# CHRONIC ALCOHOLISM WITH FATTY METAMORPHOSIS OF THE LIVER

## Mitochondrial Alterations in Hepatic Cells

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Although the ultrastructural changes in liver cells of experimental animals have been studied extensively, relatively little information is available regarding comparable changes in human liver. The degree of specificity and the pathogenesis of many of the structural alterations of mitochondria in animal liver cells are still obscure. Morphologic changes in human liver mitochondria are even less clearly understood.

The purpose of this paper is to report prominent mitochondrial alterations in chronic alcoholic patients whose only consistent histologic abnormality was the presence of moderate to marked fatty metamorphosis. The nonspecificity of these alterations is indicated and their pathogenesis is briefly discussed.

## Material and Methods

Liver biopsy was performed at the patients' bedside with local anesthesia; a Menghini needle was used. The tissue was aspirated into saline and expelled onto a paraffin plate. One half of each specimen was minced immediately in cold 2 per cent osmium tetroxide buffered with s-collidine.[1] Tissue was obtained initially from 7 patients. In 2 of these biopsy was made a second time after 3 months of adequate dietary intake without alcohol, to determine possible reversibility of the lesions. Also available for comparison were samples of normal human liver, liver in subacute viral hepatitis, neonatal hepatitis, adult diabetes and in 2 cases of primary carcinoma of the liver. (One case of Bantu hepatoma was kindly supplied by Dr. W. J. Pepler, Johannesburg, South Africa.)

Tissue for electron microscopy was embedded in Epon 812,[2] and sections were cut on a Porter-Blum microtome with glass knives. Sections were mounted unsupported on titanium grids and stained with either uranyl acetate or with lead.[3] They were examined with an RCA 3G electron microscope employing an objective aperture of 35 to 40 $\mu$. Micrographs were taken at original magnifications ranging from 2,400 to 28,000. Tissue for light microscopy was fixed in neutral buffered formalin, embedded in paraffin and stained with hematoxylin and eosin, phosphotungstic acid hematoxylin and by the periodic acid-Schiff technique with and without diastase digestion.

For additional comparison, liver from the following animals was also examined: (1) Eight rats weighing 120 to 150 gm. were given 0.6 cc. of 75 per cent ethanol per 100 gm. of body weight by intragastric tube and sacrificed at 1, 2, 8 and 24 hours. (2) Five rats weighing 300 to 450 gm. were given 0.8 cc. of 75 per cent ethanol per 100 gm. body weight by intragastric tube 5 days per week for 6 weeks and sacrificed

This study was supported in part by United States Public Health Service grant CA-06792.

Accepted for publication, November 12, 1963.

at the end of that time. (3) Five rats had fatty livers following a diet deficient in choline (3 to 5 months). (4) Eight rats remained on a diet deficient in protein (4 per cent protein) for 12 weeks.

## Results

The patients sought medical attention because of ailments unrelated to cirrhosis, such as hypertension and back pain in 2 instances, or for relief of nonspecific symptoms possibly related to cirrhosis, such as anorexia, fainting spells, anxiety, weakness and weight loss. In each case, the history of excessive alcohol intake for several years and the presence of hepatomegaly were indications for liver biopsy. Duration of excessive alcohol intake ranged from 1 to 5 or more years.

The clinical data and laboratory findings in each of the 7 patients are summarized in Table I. It is apparent that although there were scattered derangements in serum constituents in each patient, no consistent abnormality was found.

Table I

SUMMARY OF LABORATORY FINDINGS AND CLINICAL DATA *

| | Patient no. | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Age | 57 | 56 | 59 | 52 | 70 | 49 | 27 |
| Sex | M | M | M | F | M | M | M |
| Hepatomegaly | ++ | ++ | ++ | ++++ | ++ | ++ | ++ |
| Thymol turbidity | 5 | 5 | N† | 4 | N | 7 | 23 |
| Serum glutamic oxalacetic transaminase (GOT units) | N | 58 | N | 139 | N | 242 | 675 |
| Total protein (gm.) | N | N | N | N | N | 4.9 | N |
| Bromsulphalein excretion (% retained at 45 minutes, using 5 mg. per kg. dose) | 27% | 24% | 17% | N | N | 12% | N |
| Total bilirubin (mg. %) | N | 1.3 | N | 3.7 | N | 1.2 | N |
| Cephalin cholesterol flocculation (Hangar) | N | 2+ | N | N | N | N | 3+ |
| Serum albumin (gm.) | N | N | N | N | 2.9 | N | N |
| Serum globulin (gm.) | N | N | N | N | 4.5 | N | N |

* Only abnormal values are recorded.
† N = normal.

## Light Microscopy

Sections of liver showed varying degrees of fatty metamorphosis ranging from small intracytoplasmic vacuoles (Fig. 1) to the formation of fatty cysts (Fig. 2). Cirrhosis, necrosis, ductular proliferation and Mallory bodies were not present. The details of the histologic features are summarized in Table II.

## Electron Microscopy

### *Normal Human Liver*

In general, the fine structure of normal human liver resembles that of the rat or mouse (Fig. 3). The cristae mitochondriales in human liver are often fewer, shorter and less well developed than in the rat or mouse. One to 5 dense granules measuring approximately 500 Å are commonly

Table II

HISTOLOGIC FEATURES OF LIVER BIOPSIES

| No. | Fibrosis | Inflammation | Necrosis | Cholestasis | Fat | Miscellaneous |
|---|---|---|---|---|---|---|
| 1 | A* | Few small foci of polys | A | A | +++ | Circular eosinophilic bodies in cytoplasm of several cells |
| 2 | A | A | A | A | ++++ | Mononuclear infiltrate in portal tracts |
| 3 | A | A | A | A | ++ | |
| 4 | A | A | A | A | ++++ ++ | |
| 5 | Increased fibrous tissue in portal tracts | A | A | A | | Granular eosinophilia in few cells |
| 6 | A | A | A | A | ++ | |
| 7 | A | A | A | A | ++ | Mononuclear infiltrate in portal tracts |

* A = absent.

seen in the mitochondrial matrix. Microbodies have a homogeneous interior, and usually do not possess irregular or circular dense nucleoids. Glycogen is present in clusters of various size, ranging from 100 to 500 Å, composed of smaller subunits of 40 to 80 Å. Granular endoplasmic reticulum is distributed as near-parallel arrays or as semicircular profiles closely adjacent to mitochondria. Golgi apparatus, peribiliary dense bodies and desmosomes are prominent.

