CLOSE TOPOGRAPHICAL RELATIONSHIP BETWEEN MITOCHONDRIA AND ERGASTOPLASM OF LIVER CELLS IN A DEFINITE PHASE OF CELLULAR ACTIVITY*

By W. BERNHARD, M.D., and C. ROUILLER, M.D.

(From the Institut de Recherches sur le Cancer du Centre National de la Recherche Scientifique, Villejuif (Seine), France)

PLATES 22 TO 25

In normal rat liver cells, though one may sometimes find a definite grouping of mitochondrial and ergastoplasmic structures, these two organelles usually do not show any special topographical relationship. This is true of other types of cells with little ergastoplasm where mitochondria and ergastoplasmic structures are not in especially close contact, and also of cells where the whole cytoplasm is ergastoplasmic in nature, such as gland or plasma cells. The mitochondria are then apparently scattered in a haphazard manner.

As early as 1952, it was shown that the basophilic "clumps" of Berg (1, 2) produced in liver after prolonged fasting and refeeding consisted of dense clots of ergastoplasm and mitochondria (3, 4). This study has been taken up again by Fawcett who with a series of excellent micrographs has confirmed this observation (5).

The present work was planned in order to study the first stages in the development of these ergastoplasm-mitochondrial units. The idea was that further information on the mode of formation of the ergastoplasm in hepatic cells could thus be gathered. In order to determine how general the phenomenon is, hepatic cells have been studied not only after fasting and refeeding but also in the course of intense regeneration following partial hepatectomy or intoxication with carbon tetrachloride.

Material and Methods

All the experiments were carried out on rats. Regeneration after fasting was secured by Lagerstedt's method (6). In all, 26 animals were fasted for 5 days and refed after this period on a protein-rich diet. They were killed 1, 6, 15, 24, 48 hours after the first meal. In another series of experiments 16 rats were partially hepatectomized (about two-thirds of the liver volume) following the usual method (7). Hepatic regeneration was studied 6, 18, 24, 30, 36 hours after the operation.

Lastly, 39 animals were injected intraperitoneally with carbon tetrachloride (60 mg. for each 100 gm. of weight) dissolved in olive oil (0.2 gm. for 1 ml.). Different stages from 30 minutes to 6 days after the injection were studied.

* This investigation was supported by a grant from the Mutuelle Générale de l'Education Nationale.

All the fragments were fixed in 1 per cent buffered osmium tetroxide (Palade), dehydrated in alcohol, and embedded in butyl-methacrylate. Sections were cut with the Porter-Blum microtome and examined under the RCA EMU-2E electron microscope.

RESULTS

(*a*) *Ergastoplasmic Regeneration in Livers of Animals Refed after Starvation.*— As previous studies have shown, cytoplasmic basophilia decreases sharply if not totally after a few days' starvation. This is accompanied by a diminution or a disappearance of the ergastoplasm (3, 4, 5). The prominence of these cytological alterations varies greatly for the same period of starvation with the strain, the age, the preceding nutritional state of the animal. Some may die after 4 days of starvation or survive up to 28 days without showing any modification in their vitality. The animals in our experiments were in a bad state after 5 days of starvation and most of their hepatic cells had lost their ergastoplasm. All their mitochondria were swollen, showing the typical aspect of the "cloudy swelling" described by Gansler and Rouiller (8). During the first hours after refeeding the majority of the mitochondria came back to normal; some remained swollen for 12 to 18 hours. Whatever the state of the mitochondria, ergastoplasmic structures can be observed to return around the 6th hour, but the best examples of ergastoplasm in the midst of regeneration are to be found in animals having been refed for 16 to 24 hours (Figs. 1 and 2). At this stage of intense synthesis, the ergastoplasmic structures form compact groups enclosing the mitochondria. They are distributed in areas along the nuclear membrane (Fig. 1) or in the midst of the hyaloplasm or again along the cell membrane (Fig. 2). Two facts must be stressed: (*a*) the ergastoplasmic membranes reappear *simultaneously* at the periphery of the cell and along the nuclear envelope; (*b*) *all* of the mitochondria are in close contact with the ergastoplasm.

