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INTRINSIC VASOMOTION IN THE DOGFISH GILL

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It has been reported that fish may match the extent of their respiration to their need for gaseous exchange by failing to ventilate certain gills. One third of the respiratory movements of the lamprey (*Lampetra fluviatilis*), when in oxygenated tap water, consist of contractions of the anterior two or three branchial chambers only. In partially deoxygenated water the anterior five and finally all of the branchial chambers become active (Wikgren, 1953). In *Raja batis* water is taken in through the spiracles during rest and perfuses only the anterior four gill pouches. When swimming, water enters through the mouth as well and perfuses the posterior gills (Darbishire, 1907). The dogfish *Scyliorhinus canicula* has a similar division of the spiracular and buccal inflows between the anterior and posterior gills (Hughes, 1960). In the eel, van Dam (1938) reports that during rest only the gills of one side may be ventilated.

As the gill circulations are in parallel the possibility exists that venous blood may pass unchanged through the unventilated gills and into the dorsal aorta there to lower the tension of oxygen in the blood passing to the tissues. The mammalian lung also consists of many respiratory units in parallel but failure to ventilate part of it does not cause systemic hypoxaemia because alveolar perfusion is precisely matched to alveolar ventilation. This adjustment is largely effected by the changes in vascular resistance that are mechanically imposed by changes in intrathoracic pressure. However, anoxia itself causes pulmonary vessels to constrict both in the intact mammal and in the isolated lung, and Fishman (1961) concludes, in his extensive review of the subject, that this constitutes a fine control mechanism that shunts the blood from poorly ventilated to well ventilated areas.

The possibility exists that a similar mechanism operates in fish so that local anoxia causes a rise in vascular resistance and a diversion of blood to better ventilated gills. Some findings on the response of the branchial vessels of the dogfish to anoxia are presented in this paper; a brief report of this work has already been published (Satchell, 1961*a*).

MATERIALS AND METHODS

Mounting and perfusion

Twenty-six specimens of *Squalus acanthias* varying from 3 to 6 lb. in weight were used. They were restrained under water in a rectangular tank by clamps on the snout and back. The mouth was sewn closed and water was fed to the pharynx through spiracular cannulae from a constant level reservoir. Water temperatures varied from

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8 to 18° C. but remained within 1° C. throughout any one experiment. The fish were anaesthetized (Tricain Sandoz 0·05 mg./kg.) and usually curarized (D-tubocurarine 1·8 mg./kg.).

Anoxia

In some initial experiments the fish was made anoxic by perfusing it for 2 min. with deoxygenated sea water in the manner described in a previous paper (Satchell, 1961*b*). It was subsequently found that identical responses could be evoked in curarized fish by stopping the flow of water to the pharynx for 2 min. The deeper and slower respiration caused by curtailing the water flow cannot occur in the curarized preparation. The hypercapnia that must accompany anoxia evoked in this way did not appear to have any separate effect and stopping the flow of water to the pharynx was the method used in all subsequent experiments.

Recording

Records were made on a four-channel pen writer; blood pressures were recorded from above and below the gill circulation. Ventral aortic pressures were sampled from a cannula inserted through one innominate artery. Dorsal aortic blood pressures were recorded from a 25 cm. length of 1 mm. polythene tubing inserted along the dorsal aorta from the tail end of the fish so that the tip of the cannula lay in the region where the epibranchial arteries enter. After the cannulation was completed the fish was heparinized. The cannulae were connected to two P23AA Statham pressure transducers. The dynamic performances of the transducers and their connexions were made similar by suitable damping and assessed by analysing the oscillographic display of a vertical pressure release achieved by bursting stretched rubber dam (MacDonald, 1960). The response was flat to 7 cyc./sec.; the heart rate varied from 6 to 38 per min. The outputs of the pressure transducers were amplified by carrier-wave amplifiers and displayed on the pen writer. The manometers were calibrated repeatedly throughout the experiment by recording known pressures above the water surface from a burette.