### *Livers with Fatty Metamorphosis*

*Mitochondrial Inclusions.* The most conspicuous mitochondrial abnormality in fatty livers was the presence of inclusions composed of strata of dense parallel lines approximately 120 Å wide, separated by spaces approximately 200 Å wide (Fig. 4). These highly ordered structures resembled crystalline inclusions [4,5] or so-called "myelin degenera-

tion" of mitochondria.[6] One or more inclusions were present in the majority of mitochondria in most sections (Figs. 5 and 6) and did not appear to be more numerous in any portion of the cell or in any zone of the hepatic lobule. The number of mitochondria with inclusions bore no apparent quantitative relation to the severity of fatty metamorphosis. The inclusions measured up to 3 $\mu$ in length, were located at random throughout the mitochondrial matrix and were oriented in variable planes such that they could be observed both in longitudinal and cross section within a single mitochondrion (Fig. 7). In cross section they appeared as roughly parallel solid dots separated by lucent interspaces (Insert, Fig. 7). Mitochondria containing inclusions were often but not invariably enlarged up to 6 $\mu$, with irregular margins and a multilobate appearance. In the interior of the mitochondria, the peripheral cristae appeared reduced in number while numerous sinuous, apparently unattached cristae were present in the matrix. Simultaneously, there appeared in the matrix annular structures with a dense interior (Fig. 4).

The cristae frequently bore a close spatial relationship to the inclusions (Fig. 8). In many areas, at regular intervals, short linear segments or, apparently, cross-sectioned profiles of cristae were present along one or both margins of an inclusion (Figs. 9 to 11). In other areas cristae appeared to merge at angles with the dense lines. Occasionally, cristae appeared subjacent to the inclusions.

A small proportion of enlarged mitochondria exhibited round or oval cavities in their matrix. These measured up to 0.4 $\mu$ and contained a granular material of faint electron density (Fig. 12). Often, enlarged mitochondria also contained dense bodies that measured up to 0.15 $\mu$ and had a somewhat laminated interior structure (Figs. 14 to 16). The mitochondrial matrix was otherwise largely filled with faint, poorly delineated structures resembling cristae (Fig. 13). In a few mitochondria, the cristae were disposed concentrically or in parallel stacks along a segment of the peripheral mitochondrial membranes or in the interior of the mitochondria (Fig. 17).

*Additional Changes.* There was rather uniform vacuolar dilatation of cisterns of both granular and agranular endoplasmic reticulum. Fat vacuoles and fatty cysts were numerous. There were no apparent alterations in other cytoplasmic organelles or in the nucleus.

### *Animal Studies*

Administration of alcohol to rats and the production of fatty livers by choline- or protein-deficient diets failed to induce mitochondrial changes resembling those in human liver.

## Discussion

Emphasis on mitochondrial abnormalities in human liver and the description of crystalline inclusions in mitochondrial matrix have been reported in acute alcoholic hepatitis,[7] in the formation of alcoholic hyalin,[8] in juvenile diabetes mellitus,[4] biliary obstruction and in viral hepatitis.[6,9] The presence of crystalline inclusions has also been described in the retina of the frog's eye,[10] brown adipose tissue,[11] rat thyroid[12] and in various other tissues.[13-18]

There has been some variation in the connotation of the terms "myelin degeneration," "myeloid" and "paracrystalline." The term myelin was used originally by Virchow[19] to describe the pattern of organic substances from brain and other organs, capable of forming parallel membranous structures when placed in water. The term has also been used to refer to (a) the chemical and structural configuration of the lipoprotein complexes of nerve sheaths,[20] (b) lamellar structures resulting from hydration of pure phospholipid *in vitro*,[21,22] (c) whorls of concentric cytoplasmic membranes derived from phospholipid occurring as a result of nonspecific cellular injury *in vivo*,[23,24] and (d) non-neural elements, in plant cells, that bear some structural similarity to nerve myelin.[25] In this report, the terms "myelin" and "inclusion" refer to alternating dark and light, parallel linear arrays in liver cell mitochondria.

The pathogenesis and composition of the inclusions is uncertain but the studies of Stoeckenius[22] and Fawcett and Ito[23,24] suggest a possible mechanism for their formation. Stoeckenius showed that a mixture of phospholipids (cephalin, lecithin and inositides), when hydrated *in vitro*, formed bimolecular layers of light and dense lines with regular spacing or dot patterns, depending upon the plane of section. Similarly, Fawcett showed that isolated guinea pig testicular cells, as they imbibed water, contained parallel membranous structures derived from preexistent endoplasmic reticulum membranes and possibly from phospholipid of the cytoplasmic matrix. Moreover, it has been determined that rat liver mitochondria contain 35 per cent of the total cellular lipid and that 93 per cent of the mitochondrial lipid is phospholipid.[26] Since it has been observed that phospholipids, upon hydration, have the capacity to form crystal-like structures, and since phospholipids represent a significant proportion of the chemical content of mitochondria, it appears likely that changes in the state of hydration of the liver cell, or in its mitochondria, could be accompanied by spatial rearrangement, condensation and crystallization of mitochondrial phospholipid. Alternatively, the presence of crystals might be regarded as a result of excessive

synthesis of phospholipid by mitochondria[27] or as an unmasking, by crystal formation, of mitochondrial phospholipid resulting from disintegration or autolysis of intramitochondrial components.[28,29]

We have attempted to trace the formation of inclusions on morphologic grounds. Detailed examination of the inclusions suggested that they were derived, at least partially, from the membranes of the cristae since mitochondria with inclusions frequently showed diminution in the number of cristae. Moreover, cristae often appeared to merge with the dense lines of the inclusions or frequently were lined up along one margin of the inclusion (Figs. 9 to 11). The presence of inclusions did not necessarily indicate preceding or impending cell death since they were frequently found in cells that showed no obvious retrogressive changes in the remaining mitochondria or in other organelles. Re-biopsy of two patients after a reliable history of 3 months on an adequate diet with elimination of alcohol intake showed that fatty change was no longer apparent and crystalline inclusions could not be detected in the blocks prepared for electron microscopy. The absence of inclusions upon rebiopsy could indicate that their formation was reversible. On the other hand, their absence in the tissue available for study might have been attributable to insufficient sampling.