From these observations it can be inferred that (*a*) no density gradient of basophilia, decreasing from nucleus to cell surface, is detectable; (*b*) the chondrioma plays an important part in the elaboration of basophilic substance as Ludford and others had already suspected (10).

It might be supposed that the development of ergastoplasmic membranes along other membranes (nuclear, cellular, or mitochondrial) is purely a physical phenomenon, the pre-existing surfaces acting merely as a mechanical support. However, these ergastoplasmic lamellae never appear in a concentric and regular pattern around the membranes and are rather grouped essentially where the concentration of mitochondria (rich, as we know, in enzymes) is greatest. Further, it is to be noted that the surface of lipide inclusions is never thus encircled by ergastoplasm.

During the first period of ergastoplasm formation around mitochondria, one can follow the details of its development. The membranes generally precede the appearance of the granules described by Palade (11, 12). Flattened vesicles

or cavities, limited by a tenuous membrane, are found in the vicinity of mitochondria (Fig. 3). Later, the granules cover the membranes, giving them the characteristic appearance of ergastoplasmic lamellae.

It seems unlikely that such membranes are derived from invaginations of the cell surface as Palade has suggested (13). The origin of the ergastoplasmic granules could well be the nucleolus since similar granules 100 to 150 A in diameter are to be found in the nucleolar region of the nucleus. However, no density gradient, decreasing from the nucleus to the periphery, is to be found in the distribution of the ergastoplasmic granules during the reappearance of basophilia (9).

(*b*) *Ergastoplasmic Regeneration after Partial Hepatectomy.*—It is known that partial hepatectomy is followed by "cloudy swelling" and pronounced lipide accumulation, after which one observes a marked cell regeneration with active mitosis and an increase in cellular basophilia (6).

18 to 20 hours after hepatectomy, the electron microscope reveals the same relationship between mitochondria and ergastoplasm as in refed liver. During the course of this regeneration period, these two organelles are grouped together in dense masses distributed along the nuclear membrane, at some distance from the nucleus, and even along the cell membrane. Here again, the development of the ergastoplasmic structures appears to be simultaneous in the center and at the periphery of the cell. The images are in no way different from those of the preceding experiment (Fig. 4). After 36 to 48 hours, the cells come back to normal and show the usual picture of lamellar ergastoplasm and mitochondria irregularly and separately scattered in the cytoplasm.

(*c*) *Ergastoplasmic Regeneration following Carbon Tetrachloride Poisoning.*—Many studies on hepatic injury due to this drug have been published. A few days after poisoning, several cells show exactly the same appearance as already described: closely associated ergastoplasmic structures and mitochondria. Sometimes, mitochondria are surrounded by structures consisting of ergastoplasmic lamellae, granules, and a homogeneous substance (Fig. 5). Logically this suggests that ergastoplasm has developed at the surface of and with the active participation of the mitochondria. Later, at more advanced regenerative stages, the cells resume their usual aspect.

DISCUSSION

This investigation was undertaken in order to study the cell reaction to various kinds of injuries. If some cell organelles such as ergastoplasm or mitochondria could disappear without the cell dying, it was thought that they would reappear when the cell resumed its normal activity. The idea was also that this recovery would happen in a similar way whatever the initial lesion might have been. This would then provide valuable information about the

physiological behavior of the organelles studied and perhaps about their origin.

After three different initial injuries, the liver cells responded in a similar manner. The ergastoplasm reappeared in close contact with the mitochondria wherever they were located, either close to the nuclear membrane or in the periphery of the cell. This topographical relationship between mitochondria and cytoplasmic basophilia in liver cells indicates without any doubt a *physiological relationship between these two cell organelles.* The role of the nucleus in the synthesis of cytoplasmic nucleoproteins is certain but it seems to us that mitochondria also play an important role, at least at certain stages of the production of ergastoplasm. It is probable that this intimate relationship between mitochondria and ergastoplasm still exists when the latter has reached its normal development, when, morphologically, the association between the two organelles is less obvious than at the definite stage of cell regeneration here studied. The reason this association is difficult to detect in static images might well be that continual protoplasmic movements allow only transitory contacts.

