Morphology of the Lateralis Canal System in the Shark Genus *Carcharhinus*[1]

ALBERT L. TESTER and JAMES I. KENDALL[2]

THE SENSORY CANAL SYSTEM of elasmobranchs has been studied by several early workers. Garman (1888) described the gross morphology of the system in several species of sharks and rays. A detailed account of the morphology and innervation of the sensory canals was given by Ewart (1891) for the shark, *Laemargus,* and by Ewart and Mitchell (1891) for the skate, *Raia batis.* Johnson (1917) described the structure and development of the lateral canals of the sharks, *Mustelus canis* and *Squalus acanthias,* and Norris and Hughes (1920) provided detailed information on the innervation of the head and lateral canals in the same two species.

The literature does not contain a description of the sensory canal system of *Carcharhinus,* which includes many species of epipelagic sharks commonly encountered in both inshore and offshore waters. The present study attempts to overcome this deficiency, providing information on the gross morphology and microanatomy of the system. It complements a study of the related free neuromast or pit organ system of sharks by Tester and Nelson (1967) and Tester and Kendall (1967). It is hoped that the information will be of value to biophysicists, neurophysiologists, and behaviorists in interpreting the results of experiments designed to increase our understanding of how the lateralis system functions as a water displacement receptor (Dijkgraaf, 1963; van Bergeijk, 1967) or other sensor.

MATERIALS AND METHODS

Specimens examined included the following: *Carcharhinus menisorrah*—several near-term pups (33 to 35 cm total length) from Eniwetok Atoll, 2 adults (132 and 133 cm) from Eniwetok Atoll, and 2 adults (125 and 162 cm) taken off Kauai; *C. falciformis*—several near-term pups (averaging 50 cm) taken off Kauai; and *C. melanopterus*—several heads from juveniles (about 30 cm) taken from the Line Islands.

The binocular microscope was used for locating modified scales associated with tubule openings and for gross dissection of the canals. The paths of the canals and their tubules were traced both by dissection and by clearing half-heads and pealed skins in glycerine after the canals had been injected with either latex or India ink. Some preparations were bleached in Clorox before clearing to render the skins more transparent.

The structure of the neuromasts and their associated epithelia were studied in freehand, frozen, and paraffin sections using freshly killed material fixed in formalin, or Bouin's, Susa's, or Regaud's fixatives. In some paraffin preparations it was necessary to decalcify the scales with Decal before sectioning. Various stains were used including Heidenhain's hematoxylin and triosin, Mallory's triple, Giemsa, Siegel's toluidine blue O and napthol yellow S, and carmine. Altmann's method was used for staining mitochondria.

Nerve fibers and their terminations were studied in frozen sections of formalin-fixed material using silver impregnation and gold toning according to the method of Gilbert (1965).

GROSS MORPHOLOGY

The sensory canal system is similar in all species of *Carcharhinus* examined. The paths of the canals and tubules are shown in Figure 1 for a *C. falciformis* pup. The names assigned to various parts of the system follow, for the most part, those of Ewart (1891) and subsequent authors and are based on both position and innervation.

[1] Contribution No. 315, Hawaii Marine Laboratory, University of Hawaii. This study was supported by the Department of the Navy, Office of Naval Research, Contract 2756(00). Manuscript received May 23, 1968.

[2] Department of Zoology, University of Hawaii, Honolulu, Hawaii 96822.

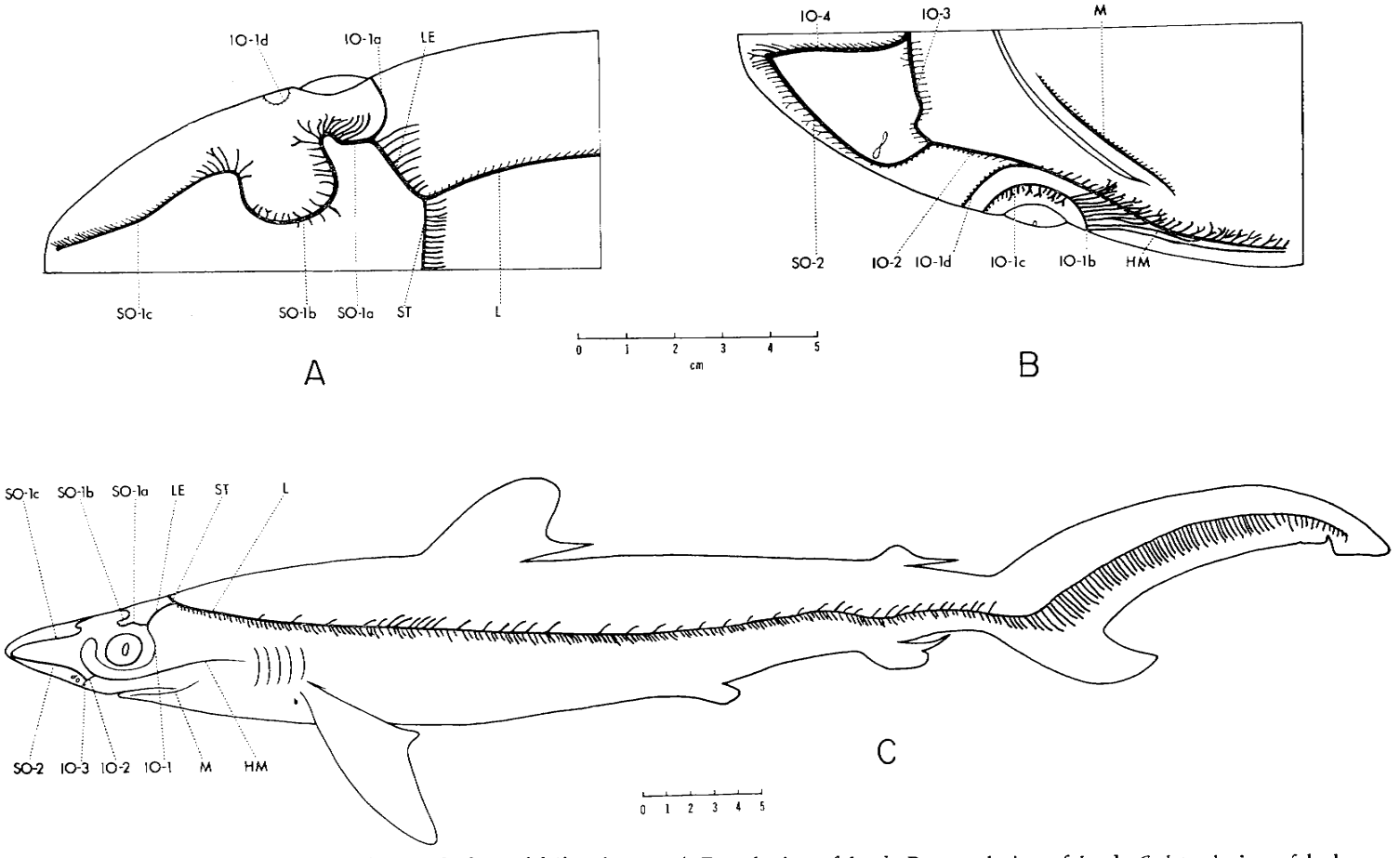

FIG. 1. The lateralis canal system of a *Carcharhinus falciformis* pup. *A,* Dorsal view of head; *B,* ventral view of head; *C,* lateral view of body.

The intercommunicating canal system includes four primary divisions: supraorbital (SO-1, SO-2), infraorbital (IO-1 to IO-4), hyomandibular (HM) and lateral (L). The infraorbital of each side is joined ventrally at the juncture of IO-3 and IO-4. The lateral of each side is joined anterodorsally by the supratemporal commissure (ST). A short length of canal from the juncture of L and ST to that of SO-1 and IO-1 has been termed the lateral extension (LE) by the present authors. Isolated mandibular canals (M) occur behind the lower jaw.

