RESULTS

For the purpose of simplicity the results were classified into :

1. Electron microscopical results .
2. Histopathological results .
3. Statistical results .

I.ELECTRON MICROSCOPICAL RESULTS

Effects of 10 minutes exposure to the CO_2 laser smoke (1st group)

Exposure of the rabbits to the CO_2 laser smoke for 10 minutes will lead to the following changes :

a-Changes in the nucleus :

No significant changes in this group.

b-Cellular changes :

1- There are massive invasion of lymphocytes inbetween the lining epithelial cells of the trachea (Fig.8).

2- Disturbed epithelial cells & loss of cilia in the region of heavy lymphocytic infiltration (Fig.8).

3- Subepithelial propial edema with swollen collagenic fibrils

4- Completely destructed cilia with loss of basal bodies (Fig.9).

5- The mitochondria are numerous, destructed and have an electron dense character (Fig.9).

6- Macrophage with cytoplasm having phagocytic bodies at various stages of deterioration. (Fig.15)

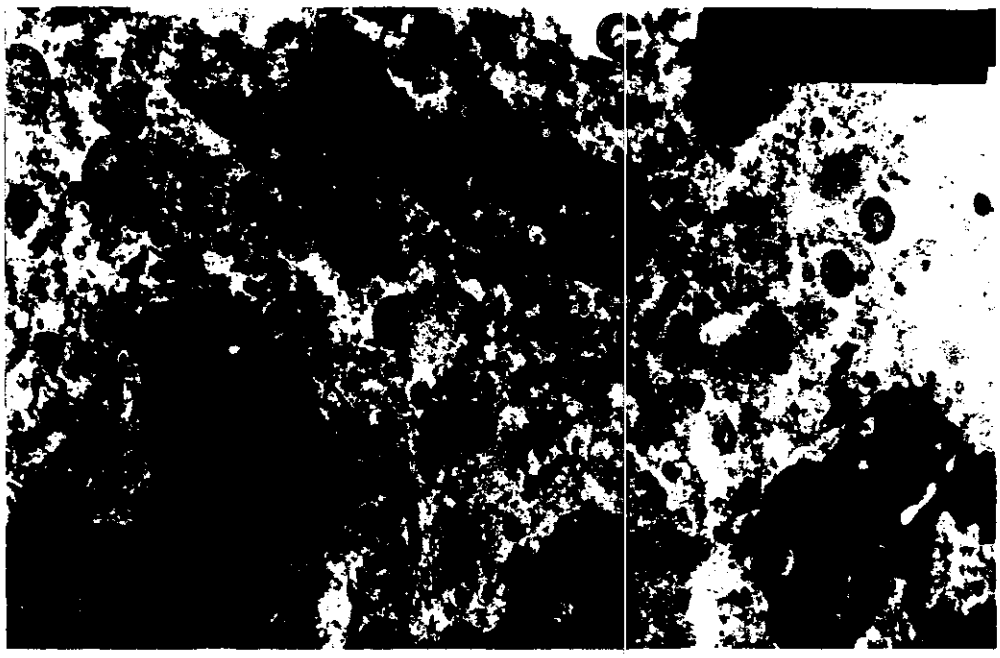


Fig. (8): An electron micrograph of the trachea of rabbit after 10 min. exposure showing massive invasion of lymphocytes (arrows), disturbed epithelium:(D), loss of cilia(C),nucleus:(n).(X6,700)

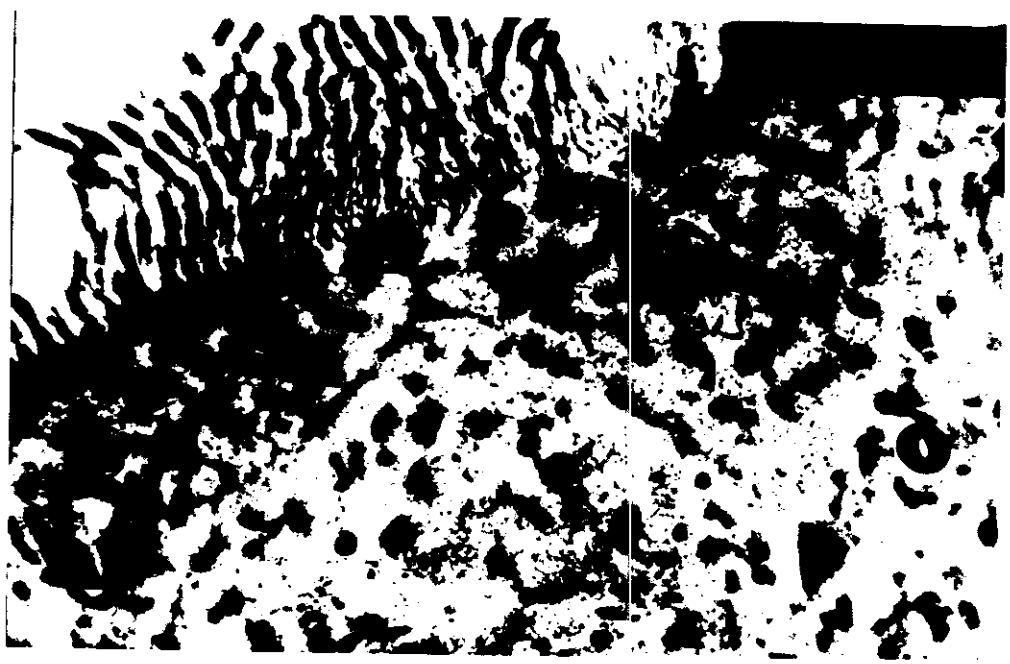


Fig. (9): An electron micrograph of the trachea of rabbit after 10min. exposure showing an intact epith. cell (A), with long normal cilia (C), other cell (O), have completely destructed cilia with loss of basal bodies (D), mitochondria (M) are numerous with electron dense character. (X8,000)

Effects of 20 minutes exposure to the CO_2 laser smoke (2nd group)

Exposure of the rabbits to the CO_2 laser smoke for 20 minutes will lead to the following changes.

1- Tracheal changes :

a- Changes in the nucleus :

Darkly stained pyknotic nucleus as seen in (Fig.10)

b-Cellular changes :

1-Disturbed epithelial cells with destructed cytoplasmic organelles including endoplasmic reticulum & mitochondria leaving scattered disturbed substance. (Fig.10)

2-An apparent intercellular irregular spaces. (Fig.10)

3-Loss of microvilli and apical cilia of the lining epithelial cells with destruction and irregularity of their apical borders. (Fig.11)

2- Bronchopulmonary changes :

a- Changes in the nucleus :

- Nuclear vacuolation in a pneumocyte type II. (Fig. 12)

b-Cellular changes :

1-Numerous oesinophilia in subepithelia propria & phagocytic vacuoles.

2-A Great alveolar cell (pneumocyte II) having a progressive stage of degeneration represented by complete destruction of most of mitochondria & numerous cytoplasmic vacuoles with homogenous lamellated bodies and destructed endoplasmic reticulum. (Fig.12)

Fig. (10) : An electron micrograph of the trachea of rabbit after 20min. exposure showing intercellular irregular spaces (I), normal nucleus (N1), darkly stained pyknotic nucleus (N2), numerous mitochondria (M). (X8,000)

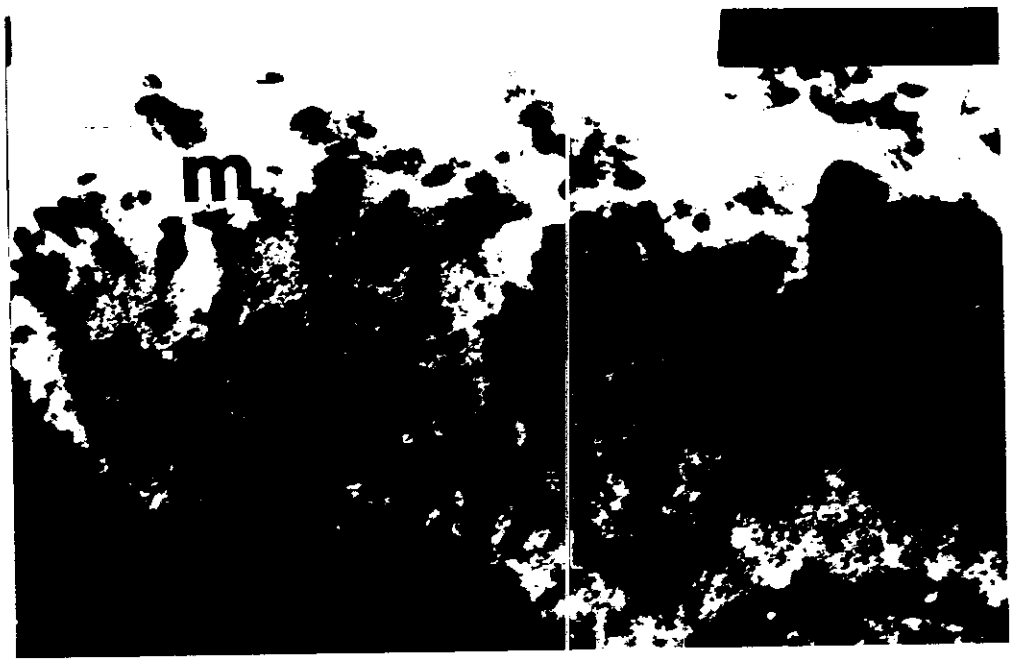


Fig. (11) : An electron micrograph of the trachea of rabbit after 20min. exposure showing the lining epithelium have lost their microvilli & cilia (m), even their apical borders (A) are destructed & irregular (X8,000).

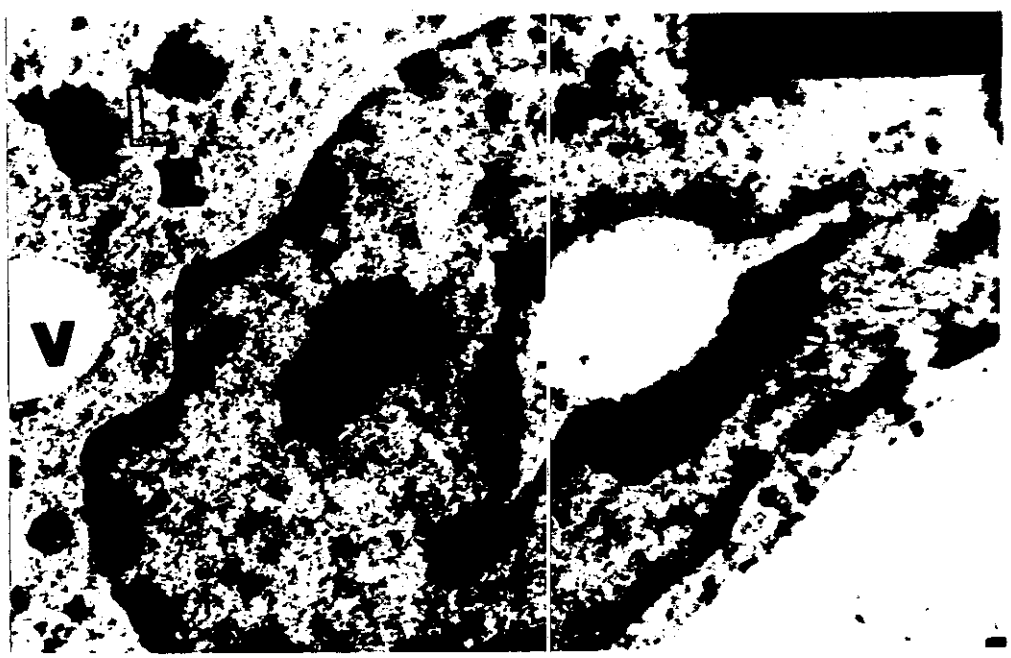


Fig. (12) : An electron micrograph of the lung of rabbit after 20min. exposure showing pneumocyte II with vacuolated nucleus (N), the cytoplasm showing large vacuoles (V) with lamellated bodies (L), destructed endoplasmic reticulum(E). (X14,000)

Effects of 30 minutes exposure to the CO_2 laser smoke (3rd group)

1- Tracheal changes :

a- Changes in the nucleus :

Two adjacent degenerated cells 1st shows an extruded pyknotic nucleus and the 2nd shows destructed cilia (Fig.13).

b- Cellular changes :

1- Loss of cilia of most tracheal cells. (Fig.14)

2- Numerous number of intracellular lysosomes with lymphocytic infiltration inbetween epithelial cell lining of the trachea. (Fig.14)

3- degenerated cell showing destruction of endoplasmic reticulum and mitochondria, there are large lysosomes inside the cytoplasm. (Fig.26)

2- Bronchopulmonary changes :

a- Changes in the nucleus :

1- Smaller disturbed nucleus was found in an interalveolar phagocytic macrophage having intracytoplasmic phagocytic matter in the form of large & small irregular vacuolated areas. (Fig.15)

2- Irregular lobulated nucleus in abnormal dust cell inside the lumen of the alveolus having an irregular lobulated nucleus with a fairly abundant euchromatin & few heterochromatin at the periphery of the nucleus with scanty cytoplasm. (Fig.16)

3- Vacuolation of the nucleus in an interalveolar septal cell. (Fig.12)

4-Hypochromatic nucleus of a great alveolar cell (pneumocyte II). (Fig.17)

b-Cellular changes :

1-Degenerated interalveolar septal cell. (Fig.37)

2-A great alveolar cell (pneumocyte II) with :

- Numerous multivesicular bodies.
- Few microvilli on its free surface.
- Disturbed mitochorndria.
- Less lamellar bodies. (Fig.17)

3-Neutrophils with :

- large azurophilic granules with vacuolation, which have lysosomal enzymes & peroixdase.
- Smaller specific granules have alkaline phosphatase & bactericidal substances. The cells appeared to be exhausted. (Fig.29, 39)

4-Sub-epithelial blood capillary with their basal lamina are surrounded with collagenic fibrils, also the cytoplasm of the endothelial cell having a lot of vacuoles. (Fig.18)

5-Interstitial hemorrhage inbetween the lung alveoli. (Fig.19)

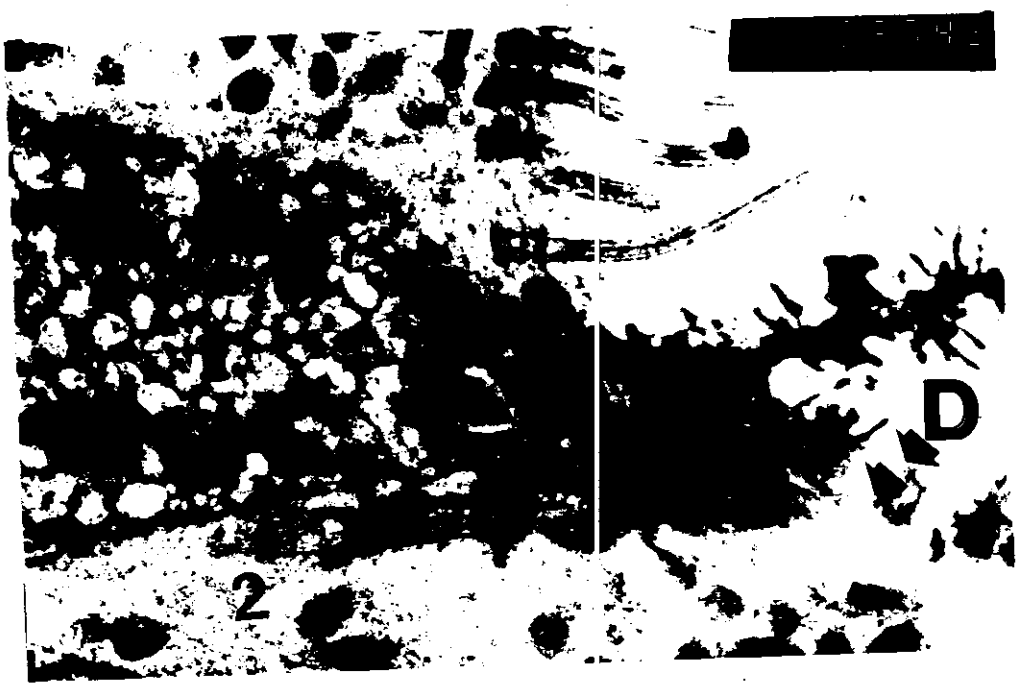


Fig. (13) : An electron micrograph of the trachea of rabbit after 30min. exposure showing adjacent two degenerated cells (1,2), the 1st shows an extruded pyknotic nucleus (K), the 2nd shows destructed cilia (D). (X10,000).

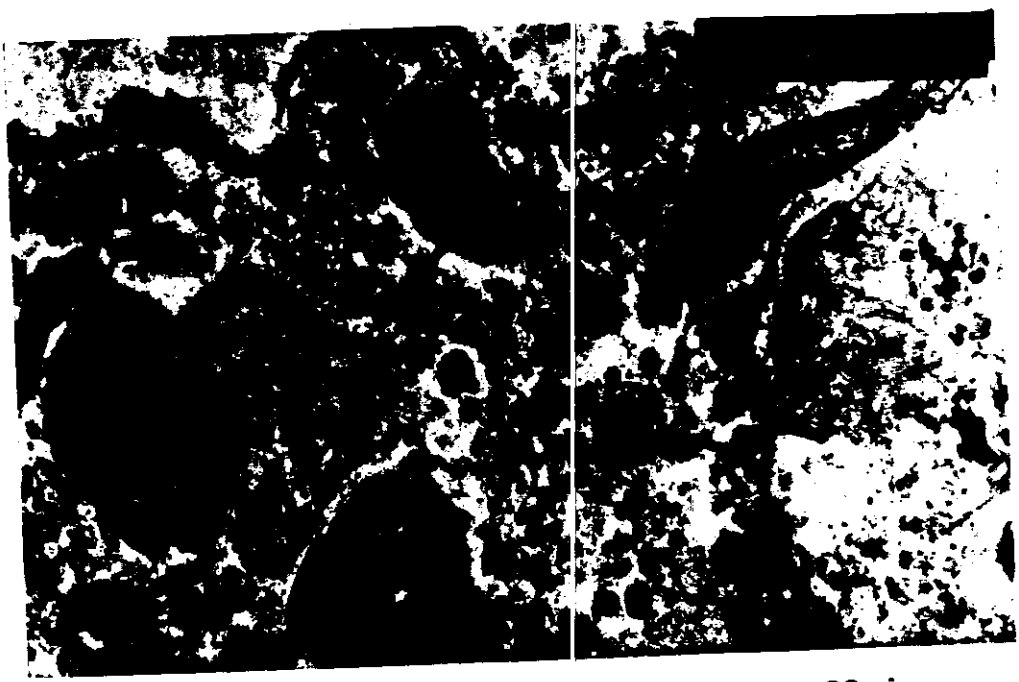


Fig. (14) : An electron micrograph of the trachea of rabbit after 30min. exposure showing lymphocytic infiltration (L), numerous number of intercellular lysosomes (l), loss of cilia of most cells (C), fibroblasts (X). (X5,000)

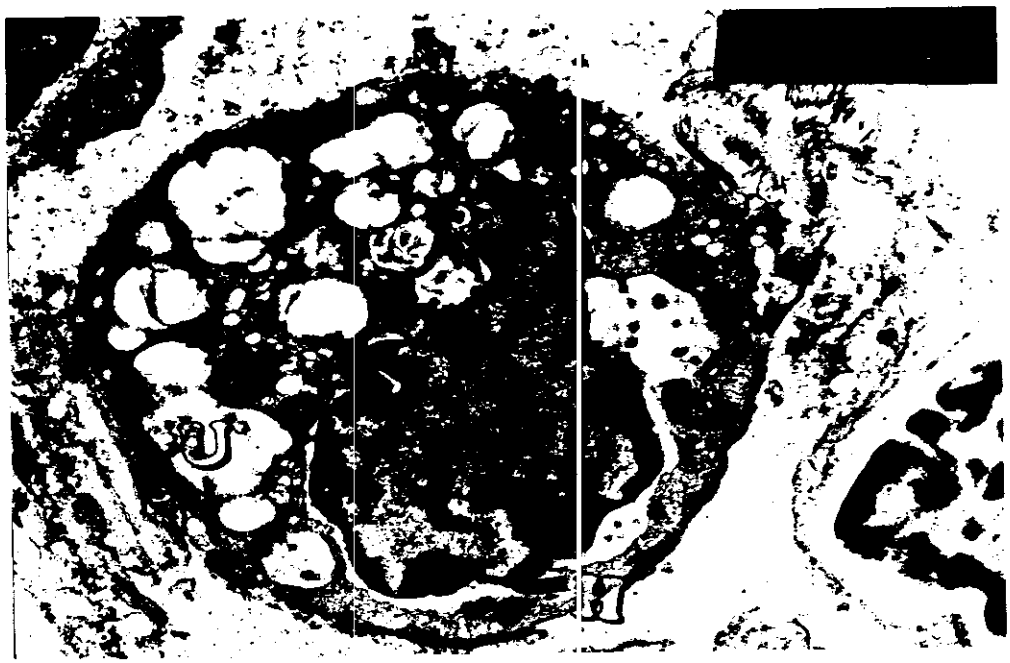


Fig. (15) : An electron micrograph of the lung of rabbit after 30min. exposure showing interalveolar phagocytic macrophage with intracytoplasmic phagocytic matter (U) form large and small irregular vacuolated areas with smaller disturbed nucleus (S) and heterochromatin (H). (X6,700).

