

ELECTRON MICROSCOPIC INVESTIGATION OF THE REGENERATING HEPATIC CELLS OF THE SENILE RAT

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ABSTRACT

Electron microscopic findings on the senile hepatic cells of rats were compared with those of young adult rats in the resting and regenerating stages 6 and 24 hours after partial hepatectomy.

In the resting senile hepatic cells, there were found some bizarre mitochondria, and mitochondria were significantly larger in size, and also slightly decreased in number than in the young adult.

In the regenerating stage, especially 6 hours after removal, mitochondria of the hepatic cells became irregular in shape more markedly in the senile cases, and in some of the cases, degenerative pictures of mitochondria were occasionally observed in this period.

In the regenerating hepatic cells, mitochondria do not increase in size, but are rather reduced. The decrease in size of mitochondria was more marked in the liver of senile cases 24 hours after partial hepatectomy. The decrease in number of mitochondria was observed 24 hours after removal, and the decrease was somewhat marked in the senile cases.

In this experiment, the pictures of so-called transverse division of mitochondria were found without any significant difference due to age and the stage of regeneration of hepatic cells.

The small osmiophilic bodies considered to be the precursor of fat droplets appeared in the space of Disse, and in the cytoplasm. Their appearances were more markedly noted in the aged than in the young adult at 6 hours of regeneration.

INTRODUCTION

Many investigations have been made on the senile changes of tissues and cells. On the hepatic parenchymal cells of the aged, there are a few histological and cytological reports. Andrew¹⁾ described the presence of giant nuclei in the hepatic cells of aged rats and human, and also the increase of nucleocytoplasmic ratio in the hepatic cells of aged rats. Tauchi *et al.*²⁾³⁾ reported that senile atrophy of the liver was not due to a decrease in volume of the

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hepatic cells, but due to a decrease in their number, and that there was noted an increase in number of binucleate and giant nuclear hepatic cells in the liver of the aged, and they considered that the senile hepatic cells seemed, in a sense, to be active. They⁴⁾ also noted that mitochondria of the hepatic cells of senile rats were decreased in number and increased in size. On the other hand, some authors⁵⁾ revealed that no difference in activity of the mitochondrial enzyme (Succinic dehydrogenase) was recognized between old and young adult liver cells.

There are many reports⁶⁾⁷⁾⁸⁾⁹⁾ on the regeneration of hepatic cells, but on the regeneration of the senile there are only a few reports¹⁰⁾¹¹⁾¹²⁾, and many problems remain to be clarified.

Electron microscopic studies of senile hepatic cells have been made by some authors²⁾¹³⁾, but they are only a few on the clarification of senile changes of the hepatic cells, and no electron microscopic study on the regenerating cells of the aged.

The present author made electron microscopic observations of the hepatic cells of senile rats in the resting and in the earlier stages of regeneration.

MATERIALS AND METHODS

A total of 20 Wistar strain rats were used in this study. They were bred and maintained in our laboratory. Ten rats of age above 27 months, weighing from 180 to 250 g. were used as the senile group, and 10 rats of 6 months of age, weighing from 200 to 350 g, as young adult. They were fed with the standard pellet and water *ad libitum* throughout the experiment.

They were subjected to two-thirds partial hepatectomy by the method of Higgins and Anderson¹⁴⁾ without anesthesia. Experimental animals were killed at periods of 6 and 24 hours after partial hepatectomy, and the sacrifices were made between 10 A.M. and 2 P.M. in consideration of the diurnal variation in mitotic rate and amount of glycogen¹⁵⁾.

The resting liver tissue removed at hepatectomy, and the regenerating tissue at the time of sacrifice were examined.

For electron microscopy, small blocks of the tissues were fixed for 1 hour in 1% osmium tetroxide with acetate veronal buffer (pH 7.4), to which was added sucrose to make an isotonic solution. Parallel blocks were fixed in 10% formalin for light microscopy. The blocks were dehydrated in alcohol and embedded in Epon 812¹⁶⁾. Sections were cut with a Porter-Blum and JUM-5 type ultramicrotome using glass knives. Thin sections stained with lead hydroxide¹⁷⁾ for 10 minutes were examined by a Hitachi HU-11 A type electron microscope at 50 kV.

Almost all electron micrographs were taken at a magnification of $\times 10,000$. Photographic enlargements (final magnification $\times 20,000$) of these micrographs

