"MICROBODIES" AND THE PROBLEM OF MITOCHONDRIAL REGENERATION IN LIVER CELLS*

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

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

PLATES 119 TO 121

Hepatic cells contain, besides well defined organelles—mitochondria, ergastoplasm, and Golgi apparatus—round or ovular structures which recall the "microbodies" described by Rhodin (1) in the brush border kidney cells of the mouse. This work aims to define these organelles, to study their behavior under various experimental conditions and their relation to mitochondria.

Rat liver, either normal or subjected to three types of experiments, refeeding after starvation, partial hepatectomy, carbon tetrachloride poisoning have been studied; the latter stages of these show hepatic regeneration more particularly marked in the last two cases. A few livers from animals fed on the carcinogenic diet were examined before and after formation of hepatomas.

Material and Method

Eighty-seven rats were used in these experiments.

Feeding after Starvation Period.—32 rats were starved for 1 or 5 days, then killed 2 to 48 hours after refeeding with Lagerstedt's diet (2).

Partial Hepatectomy.—16 rats were killed 6 to 36 hours after removal, by Drochmans' method (3), of about two-thirds of the liver.

Carbon Tetrachloride Poisoning.—39 rats were given a single intraperitoneal injection of carbon tetrachloride (60 mg. per 100 gm. weight) in olive oil solution (0.2 gm. for 1 ml.). They were killed 2 hours to 6 days after the injection.

The liver fragments were fixed with osmic acid buffered according to Palade's method, dehydrated with alcohol, and embedded in butyl methacrylate. Sections were cut with the Porter-Blum microtome and observed under the electron microscope (RCA EMU-2E).

RESULTS

In the cytoplasm of normal hepatic cells, ovoid or less frequently round dense granules, between 0.1 and 0.5 μ in diameter, much less numerous than mitochondria, can be observed. They are limited by a single well defined membrane and include a dense, finely granulated substance. A densely opaque and homogeneous core occupies their center (Fig. 1). The dense granules are to be found either near mitochondria or near the bile canaliculi. Like mitochondria they are

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sometimes seen in close association with an ergastoplasmic membrane, thus giving the erroneous impression of a double membrane.

These formations can be studied with greater ease in regenerating hepatic cells where they are infinitely more numerous. Groups of mitochondria and ergastoplasmic formations, massed in compact blocks, are a typical aspect of the hepatic cells of rats refed between 6 and 32 hours after starvation periods of various lengths.[1] They are most frequently encountered near nuclear and cellular membranes. Clear areas of finely reticular hyaloplasm occupy the spaces between these blocks. The dense granules are to be found within the mitochondria-ergastoplasm complex (Fig. 2).

In hepatic regeneration, appearing from 18 to 48 hours after partial hepatectomy, or from 3 to 6 days after carbon tetrachloride poisoning (Figs. 3 to 8), the dense granules are more polymorphic than in normal liver. Their size is more variable. While some are very small—less than 100 mμ in length—others average 800 mμ. The membrane is in some cases barely visible, when not entirely absent (Fig. 5). In other cases the granules are coated by several layers of complex membranes (Fig. 4). The substance of the granules may be very dense and made up of grains arranged in minute chains, or in small fuzzy formations (Fig. 7) among which structures resembling the cristae of mitochondria can be observed. The opaque core expands to fill the greater part of the organelle. These changes lead to the formation of bodies comparable in size to mitochondria. They are very dense and their walls include 2 or even several sheaths (Fig. 4). These formations appear most frequently embedded in a hyaloplasm which includes a great many small irregular agglomerations of granules, 20 mμ in diameter (Fig. 3).

The central core of the granules sometimes shows a special structure (Fig. 8) in the form of parallel double membranes, 15 mμ apart. The two membranes of each doublet are themselves 10 mμ apart.

In the hepatic cells of rats fed on a carcinogenic diet, whether a hepatoma has developed or not, a high proportion of dense granules can be observed. These cells also contain a great number of small mitochondria of the same size as the granules, very dense, with ill defined cristae and walls. It is difficult with the numerous intermediate forms which exist to differentiate between these two organelles.