The behavior of mitochondria and ergastoplasm in hepatic cells under other experimental conditions remains to be studied. Nothing is yet known of the ergastoplasm-mitochondria relationship in other cells.

SUMMARY

1. In most rat liver cells, no special topographical relationship between mitochondria and ergastoplasmic lamellae is to be observed. In some cells, nevertheless, the two organelles are grouped together in dense zones clearly separated from the hyaloplasm.

2. Such an association can be produced at will in the livers of animals refed after prolonged fasting, or in the regenerative phases after partial hepatectomy and intoxication with carbon tetrachloride. In all these cells, the ergastoplasm, after having disappeared, suddenly reappears in the cytoplasm, either along the nucleus or cell membranes, where the mitochondria are grouped.

3. It may be supposed that these topographical relationships between mitochondria and ergastoplasm during a definite period of cellular activity indicate a close functional link between chondrioma and basophilic structures. Mitochondria seem to play an important part in the elaboration of hepatic ergastoplasm.

BIBLIOGRAPHY

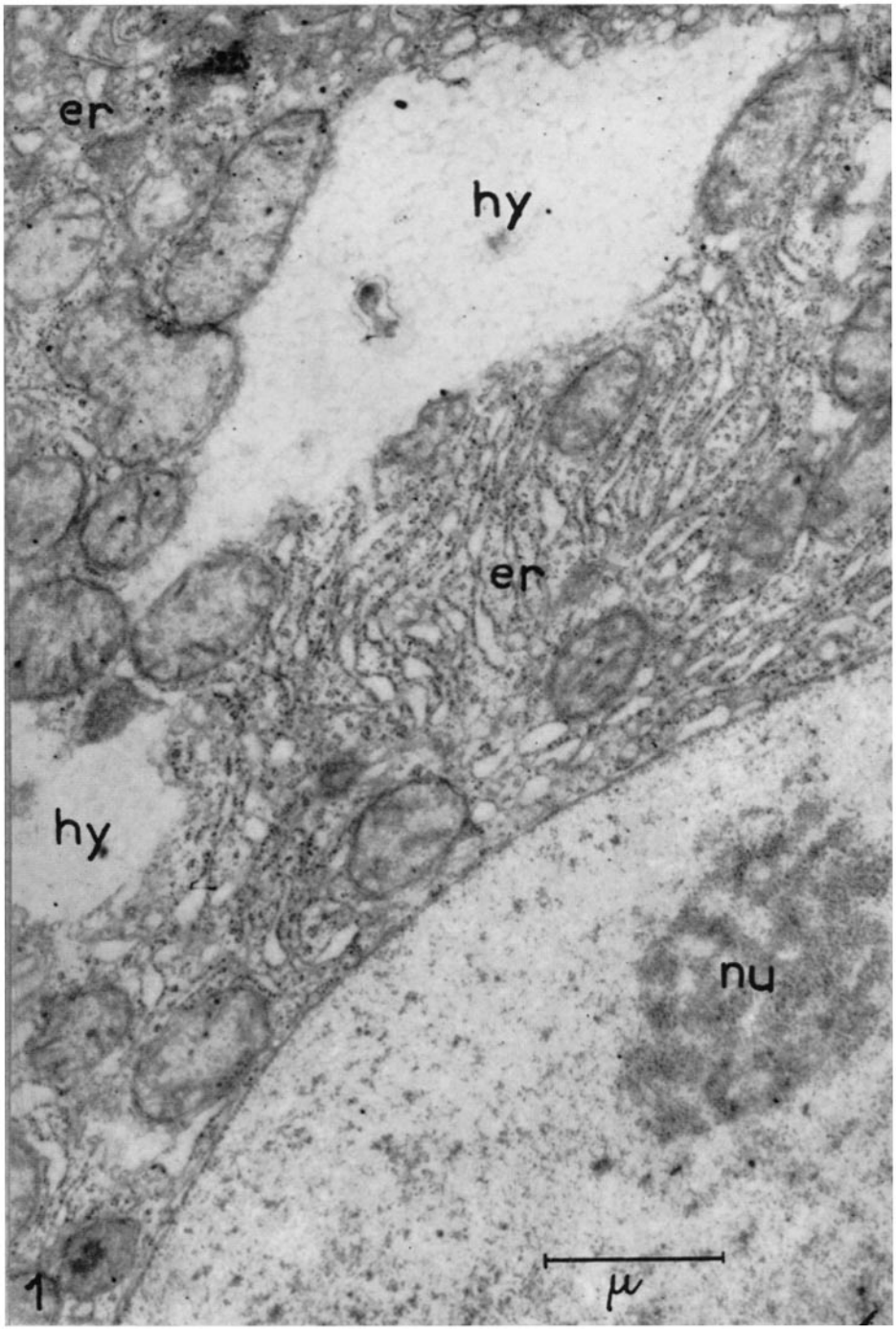
1. Berg, W., *Biochem. Z.*, 1914, **61,** 428.
2. Berg, W., *Arch. mikr. Anat.*, 1920, **94,** 518.
3. Bernhard, W., Haguenau, F., Gautier, A., and Oberling, C., *Z. Zellforsch.*, 1952, **37,** 281.