The changes in blood content in the gills were followed by using an OCP71 phototransducer (maximum sensitivity at 1·55 μ) and a Perspex rod illuminator placed across the 2nd gill lamella. The output, after d.c. amplification, was displayed on the pen writer. Anoxia never failed to evoke an apparent increase in the amount of light transmitted through the gill to the phototransducer. The possibility that such a result might be due to a spectral change involved in the conversion $HbO_2 \rightarrow Hb$, was excluded in a preliminary experiment in which a narrow-band filter (Eel 626) with a peak of transmission at 573 mμ was placed in the light path. With light of this wavelength the conversion $HbO_2 \rightarrow Hb$ would lead to greater absorption and a drop in the signal from the transducer as if the gill had become more opaque. In fact the signal increased during anoxia, as before, and it was concluded that if spectral changes are recorded they are overridden by the larger change in blood content.

The electrocardiogram was recorded in the manner described in an earlier paper (Satchell, 1960) and displayed on the fourth channel of the pen writer.

RESULTS

The trace reproduced in Fig. 1 shows, from above downwards, the changes in dorsal aortic blood pressure, ventral aortic blood pressure, gill opacity and heart rate, evoked by stopping the water flow to the pharynx of an anaesthetized curarized dogfish for 2 min.

The heart slowed from 38 to 20 per min. and the dorsal aortic mean blood pressure, calculated as diastolic plus one-third of the pulse pressure, fell from 16·3 to 12·4 mm Hg. The systolic pressure fell from 17·3 to 14·1 mm Hg. Following the return of perfusion, the heart returned to its normal rate within 3 min., and the dorsal aortic blood pressure increased to a peak value (mean pressure 22·2 mm. Hg, systolic pressure

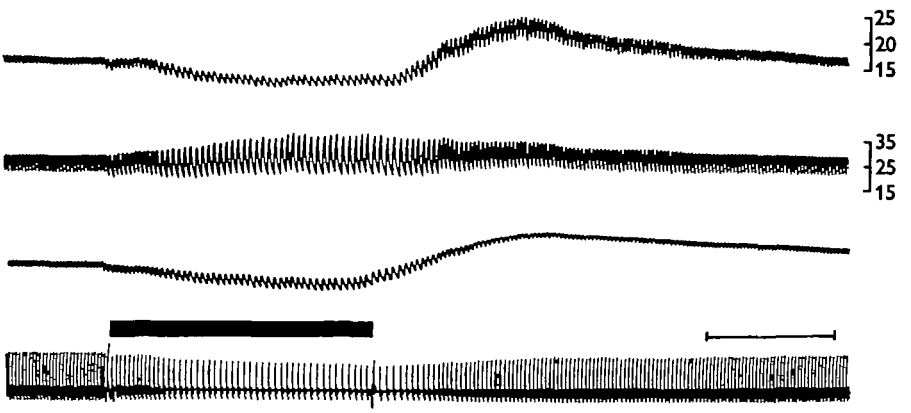

Fig. 1. Cardiovascular response of *Squalus acanthias* to 2 min of anoxia. Traces from above down, dorsal aortic blood pressure, ventral aortic blood pressure, gill opacity (increasing opacity upwards), E.C.G. Black inset = duration of anoxia. Time calibration = 1 min. Pressure calibrations = mm. Hg. 18° C.

24·9 mm. Hg) during the second minute and then slowly returned to normal. Pulse pressure in the dorsal aorta increased greatly from 1·4 mm Hg before anoxia to 4·0 mm Hg during the peak of this post-anoxic hypertension. These changes resemble closely those described as following the perfusion of the pharynx with deoxygenated water for two minutes (Satchell, 1961*b*). They differ chiefly in the failure of the heart to accelerate beyond the resting rate during the post-anoxic period; in many of the experiments in this series this acceleration was seen. The maximum rate attained doubtless varies from fish to fish with the overall level of vagal tone.

Ventral aortic blood pressure increased during the 2 min. of anoxia (mean 24·1–27·1 mm Hg, systolic 29·6–37·9 mm Hg). In this record diastolic pressures showed a slight fall during anoxia; in other experiments diastolic pressure either fell, rose, or remained the same, depending on the intensity of the bradycardia. Following the return of perfusion and the acceleration of the heart, ventral aortic mean pressure increased further to 28·5 mm. Hg during the peak of the post-anoxic hypertension. At this time the ventral aortic systolic pressure had fallen to 35·3 mm. Hg, the higher mean pressure arising from the increase in the diastolic levels.

