent hepatocyte on all margins. Several small lipid droplets are present in the cytoplasm. There is slight dilatation of the nuclear envelope and endoplasmic reticulum. Nine (9) probable peroxisomes counted.

Z16313. Liver. Hepatocyte. Two medium-sized and several smaller lipid droplets are evident in the cytoplasm. At the bottom left of the photograph are some interstitial collagen fibers. A bile canaliculus is present at the top right margin of the cell. There is slight dilatation of the endoplasmic reticulum, especially the RER, and the nuclear envelope. A large lysosome is present in close proximity to the noted bile canaliculus; it contains some electron-dense material. Fourteen (14) peroxisomes counted.

Conclusions: Essentially normal hepatocytes. No substantial increase in lipid droplets noted. Average 17.0 peroxisomes per hepatocyte.

003652

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05507

Tissue: Liver

Treatment Group: 0.75 mg/kg (hi-dose)

Block No(s).: 99.76-9

Sex: Male

43411 -
Photo/Negative No(s).: 43415

Significant Lesions (check one): X Yes No

Interpreting Pathologist (signature and date): _____

Features: 43411: Liver. Hepatocyte containing a single very large clear lipid droplet and many small to medium-size clear lipid droplets. There appears to be an increase in the amount of glycogen α -rosette granules. Several foci of ER appear irregularly dilated. A few scattered lysosomes and peroxisomes noted. Nine (9) peroxisomes counted.

43412: Liver. Hepatocyte contains a large number of small to medium-sized clear lipid droplets in the cytosol (approximately 125), most being very small. Abundant glycogen as α -rosettes is present in the cytoplasm. Twenty-six (26) peroxisomes counted.

43413: Liver. Hepatocyte containing several very large clear lipid droplets and many smaller droplets. Some interstitial tissue containing some collagen fibers is present at the lower left of the photograph. Some residual bodies present. Many glycogen rosettes present in cytoplasm. Three (3) peroxisomes counted.

43414: Liver. Several mitochondria are shown which appear essentially normal. Some normal dense granules are present in the matrix and the cristae and enveloping membrane appear normal. In the cytoplasm there are multiple small lipid droplets, usually surrounded by fine dense granules (glycogen or ribosomes).

43415: Liver. Hepatocyte with a bile canaliculus at the bottom margin and an interstitial fibroblastic cell at the upper right. The hepatocyte contains abundant small to medium-sized clear lipid droplets (>170). Random residual bodies are present. Six (6) peroxisomes counted.

Conclusions: Lipid accumulation, moderately-severe, hepatocellular. Increased glycogen. Average 11.0 peroxisomes per hepatocyte.

003653

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05534

Tissue: Liver

Treatment Group: .75 mg/kg (hi-dose)

Block No(s): 99.76-11

Sex: Female

L4798 -

Photo/Negative No(s): L4802

Significant Lesions (check one): X Yes No

Interpreting Pathologist (signature and date): _____

Features: L4798: Liver. Hepatocyte surrounded by adjacent hepatocytes. It contains abundant numbers of mitochondria and ER. There is mild dilatation of the nuclear envelope and of some ER. Scattered residual bodies are present. Just one distinct lipid droplet is evident. Four (4) peroxisomes counted.

L4799: Liver. Enlarged view of five mitochondria. Mitochondria appear essentially normal. Several dense granules are present in the matrix. The cristae and limiting membranes appear normal. At the right side is the mildly dilated nuclear envelope and the nucleus.

L4800: Liver. Hepatocyte. A few small clear lipid droplets are present in the cytoplasm. There are also abundant glycogen granules in the cytoplasm. A number of "clear areas" in the cytoplasm appear to contain glycogen granules without a distinct lipid droplet margin. Two (2) peroxisomes counted.

L4801: Liver. Hepatocyte. There are numerous small to medium-sized rarefied "pockets" in the cytoplasm (approximately 57). Some contain some wispy lamellar material, and some glycogen granules appear to be in these spaces. The margins of these droplets are not as sharply defined as clear round lipid droplets usually are. Four (4) peroxisomes counted.

L4802: Liver. Hepatocyte appears similar to L4801 with some small to medium-sized lipid droplets and other rarefied areas with glycogen in the cytoplasm (approximately 29). Some contain some wispy lamellar material. There are also abundant glycogen granules throughout the cytoplasm. Twelve (12) peroxisomes counted.

Conclusions: Increased glycogen in this animal. Increased number of glycogen granules. Average 5.5 peroxisomes per hepatocyte.

003654

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05540

Tissue: Liver

Treatment Group: .75 mg/kg (hi-dose)

Block No(s).: 99.76-13

Sex: Female

Photo/Negative No(s).: L4813

Significant Lesions (check one): X Yes No

Interpreting Pathologist (signature and date): _____

Features: L4809: Liver. Hepatocyte. Only a small segment of nucleus is present in this photograph. Abundant ER and glycogen granules are present. There are scattered small to large lipid droplets, but not too many in this hepatocyte (approximately 15). A few dark residual bodies are evident. Fifteen (15) peroxisomes counted.

L4810: Liver. Enlarged photograph of several mitochondria. No abnormalities noted. Cytoplasm contains some ER and scattered glycogen granules.

L4811: Liver. Hepatocyte contains multiple medium to large clear lipid droplets (approximately 41). There is abundant glycogen in the cytoplasm. Five (5) peroxisomes counted.

L4812: Liver. Hepatocyte. Similar to L4811. Hepatocyte contains multiple small to large clear lipid droplets, too numerous to count. There is abundant glycogen in the cytoplasm. Twenty-eight (28) peroxisomes counted.

L4813: Liver. Hepatocyte contains abundant mitochondria and ER. Only a few clear lipid droplets are present. An endothelial cell is present at the top of the photograph. There is minimal to mild dilatation of the ER and nuclear envelope. Scattered residual bodies are present. Two (2) peroxisomes clearly identified.

Conclusions: Mild to moderate increase in cytoplasmic lipid droplets. Increased glycogen in most hepatocytes. Average 12.5 peroxisomes per hepatocyte.

003655

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05551

Tissue: Liver

Treatment Group: .75 mg/kg (hi-dose)

Block No(s): 99.76-14

Sex: Female

L4814 -

Photo/Negative No(s): L4818

Significant Lesions (check one): X Yes No

Interpreting Pathologist (signature and date): _____

Features: L4814. Liver. Hepatocyte. There is a dilated canaliculus at the top right of the hepatocyte. In the cytoplasm there are multiple rarefied areas that mostly appear to contain glycogen granules. However, a few have some lamellar material in them. There is slight dilatation of the nuclear envelope and ER. Some residual bodies are present. No distinct lipid droplets are present in this hepatocyte, however, there are several lipid droplets in the perisinusoidal fat-storing cell (Ito cell) at the top left. Nine (9) peroxisomes counted.

