Ultrastructural and Biochemical Aspects of Liver Mitochondria During Recovery From Ethanol-Induced Alterations

Experimental Evidence of Mitochondrial Division

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To study the morphologic and biochemical changes occurring in liver mitochondria during recovery from ethanol-induced injury, rats fed a 6-month high-alcohol regimen plus a nutritionally adequate diet which did not induce fatty liver were compared with isocalorically fed controls. After this period the alcohol-fed animals displayed striking ultrastructural changes of liver mitochondria and a decreased respiratory activity with succinate or malate-glutamate as substrate. On the contrary, the respiratory rate with l-glycerophosphate was 50% increased. Regression changes were studied after alcohol was withdrawn from the diet. Enlarged mitochondria rapidly disappeared (in 24 hours), although a few megamitochondria were still present after 8 days of abstinence. A similar recovery was observed for the functional alterations. At the end of the experimental period, only a slight decrease of the maximal respiratory rate using malate–glutamate as substrate was noted. The ultrastructural findings and the morphometric data suggest that the way in which mitochondrial normalization takes place is based on partition of these organelles. (Am J Pathol 90:325–344, 1978)

THE EFFECTS OF CHRONIC ALCOHOLISM on the ultrastructure of liver mitochondria in both humans and experimental animals are well known.[1,2] In rats, it was found that after 2 to 4 months of high-alcohol regimens, the hepatic mitochondria had undergone striking changes in size, sometimes becoming greatly enlarged (megamitochondria). In addition, both normal-sized mitochondria and megamitochondria display many configurational changes and other atypical features such as few and short cristae and expansion of the matrix. These morphologic changes are associated with the following biochemical modifications: a) a decreased rate of respiration;[3,4] b) a decreased level of cytochromes a–a_3 and b;[3,5] c) reduced activity of cytochrome oxidase;[5] d) increased mitochondrial fra-

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gility;[6] and e) a decreased rate of biosynthesis of mitochondrial protein.[3,4] The present investigation was undertaken to determine whether the morphologic and biochemical injury of liver mitochondria induced by ethanol is reversible and to describe the manner in which the mitochondrial normalization takes place.

Materials and Methods

Animals and Diets

Forty Wistar male albino rats (of initial body weight 85 to 133 g) were allotted to two groups of 20 animals each and fed as follows: One group (alcohol group) was offered *ad libitum* a basal diet highly supplemented with lipotropes and vitamins, as previously described,[4] and, separately, a mixture of 32% alcohol/25% sucrose (w/v) in Richter drinking tubes, also *ad libitum*. The total caloric intake by the animals as derived from both the solid food and the alcohol–sucrose mixture was recorded daily. Rats of the second group (control group) were offered water as the only drinking fluid and fed a solid diet based on the final regimen (basal diet + alcohol–sucrose solution) consumed by the alcohol group, but in which alcohol was isocalorically replaced by sucrose. To adapt the animals to the high alcohol mixture, during the first 3 weeks they were offered different alcohol–sucrose solutions with increasing concentrations of both components (15%–10%, 20%–15%, and 25%–20%). All animals were housed in wire-bottomed individual cages in air-conditioned rooms. Hours of light and darkness were controlled. Body weight was recorded once a week. After 6 months, 4 animals from each group were selected at random and killed by decapitation (0 time). Their livers were excised and processed for morphologic and biochemical analysis. After this period, the alcohol–sucrose solution was withdrawn from the diet and both groups of animals were fed on the basal diet plus water. Four animals from each group were killed at 1, 2, 4, and 8 days thereafter. The livers were removed and processed by the same methods as those for animals killed at 0 time.

The caloric percentages of ingredients in the final regimen (basal diet + alcohol–sucrose solution) consumed by animals of the alcohol group during the first 6 months of the experiment were as follows: alcohol, 37%; carbohydrate, 44%; fat, 8%; and protein, 11%. The composition of the diet of the sucrose control group was the same except that the alcohol-derived calories were replaced by sucrose. The lipotropic value of both alcohol and sucrose control diets was 178 mg of choline per 100 kcal. The final regimens consumed by both groups, despite the low protein level, provided more than the recomended daily allowances of vitamins, lipotropes, and other essential food factors. The caloric proportions of the basal diet consumed by both groups during the regression period were as follows: carbohydrate, 54%; fat, 20%, and protein, 26%. The lipotropic value was 424 mg of choline per 100 kcal.

Light and Electron Microscopy

Tissue samples were taken for light and electron microscopy studies and were processed by the methods previously described.[4]

Planimetric Determinations

Liver planimetric analyses were performed on both groups of animals killed at the different periods. To establish quantitatively the presence of megamitochondria in paraffin sections stained with chromotrope 2 R, 20 microscopic fields at a magnification of 300 × were selected at random and were analyzed from each liver. To determine the mitochondrial

volumetric density (VVM), numerical density (NMUV), and particulate volume (PVM), electron micrographs were analyzed by the methods and formula described by Weibel et al.[7] Four blocks from each liver were studied and 12 to 16 electron micrographs were obtained from each of them (total, 1140). The fields were selected at random and the original magnification of the photographs was 5000 ×. The analyses were performed in final prints at 15,000 ×.

Mitochondrial Respiration

Mitochondria were prepared by the method of Schneider.[8] The homogenization medium was 0.25 M sucrose, 1 mM tris-HCl (pH 7.4). The mitochondria were suspended in the homogenization medium and the respiratory activity was measured no later than 3 hours after the end of the preparation. Mitochondrial respiration was determined polarographically at 30 C with a vibrating platinum electrode (model K Oxigraph, Gilson Medical Electronics, Middleton, Wis). The basic reaction medium contained 0.24 M sucrose, 34 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA, 9 mM tris-HCl (pH 7.4), and 5 mM K_2HPO_2 (pH 7.4). Substrates were used as follows: 10 mM succinate; 5.0 mM L-malate, 5.0 mM L-glutamate, and 2.5 mM malonate (briefly, MAL–GLUT mixture); and 10.0 mM 1-glycerophosphate. The mitochondrial protein (1 to 2 mg/ml) and ADP (0.2 to 0.3 mM) were added successively. The final volume of the reaction mixture was 2 ml. The oxygen concentration in the reaction media was 0.22 mM. The ADP:O ratio was determined according to Estabrook.[9] The protein of mitochondrial preparations was determined by the biuret method.[10]

Statistical Analysis

The data were statistically analyzed by the *t* test for differences between means; the level of confidence was 95%.

