RESULTS

** *R E S U L T S* **

I- CLINICAL STUDY :

A total of 39 patients (60 eyes) suffering from primary open angle glaucoma (21 bilateral and 18 unilateral), of whom 15 males (22 eyes) and 23 females (38 eyes) were included in this study.

Another group of normal individuals (5 patients - 10 eyes) were also included as controls (2 females and 3 males).

The age of all the cases was ranging from 40 to 50 years with a mean of 47.47 years and standard deviation $\pm$ 1.98 .

All glaucomatous patients have been discovered recently during routine ophthalmic examination in the outpatient clinic of the Institute of Ophthalmic Research, and all are of the primary simple glaucoma type with open angles and I.O.P. ranging between 24 mmHg and 30 mmHg and have received no treatment as regard glaucoma. Also all patients and controls have healthy corneas with no previous history of intra-ocular inflammation or surgical procedures.

I-Comparison between means of glaucomatous patients & controls before treatment :

A) I.O.P.(mmHg) :

Table "1"

	Number	Mean	S.D.
* Patients	60	27.2833	2.4570
* Controls	10	15.6000	2.8752

P = 5.0 E-14 (P < 0.001)

- There is highly significant difference between I.O.P. in glaucomatous patients in comparison with controls before therapy.

B) Corneal density (cells/mm^2) , Mean cell area (Um^2/cell) and Corneal thickness (mm) :

Table "2"

	Patients (60)	Controls (10)	Diff.	P	Signif.
Corneal density	2593.425	2945.000	351.575	0.0427	S.
Mean cell area	396.481	342.812	53.669	0.0182	S.
Corneal thickness	0.523	0.450	0.073	<0.001	H.S

S= Significant NS= Nonsignificant HS= Highly significant

There was significant increase in mean central corneal thickness, increase in mean cell area and decrease in mean central corneal density of glaucomatous patients as compared to controls before medical therapy.

II- Comparison between the effect of different types of anti-glaucoma drugs and preservative on the corneal endothelium before and after 3 & 6 months of therapy :

A) Effect on corneal endothelial density

1- Pilocarpine 1%

Table "3"

	Mean	S.D. (Standard deviation)
Pre-ttt	2442.43	± 536.122
3 months	2553.55	± 329.081
6 months	2568.74	± 320.847

P_1 = 0.1708 (P>0.05)
P_2 = 0.1406 (P>0.05)

N.B:

P_1 = Probability of significance between pre-ttt & 3 months
P_2 = Probability of significance between pre-ttt & 6 months

No significant difference was found between corneal endothelial density before and after 3 and 6 months of therapy indicating no effect of pilocarpine 1% on the corneal endothelial cell density after 3 and 6 months of using this medication.

2- Pilocarpine 2%

Table "4"

	Mean	S.D.
Pre-ttt	2689.53	± 363.111
3 months	2703.30	± 399.620
6 months	2724.97	± 410.030

P_1 = 0.3583 (P>0.05)
P_2 = 0.3188 (P>0.05)

No significant difference was found between corneal endothelial density before and after 3 and 6 months of therapy indicating no effect of pilocarpine 2% on the corneal endothelial cell density after 3 and 6 months of using this medication.

3- Timolol maleate 0.25%

Table "5"

	Mean	S.D.
Pre-ttt	2465.00	± 330.350
3 months	2492.40	± 422.620
6 months	2538.25	± 465.950

P_1 = 0.2393 (P>0.05)
P_2 = 0.1066 (P>0.05)

No significant difference was found between corneal endothelial density before and after 3 and 6 months of therapy indicating no effect of timolol maleate 0.25% on the corneal endothelial cell density after 3 and 6 months of using this medication.

4- Timolol maleate 0.5%

Table "6"

	Mean	S.D.
Pre-ttt	2716.21	± 270.880
3 months	2739.50	± 250.427
6 months	2763.00	± 185.27

P_1 = 0.1418 (P>0.05)
P_2 = 0.167 (P>0.05)

No significant difference was found between corneal endothelial density before and after 3 and 6 months of therapy indicating no effect of timolol maleate 0.5% on the corneal endothelial cell density after 3 and 6 months of using this medication.

