Occurrence of Centrioles in Interphasic Hepatocytes of Bat and Chicken

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Summary. Occurrence of centrioles in non-dividing hepatocytes was examined by electron microscopy in the bat, Miniopterus schreibersi (Kuhl) and Myotis macrodactylus (Temminck), and the chicken. In both species centrioles were mostly found in the apical cytoplasmic area of the hepatocyte near the bile canaliculus. They were invested in a hyaloplasmic halo which was distinct in the chicken but rather indistinct in the bat. There was no difficulty in locating centrioles in the hepatocytes of either species. In the chicken centrioles were found in the attenuated apical cytoplasmic areas of 4–6 hepatocytes surrounding the bile canaliculus, giving us the impression that the occurrence of centrioles in non-dividing hepatocytes might be more frequent in the chicken than in the bat. The centrioles found in non-dividing bat and chicken hepatocytes apparently formed diplosomes. Neither multiple centrioles (comprising more than three) nor centriolar replications were found. Single cilium formation from the centrioles was not observed in either species.

Since the histological and cytological applications of the electron microscope, the occurrence of centrioles in cells not undergoing division has been reported in various types of vertebrate cells. However, as pointed out by Wheatley (1968), centrioles have rarely been reported in interphasic hepatocytes from adult mammalian liver. After Wheatley’s (1968) electron microscope observation of the centriole in normal and regenerating (hepatectomized) rat liver parenchymal cells, there have been, so far as we know, no reports on this subject. In the present electron microscopic study we report the occurrence of centrioles in interphasic hepatocytes of normal bats and chickens.

Material and Method

Two kinds of wild bats, Miniopterus schreibersi (Kuhl) captured in December and February and Myotis macrodactylus (Temminck) captured in August and two chickens (adult male White Leghorn) were used. They were sacrificed with ether, and the livers were fixed by perfusion through the portal vein with a cold (0°C) fixative containing 2.5% glutaraldehyde and 0.1 M phosphate buffer at pH 7.4. After the perfusion fixation, the livers were excised and thin slices of the livers were cut, under a drop of the fixative, into minute blocks. After 2 hr fixation, the blocks were rinsed several times in a cold 0.1 M phosphate buffer containing 5% sucrose at pH 7.4 and left overnight in the same buffer at 5°C. They were postfixed for 90 min by immersion in a 1% osmium tetroxide solution in a 0.1 M phosphate buffer (pH 7.4) at 0°C. Following dehydration in graded ethanol, tissues were embedded in Epon 812 and sectioned on a Porter-Blum Ultra-Microtome MT2-B. Ultrathin sections were
stained with saturated uranyl acetate and Sato's lead solution. Microphotographs were taken with a JEM-100C electron microscope.

Observations

A. Bat hepatocyte

In the bat liver, bile canaliculi are usually bordered by the short apical surfaces of two adjacent hepatocytes; the bile canaliculi contain small numbers of short finger-shaped microvilli arising from the thin apical hyaloplasm or ectoplasm of hepatocytes (Fig. 1, 2). The longer basal surfaces of the hepatocytes line the perisinusoidal or Disse's space, into which numerous microvilli are sent out. The Golgi complex of bat hepatocytes is usually located in the apical cytoplasm near the bile canaliculi.

In normal bat livers, hepatocytes containing centrioles are occasionally encountered. In the majority of cases they are found in the apical cytoplasm near the bile canaliculi (Fig. 1–3), within or close to the Golgi complex. In rare cases the centrioles occur near the apical end of the hepatocytic nucleus, away from the bile canaliculus. Most centrioles are surrounded by a narrow distinct hyaloplasmic area (Fig. 1–3). Strictly longitudinal or transverse sections of centrioles are rare (Fig. 1, 2). Hepatocyte centrioles are hollow cylinders measuring, on the average, approximately 490 mμ in length and 240 mμ in diameter. The electron dense wall of the hollow cylinder contains 9 sets microtubules (fibers) arranged in triplets and arranged longitudinally at regular intervals (Fig. 1, 2), while the cavity of the hollow

