THE PATHOGENESIS OF EXPERIMENTAL
DIETARY SIDEROSIS OF THE LIVER

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In 1959 Gillman, Hathorn and Canham\(^1\) induced siderosis in rats by
feeding them iron-enriched maize-meal diets. The liver lesions in these
rats closely resembled the siderosis found at necropsy examination in
approximately 70 per cent of adult Bantu males in South Africa.\(^2\)

Recent studies have indicated that the iron deposits found in the liver
and other parenchymal organs of patients with Bantu siderosis are not
completely innocuous. For example, in some of these patients the clinical
features and pathologic changes closely resembled those found in idio-
pathic hemochromatosis.\(^3\) Furthermore, it has been concluded that “the
excessive iron deposits found in siderosis give rise to a sometimes refrac-
tory vitamin-C deficiency.”\(^4\)

In view of the increased importance now attached to the iron over-
load in Bantu siderosis and the fact that the pathologic changes seen in
these patients can be readily induced in experimental animals (rats), it
seemed desirable to study more closely the pathogenesis of the liver
lesions in experimental dietary siderosis. In this paper light and electron
microscopic studies and biochemical investigations of the livers of rats
fed iron-enriched maize-meal diets will be described.

MATERIAL AND METHODS

Forty male weanling albino rats were divided into 4 groups of 10 rats each. The
rats were fed the following diets\textit{ad libitum}: uncooked maize-meal (group I); un-
cooked maize-meal containing 4 parts per 100 by weight of ferric citrate (group II);
control diet (group III); control diet containing 4 parts per 100 of ferric citrate
(group IV). The composition of the control diet was as follows: casein, 20.6 per
cent; butterfat, 2 per cent; agar, 2 per cent; dextrin, 46.5 per cent; wheat germ oil,
1.5 per cent; cod liver oil, 2 per cent; sucrose, 18.4 per cent; vitamin mixture, 2 per
cent and salt mixture, 5 per cent.

The composition of the vitamin and salt mixtures was described in a previous pub-
lication.\(^5\) The vitamin mixture contained all the known water-soluble vitamins. The
food consumption of the rats was determined every day and they were weighed
twice weekly.

On the ninth day of the experiment half the rats in each group were subjected
to laparotomy and small pieces of liver removed. Liver biopsy specimens were there-
after procured at irregular intervals, depending on the postoperative growth response

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of the rat and the rapidity with which the laparotomy wound healed. None of the intervals was longer than 14 days. All the rats were killed on the 30th day of the experiment and the following organs removed: liver, spleen, duodenum, kidney, adrenal, testis, lung, heart and pancreas.

Preparation of Liver Biopsy Specimens for Microscopy

The small piece of liver removed at laparotomy was divided into two parts. A small block of tissue (1 cu. mm.) was fixed in cold 1 per cent OsO₄ solution buffered at a pH of 7.3 to 7.4 with acetate veronal buffer, and prepared for electron microscopy according to methods previously described. Electron microscopy was performed with a Phillips EM 100 electron microscope at 60 kv.

The rest of the liver specimen was fixed in 10 per cent formalin, embedded in a mixture of paraffin and celloidin, and sections were cut for light microscopy. The sections were stained with hematoxylin and eosin and with the ferrocyanide method for iron as described by Dry. In addition to these methods, sections from a small piece of the liver removed at the end of the experiment (39 days) were stained for acid phosphatase activity with the glycerophosphate-lead procedure of Gomori after fixation in cold formol-calcium. The rest of the liver removed at the termination of the experiment was used for the biochemical studies.

Biochemical Methods

For the biochemical studies the livers from 5 group II rats (iron-enriched maize-meal diet) and 5 group III rats (control diet) were pooled.

Fractionation of Rat Liver Homogenates. The fractionation of liver homogenates obtained from the 2 groups of rats was carried out according to the method of Dounce, Witter, Monty, Pate and Cottone, with certain slight modifications. Rats were killed by decapitation and their livers quickly removed and placed in an iced-cold mortar. After being pulped to a fine paste, the liver (7 gm.) was homogenized in 28 ml. of 0.44 M sucrose containing 0.63 ml. of 0.1 M citric acid and then centrifuged at 1,800 r.p.m. for 15 minutes.

