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FINE STRUCTURAL CHANGES PRODUCED IN RAT LIVER BY PARTIAL STARVATION

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In the assessment of liver changes produced under those experimental conditions in which food consumption is commonly depressed, such as during drug administration or following surgical procedures, it is essential to distinguish specifically induced alterations from those caused by reduced food intake *per se*. With regard to fine structural changes, this distinction is especially important because of the other inherent difficulties in evaluating such alterations.[1-4] Accordingly, in a group of young rats we have investigated the effects of reducing their intake of standard laboratory food to such an extent that they failed to gain body weight. The present report deals with the liver changes produced by this simple dietary restriction, with particular reference to fine structural alterations, and also draws attention to some ultrastructural variations observed in livers of control rats on unrestricted diet.

MATERIAL AND METHODS

Fifty male Sprague-Dawley rats, 2 months old and weighing about 130 gm. each, were divided into 2 equal groups. One group had their daily intake of ground Rockland rat diet restricted to one third of normal, which from a preliminary investigation was known to prevent the animals from gaining weight (P.J.G., unpublished data), and are referred to as the "restricted group"; the other, denoted as the "unrestricted group," had free access to the same type of food, the actual amounts consumed being recorded daily. Both groups were allowed water *ad libitum*.

The Rockland mouse/rat diet (Teklad, Inc., Monmouth, Ill.) contained crude

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protein, 24.0 per cent minimum; crude fat, 4.0 per cent; and crude fiber, 6.0 per cent. The ingredients: soybean meal, ground yellow corn, fish meal, pulverized barley, wheat midlings, ground wheat, dehydrated alfalfa meal, pulverized oats, feeding oatmeal, dried skimmed milk, 1 per cent animal fat (preserved with propylene glycol, butylated hydroxy toluene, citric acid), vitamin A palmitate, irradiated dried yeast, niacin, calcium pantothenate, riboflavin supplement, menadione, vitamin B_{12} supplement, 1 per cent calcium carbonate, 0.5 per cent dicalcium phosphate, 1 per cent salt, and traces of magnesium oxide, copper oxide, cobalt carbonate, iron carbonate, zinc oxide, calcium iodate.

The rats were weighed 3 times a week and immediately before being killed. Five rats from each group were killed by cervical dislocation on the first, second, fifth, 14th and 28th days after commencement of the experiment. At necropsy, which was always performed between 9 a.m. and 11 a.m. in order to reduce variations caused by diurnal feeding cycles, the liver was excised, lightly blotted, and weighed. A sample from a constant site on the right lateral lobe was removed for light and electron microscopy, and samples were taken for determination of water, fat, glycogen and nitrogen.

Light and Electron Microscopy

Tissues for conventional light microscopy were fixed in both aqueous 10 per cent formalin, buffered with phosphate to pH 7.0, and absolute alcohol, and after paraffin or carbowax embedment, were sectioned and stained by standard techniques with hematoxylin and eosin, Masson trichrome, oil red O,[5] and by the periodic-acid Schiff (PAS) method[6] both before and after digestion with α-amylase.

For light microscopy of thin sections, and for electron microscopy, tissues were cut under cold phosphate-buffered osmic acid[7] and after fixation at 4° C. for 60 to 90 minutes, were dehydrated at room temperature through graded alcohols and embedded in Epon 812, in the manner of Luft.[8] Sections were cut on a Porter-Blum MT-1 ultramicrotome using glass knives. From each block, sections 0.5 μ thick were mounted on glass microscope slides, stained with toluidine blue[4] and examined with the light microscope before proceeding with electron microscopy. Ultrathin sections were mounted on plain, formvar-coated, or carbon-coated[9] "Athene" or "Effa" copper grids, stained with lead,[10] or uranyl acetate, using a modification[11] of Watson's method,[12] or left unstained, and examined in an RCA (Model EMU-3C) electron microscope.

Biochemical Studies

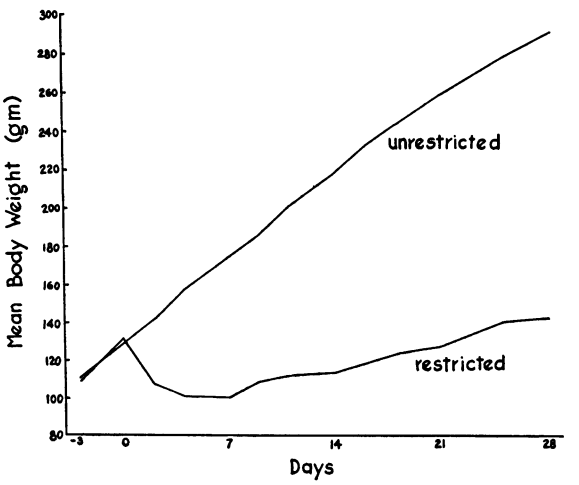
Water and fat content of the livers was determined by previously described methods.[13] Nitrogen was estimated by a micro-Kjeldahl technique, modified after Hiller, Plazin and van Slyke,[14] and glycogen was determined using the method of Good, Kramer and Somogyi,[15] modified by Keston[16] and Teller.[17]

RESULTS

The mean daily food consumption of the restricted group of rats, which had been calculated from a pilot study to be 1/3 of normal, proved in fact to vary from 1/6 to 1/3 that of the freely fed rats during the definitive 28-day experiment (Text-fig. 1). Mean body weights in the restricted group initially fell from 130 gm. to 100 gm. and then slowly increased to 140 gm. by the 28th day, whereas the unrestricted animals gained weight at a normal rate[18] as expected (Text-fig. 2). Concomitantly, mean absolute liver weights of the restricted rats were less from the first day, and by the 28th day of the experiment were about 2/5 those

of the unrestricted animals (Text-fig. 3). Expressed relative to body weight (Text-fig. 4), the relative liver weights of the restricted group were only about 3/5 of the expected value for rats of their body weight [19,20] by the 28th day, and were consistently significantly less than those of the freely fed animals which were in the normal range.[19–21]

TEXT-FIG. 1. Comparison of mean daily food consumption in the 2 groups of rats.

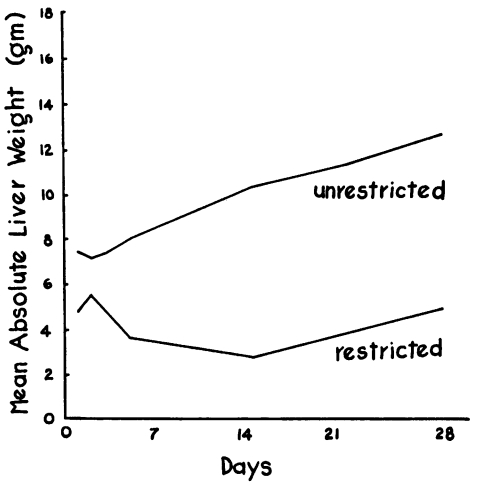


TEXT-FIG. 2. Comparison of mean body weights in the 2 groups of rats.

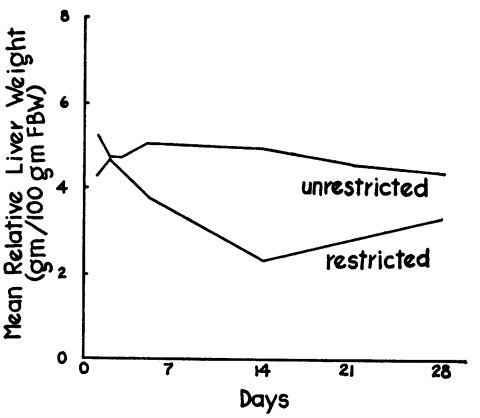
Throughout the experiment, the concentration of water in the livers of both groups varied only between 69.3 and 71.8 per cent. Likewise, in both groups the nitrogen concentration did not vary significantly, being always between 0.026 and 0.036 mg. per mg. wet tissue. The fat content of livers from rats on restricted diet was significantly lower than the freely fed controls except on the second and 28th days when normal values were recorded (Text-fig. 5). Chemical estimation of glycogen showed

an over-all depression in the livers of the restricted animals, though there was an unexpected rise to control values on both the fifth and 28th days of the experiment (Text-fig. 6).

Observations on the morphology of livers in the restricted and unrestricted animals will be considered separately.



TEXT-FIG. 3. Comparison of mean absolute liver weights in the 2 groups of rats.



