Cilium-like Structures Found by Electron Microscopy in the Glandular and the Small Ductal Lumina of the Toad Pancreas

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EKHOLM et al. (1962) and HERMAN et al. (1964) observed occasional circular formations which have an inner structure composed of one central and seven marginal annuli in glandular lumina and ductal lumina of rat pancreas. WINBORN (1963) also found the same structures in the glandular and in the intercalary ductal lumina of the Saimili monkey pancreas, and they thought these circular formations represent the cross-sections of the microvilli. In the toad pancreas the author also found the similar structures and made a few remarks on them in the previous paper (KOBAYASHI 1966). But, as it seemed that they might be in their ultrastructural properties different from the microvilli, since they contain internal annuli, the author has continued the electron microscope observations on them and obtained following important results which can clarify the nature of these structures.

Materials and Methods

Adult toads (Bufo vulgaris formosus) captured in December and March were used for this study. Immediately after decapitation, the pancreatic tissue was cut out. The fixative used was 1% osmium tetroxide solution buffered at pH 7.4 with acetate-veronal and made isotonic with sucrose as introduced by CAULFIELD (1957). The tissue blocks were fixed in the fixative for about one and half hours at about 0°C, and dehydrated in a series of cold ethanol of increasing concentrations and embedded in Epon 812 resin (LUFT 1961). Ultrathin sections were cut with glass knives on JUM-5A ultramicrotome and picked up on copper grids. Staining was carried out by floating the grids on a saturated aqueous solution of uranyl acetate, then by immersing in the lead acetate or in the solution of REYNOLDS (1963), and they were then examined in JEM-5G and JEM-7 electron microscope.

Observations

In the cytoplasm of the centroacinar cells and epithelial cells of the intercalary duct, mitochondria, many tonofilaments, a considerable number of glycogen granules and occasional elliptic dense bodies are demonstrated. Adjacent to the lumen there appear terminal bar complexes between the neighboring cells, and a large number of microvilli are protruded into the small lumen from the apical parts of the centroacinar, acinar and intercalary duct cells (Fig. 1—4, 7—9). At the tips of the microvilli, polypoid swellings are occasionally observed (Fig. 1). Six circular formations which have an inner structure composed of one central and six marginal annuli having
a diameter of about 15–25 mμ are found side by side at the center of the intercalary duct lumen illustrated in Fig. 1. The diameter of the circular formations is about 100–170 mμ and they are slightly larger than microvilli of the acinar, centro-
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In Fig. 2, several longitudinal or oblique sections of cilium-like structures (110 μm in diameter) containing inner longitudinally oriented small tubuli (20 μm in diameter) run along the long axis of the glandular lumen among a large number of microvilli from the acinar and intercalary duct cell (70–100 μm in diameter). In Fig. 2, several longitudinal or oblique sections of cilium-like structures (110 μm in diameter) containing inner longitudinally oriented small tubuli (20 μm in diameter) run along the long axis of the glandular lumen among a large number of microvilli from the acinar cell. Among a large number of microvilli there appear several longitudinal sections of cilium-like fibers with inner tubular structures (†). ER granular endoplasmic reticulum, F tonofilaments, GL glycogen granules, M mitochondria, R free ribosomes, Z zymogen granule. × 28,800
and centroacinar cells. Although the microvilli show some internal fine filamentous structures, they do not contain small tubular structure as found in the former. These findings suggest that the above mentioned circular formations with annuli found in the lumen of an intercalary duct may correspond to the cross-sections of these cilium-like structures containing inner tubuli. These cilium-like structures appear not only

Fig. 3. Longitudinal or oblique sections of the cilium-like fibers (↑) with inner small tubuli in an intercalary duct lumen. The inner tubuli curve or twist remarkably.

