# ULTRASTRUCTURAL EXAMINATION OF THE MORPHOLOGICAL CHANGES IN THE RAT LIVER FOLLOWING CHOLEDUCT LIGATION

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**S.Arbak, Ph.D.** / T. Erbengi, Ph.D.***

* *Professor, Department of Histology and Embryology, Faculty of Medicine, Marmara University, İstanbul, Turkey.*

** *Instructor, Department of Histology and Embryology, Faculty of Medicine, Marmara University, İstanbul, Turkey.*

## SUMMARY

To investigate the effects of common bile duct ligation in rat liver, 20 female Wistar Albinor rats were divided into 1 control and 4 experimental groups in which common bile ducts were ligated successively for 1, 2, 3 and 4 weeks.

Electronmicroscopical investigation showed hepatocyte degeneration and fibrosis. We evaluated those morphological changes as follows : invaginations at hepatocyte nuclei, swollen mitochondria, fingerprint formation of hypertrophied granular endoplasmic reticulum within the hepatocyte cytoplasm, dense collagenous fibers at both the Space of Dissé and portal triad. We concluded as those ultrastructural changes showed an augmentation with increasing ligation period.

**Key Words** : Common bile duct ligation, hepatocyte, hepatic fibrosis

## INTRODUCTION

It is reported that obstruction or ligation of the common bile duct causes several changes in liver structure (1). Meanwhile, kidney and stomach are said to be affected from this prevention of the bile flow ( 1-4) Ligation of the common bile duct mainly influences the portal triads and parenchyme of the liver tissue ( 1,5-8). A prominent increase in fibrous tissue especially at portal triads is reported as one of the most important result of this obstruction ( 1,4-14).

In this present study we want to form an experimental model in order to examine the ultrastructural changes in liver due to the ligation of the common bile duct in rat.

## MATERIALS AND METHOD

20 female Wistar albino rats of 180-200 gr. average body weight were used and they were accomodated in the same room and fed on a regular diet and water ad libitum. Rats were divided into 5 groups : Group I (n=4) : control rats were applied sham operations: Group II (n=4) : Common bile ducts of that rat group were remained ligated for 1 week; Group III (n=4) : for 2 weeks ; Group IV (n=4) : for 3 weeks; Group V (n=4) : for 4 weeks respectively.

In every experimental group, rats were sacrified by decapitation at the end of $1^{st}$, $2^{nd}$, $3^{rd}$ and $4^{th}$ weeks. Ligation procedure was carried out by making a loop around the midportion of the common bile duct and securing it with two or three ties of fine surgical silk.

Liver materials taken from all groups were fixed in 2.5% phosphate-buffered glutaraldehyde and then postfixed in 1% $OsO_4$ solution for one hour. The sections taken from Vestopal W blocks (400-600 A°) were stained with Uranyl acetate and Reynold's method. All sections were observed at Jeol 100 C electronmicroscope.

## RESULTS

In control group, liver parenchyme hepatocytes nuclei appeared with their prominent chromatin structure. Mitochondria, granular and agranular endoplasmic reticulum, Space of Dissé and sinusoidal wall were observed in their normal ultrastructure (Fig.1).

In group II where the common bile ducts remained ligated for 1 week, some invaginations at hepatocytes nuclei, swollen mitochondria with severely damaged cristae and hypertrophy at granular and agranular endoplasmic reticulum were noted. Sinusoidal wall appeared normal in that group (Fig.2).

In group III where the common bile ducts remained ligated for 2 weeks, many invaginations at hepatocytes nuclei and swollen mitochondria were still present. Meanwhile, granular endoplasmic reticulum membranes formed many parallel curves. Sinusoids and endoplasmic reticulum membranes were seen dilated in that group (Figs. 3 and 4).

