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An electron microscope study of liver cell in carbon tetrachloride intoxication, significance of “opaque area”*

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Abstract

Electron microscope study on the rat liver cells of carbon tetrachloride poisoning has been reported. Observations have been made on the osmic fixed tissue sections obtained from the liver at early stages of poisoning, 5 to 22 hours after carbon tetrachloride oral administration, 0.25ml. per 100g. body weight. Special attention is paid on the appearance of electron dense area, opaque area, in cytoplasm, which is compased of fibrous components, probably originated from endoplasmic reticulum. This will be an important sign of cell degeneration. Toluidine blue, PAS and methyl green-pyronin stainings of the thicker sections from the same samples as used for electron microscopy revealed that the opaque area is stained by toluidine blue and pyronin but not by PAS. The opaque areas appear already five hours after the carbon tetrachloride administration and show some continuity with elongated filaments of endoplasmic reticulum. At an advanced stage of paisoning the opaque area increases in its number and size, but some of them are shrunk as a mass, being separated from the surrounding cytoplasm with scanty area. Often they form denser masses in the center and loak like the lipid deposition. The picture suggests formation of lipid droplets in the case of fatty degeneration of the liver cell.

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AN ELECTRONMICROSCOPE STUDY OF LIVER CELL IN CARBON TETRACHLORIDE INTOXICATION, SIGNIFICANCE OF "OPAQUE AREA"

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In the previous paper[1] one of us reported an electron microscope study on the rat liver cells of carbon tetrachloride poisoning, pointing the appearance of electron dense area in cytoplasma, which is composed of fibrous component and observed in the early stage of poisoning. This electron dense area, as reported already, is distinctly different from microbody, dense mitochondria or fat droplets, then is provisionally designated as "opaque area". In this paper the findings accumulated on the opaque area since the first report are presented with consideration of its origin and fate.

MATERIAL AND METHOD

Fourteen male adult rats were used. Seven of them were given 0.25 ml. of carbon tetrachloride per 100 g. body weight by oral administration by means of a fine gum catheter. Other animals were pair fed controls and received no treatment. They were sacrificed for observation of their livers at early stages from one and a half to twenty-two hours after the carbon tetrachloride administration. Two animals at one time, one given carbon tetrachloride and another one was of control. The liver tissues, one cu. mm. in size, were fixed in 1.0 per cent osmic acid solution (phosphate buffer, pH 7.38), dehydrated through 70, 90, 99 per cent, 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. For sectioning ultramicrotome of type JUM-5, Japan Electron Optics Laboratory Co. was used.

For the electron microscopic observation sections of 200-300 Å in thickness were picked on formvar film and observed by the electron microscope of Hitachi Co., HU-10 A. Staining of sections for electron microscopy with heavy metals was carried out by floating the grids, section side down, on the surface of a saturated solution of lead acetate for the period of one hour as described by WATSON.[2,3]

For the light microscope observation the thicker sections of about 5 μ in thickness from the same samples as used for electron microscopy were prepared

by the same microtome used for electron microscopy, placed on slide glass, removed of methacrylate polymer through isoamyl acetate solution and stained with 0.5 per cent toluidine blue and one per cent methylene blue for demonstrating the cytoplasmic basophilia.[4,5] Glycogen was observed by staining the sections with the periodic acid Shiff (PAS) reaction.[6] Another series of sections were stained with methyl green-pyronin[4].

OBSERVATIONS

The opaque area, which had been introduced in the previous paper[1], was in general observed from about five hours after the poisoning (Figs. 1-10, 12). They are irregular in size and shape and have obscure boundaries separating them from the surrounding area, by which they can be distinguished from other cellular components like lysosomes, mitochondria, etc. Their contents are of fibrous or vacuolar materials and their high density in electron microscope is due to the compact arrangement of each component (Figs. 1, 2). Opaque areas appear often in contact with the outer layers of mitochondria but no continuity in both structures can be observed, while endoplasmic reticulums are found often to be continuous with the fibrous or vacuolar components of opaque area.