L4815: Liver. Enlargement of a cluster of mitochondria. These organelles appear essentially normal.

L4816: Liver. Hepatocyte contains a single medium-sized clear lipid droplet. The cytoplasm contains abundant glycogen granules. Several residual bodies present. Four (4) peroxisomes clearly identified. Several small dark bodies appear to be tips of mitochondria.

L4817: Liver. Hepatocyte appears similar to L4814. There is abundant glycogen in the cytoplasm, often making clear areas containing just glycogen granules. Several clusters of RER present. One small lipid droplet present. Fourteen (14) peroxisomes counted.

L4818: Liver. Hepatocyte contains multiple rarefied "pockets" of glycogen granules. No lipid droplets present. Eleven (11) peroxisomes counted.

Conclusions: Increased glycogen. Average 9.5 peroxisomes per hepatocyte.

003656

III. Transmission Electron Micrographs

003657

Figure Legends

Magnification. Print magnification is indicated on the back of each photograph. The measurement bar on each photomicrograph equals one micron (0.001mm).

Figure 1. Liver. Hepatocyte showing normal cytoplasmic organelles. Some peroxisomes are identified and other normal organelles. Ventrally the microvillous border extends into the perisinusoidal space. Animal No. I05517, 0 mg/kg/day (control male), 10,920X magnification.

Figure 2. Liver. Hepatocyte. Several lipid droplets are present. There is slight artifactual dilatation of some rough endoplasmic reticulum. Some extracellular collagen is present in the interstitium in the upper right corner. An endothelial cell nucleus is present lining the sinusoid in the lower right corner of the photograph. Animal No. I05544, 0 mg/kg/day (control female), 11,200X magnification.

Figure 3. Liver. Hepatocyte. Mitochondria showing outer membrane, cristae, matrix and granules. These granules within the mitochondrial matrix are normal and are sites of calcium accumulation. Animal No. I05519, 0 mg/kg/day (control male), 112,000X magnification.

Figure 4. Liver. Hepatocyte showing increased lipid droplet accumulation. Lipid droplets vary from small to very large. Several residual bodies are present. Peroxisomes are scarce. Animal No. I05507, 0.75 mg/kg/day (high-dose male), 10,920X magnification.

Figure 5. Liver. Hepatocyte showing numerous lipid droplets and peroxisomes. Several peroxisomes are labeled. Animal No. I05512, 0.75 mg/kg/day (high-dose male), 10,920X magnification.

Figure 6. Liver. Hepatocyte with many small to large lipid droplets. Representative peroxisomes are labeled. Desmosomes and a bile canaliculus is evident at the right. Animal No. I05536, 0.75 mg/kg/day (high-dose female), 11,200X magnification.

Figure 7. Liver. Hepatocyte with multiple lipid droplets and abundant glycogen granules in the cytoplasm. Animal No. I05540, 0.75 mg/kg/day (high-dose female), 11,200X magnification.

Figure 8. Liver. Hepatocyte containing numerous "pockets" of glycogen. Animal No. I05551, 0.75 mg/kg/day (high-dose female), 11,200X magnification.

Figure 9. Liver. Hepatocyte. Mitochondria showing outer membrane, cristae and matrix. Small lipid droplets are present in the surrounding cytosol. Animal No. I05512, 0.75 mg/kg/day (high-dose male), 112,000X magnification.

Figure 10. Liver. Hepatocyte. Mitochondria showing outer membrane, cristae, matrix, and granules. The nucleus is present on the right. Animal No. I05534, 0.75 mg/kg/day (high-dose female), 95,200X magnification.

Figure 11. Liver. Hepatocyte. There is moderate accumulation of small to medium-sized lipid droplets in this hepatocyte. Only a small nuclear profile is evident in this cell. The endoplasmic reticulum is slightly dilated. Animal No. I05514, 0.03 mg/kg/day (low-dose male), 11,200X magnification.

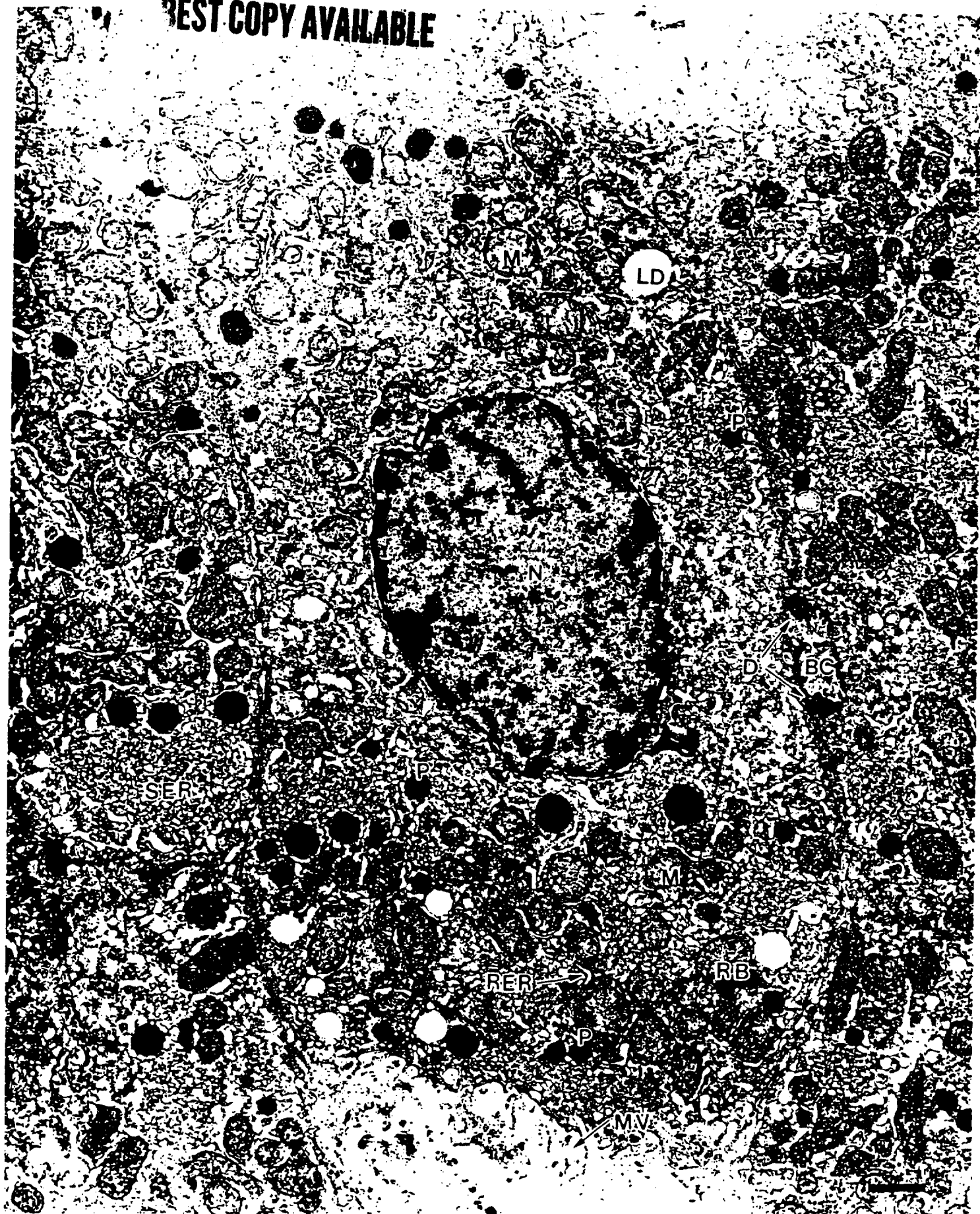
Figure 12. Liver. Hepatocyte. Mitochondria, endoplasmic reticulum and some residual bodies make up the majority of this hepatocyte's cytoplasm. A few small dark mitochondria are present, but no clearly identifiable peroxisomes are present. No lipid droplets are present in this cell, although a single lipid droplet is present in the subjacent cell at the bottom of the photograph. Animal No. I05545, 0.15 mg/kg/day (mid-dose female), 11,200X magnification

