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Effects of O2 Breathing on the Diaphragm's and the Lungs' Ultra-structural Pathological Changes in Relation to Free Radicals Accumulation

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BACKGROUND

Positive Side
Oxygen Therapeutic Clinical Applications

- **Respiratory insufficiency**
- **Myocardial infraction**
- **Ischemic brain damage**
- **Hypothyroidism**
- **Liver diseases**
- **Pancreatitis**
- **Tissue oxygenation, in hypoxic related and age-related diseases.**
- **Following surgery to reduce infection**

Negative Side

Free Radical (FR) and Reactive Oxygen Species

- Free Radical (*FR) is a molecule with unpaired electron (* = unpaired e)
  - O2 molecule is made of two atoms of oxygen has 16 electron, if one electron is added to it, the electron will be unpaired and it becomes ***superoxide radical,***

$$O_2 + e \longrightarrow {}^*O_2^-$$

  - During the partial reduction of reactive oxygen species (ROS) are produced.

- **Reactive oxygen Species (ROS) are highly reactive substances.**
  - **Examples are: Hydroxyl radical (*OH) hydrogen peroxide (H_2O_2), peroxynitrite anion ($^*ONOO^-$).**

Partial Reduction of Oxygen
ROS During Metabolism in the Mitochondria

$$O_2 + e \longrightarrow {}^*O_2^-$$

$$O_2\text{-} + e \;\; 2H \longrightarrow H_2O_2$$

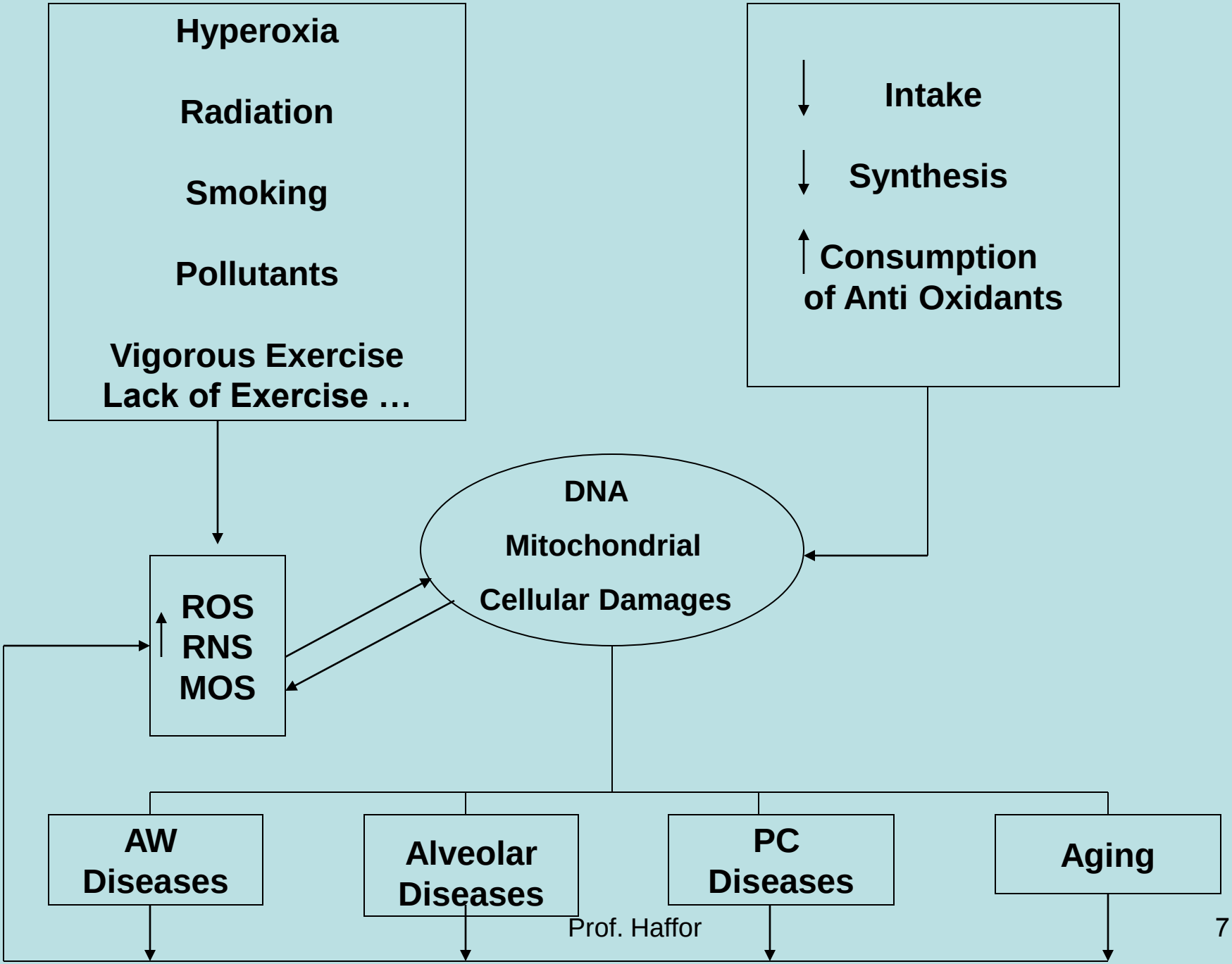
$$H_2O_2 + e \longrightarrow OH\text{-} + {}^*OH$$

$$NO + {}^*O2^- \longrightarrow {}^*ONOO^-$$

ROS are Elevated During Inflammation

Inflammatory Lungs Diseases in:

- **Airways:**
  - **Allergy, Asthma, COPD (Emphysema & Chronic Bronchitis), Cystic Fibrosis.**
- **Alveoli:**
  - **Alveolar Edema, TB, Lung Cancer, ARDS, Asbestosis. Perhaps Emphysema too.!**
- **Pulmonary Capillary:**
  - **Interstitial Edema, Sarcoidosis.**


Hyperoxia
Radiation
Smoking
Pollutants
Vigorous Exercise
Lack of Exercise …
↓ Intake
↓ Synthesis
↑ Consumption of Anti Oxidants
DNA
Mitochondrial
Cellular Damages
↑ ROS
RNS
MOS
AW Diseases
Alveolar Diseases
PC Diseases
Aging

Why is the diaphragm produce more ROS?

– High surface to volume ratio, hence high metabolic rate and elevated ROS production.

– *NO radicals are elevated for diaphragmatic because blood vessels dilation.

Purpose of the Study

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- The purpose of the present study was to examine the effect of **Oxygen Breathing** on the ultrastructural pathological alterations in the diaphragm and the lungs in relation to free radicals accumulations.

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METHODS

I- Experimental Design

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- 24 **Wiser Albino Rats (*Rattus Nervigicus), with an average weigh of 190-210 Gm and mean age 4.5 months*** were randomly assigned into two groups; control (C) and oxygen breathing (OB); 12 animals each.
  - Animals of the control group were kept at the same laboratory and dietary conditions.
  - Animals of the Oxygen breathing group were exposed to (100%O_2, medical Grade) for 72 hrs.

HYPEROXIA EXPOSURE

II. FR Measurement and Test Principle

- Free radical was measured, using the d-ROM-2 (FRAS-II, Italy)
- The test measures the levels of hydroperoxides (R-OOH) which are generated by peroxidation of biological compounds; lipid, amino acids, nucleic acids; according to **Fenton's Reaction** as follows:

$$H_2O_2 + Fe^{++} = *OH + OH^- + Fe^{++}$$

- This test is based on the principle of the ability of hydrogen peroxides to generate free radicals after reacting with some transitional metals (Fe2+ / N, N-diethyl-phenylendiamine).

