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Abstract

To reveal the mechanism of liver damage by taking CCl4 the author observed the liver tissues from rats at 1.5, 5, 6, 10, 17, 20, and 22 hours after the CCl4 administration, both by light microscope and electron-microscope. 1. Light microscope observation revealed the swelling of liver cells in the carly stage, the appearance of centrolobular fatty degeneration, focal degeneration area and the appearance of balloon cells, with the circulatory disturbances in accompanying stages and hemorrhage in the later stage. 2. Electron-microscope observation revealed the swelling of mitochondria, appearance of the files of thin ER's in the early stage and the regeneration and degeneration of mitochondria with an increase of microbodies in number. Fat droplets are developed from small ones probably from some microbodies without correlation with mitochondria. 3. From these observations the author is of the opinion that CCl4 arrests the cells at first inducing the swelling of cells and their mitochondria, but later the degenerative changes will become severe being complicated by the anoxia which is induced by the circulatory disturbances caused by the compression of vessels with the swollen cells.

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MORPHOLOGY OF MITOCHONDRIA AND CELL RESPIRATION

1. MORPHOLOGIC STUDIES ON THE RAT LIVER AND ITS MITOCHONDRIA IN CARBON TETRACHLORIDE POISONING

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The carbon tetrachloride (CCl_4) poisoning causes fatty degeneration in the liver with resultant necrosis in the central part of the liver lobules as is well known ever since the report of DOCHERTY and NICHOLS[1] (1923) on three autopsy cases (LAMSON[2], MARTIN[3], WOODS[4], GRAY[5], MOON[6], STROUSE[7]). About the same time the experimental pathology on CCl_4 poisoning seems to have been established,[8–12] but by 1936 on the heel of the reports by CAMERON, KARUNARATNE[13], many studies on this problem appeared rapidly one after another, presenting two conflicting theories on the developmental mechanism of the pathological changes caused by the poisoning.[14–31] Namely, the one points out circulatory disturbances in the liver, while the other upholds the view that the changes are brought about by direct injury on liver cells themselves. The former attributes the severe necrosis found in the central part of the lobules to oxygen deficiency because a less toxic substance are found in the central part than in the periphery of lobules while the cellular damage is more marked in central area than in periphery and yet the disappearance of vessel lumens in the central area of lobules is generally the phenomenon.[19,21] On the other hand, the latter claims the direct attack on liver parenchymal cells by CCl_4 asserting that liver necrosis can develop without blockage of the blood vessels.[14,29] Despite numerous observations reported by these predecessors the true pathophysiology of the CCl_4 poisoning had remained unclarified as pointed out by CHRISTIE and JUDAH[72]. Recently MYREN[30] has offered a compromising theory for these conflicting opinions stating that those liver cells in the intermediate zone of the lobules are directly affected by CCl_4 while those cells in the central zone are damaged by the disturbances in the blood circulation.

More recently the efforts have been made using electron-microscope to solve this conflicting problem by observing the structural change of cell organellae in the liver affected by CCl_4. Thus, ONOE[32] and MÖLBERT[33], etc., reported the swelling of mitochondria with the enlargement of the inner space and destruc-

tion of endoplasmic reticulum (ER) in the liver cells of mice and rats given CCl_4. On the other hand, there are reports attributing the genetic factor of cloudy swelling of cells including the case of CCl_4 poisoning to the damage of respiratory enzymes[34].

Therefore, the author performed a series of experiments for the purpose to clarify the morphologic change of liver cell especially of mitochondria responsible for the lowering of respiration of the cells and cloudy swelling to degeneration in CCl_4 poisoning. In this paper the findings by light microscope and electron-microscope observations on rat liver in the early stages of CCl_4 poisoning are presented.

MATERIALS AND METHODS

Forty-two hybrid male adult rats weighing 100—150 g. served as materials. They were fed on rice, fresh cabbage and water. Twenty-eight of them were given 0.25 ml. of CCl_4 per 100 g. body weight by oral administration by means of a fine gum catheter. After that the animals were kept in a relative starvation giving water only. Other 14 animals were pairs of fed controls. Three animals, two fed with CCl_4 and one control, were sacrificed by decapitation for each observation at 1.5, 5, 6, 10, 17, 20, and 22 hours after the CCl_4 administration. The livers were removed immediately and fixed for morphologic observations.

For the light microscope observation two tissue blocks from each liver were fixed in a 10% formalin solution. One was embedded in paraffin and stained with hematoxylin-eosin, and the other one was frozen-sectioned and stained with Sudan IV for fat. For the electron-microscopic observation, the livers were fixed in an 1.0% osmic acid solution (phosphate buffer, pH 7.38), dehydrated through 70, 90, 99%, and absolute alcohol and embedded in the mixture of n-butyl methacrylate and methyl methacrylate (9 : 1), polymerised by adding benzoyl peroxide in two per cent. Sections of about 200 Å in thickness were made by using the ultra-microtome of type K, Shimazu Co., picked on collodion film and observed by the electron-microscope of Hitachi Co., HU-10A.

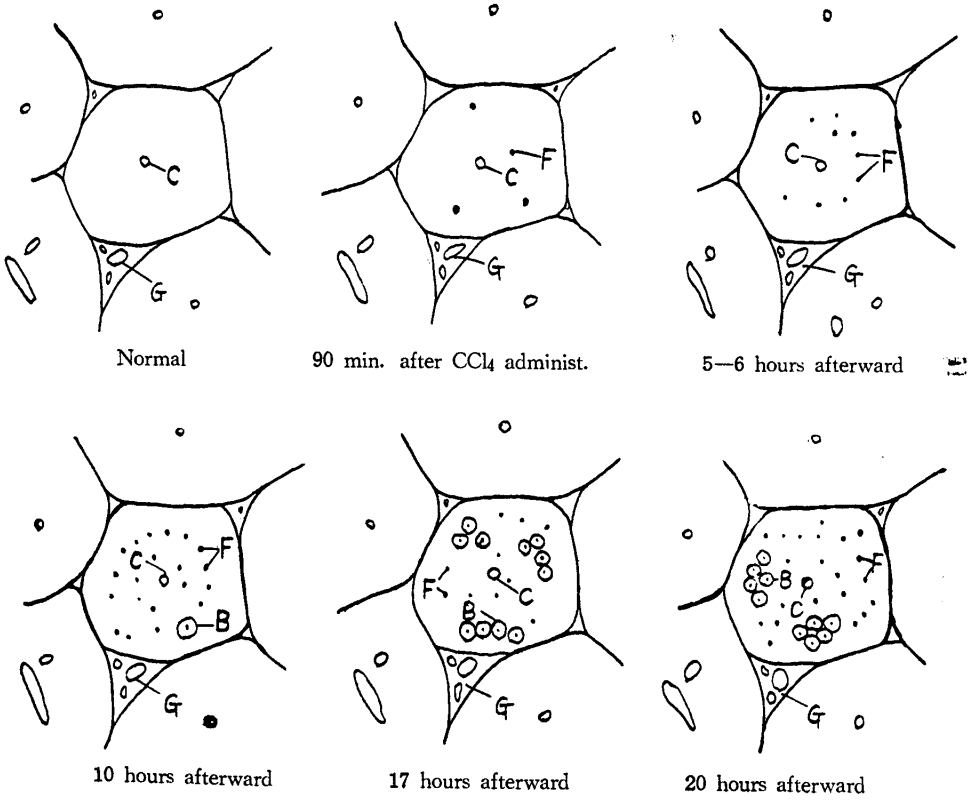
RESULTS

Patho-anatomic and histologic findings: Macroscopically, the livers of the adult rats in the control group had a brilliant reddish brown color, and showed no congestion on the cut-surface with the distinct picture of lobules. The livers taken one and half hours after the oral administration of CCl_4 were almost normal in color but somewhat enlarged. At the fifth hour after CCl_4 administration the livers presented yellowish color showing the onset of fatty

degeneration. This change in color became marked in more advanced stages.