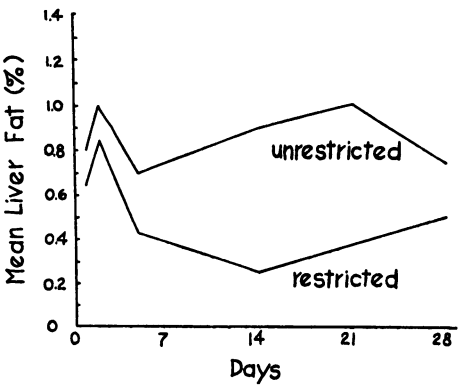
TEXT-FIG. 4. Comparison of mean relative liver weights in the 2 groups of rats.

Unrestricted Control Group

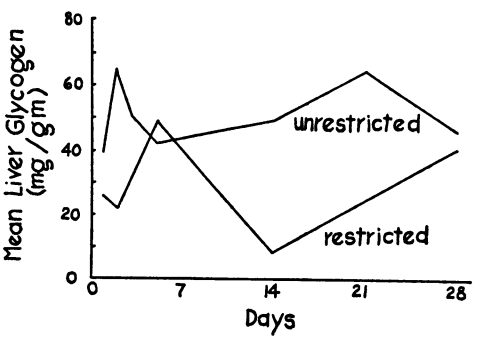
Light Microscopy. The livers in all the freely fed animals were of normal histologic appearance (Fig. 1). An average of 1 parenchymal cell in every 2 or 3 lobules showed mitotic activity. Sections 0.5 μ thick from osmium-fixed, Epon-embedded material stained with toluidine blue

showed only minimal variations in staining intensity from cell to cell (Fig. 2). Hepatocytes had an average cell diameter of 23 μ; the nuclei stained lightly, and the nucleoli were prominent as darker-staining bodies, while the cytoplasm was purplish, with greenish blue, refractile lipid inclusions, and dark-staining areas which represented the rough endoplasmic reticulum (ERR) and the mitochondria.[2,4,22,23]

Electron Microscopy. The fine structure of livers in this control group (Figs. 5 and 6) conformed in general to previous descriptions of normal rat livers,[2,23-26] and of livers from rats on a special basal diet,[3] but occasionally, cells varying considerably from the usual appearance were seen (Figs. 7 to 10). The ERR sometimes showed considerable variation from the usual well-ordered parallel orientated groups of cisternal profiles (Figs. 5 and 6), with irregular dilation and lack of obvious polarity (Figs. 7 and 8), and even frank vacuolation in some cases (Fig. 7). Some cells showed unusually extensive areas of smooth endoplasmic reticulum (ERS) which at least in the plane of section were not in proximity to the



TEXT-FIG. 5. Comparison of mean fat content (percentage w/w wet tissue) in the livers of the 2 groups of rats.



TEXT-FIG. 6. Comparison of mean glycogen content (mg./gm. wet tissue) in the livers of the 2 groups of rats.

Golgi apparatus (Fig. 10). The structure of glycogen also exhibited a wide spectrum (Figs. 5, 6, 7, 9, 11A). In some cells (Fig. 11A) it occurred in diffusely mottled areas,[3,27] while in others it appeared as dense, roughly stellate particles from 40 to 150 mμ in diameter, either aggregated (Fig. 7) or more widely dispersed (Figs. 5 and 9). The explanation of these differing appearances of glycogen in tissues treated identically, was not clear[27]; some of the variations seemed to reflect the amount of glycogen present, but the appearance of individual particles was probably related both to the amount of electron bombardment received by the specimen,[27] and to the state of polymerization of the polysaccharide. The over-all electron density of hepatocytes sometimes varied, with neighboring cells appearing "dark" and "light" (Fig. 9). This phenomenon, which could be seen in both stained and unstained sections, seemed to result from an unusually compact arrangement of the cytoplasmic organelles in the "dark" cells, rather than from any specific differences in the structure or amount of individual organelles.

Because of cell heterogeneity within the hepatic lobule,[28-31] some of these dissimilarities could in fact have represented the appearances of cells in particular sites of the lobule. However, it was possible in only some thin sections to orientate the individual hepatocyte relative to a portal tract or hepatic vein, and these variations, which were recorded by taking a large number of micrographs at random, are reported simply because of their importance in the interpretation of experimentally induced fine structural changes in rat liver.

Occasionally in the nuclei of the parenchymal cells, and more rarely in those of the Kupffer cells, 1 or sometimes 2 round structures measuring from 200 to 450 mμ were seen (Figs. 11A, B, C, D, E). These intranuclear inclusions were observed both in mononuclear hepatocytes, and in one or both of the nuclei of binuclear cells, and also were seen from time to time in the nuclei of the "restricted group" livers. Furthermore, they occurred in nuclei in which the nucleoli were obvious, as well as in those lacking clumps of chromatin material (Figs. 11A, B, C). Though randomly situated within the nucleus, they were always at a distance, and apparently quite distinct, from the nucleoli. They lacked a definable limiting membrane, and in some instances their matrices were distinctly granular, while in others, the limiting dense zones shaded off to a relatively clear central area. It is noteworthy that at least in the hepatocytes, they occurred in nuclei whose limiting membranes did not show any indentations in the plane of section.

Restricted Food Intake Group

Light Microscopy. Histologically, the liver architecture was preserved in these semi-starved rats, but the parenchyma was very compact

throughout the 28 days of restricted food consumption (Fig. 3), with loss of the normal distinction between the relatively compact cytoplasm of central cells and the more vacuolated cytoplasm of periportal cells. The basophilic components were less prominent, being diffusely distributed throughout the cytoplasm in all the parenchymal cells. The hepatocytes were small, with more obvious intercellular channels between central cords of parenchymal cells than usual. Except on the fifth and 28th days, when glycogen was present in the periportal zones, generalized glycogen depletion was demonstrated by PAS-diastase methods throughout the experiment. Likewise, oil red O staining showed very little fat at all times apart from the second and 28th days, when normal distribution of fat was seen. After the first day, mitotic figures were not observed in this group at any time during the experiment, and there was no evidence of inflammation, necrosis, fibrosis or hyperplasia.

The small size of hepatocytes in this group was well demonstrated in Epon sections 0.5 μ thick; on the 28th day their average diameter was 15 μ, about 2/3 that of control cells. From the second day, these sections showed distinct variations in the staining of many of the hepatocytes (Fig. 4). Prominent cords of dark cells were seen, frequently near portal or hepatic veins, with very dense and abnormally homogeneous cytoplasm, and nuclei which stained somewhat darker than usual. In addition, about 10 per cent of the hepatocytes were lighter than normal and rather larger than the dark cells; the chromophilic component of their cytoplasm appeared as unusually discrete granules, while their nuclei and nucleoli were unremarkable.

Electron Microscopy. Whereas variations from normal were found uncommonly in the controls, fine structural abnormalities were observed to a greater or lesser degree in virtually all the parenchymal cells of the partially starved rats. The cells were smaller than usual, exemplified by the unusual frequency with which nuclei were seen close to lateral cell membranes, but normal apposition of cells was preserved. In this group, much greater variations in electron density from cell to cell than those found in freely fed controls were first noted on the second day, and were seen in some areas of each section for the remainder of the experiment, being especially obvious on the 14th and 28th days (Figs. 13 and 14). Those cells which appeared light or dark in toluidine blue-stained material were respectively "light" and "dark" electron microscopically, whether the sections had been stained with lead or uranyl acetate, or left unstained. Although the "dark" cells were usually smaller than the "light," the nuclei and nucleoli were of normal size and appearance in both. The phenomenon seems to be an exaggeration of the variation in electron density occasionally seen in the control animals; in the "dark"

cells, cytoplasmic organelles were abnormally compact; in the "light" cells they were unusually widely dispersed.

There was a generalized lack of the usual orientated parallel grouping of ERR cisternae in all parenchymal cells. In those of normal electron density, the cisternae were scattered and individual profiles were frequently contoured about mitochondria (Figs. 12, 16 and 18). In the "dark" cells, the ERR was dense, appearing either as masses of poorly orientated closely packed membranes, or frequently, where the cisternae had been sectioned "full face," as ribosomes having the usual forms of double rows, loops, spirals, circles and rosettes[32] (Figs. 13, 14 and 19). Extensive swelling and vesiculation of the cisternae was seen in the "light" cells (Figs. 13 and 14).