Opaque areas appearing in the early stage of poisoning (5-6 hours after the carbon tetrachloride administration) are rather low in density comparing to these appearing in the later stage, so we could see easily the continuity of them with the surrounding endoplasmic reticulum especially in those samples of early stage of intoxication. The opaque areas increase in both number and density with the lapse of time after the poisoning, being encountered with a high frequency in the specimen from the animals killed 20-22nd hour afterwards (Figs. 3-6). At 10-17th hour after the poisoning, the cells contain the opaque area very often in cytoplasma but in these stages some of opaque areas show themselves as shrinking masses, being separated from the surrounding cytoplasma by scanty area (Figs. 10, 11). In these cells the opaque area has no relationship with any cell organellae. But even in later stages of the poisoning the areas are generally not so sharply demarkated from the adjacent cytoplasma, though they increase both in size and number.

The picture of some opaque areas appearing as the shrinking masses suggests that they are condensed to form lipid droplets (Fig. 11), i. e. the central part of opaque areas increases in density (Fig. 10), appears homogenous (Fig. 11) and finally shows the striped pattern which is specific in lipid droplets.

Besides these, there appear a number of typical osmiophilic lipid droplets in cytoplasm, which increase in number and size at an advanced stage of poisoning. Some of them appear sharply demarkated from the adjacent cytoplasm but in the early stage most of the lipid droplets have some irregular projections

with which the surface of droplets are connected to the cytoplasmic matrix, the endoplasmic reticulum, as to be seen in Figs. 8-9.

Observations by staining the sections with heavy metal give also the same result as in the cases observed without staining. In the liver of carbon tetrachloride intoxication the effect of electron staining can only slightly be recognized. (Figs. 5-6).

Then toluidine blue, methylene blue, methyl green-pyronin and PAS stainings of the thicker sections from the same samples employed for electron microscopy reveal that the opaque area is stained by toluidine blue, methylene blue and pyronin the same as in the case of nucleolei but not by PAS (Fig. 13). Methyl green-pyronin staining gives the same result as in the case of simple pyronin staining. No nucleus can be stained by methyl green. PAS staining shows PAS-positive filamentous structures surrounding the osmiophilic granules (Fig. 14). Furthermore, in the methylene blue and toluidine blue stainings opaque area is stained but hardly shows any metachromasia. The cytoplasm, excepting opaque area, is generally stained a little by the color tone of staining fluid themselves.

DISCUSSIONS

The pictures of the opaque areas in the liver of carbon tetrachloride poisoning presented in this paper clearly show that they have some correlation with ER in its structure, though it seems not to have RNA, and on the other hand this structure is concerned with the formation of lipid droplets in fatty degeneration of liver cells by carbon tetrachloride intoxication. PORTER has also reported the similar structure of agglomerated filamentous masses in rat liver of early stage of 3′-Me-DAB feeding. He described them as the mass of agglomerated smooth surfaced endoplasmic reticulum[6]. However, he did not see any correlation of this structure with the formation of lipid droplets. ONOE's experiment on the rat fed DAB showed again the same results[7]. In this case, too, no correlation with lipid droplets was observed. But comparing the pictures that they showed with those of ours, especially with those of early stages of carbon tetrachloride poisoning, it can be said that all these will be the same structure and show a sign of cell degeneration. The reason why PORTER and ONOE failed to see the connection between the opaque area and lipid droplets will probably be due to that in the case of DAB poisoning the cell degeneration proceeds very slowly and only the completed form of the lipid droplets can be observed. On the other hand, in the case of carbon tetrachloride poisoning the destruction of the cytoplasm of liver cell and the formation of lipid droplets proceed very rapidly, and subsequently the process of the formation of lipid droplets can clearly be persued. Clear area found surrounding the fat droplets will probably be formed

by shrinkage of the droplets at fixation or dehydration. In the lipid droplets which are supposed to be newly formed ones there can be recognized a gradual increase in the density toward the central part of droplets. This suggests that the degeneration of the structure with the release of lipids and the released lipids agglomerate to gather forming homogeneous masses. This may be the third way for the formation of fat droplets revealed up to present. The first one is of the formation from microbody as proposed by BERNHARD[8], the second is the development of small lipid droplet in the interspace of ER as revealed by ODA[9] and FAWCETT[10] respectively on brown adipose tissue of mice. The old concept of lipophanerosis in degeneration may be accounted submicroscopically as the mechanism present. However, there are still problems to be clarified to reach a final conclusion of the proposed way for fat droplet formation because this picture shows actually the submicroscopic picture of lipophanerosis, the same picture should be revealed in other cases of fatty degenerations which will be supposed to be caused by lipophanerosis.