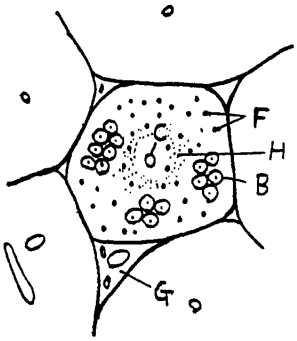
Histologically, one and half hours after the CCl_4 administration the liver cells lying near the central veins revealed small fat droplets in cytoplasm, but otherwise showing no marked changes, the major portion was still normal. In the livers taken 5—6 hours after the CCl_4 administration all the liver cells had more or less an increased number of fat droplets in the cytoplasm of cells in the centrolobular area. At the tenth hour of poisoning the picture of fatty degeneration appeared especially marked in the region surrounding the central veins, showing the tendency to extend gradually toward the peripheral area. In the cells deposited profusely with fat the nuclei were swollen or pyknotic, and fat granules in cytoplasm fused themselves in the whole area of cytoplasm with some gross ones which were formed probably by agglomeration (Fig. 1). At the seventeenth hour, a number of balloon cells, the severely swollen ones with scanty cytoplasm, appear among the cells loaded with heavy fat accumulation in cyto-

Fig. I. Schematic Representation of Liver Cell undergoing Histologic Changes with Lapse of the Time after the Carbon Tetrachloride Administration



Normal | 90 min. after CCl_4 administ. | 5—6 hours afterward

10 hours afterward | 17 hours afterward | 20 hours afterward



C: Central veins
F: Fat droplets
B: Balloon cells
H: Micro-hemorrhage
G: Glisson's capsule

22 hours afterward

plasm. At the twentieth hour on advanced fatty degeneration with increase l balloon cells they presented actually the picture of necrobiosis. At the twenty-second hour, in addition to those changes just mentioned, the dilatation of sinusoid and micro-hemorrhage were observed but no necrotic area. The findings worthy of further notice are that the primary pathological changes of the fatty degeneration do not always appear only in the central part of the liver lobule but also start often from the peripheral part, sometimes developing to the picture of an irregulary distributed focal degeneration at the fifth hour. Summarizing, the pathological changes proceed with cloudy swelling with lipophanerotic change and develop to fatty degeneration and necrobiosis within 22 hours after the CCl_4 administration. No signs of the regeneration of the liver cell can be observed during this period. The schematic drawing of these changes are illustrated in Fig. 1.

Electron-microscope findings:

i) Under electron-microscope the parenchymal cells of normal rat liver appear polygonal having round or oval nuclei with distinct nucleoli (Fig. 1). The nuclear substances show irregular distribution of opaque areas both of which consist of fine granules and fibriles and surrounded by a double-layered nuclear membrane. The cytoplasm has mitochondria rather uniform in width, about one μ, but varied in length of the profile, either oval, elliptic, or rod-like, and some of long ones appear like having one or two constrictions probably by the occasion of cut plane on the winding mitochondria (Figs. 1, 2). Occasionally, there can be encountered mitochondria in the shape as if two rods were connected with one another at one end, but since these are on the cut-surface and their true configuration is unknown. These mitochondria are distributed rather evenly in cytoplasm. The so-called cristae mitochondriales[35] appear as highly electron dense striations of double-membrane structure, extending roughly at right angle from the limiting membrane, subsequently some radial arragement like the spokes of a wheel in the mitochondria appears round or oval. The membranes

surrounding mitochondria seems to be originally of double-layer structure but appear rather indistinct. The cytoplasm also has endoplasmic reticulum[36] (ER). They are of small vesicular in shape with some elongated ones, and have a few Palade's granules. Besides these, there appear fairly large areas distributed irregularly in cytoplasm, in which no mitochondria and ER can be found but filled with moderately dense material, probably glycogen deposit. The cell membrane is generally smooth but has a numerous projections forming microbili at the surfaces toward the bile canaliculi and at some part communicating with kupper cells.

ii) In the liver one and half hours after the CCl_4 administration the shape of the nucleus presents not much change, but in some nuclei the nuclear substance is slightly electron dense near the nuclear membrane and along with it the nuclear membrane also presents a slightly rugged line (Fig. 3). Mitochondria appear usually oval or elliptic in shape, and hardly any long ones can be observed different from those seen in the normal cells (Figs. 3, 4). Cristae mitochondriales maintain their radial arrangement in some mitochondria but generally they seem to be decreased in number and in length accompanied by a slight disorder in the arrangement. However, the double-membrane structure of each cristae is well preserved (Fig. 3, M). The density of the inner spaces of mitochondria shows no remarkable change from that of the normal. Besides these, in cytoplasm there appear small round dense bodies which are rather smaller in size than mitochondria and have some ambiguous inner structure (Figs. 3, 4, Mb). These seem to be the so-called lipid granules or microbodies[37], but at present they remain unidentified. ER's of vesicular form are reduced markedly but those arranged in several lines of double layer appear to be increasing in number, most of them are distributed densely around the nuclear membrane and mitochondria as if to envelope them. In these area Palade's granules appear being distributed densely (Fig. 4). Other cellular components such Golgi's apparatus, glycogen depositing area and the cell membrane show no striking change.

iii) In the liver cells from the materials taken five hours after the CCl_4 administration the nuclear substances are almost normal in its granular and fibrous structures and their arrangement but the nuclear membrane shows a slightly-rugged line with some vesicular transformation of double-layer membrane (Figs. 5, 6. Vc). There are one or two nucleoles grown slightly bigger with a distinct nucleolonema structure (Fig. 5. Nc). In cytoplasm the mitochondria are greatly swollen, presenting a balloon shape. Their limiting membranes are smooth and well preserved. The central part appears rather vague but the cristae having double-layer structure can be seen mainly arranged radial just inside the limiting membrane. The length of cristae looks rather short by the increased diameter of mitochondria themselves. At a glance the number of mitochondria seems to

be increased, but on the actual count it is not much different from the normal. This is due to the reduction of cytoplasmic space being occupied by the enormously swollen mitochondria. Among these swollen mitochondria there can also be recognized some mitochondria on the process of disintegration (Figs. 5, 6.). They lose their limiting membrane partially but the double-membrane structure of cristae is found to be still preserved. In the vicinity of swollen mitochondria there are numerous round dense bodies with ambiguous limiting membrane, probably Bernhard's microbodies[38]. Some of them show a cristae-like inner structure suggesting the transformation into mitochondria. ER's, which appeared mainly lineal one and half hours after the CCl_4 administration, assume again vesicular structure and some of them seem to be on the way to disintegration. Palade's granules can still be recognized. Some ER's, which are arranged encircling swollen mitochondria, appear still linear. In some cytoplasmic area there appear a quantity of vacuolated structure probably by the swollen ER's with some lipid granules. The cell membrane presents no marked change.