Following one day of food restriction, the mitochondria were moderately enlarged and frequently of unusual form (Fig. 12), and by the fifth day the cytoplasm of most cells was crammed with mitochondria, many of which were very large and pleomorphic (Figs. 15 to 18). Their internal structure was preserved, with normal-looking cristae mitochondriales, mitochondrial granules, and background matrices, and presumably the increase in their size was not merely from imbibition of water.[2,33] In many instances, one mitochondrion was partially encircled by one or more of its fellows (Figs. 15, 16 and 18), and examples were seen of apparent coalescence between two mitochondria (Fig. 17), though whether this represented fusion or fission, or resulted from sections through bizarre-shaped mitochondria was difficult to determine. Individual mitochondria were more prominently outlined by the double profile of a rough endoplasmic cisterna than in normal hepatocytes.[2,34–36] Where two mitochondria were in close proximity but separated by this surrounding ERR, the limiting membranes of the intervening cisternae became very closely apposed and lost their ribosomes. In some instances the cisternal membranes seemed actually to fuse with the mitochondrial limiting membranes (Figs. 16 to 18) though this appearance probably resulted from oblique sectioning. On the 14th day the mitochondria were still large but less pleomorphic, and by the 28th day they were predominantly round in cross section, and less enlarged (Fig. 19).

Assessment of variations in the Golgi complex is notoriously difficult.[3] This labile component was flattened and rather less obvious than usual after one day of food restriction (Fig. 12); on the second day the profiles of large, very closely packed sacs were frequently observed (Fig. 13), but thereafter no striking abnormalities of this organelle were seen. Considerable quantities of glycogen were noted in most hepatocytes on the fifth and 28th days of the study, but at other times, very little glycogen was present. In view of current speculation on the role

of ERS in glycogenesis or glycogenolysis,[2,3,27,37,38] it is of interest that ERS appeared normal throughout the 28 days of partial starvation, despite this unexpected fluctuation in glycogen content. Normal-looking lipid inclusions were seen occasionally on the first, second and 28th days, but at other times were observed infrequently, as opposed to the increased amount of lipid seen after 5 days of acute starvation.[2]

The plasma cell membranes, including bile canaliculi and sinusoidal and lateral surfaces, were unremarkable in parenchymal cells of both normal and abnormal electron density. The number and structure of lysosomes[39] and microbodies[40] appeared to be unaltered throughout the experiment, but on the first day, rather larger structures consisting of variegated material enclosed within a single limiting membrane and usually situated close to bile canaliculi, were seen (Fig. 12). Apart from the occasional nuclear inclusions already described, the nuclei and nucleoli of all hepatocytes were unremarkable. The Kupffer cells showed no significant alterations.

Discussion

This study demonstrates that simple dietary restriction sufficient to prevent growth induces marked liver changes in young rats. The livers of the partially starved animals are not only smaller than those of control rats having free access to the same type of food, but also are only about 3/5 of the expected relative weight for normal rats of comparable body weight.[19,20] This diminished liver size is due to the individual hepatocytes becoming smaller through a decrease in cytoplasmic mass, and not to necrosis or fibrosis. Furthermore, mitotic activity is greatly reduced compared with the normal rate seen in controls,[41] although usually, parenchymal cell proliferation occurs more frequently in rats of low body weight.[41]

Fine structural alterations are produced by this degree of partial starvation, the most striking of which are the appearance of so-called "light" and "dark" cells, together with generalized disorganization and vesiculation of ERR, and abnormally pronounced enwrapping of mitochondria by endoplasmic cisternae. After 5 days of food restriction, bizarre-shaped mitochondria are prominent, together with examples of apparent coalescence of these organelles.

We have not attempted to elucidate whether the stunting of growth or the liver changes induced by this severe food restriction reflect a lack of any particular element, or a deficiency of several essential components of the diet. Mikata and Luse[42] have observed, in rats fed a basal diet at a level which likewise inhibited increase of body weight, less marked fine structural alterations than those which we have seen during restric-

tion of Rockland rat diet; these findings may well be due to differences in the relative imbalance of dietary components.

Fine structural alterations of a similar nature to those we have observed have been reported under a variety of experimental conditions, many of which also produce more extensive abnormalities. Thus, "light" and "dark" cells have been seen in rat liver following administration of ethionine,[43,44] α-naphthyl isothiocyanate,[45] carbon tetrachloride,[46] thioacetamide[47]; after liver circulatory arrest,[48] and common bile duct ligation[49]; in dog liver following portal vein occlusion[50]; and in ostensibly normal rabbit liver.[51] Varying degrees of disruption of ERR, with or without an unusually close association of endoplasmic cisternae and mitochondria have been reported in rat liver after administration of 3′-methyl-4-dimethylaminobenzene,[3] α-naphthyl isothiocyanate,[45] carbon tetrachloride,[46,52-54] thyroxin,[55] dimethylnitrosamine,[56] β-3-thienylalamine,[57] phosphorous,[58] cysteine,[59] thioacetamide[47,60]; following partial hepatectomy, portocaval shunt and in sham-operated pair-fed controls[61]; in acute starvation[2,62-64]; in dietary necrotic liver degeneration[65,66]; after ligation of the common bile duct,[49] and occlusion of liver circulation[48]; in ammonia intoxication[67]; in mouse liver after fasting,[68] in virus infection,[69] and during *in vitro* necrosis[34]; in guinea pig liver with acute hypoxia[70]; and in human liver in biliary atresia and obstruction,[71] and in kwashiorkor.[72] And apparent mitochondrial coalescence has been seen after occlusion of liver circulation,[48] and with carbon tetrachloride administration.[52] Clearly, the finding that hepatic fine structure can be modified in a basically similar way by partial starvation emphasizes the nonspecific nature of these alterations.

It is unlikely that the distinctive "light" and "dark" cell appearances seen in osmium-fixed material are of the same nature as the controversial light and dark cells of light microscopists, which were first described by Cohn in 1892,[73] exhaustively reviewed by Clara,[74,75] and ascribed simply to cutting and fixation artifacts by Scharrer.[76] In normal rat liver, fixed in osmium and embedded in Epon, variations in toluidine blue staining and electron density are neither as marked nor nearly as extensive as those in livers of partially starved rats. However, "light" and "dark" cells are not seen after either potassium permanganate fixation, or freeze-drying and post-osmication, with subsequent Epon embedding (P.B.H., personal observation). Thus, the distinctive appearances may be related to osmium fixation of fresh tissue, but there seems no doubt that at the moment of fixation there is a real difference in the contents or structure of these cells, which is revealed by osmication. These marked variations in electron density from cell to cell may reflect differing degrees of hydration, although somewhat against this hypothesis is the lack

of discernible fine structural variation in any region of the plasma cell membranes. Nevertheless, it is conceivable that although the percentage of water in these livers is unchanged, there could be significant variations in the hydration of individual cells, with the average percentage remaining normal.

The intranuclear structures found from time to time in both groups of rats do not resemble the type of cytoplasmic invaginations described by Leduc and Wilson,[77] in that the inclusions which we observed are smaller, of relatively constant size, lack a definable limiting membrane, and occur in nuclei whose nuclear membranes are not indented or irregular, at least in the plane of section. They are bigger than the perichromatin granules reported by Watson,[78] which are not recognizable with confidence in osmium-fixed tissue, and possibly represent rather well-defined examples of the "pore-complex" described by the same author.[79]

Summary

When the daily food intake of young rats was restricted to approximately 1/3 of normal for 28 days, the animals failed to gain body weight. Their livers were small, as a result of decreased cytoplasmic mass, and the parenchymal cells showed fine structural alterations. These changes chiefly involved the rough endoplasmic reticulum, which became dispersed and vesiculated, and the mitochondria, which enlarged, assumed bizarre shapes and were prominently enveloped by endoplasmic cisternae. There was a general depletion of glycogen and lipid, and "light" and "dark" hepatocytes were seen. Intranuclear structures, probably "pore-complexes," were observed from time to time in the livers of both partially starved and control animals. The importance of these alterations, together with variations observed in normal rat liver, is emphasized relative to assessing experimentally induced liver changes, especially under circumstances of reduced food consumption.