5- Dipivefrin hydrochloride 0.1%

Table "7"

	Mean	S.D.
Pre-ttt	2623.66	± 392.454
3 months	2627.85	± 404.130
6 months	2614.80	± 393.300

P_1 = 0.446 (P>0.05)
P_2 = 0.3912 (P>0.05)

No significant difference was found between corneal endothelial density before and after 3 and 6 months of therapy indicating no effect of dipivefrin hydrochloride 0.1% on the corneal endothelial cell density after 3 and 6 months of using this medication.

6- Controls [Benzalkonium chloride 0.01%]

Table "8"

	Mean	S.D.
Pre-ttt	2945.00	± 302.514
3 months	2932.10	± 301.079
6 months	2954.50	± 334.518

P_1 = 0.2981 (P>0.05)
P_2 = 0.3859 (P>0.05)

No significant difference was found between corneal endothelial density before and after 3 and 6 months of therapy indicating no effect of benzalkonium chloride 0.01% on the corneal endothelial cell density after 3 and 6 months of using this medication.

B- Effect on mean cell area :

1- Pilocarpine 1%

Table "9"

	Mean	S.D.
Pre-ttt	436.68	± 144.150
3 months	397.43	± 50.323
6 months	394.691	± 48.142

P_1 = 0.1711 (P>0.05)
P_2^1 = 0.1544 (P>0.05)

It was found that, there was no significant difference in the mean endothelial cell area before and after 3 and 6 months of treatment with pilocarpine 1%, indicating no significant effect of this drug on the corneal endothelial mean cell area either after 3 or 6 months of using this drug.

2- Pilocarpine 2%

Table "10"

	Mean	S.D.
Pre-ttt	378.88	± 58.690
3 months	378.25	± 63.137
6 months	374.97	± 59.390

P_1 = 0.4507 (P>0.05)
P_2 = 0.3534 (P>0.05)

It was found that, there was no significant difference in the mean endothelial cell area before and after 3 and 6 months of treatment with pilocarpine 2%, indicating no significant effect of this drug on the corneal endothelial mean cell area either after 3 or 6 months of using this drug.

3- Timolol maleate 0.25%

Table "11"

	Mean	S.D.
Pre-ttt	412.21	± 54.350
3 months	411.68	± 68.710
6 months	405.92	± 77.750

P_1 = 0.4619 (P>0.05)
P_2 = 0.2032 (P>0.05)

It was found that, there was no significant difference in the mean endothelial cell area before and after 3 and 6 months of treatment with timolol maleate 0.25% , indicating no significant effect of this drug on the corneal endothelial mean cell area either after 3 or 6 months of using this drug.

4- Timolol maleate 0.5%

Table "12"

	Mean	S.D.
Pre-ttt	371.79	± 40.677
3 months	368.15	± 38.185
6 months	363.47	± 25.705

P_1 = 0.1117 (P>0.05)
P_2 = 0.14195 (P>0.05)

It was found that, there was no significant difference in the mean endothelial cell area before and after 3 and 6 months of treatment with timolol maleate 0.5% , indicating no significant effect of this drug on the corneal endothelial mean cell area either after 3 or 6 months of using this drug.

5- Dipivefrin hydrochloride 0.1%

Table "13"

	Mean	S.D.
Pre-ttt	389.65	± 60.385
3 months	389.41	± 61.130
6 months	390.98	± 60.170

P_1 = 0.3742 (P>0.05)
P_2 = 0.3742 (P>0.05)

It was found that, there was no significant difference in the mean endothelial cell area before and after 3 and 6 months of treatment with dipivefrin hydrochloride 0.1% , indicating no significant effect of this drug on the corneal endothelial mean cell area either after 3 or 6 months of using this drug.