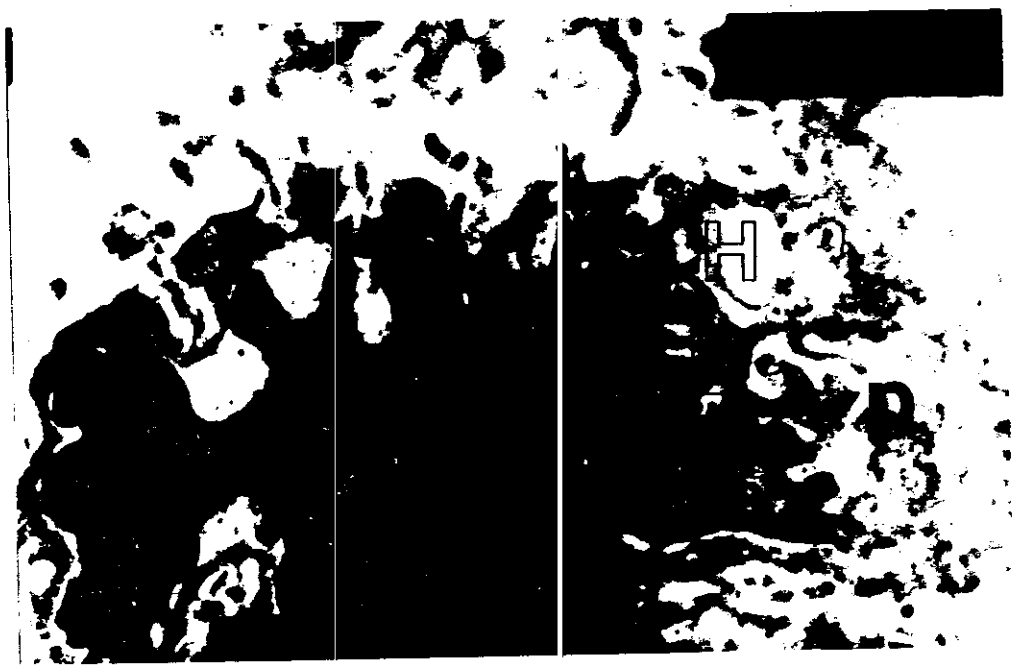


Fig. (16) : An electron micrograph of the lung of rabbit after 30min. exposure showing abnormal dust cell with an irregular lobulated nucleus (N), with abundant euchromatin (E) and few heterochromatin (H) with scanty cytoplasm (P). (X14,000)

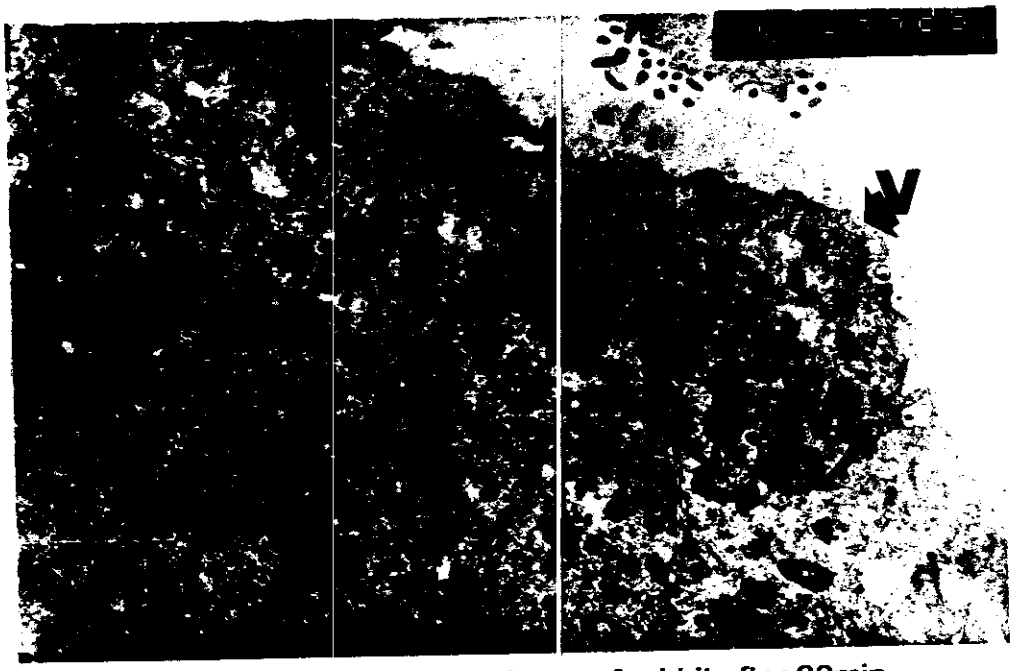


Fig. (17) : An electron micrograph of the lung of rabbit after 30min. exposure showing pneumocyte (II) with hypochromatic nucleus (H), less lamellar bodies (b), disturbed mitochondria (T), numerous multivesicular bodies (M) & few microvilli (V). (X6,700).

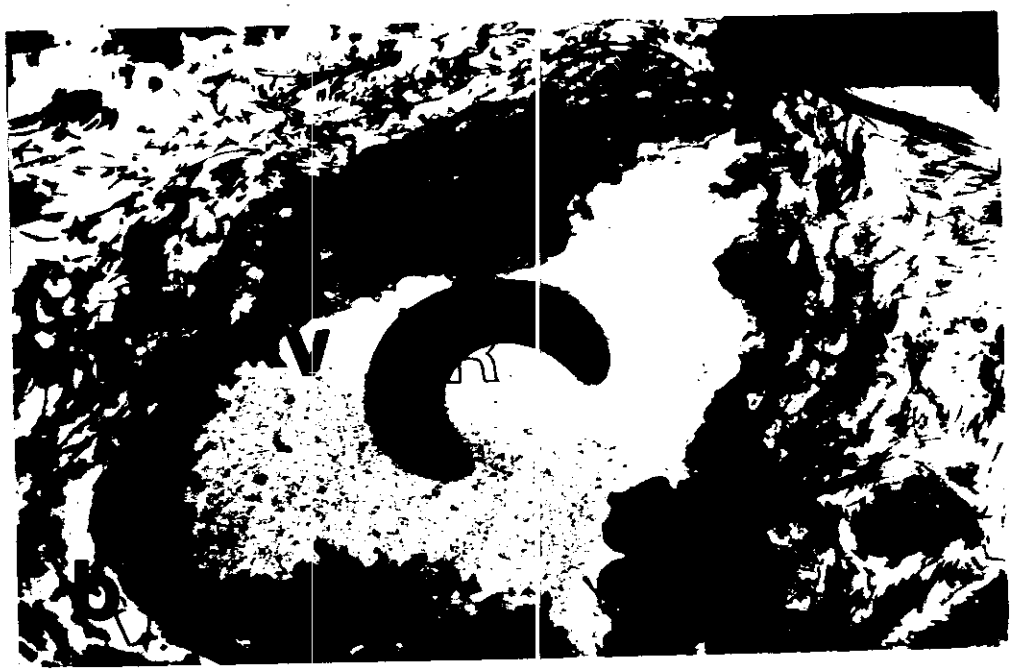


Fig. (18) : An electron micrograph of subepithelial blood capillary 30min.exposure surrounded with a lot of collagenic fibrils (F), the cytoplasm of endothelial cell having a lot of vacuoles (V), normal nucleus (N), irregular nucleus (I), basal lamina (B), red blood cell (R).(X5,000).

Effects of Chronic exposure to the CO_2 laser smoke (4th group)

Repeated exposure of male shenshilla Rabbits 1-1.1 kg 15 minutes for 3-4 weeks by the use of CO_2 laser at a power of 4 watts, 200 hz frequency at a continuous mode by the use of a hand piece we found the following results :

1- Tracheal changes :

a- Changes in the nucleus :

1- Dividing basal cell while the other adjacent cell reveals pyknotic nucleus. (Fig.20)

2- Sub-epithelial dividing cell in the telophase stage, their cytoplasm has numerous normal & abnormal mitochondria and vacuolar cytoplasm. (Fig.21)

3- A special type of destruction to the nuclei of lining epithelium of the trachea in the form of boundary vacuolation of the nuclei. (Fig..22)

4- A goblet cell having no nucleus which may be suffered from karyorrhexis & karyolysis. (Fig.28)

5- We found a cell division at its later stage of division with surrounding collagenic fibrils. (Fig.23)

6- Final division of one cell in the subepithelia propria revealing the new two nuclei, those have a dense hetero chromatin (hyperchromatic). (Fig.24)

7- An Extruded pyknotic nucleus from degenerated columnar ciliated cell with destruction of apical cilia (see the adjacent two normal columnar ciliated cells). (Fig.25)

8-Complete extrusion of the nucleus with vacuolated appearance of its remained place (karyolysis of the nucleus). (Fig.26)

b-Cellular changes :

1-Degenerated mitochondria and numerous vacuolated and dilated cisternae of endoplasmic reticulum. (Fig.20, 37)

2-Disturbed goblet cell revealing :

- Very large irregular vacuole.
- Disturbed apical microvilli of goblet cell. (Fig.27)

3-Destructed mucous granules and distorted mitochondria, distended endoplasmic reticulum (Fig.20, 21).

4-Atrophy of goblet cell with few mucous granules also the adjacent cells have lost most of their intracytoplasmic organelles. (Fig.28)

2- Bronchopulmonary changes :

a- Changes in the nucleus :

1-Exhausted neutrophil with fainter electron lucent heterochromatin in the nuclei with peri-nuclear halo. (Fig.29)

2-Inter-alveolar septal cell suffering from degenerative changes, pyknotic nucleus & irregularity in shape, deviated to one side. (Fig.10, 20)

3-An active macrophage having an irregular nucleus with destructed engulfed particles. (Fig.15)

4-A special type of degeneration of the nucleus of septal macrophage having peripheral vacuolation of the nucleus, with remaining digested carbon particles. (Fig.30)

5-Another form of special nuclear vacuolar degeneration. (Fig.31)

6-Interalveolar giant cell with irregular nucleus. (Fig.32)

7-Dividing cell at telophase stage. (Fig.33)

8-Large Clara cell having a small irregular abnormal nucleus. (Fig.34)

9-Beginning of formation of peri-nuclear halo. (Fig.35)

b-Cellular changes :

The following changes were notified :

1-Two uniting macrophages having intracytoplasmic smoke black granules of variable sizes & shapes in the interalveolar septum. (Fig.36)

2-Roughness or thickness of the endothelial cell surface may → clotting formation. (Fig.18)

3-Specific granules suffered from vacuolation other specific granules were granulated. (Fig.29)

4-Severely degenerated & vacuolated cytoplasm with large vacuoles. (Fig.26)

5-Large septal interalveolar giant cell macrophage engulfed red blood cell from hemorrhagic areas and wide cisternae of endoplasmic reticulum, the cell is surrounded with numerous collagenic fibrils. (Fig.37)

6-Abnormally formed collagenic fibrils which are arranged in the interalveolar septa. (Fig.37)

7- An active fibroblast which forms new collagenic fibrils which appeared adherent to the cell in the interstitium between the alveoli. (Fig.38)

8- An active septal macrophage having engulfed carbon particles and destructed engulfed particles. (Fig.30, 36)

9- Interalveolar septal cell having intracytoplasmic secondary lysosomes as phagocytosed & digested matters and few mitochondria and short microvilli. (Fig.35)

10- Interalveolar giant cell with intracytoplasmic specific abnormal fibrils, the cell is in its way to invade through the alveolar epithelium to the lumen. (Fig.32)

11- An alveolar duct lined with an epithelium showing sloughed one cell to the lumen. (Fig.33)

12- Large Clara cell with destructed most of mitocondria & endoplasmic reticulum and few cytoplasmic electron dense granules, with subepithelial collagenic fibrils. (Fig.34)

13- Squamous metaplasia, the columnar ciliated epithelium was changed into flattened ciliated epithelium and subepithelial neutrophil having azurophilic granules with vacuolation & smaller specific granules. (Fig.39)

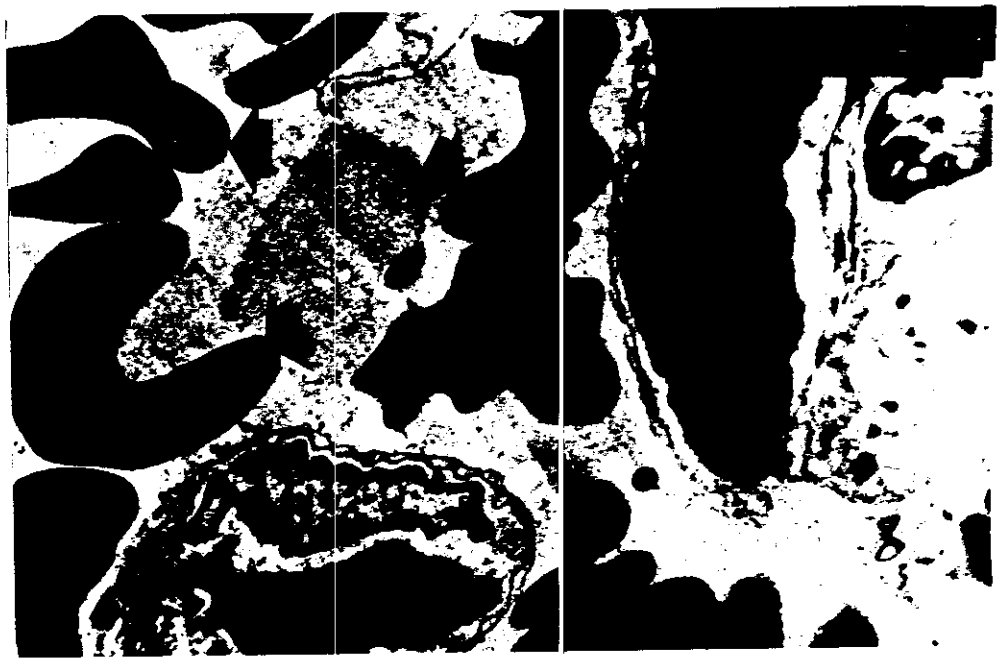


Fig. (19) : An electron micrograph of the lung of rabbit after 30min. exposure showing interstitial hemorrhage (arrows) in between lung alveoli blood cap. (C), having 1 red blood cell inside its lumen. (X4000).

Fig. (20) : An electron micrograph of the trachea of rabbit after chronic exposure showing dividing basal cell (B) while the other adjacent cell (D) reveals pyknotic Nucleus (P) with degeneration of mitochondria (M) and numerous vacuoles (U) & dilated cisternae of the endopl. Ret. (E). (X 8,000)

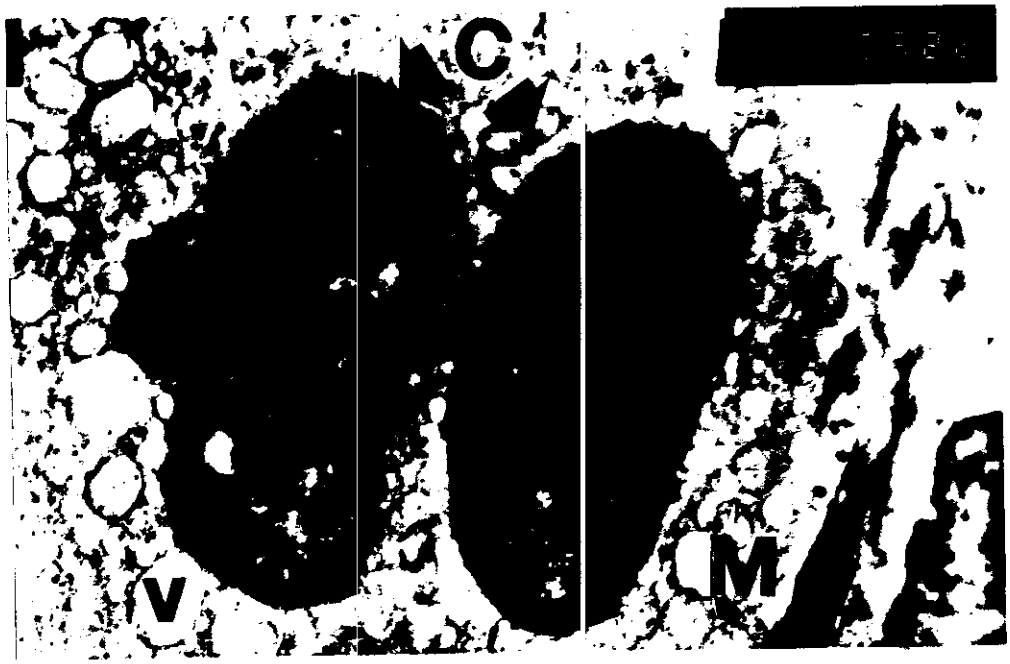


Fig. (21) : An electron micrograph of the trachea of a rabbit after chronic exposure showing :a Subepithelial dividing cell (C) in the telophase, with vacuolar cytoplasm (V)and numerous abnormal swollen mitochondria (M). (X 10.000).

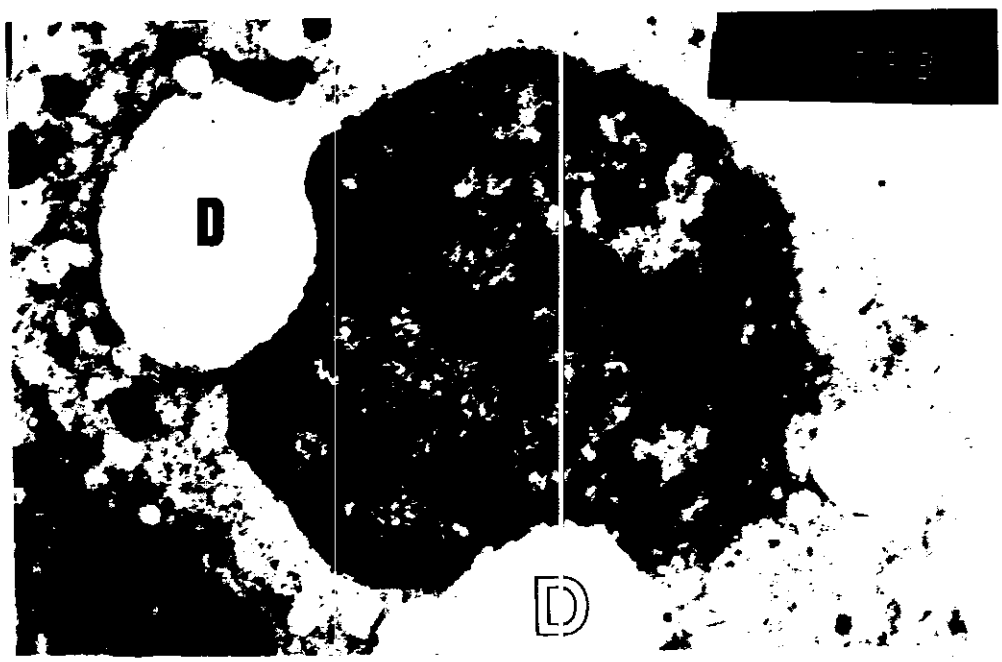


Fig. (22) : An electron micrograph of the trachea of a rabbit after chronic exposure showing a special type of destruction (D) to the nucleus (S) in the form of boundary vacuolation. (X14,000).

