Light and Electron Microscope Studies on the Saccus Vasculosus of the Ray (*Dasyatis akajei)*

Akira Watanabe**

Received August 5, 1966

The saccus vasculosus is the organ situated at the base of the diencephalon and occurs only in the fish. The organ is associated embryologically with the caudal wall of the infundibulum and the cavity in the organ is continuous to the third ventricle. It consists of the epithelial layer and the subjacent blood capillaries. Bundles of nerve fibers connect the saccus vasculosus to the brain proper, but the function of this organ is still unknown.

Collins (1685) first described on this peculiar organ of the fish, and Gottsch (1835) named it 'saccus vasculosus'. Since then many morphological studies were published and most authors inclined to the opinion that this organ might secrete something according to the histological and embryological investigations. In 1910, however, Dammerman suggested that the saccus vasculosus might be a sensory organ and described: 'The saccus vasculosus of fishes are receptive nervous organ and not a gland' (Boeke and Dammerman 1910). This theory had been supported by many investigators for a long period (see Dorn 1955), but rather recently the former theory suggestive of the secretory nature was proclaimed again by several authors such as Kamer and Schuurmans (1953), Bargmann (1954) and Dorn (1954). Certain histochemical studies also supported the secretion theory of the saccus vasculosus (Kamer et al. 1960, Sundararaj and Frasad 1964).

As to detailed cytological studies, only two short electron microscopic works have appeared treating of the organ in teleosts (Bargmann and Knoop 1955, Kurotaki 1961).

Using a species of the elasmobranch, which has the best developed saccus vasculosus, the author tried to observe the organ with both the light and electron microscopes, in order to elucidate the confusing problems concerning the function of this organ.

Materials and Methods

Four individuals of the ray (*Dasyatis akajei*) captured in June and November were used as the material. For light microscopy, the specimens were fixed in Levi and Bouin solutions and embedded in paraffin. Serial sections 3—4 μ thick were stained with anilin fuchsin-aurate after Kull, azan and iron-hematoxylin of Heidenhain, Gomori's chrome alum hematoxylin-phloxin (CHP) and aldehyde fuchsin (AF). For demonstration of Golgi apparatus, osmium impregnation of Kolatschev was used. Glycogen was detected by periodic acid-Schiff (PAS) reaction and ascertained by digestion test with saliva.

For electron microscopy, two different fixation methods were used: 1) double fixation with 6% glutaraldehyde adjusted at pH 7.4 with phosphate buffer for 24 hours followed by 1% osmium tetroxide for 1 1/2 hours, 2) simple fixation with 1% osmium tetroxide adjusted pH 7.4 with phosphate buffer. The specimens were dehydrated through a series of ascending
concentrations of ethanol and embedded in epon. Thin sections were obtained with a Leitz ultrotome equipped with glass knives, and stained with lead hydroxide. They were examined with the electron microscope (JEM-5G) and photographed under 3,000—10,000 times of direct magnification.

Observation

A. Light microscopic findings

The saccus vasculosus consists of follicular structures which are lined by an epithelium with different levels of cell nuclei (Fig. 1). The cavities of the e follicles are continuous to the third ventricle of the brain. The interstitial tissue intervening among the follicles contains a large number of blood capillaries and nerve fibers (Fig. 1). Bundles of nerve fibers become thinner as they enter the epithelium to end there (Fig. 2). Acidophilic fine granules were observed in the nerve bundles, but CHI-positive (Gomori-phile) granules could not be found in the nerve bundles. Myelinated nerves are also observed though they are small in number.

The epithelium of the saccus vasculosus consists of two cell types: the coronet cell with a cylindrical cell body containing a round nucleus, and the supporting cell with a triangular nucleus situated at the apical end in most cases, though either at middle or basal parts in lesser cases, and with a small amount of cytoplasm (Fig. 3).

The coronet cell which characterizes this peculiar epithelium contains a round nucleus. The cell is characterized by a papillary cytoplasmic protrusion from the apical surface. This part of cytoplasm is provided with many radiating processes, each ending in a somewhat swollen terminal or globule (Fig. 4, 5). The globule is faintly stained and its membranous covering is well discernible. The shaft of these small pin-like processes has been often called “the hair” and possesses a swelling at the base inserted into the apical protrusion. Such a basal swelling
Saccus Vasculosus of the Ray

is stained red with azan or anilin fuchsin and also intensely tinted with iron-hematoxylin, and resembles the basal body of the cilium. In extremely rare cases, these pin-like processes are very few in number and the terminal globules become larger (Fig. 6).