CONCLUSION

Electron microscope study on the rat liver cells of carbon tetrachloride poisoning has been reported. Observations have been made on the osmic fixed tissue sections obtained from the liver at early stages of poisoning, 5 to 22 hours after carbon tetrachloride oral administration, 0.25 ml. per 100 g. body weight. Special attention is paid on the appearance of electron dense area, opaque area, in cytoplasm, which is composed of fibrous components, probably originated from endoplasmic reticulum. This will be an important sign of cell degeneration. Toluidine blue, PAS and methyl green-pyronin stainings of the thicker sections from the same samples as used for electron microscopy revealed that the opaque area is stained by toluidine blue and pyronin but not by PAS. The opaque areas appear already five hours after the carbon tetrachloride administration and show some continuity with elongated filaments of endoplasmic reticulum. At an advanced stage of poisoning the opaque area increases in its number and size, but some of them are shrunk as a mass, being separated from the surrounding cytoplasm with scanty area. Often they form denser masses in the center and look like the lipid deposition. The picture suggests formation of lipid droplets in the case of fatty degeneration of the liver cell.

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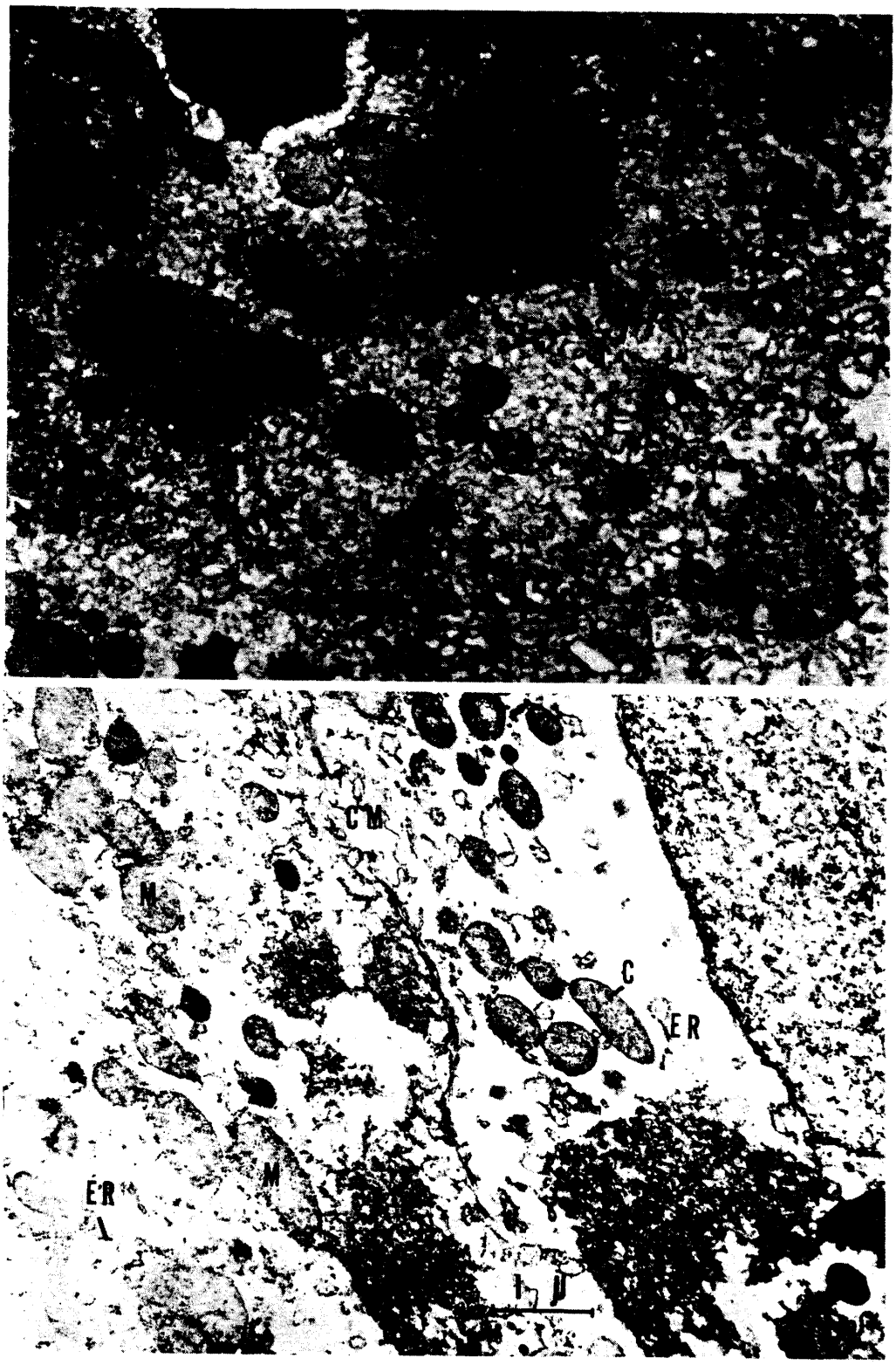
EXPLANATIONS OF FIGURES