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[*Illustrations follow*]

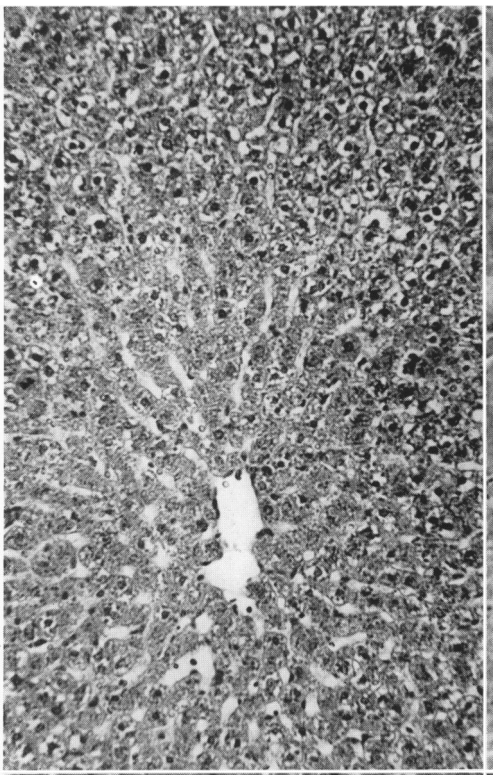
Legends for Figures

Fig. 1. Liver from a freely fed control rat, showing normal histologic appearance. The cytoplasm of central hepatocytes is compact, while that of peripheral cells is vacuolated. Hematoxylin and eosin stain. × 130.

Fig. 2. Liver from a freely fed control rat, osmium-fixed, embedded in Epon, stained with toluidine blue, showing normal histologic appearance. Average parenchymal cell diameter is approximately 23 μ. × 330.

Fig. 3. Liver from a rat after 14 days of partial starvation. The uniformly compact appearance of all parenchymal cells was seen in this group throughout the study. Hematoxylin and eosin stain. × 130.

Fig. 4. Liver from a rat after 28 days of partial starvation, osmium-fixed, embedded in Epon and stained with toluidine blue. Prominent light and dark-staining hepatocytes are evident. Average parenchymal cell diameter is approximately 15 μ. × 330.



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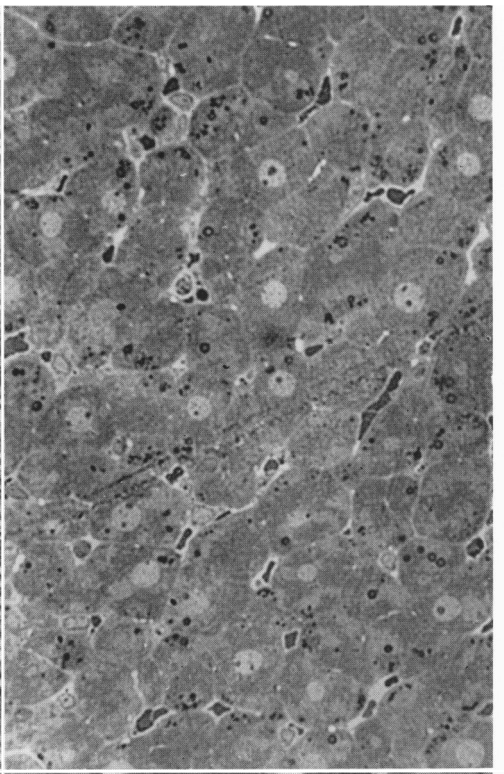

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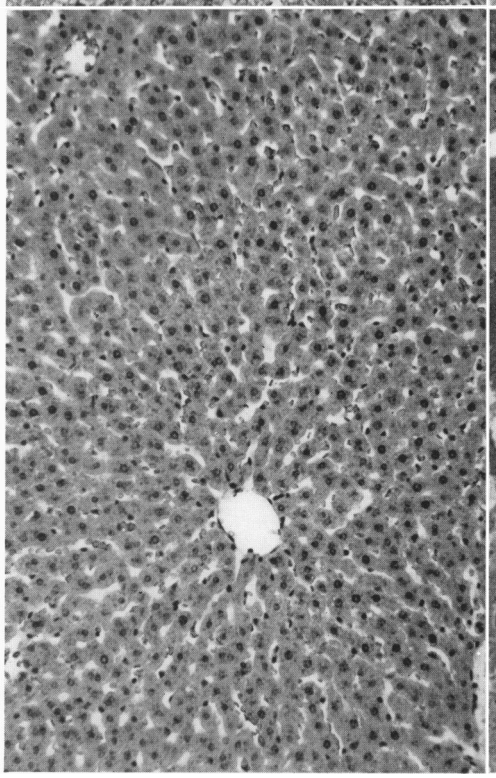

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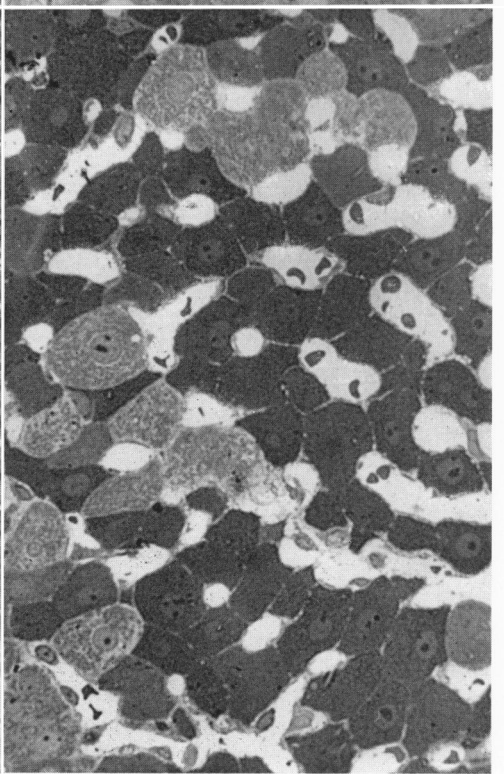