In the infranuclear zone of the coronet cell, there exists a mass of homogeneous hyaline substance which is weakly stained with PAS-method, appears pale after azan staining and dark orange yellow stained with aurantia by KULL-method (Fig. 4, 7). The cytoplasm shows a slight positive PAS reaction either generally or partly, and is tinged weakly in aldehyde fuchsin or CHP.

Extending from the supranuclear region to the apical protrusion, many small granules may occur. They are strongly stained with PAS method and some other staining techniques, and are thought to correspond to the lysosomes which may be revealed by electron microscopy as will be described later. (Fig. 4, 7, 8).

Filamentous mitochondria are distributed through the cytoplasm, and do not tend to
A. Watanabe:

cluster in the apical region but occur around
the infranuclear homogeneous hyaline mass,
in which they are very few (Fig. 8). The
Golgi apparatus consists of several strands
blackened with osmium, running parallel
to the long axis of the cell with a slight
curvature in the supranuclear zone, a little
apart from the nucleus. These strands may
be anastomosed to each other, but do not
form a definite network (Fig. 9).

There are only two previous reports on
these two kinds of organelles in the teleost
coronet cells: BROUSSY (1933) demonst-
rated a concentric arrangement of rod-like
mitochondria around the nucleus and the
Golgi apparatus forming a delicate network at the supranuclear region, and SUNDARARAJ and
PRASAD (1964) reported that the mitochondria were accumulated in the perinuclear part and
the apical end of the cytoplasm extending to the globules of pin-like processes, and that the
Golgi apparatus was observed not only in the characteristic Golgi zone but also around the
nucleus.

In some coronet cells, a large amount of glycogen can be detected in accordance with the
result reported by SUNDARARAJ and PRASAD (1964), but does not form a crescent which was
found at the apical part of the cell after pilocarpine injection by KAMER et al. (1960) (Fig. 10).
There is no fat droplet in the coronet cell.

There are smaller cells somewhat different from the above mentioned coronet cells. They
are flask-like in shape and contain the nucleus in the basal swollen part. The apical part of
Fig. 7. Coronet cells of the saccus vasculosus. Each coronet cell contains a large mass of homogeneous hyaline substance stained pale blue and situated in the infranuclear region (\(\wedge\)). There are many small granules stained red in the supranuclear region to the apical protrusion (\(\ddagger\)). Levi-fixed, azan-stained. \(\times1,100\)

Fig. 8. Mitochondria in the epithelial cells of the saccus vasculosus. They surround the infranuclear homogeneous hyaline mass of the coro-
net cell. Acidophilic granules are accumulated in the cytoplasm sub-
jacent to the apical surface. Er erythrocytes in the capillary. Levi-
fixed, Kull-stained. \(\times1,100\)

Fig. 9. Golgi apparatus of the coronet cells situated in the supranuclear region. The pin-like processes are also blackened by osmium impregnation. Champy-
fixed, postosmicated by Kolatchev method. \(\times1,100\)
432 A. WATANABE:

the cell protrudes into the lumen, but is not provided with pin-like processes (Fig. 11). They are scattered among the coronet cells. It is not clear, however, whether they belong to a different cell type from the coronet cell or are nothing but a functional modification of the latter.

B. Electron microscopic findings

1. The coronet cell

a) The nucleus: The nucleolus consists of particles of the same size and density as the ribosomes and appears to be a tightly packed cluster (Fig. 12). The nuclear matrix is a little higher in electron density than that of the cytoplasm, but in some cases the karyoplasmic matrix is less dense. Pores through the nuclear envelope are remarkable (Fig. 13). Ribosomes are often situated in clusters along the outer surface of the nuclear envelope, but not arranged regularly on the outer nuclear membrane.

b) Pin-like processes: The head of the processes, or the globule, is either spherical or oval and contains tightly packed vacuoles and vesicles of various sizes (Fig. 14, 15, 16). However, the tubuli and balloon-like swelling as described by BARGMANN and KNOOP (1955) could not be observed. The shaft of the process contains nine pairs of peripheral tubules like a cilium, but is lacking in the central two (Fig. 14). Some tubules are seen just beneath the plasma membrane covering the globule (Fig. 14, 15).