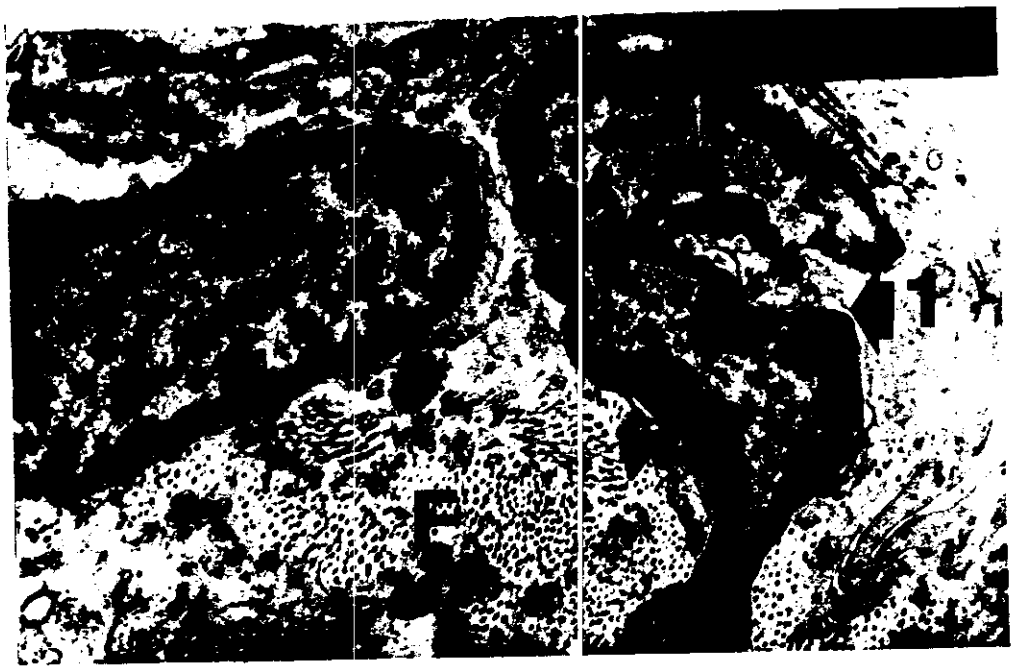


Fig. (23) : An electron micrograph of the trachea of a rabbit after chronic exposure showing : subepithelia propria with a dividing cell (1) at its later stage of final division surrounded with. collagenic fibrils (F) and non dividing cell (2). (X10,000).

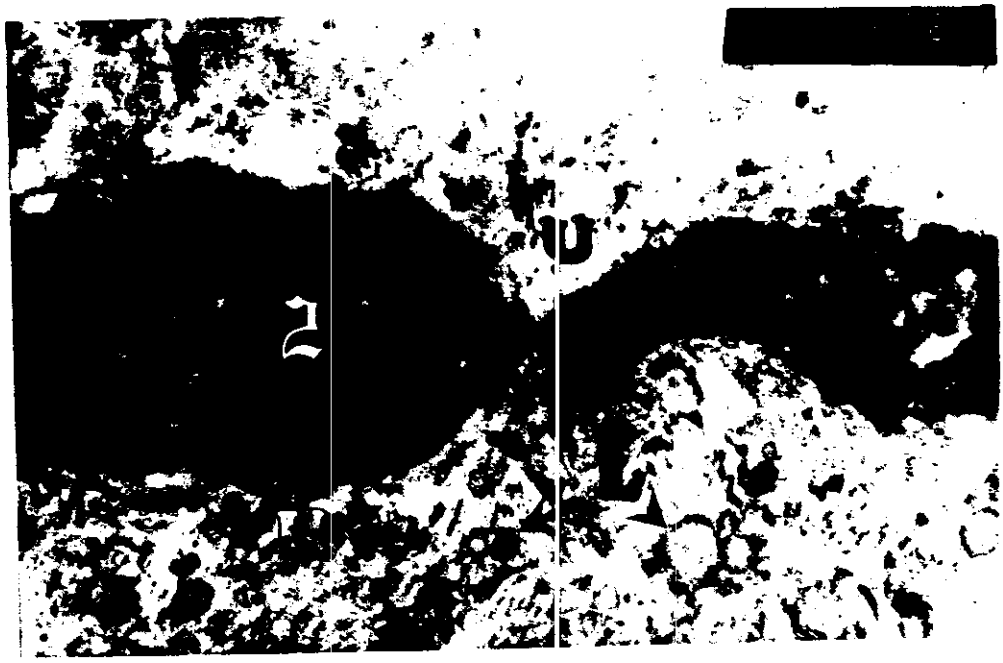


Fig. (24) : An electron micrograph of the trachea of a rabbit after chronic exposure showing : a final division of one cell in the subepithelia propria, revealing the two new nuclei (N1) & (N2) with dense heterochromatin (H) [hyperchromatic]. vacuolation of cytoplasm (U) intracellular lysosomes (L). (X14,000).

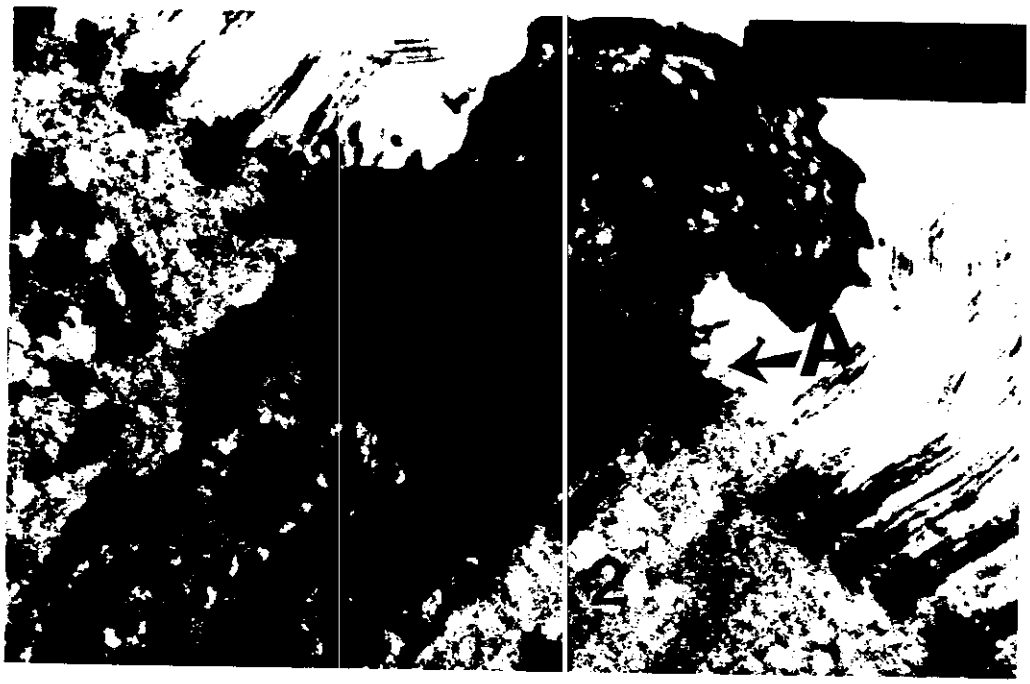


Fig. (25) : An electron micrograph of the trachea of a rabbit after chronic exposure showing : an extruded pyknotic nucleus (P) from degenerated columnar ciliated epith. cell (C). with destruction of the apical cilia (A) with adjacent two normal columnar ciliated cells (1),(2). (X10,000).

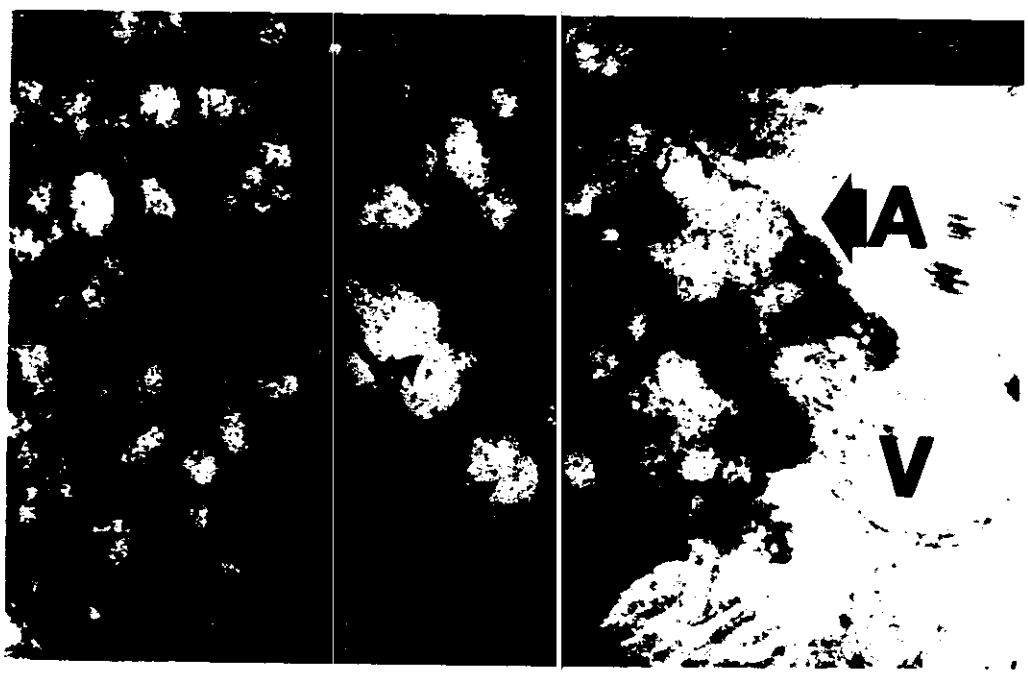


Fig. (26) : An electron micrograph of the trachea of a rabbit after chronic exposure showing : complete destruction and extrusion of the nucleus with vacuolated appearance (V) (karyolysis) & destruction of the apical cilia (A). Large lysosome (L). Destructed Endo. Ret. (E). (X10,000).

Fig. (27) : An electron micrograph of the trachea of a rabbit after chronic exposure showing : An apical border of disturbed goblet cell revealing very large irregular vacuole (I), with destructed apical microvilli (M). (X14,000).

Fig. (28) : An electron micrograph of the trachea of a rabbit after chronic exposure showing : an atrophic goblet cell (G), the adjacent cells (1), (2) have lost most of their intracytoplasmic organelles. (X8,000).

Fig. (29) : An electron micrograph of the lung of a rabbit after chronic exposure showing : Exhausted interalveolar neutrophil with electron lucent heterochromatin in the nuclei (K), with azurophilic granules (F) & specific granules suffered from vacuolation (V) or granulation (G), peri-nuclear halo (H) means liquefaction of cytoplasm. (X14,000).

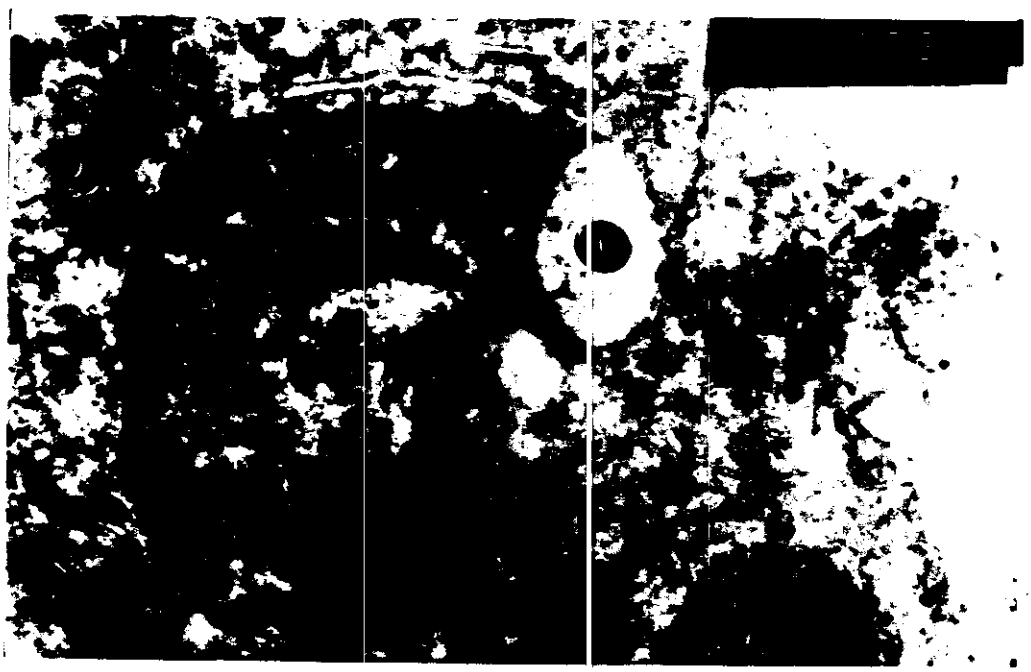


Fig. (30) : An electron micrograph of the lung of a rabbit after chronic exposure showing : A special type of degeneration of the nuclei of septal macrophage having peripheral vacuolation of the nucleus (O), with remaining of digested carbon particles (P). (X10,000).

Fig. (31) : An electron micrograph of the lung of a rabbit after chronic exposure showing :another form of special nuclear vacuolation (V), by karyorrhexis & karyolysis. (X14,000).

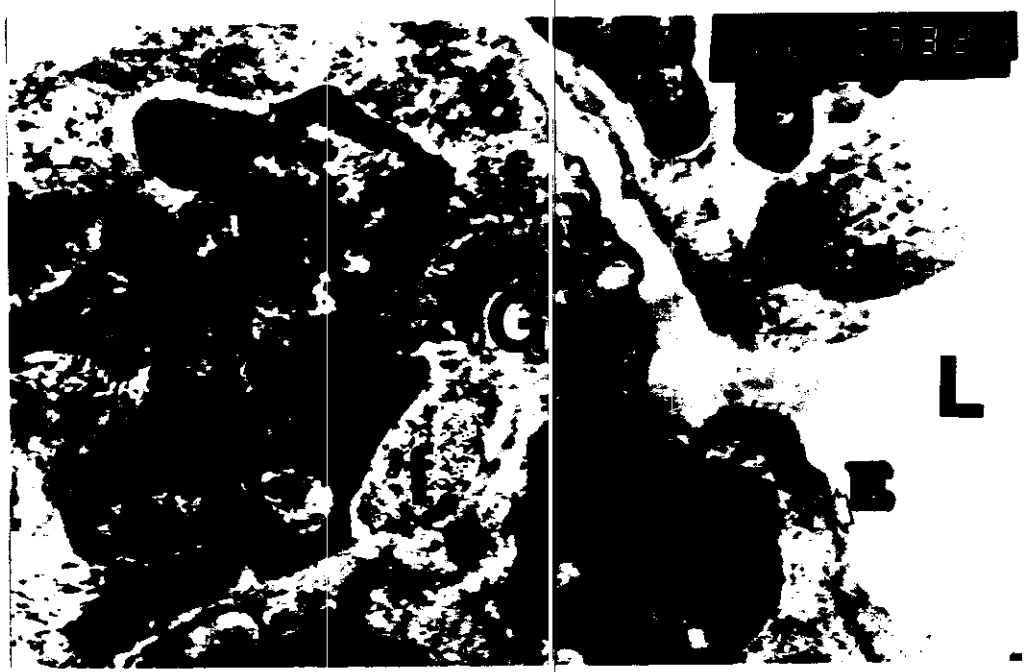


Fig. (32) : An electron micrograph of the lung of a rabbit after chronic exposure showing : interalveolar giant cell (G), with irregular nucleus (I) & intracytoplasmic abnormal fibrils(F), the cell has its way to invade through the alveolar epithelium (E) to the lumen (L). (X8,000).

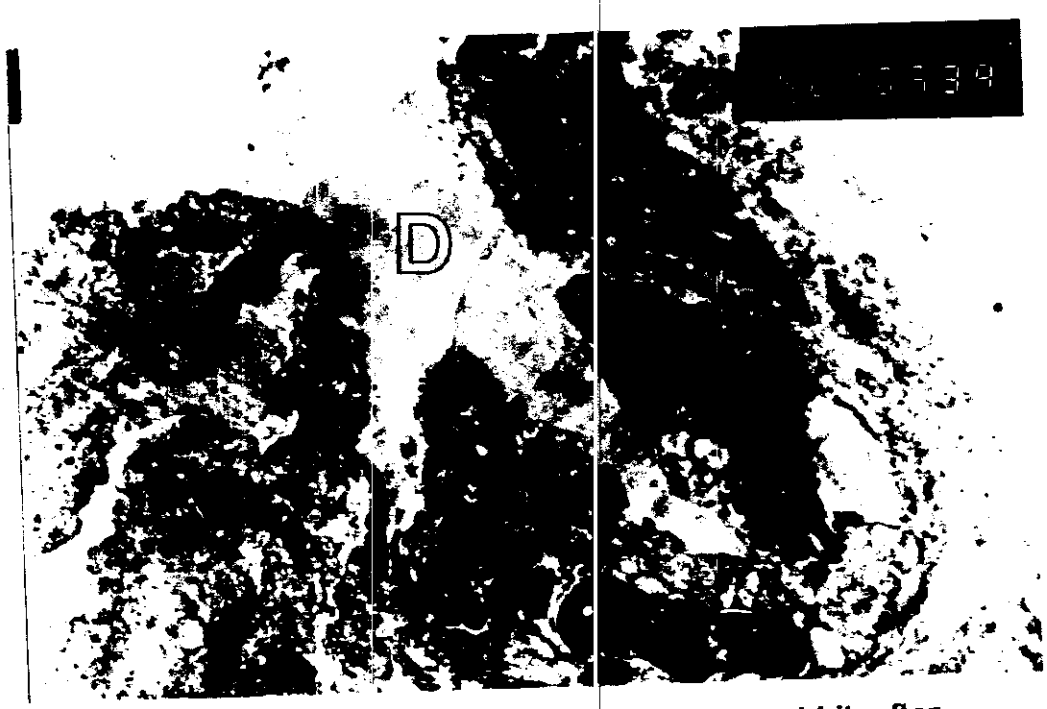


Fig. (33) : An electron micrograph of the lung of a rabbit after chronic exposure showing :an alveolar duct (D) lined with an epithelium showing sloughed one cell (C) to the lumen, also dividing another cell at telophase (T) (X5,000).

Fig. (34) : An electron micrograph of the lung of a rabbit after chronic exposure showing : large Clara cell (C) having a small irregular abnormal nucleus (N) with destruction of mitochondria & Endopl. Ret. with few electron dense granules (E), an adjacent cell (S) lining the bronchiole with subepith. collagenic fibrils (O). (X6,700).

Fig. (35) : An electron micrograph of the lung of a rabbit after chronic exposure showing : interalveolar septal cell having intracytoplasmic 2ry lysosomes as phagocytosed digested matters (P) few mitochondria (r), beginning of formation of peri-nuclear halo (O), few short microvilli (L). (X10,000).

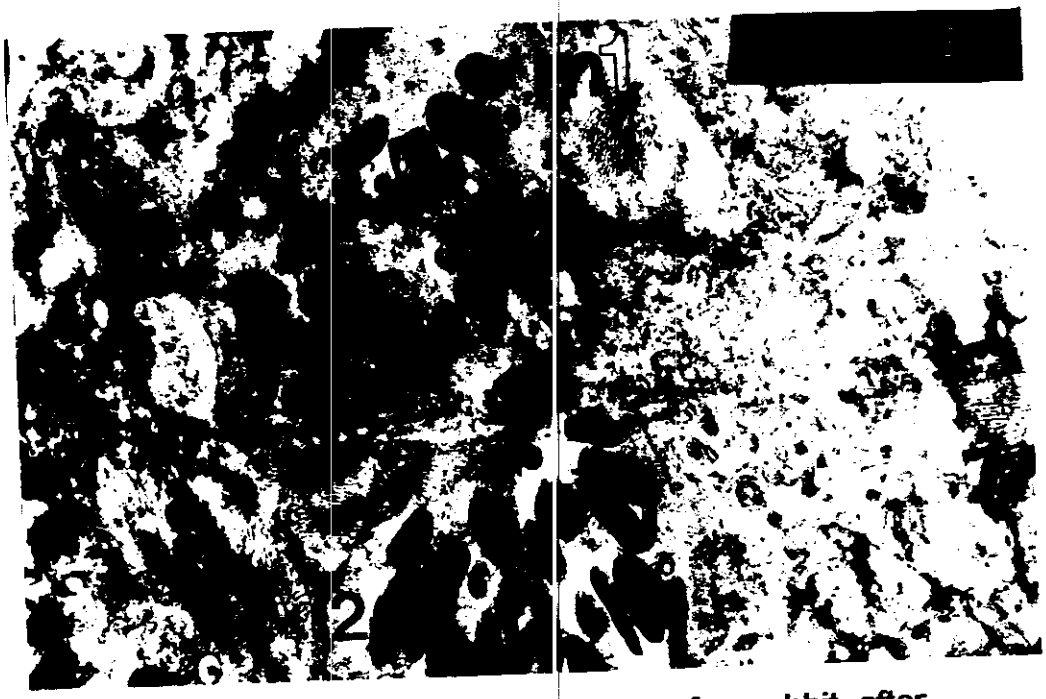


Fig. (36) : An electron micrograph of the lung of a rabbit after chronic exposure showing : two uniting macrophage m1, m2 having intracytoplasmic smoke black granules (g) of variable sizes & shapes in the interalveolar septum. (X6,700).