For the most part, the canals are embedded in or closely associated with the compact layer of the dermis. An exception is that part of the system comprised of LE, SO-1a and IO-1a, behind the eye, which lies well below the dermis and is covered in part by subcutaneous connective tissue, musculature, or ampullar tubes. IO-1a has a thick fibrous wall which is closely associated with the postorbital process. The head canals SO-1c, SO-2 and IO-4 are deeply embedded in subcutaneous connective tissue adjacent to the dermis and follow fibrous septa which separate groups of ampullae of Lorenzini.

Attention is called to certain peculiarities of the system which also occur to a greater or lesser extent in other shark genera, namely the pronounced supraorbital loop (SO-1b) on the dorsal surface of the head, the pronounced infraorbital loop (IO-2b) in front of the eye, the slight loop toward the nostril of IO-3, and the peculiar S-shaped curve of the lateral (L) below the second dorsal fin. The loops concentrate the tubule openings in these areas; the biological significance of this is uncertain.

In cross section, the canals are generally oval in shape. Measurements of the inner diameters of the fibrous sheath surrounding the epithelium and lumen are given in Table 1 for a 35-cm pup and a 162-cm adult *C. menisorrah*. The cross-sectional areas ($\pi\ r_1 r_2$), which include the epithelium, are somewhat greater than the areas of the lumen proper, particularly in the anterior head canals where the epithelium distal to the neuromast is thickened and convoluted. Although there is considerable variation, in general the more anterior head canals (SO-1, SO-2, and IO-4) have the larger bores. That of the lateral extension is also relatively large. Those of both the hyomandibular and the lateral decrease caudad. The maximum bore of the isolated mandibular, at its mid-point, is relatively small compared with that of the intercommunicating

TABLE 1

SHORT AND LONG DIAMETERS OF THE LUMEN (INCLUDING EPITHELIUM), AND THE BORE (CROSS-SECTIONAL AREA INCLUDING EPITHELIUM), FOR SENSORY CANALS OF A 35-CM PUP (PARAFFIN SECTIONS) AND A 162-CM ADULT (FREEHAND OR FROZEN SECTIONS) OF *Carcharhinus menisorrah*

	PUP			ADULT		
	DIAMETERS (mm)		BORE	DIAMETERS (mm)		BORE
CANALS*	SHORT	LONG	(mm^2)	SHORT	LONG	(mm^2)
SO-1a	0.38	0.42	0.123	0.8	1.4	0.88
SO-1b	0.30	0.42	0.099	0.9	1.4	0.99
SO-1c	0.30	0.42	0.099	0.9	1.0	0.71
SO-2	0.41	0.57	0.184	0.9	1.4	0.99
IO-1a	0.28	0.38	0.083	0.7	0.9	0.49
IO-1b	0.26	0.30	0.061	0.6	0.7	0.33
IO-2	—	—	—	0.7	0.9	0.49
IO-3	0.26	0.33	0.067	0.6	1.3	0.61
IO-4	0.22	0.68	0.118	1.1	1.4	1.21
HM (start)	0.26	0.30	0.061	0.7	0.9	0.49
M (max)	—	—	—	0.5	0.5	0.20
LE	0.22	0.66	0.114	0.8	1.2	0.75
ST	0.18	0.26	0.037	0.5	0.9	0.35
L (start)	0.27	0.30	0.064	0.7	0.9	0.49
L (1st dorsal)	0.13	0.17	0.017	0.5	0.6	0.23

* For identification of the canals see Figure 1.

canals. The canal bores increase with size of shark. When length of shark increases by a factor of 4.6, the average bore increases by a factor of 7.7. However, this is a smaller increase than would be expected with isometric growth (factor 21.4). The comparison of canal bores between sizes of shark may err slightly because of the difference in method of preparation (paraffin vs. freehand or frozen sections).

The canal tubules present an unexpected complexity in relative frequency, length, and orientation. They are difficult to map even when the canals are injected with opaque substances, for there is no assurance that impregnation has been complete. However, we believe that their representation in Figure 1 for the *C. falciformis* pup is reasonably accurate and complete.

In front of the eye, both dorsally and ventrally, most of the tubules of the canals are directed outward and forward, either anterolaterally (SO-1c and SO-2) or anteromedially (IO-4). However, some are directed straight outward, following the shortest possible route to the surface (posterior part of SO-2, IO-2, and IO-1d). A few tubules are directed posteriorly (IO-3) or posteromedially (SO-1b). In general the bore of the tubules is about one-quarter that of the canal but increases to more than half that of the canal in the most anterior tubules. A few tubules are branched.

In the vicinity of the eye, the tubules of the supraorbital (SO-1a) and part of the infraorbital (IO-1c) are directed toward the eye to partially surround it with pores. The tubules of IO-1c branch profusely. Noteworthy are the very long tubules arising from the infraorbital canal back of the eye (IO-1b) which are directed backward, branch profusely, and end in pores scattered among those of the hyomandibular. These long tubules do not form commissures with the hyomandibular but pass over it distally.

Behind the eyes, the tubules are generally directed outward and backward. Those of the lateral extension and the supratemporal are relatively long and their pores are distributed across the back. Those of the isolated mandibular are very short and are directed posteriorly. The tubules of the laterals are initially relatively short but increase in length to the fifth gill opening, and maintain this length over most of the body. Most of the lateral canal tubules are directed outward and posteroventrally; a smaller number, irregularly spaced, are directed outward and posterodorsally. On the caudal fin the tubules are relatively long and are directed outward and posteroventrally. Along the body, and particularly on the caudal fin, the posteroventrally directed tubules tend to vary slightly in length in an alternating fashion. Their bore averages about one-third that of the canal, thus progressively decreasing as canal size decreases along the body. On the caudal fin, the tubule bore becomes relatively larger; although still small, it approaches that of the canal caudad.

There are short lengths of canal without tubules (IO-1a and part of SO-1a) where the canals have thick fibrous walls and lie deep below the dermis. It may be noted here, however, that these canals contain neuromasts.

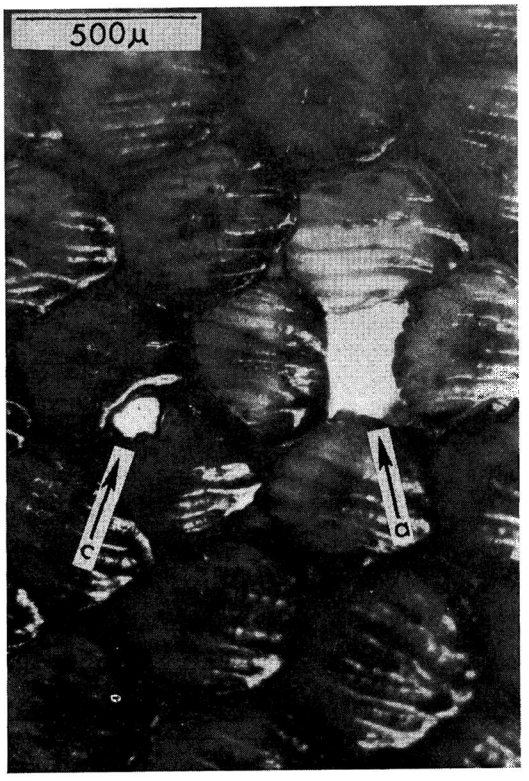

FIG. 2. Pore of head canal tubule (*c*) bounded by notched scales in contrast with larger pore of ampulla of Lorenzini (*a*) in a *Carcharhinus menisorrah* adult.

