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MICROBODIES OF REGENERATING LIVER CELLS IN RATS AFTER PARTIAL HEPATECTOMY

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Microbodies (peroxisomes) have been established as a distinct class of cytoplasmic organelles, though their functions have not been fully clarified as yet (7, 8, 15). In vertebrates, their distribution is limited to the hepatic parenchymal cells and proximal tubular cells of the kidney, suggesting that their function is characteristic of these particular cell types. Further, it was reported that microbodies were present in slowly growing well differentiated hepatoma cells, while absent in fast growing undifferentiated ones (3, 4, 9, 14, 22). In this respect, the function of microbodies might bear some correlation with cell differentiation.

Regenerating and proliferating liver cells after partial hepatectomy seem to be a counterpart of hepatoma cells, in that the former cells are in an actively multiplying state without malignancy, while the latter are in a growing state with malignancy. In this respect, increased attention is being paid to the morphological and biochemical characteristics of the regenerating liver cells after partial hepatectomy. However, as to the changes in microbodies, most of the investigations hitherto have given only

brief description of the numerical changes of the organelles (1, 5, 6, 10, 11, 16, 17, 23, 24, 26, 28).

Thus, the present study was made to ascertain the numerical changes of microbodies in regenerating and proliferating liver cells of rats after partial hepatectomy by estimating the number of the organelles in electron micrographs. Biochemical examinations on activities of catalase and urate oxidase were also made to assess the numerical changes, since these two enzymes were reported to be localized in these organelles.

Materials and Methods

Male rats of Wistar/MK strain weighing between 210 and 230 g were used. The animals were kept at 22 $\pm$ 1°C, fed on a compressed diet (Oriental, No. NMF), and given drinking water ad libitum. Partial hepatectomy was carried out according to the procedure of Higgins and Anderson (12) under light anesthesia with ether. All animals were sacrificed by decapitation at intervals of 1, 2, 3, 5, 7, 10, and 14 days after the operation. Electron microscopic examinations were made according to the procedure previously described (21, 27). Micrographs were taken at random on the sections and calculations of microbodies were made on approximately 2,000 μ^2-cytoplasmic areas on each rat. Determinations of activities of catalase and urate oxidase were done according to the method reported previously (21, 27).

Results

Electron Microscopic Findings

Normal Rats. Hepatic microbodies of normal rats were round or ovoidal in shape, generally smaller than mitochondria in size, and surrounded by a single limiting membrane, showing a slightly scalloped contour. Their matrices were finely granular and somewhat denser than those of mitochondria. Over half of the microbodies showed highly organized crystalloid core in their matrices. The organelles were generally distributed throughout the cytoplasms either singly or in clusters, although they were occasionally found in the vicinity of glycogen areas (Fig. 1). Furthermore, these were occasionally observed being surrounded by cuffs of vesicular of tubular profiles of smooth endoplasmic reticulum (sER). Direct connection between microbodies and sER was encountered but seldomly.

One Day after Hepatectomy. Prominent ultrastructural changes of the liver cells at this stage were seen in the accumulation of fat droplets, disappearance of glycogen granules, reduction of organized rough endoplasmic reticulum (rER), and appearance of cytosomes including spherical bodies. The fine structure of microbodies was found to be almost similar to those in normal liver; however, the number of the organelles had decreased with a statistical significance ($p<0.01$), showing about 60% of normal (Table 1). The microbodies were frequently found in groups (Fig. 2), although seen scattered among the increased fat droplets in some cytoplasmic areas.

Two Days after Hepatectomy. Fat droplets and spherical bodies were still encountered, and profiles of rER were found to be increased and reorganized to some extent. Microbodies were more numerous than those on the first post-operative day, although they were still in a smaller number as compared with those in normal (Table I). Small microbodies lacking cores were frequently encountered (Fig. 3), and in some cells, large microbodies were seen exhibiting rarified matrices and irregular shapes (Fig. 5). Cuffing of microbodies with vesicular profiles of sER were found more occasionally (Figs. 4, 5, 6, 7), and the connection between these two was observed in an increased frequency (Figs. 8, 9), when compared with those in normal. Some microbodies showed ring- or hook-like protrusions of their limiting membranes (Fig. 10, 11). A tendency of microbodies to cluster was also noted in many of the liver cells (Figs. 3, 4, 5).

Three Days after Hepatectomy. On the 3rd day after the operation, appearances of cytoplasms returned to those in normal. Fat droplets and spherical bodies were seldom encountered. RER was reorganized and the glycogen area reappeared. However, the number of microbodies remained significantly reduced when compared with that in normal, although it increased evidently as compared with that on the 2nd day after the operation. They were scattered throughout the cytoplasms (Fig. 12), although, in some cells, they were located mainly in the vicinity of glycogen area (Fig. 13). Occasionally, small microbodies were found. The outline of microbodies was less irregular and their matrices were

Table I. Liver Weight and Number of Microbodies of Rats after Partial Hepatectomy

Days after hepatectomy	Number of rats	Liver weight(g)	Number of microbodies*
0	4	10.2 ± 0.74**	6.79 ± 0.69**
1	5	3.5 ± 0.35	4.11 ± 0.14
2	5	5.4 ± 0.57	4.55 ± 0.41
3	5	6.6 ± 0.61	4.65 ± 0.24
5	6	9.0 ± 0.63	5.31 ± 0.34
7	3	9.1 ± 1.10	5.41 ± 0.11
10	5	9.3 ± 0.95	5.69 ± 0.40
14	3	9.4 ± 0.44	6.20 ± 0.50

* Number of microbodies per 100 μ^2-cytoplasmic area.

** Mean ± standard deviation.

denser than those on the 2nd day.

Five to Fourteen Days after Hepatectomy. Microbodies at these stages were approximately normal in their ultrastructural appearances. The number of the organelles appear still smaller than normal level; however, there might be no difference between experimental and normal statistically, as in the case of the liver weights (Table 1). Furthermore, no particular intracytoplasmic localization of the organelles was observed (Figs. 14, 15, 16).

Biochemical Results

Table II depicts activites of catalase and urate oxidase in normal and regenerating livers. There was a significant decrease in catalase activity already on the first postoperative day. The activity dropped further on the 2nd day, reaching its minimum, which was about 50% of normal. This decreased activity was continued until the 5th day after the operation. Thereafter, the activities increased gradually, although it amounted to about 85% of normal value on the 14th day.

Urate oxidase activity also decreased up to the 2nd post-operative day as seen in the catalase activity; however, the degree of the decrease in the former was less than that in the latter. The activity increased thereafter and recovered to normal values on the 7th day after the operation.

