REVIEWS

Liver Cells in a Comparative Morphological Series of Animals: Ultrastructural Features and Their Significance

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A detailed ultrastructural analysis of liver cells in a comparative morphological series of animals from various classes has provided insight into how liver structure was perfected in the course of evolution. Specific features of the adaptive responses acquired by hepatocytes in different animal classes for maximizing their many functions in the framework of the existing organ structure are identified. It is shown that the adaptations found in lower animals also normally occur in more highly organized animals, and that the same adaptations developed by hepatocytes during phylogeny are used in responding to various influences exerted upon the organism.

Key Words: *hepatocyte; ultrastructure; adaptations; phylogeny*

Perusal of the relatively large body of published ultrastructural data on liver cells of fish, amphibians, reptiles, birds, and mammals [16-27] shows that although liver cells in animals of different classes share a common set of organelles, these cells vary in structure both between and within classes. However, the specific features of their fine structure have rarely been discussed.

Drawing on the reported evidence and on our own numerous findings from studies of liver cells in various animal classes, we propose here to analyze comparative ultrastructural features of liver cells with particular reference to the adaptations these cells have developed. The basic elements we have taken for comparison are the hepatocyte (HC), bile capillary, sinusoidal cell, and sinusoid, i.e., the elements which, by combining in different ways in the livers of animals from different classes, make up this complex multifunctional organ. Analysis of liver cell ultrastructure in a comparative morphological series of animals may be expected to shed light on how the structure of such a complex and perfect organ as the mammalian liver was refined and improved in the course of phylogeny and to identify the attributes with which liver cells of different animals have been endowed for maximizing their many functions in the framework of the organ structure existing in particular animals.

Livers of the following animals were examined under the electron microscope: lancelet (*Amphioxus*); sexually mature perch, pike, grass carp (*Ctenopharyngodon idella*), and silver carp (*Hypophthalmichthys molitrix*); brown frog (*Rana temporaria*) and Siberian salamander (both adult individuals and those undergoing metamorphosis); sand lizard (*Lacerta agilis*) and polydont snake; great black racer (during embryogeny); chicken (adults and embryos); pigeon; rat (adults and embryos); musk rat, least shrew (*Sorex minutissimus*), polar bear, musk deer, elk (*Alces alus*),

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European bison, and American manatee (*Trichechus manatus*).

The lancelet liver appears as a blind hollow outgrowth which opens into the midgut lumen and is lined with large hepatic cells whose height exceeds their width 4 to 5 times and where a basal and an apical zone are clearly distinguishable. The cytoplasm in the middle part of the cell contains a nucleus over which a Golgi complex is located, as well as a few small mitochondria and cisterns of the granular endoplasmic reticulum (GER). The apical zone contains inclusions, which are bile products, and typical cilia; the cell membrane in this zone gives off microvilli. The cell membrane in the basal zone is smooth and rests on a thick basal layer behind which endothelial cells are seen; lateral cell membranes in this zone are interlocked. The large liver cells, the thick basal layer, and the absence of folds in the basement membrane create serious obstacles to rapid movement of metabolites from the blood to the gland's cavity. Adaptive devices in the lancelet liver are probably the broad intercellular spaces, which we discovered in the apical zone and which extend over two-thirds of the cell's height to serve as a medium for the movement of metabolites, and also the microvilli and cilia present on the apical surface to facilitate the movement of contents from the gland.

Starting with the Pisces, the liver is a parenchymal organ consisting of several unequally sized lobes; in snakes it extends as a strand along the entire body.

