

ASPECTS OF THE FINE STRUCTURE OF LIGHT AND DARK CELLS IN THE INTRAHEPATIC BILE DUCT EPITHELIUM OF THE MOUSE*

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Electron microscopic observations have been made of the two epithelial cell types, light and dark cells in the intrahepatic bile ducts of the mouse.

The cytoplasm of the light cells is found to be low in electron opacity and contain more or less degenerative mitochondria and elements of granular endoplasmic reticulum, rather copious Golgi apparatus and numerous lysosome-like dense bodies. These ultrastructural aspects of the light cells are conceived to indicate that they represent a degenerative cell type with declining functional activity.

The dark cells are characterized by an electron opaque cytoplasm in which an appreciable number of mitochondria, an abundance of free ribosomes and numerous bundles of intracellular fibrils are detected. These and other features of the dark cells are thought to imply that they are a peculiar cell type with relatively high synthetic and metabolic activities. It remains, however, to be elucidated what substances are synthesized actively in this cell type.

In his review based upon light microscopy of the liver and bile drainage system of vertebrates, Pfuhl¹¹⁾ recorded that light and dark cells are demonstrable among ordinarily stained cells in the intrahepatic bile duct epithelium. Since the advent of precise cytological techniques using electron microscopy, however, very little has been known about the ultrastructure of these two unique epithelial cell types. To the present author's knowledge, the only related reports are concerned with the electron microscopy of a dark cell with cytoplasm and nucleus of high electron opacity induced by various experimental means in the intrahepatic bile ductular and ductal epithelium of rodents (Carruthers and Steiner¹⁾, Grisham and Porta³⁾, Steiner, Carruthers and Kalifat¹⁵⁾, Steiner¹⁶⁾ Steiner and Carruthers¹⁷⁾).

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In the course of the author's recent electron microscopic observations on the intrahepatic bile duct epithelium of normal mice, the presence of light and dark cells were recognized. In view of the little information on this line of the biliary epithelium cytology, the present study describes some aspects of the ultrastructure of the two unique epithelial cell types occurring in the intrahepatic bile duct epithelium of normal mice, and discusses their functional activities on the basis of the results obtained.

MATERIALS AND METHODS

Fourteen adult mice of both sexes from two inbred strains (BUA and BUB) were used in the present study. Tissue pieces were dissected out from the deep and hilar regions of the liver of the donors and put into drops of chilled 1.25 percent phosphate buffered glutaraldehyde fixative (Modification of Sabatini, Bensch and Barnett¹⁴), on a sheet of dental wax and cut into tiny pieces of about a cubic millimeter. The tissue pieces were immersed in the same fixative for 20 minutes under constant agitation. They were then refixed for 2 hours in chilled 2 percent phosphate buffered osmium tetroxide solution (Millonig⁷) also under agitation. The tissues were dehydrated through an acetone series of ascending concentration and embedded in Epoxy-Epon mixture by the methods recommended by Luft⁶.

In order to identify the tissue blocks which contain relatively large intrahepatic bile ducts with diameters of approximately 70 to 150 μ , thick sections (0.5 to 2.0 μ) of the full face of the blocks were cut with glass knives on a Porter-Blum microtome, mounted on glass slides and stained with 0.1 percent toluidine blue in 2.5 percent sodium carbonate according to the technique devised by Trump, Smuckler and Benditt¹⁸. After trimming of the blocks identified to contain the bile ducts, silver grey colored thin sections were cut from the blocks on the same microtome and mounted on naked grids. The sections were stained with lead hydroxide (Karnovsky⁹) or with lead tartrate (Millonig⁸). The specimens were then examined in HITACHI HU 11 A and RCA EMU 3 F electron microscopes. Electron micrographs were taken at original magnifications of 3,000 to 10,000 times and were enlarged according to needs for individual observations.

RESULTS

Light microscopy:

In the mouse the epithelium lining the intrahepatic bile ducts consists of a single layer of low columnar or cuboidal cells, as illustrated in Fig. 1. The epithelial cells are uninucleate and the majority of them have nucleus and cytoplasm which exhibit moderate toluidine blue stainability and deserve the desig-

nation of "ordinary epithelial cells". Among these ordinary cells, there occur two types of unique cells showing different stainability of their cytoplasm and nucleus. One type is appropriately called "light cells", since its nucleus and cytoplasm reveal a lighter staining than those of ordinary cells (Fig. 1). The other cell type has deeply stained nucleus and cytoplasm and may hence be named "dark cells" (Fig. 1). In addition to the peculiar stainability of their nucleus and cytoplasm, the both cell types tend to show shapes which likewise characterize them. Thus, light cells tend to be stout and barrel-shaped, whereas dark cells are often thin and rod-shaped.