Index of Labels for Ultrastructural Structures

<u>Abbreviation</u>	<u>Structure</u>
BC	Bile canaliculus
CO	Collagen
CR	Cristae
D	Desmosome
EC	Endothelial cell
G	Glycogen
GR	Granules
LD	Lipid droplet
M	Mitochondria
MV	Microvilli
MX	Matrix
N	Nucleus
OM	Outer membrane
P	Peroxisome
RB	Residual body
RER	Rough endoplasmic reticulum
SER	Smooth endoplasmic reticulum

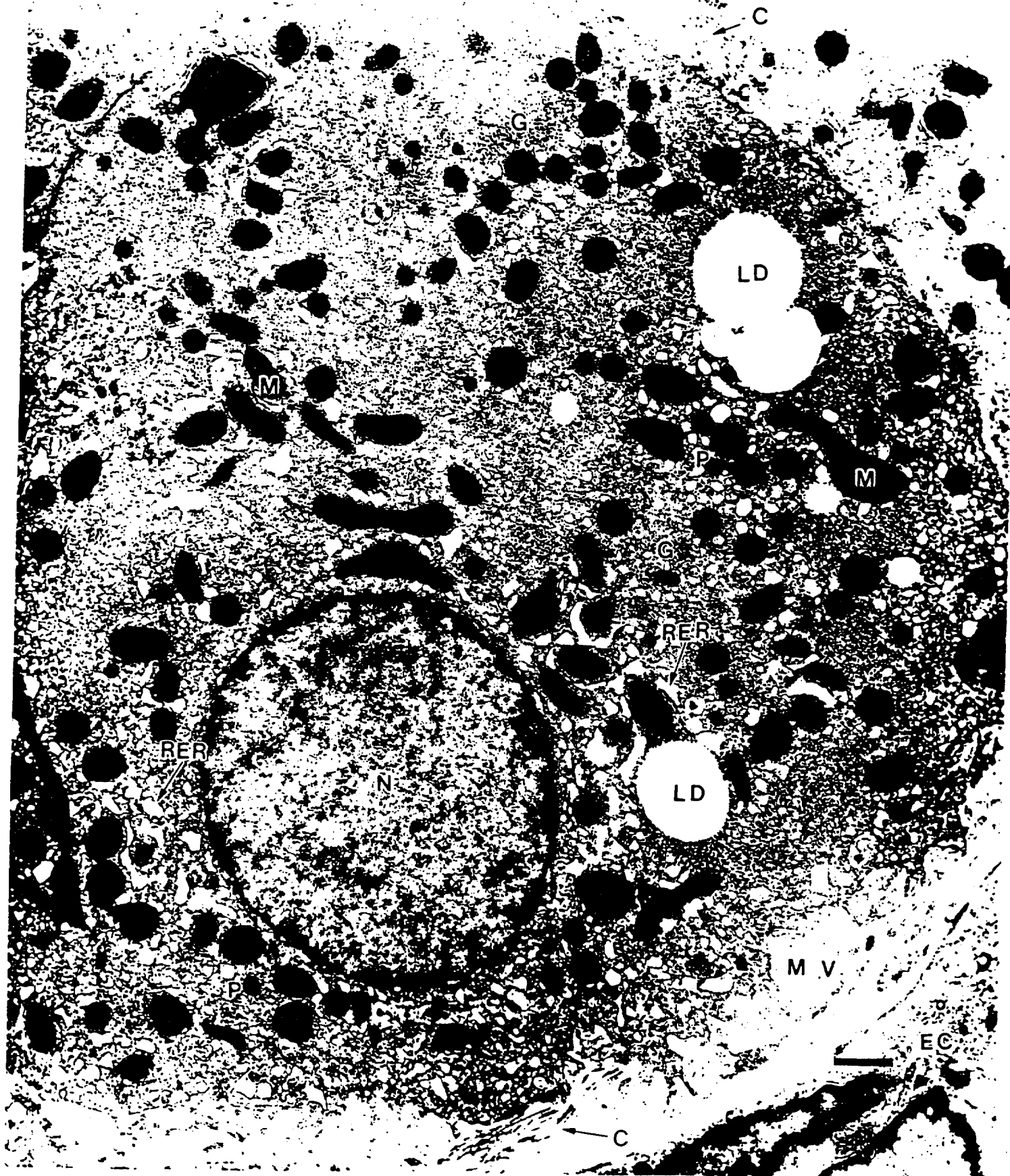
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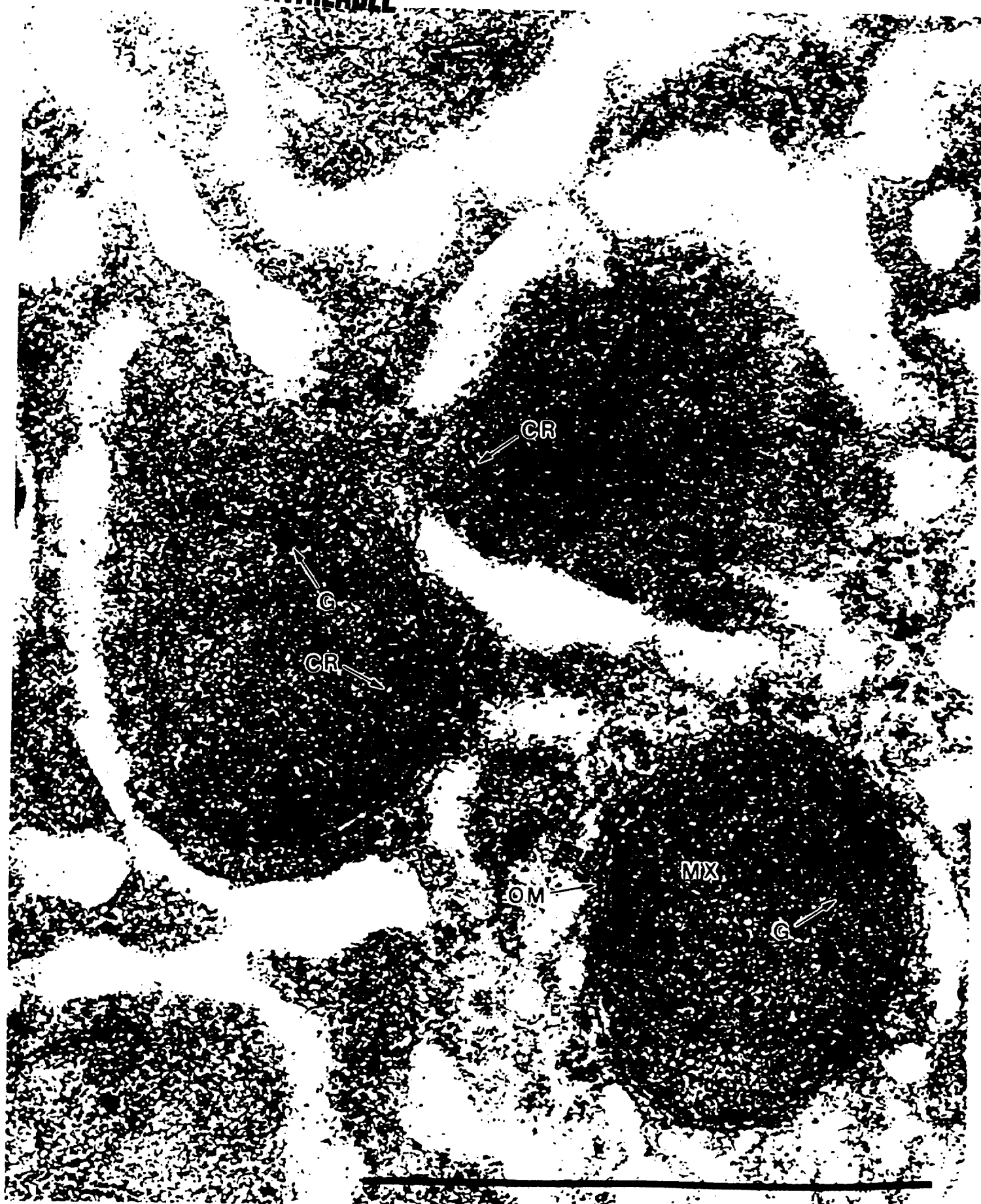
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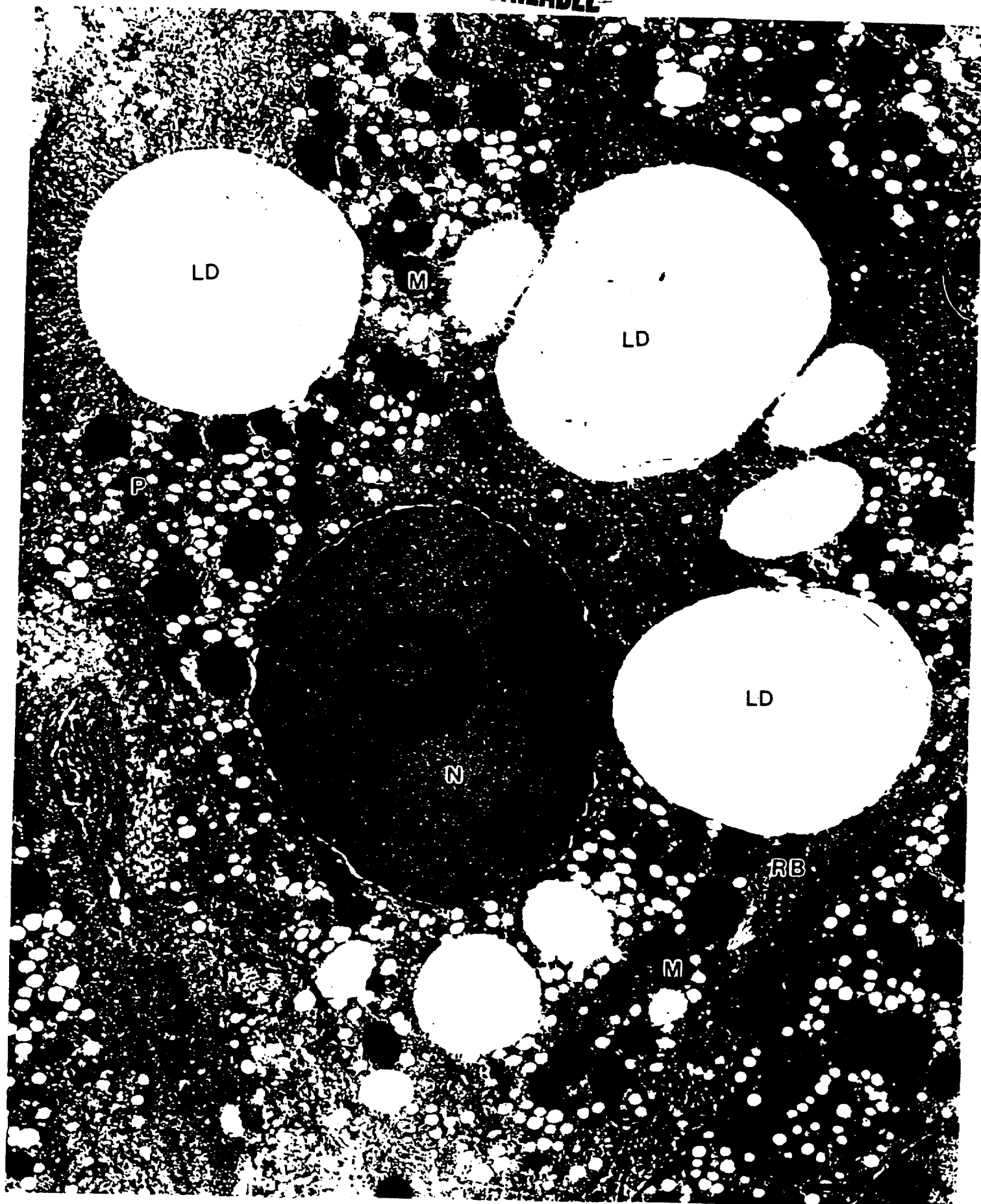