The occurrence of crystal-like formations in several species and their presence in a variety of human diseases indicates their nonspecific nature. They are very infrequent in normal human liver and were not observed in our specimens of neonatal hepatitis, subacute hepatitis or in primary carcinoma of the liver. They were present in two cases of adult diabetes mellitus; this finding extends the observations of Laguens and Bianchi,[4] who reported them in juvenile diabetes. Although previous reports describe crystalline inclusions in cholestasis, jaundice and hepatocellular necrosis with inflammation, the patients in this study had none of these conditions. It would appear, therefore, that necrosis, bile stasis or inflammation are not necessary antecedent or coexistent conditions in the formation of inclusions. Although the livers in all patients in this study had fatty metamorphosis, the experimental induction of fatty liver by choline- or protein-deficient diets in rats was not characterized by mitochondrial alteration.

It is evident that one of the remote causes of inclusions is related, though not directly or exclusively, to the consumption of large amounts of alcohol over a prolonged period. Although alcohol has been shown to affect mitochondrial enzymes,[30,31] particularly alcohol dehydrogenase and glutamate and pyruvate oxidation,[32,33] no abnormality of mitochondrial structure was demonstrable in rats given large doses of alcohol for 6 weeks. It is possible that the effects of alcohol on mitochondria *in vivo* are mediated through intermediate nutritional factors. In this

regard, Theron, Hawtrey, Liebenberg and Schirren[34] pointed out that alterations in diet may influence size and structure of hepatic mitochondria, and Century and Horwitt[35] have shown that alterations in dietary fat can influence mitochondrial function.

A relationship of large granules in the mitochondrial matrix to cellular ionic composition is suggested by the study of Weber, Usenik and Shipp.[36] They observed dense intramitochondrial bodies measuring up to 1 $\mu$ in association with loss of cristae in the zona glomerulosa of calves with uncompensated loss of body sodium. Weiss[37] observed an increase in mitochondrial granules in duodenal cells after administration of NaCl or KCl to thirsty animals. Greenawalt, Lehninger and Rossi[38] have reported granules measuring 1,000 Å in diameter in isolated mitochondria incubated to promote uptake of ionic calcium.

The rearrangement of cristae, such as concentric orientation and peripheral or central stratification, is similar to that observed in a Bantu hepatoma,[39] in livers following ligation of the common bile duct[40] and in essential fatty acid deficiency.[41] Cavitation or development of "holes" in the mitochondrial matrix appears also to be a nonspecific degenerative change.[42]

Various forms of mitochondrial alteration in pathologic conditions have been described previously. These include enspherulation, swelling, rupture, the formation of evaginations and the appearance of budding or dividing forms. Also reported are an increase in density or focal lucency of the matrix, a decrease or increase in cristae or their abnormal configuration or orientation, and loss or increase in the size and number of matrix granules. It would appear that mitochondrial alterations in fatty metamorphosis of the liver, i.e., crystal-like inclusions, cavitation, large matrix dense bodies, and disorientation of cristae, are part of the spectrum of mitochondrial response to injury and possibly represent fundamental pathologic responses of these organelles to an altered chemical or physical milieu.

## Summary

Electron microscopic study of needle biopsy specimens from the livers of patients with moderate to marked fatty metamorphosis and evidence of excessive alcohol consumption for several years disclosed a number of abnormalities in mitochondrial structure. The most conspicuous change was the presence of crystal-like inclusions resembling "myelin degeneration," often accompanied by marked mitochondrial enlargement. Other changes included the presence of abnormally large dense bodies in the mitochondrial matrix, cavitation of mitochondria and rearrangement of cristae.

Crystalline inclusions in mitochondria may be related to alterations

in the hydration state of liver cells or to disordered phospholipid metabolism in mitochondria. They are found in a variety of human liver disorders and, like the other mitochondrial changes described in this study, probably represent a nonspecific degenerative phenomenon.

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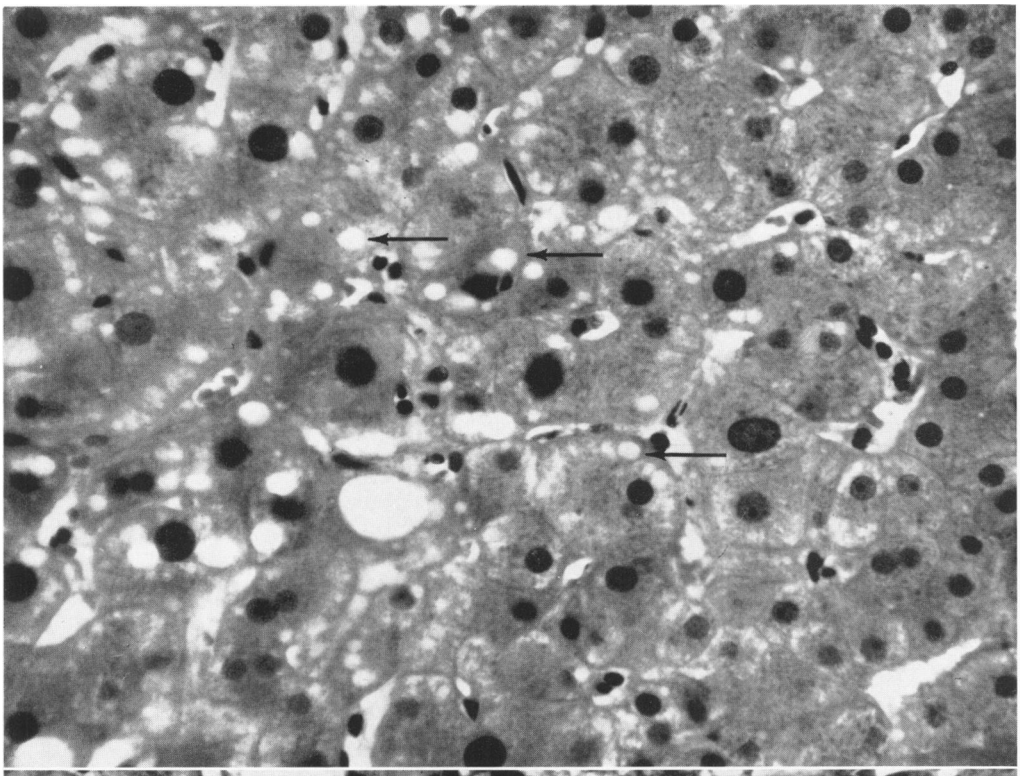
## LEGENDS FOR FIGURES

Unless otherwise stated, electron micrographs were prepared from sections stained with lead.