Tracing interclass differences in the relationships of HCs with one another, with bile capillaries, and with blood was facilitated by our ultrastructural studies of liver cells in various physiological states and at different stages of ontogeny. The livers of reptiles and birds in embryogeny, of fish in early postnatal ontogeny, and of tailless amphibians during metamorphosis all have a well-defined tubular structure, with transversely cut tubules made up of 3-5 HCs each and having a bile capillary in the center that is clearly seen under the electron microscope against a background of broad sinusoids; the HCs have an obviously bipolar structure. The tubular structure of the liver persists in these animals throughout the postnatal ontogeny, but the sinusoids become narrower because of an increase in the mass of hepatic parenchyma, while the distance between the tubules progressively shortens and the tubules come in contact with each other; in the area of their junction, HC membranes form new bile capillaries and the HCs become multipolar.

A feature of fish livers is their highly developed depositing function: glycogen in large amount and numerous lipid droplets occupy most of the HC cytoplasm, while mitochondria and GER cisterns are scarce and the Golgi complex is small and is situated around the nucleus and at the periphery of the HC. In the grass and silver carp, which are herbivores, glycogen was detected not only in the HC cytoplasm, but also in the intercellular spaces and, as previously noted by other workers [20], sinusoidal cells. Bile capillaries and sinusoids were not always apparent in ultrathin sections of livers from adult fish. It looked as if not all HCs were in contact with blood, particularly in specimens where the center of the tubules composed of HCs was a sinusoid with HCs lying around it in several layers. This arrangement was best defined in the livers from hungry fish because their HCs are smaller and more widely separated from one another than in fed fish [21]. In hungry fish, HCs appear to make contact with each other only at sites where bile capillaries are formed and surrounded by powerful desmosomes with tonofilaments penetrating into the cytoplasm. The formation of intercellular spaces may be interpreted as an adaptation to permit movement of metabolites from HCs to sinusoids, for not every HC is in direct contact with blood in fish. Desmosomes, too, may be viewed as an adaptation, since they help to preserve the integrity of bile capillaries even when the fish is hungry and the HCs are further apart. The separation of HCs to form wide intercellular spaces appears to be associated with the poor development of the hepatic stroma in the fish species studied. This view is supported by the observations of other authors that desmosomes between liver cells are absent in fish species where Disse's spaces contain many collagen fibers but are abundant in those whose Disse's spaces have no collagen fibers [23]. Another manifestation of adaptive responses in the liver of hungry fish is, in our view, clasmatosis of glycogen-containing cytoplasmic fragments into sinusoid lumens [4], since in view of the fact that their depositing function is highly developed, fish livers are low in enzymes of glycogenolysis and so glycogen is expended slowly, even during starvation [12].

A notable feature of fish livers is absorption by sinusoidal cells of undigested HC remnants (cytosegresomes) released into Disse's spaces from the cytoplasm. Although in fish, as in higher vertebrates, cytosegresomes are predominantly released into the bile via bile capillaries, this function appears to be taken up also in part by sinusoidal cells as the number of bile capillaries is relatively small in fish. This adaptive feature of fish HCs appears to have a bearing on the origin of melanomacrophagal centers. In the spleen, kidneys, and liver of fish, these centers are composed of sinusoidal macrophages that have engulfed foreign material or remnants of dead erythrocytes and other cells [26].

Livers of herbivores such as grass and silver carp were found to have a bile secretion system quite different from that in the livers of pike, perch, and many other carnivorous fish species in which bile capillaries are formed by membranes of adjacent HCs [5]. In the herbivores studied, each bile capillary is formed by the membrane of only one HC, and the bile products being released into the capillaries enter bile ducts formed by peculiar smaller cells, i.e., the bile excreting system is autonomous even at the very beginning of bile outflow. Probably, this is also a form of adaptation, for the excretory ducts, being autonomous, are not adversely affected by starvation, which results in greatly decreased liver cell sizes and a consequent widening of the intercellular spaces. The bile excretion system in the class Pisces is thus variable.