Fig. 1. Longitudinal section of a centriole (LC) in a bat hepatocyte close to the bile canaliculus (BC). The centriole is surrounded by a hyaloplasmic halo lying in the Golgi complex (G). J hepatocyte junction, MV microvilli, R ribosome. Miniopterus schreibersi. ×52,000
The cylinder is electron lucent and exhibits almost no structure. From the triplet fibers slightly electron dense rays radiate into the hyaloplasmic halo, some of which resemble, as seen in the longitudinal section of the centriole, a basal foot or satellite of the ciliary basal body, originating from the middle of the centriole (Fig. 1). In the apical cytoplasm of bat hepatocytes a few microtubules are found running in random directions, and no topographical relation between the centriole and microtubules has been discovered. No cytological signs of cilium formation from the centrioles have been observed in bat hepatocytes. Rootlets, as seen in the ciliary basal body, never develop from the bat hepatocyte centriole.

As observed in Figure 3, about 50% of the centrioles found in hepatocytes are diplosomes, located mainly in the apical cytoplasm close to the bile canaliculus, but occasionally found in the cytoplasm near the apical pole of the nucleus. The long axis of one centriole of the diplosome

Fig. 2. Transverse section of a centriole (TC) in a bat hepatocyte close to the bile canaliculus (BC). It is surrounded by a hyaloplasmic halo and lies within the Golgi complex (G). MV microvilli, R ribosomes. *Miniopterus schreibersi* ×52,000

Fig. 3. A diplosome (PC) in a bat hepatocyte area close to the bile canalculus (BC). D dense body, J interhepatocytic junction. *Miniopterus schreibersi* ×26,000
usually intersects that of the other at variable angles, and only rarely at right angles.
Neither multiple centrioles composed of more than two, nor morphological evidence
of centriolar replication was observed.

B. Chicken hepatocyte

Chicken hepatic parenchyme (lobule) is composed of networks of twisting and
branching hepatocyte cords which may correspond to the long terminal portions of
the tubular gland. Differing from the mammalin “one cell thick” hepatic cell plates
(ELIAS, 1949, 1963), chicken hepatic cell cords are composed of much more numerous
hepatocytes longitudinally arranged in rows of 4-6 cells to make the wall of the
tubular terminal portion surrounding a central narrow lumen that is the bile cana-
iculus (KITAGAWA, 1960). In the transverse section of the hepatic cords, 4-6 hepatocytes
of pyramidal shape are radially arranged surrounding a narrow bile canaliculus
(Fig. 4); the narrow surface of the attenuated apical cell part borders the central bile
canaliculus, and sends out several finger-shaped microvilli from the thin apical hyalo-
plasm (ectoplasm). Abutting on the lumen of the bile canaliculus, several electron
dense junctional complexes are observed between hepatocytes (Fig. 4). The wide
basal surface of the hepatocytes borders the perisinusoidal or Disse’s space protrud-
ing numerous microvilli in the latter.

![Fig. 4. A central part of the cross section of a tubular hepatic cell cord of the chicken liver. A bile canaliculus (BC) is surrounded by cytoplasmic areas of four hepatocytes. In one there is an oblique section of a centriole (OC) close to the bile canaliculus. In these apical cytoplasmic areas, numerous free poly-
somes (R), scattered glycogen particles (arrows), mitochondria with inclusions (M), Golgi complex
(G), microbody (MB), sparse cisterns of the rough endoplasmic reticulum (RER) are seen. J inter-
hepatocytic junction. ×26,000](image-url)
In the chicken, hepatocytes with centrioles are apparently more numerous than in the bat. The centrioles are easily found it the chicken hepatocytes, because they are usually in the attenuated apical cytoplasm close to the bile canaliculus (Fig. 5). Centrioles farther away from the bile canaliculus are rarely found (Fig. 6) and are absent near the apical pole of the nucleus.

The centriole found in non-dividing chicken hepatocytes is a hollow cylinder measuring, on the average approximately 280 mµ in diameter and 510 mµ in length. It has an electron dense wall with 9 sets of triplet tubules (fibers) regularly arranged in a longitudinal fashion (Fig. 5). From the triplet fibers, faint electron dense rays radiate into the narrow hyaloplasmic halo surrounding the centriole. (Some of which are conspicuous as seen in Figure 5). No cytological evidence of single cilium and rootlet formation from the centriole was observed.

As in the bat, about 50% of the centrioles detected in chicken hepatocytes are diplosomes (Fig. 6). They were frequently found being closely disposed end to end (Fig. 6), but their disposition at right angles is rarely observed.