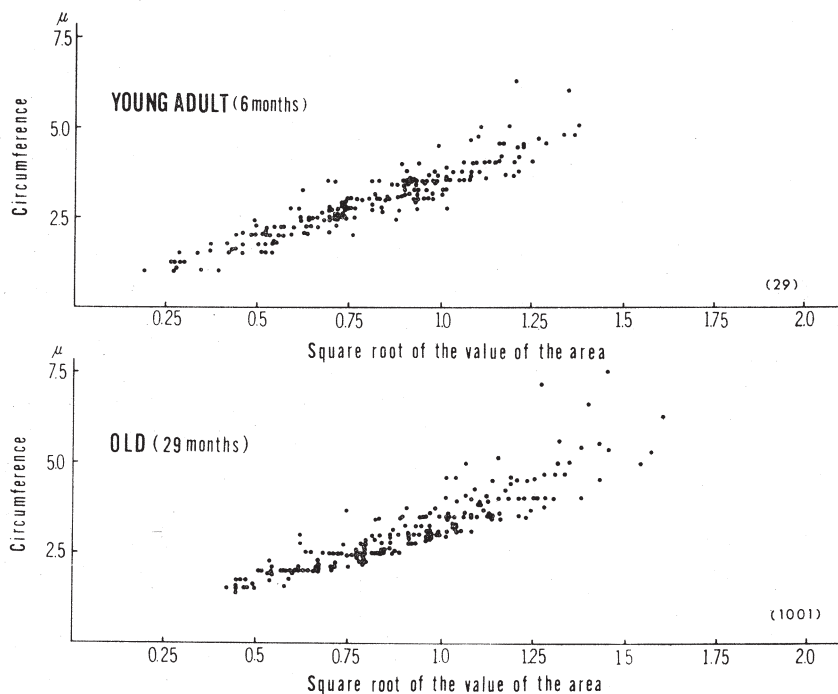
were used for counting the number of mitochondria and for measurement of their size. As mitochondrial area the product of length and width of each mitochondrion was used, and the circumference of the mitochondria was also measured. Measurements were made on 200 mitochondria in each case.

OBSERVATIONS

1) *Electron microscopic findings in the resting stage*

In the resting stage, there was generally found the usual pattern of hepatic cell organelles. On the whole, mitochondria were round, oval and elongated with a distinct limiting membrane and parallel cristae, but in the senile cases, they were sometimes bizarre and gigantic in shape (Chart 1). Changes of mitochondrial membrane were not observed, but only a few mitochondria showed a picture of so-called transverse division of existing mitochondria¹⁸⁾, namely the presence of a transverse double membrane continuous at both ends with the inner layer of their envelope. And these pictures were observed in 7 cases of senile, and in 3 cases of the young adult (Fig. 1).

Sometimes, lysosomes or lysosome-like bodies were observed in senile cases.



Mitochondrial areas were represented by the products of length and width.

CHART 1. Correlation between mitochondrial area and the circumference in the resting hepatic cells

These bodies were scattered near the bile canaliculi, and some of them existed near the Golgi field (Fig. 2).

There were, at this stage, no other special findings to be noted in the cellular organelles.

Measurement of mitochondrial size and number in the hepatic cells

Using electron micrographs, the size and number of mitochondria were measured. Average sizes of mitochondria in the young adult and senile cases were 0.826 and 0.889 respectively (Table 1). The size of mitochondria of senile rat hepatic cells was significantly larger than that of the young adult. The number of mitochondria in the young adult cases averaged 38.0, and that in senile cases 34.8 in a given area of cytoplasm, being somewhat decreased in senile cases (Table 3).

TABLE 1. Age difference in size of mitochondria of the resting hepatic cells of Wistar strain rats

	No. of Cases	No. of Mitoch. Examin	Square Root of the Value of Mitoch. Area
Young adult	10	2,000	$0.826 \pm 0.005^*$
Old	10	2,000	$0.889 \pm 0.005^{**}$

Difference between * and ** was statistically significant at 5% level. (F-test after analysis of variance)

2) *Electron microscopic findings in the regenerating stage after partial hepatectomy*

a) *Six hours after partial hepatectomy*

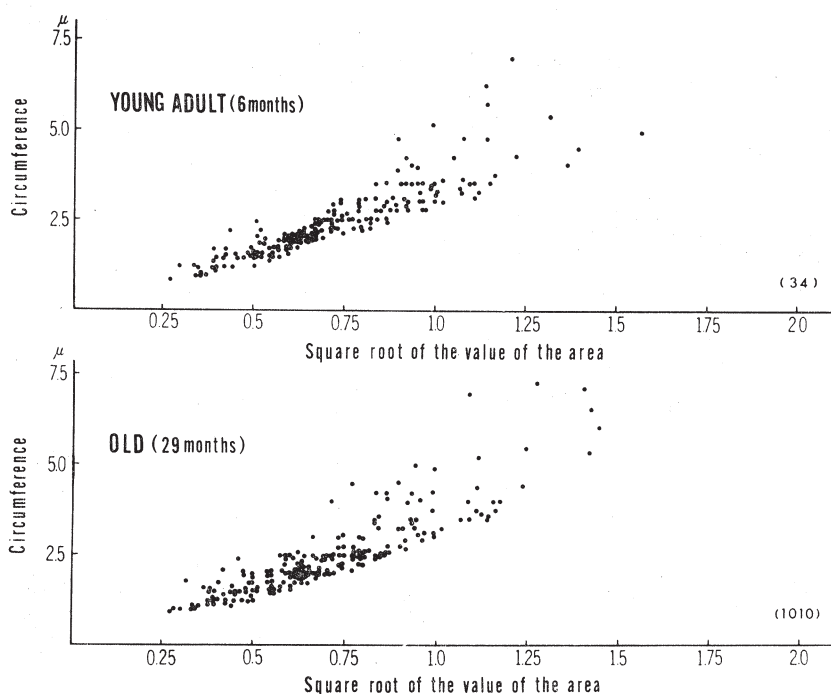
Six hours after partial hepatectomy, mitochondria were more irregular in shape than in the resting stage. And their irregularity in shape was more prominent in the senile hepatic cells (Chart 2). The transverse division of mitochondria was also observed at this period, and there were noted some cases where the picture of division was found not only in the regenerating stage but also in the resting stage. In the majority of mitochondria, the intramitochondrial granules disappeared, and mitochondria with loss of granules were observed in all cases at this regenerating period (Fig. 4). Also, at this period, the matrix of most of the mitochondria became more pale and some mitochondrial cristae showed more marked sac-like structures. In senile cases, some mitochondria showed degenerative pictures (Fig. 7).

