Results :

Nuclei appeared blue.

Cytoplasm appeared pink.

B) ELECTRON MICROSCOPY :

This was carried out to verify the data obtained by the light microscopy.

Reagents : ***(Millonig, 1961; Reynold, 1963; Sabatin, Bench and Barret, 1963).***

1- BUFFER :

Phosphate buffer (pH 7.4) :

- Sodium dihydrogen phosphate ..... 6.41 gm.
- Disodium hydrogen phosphate ..... 41.3 gm.
- Distilled water ..... 1000 ml.

2- FIXATIVES :

a) Buffered glutaraldehyde (3%) :

- Glutaraldehyde 255% .....5ml.
- Buffer solution .....15 ml.
- Distilled water ..... 20ml.

b- Buffered osmic acid (1%)

- Osmic acid ..... 0.1gm.
- Buffer solution ..... 4ml.
- Distilled water ..... 6ml.

2-Swirl the mixture and let sit from 30 to 60 minute.

3-Add the same amount of embedding media to the existing infiltration media, swirl, and let sit for another 30 minutes.

4-Pour and drain the mixture and add more embedding media.

5-Oven dry gelatin capsules were filled and left to polymerise in an oven for 24- 36 hours.

(4) Sectioning :

Survey sections were cut, one micron thick (semithin section) using ultramicrotome, ***(Reichert Jung)***, with glass knives (made on L.K.B. knife maker). The sections were stained with toluidine blue and examined under the light microscope to confirm the presence of liver tissue with similar findings of haemtoxylin and eosin sections. Selected areas were further trimmed for ultrathin sections. These ultrathin sections (60 - 90nm) were mounted on copper grids.

(5) Staining :

Double staining of the sections was carried out using uranyl acetate for 10-20 minutes in the dark followed by washing in distilled water

- Lead citrate, for 5-15 minutes.
- After staining wash in 0.02 N sodium hydroxide then in distilled water.
- Dry the girds on filter paper.

(6) Examination :

The stained sections were examined by Phillips 400 Transmission electron microscope at 80 K.V. Photographs were taken, developed, printed and examined.

RESULTS

RESULTS

1- Light microscopy :

Group I :

Examination of liver section of control rats revealed that the liver consisted of lobules which were separated by fine indistinct connective tissue septa (Fig. 1). The lobules consisted of hepatocytes arranged as interconnected flat plates of one or two cells, radiating from the central veins towards the periphery and separated by blood sinusoids which were lined by flat endothelial cells (Fig. 2). The portal tracts were found between the hepatic lobules containing a branch of hepatic artery with a narrow lumen and a thick wall, a wider and thinner walled branch of the portal vein and a small branch of the bile duct recognized by its lining of simple cuboidal epithelium (Fig. 3). The hepatocytes were polyhedral with large central vesicular nuclei. Some cells were binucleated (Fis. 2 & 3).

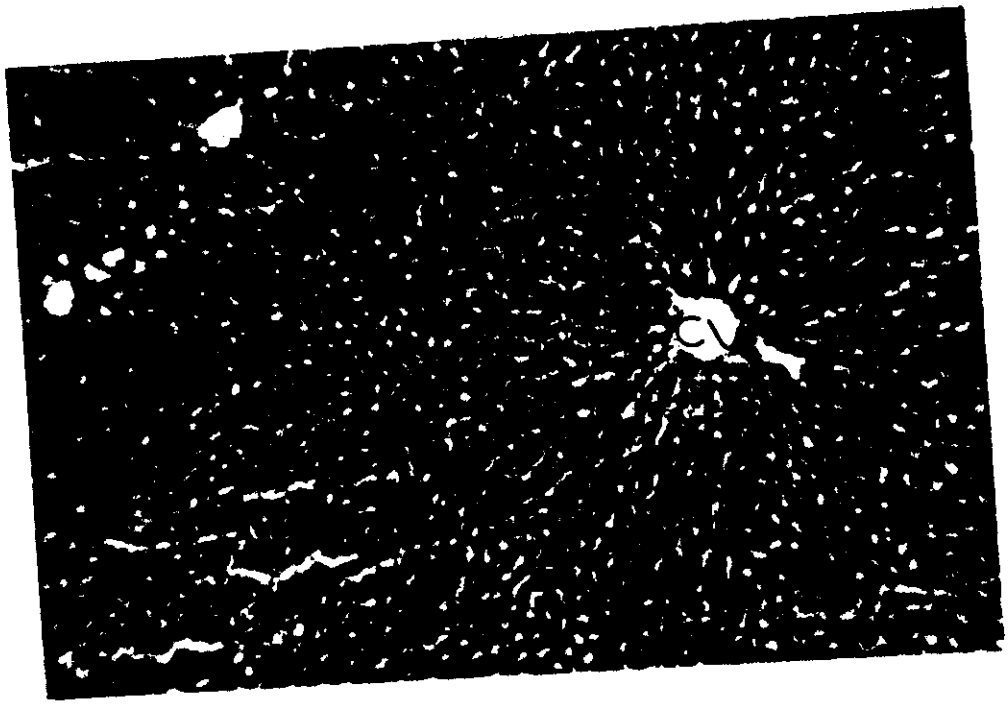

Fig. (1) : A photomicrograph of a section in the liver of a control rat showing indistinctive lobulation and cords of hepatocytes (H) radiating from a central vein (C.V)

(Hx . & E X100).

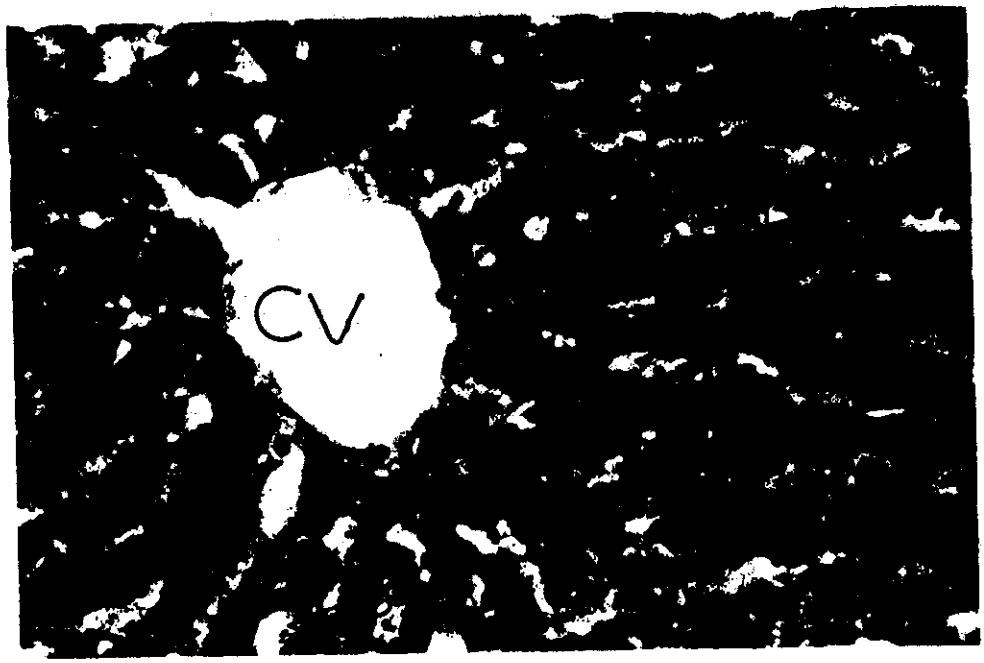

Fig. (2) : A photomicrograph of a section in the liver of a control rat showing polyhedral hepatocytes (H) with vesicular nuclei (N) radiating from a central vein (CV) and separated by blood sinusoids (S) with flat endothelial cells (E) lining the blood sinusoids. Notice the binucleated hepatocytes (bH).

(Hx. & E. X 400)

Group II :

Examination of liver sections of rats given terbinafine for two weeks showed that the hepatic lobular architecture was preserved. Few hepatocytes showed pale vacuolated cytoplasm. Mild dilated central veins were seen having a mild infiltration with inflammatory cells (Fig. 4). The portal tracts were nearly normal with a mild infiltration with inflammatory cells (Fig. 5).

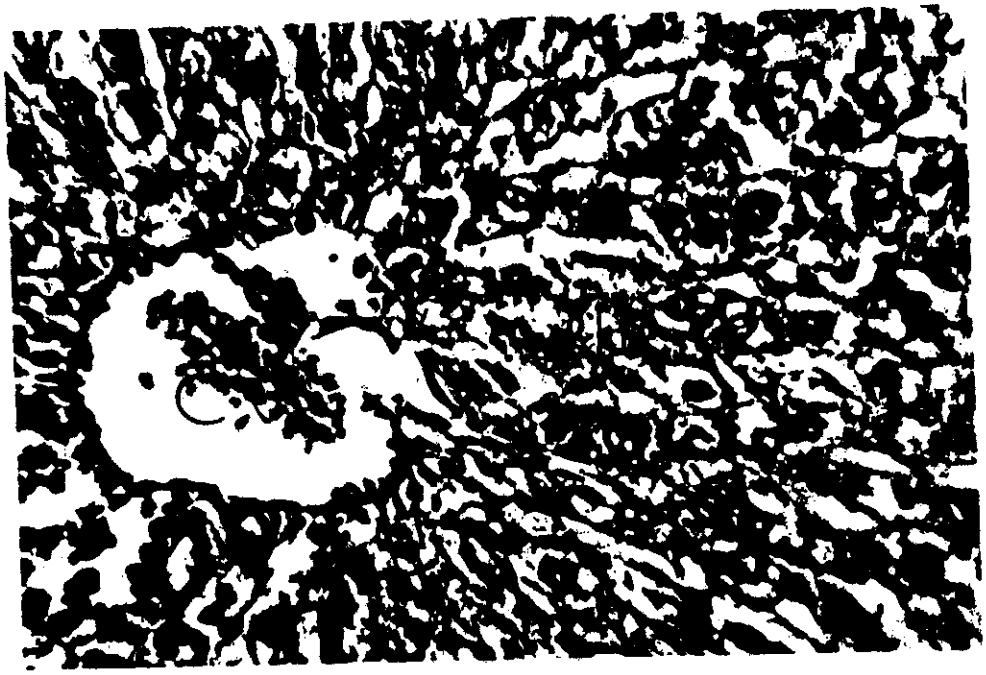

Fig. (4) : A photomicrograph of a liver of a rat given terbinafine for two weeks showing mildly dilated central vein (CV), some inflammatory cells arround it (I). Hepatocytes (H) showing pale vacolated cytoplasm.