FIG. 1. Patient with mild fatty metamorphosis. The liver cells contain several small intracytoplasmic vacuoles (arrows) and one fatty cyst. Hematoxylin and eosin stain. × 425.

FIG. 2. A severely affected liver contains numerous fatty cysts and scattered mononuclear cells. Hematoxylin and eosin stain. × 125.



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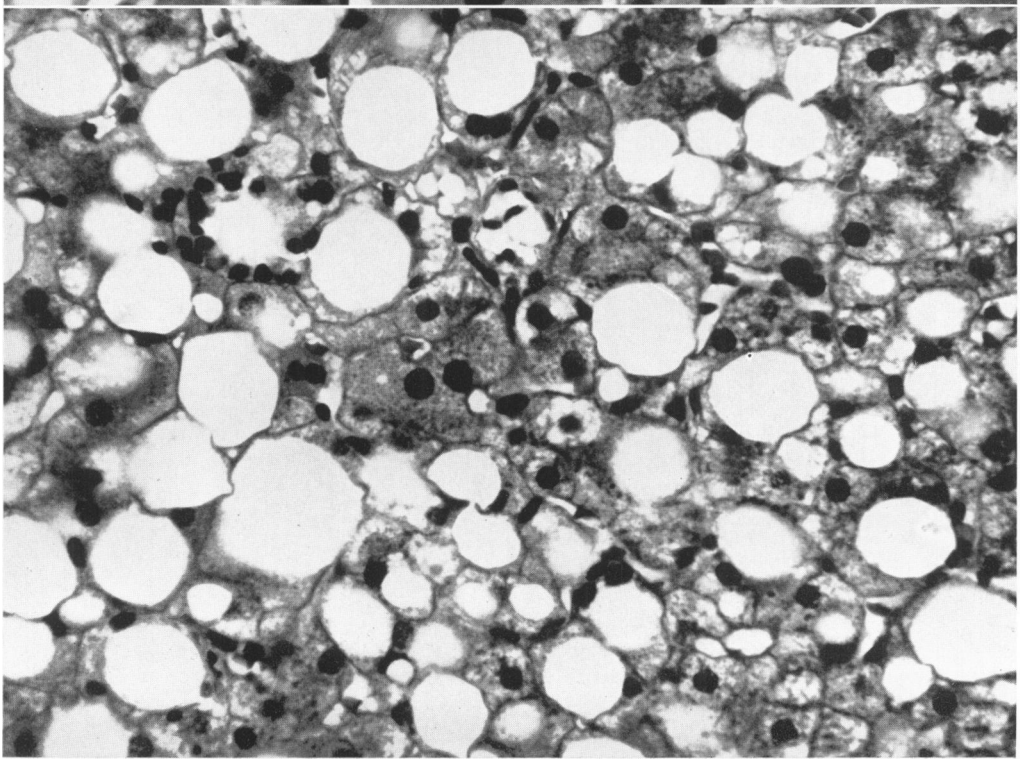

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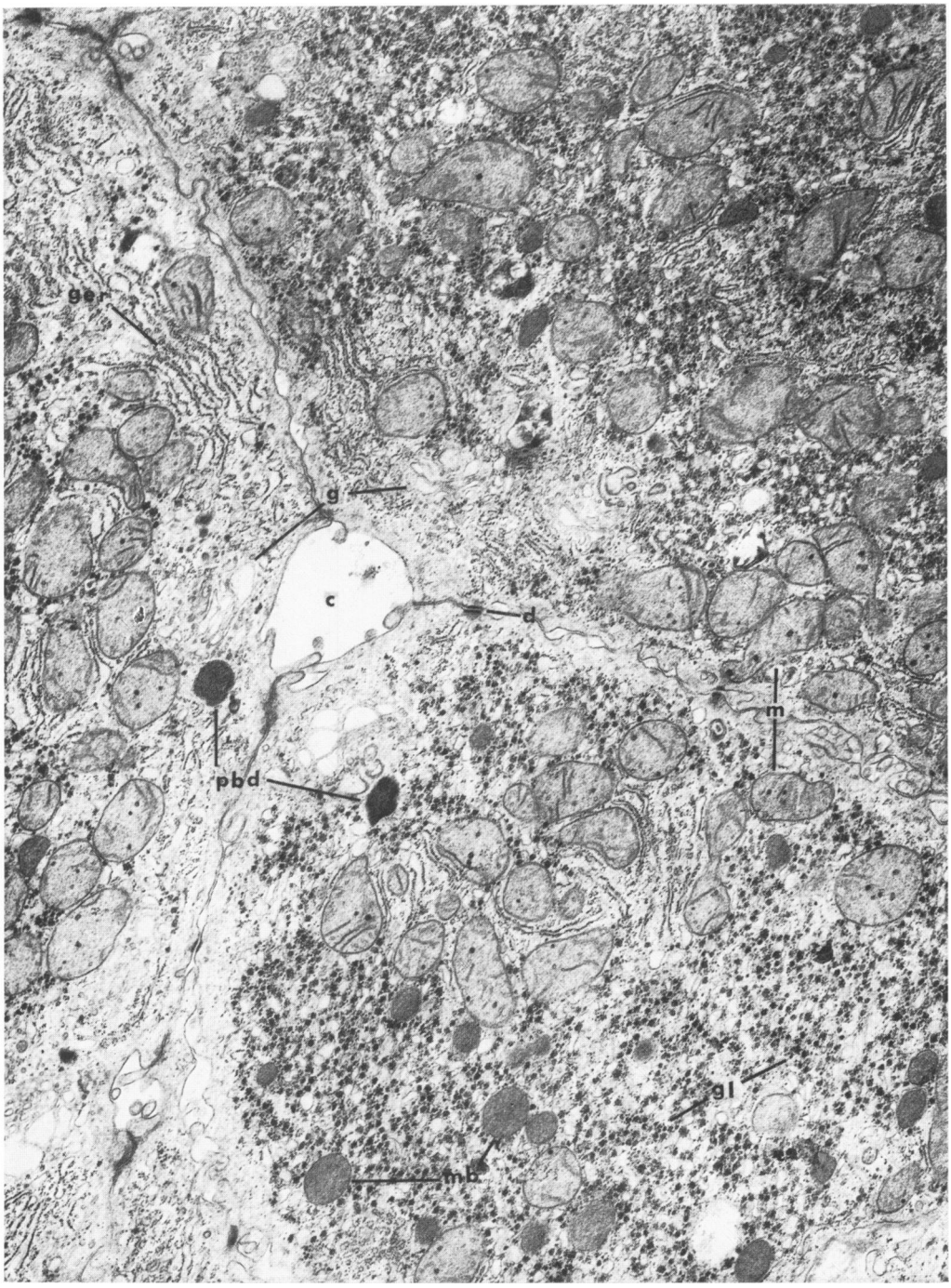
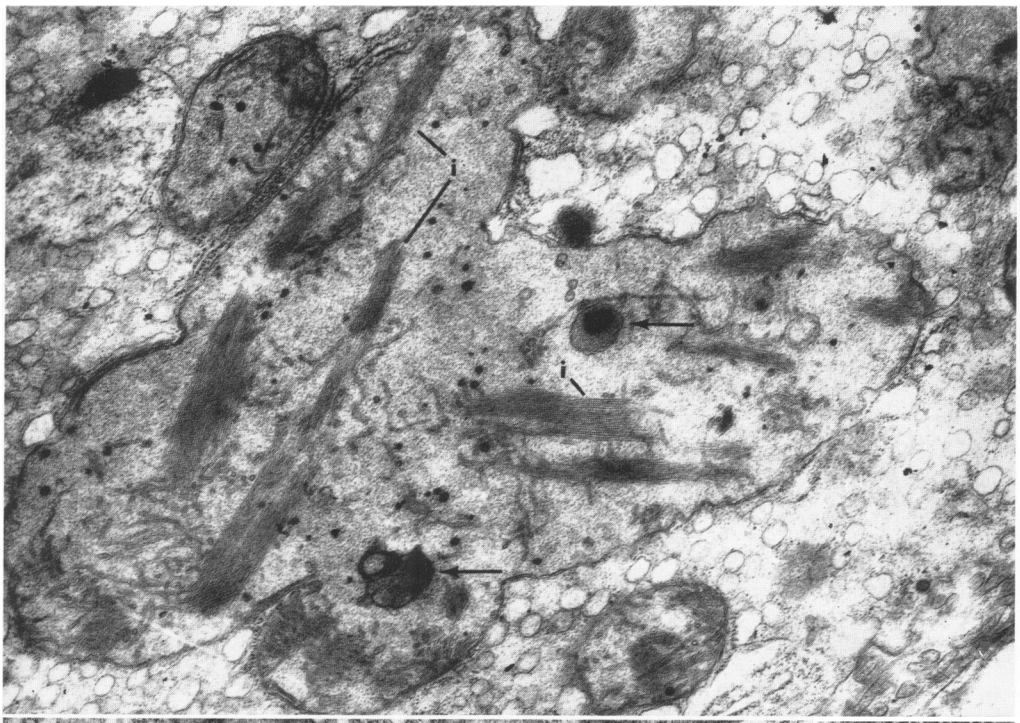