4. Bernhard, W., Gautier, A., and Rouiller, C., *Arch. anat. micr. et morphol. exp.*, 1954, **43,** 236.
5. Fawcett, D. W., *J. Nat. Cancer Inst.*, 1955, **15,** No. 5, suppl.
6. Lagerstedt, S., *Acta Anat.*, 1949, **1,** suppl., 9, 1.
7. Drochmans, P., *Arch. biol.*, 1950, **61,** 475.
8. Gansler, H., and Rouiller, C., *Schweiz. Z. Pathol. u. Bakt.*, 1956, in press.
9. Caspersson, T., Cell Growth and Cell Function, New York, W. W. Norton & Co., 1950.
10. Ludford, R. J., Smiles, J., and Welch, F. V., *Nature*, 1948, **162,** 650.
11. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 59.
12. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1955, **6,** 567.
13. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 85.

EXPLANATION OF PLATES

PLATE 22

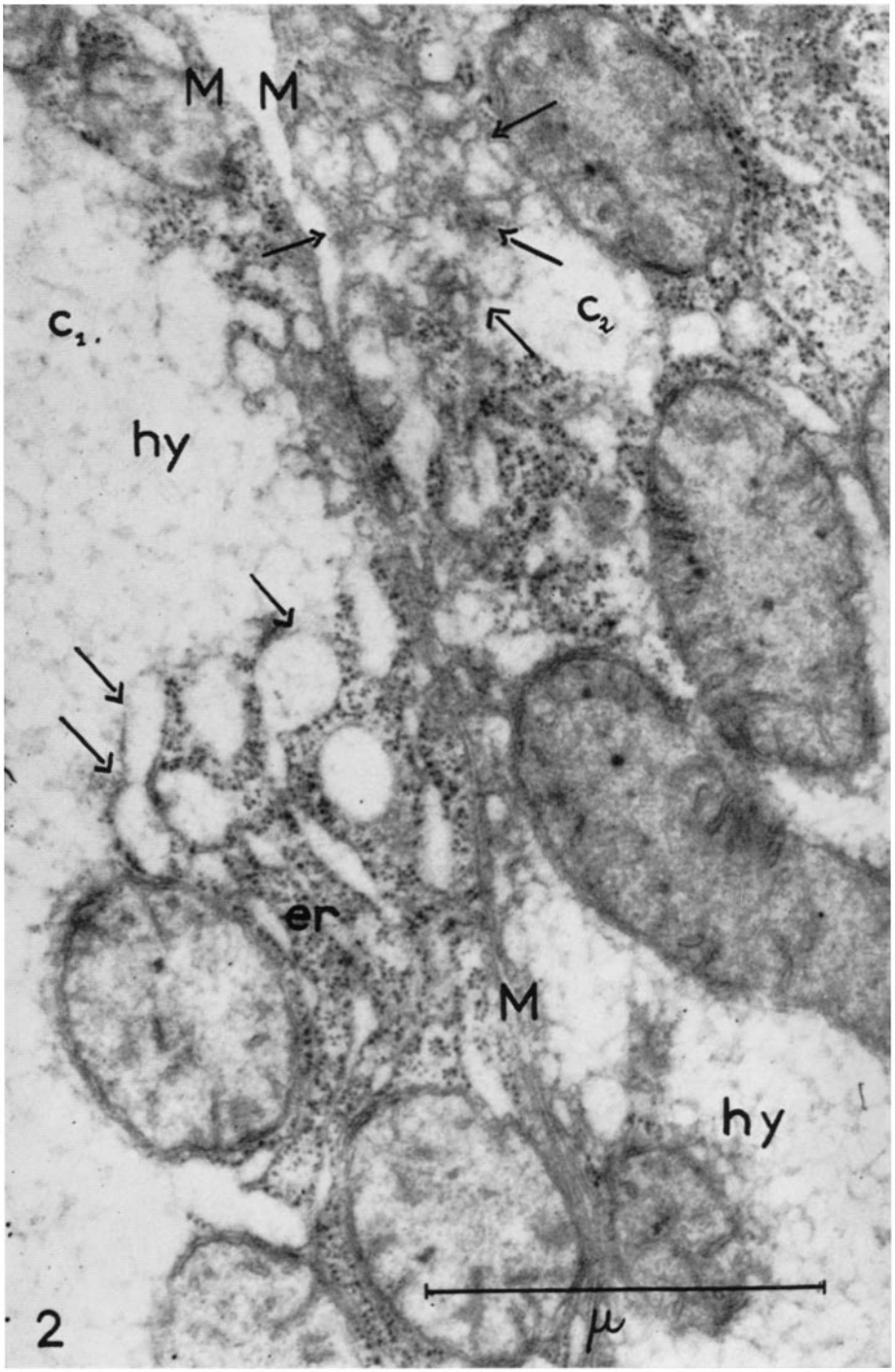
FIG. 1. Liver of rat starved for 5 days and refed after 24 hours. Ergastoplasm (*er*) reappears in limited areas along the nuclear membrane and also around the peripheries of the mitochondria. Note the hyaloplasm (*hy*) free of both organelles. *nu*, nucleolus. × 25,000.



(Bernhard and Rouiller: Mitochondria and ergastoplasm of liver cells)

PLATE 23

FIG. 2. Liver of rat starved for 5 days and refed after 15 hours. Peripheral portion of two parenchymatous cells (c_1 and c_2) in order to show the reappearance of the ergastoplasmic structures along both the cell membrane (*M*) and the mitochondrial surfaces. Arrows point to show initiating ergastoplasm. Membranes outlining clefts or cavities appear first. They are later covered with ergastoplasmic granules. *hy*, hyaloplasm; *er*, ergastoplasm. × 54,000.

