

THE VASCULAR SYSTEM OF THE COMMON SQUID, *LOLIGO PEALII*.

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THE knowledge of the histology of the vascular system of the decapod mollusks is very incomplete. The nature and extent of the capillary system especially have been so little understood that two contrary opinions find expression in the text-books of zoölogy. It has been maintained by Lang that the arterial and venous systems are connected partly by capillaries and partly by lacunæ, which are portions of the primary body cavity, while Parker and Haswell affirm that the blood flows through a complete system of capillaries. I have been unable to find any published work which decides between these views. All agree that the vascular system is highly developed and that the capillary system is extensive, but no one seems to have investigated the structure of the vessels or to have determined the exact nature of the so-called lacunæ or sinuses. Prof. Ulric Dahlgren called my attention to this subject, and under his direction I worked upon the vascular system of the squid at Woods Hole during the summers of 1899 and 1900, and at Princeton University during the intervening time. The material, *Loligo pealii*, was furnished me by the Marine Biological Laboratory through the kindness of Professor Dahlgren, and by the United States Fish Commission through the kindness of Dr. H. C. Bumpus, and further material was obtained from the traps at Elberon, N. J.

The extent of the capillary system was determined by injecting the vessels with a saturated aqueous solution of Berlin blue. The injected tissues were studied while fresh and transparent or were fixed in acetic corrosive sublimate or 95 per cent alcohol and mounted in serial sections. The structure of the walls of the vessels was studied after impregnating them with silver in order to show the outlines of the endothelial or other

lining cells. Before injecting the solution of silver nitrate it was necessary to remove the chlorides from the vessels by irrigating them with a 5 per cent solution of potassium nitrate. This solution was followed by a $\frac{1}{4}$ per cent solution of silver nitrate, and after a few moments this was removed and the tissues fixed by the injection of 95 per cent alcohol. Small pieces of the several organs were dehydrated, cleared in creosote, and mounted. The contraction of the intrinsic muscles of the vessels renders complete injection very difficult, and to obviate this difficulty by dilating the vessels the injection fluids and the water in which the squid were kept were saturated with amyl nitrite. This method was not uniformly successful.

The general arrangement of the vascular system of the squid will be recalled by a glance at the diagram (Fig. 1) of

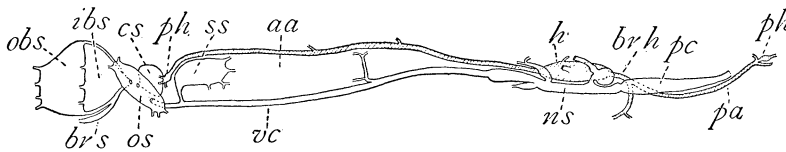


FIG. 1.—Diagram of the vascular system of the squid as seen from the left. *aa*, anterior aorta; *brh*, branchial heart; *brs*, brachial sinus; *cs*, cerebral sinus; *h*, heart; *ibs*, inner buccal sinus; *ns*, nephridial sinus; *obs*, outer buccal sinus; *os*, optic sinus; *pa*, posterior aorta; *pc*, postcava; *ph*, peripheral heart; *ss*, salivary sinus; *vc*, vena cava.

the system seen from the left. Arterial blood enters the one-chambered heart from the gills through the two branchial veins and is forced out through the anterior, posterior, and genital aortæ. The anterior aorta forks near the head, and each branch bears a muscular enlargement, called a peripheral heart, at the point where it breaks up into the branches which supply the head. The two branches of the posterior aorta which pass out of the mantle to the fins also have peripheral hearts. The latter are vascular sphincters, which probably contract synchronously with the mantle, and so prevent the transmission of an excessive blood pressure to the extra-pallial vessels. The blood returning from the arms through the brachial veins reaches the brachial sinus, which is connected with five sinuses, that partially enclose the pharynx, the eyes, and the brain. These sinuses open into the anterior vena cava, which also receives a vein from the sinus that encloses the salivary gland. The anterior

