2×10^{-5}M veratridine produced a peak increase to 1.23 ± 0.13 ml/g.h.; the secretory rate two hours after initiating the infusion was 0.565 ± 0.061 ml/g.h., still well above the control level of 0.088 ± 0.011 ml/g.h.

If the effects of veratridine on secretion are due to prolonged opening of Na channels like those involved in nerve propagation, the effect should be blocked by tetrodotoxin. Figure 2 shows two types of experiments carried out to test this point. In the experiment of Figure 2A, an infusion with veratridine (2×15^{-5}M) was initiated after the initial control period. Once the effect of veratridine had reached a stable level, tetrodotoxin (10^{-6}M) was also included in the perfusion fluid. The addition of tetrodotoxin caused a rapid reduction in the rate of secretion. When tetrodotoxin was washed out the rate of secretion increased to levels close to those observed before its addition. In the experiment in Figure 2B after the control period tetrodotoxin was infused alone it was followed by perfusion solution containing both tetrodotoxin and veratridine. In contrast with control experiments the addition of veratridine in the presence of tetrodotoxin did not stimulate secretion for up to 20 minutes. However when the perfusion was switched to a solution containing only veratridine, the rate rapidly increased to values above the control level.

Measurements of Cl^- content in the secreted fluid showed that Cl^- was not different in control and stimulated secretions (450 ± 21 mM and 447 ± 15 mM respectively).

These observations, showing that veratridine stimulates the secretion of the rectal gland through a tetrodotoxin sensitive mechanism, strongly suggest that depolarization of nerve fibers within the gland leads to activation of the Cl^- secretory mechanism. The depolarization very likely would act through the release of a transmitter that in turn activates the Cl^- secretion mechanism. Two alternative hypothesis to explain the effects of veratridine can be suggested: a) the effects are due to depolarization of a paracrine cell and not of nerve fibers; b) The effect is due to an increase of Na permeability of the Cl^- secreting cell itself. These hypothesis are, for the time being, less likely than the nerve depolarization proposal. When we consider the paracrine cell suggestion, it turns out that there is thus far no anatomical evidence showing the presence of such cells within the gland. While the possibility that an increased Na permeability of the secretory cells would, by itself, cause increased secretion runs contrary to our present understanding of the mechanism of Cl^- secretion by the gland (Silva et al., Am. J. Physiol. 233:F298-F206, 1977). Supported by the New York Heart Association.

EFFECTS OF ACETYLCHOLINE AND EPINEPHRINE ON DOGFISH GILL FILAMENT VASCULAR COMPARTMENT SIZE

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Blood entering the afferent filamental arteries of the dogfish gill either perfuses the respiratory lamellae to provide oxygenated blood to the systemic circulation or flows into the interlamellar and collateral vessels which drain back into the venous side of the circulation. The function of the latter pathway is not clear, but the vessels do course beneath the interlamellar filamental and water channel epithelium reported to contain chloride-like cells. A role in osmoregulation is implied. When the fish is exposed to a low oxygen environment, resistance to blood flow across the gill is known to increase (Kent & Pierce, Comp. Biochem. Physiol., 60C:37-44, 1978). This effect is mimicked by acetylcholine and blocked by atropine (Kent et al., MDIBL Bull. 20:109-111, 1980). Epinephrine, on the other hand, does not raise gill resistance, but increases systemic vascular resistance. The present study was undertaken to determine possible anatomical correlates to the change in gill resistance brought about by acetylcholine and epinephrine. The effect on the size of the two major blood compartments in the gill, the lamellar and intrafilamental-collateral, were studied by scanning electron microscopic analysis of methylmethacrylate corrosion replicas of the gill vasculature.