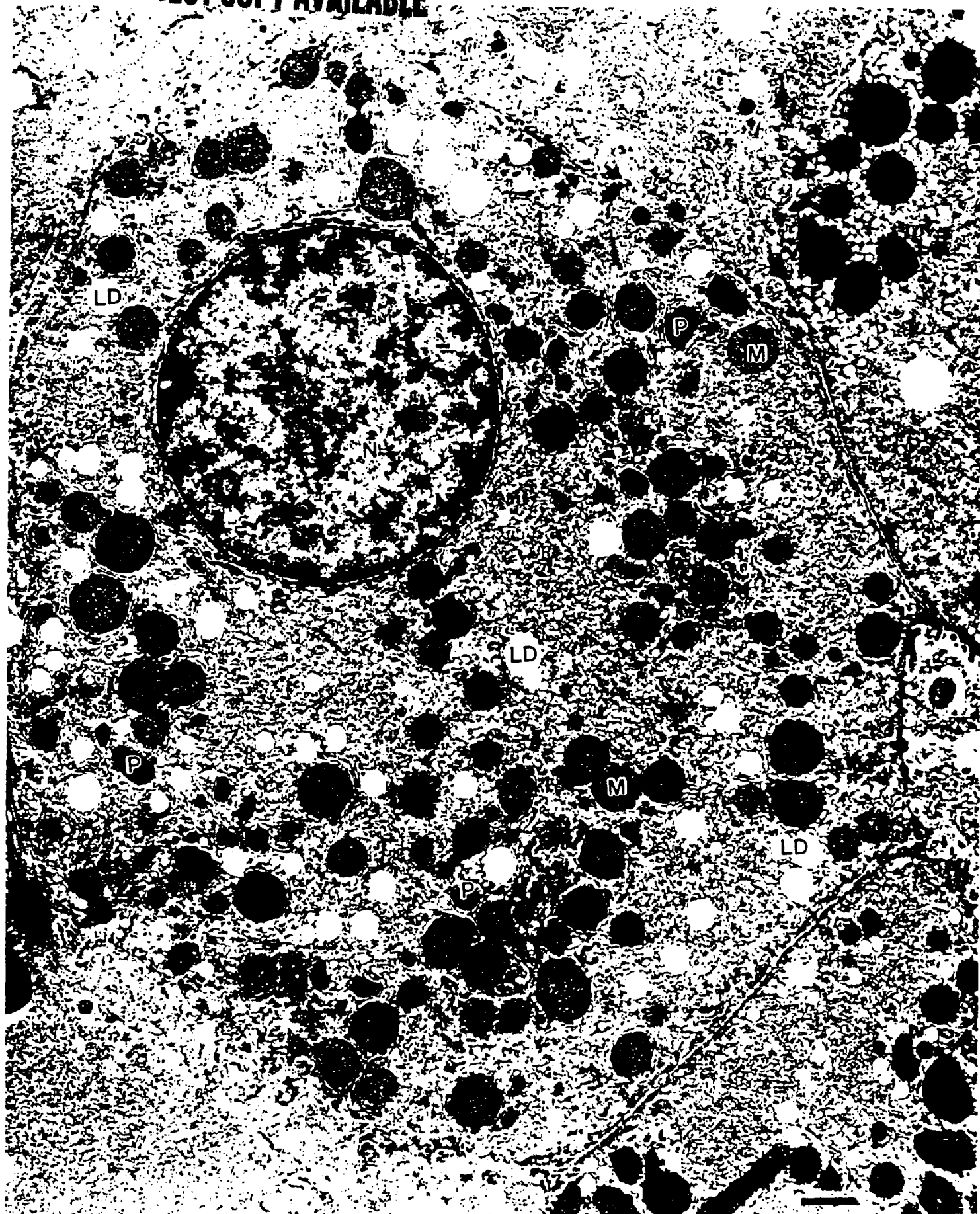
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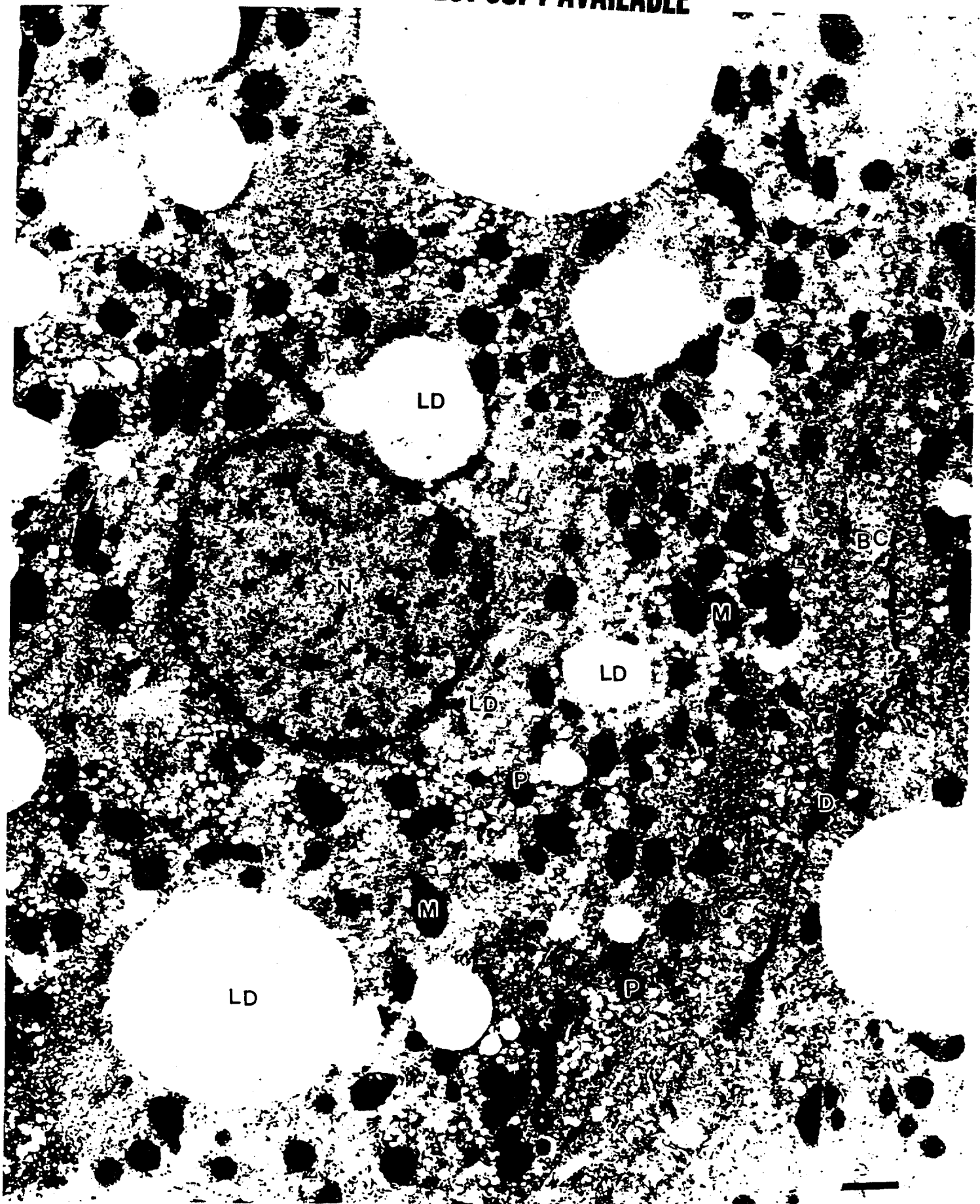