vena cava opens into the apex of the V-shaped nephridial sinus which is surrounded by the nephridial chamber. The venous blood from the dorsal end of the mantle returns through the two postcavæ which open into the ends of the arms of the nephridial sinus. This sinus opens at each side into a branchial heart which forces the blood through the gill to the systemic heart. The sinuses are not interposed between the veins and arteries but receive blood from the veins and return it to the veins. The aortæ, the larger arteries, the branchial arteries and veins, the anterior vena cava, and the postcavæ are all peristaltically contractile. The wave of contraction passes slowly away from the heart along the arteries, and toward the heart along the veins. The peristalsis of the vessels continues long after the stoppage of the hearts and the apparent death of the animal.

The Structure of the Arteries and Veins.—The systemic or arterial heart is composed of smooth muscle fibers. The external surface is firm, but the inner surface is formed by a latticework of interlaced fibers. The great irregularity of this surface interferes with the success of silver impregnations, and although some impregnations and microtome sections indicate that there is an endothelium, such a large number have yielded only negative results that it is probable that an endothelium is not present in the heart. The arterial blood which passes to the tissues of the heart from its lumen becomes venous and is carried to the nephridial sinus by two or three small veins. It is probable that these veins are separated from the lumen of the heart by capillaries. The vessels through which the blood passes are certainly as small as capillaries, but it was impossible to demonstrate an endothelium.

The branchial hearts are quite different from the systemic heart in that the muscle fibers are cross striated and are separated by numerous clusters of apparently glandular polygonal cells, which possibly have a function in connection with the pericardial gland that is attached to the inner edge of the heart and receives a large blood supply from it. Silver impregnation shows that an endothelium is not present and that the muscular and glandular cells are in contact with the blood.

A branch of the posterior aorta brings arterial blood to the tissues of the branchial heart.

The aortæ and the larger arteries are peristaltically contractile and thick walled. Their walls are formed by a thick sheet of peculiar muscular tissue, which is enclosed by two thin coats of connective tissue. The muscular tissue is formed of fusiform cells whose large oval nuclei have a small quantity of protoplasm at each end, but the greater portion of each cell consists of radiating fibers which interlace with those of neighboring cells. The muscle resembles connective tissue more than muscle, but it is actively contractile, for stimuli can be so applied that the peristaltic wave will move in the opposite direction to the blood current, and the peristalsis continues long after the final stoppage of the hearts.

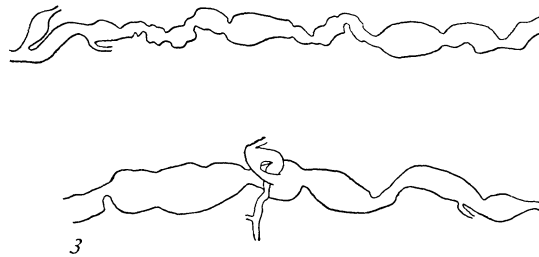


FIG. 2. — Arterioles which show the effect of the contraction of the scattered intrinsic muscle fibers of the vessels.

The muscular coat of the smaller arteries is much thinner, and the peculiar muscle cells are replaced by simple fibers, which are so irregularly distributed upon the smallest arteries that their contraction makes the vessels moniliform, as is shown in Fig. 2. Arterioles arising as lateral branches of an artery commonly have a strong band of muscle fibers at their origin.

The aortæ and their largest branches may have an endothelium, but the unavoidable contraction of their muscular walls throws the inner surface into longitudinal wrinkles, between which the silver is deposited in streaks that obscure the cell outlines. It is probable that an endothelium is present in these vessels; for, although almost all the evidence is negative, a few preparations seem to show the endothelium. The intermediate arteries are lined by an endothelium composed of

flat elongate cells that encircle the vessel. This endothelium gradually merges into the typical endothelium lining the smaller arteries. Bergh¹ found a similar arrangement in the arteries of the pulmonate gasteropods, except that he ascertained definitely that the larger arteries and the heart do not possess an endothelium but are lined by muscle fibers. He concludes that the endothelial cells are undifferentiated (*ungeformte*) muscle cells. There is nothing in the squid which tends to support such an explanation, for the larger vessels are almost certainly lined either by connective tissue or endothelium.