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RESULTS

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I. Free Radical Findings

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1) Descriptive Findings: Free Radical

Measure	N	Mean (CARR)	Std. Deviation	Std. Error
Serum of the control (SC)	12	221.83	36.24872	10.46410
Serum of oxygen breathing (SOB)	12	328.00	67.69182	19.54094
Diaphragm control (DC)	12	249.75	48.82459	14.09445
Diaphragm oxygen breathing (DOB)	12	386.17	43.65741	12.60281
Lung control (LC)	12	183.67	28.25962	8.15785
Lungs oxygen breathing (LOB)	12	256.33	65.37491	18.87211

*** 1 CARR = 0.08 g H_2O_2 /dL**

2) Inferential Findings
One Way Analysis of Variance

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	326681.96	5	65336.39	25.69*	.000
Within Groups	167842.917	66	2543.07		
Total	494524.875	71			

*P<0.05

LSD Multiple Comparisons

Measure	N	Mean	Absolute Means Difference SC vs SOB	Absolute Means Difference DC vs DOB	Absolute Means Difference LC vs LOB
Serum of the control (SC)	12	221.83			
Serum of oxygen breathing (SOB)	12	328.00	106.17*		
Diaphragm control (DC)	12	249.75			
Diaphragm oxygen breathing (DOB)	12	386.17		136.42*	
Lung control (LC)	12	183.67			
Lungs oxygen breathing (LOB)	12	256.33			72.66*

*p<0.05

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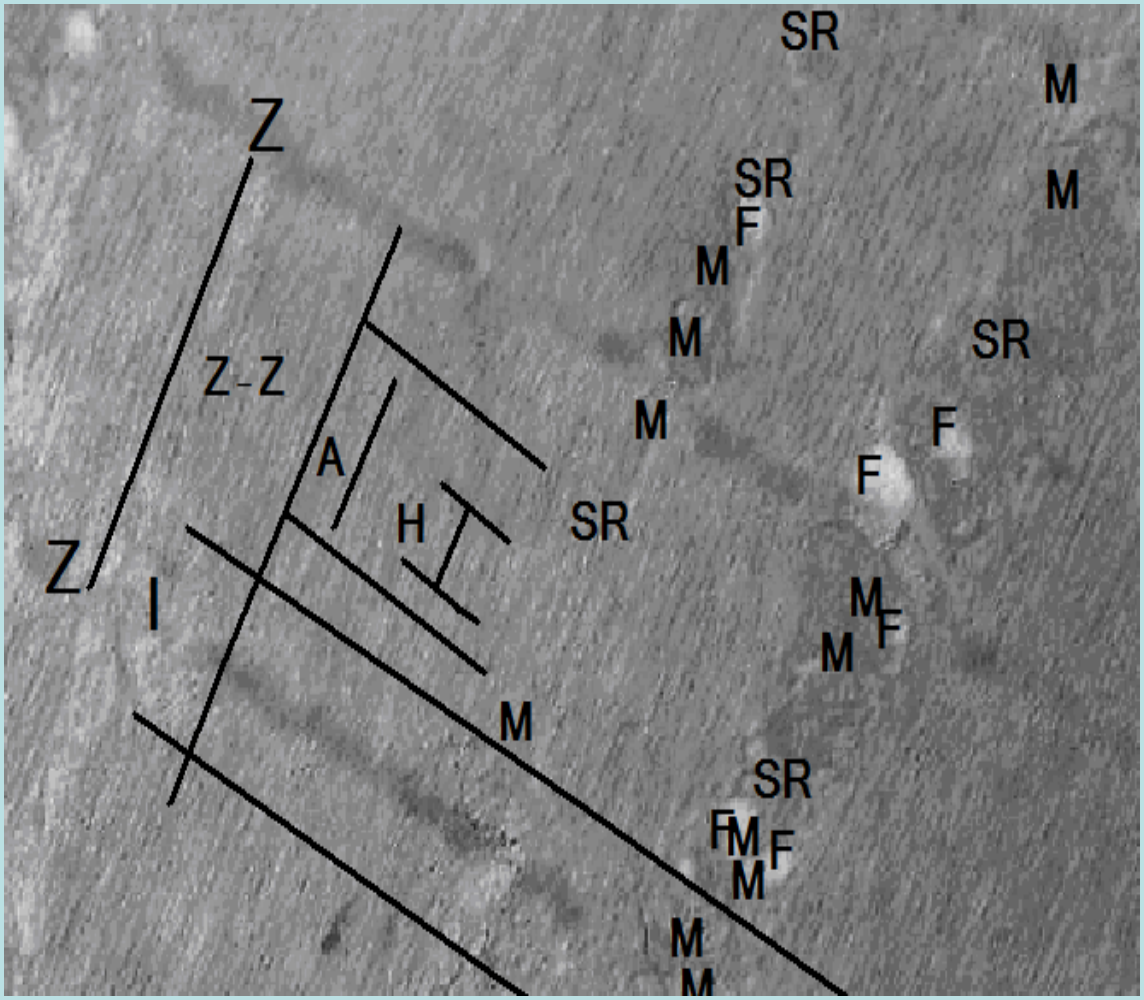
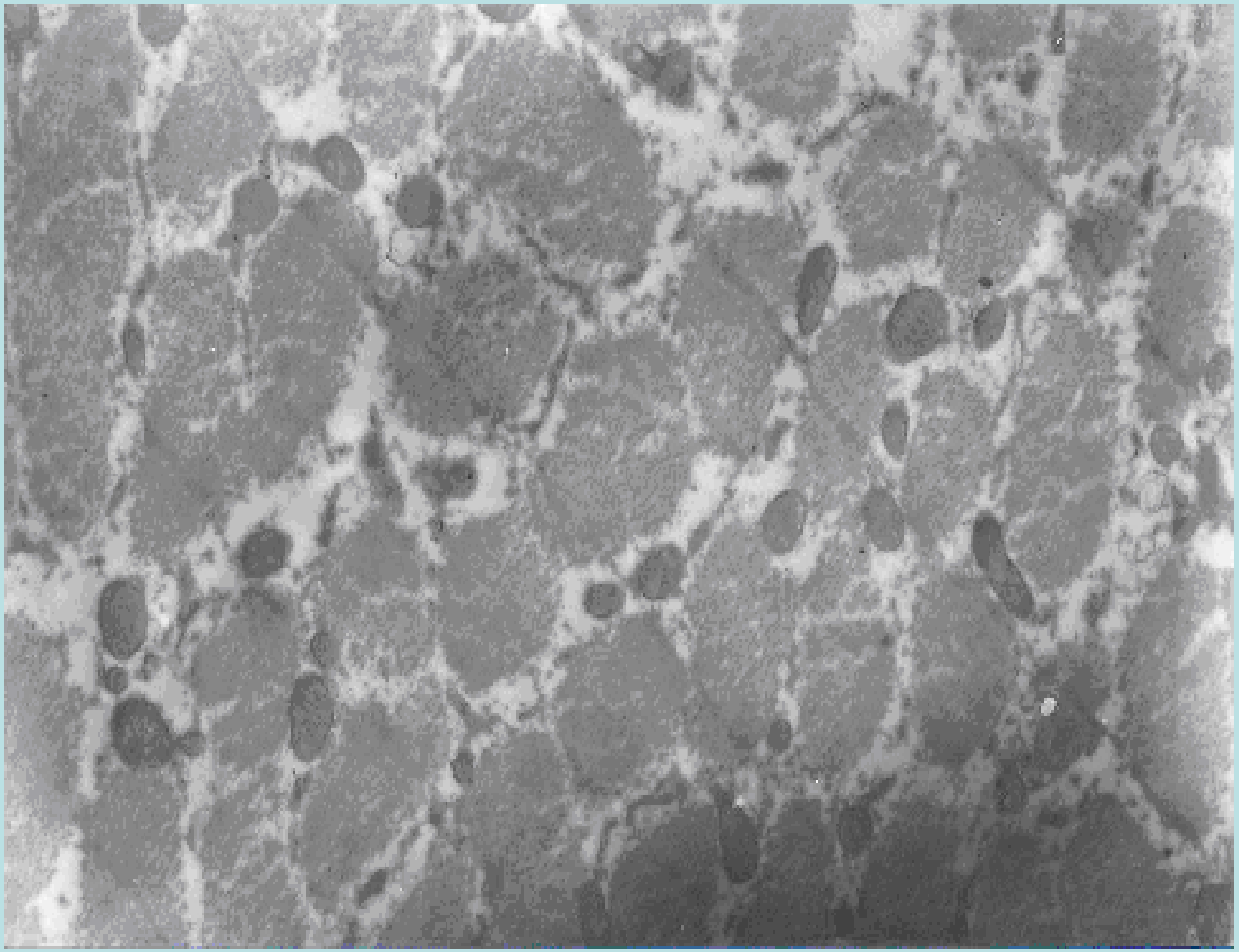
II. Ultrastructure Findgs