MV microvilli, F tonofilaments, GL glycogen granules. × 36,400
in the small ductal lumina (Fig. 1, 3, 5, 7, 9) but also in glandular lumina bordered by acinar and centroacinar cells (Fig. 2, 4, 8). They run singly or making small bundles along the long axis of the winding glandular or ductal lumina. As shown in Figures 3 and 4, these ciliary fibers and internal tubuli may presumably curve or twist at some points, they sometimes show ampullar swellings in which inner tubuli

Fig. 4. A portion of the glandular lumen containing longitudinal sections of the cilia-
like fibers (↑), they contain curved tubuli. A basal corpuscle (BC) is found at the bottom of a deep hollow depressed from the luminal surface of a centroacinar cell (CA). AC acinar cell. × 25,600
are arranged irregularly and increase in number (Fig. 5). In cross-sections of the cillum-like fibers, the internal tubuli are cut transversely and appear as the small annuli, and we can observe easily their number and arrangement in the matrix of the ciliary fibers. Although the arrangement of the annuli is not always regular, one central (sometimes disappeared) and seven, six, five or less marginal ones are distin-

Fig. 5. A portion of the intercalary duct lumen. The ampullar swelling (†) of a cillum-like fiber is observed, in which the inner tubuli increase in number and are arranged irregularly. Some cross-sections of the same fiber appear surrounding it. × 20,000
guished (Fig. 6-a and -b). In the Fig. 6-c, a cross-section of the fiber contains six pairs or doublets of annuli arranged peripherally surrounding one central pair, this finding supports the suggestion, that these cilium-like fibers should be nothing but genuine cilia from epithelial cells. It seems to be an interesting problem from where these fibers originate. Single basal bodies are sometimes observed beneath the apical surfaces of the centroacinar (Fig. 4, 8) and intercalary duct cells (Fig. 7, 9), they are often located at the bottom of the deep hollow found on the luminal surfaces of the centroacinar and intercalary duct cells (Fig. 4, 8, 9). Fortunately, a very long longitudinal section of a cilium-like fiber was found arising from such a basal body in the cytoplasm of an intercalary duct cell (Fig. 9). Immediately after protrusion from the cell surface the fiber runs in the hollow cavity toward the opposite lining of the duct to come into the ductal lumen, then the fiber curves at about right angle to one direction, presumably to distal direction to run along the long axis of the lumen. The length of the fiber is measured about 5 μ, but the real length of it seems to be far longer than this. The diameter of the fiber measures in the proximal part 170 mμ and in the distal part 150 mμ. The peri-

Fig. 6. a. A high magnification of the cross-sections of cilium-like fibers in the Fig. 1. Two of them have six peripheral and one central annuli (tubuli), in others the number of annuli is variable. × 77,000
b. Two cross-sections of the fiber having six and seven peripheral and one central annuli. × 46,900
c. A cross-section of the fiber containing six pairs (doublets) of peripheral and one pair of central annuli. × 56,000
Pheral tubuli in the fiber are continuous with the basal body. The fiber is similar in ultrastructural properties to a motile cilium, but the ultrastructural pattern and number of the internal tubuli contained in the proximal portions of the fiber are obscure, since the cross-sections of them have not been found in the present study. It may be possible that the ultrastructural pattern and number of the tubuli can be variable.

Fig. 7. Several longitudinal and oblique sections of cilium-like fibers running in the small intercalary duct lumen. A basal corpuscle (BC) of the cilium is found immediately beneath the luminal surface of an epithelial cell. G Golgi apparatus, GL glycogen granules, M mitochondria, MB multivesicular body, N nucleus. × 28,800
toward the distal portions of the fiber, and that in the distal or peripheral portions
the tubuli can decrease in number as it has been confirmed in the cross-sections of
the fibers (the circular formations with tubuli) found in the glandular and ductal
lumina (Fig. 1, 6a, b and c). In the present study it was revealed that the cilium-

Fig. 8. A portion of the glandular lumen. Several sections of the cilium-like fibers (†)
are found among a large number of microvilli from the centroacinar cells. A basal
corpuscle (BC) is observed at the bottom of a hollow found on the luminal surface of a
centroacinar cell. AC acinar cell, DS desmosome, DB dense body, G Golgi apparatus,
GL glycogen granules, M mitochondria, Z zymogen granule. × 28,800
Fig. 9. A portion of the intercalary duct lumen containing a longitudinal section of a long cilium-like fiber in association with the basal corpuscle (BC). The basal corpuscle lies at the bottom of a deep hollow found on the luminal surface of an intercalary duct cell. The fiber (cilium) originates from this basal corpuscle, ascends in the hollow cavity to come into the intercalary duct lumen, then it curves to run along the long axis of the latter. DB dense bodies, F tonofilament, GL glycogen granules, M mitochondria, MB multivesicular body, N nucleus of the intercalary duct cell. × 33,600
like structures found in the glandular and small ductal lumina of the exocrine pancreas may represent very long cilia originating from single basal corpuscles located beneath the luminal surfaces of the centroacinar cells or intercalary ductal cells, but it can not be confirmed whether these cilium-like fibers may ultrastructurally correspond to ordinary motile cilia or modified ones.