Table II. Activities of Catalase and Urate Oxidase in Liver of Rats after Partial Hepatectomy

Days after hepatectomy	Catalase*	Urate oxidase**
0	111.4 ± 8.45***	2.65 ± 0.42***
1	72.2 ± 7.28	2.18 ± 0.17
2	58.3 ± 12.77	2.00 ± 0.41
3	60.1 ± 6.35	2.19 ± 0.53
5	60.8 ± 12.70	2.39 ± 0.19
7	75.0 ± 10.61	2.68 ± 0.69
10	85.3 ± 9.44	2.66 ± 0.18
14	95.6 ± 14.24	2.61 ± 0.32

* Catalase activity: k/min/100 mg dry tissues.

** Urate oxidase activity: uric acid oxidized, μg/min/mg dry tissues.

*** Mean ± standard deviation.

Discussion

From the results of the present investigations, it is apparent that microbodies of regenerating liver cells of rats after partial hepatectomy decreased considerably in number per unit cytoplasmic area. A marked decrease in the number was observed already on the first post-operative day, followed by a gradual increase as the post-operative days elapsed. Eventually, the number of the organelles was restored approximately to the normal level on or around the day where the weight of the liver also reached the normal level. The changes in activities of catalase as well as urate oxidase were found to be roughly in parallel with those in the number of microbodies. Since catalase activity of the liver is considered to be more or less influenced by surgical operation itself, it might be conceivable that the decrease in catalase activity was more significant and the subsequent restoration was further retarded than those in urate oxidase activity. It is suggested that the electron microscopic observations were verified by the biochemical examinations.

Since Rouiller and Bernhard (23) reported a remarkable increase in the number of microbodies in the liver cells of rats after partial hepatectomy, a number of investigations were reported describing the numerical changes of microbodies as listed in Table III. A great majority of these reports assumed an appreciable increase in the number of the organelles; however, no calculation of the number or supporting figures for the increase was provided in these reports. Since microbodies in liver cells were found distributed in cytoplasms, either dispersedly or

Table III. Numerical Changes of Microbodies of Liver Cells of Rats after Partial Hepatectomy

Changes	Hours after hepatectomy	Authors
infinitely more numerous	6-36	Rouiller & Bernhard(23)
increase	48	Takahashi(26)
great increase	12	Davis(6)
increase	6	Fisher & Fisher(10)
no change	1-96	Jordan(16)
numerous in dark cells	48-96	Bartók & Virágh(1)
no change	4	Franke & Goetze(11)
abundant	24-120	Stenger & Confer(24)
occasionally many	--	Virágh & Bartók(28)
deutlich vermehrt	24	David & Uberlings(5)
increase	12	Kimura(17)

in clusters, and since the number of the organelles was reported to vary according to the location of the cells in hepatic lobules (20), evaluation for the numerical changes might necessarily await the calculation of the organelles on a considerable number of electron micrographs taken at random, or should be made from observation on the cells of similar intralobular location.

A marked contrast between the present results and reports hitherto published may be attributed to the approach of the observations and the basis of the evaluations. Since there was no evidence to suggest an enlargement of cytoplasmic volumes, the decrease in the number of microbodies might not be ascribed to a dilution phenomenon, but to an actual decrease caused by elimination of the organelles from the cytoplasms. Microbodies are considered to be eliminated from cytoplasms in some processes or other, which occur during a relative short period, since they decreased rapidly by the first post-operative day. One of the mechanisms for the removal of microbodies in injured cells was suggested by Hruban et al. (13) and Swift and Hruban (25), who reported that the damaged portion of the cytoplasms was sequestered, resulting in the formation of autophagosomes. Immediately following the operation, the remaining liver cells are probably damaged by the mechanisms involving an overflow of portal blood, surgical operation itself, or metabolic overload. In the present experiments, it was noted that appearances of autophagosomes were evident on the first post-operative day, as reported by Becker and Lane (2) in the early hours after the operation. The

decrease in the number of microbodies is thus explained as the elimination of the damaged organelles by autophagocytosis.

On the second day after the operation, tubular of hook-like protrusions of the limiting membranes of microbodies, and cuffs of tubular or vesicular images of sER surrounding microbodies were conspicuous. These may be one of the routs for the transportation of intracellular substances between microbodies and ER, and suggested as one of the morphological expressions of an elevated metabolic activity of microbodies.

In regenerating liver, synthetic activities essential to the cell multiplication might surpass activities associated with the differentiated functions. Microbodial enzymes so far have been identified may not participate in the synthetic activities of the cell constituents necessary for cell multiplication. Thus, neither any particular increase in the number of microbodies nor in their enzymatic activities might necessarily occur during cell multiplication. This assumption is considered to be supported by the observations on Tetrahymena pyriformis, in which the activities of microbodial enzymes were found to be supressed during the rapid cell multiplication, while elevated when the cell maturation proceeded (18, 19). In partially hepatectomized rats, the liver cells were observed to multiply most actively for 30 - 36 hours after the operation, followed by their maturation during the successive periods. Accordingly, it is conceivable that the number of microbodies and activities of the enzymes involved were not increased by 48 hours after the operation. The increase during the following periods is suggested to correspond to the maturation

of the cells, although convincing evidence to substantiate morphogenetic processes of microbodies was not seen electron microscopically.

The liver cells of sham-operated rats were examined, and a decrease in the number of microbodies as well as in activities of catalase and urate oxidase were also found to some extent on the first post-operative day (unpublished data). This may be attributed to elimination of microbodies from the cytoplasms resulted from damage of the cells due to the operation.

Summary

Microbodies of regenerating liver cells of partially hepatectomized rats were studied electron microscopically and biochemically, at intervals of 1, 2, 3, 5, 7, 10, and 14 days after the operation. The number of microbodies per unit area of cytoplasms was markedly decreased one day after the operation, increasing gradually up to 14 days after hepatectomy, but it did not return to control value. The activities of catalase and urate oxidase, microbodial enzymes, were parallel with the numerical changes of microbodies. Morphologically altered microbodies were markedly found on the 2nd day after the operation.