In the opacity record, increase in opacity caused an upward deflexion of the pen,

and each ventricular contraction can be seen to have caused a transient increase as the compliant lamellae were briefly distended. During anoxia these rhythmic increases in opacity were set on a steadily descending base line as the gill became paler. Following the return of perfusion the gill became steadily more opaque as though flushed with blood. The return to the pre-anoxic level of opacity had not been fully achieved by the end of the record presented in Fig. 1. Similarly, the pulse pressures in the dorsal aorta were still slightly greater than before anoxia. Full recovery usually occurred by the eighth minute.

The divergent trends in the pressure changes in the dorsal and ventral aorta could be repeatedly elicited and were always observed. They were also present in uncurarized

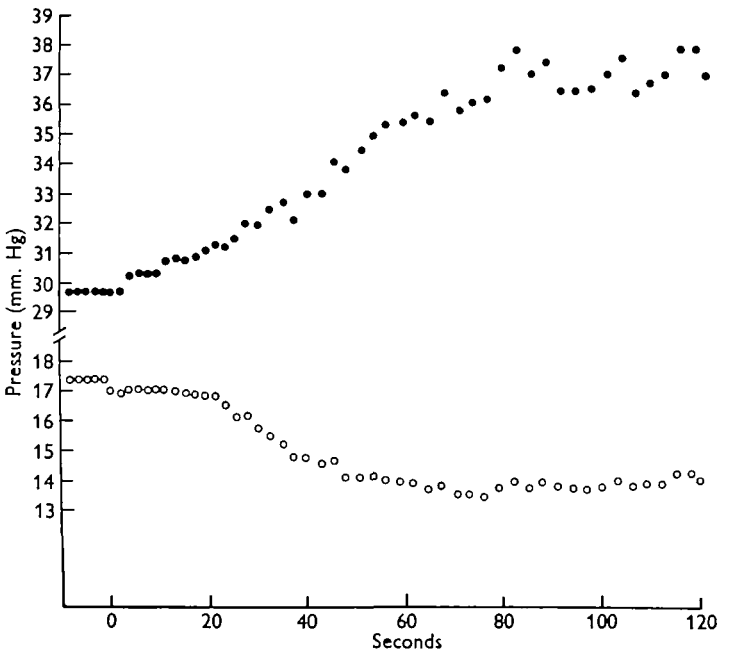

Fig. 2. Changes in systolic pressures in the dorsal and ventral aorta during 2 min. of anoxia. ●, dorsal aorta; ○, ventral aorta. 18° C.

fish breathing normally. They are presented as a graph in Fig. 2. which shows the dorsal and ventral aortic systolic pressures throughout a 2 min. period of anoxia. The vertical distance between the two curves reflects the magnitude of the pressure drop across the gills. The drop in systolic pressure increased from 12·3 to 23·1 mm. Hg (88% increase). The drop in mean pressure increased from 7·9 to 14·7 mm. Hg (86% increase). Almost all of the divergence in pressure was achieved by the end of the first minute of anoxia.

These findings point to a remarkable disparity between the changes in pressure in two parts of a continuous haemodynamic system separated only by the branchial circulation. This was further demonstrated when pairs of dorsal and ventral aortic pressure pulses were recorded at faster paper speeds. In Fig. 3, pairs of such pulses recorded (1) before, (2) at the end of the anoxic period, and (3) 4·3 min. after this, have been photographically superimposed, using the QRS deflexion of the E.C.G. and

the 20 mm. Hg pressure calibration line for alignment. The traces have been deliberately chosen from an experiment in which the fall of dorsal aortic pressure was less intense than usual in order that the declining phases of the dorsal aortic pulses before and during anoxia might span a similar range of pressures. The instant when the aortic valves closed is visible as the incisura in the lower traces. Following this, pressures in the dorsal aorta could only decline by the outflow of blood through the peripheral capillaries. Thus the slope of the declining phase of the dorsal aortic pulse reflects the rate at which blood is leaving the arterial system and hence the peripheral resistance. This point will be taken up again in the discussion. Fig. 3 shows that the dorsal aortic trace taken during anoxia (2), though starting from a lower systolic peak, declined more slowly than the pre-anoxic record (1) and after 5 sec. intersected it. The inference is that during anoxia there was an increase in peripheral resistance resulting in a slower outflow of blood from the arteries to the veins.