1. Diaphragm

Control

- Regularity of banding
- The mitochondria are organized as networks around the I band regions, near the Z-discs.
- SR at around H band and Ca^{2++} is packed into the SR by SERCA.
- Fat droplet are seen
  - Suggesting that metabol energy is derived from oxidative phosphorylatio of fat.





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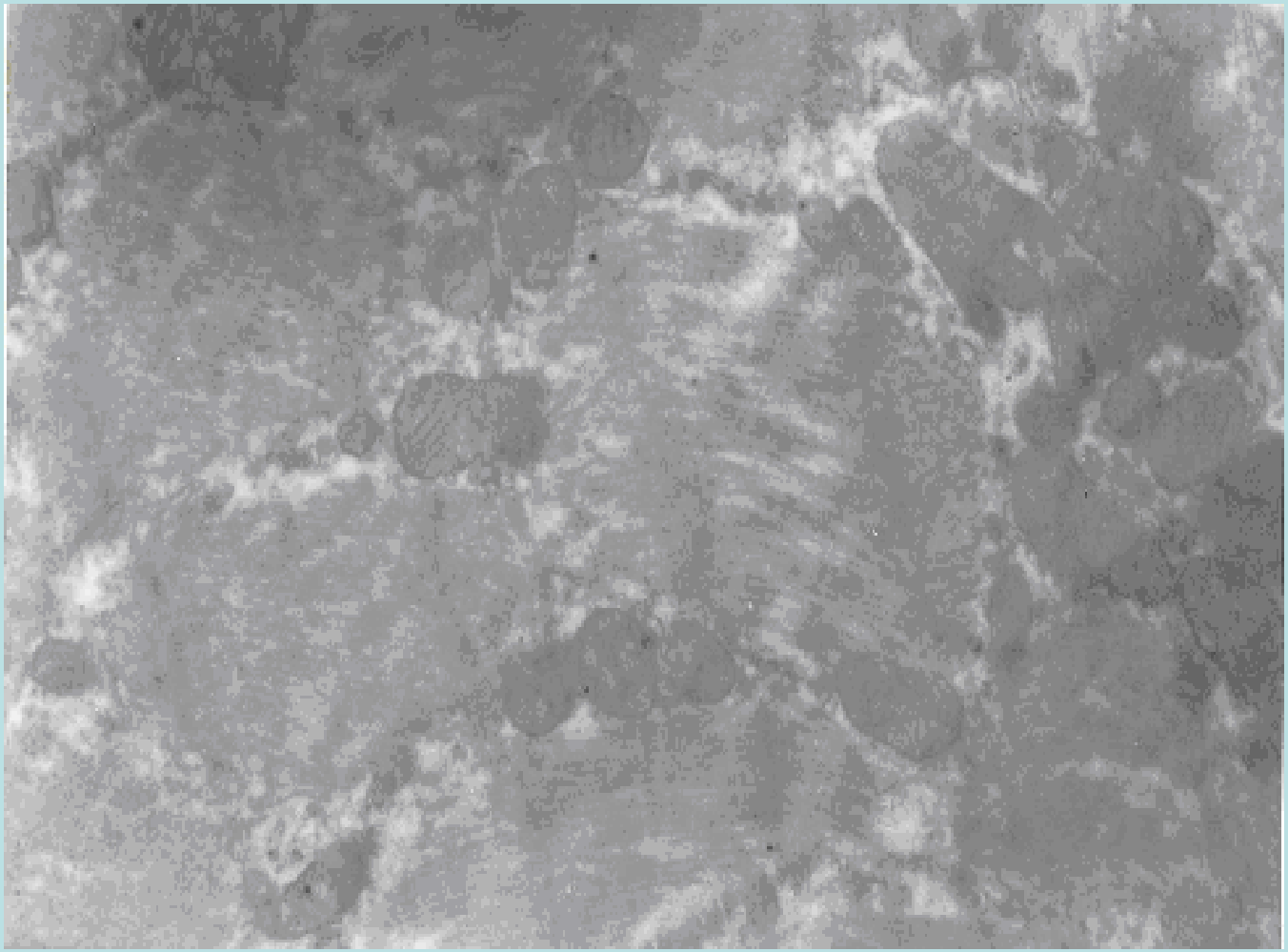
Mitochondria Discontinuity and Disruption Leading to Myolysis



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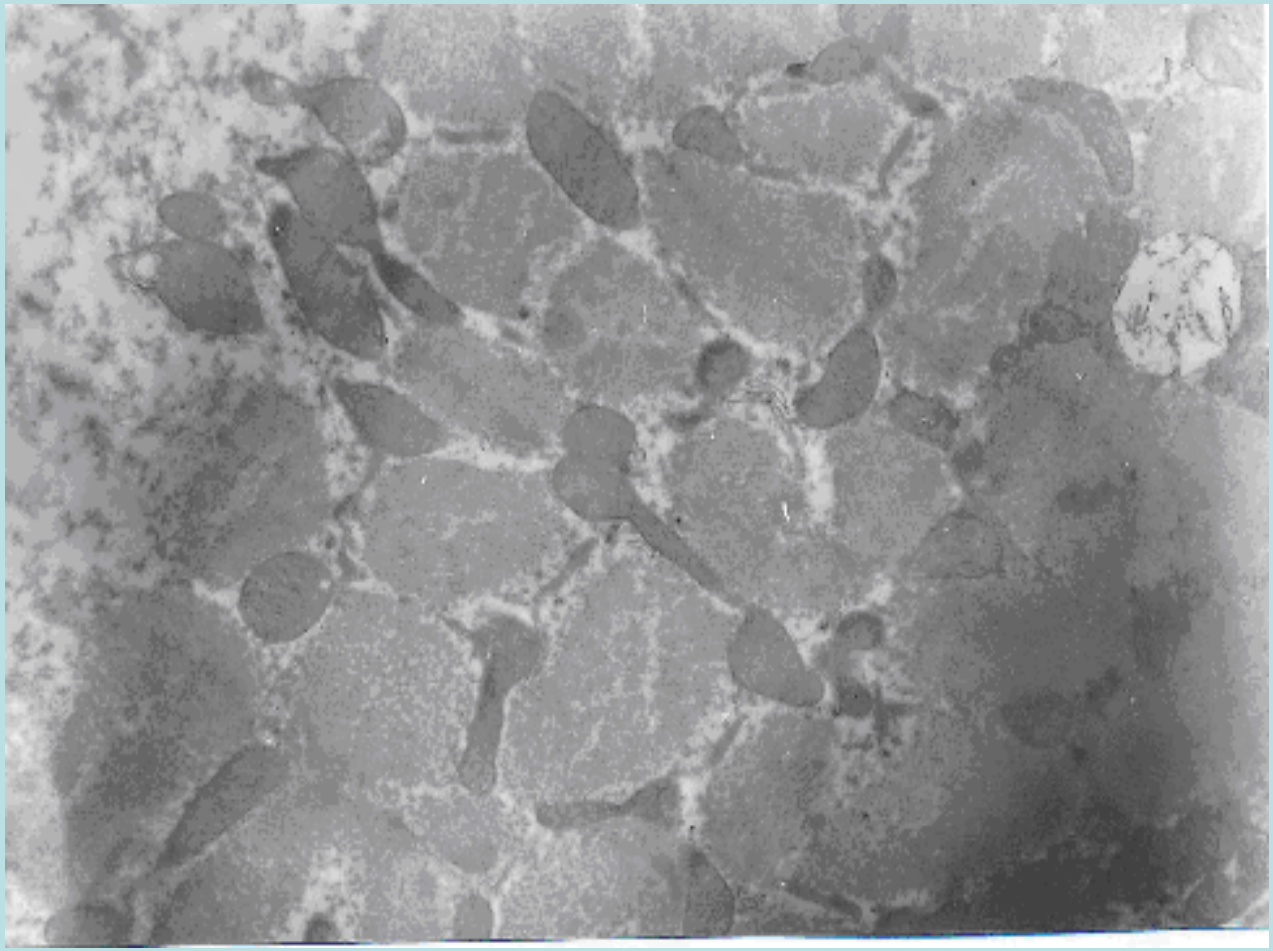
High Magnification