Numerous small osmiophilic bodies appeared at this period. They were present in the space of Disse and also in the cytoplasm of cells (Figs. 4, 5, 6). In the latter, each was located within a vesicle, some of which were connected with the endoplasmic reticulum (Fig. 6). Some of these bodies might be seen in association with the Golgi complex. These bodies were generally observed

in the space of Disse and vesicles more apparently in the senile hepatic cells than in the young adult.

In all regenerating cases, the granular endoplasmic reticulum decreased in amount, and the picture of parallel array was lost. Some parts of the membrane were fragmented, dilated, and formed small vacuoles. Ribosomes were detached from the granular endoplasmic reticulum, and there was observed an increase in number of free ribosomes.

Sometimes, few fat droplets were present in the cytoplasm at this period, and nuclei showed no significant changes.



Mitochondrial area were represented by the products of length and width.

CHART 2. Correlation between mitochondrial area and the circumference in the hepatic cells 6 hours after partial hepatectomy

b) *Twenty-four hours after partial hepatectomy*

The changes in mitochondrial shape at this period became generally less marked than seen 6 hours after operation. Mitochondria were almost normal in shape, but there were seen some bizarre shaped mitochondria in senile hepatic cells (Fig. 8). The mitochondrial matrix recovered its density, and intramitochondrial granules gradually appeared, but there were still recognized a few mitochondria without the granules. The cisternae of granular endo-

plasmic reticulum were dilated, and formed vacuoles (Fig. 9). The decrease of ribosomes on the surface of the endoplasmic reticulum, and the increase of free cytoplasmic ribosomes, were markedly noted in the senile hepatic cells.

Numerous fat droplets were present in the cytoplasm of most hepatic cells (Figs. 10, 11). On the other hand, small osmiophilic bodies almost disappeared, but in some senile hepatic cells, they still remained in the space of Disse and vesicles in the cytoplasm, but they were fewer in number than those observed 6 hours after partial hepatectomy.

Measurement of mitochondrial size and number in the hepatic cells

The size and number of mitochondria in the regenerating hepatic cells were measured. Table 2 shows the mean value of the size of mitochondria in the regenerating stages in comparison with that in the resting stage.

The decrease in size of mitochondria was recognized at 24th hour after partial hepatectomy. These changes were more significant in the senile hepatic cells, but hardly observed at 6th hour in all cases.

The number of mitochondria was decreased 24 hours after removal, and the decrease was somewhat marked in the senile cases, but not observed 6 hours (Table 3).

TABLE 2. Size of mitochondria in the resting and regenerating hepatic cells

	No. of Cases	Square Root of the Value of Mitoch. Area (Rest. Stage)	Square Root of the Value of Mitoch. Area (Regenerat. Stage)	
			6 hr	24 hr
Young adult	5	0.821±0.007	0.828±0.008	0.808±0.008
	5	0.830±0.008		
Old	5	0.882±0.007	0.880±0.010	0.839±0.009
	5	0.895±0.007		

Messurements were made on 1,000 mitochondria in each group.

TABLE 3. Number of mitochondria in the resting and regenerating hepatic cells of the Wistar strain rat

	No. of Cases	Number of Mito. in Rest. Stage /100 μ^2 of cytoplasm	Number of Mito. in Regenerat. Stage /100 μ^2 of cytoplasm	
			6 hr	24 hr
Young adult	5	40.0	42.4	32.8
	5	36.0		
Mean Value		38.0		
Old	5	31.6	32.0	32.4
	5	38.0		
Mean Value		34.8		

The number of mitochondria was obtained by the counting on the fields of many electron micrographs.

DISCUSSION

5) *Changes in mitochondria*

Morphological changes in the mitochondria of hepatic cells are found under various pathological conditions¹⁸⁾, and there are several investigations⁴⁾²⁰⁾ concerned with the age changes of mitochondria of hepatic cells.

There are a few reports on the decrease in number of mitochondria in the hepatic cells of old individuals. Tauchi⁴⁾ reported in their light microscopic observation that the mitochondria of the hepatic cells of aged rats decrease in number both in the central and peripheral areas of the hepatic lobule. The present study of counting by many electron photomicrographs also revealed that the mitochondria of the hepatic cells were decreased in number in old rats.

It is interesting to know if the shape of mitochondria has some connection with the function of the mitochondria. Some oxidative enzymes including succinic dehydrogenase are believed to be bound to the surface of the mitochondrial membrane.