In group IV where the common bile ducts remained ligated for 3 weeks, the findings were similar to those of the group III. Close relationship of the granular endoplasmic reticulum with swollen mitochondria and hypertrophy in granular endoplasmic reticulum arranged in parallel curves were still noted. In parenchyme, connective tissue cells, collagenous fibers around the hepatocytes and cell infiltration were observed. Increased number of bile canaliculi with few microvilli and dilation at the Space of Dissé were among the ultrastructural findings of that group (Fig. 5).

In group V where the common bile ducts remained ligated for 4 weeks, invaginated hepatocyte nuclei, giant mitochondria, fingerprint formation of the granular endoplasmic reticulum and hypertrophied agranular endoplasmic reticulum implied a high degree of degeneration in hepatocytes. Dense collagenous fibers still existed both at the dilated Space of Dissé and the parenchyme (Figs. 6 and 7).

## DISCUSSION

Ultrastructural changes in liver cells due to the ligation of the common bile duct were evaluated in this study.

We know from literature that ligation or obstruction of the common bile duct results in severe changes at the liver structure. Some of the investigators (15,16) used chemicals to obliterate the bile flow and reported the highly hypertrophied bile canaliculi as a result of their experiments. Steiner (16) who used ANIT in rats for obstruction of the bile flow declared the hypertrophy in agranular endoplasmic reticulum, fingerprint structure of the granular endoplasmic reticulum showing close relationship with swollen mitochondria. We also observed parallel findings especially in group IV and V. So by increasing period of ligation, these effects became evident. Cavali et al. (17) reported in their study the shortened microvilli at the dilated bile canaliculi in case of acute hepatitis. This means dilations at the bile canaliculi. We also noted it especially in group IV and V.

Parallel to our observations in group IV and V, investigators (18-20) also reported a high increase in fibrous tissue within both the parenchyme and Space of Dissé. They also claimed that fibrous tissue increase resulted in the dilation of the Space of Disse. Many investigators (2-4,8,9,11,21) tend to search the source of hepatic fibrosis in case of obstruction of the common bile duct. They pretend that the aggregated old fibers or newly formed fibers cause this intense connective tissue within the parenchyme and around the portal triads. They also report the peripheral situation of the fibroblasts around the sinusoids to form here the intense fibrous tissue. Similar observations were declared in another study (9) in which the effects of obstructive jaundice were discussed. They especially pointed out edema in parenchyme and dilated bile canaliculi.

So, we showed in this experimental model, the extensive hepatic necrosis with increasing ligation period of the common bile duct in rats. We think all these degenerations were due to the obliterated bile flow in the liver.