6- Controls [Benzalkonium chloride 0.01%]

Table "14"

	Mean	S.D.
Pre-ttt	342.81	± 35.320
3 months	344.45	± 36.929
6 months	342.47	± 39.580

P_1 = 0.2911 (P>0.05)
P_2 = 0.4647 (P>0.05)

It was found that, there was no significant difference in the mean endothelial cell area before and after 3 and 6 months of treatment with benzalkonium chloride 0.01% , indicating no significant effect of this drug on the corneal endothelial mean cell area either after 3 or 6 months of using this drug.

C- Effect on central corneal thickness :

1- Pilocarpine 1%

Table "15"

	Mean	S.D.
Pre-ttt	0.543	± 0.043
3 months	0.535	± 0.044
6 months	0.535	± 0.045

P_1 = 0.00535 (P<0.05)
P_2 = 0.0112 (P<0.05)

There was a significant difference in the corneal thickness before and after 3 & 6 months of pilocarpine 1% therapy, this means that there is a decrease in corneal thickness of the patients receiving pilocarpine 1% eye drops for 3 and 6 months as compared with the corneal thickness of the same group of patients, before receiving therapy.

2- Pilocarpine 2%

Table "16"

	Mean	S.D.
Pre-ttt	0.517	± 0.050
3 months	0.522	± 0.055
6 months	0.521	± 0.059

P = 0.0691 (P>0.05)
P = 0.2955 (P>0.05)

As regards the effect of pilocarpine 2% on the corneal thickness, there was no significant difference before and after 3 and 6 months of therapy, indicating no significant effect of this drug on the corneal thickness either after 3 or 6 months of using this drug.

3- Timolol maleate 0.25%

Table "17"

	Mean	S.D.
Pre-ttt	0.548	± 0.057
3 months	0.555	± 0.044
6 months	0.551	± 0.049

P_1 = 0.2888 (P>0.05)
P_2 = 0.4175 (P>0.05)

As regards the effect of timolol maleate 0.25% on the corneal thickness, there was no significant difference before and after 3 and 6 months of therapy, indicating no significant effect of this drug on the corneal thickness either after 3 or 6 months of using this drug.

4- Timolol maleate 0.5%

Table "18"

	Mean	S.D.
Pre-ttt	0.514	± 0.026
3 months	0.526	± 0.047
6 months	0.524	± 0.036

P_1 = 0.1566 (P>0.05)
P_2 = 0.1144 (P>0.05)

As regards the effect of timolol maleate 0.5% on the corneal thickness, there was no significant difference before and after 3 and 6 months of therapy, indicating no significant effect of this drug on the corneal thickness either after 3 or 6 months of using this drug.

5- Dipivefrin hydrochloride 0.1%

Table "19"

	Mean	S.D.
Pre-ttt	0.509	± 0.058
3 months	0.510	± 0.054
6 months	0.513	± 0.055

P_1 = 0.3676 (P>0.05)
P_2 = 0.2361 (P>0.05)

As regards the effect of dipivefrin hydrochloride 0.1% on the corneal thickness, there was no significant difference before and after 3 and 6 months of therapy, indicating no significant effect of this drug on the corneal thickness either after 3 or 6 months of using this drug.

6- Controls [Benzalkonium chloride 0.01%]

Table "20"

	Mean	S.D.
Pre-ttt	0.450	± 0.036
3 months	0.448	± 0.036
6 months	0.448	± 0.037

P_1 = 0.0839 (P>0.05)
P_2 = 0.1717 (P>0.05)

As regards the effect of benzalkonium chloride 0.01% on the corneal thickness, there was no significant difference before and after 3 and 6 months of therapy, indicating no significant effect of this drug on the corneal thickness either after 3 or 6 months of using it.

II- EXPERIMENTAL STUDY IN RABBITS

1- Pilocarpine hydrochloride 1% :

Table "21"

	Pre-ttt	3 months	6 months
Cell density	2050.0	2166.6	2366.6
Cell area	487.8	461.6	422.5
Corneal thickness	0.27	0.40	0.44

There was an average increase in central endothelial cell density by 5.7% and 15.5% after 3 and 6 months of using pilocarpine 1% 4 times daily respectively. Consequently, mean cell area decreased by an average of 5.3% and 13.4% after 3 and 6 months of using this drug respectively.