Nuclei. The pellet obtained from the above centrifugation (nuclei plus cell wall material, etc.) was resuspended in 56 ml. of 0.44 M sucrose plus 1.26 ml. of 0.1 M citric acid, filtered through 2 layers of cheese cloth, rehomogenized and then centrifuged at 1,300 r.p.m. for 10 minutes. The resulting supernatant was discarded and the nuclear pellet subjected to a further washing procedure as described above except that a speed of 1,000 r.p.m. for 10 minutes was used. The nuclei were finally suspended in 2.5 ml. of 0.44 M sucrose.

Mitochondria. These were obtained from the original nuclear supernatant by centrifugation at 17,000 r.p.m. for 10 minutes. They were washed once in 0.44 M sucrose and once in 0.25 M sucrose and finally suspended in 0.25 M sucrose (6 ml.).

Microsomes. These were obtained from the mitochondrial supernatant by centrifugation at 40,000 r.p.m. (105,000 × g) for 60 minutes in a Spinco Ultracentrifuge. The microsomal pellet was carefully rinsed with 0.44 M sucrose and resuspended in the same medium (3 ml.).

High-speed Supernatant Fraction. This was the fraction (supernatant) which remained after removal of the microsomal pellet.

Density Gradient Centrifugation of Mitochondria. This was carried out according to the method of Spiro and Ball. A lusteroid centrifuge tube (10 ml. volume) was filled successively with layers of 1.5, 1.35, 1.2, 1.05 and 0.9 M sucrose. A twice-washed mitochondrial suspension in 0.25 M sucrose was carefully layered on top of the gradient, and the tube centrifuged at 26,000 × g for 60 minutes in the swinging bucket rotor of the Spinco Ultracentrifuge.

Protein was estimated in all fractions with the method of Cleland and Slater, ovalbumin being used as a standard.
Iron was estimated according to the method of Snell and Snell.  

Enzyme Activities of Isolated Mitochondria. The activities of the following enzymes were determined in mitochondria isolated from the livers of group II and group III rats: isocitric, malic and L-glutamate dehydrogenases, DPNH diaphorase, DPNH cytochrome C reductase and DPNH oxidase. Each reaction cuvette contained the following in a final volume of 3.0 ml.: 0.10 M tris acetate buffer (pH 7.4); 0.001 M KCN (for the dehydrogenase, DPNH diaphorase and cytochrome C reductase measurements); 0.003 M MgCl₂; 0.02 M nicotinamide; 3.6 x 10⁻⁵ M 2,6-dichlorophenolindophenol as electron acceptor for the dehydrogenase and DPNH diaphorase measurements; 2 x 10⁻⁴ M TPN (isocitric dehydrogenase); 2 x 10⁻⁴ M DPNH (DPNH diaphorase, DPNH cytochrome C reductase and DPNH oxidase); 0.3 mg. cytochrome C (DPNH cytochrome C reductase). Mitochondrial preparations in 0.25 M sucrose were diluted and mixed with an equal volume of ice-cold water before use. All substrates were neutralized to pH 7.4 with 1 N KOH before use.

Results

Light Microscopy

The microscopic features of the liver and other organs have been fully described in a previous paper. Suffice it to say here that histochemically demonstrable iron first appeared in the parenchymal liver cells of the rats fed the iron-enriched maize-meal diet (group II) approximately on the 16th day of the experiment. At later stages the hepatocellular siderosis became more marked and the Kupffer cells were also affected. At all stages iron deposition was more marked in the cells around the portal tracts than in the central parts of the liver lobules. In contrast to the group II rats (iron-enriched maize-meal), the livers of the rats which received the other diets did not contain any histochemically demonstrable iron. Even the rats of group IV (control diet plus iron) did not show abnormal amounts of iron pigment in the liver. These results are in agreement with those of Gillman and co-workers.