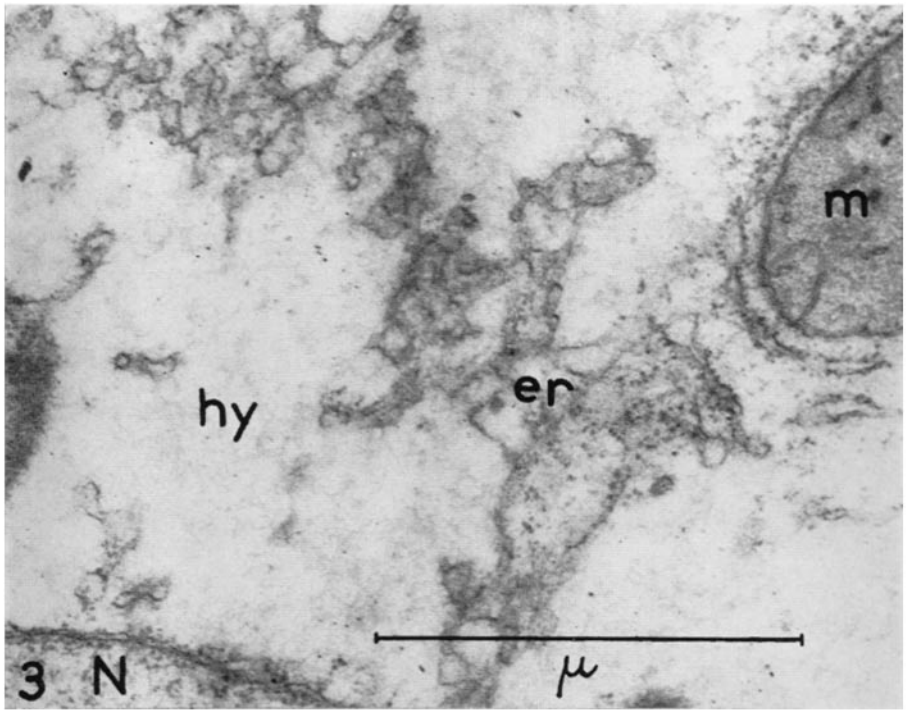


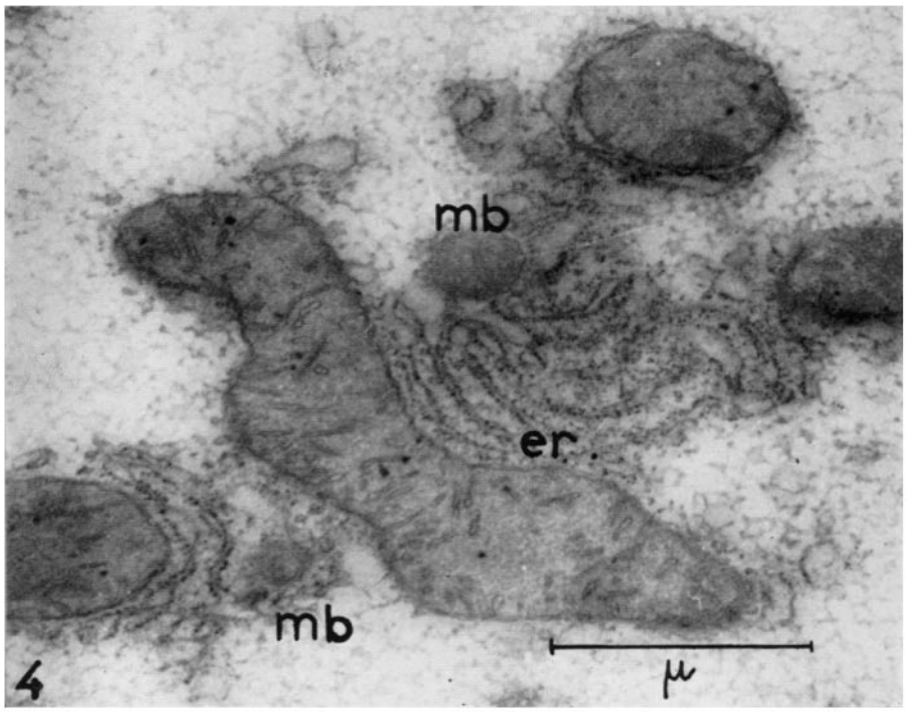
(Bernhard and Rouiller: Mitochondria and ergastoplasm of liver cells)

PLATE 24

FIG. 3. Liver of rat starved for 5 days and refed after 15 hours. Developing ergastoplasmic formations still almost entirely free of nucleoprotein granules. *N*, nucleus; *er*, ergastoplasm; *m*, mitochondria; *hy*, hyaloplasm. × 56,000.

FIG. 4. Rat liver 24 hours after partial hepatectomy. Reappearance of ergastoplasmic structures (*er*) in contact with and close to mitochondria. *mb*, microbodies. × 34,000.