The tubule openings on the head are small and inconspicuous compared with those of the ampullae of Lorenzini (Fig. 2). They occur between the crowns of scales which are notched to conform with the circular shape of the pore. The pores are not covered by overhanging scale crowns. On the side of the body, the scale crowns above the pore are also notched to provide an opening for the tubule; they are narrower and thicker than those of normal scales and often have a rosette-like arrangement. Frequently the pore is covered completely or in part by an overhanging crown.

It has been noted that the tubules of the more anterior head canals are of relatively large bore and generally are directed forward, whereas those of the more posterior head canals and the laterals are of progressively smaller bore and generally are directed backward. This arrangement should produce a slow flow of seawater caudad along the system because of water pressure on the head during forward swimming. We have demonstrated this flow in some shark genera[3] but not yet in *Carcharhinus* because of the lack of small live specimens. Doubtless it occurs. Smith (1930, 1933) has shown a flow in the canal system of some but not all of the teleosts that he studied.

MICROANATOMY

Sensory Epithelium

As found by Johnson (1917) in *Squalus* and *Mustelus,* the epithelium of the canal in *Carcharhinus* is basically two-layered. For discussion, it may be divided into sensory and nonsensory regions. Its structure is illustrated diagrammatically in Figures 3 and 4.

Within the canal opposite the tubule openings (i.e., medial to the dermis) the epithelium is modified to form the sense organ—the neuromast or macula (Figs. 3*A* and *B*). This consists of sensory hair cells, supporting cells, cupula, and a peripheral layer of mantle cells. The neuromast is attached to a definite basement membrane, supported by a loose fibro-elastic connective tissue incorporating vascular and neural elements. Nerve fibers form a fiber zone in the connective tissue region before penetrating the basement membrane to innervate the sensory cells of the neuromast. The connective tissue sheath of the incoming nerve spreads within the thick fibrous sheath of the canal to form the loose vascular connective tissue supporting the non-sensory epithelium. The basement membrane in this region is absent or incomplete.

The sensory hair cells, with a prominent round or oval nucleus, are flask- or barrel-shaped (Fig. 3*C*). They extend from the distal surface to about two-thirds of the distance to the basement membrane. However, in some sensory cells of the pup neuromast, a strand of cytoplasm extends from the proximal end of the cell to the basement membrane, indicating an origin from cells extending the entire height of the organ. The "hair," about 6 μ long and tapering from about 1 μ wide at the base, is seen in some preparations (Fig. 5). Presumably it is a bundle including a kinocilium and several stereocilia as shown in teleosts by the electron microscope (e.g., by Flock, 1967).

Slender columnar or fusiform supporting cells surround the sensory cells and extend from the free surface to the basement membrane. Their nuclei are oval and are located in the proximal portion of the cells. The distal ends of the sensory cells and the surrounding supporting cells are joined by intercellular cement showing clearly as terminal bars with Heidenhain's hematoxylin. Giemsa staining shows metachromatic granules in the distal cytoplasm of the supporting cells and extracellularly at their distal surface, indicating that these cells are actively secreting mucopolysaccharides. Degenerating or necrotic cells with pycnotic nuclei and shrunken cytoplasm are of frequent occurrence. Mitotic figures (Fig. 6) occur occasionally above or near the level of the sensory cell nuclei.

Using primarily the above information, Tester

[3] A juvenile *Sphyrna lewini* (58 cm) was forced to swim in a weak (about 0.05%) solution of trypan blue for 50 minutes. Sectioning showed dye penetration in the head canals and along the laterals to the region of the second dorsal fin, but not beyond this point. Similar results were obtained with two juvenile *Ginglymostoma cirratum* (42 and 48 cm), although the rate of flow was very slow in these sluggish sharks. In one, the dye penetrated the head canals and along the laterals to the gill region in 2 hours; in the other, it penetrated the head canals and along the laterals to the region of the second dorsal fin in 4 hours.

and Kendall (1968) showed that in sharks the supporting cells secrete columns of mucoid material comprising the cupula and producing its typical vertically-striated appearance. They conclude that cupula production is a continuing process, accompanied by discharge, discarding, and regeneration of supporting cells and by continual loss of cupular material at the distal free

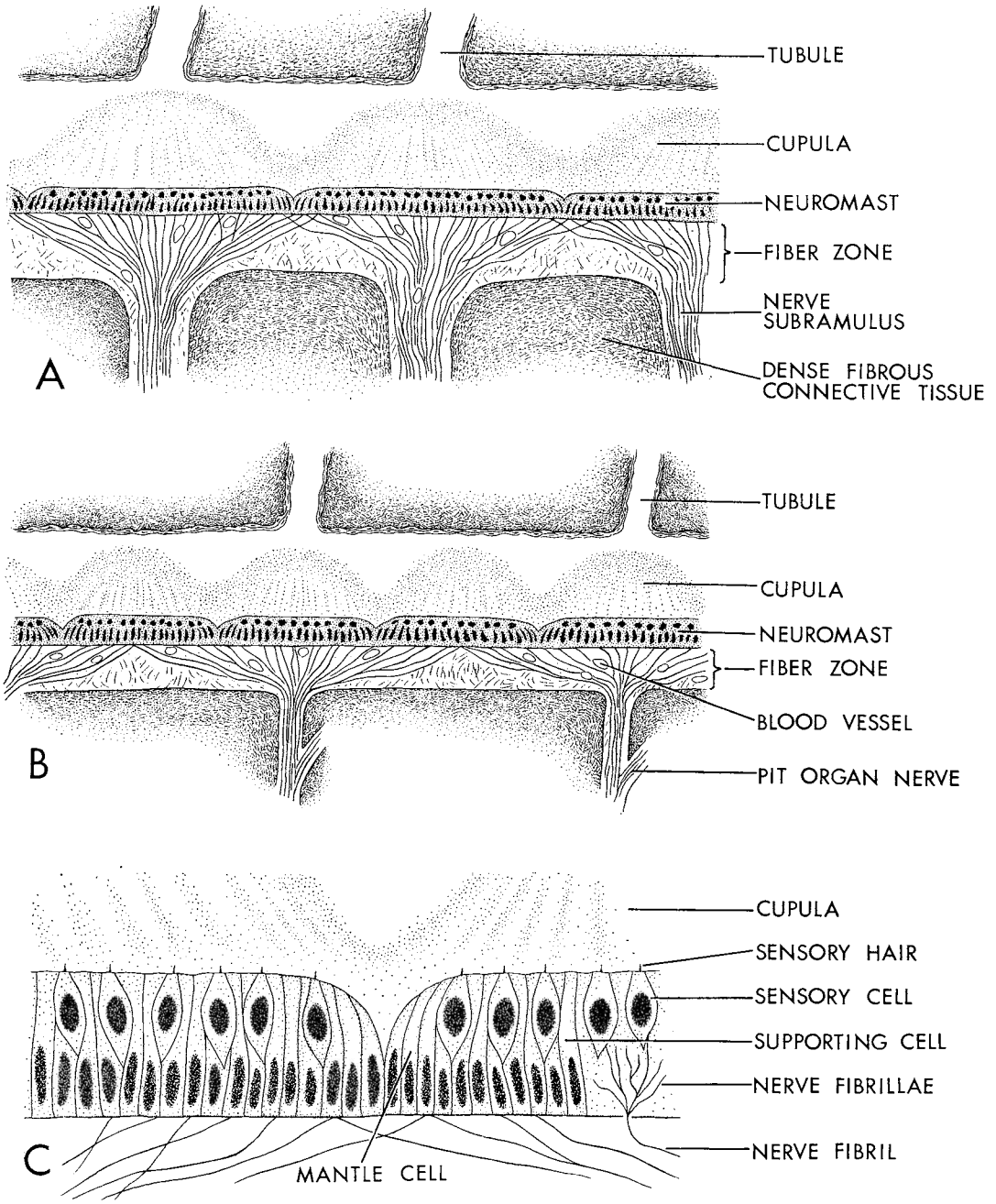

FIG. 3. Diagrams of longitudinal sections of canals in a *Carcharhinus menisorrah* pup. *A*, Head canal (ca. × 75); *B*, lateral canal (ca. × 75); *C*, head canal (ca. × 600).

surface. The delicate cupula is often lost during routine fixation and slide preparation. Even when retained, it shrinks considerably (Fig. 7). In fresh-frozen sections of carefully handled, freshly killed specimens it appears to reach nearly to the top of the canal.