Electron microscopy:

When the epithelial tissues are examined by electron microscopy, three cell types, ordinary, light and dark cells are observed, which must be comparable to those recognized light microscopically.

The ordinary epithelial cells are found to have nuclear and cytoplasmic ultrastructures which are not very unusual (Fig. 2). The nucleus is, however, irregularly contoured, ovoid in shape and occupies a considerably large portion of the cell body (Fig. 2). The cytoplasm is enclosed by a plasma membrane undergoing microvillous specializations, and contains not uncommon components such as mitochondria, free ribosomes, elements of granular endoplasmic reticulum, components of the Golgi apparatus, lysosome-like dense bodies and so forth (Fig. 2). In the present observations, detailed descriptions will not be made of the ultrastructure of such ordinary epithelial cells of the intrahepatic bile ducts, because they have been reviewed well in a recent record by Rouiller and Jézéquel¹³. The following observations are therefore directed mainly to the ultrastructural appearances of light and dark cells occurring in the bile duct epithelium.

(A) The ultrastructure of light cells:

With a low magnification of the epithelium light cells are found to have a rather voluminous clear cytoplasm containing a relatively weakly or moderately stained nucleus which is irregularly ovoid in shape (Fig. 3). Besides its low electron opacity, the cytoplasm appears to be characterized by the presence of relatively numerous lysosome-like dense bodies and poorly developed cell organelles such as mitochondria and elements of endoplasmic reticulum (Fig. 3).

High magnifications of this cell type disclose that many cell organelles are more or less degenerative throughout the cytoplasm (Figs. 4, 5 and 6). In both the distal (Figs. 4 and 5) and basal (Figs. 5 and 6) cytoplasm, mitochondria are relatively few in number and undergo various types of degeneration and disintegration; some mitochondria are rugged in outline and exhibit irregularly distorted arrangement of their cristae and lowered density of their matrix

(Figs. 4 and 5), while others display a vacuolar swelling of their intercrystal compartments (Fig. 6). Likewise, the elements of the granular endoplasmic reticulum are not well developed, and their usual form is not of flattened cisternae, but of vesicles of different sizes disseminated in the distal (Fig. 4) and basal (Fig. 6) cytoplasm. Throughout the cytoplasm the amount of free ribosomes is variable, and in some cytoplasmic loci ribosomes are distributed fairly densely, whereas in the rest of the cytoplasm they are not very concentrated and occasionally absent (Figs. 4, 5 and 6). In contrast to the ultrastructural features of these cell organelles, the Golgi apparatus of the cells is relatively pronounced in development. Thus, this organelle is detected mostly at the supranuclear area and consists usually of an aggregation of vesicles of different diameters (Fig. 4). The substances enclosed in these Golgi vesicles varies in electron density; however, smaller vesicles tend to contain a denser substance than larger ones. The cytoplasm of light cells shows another ultrastructural feature which characterizes them. This is an abundance of lysosome-like dense bodies in the cytoplasm (Figs. 4 and 5). These bodies are mostly spherical or oval in shape and measure an average diameter of 400 to 600 m μ . They are naked or membrane limited and contain dense granules and vesicles imbedded in a little less dense matrix. They occur not only in the distal (Fig. 4) but basal (Figs. 4, 5 and 6) cytoplasm, although the cytoplasmic loci of their most frequent occurrence are the Golgi and associated areas (Fig. 4). In these cytoplasmic areas a number of figures are seen which suggest various stages of lysosome-like body formation (Fig. 4). It seems likely that at the beginning of the formation Golgi vesicles containing a dense substance are accumulated and surrounded by a dense cytoplasmic ground substance and such combination of Golgi and cytoplasmic ground substances must be a core of lysosome-like dense bodies which appears to develop into their mature form following further accumulation and addition of the substances. In the distal cytoplasm of some light cells multivesicular bodies occur and they consist of a number of vesicles imbedded in a moderately dense matrix, limited by a membrane and surrounded by variously sized vesicles which may perhaps belong to components of the Golgi apparatus (Fig. 5). Since some vesicles of such multivesicular bodies are very high in electron opacity, it is likely that these bodies also represent an immature stage of lysosome-like dense bodies.

In addition to the cell organelles above mentioned, the cytoplasm of light cells is at times loaded with lipid droplets of an average diameter comparable to that of lysosome-like dense bodies (Fig. 6). In occasional cytoplasmic loci of light cells, further, fine fibrillar textures of the ground substance are discernible (Fig. 6).