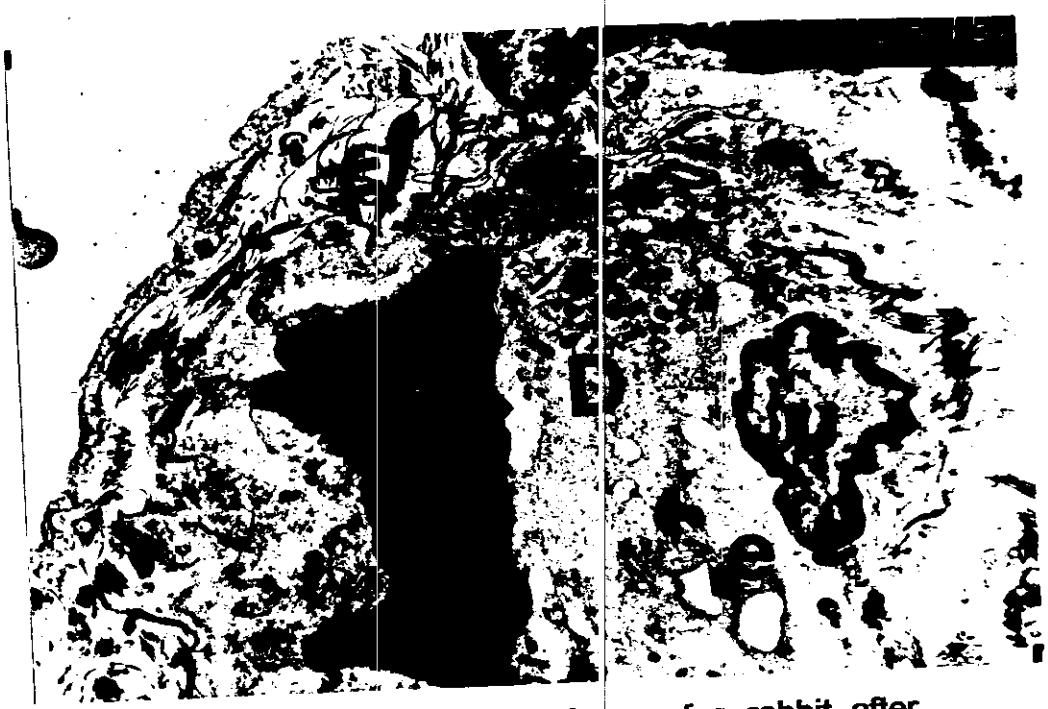


Fig. (37) : An electron micrograph of the lung of a rabbit after chronic exposure showing :a large septal interalveolar cell having small nucleus (U) with engulfed red blood cells (R) & wide cisternae of Endop. Ret. (e), it is surrounded with abnormal collagenic fibrils (F) & destructed mitochondria (D). (X5,000).

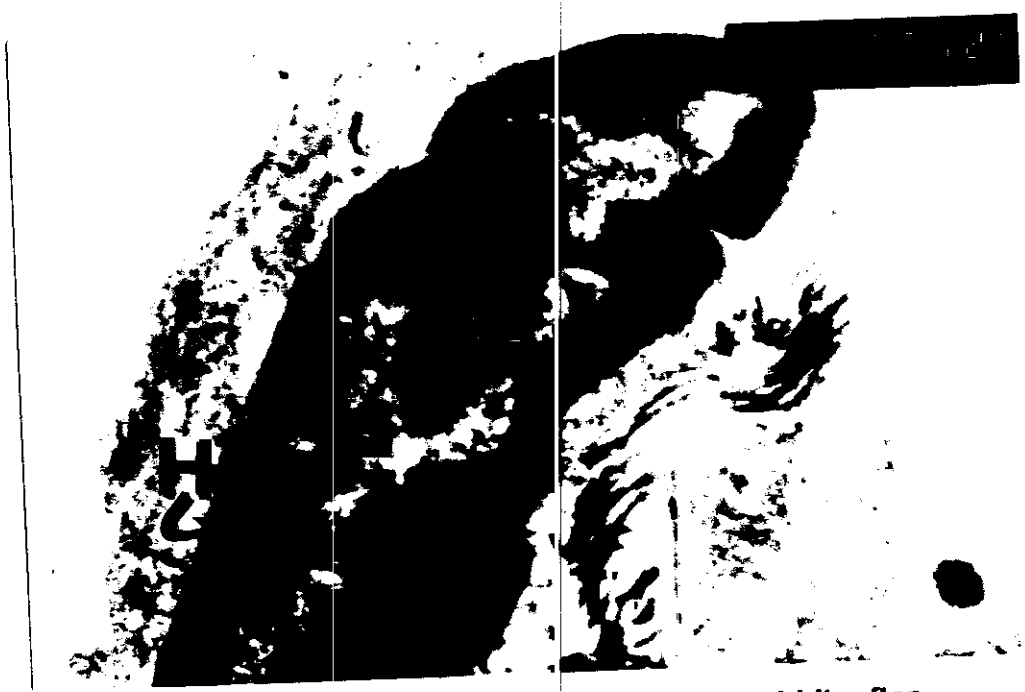


Fig. (38) : An electron micrograph of the lung of a rabbit after chronic exposure showing : an active fibroblast which forms new collagenic fibres (F), which appear adherent to the cell. Hterochromatin (H), Euchromatin (E). (X20,000).

● Control Group :

Electron microscopical micrograph of the control rabbit were observed in the following figures :

Fig (40) showing the lung control with intra- alveolar phagocyte.

Fig (41) showing normal blood vessel.

Fig (42) showing normal trachea.

Fig (43) showing normal goblet cell & normal columnar epithelial cells in the trachea .

Fig (44) showing normal lung of control with mature septal macrophage.

Fig (45) showing normal lung of control with two normal septal fibroblasts.

Fig (46) showing normal trachea of control with normal epithelial lining & normal goblet cell.

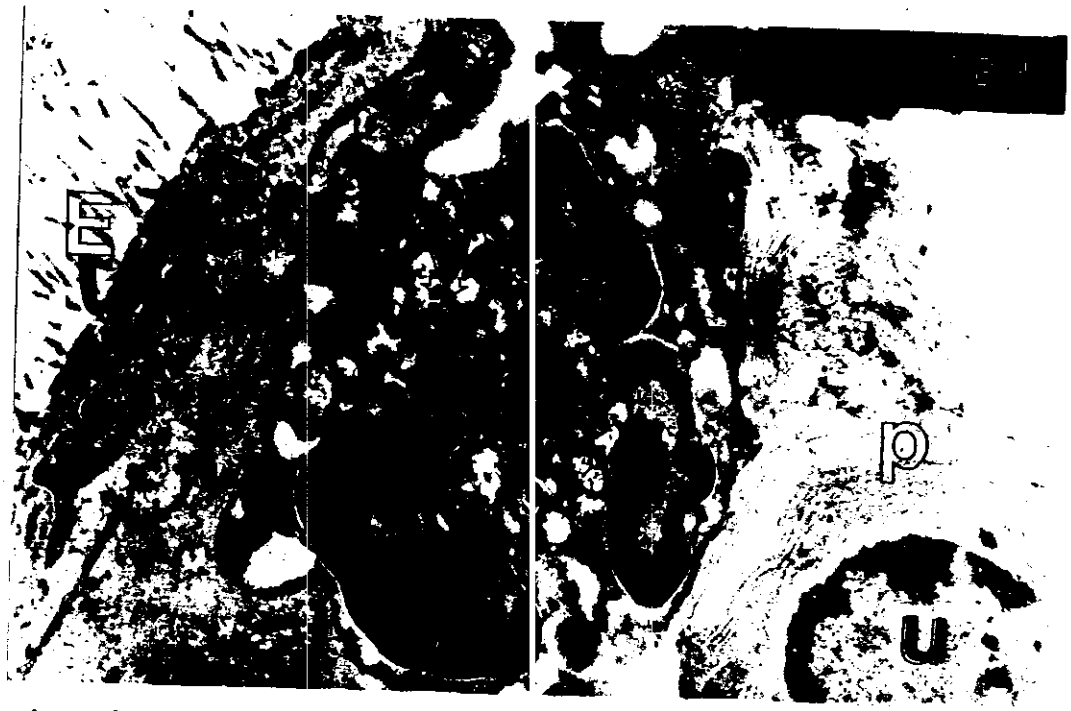


Fig. (39) : An electron micrograph of the lung of a rabbit after chronic exposure showing : squamous metaplasia the columnar ciliated epith. was changed into flattened ciliated epith. (E) & subepith. neutrophil (N) having azurophilic granules with vacuolation (V) & smaller specific granules (S). plasma cell (P) having a nucleus (U) of chart-wheel appearance. (X6,700).

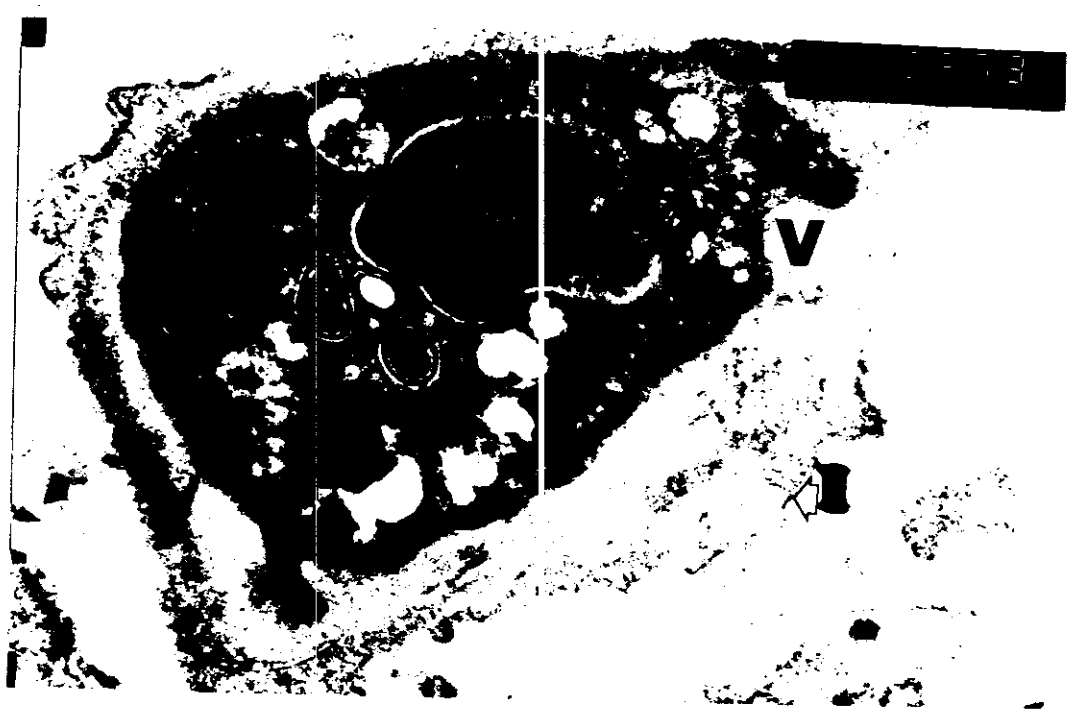


Fig. (40) : An electron micrograph of the lung of control rabbit showing an intra-alveolar phagocyte dust cell having less cytoplasmic vacuoles (V), 2ry lysosomes (S), Microvilli on its surface (I), normal nucleus (n). (X8,000).

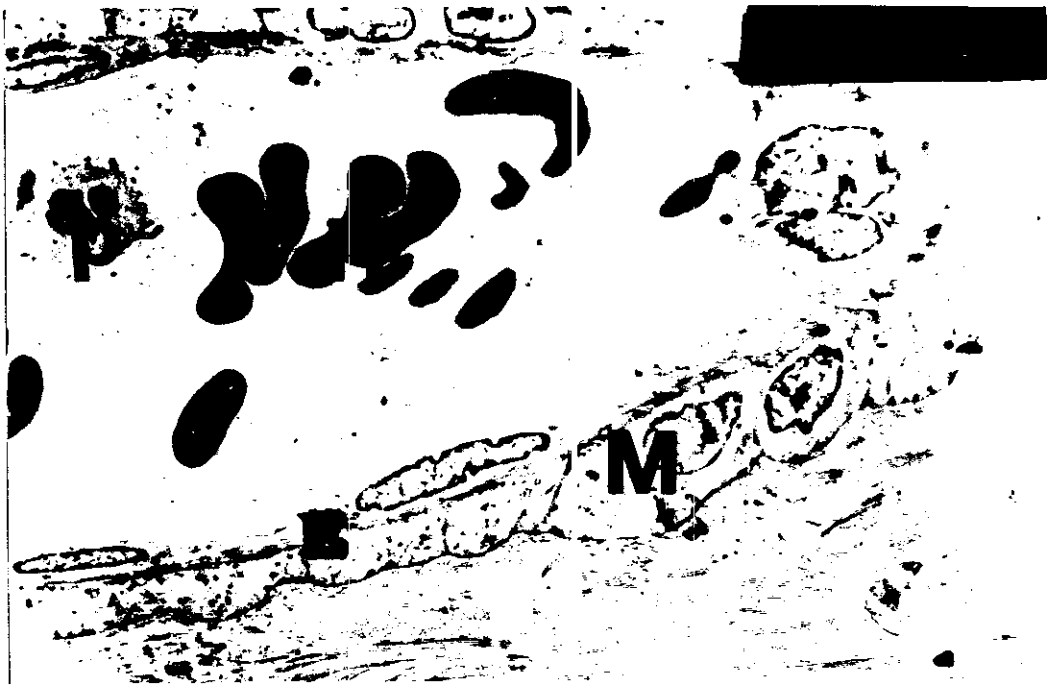


Fig. (41) : An electron micrograph showing normal blood vessel lined with normal endothelial cells (E) & muscle cells (M), RBCs inside the lumen (R), neutrophil (P). (X2,000).

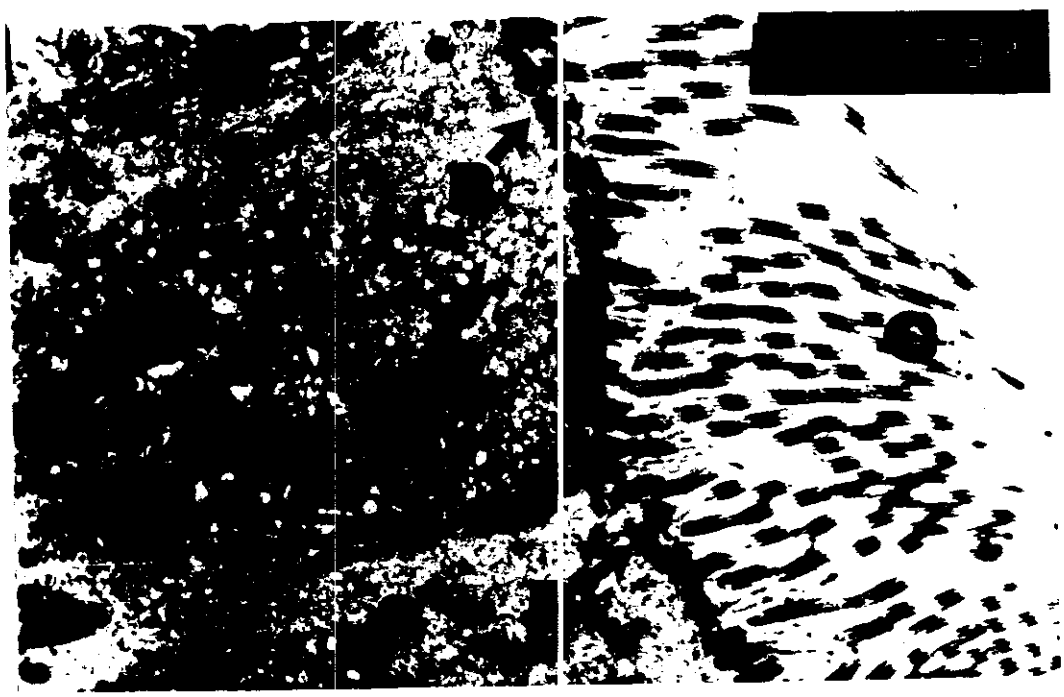


Fig. (42) : An electron micrograph of the trachea of control rabbit showing : normal ciliated two columnar epith. cells (1)&(2), long normal cilia (C), having normal basal bodies (B) & normal mitochondria (m). (X6,700).

Fig. (43) : An electron micrograph of the trachea of control rabbit showing : the epithelial lining has two types of cells 1st goblet cell (G), with microvilli (V) & secretory granules (S), 2nd columnar cell with long cilia (_) & the cytoplasm is rich in normal mitochondria (m). (X8,000).

Fig. (44) : An electron micrograph of the lung of control rabbit showing mature septal macrophage with normal nucleus (U), primary lysosomes (P) & 2ry lysosomes (L) inbetween alveoli. (X6,700).

Fig. (45) : An electron micrograph of the lung of control rabbit showing the lumen of the alveolus (A) & two septal fibroblasts (1), (2). with normal nuclei. In cell (1), the mitochondria (M) & and crossly cut normal fibrils (F). In cell (2) intracytoplasmic normal fibrils are present. (X5,000).

Fig. (46) : An electron micrograph of the trachea of control rabbit showing normal lining epithelium having ciliated columnar cell (r), normal goblet cell (G) & normal basal cell (B) with normal nuclei (I). (X2,700).

STATISTICAL RESULTS

● Statistical Analysis :

We use the Chi-square as a test for significance.

The data were entered to an EPI-INFO file using EPI-INFO version 6.02 software computer package.

Univariate analysis i.e " Chi-square (X^2) test " : was used in the assessment of difference in qualitative variables between different groups.

$$X^2 = \sum \frac{(O-E)^2}{E}$$

$$D.F = (r-1)(c-1)$$

where :

O = Observed.

E = Expected.

r = row total

c = column total

(WHO, 1994)

According to the previous classification statistical results were summarized as follows :

● Electron Microscopical Data :

a- Nuclear Changes :

As regards the significant nuclear changes the following were found :

1- Cell division *Table 1*
2-Active fibroblasts . *Table 2*
3- Extruded nuclei . *Table 3*
4- Nuclear destruction . *Table 4*
5-Abnormal collagenic fibrils . *Table 5*
6- Smoke granules . *Table 6*
7- Increased interacellular lysosomes . *Table 7*
8- Goblet cell atrophy . *Table 8*
9- Squamous metaplasia . *Table 9*
10- Blood capillary roughness . *Table 10*
11- Nuclear vacuolation . *Table 11*
12- Pyknotic nucleus . *Table 12*
13- Hypochromatosis . *Table 13*

b- Cellular Changes :

As regards the significant cellular changes the following were found :

1-Lymphocytosis . *Table 14*
2-Loss of cilia & microvilli . *Table 15*
3-Destructed mitochondria . *Table 16*

But we can summarize the **non significant** statistical results as following :

I) Non significant nuclear changes : *Table 17*

1- Nuclear elongation .
2- Hyperchromatosis .
3- Irregular nuclei .
4- Peri-nuclear halo .

II) Non significant cellular changes : *Table 18*

1- Proprial edema .
2- Phagocytic bodies .
3- Destructed endoplasmic reticulum .
4- Increased intercellular spaces .
5- Vacuolization of cytoplasm .
6- Oesinophilia .