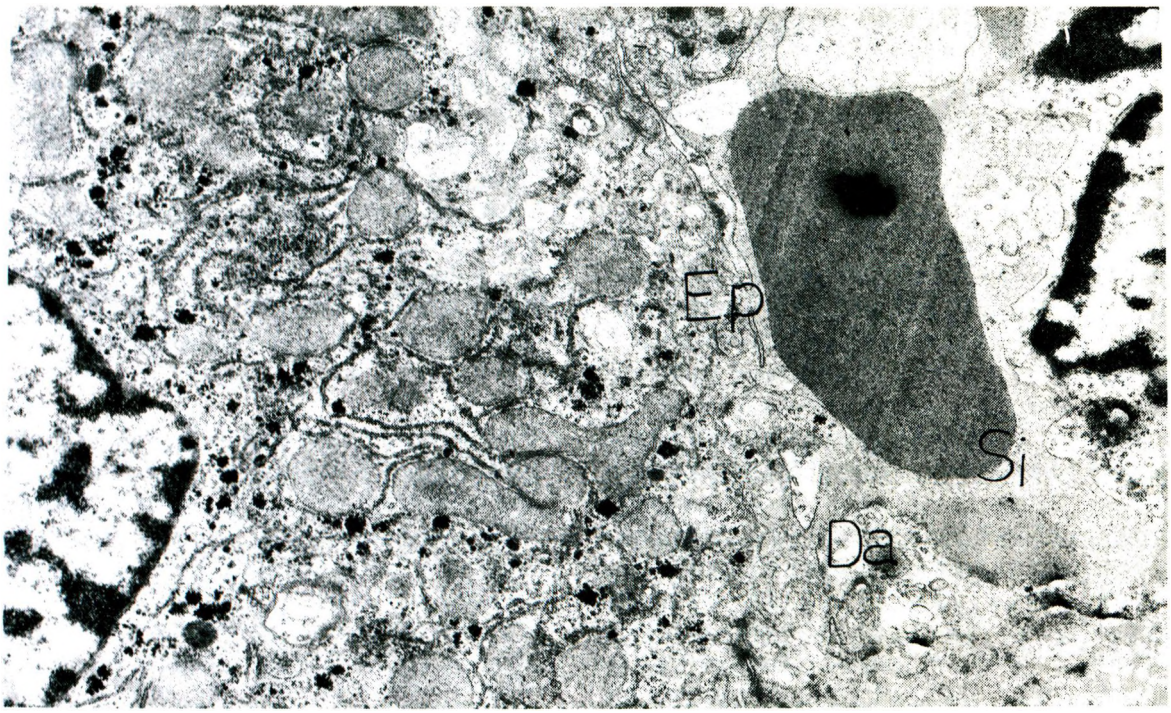


**FIG.1:** In control group electronmicrograph, hepatocyte, Space of Dissé and sinusoidal wall are present. SI: Sirosoild; Da: Space of Dissé; Ep: Endothelial cell pore, x 13.200.