As regards the surface specialization in plasma membrane, such as microvilli and intercellular digitation, the light cells are nearly identical with ordinary

epithelial cells. However, in the apical cytoplasm there are occasionally seen accumulations of vesicles which may perhaps be induced by the apical pinocytotic activity of the cells (Figs. 4 and 5).

(B) *The ultrastructure of dark cells:*

When examined with a low magnification, the dark cells of the intrahepatic bile duct epithelium are easily recognized by the high electron density of their nucleus and cytoplasm (Fig. 7). Although these cells are prone to be thin and rod-shaped, this is not necessarily the case with all individuals of this cell type. Some of the cells are not thin, and have instead a width of columnar cell body which falls within the normal range of ordinary epithelial cells.

With higher magnifications, it seems possible to elucidate what ultrastructures contribute to the high electron opacity of the cytoplasm. In the distal (Figs. 8 and 9), paranuclear (Fig. 10) and basal (Fig. 11) cytoplasm of dark cells, three morphoplasmic components are noted which are responsible for the high electron density of the cytoplasm. These are (1) mitochondria, (2) free ribosomes and (3) intracellular fibrils.

As illustrated in Figs. 8 and 11, mitochondria are spherical, rod-like or oval in shape and are apparently appreciable in number in the distal and basal cytoplasm. They are, however, not unusual in ultrastructures such as cristalline arrangements and matrix density. The distal (Fig. 8) and basal (Fig. 11) cytoplasm of dark cells is packed with free ribosomes, while occasionally the apical part of the cytoplasm being nearly devoid of them (Fig. 8). Another prominent feature of the cytoplasm of dark cells is the presence of numerous bundles of intracellular fibrils (Fig. 9). Although such fibrillar bundles are not abundant in every dark cell, they are, when present in a substantial amount, certainly responsible for providing the cytoplasm with a high electron opacity. As is shown in Fig. 9, these fibrils are running in every direction in the cytoplasm and tend to surround cell organelles such as mitochondria and cisternae of granular endoplasmic reticulum (Fig. 9). While the three types of cytoplasmic constituents mentioned are responsible for the high electron density of the dark cell cytoplasm, it seems likely that the ground substance itself is, likewise, of a high density, giving rise to the darkness of the cytoplasm (Figs. 8, 9 and 11).

In the cytoplasm of dark cells the elements of granular endoplasmic reticulum appear as flattened cisternae which sometimes display branchings (Figs. 8 and 9). Occasional cisternae of this organelle are dilated to a varying degree and enclose a homogeneous substance of low density in which tiny granular patches are at times demonstrated (Fig. 9). The granular endoplasmic reticulum cisternae are often in close topographical relation to mitochondria; the former being in direct contact with or in close vicinity to the latter (Figs. 8

and 9). In the supra- and paranuclear cytoplasm of dark cells the Golgi apparatus is discerned which is composed of flattened sacs and associated vesicles and small vacuoles (Figs. 8 and 10). The flattened sacs are in continuity with some vesicles and vacuoles at both extremities. The substances within the vacuoles tend to be lower in density than those included by vesicles (Fig. 10). In the cytoplasmic areas close to the Golgi apparatus membrane-limited spherical bodies are often found. They are of a diameter ranging from 200 to 700 $m\mu$. They may broadly be grouped into two types on the basis of their ultrastructural appearances. One type deserves the name of lysosome-like bodies, inasmuch as it comprises very dense granules and droplets imbedded in a similarly dense matrix (Fig. 8). The other may appropriately be designated to be multivesicular or multigranular bodies, based upon its content (Fig. 10). The vesicles and granules within the bodies exhibit different electron opacities and the matrix shows a variety of local density (Fig. 10). In view of their features, these multivesicular and multigranular bodies may perhaps be an immature form of the lysosome-like dense bodies, as in the case with light cells.

The plasma membrane enclosing the cytoplasm of dark cells undergoes various specializations common to those of ordinary epithelial cells. Similar to light and ordinary epithelial cells, the dark cells appear to perform resorptive function by means of pinocytosis, because pinocytotic invaginations and resultant vesicles are often seen at the apex of the cytoplasm (Figs. 8 and 9).