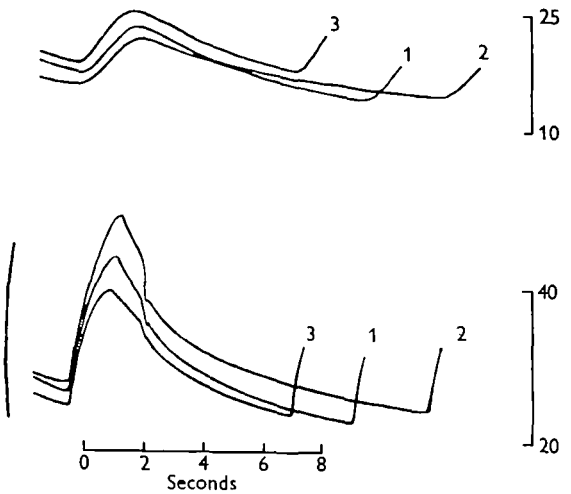

Fig. 3. Superimposed pairs of pressure pulse recordings taken in the dorsal aorta (upper traces) and ventral aorta (lower traces). 1, Before anoxia, 2, at end of 2 min anoxia; 3, 4·3 min. after anoxia. Calibrations = mm. Hg. Line of pen curvature on left. 11° C. Traces retouched to make good losses incurred in photography.

The pulse traces in Fig. 3 also demonstrate the reciprocal nature of the pressure changes in the dorsal and ventral aortae. The ventral aortic pressure during anoxia (trace 2) was consistently higher than traces 1 and 2 at each phase of the cardiac cycle whilst for the dorsal aorta trace 2 lay below the other two traces for more than half its course. During recovery from anoxia this relationship between aortic pressures was reversed (trace 3), so that the ventral aortic pressure was now lower than, and the dorsal aortic pressure higher than the pre-anoxic levels.

By subtracting the dorsal aortic blood pressure measured at 0·2 sec. intervals throughout a cardiac cycle from the simultaneously occurring ventral aortic blood pressure, curves of the change in pressure drop across the branchial circulation have been constructed. Fig. 4 shows three such curves constructed from records taken before, during, and after anoxia. During anoxia (B) the pressure drop was greater throughout the cardiac cycle; conversely after anoxia (C) the pressure drop was less

throughout the cardiac cycle. The instant at which the pressure in the ventral aorta was 30 mm. Hg has been marked on the curves by an arrow. This demonstrates that similar perfusion pressures driving blood through the branchial circulation were accompanied by an increased pressure drop during anoxia and a decreased pressure drop after it. The ventral aortic pressure fell from 30 to 20 mm. Hg in 3·1 sec. before anoxia, 4·8 sec. during anoxia and 3·7 sec. after anoxia, showing that the slowest rate of decline of ventral aortic pressure accompanied the greatest drop of pressure across the gills.