DISCUSSION

Owing to their characteristics—size, single membrane, dense and finely granular matrix—the granules described above are easily distinguished from

[1] The fasting experiments were done on young rats unable to stand more than a 4- to 5-day period of starvation. Recent observations in adult rats fed on a specially devised diet allowed these animals to starve for 25 to 30 days. The results were then different: very prolonged periods of starvation seem indeed to be accompanied by a considerable increase in the number of microbodies and small liver mitochondria (in course of study).

the mitochondria of normal hepatic cells. They resemble neither the Golgi apparatus vesicles, nor some inclusions containing compact or annular bodies and visible near the bile canaliculi (4) which probably correspond to secretion products. They are also different from the granules sprinkled with extremely opaque particles observed by Novikoff (5). They bear a very close resemblance to the "microbodies" described by Rhodin in the brush border kidney cells of the mouse (1). Like them, they are surrounded by a single membrane and contain a finely granular material.

Some details, however, are not the same. For example, in liver cells the granules are often larger and frequently have a homogeneous core, not found in kidney microbodies. We believe that these discrepancies are not significant, and that it is justified to equate these liver granules to the microbodies.

The following facts are revealed through the study of these microbodies in livers of rats killed after hepatectomy or carbon tetrachloride poisoning: (*a*) increase in the number and size, (*b*) organized elements often present in their centers, (*c*) degenerative forms, and (*d*) a close relationship with mitochondria. These phenomena are less apparent in hepatic regeneration when the animal is refed after fasting. The mildness of injury in this last experiment is possibly responsible for these differences.

The core of the microbodies, usually homogeneous, sometimes shows a series of double membranes recalling mitochondrial cristae.

Another modification of the microbodies' central core is their growth at the expense of the granular matrix. This process, often associated with an increase in the size of the organelle, results in the formation of large rounded vesicles. It is highly probable that the invading substance corresponds to some fatty deposit and this is perhaps an example of *degeneration* in a cytoplasmic organelle. It is well known that partial hepatectomy and carbon tetrachloride poisoning are accompanied by steatosis. According to our observations, the fatty deposits in cells seem to appear either in the hyaloplasm itself as small droplets fusing into large homogeneous areas, or in the microbodies and the mitochondria, which increase in size and form vesicles surrounded by a sheath often composed of several layers.

The most interesting problem, in our opinion, is that of the *relations between microbodies and mitochondria.* In regenerative cells, the two organelles are in close topographical relationship to one another and to the ergastoplasm. The early form of the ergastoplasm can be observed surrounding them, an observation which seems to favor the hypothesis that an identical part is played by mitochondria and microbodies in the formation of the ergastoplasm.

While the two organelles in normal hepatic cells are easy to differentiate, such a distinction becomes difficult and often even impossible in degenerative cells of rats fed on a carcinogenic diet and in hepatomas. On one hand, the microbodies increase in size, their less homogeneous matrix contains

structure reminiscent of cristae, and their outside membrane is less clearly defined. On the other hand, a large number of the mitochondria are smaller and denser than normally, with short and rather indistinct cristae and a double, not very clear cut, outer membrane. These observations suggest that *transition stages between microbodies and mitochondria do exist.*

These intermediate stages may correspond either to mitochondria in the process of degeneration or to mitochondrial precursors. There are several arguments in favor of the second hypothesis. First, the transitional forms are best observed in the later recovery stages in rats having undergone hepatectomy or having been poisoned with carbon tetrachloride. They correspond to a phase of cellular regeneration and not to the phase of destruction. Morever, we are dealing with experiments in which the early stages of response are characterized by intense mitochondrial destruction, a phenomenon which brings about a proportionally greater regeneration afterwards. Evidence for this regeneration is found in the great number of small dense mitochondria. In the case of rats refed after fasting—an experiment in the course of which the majority of the mitochondria modifications are reversible after 24 hours—the transition forms are, on the contrary, rare.

Mitochondria in the process of degeneration are easily recognizable in the early stages of these experiments. They do not resemble the transition forms described here. They are not shrivelled up but always much larger in size. They contain a clear matrix, sometimes coarsely reticular or, in the cases of fatty degeneration, uniformly dense and homogeneous (6).

In conclusion, transition forms between microbodies and mitochondria do not seem to represent stages of degeneration. They enable us, on the contrary, to assume that *microbodies are the precursors of mitochondria.*

This hypothesis is not in agreement with the observations of Rhodin (1), who declares himself unable to show any possible kinship between microbodies and mitochondria. These discrepancies are possibly due to different experimental conditions. Moreover, partial hepatectomy and carbon tetrachloride poisoning, producing large scale destruction of chondrioma, are followed by a regeneration all the more easily observed as it is intense.