There was an increase in central corneal thickness by 48.1% after 3 months and 63% after 6 months of using this drug.

2- Pilocarpine hydrochloride 2% :

Table "22"

	Pre-ttt	3 months	6 months
Cell density	2066.6	2200.0	2457.14
Cell area	483.9	454.5	406.98
Corneal thickness	0.32	0.42	0.42

There was an average increase in central endothelial cell density by 6.5% and 18.9% after 3 and 6 months of using pilocarpine 2% 4 times daily respectively. Consequently, mean cell area decreased by an average of 6.1% and 15.9% after 3 and 6 months of using this drug in rabbit respectively.

As regards central corneal thickness, there was an increase by 31.25% after both 3 and 6 months of using this drug.

3- Timolol maleate 0.5% :

Table "23"

	Pre-ttt	3 months	6 months
Cell density	2040.0	2257.1	2480.0
Cell area	490.2	443.05	403.2
Corneal thickness	0.27	0.42	0.42

There was an average increase in central endothelial cell density by 10.6% and 21.5% after 3 and 6 months of using timolol maleate 0.5% twice daily respectively. Consequently, mean cell area decreased by an average of 9.6% and 17.75% after 3 and 6 months of using this drug in rabbit respectively.

There was an increase in central corneal thickness by 55.5% after both 3 and 6 months of using this drug.

4- Dipivefrin hydrochloride 0.1% :

Table "24"

	Pre-ttt	3 months	6 months
Cell density	2166.6	2320.0	2450.0
Cell area	461.6	431.03	408.2
Corneal thickness	0.29	0.48	0.52

In this study, there was an average increase in central endothelial cell density by 7.0% and 13.1% after 3 and 6 months of using dipivefrin hydrochloride 0.1% twice daily respectively. Consequently, mean cell area decreased by an average of 6.6% and 11.56% after 3 and 6 months of using this drug in rabbit respectively.

As regards central corneal thickness, there was an increase by 65.5% and 79.3% after 3 and 6 months of using this drug respectively.

5- Benzalkonium chloride 0.01% :

Table "25"

	Pre-ttt	3 months	6 months
Cell density	1828.5	2033.3	2328.5
Cell area	546.9	491.8	429.5
Corneal thickness	0.27	0.36	0.42

In this study, there was an average increase in central endothelial cell density by 11.2% and 27.34% after 3 and 6 months respectively of using benzalkonium chloride 0.01% 3 times daily. Consequently, mean cell area decreased by an average of 10.1% and 21.46% after 3 and 6 months of using this drug in rabbit respectively.

There was an increase in central corneal thickness by 33.3% and 55.5% after 3 and 6 months of using this drug respectively.

**** Histo-pathological results in rabbits :***

Light microscopic examination of the cornea after treatment with ; pilocarpine 1% & 2% , timolol maleate 0.5%, dipivefrin hydrochloride 0.1% & benzalkonium chloride 0.01% revealed a variety of pathological changes in different specimens of all drug groups. There was increased anteroposterior thickness of the cornea (Fig."6"). The corneal stroma was edematous, the collagen fibres appeared separated and wavy (Fig."6"), some keratocytes were swollen with long processes extending between the lamellae (Fig."7"). The endothelial cells appeared vacuolated (Fig."7","8","9") cell degeneration was evident and some cells had ruptured posterior cell membrane (Fig."6","9") with complete absence of the nucleus. The nucleus has lost its flattened appearance and showed irregular contour (Fig."9").

By electron microscopy, the endothelial nuclei appeared swollen, irregular in shape and faint (Fig."11"). The cytoplasm contained vacuoles of variable sizes. Some of these vacuoles containing fibrogranular material (Fig."10")

while others were clear and large. Mitochondriae appeared swollen, irregular in shape and had lost their cristae and most of them were degenerated (Fig."10","11"). Rough endoplasmic reticulum was abundant (Fig."10"). The tight junction between most endothelial cells was absent.