iv) In the liver six hours after the CCl_4 administration (Figs. 7, 8), the nuclear substance looks almost normal surrounded by the double-layer of nuclear membrane. In cytoplasm mitochondria are irregular in size but mostly round in shape. In some area there appear a number of small round mitochondria of electron dense and microbodies among the swollen mitochondria (Figs. 7, 8). These small ones will probably be newly formed ones. The mitochondria on disintegration are large in size and scanty in crescent shape. The dense mitochondria are generally surrounded by linear ER's. Besides these, there are vacuoles and fat droplet in various size (Fig. 7). The degree of these changes is varied according to individual cells and not uniform.

v) At the tenth hour after the CCl_4 administration (Figs. 9, 10), the swollen mitochondria appear almost scanty in their contents, though some of them still a few double-layer cristae. Among them a number of dense mitochondria appear showing a marked variety in size (Fig. 9). These will be the regenerating ones. The cytoplasm are of vacuolated structure with the swollen ER's and the swollen mitochondria and contain a number of lipid droplets varied in size. There appear also a number of microbodies, some of which develop clearly the cristae structure (Fig. 10). Nuclear structures show no marked change.

vi) The livers 17 hours after the CCl_4 administration (Figs. 11. 12), show again the swelling of mitochondria with the decreased density suggesting the degeneration and disintegration of the regenerated mitochondria. Some of them are broken, in their limiting membrane and inner structure, but generally the swollen ones still retain the double-membrane structure of cristae, though some decrease in number (Fig. 12).

Only a few ER's can be recognized on the outside of a markedly swollen

vacuoles or around mitochondria. In addition, many vacuoles of various size and a few giant fat droplets can be recognized in cytoplasm. Microbodies are seen in a fairly large number. Nucleus and Golgi apparatus show no specific change.

vii) In the case 20 hours after the CCl_4 administration (Figs. 13, 14), the change in mitochondria do not differ much from those in the case 17 hours afterward, though the swelling and disintegration seem to be more marked. The cristae seem to be reduced and fragmented, but retain their double-layer structure. Besides these, there appear some moderately opaque substance in cytoplasm (Fig. 13). These are irregular both in shape and size and composed of the thin double-layer fibriles agglomerated or arranged somewhat parallel. These may be the regenerating ER's but they show the tendency to agglomerate forming denser structures and are not identified. In the peripheral region of the cell there are many vacuoles and fat droplets and the cell membrane is rather indistinct.

viii) In the liver 22 hours after the administration (Figs. 15, 16), the mitochondria of various size still maintain their double-membrane cristae which are rather fragmented. Some mitochondria show a picture ready to be split. ER's having granules can be seen. Beside these, the formation of vacuoles of various size, an increase in the number of fat droplets and the moderately dense unidentified substances can be seen (Fig. 15. 0), which will coincide with the substances appearing in Fig. 13, 0.

In summarizing, the changes occurring in mitochondria at the time of CCl_4 poisoning are swelling, deformation and disintegration. A marked regeneration seems to occur 5—6 to 10 hours after the CCl_4 administration but these are also swollen and degenerate afterward. The cristae mitochondriales seem to be rather resistant and retain their double-layer structure even in the marked swelling of mitochondria. ER's temporarily increase in the number at an early stage and followed by a reduction in number. The development of a greater number of vacuoles and fat droplets are the sign of later stage.

DISCUSSION

As described above, my observations by light microscope on the liver after the CCl_4 administration suggest that some circulatory disturbances would be responsible for the tissue damage, especially for the severe fatty degeneration appearing in the centrolobular area or appearing as several focal degenerations, because the formation of small focal necrotic area in the liver is generally known as the results of capillary thrombosis. But the thrombosis had not been detected. The disturbance of circulation would be caused by the narrowing of the capillary lumen by the swelling of cells. Macroscopically the liver affected by CCl_4 are swollen enormously and the tissue is rather anemic in some part and in other

part it shows blood congestion and bleeding. These changes become marked after 20 hours and will be the result of compression of blood capillaries by the swollen parenchymal cells. Therefore, the swelling, cloudy swelling, should be the first change of CCl_4 intoxication. This will be of the direct arrest of cells by the poison, CCl_4, and may develop to the irreversible degeneration and necrosis indepedently from the circulatory disturbances.

As described in the introduction of this paper briefly, opinions of the mechanism of CCl_4 intoxication differ by the authors. However, in each report the species of animals used, the method of administration and quantity of CCl_4 administered, and the length of observation time markedly differ according to different workers.[8-20,22-27,29,31-33] Therefore, it is rather difficult to make the comparison of their results fairly and exactly with the present experiment. However the efforts to find the phenomenon common in these experiments have led us to the reasonable understanding of the mechanism of CCl_4 intoxication.

The author would like to agree with MYREN[30] (1956), in his opinion that the liver damage by CCl_4 is due to both the direct arrest of liver cells by CCl_4 and the circulatory disturbances, but can not concur in the point that there are some areas which are more susceptible to the poisonous effect of CCl_4. From the author's own observation it is rather reasonably certain that all liver cells are damaged at first showing the cloudy swelling and subsequent disturbances of circulation by the compression of capillary with the swollen cells, which induce secondarily the focal or centrolobular severe degenerations presenting a complicated picture of mosaic form distributed with severely damaged area in slightly-degenerated parenchyma. Myren's observation that with a small dose of CCl_4 the severity of liver injury is dependent upon the amount of CCl_4 but in the case of a larger dose, the amount is not associated with the severity, supports the author's view. It seems to show that there is a dose of CCl_4 to induce the swelling of cells enough to give rise to the compression of the blood vessels and at the dosage of CCl_4 over this quantity the damage of liver always appears as the damage by the circulatory disturbances, showing no difference by the quantity of CCl_4 given to the animals. The dosage used in the present experiment is of course considerably large in the scheme of MYREN[30].

Thus the severe central degeneration, which has been noticed by many workers,[9,11,16,19,26,29] may be explained as the result of insufficient blood supply by the narrowing of the capillaries lying in the periphery of lobules. Of course, the focal necrobiosis can appear in other places by the localized atresia of the vessel. The balloon cells appear most frequently in the intermediate zone and yet they can be often seen where the fatty degeneration is not so severe. This is the specific type of degeneration and the site of appearance of these cells may be correlated with the area escaping from the blood supply of both portal vein

and arteria hepatica in the case of moderate constriction of capillaries by which the peripheral area of lobules may have a fairly good supply of portal vein blood and the central part by the blood from arteria hepatica. Such cases can possibly be considered from the anatomical structure of liver lobules but the true mechanism of the appearance of balloon cells is unknown.