Disorganized microfilaments associated with the absence of banding and hyperplastic mitochondria



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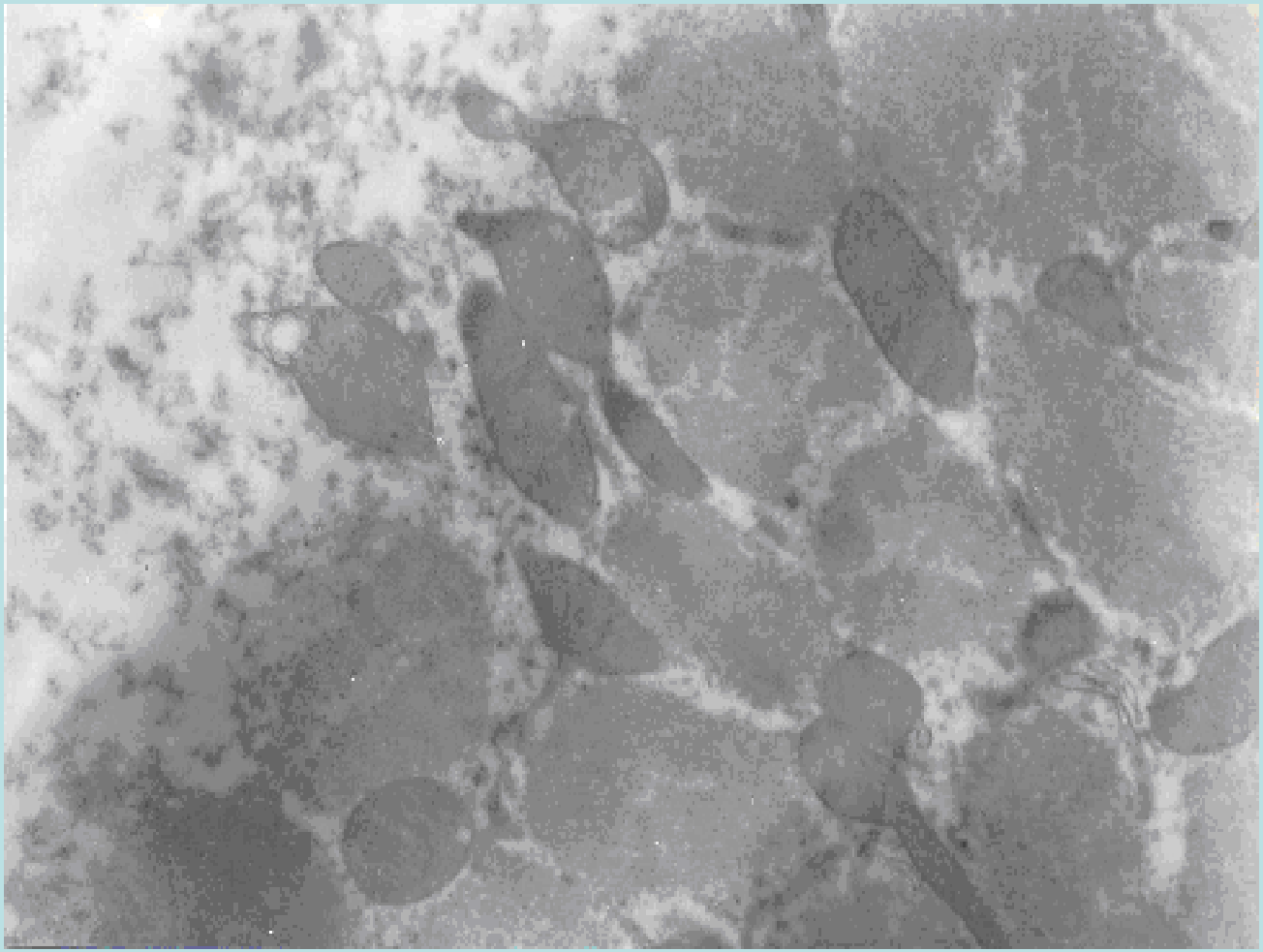
Mitochondria proliferation associated with elongation and apparent constrictions.

Indistinguishable myofibrils banding at the site of mitochondria hyperplasia



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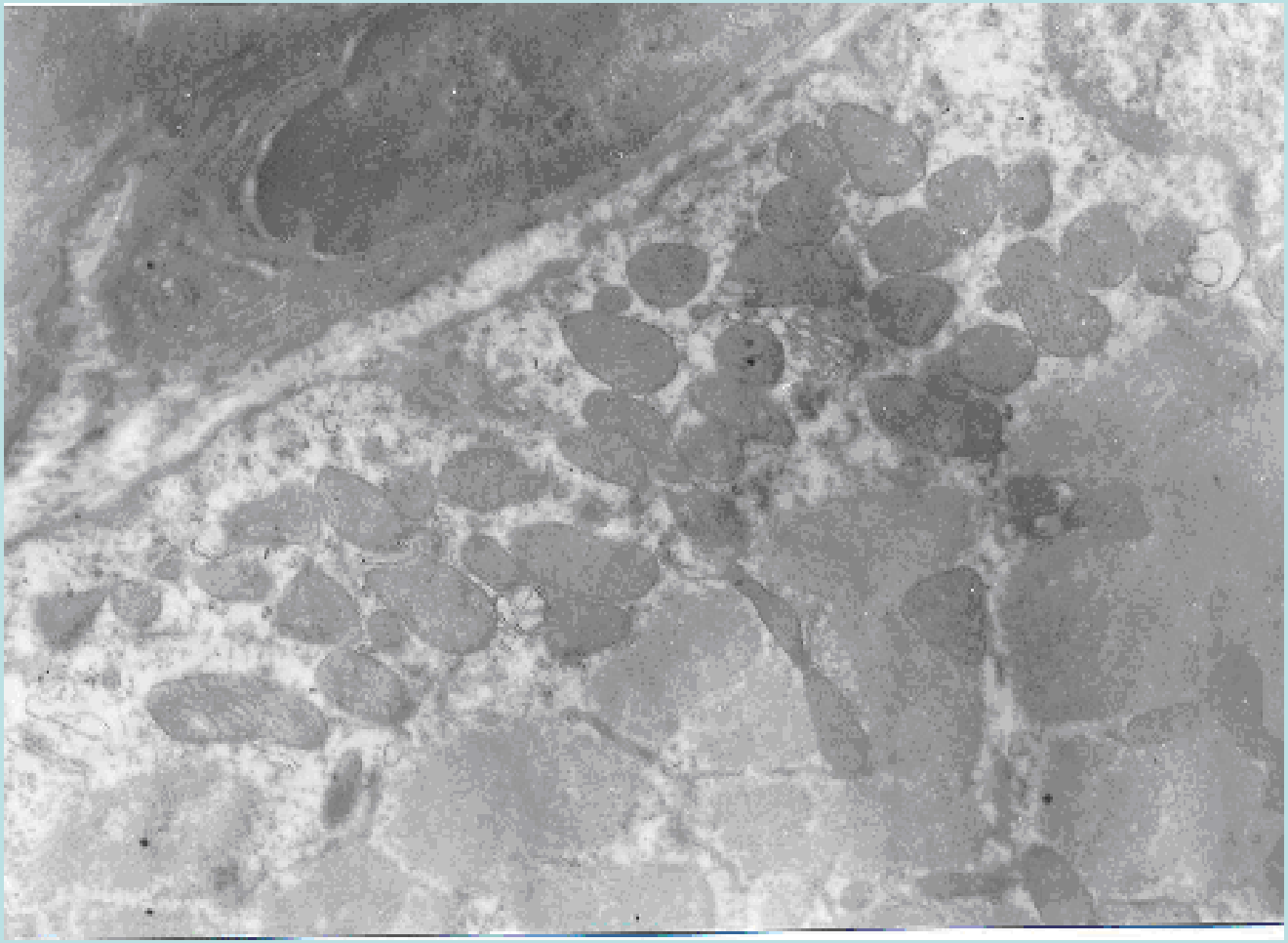
Higher magnification