FIG. 3. Normal human liver. Numerous round, oval or irregular mitochondria are apparent (m). Arrays of granular endoplasmic reticulum, ger; abundant glycogen clusters, gl. Microbodies (mb) lack a central nucleoid. Peribiliary dense bodies (pbd), Golgi areas (g), desmosomes (d) and a bile canaliculus (c) are also illustrated. × 8,900.

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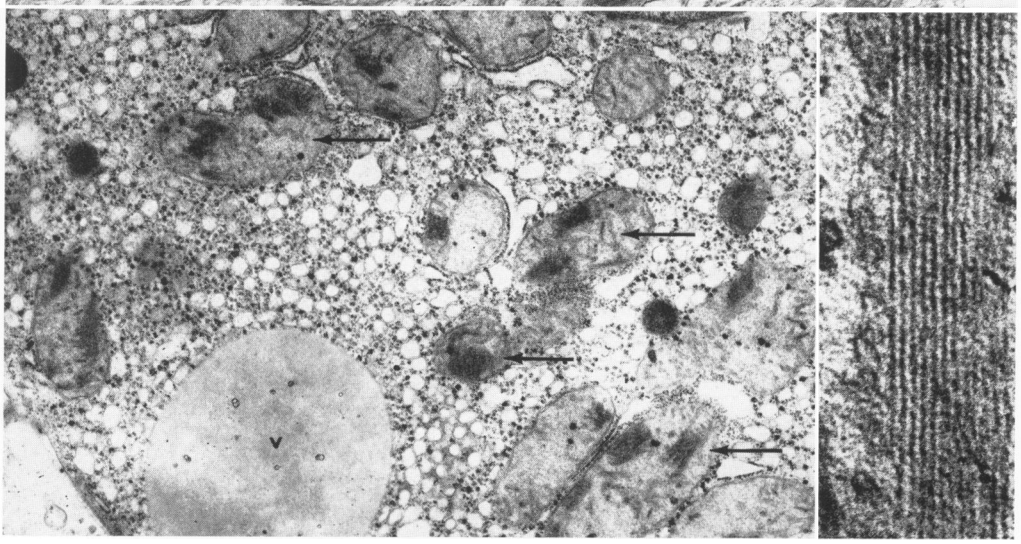


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6

FIG. 4. An enlarged mitochondrion (compare with those shown in Fig. 5) with a multilobate profile contains several crystalline inclusions (i) and annular structures (arrows), some with dense interiors. × 19,000.

FIG. 5. Several mitochondria in a single field contain inclusions (arrows). There is rather uniform vacuolar dilatation of endoplasmic reticulum. A larger cytoplasmic vacuole (v) is manifest. × 19,000.

FIG. 6. A higher magnification of an inclusion exhibits an array of dense parallel lines approximately 120 Å wide separated by lucent spaces with an average width of 200 Å. × 86,000.