Electron-microscope observation may give a reliable information to the mechanism of damage of liver cell by CCl_4 intoxication. At an early stage of CCl_4 administration, where any noticeable change has been recognized by light microscope observation, the organellae of liver cells show a recognizable change under electron-microscope. The mitochondria show no striking changes but they are rather round or oval, and elongated ones as in normal liver have not been recognized. ER's of elongated and having a narrow lumen are seen to be arranged parallel densely in some area. Slight increase in the electron density can be observed just inside the nuclear membrane. MÖLBERT[33] explained this picture as a sign of the accelerated activity of basophilia formation in cytoplasm. But the light microscopic observations do not show any increase in the basophilic substance of cytoplasm and it seems that these pictures are only the changes in shape and distribution of nuclear substance and ER's. Beside these changes, there could be observed an increase of fine granules in number that are smaller in size but higher in density than mitochondria, which correspond to the microbodies of RHODIN[38] and BERNHARD[39] and it is recognized that they generally appear numerously in the recovery period of degeneration and transform into mitochondria. MÖLBERT[33] considers these granules to be precursors of mitochondria and designate them as young mitochondria or pro-mitochondria. He further goes on to say that these granules are produced during the period when the cytoplasmic activity is being elevated and if the injury persists, mitochondria become degenerated, swollen and deformed so that these granules are produced as the compensatory measure for the mitochondrial degeneration. On the other hand, BERNHARD[39] has demonstrated electron-microscopically that fat droplets are also produced from these microbodies. Therefore, it seems that these fine granules are transformed into fat droplets or into mitochondria under the condition of degeneration or of regeneration. Moreover, what KOPROWSKI *et al*[37]. call as L-bodies seem to be lipid granules derived from these very granules.

By the fifth to sixth hour after the CCl_4 administration mitochondria are markedly swollen becoming round or oval and vague in the center. The cristae are formed and adhered to the limiting membrane with some irregular arrangement and seem to be decreased in number. Swelling of mitochondria will be due to the hypotonic condition of cytoplasm by the invasion of water.[33,34] MOORE[40] observed the similar change in mitochondria in his experiment with the peroneal muscle of the mouse ligated of its blood vessels. This suggests that the swelling

of mitochondria in the case of CCl_4 intoxication may be due to the hypoxia induced by the circulatory disturbance. Accordingly the cells are always pumping out water from their surface and the energy is supplied by the consumption of ATP, and the swelling of cell is induced by deficiency of the ATP production. Therefore, the swelling of mitochondria may be directly correlated to the ATP deficiency by hypoxia. But there is another possibility that the swelling of mitochondria is the primary change caused directly by the CCl_4 and the swelling of cells may be followed, because the main source of ATP is mitochondria. This point will be touched upon in the next report. The swollen mitochondria will be degenerated and substituted by newly-formed ones as was seen in the later stage, 10 hours after the CCl_4 administration, as was also pointed out by MÖLBERT[38].

The changes of ER's can also be seen in the early stage of intoxication. Later they are swollen forming vesicles but those having distinct double-layer and Palade's granules can be seen surrounding the mitochondria whose inner structure is retained, suggesting a close relationship between mitochondria and the development of ER. BERNHARD[39,41] and ONOE *et al.*[32,42] also noticed the relationship between ER and mitochondria in the regenerating liver. Further observation on the electron dense body appearing in Figs. 13 and 15 may give some information. But from the author's own observations it is not possible to give any explanation on the relation between the change of ER and the action of CCl_4. The appearance of fat droplets has been interpreted as the result of lipophanerosis, but electron-microscope observation proves that the fat droplets start from a small body probably from a microbody in the case of brown fat tissue of mice as observed by ODA[43] and FAWCETT[44]. Mitochondria will not be responsible for the formation of fat droplets, though ONOE[32], TSUJIMURA[45], and GANSLER[46] claim the importance of mitochondria for the formation of fat droplets. BELT[47] also disagrees with the idea that lipids are formed from mitochondria, from his observations on rat adrenal cortex.

CONCLUSION

To reveal the mechanism of liver damage by taking CCl_4 the author observed the liver tissues from rats at 1.5, 5, 6, 10, 17, 20, and 22 hours after the CCl_4 administration, both by light microscope and electron-microscope.

1. Light microscope observation revealed the swelling of liver cells in the early stage, the appearance of centrolobular fatty degeneration, focal degeneration area and the appearance of balloon cells, with the circulatory disturbances in accompanying stages and hemorrhage in the later stage.

2. Electron-microscope observation revealed the swelling of mitochondria, appearance of the files of thin ER's in the early stage and the regeneration and

degeneration of mitochondria with an increase of microbodies in number. Fat droplets are developed from small ones probably from some microbodies without correlation with mitochondria.

3. From these observations the author is of the opinion that CCl_4 arrests the cells at first inducing the swelling of cells and their mitochondria, but later the degenerative changes will become severe being complicated by the anoxia which is induced by the circulatory disturbances caused by the compression of vessels with the swollen cells.