Aknowledgement

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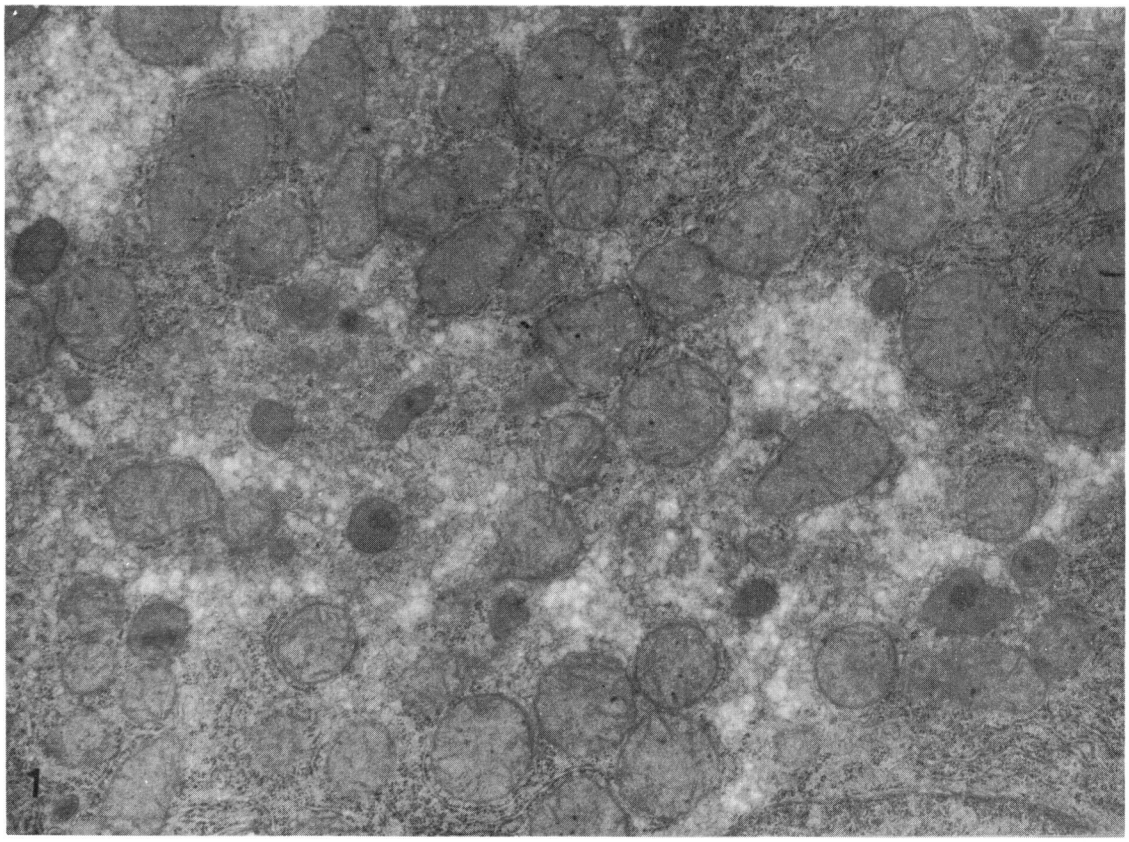
Explanations of Figures

Fig. 1. Control hepatocytes. Twelve microbodies are seen in or around the glycogen area. X 11,000

Fig. 2. Hepatocyte one day after the operation. Fat droplets (F) and spherical body (SB) are seen. Seven microbodies are found in clusters around conglomerated sER. X 11,000



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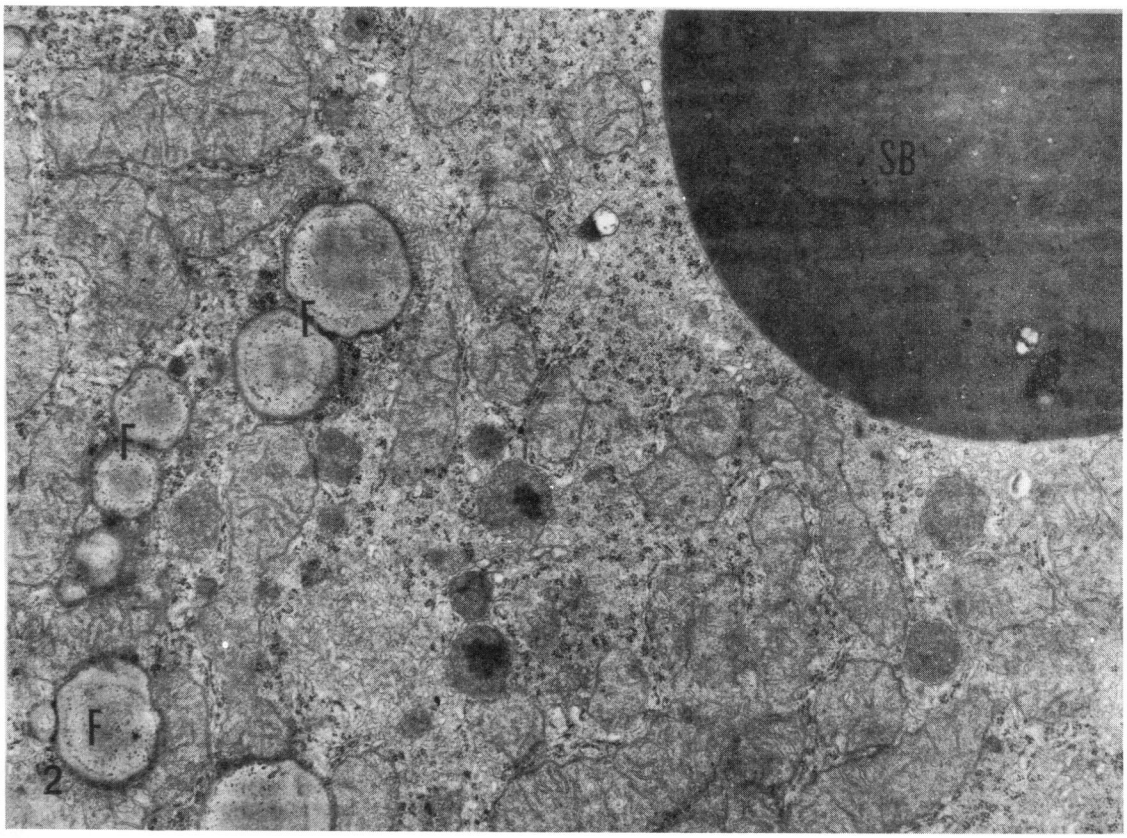