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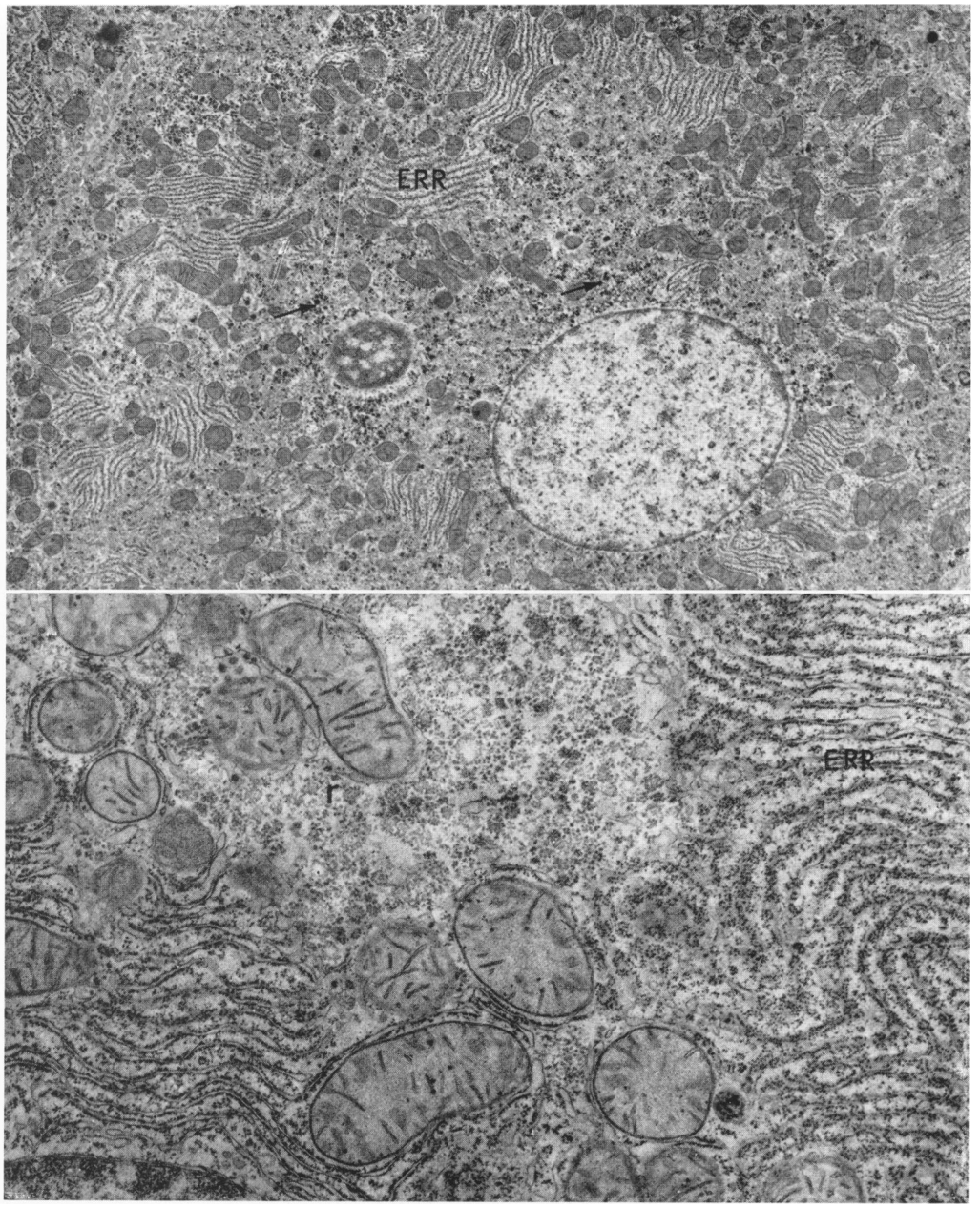
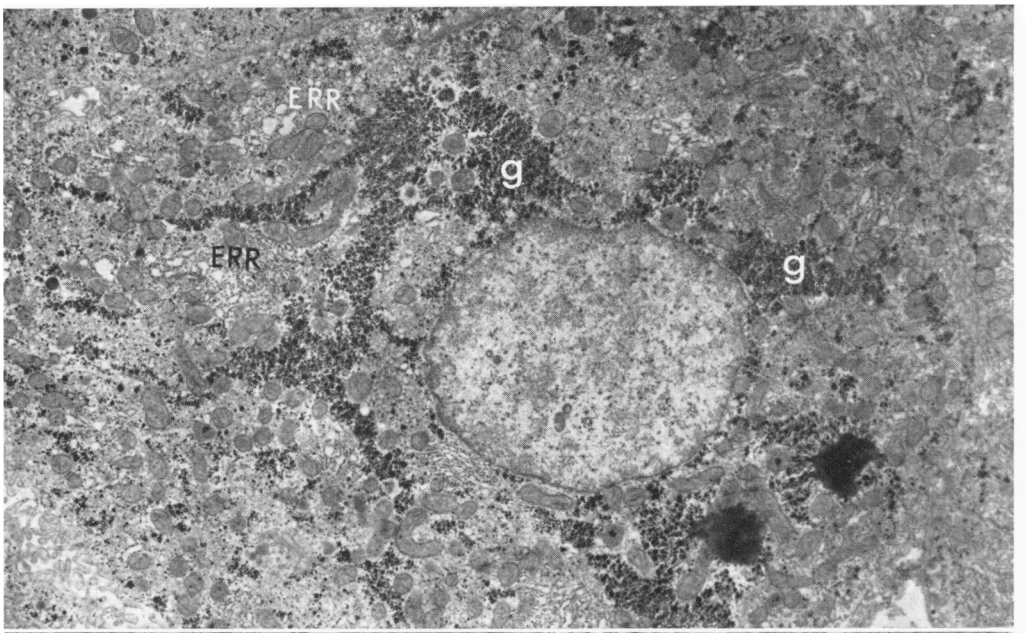


Figures 5 to 10: Hepatocytes from freely fed control rats.

FIG. 5. A parenchymal cell, showing the usual appearance, with well orientated groups of rough endoplasmic reticulum (ERR), normal mitochondria, and dispersed glycogen particles (arrows). This is a binuclear cell, the section having shaved one nucleus and passed nearer the meridian of the other. × 3,600.

FIG. 6. Portion of a parenchymal cell, showing part of the nucleus, well-ordered parallel grouping of endoplasmic cisternae (ERR), normal mitochondria, and ribosomes (r) in the usual forms of circles, spirals and rosettes where cisternae have been sectioned full face. × 17,400.



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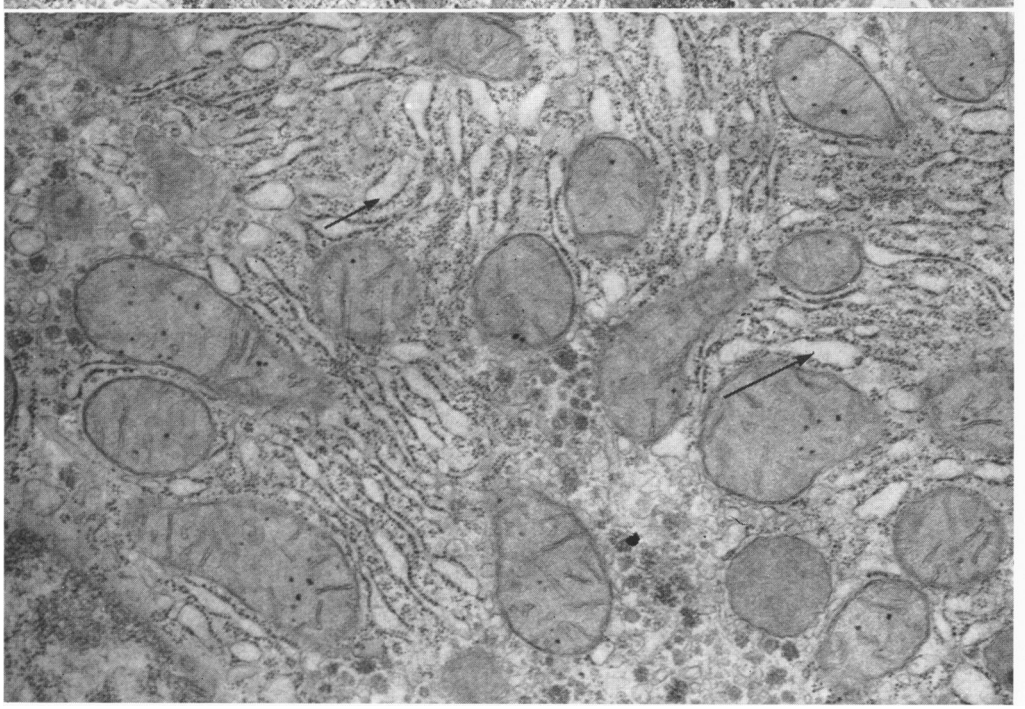

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FIG. 7. A parenchymal cell in which there is considerable dilatation of ERR cisternae with lack of polarity, and in which glycogen particles (g) are aggregated. × 3,800.

FIG. 8. Portion of a parenchymal cell in which the endoplasmic cisternae are moderately dilated (arrows). × 17,400.