Results

Body Weight

The growth rate of animals from both groups at the end of the first part of the experiment (6 months) was as follows (average ± SE values, in grams per day): alcohol, 1.56 ± 0.07; control, 1.71 ± 0.07. After the animals were transferred to the nonalcoholic regimen, a marked increase in growth rate was observed, particularly in animals from the alcohol group. Thus, after 8 days of abstinence, growth rates were as follows (expressed as above): alcohol, 3.17 ± 0.30; control, 2.38 ± 0.19.

Light Microscopy

Livers from rats of the alcohol group killed after the 6-month period of ethanol intake (0 time) showed a few fatty changes in the form of tiny droplets located specially in centrilobular hepatocytes. These fatty changes began to disappear after the removal of alcohol from the diet and were scarcely detectable after the 8-day abstinence period. Megamitochondria were observed in animals from this group during the first part of the experiment. They were numerous and large (sometimes even larger

than nuclei) and mainly located in midzonal areas, although they were also found in centrilobular hepatocytes. The number of megamitochondria was drastically reduced as soon as the alcohol was excluded from the diet. This reduction was more evident in the smaller megamitochondria (2 to 4 μ) than in the larger (8 to 10 μ). After the 8-day abstinence period, some large megamitochondria were still visible at the periphery of the liver lobule. The quantitative analyses of the number of megamitochondria per unit area and per hundred hepatocytes are presented in Table 1.

Sections from livers of the control group at the end of the first part of the experiment displayed fatty changes similar to those of the alcohol group, but the hepatocytes affected were in the periportal and midzones. After the new regimen was imposed, these fatty changes practically disappeared. No megamitochondria were observed in this group.

Electron Microscopy

Alterations in the ultrastructural appearance of liver mitochondria were evident in all the rats from the alcohol group killed after the 6 months of alcohol treatment. These organelles were generally enlarged and the configuration of many was bizarre. They appeared curved, U-shaped, annular, often encircling other mitochondria (Figure 1). Except in those exhibiting elongated shapes, cristae were scanty, but the density of their matrices and the number of dense granules did not differ from normal. Megamitochondria were also seen in some hepatocytes, particularly in those of central or midzonal areas. They were characterized by moderately dense and uniform matrices with very few and short peripheral cristae (Figure 2). These giant mitochondria closely conformed in size and appearance to those produced under almost similar conditions as pre-

Table 1—Number of Megamitochondria in Rats From the Alcohol Group

Days of abstinence	N	Number of megamitochondria	
		Per unit area (Mean ± SE)	Per hundred hepatocytes (Mean ± SE)
0	4	15.18 ± 4.37	38.09 ± 11.48
1	4	6.29 ± 1.57	17.27 ± 5.12
2	4	2.71 ± 0.30*	7.31 ± 1.16†
4	4	2.58 ± 0.85*	6.93 ± 1.99†
8	4	0.58 ± 0.15‡	1.53 ± 0.44*

* $P < 0.025$ (difference with 0 time).
† $P < 0.05$ (difference with 0 time).
‡ $P < 0.001$ (difference with 0 time).

viously reported.[11-13] Scarcely any changes were noticeable in other subcellar organelles, ie, nuclei and littoral cells.

The ultrastructural aspect of liver mitochondria after the first day of abstinence was essentially similar to that described during the alcohol feeding period, although the number of mitochondria with configurational changes was drastically reduced. Many examples of mitochondrial partition were distributed throughout the liver lobule. Dividing mitochondria were characterized by a septum which separated their matrix into two compartments (Figures 3 through 6). The partition septums were in direct continuity with the inner mitochondrial membrane and were similar in appearance to mitochondrial cristae. In some dividing mitochondria of normal or slightly enlarged size, the partition septum had a median location, dividing the organelle into similar halves. In contrast, in the dividing megamitochondria the partitions were excentrically located, sequestering portions of the organelle equal in size to normal mitochondria (Figures 7 and 9). It was often possible to discover some megamitochondria with several partition areas, characterized by the presence of normal cristae, both in configuration and number. The normal and enlarged mitochondria were constricted at the level of partition, and in a few cases it was possible to distinguish a marked matrical rarefaction in these areas (Figure 5). Particularly interesting was the ubiquitous location of the rough endoplasmic reticulum in the areas where organelle division prevailed. The corresponding membranes were located close together in the dividing zone and were sometimes even aggregated to the outer mitochondrial membrane (Figure 8). In this case, the endoplasmic reticulum membranes appeared detached from ribosomal granules. Interposition of the endoplasmic reticulum membranes to megamitochondria on the one hand and to normal mitochondria on the other (an already divided mitochondria?) could frequently be observed (Figure 10). In some of these structures it was almost impossible to distinguish the origin of any of these stacks of membranes (Figures 7 through 9). In general, no more than one or two dividing organelles were present in any cell section; many cells showed no evidence of mitochondrial division whatsoever, although giant mitochondria were present.

At the end of the second day of abstinence, the mitochondrial abnormalities showed a remarkable decrease. The majority of the mitochondria were almost normal in size and configuration, with a marked increase in the number of their cristae. However, in some areas the ultrastructural aspects remained similar to those observed before the withdrawal of alcohol from the diet and megamitochondria were still present. Dividing mitochondria were seen with similar or even increased frequency, as

compared with the findings on the first day of abstinence (Figure 6). The distribution of rough endoplasmic reticulum over the area of dividing mitochondria was similar to that described above. A constant feature of these membranes was the disappearance of ribosomal granules in the intermixed areas (Figure 10).

After 4 or 8 days of abstinence the mitochondria displayed a tendency toward a more spherical shape than usual, but in other respects they were practically normal. Megamitochondria were scarcely detectable, although on the eighth day, some of them still remained surrounded by many normal organelles (Figure 11). Partition figures were rarely seen except in giant mitochondria. In addition to these changes, a few cells contained degenerating mitochondria, which were to be found invariably within autophagic vacuoles. Most of these degenerating organelles were of normal size, although some enlarged degenerating ones were occasionally seen. In many megamitochondria the outer space was widened by the presence of multiple helical filaments (Figures 8, 10, and 11). This change, although it was most conspicuous during the abstinence period, was also observed during the alcohol feeding period. A constant observation consisted in a fairly characteristic encircling of the mitochondria by the granular endoplasmic reticulum (Figure 12). Besides this disposition, many flattened sacs displayed their characteristic parallel arrangement. Other subcellular components of the hepatocytes were normal, except for some decrease in the contents of glycogen granules.