The presumed function of the cupula in sharks is similar to that in teleosts. It is believed to move or "glide" slightly over the surface of the sensory epithelium under the influence of a displacement of the canal fluid, thereby bending the hairs embedded in it (Flock, 1967). Bending of the hair triggers electrogenesis in the hair cell by an intracellular transducing process not yet clearly understood.

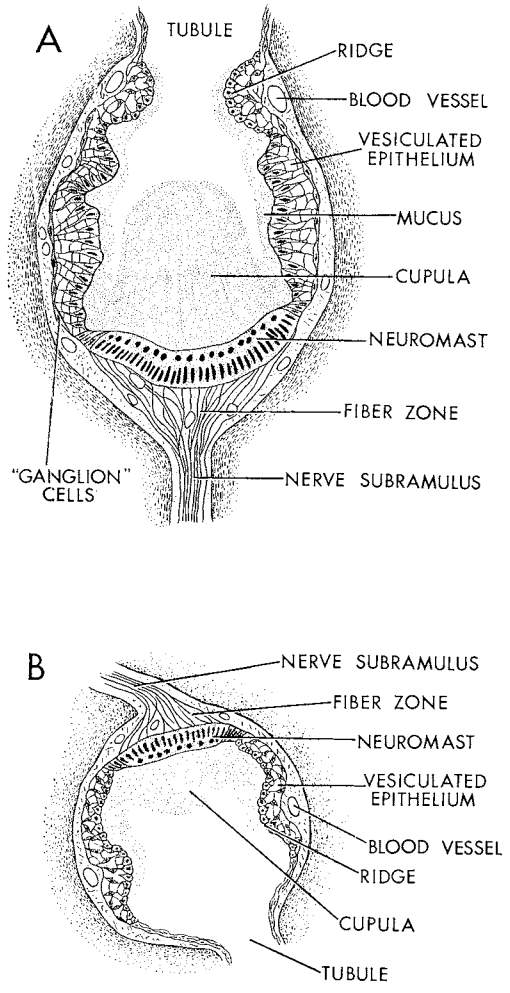

FIG. 4. Diagrams of cross sections of canals in a *Carcharhinus menisorrah* pup. *A*, Head canal (ca. × 75); *B*, lateral canal (ca. × 75).

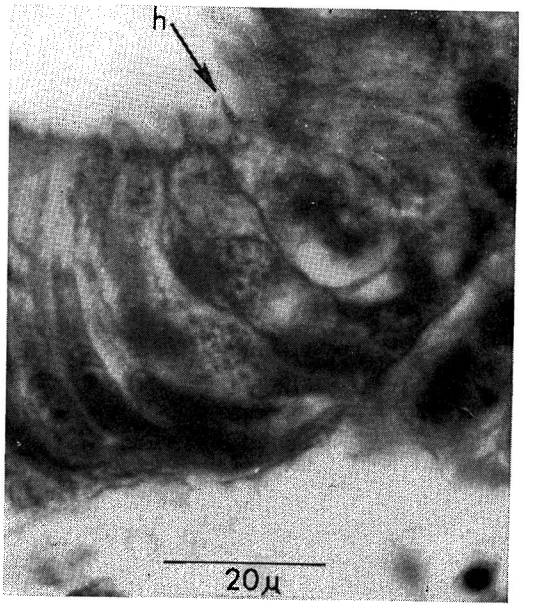

FIG. 5. Sensory cells in a pocket of the epithelium of the supratemporal canal in a *Carcharhinus menisorrah* pup showing sensory hairs (*h*). The hairs are usually lost during preparation. (Bouin's fixative, Mallory's stain, oil immersion.)

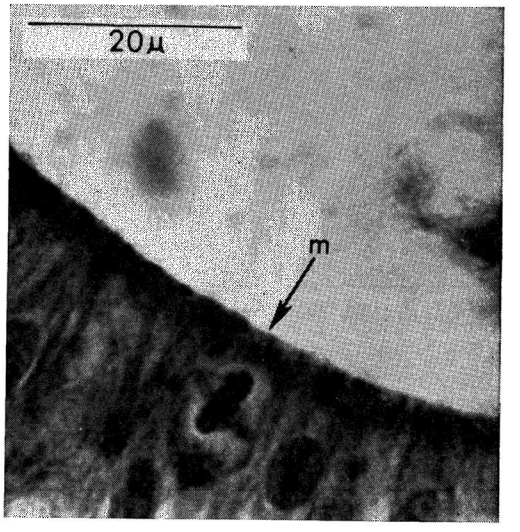

FIG. 6. Cross section through the infraorbital canal neuromast of a *Carcharhinus menisorrah* pup showing a mitotic figure (*m*) in metaphase among the sensory cell nuclei. (Bouin's fixative, Mallory's stain.)

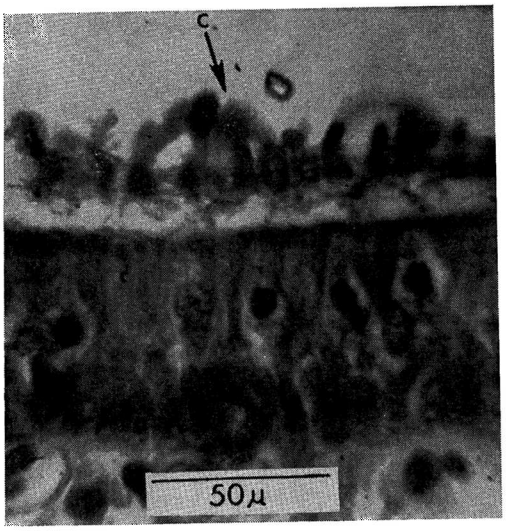

FIG. 7. Longitudinal section through lateral canal neuromast of a *Carcharhinus melanopterus* pup showing shrunken, striated cupula (*c*) partially torn from the neuromast surface. (Bouin's fixative, toluidine blue stain.)

The sensory epithelium is surrounded by a peripheral layer of inwardly curving supporting cells called "mantle" cells (Dijkgraaf, 1963). Metachromatic staining reveals the presence of mucus-producing cells which are contributing to the cupula.

In the pup and juvenile, the neuromasts of the canals are generally butted end to end, forming an almost continuous sensory epithelium. In longitudinal section (Figs. 3*C* and 8) the point of division between neuromasts can be identified by the opposing curvature of the mantle cells, producing a V-shaped indentation in the epithelial surface. The thickness of the epithelium decreases slightly (SO and IO) or abruptly (L) at the point of division. In the posterior part of the hyomandibular and in the mandibular, the neuromasts are separated by a few cuboidal or columnar cells, similar to those of the side walls, between the mantle cells. This situation also occurs in the posterior part of the lateral canals, particularly in the caudal region where the neuromasts are spaced by a distance equal to about half their length. In the larger specimens also, the neuromasts form an almost continuous epithelium. However, the spacings which occur are somewhat greater than in the pup and juvenile.