Figs. 1~12. Electron-micrograms of the rat liver cells in the carbon tetrachloride poisoning. Symbols: N: nucleus, M: mitochondria, C: cristae mitochondriales, ER: endoplasmic reticulum, Mb: microbody, Os: osmiophilic granule, O: opaque area, G: glycogen area, CM: cell membrane. (see text)

Figs. 13 and 14. Light-micrograms of the rat liver cells fixed by osmic acid and stained later by various dyes.

Fig. 1. The cell at five hours after the CCl_4 administration. Two opaque areas having the continuities with the fibrous component of endoplasmic reticulum can be seen.

Fig. 2. The cell at 6 hours after the CCl_4 administration. Two large and one small opaque areas can be seen. Their contents are of fibrous or vacuolar materials and their high density in electron microscope is due to the compact arragement of each component.


CM
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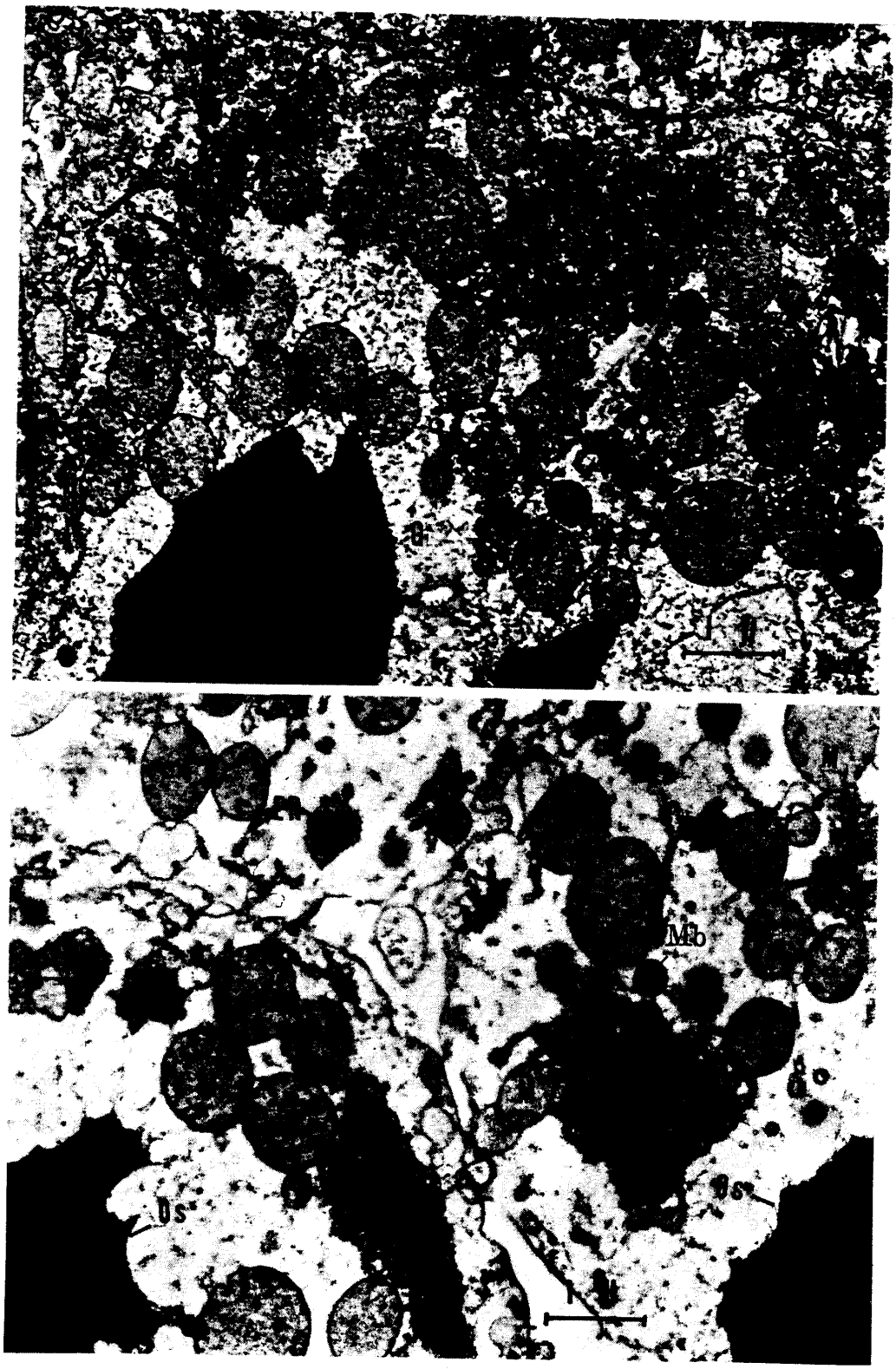
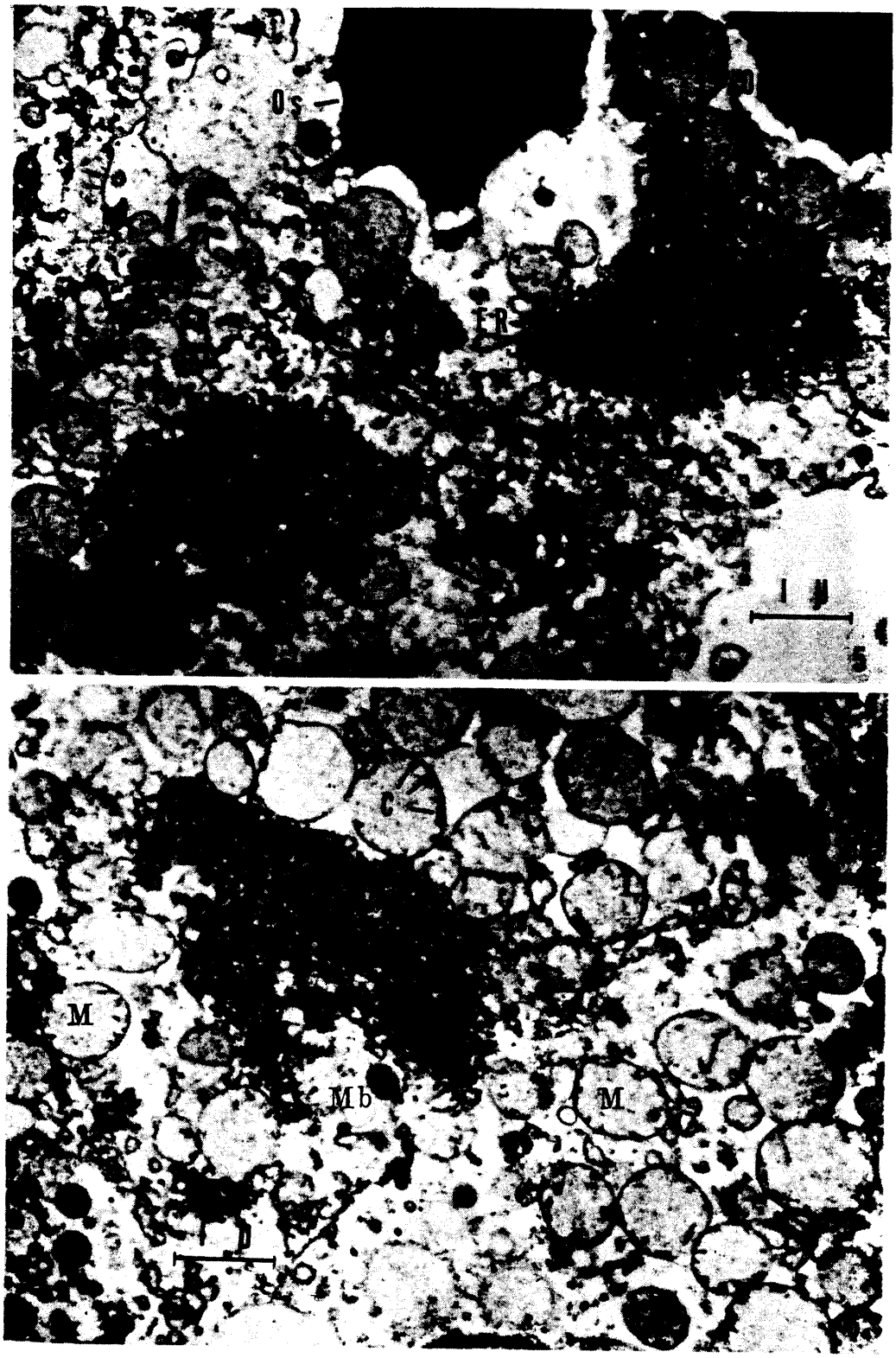