There have been some studies on the succinic dehydrogenase activity of old rat liver. The biochemical study reported by Kitani²¹⁾ revealed a decrease in activity of succinic dehydrogenase in old rat liver, and Barrows *et al.*⁵⁾ reported no change in succinic dehydrogenase activity in rat liver of different age. We⁴⁾ previously reported that no significant difference could be found in the activity of succinic dehydrogenase between old and young adult livers in the resting stage. Therefore, an increase in mitochondrial size in the senile hepatic cells seems to be a change, brought about by a decrease in their number, namely it may be said that in the resting stage each mitochondrion of the aged hepatic cells is more actively functioning than that of the young adult.

A few reports on the ultrastructures of the regenerating liver have been made by Aterman²²⁾, Takahashi²³⁾, Jordan²⁴⁾, Virágh²⁵⁾ and Stenger²⁶⁾, but no reports on the regenerating liver of the aged. In the electron microscopic observations on the regenerating rat liver, Takahashi²³⁾ reported swelling of mitochondria 6 hours after partial hepatectomy, and Jordan²⁴⁾ also observed the swelling of mitochondria in the early stage of regeneration. According to the present study on the regenerating liver in the early stage, mitochondria did not increase in size, but rather reduced 24 hours after partial hepatectomy. And the decrease in size was more markedly noted in the senile cases.

The number of mitochondria appeared to be decreased 24 hours after removal, as reported by Allard *et al.*²⁷⁾ using tissue homogenates, and the decrease was rather marked in the senile cases.

There have been some biochemical and histochemical studies on the change of mitochondrial enzymes, especially of succinic dehydrogenase activity in the regenerating hepatic cells. These studies revealed that the activity was markedly

decreased in the regenerating stage. Novikoff⁶⁾, Kitani²¹⁾, and Tsuboi⁸⁾ recognized in their biochemical studies a decrease of enzyme activity in the adult liver during the regenerating process. On the age changes of succinic dehydrogenase activity in the regenerating liver, Kobayashi¹²⁾ reported histochemically that the decrease in activity in the regenerating liver was generally more marked in senile hepatic cells. Biochemical study by Kitani²¹⁾ also revealed a more marked decrease in activity in senile rats.

The morphological behavior of mitochondria observed in the present study could give some support to these biochemical and histochemical findings of the lower activity of succinic dehydrogenase in regenerating liver cells. Namely, the mitochondria of the hepatic cells in the aged seem to be more sensitive to the regenerating process than in the young adult. The irregularity in shape of mitochondria which was more frequently noticed in senile hepatic cells of both resting and regenerating stages than in the young adult, is considered to be also an important indication of the character of mitochondria, especially the mitochondrial membrane system, of the senile hepatic cells. Weinbach²⁰⁾ also suggested, in his investigations of oxidation and phosphorylation in the liver mitochondria of the aged rat, that liver mitochondria from aged rats were more fragile than those from the young.

From the above mentioned findings on the morphological changes in the mitochondria in the resting and regenerating stages, the mitochondria of senile hepatic cells are considered to be more unstable when compared with the young adult.

The appearance of so-called transverse divided mitochondria in the regenerating rat liver was reported by Takahashi²³⁾. Such transverse divided mitochondria were noted by Fawcett²⁸⁾ in the hepatic cells of rats during both fasting and refeeding, and by Lafontaine²⁹⁾ in the hepatic cells of rats fed the azo dye 2-Me-DAB.

In the present study, the picture of so-called transverse division of mitochondria was observed in some cases in the resting and regenerating stages, relatively frequently in senile cases, however, their appearances in each case were generally less marked. The so-called transverse division of mitochondria has been considered by some authors¹⁸⁾²³⁾²⁸⁾²⁹⁾ to be a picture of process of mitochondrial division. The present author also observed a similar characteristic picture, which suggests this possibility (Fig. 3). In the appearances of the picture of transverse division of mitochondria, the present author could not notice any special differences according to age of the host and to stage of regenerating of hepatic cells. From the finding that they are found in the normal resting hepatic cells, their appearance seemed to be connected with the physiological condition of the liver tissue and cells in each individual.

2) *Appearances of small osmiophilic bodies and fat droplets*

After partial hepatectomy, there were found numerous small osmiophilic bodies in the space of Disse, and in the vesicles and Golgi vacuoles of the hepatic cells. These bodies have been observed by other investigators, in the regenerating liver of rats²⁴⁾ and mice^{30,31)}, the liver of mice fed choline deficient diet³²⁾, and also in the rat and guinea pig liver subjected to CCl₄ poisoning³³⁾. The origin, nature, and fate of small osmiophilic bodies have been discussed.

In the regenerating hepatic cells, some authors^{24,30)} considered that they were lipid-containing bodies and the precursors of fat droplets. Jordan²⁴⁾ recognized in the rat hepatic cells that these bodies appeared in the space of Disse 2 hours after partial hepatectomy. Trotter³⁰⁾ also reported that they appeared in the mouse hepatic cells 3.5 hours after removal, and that they increased in number, reaching the maximum value 7 hours after removal and decreased thereafter. In this study 6 hours after partial hepatectomy, the osmiophilic bodies in the space of Disse were observed markedly in the senile cases, but scarcely in the young adult ones, and 24 hours after removal they were not observed in all cases. Concerning these bodies in the space of Disse and fat droplets in the cytoplasm, many authors^{24,30,31)} have suggested that the bodies enter the hepatic cells by the process of pinocytosis, and they are transported through the membrane system in the cytoplasm, *e.g.* through vesicle and Golgi complex, and fat droplets are formed by their concentration. On the formation of these bodies, some authors²⁴⁾ also suggested that the re-esterification of non-esterified fatty acid in the blood may take place at the cell surface. At any rate, it has been considered that the cell membrane and membrane system in cytoplasm may play a role in the formation and mobilization of these bodies.