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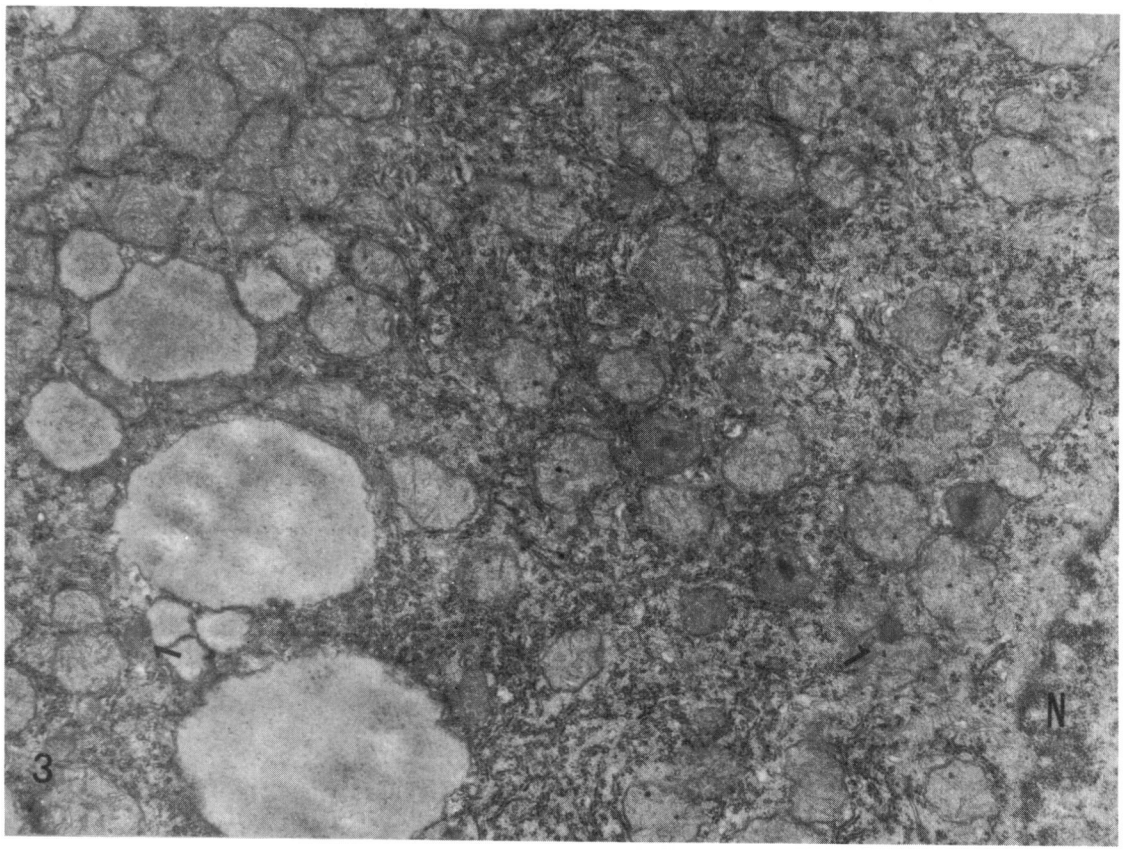
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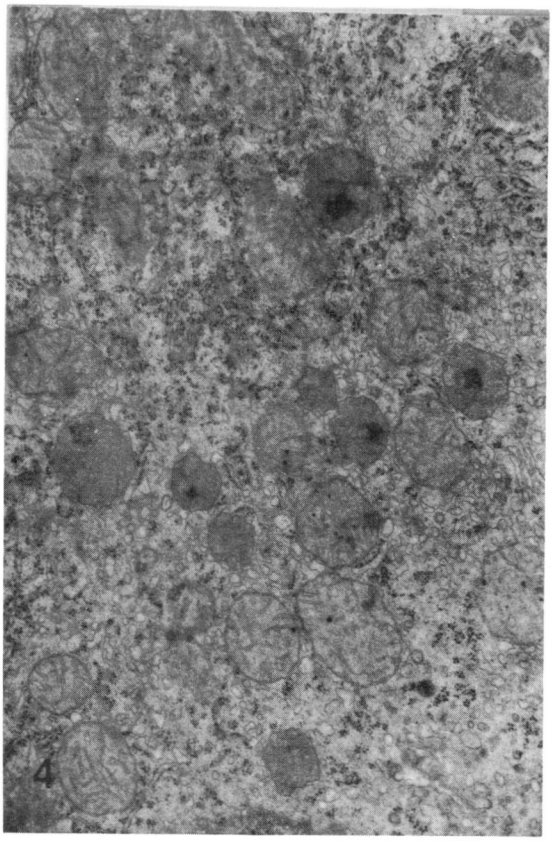
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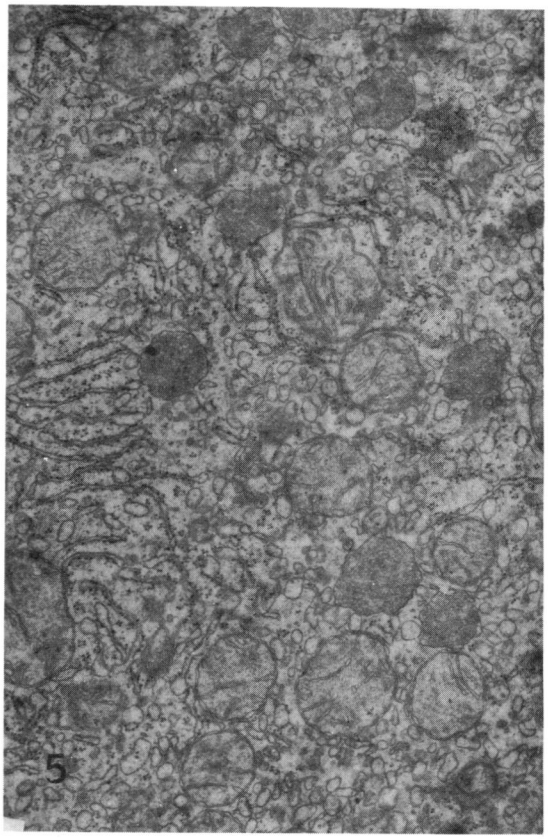
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Fig. 3. Hepatocyte two days after the operation. Microbodies are encountered in group near nucleus (N). Small microbodies (arrow) are frequently found. X 11,000