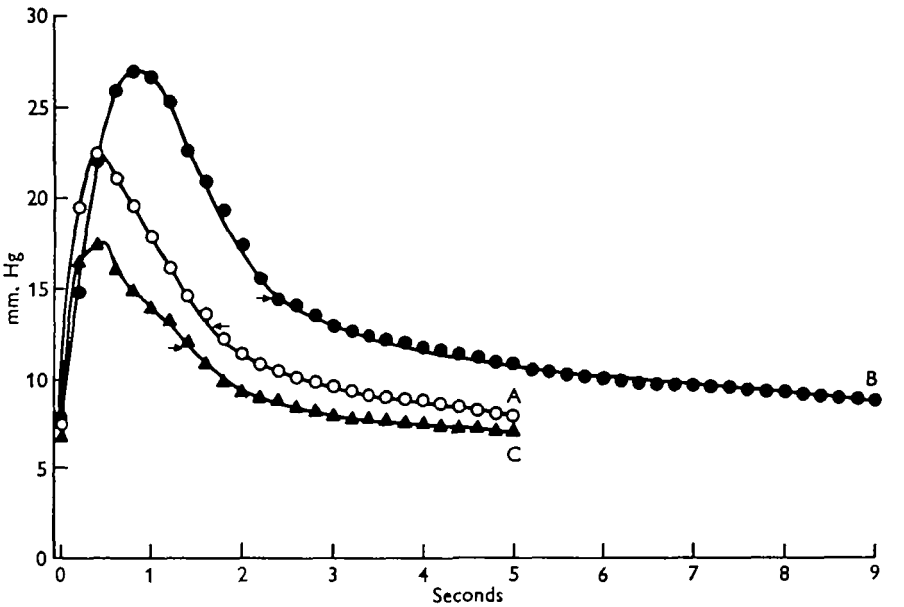

Fig. 4. Curves of differential blood pressure across the gills of *Squalus acanthias*, derived by subtracting successively determined dorsal aortic blood pressures from their corresponding values of ventral aortic pressure. A, Before; B, during; C, after anoxia. Arrow in each curve shows the instant at which the blood pressure in the ventral aorta was 30 mm. Hg. 11° C.

The pulse pressure in the dorsal aorta may be expected to increase if the heart slows or the stroke volume increases. The records of gill opacity suggest that the stroke volume does increase during the bradycardia and this was confirmed qualitatively by visual inspection of the exposed heart. Conversely, branchial vasoconstriction may be expected to diminish the dorsal aortic pulse pressure; peripheral vasoconstriction will also diminish it by decreasing the amount of blood that can flow away in each cardiac cycle. The pulse pressure is thus subject to four cardiovascular changes two of which will tend to increase it and two to decrease it. In some experiments (but not in that portrayed in Fig. 1) the pulse pressure in the dorsal aorta diminished during the period of anoxia as that in the ventral aorta increased. This is demonstrated in Fig. 5 and offers a further example of the reciprocal nature of the pressure changes in the dorsal and ventral aorta. The two are inversely correlated ($r = -0{\cdot}95$, $n = 13$, $P > 0{\cdot}001$). The probable reason that this inverse correlation is not demonstrated in all experiments is that in some the increases in pulse pressure caused by bradycardia and increased systolic ejection override the decrease due to resistance changes.

The role of the vagus in the response to anoxia

The orthosympathetic nervous system does not extend into the head in elasmobranchs (Young, 1933). Thus any changes in the gill vessels that might be effected by the autonomic nervous system must be due to parasympathetic activity and hence mediated by the IXth and Xth cranial nerves. Their role was investigated in two types of experiment. In some fish, all the branchial branches of the IXth and Xth nerves

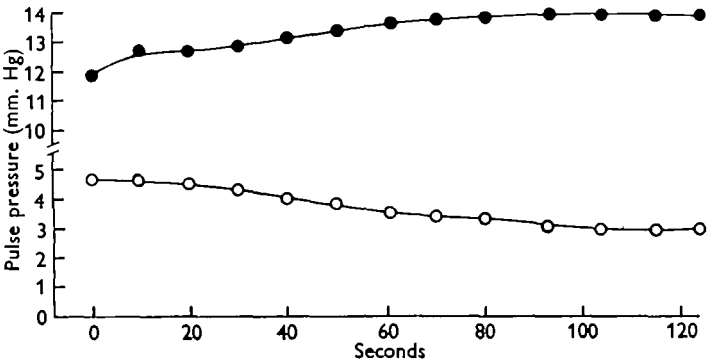

Fig. 5. Changes in pulse pressure in dorsal and ventral aorta during 2 min. anoxia. ●, Ventral aorta; ○, dorsal aorta. 9° C.