At the end of the experimental period, liver sections of the rats fed the iron-enriched maize-meal diet (group II) showed increased acid phosphatase activity in comparison with sections from the control rats (group III) (Figs. 1 and 2). In the former group of rats the activity was markedly increased in both the parenchymal liver cells and the Kupffer cells (Fig. 3).

Electron Microscopy

Approximately on the ninth day of the experiment numerous electron-dense particles appeared in the sinusoids and in the adjacent cytoplasm of the parenchymal liver cells in the rats fed the iron-enriched maize-meal diet (Fig. 4). Two types of particles could be discerned: (a) relatively large (250 to 300 Å diameter), uniformly dense homogeneous particles (Fig. 4, L) which could be regarded as lipid material on the basis of their size, electron microscopic appearance and close resemblance to the fat droplets described in studies on experimentally induced fatty in-
filtration of the liver;\textsuperscript{12,13} (b) smaller (30 to 60 Å diameter) electron-dense particles corresponding in appearance to the ferritin deposits which have been identified in human\textsuperscript{14} and rat liver cells\textsuperscript{15} (Fig. 4, F).

In the cytoplasm of the liver cells the fat droplets were found exclusively in cytoplasmic vesicles (Fig. 5), while the ferritin usually occurred free in the cytoplasm. Occasional ferritin particles were, however, seen in membrane-bound vesicles (Fig. 5). At this stage (i.e., about the ninth day) the mitochondria of the liver cells were normal in appearance (Fig. 4).

In biopsy specimens from the group II rats taken at later stages of the disease (16th to 25th day), the ferritin was present in large aggregates, each surrounded by a single membrane (Fig. 6). These aggregates were always found in the area of cytoplasm near the Golgi apparatus and the bile canaliculi (Fig. 6). The contents of these membrane-bound clumps of ferritin were often condensed into structures which were indistinguishable from the pericanalicular dense bodies or lysosomes (Fig. 7). There was a marked increase in the number of ferritin-containing lysosomes in the liver cells on the 16th day of the experiment (Fig. 8). It is noteworthy that in these specimens the other cytoplasmic organelles (mitochondria, endoplasmic reticulum) showed no significant structural changes (Fig. 8) and that "normal" lysosomes without any ferritin were also present (Fig. 8).

At this stage it was not uncommon to find bile canaliculi filled with masses of electron-dense, granular material (Fig. 9) which corresponded in appearance to the ferritin found in the dense bodies of the cytoplasm. This finding may be regarded as presumptive evidence of excretion of ferritin into the bile.

\textit{Degenerative Changes in Mitochondria.} From the ninth day of the experiment onwards a series of degenerative changes was apparent in the liver cell mitochondria of the rats fed the iron-enriched maize-meal diet (group II). These are illustrated in Figure 10 (A to F) and can be divided into the following stages for descriptive purposes:

Stage I (Fig. 10A): These mitochondria contained one or more groups of structures which consisted of parallel osmiophilic lamellas approximately 30 to 50 Å thick, separated by clear spaces of 60 to 130 Å. The lamellas seemed to be distinct from the cristae mitochondriales.

Stage II (Fig. 10B): In the matrix of these mitochondria there were localized electron-dense areas which consisted of material with a fibrillar structure.

Stage III (Fig. 10C): The matrix of these mitochondria contained several areas of increased electron density.

Stage IV (Fig. 10D): The whole matrix of these mitochondria was
homogeneously dense, but the faint outlines of the cristae could still be seen (see also Fig. 9).

Stage V (Fig. 10E): Areas of vacuolation were evident within the mitochondria. These vacuoles were initially located within the cristae and were limited by the membrane which demarcated each crista.