(Hx. & E. X 400).

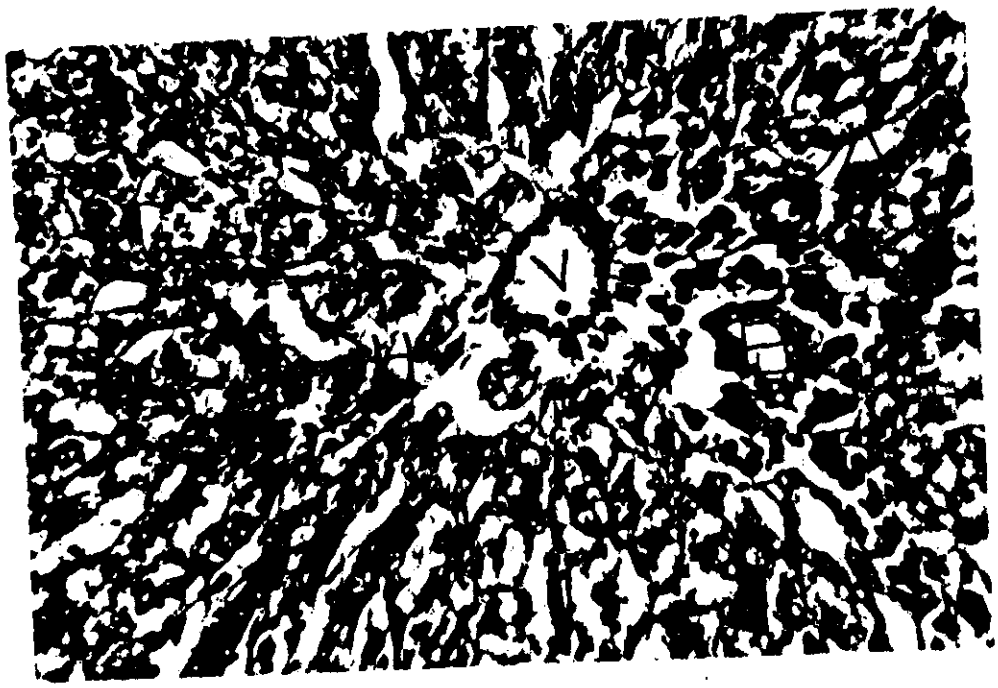

Fig. (5) : A photomicrograph of a liver section of a rat given terbinafine for two weeks showing few periportal inflammatory cells (arrow) but normal bile ductules, (B), branch of portal vein (V) and branch of hepatic artry (A). Many hepatoytes show vacuolated cytoplasm (H).

(Hx. & E. X 400).

Group III :

Examination of liver sections of rats given terbinafine for six weeks showed that the hepatic lobular architecture was still preserved. Most of the hepatocytes showed pale vacuolated cytoplasm. The central veins were dilated and having mild infiltration with inflammatory cells (Fig. 6). Moderately dilated portal vessels were seen. Moderate periportal inflammatory infiltrate was apparaent (Fig. 7).

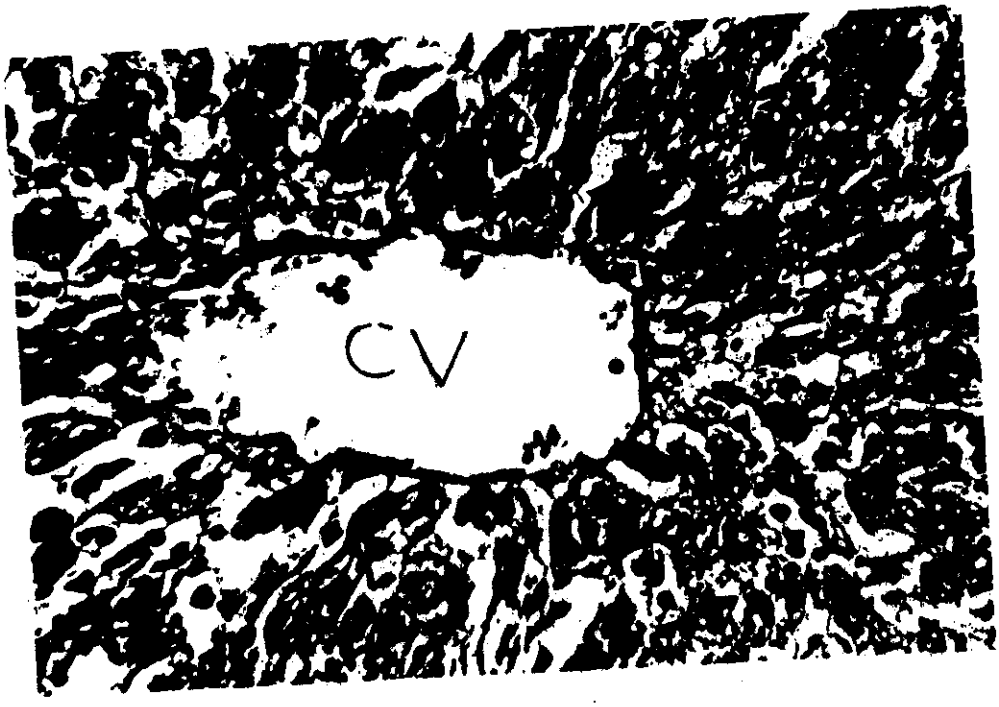

Fig. (6) : A photomicrograph of a liver section of a rat given terbinafine for six weeks showing dilated central vein (CV) and inflammatory infiltrate around it (↑↑). More vacuolated hepatocytes are seen (H).

(Hx. & E. X 400).

Group IV :

Examination of liver sections of rats given terbinafine for twelve weeks revealed marked dilation of the central veins. Also, a moderately inflammatory cellular infiltrate around the central veins was apparant Most of the hepatocytes were pale with vacuolated cytoplasm (Fig. 8). Marked portal inflammatory cellular infiltrate was seen small areas of focal necrosis were seen(Fig. 9).

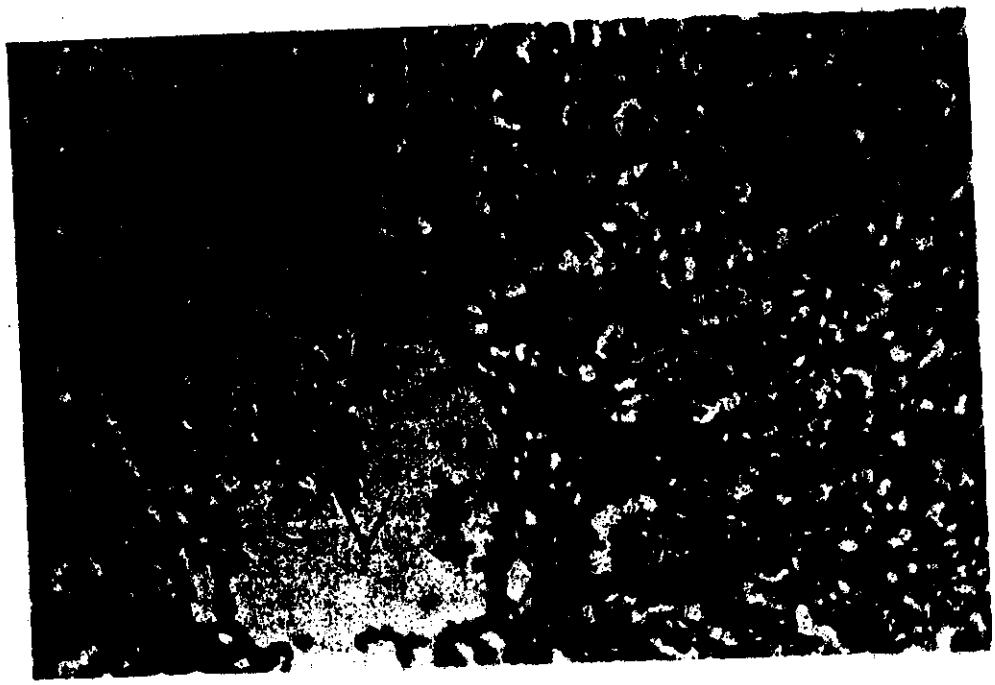

Fig. (8) : A photomicrograph of a liver section of a rat given terbinafine for twelve weeks showing marked dilatation of the central vein (CV), Moderate inflammatory infiltrate (↑↑) inflammatory cells around the central vein. Most of the hepatocytes (H) are vacuolated. Some hepatocytes have pyknotic nuclei and others have even nuclei showing karyloysis & Karyorrhexis(*).