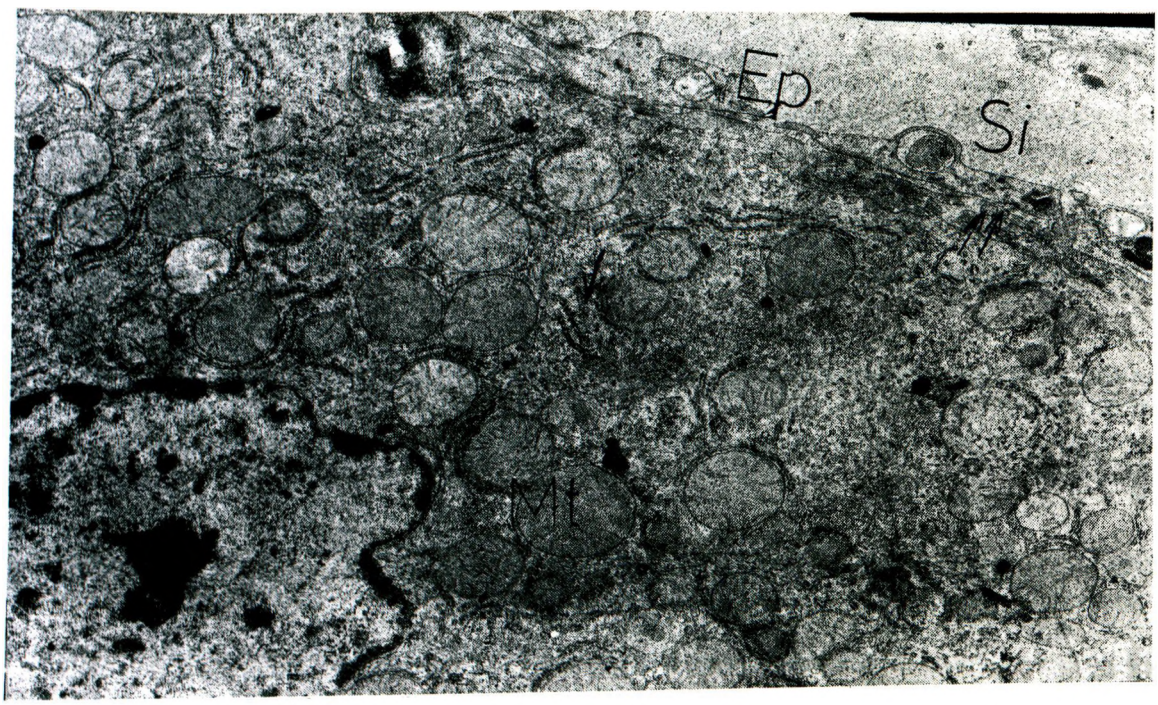


**FIG.2:** In group II where choleducts remained ligated for I week mitochondria with granular endoplasmic reticulum (arrow) surrounding them, microvilli (double arrows) the Space of Dissé and pores at the endothelial) lining of sinusoidal wall are obseved. Mi; Mitochondrion, Si; Sinusoid; Ep: Endothelial cell pore x 10.400.

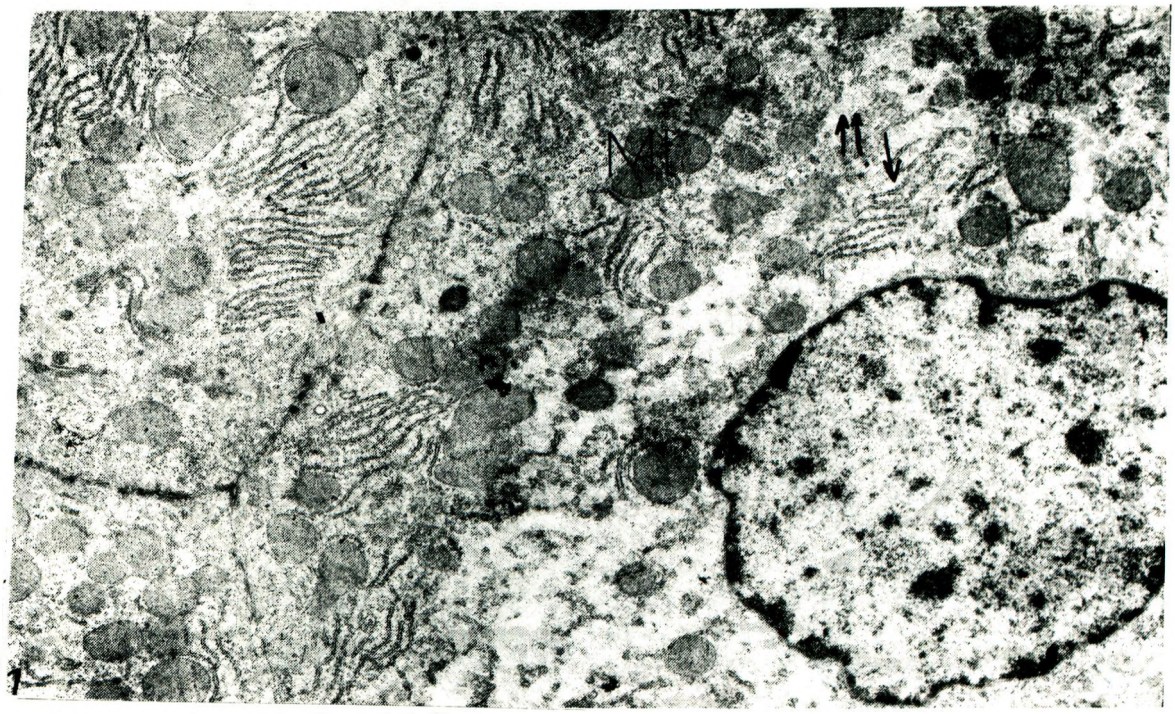

**FIG.3:** In group II electronmicrograph; decreased number of cristai within the mitochondria, parallel arrangements as granullar endoplasmic reticulum (arrow) and tubulovesicular structure of agranular endoplasmic reticulum (double arrows). x 13.200.