On the contrary, our hypothesis is in favor of the classical theories, which contend that mitochondria, besides multiplying by division, can originate in the cytoplasm from particles invisible in the optical microscope. It also agrees with Zollinger's hypothesis (7), which states that mitochondria can rise from granules the size of which is at the limit of visibility. However, we cannot agree with this author when he assumes these small corpuscles to be the microsomes of Claude. The small particles the existence of which is assumed by classical authors and those seen by Zollinger probably correspond to microbodies, the smallest of which are beyond and the largest of which are within the limit of the resolution power of the optical microscope.

Lastly, Oberling and his colleagues (8, 9), examining under the electron microscope preparations of spread cells—leukemic and normal (granulocytes, monocytes, and macrophages)— have observed granules and rods comparable to mitochondria, but smaller. They have given the name "ultrachondrioma" to this whole group of formations and postulated a relationship between the latter and mitochondria. The same organelles have been found by Selby and Berger (10) in tissue cultures of human carcinomas and earlier by Porter and Kallman (11) in rat fibroblasts derived from embryos or sarcomas (growth granules). It is most probable that a very close relationship, if not similarity, exists between ultrachondrioma, growth granules, and microbodies. A study with ultrathin sections of such biological material, observed up to now only as spread, uncut cells, should permit a solution of the problem.

SUMMARY

The cytoplasm of the hepatic cell contains, besides the well known organelles, microbodies, characterized by a single membrane, a finely granular matrix, and average dimensions below those of mitochondria.

Microbodies, rare in normal cells, become more numerous in regenerative livers and in various pathological conditions. In these cases, they either evolve towards degenerative forms or else show structural modifications corresponding to transitional forms between them and mitochondria. This suggests to us that the microbodies can be precursors of mitochondria.

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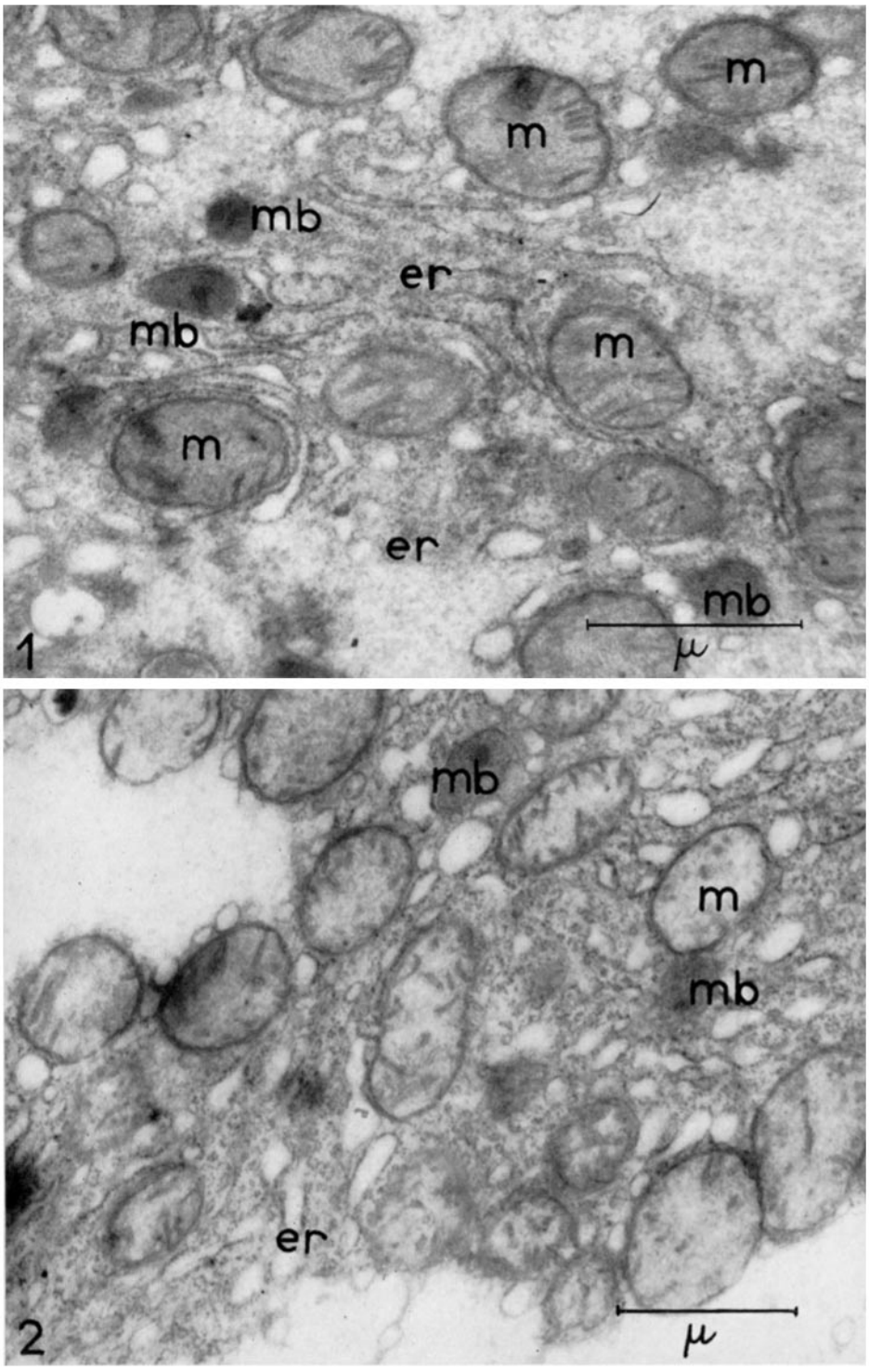
EXPLANATION OF PLATES