(Hx. & E. X 400).

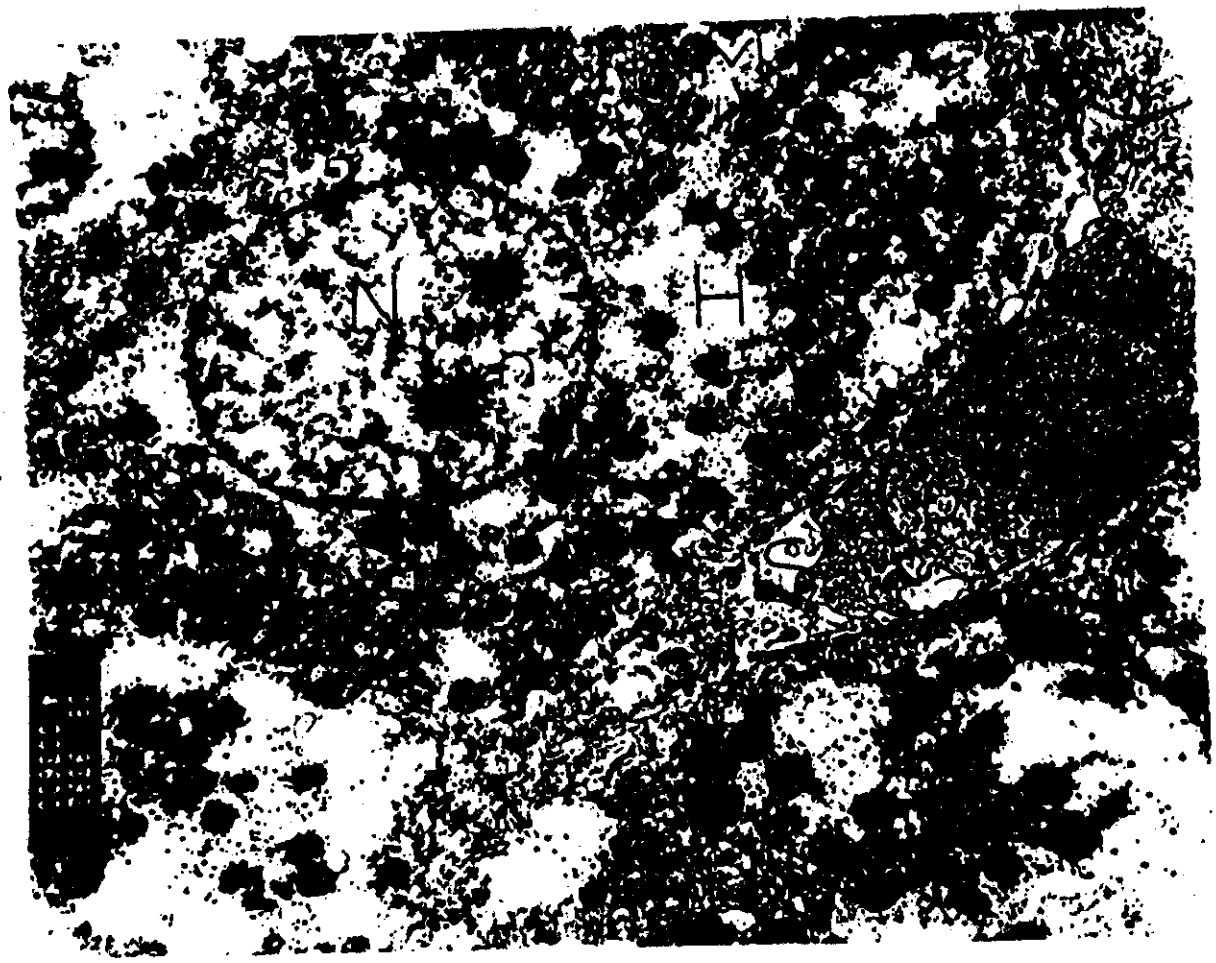

Fig. (11) : An electron micrograph of a liver of a control rat showing two hepatocytes (H). The cytoplasm is rich in cell organelles. The nucleus (N) is centrally located and oval in shape with two peripheral nucleoli (n). Mitochondria (M) are scattered allover the cell. A blood sinusoid (S) is seen between the hepatocytes.

RER = Rough endoplasmic reticulum.

RBC = Red blood cell in blood sinusoid.

(X 3600).

Fig. (16) : An electron micrograph of a liver of a control rat showing a blood sinusoid (S) with a lining Kupffer cell (K). Notice its large irregular nucleus (n), abundant cytoplasm with the phagocytic vacuoles (V) and villous projections of its cytoplasmic membrane. MV = Microvilli of hepatocyte projecting into Disse space (DS). (↑) arrows point to gaps in the endothelial lining of the blood sinusoid.

(X 10000).

Group II :

Examination of liver sections of rats given terbinafine for two weeks showed a mild increase in the lipid droplets and lysosomes of hepatocytes. The nuclei were normal. Howevere some showed condensed peripheral nucleoli (Fig. 17). A mild on change in mitochondria could be detected in the form of pleomorphism. Mild dilatation of endoplasmic reticulum was seen. (Fig. 18).

Glycogen particles were scattered all over the cytoplasm. Small areas of degeneration were apparent. The bile canaliculi were normal in structure (Fig. 19). The blood sinusoids showed Kupffer cells with an increase in their phagocytic vacuoles (Fig. 20).

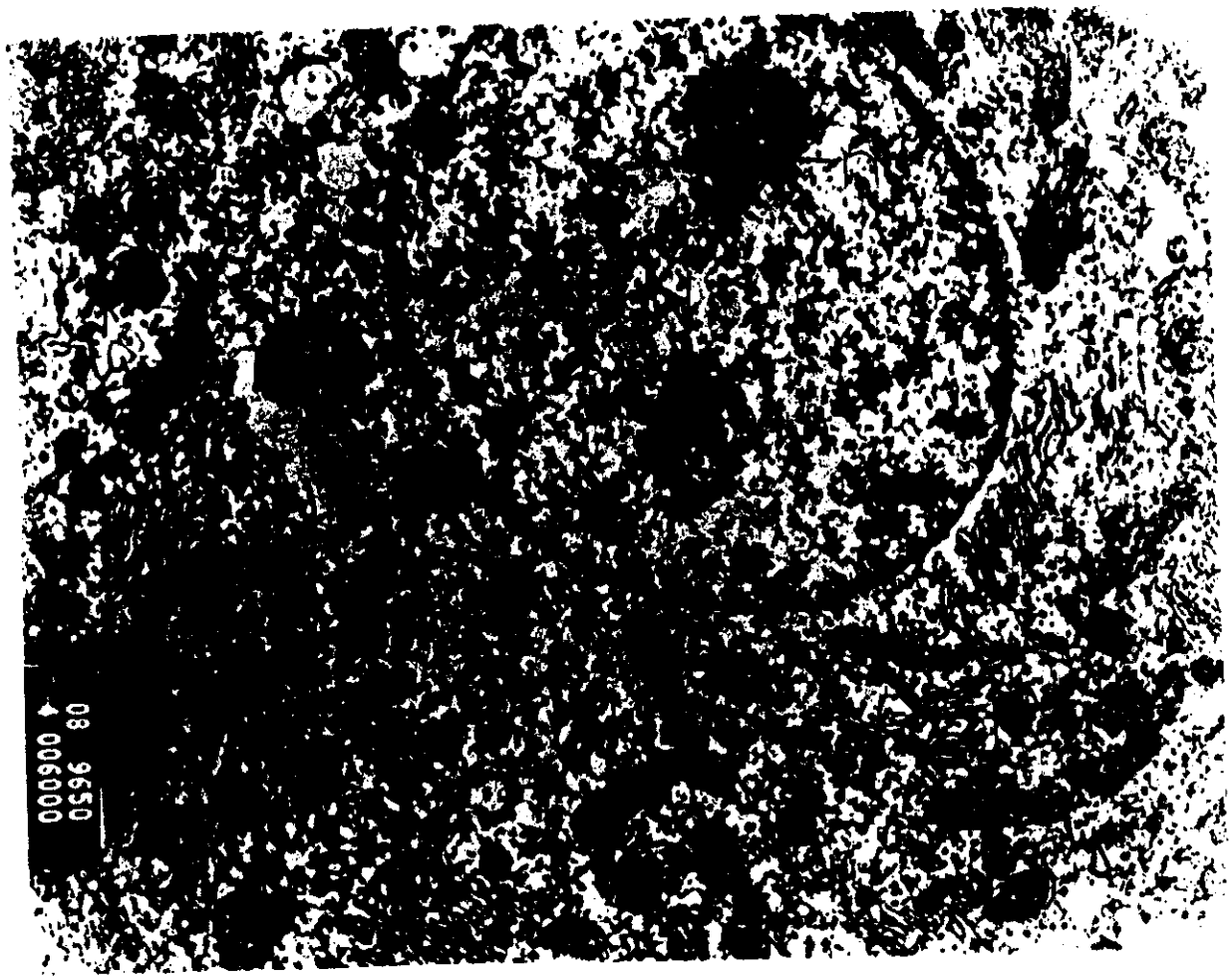

Fig. (17) : An electron micrograph of a liver of a rat given terbinafine for two weeks showing the nucleus (N) with peripheral nucleoli (n) a mild increase in rough endoplasmic reticulum (RER), Mitochondria (M) are of different shapes lipid droplets (li) lysosomes (ly).

(X 6000).