In the present observations, in spite of the notable appearance of these bodies in senile hepatic cells 6 hours after removal, their appearance in the young adult was far less marked. In almost all cases 24 hours after removal, there was observed a decrease in number of these bodies and the formation of fat droplets in the cytoplasm. On the other hand, Yamada¹¹⁾ reported by light microscopic observation that age-related difference was not so marked in the appearance of the Sudan III positive substances in the regenerating hepatic cells.

These findings seem to show the age-related difference in the time of appearance and amount of precursors of osmiophilic bodies in circulation, or in the times of mobilization of these bodies from the cell surface to fat droplets.

3) *Other organelles in the cytoplasm*

Endoplasmic reticulum is very sensitive to many physiological, pathological and some intoxicating conditions. As changes in the endoplasmic reticulum, there have been generally noted vacuolation, fragmentation, and occasionally

complete destruction of the membrane, and these changes are very similar to those reported²³⁾²⁴⁾ in the hepatic cells in the early stage of regeneration. Picture of regenerative endoplasmic reticulum described by Jordan²⁴⁾ was occasionally seen in this stage, however, disorganization of the endoplasmic reticulum was fairly seen 24 hours after partial hepatectomy, especially in the senile cases.

In the regenerating liver, Rouiller *et al.*³⁵⁾ described an increase in number of microbodies, but the present author could not find an increase.

In this study, there was found no distinct age-related difference in the findings of the endoplasmic reticulum and microbodies in the regenerating hepatic cells.

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EXPLANATION OF FIGURES

- FIG. 1. An electron micrograph of a senile rat hepatic cell in the resting stage. Mitochondria (m) are round or oval, and somewhat irregular in shape. So-called transverse divided mitochondrion (arrow) can be seen. mb, microbody; n, nucleus; ger, granular endoplasmic reticulum. $\times 23,000$.
- FIG. 2. A portion of the hepatic cell of the senile case in the resting stage. Golgi field (Go) and lysosomes (ly) are shown. Golgi sacs contain numerous small dense bodies. n, nucleus; mb, microbody. $\times 15,000$.
- FIG. 3. Mitochondria of the hepatic cell of the young adult in the resting stage. There are shown the single mitochondrial membrane at the part where two mitochondria face each other (arrow). These mitochondria seem as if resulting from so-called transverse divided mitochondrion. $\times 32,000$.
- FIG. 4. The hepatic cells of the senile case 6 hours after removal. Numerous small osmiophilic bodies can be seen in the space of Disse. Intramitochondrial granules have disappeared. m, mitochondria; Ku, Kupffer cell. $\times 15,000$.
- FIG. 5. A higher power micrograph of the part shown in Figure 4. Osmiophilic bodies (ob) are shown in the space of Disse (Di), and can be seen also in the vesicle (v) within the cytoplasm. $\times 20,000$.
- FIG. 6. The hepatic cell of the senile case 6 hours after removal. Osmiophilic bodies are shown in the vesicles (arrow), some of which are connected with granular endoplasmic reticulum (ger). $\times 21,000$.
- FIG. 7. A part of the hepatic cell of the senile case 6 hours after removal. The clustered mitochondria show apparently degenerating figures. Go, Golgi field. $\times 20,000$.
- FIG. 8. The hepatic cell of the senile case 24 hours after removal. Mitochondria (m) are irregular and bizarre in shape. mb, microbody. $\times 20,000$.
- FIG. 9. The hepatic cell of the senile case 24 hours after removal. Mitochondria (m) are somewhat irregular in shape. There are numerous vesicles (v) and free ribosomes (rb) in the cytoplasm. f, fat droplet. $\times 20,000$.
- FIG. 10. The hepatic cell of the senile case 24 hours after removal. There are shown some vesicles in the cytoplasm, and small osmiophilic bodies present in these vesicles (arrows). f, fat droplet; m, mitochondria. $\times 15,000$.
- FIG. 11. The hepatic cell of the young adult case 24 hours after removal. Mitochondria (m) are round or rod in shape. Fat droplets (f) are seen in the cytoplasm. $\times 15,000$.

