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Summary. Following our observations of the fine structure of the pharyngeal villiform processes of the hibernating soft-shelled turtle, *Trionyx sinensis japonicus* (Yokosuka et al., 2000), this paper deals with a scanning electron microscope study of the resin casts of blood vessels supplying those processes.

Each villiform process contained arterioles and venules which ran in the axial portion of the process; capillaries formed a network at the periphery of the connective tissue core of the villus. In the distal portions of the villus, the capillaries increased markedly in their caliber to form sinusoidal capillaries. Such a vascular architecture supports the view that the villiform processes serve in the aquatic respiration of the soft-shelled turtle.

The casts indicated an occurrence of sphincters in the vascular bed of the villi.

The pharyngeal mucosa in soft-shelled turtles is densely covered with "villiform processes" (designation by Grgis, 1961). Physiological studies (Gage and Gage, 1886; Dunson, 1960; Wang et al., 1989) suggested that the processes represented an aquatic respiratory organ utilized by the turtles when remaining underwater for a long time.

Although some brief morphological reports concerning the villiform processes have been available (Grgis, 1961; Dunson and Weymouth, 1965; Winokur, 1988), ours was the first report on the detailed structure of the pharyngeal villiform processes in hibernating turtles, *Trionyx sinensis japonicus*, by light microscopy, scanning, and transmission electron microscopy (Yokosuka et al., 2000). That report included the vascular supply of the processes, as observed in cross-sections of individual villi by light and electron microscopy. While it had been known only that the villiform processes were richly supplied with blood vessels, we established that arterioles and venules coursed through the axial portion of the villi, whereas capillaries occupied the peripheral portion. In the distal portion of the villi the capillaries were swollen, forming what deserved to be called the sinusoidal capillaries, and invaginated deep into the epithelium, which was attenuated. Thus, our previous findings on the construction of blood vessels and their relation to the epithelium supported the idea that they are structures involved in the aquatic respiration of turtles (Yokosuka et al., 2000).

The present scanning electron microscope study of vascular casts aims to demonstrate more directly and precisely the construction of the blood vessels in the villiform processes. Only turtles caught in their hibernation season (December-January) were used, as our preliminary study indicated that this is the only time villiform processes are well-developed.

MATERIALS AND METHODS

Four male and four female hibernating turtles (*Trionyx sinensis japonicus*) were purchased in this season, i.e., in December and January. Tissue was taken under anesthesia with an intraperitoneal injection of pentobarbital, after being introduced by ether.

Under general anesthesia, the thorax of each turtle was opened in order to expose the heart and principal
Fig. 1. Scanning electron micrograph of a vascular cast of villiform processes. Specimen taken from the ventral wall of the pharynx of a soft-shelled turtle. Essentially all process casts in this figure are of the complex type. P the primary process, S secondary process, T tertiary process. ×70

Fig. 2. a. Scanning electron micrograph of a vascular cast replicating several villi. A indicates arterioles and V, venules. They are axially located and superficially covered by a network of capillaries (arrows). b. A micrograph from the same villus as shown in Figure 2a was artificially colored. The arteriole, venule and capillaries are tinged red, blue and yellow, respectively. a: ×200, b: ×130
Fig. 2. Legend on the opposite page.
vessels. A polyethylene tube was inserted into the aortic arch. Following a 0.9% Ringer solution, methyl methacrylate resin (Melcox, Dainippon Ink & Chem., Inc. Tokyo) was infused into the blood vessels and left to be polymerized at room temperature. The head portion including the pharynx was removed to be further polymerized in warmed water (60°C). The specimens were macerated in 20% NaOH (60°C) for 12 h. After the soft tissues were removed by repeated washing, the vascular casts were trimmed into small blocks which were evaporation-coated with platinum-palladium. Observation was made with a scanning electron microscope (SEM), Hitachi S-800.

RESULTS

Each cast specimen under the dissection microscope represented a complete replica of the pharyngeal villous structure, as resin filled the blood vascular bed to the tips of the villi. As our SEM observation of the mucous surface indicated two types of the villiform processes, i.e., simple and complex types (YOKOSUKA et al., 2000), the vascular casts showed the corresponding structures (Fig. 1).

The vasculature of each villiform process comprised arterioles and venules (20-50 μm in the thickness of casts) twisting round each other in the axis, and a network of thinner (4-10 μm) capillaries on the surface (Figs. 1, 2).

The casts of the arterioles — which were thinner than those of the venules — branched here and there and issued capillaries. The venules received capillaries at places. The capillaries course mainly in a longitudinal direction but partly circularly and obliquely; in branching and anastomosing, they formed a reticular pattern. A sharp constriction was frequently recognized at the beginning of a capillary from an arteriole and at the joining of a capillary to a venule, suggesting the occurrence of a sphincter (Figs. 3a, b).

Towards the tip of the villiform process, the capillaries became rather thicker and took a hairpin turn; they tended to form a network (Fig. 3c) incorporating the terminal portions of the axial arterioles and venules.

At high magnifications, the casts of arterioles revealed striated impressions of the circular muscle fibers in the tunica media (Fig. 3d). The casts of both arterioles and venules often showed oval or fusiform impressions of endothelial nuclei (Fig. 3a).

DISCUSSION

The close association of blood capillaries with the attenuated epithelium is a structural feature typical of a gas exchanging barrier between the blood and the exterior environment, in this case the water in which turtles spend their hibernation period. It has been recorded that soft-shelled turtles move their buccopharyngeal muscles to effect an intrapharyngeal water flow about 16 times per minute (GAGE and GAGE, 1886).

The efficiency of the blood-water gas exchanging barrier seems elevated towards the tip of each villi where water can be contacted more efficiently. In the terminal portion of the villi the capillaries are swollen, assuming the shape of sinusoidal capillaries. They are more conspicuously invaginated into the epithelium than in the basal portion, and the epithelium is more attenuated. Taking all this into consideration the pharyngeal villi of soft-shelled turtles in winter represent an ideal structure for the function of gas exchange comparable to the barriers in the vertebrate lung, mammalian placental villi, and piscine gill.

The incisions on the vascular casts most likely indicate the occurrence of sphincters at the sites. The occurrence of an arteriovenous anastomosis was suggested at certain parts of our casts, but the scanning images were not sufficiently convincing. The possible regulation of the microcirculation in the villiform processes by sphincters and arteriovenous anastomoses remain to be elucidated. A very thick cast of a venule frequently found filling a large axial portion of a villus may presumably cause a kind of erection.

Fig. 3. Closer SEM images showing different parts of vascular casts in the villiform processes. a. Vascular structure at a site shortly distal to the middle level of the process. A conspicuous notch is seen at the boundaries between the arteriole or venule and capillaries, suggesting the existence of a pre-capillary (arrows) or post-capillary (arrowhead) sphincter. Impressions of endothelial nuclei are seen (asterisks). b. Closer view of a post-capillary incision in the cast implying a sphincter (arrow). c. A view at the tip of a villus. Thick vessels forming a basket-like structure correspond to sinusoidal capillaries (asterisk). d. A high power view shortly distal to the middle level of a villus. The cast of an arteriole reveals striated impressions (arrowheads) by the circular muscle fibers in the tunica media. A arterioles, V venules, C capillaries. a: ×930, b: ×2300, c: ×1000, d: ×2600
Fig. 3. Legend on the opposite page.
REFERENCES


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