In Figs. 14 and 15, one can observe tubular structures running parallel to the periphery of the globule, but it is difficult to determine whether they are identical with the peripheral tubules of the shaft. Furthermore, it is not clear whether they correspond to the "tubuli"
Saccus Vasculosus of the Ray

It is also difficult to trace up the end of tubules.

Fig. 12. Electron micrograph of the central part of a coronet cell. The nucleus (N) containing the nucleolus (Ncl) is illustrated in the left half. The space between the two nuclear membranes is relatively dilated. Ribosomes (Ri) are accumulated in the immediate outside of the nuclear membrane, but not regularly attached on it. Abundant filaments and glycogen particles occupy the entire cytoplasm, which is penetrated by an irregular network of the smooth endoplasmic reticulum. Glutaraldehyde-osmium-fixed. ×35,000

designated by Bargmann and Knoop (1955).
The shaft of the process is inserted in the cytoplasm of the apical protrusion and forms a basal body just beneath the surface plasma membrane. Sometimes rootlets with faint cross-striations extend deeply into the cytoplasm from the basal body (Fig. 14). No associated
Saccus Vasculosus of the Ray

centriole can be observed other than the basal body. Typical cilia with central paired tubules are rarely found. The surface of the pin-like process as well as that of the cilia are covered by the extension of plasma membrane covering the apical papillary protrusion. A few delicate microvilli are found among the shafts of pin-like processes (Fig. 14).

c) The cytoplasm: Extending from the supranuclear region to the apical cytoplasmic protrusion, a complicated network of the smooth endoplasmic reticulum, microtubules, vesicles, vacuoles and lysosomes are distributed (Fig. 14—17). The microtubules are laid in various, rather irregular directions, but there is a general tendency to be oriented parallel to the cell. Delicate filaments packed among them show a similar tendency in arrangement.

The rough endoplasmic reticulum is almost absent, but free ribosomes occur in a considerable amount and are scattered in clusters. One can also observe glycogen particles randomly distributed (Fig. 17, 18). The number of vesicles and vacuoles differs from cell to cell. The Golgi apparatus consisting of lamellae, vacuoles and vesicles is located in the supranuclear region a little apart from the nucleus and rather weakly developed.

Mitochondria are mostly rod-like or filamentous in shape and abundantly distributed in
the peri- and infranuclear regions, while scanty in the supranuclear part; the papillary projection contain mitochondria only in its peripheral part. The cristae mitochondriales are mainly arranged in a transverse direction.

The microtubules are rich in the apical region and almost absent in the infranuclear part. Instead, the filaments are abundantly observed in the latter part and form strong bundles running in various directions, sometimes making a large mass filling the entire infranuclear region. Complicated networks of the smooth endoplasmic reticulum and glycogen particles are scattered among these tightly packed filaments (Fig. 12, 13). The filaments and microtubules are well preserved after the double fixation with glutaraldehyde and osmium tetroxide. It is evident that the specific infranuclear area consisting of the filaments and smooth endoplasmic reticulum corresponds to the homogeneous hyaline mass observed with the light microscope. Mitochondria are distributed around this area but a few invade into the area as revealed by both light and electron microscopy (Fig. 19, 20).

The coronet cell possesses a well developed smooth endoplasmic reticulum spreading over the entire cytoplasm from the apical protrusion to the basal cytoplasmic part. It consists of
irregularly shaped tubules and cisterns, which anastomose to each other and form a network. The smooth endoplasmic reticulum varies in form extensively: the cavity of each cistern may be very narrow, the reticulum consisting exclusively of tubules (Fig. 12, 21), or the cavity may be considerably dilated (Fig. 18, 19); in certain cases the cisterns are enormously dilated and the network is no longer discernible (Fig. 17). These morphological changes of the smooth endoplasmic reticulum may be related to the functional fluctuation. Therefore, vacuoles and vesicles occurring in various parts of the cytoplasm, may, at least partly, belong to the smooth endoplasmic reticulum. Distribution of glycogen particles is parallel to that of elements of the smooth endoplasmic reticulum.