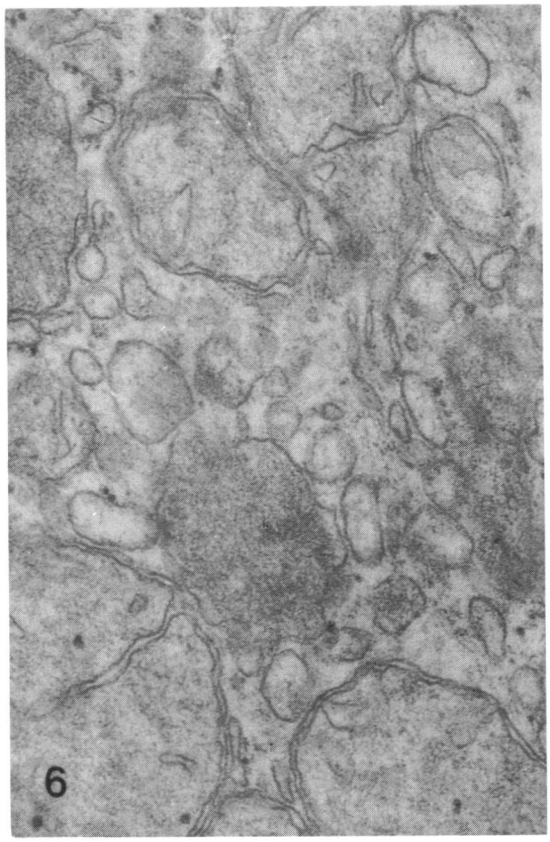
Fig. 4. Hepatocyte two days after the operation. Nine microbodies are seen, showing variety in size and irregularity in shape. X 11,000

Fig. 5. Hepatocyte two days after the operation. Large microbodies showing rarified matrices are seen in clusters. The majority have no core. Their contours are irregular. Vesicular profiles of sER are prominent around these microbodies. X 11,000

Figs. 6 - 11. Microbodies of hepatocytes two days after the operation. X 40,000

Figs. 6 and 7. Cuffs of vesicular or tubular profiles of sER are prominent. These microbodies are irregular in shape.