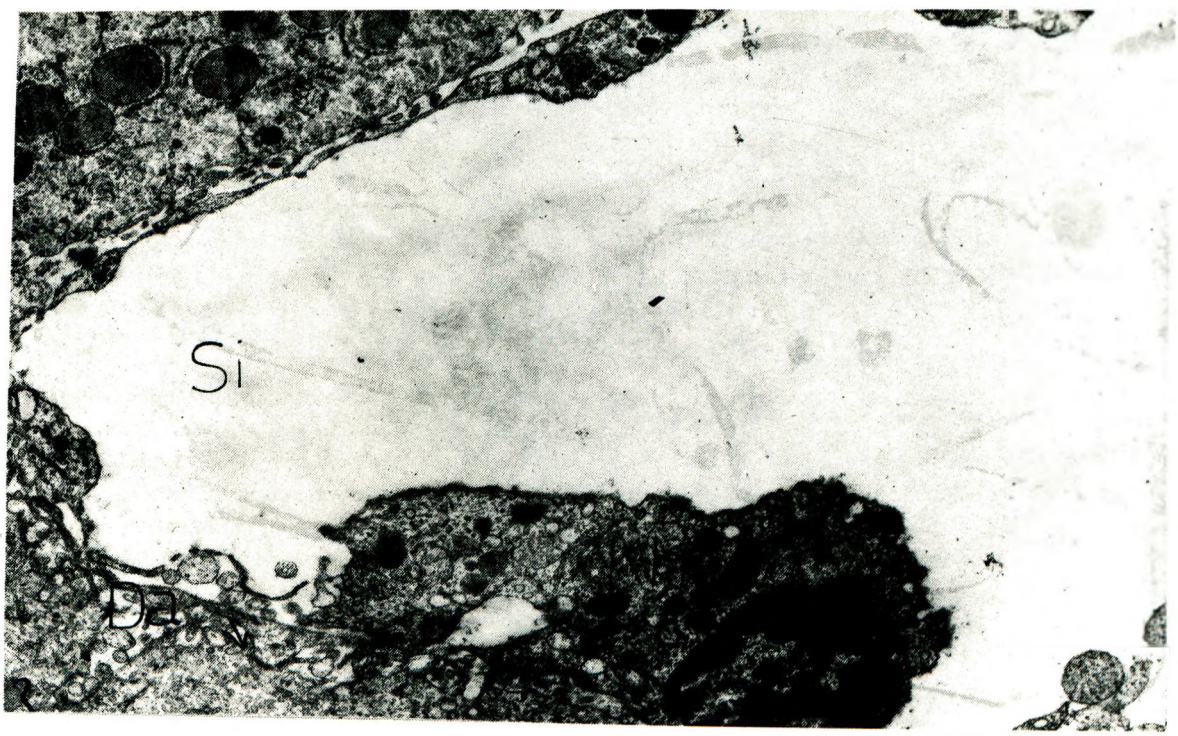


**FIG.4:** In group II sinusoidal wall together with Kupffer cell and microvilli (arrow) within the Space of Dissé are observed. Si: Sinusoid; Kh: Kupffer cell; Da: Space of Dissé x 10.400.