There is a clear cytoplasmic zone between the basal plasma membrane and the infranuclear specific area filled with filaments and cisterns of the smooth endoplasmic reticulum surrounded by mitochondria (Fig. 19, 20). This basal cytoplasmic zone contains only a small amount of fine filamentous substance, but none of other structural elements can be seen. The basal plasma membrane of the coronet cell never abuts on the basement membrane, as the thinly attenuated cytoplasm of the supporting cell always intervenes between the basal surface of
the coronet cell and the basement membrane (Fig. 19, 20). However, plasma membranes of
the cell boundary between the coronet and supporting cells in the basal part of the epithelium
are often ruptured and disappear (vide infra). In such a case, the coronet cell seems to reach
to the basement membrane (Fig. 19).
Lysosomes are mostly distributed in the supranuclear region, especially between the base of the apical protrusion and the superior pole of the nucleus (Fig. 15–18, 22). It is rare to find them in the apical protrusion, and, if present, they are situated at the base where no
pin-like processes are attached (Fig. 16, 22). The lysosomes are few in the para- and infra-nuclear parts, and none of them occur within the specific area beneath the nucleus.

The lysosomes are generally spherical and vary considerably in size. Their electron density is high in many cases, though also variable. Figures 17 and 18 illustrate the supranuclear region of the coronet cell in a considerably high magnification. There are many pleomorphic lysosomes in these figures. Each of them is covered with a smooth limiting membrane and contains fine granular or filamentous substance of high electron density. Some of them contain fragments of tubules, granules or vesicles (Fig. 17). Sometimes the lysosomes are difficult to be distinguished from the multivesicular body (Fig. 18). These are probably derived from a part of the cytoplasm trapped into the membranous sac, and reasonably correspond to the so-called cytolysomes.

There are many vacuoles and vesicles in the terminal globules of the pin-like processes and in the papillary protrusion, and they are frequently situated close to the luminal surface.
However, none of signs is suggestive of pinocytosis or inverse pinocytosis (secretion).

2. The supporting cell

The cell body of the supporting cell expands both at the apical and basal ends, the nucleus being contained more frequently in the former part of cell and less frequently in the latter. The nucleus is smaller than that of the coronet cell, and oval or triangular in shape (Fig. 22). The middle portion of this cell is generally slender as if pressed by the adjacent coronet cells, though it may rarely be rather thick and contains the nucleus. Nuclear pores are abundant. The cell often extends thin cytoplasmic sheets between the coronet cells. Thus, most coronet cells are enclosed incompletely by the cytoplasmic sheets of the supporting cell, though they, in certain parts of cell body, come into direct contact with adjacent coronet cells. In either case, the so-called intercellular interdigitation cannot be observed. The terminal bars are observed around the neck of the cytoplasmic protrusion, where the coronet cell joins to the surrounding supporting cells, but the desmosomes cannot be found at any place (Fig. 15, 16, 22). There are only terminal bars at the luminal ends of the junction between two adjacent

![Fig. 20 and 21. The basal half of the saccus epithelium and the underlying blood capillary (Cap). The basement membranes (BM) intervene the capillary and the epithelial cells. Attenuated extensions of the supporting cells (SC) are inserted in between the coronet cells and the blood capillary and between the two adjacent coronet cells. Mitochondria (M) surround the infranuclear specific area of the coronet cell. Note the tubular endoplasmic reticulum (confer the vacuolar endoplasmic reticulum in Fig. 19). N coronet cell nuclei, Er erythrocyte. Fig. 20: ×5,600. Fig. 21: ×15,000]
supporting cells.

The free surface of the supporting cell facing the lumen is generally smooth with a few elevations and depressions (Fig. 15, 16, 22). A small number of microvilli and a single cilium may occur (Fig. 22). In fine-structural organization, therefore, the surface of the supporting cell resembles that of the papillary cytoplasmic protrusion of the coronet cell.