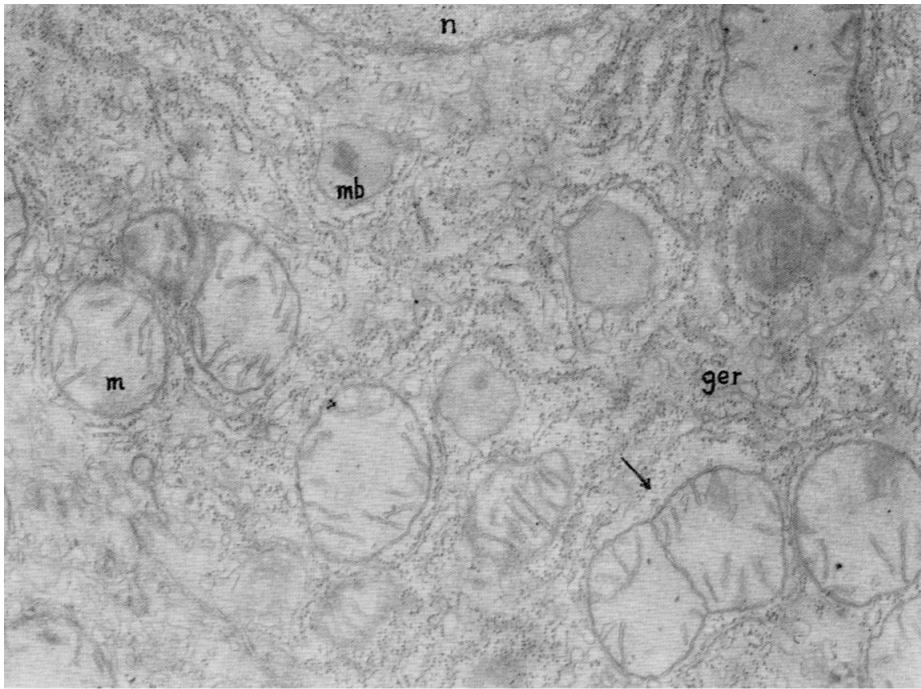


FIG. 1

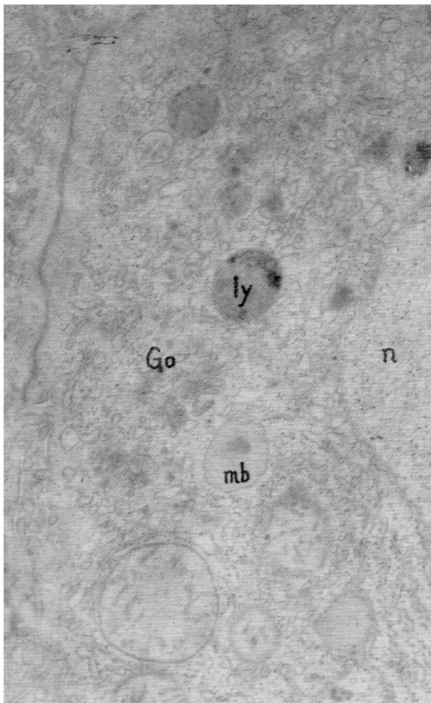


FIG. 2

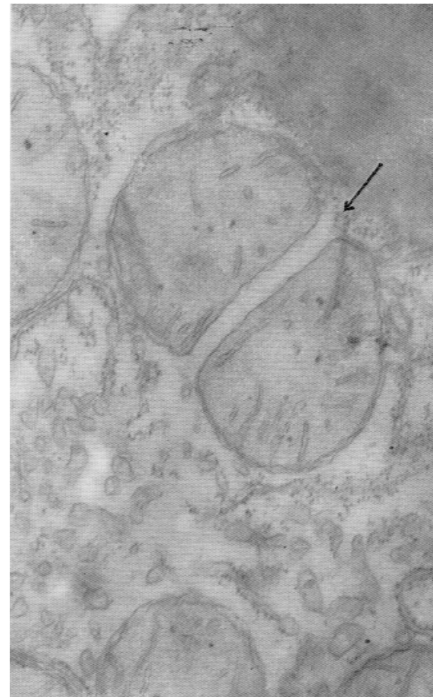


FIG. 3

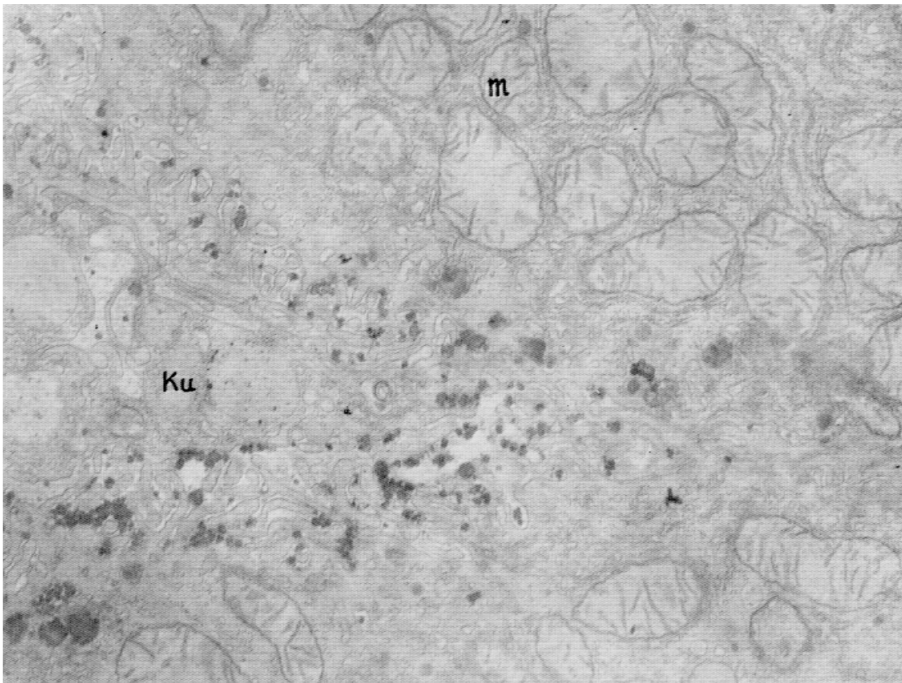