Eight dogfish pups weighing approximately 200 grams were anesthetized with MS 222. The conus arteriosus was cannulated and the fish were perfused with 200 ml heparinized dogfish Ringers bubbled with 100% O_2. Perfusion pressure ranged from 42 cm saline to 12 cm saline for each fish. The sinus venosus was opened to atmosphere so blood escaped and was replaced by the Ringer's solution. In 5 fish 10^{-5}M epinephrine was added to the perfusate and in 3, 4×10^{-7}M acetylcholine was added. When no more red cells were noticeable in the venous effluent, the Ringer's perfusion was stopped and followed immediately by perfusion with 10 ml methylmethacrylate resin and catalyst (Mercox) at a pressure of 20 cm saline. Perfusion stopped when the methacrylate began to polymerize. After a 60 minute period, the fish was immersed in 20% NaOH for tissue maceration. Vascular replicas of the filamental circulation were dissected using a Swift light microscope and SEM studies were carried out using an ISI 40 scanning electron microscope.

On gross examination both the gill and systemic circulations of the two groups seemed well filled with the casting material. However, in two of the three fish preperfused with acetylcholine the renal vasculature was not filled at all. Also, in four of the five fish preperfused with epinephrine, the internal jugular vein which collects venous drainage from the ventral portion of the gill was not filled. In general there was much more venous engorgment in the gill arches from fish pretreated with acetylcholine. The venous septal sinus which runs in the opercular skin was engorged in these fish but was absent in epinephrine pretreated ones. In dissecting lamellae away from individual filaments it was noticed that pre- and post- lamellar arterioles were broken with difficulty from filaments preperfused with epinephrine but lamellae from acetylcholine treated filaments could be removed quite easily.

Figure 1 shows a scanning electron micrograph of a segment of a cast from a filament of a fish preperfused

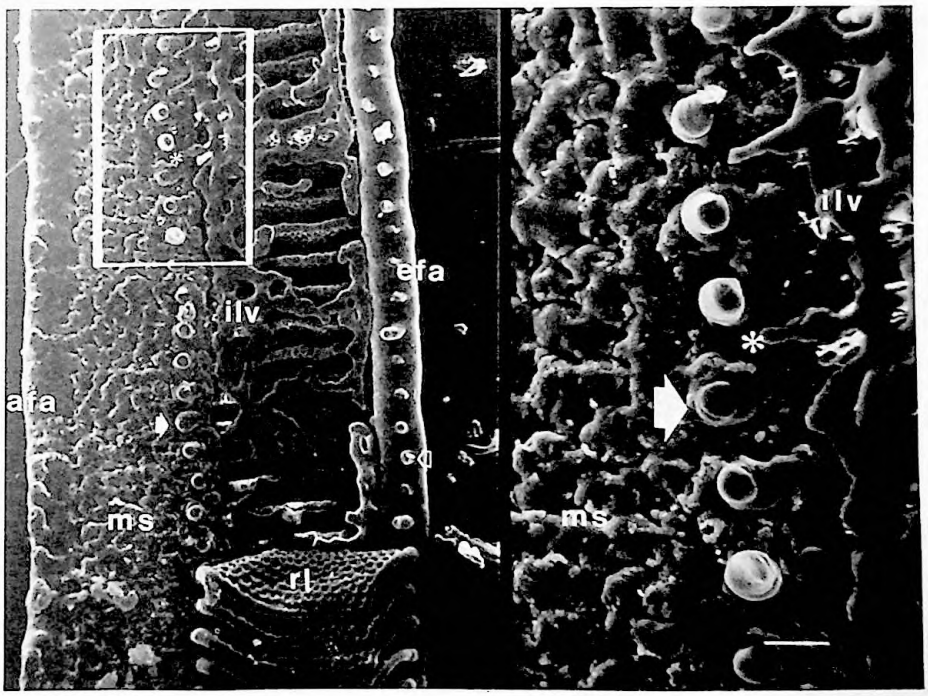

Figure 1.--SEM, x70, insert x210, bar in right hand panel = 100 microns on left panel and 33 microns on right. Methylmethacrylate replicas of gill filament preperfused with 10^{-5}M epinephrine. rl - respiratory lamella; ms = medial sinus; ilv = interlamellar vessels; efa = efferent filamental artery; afa = afferent filamental artery; closed arrow = prelamellar arteriole; open arrow = post lamellar arteriole; * = origin of collateral sinus (not filled).