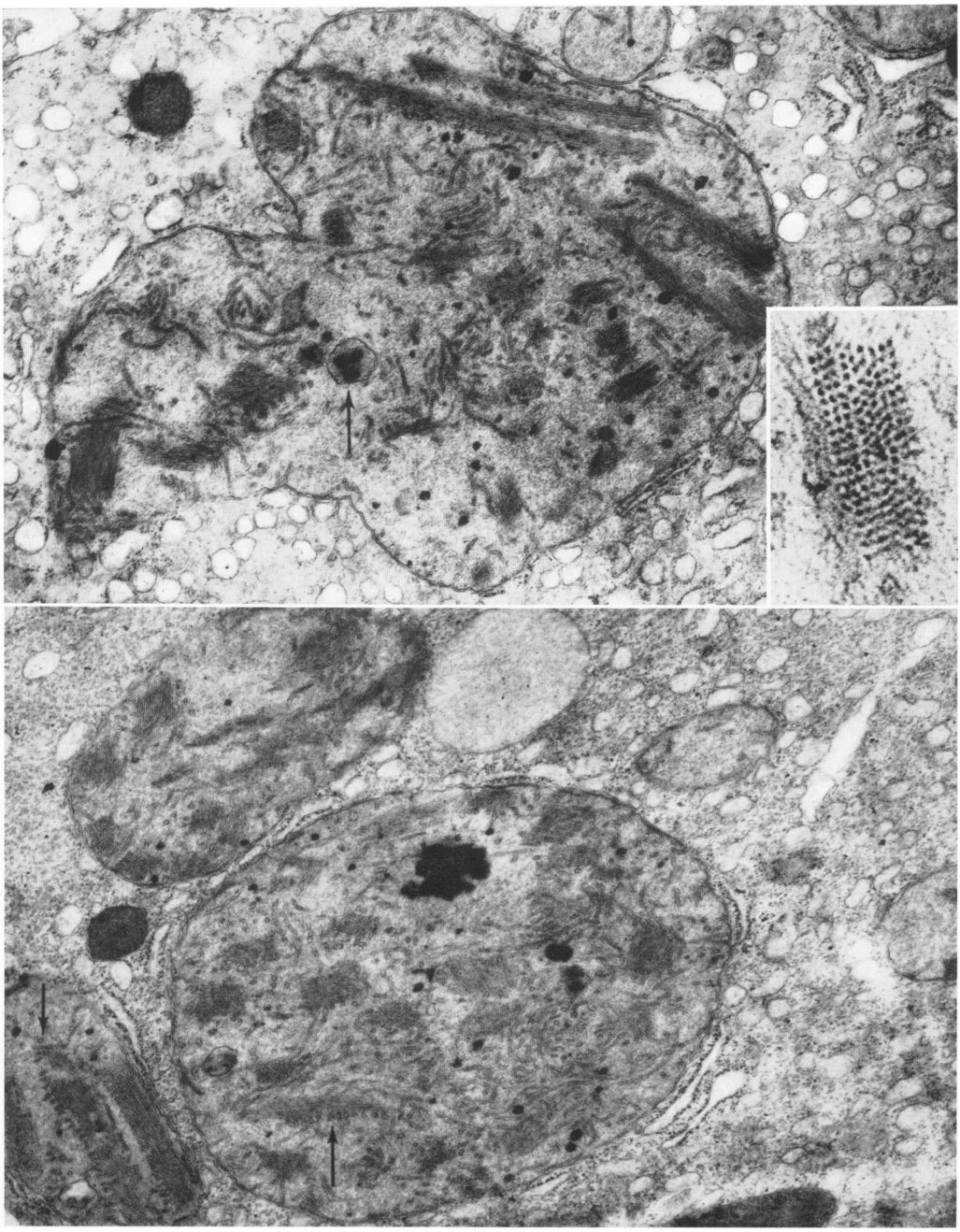
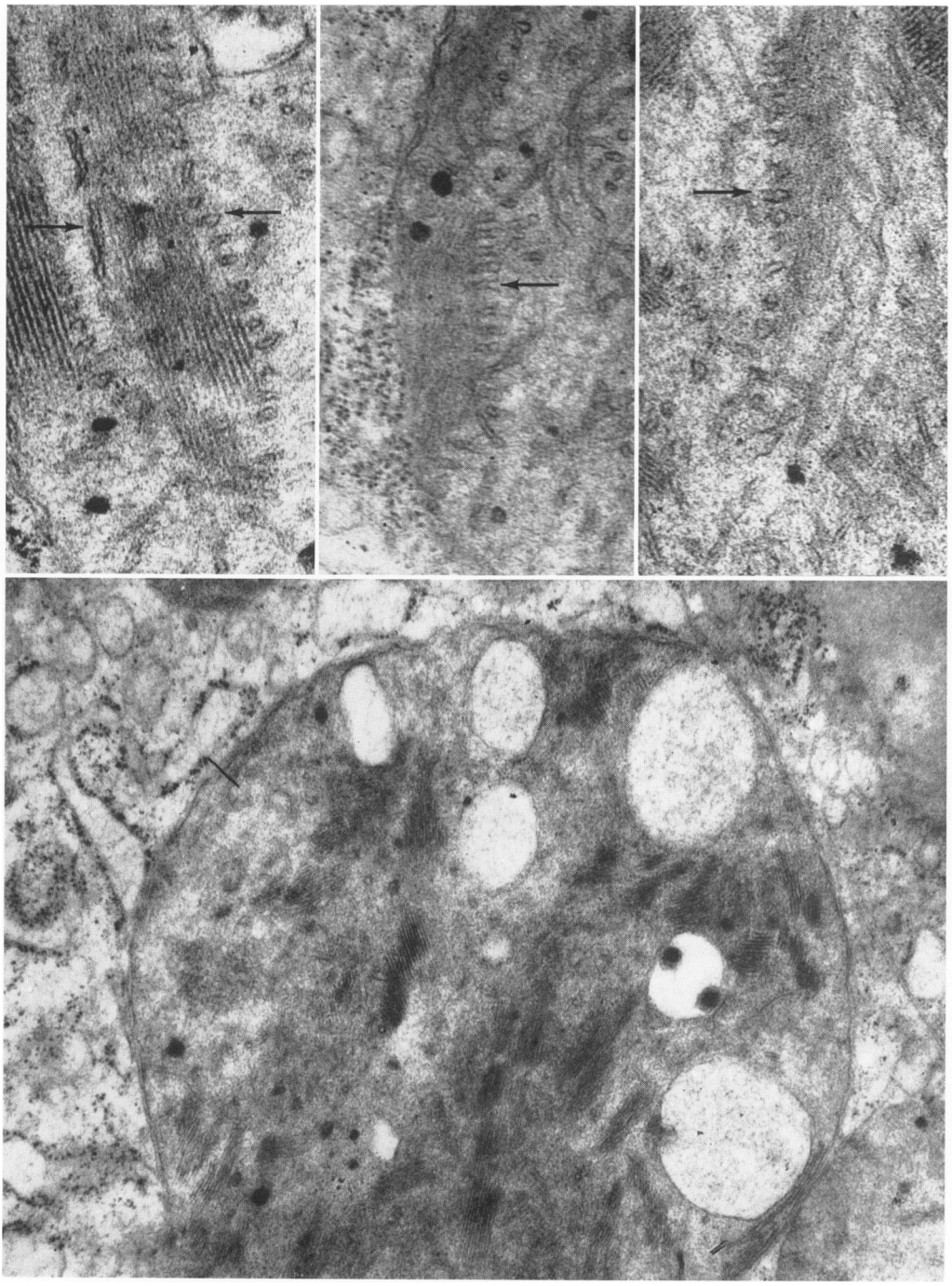