Figs. 8 and 9. These microbodies show the connection with sER.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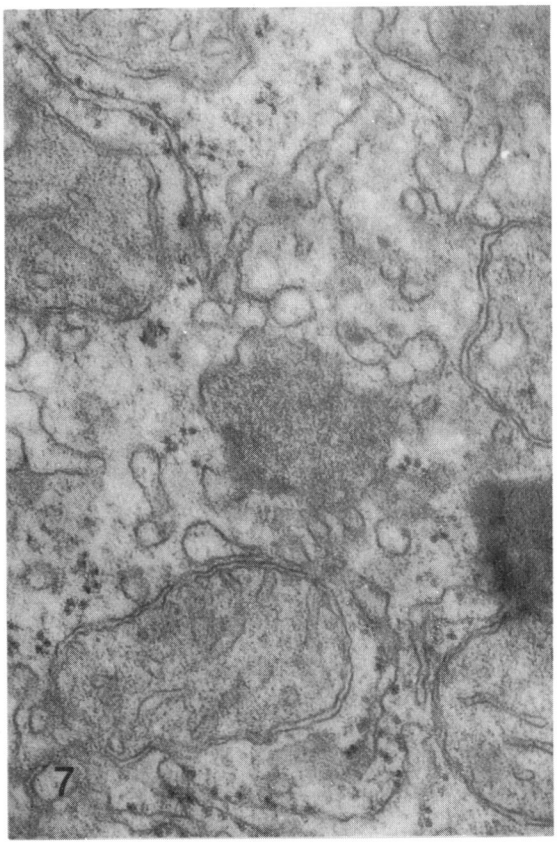
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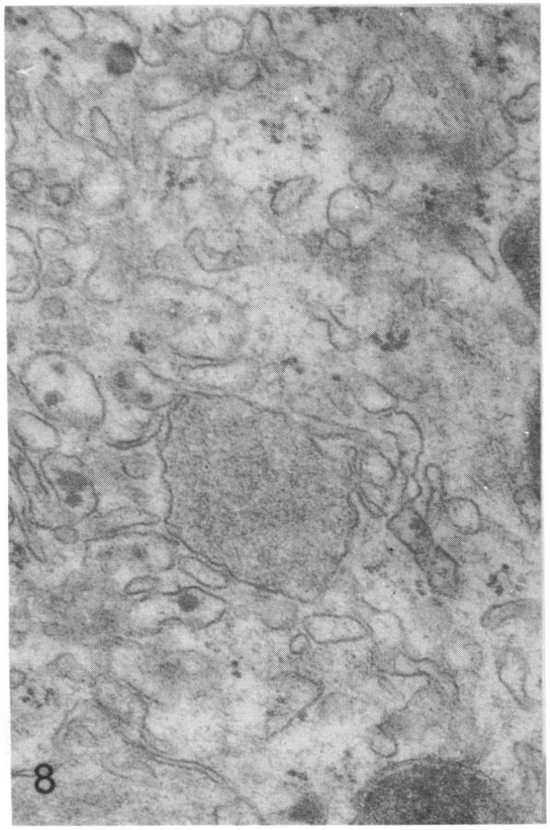
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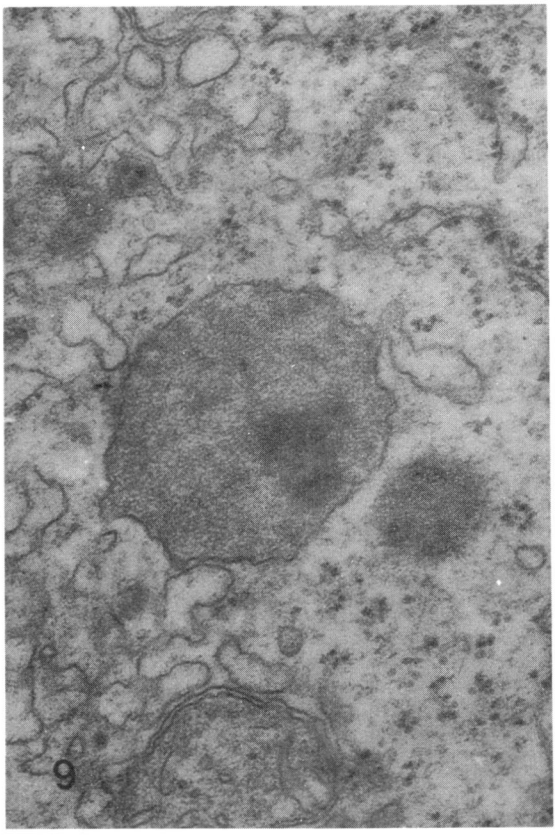
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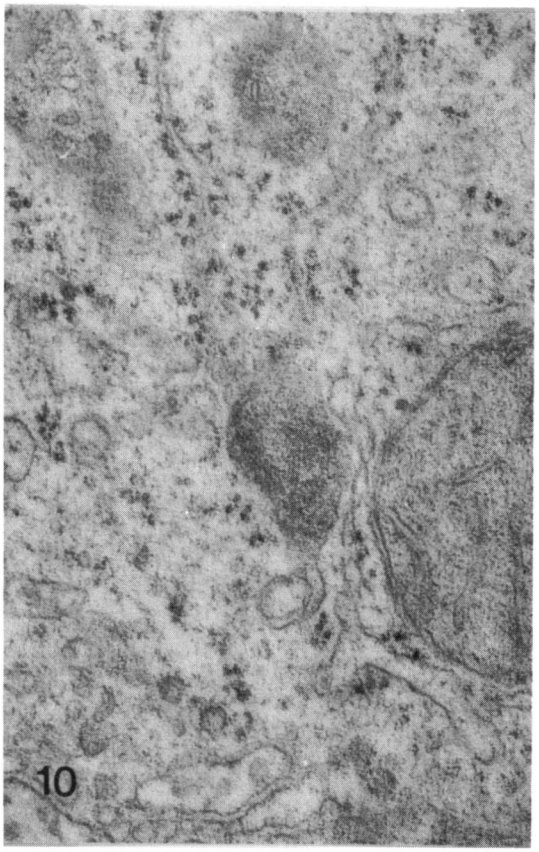
