THE ULTRASTRUCTURE OF ACIDOPHILIC “COUNCILMAN-LIKE” BODIES IN THE LIVER

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Electron microscopic studies suggest that acidophilic bodies in liver tissue represent focal alterations of both hepatocellular organelles and cytoplasm, and therefore originate from necrotic hepatocytes.1-4 Councilman observed vacuolated acidophilic bodies in yellow fever5 and subsequently another variety was described by Masshoff6 in the liver of a patient who died following an incompatible transfusion. Homogeneous acidophilic bodies have also been seen in human7 and animal viral hepatitis,8 in hepatomas9 and in drug reactions associated with hepatocellular damage.10 Here they appear condensed, stain bright red with eosin and lie within sinusoids or adjacent to plates of hepatocytes.11 Discrepancies in the various electron microscopic reports suggest that different stages in the formation of these bodies have been observed. In the course of electron microscopic studies of viral hepatitis and hepatomas in man and homotransplant rejection in dogs, we have observed structures with features resembling those of acidophilic bodies similar to those described by light microscopy. Their appearance and morphogenesis from entire cells suggested their origin as a form of cell death.

MATERIAL AND METHODS

Five adults with hepatitis, 2 children with hepatoma and 3 mongrel dogs, 6 to 8 days after an auxiliary liver was transplanted into the pelvis, provided tissues for study.12 In all instances liver tissue was obtained by biopsy with the Menghini needle. The tissue was fixed immediately in ice-cold 1 per cent osmium tetroxide, buffered with veronal acetate and sucrose and embedded in Epon 812. Sections were cut with a Porter-Blum Ultra-microtome, or an LKB Ultrotome, and stained with lead hydroxide or citrate. They were examined with a Hitachi HS7 or HU II A electron microscope. Thicker sections for light microscopic study were stained with toluidine blue and the periodic acid-Schiff (PAS) reagent.

RESULTS

In both canine and human tissue discrete structures resembling intact but altered hepatocytes were observed. These lay free within sinusoids, but were occasionally found in tissue spaces adjacent to

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plates of hepatocytes. Their most striking feature was increased cytoplasmic density, causing them to stand out as dark cells in contrast to adjacent tissue (Fig. 1). Sinusoidal microvilli were absent in the bodies not near normal-appearing hepatocytes; a distinct plasma membrane was, however, present and intact.

Organelles, easily recognizable within the bodies, were tightly packed. Very little glycogen was apparent, although even in the most dense bodies, a few small clusters of particulate glycogen could still be identified. Viral particles were not seen in the specimens from patients with hepatitis. Rough endoplasmic reticulum was frequently aligned in many parallel rows and free ribosomes or polysomes were common on or near these. Small vesicles of smooth endoplasmic reticulum, though present, were greatly reduced in number. Rarely there appeared vacuoles presumably derived from cisternae of endoplasmic reticulum. In addition, there were osmiophilic pigment resembling lipofuscin, fat, and vacuoles containing dense strands shown to be components of bile (Fig. 2).

Mitochondria varied in size and mitochondrial membranes were irregular and their cristae shortened. Occasional bodies were composed principally of clumped mitochondria.

The acidophilic bodies found in hepatomas displayed similar morphologic characteristics. Here, however, giant mitochondria with few cristae and a granular matrix were frequent (Fig. 3). Interspersed between the endoplasmic reticulum and mitochondria was a dense hyaloplasm; this appeared to be especially responsible for the dark appearance of the cytoplasm (Fig. 4).

Although a well defined nucleus and intact nuclear membrane were occasionally present and nucleoli were well preserved, various stages of nuclear alteration were observed. At times the nuclear membrane was irregular and indistinct and the nucleolar substance dispersed. In some instances nuclear material had reduced electron opacity and no nuclear membrane could be identified.

Although these features characterized well developed acidophilic bodies within sinusoids, "dark" hepatocytes with similar cytoplasmic and organelar abnormalities were observed within the liver cell plates. Others were separated from adjacent cells, but with microvilli on all surfaces. Some bodies were reduced in size and contained few recognizable organelles; smaller bodies, 3 to 8 μ in diameter, contained large, electron transparent areas. These were not surrounded by a membrane and had no resemblance to glycogen. There were, in addition, clumps of dense material which appeared to be fragments of acidophilic bodies. A patient with an aregenerative anemia who received multiple transfusions later developed acute serum hepatitis, and clusters of iron pigment appeared in the acidophilic bodies (Fig. 5).
Well developed acidophilic bodies were often surrounded by several Kupffer cells or large macrophages and a few lymphocytes. Portions of the macrophage cell membranes closest to the acidophilic body were extensively interdigitated, but other macrophage surfaces appeared smooth (Fig. 6). A few bodies were completely engulfed by single macrophages and the fragments were usually, but not necessarily within macrophages (Figs. 7 and 8).

