DIFFERENTIAL BLOOD FLOW THROUGH THE AFFERENT BRANCHIAL ARTERIES OF THE SKATE, *RAJA RHINA**

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It has been reported that in the rainbow trout, *Salmo gairdneri*, exercise brings about almost a fivefold increase in the transfer factor of the gills (Randall, Holeton & Stevens, 1967). Five times as much oxygen diffuses from the water to the blood per unit of concentration difference. This suggests that during exercise there is an increase in the area of the functional respiratory surface; this could be achieved if there was an increase in the ventilation and perfusion of some or all of the gills.

Wikgren (1959) reported that one-third of the respiratory movements of *Lampetra fluviatilis* involve only the two or three anterior gill chambers in oxygenated tap water, but that in partially deoxygenated water the anterior five and finally all of the chambers become active. It is unknown whether there are comparable changes in the flow of blood through the arteries afferent to these gills. There are no published records of the simultaneous flow of blood through two afferent branchial arteries in fish; the arteries to the third, fourth and fifth gills in the majority of elasmobranch fishes are located beneath the coracobranchial muscle and are difficult to expose without considerable trauma. Skates, however, are particularly favourable for making such records because their dorsoventral flattening makes the posterior arteries more superficial; moreover the five afferent branchial arteries arise from only two stem vessels on each side. On each side the anterior stem or innominate artery supplies blood to vessels associated with the first two gill slits while the posterior stem artery arises at the base of the ventral aorta and runs laterad close to the pericardium before giving rise to the third, fourth and fifth afferent branchial arteries. Thus the whole length of the ventral aorta lies between the origins of the two pairs of stem vessels. In most elasmobranch fishes the first two gill slits receive the water inspired through the spiracle, and the third, fourth and fifth gill slits receive the water taken in through the mouth (Darbishire, 1907; Hughes, 1960). It is likely that during swimming a higher proportion of water enters the mouth than enters the spiracles. The two stem vessels thus supply gill slits which might be expected in these flattened, bottom-living skates to exhibit some differential changes in ventilation and perfusion during swimming. This paper presents such records from nine specimens of the long nose skate, *Raja rhina*.

MATERIAL AND METHODS

Nine skates (*Raja rhina*) ranging in weight from 1·5 to 7·5 kg. were collected by otter trawl near Orcas Island in Puget Sound. They were transferred to marine aquaria at the University of Washington in Seattle. The skates did not feed but remained in good condition for periods up to 2 weeks before they were used for experiments.

During surgery the fish were in air in supine position on a tilted board. Sea water was pumped past the gills by means of a tube inserted in the mouth. Two per cent Xylocaine (Astra) was injected subcutaneously in the surgical area. No other restraint or anaesthesia was employed.

The right innominate artery and the common stem of the right third, fourth and fifth afferent branchial arteries were exposed surgically. The vessels were fitted with electromagnetic flow probes from two Micron Model RC 1000 flowmeters. The incisions were then closed with sutures and the fish was returned to an aquarium, where it was free to swim while records were taken.

The relative distribution and flow profiles of the respiratory water current across the gills of a few supine restrained skates in water were evaluated from measurements of water velocity at the gill slits using a Thermostrohmuhr technique (heated thermistor probe manufactured by High Temperature Instruments Corp.).

In four of the nine skates, flanged catheters (PE-50) were inserted by non-occlusive means into the ventral aorta to permit measurement of pressures in conjunction with the blood flows. The pressures were measured by means of Statham P 23 BB pressure transducers. All flows and pressures were recorded using a Type R Beckman Dynograph.

RESULTS

1. *Cardiac output*

The successful placement of flow probes which could monitor the entire output of the afferent branchial arteries of one side of the fish provided an opportunity to determine cardiac output directly. The method is subject to two sources of error. The surgery necessary to implant the probes may depress cardiac output; we believe that this error is small as fish remained active and survived for more than a week. The flow probes may constrain the arteries of one side and cause the blood to flow more to the opposite side. There is no way to assess the magnitude of this error. Great care was taken to choose flow probes which caused minimal constriction of the vessel yet provided recordings free from artifacts caused by movement of the vessel within the lumen of the probe.

The cardiac output determined from three resting fish was $21·2 \pm 6·4$ ml./kg./min. This figure agrees well with values for fish published by Holeton & Randall (1967a) and by Hanson & Johansen (1970).

2. *Resting flow through the anterior and posterior arteries*

There was a considerable variation in the proportion of the stroke volume which flowed through the anterior and posterior arteries at rest (Table 1). However, the mean resting flow through the anterior arteries was $11·0 \pm 4·8$ ml./kg./min., which was only slightly greater than the mean flow through the posterior arteries, $10·2 \pm 4·1$ ml./kg./min.