Amphibian livers, like those of fish, consist of intertwining and anastomosing tubules, but bile capillaries and sinusoids occur in larger numbers and can always be seen in an ultrathin section. The basal layer of sinusoids is discontinuous, as it is in fish, but microvilli on the sinusoidal HC surface are invariably present — in contrast to livers of herbivorous fish, where pinocytotic vesicles provide the only evidence that substances are absorbed from the sinusoids. The junctions between HCs are much stronger than in fish and, moreover, starvation does not cause the formation of large intercellular spaces, probably because the reticular stroma is larger and stronger. The decrease in liver cell size during starvation is accompanied by a rise in the number of minute "locks" formed by adjacent HCs from folds of their membranes [7]. The HC contains relatively large amounts of glycogen and fat, particularly in the summer and fall, i.e., the liver continues to function as a store of reserve substances in amphibians too. In the Siberian salamander, virtually all of the HC cytoplasm is filled with glycogen, which is an adaptation to life under harsh environmental conditions [7].

A hallmark of amphibian livers is the presence of numerous pigment cells whose number is greater in winter than in summer. Although pigment is known to be synthesized and to accumulate in sinusoidal Kupffer's cells [17], the reasons for the formation of pigment cells are not clear. In our ultrastructural studies of liver cells from hibernating frogs or those undergoing metamorphosis, during which tadpole erythrocytes are completely replaced by erythrocytes found in adult frogs, a variety of morphological pictures were observed, indicating that Kupffer's cells take up dead erythrocytes, transform them, and then become converted into pigment cells themselves [6]. In comparing our morphological findings with reported data on the synthesis and distribution of melanin granules in Kupffer's cells, we came to the conclusion that at least one source of pigment granules is erythrophagocytosis. This conclusion is supported by the presence in pigment granules, in addition to melanin, of hemosiderin, which arises from ferritin as a hemoglobin degradation product. The role of pigment cells as a depot for catabolic products thus becomes evident. The metabolic end-product melanin is a powerful antioxidant and protects liver cells from free oxygen radicals by acting similarly to superoxide dismutase, an enzyme which performs this protective function in unpigmented cells.

The sand lizard, polydont snake, and crocodile [24] livers that have been examined all have a well-defined tubular structure, with bile capillaries being each formed by 2-4 HCs. In the sand lizard, the depositing function of the liver is better expressed than in the polydont snake: glycogen and fat occupy the central part of the HC cytoplasm, while the mitochondria and GER are concentrated around the nucleus, sinusoid, and bile capillary. Our observations and those of other workers [24] indicate that reptilian livers have a discontinuous basal layer. In our studies pigment cells were rarely found, whereas erythrophagocytosis was repeatedly observed.

In birds, the liver also consists of intertwining and anastomosing tubules at whose junctions bile capillaries are formed by HCs. In embryogeny, the organ is represented by classic tubules each made up of 6-8 trapezoidal HCs and having a bile capillary in the center. The livers of adult birds have many bile capillaries between contacting HCs because of a very high degree of tubular intertwining; inter-HC junctions are strong, in the form of "locks", and the HCs are polygonal. A substantial progressive change seen in the birds we studied is the disappearance of the basal layer in the sinusoids, which considerably facilitates exchanges between the HCs and blood, although a basal layer is present in the liver of certain birds [22].

Liver structure in mammals is different. Tubular in early embryogeny, mammalian livers acquire a laminar structure in the course of postnatal ontogeny, with bile capillaries being predominantly formed by the membranes of two HCs (rather than 2 to 5 as in the preceding species) and, as seen in ultrathin sections, with each HC contacting a sinusoid on several sides; the number of capillaries per cell is also larger. Except in ruminants [16], there is no basal layer in the liver. Glycogen in avian and mammalian livers is found only in limited areas of the perisinusoidal zone of the HC cytoplasm, which suggests a very dynamic supply and expenditure of this polysaccharide.