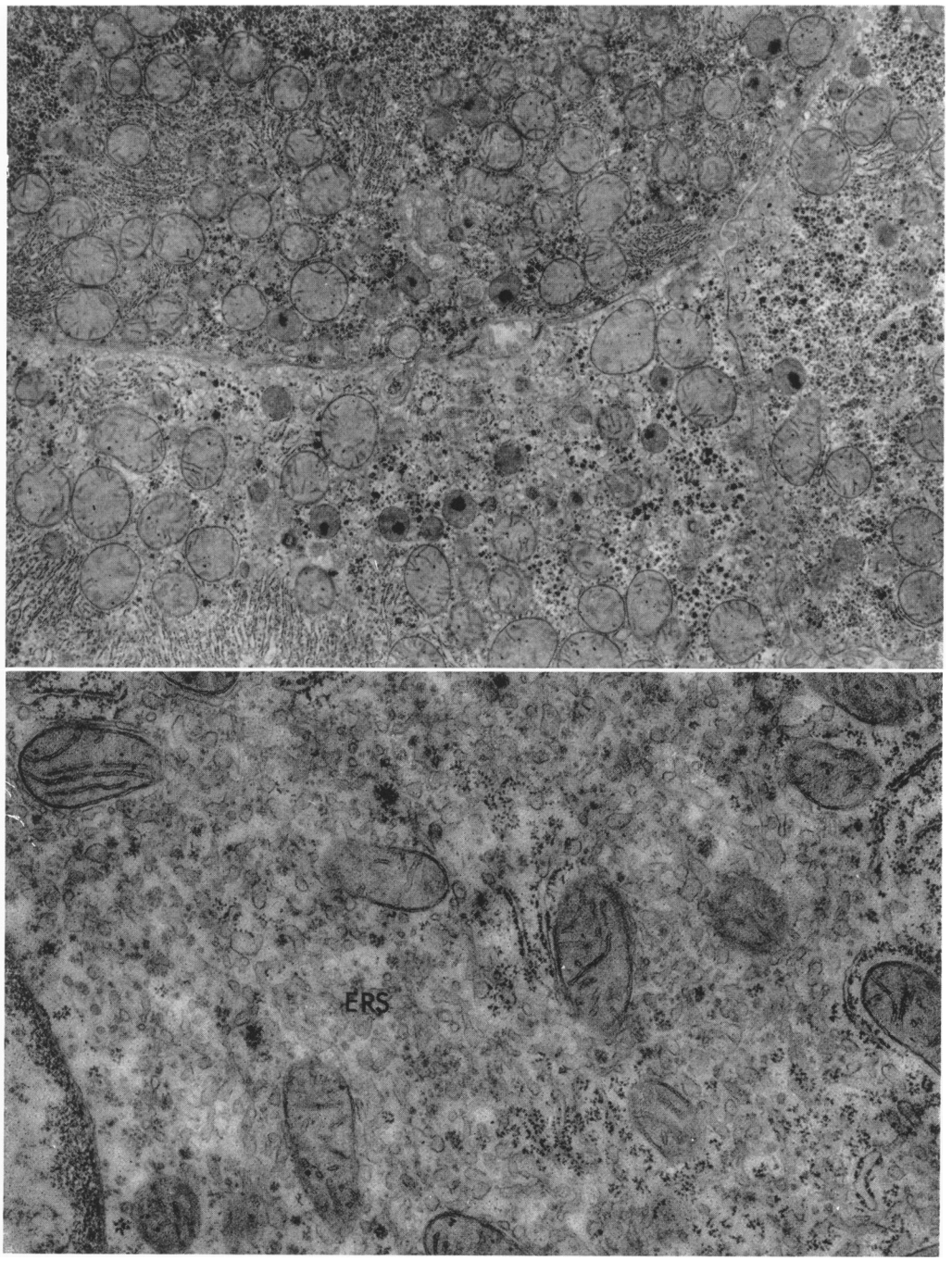

FIG. 9. Portions of 3 hepatocytes, 1 of which is "darker" with more compactly arranged cytoplasmic organelles than the other 2. × 8,600.

FIG. 10. Portion of a parenchymal cell showing part of the nucleus, and prominent smooth endoplasmic reticulum (ERS). × 17,200.

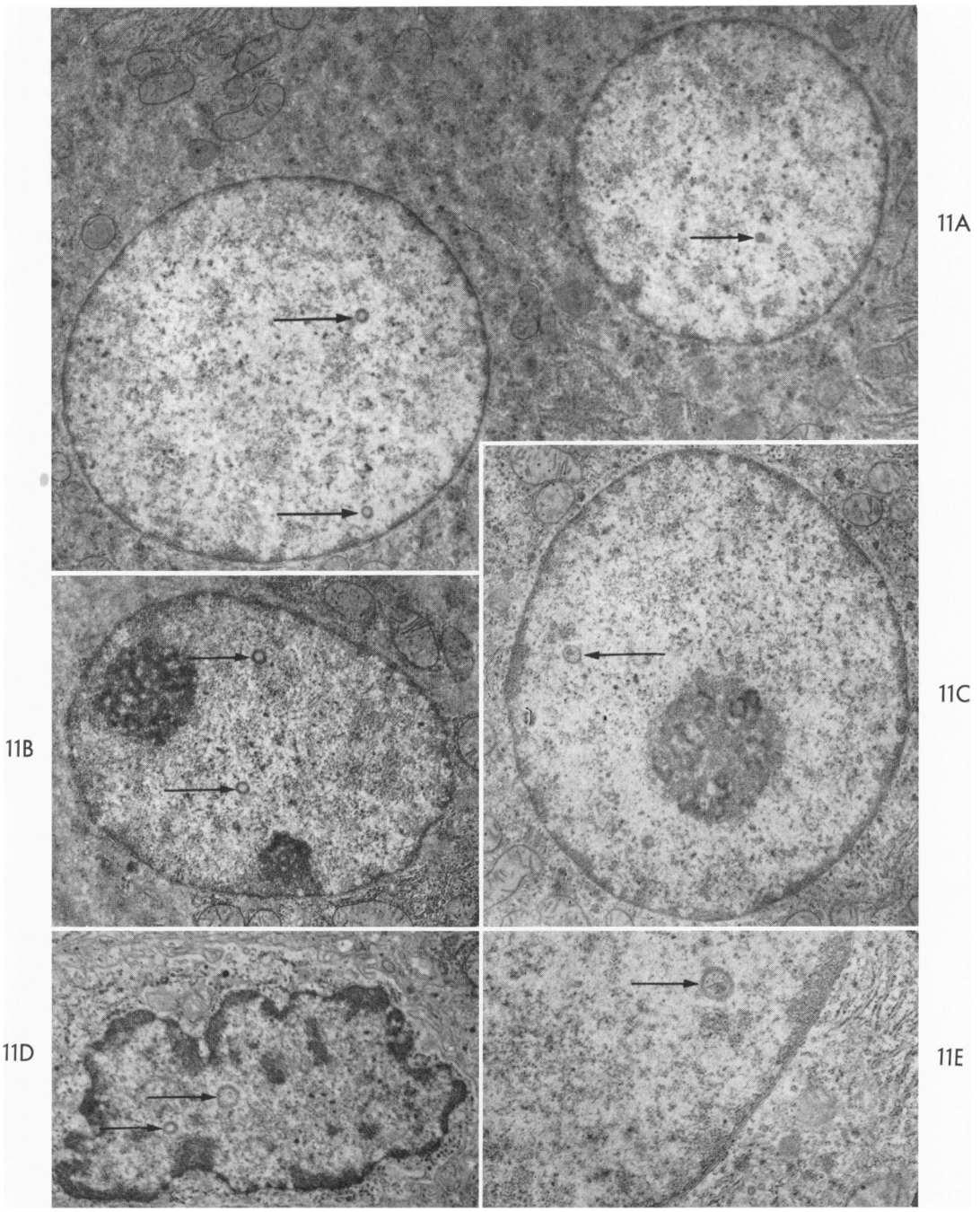

FIG. 11. Examples of the intranuclear inclusions (arrows) seen from time to time in parenchymal cells (A, B, C, E) and occasionally in Kupffer cells (D). They are found in nuclei with obvious nucleoli (B, C), and in those lacking chromatin clumps (A). In the parenchymal cells, they occur in nuclei which do not have any indentations of their nuclear membranes in the plane of section. A, × 7,400. B, × 7,200. C, × 8,100. D, × 6,300. E, × 13,500.

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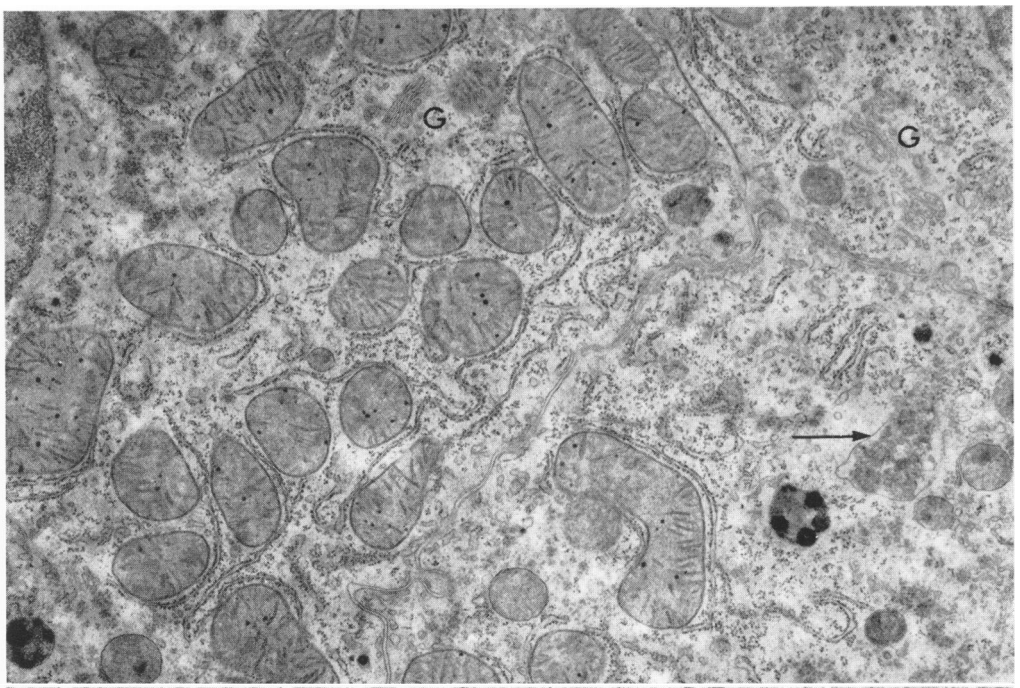