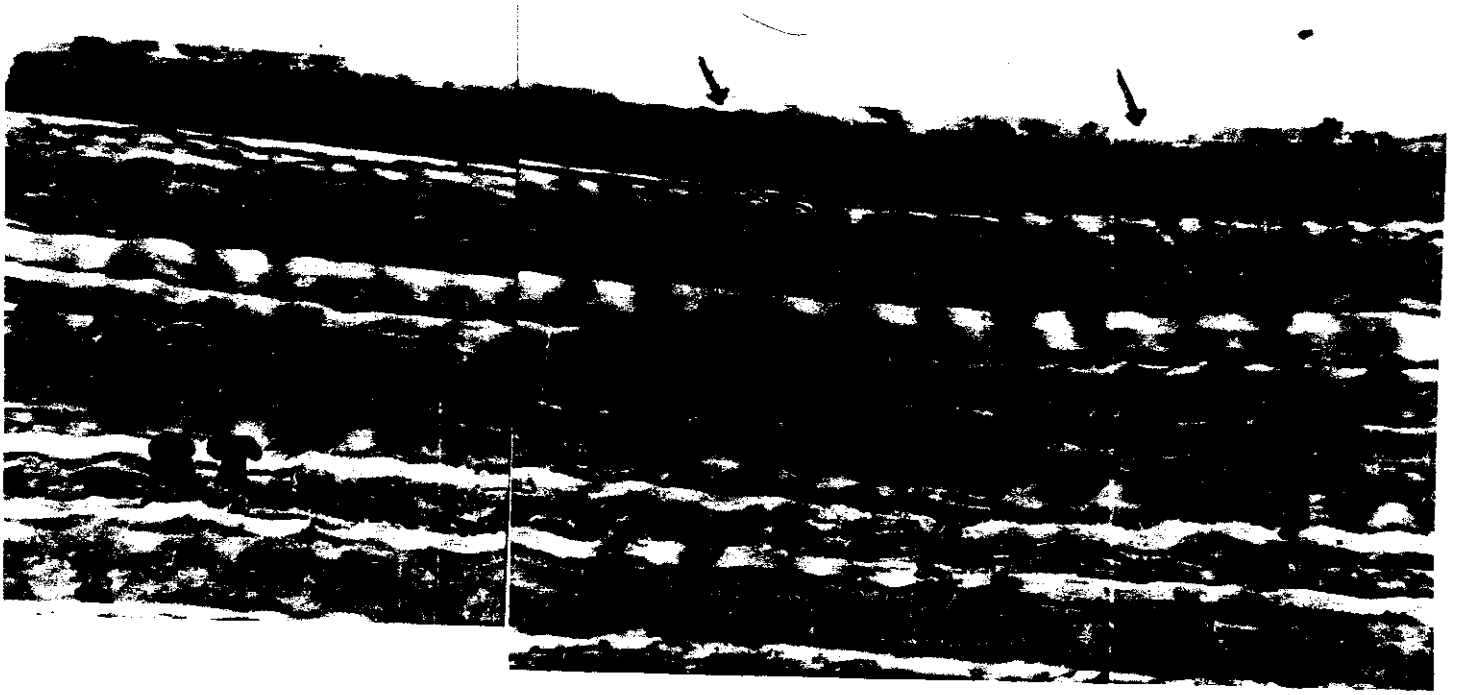

Fig. "6" : Light micrograph of the posterior part of the cornea showing the stromal lamellae with its wavy appearance (st) and rupture of the endothelial cell membrane (arrows).

[Stain : Toluidine blue]

[Mag. : X 500]