FIG. 4

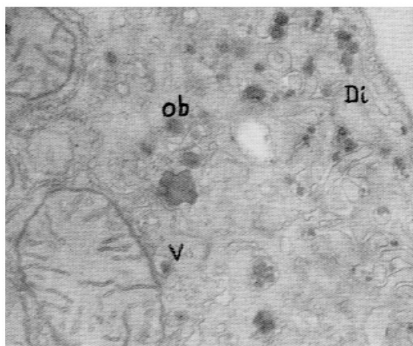


FIG. 5

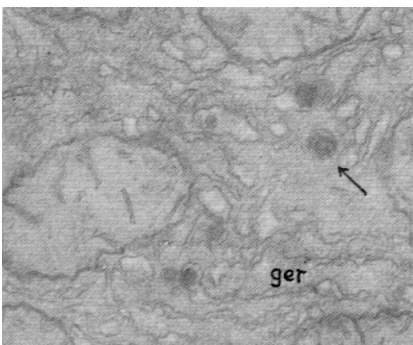


FIG. 6

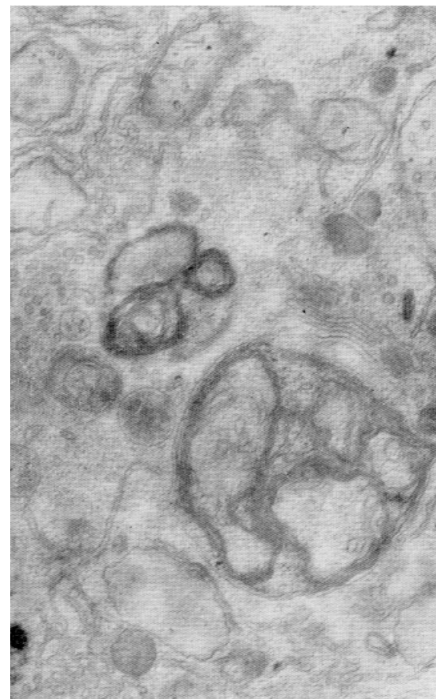


FIG. 7