The electron microscopic configuration of hepatocytes from the control group at the different periods studied was essentially normal. A very exhaustive search for partition figures in normal mitochondria yielded only a very few of these in some hepatocytes (Figure 13). As in dividing enlarged mitochondria, these organelles usually showed a furrowing at the level of the partition septum.

Planimetric Determinations

The percentage of cytoplasmic volume made up of the VVM in animals from the alcohol group during the alcoholic feeding was slightly increased as compared with the figures for control rats (Table 2). The NMUV and the PVM of these organelles were also slightly increased, although the differences were not statistically significant. During the abstinence period a steady decrease in the VVM was observed; the data at the end of the period approached the control values.

Particularly interesting were the changes detected in the NMUV. During the first day of abstinence the numerical density was reduced by 20%; in the subsequent 24 hours the number of mitochondria significantly

Table 2—Quantitative Morphologic Analysis of Liver Mitochondria From Alcohol and Control Groups

Group	Days of abstinence	VVM (Mean ± SE)	NMUV (Mean ± SE)	PVM (Mean ± SE)
Control	0	20.76 ± 1.14	31.63 ± 2.18	0.70 ± 0.04*
Alcohol	0	24.88 ± 1.59	34.54 ± 2.45	0.76 ± 0.02
	1	23.39 ± 2.22	27.66 ± 1.77	0.86 ± 0.04
	2	23.35 ± 1.19	38.67 ± 3.15*	0.64 ± 0.05†
	4	22.60 ± 0.83	34.37 ± 0.56†	0.70 ± 0.03†
	8	21.70 ± 1.38	37.64 ± 2.49†	0.60 ± 0.05‡

* $P < 0.05$ (difference with alcohol group [Day 1]).
† $P < 0.02$ (difference with alcohol group [Day 1]).
‡ $P < 0.01$ (difference with alcohol group [Day 1]).

increased to values even higher than those observed before the alcohol withdrawal. During the remainder of the abstinence period, the NMUV remained high. The PVM clearly reflects these modifications in their number. It increased during the first day of abstinence but decreased during the following days. After the 8 days of abstinence, the individual size of mitochondria in the alcohol group was even smaller than that in the control animals.

Mitochondrial Respiration

The respiratory activities of mitochondria from rats fed the different diets during the first part of the experiment are presented in Table 3. With succinate as substrate, State 3 of mitochondrial respiration for the alcohol-treated animals was significantly lower than that observed in the control group. The control respiratory values and the ADP:O ratio were unaffected. The alterations detected with malate–glutamate as substrate were similar. In this case, the alcohol-fed animals exhibited reduced maximal respiratory activity (State 3), respiratory control, and P:O values. In contrast, with 1-glycerophosphate as substrate, the rate of the ADP-activated respiration (State 3) from the alcohol group was significantly higher (50%) than that observed with mitochondria from the control rats. After removal of alcohol from the diet, mitochondrial respirations returned to their normal level; thus, with succinate or 1-glycerophosphate as substrates, they were normal after 4 days and 1 day of abstinence, respectively. With malate–glutamate the normalization was very similar. The control respiratory values and the P:O ratio did not differ between groups after the first 24 hours of the new regimen. At the end of the 8-day abstinence period, there were no differences between the two groups except for a small, although significant, difference in the maximal respiratory activity with malate–glutamate as substrate.

Table 3—Phosphorylation and Substrate Oxidation in Mitochondria From Alcohol and Control Rats*

Group	Days of abstinence	Maximal rate of respiration (ng-at O/min/ mg of protein)	ADP:O	Respiratory control	
				Before†	After‡
		Substrate: succinate			
Alcohol	0	97 ± 7§	1.60 ± 0.05	3.5 ± 0.3	3.6 ± 0.2
Control		171 ± 13	1.76 ± 0.12	3.5 ± 0.1	3.8 ± 0.2
Alcohol	1	104 ± 11§	1.53 ± 0.01‖	3.0 ± 0.1	3.7 ± 0.2
Control		172 ± 4	1.58 ± 0.01	3.4 ± 0.1	3.8 ± 0.3
Alcohol	2	139 ± 11§	1.53 ± 0.05	3.4 ± 0.3‖	3.2 ± 0.3§
Control		232 ± 12	1.50 ± 0.04	4.4 ± 0.3	5.5 ± 0.3
Alcohol	4	120 ± 18	1.52 ± 0.10	3.6 ± 0.3	4.0 ± 0.5
Control		156 ± 13	1.64 ± 0.02	3.6 ± 0.1	4.0 ± 0.2
Alcohol	8	200 ± 9	1.77 ± 0.03	5.1 ± 0.3	5.9 ± 0.3
Control		218 ± 12	1.76 ± 0.04	4.8 ± 0.2	5.5 ± 0.2
		Substrate: malate-glutamate-malonate			
Alcohol	0	49 ± 2¶	2.03 ± 0.02 ¶	2.9 ± 0.2§	2.6 ± 0.1#
Control		92 ± 5	2.43 ± 0.06	5.1 ± 0.3	4.2 ± 0.4
Alcohol	1	69 ± 2‖	2.51 ± 0.06	3.6 ± 0.2‖	4.3 ± 0.3
Control		103 ± 8	2.44 ± 0.02	4.9 ± 0.4	5.0 ± 0.6
Alcohol	2	101 ± 8#	2.18 ± 0.04	4.3 ± 0.2‖	4.8 ± 0.2
Control		124 ± 9	2.25 ± 0.06	5.2 ± 0.1	5.3 ± 0.3
Alcohol	4	73 ± 9‖	2.81 ± 0.02	5.2 ± 0.6	4.1 ± 0.2 ¶
Control		132 ± 11	2.89 ± 0.04	6.8 ± 0.6	6.0 ± 0.3
Alcohol	8	103 ± 4‖	2.86 ± 0.04	6.1 ± 0.5	6.4 ± 0.3
Control		127 ± 6	2.89 ± 0.02	6.4 ± 0.5	6.7 ± 0.2
		Substrate: 1-glycerophosphate			
Alcohol	0	28 ± 2§	1.45 ± 0.06	1.9 ± 0.1#	1.7 ± 0.1
Control		19 ± 1	—	1.5 ± 0.1	—
Alcohol	1	28 ± 2	—	1.5 ± 0.1	—
Control		28 ± 2	—	1.4 ± 0.1	—
Alcohol	2	30 ± 3	—	1.6 ± 0.1	—
Control		26 ± 1	—	1.4 ± 0.1	—
Alcohol	4	22 ± 3	—	1.8 ± 0.1	—
Control		20 ± 2	—	1.6 ± 0.1	—

* Mean values of 4 animals ± SE.
† Ratio of respiratory rate in the presence of ADP vs the respiratory rate before ADP addition.
‡ Ratio of respiratory rate in the presence of ADP vs the respiratory rate after the expenditure of ADP.
§ $P < 0.05$.
‖ $P < 0.025$.
¶ $P < 0.001$.
# $P < 0.01$.