Fig. (18) : A higher magnification of (Fig. 17) showing the Hepatocyte with a part of its nucleus (N). Mild increase in rough endoplasmic reticulaum (RER). Mitochondria (M) are different shapes. Lipid droplets (Li) and proliferation of lysosomes (Ly) were apparent.

(X 10000).

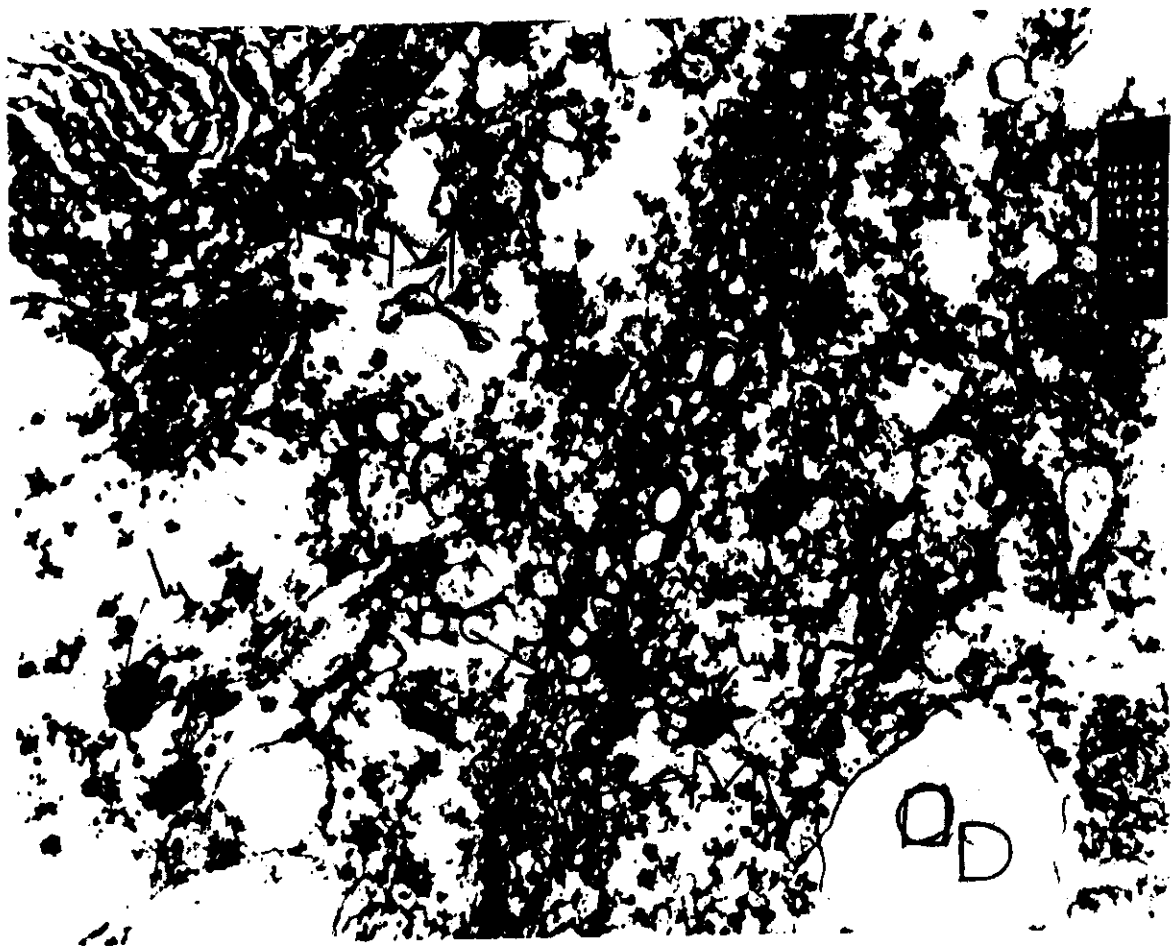

Fig. (19) : An electron micrograph of a liver of a rat given terbinafine for two weeks showing a bile canaliculus (bc) of normal size and normal microvilli (MV) projecting through it. Lysosome (Ly). A giant mitochondrion (M) is seen in the upper left corner of the field. D = areas of cytoplasmic degeneration. (X 13000).

Fig. (22) : An electorn micrograph of the liver of a rat given terbinafine for six weeks showing a hepatocyte nucleus (N), normal in shape, normal nuclear envelope but with hypertrophy of its nucleolus (n) & clumped chromatin (ch), proliferated and dilated rough endoplasmic reticulum (RER), proliferated and vesiculated smooth endoplasmic reticulum (SER), pleomorphic mitochondria (M), some areas of degeneration in the cytoplasm (D), proliferated peroxisomes (P) and lysosomes (ly).

(X 8000)

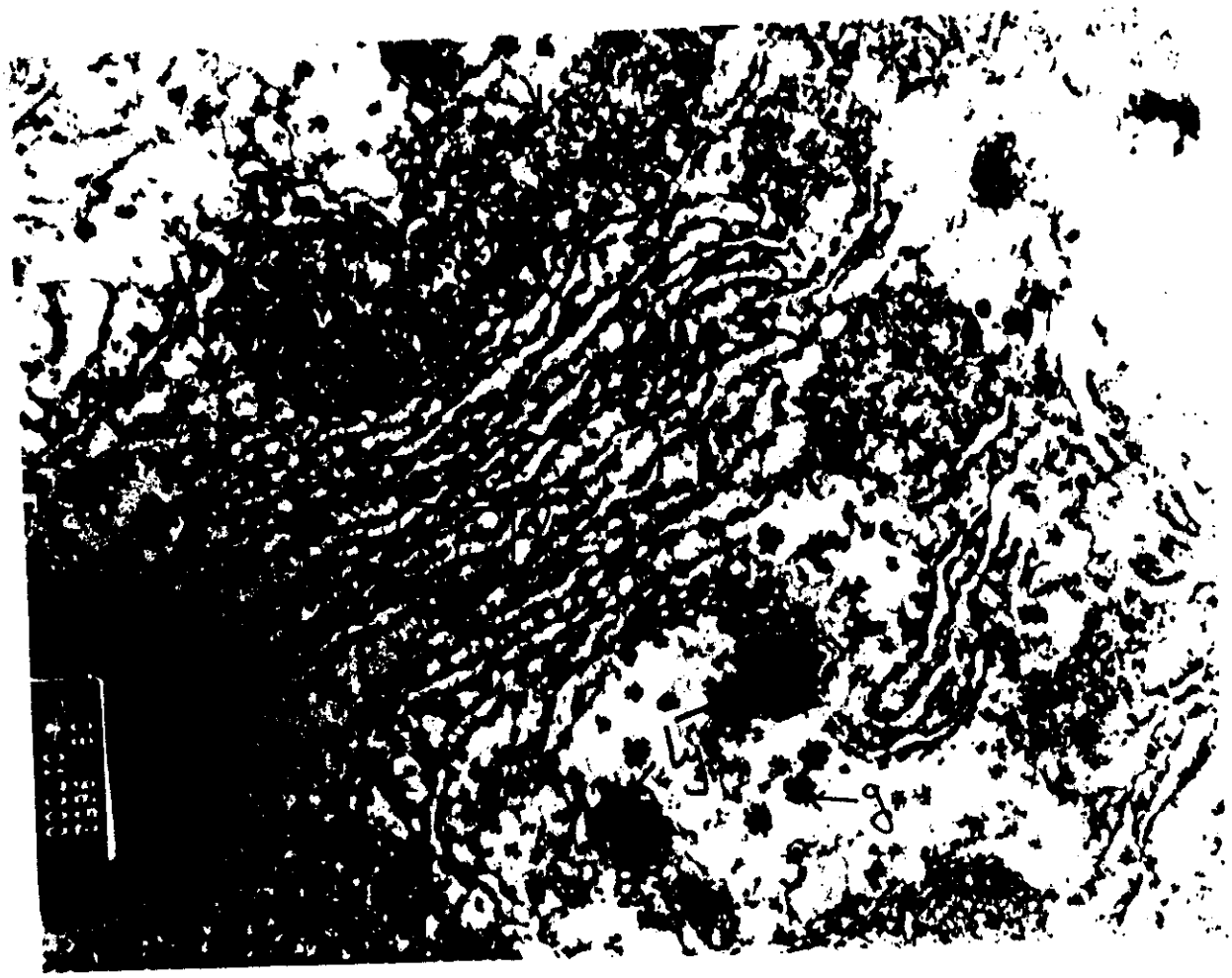

Fig. (23) : An electron micrograph of the liver of a rat given terbinafine for six weeks showing dilated proliferated rough endoplasmic reticulum (RER), megamitochondria (M) with some destruction in its outer membrane, (arrow) and scattered glycogen (g) all over the cytoplasm forming rosettes. (Ly) is a lysosome.

(X 17000).