The topographical relationship between the centriole and the Golgi complex is obscure. Multiple centrioles, comprising more than two, were not found. In chicken
hepatocytic cytoplasm no microtubules have been demonstrated.

Discussion

As pointed out by Afzelius and Schoental (1967) and by Wheatley (1968), the presence of centrioles in non-dividing mammalian hepatocytes is a very rare occurrence, in spite of many hundreds of publications dealing with the electron microscopic observations of hepatocytes. Prior to the above authors, Bernhard and Harvard (1958) observed a centriole in a hepatocyte of a mouse in which the liver had been invaded by leukemic cells, and David (1964) found centrioles in the liver cells of embryonic guinea pigs and adult guinea pigs recovering from prolonged fasting. Afzelius and Schoental (1967) found a single or three probably multiple centrioles in enlarged hepatocytes of weanling rats after the administration of a single oral dose of retrorsine. Wheatley (1968) found centrioles in non-dividing hepatocytes in normal adult rats as well as in regenerating livers in partially hepatectomized adult rats. Thus, the search for non-dividing hepatocytes has been restricted to the livers of rats and guinea pigs. It has been considered worthwhile to extend the search for centrioles in non-dividing hepatocytes to a wider variety of vertebrates. In the present study normal adult bats and normal adult chickens were used; in both species no investigation appears to have been reported concerning centrioles in non-dividing hepatocyte. Centrioles in both species could be detected in interphasic hepatocytes not so rarely as had previously been supposed before this investigation. In chicken livers centrioles in hepatocytes could be found especially easily.

Wheatley (1968) presented the following four reasons why the presence of centrioles in non-dividing mammalian hepatocytes had only rarely been reported: 1) Many worker's attentions have been drawn to the multiplicity of ultrastructural features of hepatocytes and few have concentrated their efforts to the search for centrioles. 2) Because of the large size of the hepatocyte and its small nucleo-cytoplasmic ratio, the chances of cutting centrioles are far less than in other smaller cells. 3) The liver cell does not have a definite region where centrioles are readily found. 4) Hepatocytes contain many organelles and inclusions, some of which are electron dense and tend to obscure the centrioles.

In both bat and chicken hepatocytes, centrioles usually occur in the apical cytoplasm, being surrounded by a hyaloplasmic halo, and cell organelles which might obscure the centrioles, are lacking. Especially in chicken livers, bile canaliculi are surrounded by four or six attenuated apical parts of the hepatocytes composing the tubular hepatic cell cord (terminal portion). Centrioles are for the most part located in these attenuated cytoplasmic areas close to the bile canaliculi, and are easily found, if these narrow pericanalicular areas are examined.

About half of the centrioles found in the non-dividing hepatocytes both in the bat and chicken were diplosomes. This finding makes it probable that all centrioles may diplosomes as proposed by Wheatley (1968), because even in the case of a seemingly single centriole, its partner may quite possibly reside in the adjacent plane of sectioning.

Afzelius and Schoental (1967) proposed the possible existence of multiple centrioles, comprising more than three in enlarged hepatocytes produced in weanling rats by means of administration of a single oral dose of retrorsine. Wheatley (1968) observed three centrioles in non-dividing hepatocytes from normal 60 day rat livers,
and said that occurrence of more than 2 centrioles is not entirely surprising. In the present study no more than two centrioles per hepatocyte were observed in either the normal bat or chicken. Afzelius and Schoental (1967) assumed the replication of centrioles from procentrioles in experimentally induced enlarged hepatocytes in immature rats, and Wheatley (1968) actually observed the replication of centrioles by budding in regenerating hepatocytes in partially hepatectomized rats. In normal bats and chickens, however, evidence of centriolar replications was not found, in agreement with the result by Wheatley (1968).

Single cilia originating from hepatocytic centrioles as suggested by Grisham (1963) and Wheatley (1968) have never been detected, although the presence of single cilia developing into the ductal lumen from the apical centrioles (basal bodies) of epithelial cells of the intrahepatic biliary ducts has been demonstrated using scanning and/or transmission electron microscopy (Grisham, 1963; Motta and Fumagalli, 1974; Brooks et al., 1975; Tanuma and Ohata, 1978 etc.).

References


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