with epinephrine. About 3/4 of the lamellae have been removed from one side of the filament to reveal the underlying interlamellar vessels. The interlamellar circulation was only sparsely filled when fish were pretreated with epinephrine. As illustrated in Figure 1, the basal portions of the lamellae from the opposite side of the filament can be easily seen behind the thinly filled interlamellar vessels. The collateral sinus underlying the water channels was also poorly filled or absent in these fish. On the right panel of Figure 1 the beginnings of the collateral sinus can be seen to end at the asterisk between the prelamellar arterioles. In the 60 or more filaments examined from 5 fish preperfused with epinephrine, very few prelamellar arterio-venous anastomoses between the medial sinus and the interlamellar vessels were found. The interlamellar vessels closest to the efferent filamental artery were better filled than those near the medial sinus, suggesting the nutritive vessels from the efferent circulation were open. The lamellae of these fish were very well filled as were the medial sinus and afferent and efferent filamental arteries.

When fish were preperfused with acetylcholine several differences in vascular compartment size were seen (Figure 2). The interlamellar vessels, collateral sinuses and septal venous sinuses were engorged. The respiratory

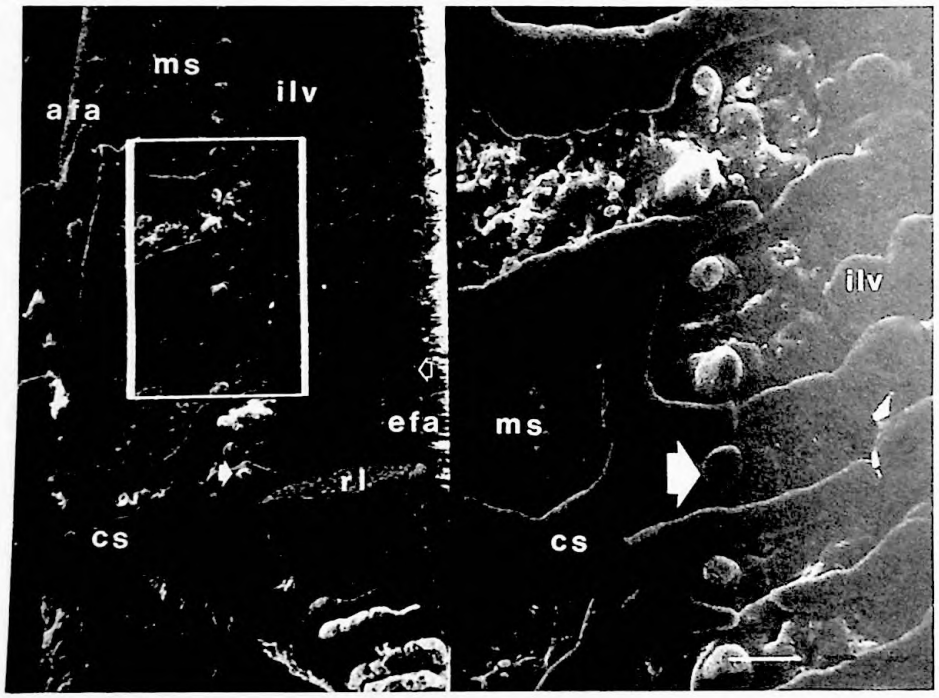

Figure 2.--SEM, x70, insert x210, bar in right hand panel 100 microns on left panel and 33 microns on right. Methylmethacrylate replicas of gill filament preperfused with 4×10^{-7} M acetylcholine. Symbols same as Figure 1. Additionally, cs = collateral sinus.

lamellae on the other hand were often shrunken in appearance and with out outer marginal channels indicating incomplete filling. Prelamellar arteriovenous anastomoses were more numerous and the diameter of the efferent filamental artery was less than that of the afferent filamental artery at the same level of the filament.