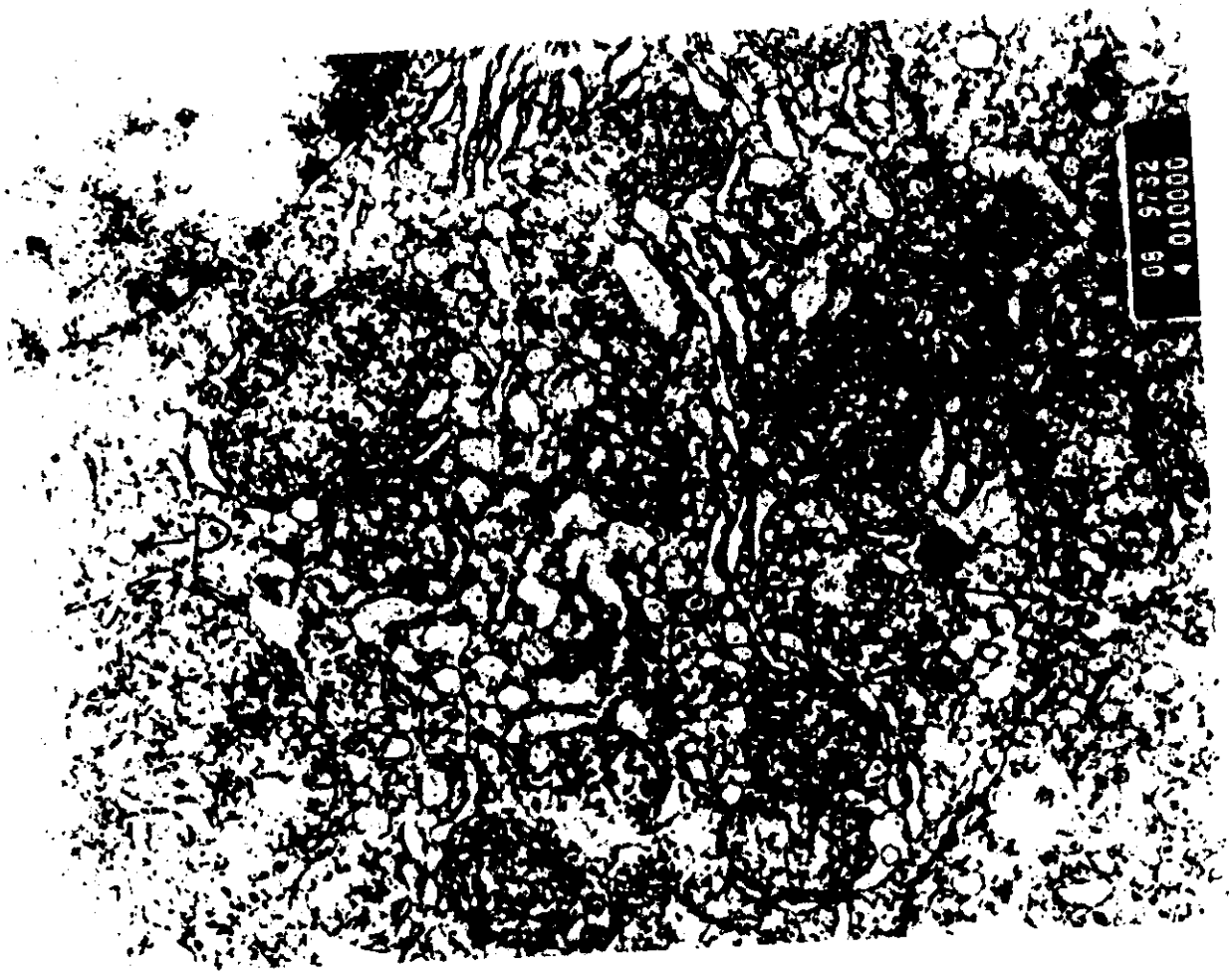

Fig. (24) : An electron micrograph of the liver of a rat given terbinafine for six weeks showing part of nucleus (N), bile canaliculi (bc) of normal size, normal microvilli (MV) projecting through it. The tight junction (J) bounding the canaliculus is also seen. Dilated proliferated rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER), pleomorphic mitochondria (M) and peroxisomes (P) are also seen.

(X 10000).

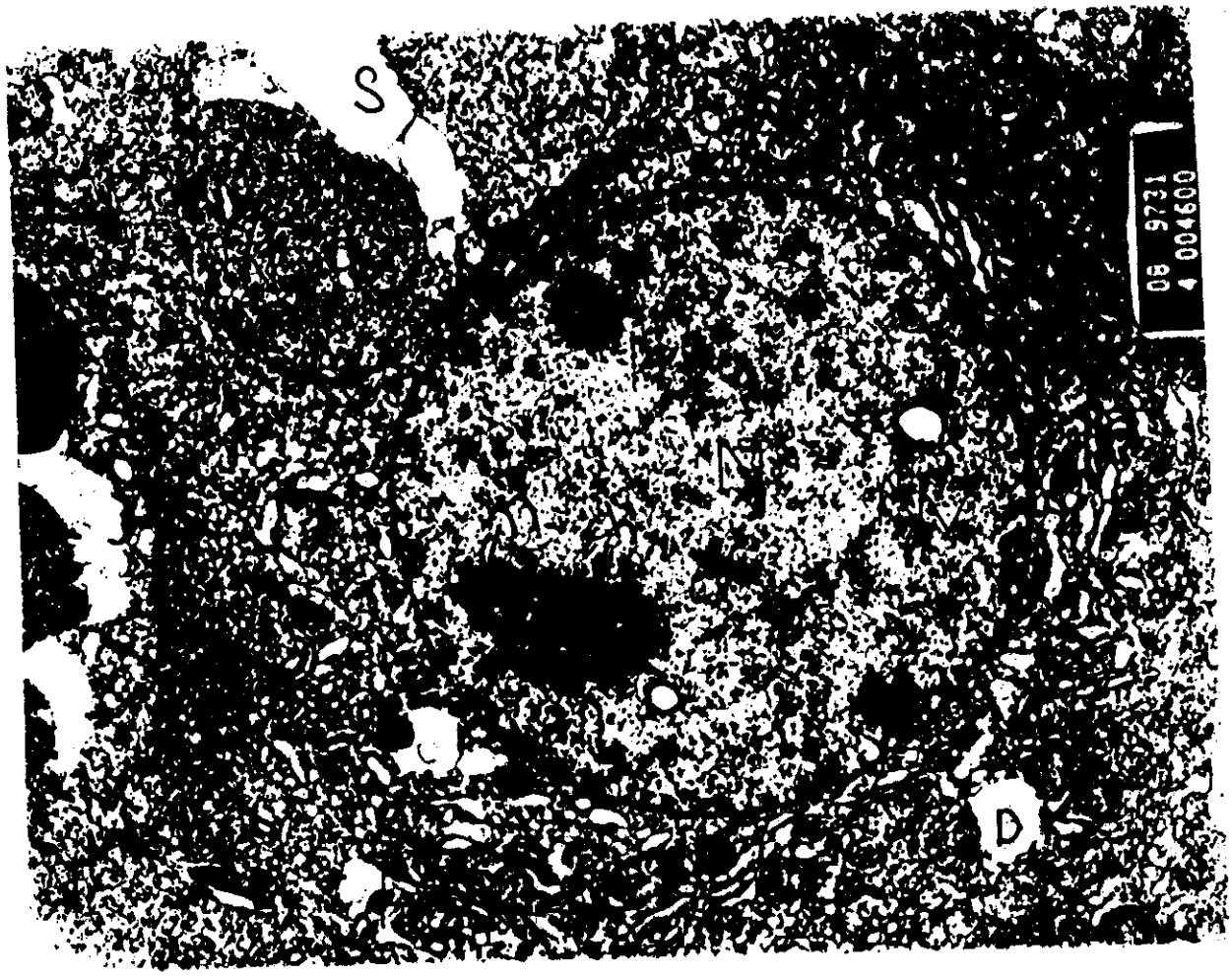

Fig. (27) : An electron micrograph of the liver of a rat given terbinafine for twelve weeks showing a hepatocyte (H) a part of a blood sinuosid (S) contain a red blood cell (RBC) in its lumen. The nucleus of the hepatocyte (N) showed nculeolar fragmentation (n), marked condensation of the nucleolonema, chromatin clumps (ch) and intranuclear vacuoles (NV). Marked proliferation and dilatation of rough and smooth endoplasmic reticulaem (RER & SER) is evident. The cytoplasm of the hepatocyte showed areas of degeneration (D).

(X 4600).