Comparing the results at 0 time with those obtained after the regression period was completed (Day 8), it is clear that the respiratory activity of mitochondria from the alcohol animals was almost doubled. The oxidative phosphorylation was considerably improved, since the values from Day 8 were significantly higher than those from Day 0 (malate–glutamate: $P < 0.001$; succinate: $P < 0.05$). It is interesting that the mitochondrial respiration in State 3 was also significantly improved in the control animals after the 8-day regression period was finished (malate–glutamate: $P < 0.005$; succinate: $P < 0.05$).

Discussion

The abnormal morphologic and biochemical data obtained from liver mitochondria of animals of the alcohol group during the alcohol treatment period were almost similar to those reported previously.[2,4,12-16] Particularly interesting was the 50% increased 1-glycerophosphate oxidation in liver mitochondria from the alcohol-fed rats. This observation is in agreement with previous reports showing increased 1-glycerophosphate supported oxygen uptake [17] and increased 1-glycerophosphate dehydrogenase activity [19] in ethanol-fed animals. However, our results differ from the results of other investigators [15,20] who failed to show increase in either oxygen uptake with 1-glycerophosphate or glycerophosphate dehydrogenase activity, in all likelihood due to the short period of alcohol intake which did not allow the fully adaptive physiologic response to ethanol.

As soon as ethanol was removed from the diet there were a marked recovery of the morphologic and biochemical alterations of liver mitochondria. In only 2 days of abstinence the number and size of megamitochondria were markedly reduced. The respiratory activities were also practically normal. After the 8 days of abstinence, the hepatic ultrastructure and the functional determinations showed an almost complete regression, although it was still possible to detect a few megamitochondria in the liver sections and a slight reduction in the ADP-activated respiration.

Since an improvement in the mitochondrial respiratory activities were also demonstrated in rats from the control group, the normalization of liver mitochondria in the alcohol group may have been the consequence of two combined processes: alcohol abstinence and nutritional enhancement. In this regard, the increase in body weights observed in both groups during the second part of the experiment shows that the regimens consumed during the ethanol-feeding period were abnormally low in their protein content.

According to the morphologic evidence, it seems clear that the way in which mitochondrial normalization takes place is based on partition of

these organelles. It has been claimed that partitioned mitochondria signify organelle division.[21,22] They have been described in liver mitochondria under different experimental conditions [21-26] and in a variety of other cells as well.[27-29] It is, of course, difficult to demonstrate solely by electron microscopy that mitochondria division has actually taken place. Nevertheless, in the presence of reductions in size and increases in mitochondrial population, and in the absence of redundant mitochondrial membranes or mitochondrial breakdown, an alternative interpretation is not readily apparent.

It seems paradoxic that during the first 24 hours of abstinence the number of mitochondria should not have increased. On the contrary, their number was even reduced. This apparent contradiction may be explained by the morphologic aspects of the mitochondria. During the alcohol-consuming period, these organelles were markedly distorted in their configuration. In such a case, the bidimensional figure provided by the electron microscope and used for planimetric measurement may give, in the same plane, different sections of the same tortuous mitochondria. If this was so, the true numerical density of these organelles may be below the figures obtained. Since the first change actually detected during the alcohol withdrawal was the normalization of the mitochondrial configuration, the reduction in the numerical density observed during the first day of abstinence may simply be the planimetric evidence of this normalization.

During the regression period a marked improvement was also observed in the number of mitochondrial cristae. For this process to take place, the mitochondrial protein synthesis, described as markedly reduced during chronic alcoholism,[3,5,15,30] must have been restored to its normal level. The cytoplasmic biosynthetic mechanism may also be functionally reactivated, since only a very small fraction of the total mitochondrial components are dependent on the mitochondrial genome.[31] In this regard, an observation of great interest is the strategic location of the rough endoplasmic reticulum between the apparently dividing zones or encircling the organelles. The highly suggestive images described here may be the morphologic expression of the transfer of enzymes between the two membranes or even the apposition of preassembled molecules to constitute parts of the outer mitochondrial membrane. It is pertinent to recall that the phospholipids and protein components of this outer membrane do not depend on the mitochondrial biosynthetic mechanism.[31] Moreover, the exchange of molecules between both endoplasmic reticulum and outer mitochondrial membranes has been repeatedly demonstrated.[32,33]

Besides the return of giant mitochondria to normal size by repeated

division based on partition and organelle constriction as indicated by our data, we cannot rule out other forms of fission involving progressive medial attenuation, as was described during recovery from cuprizone intoxication [22] or galactoflavine treatment.[34] The marked elongations and tortuousity induced in mitochondria by the long-term intake of ethanol made it almost impossible to detect an increment in mitochondrial division based on this mechanism. In fact, some of the images found suggest that this process may also have taken place.

It has been postulated [28,35,36] that the mode of formation of megamitochondria seems to involve organelle fusion as well as growth. If mitochondrial fusion proceeds during the alcohol feeding at a rate faster than that of division, then it should inevitably lead to organelle enlargement. Therefore, the giant conformations of liver mitochondria in chronic alcoholism may be produced by any of the following mechanisms: a) an increased mitochondrial fusion of otherwise normal organelles; b) a limited ability to divide with a normal or exaggerated growth; and c) a combination of these. The observation of numerous bizarre mitochondrial configurations, with polarization of their cristae in the narrowest portion of the organelle or below a large crista which clearly delimited different mitochondrial portions, suggests that the mitochondrial fission may be impaired.