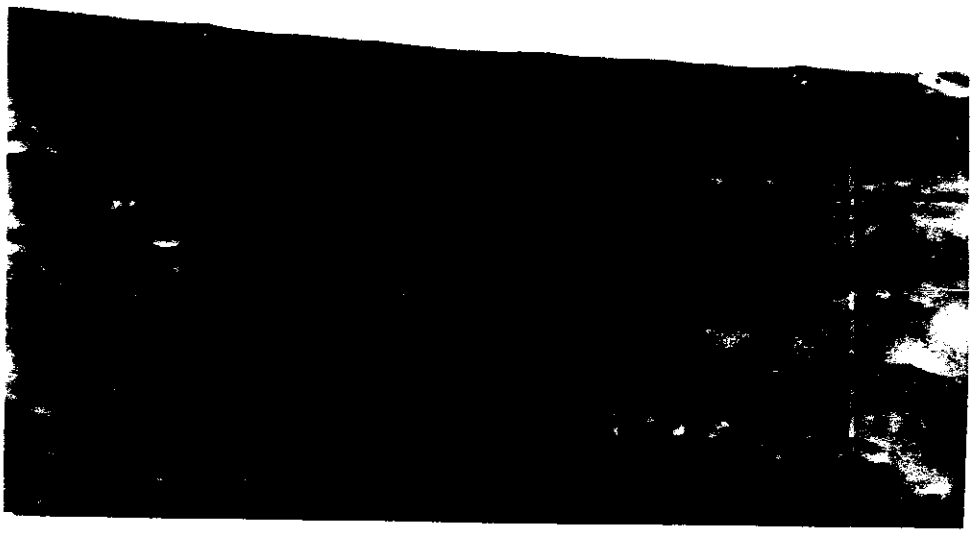

Fig. "7" : Light micrograph of the cornea showing swollen keratocytes with long processes extending between the lamellae (arrows).

[Stain : Toluidine blue]

[Mag. : X 500]

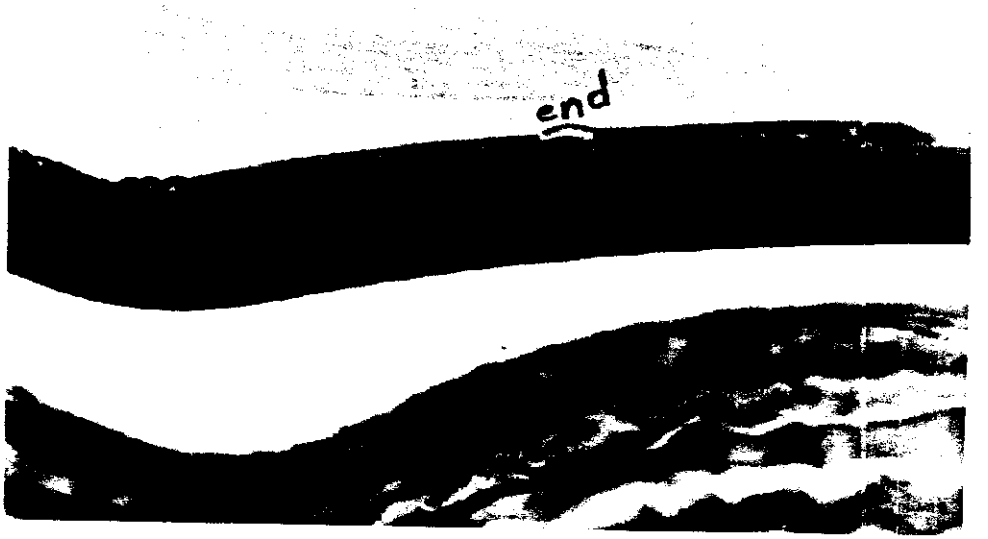

Fig. "8" : Light micrograph of the cornea showing swelling of the endothelial nucleus and vacuolation of the endothelial cell with rupture of the endothelial cell membrane (end).

[Stain : Toluidine blue]

[Mag. : X 500]

(a)

(b)

Fig."9" (a & b) : Light micrograph of the posterior part of the cornea showing vacuolation of the endothelial cell (v) and rupture of the cell membrane (arrow).