Stage VI (Fig. 10F): These mitochondria showed large vacuolated areas, demarcated by extremely dense material in which ferritin particles were visible. The arrangement of the vacuoles suggested an intracristal origin. These altered ferritin-containing mitochondria were comparable in appearance with the “siderosomes” of Richter.18

The stage VI mitochondria (siderosomes) were usually considerably enlarged and were often several microns in diameter (Fig. 11). The ferritin in these mitochondria was liberated into the cytoplasm through gaps or through vesicles formed by the outer membrane of the siderosome (Fig. 12). It must be emphasized that all the mitochondria were not uniformly affected by this process of degeneration to siderosomes. Stage VI mitochondria often occurred in the same liver in association with mitochondria which showed no obvious structural changes (Fig. 13).

Although ferritin was usually found only in mitochondria in the late stages of degeneration (stage VI), occasional mitochondria in the earlier degenerative stages also contained finely dispersed deposits of this substance (Fig. 14). It must be emphasized, however, that ferritin was never detected in structurally normal mitochondria.

Slightly aberrant degenerative changes were occasionally found in some mitochondria. In Figure 15 a stage I mitochondrion can be seen with the dense osmiophilic lamellas arranged parallel to the outer mitochondrial membrane.

In the final stage of the experiment (39 days) most liver cells in the group II rats contained large numbers of stage VI mitochondria (siderosomes) together with mitochondria which were structurally normal or in the earliest stages of degeneration (Fig. 16). In liver specimens taken from approximately the 25th day of the experiment the Kupffer cells were also affected and usually contained large aggregates of ferritin which were found in altered phagosomes or free in the cytoplasm (Fig. 17). Another change which was noted in the livers of the group II rats was the presence of collagen fibers in the space of Disse (Fig. 18). These fibers were only found in the later stages of the experiment when there was a well-developed siderosis of the liver.

No significant structural changes were encountered in the liver cells of rats which had received the control diet (group III) or the control diet plus iron (group IV). In the group I rats (maize-meal diet) certain abnormalities were noted in the form of disorganization of the endo-
plasmic reticulum, swelling of the mitochondria and a moderate fatty infiltration of the liver. These have been fully described in a previous publication.5

**Biochemical Results**

The results of the enzyme measurements are presented in Table I. It can be seen that the isocitric, malic and L-glutamate dehydrogenase activities in the group II mitochondria (iron-enriched maize-meal diet) were much lower than those in group III (control diet). A similar result was obtained for DPNH diaphorase activity measured with 2,6-dichlorophenolindophenol as an electron acceptor (Table I).

### Table I

**Enzyme Activities of Mitochondria Isolated from Livers in Rats Fed Control (Group III) and Iron-Enriched Maize-Meal (Group II) Diets**

<table>
<thead>
<tr>
<th>Enzyme Activity</th>
<th>Group II (Iron-enriched maize-meal)</th>
<th>Group III (control)</th>
<th>Group II sucrose gradient fraction 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydrogenases:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DL-isocitrate (0.006 M)</td>
<td>0.042</td>
<td>0.088</td>
<td></td>
</tr>
<tr>
<td>ΔOD 660 μA/min./mg. protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malate (0.006 M)</td>
<td>0.046</td>
<td>0.083</td>
<td></td>
</tr>
<tr>
<td>ΔOD 660 μA/min./mg. protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-glutamate (0.006 M)</td>
<td>0.042</td>
<td>0.087</td>
<td></td>
</tr>
<tr>
<td>ΔOD 660 μA/min./mg. protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPNH diaphorase</td>
<td>0.452</td>
<td>0.759</td>
<td></td>
</tr>
<tr>
<td>ΔOD 340 μA/min./mg. protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPNH cytochrome C reductase</td>
<td>0.360</td>
<td>1.060</td>
<td></td>
</tr>
<tr>
<td>ΔOD 550 μA/min./mg. protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPNH oxidase</td>
<td>0.144</td>
<td>0.003</td>
<td>0.055</td>
</tr>
<tr>
<td>ΔOD 340 μA/min./mg. protein</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results in Table I also show the DPNH cytochrome C and DPNH oxidase activities in group II and group III mitochondria. In respect to these enzymes the activity of group II mitochondria (iron diet) was considerably greater than that of the normal or group III mitochondria. The difference was especially striking in the case of DPNH oxidase. In this connection it is of interest to note that the iron/protein ratio for group II mitochondria was 21.7 as compared with 0.8 for group III mitochondria (Table II). This suggests that there was a direct correlation between mitochondrial iron content and DPNH oxidase activity.