Some of these proliferated mitochondria exhibit partial lamellation of their inner membranes, implying destruction of the respiratory chain.



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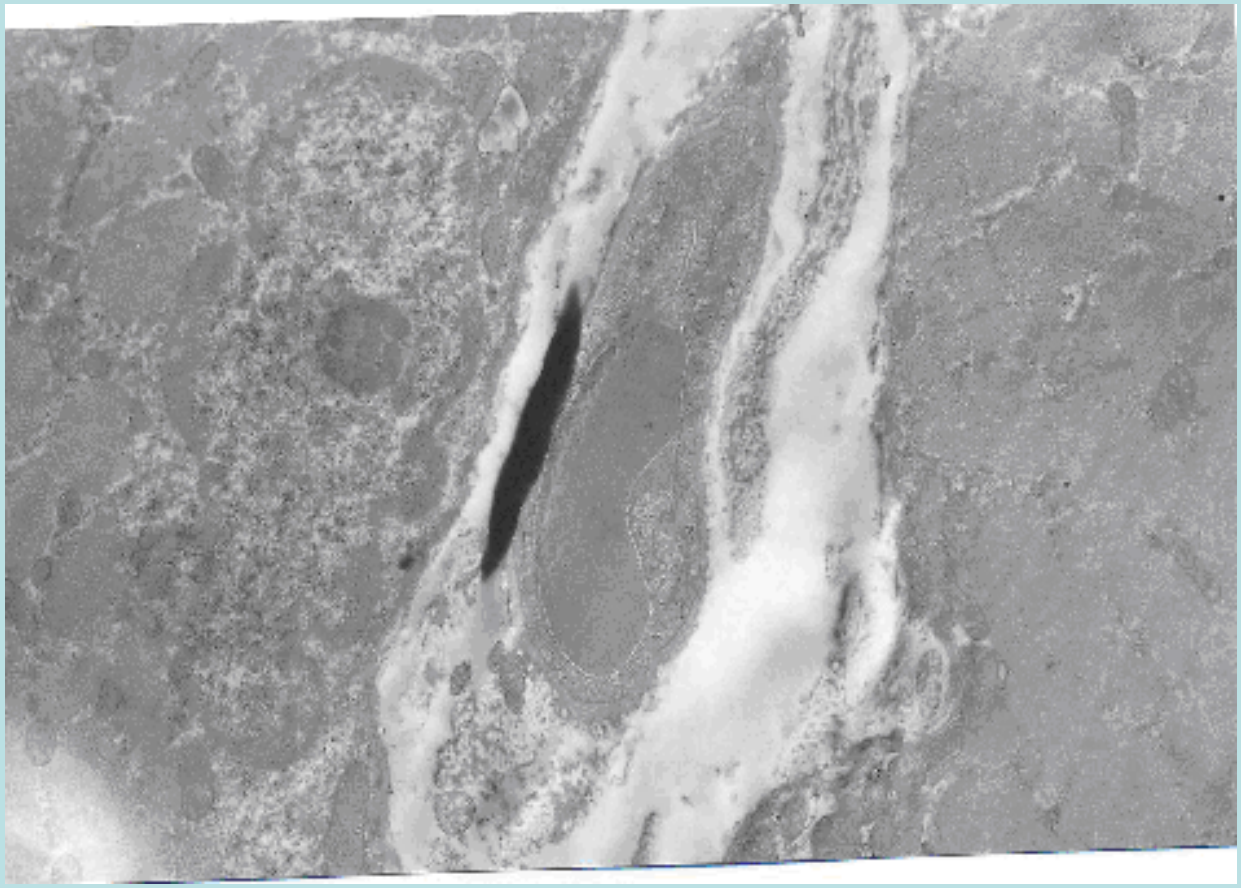
MEP

Mitochondria Hyperplasia nearby the Motor End Plate



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Interstitial blood capillary showing a marked swelling of the endothelial cell which occluded the capillary lumen and organelles damage including Mitochondria and Degenerated Nucleus

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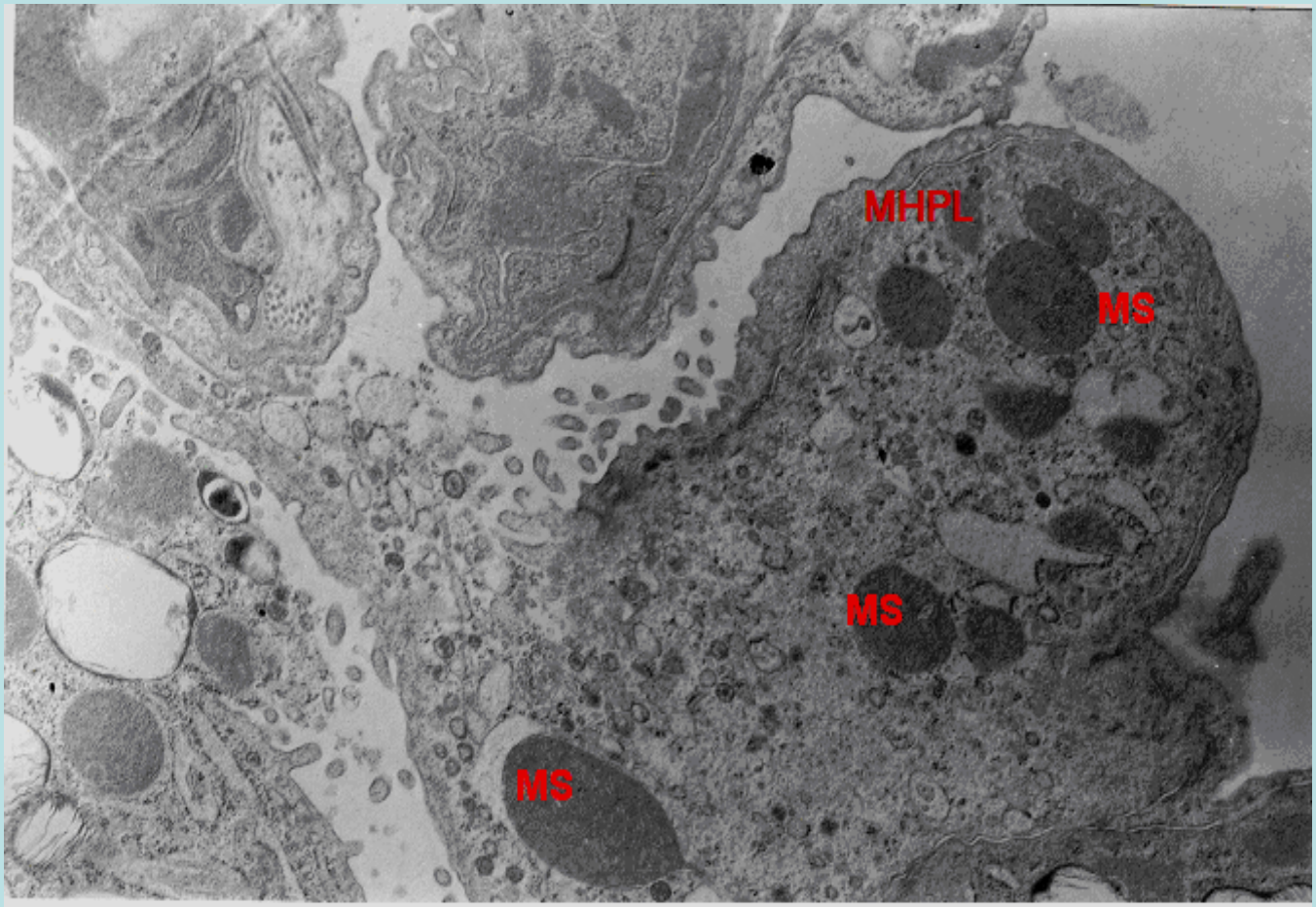
II. Ultrastructure Findgs