FIG. 7. An enlarged mitochondrion contains several inclusions in longitudinal section as well as the dot pattern produced by transverse section. Three matrix granules are limited by a membrane (arrow). × 19,000. Insert shows the transverse section to be composed of solid, roughly parallel dots separated by lucent interspaces. × 96,000.

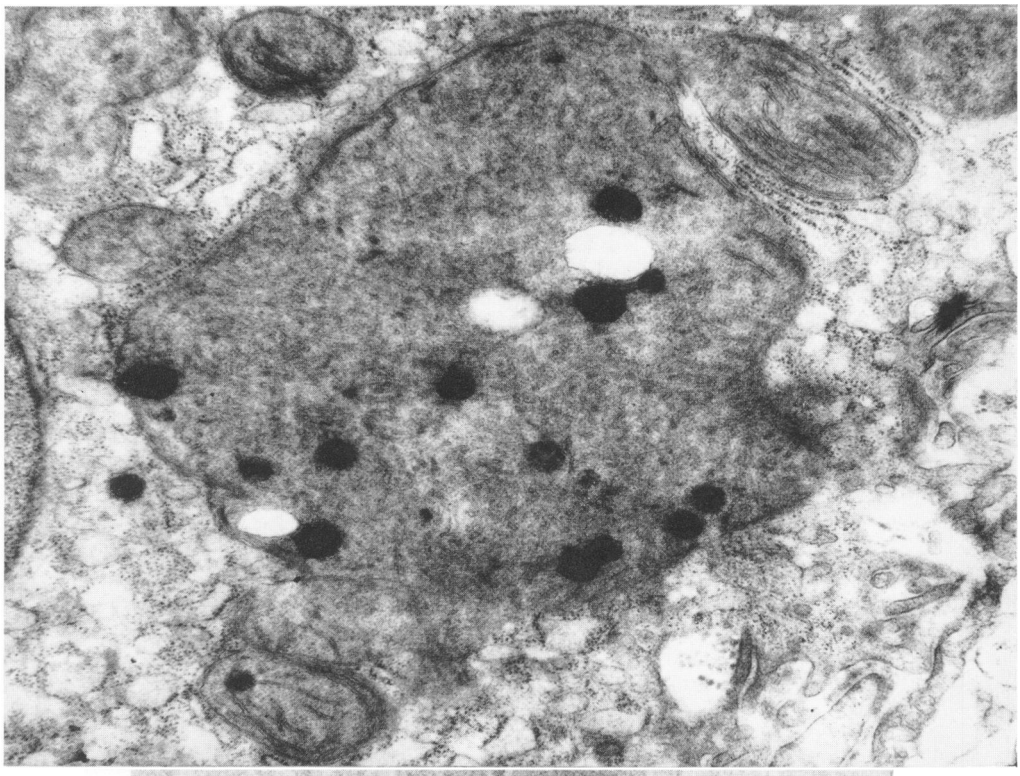
FIG. 8. Enlarged mitochondria with inclusions illustrate the close spatial relationship of fragments of cristae to the inclusions (arrows). Large, irregularly shaped granules appear in the matrix. × 19,000.




FIGS. 9 to 11. Portions of cristae (arrows) lie in close apposition to margins of the inclusions. × 67,000.

FIG. 12. A large mitochondrion exhibits oval or round cavities (up to 0.4 $\mu$) which contain dense granules or faint, fibrillar material. × 48,000.