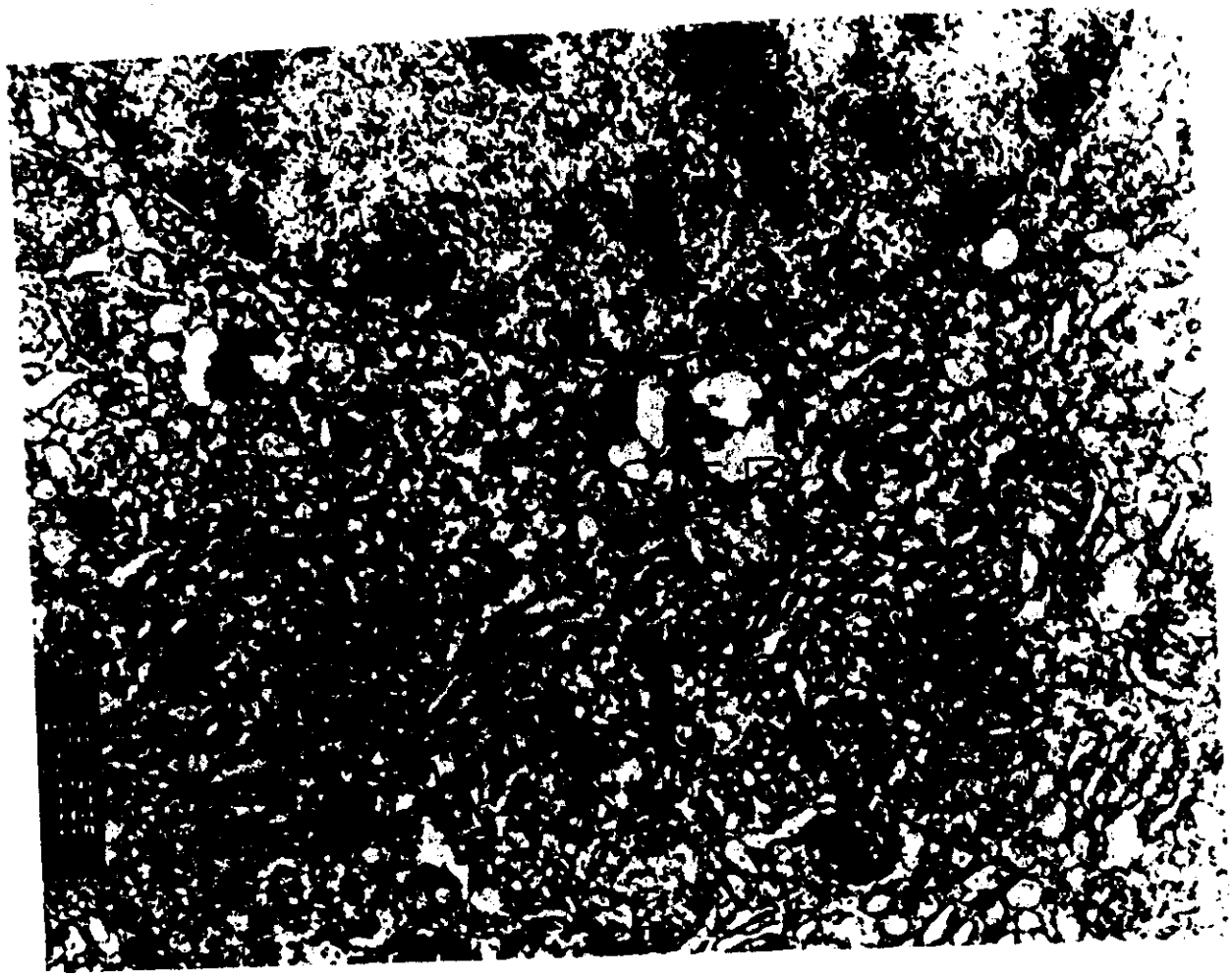

Fig. (28) : An electron micrograph of the liver of a rat given terbinafine for twelve weeks showing a higher magnification of a hepatocyte with a part of its nucleus (N), the marked proliferation and dilatation of rough endoplasmic reticulum (RER) and smooth endoplasmic reticulum (SER). Marked proliferation of peroxisomes (P) and lysosomes (Ly).

(X 10000).

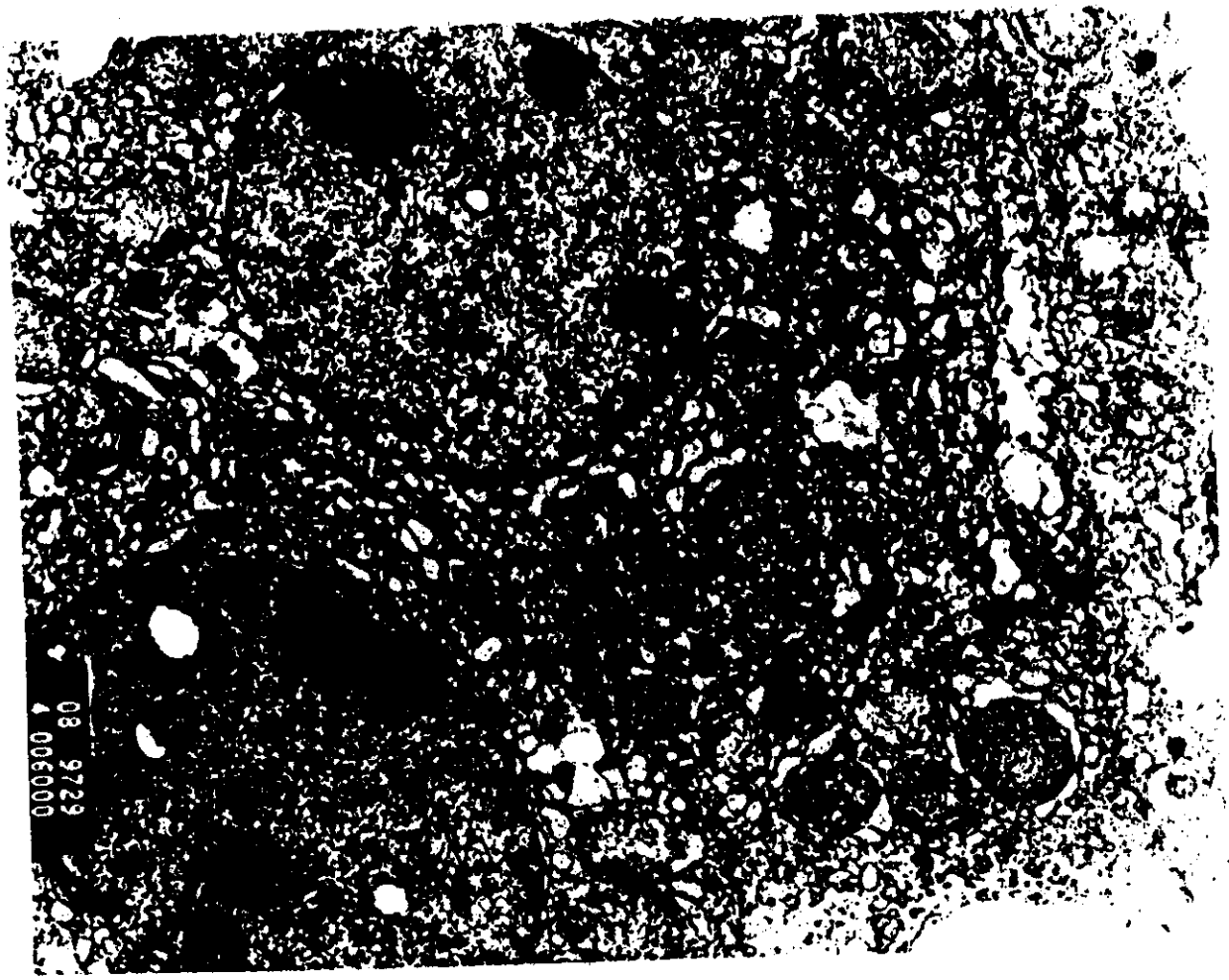

Fig. (30) : An electron micrograph of the liver of a rat given terbinafine for twelve weeks showing hepatocytes (H) and bile canaliculi (bc). The bile canaliculi showed a mild dilatation and were surrounded by pericanalicular biliary deposits (bd). A nucleus (N) of a hepatocyte showed fragmented nucleoli (n), chromatin clumps and interanuclear vacuoles (NV). Proliferated and dilated rough endoplasmic reticulum (RER) and smooth endoplasmic reticulum (SER) are evident.

(X 6000).

Fig. (32) : An electron micrograph of the liver of a rat given terbinafine for twelve weeks showing a dilated blood sinusoid filled with red blood corpuscles (RBC). The endothelial cell of the blood sinusoid (E) appears hypertrophied. The Disse space (DS) is wide and contains an Ito cell (Ic) which is filled with lipid droplets. The plasma membrane microvilli are blunt.

(X 4600).