[Stain : Toluidine blue]
["b" high power of "a"]

(a) Mag. X 500
(b) Mag. X 1250

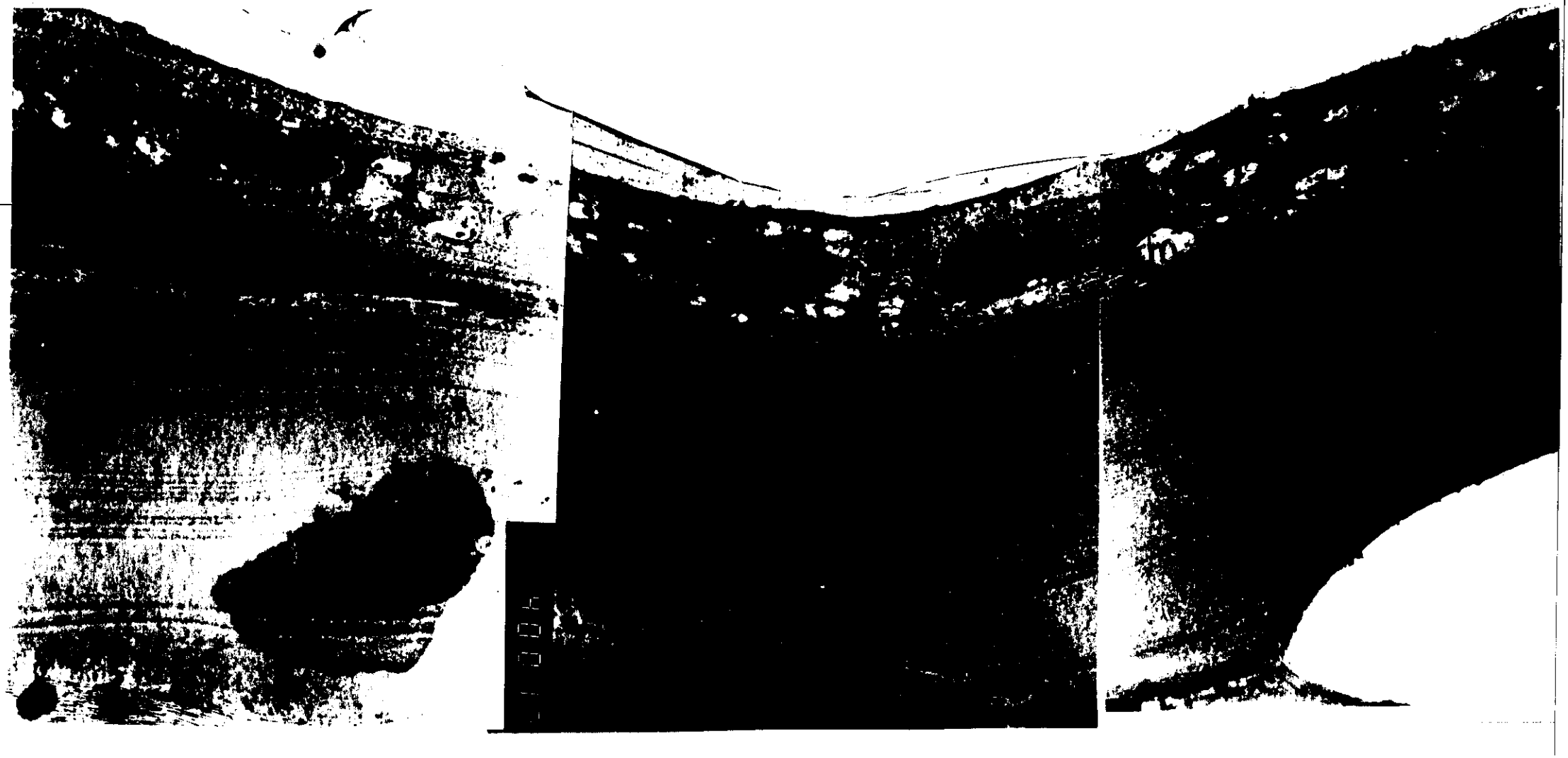

Fig."10" : Electrom micrograph of endothelium showing irregularity in the shape of the nucleus (n) degenerated mitochondria (m), rough endoplasmic reticulum (r) and vacuoles containing fibrogranular material (v).