13



14

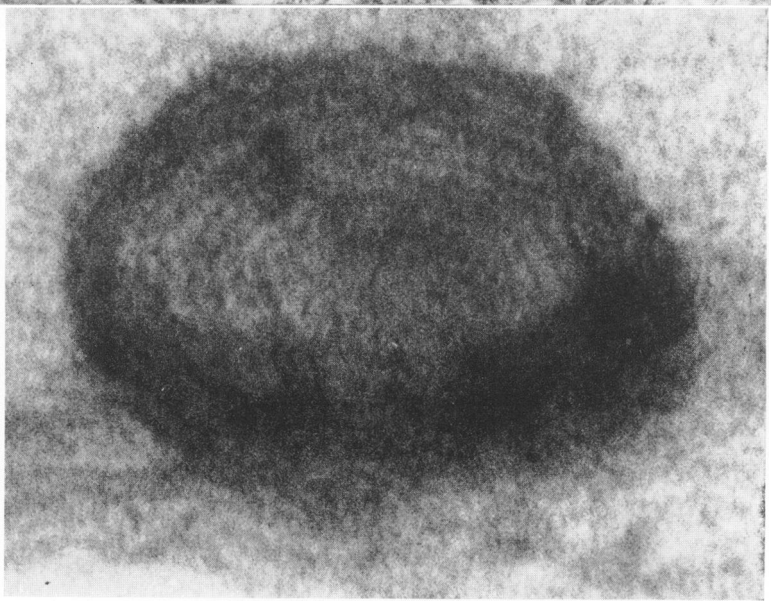
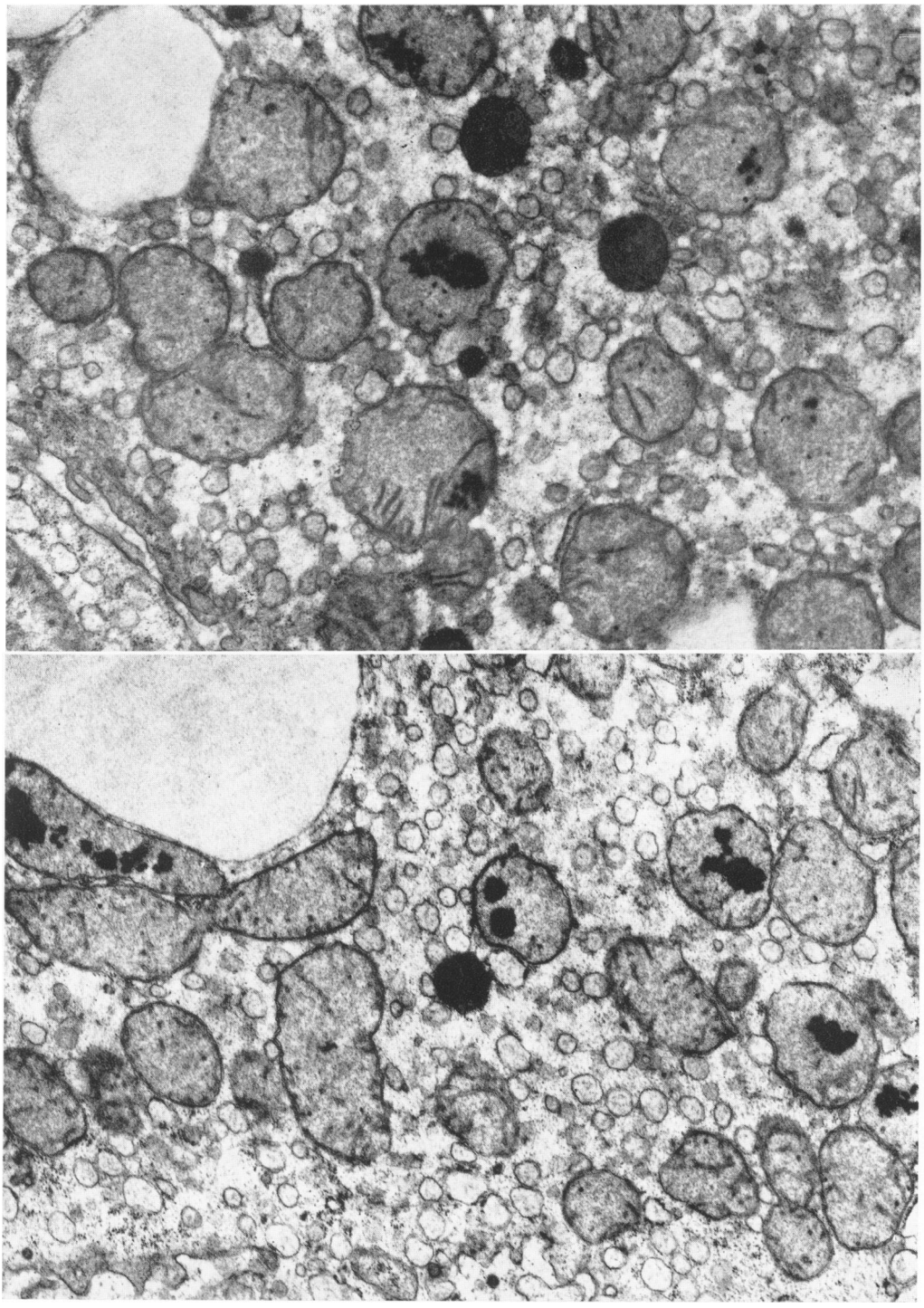


FIG. 13. A large mitochondrion contains oval cavities and large round dense bodies that measure approximately 0.15 $\mu$. The matrix is otherwise occupied with poorly delineated structures resembling cristae. × 44,000.

FIG. 14. A dense body, enlarged from Figure 13, contains a faintly laminated interior substructure. × 275,000.



15

16

FIGS. 15 and 16. Large dense bodies of irregular shape appear in mitochondria which contain few normal-sized matrix granules. Uranium stain. × 11,000.

17

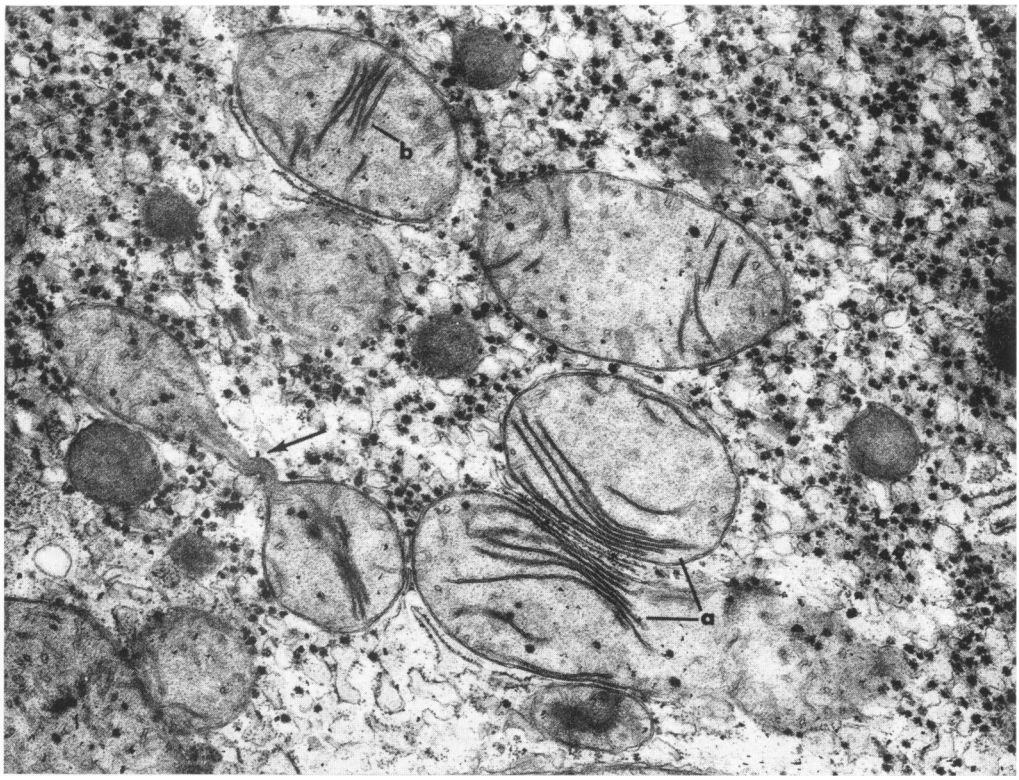



FIG. 17. Cristae are disposed in parallel stacks along a segment of the periphery of mitochondria (a) or in the interior (b). The arrow points to a tortuous portion of a mitochondrial profile. Cisterns of endoplasmic reticulum are dilated. × 66,000.