Table (1) : Distribution of cell division as regards to time of exposure :

Cell Division	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	4 100%	4 100%	4 100%	0 0.0%	12 75%
+1	0 0.0%	0 0.0%	0 0.0%	2 50%	2 12.5%
++2	0 0.0%	0 0.0%	0 0.0%	2 50%	2 12.5%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 16 Degrees of freedom = 6
P value = 0.01375397 ← ***Significant***

Table (2) : Distribution of Active Fibroblast as regards to time of exposure

Active Fibroblast	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	4 100%	4 100%	4 100%	0 0.0%	12 75%
+1	0 0.0%	0 0.0%	0 0.0%	3 75%	3 18.8%
++2	0 0.0%	0 0.0%	0 0.0%	1 25%	1 6.3%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 16 Degrees of freedom = 6
P value = 0.01375397 ← ***Significant***

Table (3) : Distribution of Extruded Nuclei as regards to time of exposure :

Extruded Nuclei	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	4 100%	4 100%	2 50%	0 0.0%	10 62.5%
+1	0 0.0%	0 0.0%	2 50%	4 100%	6 37.5%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 11.73 Degrees of freedom = 3
P value = 0.00835478 ← ***Significant***

Table (4): Distribution of Nuclear Destruction as regards to time of exposure :

Nuclear Destruction	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	4 100%	4 100%	3 75%	0 0.0%	11 68.8%
+1	0 0.0%	0 0.0%	1 25%	0 0.0%	1 6.3%
++2	0 0.0%	0 0.0%	0 0.0%	2 50%	2 12.5%
+++3	0 0.0%	0 0.0%	0 0.0%	2 50%	2 12.5%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 18.91 Degrees of freedom = 9
P value = 0.02597707 ← ***Significant***

Table (5) : Distribution of Abnormal Collagenic Fibrils as regards to time of exposure :

Abnormal Collagenic Fibrils	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	4 100%	4 100%	3 75%	0 0.0%	11 68.8%
+1	0 0.0%	0 0.0%	1 25%	0 0.0%	1 6.3%
++2	0 0.0%	0 0.0%	0 0.0%	2 50%	2 12.5%
+++3	0 0.0%	0 0.0%	0 0.0%	2 50%	2 12.5%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 18.91 Degrees of freedom = 9
P value = 0.02597707 ← **Significant**

Table (6) : Distribution of the Smoke Granules as regards to time of exposure :

Smoke Granules	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	4 100%	4 100%	0 0.0%	0 0.0%	8 50%
+1	0 0.0%	0 0.0%	3 75%	0 0.0%	3 18.8%
++2	0 0.0%	0 0.0%	1 25%	2 50%	3 18.8%
+++3	0 0.0%	0 0.0%	0 0.0%	2 50%	2 12.5%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 26.67 Degrees of freedom = 9
P value = 0.00158776 ← **Significant**

Table (7) : Distribution of Intracellular Lysosomes as regards to time of exposure :

Intracell. Lysosomes	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	4 100%	4 100%	1 25%	0 0.0%	9 56.3%
+1	0 0.0%	0 0.0%	2 50%	1 25%	3 18.8%
++2	0 0.0%	0 0.0%	1 25%	3 75%	4 25%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 15.33 Degrees of freedom = 6
P value = 0.01781669 ← ***Significant***

Table (8) : Distribution of Goblet Cell Atrophy as regards to time of exposure :

Goblet Cell Atrophy	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	4 100%	4 100%	2 50%	0 0.0%	10 62.5%
+1	0 0.0%	0 0.0%	2 50%	0 0.0%	2 12.5%
++2	0 0.0%	0 0.0%	0 0.0%	4 100%	4 25%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 22.40 Degrees of freedom = 6
P value = 0.00102447 ← ***Significant***

Table (9) : Distribution of Squamous Metaplasia as regards to time of exposure :

Squamous Metaplasia	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	4 100%	4 100%	3 75%	0 0.0%	11 68.8%
+1	0 0.0%	0 0.0%	1 25%	4 100%	5 31.3%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 12.51 Degrees of freedom = 3
P value = 0.00582796 ← **Significant**

Table (10) : Distribution of Blood Capillary Roughness as regards to time of exposure :

Capillary Roughness	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	4 100%	4 100%	2 50%	0 0.0%	10 62.5%
+1	0 0.0%	0 0.0%	2 50%	3 75%	5 31.3%
++2	0 0.0%	0 0.0%	0 0.0%	1 25.%	1 6.3%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 12.80 Degrees of freedom = 6
P value = 0.04632422 ← **Significant**

Table (11): Distribution of Nuclear Vacuolation as regards to time of exposure

Nuclear vacuolation	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	4 100%	2 50%	2 50%	0 0.0%	8 50.5%
+1	0 0.0%	2 50%	1 25%	0 0.0%	3 18.8%
++2	0 0.0%	0 0.0%	1 25%	2 50%	3 18.8%
+++3	0 0.0%	0 0.0%	0 0.0%	2 50%	2 12.5%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 17.33 Degrees of freedom = 9
P value = 0.04374481 ←***Significant***

Table (12) : Distribution of Pyknosis as regards to time of exposure :

Pyknosis	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	4 100%	0 0.0%	0 0.0%	0 0.0%	4 25%
+1	0 0.0%	4 100%	1 25%	0 0.0%	5 31.3%
++2	0 0.0%	0 0.0%	3 75%	2 50%	5 31.3%
+++3	0 0.0%	0 0.0%	0 0.0%	2 50%	2 12.5%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 32 Degrees of freedom = 9
P value = 0.00019913 ←***Significant***

Table (13) : Distribution of Hypochromatosis as regards to time of exposure

Hypochro.	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	4 100%	3 75%	3 75%	0 0.0%	10 62.5%
+1	0 0.0%	1 25%	1 25%	4 100%	6 37.5%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 9.60 Degrees of freedom = 3
P value = 0.02229098 ← ***Significant***

Table (14) : Distribution of Lymphocytosis as regards to time of exposure :

Lympho-cytosis	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	3 75%	0 0.0%	0 0.0%	0 0.0%	3 18.8%
+1	1 25%	4 100%	2 50%	0 0.0%	7 43.8%
++2	0 0.0%	0 0.0%	1 25%	0 0.0%	1 6.3%
+++3	0 0.0%	0 0.0%	1 25%	4 100%	5 31.3%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 25.6 Degrees of freedom = 9
P value = 0.00237444 ← ***Significant***

Table (15) : Distribution of Destructed Cilia and Microvilli as regards to time of exposure :

Destructed Cilia & Microvilli	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	3 75%	0 0.0%	0 0.0%	0 0.0%	3 18.8%
+1	1 25%	4 100%	2 50%	0 0.0%	7 43.8%
++2	0 0.0%	0 0.0%	2 50%	1 25%	3 18.8%
+++3	0 0.0%	0 0.0%	0 0.0%	3 75%	3 18.8%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 26.67 Degrees of freedom = 9
P value = 0.00158776 ← ***Significant***

Table (16) : Distribution of Mitochondria Destruction as regards to time of exposure :

Mitoch. Destruction	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	3 75%	0 0.0%	0 0.0%	0 0.0%	3 18.8%
+1	1 25%	4 100%	2 50%	0 0.0%	7 43.8%
++2	0 0.0%	0 0.0%	1 25%	2 50%	3 18.8%
+++3	0 0.0%	0 0.0%	1 25%	2 50%	3 18.8%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 21.33 Degrees of freedom = 9
P value = 0.01124984 ← ***Significant***

Table (17) : Distribution of Non-significant Nuclear Changes as regards to time of exposure :

	1st Group 10 min. Exp.	2nd Group 20 min. Exp.	3rd Group 30 min. Exp.	4th Group Chronic Exp.	Total	Chi - Square (χ^2)	P-Value
(1) Nuclear Elongation						10.40	0.108*
0 (-ve)	4 100%	3 75%	3 75%	0 0.0%	10 62.5%		
+1	0 0.0%	1 25%	1 25%	3 75%	5 31.3%		
++2	0 0.0%	0 0.0%	0 0.0%	1 25%	1 6.3%		
Total	4 25%	4 25%	4 25%	4 25%	16		
(2) Hyper-chromatosis						10.22	0.115*
0 (-ve)	4 100%	2 50%	3 75%	0 0.0%	9 56.3%		
+1	0 0.0%	2 50%	1 25%	3 75%	6 37.5%		
++2	0 0.0%	0 0.0%	0 0.0%	1 25%	1 6.3%		
Total	4 25%	4 25%	4 25%	4 25%	16		

	1st Group 10 min. Exp.	2nd Group 20 min. Exp.	3rd Group 30 min. Exp.	4th Group Chronic Exp.	Total	Chi - Square (χ^2)	P-Value
(3) Nuclear Irregularity						12.09	0.208*
0 (-ve)	4 100%	3 75%	2 50%	0 0.0%	9 56.3%		
+1	0 0.0%	1 25%	2 50%	2 50%	5 31.3%		
++2	0 0.0%	0 0.0%	0 0.0%	1 25%			
+++3	0 0.0%	0 0.0%	0 0.0%	1 25%	1 6.3%		
Total	4 25%	4 25%	4 25%	4 25%	16		
(4) Peri-Nuclear Halo						2.29	0.515*
0 (-ve)	4 100%	4 100%	3 75%	3 75%	14 87.5%		
+1	0 0.0%	0 0.0%	1 25%	1 25%	2 12.5%		
Total	4 25%	4 25%	4 25%	4 25%	16		

* ← ***Non Significant***

Table (18) : Distribution of Non-significant Cellular Changes as regards to time of exposure :

	1st Group 10 min. Exp.	2nd Group 20 min. Exp.	3rd Group 30 min. Exp.	4th Group Chronic Exp.	Total	Chi - Square (χ^2)	P-Value
(1) Proprial Oedema						11.73	0.228*
0 (-ve)	3 75%	2 50%	0 0.0%	0 0.0%	5 31.3%		
+1	1 25%	2 50%	2 50%	1 25%	6 37.5%		
++2	0 0.0%	0 0.0%	1 25%	1 25%	2 12.5%		
+++3	0 0.0%	0 0.0%	1 25%	2 50%	3 18.8%		
Total	4 25%	4 25%	4 25%	4 25%	16		
(2) Phagocytic Bodies						6.00	0.423*
+1	4 100%	2 50%	3 75%	2 50%	11 68.8%		
++2	0 0.0%	2 50%	1 25%	1 25%	4 25%		
+++3	0 0.0%	0 0.0%	0 0.0%	1 25%	1 6.3%		
Total	4 25%	4 25%	4 25%	4 25%	16		

	1st Group 10 min. Exp.	2nd Group 20 min. Exp.	3rd Group 30 min. Exp.	4th Group Chronic Exp.	Total	Chi - Square (χ^2)	P-Value
(3) Destructed Endoplasmic Reticulum						12.89	0167*
0 (-ve)	2 50%	0 0.0%	0 0.0%	0 0.0%	2 12.5%		
+1	2 50%	3 75%	3 75%	1 25%	9 56.3%		
++2	0 0.0%	1 25%	0 0.0%	2 50%	3 18.8%		
+++3	0 0.0%	0 0.0%	1 25%	1 25%	2 12.5%		
Total	4 25%	4 25%	4 25%	4 25%	16		
(4) Intercellular Spaces						10.40	0.108*
0 (-ve)	3 75%	2 50%	0 0.0%	0 0.0%	54 31.3%		
+1	1 25%	1 25%	4 100%	3 75%	9 56.3%		
++2	0 0.0%	1 25%	0 0.0%	1 25%	2 12.5%		
Total	4 25%	4 25%	4 25%	4 25%	16		

	1st Group 10 min. Exp.	2nd Group 20 min. Exp.	3rd Group 30 min. Exp.	4th Group Chronic Exp.	Total	Chi - Square (χ^2)	P-Value
(5) Cytoplasmic Vacuolization						11.86	0.065*
0 (-ve)	4 100%	3 75%	1 25%	0 0.0%	8 50.0%		
+1	0 0.0%	1 25%	3 75%	3 75%	7 43.8%		
++2	0 0.0%	0 0.0%	0 0.0%	1 25%	1 6.3%		
Total	4 25%	4 25%	4 25%	4 25%	16		
(6) Oesinophilia						9.23	0.416*
0 (-ve)	1 25%	0 0.0%	0 0.0%	0 0.0%	1 6.3%		
+1	3 75%	4 100%	3 75%	3 75%	13 81.3%		
++2	0 0.0%	0 0.0%	1 25%	0 0.0%	1 6.3%		
+++3	0 0.0%	0 0.0%	0 0.0%	1 25%	1 6.3%		
Total	4 25%	4 25%	4 25%	4 25%	16		

* ← ***Non Significant***

II.HISTOPATHOLOGICAL RESULTS

Effects of 10 minutes exposure to the CO_2 laser smoke (1st group)

Exposure to CO_2 laser smoke for 10 minutes will result in the following :

1- Pulmonary inflammatory response as an interstitial congestion of the lung tissue with inflammatory cellular infiltrates in the interalveolar tissue. (Fig.47)

2- Congested blood vessels with edematous thick wall.(Fig.48)

3- Some alveoli become narrowed as a result of congestion. (Fig.49)

4- Distended alveolar sacs & alveolar ducts. (Fig.49)

Effects of 20 minutes exposure to the CO_2 laser smoke (2nd group)

Accentuated features as in the 1st group but we have also the following :

1- Interalveolar hemorrhage becomes more extensive with haemosiderin deposits. (Fig.50)

2- Dilated broncheoles. (Fig.51)

3- Peri-bronchial lymphocytic aggregation. (Fig.50)

4- More distended alveolar sac with detached epithelium. (Fig.52)

5- Focal areas of interstitial pneumonia with congested alveoli. (Fig.47)

6- Interstitial congestion with atrophy of some alveoli but dilated others. (Fig.50)

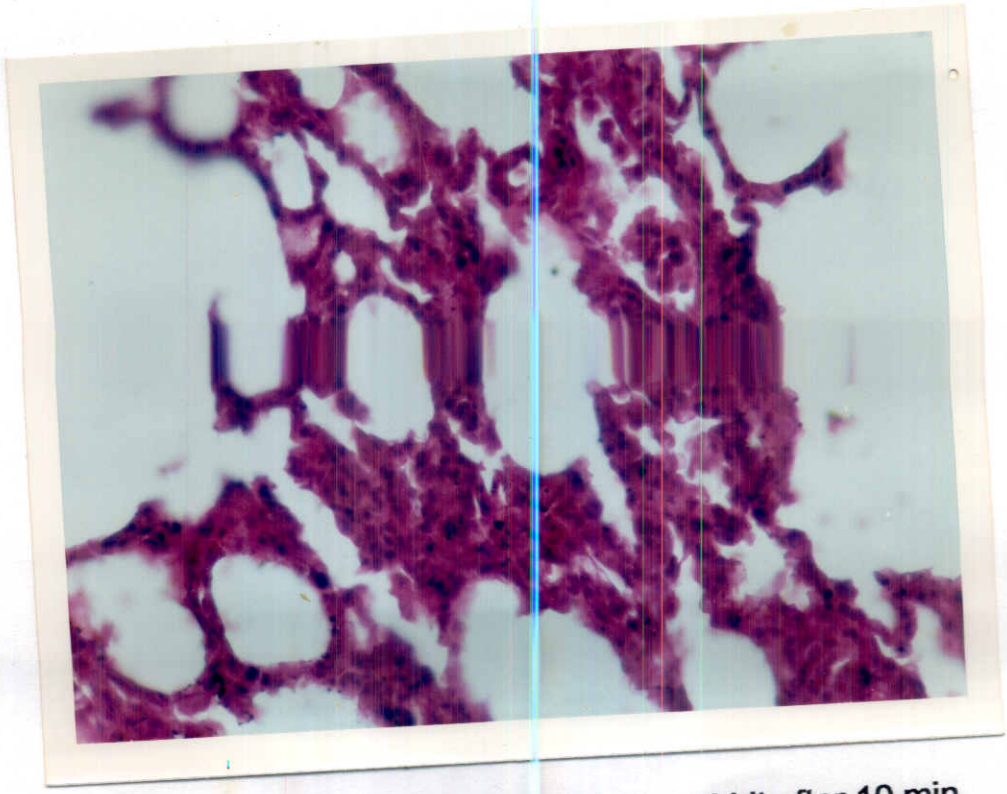


Fig. (47) : Histopathological picture of the lung of a rabbit after 10 min. exposure showing : pulmonary inflammatory response & pneumonia with inflammatory cellular infiltrates (I). (X200).

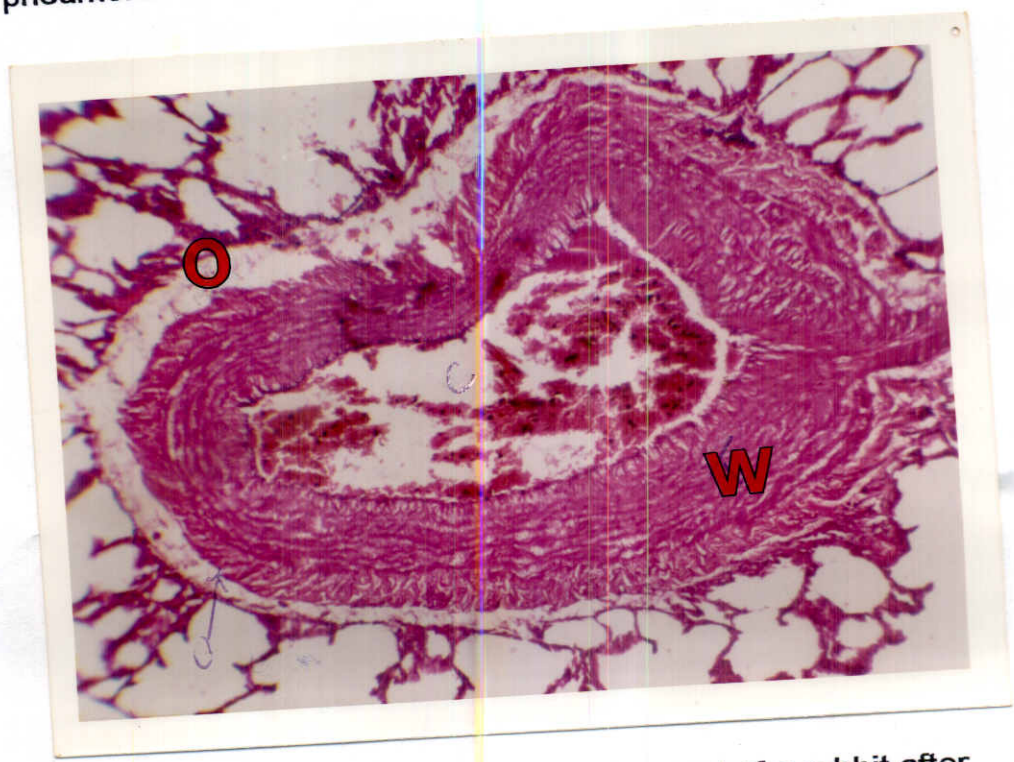


Fig. (48) : Histopathological picture of the blood vessel of a rabbit after 10 min. exposure showing :congested blood vessel with edematous thick wall (W), perivascular (O). (X200).

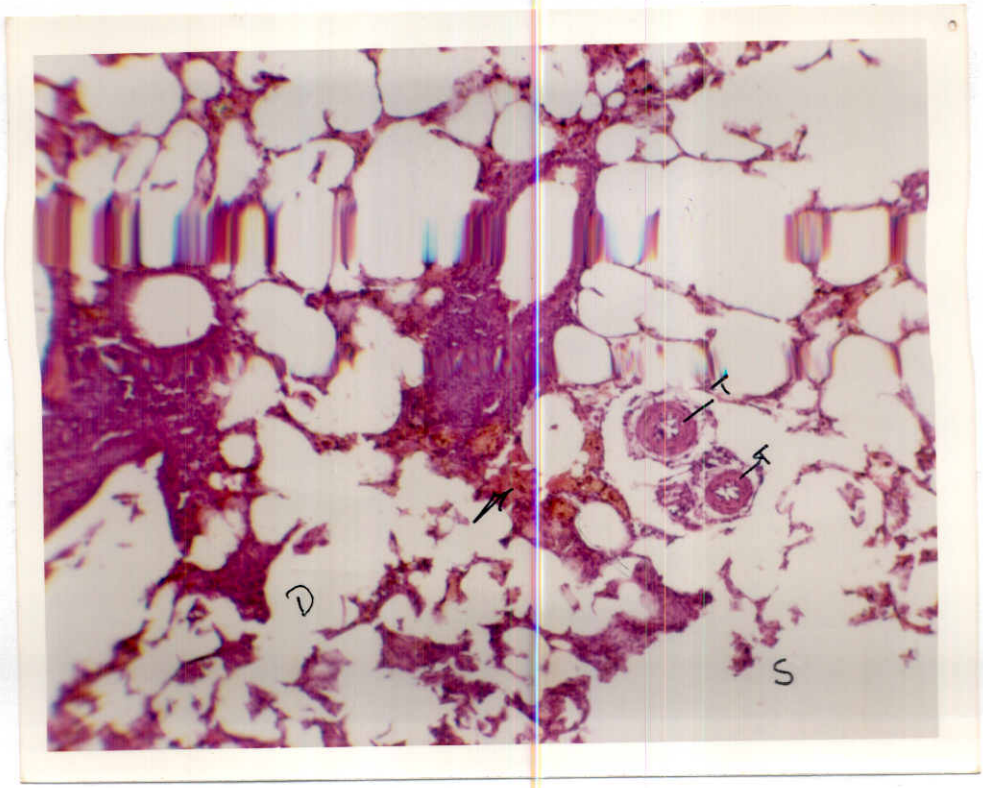


Fig. (49) : Histopathological picture of the lung of a rabbit after 10 min. exposure showing : patchy areas of congestion (arrows) & distended alveolar sac (S) & alveolar duct. (D), thick blood vessel (T). (X100).