According to the results presented here, mitochondrial enlargement could be another expression of the effect of alcohol on mitochondrial protein synthesis, which may be added to the decrease in enzymes partly dependent on the mitochondrial genome (cytochrome oxidase, cytochromes a–a_3 and b).[3,5,15] In this sense, ethanol has an effect on the mitochondria wholly similar to that described for cloramphenicol or other inhibitors of mitochondrial protein synthesis.[37,38]

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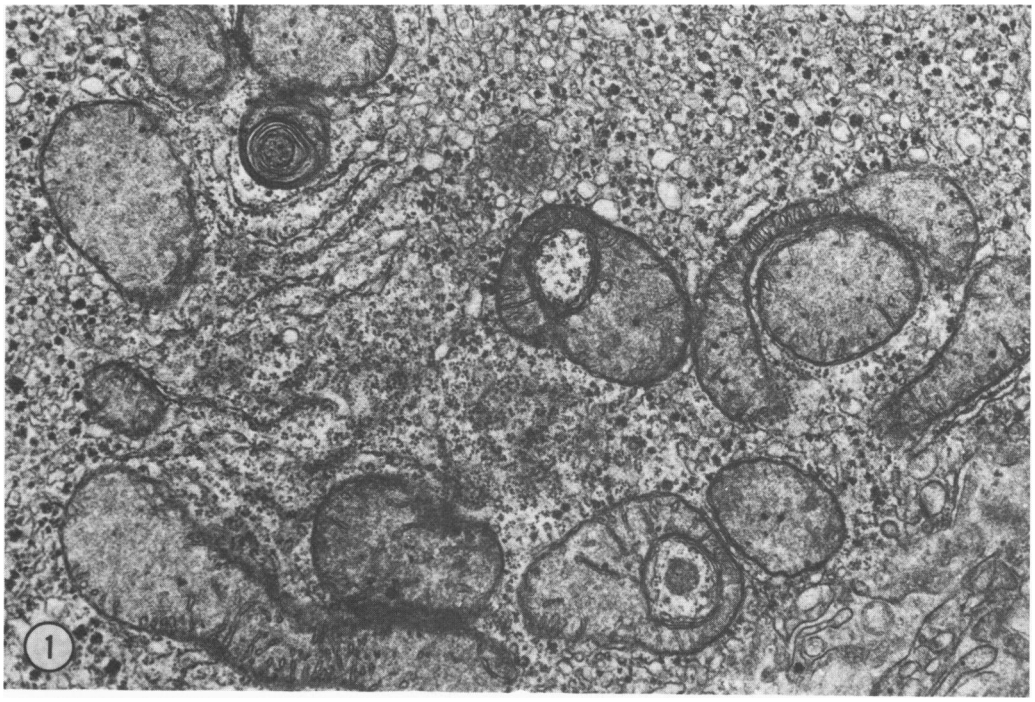
[*Illustrations follow*]



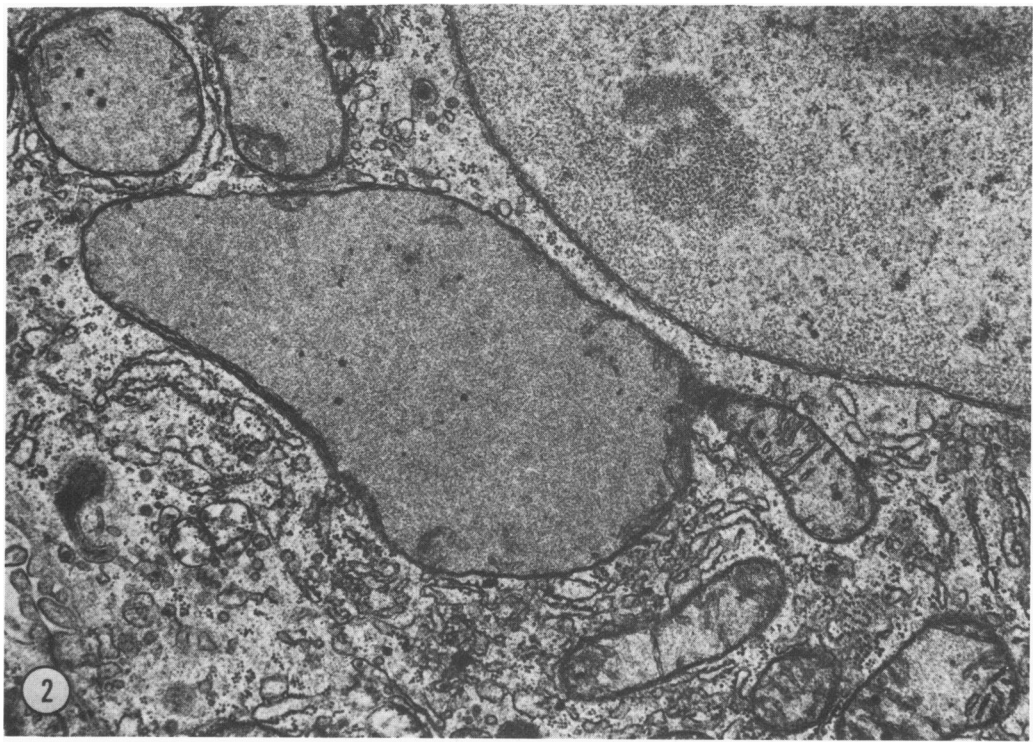


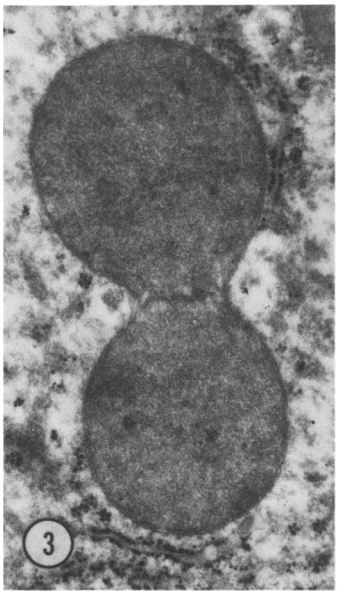
Figure 1—Configurational alterations of liver mitochondria from animals fed the alcohol regimen during 6 months. The cristae are shorter and diminished in number except in the narrow portions of the organelles. (Lead citrate, × 12,800) **Figure 2**—Marked mitochondrial enlargement (megamitochondria) in the liver of rats fed the alcohol regimen during 6 months. (Lead citrate, × 12,800)