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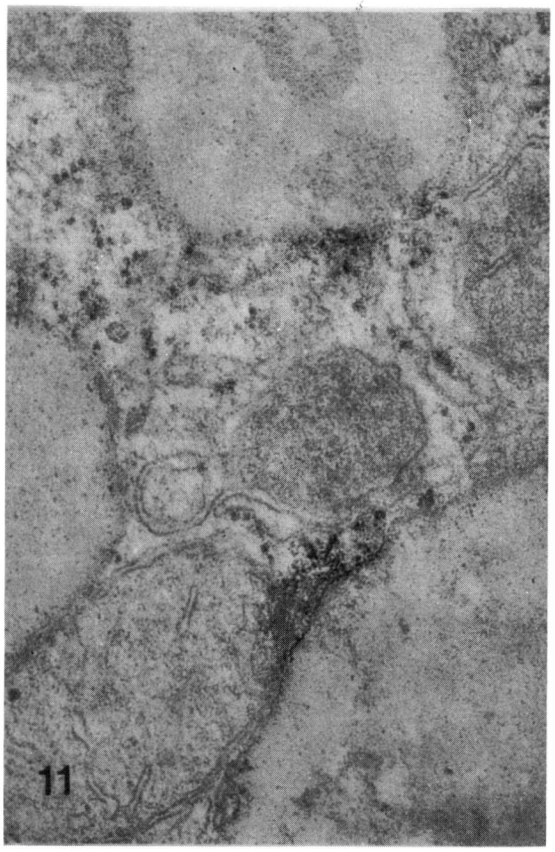
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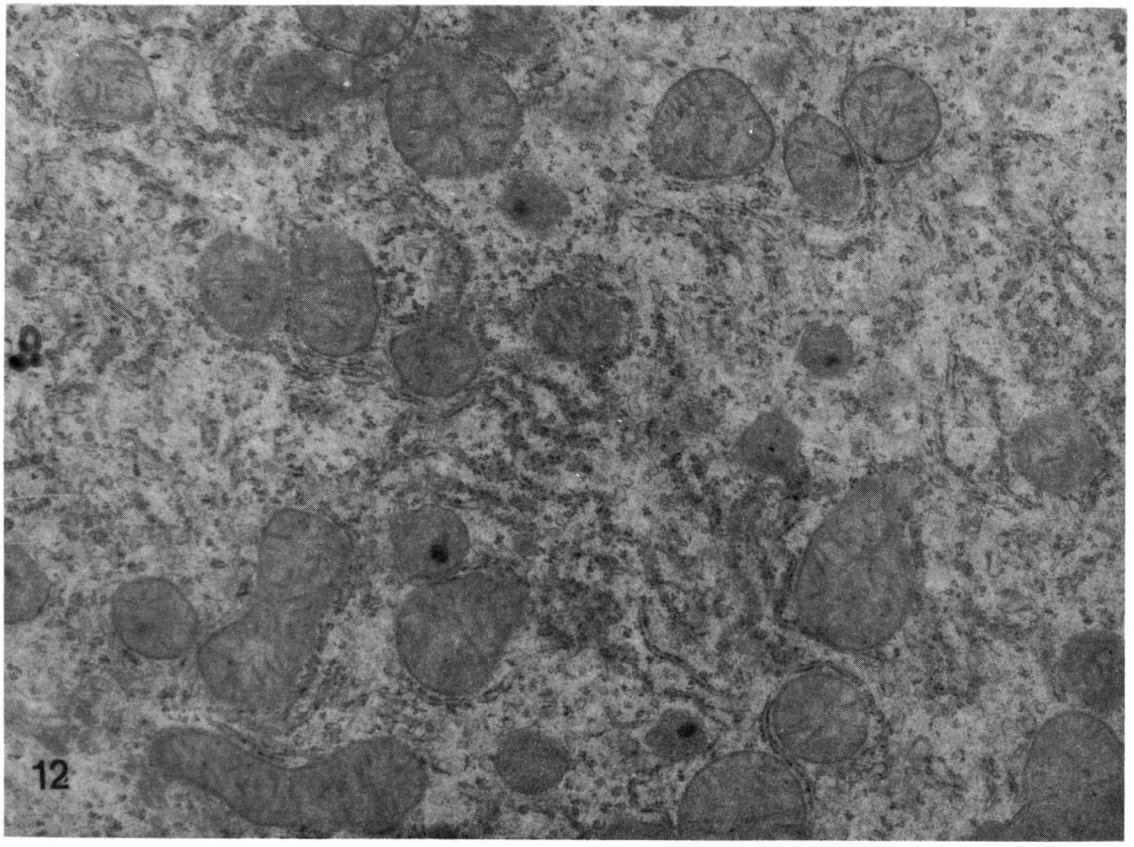
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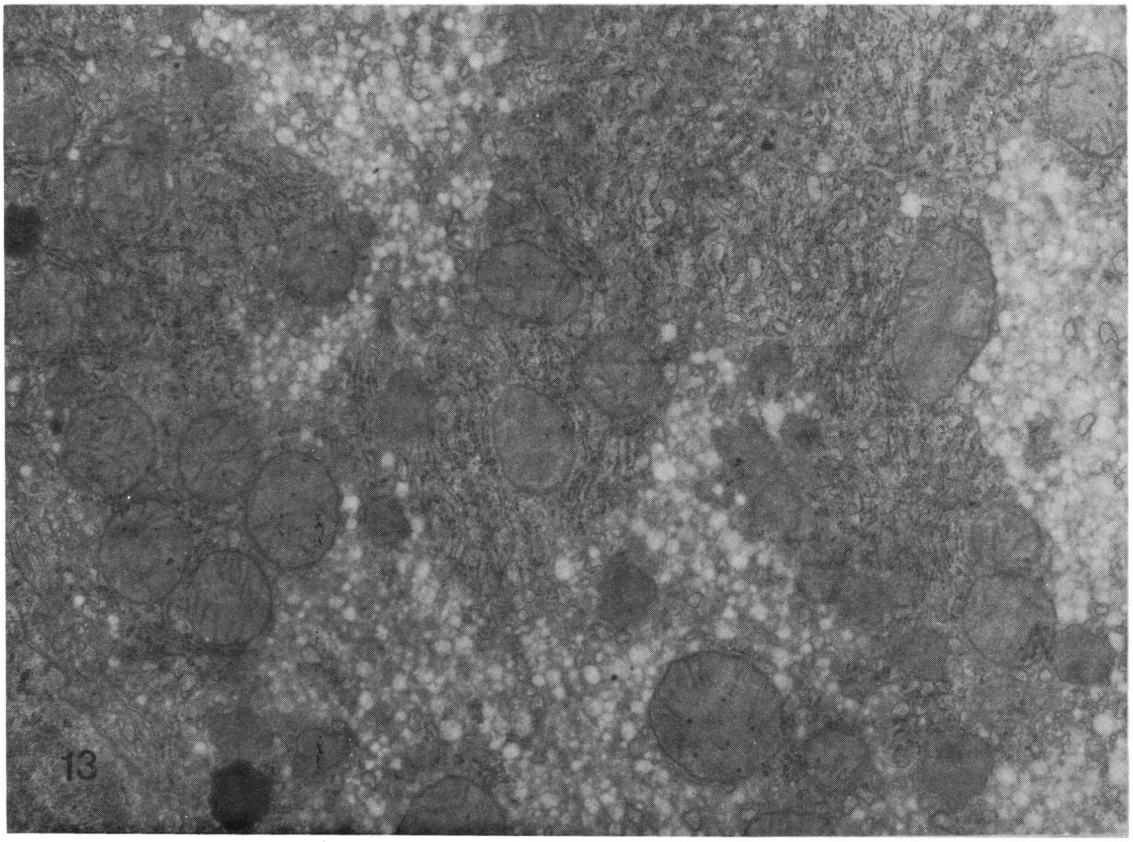
Figs. 10 and 11. These microbodies have ring-like protrusion of their limiting membrane.