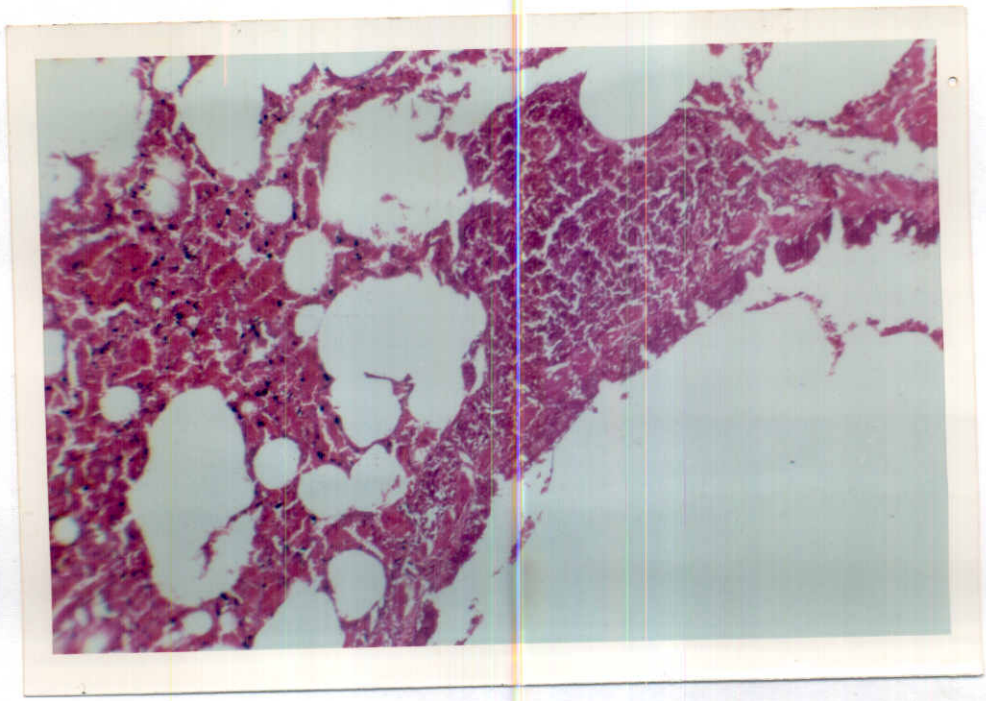


Fig. (50) : Histopathological picture of the lung of a rabbit after 20 min. exposure showing : interalveolar hemorrhage (H), with hemosidren deposits, narrowed alveoli due to compression atrophy (A) with peri bronchial lymphocytic aggregation (P). (X100).

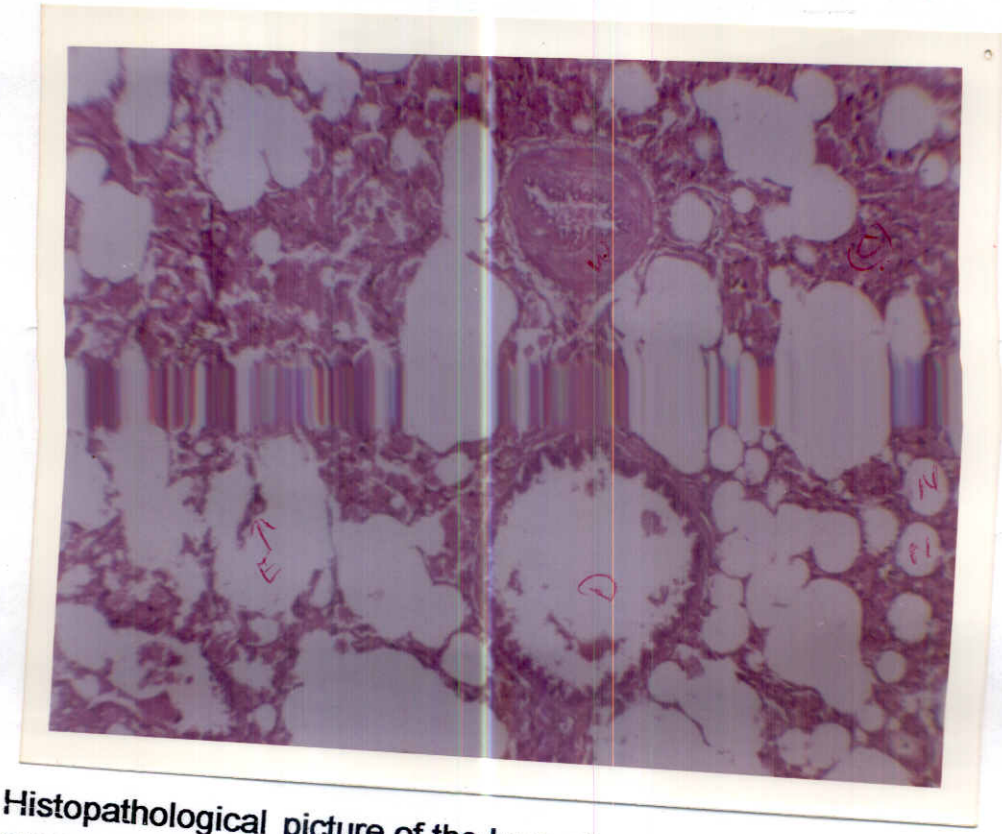


Fig. (51) : Histopathological picture of the lung of a rabbit after 20 min. exposure showing : dilated bronchiole(D), edematous blood vessel wall (W) with detached epith. in the alveolar sac (E) & narrowed alveoli (I) as a result of congestion. (X100).

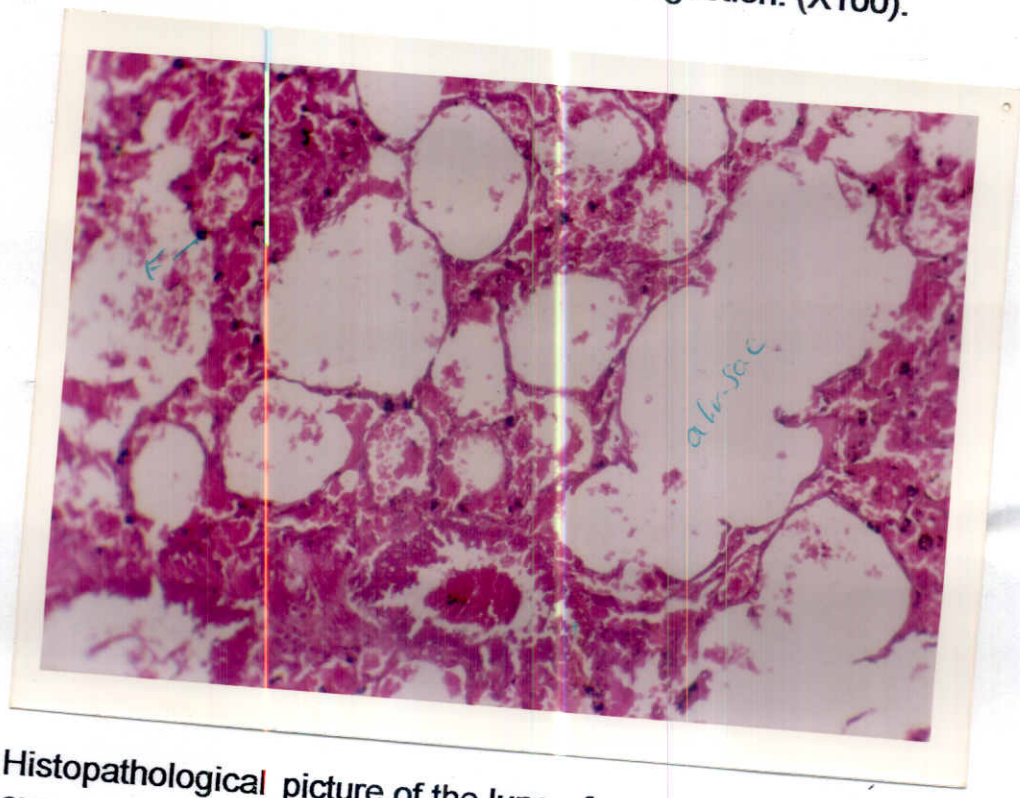


Fig. (52) : Histopathological picture of the lung of a rabbit after 20 min. exposure showing :distended alveolar sac (S), with detached epith. (E), & narrowed some alveoli (I). (X100).

Effects of 30 minutes exposure to the CO_2 laser smoke (3rd group)

More accentuated features were notified as follows :

1- Detached endothelium with hyalinization & swollen wall, perivascular edema with acute & chronic cellular infiltration. (Fig.52,53,61)

2- Areas of emphysematous changes and dilated broncheoles. (Fig.54)

3- More lymphocytic aggregation with lymphoid follicle formation. (Fig.56)

4- Desquamation of the lining epithelium inside the alveoli. (Fig.55)

5- Intra-alveolar migration of phagocytic cells inside the alveoli. (Fig.55)

6- Peribronchial inflammatory cellular infiltration with compensatory emphysema (Fig.54,57) & interstitial pneumonia. (Fig.47)

7- Goblet cells becomes more thin with atrophic changes. (Fig.56)

8- Thrombosed pulmonary capillaries. (Fig.57)

9- Thickened edematous wall of blood vessels (arterioles) with detached endothelium. (Fig.53, 54, 61)

10- Destructed cilia with detached epithelial layer and perivascular edema in subepithelium. (Fig.58)

Fig. (55) : Histopathological picture of the lung of a rabbit after 30 min. exposure showing : desquamation of epith. lining inside the alveoli (d) & intra-alveolar migration of phagocytic cells (P). (X100).

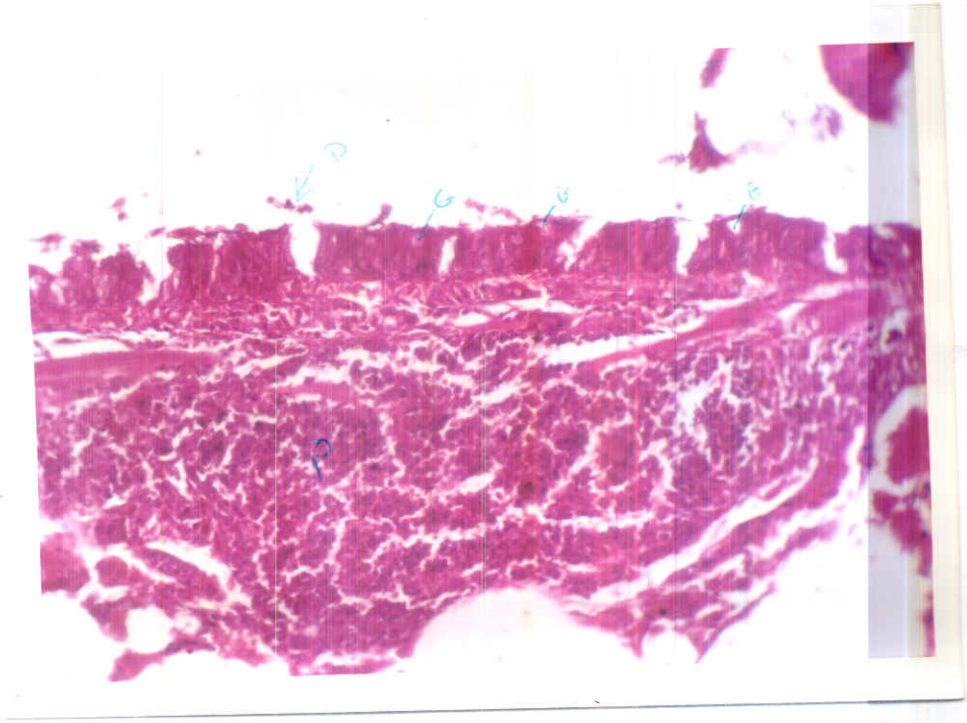


Fig. (56) : Histopathological picture of the lung of a rabbit after 30 min. exposure showing :goblet cells (G), becomes more thin with atrophic changes. Peri-bronchial lymphocytic infiltration (B) & destructed cilia (D). (X100).

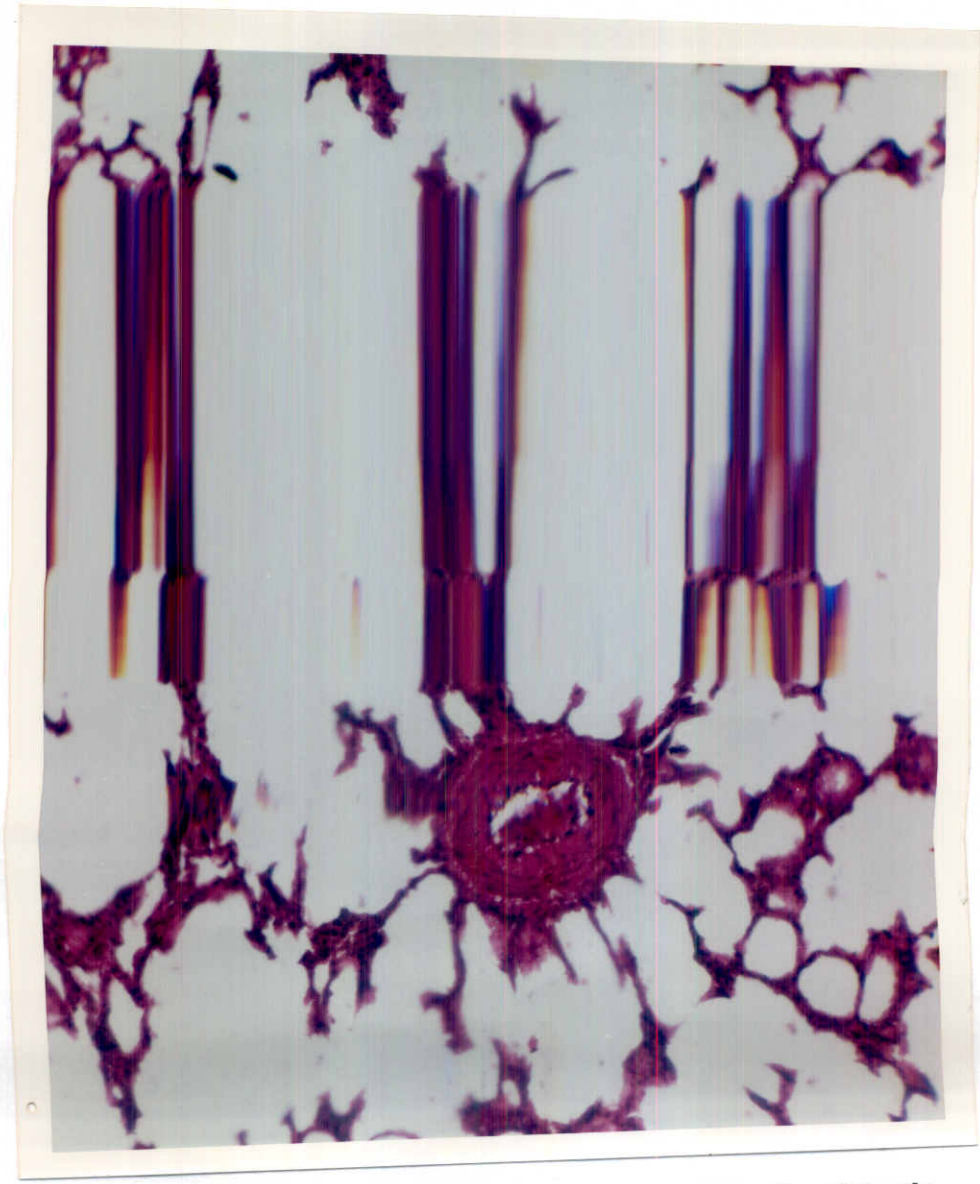
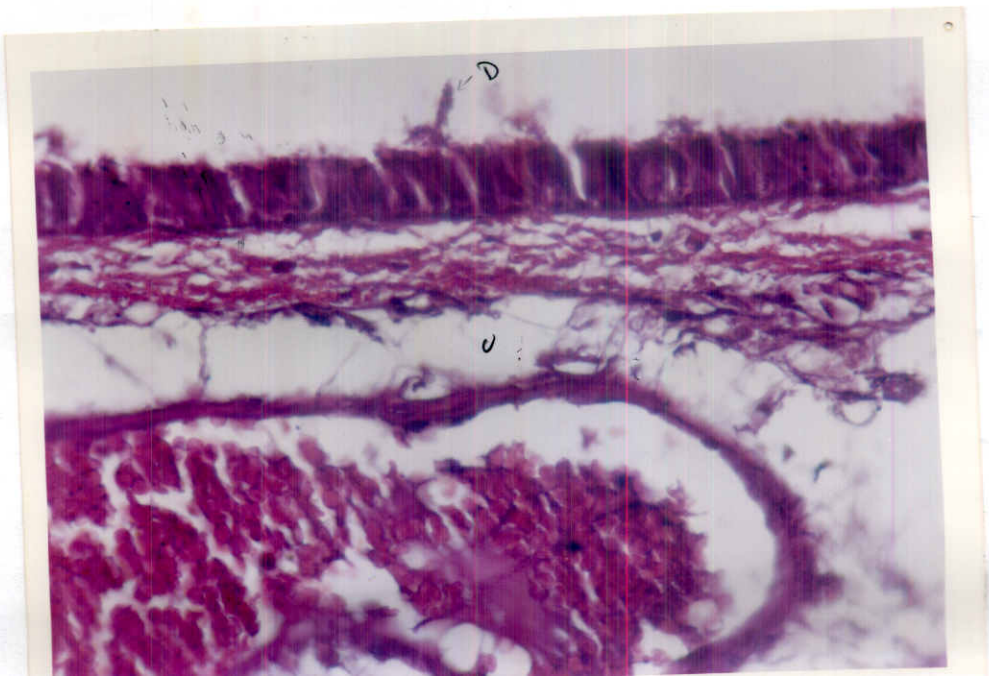


Fig. (57) : Histopathological picture of the lung of a rabbit after 30 min. exposure showing :thrombosed pulmonary capillaries (T), hyalinization of blood vessel wall (H) with emphysema (E). (X100).



Effects of Chronic exposure to the CO_2 laser smoke 4th group

1- Emphysema becomes more extensive. (Fig.59)

2- Thickened blood vessel walls with hyaline degenerative changes of the intima. (Fig.60, 61)

3- Abnormal chondrocytes in the cartilage plates of bronchi we find degenerative cells, (karyolysis, stellate chondrocytes i.e suffering from different stages of atrophy). (Fig.62)

4- **Metaplastic changes** :

a- Blood vessels :

1- The endothelium shows patchy areas of perpendicular endothelial cells. (Fig.63)

2- Stratification of endothelium in the pulmonary artery branches, the simple squamous epithelium changed into stratified squamous epithelium. (Fig.64)

b- Lung :

- Beginning of metaplastic changes of epithelial lining of broncheoles, the simple columnar epithelium changed into pseudostratified columnar epithelium with cilia, with formation of fibrin threads & peribroncheolar inflammatory lymphocytic aggregates (Fig.65)

c- Trachea :

- Blebs protrusions in the apical border of most upper cells of metaplastic epithelium in the trachea. (Fig.66)

5- Lung collapse and fibrosis. (Fig.67)

6- Thick layer of subepithelial fibrin formation in the bronchus. (Fig.68)

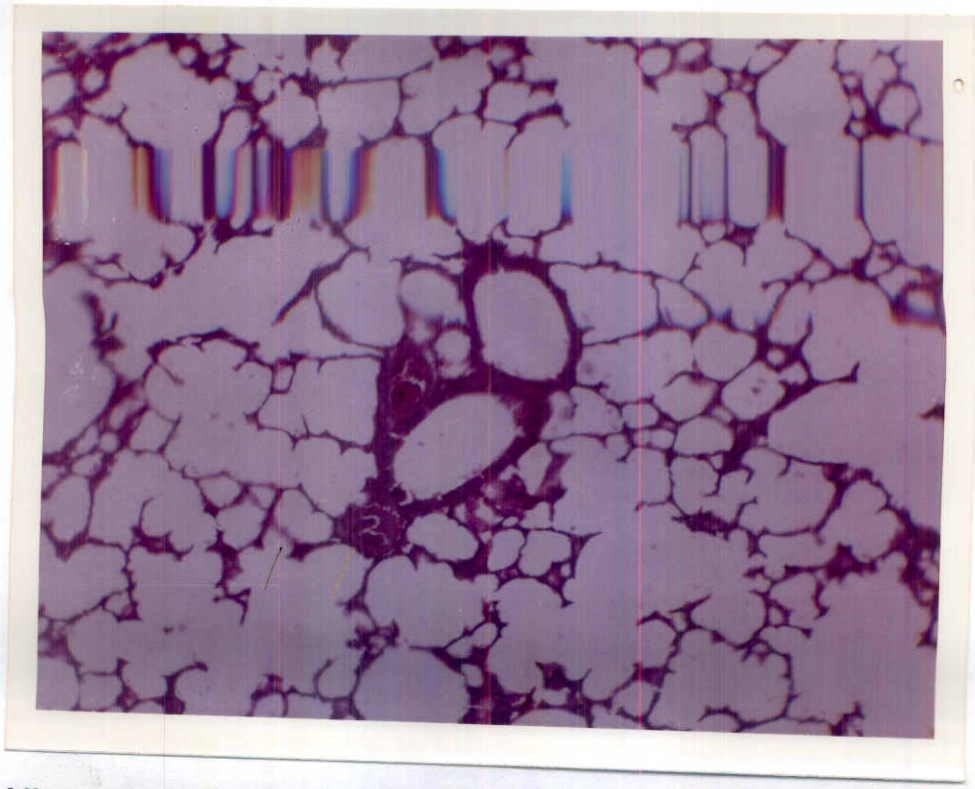


Fig. (59) : Histopathological picture of the lung of a rabbit after chronic exposure showing :extensive emphysema (E) with thrombosed pulmonary arterioles (T). (X100).