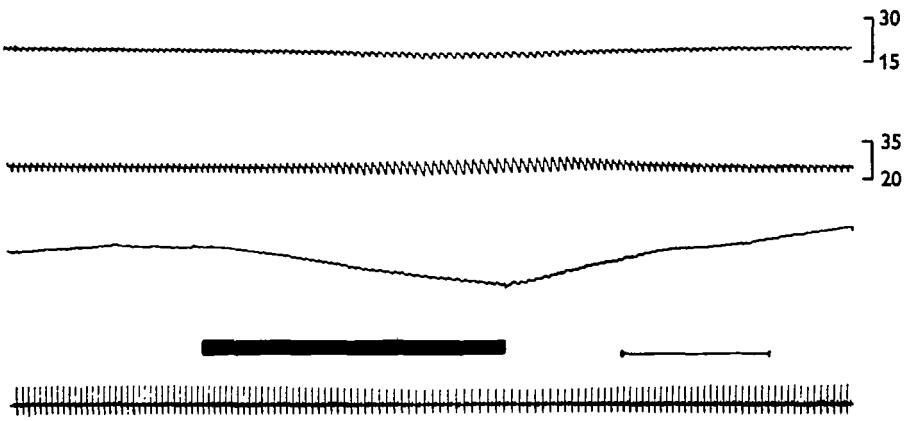

Fig. 6. Cardiovascular response of *Squalus acanthias* to two min. of anoxia; branchial branches of IX and X nerves cut. Traces from above downwards, dorsal aortic blood pressure, ventral aortic blood pressure, gill opacity, E.C.G. Black inset = duration of anoxia. Time calibration = 1 min. Pressure calibrations = mm. Hg. 10° C.

were cut and the response to anoxia was re-assessed. In others, parasympathetic activity was blocked with atropin (0·02 mg./kg.). Fig. 6 shows the response in a fish with the branchial nerves cut. The bradycardia in these circumstances was less intense and slower in onset (Satchell, 1961*b*) but there was still an elevation of blood pressure in the ventral aorta and a fall in blood pressure in the dorsal aorta; the increase in gill transparency was still present. Similar changes in pressure and opacity were seen in atropinized fish. In a previous paper (Satchell, 1961*b*) it was stated that blood pressure in the dorsal aorta did not fall during anoxia in the atropinized fish. There is, however,

a slight fall which is scarcely manifest in some preparations and was previously overlooked. The changes are much less obvious than in the normal fish but the fact that they occur at all when the branchial nerves have been cut suggests either that they originate in some part of the vascular system other than the gills, or that they are an intrinsic response of the gill vasculature. If the response is intrinsic to the gill, the evidence from the atropinized fish implicates the vascular smooth muscle directly rather than the operation of some local reflex.

DISCUSSION

When a dogfish is subjected to anoxia for 2 min. the cardiovascular responses include bradycardia, a rise in mean pressure in the ventral aorta, a fall in mean pressure in the dorsal aorta, and, in consequence, an increase in the pressure drop across the gill circulation. These findings are consistent with an increase in the resistance of the gill vessels; it remains to be discussed whether they could satisfactorily be explained by any other combination of cardiovascular changes. A possible alternative hypothesis is that anoxia causes peripheral vasodilation and hence the drop in dorsal aortic blood pressure, accompanied by an increase in cardiac output which in turn could account for both the rise in ventral aortic pressure and the increased pressure drop across the gill circulation.

It has already been noted that the declining phase of the dorsal aortic pressure pulse has a more gentle slope during anoxia than before, suggesting vasoconstriction rather than vasodilation in the peripheral vascular beds. But a more decisive argument against the existence of a peripheral vasodilation is provided by those experiments in which the dorsal aortic pulse pressure diminished during anoxia. If it is argued that peripheral vasodilation occurred, then three influences all tending to increase the pulse pressure would be operating at once. The first, bradycardia, is well established and operates by increasing the time available for blood to leave the arterial tree. The second, increase in stroke volume, has been directly observed and increases the pulse pressure because the increased systolic discharge raises the systolic pressure. The third, vasodilation, is postulated and would increase pulse pressure by permitting a faster outflow and thus a lower diastolic pressure. Indeed Wiggers (1949) placed decreased peripheral resistance, decreased heart rate, and increased systolic discharge at the head of his table of changes that increase the pulse pressure of the mammal. The established fact that pulse pressures decrease suggests that the unknown change in peripheral resistance is in such a direction and of such an intensity as to counteract the other two, i.e. that vasoconstriction occurs. Possibly relevant here are the observations of Rodionov (1959) whose drop-recorder studies of blood flow through the gut of the catfish strongly suggest an autonomic control of the visceral blood vessels, and of Hall (1929) that anoxia causes contraction of the smooth muscle of the spleen of the menhaden, *Brevoortia tyrannus*.