2. Lungs



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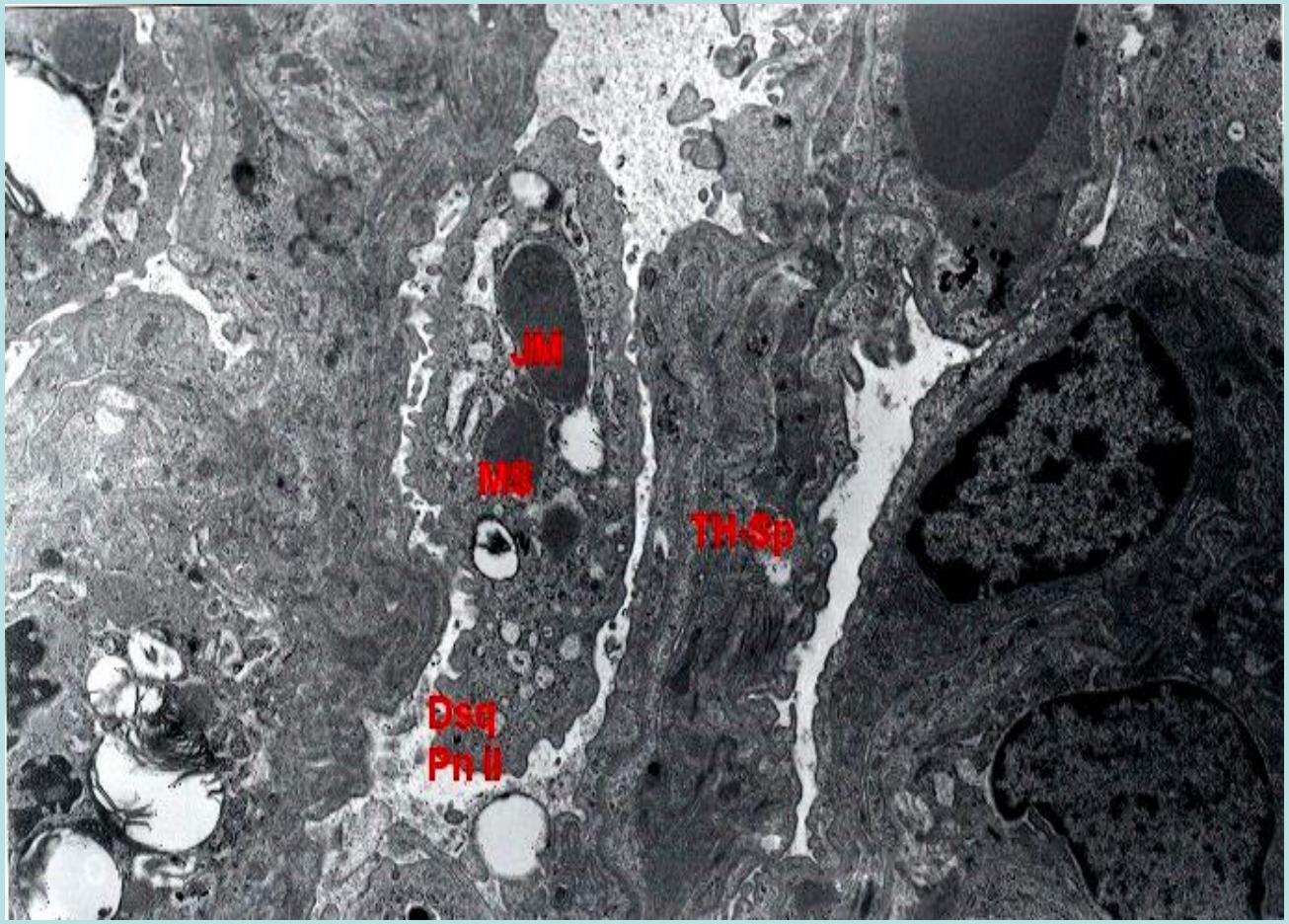
AE-I

Degenerated Alveolar Epithelia Type-I, showing mitochondrial swelling (MS), mitochondria hyperplasia (MHPL), dilated RER; note, cell lost its squmous form and became swollen and projected into the alveolar lumen.