DISCUSSION

The Pfuhl's description¹¹⁾ of light and dark cells in the intrahepatic bile duct epithelium was derived from the light microscopic observations, and few authors have since suspected the two types of cells to be a result of artefacts due to fixation in the course of tissue preparation. A recent study on the pancreatic acinar and ductal tissues of the guinea pig and mouse (Ichikawa¹⁴⁾) raised the possibility that light and dark cells in electron microscopic specimens may be a product of fixation artefact during the tissue preparation procedures. In the present study, however, light and dark cells are found to exhibit qualitative peculiarities such as the presence of numerous lysosome-like dense bodies and few degenerative mitochondria in the former, and the occurrence of a substantial amount of free ribosomes and numerous mitochondria in the latter. It is almost impossible to regard such qualitative characteristics in ultrastructure as an artefact due to fixation. Therefore, the above possibility is to be excluded, as far as the present two types of the bile duct epithelial cells are concerned.

In the light cells observed here, the more or less degenerative features of cell organelles such as mitochondria and elements of granular endoplasmic reticulum are taken to imply a lowered metabolic and synthetic activity in the

cytoplasm. The rather pronounced development of the Golgi apparatus in the cytoplasm seems inconsistent with this concept. In the cytoplasm of the light cells, however, an abundance of lysosome-like dense bodies together with figures suggestive of their formation in close association with the Golgi components are observed. Therefore, the relatively pronounced development of the Golgi apparatus must be correlated with the presence of numerous lysosome-like dense bodies. As has been advocated by Novikoff⁹⁾ and Novikoff, Essner, Goldfischer and Hues¹⁰⁾, the components of the Golgi apparatus are believed to be an origin of lysosomes. Thus, it appears certain that the present lysosome-like bodies are really lysosomes. If this presumption is correct, the latent activity of intracellular digestion must be high in the cytoplasm of light cells, in view of the lytic enzyme content of lysosomes (De Duve²⁾). Such high lytic activity potential of the light cells is, in turn, compatible with their degenerative organelles, which may possibly be digested by the numerous lysosomes and may thus suggest the cell death in not distant future.

From what has been observed and discussed of the light cells of the bile duct epithelium, it may be stated that they represent a degenerative cell type with declining functional activity. A light cell containing a number of lysosomes of pinocytotic origin has been reported to exist in the gall bladder epithelium of the mouse and concluded to be degenerative in nature on the basis of not only histochemical (Yamada¹⁹⁾) but electron microscopic (Yamada²⁰⁾) observations on it. While the light cells of the gall bladder and bile duct epithelia are different from each other in the origin of the majority of their lysosomes and consequently in degree of cytoplasmic hydration, it deserves attention that they show a common tendency to be degenerative. In this connection, it seems safe to conclude that the epithelial cells of the bile drainage system of the mouse display similar ultrastructural and functional aspects, when they, after repeated cycles of their functioning, are degenerated and disintegrated.

In the intrahepatic bile ductular and ductal epithelium of the rat, dark cells with cytoplasm and nucleus of high electron opacity have been described following biliary obstruction (Carruthers and Steiner¹⁾, Grisham and Porta³⁾, Steiner, Carruthers and Kalifat¹⁵⁾, Steiner¹⁶⁾) after carbon tetrachloride administration (Carruthers and Steiner¹⁾) and following various dietary injuries and partial hepatectomy (Grisham and Porta³⁾, Steiner¹⁶⁾, Steiner and Carruthers¹⁷⁾). The increased density of the cytoplasm of these cells was reported to be due to a large amount of such cytoplasmic components as ribonucleoprotein particles and intracellular fibrils. The cells were conceived to occur as a result of a sudden discharge of secretion on the one hand (Steiner, Carruthers and Kalifat¹⁵⁾), while they were regarded as atrophying cells on the other (Grisham and Porta³⁾). In the dark cells of the present study an appreciable number of mitochondria,

an abundance of free ribosomes and numerous bundles of intracellular fibrils are observed. The abundance of free ribosomes and numerous bundles of the fibrils are common to the cytoplasm of the dark cells reported to occur in the biliary epithelium under various experimental conditions. Accordingly, it is possible that the present dark cells are similar in some functional activities to those of the epithelium reported previously. The previous concept that the dark cells are regarded as being induced by a sudden discharge of secretion¹⁵⁾ or atrophying cells⁹⁾, is, however, not necessarily true with the present dark cells. It is, instead, reasonable to regard them as being relatively high in metabolic and synthetic activities, in view of the presence of an appreciable number of mitochondria and a substantial amount of free ribosomes in the cytoplasm. The presumably high synthetic activity may be reflected in the particular ultrastructural features of the granular endoplasmic reticulum cisternae undergoing a varying degree of dilatation with homogeneous and granular substances, because of the role of storing and transferring cellular synthetic products to other cytoplasmic loci played by the reticulum (Porter¹²⁾).