Our electron microscopic studies revealed adaptive features at the HC level in various mammals. The

GER in HCs of omnivores is developed much better than the agranular endoplasmic reticulum (AER), whereas the reverse is true of HCs in all the herbivorous mammals examined (American manatee, musk rat, musk deer, European bison, and elk), which possess an abundant AER and numerous inclusions of various kinds [8,9]. The buildup of AER is, in our opinion, an adaptive response of significance not only for carbohydrate and lipid metabolism but also, and mainly, for the detoxification of substances (alkaloids, glycosides, tannins, estrogens, etc.) that accumulate in large quantities and become toxic after the animal has consumed large amounts of plant food. The presence of various inclusions (sucrose vacuoles, electron-dense granules, needle-shaped cavities with fibrillary contents) may be attributed to the consumption of food widely differing in chemical composition. The aquatic vegetation on which manatees and musk rats feed and the lichens eaten by musk deer are all rich in carbohydrate that is converted to sucrose which accumulates in the HCs where it appears as large, clear vacuoles. The close topographical contact of sucrose vacuoles with mitochondria, AER, and peroxisomes is an indication that these structures are involved in sucrose metabolism. In the manatee and musk rat, the electron-dense inclusions, which arise from particles (probably metals contained in aquatic plants) entering HCs from the blood, move to the bile capillaries and are excreted together with the bile. In the elk and European bison, which feed on protein-rich branches of trees and shrubs, the HC cytoplasm is the site where glycogen is deposited and needle-shaped cavities containing a finely fibrillary substance (probably protein) are formed.

The polar bear was found to have an abundant supply of vitamin A in its liver — this animal feeds on the fat and meat of pinnipeds, which in turn consume fish rich in vitamin A. This vitamin is known to occur in the liver in the form of esters located in fat droplets present in large numbers in the HC cytoplasm. Our observation that mitochondria, peroxisomes, and AER cisterns and vesicles are in close topographical contact with lipid droplets undergoing various stages of transformation attests to the participation of these organelles in lipid and vitamin A metabolism. This is one of the factors favoring the survival of the polar bear at low temperatures and without food for prolonged periods [3].

Manifestations of adaptive responses in the liver of the least shrew (a very small animal that is on the move much of the day in search of food, which it consumes in quantities 2 to 4 times its body weight) are an abundance of mitochondria with very large numbers of cristae, broad, open sinusoids containing erythrocytes in their lumens, and the release of cytosegresomes from HCs not only into bile capillaries but also into sinusoids, where they undergo phagocytosis by Kupffer's cells and macrophages [10]. The abundance of mitochondria with numerous cristae in the shrew HCs testifies to a high level of energy metabolism in these cells. A similar observation was made for HCs of the hummingbird, which, like the least shrew, is extremely mobile [25]. The release of cytosegresomes into both capillaries and sinusoids speeds up the removal of undigested metabolic products from the HCs. The open sinusoids and the presence of erythrocytes even in the smallest of them are indications that each HC is well supplied with blood and oxygen and that the metabolic activity of the liver is high, which agrees with our physiological data pointing to a high level of oxygen consumption by the least shrew [10].

It is evident from the foregoing descriptions of mammalian HC ultrastructures that while HCs of different animals contain the same set of organelles, the quantities and proportions of these vary between animals, each variant reflecting the prevalence of one particular function or aspect thereof — either detoxification (in herbivores), high levels of energy and substance metabolism (in the least shrew), or intensive metabolism of fat-soluble vitamin A (in the polar bear). Morphological correlates of particular functional states have thus been obtained.

The evidence in hand leads to the conclusion that although the cellular makeup of the liver has remained unchanged in the course of evolution (sinusoidal cells, Kupffer's cells, and Ito's cells have been found in animals from all classes [16]), in the hepatic parenchyma the HCs drew closer and closer to the blood, primarily through a regrouping or "recombination" [13] of these cells among themselves. Thus, tubules composed of 3-5 HCs came in contact, new bile capillaries appeared at their junctions, and HCs became transformed from bipolar to multipolar cells; this was followed by another recombination whereby mammalian HCs became arranged in lamellae in which bile capillaries were formed by membranes of only two HCs and the lamellae then intertwined, with the result that not only did the number of bile capillaries per cell increase, but so did the area of contact of HCs with blood. In addition, the disappearance of the basal layer from Disse's space in most mammals and the emergence of microvilli on the sinusoidal surface of HCs led to a further increase in the area of their contact with blood, thus allowing the liver to perform its many functions in maintaining homeostasis in the body. A progressive increase in the complexity of liver structure in the process of evolution was necessary because

of the need to intensify liver function, since this is an organ where all types of metabolism take place.