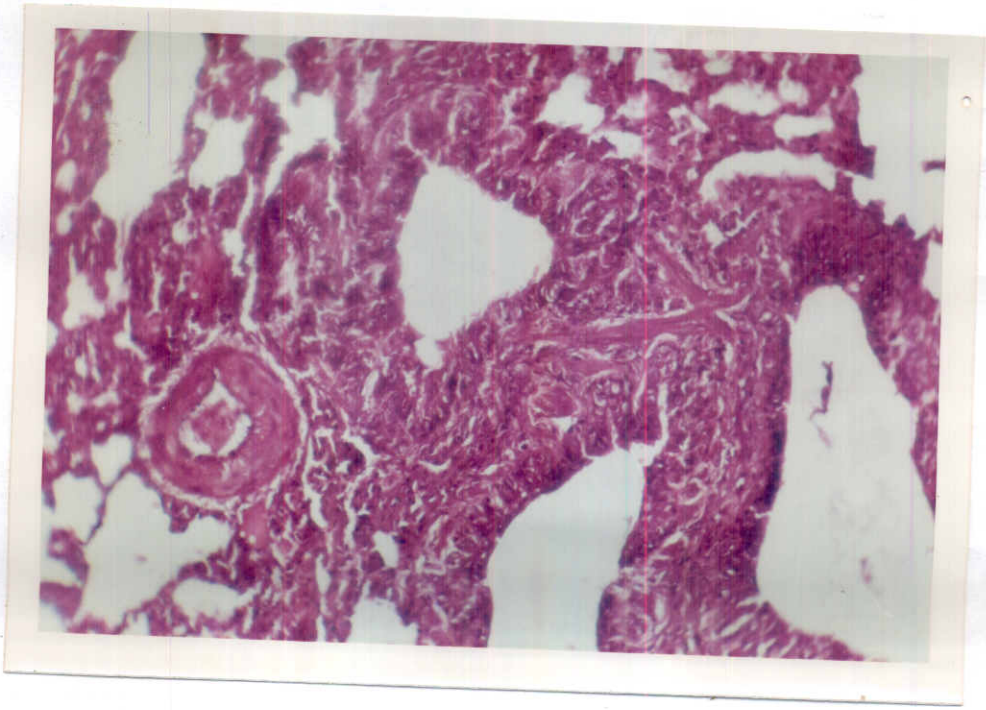


Fig. (60) : Histopathological picture of the lung of a rabbit after chronic exposure showing : thickened blood vessel walls with hyaline degenerative changes (H) & peribronchial fibrosis (F). (X100).

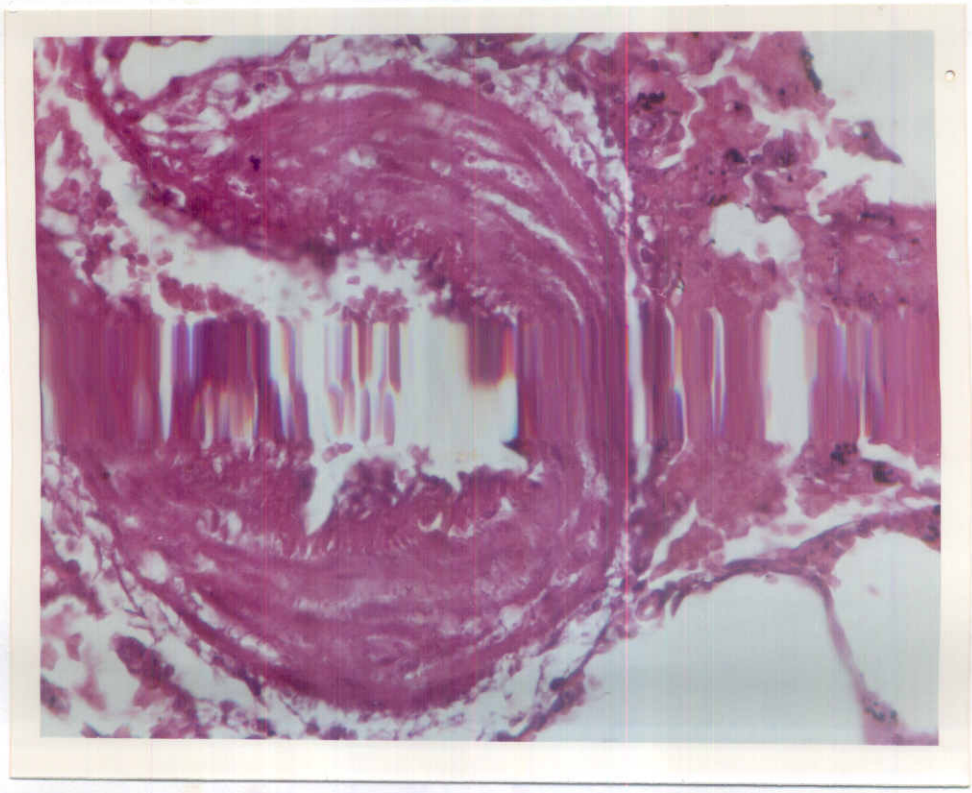


Fig. (61) : Histopathological picture of the blood vessel of a rabbit after chronic exposure showing : thickened wall with hyaline degeneration (H) & detached endothelium (t). (X100).

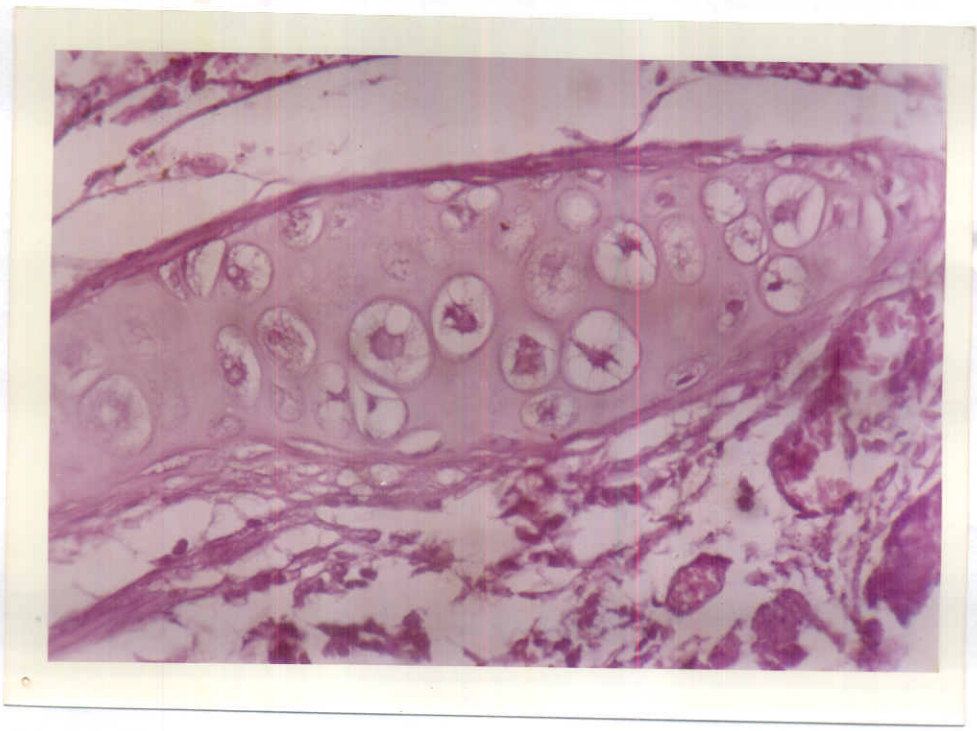


Fig. (62) : Histopathological picture of the bronchus of a rabbit after chronic exposure showing : abnormal chondrocytes (d) with degenerative changes : Karyolysis (K) & stellate chondrocytes (S). (X200).

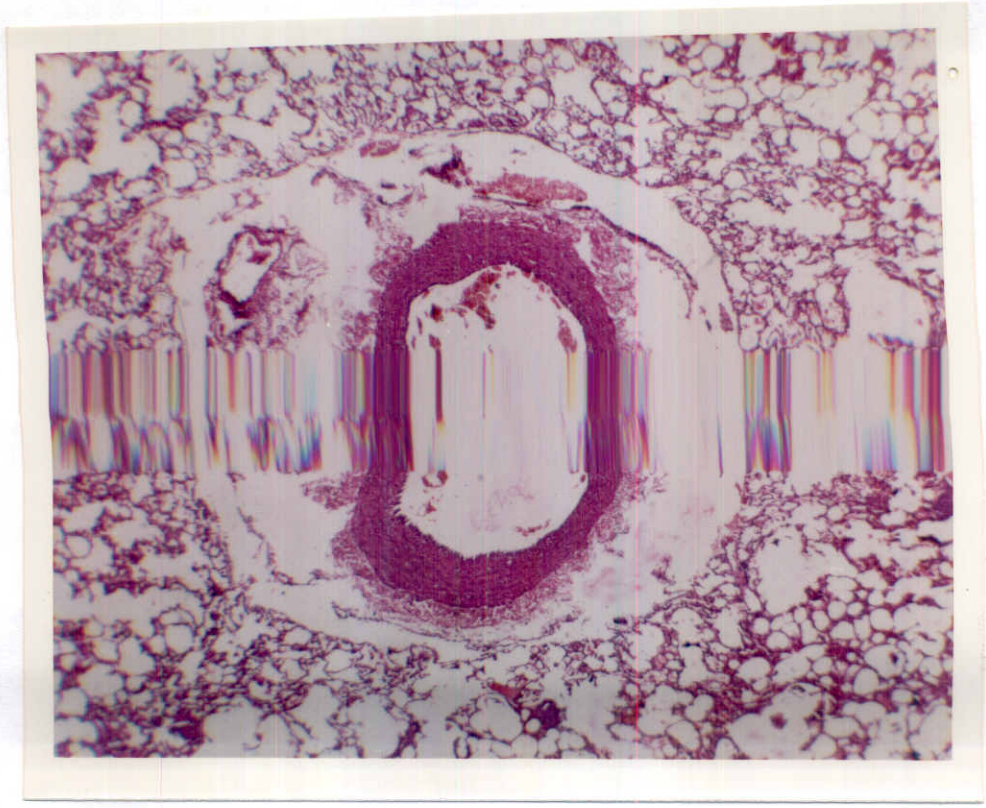


Fig. (63) : Histopathological picture of the blood vessel of a rabbit after chronic exposure showing : peri-arterial edema (E), thickened endothelium (T) & patchy areas of perpendicular endothelial cells (arrows). (X100).

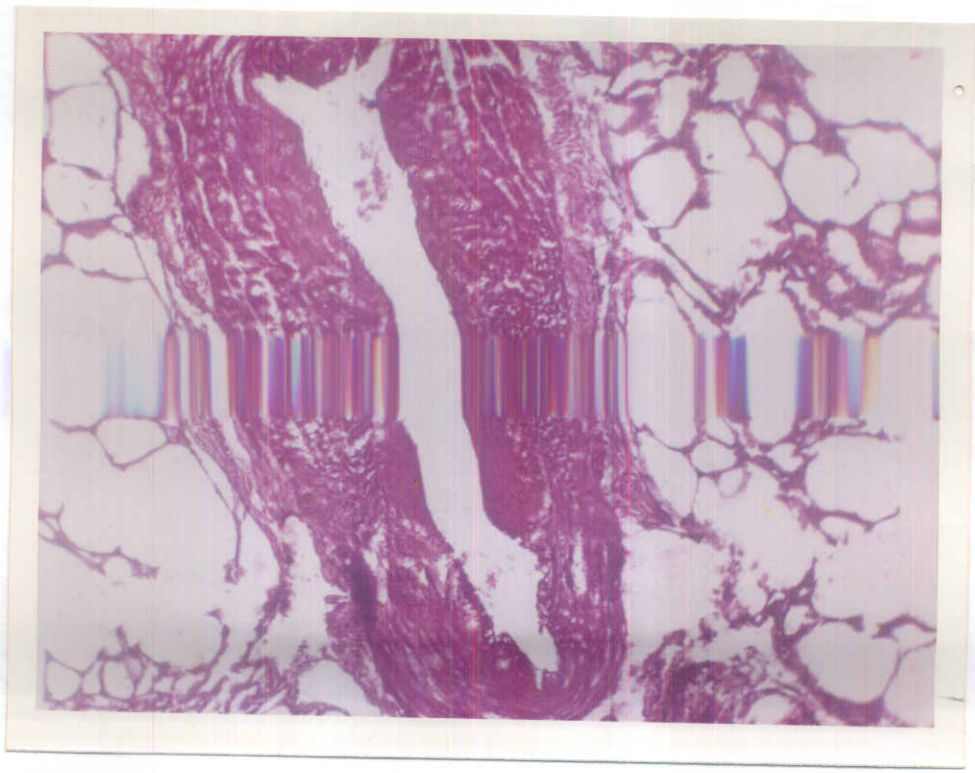


Fig. (64) : Histopathological picture of the blood vessel of a rabbit after chronic exposure showing : stratification of endothelium in the pulmonary artery branches (S) i.e metaplasia of endothelium the simple squamous is changed into stratified squamous.(X100).

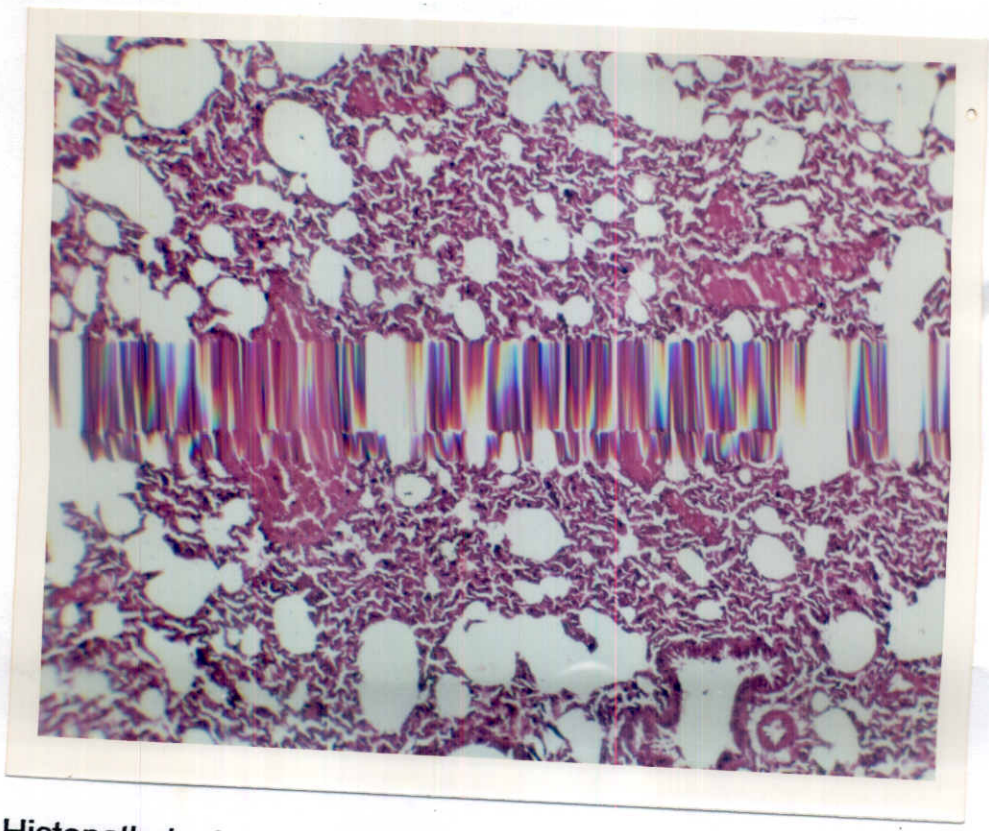


Fig. (67) : Histopathological picture of the lung of a rabbit after chronic exposure showing : lung collapse &fibrosis
(A) : narrowed alveoli.
(I) : Interstitial fibrosis. (X100).

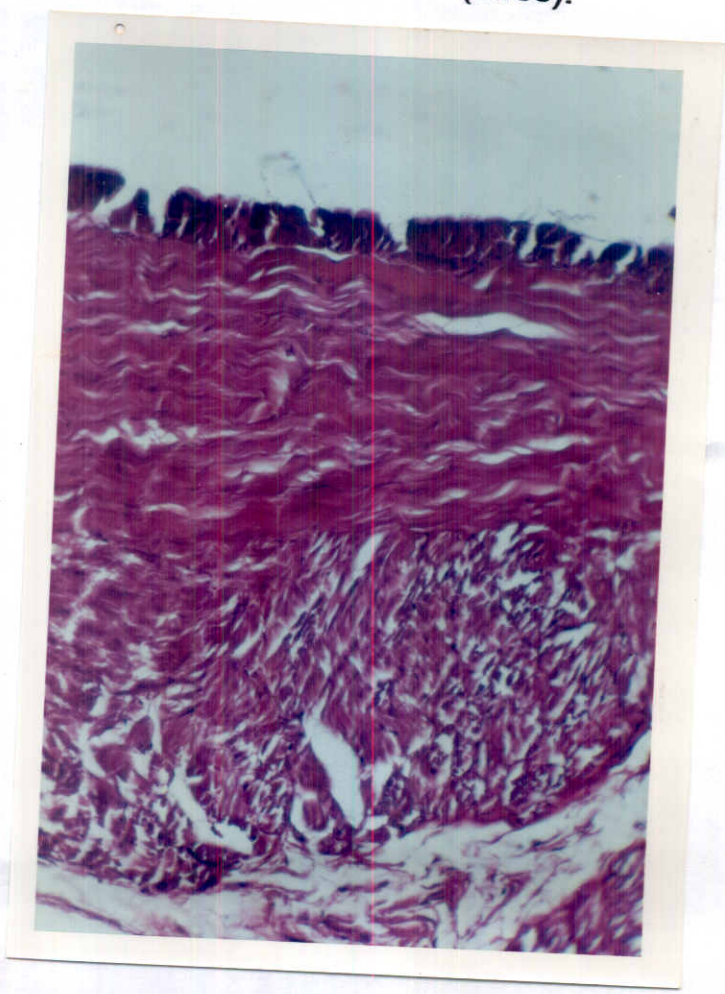


Fig. (68) : Histopathological picture of the lung of a bronchus of rabbit after chronic exposure showing : thick layer of subepith. fibrin formation in the bronchus (K). (X100)

● Control Group :

Histopathological picture of control group were observed in the following figures :

Fig. (69) : showing the lung Control with normal blood vessel.
Fig. (70) : showing the normal brouchus.
Fig. (71) : showing the normal chondrocytes.
Fig. (72) : showing the normal Trachea.