No investigator has yet devised a satisfactory method of determining the cardiac output of a fish, but certain conclusions may be tentatively drawn despite this. Once the aortic valves have closed, an event indicated in Fig. 3 by the incisura on the ventral aortic trace, all blood that is to pass through the branchial circulation before the next cardiac cycle must come from the emptying of the elastic reservoir of the ventral aorta

and branchial arteries. If it is assumed that the volume decrease per unit of pressure drop is not changed by anoxia then the decline from one particular value of ventral aortic pressure to another lower one represents the outflow of a specific though unknown quantity of blood. It has been noted (Fig. 4) that the decline from 30 to 20 mm. Hg took 3·1 sec. before anoxia and 4·8 sec. during anoxia. Thus a reduced flow of blood during this part of the cardiac cycle was associated with an enhanced pressure drop across the gills. The argument assumes that anoxia does not alter the volume-elasticity relations of the elastic reservoir. Remington, Hamilton, Wheeler & Hamilton (1949) infer that a variety of pharmacological agents active on smooth muscle do not alter the elastic properties of the arteries of the dog to any significant extent. However, if the reservoir vessels of the fish were themselves to participate in the vasoconstrictor response which it is argued occurs beyond them, the volume decrease per unit of pressure drop would be even less, and the flow during ventricular diastole even smaller.

The most satisfactory hypothesis thus remaining is that anoxia constricts some part of the branchial vasculature. Because the increase in resistance lies between the dorsal and ventral aortae the reciprocal nature of the pressure changes at these two sites is explained. It remains to enquire what parts of the branchial circuit undergo constriction. The phototransducer traces offer some evidence on this. It is likely that the gill opacity record is dominated by changes in blood content of the respiratory lamellae. These closely packed thin-walled, transparent, blood-filled leaflets are arranged with their long axes in the light path, there being a complete series on each side of the gill. The changes in the gill opacity (Fig. 1) so closely follow the change in dorsal aortic blood pressure as to suggest that the constrictor zone is pre- rather than post-lamellar. Were the constriction occurring beyond the lamellae their opacity might be expected to follow the ventral aortic blood-pressure change and to increase during anoxia. In 1882 Dröscher described a series of small muscular bulbous swellings on the fine arteries leading to the gill lamellae of elasmobranchs and these may well be the sphincters that mediate the vasoconstriction of anoxia. They would thus correspond to the pre-capillary sphincters of the mammalian lung which are believed to be responsible for the constriction that occurs in response to perfusion with venous blood of very low oxygen content (Fishman, 1961).

In mammals local anoxia is known to dilate vascular beds as diverse as the cerebral circulation (Opitz & Schneider, 1950), skin (Krogh, 1929), the coronary vessels (Hilton & Eicholtz, 1925), intestine (Bean & Sidky, 1957) and muscle (Golliwitzer-Meier & Dunker, 1953). That it should constrict the vascular beds of both lungs and gills suggests that the response is characteristic of organs that supply oxygen rather than consume it.

SUMMARY

1. The circulatory response of anaesthetized curarized *Squalus acanthias* to anoxia of 2 min. duration is described. Anoxia was evoked either by perfusing the pharynx with deoxygenated sea water or by stopping the flow of normal sea water.

2. Ventral aortic blood pressures increased (systolic: 29·6–37·9 mm. Hg; mean: 24·1–27·1 mm. Hg) during a period of 2 min. Concurrently dorsal aortic blood pressures fell (systolic: 17·3–14·1 mm. Hg; mean: 16·3–12·4 mm. Hg). The heart

slowed from 38 to 20 per min. The opacity of the gill, recorded with a phototransducer, decreased.