Figure 3—A dividing mitochondria of normal size. The organelle is traversed by a median partition and shows a narrowing at the level of partition. 6 months ethanol, 1 day recovery. (Uranyl acetate-lead citrate, × 15,000)

Figure 4—A dividing mitochondria of normal size. The partition septum is in direct continuity with the inner mitochondrial membrane. 6 months ethanol, 1 day recovery. (Uranyl acetate-lead citrate, × 15,000)

Figure 5—A dividing mitochondria of normal size. Marked rarefaction at the level of partition. 6 months ethanol, 1 day recovery. (Uranyl acetate-lead citrate, × 15,000)

Figure 6—Several dividing mitochondria (*arrows*) are apparent in this single cell. 6 months ethanol, 2 days recovery. (Lead citrate, × 13,800)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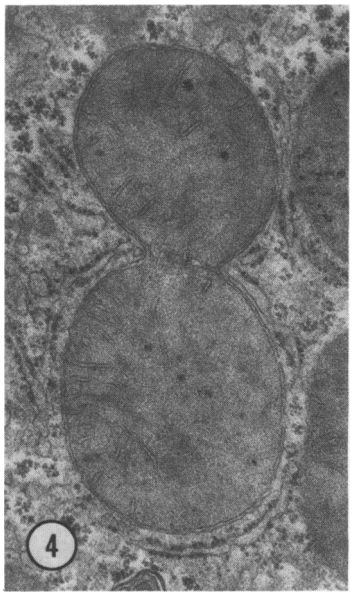
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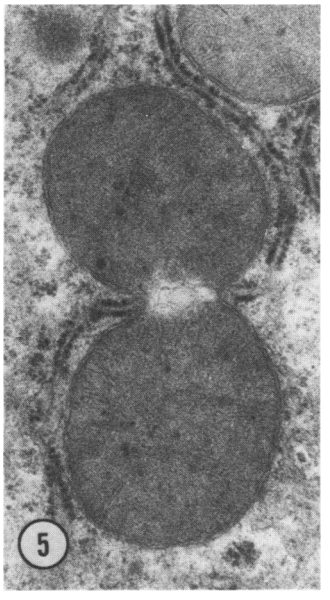
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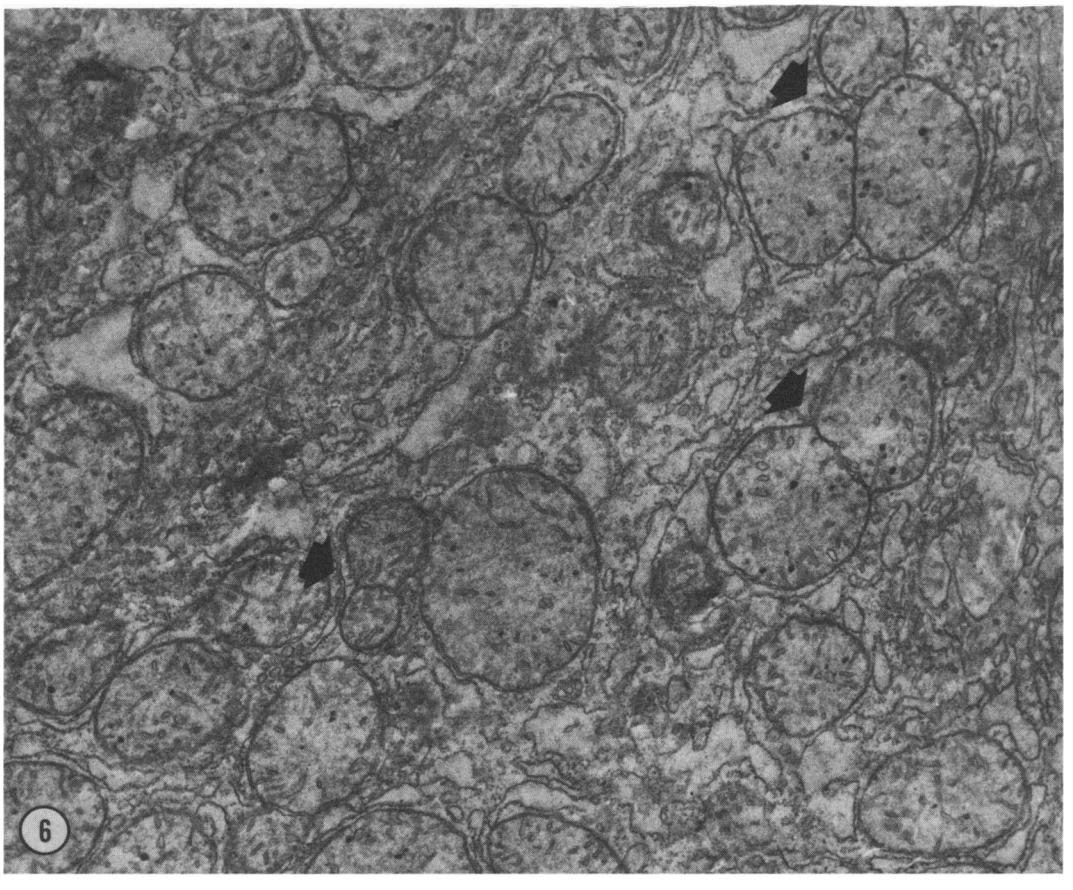
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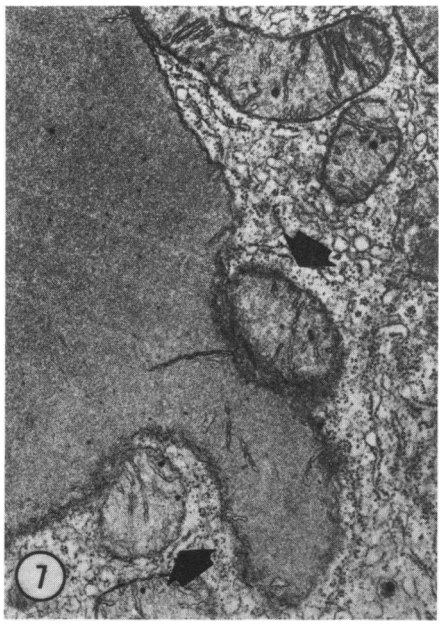
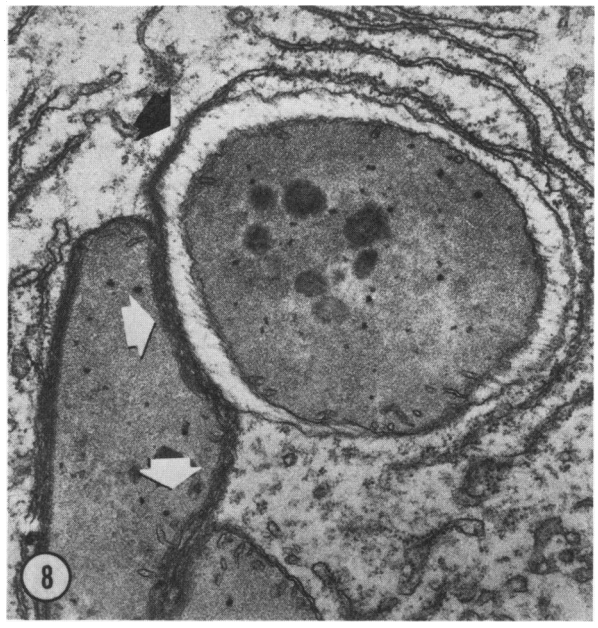
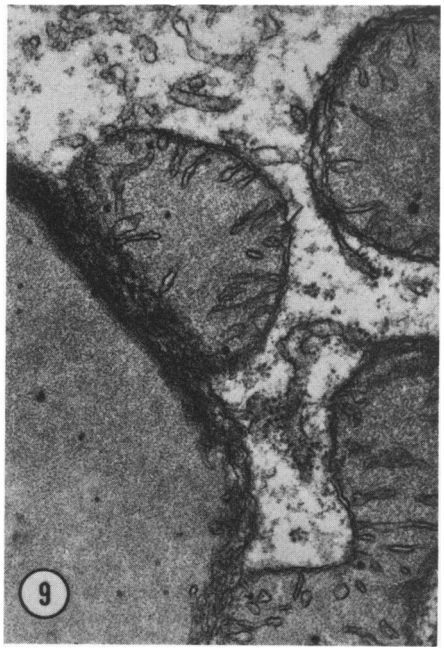