The data presented above admirably illustrate Severtsov's doctrine of methods by which organs undergo phylogenic transformation (a doctrine resting on the principles of organ multifunctionality and function intensification) [14] and Zavarzin's law of parallel series in tissue evolution [2]. The HC, bile capillary, sinusoidal cell, and sinusoid are the "building blocks" making up the liver of any vertebrate, although there are variations of HC ultrastructure even among animals of the same class. This testifies to the validity of Darwin's statement that "nature is prodigal in variety, though niggard in innovation" [1].

To summarize the discussion on ultrastructural features of liver cells in phylogeny, it may be said that all forms of adaptation are aimed at providing such conditions for HCs that would enable them to execute their many functions to the maximum possible extent in the framework of the existing organ structure and to maintain bodily homeostasis in periods of drastic change in functional activity (e.g., during starvation or metamorphosis).

As detected at the ultrastructural level, the relationships of the HC with the bile capillary, sinusoid, and sinusoidal cell in animals of different classes may serve as a striking example of how a new quality emerges in liver structure on the basis of "recombinant transformations and quantitative additions" [13].

Having arrived at ultrastructural correlates of particular HC states reflecting the adaptation of these cells to specific conditions of functioning, we may be state that such correlates are characteristic not only of lower animals but also of more highly organized ones. In responding to various influences exerted upon the organism, HCs make use of the adaptive devices they have acquired in the course of phylogeny. Thus, intercellular spaces are encountered in the livers of both lancelets and fish, while desmosomes, which occur around bile capillaries in fish, are also present around bile capillaries (as well as further away from them) in the livers from old rats, where the distances between HC membranes are larger than in fish, probably because the reticular stroma is weaker.

The phenomenon of clasmatosis seen in fish was also repeatedly observed in avian, reptilian, and mammalian livers. In the embryogeny of the chicken and great racer, for example, HCs were seen to shed large portions of cytoplasm with ribosomes into sinusoidal lumens to saturate the blood with protein products during the period of endogenous nutrition, when the liver is actively involved in the processing of albumen and yolk products [4]. In a similar vein, our studies of erythrocyte maturation in rat embryos demonstrated that enucleation was preceded by the removal of organelles from the erythroblast through clasmatosis of cytoplasmic fragments together with mitochondria and ribosomes [4].

In all cases, the "rationale" for clasmatosis was to ensure a rapid supply of the body with substances it needs or the rapid removal (as in the case of erythroblasts) of superfluous material from the cell.

Release of cytosegresomes into both bile capillaries and sinusoids was observed not only in fish, but also in the least shrew. However, whereas the release of cytosegresomes into sinusoids in fish may be regarded as an adaptive response developed because the number of bile capillaries is small, the occurrence of this phenomenon in the shrew can probably be explained by the very high metabolic rates resulting in the death of some organelles, so that the cells strive to free themselves from cytosegresomes as soon as possible by removing them via both bile capillaries and sinusoids [10].

HCs of rats that had been exercising on a treadmill were found to have markedly increased numbers of mitochondria and mitochondrial cristae [11], comparable to those observed in the normally highly mobile least shrew [10]. Similarly, when rats, which do not normally absorb concentrated sucrose solutions in large amounts, were experimentally compelled to metabolize sucrose at a high rate, they "packed" it into vacuoles [27] in the same way as is normally done by American manatees and musk rats [8,9].

The data reviewed here lend concrete meaning to N. G. Khlopin's words [15] about how "the liver has become transformed from an organ of the ordinary glandular type into one of predominantly endocrine structure".

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