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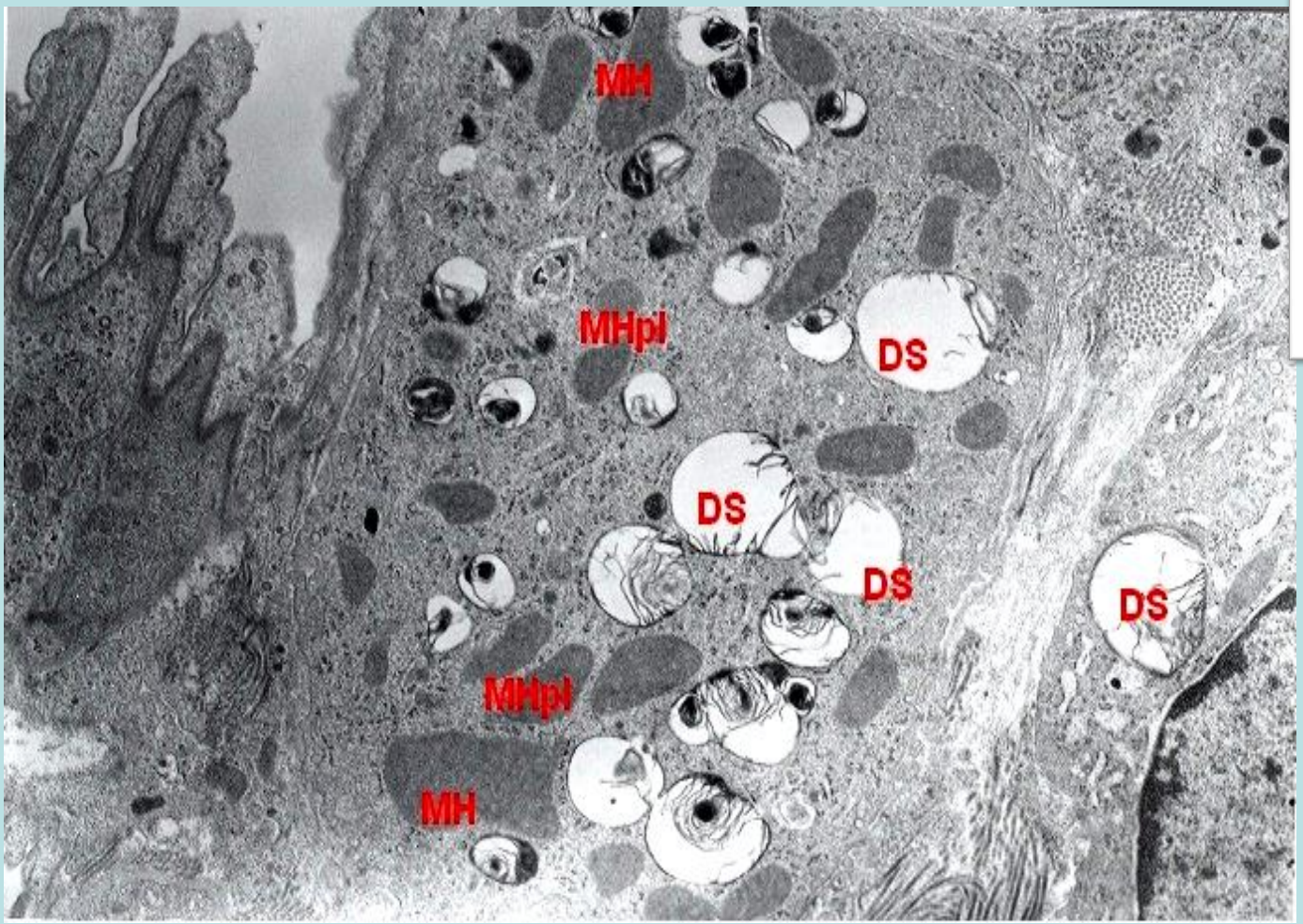
AE-II

Desquamated Alveolar Epithelial-II (DsqPn II), falling in the alveolar lumen; Jiant mitochondria (JM); swelled mitochondria (MS); thickening the alveolar septum (TH-Sp).



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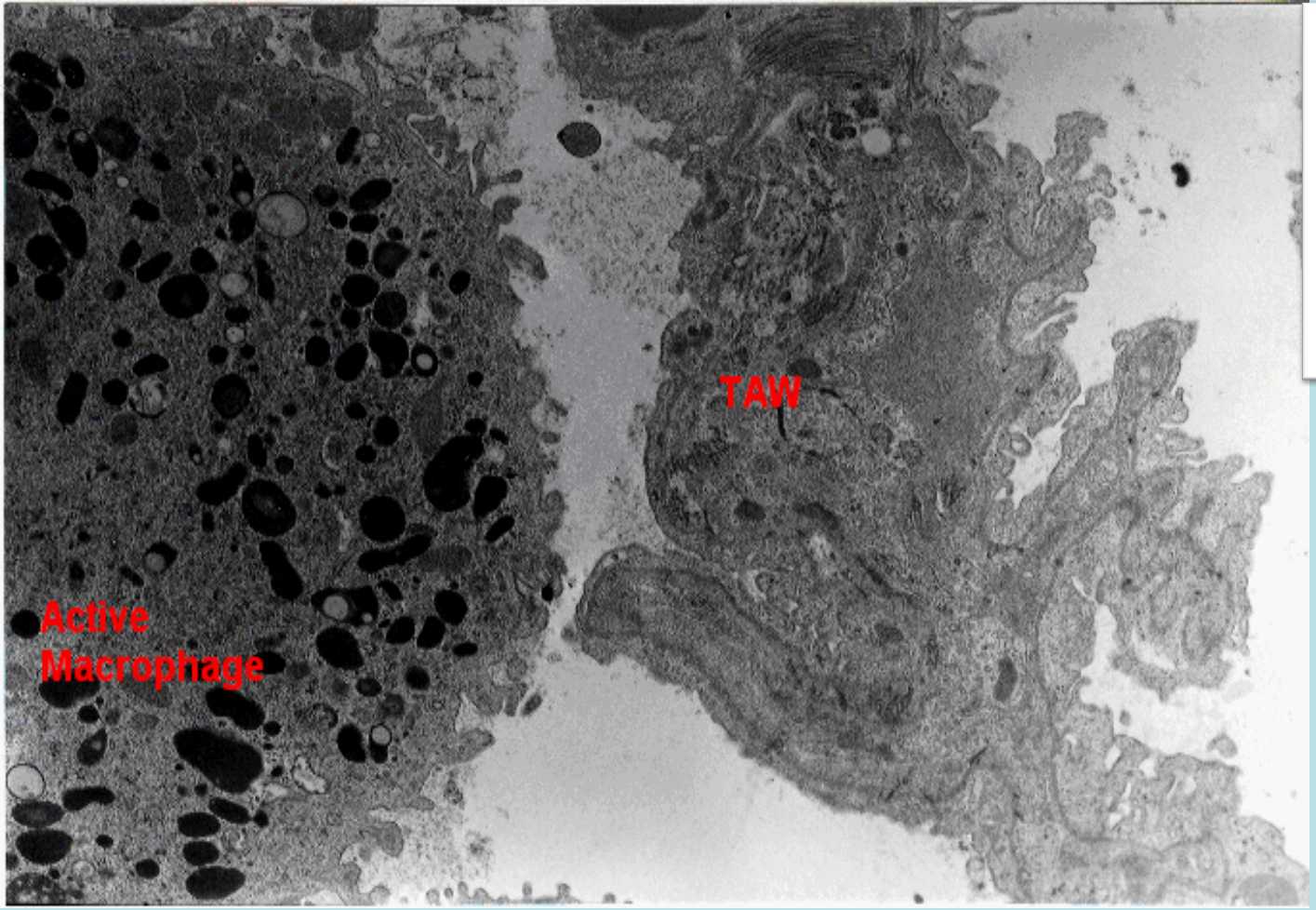
AE-II

AE-Type II showing depletion of the surfactant material (DS) as evident by its observed density; organelle degeneration in terms of mitochondrial hyperplasia and swelling.