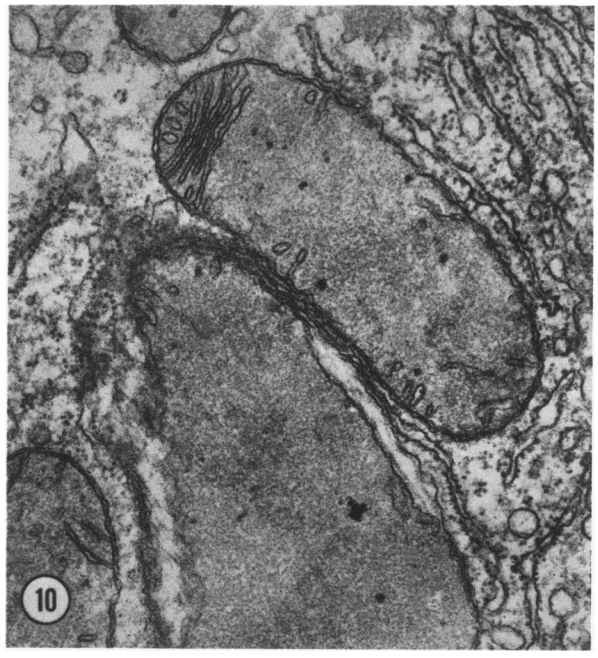
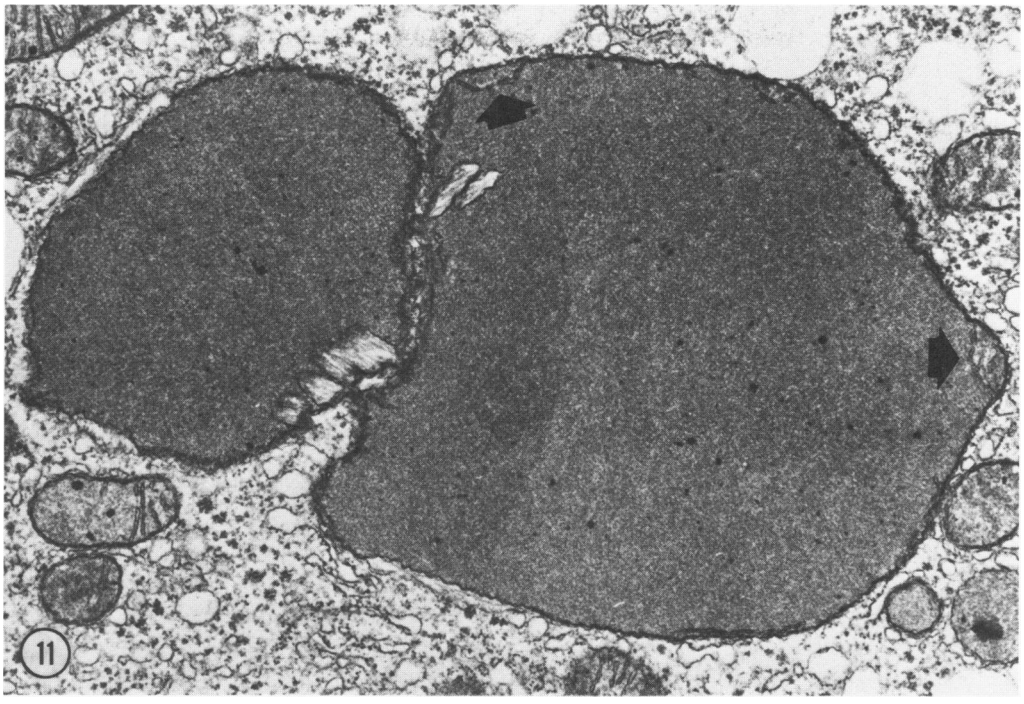