Preperfusion of the gills of the dogfish with epinephrine or acetylcholine clearly resulted in changes in the vascular anatomy as reflected in the methylmethacrylate replicas. Epinephrine favored filling of the respiratory circulation at the expense of the collateral and acetylcholine brought about engorgment of the non-respiratory circulation without fully filling the lamellae. A simplified schematic of the dogfish circulation is shown in

Figure 3. The epinephrine-induced increase in resistance in the systemic circulation would be expected to increase

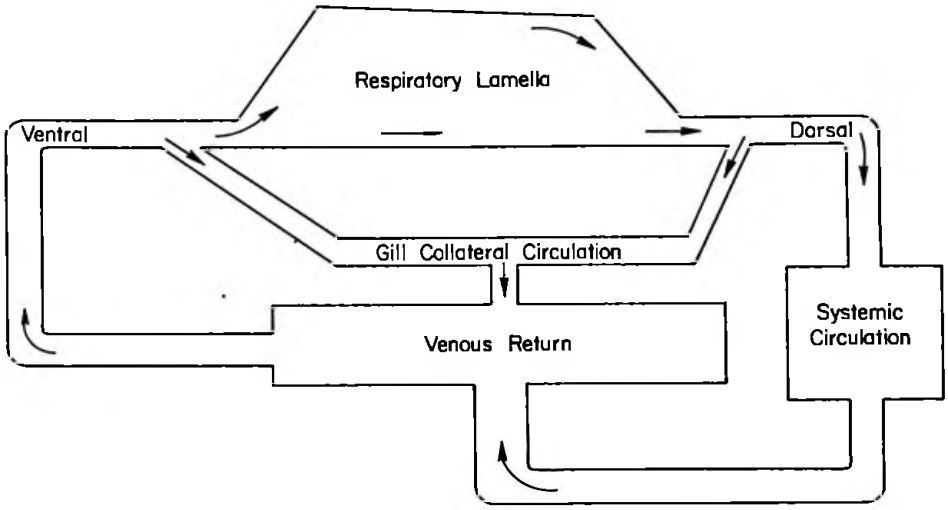

Figure 3.--Schematic of dogfish circulation.

post-gill or dorsal aortic pressure which in turn would favor the filling of the gill circulation. Epinephrine is also known to enhance venous return by relaxing venous sphincters. A resistance drop between the gill collateral circulation and the central venous return channels (Figure 3) would tend to decompress the collateral circulation. The inner margins of the well filled, relatively high pressure respiratory lamellae might well buldge into the interlamellar space and impede flow in the interlamellar vessels by a sluice type effect. In addition, since epinephrine causes an increased flow in isolated dogfish gills perfused at constant pressure (Davies & Rankin, Comp. Gen. Pharmac. 4:139-147, 1973), it may be that locally acting vasoactive catecholamine receptors cause changes within the filamental circulation itself. For instance, receptor-mediated constriction of the pre-lamellar arterio-venous anastomoses and dilation of prelamellar arterioles could also account for the vascular compartment size change. The seeming preferential flow pattern to the respiratory circulation in the gill with epinephrine shown by the methacrylate vascular casts is in accord with its physiological effects and is also in agreement with similar findings using catfish gills (Olson, SEM/1980/III 357-372, 1980).

The shrunken appearance of the replicas of the respiratory lamellae after acetylcholine treatment suggests an impediment to flow in that pathway and is consistent with the physiological observation of an increase in gill resistance. The lack of complete filling of the lamellae with the preference on filling the basal portion where little oxygen exchange takes place may explain the decreased oxygen exchange efficiency in gills from fish treated with acetylcholine (Kent, et al., MDIBL Bull 20:109-111, 1980). Acetylcholine is known to close venous sphincters (Johansen & Hanson, J. Exp. Biol. 46:195-203, 1967) and inhibit venous return. The engorgment of the non-respiratory circulation in the gill may indicate backing up of perfusate as a result of venous sphincter closing downstream. Since acetylcholine is the parasympathetic mediator in the dogfish, the vascular space patterns in the gill found after acetylcholine infushion may reflect events occurring during vagal stimulation which is caused by hypoxia. Coupled with the hemodynamic responses of bradycardia and lowered cardiac output, the shift away from the respiratory pathways may be a mechanism for minimizing exposure of blood to the thin lamellar exchange surfaces during times of low ambient oxygen tension or it could be a mechanism for local regulation to match blood flow with gill filament irrigation in various sections of the gill arch.