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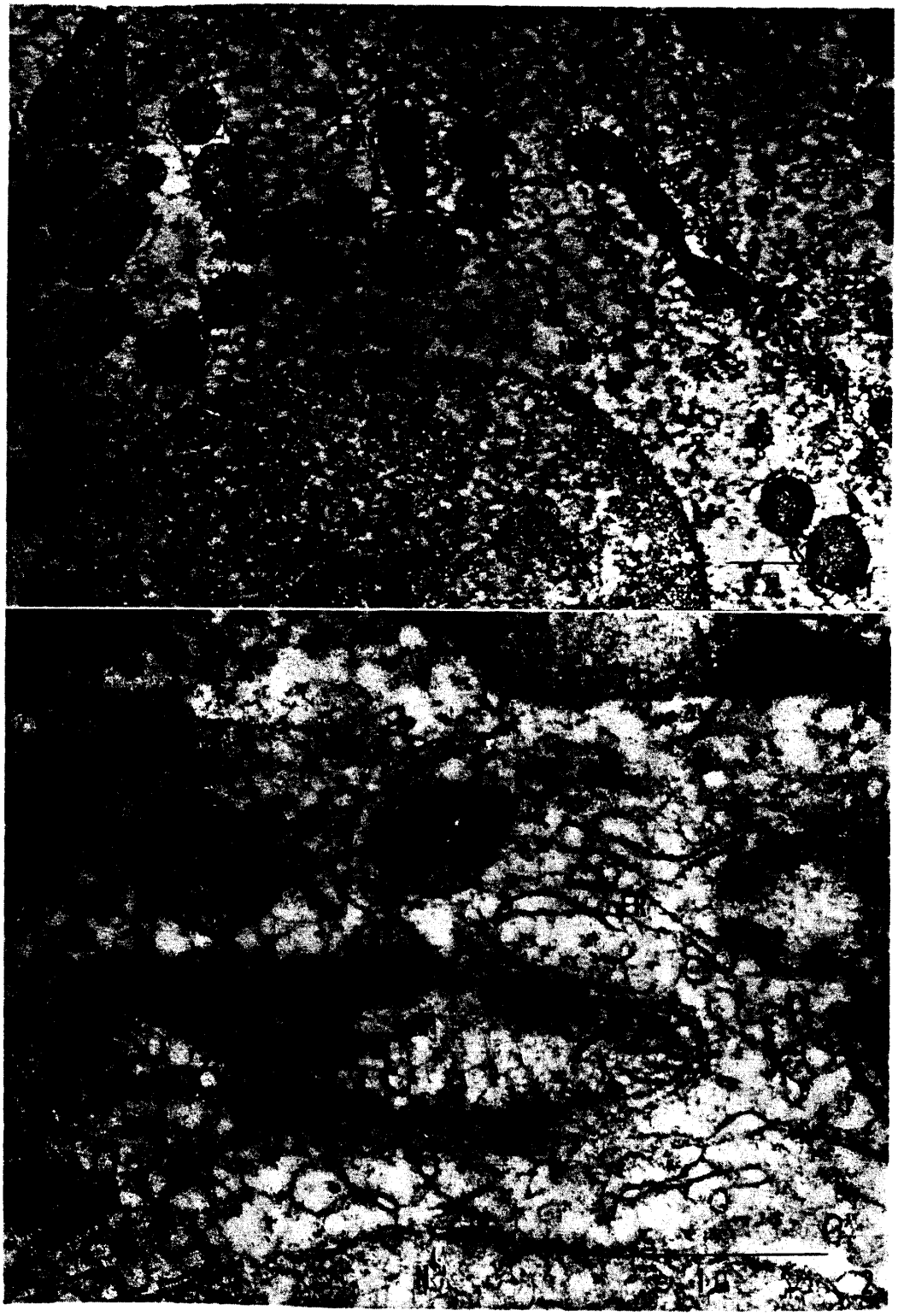
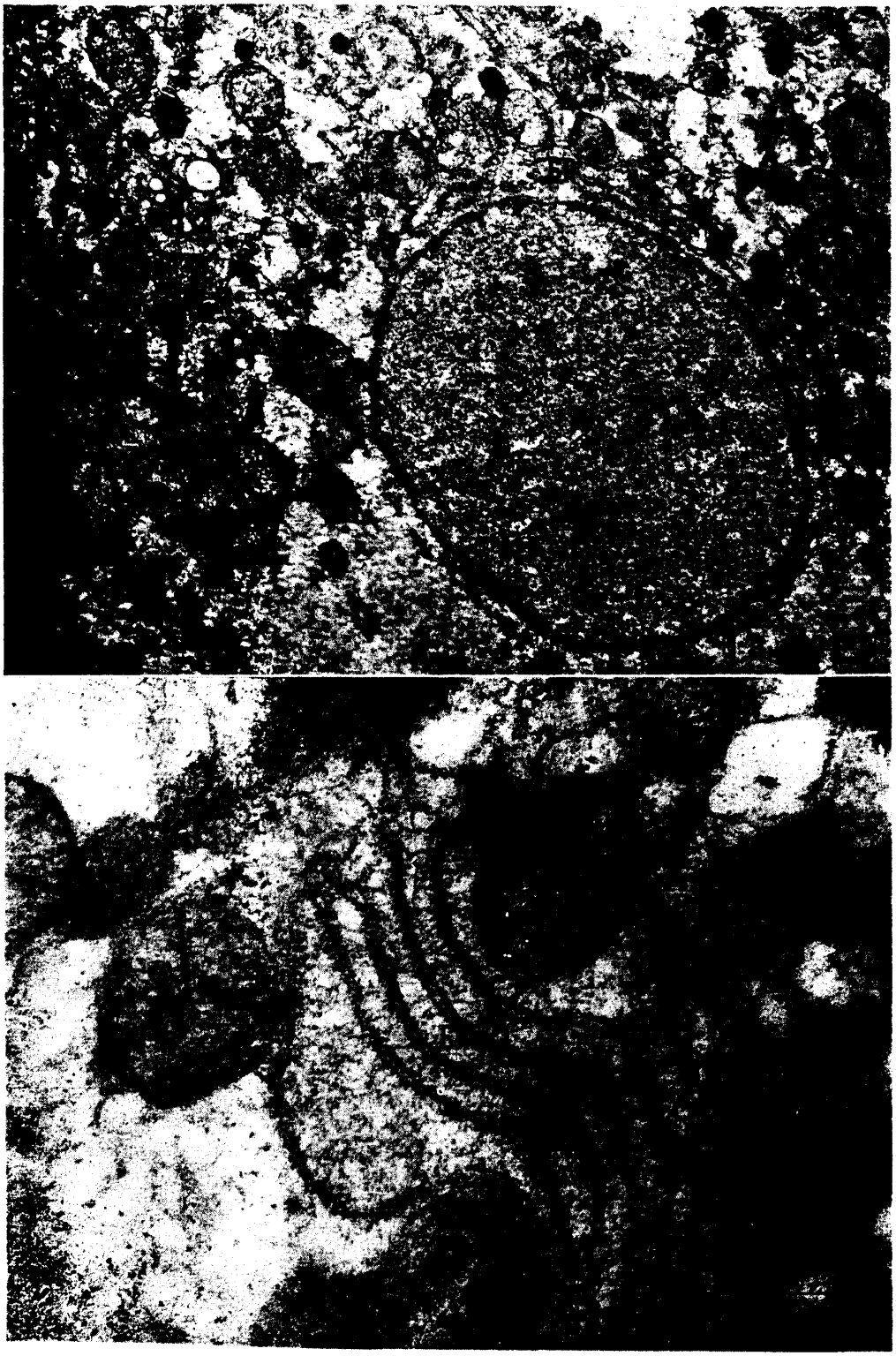
EXPLANATIONS OF FIGURES

Figs. 1—16. Electron-micrograms of the rat liver cells. Odd number is of low magnification and even number, of high magnification. Symbols: N: nucleus, Nc: nucleole, M: mitochondria, C: cristae mitochondriales, ER: endoplasmic reticulum, Mb: microbody, Md: mitochondria with destroyed cristae mitochondriales, CM: cell membrane, BC: bile canaliculi, Os: osmiophilic granule, V: vacuole, O: opaque area, Vc: Vesicular formation. (see text)