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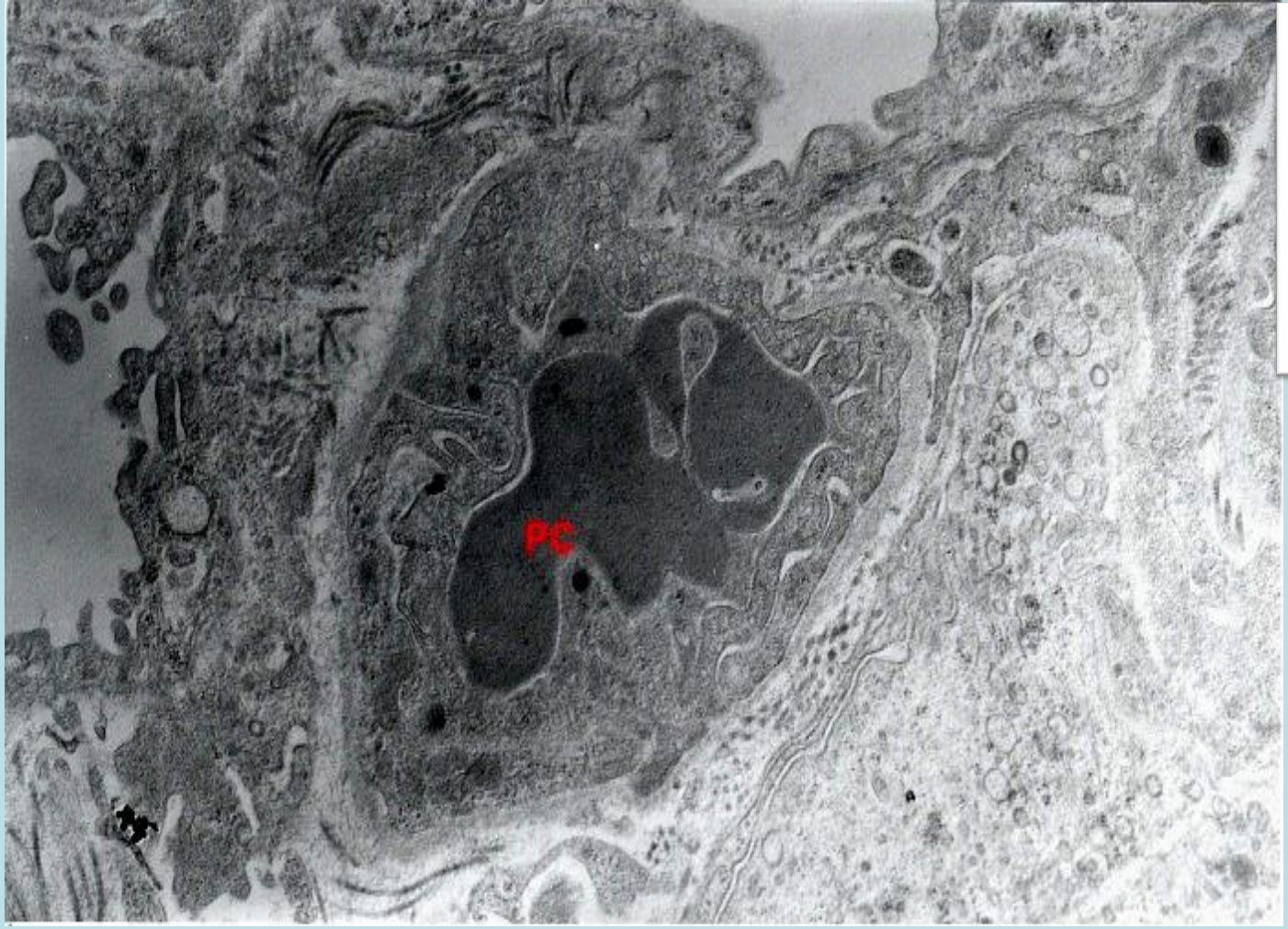
Alveolar Macrophages

Active macrophage laid free in the alveolar lumen, the cell contains secondary lysosomal structures while facing inflamed and thick alveolar wall (TAW).



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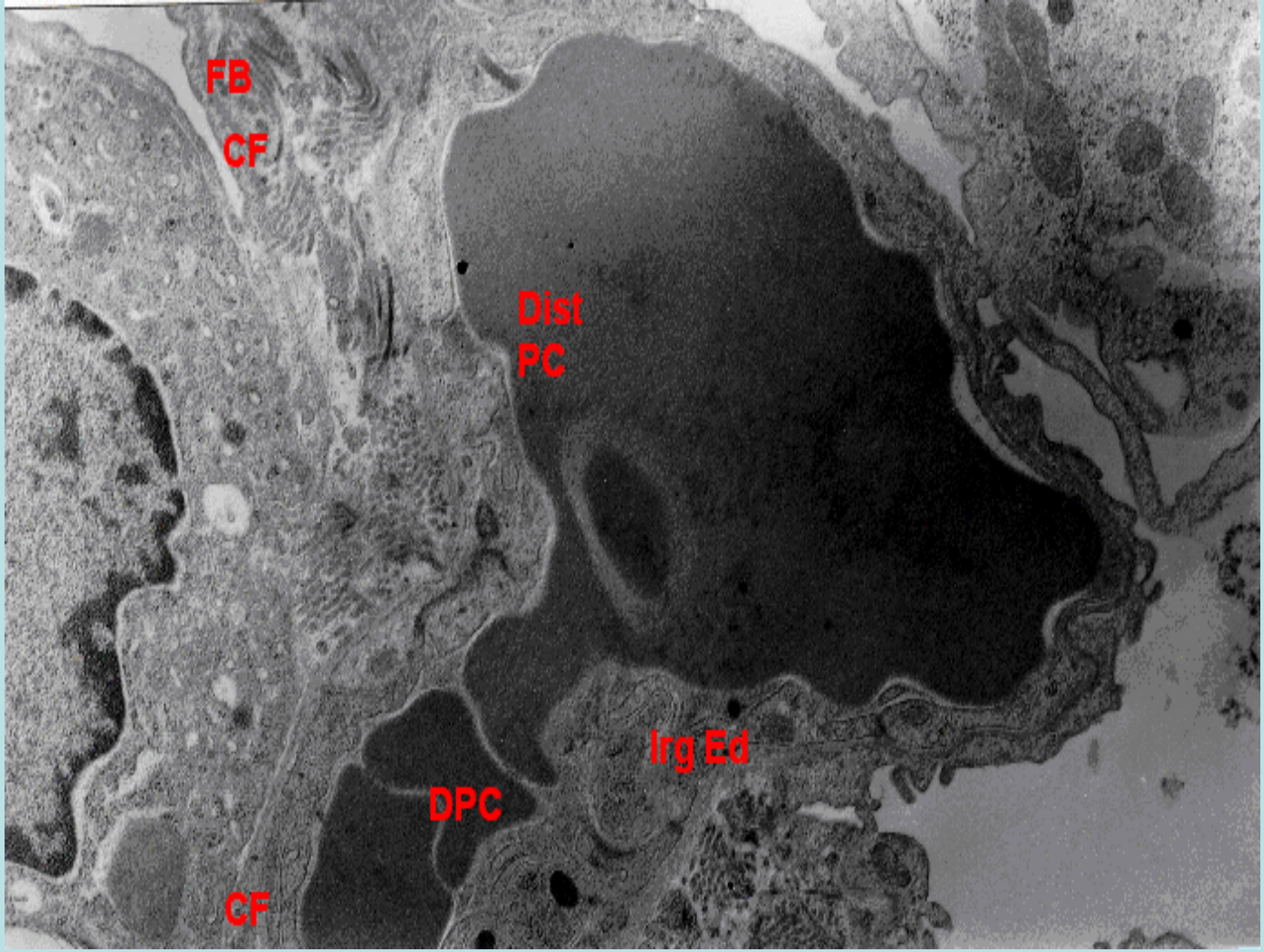
Alveolar Capillary

Alveolar capillary showing projections and blebs extended into its lumen which became irregular and narrower pulmonary capillary (PC).



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Distended Capillary

Distended pulmonary capillary (DistPC), irregular endothelia (Irg Ed), collagen fibers (CF) in the alveolar wall and fibroblast and loss of myofibroblastic structure (FB).

CONCLUSIONS

- Based on the results of the present study it can be concluded that:
  - OB for 72 hr inducted mitochondrial pathological changes that resulted in the buildup of ROS, leading to cellular injury in the nearby tissues, in the diaphragm and the lungs.
  - As cellular and tissue damages (myolysis), involved regions nearby MEP and capillary endothelium, thus OB induced-super oxide was boosted by peroxinitrates radicals (*$ONOO^-$ *ON) from both endothelial cells and nerve ending.

RECOMMENDATIONS

- Oxygen therapeutic clinical applications requires patient's specific protocol to determine the minimum FIO2 that yield the optimal SaO2 and PaO2.

- Oxygen therapeutic clinical applications requires antioxidants supplements.

END!

Special Thanks and Appreciation for Dr. Mohammed Gamal, Lecturer in the College of Medicine for his help to produce this Video