PLATE 119

FIG. 1. Portion of liver cytoplasm in a normal rat. In between the mitochondria (*m*) one can see the ergastoplasm (*er*) composed of flattened membranes edged with ergastoplasmic granules and microbodies (*mb*). × 29,000.

FIG. 2. Portion of liver cytoplasm in a rat starved for 4 days and sacrificed 15 hours after refeeding. The microbodies are unchanged. Note the opaqueness of the central core of a microbody at top center (*mb*). At this stage of regeneration, the mitochondria are more transparent than normally and are sometimes still swollen. The ergastoplasm is rich in rosette-like arrangements of the ergastoplasmic granules. × 21,000.



(Rouiller and Bernhard: Mitochondrial regeneration in liver cells)

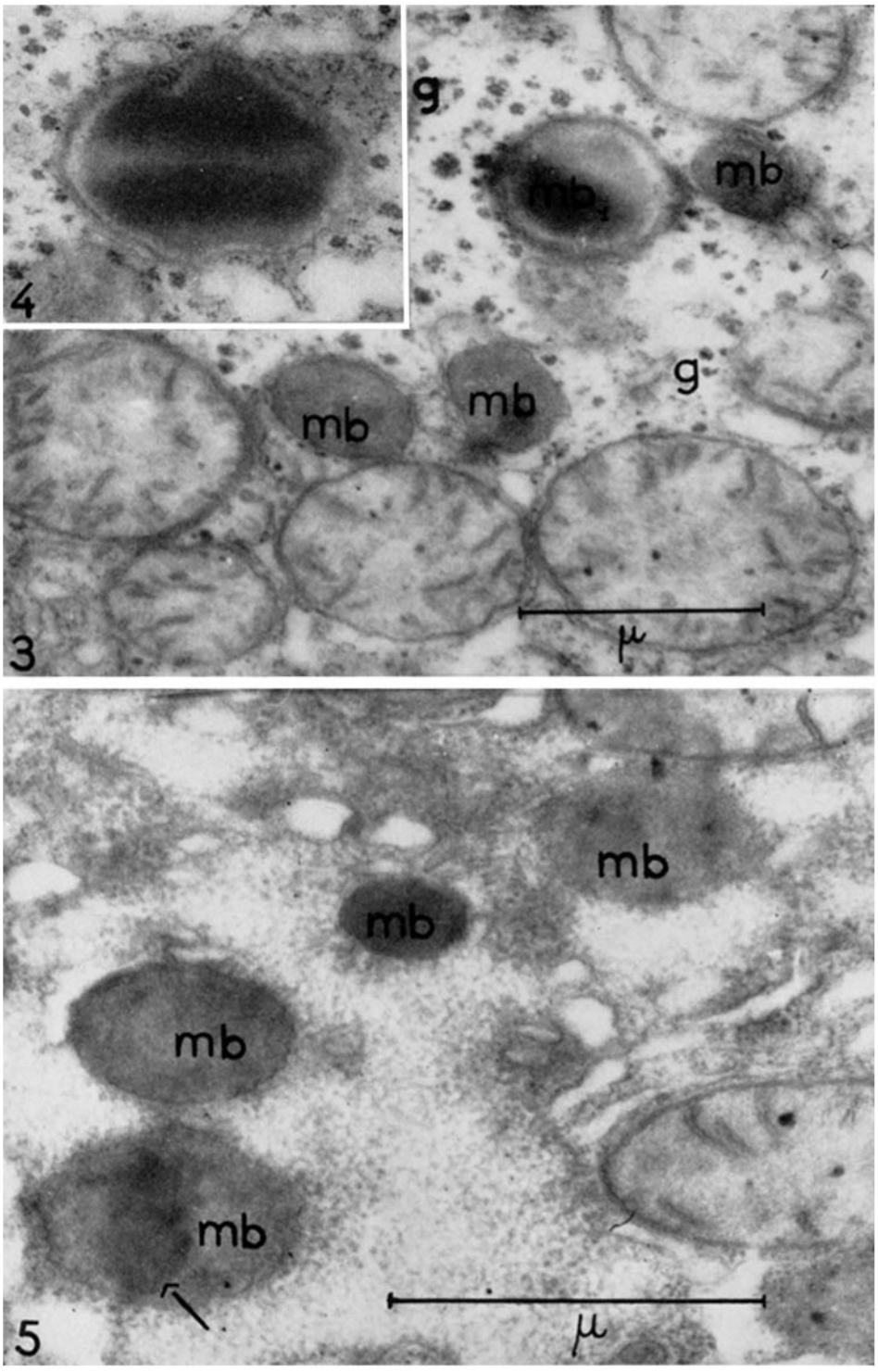
PLATE 120

FIG. 3. Portion of liver cytoplasm in a rat killed 18 hours after partial hepatectomy. *mb* marks microbodies. Mb_1 represents "degenerating" microbody. Its volume is increased. The external envelope or sheath consists of a few delicate membranes; the central core occupies most of the structure. The mitochondria are clear and swollen and still in the "cloudy swelling" state.

The hyaloplasm is strewn with small irregular particles, grouped together. They offer more variation in form, and size, and greater opaqueness than ergastoplasmic granules. They probably correspond to fatty deposits. × 30,000.

FIG. 4. From same liver as Fig. 3; shows another aspect of a "degenerating" microbody. × 54,000.

FIG. 5. Portion of a liver cell from a rat killed 6 days after one intraperitoneal injection of carbon tetrachloride. The microbodies (*mb*) of different sizes are very opaque and may have a central core. They are identical to those observed in hepatectomized rats. × 54,000.