3. Comparison of simultaneous pulse traces from the dorsal and ventral aortae demonstrated that the pressure drop across the gills was increased during anoxia and decreased after it.

4. In some, but not all, experiments the dorsal aortic pulse pressure diminished as the ventral aortic pulse pressure increased.

5. Neither cutting all the branchial nerves nor atropinization completely abolished these responses.

6. Possible explanations of these findings are discussed; it is concluded that anoxia evoked a constriction of some prelamellar elements in the branchial vessels and that the response was, at least in part, intrinsic to the gill.

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REFERENCES

BEAN, J. W. & SIDKY, M. M. (1957). Effect of low oxygen on intestinal blood flow, tonus and motility. *Amer. J. Physiol.* **189**, 541–7.

van DAM, L. (1938). On the utilization of oxygen and regulation of breathing in some aquatic animals. Dissertation. Gröningen.

DARBISHIRE, A. D. (1907). On the direction of the aqueous current in the spiracle of the dogfish. *J. Linn. Soc. Lond. Zool.* **80**, 86–94.

DRÖSCHER, W. (1882). Beiträge zur Kenntnis der histologischen Strucktur der Kiemen der Plagiostomen. *Arch. Naturgesch.* **48**, 120–77.

FISHMAN, A. P. (1961). Respiratory gases in the regulation of the pulmonary circulation. *Physiol. Rev.* **41**, 214–80.

GOLLWITZER-MEIER, K. & DUNKER, E. (1953). Einfluss des Sauerstoffmangels auf die Reaktionsänderungen und auf die Durchblutung des arbeitenden Warmblutermuskels. *Pflüg. Arch. ges. Physiol.* **256**, 288–95.

HALL, F. G. (1928). Blood concentration in marine fishes. *J. Biol. Chem.* **76**, 623–31.

HILTON, R. & EICHOLTZ, F. (1925). The influence of chemical factors on the coronary circulation. *J. Physiol.* **59**, 413–25.

HUGHES, G. M. (1960). The mechanism of gill ventilation in the dogfish and skate. *J. Exp. Biol.* **37**, 11–27.

KROGH, A. (1929). *The Anatomy and Physiology of the Capillaries*, 422 pp. New Haven: Yale University Press.

McDONALD, D. A. (1960). *Blood Flow in Arteries*, 328 pp. London: Edward Arnold.

OPITZ, E. & SCHNEIDER, M. (1950). Über die Sauerstoffversorgung des Gehirns und den Mechanismus von Mangelwirkungen. *Ergebn. Physiol.* **46**, 126–260.

REMINGTON, J. W., HAMILTON, W. F., WHEELER, N. C. & HAMILTON, W. F., Jr. (1949). Validity of pulse contour method for calculating cardiac output of the dog with notes on effect of various anaesthetics. *Amer. J. Physiol.* **159**, 379–84.

RODIONOV, I. M. (1959). Reflex regulation of cardiac activity in fish. II. Reflex influences on the heart and gut vessels, caused by stimulating the baroceptors of the branchial vessels. (In Russian.) *Biul Eksp. Biol. Med.* **48**, 11–14.

SATCHELL, G. H. (1960). The reflex co-ordination of the heart beat with respiration in the dogfish. *J. Exp. Biol.* **37**, 719–31.

SATCHELL, G. H. (1961*a*). Vasoconstriction in the gills of the dogfish in response to anoxia. *Proc. Univ. Otago med. School.* **39**, 32–4.

SATCHELL, G. H. (1961*b*). The response of the dogfish to anoxia. *J. Exp. Biol.* **38**, 531–43.

WIGGERS, C. J. (1949). *Physiology in Health and Disease*, 1242 pp. Philadelphia: Lea and Febiger.

WIKGREN, B. (1953). Osmotic regulation in some aquatic animals with special reference to the influence of temperature. *Acta zool. fenn.* **71**, 1–102.

YOUNG, J. Z. (1933). The autonomic nervous system of Selachians. *Quart. J. Micr. Sci.* **15**, 571–624.