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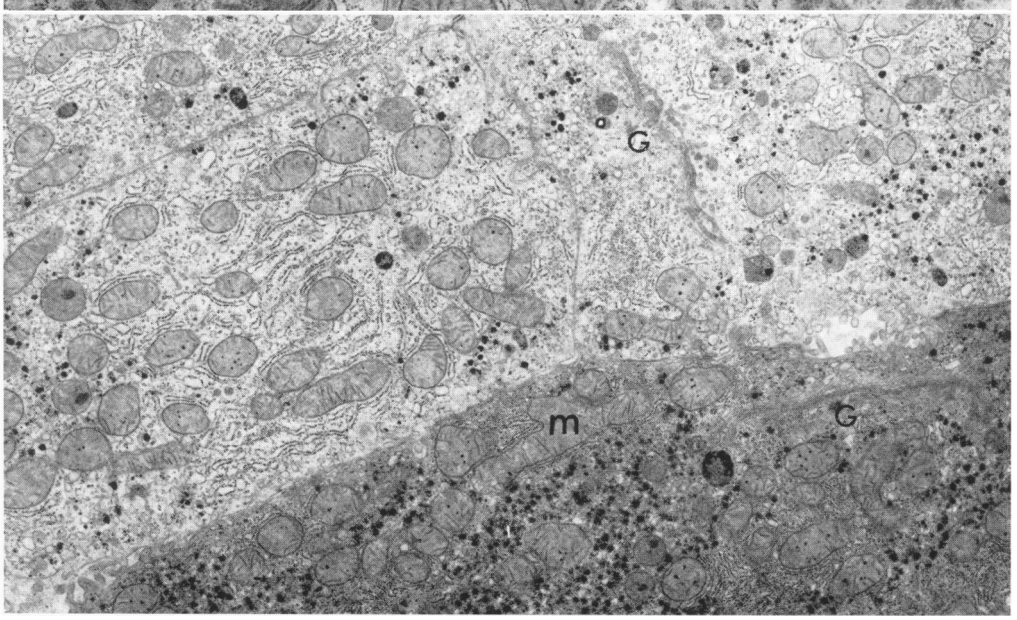
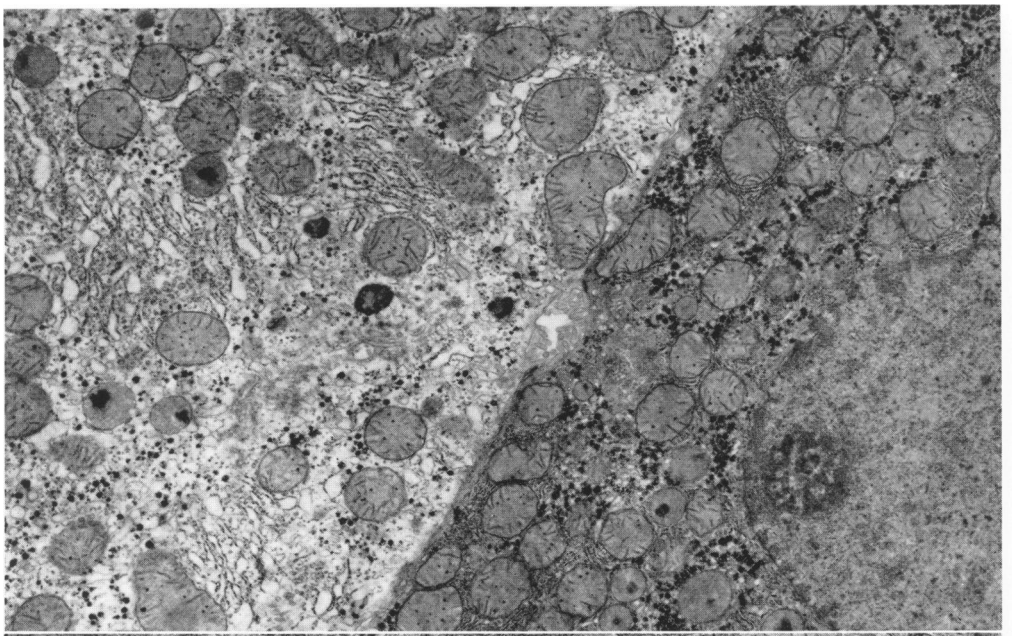


FIG. 12. Portions of 3 hepatocytes after 1 day of food restriction. The ERR appears as individual cisternae, and occasional, relatively large membrane-bound structures are seen; these contain variegated material (arrow). Golgi complex, G. × 13,300.

FIG. 13. Portions of 4 parenchymal cells, after 2 days of food restriction. One cell is markedly darker than the others, with closely packed endoplasmic cisternae. All the cells lack orientated groupings of cisternae, and a large mitochondrion is seen (m). Golgi complex, G. × 6,500.



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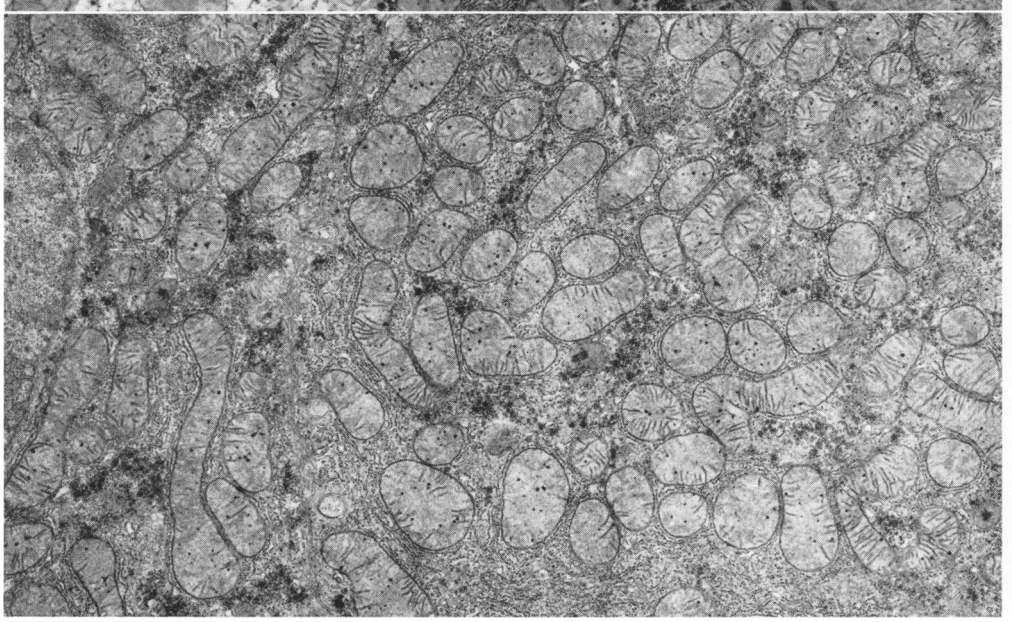

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FIG. 14. Portions of 2 parenchymal cells after 28 days of food restriction, showing distinct variation in electron density. Both cells lack the usual well-ordered parallel groups of ERR cisternae; in the "dark" cell, the cisternae, together with other cytoplasmic organelles, are closely packed, while in the "light" cell, there is considerable swelling and vesiculation of the cisternae. × 7,700.

Figures 15 to 18: After 5 days of food restriction.

FIG. 15. Portions of 2 hepatocytes, showing the cytoplasm crammed with mitochondria, some of which are abnormally large. × 7,400.