Mb
Os
Os

Fig. 3. Opaque areas at 20 hours after the CCl_4 administration can be seen.

Fig. 4. Opaque areas at 22 hours after the CCl_4 administration. Opaque areas increase in both number and density with the lapse of time after the poisoning.

Figs. 5 and 6. Micrographs of a sandwiched section of rat liver at 22 hours after the CCl_4 administration, after staining an hour in a saturated solution of lead acetate. The effect of electron staining could only slightly be recognized, then we cannot conclude opaque areas consist of agglomerates of rough endoplasmic reticulum.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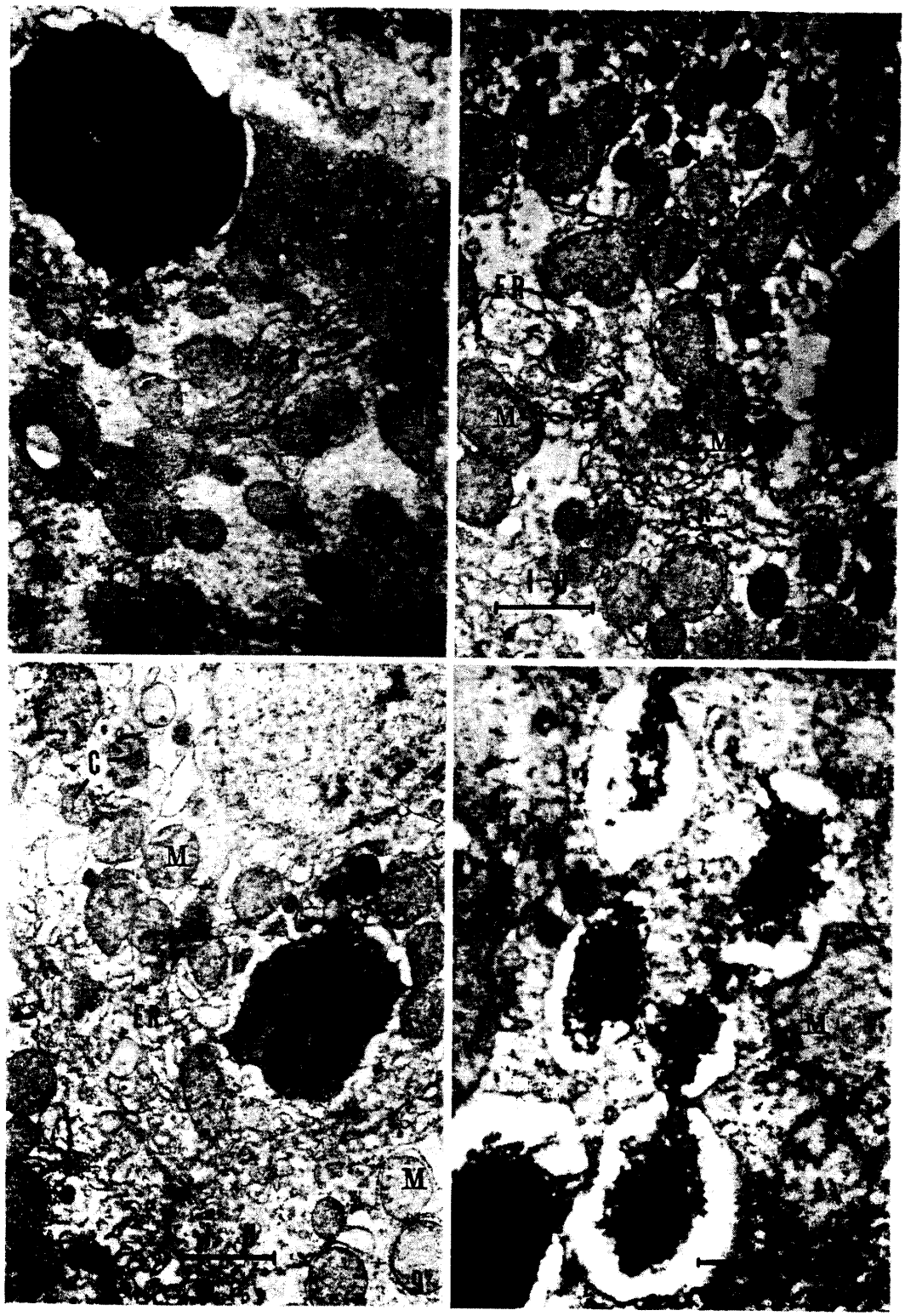
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Fig. 7. Micrograph of rat liver cell at 5 hours after the CCl_4 administration. Opaque area has the continuity with endoplasmic reticulum.

Figs. 8 and 9. Five hours after the CCl_4 administration. Lipid droplets have some irregular projections with which the surface of droplets are connected to the cytoplasmic matrix, the endoplasmic reticulum.