Fig. 12. Hepatocyte three days after the operation. Microbodies are seen scattered in the cytoplasm. X 11,000

Fig. 13. Hepatocyte three days after the operation. Microbodies are seen around reappeared glycogen area. X 11,000

Fig. 14. Hepatocyte five days after the operation. Microbodies are found near Golgi apparatus, in the vicinity of glycogen area, and near nucleus. The appearences of microbodies are similar to those of control. X 11,000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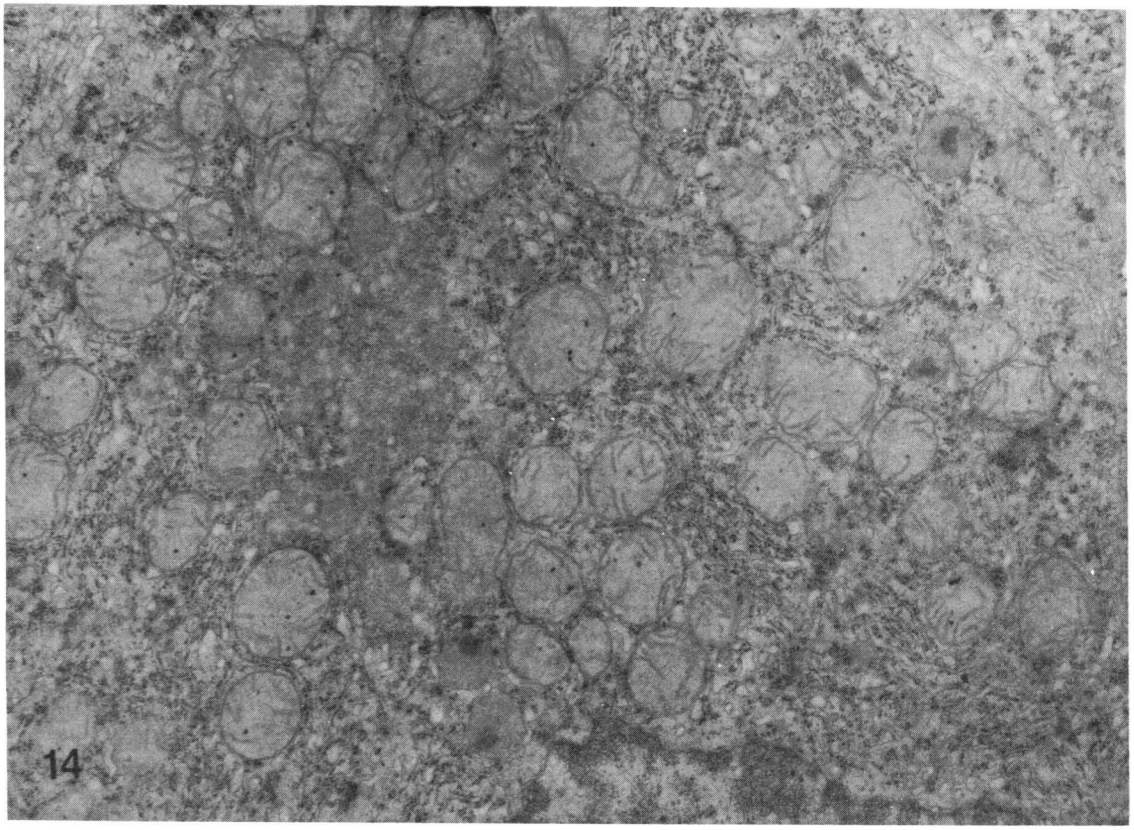
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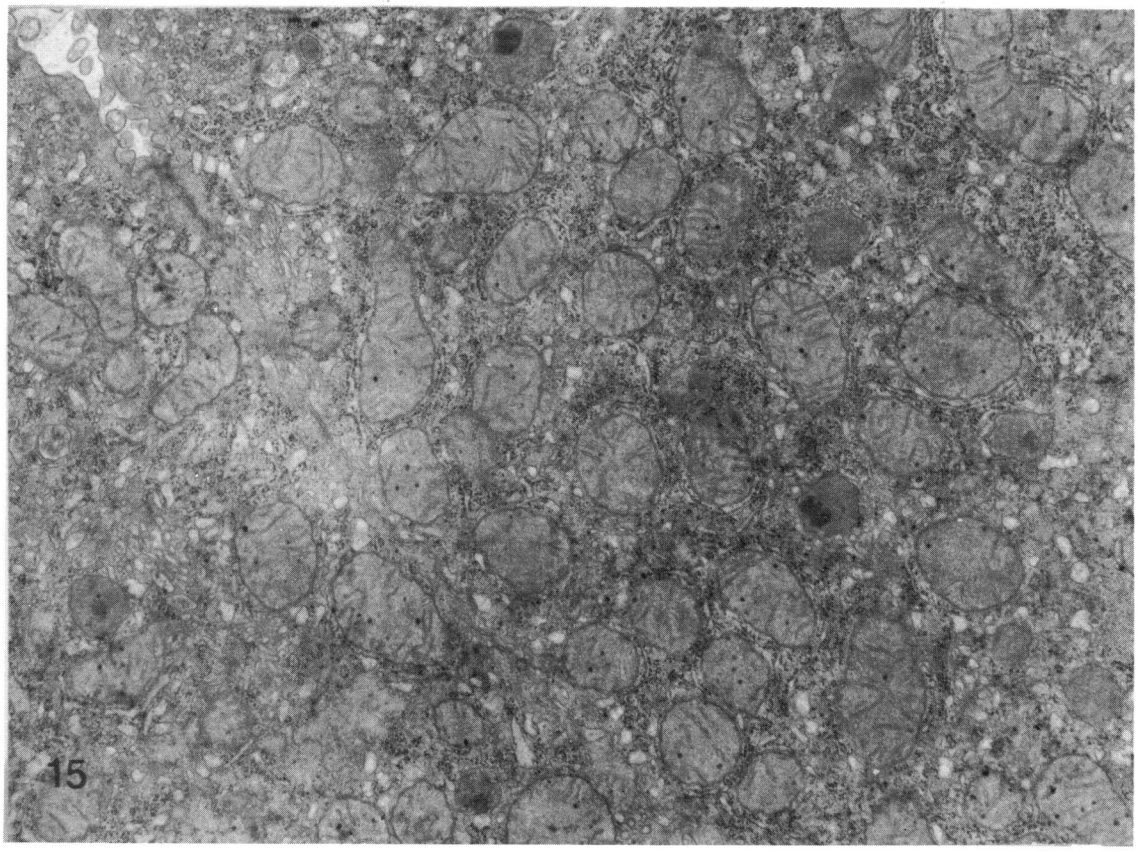
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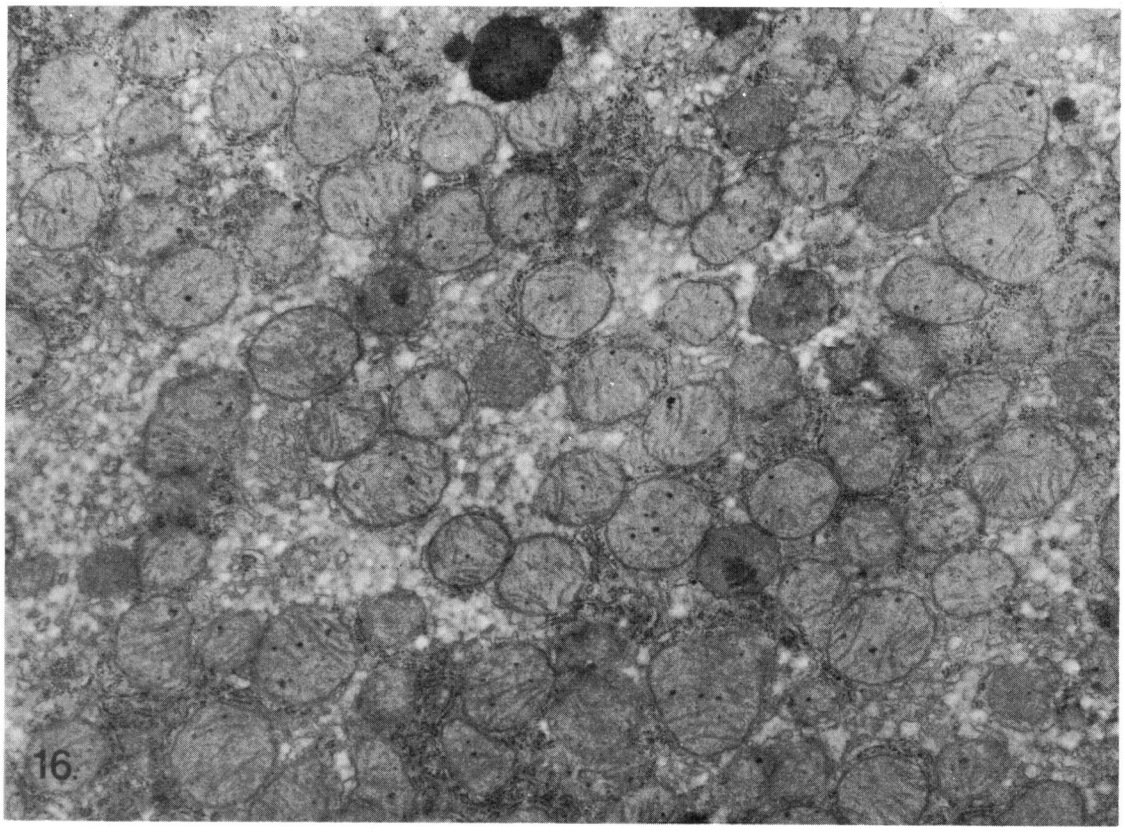
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Fig. 15. Hepatocytes seven days after the operation. Nine microbodies are seen scattered randomly in the cytoplasm. X 11,000

Fig. 16. Hepatocyte fourteen days after the operation. Ten large microbodies are seen in or around the glycogen area. X 11,000