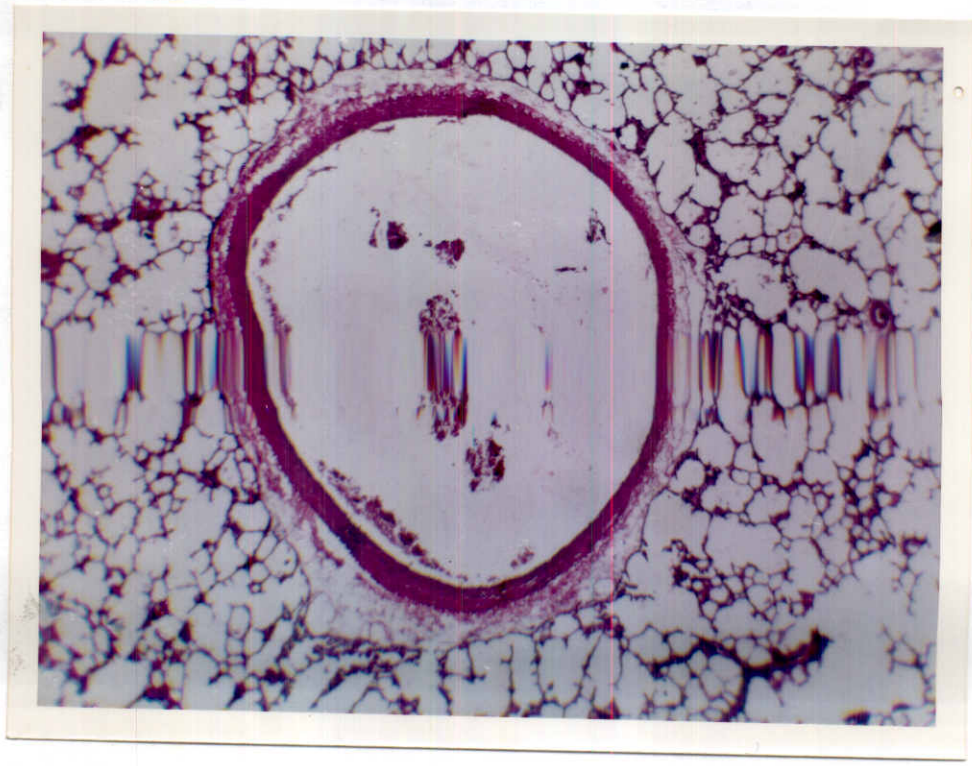


Fig. (69) : Histopathological picture of the lung of control rabbit showing : normal alveoli (A).
normal pulmonary vessel (P).(X100).

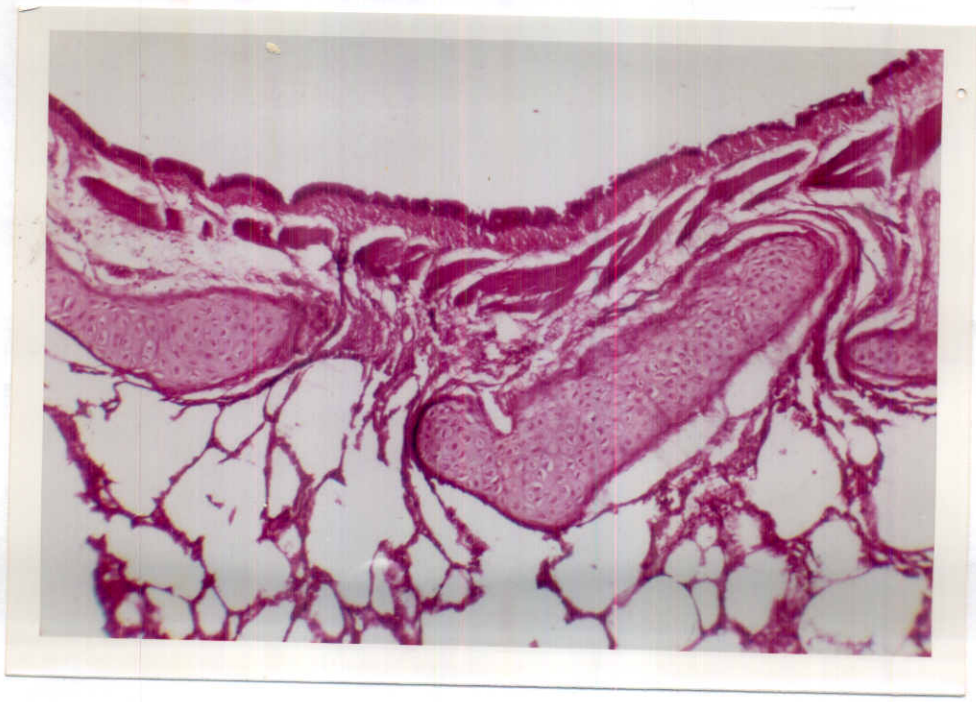


Fig. (70) : Histopathological picture of the lung of control rabbit showing : normal bronchus (B). (X100).

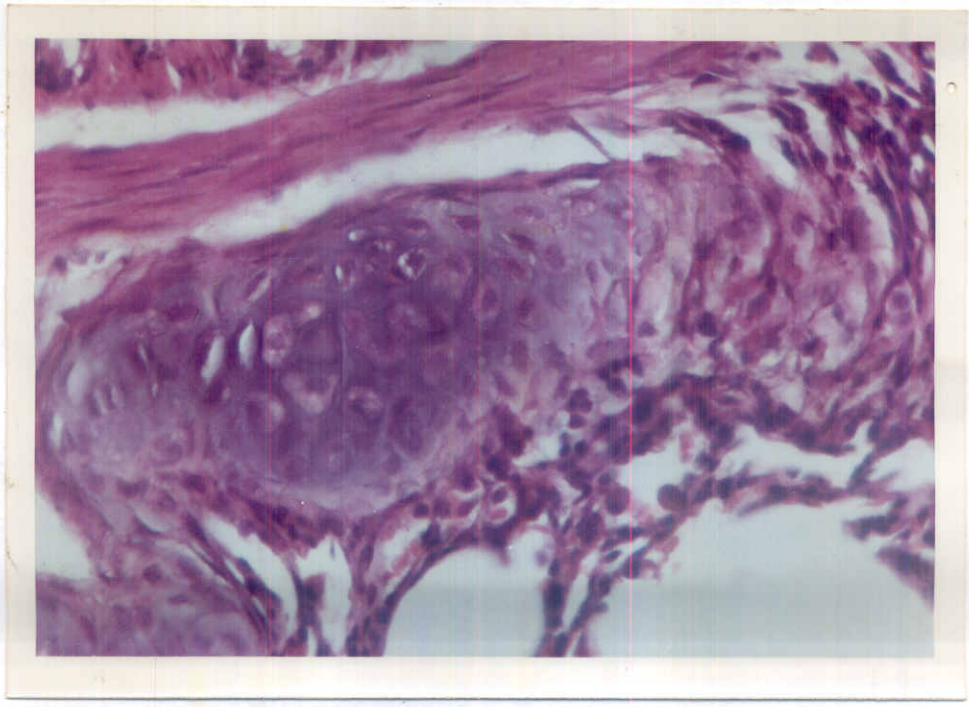


Fig. (71) : Histopathological picture of the bronchus of control rabbit showing : normal chondrocytes (Y). (X100).

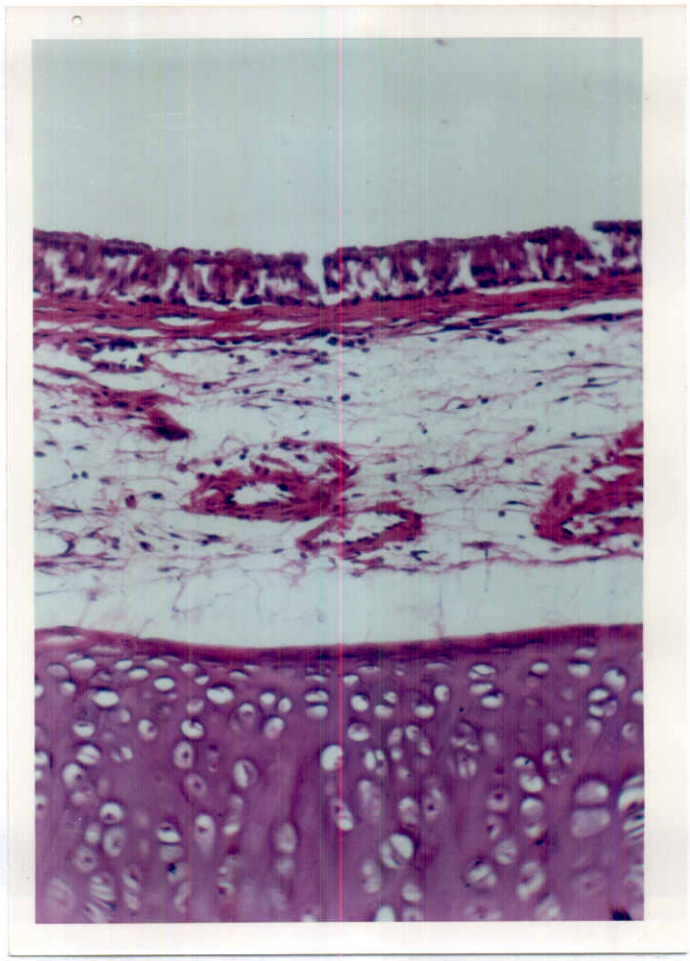


Fig. (72) : Histopathological picture of the normal trachea of control rabbit. (X100)

STATISTICAL RESULTS

● Histopathological Data :

We get the following **significant** data :

1- Emphysema.	*Table 19*
2- Lymphocytic aggregation.	*Table 20*
3- Hyalinization of blood vessel walls.	*Table 21*
4- Destructed cilia.	*Table 22*
5- Atrophic thin goblet cells.	*Table 23*
6- Interstitial fibrosis.	*Table 24*
7- Phagocytic cell migration.	*Table 25*
8- Interstitial congestion.	*Table 26*
9- Thrombosed pulmonary capillaries & arterioles	*Table 27*
10- Metaplastic changes.	*Table 28*
11- Inter-alveolar hemorrhage.	*Table 29*
12- Desquamation of epithelial cells.	*Table 30*

The **non-significant** histopathological data were : *Table 31*

1- Abnormal chondrocytes.
2- Narrowed alveoli.
3- Distended alveolar sacs and ducts.
4- Interstitial pneumonia.
5- Inflammatory cellular infiltration.

Table (19) : Distribution of Emphysema as regards to time of exposure :

Emphysema	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	4 100%	4 100%	3 75%	0 0.0%	11 68.8%
+1	0 0.0%	0 0.0%	1 25%	0 0.0%	1 6.3%
++2	0 0.0%	0 0.0%	0 0.0%	3 75%	3 18.8%
+++3	0 0.0%	0 0.0%	0 0.0%	1 25%	1 6.3%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 18.91 Degrees of freedom = 9
P value = 0.02597707 ← ***Significant***

Table (20) : Distribution of Lymphocytic Aggregation as regards to time of exposure :

Lymphocytic Aggregation	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	3 75%	0 0.0%	0 0.0%	0 0.0%	3 18.8%
+1	1 25%	4 100%	2 50%	0 0.0%	7 43.8%
++2	0 0.0%	0 0.0%	2 50%	3 75%	5 31.3%
+++3	0 0.0%	0 0.0%	0 0.0%	1 25%	1 6.3%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 22.40 Degrees of freedom = 9
P value = 0.0769412 ← ***Significant***

Table (21) : Distribution of Hyalinization of blood Vessel Wall as regards to time of exposure :

B.V Hyalinization	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	4 100%	4 100%	2 50%	1 25%	11 68.8%
+1	0 0.0%	0 0.0%	2 50%	0 0.0%	2 12.5%
++2	0 0.0%	0 0.0%	0 0.0%	3 75%	3 18.8%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 17.45 Degrees of freedom = 6
P value = 0.00775018 ← ***Significant***

Table (22) : Distribution of Destructed Cilia as regards to time of exposure :

Destructed Cilia	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	3 75%	0 0.0%	0 0.0%	1 25%%	4 25%
+1	1 25%	4 100%	4 100%	1 25%	10 62.50%
++2	0 0.0%	0 0.0%	0 0.0%	2 50%	2 12.5%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 15.60 Degrees of freedom = 6
P value = 0.01606981 ← ***Significant***

Table (23) : Distribution of Thin Goblet Cells as regards to time of exposure :

Thin Goblet Cells	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	4 100%	4 100%	3 75%	1 25%%	12 75%
+1	0 0.0%	0 0.0%	1 25%	3 75%	4 25%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 8.00 Degrees of freedom = 3
P value = 0.04601171 ←***Significant***

Table (24) : Distribution of Interstitial Fibrosis as regards to time of exposure :

Interstitial Fibrosis	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	4 100%	4 100%	3 75%	0 0.0%	11 68.8%
+1	0 0.0%	0 0.0%	1 25%	0 0.0%	1 6.3%
++2	0 0.0%	0 0.0%	0 0.0%	3 75%	3 18.8%
+++3	0 0.0%	0 0.0%	0 0.0%	1 25%	1 6.3%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 18.91 Degrees of freedom = 9
P value = 0.02597707 ←***Significant***

Table (25) : Distribution of Phagocytic cell migration as regards to time of exposure :

Phagocytic Cell Migration	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	0 0.0%	0 0.0%	2 50%	3 75%	5 31.3%
+1	4 100%	2 50%	0 0.0%	0 0.0%	6 37.5%
++2	0 0.0%	2 50%	2 50%	1 25%	5 31.3%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 14.93 Degrees of freedom = 6
P value = 0.020778162 ← ***Significant***

Table (26) : Distribution of Interstitial Congestion as regards to time of exposure :

Interstitial Congestion	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	1 25%	1 25%	4 100%	4 100%	10 62.5%
+1	3 75%	0 0.0%	0 0.0%	0 0.0%	3 18.8%
++2	0 0.0%	3 75%	0 0.0%	0 0.0%	3 18.8%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 21.60 Degrees of freedom = 6
P value = 0.00143041 ← ***Significant***

Table (27):Distribution of Thrombosed Pulmonary Capillaries and Arterioles as regards to time of exposure :

Thrombosed Pulmonary Capillaries	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	4 100%	4 100%	3 75%	1 25%	12 75%
+1	0 0.0%	0 0.0%	1 25%	0 0.0%	1 6.3%
++2	0 0.0%	0 0.0%	0 0.0%	3 75%	3 18.8%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 14.00 Degrees of freedom = 6
P value = 0.02963616 ←***Significant***

Table (28) : Distribution of Metaplastic Changes as regards to time of exposure :

Metaplastic Changes	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	4 100%	4 100%	4 100%	1 25%	13 81.3%
+1	0 0.0%	0 0.0%	0 0.0%	3 75%	3 18.8%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 11.08 Degrees of freedom = 3
P value = 0.01131711 ←***Significant***

Table (29) : Distribution of Interalveolar Hemorrhage as regards to time of exposure :

Interalveolar Hemorrhage	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	2 50%	1 25%	2 50%	4 100%	9 56.3%
+1	2 50%	0 0.0%	0 0.0%	0 0.0%	2 12.5%
++2	0 0.0%	3 75%	2 50%	0 0.0%	5 31.3%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 13.51 Degrees of freedom = 6
P value = 0.03560052 ← ***Significant***

Table (30) : Distribution of Desquamation of Epithelium as regards to time of exposure :

Desquamation of Epithelium	1st 10 min.	2nd 20 min.	3rd 30 min	4th Chr. Exp.	Total
0 (-ve)	3 75%	3 75%	2 50%	0 0.0%	8 50%
+1	1 25%	1 25%	2 50%	0 0.0%	4 25%
++2	0 0.0%	0 0.0%	0 0.0%	3 75%	3 18.8%
+++3	0 0.0%	0 0.0%	0 0.0%	1 25%	1 6.3%
Total	4 25%	4 25%	4 25%	4 25%	16

Chi square = 17.00 Degrees of freedom = 9
P value = 0.04871598 ← ***Significant***

Table (31) : Distribution of Non-significant Histopathological Changes as regards to time of exposure :

	1st Group 10 min. Exp.	2nd Group 20 min. Exp.	3rd Group 30 min. Exp.	4th Group Chronic Exp.	Total	Chi - Square (χ^2)	P-Value
(1) Abnormal Chondrocytes						11.08	0.270*
0 (-ve)	4 100%	4 100%	4 100%	1 25%	13 81.3%		
+1	0 0.0%	0 0.0%	0 0.0%	1 25%	1 6.3%		
++2	0 0.0%	0 0.0%	0 0.0%	1 25%	1 6.3%		
+++3	0 0.0%	0 0.0%	0 0.0%	1 25%	1 6.3%		
Total	4 25%	4 25%	4 25%	4 25%	16		
(2) Narrowed Alveoli						8.00	0.238*
0 (-ve)	2 50%	2 50%	4 100%	4 100%	12 75%		
+1	2 50%	1 25%	0 0.0%	0 0.0%	3 18.8%		
++2	0 0.0%	1 25%	0 0.0%	0 0.0%	1 6.3%		
Total	4 25%	4 25%	4 25%	4 25%	16		

	1st Group 10 min. Exp.	2nd Group 20 min. Exp.	3rd Group 30 min. Exp.	4th Group Chronic Exp.	Total	Chi - Square (χ^2)	P-Value
(3) Distended Alveolar Sacs and Ducts						9.33	0.155*
0 (-ve)	2 50%	0 0.0%	2 50%	4 100%	8 50%		
+1	2 50%	3 75%	1 25%	0 0.0%	6 37.5%		
++2	0 0.0%	1 25%	1 25%	0 0.0%	2 12.5%		
Total	4 25%	4 25%	4 25%	4 25%	16		
(4) Interstitial Pneumonia						8.33	0.214*
0 (-ve)	3 75%	2 50%	0 0.0%	3 75%	8 50%		
+1	1 25%	2 50%	3 75%	0 0.0%	6 37.5%		
++2	0 0.0%	0 0.0%	1 25%	1 25%	2 12.5%		
Total	4 25%	4 25%	4 25%	4 25%	16		
(5) Inflammatory Cellular Infilteration						9.00	0.173*
0 (-ve)	1 25%	0 0.0%	1 25%	2 50%	4 25%		
+1	2 50%	0 0.0%	2 50%	0 0.0%	4 25%		
++2	1 25%	4 100%	1 25%	2 50%	8 50%		
Total	4 25%	4 25%	4 25%	4 25%	16		

* ← ***Non Significant***

To summarize the results :

10 min. exposure to the Co_2 laser smoke

Electron Microscopically	Histopathologically
●Massive invasion of lymphocytes ●Loss of cilia & microvilli ●Subepithelial edema ●Swollen collagenic fibrils ●Destructed mitochondria ●Macrophages with numerous phagocytic bodies	●An interstitial congestion of lung tissue . ●Congested blood vessel with edematous thick wall ●Distended alveolar sacs & ducts.

20 min. exposure to the Co_2 laser smoke

Electron Microscopically	Histopathologically
●Darkly stained pyknotic nuclei in some cells. ●Completely destructed cytoplasmic organelles & mitochondria ●Intercellular irregular spaces ●Complete loss of cilia & microvilli ●Numerous oesiophilia ●Nuclear vacuolation of some nuclei. ■Interalveolar hemorrhage.	●Interalveolar hemorrhage with hemosidrin deposits ●Dilated broncheoles ●Lymphocytic aggregation ●Interstitial pneumonia ●Atrophy of some alveoli but dilated the others

30 min. exposure to the Co_2 laser smoke

Electron microscropically	Histophathologically
●Extruded pyknotic nuclei ●Numerous number of inter-cellular lysosomes ●Irregular lobulated nuclei ●Hypochromatic nuclei ●The subepithelial blood capillaries are surrounded by collagenic fibrils ●Interstitial hemorrhage in-between the lung alveoli ●Goblet cell atrophy.	●Detached endothelium with hyalinization swollen blood vessel walls. ●Lmphoid follicle formation ●Thrombosed pulmonary capillaries ●Desquamation of the lining epithelium . ●Atrophic thin goblet cells.

Chronic exposure to the Co_2 laser smoke

1- Electron microscopical results

Nuclear changes	Cellular changes
● Mitotic cell division activity . ● Destructed vacuolated nuclei. ● Karyolysis (no nucleus) of some cells. ● Extruded pyknotic nuclei ● Peri-nuclear halo-formation ● Hypochromatosis .	● Abnormal mitochondria ● Goblet cell atrophy ● Active macrophage with engulfed carbon particles ● Intracytoplasmic smoke black granules ● Abnormal aggregation of blood platlets ● Active fibroblasts with abnormal collagenic fibrils. ● Squamous metaplasia. ● Increased intracytoplasmic lysosomes

2- Histopathological results

- Emphysema
- Thickened blood vessel walls with hyaline degeneration
- Abnormal chondrocytes
- Metaplastic Changes in the form of :
  - Patchy areas of perpendicular endothelial cells.
  - Stratification of endothelium.
  - Blebs protrusion in the apical border of metaplastic epith. in the trachea.
- Lung collapse & fibrosis with hyalinization of brocheoles & bl. vessel walls.