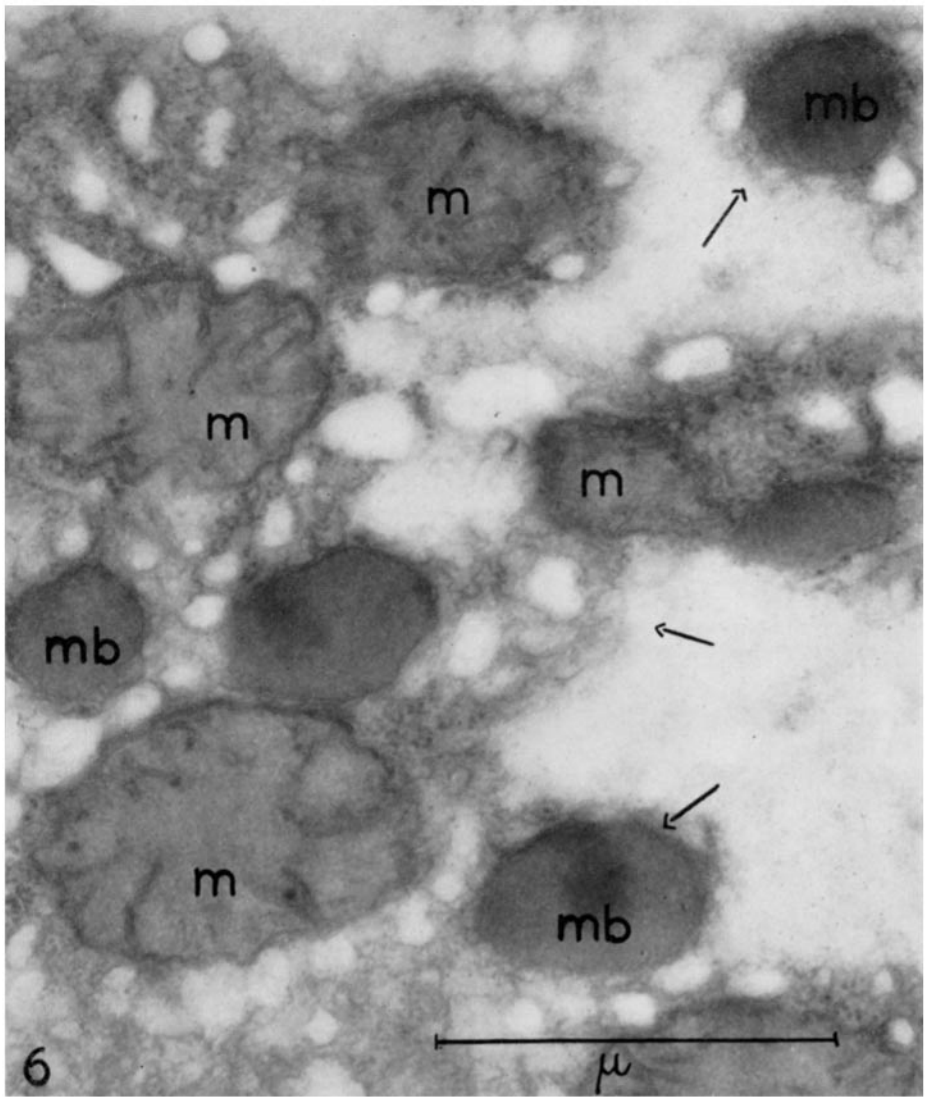
(Rouiller and Bernhard: Mitochondrial regeneration in liver cells)

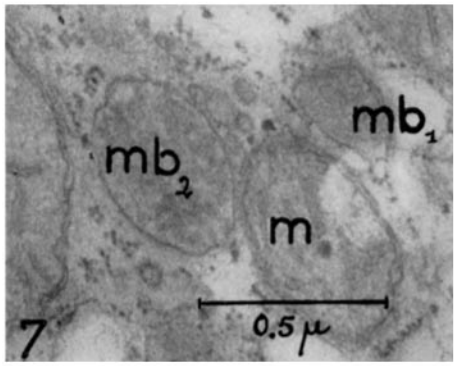
PLATE 121

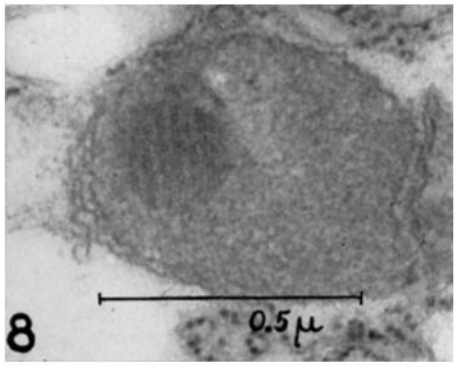
FIG. 6. Portion of the liver cytoplasm in a rat killed 36 hours after hepatectomy. Regenerating cell. Mitochondria (*m*) and microbodies (*mb*) are often enclosed in surrounding ergastoplasmic membranes. × 54,000.

FIG. 7. From liver cell of a rat killed 18 hours after partial hepatectomy showing possible transformation of microbodies into mitochondria. mb_1 marks microbody filled with a granular or delicate filamentous substance. mb_2 represents microbody with beginning of organization in the matrix. *m* represents small mitochondria at the stage where the cristae are still ill defined. × 54,000.

FIG. 8. Same animal. Microbody with parallel double membrane structure. The distance between each is 15 mμ. × 72,000.







(Rouiller and Bernhard: Mitochondrial regeneration in liver cells)