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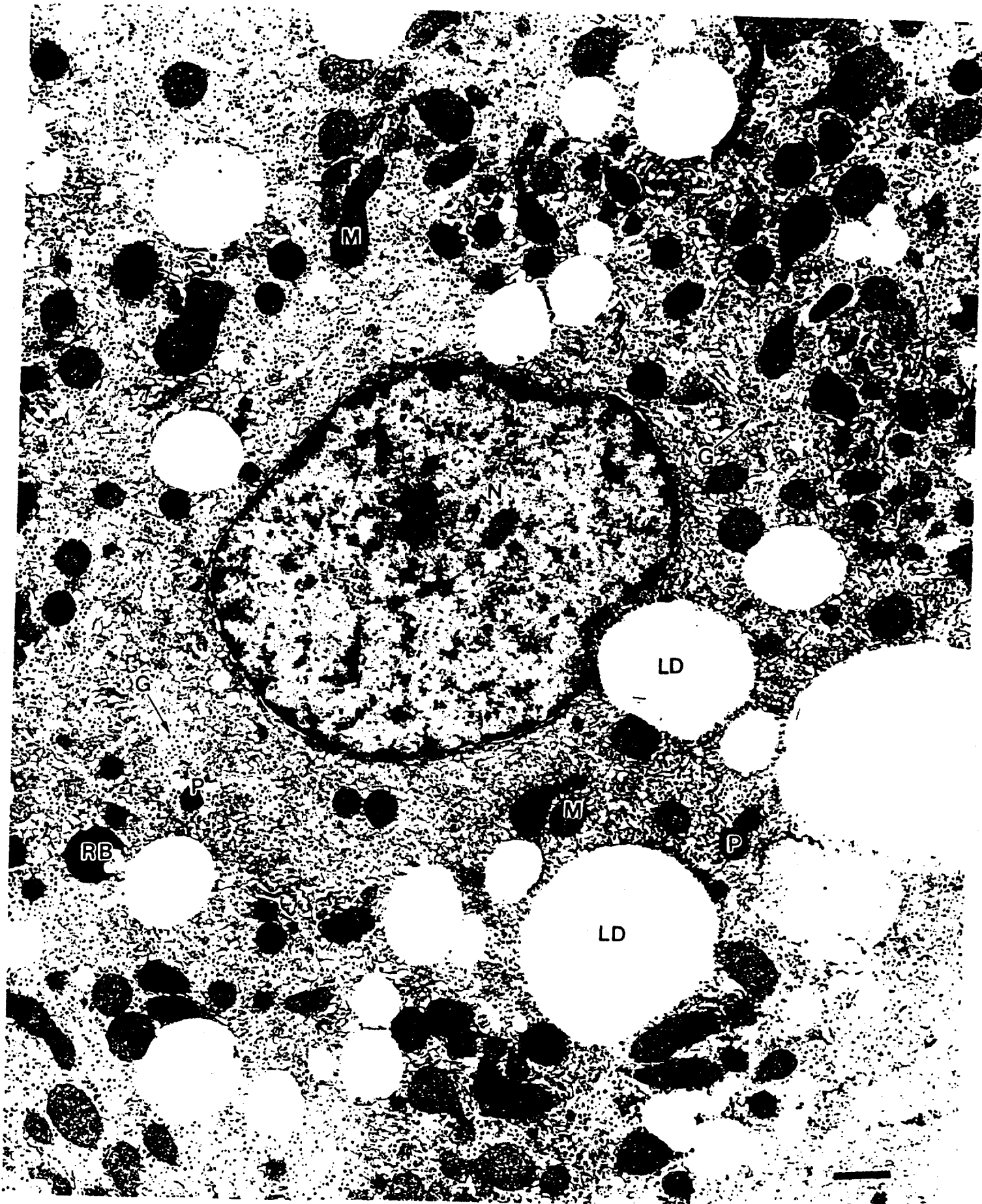