The base of the supporting cell is extended along the basement membrane to separate the basal surface of the coronet cells from the basement membrane. However, fragmentation of the apposed plasmatic membranes of coronet and supporting cells frequently occur probably due to an artefact during the specimen preparation, and a seemingly free communication between both cells may be observed (Fig. 19, 20). Sometimes, the apposed plasma membranes of the adjacent cells of either the same or different cell types may turn into a row of small vesicles (Fig. 19), which is reminiscent of the aligned vesicles found in the spiral ligament of the bat inner ear (Watanabe 1964). In the cited paper, the author could not decide whether this feature was artefact or not.

The cytoplasm of the supporting cell is generally less dense than that of the coronet cells. There is an endoplasmic reticulum of rough-surface type, and free ribosomes are scattered through the cytoplasm forming small clusters. The smooth endoplasmic reticulum is less developed than in the coronet cell. There are many vesicles of various sizes at the apical end of the cell (Fig. 22), but the pinocytosis or inverse pinocytosis cannot be recognized. At the basal
part of the cell, a few vesicles are observed, but also here no evidence can be found for the pinocytosis.

Relatively small Golgi apparatus is situated at the supranuclear region, and consists of lamellae, vesicles and vacuoles. The Golgi vacuoles are most remarkable. Mitochondria are distributed through the entire cytoplasm, but numerous above the nucleus.

The microtubules are completely absent in the supporting cell unlike in the coronet cell. This fact and the less developed smooth endoplasmic reticulum characterize the supporting cell. However, filaments are seen is every place of the cytoplasm, and lysosomes which resemble those in the coronet cell are distributed in the supranuclear region.

3. The relationship between the coronet cells and nerve fibers

Bundles of fine nerve fibers are often observed among cells in the base of the saccus epithelium. Fig. 23 shows a nerve bundle composed of six unmyelinated fibers found in the intercellular space between the basal cytoplasmic parts of coronet cells; Fig. 24 reveals it in higher magnification. In one of these unmyelinated axons, a few vesicles are seen in the matrix, and the plasma membranes adjoining the coronet cell is slightly thickened. This seems to suggest

Fig. 22. The apical part of the saccus epithelium. The coronet cells bear the papillary cytoplasmic protrusions (Pcp) with pin-like processes which end in globules (G). Lysosomes (Ly) are contained in the coronet cells. At the luminal edge of the intercellular boundaries, terminal bars are observed, but no desmosome can be seen. The nuclei (N') of the supporting cells are situated in various level of the epithelium. ×5,600
Fig. 23. The basal part of the epithelium in the saccus vasculosus. The arrow indicates a bundle of unmyelinated nerve fibers situated in the intercellular clef between the coronet cells. N nucleus, M mitochondria of the coronet cell, Cap blood capillary $\times$1,5000

Fig. 24. A higher magnification micrograph of the bundle of six unmyelinated axons shown in Fig. 23. One of the axons contains a few vesicles and possesses a thickened plasma membranes suggesting a synapse, M mitochondria. $\times$23,000
the existence of a synapse in this place. As shown in these micrographs, fragmentation and partial disappearance of the plasma membranes occur at the apposition of the coronet cells to the supporting cells.

**Discussion**

The saccus vasculosus is the specific organ only found in the fish, and it has been known that the organ is more developed in the elasmobranches than in the teleosts. The function of this organ is still in debate; two major theories, i.e. the sensory and secretory theories have been repeatedly proposed (see DORN 1955).

Detailed cytological descriptions concerning the fine structure of the coronet cell, which is believed to be most important to elucidate the function of this organ, are very few, and only two reports by electron microscopy (BARGMANN and KNOOP 1955, KUROTAKI 1960) have appeared, both treating of only the teleost. Since these studies were performed in the days when the electron microscopic techniques had not been advanced as today, the results seem not satisfactory. Therefore, it may be worth to discuss again on the functional significance of this peculiar organ of the fish, standing upon the results obtained in the specimens made through the current technique with epon-embedding and lead-staining. Furthermore, as the recent progress in histochemistry seems also effective for elucidation of this problem (KAMER et al. 1960, SUNDARARAJ and PRASAD 1964), the light microscopical cytology should be simultaneously studied on this organ.

The terminal globules of the pin-like processes of the coronet cell are always spherical or oval in shape, and contain vacuoles and vesicles. The modification of the globules into a balloon-like swelling reported by BARGMANN and KNOOP (1955) as one of the evidences for the secretory hypothesis could not be recognized in the present observation. Moreover, no formed elements probably detached from the coronet cells as observed with the light microscope by BARGMANN (1954) could be found in the follicular lumen.