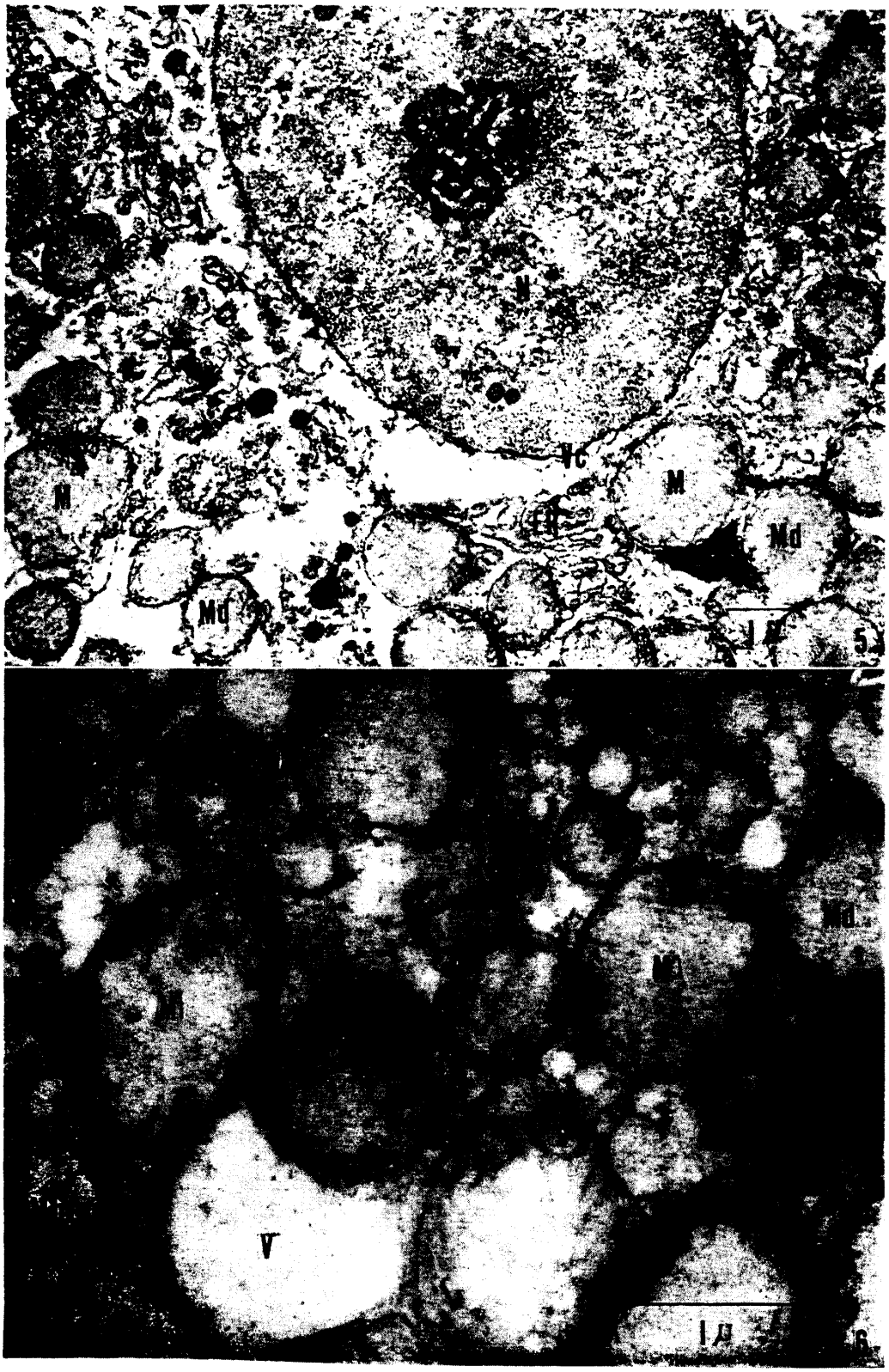
Figs. 1—2. A normal rat liver cell. Mitochondria (M) have a fairly uniform shape but some of them are of irregular shape depending on the direction of cross-section. Cristae (C) have a distinct double-membrane structure and are extending roughly at right angle from the mitochondrial membrane. Microbodies (Mb) can be observed.



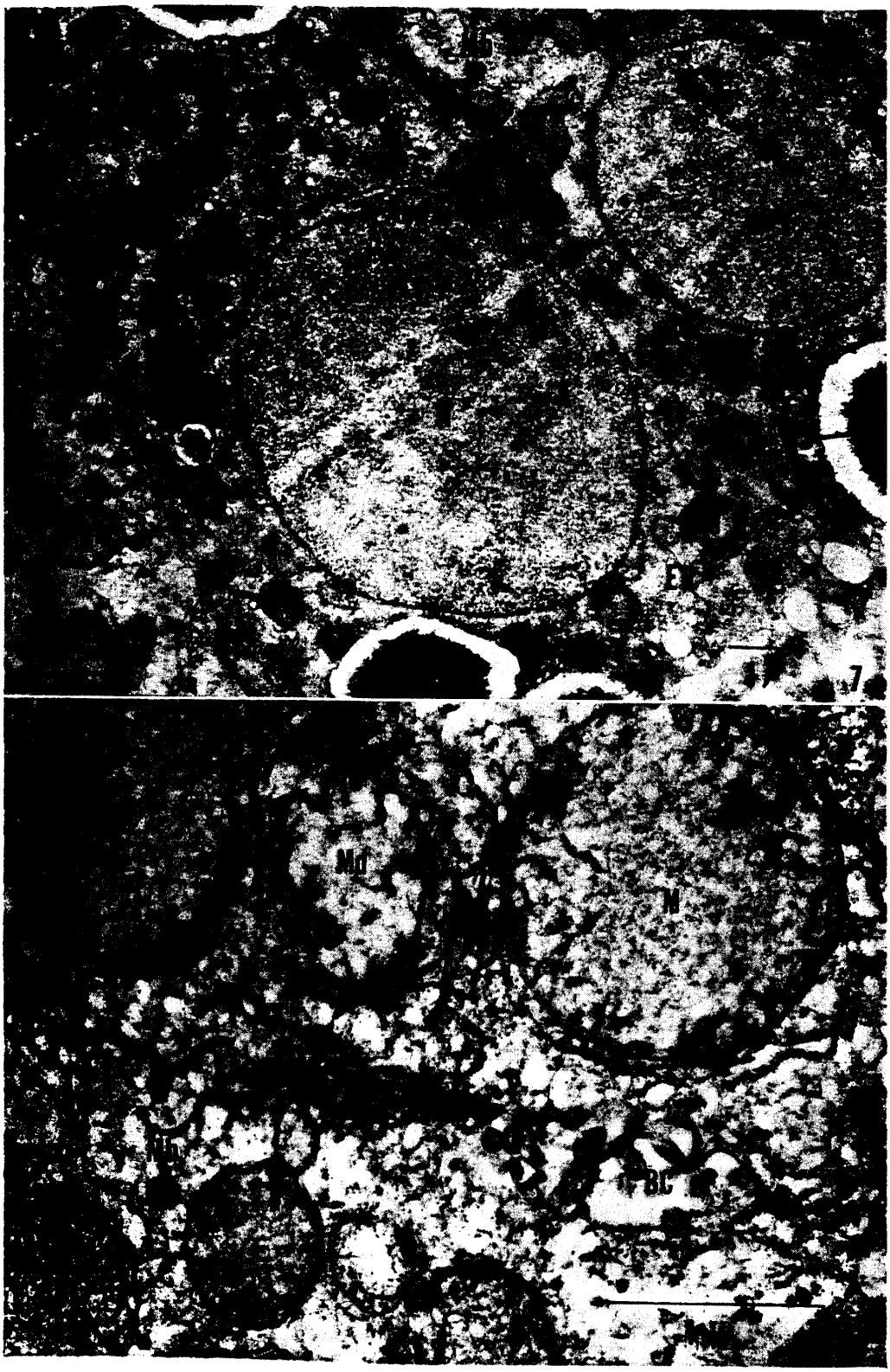
Figs. 3—4. The cell at 1.5 hours the after the CCl_4 administration. No appreciable change can be recognized in M, and some of C are in the shape of spokes of a wheel and they are decreased in number and their arrangement is in disorder. The double-membrane structure is well preserved. Some of ER's have narrow inner space while others dilated one. Those with narrow inner space present a layer appearance. In low power field, many Mb's can be observed.