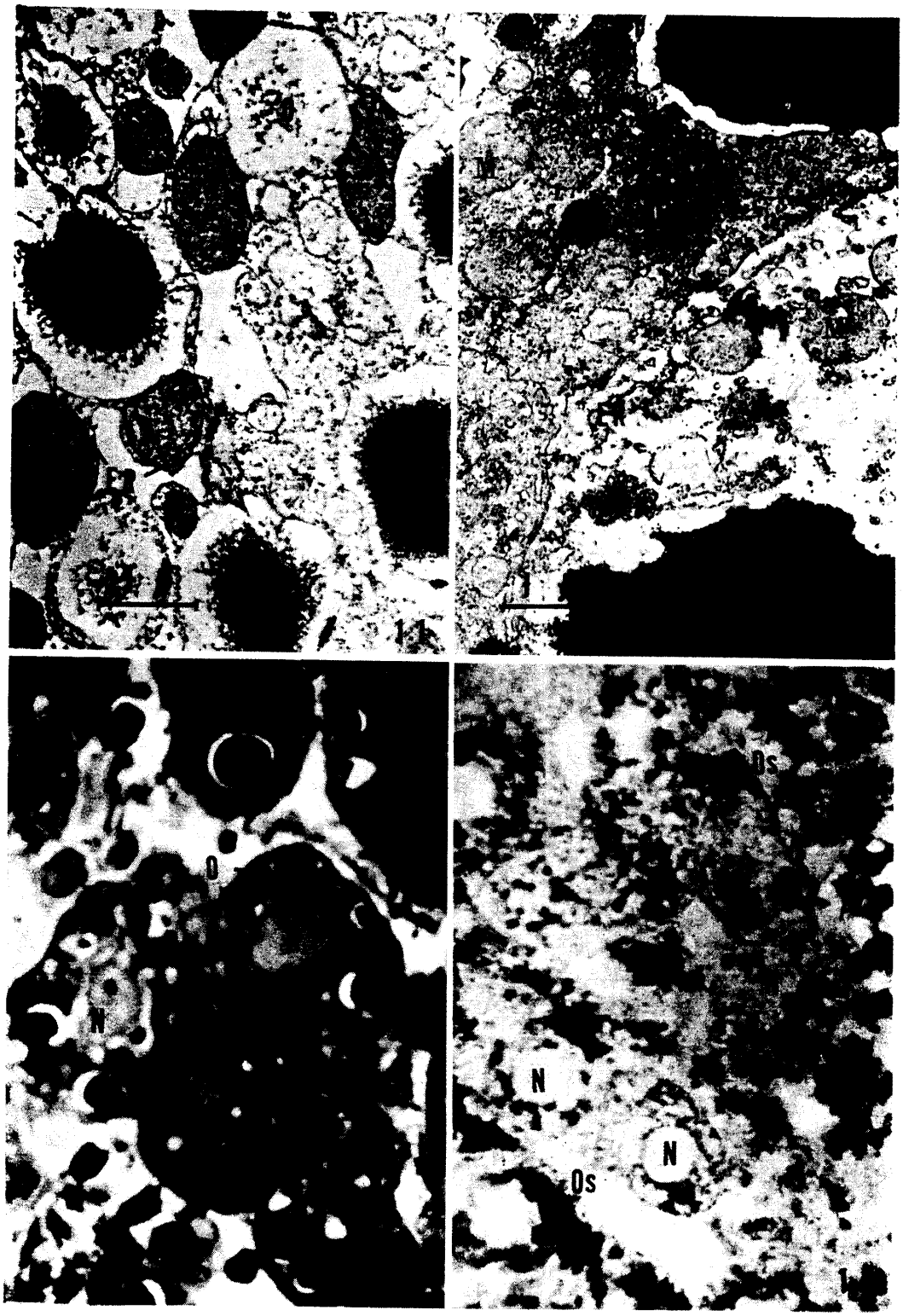
Fig. 10. Six small opaque areas as shrinking masses, being separated from the surrounding cytoplasm with scanty area at 20 hours after the CCl_4 administration. The central parts of opaque areas increase in density.

Fig. 11. Micrograph of balloon cell of rat liver at 17 hours after the CCl_4 administration. This picture of some opaque areas appearing as the shrinking masses suggests that these areas are connected to form lipid droplets, i. e. the central part of some opaque areas appears homogenous and of the other shows the striped pattern which is specific in lipid droplets.

Fig. 12. Micrograph of balloon cells of rat liver at 22 hours after the CCl_4 administration. Small opaque area can be seen.

Fig. 13. Light micrograph of rat liver cells at 22 hours after the CCl_4 administration. Fixed in OsO_4, embedded in methacrylate, and stained with methyl green-pyronin. Opaque area is represented by the light pink materials in the cytoplasm. Nucleoli are stained by pyronin, too, but no nuclei could be stained by methyl green. ($\times$1500)

Fig. 14. Light micrograph of rat liver cells at 22 hours after the CCl_4 administration. Fixed in OsO_4, embedded in methacrylate, and stained with PAS. No opaque area is stained by PAS. ($\times$1000)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