The veins have the same structure as the arteries except that their muscular coat is thinner or wanting and that all have a typical endothelium (Fig. 3). They may readily be distinguished from the arteries by their thinner walls. In many places an artery is accompanied by a pair of veins which anastomose frequently. Fig. 4 represents a portion of such an artery with its companion veins; the narrow initial caliber of the branches of the artery, due to the contraction of the intrinsic muscles of the vessels, should be noted.

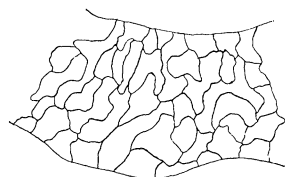


FIG. 3.—Endothelium of a vein.
× 260.

The nephridial sinus passes through the nephridial cavity and is invested by the secretory nephridial epithelium. Both surfaces of the sinus wall are increased by interdigitating evaginations from the lumen of the vessel and from the nephridial cavity. The consequent irregularity of the surface of the walls causes many artifacts in silver preparations, so that the cell outlines cannot be followed certainly; nevertheless, sections indicate that an endothelium is present. The nephridial epithelium of the sinus is columnar and is supported by a membrane, the proper wall of the sinus, which carries the nutrient arteries of the wall.

The Capillaries.—The Berlin-blue injections which were intended to demonstrate the extent of the capillary system were perfectly successful. The injection fluid passed from the arteries through the tissues until it filled and flowed from

¹ Bergh, R. S. *Anat. Hefte*, I. Abth., Bd. x.

the veins, so that it is certain that the vascular system was fully injected. Serial sections of many portions of the squid, and gross mounts of portions of the skin, mesentery, and intestinal wall show conclusively that there is a perfect capillary network which connects the arteries and veins in all parts of the body. Like other capillaries, these (Fig. 4) branch and anastomose frequently without altering their diameter, and their walls are formed by an endothelium composed of flat oval cells with sinuous margins. The silver impregnations of the capillaries, though rarely successful, leave no doubt as to the nature of the wall.

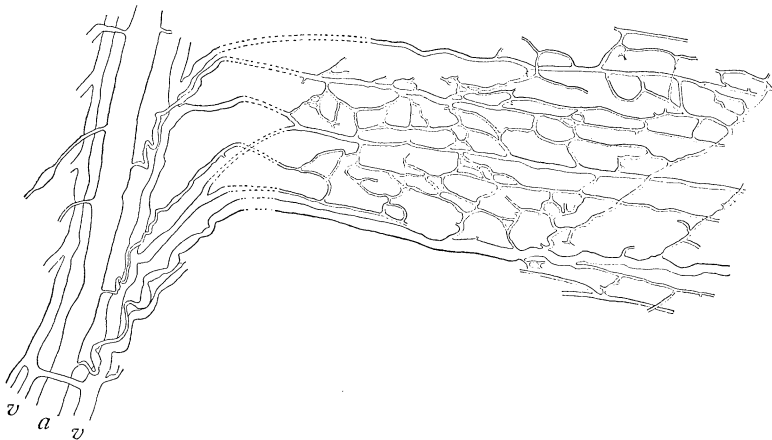


FIG. 4. — Artery, companion veins, and connecting capillaries sketched from the supporting membrane of the gill. *a*, artery; *v*, veins.

Some arterioles and capillaries are connected with lacuna-like cavities which may be called the end sinuses of the veins. Fig. 5 is a camera sketch of such a sinus situated in the visceral body wall. Similar sinuses have been found in the testis, glands, and muscles, and it is very probable that they are more widely distributed. Each sinus is a small irregular cavity, into which an arteriole passes and then breaks up into branches; the latter pass out of the sinus and communicate with the surrounding capillary plexus, some of whose branches open into the sinus, so that the blood must pass through the perforating arteriole and then through the capillaries into the

sinus.¹ The sinuses are connected with the veins by very small vessels. In partially successful injections the colored arteriole can be seen surrounded by a space filled with blood corpuscles, but in perfect injections both the arteriole and the sinus are filled with the injection mass. The arteriole, capillaries, and the sinus are all lined by endothelium. It was impossible to determine whether the endothelium of the sinus was reflected over the surface of the perforating arteriole. It is worthy of note that when the blood had not been driven out of the sinus the proportion of corpuscles in the blood of the sinus was six or eight times as great as in the other vessels, — a fact which