**Discussion**

EKHOLM et al. (1962) reported that glandular lumina bordered by acinar and centroacinar cells, and ductal lumina of the rat pancreas contain occasional circular formations which have an inner structure composed of one central and seven marginal annuli. WINBORN (1963) also demonstrated the structure with the same arrangement of annuli, seven peripheral and one central in the Saimili monkey pancreas. They thought these formations represent microvilli. HERMAN et al. (1964) have found similar structure in a ductal lumen of the rat pancreas, and they regarded these structure as microvilli too. They observed, however, only cross-sections, and thought these were microvilli with internal tubuli. But in the present study on the toad pancreas, the longitudinal sections of the circular formations were observed, and it was revealed that they have some different ultrastructural characteristics from the microvilli: They are considerably long fibers containing several tubuli within them, whereas the microvilli are not so long and do not have definite tubuli except for some fine filaments. The fibers have somewhat larger diameter than the microvilli, running along the long axis of the narrow lumen close to the microvilli. They are found singly but occasionally making a small bundle. ZEIGEL (1962) has reported the presence of cilia arising from acinar, centroacinar and duct cells of embryonic and posthatched chick pancreas. FAWCETT (cited by SOROKIN 1962 and HERMAN 1964) has observed cilia in association with centroacinar cells in the bat pancreas, which, unlike the motile cilium with 9 peripheral and 2 central fibrils (9+2), have a (9+0) pattern. Recently, OKANO (1965) has reported fine structure of the canine olfactory hairlets arising from basal corpuscles in the apical end of the olfactory cell. He observed that the thick portion of the extraordinarily long hairlets is ultrastructurally analogous to the mammalian epithelial cilia; however, in the transitional region to the thin portion the nine peripheral doublets become single tubuli and in the thin portion, which has an ampullar swelling, ten or more than ten to one single are observed, being arranged irregularly in the matrix.

In the toad pancreas, basal bodies were frequently found at the apical part of the centroacinar and intercalary duct cells, however, only in one case a longitudinal section (about 5µ in length) of a long cilium-like fiber was demonstrated in association with a basal corpuscle found in the apical part of an intercalary duct cell, this is the important finding which reveals that the cilium-like fibers found both in the glandular and in the small ductal lumina should be the cilia originating from the lining epithelial cells of these glandular portions. The internal tubuli running along the long axis of the cilium-like fibers are in the most cases single and their arrangement in the matrix is not always regular. Their number is variable from fiber to fiber; one central (sometimes disappeared) and seven, six, five or less peripheral tubuli were distinguished. In only one case the six peripheral paired tubuli or doublets were confirmed surrounding one central pair. In these respects the cilium-
like fibers found in the glandular and small duct lumina of the toad pancreas are similar to olfactory hairlets described by Okano (1965). The circular formations found in the glandular and ductal lumina of the toad pancreas, in which the patterns of the inner single tubuli were observed, might be probably the cross-sections of the distal portions of the long cilium-like fibers, and the one in which six peripheral doublets and one central pair were found, might be a cross-section of a somewhat proximal portion. The cross-sections of the extremely proximal portions nearest to the basal corpuscle were not found in the present study, therefore the internal structure of the long cilia running along the long axis of the glandular and small ductal lumina probably toward the opening of the main duct was not completely clarified in the present study. The functional significance of the cilium-like fibers found in the glandular and small ductal lumina of the toad pancreas is still obscure, but, judging from the ultrastructural similarity of the fiber with the canine olfactory hairlets (Okano, 1965), the following speculation might be possible that these fibers might represent sensory cilia acting as chemoreceptors protruded from the epithelial cells into the lumen of the most proximal part of the pancreatic duct.

**Summary**

Cilium-like fibers with internal tubular structures were found in the glandular and in the intercalary duct lumina of the toad pancreas. In the present study it was revealed that they are extraordinary long cilia originating from the basal corpuscle found in the apical cytoplasm of the centroacinar and the intercalary duct cells. However, the ultrastructural pattern and the number of inner tubuli contained in the matrix of the fibers were not completely clarified in the present study.

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**References**