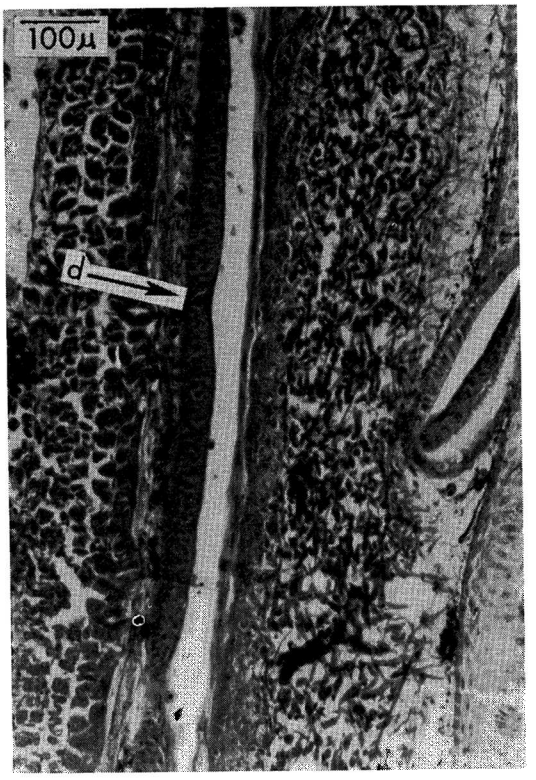

FIG. 8. Longitudinal section through lateral canal neuromasts of a *Carcharhinus menisorrah* pup showing V-shaped division (*d*) between two neuromasts. (Bouin's fixative, hemotoxylin-triosin stain.)

The neuromast is oval in facial section with its long axis parallel to that of the canal (Fig. 9). Measurements of the sensory epithelium in a 33-cm pup and a 125-cm "adult" (apparently still immature) *C. menisorrah* are included in Table 2. For each canal section there are averages of 2 or 3 measurements in each of 1 to 3 preparations. Length measurements are few because of the difficulty of obtaining perfectly oriented longitudinal sections. The measurements of the pup and adult are not strictly comparable because of a possible difference in shrinkage between paraffin and frozen sections.

In general, in both pup and adult, there appears a slight decrease in thickness, a somewhat larger decrease in width, and a still larger decrease in length of the neuromast caudad from the most anterior head canals (SO-1 and IO-4). There also appears to be an increase in size of neuromast with growth. With increase in

TABLE 2

MAXIMUM WIDTH AND THICKNESS (IN CROSS SECTIONS) AND LENGTH OF CANAL NEUROMASTS OF A 33-CM PUP (PARAFFIN SECTIONS) AND A 125-CM ADULT (FROZEN SECTIONS) *Carcharhinus menisorrah* (All measurements in microns)

CANAL	PUP			ADULT		
	WIDTH	THICKNESS	LENGTH	WIDTH	THICKNESS	LENGTH
SO-1a	160	30	—	330	45	—
SO-1b	163	30	—	330	60	—
SO-1c	171	30	504	432	42	1800
SO-2	217	38	450	425	38	—
IO-1	103	27	—	300	38	—
IO-3	171	34	—	330	—	—
IO-4	190	38	900	675	42	—
LE	106	30	—	300	42	—
HM	95	27	300	—	—	—
M	76	27	—	—	—	—
L (start)	84	30	—	225	34	—
L (above gills)	76	30	—	—	—	—
L (1st dorsal)	76	34	300	201	38	—
L (2nd dorsal)	—	—	—	152	38	—
L (caudal peduncle)	76	27	—	—	—	600
L (mid caudal fin)	60	19	—	—	—	—

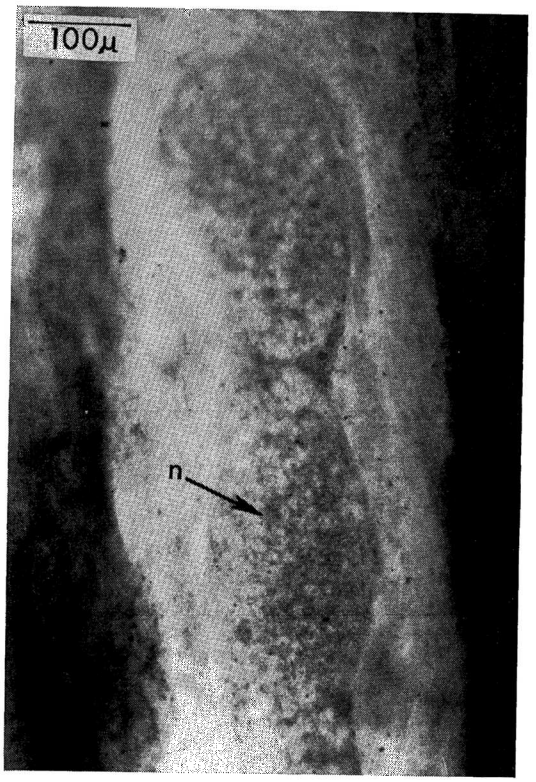

FIG. 9. Facial view of lateral canal neuromasts (*n*) from caudal peduncle of an adult *Carcharhinus menisorrah* showing their oval shape. (Formalin fixed frozen section, Giemsa stained.)

length of shark by a factor of 3.8, the neuromast increased slightly in thickness (average factor 1.3), considerably in width (average factor 2.5), and, from the meager data, extensively in length (factor 3.6 for SO-1c, only). It is unlikely that these differences are due entirely to differential shrinkage between paraffin and frozen sections. Since the average maximum diameter of a sensory cell appears to be similar (about 7 to 8 μ) in both pup and adult and since the ratio of sensory to supporting cells seems to remain about the same (about 1 to 5), it may be inferred that the number of sensory cells increases with growth.

Rough calculations may be made of the number of sensory cells per neuromast. The distance (d) between several sensory cells (n) in one plane (in sharp focus) is determined. The area occupied by one sensory cell and its surrounding supporting cells may be calculated as d^2/n^2. Based on 10 determinations (SO-1c and L) the

average is 39 μ^2 (range 20 μ^2 to 56 μ^2) for the *C. menisorrah* pup. Dividing this average into the area of the neuromast surface (calculated from Table 2) provides the following rough estimates (rounded to the nearest hundred) of the number of sensory cells per neuromast: IO-4–3,400; SO-2–2,000; SO-1c–1,400; HM–600; L (first dorsal fin)–500. These estimates agree in order of magnitude with those obtained by counting the number of sensory cell nuclei at all depths of focus in a 10-μ cross section and multiplying by the number of sections in the neuromast length. In the adult, assuming the same unit area, for SO-1c (the only canal for which data are available) the estimated number of sensory cells per neuromast is about 10 times greater than in the pup (15,700 compared with 1,400).

Non-sensory Epithelium

In the head canals, the epithelial lining adjacent to the neuromast has a peculiar "vesiculated" appearance (Figs. 4*A* and 10). It consists of columnar or wedge-shaped cells which are joined by distinct cytoplasmic processes enclosing well defined intercellular spaces. The cell nuclei are elongated and generally lie closer to the distal than to the proximal end of the cell. Staining with Giemsa stain shows mucus on the surface but not within the interior of the cells or in intercellular vesicles. Staining with Altmann's technique shows mitochrondria densely aggregated near the distal end of the cells (Fig. 11). The distal cell membrane has a striated appearance. Staining with naphthol yellow S (Siegel's stain) shows a finely granular content indicating the presence of residual proteins within the intercellular vesicles, in adjacent blood vessels, and in the lumen of the canal. The staining responses show that the cells are secretory in nature, but are not producing mucus. The accumulation of mitochondria in the distal portion of these cells in the absence of mucus secretion suggests a possible similarity to the "chloride cell" described by Copeland (1948) in the gills of *Fundulus heteroclitus,* particularly in those fish which he immersed in hypertonic seawater. Tests for chloride and other ions (Na, K) await the availability of living specimens.