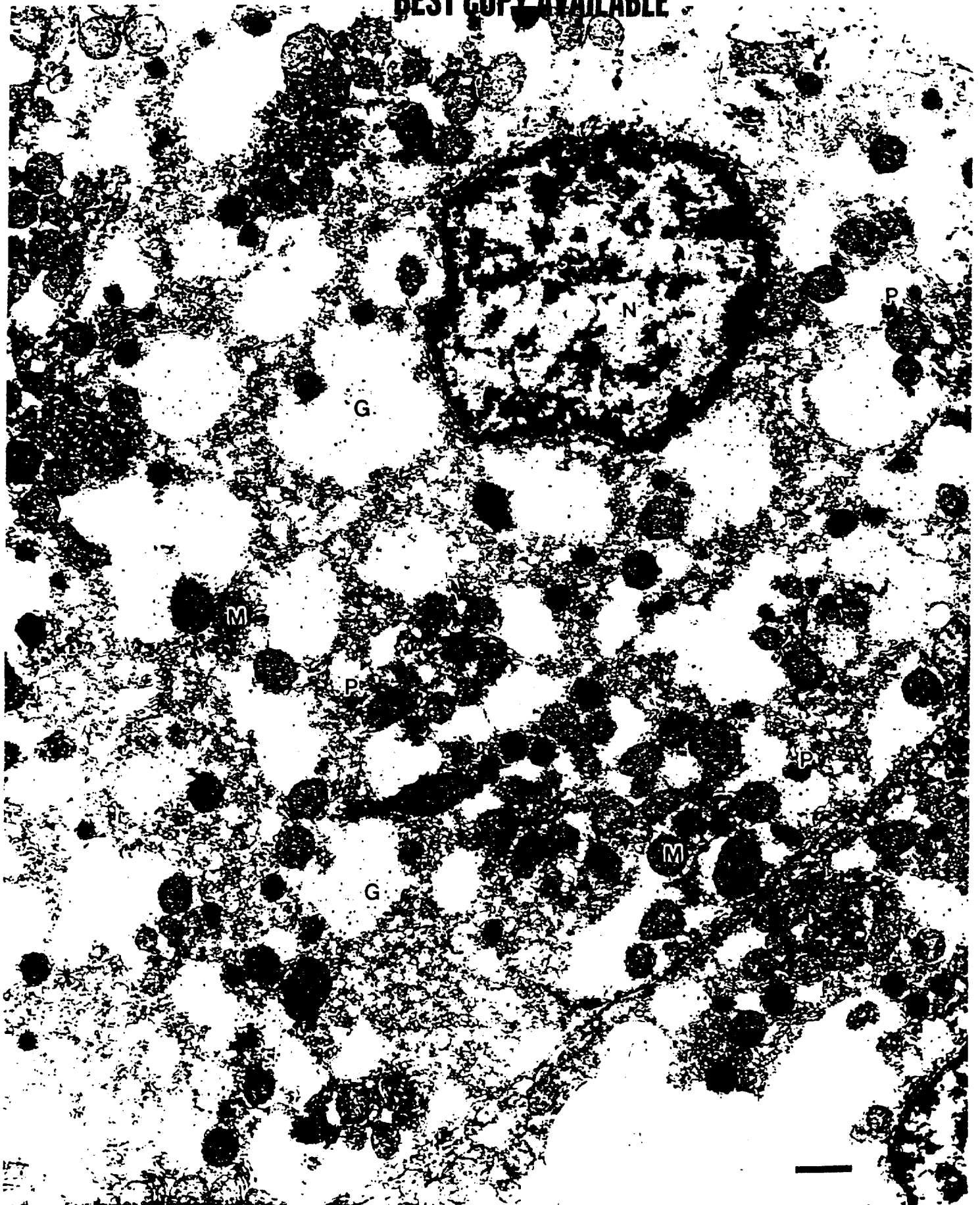
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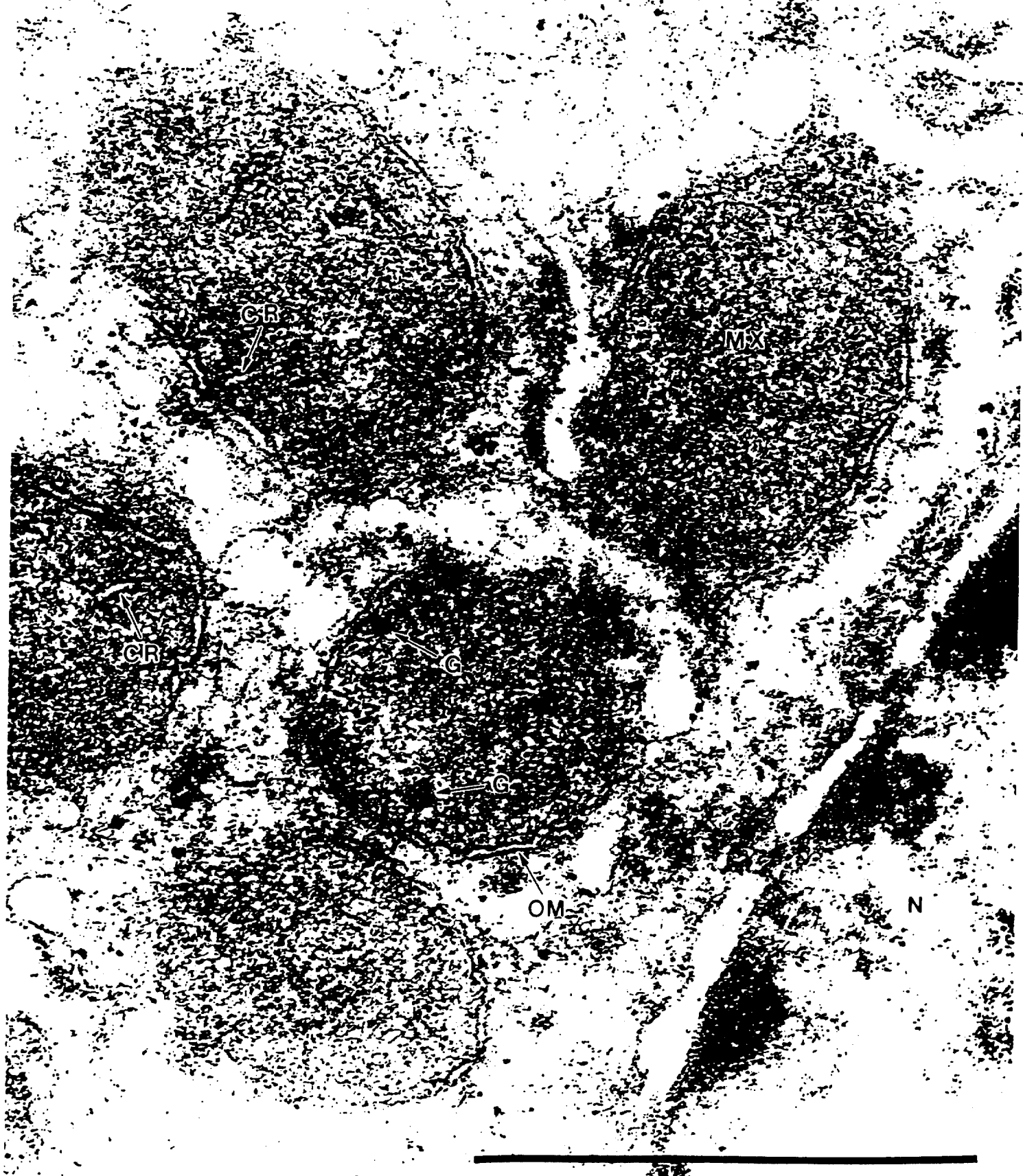
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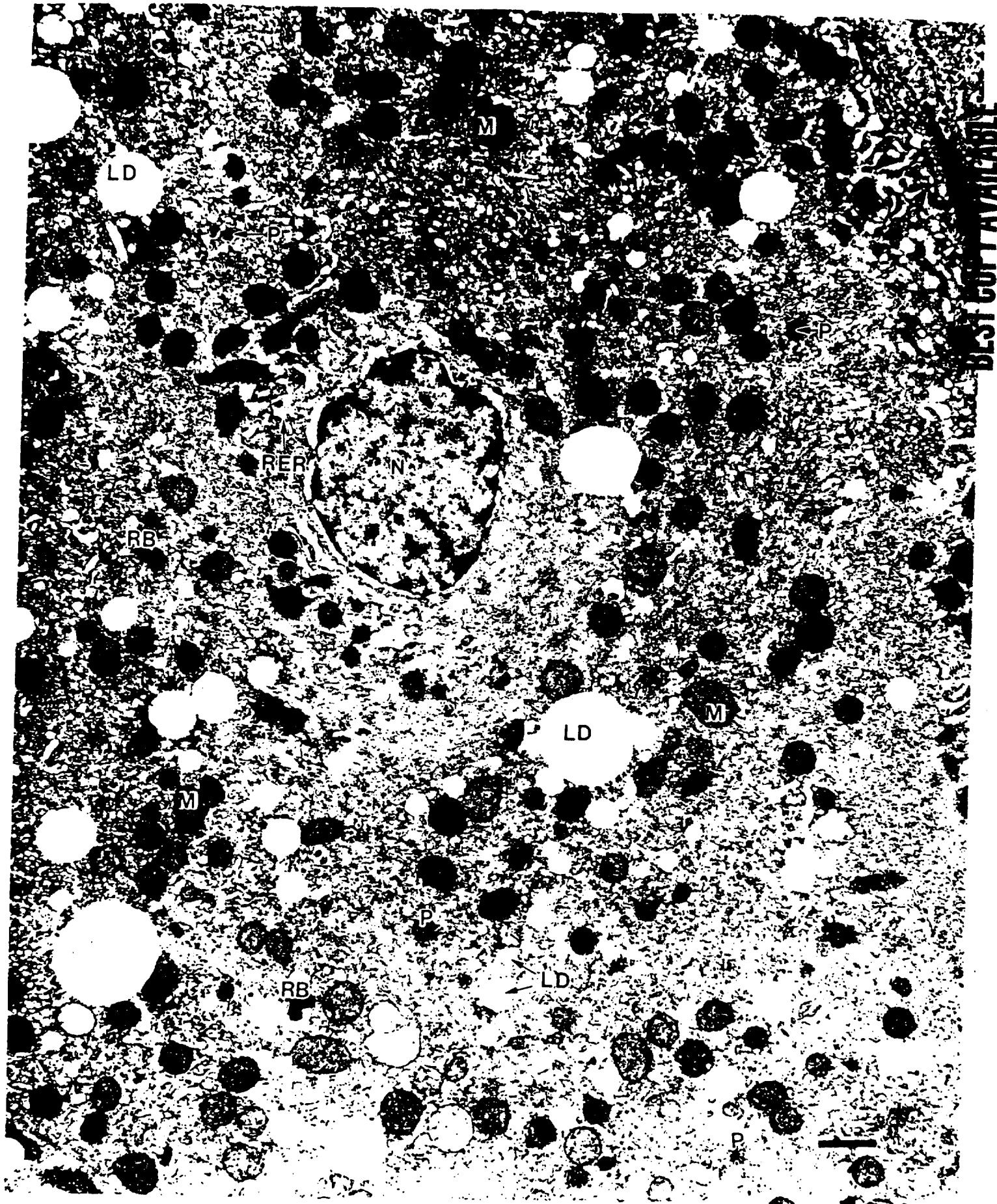