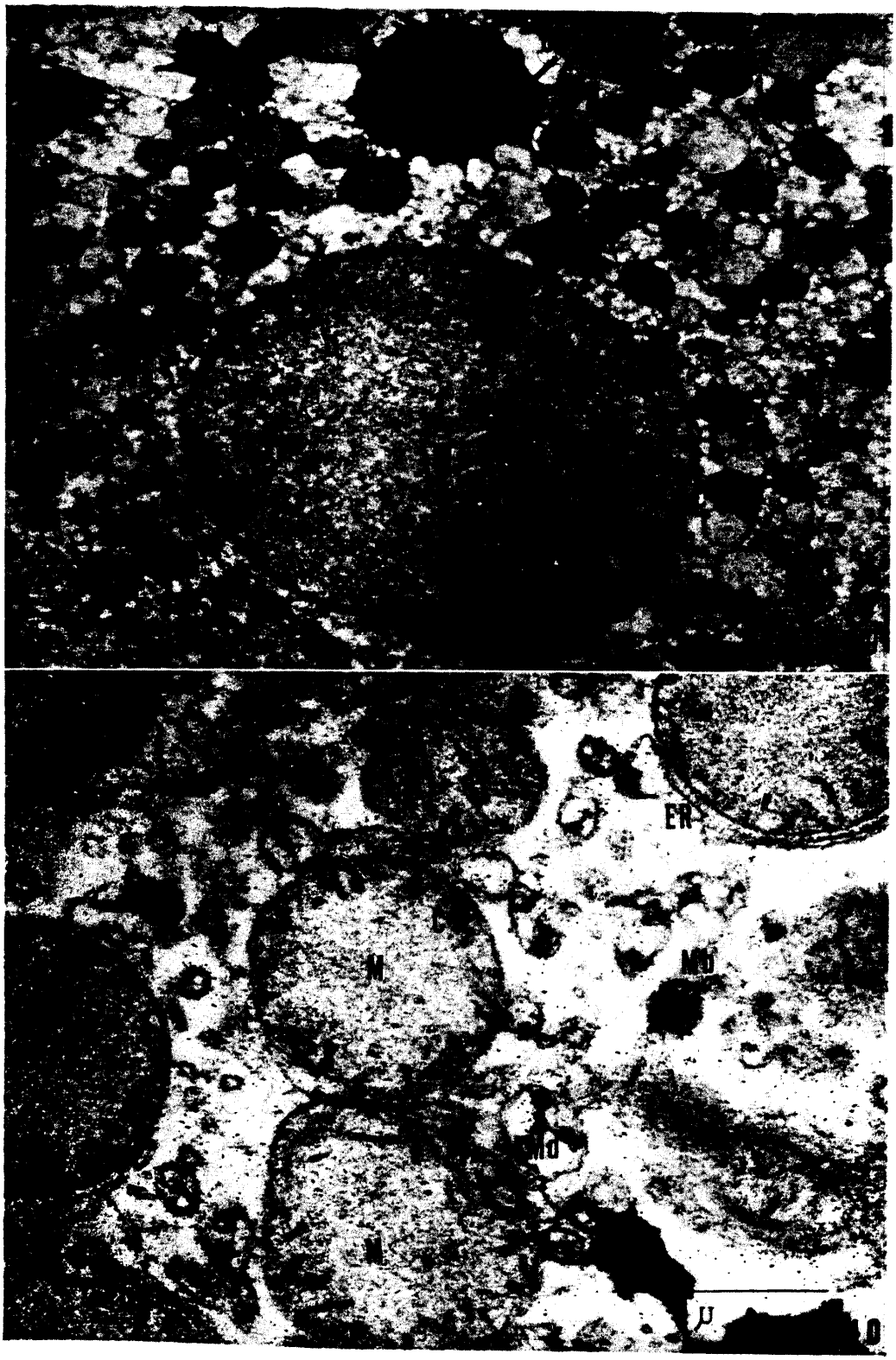
Figs. 5—6. The cell five hours after the administration. Mitochondria with destrored cristae mitochondriales (Md) are markedly swollen, presenting a balloon shape. ER's are decreased in number and enlarged, making vacuoles (V). In high power field, two Mb's can be observed.


M
Vc
M
M
Md
Md
1 μ
5
Md
M
V
1 μ
6

Figs. 7—8. Six hours after the CCl_4 administration. Mitochondria are either markedly swollen, deformed or degenerated. Cristae are decreased in number but the double-membrane structure is still well preserved. In low power field big osmiophilic granules (Os) can be seen. In high power field, distinct bile canaliculi (BC) can be observed.