In the more anterior head canals of the pup,

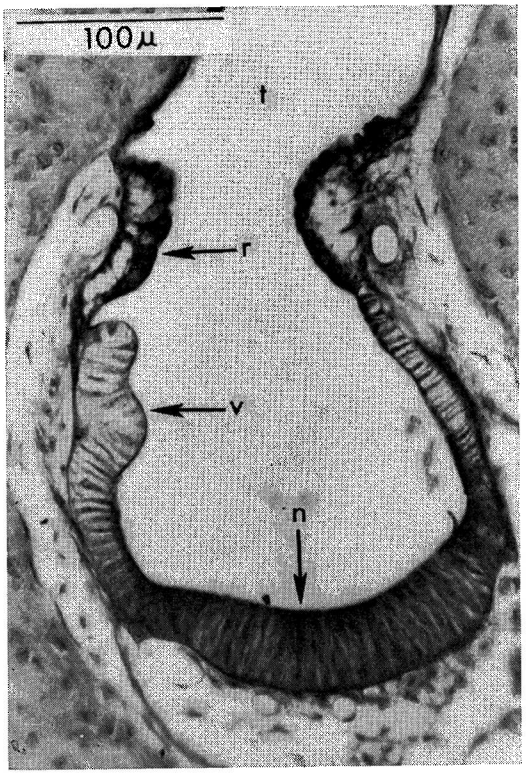

FIG. 10. Cross section of infraorbital head canal of a *Carcharhinus menisorrah* pup showing, from top to bottom, tubule (*t*) entering the canal, ridge area (*r*), vesiculated epithelium (*v*), and neuromast (*n*). (Bouin's fixative, Giemsa stained.)

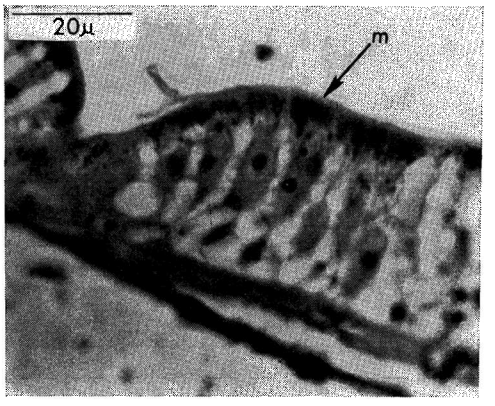

FIG. 11. Longitudinal section of the supraorbital head canal in an adult *Carcharhinus menisorrah* showing mitochondria (*m*) aggregated at distal end of cells of the vesiculated epithelium. (Altmann's staining method.)

those with forward-directed tubules (SO-1c, SO-2, and IO-4 in particular), the vesiculated area is elaborated into folds and finger-like projections (Figs. 4*A* and 10). In the more posterior head canals only the folds are present. In the laterals, it is reduced anteriorly and disappears posteriorly (Fig. 4*B*).

In juvenile and adult *Carcharhinus* the epithelium is similarly modified. In the head canals the elaboration into folds and finger-like projections is even more pronounced than in the pup and may be seen under the binocular microscope in gross dissection of the canal.

The function of this vesiculated epithelium will be discussed elsewhere. In brief, it is postulated that it may serve to regulate the ionic content (Na, K) of the canal fluid and tissues against a slow incursion of seawater through the anteriorly-directed tubules to facilitate electrogenesis in the neuromast. By controlling pH it may also play a role in polymerization of the polysaccharides involved in the cupular mucus.

Between the lining layer of cells and vesicles and the vascular connective tissue there is a layer of ganglion-like cells (Fig. 4*A*) which may be differentiated from smaller connective tissue cells. They are thin and elongate in cross sections of the canal but flattened and stellate in shape in longitudinal sections. They have a relatively large, round nucleus containing peripherally organized chromatin material and a prominent nucleolus. Vacuoles and fine granules and fibrils appear under the phase microscope; also cytoplasmic extensions link the cells into an anastomozing network. However, no neurofibrillae or neural connections to the neuromast nerve supply have been seen. It is possible that these ganglion-like cells exert neurohumeral control of the overlying secretory cells of the vesiculated area.

The vesiculated area extends from the mantle cells to the squamous or low cuboidal epithelium which lines the remainder of the canal and the canal tubules. However, just below the level of the tubule openings, both cells and intercellular vesicles become irregular in shape and are covered by a lining layer of cuboidal or low columnar cells to form a ridge (Figs. 4*A* and 10). The ganglion-like cells adjacent to the basement membrane are no longer present. This thickened ridge is supported by vascularized connective tissue, including a prominent blood vessel.

The epithelial cells of the ridge area have a dense, finely granular cytoplasm with a prominent round nucleus. The Altmann staining method shows mitochondria condensed below a distal vacuolation in which metachromatic granules are seen following Giemsa staining. The presence of mucopolysaccharides, both on the distal surface and as granules within the cells, shows that they are producing mucus.

The ridges are well developed in the more anterior head canals and may form one or several folds. Sometimes they extend into the canal tubule openings, forming valve-like flaps in cross section. The ridges are less well developed in the more posterior head canals, forming only a bulge or thickening of the epithelium. In the laterals they are progressively reduced caudad, persisting as a double row of cells adjacent to the neuromast and replacing the area occupied more anteriorly by the lining layer of vesiculated epithelium (Fig. 4*B*). They are thickened into a weak ridge only in the more anterior part of the lateral canal and only in the vicinity of the canal tubules. It seems likely that in the more anterior head canals with forward-directed tubules the flaps serve to impede or regulate a slow flow of seawater into the canal, and the contiguous ridges serve to direct the flow along the surface of the canal distal to the neuromast, thus minimizing the effect of flow on the cupula. They also secrete a mucous cover for the epithelial surface from the tubule openings to the neuromast.

The epithelium between the ridges in the canal and lining the canal tubules is also two-layered but is relatively thin and low-cuboidal or squamous. Caudad, with the retreat of the ridge cells toward the neuromast there is an increased circumference lined with this thin epithelium; it continues along the tubules until the transition to the many-layered stratified squamous epithelium of the epidermis (Fig. 4*B*). Secretion of mucus occurs in small patches of cuboidal cells along the course of the tubules.

Cephalic Glands

In close approximation to the more anterior head canals and the sacs of the ampullae of Lorenzini, there are numerous vascularized

gland-like pouches (Daniels, 1967) which, like the canals, open to the exterior by one or more tubules ending in pores among the scales (Fig. 12). The tubules are shorter than those of the canals and proceed directly to the surface. In one section, a small pouch opens into a canal tubule. The pore openings vary in size but are generally smaller than those of the canal tubules.

The glands have neither a neuromast nor a nerve supply. Otherwise their epithelium is very similar to that of the more anterior head canals, with folds of vesiculated epithelium resting on a thin layer of stellate, ganglion-like cells, with a smaller, lower ridge of mucus-producing cuboidal cells resting on irregularly shaped cells and vesicles, and with a thin, two-layered squamous or low cuboidal epithelium lining the neck of the pouch and continuing into the tubules until it joins the stratified squamous epithelium of the epidermis. The glands appear to be partially developed anlagen of head canals which have remained as isolated units with neither neuromast nor innervation. The lumen is filled with a secretion containing mucus which, in paraffin section, is much shrunken. This mucus resembles that which covers the vesiculated area of the canal and is presumably derived from the mucus-secreting ridge cells. It differs in density and lacks the striations found in the cupular covering of the neuomast. Residual proteins, demonstrated by naphthol yellow S both in the lumen of the gland and in the intracellular vesicles and blood vessels, may have been added to the lumen by the columnar and wedge-shaped cells of the vesiculated area. As in the head canals, the vesiculated area increases in size and in elaboration of folds from pup to juvenile and adult.