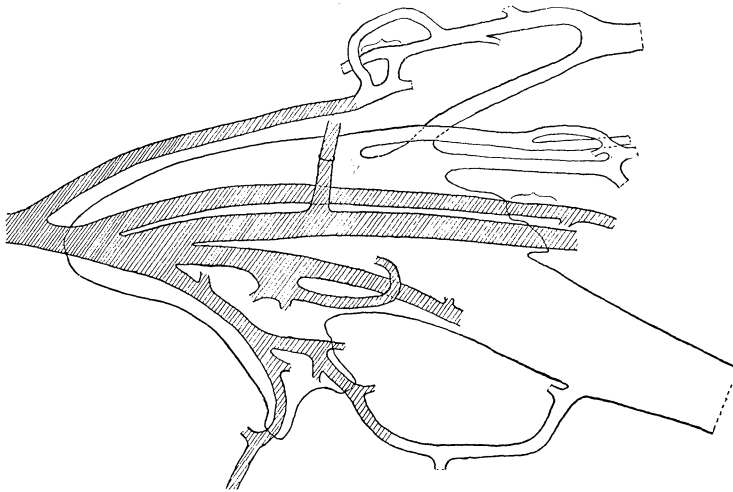


FIG. 5. — End sinus of vein, from visceral body wall.

may be due to the filtering out of the corpuscles as the blood was driven through the vessels by the injection fluids.

The Capillaries of the Gills. — The gills are not easily injected because a valve at the origin of the branchial artery prevents the free flow of injection fluids in either direction. The valve is formed by four tubercles which project in the direction of the blood current into the lumen of the artery. A band of muscle fibers forms a sphincter whose contraction forces the tubercles together so that the lumen of the vessel may be closed.

¹ The connection between these vessels and the veins has been inferred from the course of the injection fluids through the sinus.

Notwithstanding the resistance of this valve, successful capillary injections of Berlin blue were obtained as well as good silver impregnations of the veins and arteries. The capillaries are enclosed between epithelial plates which form the upper and lower surfaces of the gill and are separated from one another by small columns of cells which bind together the gill surfaces. The vascular endothelium could be traced only to the beginning of the capillaries, but microtome sections leave little doubt that the endothelium also extends into the capillaries.

The salivary sinus and the sinuses of the head (brachial, inner and outer buccal, optic, and cephalic), which are usually cited as examples of lacunæ, must be considered to be enormous dilations of the veins. They are so large that it is impracticable to examine every portion of their walls, but silver impregnations of portions of several sinuses show that they are lined by an endothelium. All the sinuses have essentially the same structure, so that the description of one sinus will convey a true conception of all. The outer buccal sinus surrounds the sides and dorsal surface of the oval pharynx as the pericardium invests the heart. The outer wall of the sinus is supported by the muscular ring at the base of the arms, and the inner wall is supported by the pharynx and by the muscular septum which separates the inner buccal sinus from the outer. The walls are formed by connective tissue and by the endothelium which it supports. Ten or fifteen small veins which arise in the peristomial membrane open into the ventral end of the sinus, and the brachial sinus opens into the dorsal end. The blood passes from the buccal sinus through the optic sinuses to the vena cava. Beside having definite walls, these sinuses, unlike lacunæ, do not connect arteries and veins, but receive blood from the veins and return it to the veins. The wide distribution of the capillary vessels, the presence of an endothelium around every blood-containing cavity, except possibly the hearts, and the absence of demonstrable lacunæ, all lead to the conclusion that the arterial and venous vessels of the squid are connected by capillaries so that the vascular system is closed.

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