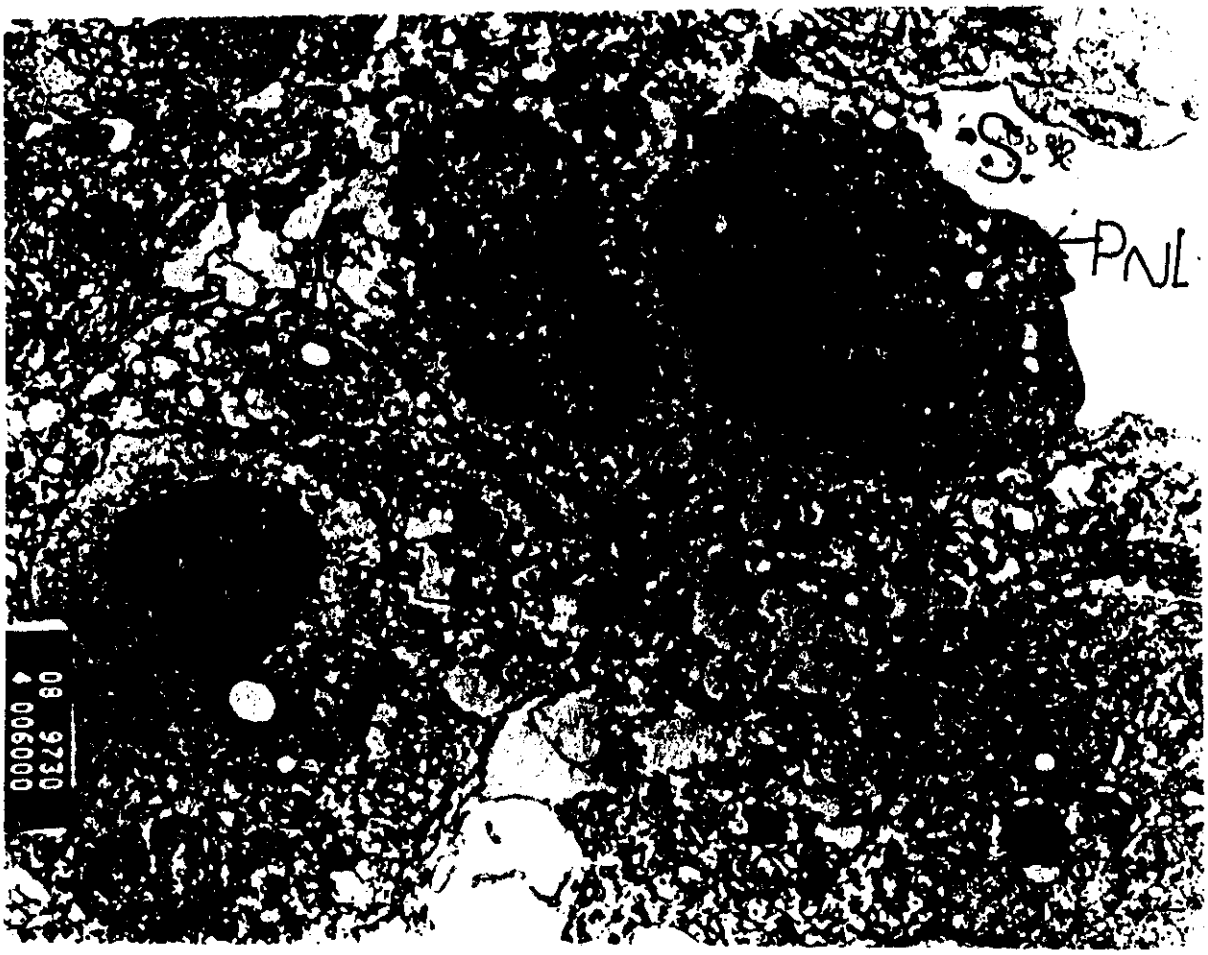

Fig. (33) : An electron micrograph of the liver of a rat given terbinafine for twelve weeks showing a blood sinuosid (S) infiltrated by inflammatory cells as lymphocytes (Lym) macrophage (M) and polymorph (PNL), the adjoining hepatocytes (H) appear degenerated with blunt vascular surface.

(X 6000).

Group V :

Liver of rats after one month of stopping terbinafine showed a general regression of all changes in the previously treated groups and return to the normal control pciture. The cytoplasm showed lipid. The nuclei appeared normal (Figs. 34 & 36). Some nuclei showed condensed peripheral nucleoli. Lipid droplets of variable size were seen. The mitochondria were nearly normal. Normal cisternae of rough endoplasmic reticulum were seen (Fig. 35). Glycogen particles were seen scattered in the cytoplasm (Figs. 35 & 36).

The blood sinusoids with their endothelial lining and Kupffer cells appeared normal. Inflammatory infiltrates disappeared (Figs. 36 & 37). The bile canaliculi were normal in structure (Fig. 38).

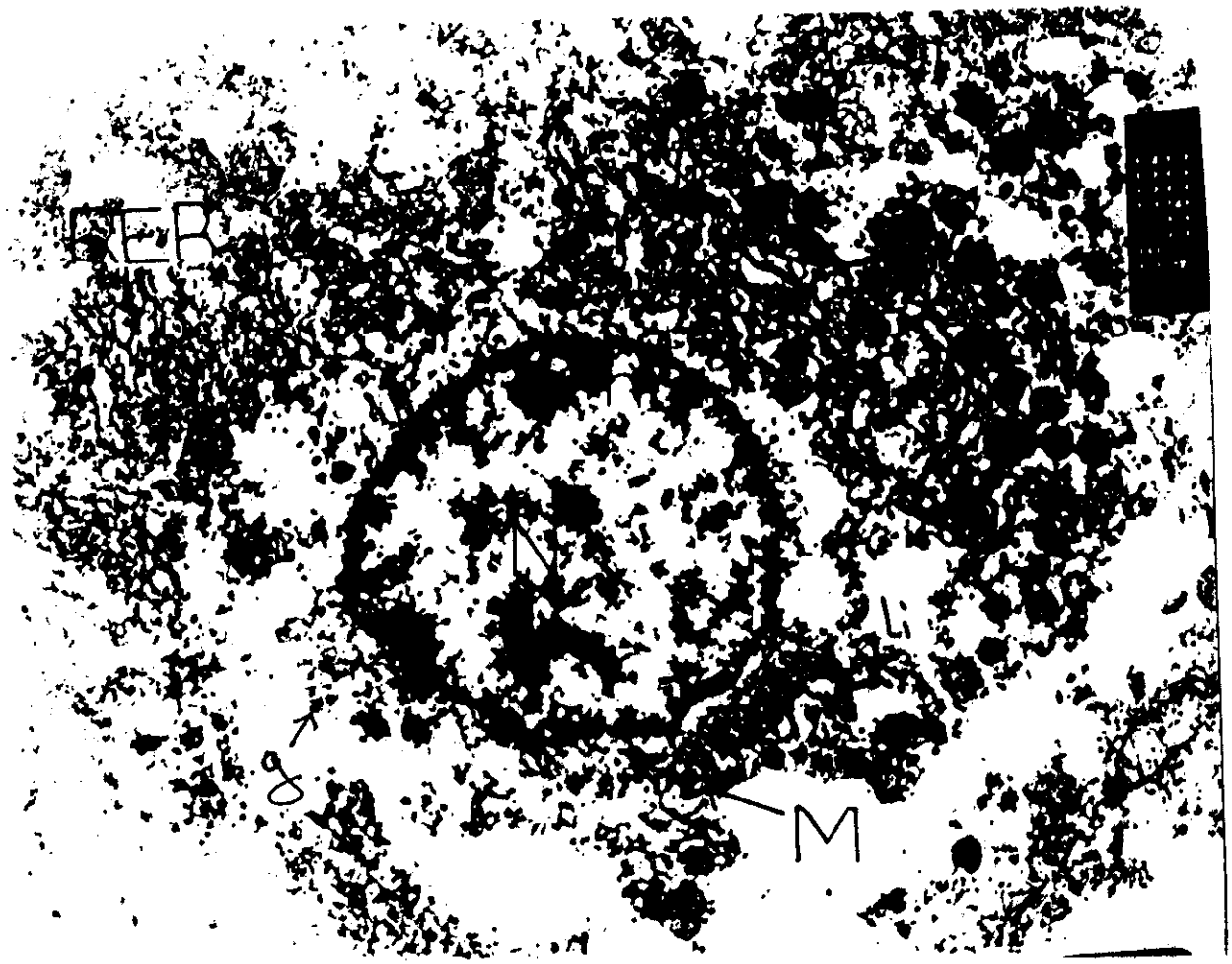

Fig. (34) : An electron micrograph of the liver of a rat after one month of stopping terbinafine showing the hepatocytes nucleus (N) with normal peripheral nucleoli (n), normal rough endoplasmic reticulum (RER), few lipid droplets (Li) and normal mitochondria (M). Glycogen is scattered in the cytoplasm (g).

(X 6000).

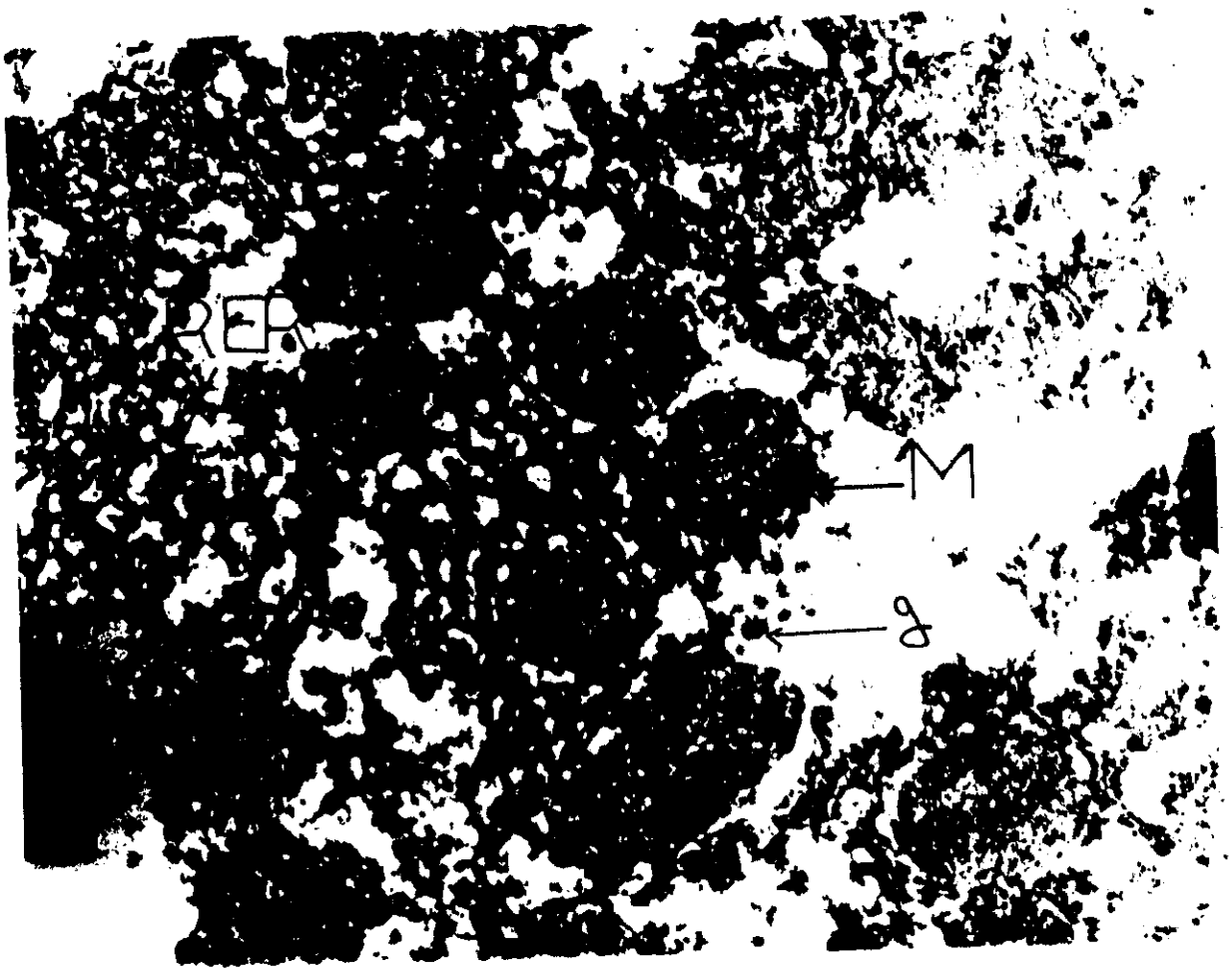

Fig. (35) : An electon micrograph of the liver of a rat after one month of stopping terbinafine showing. Rough endoplasmic reticulum (RER) and mitochandria (M) are normal. Glygoen particles are seen all over the cytoplasm forming rosettes (g).