The results of the sucrose gradient fractionation in group II and group III mitochondria are illustrated in Text-figure 1. Differences in the sedimentation rates of the different mitochondrial fractions can be seen. These were obviously related to the iron content of the mitochondrial subfractions.
The results of iron determinations carried out on the subcellular fractions from the livers of rats fed control (group III) and iron-enriched maize-meal diets (group II) are presented as iron/protein ratios in Table II. It is obvious that the iron content of all the fractions from

Mitochondria Sucrose Gradient,

- IRON DIET
- NORMAL DIET

![Density gradient centrifugation of liver mitochondria in rats fed a control and a high-iron diet.](image)

TEXT-Fig. 1. Density gradient centrifugation of liver mitochondria in rats fed a control and a high-iron diet.

**Table II**

<table>
<thead>
<tr>
<th>Liver cell fraction</th>
<th>Iron/protein ratio × 10⁻⁸</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group II (iron-enriched maize-meal)</td>
</tr>
<tr>
<td>Nuclei</td>
<td>28.0</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>21.7</td>
</tr>
<tr>
<td>Mitochondrial subfraction 2*</td>
<td>12.0</td>
</tr>
<tr>
<td>Mitochondrial subfraction 3*</td>
<td>12.0</td>
</tr>
<tr>
<td>Mitochondrial subfraction 4*</td>
<td>440.0</td>
</tr>
<tr>
<td>Microsomes</td>
<td>35.8</td>
</tr>
<tr>
<td>High speed supernatant</td>
<td>162.0</td>
</tr>
</tbody>
</table>

* See Text-figure 1.

The group II rats was increased in comparison with that of the control animals. This result agrees with the electron microscopic studies, which indicated that in the final stage of the experiment iron was present both in the various cytoplasmic organelles and free in the cytoplasm.

**Discussion**

On the basis of the results of the present investigation, the pathogenesis of experimental dietary siderosis in rats may be presented as indicated in Text-figure 2.
As suggested by the finding of Gillman and co-workers, it appears that in order to provoke dietary siderosis, a high iron intake must function in the context of a grossly deficient (e.g., maize-meal) diet. Although the daily iron intake of the rats which received the control diet plus iron (group IV) was much higher than that of the rats in group II (maize-meal plus iron), as a result of the higher food intake of the former group, the group IV rats did not develop siderosis of the liver under the experimental conditions described. Gillman and co-workers used diets comparable to those selected for the present investigation and obtained identical results.

The numerous ferritin particles found in the liver sinusoids of the rats which received the iron-enriched maize-meal diet are presumably an indication of increased absorption of iron by the gastrointestinal tract in these rats, leading to an increased ferritin content of the portal blood.

The exact mechanism of uptake of ferritin molecules by the parenchymal liver cells in our rats was not completely clear. In their studies on ferritin uptake by bone marrow erythroblasts, Bessis and Breton-
Gorius\textsuperscript{18} described a process which they called "rhopheocytosis" and which was characterized by the adherence of ferritin molecules to the surface of the erythroblasts, followed by the appearance of small invaginations in the cell membrane which eventually formed vacuoles into which the ferritin molecules were incorporated. In the final stage the membranes of the vacuoles ruptured and the ferritin was released into the cytoplasm. It is obvious from Figure 4 that in the earlier stages of our experiments the peripheral cytoplasm of the parenchymal liver cells contained numerous vacuoles. As emphasized previously and as can be seen in Figure 5, however, most of the ferritin molecules were free in the cytoplasm and were not contained in vacuoles. It is, of course, possible that because of the relatively long intervals between biopsies, we only witnessed the later stages of the process of rhopheocytosis.