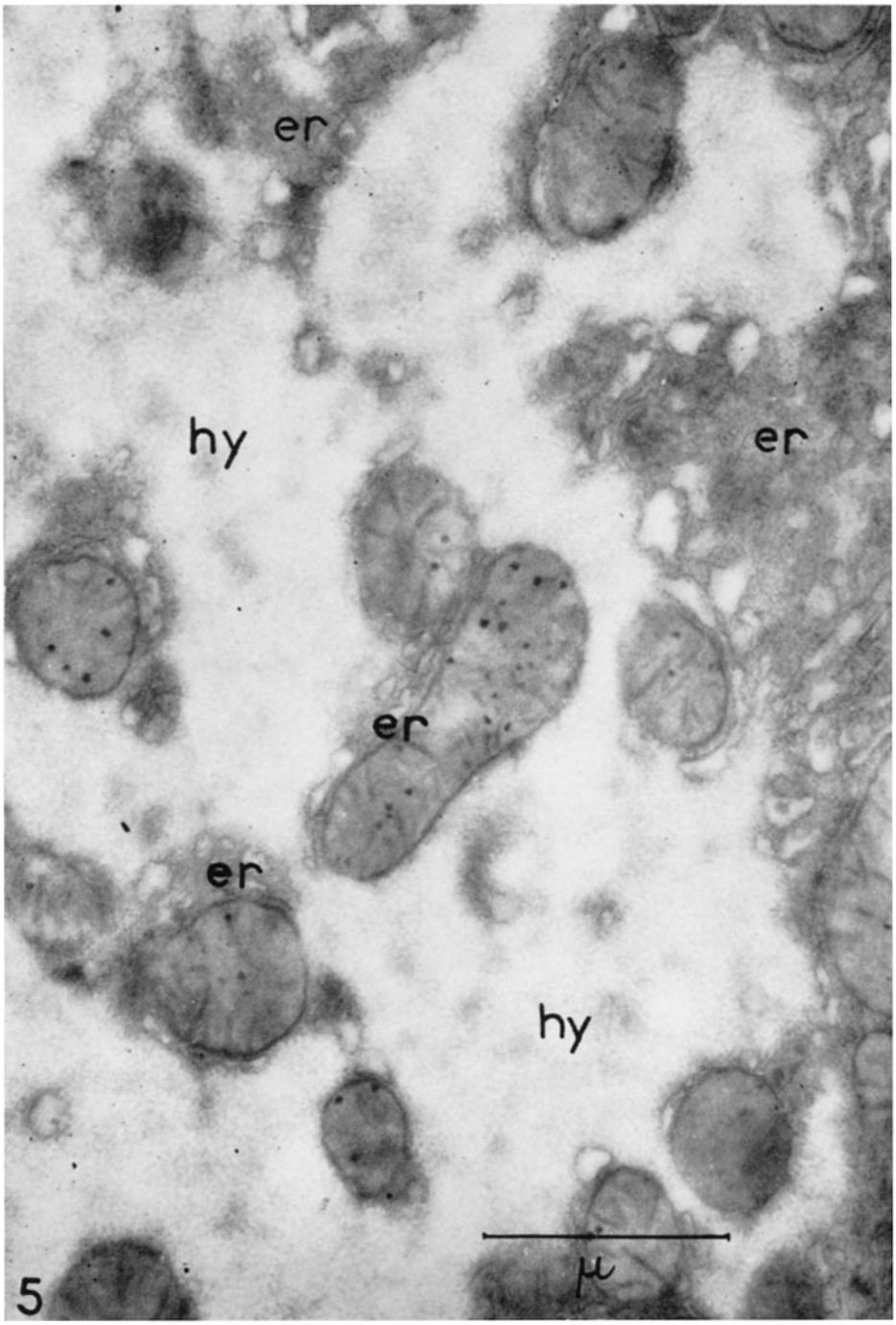




(Bernhard and Rouiller: Mitochondria and ergastoplasm of liver cells)

PLATE 25

FIG. 5. Rat liver 6 days after carbon tetrachloride poisoning. In the hyaloplasm, ergastoplasm (*er*) appears first around scattered mitochondria. × 35,000.



(Bernhard and Rouiller: Mitochondria and ergastoplasm of liver cells)