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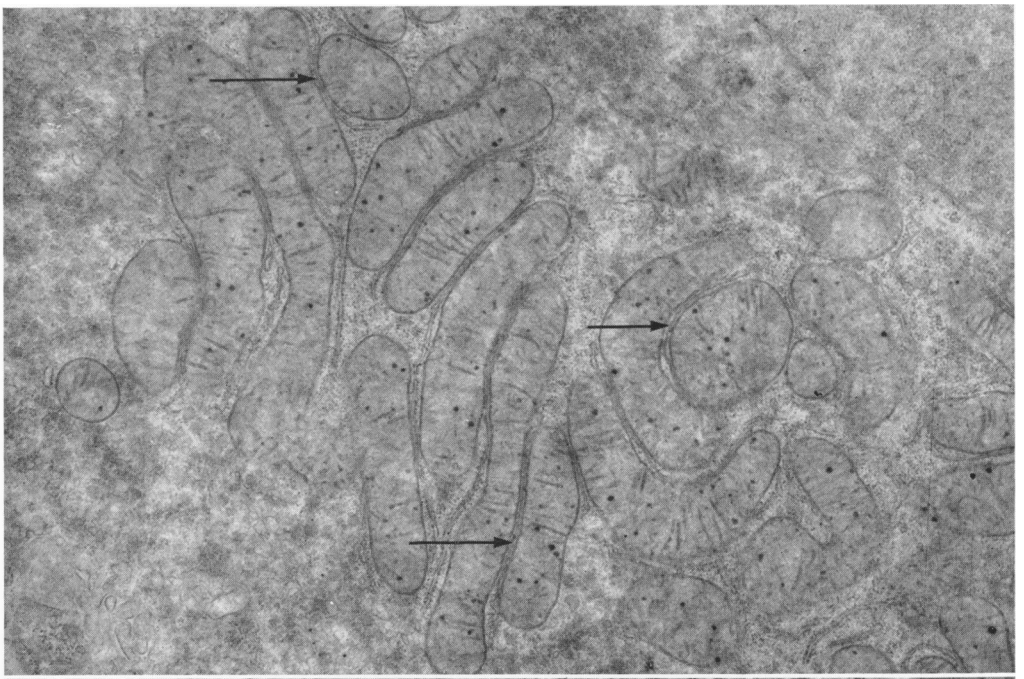

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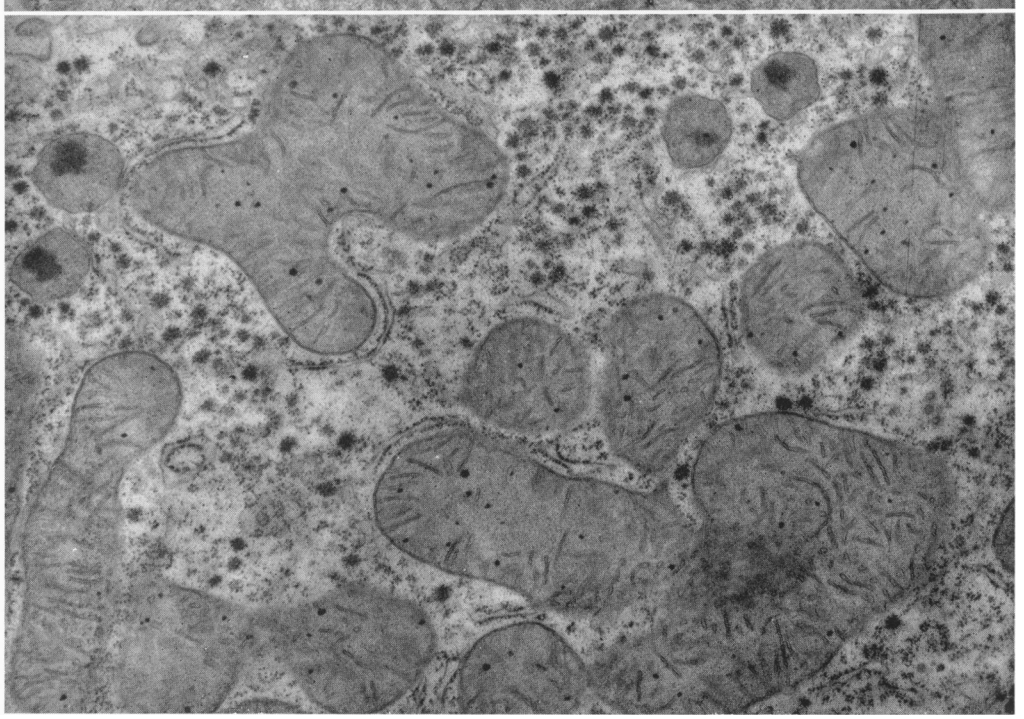

FIG. 16. Closely packed enlarged mitochondria. Endoplasmic cisternae lose their ribosomes between closely opposed mitochondria (arrows). × 12,500.

FIG. 17. Pleomorphic mitochondria, with apparent coalescence. × 17,200.

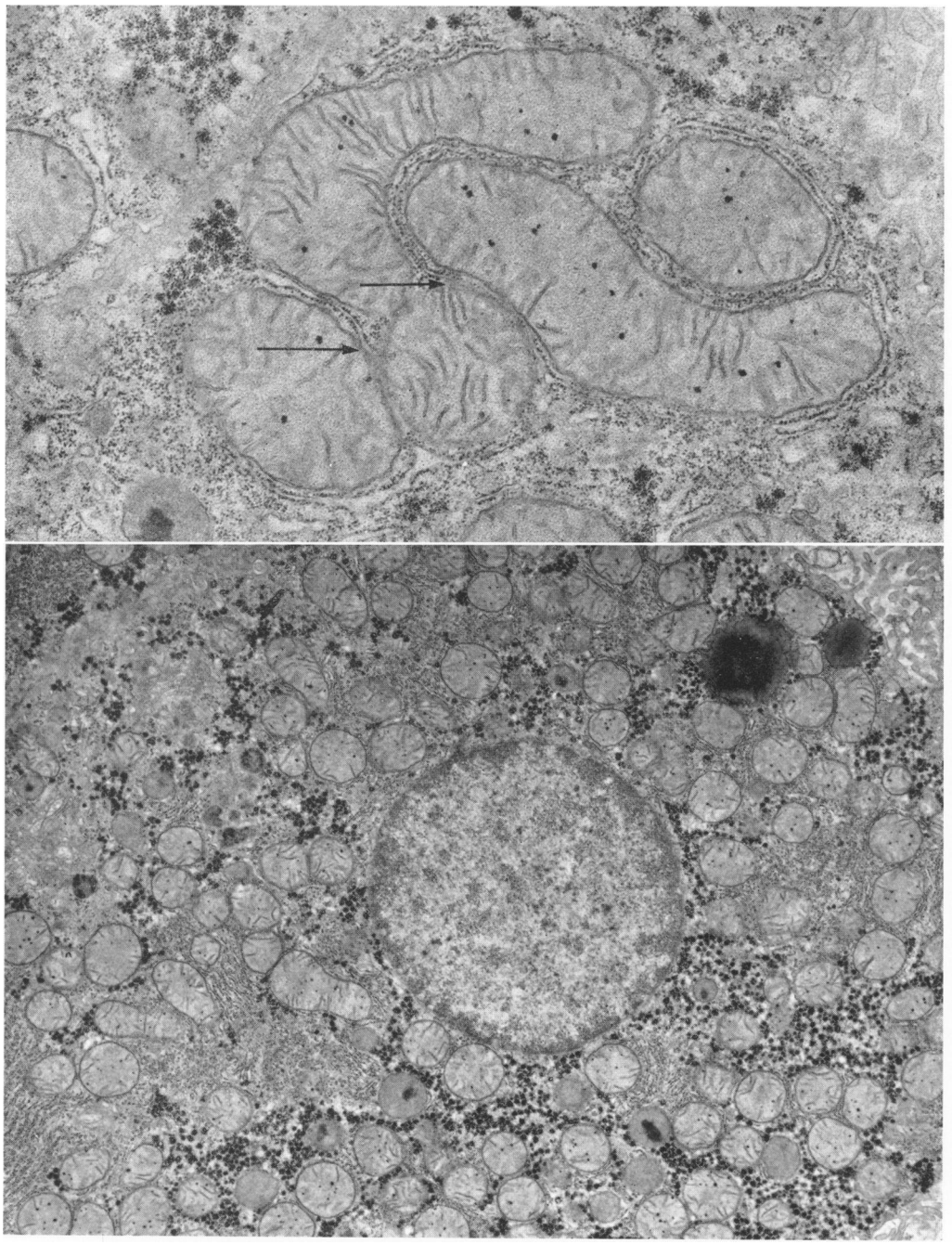

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FIG. 18. Enlarged mitochondria, each closely enwrapped by an endoplasmic cisterna. The apparent fusion of cisternal and mitochondrial membranes (arrows) is probably due to oblique sectioning. × 17,200.

FIG. 19. A typical hepatocyte after 28 days of partial starvation, showing compact arrangement of cytoplasmic organelles. The mitochondria are predominantly round in cross section and the endoplasmic cisternae are closely packed. × 8,600.