The methylmethacrylate casting material used is thought to be vasoactively inert. It is replacing an active biological tissue (blood) and the consequences of this on the resulting vascular compartment sizes are not known. This study shows that large discernible differences in the effects of acetylcholine and epinephrine on the microcirculation can be seen using this casting material and technique. This work was supported by departmental funds from the Department of Surgery, Mt. Sinai School of Medicine and by V.A. research funds.

40 ELECTROLYTE TRANSPORT BY THE ALKALINE GLAND OF THE LITTLE SKATE, RAJA ERINACEA. MECHANISM OF LUMINAL ALKALINIZATION

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Prior studies with the alkaline gland of the little skate Raja erinacea demonstrated that this tissue: (1) maintains a lumen negative potential difference of 16 mV with respect to the serosal solution; (2) actively transports Cl from the serosal to mucosal solution under short circuit conditions; and (3) alkalinizes the luminal contents to a pH of 9.2 (Maren et al., Comp. Biochem. Physiol. 10:1, 1963). To further investigate the transport properties of this tissue, we measured: (1) transepithelial unidirectional fluxes of Na and Cl across short-circuited alkaline gland in vitro; (2) luminal alkalinization using a pH stat technique with alkaline gland mounted in Ussing chambers; and (3) transapical membrane potentials using conventional intracellular microelectrode techniques.

METHODS

Male skates, Raja erinacea, were caught in nets in Frenchman Bay and maintained in running seawater tanks until used. Skates were sacrificed by transection of the spinal cord, the pair of alkaline glands removed from their surrounding connective tissue using fine curved forceps, opened along the epididymal border and placed in ice cold Ringer solution containing (mmols/l): Na,290; K,5; Ca,3.8; Mg,3.3; Cl,299; HCO_3,10; urea,350; and glucose,5. The pH was 8.4 when gassed with air.

Transepithelial Na and Cl fluxes were determined under short-circuit conditions as previously described (Field et al., J. Memb. Biol. 41:265, 1978). Luminal alkalinization was measured with tissues mounted in standard Ussing chambers and bathed by 10 ml of normal Ringer on the serosal surface and 10 ml of HCO_3-free Ringer on the mucosal surface (NaCl substituted for $NaHCO_3$ buffered with 0.5 mM HPO_4-H_2PO_4). The serosal solution pH was 7.6 when gassed with 1% CO_2 in O_2. The luminal bathing solution was maintained at pH 5.5 using a pH stat (Radiometer, Copenhagen). The titrant was 3.98 mM H_2SO_4.

Measurement of the electrical potential profile was similar to that described by Welsh et al., (submitted for publication).

RESULTS AND DISCUSSION

Electrical and flux measurements. Alkaline glands bathed on both surfaces by normal Ringer, bubbled with air and maintained at 15°C had a transepithelial potential (ψ_t) of 6.9 ± 0.6 mV (n = 28) serosa positive with respect to the luminal solution. ψ_t increased during the first 45-60 min. after mounting and remained stable for up to 8 hrs.

Unidirectional and net Na and Cl fluxes, short-circuit current (I_{sc}) and transepithelial conductance (G_t) are presented in Table 1.

Under short-circuit conditions, Na fluxes were equal in both directions and I_{sc} could be accounted for entirely by a net movement of Cl from serosa to mucosa. Table 1 also shows that Cl secretion is dependent on the presence of Na in the bathing solutions. These results suggest that Cl secretion by alkaline gland may involve a neutral coupled NaCl cotransport process at the basolateral membrane consistent with the model described by Frizzell et al., (Am. J. Physiol., 236:F1, 1979). Support for this hypothesis is provided by the finding that ouabain (10^{-4}M) added to the serosal bathing medium completely abolished I_{sc}.