It is evident that the pin-like processes are nothing but a modification of the cilia, though the two central tubules were not observed in the shaft of the former. This view coincides with those of BARGMANN and KNOOP (1954) and of KUROTAKI (1960). In the ray, however, the peripheral tubules do not enter the central part of the globule unlike those described by these authors, but are situated at the peripheral zone of the globule. This seems to be one of the important differences in structure of the coronet cells between the teleosts and elasmobranches. KAMER (1960) supported the secretion hypothesis from his result that glycogen occurred at the cell apex after pilocarpine injection. In the ray saccus vasculosus, however, various amounts of glycogen can be detected throughout the cytoplasm already in the normal case, in coincidence with the finding of SUNDARARAJ and PRASAD (1960).

The apical papillary protrusion of the coronet cell resembles the distal projection of the olfactory cell (olfactory rod) extending into the nasal cavity (RHODIN 1963, REESE 1965, STOCKINGER and CIRELI 1965, GRAZIADELI 1965, OKANO 1965, BANNISTER 1965). By these authors it is elucidated that the cytoplasm of the olfactory cell is penetrated by longitudinally oriented microtubules, vesicles and filaments which enter into the distal projection. There are slight differences in form and ultrastructure between the olfactory hairlets radiating from the olfactory rod and the pin-like processes similarly radiating from the apical papillary protrusion of the coronet cell, both of which are considered to be modified cilia.

The apical protrusion with pin-like processes of the coronet cell has a certain resemblance
to the inner and outer segments of cone and rod cells of the retina as suggested by KUROTAKI (1960), though the former does not contain an accumulation of mitochondria like the ellipsoid of the visual cells. These structural resemblance of the coronet cells to the olfactory or visual cells seems to indicate that the cells probably are sensory in nature. Furthermore, light microscopy reveals a number of nerve bundles at the basal region of the epithelium. No report has been published concerning the relationship between the epithelial cells and nerve fibers in the teleost saccus vasculosus. In the ray organ, however, small bundles of axons were observed in the intercellular spaces between the basal parts of the epithelial cells, and the synaptic vesicles and synaptic membranes were identified in an axon abutting on the basal part of a coronet cell. These findings strongly support the sensory hypothesis, though the problem has not yet been decisively resolved.

Longitudinal fibers in the coronet cell cytoplasm demonstrated by silver impregnation (BARGMANN 1954) and phase contrast microscopy (DORN 1954) probably correspond to the microtubules found in the present electron micrographs.

Although, as mentioned above, it is clear that the pin-like processes are modified type of cilia as suggested by YAMADA (1961), the origin of abundant vacuoles and vesicles contained in them could not be elucidated. The vacuoles scattered through the general cytoplasm except for the pin-like processes are evidently formed by the dilatation of the smooth endoplasmic reticulum, and vary considerably according to the functional state. As the vacuoles in the globules of the processes do not show any relationship with the smooth endoplasmic reticulum and do not change their form and size accompanying the functional fluctuation, they are reasonably thought to be different in nature from the vacuoles in the main cell body.

REESE (1965), OKANO (1965) and other authors reported the occurrence of small vesicles in the olfactory hairlets, but the vesicles in the globules of the coronet cell are too large and numerous to be compared with them. As far as this problem is concerned, the coronet cell seems to stand nearer to the visual cell than to the olfactory cell.

It was noted by previous authors that the teleost coronet cells contained numerous tubular elements of the smooth endoplasmic reticulum (BARGMANN and KNOOP 1954), many vesicles and a few vacuoles (KUROTAKI 1961). In the present observation on the saccus vasculosus of the ray, a well developed network of the smooth endoplasmic reticulum, many filaments and microtubules were found, and the vacuoles and vesicles were assumed to be derived from the tubular smooth endoplasmic reticulum.

Light microscopy indicated the presence of a mass of homogeneous hyaline substance in the infranuclear region, and this structure was revealed by electron microscopy to be composed of a thick feltwork of filaments and a complicated network of the smooth endoplasmic reticulum distributed among the filaments. It is interesting to note that the morphology of the smooth endoplasmic reticulum in this specific area may vary considerably according to the functional state of the cell. Also conspicuous is the absence of microtubules in the infranuclear specific area, around which mitochondria were arranged as revealed by both light and electron microscopy. The functional significance of this area could not be clarified.