**Discussion**

The ultrastructure of acidophilic bodies clearly reaffirms their hepatocellular origin since they contain rosettes of particulate glycogen and organelles similar to those in normal hepatocytes.\(^{13}\) In the course of alteration the cytoplasm of affected hepatocytes appears to become progressively more electron dense and compact, probably as a result of water loss. The dehydrated cells become reduced in size and exhibit a reduced content of glycogen and ribosomes. Eventually condensation of the cytoplasm results in a mummified cellular appearance. The cells become separated from their neighbors and then lose continuity with the hepatic cell plate. Thus they are found free in the tissue spaces or at times even in sinusoids.

Neither autophagic vacuoles nor focal alteration of hepatocellular elements were observed, suggesting that the formation of the acidophilic body involved a sudden change of the whole cell and did not result from sequential focal degeneration of cytoplasm.

Nuclear alterations tended to parallel the degenerative changes in the cytoplasm. Nuclei, however, were frequently preserved within well developed acidophilic bodies. Therefore, alteration of nuclear function did not appear to be a primary event in the formation of the body as has been suggested.\(^{8}\)

Macrophages in close proximity to the bodies with cytoplasm applied to their surfaces probably take part in the reabsorption of water and possibly other soluble cytoplasmic constituents.

After advanced reduction in size, the bodies appear to break up and their fragments are engulfed and digested. The remnants appear as phagosomes in macrophages. The formation of acidophilic bodies is therefore a form of cell death, although it is impossible to define the stage in its formation when loss of viability actually occurs. They are not unique to man nor do they appear to reflect a specific etiologic agent particularly since no viral particles were found. A relationship between the “true” Councilman body found in yellow fever and the acidophilic bodies described here cannot be ascertained because of the lack of published detailed electron microscopic studies in yellow fever. The acidophilic body is an uncommon form of cell damage, possibly the end result
of irreversible cytoplasmic dehydration, which in its reversible stages appears as a “dark cell.”

Summary

Acidophilic bodies were examined by electron microscopy in liver tissue in human viral hepatitis, human hepatoma and in dogs with auxiliary hepatic transplants. The bodies apparently represent a form of hepatocellular injury which can occur in species other than man and which does not indicate any specific etiologic agent. Acidophilic bodies constitute entire cells containing condensed hyaloplasm. These are separated from their neighbors and surrounded by macrophages and may represent a later stage of the so-called “dark cells.”

References

[Illustrations follow]
LEGENDS FOR FIGURES

FIG. 1. Hepatic homotransplant, dog. A discrete body appears more dense and partially separated from a surrounding plate of parenchymal cells. Mitochondria and elements of endoplasmic reticulum can be identified within the body. There is also a pigment resembling lipofuscin (arrow). Lead citrate stain. × 3,800.

FIG. 2. Human viral hepatitis. A typical "Councilman body" contains a well-defined nucleus and dispersed nucleolar material. The endoplasmic reticulum is aligned in parallel rows and a few clusters of particulate glycogen are still present (arrow). Osmiophilic strands resembling bile pigment are also found. Lead hydroxide stain. × 8,000. Inset: A thick section shows an acidophilic body and an adjacent macrophage within the sinusoidal space. PAS and toluidine blue stain. × 1,000.
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Fig. 3. Human hepatoma. Mitochondria vary in size and large mitochondria with granular matrices and few cristae are prominent. Lead citrate stain. \( \times 15,000 \).

Fig. 4. Hepatic homotransplant, dog. Both smooth and rough endoplasmic reticulum are preserved. Between packed mitochondria is a dense hyaloplasm. Lead citrate stain. \( \times 8,000 \). Inset: Conventional microscopy of the same tissue shows an acidophilic body within a sinusoid. A macrophage lies in close proximity. PAS and toluidine blue stain. \( \times 1,000 \).
Fig. 5. Viral hepatitis, systemic iron overload. Two bodies, 3 to 5 μ, contain large transparent areas resembling neither glycogen nor clumps of dense material. Distinct organelles can no longer be identified; iron granules, however, are frequent. Lead citrate stain. \( \times 50,000 \).

Fig. 6. Viral hepatitis. Macrophages adjacent to an acidophilic body exhibit extensive membrane interdigititation. Lead citrate stain. \( \times 18,000 \).
Fig. 7. Viral hepatitis. A portion of a macrophage contains many engulfed fragments of acidophilic bodies. These are surrounded by membranes and resemble phagosomes. Lead citrate stain. × 50,000.

Fig. 8. Viral hepatitis. A large macrophage with many interdigitating membranes contains a condensed fragment of an acidophilic body. A few elements of endoplasmic reticulum remain. In the same field the cytoplasmic processes of a macrophage are closely applied to a portion of a well developed, acidophilic body. Lead hydroxide stain. × 10,000.