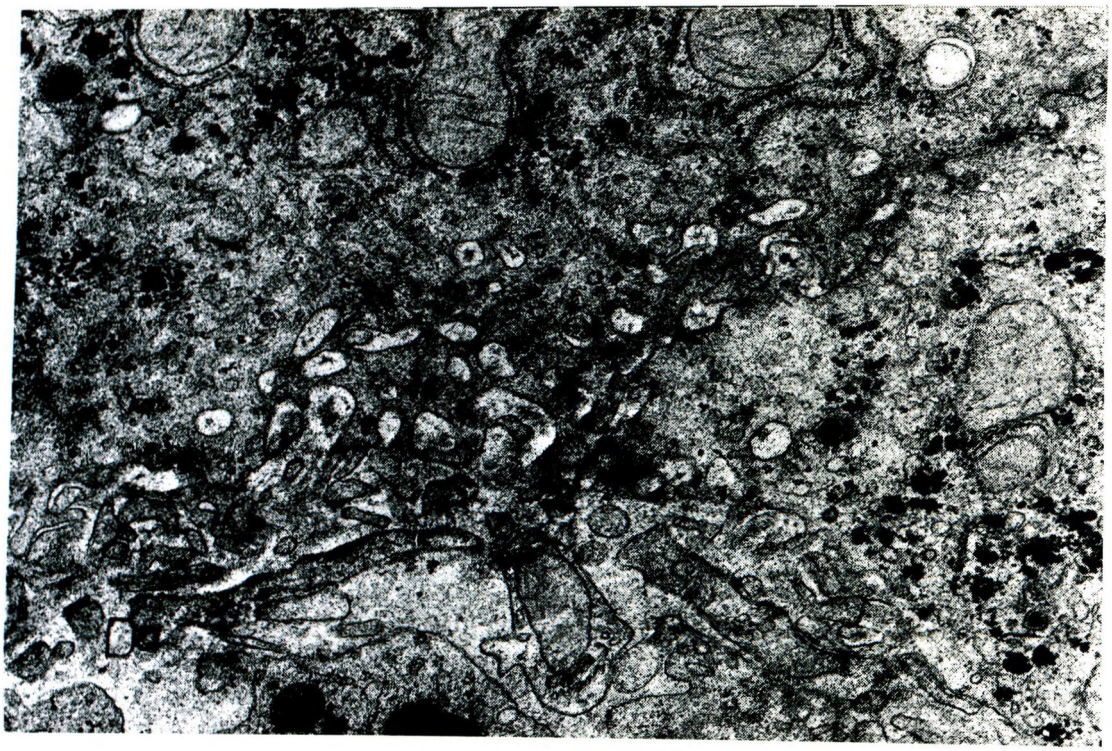

**FIG.5:** In group III (choleducts were remained ligated for 3 weeks) dilations at the Space of Dissé with microvilli within it are present. Da: Space of Dissé x 10.400.