It seems obvious from the results of the present investigation that in the earlier stages of experimental siderosis most of the ferritin was incorporated into structures which, on the basis of their electron microscopic appearance and arrangement in the liver cells, were indistinguishable from pericanalicular dense bodies or lysosomes. This conclusion was further strengthened by the evidence of increased acid phosphatase activity in the liver cells of rats which received iron-enriched maize-meal diets. It also agrees with the results of Essner and Novikoff,\textsuperscript{17} who concluded that iron-containing granules in human liver cells were derived from lysosomes.

Although lysosomes play an important role in the pathogenesis of experimental dietary siderosis, our results indicated that, especially in the later stages, iron derived from altered mitochondria contributed toward the increased iron content of the liver cell. This finding conformed to the suggestion of Gillman and Gillman\textsuperscript{18} that "the earliest detectable lesions in African siderosis seemed to be in and around the hepatic mitochondria." The electron microscopic studies of Richter\textsuperscript{15} also indicated that dense aggregates of hemosiderin granules were often situated in "specialized cytoplasmic structures which may be derivatives of mitochondria," and for which he suggested the term "siderosomes." As has been previously remarked, the stage VI mitochondria described in the present study were comparable with the siderosomes of Richter.

There are indications in the literature that alterations in the diet may influence the size and structure of hepatic mitochondria.\textsuperscript{19} In the intact cells and in isolated cell fractions of the brown fat tissue and liver of starved rats, Lever and Chappell\textsuperscript{20} described vacuolated mitochondria which resembled the stage V mitochondria in the present study. It was furthermore of interest to note that mitochondria resembling our stage I were described by Jezequel\textsuperscript{21} in liver cells of patients with bile duct
carcinoma and viral jaundice. According to this worker the laminated structures in the mitochondria were comparable with myelin figures (dé-générescence myelinique). It seems possible that the profound dietary and metabolic changes which usually occur in patients with malignant neoplastic disease or long drawn-out illness (viral jaundice) may influence the ultrastructure of the hepatic mitochondria. Degenerated mitochondria which structurally resembled our stage VI mitochondria were described by Leduc and Wilson in the intranuclear inclusions of enlarged liver cells in mice fed a diet containing bentonite.

The above observations indicate that dietary factors may provoke ultrastructural alterations in liver mitochondria comparable with some of the degenerative changes observed in these organelles in the present investigation. It was therefore possible that the grossly deficient high-iron maize-meal diet may have initiated a series of alterations in the ultrastructure of the hepatic mitochondria which eventually led to the formation of siderosomes (stage VI mitochondria) and the release of ferritin, possibly from intramitochondrial iron-containing enzymes.

The results of the biochemical studies indicated that the structural changes in the mitochondria in the rats fed the iron-enriched maize-meal diet were accompanied by a reduction in the dehydrogenase activity of the mitochondria. It is not clear from the present investigation whether or not this decrease was more marked in those mitochondria which showed the most profound degenerative changes (i.e., stage VI mitochondria). Studies designed to test this possibility are in progress.

The reasons for the increased DPNH oxidase activity in the mitochondria of the rats fed the high-iron, maize-meal diet are unknown. It is possible, however, that the ferritin or inorganic iron in these mitochondria may have acted as a non-enzymatic catalyst in the electron transfer between DPNH and oxygen.

**Summary**

Light and electron microscopic observations of the livers in rats fed high-iron, maize-meal diets revealed that the experimental animals developed a marked siderosis of the liver. The pathogenesis of the siderosis was characterized by an increased uptake of iron by the parenchymal liver cells and the initial localization of the iron in lysosomes. A series of degenerative changes occurred within the liver cell mitochondria. These changes eventually led to the release of ferritin from the degenerated mitochondria. The structural changes were accompanied by a loss of dehydrogenase activity and an increased DPNH oxidase activity of the mitochondria.
References


The authors wish to thank Dr. F. W. E. Strelow of the National Chemical Research Laboratory for the iron analyses.