The profiles of the velocity of flow in the two vessels showed two small but consistent differences. Following the opening of the conal valves more of the blood passed into the anterior than into the posterior vessel (Fig. 1). During the initial tenth of the cardiac cycle, $14 \cdot 7 \pm 2 \cdot 6\%$ of the stroke volume passed into the anterior artery and $10 \cdot 0 \pm 1 \cdot 9\%$ passed into the posterior artery. As pressure rose in the ventral aorta, the flow into the posterior vessel came to equal or exceed that into the anterior vessel. If we consider the flow during the fourth tenth of the cycle the figures were $21 \cdot 9 \pm 1 \cdot 3$ and $23 \cdot 0 \pm 0 \cdot 1\%$ respectively.
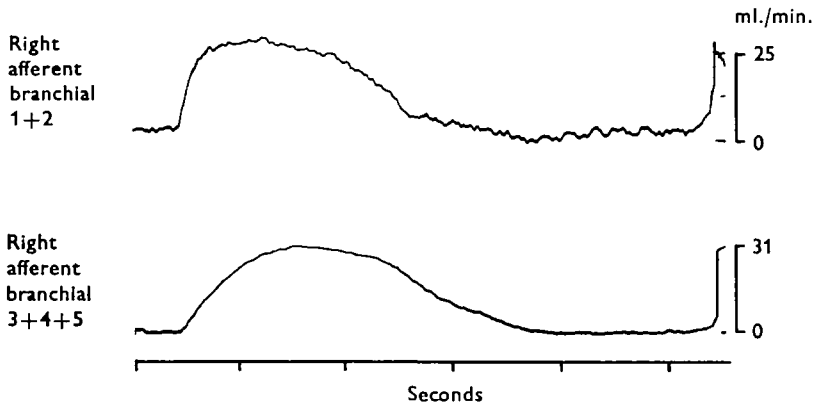

Fig. 1. Simultaneously measured flows of blood in the two stem arteries to the right gills in *Raja rhina* at 10° C. Note the more rapid rise of velocity in the stem vessel for afferent branchial arteries 1 and 2 than in the stem vessel for 3, 4 and 5.

Table 1. *Changes of blood flow and blood distribution to anterior and posterior gills in* Raja rhina *during rest and swimming*

(A higher proportion of the blood passes through the posterior three gills during swimming than at rest.)

Record	Duration of swim (sec.)	Flow at rest (ml./kg./min.)		$(3+4+5)$ % of total	Flow during exercise (ml./kg./min.)		$(3+4+5)$ % of total	% Increase in flow through $(3+4+5)$
		$(1+2)$	$(3+4+5)$		$(1+2)$	$(3+4+5)$		
1	136	16·6	4·1	19·8	13·6	8·3	37·9	18·1
2	120	11·8	7·5	38·9	8·9	10·0	52·9	14·0
3	115	8·1	14·7	64·5	7·0	16·3	70·0	5·5
4	105	6·9	6·0	46·5	4·6	6·5	58·6	12·1
5	98	16·0	13·3	45·4	10·1	14·1	58·3	12·9
6	86	15·4	11·6	43·0	12·8	10·8	45·7	2·7
7	82	14·2	8·3	36·9	7·7	5·8	43·6	6·7
8	77	14·0	17·9	56·1	11·6	21·2	64·6	8·5
9	72	7·1	10·0	58·4	3·2	7·1	68·9	10·5
10	62	6·8	11·4	62·6	3·5	9·1	72·2	9·6
11	60	3·9	7·7	66·4	1·9	5·5	74·3	7·9

3. The effect of exercise

In the laboratory the fish seldom swam for more than a few minutes. In Table 1 the flow of blood through the anterior and posterior afferent arteries at rest and during eleven brief periods of swimming are compared. Swimming is often accompanied by a brief period of cardiac inhibition, and in nine of these eleven periods the cardiac

output decreased; in two it increased. Despite these differences the proportion of the total flow that passed through the posterior artery to the last three gill slits increased in every instance (Table 1). The magnitude of the increase was roughly related to the duration of the period of swimming; it had a mean value of 10 % ± 4·4 % of the total flow. In all instances the absolute flow through the anterior artery decreased; in six of the eleven the absolute flow through the posterior artery increased. This proportionate increase in flow through the posterior artery during swimming was observed in other fish but baseline shifts of calibration have excluded them from the data.

The response suggests that during swimming there is a decrease in the relative resistance of the gill vasculature supplied by the posterior artery compared with the anterior artery. Either the resistance of posterior gill vessels diminishes or that of the anterior vessels increases. Either change would cause a rise in the flow through the posterior artery and a fall in the flow through the anterior artery.