Figure 7—A dividing megamitochondria. The elongated, transversely oriented crista at the base of a small protrusion probably represents an early stage in the formation of a partition. The *arrows* indicate two probably divided mitochondria with normal aspect of their cristae. 6 months ethanol, 1 day recovery. (Lead citrate, × 11,000) **Figure 8**—Typical attachment of membranes in partition areas (*white arrows*). The outer mitochondrial space is widened by the presence of helical filaments; some osmiophilic bodies are also present in the matrix. The *black arrow* indicates the aposition of rough endoplasmic reticulum to the outer mitochondrial membrane. 6 months ethanol, 2 days recovery. (Lead citrate, × 13,800) **Figure 9**—High magnification of small mitochondria buds detached from a megamitochondria by a stack of parallel membranes. The number and length of cristae in the mitochondrial buds are almost normal. 6 months ethanol, 2 days recovery. (Lead citrate, × 20,000) **Figure 10**—Rough endoplasmic reticulum interposition between a megamitochondria (*bottom*) and a normal-sized mitochondria (an already divided mitochondria ?). Note detachment of ribosomal granules in the intermixed area and the helical filaments in the outer mitochondria space. 6 months ethanol, 2 days recovery. (Lead citrate, × 20,000)



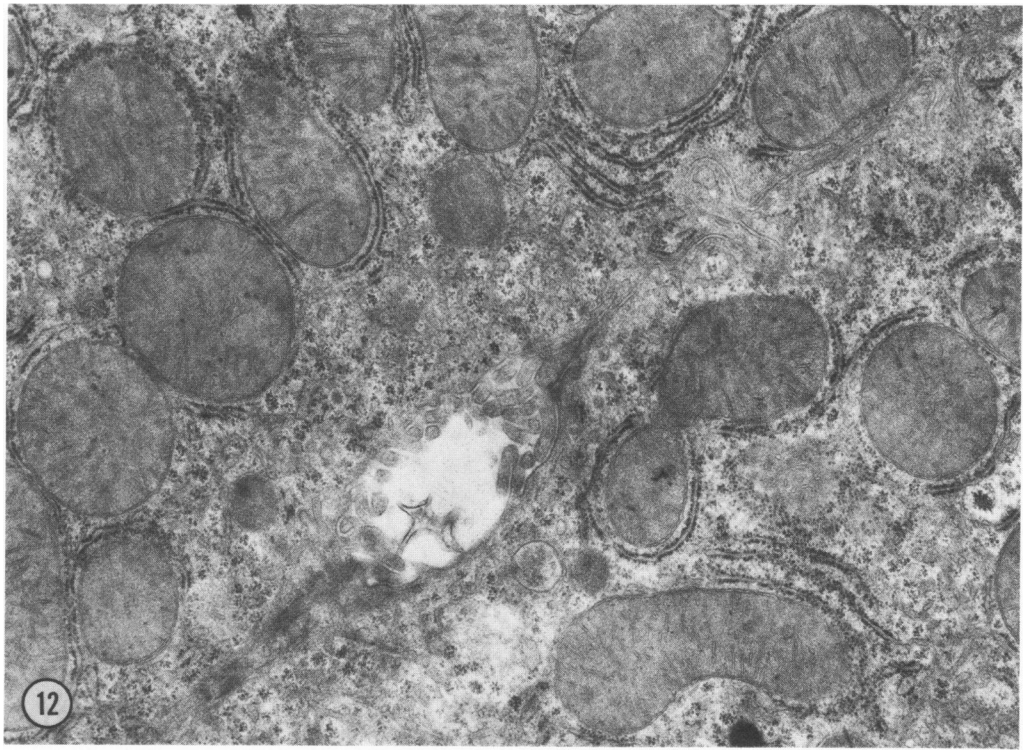


Figure 11—A dividing megamitochondria after 8 days of recovery. Note helical filaments and fairly characteristic transversely oriented long cristae conforming a dividing area (*arrows*). (Lead citrate, × 13,800) **Figure 12**—Normal aspects of mitochondria from rats after 8 days of abstinence. The rough endoplasmic reticulum appears encircling these organelles. (Uranyl acetate–lead citrate, × 20,000)

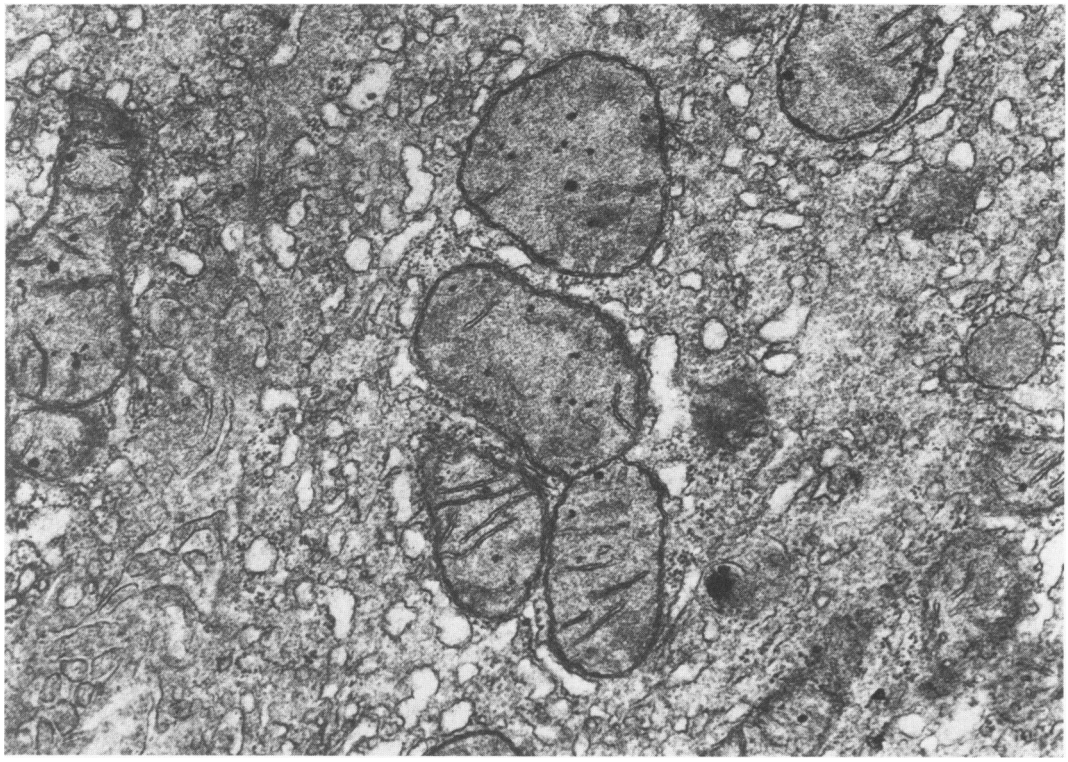


Figure 13—A dividing mitochondria observed in a hepatic cell from a rat of the control group. (Lead citrate, × 10,000)