In the biliary epithelial cells the functional significance of the intracellular fibrils remains uncertain, except for a tentative idea that they may be contractile in function to assist the transport of bile (Yamamoto²¹⁾). It is, therefore, difficult to correlate the numerous bundles of intracellular fibrils reasonably with the presumably high metabolic and synthetic activities in the dark cells. Furthermore, it awaits further study to elucidate what substances are being synthesized rather actively in the dark cells observed here. As a possible proteinaceous substance produced as a result of the high synthetic activity, lysosomal substances may be proposed, in view of the fact that mature and immature lysosome-like dense bodies are often found in the cytoplasmic areas close to the Golgi apparatus of the dark cells.

On the basis of all the available evidences of the dark cells, it can be advocated that they are a peculiar cell type characterized by rather high synthetic and metabolic activities.

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EXPLANATION OF FIGURES

- Bl: Basal lamina
- Ct: Connective tissue
- Dc: Dark cell
- Er: Granular endoplasmic reticulum
- F: Intracellular fibrils
- G: Golgi apparatus
- Hp: Hepatic parenchyma
- L: Lumen
- Lc: Light cell

- Ld: Lipid droplet
 Ly: Lysosome-like dense body
 M: Mitochondria
 Mb: Multivesicular body
 Mv: Microvilli
 N: Nucleus
 Oc: Ordinary epithelial cell
 Pv: Pinocytotic vesicle
 Rnp: Free ribosome
 T: Terminal bar

- FIG. 1. Part of a portal canal of the liver in a mouse. An oblique section of an intrahepatic bile duct is shown, and in the lining epithelium light and dark cells are interposed between ordinary epithelial cells. Toluidine blue stained photomicrograph. $\times 450$.
- FIG. 2. Part of the epithelium lining the intrahepatic bile duct of a mouse. Two ordinary epithelial cells are seen, which have a cytoplasm exhibiting not very unusual ultrastructures. Lead hydroxide stained electron micrograph. $\times 7,000$.
- FIG. 3. Part of the epithelium lining the intrahepatic bile duct of a mouse. Light cells are found to have a clear cytoplasm in which lysosome-like dense bodies are numerous. Lead tartrate stained electron micrograph. $\times 6,800$.
- FIG. 4. Part of the cytoplasm of a light cell. In the distal half of the cytoplasm degenerative mitochondria and distinguished Golgi vesicles are noted, whereas free ribosomes and lysosome-like dense bodies are distributed throughout the cytoplasm. Lead hydroxide stained electron micrograph. $\times 20,000$.
- FIG. 5. Part of the cytoplasm of a light cell. In the cytoplasm few degenerative mitochondria, vesicles of granular endoplasmic reticulum, free ribosomes, numerous lysosome-like dense bodies and three multivesicular bodies are demonstrated. Lead hydroxide stained electron micrograph. $\times 23,500$.
- FIG. 6. Basal part of the cytoplasm of a light cell. Mitochondria undergoing a vacuolar swelling of their intercrystal compartments, vesicles of granular endoplasmic reticulum, free ribosomes, lysosome-like dense bodies are distinguished. Lead hydroxide stained electron micrograph. $\times 26,600$.
- FIG. 7. Part of the epithelium lining the intrahepatic bile duct of a mouse. A dark cell is in abutment upon ordinary epithelial cells. Its nucleus and cytoplasm are higher in electron density than those of ordinary cells. Lead hydroxide stained electron micrograph. $\times 6,800$.
- FIG. 8. Distal and paranuclear parts of the cytoplasm of a dark cell abutting upon an ordinary epithelial cell. The cytoplasm is characterized by the presence of numerous mitochondria and an abundance of free ribosomes. Lead hydroxide stained electron micrograph. $\times 28,500$.
- FIG. 9. Distal part of the cytoplasm of a dark cell. Mitochondria, free ribosomes, dilated cisternae of granular endoplasmic reticulum and bundles of intracellular fibrils occupy the majority of this part. Arrow indicates a pinocytotic invagination. Lead hydroxide stained electron micrograph. $\times 32,500$.
- FIG. 10. Paranuclear part of the cytoplasm of a dark cell adjacent to an ordinary epithelial cell. In the upper half of the figure Golgi apparatus with its related bodies of multivesicular appearance is observed. Lead hydroxide stained electron micrograph. $\times 26,000$.
- FIG. 11. Basal part of the cytoplasm of a dark cell adjoining an ordinary epithelial cell. The cytoplasm is packed with free ribosomes and mitochondria. Lead tartrate stained electron micrograph. $\times 26,800$.