DISCUSSION

The postulated increase in the functional surface area of respiratory epithelium which has been invoked to explain the increase in the transfer factor of the gills during exercise (Randall *et al.* 1967) could operate in two ways. Within the individual secondary lamellae the relaxation of the contractile elements in pillar cells (Hughes & Grimstone, 1965) might cause blood to be channelled to pathways nearer the periphery and more accessible to the respiratory stream (Steen & Kruysse, 1964). Relaxation of the smooth muscle of arterioles leading to the secondary lamellae might cause entire gill filaments to be perfused which had been closed down at rest. J. C. Davis (personal communication) finds that in quiescent *Salmo gairdneri* the P_{O_2} of the expired water varies with the placement of the cannula behind the operculum used to collect the sample. Placements which collect samples efferent to the more dorsally located filaments often have a higher P_{O_2} than water sampled more ventrally at rest. This suggests that some of the dorsally located filaments, remote from the origin of the branchial artery, are excluded from perfusion. A combination of these responses might serve to enhance contribution to the total respiration of an entire gill or gills. The evidence presented in this paper suggests that this occurs in the skate.

It has been established that hypoxia causes an increase in the resistance of the gill circulation both in *Salmo gairdneri* (Holeton & Randall, 1967b) and in *Scyliorhinus stellaris* (Baumgarten & Piiper, 1969). Satchell (1962) reported that in *Squalus acanthias* some of this response remained after denervation of the gills, and suggested that it was an intrinsic response of branchial vascular smooth muscle to lowered oxygen tension. Whether it is intrinsic or mediated by the autonomic nervous system, the response could provide an explanation of why flow through the posterior branchial artery increases during swimming. It is known (Darbishire, 1907; Hughes, 1960) that in the elasmobranch fish which possess a well-developed spiracle, water that enters the spiracle tends to be directed to the anterior gill slits. The subject is much in need of investigation, and there is probably some overlap of the two streams. Skates close the mouth when resting on the bottom, and open it when they swim. It is suggested that this causes an increase in the flow of water through the posterior three gill slits which increases the P_{O_2} of the water surrounding the gills, and this brings about the fall in gill resistance that enhances the flow of blood through them.

Two features of the anatomy of the circulatory system are worthy of comment. The origin of the efferent pseudobranchial artery from the rostral side of the epibranchial loop which encircles the first gill slit ensures that the blood supply to the retina and brain is derived from a gill slit which is ventilated both at rest and during activity.

If we examine the spacing of the five or six afferent branchial arteries along the length of the ventral aorta in elasmobranch fish it appears that in bottom-living forms such as skates and rays the first two vessels are separated from the last three by the entire length of the ventral aorta. Active swimming forms like *Mustelus* (Parker, 1887) and *Heptanchus* (Daniel, 1934) have their afferent branchial arteries more evenly spaced at their origins. These differences may have a functional significance. We may speculate that at rest the blood that is ejected from the conus arteriosus will tend to be thrown forwards into the first and second afferent branchial arteries, thereby favouring the perfusion of the first two gill slits ventilated by the spiracle. The location of the common stem of the posterior three arteries at each side of the base of the ventral aorta and the oblique angle of its entry into this vessel may hinder the passage of blood to the third, fourth and fifth gill slits. The rise in ventral aortic blood pressure which exercise is known to cause, and the concomitant decrease in vascular resistance of the posterior gills which is postulated in this paper, ensures (we may suppose) an increase in the flow of blood through the gills when the respiratory demands of the mass of swimming muscle that lies behind them increases. Pelagic species which swim for a greater part of their lives have the spiracle reduced or absent; their respiratory and circulatory systems do not show this subdivision into anterior and posterior regions associated respectively with rest on the substratum and forward progression through the water.

SUMMARY

1. In *Raja rhina* the entire cardiac output is distributed to the afferent branchial arteries by two stem arteries on each side. In each of nine skates (1·5 to 7·5 kg.) the total blood flow to gills on one side was measured simultaneously by means of two electromagnetic flow probes implanted on the stem arteries.

2. At rest the average cardiac output was about 21 ml./kg. min. and blood flow was distributed almost equally to the anterior and posterior stem arteries.

3. During short periods of free swimming the proportion of blood flow through the posterior stem invariably increased. This increase is apparently correlated with increased water flow past these gills during swimming.

4. It is postulated that the selective change of blood flow with short exercise is caused by a decrease of vascular resistance of the posterior gills.

5. The data are discussed in relation to the bottom-living habits of skates and the alternate pathways of the respiratory water current through the spiracles at rest and through the mouth and spiracles during swimming. Comparisons are made with other elasmobranchs in regard to the configuration of the branchial vessels in relation to probable patterns of water flow past their gills.

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