The basal portion of the supporting cell extends a thin cytoplasmic sheet along the basement membrane to separate the coronet cells from it. There are found ruptures and fragmentations in the apposed plasma membranes of coronet and supporting cells and the coronet cell seems to reach the basement membrane in such a place. Instead of ruptures, the apposed membranes sometimes form a row of numerous vesicles. This feature coincides with the finding in the spiral ligament cells of the bat inner ear (WATANABE 1964). It is not yet determined
whether this feature is an artefact or not. It may be worth to add that such a feature can be observed not only at the basal part of coronet cells, but also at the level of their nuclei.

As in the teleost coronet cells reported by Bargmann (1954), PAS-positive granules occur in the supranuclear region and the apical protrusion in the ray coronet cells. These granules correspond to the so-called lysosomes found by electron microscopy. They are surrounded by a single smooth membrane and contain various particles probably fragmented parts of the cytoplasm and are thus thought to be the cytolyosomes. Some lysosomes are similar in structure to the multivesicular body. Recently Merker (1965) classified the multivesicular body as a type of lysosomes. It must be noted that the lysosomes are never observed in the infranuclear cytoplasmic region.

The supporting cell shows no remarkable characteristics. Although some vesicles may be accumulated subjacent to the surface plasma membrane, none of secretory signs could be recognized.

By light microscopy, another cell type was found, showing a smaller flask-like cell body with a slight elevation of apical cytoplasm without pin-like processes. Sometimes, the apical elevation of this cell type possesses a single thick flagellum (or cilium) which is similar to that of the coronet cell. It is regrettable that this cell could not be successfully observed with the electron microscope in this study. It should be elucidated in the near future whether this cell represents a functional phase either of the coronet cell or of the supporting cell, or a completely independent cell type.

Through the present light and electron microscopic studies on the saccus vasculosus of the ray, a species of the elasmobranch, no evidence favorable to the secretion hypothesis of this organ was obtained. On the contrary, the coronet cells possess some resemblances to certain sensory cells and show a close relationship to the nerve fibers. These findings strongly support the receptor hypothesis of this organ first proclaimed by Dammerman (1910).

**Summary**

The saccus vasculosus of the ray, *Dasyatis akajei* (elasmobranch) was observed with the light and electron microscopes.

The light microscopy revealed that the saccus vasculosus consists of follicles whose walls are lined with the epithelium made up of two cell types: the coronet cells with an apical cytoplasmic protrusion provided with many pin-like processes and the supporting cells distributed among the former. There are many blood capillaries and nerve bundles in the interstitial tissue among follicles. The general structures of the coronet cells, their Golgi apparatus, mitochondria, infranuclear homogeneous hyaline substance, PAS-positive granules and the intracellular glycogen were described. A special type of epithelial cell, flask-like in shape and filled with many mitochondria, were scattered in the saccus vasculosus. This cell type was recognized only by light microscopy, and failed to be observed under the electron microscope.

The detailed ultrastructure of the coronet cell was observed by electron microscopy. The papillary cytoplasmic protrusion on the apical surface is provided with many pin-like processes whose tips are called globules and contain many vacuoles and vesicles. These pin-like processes are regarded as modified cilia. The homogeneous hyaline substance filling the infranuclear cytoplasm found by light microscopy is a peculiar cytoplasmic area composed of a thick layer or a network of fine filamentous bundles.

Besides wide-spread smooth endoplasmic reticulum and filaments, electron microscopy
revealed in the coronet cell cytoplasm also microtubules, vacuoles, vesicles, lysosomes, mitochondria and a Golgi apparatus. Small nerve bundles frequently penetrate into the epithelium and make a synapse-like structure adjacent to the coronet cell.

Any evidence was found for secretory function neither in the coronet nor in the supporting cells. From the similarity in structure of the papillary protrusion with pin-like processes of the coronet cells to the olfactory rod with hairlets, the probable function of the saccus vasculosus was presumed to be sensory.

References


Saccus Vasculosus of the Ray

---