(X 17000).

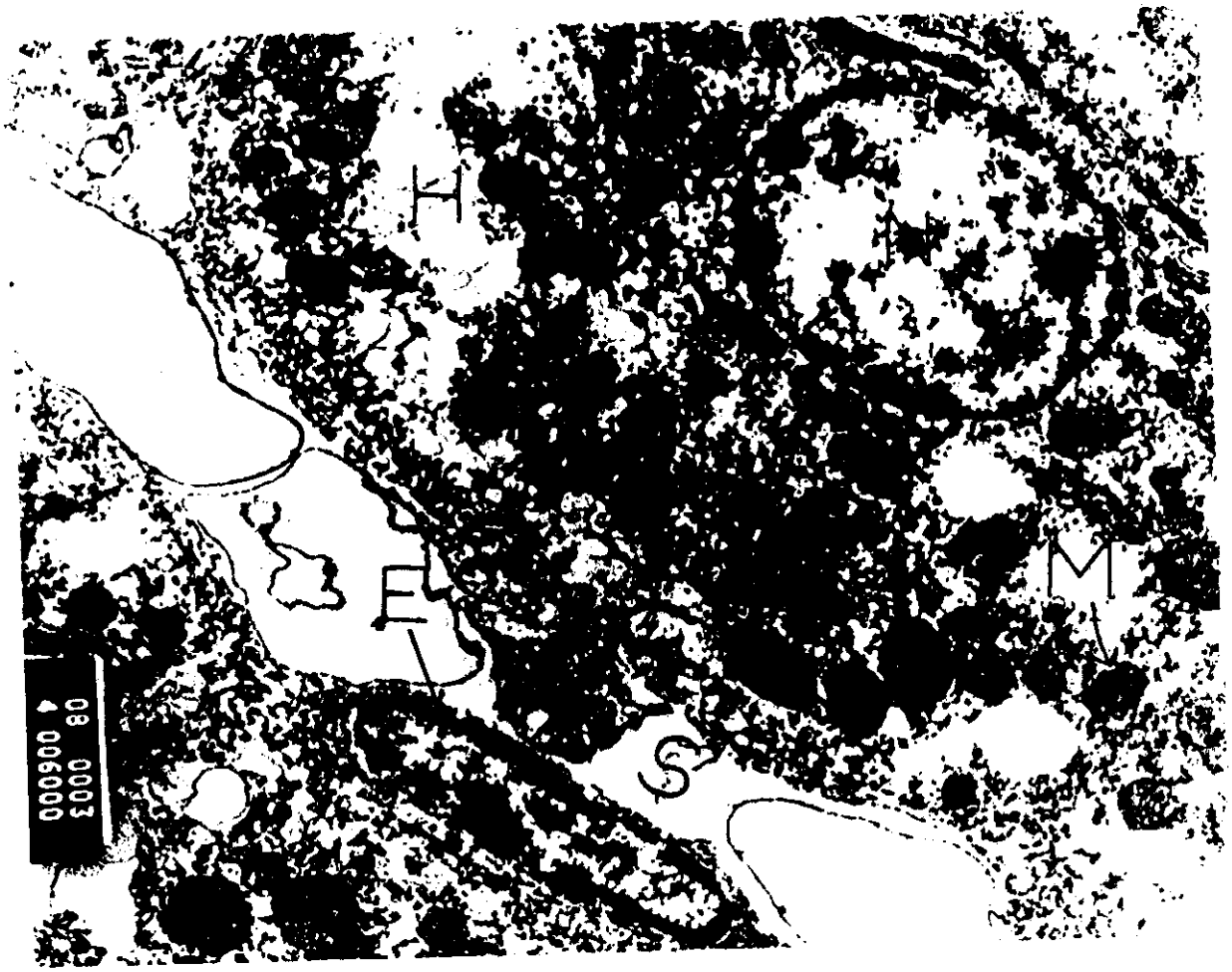

Fig. (36) : An electron micrograph of the liver of a rat after one month of stopping terbinafine showing a blood sinusoid (S) with a lining endothelial cell (E). Notice its return to normal sturcture. The hepatocyte (H) has normal nucleus (N) and its vascular surface shows microvilli and normal mitochondria (M). Glycogen particles (g) are scattered all over the cytoplasm.

(X 6000).

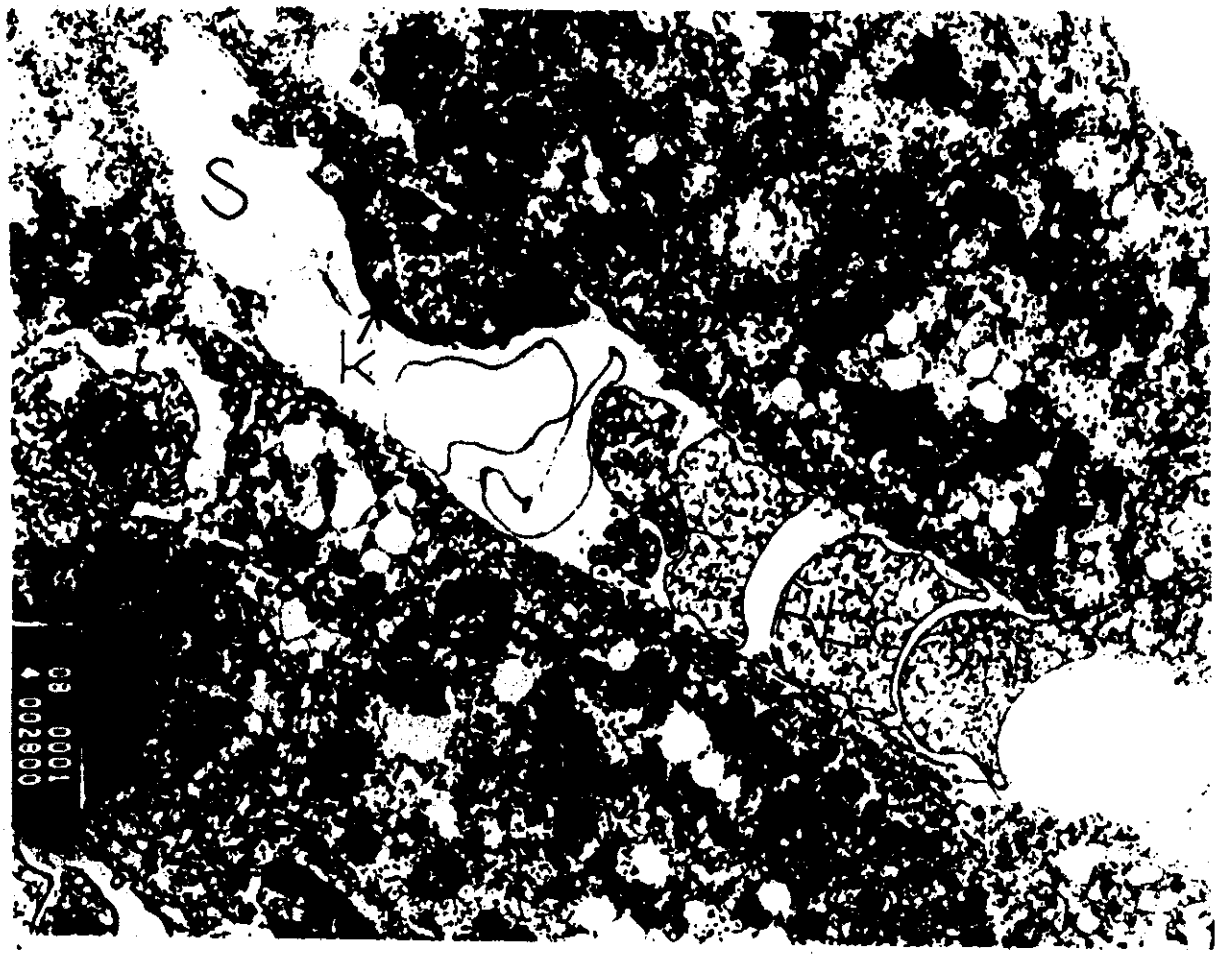

Fig. (37) : An electron micrograph of the liver of a rat after one month of stopping terbinafine showing a normal blood sinusoid (S) with a normal Kupffer cell (K) and Disse space (S) (RBC) is a red blood cell in the lumen of sinusoid.

(X 2800).