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**Legends for Figures**

Figures 1 to 3 were prepared from sections stained with the glycerophosphate-lead procedure of Gomori.

**Fig. 1.** Acid phosphatase activity in the liver of a control (group III) rat. × 85.

**Fig. 2.** Increased acid phosphatase activity in the liver of a rat fed the iron-enriched maize-meal diet (group II). Compare with Figure 1. × 85.

**Fig. 3.** Increased acid phosphatase activity in the parenchymal and Kupffer cells (arrow) in a rat fed the iron-enriched maize-meal diet (group II). × 120.

Figures 4 to 19 were all prepared from sections of the livers of the rats fed the iron-enriched maize-meal diet (group II).

**Fig. 4.** Lipid (L) and ferritin (F) particles in the sinusoid of a parenchymal liver cell. The mitochondria (M) are normal. × 20,000.
FIG. 5. Higher magnification of part of the cytoplasm of the parenchymal liver cell shown in Figure 4. Fat droplets (L) are present in cytoplasmic vesicles, while the ferritin is sometimes free in the cytoplasm (F₁) and sometimes contained in cytoplasmic vesicles (F₂). × 150,000.

FIG. 6. Aggregates of ferritin (F) are each surrounded by a single membrane in the cytoplasm of a parenchymal liver cell near the Golgi apparatus (G) and bile canaliculi (B). N = nucleus. M = mitochondria. × 25,000.
Fig. 7. A ferritin-containing lysosome (F) appears in the cytoplasm of a parenchymal liver cell. M = mitochondrion. ER = endoplasmic reticulum. × 35,000.

Fig. 8. Large numbers of ferritin-containing lysosomes (F) are present in the cytoplasm of two adjacent parenchymal liver cells. The mitochondria (M), endoplasmic reticulum (ER) and nonferritin containing lysosomes (D) are normal. B = bile canaliculus. S = sinusoid. × 15,000.
Fig. 9. A bile canaliculus (B) contains ferritin particles. The mitochondria (M) show signs of degeneration (stage IV mitochondria). G = Golgi apparatus. N = nucleus. × 26,000.

Fig. 10. Degenerative changes in mitochondria. A. Parallel osmiophilic lamellas (O) appear in the matrix of a mitochondrion. × 37,500. B. A fibrillar electron-dense area (A) is apparent in a mitochondrion. × 30,000. C. Areas of increased electron-density may be observed in a mitochondrion. × 32,000. D. The whole matrix of the mitochondrion is extremely dense. × 40,000. E. Areas of vacuolation (V) are manifest within the mitochondrion. × 25,000. F. Large vacuolated areas are demarcated by dense material containing ferritin particles. × 31,000.
FIG. 11. A large stage VI mitochondrion (M) contains ferritin particles. M1 = stage V mitochondrion. × 28,000.

FIG. 12. A higher magnification of part of the area shown in Figure 11. Ferritin particles (F) are liberated into the cytoplasm from the stage VI mitochondrion (M) or siderosomes, through gaps (arrow) or through vesicles (double arrows) formed by the outer membrane of the siderosome. × 70,000.
**Fig. 13.** Stage VI mitochondria or siderosomes (S) appear in a liver cell together with structurally normal mitochondria (M). \( \times 16,000 \).

**Fig. 14.** A stage III mitochondrion contains ferritin (F) which is more finely dispersed than the granular ferritin in the lysosomes (D). \( \times 49,000 \).
Fig. 15. Dense osmiophilic lamellas (O) are arranged parallel to the outer membrane of the mitochondrion. ER = endoplasmic reticulum. × 50,000.

Fig. 16. Large numbers of stage VI mitochondria or siderosomes (S) are present in most liver cells. Note the structurally normal mitochondria (M). × 4,000.

Fig. 17. A Kupffer cell (K) contains aggregates of ferritin in altered phagosomes (F) and free in the cytoplasm (F1). × 17,000.

Fig. 18. Collagen fibers (C) are present in the space of Disse. S = sinusoid. N = nucleus. M = dense stage IV mitochondrion. × 12,000.