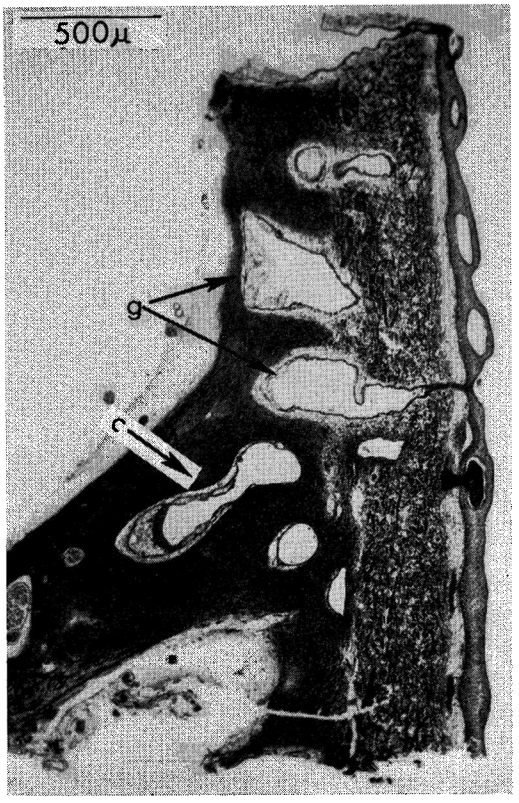

FIG. 12. Cross section through the infraorbital head canal (IO-4) of a *Carcharhinus menisorrah* pup showing the canal (*c*) and isolated cephalic glands (*g*). (Bouin's fixative, Mallory's stain.)

The function of the glands is unknown. It may be postulated that they serve to control the ionic concentration and polarity of the tissues adjacent to the sensory epithelia of the ampullae of Lorenzini.

INNERVATION

The gross innervation of the head canals in *Carcharhinus* has not been studied, but presumably it is similar to that described by Norris and Hughes (1920) in *Squalus acanthias*. The main nerves supplying the various canals in that species are summarized in Table 3.

It may be noted that some of the rami of the facial (VII) also innervate ampullae of Lorenzini, that some are intimately associated with corresponding branches of the trigeminal (V) which may contain general cutaneous fibers, and that one (r.m.e. VII) has a slender branch innervating the mandibular row of pit organs. Also, the rami of the vagus (X) innervate both canal and pit organs.

On leaving their ganglia, the nerves supplying the head canals proceed to a position just below the dermis. On reaching a canal, they follow its course, giving off ramuli which frequently divide into subramuli. These enter the canal midway along the length of a neuromast (Figs. 3*A* and 3*B*). As seen in silver-impregnated, gold-toned frozen sections, they consist of a relatively

TABLE 3

SUMMARY OF INNERVATION OF SENSORY CANALS FOR *Squalus acanthias**

CANAL	MAIN NERVE BRANCH
Supraorbital (SO)	Ramus ophthalmicus superficialis (VII)
Infraorbital (IO)	Ramus buccalis (VII)
Hyomandibular (HM)	Ramus mandibularis externis (VII)
Mandibular (M)	Ramus mandibularis externis (VII)
Lateral extension (LE)	Ramus oticus (VII)
	Ramus supratemporalis (IX)
	Ramus supratemporalis (X)
Supratemporal (ST)	Ramus supratemporalis (X)
Lateral (L)	Ramus dorsalis (X)
	Ramus lateralis (X)

* Compiled from Norris and Hughes (1920).

large number (ca. 25) of myelinated nerve fibers. Most of the axis cylinders are relatively thick (about 2 to 5 μ) in the 33-cm pup but with a few very thin fibers among them. After penetrating the canal wall, at least some of the fibers divide once, each branch proceeding in an opposite direction along the canal, as also noted by Johnson (1917) in *Squalus acanthias*. The fibrils form a thick fiber zone in the vascular connective tissue below the sensory epithelium. Although for the most part one neuromast seems to be innervated by the fibrils of one subramulus, the fibrils from adjacent subramuli overlap each other at the ends of adjacent neuromasts.

The innervation of the laterals (Tester and Kendall, 1967) is similar to that of the head canals but differs in detail. Over most of its length the ramus lateralis lies close to the vertebral column, moving to a more superficial position adjacent to the canal in the caudal peduncle and caudal fin. Ramuli are given off segmentally and proceed posterodorsally in the connective tissue sheath between the muscle segments. On reaching the lateral canal, which lies in the compact layer of dermis opposite the juncture of the dorsal and ventral muscle masses, the ramuli divide into subramuli which, after giving off fine nerves to the pit organs, enter the canal. The subramuli contain fewer fibers than those of the head canals (about 15 in the region of the gills, about 9 in the region of the dorsal fin, and fewer more posteriorly) and form a correspondingly thinner fiber zone in the connective tissue supporting the neuromast. One bundle of fibers proceeds anteriorly and another posteriorly, instead of each fiber dividing into two at the point of entry, as in the head canals. The fibers of one subramulus usually supply two neuromasts—one immediately adjacent to the subramulus, and one-half of each neuromast on either side. Occasionally the fibers of one subramulus will supply 3 neuromasts—one opposite the point of entry and one on either side (Fig. 13).

In both head and lateral canals, the fibrils of the fiber zone at intervals give off branches on approaching the basement membrane. The myelinated fibers appear to lose their myelin sheath on penetrating the basement membrane. After penetrating the membrane the fibrils are seen to branch repeatedly into fine fibrillae

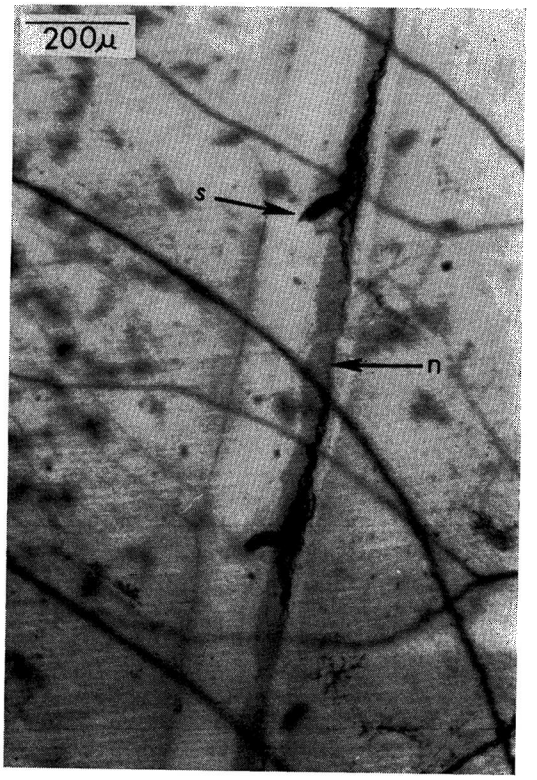

FIG. 13. Longitudinal section of lateral canal of a *Carcharhinus menisorrah* pup showing two subramuli (*s*), cut after leaving the canal, each innervating three neuromasts (*n*). (Gilbert's silver method.)

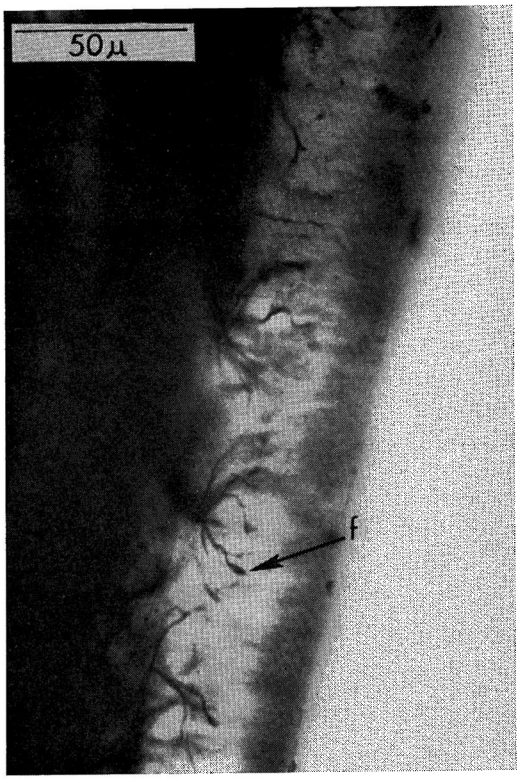

FIG. 14. Longitudinal section of the supraorbital canal of a *Carcharhinus menisorrah* pup showing nerve fibers dividing into fibrillae (*f*) among the sensory cells of the neuromast. (Gilbert's silver method.)