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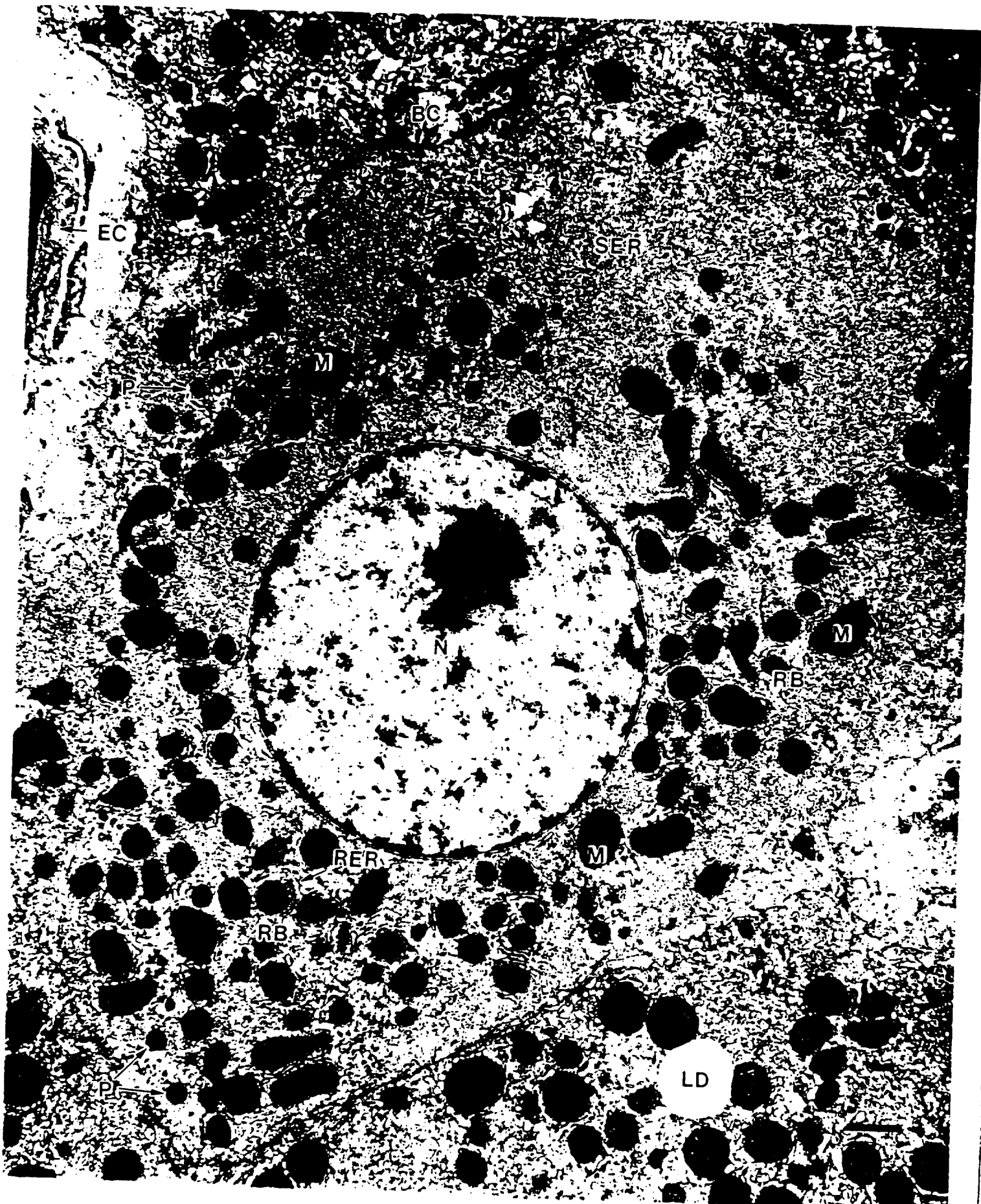


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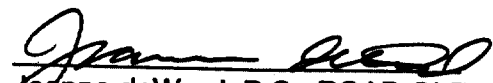
IV. Quality Assurance Statement



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>
4/1, 2/99	Data Audit	4/2/99
4/2/99	Draft Report Audit	4/2/99
7/13/99	Audit of Data from Additional Groups	7/13/99
7/13/99	Final Report Audit	7/13/99


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

7/13/99
Date

Sponsor: 3M Corporate Toxicology

Test Article: PFOS

Study Number: 6329-223, PAI Study EM-99.76

003674