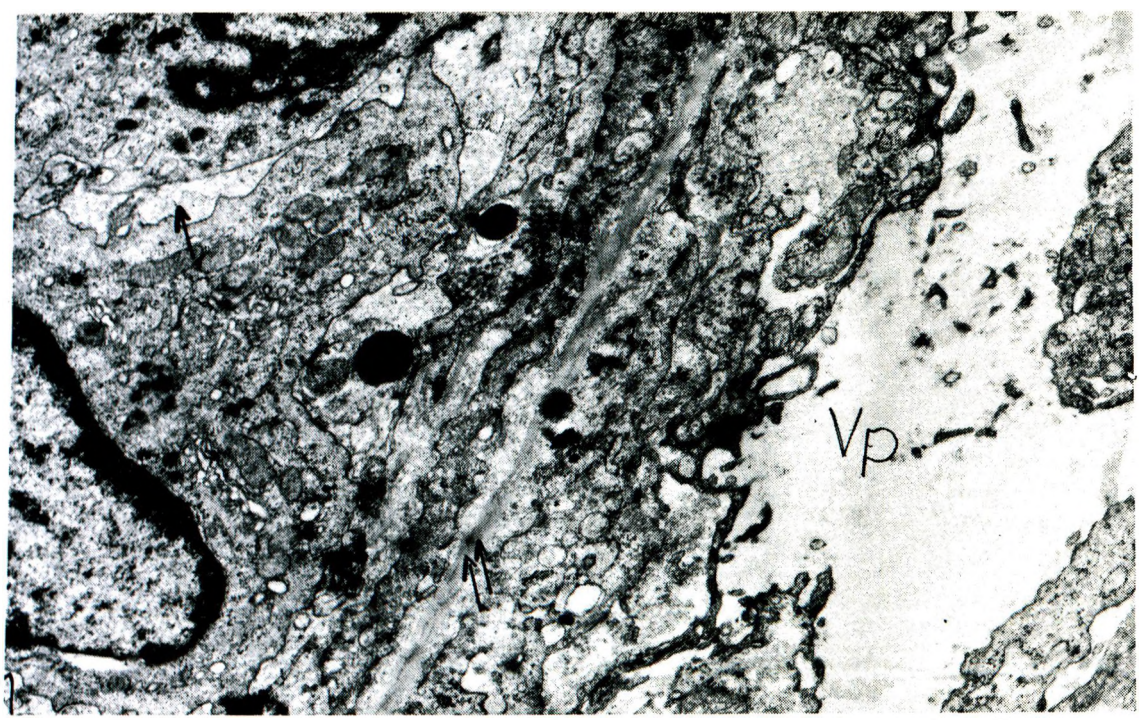


**FIG.6:** In rats (choleducts remained ligated for 4 weeks), venulae perilobularis, peripheral space (arrow) and many collagenous fibers (double arrows) are observed. Vp: Venulae perilobularis x 13.400.

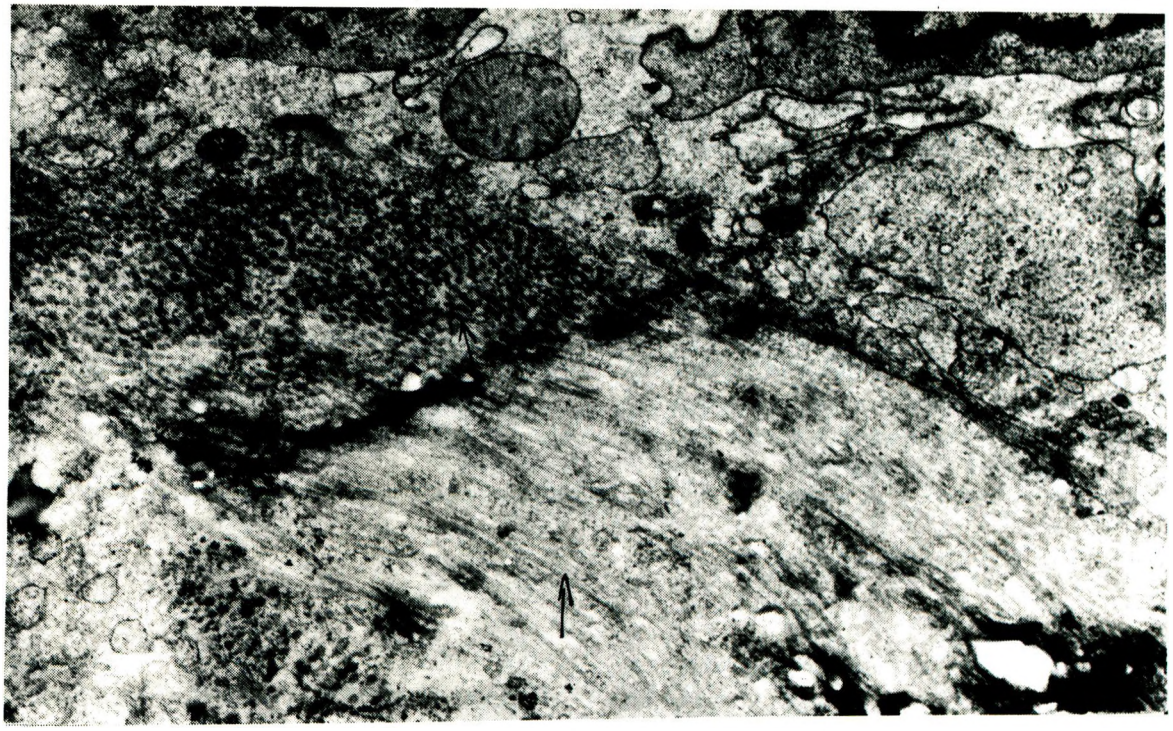

**FIG.7:** In group V electronmicrograph, dense collagenous fibers (arrow) at the portal triad are seen x 20.000.

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