(Figs. 3C and 14). These end among the hair cells, presumably in contact with the cell membranes, as has been shown in teleosts by electron microscopy (Hama, 1965; Flock, 1965; and others).

It may be pointed out that, by profuse branching, one nerve fiber supplies a large number of hair cells (possibly 50 or more in the *Carcharhinus menisorrah* pup and perhaps several times that number in the adult). Moreover, there seems to be overlapping innervation between neuromasts, but we could not determine whether the overlap involves one fiber or separate fibers from one subramulus. Frequently several unbranched fibrils are seen passing from one neuromast to another. Electrophysiologists may be able to determine the significance of these observations.

SUMMARY

The lateralis sensory canal system in *Carcharhinus* consists of intercommunicating supraorbital, infraorbital, hyomandibular, lateral extension, and lateral canals, and isolated mandibular canals. The bore is largest in the more anterior head canals and decreases caudad. The canal tubules of the more anterior head canals generally are relatively large and are directed forward, whereas those of the more posterior canals are generally smaller and are directed backward. This allows a slow perfusion of seawater caudad through the system as the shark swims forward.

The sensory epithelium consists of neuromasts with typical hair cells embedded among supporting cells, covered distally by a mucous cupula and surrounded peripherally by mantle cells. The neuromasts are oval in facial section and, butted end to end, form an almost continuous sensory epithelium over most of the system. They are somewhat more discrete in the mandibular canals and in the posterior part of the hyomandibular and lateral canals. The neuromasts decrease in length and width caudad, and there is a corresponding decrease in the number of sensory cells per neuromast. The neuromasts in the adult are larger than in the pup. Since size and arrangement of the sensory and supporting cells appears to remain the same, it is inferred that the number increases with growth of the shark.

Adjacent to the neuromast, the columnar epithelium of the head canals is vesiculated and elaborated into folds. It rests on a layer of stellate "ganglion-like" cells. The vesiculated areas are reduced caudad. There is evidence that they are secretory in function, possibly adjusting the ionic potential of the canal fluid and tissues against an influx of seawater. The ganglion-like cells may exert neurohumeral control of the secretion.

Distal to this vesiculated area, the epithelium of the head canals is further modified to form a thickened ridge of cuboidal mucus-secreting cells resting on irregularly shaped cells separated by vesicles. The ridge may serve to direct the slow influx of seawater along the surface of the canal distal to the neuromast. It decreases in

prominence and retreats toward the neuromast caudad.

On the head, adjacent to the ampullar sacs, there are numerous cephalic glands which open to the exterior by one or more short tubules and which contain mucus. Their epithelium is similar to that of the adjacent head canals except that they lack a neuromast and lateralis innervation. Their primary function may be to adjust tissue osmotic pressure and potential.

The neuromasts of the canals are innervated by various branches of the cranial nerves (VII, IX, and X). The nerve ramuli and subramuli enter the canal opposite the canal tubules and adjacent to the center of a neuromast. Below the sensory epithelium the nerve fibers form a more or less continuous fiber zone, from which fine branches arise, lose their myelin sheath, penetrate the basement membrane, and branch repeatedly into fine fibrillae which end among the hair cells. One fiber may innervate 50 or more hair cells. Fibers from one nerve innervate the neuromast opposite to its point of entry and also the proximal ends of adjacent neuromasts. Both the number of fibers and the thickness of the fiber zone decrease caudad, corresponding to the decrease in size and number of sensory cells per neuromast.

It is hoped that the information included in this report will be of use to biophysicists, electrophysiologists, and behaviorists in designing models and experiments to clarify the function and mode of operation of the lateralis system in sharks.

LITERATURE CITED

COPELAND, D. E. 1948. The cytological basis of chloride transfer in the gills of *Fundulus heteroclitus*. Journal of Morphology, vol. 82, pp. 201–228.

DANIELS, C. I. 1967. The distribution, morphology, and innervation of the ampullae of Lorenzini in the hammerhead shark and other species. M. S. thesis, University of Hawaii, January 1967.

DIJKGRAAF, S. 1963. The functioning and significance of the lateral-line organs. Biological Reviews, vol. 38, pp. 51–105.

EWART, J. C. 1891. The lateral sense organs of elasmobranchs. I. The sensory canals of *Laemargus*. Transactions of the Royal Society of Edinburgh, vol. 37, pp. 59–85.

EWART, J. C., and J. C. MITCHELL. 1891. The lateral sense organs of elasmobranchs. II. The sensory canals of the common skate (*Raia batis*). Transactions of the Royal Society of Edinburgh, vol. 37, pp. 87–105.

FLOCK, Å. 1965. Electron microscopic and electrophysiological studies on the lateral line canal organ. Acta Oto-Laryngologica (Stockholm), Supplement 199, 90 pp.

——— 1967. Ultrastructure and function in the lateral line organs. In: P. H. Cahn, ed., Lateral line detectors. Bloomington, Indiana University Press. Pp. 163–197.

GARMAN, S. 1888. On the lateral canal system of the Selachia and Holocephala. Bulletin of the Museum of Comparative Zoology (Harvard), vol. 17, no. 2, pp. 57–119.

GILBERT, B. 1965. Triple silver impregnation for selective staining of avian nerves. Stain Technology, vol. 40, no. 5, pp. 301–304.

HAMA, K. 1965. Some observations of the fine structure of the lateral line organ of the Japanese sea eel *Lyncozymba nystromi*. Journal of Cell Biology, vol. 24, no. 2, pp. 193–210.

JOHNSON, S. E. 1917. Structure and development of the sense organs of the lateral canal system of selachians (*Mustelus canis* and *Squalus acanthias*). Journal of Comparative Neurology, vol. 28, no. 1, pp. 1–74.

NORRIS, H. W., and S. P. HUGHES. 1920. The cranial, occipital and anterior spinal nerves of the dogfish (*Squalus acanthias*). Journal of Comparative Neurology, vol. 31, no. 5, pp. 293–402.

SMITH, G. W. 1930. A mechanism of intake and expulsion of colored fluids by the lateral line canals as seen experimentally in the goldfish (*Carassius auratus*). Biological Bulletin, vol. 59, pp. 313–321.

——— 1933. The mechanism of intake and outflow of fluids in the lateral line canal of fishes. Anatomical Record, vol. 56, no. 4, pp. 365–371.

TESTER, A. L., and J. I. KENDALL. 1967. Innervation of free and canal neuromasts in the

sharks *Carcharhinus menisorrah* and *Sphyrna lewini*. In: P. H. Cahn, ed., Lateral Line Detectors. Bloomington, Indiana University Press. Pp. 53–69.

——— 1968. Cupulae in shark neuromasts: composition, origin, generation. Science, vol. 160, no. 3829, pp. 772–774.

TESTER, A. L., and G. J. NELSON. 1967. Free neuromasts (pit organs) in sharks. In: P. W. Gilbert, R. F. Mathewson and D. P. Rall, eds., Sharks, skates and rays. Baltimore, The Johns Hopkins Press. Pp. 503–531.

VAN BERGEIJK, W. A. 1967. Introductory comments on lateral line function. In: P. H. Cahn, ed., Lateral line detectors. Bloomington, Indiana University Press. Pp. 73–81.