M
M
BC

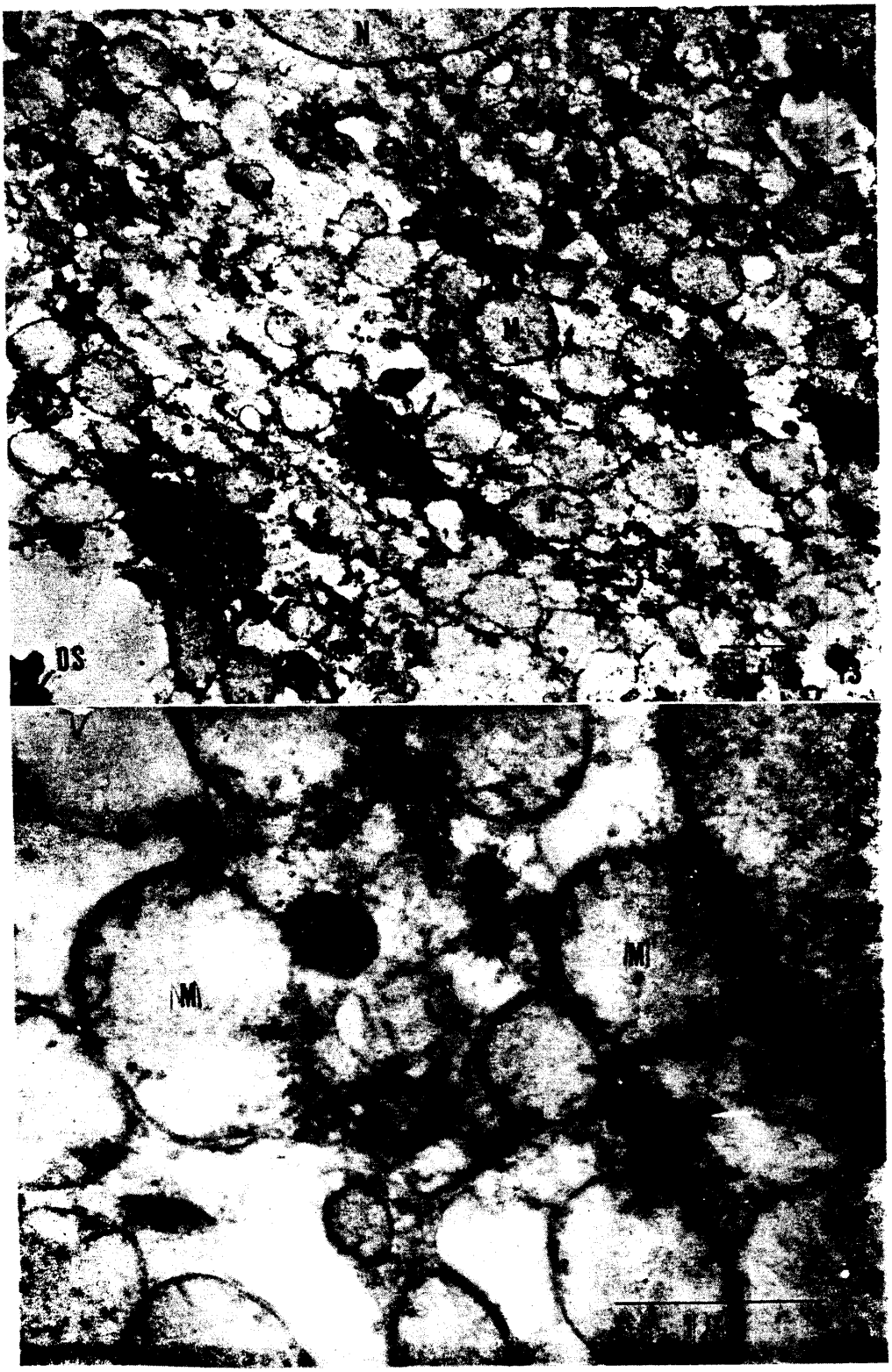
Figs. 9—10. Ten hours after the CCl_4 administration. Mitochondria show a variety of changes in size and shape; these mitochondia seem to be a mixture of degenerated and regenerated ones. Those swollen ones show a well-preserved double-membrane structure of cristae. These degenerated ones show cristae in a ring form or crescent shape (Md).


ER
M
MD
MD
M
μ

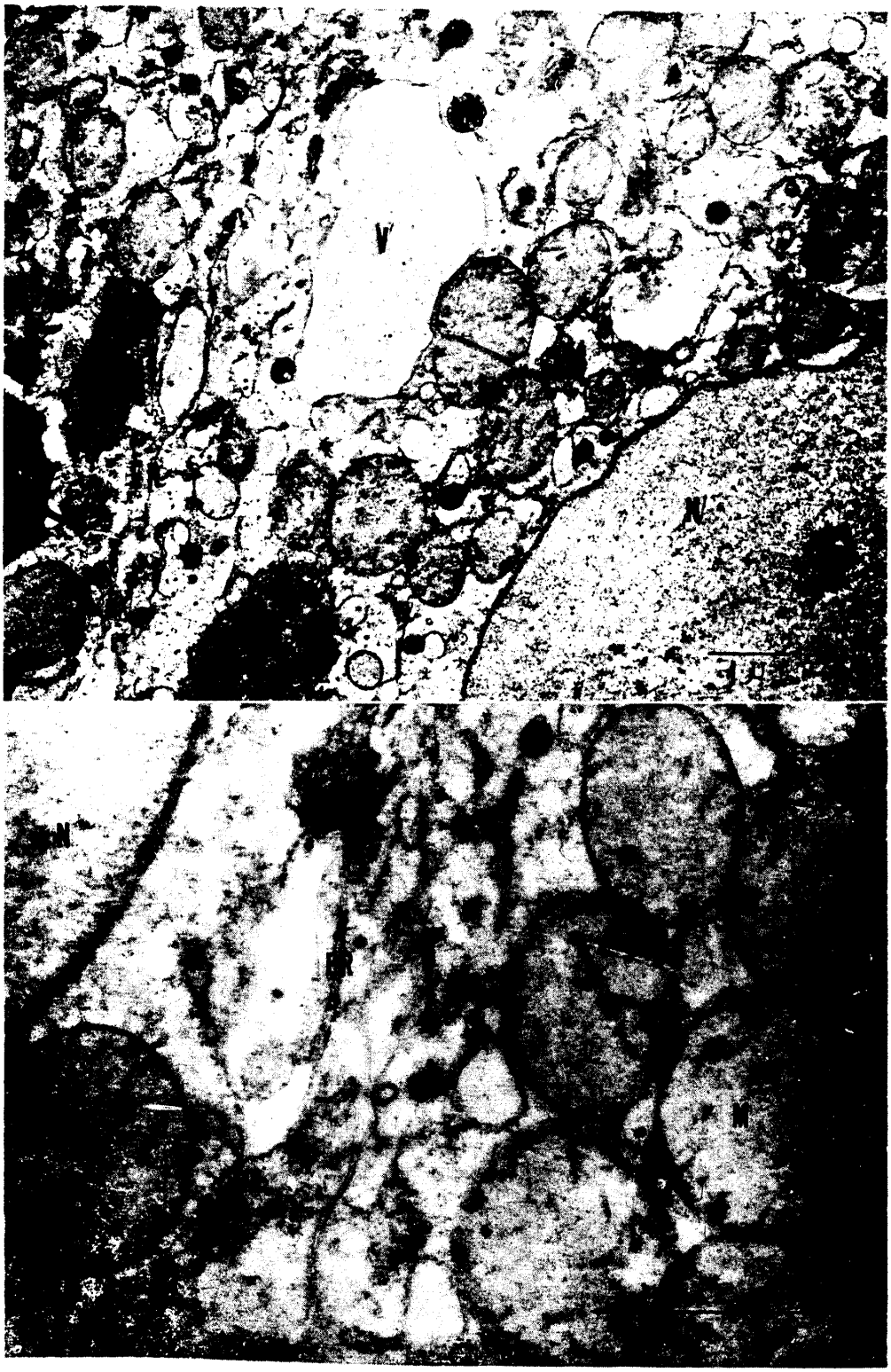
Figs. 11—12. Seventeen hours afterward. Degenerated of various size still maintain distinct double-membrane structure of cristae. Microbody of high density can be seen and osmiophilic substance becomes increasingly bigger. ER's are almost lost.


Ma
Mb

Figs. 13—14. Twenty hours afterward. Markedly swollen and degenerated M and vacuoles, that grow increasingly bigger, can be observed. In low power field, opaque areas can be seen.


N
OS
M
M

Figs. 15—16. Twenty-two hours afterward. Nuclear membrane shows rugged outline, and vacuoles, osmiophilic substance and opaque areas are enlarged and increased in number. M are swollen and degenerated but C still show well-preserved double-membrane structure.


V
N
N
M