[Stain : Uranyl actate + Lead citrate] [Mag. : X 4000]

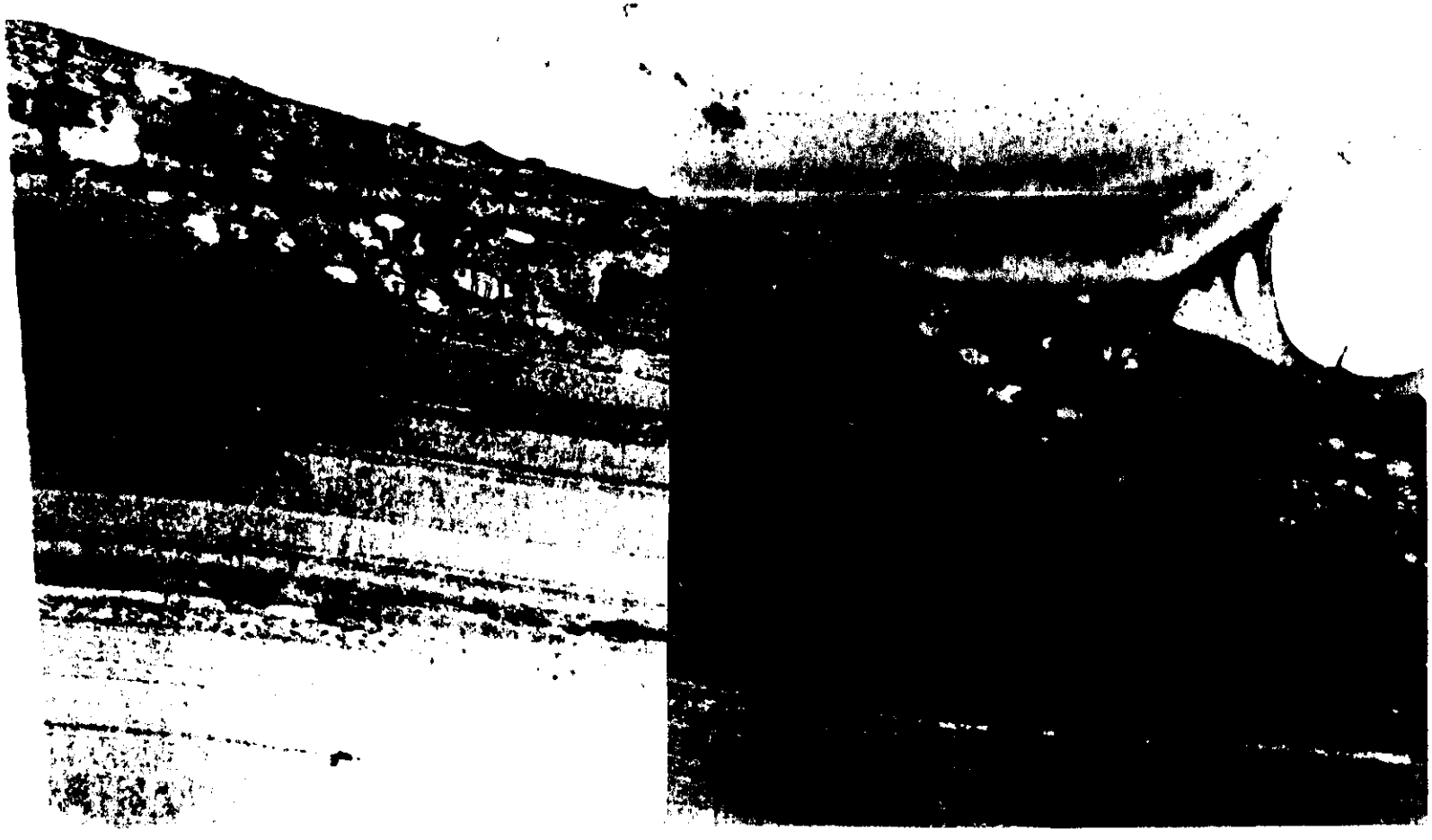

Fig."11": Electrom micrograph of the endothelium showing irregularity of the nucleus with faintness (n) swollen mitochondria (degenerated) (m) and vacuoles (v).

[Stain : Uranyl actate + Lead citrate]

[Mag. : X 4000]