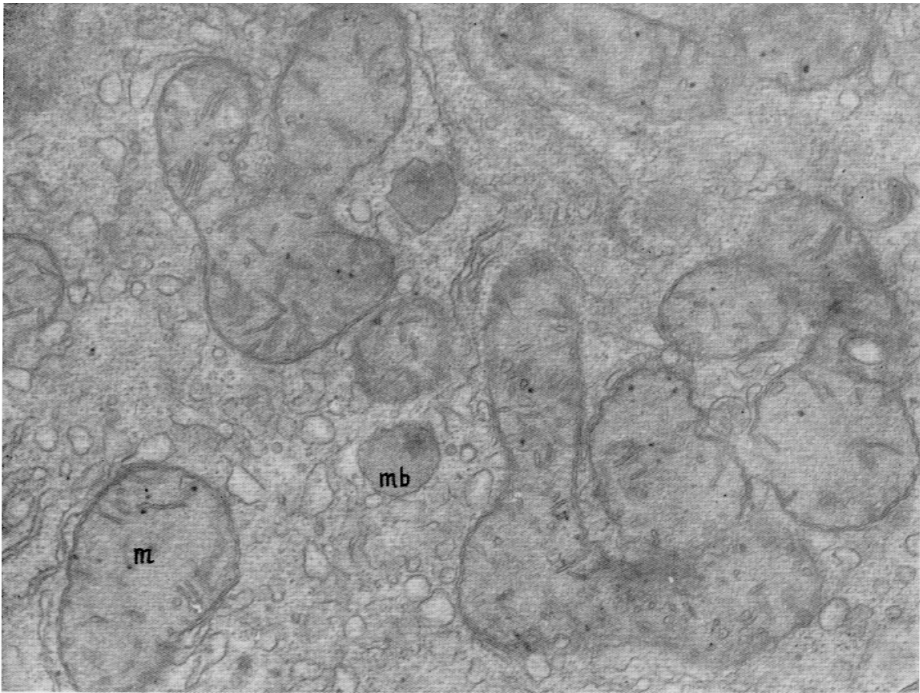


FIG. 8

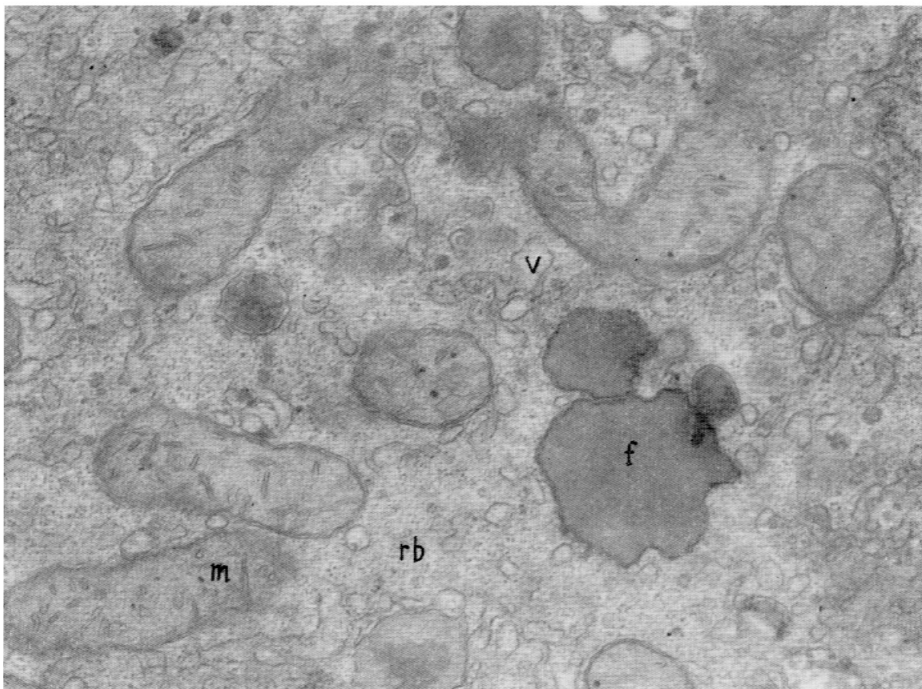


FIG. 9

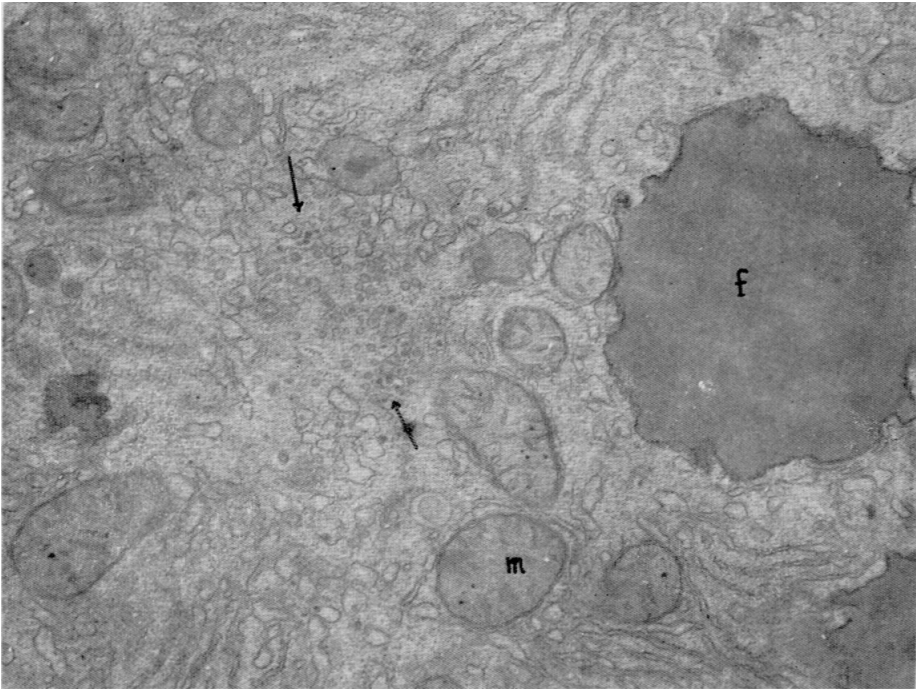


FIG. 10

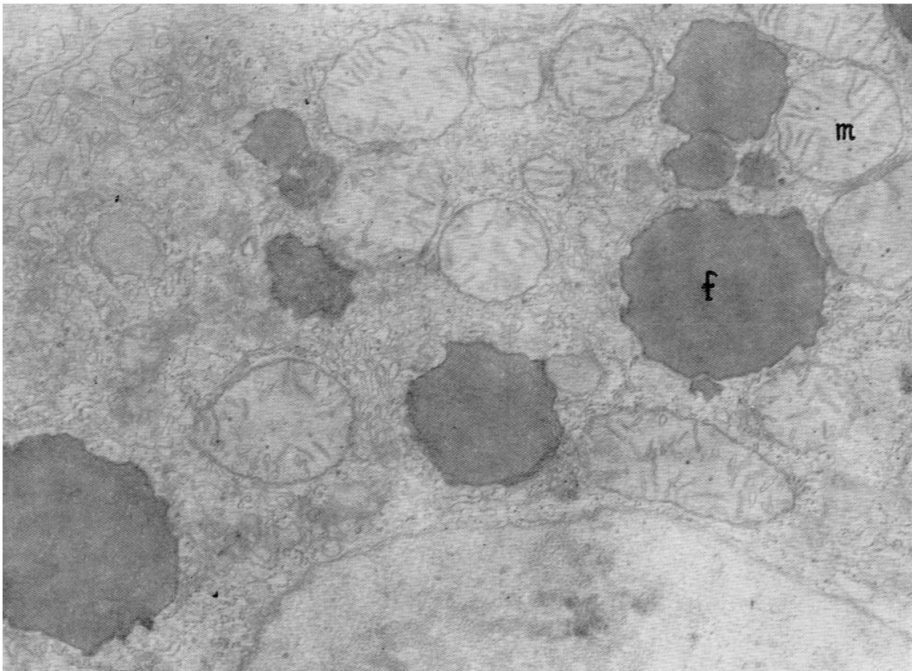


FIG. 11